



Adaptability of protein structures to enable functional interactions and evolutionary implications

Turkan Haliloglu^{1,2} and Ivet Bahar²



Several studies in recent years have drawn attention to the ability of proteins to adapt to intermolecular interactions by conformational changes along structure-encoded collective modes of motions. These so-called soft modes, primarily driven by entropic effects, facilitate, if not enable, functional interactions. They represent excursions on the conformational space along principal low-ascent directions/paths away from the original free energy minimum, and they are accessible to the protein even before protein–protein/ligand interactions. An emerging concept from these studies is the evolution of structures or modular domains to favor such modes of motion that will be recruited or integrated for enabling functional interactions. Structural dynamics, including the allosteric switches in conformation that are often stabilized upon formation of complexes and multimeric assemblies, emerge as key properties that are evolutionarily maintained to accomplish biological activities, consistent with the paradigm sequence → structure → dynamics → function where ‘dynamics’ bridges structure and function.

Addresses

¹ Department of Chemical Engineering and Polymer Research Center, and Center for Life Sciences and Technologies, Bogazici University, 34342 Istanbul, Turkey

² Department of Computational and Systems Biology, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15260, USA

Corresponding author: Bahar, Ivet (bahar@pitt.edu)

Current Opinion in Structural Biology 2015, 35:17–23

This review comes from a themed issue on **Protein–protein interactions**

Edited by **Ozlem Keskin** and **Alexandre Bonvin**

<http://dx.doi.org/10.1016/j.sbi.2015.07.007>

0959-440/© 2015 Elsevier Ltd. All rights reserved.

Introduction

With the accumulation of structural and dynamic data and the rapid advances in the visualization of the spatio-temporal dynamics of protein–protein interactions [1] as well as the conformational dynamics of proteins in living cells [2], and with the availability of efficient models and methods for analyzing structural dynamics and allostery [3,4[•],5], there is increasing support for the significance of structure-encoded dynamics as a major

determinant of protein–protein and protein–ligand interaction mechanisms.

Structure-encoded dynamics, also called intrinsic dynamics, represents the conformational motions, or the spectrum of modes, uniquely defined by the 3-dimensional structure. The most favorable modes, also called ‘soft modes’ are usually distinguished by their cooperativity, hence their involvement in allosteric switches or global changes in structure [3,4[•],6]. The functional significance and robustness of these modes of motions suggest new design and engineering principles, such as the need to enjoy suitable conformational flexibility, or substrate adaptability, rather than a high stability exclusively. Conformational flexibility appears to be essential to optimizing protein–substrate interactions [7,8], enabling allosteric responses [9] or mediating multispecificity [10–12]. In line with these concepts, the intrinsic dynamics of proteins is emerging as a factor closely related to the evolutionary selection of structures [13,14[•],15[•]].

We present here recent studies, in support of the significance of structural dynamics in determining binding geometry, assembly and/or oligomerization mechanisms and facilitating allostery. We also highlight recent work on the relationship between the evolutionary selection of structures and their intrinsic dynamics.

The functional motions of proteins are not random: they are robustly favored by the structure

Proteins engage in many complex interactions in the cell. These are usually accomplished by changes in their structure, varying over a broad range, from highly localized movements at the level of single-residues, to cooperative rearrangements of multiple domains or subunits. While conformational changes have been broadly described as ‘wiggings and jiggings’, this description falls short of reflecting the cooperative nature of many functional interactions. In particular molecular machines require precise integration of functional movements (often driven by ATP binding). Increasing evidence supports the propensities of many complexes and assemblies to undergo non-random changes in their structures. These changes are usually predictable by simple models such as elastic network models (ENMs) which take account of the cooperative nature of biomolecular dynamics [16].

A few principal modes of motion, also called soft modes, mediate intermolecular interactions

The old concept of a single ‘native’ structure has long given way to that of an ‘ensemble of substates in the native state’ which usually share the same fold. The protein essentially samples a multitude of conformers, which are transiently stabilized during its biological activity. These conformers are accessible through local changes in structure (e.g. loop motions or side chain rotations) or global rearrangements (domain/subunit movements). Yet, these are all ‘native’ substates for a given protein, the relative probabilities of which change under different conditions, or at different stages of the biological processes (e.g. allosteric cycle) in which they take part, or in the presence or absence of their natural substrates — a phenomenon usually referred to as ‘conformational shift’. Such shifts between pre-existing states may also occur due to mutations. There is increasing attention on the opportunities (and limitations) of modulating conformational shifts for controlling binding affinities and/or biomolecular functions [17].

An important observation is that these different conformers are along a few ‘principal modes of motion’ intrinsically accessible to the fold that they share [3,4^{••},18–21]. One of the early studies demonstrating that experimentally observed structural variations simply represent reconfigurations of different sizes along one or two principal modes encoded by the structure is that of de Groot, Gresinger and coworkers [22] for ubiquitin. This highly versatile protein adopts a variety of conformations while binding its substrates, and these are simply those sampled along one or two principal directions of motions accessible to the unbound ubiquitin, also seen by NMR residual dipolar coupling. A more recent example is the single-molecule Förster resonance energy transfer analysis of phosphoglycerate kinase (PGK) dynamics by Fitter and coworkers [23[•]]. In that study, Fitter and coworkers elegantly showed that first, the experimentally detected functional (hinge-bending) motions of the enzyme are encoded by the fold, as predicted by ENMs, and second, those motions are already performed in the ligand-free state of PGK domains, before substrate-binding.

Soft modes define pre-existing pathways of reconfiguration selected for modulating binding, assembly or multimerization

For a better visualization of the conformational space and accessible conformers, let us consider the free energy surface in Figure 1. The surface depicts the most favorable region, or the global minimum, of a much broader energy landscape. In principle, the protein (P) would sit at the lowest energy well, for example, position ‘2’ on the landscape. But because of the different crystallization conditions and the inherent conformational flexibility of P, the structures resolved for P (as well as the models

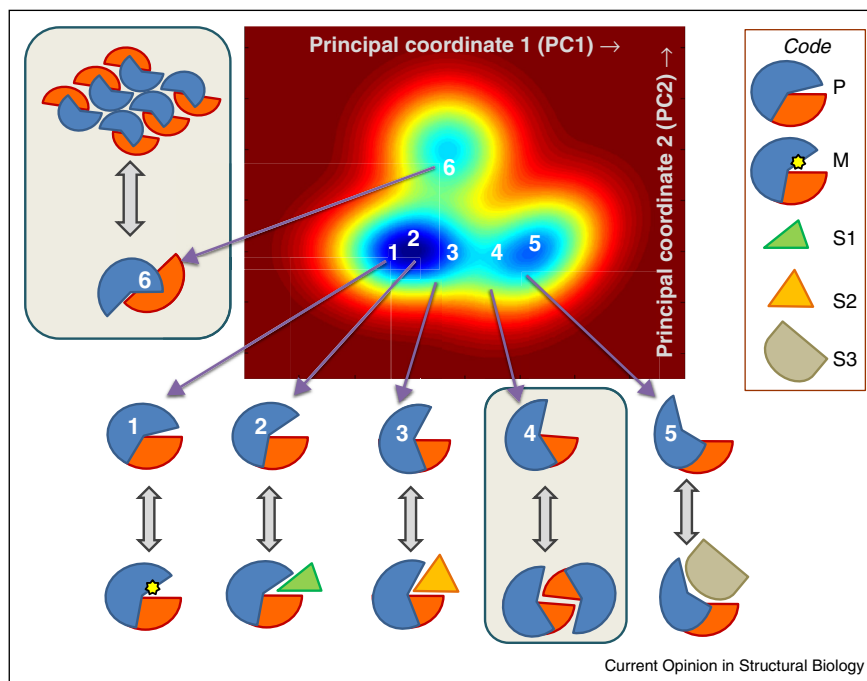
determined by NMR) would be distributed in the vicinity of this well. The conformers designated as ‘1’–‘6’ display such alternative structures, or *substates* of the native state. The important observation, from experiments and computations, is that these substates are not randomly distributed in space, but more or less aligned along a few principal directions of reconfiguration and these directions are nothing else than the soft/principal modes of motions accessible to P.

The soft modes may therefore be viewed as pre-existing paths or valleys on the conformational energy landscape, away from the lowest energy minimum [24]. Some are steeper; others are easy or soft. In the same way as these regions will be the first to be flooded when there is a rise in the water level, these modes are the first to be ‘recruited’ in response to a perturbation (substrate binding, mutation, etc.). In other words, the conformational changes undergone by the protein upon formation of multimers or complexes with different substrates (S1–S3), or in the presence of mutations (M), schematically illustrated in the last row (Figure 1) are simply those conformers already accessible to the (unbound) protein via deformations along its softest modes/paths.

In summary, the emerging picture is the following. The alternative structures resolved for a given protein — for example, ligand-bound/unbound, active/inactive, open/closed, outward-facing/inward-facing, or at different stages along an allosteric cycle — usually represent substates accessible via soft modes [18,21,25,26] predictable with the help of physics-based approaches such as ENMs and normal mode analysis [3,4^{••}]. Excursions on the conformational energy landscape thus define the type of substates accessible to the protein to adapt to its interactions. Allosteric effects often arise by triggering or altering these pre-existing modes upon ligand binding.

We present here a few recent studies supporting these concepts: Dima and coworkers showed that the conformational diversity attained via excursions on the conformational landscape underlie the mechanosensing functionality of a muscle anchoring complex observed in atomic force microscopy (AFM) [27]; Gur *et al.* showed that the reconfiguration of adenylate kinase between its open and closed forms upon ligand binding takes place along such valleys of the conformational space [28]. Further, the conformational pathways described by a single mode starting from the open state were shown to successfully predict the closed state for a set of proteins that undergo large hinge-bending motions [29]. Shi and coworkers showed that the changes induced by Na⁺ binding on the intramolecular interaction network correlate well with the principal mode of motion intrinsically accessible to the dopamine D2-like receptor [30]. Similarly, an evolutionary conserved interaction network was shown to connect Na⁺ binding to global conformational

Figure 1



Schematic representation of conformers accessible to a given protein under physiological conditions, and pre-disposition to bind different substrates or favor different multimerization states. The energy landscape in the middle represents the vicinity of the native state or global energy minimum for a hypothetical protein P. It represents the projection of this region onto the subspace spanned by two principal coordinates, PC1 and PC2. Numbers 1–6 on the surface depict the location of various structures, or substates that might be resolved for the same protein. They would be distinguished by the type and extent of rearrangements between the two subunits (colored *red* and *blue*) of the protein. There is a series of conformers along PC1, differing in the extent of ‘opening’ of the cleft between the two subunits, from the most compact (labeled ‘1’; leftmost) to the most exposed (‘5’, *rightmost*), predisposed to bind different substrates (S1–S3). Structure ‘6’ shows a different inter-subunit packing arrangement, for example, a twisting motion, defined by PC2. Some of the structures are pre-disposed to form multimers (e.g. ‘4’ favors dimer formation, and ‘6’ favors hexamer formation). The mutant M structure resembles an already accessible structure (‘2’). Thus, the protein may accommodate different substrate binding (multispecificity) or adapt to different oligomerization states by reconfigurations along two principal coordinates. PC1 and PC2 are generally along the two softest modes intrinsically accessible to P, predictable ENM analysis. The soft modes thus represent pre-existing directions of structural change, probabilistically accessible under equilibrium conditions, and can be selected for mediating ligand binding, mutations or oligomerization.

changes crucial to neurotransmitter transport [31]. The transition of aspartate transporter between inward-facing and outward-facing states is essentially accommodated by a single mode predicted by the ENM in the presence of membrane environment [32]. Modulation of soft modes underlies allosteric regulation in CRP/FNR family of transcription factors [33–35]. Residues acting as hinges in the softest mode of ASC protein allosterically modulate the binding surface to promote the formation of ASC speck assembly [36^{*}]; CO₂ binding stabilizes the open form of connexin26 by interfering with the softest mode accessible to the protein (which otherwise drives the opening/closing of the hemichannel) [37]. ENM modes that enable the contraction/dilation of the extracellular vestibule in a series of GPCR family members correlate with the formation of the cavity for G-protein binding on the intracellular side [38^{*}]. Also, the global modes of motion provide mechanistic insights into how the function of voltage-gated potassium channel Kv7.1 is

regulated by the binding of its auxiliary subunit KCNE [39]. Finally, the binding of the transactivation domain of MLL protein and c-Myb to CREB KIX domain is enabled by reconfiguration along soft modes [40].

Binding is mediated by not only local interactions but global dynamics that alter surface properties and regulate allosteric responses

Several observations point to the interplay between stability and dynamics in shaping protein’s binding landscape. The significance of shape complementary and local physiochemical characteristics is well-established. But binding is not necessarily a local phenomenon. It also entails global changes in conformation that may allosterically alter surface properties at distal regions. Examples are pre-existing structural fluctuations that expose binding epitopes or drive the formation of a cavity for substrate binding [41^{*}], stabilization of the selected conformational

shift that enables binding and controls the binding affinity [17], or the modulation of the global dynamics upon complex formation [33].

We anticipate that design studies that involve substrate binding will increasingly require a thorough understanding of the nature of the global modes accessible to the unbound protein, in addition to the usual examination of its structure and surface properties. On a local scale, protein–protein interfaces show dynamic patterns with distinct thermal fluctuations [42]; on a global scale, allosteric changes in structure may explain some of the entropic gain in binding [43]; a distant mutation that induces a large conformational change may disrupt the dimerization of an enzyme [44]; or, the functional effect of *N*-glycosylation is not through changes in protein structure but decreases in protein dynamics, and an increase in protein stability modulates the oligomerization and aggregation states of the glycosylated protein [45]. Likewise, a nucleotide-mimetic was shown to modulate the oligomerization state of the oncoprotein repton by altering its global conformation and protein-binding activity [46^{*}]. Finally, an approach based on the maximization of information entropy change associated with the global modes between bound and unbound structures significantly helps in distinguishing the native protein complex structures from the designed complex structures [47].

Triggering or altering of pre-existing dynamics is a means of modulating biological activity

Proteins present key sites that have the capacity to trigger or alter global modes of motion. Hinge regions are such sensitive sites [3,29]. Hinge-bending and large twisting/untwisting motions are common mechanisms of allosteric regulation, as shown in numerous ENM applications. Enhanced hinge flexibility facilitates kinase activation [48]; conversely, substrate coevolution in HIV-1 protease restore the hinge axis deformed by drug resistant mutations, highlighting the functional importance of hinge motions [49]. Allosteric hot spots constitute another group of target sites that modulate protein–protein interactions [50]. Notably, an allosteric inhibitor recently discovered for neuropeptidases presumably disrupted activity by preventing a hinge-like motion associated with substrate binding and catalysis [51^{*}]. In some cases, key sites may be on the surface, for example, some residues serve as sensors, and others as effectors for efficiently sensing and rapidly communicating perturbations. A recent study invited attention to such residues on the ATPase domain of Hsp70, which mediate interdomain allostery [52^{*}]. In the case of the PyrR family of pyrimidine operon attenuators, key mutations all distant from the interface and outside ligand-binding pockets were identified to control the oligomeric state; these mutations introduced structural changes comparable to the conformational shift observed between the unbound and nucleotide-bound

conformations of the protein [14^{**}]. Distant dynamic couplings between variable (V_H) and constant (C_{H2}) domains and the hinge region (C_{H1} – C_{H2} interface) were also observed within an IgG1 monoclonal antibody during its reversible self-association [53]. Allostery through DNA is also an important modulator of DNA functions; the coalescence of protein-induced DNA bubbles was suggested to regulate DNA's flexibility and the assembly of the transcription machinery [54]. Binding of an antibiotic 60 Å away from the DD-transpeptidase active site has been shown to allosterically stimulate the opening of the active site, predisposing the penicillin binding protein 2a to inactivation [55].

Evolution of sequences and structures to enable intrinsic dynamics in favor of functional interactions

Emphasis in classical studies has been on the requirement to conserve biochemical (e.g. catalytic) activity and overall stability, and on the evolution (or conservation) of amino acids to ensure them. However, with the emergence of structural dynamics as a major determinant of mechanisms of interaction, it is clear that the conservation of the conformational mechanics (not only chemistry) is another equally important evolutionary requirement. ENM-based normal mode analyses have helped elucidate the shared dynamics of homologous proteins starting from the original work of Echave and coworkers [56] and Ortiz and coworkers [57]. An important observation has been the correlation between the structural core change among family members (for a series of protein families) and the soft modes intrinsically favored by the shared architecture of family members [57]. A more recent study helped elucidate the conserved ENM modes crucial to the switching function of Ras GTPase family, as well as the modes specific to particular family members [58].

Systematic study of sequence conservation patterns and conformational mobilities demonstrate that regions that enjoy higher conformational mobility are also sequentially variable, and vice versa [59,60]. Furthermore, not only conserved residues, but also co-evolving pairs of residues are of interest toward gaining a better understanding of the functional interactions (intramolecular or intermolecular) that are presumably maintained by compensating mutations. These analyses clearly demonstrate that co-evolving pairs of residues relate to 3-dimensional contacts [59,61–65]. Furthermore, coevolving pairs of residues often populate conformational flexible regions such as substrate-recognition sites [52^{*},59,66^{**}], suggestive of the need to modulate specificity by sequence coevolution. Finally, proteome-wide analysis of conformational dynamics indicates that the interface sites enriched in disease-associated non-synonymous single nucleotide variants play a crucial role in functional dynamics [67]. It remains to be seen if such studies can assist in the identification of allosterically coupled sites and in the

design of allosteric inhibitors. Efforts to push forward these efforts combined with druggability considerations [68] may open new avenues for identifying potential sites for allosteric regulation.

An interesting observation is that pathways of assembly are also under evolutionary pressure [13,69]. In a recent review, Marsh and Teichmann emphasized how local protein flexibility and disorder, as well as large-scale motions and quaternary structure assembly correlate with evolutionary changes in protein sequence and structure [70]. Electrospray mass spectroscopy experiments show assembly intermediates that are in accord with those observed evolutionarily [13]. Teichmann and coworkers also showed that the conformational changes allosterically induced by selected mutations (called *allosteric mutations*) are similar to those stabilized upon ligand binding or by intersubunit geometry changes occurring upon oligomerization [14**]. This observation again highlights the intrinsic preferences of the original structure to undergo changes along well-defined (soft) modes of deformation, as illustrated in Figure 1. The relationship between intrinsic dynamics and evolution appears to be twofold: evolution selectively maintains the structures that lend themselves to functional intrinsic dynamics, and evolution employs the intrinsic dynamics of the protein to promote allosteric switches or oligomerization mechanisms.

Conclusion

A wide range of events/processes implicated in a given protein's interactions, function and evolution appear to proceed via similar mechanisms: allosteric response of the protein to specific substrate binding, its structural changes triggered or stabilized by ligand-binding or drug-binding, its evolutionarily selected modes of assembly or oligomerization. Our current understanding is that these are 'similar' because they represent all excursions along a few dominant directions on the energy landscape: the soft modes of motions uniquely defined by the protein architecture. Methods that exploit the intrinsic dynamics of proteins are probably to open the way to new strategies that for design, discovery and therapeutics.

Acknowledgements

Support from NIH awards P30 DA035778 and U54 HG008540 is gratefully acknowledged by IB. TH acknowledges TUBITAK project no: 110T088.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Tchekanda E, Sivanesan D, Michnick SW: **An infrared reporter to detect spatiotemporal dynamics of protein-protein interactions.** *Nat Methods* 2014, **11**:641-644.
2. Kim TJ, Zheng S, Sun J, Muhamed I, Wu J, Lei L, Kong X, Leckband DE, Wang Y: **Dynamic visualization of alpha-catenin reveals rapid, reversible conformation switching between tension states.** *Curr Biol* 2015, **25**:218-224.
3. Bahar I, Lezon TR, Yang LW, Eyal E: **Global dynamics of proteins: bridging between structure and function.** *Annu Rev Biophys* 2010, **39**:23-42.
4. Fuglebakk E, Tiwari SP, Reuter N: **Comparing the intrinsic dynamics of multiple protein structures using elastic network models.** *Biochim Biophys Acta* 2015, **1850**:911-922.
- This is an excellent review on computational strategies for evaluating the intrinsic dynamics of proteins using ENMs, with several illustrative examples.
5. Feher VA, Durrant JD, Van Wart AT, Amaro RE: **Computational approaches to mapping allosteric pathways.** *Curr Opin Struct Biol* 2014, **25**:98-103.
6. Dobbins SE, Lesk VI, Sternberg MJ: **Insights into protein flexibility: the relationship between normal modes and conformational change upon protein-protein docking.** *Proc Natl Acad Sci U S A* 2008, **105**:10390-10395.
7. Landes CF, Rambhadran A, Taylor JN, Salatan F, Jayaraman V: **Structural landscape of isolated agonist-binding domains from single AMPA receptors.** *Nat Chem Biol* 2011, **7**:168-173.
8. Phillips AH, Zhang Y, Cunningham CN, Zhou L, Forrest WF, Liu PS, Steffek M, Lee J, Tam C, Helgason E, Murray JM, Kirkpatrick DS, Fairbrother WJ, Corn JE: **Conformational dynamics control ubiquitin-deubiquitinase interactions and influence in vivo signaling.** *Proc Natl Acad Sci U S A* 2013, **110**:11379-11384.
9. Cronin M, Coolbaugh MJ, Nellis D, Zhu J, Wood DW, Nussinov R, Ma B: **Dynamics differentiate between active and inactive inteins.** *Eur J Med Chem* 2015, **91**:51-62.
10. James LC, Roversi P, Tawfik DS: **Antibody multispecificity mediated by conformational diversity.** *Science* 2003, **299**:1362-1367.
11. Tawfik DS: **Accuracy-rate tradeoffs: how do enzymes meet demands of selectivity and catalytic efficiency?** *Curr Opin Chem Biol* 2014, **21**:73-80.
12. Tokuriki N, Tawfik DS: **Protein dynamism and evolvability.** *Science* 2009, **324**:203-207.
13. Marsh JA, Teichmann SA: **Parallel dynamics and evolution: protein conformational fluctuations and assembly reflect evolutionary changes in sequence and structure.** *Bioessays* 2014, **36**:209-218.
14. Perica T, Kondo Y, Tiwari SP, McLaughlin SH, Kemplen KR, Zhang X, Steward A, Reuter N, Clarke J, Teichmann SA: **Evolution of oligomeric state through allosteric pathways that mimic ligand binding.** *Science* 2014, **346**:1254-1256.
- This is an outstanding study exposing the similarities between the conformational shifts triggered by small molecule binding and those selected by evolution to stabilize different oligomerization states. In both cases, the structural changes are in line with the intrinsic dynamics of the protein.
15. Toth-Petroczy A, Tawfik DS: **The robustness and innovability of protein folds.** *Curr Opin Struct Biol* 2014, **26**:131-138.
- This is an insightful reflection essay on the co-existence of opposite features — structural stability and conformational flexibility (or plasticity), for enabling promiscuous protein's robustness (to sequence variations) and innovability (to accomplish new functions).
16. Leioatts N, Romo TD, Grossfield A: **Elastic network models are robust to variations in formalism.** *J Chem Theory Comput* 2012, **8**:2424-2434.
17. Michielssens S, de Groot BL, Grubmüller H: **Binding affinities controlled by shifting conformational equilibria: opportunities and limitations.** *Biophys J* 2015, **108**:2585-2590.
18. Yang L, Song G, Carriquiry A, Jernigan RL: **Close correspondence between the motions from principal component analysis of multiple HIV-1 protease structures and elastic network modes.** *Structure* 2008, **16**:321-330.
19. Yang LW, Eyal E, Bahar I, Kitao A: **Principal component analysis of native ensembles of biomolecular structures**

(PCA_NEST): insights into functional dynamics. *Bioinformatics* 2009, **25**:606-614.

20. Bahar I: **On the functional significance of soft modes predicted by coarse-grained models for membrane proteins.** *J Gen Physiol* 2010, **135**:563-573.
 21. Skjaerven L, Martinez A, Reuter N: **Principal component and normal mode analysis of proteins; a quantitative comparison using the GroEL subunit.** *Proteins* 2011, **79**:232-243.
 22. Lange OF, Lakomek NA, Fares C, Schroder GF, Walter KF, Becker S, Meiler J, Grubmuller H, Griesinger C, de Groot BL: **Recognition dynamics up to microseconds revealed from an RDC-derived ubiquitin ensemble in solution.** *Science* 2008, **320**:1471-1475.
 23. Gabba M, Poblete S, Rosenkranz T, Katranidis A, Kempe D, Zuchner T, Winkler RG, Gompfer G, Fitter J: **Conformational state distributions and catalytically relevant dynamics of a hinge-bending enzyme studied by single-molecule FRET and a coarse-grained simulation.** *Biophys J* 2014, **107**:1913-1923.
- Using smFRET, normal mode analysis and mesoscopic simulations, the authors showed that the functional motions of PGK are encoded in the topology of its ligand-free state, and that ligand binding only slightly affects the orientation of PGK domains and locks the the motions along a pre-existing path.
24. Meireles L, Gur M, Bakan A, Bahar I: **Pre-existing soft modes of motion uniquely defined by native contact topology facilitate ligand binding to proteins.** *Protein Sci* 2011, **20**:1645-1658.
 25. Tobi D, Bahar I: **Structural changes involved in protein binding correlate with intrinsic motions of proteins in the unbound state.** *Proc Natl Acad Sci U S A* 2005, **102**:18908-18913.
 26. Bakan A, Bahar I: **The intrinsic dynamics of enzymes plays a dominant role in determining the structural changes induced upon inhibitor binding.** *Proc Natl Acad Sci U S A* 2009, **106**:14349-14354.
 27. Bodmer NK, Theisen KE, Dima RI: **Molecular investigations into the mechanics of a muscle anchoring complex.** *Biophys J* 2015, **108**:2322-2332.
 28. Gur M, Madura JD, Bahar I: **Global transitions of proteins explored by a multiscale hybrid methodology: application to adenylate kinase.** *Biophys J* 2013, **105**:1643-1652.
 29. Uyar A, Kantarci-Carsibasi N, Haliloglu T, Doruker P: **Features of large hinge-bending conformational transitions. Prediction of closed structure from open state.** *Biophys J* 2014, **106**:2656-2666.
 30. Michino M, Free RB, Doyle TB, Sibley DR, Shi L: **Structural basis for Na(+)-sensitivity in dopamine D2 and D3 receptors.** *Chem Commun (Camb)* 2015, **51**:8618-8621.
 31. Stolzenberg S, Quick M, Zhao C, Gotfryd K, Khelashvili G, Gether U, Loland CJ, Javitch JA, Noskov S, Weinstein H, Shi L: **Mechanism of the association between Na+ binding and conformations at the intracellular gate in neurotransmitter:sodium symporters.** *J Biol Chem* 2015, **290**:13992-14003.
 32. Lezon TR, Bahar I: **Constraints imposed by the membrane selectively guide the alternating access dynamics of the glutamate transporter Gltp_h.** *Biophys J* 2012, **102**:1331-1340.
 33. Rodgers TL, Townsend PD, Burnell D, Jones ML, Richards SA, McLeish TC, Pohl E, Wilson MR, Cann MJ: **Modulation of global low-frequency motions underlies allosteric regulation: demonstration in CRP/FNR family transcription factors.** *PLoS Biol* 2013, **11**:e1001651.
 34. Townsend PD, Rodgers TL, Pohl E, Wilson MR, McLeish TC, Cann MJ: **Global low-frequency motions in protein allostery: CAP as a model system.** *Biophys Rev* 2015, **7**:175-182.
 35. Aykac F, Tutar Y, Haliloglu T: **Dynamic fluctuations provide the basis of a conformational switch mechanism in apo cyclic AMP receptor protein.** *PLoS Comput Biol* 2013, **9**:e1003141.
 36. Sahillioglu AC, Sumbul F, Ozoren N, Haliloglu T: **Structural and dynamics aspects of ASC speck assembly.** *Structure* 2014, **22**:1722-1734.
- Hinge residues involved in soft modes were shown to modulate the formation of ASC speck assembly — a micrometer-sized perinuclear structure serving as a platform for caspase-1 activity. Mutations at the hinge sites were seen to alter the assembly of ASC proteins from SPECK structures into fibrils by exposing alternative binding modes.
37. Meigh L, Greenhalgh SA, Rodgers TL, Cann MJ, Roper DI, Dale N: **CO(2) directly modulates connexin 26 by formation of carbamate bridges between subunits.** *Elife* 2013, **2**:e01213.
 38. Kolan D, Fonar G, Samson AO: **Elastic network normal mode dynamics reveal the GPCR activation mechanism.** *Proteins* 2014, **82**:579-586.
- The authors showed that ENM normal modes help establish the communication between the extracellular vestibule and the G-protein binding cavity (at the intracellular region), for a series of GPCR family members.
39. Gofman Y, Shats S, Attali B, Haliloglu T, Ben-Tal N: **How does KCNE1 regulate the Kv7.1 potassium channel? Model-structure, mutations, and dynamics of the Kv7.1-KCNE1 complex.** *Structure* 2012, **20**:1343-1352.
 40. Korkmaz EN, Nussinov R, Haliloglu T: **Conformational control of the binding of the transactivation domain of the MLL protein and c-Myb to the KIX domain of CREB.** *PLoS Comput Biol* 2012, **8**:e1002420.
 41. Bohnuud T, Kozakov D, Vajda S: **Evidence of conformational selection driving the formation of ligand binding sites in protein-protein interfaces.** *PLoS Comput Biol* 2014, **10**:e1003872.
- The authors examined the druggability properties of protein-protein interfaces using computational solvent mapping technique with small probes, to show that peptide-binding or ligand-binding invariably occurs with the help of a selection from a set of pre-existing conformations.
42. Lin JJ, Lin ZL, Hwang JK, Huang TT: **On the packing density of the unbound protein-protein interaction interface and its implications in dynamics.** *BMC Bioinformatics* 2015, **16**(Suppl 1):S7.
 43. Visscher KM, Kastiris PL, Bonvin AM: **Non-interacting surface solvation and dynamics in protein-protein interactions.** *Proteins* 2015, **83**:445-458.
 44. Jones MR, Liu C, Wilson AK: **Molecular dynamics studies of the protein-protein interactions in inhibitor of κ B kinase- β .** *J Chem Inf Model* 2014, **54**:562-572.
 45. Lee HS, Qi Y, Im W: **Effects of N-glycosylation on protein conformation and dynamics: Protein Data Bank analysis and molecular dynamics simulation study.** *Sci Rep* 2015, **5**:8926.
 46. Healy AR, Houston DR, Remnant L, Huart A-S, Brychtova V, Maslon MM, Meers O, Muller P, Krejci A, Blackburn EA, Vojtesek B, Hernychova L, Walkinshaw MD, Westwood NJ, Hupp TR: **Discovery of a novel ligand that modulates the protein-protein interactions of the AAA + superfamily oncoprotein reptin.** *Chem Sci* 2015, **15**:3109-3116.
- The authors discovered a nucleotide-mimetic with the help of an *in silico* screen coupled with chemical optimization, which was shown to modulate the global conformation, binding activity and oligomerization state of the oncoprotein reptin.
47. Fleishman SJ, Whitehead TA, Strauch EM, Corn JE, Qin S, Zhou HX, Mitchell JC, Demerdash ON, Takeda-Shitaka M, Terashi G, Moal IH, Li X, Bates PA, Zacharias M, Park H, Ko JS, Lee H, Seok C, Bourquard T, Bernauer J, Poupon A, Aze J, Soner S, Ovali SK, Ozbek P, Tal NB, Haliloglu T, Hwang H, Vreven T, Pierce BG, Weng Z, Perez-Cano L, Pons C, Fernandez-Recio J, Jiang F, Yang F, Gong X, Cao L, Xu X, Liu B, Wang P, Li C, Wang C, Robert CH, Guharoy M, Liu S, Huang Y, Li L, Guo D, Chen Y, Xiao Y, London N, Itzhaki Z, Schueler-Furman O, Inbar Y, Potapov V, Cohen M, Schreiber G, Tsuchiya Y, Kanamori E, Standley DM, Nakamura H, Kinoshita K, Driggers CM, Hall RG, Morgan JL, Hsu VL, Zhan J, Yang Y, Zhou Y, Kastiris PL, Bonvin AM, Zhang W, Camacho CJ, Kilambi KP, Sircar A, Gray JJ, Ohue M, Uchikoga N, Matsuzaki Y, Ishida T, Akiyama Y, Khashan R, Bush S, Fouches D, Tropsha A, Esquivel-Rodriguez J, Kihara D, Stranges PB, Jacak R, Kuhlman B, Huang SY, Zou X, Wodak SJ, Janin J, Baker D: **Community-wide assessment of protein-interface modeling suggests improvements to design methodology.** *J Mol Biol* 2011, **414**:289-302.

48. Sours KM, Xiao Y, Ahn NG: **Extracellular-regulated kinase 2 is activated by the enhancement of hinge flexibility.** *J Mol Biol* 2014, **426**:1925-1935.
49. Ozer N, Ozen A, Schiffer CA, Haliloglu T: **Drug-resistant HIV-1 protease regains functional dynamics through cleavage site coevolution.** *Evol Appl* 2015, **8**:185-198.
50. Ma B, Nussinov R: **Druggable orthosteric and allosteric hot spots to target protein-protein interactions.** *Curr Pharm Des* 2014, **20**:1293-1301.
51. Hines CS, Ray K, Schmidt JJ, Xiong F, Feenstra RW, Pras-Raves M, de Moes JP, Lange JH, Melikishvili M, Fried MG, Mortenson P, Charlton M, Patel Y, Courtney SM, Kruse CG, Rodgers DW: **Allosteric inhibition of the neuropeptidase neurolysin.** *J Biol Chem* 2014, **289**:35605-35619.
- A newly discovered allosteric inhibitor is shown here to disrupt neuropeptidase neurolysin activity by preventing a hinge-like motion required for substrate binding and catalysis.
52. General IJ, Liu Y, Blackburn ME, Mao W, Gierasch LM, Bahar I: **ATPase subdomain IA is a mediator of interdomain allostery in Hsp70 molecular chaperones.** *PLoS Comput Biol* 2014, **10**:e1003624.
- Using a combination of ENM-based perturbation and sequence coevolution analyses, the authors showed that the Hp70 molecular chaperones adapt to co-chaperone recognition and activity via coevolving residues, whereas their interdomain allostery is supported by conserved interactions.
53. Arora J, Hickey JM, Majumdar R, Esfandiary R, Bishop SM, Samra HS, Middaugh CR, Weis DD, Volkin DB: **Hydrogen exchange mass spectrometry reveals protein interfaces and distant dynamic coupling effects during the reversible self-association of an IgG1 monoclonal antibody.** *MAbs* 2015, **7**:525-539.
54. Traverso JJ, Manoranjan VS, Bishop AR, Rasmussen KO, Voulgarakis NK: **Allostery through protein-induced DNA bubbles.** *Sci Rep* 2015, **5**:9037.
55. Otero LH, Rojas-Altuve A, Llarrull LI, Carrasco-Lopez C, Kumarasiri M, Lastochkin E, Fishovitz J, Dawley M, Heseck D, Lee M, Johnson JW, Fisher JF, Chang M, Mobashery S, Hermoso JA: **How allosteric control of *Staphylococcus aureus* penicillin binding protein 2a enables methicillin resistance and physiological function.** *Proc Natl Acad Sci U S A* 2013, **110**:16808-16813.
56. Maguid S, Fernandez-Alberti S, Ferrelli L, Echave J: **Exploring the common dynamics of homologous proteins. Application to the globin family.** *Biophys J* 2005, **89**:3-13.
57. Leo-Macias A, Lopez-Romero P, Lupyan D, Zerbino D, Ortiz AR: **An analysis of core deformations in protein superfamilies.** *Biophys J* 2005, **88**:1291-1299.
58. Raimondi F, Orozco M, Fanelli F: **Deciphering the deformation modes associated with function retention and specialization in members of the Ras superfamily.** *Structure* 2010, **18**:402-414.
59. Liu Y, Bahar I: **Sequence evolution correlates with structural dynamics.** *Mol Biol Evol* 2012, **29**:2253-2263.
60. Seeber M, Felline A, Raimondi F, Mariani S, Fanelli F: **WebPSN: a web server for high-throughput investigation of structural communication in biomacromolecules.** *Bioinformatics* 2015, **31**:779-781.
61. Jones DT, Buchan DW, Cozzetto D, Pontil M: **PSICOV: precise structural contact prediction using sparse inverse covariance estimation on large multiple sequence alignments.** *Bioinformatics* 2012, **28**:184-190.
62. Jones DT, Singh T, Kosciulek T, Tetchner S: **MetaPSICOV: combining coevolution methods for accurate prediction of contacts and long range hydrogen bonding in proteins.** *Bioinformatics* 2015, **31**:999-1006.
63. Mao W, Kaya C, Dutta A, Horovitz A, Bahar I: **Comparative study of the effectiveness and limitations of current methods for detecting sequence coevolution.** *Bioinformatics* 2015, **31**:1929-1937.
64. Morcos F, Pagnani A, Lunt B, Bertolino A, Marks DS, Sander C, Zecchina R, Onuchic JN, Hwa T, Weigt M: **Direct-coupling analysis of residue coevolution captures native contacts across many protein families.** *Proc Natl Acad Sci U S A* 2011, **108**:E1293-E1301.
65. Ovchinnikov S, Kamisetty H, Baker D: **Robust and accurate prediction of residue-residue interactions across protein interfaces using evolutionary information.** *Elife* 2014, **3**:e02030.
66. Jeon J, Nam HJ, Choi YS, Yang JS, Hwang J, Kim S: **Molecular evolution of protein conformational changes revealed by a network of evolutionarily coupled residues.** *Mol Biol Evol* 2011, **28**:2675-2685.
- The authors investigated the relationship between sequence evolution and proteins conformational change and found that coevolving residues are clustered in the flexible regions of proteins. Their correlated mutations is suggested to cooperatively facilitate conformational motions.
67. Butler BM, Gerek ZN, Kumar S, Ozkan SB: **Conformational dynamics of nonsynonymous variants at protein interfaces reveals disease association.** *Proteins* 2015, **83**:428-435.
68. Nichols SE, Hernandez CX, Wang Y, McCammon JA: **Structure-based network analysis of an evolved G protein-coupled receptor homodimer interface.** *Protein Sci* 2013, **22**:745-754.
69. Marsh JA, Teichmann SA: **Protein flexibility facilitates quaternary structure assembly and evolution.** *PLoS Biol* 2014, **12**:e1001870.
70. Marsh JA, Teichmann SA: **Structure, dynamics, assembly, and evolution of protein complexes.** *Annu Rev Biochem* 2014, **84**:551-575.