View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by University of Brighton Research Portal

Applied Biochemistry and Biotechnology

Modulating mobility: a paradigm for protein engineering?

--Manuscript Draft--

Manuscript Number:	ABAB-D-16-00805R1
Full Title:	Modulating mobility: a paradigm for protein engineering?
Article Type:	Commentary
Keywords:	enzyme engineering; molecular dynamics; protein flexibility; active site; biocatalysis; conformational change
Corresponding Author:	David J Timson University of Brighton Brighton, UNITED KINGDOM
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	University of Brighton
Corresponding Author's Secondary Institution:	
First Author:	Margaret McAuley
First Author Secondary Information:	
Order of Authors:	Margaret McAuley
	David J Timson
Order of Authors Secondary Information:	
Funding Information:	Department of Employment and Learning, Northern Ireland — Miss Margaret McAuley
Additional Information:	
Question	**Response**
Please select a section/category for your manuscript.	Biocatalysis for organic synthesis - Novel and significant advances in biocatalysis for organic synthesis, including chemo-, regio- and enantioselective biotransformation, screening and engineering of novel enzymes for biocatalysis, biocatalyst immobilization, and nonaqueous biocatalysis.
Abstract:	Proteins are highly mobile structures. In addition to gross conformational changes occurring on, for example, ligand binding, they are also subject to constant thermal motion. The mobility of the protein varies through its structure and can be modulated by ligand binding and other events. It is becoming increasingly clear that this mobility plays an important role in key functions of proteins including catalysis, allostery, cooperativity and regulation. Thus, in addition to an optimum structure, proteins most likely also require an optimal dynamic state. Alteration of this dynamic state through protein engineering will affect protein function. A dramatic example of this is seen in some inherited metabolic diseases where alternation of residues distant from the active site affects the mobility of the protein and impairs function. We postulate that using molecular dynamics simulations, experimental data or a combination of the two it should be possible to engineer the mobility of active sites. This may be useful in, for example, increasing the promiscuity of enzymes. Thus, a paradigm for protein engineering is suggested in which the mobility of the active site is rationally modified. This might be combined with more "traditional" approaches such as altering functional groups in the active site.

Powered by Editorial Manager® and ProduXion Manager® from Aries Systems Corporation

Manuscript #ABAB-D-16-00805
Modulating mobility: a paradigm for protein engineering?
Margaret McAuley and David J. Timson

Reviewer: Flexibility of protein plays an important role in protein functions such as activity and stability. Up to now, a number of studies strategized for engineering the protein towards desirable properties are published. The manuscript has a great summary of recent studies based on modulating mobility of protein especially in activity aspect. I recommend its publication after addressing the following issue.

Response: We thank the reviewer for his/her support and encouraging remarks.

There are also many reports about the improvement of stability of protein by modulating the flexibility of protein. Such as, "Park.H.J et al, Computational approach for designing thermostable Candidaantarctica lipase B by molecular dynamics simulation; Zhu, F., et al, Rational substitution of surface acidic residues for enhancing the thermostability of thermolysin; Chen, J et al, Improving stability of nitrile hydratase by bridging the salt-bridges in specific thermal-sensitive regions, and so on". It would be attractive if the authors could incorporate some examples that explain the relationship between activity and stability modulated by flexibility of protein.

Response: We thank the reviewer for this suggestion. We have added some material on this topic, including the references suggested.

In addition, we have taken the opportunity to make some small, typographical corrections to the manuscript. All changes are highlighted.

Manuscript

Click here to download Manuscript enzyme flexibility commentary ABB 2016 R1.doc

Click here to view linked References

Modulating mobility: a paradigm for protein engineering?

Margaret McAuley[1] and David J. Timson[1,2*]

[1] School of Biological Sciences, Queen's University Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast, BT9 7BL. UK.

[2] School of Pharmacy and Biomolecular Sciences, University of Brighton, Huxley Building, Lewes Road, Brighton BN2 4GJ. UK.

* Corresponding author.

Address: School of Pharmacy and Biomolecular Sciences, University of Brighton, Huxley Building, Lewes Road, Brighton BN2 4GJ. UK.

Telephone +44(0)1273 641623

Fax +44(0)1273 642090

Email d.timson@brighton.ac.uk

Abstract

Proteins are highly mobile structures. In addition to gross conformational changes occurring on, for example, ligand binding, they are also subject to constant thermal motion. The mobility of the protein varies through its structure and can be modulated by ligand binding and other events. It is becoming increasingly clear that this mobility plays an important role in key functions of proteins including catalysis, allostery, cooperativity and regulation. Thus, in addition to an optimum structure, proteins most likely also require an optimal dynamic state. Alteration of this dynamic state through protein engineering will affect protein function. A dramatic example of this is seen in some inherited metabolic diseases where alternation of residues distant from the active site affects the mobility of the protein and impairs function. We postulate that using molecular dynamics simulations, experimental data or a combination of the two it should be possible to engineer the mobility of active sites. This may be useful in, for example, increasing the promiscuity of enzymes. Thus, a paradigm for protein engineering is suggested in which the mobility of the active site is rationally modified. This might be combined with more "traditional" approaches such as altering functional groups in the active site.

Keywords: enzyme engineering; molecular dynamics; protein flexibility; active site; biocatalysis; conformational change

Introduction: protein mobility

It is a truth universally acknowledged that protein structure determines function. Indeed this postulate is a key feature of introductory, undergraduate biochemistry programmes. This fact also underpins structure-based drug design and many aspects of enzyme engineering. It is often clear from looking at protein structures that their structure fits them for binding to small molecule ligands, macromolecular binding partners (e.g. other proteins, nucleic acids) and for catalytic or energy transducing applications. Consequently, structural biology has been highly successful in furthering our understanding of how proteins work (46, 5, 43).

However, it is also clear that proteins are not static structures. That they must undergo conformational changes in order to function as catalysts, molecular motors and signalling molecules is well-established. Furthermore, like all molecules, they are subject to fluctuations and vibrations arising from the thermal energy of the protein and surrounding solvent molecules. It is becoming increasingly clear that the various motions that proteins undergo are vital to their functions. Indeed it is likely that these motions are at least as important as the overall, three-dimensional structures in mediating these functions (16, 47). It is important to be clear about the scale of molecular motions being considered. In general, the term "flexibility" is used to refer to larger scale motions occurring over a timescale of 10^{-7} s to 10 s and includes the motions required for protein folding, allosteric regulation and ligand binding. In contrast "dynamics" refers to faster motions (10^{-12} s to 10^{-8} s) such as side chain rotations (47). Understanding the links between these levels of mobility remains a considerable challenge in protein science.

Within an individual protein molecule there is considerable variation in the magnitude of the molecular motions present. While some parts may show very limited motions, others may be highly mobile. There are various measures of these motions. The B-factor or the Debye-Waller factor is a measure of thermal motion usually determined during X-ray crystallography (10, 52).

Root mean square deviation (RMSD) and root mean square fluctuation (RMSF) are measures of deviation from a reference structure. RMSD is usually measure of deviation from the initial structure whereas RMSF is a measure of deviation from an average structure (4). Normal mode analysis (NMA) and principal component analysis (PCA) have emerged as useful tools for the study of conformational changes in proteins. NMA utilises a harmonic model to determine possible molecular deformations in a particular equilibrated system (27, 29, 44). PCA extracts the contribution of fluctuations from a large selection of conformations (usually from molecular dynamic simulations) to the overall dynamics of the molecule (44, 23, 26). These approaches are reviewed in more detail elsewhere (44, 4).

There are a variety of motions which proteins can undergo. These include large scale domain movements where domains move relative to each other over a scale of tens of angstroms. Such motions dramatically re-arrange the overall structure of the protein and have been observed in enzymes such as hexokinase (EC 2.7.1.1) (1-3, 32) and molecular motors such as myosin (25). More subtle changes include the local rearrangement of residues, for example in induced fit around enzyme substrates or other ligands. For example, the interaction between phosphodiesterase 5 (PDE5; EC 3.1.4.35) and sildenafil (Viagra, Revatio) requires mutual conformational changes in the protein and the ligand to create a complementary binding site. Other PDE isoforms are unable (or less able) to undergo this conformational change, hence the selectively of the drug for this isoform despite high levels of similarity between PDE isoforms (53). There are also other subtle changes such rotations of side chains. Surface-exposed side chains which are not bonded to other molecules can usually rotate freely and rapidly. This can be constrained by binding to other parts of the protein (or other ligands) or by steric hindrance. The activation energy barriers between the different conformers is often small, hence the rapid rates. Therefore, in addition to flipping between two (or more) conformational states, we also need to consider changes that result in side chains altering the fraction of time spent in various states. These changes can have profound influences on

ligand binding and protein function (e.g. (41, 28)).

Recent studies have suggested that the natural vibrational properties of protein molecules can be important in mediating catalysis and intra-molecular communication (16). Thus, these vibrational motions can be "tuned" at a local level by events such as ligand binding or changes in the external environment of the protein and may be critical for enzyme catalysis. However, this hypothesis is not accepted by all authors. For example, some studies consider that there is no experimental proof linking molecular motions with catalysis (22). These authors propose that electrostatic pre-organisation and consequent alterations in free energies of activations provide a better explanation (with greater experimental evidence) of the catalytic power of many enzymes (22, 15).

One key group of proteins which are postulated to use coupled molecular motions, are those which demonstrate cooperativity in ligand binding. Typically these proteins have at least two ligand binding sites. While these sites may appear structurally similar in x-ray or NMR studies, they do not function independently. The binding of a ligand at one site, will affect the affinity at the other site. Two forms of cooperativity are recognised – positive (in which binding at the first site increases the affinity at the second site) and negative (in which binding at the first site makes interaction at the second one less likely (13, 48)). Both forms of cooperativity generally require some form of allosteric communication between the active sites. Most obviously this can be achieved through conformational changes which ramify through the structure linking one active site to the other (eg haemoglobin (34)). However, it is also possible to achieve this communication through modulation of the mobility of the protein, e.g. in glutamate dehydrogenase (51). Recent models of allosteric communication suggest that there are many different structural forms of the enzyme present (possibly including some intrinsically disordered states) (33).

That protein motions can affect functions of enzymes has been well established experimentally. In

dihydrofolate reductase (DHFR; EC 1.5.1.3), alterations in the dynamic behaviour have been mapped to various stages of the catalytic cycle (42). In the prolyl *cis-trans* isomerase cyclophilin A (CypA; EC 5.2.1.8), NMR measurements demonstrated that motions required for catalysis also occur in the free enzyme. This suggested that the enzyme's intrinsic motions are evolved for catalysis (12). Similar observations were made with adenylate kinase (EC 2.7.4.3) (17). In ribonuclease A (EC 3.1.27.5) motions in the RNA binding site and the catalytic site are coupled (9). In addition, the motions of a surface loop distant from the active site are also correlated: substitution of the this loop with a shorter sequence from a structurally similar protein resulted in slower loop motions, reduced active site dynamics and a lower turnover number (11).

Towards an alternative paradigm for protein engineering

The engineering of proteins to enhance or alter their activity is now well-established (39, 6). The aspiration of such studies is to adopt an engineering approach in which a well-understood enzyme is redesigned for a specific role. For example, for an enzyme with a structurally characterised active site and well defined catalytic mechanism, the aim would be to alter the side chains in order to provide altered chemical functionality and, consequently, altered function. In theory, this appears quite straightforward especially if combined with molecular modelling approaches to predict the architecture of the redesigned active site. Using such approaches it ought to be possible to redesign active sites around specific, novel substrates and produce enzymes capable of catalysing non-physiological reactions with activities sufficient to be commercially viable. While there have been some considerable successes in the design of novel biocatalysts, practical considerations have limited the application of enzyme redesign. Such considerations include our current limited ability to predict the structural consequences for the whole protein of even single amino acids changes. Current modelling techniques also struggle to account for entropic changes (e.g. resulting from solvation/desolvation in the active site) of such alterations. Linked to both of these factors, is the need to consider not just alterations to the mean structure of the protein but also to the mobility of

the molecule, especially around the active site. It is clear from some studies on inherited metabolic diseases that single point mutations which alter one amino acid (normally away from the active site) can have profound effects on structure, folding and consequently activity. In some cases, these changes have been shown to result in increased or decreased flexibility of the protein, e.g. galactose 1-phosphate uridylyltransferase (EC 2.7.7.12), UDP-galactose 4'-epimerase (EC 5.1.3.2), phosphoglycerate kinase 1 (EC 2.7.2.3), cystathionine β-synthase (EC 4.2.1.22) and mevalonate kinase (EC 2.7.1.36) (31, 49, 38, 37, 18, 7). Such studies strongly suggest the an optimum amount of flexibility is required for maximum activity, Indeed, it seems likely that, in addition to optimal three-dimensional structures, proteins also require optimal dynamics to fit them for their functions.

Recognition of the need for optimal dynamics as well as structure suggests a paradigm in protein engineering. Instead of (or as well as) attempting to alter the chemical functionality of active sites, it should be possible to alter their flexibility. In one early study, the hinge regions of various immunoglobulins were engineered with consequent effects on the flexibility and function of the molecules (40). Modulation of the flexibility of a hinge region in triose phosphate isomerase (TPI; EC 5.3.1.1) markedly affected the steady-state kinetic parameters (45). There has also been considerable success in the engineering the mobility of proteins such that their overall stability is enhanced. In the case of nitrile hydratase (EC 4.2.1.84), molecular dynamics (MD) simulations were used to identify regions responsible for initial thermal unfolding. Residues in these regions were then altered in order to introduce additional salt bridges into the protein. When additional salt bridges were introduced into the C-terminal region of the β-subunit, there was a substantial increase in stability without loss of activity (8). MD simulations were also used to investigate the role of surface-exposed glutamate and asparate residues in thermolysin (EC 3.4.24.27). Substitution of two of these (Asp37 and Glu119) resulted in a more thermally stable protein (58). Lipase B (EC 3.1.1.3) was redesigned following MD simulations which identified highly mobile regions. One variant (A251E) was approximately 2.5 times more stable than the wild-type protein (36). In a

separate approach, rigidification of active site residues in this enzyme also resulted in a more thermally stable protein (54). MD simulations have also been carried out to investigate the effects of organic solvents on lipase B. These solvents are predicted to reduce the flexibility of the enzyme (50). A site on the surface of the enzyme which is affected by methanol was identified by MD simulations and then altered resulting in a protein with higher stability in this solvent (35). In a number of these cases, greater stability was initially obtained at a cost to the enzyme's activity. Further rounds of simulations and/or experiments were often required to generate a more stable protein with comparable activity to wild-type.

It might be expected that an active site with increased flexibility compared to the wild-type may accommodate a wider range of substrates. This has been suggested in the case of galactokinase (EC 2.7.1.6), where alterations to the sequence of a β-sheet structure distant from the active site resulted in a broader range of substrates being accepted and processed by the enzyme (19, 20, 55-57). Preliminary molecular modelling studies suggested that this may be due to increased mobility of two loop structures in the active site. This prediction is consistent with the kinetic data which demonstrated widely increased range of monosaccharides which were recognised by the enzyme, but also reduced activity compared to the wild-type (24). The increased mobility of the active site will result in a greater range of conformations adopted by this part of the structure. In turn, this will enable a greater range of complementary interactions with sugar molecules, but also decrease the time spent by the active site in a catalytically competent conformation. We have previously noted that combining this approach with methods to increase the overall stability of the enzyme may result in galactokinases with broader specificity and higher activities (30).

Engineering the dynamics of proteins requires a detailed knowledge of their motions. While this can sometimes be obtained experimentally for smaller proteins (typically < 25 kDa) by NMR spectroscopy it is generally only possible to obtain limited information experimentally. However, *in*

silico approaches to model the dynamics of proteins are well-established. These can provide a model of the overall dynamic behaviour of a protein combined with estimates of the numerical measures of mobility described above. Extending these methods permits the prediction of the effects of alteration of amino acid residues on local and global mobility. However, molecular dynamics methods are computationally expensive and time-consuming. The generation of 100 ns of simulation typically requires weeks of computer time. Therefore, it is not currently realistic to undertake computational studies in which the alteration of large numbers of residues are evaluated for their effects on active site flexibility. Intelligent analysis of MD data can, however, enable sensible predictions to be made about which residues might be altered. For example, the use of MD data to identify regions of high or low mobility which correlate with that of the active site may suggest regions to target for alteration. The existence of tables of mean residue flexibility (e.g. (21)) facilitate the alteration of residues to ones which impart more or less mobility that the wild-type. At the extremes of flexibility are glycine (generally the most flexible) and proline (normally highly conformationally constraining).

Over 20 years ago, Alan Fersht proposed "rules" for enzyme engineering (14). In essence these rules suggested that small changes may be more likely to result in successful outcomes. For example, alteration of a glutamate residue to asparate (to create more space) or to a glutamine (to abolish the charge) may be more successful than alteration to lysine (to reverse the change) or phenylalanine (to abolish the charge, increase size and generate a more hydrophobic environment). We suggest that similar "rules" may apply when modulating mobility. Alteration of glycine to proline is likely to result in greatly enhanced rigidity (and the imposition of a very limited range of conformations). This will have effects beyond the local region and may well result in misfolded, non-functional protein. In this case, alteration to the next most flexible residue (serine) may be more appropriate. Further theoretical and experimental studies to understand the links between local and global changes in mobility will be required before more sophisticated "rules" can be

proposed.

The approaches suggested above have an element of trial and error. They involve the prediction of residues likely to affect flexibility on the basis of MD simulations (or experimental data) followed by further MD work and, ultimately, experimental testing. For a more design-orientated approach, it would be desirable to decide where more mobility was required (e.g. in the active site) and then use computational methods to predict where changes should be made to best achieve this. This may require the development of novel MD approaches and analyses. Doing so would open up new possibilities of protein engineering and should increase the success rate.

Overall, we consider that the dynamic behaviour of proteins should be considered to be at least as important as their three-dimensional structures. We further emphasise that this dynamic behaviour is critical in the redesign of proteins and is an important factor in the success or failure of protein engineering projects. Finally, we postulate a paradigm for protein engineering in which the mobility of active sites (and other critical parts of proteins) are redesigned based on MD simulations and/or experimental data.

Acknowledgments

MM thanks the Department of Employment and Learning, Northern Ireland (DELNI, UK) for a PhD studentship.

References

1. Bennett, W. S., Jr. and Steitz, T. A. (1978) Glucose-induced conformational change in yeast hexokinase. Proc Natl Acad Sci U S A, **75,** 4848-4852.
2. Bennett, W. S., Jr. and Steitz, T. A. (1980) Structure of a complex between yeast hexokinase A and glucose. I. Structure determination and refinement at 3.5 Å resolution. J Mol Biol, **140,** 183-209.
3. Bennett, W. S., Jr. and Steitz, T. A. (1980) Structure of a complex between yeast hexokinase A and glucose. II. Detailed comparisons of conformation and active site configuration with the native hexokinase B monomer and dimer. J Mol Biol, **140,** 211-230.
4. Benson, N. C. and Daggett, V. (2012) A comparison of multiscale methods for the analysis of molecular dynamics simulations. The journal of physical chemistry. B, **116,** 8722-8731.
5. Berman, H. M., Coimbatore Narayanan, B., Di Costanzo, L., Dutta, S., Ghosh, S., Hudson, B. P., Lawson, C. L., Peisach, E., Prlic, A., Rose, P. W., Shao, C., Yang, H., Young, J. and Zardecki, C. (2013) Trendspotting in the Protein Data Bank. FEBS Lett, **587,** 1036-1045.
6. Brannigan, J. A. and Wilkinson, A. J. (2002) Protein engineering 20 years on. Nat Rev Mol Cell Biol, **3,** 964-970.
7. Browne, C. and Timson, D. J. (2015) *In Silico* Prediction of the Effects of Mutations in the Human Mevalonate Kinase Gene: Towards a Predictive Framework for Mevalonate Kinase Deficiency. Ann Hum Genet, **79,** 451-459.
8. Chen, J., Yu, H., Liu, C., Liu, J. and Shen, Z. (2012) Improving stability of nitrile hydratase by bridging the salt-bridges in specific thermal-sensitive regions. J Biotechnol, **164,** 354-362.
9. Cole, R. and Loria, J. P. (2002) Evidence for flexibility in the function of ribonuclease A. Biochemistry, **41,** 6072-6081.
10. Debye, P. (1913) Interferenz von Röntgenstrahlen und Wärmebewegung. Annalen der Physik, **348,** 49-92.
11. Doucet, N., Watt, E. D. and Loria, J. P. (2009) The flexibility of a distant loop modulates active site motion and product release in ribonuclease A. Biochemistry, **48,** 7160-7168.
12. Eisenmesser, E. Z., Millet, O., Labeikovsky, W., Korzhnev, D. M., Wolf-Watz, M., Bosco, D. A., Skalicky, J. J., Kay, L. E. and Kern, D. (2005) Intrinsic dynamics of an enzyme underlies catalysis. Nature, **438,** 117-121.
13. Ferrell, J. E., Jr. (2009) Q&A: Cooperativity. Journal of Biology, **8,** 53.
14. Fersht, A. (1999) Structure and mechanism in protein science: a guide to enzyme catalysis and protein folding. ed. Macmillan.
15. Glowacki, D. R., Harvey, J. N. and Mulholland, A. J. (2012) Taking Ockham's razor to enzyme dynamics and catalysis. Nature chemistry, **4,** 169-176.
16. Goodey, N. M. and Benkovic, S. J. (2008) Allosteric regulation and catalysis emerge via a common route. Nature chemical biology, **4,** 474-482.
17. Henzler-Wildman, K. A., Thai, V., Lei, M., Ott, M., Wolf-Watz, M., Fenn, T., Pozharski, E., Wilson, M. A., Petsko, G. A., Karplus, M., Hubner, C. G. and Kern, D. (2007) Intrinsic motions along an enzymatic reaction trajectory. Nature, **450,** 838-844.
18. Hnizda, A., Majtan, T., Liu, L., Pey, A. L., Carpenter, J. F., Kodicek, M., Kozich, V. and Kraus, J. P. (2012) Conformational properties of nine purified cystathionine beta-synthase mutants. Biochemistry, **51,** 4755-4763.
19. Hoffmeister, D. and Thorson, J. S. (2004) Mechanistic implications of *Escherichia coli* galactokinase structure-based engineering. Chembiochem, **5,** 989-992.
20. Hoffmeister, D., Yang, J., Liu, L. and Thorson, J. S. (2003) Creation of the first anomeric D/L-sugar kinase by means of directed evolution. Proceedings of the National Academy of Sciences of the United States of America, **100,** 13184-13189.
21. Huang, F. and Nau, W. M. (2003) A conformational flexibility scale for amino acids in peptides. Angewandte Chemie (International ed.in English), **42,** 2269-2272.

22. Kamerlin, S. C. and Warshel, A. (2010) At the dawn of the 21st century: Is dynamics the missing link for understanding enzyme catalysis? Proteins, **78,** 1339-1375.
23. Kitao, A., Hirata, F. and Gō, N. (1991) The effects of solvent on the conformation and the collective motions of protein: Normal mode analysis and molecular dynamics simulations of melittin in water and in vacuum. Chemical Physics, **158,** 447-472.
24. Kristiansson, H. and Timson, D. J. (2011) Increased promiscuity of human galactokinase following alteration of a single amino acid residue distant from the active site. Chembiochem : a European journal of chemical biology, **12,** 2081-2087.
25. Kull, F. J. and Endow, S. A. (2013) Force generation by kinesin and myosin cytoskeletal motor proteins. J Cell Sci, **126,** 9-19.
26. Levy, R. M., Srinivasan, A. R., Olson, W. K. and McCammon, J. A. (1984) Quasi-harmonic method for studying very low frequency modes in proteins. Biopolymers, **23,** 1099-1112.
27. Ma, J. and Karplus, M. (1998) The allosteric mechanism of the chaperonin GroEL: a dynamic analysis. Proc Natl Acad Sci U S A, **95,** 8502-8507.
28. Maltsev, A. S. and Oswald, R. E. (2010) Hydrophobic side chain dynamics of a glutamate receptor ligand binding domain. J Biol Chem, **285,** 10154-10162.
29. Marques, O. and Sanejouand, Y. H. (1995) Hinge-bending motion in citrate synthase arising from normal mode calculations. Proteins, **23,** 557-560.
30. McAuley, M., Kristiansson, H., Huang, M., Pey, A. L. and Timson, D. J. (2015) Galactokinase promiscuity: a question of flexibility? Biochem.Soc.Trans., **44,** 116-122.
31. McCorvie, T. J., Gleason, T. J., Fridovich-Keil, J. L. and Timson, D. J. (2013) Misfolding of galactose 1-phosphate uridylyltransferase can result in type I galactosemia. Biochimica et biophysica acta, **1832,** 1279-1293.
32. McDonald, R. C., Steitz, T. A. and Engelman, D. M. (1979) Yeast hexokinase in solution exhibits a large conformational change upon binding glucose or glucose 6-phosphate. Biochemistry, **18,** 338-342.
33. Motlagh, H. N., Wrabl, J. O., Li, J. and Hilser, V. J. (2014) The ensemble nature of allostery. Nature, **508,** 331-339.
34. Otsuka, J. and Kunisawa, T. (1978) Conformational change and cooperative ligand binding in hemoglobin. Advances in biophysics, **11,** 53-92.
35. Park, H. J., Joo, J. C., Park, K., Kim, Y. H. and Yoo, Y. J. (2013) Prediction of the solvent affecting site and the computational design of stable *Candida antarctica* lipase B in a hydrophilic organic solvent. J Biotechnol, **163,** 346-352.
36. Park, H. J., Park, K., Kim, Y. H. and Yoo, Y. J. (2014) Computational approach for designing thermostable *Candida antarctica* lipase B by molecular dynamics simulation. J Biotechnol, **192 Pt A,** 66-70.
37. Pey, A. L. (2013) The interplay between protein stability and dynamics in conformational diseases: the case of hPGK1 deficiency. Biochim Biophys Acta, **1834,** 2502-2511.
38. Pey, A. L., Maggi, M. and Valentini, G. (2014) Insights into human phosphoglycerate kinase 1 deficiency as a conformational disease from biochemical, biophysical, and in vitro expression analyses. Journal of inherited metabolic disease, **37,** 909-916.
39. Quin, M. B. and Schmidt-Dannert, C. (2011) Engineering of biocatalysts - from evolution to creation. ACS catalysis, **1,** 1017-1021.
40. Roux, K. H., Strelets, L., Brekke, O. H., Sandlie, I. and Michaelsen, T. E. (1998) Comparisons of the ability of human IgG3 hinge mutants, IgM, IgE, and IgA2, to form small immune complexes: a role for flexibility and geometry. J Immunol, **161,** 4083-4090.
41. Schnell, J. R., Dyson, H. J. and Wright, P. E. (2004) Effect of cofactor binding and loop conformation on side chain methyl dynamics in dihydrofolate reductase. Biochemistry, **43,** 374-383.
42. Schnell, J. R., Dyson, H. J. and Wright, P. E. (2004) Structure, dynamics, and catalytic function of dihydrofolate reductase. Annu Rev Biophys Biomol Struct, **33,** 119-140.
43. Shi, Y. (2014) A glimpse of structural biology through X-ray crystallography. Cell, **159,** 995-

1014.
44. Skjaerven, L., Martinez, A. and Reuter, N. (2011) Principal component and normal mode analysis of proteins; a quantitative comparison using the GroEL subunit. Proteins, **79,** 232-243.
45. Sun, J. and Sampson, N. S. (1999) Understanding protein lids: kinetic analysis of active hinge mutants in triosephosphate isomerase. Biochemistry, **38,** 11474-11481.
46. Sussman, J. L., Abola, E. E., Lin, D., Jiang, J., Manning, N. O. and Prilusky, J. (1999) The protein data bank. Bridging the gap between the sequence and 3D structure world. Genetica, **106,** 149-158.
47. Teilum, K., Olsen, J. G. and Kragelund, B. B. (2009) Functional aspects of protein flexibility. Cell Mol Life Sci, **66,** 2231-2247.
48. Timson, D. J. (2015) Quantitative enzymology. Current Enzyme Inhibition, **11,** 12-31.
49. Timson, D. J. and Lindert, S. (2013) Comparison of dynamics of wildtype and V94M human UDP-galactose 4-epimerase-A computational perspective on severe epimerase-deficiency galactosemia. Gene, **526,** 318-324.
50. Trodler, P. and Pleiss, J. (2008) Modeling structure and flexibility of *Candida antarctica* lipase B in organic solvents. BMC Struct Biol, **8,** 9.
51. Wacker, S. A., Bradley, M. J., Marion, J. and Bell, E. (2010) Ligand-induced changes in the conformational stability and flexibility of glutamate dehydrogenase and their role in catalysis and regulation. Protein Sci, **19,** 1820-1829.
52. Waller, I. (1923) Zur Frage der Einwirkung der Wärmebewegung auf die Interferenz von Röntgenstrahlen. Z. Physik, **17,** 398-408.
53. Wang, H., Liu, Y., Huai, Q., Cai, J., Zoraghi, R., Francis, S. H., Corbin, J. D., Robinson, H., Xin, Z., Lin, G. and Ke, H. (2006) Multiple conformations of phosphodiesterase-5: implications for enzyme function and drug development. J Biol Chem, **281,** 21469-21479.
54. Xie, Y., An, J., Yang, G., Wu, G., Zhang, Y., Cui, L. and Feng, Y. (2014) Enhanced enzyme kinetic stability by increasing rigidity within the active site. J Biol Chem, **289,** 7994-8006.
55. Yang, J., Fu, X., Jia, Q., Shen, J., Biggins, J. B., Jiang, J., Zhao, J., Schmidt, J. J., Wang, P. G. and Thorson, J. S. (2003) Studies on the substrate specificity of *Escherichia coli* galactokinase. Organic letters, **5,** 2223-2226.
56. Yang, J., Fu, X., Liao, J., Liu, L. and Thorson, J. S. (2005) Structure-Based Engineering of *E. coli* Galactokinase as a First Step toward In Vivo Glycorandomization. Chemistry & biology, **12,** 657-664.
57. Yang, J., Liu, L. and Thorson, J. S. (2004) Structure-based enhancement of the first anomeric glucokinase. Chembiochem : a European journal of chemical biology, **5,** 992-996.
58. Zhu, F., Zhuang, Y., Wu, B., Li, J. and He, B. (2016) Rational Substitution of Surface Acidic Residues for Enhancing the Thermostability of Thermolysin. Appl Biochem Biotechnol, **178,** 725-738.