CHAPTER 5

Static and dynamic response of microscopic conformational variables of ligand binding residues in different proteins

5.1 Introduction

The rate of chemical processes in a medium generally shows inverse dependence on medium viscosity, $\eta^{-1}$ known as the Stoke’s behaviour.\textsuperscript{44, 45} However, marked deviations from the Stoke’s law have been reported in many experiments with bio-molecular systems.\textsuperscript{145-161} For instance, rate of folding of many peptides show $\eta^{-\alpha}$ dependence with $\alpha < 1$. The rates of catalysis\textsuperscript{146, 154, 159} of enzymes and ligand binding rates\textsuperscript{151} report both $\alpha < 1$ and $\alpha > 1$. For peptide folding cases where deviations from Stoke’s behaviour have been observed, detailed simulation studies reveal characteristic involvement of internal degrees of freedom, like dihedral angles\textsuperscript{22-24, 34}. Dihedral angles undergo transitions between isomeric states as conformational state of the protein changes.\textsuperscript{6, 23} These isomeric states are separated by energy barriers, also termed as internal friction\textsuperscript{46, 47}. Since protein function is modulated by conformational changes\textsuperscript{162}, it is pertinent to ask: Can the diverse experimental reports on deviations of rates from Stoke’s behaviour be unified in terms of dihedral transitions? Here we address this question which is expected to throw light to microscopic mechanisms of kinetics of variety of bio-molecular processes.
In this chapter, we propose a mathematical model (see Methods and Appendix I) to understand the role of energy barrier in governing dihedral angle transitions between isomeric states. We find that dihedral angle relaxation is slow whenever the isomeric states are separated by low energy barrier \( f_B \) with a characteristic time-scale, \( \tau \propto f_B^{-1} \). We further show that participation of several dihedral angles with a distribution of barrier heights account for observed deviation from Stoke’s law. We perform MD simulations of different calcium (Ca\(^{2+}\)) binding proteins to extract the barrier height distribution of the dihedral angle isomerisation states. We find that the distribution shows exponential decay in energy. We also compute dihedral auto-correlation function from the MD simulated trajectories, which yields the \( \tau \propto f_B^{-1} \) dependence consistent with our modelling results.

5.2 Methods

We perform explicit solvent MD simulation using standard protocols (see Appendix I and II, chapter 2) for (i) calmodulin (CaM)
\(^{22,23}\), (ii) troponin C (TnC)
\(^{40}\), (iii) \( \alpha \)-Lactalbumin (aLA)
\(^{34}\) and (iv) calbindin (CALB).

Radius of gyration

The radius of gyration, \( R_g \) is calculated as the average distance of the C-\( \alpha \) atoms from their centre of mass \( \bar{R}_{CM} \), \( \bar{R}_{CM} = \sum_i m_i \vec{r}_i / \sum_i m_i \), where \( m_i \) and \( \vec{r}_i \) are the mass and position vectors of the \( i^{th} \) C-\( \alpha \) atom. The squared \( R_g \) is then calculated as:

\[
R_g^2 = \sum_i m_i \left( \vec{r}_i - \bar{R}_{CM} \right)^2 / \sum_i m_i ,
\]

(1)

The \( R_g \) corresponding to the calcium binding loops is computed for every structure in the
ensemble and a histogram is generated for each of the loops in the apo and the holo states.

**Dihedral autocorrelation function**

We compute the histograms of the dihedral angles, $\phi$, $\psi$ and $\chi_1$ of the loop residues, from the ensemble. The dihedral auto-correlation function (DACF) in time,

$$C_\theta(t) = \frac{\langle (\cos \theta(t) + \tau) - \langle \cos \theta \rangle \rangle (\cos \theta(t) - \langle \cos \theta \rangle)}{\langle (\cos \theta(t) - \langle \cos \theta \rangle)^2 \rangle}$$

of fluctuations of any dihedral $\theta$ is calculated for a set of initial conditions $\tau$ with $t = 0.01$ ns. The $\cos \theta(t)$ and $\cos \theta(t + \tau)$ terms are the cosine values of the dihedral $\theta$ at some initial time $\tau$ and after some time interval $t$, while $\langle \cos \theta \rangle$ is the time-averaged cosine value of the dihedral angle$^{28,163}$. The outer angular brackets indicate averaging over the initial conditions chosen from the equilibrated trajectory. The DACFs from MD trajectory are computed as follows:

For any given time-difference $t = |t_2 - t_1|$, we compute the product from the series of conformations starting with the $i^{th}$ initial condition $A_i(t_2, t_1) = (\cos \theta(t_2) - \langle \cos \theta \rangle)(\cos \theta(t_1) - \langle \cos \theta \rangle)$. The average $\langle A_i(t_2, t_1) \rangle$ is computed over all possible combinations of $t_1$ and $t_2$ in the given time series giving rise to same $t$. We then choose several initial conditions from various parts of the trajectory to calculate

$$C_\theta(t_2, t_1) = \frac{1}{n} \sum_i \langle A_i(t_2, t_1) \rangle$$

where $n$ is the number of independent initial conditions. The stationarity of the system in the equilibrium allows considering $t_1 = 0$ and $t_2 = t$, leading to $C_\theta(t)$. The computation is done till $C_\theta(t)$ approaches zero for sufficiently large $t$. 
Mathematical modelling

We formulate a mathematical model to relate the equilibrium bimodal dihedral distributions to the corresponding DACFs. The dynamics of dihedral angles can be modelled through the Langevin equation of a damped oscillator\textsuperscript{28,163}.

\[ I \ddot{\theta} + \frac{f_B}{\omega_0} \dot{\theta} + k \theta(t) = f(t) \]  
\[ \text{(4)} \]

where, \( \theta(t) \) is the torsional displacement, \( I \) is the moment of inertia about the torsional axis, \( f_B \) is the energy barrier between transition from one peak to other of a bimodal distribution, \( \omega_0 = \sqrt{\frac{k}{I}} \) is the vibrational frequency and \( k \) is the associated force constant. The \( f(t) \) is random torque due to environmental fluctuations and considered as Gaussian noise. The detailed algebra is shown in Appendix I.
5.3 Results

We work out a mathematical model to understand the role of energy barrier in governing dihedral angle transitions from one isomeric state to another. We also perform MD simulations of different calcium (Ca$^{2+}$) binding proteins in apo and holo state to support the mathematical model.

A. Mathematical Model

We characterize the dynamics of dihedral angles in the presence of an energy barrier. We consider a free energy profile of a dihedral $\theta$ having two minima, one primary and the other secondary, separated by a barrier, $f_B$ (see Fig 5.1 for schematic). Let us consider the situation that the dihedral angle undergoes isomerisation by transition from the primary minimum to the secondary one. The barrier hinders the motion which can be considered as frictional force, given by $\frac{f_B}{\omega_0}$ where $\omega_0^{-1}$ is a typical time-scale associated with the attempts to cross the barrier in presence of thermal noise $f(t)$. The noise, $f(t)$ is taken to have Gaussian statistics.

\[
\text{Fig 5.1. Schematic representation of free energy profile of a dihedral, } \theta \text{ having a primary and a secondary minima separated by an energy barrier, } f_B.
\]

The dynamical characteristic is given in terms of auto-correlation function\textsuperscript{28}, $\langle \theta(t)\theta(0) \rangle$ in time. The function reflects how the values of a dihedral angle at time $t$ is correlated to its
initial value. Physically, it describes the decay behaviour of dihedral angle dynamics, namely, for a given dihedral angle at its primary minima, it reflects the time-scale of transition to its secondary minima across an energy barrier. We calculate the auto-correlation function (see Appendix I) from the Langevin equation, $\langle \theta(t)\theta(0) \rangle = A\exp(-\frac{t}{\tau})\sin(\omega_n t)$. Here $\tau = \frac{2k}{f_B}\omega_o$ denotes the overall decay time of dihedral fluctuations and $\omega_n = \sqrt{1 - \left(\frac{f_B}{2k}\right)^2}\omega_o$, $k$ being the force constant given by the inverse width at the primary peak. Here, $\omega_n$ is a finite number if $f_B < 2k$ and becomes imaginary for large barrier ($f_B > 2k$). Thus the sinusoidal oscillation with frequency, $\omega_n$ represents the barrier crossing events between two isomeric states.

Now we consider a binding process involving a pair of bio-molecules with internal degrees of freedom given by their respective dihedral angles. Let the binding event be accompanied by isomerisation of dihedral angles at the region of binding. These two bio-molecules approach each other in presence of a solvent, governed by diffusion controlled rate, $\Gamma$ inversely proportional to solvent viscosity ($\sim \eta^{-1}$). We have shown that the dihedral relaxation time-scale across barrier separated isomerisation states is given by $\tau$. Hence, the rate of dihedral angle isomerisation will be $\tau^{-1}$. The total time for overall process is given by, $t_{tot} = (\Gamma + \tau^{-1})^{-1}$.

Suppose a large number of dihedral angles at the binding region undergo isomerisation in the process via various energy barriers, which contributes to the overall time scale of binding process. Let the energy barriers have a probability distribution, $p(f_B)$. The overall time scale is then given by integrating over the distribution of barrier heights, $t_{tot} = \int df_B p(f_B) \left(\Gamma + \frac{f_B\omega_o}{2k}\right)^{-1}$. The integration yields a dependence of overall time-scale, $t_{tot} \sim \eta^{\alpha}$ (see Appendix I), if the
barrier height distribution shows a power law behaviour which falls off algebraically,
\[ p(f_B) \propto f_B^{-\alpha}. \]
Another interesting situation arises for exponential distribution,
\[ p(f_B) \propto \exp\left(-\frac{f_B}{\lambda}\right) \]
where \( \lambda \) is a decay constant in energy. This yields a time-scale \( t_{tot} \sim \eta^2 \) in
the high viscosity regime (see Appendix I). Physically, the overall time scale is governed by the
convolution over all the individual dihedral angle relaxation in combination with solvent
viscosity mediated diffusion.

B. Distribution of barrier heights from MD simulations

Our mathematical analysis shows that the distribution of barrier heights separating the
isomeric states of dihedrals decide the overall dependence of binding rate on viscosity. We
examine the barrier heights between isomeric states of dihedral angles of binding residues in an
important class of biomolecular process called molecular recognition of ligand binding to
proteins. The different models for molecular recognition of protein-ligand systems
include Fischer’s ‘lock and key’ (LK), Koshland’s ‘induced fit’ (IF) and conformational
selection (CS). The LK model states that the ligand-free conformation of the protein is
capable of binding to the ligand without undergoing any conformational change. According to
the IF mechanism, ligand binding induces conformational changes in the protein leading to
stability of the complex. However, experimental evidences suggest that proteins intrinsically
span a wide range of conformations in the ligand free state and the ligand chooses a
conformational state compatible to its binding through the CS mechanism. Recent developments
in this field indicate a hybrid recognition mechanism where CS is followed by an adjustment
through IF (CS-IF).

Radius of gyration distribution: We consider a simple case of ligand binding, specially
calium (Ca\(^{2+}\)) ion binding to different metallo-proteins, like calmodulin (CaM), calbindin
skeletal muscle Troponin C (TnC) and alpha-Lactalbumin (aLA). The nature of Ca$^{2+}$ ion binding residues in all these proteins is similar, either acidic or polar. All atom MD simulations are performed for both Ca$^{2+}$ ion free (apo) and bound (holo) proteins. We specify the conformational states through equilibrium distribution of radius of gyration $P(R_g)$ of the Ca$^{2+}$ ion binding loops in both apo and holo states. We find that the mean $\langle R_g \rangle$ in holo state is always smaller compared to that in apo state due to strong electrostatic interaction between the Ca$^{2+}$ ion and acidic residues of these loops. $P(R_g^{CaM-L1})$ (Fig 5.2 a) shows a bimodal apo state distribution with a major peak at 7.3 Å and a minor peak at 6.4 Å. The holo state distribution, in contrast, shows a single sharp peak at 5.8 Å in the vicinity of the less populated apo peak, indicating CS-
IF. On other hand, the distributions, $P(R_{g}^{CaM-L2})$, $P(R_{g}^{CaM-L3})$ and $P(R_{g}^{CaM-L4})$ show a new conformational state upon Ca$^{2+}$ ion binding, indicative of the IF mechanism. The distribution of radius of gyration of both loop 1 and loop 2 in TnC (Fig 5.2 b) indicate conformational changes governed by IF mechanism upon Ca$^{2+}$ ion binding. In contrast, $P(R_{g}^{aLA-L1})$ exhibits sharp single peak in both apo and holo-aLA with minor shift of 0.2 Å, indicating LK mode of recognition (Fig 5.2 c). The $P(R_{g}^{CALB-L1})$ (Fig 5.2 d) in apo state shows maxima around 7.8 Å with a shoulder at 8.2 Å indicating presence of two populations. The holo state population shows a new peak at 5.8 Å with a tail extending towards the apo population. The apo state distribution of the other CALB loop, $P(R_{g}^{CALB-L2})$ shows a single peak around 6.8 Å. In the holo state we find a primary maxima at about 5.6 Å and another secondary peak at 6.4 Å. The holo state distributions $P(R_{g}^{CALB-L1})$ and $P(R_{g}^{CALB-L2})$ indicate new conformation through IF mechanism (Fig 5.2 d). Although the conformational states of the loops change upon metal ion binding, the secondary structural elements remain largely unchanged (Fig 5.3 for representative Ramachandran plots).

![Ramachandran plots](image)

Fig 5.3. Ramachandran plot of different calcium binding residues in apo (black) and holo (grey) state of proteins: CaM, TnC, aLA and CALB.
Dihedral angle distribution: However, we observe changes in dihedral angles of the binding residues, while the conformational state changes from apo to holo. The peaks of equilibrium distribution \( p_R^i(\theta) \) of dihedral \( \theta \) for residue \( R \) in protein “i” represent different isomeric states (Fig 5.4). There are four patterns of isomeric transitions between apo and holo states. For instance, \( p_{CaM}^{A103}(\phi) \) (Fig 5.4 a) exhibits overlapping unimodal distributions in both apo- and holo state. We denote such overlapping single peaked distributions as SP1. \( p_{TnC}^{D65}(\psi) \) (Fig 5.4 b) is also unimodal in both apo and holo states but having large amount of peak shift which we denote as SP2. On the other hand, \( p_{CaM}^{D20}(\phi) \) (Fig 5.4 c) is bimodal with peaks at -117° and -62° in apo state, while in holo-CaM the population shifts towards the minor peak at -62°. We denote the cases where the holo state population grows in vicinity of an apo population corresponding to a multimodal apo distribution as MP1. The side-chain dihedrals however show different behaviour, where both distributions in ligand free and bound state are flat.

The free energy profiles, \(-k_BT\ln(p(\theta))\) corresponding to the equilibrium distributions of the dihedrals in apo state are also shown in Fig 5.4 d-f. For instance, the free energy profile of the unimodal distribution of \( p_{A103}^{CaM}(\phi) \) (Fig 5.4 d) shows a single minimum. Similar free energy profile is also observed for \( p_{D65}^{TnC}(\psi) \) (Fig 5.4 e). Let us now consider the MP1 cases. For instance, \( p_{D20}^{CaM}(\phi) \) (see Fig 5.4 f) in apo state has a primary peak (\( \theta_{\text{max}} \)) at -117° and a secondary peak at -62° separated by a minima (\( \theta_{\text{min}} \)) at -102°. The secondary apo peak shows population growth in holo-CaM through population shift from \( \theta_{\text{max}} \) across the minima, \( \theta_{\text{min}} \). So we define the barrier separating two apo peaks by

\[
f_B = -k_BT \ln \left( \frac{p(\theta_{\text{max}})}{p(\theta_{\text{min}})} \right).
\]
Fig 5.4. The equilibrium distributions, $p^i_R(\theta)$ of dihedral angles “$\theta$” of residue “$R$” in protein “$i$” for apo (solid line) and holo (dashed line). (a) $p^\text{CaM}_{A103}(\phi)$, (b) $p^\text{TnC}_{D65}(\psi)$ and (c) $p^\text{CaM}_{D20}(\phi)$. The corresponding free energy profiles of (d) $p^\text{CaM}_{A103}(\phi)$, (e) $p^\text{TnC}_{D65}(\psi)$ and (f) $p^\text{CaM}_{D20}(\phi)$.

Fig 5.5. The equilibrium distributions, $p^\text{CaM}_{T26}(\psi)$ generated from five different ensembles corresponding to different starting configurations of apo-CaM loop 1. The variation in barrier height, $f_B$ for different ensembles reflects the stochasticity in distribution.

The experimental determination of rate of any bio-molecular process represents an ensemble averaged measurement. In order to mimic this situation we perform MD simulation of apo-CaM loop1 with different starting structures and generate separate ensembles representing
different molecules of the same species. We calculate the barrier heights of backbone dihedral
angles from each of these ensembles. For instance, the barrier heights calculated from the
dihedral angle distributions, $p_{T_{26}}^{CaM}(\psi)$ for different ensembles show statistical variation (Fig 5.5).
These statistical variations account for stochasticity in the distribution of barrier heights for a
bio-molecular system. We calculate the $f_B$ between dihedral isomeric states for different MP1
cases from all the loops we have simulated. The distribution of barrier height, $p(f_B)$ for these
cases, shown in Fig 5.6 a, exhibits an exponential behaviour $\exp\left(-\frac{f_B}{\lambda}\right)$ with $\lambda = 3.6 \ k_B T$.

**Dihedral autocorrelation functions:** We compute the dynamic response of the dihedral
angles in apo state using the MD simulation trajectories. We compute the dihedral
autocorrelation function, $C^i_r(\theta,t)$ for dihedral angle fluctuations about its equilibrium value as
the product of the value of $\delta \cos \theta(t_0)$ at some initial time $t_0$ and the value after some time
interval $t$, $\delta \cos \theta(t+t_0)$, averaged over a set of initial conditions $t_0$.

The DACFs for SP1 cases, like $C^{CaM}_{A103}(\phi,t)$ (Fig 5.6 b) and SP2 cases, like $C^{TnC}_{D65}(\psi,t)$ (Fig
5.6 c) show fast decay in apo state. However, for MP1 and MP2 cases, the apo state auto-
correlation amplitude is characterized by initial slow decay along with significant anti-
correlation at long time. This is shown for ligand free DACF, $C^{CaM}_{D20}(\phi,t)$ (Fig 5.6 d) as a
representative for MP1 case. Such apo state DACFs bear signature of damped oscillations
characterized by an oscillation frequency, $\omega_n$ having an overall decay time constant, $\tau$. 

Fig 5.6. (a) The distribution of barrier height, \( p(f_B) \) separating dihedral isomerisation states for MP1 cases. The DACF response of the dihedral angles corresponding to apo (black) states illustrating different time-scales for (b) \( C_{A103}^{C_{\alpha M}}(\phi,t) \), (c) \( C_{D65}^{C_{\alpha M}}(\psi,t) \) and (d) \( C_{D20}^{C_{\alpha M}}(\phi,t) \). (e) Correlation plot of barrier height, \( f_B \) with overall decay time constant \( \tau \). (f) Correlation plot of overall decay time constant \( \tau \) of backbone dihedral angles with viscosity, \( \eta/\eta_0 \).

We fit the DACFs from simulation with the functional form, \( \exp\left(-\frac{t}{\tau}\right)\sin(\omega_n t) \) considering \( \omega_n \) and \( \tau \) as parameters for best fit. The fitted DACFs are shown in Fig 5.7. Similar slow decaying DACFs are observed for multimodal holo state distributions as in the apo cases.
Fig 5.7. The apo state DACFs (black) fitted (grey) with the expression, \( \exp\left(\frac{-t}{\tau}\right)\sin(\omega_n t) \).

We show in Fig 5.6 that the overall decay time constant, \( \tau \) decreases linearly with increasing \( f_B \). This is in agreement to the results of mathematical analysis. The dihedral relaxation between isomeric states occurs at slower time-scales when the barrier between them is less. The underlying physical picture is that whenever two isomeric states are separated by a low energy barrier, frequent barrier crossing events occur as a result of which the dihedral fluctuations exhibit slow decay profile.

*Dependence on solvent viscosity:* We examine the dependence of \( \tau \) of dihedral angle fluctuations of the Ca\(^{2+}\) binding residues on solvent viscosity. We choose MP1 predominant apo-CaM loop 1 for various solvent viscosities through rescaling the mass of explicit water molecules.\(^{177, 178}\) We consider individual backbone dihedral angles of apo-CaM loop 1 to estimate the respective decay time constants \( \tau \) at different viscosities. The correlation plot of \( \tau \)
versus $\eta/\eta_0$ (Fig 5.6 f) shows little correlation ($R^2 \approx 0.046$). The decay time constant $\tau$, governed by the barrier between isomeric states of dihedral angles, is thus observed to be independent of solvent viscosity. This is in agreement to earlier studies$^{177, 178}$, where the time constant of auto-correlation function averaged over backbone dihedral angles representing internal friction time-scale is reported to be independent of solvent viscosity. We show representative dihedral angle distributions of residues from apo-CaM loops under different viscosity conditions in Fig 5.8.

![Graphs showing dihedral angle distributions](image)

Fig 5.8. The equilibrium distributions, $p_R^{CaM}(\theta)$ of dihedral angles “$\theta$” of residue “$R$” in apo-CaM loop 1 at different viscosities.
Connection to experimental results: Table 5.1 summarizes different bio-molecular processes with rates deviating from Stoke’s law. We consider a diversity of bio-molecular rate processes, which include ligand binding and dissociation, protein folding-unfolding, electron transfer, proton exchange and enzyme catalysis. The binding and dissociation rates of carbon mono-oxide and oxygen to heme proteins like haemoglobin, hemerythrin and myoglobin exhibit fractional power law in viscosity.\(^\text{145, 151, 152}\) The folding rates\(^\text{150, 156-158}\) of various proteins and peptides and enzyme catalysed reaction rates\(^\text{146, 148, 153, 154, 159}\) also show fractional power dependence, \(\alpha \leq 1\) on solvent viscosity. Such dependence on solvent viscosity has been observed for intra-electron transfer rates in sulphite oxidase\(^\text{147}\) and proton exchange rates of Cod PA III\(^\text{160}\) as well. Our mathematical analysis accounts for such behaviour when the distribution of barrier heights exhibits power law dependence as \(p(f_B) \propto f_B^{-\alpha}\), where \(\alpha \leq 1\). Table 5.1 further shows exponent \(\alpha > 1\) in many cases. For instance, unfolding\(^\text{161}\) and electron transfer rates\(^\text{155}\) of Cytochrome C, amide proton exchange rates\(^\text{149}\) of lysozyme and kinetics of human S4 enzyme\(^\text{161}\) show such behaviour at very high viscosities. In these cases the exponent is close to \(\sim 2\). Our mathematical modelling shows that exponents \(\sim 2\) is possible for high solvent viscosity with an exponential distribution of barrier heights. Our analysis suggests that Stoke’s behaviour arises when there is no contribution from internal degrees of freedom or in absence of internal degrees of freedom. However, even in the presence of internal degrees of freedom, Stoke’s law can still hold, if \(p(f_B) \propto f_B^{-1}\) with \(\alpha = 1\).
Table 5.1. The power law exponent in viscosity, $\eta^{-\alpha}$ dependence of rates of bio-molecular processes,

<table>
<thead>
<tr>
<th>Biomolecular processes</th>
<th>$\alpha$</th>
</tr>
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<tbody>
<tr>
<td><strong>Enzyme catalysis</strong></td>
<td></td>
</tr>
<tr>
<td>Carboxypeptidase A</td>
<td>0.4-0.6</td>
</tr>
<tr>
<td>Subtilisin BPN</td>
<td>0.65</td>
</tr>
<tr>
<td>Glutathionine transferase</td>
<td>0.8</td>
</tr>
<tr>
<td>Dihydrofolate reductase</td>
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</tr>
<tr>
<td>Human S4</td>
<td>2.1</td>
</tr>
<tr>
<td><strong>Electron transfer/Proton exchange</strong></td>
<td></td>
</tr>
<tr>
<td>Cytochrome C</td>
<td>2.4</td>
</tr>
<tr>
<td>Sulfite oxidase</td>
<td>0.7</td>
</tr>
<tr>
<td>Cod PA III</td>
<td>0.63</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>1.4</td>
</tr>
<tr>
<td><strong>Ligand binding/dissociation</strong></td>
<td></td>
</tr>
<tr>
<td>Myoglobin (O2 diss)</td>
<td>0.4</td>
</tr>
<tr>
<td>Myoglobin (CO binding)</td>
<td>0.45</td>
</tr>
<tr>
<td>Hemerythrin (O2 diss)</td>
<td>0.5</td>
</tr>
<tr>
<td>Haemoglobin (O2/CO exchange)</td>
<td>0.26</td>
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<tr>
<td><strong>Protein folding/unfolding</strong></td>
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</tr>
<tr>
<td>Trp Cage</td>
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<tr>
<td>alpha-helix</td>
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<tr>
<td>beta-hairpin</td>
<td>0.93</td>
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<td>Ig-G (protein L)</td>
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<td>A3D (73 residue alpha-helical protein) folding</td>
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</tr>
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</table>

*Relation with molecular recognition:* The instances of different isomerisation patterns, SP1, SP2, MP1 and MP2 for different proteins are summarized in Table 5.2. We now relate these isomerisation patterns to the molecular recognition processes: CS, IF and LK as determined from the $P(R_g)$ of the Ca$^{2+}$ binding loops. The molecular recognition process in CaM loop 1 is a hybrid mechanism of CS and IF. We find that MP1 (~ 49%) is predominant in CaM loop 1. In
CaM loop 2 and loop 3 SP1 (~37 %) cases are predominant, while CaM loop 4 shows preference for SP2 (~53 %) patterns. The two loops of TnC indicating an IF mechanism show a predominance of SP2 isomerisation pattern (>40 %). Similarly, the SP2 pattern is also predominant (~40 %) for the Ca\(^{2+}\) binding loop of aLA, which is governed by LK. On other hand molecular recognition is governed by IF mechanism in CALB loops, where MP1 (~40 %) pattern is prevalent in loop 1 and SP1 (~35 %) in loop 2.

Since MP1 cases are more abundant in the Ca\(^{2+}\) ion free CaM loops 1 and 2 than the other loops (see Table 5.2), the relaxation of the corresponding dihedral angles are slower compared to that in loops 3 and 4. Our data on time-correlation thus suggests that Ca\(^{2+}\) ion binding occurs first at the C-terminal loops and then the N-terminal loops get saturated, which is consistent with experimental results.\(^{179, 180}\) On a similar note, one would expect slow Ca\(^{2+}\) ion binding to the CALB loops compared to the kinetics of Ca\(^{2+}\) ion binding to TnC or aLA.

Table 5.2. The percentage (%) of SP1, SP2, MP1 and MP2 observed for dihedral angles in different loops of CaM, TnC, CALB and aLA.

<table>
<thead>
<tr>
<th></th>
<th>SP1 %</th>
<th>SP2 %</th>
<th>MP1 %</th>
<th>MP2 %</th>
</tr>
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<tbody>
<tr>
<td>CaM L1</td>
<td>20</td>
<td>22.8</td>
<td>48.6</td>
<td>8.6</td>
</tr>
<tr>
<td>CaM L2</td>
<td>36.7</td>
<td>20</td>
<td>33.3</td>
<td>10</td>
</tr>
<tr>
<td>CaM L3</td>
<td>37.5</td>
<td>21.9</td>
<td>21.9</td>
<td>18.7</td>
</tr>
<tr>
<td>CaM L4</td>
<td>14.7</td>
<td>53</td>
<td>11.7</td>
<td>20.6</td>
</tr>
<tr>
<td>TnC L1</td>
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<td>43.75</td>
<td>21.9</td>
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<td>47.0</td>
<td>14.7</td>
<td>11.8</td>
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<tr>
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<td>13.9</td>
<td>41.7</td>
<td>13.9</td>
</tr>
<tr>
<td>CALB L2</td>
<td>35.3</td>
<td>20.6</td>
<td>20.6</td>
<td>23.5</td>
</tr>
<tr>
<td>aLA</td>
<td>36.7</td>
<td>40</td>
<td>13.3</td>
<td>10</td>
</tr>
</tbody>
</table>
5.4 Conclusion

We provide a theory to account for the anomalous dependence of different bio-molecular rate processes on solvent viscosity. Our mathematical modelling explains the deviation from Stoke’s law and provides a way to understand power law dependence with $\alpha \neq 1$ for rates of different bio-molecular processes on solvent viscosity based on the dihedral relaxation time-scales. The decay time constant, $\tau$ is observed to decrease linearly with increasing barrier height. We show that the distribution of barrier heights accounts for the observed deviation from Stoke’s law.

We further study a particular class of bio-molecular process, namely $\text{Ca}^{2+}$ ion binding to different proteins to explore the behaviour of dihedral transitions. To this end we perform MD simulation of $\text{Ca}^{2+}$ binding proteins in apo and holo state. We compute the dihedral angle distributions and generate a probability distribution of barrier heights separating the dihedral angle isomerisation states of $\text{Ca}^{2+}$ binding residues. The distribution of barrier heights shows an exponential decay with increasing energy. We also estimate the time-scales of dihedral angle relaxation and find that $\tau \propto f_B^{-1}$ from the simulation data consistent with mathematical analysis. This time-scale is further observed to be independent of solvent viscosity. The most important aspect of our work is to provide a unified understanding of rate of various bio-molecular processes in terms of microscopic variables.
Appendix I

Taking the Fourier transform of \( I \dddot{\theta} + \frac{f_B}{\omega_0} \ddot{\theta} + k \theta(t) = f(t) \) yields \( \theta(\omega) \) and the DACF in the frequency domain

\[
\langle \theta(\omega) \theta(-\omega) \rangle = \frac{\langle f(\omega)f(-\omega) \rangle}{(k - I\omega^2)^2 + \left( \frac{af_B}{\omega_0} \right)^2} \tag{1}
\]

The inverse Fourier transform of equation (2) yields

\[
\langle \theta(t)\theta(0) \rangle = \int_{-\infty}^{+\infty} \frac{\exp(-i\omega t)}{(k - I\omega^2)^2 + \left( \frac{af_B}{\omega_0} \right)^2} d\omega \tag{2}
\]

Solving the integral we find that

\[
\langle \theta(t)\theta(0) \rangle = A \sin \left( \sqrt{1 - \left( \frac{f_B}{2I\omega_0} \right)^2} \omega_0 t \right) \exp \left( -\frac{f_B}{2I\omega_0} t \right) = A \sin(\omega_n t) \exp \left( -\frac{t}{\tau} \right) \tag{3}
\]

We use equation (3) for fitting the DACFs.

The rate process of bio-molecular function is governed by both contribution from external friction arising out of solvent viscosity and internal friction of dihedral transitions. Let the rate associated with external friction be \( \Gamma \) and that with internal friction is \( \tau^{-1} \). The overall time scale is then calculated by integrating over the distribution of barrier heights, \( \int df_B p(f_B)(\Gamma + \tau^{-1})^{-1} \) We assume a power law dependence of barrier height distribution given by \( p(f_B) \propto f_B^{-\alpha} \) and replace \( \tau^{-1} = \frac{f_B\omega_0}{2k} \) in the above equation. Taking the inverse Laplace transform we get

\[
\int_{0}^{\infty} \exp(-s\Gamma) \int_{0}^{\infty} df_B \exp(-sf_B\omega_0/2k)f_B^{-\alpha} \cdot \text{Solving the inner integral within the}
\]
limits yields \( \int_0^\infty ds \exp(-s\Gamma) \left( \frac{s\omega_0}{2k} \right)^{\alpha-1} \). This finally gives 
\[
\left( \frac{\omega_0}{2k} \right)^{\alpha-1} \frac{1}{\Gamma^\alpha} \int \exp(-z) z^{\alpha-1} dz ,
\]
where 
\( z = s\Gamma \). Our mathematical modelling thus indicate \( \Gamma^\alpha \) dependence of rate processes if 
\[
p(f_B) \propto f_B^{-\alpha} .
\]

Now if \( p(f_B) = \exp\left(-\frac{f_B}{\lambda}\right) \), the inner integral 
\[
\int_0^\infty df_B \exp\left[-f_B \left( \frac{s\omega_0}{2k} + \frac{1}{\lambda} \right) \right]
\]
yields 
\[
\left( \frac{s\omega_0}{2k} + \frac{1}{\lambda} \right)^{-1} .
\]
Solving the full integral, we get 
\[
\frac{\omega_0}{2k\Gamma^{\alpha-1}} + \frac{1}{\Gamma\lambda} .
\]
At large \( \Gamma \), the second term predominates, while at low \( \Gamma \) \( (0 < \Gamma < 1) \) the first term is predominant.