Computational perspective and evaluation of plausible catalytic mechanisms of peptidyl-prolyl*cis-trans*isomerases

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Abstract

Background: Peptidylprolylcis-trans isomerization of the protein backbone is involved in the regulation of many biological processes. Cis-trans isomerization is notoriously slow and is catalyzed by a family of cis-trans peptidylprolyl isomerases (PPIases) that have been implicated in many diseases. A general consensus on how these enzymes speed up prolyl isomerization has not been reached after decades of both experimental and computational studies.

Scope of review: Computational studies carried out to understand the catalytic mechanism of the prototypical FK506 binding protein 12 (FKBP12), Cyclophilin A (CypA) and Peptidyl-prolylcis-trans isomerase NIMA-interacting 1 (Pin1) are reviewed. A summary and an evaluation of the implications of the proposed mechanisms from computational studies are presented.

Major conclusions: The analysis of computational studies and evaluation of the proposed mechanisms provide a general consensus and a better understanding of PPIases catalysis. The speedup of the rate of peptidyl-prolyl isomerization by PPIases can be best described by a catalytic mechanism in which the substrate in transition state configuration is stabilized. The enzymes preferentially bind the transition state configuration of the substrate relative to the cis conformation, which in most cases is bound better than the trans conformation of the substrate. Stabilization of the transition state configuration of the substrate leads to a lower free energy barrier and a faster rate of isomerization when compared to the uncatalyzed isomerization reaction.

General significance: Fully understanding the catalytic mechanism of PPIases has broad implications for drug design, elucidation of the molecular basis of many diseases, protein engineering, and enzyme catalysis in general.

Introduction

Biological processes on the molecular level normally involve conformational transitions that are at times regulated by transient protein-protein and enzyme-protein interactions. Peptidyl-Prolylcis-trans isomerization of the protein backbone is involved in many biological processes, including protein folding, biomolecular assembly, and molecular switches, due to additional conformational variability of the cis and trans isomers.[1] *Cis-trans* isomerization of the peptidyl-propyl bond is, however, notoriously slow, with a free energy barrier of ~20 kcal/mol and a time scale of approximately hundreds of second to minutes.[2] The rate of cis-trans isomerization is therefore catalyzed by a family of structurally unrelated classes of enzymes, typically referred to as rotamases or peptidyl-prolylcis-trans isomerases (PPIases).[3] There are three main classes of PPIases: the FK506 binding proteins (FKBPs), the cyclophilins, and pervulins, of which Pin1 is a member and a special case. FKBPs and cyclophilins are somewhat promiscuous and reversibly catalyze cis-trans rotation around any -X-Pro- peptide bond, where X is any amino acid residue. On the other hand, Pin1 is a phosphorylation-dependent cis-trans isomerase, which specifically and reversibly catalyzes phosphorylated-Serine/Threonine-Proline peptide bonds.[4, 5] PPIases regulate numerous biological processes and have been implicated in many diseases, including cancer and Alzheimer's.[6-9]

Peptidyl-prolylcis-trans isomerases have been the subject of intense experimental and computational research over the years. Over the years, many computational studies have been carried out on the prototypical FKBP12, Cyclophilin A (CypA), and Pin1. It is generally accepted that catalysis by PPIases occurs without any bond breakage or formation to achieve a speed up of the rate of isomerization of about five orders of magnitude.[3] However, a general consensus on

how these enzymes accomplish such a speedup has not been reached and many groups are still actively carrying out computational studies to fully understand their mechanisms of action. One of the major drawbacks is the time scale limitation of current computational simulation methods. Regular classical molecular dynamics simulations are limited to microsecond simulation timescale, and hybrid quantum mechanical molecular mechanics simulations are even limited to a much shorter timescale. However, the uncatalyzed and catalyzed *cis-trans* isomerization reactions occur beyond second and millisecond time scales, respectively, which had hampered more detailed study and progress in the field. More recently, advanced simulation methods have been used to directly simulate the isomerization process free in solution and during catalysis, providing a more direct observation of the catalytic process and complementing experimental findings.

In this review, a summary of computational studies that have been carried out on the prototypical FKBP12, CypA, and Pin1 is presented. Additionally, a broad overview of their biological importance is included to illustrate their physiological significance. In general, there are three schools of thoughts that have emerged from many years of computational studies of PPIases. The three major conclusions of these studies have mainly ascribed the catalytic power and efficiency of PPIases to stabilization of the transition state, destabilization of the ground *cis* or *trans* state, and the dynamics of the enzyme.[10, 11] Majority of the computational studies have been carried out on CypA, one of the first PPIases to be co-crystallized with its natural substrate or substrate analogues. We discuss the computational studies that have been carried out on each class of PPIases. We evaluate the origin of the rate enhancement during catalysis and the role of transition state stabilization in the proposed mechanisms. Analysis of the computational studies

on PPIases and evaluation of the major conclusions provide a general consensus and a better understanding of the necessary attributes required for catalysis in PPIases and enzymes in general.

FK506 Binding Protein (FKBP) 12

FKBP12, a member of the FK506 binding protein (FKBP) family, is a 107 amino acid PPIase (~1700 atoms; 12kDa). FKBP12 is classified as an $\alpha + \beta$ structural protein (Figure 1). It has an overall barrel shape with five antiparallel β -strands wrapped around a short α -helix. The active site of this enzyme includes a hydrophobic pocket and loop regions with mainly conserved aromatic residues (Figure 1B; Tyr26, Asp 37, Phe36, Phe46, Val55, Ile56, Trp59, Tyr82, His87, Ile90, Ile91, Leu 97, Phe99).[10-14]

FKBPs bind several immunosuppressant drugs, including FK506, rapamycin and ascomycin.[10-13, 15] The complex interacts with calcineurin to form a ternary complex, impeding the phosphatase activity of calcineurin and preventing the transcription of genes encoding for cytokines. Binding of FK506 by FKBP12 assists in averting graft rejections and autoimmune disorders.[6-8] The higher presence of FKBP12 in the brain than immune tissues is suggestive of its neurotrophic properties.[6] Development of new classes of FKBP inhibitors may, therefore, serve as treatment for neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases. A detailed understanding of the catalytic mechanism of FKBPs and other PPIases and their molecular interactions will assist and advance the design of new classes of inhibitors.[13, 16-20]

One of the earliest studies of the catalytic mechanism of FKBP by Fischer et al., using computational modeling, showed that a peptide (N-acetyl-Leu-Pro-Phe-methylamide) substrate analogue interacted with FKBP by adopting a type VIa proline turn conformation.[11] A combination of hydrophobic and hydrogen bonding interactions were responsible for the favorable free energy of FKBP-substrate complex formation.[10, 11] The conformation of the substrate bound to FKBP was found to be significantly different with the conformation of the substrate bound to CypA, according to an earlier study by Kallen et al.[21] In CypA, the conformation of the substrate was mainly in the type VIb proline turn. These variations resulted in different types of interactions between each of these enzymes and their corresponding substrates. Hence, both FKBP and CypA were suggested to show some divergent properties in their catalytic mechanisms in relation to the preferential conformations of their bound substrates.[11] Nonetheless, cisprolines are frequently located on protein surfaces, where they are found in either type VIa or type VIb turns. The logical and interesting notion of the evolution of two different classes of PPIases to recognize corresponding protein backbone turns came in the forefront.[11] Recognition of a particular type VI polypeptide turn was hypothesized to be significant for PPIase activity. The twisted conformation of the type VIa turn in the active site of FKBP positioned the amide hydrogen of the residue following proline close to the imide nitrogen of proline that was partially pyramidalized. Fischer et al. concluded that the hydrogen bonding interaction between the amide hydrogen and the imide nitrogen could stabilize the transition state and promote substrate autocatalysis by weakening the pseudo-double C-N bond.[11]

Around the same time as Fischer et al., Orozco et al.[10] used molecular dynamics simulations to investigate the interactions between FKBP and a different substrate analogue (Ac-AAPF-Nme)

and concluded that catalysis was mainly due to stabilization of the twisted transition state ($\omega \sim 90^\circ$) conformation of the substrate relative to the cis conformation [10]. Stabilization of the transition state was aided by several factors, including favorable non-bonded interactions. They also were one of the first to use computational simulations to show that catalysis could not occur via a covalent adduct. The conserved hydrophobic active site residues were shown to play a major role in both specificity and catalysis. [10, 13] Experimentally, the energy barrier of peptidylprolyl isomerization was shown to be approximately 2 kcal/mol lower in a hydrophobic environment [3, 22]. One-third of the influence of FKBP on isomerization was, therefore, credited to the hydrophobic environment of the active site. This hydrophobic effect was speculated as being common amongst PPIases, since the active sites of cyclophilins are also mainly hydrophobic. It was also shown that the FKBP-transition state complex initiated an intra-substrate hydrogen bonding between the HN of Phe and N of Pro, similar to the observation by Fischer et al. [11] This specific interaction persisted throughout their simulations when the substrate was in the transition state in the active site of FKBP. This intra-substrate hydrogen bond was suggested to stabilize the transition state and promote a possible enzyme-induced autocatalysis by the substrate. The major contribution to catalysis was suggested to come from a reduction in the free energy barrier and stabilization of the transition state by approximately 6-7 kcal/mol, in good agreement with experiments. [23]

Fischer et al. made similar inferences when studying catalytic mechanism of FKBP. [11] The interaction between the enzyme and the transition state conformation of the substrate was suggested to be more stable than the interaction between the enzyme and the ground state cis conformation. However, the interaction energy, not free energy, was found to only change by -

1.5 kcal/mol from cis to the transition state ($\omega \sim 90^\circ$) and -2.3 kcal/mol from trans to the transition state between the aromatic residues of the enzyme and the amide carbonyl group of the peptidylprolyl bond of the substrate.[11] Van Duyne et al.[24] also examined the role of the hydrogen bonding interaction between the carbonyl oxygen of the peptidylprolyl bond of the substrate and the enzyme in stabilizing the transition state. The stabilizing interactions between the enzyme and the transition state of the substrate were suggested to be substantial. The more favorable interactions between the enzyme and the substrate were gradually achieved upon twisting the peptidylprolyl bond angle toward the transition state.

In general, these initial computational studies on FKBP suggested a lower free energy barrier during catalysis, compared to the free energy barrier of the uncatalyzed isomerization. The lower energetic barrier was mainly attributed to more favorable interactions between FKBP and the twisted transition state of the substrate than that of the cis and trans ground states. The ground state conformations of the substrate that binds to FKBP was suggested to be slightly twisted compared to that of the most populated ground state conformation of the free substrate.[10, 11] Due to the lack of computer power and limitation in the methods, very limited conformations were sampled or used to analyze the interaction energies in these earlier computational studies. Nevertheless, many relevant and thought provoking ideas were discovered and the results were somewhat consistent with experiments.

Cyclophilin A (CypA)

The monomeric 18-kDa Cyclophilin A (CypA) is a cytosolic member of the Cyclophilin family of about 17 human isoforms.[1, 25] The enzyme consists of an eight-stranded antiparallel β -

barrel with two α -helices flank on each side. Within the barrel, there exist seven aromatic and hydrophobic residues that form a compact hydrophobic core.[26]CypA has been shown to play a critical role in protein folding and regulation of many biological processes.[27, 28]Cyclophilins are involved in gene expression,[29] cellular signaling[30] and the immune response.[31]CypA was also discovered to be significant in various pathological condition such as infection by human immunodeficiency virus HIV-1 and cancer.[1, 9, 25, 32]Following its discovery,[33, 34] increasing number of experimental and computational studies have ignited many debates over the exact role of CypA in catalysis.[35-40]Hence, the results of representative computational studies focused on intermolecular interactions and dynamical effects of CypA in catalyzing cis-trans isomerization of the peptidylprolyl bond are summarized below.

Intermolecular interactions in catalysis by CypA

The catalyzed cis-trans isomerization has a highly accelerated rate of more than five orders of magnitude in comparison to the uncatalyzed reaction.[35]Computational studies have attributed the peptidyl-propyl isomerization mechanism of CypA to different factors.[34, 41-44]Although each mechanism can rationalize some of the catalytic activity of CypA, none of them, except transition state stabilization, can quantitatively explain the observed high isomerization speed-up. A detailed description of the mechanism of transition state stabilization in CypA was presented in the work by Hamelberg and McCammon,[45] where isomerization of free and CypA-bound complex of a substrate analogue (Ace-HAGPIA-Nme) was evaluated using accelerated molecular dynamics.[46]A more detailed review of the accelerated molecular dynamics method can be found elsewhere.[47]The free energy profiles along the peptidylprolyl bond of the uncatalyzed and catalyzed isomerization suggested stabilization of the transition state by

CypA. More recently, Ladani and Hamelberg[48] and McGowan and Hamelberg[49] observed similar results using a different substrate and an updated molecular mechanics force field.[50] The cis conformation of the substrate was shown to bind more strongly than the trans to CypA, in excellent agreement with experiments.[21, 51, 52] From the free energy profiles, one can create a thermodynamics cycle to assess the relative free energy of binding the trans, cis and transition state configurations of the substrate by CypA.[45] It was clear to see that the free energy of binding the transition state ($\omega \sim 90^\circ$), ΔG_{ts} , was less than that of the cis, ΔG_{cis} , which was less than that of the trans, ΔG_{trans} ($\Delta G_{ts} < \Delta G_{cis} < \Delta G_{trans}$; Figure 2). The stabilizing interactions were mainly due to a combination of very long lasting hydrogen bonds involving Arg55, Asn102 and few other residues and a favorable hydrophobic effect between the side chain of proline and the non-polar proline-binding pocket. The study also revealed that the stability of the enzyme-substrate complex was directly correlated to the interaction between the substrate and Arg55, which was identified as one of the key residues in stabilizing the transition state.[45, 53] CypA binds the transition state configuration of the substrate more than 6 kcal/mol better than the trans state, enough to speed up the rate of isomerization by almost five orders of magnitude, in good agreement with experiments.[35] The free energy of binding the substrate in the different states was also calculated using the molecular dynamics simulation trajectories of the different complexes as shown in Figure 2D.[54] It was also shown that the transition state configuration of the substrate binds better than the cis configuration, which also binds better than the trans configuration (Figure 2D), consistent with the accelerated molecular dynamics simulations.

In line with previous crystal structure analysis,[55]the accelerated molecular dynamics simulations revealed that, during catalysis, rotation of the peptidylprolyl bond was achieved through the rotation of the carbonyl oxygen on the N-terminal of the prolyl peptide bond in a predominately unidirectional fashion along the positive angle[45], settling a longstanding debate.[55, 56]This direction of rotation of the carbonyl oxygen of the peptidylprolyl bond led to a favorable interaction between the carbonyl oxygen and the backbone NH of Asn102 at the twisted transition state. These observations were in contrast with conclusions an NMR experiment,[56] suggesting a C-terminal rotation during catalysis. The C-terminal of the peptide bond, which comprised the proline ring, did not rotate during catalysis and stayed firmly bound in the hydrophobic pocket of the active site. Several groups had also observed similarly enzyme-substrate interactions[53, 57]. Trzesniak et al.[58]had shown, using several regular molecular dynamics simulations,the N-terminal rotation, and not the C-terminal rotation,was consistent with the NMR experimental data. They also showed that both the cis and the trans conformations of the substrate bind to CypA with little or no distortion of the planarity of the peptidylprolyl bond angle.

However, Hur and Bruice[43] suggested that CypA recognized a slightly twisted conformation of the cis substrate ($\omega \sim 20^\circ$). The ω angle can fluctuate quite a lot in the free substrate in solution, varying up to $\pm 50^\circ$ in both the cis and trans states as was shown.[59] The fluctuation within the active site of the CypA is also significant with a minor shift in the bottom of the well when compared to the free substrate (Figure 2B). Hur and Bruice defined Near Attack Conformations of the substrate bound to the enzyme as conformations with $\omega \sim 20^\circ$ and hydrogen bond distance of about 4 Å between the carbonyl oxygen of proline and Arg55. They suggested that thermal

motions could simply generate the CypA-transition state ($\omega \sim 90^\circ$) complex from the near attack conformation, suggesting that the free energy of the CypA-near attack complex would already be closer to the CypA-transition state complex.[43] The study therefore concluded that the affinity for the transition state of the substrate by CypA was similar to that of the cis conformation. It was however not clear how the free energy barrier of the catalytic process would be different from that of the uncatalyzed reaction if the enzyme has similar affinity for both the transition state and cis state, as we have shown above (Figure 2). A recent detailed computational evaluation of the near attack hypothesis has ruled out its involvement in the HIV-1 protease enzyme.[60]

Li and Cui[53] carried out hybrid quantum mechanical and molecular mechanics simulation to specifically probe the role of Arg55 in the catalytic mechanism of CypA. In addition to wild type CypA, they studied the Arg55Ala and Arg55Lys mutants. They also carried out a simulation by zeroing out the charges on Arg55. They showed that Arg55 is mainly responsible for stabilization of the transition state of the substrate through electrostatic interactions. The Arg55Lys CypA mutant was suggested to be almost inactive due to the lack of the proper positioning of Lys to electrostatically stabilize the transition state. Li and Cui ruled out the relevancy of the proposed near attack conformations to catalysis by Hur and Bruice[43], showing that wild-type CypA preferentially binds the transition state.[53] McGowan and Hamelberg[49] identified about five hydrogen bonds and favorable non-polar interactions between the substrate and CypA that are responsible for recognition and stabilization of the transition state (Figure 2A). The hydrogen bonds were formed between all the residues of the substrate and Arg55, Gln63, Asn 102, and Trp 121 of CypA (Figure 2A). As a whole, the hydrogen bonds

were formed and broken when the substrate was in the cis and trans states but well formed when the substrate was in the transition state configuration.[49] The developing CypA-substrate hydrogen-bonding network was extremely sensitive to the configuration of the substrate.[49]The loosely formed interactions between CypA and substrate in ground states became considerably well established in the transition state that contributed to the lower free energy of binding the transition state compared to that of the cis and trans states.

Ladani and Hamelberg[48] also recently showed that during catalysis the conformations of the substrate (-AAPF-) in the active site of CypA were more confined to the β -region. In the free substrate in solution, the trans and cis configurations populated either the β - and α -helical regions, resulting in four distinct free energy basins, with the trans configuration more stable than the cis in line with experiments. As the substrate transitions from, say, the cis state to the transition state, the loss in entropy during catalysis is therefore less than that free in solution. The relative change in the entropy from the cis state to the transition state is therefore positive and favorably contributes to the stabilization of the transition state in the active site of CypA. The stabilization of the transition state was found therefore to be due to both energetic interactions (enthalpic) and a favorable entropic component.[48]

One of the limitations of the widely used classical molecular mechanics force field is the use of fixed partial charges. How accurately can the force field describe the electrostatics of the isomerization process, especially around the transition state where there is a redistribution of the electrons? Ladani and Hamelberg[48] carried out detailed free energy calculation using molecular dynamics simulations to investigate the effect of intra-molecular polarization during catalysis of

cis-trans isomerization. Intra-molecular polarization is a result of the loss of pseudo double bond character of the C-N peptide bond at the transition state, which represents the maximum deviation from the cis and trans substrate conformations and localization of the electrons on C and N. The electrostatic potentials of the substrate at the trans state and transition state were calculated using detailed quantum mechanical calculations and used to develop the partial charges for the molecular mechanics force field. Interestingly, the binding free energy of the transition state configuration of the substrate by CypA was found to be less than -1.0 kcal/mol more favorable when intra-molecular polarization was taken into consideration. Considering that the free energy barrier of isomerization free in solution is ~20 kcal/mol and that of the catalyzed process is ~10-13 kcal/mol, less than 1 kcal/mol is within the error of the method. The results therefore validated the use of molecular mechanics force fields, with fixed partial charges, to reliably describe these types of systems, in which the active site of the enzyme is essentially hydrophobic and the overall charge of the substrate does not change during the isomerization process.

The simulation results by Ladani and Hamelberg[48] mirrored earlier results by Leone et al.,[57] who investigated the mechanism of action of CypA using metadynamics with a slightly older generation of the force field parameters. The free energy of the uncatalyzed and catalyzed isomerization processes was projected on two similar reaction coordinates of the substrate, ζ and ψ of the proline residue. ζ is a pseudo-angle of the ω peptidylprolyl angle, which was suggested to be a slightly better reaction coordinate by Fischer et al.[61] Leone et al.[57] found that the dominate transition during catalysis was between the $\text{trans}_{180}(\zeta \sim \pm 180^\circ \text{ and } \psi \sim \pm 180^\circ)$ to $\text{cis}_{180}(\zeta \sim 0^\circ \text{ and } \psi \sim 180^\circ)$ and vice versa in the active site of CypA. They showed that the free energy barrier during catalysis was less than that of the free substrate in solution. They were also

able to explain the drop in catalytic activity as experimentally measured by k_{cat}/K_m of several mutants of CypA. Notably, the decrease in the catalytic activity of the R55A mutant was suggested to be due to the reduction of the trans_{180} population of the substrate when R55 was mutated to alanine.

A recent application of a combination of NMR experiments, molecular dynamics simulations, and quantum mechanical DFT calculations by Carlo Camilloni et al.[40] took another approach in pointing to the essential role of electrostatic interaction in the catalytic mechanism of CypA. They concluded that CypA provided a catalytic environment that acted on the substrate through an electrostatic handle mechanism. The electrostatic field in the catalytic site tuned the electric dipole associated with the carbonyl group of the amino acid preceding the proline in the substrate, thus causing rotation of the peptide bond between the two residues. The electrostatic handle was validated by replacing the oxygen atom of this carbonyl group with a sulfur atom. This replacement, which would also affect the stabilizing hydrogen bonding interaction between the Asn 102 of the enzyme and the carbonyl oxygen of the substrate at the transition state, resulted in significant reduction in the catalytic activity of CypA.[40] The enzyme therefore induces a smaller free energy barrier of rotation, stabilizing the transition state.

Role of Enzyme Dynamics in CypA Catalysis

It is a well-known fact that enzymes, and the substrates, are dynamic, undergoing thermal fluctuations and conformational transitions during catalysis. However, it is easy for us to interpret experimental results and catalytic mechanisms from a static view of the crystal structure of enzymes, ignoring the vast ensemble of conformations that could possibly exist. Experimental observations, free energies, NMR chemical shifts and kinetic rates, for example, are ensemble

averages that are due to orders of magnitude of molecules and events in solution. The dynamical motions of enzymes have therefore been suggested to play a vital role in catalysis and, in some studies, suggested to promote catalysis, since the enzyme and substrate are constantly bumping into each other. This idea is very controversial, underscored by intense debates in the literature over the years.[62-66] Interestingly, Cyclophilin A is one of the model systems that has been used to probe the role of enzyme dynamics in catalysis.

Agarwal et al.[44, 67] identified a network of vibrational motions in CypA ranging from picosecond to microsecond–millisecond time scales using molecular simulations. The essential parts of this dynamical network were found to be located in the highly flexible loops of CypA, extending from surface regions of the enzyme to the active site and coupled to substrate turnover. These vibrations had the ability to change critical hydrophobic and hydrophilic interactions that were shown to be necessary for the catalytic action of CypA. The conservation of crucial parts of this network in ten cyclophilin structures from six different species[67] was therefore indicative of its possible role in promoting catalysis.[68] In addition, NMR relaxation studies revealed millisecond time scale motions in Arg55, Lys82, Ala101, Asn102, Ala103, and Gly109 during substrate turnover, suggestive of the importance of such dynamical network during catalysis.[39] The conformational fluctuations of the interactions between the active site of CypA and the substrate near the transition state were found to correlate with barrier crossings.[44] Catalysis was also shown to be sensitive to the first solvation shell around the CypA-substrate complex, transferred through the fluctuations of vibrational network in the enzyme.[44]

Although the emerging evidences were supportive of functionally relevant sub-states of CypA conformations as being relevant for promoting catalysis, the transient nature and low populations of these sub-states significantly restricted their characterization.[69, 70] In finding these low population sub-states, Ramanathan et al. applied quasi-anharmonic analysis, as opposed to the widely used principal component analysis,[71, 72] to analyze the molecular simulation trajectories.[69, 70] Quasi-anharmonic analysis utilized higher-order statistics to identify sub-states on the conformational landscape of CypA with anharmonic internal motions. They developed a hierarchical description of the sub-states along the progress of the isomerization reaction, and identified sub-states with structural and dynamical features critical for stabilizing the transition state.

The role of CypA conformational dynamics was also studied by Doshi et al.[54] in an attempt to resolve the longstanding debate that has persisted in part due to the lack of realistic atomistic models in interpreting some ambiguous experimental results. The rate of trans to cis isomerization of the free substrate in solution was simulated and calculated. For a single barrier process in solution, the decay of the survival probability with time is normally a single exponential. Doshi et al.[54] observed a single exponential decay of the survival probability of the free substrate in water. Interestingly, the decay of the survival probability from trans to cis during catalysis (of the substrate in the active site of CypA) was found to fit to a multi-exponential, not a single exponential. This observation was indicative of dynamical coupling of CypA motions to the chemical step during catalysis. Further evidence supporting the intricate coupling between the motions of CypA and the substrate during catalysis came from accelerating the dynamics of CypA alone using accelerated molecular dynamics and observing the effect on

the rate of trans to cis isomerization of the substrate in the active site of CypA. A concomitant increase in the rate of trans to cis isomerization of the substrate was observed, suggesting a direct coupling between the dynamics of CypA and the substrate.[54]Ladani and Hamelberg[73] also showed that the dynamics of the solvent could also affect the catalytic rate in the active site of CypA. The dynamics of the solvent seemed to affect enzyme motions, which is in turn directly coupled to the catalysis process. Nonetheless, the real nature of this intricate enzyme-substrate coupling and the involvement of enzyme dynamical motions towards enhancing the rate of isomerization were ambiguous. Do dynamical motions of the enzyme enhance the rate of isomerization?

Doshi et al.[54] used Kramers kinetic rate theory[74, 75] in the high-friction regime to interpret their data and to estimate the effective diffusion coefficient, D_{eff} , during isomerization of the substrate free in solution and during catalysis. Kramers kinetic rate theory takes into account the effects of the environment of the process and has been shown to be applicable to biological systems and processes.[76-79] Interestingly, they estimated a smaller D_{eff} of the isomerization process in the active site of the enzyme than free in solution, suggesting that the motions of the enzymes are effectively limiting that of the substrate during catalysis. The decrease in D_{eff} in the enzyme was later confirmed by the decay of the autocorrelation function of the ω -bond in the solution and the active site of CypA.[73] The effective diffusion in the active site environment of CypA was ~ 14 times smaller than that of the free substrate in solution, in good agreement with the estimated ~ 13 times smaller D_{eff} from Kramers rate theory.[54] A rate enhancement during catalysis of $\sim 4.5 \times 10^5$ times the rate of the isomerization free in solution was estimated using Kramers rate theory, in excellent agreement with the experimental observed rate

enhancements. This rate enhancement was therefore observed to come purely from the decrease in the free energy barrier during catalysis that was a result of the stabilization of the transitions state by CypA.[45]

Are the conformation dynamics of CypA therefore important for catalysis? The answer is yes. These conformational changes may not enhance the rate of isomerization, but they are necessary to shepherd or guide the substrate through a lower free energy path. McGowan and Hamelberg[49] showed that the conformational dynamics of the active site residues of free CypA sampled a very broad conformational space and the conformations of the active site residues of bound CypA were inherent in the free enzyme. Hence, CypA tends to bind its substrate via conformational selection, where rearrangements of certain key active site residues and loops are necessary for substrate recognition. The conformational ensemble of the active site residues of CypA that recognized the substrate in the transitions state was slightly different and more compact than those used by the enzyme to bind the cis and trans states of the substrate. The active site was intrinsically plastic during catalysis and could readily change its conformation to accommodate the substrate in the different states, which are slightly different during the progress of the reaction.[49] As noted above, the loosely formed interactions between CypA and the substrate in the ground cis and trans states became considerably well formed in the transition state.

What are some of the possible implications of the conformational dynamics of CypA in drug design? Nagaraju et al.[80] hypothesized that the collective enzyme conformations most relevant for function should be used for structure based drug design. Although static x-ray crystal

structures are valuable starting points for modeling, they lack critical information about the ensemble and the functional dynamics of the enzyme. Nagaraju et al. showed that the conformations of the active site of CypA, bound to several known active, non-peptidomimetic inhibitors were similar to those that recognized the natural substrate in its different states.[80] The results suggested that the conformations of the active site residues of CypA that recognize the substrate in the tightly bound trans and cis states are more relevant in de novo structure-based drug design. Interestingly, slightly less than half of the ensemble of conformations of the active site of the free enzyme was used for recognizing the substrate in the different conformations.[80]

Peptidyl-prolyl *cis-trans* isomerase NIMA-interacting 1 (Pin1)

Pin1 is a member of the parvulin family of PPIases and unique amongst this class.[4] It has two domains, a catalytic domain and a WW domain, and specifically binds phosphorylated Ser/Thr (pSer/pThr)-Pro motifs of its substrates and catalyzes *cis-trans* isomerization of the peptidyl-prolyl ω -bond reversibly.[4, 5] The WW domain does not have catalytic function and binds only the trans configuration of the substrate.[81] The catalytic domain maintains almost all of its activity *in vitro* when the WW domain is not present.[82, 83] The role of the WW-domain in Pin1 function is not well understood. Regulation of phosphorylation dependent prolyl *cis-trans* isomerization by Pin1 is involved in several cellular signaling pathways, including cell growth and division, DNA repair, apoptosis, and transcription. Pin1 has directly been implicated in many diseases, including cancer[9, 32] and Alzheimer's.[84-86]

Structural analyses have revealed that, unlike CypA and FKBP, Pin1 contains polar residues deep in the active site[87] Two His residues (His59 and His157) are found side by side at the central

core of the active site. Cys113 and Ser154 act as “gate keepers” and are located at either ends of the substrate-binding site. The overall involvement of these residues in Pin1 catalysis remains largely unknown. Cys113 was suggested to participate in a covalent mechanism by Ranganathan et al.[88] Behrsin et al. called the covalent catalytic mechanism into question when the enzyme was found to retain its activity after mutating Cys113 to Asp.[89] A non-covalent mechanism was therefore hypothesized to speed up the catalytic rate of cis-trans isomerization. In spite of numerous experimental and computational studies, proposing a sequence of steps involved in the enzymatic mechanism of Pin1 is still a challenge.

The orientations of His59 and His157 were found to be ambiguous from many x-ray crystal structures of Pin1 in the Protein Data Bank. The identity of the nitrogen and carbon atoms in the imidazole side chains was not obvious from especially low-resolution x-ray crystal structures. The MD simulation study with detailed free calculations by Barman and Hamelberg aimed to resolve the most probable orientation of His59 and His157.[90] The study revealed that these residues form an extended hydrogen-bonding network in the active site with the conserved Cys113 and Thr152, similar to the hydrogen bonding network observed for Par14 from a high-resolution x-ray crystal structure by Mueller et al.[91] Barman and Hamelberg proposed a dynamic hydrogen-bonding network that was modulated by the protonation state of Cys113, His59, and His157. The pK_a of Cys113 was significantly perturbed in the absence of the substrate. Cys113 was unprotonated in the free Pin1 while neutral in the presence of the substrate during catalytic turnover, providing a detailed atomistic explanation for the reactive properties of free Pin1.

Recent quantum mechanical analysis of Pin1 by Voringer-Martinez et al. suggested a correlation between certain active site properties and catalysis.[92] According to their findings, both structural and electrostatic characteristics of active site have significant effects on the mechanism of isomerization. The free energy barrier between the cis and trans state of the uncatalyzed phosphorylated substrate was calculated to be ~20 kcal/mol, similar to the isomerization free energy barrier of the unphosphorylated peptidylprolyl bond.[83, 93] Voringer-Martinez et al. concluded that Pin1 did not reduce the barrier from cis to trans, and almost specifically catalyzed the substrate from trans to cis through a barrier-less process.[92] The authors proposed a mechanism through ground state destabilization of the substrate. However, these conclusions contradicted recent NMR experiments of the catalytic process that showed the rates of cis to trans and trans to cis transitions to be in the millisecond time scale, representing an appreciable barrier separating the cis and trans state during catalysis.[83] Also, their proposed mechanism contradicts the fact that Pin1 has only a slightly higher affinity for the cis state than the trans state.[81, 83, 94] According to their uncatalyzed and catalyzed free energy profiles, using a thermodynamic cycle such as that shown above for CypA (Figure 2C), one would estimate the free energy of binding the substrate in the trans configuration, ΔG_{trans} , to be similar to the free energy of binding the substrate in the transition state configuration, ΔG_{ts} ($\Delta G_{\text{trans}} \approx \Delta G_{\text{ts}}$) and about 20 kcal/mol more favorable than the free energy of binding the cis configuration of the substrate, ΔG_{cis} ($\Delta G_{\text{trans}} \ll \Delta G_{\text{cis}}$).

In a follow-up study of Pin1, Voringer-Martinez et al. proposed an explanation for the role of Cys113, Ser154 and Gln131 in catalysis.[95] They suggested a “jump rope” type of isomerization process from the trans to the cis state through the barrier-less process. They suggested that one

end of the peptide (phosphate group of pThr) was anchored to the basic triad (Lys63, Arg68, Arg69) and the other end was restricted through enzyme-substrate hydrogen bonding interactions. Ser154 in association with the Gln131 stabilized the rotation of the trans conformation and assisted in the isomerization from trans to cis. Cys113, on the other hand, was suggested to play a structural role during the isomerization of the cis isomer. However, the barrier from cis to trans was as high as the uncatalyzed process. Cys113 was found to form a hydrogen bond with the carbonyl oxygen of the peptidylprolyl bond when the substrate was in the cis conformation. Voringer-Martinez et al.[92, 95] stated that their proposed mechanism was supported by recent kinetic isotope effects study by Mercedes-Camacho et al., in which Pin1 was suggested to bind a twisted conformation of the substrate.[96] However, it is not clear how the kinetic isotope effect experiments directly support the destabilization mechanism proposed by Voringer-Martinez et al. Pin1 can certainly bind a twisted ground state configuration of the substrate due to conformational selection or induced fit, but the overall free energy of the enzyme-substrate complex cannot be higher than the sum of the free energies of the enzyme and the substrate as we demonstrate below.

Similar to the approach used to study CypA, Velazquez and Hamelberg studied the catalytic mechanism of Pin1 by directly simulating the isomerization free in solution and in the active site of Pin1 using accelerated molecular dynamics simulations (Figure 3).[97] Initially, Velazquez and Hamelberg had shown that Pin1 recognized its substrate in a particular backbone configuration via conformational selection.[98] Pin1 selectively bound sub-microstates of the substrate amongst several microstates of the substrate free in solution. The simulation results suggested that the isomerization process and Pin1-substrate interactions were intricately coupled

to the conformational dynamics of the enzyme during catalysis, similar to the results observed for CypA.[49, 98] Many key interactions, including the interactions between the phosphate moiety of the phosphorylated substrate and the residues of the phosphate-binding pocket of Pin1 (Figure 3; Lys63, Arg68, and Arg69), were sensitive to the state of the substrate during catalysis. For example, the interactions between the phosphate group of the substrate and Pin1 were found to be more favorable when the substrate was in the transition state configuration ($\omega \sim 90^\circ$). Arg68 was not as sensitive to the state of the substrate as Arg69 and Lys63, in excellent agreement with experiment. Experimentally, it had been shown that mutation of Arg68 to Ala did not drastically affect binding and catalysis.[82] Cys113 was also shown to favorably interact with the carbonyl oxygen of the peptidylprolyl bond of the substrate in both cis,[90] similar to the observation by Voringner-Martinez et al.,[95] and the transition state configuration. The more favorable interactions between Pin1 and the transition state of the substrate resulted in Pin1 binding the transition state configuration better than the cis and trans ground states (Figure 3).[97] The cis configuration was found to also bind better to Pin1 than the trans configuration, in agreement with all of the experimental findings.[81, 83, 94] The preferential binding of the transition state of the substrate led to a calculated catalytic isomerization barrier of ~ 13 kcal/mol, a reduction of ~ 7 kcal/mol in the uncatalyzed free energy barrier, in excellent agreement with experiments.[83, 93] These computational studies provided additional support for the non-covalent mechanism of Pin1 facilitated by stabilization of the transition state due to key interactions between the enzyme and the substrate.

Another recent computational study of the catalytic mechanism of Pin1 by Di Martino et al. used molecular dynamics simulations and umbrella sampling to drive the isomerization process along

the peptidylprolyl dihedral angle.[99] They also showed that the bound conformational space of the substrate was much reduced. Their results suggested that the active site of Pin1 was pre-organized to accommodate the substrate in a perturbed ground state conformation that positioned an amide hydrogen of the substrate to the prolyl nitrogen of the peptidylprolyl bond. Pyramidalization of the nitrogen of the peptidylprolyl bond and the possible hydrogen bond with an amide hydrogen could lead to a possible autocatalysis, similar to the earlier suggestion by Fischer et al. in the context of FKBP[11]. They therefore suggested a possible autocatalytic mechanism of Pin1 that resulted in the stabilization of the transition state by also ~ 6 kcal/mol through additional stabilizing interactions between Pin1 and the substrate.

Can catalysis occur without stabilizing the transition state?

Experimental and computational studies carried out on peptidylprolyl *cis-trans* isomerases in the last several decades have facilitated discussions and speculations on the contributing factors in enzyme catalysis. Amongst these suggestions, the most common in the literature involve stabilization of the transition state of the substrate due to preferential binding by the enzyme, a combination of binding a twisted conformation of the substrate by the enzyme and transition state stabilization, and substrate destabilization, which posits that the enzyme binds a substrate conformation that is closer to the transition state such that the catalytic barrier is lower without the enzyme stabilizing the transition state. Ultimately, the thermodynamic free energies, not the energy or enthalpy, have to be taken into account in order to rationalize the catalytic mechanism. A favorable interaction (negative change in free energy) between the enzyme and the substrate will always lead to a lower free energy of the complex compared to the sum of the free energies of the enzyme and substrate separately. Otherwise, binding will not occur and the enzyme will

not have an affinity for its substrate, which is counterintuitive and leads to an ineffective enzyme.

Let's first take a look at the classical representation of enzyme catalysis, schematically shown in Figure 4A. Wolfenden and Snider have presented a more detailed review on this subject.[100] The y-axis represents the free energy and the x-axis represents the progress of the reaction from cis to trans along the peptidylprolyl angle. The cis configuration is $\sim 0^\circ$, the transition state is $\sim 90^\circ$, and the trans conformation is $\sim 180^\circ$. The free energy profile of the uncatalyzed reaction (black line) goes from the cis to a higher free energy transition state to a lower free energy trans state, assuming cis is the reactant and trans is the product. The free energy barrier is the difference between the free energy of the transition state and the cis state that determines the rate of the reaction -the larger the free energy barrier the slower the rate and vice versa. When the enzyme binds the reactant (cis configuration) the free energy of the complex becomes lower than the sum of the free energies of the enzyme and the reactant and the difference in the free energies is the change in binding free energy. It is assumed in Figure 4A that the enzyme binds the dominant conformation of free cis in solution. In order for the catalyzed free energy barrier to be lower than that of the uncatalyzed barrier, the difference between the free energy of the enzyme-transition state complex and the sum of the free energies of the enzyme and substrate at the transition state has to be much larger (more negative) than that of the binding free energy between the enzyme and the reactant as illustrated in Figure 4A. PPIases bind the cis and trans isomers of their substrates favorably with a negative free energy change.[81-83, 101] The enzyme therefore has to bind the transition state configuration of the substrate much better than the cis conformation of the substrate. If the enzyme's affinity for the transition state is similar to

that of the substrate in the cis (reactant), then the free energy barrier of the catalyzed process will simply be that of the uncatalyzed process, with no observed rate enhancement (Figure 4B).

Similarly, if the enzyme binds a twisted or destabilized cis conformation of the substrate, which is likely, the overall change in the binding free energy has to also be negative, as observed experimentally, and the free energy of the complex has to be lowered (Figure 4C). Similar to the previous scenario (Figure 4A), a lower catalytic barrier and enhanced rate can therefore only be achieved if the catalytic free energy barrier is lower than the uncatalyzed free energy barrier due to preferential binding of the transition state configuration of the substrate by the enzyme. If the enzyme does not favorably bind the transition state (Figure 4D), the catalytic free energy barrier will be similar to that of the uncatalyzed free energy barrier without any rate enhancement. Therefore, even when the enzyme binds a conformation of the substrate that is twisted or away from its dominant conformation in solution (the so called destabilized substrate), the enzyme has to also preferentially bind the transition state in order to increase the rate.

What is the limit of the twisted or destabilized conformation of a substrate that an enzyme can bind? If the enzyme binds the twisted substrate by selecting the twisted free substrate out of solution, $E + S \rightleftharpoons E + S^* \rightleftharpoons E:S^*$, where S^* is the twisted conformation, the rate of $S \rightarrow S^*$ will be rate limiting if S^* has a very high free energy and is close to the transition state. In the limiting case when the enzyme directly binds the transition state, as illustrated in Figure 4E, the process will be downhill after binding, but the rate-limiting step for catalysis will be the rate of $S \rightarrow S_{ts}$, which is simply the uncatalyzed rate and is counterintuitive.

Another argument that is counterintuitive is the suggestion that the free energy of the enzyme-substrate complex is higher than the sum of the free energies of the enzyme and the substrate, leading to a smaller barrier without stabilizing the transition state (Figure 4F). The main reason why this argument is counterintuitive is because complex formation always leads to a negative binding free energy, as shown experimentally, hence a lower free energy of the complex (Figure 4C, D).

It is therefore hard to imagine the scenario where the enzyme does not have a higher affinity for the transition state in order to have a lower catalytic free energy barrier and a faster catalytic rate. As stated above, the enzyme can bind a conformation of the substrate that is twisted, but the binding free energy has to ultimately be negative, which will also suggest an even greater affinity for the transition state configuration. In answering the question posed in the subtitle, it is therefore not clear how catalysis can occur in PPIases without stabilizing the transition state of the substrate.

Concluding Remarks

Computational studies on PPIases have progressively contributed to our understanding of the mechanism of action and nature of this particular family of enzymes and enzymes in general. These studies have provided atomistic and molecular level details that are beyond the limit of current experimental techniques. Computational approaches have allowed us to rigorously probe the validity of several proposed mechanisms and put forward many testable hypotheses. It is now clear and well accepted that catalysis by PPIases occurs without any bond formation or breakage, partly due to detailed atomistic computational studies. Among several proposed mechanisms of

PPIases, stabilization of the transition state configuration of the substrate due to preferential binding by the enzymes gives rise to results that are in accord with both thermodynamic intuition and experimental data. A consensus mechanism can therefore be summarized by Figure 4A or Figure 4C, depending on the conformation of the substrate that is recognized by the enzyme. Preferential binding of the transition state by PPIases during catalysis is suggestive of their functional similarity with implications for designing efficient inhibitors that are not only transition state mimics but also that can target the ensemble of functional enzyme conformations.

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References

- [1] P. Wang, J. Heitman, The cyclophilins, *Genome Biology*, 6 (2005) 226.
- [2] J.F. Brandts, H.R. Halvorson, M. Brennan, Consideration of the possibility that the slow step in protein denaturation reactions is due to cis-trans isomerism of proline residues, *Biochemistry*, 14 (1975) 4953-4963.
- [3] J. Fanghanel, G. Fischer, Insights into the catalytic mechanism of peptidyl prolyl cis/trans isomerases, *Frontiers in Bioscience*, 9 (2004) 3453-3478.

- [4] M.B. Yaffe, M. Schutkowski, M. Shen, X.Z. Zhou, P.T. Stukenberg, J.-U. Rahfeld, J. Xu, J. Kuang, M.W. Kirschner, G. Fischer, L.C. Cantley, K.P. Lu, Sequence-Specific and Phosphorylation-Dependent Proline Isomerization: A Potential Mitotic Regulatory Mechanism, *Science*, 278 (1997) 1957-1960.
- [5] K. Ping Lu, S.D. Hanes, T. Hunter, A human peptidyl-prolyl isomerase essential for regulation of mitosis, *Nature*, 380 (1996) 544-547.
- [6] J.P. Steiner, T.M. Dawson, M. Fotuhi, C.E. Glatt, A.M. Snowman, N. Cohen, S.H. Snyder, High brain densities of the immunophilin FKBP colocalized with calcineurin, *Nature*, 358 (1992) 584-587.
- [7] F. Sun, P. Li, Y. Ding, L. Wang, et al., Design and Structure-Based Study of New Potential FKBP12 Inhibitors, *Biophysical Journal*, 85 (2003) 3194-3201.
- [8] C. Sich, S. Improta, D.J. Cowley, C. Guenet, J.-P. Merly, M. Teufel, V. Saudek, Solution structure of a neurotrophic ligand bound to FKBP12 and its effects on protein dynamics, *European Journal of Biochemistry*, 267 (2000) 5342-5355.
- [9] M. Theuerkorn, G. Fischer, C. Schiene-Fischer, Prolyl cis/trans isomerase signalling pathways in cancer, *Curr. Opin. Pharmacol.*, 11 (2011) 281-287.
- [10] M. Orozco, J. Tirado-Rives, W.L. Jorgensen, Mechanism for the rotamase activity of FK506 binding protein from molecular dynamics simulations, *Biochemistry*, 32 (1993) 12864-12874.
- [11] S. Fischer, S. Michnick, M. Karplus, A mechanism for rotamase catalysis by the FK506 binding protein (FKBP), *Biochemistry*, 32 (1993) 13830-13837.

- [12] I. Nakanishi, D.G. Fedorov, K. Kitauro, Molecular recognition mechanism of FK506 binding protein: An all-electron fragment molecular orbital study, *Proteins: Structure, Function, and Bioinformatics*, 68 (2007) 145-158.
- [13] Y. Xu, R. Wang, A computational analysis of the binding affinities of FKBP12 inhibitors using the MM-PB/SA method, *Proteins: Structure, Function, and Bioinformatics*, 64 (2006) 1058-1068.
- [14] S. Szep, S. Park, E.T. Boder, G.D. Van Duyne, J.G. Saven, Structural coupling between FKBP12 and buried water, *Proteins*, 74 (2009) 603-611.
- [15] M.K. Rosen, R.F. Standaert, A. Galat, M. Nakatsuka, S.L. Schreiber, Inhibition of FKBP Rotamase Activity by Immunosuppressant FK506: Twisted Amide Surrogate, *Science*, 248 (1990) 863-866.
- [16] O. Acevedo, Z. Ambrose, P.T. Flaherty, H. Aamer, P. Jain, S.V. Sambasivarao, Identification of HIV inhibitors guided by free energy perturbation calculations, *Current pharmaceutical design*, 18 (2012) 1199-1216.
- [17] T. Hediger, W. Frank, M. Schumann, G. Fischer, M. Braun, Aryl hetaryl ketones and thioketones as efficient inhibitors of peptidyl-prolyl cis-trans isomerases, *Chemistry & biodiversity*, 9 (2012) 2618-2634.
- [18] S.V. Sambasivarao, O. Acevedo, Computational insight into small molecule inhibition of cyclophilins, *Journal of chemical information and modeling*, 51 (2011) 475-482.

- [19] S. Daum, M. Schumann, S. Mathea, T. Aumuller, M.A. Balsley, S.L. Constant, B.F. de Lacroix, F. Kruska, M. Braun, C. Schiene-Fischer, Isoform-specific inhibition of cyclophilins, *Biochemistry*, 48 (2009) 6268-6277.
- [20] S. Daum, F. Erdmann, G. Fischer, B. Feaux de Lacroix, A. Hessamian-Alinejad, S. Houben, W. Frank, M. Braun, Aryl indanyl ketones: efficient inhibitors of the human peptidyl prolyl cis/trans isomerase Pin1, *Angewandte Chemie*, 45 (2006) 7454-7458.
- [21] J. Kallen, M.D. Walkinshaw, The X-ray structure of a tetrapeptide bound to the active site of human cyclophilin A, *FEBS Letters*, 300 (1992) 286-290.
- [22] E.M. Duffy, D.L. Severance, W.L. Jorgensen, Solvent effects on the barrier to isomerization for a tertiary amide from ab initio and Monte Carlo calculations, *J. Am. Chem. Soc.*, 114 (1992) 7535-7542.
- [23] S.T. Park, R.A. Aldape, O. Futer, M.T. DeCenzo, D.J. Livingston, PPIase catalysis by human FK506-binding protein proceeds through a conformational twist mechanism, *Journal of Biological Chemistry*, 267 (1992) 3316-3324.
- [24] G.D. Van Duyne, R.F. Standaert, P.A. Karplus, S.L. Schreiber, J. Clardy, Atomic Structures of the Human Immunophilin FKBP-12 Complexes with FK506 and Rapamycin, *Journal of Molecular Biology*, 229 (1993) 105-124.
- [25] T.L. Davis, J.R. Walker, V. Campagna-Slater, P.J. Finerty, Jr., R. Paramanathan, G. Bernstein, F. MacKenzie, W. Tempel, H. Ouyang, W.H. Lee, E.Z. Eisenmesser, S. Dhe-Paganon, Structural and Biochemical Characterization of the Human Cyclophilin Family of Peptidyl-Prolyl Isomerases, *PLoS Biol*, 8 (2010) e1000439.

- [26] H.M. Ke, L.D. Zydowsky, J. Liu, C.T. Walsh, Crystal structure of recombinant human T-cell cyclophilin A at 2.5 Å resolution, *Proc Natl Acad Sci U S A*, 88 (1991) 9483-9487.
- [27] K.N. Brazin, R.J. Mallis, D.B. Fulton, A.H. Andreotti, Regulation of the tyrosine kinase Itk by the peptidyl-prolyl isomerase cyclophilin A, *Proceedings of the National Academy of Sciences*, 99 (2002) 1899-1904.
- [28] S.A. Helekar, D. Char, S. Neff, J. Patrick, Prolyl isomerase requirement for the expression of functional homo-oligomeric ligand-gated ion channels, *Neuron*, 12 (1994) 179-189.
- [29] M. Li, H. Wang, F. Li, W.E. Fisher, C. Chen, Q. Yao, Effect of cyclophilin A on gene expression in human pancreatic cancer cells, *American Journal of Surgery*, 190 (2005) 739-745.
- [30] V. Yurchenko, G. Zybarth, M. O'Connor, W.W. Dai, G. Franchin, T. Hao, H. Guo, H.-C. Hung, B. Toole, P. Gallay, B. Sherry, M. Bukrinsky, Active Site Residues of Cyclophilin A Are Crucial for Its Signaling Activity via CD147, *Journal of Biological Chemistry*, 277 (2002) 22959-22965.
- [31] J. Colgan, M. Asmal, M. Neagu, B. Yu, J. Schneidkraut, Y. Lee, E. Sokolskaja, A. Andreotti, J. Luban, Cyclophilin A Regulates TCR Signal Strength in CD4⁺ T Cells via a Proline-Directed Conformational Switch in Itk, *Immunity*, 21 (2004) 189-201.
- [32] K.P. Lu, X.Z. Zhou, The prolyl isomerase PIN1: a pivotal new twist in phosphorylation signalling and disease, *Nat. Rev. Mol. Cell. Biol.*, 8 (2007) 904-916.

- [33] G. Fischer, H. Bang, C. Mech, Determination of enzymatic catalysis for the cis-trans-isomerization of peptide binding in proline-containing peptides, *Biomed Biochim Acta*, 43 (1984) 1101.
- [34] G. Fischer, B. Wittmann-Liebold, K. Lang, T. Kiefhaber, F.X. Schmid, Cyclophilin and peptidyl-prolyl cis-trans isomerase are probably identical proteins, *Nature*, 337 (1989) 476-478.
- [35] D. Kern, G. Kern, G. Scherer, G. Fischer, T. Drakenberg, Kinetic-Analysis of Cyclophilin-Catalyzed Prolyl Cis/Trans Isomerization by Dynamic Nmr-Spectroscopy, *Biochemistry*, 34 (1995) 13594-13602.
- [36] F.F. Vajdos, S. Yoo, M. Houseweart, W.I. Sundquist, C.P. Hill, Crystal structure of cyclophilin A complexed with a binding site peptide from the HIV-1 capsid protein, *Protein Science*, 6 (1997) 2297-2307.
- [37] E.Z. Eisenmesser, O. Millet, W. Labeikovsky, D.M. Korzhnev, M. Wolf-Watz, D.A. Bosco, J.J. Skalicky, L.E. Kay, D. Kern, Intrinsic dynamics of an enzyme underlies catalysis, *Nature*, 438 (2005) 117-121.
- [38] S. Yoo, D.G. Myszka, C.-y. Yeh, M. McMurray, C.P. Hill, W.I. Sundquist, Molecular recognition in the HIV-1 capsid/cyclophilin A complex, *J. Mol. Biol.*, 269 (1997) 780-795.
- [39] P.K. Agarwal, *Enzymes: An integrated view of structure, dynamics and function*, Microbial Cell Factories, 5 (2006).
- [40] C. Camilloni, A.B. Sahakyan, M.J. Holliday, N.G. Isern, F.L. Zhang, E.Z. Eisenmesser, M. Vendruscolo, Cyclophilin A catalyzes proline isomerization by an electrostatic handle

mechanism, *Proceedings of the National Academy of Sciences of the United States of America*, 111 (2014) 10203-10208.

[41] R.K. Harrison, R.L. Stein, Mechanistic studies of peptidyl prolyl cis-trans isomerase: evidence for catalysis by distortion, *Biochemistry*, 29 (1990) 1684-1689.

[42] E.S. Eberhardt, S.N. Loh, A.P. Hinck, R.T. Raines, Solvent Effects on the Energetics of Prolyl Peptide Bond Isomerization, *Journal of the American Chemical Society*, 114 (1992) 5437-5439.

[43] S. Hur, T.C. Bruice, The Mechanism of Cis–Trans Isomerization of Prolyl Peptides by Cyclophilin, *Journal of the American Chemical Society*, 124 (2002) 7303-7313.

[44] P.K. Agarwal, Role of protein dynamics in reaction rate enhancement by enzymes, *Journal of the American Chemical Society*, 127 (2005) 15248-15256.

[45] D. Hamelberg, J.A. McCammon, Mechanistic insight into the role of transition-state stabilization in cyclophilin A, *Journal of the American Chemical Society*, 131 (2009) 147-152.

[46] D. Hamelberg, J. Mongan, J.A. McCammon, Accelerated molecular dynamics: a promising and efficient simulation method for biomolecules, *The Journal of chemical physics*, 120 (2004) 11919-11929.

[47] U. Doshi, D. Hamelberg, Towards fast, rigorous and efficient conformational sampling of biomolecules: Advances in accelerated molecular dynamics, *Biochimica et biophysica acta*, (2014).

- [48] S.T. Ladani, D. Hamelberg, Entropic and Surprisingly Small Intramolecular Polarization Effects in the Mechanism of Cyclophilin A, *Journal of Physical Chemistry B*, 116 (2012) 10771-10778.
- [49] Lauren C. McGowan, D. Hamelberg, Conformational Plasticity of an Enzyme during Catalysis: Intricate Coupling between Cyclophilin A Dynamics and Substrate Turnover, *Biophysical Journal*, 104 (2013) 216-226.
- [50] U. Doshi, D. Hamelberg, Reoptimization of the AMBER force field parameters for peptide bond (Omega) torsions using accelerated molecular dynamics, *The journal of physical chemistry. B*, 113 (2009) 16590-16595.
- [51] L.T. Kakalis, I.M. Armitage, Solution Conformation of a Cyclophilin-Bound Proline Isomerase Substrate, *Biochemistry*, 33 (1994) 1495-1501.
- [52] H.M. Ke, D. Mayrose, W. Cao, Crystal-Structure of Cyclophilin-a Complexed with Substrate Ala-Pro Suggests a Solvent-Assisted Mechanism of Cis-Trans Isomerization, *Proceedings of the National Academy of Sciences of the United States of America*, 90 (1993) 3324-3328.
- [53] G.H. Li, Q. Cui, What is so special about Arg 55 in the catalysis of cyclophilin A? Insights from hybrid QM/MM simulations, *Journal of the American Chemical Society*, 125 (2003) 15028-15038.
- [54] U. Doshi, C.L. McGowan, S. Tork Ladani, D. Hamelberg, Resolving the complex role of enzyme

conformational dynamics in catalytic function, Proceedings of the National Academy of Sciences of the United States of America, Early Edition (2012).

[55] B.R. Howard, F.F. Vajdos, S. Li, W.I. Sundquist, C.P. Hill, Structural insights into the catalytic mechanism of cyclophilin A, *Nat Struct Mol Biol*, 10 (2003) 475-481.

[56] E.Z. Eisenmesser, D.A. Bosco, M. Akke, D. Kern, Enzyme Dynamics During Catalysis, *Science*, 295 (2002) 1520-1523.

[57] V. Leone, G. Lattanzi, C. Molteni, P. Carloni, Mechanism of action of cyclophilin a explored by metadynamics simulations, *PLoS Comput Biol*, 5 (2009) e1000309.

[58] D. Trzesniak, W.F. Van Gunsteren, Catalytic mechanism of cyclophilin as observed in molecular dynamics simulations: Pathway prediction and reconciliation of X-ray crystallographic and NMR solution data, *Protein Science*, 15 (2006) 2544-2551.

[59] Q. Johnson, U. Doshi, T.Y. Shen, D. Hamelberg, Water's Contribution to the Energetic Roughness from Peptide Dynamics, *J Chem Theory Comput*, 6 (2010) 2591-2597.

[60] K.S. Sadiq, P.V. Coveney, Computing the Role of Near Attack Conformations in an Enzyme-Catalyzed Nucleophilic Bimolecular Reaction, *J Chem Theory Comput*, (2014) 10.1021/ct5008845.

[61] S. Fischer, R.L. Dunbrack, M. Karplus, Cis-Trans Imide Isomerization of the Proline Dipeptide, *Journal of the American Chemical Society*, 116 (1994) 11931-11937.

[62] G.G. Hammes, Multiple Conformational Changes in Enzyme Catalysis†, *Biochemistry*, 41 (2002) 8221-8228.

- [63] K.A. Henzler-Wildman, M. Lei, V. Thai, S.J. Kerns, M. Karplus, D. Kern, A hierarchy of timescales in protein dynamics is linked to enzyme catalysis, *Nature*, 450 (2007) 913-916.
- [64] A.V. Pisliakov, J. Cao, S.C.L. Kamerlin, A. Warshel, Enzyme millisecond conformational dynamics do not catalyze the chemical step, *Proceedings of the National Academy of Sciences*, 106 (2009) 17359-17364.
- [65] M. Karplus, Role of conformation transitions in adenylate kinase, *Proceedings of the National Academy of Sciences*, 107 (2010) E71.
- [66] S.C.L. Kamerlin, A. Warshel, Reply to Karplus: Conformational dynamics have no role in the chemical step, *Proceedings of the National Academy of Sciences*, 107 (2010) E72.
- [67] P.K. Agarwal, A. Geist, A. Gorin, Protein Dynamics and Enzymatic Catalysis: Investigating the Peptidyl-Prolyl Cis-Trans Isomerization Activity of Cyclophilin A†, *Biochemistry*, 43 (2004) 10605-10618.
- [68] A. Ramanathan, P.K. Agarwal, Evolutionarily Conserved Linkage between Enzyme Fold, Flexibility, and Catalysis, *Plos Biology*, 9 (2011).
- [69] A. Ramanathan, A.J. Savol, C.J. Langmead, P.K. Agarwal, C.S. Chennubhotla, Discovering Conformational Sub-States Relevant to Protein Function, *Plos One*, 6 (2011).
- [70] A. Ramanathan, A. Savol, V. Burger, C.S. Chennubhotla, P.K. Agarwal, Protein Conformational Populations and Functionally Relevant Substates, *Accounts of Chemical Research*, 47 (2014) 149-156.

- [71] M.A. Balsera, W. Wriggers, Y. Oono, K. Schulten, Principal Component Analysis and Long Time Protein Dynamics, *The Journal of Physical Chemistry*, 100 (1996) 2567-2572.
- [72] G.G. Maisuradze, A. Liwo, H.A. Scheraga, Principal Component Analysis for Protein Folding Dynamics, *Journal of Molecular Biology*, 385 (2009) 312-329.
- [73] S. Tork Ladani, D. Hamelberg, Intricacies of interactions, dynamics and solvent effects in enzyme catalysis: a computational perspective on cyclophilin A, *Molecular Simulation*, (2014) 1-12.
- [74] H.A. Kramers, Brownian motion in a field of force and the diffusion model of chemical reactions, *Physica*, 7 (1940) 284-304.
- [75] P. Hänggi, P. Talkner, M. Borkovec, Reaction-rate theory: fifty years after Kramers, *Reviews of Modern Physics*, 62 (1990) 251-341.
- [76] H.X. Zhou, Rate theories for biologists, *Quarterly Reviews of Biophysics*, 43 (2010) 219-293.
- [77] N.D. Socci, J.N. Onuchic, P.G. Wolynes, Diffusive dynamics of the reaction coordinate for protein folding funnels, *The Journal of Chemical Physics*, 104 (1996) 5860-5868.
- [78] R.B. Best, G. Hummer, Diffusive Model of Protein Folding Dynamics with Kramers Turnover in Rate, *Physical Review Letters*, 96 (2006) 228104.
- [79] H.S. Chung, W.A. Eaton, Single-molecule fluorescence probes dynamics of barrier crossing, *Nature*, 502 (2013) 685-688.

- [80] M. Nagaraju, L.C. McGowan, D. Hamelberg, Cyclophilin A Inhibition: Targeting Transition-State-Bound Enzyme Conformations for Structure-Based Drug Design, *Journal of chemical information and modeling*, 53 (2013) 403-410.
- [81] A.T. Namanja, X.J. Wang, B. Xu, A.Y. Mercedes-Camacho, K.A. Wilson, F.A. Etzkorn, J.W. Peng, Stereospecific gating of functional motions in Pin1, *P. Natl. Acad. Sci. USA*, 108 (2011) 12289-12294.
- [82] S. Daum, J. Fanghanel, D. Wildemann, C. Schiene-Fischer, Thermodynamics of phosphopeptide binding to the human peptidyl prolyl cis/trans isomerase Pin1, *Biochemistry*, 45 (2006) 12125-12135.
- [83] A. Greenwood, M. Rogals, S. De, K. Lu, E. Kovrigin, L. Nicholson, Complete determination of the Pin1 catalytic domain thermodynamic cycle by NMR lineshape analysis, *J. Biol. NMR*, 51 (2011) 21-34.
- [84] A. Bulbarelli, E. Lonati, E. Cazzaniga, M. Gregori, M. Masserini, Pin1 affects Tau phosphorylation in response to A β oligomers, *Mol. Cell. Neurosci.*, 42 (2009) 75-80.
- [85] P.-J. Lu, G. Wulf, X.Z. Zhou, P. Davies, K.P. Lu, The prolyl isomerase Pin1 restores the function of Alzheimer-associated phosphorylated tau protein, *Nature*, 399 (1999) 784-788.
- [86] L. Pastorino, A. Sun, P.-J. Lu, X.Z. Zhou, M. Balastik, G. Finn, G. Wulf, J. Lim, S.-H. Li, X. Li, W. Xia, L.K. Nicholson, K.P. Lu, The prolyl isomerase Pin1 regulates amyloid precursor protein processing and amyloid-[beta] production, *Nature*, 440 (2006) 528-534.

- [87] Y. Zhang, S. Daum, D. Wildemann, X.Z. Zhou, M.A. Verdecia, M.E. Bowman, C. Lücke, T. Hunter, K.-P. Lu, G. Fischer, J.P. Noel, Structural Basis for High-Affinity Peptide Inhibition of Human Pin1, *ACS Chem. Biol.*, 2 (2007) 320-328.
- [88] R. Ranganathan, K.P. Lu, T. Hunter, J.P. Noel, Structural and Functional Analysis of the Mitotic Rotamase Pin1 Suggests Substrate Recognition Is Phosphorylation Dependent, *Cell*, 89 (1997) 875-886.
- [89] C.D. Behrsin, M.L. Bailey, K.S. Bateman, K.S. Hamilton, L.M. Wahl, C.J. Brandl, B.H. Shilton, D.W. Litchfield, Functionally Important Residues in the Peptidyl-prolyl Isomerase Pin1 Revealed by Unigenic Evolution, *J. Mol. Biol.*, 365 (2007) 1143-1162.
- [90] A. Barman, D. Hamelberg, Cysteine-Mediated Dynamic Hydrogen-Bonding Network in the Active Site of Pin1, *Biochemistry*, 53 (2014) 3839-3850.
- [91] J.W. Mueller, N.M. Link, A. Matena, L. Hoppstock, A. Rüppel, P. Bayer, W. Blankenfeldt, Crystallographic Proof for an Extended Hydrogen-Bonding Network in Small Prolyl Isomerases, *J. Am. Chem. Soc.*, 133 (2011) 20096-20099.
- [92] E. Vöhringer-Martinez, F. Duarte, A. Toro-Labbé, How Does Pin1 Catalyze the Cis–Trans Prolyl Peptide Bond Isomerization? A QM/MM and Mean Reaction Force Study, *J. Phys. Chem. B*, 116 (2012) 12972-12979.
- [93] M. Schutkowski, A. Bernhardt, X.Z. Zhou, M. Shen, U. Reimer, J.-U. Rahfeld, K.P. Lu, G. Fischer, Role of Phosphorylation in Determining the Backbone Dynamics of the Serine/Threonine-Proline Motif and Pin1 Substrate Recognition†, *Biochemistry*, 37 (1998) 5566-5575.

- [94] X.J. Wang, B. Xu, A.B. Mullins, F.K. Neiler, F.A. Etzkorn, Conformationally Locked Isostere of PhosphoSer–cis-Pro Inhibits Pin1 23-Fold Better than PhosphoSer–trans-Pro Isostere, *J. Am. Chem. Soc.*, 126 (2004) 15533-15542.
- [95] E. Vöhringer-Martinez, T. Verstraelen, P.W. Ayers, The Influence of Ser-154, Cys-113, and the Phosphorylated Threonine Residue on the Catalytic Reaction Mechanism of Pin1, *The Journal of Physical Chemistry B*, 118 (2014) 9871-9880.
- [96] A.Y. Mercedes-Camacho, A.B. Mullins, M.D. Mason, G.G. Xu, B.J. Mahoney, X. Wang, J.W. Peng, F.A. Etzkorn, Kinetic Isotope Effects Support the Twisted Amide Mechanism of Pin1 Peptidyl-Prolyl Isomerase, *Biochemistry*, 52 (2013) 7707-7713.
- [97] H.A. Velazquez, D. Hamelberg, Conformation-Directed Catalysis and Coupled Enzyme–Substrate Dynamics in Pin1 Phosphorylation-Dependent Cis–Trans Isomerase, *J. Phys. Chem. B*, 117 (2013) 11509-11517.
- [98] H.A. Velazquez, D. Hamelberg, Conformational Selection in the Recognition of Phosphorylated Substrates by the Catalytic Domain of Human Pin1, *Biochemistry*, 50 (2011) 9605-9615.
- [99] G.P. Di Martino, M. Masetti, A. Cavalli, M. Recanatini, Mechanistic insights into Pin1 peptidyl-prolyl cis-trans isomerization from umbrella sampling simulations, *Proteins: Structure, Function, and Bioinformatics*, (2014) n/a-n/a.
- [100] R. Wolfenden, M.J. Snider, The Depth of Chemical Time and the Power of Enzymes as Catalysts, *Accounts of Chemical Research*, 34 (2001) 938-945.

[101] J.S. Fraser, M.W. Clarkson, S.C. Degnan, R. Erion, D. Kern, T. Alber, Hidden alternative structures of proline isomerase essential for catalysis, *Nature*, 462 (2009) 669-673.

Figure 1. X-ray crystal structure of FKBP12 (PDB ID: 2PPN). (A) Structure of the prototypical FK506 binding protein with the residues of the active site rendered as surface. (B) The active site of FKBP12 with hydrophobic residues is colored white, Tyr26 and Tyr82 are colored green, Asp37 is colored red and His 87 is colored blue. The active site is mainly hydrophobic.

Figure 2. Free energy and interactions between cyclophilinA and a substrate analogue (AAPF). (A) Hydrogen bonding interactions between the CypA and a substrate analogue in the transition state configuration. (B) The free energy profiles along the peptidylprolyl bond between Ala and Pro when the substrate is free in solution (black) and in the active site of CypA (red). (C) A thermodynamic cycle of the isomerization and binding of the substrate analogue to assess the relative free energies of binding different states of the substrate by CypA. (D) Distributions of the free energies of binding the different states of the substrate to CypA. The results of the molecular dynamics studies are from McGowan and Hamelberg (2013), Ladani and Hamelberg (2012), and Doshi et al. (2012).

Figure 3. Catalytic mechanism of Pin1. (A) Interactions between the substrate analogue and Pin1. The phosphate binding pocket is colored green. The peptidyl prolyl bond and backbone dihedral angles of the proline and phosphorylated serine are labeled. (B) The free energy profiles along the peptidyl prolyl bond between phosphorylated Serine and Pro when the substrate is free in solution (black) and in the active site of Pin1 (red). (C) The dominant states of the substrate analogue projected on the peptidyl prolyl dihedral angle and the backbone angle of proline during catalysis from cis to trans and vice versa. (D) The dominant states of the substrate analogue projected on the peptidyl prolyl dihedral angle and the backbone angle of phosphorylated Serine during catalysis from cis to trans and vice versa. (E) A thermodynamic cycle of the isomerization and binding of the substrate analogue to assess the relative free energies of binding the different states of the substrate by Pin1. The results of the molecular dynamics studies are from Velazquez and Hamelberg (2013).

Figure 4. Schematic representations of free energy profiles of the uncatalyzed and possible catalyzed (relative to the uncatalyzed free energy profile) isomerization reactions, assuming that catalysis occurs from cis to trans. (A) The enzyme binds the dominant conformation of the free cis substrate in solution and binds the transition state configuration better than the cis conformation. (B) Similar to (A) but the enzyme does not bind the transition state configuration of the substrate any better than the cis conformation. (C) The enzyme binds a low population of the free substrate (twisted conformation with $\omega \sim \theta$) and binds the transition state configuration better than the cis conformation. (D) Similar to (C) but the enzyme does not preferentially bind the transition state configuration of the substrate over the cis state. (E) A limiting case of (C) where the enzyme directly binds a transition state-like conformation of the substrate (highly twisted; $\omega \sim 90^\circ$). (F) The enzyme binds the cis conformation of the substrate with a positive free energy of binding and does not preferentially bind the transition state of the substrate.

Graphical Abstract