

# **multitemperature** data and diffuse scattering to reveal protein allostery

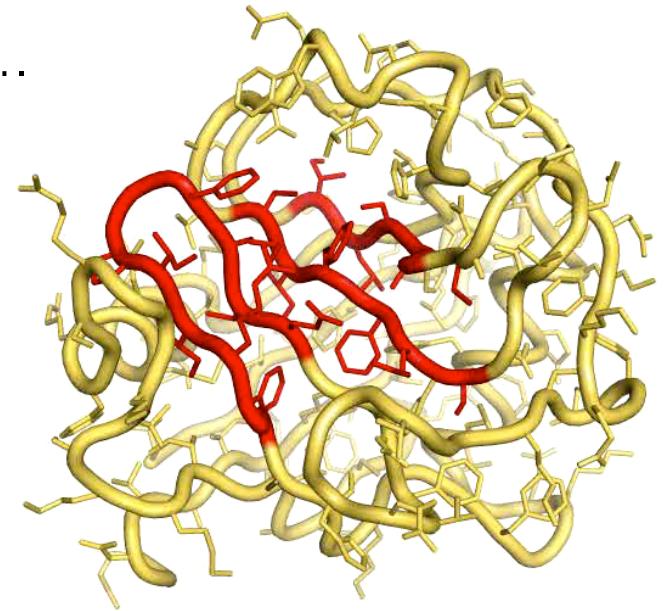
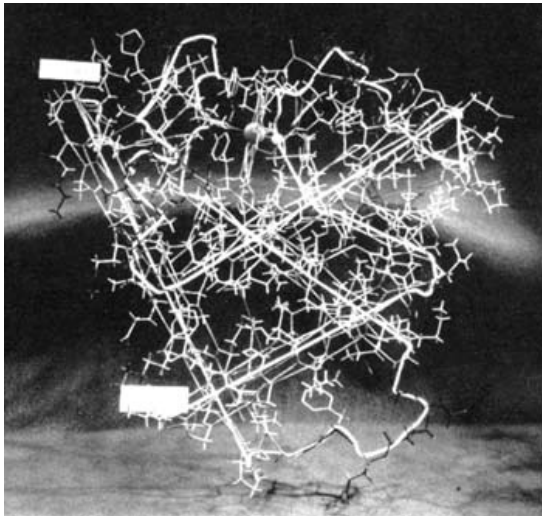
ALS Structural Biology Review

831 User Talk - James Fraser - UCSF

 @fraser\_lab

# From assemblies to molecular mechanism...

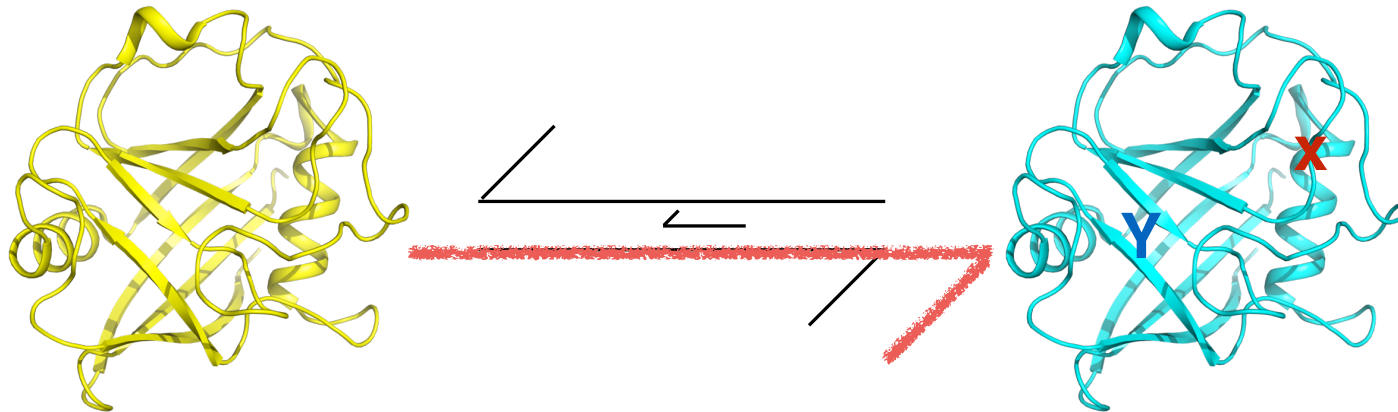
We are transitioning from static structural biology....



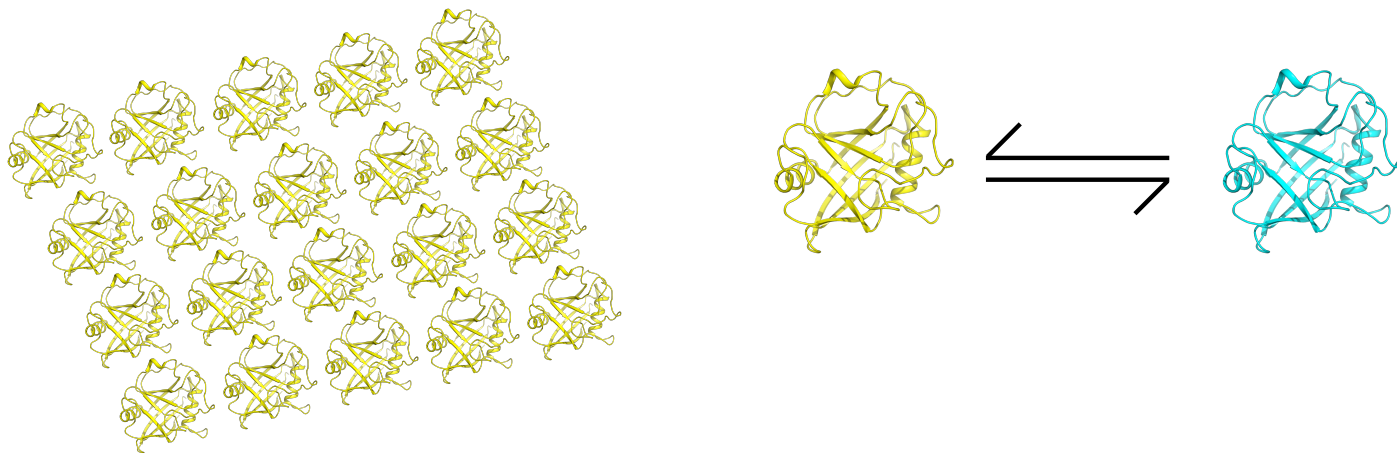
...to dynamic structural biology



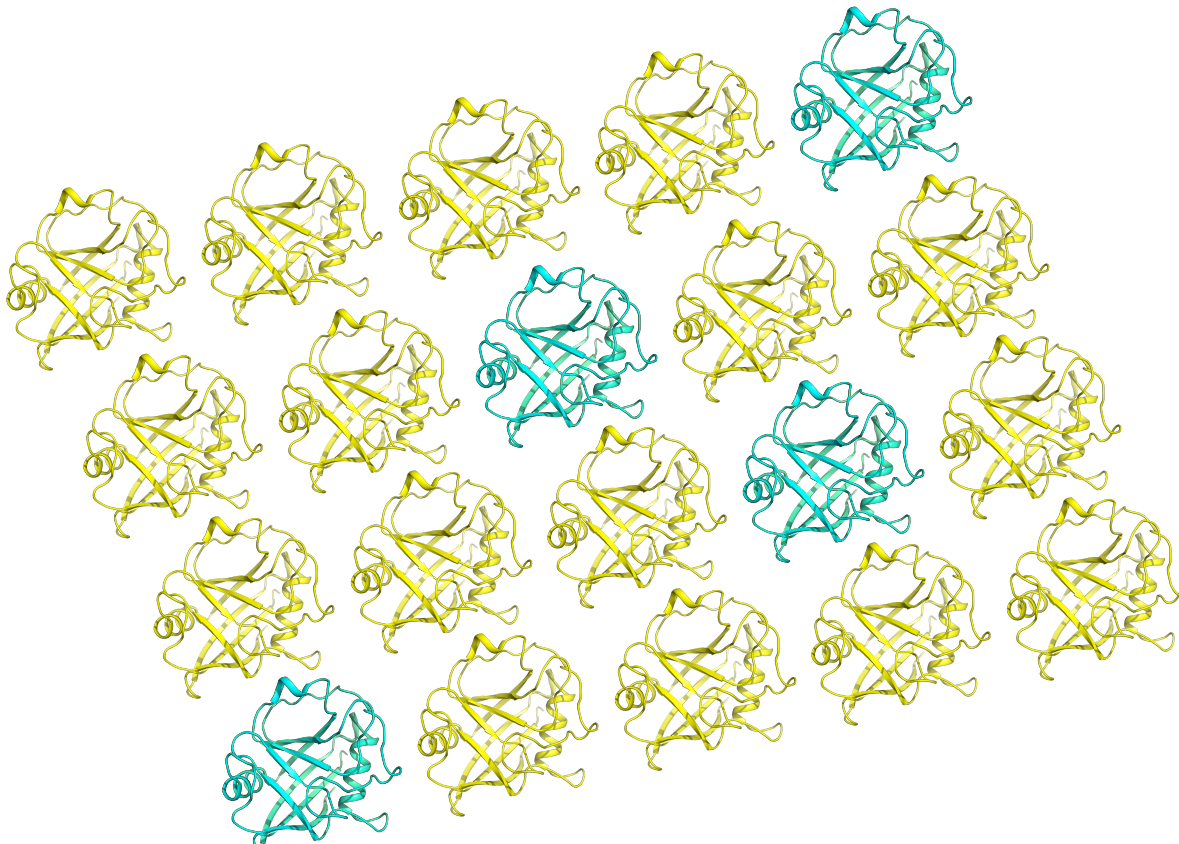
How are **allosteric** perturbations communicated intramolecularly to alter protein function?



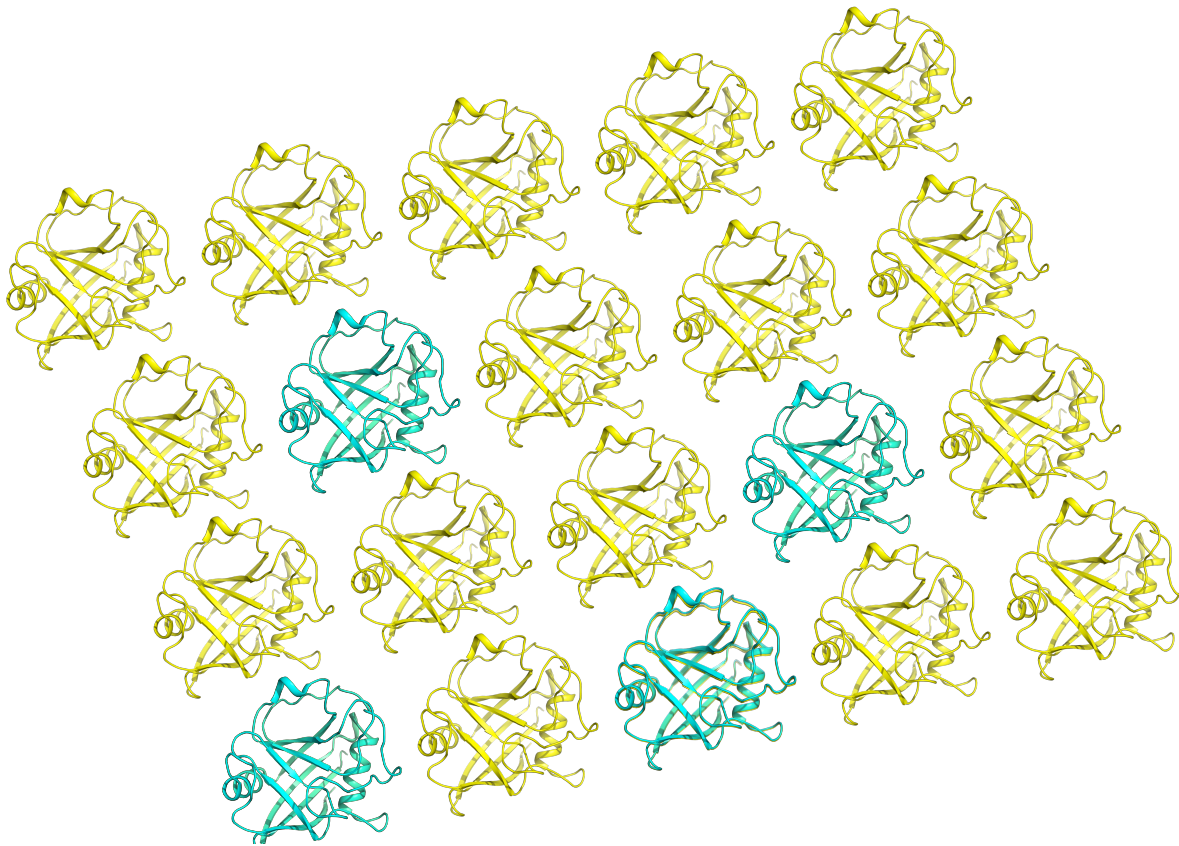
Proteins often populate  
**multiple conformations** in crystals



Conformational heterogeneity  
can be **static** or **dynamic**

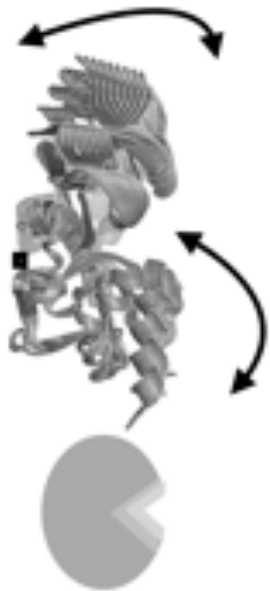


Conformational heterogeneity  
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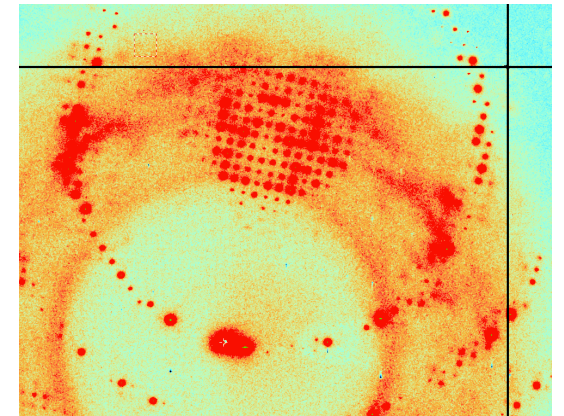
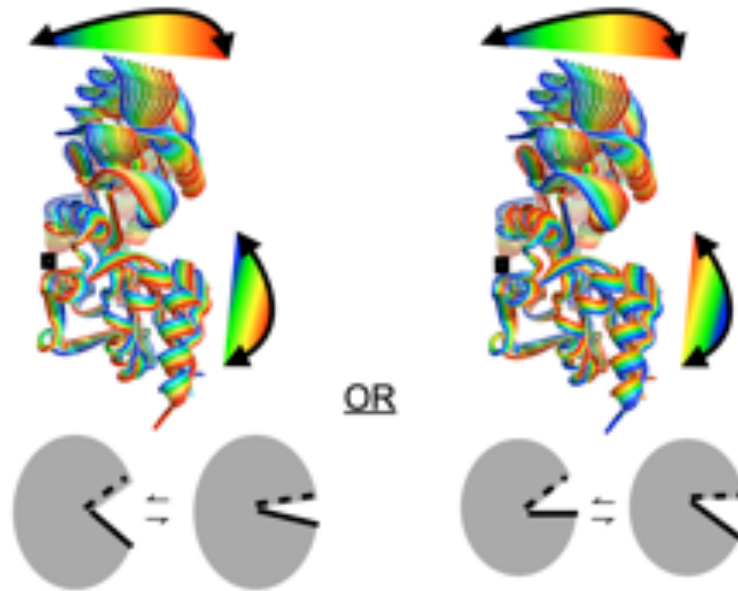


Diffuse scattering can **distinguish** different models of coupled heterogeneity

Bragg Scattering



Diffuse Scattering



Small features - **between** unit cells | Large features - **within** unit cells

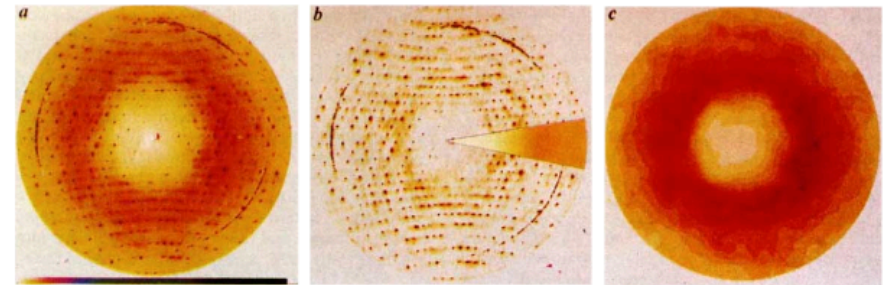
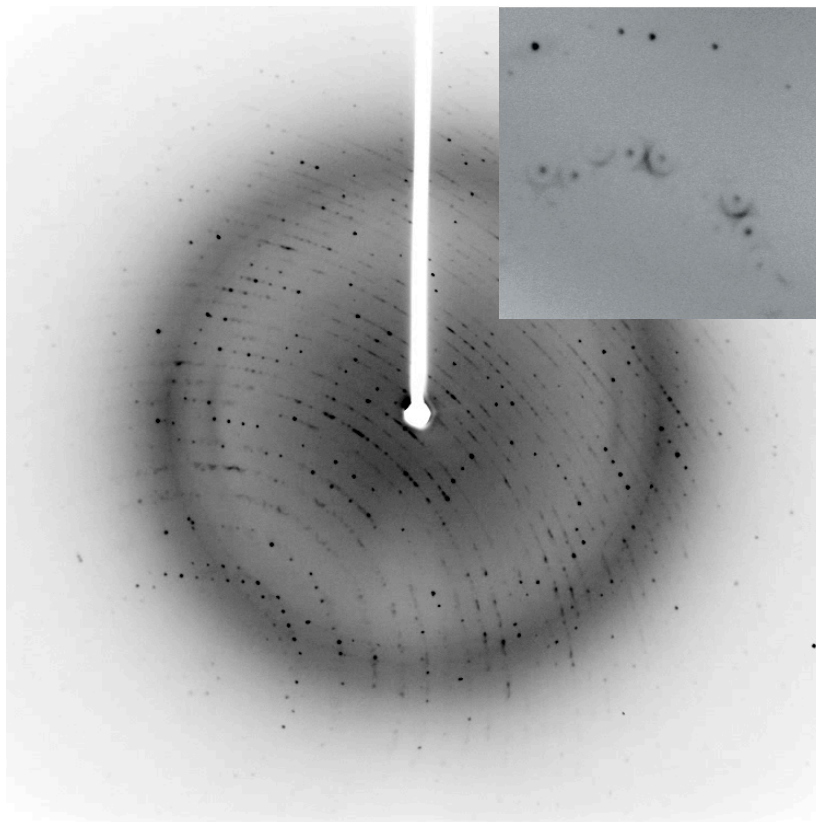


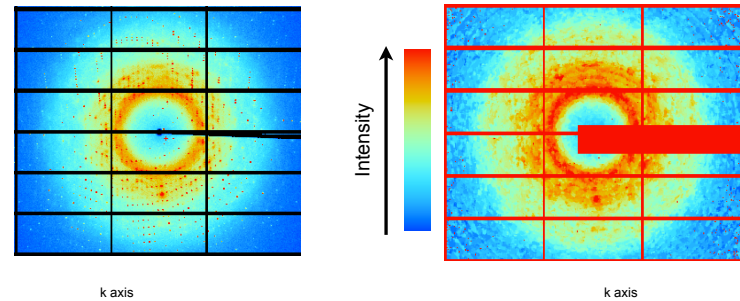
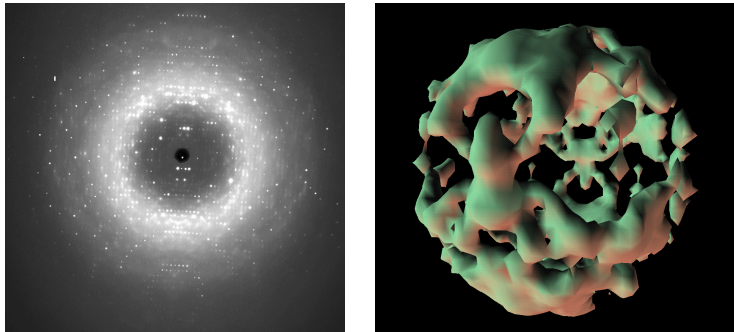
Fig. 1 X-ray diffraction data from insulin. *a*, A 40-h exposure of a stationary crystal (3-fold axis nearly vertical) recorded with focused, monochromatic Cu  $K_{\alpha}$  radiation from an Elliott rotating anode. The data (corrected for camera background and polarization) are displayed out to radius  $R = 0.45 \text{ \AA}^{-1}$ . Bragg reflections are overexposed. The sharp arcs are due to diffraction from the Al foil window of the He beam tunnel. The colour table (optical density range 0–2 OD units) was constructed to distinguish small variations in intensity up to 0.5 OD units. *b*, Bragg reflections and haloes digitally separated by subtracting the smoothly-varying diffuse scattering component from the film data. The inset wedge shows the estimated circularly symmetrical Compton-plus water scattering. *c*, Variational scattering evaluated from the difference between *a* and the two components in *b*. The colour table scale in *b* and *c* is  $1.5 \times$  that in *a*. Each intensity step in *c* equals 0.02 OD units in the data.

Caspar et al, Nature, 1988

Welberry et al, Acta B, 2011



# New data sets are needed to advance diffuse scattering



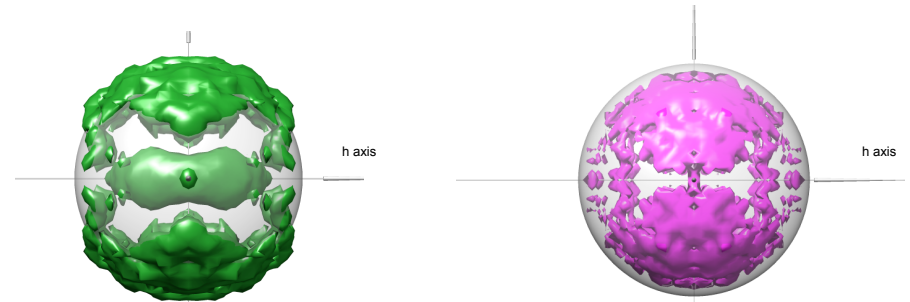
Wall, Ealick, and Gruner, PNAS 1997



Andrew  
VanBenschoten



Michael  
Wall (LANL)



Map	CC <sub>Friedel</sub>	CC <sub>Sym</sub>
CypA	0.9	0.7
Trypsin	0.95	0.69



TLS models are poor at explaining diffuse intensities, but **normal modes** or liquid like motions are better

Whole molecule



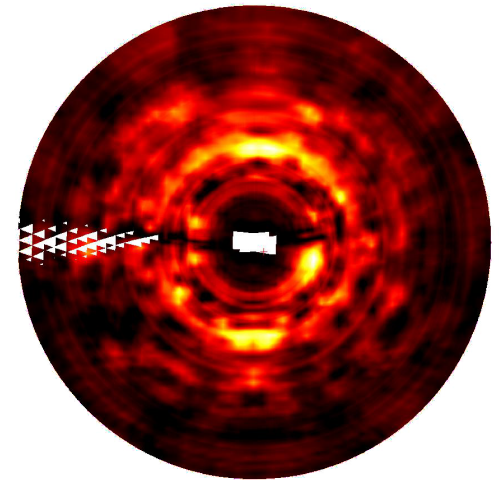
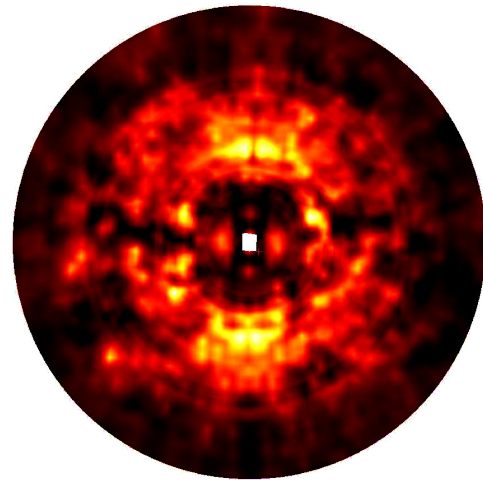
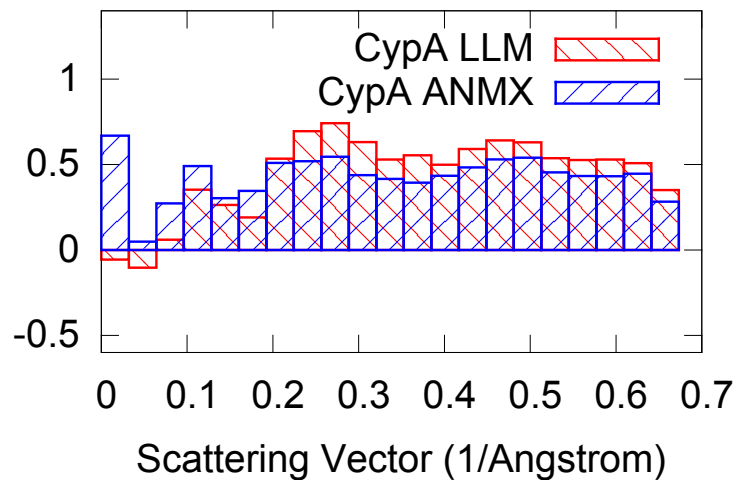
Phenix



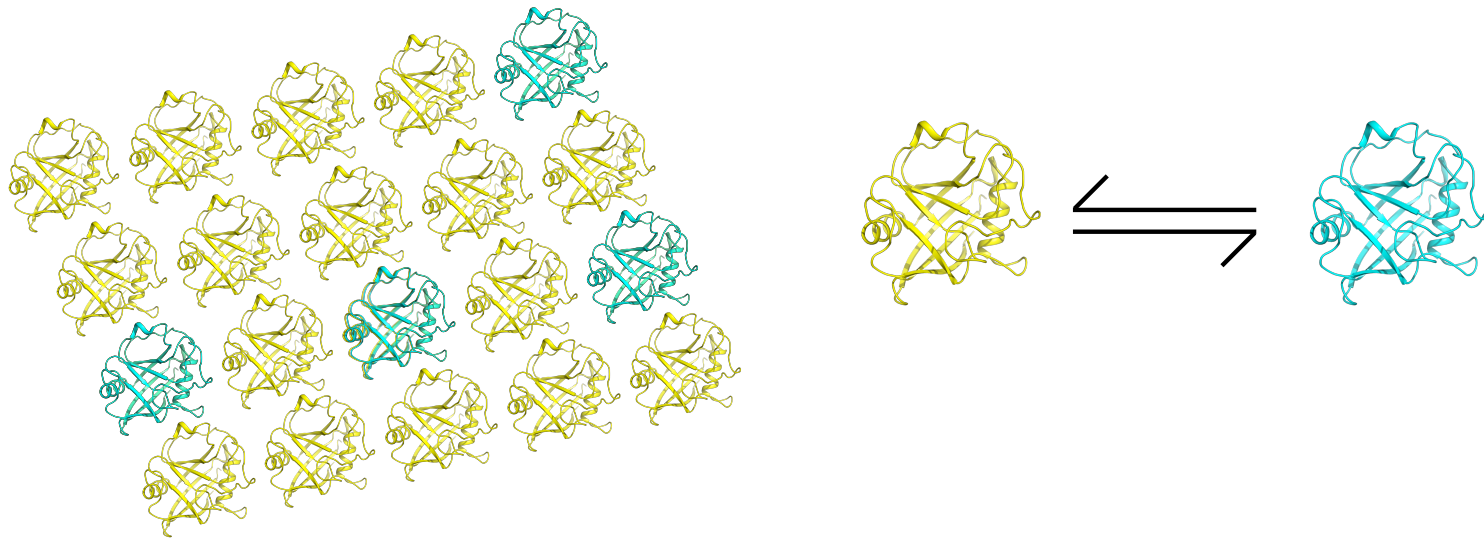
TLSMD



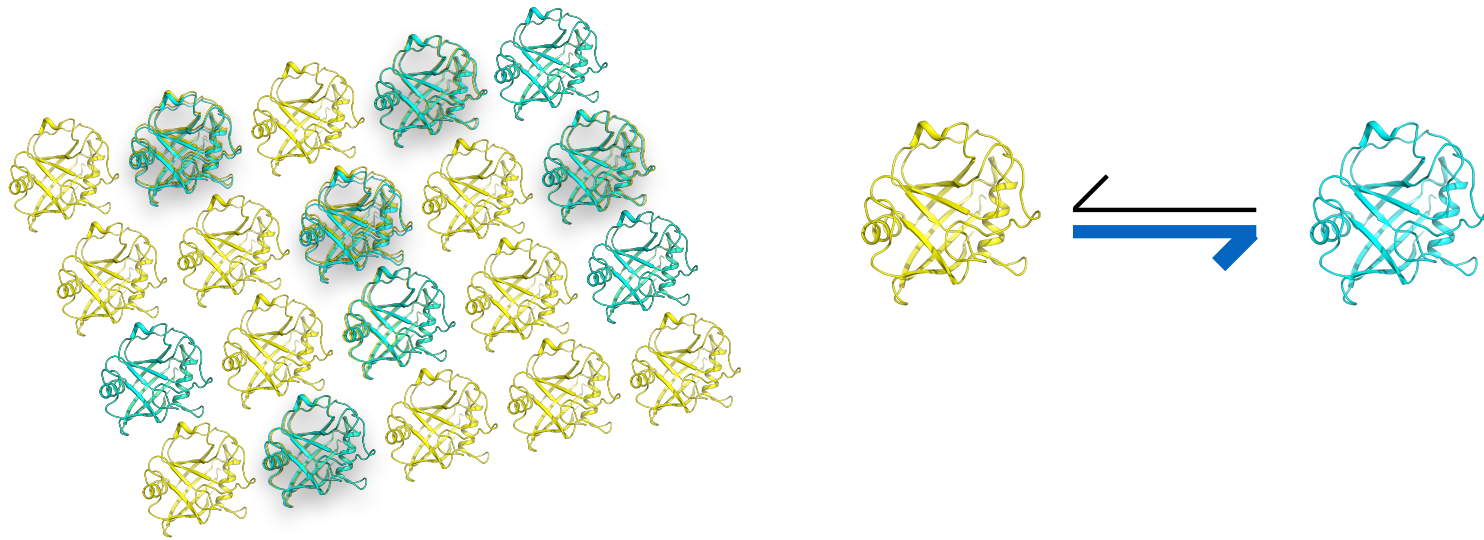
Correlation with Data



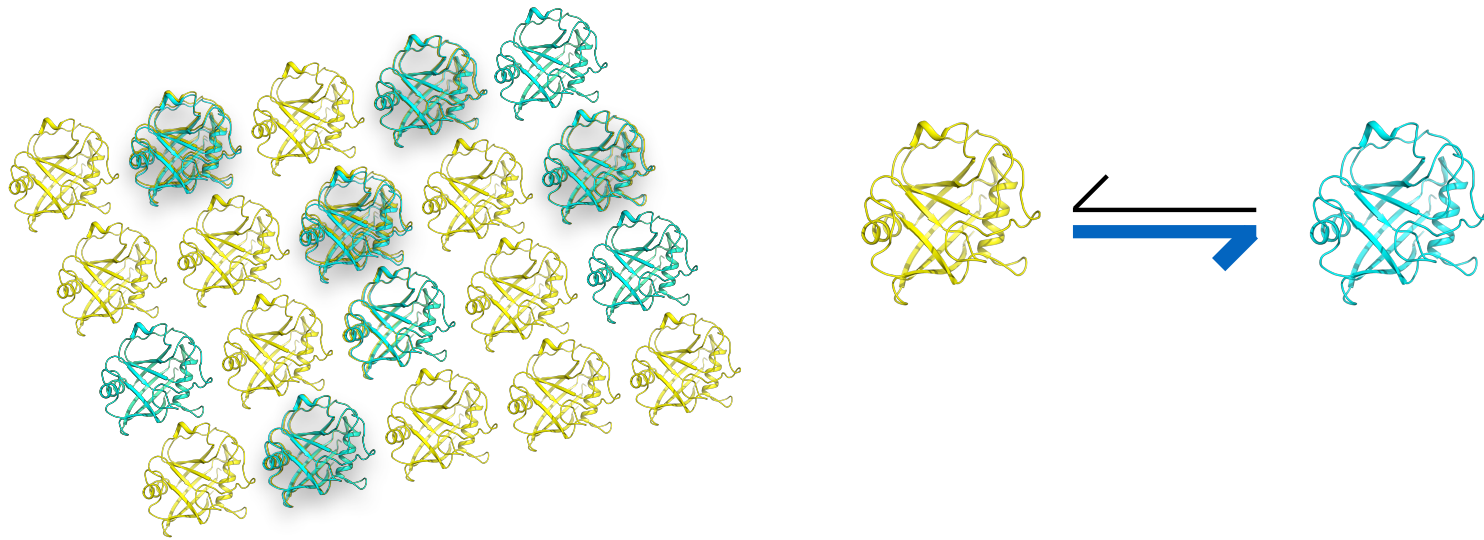
Temperature can **shift** the relative populations of conformations in the crystal



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**Hypotheses:** (1) shifting temperature exposes conformations near the “ground” state;  
(2) these new conformations are used by the protein in physiological mechanisms

Conformational dynamics are at the core of  
**three critical problems in biology**

We want to:

**design** macromolecules with new (unnatural) functions

understand how mutations alter protein function in **disease**

discover small molecules **drugs** to modulate protein function

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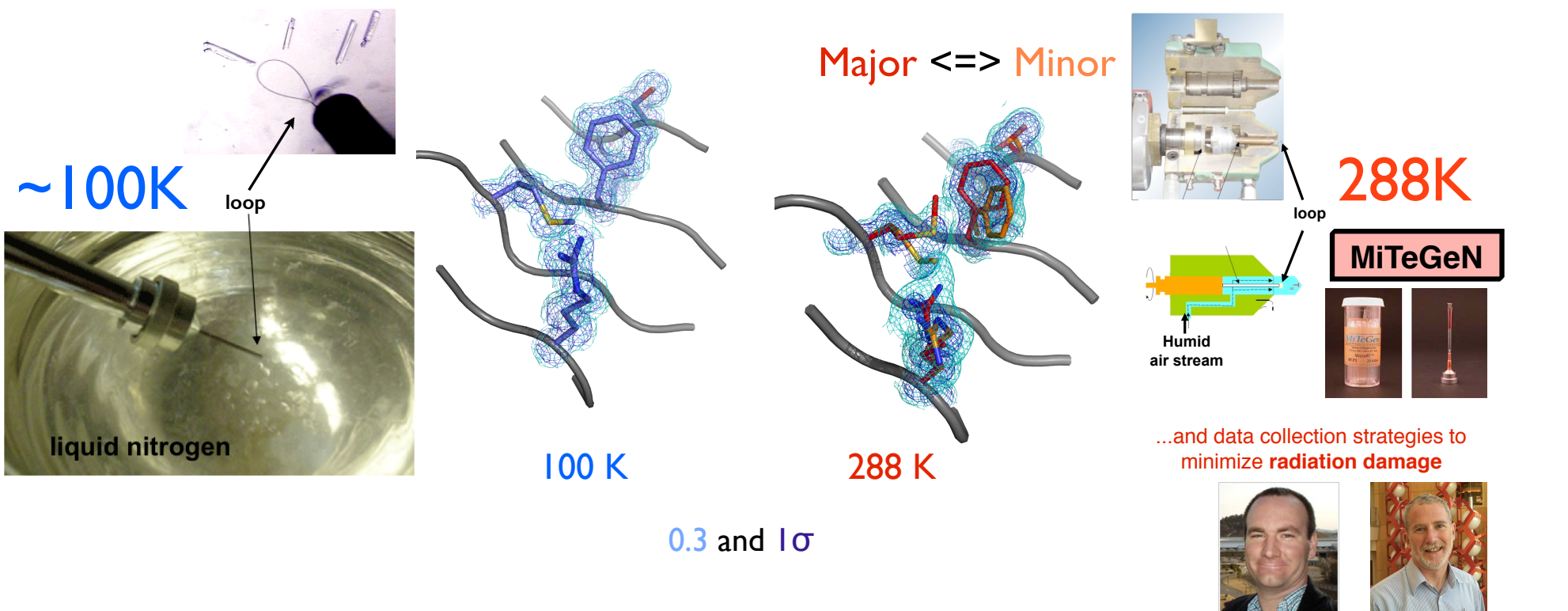
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**Cryocooling** has been amazing for static structural biology  
 - but **limits opportunities** for dynamic structural biology!



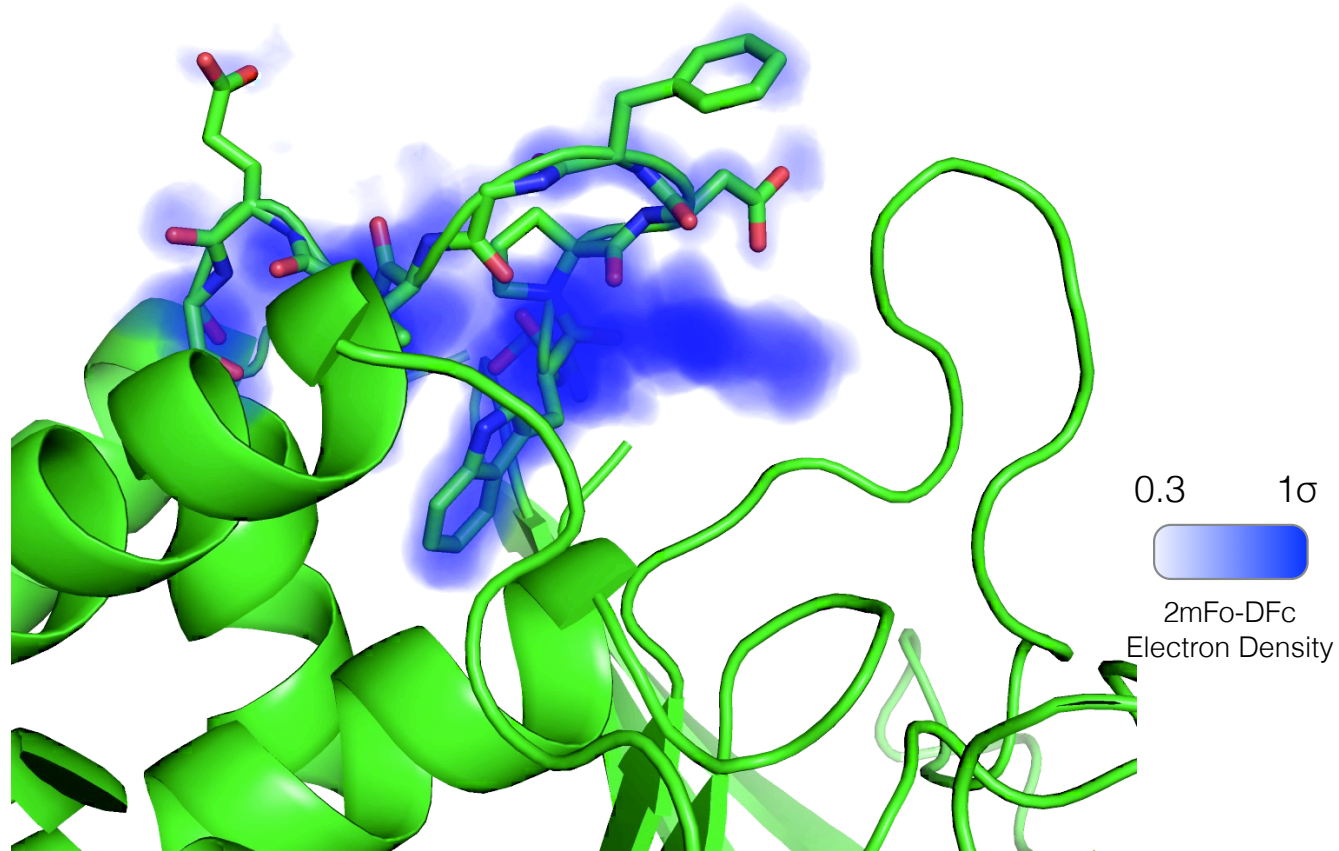
0.3 and  $1\sigma$

Fraser...Alber, *Nature*, 2009

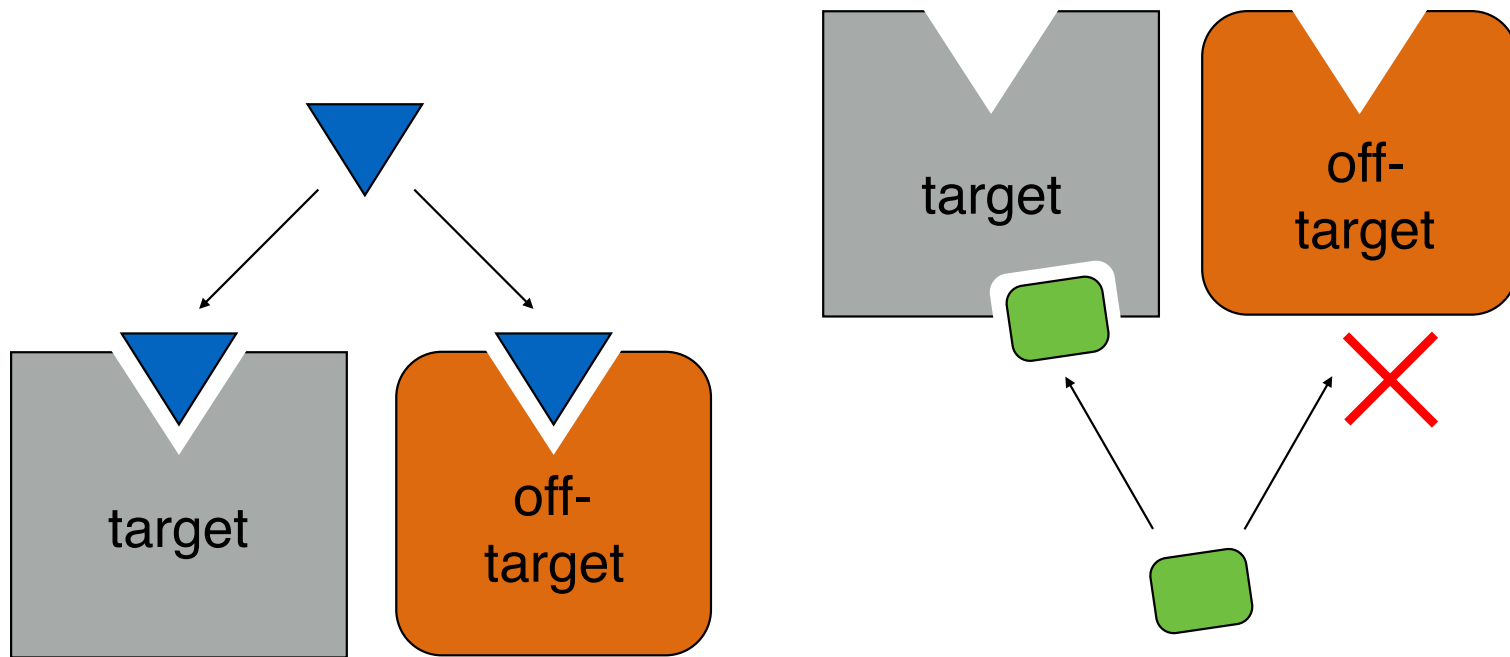
Fraser...Holton...Alber, *PNAS*, 2011



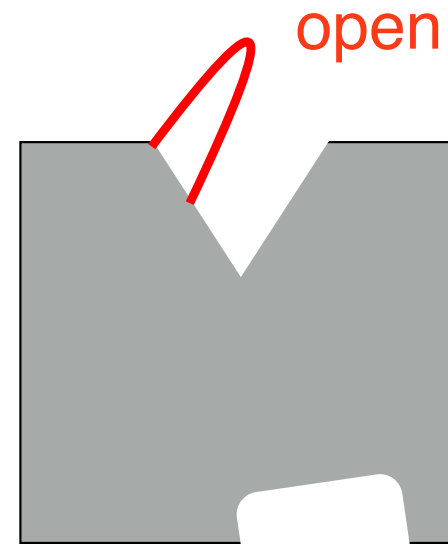
**Low-occupancy** features present at **room temperature** are dynamically accessed conformations and can provide new mechanistic insights



An **allosteric inhibitor** for PTP1B can be more **specific** and **bioavailable**



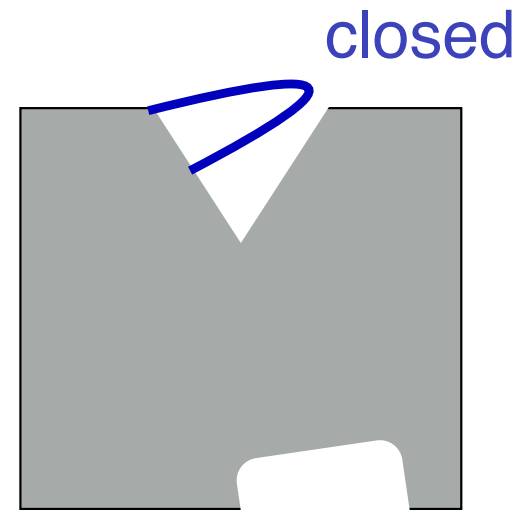
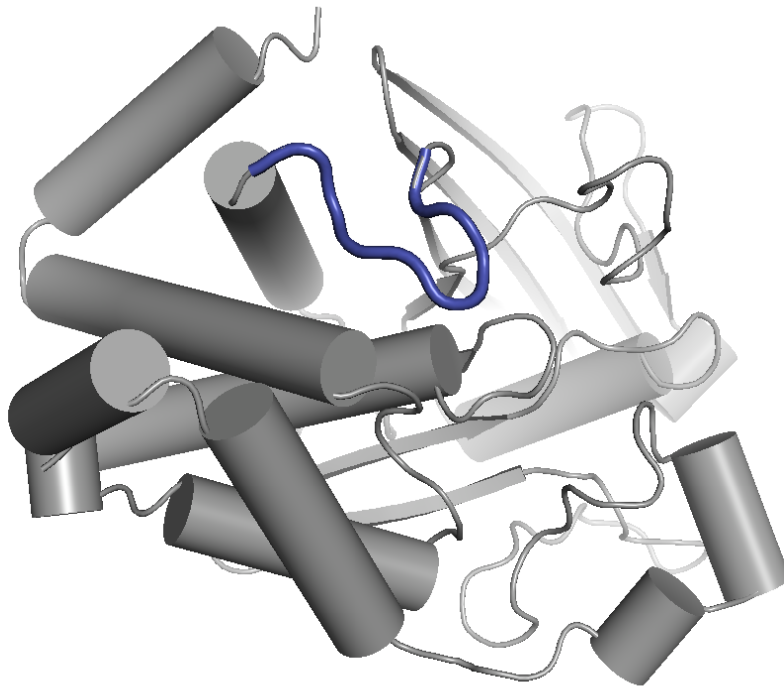
The active-site **WPD loop** in PTP1B  
opens & closes during catalysis



apo  $k_{\text{close}} \sim 20 \text{ s}^{-1}$   
apo  $k_{\text{open}} \sim 900 \text{ s}^{-1}$

Whittier et al.  
*Science* (2013)

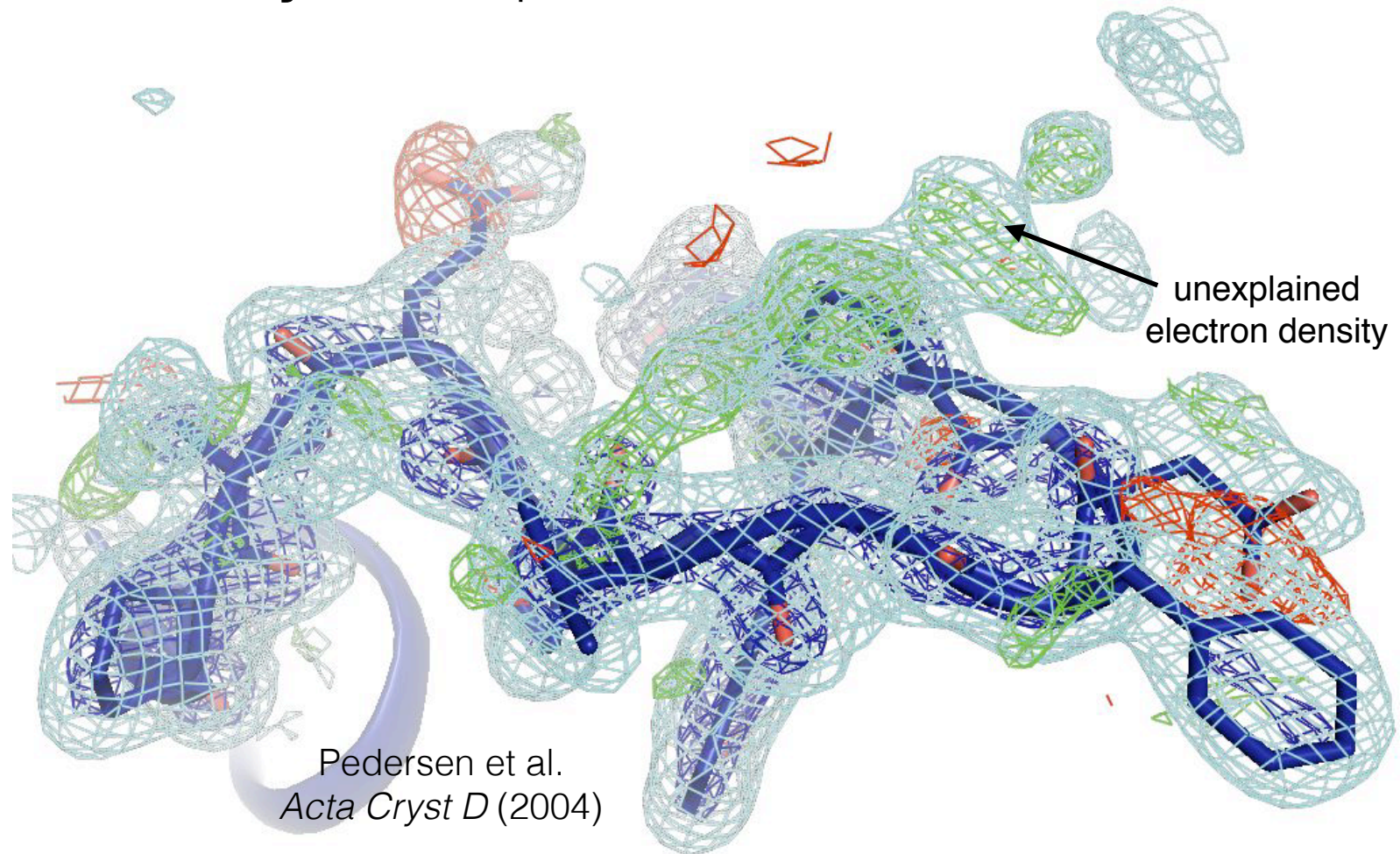
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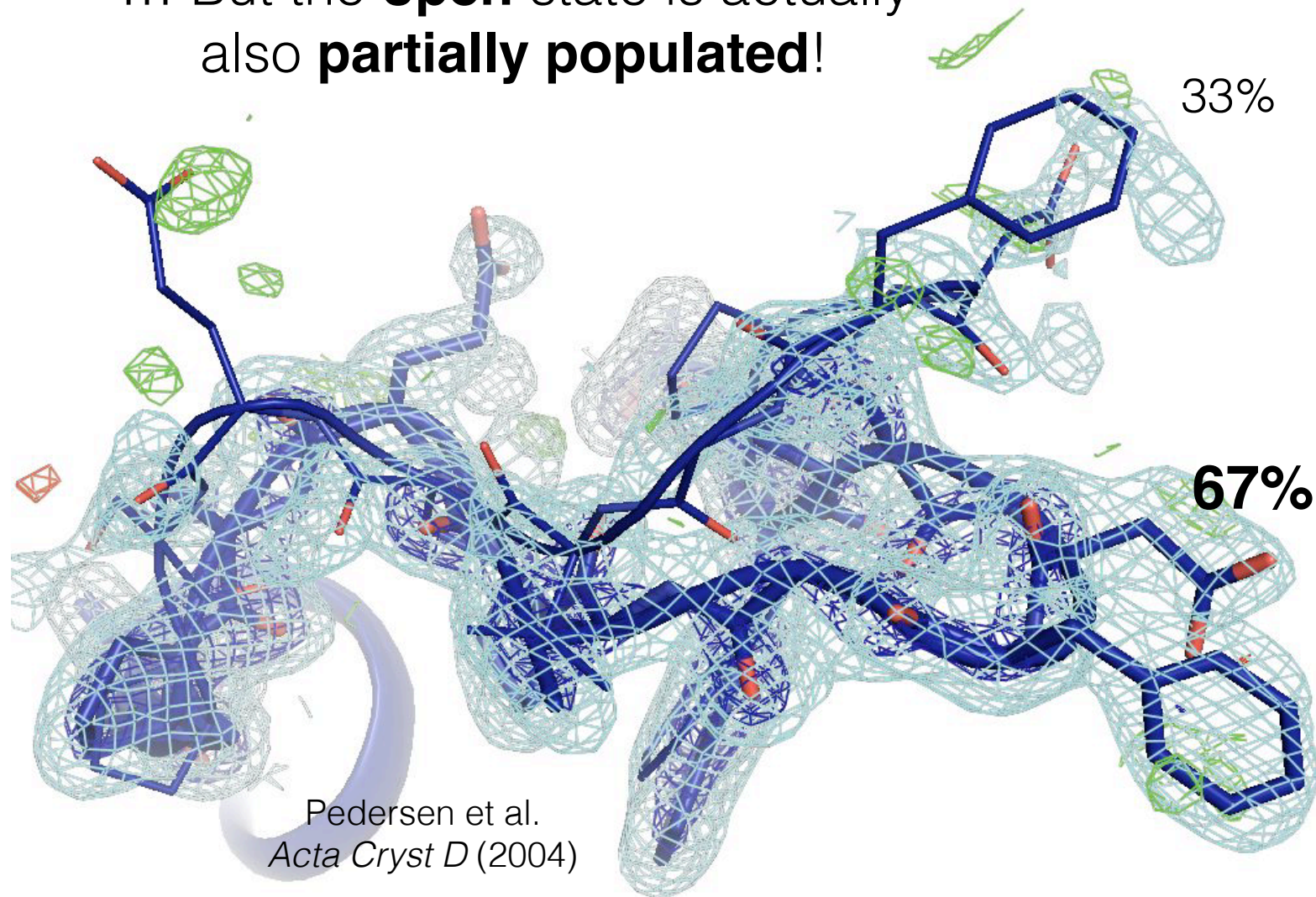
Whittier et al.  
*Science* (2013)

The WPD loop was **modeled** as **closed**  
**only** in the apo structure...



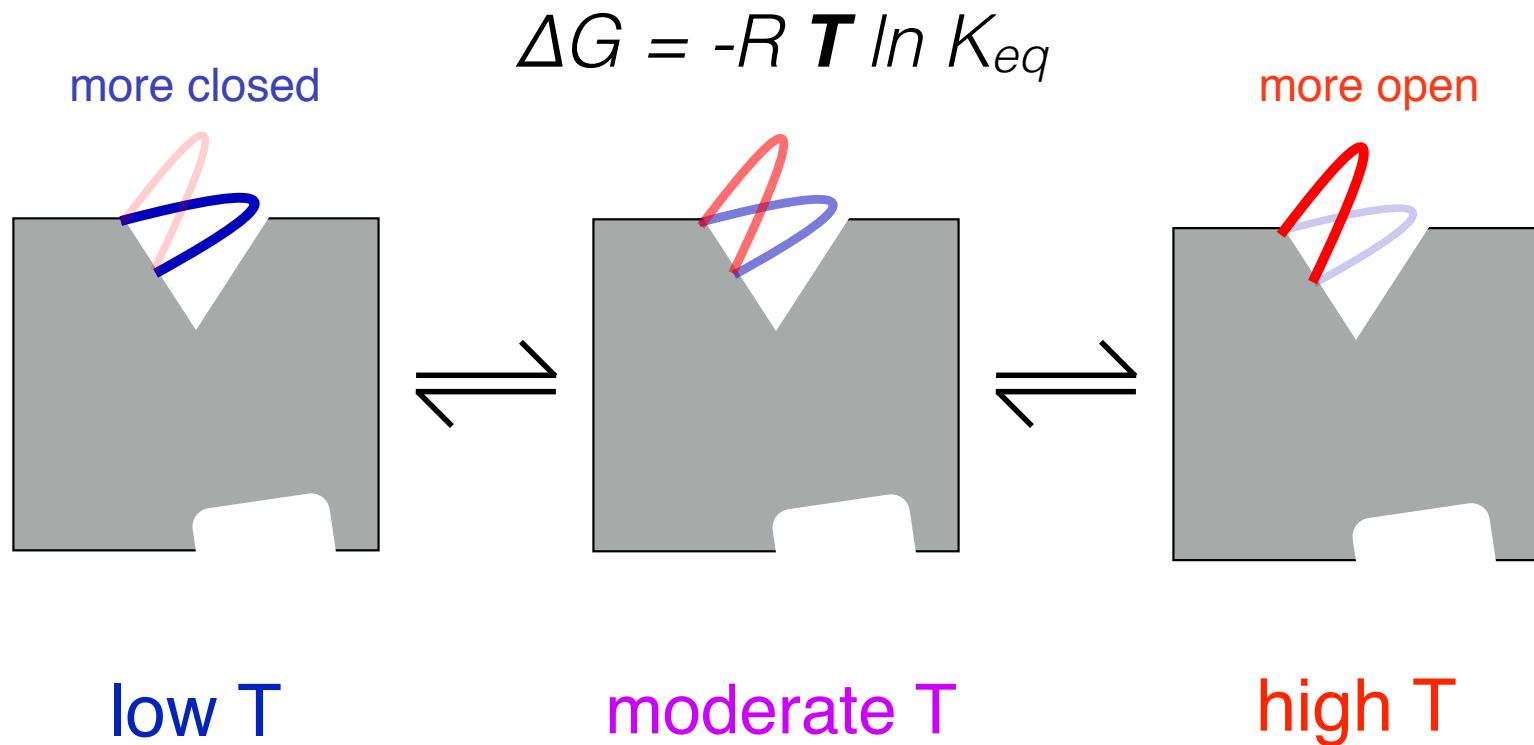


... But the **open** state is actually also **partially populated!**



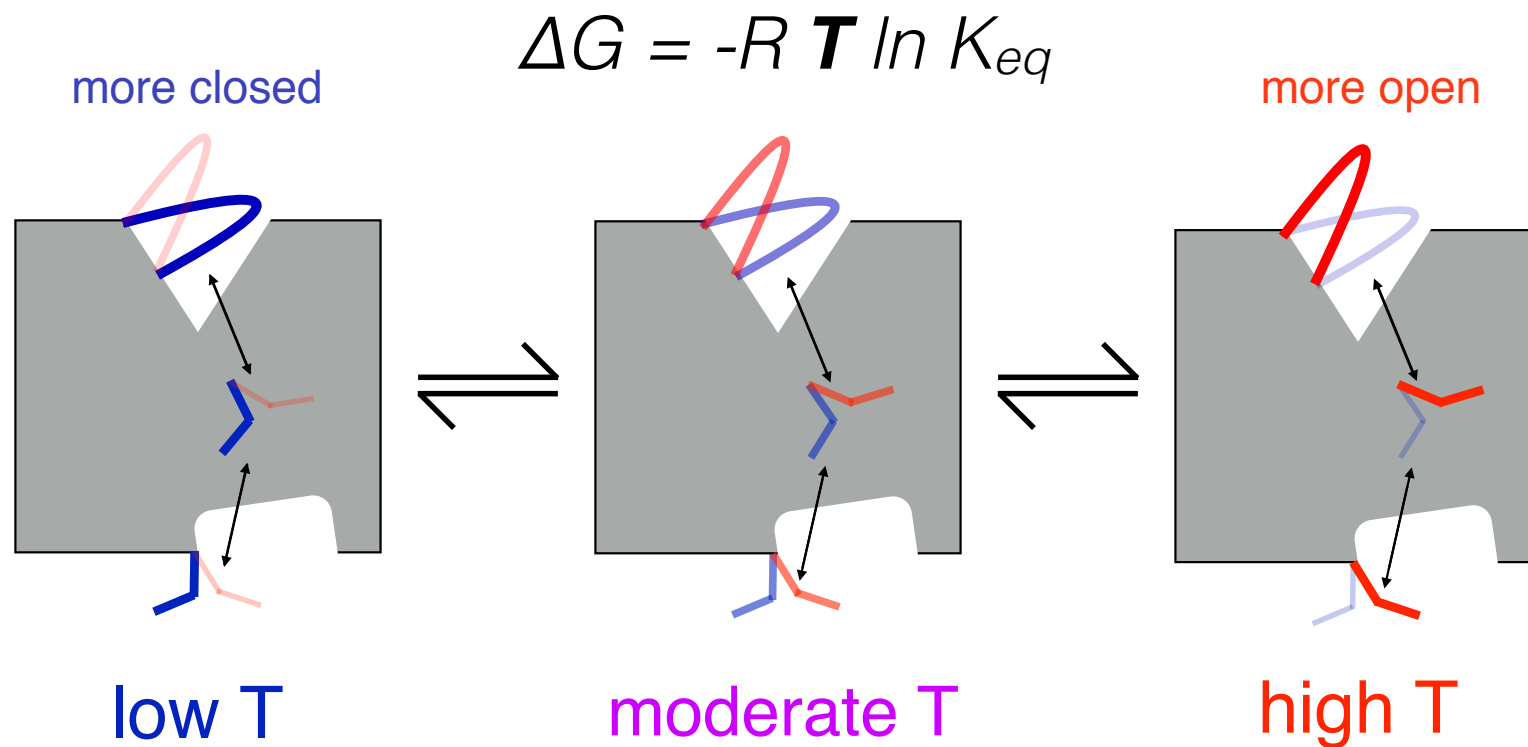
Pedersen et al.  
*Acta Cryst D* (2004)

**Temperature** should modulate the WPD loop's **open-closed** equilibrium

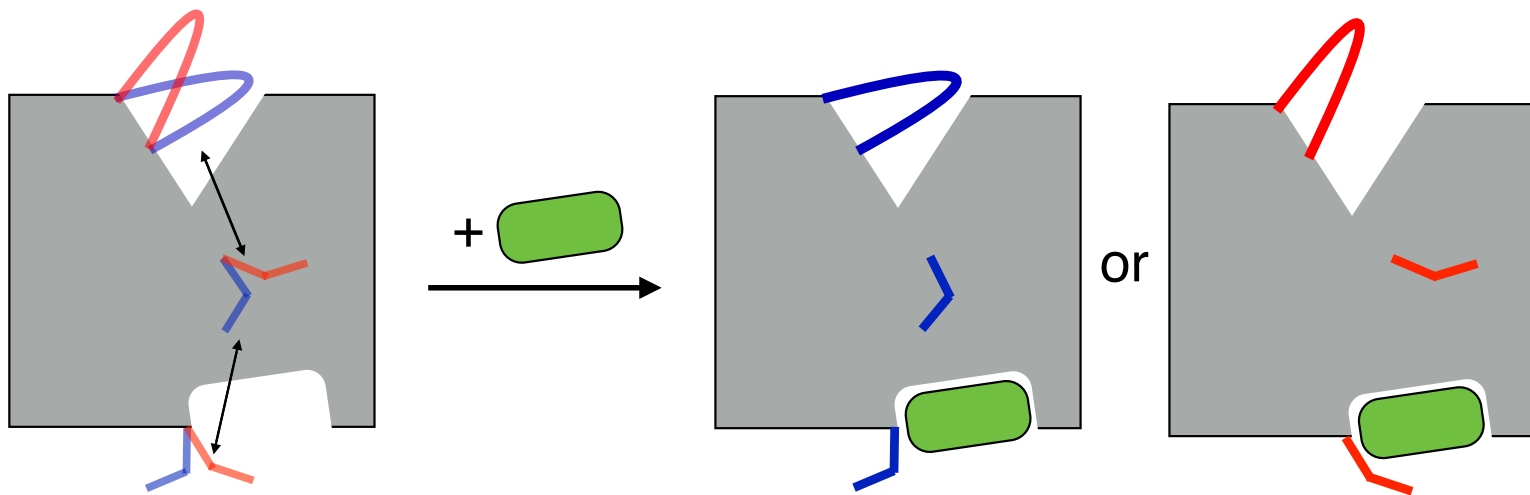




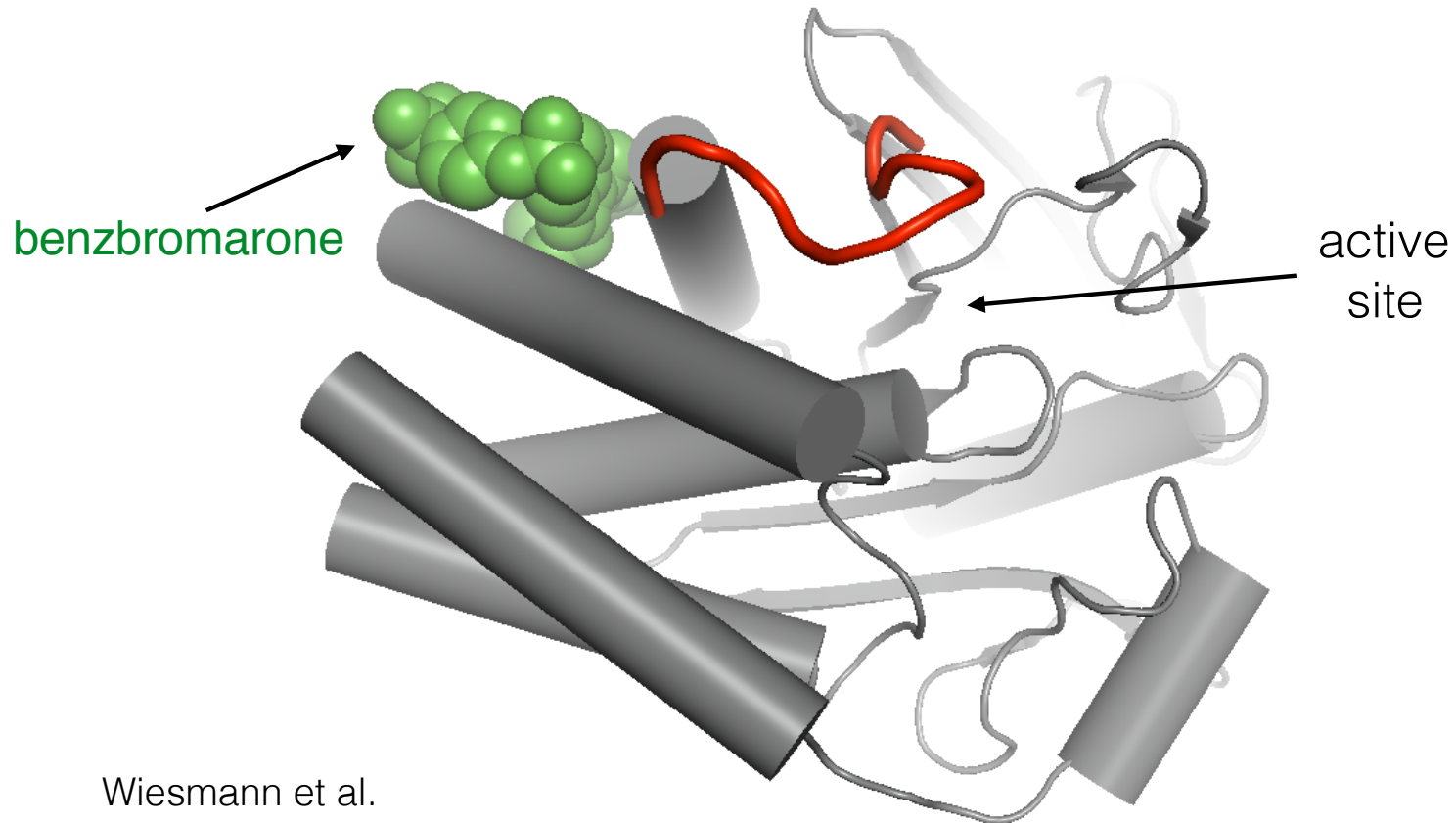
Residues that warm up **in sync** with the WPD loop may be **energetically coupled**



An **allosteric drug** could **topple the dominos**  
to remotely lock the WPD loop



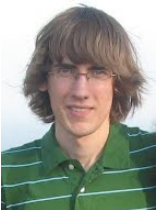
A known allosteric small-molecule inhibitor is good **proof of principle** (but inhibits weakly)



Wiesmann et al.  
*Nat Struct Mol Biol* (2004)

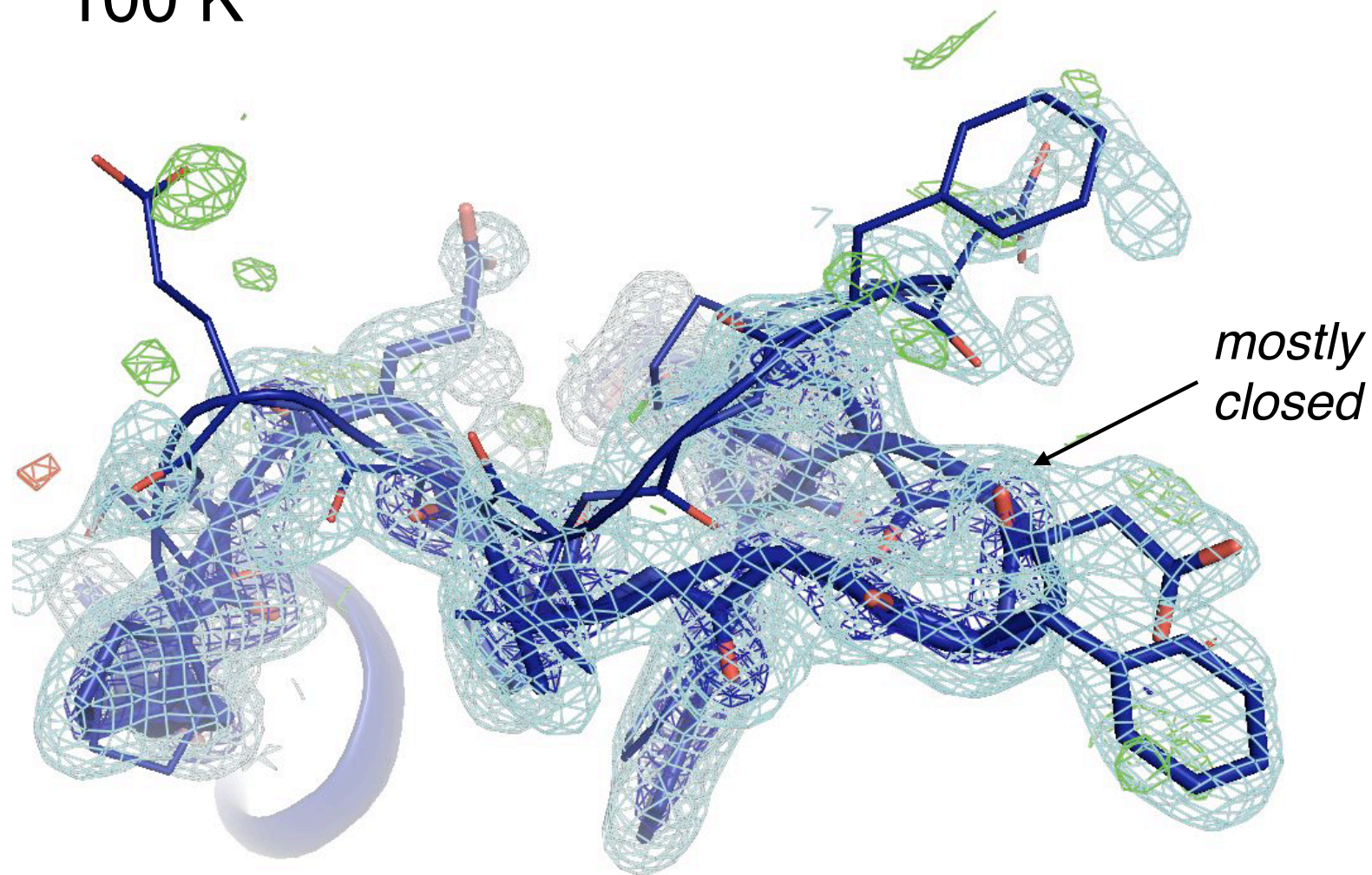
Apo X-ray datasets  
across a wide **temperature range**

Temperature	Source	Resolution
100 K	PDB: 1sug	1.95 Å
180 K	<i>new data</i>	1.84 Å
240 K	<i>new data</i>	1.87 Å
278 K	<i>new data</i>	1.78 Å



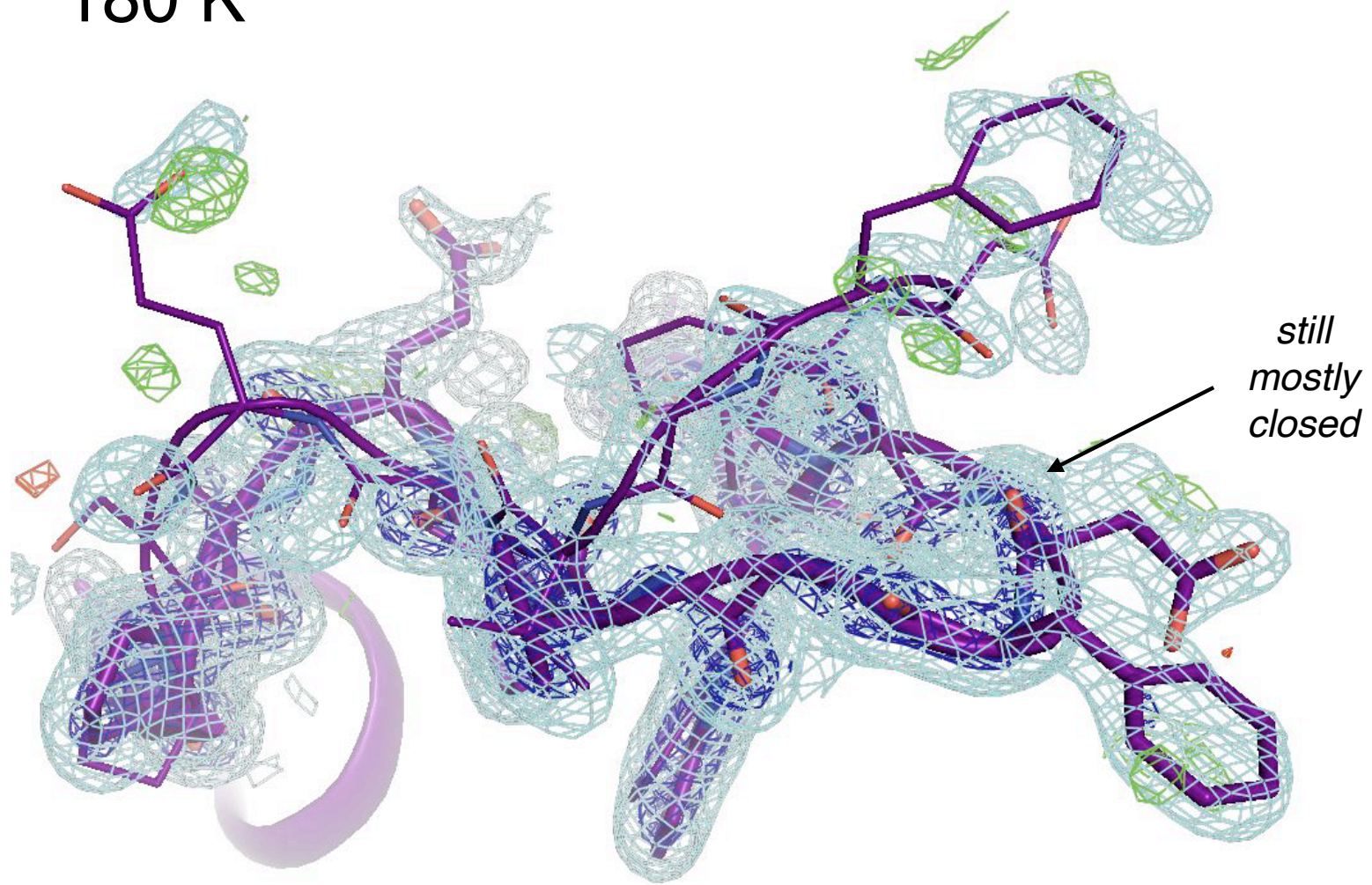
Daniel Keedy

100 K

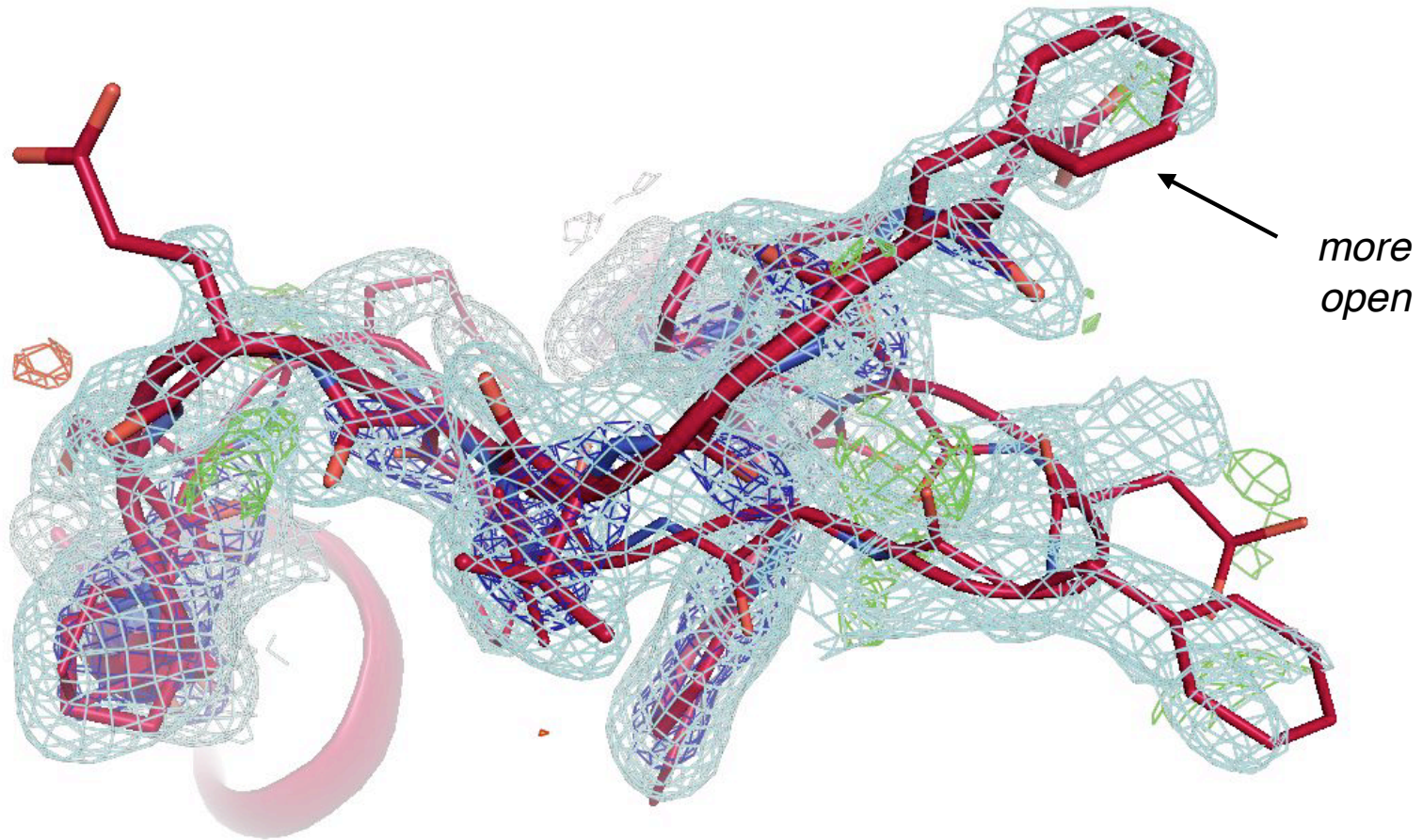




180 K

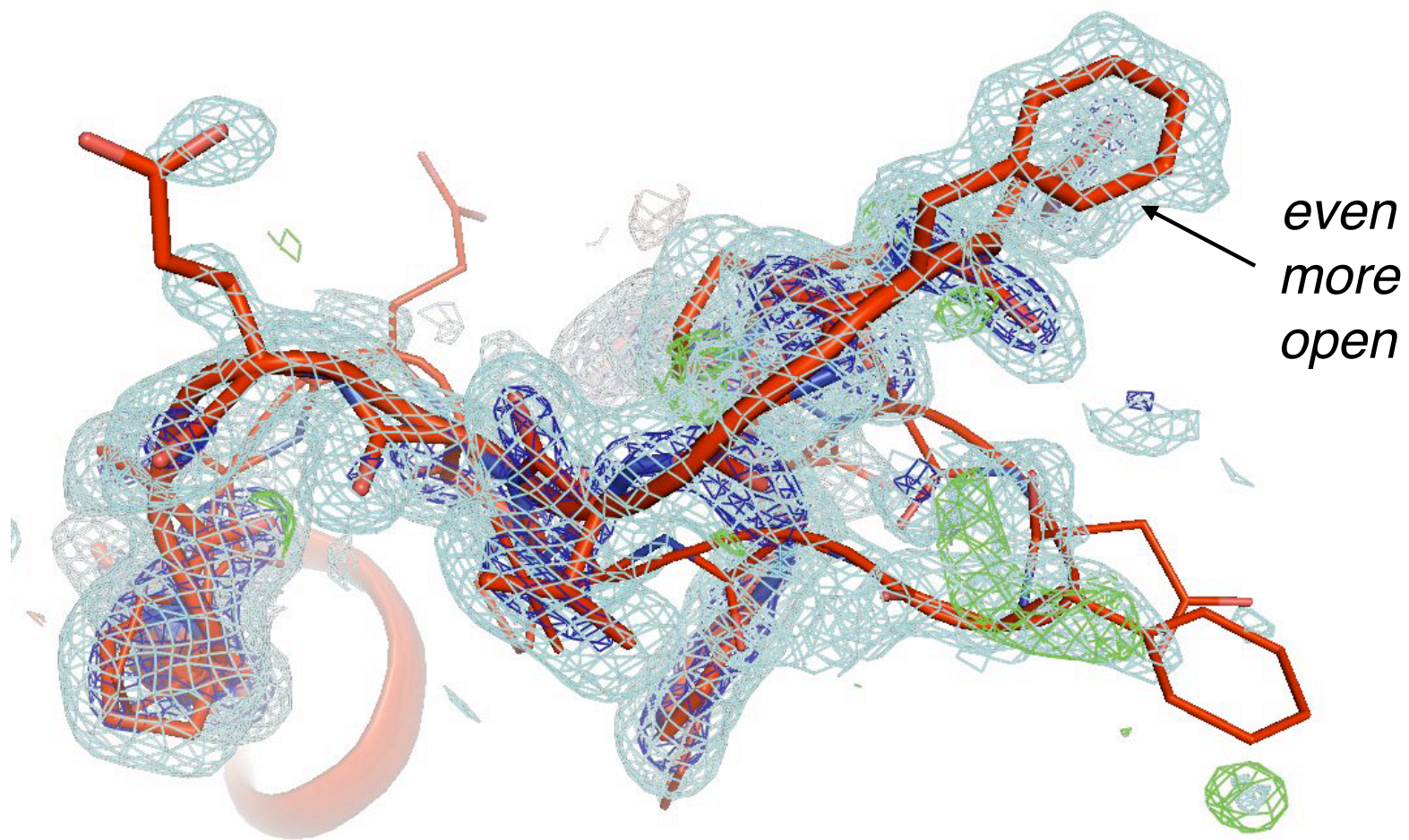


240 K

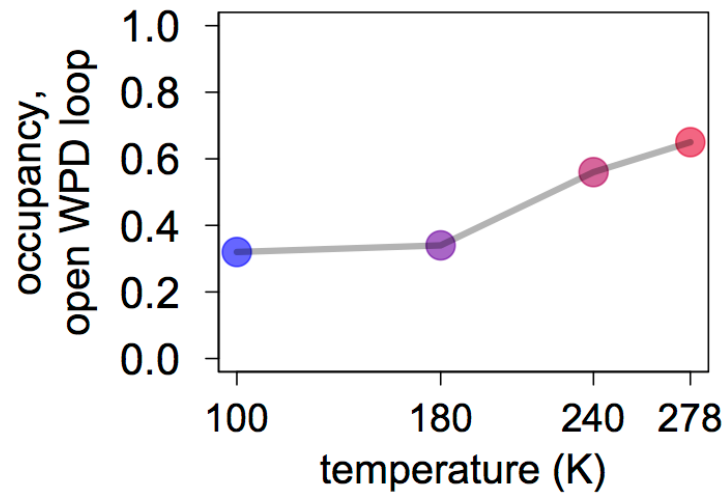




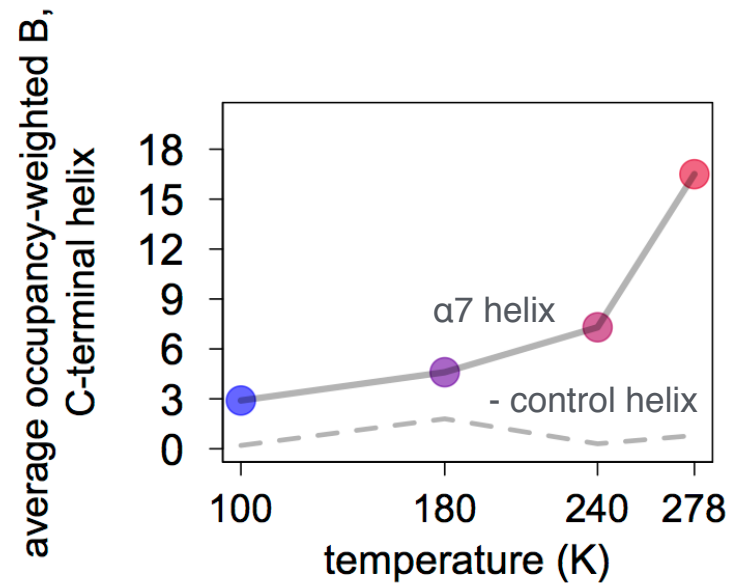
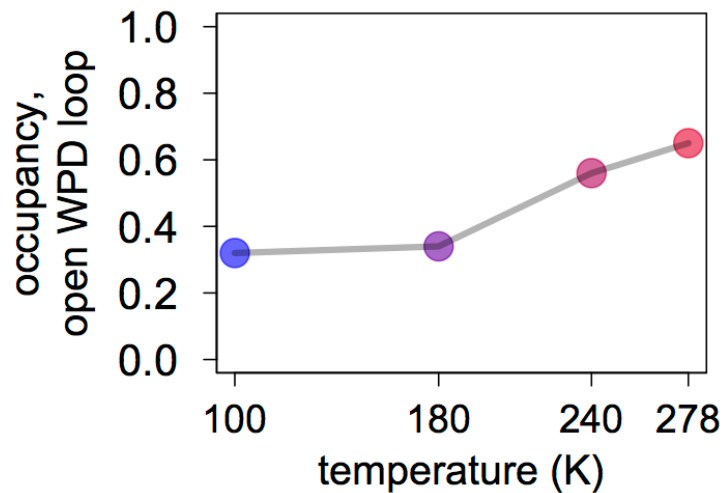
278 K



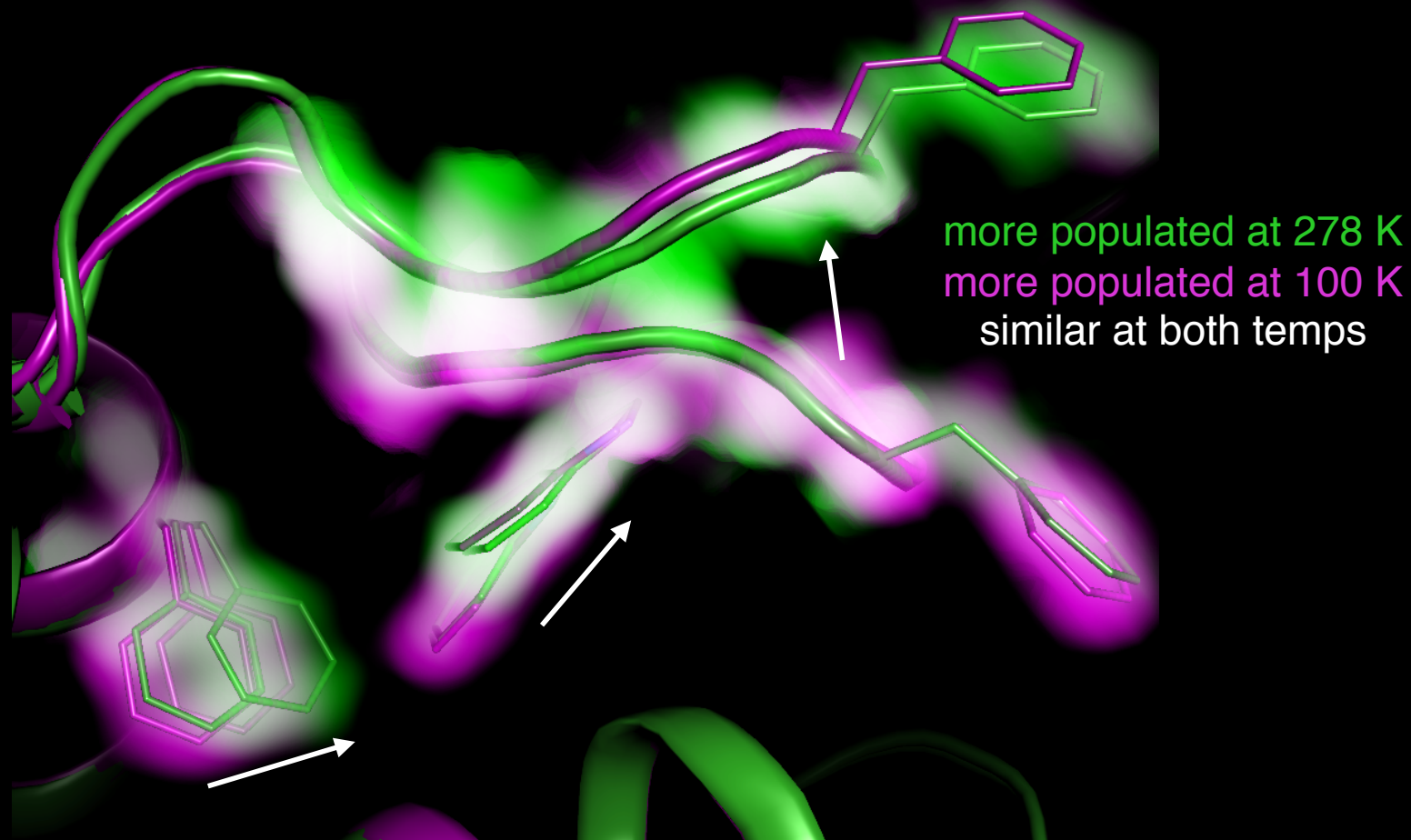
The WPD loop **opens** as temperature increases



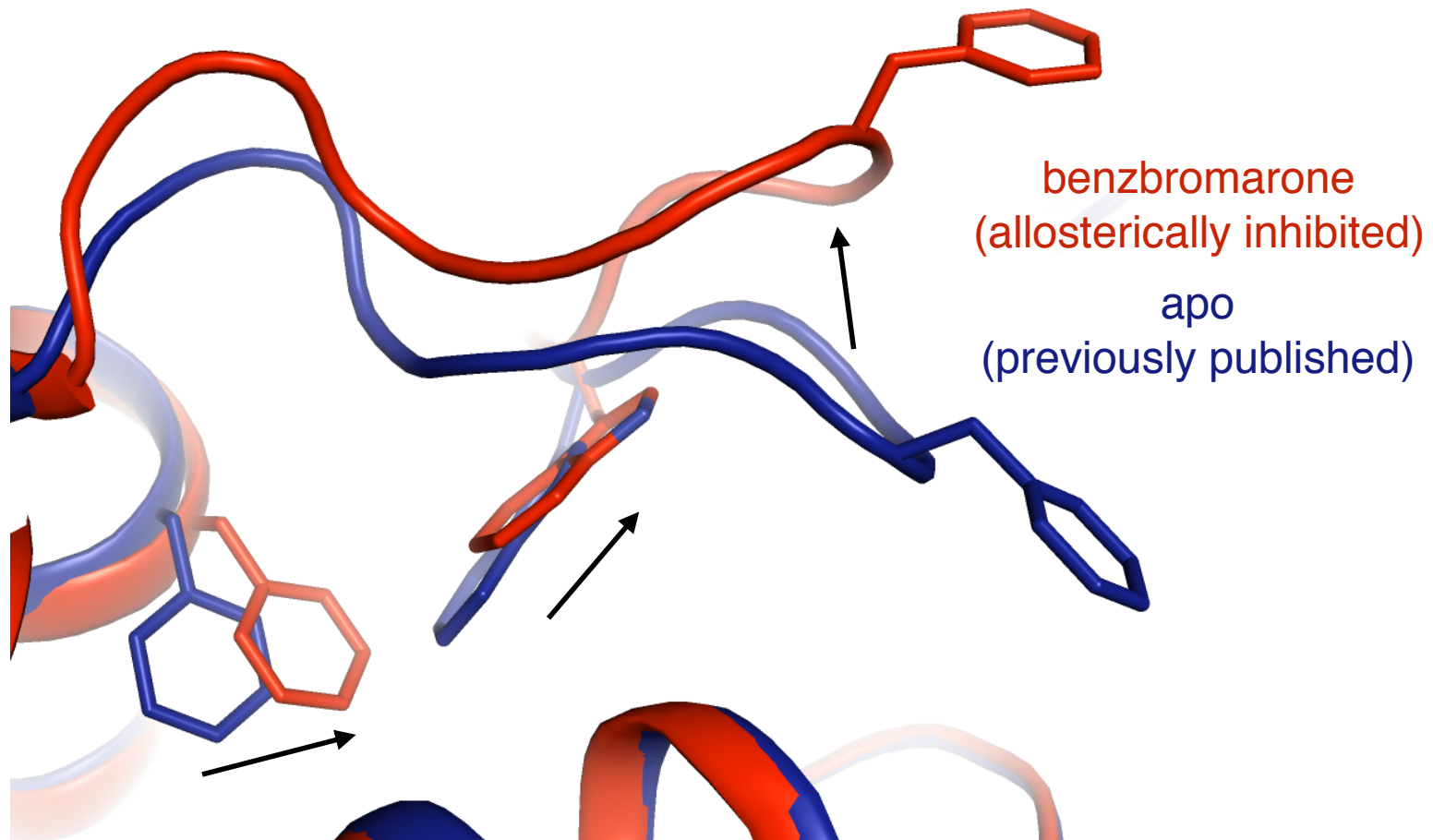
As the WPD loop **opens**,  
the C-terminal  $\alpha 7$  helix **undocks**



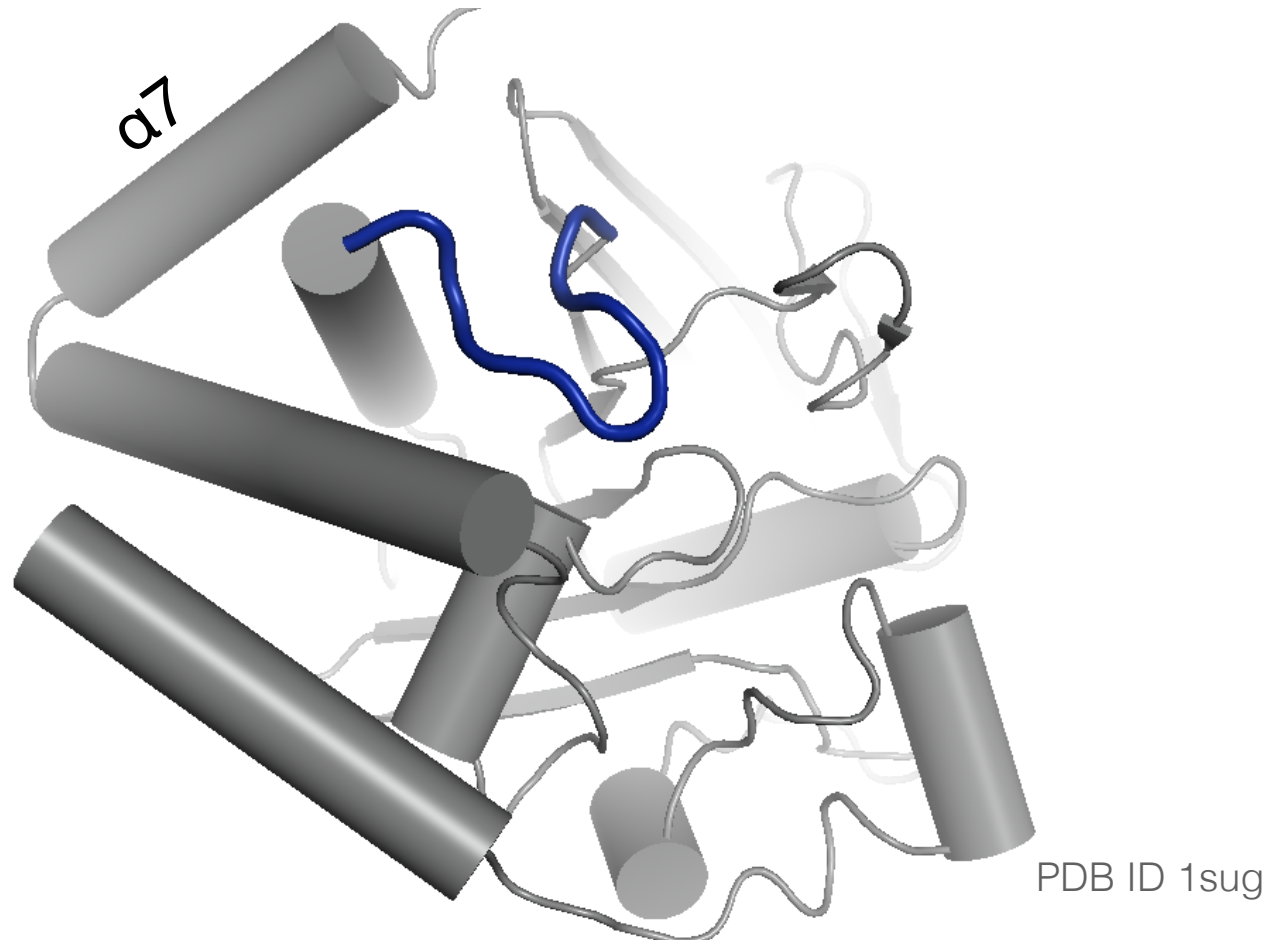
It stabilizes **pre-sampled**  
minor conformations!



Benzbromarone does not simply **induce** conformational change...



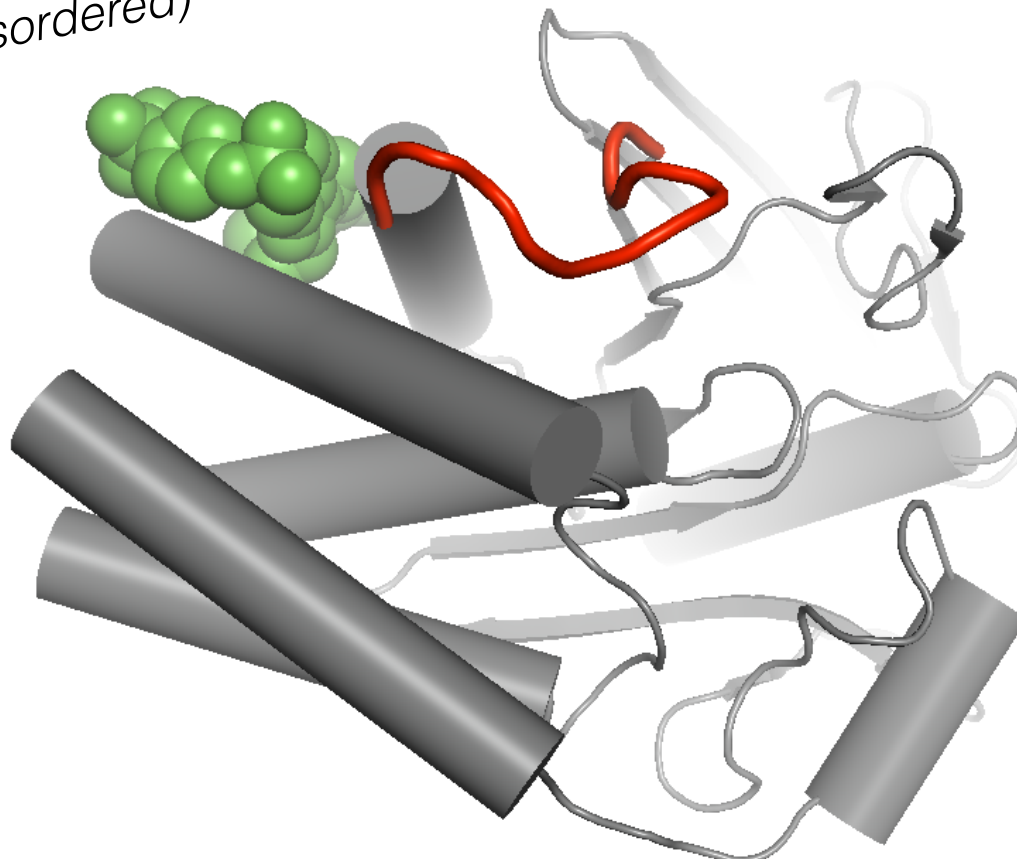
The  $\alpha 7$  helix also undocks when **benzbromarone** binds





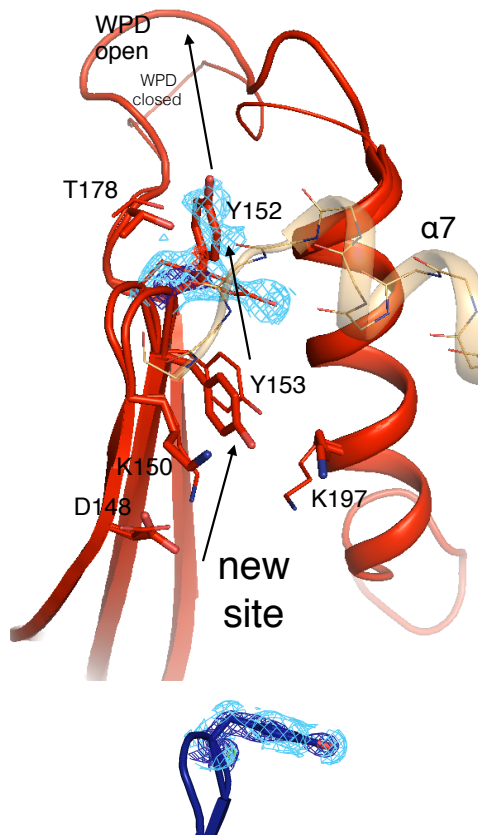
The  $\alpha 7$  helix also undocks  
when **benzbromarone** binds

( $\alpha 7$  disordered)

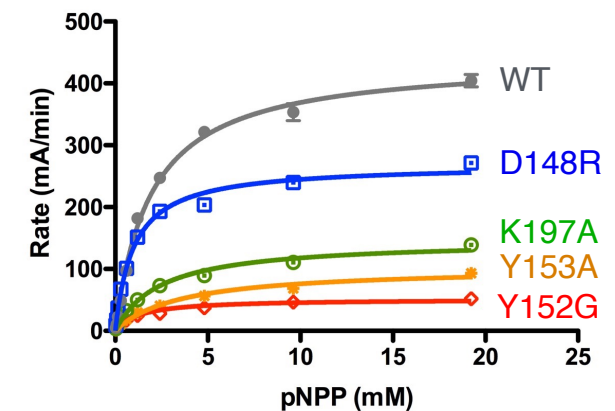
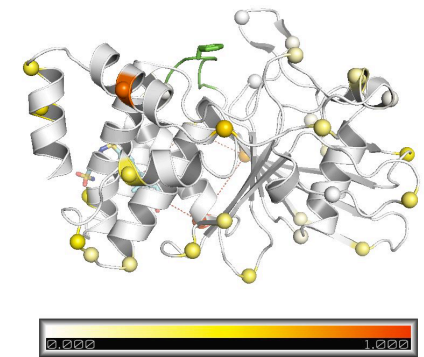


PDB ID 1t49

We have also discovered a new allosteric site by **multitemperature** mapping



- **specific**: residues not conserved in homologs
- **“bindable”**: binds cryoprotectants in existing structures AND is identified as a hotspot in tethering screen
- **functional**: mutations along the path to the new site impair catalysis



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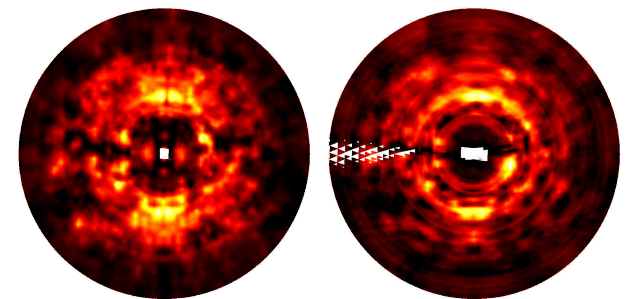
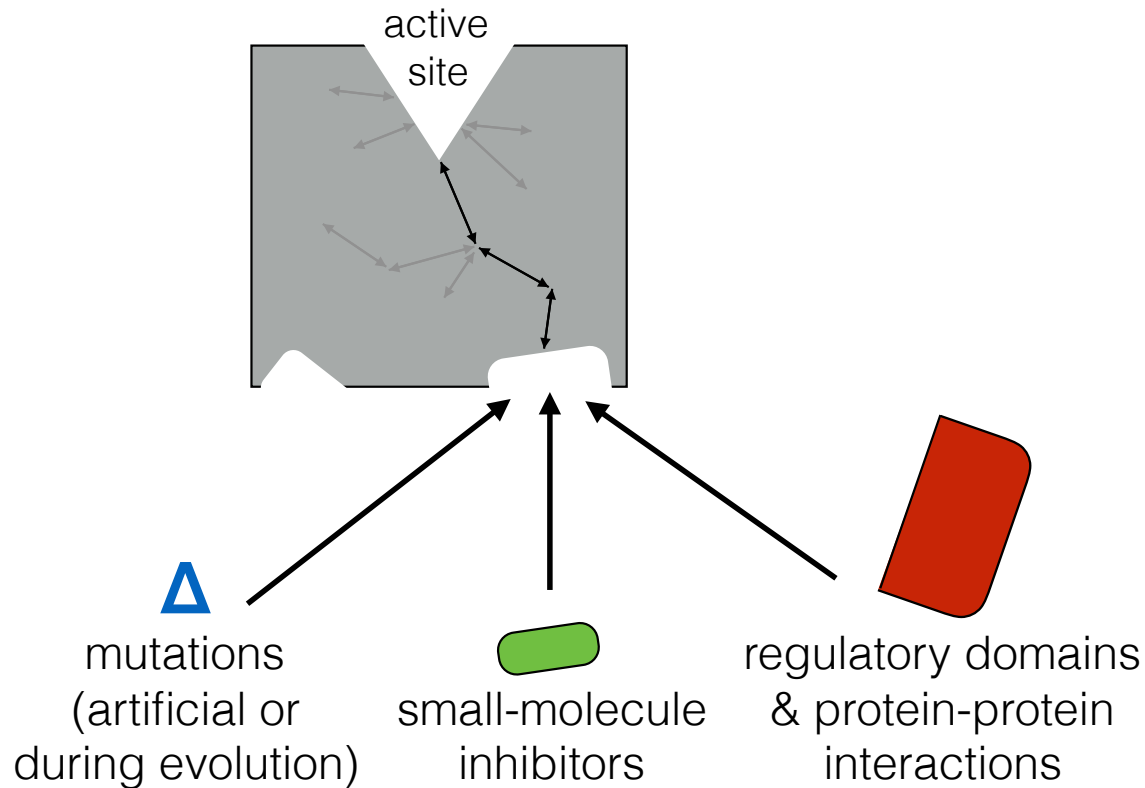
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**Different perturbations** can tap into the intramolecular nervous system of proteins... including global, physical perturbations, like **temperature**



... and diffuse scattering will extend our ability to model the response to perturbations

# FRASER LAB



WE ARE RESEARCH

BEER OR SOCCER?

RIDES THE LIN-MOBILE IN STYLE

HAS A KILLER NARWHAL FIGURINE

TOO MANY COMPUTERS? NEVER.

VEGETARIAN.

HOGS THE LABEL MAKER

ANSWERS THE MYSTERIOUS LAB PHONE

OBSESSED WITH RUNNING

ARRIVES EARLY TO PUMP IRON

HONESTLY "THE GREATEST EVER"



David Mavor

Dr. Lin Liu

Dr. Daniel Keedy

Ben Barad

Justin Biel

Rahel Woldeyes

Dr. Mike Thompson

Erin Poss

Alex Wolff

Pooja Suresh

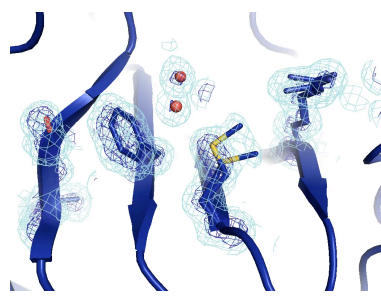


## Collaborators:

- Henry van den Bedem (SLAC)
- Jim Wells (UCSF)
- James Holton (LBL/UCSF)



- Michael Wall, Tom Terwilliger (LANL)
- Paul Adams, Nick Sauter, Aaron Brewster (LBL)



@fraser\_lab





# Why my lab really loves 831

