SHAPING THE INFORMATION CHANNEL: MOLECULES, CELLS, AND EXPERIMENTS

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A Dissertation
Presented to the Faculty
of Princeton University
in Candidacy for the Degree
of Doctor of Philosophy

RECOMMENDED FOR ACCEPTANCE
by the Department of
Physics

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September 2016
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Abstract

An important and ubiquitous question in the physics of biological systems is how an information transmission channel is shaped and optimized by a careful exploitation of the structural details of the underlying system. This dissertation explores three instances of this central question, at the levels of molecules, cells, and scientific experiments.

The first chapter is focused on the molecular gateway of cellular signaling, where a ligand concentration reflects some extracellular condition, and a receptor works as an information channel by binding the ligand and consequently activating the downstream signaling pathway. In this sense, the ligand-receptor binding event is the initial information channel of the signaling process, setting an upper bound on the final amount of information transmitted. We investigate how the information flow through the ligand-receptor binding can be optimized by the choice of the kinetic parameters, and how it is limited by various constraints of the cellular environment.

Once the signal is initiated, it needs to be relayed until it reaches the final target in the cell. Such signal transduction pathways are built on a network of specific protein-protein interactions, and one of the important determinants of interaction specificity is shape complementarity. In the second chapter, we aim to characterize the statistical properties of the ensemble of proteins in the cell, in terms of the shapes of protein surfaces. We study the intrinsic dimensionality of the space of surfaces, and discuss how it is linked to the properties of individual protein surfaces, revealing the non-trivial organization of the shape space.

The third chapter concerns the optimization of the design of a scientific experiment, viewing the experiment as an information channel through which the scientist collects data about the natural world. Specifically, we consider a behavioral neuroscience experiment where the aim is to infer the psychometric function, which governs the stimulus-dependent decision-making behavior of an animal. We demonstrate how the experimental design can be optimized to reach the desired precision of measurement with a minimal amount of data,
using an adaptive, closed-loop algorithm that selects the most informative stimulus at each trial.
Acknowledgements

I am forever grateful to Bill Bialek for attracting me into the ever-exciting field of biophysics, and for always being the finest example of what and how much a physicist can do for biology. Every meeting we had was a substantial jump in my learning curve, where questions were developed carefully and to the fullest. I am also indebted to Bill for letting me navigate freely through different questions over the years, and for supporting me to attend meetings and conferences during my graduate study, through which I expanded my view and learned how to communicate.

I am deeply grateful to Jonathan Pillow, who showed me how research was done in real time, and guided me through a most rewarding learning experience. The collaboration was always lively and stimulating, and quickly evolved into a very interesting series of projects, the whole path of which I can only call fortunate; it was with Jonathan that I rediscovered my strengths. I thank Jonathan for the weekly meetings, for the timely doses of encouragement, and for the genuine enthusiasm that was infectious.

I thank Anne-Flo Bitbol, who has been an indispensable friend and mentor since when we started the protein surface project together, for sharing so much of her time, fun, and wisdom. It was a joy to have a collaboration in which you could discuss every detail of what you were thinking about, and always knew that you would get the most thoughtful feedback. I thank Ned Wingreen, who cared to come and hear, and was there to witness the days and milestones of my graduate research. I also thank Curt Callan and Josh Shaevitz for their insightful advice.

At the Icahn lab, I was lucky to be among the amazing peers of the biophysics theory group: I learned from Gordon Berman, Farzan Beroz, David Borenstein, Chase Broedersz, Michelle Castellana, Mark Ioffe, Dima Krotov, Zhiyuan Li, Ben Machta, Leenoy Meshulam, Armita Nourmohammad, Kanaka Rajan, David Schwab, Zach Sethna, DJ Strouse, Thibaud Taillefumier, Misha Tikhonov, and Bin Xu. At the Neuroscience Institute, I am grateful for the sense of community I enjoyed from the interaction with the wonderful people in the Pillow
group: Mikio Aoi, Adam Charles, Lea Duncker, Angela Langdon, Nick Roy, and Anqi Wu.

On a separate note, I thank Jung Yoon Choi for the numerous lunch-table conversations, which eventually led me to venture into the field of neuroscience, and have now evolved into a most exciting collaboration, which is not a part of this dissertation. I also thank Ilana Witten for being an encouraging mentor on this project.

All this would not have been possible without the endless love and support from my family, to whom I owe my optimism. Finally, Sung-Jin, thank you for believing in me much more than I do myself.
Prior presentations

There is no publication or preprint associated with this dissertation at the point of submission. However materials from this dissertation have been publicly presented at the following conferences:

1. Bak JH and Bialek W. “What limits information flow through ligand-receptor binding?”
   - International Physics of Living Systems (iPoLS) Meeting, Munich, Germany, July 2014
   - American Physical Society (APS) March Meeting, Baltimore, MD, March 2013

2. Bak JH, Bitbol AF, and Bialek W. “Characterizing the statistical properties of protein surfaces”
   - APS March Meeting, Baltimore, MD, March 2016
   - BPS Annual Meeting, Los Angeles, CA, March 2016
   - q-bio Conference, Blacksburg, VA, August 2015
   - APS March Meeting, San Antonio, TX, March 2015

3. Bak JH and Pillow JW. “Active learning of psychometric functions with multiple-alternative responses”
   - Computational Neuroscience Society (CNS) Annual Meeting, Jeju Island, South Korea, July 2016
   - Computational and Systems Neuroscience (CoSyNe) Meeting, Salt Lake City, UT, February 2016
I would like to separately thank my collaborators William Bialek, Anne-Florence Bitbol, and Jonathan Pillow, as well as Anne Churchland who provided data for the active learning project. I also would like to acknowledge support through the Samsung Scholarship, the NSF program Physics of Living Systems (PoLS), and the Compton Fund from the department of physics at Princeton.
To my family, old and new.


## Contents

Abstract . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . iii  
Acknowledgements . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . v  
Prior presentations . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . vii  
List of Tables . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . xiv  
List of Figures . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . xv  

**Introduction**  

1 Limits of information flow through ligand-receptor binding  

1.1 Introduction . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 3  
1.2 The simplest versions of the problem . . . . . . . . . . . . . . . . . . . . . . . 5  
   1.2.1 The equilibrium case . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 8  
   1.2.2 More sites, more states . . . . . . . . . . . . . . . . . . . . . . . . . . . 9  
   1.2.3 The kinetic case . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 12  
1.3 Crosstalk . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 14  
   1.3.1 Formulating the problem . . . . . . . . . . . . . . . . . . . . . . . . . . 14  
   1.3.2 Optimizing the specific information . . . . . . . . . . . . . . . . . . . . 18  
1.4 Output noise . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 23  
   1.4.1 The catalytic rate . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 24  
   1.4.2 The diffusion rate . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 26  
1.5 The cooperative dimer . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 29
1.5.1 At equilibrium ....................................................... 30
1.5.2 Kinetic case ......................................................... 32
1.6 Discussion ............................................................. 36

Technical Notes .......................................................... 40
1.A Small noise approximation for non-monotonic response .......... 40
1.B Computing the time-averaged variance ............................. 43
  1.B.1 The monomer .................................................... 44
  1.B.2 The cooperative dimer .......................................... 45
1.C Cooperativity in the kinetic case .................................. 47
  1.C.1 Small $F$ limit .................................................. 47
  1.C.2 Large $F$ limit ................................................ 49
1.D Integrals in the crosstalk approximation .......................... 52
1.E Fluctuation-dissipation of target activation ...................... 54

2 Characterizing the statistical properties of protein surfaces 57
  2.1 Introduction ....................................................... 57
  2.2 Surface objects and operations ................................ 61
    2.2.1 Scope of dataset .......................................... 61
    2.2.2 Working with triangulated surfaces ...................... 66
  2.3 Intrinsic dimensionality ....................................... 69
    2.3.1 Measuring the intrinsic dimensionality of the dataset ... 70
    2.3.2 Dimensionality of the space of surface objects .......... 73
    2.3.3 Dimensionality of synthetic datasets ..................... 78
  2.4 Scales of shape variation ...................................... 85
    2.4.1 Measuring surface curvature at finite scopes ............ 86
    2.4.2 Surface curvature of proteins ............................. 88
  2.5 Discussion ........................................................ 99
2.A Geodesic path on the triangular mesh ........................................ 103
2.B Sampling the shape space ...................................................... 105
2.C Comparing two surface objects: the Procrustes distance ................. 108
2.D Comparing multiple surface objects: Alignment .......................... 113
2.E More on dimension estimate .................................................. 115
   2.E.1 The issue of sampling ...................................................... 117
   2.E.2 Implementation with real data: the scale matters ..................... 118
   2.E.3 The systematic error: effect of the dataset size $N$ .................... 123
2.F More on the synthetic curves ................................................. 124
   2.F.1 Generating planar curves as Gaussian random variables .............. 124
   2.F.2 Generating polymers with ideal chain models ......................... 125
2.G Scope-dependent surface curvature: error from random fluctuation .... 128
3 Active learning of psychometric functions ................................... 132
  3.1 Introduction ........................................................................... 132
  3.2 Multinomial logistic model ................................................... 135
     3.2.1 Classical MNL .............................................................. 135
     3.2.2 MNL with lapse .......................................................... 138
  3.3 Posterior inference .................................................................. 139
     3.3.1 Gaussian approximation to posterior .................................... 140
     3.3.2 Sampling the posterior .................................................... 142
  3.4 Choosing the most informative stimulus .................................... 148
     3.4.1 InfoMax with Laplace approximation ................................. 148
     3.4.2 Sampling-based InfoMax ................................................ 152
  3.5 Results ................................................................................. 153
     3.5.1 Test with simulated data ................................................ 153
     3.5.2 Application to psychological experiments ............................ 156
List of Tables

2.2.1 List of protein structures (1/3) ............................................. 63
2.2.2 List of protein structures (2/3) ............................................. 64
2.2.3 List of protein structures (3/3) ............................................. 65
<table>
<thead>
<tr>
<th>Figure</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Ligand-receptor kinetics for a single-sited receptor (monomer)</td>
<td>6</td>
</tr>
<tr>
<td>1.2</td>
<td>Monomer with a finite catalytic rate</td>
<td>24</td>
</tr>
<tr>
<td>1.3</td>
<td>Schematic diagrams of the receptor: monomer and dimer</td>
<td>29</td>
</tr>
<tr>
<td>1.4</td>
<td>Cooperative dimer at equilibrium</td>
<td>30</td>
</tr>
<tr>
<td>1.5</td>
<td>Catalytic rates of a receptor with multiple sites</td>
<td>32</td>
</tr>
<tr>
<td>1.6</td>
<td>Cooperative dimer with time-averaged information</td>
<td>34</td>
</tr>
<tr>
<td>1.7</td>
<td>Informative regimes of a cooperative dimer</td>
<td>36</td>
</tr>
<tr>
<td>1.8</td>
<td>Optimizing information capacity of a cooperative dimer</td>
<td>37</td>
</tr>
<tr>
<td>2.1</td>
<td>Definition of surface objects</td>
<td>66</td>
</tr>
<tr>
<td>2.2</td>
<td>Intrinsic dimensionality</td>
<td>70</td>
</tr>
<tr>
<td>2.3</td>
<td>Errors in estimating intrinsic dimensionality</td>
<td>74</td>
</tr>
<tr>
<td>2.4</td>
<td>Dimensionality of protein surface curves</td>
<td>77</td>
</tr>
<tr>
<td>2.5</td>
<td>Correlation length and the intrinsic dimensionality</td>
<td>80</td>
</tr>
<tr>
<td>2.6</td>
<td>Synthetic polymer model</td>
<td>82</td>
</tr>
<tr>
<td>2.7</td>
<td>Scope-dependent surface curvature of a protein</td>
<td>89</td>
</tr>
<tr>
<td>2.8</td>
<td>Curvature statistics at different scales</td>
<td>90</td>
</tr>
<tr>
<td>2.9</td>
<td>Distribution of mean surface curvature</td>
<td>92</td>
</tr>
<tr>
<td>2.10</td>
<td>Joint distribution of principal curvatures</td>
<td>93</td>
</tr>
<tr>
<td>2.11</td>
<td>Scales of surface curvature variation, from the correlation function</td>
<td>96</td>
</tr>
<tr>
<td>2.12</td>
<td>Oscillations in the correlation function of curvature</td>
<td>98</td>
</tr>
</tbody>
</table>
2.S1 Variance of aligned curves ........................................... 114
2.S2 Dimensionality of surface patches ................................. 116
2.S3 Mesh density: how fine is fine enough? ............................ 118
2.S4 Different methods for dimension estimates ....................... 119
2.S5 Regression examples for the dimension estimates ............... 121

3.1 Inferring the psychometric function ............................... 143
3.2 Semi-adaptive MCMC for sequential data ......................... 145
3.3 InfoMax active leaning ............................................... 150
3.4 Performance of the algorithm: simulation ......................... 154
3.5 Performance of the algorithm: subsampling actual dataset ....... 157
3.6 Exploiting the full stimulus space ................................. 158
Introduction

I had a great fortune of entering and exploring the field of theoretical biophysics at its very exciting moment, and of being encouraged to navigate through different ideas as I developed my own set of interests. During my time at Princeton I worked on three different research projects at the boundary of physics and biology: on the limits of information flow through ligand binding, on the statistical properties of protein surfaces, and on the optimal design of psychophysical experiments based on Bayesian inference. Although these projects seemed detached from one another at their beginnings, featuring a wide array of traditional disciplines from molecular biology to behavioral neuroscience, gradually it became clear they were all resonating around a central theme.

The theme of my graduate research, or the hyperplane in the question space that spans my three vertices, is to understand how biological information transmission is connected to the structural details of the system. For example, it is remarkable how in a cell crowded with numerous proteins and organelles, every protein is doing exactly what it should be doing, so as to keep the life going. How, and how accurately, can a cell ensure that relevant information reaches the correct target? What is the limit of information transmission, set by the physical details of the interaction? This family of questions led me to work on the first two problems discussed in this dissertation (Chapters 1 and 2). As in many other problems in biological systems, the problem of cellular information transmission naturally spans multiple scales: in this dissertation I focus on investigating the structures of the building blocks of the signaling
pathway at the molecular level (first the ligand-receptor binding, then the protein-protein interaction), to eventually answer questions about the limit on performances at the cell level.

On the other hand, the task of efficient information processing is not the job of the biological molecules or cells only; it is also how science works. In biological science, this reverse process is often very critical, in which scientists try to learn about natural systems by conducting experiments and accumulating data. In the later part of my graduate research, I have extended my interest to the problem of how to collect information efficiently through experiments, in the context of the decision-making behavior of animals, by actively designing the structure of data acquisition (Chapter 3). Although in a very different context compared to the other two problems, this problem provides another important and interesting case where the information transmission process is optimized by a careful exploitation of the structure of the system.

The main part of this dissertation is written in the first person plural “we”, emphasizing the fact that each chapter of the dissertation is the result of a wonderful collaboration. My use of “we” includes Bill in Chapter 1, Anne-Flo and Bill in Chapter 2, and Jonathan in Chapter 3, without any one of whom this dissertation would not have been possible.
Chapter 1

Limits of information flow through ligand-receptor binding

1.1 Introduction

Cells monitor their environment by maintaining concentrations of certain signaling particles, so that external changes are transmitted into the cell in the form of changes in the concentration. There are specialized proteins (the “receptors”) that recognize and bind these signaling particles (the “ligands”). For example, the calcium signaling system plays an important role in many contexts in the body (Berridge et al., 2000), where the signaling pathway involves calmodulin, a ubiquitous receptor for calcium (Pepke et al., 2010; Faas et al., 2011), although there are many other calcium-binding proteins with similar structures (Lewit-Bentley and Réty, 2000).

In many cases, the ligand-receptor binding is the first event in a much larger cellular signaling pathway, which provides a channel between the input signal (ligand concentration) and the output response (catalyzed by the activated receptors). The amount of information that flows through this channel can be quantified by the mutual information, defined in terms of the joint distribution of the input and output variables (Shannon, 1948; Cover and Thomas,
And because information can only be lost as it is passed further on, this initial step sets an upper limit to all downstream information. Therefore a meaningful problem to consider is to quantify the capacity of the information channel given the joint distributions, and to investigate how the information capacity can be attained by optimizing the parameters of the model (Bialek, 2012). Also, because the number of the receptor molecules at work are often small, and the molecular processes involved are inherently noisy, there may be a pressure for the signaling system to develop efficient mechanisms to extract the relevant information from a limited number of molecules and with noisy signals, which might operate near the physical limits of sensing (Berg and Purcell, 1977; Bialek and Setayeshgar, 2005; Mora and Wingreen, 2010). Beneath this parsimonious picture is also the assumption that the system would be limited by the costs of computation (Mehta and Schwab, 2012; Lan et al., 2012; Sartori et al., 2014), although we do not consider the energetic constraints explicitly here, except by placing constraints in the number of receptor molecules.

The optimization problem for the capacity of the information channel has received a considerable attention in the past decade, and was studied in depth especially in the context of genetic regulation (Tkačik et al., 2008a,b,c, 2009; Walczak et al., 2010; Tkačik et al., 2012; Sokolowski and Tkačik, 2015), reviewed by Tkačik and Walczak (2011). There are also a rich volume of works that studied information transmission in biochemical networks, in cases where the signal is non-stationary (Tostevin and ten Wolde, 2009, 2010; Mugler et al., 2010; Govern and ten Wolde, 2012; Mancini et al., 2013) or multiplex (de Ronde et al., 2011).

Our work is aligned to the previous efforts, but lays an important additional groundwork by examining the formulation of the problem and highlighting important features of the problem that were often neglected in earlier works. Specifically, we observe that the simple versions of the problem are ill-posed, in the sense that the information-optimizing solutions are driven to pathological limits. In particular, we demonstrate that optimization in realistic versions of the problem may be limited by various factors, such as the finite catalytic rate of the output reaction (Pepke et al., 2010), the finite diffusion rates of the molecules involved.
(Bialek and Setayeshgar, 2005; Aquino and Endres, 2010), or the presence of spurious interactions (Cepeda-Humerez et al., 2015; Mora, 2015; Friedlander et al., 2015). Furthermore, armed with the insights from the case of the receptor with a single binding site, we consider the case of the cooperative dimer and elucidate how the information flow can be optimized in a qualitatively different way by exploiting the additional layer of structure. We demonstrate that the effect of cooperativity is more than just scaling and “sensitizing” the response curve. We also show that although the solution is still subject to the limits set by the finite timescales of related events, cooperativity lets the receptor perform near the physical limits by working in a new regime with a greater flexibility, which also echoes with the observations of Bialek and Setayeshgar (2008).

1.2 The simplest versions of the problem

Let us consider the simplest receptor, with a single binding site for a ligand; this receptor has only two states, either occupied or unoccupied. The ligand is assumed to be present at a fixed concentration $c$, which is unaffected by the binding events (providing an infinite bath). Let us say that the binding rate is $k_+ c$, and the unbinding rate $k_-$, where $k_+$ and $k_-$ are kinetic parameters of the ligand-receptor interaction.

\[
\text{Receptor } \xrightarrow{k_+ c} \xleftarrow{k_-} \text{(Receptor + Ligand)}
\]  

(1.1)

If there are $N$ such receptors, the natural way to quantify the response of the system is to count the number of occupied receptors as a function of ligand concentration, $n(c)$. The ligand-receptor system is governed by the simple equation

\[
\frac{dn(t)}{dt} = k_+ c(N - n(t)) - k_- n(t).
\]  

(1.2)
We can write down the equilibrium response as

\[ \bar{n}(c) \equiv \frac{Nc}{K + c} \equiv Np, \tag{1.3} \]

where \( K \equiv k_- / k_+ \) is the concentration scale of binding, and \( p \) denotes the equilibrium probability for each receptor to be occupied. Even in a steady state, however, the actual response of any given receptor population \( n(c) \) would keep fluctuating around the equilibrium, due to the stochastic nature of the molecular process. In other words, the ligand-receptor channel is inherently noisy.

How much information can be transmitted through this noisy channel? This can be quantified by the mutual information between the input concentration \( c \) and the output response \( n \), defined in terms of their joint distribution as

\[ I(n; c) = \int dn \int dc P(n, c) \cdot \log \left( \frac{P(n, c)}{P_n(n) P_c(c)} \right), \tag{1.4} \]

where \( P_c(c) \) and \( P_n(n) \) are the marginal distributions of \( c \) and \( n \) respectively (Shannon, 1948). Because the joint distribution can be decomposed as \( P(n, c) = P(n|c)P_c(c) \), maximizing the information transmission is equivalent to finding the optimal match between the two distributions: the marginal input distribution \( P_c(c) \), and the input-output relation \( P(n|c) \) determined by the ligand-receptor interaction (Bialek, 2012). For our formulation, it will be
convenient to write the mutual information as the difference of two entropies,

$$I(n; c) = S[P_n(n)] - \langle S[P(n|c)] \rangle_{P_c(c)}$$

(1.5)

which also makes it clear that information is quantified by the amount of reduced uncertainty.

For analytical tractability we will work in the small-noise approximation, which was already studied in the context of gene regulation and was shown to place a lower bound on the true capacity of the channel (Tkačik et al., 2008a). Let us start by reviewing the original formulation, which assumes that the mean response $\bar{n}(c)$ is a monotonic function of the input $c$. If the conditional distribution $P(n|c)$ is narrow (small noise) and symmetric around the mean $n = \bar{n}(c)$, the output entropy can be approximated to the leading order as

$$S[P_n(n)] = -\int dc P_c(c) \int dn P(n|c) \log P_n(n)$$

$$\approx -\int dc P_c(c) \log P_n(\bar{n}(c)).$$

(1.6)

On the other hand, the probability distribution can be written in terms of the mean output $\bar{n}$ under the small-noise approximation, to give $dc P_c(c) = d\bar{n} P_n(\bar{n})$. For a more careful discussion see Tkačik et al. (2008a). In order to keep the problem analytically tractable, the conditional distribution $P(n|c)$ is approximated as a Gaussian with the variance $\sigma_n^2(c)$. The entropy of the input-output relation then evaluates to $S[P(n|c)] = \frac{1}{2} \log(2\pi e \sigma_n^2(c))$. Also, rewriting the output noise $\sigma_n$ in terms of its mean $\bar{n}$, the mutual information is

$$I(n; c) \approx -\int d\bar{n} P_n(\bar{n}) \left( \log P_n(\bar{n}) + \log \sqrt{2\pi e \sigma_n(\bar{n})} \right).$$

(1.7)

Varying $P_n(\bar{n})$, one can find that the optimal distribution is inversely related to the noise level, $P_{opt}(\bar{n}) \propto 1/\sigma_n(\bar{n})$. The information (in bits) is optimized to be $I_{opt}(n; c) = \log_2(Z/\sqrt{2\pi e})$, where $Z = \int d\bar{n}/\sigma_n$ is the information partition function. Under the small noise approximation, further optimization of the information transmission
is equivalent to maximizing $Z$. The partition function $Z$ can also be written in terms of the effective noise level $\delta_{\text{c eff}}$ of the input,

$$Z = \int_{0}^{c_{\text{max}}} \frac{dc}{\delta_{\text{c eff}}}, \quad \frac{1}{\delta_{\text{c eff}}} \equiv \left| \frac{\partial \bar{n}}{\partial c} \right| / \sigma_{n},$$

(1.8)

where $c_{\text{max}}$ parametrizes the upper limit of the dynamic range of the input (lower limit is automatically at zero ligand). Intuitively, $Z$ can be interpreted as the number of distinguishable input or output levels.

An extended treatment for the case of a non-monotonic response is provided in Section 1.A, which will be important later, when we consider the possibility that a receptor with multiple binding sites is activated by a partial occupation of its sites. But for now, the classical version will serve our purpose.

1.2.1 The equilibrium case

Now we examine the information-optimizing solutions in various scenarios of ligand-receptor interaction, where the problem has just been reduced to maximizing the partition function $Z$.

Let us first consider the “snapshot” of the ensemble, where the effective output of the channel is determined by the number of ligand-bound receptors at a single instant of time. In order to calculate the channel capacity under the small-noise approximation, we want to characterize the output noise with its variance $\langle (\delta n)^2 \rangle$, and translate it as an effective noise level $\delta_{\text{c eff}}$ of the input concentration as

$$\frac{1}{(\delta_{\text{c eff}})^2} = \frac{1}{\langle (\delta n)^2 \rangle} \left| \frac{\partial \bar{n}}{\partial c} \right|^2.$$

(1.9)

At equilibrium, the noise variance of the two-state system is given by

$$\langle (\delta n)^2 \rangle = Np(1 - p),$$

(1.10)
where \( p = c/(c + K) \) is the mean response of the single receptor molecule, introduced in equation (1.3). The first derivative (the “sensitivity”) of the response with respect to the input is

\[
\frac{\partial \bar{n}}{\partial c} = \frac{N}{c} p(1 - p).
\] (1.11)

Note the scaling \( \delta c_{\text{eff}} \propto 1/\sqrt{N} \) for \( N \) independent receptor molecules. Putting together, the information partition function is computed as

\[
Z = \sqrt{N} \int_0^{c_{\text{max}}} dc \frac{1}{\sqrt{p(1 - p)}} \frac{1}{c} p(1 - p)
= \sqrt{N} \int_0^{c_{\text{max}}} dc \left[ \frac{cK}{(c + K)^2} \right]^{1/2}
= \sqrt{N} \int_0^{c_{\text{max}}/K} dx \frac{1}{\sqrt{x} (1 + x)};
\] (1.12)

the integral has a closed-form solution, which is

\[
Z = \sqrt{N} \cdot 2 \tan^{-1} \sqrt{\frac{c_{\text{max}}}{K}}.
\] (1.13)

This \( Z \) is maximized when \( K \to 0 \), at which \( Z \to \sqrt{N}\pi \), meaning that we get more information by maintaining strong binding and focusing on the lower part of the dynamic range. At the same time, the maximal value of \( Z \) at \( K \to 0 \) is independent of the dynamic range \( c_{\text{max}} \), because the optimal solution only focuses on the range which is far below the upper bound. This solution means that information transmission is maximized in a pathological limit where binding is infinitely strong, and is not limited by the small concentration of signaling molecules.

### 1.2.2 More sites, more states

More generally, we can consider the case of receptors with multiple states, each corresponding to a different number of bound molecules. Solving the full optimization problem becomes
intractable very quickly due to the increased number of parameters, but by looking at a snapshot of the system at equilibrium, we can make a simple yet strong argument based on properties of the canonical ensemble. Let us consider the Hamiltonian

$$H = \sum_s n_s \epsilon_s - \frac{1}{\beta} \sum_s n_s b_s \log c,$$

where $n_s$ is the number of receptors, $\epsilon_s$ the energy level, and $b_s$ the number of bound ligands in state $s$. The input variable $c$ in the logarithm can always be made dimensionless, in units of some reference concentration. We assume that the output of the system is some linear combination of the individual states, $A \equiv \sum_s a_s n_s$, which is already a reasonable assumption but will be further justified later in Section 1.4. From the canonical ensemble, it is straightforward to show that

$$\frac{\partial \langle A \rangle}{\partial c} = \frac{1}{c} \left[ \langle AB \rangle - \langle A \rangle \langle B \rangle \right] = \frac{1}{c} \sigma_{AB}^2$$

where $B \equiv \sum_s b_s n_s$ arises naturally from the structure of the Hamiltonian. The symbol $\sigma_{AB}^2 \equiv \langle \delta A \cdot \delta B \rangle$ denotes the non-diagonal element in the covariance matrix. Then

$$\frac{1}{(\delta c_{\text{eff}})^2} = \frac{|\partial \langle A \rangle / \partial c|^2}{\sigma_{AA}^2} = \frac{\sigma_{BB}^2}{c^2} \left( \frac{(\sigma_{AB}^2)^2}{\sigma_{AA}^2 \cdot \sigma_{BB}^2} \right),$$

and Cauchy-Schwarz inequality states that the last factor $(\sigma_{AB}^2)^2/\sigma_{AA}^2 \cdot \sigma_{BB}^2$ is maximized to 1 if and only if the two quantities $A$ and $B$ are proportional to each other. Because the mutual information $I$ is a monotonic function of $Z$ which is the integral of $1/\delta c_{\text{eff}}$, we have

$$I \left( \sum a_s n_s; c \right) \leq I \left( \sum b_s n_s; c \right),$$

meaning that information readout is maximized when the output counts the total number of bound ligands.
On the other hand, we can show that this optimal output is a monotonic function of $c$ when all the binding sites are symmetric. For a single receptor with $m$ binding sites, the probability that it is in state $s$ can be written from the Hamiltonian as $ar{n}_s(c) \propto \exp(-\beta \epsilon_s + b_s \log c) \equiv \kappa_s c^{b_s}$, where we write $\kappa_s \equiv \exp(-\beta \epsilon_s)$. Assuming that the sites are fully symmetric (indistinguishable), we can write in terms of the number of sites occupied. The probability that $b$ out of $m$ sites of the receptor are occupied is

$$\bar{n}_b(c) = \frac{1}{W} \binom{m}{b} \kappa_b c^b,$$

where $W = \sum_{b'} \binom{m}{b'} \kappa_{b'} c^{b'}$. (1.18)

In this case, it is a straightforward exercise to show that the optimal output $\bar{A} = \sum_b b \cdot \bar{n}_b(c)$ is a monotonically increasing function of $c$:

$$\frac{\partial \bar{A}}{\partial c} = \frac{1}{c} \sum_b \left(b - \sum_{b'} b' \cdot \bar{n}_{b'}(c)\right)^2 \cdot \bar{n}_b(c) = \left\langle b - \left\langle b\right\rangle \bar{n}_b(c)\right\rangle \bar{n}_b(c) \geq 0,$$

where the final inequality is based on the fact that at fixed $c$, the set of values $\{\bar{n}_b(c)\}$ is a probability distribution over the set of possible values of $b$.

It follows from equation (1.15) that the optimal output $A$ satisfies $\frac{\partial A}{\partial c} = \langle (\delta A)^2 \rangle / c$. Note that the result from the small-noise approximation (1.8) is not limited to the monomer case; it can be applied here as long as the mean response is monotonic. Since the optimal output $\bar{A}(c)$ was just shown to be monotonic, we get

$$Z = \sqrt{N} \int_0^{c_{\text{max}}} dc \frac{1}{\sqrt{\langle (\delta A)^2 \rangle / c}} = \sqrt{N} \int_0^{c_{\text{max}}} dc \frac{1}{c \sqrt{\langle (\delta A)^2 \rangle / c}}.$$  

(1.20)

In the trivial limit where the binding sites are independent and identical, the energy level would be proportional to the number of bound ligand $b$ up to a constant, as $\epsilon_b \sim b \epsilon_1$. Consequently, we may write $\kappa_b = \exp(-\beta \epsilon_b) \equiv (\kappa_1)^b$, where $\kappa_1$ is a constant independent of the occupation state. Then it is possible to renormalize the input variable as $c \rightarrow c / \kappa_1$, where $\kappa_1$ corresponds to the concentration scale of binding $K$ of a monomer, which reflect the
assumption that the receptor is effectively a package of \( m \) independent monomers. Therefore the mean occupancy of any state \( \bar{n}_b \) is a function of the renormalized variable \( c/K \), and so is the linear combination \( \bar{A} \); in this trivial limit \( Z \) is still maximized at \( K \to 0 \). The solution is no longer trivial when the energy levels associated to the occupations of the sites are no longer independent of one another. We return to this problem in Section 1.5, where we focus on the effect of cooperativity.

1.2.3 The kinetic case

Now let us get back to the case of the single-site receptor, but consider the time average of the state of the receptors over time \( T \), rather than a single snapshot of the ensemble. Intuitively, everything is the same as before except the noise variance is reduced by a factor \( \sim \tau_c/T \), where \( \tau_c \) is the correlation time of the receptor occupancy. More precisely, in the long-time limit \( T \to \infty \), the noise variance is dominated by the zero frequency component of the power spectrum (see Section 1.B for details)

\[
\langle (\delta n)^2 \rangle = \frac{S_n(\omega = 0)}{T} = Np(1-p) \frac{2\tau_c}{T},
\]

(1.21)

where the correlation time of the single binding site is

\[
\frac{1}{\tau_c} = k_+ c + k_- = k_+ (c + K).
\]

(1.22)

Because the binding rate \( k_+ \) is ultimately limited by the rate of diffusion of the ligands (Berg and Purcell, 1977), we may hold \( k_+ \) constant as fixed by the environment, and view \( K = k_-/k_+ \) as the only parameter of receptor kinetics in this case. So we write

\[
Z = \sqrt{\frac{NTk_+}{2}} \int_0^{c_{\text{max}}} \frac{dc}{c} \left[ \frac{cK}{(c + K)^2} \right]^{1/2} [c + K]^{1/2}
\]

(1.23)

\[
= \sqrt{\frac{NTk_+}{2}} \sqrt{K} \int_0^{c_{\text{max}}/K} \frac{dx}{\sqrt{x} \sqrt{1 + x}}.
\]

(1.24)
Note that now there is a factor of \( \sqrt{K} \) that scales the overall size of \( Z \), which reflects the advantage of having a shorter correlation time, and therefore a larger number of independent measurements in a given time window. This effect of having a shorter correlation time wins over the effect of reading from a wider dynamic range. To see this, we can evaluate \( Z \) in the small-\( K \) limit (\( K \ll c_{\text{max}} \)):

\[
Z \approx \sqrt{N T k_+ \sqrt{K}} \int_{c_{\text{max}}/K}^{c_{\text{max}}/K} \frac{dx}{x} \approx \sqrt{N T k_+ \sqrt{K}} \log \left( \frac{c_{\text{max}}}{K} \right),
\]  

(1.25)

and in this regime the square root wins over the log, driving the optimum outside the regime of small \( K \). On the other hand, in the large-\( K \) limit (\( K \gg c_{\text{max}} \)), we get

\[
Z \approx \sqrt{N T k_+ \sqrt{K}} \int_{0}^{c_{\text{max}}/K} \frac{dx}{\sqrt{x}} \approx \sqrt{N T k_+ c_{\text{max}}}.  
\]

(1.26)

More careful calculations show that \( Z \) can actually be written as a closed-form solution

\[
Z = \sqrt{2 N T k_+ K} \cdot \log \left( 1 + \frac{2 c_{\text{max}}}{K} + \sqrt{\left( 1 + \frac{2 c_{\text{max}}}{K} \right)^2 - 1} \right)
\]

(1.27)

which is a monotonically increasing function of \( K \), saturating at \( K \to \infty \) to

\[
Z_{\text{max}} = 2 \sqrt{2 N T k_+ c_{\text{max}}}. 
\]

(1.28)

This solution means that in the kinetic case, information is limited by the maximum rate at which molecules can arrive (\( k_+ c_{\text{max}} \)), which in turn is limited by the diffusion of ligands.

In this limit, \( Z \) counts the effective number of binding events that occurs within the integration time, and the system is driven to the linear response regime as \( K \to \infty \). In this sense we get an “event-counting” solution. In particular, this solution requires that there is a separation of scales

\[
c_{\text{max}} \ll K,
\]

(1.29)
or equivalently $1/k_+ c_{\text{max}} \gg 1/k_-$, such that unbinding occurs much faster than binding (each binding event is sharply distinguishable). In other words, to reach the bound of this solution requires another pathological limit in which the unbinding rate is infinitely fast ($K \to \infty$). Because the lifetimes of the ligand-receptor complexes are infinitely brief, taking this solution seriously, information transmission in this regime must depend on being able to have finite effect in infinitesimal time.

### 1.3 Crosstalk

Within the simple pictures, we were led to conclude that the information transmission was maximized at some extreme limits of receptor kinetics. But would these solutions be robust under realistic constraints, for example, in the presence of spurious and non-specific interactions that creates a “crosstalk” across different channels?

#### 1.3.1 Formulating the problem

Let us suppose that there are two types of receptor-ligand pairs, with subscripts 1 and 2 respectively, that interact specifically within each type. In general, the activity of the receptor $n_1$ may depend not only on its cognate ligand concentration $c_1$, but also on $c_2$. These cross-interactions are non-specific, and it is assumed that there is a finite free energy difference $s$ between the the specific and non-specific interaction. For simplicity, let us also assume that the two specific interactions and the two non-specific interactions have the same affinities ($K$ and $sK$) respectively. Let us also work with single receptors of respective types ($N = 1$), knowing the relation $Z \propto \sqrt{N}$ in the current framework. Then the mean occupancies of the two receptors are

$$
\bar{n}_1 = \frac{c_1/K + c_2/sK}{1 + c_1/K + c_2/sK}, \quad \bar{n}_2 = \frac{c_2/K + c_1/sK}{1 + c_2/K + c_1/sK}.
$$

(1.30)
Generalizing this model to multiple receptor-ligand pairs, we may write

\[ \bar{n}_i = \frac{\sum_j c_j / s_{ij} K}{1 + \sum_j c_j / s_{ij} K} \]  

(1.31)

with \( s_{ii} = 1 \) for the correct pairs. It is clear that the system can be decoupled in terms of a new set of coordinates \( w_i = \sum_j c_j / s_{ij} \), with which \( \bar{n}_i = (w_i / K) / (1 + w_i / K) \).

We will start by showing that maximizing the full joint mutual information, between the all-ligand input and the all-receptor output, is in fact trivial because of this decomposition. The joint mutual information \( I(n; c) \) between \( n = \{n_1, \cdots n_m\} \) and \( c = \{c_1, \cdots c_m\} \) is

\[ I_{\text{joint}} = I(n; c) = H(n) - \langle H(n|c) \rangle_{P(c)} . \]

(1.32)

Assuming that the conditional distribution \( P(n|c) \) is Gaussian with the mean \( \bar{n} \) and the covariance matrix \( \Sigma_n(n) \), its entropy is \( H(n|c) = \log \sqrt{(2\pi e)^m \det \Sigma_n} \). Since different \( n_i \)'s correspond to the states of different receptors, the output noises \( \delta n_i \) are uncorrelated, and the determinant of the covariance matrix is simply \( \det \Sigma_n = \prod_{i=1}^m \langle (\delta n_i)^2 \rangle \). Working in the small-noise limit, we can expand the integrals near \( \bar{n} \) as in the single-interaction case. We can also assume \( d^m \bar{n} P(\bar{n}) = d^m c P(c) \). In this limit, the joint information is

\[ I_{\text{joint}} = I(n; c) = -\int d^m \bar{n} P(\bar{n}) \log P(\bar{n}) \]

\[ -\int d^m c P(c) \frac{1}{2} \log ((2\pi e)^m \det \Sigma_n) + \cdots \]

\[ = -\int d^m \bar{n} P(\bar{n}) \log \frac{P(\bar{n})}{\sqrt{(2\pi e)^m \det \Sigma_n}} + \cdots \]  

(1.33)

where (\( \cdots \)) are terms that vanish as \( \det \Sigma_n \) decreases. As a direct multivariate generalization of the simple problem, this joint information is optimized with

\[ P(\bar{n}) = \frac{1}{Z} \frac{1}{\sqrt{\det \Sigma_n(\bar{n})}} , \quad Z = \int d^m \bar{n} \frac{1}{\sqrt{\det \Sigma_n}} \]  

(1.34)
where $I_{\text{joint}} = \log_2[Z/(2\pi e)^{m/2}]$. Changing the variables to the decoupled coordinates $w$, and using the Jacobian matrix $J = \partial \tilde{\mathbf{n}} / \partial \mathbf{w}$, we can rewrite $Z$ as

$$Z = \int d^m w \frac{\det J}{\sqrt{\det \Sigma_n}} = \prod_{i=1}^m \int dw_i \frac{d\tilde{n}_i/dw_i}{\sqrt{\langle (\delta n_i)^2 \rangle}}. \quad (1.35)$$

Since both $J$ and $\Sigma_n$ are diagonal, $Z$ becomes a product of $m$ independent integrals. Mathematically, this is equivalent to having $m$ independent, crosstalk-free receptor-ligand pairs.

We already know the solution for this case: at equilibrium, it is optimal to have $K \to 0$ (tight binding).

However, the joint information is only relevant when it can be assumed that different receptor-ligand pairs actually work collectively, as if they form a set of “combined channels” distributed over multiple pairs. In real systems, different ligands may mean entirely different signals, and different receptors may connect to entirely different pathways. A more plausible scenario is where the system is interested in maximizing the specific information, which is the sum of mutual information through each “correct” interaction averaged over all crosstalk (the “incorrect” inputs).

$$I_{\text{specific}} = \sum_{i=1}^m \langle I(n_i; c_i) \rangle_{P(\{c_{j\neq i}\}|c_i)}. \quad (1.36)$$

In general, $n_i = n_i(c)$, meaning that the activity of a receptor may depend on the concentrations of all ligands in the system, not only its cognate partner’s. But here we are interested in the channel-specific information averaged over all other effects, which is the marginal input-output relation of the specific pair. Let us define the marginal conditional distribution $P(n_i|c_i)$ in the following sense,

$$P(n_i) = \int d^m c P(n_i|c) P(c) = \int dc_i P(n_i|c_i) P(c_i),$$
which leads to

\[ P(n_i|c_i) \equiv \int d^{n-1}\{c_j \neq i\} P(n_i|c)P(\{c_j \neq i\}|c_i). \quad (1.37) \]

The small-noise approximation in this context is to assume that \( P(n_i|c_i) \) is narrow around the mean receptor activity \( \bar{n}_i(c_i) \). It naturally includes the small crosstalk condition, because the conditional distribution is marginalized over all effects of crosstalk. In other words, we are assuming that the relevant information is mostly conveyed through the specific interactions between the correct ligand-receptor pairs, while the non-specific interactions are small perturbations.

Let us further assume that \( P(n_i|c_i) \) is Gaussian with variance \( \sigma_i^2(c_i) \). Expanding the integrals in the limit where \( \sigma_i \) is small, and assuming the small-noise condition \( d\bar{n}_i P(\bar{n}_i) = dc_i P_i(c_i) \), we may write

\[
I(n_i; c_i) = -\int d\bar{n}_i P(\bar{n}_i) \log P(\bar{n}_i) - \int dc_i P_i(c_i) \log \sqrt{2\pi e\sigma_i^2(c_i)} + \cdots
\equiv -\int dc_i P_i(c_i) \left[ \log P_i(c_i) - \log \frac{f_i(c_i)}{\sqrt{2\pi e}} \right] + \cdots \quad (1.38)
\]

where (\cdots) are terms that vanish as \( \sigma_i^2 \) (the marginal noise) decreases. Here \( f_i(c_i) \) are known properties of the receptor-ligand kinetics, corresponding to (the inverse of) what we have called the effective noise level \( \delta_{\text{eff}} \) in the simple problem.

\[
f_i(c_i) \equiv \frac{\partial \bar{n}_i}{\partial c_i} \frac{1}{\sqrt{\sigma_i^2(c_i)}} \quad (1.39)
\]
The specific information in the small-noise limit is

\[ I_{\text{specific}} = -\sum_i \int dc_i P_i(c_i) \left[ \log P_i(c_i) - \log \frac{f_i(c_i)}{\sqrt{2\pi e}} \right] \]

\[ = -\sum_i \int dc_i P_i(c_i) \left[ \log \frac{P_i(c_i)}{f_i(c_i)/Z_i} - \log \frac{Z_i}{\sqrt{2\pi e}} \right] \]

\[ = \sum_i \log \frac{Z_i}{\sqrt{2\pi e}} - \sum_i D_{KL} \left( P_i(c_i) \bigg\| \frac{f_i(c_i)}{Z_i} \right) \]  

(1.40)

where \( Z_i = \int dc_i f_i(c_i) \) is the partition function specific to channel \( i \), and \( D_{KL} \) is the Kullback-Leibler divergence. The specific information is maximized when all the Kullback-Leibler divergences vanish, that is, when \( P_i(c_i) = f_i(c_i)/Z_i \) for each channel \( i \). Therefore, optimizing the specific information is equivalent to having each channel optimize its own information marginalized over all other inputs. If \( f_i(c_i) \) is symmetric over channels, then the solution \( P_i(c_i) \) is also symmetric.

### 1.3.2 Optimizing the specific information

What can the receptors do to maximize the specific information, under the presence of crosstalk? For simplicity, let us consider a system of two receptor-ligand pairs, labeled 1 and 2 respectively, and optimize the marginalized information for the pair 1. Generalization to multiple pairs is straightforward and will be discussed at the end of this section.

Recall that the specificity parameter \( s \) stands for the free energy difference between the specific and the non-specific interactions. Assuming that the effect of crosstalk is small, we may expand in \( 1/s \):

\[ \bar{n}_1(c_1) = \int dc_2 P_2(c_2) \frac{c_1/K + c_2/sK}{1 + c_1/K + c_2/sK} \]

\[ = p_1 + \frac{c_2}{sK} (1 - p_1)^2 + \cdots \]  

(1.41)
where \( p_1 = (c_1/K) / (1 + c_1/K) \). When the response is itself very weak, however, the effect of crosstalk may become comparable to the size of the cognate response, and the expansion over \( 1/s \) would break down. The small-crosstalk approximation is thus only valid above some cutoff

\[
c > \epsilon K, \quad \epsilon \approx \frac{\langle c_2 \rangle}{sK}.
\]

In the small-crosstalk limit, the marginal sensitivity is

\[
\frac{\partial \bar{n}_1}{\partial c_1} = \frac{p_1(1 - p_1)}{c_1} \left[ 1 - \frac{\langle c_2 \rangle}{sK} \cdot 2(1 - p_1) \right].
\]

The marginal variance can also be calculated from \( \sigma_1^2(c_1) = \langle (\delta n_1)^2 \rangle \), where we may average either over the ensemble or over time.

In order to simplify, we may assume that each receptor-ligand interaction has an identical kinetics \( f_1(c) = f_2(c) \), which means \( P_1(c) = P_2(c) = P(c) \) and \( \langle c_1 \rangle = \langle c_2 \rangle = \langle c \rangle \), allowing us to drop the subscripts when there is no confusion.

**The equilibrium case** Let us start with the snapshot at equilibrium. We are considering the variance of \( n_1(c_1) \), which is evaluated at a fixed \( c_1 \), and averaged over a variable \( c_2 \).

There are two contributions to the variance in this case, the shot noise of the output \( n_1 \) and the noise coming from the fluctuation of \( c_2 \):

\[
\langle (\delta n_1)^2 \rangle = \bar{n}_1(1 - \bar{n}_1) + \left| \frac{\partial \bar{n}_1}{\partial c_2} \right|^2 \langle (\delta c_2)^2 \rangle.
\]

But we can see from (1.41) that the contribution from the fluctuation of \( c_2 \) scales as \( 1/s^2 \), and is small compared to the shot noise in the small crosstalk limit. Dropping the subscripts, the leading terms of the output variance can be written as \( \langle (\delta n)^2 \rangle \approx \bar{n}(1 - \bar{n}), \) or

\[
\langle (\delta n)^2 \rangle \approx p(1 - p) \left[ 1 + \frac{\langle c \rangle}{sK} \frac{(1 - p)(1 - 2p)}{p} \right].
\]
Substituting from (1.39), (1.43) and (1.45), we compute

\[ f(c) \approx \frac{1}{c} \sqrt{p(1 - p)} \cdot \left[ 1 - \frac{\langle c \rangle (1 - p)(1 + 2p)}{2sKp} \right] \]

\[ \approx \frac{1}{c} \sqrt{\frac{c/K}{(1 + c/K)^2}} \cdot \left[ 1 - \frac{\langle c \rangle (1 + 3c/K)}{2sK(c/K)(1 + c/K)} \right], \]

which gives

\[ Z \approx Z^{(0)} - \frac{\langle c \rangle}{2sK} \int_{c_{\text{max}}/K}^{c_{\text{max}}/K} dx \cdot \frac{1}{\sqrt{x}(1 + x)} \approx \frac{2}{\pi} \sqrt{\frac{c_{\text{max}}}{K} \cdot K}, \quad (1.46) \]

with the lower cutoff \( \epsilon = \langle c \rangle /sK \) for the small crosstalk regime. We are interested in the limit where \( c_{\text{max}} \gg K \), in which the crosstalk-free information is maximized as \( Z^{(0)} \approx \pi - 2\sqrt{K/c_{\text{max}}}. \) We can calculate the last term in (1.46) in the same limit. It is enough to evaluate the expectation value \( \langle c \rangle \) from the crosstalk-free distribution

\[ \langle c \rangle \approx \frac{K}{Z^{(0)}} \int_0^{c_{\text{max}}/K} dx \cdot \frac{1}{\sqrt{x}(1 + x)} \approx \frac{2}{\pi} \sqrt{\frac{c_{\text{max}}}{K} \cdot K}, \quad (1.47) \]

and the integral evaluates to

\[ \int_{c_{\text{max}}/K}^{c_{\text{max}}/K} dx \cdot \frac{1 + 3x}{x^{3/2} (1 + x)^2} \approx \frac{2}{\sqrt{\epsilon}}. \]

Exact solutions for all integrals in this section are provided in Section 3.B. The divergence of this integral at the lower bound, as \( \epsilon \to 0 \), is eventually taken care of by the pre-factor \( 1/s \). Taken together, the effect of crosstalk in \( Z \) scales with \( 1/\sqrt{s} \) in the small-crosstalk limit.

Let us take a moment to make sure that the large-crosstalk contribution to the integral (below the cutoff \( \epsilon \)) is negligible. When \( c_1 < c_2/s \), the marginalized mean response expands differently as

\[ \bar{n}_1(c_1) \approx \left\langle \frac{c_2/sK}{1 + c_2/sK} \right\rangle + \frac{c_1}{K} \left\langle \frac{1}{(1 + c_2/sK)^2} \right\rangle \]

\[ \approx \langle p_2 \rangle + \frac{c_1}{K} \left\langle (1 - p_2)^2 \right\rangle \quad (1.48) \]

20
where the averages are over $P_2(c_2)$. It follows that $f_1(c_1)$ is a constant to the leading order in $c_1$. The contribution from this large-crosstalk limit is therefore $\int_0^\epsilon (\text{const.}) \sim \epsilon$. Since this large-crosstalk contribution scales linearly with $1/s$, it is indeed negligible compared to the small-crosstalk contribution that scales with $1/\sqrt{s}$.

Finally, in the limit where $c_{\text{max}} \gg K$, and when the effect of crosstalk is small, the partition function is

$$Z \approx \pi - 2\sqrt{\frac{K}{c_{\text{max}}}} - \sqrt{\frac{2}{\pi s}} \left(\frac{c_{\text{max}}}{K}\right)^{1/4}.$$  \hfill (1.49)

The optimal value of $K$ is finite and is determined weakly by crosstalk parameter $s$,

$$K_{\text{opt}} \approx \frac{c_{\text{max}}}{4(\pi s)^{2/3}},$$  \hfill (1.50)

where the information loss is $\Delta Z = (\pi - Z_{\text{opt}}) \approx 3/(\pi s)^{1/3}$. In the perfect specificity limit $s \to \infty$, we recover the solution for the simple problem, $K \to 0$ and $Z \to \pi$.

**The kinetic case** On the other hand, in the kinetic case where we average the state of the receptor over a window of time $T$, the output variance for a single receptor is $\langle (\delta n_1)^2 \rangle_T = (2\tau_c/T) \cdot \bar{n}_1 (1 - \bar{n}_1)$. Once again, the contribution from the fluctuation of $c_2$ is small compared to the shot noise. In the small-crosstalk limit, the correlation time marginalizes to

$$\tau_c = \frac{1}{k+K} \left\langle \frac{1}{(1 + c_1/K + c_2/sK)} \right\rangle_{P_2(c_2)} \approx \frac{1}{k+K} (1 - p_1) \left[ 1 - \frac{\langle c_2 \rangle_s}{sK} (1 - p_1) \right],$$  \hfill (1.51)

and the variance is (dropping the subscripts)

$$\langle (\delta n)^2 \rangle_T = \frac{2\tau_c}{T} \cdot \bar{n} (1 - \bar{n}) \approx \frac{2p(1 - p)^2}{Tk+K} \left[ 1 + \frac{\langle c \rangle}{sK} \frac{(1 - p)(1 - 3p)}{p} \right].$$  \hfill (1.52)
The rest of the calculation is similar to what was done in the equilibrium case. Substituting from equations (1.39), (1.43) and (1.52),

\[ f(c) \approx \sqrt{\frac{T_{k+K}}{2} \frac{\sqrt{p}}{c}} \left[ 1 - \frac{\langle c \rangle (1 - p)(1 + p)}{2sK} \right] \]

we get

\[ Z \approx Z^{(0)} - \frac{\langle c \rangle}{2sK} \sqrt{\frac{T_{k+K}}{2}} \int_{\epsilon}^{c_{\text{max}}/K} \frac{dx}{x^{3/2}} \frac{(1 + 2x)}{(1 + x)^{3/2}}. \]  

(1.53)

Evaluating at \( c_{\text{max}} \ll K \) where the crosstalk-free information \( Z^{(0)} \) is maximized, we get

\[ Z \approx \sqrt{2Tk_{+}c_{\text{max}}} \left( 1 - \frac{c_{\text{max}}}{2K} \right) - \frac{\epsilon}{2} \sqrt{\frac{T_{k+K}}{2}} \cdot \frac{2}{\sqrt{\epsilon}} \]

\[ \approx \sqrt{2Tk_{+}c_{\text{max}}} \left( 1 - \frac{c_{\text{max}}}{2K} - \frac{1}{\sqrt{8s}} \right), \]  

(1.54)

again with the small crosstalk cutoff \( \epsilon = \langle c \rangle / sK \). See Section 3.B for details of carrying out the integral. The expectation value evaluates to \( \langle c \rangle \approx c_{\text{max}} / 2 \) based on the crosstalk-free distribution. Once again, the large-crosstalk contribution scales with \( \sim 1/s \) and is negligible compared to these leading terms.

Contrary to the equilibrium result, to this order of approximation, \( Z \) is still maximized in the \( K \rightarrow \infty \) limit which was the solution for a single ligand-receptor pair. Even in the presence of crosstalk, the event-counting solution still maximizes the information flow, although it now ends up counting some spurious events that results in an information loss of \( \Delta Z \sim s^{-1/2} \).

**Multiple pairs** Finally, we show that this formulation generalizes to the case of multiple ligand-receptor pairs in a straightforward manner. When there are \( m \) types of ligand-receptor
pairs in the system, rather than just two, the mean occupation state of the receptor 1 is

\[ \bar{n}_1 = \left\langle \frac{c_1/K + \sum_{j=2}^m c_j/s_j K}{1 + c_1/K + \sum_{j=2}^m c_j/s_j K} \right\rangle \tag{1.55} \]

where \( c_1 \) is the concentration of its cognate ligand, and the average is over the distribution of all the other ligands, \( P(c_2, \cdots, c_m) \). In the small-crosstalk limit we have

\[ \bar{n}_1 = p_1 + (1 - p_1)^2 \frac{\langle c_j \rangle}{s_j K}, \quad p_1 = \frac{c_1/K}{1 + c_1/K}. \tag{1.56} \]

Because our formulation of the problem ensures the symmetry of the solutions, \( P_i(c_i) = P(c) \) and \( \langle c_i \rangle = \langle c \rangle \) for each input source \( i \), we can drop the subscripts and recover the two-pair-like result

\[ \bar{n} = p + (1 - p)^2 \frac{\langle c \rangle}{s K} \tag{1.57} \]

where \( 1/s = \sum_{i=2}^m (1/s_i) \) is the total strength of nonspecific interaction. In other words, even when there are multiple sources of crosstalk, in the current formulation they can be treated effectively as a single source with the combined strength.

### 1.4 Output noise

We have just shown that the \( K \to \infty \) solution in the kinetic case is robust under spurious interactions. Yet we need to remember that optimizing the amount of information encoded in the state of the receptor is not the end of this story. In order for the information to be accessible by the downstream pathways, something must happen to transduce the activity of the receptor. For example, if the lifetime of each ligand-receptor complex is extremely short, it may not be able to catalyze a reaction in the physiological timescale. In this sense, the simple version of the problem in the kinetic case is leaving out another important ingredient.
1.4.1 The catalytic rate

So far with the monomeric (single-site) receptors, our implicit assumption was that a receptor becomes “activated” immediately when it is occupied by a ligand. In fact, however, the receptor is only active in the sense that it can catalyze a reaction for its target, relaying the signal downstream. In order to fully account for the catalytic process as part of the information transmission problem, let us suppose that the ligand-bound receptor catalyzes a reaction at some rate $R$ (Figure 1.2A),

$$\dot{Q} = Rn. \tag{1.58}$$

Then the catalysis of the product $Q$ has at least two sources of noise: one from the fluctuations in receptor occupancy ($\langle \delta n^2 \rangle$), another from the shot noise of the catalytic reaction ($\sigma_\dot{Q} \sim Q$). Averaged over time $T$, the variance of the final product involves the following
two components (also see Section 1.E)

\[ \langle \delta Q^2 \rangle \sim T^2 R^2 \left( \langle \delta n^2 \rangle + \frac{2}{T R} \bar{n} \right) \]  

which is reflected conveniently in an effective variance of receptor activation

\[ \langle \delta n^2 \rangle_R = \langle \delta n^2 \rangle + \frac{2}{T R} \bar{n} \]  \hspace{1cm} (1.60)

\[ = \frac{2}{T k_+ K} \bar{n}(1 - \bar{n})^2 \left[ 1 + \frac{k_+ K}{R} \frac{1}{(1 - \bar{n})^2} \right] \]  \hspace{1cm} (1.61)

because \( \langle \delta n^2 \rangle = \bar{n}(1 - \bar{n}) \cdot 2 \tau_c/T \) and \( \tau_c = (1 - \bar{n})/k_+ K \). Note that we are still with a single receptor, \( N = 1 \), while assuming \( Z \propto \sqrt{N} \) for an ensemble of receptors. In this case it is useful to write \( Z \) in terms of the mean output noise \( \bar{n} \) directly, as

\[ Z = \int_{n_{\min}}^{n_{\max}} \frac{d\bar{n}}{\sqrt{\langle \delta n^2 \rangle_R}}, \]  \hspace{1cm} (1.62)

although for completeness, we also write down the expression of \( Z \) in terms of the input concentration \( c \),

\[ Z = \sqrt{\frac{T k_+ K}{2}} \int_0^{c_{\max}} \frac{dc}{\sqrt{c(c + K)}} \cdot \left( 1 + \frac{(c + K)^2}{(R/k_+) \cdot K} \right)^{-1/2} \]  \hspace{1cm} (1.63)

Evaluating in the weak response limit \( (\bar{n}_{\max} \ll 1) \), where information in the simple kinetic picture was maximized, we get (although a more careful expansion can be made in terms of the input)

\[ Z \approx \frac{\sqrt{T k_+ K/2}}{\sqrt{1 + k_+ K/R}} \int_{\bar{n}_{\min}}^{\bar{n}_{\max}} \frac{d\bar{n}}{\sqrt{\bar{n}}} \left( 1 + \frac{\bar{n}}{1 + k_+ K/R} \right) \]

\[ \approx \sqrt{2Tk_+ K} \sqrt{\bar{n}_{\max}} \frac{1}{\sqrt{1 + k_+ K/R}} + \cdots \]
In this limit $\bar{n}_{\text{max}} \approx c_{\text{max}}/K$. Keeping only the leading term, $Z$ is also written as

$$Z \approx \sqrt{2T k_{+} c_{\text{max}}} \frac{1}{\sqrt{1 + k_{+} K/R}}.$$  \hspace{1cm} (1.64)

As long as the catalysis happens faster than the receptor kinetics ($R \gg k_{+} K$), we recover the event-counting solution where information is limited only by the binding rate $k_{+} c_{\text{max}}$. But as we further increase $K$ such that $R \ll k_{+} K$, the rate of catalysis $R$ starts to limit information transmission. We learn that the receptor kinetics should be faster than the rate at which ligands can bind to the receptor, $k_{-} > k_{+} c_{\text{max}}$, but slower than the rate at which the target can recognize the receptor, $k_{-} < R$. Hence the information flow is optimized when

$$c_{\text{max}} \ll K \ll \frac{R}{k_{+}},$$  \hspace{1cm} (1.65)

where $R/k_{+}$ is the effective concentration scale of target activation (Figure 1.2B). Given a finite rate of catalysis $R$, the optimal $K$ would take a finite value instead of being pushed to the extreme. However, we note that the quantitative effect of catalytic rate on $K_{\text{opt}}$ is weak. Although $K_{\text{opt}}$ increases as $R$ increases, there is a diminishing return of information gain (Figure 1.2C).

### 1.4.2 The diffusion rate

We have just learned that information is limited by the rate at which the ligand-receptor complex can catalyze a reaction by interacting with a target molecule (or the “substrate” if we view this like an enzyme-substrate reaction). But the information flow is also limited by the diffusion of the target molecules. When we take into account the effect of target diffusion, the catalytic noise is actually written in the form (see section 1.E for the derivation)

$$\sigma_{Q}^{2} = 2TR\bar{n} \left(1 + \frac{R\bar{n}}{\rho}\right).$$  \hspace{1cm} (1.66)
where $\rho$ characterizes the rate at which the target molecules arrive at the target/receptor interface. With this diffusion term, the effective variance of receptor activation is

$$\langle \delta n^2 \rangle_{\text{eff}} = \langle \delta n^2 \rangle + \frac{2}{T R} \bar{n} + \frac{2}{T \rho} \bar{n}^2$$  \hspace{1cm} (1.67)$$

where $\langle \delta n^2 \rangle = 2 \bar{n}(1 - \bar{n})/Tk_{+}K$ is the noise of the binding/unbinding kinetics. When the noise-to-signal ratio $(\delta n)/\bar{n}$ is considered,

$$\left( \frac{\delta n_{\text{eff}}}{\bar{n}} \right)^2 = \frac{\langle \delta n^2 \rangle}{\bar{n}^2} + \frac{2}{TR\bar{n}} + \frac{2}{T\rho},$$

there is a lower bound that depends only on diffusion, as emphasized by Bialek and Setayeshgar (2005):

$$\frac{\delta n_{\text{eff}}}{\bar{n}} > \sqrt{\frac{2}{T\rho}}.$$  \hspace{1cm} (1.69)$$

Note that in equation (1.68), whereas the noise-to-signal ratio contribution from the kinetic fluctuation decreases as the mean response $\bar{n}$ becomes stronger, the contribution from diffusion is a constant of $\bar{n}$. Consequently, the total effective output noise approaches to the diffusion-dependent lower bound when $\bar{n}$ is large. We can write this condition as $\bar{n} \gg \bar{n}_{\text{diff}}$, identifying the cutoff $\bar{n}_{\text{diff}}$ at the boundary of the inequality $\langle \delta n^2 \rangle < 2\bar{n}^2/T\rho$, which is guaranteed to exist in the range $(0,1)$:

$$\bar{n}_{\text{diff}} = \left( 1 + \frac{k_{+}K}{2\rho} \right) - \sqrt{\left( 1 + \frac{k_{+}K}{2\rho} \right)^2 - 1}.$$  \hspace{1cm} (1.70)$$

The cutoff scales as $\bar{n}_{\text{diff}} \approx \rho/k_{+}K$ in the slow diffusion limit (when $\rho/k_{+}K \ll 1$) and as $\bar{n}_{\text{diff}} \approx 1 - \sqrt{k_{+}K/\rho}$ in the fast diffusion limit (when $\rho/k_{+}K \gg 1$).

This means that when $\bar{n}_{\text{max}} > \bar{n}_{\text{diff}}$, the dynamic range can be divided into two regimes at the diffusion cutoff $\bar{n}_{\text{diff}}$. Below the cutoff, the effective output noise $\langle \delta n^2 \rangle$ has more contribution from the binding kinetics than from the diffusion of the target. Above the cutoff, on the other hand, the effect target diffusion dominates the output noise and masks the
contribution from the receptor kinetics. The diffusion rate of the target molecule, therefore, effectively places an upper cutoff on the level of informative response $\bar{n}$. Whereas the actual dynamic range of the output can be arbitrarily large, the signal that results in anything beyond $\bar{n}_\text{diff}$ is ultimately limited by the availability of the target molecules, which is powered by diffusion. We could also translate this cutoff in terms of the input $c_{\text{diff}}$, at least in the monotonic response regime, such that only the signals below $c_{\text{diff}}$ can be transmitted effectively through this diffusion-limited bottleneck. In other words, the effect of finite diffusion rates can be incorporated into the problem by assuming a finite $c_{\text{max}}$. If we denote the actual (externally regulated) dynamic range by $c^0_{\text{max}}$, the effective dynamic range is $c_{\text{max}} = \min\{c^0_{\text{max}}, c_{\text{diff}}\}$. Even when the actual $c^0_{\text{max}}$ is large, when diffusion slow, the dynamic range may effectively be determined by the rate of diffusion as $c_{\text{max}} = c_{\text{diff}}$.

To close the section on the output noise, we note that analysis in this section shares the spirit of what Tkačik et al. (2009) observed about the additive interaction of the diffusive noise of the input and statistical noise of the output, in the context of gene expression (output) by transcription factors (input). In our context of ligand-receptor signaling, the rate of ligand (input) diffusion has been fixed indirectly by assuming a fixed binding rate $k_{+}$. On the other hand, because the signaling pathway continues beyond the event of ligand-receptor binding, it is important that the ligand-receptor complex catalyzes a reaction for the next-level target, which is the ultimate output of this channel. We thus considered the statistical noise of the formation of the (ligand-)receptor-target complex assuming a finite rate of catalysis, as well as the noise that comes from the finite rate of diffusion of the target molecule, and showed how the information capacity of the channel is limited by a combination of all of these factors.
1.5 The cooperative dimer

Now that we understand the single-site receptor, we are ready to revisit the case of the multi-site receptor and ask about the role of cooperativity beyond the simple equilibrium problem considered in Section 1.2. When the receptor has more than one binding sites, the sites may interact such that the occupation state of one site affects the binding kinetics of another site. As the simplest multi-site example, let us consider a receptor with two identical binding sites (the cooperative dimer), as shown in Figure 1.3. The cooperativity changes the binding and unbinding rate by factors $F_+$ and $1/F_-$ respectively. If we denote by $n_j$ the number of receptors with $j$ bound ligands, the governing equations are

\[
\frac{dn_1}{dt} = 2k_+cn_0 - (k_- + F_+k_+c)n_1 + \frac{2k_-}{F_-}n_2 \quad (1.71)
\]

\[
\frac{dn_2}{dt} = F_+k_+cn_1 - \frac{2k_-}{F_-}n_2 \quad (1.72)
\]

where $n_0 + n_1 + n_2 = N$. In particular, the equilibrium solutions only depend on the total cooperativity $F = F_+F_-$, aside from the concentration of binding $K$:

\[
\bar{n}_1 = \frac{N \cdot 2Kc}{K^2 + 2Kc + Fc^2}, \quad \bar{n}_2 = \frac{N \cdot Fc^2}{K^2 + 2Kc + Fc^2}. \quad (1.73)
\]

The cooperativity factors can have any value in $(0, \infty)$, although $F \in (0, 1)$ would mean a “negative” or “anti”-cooperativity in the conventional terms. High cooperativity gives a more
strongly sigmoidal response, and the conventional wisdom is that cooperativity “sensitizes” the response by making the response curve steeper. We could ask questions like, for example, whether the effect of cooperativity is merely scaling the response curve, or whether it is more than just scaling.

1.5.1 At equilibrium

At equilibrium, evaluating for the optimal readout $A \propto (n_1 + 2n_2)$ as discussed in Section 1.2.2 above, the information partition function $Z$ is

$$Z = \int_0^{c_{\text{max}}} \frac{dc}{\delta c_{\text{eff}}} \propto \int_0^{c_{\text{max}}/K} \frac{dx}{\sqrt{x}} \cdot \frac{\sqrt{1 + 2Fx + Fx^2}}{1 + 2x + Fx^2}$$
up to a constant factor. Let us evaluate the integral in the limits where \( F \) is small and large. When \( F \gg 1 \):

\[
\int_0^{K/F} \frac{dc}{\delta c_{\text{eff}}} \sim \int_0^{1/F} \frac{dx}{\sqrt{x}} \sim \frac{2}{\sqrt{F}}; \quad (1.74)
\]

\[
\int_{K/F}^{K/\sqrt{F}} \frac{dc}{\delta c_{\text{eff}}} \sim \int_{1/F}^{1/\sqrt{F}} \frac{dx}{\sqrt{x}} \sqrt{2Fx} \sim \sqrt{2}; \quad (1.75)
\]

\[
\int_{K/\sqrt{F}}^{K} \frac{dc}{\delta c_{\text{eff}}} \sim \int_{1/\sqrt{F}}^{1} \frac{dx}{\sqrt{x}} \sqrt{Fx^2} \sim 1; \quad (1.76)
\]

\[
\int_{K/F}^{K} \frac{dc}{\delta c_{\text{eff}}} \sim \int_{1/F}^{1/\sqrt{F}} \frac{dx}{\sqrt{x}} \sqrt{Fx^2} \sim 1/\sqrt{F}. \quad (1.77)
\]

In this limit, the dominant contribution to the integral comes from the narrow interval centered at \( K/\sqrt{F} \), bounded by \((K/F, K)\). On the other hand, when \( F \ll 1 \):

\[
\int_0^{K} \frac{dc}{\delta c_{\text{eff}}} \sim \int_0^{1} \frac{dx}{\sqrt{x}} \sim 2; \quad (1.78)
\]

\[
\int_{K/\sqrt{F}}^{K} \frac{dc}{\delta c_{\text{eff}}} \sim \int_{1/\sqrt{F}}^{1} \frac{dx}{\sqrt{x}} \frac{1}{\sqrt{2x}} \sim 1; \quad (1.79)
\]

\[
\int_{K/\sqrt{F}}^{K/F} \frac{dc}{\delta c_{\text{eff}}} \sim \int_{1/\sqrt{F}}^{1/F} \frac{dx}{\sqrt{x}} \sqrt{F/x^2} \sim 1; \quad (1.80)
\]

\[
\int_{K/F}^{K} \frac{dc}{\delta c_{\text{eff}}} \sim \int_{1/F}^{1/\sqrt{F}} \frac{dx}{\sqrt{x}} \sqrt{Fx^2} \sim 2. \quad (1.81)
\]

There are finite contributions from all intervals in this case, while the last integral is controlled by the lower bound \( K/F \). Hence at small \( F \), as the total value of the integral would increase as the dynamic range increases up to \( \sim K/F \). This is contrary to the previous case at large \( F \), where only the weak response range was informative, and the information stayed saturated as the dynamic range extends further. Furthermore, we can see that the maximum number of bits that can be transmitted (in this case, when integrated over the full dynamic range) is larger in the small-\( F \) limit (Figure 1.4C). But when the dynamic range is limited \((c_{\text{max}}/K \text{ is small})\), a large \( F \) would be more advantageous, as it can keep the most informative part of the response localized within the dynamic range (Figure 1.4D). Importantly,
Figure 1.5: A receptor with multiple binding sites has multiple states, which might catalyze the target reaction at different rates.

we can see that the cooperativity $F$ does not only scale the response curve, but changes the way information is distributed over the dynamic range.

1.5.2 Kinetic case

In the kinetic case, the cooperativity affects the distribution of information over the dynamic range in a similar way. However there is a factor of $\sqrt{K}$ that comes from the timescale, which acts against having a large dynamic range $c_{\text{max}}/K$, as shown in the case of the monomer. Let us start by setting up a proper model of the dimer in the kinetic case, and then proceed to investigate different regimes of cooperativity.

We saw from the monomer problem that the catalytic rate plays an important role in this information transmission system. Now if the receptor has multiple states, receptors in different states may catalyze a given target at different rates. In the case of a dimer, the number of target-receptor complexes (the product) $Q$ would build up with $\dot{Q} = R_1 n_1 + R_2 n_2$. 

32
In terms of an overall rate \( R = R_1 + R_2 \), we may write this as

\[
\dot{Q} = RA, \quad A = an_1 + (1 - a)n_2
\]  

(1.82)

where \( a = R_1/(R_1 + R_2) \). We have used similar definitions of the output activity \( A \) in Section 1.2.2 already, but now it becomes clearer that this is the natural way to summarize the state of the receptor. More generally, the (target-dependent) activity of a multi-site receptor is written in terms of a weighted sum of the individual states, weighted according to the catalytic rates.

Integrated over time \( T \), the variance of \( Q \) is

\[
\langle (\delta Q)^2 \rangle \sim T^2 R^2 \left( \langle (\delta A)^2 \rangle + \frac{1}{TR} \bar{A} \right),
\]  

(1.83)

where the effect of diffusion is already incorporated into the constraint on the dynamic range, \( c_{\text{max}} \). In the following, it is sufficient to consider the effective variance

\[
\langle (\delta A)^2 \rangle_R \equiv \langle (\delta A)^2 \rangle + \bar{A}/TR,
\]  

(1.84)

such that

\[
\frac{1}{\delta c_{\text{eff}}^2} = \frac{1}{\langle (\delta A)^2 \rangle_R} \left| \frac{\partial \bar{A}}{\partial c} \right|^2.
\]  

(1.85)

The partition function \( Z \) is obtained by integrating \( 1/\delta c_{\text{eff}} \) as before. When dealing with the receptor with multiple states, however, one needs to be careful with the integration range because the mean response \( \bar{A}(c) \) may no longer be monotonic. See Section 1.A for more discussion.

**The monomer-like limit**

We can calculate \( \bar{A} \) and \( \langle (\delta A)^2 \rangle \) for the cooperative dimer, and evaluate \( Z \) at different points in the parameter space. Details of the calculation are presented in Section 1.C. It turns out
Figure 1.6: The cooperative dimer with time-averaged information, with catalytic rates (A) \( R/k_c^{\text{max}} = 10^3 \) and (B) \( R/k_c^{\text{max}} = 10^{-3} \). Plotted is the contour of \( Z \) on the cooperativity plane, calculated at different values of \( K/c_{\text{max}} \) and \( a \). The color scale goes from blue (low) to red (high), relative within each panel. The diagonal line indicates \( F^+ = F^- \).

that depending on the target-specific parameters \( R \) and \( a \), the information is maximized either at the \( F\rightarrow 0 \) limit (monomer) or at a peak at finite cooperativity (tuned dimer).

In the monomer limit, the second binding is prohibited due to the strong anti-cooperativity \( (F\rightarrow 0) \), and each receptor utilizes only one binding site. The conditions that the receptor functions like a monomer are (details in Section 1.C)

\[
\sqrt{F} \ll 1, \quad \frac{(1-a)}{a} \sqrt{F} \ll 1, \quad \frac{\sqrt{F} c_{\text{max}}}{K} \ll 1. \tag{1.86}
\]

In this regime the information capacity no longer depends on \( F \), and is maximized at some \( K/c_{\text{max}} \) that depends on \( R \), just as in the case of the simple monomer.

The tuned dimer

When the receptor exploits both binding sites (when \( F \) is not too small), there exists a non-trivial set of conditions at which the information flow is optimized. In order to reach this peak, the cooperativity \( F \) should be large enough to allow both binding sites to be functional,
but small enough to keep the correlation time short. So there exists an optimal level of cooperativity the dimer should be tuned at, and we can derive the conditions analytically. When $F_+ = F_- = \sqrt{F}$, the information is optimized when (details in Section 1.C)

$$\sqrt{F} \gg 1, \quad \frac{(1-a)}{a} \sqrt{F} \gg 1, \quad \frac{\sqrt{F}c_{\text{max}}}{K} \approx 1,$$

providing a finite-$K$ solution even in the $R \to \infty$ limit. Interestingly, the optimal dynamic range is just enough to host the second binding, the concentration scale of which is $K/\sqrt{F}$. It also means that the input-output response stays mostly in the monotonic regime.

Looking at the diagonal $F_+ = F_-$ is a choice of convenience, by which we could get an idea of the approximate structure of the information landscape while avoiding the cost of considering the full parameter space. Although the peak does not lie exactly on the diagonal, it turns out that the tuned dimer is not very far off from this diagonal $F_+ = F_-$ (Figure 1.6), and that the information peak captured along the diagonal is not too different from the actual peak (Figure 1.7.)

When the catalytic rate $R$ is high enough, the monomer-like solution is capable of conveying more information (Figure 1.7A). But then why the cell would ever bother to have receptors with multiple sites? The reason may be twofold. First, when the catalytic rate is limiting (small $R$), the dimer solution can provide more information (Figure 1.7B). This observation is made quantitative in Figure 1.8, which plots the information peak height and position (in terms of $K_{\text{opt}}$) as functions of the catalytic rate $R$. In the large-$R$ limit where the information capacity is maximized, the optimal $K_{\text{opt}}$ for the dimer solution depends only on $F$, but not on $R$, as long as $R$ is large enough. On the other hand, the optimal $K_{\text{opt}}$ for the monomer-like solutions increases with $R$, with a diminishing return in the information capacity. Second, the region of the parameter space achievable in the physiological conditions may not always allow for the monomer-like solution. For example, while it is critical to attain the optimal value of $K$ (given $R$ and $c_{\text{max}}$), there may be situations where it is not
possible to maintain this optimal $K$, due to energetic constraints that limits the timescale of reaction.

### 1.6 Discussion

In this work we examined how the information capacity of the ligand-receptor system is optimized under different scenarios, and how it is limited by multiple components of the information transmission pathway. This work builds on the theoretical framework that has been developed through the past decade, but it steps further to highlight how the problem should be more carefully formulated.

The signal comes through the concentration of ligand, the receptor binds the ligand (with a concentration scale of binding $K$), and relays the signal to the downstream pathways; the ligand-bound receptor is “active” in the sense that it is capable of activating the target molecule. In the simplest and the most conventional versions of the problem, where we
Figure 1.8: The information capacity of a cooperative dimer is optimized differently at different catalytic rate $R$.

Only considered the ligand-receptor interaction in isolation, we found that the solutions to the information optimization at some extreme limits. In the case where we considered a snapshot of the receptor activity at a single instant in time, the solution was driven to the $K \rightarrow 0$ limit where the unbinding rate is infinitely slow (once a ligand binds the receptor, it stays there indefinitely), and the optimized receptors are highly occupied no matter how small the ligand concentration is. In the kinetic scenario where the activity is averaged over time, the solution was driven to $K \rightarrow \infty$, meaning that the unbinding rate is infinitely fast (the lifetime of a ligand-receptor complex is extremely short), where each binding event occurs at discrete points in time, and the resulting information is essentially a count of such binding events within the integration timescale.

However, these extreme solutions correspond to some pathological limits that are unrealistic. We first demonstrated that the equilibrium solution $K \rightarrow 0$ (strong binding limit) breaks down in the presence of spurious interaction partners, with a possibility of crosstalk between different ligand-receptor pairs. In this case the strong-binding strategy is detrimental because whenever a receptor binds a wrong ligand, it will hold on the “wrong” signal for
an indefinitely long time. Indeed, our calculation in the presence of crosstalk showed that there are competing terms of binding strongly to the correct partner and suppressing the effects of incorrect partners, forcing the optimal $K$ to be balanced at some finite value that depends on the strength of crosstalk.

On the other hand, the kinetic solution $K \to \infty$ (weak binding limit) suffers from different sources of perturbations. Although this solution is robust under the presence of crosstalk, we highlighted that this weak-binding, event-counting solution has a fundamental pitfall that its performance depends on the ability of having a finite effect within the infinitesimal timescale during which the ligand-receptor complex remains bound. In particular, we demonstrated that it should be explicitly taken into account that the receptor is only active in the sense that it can catalyze a reaction on its target’s side, which should happen at some finite rate $R$. With the finite rate of catalysis, the kinetic solution was amended such that $K$ goes as large as possible while not exceeding the effective concentration scale of target activation, and now always sits at some finite value. The insight made clear in this case of finite catalytic rate is also aligned with the observation of Tikhonov et al. (2015) that only the accessible information is useful, and of Tkačik et al. (2009) which emphasized that the interaction of the input and output noise was essential for understanding the non-trivial solution of the information channel.

Then we took a further step in investigating the output end of the channel. Unlike in the previous study in the context of genetic network (Tkačik et al., 2009), the ligand-receptor signaling is only complete when the ligand-bound receptor interacts with some target, forming a (ligand-)receptor-target complex which would convey the signal downstream. So even if the receptor is bound to the ligand and ready to activate the target, the final catalysis depends on the availability of fresh target molecules – which emphasizes the role of the rate of target diffusion. As was made clear in our calculation, a finite rate of target diffusion masks the high activity of the receptor, which corresponds to a high level of input concentration, effectively placing an upper cutoff to the dynamic range of the signal. There is a
general lesson from this exercise: the effective dynamic range of the problem reflects not only how many ligand molecules there are per unit volume, but rather how many of them can actually interact with the receptor, and induce a meaningful downstream response in the target, given the finite timescales at which all these events take place.

Finally, we considered the cooperative dimer, and demonstrate how the information flow can be optimized in qualitatively different manners depending on the structures of the ligand-receptor interaction. Our result showed that, a receptor with two interacting binding sites may have an information-optimizing solution that is unattainable by its single-site counterpart, when its kinetic parameters are in the appropriate regime (the “tuned dimer”). Importantly, we considered the possibility that the singly- and doubly-bound states of the dimer may catalyze the target reaction at different rates, with some target-specific ratio $a$. The tuning requires a non-trivial balance between the cooperativity $F$, the catalytic rate $R$, the receptor kinetics $K$ and the relative strength of activation $a$; the cooperativity of the two binding sites should be large enough to allow both binding sites to be functional (whereas in the “anti-cooperative” regime, either of the binding site may never be occupied, and the problem falls back in to the monomer-like regime), but small enough to keep the correlation time short, as well as to stay in the monotonic response regime. We also found that the information capacity of the tuned dimer solution is at most comparable to that of the monomer-like solution in non-limiting situations, but is much more flexible under limiting catalytic rates $R$ and/or the dynamic range of the input signals $c_{\text{max}}$. In other words, cooperativity lets the receptor to operate near the limits set by the environmental constraints, which is an insight shared with Bialek and Setayeshgar (2008). By exploiting the next level of complexity in the structure, the receptor gains the capability to switch between two modes depending on the environment; the “productivity mode” which performs optimally on a well-paved pathway, and the “flexibility mode” which performs much more robustly under more complicated sets of constraints.
Technical Notes

1.A Small noise approximation for non-monotonic response

In the main text, we have discussed how the mutual information \( I(n; c) \) can be conveniently re-written under the small noise approximation

\[
I(n; c) \approx - \int dc P_c(c) \left[ \log P_n(\bar{n}(c)) + L(c) \right] \\
\approx - \int d\bar{n} P_n(\bar{n}) \left[ \log P_n(\bar{n}) + L(\bar{n}) \right],
\]

(1.88)

where \( L(c) = S(n|c) \). However, the approximation \( dc P_c(c) = d\bar{n} P_n(\bar{n}) \) assumes that the mean response \( \bar{n}(c) \) is an invertible function. This assumption is true when the output is the occupation state of a single-site receptor, for which \( \bar{n}(c) \) is monotonic.

When we deal with receptors with more than one binding sites, the mean response \( \bar{A} = g(c) \) may no longer be monotonic, depending on how the individual states are weighted in the final readout. In this more general situation, the correct thing to assume is

\[
d\bar{A} P_\bar{A}(\bar{A}) = \sum_{\bar{A} = g(c)} |dc P_c(c)|.
\]

(1.89)
Again assuming that \( P(A|c) \) is narrow and symmetric around the mean, the second term in the mutual information is

\[
\int dc P_c(c) L(c) = \int dc \left[ \int d\bar{A} \delta_A (\bar{A} - g(c)) \right] P_c(c) L(c)
\]

\[
\approx \int d\bar{A} P_A(\bar{A}) \left[ \int dc \frac{\delta_c (\bar{A} - g(c)) P_c(c) L(c)}{|\partial g / \partial c|} \right]
\]

\[
\approx \int d\bar{A} P_A(\bar{A}) L_{\text{eff}}(\bar{A}).
\] (1.90)

The two delta functions (\( \delta_A \) and \( \delta_c \)) are connected under the small noise approximation, when \( \partial g / \partial c \neq 0 \). We have introduced \( L_{\text{eff}}(\bar{A}) \) above, such that the mutual information can be written as

\[
I \approx -\int d\bar{A} P_A(\bar{A}) \left[ \log_2 P_A(\bar{A}) + L_{\text{eff}}(\bar{A}) \right].
\] (1.91)

The integral in \( L_{\text{eff}}(\bar{A}) \) can be replaced by a discrete sum over all values of \( c \) that satisfies the condition \( \bar{A} = g(c) \):

\[
L_{\text{eff}}(\bar{A}) \approx \int dc \delta_c (\bar{A} - g(c)) \frac{P_c(c) / P_A(\bar{A})}{|\partial g / \partial c|} L(c)
\]

\[
\approx \sum_{g(c)=\bar{A}} \left| \frac{\partial g}{\partial c} \right|^{-1} \frac{P_c(c)}{P_A(\bar{A})} L(c).
\] (1.92)

In effect, the small noise approximation breaks down the optimization problem over the full joint distribution \( P(A,c) \) to a two-step optimization

\[
\max_{P(A,c)} \mathcal{I}[P(A,c)] \implies \max_{P_A(\bar{A})} \max_{P(c|\bar{A})} \mathcal{I}\left\{ P_A(\bar{A}), P(c|\bar{A}) \right\}.
\] (1.93)

When the mapping \( c \mapsto \bar{A}(c) \) is invertible, the first layer could be suppressed because \( c = g^{-1}(\bar{A}) \) is unique, and the information would simply be optimized over \( P_A(\bar{A}) \) to give

\[
I_{\text{opt}} = \log_2 Z,
\]

\[
Z = \int d\bar{A} \exp \left[ -L_{\text{eff}}(\bar{A}) \right].
\] (1.94)
However, with non-monotonicity taken into account, we need to pick up the additional optimization over $P(c|\bar{A})$. Because information is optimized when $Z$ is maximized, the goal here is to minimize $L_{\text{eff}}(\bar{A})$. Since $L_{\text{eff}}(\bar{A})$ is effectively a convex sum of $L(c)$ over a discrete set of $c$, the minimization is done by

$$
\min_{P(c|\bar{A})} L_{\text{eff}}(\bar{A}) = \min_c \{ L(c) | g(c) = \bar{A} \}.
$$

(1.95)

All other input values $c_{\text{other}}$ that map to the same output $A$ are abandoned by setting $P(c_{\text{other}}) = 0$. Let us now introduce the optimal support of the input distribution $P(c)$,

$$
\text{supp}(P_c) = \left\{ c \, \bigg| \, \text{L}(c) = \min_{g(c')=g(c)} L(c') \right\}
$$

(1.96)

which is a subset of the dynamic range of $c$. When the response is monotonic, $\text{supp}(P_c)$ simply equals the entire dynamic range.

If we further assume that $P(A|c)$ is Gaussian with the variance $\sigma_A^2(c)$, the final solution to the information-optimizing problem is

$$
Z = \frac{1}{\sqrt{2\pi e}} \int_{\text{supp}(P_c)} \frac{dc}{\sigma_A(c)},
$$

(1.97)

where the optimal support corresponds to the least-noise channels,

$$
\text{supp}(P_c) = \left\{ c \, \bigg| \, \sigma_A(c) = \min_{g(c')=g(c)} \sigma_A(c') \right\}.
$$

(1.98)

In the case of the cooperative dimer, the mean response is given as

$$
\bar{A}(c) = \frac{2aKc + (1-a)Fc^2}{K^2 + 2Kc + Fc^2}.
$$

(1.99)
When \( a > 1/2 \), it has a single peak at

\[
\frac{c_{\text{peak}}}{K} = \frac{(1 - a)}{2(2a - 1)} \left( 1 + \sqrt{1 + \frac{4a(2a - 1)}{(1 - a)^2 F}} \right).
\]  
(1.100)

This peak is bounded below by \( c_{\text{peak}} \geq K/\sqrt{F} \), touching the minimum at \( a = 1 \). When \( a \leq 1/2 \), the response curve is monotonically increasing, so \( c_{\text{peak}} = \infty \). The problem falls into the non-monotonic regime when \( c_{\text{max}} > c_{\text{peak}} \). The single-peaked curve means that in the non-monotonic regime, two values of \( c \) may yield the same level of \( \bar{A} \). Such a “conjugate” point is found at

\[
\text{conj}(c) = \frac{K^2}{Fc} \cdot \frac{1}{1 - (1 - a)/\bar{A}(c)}.
\]  
(1.101)

To implement the numerical integration of \( Z \) in a point-wise manner, at each \( c_i \) within the dynamic range, one needs to compute the conjugate point \( \hat{c}_i = \text{conj}(c_i) \) and (if applicable) the variances at both \( c_i \) and \( \hat{c}_i \). Let \( c_i \) contribute to the integral only if it has no conjugate \( \hat{c}_i < c_{\text{max}} \) with a smaller variance.

### 1.B Computing the time-averaged variance

In a dynamical situation where the system integrates the signal over a long window \( T \), the time-averaged variance of \( A \) can be written as a weighted integral of the power spectrum \( S_{AA}(\omega) = \langle \delta A^*(\omega) \delta A(\omega) \rangle \). Fourier transforming from the time space,

\[
\langle (\delta A)^2 \rangle = \frac{1}{T^2} \int_0^T \int_0^T dt \, dt' \langle \delta A(t) \delta A(t') \rangle
\]

\[
= \frac{1}{T^2} \int_{-\infty}^{\infty} \frac{d\omega}{2\pi} S_{AA}(\omega) \int_0^T \int_0^T dt \, dt' e^{i\omega(t-t')}
\]

\[
= \int_{-\infty}^{\infty} \frac{d\omega}{2\pi} S_{AA}(\omega) \left| \frac{1}{i\omega T} (1 - e^{-i\omega T}) \right|^2
\]

\[
= \frac{1}{T} \int_{-\infty}^{\infty} \frac{d\omega}{2\pi} \left[ T \frac{\sin^2(\omega T/2)}{(\omega T/2)^2} \right] S_{AA}(\omega),
\]  
(1.102)
where the kernel $[,]$ becomes a delta function as $T \to \infty$, giving $\langle \delta A^2 \rangle_T \approx S_{AA}(0)/T$.

1.B.1 The monomer

In the case of the monomer, the receptor has only two states: either occupied or not. If there are $N$ such monomers, and $n$ is the number of monomers in the occupied state, we may write:

$$\frac{dn}{dt} = k_+ c (N - n(t)) - k_- n(t) + \xi(t), \quad (1.103)$$

where $\xi(t)$ is the random Langevin noise $\langle \xi(t) \xi(t') \rangle = v \delta(t - t')$, with the noise strength $v = k_+ c (N - \bar{n}) + k_- \bar{n}$ given in the random-walk sense. The equilibrium solution is $\bar{n} = Nk_+ c/(k_+ c + k_-)$. Linearizing around the equilibrium solution and Fourier transforming to the frequency space, we get

$$-i\omega \delta n(\omega) = -(k_+ c + k_-) \delta n(\omega) + \xi(\omega), \quad (1.104)$$

where $\langle \xi^*(\omega) \xi(\omega) \rangle = v = 2\bar{n}(N - \bar{n})/(N\tau_c)$ where $\tau_c \equiv 1/(k_+ c + k_-)$ is the correlation time. The power spectrum of $n$ is

$$S_n(\omega) \equiv \langle \delta n^*(\omega) \delta n(\omega) \rangle \equiv \frac{\langle \xi^*(\omega) \xi(\omega) \rangle}{\| (1/\tau_c) - i\omega \|^2} = \frac{1}{N} \bar{n}(1 - \bar{n}) \frac{2\tau_c}{1 + (\omega\tau_c)^2}. \quad (1.105)$$

In the long-time-average limit, the effective variance is

$$\langle \delta n^2 \rangle_T \approx \frac{S_n(0)}{T} = \frac{1}{N} \bar{n}(1 - \bar{n}) \cdot \frac{2\tau_c}{T}. \quad (1.106)$$
1.B.2 The cooperative dimer

We now repeat the same process in the case of the cooperative dimer with two symmetric binding sites:

\[
\begin{align*}
\frac{dn_1}{dt} &= 2k_+cn_0 - (k_- + F_+k_+c)n_1 + \frac{2k_+}{F_-}n_2 + \xi_1 \\
\frac{dn_2}{dt} &= F_+k_+cn_1 - 2\frac{2k_-}{F_-}n_2 + \xi_2,
\end{align*}
\]

(1.107) (1.108)

where \( n_0 + n_1 + n_2 = N \). The equilibrium solutions are

\[
\bar{n}_0 = N\frac{K^2}{W}, \quad \bar{n}_1 = N\frac{2cK}{W}, \quad \bar{n}_2 = N\frac{F_+F_-c^2}{W}
\]

(1.109)

where \( K \equiv k_-/k_+ \) and \( W = K^2 + 2cK + F_+F_-c^2 \). The noise \( \xi_i \) is assumed to be random and independent, \( \langle \xi_i(t) \xi_j(t') \rangle = v_i \delta_{ij} \delta(t - t') \), with the noise strengths

\[
\begin{align*}
v_1 &= 2(k_- + F_+k_+)\bar{n}_1 = \frac{4Nk_+Kc}{W}(K + F_+c), \\
v_2 &= 2\cdot \frac{2k_-}{F_-}\bar{n}_2 = \frac{4Nk_+Kc}{W}(F_+c).
\end{align*}
\]

(1.110) (1.111)

Linearizing and rearranging in the frequency space,

\[
\vec{\delta n}(\omega) = (M - i\omega I)^{-1} \vec{\xi}(\omega),
\]

(1.112)

where \( M \) is the rate matrix

\[
M = k_+ \begin{pmatrix} K + (2 + F_+)c & 2c - 2K/F_- \\ -F_+c & 2K/F_- \end{pmatrix}.
\]

(1.113)
With $\langle \xi_k(\omega) \xi_l^*(\omega') \rangle = v_k \delta_{kl} \delta(\omega - \omega')$, the power spectrum is

$$S_{ij}(\omega) = \langle \delta n_i(\omega) \delta n_j^*(\omega) \rangle = \sum_k (M - i\omega I)_{ik}^{-1} (M + i\omega I)_{jk}^{-1} v_k.$$ (1.114)

We are only interested in the zero-frequency components $S_{ij}(\omega = 0) = \sum_k M_{ik}^{-1} M_{jk}^{-1} v_k$, which are readily calculated from the noise strengths $v_i$ and

$$M^{-1} = \frac{F_-}{2k_+W} \begin{pmatrix} 2K/F_- & 2K/F_- - 2c \\ F_+ c & K + (2 + F_+) c \end{pmatrix}.$$ (1.115)

Collecting the terms, the time-averaged variance of the output activity $A = an_1 + (1 - a)n_2$ is

$$\langle (\delta A)^2 \rangle = \left( a^2 \frac{S_{11}(0)}{T} + 2a(1 - a) \frac{S_{12}(0)}{T} + (1 - a)^2 \frac{S_{2}(0)}{T} \right)$$

$$= \frac{N}{k_+K^2} \frac{K^2c \cdot (\Gamma_0 K^3 + \Gamma_1 K^2 c + \Gamma_2 K c^2 + \Gamma_3 c^3)}{W^3}.$$ (1.116)

where

$$\Gamma_0 = 4a^2,$$ (1.117)

$$\Gamma_1 = F_+ \left[ 8a^2 + 8a(1 - a)F_- + (1 - a)^2 F_-^2 \right],$$ (1.118)

$$\Gamma_2 = F_+ F_- \left[ -8a^2 + 4a(1 - a)(2 + 2F_+ - F_-) + (1 - a)^2 (4 + 3F_+)F_- \right],$$ (1.119)

$$\Gamma_3 = 2F_+ F_-^2 \left[ 2a^2 - 2a(1 - a)(2 + F_+) + (1 - a)^2 (2 + 2F_+ + F_-^2) \right].$$ (1.120)

Introducing $\Gamma' = (\Gamma_0 K^3 + \Gamma_1 K^2 c + \Gamma_2 K c^2 + \Gamma_3 c^3)/K^3$, the effective variance with the catalytic rate $R$ is

$$\langle (\delta A)^2 \rangle_R = \langle (\delta A)^2 \rangle + \frac{1}{TR} \tilde{A} = \frac{N}{k_+K^2} \left( \frac{c}{K} \frac{K^6 \Gamma'}{W^3} + \frac{k_+K}{R} \tilde{A} \right).$$ (1.121)
1.C Cooperativity in the kinetic case

Let us restrict our discussion to the special case where \( F_+ = F_- = \sqrt{F} \) (along the diagonal of the cooperativity space). Then the mean response is

\[
\bar{A} = \frac{aK^2c}{W K} \left[ 2 + \left( \frac{1 - a}{a} \sqrt{F} \right) \frac{\sqrt{Fc}}{K} \right]
\]  

(1.122)

and its sensitivity

\[
\frac{\partial \bar{A}}{\partial c} = \frac{N 2 a K^4}{K W^2} \left[ 1 - \left( \frac{\sqrt{Fc}}{K} \right)^2 \right] + \left( \frac{1 - a}{a} \sqrt{F} \right) \frac{\sqrt{Fc}}{K} \left( 1 + \frac{c}{K} \right).
\]  

(1.123)

Combining the last equation with (1.117–1.120), (1.122), we notice that different regimes of cooperativity should be identified relative to the following important scales:

\[
\sqrt{F}, \quad \frac{1 - a}{a} \sqrt{F}, \quad \frac{\sqrt{Fc\text{max}}}{K}.
\]  

(1.124)

For the rest of the section, let us introduce shorthands for the two dimensionless constants \( \alpha = N/k_+KT \) and \( \beta = k_+K/R \).

1.C.1 Small \( F \) limit

In the limit where \( F \) is small, the cooperative dimer problem reduces to that of the monomer. To be in this monomer-like regime \( F \) should satisfy

\[
F \ll 1 \quad \text{and} \quad \frac{(1 - a)}{a} \sqrt{F} \ll 1,
\]  

(1.125)

where

\[
\Gamma' \approx 4a^2 \left[ 1 + 2 \frac{\sqrt{Fc}}{K} - 2 \left( \frac{\sqrt{Fc}}{K} \right)^2 + \left( \frac{\sqrt{Fc}}{K} \right)^3 \right].
\]  

(1.126)
Evaluating at \( c \) at which \( \sqrt{Fc/K} < 1 \),

\[
\Gamma' \approx \Gamma_0, \quad W \approx K^2 + 2cK, \quad \frac{\bar{A}}{N} \approx \frac{2ac}{K + 2c}, \tag{1.127}
\]

and we have

\[
\langle (\delta A)^2 \rangle_R \approx \alpha \cdot 4a^2 \left( \frac{K^2c}{(K + 2c)^3} + \frac{\beta c}{2a K + 2c} \right), \tag{1.128}
\]

\[
\frac{\partial \bar{A}}{\partial c} \approx \frac{N \cdot 2aK^2}{K (K + 2c)^2}. \tag{1.129}
\]

This is equivalent to the monomer problem (recall that \( \beta/a = k_+K/R_1 \)), only scaled by the factor of 2 that comes from the binding site degeneracy.

To complete the discussion we consider the other extreme. Evaluating at \( c \) at which \( \sqrt{Fc/K} > 1 \),

\[
\Gamma' \approx 4a^2 \left( \frac{\sqrt{Fc}}{K} \right)^3, \quad W \approx Fc^2, \tag{1.130}
\]

and

\[
\frac{\bar{A}}{N} \approx \frac{2aK}{Fc} \quad \text{when} \quad \frac{\sqrt{Fc} \left( \frac{1 - a}{a} \sqrt{F} \right)}{K} < 1; \tag{1.131}
\]

\[
\approx (1 - a) \quad \text{when} \quad \frac{\sqrt{Fc} \left( \frac{1 - a}{a} \sqrt{F} \right)}{K} > 1. \tag{1.132}
\]

The variance when \( 1 < \sqrt{Fc/K} < a/(1 - a)\sqrt{F} \) is

\[
\langle (\delta A)^2 \rangle_R \approx \frac{N \cdot \bar{A}}{TRN} \approx \frac{N \cdot 2aK}{TR \cdot Fc}, \tag{1.133}
\]

and when \( a/(1 - a)\sqrt{F} < \sqrt{Fc/K} \)

\[
\langle (\delta A)^2 \rangle_R \approx \frac{N \cdot c \cdot K^6 \Gamma'}{Tk_+K^3W^3} \approx \frac{N \cdot 4a^2K^2}{Tk_+K^3F^{3/2}c^2}. \tag{1.134}
\]
In both cases, noise diverges as $F \to 0$, whereas the noise in the small $c/K$ limit (1.128) is independent of $F$. Hence when there is non-monotonicity, knowing that the mean response peak is at $\sqrt{F}c/K = 1$, the least-noise channel is always on the lower side of the dynamic range. From Section 1.A, it suffices to consider the range $c \in (0, K/\sqrt{F})$ which is the optimal support for the input distribution.

1.C.2 Large $F$ limit

In the limit where $F$ is large, where

$$F \gg 1 \text{ and } \frac{1-a}{a} \sqrt{F} \gg 1,$$

we have

$$\Gamma' \approx \frac{4}{a^2} + \left( \frac{1-a}{a} \sqrt{F} \right)^2 \frac{\sqrt{F}c}{K} \left( 1 + \frac{\sqrt{F}c}{K} \right) \left( 1 + 2 \frac{\sqrt{F}c}{K} \right).$$

Let us evaluate the effective noise level at different limits in the dynamic range of $c$. For brevity, let us introduce two dimensionless quantities

$$\theta_1 = \left( \frac{1-a}{a} \sqrt{F} \right) \sqrt{F}, \quad \theta_2 = \left( \frac{1-a}{a} \sqrt{F} \right)^2 \sqrt{F}.$$ 

with $\theta_2 > \theta_1 > \sqrt{F} > 1$ in this regime. It will be useful to note that

$$\frac{\theta_2}{\theta_1} = \frac{\theta_1}{\sqrt{F}} = \sqrt{\frac{\theta_2}{\sqrt{F}}} = \frac{1-a}{a} \sqrt{F}.$$ 

When $\sqrt{F}c/K < 1$, we have $W \approx K^2$ and

$$\Gamma' \approx a^2 \left( 4 + \frac{\theta_2c}{K} \right), \quad \frac{\tilde{A}}{N} \approx \frac{ac}{K} \left( 2 + \frac{\theta_1c}{K} \right).$$
\[ \langle (\delta A)^2 \rangle_R \approx \alpha \cdot \frac{a^2 c}{K} \left[ \left( 4 + \frac{2\beta}{a} \right) + \left( \theta_2 + \frac{\beta}{a} \theta_1 \right) \frac{c}{K} \right], \quad (1.140) \]

\[ \frac{\partial \tilde{A}}{\partial c} = \frac{2N}{K} \cdot a \left( 1 + \frac{\theta_1 c}{K} \right), \quad (1.141) \]

\[ \frac{K}{\delta c_{\text{eff}}} \approx \frac{2N}{\sqrt{\alpha}} \sqrt{\frac{K}{c}} \left( 1 + \frac{\theta_1 c}{K} \right) \left[ \frac{4 + \theta_2 c}{K} + \frac{\beta}{a} \left( 2 + \frac{\theta_1 c}{K} \right) \right]^{-1/2}. \quad (1.142) \]

When \( \sqrt{F} c / K > 1 \),

\[ \Gamma' \approx 2(1 - a)^2 \frac{F^{5/2} c^3}{K^3}, \quad W \approx F c^2, \quad \frac{\bar{A}}{N} \approx (1 - a), \quad (1.143) \]

\[ \langle (\delta A)^2 \rangle_R \approx \alpha \cdot (1 - a)^2 \left[ \frac{2 K^2}{\sqrt{F} \ c^2} + \frac{\beta}{(1 - a)} \right], \quad (1.144) \]

\[ \frac{\partial \tilde{A}}{\partial c} \approx \frac{2N}{K} \left( \frac{K}{c} \right)^2 \frac{1}{F} \left[ -a + (1 - a) \frac{K + c}{c} \right]. \quad (1.145) \]

\[ \frac{K}{\delta c_{\text{eff}}} \approx \frac{2N}{\sqrt{\alpha} F} \left( \frac{K}{c} \right)^2 \left[ - \frac{a}{1 - a} + \frac{K + c}{c} \right] \left[ \frac{2 K^2}{\sqrt{F} \ c^2} + \frac{\beta}{(1 - a)} \right]^{-1/2}. \quad (1.146) \]

We can now evaluate segments of the integral \( Z \) over sub-intervals of the dynamic range:

\[ \int_0^{K/\theta_2} dc \frac{dc}{\delta c_{\text{eff}}} = \int_0^{1/\theta_2} d(c/K) \frac{dc}{\delta c_{\text{eff}} / K} \]

\[ \approx \frac{2N}{\sqrt{\alpha}} \int_0^{1/\theta_2} dx \frac{1}{\sqrt{x} \sqrt{4 + 2\beta/a}} \]

\[ \approx \frac{2N}{\sqrt{\alpha} F^{1/4}} \cdot \frac{1}{\sqrt{1 + \beta/2a}} \cdot \frac{\theta_1}{\theta_2}. \quad (1.147) \]
In the limit where $F \to \infty$, the only non-vanishing contribution comes from the interval $(K/\sqrt{F}, K)$, controlled by the lower bound $K/\sqrt{F}$. Hence the integral is dominated by the narrow region centered at $c = K/\sqrt{F}$. On the other hand, each segment of the interval is
bounded by the factor

\[ \frac{N}{\sqrt{\alpha F^{1/4}}} = \sqrt{N k_{c_{\text{max}}}} \frac{K}{F^{c_{\text{max}}}}, \]  

(1.152)

which suppresses the integral \( Z \) when \( \sqrt{F} c_{\text{max}} / K \gg 1 \). Putting all together, in the large-\( F \) limit, \( Z \) is maximized at

\[ \frac{\sqrt{F} c_{\text{max}}}{K} \approx 1. \]  

(1.153)

### 1.D Integrals in the crosstalk approximation

The following integrals can be solved exactly by substituting \( x = \tan^2 \theta \).

\[ \int_{0}^{x_{\text{max}}} \frac{dx}{\sqrt{x(1 + x)}} = 2 \tan^{-1} \sqrt{x_{\text{max}}} \approx \pi - \frac{2}{\sqrt{x_{\text{max}}}}, \quad x_{\text{max}} \gg 1. \]  

(1.154)

\[ \int_{0}^{x_{\text{max}}} \frac{dx}{(1 + x)} = \int_{0}^{\theta_{\text{max}}} d\theta \tan^2 \theta = \int_{0}^{\theta_{\text{max}}} (\sec^2 \theta d\theta - d\theta) \\
= \sqrt{x_{\text{max}}} - \tan^{-1} \sqrt{x_{\text{max}}} \\
\approx \sqrt{x_{\text{max}}}, \quad x_{\text{max}} \gg 1. \]  

(1.155)

\[ \int_{x_{\text{min}}}^{x_{\text{max}}} \frac{dx}{x^{3/2} (1 + x)^2} = 2 \int_{\theta_{\text{min}}}^{\theta_{\text{max}}} \left( \cot^2 \theta + 2 \cos^2 \theta \right) d\theta \\
= \int_{\theta_{\text{min}}}^{\theta_{\text{max}}} [-2d(\cot \theta) + d(\sin 2\theta)] \\
= 2 \left( \frac{1}{\sqrt{x_{\text{min}}}^3} - \frac{1}{\sqrt{x_{\text{max}}}^3} + \sqrt{x_{\text{max}}} \right) \\
\approx \frac{2}{\sqrt{x_{\text{min}}}}, \quad x_{\text{max}} \gg \{1, x_{\text{min}}\}. \]  

(1.156)
\[
\int_{0}^{x_{\text{max}}} \frac{dx}{\sqrt{x(1+x)}} = 2 \int_{0}^{\theta_{\text{max}}} \sec \theta d\theta = 2 \log (\tan \theta_{\text{max}} + \sec \theta_{\text{max}})
\]
\[
= 2 \log (\sqrt{x_{\text{max}}} + \sqrt{1+x_{\text{max}}})
\]
\[
\approx 2\sqrt{x_{\text{max}}} \left(1 - \frac{1}{2} x_{\text{max}}\right), \quad x_{\text{max}} \ll 1. \quad (1.157)
\]

\[
\int_{0}^{x_{\text{max}}} \frac{dx}{\sqrt{x(1+x)}} \cdot x = 2 \int_{0}^{\theta_{\text{max}}} \tan^{2} \theta \sec \theta d\theta = 2 \int_{0}^{\theta_{\text{max}}} (\sec^{3} \theta d\theta - \sec \theta d\theta)
\]
\[
= 2 \left[\frac{1}{2} \sec \theta_{\text{max}} \tan \theta_{\text{max}} - \frac{1}{2} \log(\sec \theta_{\text{max}} + \tan \theta_{\text{max}})\right]
\]
\[
= \left[\sqrt{x_{\text{max}}} (1 + x_{\text{max}}) - \log (\sqrt{x_{\text{max}}} + \sqrt{1+x_{\text{max}}})\right]
\]
\[
\approx (x_{\text{max}})^{3/2}, \quad x_{\text{max}} \ll 1. \quad (1.158)
\]

\[
\int_{x_{\text{min}}}^{x_{\text{max}}} \frac{dx}{x^{3/2} (1+x)^{3/2}} = 2 \int_{\theta_{\text{min}}}^{\theta_{\text{max}}} \frac{d\theta}{\sec \theta} \frac{1+2 \tan^{2} \theta}{\tan^{2} \theta}
\]
\[
= 2 \int_{\theta_{\text{min}}}^{\theta_{\text{max}}} d(\sin \theta) \left(\frac{1}{\sin^{2} \theta} + 1\right)
\]
\[
= \left[-\frac{2\sqrt{1+x}}{\sqrt{x}} + \frac{2\sqrt{x}}{\sqrt{1+x}}\right]_{x_{\text{min}}}^{x_{\text{max}}}
\]
\[
\approx -\frac{2}{\sqrt{x_{\text{max}}}} + \frac{2}{\sqrt{x_{\text{min}}}}, \quad \{x_{\text{max}}, x_{\text{min}}\} \ll 1. \quad (1.159)
\]
Note that the log approximations in (1.157) and (1.158) should be obtained by expanding up to two leading terms, not only one:

\[
\log \left( \sqrt{x_{\text{max}}} + \sqrt{1 + x_{\text{max}}} \right) \approx \log \left( 1 + \left( \sqrt{x_{\text{max}}} + \frac{1}{2} x_{\text{max}} \right) \right)
\]

\[
\approx \left( \sqrt{x_{\text{max}}} + \frac{1}{2} x_{\text{max}} \right) - \frac{1}{2} \left( \sqrt{x_{\text{max}}} + \frac{1}{2} x_{\text{max}} \right)^2
\]

\[
\approx \sqrt{x_{\text{max}}} - \frac{1}{2} \left( x_{\text{max}} \right)^{3/2}.
\]

### 1.E Fluctuation-dissipation of target activation

Suppose that the target-receptor complex \( Q \) is synthesized as \( \dot{Q} = Rn \). Because the level of target activation is the true output of the information channel, if we only consider the noise of receptor occupation in solving the information maximization problem, it is equivalent to assuming \( \langle (\delta Q)^2 \rangle \sim TR \langle (\delta n)^2 \rangle \). However, there are additional terms in the product fluctuation \( \delta Q \). For example, it is important to consider the effect of thermal fluctuation. To establish the fluctuation-dissipation formulation, let us imagine that there is a degradation process with rate \( r_- Q \) which balances the concentration of \( Q \) at some equilibrium. Then the master equation is \( \dot{Q} = Rn - r_- Q \), with the equilibrium solution \( Q = (R/r_-) \bar{n} \). Near this equilibrium we can write, following the approach of Bialek and Setayeshgar (2005) in general:

\[
\frac{d}{dt} \delta Q(t) = R\delta n(t) - r_- \delta Q(t) + R\bar{n} \left( \frac{\delta F}{k_B T} \right)
\]

(1.161)

where the last fluctuation term comes from the detailed balance condition \( R/r_- = \exp(F/k_B T) \).

We could also take into account the effect of target (substrate) diffusion, by realizing that the catalytic rate can actually be written as \( R = r_+ b \), such that \( b(\vec{x}, t) \) is the concentration.
of the substrate. Then

\[
\frac{d}{dt} \delta Q(t) = R\delta n(t) - r_+ \delta Q(t) + r_+ \bar{n} \delta b(t) + R\bar{n} f(t)
\]  

(1.162)

where \( f(t) = \delta F(t)/k_B T \). The substrate fluctuation \( \delta b \) is driven by diffusion and is connected to the product fluctuation,

\[
\frac{\partial}{\partial t} b(\vec{x}, t) = D \nabla^2 b(\vec{x}, t) - \delta(\vec{x} - \vec{x}_0) \frac{dQ}{dt}
\]  

(1.163)

where \( \vec{x}_0 \) is the position of the receptor. Linearizing and Fourier transforming both in time and space,

\[
- \omega \delta b(\vec{k}, \omega) = -Dk^2 \delta b(\vec{k}, \omega) - e^{-\vec{k} \cdot \vec{x}_0} (-i \omega \delta Q(\omega));
\]  

(1.164)

with an inverse Fourier transform in space, we get

\[
\delta b(\vec{x}_0, \omega) = i \omega \delta Q(\omega) \int \frac{d^3 k}{(2\pi)^3} \frac{1}{(-i \omega + Dk^2)}.
\]  

(1.165)

This gives

\[
(-i \omega [1 + \Lambda(\omega)] + r_-) \delta Q(\omega) = R\delta n(\omega) + R\bar{n} f(\omega)
\]  

(1.166)

where

\[
\Lambda(\omega) = r_+ \bar{n} \int \frac{d^3 k}{(2\pi)^3} \frac{1}{(-i \omega + Dk^2)}.
\]  

(1.167)

For later use, we evaluate the low frequency limit of \( \Lambda(\omega) \)

\[
\Lambda(0) = r_+ \bar{n} \int \frac{dk}{2\pi} \int \frac{k^2 d\Omega}{(2\pi)^2} \frac{1}{Dk^2} = \frac{R\bar{n}}{bDl}
\]  

(1.168)

where \( l = (\int dk/2\pi)^{-1} \) is the linear dimension of the system. The denominator \( bDl \) has a dimension of inverse time, and may be interpreted as the rate at which the target molecules reach the receptor-target interface by diffusion.
We have a linear response theory

\[ \delta Q(\omega) = \left( \frac{R}{-i\omega [1 + \Lambda(\omega)] + r} \right) \delta n(\omega) + \alpha_Q(\omega) f(\omega) \] (1.169)

where \( \alpha_Q \) is the susceptibility

\[ \alpha_Q(\omega) = \frac{R\bar{n}}{-i\omega [1 + \Lambda(\omega)] + r}. \] (1.170)

Using the fluctuation-dissipation theorem, the noise power spectrum of \( Q \) can be written as

\[ S_Q(\omega) = \frac{R^2 S_n(\omega)}{\omega^2 [1 + \Lambda(\omega)]^2 + r^2} + \frac{2}{\omega} \text{Im} [\alpha_Q(\omega)] \]

\[ = \frac{R^2}{\omega^2 [1 + \Lambda(\omega)]^2 + r^2} \left( S_n(\omega) + \frac{2\bar{n}}{R} [1 + \Lambda(\omega)] \right). \] (1.171)

We can think in terms of the effective noise of \( n \),

\[ S_n^{\text{eff}}(\omega) = S_n(\omega) + \frac{2\bar{n}}{R} [1 + \Lambda(\omega)], \] (1.172)

such that the variance in the long-time limit is

\[ \langle \delta n^2 \rangle_{\text{eff}} = \langle \delta n^2 \rangle + \frac{2\bar{n}}{TR} \left[ 1 + \frac{R\bar{n}}{bDL} \right]. \] (1.173)
Chapter 2

Characterizing the statistical properties of protein surfaces

2.1 Introduction

Proteins and their interactions form the body of the signaling transduction pathway in many living systems. In order to ensure the accuracy as well as the specificity of signaling, it is crucial that proteins recognize their correct interaction partners; in other words, maintaining the specificity of protein-protein interactions is a critical problem every cell needs to solve. How difficult, then, is it for a protein to discriminate its correct interaction partners from the possibly large set of other proteins it may encounter in the cell? Eventually, this is a question about the complexity of the interaction space: the interaction space cannot be too complex (high-dimensional), because the problem of finding and recognizing the specific binding partner must be a solvable one. On the other hand, if the interaction space is too simple (low-dimensional), it may not have enough room for all its constituent proteins to maintain specific interactions. The ensemble of interfaces for protein-protein interactions, in turn, must be constrained by the need for maintaining functional interactions while avoiding spurious ones. How the simultaneous requirement for specificity and capacity limits and
shapes the interaction networks of proteins by evolution, at various stages of interaction, led to a large volume of research articles (Beltrao and Serrano, 2007; Johnson and Hummer, 2011; Perica et al., 2012a; Peleg et al., 2014; Goncearenco et al., 2015) and reviews (James and Tawfik, 2003; Perica et al., 2012b; Sikosek and Chan, 2014).

An important determinant of specific interaction is shape complementarity (Katchalski-Katzir et al., 1992; Janin, 1995; Janin et al., 2008; Keskin et al., 2008). While it is generally understood that specificity of an interaction depends on both the chemical interactions (electrostatic, hydrophobic, etc.) and the geometric fit (shape) between the two interfaces, shape complementarity is believed to play a central role in the initial recognition phase of the interaction. First of all, shape complementarity is a prerequisite for specific chemical interaction (Chothia and Janin, 1975; Zhang et al., 2009). Because all interactions are short-ranged, the two interfaces must begin in a close contact (yet loose enough to allow further, finer samplings of the interaction energy landscape), also known as the encounter complex (Berg and von Hippel, 1985; Von Hippel and Berg, 1989; Kozakov et al., 2014) or the transient complex (Schreiber et al., 2009). Moreover, it was reported that a protein can interact with many proteins with structurally similar interfaces, although at different affinities depending on the amino acid sequence variation (Schreiber and Keating, 2011); a recent theoretical study also observed that shape coding of interactions has higher information capacity compared to chemical coding (Huntley et al., 2016). Besides, shape complementarity has its own entropic effect when placed in the cellular environment, leading to a protein-protein attraction induced by the entropy change of the small molecules floating between the two proteins (Li et al., 2013).

Understanding the properties of the interfaces, through which protein-protein interactions take place, has been an important interest of cell and molecular biology. Reviewing the literature with a focus on the idea of shape complementarity, previous works to characterize the interaction space are roughly divided into two branches. Works in the first branch evolved around an abstract idea of the shape space, first introduced in the immunological
context (Edelstein and Rosen, 1978; Perelson and Oster, 1979). In this picture an antigen is considered as a point in a shape space, and stimulates reaction in the cells that correspond to points within a sphere in the shape space centered at the antigen, called the ball of stimulation. Perhaps most importantly, this idea of shape space could explain cross-reactions intuitively, in terms of the intersection of two or more balls. Because the cross-reactivity between two antigens is a measurable quantity, there were attempts to characterize the parameters of the shape space using immunological data (Perelson and Oster, 1979; Smith et al., 1997; Lapedes and Farber, 2001). According to Smith et al. (1997), the shape space of antibody-antigen interaction can be fit with only about five to eight parameters when modeled as a Euclidean space, which is a remarkably small number of degrees of freedom. However, this notion of shape space is actually not only about the shape of the interface; rather, it is an intuitive tool for describing the overall functional atlas of the interaction, only implicitly involving the shape and the physicochemical properties of the interface.

The other branch of previous works attempted to find typical properties of the known interfaces directly, either in terms of the residue (amino acid) composition or in terms of shape. A majority of the studies to characterize interfaces were focused on the residue composition, as in Bogan and Thorn (1998); Keskin et al. (2005); Reichmann et al. (2007) or Yan et al. (2008), to cite only a few. On the other hand, there have also been attempts to characterize and predict the interfaces in terms of shape. Each work focused on a small number of specifically chosen structural features: for example, contact area and packing (Chothia and Janin, 1975; Janin and Chothia, 1990; Lo Conte et al., 1999), protrusion from the overall globular shape (Thornton et al., 1986; Jones and Thornton, 1997), shape correlation statistics (Lawrence and Colman, 1993), holes and knobs (Norel et al., 1994), size and flatness (Chakrabarti and Janin, 2002), distance from the center of the protein (Nicola and Vakser, 2007) or secondary structure motif (Phillips et al., 2006), although the list is certainly not exhaustive.
To look for these structural features that characterize the interfaces, or for a parsimonious parametrization of the shape space, is already to assume that there are some coarse-grained properties (Vakser et al., 1999; Zhang et al., 2009) that are relevant to the functioning of an interface, rather than to think that every fine detail of the surface matters. More generally, on the bottom of all these studies, there is a belief that there would be an effectively lower-dimensional structure that underlies the apparently high-dimensional set of observed data. This way of thinking was successfully applied to other contexts as well, including but not limited to the spaces of behavior (Stephens et al., 2008; Berman et al., 2014), olfactory perception (Magnasco et al., 2015), neural responses (Sharpee et al., 2004; Schwartz et al., 2006; Park and Pillow, 2013) or even plant traits (Díaz et al., 2015). The related systematic strategies form a large body of techniques called dimensionality reduction, with a recent boost in the context of neural dynamics (Cunningham and Yu, 2014).

In this work, we seek a more direct characterization of the statistical properties of protein surfaces. The shape space of protein surfaces, however, appears extremely high-dimensional and may be very difficult to parametrize efficiently (anything less than writing down the real-valued coordinates of all the surface points is non-trivial), which makes it much harder to work on the shape space than on the sequence space composed of a discrete and finite set of amino acids; which is why progress has mostly been on the sequence side so far. Taking this complexity seriously, we start by crystalizing the very notion of dimensionality of the space of protein surfaces, using intrinsic dimensionality estimators (Grassberger and Procaccia, 1983; Pettis et al., 1979) whose inceptions track back to problems in the chaos theory. For example, the correlation dimension estimator was first proposed for “measuring the strangeness of strange attractors” (Grassberger and Procaccia, 1983); here we aim to measure the complexity of the shape space, which at face value seems to be intractably large and ever more so as we discover more (Tuncbag et al., 2008). As will unfold to more details in the main text, it appears that there is pattern in which the dimensionality grows (the shape space becomes more complex) as larger patches of the protein surfaces are taken
into account, which points to a characteristic size scale on protein surfaces. The idea of the characteristic scale is then taken down to the level of individual protein surfaces. In order to quantify the shape variation of individual protein surfaces, with its intrinsic multi-scaled nature, we develop a generalized surface curvature measure that captures the shape feature at finite scopes of locality, and find a characteristic scale consistent with the result from the dimensionality estimate.

Unlike in most of the previous studies where the known interfaces were considered separately from known non-interfaces, we sample the space of all accessible protein surfaces, which is the biologically relevant shape space when a given protein interface is solving the problem of finding its binding partner in the cell. Indeed, it has been proposed that the boundary between interfaces and non-interfaces might not be so clear (Tonddast-Navaei and Skolnick, 2015), which makes it even more important that we look at the structural space of all protein surfaces without restriction from any (probably incomplete) functional annotation.

2.2 Surface objects and operations

2.2.1 Scope of dataset

We used a non-redundant dataset of complete high-resolution X-ray crystal structures (with resolutions better than 3Å) of proteins specific to *E. coli*. All structures were downloaded from the Protein Data Bank (Berman et al., 2000), abbreviated as PDB from this point throughout the text. The species-specific proteins were filtered by sequence homology, including only those proteins whose sequences identify with the *E. coli* proteome. Following Levy (2010), only the cytoplasmic and non-DNA-binding proteins were included, in order to focus on the protein-protein interactions but not on the protein-ligand or the protein-DNA interactions. Finally, for proteins involved in multi-protein complexes, only those with crystalized “biological assemblies” (i.e. those for which functional protein complexes have
a known structure) are considered. We take a specific subunit from each protein structure (biological assembly), as a full structure registered on PDB usually consists of linearly transformed copies of one or a small number of these units. We thus know where the actual specific interfaces are when the subunits are assembled into the functional complexes, although the interface information is not used directly in the current study. The resulting database consists of 397 E. coli protein structures, listed in Tables 2.2.1, 2.2.2 and 2.2.3.

**Rendering the protein surfaces** In the current work, the object of our interest is the solvent-excluded surface (Richards, 1977) of the protein molecules, which results from rolling a probe sphere (e.g. a water molecule) over the van der Waals surface of the atoms that constitute the protein. An analytical description is available, consisting of pieces of spheres and tori that join smoothly (Connolly, 1983). Once we have a set of protein structures (in terms of the atomic coordinates), we can render the solvent-excluded surface of each chosen protein subunit in the dataset, and store the surface information in a discrete mesh. Here we used the Maximal Speed Molecular Surface (MSMS) algorithm (Sanner et al., 1996), the output from which consists of a list of vertices and a list of triangular faces. MSMS first calculates the analytical model of the solvent-excluded surface, then construct a triangulation in which the mesh points lie on the analytical surface, while treating singularities appropriately. The algorithm allows the user to specify a mesh density, which is the number of mesh vertices per 1\(\text{Å}^2\) of surface area. We use a probe sphere radius of 1.5\(\text{Å}\), modeling a water molecule, and four different mesh densities \{1, 3, 5, 7\} points/\(\text{Å}^2\).

**Defining the shape space** We construct our shape space by sampling a set of surface “patches” of some fixed size, from all proteins in our protein surface database. Importantly, we sample surface patches randomly from all available protein surfaces in our database, rather than focusing on the known protein-protein interfaces. See Section 2.B for the details of the sampling method. Eventually we look at multiple such sets by varying the size of the surface patches collected, and see how the statistical properties change with size. For example, it is
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Table 2.2.2: List of protein structures used in the study (Part 2 of 3).
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Table 2.2.3: List of protein structures used in the study (Part 3 of 3).
natural to expect that the shape space would be more complex when larger surface patches are considered, and this idea will be made more precise as the chapter unfolds.

Alternatively, the shape space could be constructed by sampling curves of fixed length from the protein surfaces, instead of sampling surface patches. Although this curve-sampling approach is less straightforward compared to patch-sampling (because in general, curves might not retain the full shape information of the underlying surface), having a lower-dimensional sampling of the shape space can have a great advantage for the relative ease of the computation and interpretation. For the details of the sampling process also see Section 2.B.

2.2.2 Working with triangulated surfaces

We now define the surface objects (either surface patches or curves) on a more precise basis, in terms of the triangular mesh that approximates the protein surface.
Triangulated surface and sub-surface  A triangulated surface $S$ approximates the corresponding analytical surface $S_0$, with a discrete sampling of surface points or “vertices”, $\mathcal{P} = \{p_i \in S_0\}$, and a set of triangular faces $\mathcal{F} = \{\Delta_f\}$ each of which is a set of three adjacent vertices, $\Delta_f = (p_{f_1}, p_{f_2}, p_{f_3})$. In order for a triangulated surface to be well-defined, it needs to satisfy two conditions: (1) all three vertices $p \in \Delta$ of any given face $\Delta \in \mathcal{F}$ should be members of $\mathcal{P}$; (2) any given vertex $p \in \mathcal{P}$ should participate in at least one face in $\mathcal{F}$.

A triangular sub-surface $S_1$, of the surface $S = \{\mathcal{P}, \mathcal{F}\}$, is a triangular surface whose vertices are a subset of $\mathcal{P}$ and whose faces are a subset of $\mathcal{F}$. A sub-surface $S_1 = \{\mathcal{P}_1, \mathcal{F}_1\}$ of $S$ is said to be induced by a subset of points $\mathcal{P}_1 \subset \mathcal{P}$ if, for every triplet of vertices $p_i, p_j$ and $p_k$ in $S_1$, $\Delta = (p_i, p_j, p_k)$ is in $\mathcal{F}_1$ if and only if $\Delta \in \mathcal{F}$. We will denote such $\mathcal{F}_1$ by

$$\mathcal{F}_1 = F_{\text{induced}}(\mathcal{P}_1; S) = \{\Delta \in \mathcal{F} \mid p \in \mathcal{P}_1 \text{ for all } p \in \Delta\}. \quad (2.1)$$

This definition of an induced sub-surface is analogous to the definition of an induced subgraph in graph theory. If $S$ was a well-defined triangular surface satisfying the two conditions above, the induced sub-surface $S_1$ is also well-defined in the same sense.

Surface points with non-uniform weights  Although the average mesh density is specified in generating the surface mesh, the final density of vertex points sampled by MSMS is not really uniform. In particular, the point density is significantly higher in the “toric” regions that correspond to the valleys between adjacent atoms, compared to the “spheric” regions that correspond to the surface atoms. Hence, when one wants to choose a surface point randomly, treating each vertex in the mesh equally may introduce a systematic bias in favor of the the toric regions. In order to account for the systematic non-uniformity, one
can choose a vertex \( p_i \) weighted by the area associated to it:

\[
w(p) \propto \text{VertArea}(p) = \frac{1}{3} \sum_{\Delta|p \in \Delta} \text{FaceArea}(\Delta),
\]

(2.2)

where the sum runs over all triangular faces \( \Delta \) which has \( p \) as one of its three vertices. Similarly when one wants to average some point quantity \( f(p) \) over multiple surface points, the correct way to do this is

\[
\langle f \rangle_p = \frac{\sum_p f(p)w(p)}{\sum_{p'} w(p')}
\]

(2.3)

**Geodesic path on surface** For a connected pair of surface points \( p_1, p_2 \in S \) on a surface \( S \), the geodesic distance \( d_S(p_1, p_2) = d_S(p_2, p_1) \) is defined to be the length of the minimal path on \( S \) that connects \( p_1 \) and \( p_2 \). This path is called the geodesic path \( G(p_1, p_2; S) \).

Because our surfaces are triangulations of some underlying analytical surfaces, there are two approaches of thinking about the geodesic paths. First, one could imagine a true underlying analytical surface \( S_0 \), and try to approximate the geodesic path on this analytic surface, \( G(p_1, p_2; S_0) \), by a path on a triangulation \( S \) of \( S_0 \). In this case the approximated geodesic path would consist of an ordered set of mesh vertices connected by the triangular edges. Alternatively, one could directly obtain the geodesic path \( G(p_1, p_2; S) \) and the geodesic distance \( d_S(p_1, p_2) \) on the triangulated surface \( S \), treating \( S \) as if it were the “true” surface.

In this case, not only the mesh vertices but all the points that are on the surface of the triangular faces are treated as true surface points. Consequently, the path need not pass through the mesh vertices only; it can now be fully described by an ordered set of vertices or edge points that are connected by the triangular faces. For the detailed algorithms of finding the geodesic paths, see Section 2.A.

**Geodesic disk and surface patch** A geodesic disk \( D(p_0, r; S) \), on a triangulated surface \( S \), is the sub-surface of \( S \) induced by the set of all mesh points \( p \) within the geodesic distance
$r$ from the center $p_0$.

$$\mathcal{D}(p_0, r; \mathcal{S}) = \{\mathcal{P}_{\text{Disk}}, F_{\text{induced}}(\mathcal{P}_{\text{Disk}}; \mathcal{S})\},$$  \hspace{1cm} \text{(2.4)}$$

where

$$\mathcal{P}_{\text{Disk}}(p_0, r; \mathcal{S}) = \{p \in \mathcal{S} \mid d_S(p, p_0) < r\}.$$  \hspace{1cm} \text{(2.5)}$$

Note that according to this definition, a geodesic disk is not necessarily a (simply connected) disk in the topological sense, depending on the shape of the original surface $\mathcal{S}$.

In this study, a surface patch $\mathcal{D}_A(p; \mathcal{S})$ is specifically defined as a geodesic disk with a given surface area $A$ centered at a mesh point $p$ on a triangular surface $\mathcal{S}$, where the surface area is defined as

$$\text{Area}(\mathcal{D}) = \sum_{\Delta \in \mathcal{D}} \text{FaceArea}(\Delta).$$  \hspace{1cm} \text{(2.6)}$$

The surface area of a disk as a function of the geodesic radius, $A(r)$, is a monotonic but generally non-trivial function that depends on the specific shape of the underlying surface. In practice, we first generate a geodesic disk with some large enough radius $r_{\text{max}}$ such that $A(r_{\text{max}}) > A_{\text{target}}$, where $A_{\text{target}}$ is the targeted total surface area of the patch. Then it is easy to compute $A(r)$ at all $r \in (0, r_{\text{max}})$, and to find $r$ at which $A(r) = A_{\text{target}}$.

### 2.3 Intrinsic dimensionality

In this section, we aim to measure and understand the intrinsic dimensionality of the shape space of the protein surfaces. The intrinsic dimension is different from the embedding dimension, where the latter is the dimension of the space containing the shape objects, i.e., the number of parameters need to describe each shape object within this space. For example, imagine that we draw a circle on the page (Figure 2.2). The circle is embedded in a 2-dimensional plane which is the paper, and each point in the circle would in general be assigned a coordinate $(x, y)$. The dimensionality of the embedding space is not limited by the intrinsic structure of the dataset and may be arbitrarily large, depending on how the
Figure 2.2: Basic idea of intrinsic dimensionality: the circle is embedded on this two-dimensional page, but intrinsically, the set of points that form the circle has only one degree of freedom along the arc. The intrinsic dimensionality of the noisy circle can be measured if viewed at the right scale.

Data have been represented. For example, you could also hold the page in a 3-dimensional space which is your room, and assign a new coordinate \((x, y, z)\) to each point in the circle. On the other hand, the circle is an intrinsically 1-dimensional manifold, no matter how it was represented in the embedding space; this is what we mean by saying that the intrinsic dimensionality of the circle is 1. Note that the “coordinates” corresponding to the intrinsic structure of the dataset (in the case of the circle, it would be the polar angle \(\theta\)) may be nonlinear in the Cartesian coordinates of the embedding space.

2.3.1 Measuring the intrinsic dimensionality of the dataset

How can we quantify the intrinsic dimensionality of a set of objects with high embedding dimensions? Here we use two variants of the near-neighbor algorithm. The intuition is that the volume of a hyper-sphere of radius \(r\) scales as \(r^D\) in a \(D\)-dimensional space. For a point dataset \(\mathcal{X}\), it means that if we pick a point \(x \in \mathcal{X}\), the number of neighboring points \(C(r; x)\) within a distance \(r\) from \(x\) would scale as \(r^D\) on average, assuming that the points are uniformly distributed in the \(D\)-dimensional manifold.

\[
\langle C(r) \rangle = \langle C(r; x) \rangle_x \propto r^D. \tag{2.7}
\]
This is the idea of the correlation dimension (Grassberger and Procaccia, 1983), developed originally to characterize the low-dimensional behavior of chaotic systems, and to distinguish deterministic chaos from random noise. In principle, the dimension $D$ is to be obtained by

$$D_{\text{corr}} = \frac{\partial \log \langle C(r) \rangle}{\partial \log r}. \quad (2.8)$$

Based on the same idea, one could alternatively consider the average distance $\langle r_k \rangle = \langle r_k(x) \rangle_x$ to the $k$-th nearest point from all $x$'s. This leads to the definition of the regression dimension (Pettis et al., 1979)

$$k \propto \langle r_k \rangle^D \Rightarrow D_{\text{reg}} = \frac{\partial \log k}{\partial \log \langle r_k \rangle}. \quad (2.9)$$

The correlation dimension and the regression dimension differ only in the way they average over the dataset. Whereas the correlation dimension averages the count of neighboring points up to a given distance over different choice of the centers, the regression dimension considers the average distance needed to reach a given degree of neighborhood.

**Pairwise distances**

In order to compute the intrinsic dimensionality of a given dataset using the method described here, it is necessary to first compute the full set of pairwise distances for all pairs of samples within the dataset. Depending on the size and type of the dataset, calculating the pairwise distance matrix may be the computational bottleneck. The pairwise distances are then used to obtain the average number of points within the $r$-sphere, $C(r)$, and the average distance to the $k$-th neighbor, $\langle r_k \rangle$, for the correlation dimension and the regression dimension, respectively. The distance measure should be defined appropriately according to the type and structure of the dataset. If each object in the dataset can be parametrized as a vector in some high-dimensional embedding space, for example, a possible distance measure would be the Euclidean distance in the corresponding space. Note that the current method
does not depend on the chosen parametrization of the samples, but only on the definition of pairwise distance, which proves useful in high-dimensional sample spaces.

Choosing the right scale: with the two sources of error

Now we need to find $D$ by regression. We are interested in working with a real dataset, which is noisy and, more importantly, of a finite size. For example, our method should be able to determine the intrinsic dimensionality to be $D = 1$ for the noisy circle in Figure 2.2. This problem is straightforward for human eyes (and for our dimension estimators as well), but only when we are looking at the dataset at the right scale. If we look too closely, we would only see the randomness whose dimensionality is determined by the embedding space (within the thickness of the noisy circle). On the other hand, as we try to extend our scope, our count of the neighboring points would at some point be limited by the “edge” of the dataset, because a real-world dataset cannot extend indefinitely. The entire dataset might even look like a single blurry point at the extreme end.

In other words, there are two major sources of error in estimating the intrinsic dimension $D$ using the near-neighbor methods. First, there is a statistical error due to the sparsity of points in the dataset (Smith, 1988). Second, there is a more fundamental systematic geometric effect, due to the finite size of the dataset (Nerenberg and Essex, 1990; de Rover and van de Water, 1995). The two errors are illustrated in Figure 2.3A in the case of the regression dimension estimator. A real-data example is also shown in Figures 2.3B and 2.3C. Whereas the statistical error may be tamed by appropriate smoothening, the geometric effect is unavoidable and always tends to underestimate the count of the neighboring points close to the dataset boundaries, consequently underestimating the dimensionality.

Importantly, the negative systematic bias from the geometric effect becomes more severe at higher dimensions, where the presence of the boundary of the dataset becomes more significant. Assuming that the dataset size is fixed to $N$, the typical size $l$ of the dataset in the $D$-dimensional space scales as $l \sim N^{1/D}$. The edge effect can only be avoided when
\( l \gg r \) where \( r \) is our viewing scale of choice, but this condition becomes unachievable when \( D \) is large. In principle this curse of dimensionality can be overcome by increasing the dataset size \( N \), but in practice even a doubling of the number of observations in the dataset is often impossible. Figure 2.3D demonstrates the systematic error growing with the true dimensionality of the dataset, using synthetic datasets of high-dimensional vectors that are uniformly sampled within a hyper-sphere. All this emphasizes the importance of our choice of the viewing scale in measuring the intrinsic dimension \( D \) from a real dataset. In practice, in terms of the regression problem formulated above, this is an issue of choosing at which scale to evaluate the derivatives – the relevant scale would be the point-to-point distance \( r \) for the correlation dimension (equation 2.8), or the order of neighborhood \( k \) for the regression dimension (equation 2.9). See Section 2.E for more discussion. Also see Figure 2.S5 to find more example plots of \( \log C(r) \) vs. \( \log r \) (for the correlation dimension) or \( \log k \) vs. \( \log \langle r_k \rangle \) (for the regression dimension) at different scales, to supplement Figure 2.3.

There are other modern (and possibly better-performing) estimators for the intrinsic dimensionality, for example the maximum likelihood estimator suggested by Levina and Bickel (2004). Here, however, we choose to use the most straightforward definitions, which also make fewer assumptions, in the interest of making our analyses and interpretations as transparent as possible.

### 2.3.2 Dimensionality of the space of surface objects

We set out to calculate the intrinsic dimensionality of the set of surface patches at fixed sizes, sampling the space of protein surfaces. At each given size, defined by the total surface area \( A \), we randomly sample a set of \( N \) surface patches (see Section 2.B for how we generate a surface patch of a given area). The process is repeated to construct an independent dataset at each designated surface area \( A \). Naturally, we expect that the set of larger surface patches will have a more complex structure than the space of smaller surface patches. Here we aim to make this idea quantitative by measuring the dimensionality for each dataset.
Figure 2.3: Estimation errors of intrinsic dimensionality. (A) A schematic of the two sources of error in solving the regression problem. (B) A plot of log \( k \) vs. log \( \langle r_k \rangle \) plotted at all scales \( r \), calculated from the pairwise distances of a set of protein surface curves with the total length \( L = 10 \AA \). (C) The resulting regression dimension estimates from the dataset shown in (B), using two methods for taking the finite difference approximation of derivative, explained in details in Section 2.E. (D) The systematic error grows with the true dimensionality of the dataset, shown using synthetic datasets of high-dimensional vectors. Even when the dataset size \( N \) is increased by a full order of magnitude, the systematic error at high dimensions is still significant; the curse of dimensionality is not easily tamed. (E) Rationale for sampling the protein surface with geodesic curves, replacing surface patches. If \( D_2 \) is the number of independent degrees of freedom in the 2-dimensional manifold and \( D_1 \) is the number of degrees of freedom in the 1-dimensional slice that sub-samples the manifold, then one would expect \( D_2 \sim D_1^2 \) in general.
Within each dataset, we compute the pairwise distances between all pairs of surface patches. In this case the pairwise distance\(^1\) is defined in terms of the Procrustes distance between the patches, based on the superimposition of the two patches by optimal translation and rotation (see Section 2.C for details). Determining the Procrustes distance between surfaces is complicated, essentially because there is no unique parameterization of the two-dimensional surface patch (or equivalently in our case, no unique indexing scheme of the surface points in the patch), for example due to the rotational degree of freedom. In practice we had to look for the optimal superimposition starting from multiple initial configurations (different indexing of the surface points), obtained by rotating one patch around the axes that goes through the center of the patch and is normal to the best embedding plane of the patch. Therefore the pairwise distance calculation was a real computational bottleneck in the procedure, not only for the obvious reason that the computational time scales as \(O(N^2)\) where \(N\) is the number of surface objects in the dataset, but also because each pairwise distance determination involves multiple iterations of the Procrustes algorithm.

With \(N \approx 1000\) patches per dataset, we find the dimensionality of the space of patches at sizes \(A \gtrsim 50\text{Å}^2\) to be as large as \(D_{\text{patch}} \approx 30–40\) (data shown in Figure 2.S2). This large number is problematic, because it is not statistically reliable when the number of observations is too small compared to what would be needed to estimate the true dimensionality, and one would never have enough observations for such large dimensionality. In particular, Eckmann and Ruelle (1992) calculated an upper bound of dimension estimates \(D_{\text{max}}\) that can be obtained from a given dataset of \(N\) observations, which basically follows from the argument that the total size of the dataset \(l\) should be much larger than the viewing scale \(r\), or \(\rho = r/l \ll 1\). They state that the correlation dimension estimator (Grassberger and

\(^1\)To be more precise, the Procrustes distance of a pair of surface patches should be called the pairwise dissimilarity instead of the pairwise distance. The Procrustes distance is not a distance metric in the mathematical sense, because the triangle inequality does not hold.
Procaccia, 1983) will not produce dimensions larger than

\[ D_{\text{max}} = \frac{2 \log N}{\log(1/\rho)} \]  

(Eckmann-Ruelle) \hspace{1cm} (2.10)

Using the scale separation criterion value of \( \rho \approx 0.1 \), the Eckmann-Ruelle bound suggests that the largest reliable estimate of the intrinsic dimensionality is only \( D_{\text{max}} \approx 6 \) for our surface patch dataset \( (N = 10^3) \). Note, however, that this is a pessimistic bound that cares only of the systematic error, which in principle can be corrected if the dataset is well-behaved (see Figure 2.3D and Section 2.E).

**Set of geodesic curves on protein surfaces**

In order to get around the curse of dimensionality, we consider the set of geodesic curves (of a fixed length \( L \)) to sample the space of the protein surfaces. The idea is to benefit from the fact that the geodesic curves are simpler surface objects: whereas each surface patch is a 2-dimensional object, each geodesics curve is a 1-dimensional object.\(^2\) In the limit where surface is constructed with uncorrelated patches, as illustrated in Figure 2.3E, we would expect \( D_2 \sim D_1^2 \) where \( D_2 \) is the dimension of the set of surface patches and \( D_1 \) is the dimension of the set of geodesic curves. Because a set of geodesic curves is lower-dimensional, the systematic error is expected to be less severe compared to the case of surface patches. Moreover, it is easier to tackle larger datasets with the curves, because dealing with curves is computationally less costly than dealing with patches (no more rotational degree of freedom).

Similarly as with the surface patches, we can construct a set of geodesic curves at each fixed length of the curves \( L \). See Section 2.B for how we sample a random geodesic curve from the set of protein surfaces. Once the curves are sampled, the pairwise distances are

\(^2\)Caveat: when we say that the surface patch is a 2-dimensional object, it means the dimensionality of the points that constitutes a given surface patch. This should be distinguished from our discussion of the dimensionality of the set of surface patches, where each surface patch plays the role of a high-dimensional “point”.

76
Figure 2.4: Dimension estimates for the set of protein surface curves, sampled from triangulated surfaces of different mesh densities, at a fixed curve-sampling density of 10 points/Å. The dimensionality tends to grow linearly with the length of the curves in the dataset. The result is robust over different mesh densities shown here, meaning that our current sampling of the triangulated surface at 7/Å² (and the geodesic curve at 10/Å) is fine enough in this context.

Calculated within each dataset using the Procrustes algorithm, which is now straightforward (without the multiple iterations) and fast for the curve objects. See Section 2.C for details.

Now we are ready to estimate the intrinsic dimensionality of the space of protein surfaces, sampled by the set of geodesic curves. After calculating the full pairwise distance matrix within each dataset, we can directly calculate the correlation dimension (equation 2.8) and the regression dimension (equation 2.9), at the optimal scales empirically determined for the respective estimators (Section 2.E).

Working with the geodesic curves, we can deal with a larger dataset of \( N = 10^4 \) at each curve length thanks to the reduced computational cost per pair, achieving a scale-up of a full order of magnitude compared to the surface patch dataset. This gives the Eckmann-Ruelle bound (equation 2.10) of \( D_{\text{max}} \approx 8 \). The estimated dimensionality of the set of geodesic curves turns out to be \( D_{\text{curve}} \approx 7 \) at the longest length considered (\( L = 20\text{Å} \)), which is now safely within the limit of the method.
As expected, the intrinsic dimensionality $D$ grows as the length $L$ of the curves in the dataset increases (Figure 2.4), using either of the two dimension estimators. What is unexpected is the pattern in which it grows: we observe that there are two regimes of different growth rates, with the transition taking place at $L \approx 4 - 7\text{Å}$. In particular, in the large-$L$ regime, it appears that the dimensionality $D$ grows with rate $\partial D/\partial L \sim 1/7\text{Å}$, meaning that each additional sampled length of 7Å accounts for an extra dimension in the dataset. Also, the estimated values of the curve dimensions are in a reasonable agreement with the patch dimensions, when the latter is scaled with a square root and plotted versus an effective length scale $L_{\text{eff}} = \sqrt{A/\pi}$ rather than the patch area $A$ (data shown in Figure 2.S2). This result is consistent with the assumption of $D_{\text{patch}} \sim D_{\text{curve}}^2$, which supports our practice of sampling the curve objects to study the dimensionality of the underlying surface.

One possible interpretation of the linear increase of the dimensionality $D(L)$ comes in a simplified picture where the protein surface is a mosaic of uncorrelated patches of area $A_c \sim 50\text{Å}^2$ (corresponding to uncorrelated curve segments of length $L_c \sim 7\text{Å}$). In this picture, each additional area of $A_c$ on the surface contributes to an extra dimension in the space of surface patches. If this is the case, it would open the possibility of modeling the shape space of protein surfaces in terms of the uncorrelated surface patches as the structural building blocks, which is a huge jump from a modeling regime where all coordinates of the surface atoms have to be specified.

### 2.3.3 Dimensionality of synthetic datasets

In order to have a better understanding of the results from the dimensionality estimates of protein surface objects, it would be useful to repeat the analysis on synthetic datasets with well-known properties. For example, we have already used a set of high-dimensional vectors in order to understand how the systematic error grows with the true dimensionality of the dataset (Figure 2.3D). Now, since our protein surface dataset consists of geometric objects embedded in the real space, we would like to examine the link between the intrinsic
dimensionality and the statistical properties of geometric objects. Here we will restrict to the case of curve objects.

**Synthetic planar curves (with correlated displacements)**

Let us first consider simple planar curves, described by a set of points \( \{x_i, f_i\} \) where \( \{x_i\} \) is a fixed uniform grid. In this case each curve can be described by a vector \( f = \{f_i\} \), and the pairwise distance is simply the Euclidean norm \( d(f_1, f_2) = \|f_1 - f_2\|^2 \) between two vectors \( f_1 \) and \( f_2 \). The curves are generated as Gaussian random variables (Rasmussen and Williams, 2005) where the mean (0) and variance (\( \sigma^2 \)) of the individual curves are fixed, and the smoothness of the curve is controlled through a two-point covariance function \( k(x_1, x_2) \) in which a length scale can be specified. The covariance constrains the ensemble average of the two-point correlation as

\[
\langle f_i f_j \rangle = \langle f(x_i) f(x_j) \rangle = k(x_i, x_j). \quad (2.11)
\]

We use two standard forms for \( k(x_i, x_j) \), each with a single correlation length \( \xi \): the Gaussian function \( k_g(x_1, x_2) \sim \sigma^2 \exp(-(x_1 - x_2)^2/(2\xi^2)) \) and the exponential function \( k_e(x_1, x_2) \sim \sigma^2 \exp(-|x_1 - x_2|/\xi) \). Whereas the Gaussian covariance produces smoother curves (Figure 2.5A), the exponential covariance produces noisier curves with more small-scale fluctuations because the covariance falls off more steeply (Figure 2.5B).

Going through the same analysis as with the protein data, we can make two important observations from the dimension estimates. First, we find that the dimensionality of the set of synthetic curves increases at a rate of \( \partial D/\partial L \approx 1/\xi \), where \( \xi \) is the fixed correlation length. Results from different combinations of model parameters collapse on a single trend when with a renormalized length variable \( L/\xi \) (Figure 2.5C-D). This result supports our idea that the linear increase of intrinsic dimensionality may be attributed to the existence of a single length scale. Second, we observe an interesting difference between the two types of
Figure 2.5: Examples of synthetic curves generated as Gaussian random variables with fixed correlation lengths, where the covariance function of the points in each curve was specified as either (A) Gaussian or (B) exponential. Each panel shows 7 samples drawn from the same model. (C-D) Dimensionality of synthetic datasets (A) and (B), respectively, calculated using the regression dimension estimator. Different markers indicate different combinations of model parameters: the total length of curves $L$ (different colors) and the correlation length $\xi$ (different symbols). In each case, the ensemble gains an extra dimension for every $\Delta L \sim 1/\xi$ added to the curve length (fit in the range where $L/\xi > 1$), whereas the offset dimension depends on the smaller-scale fluctuations of the curves.
synthetic curves with different smoothness, in the initial behavior of the dimensionality when the total length of the curve is smaller than the correlation length, $L < \xi$. Compared to the smoother curves (Figure 2.5C), the initial increase of the dimensionality estimates for the noisier curves (Figure 2.5D) is much steeper. This observation suggests that the initial offset of the dimensionality reflects the smaller-scale fluctuations below the correlation length. In the case of the protein surface data, the initial offset in the dimensionality plot $D(L)$ would have been reflecting the surface ruggedness below the characteristic scale, such as the protruding atomic spheres, which is fundamental to the way the protein surfaces are constructed.

**Synthetic polymers (with correlated angular displacements)**

However, the set of planar curves may not be the best null model for our data. Our protein surface curves consist of 3D space points sampled at regular intervals, where $dx, dy, dz$ are not all independent, and the intrinsic measure of length is the arc length $ds$ (such that $ds^2 = dx^2 + dy^2 + dz^2$) rather than any one linear coordinate. Moreover, the fact that they are geodesic curves on surfaces may further constrain the shape space of protein surface curves. Here we consider the ideal chain model in polymer physics (Rubinstein and Colby, 2003), where a (semi-flexible) polymer is described in terms of a chain of short rigid rods of fixed lengths, jointed linearly to form a long curvy object in space. Each joint can be fully described by a bond angle $\theta$ and an angle of torsion $\phi$ (Figure 2.6A; also see Section 2.F for details). The polymer model may be a particularly good fit to our case if we assume that the surface constraint and the geodesic constraint are summarized as some constrained distribution of these angles. Indeed, if one measures the bond angle and the torsion from the real protein curves, it appears that the (positive) bond angle $\theta$ is very narrowly distributed with a peak at zero and with the standard deviation $\sigma_{\theta} \sim 0.14$, whereas the torsion angle $\phi$ is heavy-tailed (Figure 2.6B). The peak of $\phi$ near zero (small torsion) presumably reflects the fact that our curves are geodesic paths on the surface.

81
Figure 2.6: The chain model for synthetic space curves.  

(A) Construction of the polymer chain, with the two angles.  

(B) Angles recovered from protein surface curves.  

(C) Persistence length of the set of real protein surface curves is measured to be 7.86Å, fit within the small-distance regime. The longer-range decay length, fit over the range of arc distances 5Å – 10Å after the elbow, is about 25Å (fit not shown in figure).  

(D) For a set of synthetic polymers, where the bond angles were drawn from a half-normal distribution with the standard deviation matched to that of protein surfaces curves, the persistence length is measured to be about 1Å.  

(E) Regression dimensions of the sets of 1000 synthetic polymers with different total lengths, where the bond angle θ is drawn from half-normal distributions with varied widths, and the torsion angle φ is uniformly distributed on (−π/2, π/2). Standard deviations indicated in the figure are that of the underlying normal distributions, which corresponds to σ in the text.
When generating synthetic polymers, therefore, we considered rigid rods of lengths \( \Delta s = 0.1 \text{Å} \) and let the bond angles be drawn from some distribution. Specifically, we use half-normal distributions (i.e. draw a random variable from a normal distribution centered at zero, then take its absolute value) of specified standard deviations. The half-normal distribution was chosen because it is a monotonically decreasing distribution on the positive real domain (in a qualitative match to the empirical distribution of the bond angle measured from the protein surface curves) that can be fully described by the standard deviation. It is known that the standard deviation of the half-normal distribution \( \sigma \) is related to the standard deviation of the underlying normal distribution \( \hat{\sigma} \) as \( \sigma^2 = \hat{\sigma}^2(1 - 2/\pi) \). Therefore in order to reproduce the standard deviation of \( \sigma_\theta \sim 0.14 \) as observed from real protein surface curves, for example, the generating normal distribution should have \( \hat{\sigma} \sim 0.23 \). For the torsion, we use a uniform distribution on \( \phi \in (-\pi/2, \pi/2) \), randomizing as much as possible; the only thing we control in this model is the bond angle distribution. This assumption is also called the freely rotating chain (Rubinstein and Colby, 2003).

The natural length scale in this model is the correlation length of the angular displacements, also known as the **persistence length** \( l_p \), defined by

\[
\langle \cos \theta(s) \rangle \sim \exp(-s/l_p)
\]  

(2.12)

where \( \theta(s) \) is the angular displacement (angle between the tangential vectors) of two points separated by an arc length \( s \). Interestingly, the persistence length of the protein surface curves is calculated to be \( l_p = 7.64 \text{Å} \) (Figure 2.6C), which is close to our estimate of the characteristic length obtained from the growth of intrinsic dimensionality.

Analytical treatment of the persistence length is often difficult, but we can try to approximate the persistence length scale of the synthetic model in the regime where \( \theta(s) \) is small (the worm-like chain limit), by writing \( \langle \cos \theta(s) \rangle \approx 1 - \langle \theta^2(s) \rangle /2 \). Neglecting the fact that angular displacements don’t really add up, let us go on by making a very rough approx-
imation $\langle \theta^2(s) \rangle \approx \langle \theta_1^2 \rangle (s/\Delta s)$ in the random-walk sense, where $\Delta s$ is the joint separation, and $\theta_1 \equiv \theta(\Delta s)$ is the single-joint bond angle. This approximation might hold in the limit where the joint separation $\Delta s$ is much smaller than the persistent length. The single-joint bond angle variance is $\langle \theta_1^2 \rangle = \hat{\sigma}^2$ when $\theta_1$ is drawn from a half-normal distribution with an underlying normal distribution $\mathcal{N}(0, \hat{\sigma}^2)$. Putting the assumptions together, and evaluating at $\Delta s = 0.1\text{Å}$ and $\hat{\sigma} = 0.23$ for our protein surface curves, this corresponds to a persistence length of $l_{p,\text{approx}} \approx 2\Delta s/\hat{\sigma}^2 = 3.78\text{Å}$. But once again, this approximation is crude, and neglects the fact that angular displacements do not add up in trivial ways. The resulting value should only be used to check the rough scale of the persistence length to expect, which indeed appears to be in the order of Angstroms. In practice, the angular displacement becomes uncorrelated more quickly due to the effect of torsion; the persistence length of the synthetic polymer dataset was measured to be as small as $l_{p,\text{rand}} \approx 1\text{Å}$, where the unit is set by the fixed joint separation $\Delta s = 0.1\text{Å}$.

Finally, with exactly the same process as with protein surface curves, we can estimate the regression dimensions of the sets of synthetic polymers (Figure 2.6E). First of all, we find that when the bond angle distribution of the synthetic polymers is matched to the empirically measured distribution, the resulting dimensionality estimates are very similar to what we see in the case of real protein surface curves. In particular, the dimensionality $D(L)$ grows linearly with the total length of the curves in the dataset, almost overlapping with the real data, as shown in the figure. The intrinsic dimensionality of the set of curves is almost exactly reproduced by matching the standard deviation of the bond angle distribution, while randomizing all other properties including torsion. On the other hand, the offset dimensionality $D(0) \approx 4$ of the synthetic dataset appears to be different from the offset in the protein curve dataset, $D(0) \approx 2$. This difference may be explained in terms of the distribution of curve samples in the high-dimensional shape space (see Section 2.E for more discussion), using the definition of our regression dimension estimator. This problem provides a good test case for demonstrating our understanding of how the dimension estimator works.
Overall, these results on synthetic curves reinforce our hypothesis on the connection between the scale of unit dimensionality growth and the correlation length of the samples. Going back to the result from real protein surface curves, this suggests that the linear growth of intrinsic dimensionality over increasing curve lengths reflects some characteristic length scale of protein surfaces. What would this characteristic scale mean, and can we confirm the existence of such a scale by looking at the individual shapes of our protein surfaces directly? In the next section we attempt to answer these questions by returning to the full protein surfaces.

2.4 Scales of shape variation

Shifting the interest from the ensemble to the individual protein surfaces, we would like to ask whether there is any characteristic scale of shape variation on real protein surfaces, as inferred above from the results of dimensionality estimates, and from the estimate of the persistence length of the protein surface curves. A good candidate measure of shape variation is the surface curvature: it is an intrinsic property of the surface, and an intuitively interpretable proxy for shape variation. A fundamental difficulty in thinking about the surface curvature of protein surfaces, however, is that any classical definition of surface curvature would not be relevant for our protein surfaces because it is defined point-wise and will almost always look at the atomic spheres. To avoid finding only the trivial atomic feature, and to capture the multi-scale nature of the protein surfaces (Zhang et al., 2009), we need to work with a generalized definition of surface curvature which is defined at finite and variable scales.

We define the scope-dependent surface curvature tensor \( \hat{K}(r) \), which captures the second-order variation of the shape over a locality of a finite scope \( r \), at each point on the surface. We study how this curvature \( \hat{K}(r) \) and its eigenvalues scale with the scope \( r \), and what it means in the context of protein surfaces. We also calculate the correlation function of this curvature over pairs of points on the same protein surface, and in particular, find an
oscillation in the correlation function with period $\sim 7\AA$, indicating a characteristic scale of shape variation on the protein surfaces.

### 2.4.1 Measuring surface curvature at finite scopes

We start by re-examining how we would like the surface curvature to capture the second-order properties of the surface. Given a local description\(^3\) of a surface $z = f(x,y)$, near the surface point $p_0 = (x_0, y_0, z_0)$, one can make analytic expansions near this point. If the coordinate system was chosen such that $z = z_0$ is the tangential plane, the linear terms in the expansion vanish, and the surface height can be approximated to the leading order by the quadratic form:

$$dz = f(x,y) - f(x_0, y_0) \approx -\frac{1}{2}ds^T\hat{K}ds,$$

where $ds \equiv \begin{pmatrix} dx \\ dy \end{pmatrix}$. \hspace{1cm} (2.13)

This $\hat{K}$ is called the curvature tensor. The eigenvalues and eigenvectors of $\hat{K}$ are the two principal curvatures and the principal directions, respectively, and are independent of the initial parametrization of the surface. Here we follow the sign convention where a positive curvature means a concave surface (protruding upward).

**The scope-dependent surface curvature**

For an analytic surface, as just reviewed, $\hat{K}$ is well defined in terms of the second derivatives of the surface heights at the point of interest $p_0$. However, this is neither what we can do for a triangulated surface, nor is it what we want to do for the current purposes. Now we will generalize this definition to accommodate a finite vicinity of $p_0$ which contains $N$ discrete surface points, $\{p_1, p_2, \cdots p_N\}$. We define the scope-dependent surface curvature

\(^3\)Note, however, that a map like $z = f(x,y)$ cannot be a global description of the protein surface in general, because often there are structures that “overhang”, such as a long bent protrusion that consists of one or more amino acids.
tensor $\hat{K}(p_0; N)$ to be the minimizer of the square error

$$\hat{K}(p_0; N) = \arg \min_{\hat{K}'} Err(\hat{K}'; p_0, N), \quad (2.14)$$

where

$$Err(\hat{K}'; p_0, N) = Err(\hat{K}'; \{p_i\}_{i=1}^N) = \sum_{i=1}^N \left( dz_i + \frac{1}{2} ds_i^T \hat{K}' ds_i \right)^2. \quad (2.15)$$

This is a linear regression problem which can be solved exactly. Alternatively, instead of fixing the number of points $N$, we can also specify the geodesic radius $r$ to define the locality as geodesic disk. This way we can calculate a mean scope-dependent surface curvature, or simply the mean curvature, $K(p; r)$ at each surface point $p$, given a scope of locality $r$.

Throughout the rest of the section we will be interested in three curvature quantities – the mean curvature $K = \frac{1}{2} \text{tr} \hat{K}$, and the two principal curvatures $\kappa_{\text{max}}$ and $\kappa_{\text{min}}$ sorted by the sizes. The mean scope-dependent surface curvature is simply the mean of the two principal curvatures, $K = (\kappa_{\text{max}} + \kappa_{\text{min}})/2$.

**Calculating from generic coordinates** The above formulation assumes that the point coordinates are already aligned with the local orientation of the surface, such that the $z$ coordinate is in the normal direction and the two surface coordinates are in the tangential plane. However, for a real point cloud at a finite scope, applying this formulation directly requires a two-step fitting: first find the tangential plane, then the curvature tensor. In this case the source of error is unnecessarily distributed into the two steps.

Instead, starting from an arbitrary parametrization of the 3-dimensional coordinates of the point cloud, one may fit all coefficients at once up to the quadratic term. This involves finding the vector $g$ and the matrix $H$ that minimize the error function

$$Err(g, H; \{p_i\}) = \sum_{i=1}^N \left( dz_i - g^T ds_i + \frac{1}{2} ds_i^T H ds_i \right)^2. \quad (2.16)$$
This is still a linear least-squares regression problem that can be solved exactly. The resulting curvature tensor is (Pressley, 2001)

\[
\hat{K} = \frac{1}{(1 + g_1^2 + g_2^2)^{3/2}} \begin{pmatrix}
(1 + g_2^2)H_{11} - g_1 g_2 H_{12} & (1 + g_2^2)H_{12} - g_1 g_2 H_{22} \\
(1 + g_1^2)H_{12} - g_1 g_2 H_{11} & (1 + g_1^2)H_{22} - g_1 g_2 H_{12}
\end{pmatrix},
\]  

(2.17)

which does not depend on the initial parametrization of the coordinates. We have tried using both the tangential plane method and the generic coordinate method. The results tend to agree in smaller scopes, but disagree more in larger scopes, presumably because the notion of the tangential plane becomes more irrelevant at larger scopes. In the rest of the section, we will only show the results from the method based on generic coordinates.

### 2.4.2 Surface curvature of proteins

Using the above formulation of the scope-dependent surface curvature tensor, we can measure the curvature of a given protein surface, by making quadratic fits at each point of the surface mesh, at different scopes. For clear presentation, we focus on a single small protein: all data shown in this section is from protein #4 in our dataset (PDB identifier 1JW9-D). Data from other proteins are not essentially different, in terms of the observations we will be presenting here.

**Scaling of surface curvature at different scopes**

First of all, we would like to make sense of the value of the scope-dependent surface curvature at a finite scope of locality. As the scope-dependent surface curvature tensor \( \hat{K}(p; r) \) is a quadratic property of the surface, the measured curvature quantities reflect different quadratic approximations of the surface at different scopes \( r \) (Figure 2.8A). Our surface curvature is scope-invariant only when the surface is an exact paraboloid whose vertex is at \( p \).
Figure 2.7: The mean scope-dependent surface curvature $K$ calculated for protein 1JW9-D. This is a relatively small protein in our database, with the longest dimension $\sim 41\text{Å}$ (range of the first component from a principal component analysis). The surface of the protein is colored with the value of the mean surface curvature at scopes $r = 1\text{Å}$ and $r = 5\text{Å}$ respectively. Color contrast has been enhanced by a nonlinear transform of the curvature values (color scale common to both pictures).

Remembering that surface curvature has a unit of inverse length, the most natural scaling for our scope-dependent surface curvature would be the inverse dependence on the scope of locality, which is also a measure of variation of the coordinates of the surface points considered ($r \sim |\mathbf{d}s|$ in equation (2.13)). Therefore, we would expect a scaling relation of $\hat{K} \sim 1/r$ in the case of a completely featureless surface, whereas in reality, the scope-dependence of the surface curvature should reflect the characteristic scales of actual surface features. Interestingly, we find that the scope-dependent surface curvature quantities from a real protein surface exhibit a mean scaling of $\sim 1/r$ at large scopes $r > 10\text{Å}$ (Figure 2.8B). In particular, when extrapolated to the large-$r$ limit, the larger principal curvature converges to $\bar{\kappa}_{\text{max}}(\infty) \rightarrow 1/22\text{Å}$, reflecting the global shape of the protein (also matching the size of this particular protein). On the other hand, the smaller principal curvature converges to a small positive value $\bar{\kappa}_{\text{min}}(\infty) \rightarrow 1/80\text{Å}$ in the large-$r$ limit, reflecting the fact that the overall shape of the protein is globular, whereas a local surface patch is typically saddle-like (measured average $\kappa_{\text{min}}$ is negative at all scopes up to $r = 20\text{Å}$).
Figure 2.8: The mean (blue lines) and the standard deviations (red lines) of the three curvature quantities, the two principal curvatures $\kappa_{\text{min/max}}$ and the mean scope-dependent surface curvature $K$, scaling with the scope of locality $r$. The statistics are taken across a full surface of a single protein, properly weighted by the mesh vertex area. (A) Plotted versus the scope $r$, both the mean and the standard deviation of the curvature quantities decrease in size as the scope is increased. (B) Plotted versus $1/r$, it can be observed that both the mean and standard deviations of the curvature values scale as $\sim 1/r$ at large scope $r \gtrsim 10\text{Å}$. In particular in the $r \to \infty$ limit, the two principal curvatures converge to $\kappa_{\text{max}}(\infty) \to 1/22\text{Å}$ and $\kappa_{\text{min}}(\infty) \to 1/80\text{Å}$ respectively, reflecting the global shape of the protein. Also, it appears that the standard deviation of the maximum curvature scales as $\sigma(\kappa_{\text{max}}) \propto 1/r$ with zero offset.

As we change the scope $r$ at which we approximate the surface, not only the mean but also the variance of the measured distribution of the scope-dependent surface curvature would change. In particular, the variance of our scope-dependent surface curvature measurements might come from two sources: either from the statistical fluctuations (noise) of the point samples at scales smaller than the specified scope, or from the meaningful shape variations of the surfaces. Whereas it is generally difficult to characterize the real shape features explicitly, we can look at a hypothetical limit where all variance comes from the noise. For example, in the limit where the surface is simply a random fluctuation around a quadratic surface, the variance of our measurement of the scope-dependent surface curvature would be inversely proportional to the number of surface points $N$ within the finite scope $r$, $\sigma^2 \sim 1/N \sim 1/r^2$. 
(Section 2.G), and consequently the standard deviation would scale as $\sigma \sim 1/r$. Indeed, we find a $1/r$ dependence of the standard deviation at larger scopes $r \gtrsim 10\text{Å}$, with a zero offset for the larger principal curvature $\kappa_{\text{max}}$ (Figure 2.8B). This might imply that most of the meaningful shape features of the protein surface are present below some critical scale $r_c \leq 10\text{Å}$, and are treated as noise when viewed at a larger scope $r > r_c$.

The scope-dependence of the surface curvature quantities is also manifest when we look at their probability distributions. For example, the distribution of the mean curvature $K$ at each scope $r$ (Figure 2.9A) becomes narrower and sharper as the scope $r$ increases. Again, there are two sources for this increasing “peakiness”: First, we are increasingly coarse-graining over the smaller-scale features as we increase our scope of locality, only keeping the information about the global shape eventually. Second, there is a statistical gain from the fact that at larger scopes, we have more points to fit from (Section 2.G). We have already observed that the latter (statistical) effect is more prominent at larger scopes, in which regime we would have already lost most of the meaningful surface features. When scaled by the scope $r$, the distributions of the dimensionless quantities $P(rK)$ are more comparable to one another, for instance in terms of the widths (Figure 2.9B).

**Signatures of typical surface features** From the distribution of the mean curvature $K$ alone, we already find some interesting signatures of known features of the protein surface (Figure 2.9). For example, at the smallest scope $r = 1\text{Å}$, we see a clear peak at $K = 0.53/\text{Å} = 1/1.88\text{Å}$ where 1.88Å is close to the typical van der Waals radius of the atoms that constitutes the protein. We also find a strong bias of the distribution toward positive curvatures at the largest scope $r = 20\text{Å}$, reflecting the global shape of the “globular” protein.

We can see more by looking at the joint distribution of the two principal curvatures. At each scope, we estimate the joint distribution by counting the number of pairs $(\kappa_{\text{min}}, \kappa_{\text{max}})$ within a square region centered at each grid point on the plane of the two principal curvatures. In each panel of Figure 2.10, we plot several contours of the resulting distribution, along with
Figure 2.9: The probability density functions of the mean scope-dependent surface curvature $K(r)$, at different scopes $r$, ranging from $r = 1\,\text{Å}$ to $r = 20\,\text{Å}$. Contribution from each surface point has been weighted based on the area associated. (A) The distribution $P(K)$ becomes sharper as the scope increases. (B) If we look at the distribution of the scaled dimensionless quantity $P(rK)$, the distributions are of comparable widths. The signature of atomic spheres is clearly visible as a sharp peak at a positive curvature $K_{\text{atom}} \approx 0.53/\text{Å} = 1/1.88\,\text{Å}$ at the smallest averaging scope ($r = 1\,\text{Å}$, red lines), whereas the peak at negative curvature represents toric region of the surface defined by the probe molecule of fixed radius $1.5\,\text{Å}$. These two peaks demonstrate that our generalized definition of surface curvature does reduce to the classical definition at smaller scopes. It is also clear that at a very large scope $r = 20\,\text{Å}$ (blue lines), the surface curvature tends to be positively biased, reflecting the globular shape of the protein.
Figure 2.10: The joint distributions of the two eigenvalues of the curvature tensor, at different scopes (A) $r = 1\,\text{Å}$, (B) $r = 2\,\text{Å}$, (C) $r = 5\,\text{Å}$ and (D) $r = 20\,\text{Å}$. Shown in each panel is a scatter plot of all measurements on a single protein (in gray dots), along with the contours of the empirical joint distribution calculated by counting the number of neighboring points within a small square window centered at each point. The contours are plotted at $\{1, 20, 40, 60, 80, 99\}$ per-cent levels of the peak height of the distribution (not at fixed quantiles). Note that the four panels are not drawn in the same scale: the widths of the distributions tend to narrow as $\sim 1/r$, as explained above.
all instances of the principal curvature pairs observed on the full protein surface at the given scope \( r \) (when counting, each observation was weighted based on the vertex area of the point at which the curvature was measured). At \( r = 1\,\text{Å} \) where the scope of locality is smaller than the atomic scale, the typical size of atomic spheres \( (\kappa_{\text{max}} \equiv \kappa_{\text{atom}} \approx 1/1.88\,\text{Å}) \) as well as the inter-atom valleys defined by the MSMS surface-rendering probe molecule of fixed radius 1.5Å \( (\kappa_{\text{min}} \equiv \kappa_{\text{probe}} \approx -1/1.39\,\text{Å}) \) are clearly visible from the joint distribution (Figure 2.10A). In particular, the peak of the distribution is at \( \kappa_{\text{max}} = \kappa_{\text{min}} = \kappa_{\text{atom}} \), representing the typical atomic sphere. Such clear peaks are already smoothed out at \( r = 2\,\text{Å} \) where the scope exceeds the atomic scale, although we can still find a weak trace of the atomic spheres reflected in the larger principal curvature \( \kappa_{\text{max}} \approx \kappa_{\text{atom}} \) (Figure 2.10B). At larger scopes, interestingly, the peaks of the distributions are not exactly on the diagonal but slightly above it, suggesting that a typical surface is nearly flat but slightly saddle-like. For example at \( r = 5\,\text{Å} \), the peak of the distribution lies at \( \kappa_{\text{max}} \gtrsim 0 \) and \( \kappa_{\text{min}} \lesssim 0 \) (Figure 2.10C). Conversely, at an even larger scope \( r = 20\,\text{Å} \), where one starts to see the signature of the global shape of the protein, we find the peak of the distribution at \( \kappa_{\text{max}} \gtrsim 0 \) and \( \kappa_{\text{min}} \gtrsim 0 \) (Figure 2.10D).

The correlation function

In order to capture the longer-range statistical properties of the surface, we computed the correlation function \( C_K(d; r) \) of the scope-dependent surface curvature \( K \) measured at scope \( r \) on a single protein surface \( \mathcal{S} \), based on the geodesic distances \( d = d_{\mathcal{S}}(\mathbf{p}_1, \mathbf{p}_2) \) between pairs of surface points \((\mathbf{p}_1, \mathbf{p}_2)\). It is calculated as

\[
C_K(d; r) = \langle K(\mathbf{p}_1; r)K(\mathbf{p}_2; r) \rangle - \langle K(\mathbf{p}_1; r) \rangle \langle K(\mathbf{p}_2; r) \rangle
\]

\[
= \frac{\sum_{d_{12}=d} w(\mathbf{p}_1)K(\mathbf{p}_1; r) \cdot w(\mathbf{p}_2)K(\mathbf{p}_2; r)}{\sum_{d_{12}=d} w(\mathbf{p}_1)w(\mathbf{p}_2)} - \left( \frac{\sum_{\mathbf{p}} w(\mathbf{p})K(\mathbf{p}; r)}{\sum_{\mathbf{p}} w(\mathbf{p})} \right)^2
\]
where the weight \( w(p) \) for each surface point is the area associated to the point (also see Section 2.2.2). The double sum \( \sum_{d_{12}=\tilde{d}} \) runs over all pairs of points \( p_1, p_2 \in S \) with the geodesic distance \( d_S(p_1, p_2) = \tilde{d} \). In practice, we bin the distances with a finite width \( \delta d \), and let the sums run over all pairs with \( d_S \in [\tilde{d}, \tilde{d} + \delta d) \), choosing the representative point \( \tilde{d} \) to be the left endpoint of the interval. The last term in (2.19) calculates the mean, where the single-point sum \( \sum_p \) runs over all surface points \( p \in S \).

Each correlation function (at a given scope \( r \)) was then normalized, so that each one is in the same unit where \( C_K = 1 \) means a perfect correlation and \( C_K = 0 \) means the mean level. Ideally the normalization constant should be the zero-distance (or self) correlation \( C_K(0; r) \), but the finite sampling of the surface mesh prohibits the practice. More fundamentally, the problem is twofold: first, there is no pair of two different points for which the geodesic distance is truly zero; second, from the way the surfaces are rendered, the smallest-distance pairs are often biased towards negative curvatures, corresponding to inter-atomic valleys. Instead, the correlation function was normalized by extrapolating the small-distance exponential decay (from \( d = 1\AA \) to \( d = 3\AA \)) to the zero-distance limit \( d = 0 \) (as shown in Figure 2.11A). The correlation functions for the two principal curvatures \( \kappa_{\text{min}} \) and \( \kappa_{\text{max}} \) can be obtained and normalized in the same ways.

**Important scales**  Plotting the correlation functions in log scale as in Figure 2.11A, we find two interesting scales of geodesic separation that correspond to the decays of correlations. First, we may consider the zero-correlation distance \( d_0 \) (Figure 2.11B), which is the typical separation for a pair of surface points to lose the correlation in their surface curvature, at which the correlation function falls to the mean level. Whereas the zero-correlation distance of \( \kappa_{\text{max}} \) smoothly ranges between \( d_0 \sim 4 - 10\AA \), that of \( \kappa_{\text{min}} \) shows a sharp transition. In particular, for \( \kappa_{\text{min}} \), the zero-correlation distance \( d_0 \) starts to be limited by the scope as \( d_0 \approx r \) at larger scopes \( r \gtrsim 10\AA \). We may assume the zero-correlation distance \( d_0 \) is meaningful only when \( d_0 > r \), where the correlation extends beyond the scope of local measurement; in this
Figure 2.11: Scales of shape variation, observed from the correlation functions of the scope-dependent surface curvature. (A) The correlation functions were normalized at zero distance by extrapolating the small-distance exponential decay (from $d = 1\text{Å}$ to $d = 3\text{Å}$). Shown is the correlation function of the mean curvature $K$, but correlation functions for the two principal curvatures $\kappa_{\text{max}}$ and $\kappa_{\text{min}}$ were treated similarly. The long-range decay constant of the correlation function at the largest scope $r = 20\text{Å}$, is about $d_{\text{large}} \approx 10\text{Å}$ (fit not shown in figure) when fit in the interval from $d = 6\text{Å}$ to $d = 16\text{Å}$. (B) The zero-correlation distance $d_0$, or the geodesic separation on surface where the correlation of surface curvature is lost; and (C) the initial decay lengths $\delta(r)$ as functions of the scope $r$, defined in (A) as $C_K(d; r) \sim \exp(d/\delta(r))$. Result from the mean curvature is shown together with results from the two principal curvatures.
sense, $d_0(\kappa_{\text{min}})$ is only meaningful up to $r < 10\text{Å}$, which also happens to be the scale above which the surface features are averaged over and lost from view (as in Figure 2.8). Second, we can also consider the initial decay length $\delta$ (Figure 2.11C), from the initial exponential decay in the interval from $d = 1\text{Å}$ to $d = 3\text{Å}$ as marked in Figure 2.11A. We find that at small $r$, the decay length $\delta(r)$ increases with the scope until it peaks at $r \approx 5\text{Å}$; then at large $r \gtrsim 10\text{Å}$, the decay lengths converge to a small value, for example at $\delta \approx 1.38\text{Å}$ for the mean curvature $K$. This is consistent with the observation that in Figure 2.11A, the large-scope correlation functions seem to converge to a long-range decay with a decay constant of $d_{\text{large}} \sim 10\text{Å}$, which is completely missing at smaller scopes. On the other hand, we must point out that the fall of the decay length $\delta(r)$ at a larger scope may be due to the inevitable limitations of our approach which is based on quadratic approximations; the quadratic surface ceases to be a good approximation of the real protein surface when a larger neighborhood is taken into account, where the surface curvature measure becomes less reliable. In general, one needs to be careful in interpreting any signal that is too much below $d = 2r$, since the domains where the two curvatures are calculated overlap, making a built-in correlation.

If we plot the correlation functions in linear scale, alternatively, another important scale manifests itself through the oscillation of the correlation function, pointing to a periodicity in the surface shape variation. Correlation functions of the mean scope-dependent curvature $C_K(d)$ exhibit oscillations with period $\lambda \sim 7\text{Å}$ at smaller scopes of locality $r < \lambda$ (six colored curves in Figure 2.12A). This oscillation is no longer visible at larger scopes $r > \lambda$ (gray and black curves in Figure 2.12A), which is consistent with the idea that there is an important scale near $\lambda \sim 7\text{Å}$, because all shape features smaller than the chosen scope $r$ will be averaged out and lost. The smaller-scale oscillation at period $\sim 0.4\text{Å}$ (Figure 2.12B) is an artifact from the surface mesh.

Putting together, it can be inferred that the important shape features of protein surfaces are in scales smaller than $10\text{Å}$ (from Figure 2.11), perhaps most prominently at $7\text{Å}$ (from
Figure 2.12: Oscillations in the correlation functions of surface curvature. (A) Correlation functions of the surface curvature at different scopes $r$, after being normalized as illustrated in Figure 2.11A. Oscillation at period $\sim 7\AA$ are observed from the correlations functions of surfaces curvatures at scopes $r < 7\AA$ (colored plots), but lost at scopes larger than the observed period (grayscale plots). The inset shows a closer view for the oscillation at smaller scopes only. (B) Another type of oscillation is observed at a much smaller scale, corresponding to the high-frequency “fluctuation” in the full-scale view, with periods $\sim 0.4\AA$. These smaller-scale oscillations are artifacts due to the mesh discreteness, which is clear when compared with (C) the counts of surface mesh point pairs at respective geodesic distances. Indeed, at mesh density $7/\AA^2$ used in our study, the typical distance of the neighboring mesh points is $d_{\text{mesh}} \sim (1/\sqrt{7})\AA = 0.38\AA$. 
the oscillations in Figure 2.12). This is consistent with the fact that our method based on the scope-dependent surface curvature captures meaningful signals only when we view the surface at smaller scopes (smaller than the scale of the features), but gives featureless results at larger scopes (where the features are averaged out). This provides a direct evidence that there is a characteristic scale of shape variation on protein surfaces (at about 7Å), as hypothesized based on the intrinsic dimension estimate above.

2.5 Discussion

This work is dedicated to characterizing and understanding important statistical properties of the set of protein surfaces. The statistical properties are considered at two levels. First, we looked at the level of the ensemble of samples of protein surfaces, which is a representative for the full shape space of proteins, and investigated the intrinsic dimensionality of the sampled shape space. Our main results came through a set of geodesic curves sampled from protein surfaces, which may be viewed as an indirect sampling of the space of protein surfaces (compared to directly sampling patches of surface). Importantly, however, working with the curves enables one to overcome the curse of dimensionality that exists fundamentally in the dimension estimation problem (Eckmann and Ruelle, 1992). We found that as we look at larger surface objects (the size measured by either the total area for a surface patch, or the total length for a geodesic curve), the dimensionality of the dataset grows accordingly. Specifically, the dimensionality of the geodesic curve dataset tends to grow linearly with the length of the curves considered, at a rate of one extra dimension per an additional curve length of about $L_c \approx 7\text{Å}$. Assuming that the dimensionality of the surface objects is related to the dimensionality of the curve objects by the square, $D_{\text{surface}} \sim D_{\text{curve}}^2$, we could also say that the dimensionality of the set of surface patches grows linearly with the area of the patches. This finding led us to an interesting picture where the protein surfaces consists of a mosaic of statistically uncorrelated patches of some characteristic area $A_c \approx (L_c)^2 \approx 50\text{Å}^2$. This
interpretation of the growth rate of the intrinsic dimensionality, in terms of the characteristic size scale of the shape samples in the dataset, was tested on sets of synthetic curves with controlled properties. In both of the synthetic models considered (planar maps with fixed covariances, and polymers with fixed distributions of the bond angles) we found that the growth rate of the intrinsic dimensionality is indeed a faithful indicator of the characteristic length scale of the generated curves. Interestingly, while modeling the curve using the ideal polymer model, the persistence length of the protein surface curves was measured to be about 7.9Å, in a close quantitative agreement with the characteristic length scale of dimensionality growth.

Then we moved down to the level of individual protein surfaces, in order to look more closely at the statistics of the surfaces themselves, and to look for direct evidence of the characteristic scale of shape variation that was indicated from the dimensionality study, as well as from the measurement of the persistence length. We chose to look at the surface curvature of the proteins, which is a natural measure of shape variation on surfaces, but with a fundamental point-wise properties in its conventional definition. In order to capture the inherent multi-scale structure of our data, in particular to go beyond the atomic spheres from which the protein surface builds up, we developed a new framework which we call the scope-dependent surface curvature. The scope-dependent surface curvature measures the second-order shape variation at a finite scope of locality, and is equivalent to the conventional surface curvature in the small-scope limit. A key property of the scope-dependent surface curvature is that whereas a given shape feature is captured by measuring the curvature at scopes smaller than the scale of the feature, it is effectively averaged out and becomes indistinguishable at a larger scope. By looking at the correlation of the scope-dependent surface curvatures at different scopes, we found that there is a prominent scale of shape features at geodesic length \( \sim 7\text{Å} \), which was only captured when measured at scopes smaller than this scale, but was lost at larger scopes. This scale manifested itself as the characteristic
period of the oscillation of mean surface curvature values on the protein surface, not as the decay length of the correlation function.

Our analyses show that the characteristic scale of individual surfaces is consistent with the size scale at which the ensemble gains each extra dimension. This result reveals a new insight that whereas the shapes of protein surfaces are highly variable, it may actually be viewed in a coarse-grained manner: we may view the shape of a protein surface as a mosaic composed of uncorrelated surface patches of a characteristic size, significantly reducing the overall complexity and possibly allowing a low-dimensional representation of the shape space. In particular, in a separate study\(^4\) we considered an independent spring model of protein surfaces, a simplified picture where surfaces are described by the displacements of a set of independent springs, assuming that there are no “overhangs” in the surface. Specifically, we modeled the shape of a protein-protein interface with the displacements of 20 independent springs, taking the typical area of an interface as \(\sim 1000 \text{Å}^2\) (Chakrabarti and Janin, 2002; Janin et al., 2008; Hwang et al., 2010), and using a “surface unit” of \(A_c \approx 50 \text{Å}^2\) as inferred from our study. Assuming a one-to-one and face-to-face correspondence between the springs, the interaction energy for a pair of surfaces was written as a harmonic model, where the spring constants were inferred from the temperature factors of the protein surfaces reported in the PDB entries. Using the pairwise distance distribution between the protein surface patches sampled in our current study, we could ask how many different interfaces the system were needed before the effect of non-specific interaction (between mismatching/different surfaces) becomes comparable to that of specific interaction (between matching/identical surfaces). The study points to an interesting result, predicting that up to \(\sim 10^4\) distinct interfaces can be accommodated in the system while maintaining specific interactions; remarkably, this number is comparable to the number of different proteins encoded in the \(E.\ coli\) genome, about 4000. Details of this model will be published later, along with the materials presented in this chapter.

\(^4\)The independent spring model was developed primarily by Anne-Florence Bitbol.
The picture of the modular organization of surfaces, inferred from our statistical analysis, is further supported by previous studies. Some works that aimed to determine the characteristic properties of known interfaces in terms of residue compositions consistently suggested that the interface may be a combination of some modules, although in different terminologies. Interfaces are reported to have “hot spot residues” (Bogan and Thorn, 1998; Keskin et al., 2005, 2008) which seem to be the energetically independent (Carbonell et al., 2009); “recognition patches” with cores and rims, where the typical size scale is ∼6.5Å for the core and ∼8Å with the rim included (Chakrabarti and Janin, 2002); as well as multiple, spatially separated “binding modes” (Kundrotas and Vakser, 2013). Moreover, interestingly enough, our estimate of the characteristic scale of shape variation on protein surfaces seems to be in coincidence with the cutoff scales used to tolerate the structural inaccuracies in docking studies (Vakser et al., 1999; Nicola and Vakser, 2007; Sinha et al., 2012), which are usually optimized solely based on the performance.

We hope that our findings on the statistical properties of protein surfaces will serve as a ground for more future works, towards understanding the interaction specificity in terms of the shape of the interface as well as the chemical compositions. Furthermore, once the structural information of protein surfaces are written in some low-dimensional, tractable representation, it might be possible to include shape in predictions for protein-protein interactions based on the physicochemical and evolutionary information of individual proteins (Tseng et al., 2009; Bitbol et al., 2016), or on modern bioinformatics techniques (Wiederstein et al., 2014) powered by the growing library of known interactions and structural information.
2. A Geodesic path on the triangular mesh

For any pair of surface points \( p_1 \) and \( p_2 \) on an analytical surface \( S_0 \), the geodesic distance \( d_{S_0}(p_1, p_2) = d_{S_0}(p_2, p_1) \) is uniquely defined to be the length of the minimal path on \( S_0 \) that connects \( p_1 \) and \( p_2 \). This path is called the geodesic path \( G(p_1, p_2; S_0) \). For a pair of surface mesh points on a triangulated surface \( p_1, p_2 \in S \), there are two approaches to calculate the geodesic distance on a triangular mesh.

Marching method (on mesh vertices)

One could imagine a true underlying analytical surface \( S_0 \), and try to approximate the geodesic path on this analytic surface, \( G(p_1, p_2; S_0) \), by a path on a triangulation \( S = \{ P, F \} \) of \( S_0 \). In this case the approximated geodesic path would consist of an ordered set of mesh vertices in \( P \), connected by the triangular edges in \( F \).

For example, we can use the Fast Marching Method (FMM) algorithm to approximate the geodesic distance on triangular meshes (Kimmel and Sethian, 1998). As its name suggests, the algorithm “marches” from the center point \( p_1 \in P \) with a circular front, sweeping through all the intermediate mesh points until it reaches the desired endpoint \( p_2 \in P \), effectively sampling all points \( p' \) with \( d(p', p_1) < d(p_2, p_1) \). This property actually provides an efficient method of defining a geodesic-disk-shaped surface patch. The FMM algorithm is
very useful when one only needs a reasonable estimate of the geodesic distance from a given
mesh point to other mesh point(s).

In principle, the accuracy depends greatly on the mesh density, as well as on the shapes
of the triangles (which is connected to the mesh regularity). For the triangulated protein
surfaces in our dataset, the algorithm at least seems to be consistent: the symmetries of
geodesic distances were very well preserved under exchange of the center and the endpoint
(exchange of \( p_1 \) and \( p_2 \)).

The geodesic path defined by the marching method is the shortest path that goes from
\( p_1 \) to \( p_2 \) through the mesh (an ordered set of mesh vertices, connected by the edges of the
triangular faces). Finding this path requires no more than a simple backtracking algorithm
when we have the FMM distances from \( p_1 \) to every other mesh point up to \( p_2 \).

**Variational method (on vertices and faces)**

Alternatively, one could directly obtain the geodesic path \( \mathcal{G}(p_1, p_2; S) \) and the geodesic
distance \( d_S(p_1, p_2) \) on the triangulated surface \( S \), treating \( S \) as if it were the true surface. In
this case, not only the mesh vertices but all the points that are on the surface of the triangular
faces are treated as true surface points (for the moment the surface is truly continuous,
although it is angular and not differentiable). Consequently, the path need not pass through
the mesh vertices only; it can now be fully described by an ordered set of vertices or edge
points that are connected by the triangular faces.

The geodesic path can be found using a variational algorithm that converges to the
shortest path on \( S \) between the two points \( p_1 \) and \( p_2 \). As a preliminary step, we first obtain
the backtracked path using the FMM algorithm, which passes only through the mesh vertices.
Then, starting from this backtracked path, we go through each corner of this path, which we
call the “path points”, adjusting each path point so that it lies collinear with its neighbors
on the locally unfolded surface (hence the “variational” algorithm), iterating until the path
converges. The path points are initially located at the mesh vertices in the backtracked
path, but with the variational adjustments they are allowed to move along the triangular edges, where each edge point is stored in terms of a weighted interpolation between two mesh vertices. The resulting set of path points is a sufficient representation of a curve on a triangulated surface.

This variational algorithm is useful in cases where it is important to have the geodesic path explicitly, for example when we want to sample geodesic curves as our surface objects. The resulting geodesic distance is not necessarily smaller than the FMM distance.

2.B Sampling the shape space

Sampling the surface patches In order to generate a surface patch of area $A$ that samples the shape space of protein surfaces, we do the following:

1. Randomly choose a protein surface $S = \{P, \mathcal{F}\}$ from the database, weighted by the total surface area. Then randomly choose a surface point $p \in P$ to be the center of the patch, weighted by the vertex area $w(p)$.

   - In practice we imposed a slightly ad hoc constraint that we sample as many non-overlapping patches as possible from each protein surface (up to the randomness of choosing the centers), because we wanted to cover the shape space most “efficiently” given the computational cost of having more patches. In this case the number of patches sampled per protein is roughly proportional to the total surface area, but the choice of $p \in P$ is not truly random.

2. Given a target surface area $A$, determine an auxiliary distance cutoff $d_{\text{max}} = \rho(A/\pi)^{1/2}$ where $\rho > 1$ leaves a margin to take care of the non-zero curvature of the surface. We usually used $\rho = 1.5$, and increased in the rare cases where it was not enough (cases where the patch was far from being a planar disk; for example when the patch consists of a long, finger-like protrusion).
3. Use the FMM algorithm to calculate the geodesic distances $d_S(p', p)$ from $p'$ to all neighboring points $p'$ such that $d_S(p', p) < d_{\text{max}}$. Construct a subset of $P$ consisting of these neighboring points, $P' = \{p' \mid d_S(p', p) < d_{\text{max}}\}$. Construct an induced sub-surface $S' = \{P', F_{\text{induced}}(P', S)\}$ of $S$. Denote $F' \equiv F_{\text{induced}}(P', S)$.

4. Assuming that $d_{\text{max}}$ was large enough, and the discarded area was small enough, the total surface area of $S'$ should be slightly larger than $A$. If not, increase $\rho$ and resume from step 2.

5. Order the triangular faces in $F'$. Because $P'$ is naturally ordered by the geodesic distance from the center, the faces in $F'$ can also be ordered by the minimum of the geodesic distances to its three vertices.

6. Add one triangular face at a time in this order, starting from the center, until the cumulated area reaches $A$. Note that because of the way the faces were ordered, the resulting set of faces is always some approximation of a geodesic disk.

7. Construct a final induced sub-surface $S'' = \{P'', F_{\text{induced}}(P'', S)\}$ where $P''$ is the union of all vertices in the triangular faces added before the total surface area of the patch reached $A$.

**Sampling the geodesic curves** In order to sample a geodesic curve of length $L$ from the protein surfaces, we do the following. Note that for implementation convenience, we only consider 20 lengths that are integer multiples of an Angstrom, from $L = 1\AA$ to $L = 20\AA$. A dataset is a collection of curves of the same length $L$, hence 20 datasets; each dataset is independently sampled.

1. Randomly choose a protein surface $S = \{P, F\}$ from the database, weighted by the total surface area.

2. Choose two vertices $p_1, p_2 \in P$ randomly and independently, based on the vertex area.
3. Construct a geodesic curve $\mathcal{G}(p_1, p_2)$ between the two endpoints using the variational method described in Section 2.A. This is facilitated if we already have the full interseed distance data for $S$. Otherwise the FMM algorithm should be used prior to using the variational algorithm, centered at one of the two endpoints. We abort the FMM algorithm when the geodesic distance between the pair of endpoints is too large (and discard this pair), i.e., when the FMM reached a geodesic radius of $d_S(p_1, p') > 20\text{Å}$ but still not found $p_2$.

4. Sample the curve with a set of points, starting from $p_1$ and moving along the curve, at a regular interval $\Delta s = 0.1\text{Å}$, or equivalently, at a sampling density of 10 points/Å. Cut to the longest possible integer multiple of 1Å, so that the total length $L$ is one of the 20 target values. In this work we always made the cut by discarding one end of the curve, which might have biased the curves slightly, but only very slightly (by construction, the length of the cut-out is always smaller than 1Å). Also see below for a discussion on the fairness of the sampling.

5. Put the curve in the corresponding dataset of length $L$. Discard if there is no matching dataset (e.g., $L$ is larger than 20Å).

It is often useful to represent each geodesic curve by a list of 3-dimensional point coordinates spaced at a regular interval, such that all curves in the same dataset are written in the same (large) number of coordinates. Throughout this study, we use a sampling density of 10 points/Å. The sampling density ceases to matter at some point, where the information stored in this representation is limited instead by the density of the surface mesh. The sampling interval of 0.1Å for the curve is fine enough in this sense, even on our finest mesh at 7 points/Å$^2$, where the typical point-to-point distance is $\sim 0.4\text{Å}$.

**How fair is the sampling?** There is a fundamental issue here, which is the fact that a random point on a geodesic curve is not equivalent to a random point on the underlying
surface. In other words, if we were to sample a large number of (long enough) geodesic curves out of a given surface, and color the geodesic curves such that the surface appears darker in the region where more geodesic curves pass through, the resulting shade will not be homogeneous throughout the surface. We might also say that the density of geodesic curves is inversely correlated with the magnitude of surface curvature. The consequences are:

- Once we have sampled a set of geodesic curves, the statistics of the internal points of the curve (not too close to the endpoint) is different from the statistics of the endpoints that we picked randomly on the surface. We can check this by calculating the coordinate variance of the points along the arc length after aligning the curves (more on Section 2.D and Figure 2.S1).

- The set of geodesic curve would always under-sample the surface features systematically, in the sense that the geodesic curves tend not to pass through a protruding (or intruding) region of the surface. We can check this by calculating the variance of the surface curvature along all points on our geodesic curves and comparing with the variance over all surface points. We find that the variance is significantly lower along the curves than over the full surface (data not shown).

At this point we do not have an immediate solution to this issue. It would be an important future work to seek a better understanding of the relation between the geodesic curves and the surface curvature, which seems feasible as we also have access to a measure of surface curvature (Section 2.4).

2.C Comparing two surface objects: the Procrustes distance

The Procrustes distance is a measure of difference between the shape of two objects, which is evaluated after superimposing the two objects by the optimal translation and rotation.
There are other variants of the Procrustes superimposition which allows scaling and/or
reflection, but in the current problem we are only interested in rigid and sign-preserving
transformations, because our shape objects are real protein surfaces where both the scale
and the handedness have physical meanings. Given two sets of points \( A \) and \( B \) (each with
\( N \) points, arranged into a \( 3 \times N \) matrix), the problem is to find the linear transformation
\( \Omega^* \) which most closely maps \( A \) to \( B \):

\[
\Omega^* = \arg \min_{\Omega} \| \Omega A - B \|_F, \tag{2.20}
\]

where \( \| \cdot \|_F \) denotes the Frobenius norm, defined as

\[
\| M \|_F = \sqrt{\sum_{n=1}^{N} \sum_{i=1}^{3} (M_{in})^2} = \sqrt{\text{tr}(M^T M)} \tag{2.21}
\]

where \( M \) is a real matrix.

In this case, the optimal rigid transformation \( \Omega \) can be uniquely decomposed into two
distinct steps: a translation, and a rotation around the center-of-mass. For our purpose it is
easier to first shift the center-of-mass positions of both \( A \) and \( B \) to the origin, and make a
rotation \( R \) around the origin. Actually, we can assume that the two point clouds \( A \) and \( B \) are
already mean-shifted, such that the centers of mass are both at the origin of the coordinate
system. Now the problem reduces to finding the optimal rotation matrix

\[
R^* = \arg \min_{R} \| RA - B \|_F \text{ subject to } R^T R = I, \det R = 1. \tag{2.22}
\]

It is known that there is a simple and unique solution to the \textit{orthogonal} Procrustes problem:
the orthogonal matrix \( R \) is found to be \( R = VU^T \), where \( X = U\Sigma V^T \) is the singular value
decomposition of the matrix \( X = A^T B \). (Schönemann, 1966)
Implicit in the Procrustes analysis is the assumption that the point-to-point correspondence (or alignment) between the two sets of points $A$ and $B$ is known. More fundamentally, it assumes that each set of points can be linearly indexed by $n = 1, \cdots, N$ and written down in a $N \times 3$ matrix where the columns are the 3-dimensional coordinates. The point-to-point correspondence is automatically defined when both objects are linearly indexed this way, for example in the case of a linear object (such as our geodesic curve) sampled by an indexable set of points. The only caveat is that there is a “flipping” degree of freedom that arises because the choice of the initial indexing orientation is arbitrary. While indexing does not affect the positions of the individual curves in the real space, it changes the point-to-point correspondence between the two curves. In other words, with two curve objects represented by $A = \{a_1, a_2, \cdots a_m\}$ and $B = \{b_1, b_2, \cdots b_m\}$, the natural correspondence map between the two objects is $a_i \mapsto b_i$; however, because we could also have indexed the points starting from the other end of the curve(s), there is an equally natural correspondence map $a_i \mapsto b_{m+1-i}$. In practice, given two linear objects, one would compute the two candidate Procrustes distances

$$
\begin{align*}
    d_1 &= \frac{1}{\sqrt{N}} \min_R \| RA - B \|_F, \\
    d_2 &= \frac{1}{\sqrt{N}} \min_R \| RA - B^{\text{flip}} \|_F
\end{align*}
$$

(2.23)

where $R$ is always an orthogonal matrix, and $B^{\text{flip}}$ is the matrix obtained by flipping the order of the rows (point indices) of $B$. The minimum of the two Procrustes distances $d$ is registered as the pairwise distance of the two shape objects, as well as the corresponding Procrustes superimposition $R$ when necessary.

**Objects with no well-defined indexing orientations**

However, there are problems where the point-to-point correspondence is not well-defined, for example in the case of our surface patches. In a surface patch, the points are arranged on
a 2-dimensional manifold in an essentially irregular fashion, without any natural endpoint, and consequently there is no uniquely defined indexing scheme that is robust under a re-parameterization of the 3-dimensional coordinate system, nor under a resampling of the same surface object.

Let us think of a heuristic indexing scheme to illustrate the two problems. Consider the simplest situation where our surface object is a simply connected surface patch constructed as a geodesic disk. Imagine dividing the geodesic disk into a set of annuli (areas between two concentric circles), with a fixed surface area for each annulus. Because the points have been sampled at a constant density, on average, each annulus has the same number of points. This scheme has an advantage that there is an intuitive ordering for the set of geodesic annuli, based on their geodesic distances from the center. However, there is no definitive answer as to how we should be indexing the points within each annulus, because such linear indexing requires an “endpoint” which is always arbitrary in this case (at the very least, there is a rotational degree of freedom). This is a continuous degree of freedom, making it practically impossible to resolve (unlike in the case of curves where we could choose between the two orientations).

Moreover, our representation of the surface object consists of discrete points that are randomly sampled from the surface at a given point density. Whatever better heuristic linear indexing scheme one comes up with, it would not be robust under a statistically equivalent resampling (or just a statistically unbiased perturbation) of these representative points. In other words, even if the surface points could somehow be indexed in a linear order, the point \( p_i \), which was assigned the \( i \)-th index from this scheme may no longer be assigned the same index \( i \) when the position of one of the other points is perturbed.
ICP-Procrustes

Instead of relying on arbitrarily fixed indices of the point samples, we use the iterative closest point (ICP) algorithm (Besl and McKay, 1992) to dynamically associate points between the two objects based on the proximity.

Given two surface objects $A$ and $B$ that are already mean-shifted (meaning that the center-of-mass of each object is at the origin), we can assume that we are applying rigid transformation to $A$ while fixing $B$ (we are superimposing $A$ onto $B$). At any given configuration, the point correspondence $f_{AB}$ maps each point $a \in A$ in the rotating object to the closest point $b \in B$ in the fixed object:

$$f_{AB} : a \mapsto b \in B$$

so that $\|a - b\| \leq \|a - b'\|$ for all $b' \in B$. (2.24)

where $\| \cdot \|$ is the 3-dimensional Euclidean norm. By construction, multiple points in $A$ may be assigned to the same point in $B$, and the correspondence map is no longer one-to-one.

Based on the point-to-point correspondence, a Procrustes analysis is performed to find the optimal rotation $\hat{R}$ that minimizes $\|\hat{R}A - B\|_F$. Because there is no global analytic solution in this case, the problem needs to be attacked in a hierarchical loop of minimizations at three different levels.

1. Iterative assignment of closest points — Starting from an arbitrary initial configuration of the rotating object $A_0$, iterate the following steps until a termination condition is met: Step 1, update $A_t = \hat{R}_{t-1}A_{t-1}$; Step 2, construct a new point correspondence map $f_{AB}^{(t)} : A_t \to B$; Step 3, perform Procrustes analysis to find the optimal rotation $\hat{R}_t$ and calculate the corresponding Procrustes distance $d_t$. Since there is a theoretical guarantee that the ICP converges monotonically to a local minimum (Besl and McKay, 1992), we can use a simple tolerance $\delta$ for the relative change in the resulting Procrustes distance, terminating when $(d_t - d_{t-1})/d_{t-1} < \delta$. Each iteration is locally deterministic, because the Procrustes analysis has an analytic solution given a point correspondence.
2. Repetition over multiple initial configurations — Because the iterative process is not guaranteed to find the global minimum of Procrustes distance, it may end up in one of the local minima in the configuration space. In order to deal with this problem we sample multiple initial configurations that are equivalently good a priori. After shifting the centers of the two surface objects \( A \) and \( B \) to the origin, we perform the Principal Component Analysis (PCA) on each object, identifying three principal axes \( \{\hat{u}_1, \hat{u}_2, \hat{u}_3\} \). We match the plane of the first two principal components, but vary the relative angular orientation by rotating \( A \) around the third principal axis \( \hat{u}_3 \). In practice we sample 20 initial configurations, with minimum angular differences of 18 degrees around \( \hat{u}_3 \).

3. Repetition with reversed reference between the two objects — Finally, it is important to remember that unlike the classical Procrustes analysis, this iterative process is asymmetric because of the way we map the points in one object to the points in another object. In order to find the best superimposition that gives the minimal distance, the entire process needs to be repeated with the reversed reference between the two objects: this time rotating \( B \) while fixing \( A \).

2.D Comparing multiple surface objects: Alignment

Alignment of multiple surface objects is a generalized problem of the pairwise alignment, again involving making rigid transformations (translation and rotation in the 3-dimensional space) to each surface object in the dataset, such that corresponding points across all samples in the dataset could be directly compared to one another.

Aligning a set of surface patches is hard due to the same reason that made the pairwise point correspondence problem unsolvable, because there is no natural order of indexing the points on a 2-dimensional manifold. Here we describe how we align sets of 3-dimensional curves. The raw curves can have arbitrary coordinates, depending on the coordinates in
which their mother protein structure were written. Before running any alignment algorithms, curves are first mean-shifted so that all centers-of-mass are at the origin. Then we use either of the two alignment algorithms: the generalized Procrustes analysis and the principal axes alignment.

**Generalized Procrustes analysis** This is a generalized “global” version of the classic Procrustes analysis for a set of objects, first suggested by Gower (1975). At each step, each curve in the dataset is Procrustes-superimposed to a reference curve. The reference curve is then updated to be the average of all curves in the dataset, and the entire dataset is now superimposed to the new reference curve. Again, the caveat is that at each step for each curve, two Procrustes superimpositions should be performed using both indexing orientations (forward and backward), and the minimal-distance configuration should be taken. We iterate until the reference curve converges to a fixed object (again with a small tolerance). The reference curve can be initialized with any one curve in the dataset, and the algorithm normally runs very quickly because each iteration has an analytic solution.

Figure 2.S1: The coordinate variance $\langle dx^2 + dy^2 + dz^2 \rangle$ of curves after global alignment, for (A) the protein surface curves and (B) the synthetic polymers with matching bond angle distribution. The variances were measured along the arc length, for curves with total length $L = 19\text{Å}$. This quantity is rotation and translation invariant (hence the choice of the coordinate system does not matter). Note how the protein surface curves aligns significantly better (smaller variance), reflecting the fact that these are geodesic curves on surfaces.
**Principal axes alignment**  This is a “local” alignment, in the sense the the alignment is accomplished at the level of each individual curves in the dataset, rather than comparing different curves to minimize the difference. For each curve in the dataset, we perform a principal component analysis (PCA) to identify the three principal axes of the curve, and change the basis such that the new $x, y, z$ coordinates correspond to the three principal axes in the order of descending variance.

This method leaves out an unresolved degree of freedom, namely the signs of the respective principal axes, as well as the familiar “flipping” degree of freedom. Once we have written the curves in terms of the new $x, y, z$ axes, we first fix the sign such that the $y$ coordinate at the midpoint of the curve is always positive, by selectively rotating each curve by 180 degrees around the first principal axis $x$ (to have $y \rightarrow -y$ and $z \rightarrow -z$). Because the orientations of the coordinate system is supposed to be right-handed, we only need to fix one sign. Note, however, that the specific choice of fixing the $y$-sign of the midpoint is rather arbitrary. It works nicely for the protein surface curves, but may not work as well with a set of highly fluctuating random curve objects, for example.

Finally, we fix the indexing order by making sure that the $x$ coordinates in $\{x_i\}$ tend to increase, rather than decrease, with increasing index $i$. The heuristic method works nicely in our curve dataset, because a geodesic curve on the protein surface tend to have a direction with a reasonably small curvature (because of the geodesic nature), effectively captured by the first principal axis in this case. This observation corresponds to the limit where the length of the curve is short compare to the global size of the protein.

### 2.E  More on dimension estimate

First of all we show the estimated dimensionality of the set of surfaces patches, to be compared to the dimensionality of the set of geodesic curves sampled from protein surface (Figure 2.S2).
Figure 2.S2: Dimension estimates for the set of surface patches, when scaled appropriately, appear to agree with the dimension estimates for the set of geodesic curves. In order to compare the two dimensionalities we take the square roots of the original patch dimension estimates, reflecting the assumption of $D_{\text{patch}} \sim D_{\text{curve}}^2$. The effective length scales of the patch are estimated by $L_{\text{eff}} = \sqrt{A/\pi}$, where $A$ is the surface area of the patch. For the atomic patches (which were not introduced in the main text but consist of the coordinates of surface atoms, that are “reduced” from the surface patch by tracking back which part of the surface came from which atom), the effective surface area $A_{\text{eff}} = N_{\text{atoms}} \langle A \rangle$ was calculated by multiplying the number of atoms in the patch, $N_{\text{atoms}}$, by the average surface area per atom, $\langle A \rangle \sim 10\AA^2$.

On the other hand, the dimension estimates may depend on the fineness at which the surface is sampled, as well as on the smoothing parameters in the regression. We checked that the dimensionality estimates come to a convergence at our choice of mesh density, and found an optimal method for each estimator, which is robust over a range of smoothing parameters.

We also provide an error analysis for the inevitable systematic (geometric) error of the regression dimension estimator.
2.E.1 The issue of sampling

Our study is fundamentally dependent on issues of whether we have fair sampling of the system of interest, at least at three distinct levels. First, at the largest scale, there is an issue whether the X-ray crystalized structures in our protein dataset actually reflects the native, real-time structures of the proteins, because there are known phenomena of structural fluctuation (Cooper, 1976; Tang et al., 2016) as well as studies that directly aim to characterize the conformational ensemble of the same protein (Rother et al., 2008; Lane et al., 2014). There are also reports of conformation change upon binding, as well (Marsh and Teichmann, 2011; Wang et al., 2013; Deis et al., 2015). Second, the existing collection of protein structures on the Protein Data Bank may not be a faithful and unbiased sampling of the shape space, which consists of all protein structures there are in real cellular environment – perhaps because some kind of structures are easier to freeze and crystalize, or because some family of proteins received more attention over the past decades. Finally, there is a more operational problem of rendering a given protein surface into a triangular mesh, with a fine enough mesh such that no important information about the shape is lost on the way. This last issue of loss-less sampling, out of the three that has just been discussed briefly, is probably the only place we have control of, and are responsible for making sure we have a fine enough sampling of protein surfaces.

Our operational definition of being “fine enough” is in the sense of a robust and loss-less estimation of dimensionality. As was presented in Figure 2.4 in the main text, our dimension estimates converge to very similar values at higher mesh densities \{3, 5, 7\} points/Å². On the other hand, at the lower mesh density 1 point/Å², the mesh is losing some of the degrees of freedom in the data, although only slightly: see Figure 2.S3 for comparison. In particular, the regression dimension estimate of the shortest curves \( L = 1 \) seems to be affected by the coarseness of the mesh at 1 point/Å². The curve-sampling density of 10 points/Å is already finer than the mesh, and does not limit our dimensionality estimates (data not shown).
Figure 2.S3: How fine is fine enough? Our current choice for the density of the triangulated mesh, at 7 points/Å², seems to be fine enough in the sense that the dimension estimates converge to stable values well before this mesh density is reached. Indeed, a mesh density of 7/Å² corresponds to an average nearest-point distance of $d_{\text{mesh}} \sim (1/\sqrt{7})\text{Å} \approx 0.38\text{Å}$, which appears to be much shorter than any meaningful features of the surface, even below the atomic scale.

### 2.E.2 Implementation with real data: the scale matters

It was discussed in the main text that the estimated dimensionality of a dataset may depend on the viewing scale, as well as on the smoothing parameters in the regression. In order to decide our favorite method of dimensionality estimate, we tested and compared some conventional criteria of determining the scale at which we should estimate $D$. The data usually need some smoothing at smaller scales, because of the statistical fluctuation, but care should be taken as smoothing may also affect our estimate of the dimensionality.

The two criteria considered in this study are motivated by the earlier work of Smith (1988), where it was observed that the true intrinsic dimensionality $D$ was best captured at the plateau of $\hat{D}(r)$, when $\hat{D}(r)$ is the estimate at scale $r$. The **Most-Linear criterion** looks for the most linear region of the curve by maximizing the goodness of fit (the $R^2$ value) of the linear fit (“MostLin”). The smoothness parameters include the scale ratio $SR = r_{max}/r_{min}$.
Figure 2.S4: A comparison of different methods to obtain dimension estimates. Different curves in each panel represent the estimates obtained by using different smoothing parameters. For the Most-Linear criterion, the smoothing parameter is the number of fitting points ($N_{\text{fit}}$) and the fitting range ($SR$). For the two Max-Slope criteria, the smoothing parameter is the number of bins ($N_{\text{bin}}$) in the discretization. For example, (A) shows the regression dimension estimates using the MostLin criterion, where different colors represent different $SR$ values (as in colorbar). On the other hand (E) shows the correlation dimension estimates using the MaxSlopeLog criterion, where different color represent different $N_{\text{bin}}$ values. In the four other cases (B,C,D,F) the estimates were not sensitive to the smoothing parameters used in this case. The overshoot values in (E-F) come from the fact that we are looking for the maximum slope when the counts are highly fluctuating; hence the MostLin criterion is more suitable for the correlation dimension estimator. To generate the main result we used the MaxSlopeLin criterion for the regression dimension and the MostLin criterion for the correlation dimension.
which determines the width of the fitting range in the log scale, and the number of fitting
points $N_{\text{fit}}$ which effectively determines the coarseness of the max-search grid.

On the other hand, the **Max-Slope criterion** looks for the maximum slope of the curve
(corresponding to the peak in $\hat{D}(r)$), where the slope is determined simply by comparing
the first neighbors in the discrete data. The smoothness parameter is the number of bins $N_{\text{bin}}$,
which determines the coarseness of the re-binning of the curve $\log k$ vs. $\log \langle r_k \rangle$, necessary
for obtaining the numerical derivative of the curve. In this case, in particular, we realize
that there are two ways of implementing the estimator using the numerical derivative. For
example in the case of the regression dimension estimator, we could do it with the log
envelope on (“MaxSlopeLog”)

$$
\hat{D}_{\text{Log}}(k) = \frac{\Delta \log k}{\Delta \log \langle r_k \rangle} = \frac{\log(k + 1) - \log(k)}{\log \langle r_{k+1} \rangle - \log \langle r_k \rangle}; \quad (2.25)
$$

or expand the log using the chain rule and work in terms of the linear quantities (“MaxSlopeLin”)

$$
\hat{D}_{\text{Lin}}(k) = \frac{(\Delta k)/k}{(\Delta \langle r_k \rangle)/\langle r_k \rangle} = \frac{1}{k} \frac{1}{\langle r_{k+1} \rangle / \langle r_k \rangle - 1}. \quad (2.26)
$$

Based on the comparison of the three criteria (Figure 2.S4), it appears that for the regression
dimension, the best practice is to use the MaxSlopeLin criterion; for the correlation
dimension, the MostLin criterion seems to produce robust results over a range of smoothing
parameters. Interestingly, MaxSlopeLin criterion on the regression dimension estimator
seems always to peak at the nearest-neighbor scale (Figure 2.S5), meaning that when the
binning is fine enough, it always compares the average distance to the first and second neighbor,
resulting in $\hat{D}_{\text{reg}} \approx \langle r_1 \rangle / (\langle r_2 \rangle - \langle r_1 \rangle)$. Because any one curve would (presumably) look
very much alike its first or second neighbor, this sense of dimensionality concerns how many
possible “modes” there are for the curve to be slightly deformed, rather than how many
very-differently-shaped curves there can be.
Figure 2.S5: Regression examples for the dimension estimates. (A-B) Regression dimension estimates, using sets of 10000 real protein surface curves and 1000 synthetic polymers, at total lengths (A) $L = 1\text{Å}$ and (B) $L = 10\text{Å}$. Synthetic polymer dataset is a good approximations of the protein surface curve dataset at large $L$, but there are surface-specific effects at small $L$. (C-D) Same for the correlation dimension estimates.
**Regression dimension in the small-$L$ limit**  In light of this understanding of the working of the regression dimension estimator, using the MaxSlopeLin criterion (2.26), we can actually explain the non-zero offset dimension $D(L \to 0)$ observed in the main text. We make two assumptions:

- When the curve samples are all very similar and probably only differ at the level of single-point fluctuations, for example at length scales that are much shorter than the correlation length (the short-length limit), the dimensionality of the smallest fluctuation $D_f$ is related to the embedding dimensionality of single points that sample the curve (in our case $D_f = 3$).

- We assume that the curve objects are uniformly distributed in the high-dimensional space of curves, from which we can approximate

$$
\langle r_k \rangle \approx \left( \frac{\text{Volume} \cdot k}{N} \right)^{1/D_f}, \quad \frac{\langle r_{k+1} \rangle}{\langle r_k \rangle} \approx \left( \frac{k + 1}{k} \right)^{1/D_f}
$$

(2.27)

where $N$ is the dataset size and $D_f$ is the smallest fluctuation dimension defined above.

With these assumptions, we can approximate the regression dimension estimator as

$$
\hat{D}(k) \approx \frac{1}{k} \left( \left( \frac{k + 1}{k} \right)^{1/D_f} - 1 \right)^{-1},
$$

(2.28)

which means at $k = 1$ (the nearest neighbor scale, where MaxSlopeLin usually peaks at) we get $\hat{D}(1) \approx 1/(2^{1/D_f} - 1)$. For $D_f = 3$, the small-$L$ limit (the offset) of the dimension estimate is

$$
\hat{D}_{L \to 0} \approx \frac{1}{2^{1/3} - 1} = 3.85
$$

(2.29)

which is close to what we observe from the synthetic curves (Figures 2.6E and 2.55A). Note that this rough estimate is based on the postulated value of $D_f$ (and the postulated existence of such dimensionality) for geometric curve objects in space, as well as on the uniformity
assumption. This result is not contradictory to the fact that the “offset dimension” converges to zero in Figure 2.3D, because the results shown there were calculated with sets of high-dimensional vectors for which every aspect of the vector can fluctuate independently. If we were to stick with the notion of the smallest fluctuating dimension $D_f$, we can say that $D_f = D_e$ for the $D_e$-dimensional vectors, in the absence of further constraints.

On the other hand, the uniform distribution assumption (2.27) may break down in real datasets. In particular, in the case of protein surface curves, the distance ratio is probably super-linear, or $\alpha > 1$ in $\langle r_{k+1} \rangle / \langle r_k \rangle \sim ((k + 1)/k)^{\alpha/D_f}$; that is, the average distance to the second nearest neighbor is larger than twice the average distance to the first neighbor (true when there are many super-similar objects, like similar segments of atomic spheres). A smaller offset dimension $\hat{D}_{L \rightarrow 0}$ is expected with a larger nonlinearity $\alpha$. For example, with $\alpha = 2$, we get $\hat{D}_{L \rightarrow 0} = 1.70$ which is close to what we see from the protein surface curves (Figure 2.4).

2.E.3 The systematic error: effect of the dataset size $N$

The effect of systematic (geometric) error is profound at small datasets, as demonstrated using a set of high-dimensional vectors in Figure 2.3D in the main text. Using the linear term expansion for the regression dimension (as in MaxSlopeLin), one can also calculate the effect of the systematic error, in terms of the scale parameter $k/N$ where $k$ is the viewing scale (the neighbor rank) and $N$ is the number of samples in the dataset. The derivation is similar to the work of Nerenberg and Essex (1990) that provided an error analysis for the correlation dimension estimator, although we do not present full details here.

Let $N$ be the number of samples, $k$ the neighbor rank considered, and $r(k; N)$ the average distance to the $k$-th neighbor. Here, the regression dimension estimate at rank $k$ is defined by

$$\hat{D}(k; N) \equiv \frac{1}{k} \frac{r(k; N)}{r(k+1; N) - r(k; N)}.$$  \hspace{1cm} (2.30)
Here we consider points that are uniformly distributed in a hypersphere of radius 1 in true dimension \( D \). We study the regression dimension estimate for each neighbor rank in this simple case. To first order in \((k/N)^{1/D}\), we find that\(^5\)

\[
\hat{D}(k; N) = D \left[ 1 - A(D) \frac{\Gamma(k + 2/D)}{\Gamma(k + 1/D)} N^{-1/D} \right] \approx D \left[ 1 - A(D) \left( \frac{k}{N} \right)^{1/D} \right],
\]

with

\[
A(D) = \frac{D}{2} \left[ \left( \frac{K_D}{K_{D-1}} \right)^{2/D} \Lambda(D) - 1 \right] > 0,
\]

where

\[
K_D = \frac{\pi^{N/2}}{\Gamma(1 + n/2)}
\]

is the volume of a hypersphere of radius 1 in dimension \( D \), and

\[
\Lambda(D) = \int_0^1 dx \left( \int_{-1}^x du (1 - u^2)^{(D-1)/2} \right)^{-2/D}.
\]

Using this result, it should be possible to add an appropriate scale correction for the regression dimension estimator.

### 2.F More on the synthetic curves

#### 2.F.1 Generating planar curves as Gaussian random variables

Fixing zero mean, we aim to generate an ensemble of samples with a given two-point correlation \( k(x_i, x_j) \):

\[
\langle f(x_i) f(x_j) \rangle = k(x_i, x_j)
\]

where \( \langle \cdot \rangle \) is the ensemble average. Let \( K \) be the covariance matrix defined by \( K_{ij} = k(x_i, x_j) \). Let \( V \) be a matrix whose columns are the eigenvectors of \( K \), and \( D \) a diagonal matrix whose

---

\(^5\)This error analysis was carried out by Anne-Florence Bitbol.
diagonal entries are the corresponding eigenvalues of $K$. By construction, $KV = VD$.

Instead of actually constructing the probability distribution and drawing samples from it, we can easily achieve the desired covariance by introducing a random vector $\epsilon$ which is independently and identically distributed:

$$K = VDV^T$$
$$= VD^{1/2} \langle \epsilon \epsilon^T \rangle D^{1/2} V^T$$
$$= \langle (VD^{1/2} \epsilon)(VD^{1/2} \epsilon)^T \rangle.$$  \hspace{1cm} (2.36)

### 2.F.2 Generating polymers with ideal chain models

In order to define the bond angle $\theta$ and the torsion $\phi$ which characterize a joint in the model, we first need to introduce the three unit vectors (tangential, normal, and binormal) that form the orthonormal basis of the Frenet-Serret frame in differential geometry (Pressley, 2001). In short, the unit tangent vector $t$ is defined to be pointing in the direction that is along the arc; the unit normal vector $n$ is the normalized derivative of $t$ with respect to the arc length; finally, the unit binormal vector $b$ is the cross product $t \times n$. Also see Figure 2.6A in the main text.

Within the discrete picture of the chain model, our working definition for the bond angle $\theta$ at a joint is the angular displacement between the two adjacent tangents:

$$t_{i-1} \cdot t_i \equiv \cos \theta_i.$$ \hspace{1cm} (2.37)

On the other hand, the torsion angle $\phi$ is related to the rate of rotation of the binormal vector:

$$b_{i-1} \cdot b_i \equiv \cos \phi_i.$$ \hspace{1cm} (2.38)
Note that the orientations (signs) of these angles are naturally defined by the orientation of the basis \( \{ \mathbf{t}, \mathbf{n}, \mathbf{b} \} \). Actually, we will be choosing the orientation of the basis such that the bond angle \( \theta \) is always positive, as explained more below.

**Polymer-generating algorithm:** Given the angles \( \{ \theta_i, \phi_i \} \) at each joint \( i \), a consistent polymer-generating algorithm is to iterate the following while incrementing \( i \), with some arbitrarily initialized orthonormal basis \( \{ \mathbf{t}_1, \mathbf{n}_1, \mathbf{b}_1 \} \):

1. Implement bond angle: rotate along the \( \mathbf{b} \) axis (rotate \( \mathbf{n} - \mathbf{t} \) plane)

\[
\mathbf{t}_i = \mathbf{t}_{i-1} \cos \theta_i + \nu \mathbf{n}_{i-1} \sin \theta_i; \quad \mathbf{n}_{\text{temp}} = \mathbf{n}_{i-1} \cos \theta_i - \nu \mathbf{t}_{i-1} \sin \theta_i
\]  \hspace{1cm} (2.39)

where the sign \( \nu \in \{-, +\} \) is positive for the inward normal convention and negative for the outward normal convention. Here we use the outward normal (\( \nu = -1 \)) with the protein surface application in mind.

2. Implement torsion: rotate along the new \( \mathbf{t} \) axis (rotate \( \mathbf{n} - \mathbf{b} \) plane)

\[
\mathbf{n}_i = \mathbf{n}_{\text{temp}} \cos \phi_i + \mathbf{b}_{i-1} \sin \phi_i; \quad \mathbf{b}_i = \mathbf{t}_i \times \mathbf{n}_i.
\]  \hspace{1cm} (2.40)

3. Generate the \( i \)-th vertex (joint) after progressing by \( \Delta s \) (the fixed length of a rod in chain, or equivalently the vertex spacing along the arc) along the tangent \( \mathbf{t}_i \).
It can easily be checked that the recursive algorithm (2.39-2.40) is consistent with the definition of the torsion angle (2.38), as:

\[
\begin{align*}
\mathbf{b}_{i-1} \cdot \mathbf{b}_i &= \mathbf{b}_{i-1} \cdot (\mathbf{t}_i \times \mathbf{n}_i) \\
&= \mathbf{t}_i \cdot (\mathbf{n}_i \times \mathbf{b}_{i-1}) \\
&= \mathbf{t}_i \cdot (\mathbf{n}_{\text{temp}} \times \mathbf{b}_{i-1}) \cos \phi_i \\
&= \mathbf{t}_i \cdot (\mathbf{n}_{i-1} \times \mathbf{b}_{i-1}) \cos \theta_i - (\mathbf{t}_{i-1} \times \mathbf{b}_{i-1}) \sin \theta_i \cos \phi_i \\
&= \mathbf{t}_i \cdot (\mathbf{t}_{i-1} \cos \theta_i + \mathbf{n}_{i-1} \sin \theta_i) \cos \phi_i \\
&= (\cos^2 \theta_i + \sin^2 \theta_i) \cos \phi_i \\
&= \cos \phi_i.
\end{align*}
\]

Our modeling assumption is that the angles \(\{\theta_i, \phi_i\}\) are drawn from some fixed distributions. In order to generate synthetic space curves used in this study, we used a half-normal distribution on \((0, \pi)\) for the bond angle \(\theta\), and a uniform distribution on \((-\pi/2, \pi/2)\) for the torsion angle \(\phi\), as discussed in the main text.

**Angle-recovering algorithm:** By reverse-engineering the generating algorithm, the angles can also be recovered uniquely from a given curve object, consisting of a set of points (vertices/joints) sampled along the arc lengths at a regular interval \(\Delta s\). The angle-recovering algorithm is straightforward the generating algorithm: first the unit basis vectors are recovered as

\[
\begin{align*}
\mathbf{t}_i &= \frac{\mathbf{x}_i - \mathbf{x}_{i-1}}{\| \cdot \|}, \\
\mathbf{n}_i &= \pm \frac{\mathbf{t}_i - (\mathbf{t}_i \cdot \mathbf{t}_{i-1}) \mathbf{t}_{i-1}}{\| \cdot \|}, \\
\mathbf{b}_i &= \mathbf{t}_i \times \mathbf{n}_i
\end{align*}
\] (2.41)
where the denominators indicate normalizations by the corresponding vector amplitude. The angles are recovered from

\[ \{ \mathbf{t}_i \cdot \mathbf{t}_{i-1} = \cos \theta_i , \quad \mathbf{t}_i \cdot \mathbf{n}_{i-1} = \nu \sin \theta_i \} \rightarrow \theta_i ; \quad (2.42) \]

\[ \{ \mathbf{b}_i \cdot \mathbf{b}_{i-1} = \cos \phi_i , \quad \mathbf{n}_i \cdot \mathbf{b}_{i-1} = \sin \phi_i \} \rightarrow \phi_i . \quad (2.43) \]

The orientation of the normal vector is fixed post hoc such that \( \sin \theta \geq 0 \).

### 2.G Scope-dependent surface curvature: error from random fluctuation

Our definition of the scope-dependent surface curvature is based on a quadratic surface fit over a fixed number of points, which is formulated as a linear least-squares regression problem for which we have exact solutions. However, in this case, the points being fit over are random samples of the underlying surface. If the measured surface curvatures at different sets of points is different for two sets of points, it may be that the underlying surface is truly different, but it might also be a mere reflection the random fluctuation of the points. Likewise, the variance of surface curvature would have two components: the contribution from the meaningful variation of the surface, and the contribution from the statistical fluctuation. Here we provide an error analysis of the surface curvature in the limit where the points are noisy sampling of a target surface, and show that in this limit, the (co-)variance of the fit parameters scales inversely with the number of points.

#### Line fitting on a plane

To get an idea of the problem, let us start with a simple 2-dimensional problem where we want to fit a straight line, out of a noisy sampling of a line \( y = f_0(x) = a_0 x + b_0 \). Let’s say there are \( N \) points \( \{ x_i, y_i \} \) with identical noise \( y_i = f_0(x_i) + \epsilon_i \) where \( \epsilon_i \sim \mathcal{N}(0, \sigma^2) \). The
least-squares fit of the line is the set of parameters \(\{a, b\}\) that minimizes

\[
S = \sum_{i=1}^{N} [y_i - (ax_i + b)]^2 = \sum_{i=1}^{N} [(a_0 - a)x_i + (b_0 - b) + \epsilon_i]^2.
\] (2.44)

Differentiating the objective function with respect to the two fit parameters,

\[
\frac{\partial S}{\partial a} = -2 \sum_{i=1}^{N} x_i [(a_0 - a)x_i + (b_0 - b) + \epsilon_i],
\] (2.45)

\[
\frac{\partial S}{\partial b} = -2 \sum_{i=1}^{N} [(a_0 - a)x_i + (b_0 - b) + \epsilon_i];
\] (2.46)

or in vector form

\[
\begin{pmatrix}
\frac{\partial S}{\partial a} \\
\frac{\partial S}{\partial b}
\end{pmatrix} = -2 \begin{pmatrix} x^T \\
1^T
\end{pmatrix} \left((a_0 - a)x + (b_0 - b)\mathbf{1} + \epsilon\right).
\] (2.47)

We realize that this expression can be written more generally in terms of the 2-dimensional parameter vector \(\theta = (a; b)\) and a \(N \times 2\) matrix \(U = (x \ 1)\) as

\[
\frac{\partial S}{\partial \theta} = -2 \cdot U^T (U(\theta_0 - \theta) + \epsilon).
\] (2.48)

The matrix \(U\) can be called the conjugate of the parameter vector \(\theta\) in the sense that it is defined as \(U_{ik} = -\partial \chi_i / \partial \theta_k\), where \(\chi = [y - (ax + b)]\) is the \(N\)-dimensional error vector, such that the total squared error is \(S = \chi^T \chi\). This convenient simplification is specific for a linear regression problem, where the error can be written linearly as \(\chi = U(\theta_0 - \theta) + \epsilon\).

Now we can define a \(2 \times N\) matrix \(M \equiv (U^T U)^{-1} U^T\), so that the least-squares solution can be written concisely as \(\theta = \theta_0 + M\epsilon\). The error covariance of the parameter vector is therefore written in terms of this matrix \(M\), as

\[
C_\theta = \langle (\theta - \theta_0)(\theta - \theta_0)^T \rangle = \langle M\epsilon\epsilon^T M^T \rangle = \sigma^2 MM^T
\] (2.49)
where, assuming that the noise \( \epsilon \) is independently and identically distributed, we can write 
\[
\langle \epsilon \epsilon^T \rangle = \sigma^2 I.
\]
On the other hand, 
\[
MM^T = ((U^T U)^{-1} U^T) (U (U^T U)^{-1}) = (U^T U)^{-1}.
\]
(Note that \( U \) is not a unitary matrix.) If we write down this matrix explicitly for the line fitting problem, it looks like
\[
MM^T = \begin{pmatrix}
x^T x & 1^T x \\
x^T 1 & 1^T 1
\end{pmatrix}^{-1}.
\] (2.50)
This result tells us two things. First, since each entry of the final matrix in equation (2.50) is an inner product between two \( N \)-dimensional vectors, 
\[
MM^T \sim 1/N
\]
and the covariance of the least-square error also scales inversely with the number of points as
\[
C_\theta \sim \sigma^2 / N.
\] (2.51)
Second, this is also a statement about the quality of fit. If our point samples are perfectly balanced on both sides of the origin, such that the mean is zero (\( x^T 1 = 0 \)), not only the covariance matrix is diagonal and easy to compute, but it is also non-singular (the determinant of \( MM^T \) is as large as possible). On the other hand, if all the points are concentrated on a location far from the origin, \( MM^T \) approaches a singular matrix, leading to a blowup of \( C_\theta \) (the fit fails).

**Quadratic surface fitting**

Scaling up is straightforward. Consider a problem where we want to fit a quadratic surface out of a noisy sampling of a quadratic surface, 
\[
z = f_0(x, y) = \bar{g}_0 + \bar{g}_1 x + \bar{g}_2 y + \bar{h}_{11} x^2 + 2\bar{h}_{12} xy + \bar{h}_{22} y^2.
\]
If there are \( N \) points \( \{x_i, y_i, z_i\} \) with identical noise \( z_i = f_0(x_i, y_i) + \epsilon_i \) where \( \epsilon_i \sim \mathcal{N}(0, \sigma^2) \), the least-squares method minimizes
\[
S = \chi^T \chi,
\] (2.52)
where $\chi_i$ abbreviates the error at point $i$,

$$
\chi_i = \left[ (\bar{g}_0 - g_0) + (\bar{g}_1 - g_1)x_i + (\bar{g}_2 - g_2)y_i \\
(\bar{h}_{11} - h_{11})x_i^2 + 2(\bar{h}_{12} - h_{12})x_iy_i + (\bar{h}_{22} - h_{22})y_i^2 + \epsilon_i \right].
$$

Note that this is still a linear regression problem, although the function we fit is quadratic (non-linear). Analogously as in the simple example of the linear fit, we can introduce the 6-dimensional parameter vector $\theta = (g_0; g_1; g_2; h_{11}; h_{12}; h_{22})$ and the $N \times 6$ conjugate matrix $U_{ik} = -\partial \chi_i / \partial \theta_k$. In this case, the six columns $u^{(k)}$ of the matrix $U$ is defined element-wise as

$$
u^{(1)} = 1; \quad [u^{(4)}]_i = x_i^2;$$
$$
u^{(2)} = x; \quad [u^{(5)}]_i = 2x_iy_i;$$
$$
u^{(3)} = y; \quad [u^{(6)}]_i = y_i^2.$$

Now that we can write $\chi = U(\theta_0 - \theta) + \epsilon$, the rest of the problem has exactly the same structure as in the simple example. The least-squares solution is

$$
0 = \frac{\partial S}{\partial \theta} = -2U^T \chi \quad \Rightarrow \quad \theta = \theta_0 + (U^T U)^{-1}U^T \epsilon;
$$

identifying the matrix $M = (U^T U)^{-1}U^T$, the error covariance scales as

$$
C_\theta = \langle (\theta - \bar{\theta})(\theta - \bar{\theta})^T \rangle = \langle M \epsilon \epsilon^T M^T \rangle = \sigma^2 M M^T \sim \sigma^2 / N,
$$

where $M M^T = (U^T U)^{-1}U^T U (U^T U)^{-1} = (U^T U)^{-1} \sim 1/N$. 

131
Chapter 3

Active learning of psychometric functions

3.1 Introduction

Understanding the psychophysical performance of an animal in various conditions has always been a central question in psychology. Whereas an accurate quantification of the behavior is an important goal by itself, in modern neuroscience it is also useful as a proxy of how the brain function changes, as new technologies allow direct manipulations of the animal brain; there is a growing need for methods to rapidly characterize behavior and its dependence on stimuli. In a typical experiment that measures behavior, an animal is presented with a stimulus on each trial and has to select a response among several options, where it is assumed that the animal has a fixed internal rule of choice-making. The probabilistic map between the stimulus and the response is called the psychometric function, and can be inferred from multiple observations of stimulus-response pairs. However, such experiments are usually costly, both in terms of resource (the cost of feeding and maintaining the subject animals in the lab) and time (for instance when using virus-based optogenetic techniques, the duration
of virus expression may be limited). Therefore, a problem of practical importance is to learn
the animal’s psychometric function from a minimal amount of data.

The idea of active learning, or the sequential optimal design of experiments, is that this
problem can be solved by adaptively selecting the next stimuli to be presented, in a way that
maximizes the expected “utility” of the candidate data points based on the current estimate
of the model (MacKay, 1992). In general, a Bayesian active learning method requires that
we have a model of the system being learned (in the current context the model of the
psychometric function), a prior distribution over the model parameters, and a utility function
which quantifies the usefulness of a given stimulus-response pair in learning the model, given
the data available to the point (Pillow and Park, 2016). Given the psychometric model,
one can calculate the likelihood of the observed data as a function of the parameters of the
model, which combines with the prior distribution to construct the posterior distribution.
The posterior distribution, in turn, characterizes the experimenter’s best knowledge about
the system up to the point, based on which the expected utility of a candidate stimulus must
be determined.

For the physics-oriented readers of the dissertation, we note that when mutual informa-
tion (Shannon, 1948) is used as the utility function of the Bayesian active learning, which
we do here, the problem of active learning can be formulated as a problem of maximizing
the information transmission through the communication channel, between the stimulus pre-
sented in the experiment and the model parameters to be inferred. In this sense the problem
we solve in this chapter may be thought of as a reiteration of the problem we considered in
Chapter 1, although in a different context, and subject to a different set of constraints.

Early works of active learning, applied to the problem of estimating psychometric func-
tions, include the QUEST algorithm (Watson and Pelli, 1983) which attempted to estimate
the threshold between two choices, and the Ψ method (Kontsevich and Tyler, 1999) which
introduced an information theoretic approach with a Bayesian framework, proposing the
use of the prior information to choose the stimulus that minimizes the expected posterior
entropy. The field was further powered by advances in Bayesian inference techniques on psychometric functions, such as the use of Markov chain Monte Carlo (MCMC) methods for posterior sampling (Kuss et al., 2005; Kujala and Lukka, 2006). Multi-dimensional stimulus space was also discussed: Kujala and Lukka (2006) to extended the $\Psi$ method to the 2-dimensional stimulus space, and more recently DiMattina (2015) discussed the application of adaptive algorithms on logistic models with multi-dimensional stimuli. However, all of these works have considered the binary choice behavior exclusively.

Our work extends from the previous literature and makes two fundamental contributions. First, we study tasks with more than two responses (i.e. multinomial as opposed to binary choices), which is a more flexible description of the decision-making behavior in many natural settings. We consider the multinomial logistic model, which supports multiple-alternative responses yet retains many convenient properties of the exponential family. Although there were early works on the optimal experimental design with the multinomial logistic model (Zocchi and Atkinson, 1999; Heise and Myers, 1996; Fan and Chaloner, 2004), they did not use adaptive algorithms; they rather discuss how the prior uncertainty in the model parameters can be used to design the stimulus space before the experiment starts. Second, we introduce “lapses”, the possibility that animals may occasionally make errors on easy trials due to momentary lapses in concentration or memory (Wichmann and Hill, 2001; Kuss et al., 2005), augmenting the model to reflect the realistic aspect of animal behavior. Because this departure from the ideal model calls for the development of a more flexible method for posterior inference, we develop an efficient sequential method based on Markov chain Monte Carlo (MCMC) sampling that is accurate in settings in which the log-likelihood is not concave, for example as in the presence of lapse rates, as well as being scalable to a larger number of parameters.

We develop and discuss two active learning algorithms based on two efficient methods for posterior inference, respectively, one based on a Gaussian approximation of the posterior and one based on MCMC sampling. We test our methods on simulated data, as well as
on an experimental dataset concerning the multiple-alternative choice behavior of monkeys (Churchland et al., 2008), demonstrating that active sampling of the stimulus space facilitates the learning of the psychometric function significantly, as well as suggesting that the full range of the multi-dimensional stimulus could have been exploited more efficiently using our active learning framework.

3.2 Multinomial logistic model

We use multinomial logistic (MNL) model to describe the psychometric function, where there are a set of \( k \) possible behavioral outcomes. In the classical MNL model, the probability \( p_i \) of having each outcome \( i \) is determined by the input stimulus, with a possibly non-linear carrier vector of the input, according to a set of linear weights (Chaloner and Larntz, 1989; Zocchi and Atkinson, 1999). We will start by reviewing the classical multinomial logistic models, and then add the lapse rate into the model later in the section.

3.2.1 Classical MNL

Suppose that we have a set of \( k \) possible behavioral outcomes. For a single trial, let \( y_i \) denote a binary indicator variable taking the value 1 if the animal chooses option \( i \), and the value 0 otherwise. Then the outcome can be written as a \( k \)-dimensional vector \( y = [y_1, y_2, \cdots, y_k] \) with only one 1 and all 0’s otherwise. The probability \( p_i = P(y_i = 1) \) of having each outcome state \( y_i \) is determined by the stimulus \( x \) and the weight parameters \( w_i \) for \( i \in \{1, \cdots k\} \). In general, we may write

\[
p_i = \frac{\exp(g_i^T(x)w_i)}{\sum_{h=1}^{k} \exp(g_h^T(x)w_h)}, \quad \sum_{i=1}^{k} p_i = 1 \tag{3.1}
\]

where \( g_i(x) \) is the carrier vector of the input, which may be different for each state \( i \) (Zocchi and Atkinson, 1999; Glonek and McCullagh, 1995). The set of probabilities \( \{p_i\} \equiv p \) is our
model of the psychometric function. It is worth pointing out that when there are multiple alternatives, it is important to write the psychometric function in terms of the probability of certain choice behavior, \( P(\text{choice}) \), rather than the probability of correct/incorrect choices, \( P(\text{correct}) \), because there are multiple ways to be incorrect.

Conveniently, the probabilities depend only on the linear predictors \( V_i = \mathbf{g}_i(\mathbf{x})^T \mathbf{w}_i \). Because the nonlinearity of the stimulus can be flexibly incorporated by the carrier \( \mathbf{g} \), this model naturally deals with multi-dimensional stimuli as well as multiple-alternative responses. Hence our method provides a more general description of psychophysical behavior, extending previous work from Kujala and Lukka (2006); DiMattina (2015). For theoretical development, we will keep the most general form of \( \mathbf{g}(\mathbf{x}) \) throughout this work. In later sections where we need a definite form of \( \mathbf{g}(\mathbf{x}) \), we will assume that the carrier function of the multinomial logistic model \( g_i : \mathbf{x} \mapsto \mathbf{g}_i(\mathbf{x}) \) is known; specifically, we will assume that it is a linear function \( \mathbf{g}_i(\mathbf{x}) = [1, \mathbf{x}^T]^T \) for each \( i \) identically.

For example, we may imagine in an experiment where an animal may choose between Left \((i = 1)\) or Right \((i = 2)\) based on a possibly multi-dimensional input stimulus \( \mathbf{x} \), or just do nothing (Abort; \( i = 3 \)), there are three outcomes \((k = 3)\). In the simplest case, the animal would react with some sensitivity \( \mathbf{a}_i \) to the stimulus, and possibly some bias \( b_i \) towards making each choice, leading to the probability distribution

\[
p_i(\mathbf{x}) \propto \exp(b_i + \mathbf{x}^T \mathbf{a}_i) = \exp(\mathbf{g}_i^T(\mathbf{x}) \mathbf{w}_i),
\]

where \( \mathbf{g}_i(\mathbf{x}) = [1, \mathbf{x}^T]^T \) and \( \mathbf{w}_i = [b_i, \mathbf{a}_i^T]^T \) for each \( i \).

**Calculating the log likelihood** The log likelihood of having an outcome \( \mathbf{y} \) from a single trial (where \( \mathbf{y} \) is a vector of only one 1 for the actual response and all 0’s otherwise) is

\[
L = \sum_i y_i \log p_i = \mathbf{y}^T \log \mathbf{p}, \quad \text{where} \quad \mathbf{p} = \frac{\exp(\mathbf{V})}{1^T \exp(\mathbf{V})}.
\]
It is convenient to work in terms of the linear predictor $V = \{V_i\}$ first. If $N_y \equiv \sum_{i=1}^{k} y_i = 1$ is the total number of observations, the first and second derivatives of the log likelihood with respect to $V$ are

$$\frac{\partial L}{\partial V_j} = y_j - N_y p_j \quad \Rightarrow \quad \frac{\partial L}{\partial V} = (y - N_y p)^T,$$

$$\frac{\partial^2 L}{\partial V_i \partial V_j} = N_y p_i (\delta_{ij} - p_j) \quad \Rightarrow \quad \frac{\partial^2 L}{\partial V^2} = -N_y (\text{diag}(p) - pp^T) \equiv -N_y \Gamma(p),$$

where $\text{diag}(p) = [p_i \delta_{ij}]$ is a square matrix with the elements of $p$ on the diagonal, and zeros otherwise. In particular, $L$ is concave with respect to $V$ (see Section 3.A for the simple proof).

Putting back in terms of the weight vector $w$ is easy, using the convenient linearity. Let us define the long extended weight vector $w = [w_1^T, \ldots, w_k^T]^T$ by concatenating, and introduce the input matrix $X = \bigoplus_{i=1}^{k} g_i^T(x)$ where $\bigoplus$ is a direct sum operator,

$$X = \bigoplus_{i=1}^{k} g_i^T(x) = \begin{bmatrix} g_1^T & 0 & \cdots & 0 \\ 0 & g_2^T & \cdots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \cdots & g_k^T \end{bmatrix}.$$  \hfill (3.6)

Then the $k$-dimensional linear predictor vector is written as $V = Xw$. The derivatives are easily translated in terms of the weight parameters:

$$\frac{\partial L}{\partial w} = \frac{\partial L}{\partial V} X = (y - p)^T X \equiv \Delta^T,$$

$$\frac{\partial^2 L}{\partial w^2} = X^T \frac{\partial^2 L}{\partial V^2} X = -X^T \Gamma X \equiv -\Lambda.$$  \hfill (3.8)
3.2.2 MNL with lapse

In the classical MNL model, the log likelihood is a concave function of the weights, and many machine learning methods had relied on this convenient property. In actual experiments, however, there are cases where the subject experiences a lapse in concentration, losing the stimulus, and consequently chooses the response with some stimulus-independent probability (Wichmann and Hill, 2001; Kuss et al., 2005). Whereas the lapse probability is often negligible in humans or primates on simple tasks, it is often significant in lower mammals such as rats. This effect was also known to Kontsevich and Tyler (1999), where it was called the miss rate, and was assumed fixed while the other parameters were learned. In this work we will take it more generally and let our algorithm learn the lapse probability from data, at the same time as it learns the weight parameters of the model.

Let us assume that the animal lapses (ignores the stimulus) with a probability $c_{total} = \sum_{i=1}^{k} c_i$, and when lapsing, chooses the $i$-th response with probability $c_i$. In the presence of lapse, the probability of having each response is

$$p_i = (1 - c_{total}) q_i + c_i, \quad q_i = \frac{\exp(V_i)}{\sum_h \exp(V_h)} \quad (3.9)$$

where $q_i$ is the lapse-free probability from the classical MNL. Now the full parameter vector of the model is $\theta = [w^T, c^T]^T$ including the $k$ lapse probabilities. The derivatives of the log likelihoods $L$ with respect to $V$ are

$$\frac{\partial L}{\partial V} = (t - N t q)^T, \quad (3.10)$$

$$\frac{\partial^2 L}{\partial V^2} = -N t \Gamma(q) + [\text{diag}(s) - (qs^T + sq^T) + N s q q^T] \quad (3.11)$$

where $t$ and $s$ are element-wise defined vectors $t_i \equiv y_i r_i$ and $s_i \equiv y_i r_i (1 - r_i)$, where $r_i \equiv (1 - c_{total}) q_i / p_i$. $N t = \sum_i t_i$ and $N s = \sum_i s_i$ are the sums. Since these vectors can also be written as $r_i = 1 - c_i / p_i$, $t_i = y_i (1 - c_i / p_i)$ and $s_i = y_i (c_i / p_i) (1 - c_i / p_i)$, we recover $t_i \rightarrow y_i$.
and $s_i \to 0$ in the lapse-free limit $c_i \to 0$. In particular, $N_t \to 1$ and $N_s \to 0$ in this limit, and the derivatives reduce back to the lapse-free form. When the lapse rates are finite, the log likelihood is no longer concave with respect to $V$ (details in Section 3.A).

In practice, we re-parameterize the lapse probability by taking a logit transformation, and work with the auxiliary lapse parameter $\gamma_i$ such that $c_i = e^{\gamma_i}/(1 + e^{\gamma_i})$. This way the lapse parameter $\gamma_i$ scales in the same way as the weight parameters do, which are also the logit of the multinomial probabilities. With the new parametrization, $\gamma \to -\infty$ corresponds to the lapse-free case $c_i = 0$, whereas $\gamma = 0$ corresponds to $c_i = 1/2$, meaning that the subject is randomly choosing this response half of the time. We restrict $\gamma$ to be always negative.

### 3.3 Posterior inference

Our active learning method is an adaptive algorithm, which assesses the informativeness of the next stimuli based on the current knowledge of the model parameters. Hence it is important that all of the previous observations are accurately and efficiently summarized in the form of a (Bayesian) posterior distribution. In this section we investigate two methods to infer the posterior distribution, given a set of stimulus-response pairs.

Following the Bayes rule, the posterior distribution of the model parameters combines the likelihood of the data given the model with the prior belief. In practice, starting with a weak prior distribution for the parameters may help to obtain reasonable models even when a small number of observations is available. The prior should become negligible as the data accumulates and dominates the posterior. We used a Gaussian prior centered at $w = 0$ for the weight parameters, and $\gamma_i = -5$ for each lapse parameter if being considered, with a diagonal covariance matrix with large values (wide distributions), to obtain reasonably smooth psychometric functions even when only a limited set of data is available.
3.3.1 Gaussian approximation to posterior

Let us denote by $\mathcal{D}_{1:t} = \{x_i, y_i\}_{i=1}^t$ the accumulated data up to trial $t$, where the observations are assumed to be independent and coming from the same psychometric function (i.i.d.). The maximum a posteriori (MAP) estimate of the parameter vector $\theta$ can be obtained by directly maximizing the log of the posterior distribution $P_t(\theta) = p(\theta|\mathcal{D}_{1:t})$. Up to constant terms, the log posterior is written as

$$
\log P_t = \log p(\theta|\mathcal{D}_{1:t}) = \log p(\theta) + \log p(Y_{1:t}|X_{1:t}, \theta)
= \log p(\theta) + \sum_{i=1}^t \log p(y_i|x_i, \theta),
$$

(3.12)

where $p(\theta)$ is the prior distribution, and $p(y_i|x_i, \theta)$ is the likelihood of the $i$-th observation $\{x_i, y_i\}$ given $\theta$. For later convenience let us also introduce a shorthand for the log likelihood, $L_i(w) = \log p(y_i|x_i, w)$.

**Maximum a posteriori (MAP) estimate** Because we can explicitly provide the full gradient and Hessian of the log likelihood, numerical optimization becomes a straightforward problem: we can call any standard function (for example Matlab’s fminunc). However, the numerical optimization is guaranteed to converge to the global optimum only in the case of the classical (lapse-free) multinomial logistic model, where the log likelihood is a concave function of the weight parameters. For the moment let us restrict ourselves to the lapse-free model, where $\theta = w$.

**Laplace (Gaussian) approximation** With the log-concavity, we can further simply our description of the posterior by approximating it as a Gaussian distribution centered at the posterior mode $u_t$, where the covariance matrix is obtained from the Hessian of the posterior evaluated at $u_t$:

$$
P_t(w) = p(w|\mathcal{D}_{1:t}) \approx \mathcal{N}(u_t, C_t),
$$

(3.13)
where
\[ \mathbf{u}_t = \arg \max_w P_t(w), \quad C_t = -H_t^{-1}, \quad H_t = \frac{\partial^2 P_t(w)}{\partial w^2} \bigg|_{w=\mathbf{u}_t}. \tag{3.14} \]

**Fast sequential update of the posterior** Use of the Laplace approximation was shown to be particularly useful in a sequential experiment (Lewi et al., 2009), where it can be assumed that the posterior distribution after the next trial in sequence, \( P_{t+1} \), will not be very different from the current posterior \( P_t \), which is to believe that our accumulated knowledge would be robust over any single additional observation. Rearranging from (3.12), the sequential update equation for the posterior distribution is concisely written as
\[ \log P_{t+1}(\mathbf{w}) = \log P_t(\mathbf{w}) + L_{t+1}(\mathbf{w}). \]
Applying the Laplace approximation, we have
\[ \log \mathcal{N}(\mathbf{w}|\mathbf{u}_{t+1}, C_{t+1}) \approx \log \mathcal{N}(\mathbf{w}|\mathbf{u}_t, C_t) + L_{t+1}(\mathbf{w}). \tag{3.15} \]

With this we can achieve a fast sequential update the posterior, without performing the full numerical optimization each time. Since the new posterior mode \( \mathbf{u}_{t+1} \) is where the gradient vanishes, it can be approximated from the previous mode \( \mathbf{u}_t \) by taking the first derivative of (3.15):
\[ \mathbf{u}_{t+1} = \mathbf{u}_t + C_t \Delta_{t+1}, \quad \text{where} \quad \Delta_{t+1} = \frac{\partial L_{t+1}}{\partial \mathbf{w}} \bigg|_{\mathbf{w}=\mathbf{u}_{t+1}}. \tag{3.16} \]
Similarly, the posterior covariance \( C_{t+1} \) is the negative inverse of the second derivative at the posterior mode \( \mathbf{u}_{t+1} \), and is approximated by taking the second derivative of (3.15) as
\[ C_{t+1} = (C_t^{-1} + \Lambda_{t+1})^{-1}, \quad \text{where} \quad \Lambda_{t+1} = -\frac{\partial^2 L_{t+1}}{\partial \mathbf{w}^2} \bigg|_{\mathbf{w}=\mathbf{u}_{t+1}}. \tag{3.17} \]
Using the matrix inversion lemma (Henderson and Searle, 1981), we can rewrite the posterior covariance as
\[ C_{t+1} = C_t \left[ I - (I + \Lambda_{t+1} C_t)^{-1} \Lambda_{t+1} C_t \right]. \tag{3.18} \]
In the original development of Lewi et al. (2009), the response (neural firing rate) was drawn from a continuous Poisson distribution, and the covariance matrix update had a convenient rank-one property. In our case the response is assumed to follow a multinomial distribution, which shares many convenient properties of the exponential family, but with a non-scalar linear predictor $V$ (because of the multi-nomial nature). The consequence of the multivariate-ness is that the update of the covariance matrix (3.17) is no longer rank-one, and that the integration over the parameter space will be inherently high-dimensional. On the other hand, since our response is a discrete variable (a choice out of several alternatives), integration over the response space is now straightforward, which will be useful in the following section, for calculating the mutual information.

Finally, we note that this sequential fast-update trick will only be used for calculating the expected utility of each candidate stimulus by approximating the posterior distribution at the next trial, as will be explained more in section 3.4. In order to obtain the parameter estimates after each observation, full numerical maximization will be performed using the full dataset directly each time, without the sequential approximation.

### 3.3.2 Sampling the posterior

When the concavity of the log posterior is not guaranteed, as is the case in many realistic situations where the animal lapses, a good alternative is to use the Markov chain Monte Carlo (MCMC) method to sample the posterior distribution. Once a good sampling of the posterior distribution is obtained, many properties of the distribution can be calculated through straightforward summations over the samples in the chain, conveniently replacing integrals over the parameter space.

Because each sample in the chain points to a specific set of model parameters and the corresponding psychometric function, there are different ways of taking the average. Here we choose to obtain the mean psychometric function, $\langle f(\theta) \rangle$, instead of the psychometric function characterized by the mean parameter, $f(\langle \theta \rangle)$. This is to believe is that the psycho-
Figure 3.1: Example with a binomial logistic model with two parameters, the slope $a$ and the bias $b$. (A) Estimates of the psychometric function $P(y = 1)$ using the two posterior inference methods (MAP and MCMC), where $N$ denotes the number of observations in the dataset. Observations were simulated by first presenting a randomly chosen stimulus within the stimulus space, and drawing a response from the true psychometric function, repeating $N$ times for the respective datasets. Both estimates improve when the dataset is larger. Also, the two methods are highly consistent in this simple case with binomial response and no lapse. (B) Posterior distributions in the parameter space. For the MAP estimate, the mode of the distribution is marked with a square, and the two standard deviations (“widths”) of its Gaussian approximation are shown with bars. For the MCMC estimate, the chain length is fixed at $n_s = 500$. All 500 samples of the chain are shown in dots, the sample mean is marked with a triangle, and the principle-component widths of the samples are shown with the bars. In the case of a multinomial logistic model, the parameter space would have more than two dimensions. For a $k$-alternative response space and an $l$-dimensional stimulus space, for example, the number of parameters required to describe the model would be $(k - 1) \times (l + 1)$ assuming the same form of $g(x) = [1, x^T]^T$. 
metric function is the meaningful characterization of animal behavior, not the parameters in the model. The difference between the two approaches may be significant for non-linear functions in general.

We consider the Metropolis-Hastings sampler (Metropolis et al., 1953) with a fixed number of samples $n_s$. Because the chain length $n_s$ is directly related to the computation time, one does not want to keep it unnecessarily large. On the other hand, the chain length cannot be too small, because adjacent samples in the chain may be correlated. In order to ensure a good sampling, therefore, the number of samples $n_s$ should be large enough compared to the characteristic scale of “mixing” (the scale at which the autocorrelation of the chain vanishes), if the chain mixes at all. This mixing problem is generally nontrivial: in particular, there is a known difficulty of properly choosing the proposal distribution, which determines the well-mixedness of the chain. Here we propose and use a semi-adaptive Metropolis-Hastings algorithm, developed specifically for the current context of sequential learning.

**The Metropolis-Hastings algorithm** One of the most widely used MCMC algorithms, the Metropolis-Hastings algorithm (Metropolis et al., 1953) generates a random walk (a Markov-chain sequence of samples) using a proposal density and a method to accept or reject the proposed moves.

A proposal is made at each iteration, where the algorithm randomly chooses a candidate for the next sample value $x'$ based on the current sample value $x_t$. The choice follows the proposal density function, $x' \sim Q(x'|x_t)$. When the proposal density $Q$ is symmetric, for example a Gaussian, the sequence of samples is a random walk. In general the width of $Q$ should match with the statistics of the distribution being sampled, and individual dimensions in the sampling space may behave differently in the multivariate case; finding the appropriate $Q$ can be difficult.

The proposed move is either accepted or rejected with some probability; if rejected, the current sample value is reused in the next iteration, $x' = x_t$. The probability of acceptance
is determined by comparing the values of $P(x_t)$ and $P(x')$, where $P(x)$ is the distribution being sampled. Because the algorithm only considers the acceptance ratio $r = P(x')/P(x_t) = f(x')/f(x_t)$ where $f(x)$ can be any function proportional to the desired distribution $P(x)$, there is no need to worry about the proper normalization of the probability distribution. If $r \geq 1$, the move is always accepted; if $r < 1$, it is accepted with a probability $r$. Consequently the samples tend to stay in the high-density regions, visiting the low-density regions only occasionally.

**Optimizing the sampler** One of the major difficulties in using the MCMC method is to make an appropriate choice of the proposal distribution, which may significantly affect the performance of the sampler. If the proposal distribution is too narrow, it will take a long time
for the chain to diffuse away from the starting point, producing a chain with highly correlated samples, requiring a long time to achieve independent samples. On the other hand if the proposal distribution is too wide, most of the proposed moves would be rejected, once again resulting in the chain stuck at the initial point. In either case the chain would “mix” poorly (Rosenthal, 2011). In this paper we restrict our consideration to the Metropolis-Hastings algorithm (Metropolis et al., 1953), although the issue of proposal distribution optimization is universal in most variants of MCMC algorithms, only with implementation-level differences.

The basic idea is that the optimal width of the proposal distribution would be determined in proportion to the typical length scale of the distribution being sampled. This idea was made precise in the case of a stationary random-walk Metropolis algorithm with Gaussian proposal distributions, by comparing the covariance matrix $\Sigma_p$ of the proposal distribution to the covariance matrix $\Sigma$ of the sampled chain. Once a linear scaling relation $\Sigma_p = s_d \Sigma$ is fixed, it was observed that it is optimal to have $s_d = (2.38)^2/d$ where $d$ is the dimensionality of the sampling space (Gelman et al., 1996; Roberts et al., 1997). An adaptive Metropolis algorithm (Haario et al., 2001) followed this observation, where the Gaussian proposal distribution adapts continuously as the sampling progresses. The adaptive algorithm uses the same scaling rule $\Sigma_p = s_d \Sigma$, but updates $\Sigma_p$ at each proposal where $\Sigma$ is covariance of the samples accumulated so far.

Here we use the semi-adaptive Metropolis-Hastings algorithm, which is a coarse-grained version of the original adaptive algorithm by Haario et al. (2001). The major difference in our algorithm is that the adjustment of the proposal distribution is made only at the end of each (sequential) chain, rather than at each proposal within the chain. This coarse-graining is a reasonable approximation because we will be sampling the posterior distribution many times as it refines over the course of data collection, once after each trial. Assuming that the change in posterior distribution after each new observation is small enough, we can justify our use of the statistics of the previous chain to adjust the properties of the current chain. Unlike in the fully adaptive algorithm where the proposal distribution needs to stabilize quickly
within a single chain, we can allow multiple chains until stabilization, usually a few initial observations – leaving some room for the coarse-grained approximation. This is because, for our purpose, it is not imperative that we have a good sampling of the distribution at the very early stage of the learning sequence where the accuracy is already limited by the smallness of the dataset.

For simplicity, we let our Gaussian proposal distributions to be aligned to our parametrization of the sampling space – in other words, we assume that the covariance matrix $\Sigma_p$ of the proposal distribution is always diagonal. This way our proposal distribution can be characterized by $d$ parameters (specifying a “step size” for each dimension). This simplification is particularly useful when $d$ is large, which is the case for the psychometric model when the stimulus is multi-dimensional and the response has multiple alternatives.

Kujala and Lukka (2006) also had the idea of adjusting the proposal density between trials based on the covariance of the chain, but their scaling factor were fixed and did not scale according to the sampling dimension. Based on more precise statistical observations, our method generalize well to a high-dimensional parameter space, which is typical for a multiple-alternative models. Moreover, our semi-adaptive sampling method provides an efficient and robust alternative to the particle filter implementations (Kujala and Lukka, 2006), which has the known problem of weight degeneration (DiMattina, 2015) as the posterior distribution narrows down with the accumulation of data. When applied to the sequential learning algorithm, our semi-adaptive Metropolis sampler shows a consistent well-mixing property after a few initial adjustments, with the standard deviation of each sampling dimension decreasing stably as data accumulate (Figure 3.2).
3.4 Choosing the most informative stimulus

We are now ready to discuss the active learning algorithm, which aims to choose the most informative stimulus adaptively for each observation. At trial $t + 1$, on top of the previously accumulated data $D_{1:t}$, we present a new stimulus $x_{t+1}$ and observe the outcome $y_{t+1}$, hoping to collect some more information about the model parameters. The amount of such information we expect to gain by having the new stimulus-response pair can be written as the mutual information

$$I(\theta; \{x_{t+1}, y_{t+1}\}|D_{1:t}) = \langle I(\theta; y_{t+1}|x_{t+1}, D_{1:t}) \rangle_{P(x_{t+1}|D_{1:t})},$$

(3.19)

where $P(x_{t+1}|D_{1:t})$ is actually a delta function when we specify the next stimulus $x_{t+1}$. The mutual information provides a measure of the expected “utility” of the stimulus. Now the aim is to find the stimulus $x_{t+1}$ that maximizes the mutual information (InfoMax):

$$x^*_{t+1} = \arg \max_{x_{t+1}} I(\theta; y_{t+1}|x_{t+1}, D_{1:t}).$$

(3.20)

Finding the optimal stimulus involves a high-dimensional integral over the parameter space, combined with the posterior inference that needs to be performed for each possible stimulus, coming with a huge computational cost. In this section, we present an efficient InfoMax algorithm based on each of the two methods of posterior inference: the MAP estimate with Laplace approximation, and the semi-adaptive MCMC sampling.

3.4.1 InfoMax with Laplace approximation

The problem of calculating the mutual information is greatly simplified when the posterior at each step is approximated as a Gaussian distribution, $p(\theta|D_{1:t}) \approx N(\mu_t, C_t)$, as described earlier in the case of the lapse-free model where $\theta = w$. In this case, the mutual information
in (3.20) can be expanded as the difference of two entropies as

$$x^*_{t+1} = \arg \max_{x_{t+1}} \left[ H(\theta | D_{1:t}) - E_{y_{t+1}|x_{t+1}, D_{1:t}} H(\theta | D_{1:t+1}) \right] . \quad (3.21)$$

Since the entropy of a Gaussian distribution is determined by the covariance matrix, the most informative stimulus under the Laplace approximation is the one that narrows the posterior covariance the most:

$$x^*_{t+1} = \arg \max_{x_{t+1}} \left[ \log \det C_t - E_{y_{t+1}|x_{t+1}, D_{1:t}} \log \det C_{t+1} \right] \quad (3.22)$$

$$= \arg \min_{x_{t+1}} E_{y_{t+1}|x_{t+1}, D_{1:t}} \log \det (C_t^{-1} C_{t+1}) . \quad (3.23)$$

For sequential data, we can take advantage of the fast and cost-efficient updating of the approximated posterior, as described above. From (3.18), the integrand in the last expression (3.23) is

$$\log \det (C_t^{-1} C_{t+1}) = \log \det \left[ I - (I + \Lambda_{t+1} C_t)^{-1} \Lambda_{t+1} C_t \right] . \quad (3.24)$$

Once again, the sequential approximation trick will only be used to efficiently perform the integral required for calculating the expected utility.

In the presence of lapse, the method based on Laplace approximation is not guaranteed to work. However when the lapse is small enough, one could choose to maximize the mutual information between the next observation and the weight parameters $I(w; y|x)$ instead of the full information $I(\theta; y|x)$. With respect to the weight parameters $w$, the log likelihood is asymptotically concave in the small lapse limit, as shown in (3.11). In place of the full covariance $C = - (\partial^2 P/\partial \theta^2)^{-1}$, the partial covariance $C'_{ww} = - (\partial^2 P/\partial w^2)^{-1}$ evaluated at the posterior mean should be used. As long as the effect of lapse is small, this partial covariance may be a positive semi-definite matrix and Laplace approximation may work, although the validity of the approximation is still not guaranteed.
Separation of integrals

The remaining challenge is to carry out the high-dimensional integration involved in calculating the mutual information. We observe that the integral can be separated as an integral over the response space followed by an integral over the parameter space,

\[ E_{y_{t+1}|x_{t+1}, D_{1:t}} = E_{\theta’|D_{1:t}} E_{y_{t+1}|x_{t+1}, \theta’} \]

assuming \( p(y_{t+1}|x_{t+1}, D_{1:t}) = p(\theta’|D_{1:t}) p(y_{t+1}|x_{t+1}, \theta’). \)

Integrating over the response space Let us first look at how the InfoMax integrand (3.24) depends on the next response, \( y_{t+1} \). The posterior covariance \( C_{t+1} \) is to be evaluated at the posterior mode \( u_{t+1} \) under the Laplace approximation, as well as the corresponding
Hessian $-\Lambda_{t+1}$ of the log likelihood (3.8),

$$\Lambda_{t+1} = -\left. \frac{\partial^2 L_{t+1}}{\partial w^2} \right|_{u_{t+1}} = X_{t+1}^T \Gamma(p(X_{t+1}u_{t+1}))X_{t+1}, \quad (3.26)$$

where $V = Xu$ is the linear predictor at the posterior mode. The $y_{t+1}$-dependence is in the posterior mode $u_{t+1}$, which is in turn coming from the gradient $\Delta_{t+1}$ of the log likelihood (3.7),

$$\Delta_{t+1} = \left. \frac{\partial L_{t+1}}{\partial w} \right|_{u_{t}} = X_{t+1}^T(y_{t+1} - p(X_{t+1}u_{t})). \quad (3.27)$$

Now that the integrand can be calculated explicitly, $E_{y_{t+1}}$ can be performed as a weighted sum, conveniently because the response is a discrete variable.

**Integrating over the parameter space** In order to exploit the full posterior distribution, we substitute $u_t$ in the above expression by a random variable $\theta' \sim N(u_t, C_t)$ which is distributed according to the current posterior. Now the integral $E_{\theta'}$ runs over the parameter space. Although the parameter space is in general high-dimensional, we can reduce the integration space using the linear structure of the multinomial logistic model (without lapse). Specifically, we make use of the linearity in $V = Xw$ to work on the lower dimensional $V$ space instead of dealing with the full $w$ space. Here we outline the process of reducing the integration space, and leave the details to the appendix (Section 3.B). The process consists of three steps: diagonalization, marginalization, and standardization. First we choose a new “coordinate system” of the (say $q$-dimensional) weight space, such that the first $k$ elements of the extended weight vector $w$ are coupled one-to-one to the elements of $k$-vector $V$. Then we marginalize to integrate out the remaining $(q - k)$ dimensions, effectively changing the integration variable from $w$ to $V$. Finally, we use Cholesky decomposition to standardize the normal distribution which is the posterior on $V$. The resulting integral is still multi-dimensional, due to the multinomial nature of our model. But once the distribution is standardized, there are a number of efficient numerical integration methods that can be
applied. For example, here we used the Sparse Grid method (Heiss and Winschel, 2008) based on the one-dimensional Gauss-Hermite quadrature.

### 3.4.2 Sampling-based InfoMax

In more general situations, for example when the log-concavity is not guaranteed due to the presence of lapse, another robust approach is to use the MCMC method to sample the posterior distribution and calculate the utility of the next stimulus based on it. Once we have a good sample of the posterior distribution, the chain \{\theta_m\} approximates the distribution as

\[
p(\theta | D) \approx \frac{1}{n_s} \sum_{m=1}^{n_s} \delta(\theta_m)
\]

where \(n_s\) is the chain length. In this case it is convenient to expand the mutual information the other way around, such that it is expressed as the expected uncertainty reduction in the observed response averaged over the current knowledge of the parameters \(\theta\), rather than in terms of the expected posterior entropy of \(\theta\) itself. Dropping the trial-related subscripts from \(D_t, x_{t+1}\) and \(y_{t+1}\), we may write

\[
I(y; \theta | x, D) = H(y| x, D) - \mathbb{E}_{\theta|D} H(y| \theta, x, D).
\]

Once we have computed the log likelihood at all possible \(x\) and all sampled \(\theta_m\), we can approximate

\[
p(y|x, D) \approx \frac{1}{n_s} \sum_m p(y|x, \theta_m).
\]
Using the abbreviation $L_{jm}(x) \equiv p(y_j = 1|x, \theta_m)$ that emphasizes the discrete nature of the response $y$, the marginal entropy of the response is

$$H(y|x, \mathcal{D}) \approx -\sum_j \left( \frac{1}{n_s} \sum_m L_{jm} \right) \log \left( \frac{1}{n_s} \sum_m L_{jm} \right),$$  

(3.31)

and the conditional entropy

$$\mathbb{E}_{\theta|x, \mathcal{D}} H(y|\theta, x, \mathcal{D}) \approx -\frac{1}{n_s} \sum_{j,m} L_{jm} \log L_{jm}. \quad (3.32)$$

Hence, computing the mutual information is as simple as doing the following summation

$$I(y; \theta|x, \mathcal{D}) = -\frac{1}{n_s} \sum_{j,m} L_{jm}(x) \log \frac{L_{jm}(x)}{\sum_{m'} L_{jm'}(x)/n_s}$$  

(3.33)

which makes it a straightforward job to find the maximizer $x$.

3.5 Results

3.5.1 Test with simulated data

We first tested our algorithm with simulated data, generated from a fixed trinomial probability distribution (3-alternative responses) on the 1D stimulus space. The response was drawn from the true model each time given the stimulus, much the same way as one would make observations in actual experiments. In each simulation we started with 5 initial observations (stimulus-response pairs) where the stimuli were chosen randomly, and progressively added one observation at a time by actively choosing the information-maximizing stimulus, as shown in Figure 3.3. We have run 300 independent simulations, with $N = 100$ observations each.

The performances of the algorithms are measured based on the accuracy of the inferred psychometric function, rather than of the values of the model parameters, for twofold reasons.
Figure 3.4: Estimate error (the total Kullback-Leibler divergence) during simulated learning, averaged over 300 independent trials, when the true model did not lapse (left) and when it lapsed with probabilities $c_i = 0.05$ for each of $i = 1, 2, 3$ (right). Note how the performance of the MCMC algorithm improves as the chain length improves (for example the legend “MCMC500” in the figure means MCMC the chain length of $n_s = 500$).
First, for most application it is more relevant to look at the functional quantity than the parameters in the underlying model. Second, although one could look at the evolution of any one parameter, it is difficult to compare the errors in different parameters because they are in different scales and are not comparable to one another.

We looked at two error measures, the Kullback-Leibler divergence (KLD) and the sum-of-square error (SSE). Both are functions of the true probability $p(x)$, which is known in this simulated case, and the estimated probability $p'(x)$. The Kullback-Leibler divergence of $p'$ with respect to $p$ is defined as

$$D_{KL}(x) \equiv D_{KL}[p(x)\|p'(x)] = \sum_{i=1}^{k} p_i(x) \log \frac{p_i(x)}{p'_i(x)}.$$  (3.34)

The root-sum-square error is defined as the norm in the space of probability "vectors",

$$E_{SS}(x) = \|p(x) - p'(x)\| = \left(\sum_{i=1}^{k} [p_i(x) - p'_i(x)]^2\right)^{1/2}.$$  (3.35)

The total errors are defined by integrating over the stimulus space as

$$\text{KLD} = \int d\mathbf{x} \ D_{KL}(\mathbf{x}) = \sum_{m} D_{KL}(\mathbf{x}_m), \quad \text{SSE} = \int d\mathbf{x} \ E_{SS}(\mathbf{x}) = \sum_{m} E_{SS}(\mathbf{x}_m).$$  (3.36)

For simulated data we sum over the grid points $\{\mathbf{x}_m\}$; for application to real data (next section), we sum over the actual set of stimuli that was presented in the experiment. The two different error measures reflect different aspects of the psychometric function (the probability map). By construction, the KL divergence is sensitive on the tail of the true distribution; for example, when some true binomial process has probability $p(x_1) = [0.99, 0.01]$ at some $x_1$ but is estimated to be $p'(x_1) = [0.99999, 0.00001]$, the KL divergence at this point is as large as $D_{KL}(x_1) = 0.06$ while the sum of square error is $E_{SS}(x_1) = 0.01$. On the other hand, if for a true $p(x_2) = [0.6, 0.4]$ the estimated was $p'(x_2) = [0.5, 0.5]$, we get $D_{KL}(x_2) = 0.02$ while $E_{SS}(x_2) = 0.14$. 

155
Figure 3.4 shows the performances of different learning algorithms on simulated data, with and without lapse. As expected, the algorithm based on Laplace approximation performs well for the lapse-free data, but fails in the presence of lapse. Interestingly, the overall performance tends to stumble in the early stage of learning for the Laplace-approximation-based method, presumably because the way InfoMax is based on the current estimate of the posterior distribution (exploitation of the current knowledge), which may not be accurate enough with only a few observations. This is a known phenomenon for adaptive estimate algorithms, reported for example in Lewi et al. (2009), where it was explained through the lens of exploitation-exploration tradeoff (Kaelbling et al., 1996). On the other hand, our MCMC sampling method works similarly well in both cases, and without the initial hump, suggesting that it might be the robust method under different forms of the posterior distribution and with limited amounts of data. It also demonstrates that our semi-adaptive tuning algorithm for the MCMC sampler was successful. More generally, these results emphasize that active learning can be sensitive to model mismatch and that the sampling-based methods can provide an efficient and robust platform for adaptive experiments.

3.5.2 Application to psychological experiments

Next, we applied our method on a real dataset, from an experiment on the decision-making behavior of monkeys (Churchland et al., 2008). Here the monkeys are trained to observe a group of moving dots with varying degree of coherence, and choose the collective direction of the dots from a set of four given choices, one of which is aligned to the preferred direction of the neuron being measured. This is a nice example of the type of psychometric experiments our framework is particularly relevant to.

Because the four directions in the experiment span a two-dimensional plane, as in East-West/North-South, we can characterize the moving dot stimulus as a two-dimensional stimulus vector $\mathbf{x}$, where the direction of the vector is the direction of the mean movement of the dots, and the amplitude of the vector is given by the coherence of the movement (scaled...
Figure 3.5: Performance of the active learning algorithms based on MCMC, quantified by the Kullback-Leibler divergence of the estimated psychometric functions, averaged over 300 independent runs each. The “experiment” consists of sub-sampling datapoints from the existing dataset on the 4-choice behavior of monkeys. A significant speedup is achieved by an active sampling design, compared to a randomized one. Consistent with the fact that monkeys are almost lapse-free for this task, our two models with and without lapse work almost equivalently.

from 0 to 1). By conventional practice, however, all stimulus vectors given in the experiment are aligned with either one of the two axes (also see Figure 3.6A).

With the real data, what we do at each step is to sample a stimulus-response pair from the existing database using our InfoMax criterion, effectively to construct a smaller subset of the database for the “virtual experiment”. Once we know which stimulus value $x_{t+1} = x^*$ we want to sample next, one of the stimulus-response pairs $\{(x_i, y_i) | x_i = x^*\}$ at the given stimulus is picked randomly from the dataset. Because this high-throughput monkey dataset is large enough, with more than 10,000 total observations at 29 different conditions from the 2-dimensional stimulus space, it can be assumed that the set of all observed responses at any fixed stimulus is a good statistical representation of the underlying choice probability. Consequently, this subsampling procedure effectively simulates an experiment where the stimulus was chosen at each trial adaptively and the response was freshly measured.

**Speedup achieved by active learning**  As shown in Figure 3.5, our active learning algorithm efficiently optimizes the experimental design, learning the psychometric function more quickly compared to the conventional, randomized design. The performance of each learning
Figure 3.6: Learning on a two-dimensional stimulus space. (A) Inferred psychometric function from the monkey dataset, with four alternatives. (B-D) Black dots indicate stimuli in the respective datasets, and the background colors represent the dominating response in the corresponding region of the stimulus space, according to the underlying psychometric function. (B) The limited stimulus set, as used in the monkey experiment. (C) Stimuli chosen by the InfoMax active learning algorithm from the full plane, over a single active learning sequence of 100 trials. Responses are simulated according to the psychometric function inferred from the monkey experiment. (D) InfoMax choice of stimuli as in (C), but with a more general form of the underlying function.

The algorithm is quantified by the Kullback-Leibler divergence of the estimated probability $p'$, compared to the best probability estimate $p$ (estimated using the full dataset) in place of the “true” probability in this case. Here we only show the result from the method based on the semi-adaptive Monte Carlo sampling of the posterior distribution, but the method based on Laplace approximation works as well in this case because the monkeys are almost lapse-free (it appears that the monkeys are almost lapse-free on this particular task of moving dot direction discrimination). Our algorithms with both models, with and without lapse, perform equally well on this dataset. It demonstrates that as long as the MCMC method is used, our model with lapse passes the test of the correspondence principle: when the data is lapse-free, the lapse model reproduces the result of the classical lapse-free model, despite the $k$ additional parameters to learn (where $k$ is the number of alternative responses, in this case $k = 4$) and the loss of the log concavity.

**Exploiting the full stimulus space** The success of our active learning methods may have an important implication on the design of animal psychometric experiments. It is a conventional practice in animal psychology to limit the number of different stimuli in the
stimulus set $X$ that is presented to the subject animal, in order to obtain a reasonably narrow error bars at each stimulus (which takes a large number of datapoints per stimulus). When exploring a multi-dimensional stimulus space, therefore, it was completely reasonable to stick to an axis-sampling design as shown in Figure 3.6B which is the set of stimuli actually used in the monkey experiment. However, using our Bayesian inference framework, observations at different points in the stimulus space (not only those at the same point) may add up in the form of posterior distribution, and the old constraint on the size of the stimulus set is removed. We show that the 2-dimensional stimulus plane of the monkey experiment could have been exploited more efficiently using our active learning method, resulting in a set of observations concentrated near the thresholds of different phases of dominant responses (Figure 3.6C), optimizing the information gain from the sequence of observations. Although it is not straightforward to compare the performances of the learning algorithms between the two different stimulus spaces, because the KL divergences are defined on different measures, it seems clear that the conventional axis-sampling design is losing some of the most informative stimuli. This idea of efficiently exploiting the full stimulus domain is not specific to the simple psychometric function of the monkey’s choice behavior, and may be extended to more general functions, as demonstrated in Figure 3.6D.

3.6 Discussion

We developed a Bayesian active learning algorithm for inferring animal psychometric functions described by the multinomial logistic model, with an objective of maximizing the expected informativeness of each stimulus in a sequential experiment. This work is a nontrivial extension of the previous works in several dimensions: First, we stepped beyond the binary-choice framework and considered a general setting of multiple-alternative responses. Second, we focused on developing an applicable theory of active learning, incorporating common realistic aspects of animal behavior such as the lapse probability into the model of psychometric
function, as well as testing the method on actual experimental settings. Third, we proposed and demonstrated an effective, semi-adaptive implementation of the Markov chain Monte Carlo (MCMC) sampling method that is used to infer the posterior distribution of the model parameters, developed specifically for the current context of sequential experiment.

Although the well-studied analytical method based on a local (Laplace) approximation of the posterior distribution is effective in ideal settings, it may easily break down with more realistic data (for example with lapse), and our MCMC method is a robust alternative to it. The cost of such flexibility comes in the form of increased computation time (compared to the method based on Laplace approximation); depending on the experimental paradigm, a naive implementation of the sampling method may take too long to run within a single-trial interval. It will be a meaningful future direction to further optimize the sampling algorithm for real-time applications. It might also be possible to first test the psychometric function with the more reliable MCMC method, then if it appears that the animal is lapse-free, switch to the faster method with Laplace approximation. If the animal actually has a non-zero lapse rate, on the other hand, the MCMC method would be the safe choice to go.

Our method was applied successfully both on simulated data and on a real experimental dataset, with and without lapse, achieving significant speedups in learning rates. Importantly, the success of the algorithm depends heavily on the use of the correct model; for example, it fails when a lapse-free model was used on a dataset with lapse. Within this work, however, our aim is not in solving the model selection problem for given data, but in developing an algorithm for the most efficient learning of the given family of model, which is assumed to be correct. The implications of our work extends beyond the current experimental paradigm. For example, we suggest that a multi-dimensional stimulus space could be sampled more efficiently using our Bayesian active learning algorithm, which removes the long-standing constraint on the number of observations per stimulus. We hope that this work will be a step forward to a wider application of active learning theories in the laboratories,
which would surely facilitate the development of the field by reducing the time and resource associated to each experiment.

Finally, we note that a potential limitation of the active learning framework is the possibility that the psychometric function of the animal might adapt to the distribution of stimuli presented during the experiments. In this case the system being measured is no longer stationary nor independent of the experimental design, and the current strategy breaks down fundamentally. This opens an interesting avenue for future research, with at least three ingredients: how the animal behavior is affected by the history of stimuli, responses and rewards (a problem of reinforcement learning); how the time-varying psychometric function of the animal can be estimated accurately from the behavioral data (Paninski et al., 2010; Bak et al., 2016); and finally, how an optimal experiment can be designed while taking into account the feedback loop between the experimental design and the animal behavior.
3.A Log likelihood for MNL

3.A.1 Derivatives of the log likelihood in the presence of lapse

With lapse probabilities $c_i$, the multinomial logistic model has

$$p_i = (1 - c_{\text{total}})q_i + c_i, \quad q_i = \frac{\exp(V_i)}{\sum_h \exp(V_h)}$$

with $q_i$ the lapse-free probability. Differentiating with the linear predictor $V$, we get

$$\frac{\partial q_i}{\partial V_k} = (\delta_{ik} - q_k)q_i,$$

$$\frac{\partial^2 q_i}{\partial V_j \partial V_k} = [(\delta_{ij} - q_j)(\delta_{ik} - q_k) - (\delta_{jk}q_k - q_jq_k)]q_i.$$

which leads to

$$\frac{\partial p_i}{\partial V_k} = (1 - c_{\text{total}})\frac{\partial q_i}{\partial V_k}, \quad \frac{\partial^2 p_i}{\partial V_j \partial V_k} = (1 - c_{\text{total}})\frac{\partial^2 q_i}{\partial V_j \partial V_k}.$$

We are interested in the derivatives of the log likelihood $L = y^T \log p$ with respect to $V$. Using the abbreviation $r_i \equiv (1 - c_{\text{total}})q_i/p_i$, $t_i \equiv y_i r_i$ and $s_i \equiv y_i r_i (1 - r_i)$ for notational convenience, we can write down the partial gradient as (partial because it differentiates only
respect to the weight parameters, not the lapse parameters)

\[
\frac{\partial L}{\partial V_k} = \sum_i y_i \frac{1}{p_i} \frac{\partial p_i}{\partial V_k} = (1 - c_{\text{total}}) \sum_i y_i \frac{q_i}{p_i} (\delta_{ik} - q_k)
\]

\[
= y_k r_k - q_k \sum_i y_i r_i
\]

\[
= t_k - q_k \sum_i t_i, \quad (3.41)
\]

and the partial Hessian

\[
\frac{\partial^2 L}{\partial V_j \partial V_k} = \sum_i y_i \left( \frac{1}{p_i} \frac{\partial^2 p_i}{\partial V_j \partial V_k} - \frac{1}{p_i^2} \frac{\partial p_i}{\partial V_j} \frac{\partial p_i}{\partial V_k} \right)
\]

\[
= \sum_i y_i \left[ (1 - c_{\text{total}}) \frac{q_i}{p_i} \left[ (\delta_{ij} - q_j)(\delta_{ik} - q_k) - (\delta_{jk}q_k - q_j q_k) \right] - (1 - c_{\text{total}}) \frac{q_i^2}{p_i^2} (\delta_{ij} - q_j)(\delta_{ik} - q_k) \right]
\]

\[
= \sum_i y_i \left[ r_i (1 - r_i) (\delta_{ij} - q_j)(\delta_{ik} - q_k) - r_i (\delta_{jk}q_k - q_j q_k) \right]
\]

\[
= \sum_i \left[ s_i (\delta_{ij} - q_j)(\delta_{ik} - q_k) - t_i (\delta_{jk}q_k - q_j q_k) \right]
\]

\[
= \delta_{jk} \left( s_k - q_k \sum_i t_i \right) - (q_j s_k + q_k s_j) + q_j q_k \left( \sum_i s_i + \sum_i t_i \right). \quad (3.42)
\]

In vector notation, we may write more succinctly as

\[
\frac{\partial L}{\partial V} = (t - A_t q)^T, \quad (3.43)
\]

and

\[
\frac{\partial^2 L}{\partial V^2} = \text{diag}(s - A_t q) - (qs^T + sq^T) + (A_t + A_s)qq^T \quad (3.44)
\]

\[
= -A_t \left[ \text{diag}(q) - qq^T \right] + \left[ \text{diag}(s) - (qs^T + sq^T) + A_s qq^T \right] \quad (3.45)
\]
with abbreviations $A_t = \sum_i t_i$ and $A_s = \sum_i s_i$ for the sums. Since the abbreviated variables can also be written as $t_i = y_i (1 - c_i/p_i)$ and $s_i = y_i c_i (1 - c_i/p_i)$, we recover $t_i \to y_i$ and $s_i \to 0$ in the lapse-free limit $c_i \to 0$. Hence the first square bracket in $\partial^2 L/\partial V^2$ reduces back to the lapse-free Hessian, while the second square bracket vanishes as $c_i \to 0$ (also see Appendix 3.A.2).

Now with respect to the lapse rate $c$, we have

$$\frac{\partial p_i}{\partial c_k} = \delta_{ik} - q_i, \quad \frac{\partial^2 p_i}{\partial c_j \partial c_k} = 0, \quad \frac{\partial^2 p_i}{\partial c_j \partial V_k} = -\frac{\partial q_i}{\partial V_k}, \quad (3.46)$$

$$\frac{\partial L}{\partial c_k} = \sum_i y_i \frac{1}{p_i} \frac{\partial p_i}{\partial c_k} = \sum_i y_i \frac{1}{p_i} (\delta_{ik} - q_i) = \frac{y_k}{p_k} - \frac{\sum_i t_i}{1 - c_{\text{total}}}. \quad (3.47)$$

$$\frac{\partial^2 L}{\partial c_j \partial c_k} = -\sum_i y_i \frac{1}{p_i^2} \left( \frac{\partial p_i}{\partial c_j} \frac{\partial p_i}{\partial c_k} - \frac{\partial^2 p_i}{\partial c_j \partial c_k} \right) = -\sum_i y_i \frac{1}{p_i^2} (\delta_{ij} - q_i) (\delta_{ik} - q_i)$$

$$= -\delta_{jk} \frac{y_k}{p_k^2} + \left( \frac{y_k q_k}{p_k^3} + \frac{y_j q_j}{p_j^3} \right) - \frac{\sum_i (s_i - t_i)}{(1 - c_{\text{total}})^2}. \quad (3.48)$$

$$\frac{\partial^2 L}{\partial c_j \partial V_k} = \sum_i y_i \left( \frac{1}{p_i} \frac{\partial^2 p_i}{\partial c_j \partial V_k} - \frac{1}{p_i^2} \frac{\partial p_i}{\partial c_j} \frac{\partial p_i}{\partial V_k} \right)$$

$$= -\sum_i y_i p_i \left[ 1 + (1 - c_{\text{total}}) \frac{1}{p_i} \frac{\partial p_i}{\partial c_j} \frac{\partial p_i}{\partial V_k} \right] \frac{\partial q_i}{\partial V_k}$$

$$= -\sum_i y_i \left[ 1 + (1 - c_{\text{total}}) \frac{1}{p_i} (\delta_{ij} - q_i) \right] (\delta_{ik} - q_k) q_i$$

$$= -\sum_i \frac{s_i}{1 - c_{\text{total}}} \left[ \frac{\delta_{ij} t_i}{p_i} \right] (\delta_{ik} - q_k)$$

$$= -\delta_{jk} \frac{t_k}{p_k} - \frac{s_k}{1 - c_{\text{total}}} + q_k \frac{t_j}{p_j} + q_k \frac{\sum_i s_i}{(1 - c_{\text{total}})^2}. \quad (3.49)$$
Introducing the auxiliary lapse variable \( \gamma \), defined such that \( c_i = e^{\gamma_i} / (1 + e^{\gamma_i}) \) and \( \partial c_i / \partial \gamma_i = c_i(1 - c_i) \), we can easily rewrite these derivatives with respect to \( \gamma \) as

\[
\frac{\partial L}{\partial \gamma_k} = \frac{\partial c_k}{\partial \gamma_k} \frac{\partial L}{\partial c_k}, \quad \frac{\partial^2 L}{\partial \gamma_j \partial \gamma_k} = \frac{\partial c_j}{\partial \gamma_j} \frac{\partial^2 L}{\partial c_j \partial \gamma_k}.
\]

and

\[
\frac{\partial^2 L}{\partial \gamma_j \partial \gamma_k} = \frac{\partial c_j}{\partial \gamma_j} \frac{\partial c_k}{\partial \gamma_k} \frac{\partial^2 L}{\partial c_j \partial c_k} + \delta_{jk} \frac{\partial^2 L}{\partial c_j \partial c_k} (1 - 2c_k).
\]

**3.A.2 Concavity of the log likelihood**

For the classical multinomial logistic model, it is straightforward to show that the Hessian \( H = -\text{diag}(p) + pp^T \equiv -\Gamma \) is negative semi-definite, which is equivalent to showing that \( z^T \Gamma z \geq 0 \) for an arbitrary vector \( z \).

\[
z^T \Gamma z = z^T \text{diag}(p)z - (z^T p)^2
\]

\[
= \sum_i z_i^2 p_i - \left( \sum_j z_j p_j \right)^2
\]

\[
= \sum_i z_i^2 p_i - 2 \sum_i z_i p_i \sum_j z_j p_j + \left( \sum_j z_j p_j \right)^2
\]

\[
= \sum_i p_i \left[ z_i^2 - 2z_i \sum_j z_j p_j + \left( \sum_j z_j p_j \right)^2 \right]
\]

\[
= \sum_i p_i \left[ (z_i - \sum_j z_j p_j)^2 \right] \geq 0.
\]

In the presence of lapse rates, we are interested in the negative semi-definiteness of the partial Hessian (with respect to the weight parameters but not the lapse parameters),

\[
H \equiv \frac{\partial^2 L}{\partial V^2} = -A_t \left[ \text{diag}(q) - qq^T \right] + \left[ \text{diag}(s) - (qs^T + sq^T) + A_s qq^T \right].
\]
For an arbitrary vector $\mathbf{z}$,

$$\mathbf{z}^T H \mathbf{z} = -A_t \left[ \sum_k z_k^2 q_k - \left( \sum_k z_k q_k \right)^2 \right]$$

$$+ \left[ \sum_k z_k^2 s_k - 2 \sum_k z_k q_k \sum_j z_j s_j + A_s \left( \sum_k z_k q_k \right)^2 \right]$$

$$= -A_t \left( \langle z - \langle z \rangle_q \rangle \right) q + \left[ \sum_j s_j z_j \left( z_j - \langle z \rangle_q \right) - \sum_j s_j \langle z \rangle_q \left( z_j - \langle z \rangle_q \right) \right]$$

$$= -\sum_j t_j \left( \langle z - \langle z \rangle_q \rangle^2 \right) q + \sum_j s_j \left( z_j - \langle z \rangle_q \right)^2 \tag{3.54}$$

where $\langle x \rangle_q = \sum_k x_j q_j$. There is no guarantee that the partial Hessian will be negative semi-definite, except at the lapse-free limit where $t_j \to y_j$ and $s_j \to 0$.

3.B Integrating over the parameter space: reducing the integration space

Diagonalization It is clear from (3.24)–(3.27) that all parameter-dependence in our integrand is actually in terms of the linear predictor $\mathbf{V} = \mathbf{X} \mathbf{w}$. That is, we are dealing with the integral of the form

$$F = \int d\mathbf{w}^\prime \mathcal{N}(\mathbf{w}^\prime | \mathbf{w}^\prime, C) \cdot f(\mathbf{X} \mathbf{w}^\prime), \tag{3.55}$$

where $C$ is the covariance matrix, and $\mathbf{X} = \bigoplus_{j=1}^{k} \mathbf{G}_j^T$ is a fixed matrix constructed from direct sum of $k$ vectors. It helps to work in a diagonalized coordinate system, so that we can separate out the relevant dimensions of $\mathbf{w}$. We use the singular value decomposition of the design matrix ($\mathbf{X} = \mathbf{UGV}^T$ with $U = I$ and $V = Q^T$). Because of the direct-sum construction, $\mathbf{XX}^T$ is already diagonal, and the left singular matrix is always $I$ in this case. Then

$$G = \mathbf{XQ}^T = \left[ \begin{array}{cc} G_k & G_q \end{array} \right], \tag{3.56}$$

166
where $G_k$ is a $k \times k$ diagonal matrix and $G_q$ is a $k \times (q-k)$ matrix of zeros. We can now denote $w_k = (w_1, \ldots, w_k)$ and $w_q = (w_{k+1}, \ldots, w_q)$ in the diagonalized variable $w = Qw'$, such that
\[
w = [w_k, w_q]^T, \quad Gw = G_kw_k = (g_1w_1, g_2w_2, \ldots g_kw_k).
\] (3.57)

**Marginalization**  
Now we have
\[
F = \int dw \mathcal{N}(w|\hat{w}, H^{-1}) \cdot f(Gw), \quad H^{-1} = QCQ^T
\] (3.58)
where $H$ is the inverse of the new covariance matrix after diagonalization. We can block-decompose this matrix following the above notation,
\[
H = \begin{bmatrix}
H_{kk} & H_{kq} \\
H_{qk} & H_{qq}
\end{bmatrix}, \quad H_{kq} = (H_{qk})^T.
\] (3.59)

Then the Gaussian distribution can be decomposed as (see Appendix 3.B.1 for details)
\[
\mathcal{N}(w|\hat{w}, H^{-1}) = \mathcal{N}(w_k|\hat{w}_k, H_*^{-1}) \mathcal{N}(w_q|\hat{w}_q - H_{qk}^{-1}H_{qk}w_k, H_{qq}^{-1})
\] (3.60)
where
\[
H_* = H_{kk} - H_{kq}H_{qq}^{-1}H_{qk}.
\] (3.61)

As the non-parallel part $w_q$ is integrated out, we have marginalized the integral. It is useful to note that if a variable $w \sim \mathcal{N}(\hat{w}, C)$ is Gaussian distributed, its linear transform $V = Xw$ is also Gaussian distributed as $V \sim \mathcal{N}(\hat{V}, \Sigma)$, with $\hat{V} = X\hat{w}$ and $\Sigma = XCX^T$ (derived in Appendix 3.B.2). Changing the integration variable to $V = G_kw_k$ is then straightforward:
\[
F = \int dw_k \mathcal{N}(w_k|\hat{w}_k, H_*^{-1}) \cdot f(G_kw_k)
= \int dV \mathcal{N}(V|\hat{V}, \Sigma) \cdot f(V), \quad \Sigma = G_kH_*^{-1}G_k^T.
\] (3.62)
**Standardization**  Finally, in order to deal with the numerical integration, it is convenient to have the normal distribution standardized. We can use the Cholesky decomposition for the covariance matrix,

\[ LL^T = \Sigma_{t+1}, \quad (3.63) \]

such that the new variable \( U = L^{-1}(V - \hat{V}_{t+1}) \) is standard normal distributed. From the above formulation, \( L \) can be written directly in terms of the Cholesky decomposition of \( H^* \):

\[ L = G_k R^{-1} \quad \text{where} \quad R^T R = H^*. \quad (3.64) \]

Importantly, with this transformation, each dimension of \( U \) is independently and identically distributed. The objective function to be evaluated is now

\[
F(x) = \int dV \cdot N(V|\hat{V}_{t+1}, \Sigma_{t+1}) \cdot f(V, x)
= \int dU \cdot N(U|0, I) \cdot f(\phi(U), x) \quad (3.65)
\]

where \( \phi(U) = \hat{V}_{t+1} + LU \). Once the integration is standardized this way, there are a number of efficient numerical methods that can be applied.

**3.B.1 Decomposition of a multivariate Gaussian**

Consider a multivariate Gaussian distribution \( N(w|0, H^{-1}) \) of a random vector \( w \), where

\[
w = [w_k, w_q]^T \quad \text{and} \quad H = \begin{bmatrix} H_{kk} & H_{kq} \\ H_{qk} & H_{qq} \end{bmatrix}. \quad (3.66)
\]
The quadratic form can be expanded as

\[ w^T H w = w_k^T H_{kk} w_k + 2w_k^T H_{kq} w_q + w_q^T H_{qq} w_q \]

\[ = w_k^T H_{kk} w_k + (w_q + b)^T H_{qq} (w_q + b) - b^T H_{qq} b, \quad b = H_{qq}^{-1} H_{qk} w_k \]

\[ = w_k^T (H_{kk} - H_{kq} H_{qq}^{-1} H_{qk}) w_k + (w_q + b)^T H_{qq} (w_q + b). \quad (3.67) \]

Then

\[ N(w|0, H^{-1}) = \frac{|H|^{1/2}}{(2\pi)^{q/2}} \exp \left( -\frac{1}{2} w^T H w \right) \]

\[ = \frac{|H|^{1/2}}{(2\pi)^{q/2}} \exp \left( -\frac{1}{2} w_k^T H_{kk} w_k \right) \exp \left( -\frac{1}{2} (w_q + b)^T H_{qq} (w_q + b) \right) \]

\[ = \left( \frac{|H|}{|H_{kk}| |H_{qq}|} \right)^{1/2} N(w_k|0, H_{kk}^{-1}) N(w_q| - H_{qq}^{-1} H_{qk} w_k, H_{qq}^{-1}). \quad (3.68) \]

But from the property of the block matrix, \( \det(H) = \det(H_{qq}) \det(H_*) \), the pre-factor is exactly one. Hence we can write

\[ N(w|0, H^{-1}) = N(w_k|0, H_{kk}^{-1}) N(w_q| - H_{qq}^{-1} H_{qk} w_k, H_{qq}^{-1}) \quad (3.69) \]

where

\[ H_* = H_{kk} - H_{kq} H_{qq}^{-1} H_{qk}. \quad (3.70) \]

### 3.B.2 Linear transformation of Gaussian distribution

Suppose that \( z \) is a random vector and \( A \) is a fixed matrix. The claim is that \( z \) is Gaussian distributed as \( z \sim N(\hat{z}, C) \) with with the mean \( \hat{z} \) and the covariance matrix \( C \), then \( y = Az \) is also Gaussian distributed as \( y \sim N(\hat{y}, \Sigma) \), with the mean \( \hat{y} = A\hat{z} \) and the covariance matrix \( \Sigma = ACA^T \).
Showing the transformation of the mean $\hat{y} = A\hat{z}$ is trivial. For the covariance matrix:

$$
\Sigma_{ij} = \mathbb{E} [(y_i - \hat{y}_i)(y_j - \hat{y}_j)]
= \mathbb{E} \left[ \left( \sum_l A_{il}(z_l - \hat{z}_l) \right) \left( \sum_m A_{jm}(z_m - \hat{z}_m) \right) \right]
= \sum_{lm} A_{il}A_{jm} \mathbb{E} [(z_l - \hat{z}_l)(z_m - \hat{z}_m)]
= \sum_{lm} A_{il}C_{lm} (A^T)_{mj} = [ACA^T]_{ij}.
$$

(3.71)

3.C More on InfoMax with Laplace approximation

3.C.1 Two expansions of mutual information

There are two convenient ways of expanding and rewriting the mutual information. First, one can expand the mutual information as the difference of two entropies (with the intuitive interpretation of reduced uncertainty) as

$$
I(\theta; y_{t+1}|x_{t+1}, D_{1:t}) = H(\theta|D_{1:t}) - \mathbb{E}_{y_{t+1}|x_{t+1}, D_{1:t}} H(\theta|D_{1:t+1}).
$$

(3.72)

Alternatively, the mutual information can also be written as the expectation value of the Kullback-Leibler (KL) divergence as

$$
I(\theta; y_{t+1}|x_{t+1}, D_{1:t}) = \int dy_{t+1} \int d\theta P(\theta, y_{t+1}|x_{t+1}, D_{1:t}) \log \frac{P(\theta|y_{t+1}, x_{t+1}, D)}{P(\theta|D_{1:t})}
= \int dy_{t+1} P(y_{t+1}|x_{t+1}, D_{1:t}) \int d\theta P(\theta|D_{1:t+1}) \log \frac{P(\theta|D_{1:t+1})}{P(\theta|D_{1:t})}
= \langle D_{KL}[P(\theta|D_{1:t+1}) || P(\theta|D_{1:t})] \rangle_{P(y_{t+1}|x_{t+1}, D_{1:t})}.
$$

(3.73)

The KL divergence $D_{KL}[p||q] = \langle \log(p/q) \rangle_p$ is a measure of difference between two probability distributions: it measures the information loss when the true distribution $p$ was approximated by $q$. It is minimized at zero when $q$ is a perfect estimate of $p$ (i.e., when $p = q$ almost
everywhere). In our case, maximizing the mutual information is equivalent to maximizing
the KL divergence; seeking to make the posterior $P(\theta | \mathcal{D}_{1:t+1})$ as different from the prior
$P(\theta | \mathcal{D}_{1:t})$ as possible.

In principle, the two expansions in Equations (3.72 – 3.73) are both exact representa-
tions of the mutual information and are therefore equivalent. However, under the Laplace
approximation, they give different approximated results. One can actually write the two
expansions of the mutual information in closed forms respectively, up to constant factors:

$$H[\theta | \mathcal{D}_{1:t}] - \langle H[\theta | \mathcal{D}_{1:t+1}] \rangle = \frac{1}{2} \langle - \log \det(C_t^{-1}C_{t+1}) \rangle;$$ (3.74)

$$\langle D_{KL}[P(\theta | \mathcal{D}_{1:t+1})||P(\theta | \mathcal{D}_{1:t})] \rangle = \frac{1}{2} \langle - \log \det(C_t^{-1}C_{t+1}) + \text{tr}(C_t^{-1}C_{t+1}) - N_\theta 
+ (u_{t+1} - u_t)^T C_t^{-1}(u_{t+1} - u_t) \rangle, $$ (3.75)

where $N_\theta$ is the dimensionality of the parameter space (equivalent to $k$ in the main text), and
$\langle \cdot \rangle$ is the average over the response probability distribution $P(y_{t+1} | x_{t+1}, \mathcal{D}_{1:t})$. Intuitively, the
discrepancy is due to the order in which we make the approximations and take the differences,
alogous to the problem of calculating the difference of two numbers with limited precision:

$$\text{round}(10123235) - \text{round}(10010173) \neq \text{round}(10123235 - 10010173),$$

$$(1.01 \times 10^7) - (1.00 \times 10^7) \neq (1.13 \times 10^5).$$

If we take the Laplace approximation seriously, therefore, the expansion with KL diver-
gence should be more accurate. However, the KL divergence approach is presumably more
useful when we are interested in the change in the distribution, rather than in the resulting
posterior distribution alone. In the current problem we only care about our final knowledge
on the psychometric function, and not necessarily about how we have improved from the
previous trial, and we are happy to simply take the difference of entropies and to minimize
the entropy of the posterior distribution.
Conclusion

Scrolling over the first compiled draft of my dissertation, I thought the evolution of my research interests during the graduate study was an interesting example of how a young theoretical physicist gets introduced to the biological world which is “messy” but fascinating, and gradually becomes more aware of how the biological science operates within the constraints of the real world. My research started with an almost purely theoretical problem in information theory, with simple models of ligand-receptor interaction in mind, the original form of which was a small textbook exercise of my advisor’s (Bialek, 2012). My second project, on protein surfaces, was for me the first exposure to data-driven research, and it came with a shock how noisy and sometimes imperfect the real dataset was – even when the concept of extracting relevant information from noisy signal was at the core of my first project on the abstract level. Before one could ask any question about the structure of the shape space of proteins that might have evolved to make sense of the interactions, there was a fundamental problem of how one should make sense of the data. In this light, perhaps my next move to the “science of science-doing” was not an unexpected one, because as I struggled with the protein datasets I already started noticing the parallel lines between the problems cells need to solve in order to function more efficiently, and the problems we scientists solve in order to understand better. In my third project on optimal experimental design, I worked directly in the feedback loop of theory and experiment, where the set of accumulated data brings forth a theory (in this simple case an inference of the model
parameters), and the theory provides a guide to where the experiment should target next, optimizing the structure of the information-collecting process.

The three projects were three instances of how an information channel in biology (or in biological science) is shaped by the structural details of the system it is built upon, all in different scales. In Chapter 1, the optimization of information flow through the ligand-receptor interaction was dependent on the choice of the parameters governing the kinetics of the receptor, which was at the molecular level. In Chapter 2 where we looked at the ensemble of protein surfaces, the underlying question was how the space of protein-protein interaction has been shaped, presumably by evolution, in order to meet the twofold requirement of specificity and capacity; it was about a snapshot of an optimization process that works at the cellular (or even organismal) level, in the evolutionary timescale. On the other hand, in Chapter 3 we considered how the scientific experiment, which is by definition the process of collecting information from the natural world, could be viewed as an information channel and optimized by designing the structure in which the experiment is conducted.

I was more than fortunate to have been able to sample such a broad array of starting points, which now leaves me with a great range of possible directions for future research. The works in this dissertation lay some important groundworks. For example, I believe there is much more to think about in the space of protein-protein interaction. Based on what we understood so far, such as the non-trivial organization of the protein surfaces in terms of shape, it might now be possible to construct a low-dimensional representation of the shape space, and discuss the full physicochemical properties of the interaction space at the system level. On the other hand, continuing from the active learning study, an immediate follow-up question is to ask how the system being studied (in this case the behavior of the subject animal) interacts with the experimental design, rather than staying as an independent and stationary system. This project, which involves the behavior of rats under training, is already taking place (not only theoretically but also experimentally, in the hands of Jung Yoon Choi and Ilana Witten), and I feel extremely lucky to be working at the exciting
boundary where theory can do something for the experiment, not the other way around as in the usual case. Moreover, I also expect to find productive combinations of my three starting points, by interpolating in the question space; for example, the modern techniques of Bayesian inference that were used in the active learning project could also be applied to other data-rich problems, such as in the context of proteins. There seems to be “plenty of room” in between, although I am happily looking forward to meeting new and unfamiliar questions that will emerge, as well.

Being a student in biophysics, I had a very special experience in which the science (the biological systems to be studied) and the life (the biological system which is, and surrounds, myself) interact with each other so intimately. I spent time thinking about how a cellular signaling pathway would optimize the information flow (how I would optimize my learning process), and how it is limited by various constraints (how every optimization in my life is constrained by the finiteness of my being). I looked at the real shapes of the protein surfaces, and imagined them living in an immensely high-dimensional space but with some underlying lower-dimensional structure to be revealed (like the truths of life I would like to believe I’ll find). I thought hard about how the multi-scaled details of the protein surfaces should be viewed and understood in the most relevant way (and about what relevance means). I became familiar with the tradeoff between accuracy and simplicity, in summarizing the characteristics of the protein surfaces, as well as in finding the right level of approximation for the machine learning algorithm that had to infer the model parameters in real time. But above all, I learned to appreciate the importance of asking the right and relevant question, which is a fine art that was demonstrated by my wonderful mentors at Princeton, and one I am trying very hard to grasp. I have always been a person who asks questions, which is what brought me to this point. Now I hope to be asking better and better questions as I go on, both in research and in life, past and beyond my six amazing years at Princeton.


