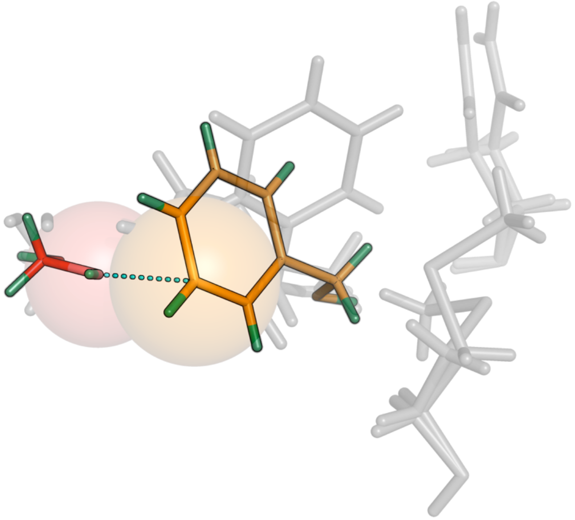
Something's Got to Give:

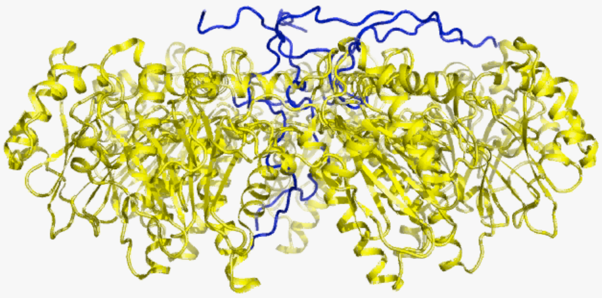
Contact Networks in Multiconformer Protein Structures



James Fraser
Department of Bioengineering and Therapeutic Sciences, QB3, UCSF

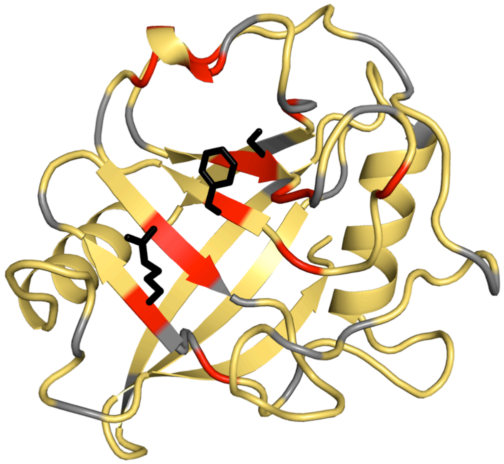
Protein functions are exerted by ensembles

Proteosome



DHFR

CypA



Kay Group

Wright Group

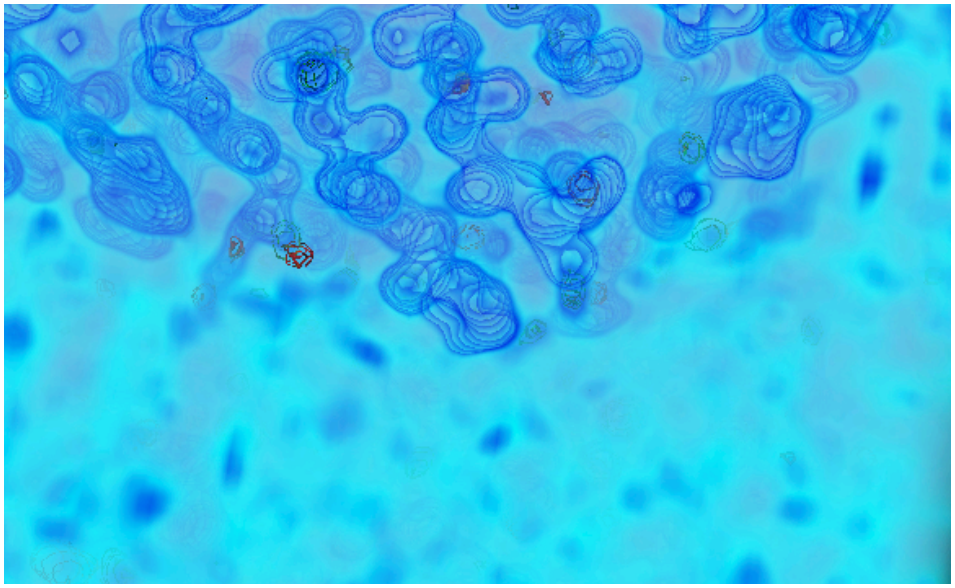
Kern Group

What is the structural basis for chemical shift perturbations?

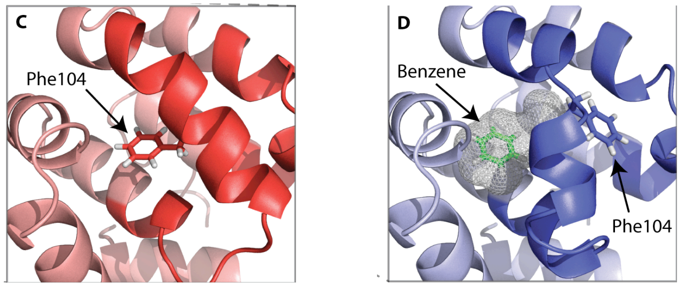
What is the “allosteric”

What is the structural basis for protein dynamics and how do they relate to catalysis?

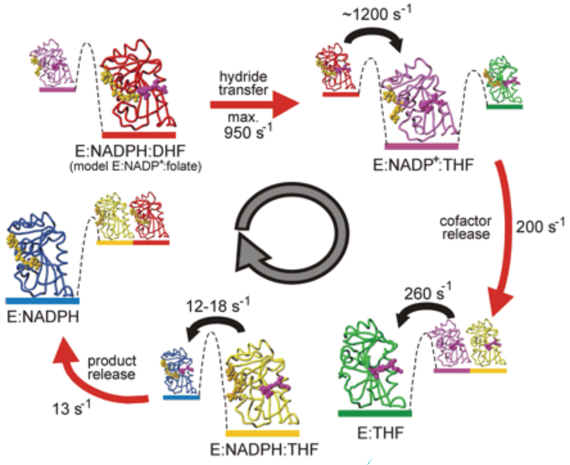
How can we discover the "structures" of alternative states?



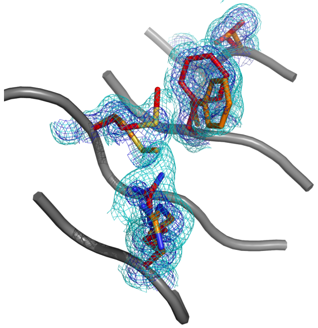
Computation, X-ray structure comparisons, and multiconformer X-ray models generate hypotheses about alternative structures



Rosetta CS guided fragment placement
(Bouvignies, Vallurupalli...Baker, Kay,
Nature 2011)



DHFR structures with different ligands
(Boehr...Wright, *Science* 2006)

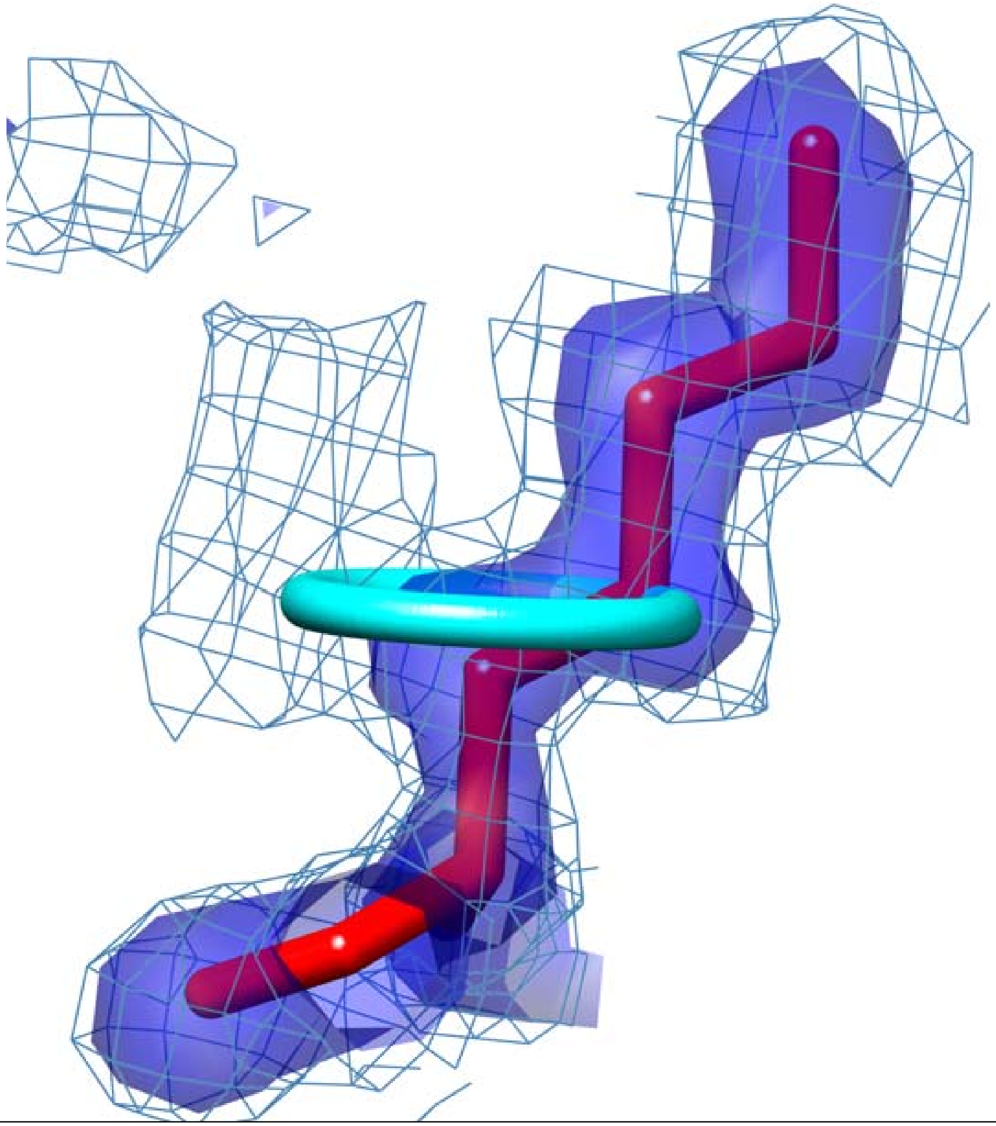


Electron density from a single X-ray dataset
(Fraser...Alber, *Nature* 2009)

↓

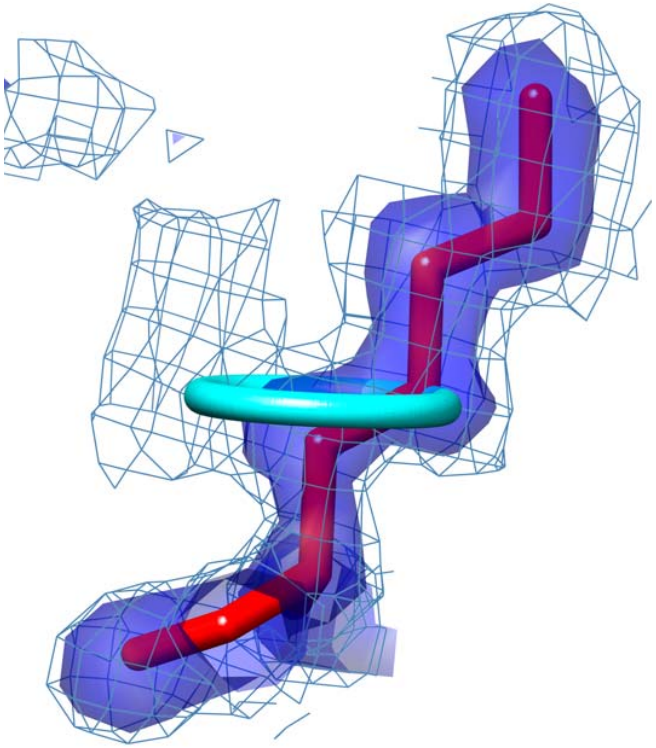
Multiconformer Contact Networks

X-ray crystallography is an ensemble experiment

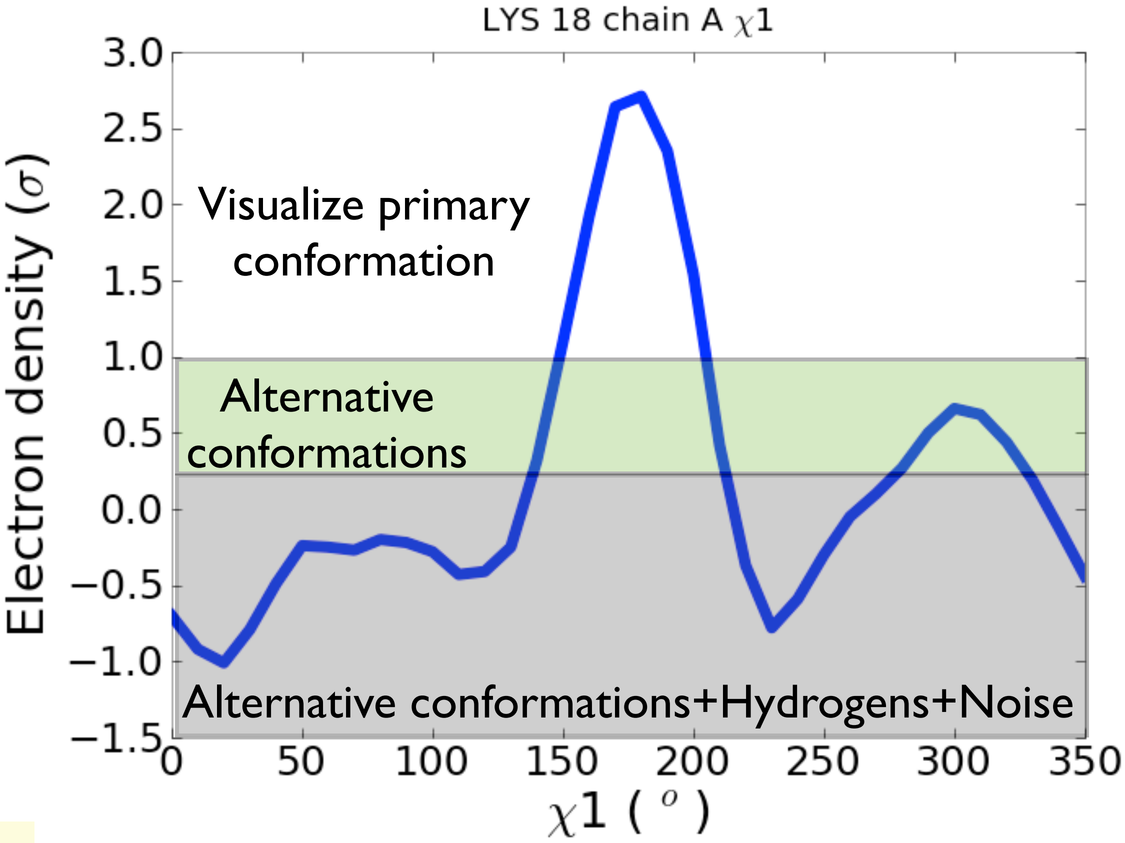


0.3 and 1σ

Ringer is an assay for electron density



0.3 and 1σ

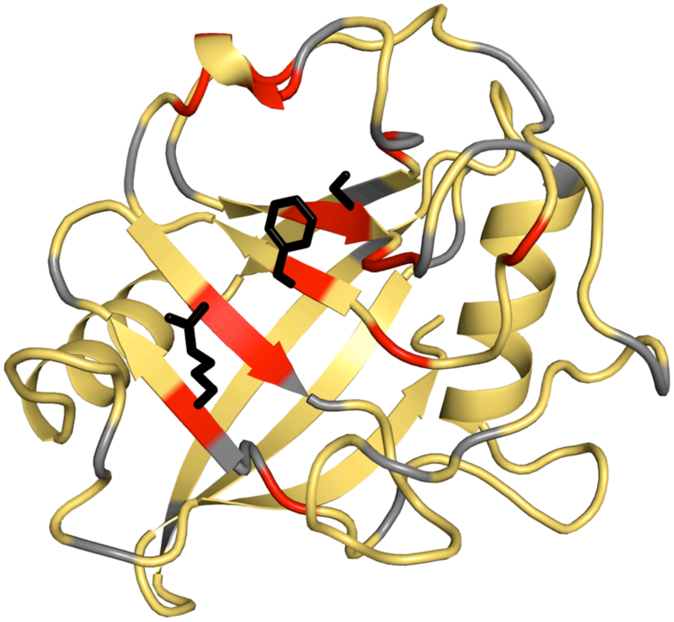


20-40% of residues are (unmodeled) polysteric

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

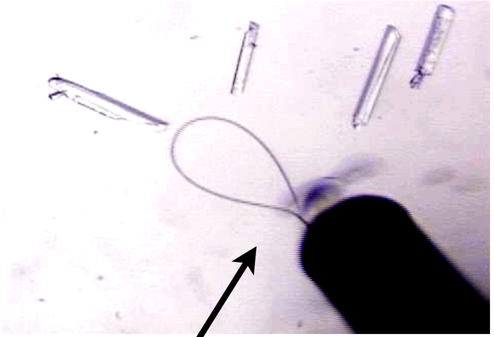
What are the alternative conformations of CypA?

Dorothee Kern

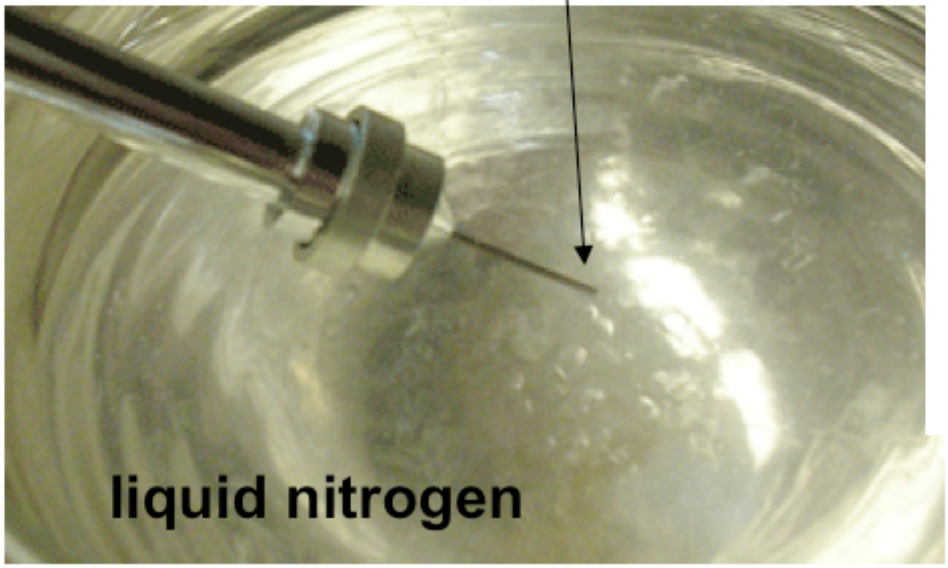


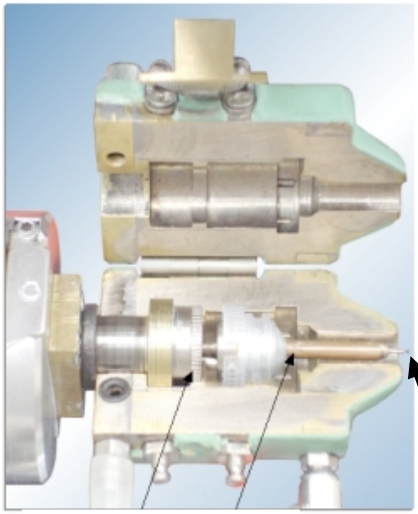
X-ray crystallography is normally done at low temperature

~100K



loop

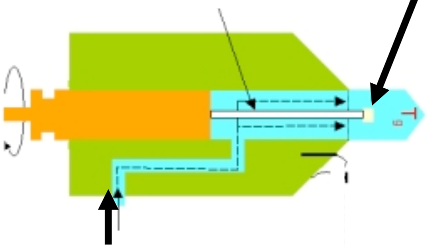




“off label” use of
Proteros FMS
BL12.3.1@ALS

288K

loop



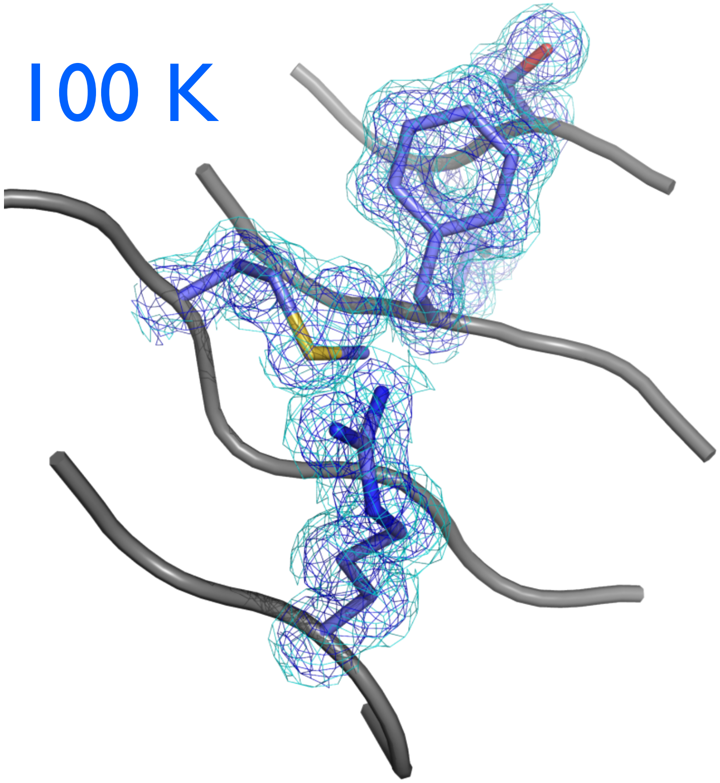
Humid
air stream

MiTeGeN

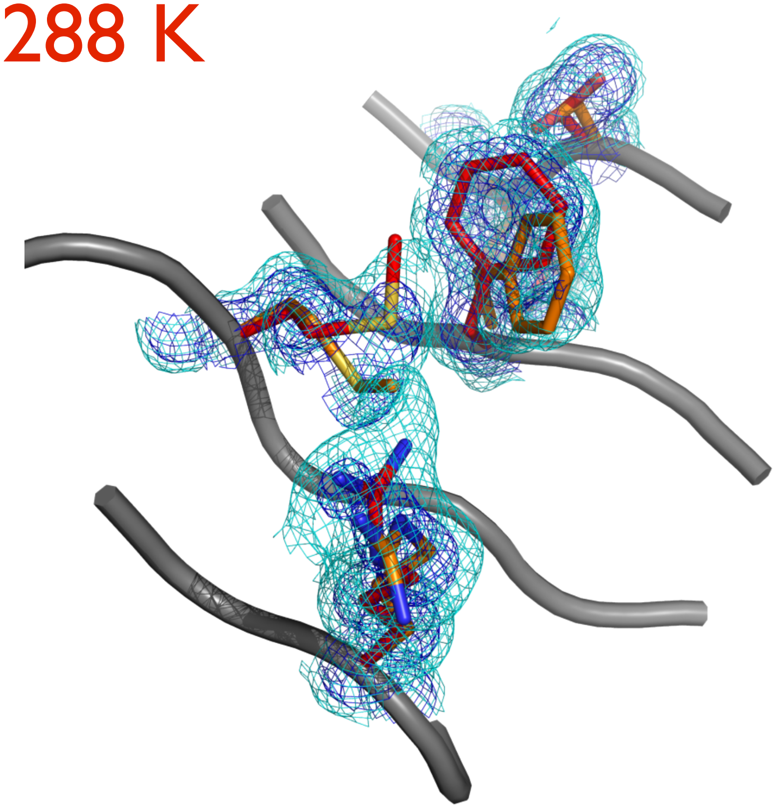
Coupled side chain motions at RT explain NMR relaxation signal

Major <=> Minor

100 K

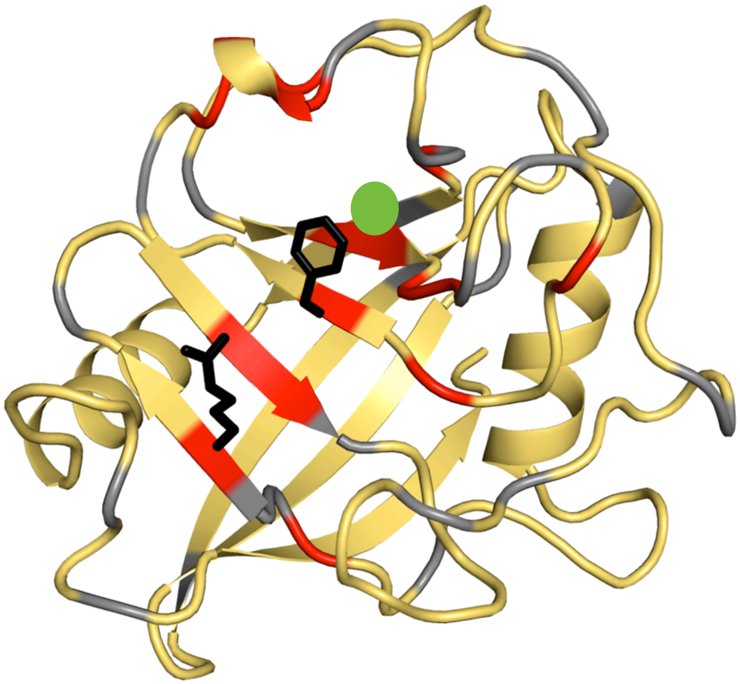


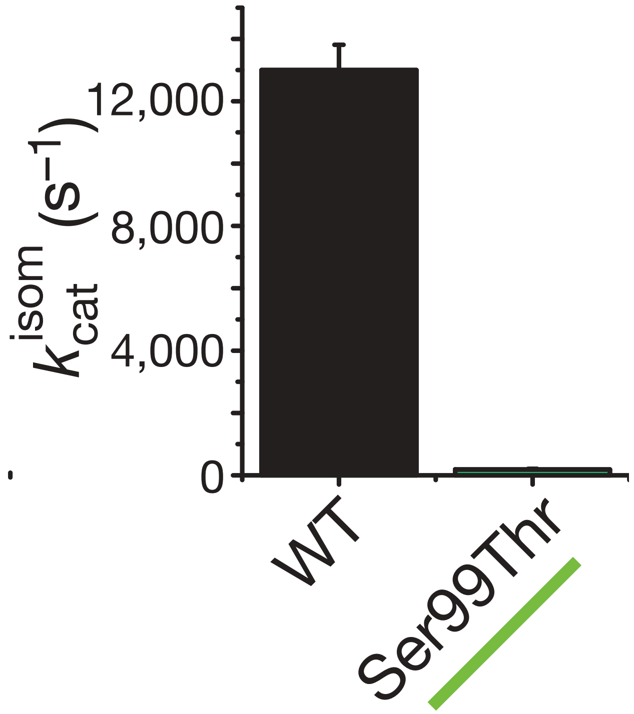
288 K



0.3 and 1σ

A 3rd-shell mutant with parallel reductions in dynamics and turnover

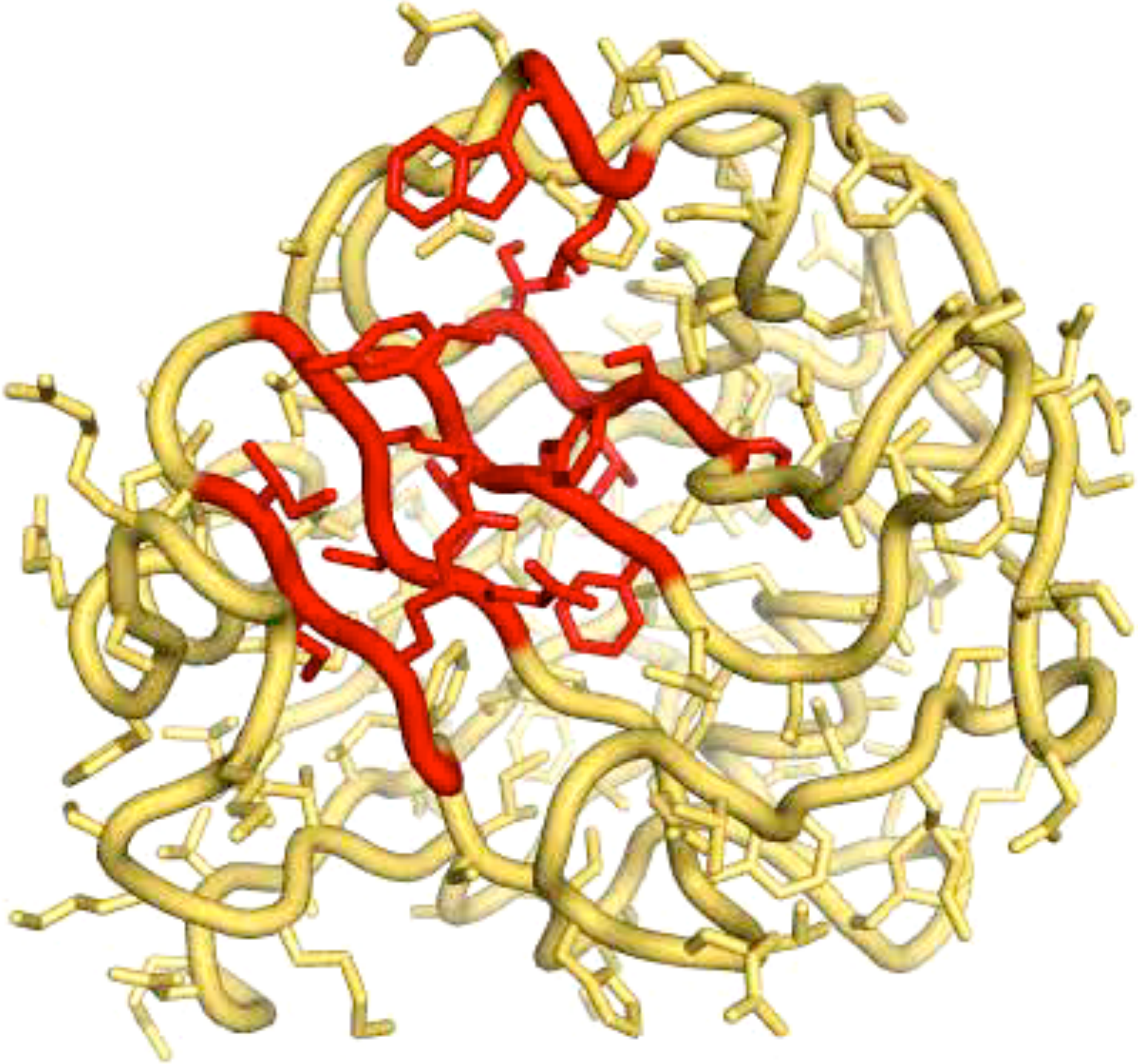




NMR dynamics for Ser99Thr reduced ~60x

Rate of catalysis for Ser99Thr reduced ~60x

From a single crystal at room temperature...



... the protein "moves" to connect
X-ray crystallography, NMR, and catalysis

Fraser... Alber
Nature, 2009

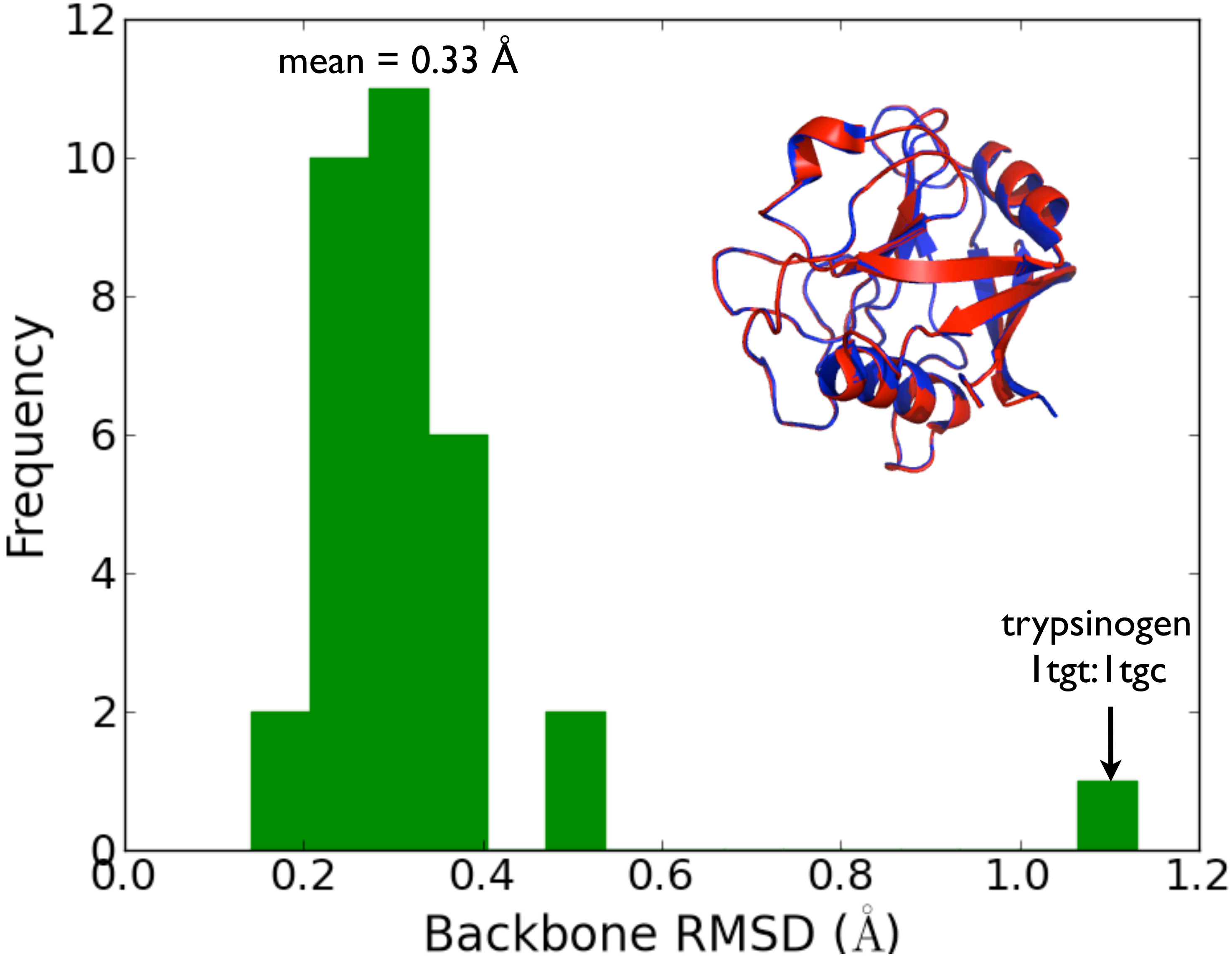
...so...how general is this
cryogenic vs.
room temperature effect?

30 X-ray datasets at both temperatures

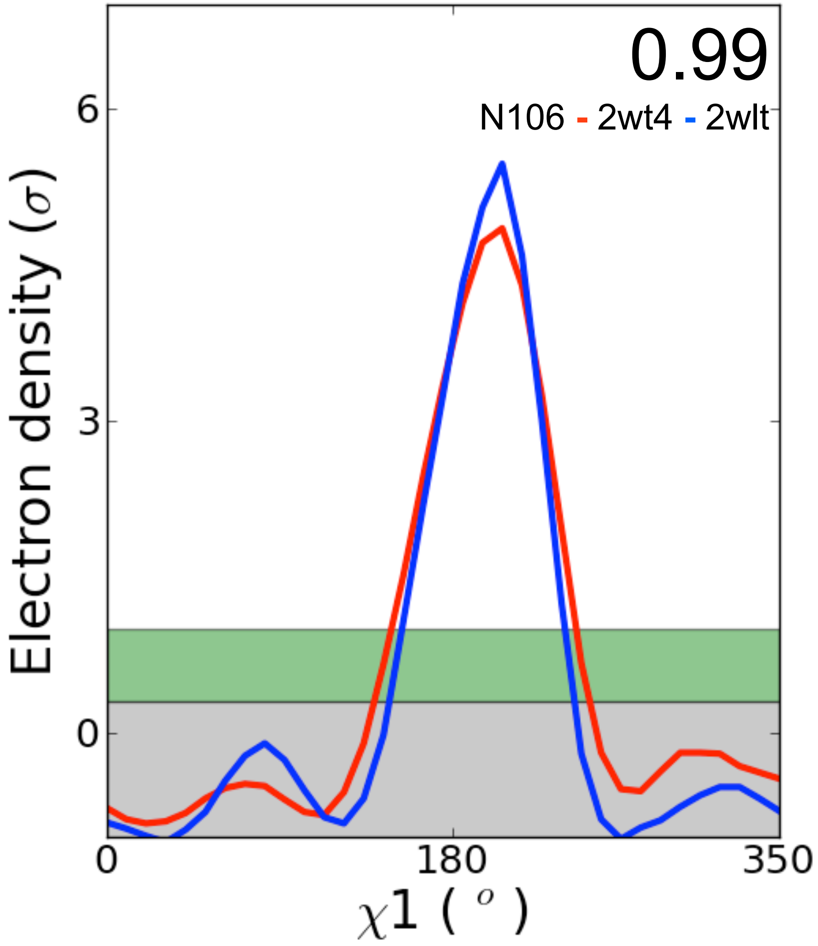
>95% of the PDB is at cryogenic temperatures

- Sequence identical pairs at cryogenic and room temperature from the PDB+our lab
    - both at high resolution (at least 2.0Å)
    - isomorphous and in the same state (ligands, etc)
    - with reflection data available so that we can re-refine and sample electron density

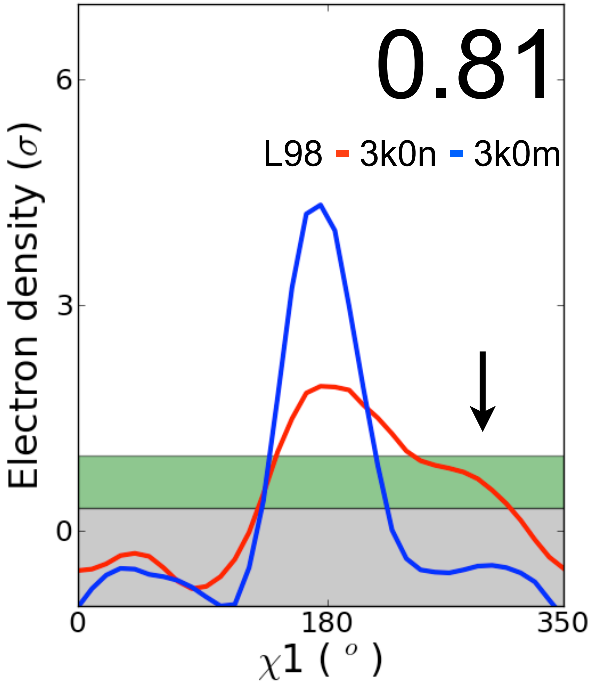
Folds are identical at cryo and room temperature



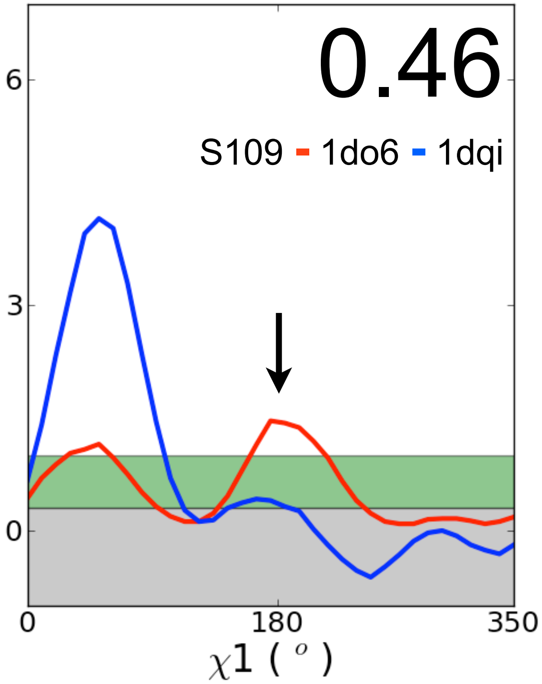
For many residues, freezing simply reduces harmonic motions



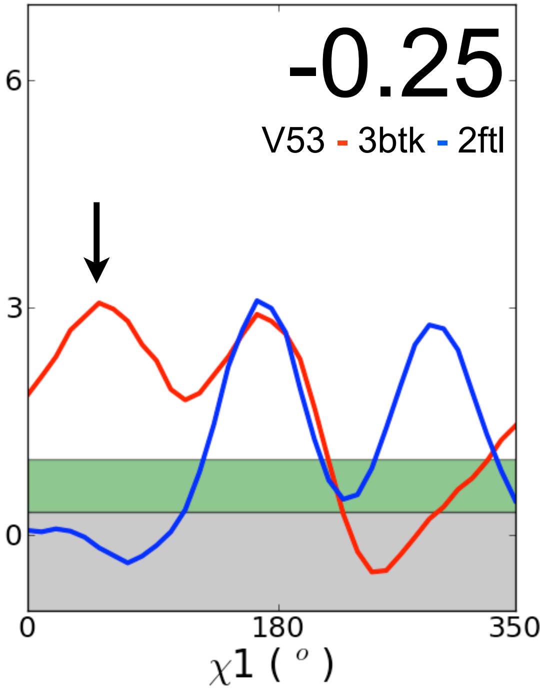
...but for other residues, freezing changes the ensemble



Alternative Conformations

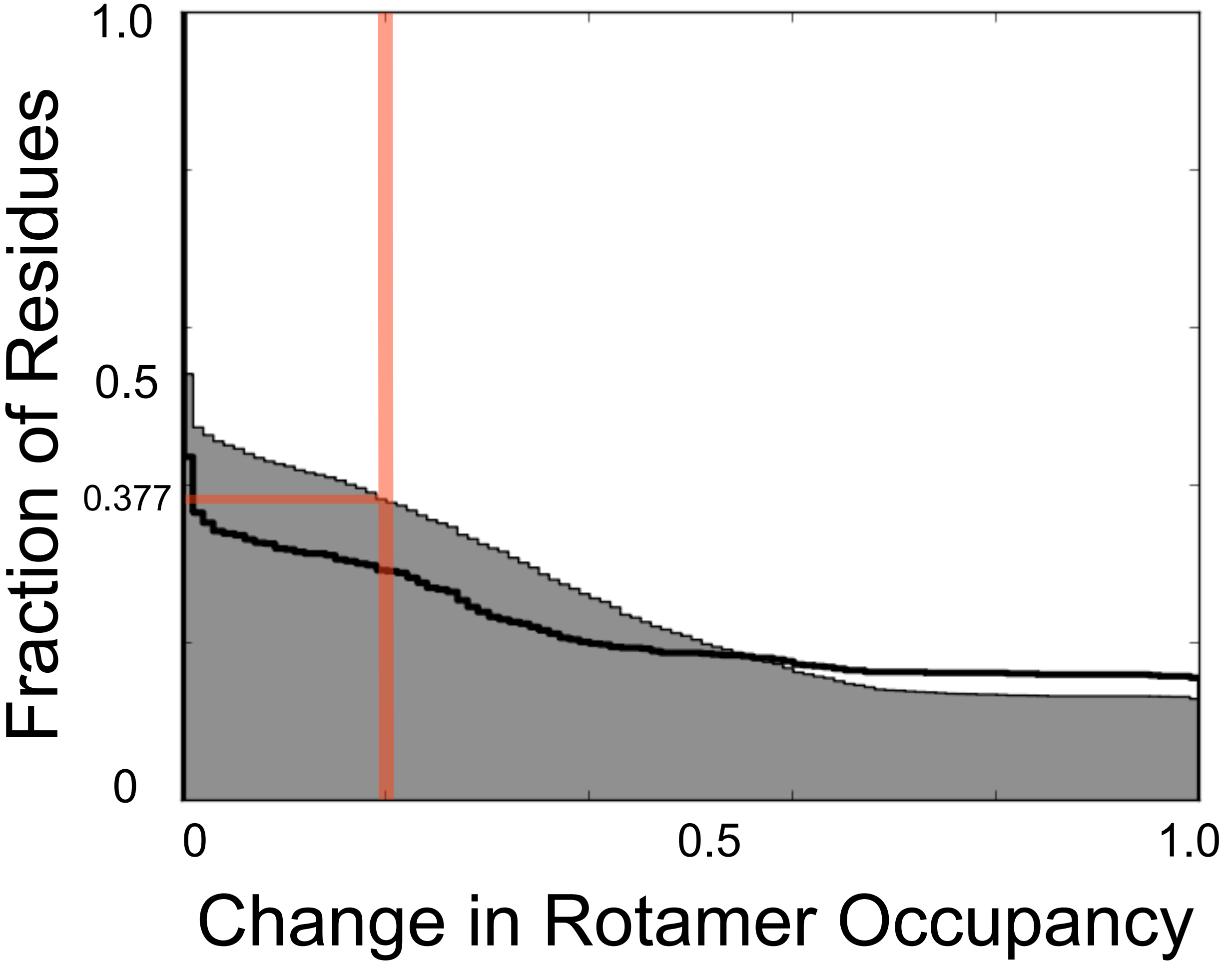


Changes in occupancy



Different Rotamers

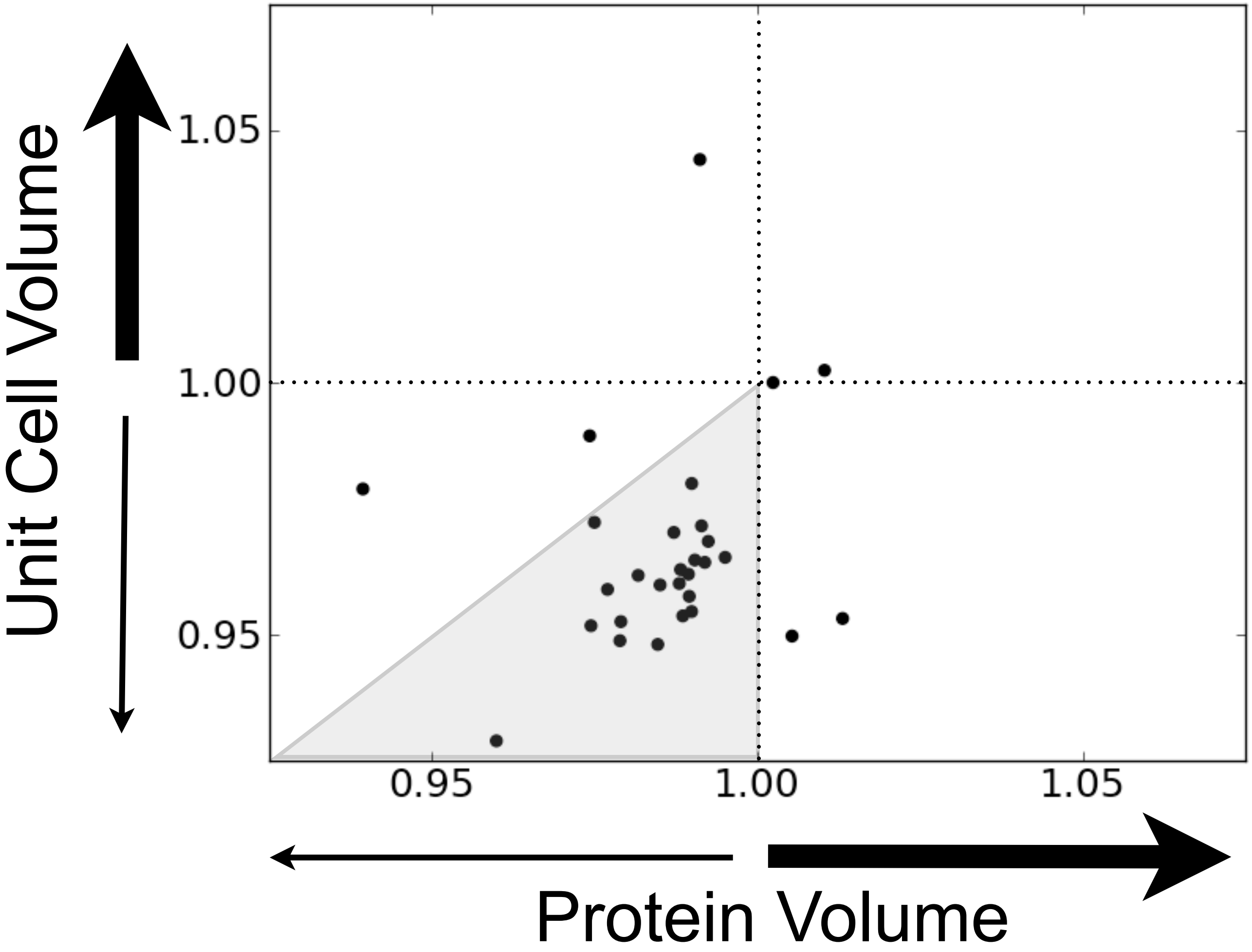
~35% of residues are remodeled by freezing



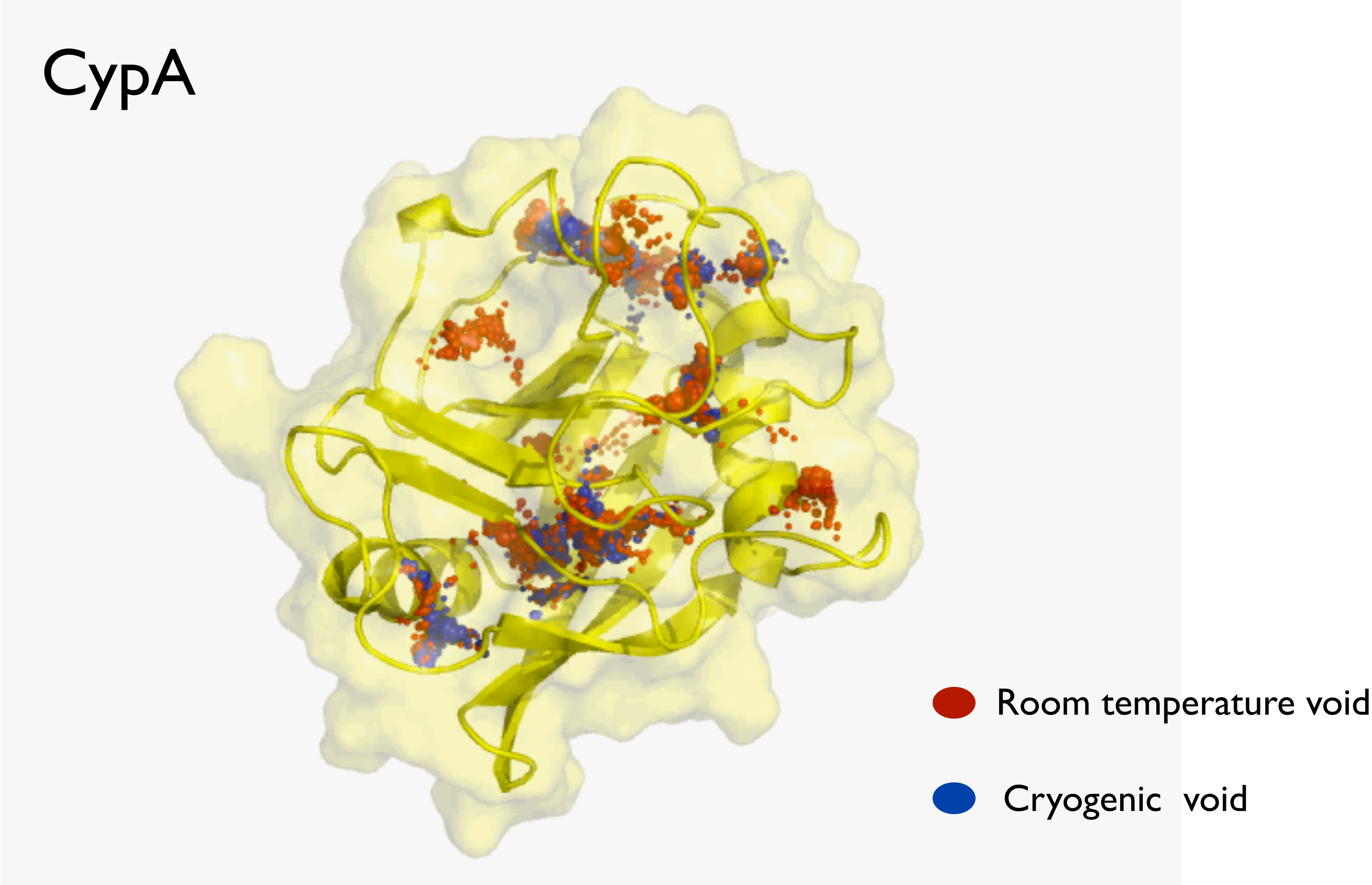
29% of all
buried residues

with Henry van den Bedem (JCSG)

Lattice contacts increase and proteins shrink upon freezing

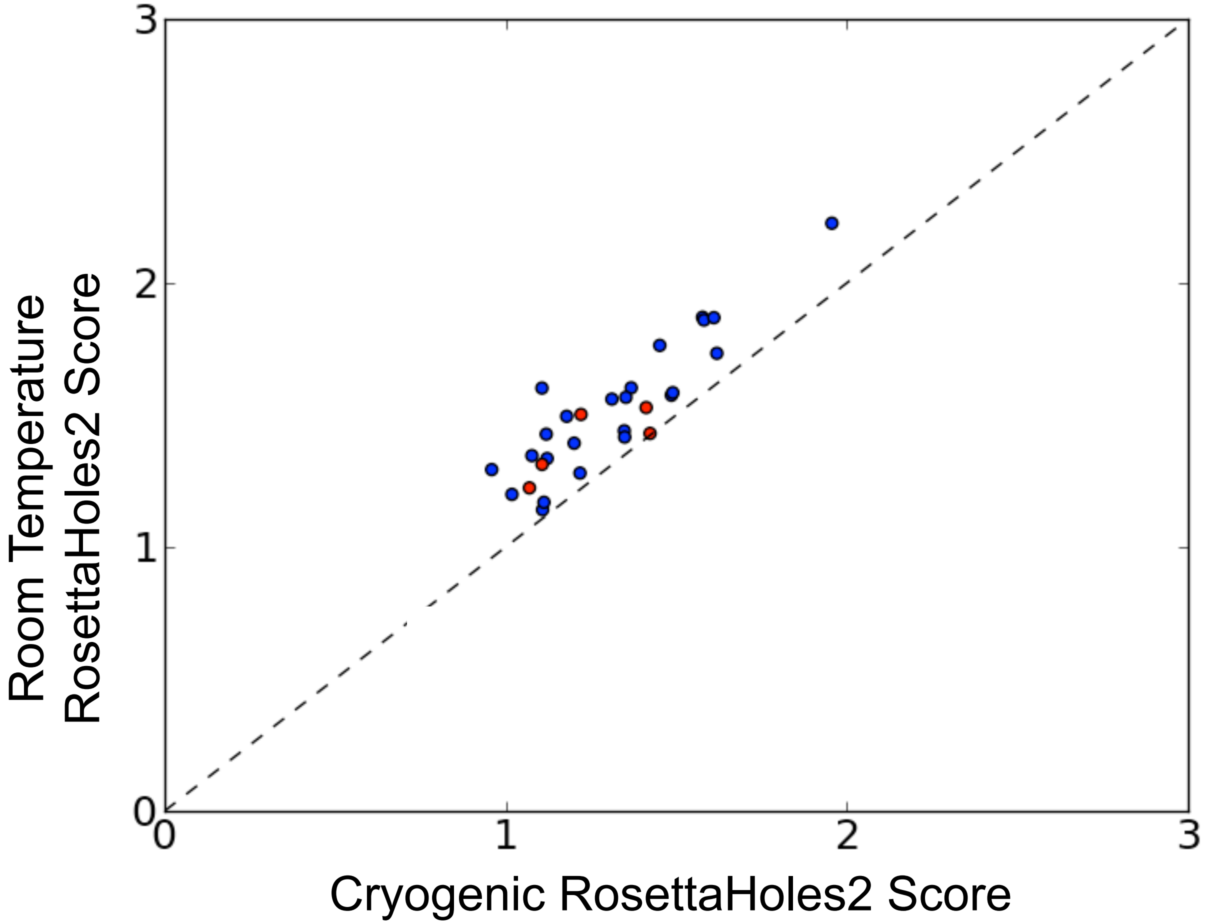


Freezing reduces packing defects



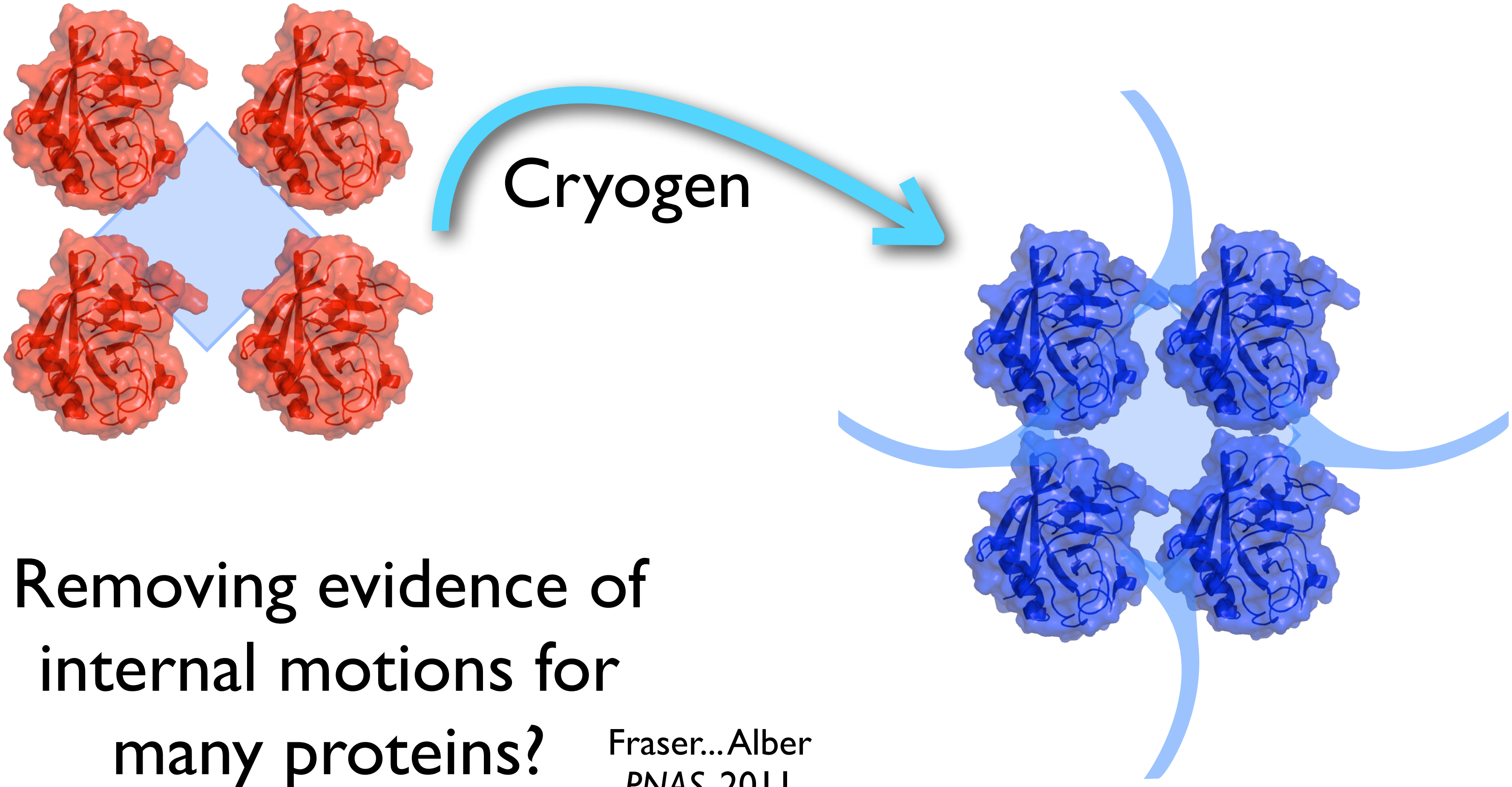
...which can be transiently filled by alternative conformations

Proteins are “better” packed when frozen



Software and help from
Will Sheffler and David Baker, UWashington

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



Removing evidence of internal motions for many proteins?

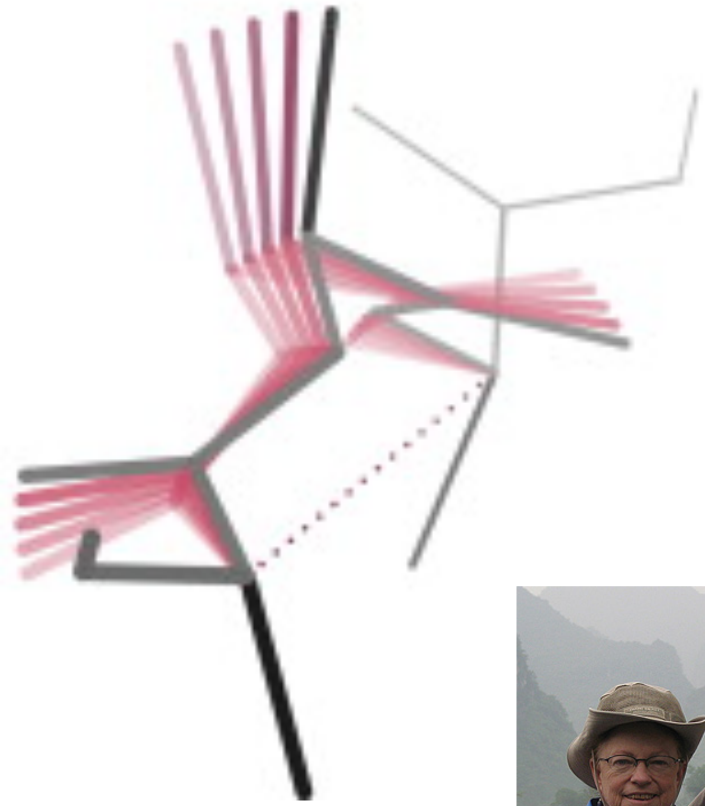
Fraser...Alber
PNAS, 2011

How do we (automatically) fit all this heterogeneity?

Henry van den Bedem
(SLAC/JCSG)

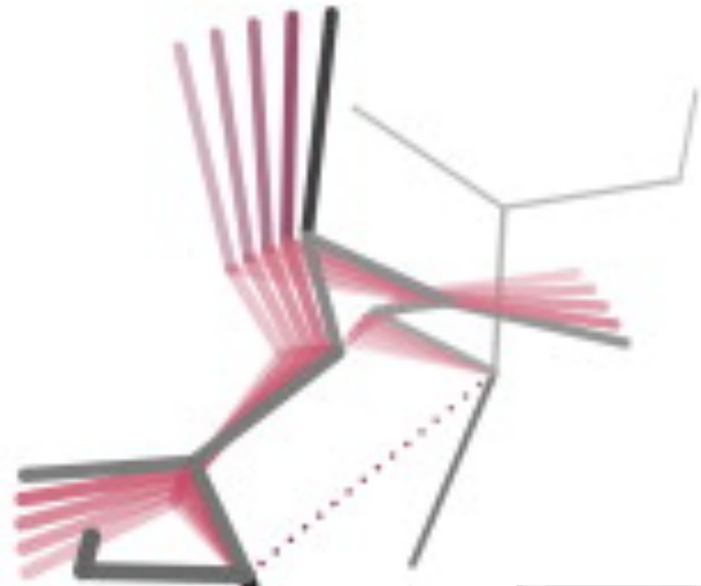
JCSG
Joint Center for Structural Genomics
Developing HT methods for Gene to Structure and Function

Describing heterogeneity requires *both* backbone and side chain movement

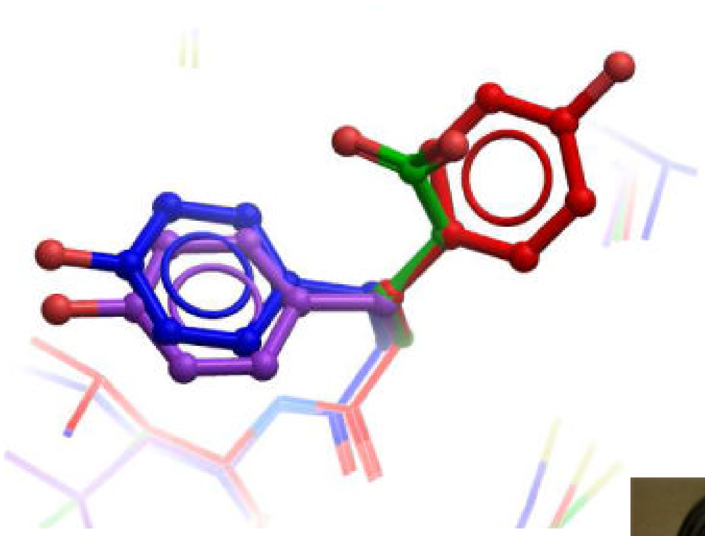


Davis...Richardson, *Structure*, 2006

Describing heterogeneity requires *both* backbone and side chain movement



WT
fixed
backrub
mutant

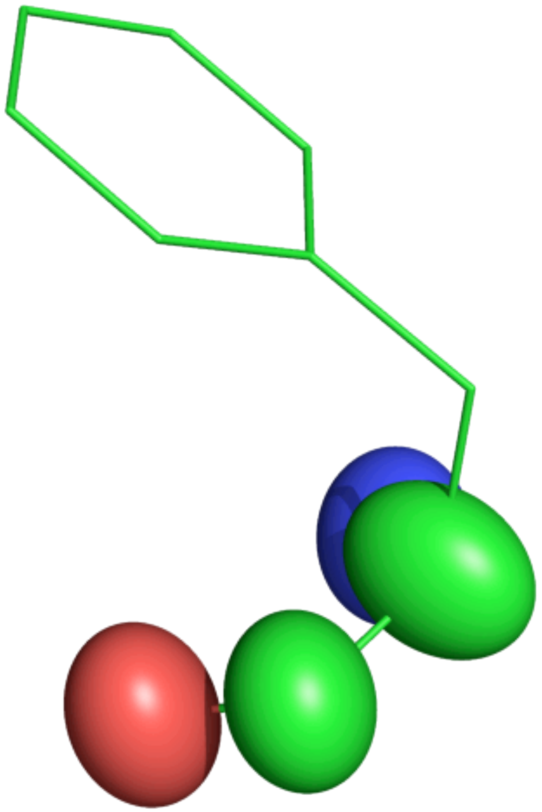


Davis...Richardson, *Structure*, 2006

Smith and Kortemme, *JMB*, 2008

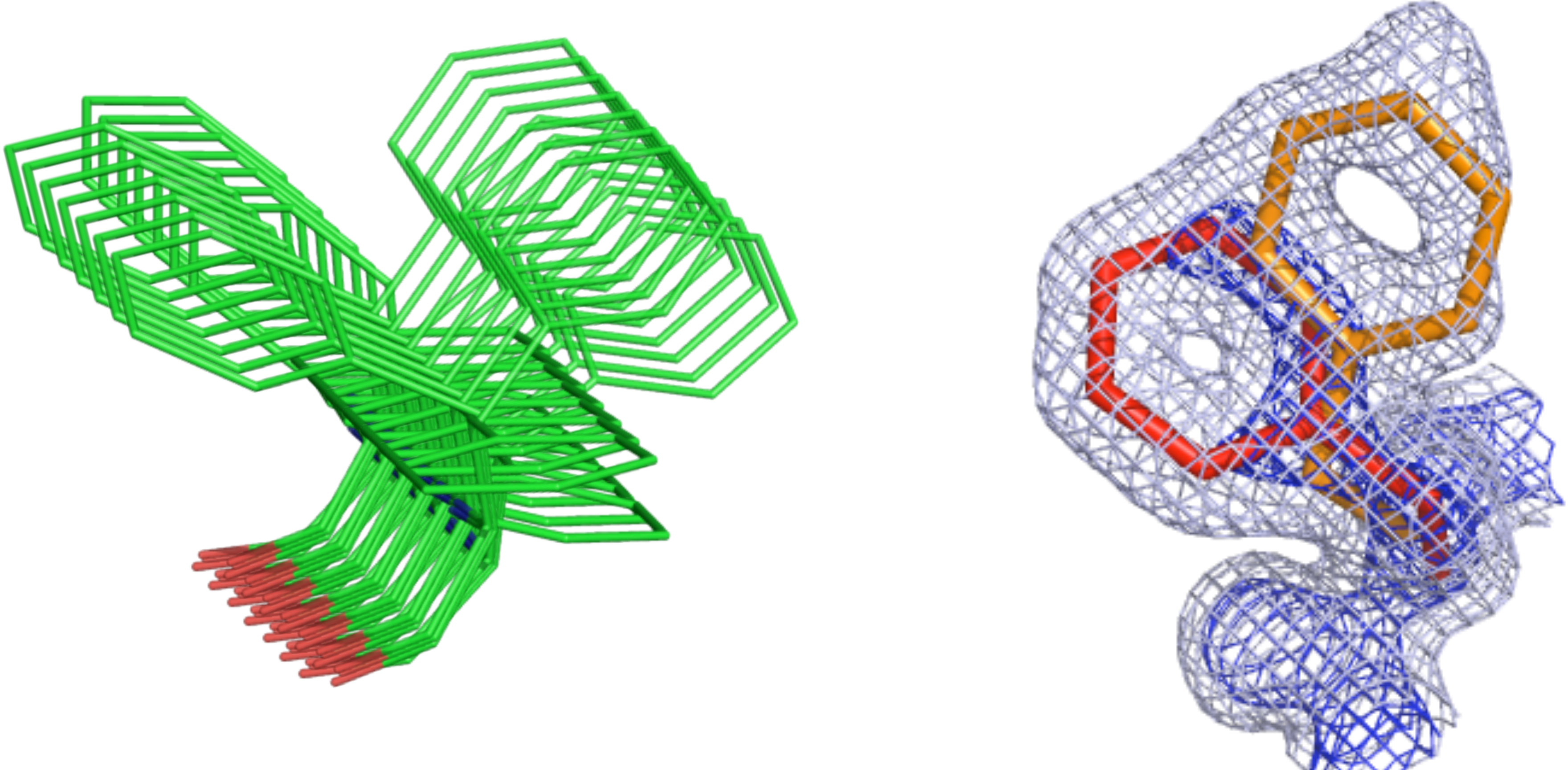
qFit starts from a traditional (unique) model

- For each residue (individually):
  - fit anisotropic B-factors for the backbone
  - seed backbone conformations into anisotropic envelope
  - sample a neighbourhood of rotamers



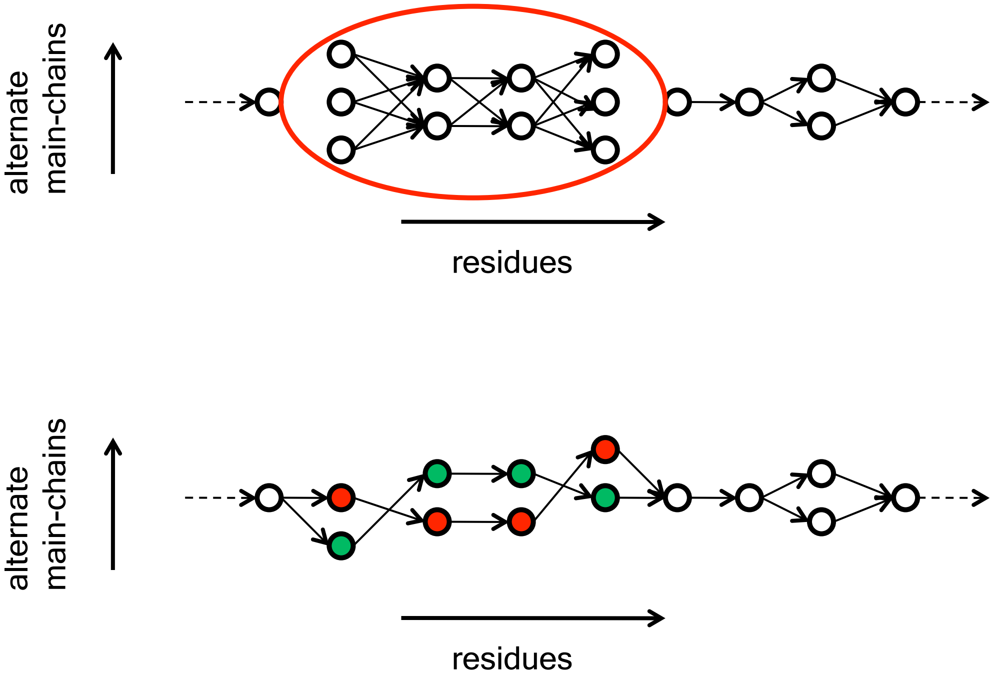
van den Bedem...Deacon, *Acta Cryst D*, 2009

compute the “optimal” fit to electron density in real space



from >500 potential conformations only 1-3 conformations are assigned non-zero occupancy

and connect residues together… so they look like backrubs!

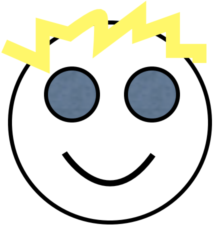


1-2% drop in Rfree (at high resolution)

van den Bedem...Deacon, *Acta Cryst D*, 2009

How can this help Rosetta?

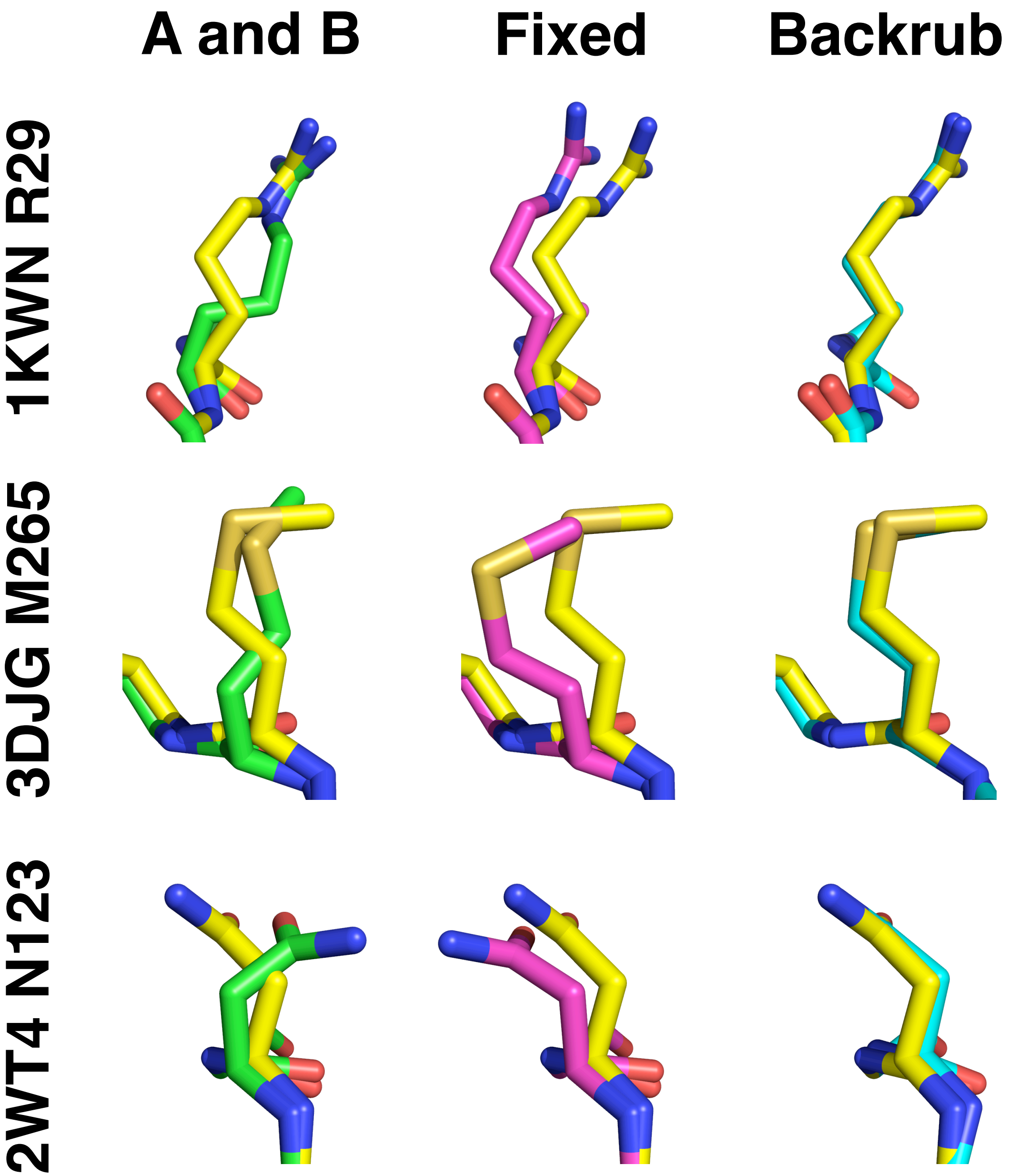
Noah Ollikainen

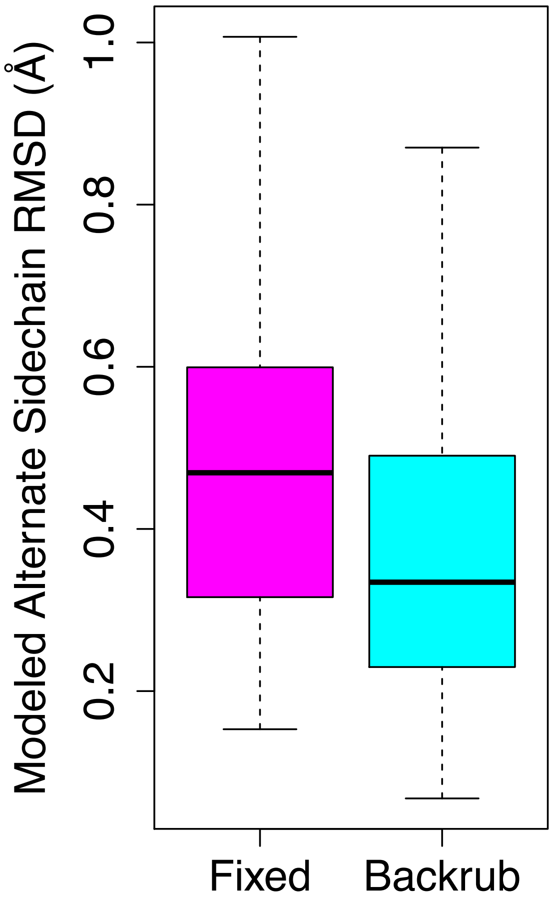


Colin Smith

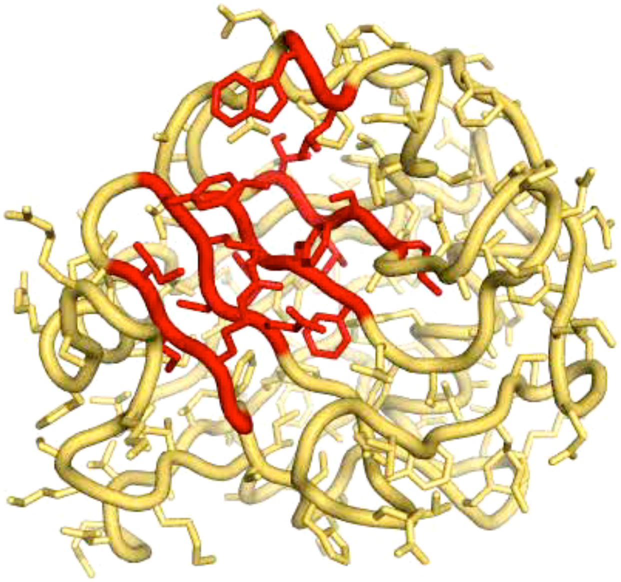
Tanja Kortemme

Use alternative conformations from RT X-ray and qFit to test sampling methods





...but these moves are in isolation...
coupled moves are harder...



Which excursions from the "structure" work together?

Henry van den Bedem

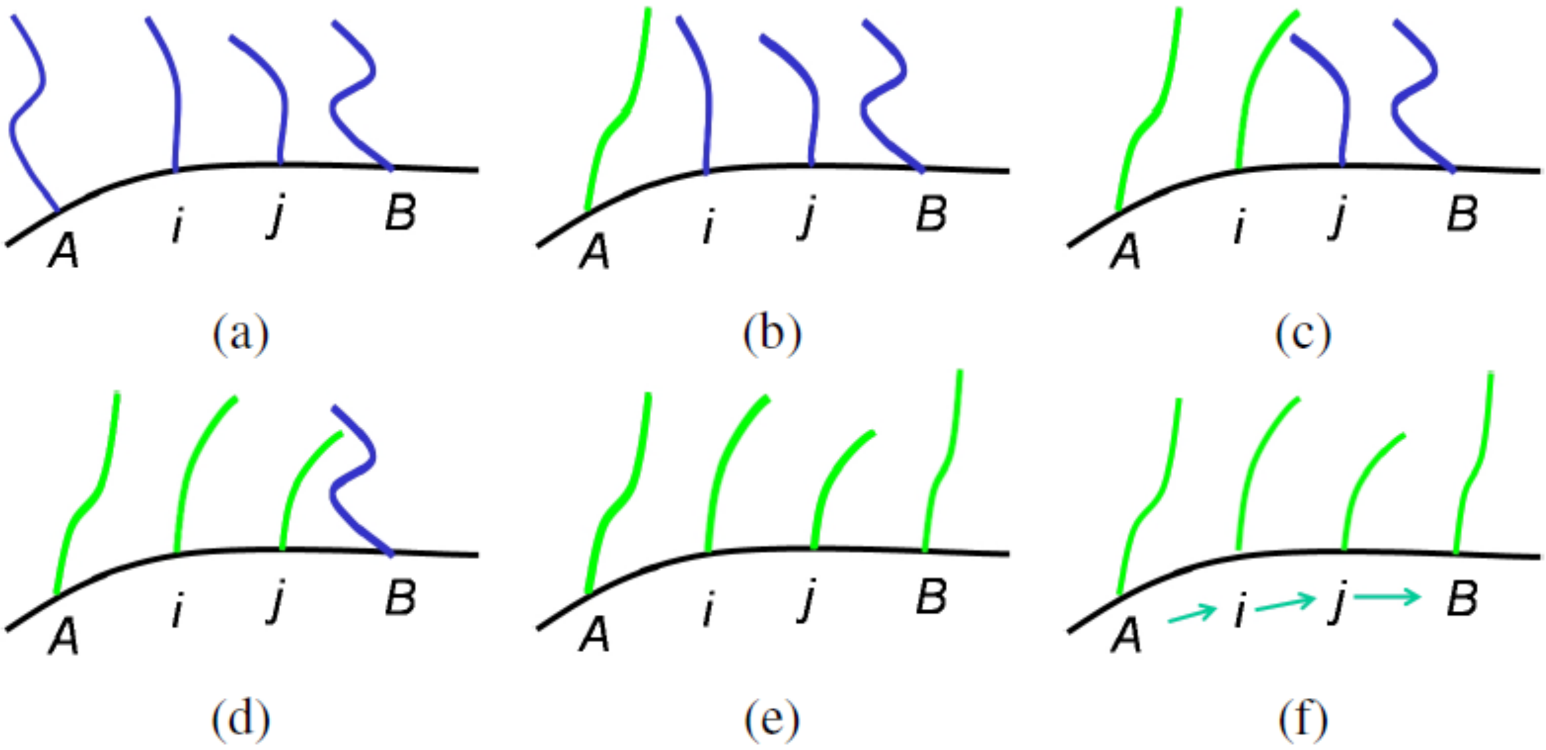
(SLAC/JCSG)

JCSG

Joint Center for Structural Genomics

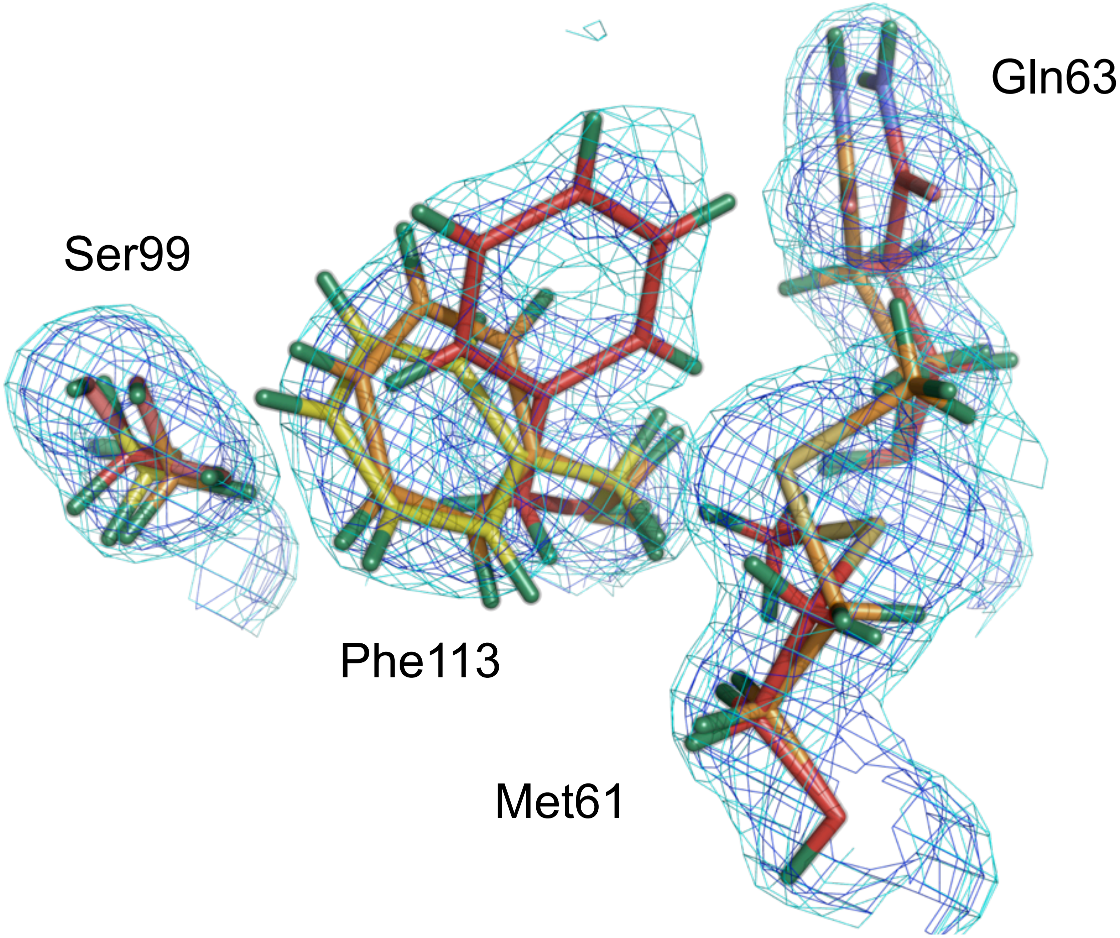
Developing HT methods for Gene to Structure and Function

Relief of steric clashes by alternative conformations identifies pathways

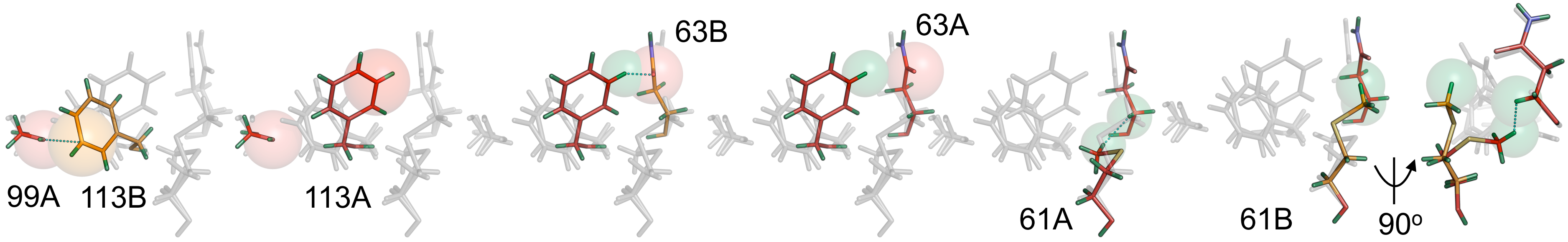


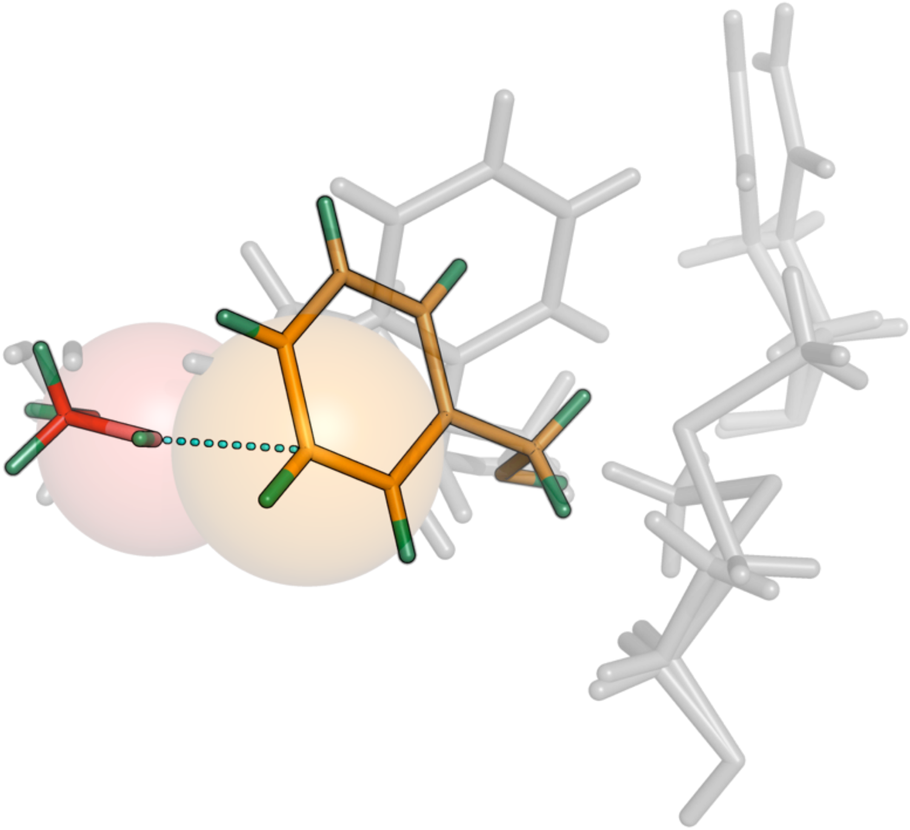
X-ray-based compliment to MD-based methods:
RIP (Agard), MutInf (Jacobson), etc

What does a pathway of clash/ relieve interactions between alternative conformations look like?



What does a pathway look like?





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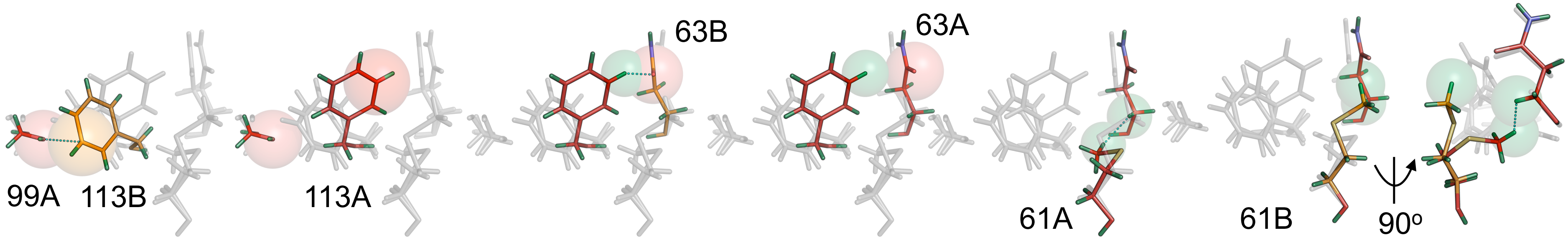
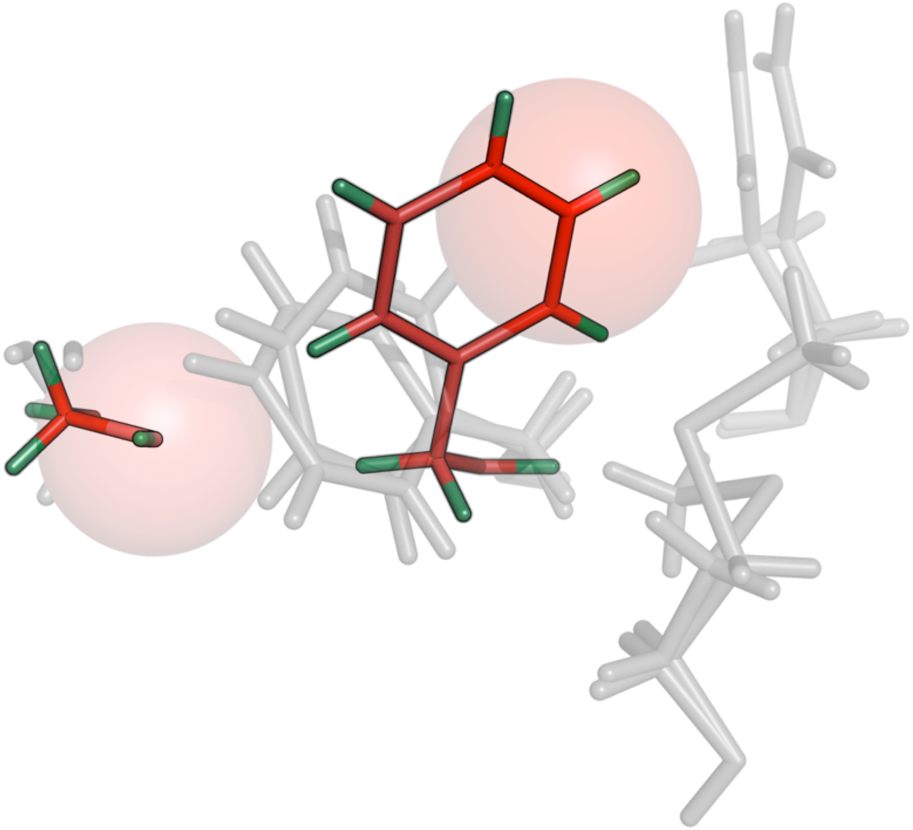
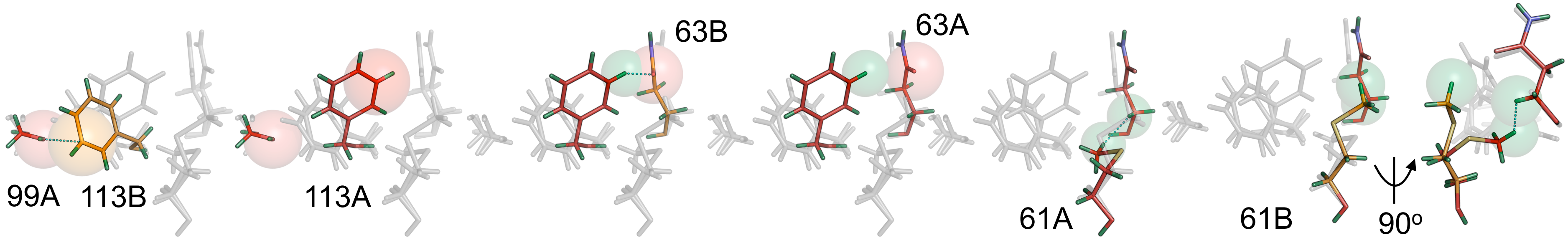
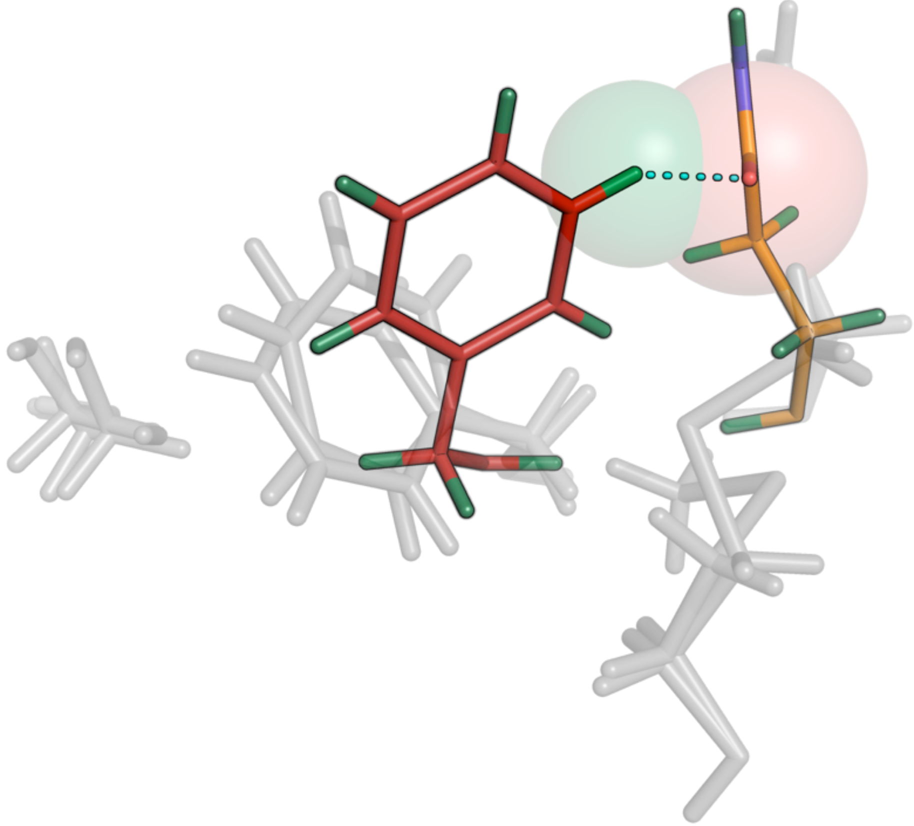




What does a pathway look like?





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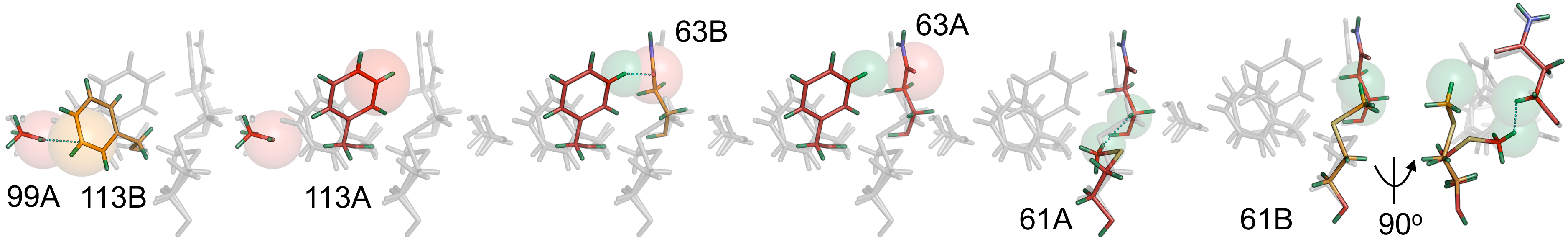

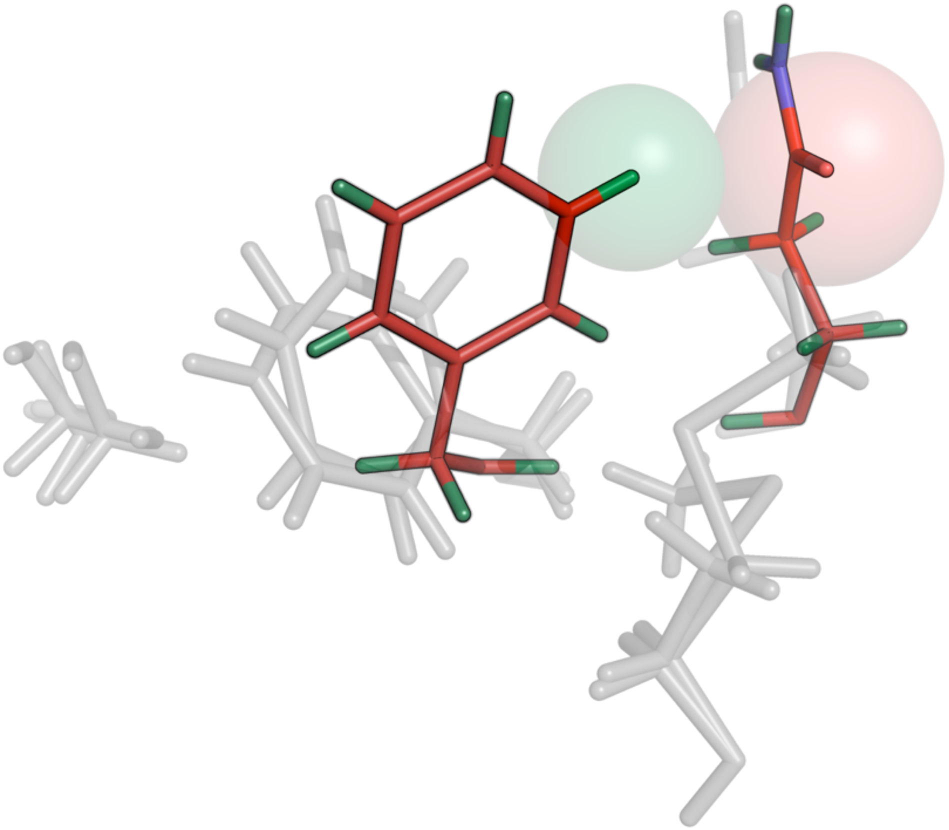

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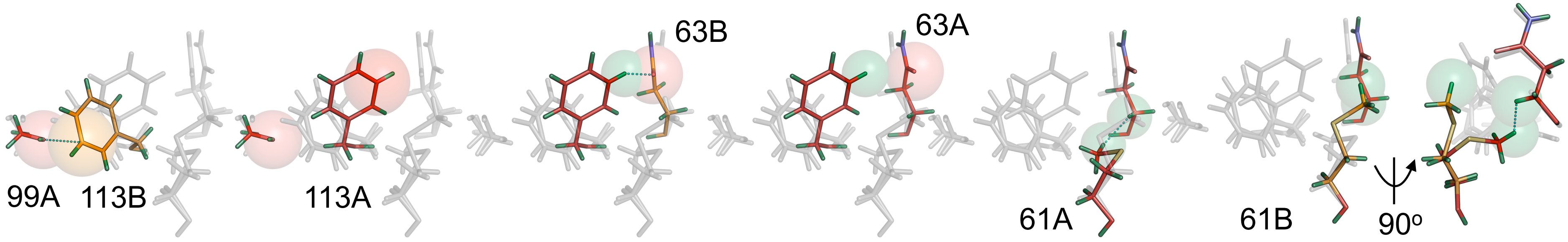

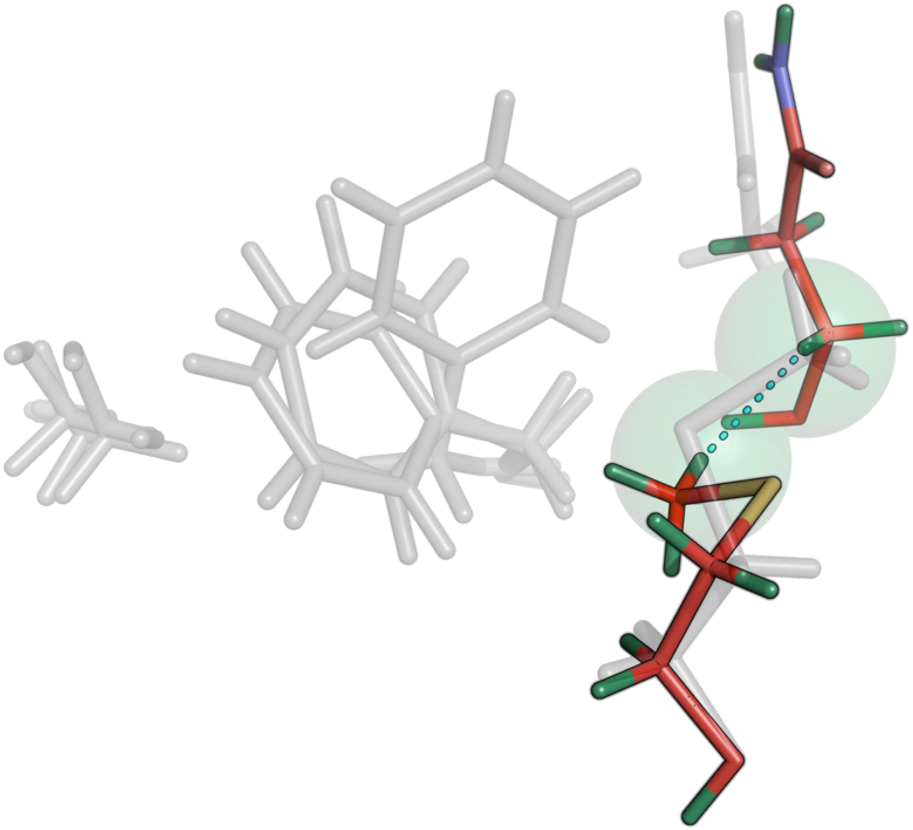

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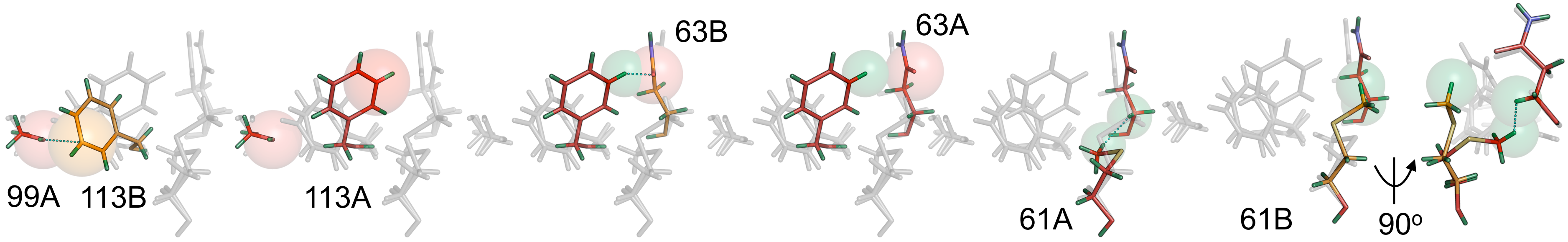

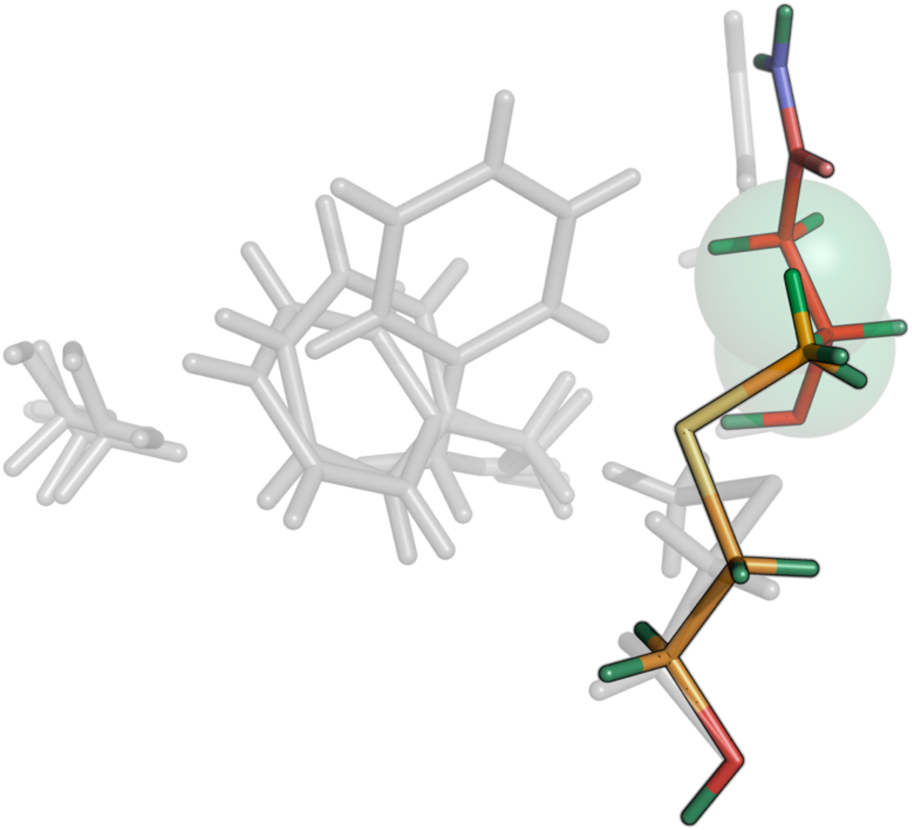

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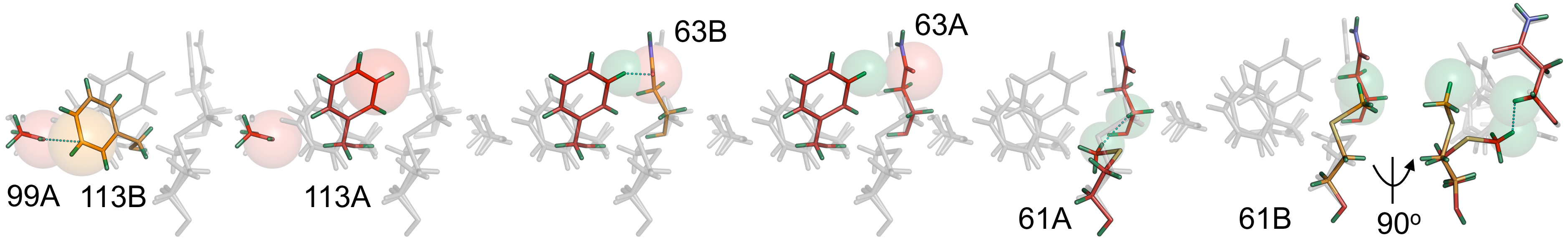
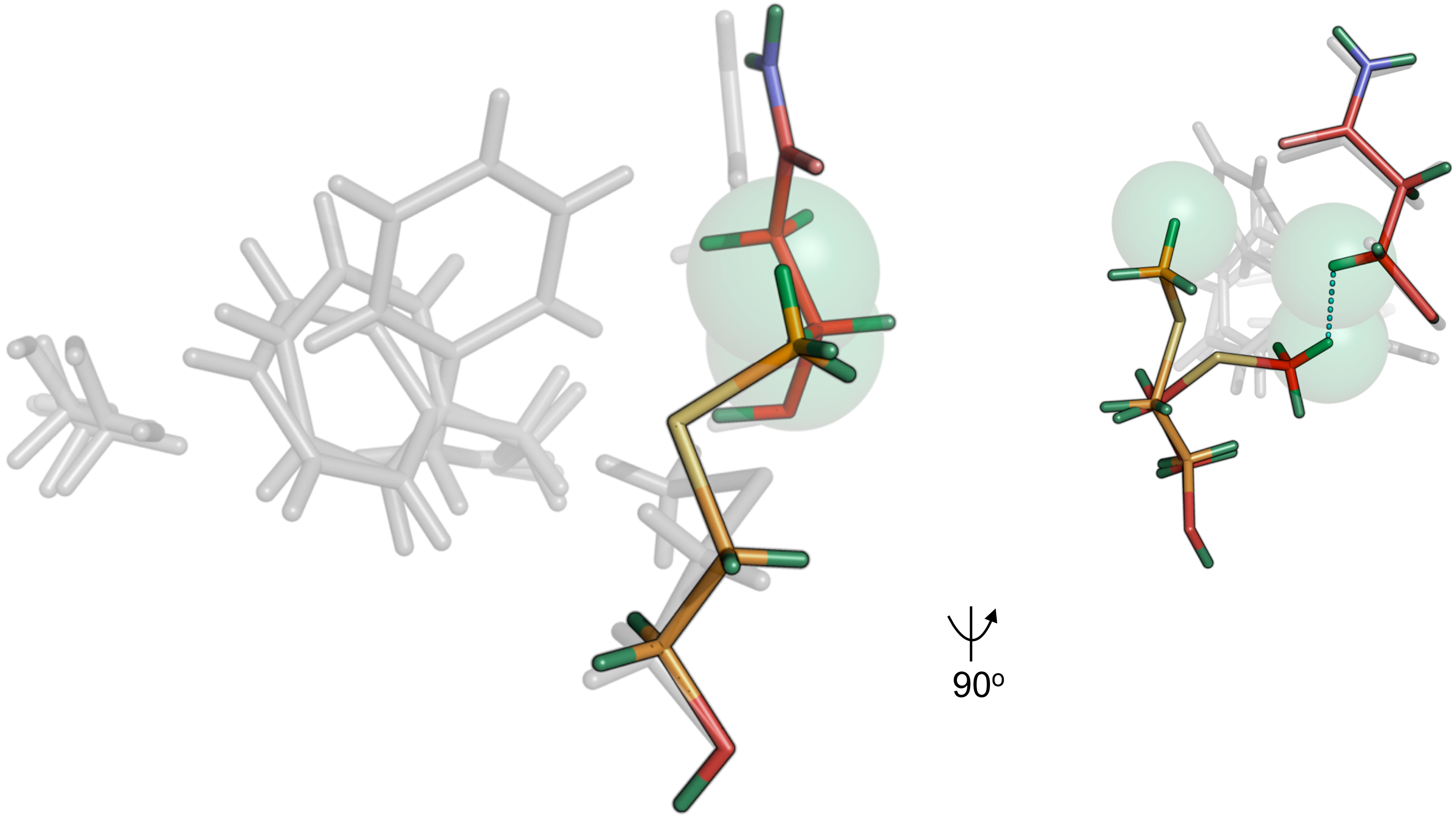
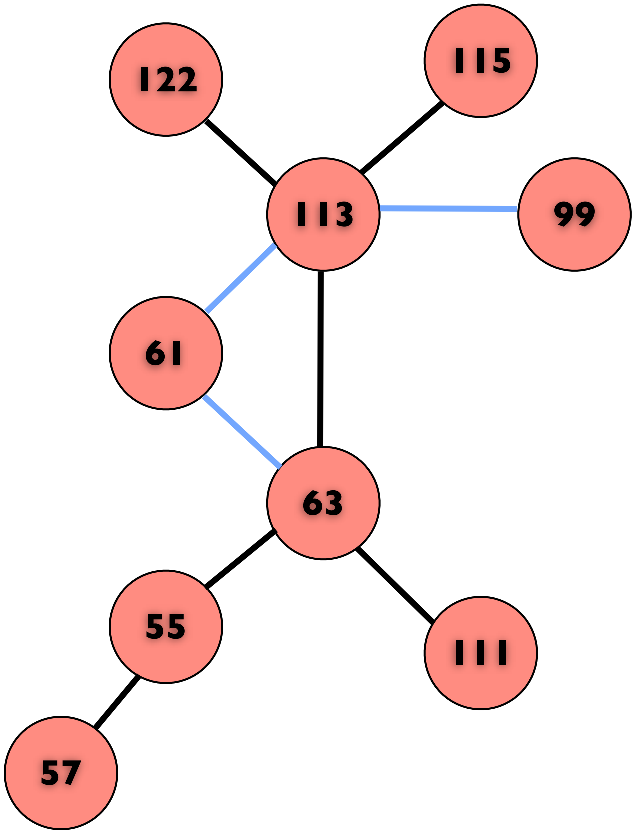
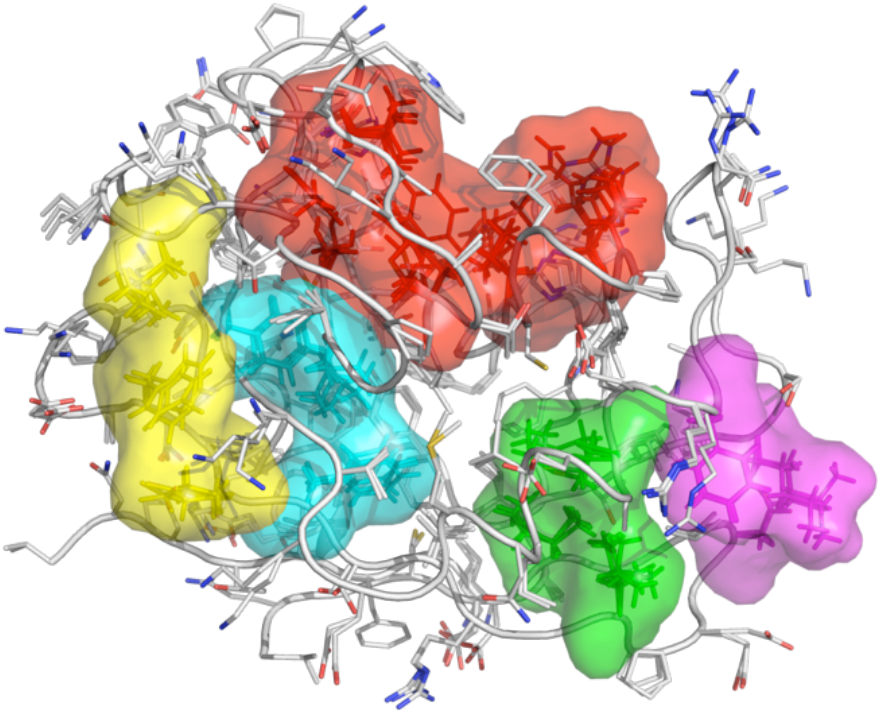




Pathways can be linked together in contact networks





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

For 40 proteins, pathways and networks are:

- more common...
  (increased number of pathways)
- more “global”...
  (increased network participation)
- and longer...
  (increased average pathway length,
  network size)

...at room temperature
(compared to paired cryogenic data)

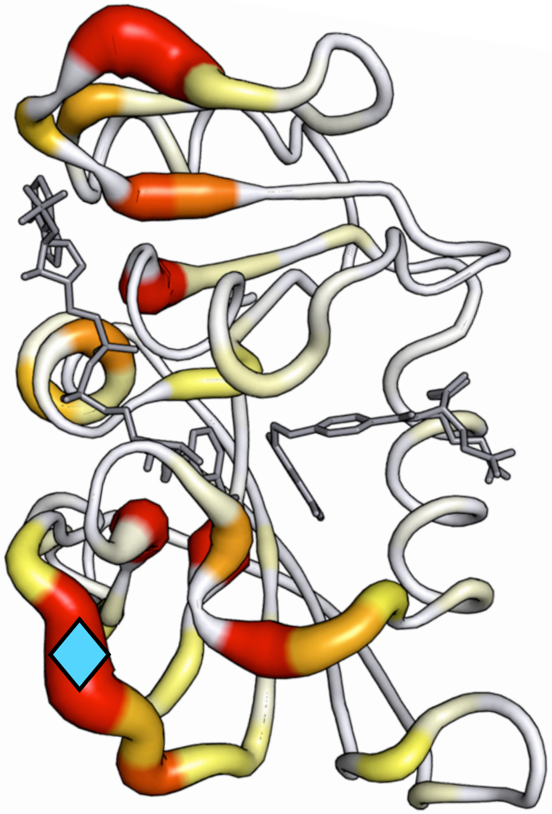
Can we use knowledge of networks
for engineering and design?

What is the structural basis for "allosteric" chemical shift perturbations upon mutation?

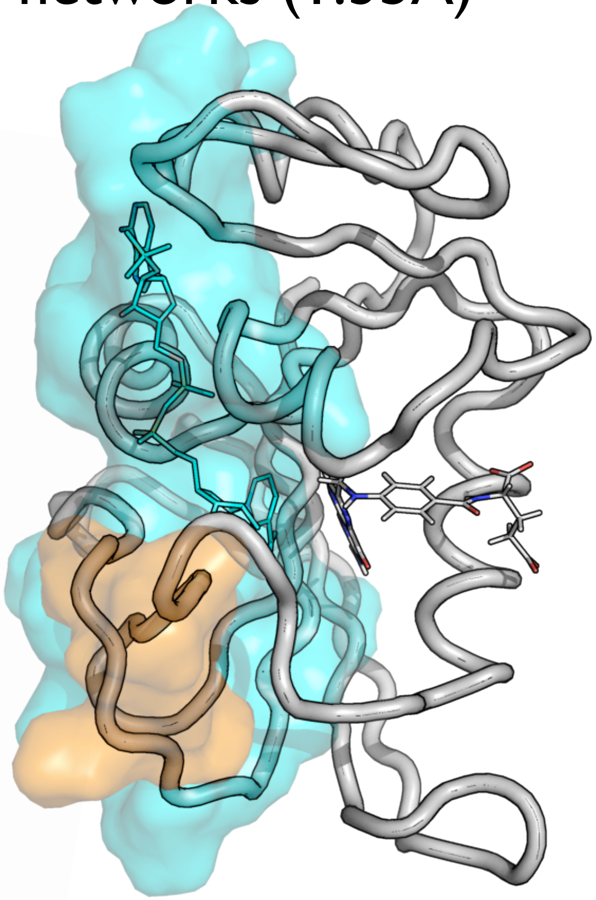
Gira Bhabha   Peter Wright

Contact networks predict chemical shift perturbations

DHFR G121V Δδ

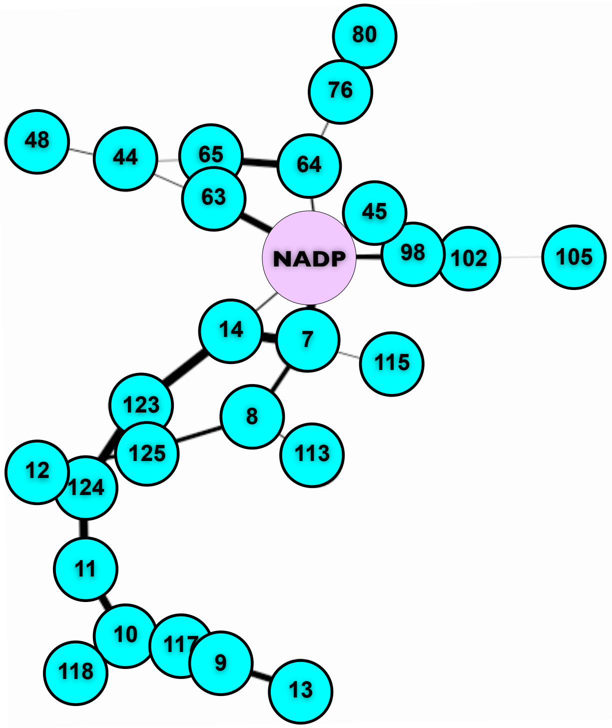


room temperature networks (1.35Å)



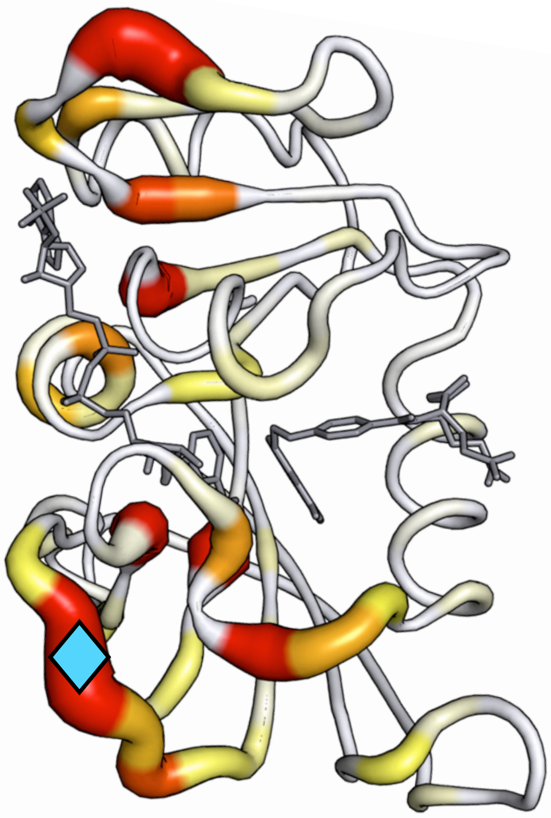
no networks connected to this area at cryo

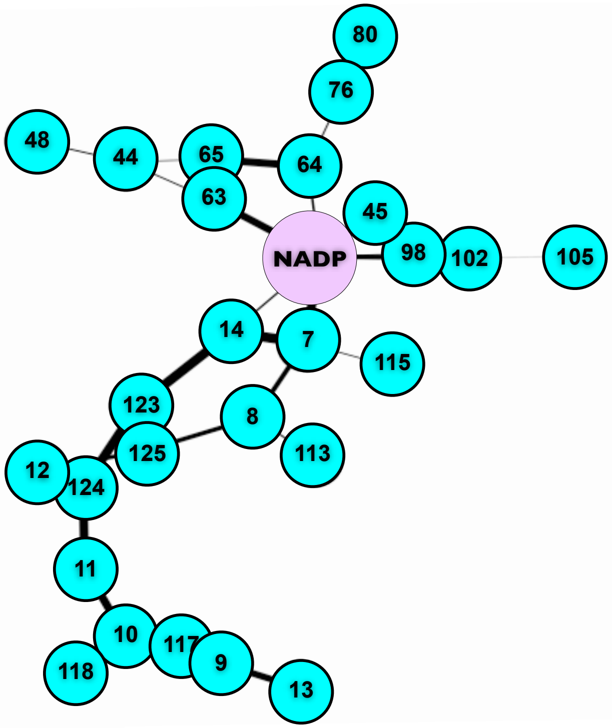
network graph



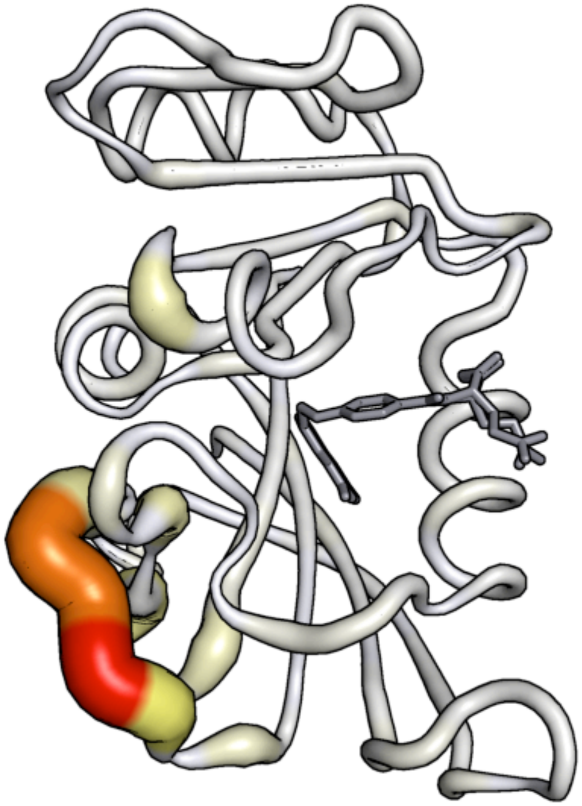
Contact network suggests NADP couples subdomains

DHFR G121V Δδ + NADP





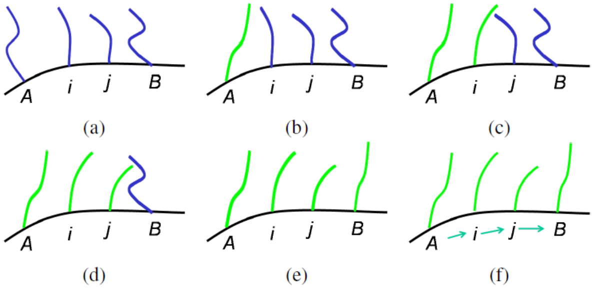
DHFR G121V Δδ - NADP



~~Structure~~
Ensemble:Function Studies

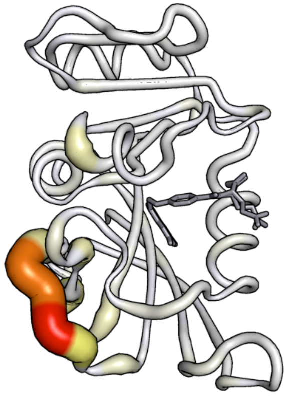
- Ensemble properties extracted from X-ray crystallography can be used to study radiation damage, cryo-protection, pressure - and to test new sampling/scoring methods in Rosetta

- Contact networks observed in RT X-ray data complement NMR chemical shift perturbations and relaxation experiments



- Discrete heterogeneity at RT, and often not cryo, implicated in ligand binding/resistance mutations (Shoichet), catalysis (Wright, Kern), and allostery (Fletterick)

- Having a structural mechanism for protein dynamics can be useful for engineering biological functions



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Electron Density Sampling

http://ucxray.berkeley.edu/**ringer.htm**

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