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Perspective

Perspective: The promises of a holistic view of proteins—impact on antibody engineering and drug discovery

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The reductionist approach is prevalent in biomedical science. However, increasing evidence now shows that biological systems cannot be simply considered as the sum of its parts. With experimental, technological, and computational advances, we can now do more than view parts in isolation, thus we propose that an increasing holistic view (where a protein is investigated as much as a whole as possible) is now timely. To further advocate this, we review and discuss several studies and applications involving allostery, where distant protein regions can cross-talk to influence functionality. Therefore, we believe that an increasing big picture approach holds great promise, particularly in the areas of antibody engineering and drug discovery in rational drug design.

Introduction

Due to natural complexity and resource limitations such as those present in technical, computational, and experimental methods, the reductionist approach in biomedical science has often reduced proteins to a mere sum of its parts, namely subunits, domains/folds, secondary and super-secondary structure elements etc. As a result, scientists have been looking at proteins in parts based on domains and functional sites while ignoring the less characterized parts with no known functions. In some cases, new artificial classifications based on the reductionist approach were also introduced.

To date, the reductionist approaches in biomedical experiments provided significant insights into the predominant region(s) associated with specific functions. Such findings have, in turn, led to significant applications. For example, antibody fragments such as antigen-binding fragment (Fab), single-chain variable fragment (scFv), or Fc, are widely used as research reagents and as potential therapeutics [1,2], and the classifications of protein domains in structural refinement and functional predictions [3]. Yet, the reductionist approach, though amenable and highly useful, ignores the bigger picture of inter-regional communications and their possible co-operative effects [3] that would be useful for further detailed analysis.

Generally, protein domain cross-talks, coined here loosely as 'allostery', have largely been neglected due to the lack of whole structures for holistic investigations. Nonetheless, allostery is increasingly shown to be essential in manipulating protein functions, especially in the area of drug discovery such as designing allosteric drugs [4–8] to affect protein function by binding to distant pockets from the protein active site. Such allosteric effects have also been found in numerous proteins [9] such as aspartate carbamoyltransferase (ATCase) [10], bovine glutamate dehydrogenase (BGDH) [11], phosphofructokinase [12], and also in antibodies [13–18].

Therefore, given advances in technologies leading to advanced experimental and computational techniques in recent years, the next level of scientific breakthroughs may require looking at proteins as holistically as possible. Calls for such an approach are already present in various specialties [19–22] with these attempts aimed at putting together insights derived from reductionist investigations.

According to Regenmortel [19], revisiting biological systems wholly as systems is important [23]. On this line of thought, while limitations in looking at whole systems are ever present, we may, nonetheless, be already reaching a saturation point for scientific breakthroughs within the reductionist approach. Thus, we propose that it is now time to re-analyze proteins in their entirety (where possible). In this article, we will focus on the issues pertaining to computational structural analysis and the bottlenecks in translating them toward experimental and possible future clinical outcomes.

To further illustrate our point, we utilized augmented reality (AR) via the use of mobile apps (see commentary [24] for details on its methodology and usage).

Antibodies and receptors

A resurgence of interest in the antibody and its regions is augmented by the 2018 Nobel Chemistry Prize awarded to Sir Gregory Winter for the ingenious phage display method that led to many antibody-based applications. In monomeric form, the whole antibody is known to be a Y-shaped molecule [25]. The two ends of the V-shaped variable (V) regions are for antigen recognition and binding. The stalk (constant or C-region), holding up the V-regions, binds and triggers immune effector cell functions via engagement of the Ig receptor [26]. Even though the antibody V(D)J recombination underlies the genetic system for antibody generation [27], structural and sequence analysis have led to an additional classification within the V-regions of frameworks (FWRs) and complementarity determining regions (CDRs), where the FWRs are scaffolds to hold up the CDR loops [28]. From this FWR-CDR classification, humanization of antibodies from rodent sources have been successfully performed, with some leading to therapeutics [29]. This technology of CDR grafting is however hindered by a high degree of trial and error given the lack of rule-based understanding. Different algorithms [30–33] do not fully agree with one another, often defining the boundaries of FWRs and CDRs differently, thereby requiring additional analysis (e.g. Ling et al. [15]) for reaffirmation.

Computational efforts to aid in *de novo* V-region design [34] hold great promise to synthesize antigen-specific antibodies from scratch, bypassing the contended animal-dependent methods. However, it is still essential to validate these *in silico* designs using *in vitro* experimental testing. There is no doubt that the structural classifications of CDR and FWRs are useful; however, neither CDRs nor FWRs alone can yield significant outcomes in isolation. In fact, recent evidence have demonstrated the interdependency of FWRs and CDRs in the binding of antigens, antibody production and purification, and even the functioning of distal antigen-binding regions [15]. To complicate things further, the C-region, typically neglected in experimental affinity maturation experiments relying on scFv or Fabs [35], can affect antigen binding as well [14,36]. Such findings highlight the need to also study the less studied antibody allotypes [37]. At the same time, the V-region FWR families of both antibody heavy (VH) and light (VL) chains were also found to affect C-region receptor binding [15], possibly modulating effector cell functions [38] (Figure 1).

Many reports [13,15–17,39] have demonstrated allosteric communications between various antibody domains (C-region and the antigen-binding regions) in IgG antibodies. Yang et al. [16] showed the allosteric co-operativity of both the V- and C-regions, rationalizing the structure–function relationship to go beyond the conventional domain-based hypothesis. In other antibody isotypes, similar findings to IgG [15,17] were also reported. Lua et al. [36] demonstrated changes in antigen engagement, where the same V-regions had equilbrium dissociation constants that indicated stronger (for IgM, due to avidity effects) or weaker (for monomeric IgD, and IgA and its subtypes) interactions by simply changing the heavy chain C-regions alone [36]. This effect was however not found when the light-chain C-regions were swapped. A follow-up study [14] focussing only on IgA further demonstrated that the allosteric signaling propagated bidirectionally between the V- and C-regions via the domain-linking hinge.

In the case of antibody-dependent enhancement (ADE), the antibody–receptor interaction-mediated endocytosis enhanced the infection of the dengue virus [40,41], which would likely be avoided by using the high avidity IgM instead [42]. Similarly in studies of other infectious diseases such as HIV [43], a systems level investigation (a holistic view) on ADE, where antibody therapeutics can be engineered not only to optimize the interaction with other molecules, but also to strike a balance between the efficacy of the drug and unwanted effects, would be important. Certainly, considering the molecule as a whole is useful especially when developing therapeutic antibodies, in which communication between the antibody and antigen/receptor play a key role. In the light of such effects across antibody regions, there is a reason to expect that detailed understanding and application requires the consideration of the whole antibody engagement to the antigen and/or Fc receptor.
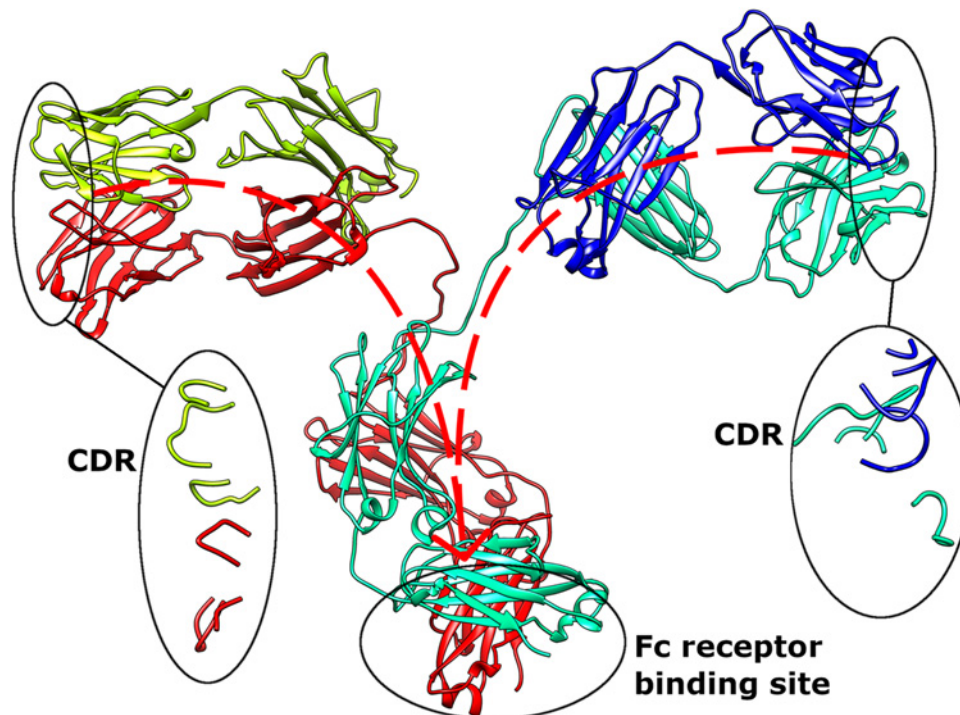
Figure 1. Visual representation of inter-domain signaling between CDR and Fc receptor binding region
The antibody structure is retrieved from Protein Data Bank [25] (PDB: 1IGT). The animated (communication) effect can be viewed using the 'APD AR Holistic Review' app, available freely on Google and Apple app stores (view the image using the app camera, see commentary [24] for more details). Alternatively, download 'HP Reveal' from the stores and access the link, 'http://auras.ma/s/wdpFQ' to view the above image with the mobile app. An alternative video of the AR representation of this figure can be found at https://www.facebook.com/APDLab/videos/2075249849390855/.

Beyond antibodies, antibody receptors have also defied the reductionist-based approach where certain sequence regions exert effects beyond their boundaries. One such example is the IgA Fc receptor FcαR (CD89). The natural variant of this receptor molecule contains a full signal peptide and extracellular (EC) domains that bind to IgA antibody. Lua et al. [44] discovered that when a natural variant of the receptor lacking only the EC1 domain responsible for binding the IgA molecule [44] but having the full signal peptide was studied, the variant was found spatially constrained intracellularly rather than extracellularly. Attempts to 'force' EC localization, using other secretory signal peptides and mutations at the signal peptide cleavage sites, yielded no success [44]. Further studying other variants (in the presence of the EC1 domain and the complete signal peptide) showed that the lack of the other EC domain, EC2 located more distantly from the signal peptide than EC1, also prevented the EC localization [45]. It may suggest that for proper localization, all EC domains of CD89 are required to be present despite their distance from the signal peptide, demonstrating that protein domain co-operation is more enigmatic than expected.

In the investigation of antibody–receptor interaction, Ling et al. [15] found that different VH-VL FWRs of IgG1 variants, with the same CDRs, exhibited different equilibrium dissociation constants to the FcγIIA IgG receptor as well differently to the antigen. In several VH–VL FWR combinations, there were compromised FcγIIA interactions but not to the antigen [15]. This raises questions if neglecting Fc receptor engagement can result in therapeutic antibodies with reduced immune effector cell engagement. Especially when further analyses demonstrated that by varying the VL pairs (not involved in direct FcR binding), FcγIIA equilibrium dissociation constants could be reinstated to that of the control antibody without compromising antigen equilibrium dissociation constants. While the underlying mechanisms of such effects are still elusive, further investigations would need to take a more holistic approach involving whole Ig–FcR complexes.
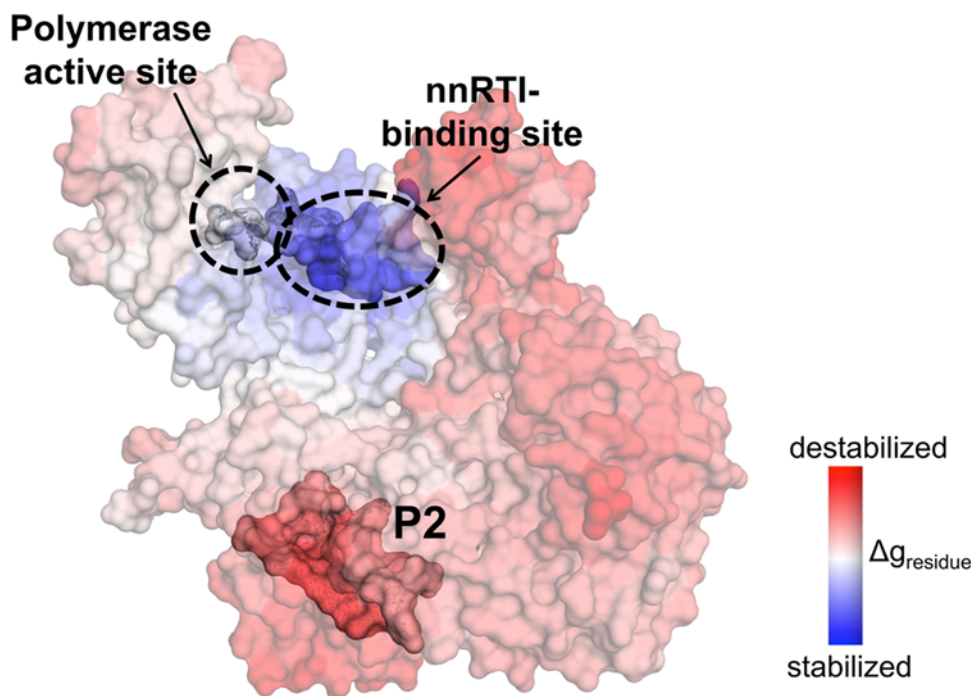
Figure 2. Allosteric communication of Pocket P2 on the polymerase active site
This figure shows the rigidifying effects caused by the potential druggable pocket P2 on the polymerase active site (blue), subsequently regulating the function of the enzyme. Figure adapted from Chiang et al. [56]. An animation of inhibitor binding to the allosteric NNRTI-binding site can be viewed using the 'APD AR Holistic Review' app, available freely on Google and Apple app stores (view the image using the app camera, see commentary [24] for more details). Alternatively, download 'HP Reveal' from the stores and access the link, 'http://auras.ma/s/wdpFQ' to view the above image with the mobile app. An alternative video of the AR representation of this figure can be found at https://www.facebook.com/APDLab/videos/2075249849390855/. Permission to use this figure has been granted by the authors.

Allostery for druggable targets and drug discovery

The use of allostery for druggable targets in intervening pathogenesis of many diseases are many, ranging from: (i) identifying allosteric targets that influence enzymatic activity; (ii) identifying allosteric epitopes/sites for targetting by antibodies [46–49] and/or inhibitors [50–55] to affect the active site; or (iii) repurposing existing natural enzymes.

Allosteric targets that influence enzymatic activity

In HIV therapy, promising results were achieved in the search for druggable pockets and potential alternative inhibitors to inhibit viral enzymes, particularly HIV-1 Reverse Transcriptase (RT) [56]. Of the two current classes of RT inhibitors (RTIs), the allosteric non-nucleoside RTIs (NNRTIs) target an allosteric pocket located 10 Å away from the polymerase active site and disrupted the active site. By studying the full structure of RT, alternative allosteric pockets away from the active site, can be identified for drug targetting [56, 57]. For example, Chiang et al. [56] performed virtual screenings for alternate allosteric pockets using AlloPred [58] and AlloSigMA [59] and detected three such pockets located on p51 subunit (but not on the active site subunit p66, shown in Figure 2). Given the increasing reports of HIV drug resistance [60] to the current Highly Active Antiretroviral Therapy (HAART), the novel allosteric sites on the RT p51 subunit opens up opportunities for novel drug sites, where various screening methods [61] such as ligand-based or structure-based virtual screening [62], can be applied.

Allosteric targets for antibodies or inhibitors

In the application toward allergy treatment, there is great promise to disrupt the IgE antibody and IgE Fc receptor (FcεRIα) interaction. Here, the identification of potential allosteric drug target sites using computational epitope and allosteric analyses (Figure 3A) can be performed. First, we performed epitope prediction using BepiPred-2.0 [63], Emini Surface Accessibility [64], and ABCPred [65] on the EC FcεRIα structure [66] (PDB: 1F2Q) followed
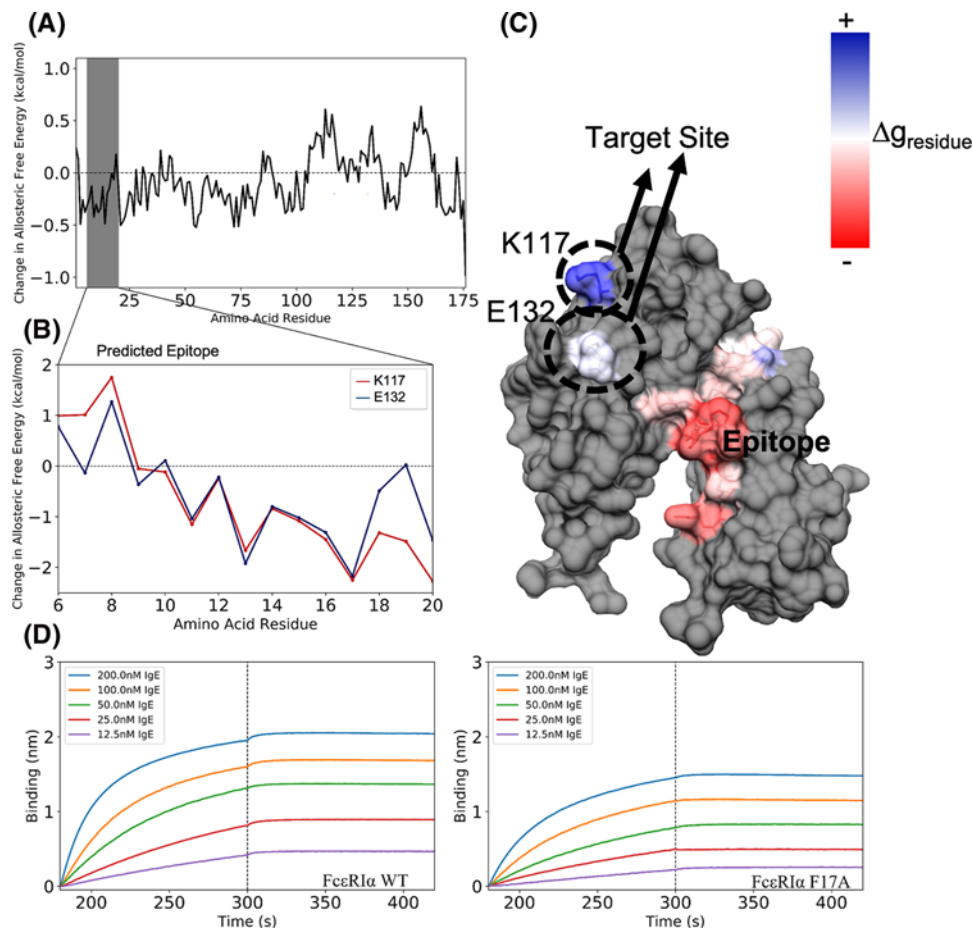
Figure 3. Analysis of allosteric communications between the identified epitope and the FcεRIα active site

(**A**) Allosteric responses at each FcεRIα residue (X-axis) in the event of IgE binding (i.e. the active site residues K117 and E132, previously identified by Cook et al. [87], were assigned as 'site' in the AlloSigMA server [59] using the FcεRIα structure PDB: 1F2Q). The predicted epitope region (from K6 to E20) is highlighted in gray. (**B**) Estimated allosteric effects on each of the two active site residues by the individual mutation on the predicted epitope (i.e. each residue of the epitope was assigned at 'UP-mutation' in the AlloSigMA server). (**C**) Structural representation of allosteric communication between the identified epitope region and the IgE-FcεRIα interacting site. (**D**) Bio-layer interferometry binding experiments of the FcεRIα wild-type (left) and the F17A FcεRIα mutant (right) to the IgE at different concentrations. The animated (communication) effect can be viewed using the 'APD AR Holistic Review' app, available freely on Google and Apple app stores (view the image using the app camera, see commentary [24] for more details). Alternatively, download 'HP Reveal' from the stores and access the link, 'http://auras.ma/s/wdpFQ' to view the above image with the mobile app. An alternative video of the AR representation of this figure can be found at https://www.facebook.com/APDLab/videos/2075249849390855/

by quantitating the allosteric communications between the individual residues of the predicted epitopes and the IgE FcεRIα-binding site using AlloSigMA [59]. We found the residue positions W13 and F17 on the FcεRIα to be potential mutation targets (Figure 3B,C). In the process of making both the mutants and the wild-type control using site-directed mutagenesis and transient transfection methods [15,36,44,67], the W13A mutant could not be produced at detectable amounts and therefore could not be subjected to subsequent experiments. Results of bio-layer interferometry (using nickel-NTA biosensors to capture the purified FcεRIα proteins followed by interacting with IgE at various concentrations from 200 to 12.5 nM) shown in Figure 3D demonstrated that the F17A mutation experimentally reduced the IgE-FcεRIα responses. This finding is consistent with a previous study by Mackay et al. [68]. Our combined methodology of *in silico* prediction and *in vitro* validation took a few weeks and were able to reproduce the previous conclusion, thus demonstrating a simplified process without tedious sequential single mutation experiments to find and validate such allosteric epitopes. Given that there would generally be a higher number of allosteric

sites to target than the active site alone [69], such an approach allows additional allosteric epitopes to be identified in a wide range of proteins, as well as the potential for reverse perturbation to fine-tune and target allosteric responses [70].

Taking a step further, allostery-induced changes can include the more roundabout way to expose buried epitopes that would enhance immune detection. By studying the dynamics of the whole protein, buried binding sites can be exposed. In one example, Fuentes et al. [71] found a Trastuzumab-induced 'cryptic epitope' on Her2 that enhanced Pertuzumab interaction in simulations. Although this effect was not shown in *in vitro* experiments [72], such an approach is worth pursuing in proteins with no clear epitopes, allosteric or otherwise. Online databases such as SYF-PEITHI [73], BIMAS [74], IEDB [75], and other such allosteric site prediction servers can help reveal buried epitopes and potential allosteric sites on the protein. This indirect approach may benefit from the combinatorial uses of small molecule inhibitors together with biologics to expose drug relevant sites/pockets/epitopes.

Nonetheless, the nature of allosteric inhibitors and therapeutic targets can be a double-edged sword. On one side of the blade, allosteric sites, having no intrinsic function of its own, may not be conserved and be easily mutated without affecting core functions of the proteins. As a result, resistance against therapeutic agents might develop quickly with mutations occurring directly on or between the allosteric site and the functional site to disrupt the allosteric communication. While it is possible to overcome the rapidly emerging mutations by screening for structurally conserved regions, this is conceptual at this point.

On the other hand, lack of direct inhibition on the active site may also allow for reduced inhibitory flexibility. The flexible nature of numerous proteins may result in partial rather than complete inhibition (see above $Fc\varepsilon RI\alpha$ example). It is advantageous to lower the selection pressure for drug resistance that may allow allosteric drugs to last longer. In addition, the partial inhibition may also allow the immune system to deal naturally with diseases that may be more beneficial in view of immune memory and natural resilience. Admittedly, this discussion of the potential uses of allosteric biologics and drugs is merely conjecture at this point, for even in the very established HIV therapy, NNRTIs are yet to reap the proposed benefits. While HIV is likely to be unique in its adaptive ability that other infectious agents or cancers are unlikely to follow, further work is certainly required.

Allosteric targets to repurpose natural enzymes

The repurposing of natural enzymes against disease agents by allosteric mutations to affect the catalytic site is the final discussed approach to use allostery in unique ways beyond that of allosteric epitopes or druggable pockets.

A proof-of-concept to this approach was found in the insulin degrading enzyme (IDE) that was modified to have catalytic activity toward an amyloid β peptide [76]. Since the target modifications were performed on natural enzymes, chances of eliciting unwanted immune responses were low. However, in those cases, it is perhaps wise to avoid sites on the protein surface to reduce immunogenicity.

In viral infections, structural modeling of the whole HIV Gag protein [77] provided functional insights into a neglected Gag domain – p6 – for potential intervention in viral maturation (Figure 4). While p6 might not be easily drugged due to its high flexibility, analysis of the full-length Gag structure showed its contribution to the Gag conformational changes during maturation. Should there be intracellular interacting partners that could be engineered to constrain p6, viral maturation could be antagonized. Also, smaller antibody fragments or other repurposed proteins could be potential p6-binding candidates.

There is a clear need for more intensive research in these areas, and such efforts promise to generate more novel biologics against a variety of diseases, particularly those involving protein aggregation and viral infections.

Challenges in considering proteins as whole

As discussed in several examples above, allostery is represented by communication effects between distal regions of proteins. Various allosteric models have been proposed, e.g. from the classical MWC model involving dynamics couplings of protein conformational changes [78] that interconvert concertedly, to the sequential KNF model where conformations of the involved domains sequentially change one at a time [79] leading to the propagation of the changes [80]. One of the most recent proposals argue the underlying allosteric mechanism to be derived from the population shift of the protein conformational ensembles, in which the more predominant conformational state drives the protein function [81–83]. Nonetheless, all the models imply the engagement of the whole protein structure, which otherwise would have been biased in any absence of the involved partners.

Such efforts are challenging when applied to a large system, e.g. antibody, multi-domain proteins, or membrane proteins etc., and when the structures of interest are far from achievable given current experimental limitations or computational bottlenecks. Besides that, 'the elephant in the room' is the poor translatability of computer predictions
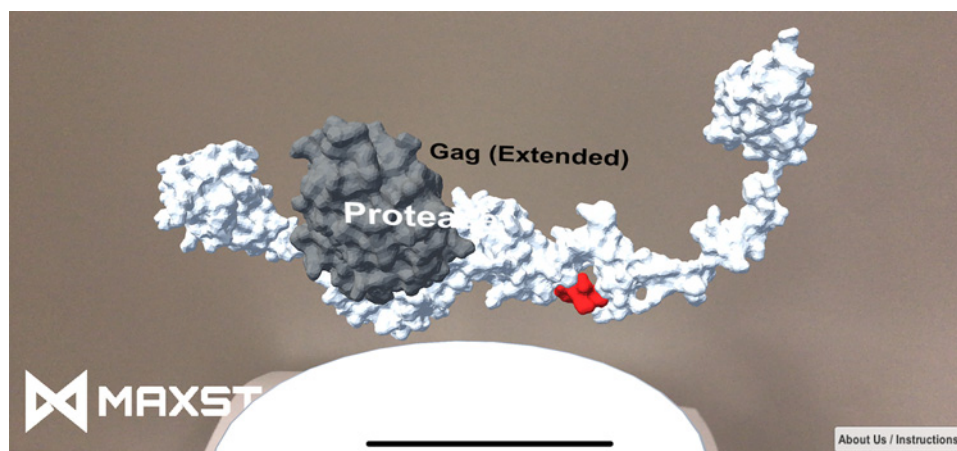
Figure 4. AR illustration of the extended conformation of the HIV-1 Gag polyprotein
More details can be explored using the 'APD AR Holistic Review' app, available on Google and Apple app stores (pointing the running app on to Figure 2 in Su et al. [77] as the target image). Alternatively, download 'HP Reveal' from the stores and access the link, 'http://auras.ma/s/wdpFQ' to view Figure 2 in Su et al. [77] as the target image with the mobile app. An alternative video of the AR representation of this figure can be found at https://www.facebook.com/APDLab/videos/2075249849390855/

to experimental observations. Apart from constraints in computing resources that have gradually been resolved with technological advances, novel insights can come from considering whole proteins with multi-scale simulations and modeling. As computing power improves, the microenvironment can be included, e.g. entire viral organisms [84]. Otherwise, the availability of coarse-grained approaches [58,59,85] certainly can be a possible alternative.

Nonetheless, as discussed in the above examples, the approach of looking at biomolecules holistically allows the study of allosteric communication and allostery-derived interventions. Allostery is likely to apply to all types of proteins [83,86], promising to identify novel druggable sites, pockets, and repurposing enzymatic/binding activity. On this basis, considering whole proteins would be beneficial in detecting more allosteric sites, and also in providing new understanding of the subject matter.

Conclusion

We are not pushing for extreme holism as warned by Regenmortel [19], which would be ineffective given current real-life resource constraints. While the reductionist approach still has great value, moving toward a more holistic approach in considering whole proteins, protein complexes, and potential microenvironments would certainly be useful, at least in the areas of antibody engineering, druggable targets, drug discovery, and enzyme repurposing.

Author contribution

S.-X.P., K.-F.C., C.T.-T.S. and S.K.-E.G. drafted the manuscript. C.T.-T.S. and S.-X.P. prepared the figures. J.-J.P., K.-F.C., and S.-X.P. prepared the AR components. S.K.-E.G. directed and supervised the writing of the entire manuscript and the associated AR parts. All authors approved the final version of the manuscript.

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Competing interests

The authors declare that there are no competing interests associated with the manuscript.

Abbreviations

ADE, Antibody-dependent enhancement; CDR, Complementarity determining region; EC, Extracellular; Fab, Antigen-binding fragment; FWR, Framework region; NNRTI, Non-nucleoside reverse transcriptase inhibitor; RT, Reverse transcriptase; RTI, Reverse Transcriptase inhibitor; scFv, single-chain variable fragment.

References

1   Ecker, D.M., Jones, S.D. and Levine, H.L. (2015) The therapeutic monoclonal antibody market. *mAbs* **7**, 9–14,
    https://doi.org/10.4161/19420862.2015.989042

2   Johnson, M. (2013) Antibody structure and fragments. *Mater. Methods* **3** (160)), https://doi.org/10.13070/mm.en.3.160

3   Ponting, C.P. and Russell, R.R. (2002) The natural history of protein domains. *Annu. Rev. Biophys. Biomol. Struct.* **31**, 45–71,
    https://doi.org/10.1146/annurev.biophys.31.082901.134314

4   Nussinov, R. and Tsai, C.-J. (2014) The design of covalent allosteric drugs. *Annu. Rev. Pharmacol. Toxicol.* **55**, 249–267,
    https://doi.org/10.1146/annurev-pharmtox-010814-124401

5   Amaro, R.E. (2017) Toward understanding "the ways" of allosteric drugs. *ACS Cent. Sci.* **3**, 925–926, https://doi.org/10.1021/acscentsci.7b00396

6   Abdel-Magid, A.F. (2015) Allosteric modulators: an emerging concept in drug discovery. *ACS Med. Chem. Lett.* **6**, 104–107,
    https://doi.org/10.1021/ml5005365

7   Berezovsky, I.N. (2013) Thermodynamics of allostery paves a way to allosteric drugs. *Biochim. Biophys. Acta* **1834**, 830–835,
    https://doi.org/10.1016/j.bbapap.2013.01.024

8   Guarnera, E. and Berezovsky, I.N. (2019) On the perturbation nature of allostery: sites, mutations, and signal modulation. *Curr. Opin. Struct. Biol.* **56**,
    18–27, https://doi.org/10.1016/j.sbi.2018.10.008

9   Guarnera, E. and Berezovsky, I.N. (2016) Structure-based statistical mechanical model accounts for the causality and energetics of allosteric
    communications. *PLoS Comput. Biol.* **12**, e1004678, https://doi.org/10.1371/journal.pcbi.1004678

10 Berg, J., Tymoczko, J. and Stryer, L. (2002) Section 10.1: Aspartate Transcarbamoylase is allosterically inhibited by the end product of its pathway.
    Biochemistry, 5th , WH Freeman, New York

11 Smith, T.J., Schmidt, T., Fang, J. et al. (2002) The structure of apo human glutamate dehydrogenase details subunit communication and allostery. *J.
    Mol. Biol.* **318**, 765–777, https://doi.org/10.1016/S0022-2836(02)00161-4

12 Traut, T.W. (2008) *Allosteric Regulation Enzymes*, Springer, U.S.A.

13 Zhao, J., Nussinov, R. and Ma, B. (2018) Antigen binding allosterically promotes Fc receptor recognition. *mAbs* **11**, 58–74 ,
    https://doi.org/10.1080/19420862.2018.1522178

14 Su, C.T.-T., Lua, W.-H., Ling, W.-L. et al. (2018) Allosteric effects between the antibody constant and variable regions: a study of IgA Fc mutations on
    antigen binding. *Antibodies* **7**, 20, https://doi.org/10.3390/antib7020020

15 Ling, W.L., Lua, W.H., Poh, J.J. et al. (2018) Effect of VH-VL families in Pertuzumab and Trastuzumab recombinant production, Her2 and FcγIIA binding.
    Front. Immunol. **9**, 469, https://doi.org/10.3389/fimmu.2018.00469

16 Yang, D., Kroe-Barrett, R., Singh, S. et al. (2017) IgG cooperativity – Is there allostery? Implications for antibody functions and therapeutic antibody
    development. *mAbs* **9**, 1231–1252, https://doi.org/10.1080/19420862.2017.1367074

17 Janda, A., Bowen, A., Greenspan, N.S. et al. (2016) Ig constant region effects on Variable region structure and function. *Front. Microbiol.* **7** (22),
    https://doi.org/10.3389/fmicb.2016.00022

18 Oda, M., Kozono, H., Morii, H. et al. (2003) Evidence of allosteric conformational changes in the antibody constant region upon antigen binding. *Int.
    Immunol.* **15**, 417–26, https://doi.org/10.1093/intimm/dxg036

19 Regenmortel, M.H.-V.V. (2004) Reductionism and complexity in molecular biology. *EMBO Rep.* **5**, 1016–1020,
    https://doi.org/10.1038/sj.embor.7400284

20 Ahn, A.C., Tewari, M., Poon, C.-S. et al. (2006) The limits of reductionism in medicine: could systems biology offer an alternative? *PLoS Med.* **3**, e208,
    https://doi.org/10.1371/journal.pmed.0030208

21 Krakauer, J.W., Ghazanfar, A.A., Gomez-Marin, A. et al. (2017) Neuroscience needs behavior: correcting a reductionist bias. *Neuron* **93**, 480–490,
    https://doi.org/10.1016/j.neuron.2016.12.041

22 Bieber, T. (2015) Concept and scientific background of personalized medicine. In *Personalized Treatment Options in Dermatology* (Bieber, T. and Nestle,
    F., eds), vol. 2015, pp. 1–5, Springer, Berlin Heidelberg

23 Albergante, L., Liu, D. and Palmer, S. (2016) Insights into Biological Complexity from Simple Foundations. In *Biophysics of Infection* (Leake, M.C., ed.),
    vol. 915, pp. 295–305, Springer International Publishing

24 Poh, J.J., Phua, S.X., Chan, K.F. et al. (2018) Commentary: Augmented reality scientific phone apps –making the APD AR Holistic Review app and using
    existing AR apps for scientific publications. *Sci. Phone Apps Mobile Devic.* **4** (4)), https://doi.org/10.30943/2018/28092018

25 Harris, L.J., Larson, S.B., Hasel, K.W. et al. (1997) Refined structure of an intact IgG2a monoclonal antibody. *Biochemistry* **36**, 1581–1597,
    https://doi.org/10.1021/bi962514+

26 Kinet, J.P. (1989) Antibody-cell interactions: Fc receptors. *Cell* **57**, 351–354, https://doi.org/10.1016/0092-8674(89)90910-0

27 Gellert, M. (2002) V,(D)J recombination: RAG proteins, repair factors, and regulation. *Annu. Rev. Biochem.* **71**, 101–132,
    https://doi.org/10.1146/annurev.biochem.71.090501.150203

28 Wu, T.T. and Kabat, E.A. (1970) An analysis of the sequences of the variable regions of Bence Jones proteins and myeloma light chains and their
    implications for antibody complementarity. *J. Exp. Med.* **132**, 211–250, https://doi.org/10.1084/jem.132.2.211

29 Scott, A.M., Wolchok, J.D. and Old, L.J. (2012) Antibody therapy of cancer. *Nat. Rev. Cancer* **12**, 278–287, https://doi.org/10.1038/nrc3236

30 Kabat, E.A., Te Wu, T. and Foeller, C. (1992) *Sequences of Proteins of Immunological Interest*, Diane Publishing Company

31 Chothia, C., Lesk, A.M., Tramontano, A. et al. (1989) Conformations of immunoglobulin hypervariable regions. *Nature* **342**, 877–883,
    https://doi.org/10.1038/342877a0

32 Abhinandan, K. and Martin, A.C. (2008) Analysis and improvements to Kabat and structurally correct numbering of antibody variable domains. *Mol.
    Immunol.* **45**, 3832–3839, https://doi.org/10.1016/j.molimm.2008.05.022

33 Honegger, A. and Plückthun, A. (2001) Yet another numbering scheme for immunoglobulin variable domains: an automatic modeling and analysis tool. *J. Mol. Biol.* **309**, 657–670, https://doi.org/10.1006/jmbi.2001.4662

34 Li, T., Pantazes, R.J. and Maranas, C.D. (2014) OptMAVEn–a new framework for the *de novo* design of antibody variable region models targeting specific antigen epitopes. *PLoS ONE* **9**, e105954, https://doi.org/10.1371/journal.pone.0105954

35 van den Beucken, T., Pieters, H., Steukers, M. et al. (2003) Affinity maturation of Fab antibody fragments by fluorescent-activated cell sorting of yeast-displayed libraries. *FEBS Lett.* **546**, 288–294, https://doi.org/10.1016/S0014-5793(03)00602-1

36 Lua, W.H., Ling, W.L., Yeo, J.Y. et al. (2018) The effects of antibody engineering CH and CL in Trastuzumab and Pertuzumab recombinant models: Impact on antibody production and antigen-binding. *Sci. Rep.* **8**, 718, https://doi.org/10.1038/s41598-017-18892-9

37 Vidarsson, G., Dekkers, G. and Rispens, T. (2014) IgG subclasses and allotypes: from structure to effector functions. *Front. Immunol.* **5**, 520, https://doi.org/10.3389/fimmu.2014.00520

38 Zhao, J., Nussinov, R. and Ma, B. (2017) Allosteric control of antibody-prion recognition through oxidation of a disulfide bond between the CH and CL chains. *Protein Eng. Des. Sel.* **30**, 67–76

39 Oda, M., Kozono, H., Morii, H. et al. (2003) Evidence of allosteric conformational changes in the antibody constant region upon antigen binding. *Int. Immunol.* **15**, 417–426, https://doi.org/10.1093/intimm/dxg036

40 Vaughn, D.W., Green, S., Kalayanarooj, S. et al. (2000) Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. *J. Infect. Dis.* **181**, 2–9, https://doi.org/10.1086/315215

41 Sasaki, T., Setthapramote, C., Kurosu, T. et al. (2013) Dengue virus neutralization and antibody-dependent enhancement activities of human monoclonal antibodies derived from dengue patients at acute phase of secondary infection. *Antiviral Res.* **98**, 423–431, https://doi.org/10.1016/j.antiviral.2013.03.018

42 Halstead, S.B. (2003) Neutralization and antibody-dependent enhancement of dengue viruses. *Adv. Virus Res.* **60**, 421–467, https://doi.org/10.1016/S0065-3527(03)60011-4

43 Burke, D.S. (1992) Human HIV vaccine trials: does antibody-dependent enhancement pose a genuine risk? *Perspect. Biol. Med.* **35**, 511–530, https://doi.org/10.1353/pbm.1992.0048

44 Lua, W.-H., Ling, W.-L., Su, C.T.-T. et al. (2017) Discovery of a novel splice variant of Fcar (CD89) unravels sequence segments necessary for efficient secretion: a story of bad signal peptides and good ones that nevertheless do not make it. *Cell Cycle* **16**, 457–467, https://doi.org/10.1080/15384101.2017.1281480

45 Phua, S.-X., Lua, W.-H. and Gan, S.K.-E. (2018) Role of FcαR EC2 region in extracellular membrane localization. *Cell Cycle* **17**, 669–670, https://doi.org/10.1080/15384101.2018.1444236

46 Bhaskar, V., Goldfine, I.D., Bedinger, D.H. et al. (2012) A fully human, allosteric monoclonal antibody that activates the insulin receptor and improves glycemic control. *Diabetes* **61**, 1263–1271, https://doi.org/10.2337/db11-1578

47 Hinke, S., Cieniewicz, A., Kirchner, T. et al. (2018) Unique pharmacology of a novel allosteric agonist/sensitizer insulin receptor monoclonal antibody. *Mol. Metab.* **10**, 87–99, https://doi.org/10.1016/j.molmet.2018.01.014

48 Hinkofer, L.C., Seidel, S.A., Korkmaz, B. et al. (2013) A monoclonal antibody (MCPR3-7) interfering with the activity of proteinase 3 by an allosteric mechanism. *J. Biol. Chem.* **288**, 26635–26648, https://doi.org/10.1074/jbc.M113.495770

49 Mukund, S., Shang, Y., Clarke, H.J. et al. (2013) Inhibitory mechanism of an allosteric antibody targeting the glucagon receptor. *J. Biol. Chem.* **288**, 36168–36178, https://doi.org/10.1074/jbc.M113.496984

50 Brecher, M., Li, Z., Liu, B. et al. (2017) A conformational switch high-throughput screening assay and allosteric inhibition of the flavivirus NS2B-NS3 protease. *PLoS Pathog.* **13**, e1006411, https://doi.org/10.1371/journal.ppat.1006411

51 Christodoulou, M.S., Caporuscio, F., Restelli, V. et al. (2017) Probing an allosteric pocket of CDK2 with small molecules. *Chem. Med. Chem.* **12**, 33–41, https://doi.org/10.1002/cmdc.201600474

52 Korczynska, M., Clark, M.J., Valant, C. et al. (2018) Structure-based discovery of selective positive allosteric modulators of antagonists for the M_2 muscarinic acetylcholine receptor. *Proc. Natl. Acad. Sci. U.S.A.* **115**, E2419–E2428, https://doi.org/10.1073/pnas.1718037115

53 Kunze, J., Todoroff, N., Schneider, P. et al. (2014) Targeting dynamic pockets of HIV-1 protease by structure-based computational screening for allosteric inhibitors. *J. Chem. Inf. Model.* **54**, 987–991, https://doi.org/10.1021/ci400712h

54 McCarthy, M., Prakash, P. and Gorfe, A.A. (2015) Computational allosteric ligand binding site identification on Ras proteins. *Acta Biochim. Biophys. Sin.* **48**, 3–10

55 Mukhametov, A., Newhouse, E.I., Ab Aziz, N. et al. (2014) Allosteric pocket of the dengue virus (serotype 2) NS2B/NS3 protease: *in silico* ligand screening and molecular dynamics studies of inhibition. *J. Mol. Graph. Model.* **52**, 103–113, https://doi.org/10.1016/j.jmgm.2014.06.008

56 Chiang, R.Z.-H., Gan, S.K.-E. and Su, C.T.-T. (2018) A computational study for rational HIV-1 non-nucleoside reverse transcriptase inhibitor selection and the discovery of novel allosteric pockets for inhibitor design. *Biosci. Rep.* **38** (2), https://doi.org/10.1042/BSR20171113

57 Kang, D., Wang, Z., Zhang, H. et al. (2018) Further exploring solvent-exposed tolerant regions of allosteric binding pocket for Novel HIV-1 NNRTIs discovery. *ACS Med. Chem. Lett.* **9**, 370–375, https://doi.org/10.1021/acsmedchemlett.8b00054

58 Greener, J.G. and Sternberg, M.J. (2015) AlloPred: prediction of allosteric pockets on proteins using normal mode perturbation analysis. *BMC Bioinformatics* **16**, 335, https://doi.org/10.1186/s12859-015-0771-1

59 Guarnera, E., Tan, Z.-W., Zheng, Z. et al. (2017) AlloSigMA: allosteric signaling and mutation analysis server. *Bioinformatics* **33**, 3996–3998, https://doi.org/10.1093/bioinformatics/btx430

60 Trotter, A.B., Hong, S.Y., Srikantiah, P. et al. (2013) Systematic review of HIV drug resistance in the world health organization Southeast Asia region. *AIDS Rev.* **15**, 162–170

61 Poongavanam, V., Namasivayam, V., Vanangamudi, M. et al. (2018) Integrative approaches in HIV-1 non-nucleoside reverse transcriptase inhibitor design. *Wiley Interdiscip. Rev.* **8**, e1328

62 Good A. Virtual Screening (2007) *Comprehensive Medicinal Chemistry II, 2007*, pp. 459–494, Elsevier, ISBN: 978-0-08-045044-5

63 Jespersen, M.C., Peters, B., Nielsen, M. et al. (2017) BepiPred-2.0: improving sequence-based B-cell epitope prediction using conformational epitopes. *Nucleic Acids Res.* **45**, W24–W29, https://doi.org/10.1093/nar/gkx346

64 Emini, E.A., Hughes, J.V., Perlow, D.S. et al. (1985) Induction of hepatitis A virus-neutralizing antibody by a virus-specific synthetic peptide. *J. Virol.* **55**, 836–839

65 Saha, S. and Raghava, G.P.S. (2006) Prediction of continuous B-cell epitopes in an antigen using recurrent neural network. *Proteins* **65**, 40–48, https://doi.org/10.1002/prot.21078

66 Garman, S.C., Kinet, J.-P. and Jardetzky, T.S. (1998) Crystal structure of the human high-affinity IgE receptor. *Cell* **95**, 951–961, https://doi.org/10.1016/S0092-8674(00)81719-5

67 Su, C.T.-T., Ling, W.-L., Lua, W.-H. et al. (2017) The role of antibody Vκ Framework 3 region towards antigen binding: effects on recombinant production and Protein L binding. *Sci. Rep.* **7**, 3766, https://doi.org/10.1038/s41598-017-02756-3

68 Mackay, G.A., Hulett, M.D., Cook, J.P. et al. (2002) Mutagenesis within human FcεRIα differentially affects human and murine IgE binding. *J. Immunol.* **168**, 1787–1795, https://doi.org/10.4049/jimmunol.168.4.1787

69 Nussinov, R. and Tsai, C.-J. (2013) Allostery in disease and in drug discovery. *Cell* **153**, 293–305, https://doi.org/10.1016/j.cell.2013.03.034

70 Tee, W.-V., Guarnera, E. and Berezovsky, I.N. (2018) Reversing allosteric communication: From detecting allosteric sites to inducing and tuning targeted allosteric response. *PLoS Comput. Biol.* **14**, e1006228, https://doi.org/10.1371/journal.pcbi.1006228

71 Fuentes, G., Scaltriti, M., Baselga, J. et al. (2011) Synergy between trastuzumab and pertuzumab for human epidermal growth factor 2 (Her2) from colocalization: an *in silico* based mechanism. *Breast Cancer Res.* **13** (3), https://doi.org/10.1186/bcr2888

72 Lua, W.-H., Gan, S.K.-E., Lane, D.P. et al. (2015) A search for synergy in the binding kinetics of Trastuzumab and Pertuzumab whole and F (ab) to Her2. *NPJ Breast Cancer* **1**, https://doi.org/10.1038/npjbcancer.2015.12

73 Rammensee, H.-G., Bachmann, J., Emmerich, N.P.N. et al. (1999) SYFPEITHI: database for MHC ligands and peptide motifs. *Immunogenetics* **50**, 213–219, https://doi.org/10.1007/s002510050595

74 Parker, K.C., Bednarek, M.A. and Coligan, J.E. (1994) Scheme for ranking potential HLA-A2 binding peptides based on independent binding of individual peptide side-chains. *J. Immunol.* **152**, 163–175

75 Vita, R., Overton, J.A., Greenbaum, J.A. et al. (2015) The immune epitope database (IEDB) 3.0. *Nucleic Acids Res.* **43**, D405–D412, https://doi.org/10.1093/nar/gku938

76 Kurochkin, I.V., Guarnera, E., Wong, J.H. et al. (2017) Toward allosterically increased catalytic activity of insulin-degrading enzyme against amyloid peptides. *Biochemistry* **56**, 228–239, https://doi.org/10.1021/acs.biochem.6b00783

77 Su, C.T.-T., Kwoh, C.-K., Verma, C.S. et al. (2017) Modeling the full length HIV-1 Gag polyprotein reveals the role of its p6 subunit in viral maturation and the effect of non-cleavage site mutations in protease drug resistance. *J. Biomol. Struct. Dyn* **36**, 4366–4377, https://doi.org/10.1080/07391102.2017.1417160

78 Monod, J., Wyman, J. and Changeux, J.-P. (1965) On the nature of allosteric transitions: a plausible model. *J. Mol. Biol.* **12**, 88–118, https://doi.org/10.1016/S0022-2836(65)80285-6

79 Koshland, D.J., Nemethy, G. and Filmer, D. (1966) Comparison of experimental binding data and theoretical models in proteins containing subunits. *Biochemistry* **5**, 365–385, https://doi.org/10.1021/bi00865a047

80 Yu, E.W. and Koshland, D.J. (2001) Propagating conformational changes over long (and short) distances in proteins. *Proc. Natl Acad. Sci. U.S.A.* **98**, 9517–9520, https://doi.org/10.1073/pnas.161239298

81 Ma, B. and Nussinov, R. (2016) Conformational footprints. *Nat. Chem. Biol.* **12**, 890–891, https://doi.org/10.1038/nchembio.2212

82 Wei, G., Xi, W., Nussinov, R. et al. (2016) Protein ensembles: how does nature harness thermodynamic fluctuations for life? The diverse functional roles of conformational ensembles in the cell. *Chem. Rev.* **116**, 6516–6551, https://doi.org/10.1021/acs.chemrev.5b00562

83 Gunasekaran, K., Ma, B. and Nussinov, R. (2004) Is allostery an intrinsic property of all dynamic proteins? *Proteins* **57**, 433–443, https://doi.org/10.1002/prot.20232

84 Marzinek, J.K., Holdbrook, D.A., Huber, R.G. et al. (2016) Pushing the envelope: dengue viral membrane coaxed into shape by molecular simulations. *Structure* **24**, 1410–1420, https://doi.org/10.1016/j.str.2016.05.014

85 Goncearenco, A., Mitternacht, S., Yong, T. et al. (2013) SPACER: server for predicting allosteric communication and effects of regulation. *Nucleic Acids Res.* **41**, W266–W272, https://doi.org/10.1093/nar/gkt460

86 Cui, Q. and Karplus, M. (2008) Allostery and cooperativity revisited. *Protein Sci.* **17**, 1295–1307, https://doi.org/10.1110/ps.03259908

87 Cook, J.P.D., Henry, A.J., McDonnell, J.M. et al. (1997) Identification of contact residues in the IgE binding site of human FcεRIα. *Biochemistry* **36**, 15579–15588, https://doi.org/10.1021/bi9713005