





[illegible]

**a**

Gibbs Free Energy

crystal form 1 crystal form 2

Conformation

active

inactive

**b**

KDM4D (space group P3221)

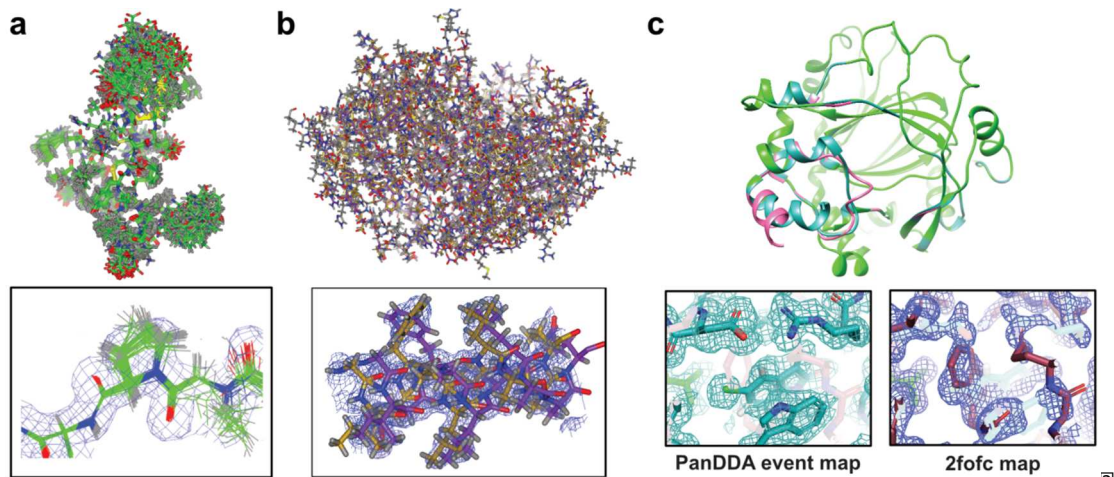
KDM4D (space group P43212)

[illegible]









The structure of the protein complex is shown in three panels. Panel (a) shows the protein structure with a green mesh overlay. Panel (b) shows the protein structure with a blue mesh overlay. Panel (c) shows the protein structure with a green mesh overlay. The inset images show close-up views of the protein structure.

?

The structure of the protein complex is shown in three panels. Panel (a) shows the protein structure with a green mesh overlay. Panel (b) shows the protein structure with a blue mesh overlay. Panel (c) shows the protein structure with a green mesh overlay. The inset images show close-up views of the protein structure.

The structure of the protein complex is shown in three panels. Panel (a) shows the protein structure with a green mesh overlay. Panel (b) shows the protein structure with a blue mesh overlay. Panel (c) shows the protein structure with a green mesh overlay. The inset images show close-up views of the protein structure.

The structure of the protein complex is shown in three panels. Panel (a) shows the protein structure with a green mesh overlay. Panel (b) shows the protein structure with a blue mesh overlay. Panel (c) shows the protein structure with a green mesh overlay. The inset images show close-up views of the protein structure.

?

The structure of the protein complex is shown in three panels. Panel (a) shows the protein structure with a green mesh overlay. Panel (b) shows the protein structure with a blue mesh overlay. Panel (c) shows the protein structure with a green mesh overlay. The inset images show close-up views of the protein structure.

The structure of the protein complex is shown in three panels. Panel (a) shows the protein structure with a green mesh overlay. Panel (b) shows the protein structure with a blue mesh overlay. Panel (c) shows the protein structure with a green mesh overlay. The inset images show close-up views of the protein structure.







Development and Innovation, Pfizer, São Paulo Research Foundation-FAPESP, Takeda, and Wellcome [106169/ZZ14/Z].

## 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. Koshland DE, Nemethy G, Filmer D: **Comparison of Experimental Binding Data and Theoretical Models in Proteins Containing Subunits**. *Biochemistry* 1966, **5**:365-385.
2. Monod J, Wyman J, Changeux JP: **On Nature of Allosteric Transitions - a Plausible Model**. *Journal of Molecular Biology* 1965, **12**:88-118.
3. Frauenfelder H, Sligar SG, Wolynes PG: **The Energy Landscapes and Motions of Proteins**. *Science* 1991, **254**:1598-1603.
4. Cooper A, Dryden DTF: **Allostery without Conformational Change - a Plausible Model**. *European Biophysics Journal with Biophysics Letters* 1984, **11**:103-109.
5. Hilser VJ, Wrabl JO, Motlagh HN: **Structural and Energetic Basis of Allostery**. *Annual Review of Biophysics*, Vol 41 2012, **41**:585-609.
6. Motlagh HN, Wrabl JO, Li J, Hilser VJ: **The ensemble nature of allostery**. *Nature* 2014, **508**:331-339.
7. Tzeng SR, Kalodimos CG: **The role of slow and fast protein motions in allosteric interactions**. *Biophys Rev* 2015, **7**:251-255.
8. Thal DM, Glukhova A, Sexton PM, Christopoulos A: **Structural insights into G-protein-coupled receptor allostery**. *Nature* 2018, **559**:45-53.
- Comprehensive review on the atomic details of allosteric transitions that govern GPCR biology.
9. Lu SY, Qiu YR, Ni D, He XH, Pu J, Zhang J: **Emergence of allosteric drug-resistance mutations: new challenges for allosteric drug discovery**. *Drug Discovery Today* 2020, **25**:177-184.
10. Gray JL, von Delft F, Brennan PE: **Targeting the Small GTPase Superfamily through Their Regulatory Proteins**. *Angewandte Chemie-International Edition* 2020, **59**:6342-6366.
11. Ludlow RF, Verdonk ML, Saini HK, Tickle IJ, Jhoti H: **Detection of secondary binding sites in proteins using fragment screening**. *Proceedings of the National Academy of Sciences of the United States of America* 2015, **112**:15910-15915.
12. Guarnera E, Berezovsky IN: **Allosteric sites: remote control in regulation of protein activity**. *Current Opinion in Structural Biology* 2016, **37**:1-8.
13. Ostrem JM, Peters U, Sos ML, Wells JA, Shokat KM: **K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions**. *Nature* 2013, **503**:548-551.
14. Kessler D, Gmachl M, Mantoulidis A, Martin LJ, Zoephel A, Mayer M, Gollner A, Covini D, Fischer S, Gerstberger T, et al.: **Drugging an undruggable pocket on KRAS**. *Proceedings of the National Academy of Sciences of the United States of America* 2019, **116**:15823-15829.
- Recent study which gives detailed account of the structure-based development process of an allosterically binding chemical probe for KRAS.
15. Chen YN, LaMarche MJ, Chan HM, Fekkes P, Garcia-Fortanet J, Acker MG, Antonakos B, Chen CH, Chen Z, Cooke VG, et al.: **Allosteric inhibition of SHP2 phosphatase inhibits cancers driven by receptor tyrosine kinases**. *Nature* 2016, **535**:148-152.

[illegible]

36. Douangamath A, Fearon D, Gehrtz P, Krojer T, Lukacik P, Owen CD, Resnick E, Strain-Damerell C, aimon A, Ábrányi-Balogh P, et al.: **Crystallographic and electrophilic fragment screening of the SARS-CoV-2 main protease.** *bioRxiv* 2020, 10.1101/2020.05.27.118117:2020.2005.2027.118117.
37. Keedy DA, Hill ZB, Biel JT, Kang E, Rettenmaier TJ, Brandao-Neto J, Pearce NM, von Delft F, Wells JA, Fraser JS: **An expanded allosteric network in PTP1B by multitemperature crystallography, fragment screening, and covalent tethering.** *Elife* 2018, **7**.
  - Seminal study which combines multi-temperature crystallography and fragment screening to illuminate the allosteric networks governing the protein tyrosine phosphatase PTP1B.
38. Murray CW, Rees DC: **The rise of fragment-based drug discovery.** *Nature Chemistry* 2009, **1**:187-192.
39. Price AJ, Howard S, Cons BD: **Fragment-based drug discovery and its application to challenging drug targets.** *Structure-Based Drug Design: Insights from Academia and Industry* 2017, **61**:475-484.
40. Spurlino JC: **Fragment Screening Purely with Protein Crystallography.** *Fragment-Based Drug Design: Tools, Practical Approaches, and Examples* 2011, **493**:321-356.
41. Owen RL, Juanhuix J, Fuchs M: **Current advances in synchrotron radiation instrumentation for MX experiments.** *Archives of Biochemistry and Biophysics* 2016, **602**:21-31.
42. Schiebel J, Krimmer SG, Rower K, Knorlein A, Wang XJ, Park AY, Stieler M, Ehrmann FR, Fu K, Radeva N, et al.: **High-Throughput Crystallography: Reliable and Efficient Identification of Fragment Hits.** *Structure* 2016, **24**:1398-1409.
  - This study gives a detailed overview of the parameters influencing hit rates of a crystallographic fragment screen on the aspartic protease endothiapepsin.
43. Erlanson DA: **Poll results: affiliation and fragment-finding methods in 2019.** Edited by; 2019.
44. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE: **The Protein Data Bank.** *Nucleic Acids Research* 2000, **28**:235-242.
45. Giordanetto F, Jin CT, Willmore L, Feher M, Shaw DE: **Fragment Hits: What do They Look Like and How do They Bind?** *Journal of Medicinal Chemistry* 2019, **62**:3381-3394.
  - Systematic study analysing binding properties of 489 protein-fragment structures in the PDB.
46. Fragalysis on World Wide Web URL: <https://diamondlightsource.atlassian.net/wiki/spaces/FRAG/overview>
47. Lang PT, Ng HL, Fraser JS, Corn JE, Echols N, Sales M, Holton JM, Alber T: **Automated electron-density sampling reveals widespread conformational polymorphism in proteins.** *Protein Science* 2010, **19**:1420-1431.
48. Keedy DA, Fraser JS, van den Bedem H: **Exposing Hidden Alternative Backbone Conformations in X-ray Crystallography Using qFit.** *Plos Computational Biology* 2015, **11**.
49. Burnley BT, Afonine PV, Adams PD, Gros P: **Modelling dynamics in protein crystal structures by ensemble refinement.** *Elife* 2012, **1**.
50. Pearce NM, Krojer T, Bradley AR, Collins P, Nowak RP, Talon R, Marsden BD, Kelm S, Shi JY, Deane CM, et al.: **A multi-crystal method for extracting obscured crystallographic states from conventionally uninterpretable electron density.** *Nature Communications* 2017, **8**.
  - Description of the PanDDA algorithm which utilises the large number of ligand-free datasets collected during crystallographic fragment screening in order to detect weakly bound ligands.
51. Grimes JM, Hall DR, Ashton AW, Evans G, Owen RL, Wagner A, McAuley KE, von Delft F, Orville AM, Sorensen T, et al.: **Where is crystallography going?** *Acta Crystallographica Section D-Structural Biology* 2018, **74**:152-166.
52. Fraser JS, Clarkson MW, Degnan SC, Erion R, Kern D, Alber T: **Hidden alternative structures of proline isomerase essential for catalysis.** *Nature* 2009, **462**:669-673.
53. Fraser JS, van den Bedem H, Samelson AJ, Lang PT, Holton JM, Echols N, Alber T: **Accessing protein conformational ensembles using room-temperature X-ray crystallography.** *Proceedings of the National Academy of Sciences of the United States of America* 2011, **108**:16247-16252.

