Simultaneous Bragg and diffuse scattering data collection from single enzyme crystals

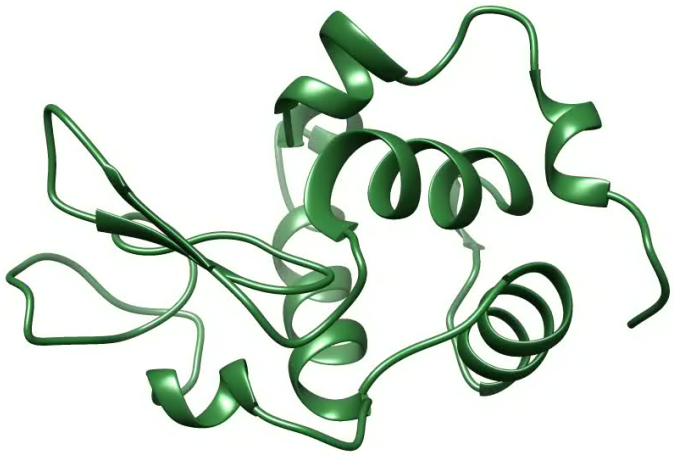
Andrew Van Benschoten

ALS Diffuse Scatter Workshop

10/9/13

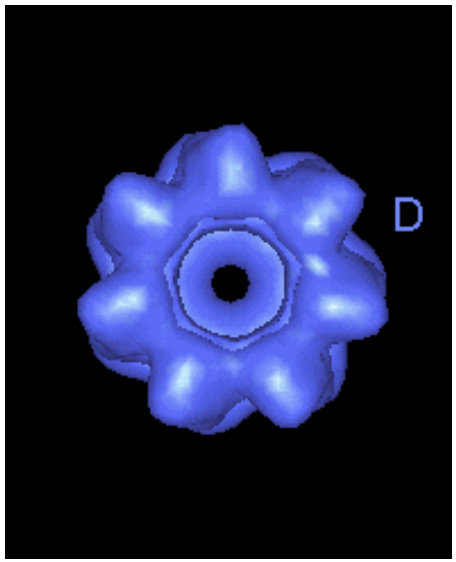
Protein function requires correlated motion

Lysozyme



Hinge bending

GroEL



Subunit rotation

Rye et.al (1999)

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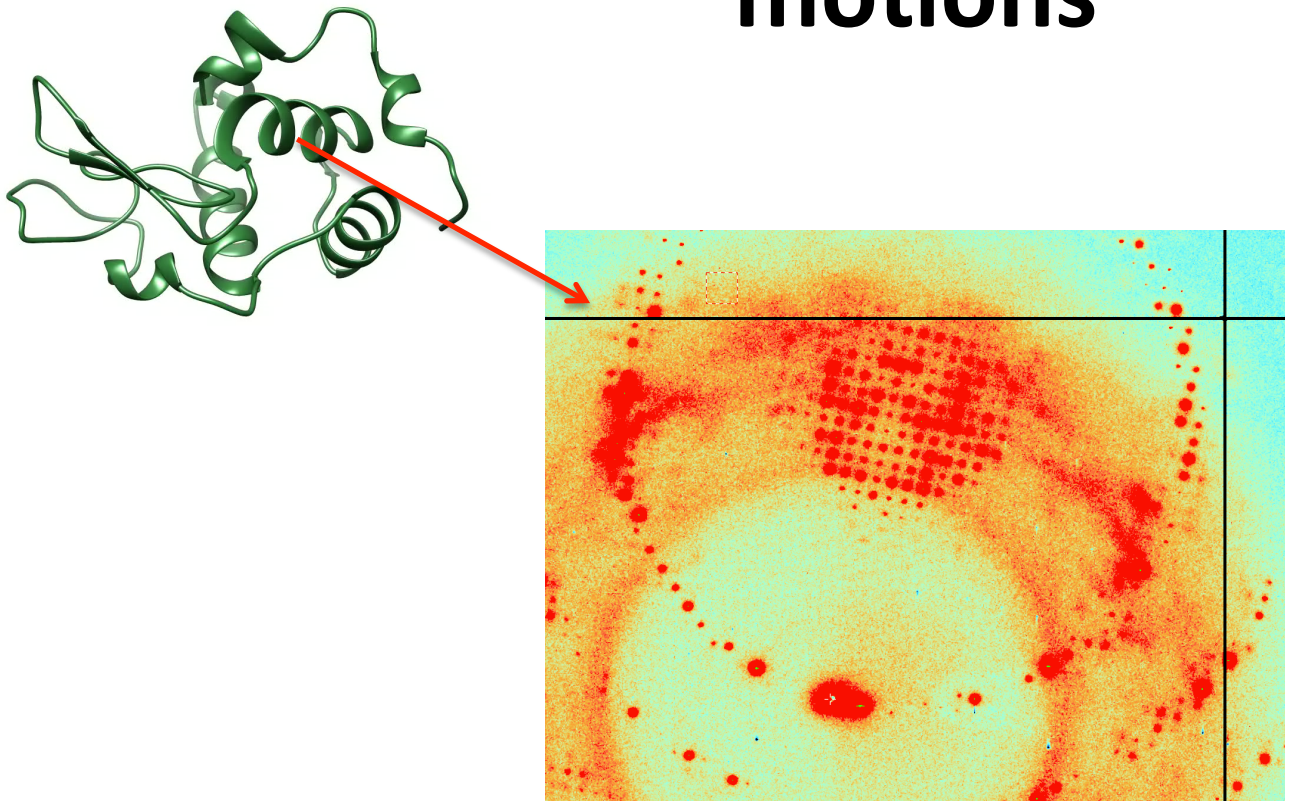
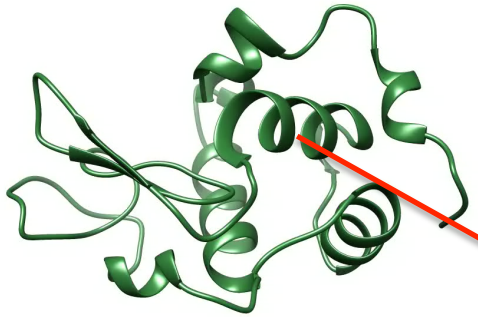
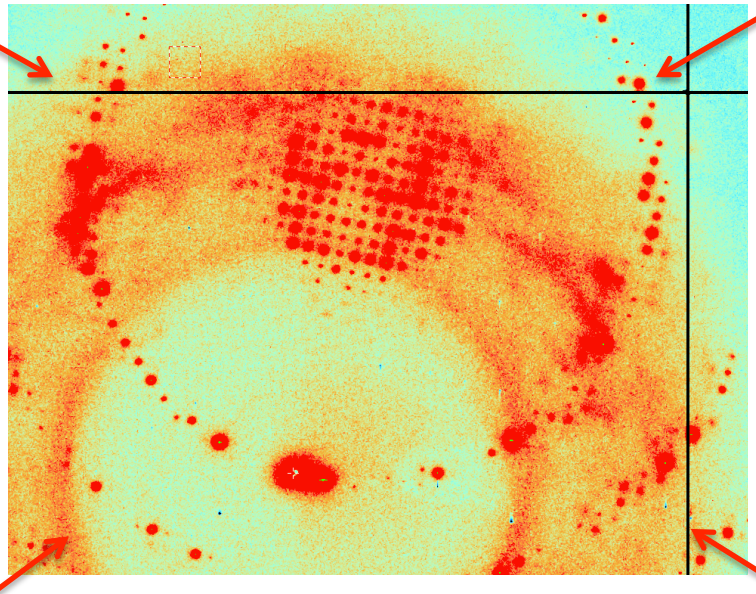
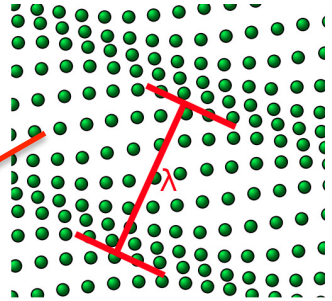
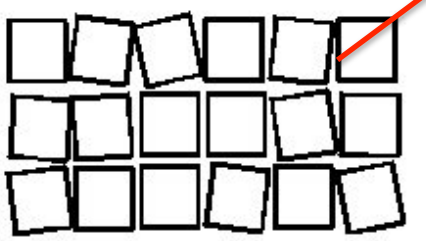


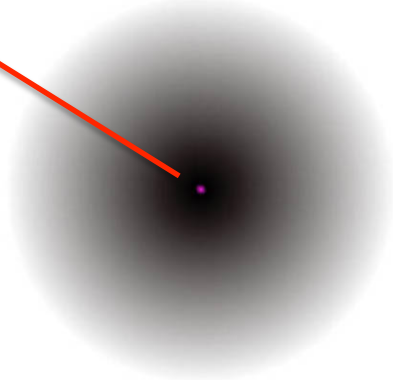
…and other crystal imperfections











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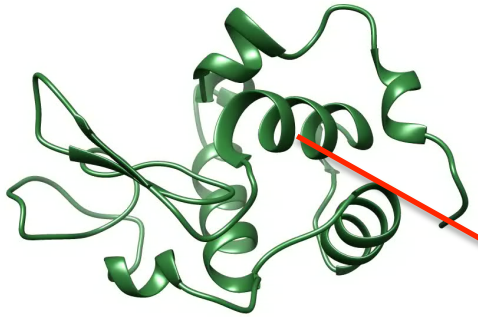

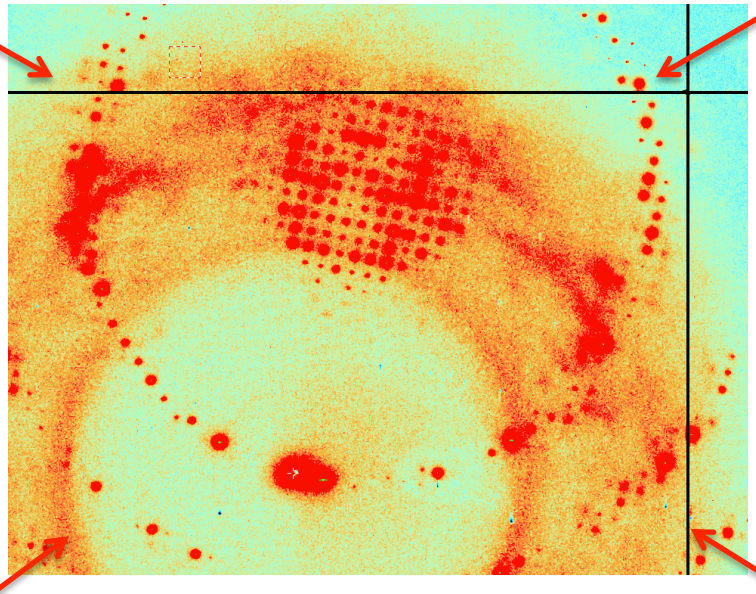

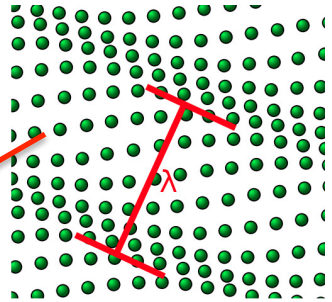

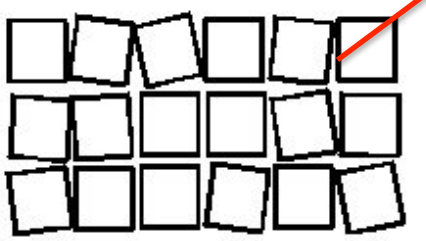

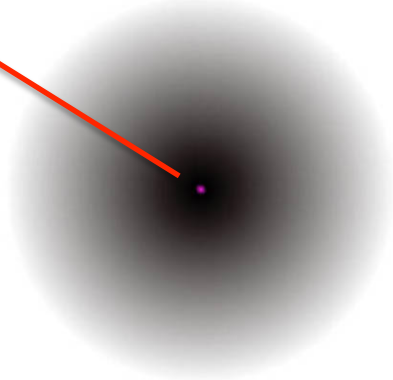

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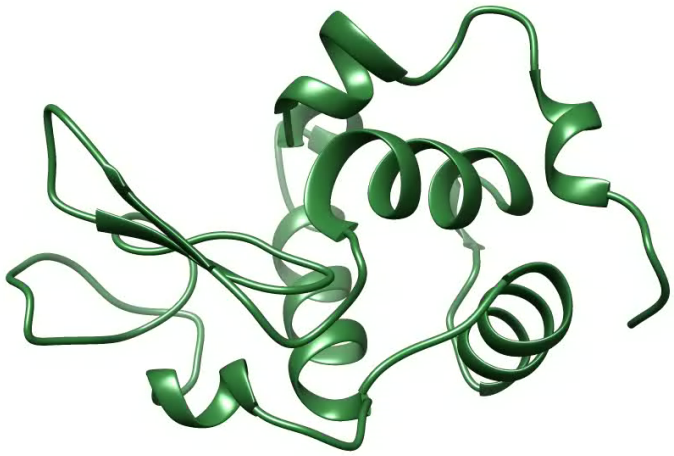

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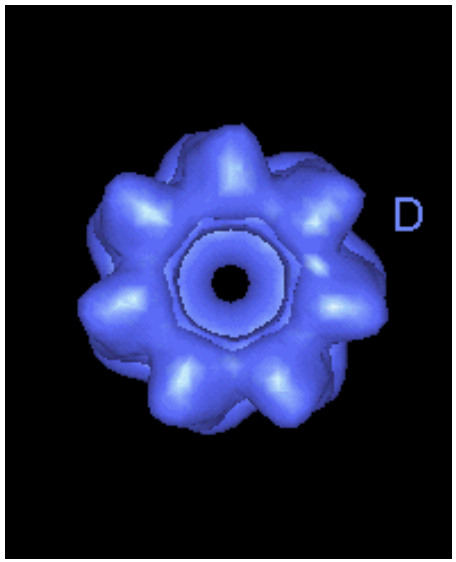

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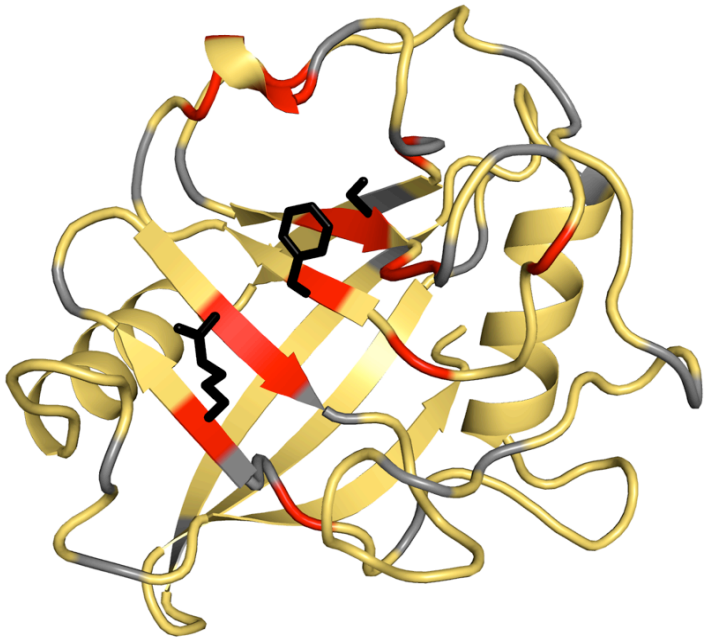
Room-temperature crystallography reveals catalytic Cyclophilin A conformations

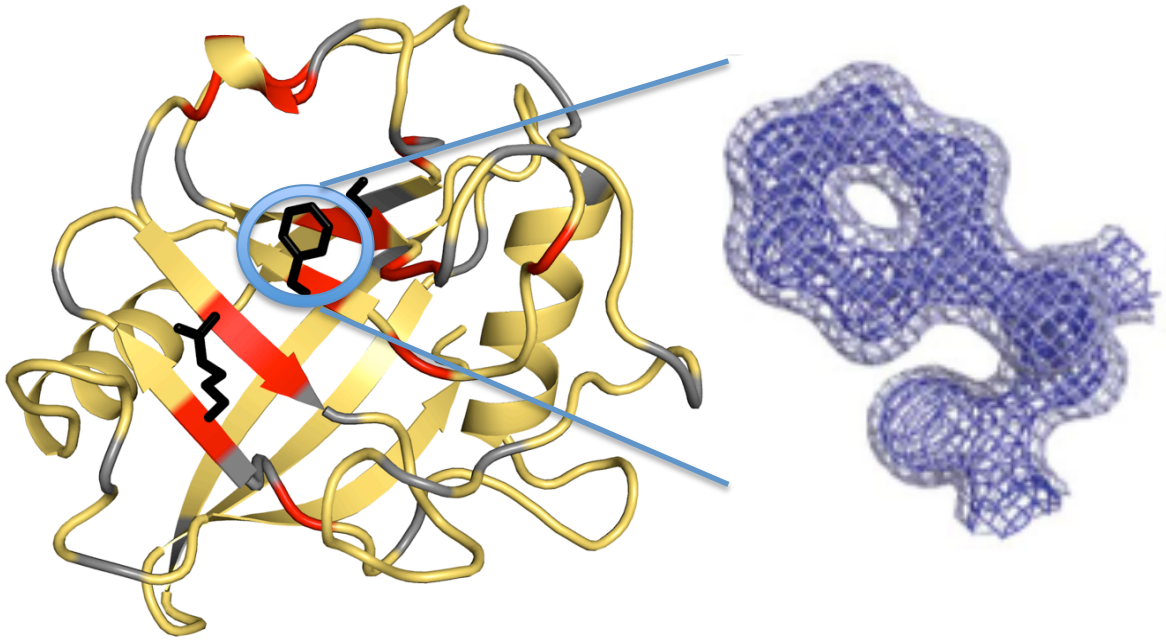


CypA major/minor conformation

Fraser et.al (2009)

Room-temperature crystallography reveals catalytic Cyclophilin A conformations

Phe 113



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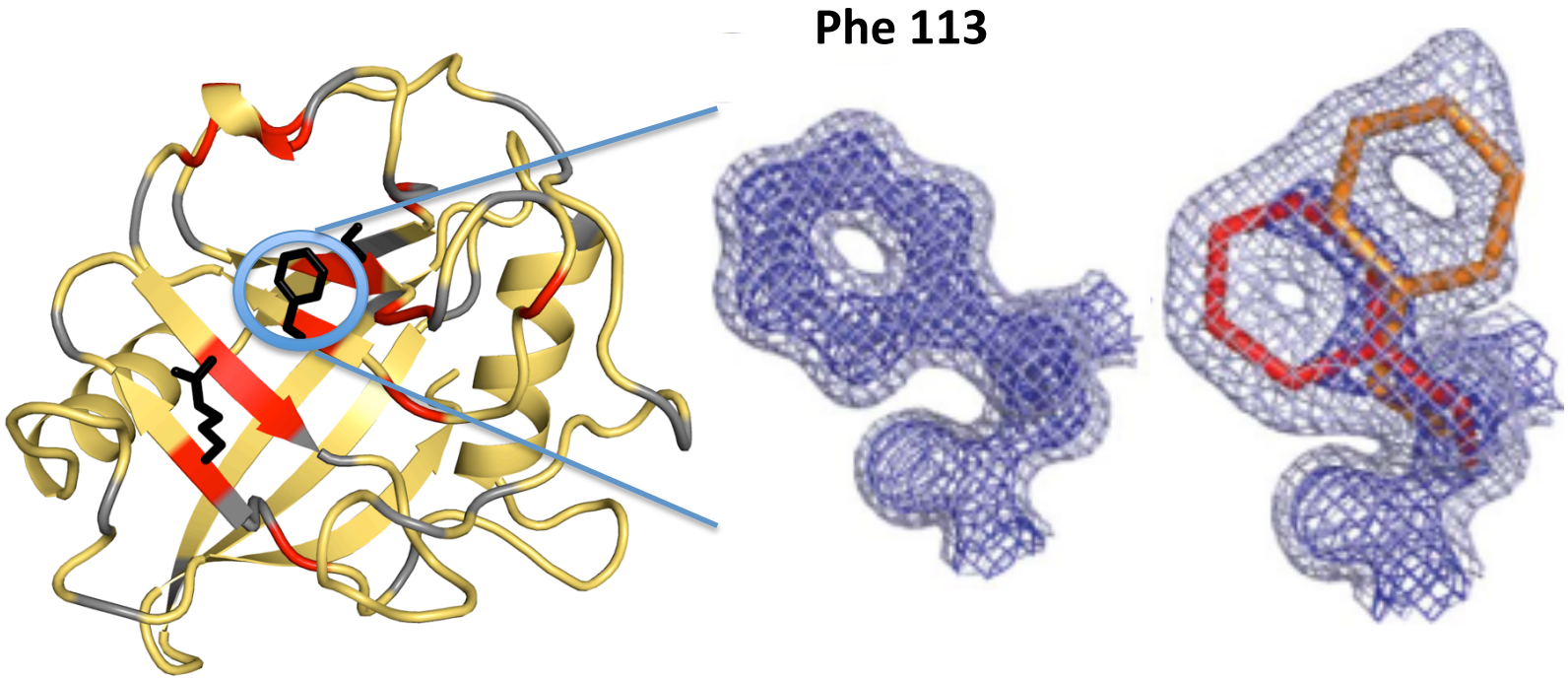

CypA major/minor conformation

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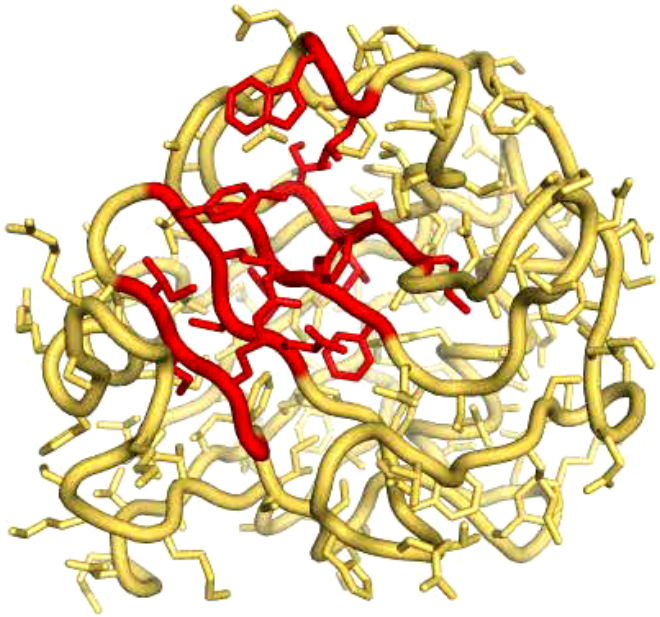
288K

Fraser et.al (2009)

Logjam mutations reduce CypA conformational exchange and catalysis



Fraser lab

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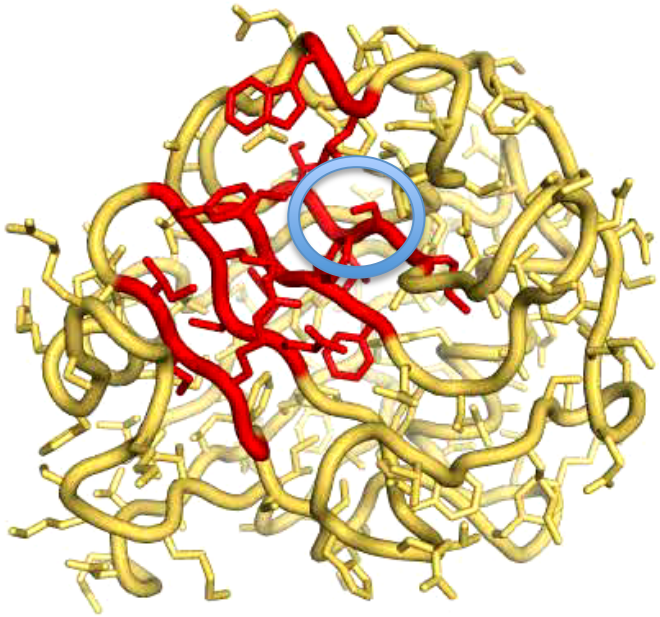

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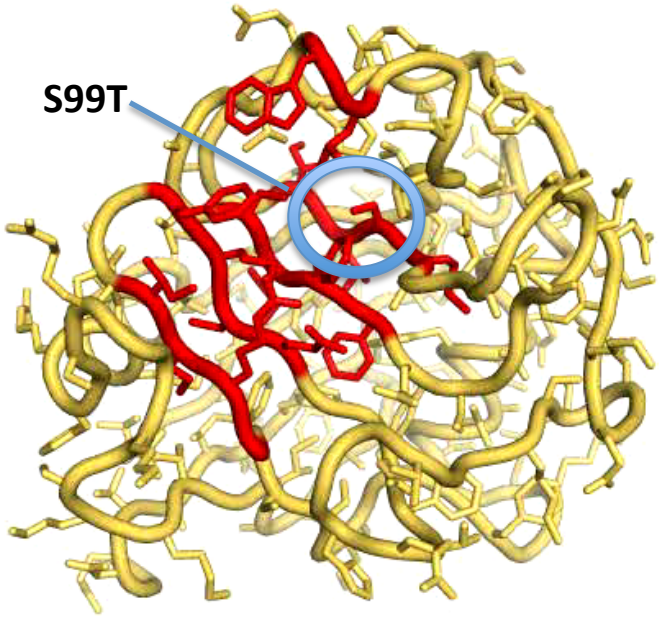

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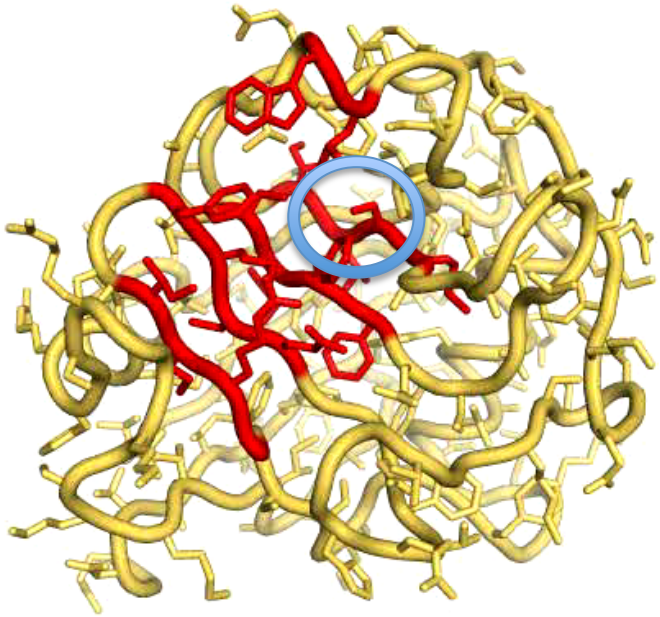

~60x decrease in NMR dynamics

Fraser lab

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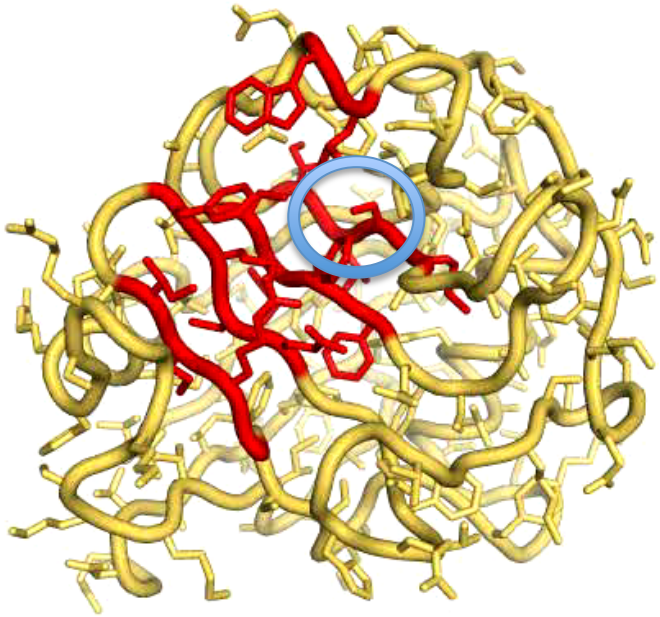
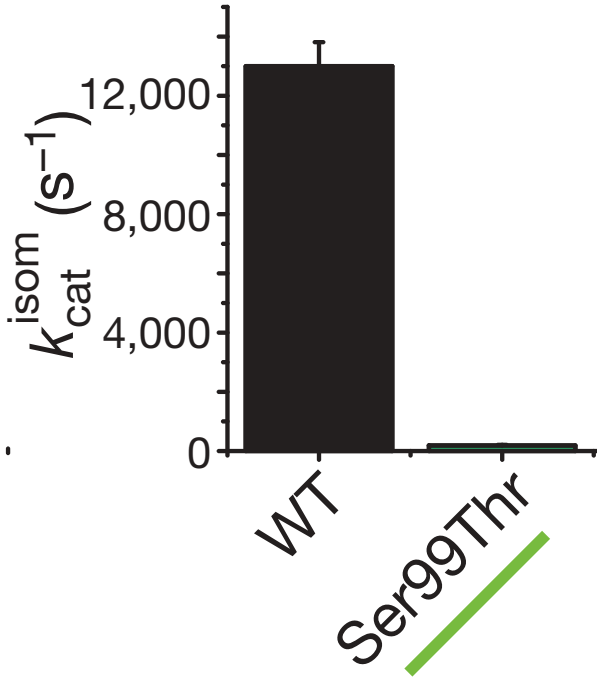


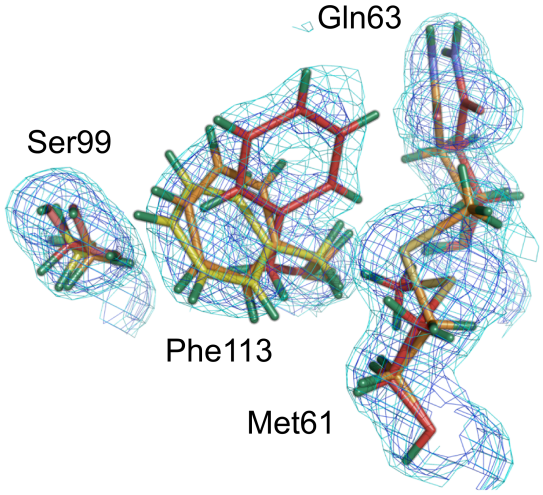


~60x decrease in NMR dynamics

~68x decrease in catalysis

Fraser lab

Computational analysis reveals connected residue networks



CONTACT (van den Bedem* et al., 2013*)

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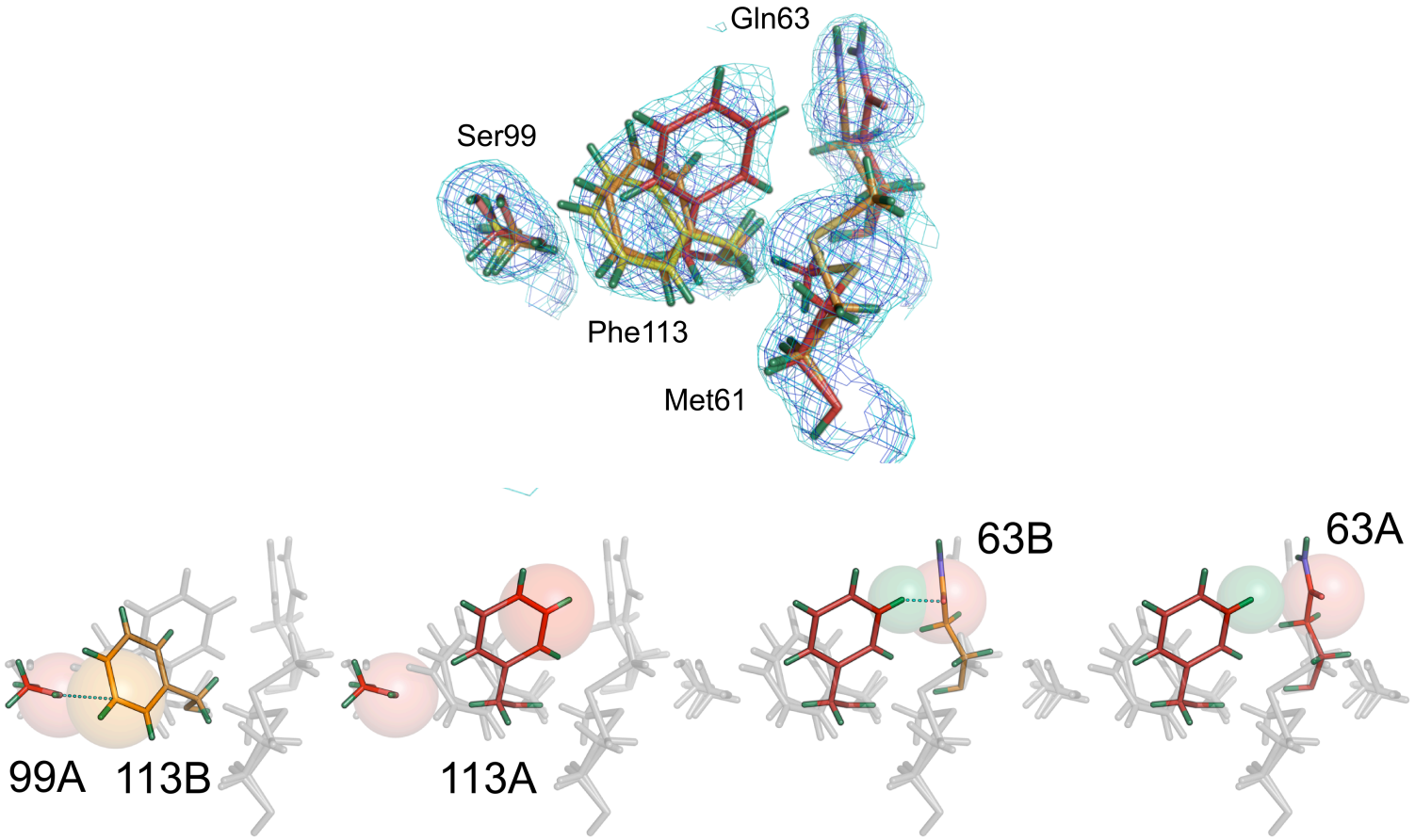

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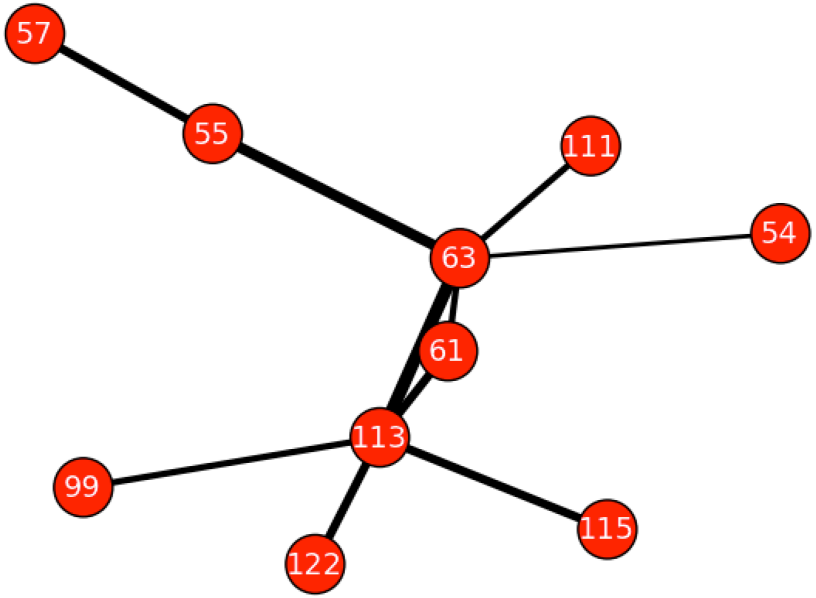

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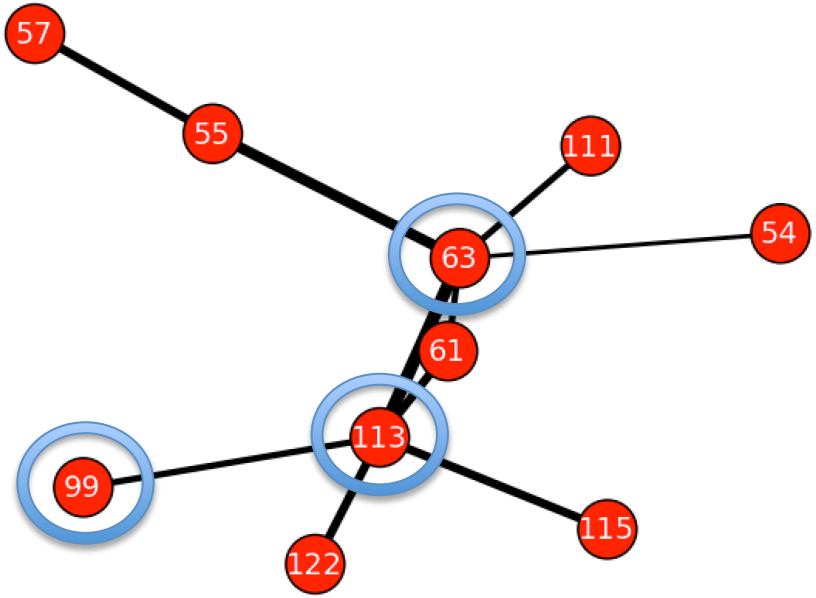

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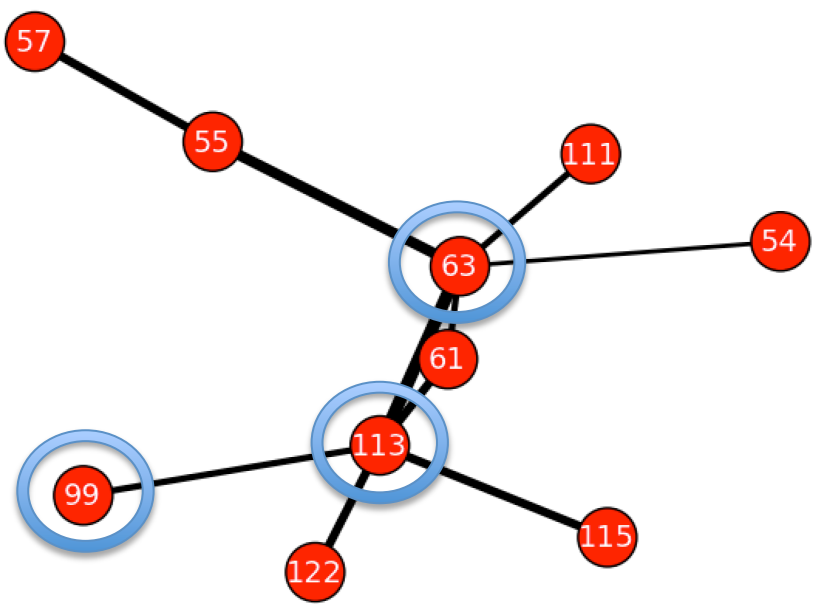

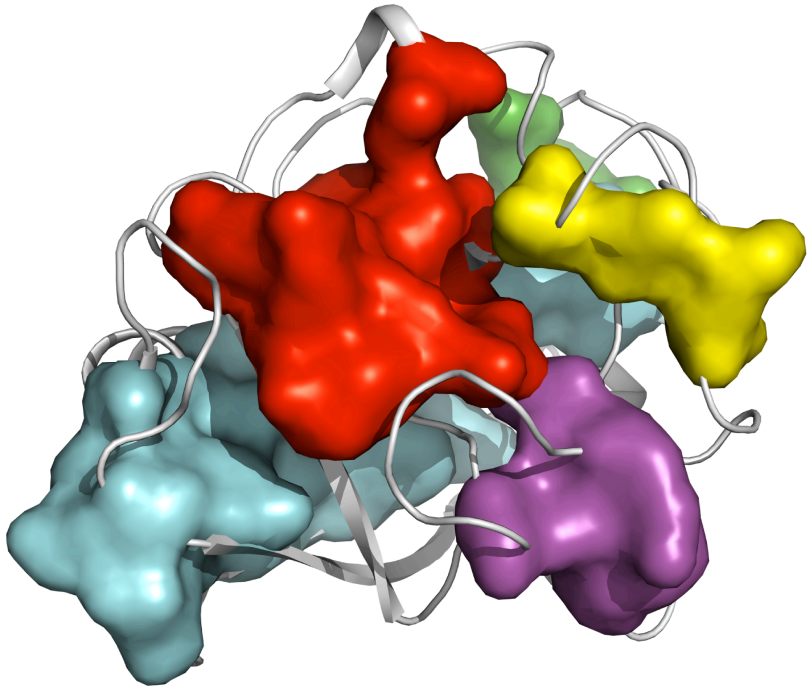

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This is precisely what diffuse scatter could measure!

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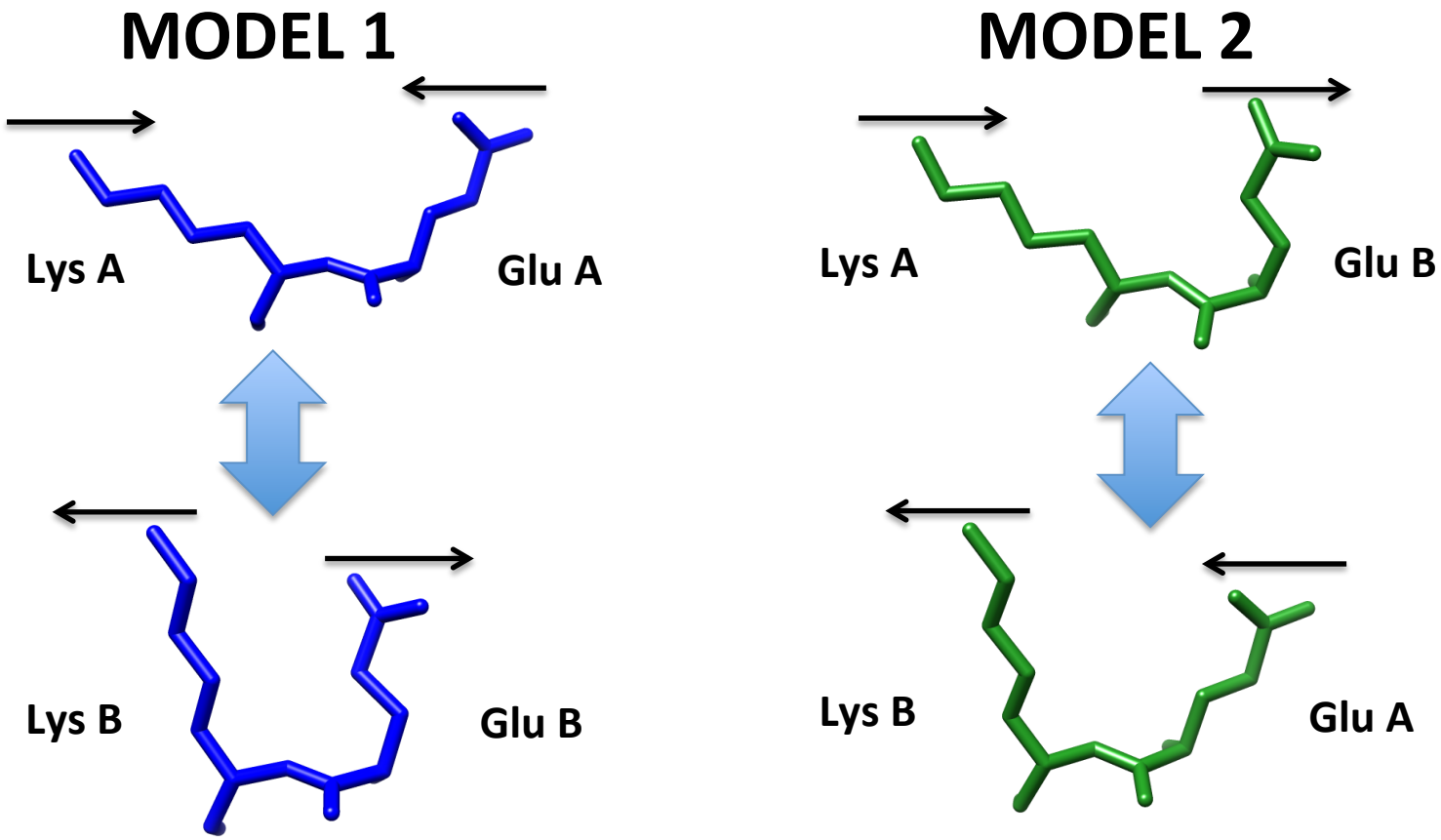
…if signal to noise was infinite

Bragg crystallography yields degenerate models



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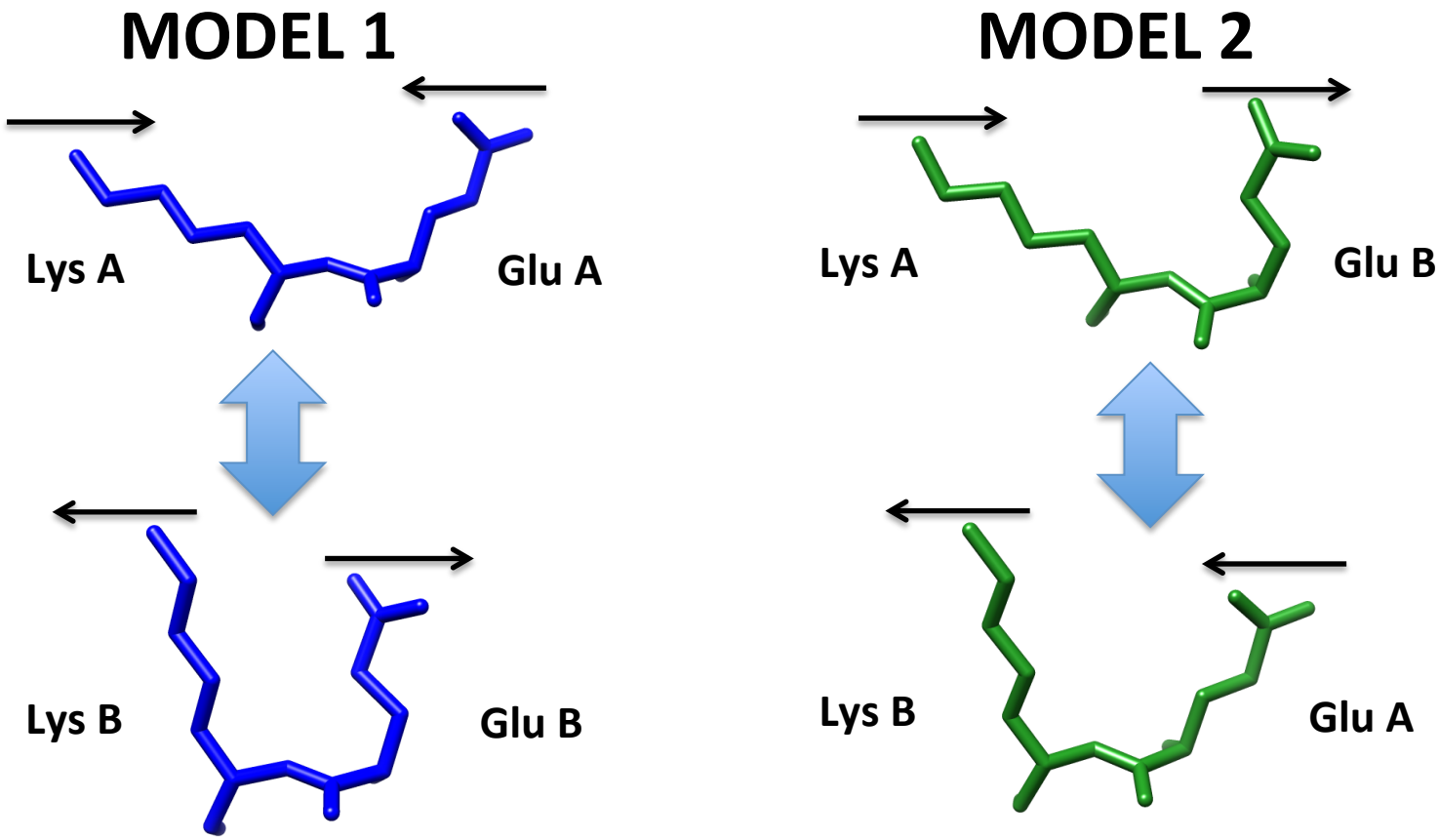
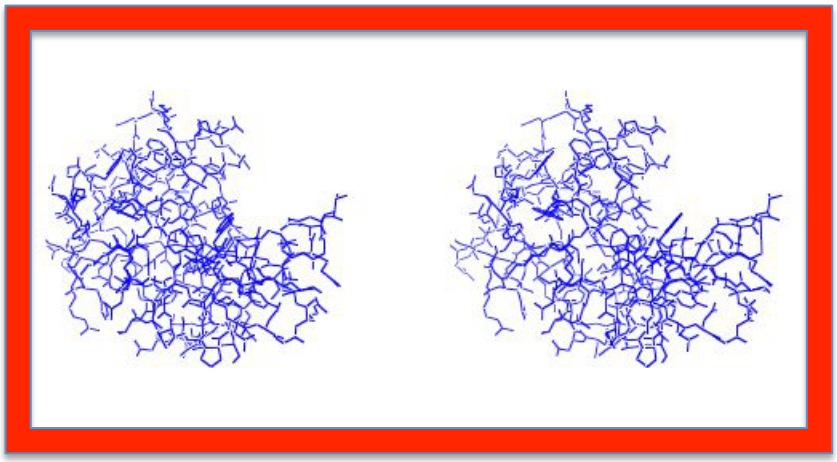


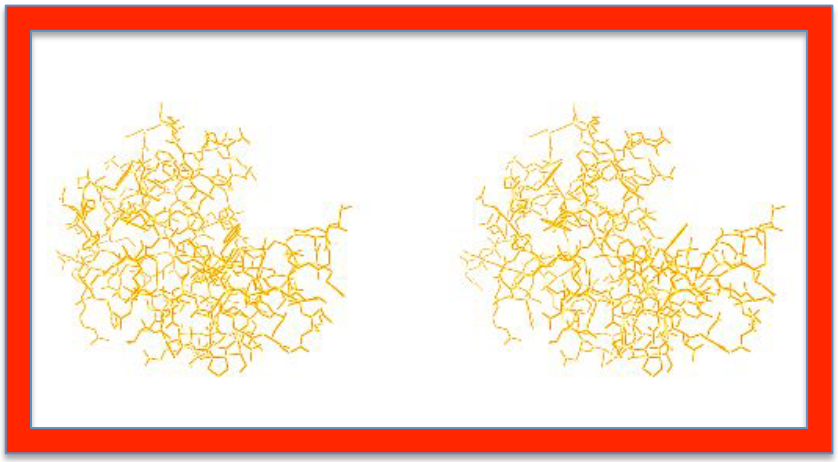
Both models have identical Bragg diffraction!

Bragg crystallography yields degenerate models

Model #1 (*correlated*)



Model #2 (*anti-correlated*)



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Diffuse scattering patterns are non-degenerate!

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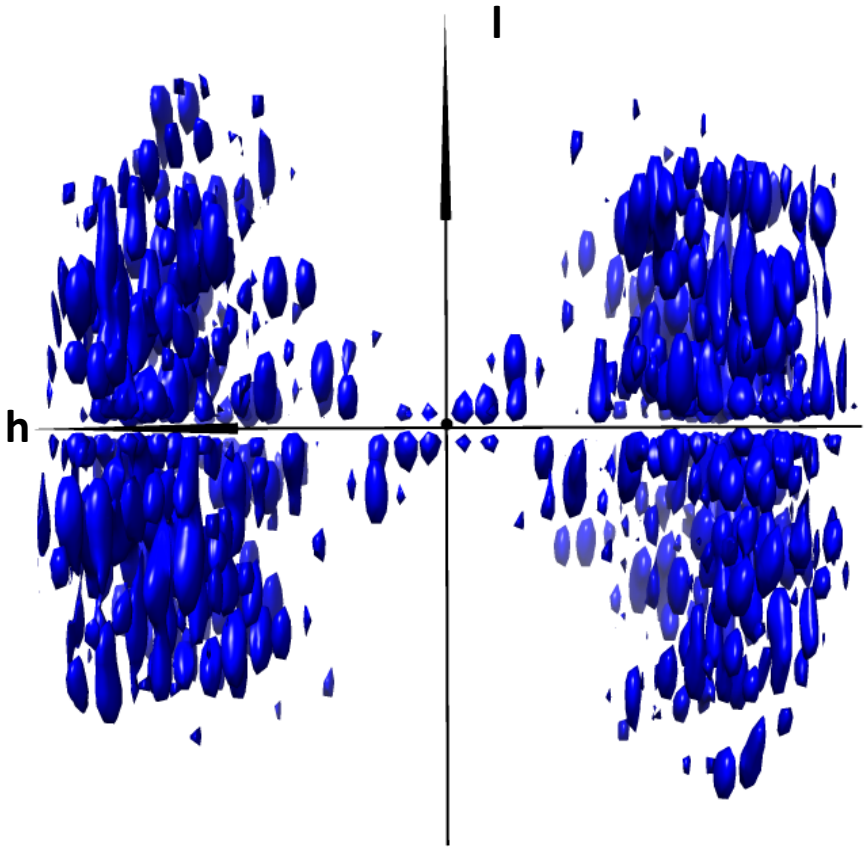

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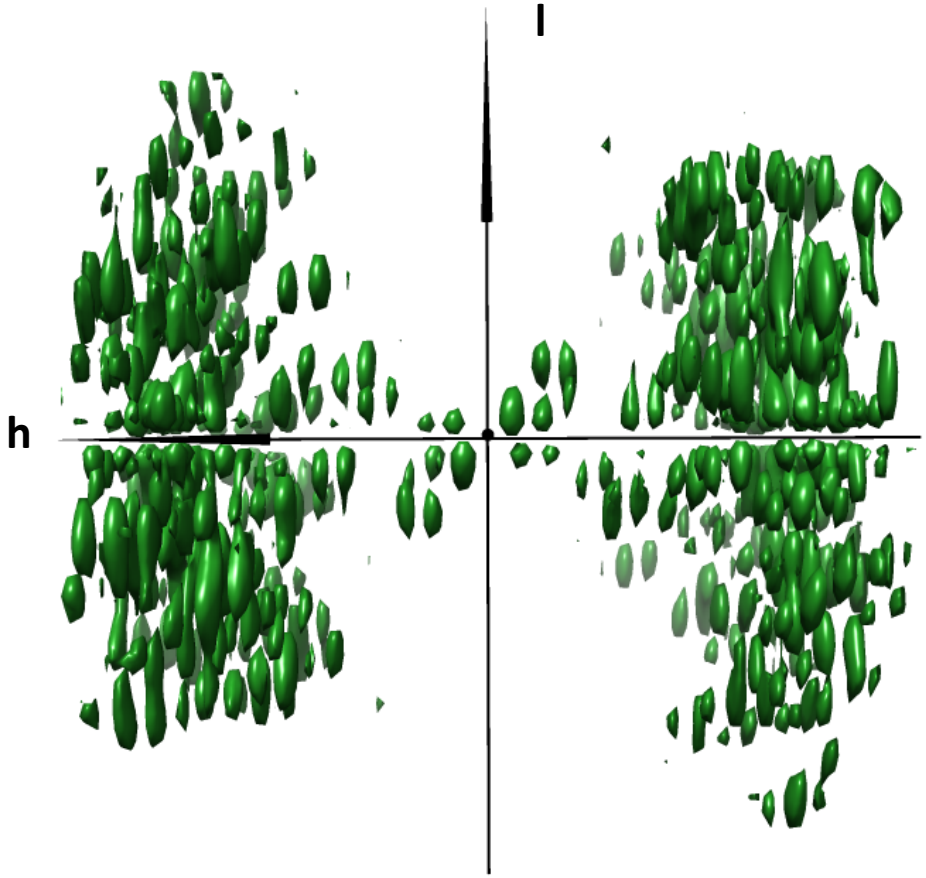

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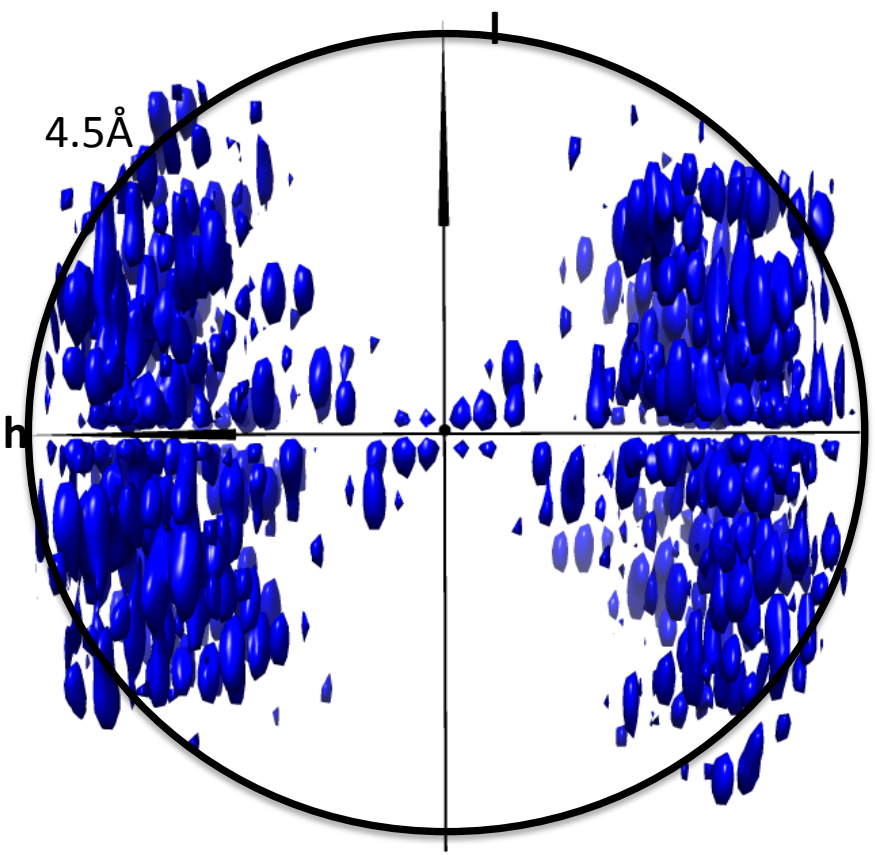

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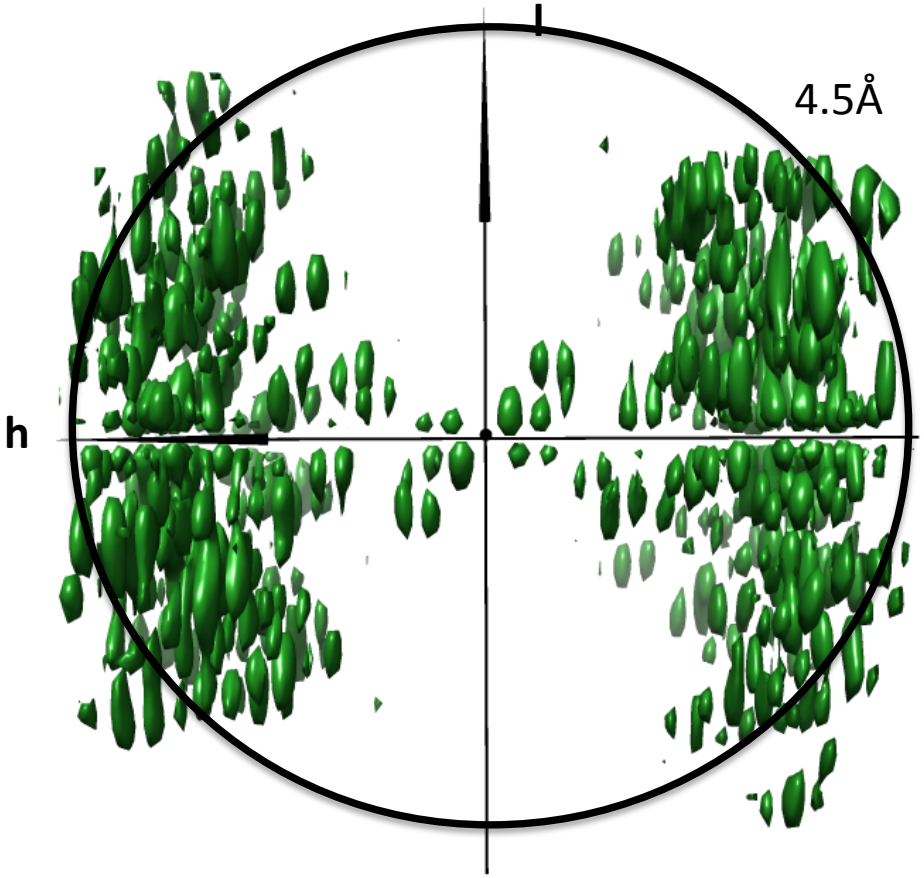

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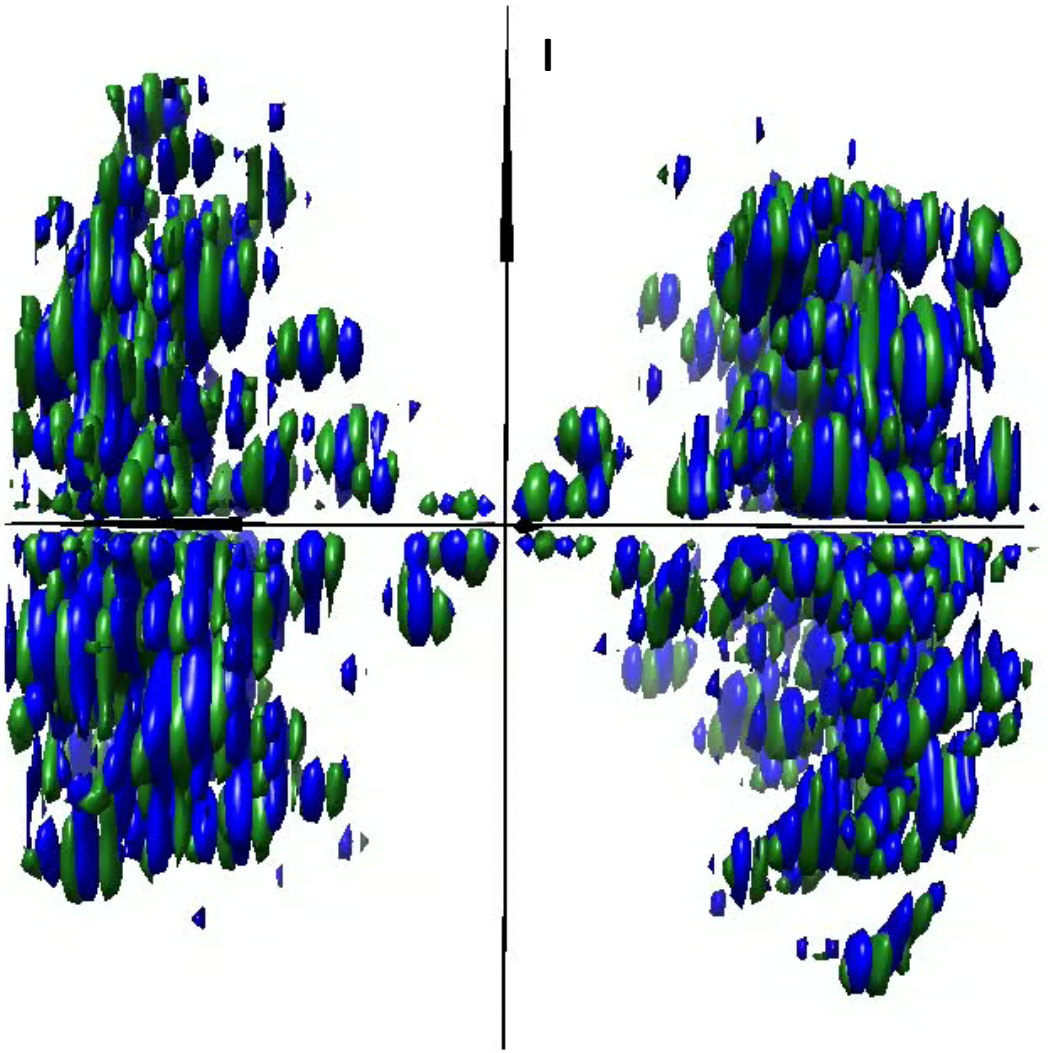


Diffuse scatter can be collected on a variety of detector platforms

Advanced Light Source

Charge-coupled device area detector

Alternate collection of Bragg frames and diffuse frames (1°/0.1° oscillation)

Crystal translation during rotation

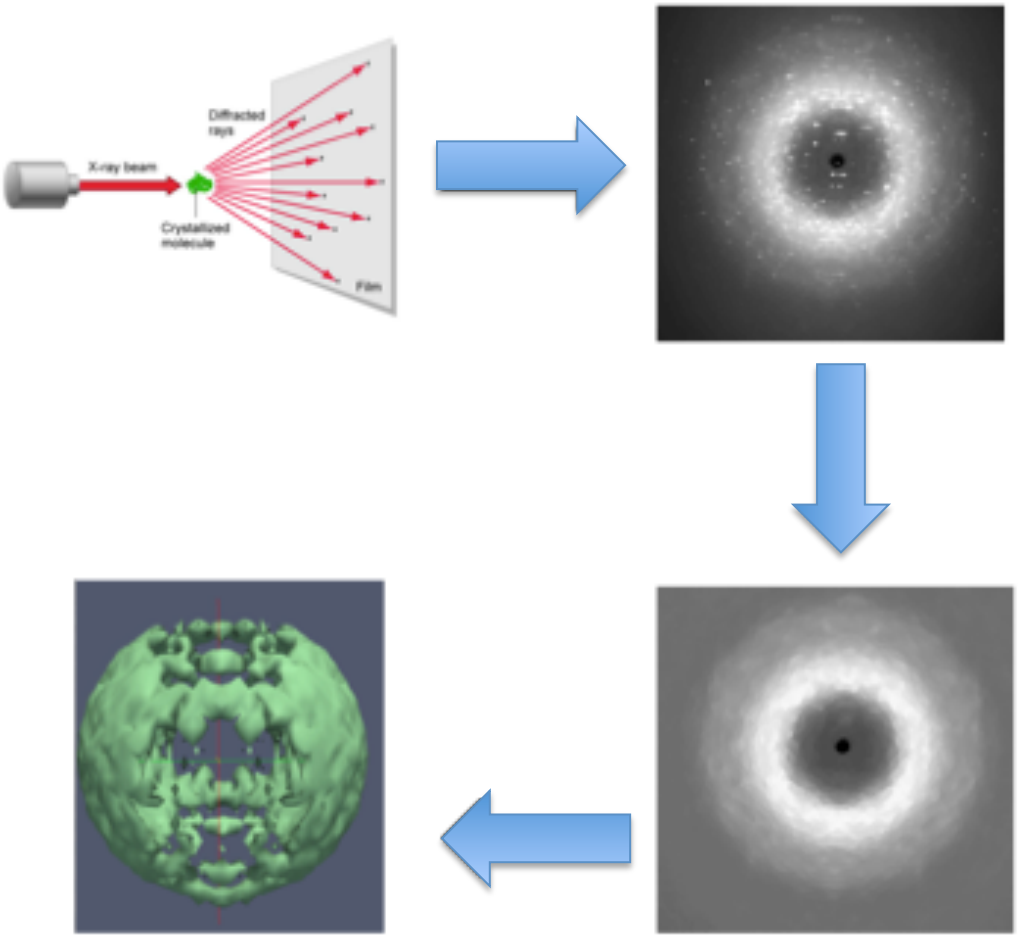
Stanford Synchrotron Radiation Lightsource

PILATUS 6m detector

Simultaneous collection of Bragg/ diffuse frames (fine phi slicing)

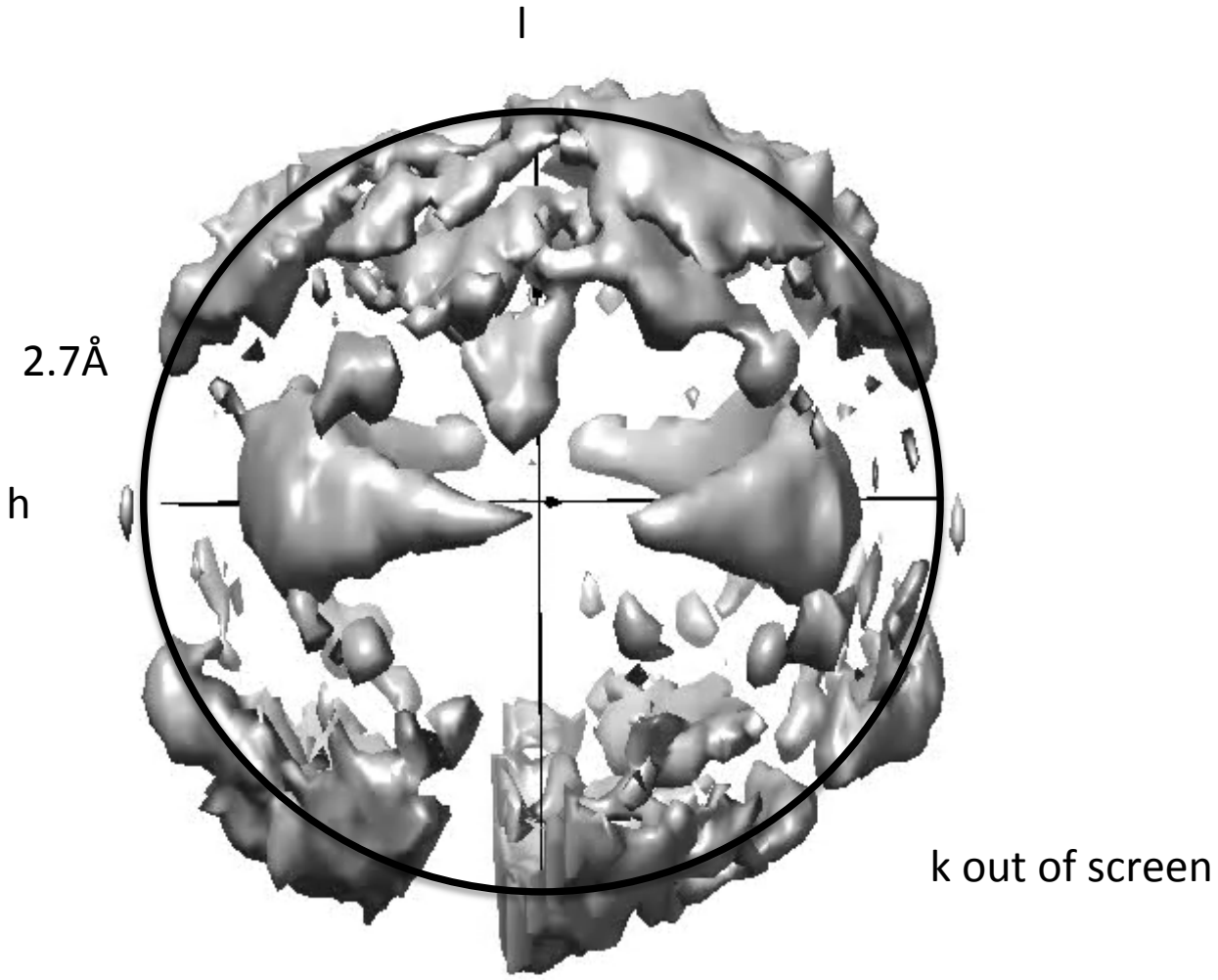
Crystal translation during rotation

Diffuse scatter maps are created using LUNUS

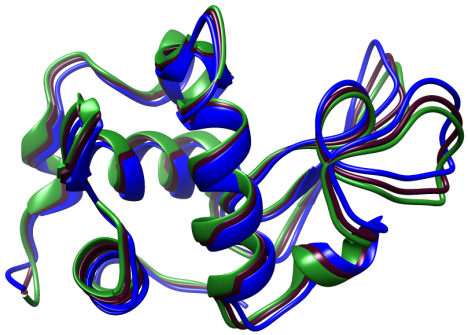


Wall et al (1997)

CypA diffuse scatter map



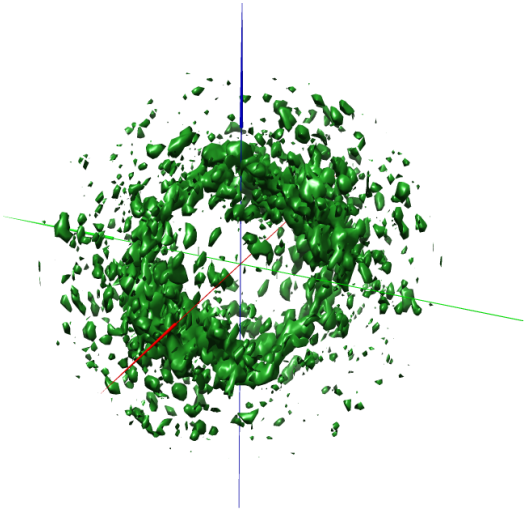
Phenix calculates diffuse scatter maps from structural ensembles



Phenix

UCSF CHIMERA

an Extensible Molecular Modeling System

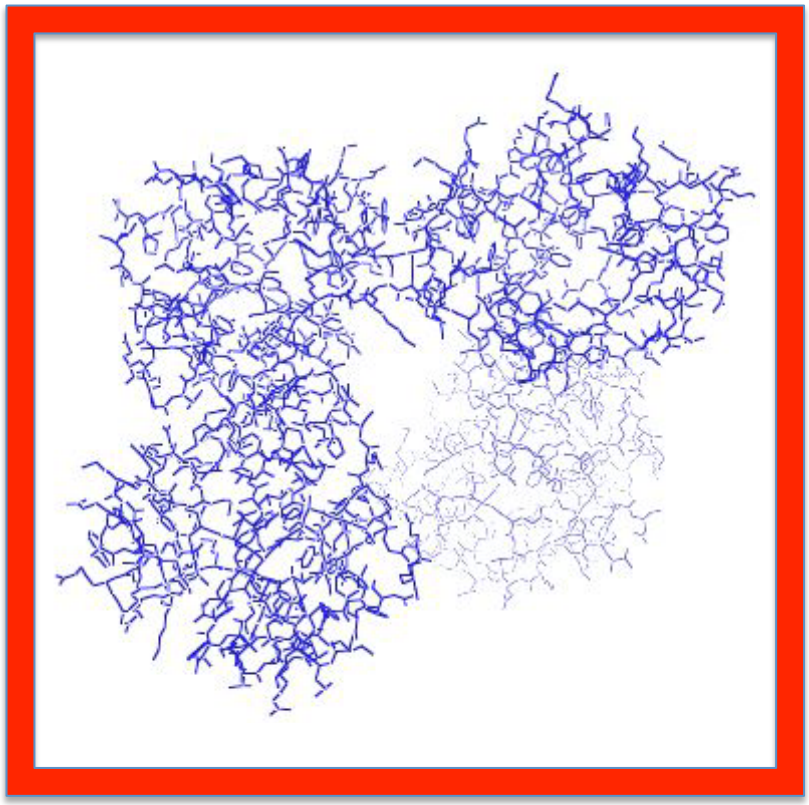


Structural ensemble

Get_struct_fact_from_md.py

3D diffuse map

Normal mode motions in CypA unit cell produce unique diffuse intensity



http://lorentz.immstr.pasteur.fr/nomad-ref.php

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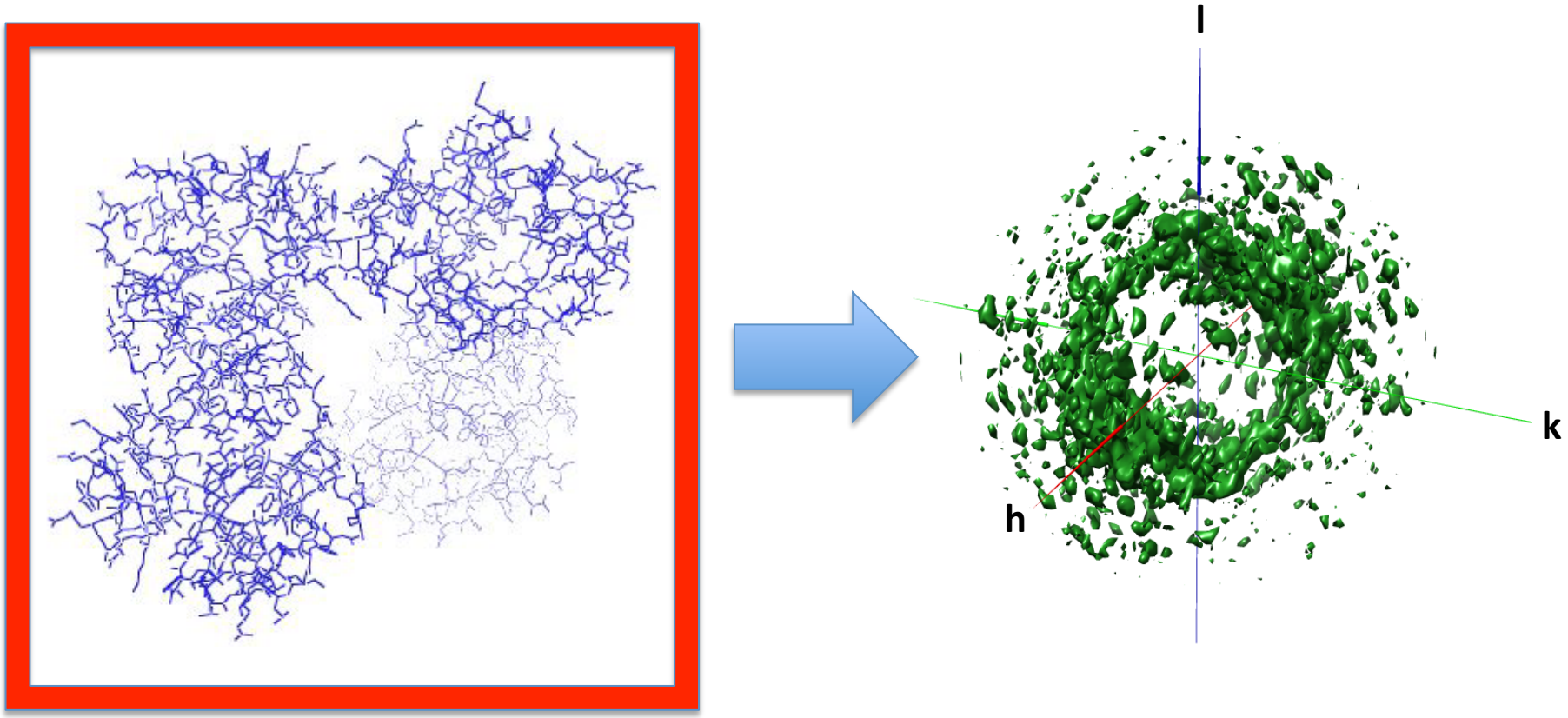

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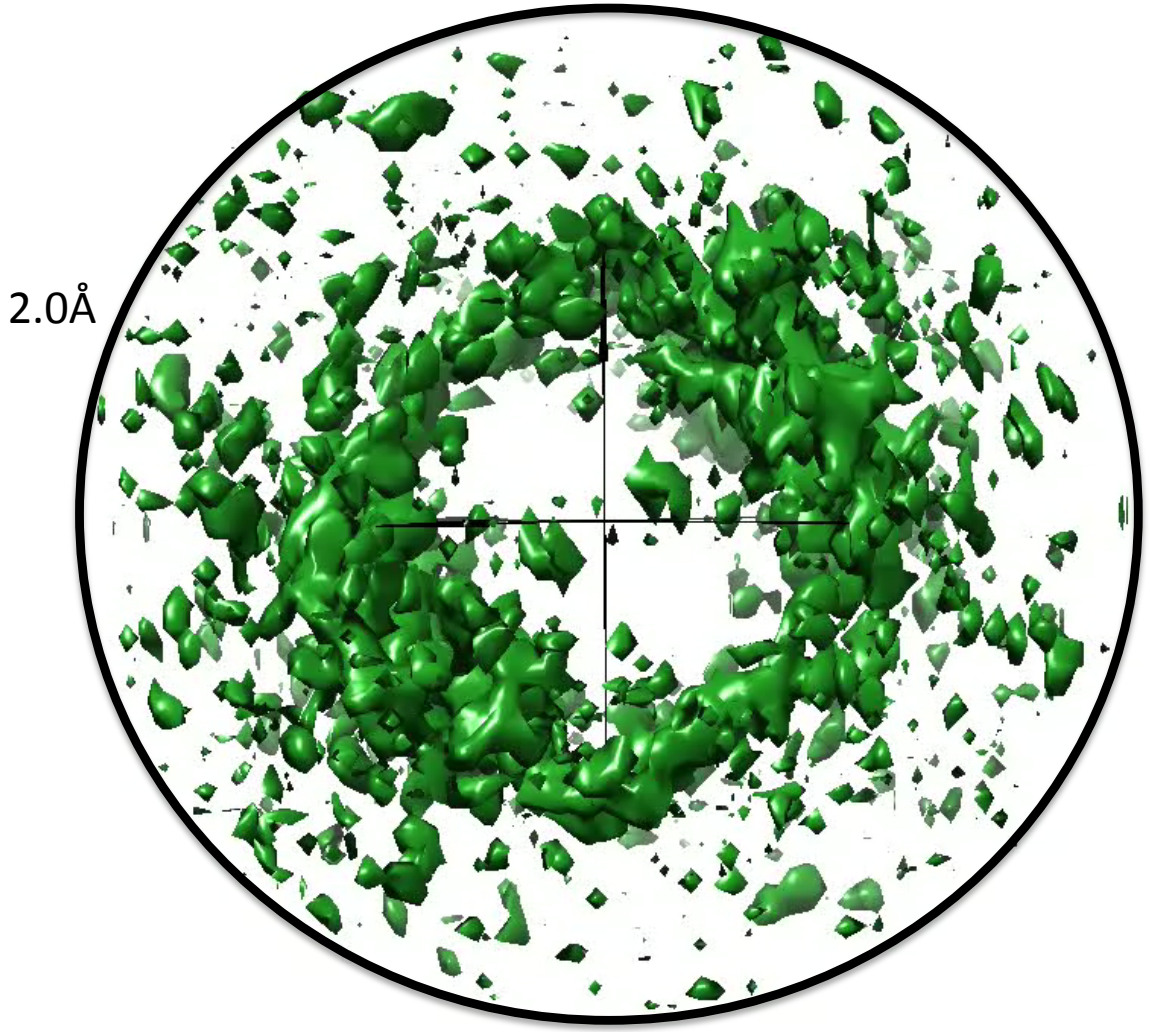
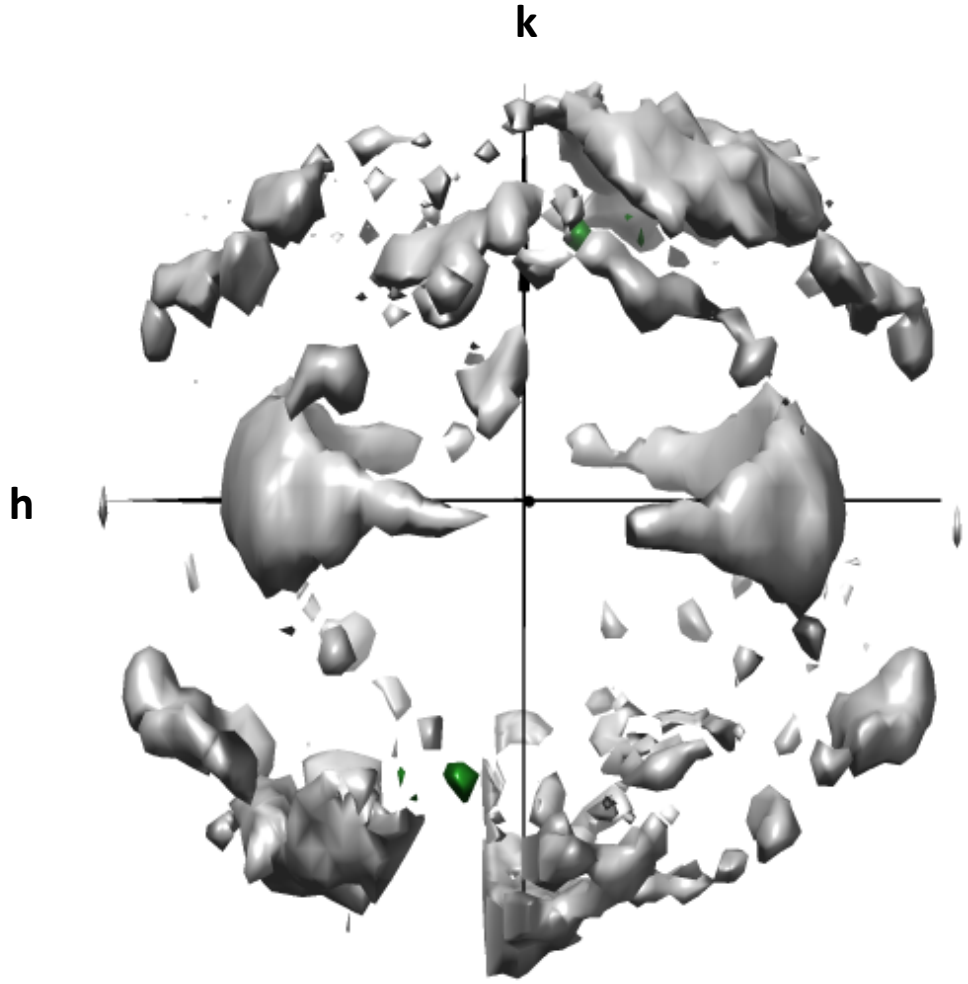
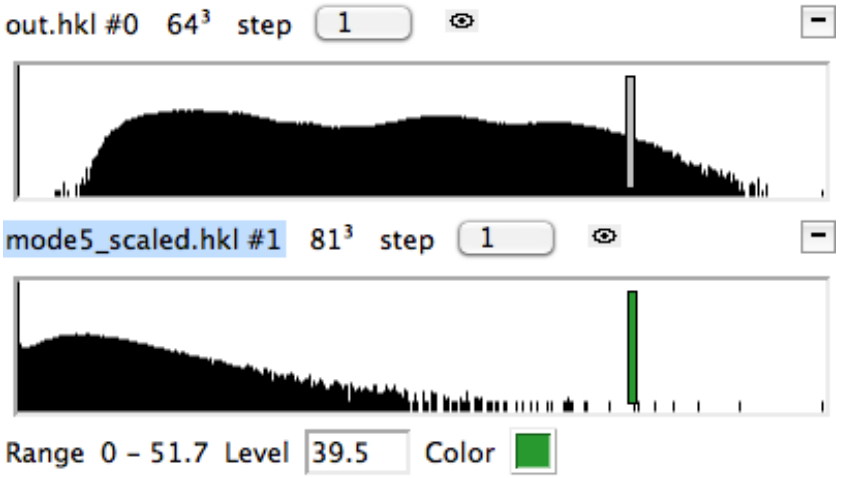


Next step: statistical comparisons



Grey: CypA experimental data
Green: Normal mode simulation



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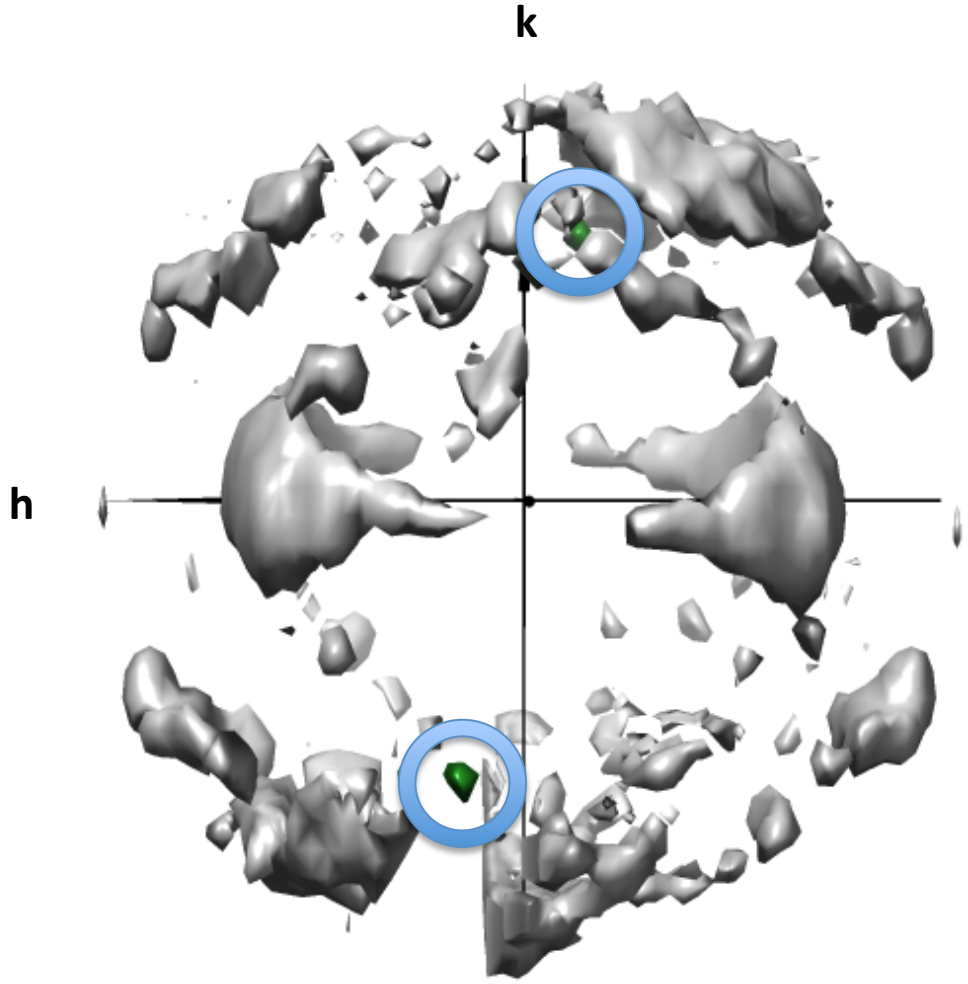

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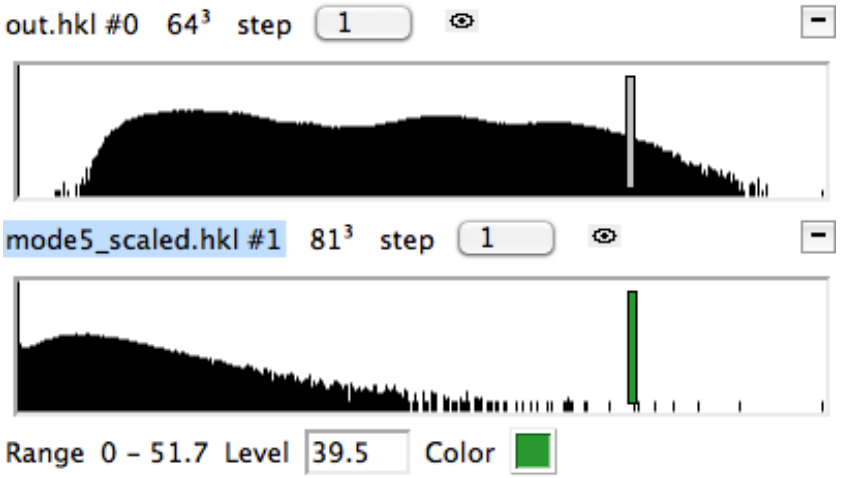

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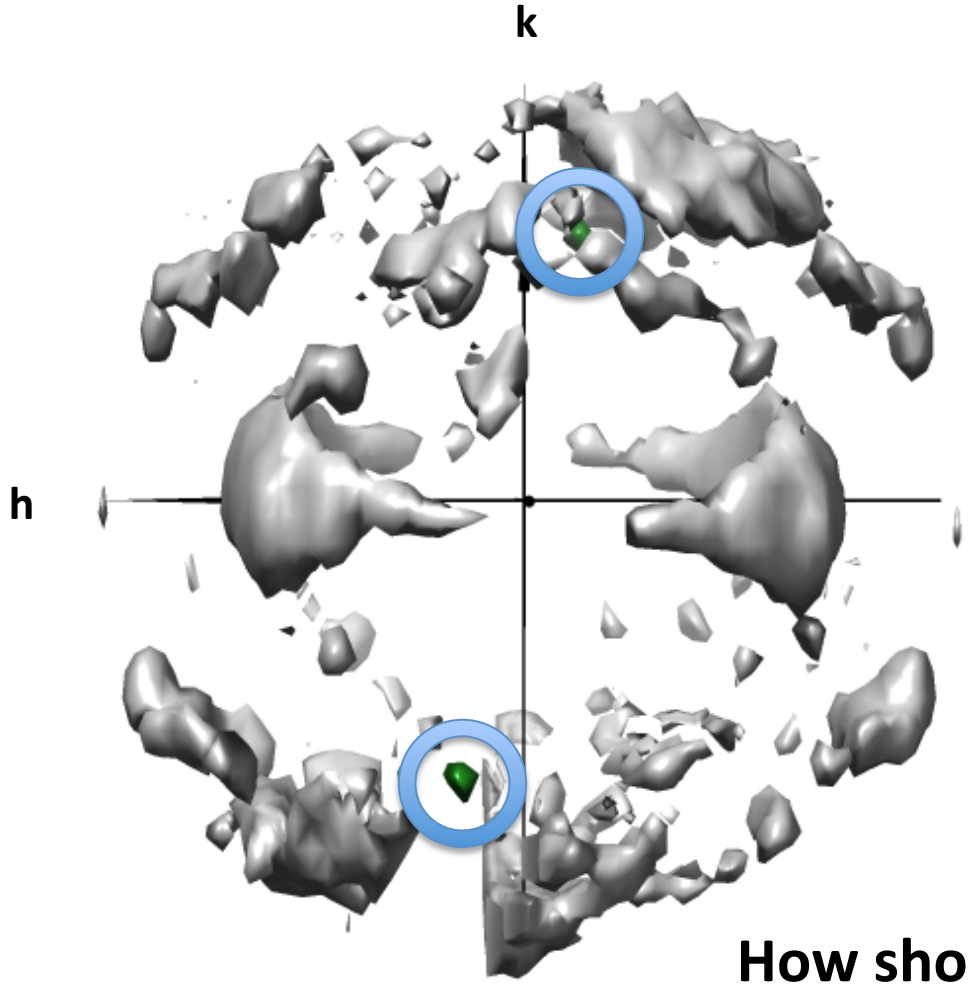

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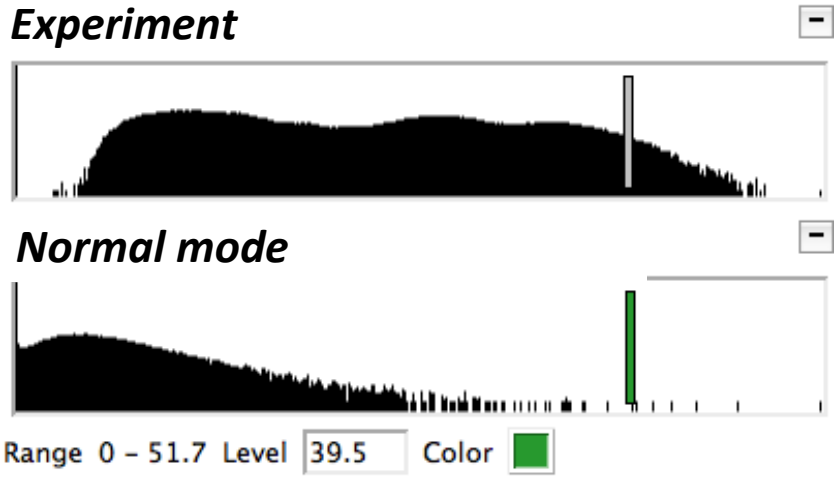
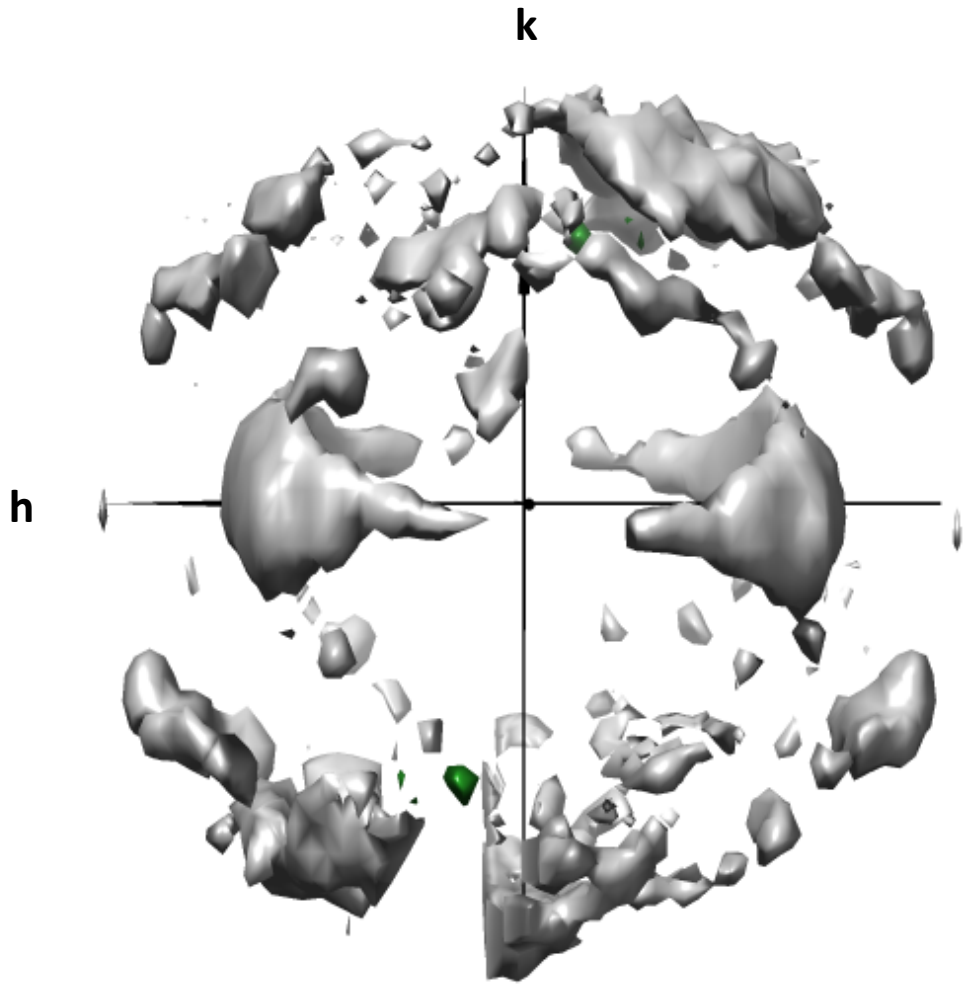
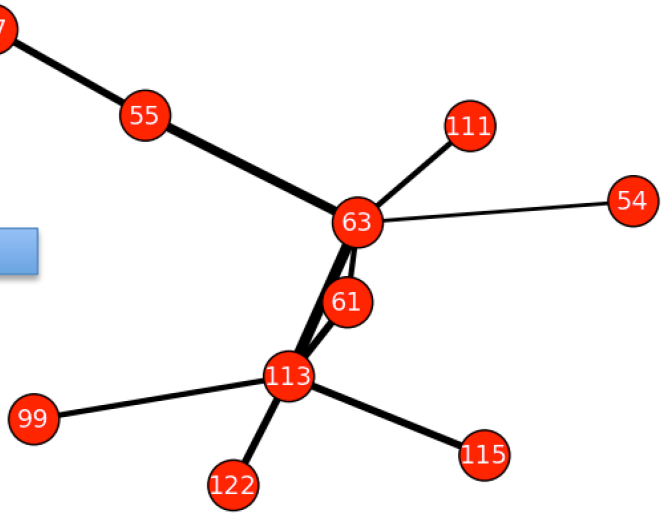


How should we compare maps? R factor?
Pearson Correlation Coefficient?

Next step: statistical comparisons

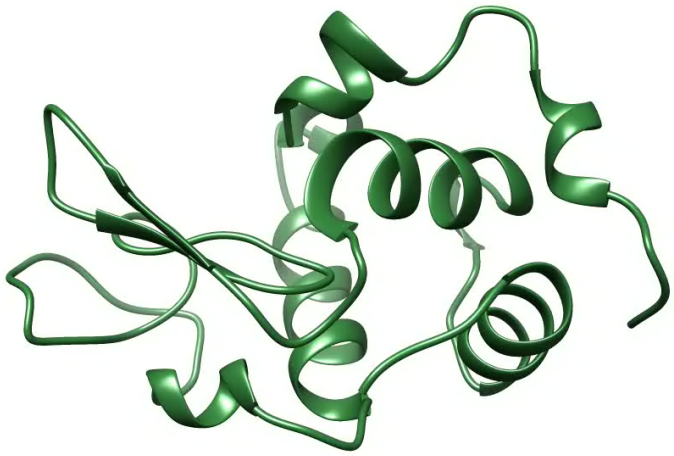




Diffuse scattering might probe links between correlated side chain motions with enzyme catalysis

Protein function requires correlated motion

Lysozyme



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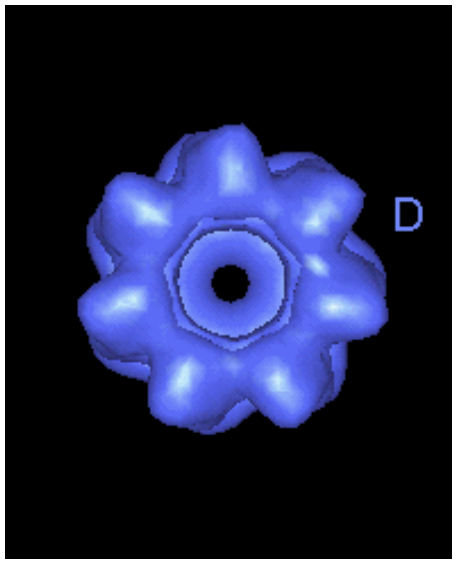
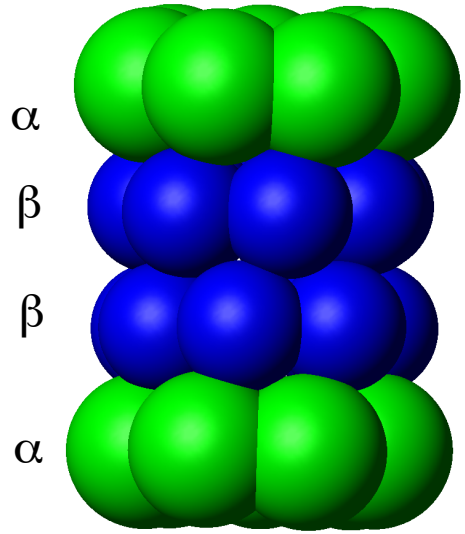


Subunit rotation

Rye et.al (1999)

Small proteins → **Large complexes**

NMR reveals protein conformational exchange in T20S proteasome



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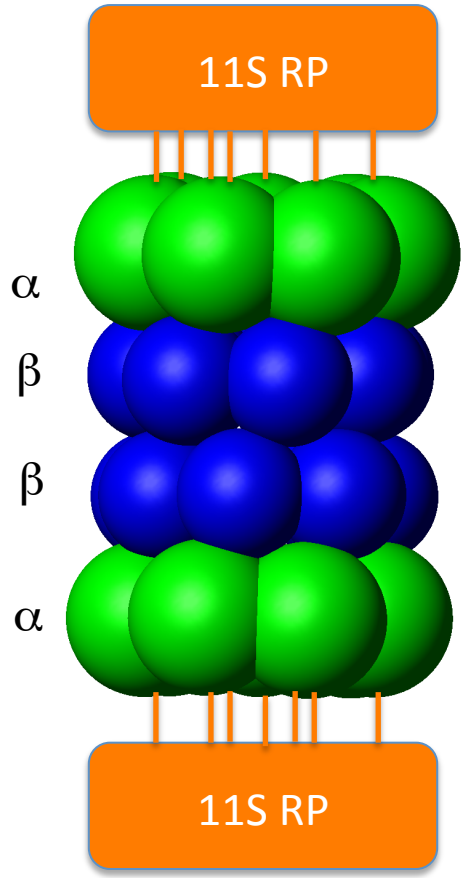

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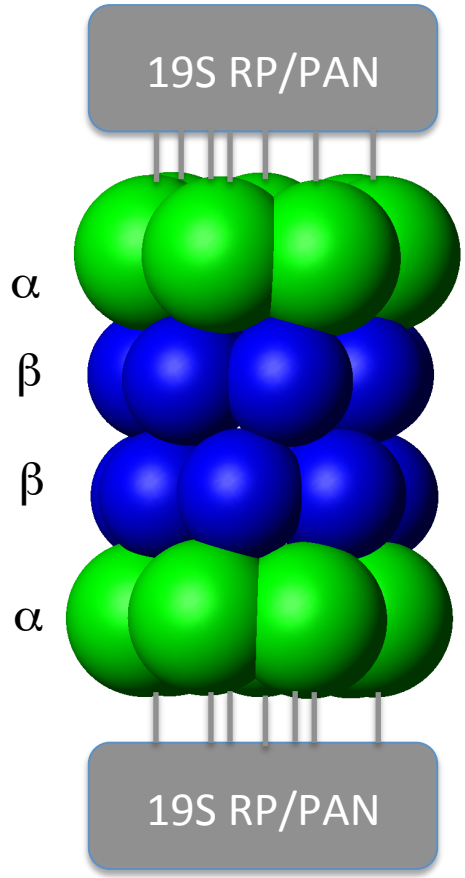

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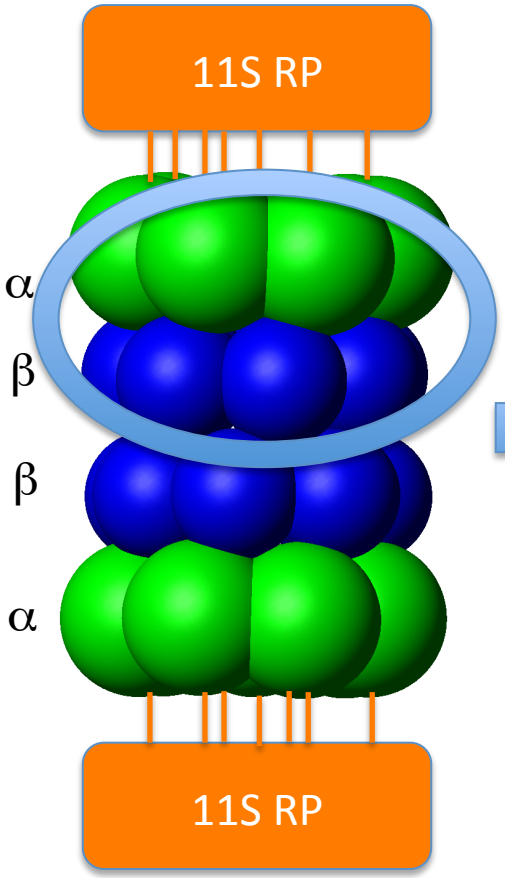
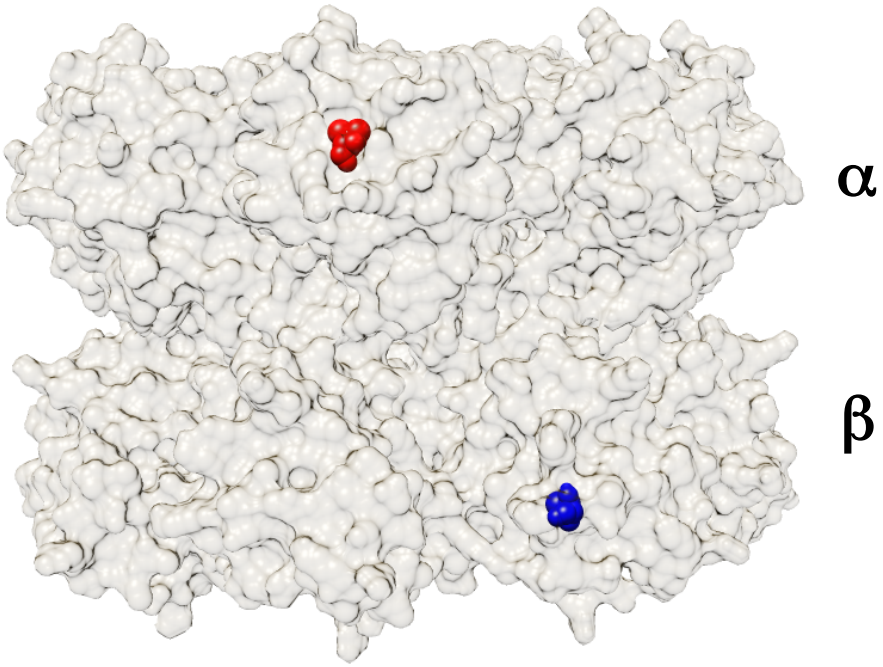
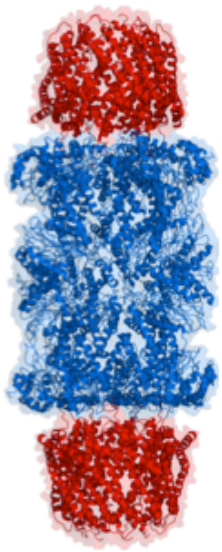




Proteasome allostery allows for multiple functions

Cell Cycle

Cell homeostasis



Apoptosis

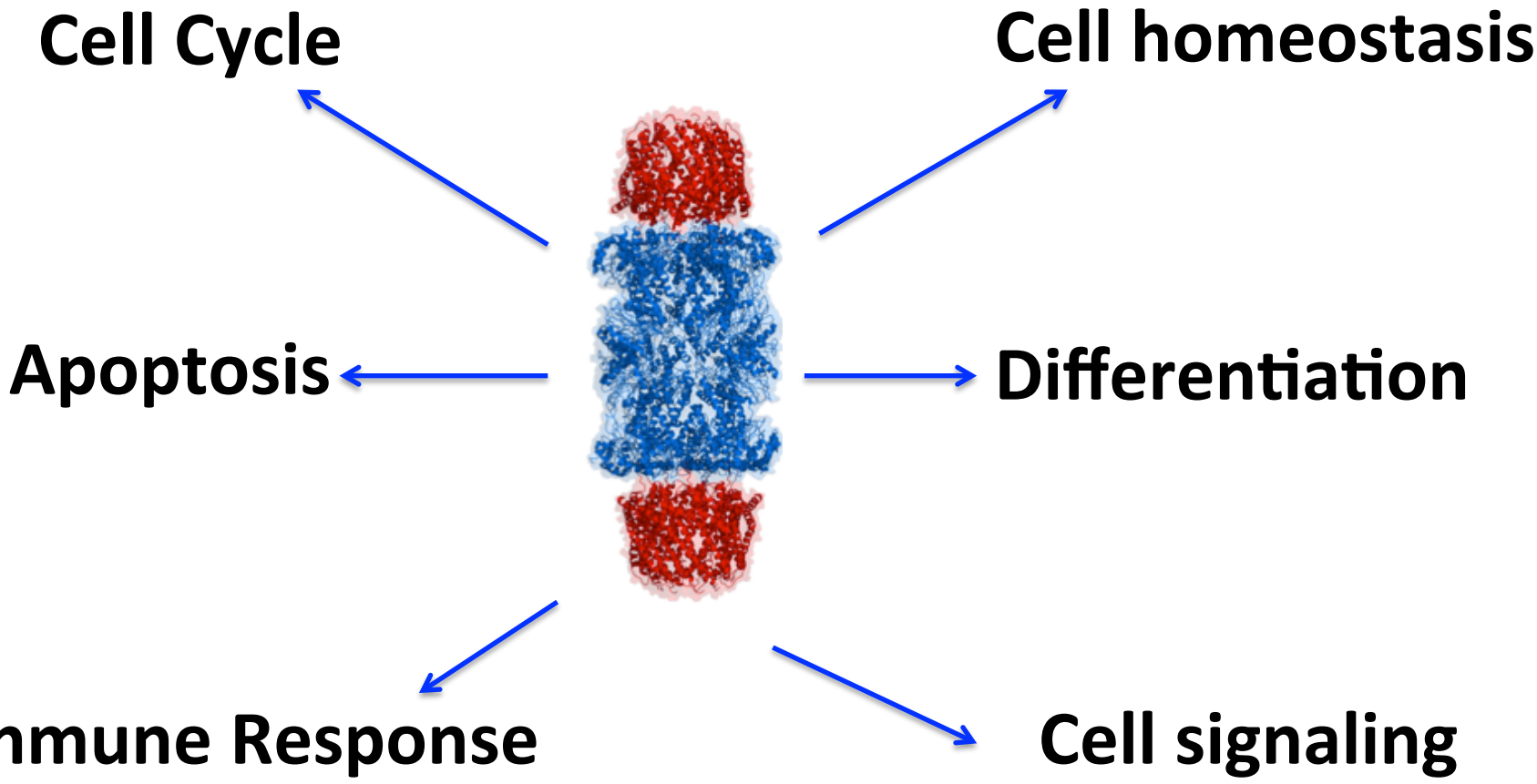
Differentiation

Immune Response

Cell signaling

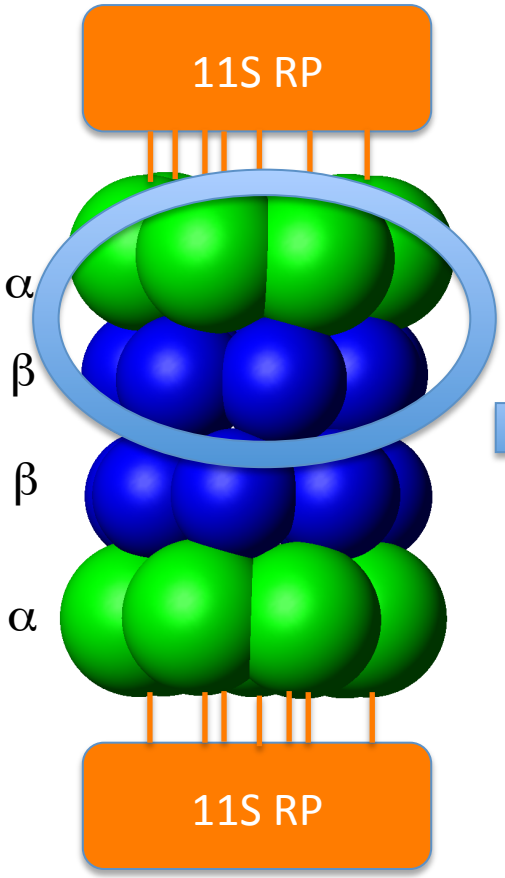
Splettstoesser (2006)

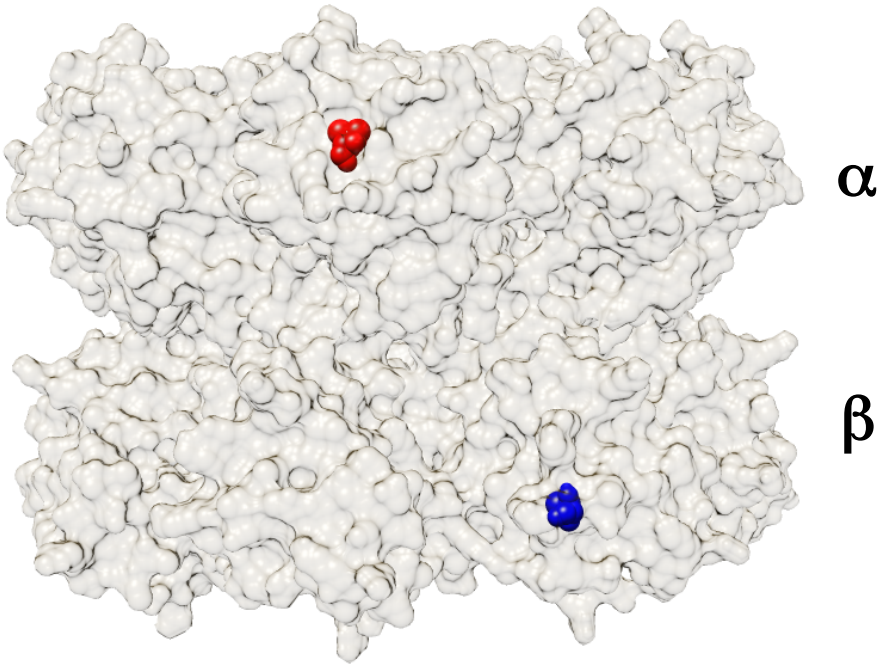
Proteasome allostery allows for multiple functions



Splettstoesser (2006)

NMR reveals protein conformational exchange in T20S proteasome





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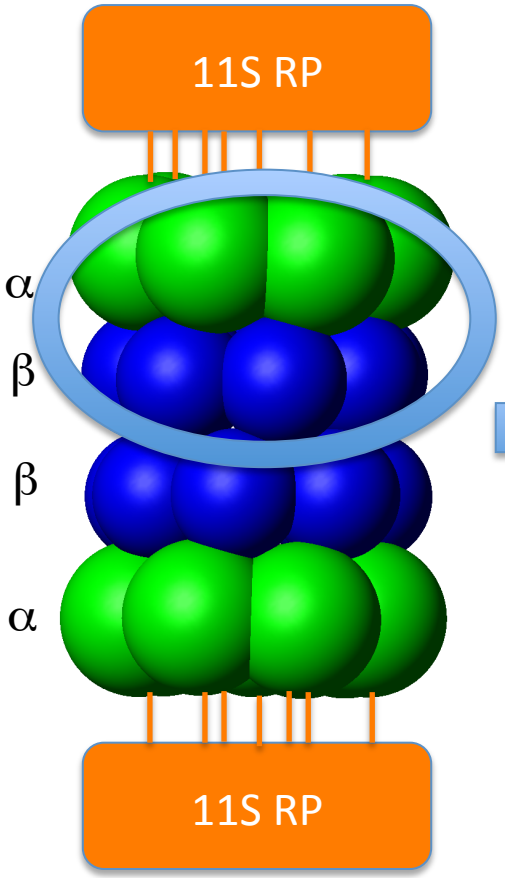
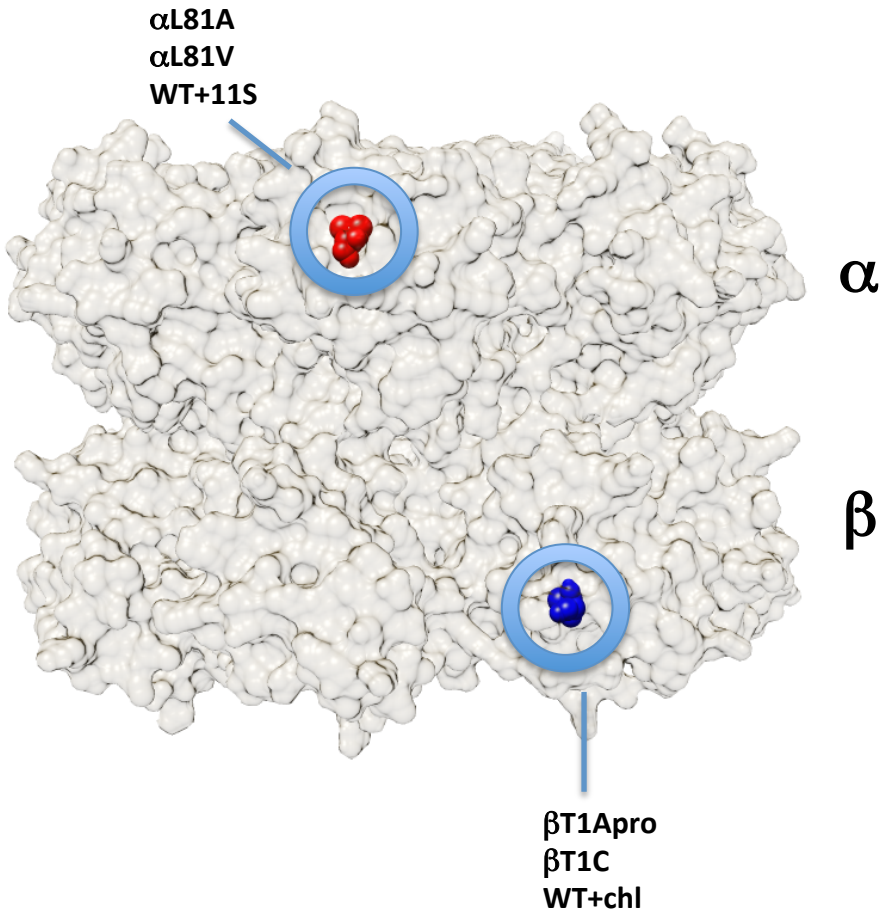
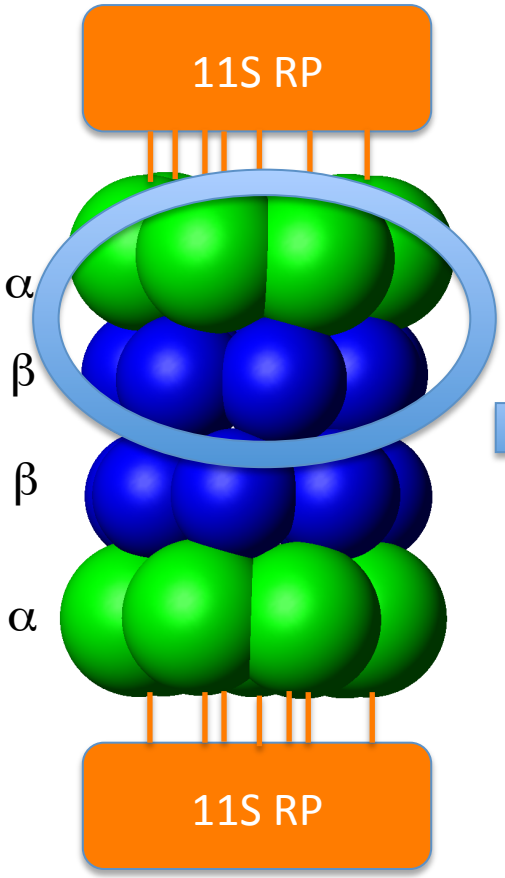
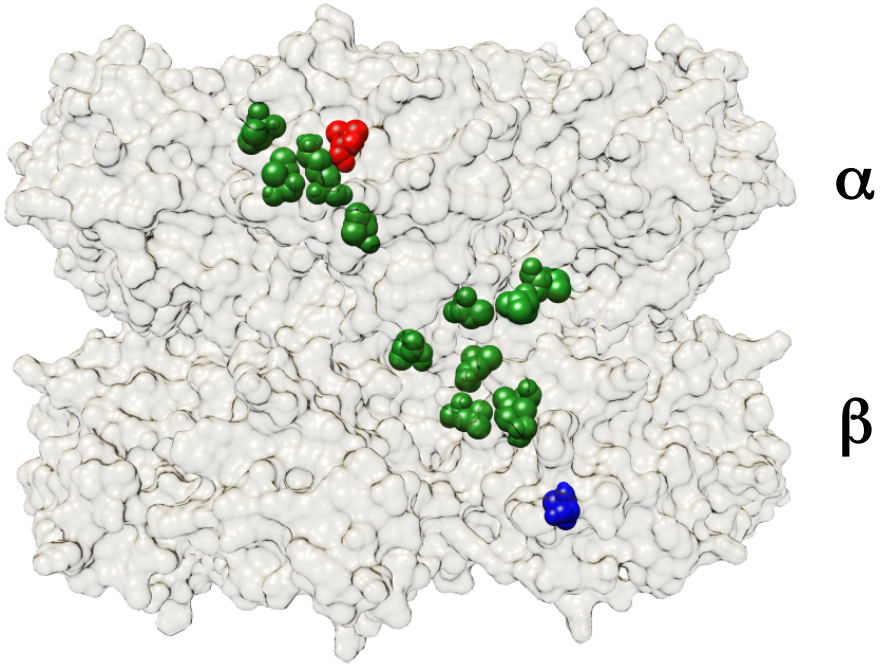




NMR reveals protein conformational exchange in T20S proteasome





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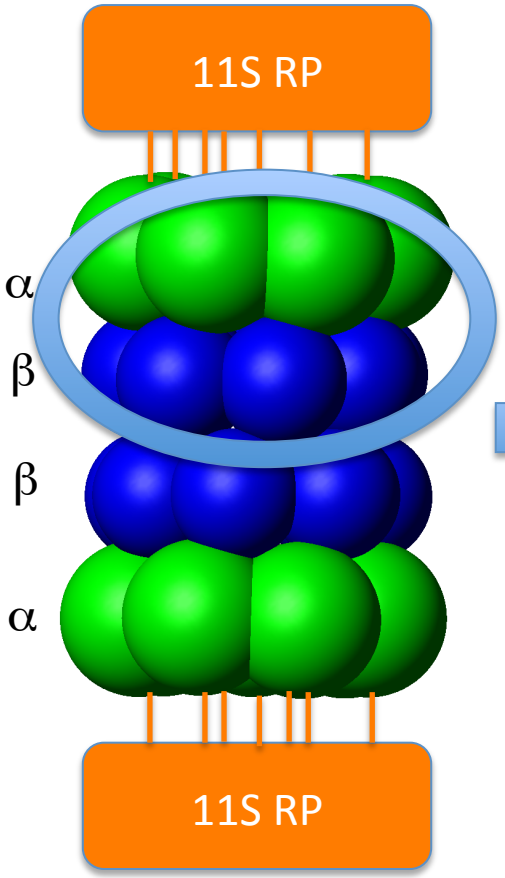

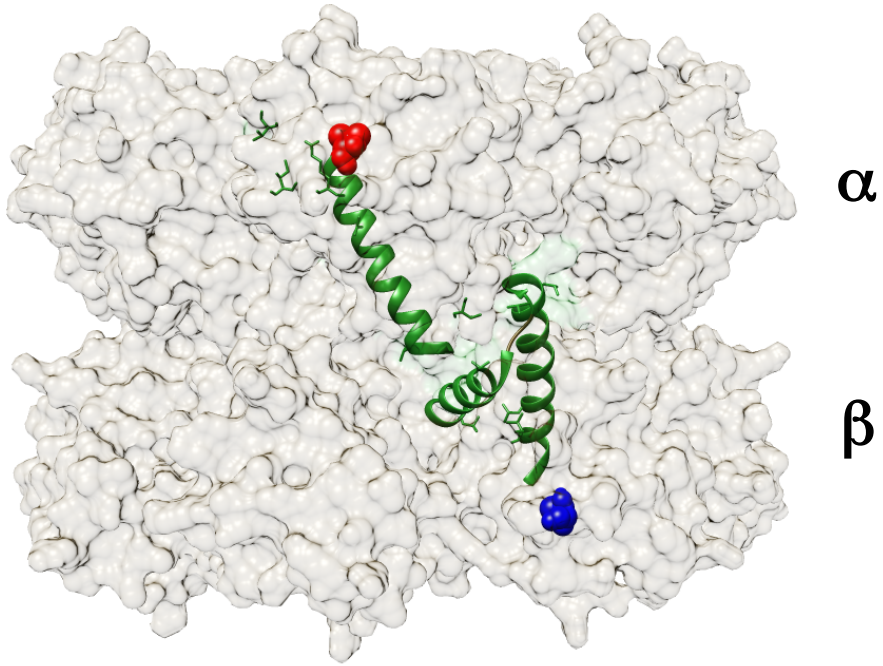

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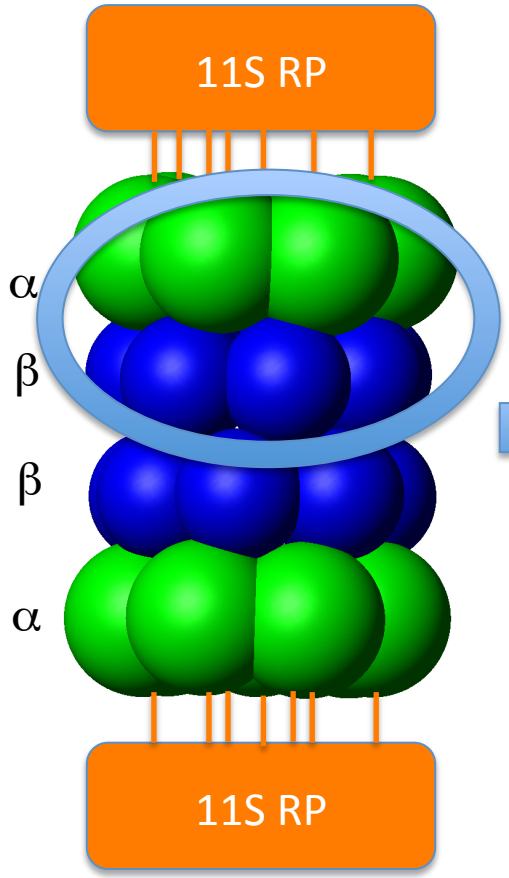

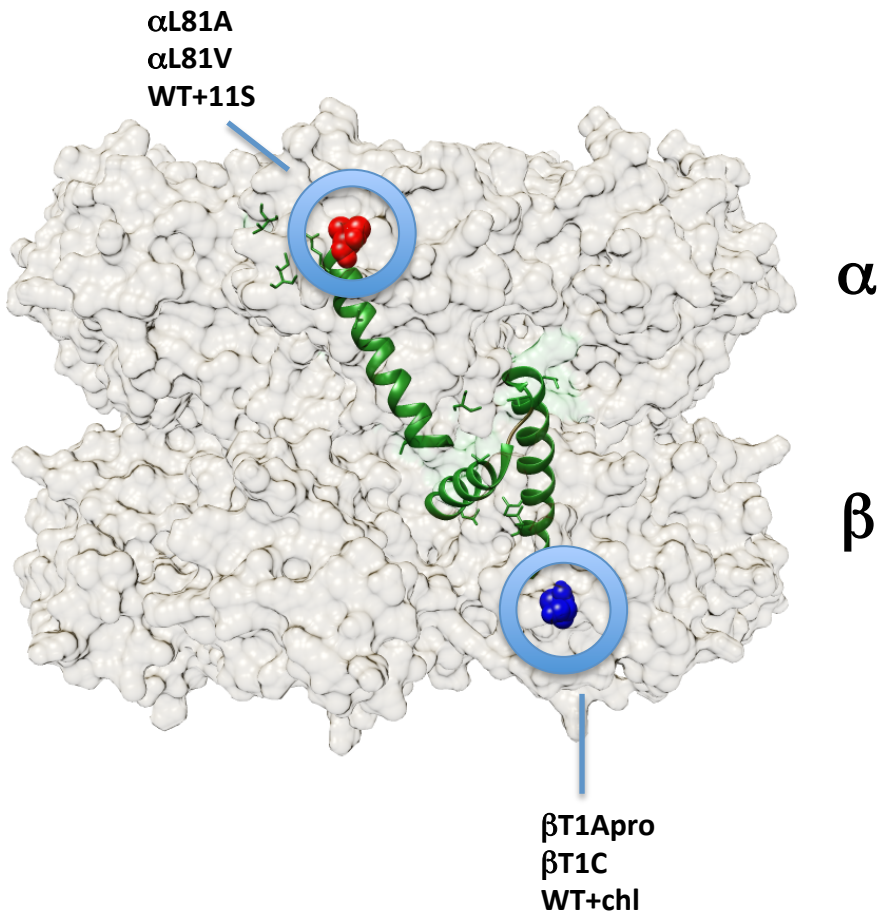

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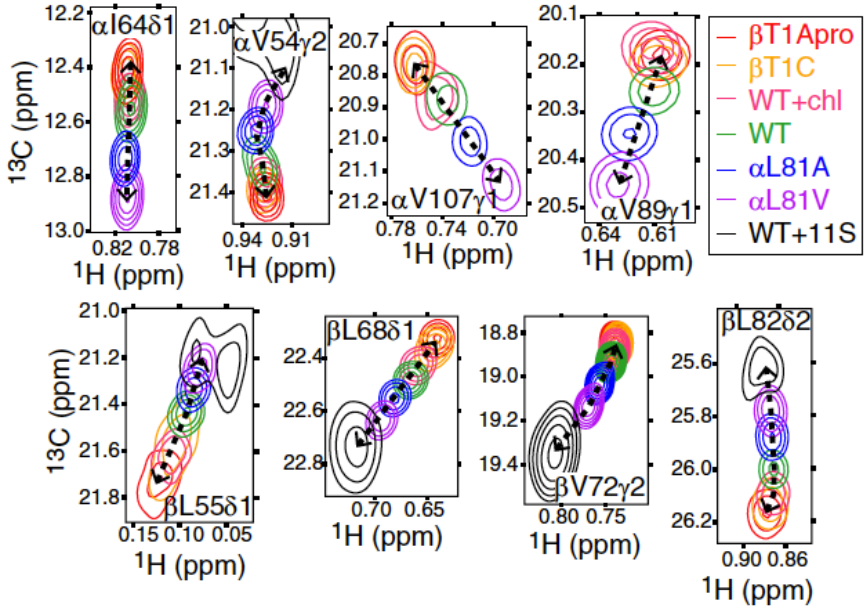

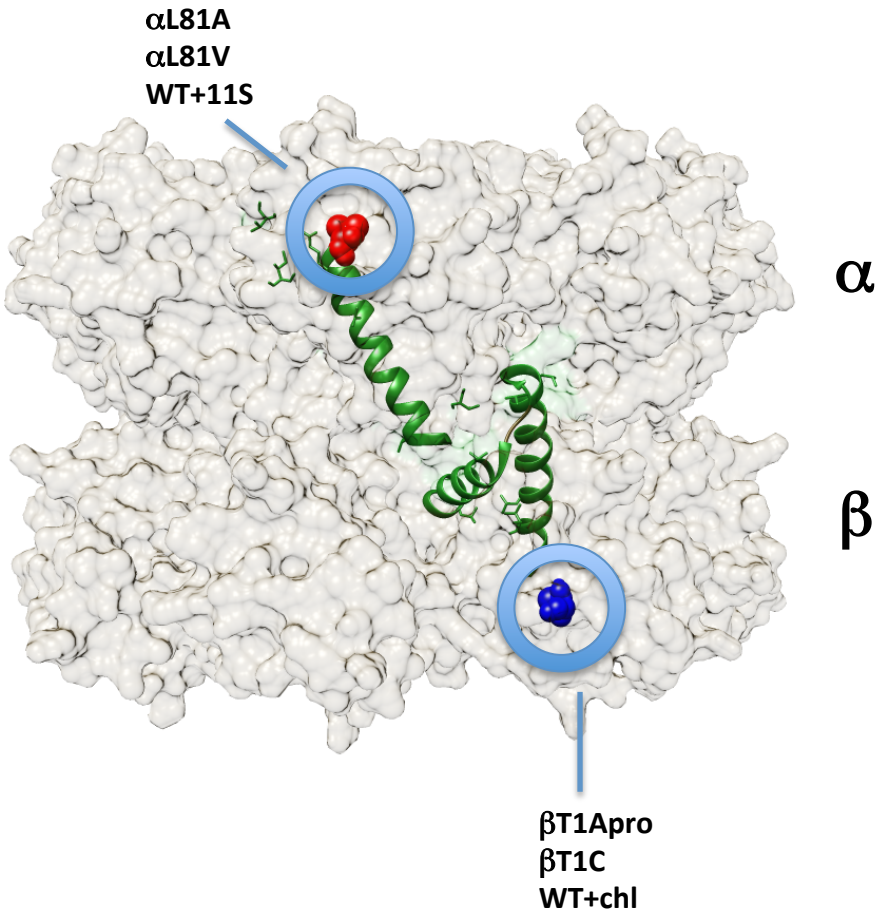

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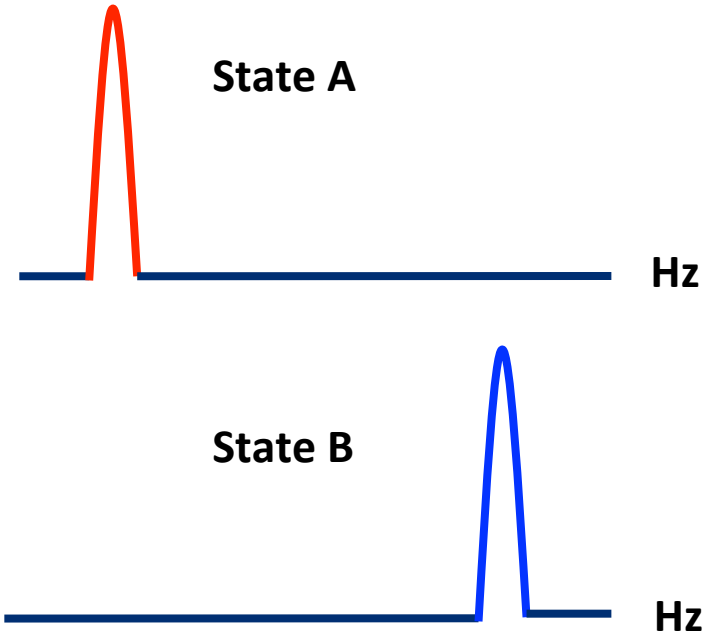
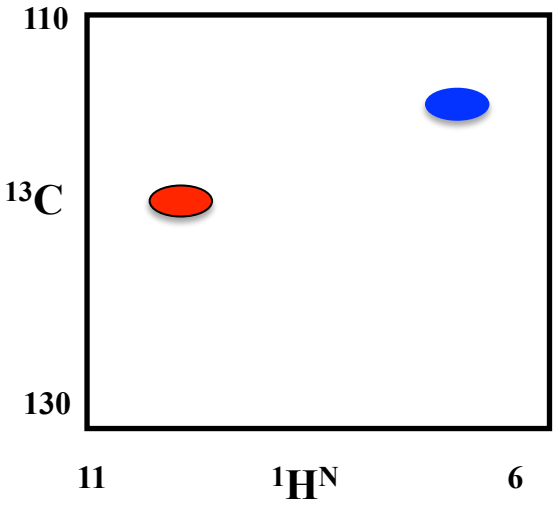
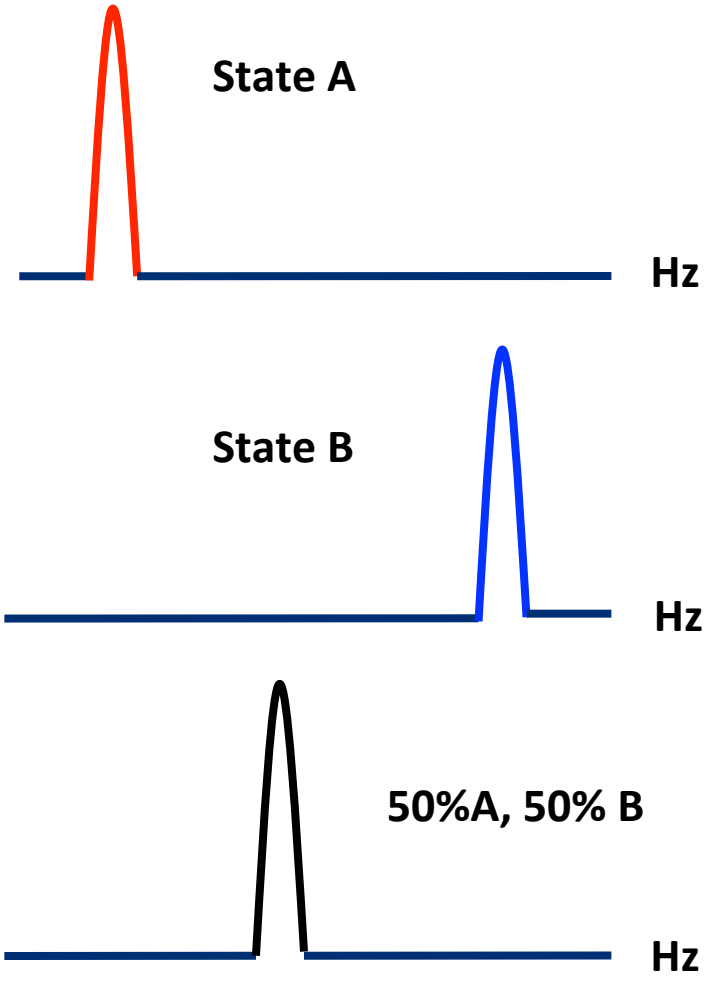
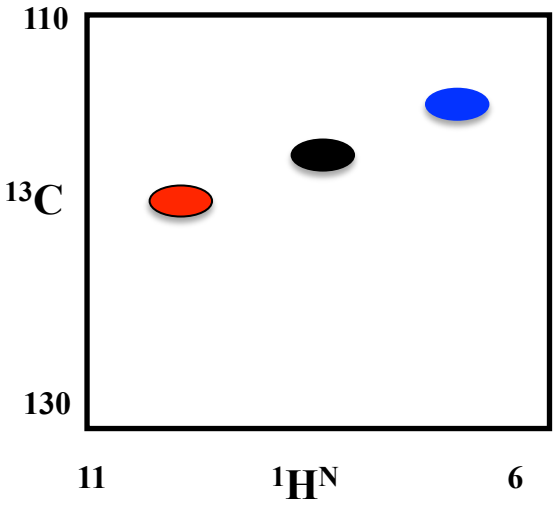




NMR reveals protein conformational exchange





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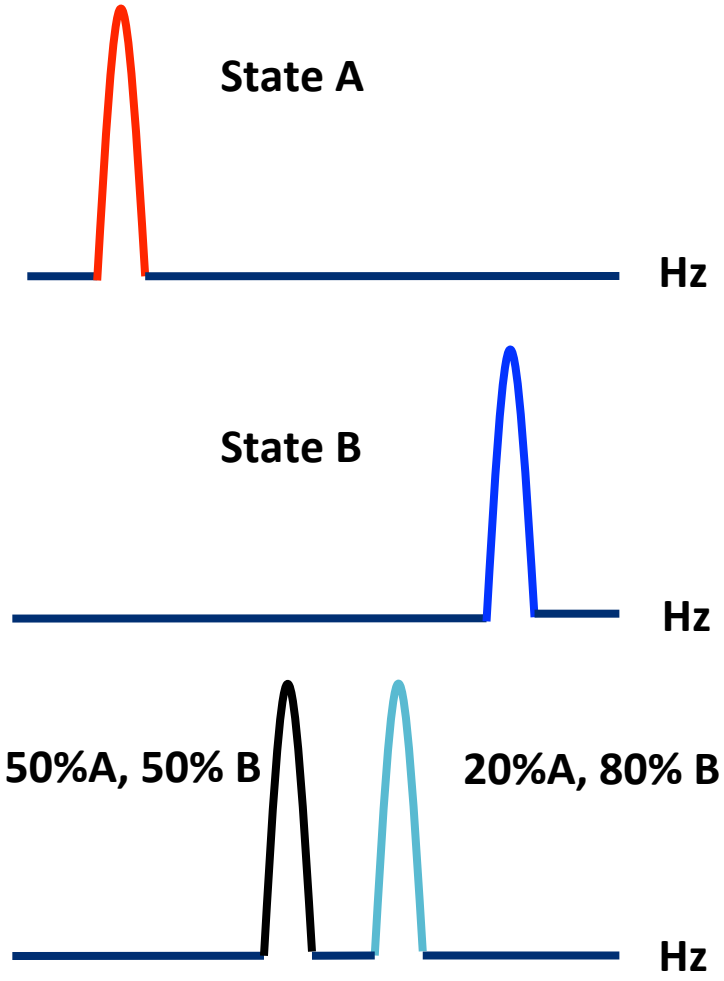
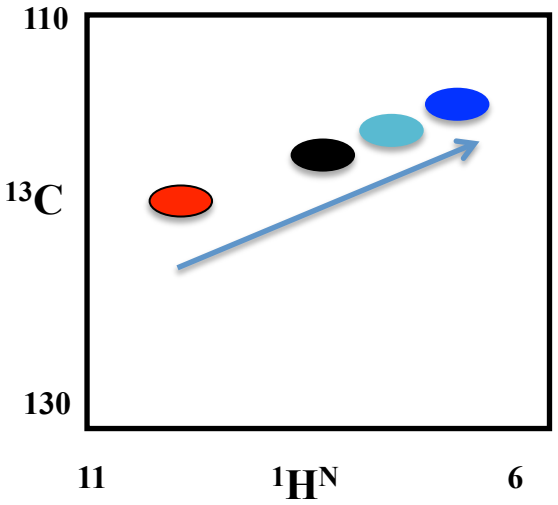
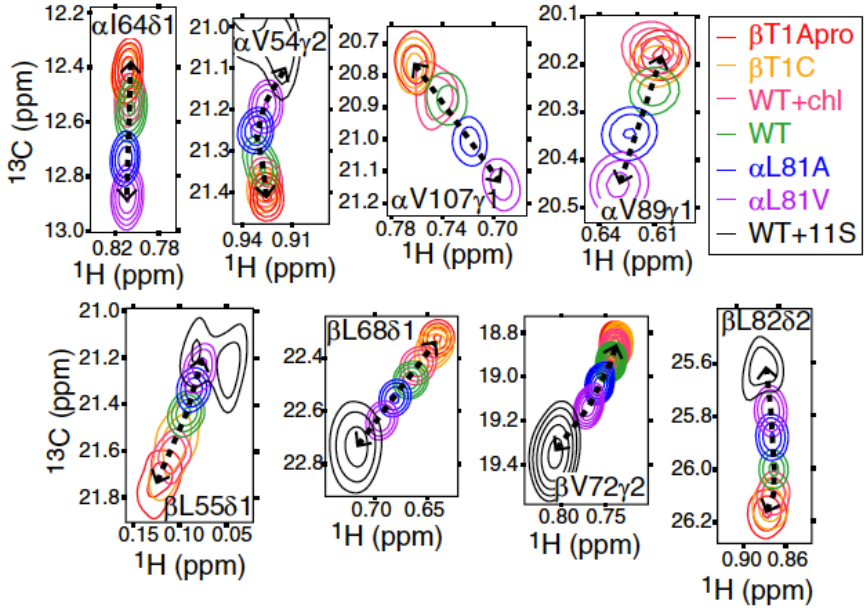
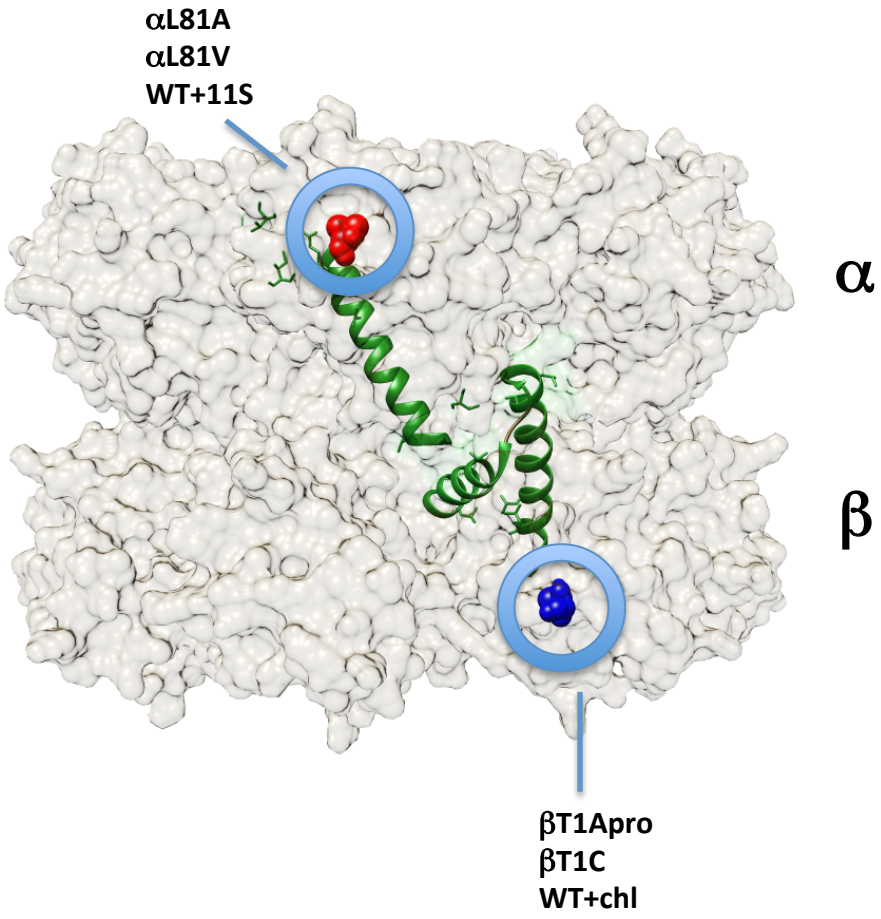


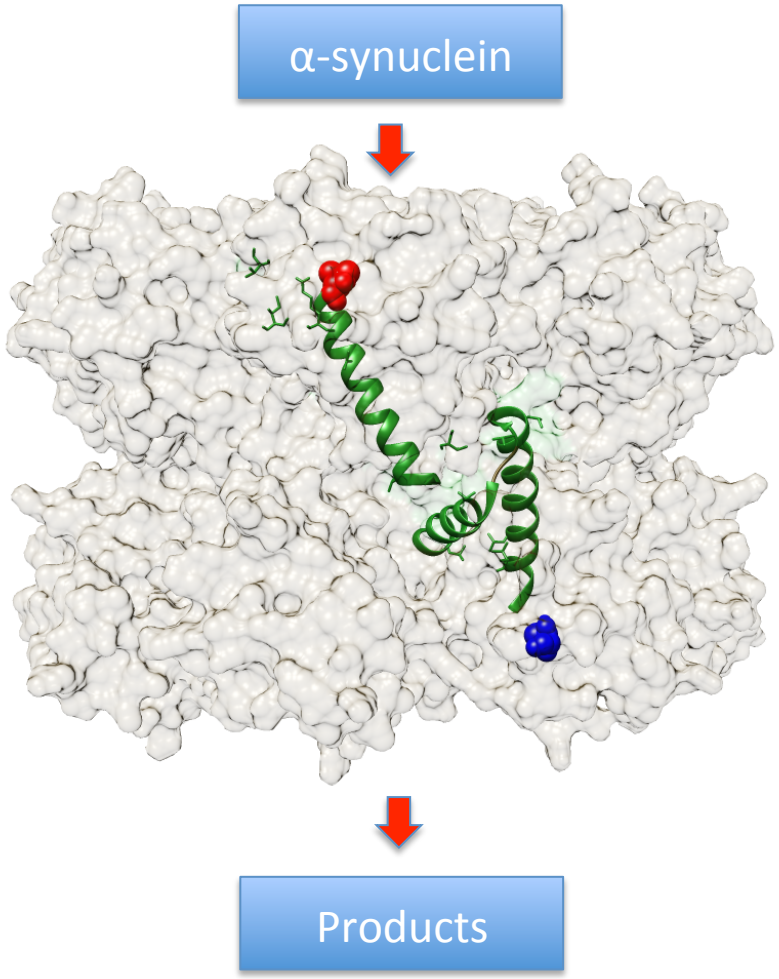


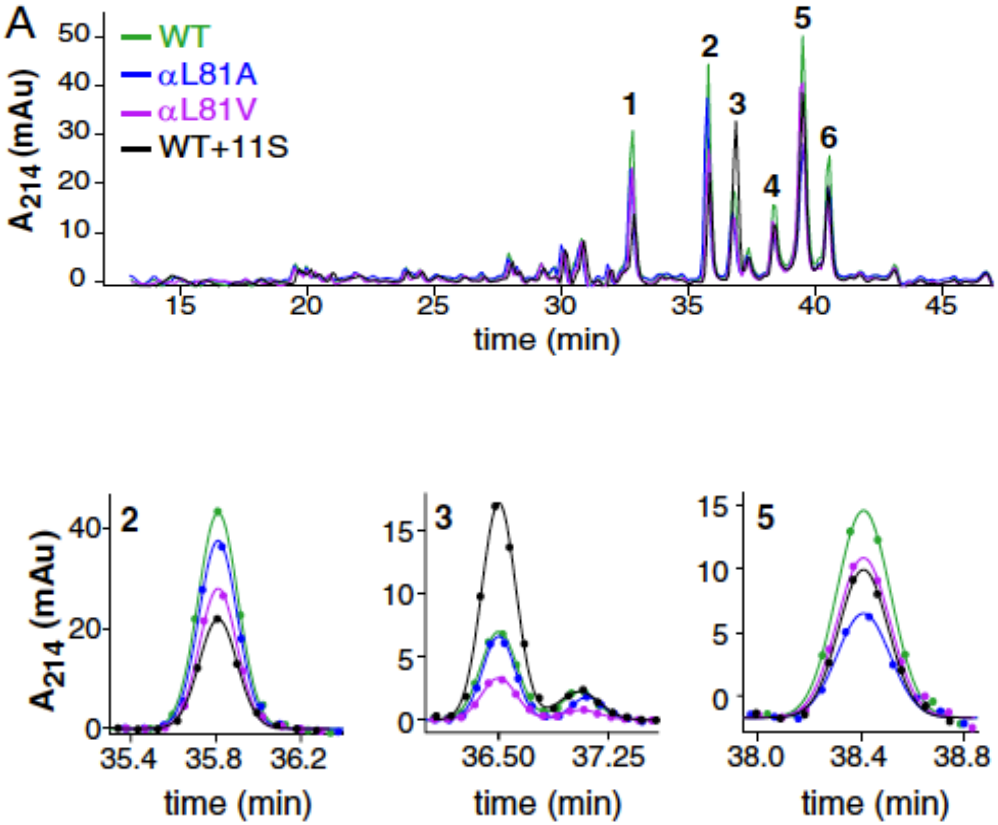
NMR reveals protein conformational exchange in T20S proteasome





T20S mutations alter degradation product ratios

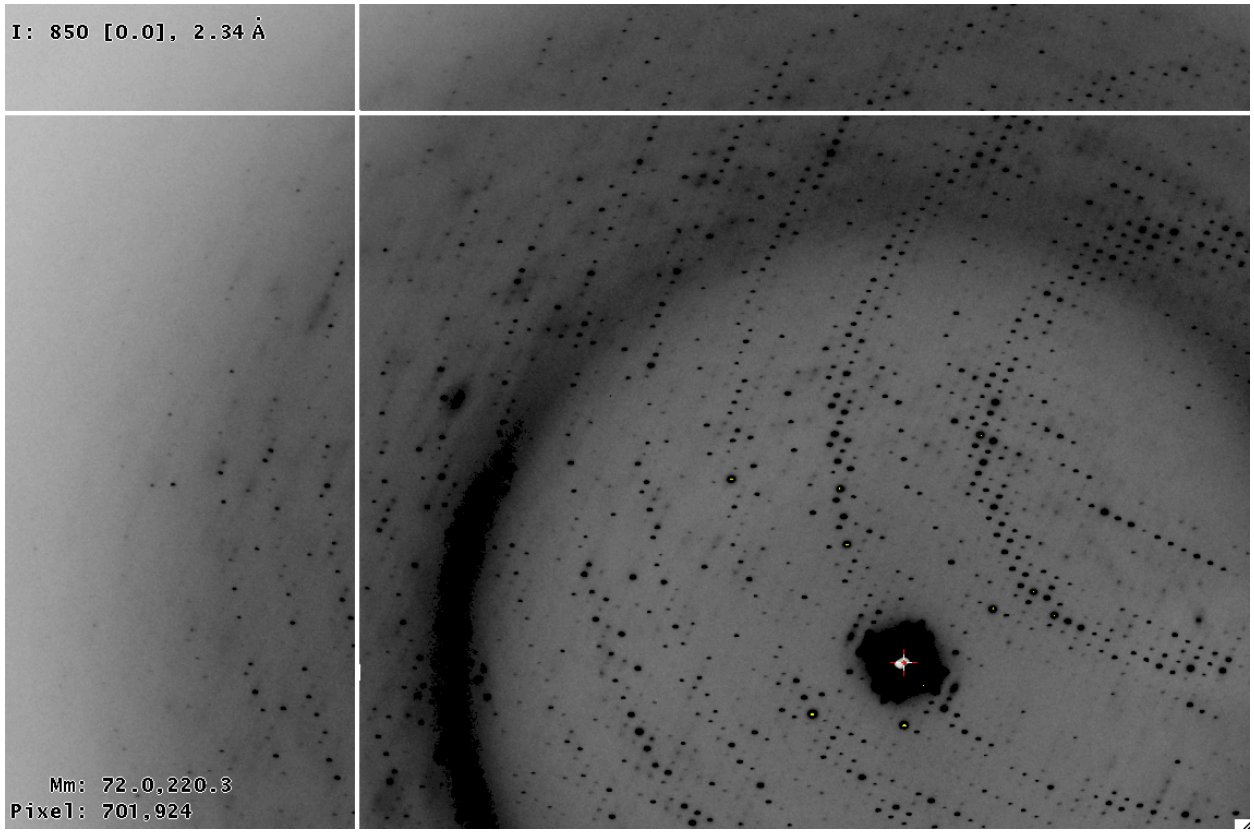




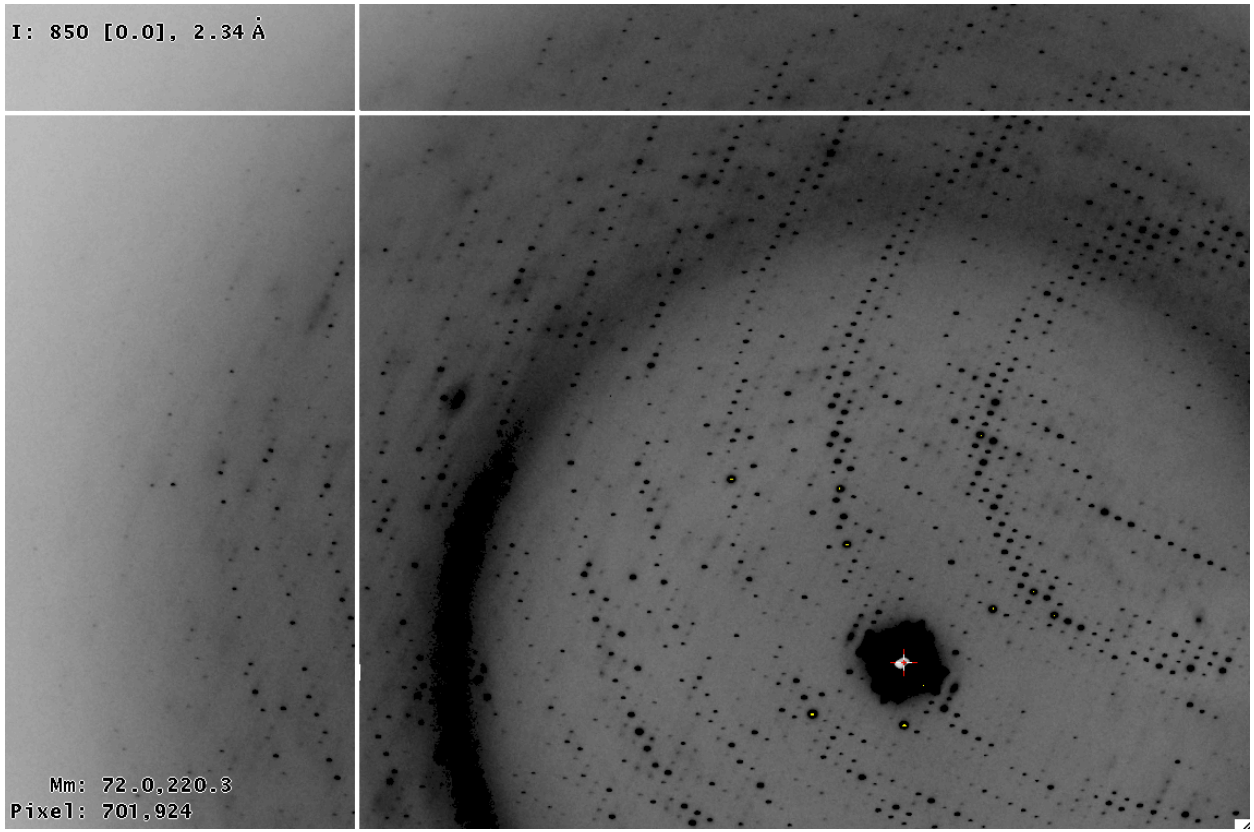
Ruschak and Kay (2012)

Diffuse scattering can help us determine the mechanism of the T20S allosteric network

T20S proteasome lacks high-resolution crystal data

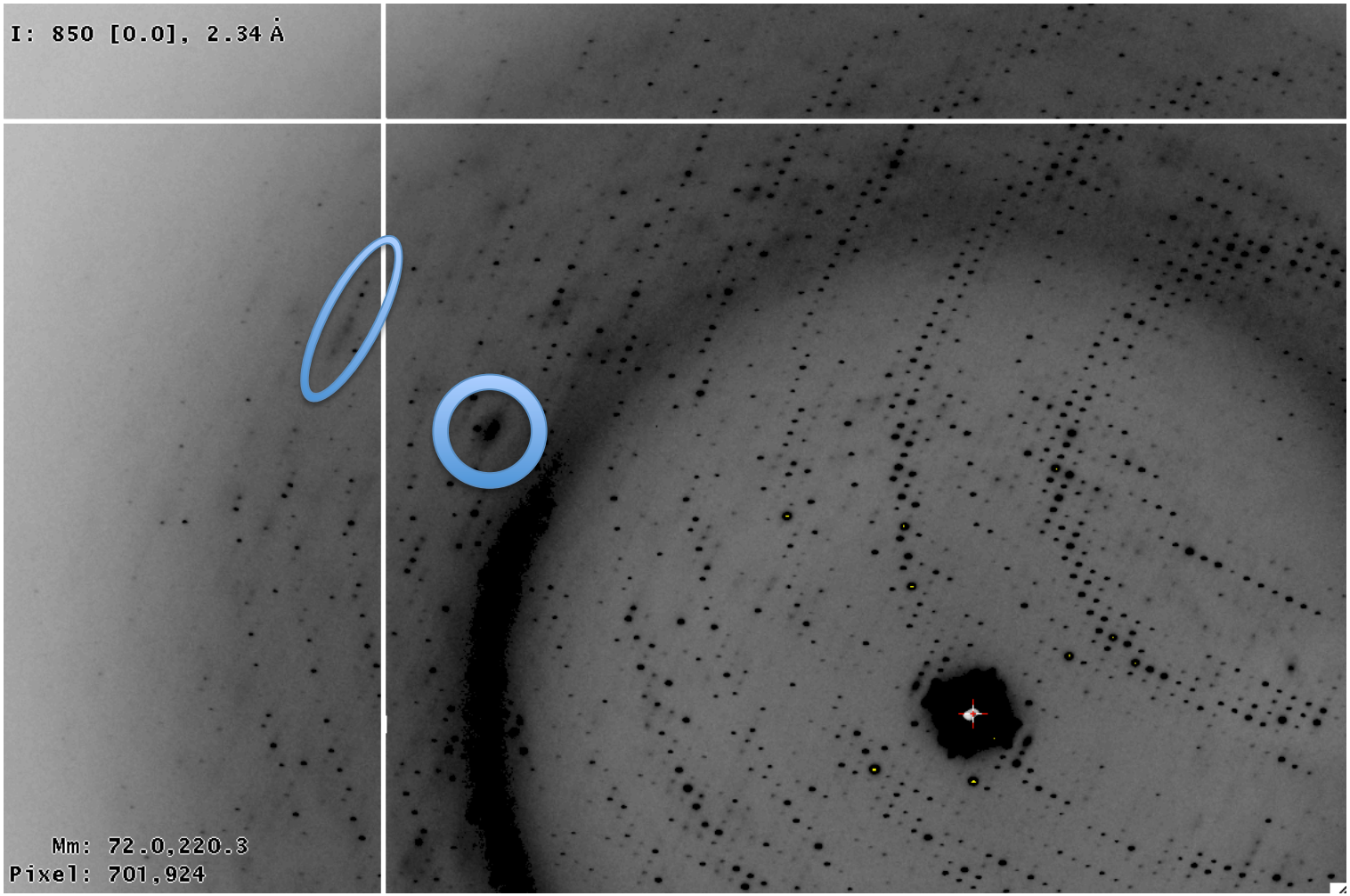


T20S proteasome lacks high-resolution crystal data

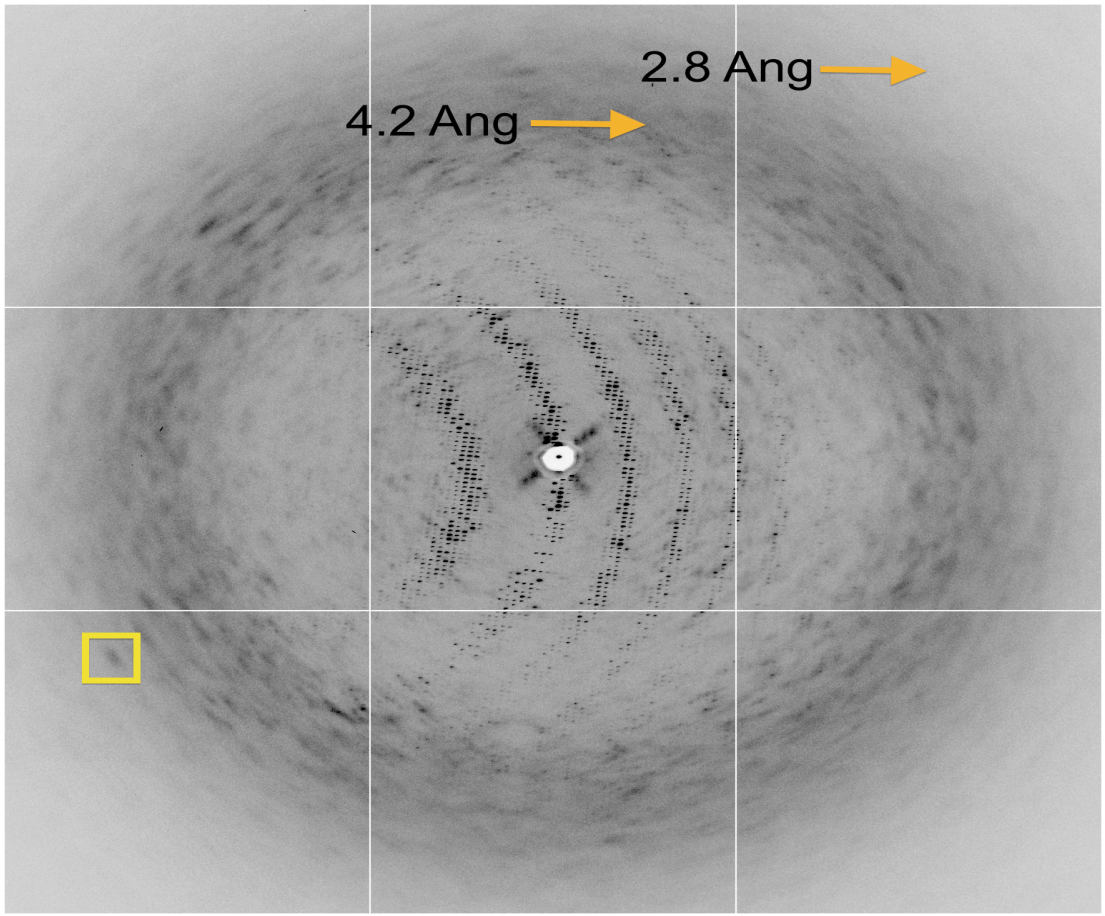


2.6Å diffraction…

Significant diffuse scattering at mid/ low resolutions for proteasome



Significant diffuse scattering at mid/low resolutions for macromolecular machines

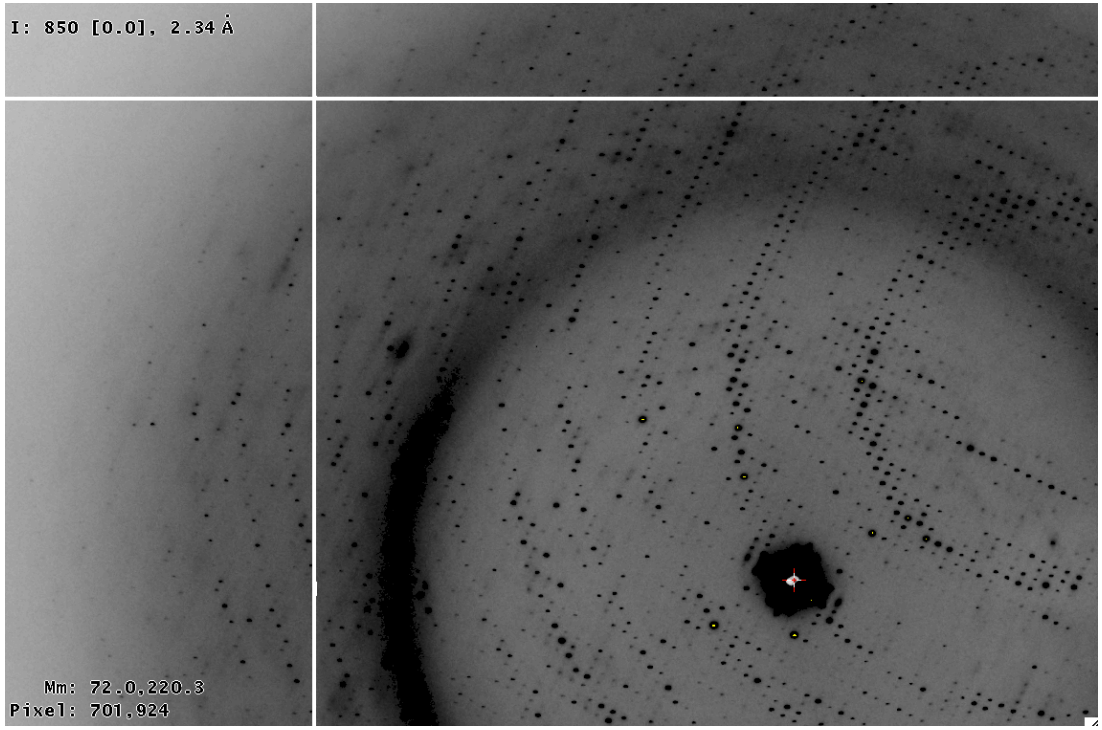


Photosystem II (4.2Å)

Sauter group (2013)

Future T20S investigation requires data and motional models

- We need complete diffuse maps of WT, L81A/V proteasome
- Maps will be fit with predictive models of proteasome allostery (TLS)



Summary

1. **Correlated protein motion underscores enzymatic function**
   - **Conversion of sidechains is rate-limiting for catalysis**
   - **Can be determined by room temperature crystallography, CONTACT**
2. **Allosteric mechanism transmits data 80Å in T20S proteasome**
   - **Co-linear chemical shift perturbations suggest correlated motions**
3. **Diffuse scatter is present at low to medium resolution**
   - **Currently we can collect data at ALS, SSRL**
   - ***How can we merge data across multiple crystals?***
   - ***What's the effect of cryo-cooling?***
4. **Diffuse scatter distinguishes between models with identical Bragg data**
   - ***How do we go from diffuse intensity to models of motion?***
   - ***What are the different motions on different length-scales?***