Reaction-Diffusion kinetics of Protein DNA Interactions

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How wonderful that we have met with a paradox. Now we have some hope of making progress.

'Niels Bohr’
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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Other work not directly related to the thesis.

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Abbreviations

ODE       Ordinary Differential Equation
PDE       Partial Differential Equation
CME       Chemical Master Equation
RDME      Reaction Diffusion Master Equation
MD        Molecular Dynamics
SBML      Systems Biology Markup Language
DNA       Deoxy Ribonucleic Acid
RNA       Ribonucleic Acid
ATP       Adenosine Tri Phosphate
E.Coli    Escherichia Coli
xD        x Dimensions
LacI      Lactose Inhibitor
COM       Center Of Mass
Introduction

This is an exciting time to be involved in the development of the life sciences! With the intention of inducing the same excitement in yourself I would like to present a few examples (i) the ongoing progress made on tracking single molecules in living cells(1-3), the prospect of resolving reaction kinetics at atomistic detail in real time by irradiating a sample with high intensity x-rays(4) or with cryo electron tomography(5-7) and the ever-increasing availability of computing power allowing even average Joe to approach biological millisecond timescales in molecular dynamics simulations(8-10). While simulations, experiments and theory are potent disciplines in their own right, the real power comes from being able to combine them. This thesis aims to do just that in order to answer the overarching question of how DNA-binding proteins search, find and bind one specific target site among millions of non-specific sites(11-16). The quest for the answer brought to light the question of the definition or the nature of reaction rates; thus elucidating this nature is another major goal of the thesis. These findings came about partly by developing an in-house tool, MesoRD(17, 18), and by using other tools such as Gromacs(19) and homebrewn Monte-Carlo simulation schemes. Therefore I will first highlight the major theoretical themes/subjects underlying these tools in section 2 which is made up of three major sections, reaction kinetics(20-31), search kinetics(14, 32-34) and dynamics at the atomistic level of detail(10). In the reaction kinetics section I will describe the theory behind modeling chemical reactions at different levels of detail (ODE, PDE, CME, RDME). In these models, the molecules are viewed as structure-less point particles, and hard spheres in the RDME case, where the individuality of these particles and spheres are taken into account by endowing chemical reactions with the theory of stochastic processes. The atomistic level section will describe how to study the dynamics of molecules at a level of detail where the motion of each constituting atom is handled. At the end of the theory section there is a general description of search kinetics governed by diffusion and a more in-depth discussion about transcription factors searching for a specific binding site on DNA. I will justify why the properties of diffusion and the geometry of the molecules naturally lead to the need to distinguish between microscopic and macroscopic reaction rates. I highly recommend reading the theory section first before sinking your teeth into the results section. In the results section I
will show how to couple the different levels of detail to simulations and experimental data to gain quantitative insight into the interaction between the LacI dimer and DNA.
Theoretical Considerations

Reaction Kinetics

The time rate of change of the average number of molecules - ODE, PDE

Consider a vial into which two types of molecules are poured, some alcohol and a carboxylic acid which can react to form ester (fragrances, oils, pheromones) and water. The concentrations of the species in the vial will change until equilibrium is reached at which point the forward and backward rates are balanced. Wouldn’t it be nice to be able to figure out the time development of the concentrations of the species and to be able to predict the amount of each species at equilibrium given the amount of chemicals that we started with? I think so, and so did Guldberg and Wage who in 1864-1867 conjectured the law of mass action based on experiments on alcohol-acid reactions. This law was shown to be derivable from first thermodynamic principles as shown by van’t Hoff later in the beginning of the 20th century. In words, this law states that the time-rate of change of the average concentration of a product is proportional to a phenomenological reaction rate constant and the average reactant concentrations raised to the power of their stoichiometric coefficients. This results in a system of ODEs (Ordinary Differential Equations) which relates the average concentrations of the reactants in the vial and which more often than not need to be solved numerically rather than analytically. In biology one employs this law to translate a graphical representation of a biochemical network to the corresponding ODEs in the hope of validating or discovering something new about the inner workings of the system of interest. The need to somehow standardize the translation of the graphical representation to the system of equations arose as more and more people acquired a taste for quantitative biology, and thus the System Biology Markup Language (SBML) was devised (35). SBML is a xml schema where species definitions, compartments, reaction rates etc. are represented by xml tags. The first step in gaining quantitative information of the biomolecular
system of interest is thus to transform it to the corresponding SBML representation and then use one of the many software solutions available to parse the SBML file and usually output the time development of the average concentration of all species (18, 36-39). One of these implementations lying close to heart is MesoRD (18), developed to be able to generate stochastic trajectories corresponding to the RDME or the CME descriptions discussed below and which can also numerically solve systems of ODEs.

The species making up biological systems are often very few in number and the chemical reactions between them are random due to the encounter between reactants being probabilistic with statistically independent positions and velocities (Boltzmanns Stosszahlansatz) (40). A consequence of this randomness is that the magnitude of the copy number fluctuations relative to the average is inversely proportional to the square root of the copy number (41). Thus, these fluctuations become substantial for low copy numbers which is a common situation in the interior of the living cell. A model which takes into account the discreetness of molecules seems therefore necessary to capture the dynamics in living cells. The ODE model on the other hand is appropriate to use when there are a large number of well stirred molecules close to equilibrium and since we have not included any spatial dependence they should be homogenously distributed. This means that an ODE model may not accurately describe a situation where molecules react immediately upon contact with each other (the diffusion limit) because this introduce depletion zones around the molecules which usually calls for an inclusion of space (42). However the ODE model works well in the other limit where the molecules have to overcome an energy barrier at contact to be able to react which is called the reaction limit. The space independent ODE description demands inclusion of space if the underlying homogeneity assumption breaks down due to fast reactions relative to the diffusion rate of the molecules. Operationally, what one does is add the diffusion operator \( (D \Delta) \) acting on the concentration of all the species which changes the time rate of change of the local concentration of molecules due to fluxes in space in addition to the time rate of change due to chemical reactions. A more rigorous derivation can be found by starting at the mesoscopic CME level of description where the addition of the diffusion operator is justified by noting that the moments are changing in time due to both reactions and diffusion (41).

The time rate of change of state probabilities – CME, RDME

The discreetness of molecules are taken into account by applying the theory of stochastic processes to the change in the number of molecules of each species, here exemplified with one species only and extended to multiple species in Eq. 6 by vectorizing an index, as time goes on due to chemical reactions. We also consider the case where the future change in the number of
molecules $X(t)$ only depends on the current state and not on how many molecules there was for $s<t$. This is appropriately called the memoryless property of the process which makes it a time-continuous Markov process with discrete states taking on integer values (the discreteness of molecules)\(^{(43)}\).

One can deduce the dwell time distribution based on the fact that jumps between states are memoryless which means that the probabilistic process of drawing dwell times from a distribution needs to restart itself. The only continuous distribution with this memoryless property is the exponential distribution. Thus, the distribution function for the dwell times in state $i$ is given by

$$F(t_i) = 1 - e^{-\nu_i t_i}$$ \hspace{1cm} (1)

where $\nu_i$ is the rate at which state $i$ is left. With this distribution, the mean time spent in state $i$ becomes.

$$<t_i> = \frac{1}{\nu_i}$$ \hspace{1cm} (2)

According to a Lemma found in\(^{(44)}\) we can also write this rate as

$$L1: \lim_{t \to 0} \frac{1 - P_{ii}(t)}{t} = \nu_i$$ \hspace{1cm} (3)

which is the probability of having left state $i$ per unit time. The transition rate from state $i$ to state $j$ is then given by

$$q_{ij} = \nu_i P_{ij}$$ \hspace{1cm} (4)

where $P_{ij}$ is the probability of transitioning from state $i$ to state $j$. According to another lemma for which the derivation may be found in \(^{(44)}\), $q_{ij}$ can also be written as the transition probability from state $i$ to $j$ per unit time:

$$L2: \lim_{t \to 0} \frac{P_{ij}(t)}{t} = q_{ij}$$ \hspace{1cm} (5)

These transition probabilities correspond to the reaction rates in graphical illustrations of biochemical networks. Based then on the Chapman-Kolmogorov equation\(^{(41)}\)(the central equation for mesoscopic kinetics) and the two lemmas (Eqs 4-5) one can derive the chemical master equation (CME) by going in the continuous time limit.
The CME is a statement of conservation of probability fluxes, i.e. the time rate of change of the probability of having \( j \) molecules is due to a positive contribution from all reactions leading to jumps from all \( i \) to \( j \) and a negative contribution due to jumps from \( j \) to all \( i \). The CME as written in Eq. 6 is easily extended to include multiple species by making index \( j \) a vector of indices and the order of the reactions will determine the dependence of the reaction rates, \( q_{ij} \), on the stochiometries. An exact solution of the CME is, but for the simplest biochemical networks, intractable’ and is usually solved using a Monte Carlo method, the Gillespie algorithm, where trajectories are generated which satisfies the properties of the CME. These properties correspond to determining the time for the next reaction event and which of all the possible reaction events that take place at this time. The time is sampled from the exponential distribution (Eq. 1) using the inverse distribution method and the reaction event is chosen based on the fact that the probability of a reaction is equal to the fractional rate of that reaction.

The idea of conservation of probability fluxes (Eq 6) can be applied to model physical phenomena in general. Usually this is done by setting up conservation laws involving currents and conserved quantities and constitutive laws, such as Ficks law, which relate the currents to the conserved quantities. By this approach one can derive the diffusion equation, which have been central in my work, like so

\[
\begin{align*}
\frac{\partial p(\vec{r},t)}{\partial t} + \nabla \cdot \vec{J} &= 0 \\
\vec{J} &= -D \nabla p(\vec{r},t)
\end{align*}
\]

(7)

where \( p \) is the probability of a particle being at position \( \vec{r} \) at time \( t \) and \( J \) is the so called current related to \( p \) by Ficks first law and \( D \) is the diffusion constant. The conservation in probability in this case is not due to reactions as in Eq 6. but due to molecules diffusing in and out across a boundary. By inserting the expression for \( J \) in the top equation we get the diffusion equation (see search kinetic section for a discussion on diffusion).

\[
\frac{\partial p(\vec{r},t)}{\partial t} = D \nabla^2 p(\vec{r},t)
\]

(8)
This is where the diffusion operator which was added to the ODE description above comes from. What about adding spatial fluxes at the CME level of description which assume that molecules diffuse very fast compared to the fastest reaction times so that they are homogenized throughout the reaction volume? The extension is made by discretizing the reaction volume at the CME level into yet smaller subvolumes, and assuming that the conditions for the CME description are satisfied in each subvolume, we get the Reaction Diffusion Master Equation (RDME). In its’ most general form the RDME can be written as

\[
\frac{dP}{dt}(a,b,c,t) = (L_h P + R_h P)(a,b,c,t)
\]

where the notation has been adopted from(45) for a bimolecular reversible reaction involving species a, b and c. The species in the equation above are vectors giving the amount of each of them in each subvolume. The diffusion and reaction operators are respectively \(L_h\) and \(R_h\) where \(R_h\) corresponds to the CME in each subvolume and \(L_h\) contains the sum over the nearest neighbors of the subvolume of interest (SI) with a positive contribution to the time rate of change due to molecules flowing into the SI and a negative contribution due to molecules flowing out from the SI as in Eq 6. Trajectories are readily generated in MesoRD, satisfying the properties of the RDME using a very efficient (O(Nlog(N)) with N being the number of subvolumes) method called the Next Subvolume Method(46). This method is this efficient because it only updates the rates for those species which have changed either due to diffusion or reaction and then updates the queue for the reaction events in a computationally efficient manner.

It is evident by now that in describing how a biochemical network works including diffusion and reaction events in the living cell one often need to take the discreeteness of the molecules into account by using, for example, the RDME model. This model have proved inconsistent in the limit of very small subvolumes where it is expected to converge to the microscopic description(47). It was recently resolved by beautiful work by Fange and coworkers(48) (and others at the more theoretical level(45, 49, 50)) where they introduced a scaling of the reaction rates depending on the size of the subvolumes. It was shown that the corrections are needed for all subvolume sizes for 2D systems. Thus, the implementation of these corrections made the 2D reaction kinetic results presented in this thesis possible. These RDME simulations of reactions on a 2D plane do have a connection to protein-DNA interactions as we will find out in the Results section of this thesis.
Search Kinetics

Background

The prototypical example of gene expression control is the repression of the lac-operon in E. coli by the protein LacI(51). The LacI protein binds to an operator region upstream of the lac operon in the absence of lactose and sterically obstructs RNA polymerase from binding the promoter region. The rate with which LacI finds and binds the operator region need to be fast to keep the cost of unnecessary expression down in the absence of lactose while rapid dissociation in the presence of lactose (allo lactose) determines the fitness of the bacteria. The binding kinetics of LacI is therefore of some interest for understanding the biology of the specific system, but more importantly the lac system has become the well established bacterial model system for gene regulation and for this reason it is appropriate for studies of binding kinetics. An experimental measurement of LacI binding to its operator site on DNA in vitro was made in the early 70s using filter-binding techniques(52) which when compared to theoretical estimates of the specific association rate constant resulted in surprising results. According to the experiment, the association rate of one LacI tetramer to one operator site per genome equivalent was estimated to be $\sim 10^{10}$ M$^{-1}$s$^{-1}$. A theoretical upper limit of the association rate can be determined using the Smoluchowski formula(47). The formula gives the rate with which two molecules react when diffusing and colliding with each other and where the reaction takes place immediately upon contact (the diffusion limit). According to the Smoluchowski formula one gets $k_a \sim 10^8$ M$^{-1}$s$^{-1}$, i.e. two orders of magnitude smaller than suggested by the filter binding assay! Although Riggs remarked on the theoretical possibility of LacI being able to slide along the DNA he quickly dismissed it as a possibility(52). Two years after that, Richter and Eigen revisited the remark and concluded based on quantitative modeling that the necessary two order of magnitude speedup could not be accounted for solely due to electrostatic attraction and sliding is necessary(135). However, they neglected the proper coupling between 3D and 1D diffusion and the time spent on nonspecific DNA before binding the specific site. Berg and coworkers recognized this and developed an extensive model on search kinetics in a series of papers for straight DNA chains(53) for coiled DNA(54) and with the inclusion of ions(55). This model has served as the foundation upon which much of my work is built. It has been expanded by simulations to investigate more realistic and complex situations where the equations becomes intractable(56). The need for complicating things may seem excessive; after all the search kinetic model have adequately explained in vitro results by predicting both the association rate dependence on the nonspecific
binding strength and the salt concentration\(^{(57)}\). However, the development of new experimental techniques lets us follow single molecules such as LacI in living complex cells. To be able to extract quantitative information from this experimental data one needs to apply a model which demands inclusion of e.g. the existence of a congested interior of the cell\(^{(58-73)}\) and reactive patches on the reacting molecules\(^{(74-83)}\).

Thermal fluctuations driving motion

My view of search kinetics, which has emerged as a result of research, is described below. The way the picture is painted is most easily conveyed by considering the physics that drives the search, i.e. diffusion. Thermal diffusion is the movement of molecules due to the numerous (~\(10^{20}\) s\(^{-1}\)) collisions with other molecules. There is a probability distribution associated with being collided with from any directions per unit time and although the distribution is uniform, the probability is nonzero of transiently being hit by say ~\(10^8\) molecules more in one direction than another. The displacement of the molecule will appear erratic when this occurs, as first witnessed by Robert Brown in 1827. A particle whose motion is driven by diffusion will after having moved a distance R from where it started at time zero have explored a volume \(4\pi R^3/3\) on average and it will have returned to its starting point many times. These properties makes diffusion take on a volume-filling character\(^{(42)}\) on average which is evident in that the sphere-sphere association rate is proportional to the linear extension of the axially symmetric reactive patch on one of the sphere and not to the area of the patch\(^{(75)}\). Based then on these properties of diffusion and the geometry of DNA being a linear chain of base pairs we can identify different dissociation and association regimes.

Microscopic dissociation/binding – Hopping

From the point of view that LacI is nonspecifically bound to DNA, one can imagine that LacI breaks its bonds to DNA and diffuses away a distance where it might be free to move along the length of the DNA chain and if it rebinds does so to the same or a nearby base pair. This mode of transfer is referred to as hopping\(^{(12, 84-92)}\). The event of LacI diffusing to the distance were hopping ensues will be referred to as a microscopic dissociation and the rebinding as a microscopic association. These events take place with rates \(\lambda\) (s\(^{-1}\)) and \(k\) (\(\mu\text{m}^3\text{s}^{-1}\)), respectively. The existence of hopping is due to the properties of diffusion discussed above and the geometry of DNA, so we need to take it into account and by doing so we are forced to properly define how far out LacI
have to diffuse before hopping occur. This definition is made by recognizing that the nonspecific association is entropic in nature(57) i.e. the magnitude of the equilibrium constant is proportional to the entropy gained by ions and water molecules when released from DNA when LacI binds. This will be exploited in the molecular dynamics simulations to figure out the microscopic dissociation distance by the LacI dimer.

**Macroscopic dissociation/binding – The experimental observable**

After a microscopic dissociation there is a possibility for LacI to leave the DNA segment altogether without rebinding. The demarcation point for when this occurs is a distance $R_c$ where the binding of LacI to any other DNA strand is equally probable. This is denoted as a macroscopic dissociation and the rate with which it occurs, $k_d$, is given by $\lambda$ (microscopic dissociation rate) times the probability of reaching $R_c$. This probability can be derived by solving the diffusion equation in cylindrical coordinates(14). Mathematically, the diffusion of LacI out to a certain distance will take on a 2D character, which makes the probability of reaching $R_c$ dependent on $R_c$ for all $R_c$. This distance dependence is manifested in the simulations of the Michaelis-Menten kinetics on a 2D plane (see Results section), which calls for a correction of the rates at all sub-volume length-scales in the simulations with MesoRD(93). The macroscopic association rate, $k_a$, is likewise given by the microscopic association rate $k$ times the probability of reaching $R_c$. This makes sense when compared to the macroscopic dissociation rate because the dynamical equations governing the search kinetics are time symmetric, a symmetry which implies microscopic reversibility(94). Applied to our system this means that $k/\lambda = k_a/k_d = K_{RD}$ which is satisfied throughout the simulations.

**Sliding – Bridging the gap**

Equipped with an understanding of the distinction between microscopic and macroscopic dissociations/rebindings and the transfer modes of hopping and macroscopic binding, we move on to describe two modes of transfer for which LacI is associated with DNA. First up is sliding whereby LacI diffuses along the length of the DNA and while doing so, tracks the phosphate backbone(14, 95-105). This means that LacI experience additional rotational friction with water molecules in relation to free diffusion, which makes the 1D diffusion coefficient $D_1$ about two orders of magnitude smaller than $D_3$, the 3D diffusion coefficient. There are also spatially localized charges on the DNA due to the negatively charged phosphate groups which serve as electrostatic “bumps in the road” and further hinders the 1D diffusion of LacI(106). There could also be an important contribution to the dynamics of LacI sliding due to the DNA
segment diffusion at these length and time scales(14, 107). This diffusion of DNA segments is partly neglected in our MD simulations due to the periodic boundary conditions employed. Water molecules and the accompanying hydrodynamic couplings are further ignored in the non-MD models which could have an impact on the result(58). The sliding of different proteins have been observed in vitro (J) but the question remained whether sliding is actually a mode of transfer in vivo where we have to consider crowding(108), DNA condensation(73) and physiological salt concentrations. Hammar et al. recently showed that the LacI dimer attached to the fluorescent protein Venus do slide in living E. coli cells(2). The rationale behind the experiments is that if sliding exists then it should bridge two operator sites when the sites are at a distance from each other less than the sliding length. Without sliding, the operator sites would be perceived as independent and the ratio between the rates of finding two sites relative to finding one site is 2 while the bridging due to sliding makes this ratio < 2. It was found that the ratio as a function of distance between operator sites showed a (1+tanh(L/2s)) dependence, with L being the distance between operator sites and s the sliding length. A fit of a function of this form with explicit dependence on the sliding length made it possible to extract a sliding length from the data of ~32bp. Another interesting finding is that the association rate of LacI to the operator site O{\text{sym}} is different from the association rate of LacI to another operator site O{\text{1}}, which adds a twist to the model in (14). This difference in association rate can be explained by introducing different specific binding probabilities(2).

Intersegment transfer – Making a sandwich

This is another mode of transfer where the LacI tetramer remains nonspecifically bound to DNA. The idea is that LacI can transiently bind to two different DNA segments at the same time and move from one segment to the other(14, 56, 109-113). A requirement for this to be a workable search mode is that the doubly bound complex should be unstable, otherwise intersegment transfer will not contribute, especially considering that the rate limiting step for this transfer mode is for two DNA segments to find each other. The physical effects of intersegment transfer are essentially equivalent to a macroscopic dissociation in that the relocation to a new position on DNA is uncorrelated with the old one not counting the negative transfer correlation to nearby sites. To my knowledge, there have never been an experimental observation of this transfer mode for the LacI tetramer in vivo nor for the LacI dimer in vitro although recent theoretical work suggests that it may play a role in facilitating the transfer of LacI to the operator site O1 from auxiliary operator sites O3 and O2(114).
Dynamics at the atomistic level of detail

The concept of Molecular Dynamics

The ODE, PDE, CME and RDME descriptions allow deducing the outcome from the numerous collision and reaction events between molecules represented as structureless point-particles ODE, PDE and CME descriptions and hard spheres in the RDME description. Wouldn’t it be cool to be able to magnify (~200000x) for example the RNAP interacting with the bound LacI; what would you see?

As philosophized by the ancient Greeks and Indians, suggested by Daltons law of multiple proportions, implicitly witnessed by Brown, calculated by Einstein(115) and experimentally proven by Perrin(116), matter is made of discrete units called atoms. The atom is composed of a positively charged core the nucleus which is surrounded by a diffuse cloud of negative charges, the electrons (the subdivision goes on but this will do for our purposes), with the nucleus being at least 1800 times more massive than the electrons. The electrons interact constructively with each other to form bonds between atoms thus making a molecule. Considering the dynamics of these subatomic particles we invoke the Born-Oppenheimer approximation stating that the dynamics of the electrons are instantaneously adjusted to the motion of the nucleus (due to separation in mass) (117). Therefore, ignoring any relativistic or quantum effects, we consider the molecule as a mechanical system of soft balls with springs holding them together where the trajectories of the atoms can be determined by the principle of least action from which the equations of motion can be derived. Dividing the N-body problem into pairwise interactions the force, \( F_i \) experienced by atom \( i \) due to the presence of other atoms \( j \) is

\[
m_i \ddot{a}_i = F_i = -\nabla_i U_{\text{pot}}
\]

where \( U_{\text{pot}} \) is the potential energy surface set up by the other atoms \( j \) and is given as a sum of bonding contributions (bonds, angles, dihedrals, improper dihedrals) and nonbonding contributions (vdw interactions and Coloumb electrostatic interactions):
Here $k_\alpha$ are force constants, $n$ is a periodicity, $\delta$ is a phase shift, $q_i$ is the partial charge on atom $i$, $\varepsilon$ is the permittivity, $r_{ij}$ is the distance between atoms $i$ and $j$, $\varepsilon_D$ is the well depth and $\sigma_{ij}$ is the distance for which the inter-particle potential is zero. The collection of constants in the potential for different atom types and the form of the potential is what is referred to as a force field and this force field in combination with a method for integrating the equations of motion for each atom in the system is the concept of Molecular Dynamics\((118)\). Usually there is a trade-off between the form of the potential, how amenable the potential is for evaluation on a computer and how transferable the force-field is to other molecules. Most of the terms in the potential are straightforward except maybe the $vdw$ expression which warrants a bit of discussion. The attractive 6-term is indicative of a transient dipole-dipole interaction and models the electronic redistribution in atoms as the molecules approach one another and is therefore, for example, proportional to the size of the atoms. This makes chlorine a gas at room-temperature whereas bromine is a liquid. The 12-term models repulsion between atoms which is a consequence of the Pauli exclusion principle\((119)\). This 12-term is an example of a tradeoff as it is particularly amenable to computation while a more accurate expression would include an exponential as suggested by the radial part of the solution to the Schrödinger equation for the hydrogenic atom. A major consequence of the 12-term and the associated steep rise in potential when atoms come close together is to make the trajectories (the coordinates of the atoms as a function of time) chaotic. This means that Bob and Alice do not get the same trajectory if they execute a MD simulation run on the same computer, for the same system, at the same time. Infinitesimal perturbations in the initial conditions due to truncation errors in the Leap Frog integrator method we use in our work\((120)\) and roundoff errors makes deviations grow at an exponential rate which makes the accuracy of an otherwise deterministic trajectory, after a couple of timesteps, not so accurate. There is no need to panic however as the macroscopic observables do not depend on the accuracy of one trajectory but correspond to ensemble averages which is to say averages over the different ways that an observation can be realized at the microscopic level. But MD takes the coordinates and updates them every femtosecond, does then each such updated configuration necessarily correspond to the same observable? The answer is no. However, assuming ergodicity, if any point in phase space
(the space that contains all that the system can be in terms of its state which for a mechanical system is fully specified by listing all the coordinates and momenta for all atoms) can be reached by any other point, then the ensemble average equals the time average. This means that if you simulate long enough, the average of the property of interest over time will correspond to the macroscopic observable. This poses a problem for those points in phase space which have a higher energy than a few kT and correspond to so called rare transitions(121, 122). To find a way of sampling this rare transition was particularly important for me because the few 100ns that molecular dynamics can achieve is not enough to sample the microscopic dissociation and sliding of LacI while bound to a nonspecific stretch of DNA. One method to do this is umbrella sampling(123) which is briefly described in the next section.

**Umbrella Sampling**

Given states A, B and an order parameter (the reaction coordinate $\xi$) which when varied advances the system from A towards the end state B, one can pick out representative configurations along the reaction coordinate (these are called windows) and restrain these configurations by the application of a harmonic potential $w$ on each atom. This corresponds to locally flattening the free energy profile, which ensures an adequate sampling of configurations high in free energy. Operationally, one equates the probability of observing the system at the reaction coordinate, $P^b_i(\xi)$, with the fraction of time the system has been in the configuration corresponding to the coordinate for each window $i$ (the ergodicity assumption at work). In each window there is an associated free energy, $A^i(\xi)$, which can be calculated from the following equation which takes into account that the sampling have been collected in a biased potential $w_i$:

$$A^i(\xi) = -(1/\beta) \ln(P^b_i(\xi)) - w_i(\xi) + F_i$$  \hspace{1cm} (12)

The $F_i$ connects the free energy curves in different windows and is an inevitable consequence of introducing the harmonic potential $w_i(\xi)$. One can estimate the $F_i$ by stating that the global unbiased probability distribution for $\xi$, $P^u(\xi)$ is a weighted average of the unbiased probability distribution for $\xi$ in each window $i$, $P^u_i(\xi)$. The relation between the sampled $P^b_i(\xi)$ and $P^u_i(\xi)$ is given by

$$P^u_i(\xi) = P^b_i(\xi) e^{\beta w_i(\xi)} < e^{-\beta w_i(\xi)} >$$  \hspace{1cm} (13)

where the average $<...>$ is over configuration space. Lastly, the weights $p_i(\xi)$ are chosen to minimize the statistical error of $P^u(\xi)$,
This is the rationale behind the method of WHAM(124) used to get the unbiased global free energy as a function of a reaction coordinate, which I have employed in my work. A nice review on methods for sampling rare events, the difference between the methods having to do mostly with whether configurations along the reaction coordinate are restrained (umbrella sampling), constrained (thermodynamic integration), slowly moved (slow growth) or rapidly moved (fast growth) etc. can be found here(123). A slowly moving restraint, commonly called steered molecular dynamics, have also been employed in our work to figure out how a protein slides along a DNA molecule (96).
Results

Is there something that connects the concepts of microscopic rate constants, macroscopic rate constants, Brownian motion, molecular dynamics, umbrella sampling, reactions in 3D vs 2D? This is the question we seek to answer in this section by means of our results. This section is a tad more technical and right to the point than the introduction so if there are any difficulties understanding it I suggest reading the introductory sections thoroughly. Let us begin.

Nonspecific Dissociation

A characteristic trait of dissociating species is that they need to separate by pure diffusion in a potential field, set up by the reacting and other species, to a distance, $\rho$, where all correlations are lost between them before being counted as dissociated. We use the idea of extracting the mean residence time a molecule spends within correlation distance of another molecule based on diffusion in a potential,

$$\frac{\partial p(r,t)}{\partial t} = D_3 \frac{\partial^2 p(r,t)}{\partial r^2} + D_3 \frac{\partial p(r,t)}{\partial r} \frac{dG_b(r)}{dr} + D_3 p(r,t) \frac{d^2G_b(r)}{dr^2}$$

(15)

with $p(r,t)$ being the probability of finding the molecule at distance $r$ at time $t$ from the other molecule, $D_3$ is the 3D diffusion coefficient and $G_b$ is the free energy where the subscript b signifies that the molecules are in a bound state i.e. a state where the molecules are correlated with each other through non-bonding interactions. Integrating the time-integrated form ($p$ with tilde in eq 16) of this expression with respect to space we get the mean residence time $\tau_d$, the inverse of which is the microscopic dissociation rate constant $k_d^{\text{micro}}$, as a function of the free energy
where $r_0$ is the minimum in the free energy profile as a function of radial distance and the integrand is the time integrated form of the diffusion equation above.

**Example: The LacI Dimer**

By using molecular dynamics and the method of umbrella sampling we get the free energy profile shown in figure 1.

![Figure 1](image)

**Fig 1.** The figure illustrated the breaking of the hydrogen bonds in D and the free energy profile as a function of the radial distance between the COM of the binding domains of LacI and the DNA axis in E. The dashed line correspond to a theoretical estimate of the free energy for an unbound LacI dimer.

This figure shows the breaking of the hydrogen bonds in the top panel as the LacI dimer dissociates from a nonspecific stretch of DNA and the free energy profile as a function of radial distance between the COM of the head domains and the center axis of DNA in the bottom panel. Here, $r_0$ is around 1.6nm and the microscopic dissociation distance $\rho$ is in the vicinity of 2.8nm. This distance is fraught with uncertainty as the number of configurations increase with the distance between the molecules which demands more sampling and more time to adequately estimate the entropic contribution to the free energy. To increase the certainty we also incorporate other pieces of information which are the distance between the molecules at which water recondense on DNA (PNAS suppl figure 4), the root mean square deviation...
(RMSD) of the binding interfaces of LacI (suppl figure 4) and an estimate of what the free energy should be at equilibrium by equating the chemical potentials of the bound and the free states (the formula in bottom panel). These pieces of information in combination with reports of binding free energies in the range $10-15k_BT(125)$ for nonspecific transcription factor – DNA interactions suggest that we are in the right ballpark. Also, the fact that there are no bumps in the free energy in going from $r_0$ to $\rho$ imply that there are no conformational changes that need to occur before dissociation which lend support to the ‘diffusion in a potential’ approach. With the integrand in equation 16 being a smooth function we numerically integrate it using a simple trapezoidal method and get a residence time $\tau_D=69\mu$s which correspond to a microscopic dissociation rate constant $k_d^{\text{micro}}=1.45\times10^4\text{s}^{-1}$.

**Macroscopic dissociation**

The microscopic rate for breaking the hydrogen bonds is so far not directly observable and the experimentalist see an average number of molecules in time and space where the microscopic rates are averaged out. There is however a connection between the observable fluxes and the microscopic rates. That connection can be made through a boundary condition to the diffusion equation (126).

$$\frac{dP_b(t)}{dt} = k_a^{\text{micro}}p(\rho, t) - k_d^{\text{micro}}P_b(t)$$

(17)

where $P_b(t)$ is the probability of the molecules being bound and $k_a^{\text{micro}}$ is the microscopic association rate constant. This equation is an instantiation of the conservation law discussed in the introductory sections. The rate of change of the conserved quantity $P_b(t)$ have a positive contribution due to binding reactions with free molecules at the reaction radius $\rho$ with a rate $k_a^{\text{micro}}$ and negative contribution due to dissociation reactions with rate $k_d^{\text{micro}}$. The boundary condition in eq. 17 together with the diffusion equation describes the diffusion and reaction of a fully reactive disk/sphere to another in continuous space and time. By discretizing the boundary condition in space one gets a master equation representation of the reaction-diffusion process which becomes amenable to Monte Carlo simulations(48). Thus we let the DNA correspond to a disk fixed at origin and the protein to be another disk which is allowed to jump between concentric circles and react within the innermost circle containing the DNA. Furthermore, we introduce Brownian rotational realizations(127) of the protein relative to the DNA and relative to its own
center. This scheme allows for determining the average number of microscopic dissociations per macroscopic dissociation by introducing an absorbing boundary at a distance $R_c$ determined by the density of DNA segments used in the experiments to which the simulations will be anchored. The macroscopic observable rate is simply the average microscopic residence time, $\tau_D$, times the average number of microscopic dissociations per macroscopic dissociation which is equivalent to

$$k_{d \text{macro}} = k_{d \text{micro}} P_{\text{diss}}$$  \hspace{1cm} (18)$$

where $P_{\text{diss}}$ is the probability of a macroscopic dissociation. Microscopic reversibility ensures us that this equation is also satisfied by the association rate constant.

Example: The LacI Dimer

Letting the size of the active patch on the protein range between $\pi/10$ and $\pi/20$ radians and the size of the patch on the DNA range between $\pi/3$ and $\pi/2$ radians one gets $700 \pm 180$ rebindings, resulting in an average macroscopic residence time of $48 \pm 12$ ms. Thus, we have a numerical value for the macroscopic dissociation rate, but is it reasonable? The best we can do in answering this question is to find consistency in independent pieces of information. One of these pieces can be experiments that as closely as possible represent the molecular dynamics setup such as an in vitro experiment reporting on the value of the sliding length ($128$). However the sliding length is defined as

$$s = \sqrt{\frac{D_1}{k_{d \text{macro}}}}$$  \hspace{1cm} (19)$$

which depends on the 1D diffusion coefficient as well as the macroscopic dissociation rate so we need information on the diffusion coefficient as well.

The 1D diffusion along DNA

The relation between the diffusion coefficient and the friction coefficient ($f$) is given by the Einstein-Smoluchowski formula
\[ D_i = \frac{k_b T}{f} \]  

where \( k_b \) is the Boltzmann constant and \( T \) is the temperature. This equation is a statement about the common origin of the friction experienced by particles in solution and the jittering (a negligible velocity autocorrelation time) of that same particle. This origin is the numerous collisions per unit time with water molecules experienced by the particle. Increase the temperature and thus the jittering frequency will make the particle search a larger volume per unit time and the diffusion constant is higher. But raising the collision frequency with water molecules also increase the rate with which the momentum of the particle is dampened out which means an increase in the friction \( f \) which lowers the diffusion coefficient. The winner of these competing effects depends on the geometry of the particle and the specific properties of the solvent they are in. Using this relation means knowing how the protein moves on DNA as it slides to accurately determine the viscous drag and consequently the friction \( f \).

Example: The LacI Dimer

While it has been implicitly shown that LacI tracks the phosphate backbone in a recent in vitro experiment we wanted to see and prove it at the atomistic level. So the idea is to pull LacI very slowly along the DNA in both directions (the different colors in Figure 2) and see whether it is prone to track the phosphate backbone (figure 2).
Fig 2. (A) The figure shows the LacI dimer (gray), the DNA molecule (orange) and the trajectory explored by the COM of the binding domains of LacI. (B) The COM of the binding domain of LacI as a force is applied in the axial direction. The colors correspond to applying the force in both directions and the black line is the ideal helical trajectory.

Sure enough, all pulling simulations fall in the vicinity of the black line in the inset figure corresponding to a helical trajectory. The blue semi-transparent blob-thing shows the trajectory taken by the center of mass of the COM of the head domains. This means we can use the correct expression for f and calculate $D_1$ and the sliding length $s$, almost. As discussed in the introductory section, the variance in the potential, $\varepsilon$, due to the localization of charges on the phosphate groups on DNA will retard the diffusion and need to be taken into account. Estimate the variance in the potential using umbrella sampling and the result of Zwanzig[106],

$$D_1 = \frac{k_b T}{f} e^{-\varepsilon^2} \quad (21)$$

where we calculate $D_1$ in the range 0.05-0.29 \(\mu\text{m}^2\text{s}^{-1}\) (~ 2-40\(\mu\text{s}\) to slide 1bp) which gives a sliding length in the range 135-345bp and is comparable to what is found experimentally (150bp) in vitro under that same salt conditions
as the MD simulation (128). By comparison, the microscopic residence time is 69μs which makes the existence of sliding as a transfer mode for the LacI dimer highly probable.

Finding The Specific Operator Site

How does this knowledge of the characteristics of LacI-DNA nonspecific interactions at the atomistic level of detail factor into the time it takes for LacI to search, find and bind the specific operator site? In particular, is sliding also present in the interior of the congested living cell? By shifting focus to the question of specific site searching we greatly expand the timescales that need to be taken into account which means that molecular dynamics is no longer a feasible method of investigation. Again, we are going to use the rationale of anchoring a simulation to a recent single molecule in vivo experiment. The simulation approach will be a realization of a continuous-time discrete state markov jump process by the searching protein on a 1D lattice occupied by other proteins on DNA that prevents sliding protein to bypass them (so called roadblocks). Although the method appear simplistic it is fully adequate in relation to the questions posed. This jump process will concurrently take into account the different modes of transfer by LacI discussed in the introductory sections, i.e. sliding, hopping, intersegment transfer and macroscopic dissociations. The in vivo experiment also suggest the possibility of LacI traversing the specific binding site which is contained in a parameter $p_{\text{bind}}$ in the simulations. The experiment suggest that the total search time for one LacI dimer coupled to Venus is between 236s and 416s and the extent to which two operator sites are correlated at distances $[25, 45, 65, 113, 203]$ bp. This information together with the parameter set defining the transfer modes and binding probability allows measuring the degeneracy of the parameter set which is a measure of how many parameter combinations satisfy the absolute search time and the sliding length constraints. The simulation of the markov jump process requires knowledge only about the probabilities of the different events which are equal to the fractional rate of the different modes of transfer. That means having an idea of how the microscopic dissociation rate constant and the probability of a macroscopic dissociation given a microscopic dissociation behaves as a function of fundamental input parameters listed in table 1.
Using the PDE model by Berg (129) which is extended to include other DNA binding proteins and specific binding probabilities (56) we find this behavior for the microscopic dissociation rate constant \( \lambda \) (60),

\[
\lambda = 2\pi D_3 c \alpha M_{\text{tot}} v e^{1-c} \frac{1-F_B}{F_B}
\]  

(22)

the probability of dissociation \( P_{\text{diss}} \) (14),

\[
P_{\text{diss}} = \frac{1}{1 + \alpha \ln\left(\frac{R_c}{\rho}\right)}
\]  

(23)

and even the total search time, \( \tau \), until one specific operator site is found (56).

\[
\tau = \frac{M_{\text{tot}}}{2F_B} \left[ 1 + \frac{1}{D_k k_{d\text{macro}}} \sqrt{\frac{\pi}{k_{d\text{macro}}}} \left( 1 + \frac{2\sqrt{D_k k_{d\text{macro}}}}{k_{sp}} \right) \right]
\]  

(24)

There is an independence assumption involved in deriving the \( \tau \) equation 24. This assumes independence in the effects of crowding, \( \nu \), and the probability of specific binding, \( p_{\text{bind}} \). Also, the effect of hopping is not included in the equation. The assumption and the negligence of hopping was seen to be valid in the parameter regions investigated by the simulations, thus we use this equation for the absolute search time to generate the blue regions in the parameter space figure 3. Additionally, there is no equation for the search time to one of two operator sites at different distances from each other in the presence of roadblocks. This situation is best studied with simulations and correspond to the level curves in figure 3.
Example: The LacI Dimer

The degeneracy of states is summarized in figure 3. In the figure, the blue region correspond to the total search time to one operator site as given by equation 24 constrained to lie within the experimentally measured interval. The level curves (yellow to black) are the goodness of fit, as measured by the $\chi^2$ test, to the degree with which the search time depends on the distance between two operator sites in the in vivo experiments and the simulations. As we demand consistency with respect to both experimental results, the acceptable parameter space correspond to the overlap between the blue region and the level curves.

Fig 3. The absolute search times for the LacI dimer satisfying the in vivo estimated search time 236-416s shown as cyan. The level curves corresponding to chi-squared values less than 3 measuring the goodness of fit of simulations to experiment for one of the two operator sites at distances 24, 45, 65, 115 and 203bp. The values are functions of the 1D diffusion coefficient $D_1$ and the degree of diffusion control $\alpha$ given the vacancy $v$, the probability of specific binding $p_{\text{bind}}$ and the fraction of time the LacI dimer stays nonspecifically bound, $F_B=0.9$.

The 4 different plots correspond to different combinations of the vacancy, $v$, and the probability of specific binding at the specific site where the vacancy is the fraction of the DNA that is unoccupied by DNA-binding proteins with different experiments arriving at different values($60, 130$). On the y-axis
there is the 1D coefficient in units μm²s⁻¹ and on the x-axis there is the degree of diffusion control α which is proportional to the ratio of the association rate to the 3 dimensional diffusion coefficient at contact between the LacI dimer and the DNA molecule. A very high α means that the protein will be absorbed immediately upon contact while a very low α correspond to a low probability of association upon contact. Based on the plots we can discuss the acceptable values of D₁ and α in an E.coli cell. Starting with D₁, an estimate of D₁ in vivo based on viscosity effects alone have been made by Tabaka where he gets ~0.025μm²s⁻¹ (131). Including Zwanzigs retardation factor with ε estimated from the MD simulations D₁ becomes even lower, around ~0.01μm²s⁻¹. So the bottom cases in the parameter space figure 3 where the occupancy on DNA is 30% by obstructing proteins is clearly not acceptable since the overlap between blue and yellow regions are for much higher D₁. In terms of the plots corresponding to a 15% occupancy one is inclined towards placing more belief in the plot with pbind=1.0 than the one where pbind=0.2 because the 1.0 case at least correspond to an expected D₁ value based on viscosity effects only, which means perhaps that the retardation effect due to a varying potential is somehow canceled out in vivo. Other explanations to the high D₁ value is that the number of searching proteins were underestimated in the experiments or that the DNA is less occupied in the neighbourhood of the operator site. This might also be the function of divergent transcription found in eukaryotic cells(132). Figure 4 shows how the ‘front’ of the semitransparent blue region moves as the number of searching proteins are increased or as the occupancy in the neighbourhood of the operator site is lower than elsewhere on DNA.
**Fig 4.** The dependence of the parameter space on the total number of searching proteins and the genome size showing how the front of the semitransparent cyan regions in Figure 2 would move under different assumptions. The blue, green and magenta lines correspond to 4, 5 and 6 searching proteins, respectively, while the black dashed line represents an 85% vacancy around the operator site (v_{OP}) and a vacancy of 70% elsewhere. The chi-square values depend weakly on the genome size as tested by simulations. The acceptable 1D diffusion constant is pushed toward lower values by either increasing the number of searching proteins or decreasing the genome size.

Clearly lower D1 values in combination with a varying potential is achieved as the number of searching proteins are increased or if the occupancy near the operator site is lower than elsewhere. The keen-eyed reader will observe that no intersegment transfer is allowed, since k_{IST} is set to zero. Intersegment transfer would actually make the front go in the opposite direction because the dependency between two operator site need to be satisfied and since this dependency is lowered by intersegment transferring it needs to be compensated for by an even higher D1 value. For this reason I do not therefore believe that intersegment transfer contribute to the total search time for the LacI-dimer coupled to Venus. Also, by visual inspection of the MD trajectories I personally feel that intersegment transfer would be hard to achieve based on steric repulsions between the LacI DNA binding domains and the rest of the protein which appear to hinder the DNA binding domains to assume a configuration such that two different DNA segments can be bound at the same time. The experimentally measured dependency between operator sites also demands that sliding exists and that the LacI dimer slides around ~40bp in vivo. As hopping explores just a few bp in comparison to sliding it will therefore not contribute to the search time. However, the geometry and the properties of diffusion dictates that it should still be there and that it might have a significant effect on the search times for proteins which do not exhibit significant sliding.

**Diffusion controlled or not?**

Based on the parameter space figures one expects a low degree of diffusion control, i.e. in the allowed space \( \alpha \sim 0.1-0.25 \). This is in contrast to what has always been assumed of the nonspecific transcription factor – DNA interaction in the literature in the past. A low \( \alpha \) means a low probability of nonspecific binding upon contact and a significant decrease of the nonspecific association rate constant from its maximal diffusion limited value. This also may
seem to be in conflict with the molecular dynamics results, where the radial monotonic free energy profile suggest a diffusion limited binding, since there is no barriers in the PMF profile. The discrepancy between the allowed alpha to get a consistent model and the expectancy can be reconciled by recognizing that only a part of LacI is reactive while the model in the parameter space results considers LacI to be a fully reactive sphere.

Steric Effects

In the last section it was suggested that a target search model without steric effects demand reaction control when compared with in vivo data for consistency. The reaction control without steric effects is interpreted as a low reactivity upon contact between the fully reactive sphere and cylinder. However, steric effects might account for the low reactivity due to inert non-reactive parts on the protein and the DNA while still allowing for a high reactivity given contact between the reactive parts so the effects of geometry needs a thorough investigation. The path of least resistance towards understanding the effect of reactive patches on the spherical protein and the cylindrical DNA molecule is to consider the magnitude of these effects in simple limits and to gradually add complexity and thus gradually close the distance between realism and the model world. We start by considering a fully reactive protein, whether it is a disk or sphere does not matter in this case. Let the geometry of the reactive patch on the cylinder DNA be either a stripe running along the length of the cylinder (Fig 5A) or rings placed periodically along the cylinder (Fig 5B). If F is the fraction of the cylinder surface covered by the reactive patch then one would expect a linear dependence of the macroscopic association rate on F in the reaction controlled limit as this limit correspond to a homogenous sampling of configuration space. Actually, in the reaction controlled limit where the association rate is independent of diffusion the macroscopic association rate becomes equal to the microscopic rate, k. Thus, we expect that \( k = 2\pi Dl\alpha F \) and the measure of the extent of diffusion control becomes \( \alpha \) times F meaning that \( \alpha F = \) constant correspond to an unchanging equilibrium constant for nonspecific binding to DNA. The small value of what was thought \( \alpha \) when compared with in vivo experiments is with inclusion of geometry a small value for \( \alpha F \) which means there can be a high value for the reactivity \( \alpha \) if F is small.
The more interesting case is the diffusion limit where it becomes more difficult to conjecture how steric effects impact the association rate because of spatial correlations between the cylinder and the protein. We know that the measure of diffusion control is $\alpha F$ and that the macroscopic association rate constant can be written as $k_a = 4\pi D \rho f(F_P)$ for a reactive patch covering the fraction $F_P$ of the surface of a sphere in a sphere-sphere association. The steric factor $f$ which embodies the effects of geometry can be represented as a scaling of the relative sizes of the spheres by scaling the reaction radius $\rho$.

Perhaps steric effects affect the sphere-cylinder association in the same way, by modifying the reaction radius $\rho$? The expression for the nonspecific association rate constant would then become

$$k_a = \frac{k}{1 + \alpha F \left( \ln \left( \frac{R_c}{\rho g(F)} \right) \right)}$$

For the simple geometries in Fig 5 one can solve the steady state diffusion equation with the assumption of a homogeneous influx of the sphere over the reactive patch on the cylinder(79) and show that this is indeed the case with $g(F)$ equal to the exponential of a steric factor $f(F)$ meaning we can write,

$$k_a = \frac{k}{1 + \alpha F \left( \ln \left( \frac{R_c}{\rho} + f(F) \right) \right)}$$

Since $k_a = k P_{\text{diss}}$, the probability of a macroscopic dissociation given a microscopic dissociation becomes
\[ P_{\text{diss}} = \frac{1}{1 + \alpha F (\ln \left( \frac{R}{\rho} \right) + f(F))} \]  

(27)

When geometry is introduced on the cylinder, i.e. \( F < 1 \) then as expected \( P_{\text{diss}} \) becomes higher and \( k_a \) becomes lower. The term in the denominator equals the ratio between the microscopic association rate constant \( k \) and the diffusion-controlled macroscopic association rate constant \( k_a \),

\[ \alpha F \left( \ln \left( \frac{R}{\rho} \right) + f(F) \right) = \frac{k}{k_a^{\text{diff}}} \]  

(28)

can be viewed as a measure of the spatial correlations between the sphere and the cylinder. This is also accurately the degree of diffusion control rather than being a measure of it as stated above for \( \alpha F \). Let’s consider the expected differences in the spatial correlation measure (eq 28) for the stripe and the ring cases. If the protein start just outside the reaction radius at time zero then it would need to diffuse a distance \( 2\pi \rho \) to go around the DNA which corresponds to ~100bp. It is therefore much more likely that the protein have sampled many sites along the length of the cylinder before having sampled sites around the DNA. For this reason the spatial correlations, which are due to the smearing of reactive sites on the cylinder that compete for binding to the protein, are expected to be larger in the stripe case than in the ring case. This is what we have observed as a smaller probability of dissociation for the stripe case than the ring case.

Let’s add another layer of complexity to the geometry due to the helical twisting of the phosphate backbone of the DNA molecule and the fact that only a subset of the amino acids constituting a protein are actually involved in the nonspecific interaction to the phosphate backbone and the grooves (Fig 6).
Fig 6. The figure shows the helix geometry on the DNA cylinder and the axially symmetric patch on the spherical protein for which the dynamics is investigated by an event driven simulation scheme.

This geometric situation is beyond the scope of my analytical capabilities and was studied using a simulation instead. The idea behind the simulation scheme is to start the protein just outside the reactive patch homogenously over the reactive patches on both the protein and the DNA as demanded by microscopic reversibility. Then the protein can dissociate with a probability given in eq 27 with \( f=0 \) and \( F=1 \). If the protein do not dissociate, then return times are sampled from a distribution. Given the return times one can sample how much the protein have rotated around the DNA, around its own center and how much it has diffused along the DNA. The simulation keep executing these events until the protein binds nonspecifically or leaves the DNA segment at which point the simulation is restarted and the coordinates reset. The probability of dissociation, \( P_{\text{diss}} \), is estimated as the fraction of nonspecific binding events out of a total of \( 4\times10^5 \) events. The details of how the simulation was performed and the coordinate systems can be found in the supplementary material in the manuscript in this thesis. The first question one might ask is if the steric effects can be incorporated by a scaling of relative sizes when also a reactive patch on the protein, \( F_P \), is introduced. If so, then \( 1/P_{\text{diss}} - 1 \) should be a linear function of \( \alpha \).
Fig 7. The x-axis is the reactivity upon alignment of the reactive patches on the protein and the DNA, $\alpha$ and on the y-axis is the inverse of the probability of macroscopic dissociation given a microscopic one, $P_{\text{diss}}^{-1}$, minus one. The plots correspond to the three different geometries and different fractions of cylinder surface coverage by reactive patches for the spherical protein with a reactive patch surface coverage, $F_P$ equal to 0.1.

Linearity appears as a good approximation and we can write

$$k_a = \frac{k}{1 + \alpha FF_p (\ln \left( \frac{R_c}{\rho} \right) + f(F, F_P))}$$

(29)

with the corresponding changes in $P_{\text{diss}}$ and the spatial correlation measure following equations 27 and 28, i.e. $f = f(F, F_P)$. Also note that the reaction rate in the reaction controlled limit becomes $k_a = k = 2\pi D l \alpha FF_p$. We can now compare the nonspecific association rates between the stripe and the helix case given a fully reactive sphere at first. If the protein enters the macroscopic dissociation distance uniformly at $R_c$ then we would expect a lesser $k_a$ for the stripe as the protein samples sites along the length of the DNA more.
readily than around it. This means that the protein will probably leave the DNA segment unless it collides with the DNA in the vicinity of the stripe. The simulation results confirm this (Fig 8A). We see that the nonspecific association rate is very similar for the helix and the ring cases and that it can be up to ~2.5 times larger in these cases compared to the stripe geometry (Fig 8B). The similarity between the ring and the helix case might raise concerns. Figure 3 in the supplemental material shows that this is a coincidence. As $F \rightarrow 1$ and the cylinder becomes fully reactive there is no difference as expected.

![Figure 8](image)

*Fig 8.* The diffusion limited ($\alpha = 16$) nonspecific association rate constant as a function of the fraction of the DNA cylinder covered by reactive patches, $F$, in A. B shows the nonspecific association rate to a helix relative to the stripe DNA cylinder geometry as a function of the reactivity, $\alpha$.

The nonspecific association rate decreases more steeply in the stripe case than for the helix case if the fraction of the cylinder surface covered with the reactive patch, $F$, is decreased. This is shown in Fig 8B as a higher $k_a$ for the helix relative to the stripe case given the reactivity $\alpha$. The reason for an increased amplification of $k_a$ in the helix case as the reactivity is increased is because $k_a$ saturates with $\alpha$ much faster for the stripe case than for the helix case. It does not really matter if $\alpha$ is increased from 10 to 15 in the stripe case as the rate limiting process is to align the reactive patches on the protein and the DNA. Next we ask if a patch on the protein covering a fraction $F_P$ of the surface would have any impact on the rate amplification in the helix case. The results are shown as an isosurface on the $F-F_P$ plane (Fig 9).
Fig 9. An isosurface showing the nonspecific association rate to the helix relative to the stripe DNA cylinder geometries as a function of the reactive patch coverage on the DNA cylinder, F, and the reactive patch coverage on the spherical protein $F_p$.

The isosurface shows that the rate amplification is more or less independent of $F_p$ as long as both $F$ and $F_p$ are larger than ~0.4. There is a negligible change in the nonspecific association rate for both geometry cases by increasing $F_p$ beyond 0.4. However $F_p$ starts to matter for small values. As previously stated, the rate limiting step for the association in the stripe case is to align the reactive patches. The protein have time to sample many rotations relative to its own center during the rotations of the protein around the DNA while it does not when diffusing along the length of the cylinder. This means that the helix case is more sensitive to a change in $F_p$ which make $k_a$ rise much more steeply upon increasing $F_p$ in this case than in the stripe case. Thus, for small $F_p$ the rate amplification is increased with increasing $F_p$. In conclusion, the steric effects are necessary to take into account to properly gauge the degree of diffusion control. The nonspecific binding is ~2 times faster to a helix reactive patch geometry than to a stripe and in the limit of very small $F$ approaches $2\pi p/h$ where $h$ is the periodicity of the patches where we used 10.4bp. This amplification rate have a direct impact on the search time especially when crowding on DNA is considered.
Reactions in 2D

The translation invariance along the DNA (at least for the stripe and the fully reactive cylinder cases) means that the mathematical description of the non-specific interaction between proteins and DNA take on a 2D character. Reactions in a 2D geometry are a bit special and important as the reaction and diffusion processes in membranes by proteins and organic molecules such as cholesterol determine the structural integrity and fluidity of the membrane, properties that are correlated with a plethora of diseases. We can study the behavior of reaction-diffusion processes on the membrane by investigating how one of the most well-known models for enzyme kinetics behave there. Consider a Michaelis-Menten system on a 2D plane which is simulated at the RDME level of detail in MesoRD. In this system, substrate molecules S are produced homogenously over the 2D surface with rate Φ where they subsequently diffuse and upon meeting an enzyme molecule E binds to form an enzyme substrate complex ES with rate $k_a$. The substrate may then be converted and released as a product P with rate $k_{cat}$ or the complex might fall apart to a free enzyme and substrate molecule with rate $k_d$. Taking the law of mass action approach one can show that the stationary flux per enzyme is given by (93),

$$\frac{\Phi}{E} = J_{ass} = \frac{k_{cat}k_a S}{k_{cat} + k_d + k_a S}$$

(30)

where the substrate concentration does not change if Φ is increased in proportion to E.
Fig 10. Long-range correlations on membranes. Right, illustration of the decrease in search time when the enzyme concentration doubles. Left, reaction scheme modeled with the indicated frameworks. The influx rate increases in proportion to the total number of degradation enzymes, $E_{tot}$.

In line with the law of mass action, figure 10 show that the number of substrate molecules is constant for the 3D CME (black dots) or PDE (red line) description of the biochemical network of reactions while it is not in 2D! The substrate to product conversion capacity for each enzyme molecule increases as the total number of enzymes increase on the membrane and the number of stationary state substrate molecules decreases monotonically. From the microscopic point of view, whenever a substrate molecule is added the distribution of molecules will be perturbed and it will take some redistributing of the molecules before a possible stationary flux is reached. This means that the association rate constant between the molecules will depend on time. In 3 dimensions the redistributions are short lived and the association rate will reach a constant value in the $\sim 1 \mu$s timescale. In 2 dimensions however the association rate constant is inversely proportional to $\ln(t)$ and will never reach a nonzero constant value. In the case of a centered enzyme molecule on an infinite plane, this means that a constant association flux can never be attained and consequently a reaction rate constant cannot be defined there. Intuitively, the depletion zone around the enzyme will continuously expand with the constant concentration at infinity not being sufficient to balance this expansion. In our Michaelis-Menten example above we have a homogenous influx of substrates over the membrane which allows for a non-trivial stationary state to be found but the spatial correlations between molecules at all distances remain. This becomes evident as the association rate constant $k_a$ is dependent through $R_c$ on the concentration of sinks/enzymes $E$,

$$k_a = \frac{2\pi D_3}{2\pi D_3 + \ln(\frac{R_c}{\rho}) - \frac{3}{4}}$$

(31)

where $R_c$ is the radius of the depletion zone around each enzyme. Thus, $\pi R_c^2 = 1/E$ and $k_a = k_a(E)$ making the reaction rate not strictly proportional to the product of the number of the reacting species thus breaking the law of mass action. In other words, a substrate molecule on a membrane never forgets where it has been before and adding another enzyme molecule on a membrane will, due to this memory effect, have a disproportionate effect on the association rate to substrate molecules making the substrate conversion capacity for each enzyme higher. Another effect of 2 dimensions is to increase the sensitivity of the number of stationary state substrate molecules to the regularity of
the enzyme molecules on the membrane. This is related to protein DNA interactions due to their 2D character where there can be up to a 20% difference in the nonspecific association rate $k_a$ between a protein and a DNA molecule depending on whether the DNA segments are modeled as a regular array or if the DNA molecule is modeled as ‘spaghetti’(56). It also means that there could be a potential for the DNA breathing(133) dynamics to regulate the nonspecific association rate constant for DNA binding proteins at a global level.
Svensk Sammanfattning

I början av 1800-talets andra hälft började kemister, matematiker och fysiker intressera sig för vad som bestämmer hur fort molekyler reagerar med varandra och hur man kan modellera denna hastighet i förhoppningen att förutspå mängden av varje typ av molekyl vid ett givet tillfälle(20). Intresset gav till en början upphov till massverkans lag som konstaterar att reaktionshastigheten är direkt proportionell mot produkten av reaktanternas koncentration i provröret(20). Denna lag visade sig senare vara härledningsbar från termodynamikens första huvudsats som implicerar att reaktionshastigheten är relaterad till energi och temperatur. Arrhenius insåg att detta exponentiella samband antydde förekomsten av ett kortlivat instabilt aktiveringskomplex och en aktiveringsenergi som reaktanterna transient behöver låna från sin omgivning för att ta sig till aktiveringstillståndet och vidare till produktstillståndet(29). Decennier av arbete visade senare att aktiveringsenergin motsvarar den fria energin för reaktioner i lösning(J34). Detta gör det möjligt att bestämma reaktionshastighetskonstanterna om man har tillgång till en fri energiprofil som tar reaktantstillståndet till produktstillståndet. En sådan profil kan man få genom att studera molekylernas dynamik på atomnivån och simulera varje atoms position över tid i enlighet med Newtons andra lag.

Vidare arbete uppdagade att de deterministiska ekvationerna som följer av massverkans lag inte räcker till för att modellera kemiska reaktioner i de fall där man har relativt stora fluktuationer i antalet molekyler. Ett exempel på detta är när man har få molekyler, vilket är en vanligt förekommande situation i levande celler. Modellering av kemiska processer i levande celler utnyttjar därför den matematiska teorin om stokastiska processer vilket leder till en evolutionsekvation i tiden för sannolikheten att ha ett visst antal molekyler av en viss typ. Denna ekvation balanserar alla utgående och inkommande reaktionsflöden och kallas för den kemiska master ekvationen. Ekvationens komplexitet kräver oftast att den löses numeriskt t.ex. genom att man realiserar trajectorier motsvarande evolutionsekvationen. Det är också möjligt att lägga till ett rumsberoende i massverkans lag och för den kemiska masterekvationen. Det senare fallet kan exempelvis realiseras genom att dela upp den totala volymen i kubiska subvolymer där varje subvolyms uppfyller masterekvationen med tillägget att man tillåter hopp ut från subvolymen och hopp in från de närmaste grannarna. Förutom att hålla koll på antalet...
molekyler måste man därför även hålla reda på i vilken subvolym de befinner sig. MesoRD är en mjukvara som simulerar kemiska reaktionssystem med hjälp av subvolym, men i likhet med andra program av den här typen stöter MesoRD på problem när subvolymerna blir mycket små. I detta gränsområde håller inte de grundläggande antagandena för den kemiska masterevaktionen och resultaten divergerar. I mitt arbete har jag vidareutvecklat MesoRD för att råda bot på detta och skapa konsistens i detta gränsområde.

Ett tillämpningsområde för teorin ovan är studiet av hur genreglerande DNA-bindande proteiner hittar fram till ett specifikt bindningsställe bland ~4.5 miljoner (E.Coli) ospecifika bindningsställen. Om ett protein letar slumpmässigt i 3 dimensioner så behöver proteinen i medeltal besöka varje ställe på DNAt innan det hittar fram. För transkriptionsfaktorn LacI skulle detta innebära i medeltal ~2.5 dagars letande vilket naturligtvis är biologiskt ohållbart. Experiment visar också att det tar 4-5 minuter för LacI att hitta sitt bindningsställe i levande E.Coli celler(2). Om man antar att de ospecifika bindningställena fungerar som fällo för så kan man skatta en nedre gräns för söktiden genom att räkna på hur lång tid det skulle ta för ett protein att hitta fram utan det ospeciska DNAt närvarande. Experiment med ospecifikt DNA visar dock att LacI hittar fram fortare än den beräknade nedre gränsen för söktiden(52), hujedamej! Detta betyder att LacI måste utnyttja sin ospecifika binding till DNA för att förkorta söktiden. Den rådande hypotesen, som stöds av experiment både in vitro och nyligen in vivo, är att LacI glider längs med DNA helixen och på så vis kombinerar tredimensionell diffusion med diffusion i en dimension(135). En annan potentiell sökstrategi, så kallad hopping, går ut på att proteinet dissocierar ut i lösning till ett avstånd där det inte känner av DNA segmentet i term av elektrostatiska eller transienta dipol-dipol växelverkningar men där proteinet ändå är spatiellt korrelerad till DNA segmentet(14). Ytterligare en modell är att ett protein binder två DNA segment samtidigt och att detta instabila komplex vid sönderfall lämnar proteinet bundet till det andra DNA segmentet, en sökstrategi som kallas för intersegment överföring.

Från den finaste detaljnivån vi har arbetat med, atomnivån, får vi den fria energiprofilen för LacI dimeren när den mikroskopiskt dissociarar från ett ospecifikt DNAsegment och även den fria energiprofilen för dimeren när den glider längs med DNAt. Genom att långsamt ”dra” LacI längs DNAt, kunde vi bekräfta den tidigare observationen att proteinet rör sig i en spiralrörelse. Från den fria energiprofilerna får vi dels den mikroskopiska associationshastighetskonstantens värde som är \( k_{\text{d, micro}} = 1.45 \times 10^4 \text{s}^{-1} \) och den 1 dimensionella diffusionshastigheten som är \( D_1 = 0.05-0.29 \text{μm}^2\text{s}^{-1} \) (det tar 2-40μs att glida ett baspar). Vi kan också skatta hur många gånger LacI återvänder innan en makroskopisk dissociation sker. Genom att multiplicera medelantalet gånger proteinet återvänder med \( k_{\text{d, micro}} \) får vi en uppskattning av
Jajamänsan! Vi kan härleda uttryck för sannolikhete att glida ett baspar längs med DNAt, att dissociera mikroskopiskt, att dissociera makroskopiskt, sannolikheten för intersegmentell överföring samt för specifik bindning i närvaron av andra DNA-bindande proteiner. Den matematiska beskrivningen av sökproblemet baseras på den stokastiska processteorin där proteinet anses hoppa mellan de olika tillstånden glidning, hopping, intersegmentell överföring, makroskopiska dissociationer och specifik binding. Numerisk simulering av processen kan identifiera begränsningar i de ingående parametrernas värde vid jämförelse med experimentella resultat för söktiden. Dessa parameterar är den specifika bindingssannolikheten pbind, graden av diffusions kontroll α, den relative mängden DNA som är fri för inbindning v, fraktionen av tiden ett protein sitter ospecifikt bundet till DNA Fp, den 1 dimensionella diffusions hastigheten D1 och intersegment överföringshastigheten kIST.

Tidigare estimat av D1 in vivo med hänsyn taget till endast viskositetseffekter ger ett värde omkring ~0.025μm²s⁻¹. Om vi även tar hänsyn till potentialfluktuationerna längs med DNAfrån våra MD simuleringar, så förväntas D1 ligga omkring ~0.01μm²s⁻¹. Vår stokastiska processsimulering kräver efter jämförelse med experiment ett D1 värde ~0.025μm²s⁻¹, att mängden uppbundet DNA ~15%, en specific bindingssannolikhet som är nära 1, att Fp är 0.9 samt att α ligger omkring 0.15. Uppseendeväckande är det höga värden på D1 och det låga värden på α (en så kallad reaktionskontrollerad association; högt α innebär en diffusionskontrollerad association). Vårt värde på D1 antyder att effekten av potentialfluktuationer längs med DNA på något sätt negeras i levande celler. Alternativa förklaringar är att vi har underskattat antalet proteiner som söker efter det specifika bindingsstället eller att det finns en mekanism i levande celler som håller området omkring det specifika bindingsstället relativt fritt från andra DNA-bindande proteiner. En sådan mechanism i eukaryota celler kan möjligtvis vara divergent transcription. Vi finner även att hopping inte bidrar nämnvärt till den totala söktiden p.g.a. den relativt obetydliga uppsnabningen i söktiden jämfört med glidning. Notera att det här inte betyder att hopping inte finns bara att det inte bidrar till söktiden i närvaro av glidningar större än ca 10 baspar. Vi finner också att införandet av intersegmentell transfer gör simuleringar resultatet inkonsistenta med experiment. Följaktligen tror vi inte att LacI utnyttjar intersegment överföring som en sökstrategi under våra experimentella
förhållanden. Vad gäller $\alpha$ så är det ett proportionerligt mått på hur sannolikt det är att ett protein binder ospecifict till ett DNA segment givet att de stöter ihop. Decennier av in vitro experiment och våra molekyldynamikssimuleringar antyder att denna sannolikhet ska vara stor och därmed att $\alpha$ ska vara stort (>1) vilket gör vårt resultat förvånande.

Förklaringen kan ligga i steriska effekter samt att den matematiska beskrivningen för protein-DNA-interaktioner vi har utnyttjat modellerar proteinet som en helt reaktiv sfär och DNA som en helt reaktiv cylinder. För att komma närmare sanningen kan vi utföra simuleringarna med olika reaktiva ytor på proteinet och DNA. De huvudsakliga resultaten från dessa simuleringar är att utan hänsyn till geometri och begränsade reaktiva ytor på både proteinet och på DNA kan en ospecific associering misstolkas som reaktionskontrollerad. Vidare så finner vi att proteinen associerar fortare till DNA om den reaktiva ytan på DNA cylindern har en helix form än om den reaktiva ytan är en strip som löper längs längden på DNA cylindern. Detta har en direkt betydelse för hur fort proteinet hittar fram till sitt specifika bindningsställe, speciellt i levande celler där andra proteiner ockuperar DNA molekylen. Simuleringsresultaten antyder också att man kan sammanfatta de steriska effekterna i en parameter, den så kallade steriska faktorn, $f$, som naturligt är en funktion av hur stor del av DNA (F) och proteins ytor (FP) som är reaktiva. Vi föreslår en generalisering av tidigare uttryck för den makroskopiska dissociations sannolikheten på följande form.

$$P_{diss} = \frac{1}{1 + \alpha FF_P (\ln\left(\frac{R_c}{\rho}\right) + f(F, F_P))}$$

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@Sälen, steak and chocolate cake 2 nights in a row => dream come true.
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Your cake bringing skills are, as your mind is, top notch!
It's very nice talking to u. I like you! Thank you for all the help with the papers and writing this thesis.

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You are such a nice person, very nice talking to u! This thesis would have been much delayed without your assistance. Thanks!

Cecilia:
Chocolate cake brought => friends 4 ever!
Vladimir:
You are such a nice and smart guy, really enjoy talking to you. You are my friend. Good luck with everything.

Fredrik:
veet => boom-chikki ~ within 1...2? orders of magnitude.

Daniel C:
Finally, I’m about to enter the realm of doctoral jokes! See you there buddy!

Daniel J:
Thanks for being unconditionally nice and good luck with everything!

Martin:
Your theoretical prowess is huge.

Ebba:
Yo! Talkin to u is awsm. Right on! The cakes brought after maternity leave were awsm.

Emil:
Your potential is humongous, looking forward to reading your papers! However, need to work on the ka-ka-ing dude! Visit grandma now and again, she'll be happy and perhaps will make cake for you to bring. "Oh no he didn't!"

Ozzy:
Lokum from turkey => friends 4 ever! Erkek cucuk yemek yior.

Prune:
Many cakes brought => ...you get it... If your sons ever need some ninja tutoring...

Petter:
Spooning in San Francisco... Oh hush you know what I'm talkin bout!

Alexi:
Lemon pie => ... Thanks for being supportive and a friend!

Magnus:
Talkin to you is super duper. Looking forward to the ribosome results. Right on.

Ivan:
Cake brought 15:30 08-19-2015! You're great! Good luck with everything.
Kalle:
What happens in London stays in London. Right Kalle? Thank you for being a friend and good luck with everything!

Jimmy:
That's right, this is what happens, the no-cake zone!
Bwaha, just kidding you're great! Looking forward to that ping-pong match!

Zahedeh:
Ouch, the no-cake zone! You are awesome anyhow! Looking forward to see all the great papers from you!

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