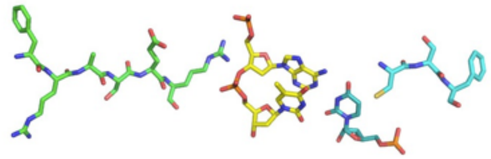
multitemperature data and diffuse scattering in X-ray crystallography



James S Fraser
UCSF
@fraser_lab

BioXFEL


Daniel Keedy


Robert Thorne (Cornell)


Henry van den Bedem (SLAC)

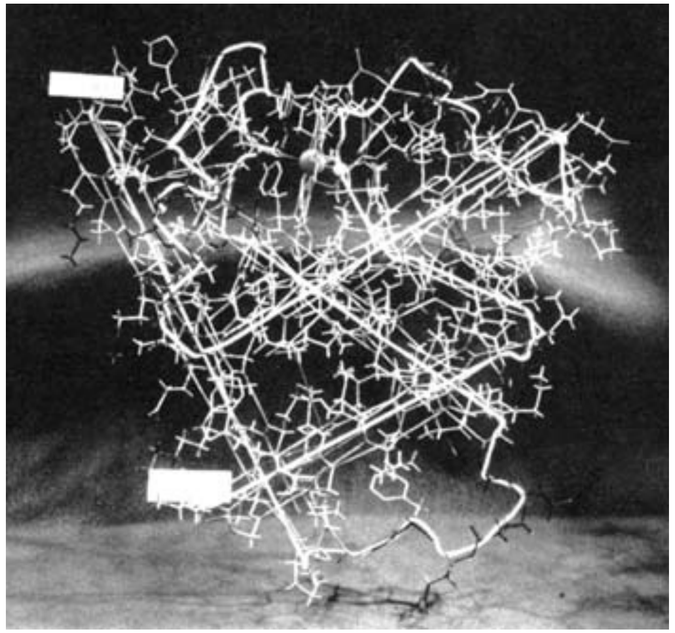

Andrew VanBenschoten

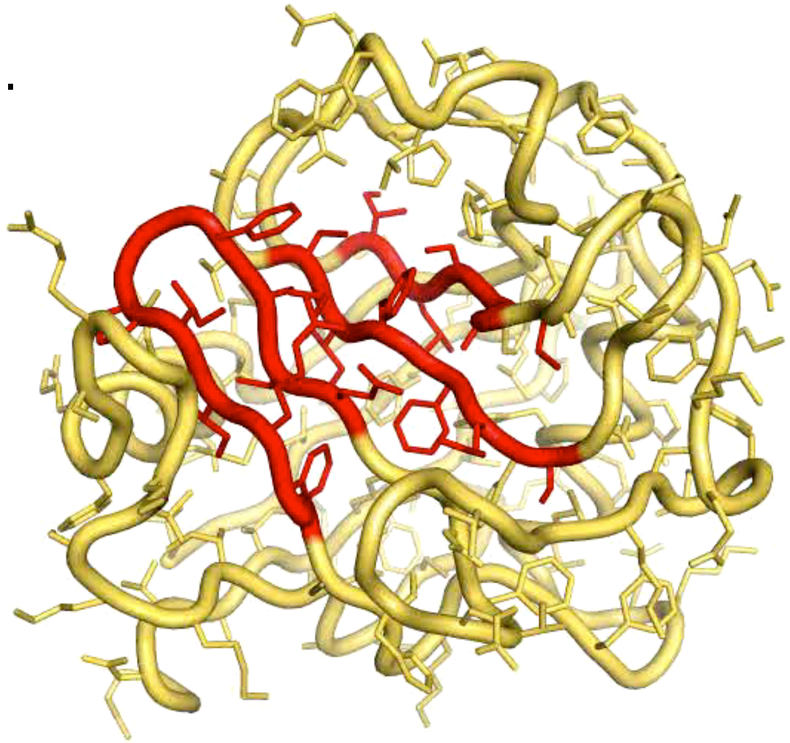

Michael Wall (LANL)

Nick Sauter, Aaron Brewster, Paul Adams, Pavel Afonine (LBNL), Tom Terwilliger (LANL), Sacha Urzhumtsev (CNRS)

XFELs and structural biology:
It's about time!

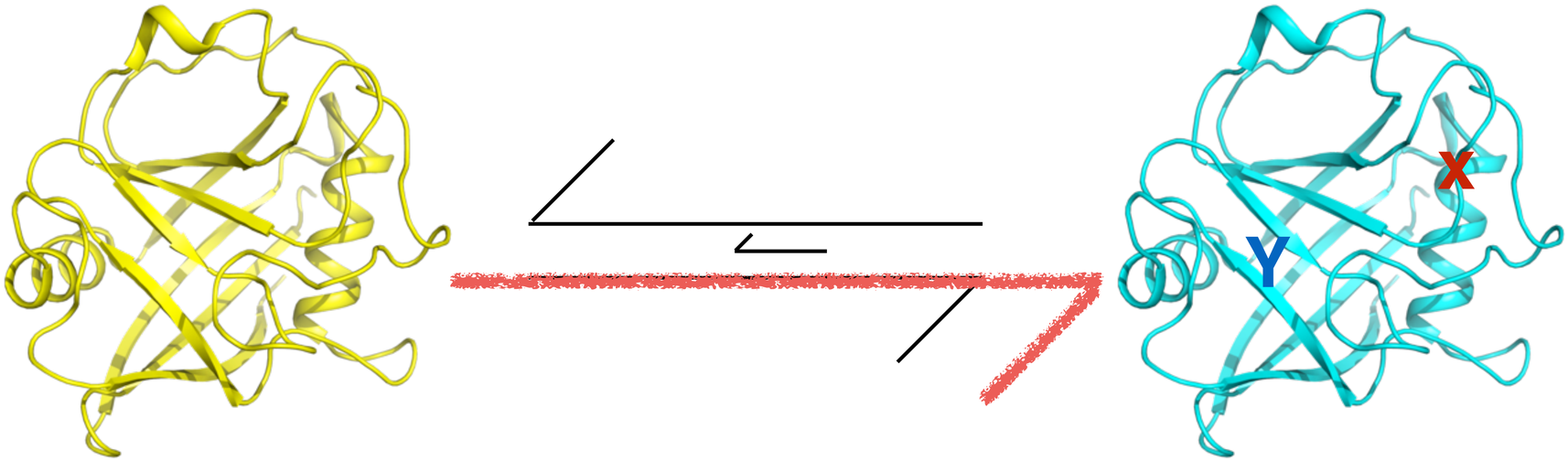
We are transitioning from static structural biology….



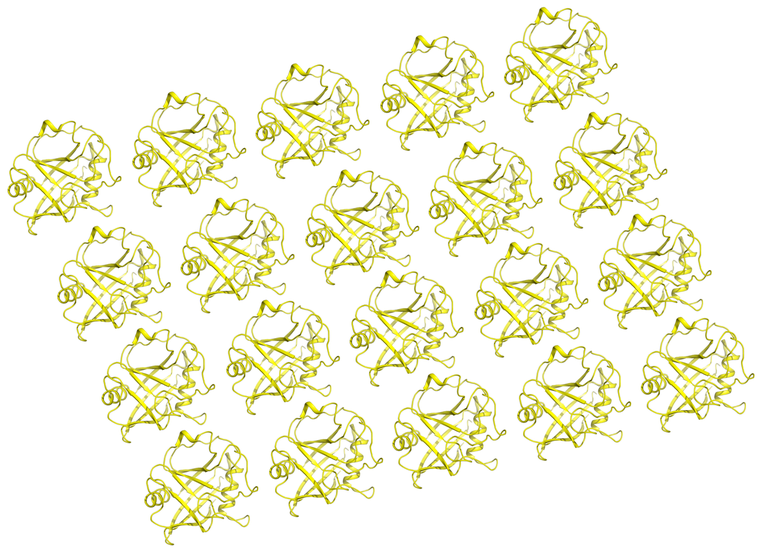


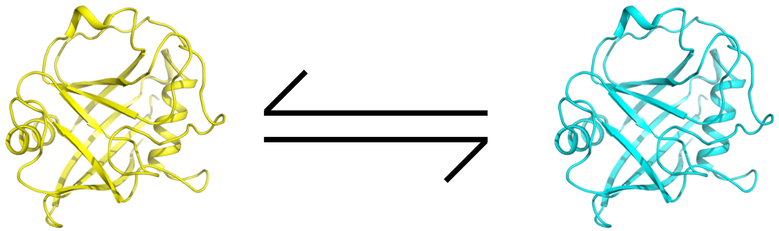
…to dynamic structural biology

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

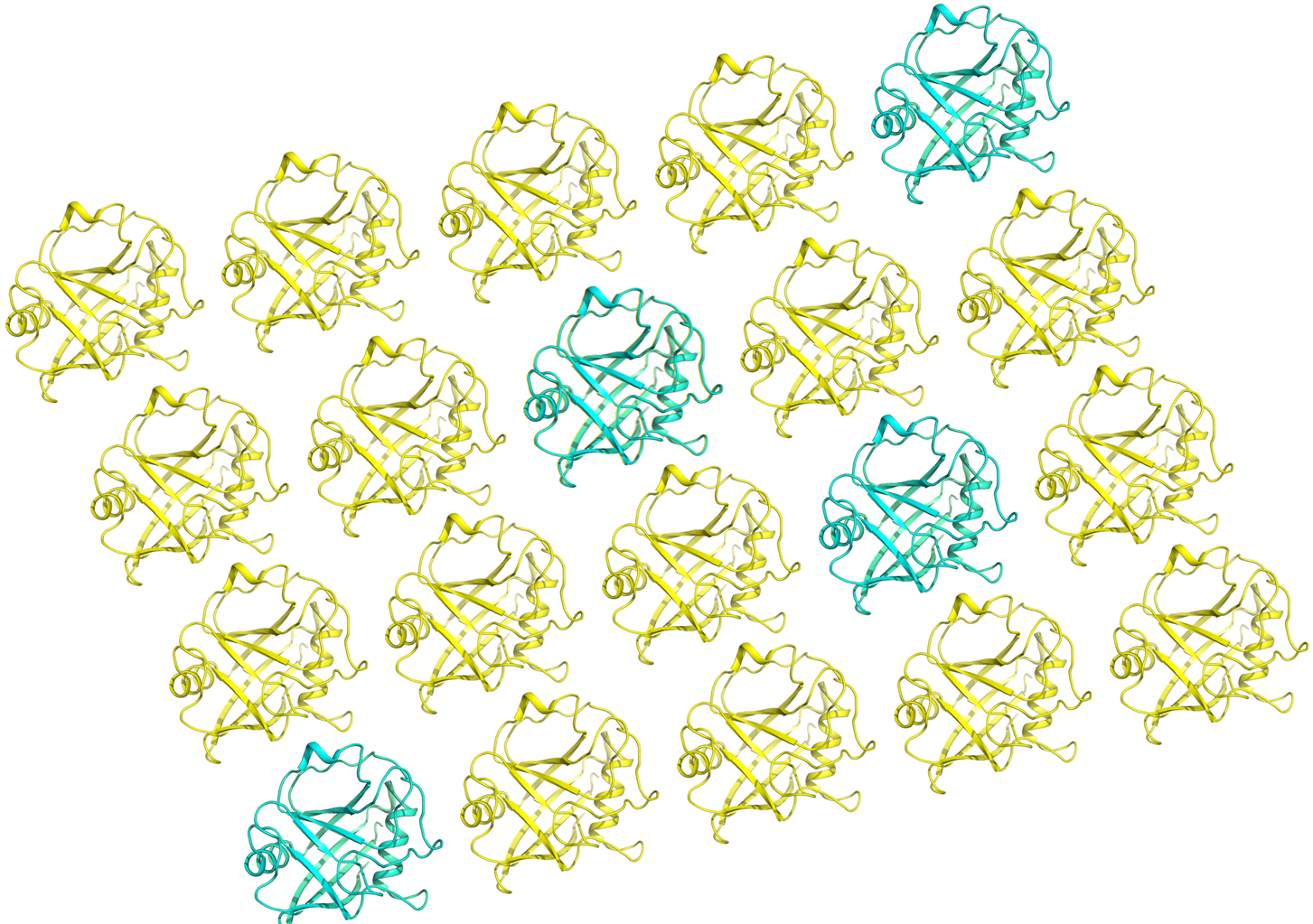


Proteins often populate **multiple conformations** in crystals





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



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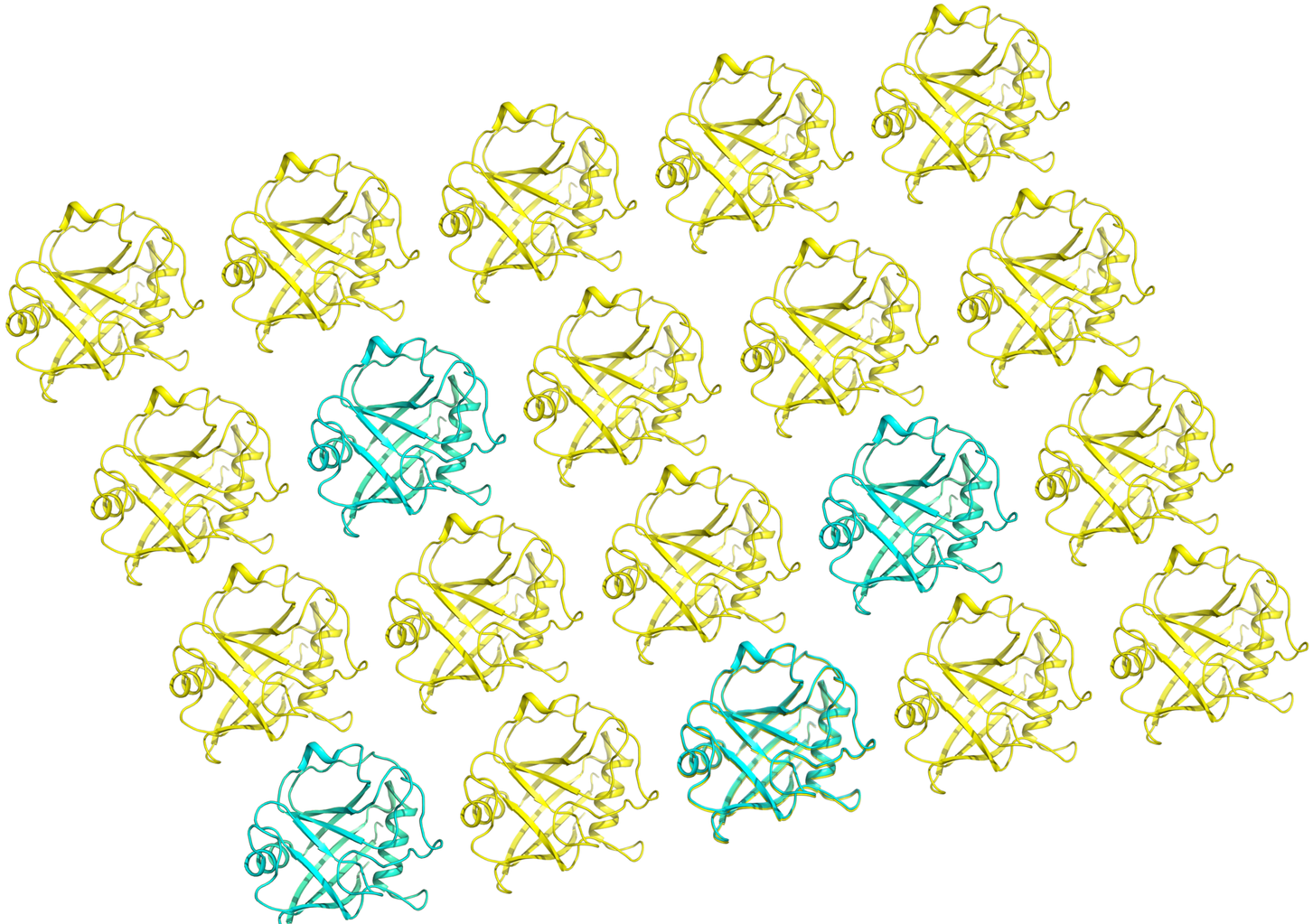
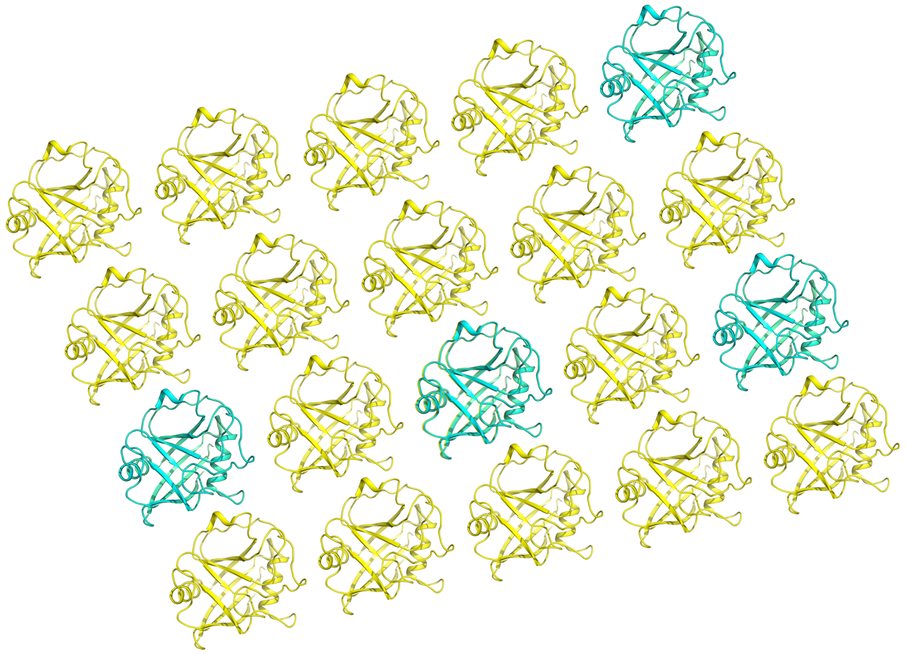
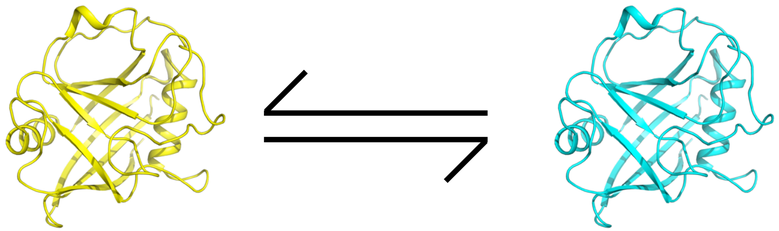


Temperature can **shift** the relative populations of confirmations in the crystal





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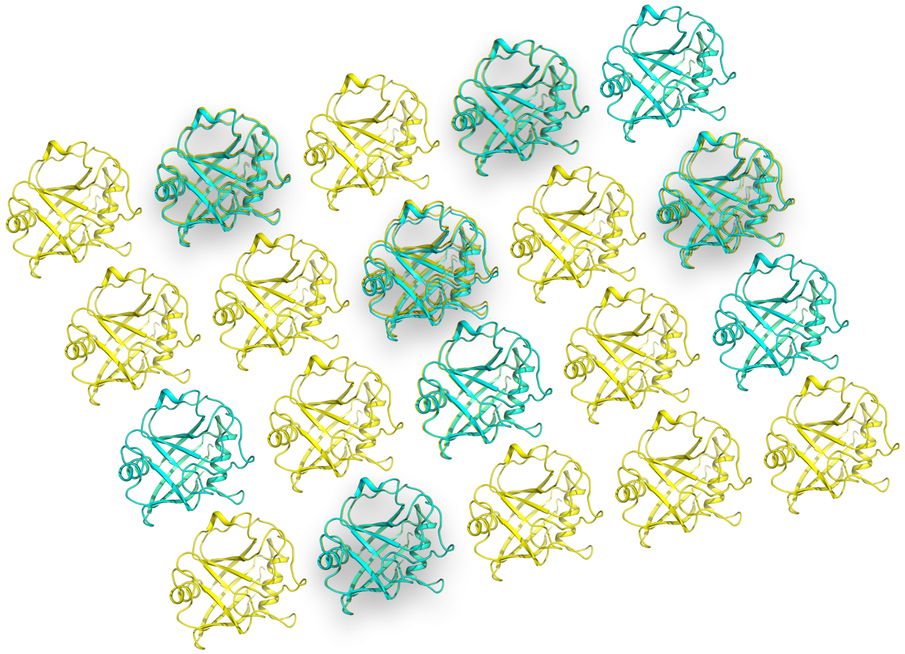

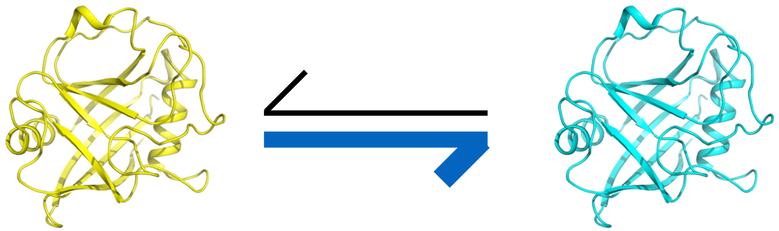

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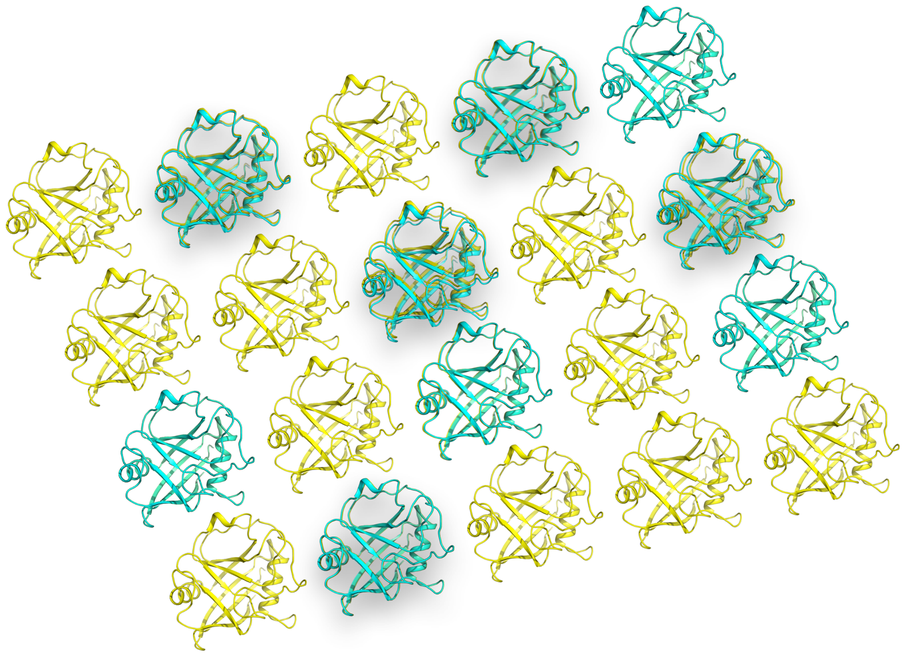

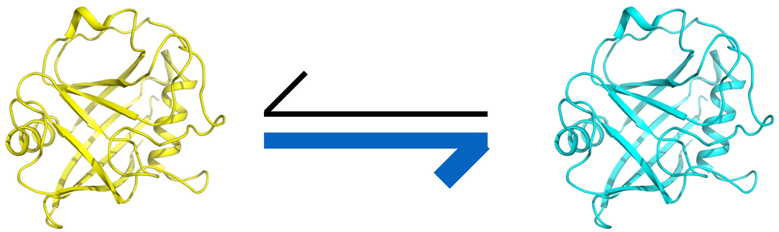

Hypotheses: (1) shifting temperature exposes conformations near the “ground” state; (2) these new conformations are used by the protein in physiological mechanisms

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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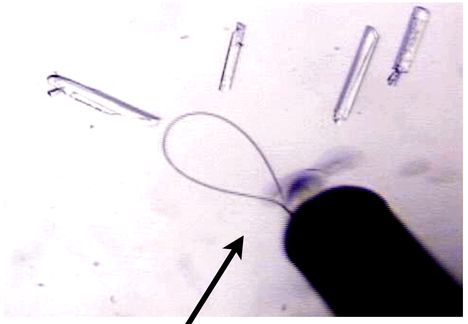
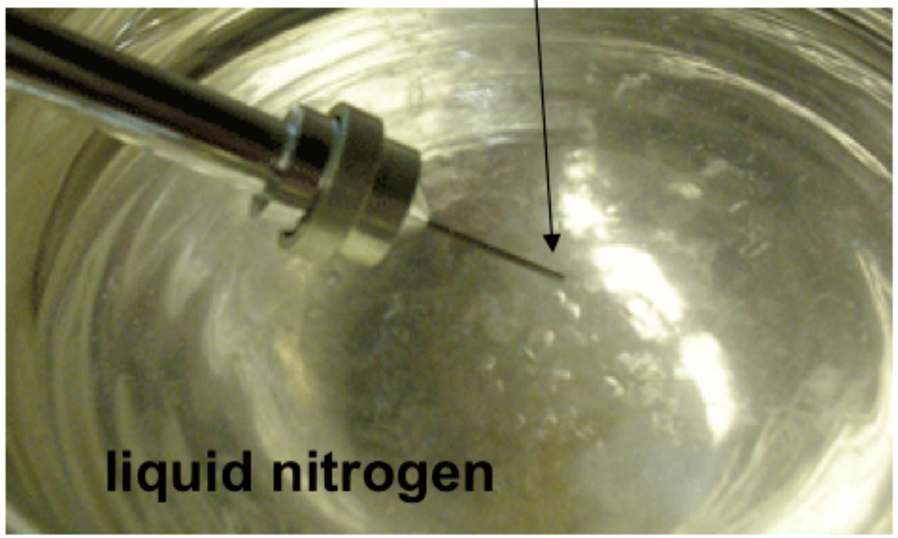
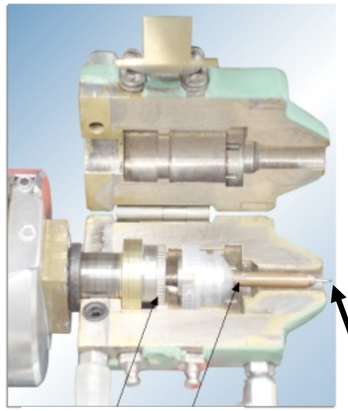
Conventional X-ray experiments are performed at **cryogenic** temperatures



~100K

loop

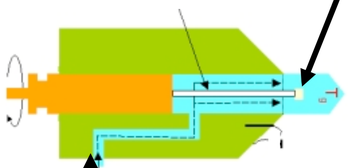




"off label" use of Proteros FMS BL12.3.1@ALS

288K

loop



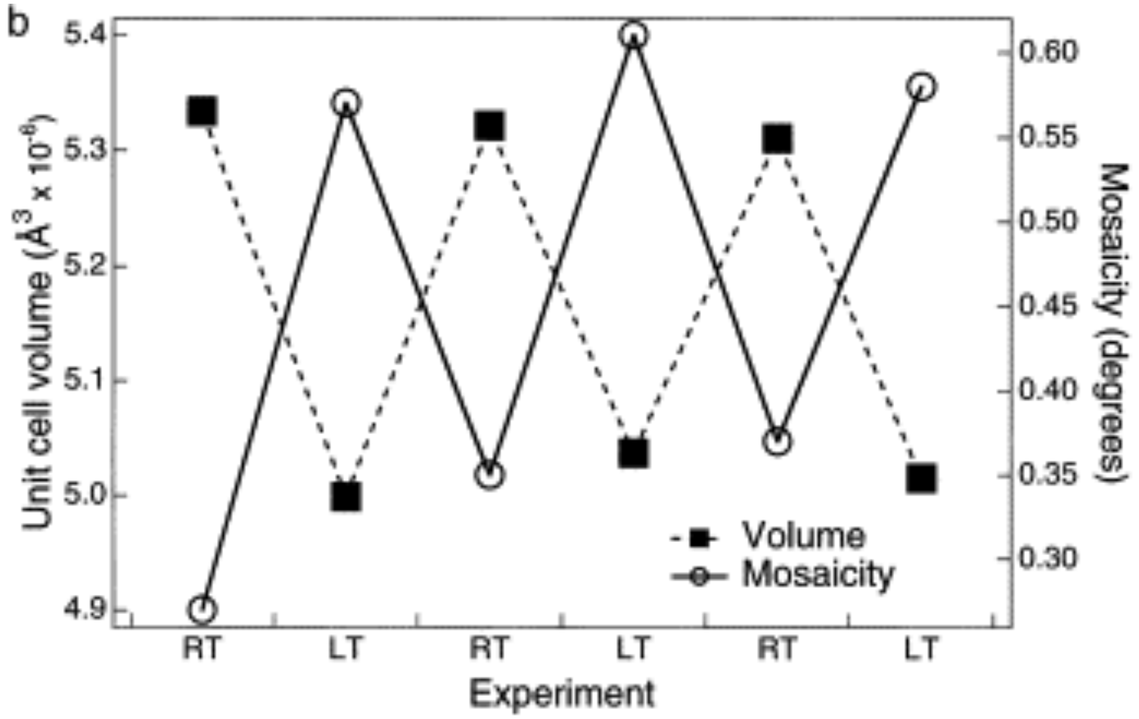
MiTeGeN

...and data collection strategies to minimize **radiation damage**

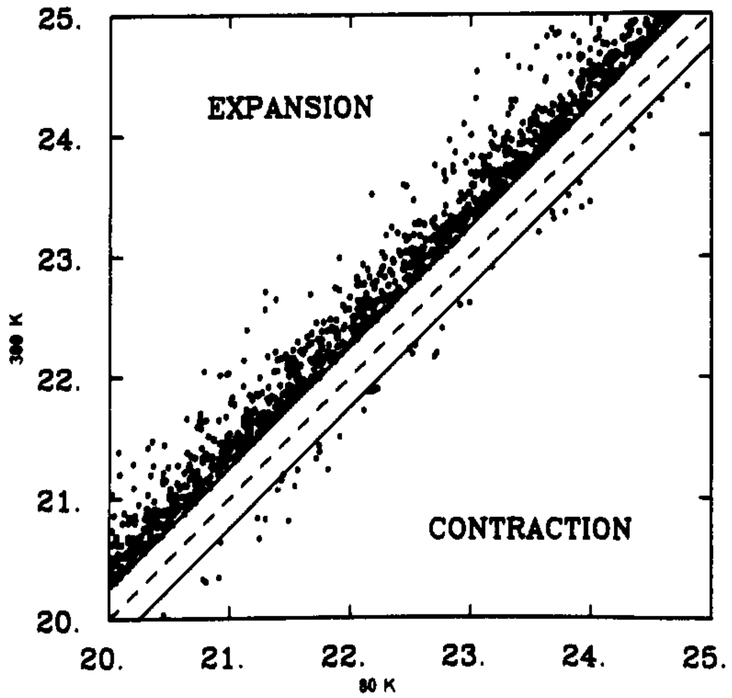
now: XFELs!

Fraser…Alber, *PNAS*, 2011

Cryocooling **contracts** the lattice - and protein

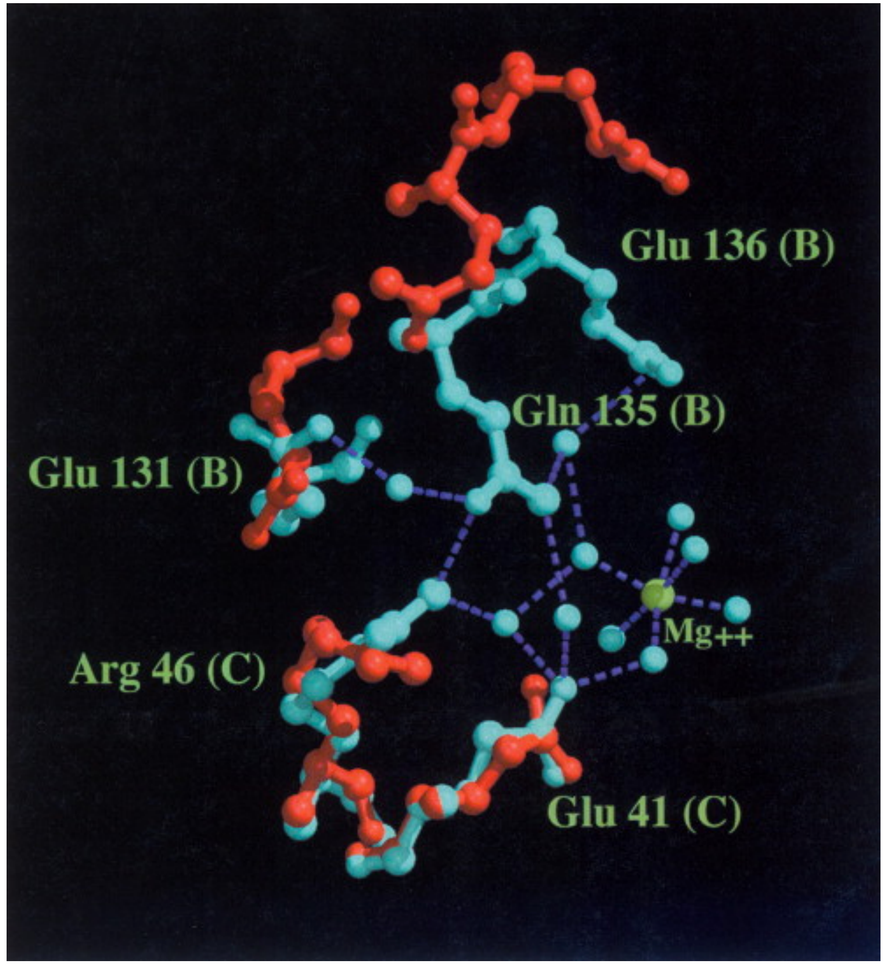


Juers and Matthews, JMB, 2002

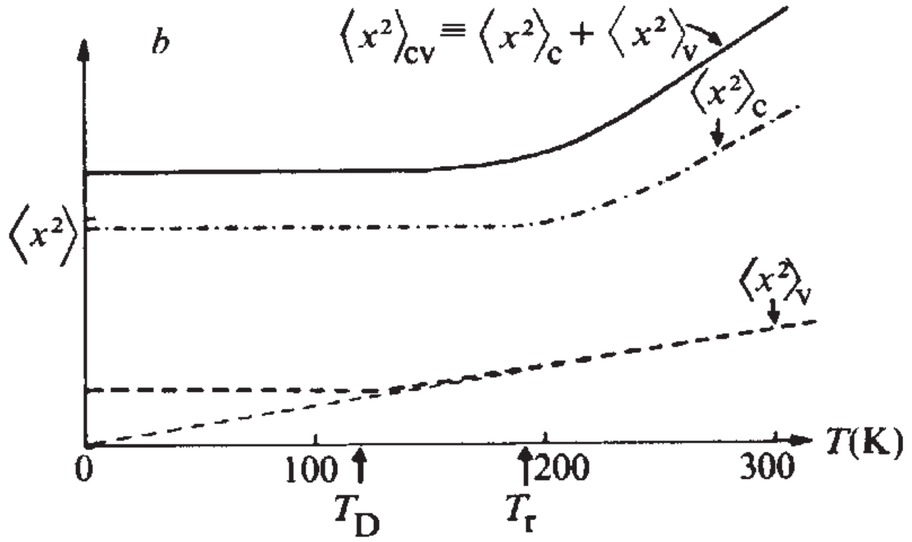


Frauenfelder et al, Biochemistry, 1987

Cryocooling causes **local changes** in structure and conformational heterogeneity



Juers and Matthews, JMB, 2002



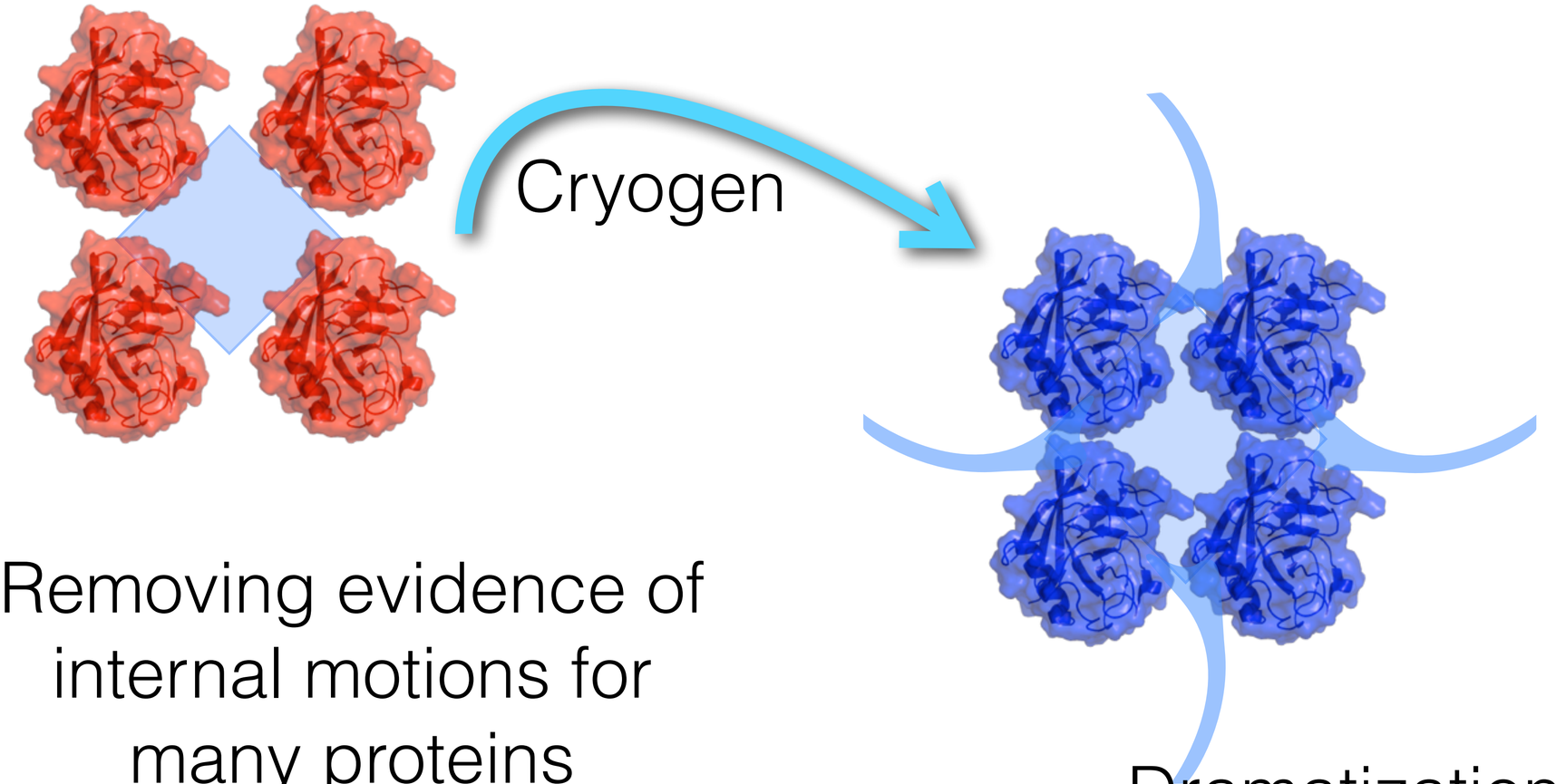
Temperature-dependent X-ray diffraction as a probe of protein structural dynamics

Hans Frauenfelder, Gregory A. Petsko* & Demetrius Tsernoglou

Department of Physics, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801
and
Department of Biochemistry, Wayne State University School of Medicine, Detroit, Michigan 48201

Nature Vol. 280 16 August 1979

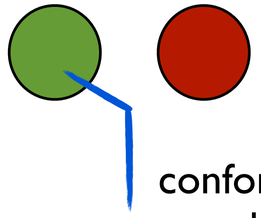
Cryocooling **slowly** shrinks proteins, remodels side chains, and improves packing



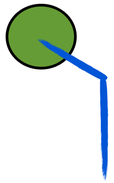
Removing evidence of internal motions for many proteins

Dramatization!!!

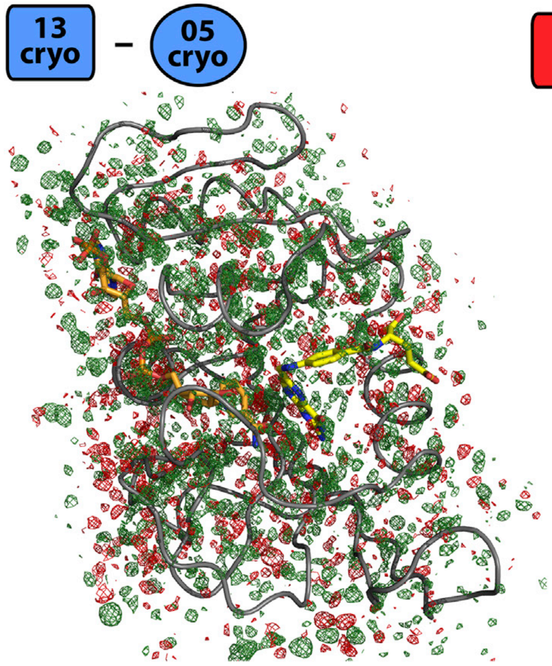
Fo-Fo map

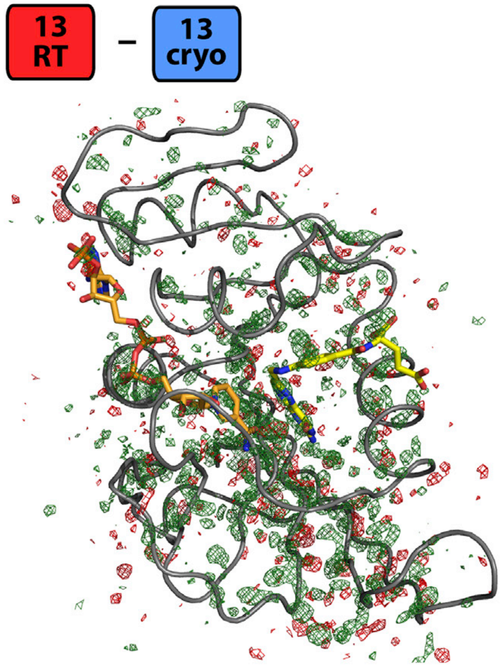


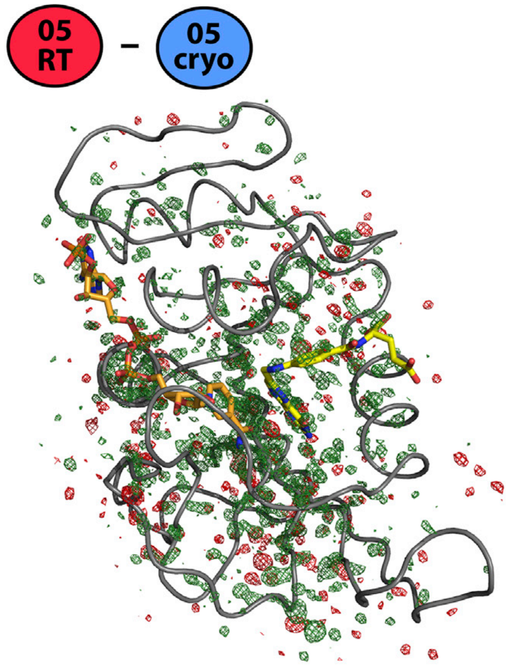
conformational change



conformational ensemble change



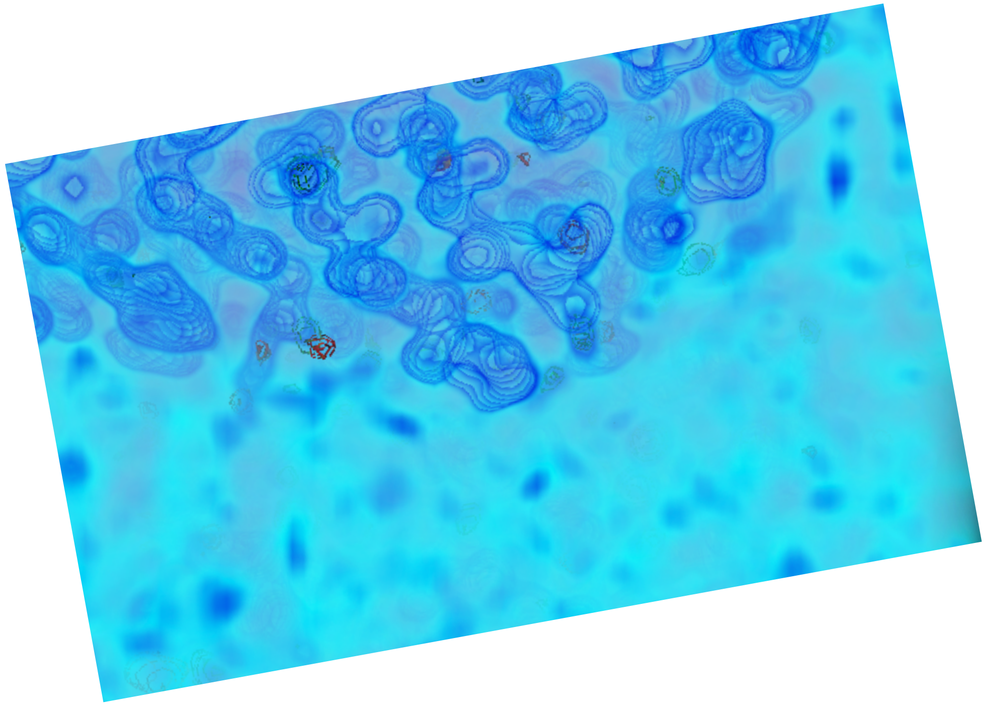


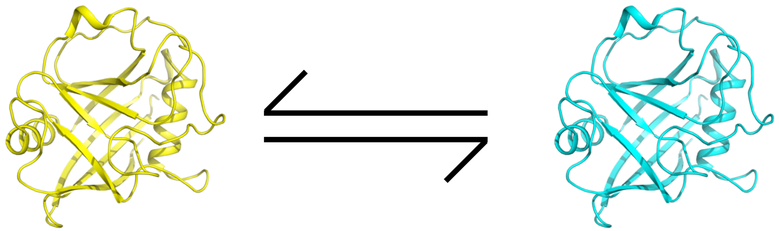


Room temperature data are more consistent than cryocooled data

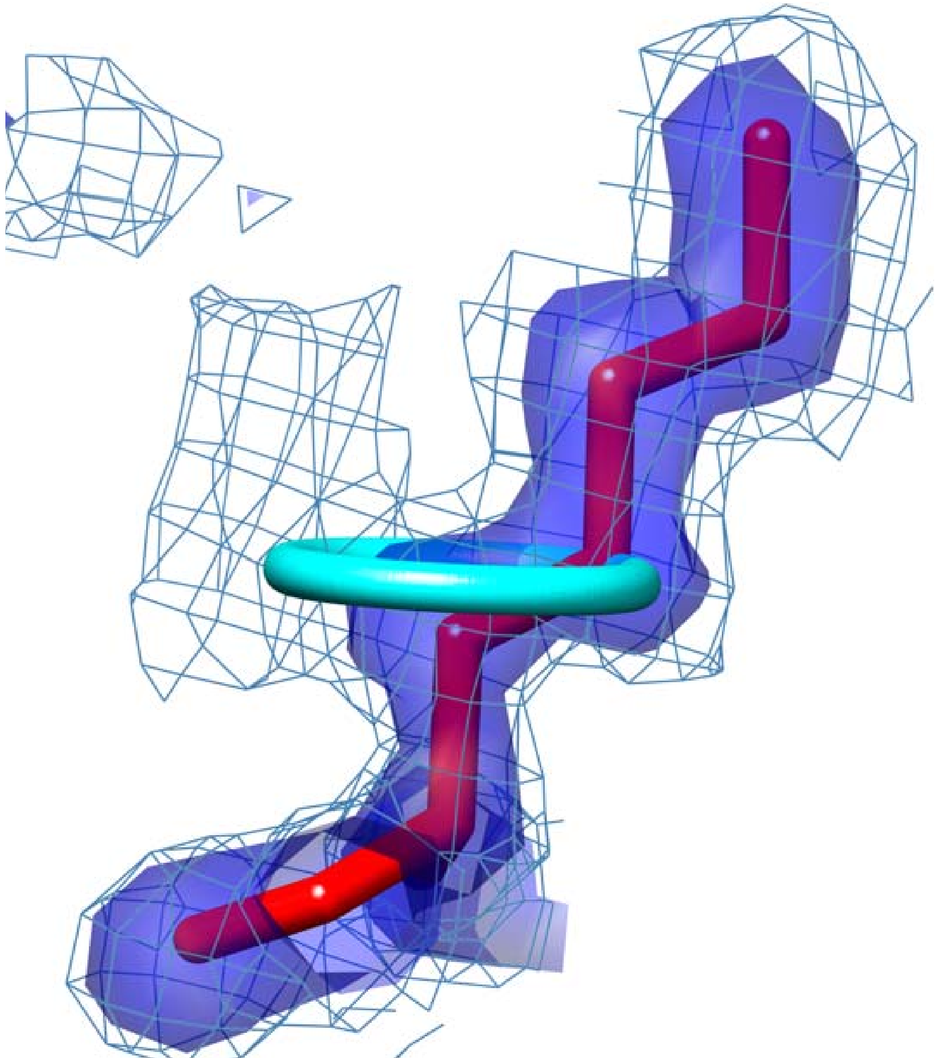
Keedy…Fraser, *Structure*, 2014

The challenge is to deconvolute multiple conformations from an **ensemble averaged** density map

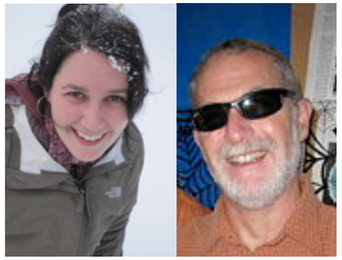




Ringer is an assay for electron density



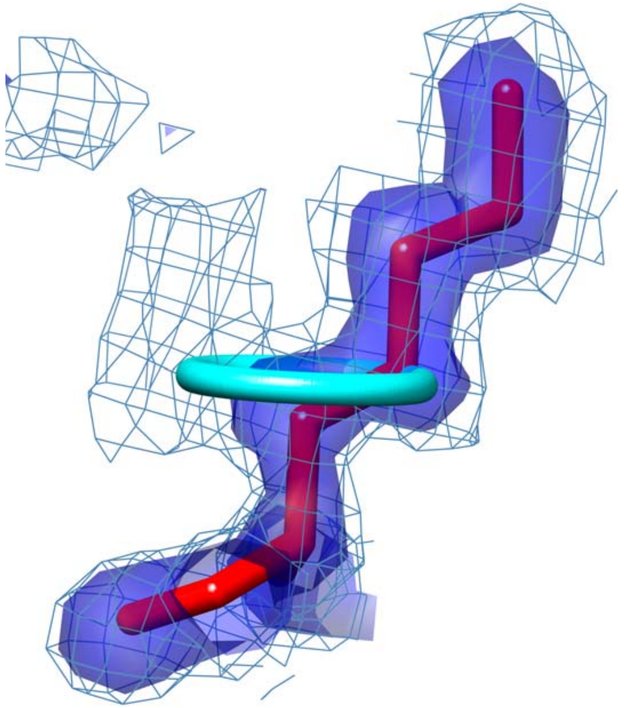
0.3 and 1σ



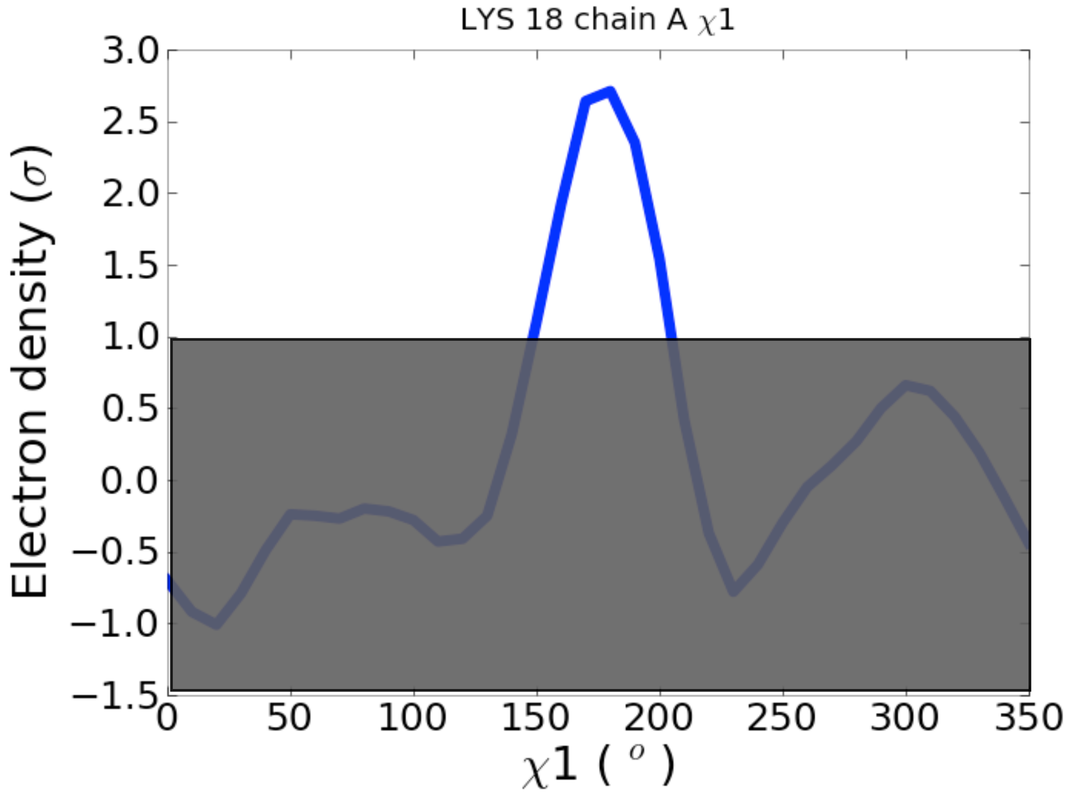
Terry Lang **Tom Alber**

UC Berkeley

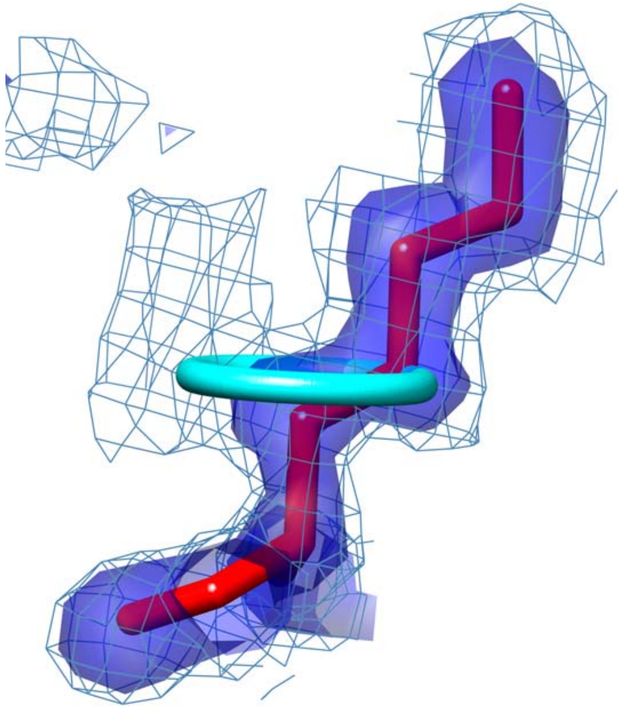
Side chains are built into **high electron density** regions



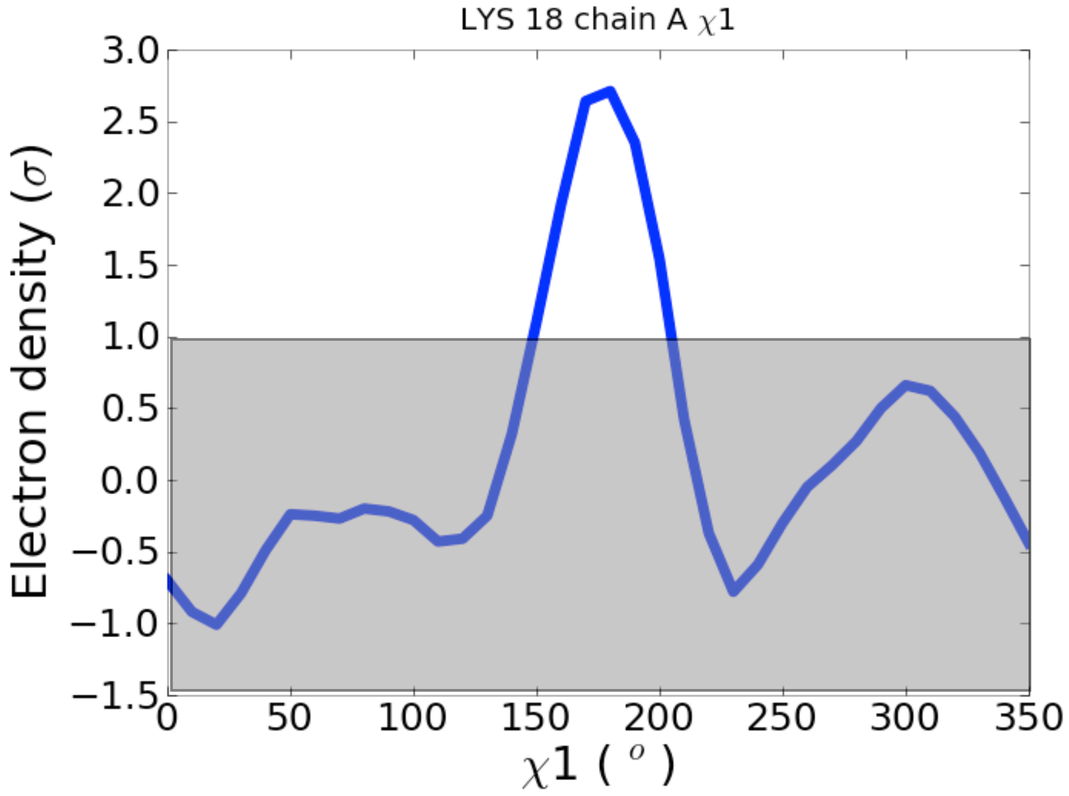
0.3 and 1σ



Only 5% of residues are modeled in **multiple conformations**

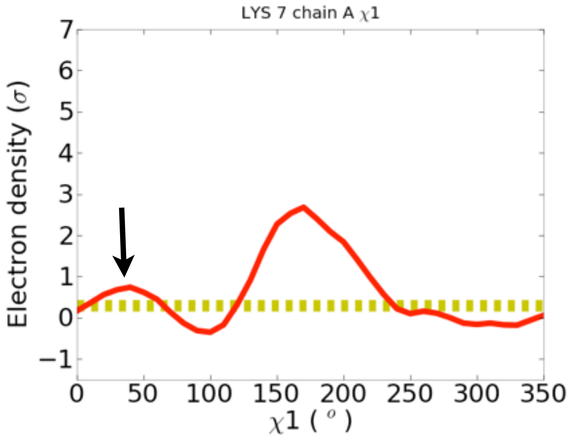


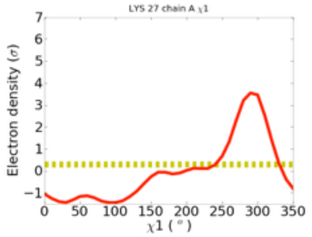
0.3 and 1σ

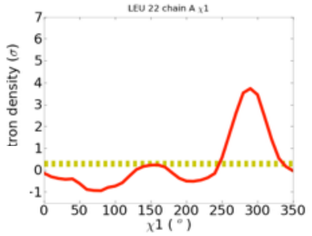


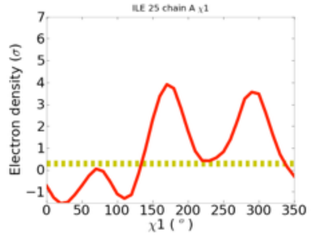
…but electron density sampling reveals multiple peaks

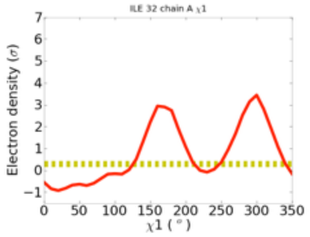
Ringer peaks are rotameric (**non-random**)

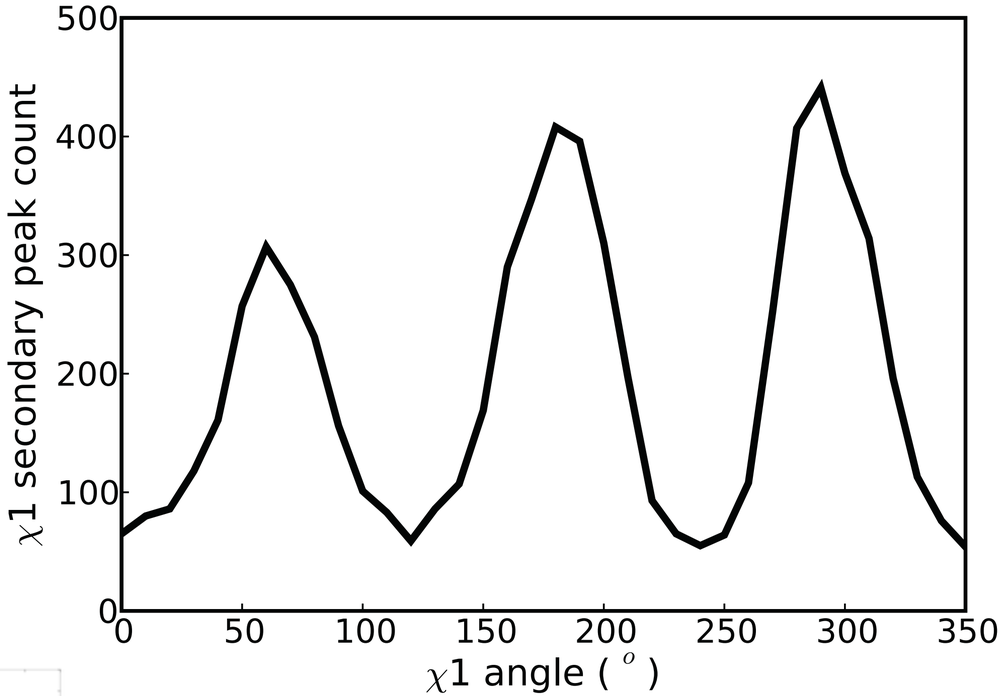






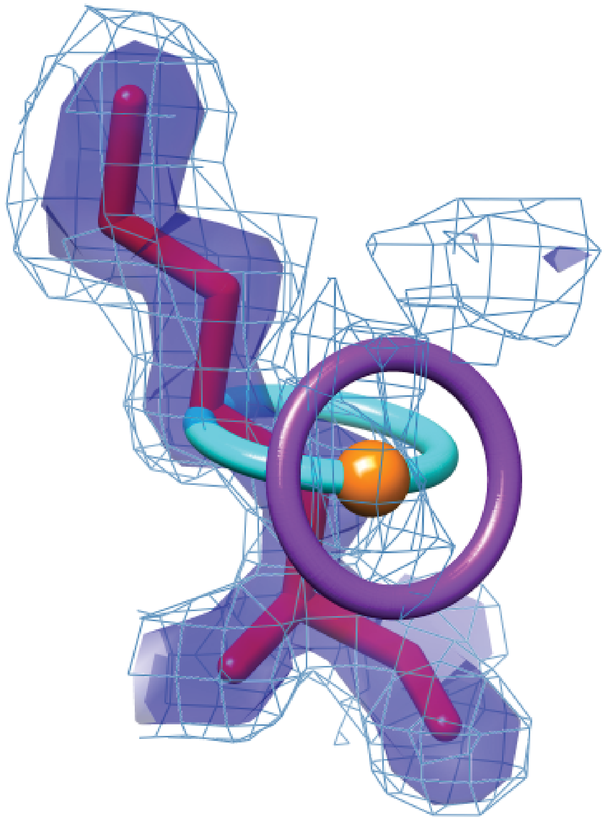




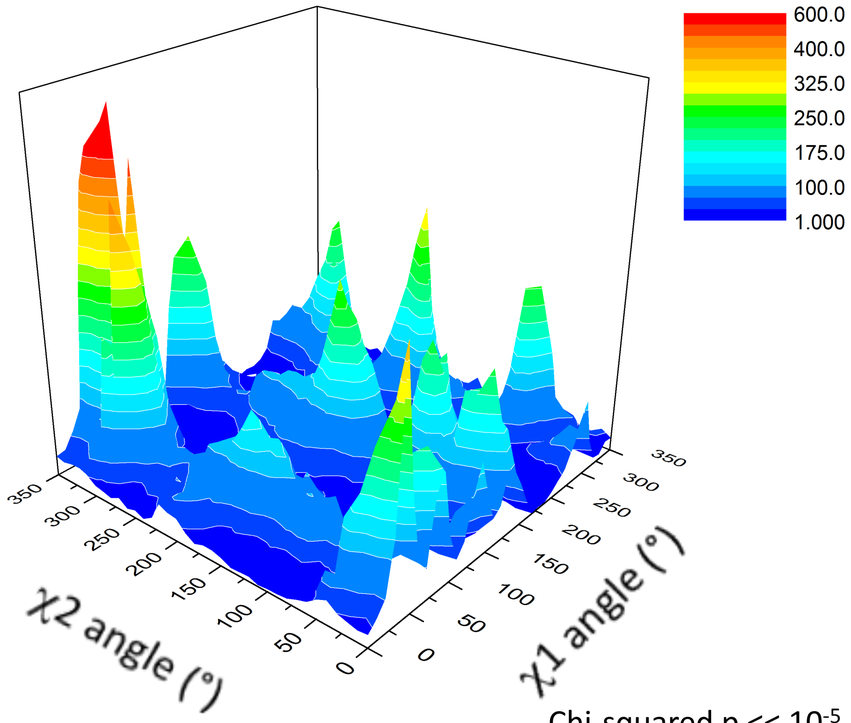


~600 structures at 1.5Å or better

Most peaks represent **hidden conformations**



0.3 and 1σ

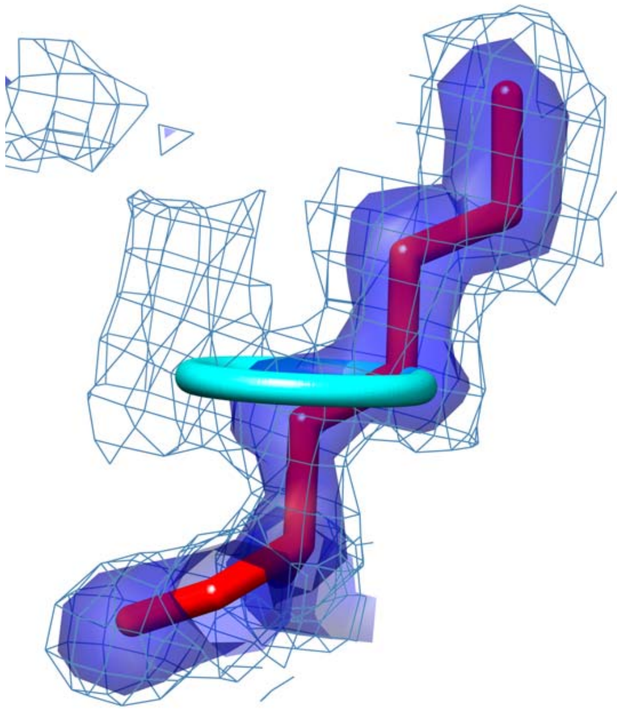


Chi-squared $p << 10^{-5}$

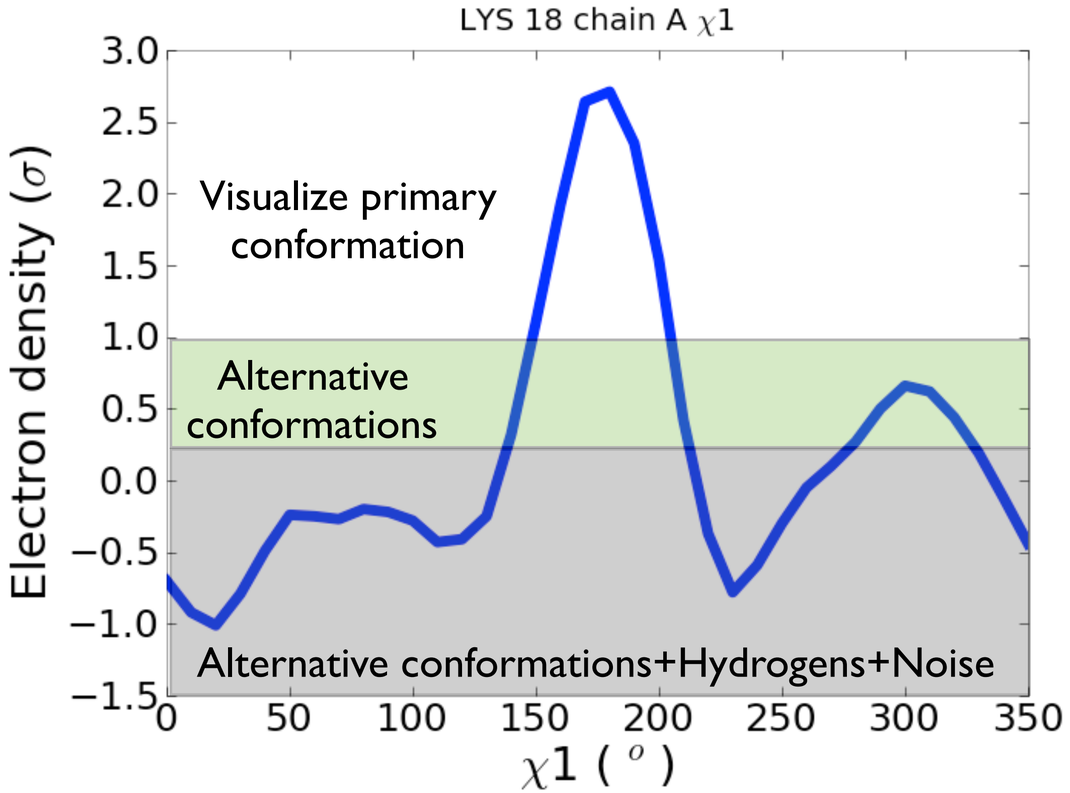
$\chi 1 - \chi 2$ correlation plot

~600 structures at 1.5Å or better

Ringer gives us **confidence** in signals at low electron density

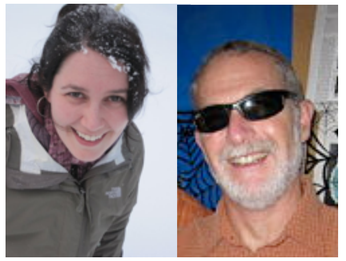


0.3 and 1σ



RINGER: Lang, Ng...Alber *Protein Science*, 2010

END/RAPID: Lang, Holton, Fraser, Alber *PNAS*, 2014



Terry Lang Tom Alber

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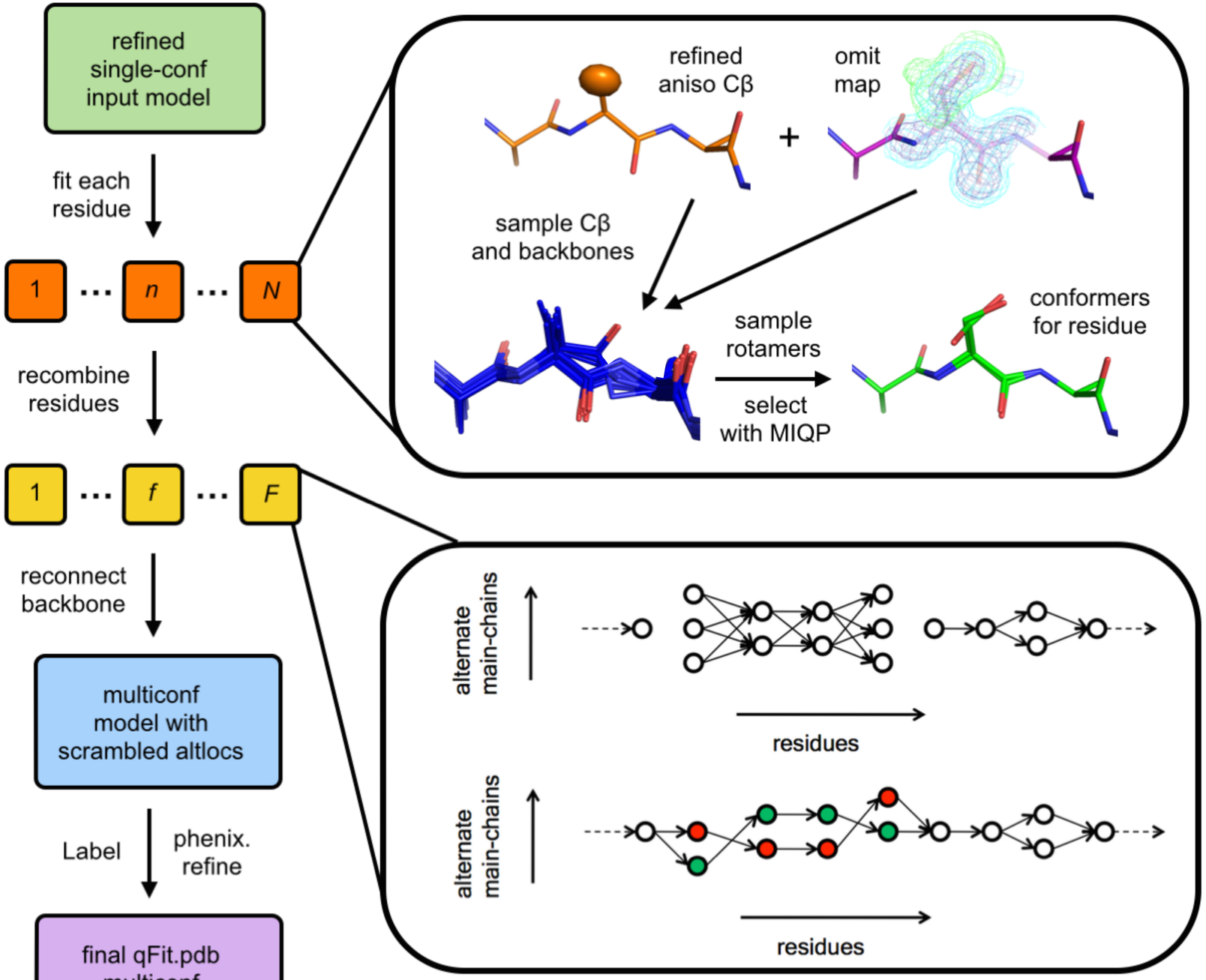
James Holton

LBL/UCSF



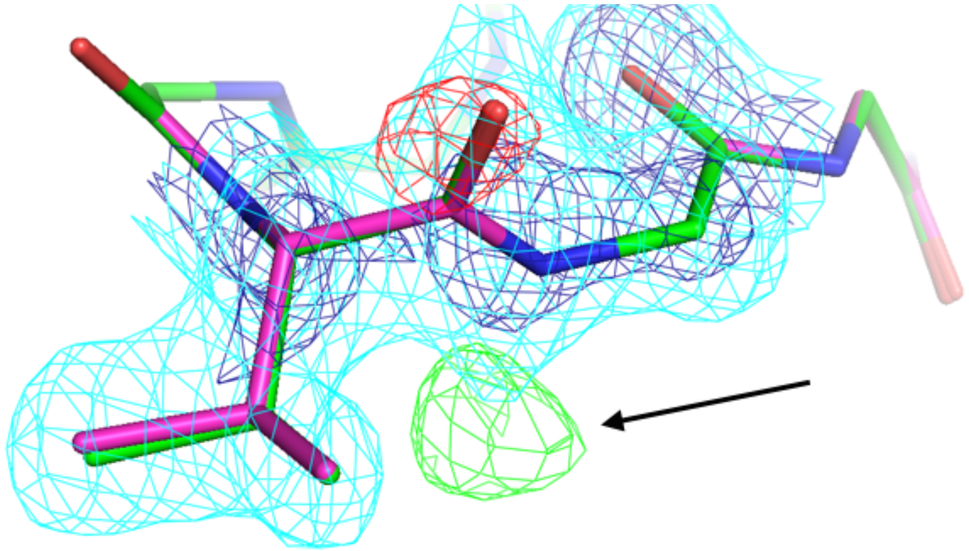
van den Bedem…Deacon, Acta D, 2009;
Keedy, Fraser, van den Bedem, PLOS CompBiol, 2015

qFit automates **multiconformer** side chain and backbone **modeling**

Henry van den Bedem
(SSRL)

Daniel Keedy

Alternative conformations (refined occupancies) model anharmonic motions better than B-factors



2mFo-DFc		mFo-DFc	
	1.0σ		-3.0σ
	0.5σ		+3.0σ

van den Bedem…Deacon, Acta D, 2009;
Keedy, Fraser, van den Bedem, PLOS CompBiol, 2015

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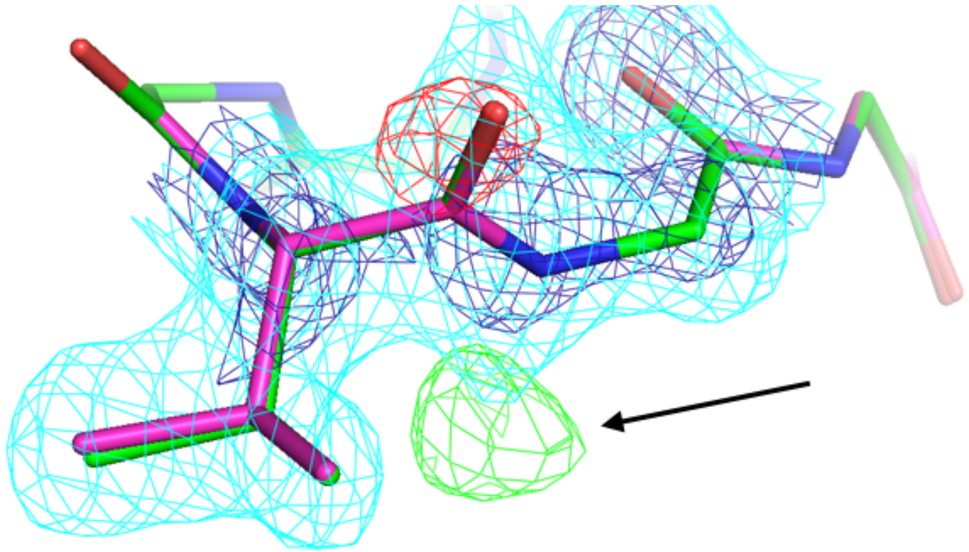
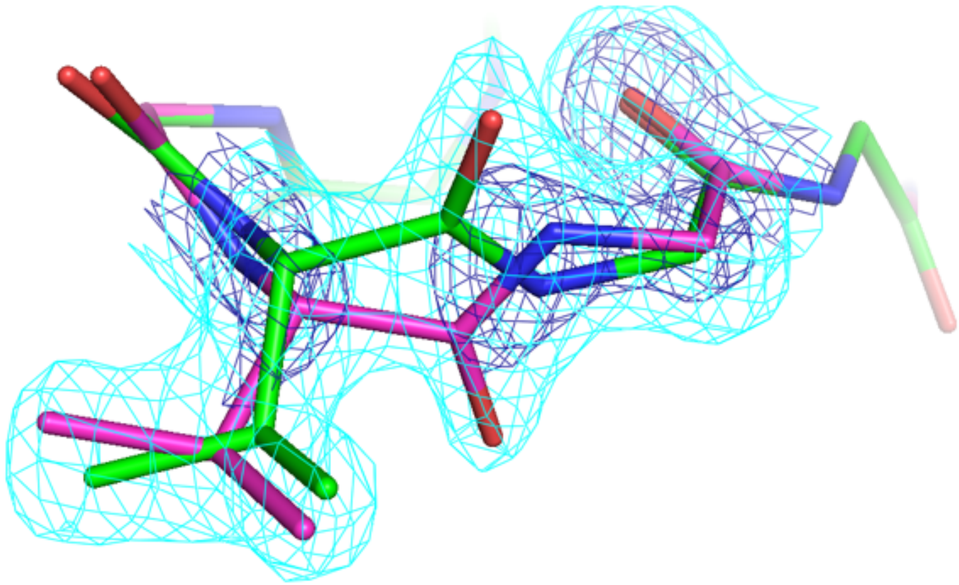




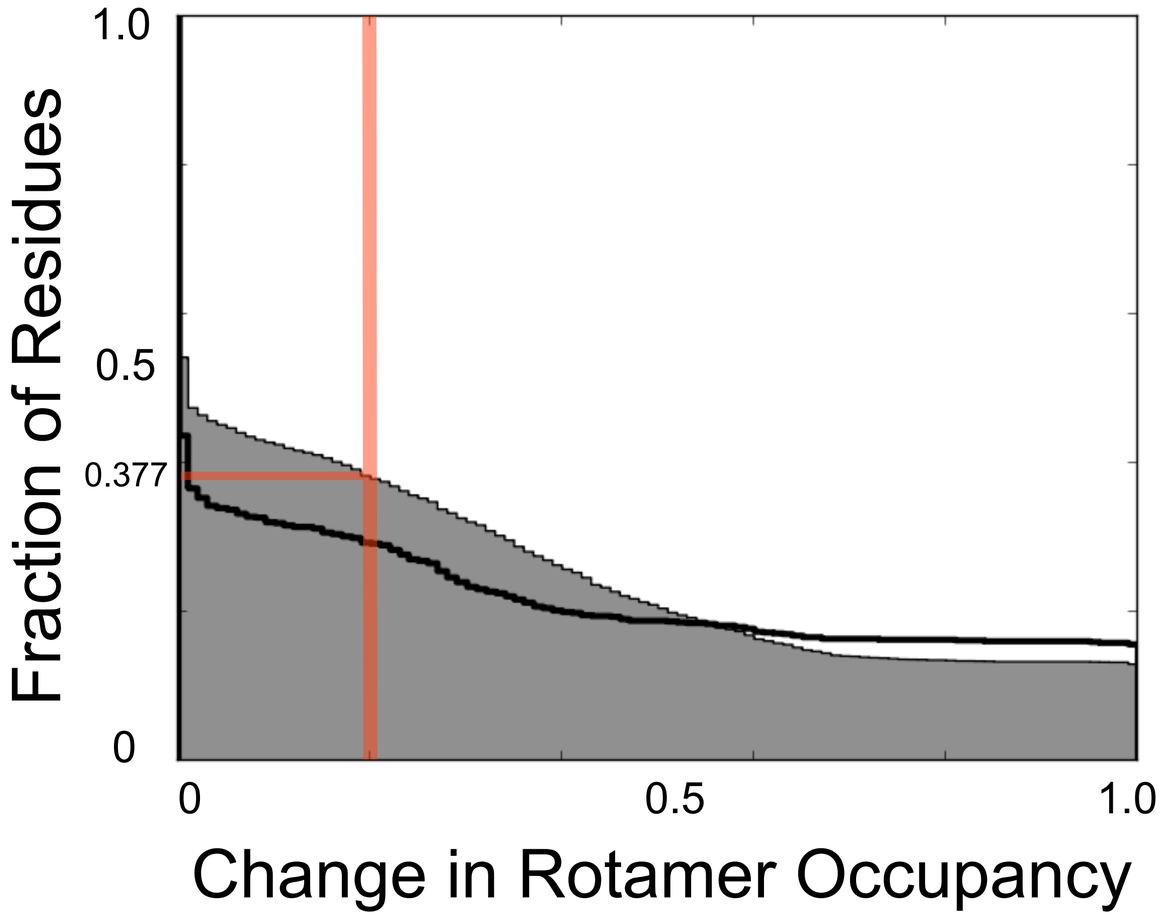
2mFo-DFc		mFo-DFc	
	1.0σ		-3.0σ
	0.5σ		+3.0σ

van den Bedem…Deacon, Acta D, 2009;
Keedy, Fraser, van den Bedem, PLOS CompBiol, 2015

Henry van den Bedem
(SSRL)

Daniel Keedy

~35% of side chain conformations are changed by **cryocooling**



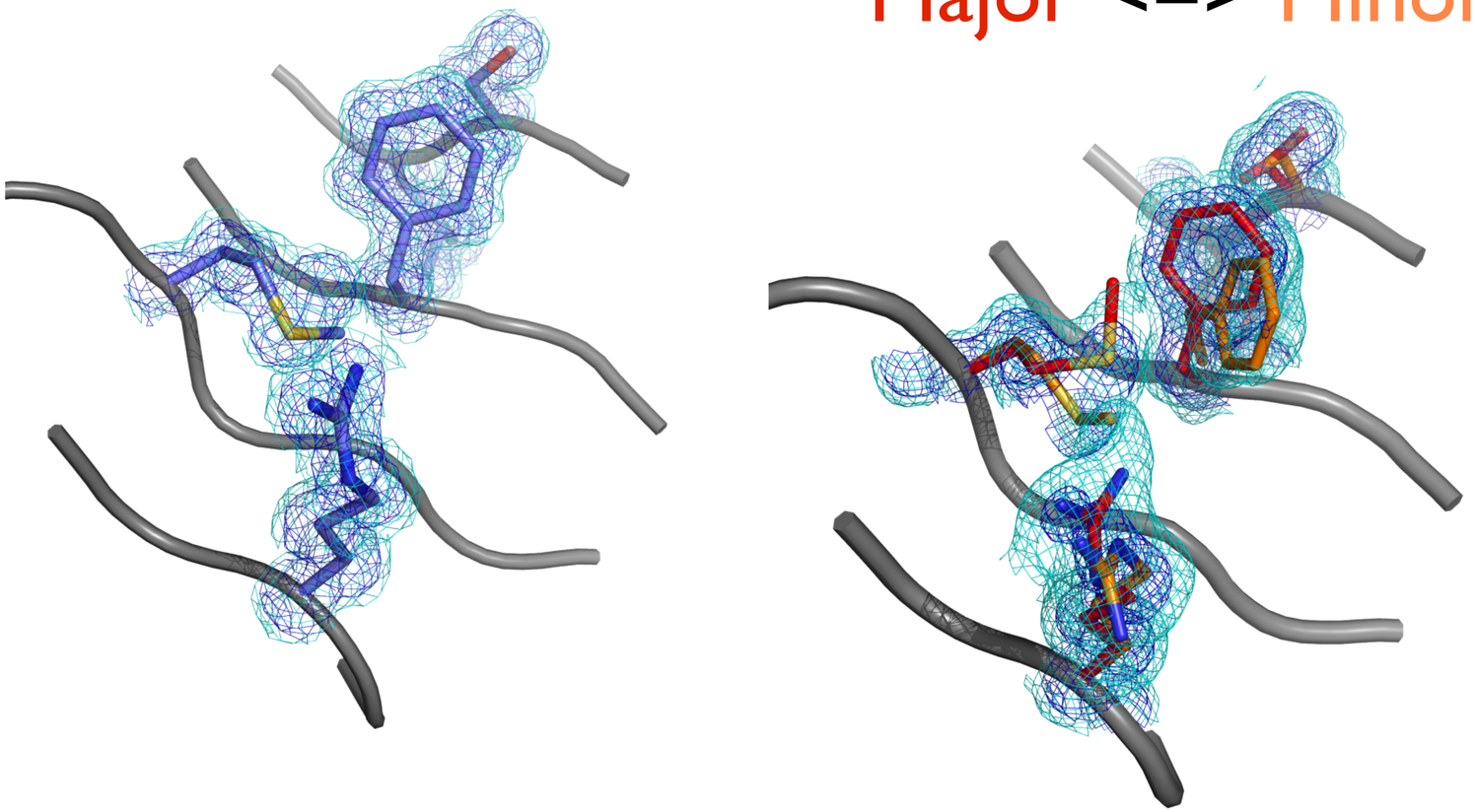
29% of all buried residues

Fraser…Alber, *PNAS*, 2011

with Henry van den Bedem (JCSG)

Coupled side chain motions explain NMR relaxation, enabling design of mutations to demonstrate **dynamics-function link**

Major <=> Minor



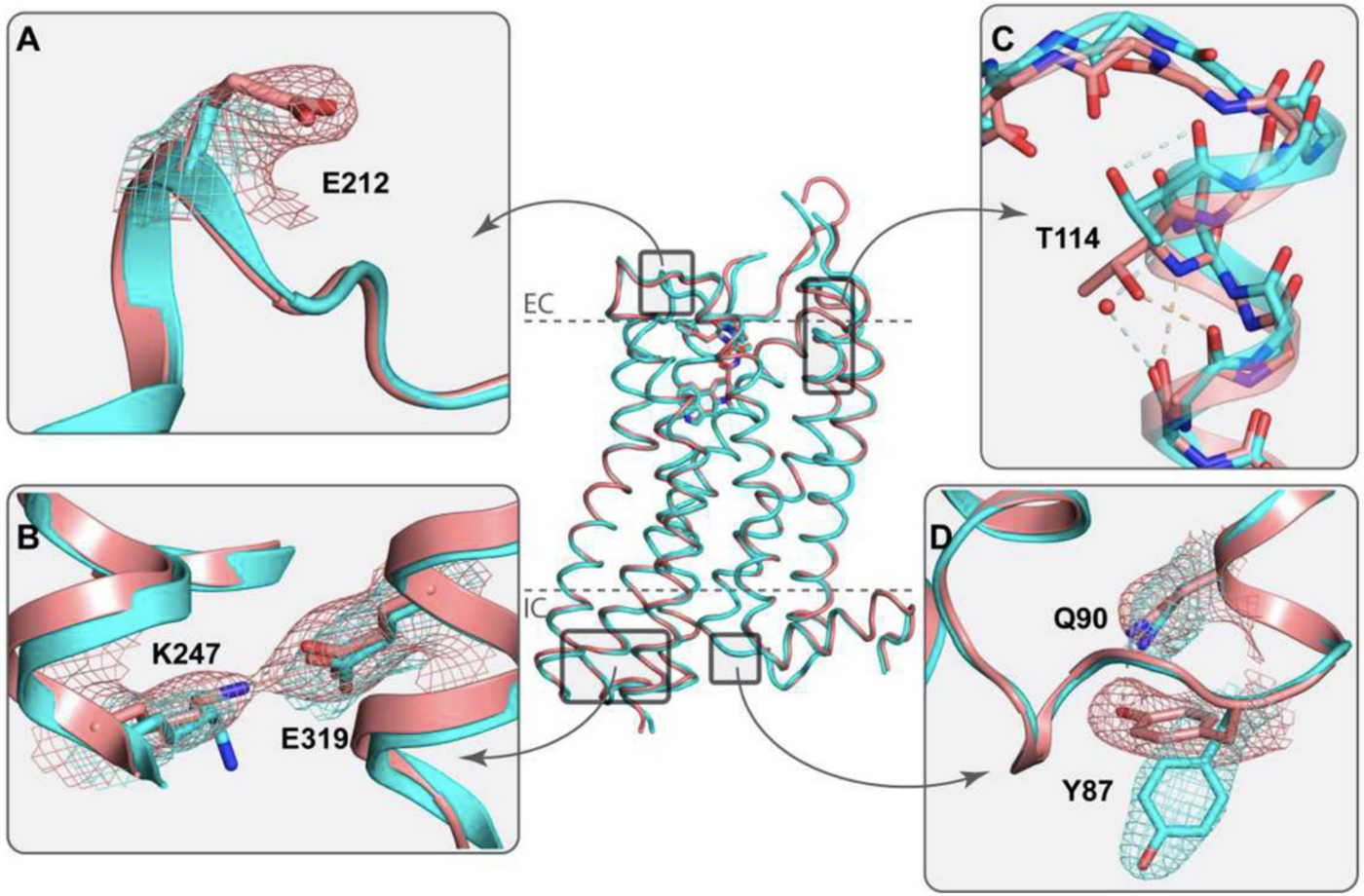
100 K

288 K

0.3 and 1σ

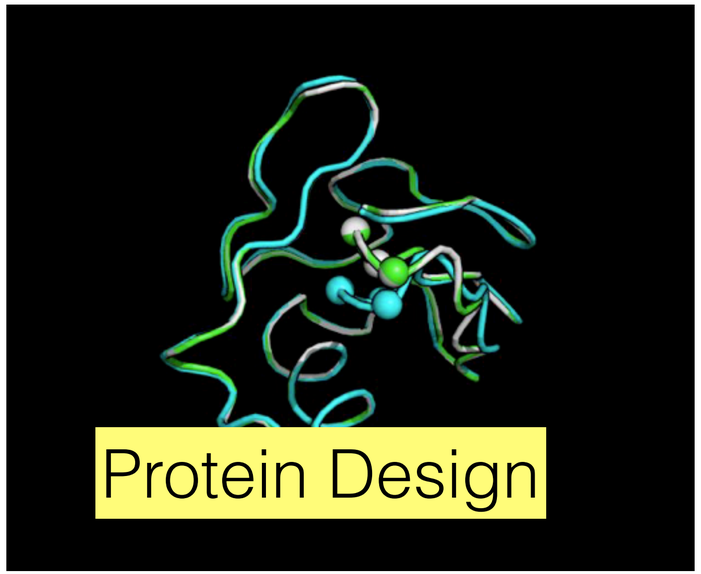
Fraser…Kern, Alber, *Nature*, 2009

Conformational differences between room temperature and cryogenic temperatures using SFX at **XFELs**

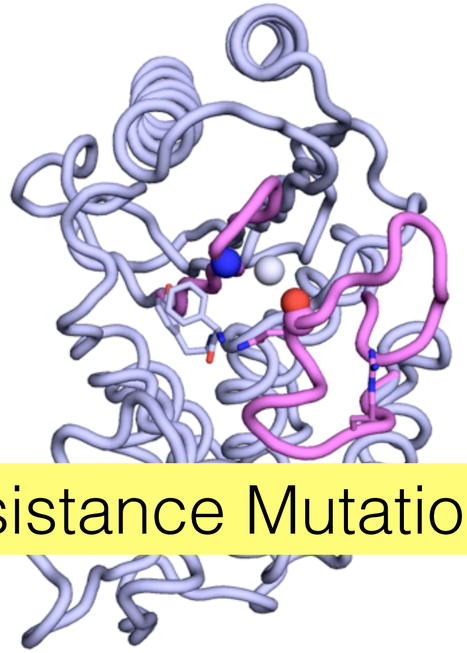


Liu…Cherezov, *Science*, 2013

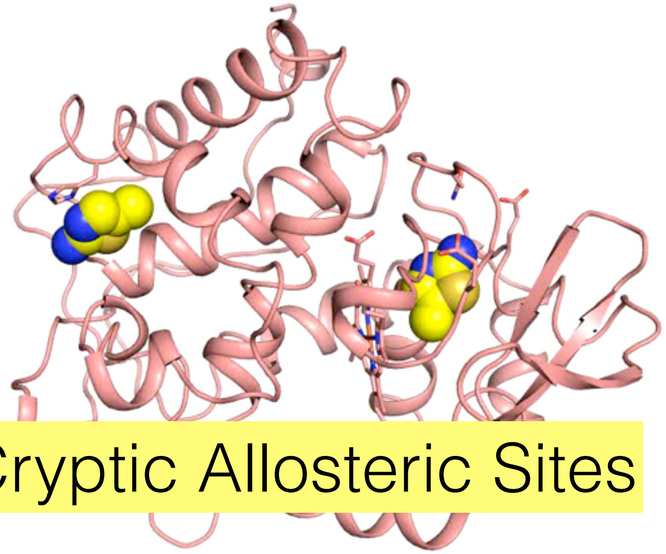
Conformational dynamics are at the core of **three critical problems in biology**



Protein Design



Resistance Mutations



Cryptic Allosteric Sites

Can temperature perturbation expose the **protein conformations** used by design, disease, and drugs?

We want to:

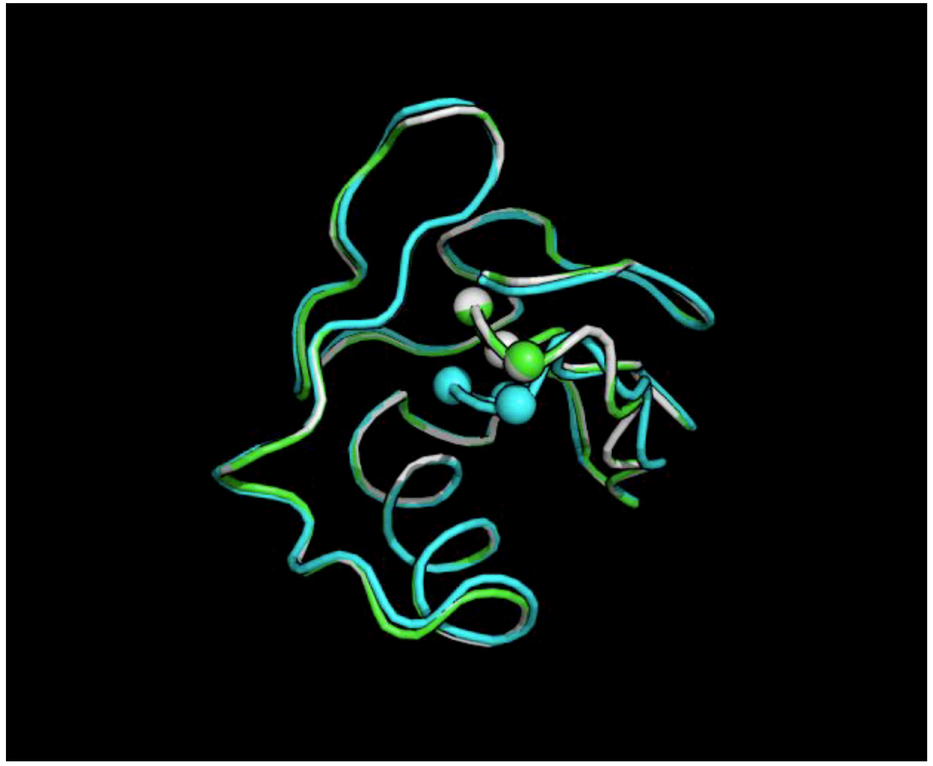
design macromolecules with new (unnatural) functions

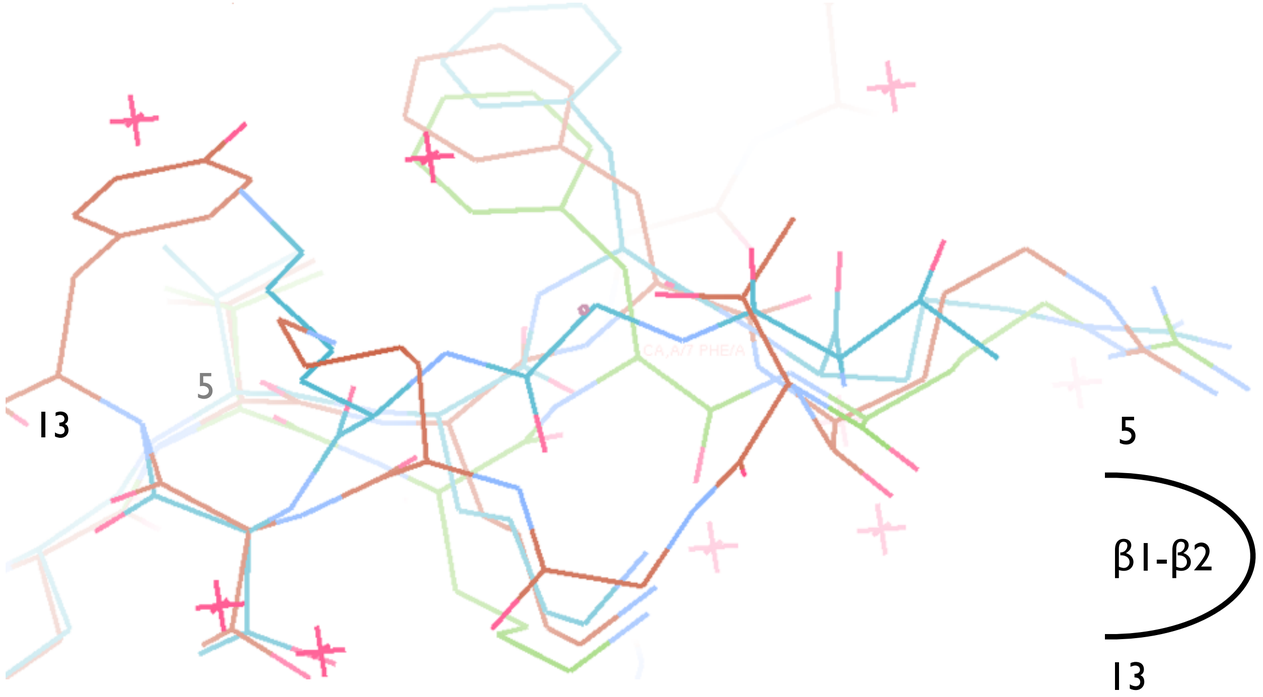
understand how mutations alter protein function in disease

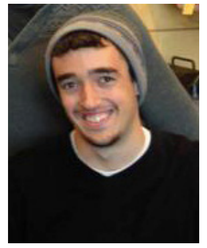
discover small molecules drugs to modulate protein function

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

Designed proteins
(e.g. Ubiquitin variants to inhibit USP7)
are currently **too dynamic**

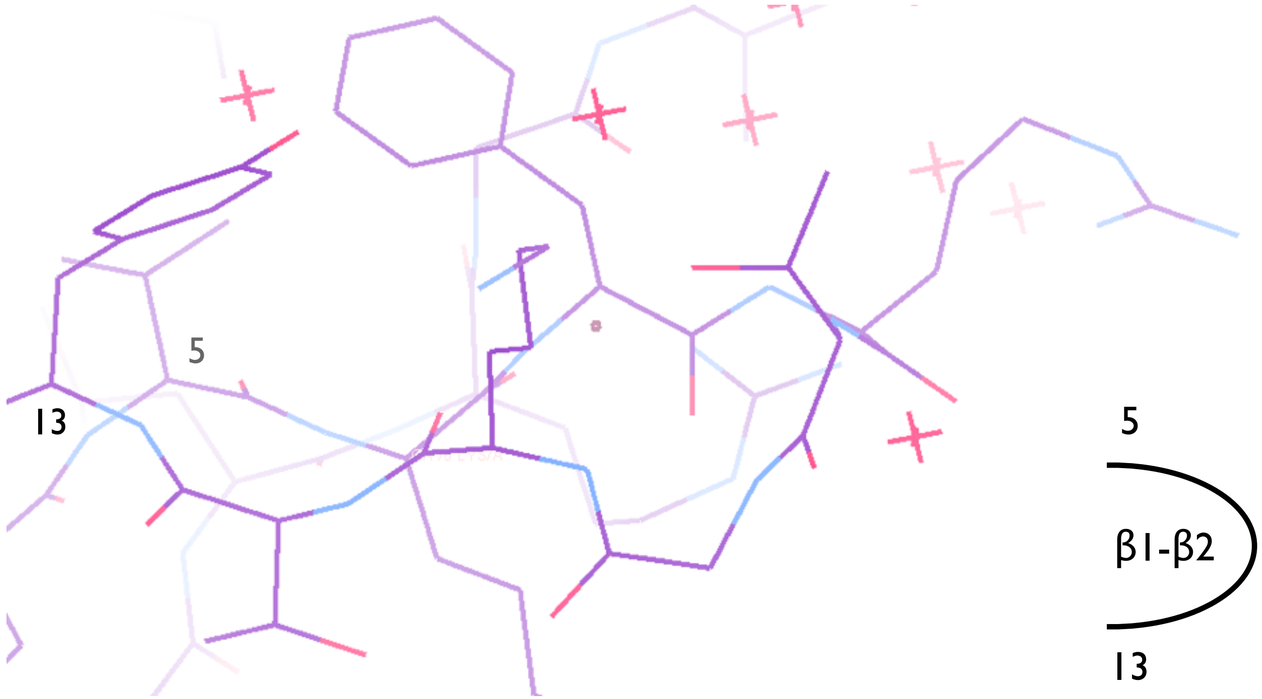


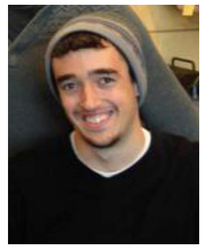




Justin Biel (Fraser Lab/UCSF); Jacob Corn (Genentech)

After **directed evolution**, dynamics are reduced….

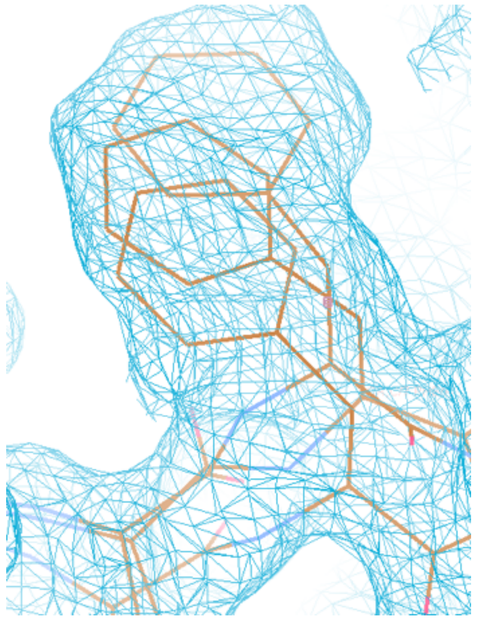




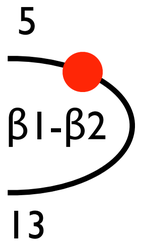
Justin Biel (Fraser Lab/UCSF); Jacob Corn (Genentech)

How **different** are dynamics of designed, artificially evolved, and natural proteins?

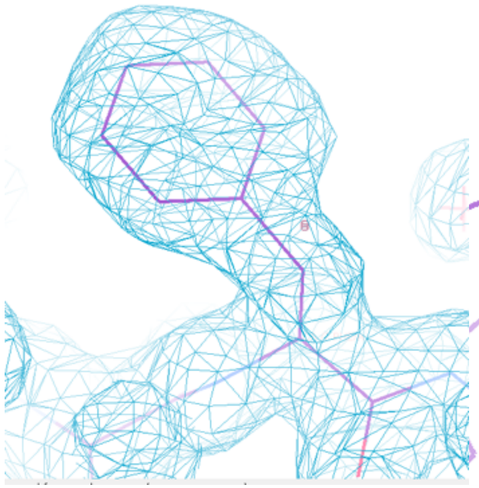
Designed mutant

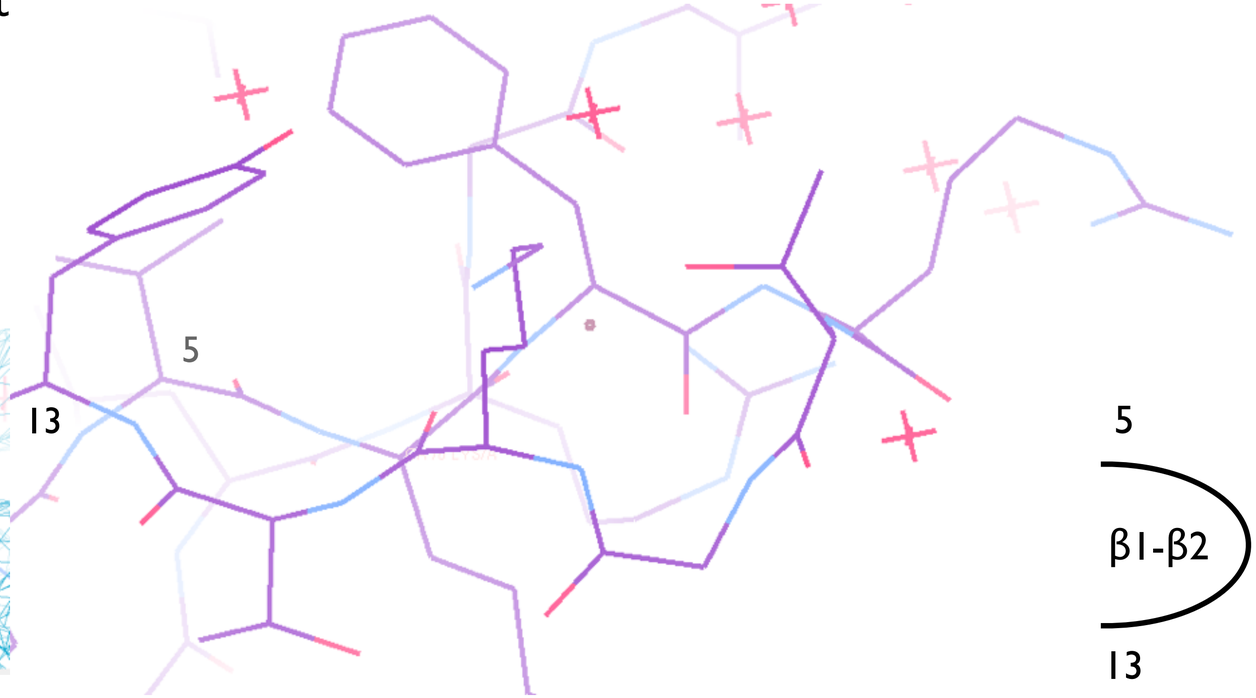


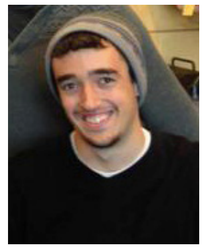
Phe-7
0.25 $e^-/Å^3$



Affinity enhanced mutant







Justin Biel (Fraser Lab/UCSF); Jacob Corn (Genentech)

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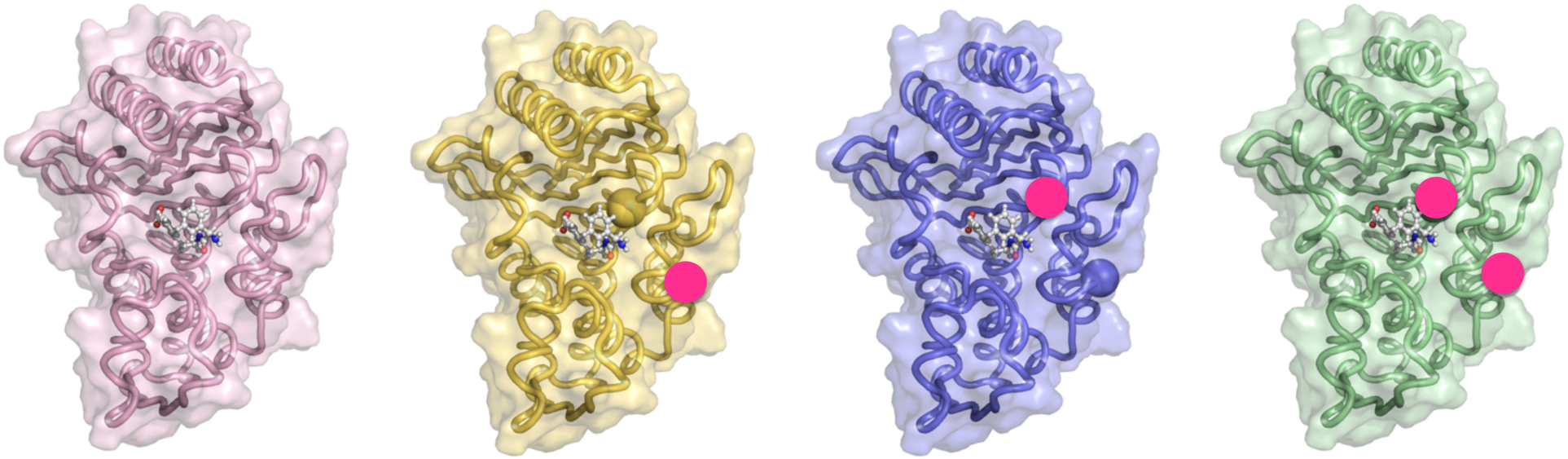
understand how mutations alter protein function in disease

discover small molecules drugs to modulate protein function

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

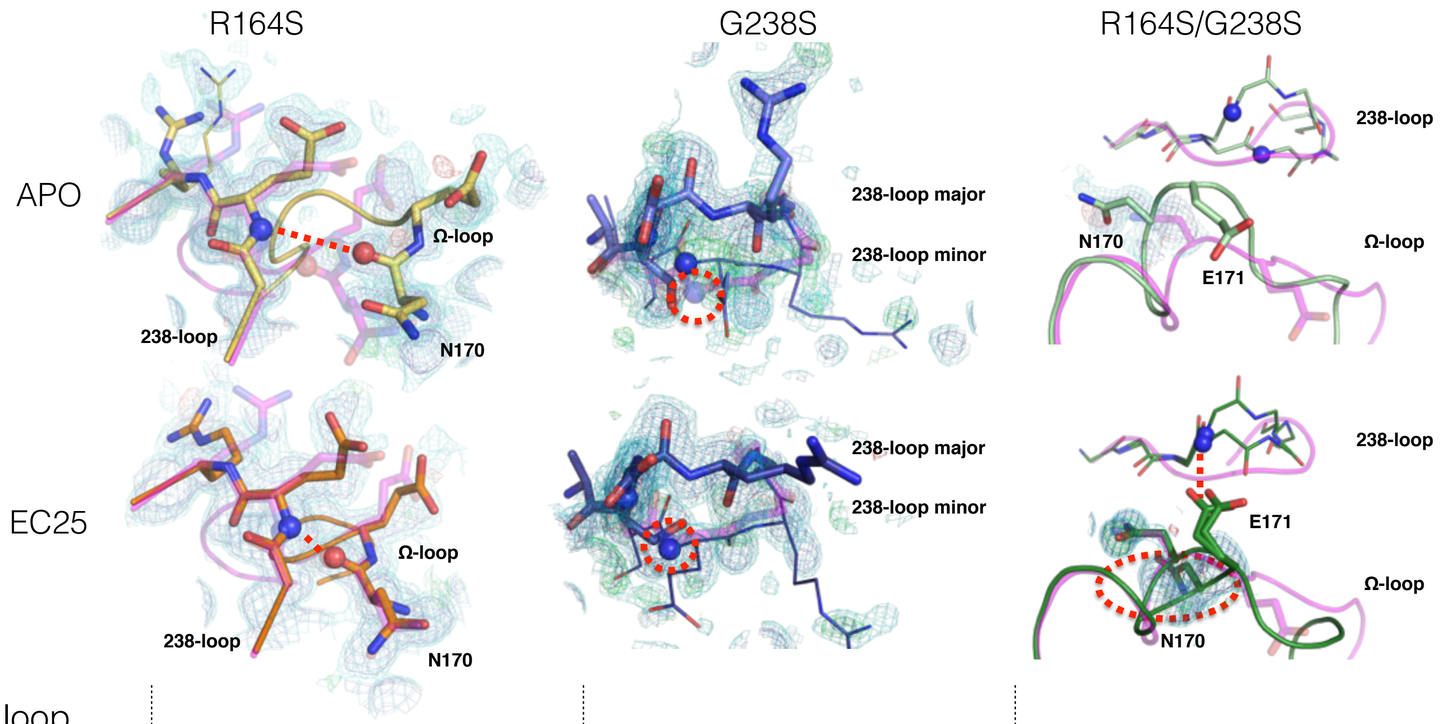
Why are these two resistance mutations in TEM-1 **incompatible**?



WT R164S G238S R164S/G238S

+/- inhibitor

Different resistance **mutants cannot be combined** due to dynamics



omega loop preorganized	**no**	yes	no
238 loop in unique conformation	yes	**no**	no
169 N interaction with 239-O	yes	yes	**no (E171 - non-native)**
N169 side chain ordered for catalysis	yes	yes	**partial occupancy**

suggests a method discover combination therapies with **huge barriers** to resistance

Eynat Dellus-Gur, Dan Tawfik (Weizmann)

Dellus-Gur…Fraser, Tawfik, JMB, 2015

We want to:

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understand how mutations alter protein function in disease

discover small molecules drugs to modulate protein function

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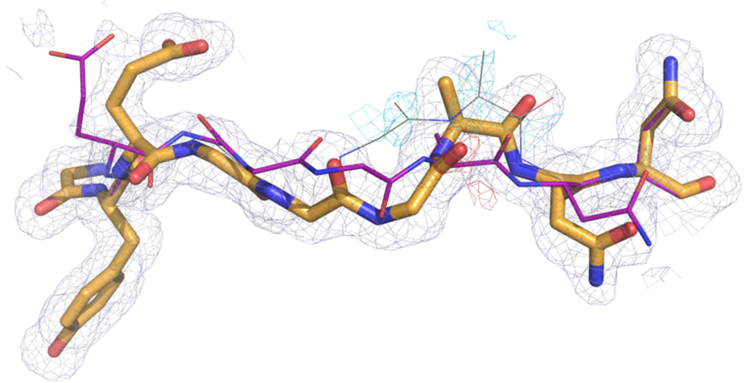
Cryptic allosteric fragment sites are quenched by cryocooling

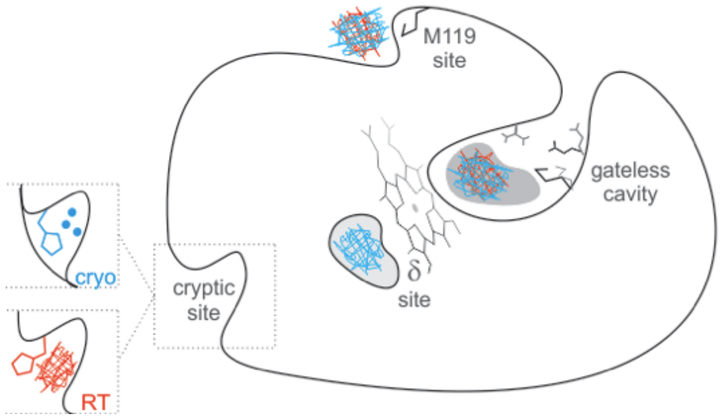
Active site heterogeneity
differs between
cryo and room temperature



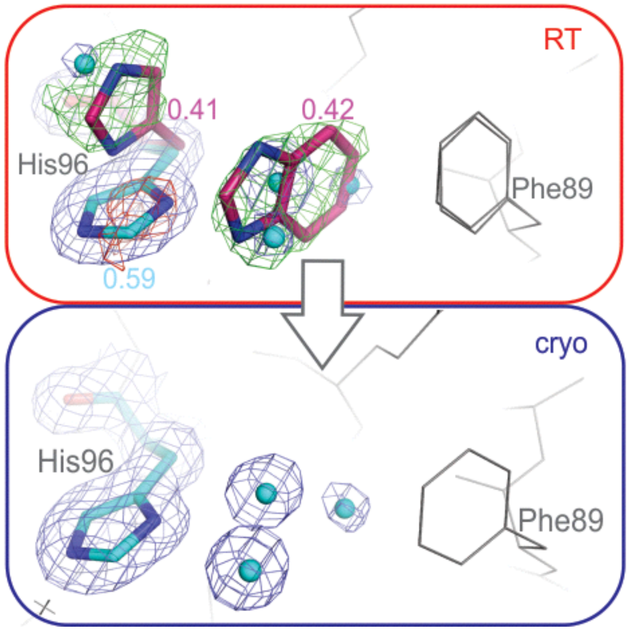
changes small molecules discovered by
in silico docking procedures

Fischer, Coleman, Fraser, Shoichet
Nature Chemistry, 2014

Cryptic site:

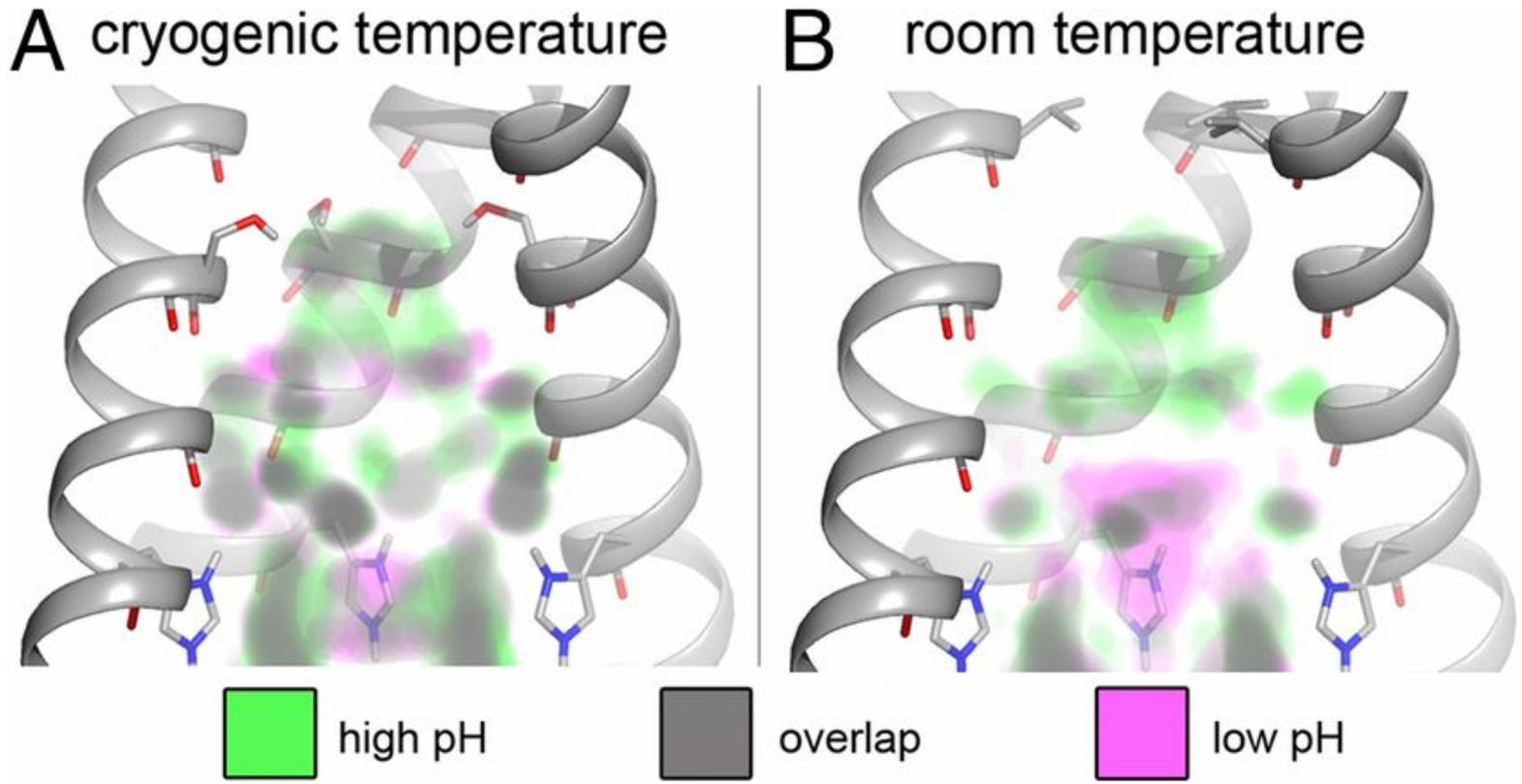


Fischer, Shoichet, Fraser
ChemBioChem, 2015



Marcus Fischer, Brian Shiochet (UCSF)

M2 Channel water density is temperature sensitive - ordered around drug binding sites and **pH sensitive**

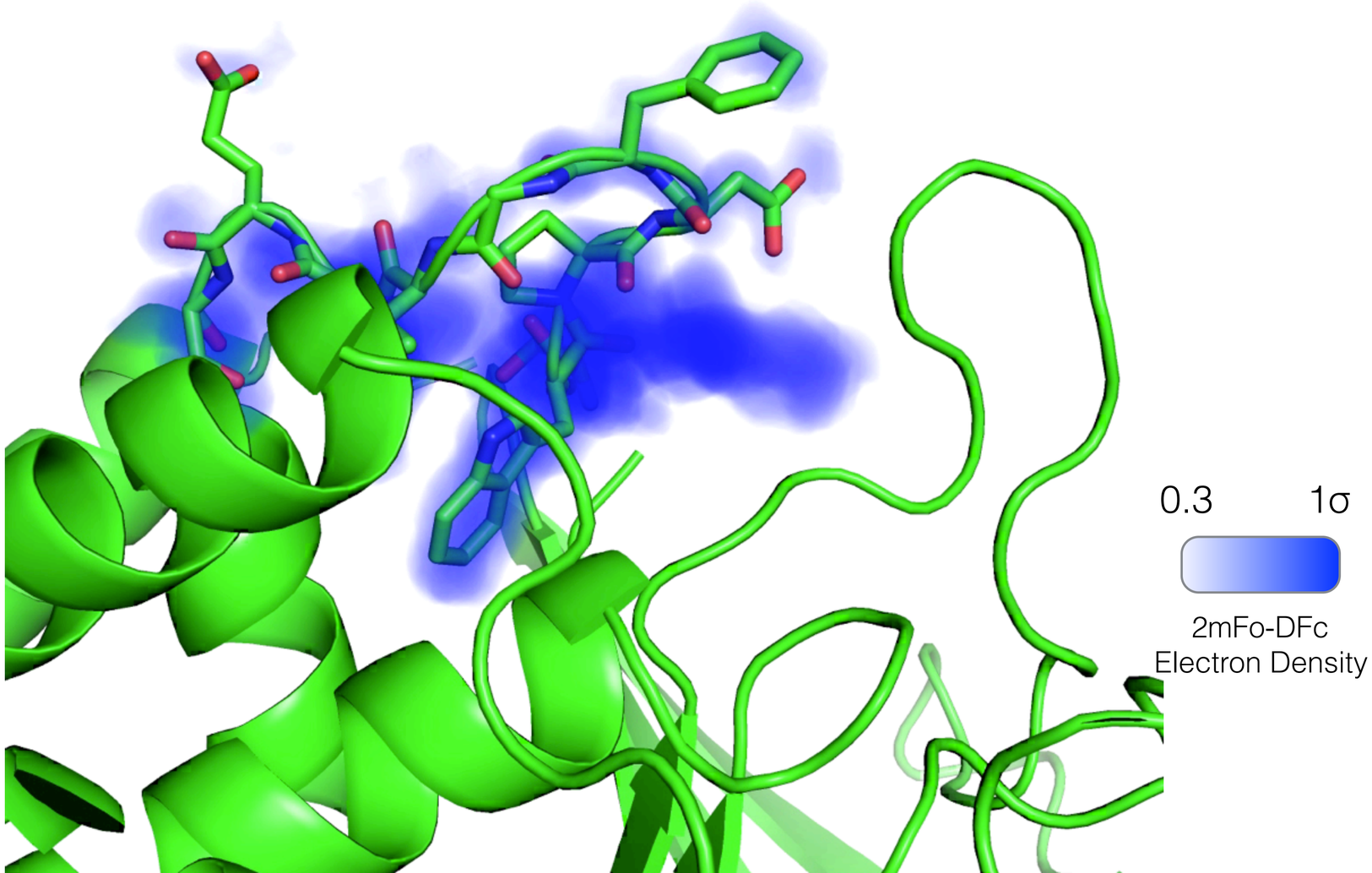


Thomaston…Woldeyes, Fraser…DeGrado, PNAS, 2015

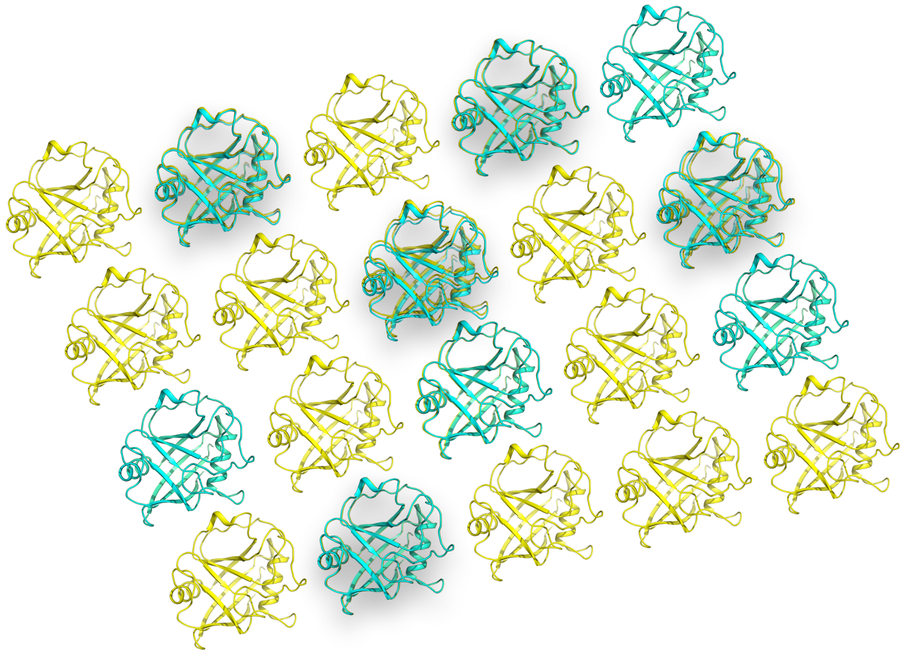
Jessica Thomaston
(DeGrado Lab)

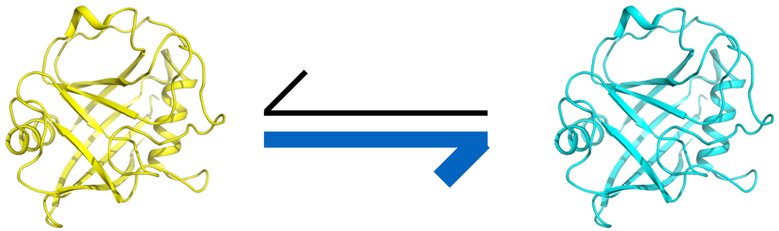
Rahel Woldeyes

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



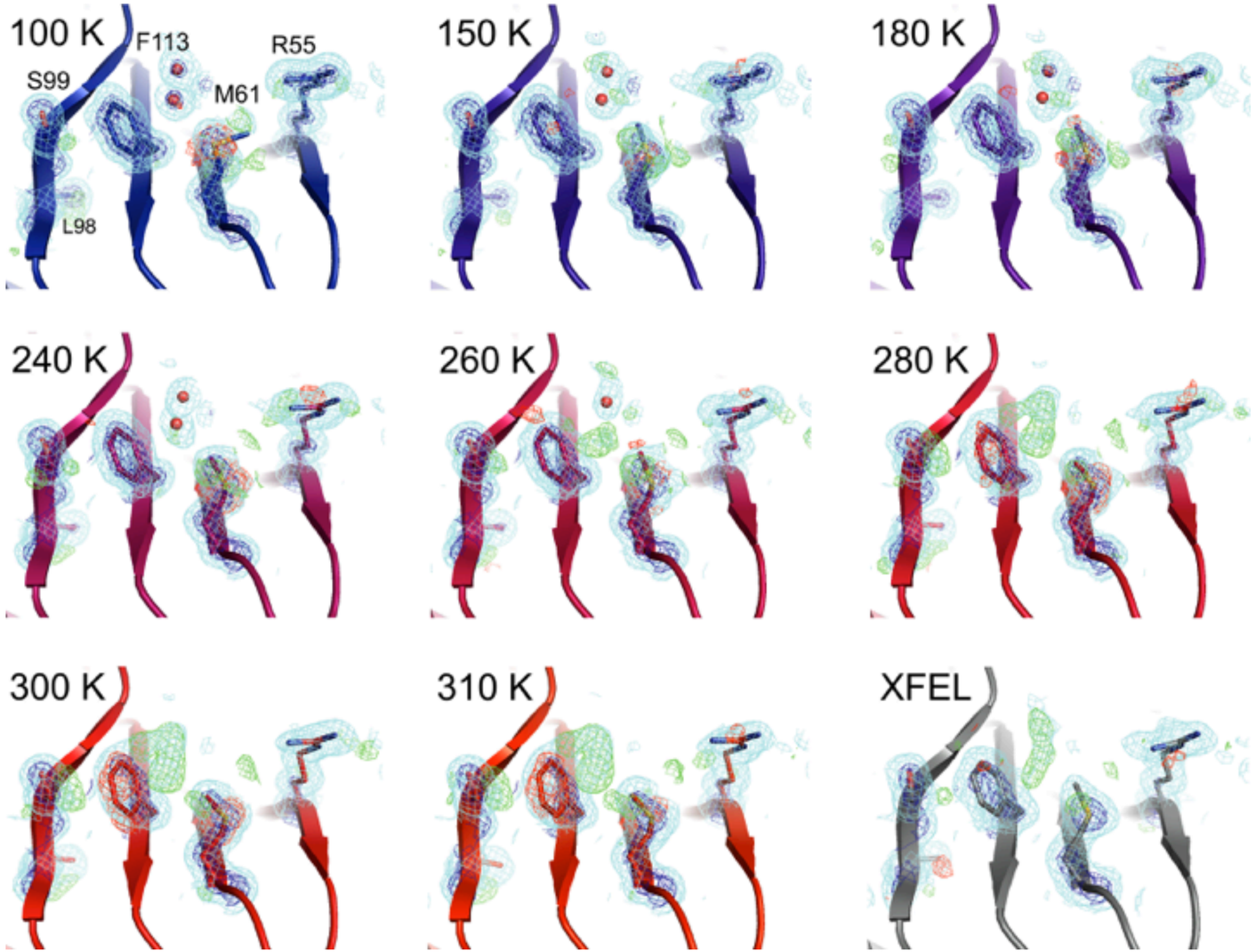
Temperature can **shift** the relative populations of confirmations in the crystal





Does this happen collectively?
How complex is the energy landscape?

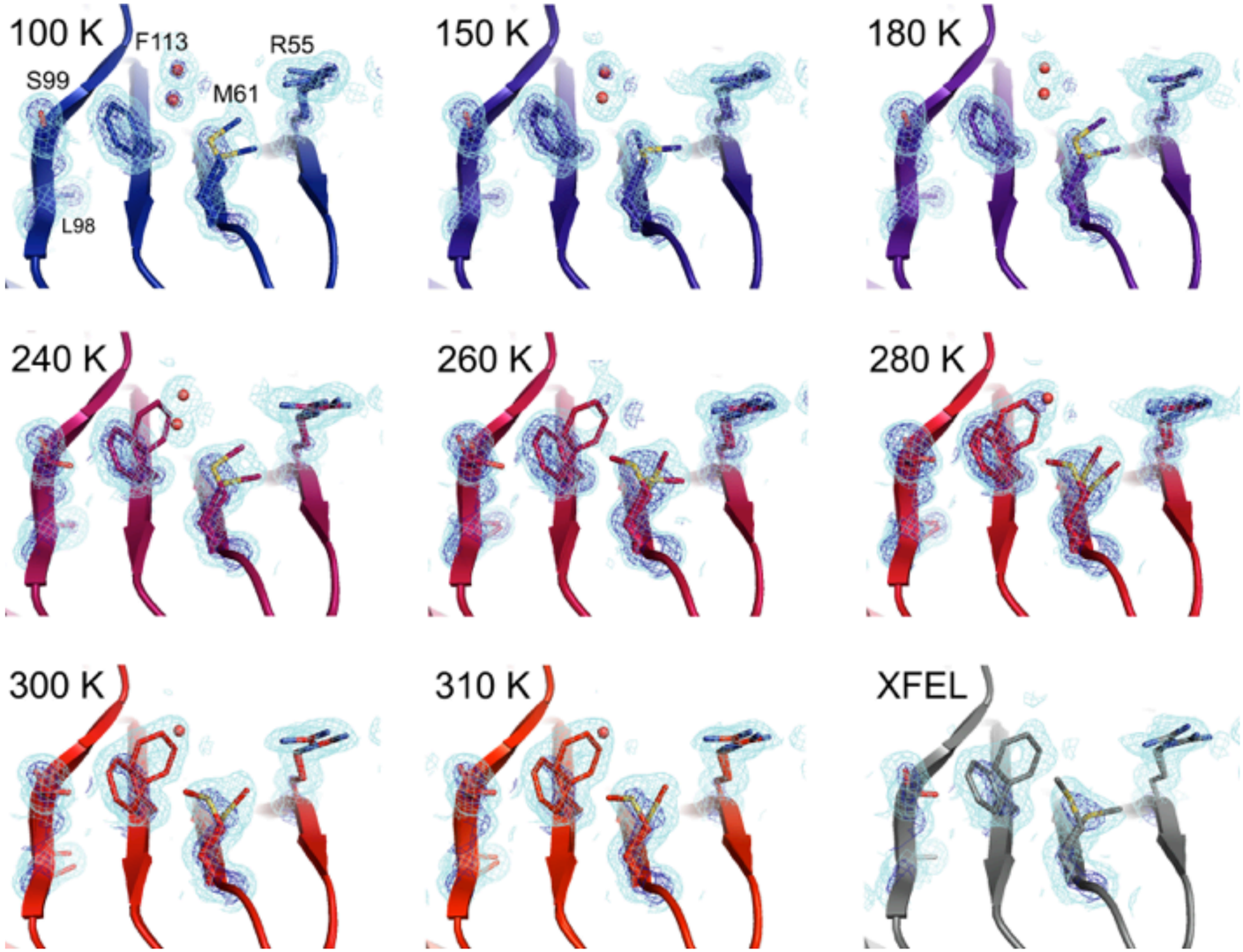
CypA conformational heterogeneity is sensitive to **temperature changes**



Daniel Keedy

Matt Warkentin,
Robert Thorne (Cornell)

CypA conformational heterogeneity is sensitive to **temperature changes**



Daniel Keedy

Matt Warkentin,
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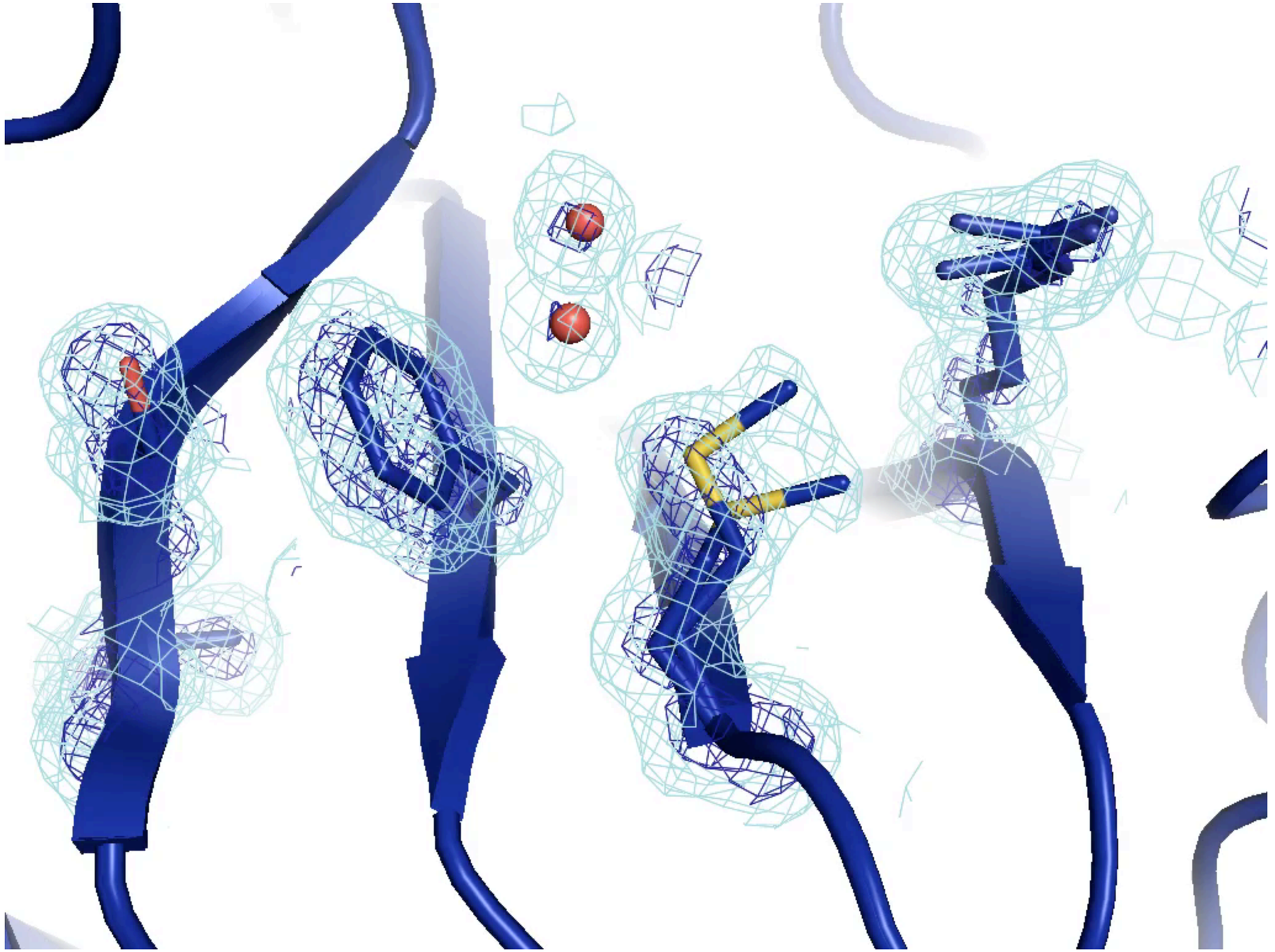
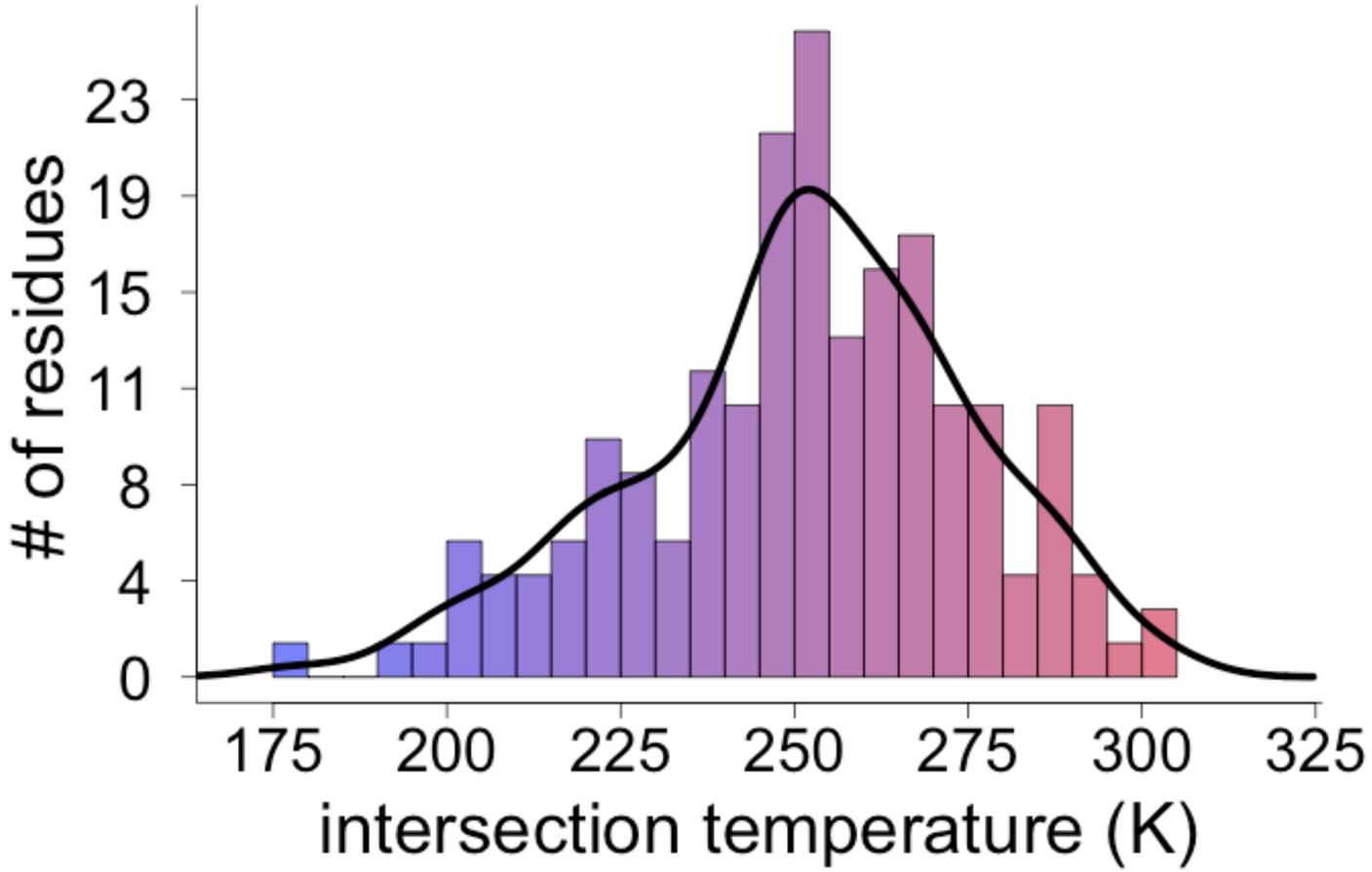
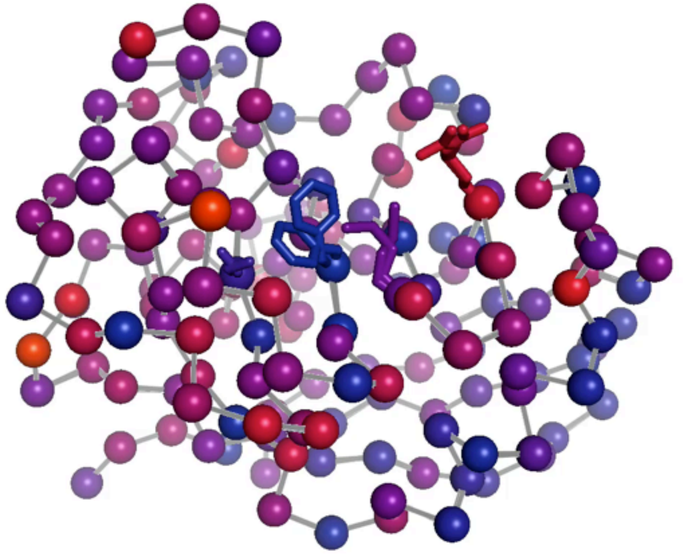


Different regions of the protein have their disorder arrested at **different temperatures**

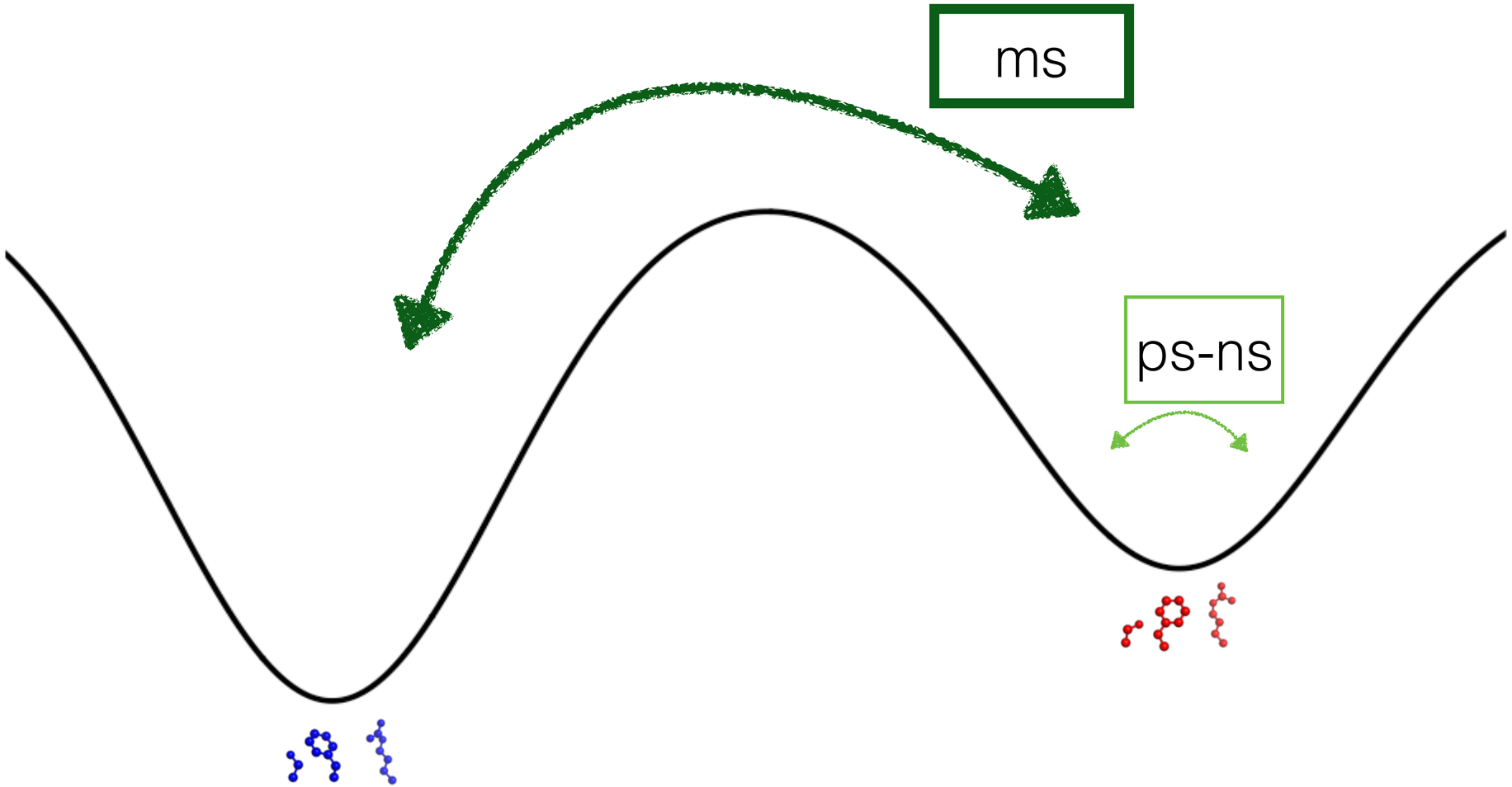




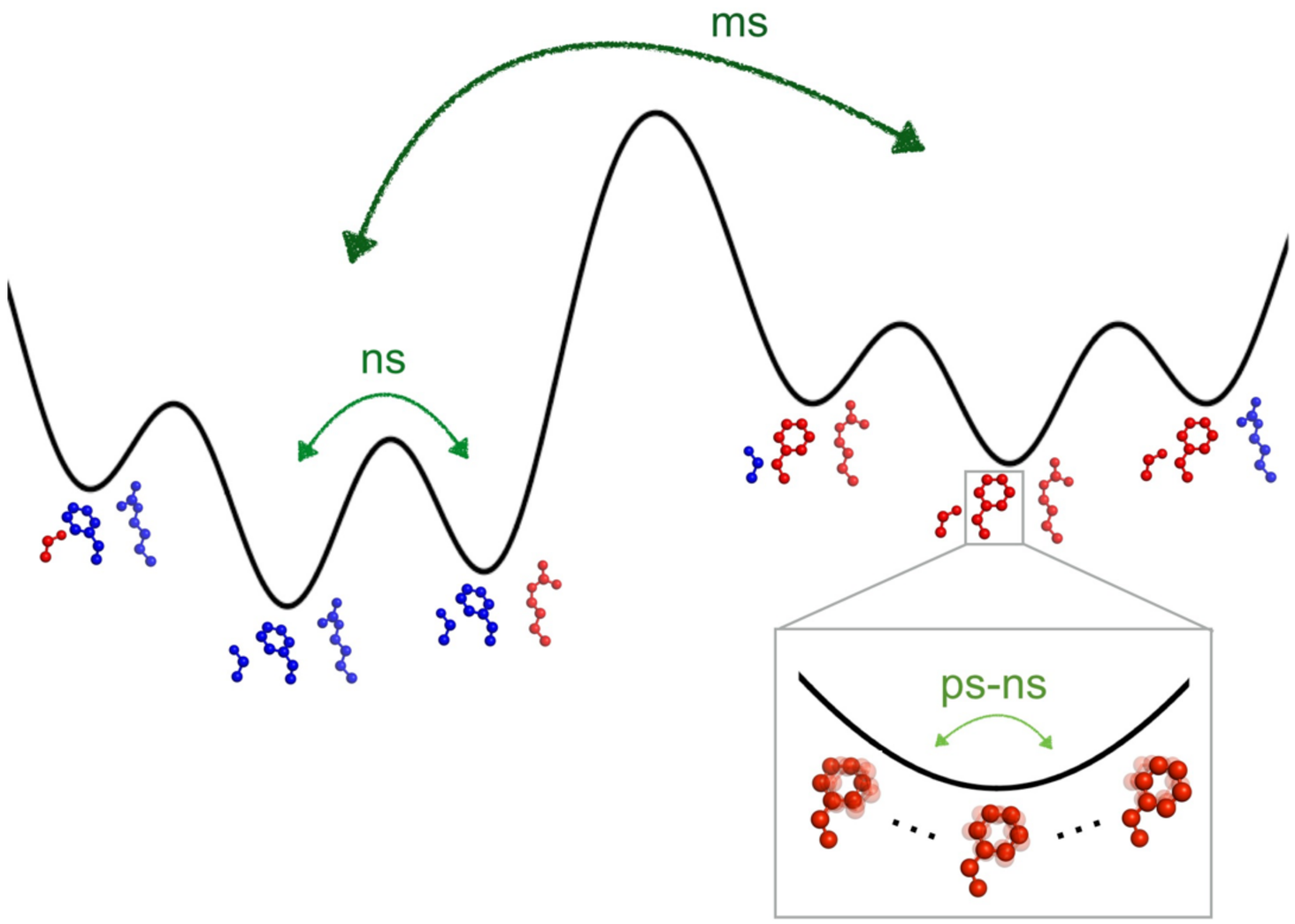
(one reason why it has been challenging for us to **extrapolate** from 100K to 293K X-ray Data)

Is the **response to temperature** correlated across the CypA “dynamic network”?

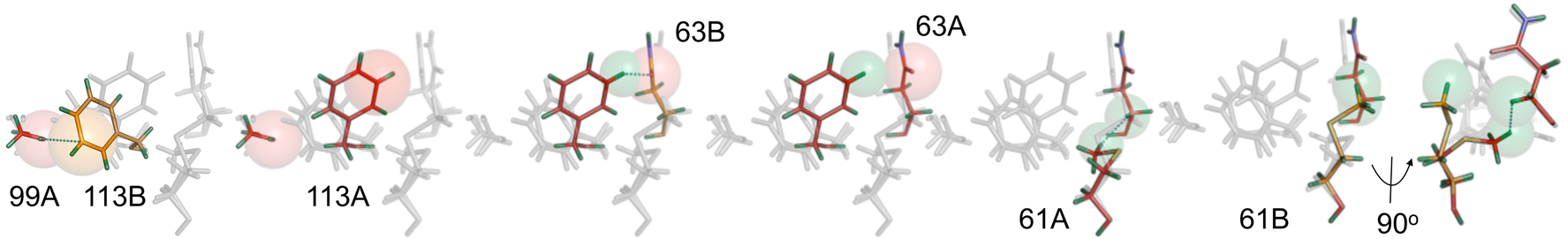
CypA temperature response does not favor a single **coupled transition**

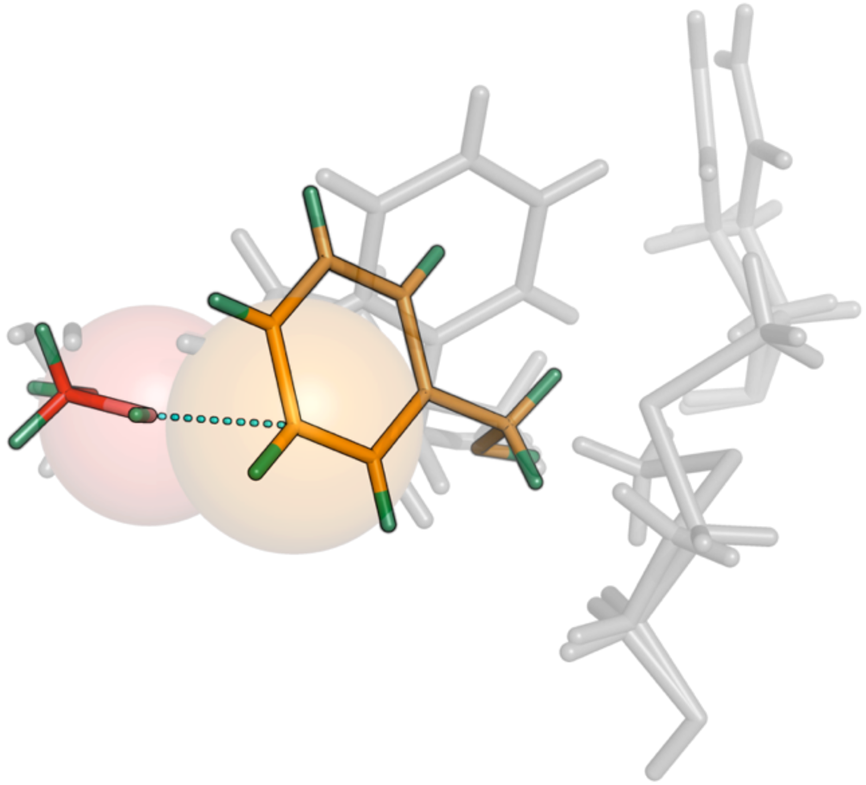


NMR relaxation dispersion experiments may sense **population shuffle** upon Phe113 rotameric switch

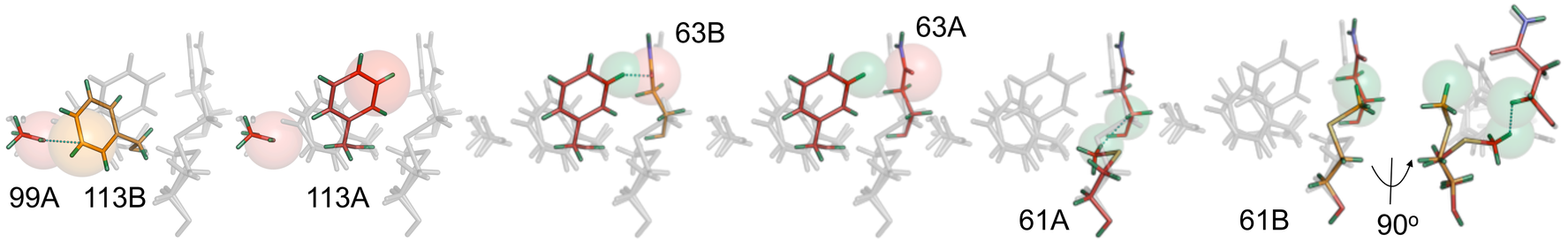


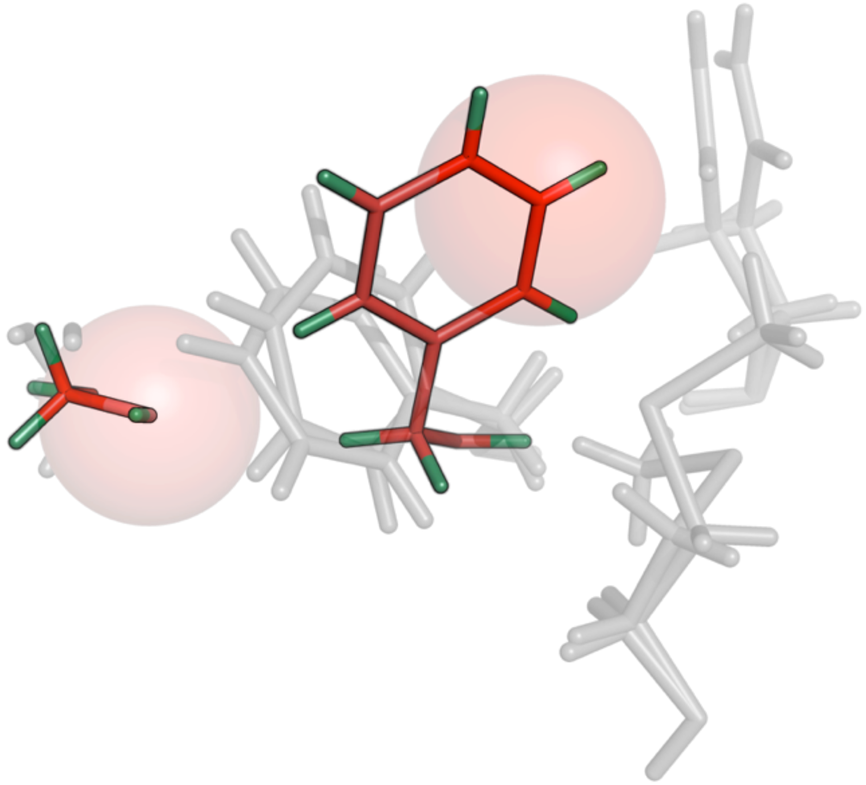
Population shuffle: alternative conformations do not segregate neatly into A/B - through “clash-relief” pathways



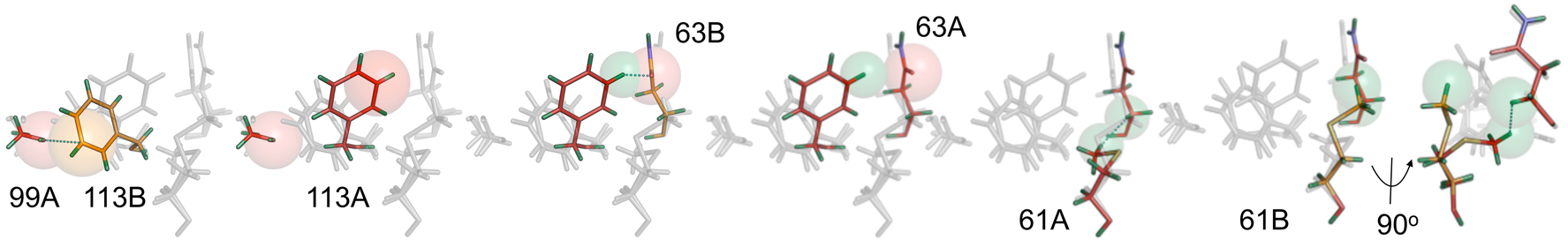


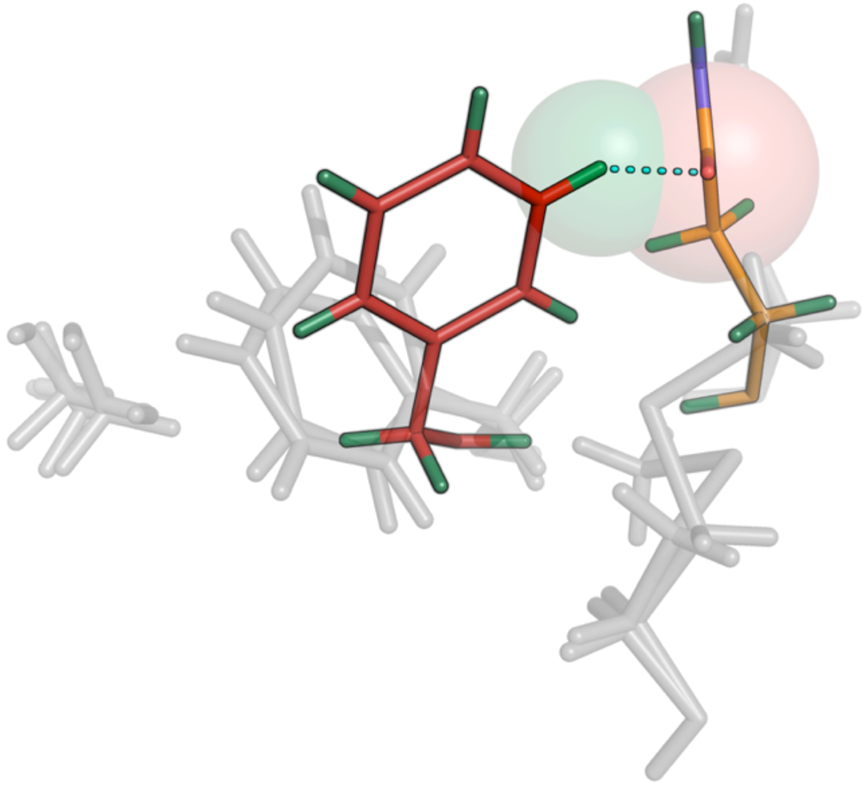
Residues connect through “clash-relief” pathways



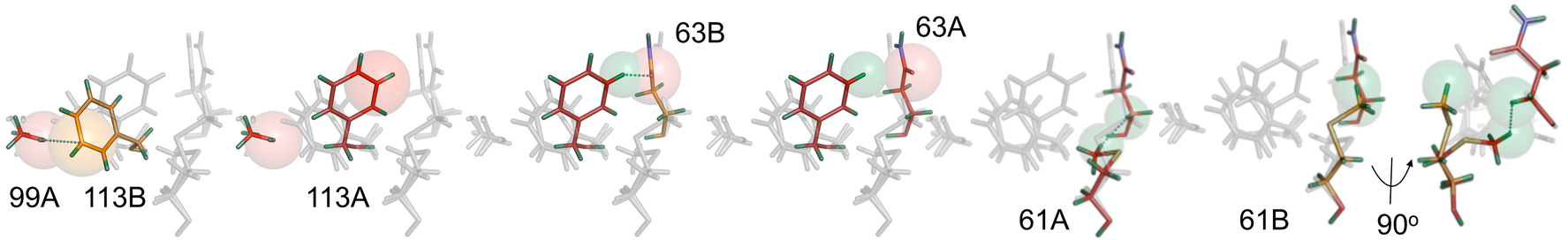


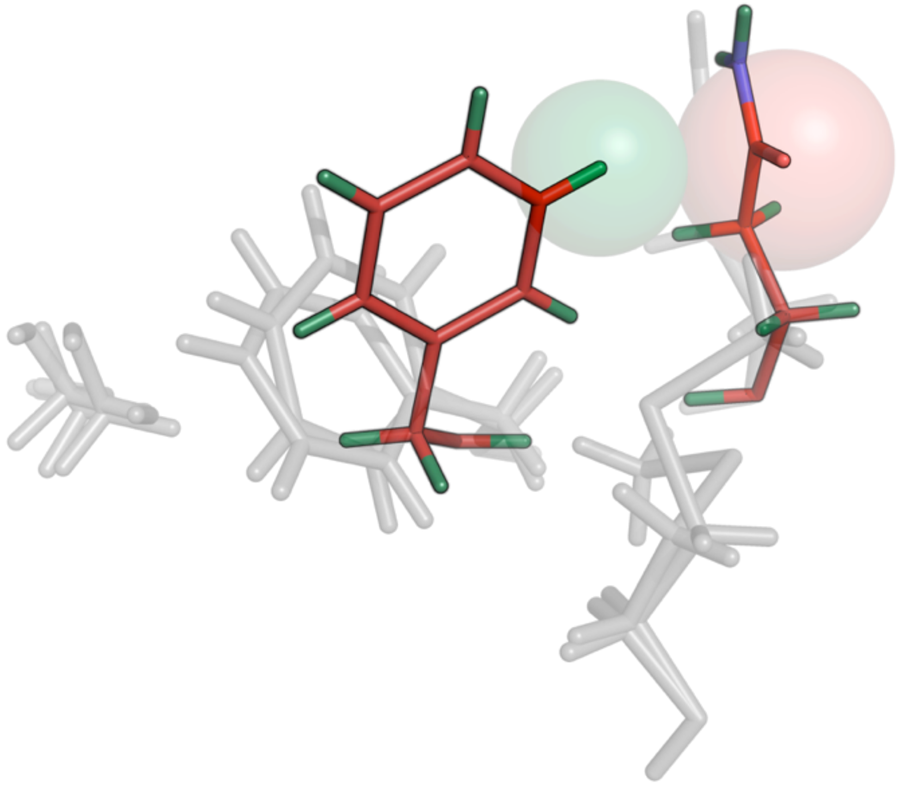
Residues connect through “clash-relief” pathways





Residues connect through “clash-relief” pathways





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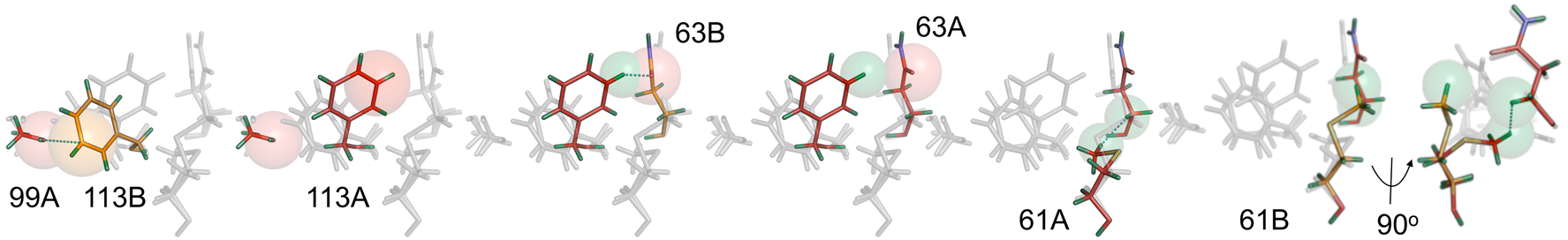
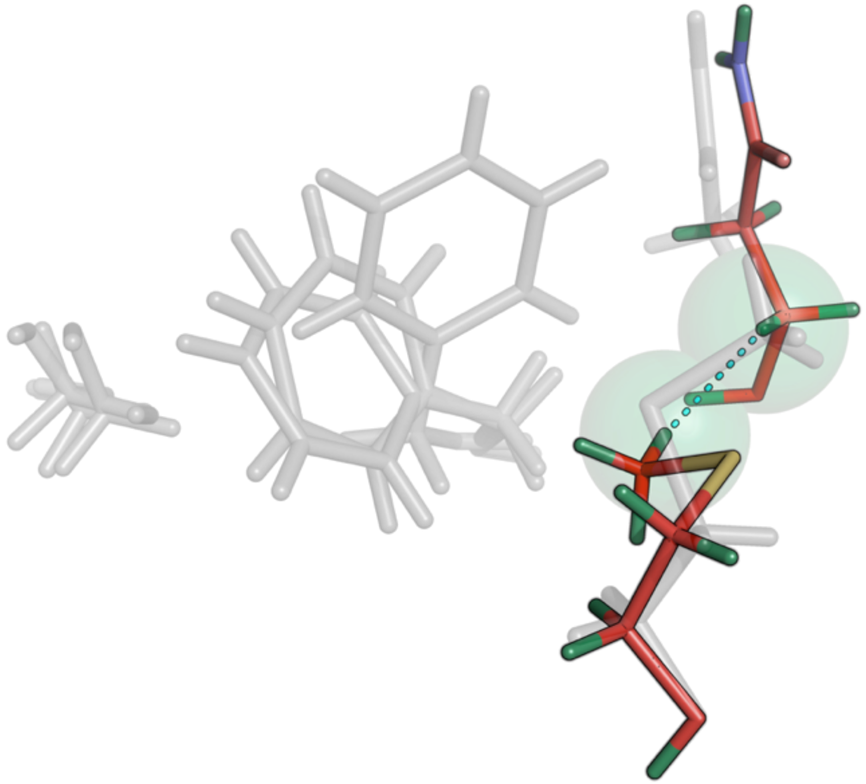
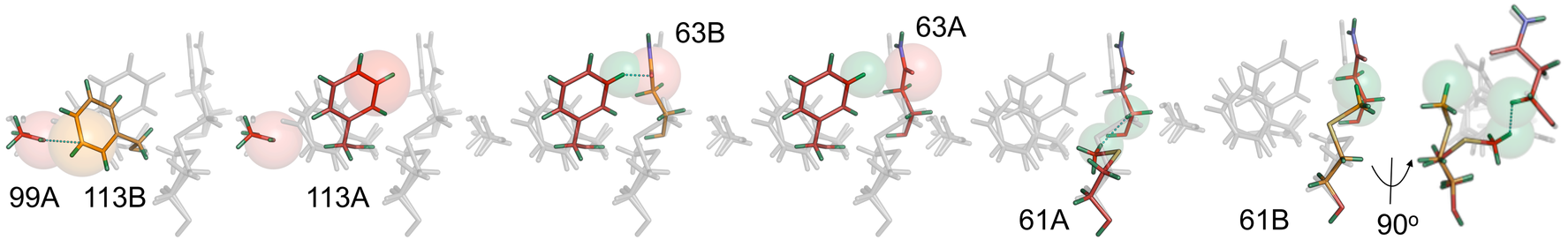
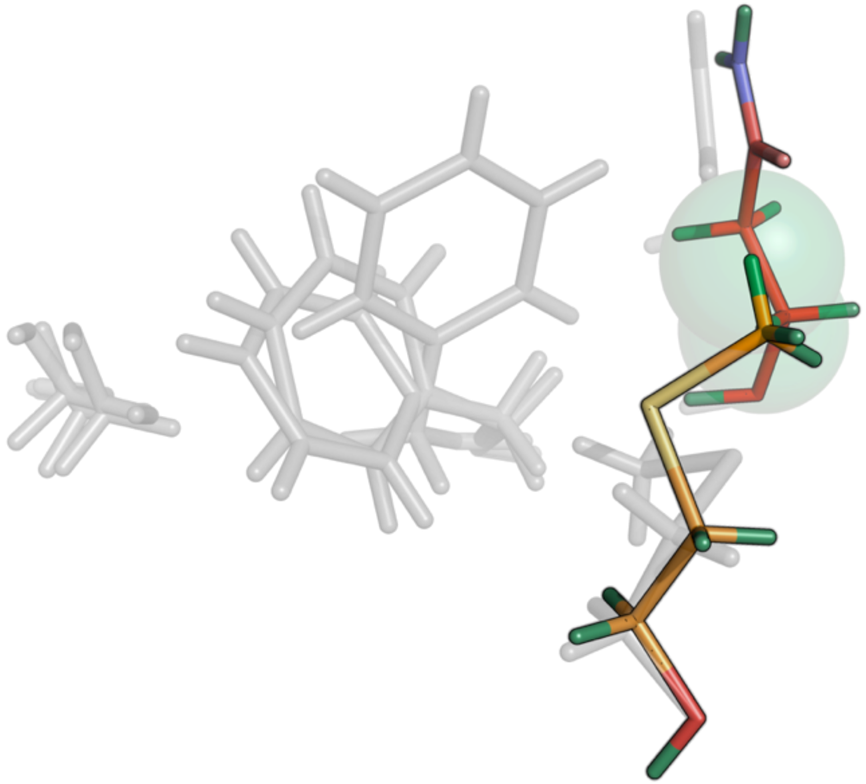


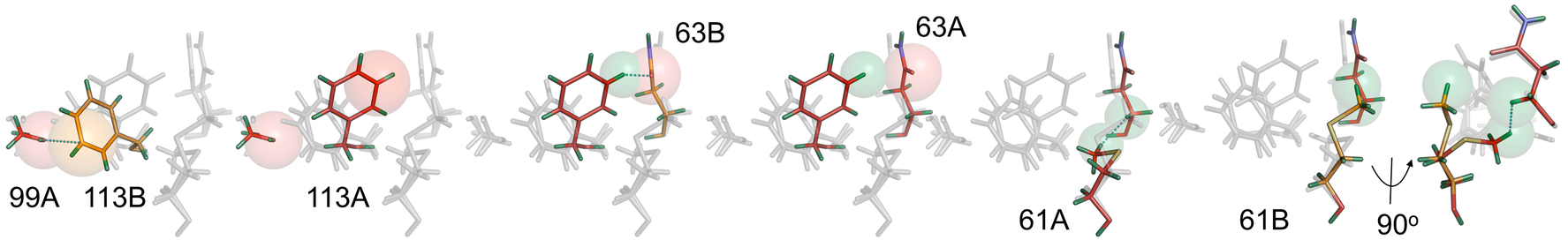


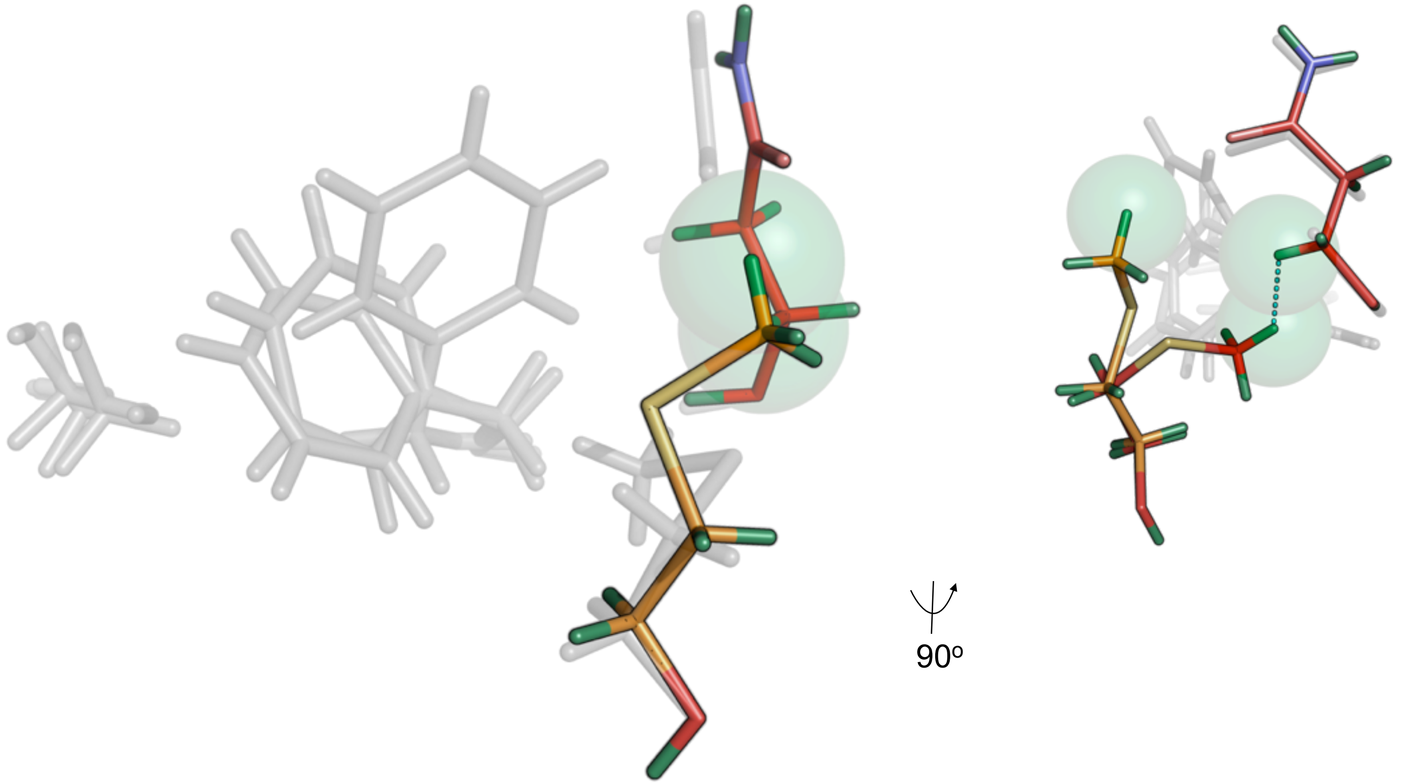
Residues connect through “clash-relief” pathways



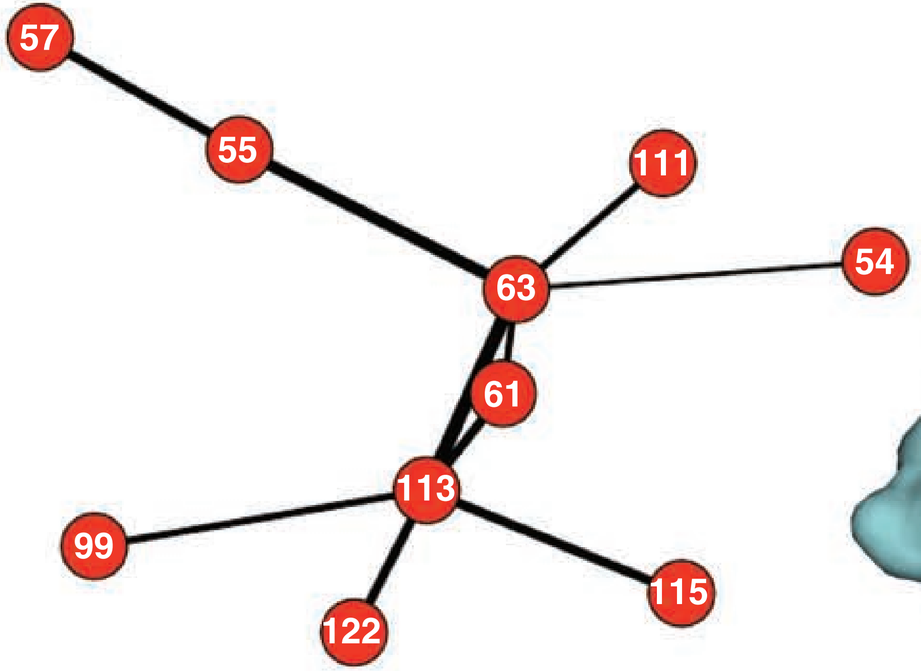


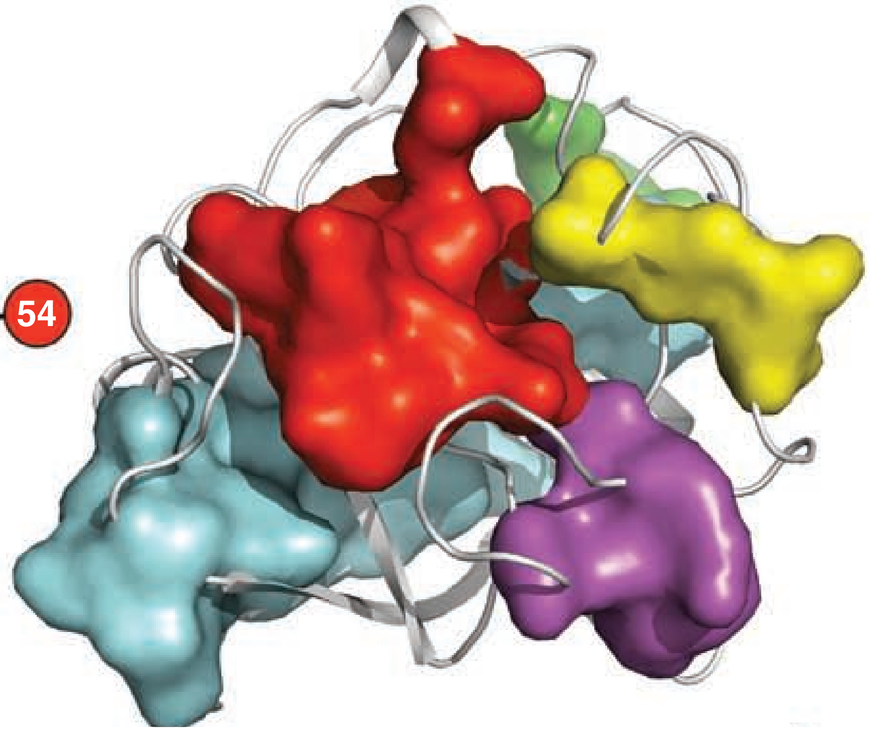
Residues connect through “clash-relief” pathways





Residues linked together in **CONTACT networks**





Henry van den Bedem
(SLAC)

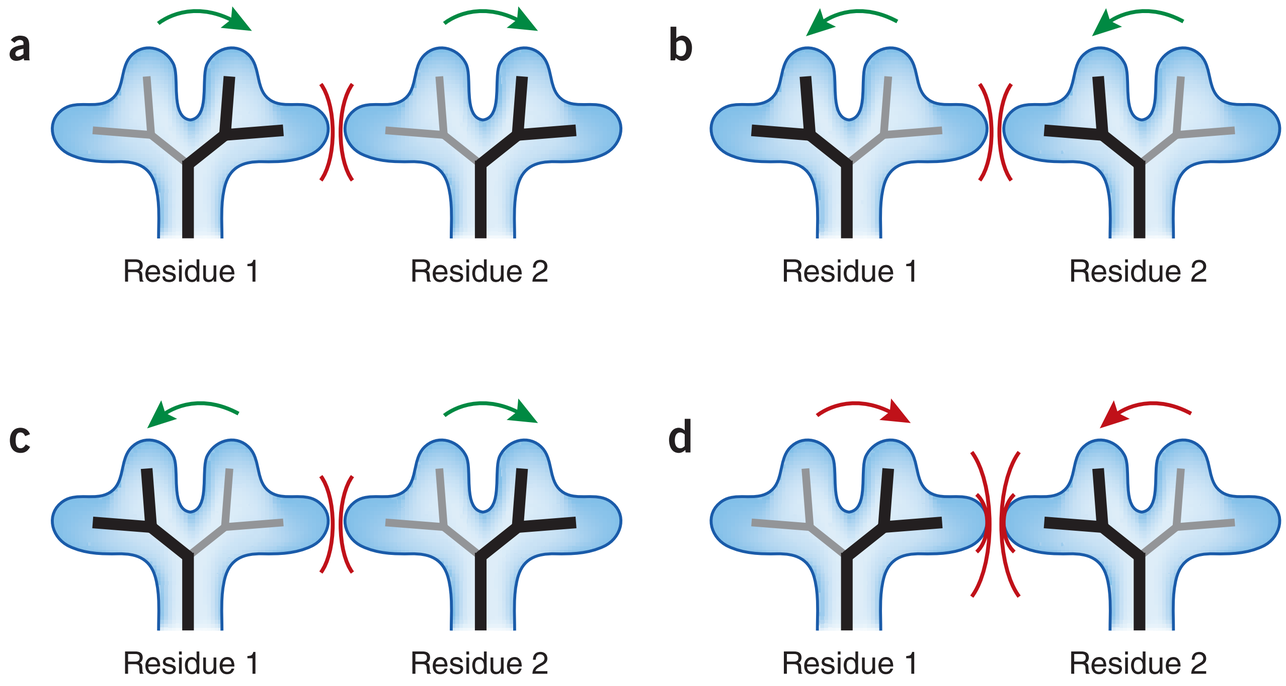
Sensitive to: ligands, mutations, temperature
Predictive of: chemical shift perturbations, collective motions

van den Bedem...Fraser, *Nature Methods*, 2013

Visualizing networks of mobility in proteins

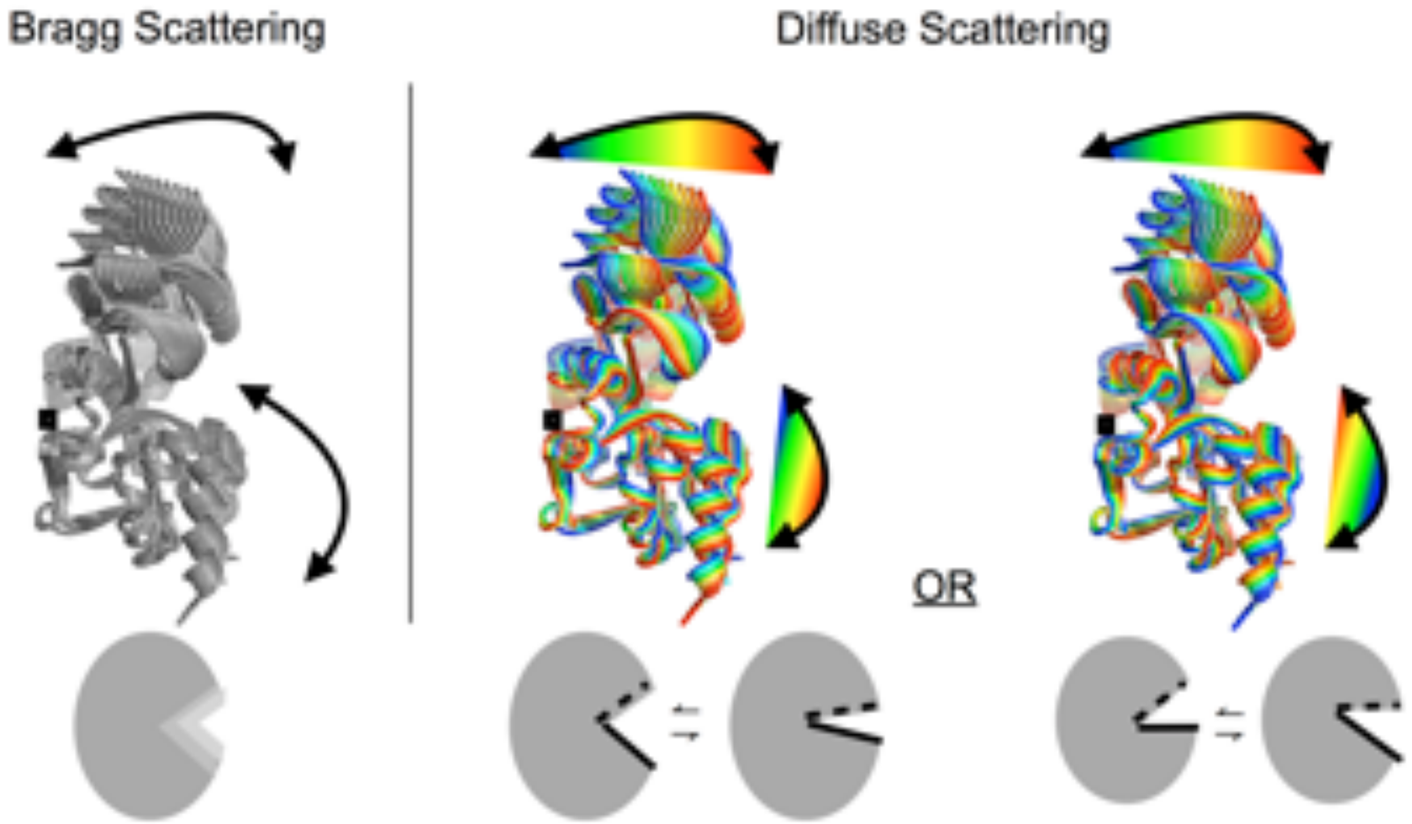
Mark A Wilson

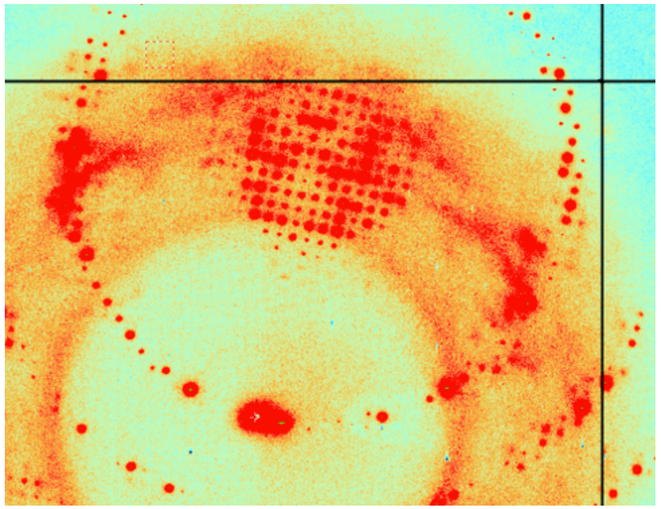
NATURE METHODS | VOL.10 NO.9 | SEPTEMBER 2013 | 835



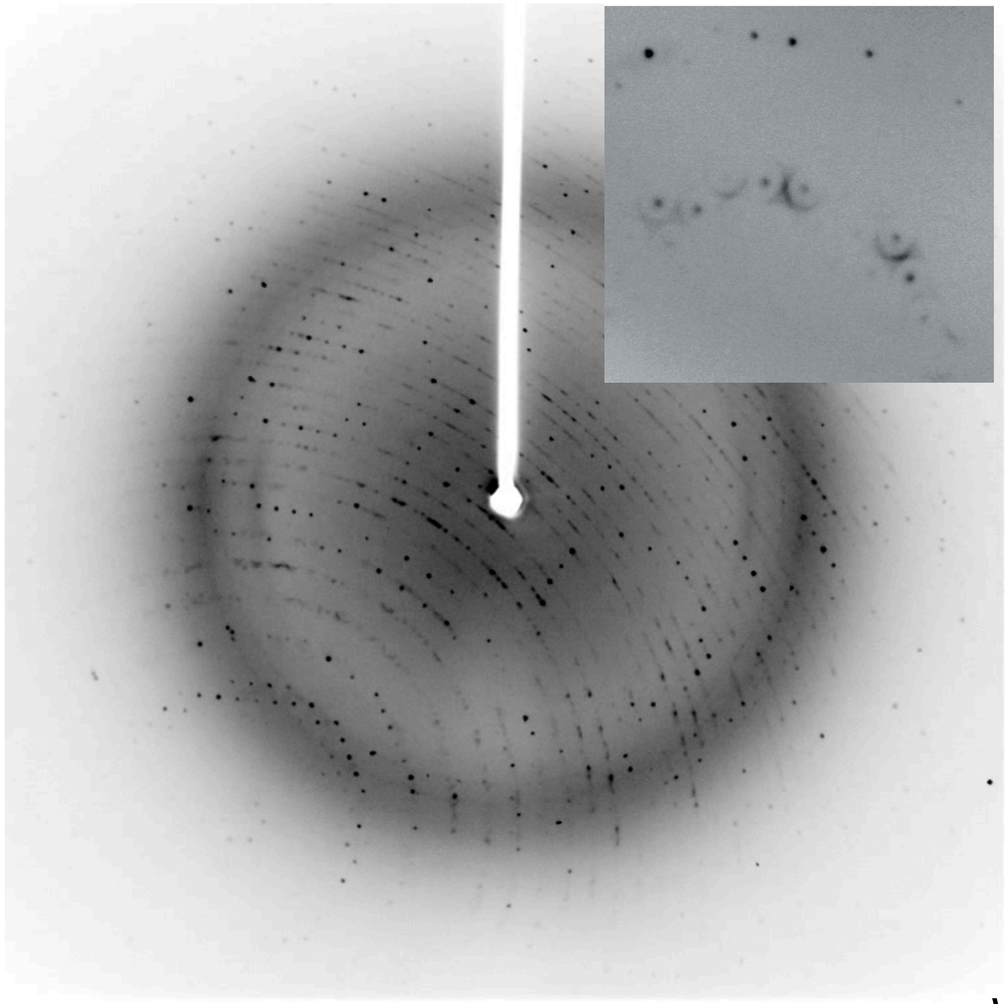
Happily for future methods developers, there is an opportunity to improve the modeling of mobility in crystallographic data. Out of necessity, van den Bedem *et al.*[1] used a stringent steric overlap criterion for determining which residues are correlated. However, more subtly correlated motions will be missed with this approach and are difficult to experimentally characterize. A possible solution to this is the use of X-ray diffuse scattering data, which contain information about all correlated motions in the crystal. The value of diffuse scattering data has been known for decades[8,9], but, owing to multiple technical challenges, these data are still routinely discarded[10]. Future improvements in this direction will allow the full extent of X-ray diffraction from crystals to be collected and analyzed, thus providing a fully integrated view of the connection between protein structure and correlated motions.

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





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



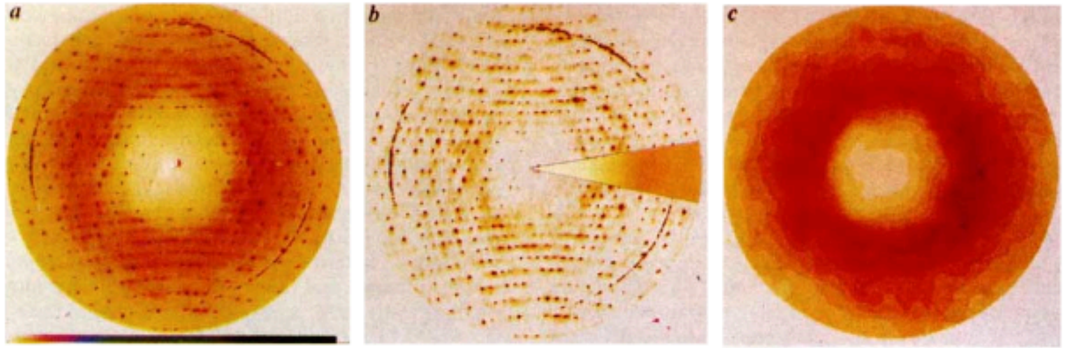


Fig. 1 X-ray diffraction data from insulin. *a*, A 40-h exposure of a stationary crystal (3-fold axis nearly vertical) recorded with focused, monochromatic Cu K_{α_1} radiation from an Elliott rotating anode. The data (corrected for camera background and polarization) are displayed out to radius $R = 0.45$ Å^{-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× 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

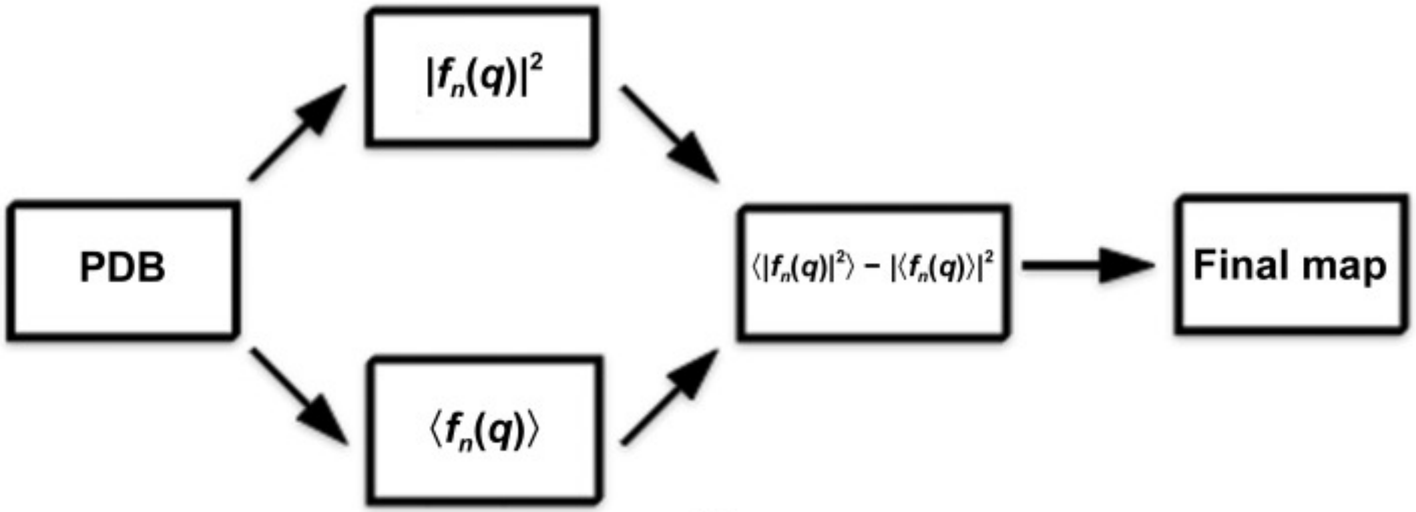
Given an ensemble, **Guinier's equation** relates large scale features (along hkl) to conformations

$$I(q)_{\text{diffuse}} = N \cdot [\langle |f_n(q)|^2 \rangle - |\langle f_n(q) \rangle|^2]$$

No. of unit cells in crystal

Complex structure factor of *n*th protein conformation unit cell

(*a*)



(*b*)

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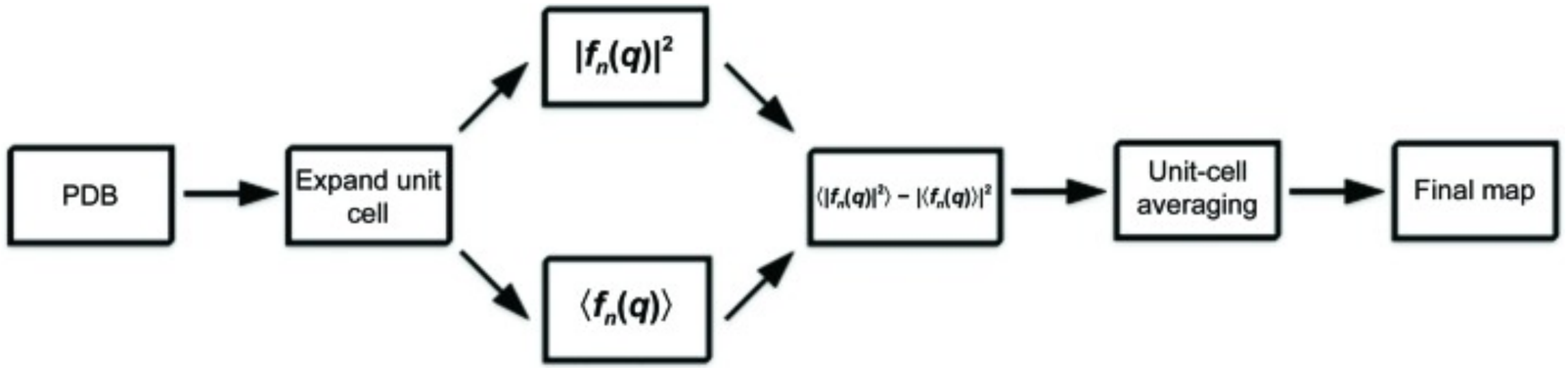
Van Benschoten…Fraser, Acta D, 2015

Given an ensemble, **Guinier's equation** relates large scale features (along hkl) to conformations



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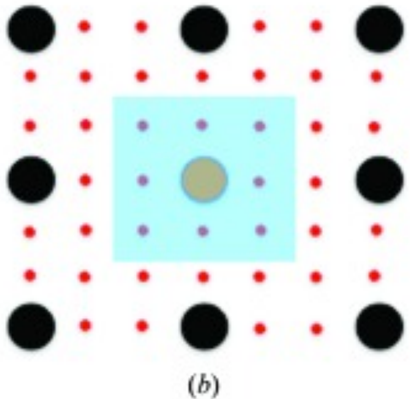
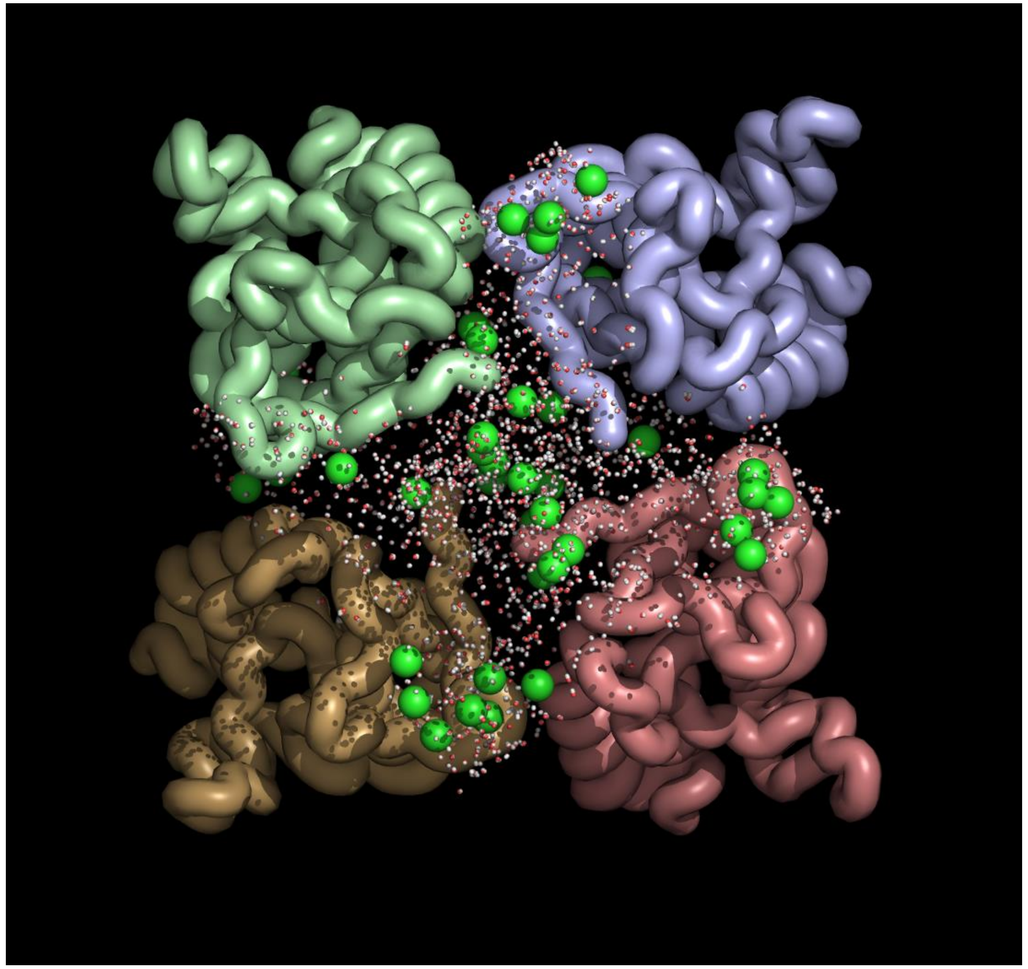
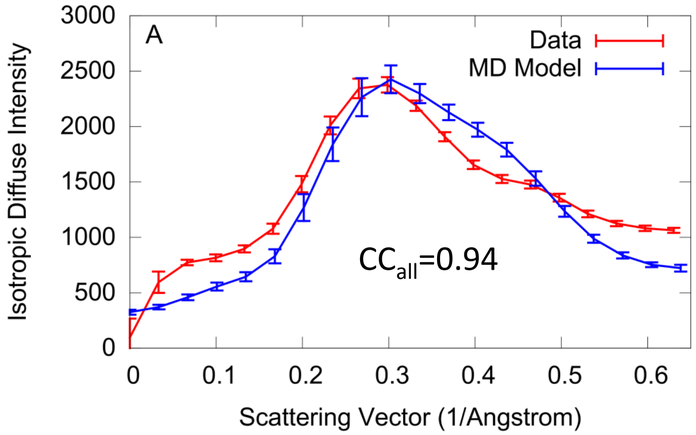
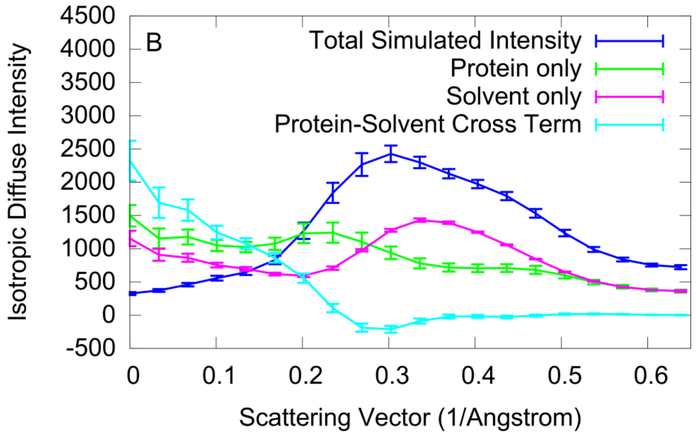


Van Benschoten…Fraser, Acta D, 2015

Long (microsecond) **molecular dynamics simulations** of crystals provide mechanisms for diffuse scattering

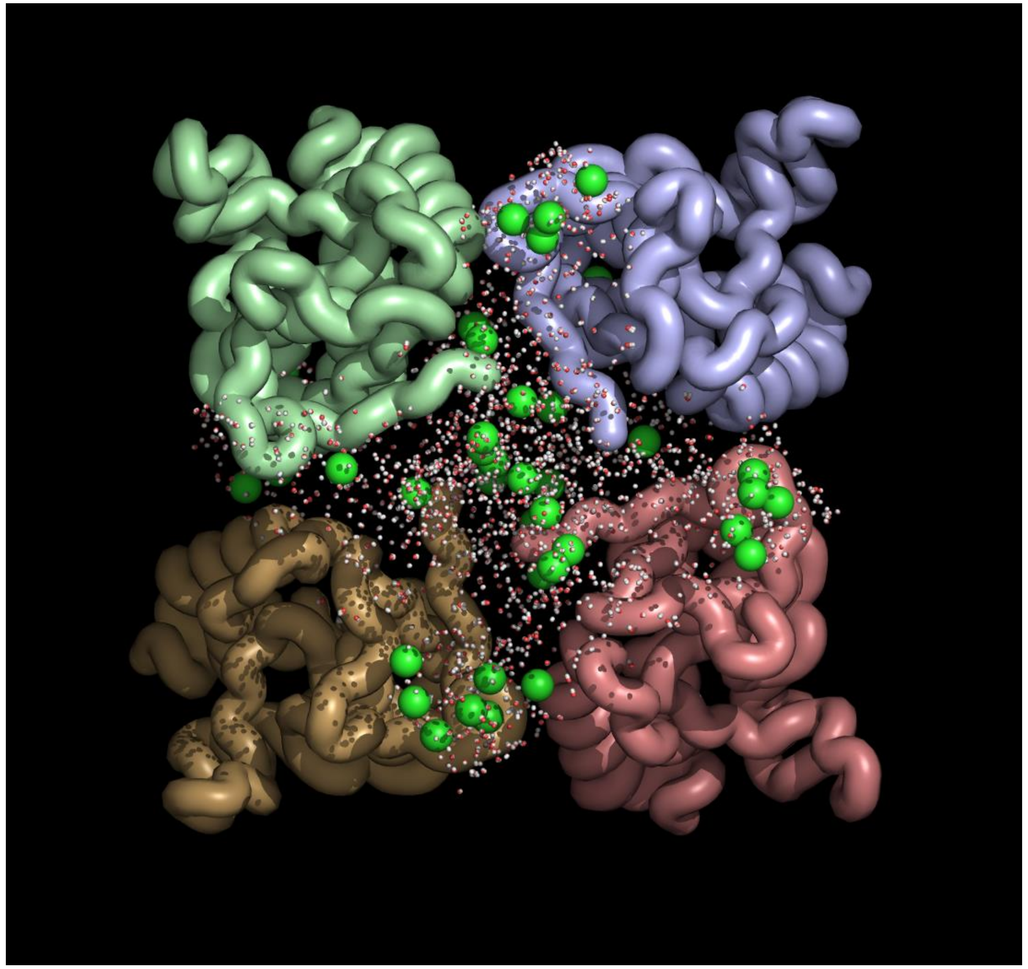


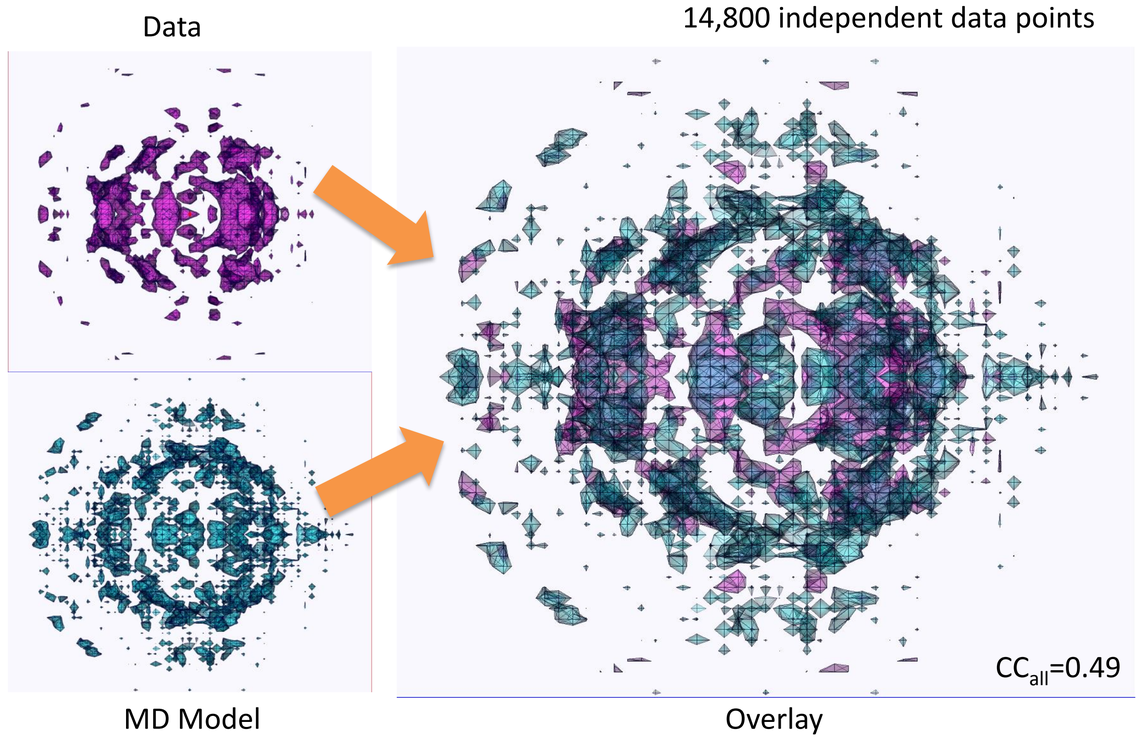




Wall et al, PNAS, 2014

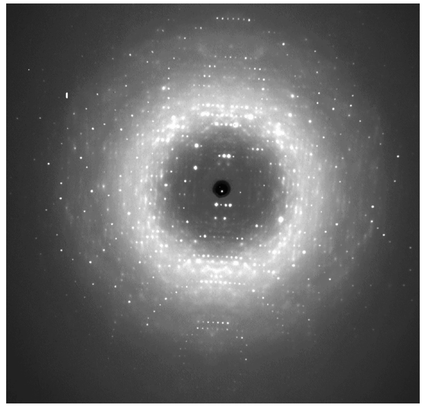
Agreement of **anisotropic features** is currently moderate, improvements needed for sampling and scoring?

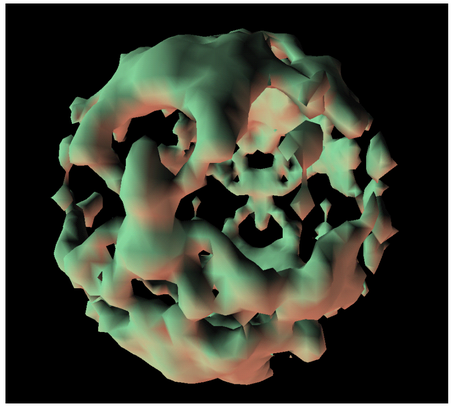




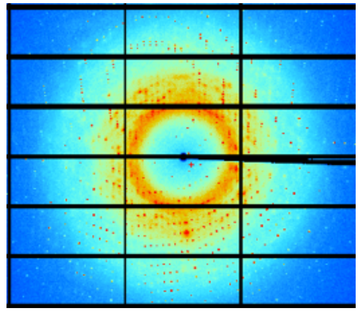
Wall et al, PNAS, 2014

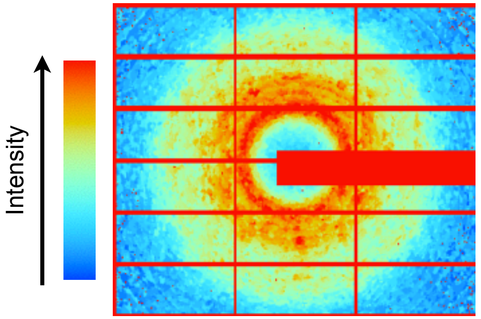
New data sets are needed to advance diffuse scattering





Wall, Ealick, and Gruner, PNAS 1997





Map	$CC_{Friedel}$	CC_{Sym}
CypA	0.9	0.7
Trypsin	0.95	0.69

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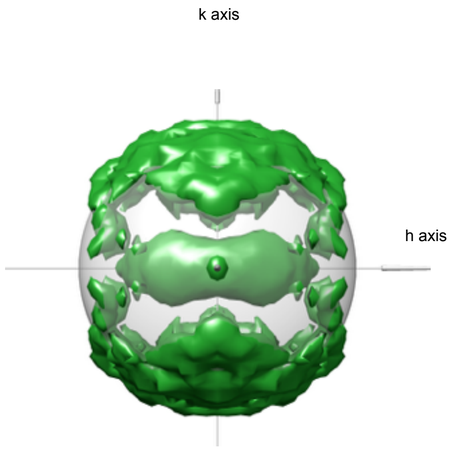
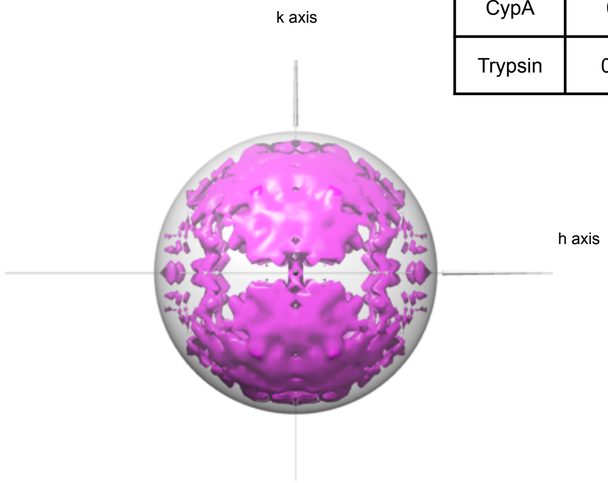
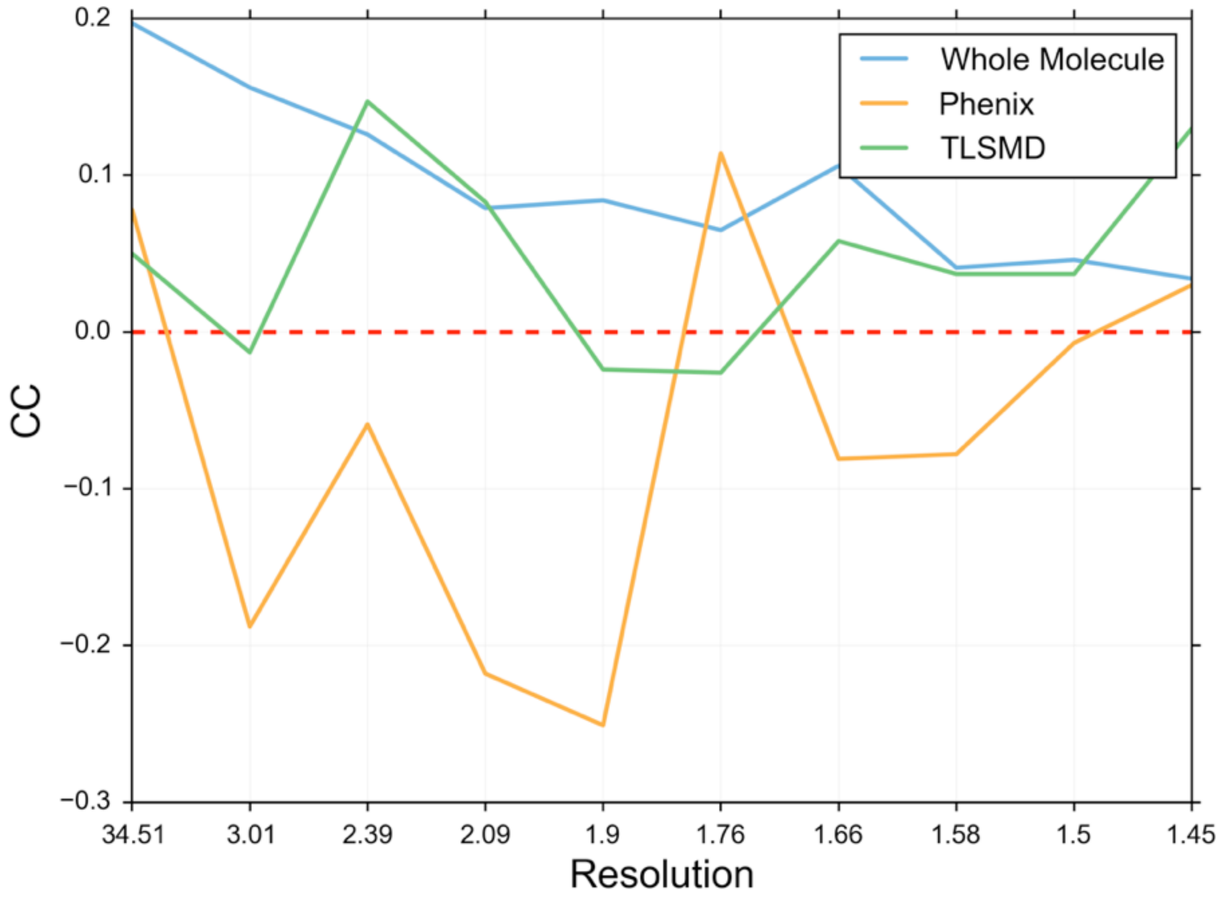
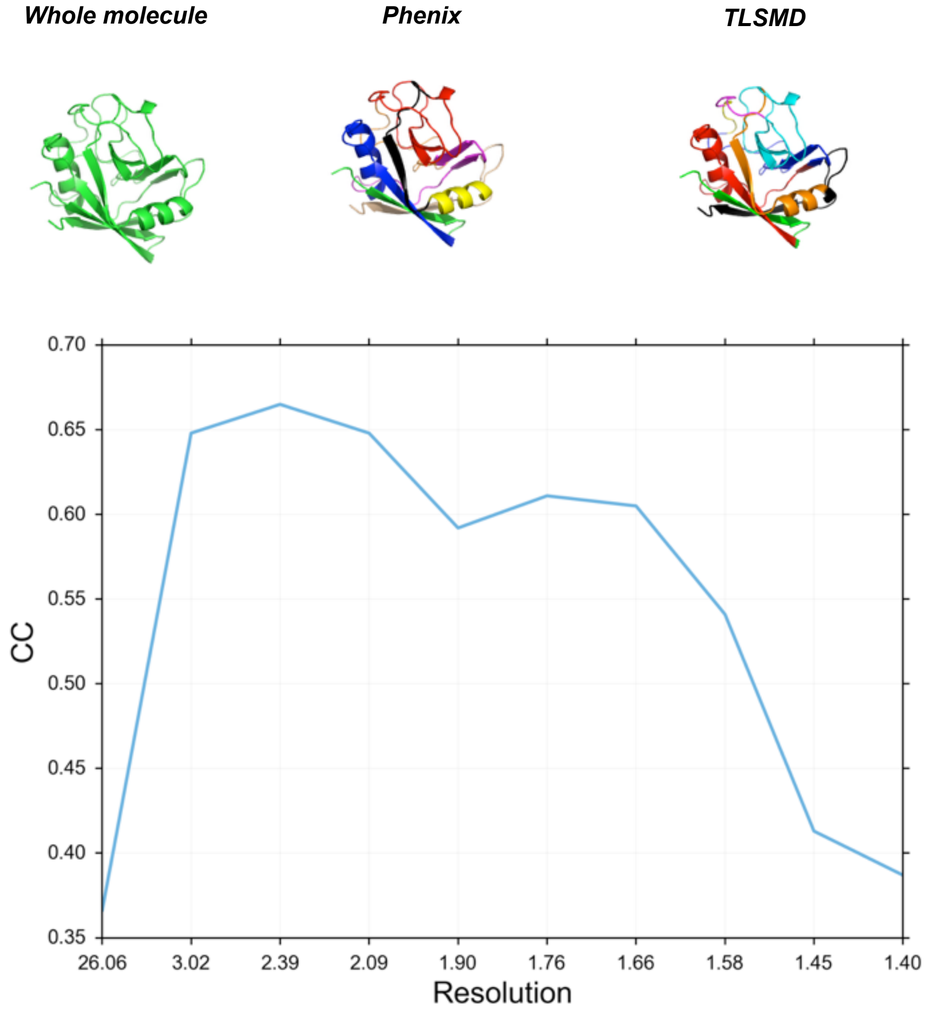




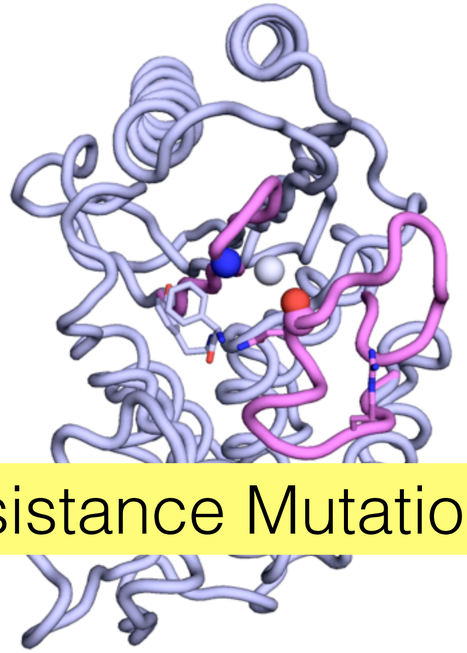
TLS models are poor at explaining diffuse intensities, but **liquid like motions** are better



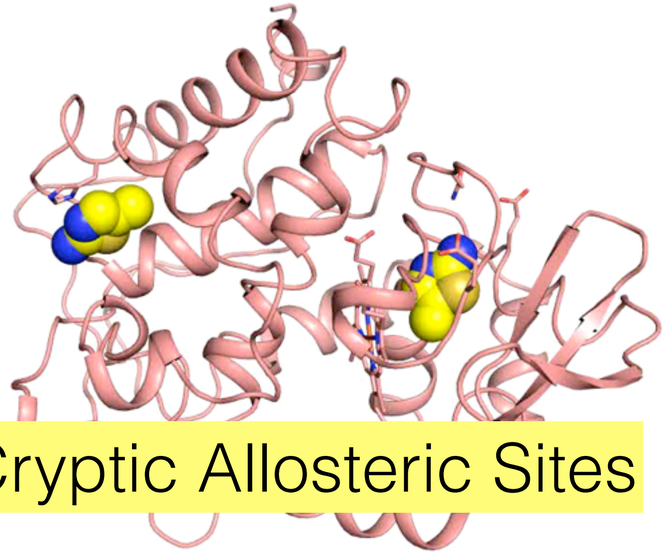


Conformational dynamics are at the core of **three critical problems in biology**

Protein Design



Resistance Mutations



Cryptic Allosteric Sites

Temperature perturbations expose the
coupled **protein conformations**
used by design, disease, and drugs

Saul Cortez

FRASER LAB

Jennifer Li

David Mavor | Dr. Lin Liu | **Dr. Daniel Keedy** | Ben Barad | Justin Biel | **Rahel Woldeyes** | **Dr. Mike Thompson** | Erin Poss | Alex Wolff | Pooja Suresh

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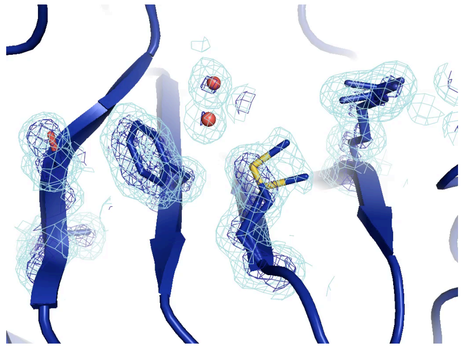

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