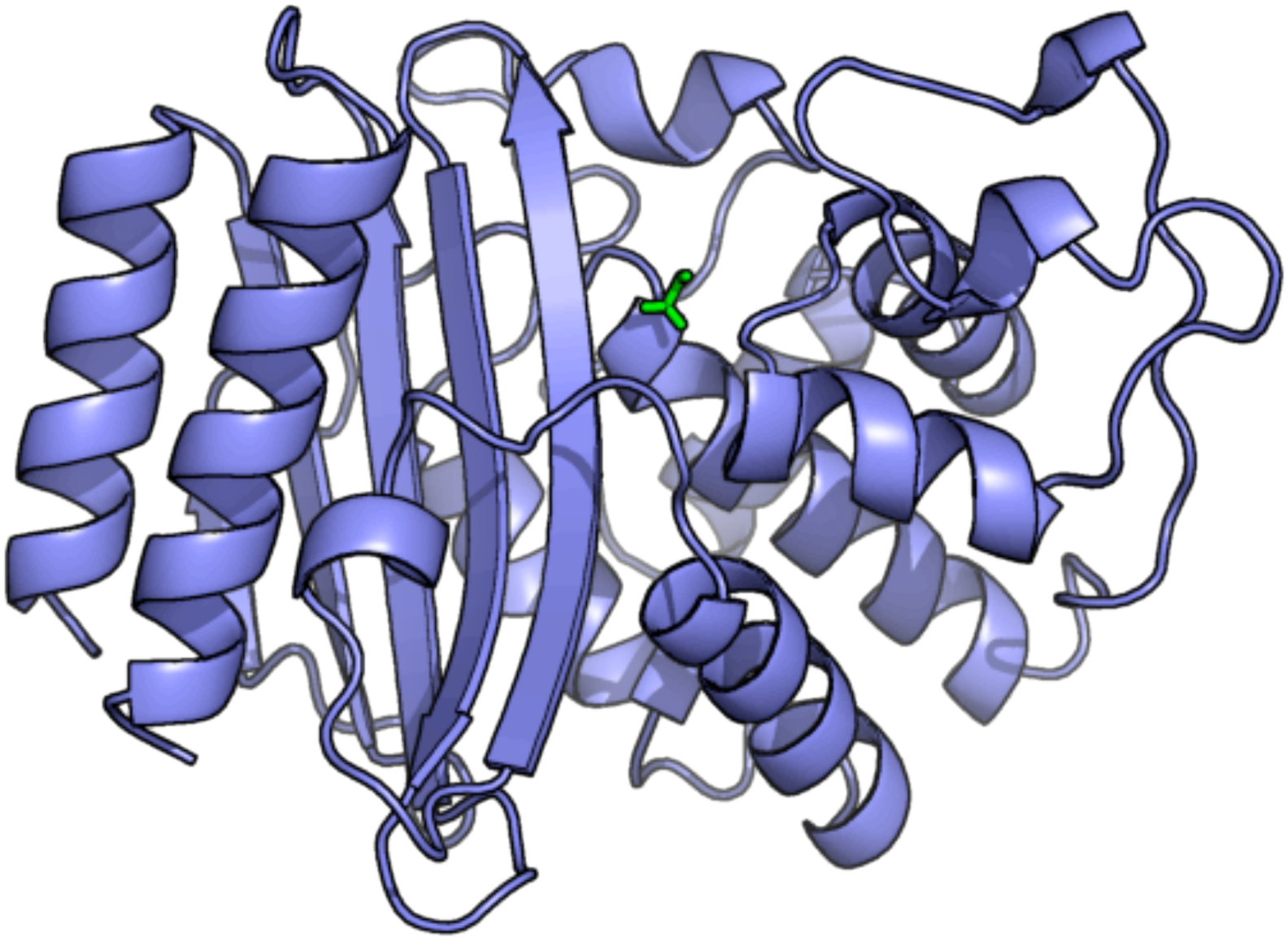
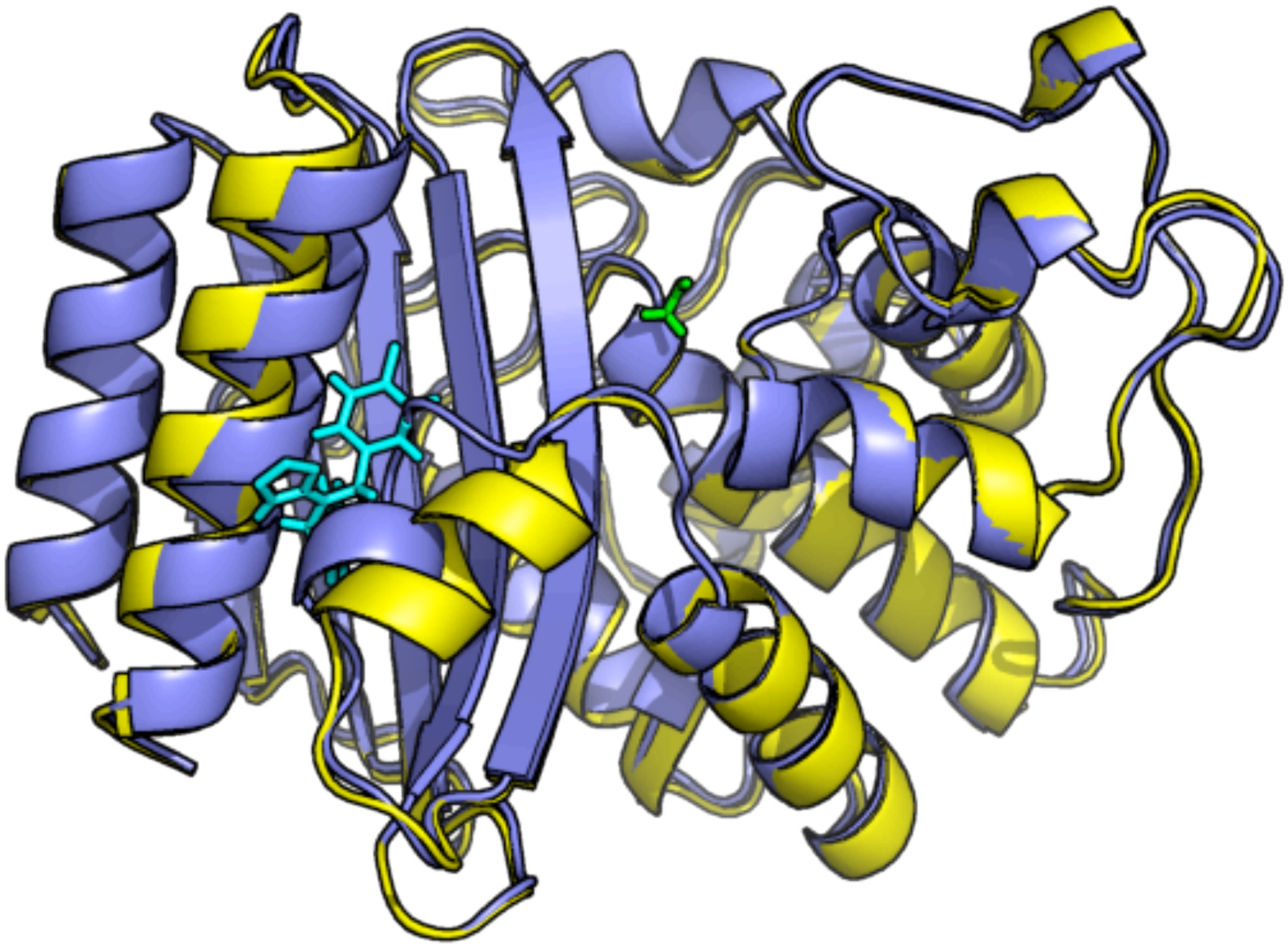
Augmenting experiments with *in silico* Markov state models

Gregory R. Bowman
Miller Research Fellow
University of California, Berkeley

Meet β-lactamase



Considering structural fluctuations opens up new possibilities



Horn and Shoichet. *JMB* 2004.

Outline

?

Computational approach

Outline

?

Computational approach

?

Understanding and predicting allostery

Outline

?

Computational approach

?

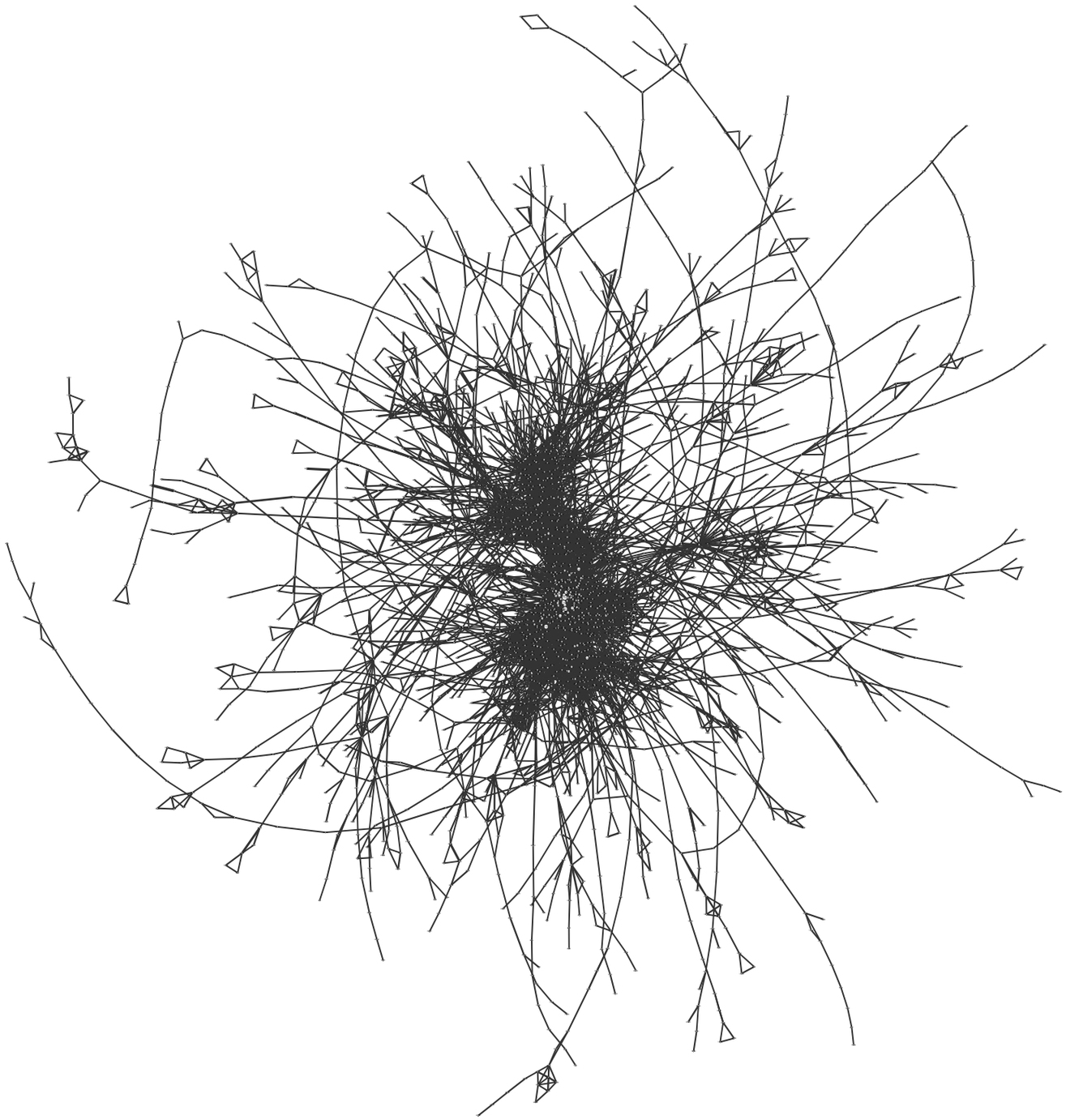
Understanding and predicting allostery

?

Experimental tests

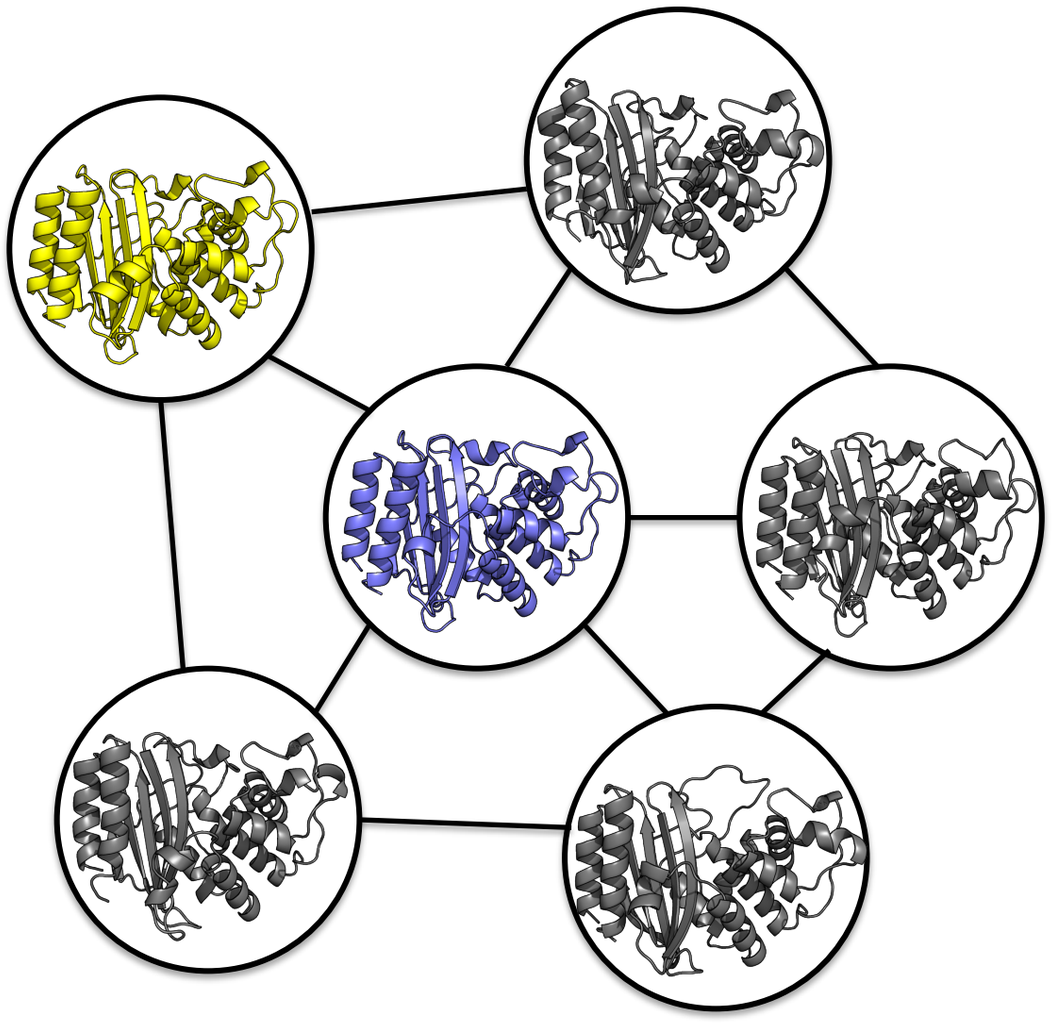
Molecular dynamics simulations capture protein fluctuations with atomic resolution

Markov state models are quantitative maps of a protein's conformational space



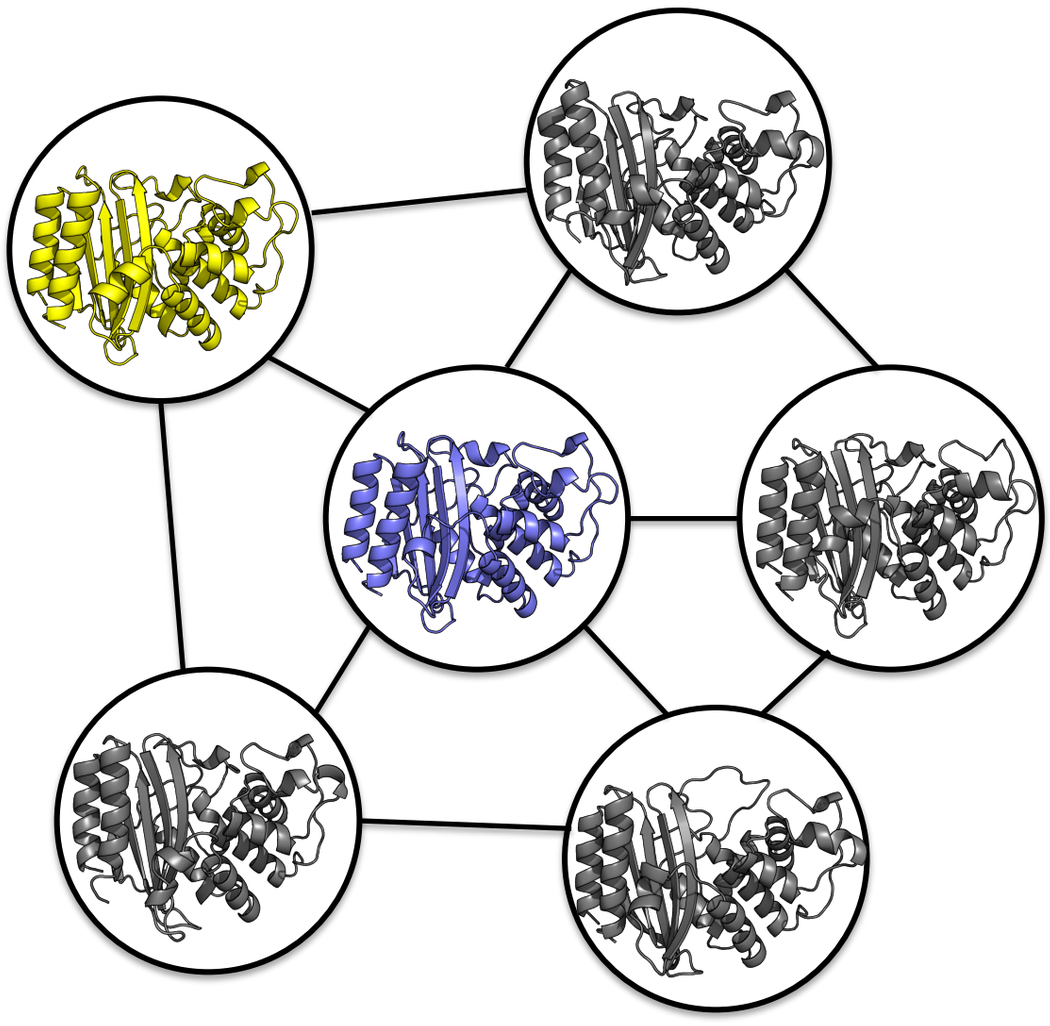
Bowman *et al*. Cell Res. 2010.
Noe *et al*. Curr Opin Struct Biol. 2008.

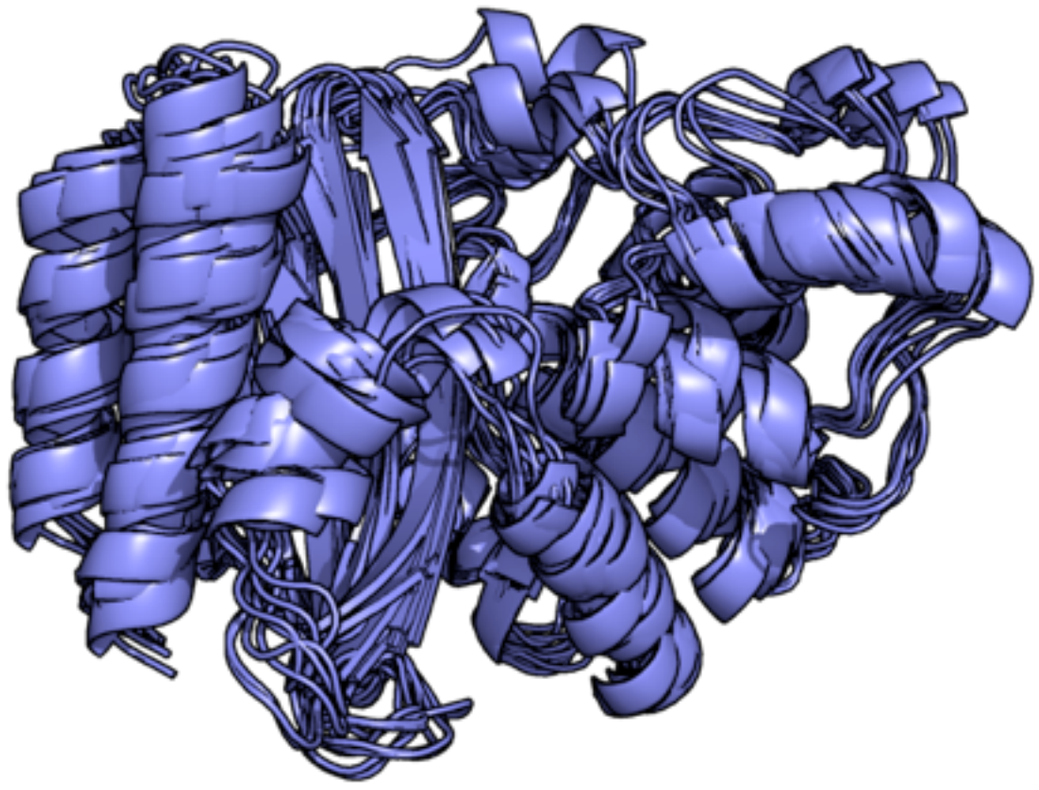
Coarse-graining Markov models facilitates understanding



Bowman. JCP 2012.
Bowman *et al*. Cell Res. 2010.
Noe *et al*. Curr Opin Struct Biol. 2008.

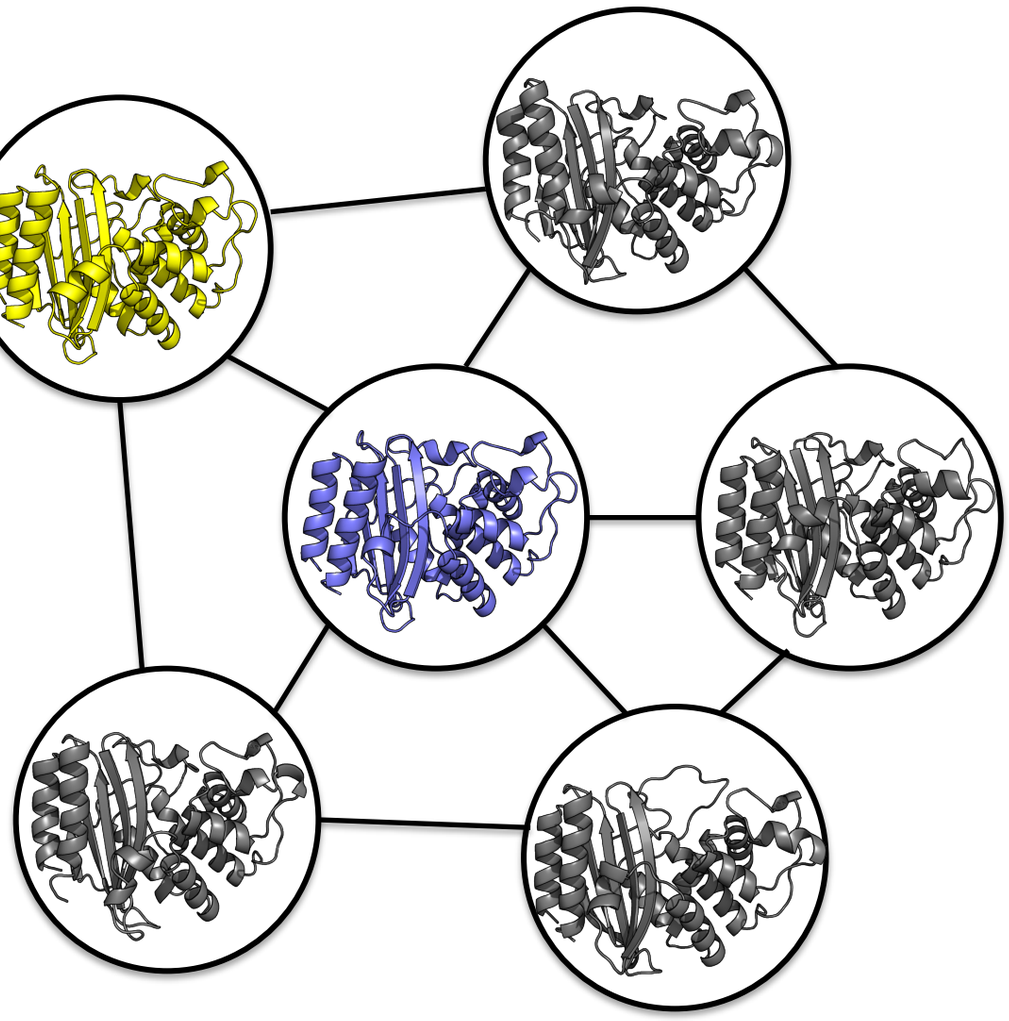
Coarse-graining Markov models facilitates understanding

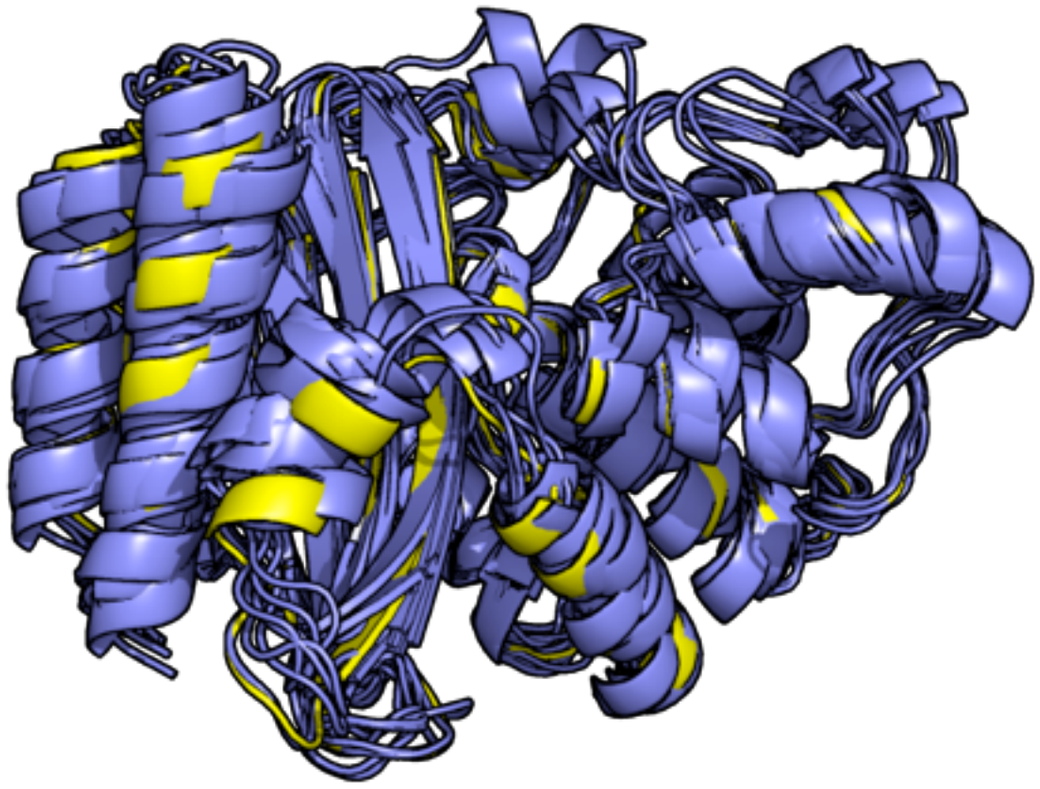




Bowman. JCP 2012.
Bowman *et al*. Cell Res. 2010.
Noe *et al*. Curr Opin Struct Biol. 2008.

Coarse-graining Markov models facilitates understanding



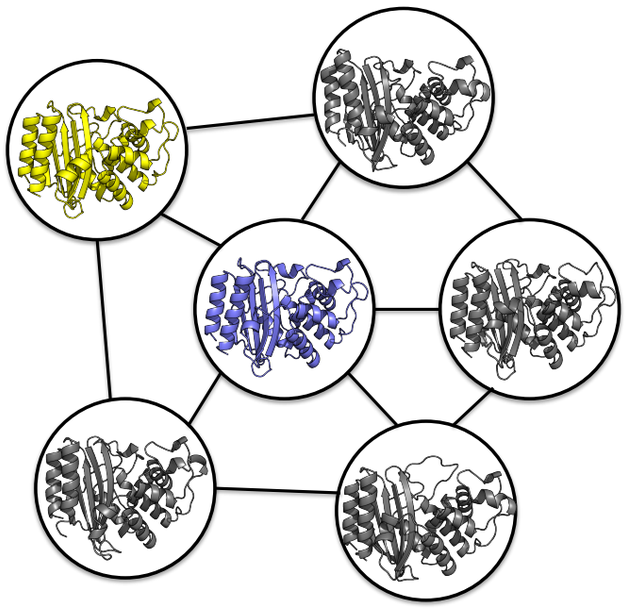


Bowman. JCP 2012.

Bowman *et al*. Cell Res. 2010.

Noe *et al*. Curr Opin Struct Biol. 2008.

Outline



Computational approach

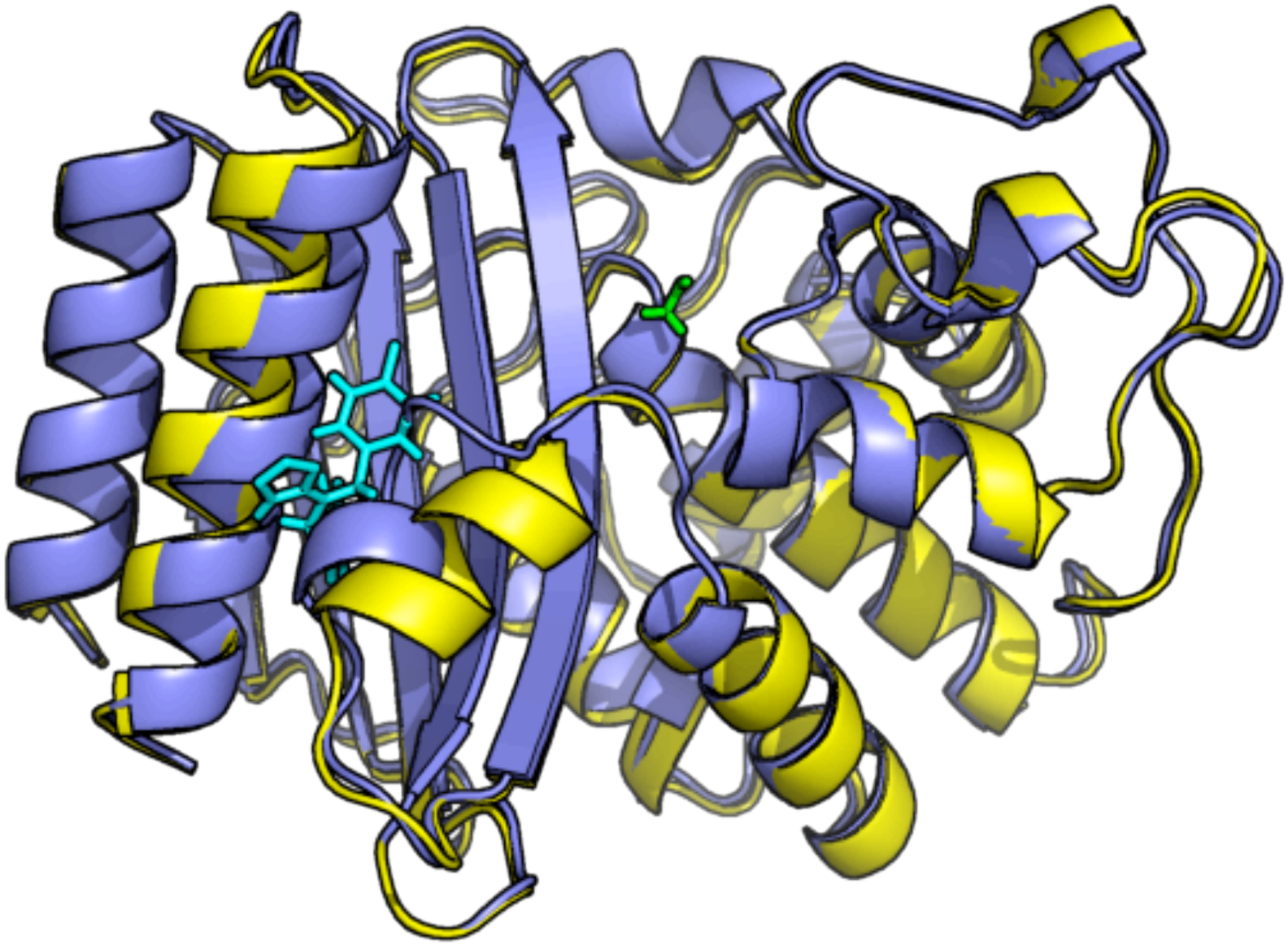
?

Understanding and predicting allostery

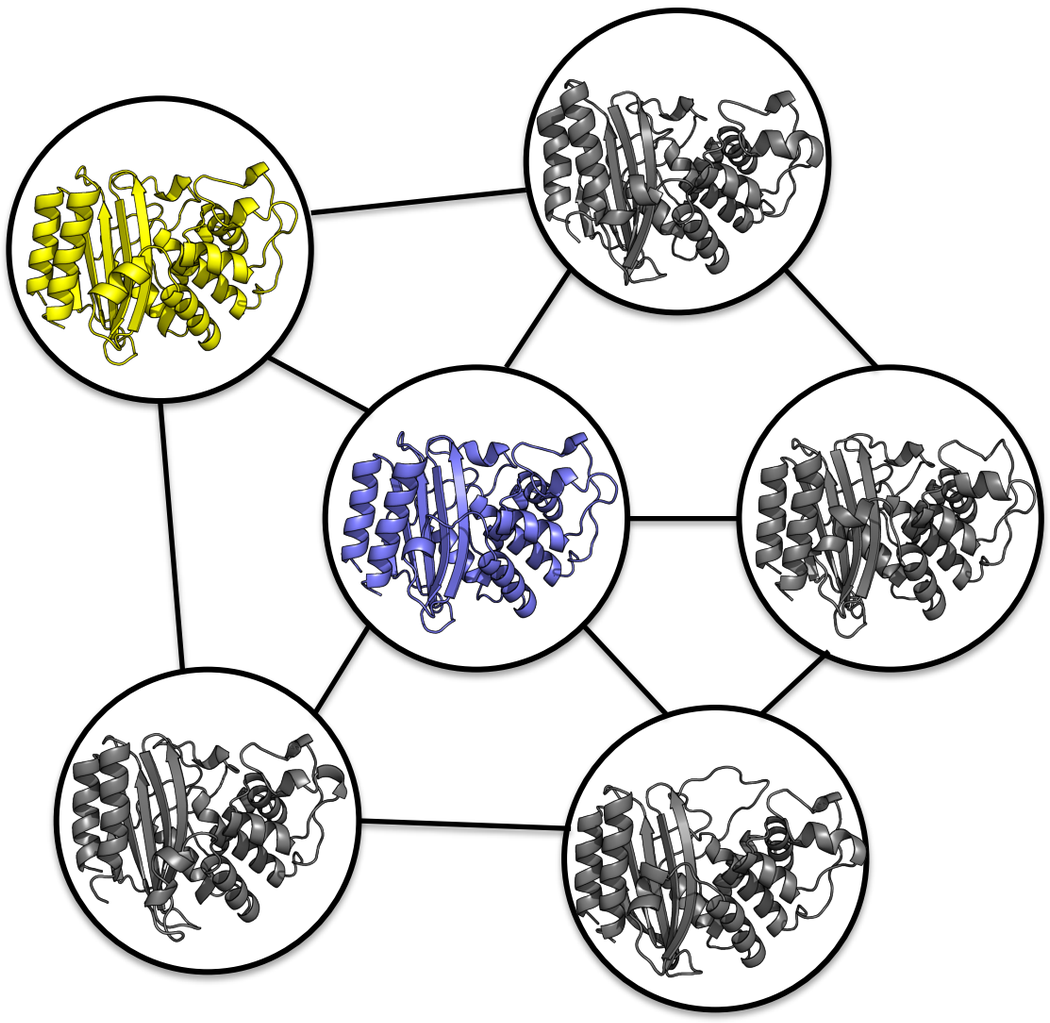
?

Experimental tests

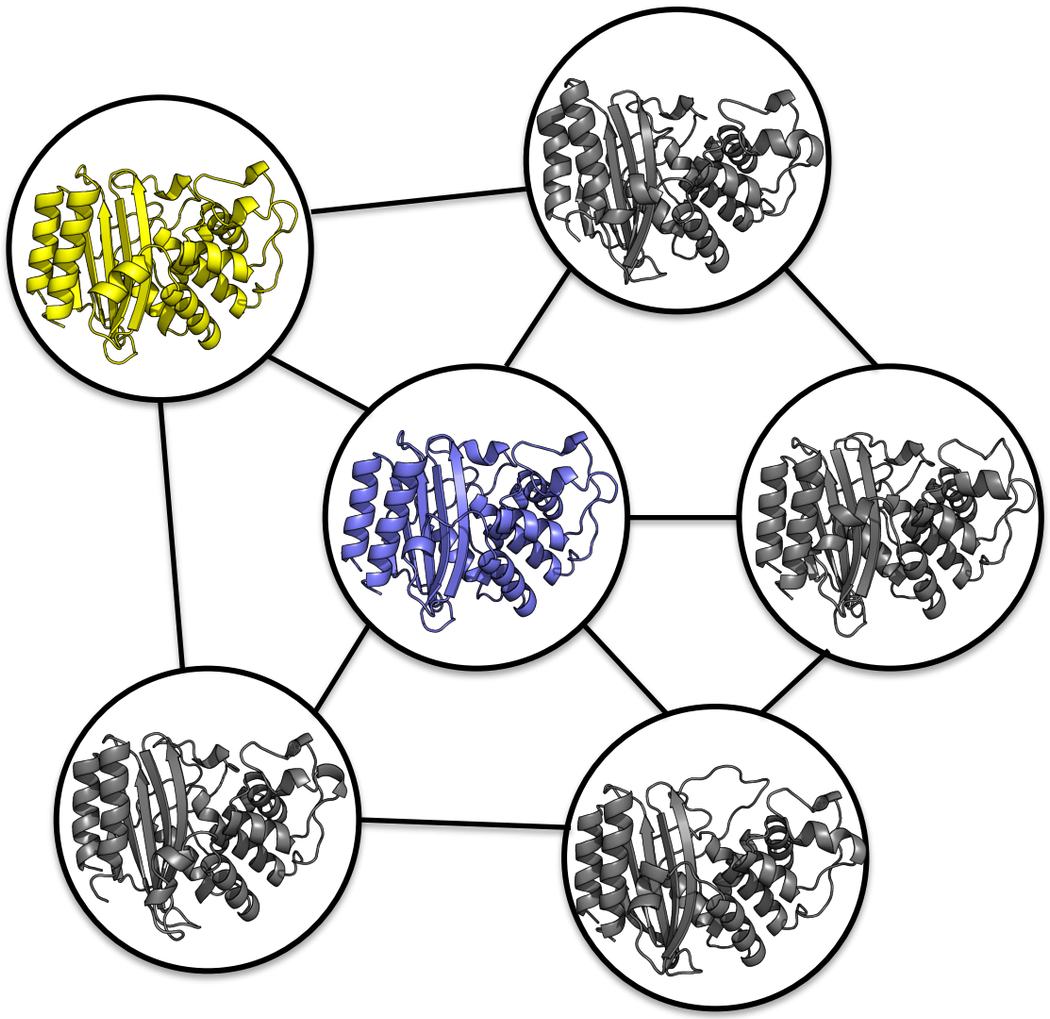
Hidden allosteric sites should be detectable on the basis of two signature structural fluctuations

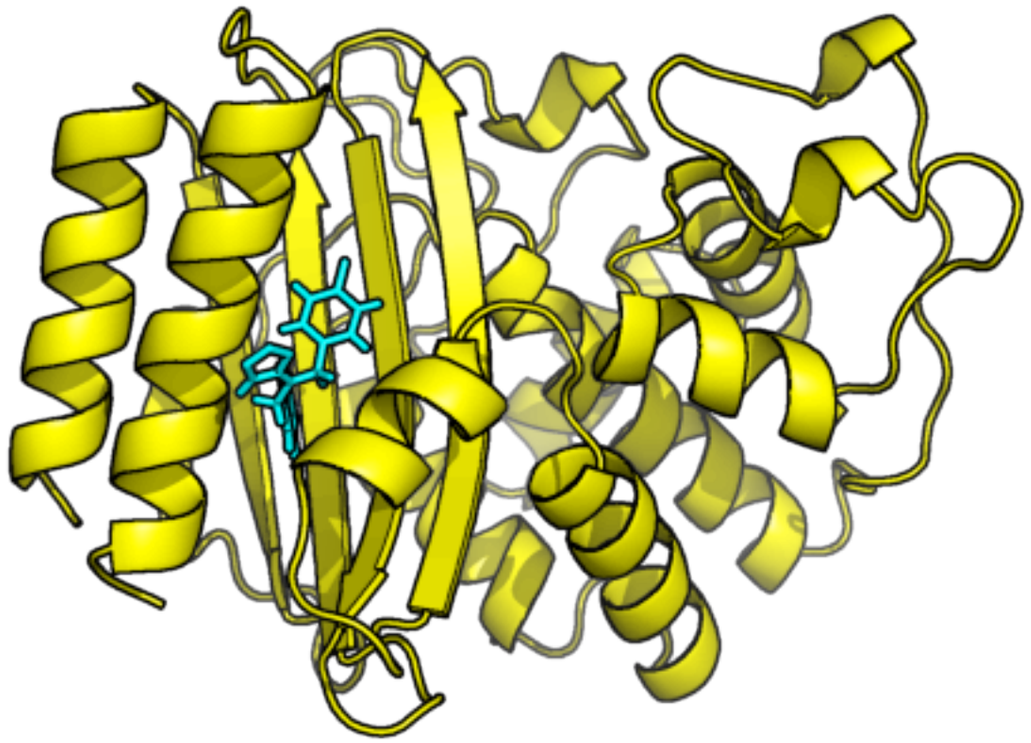


Pockets are just volumes surrounded by protein



Pockets are just volumes surrounded by protein

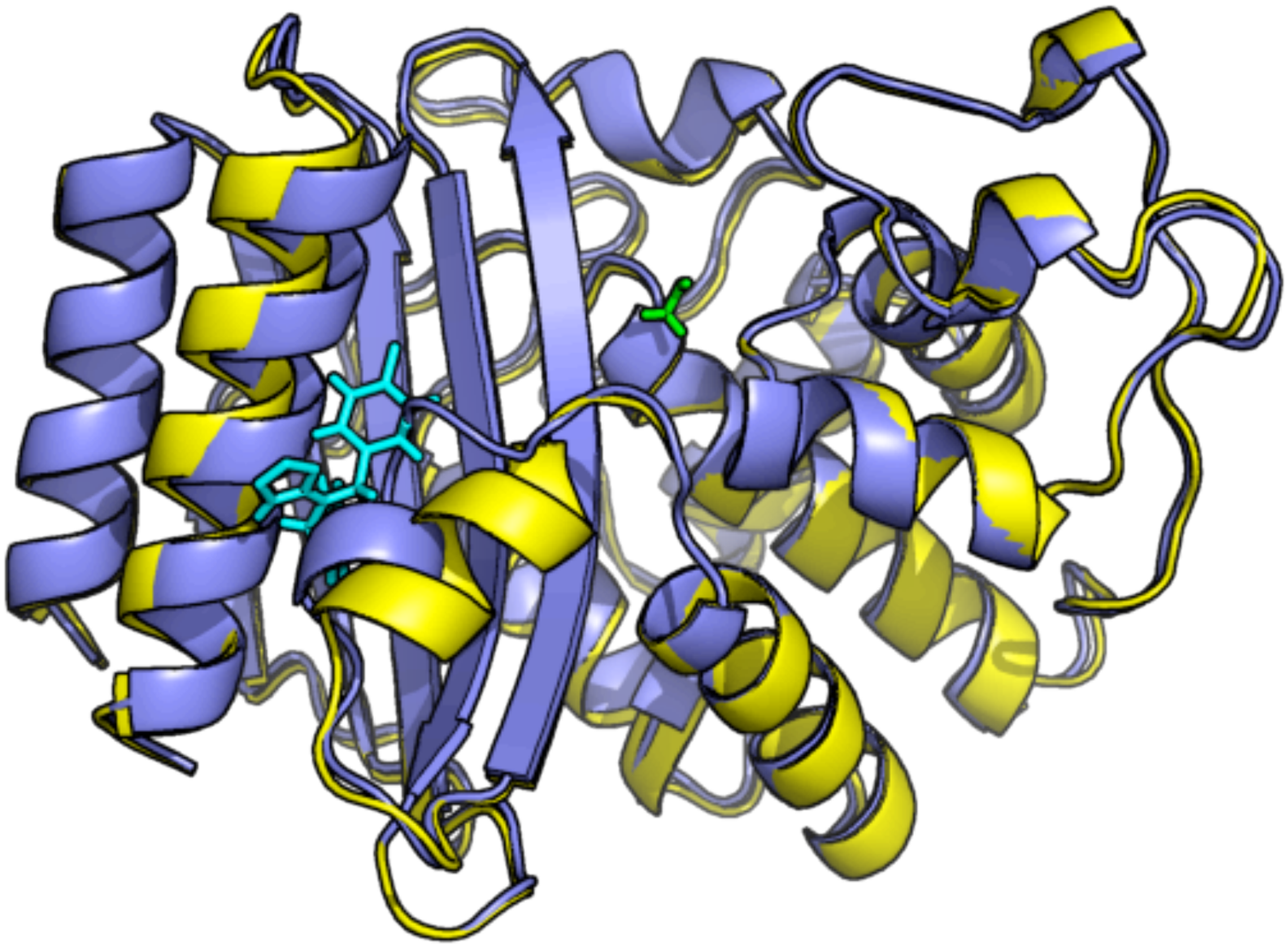




The known pocket is visible even in the absence of any ligand

The known pocket opens more widely than in the ligand-bound state

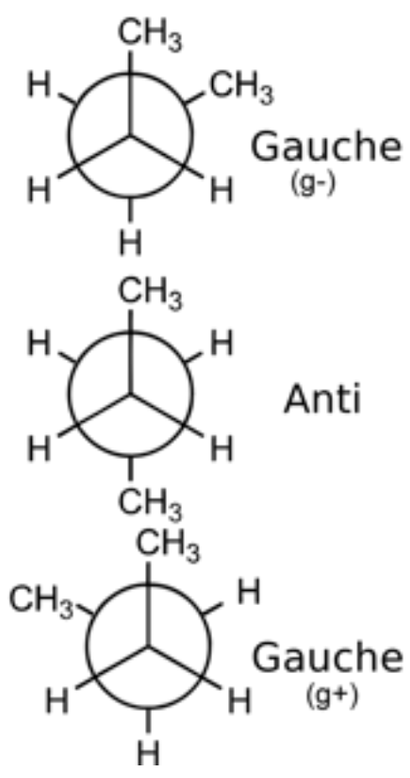
We also need some form of communication for an allosteric effect



Correlations between side-chain rotameric states allows long-distance communication

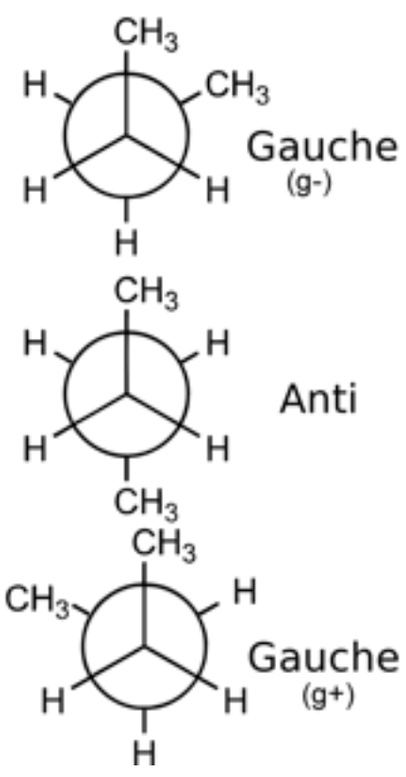
Bowman and Geissler. *PNAS* 2012.
DuBay and Geissler. JMB 2009.
McClendon et al. JCTC 2009.

Correlations between side-chain rotameric states allows long-distance communication



Bowman and Geissler. *PNAS* 2012.
DuBay and Geissler. JMB 2009.
McClendon et al. JCTC 2009.

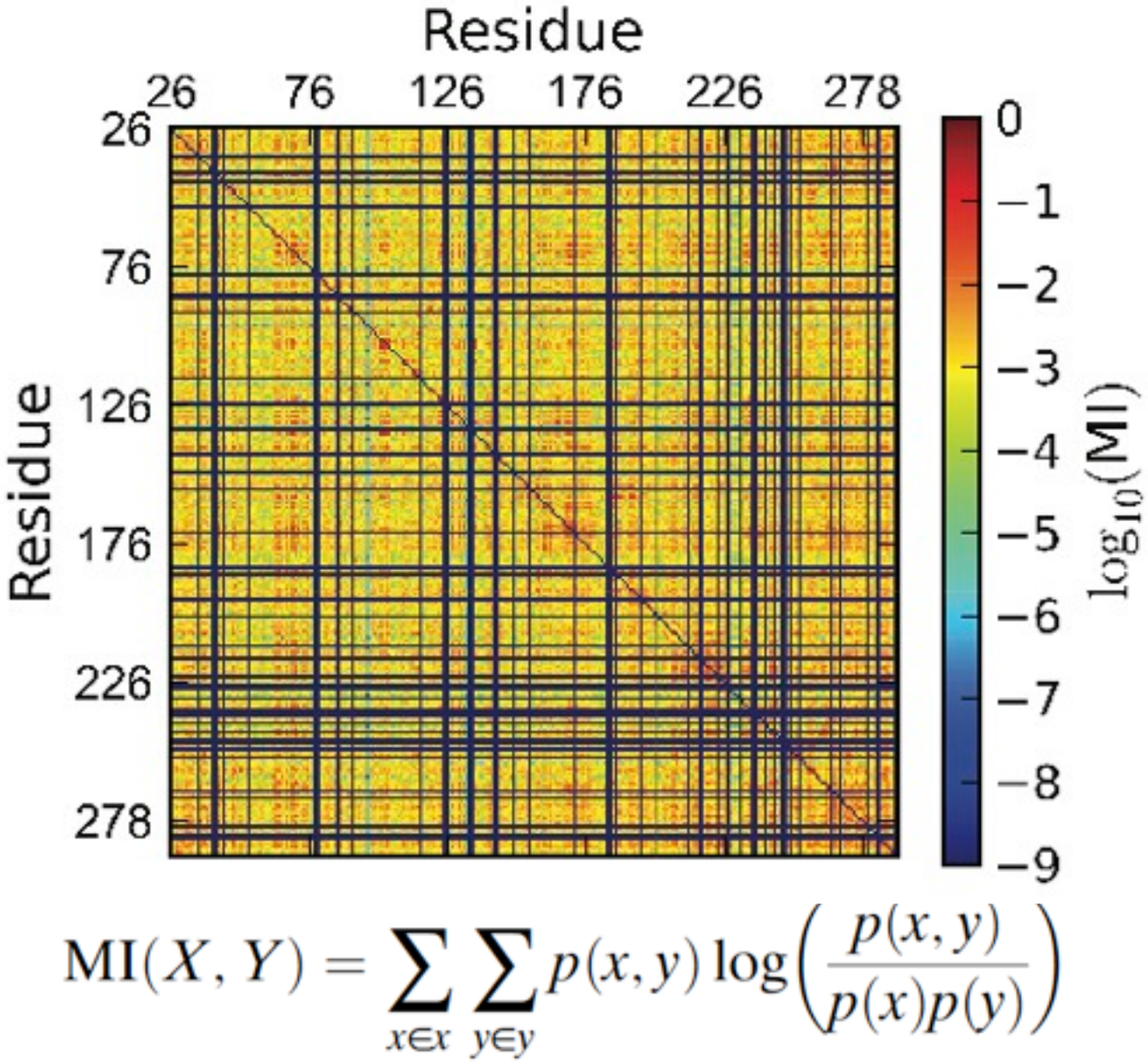
Correlations between side-chain rotameric states allows long-distance communication



$$\mathrm{MI}(X,Y) = \sum_{x \in x} \sum_{y \in y} p(x,y) \log\left(\frac{p(x,y)}{p(x)p(y)}\right)$$

Bowman and Geissler. *PNAS* 2012.
DuBay and Geissler. JMB 2009.
McClendon et al. JCTC 2009.

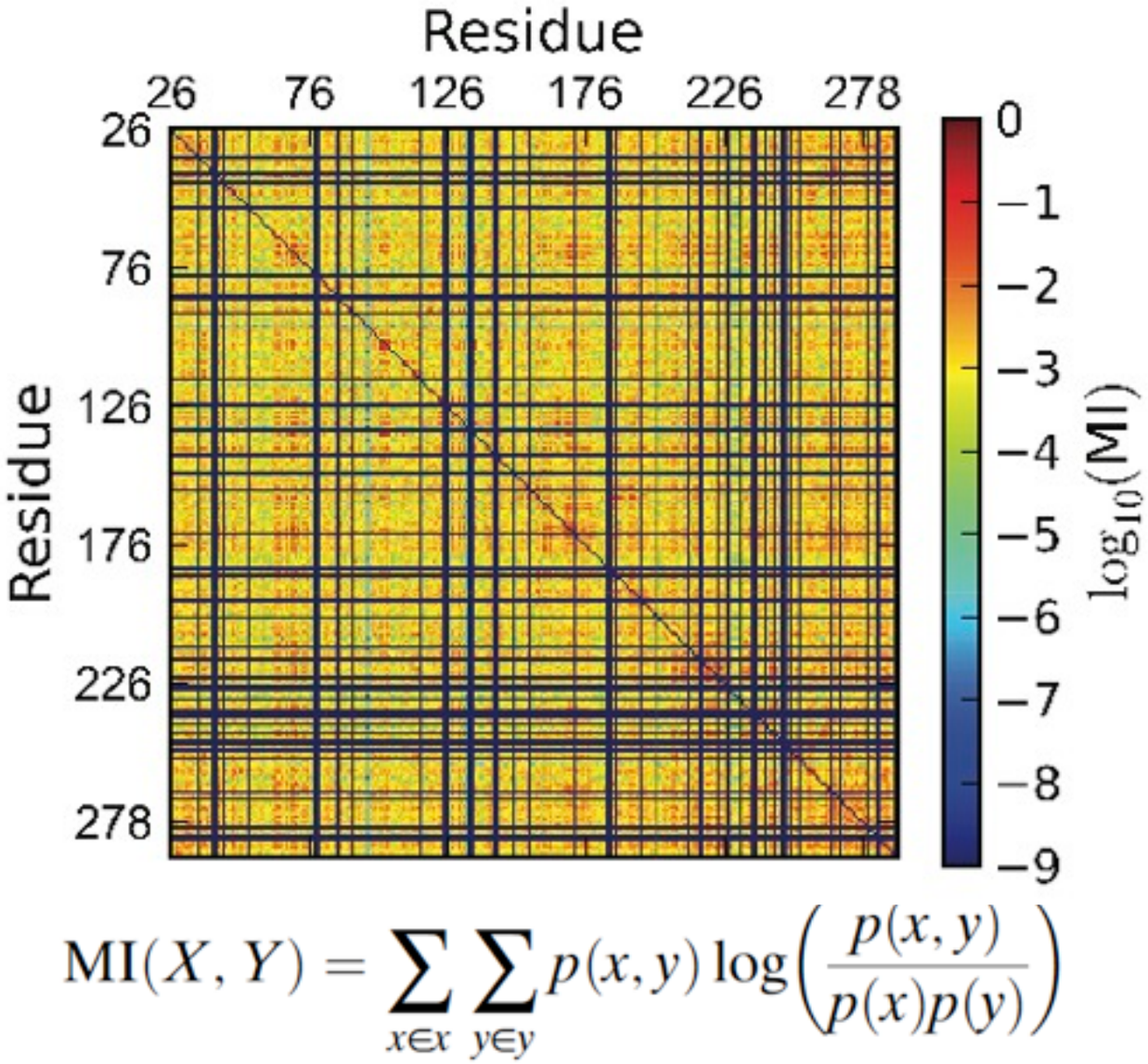
Correlations between side-chain rotameric states allows long-distance communication



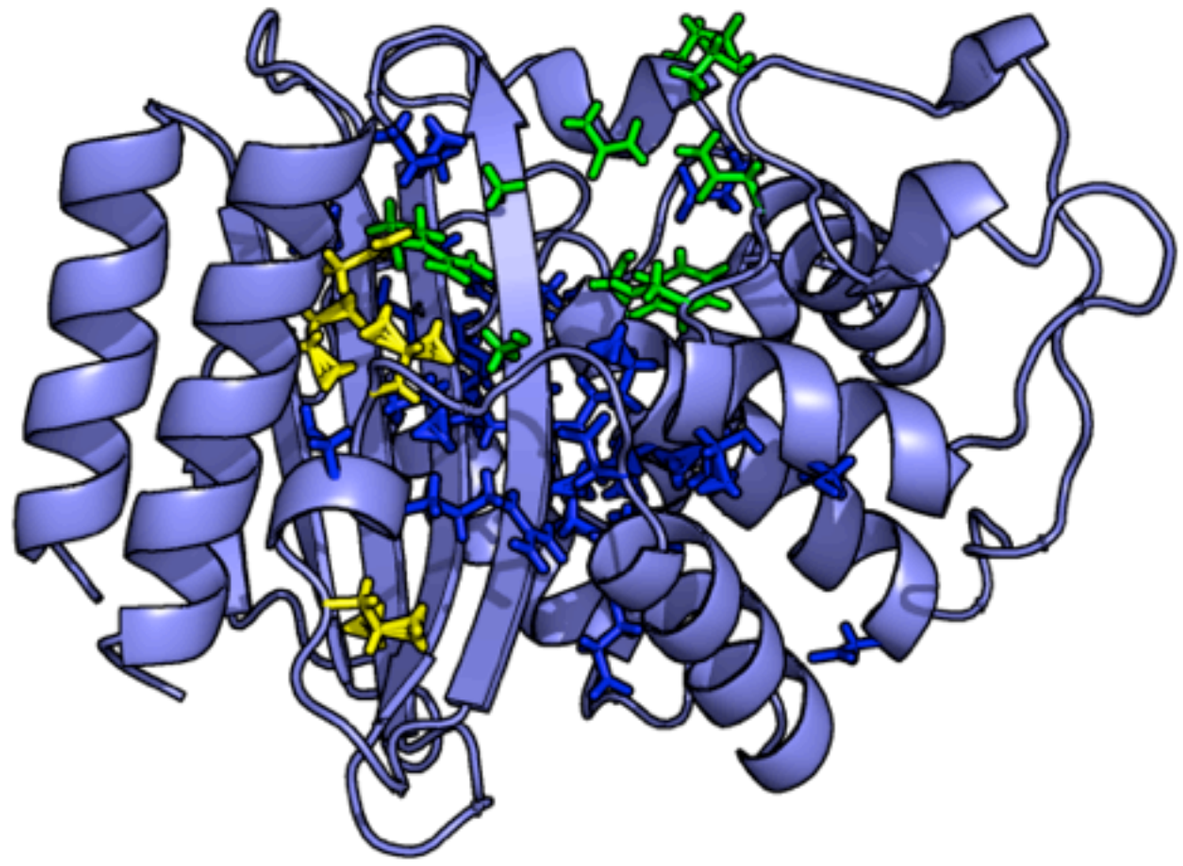
$$\mathrm{MI}(X,Y) = \sum_{x \in x} \sum_{y \in y} p(x,y) \log\left(\frac{p(x,y)}{p(x)p(y)}\right)$$

Bowman and Geissler. *PNAS* 2012.
DuBay and Geissler. JMB 2009.
McClendon et al. JCTC 2009.

Correlations between side-chain rotameric states allows long-distance communication

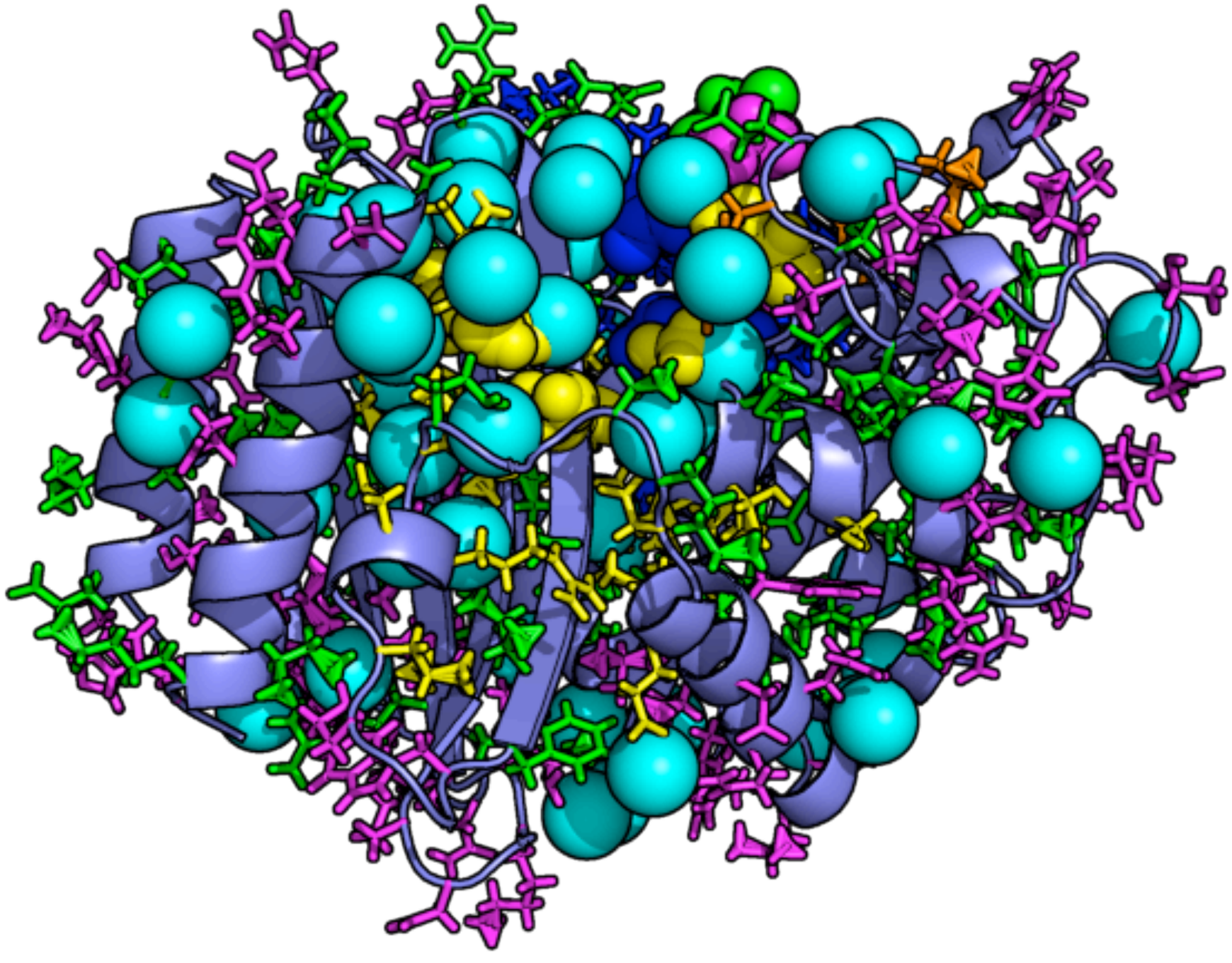


$$\mathrm{MI}(X,Y) = \sum_{x \in x} \sum_{y \in y} p(x,y) \log\left(\frac{p(x,y)}{p(x)p(y)}\right)$$



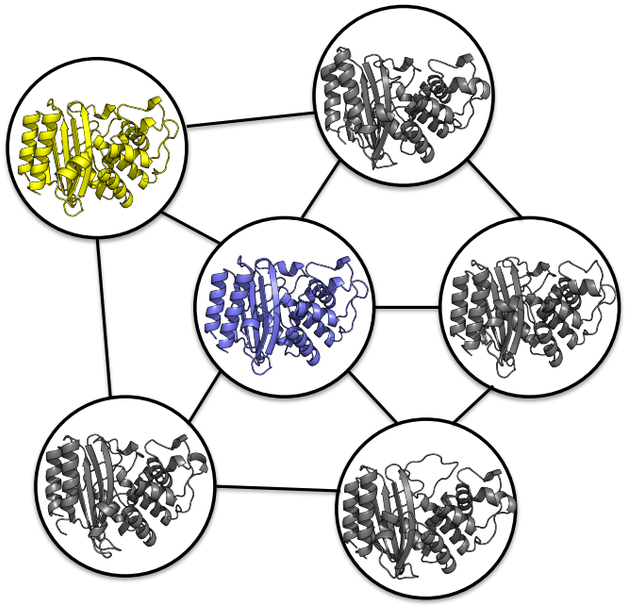
Bowman and Geissler. *PNAS* 2012.
DuBay and Geissler. JMB 2009.
McClendon et al. JCTC 2009.

There are many other potential cryptic allosteric sites

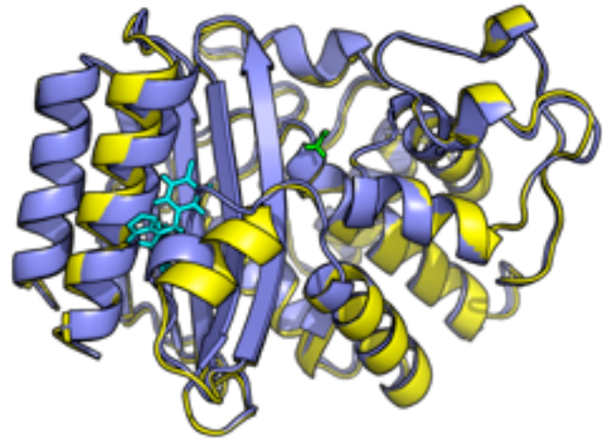


Bowman and Geissler. *PNAS* 2012.

Outline



Computational approach

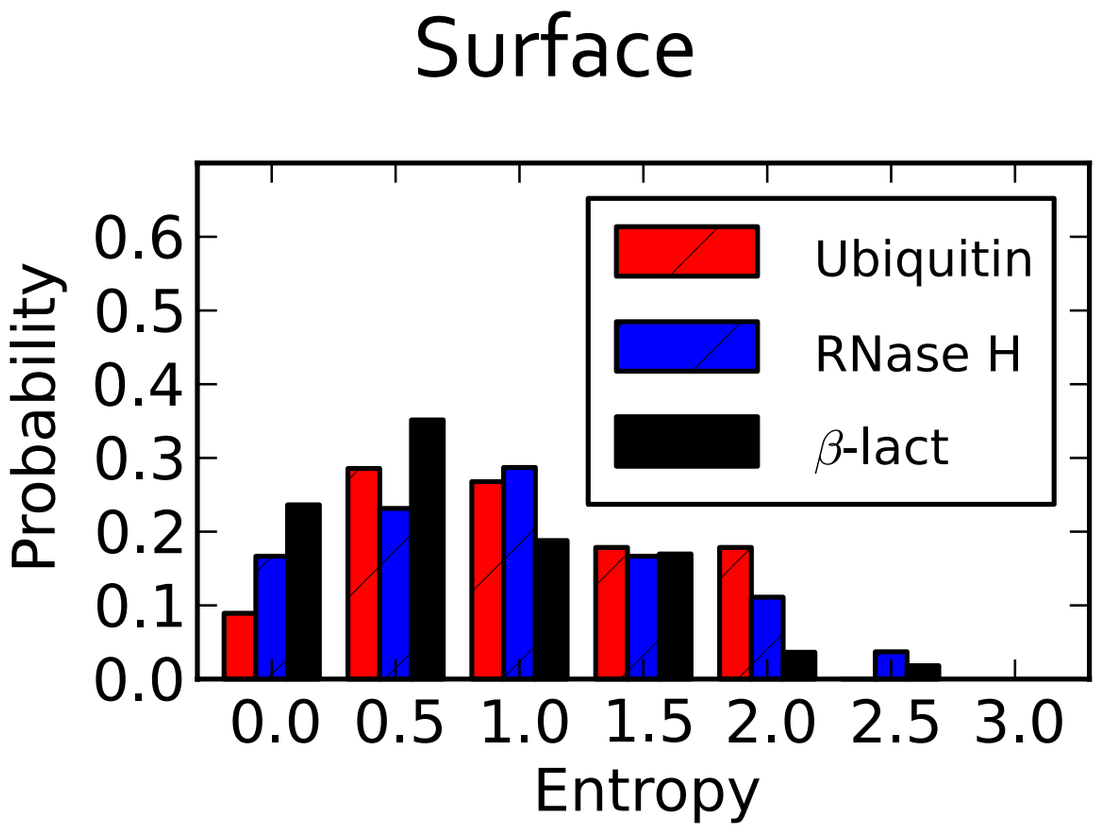


Understanding and predicting allostery

?

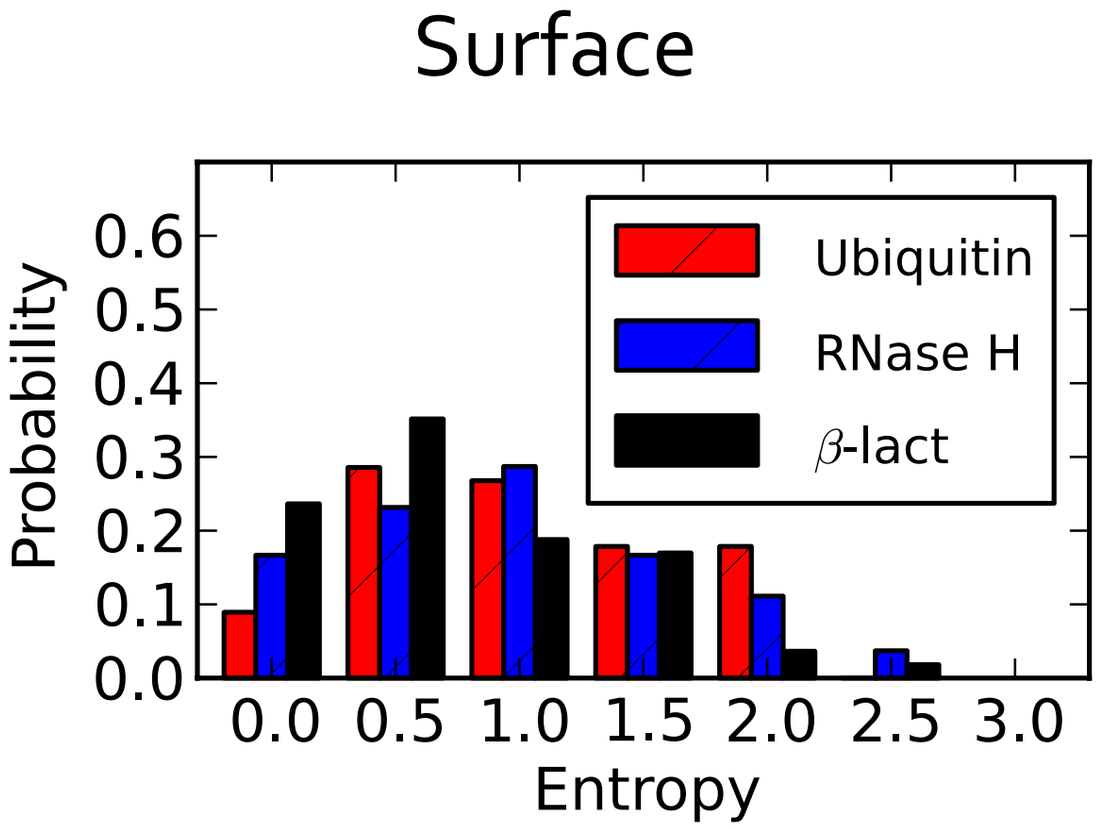
Experimental tests

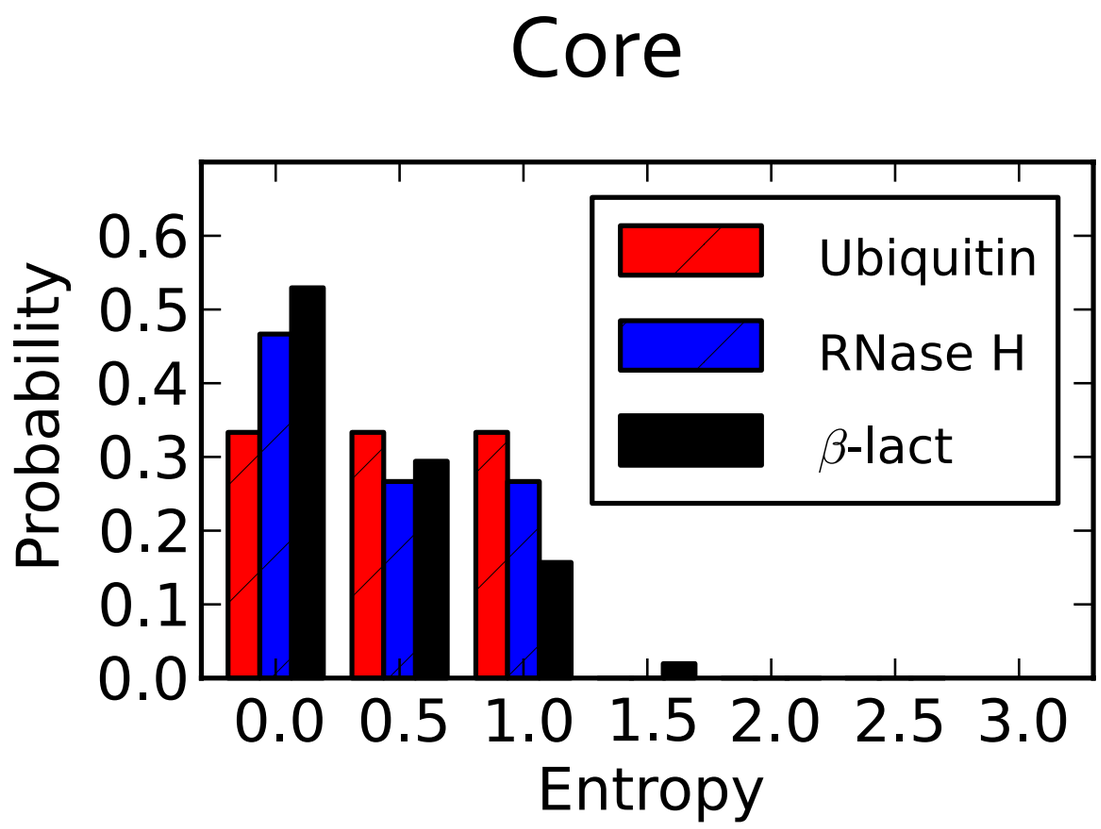
My models predict extensive side-chain heterogeneity even in proteins' cores



Bowman and Geissler. *submitted* 2013.

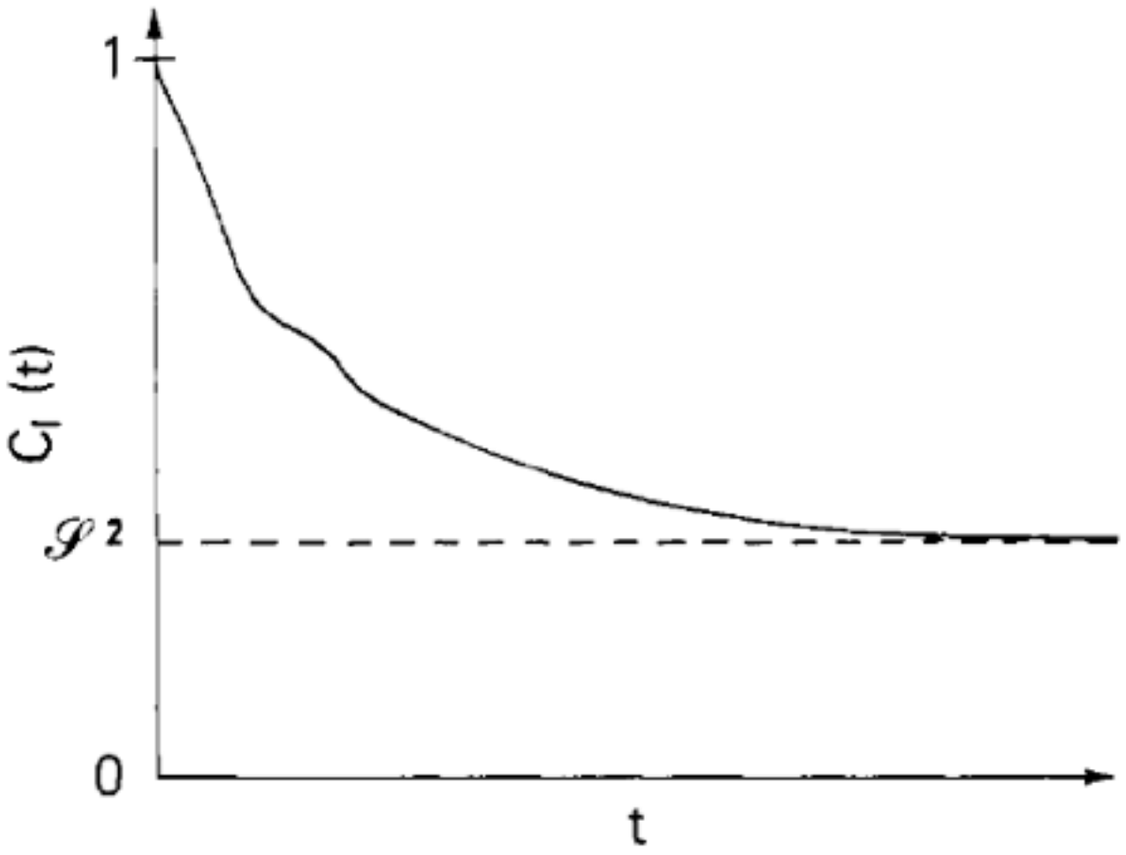
My models predict extensive side-chain heterogeneity even in proteins' cores





Bowman and Geissler. *submitted* 2013.

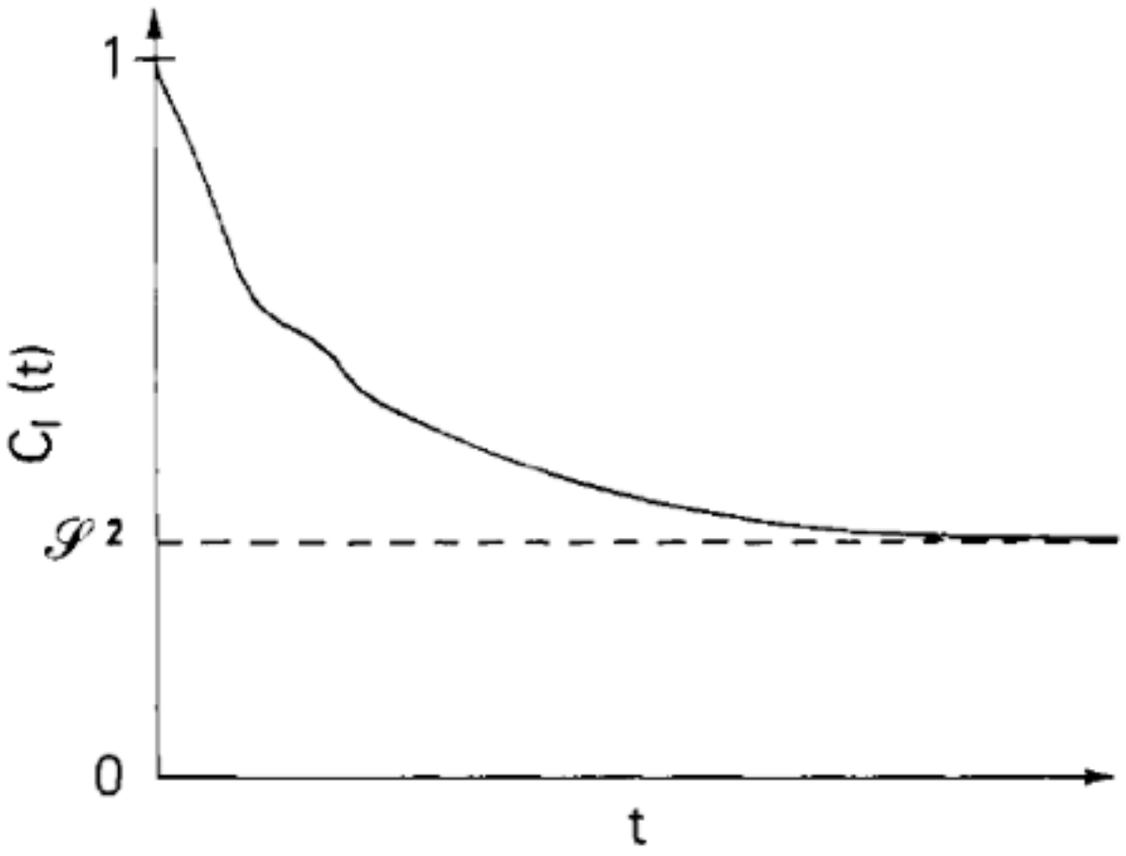
My observations are consistent with NMR order parameters

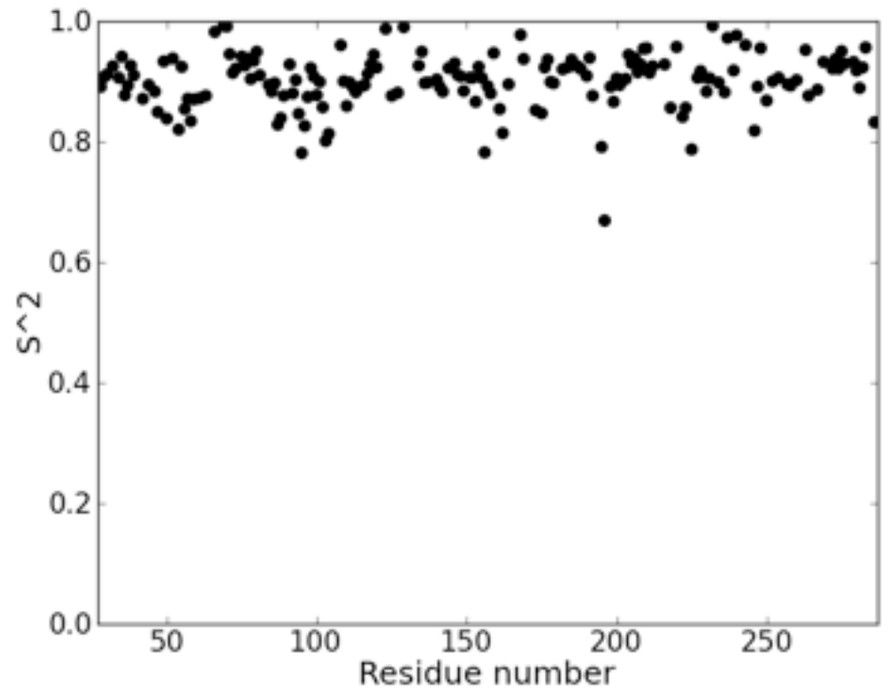


$$C_I(t) = \langle P_2(\hat{\mu}(0)\cdot\hat{\mu}(t)) \rangle$$

Savard and Gaqne. *Biochemistry* 2006.

My observations are consistent with NMR order parameters

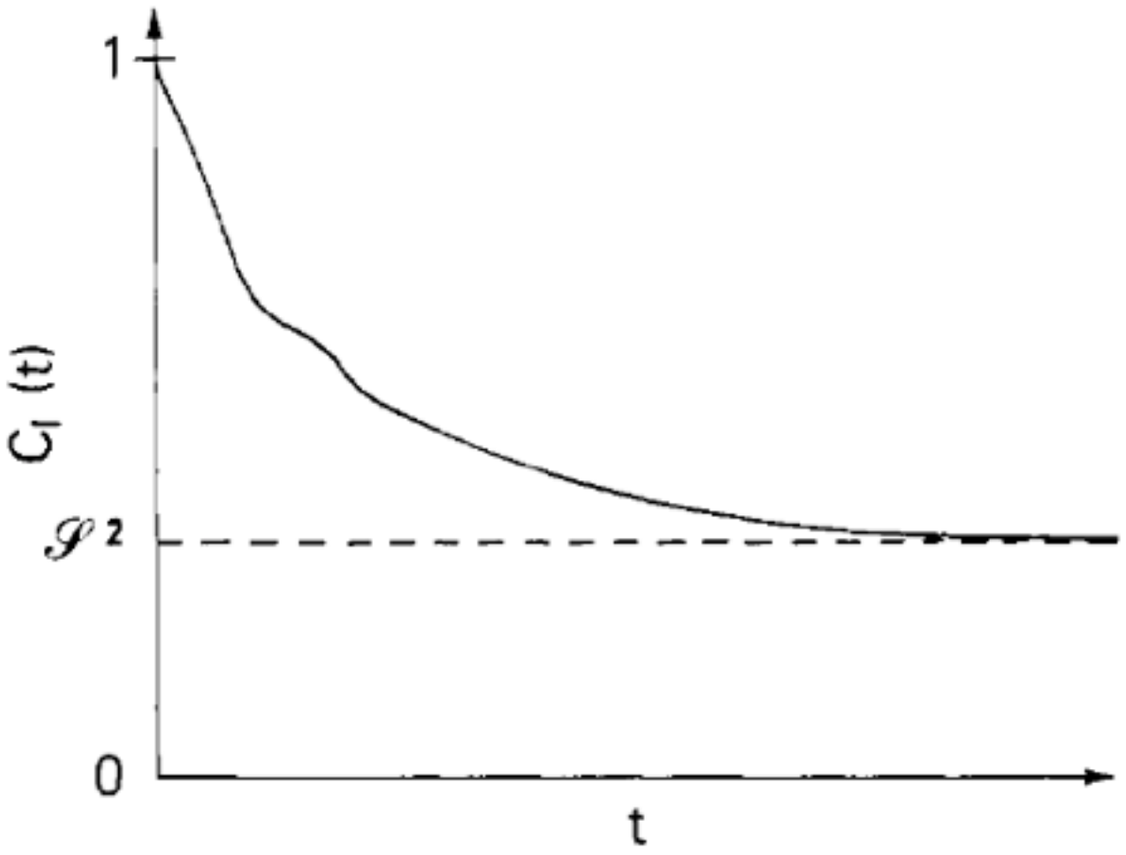


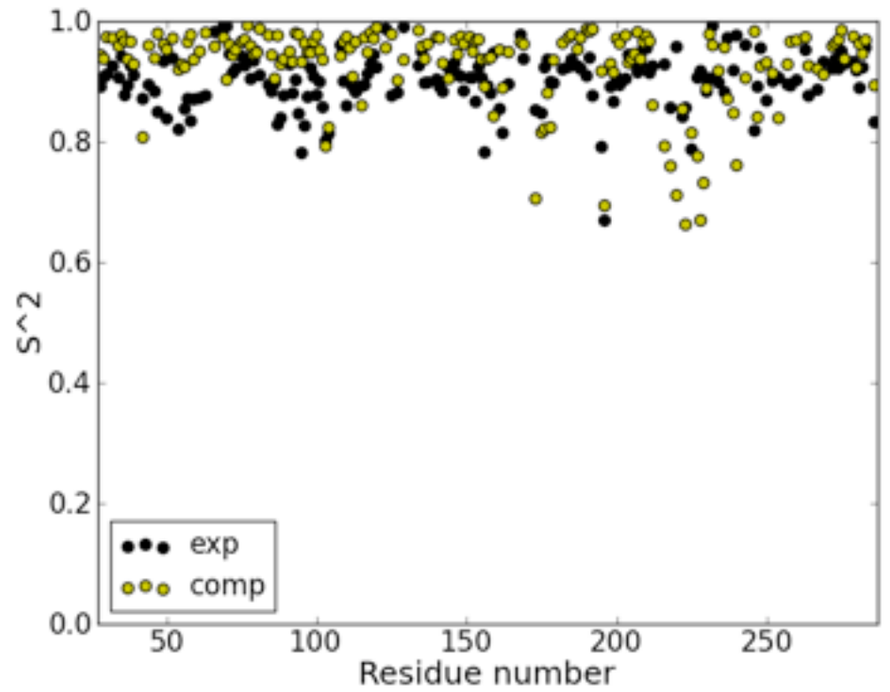


$$C_I(t) = \langle P_2(\hat{\mu}(0)\cdot\hat{\mu}(t)) \rangle$$

Savard and Gagne. *Biochemistry* 2006.

My observations are consistent with NMR order parameters

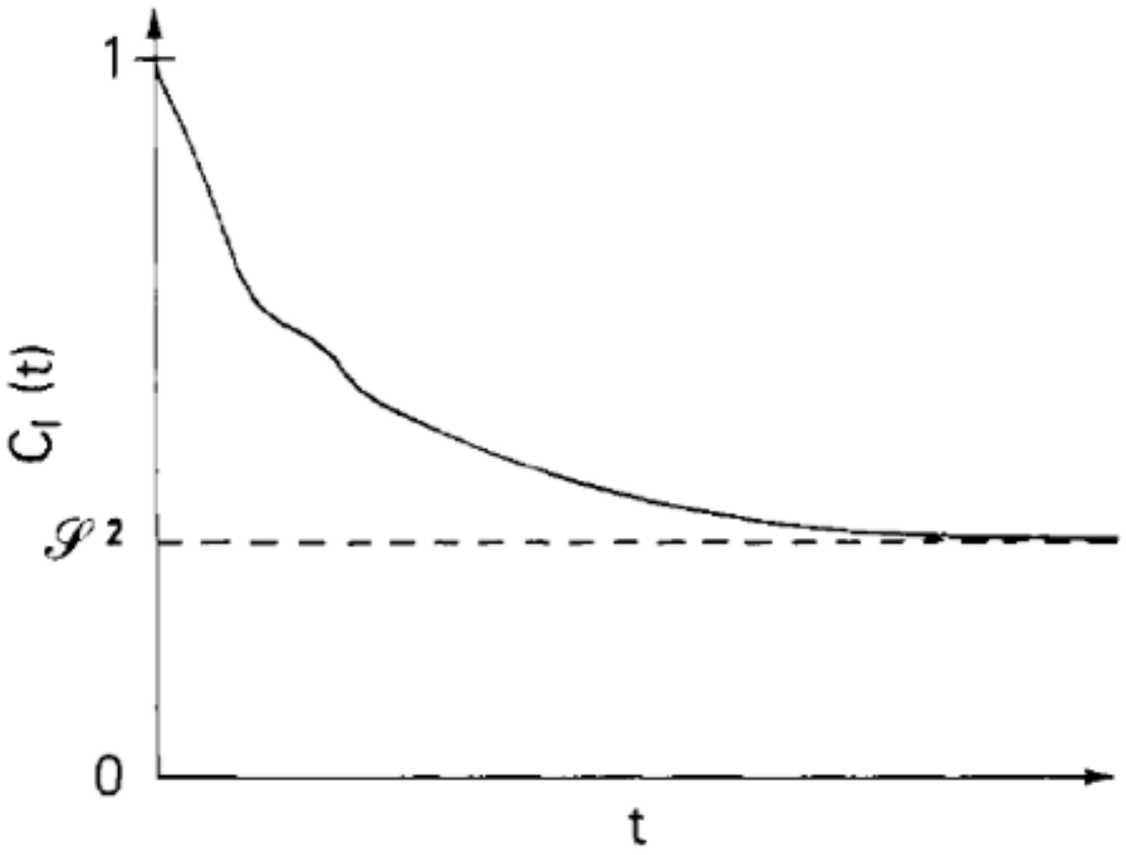




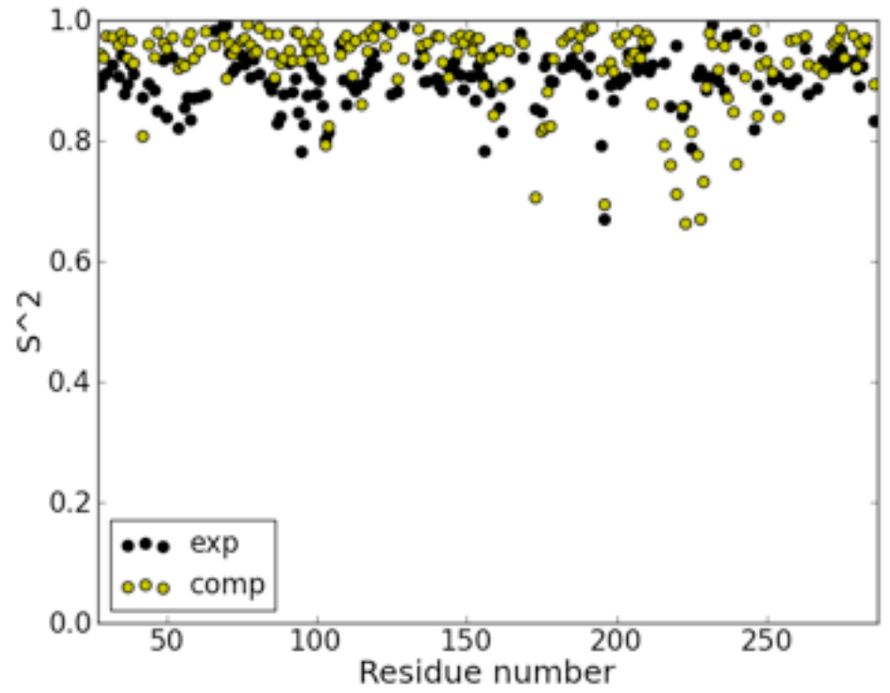
$$C_I(t) = \langle P_2(\hat{\mu}(0)\cdot\hat{\mu}(t)) \rangle$$

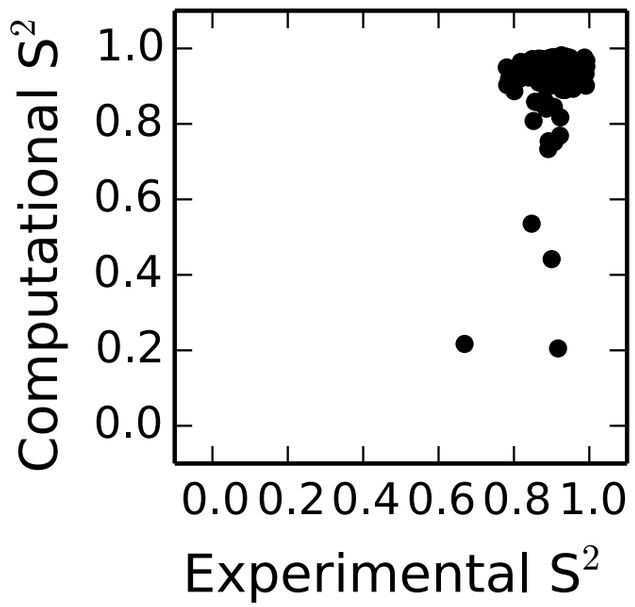
Savard and Gagne. *Biochemistry* 2006.

My observations are consistent with NMR order parameters



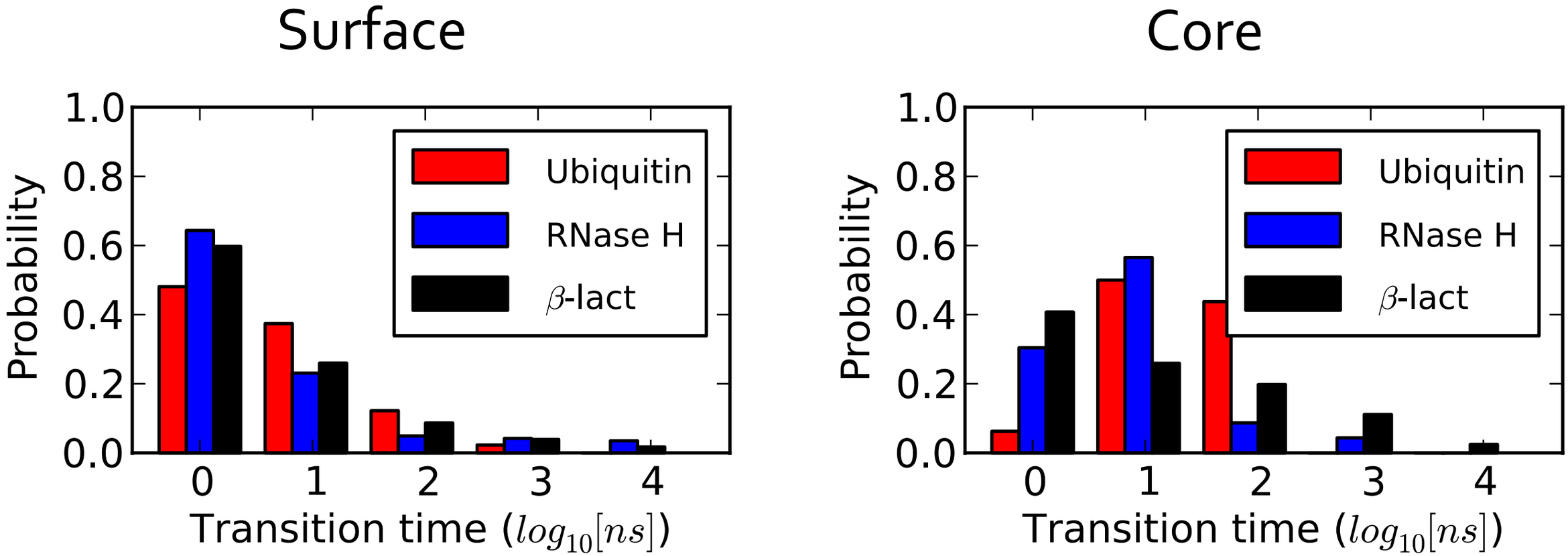
$$C_I(t) = \langle P_2(\hat{\mu}(0)\cdot\hat{\mu}(t)) \rangle$$





Savard and Gagne. *Biochemistry* 2006.

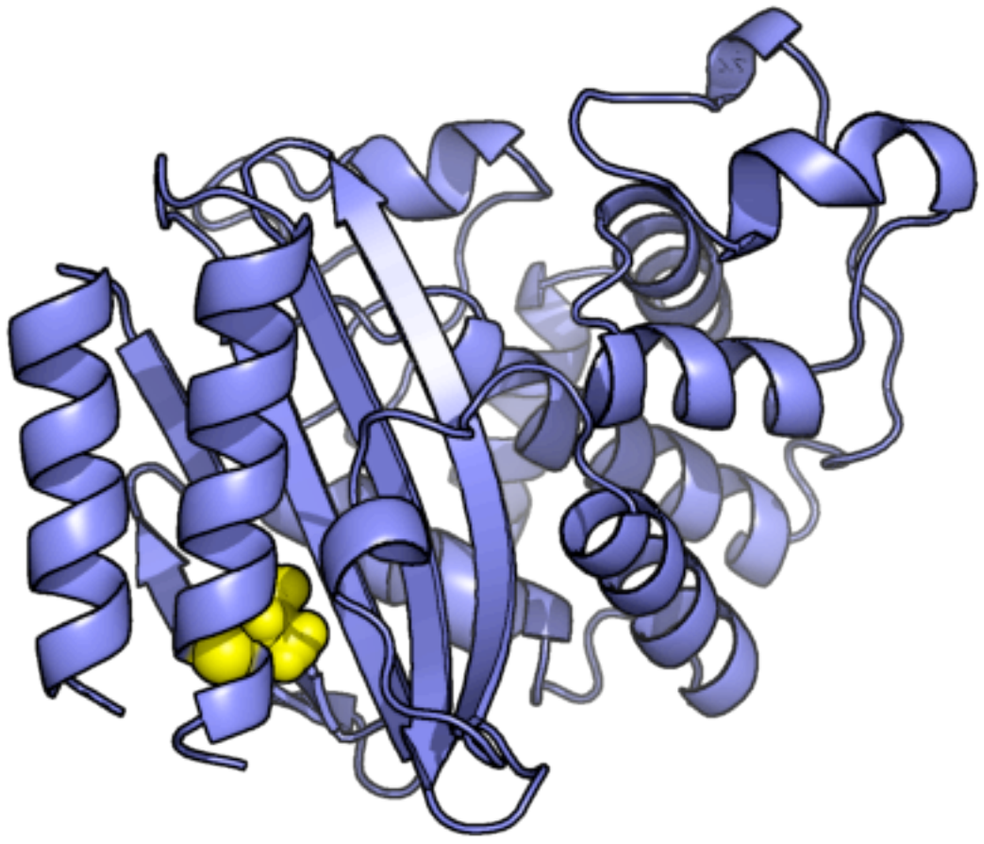
My models provide access to the timescales for side-chain dynamics



Bowman and Geissler. *submitted* 2013.

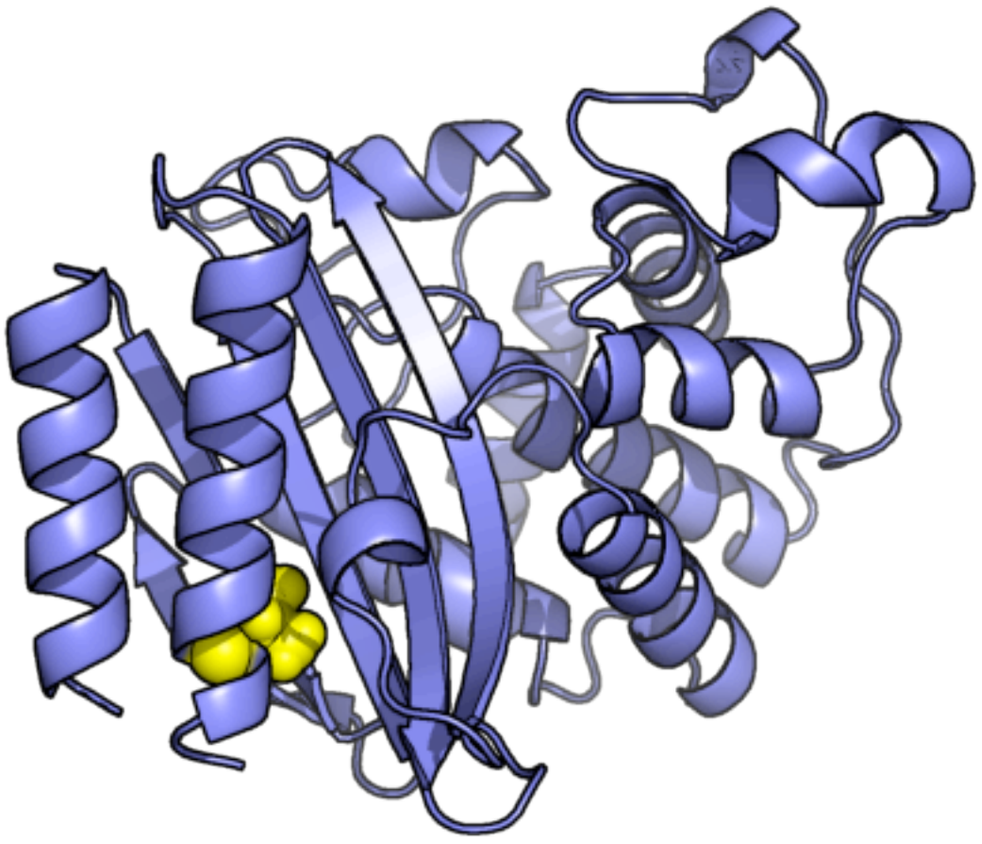
Native state thiol-exchange provides a direct test of my predicted pockets

Native state thiol-exchange provides a direct test of my predicted pockets

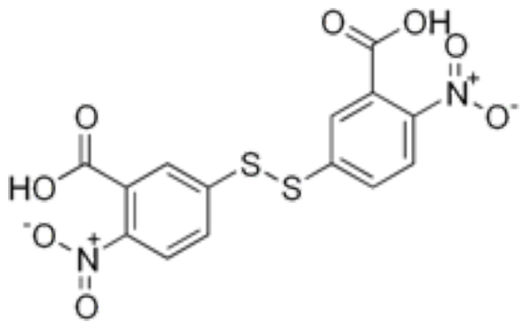


Bernstein *et al. PNAS* 2011.

Native state thiol-exchange provides a direct test of my predicted pockets

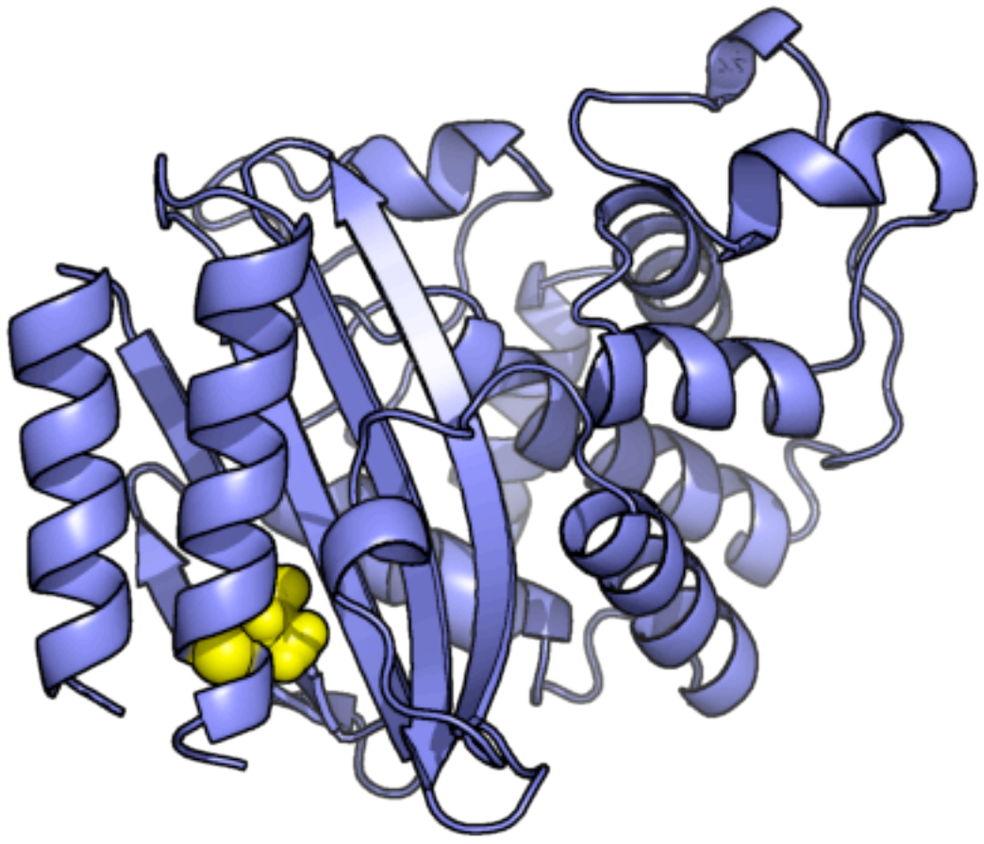


DTNB



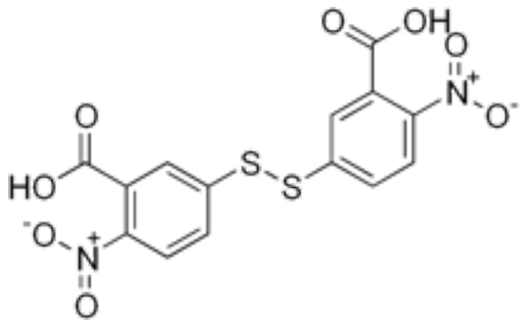
Bernstein *et al. PNAS* 2011.

Native state thiol-exchange provides a direct test of my predicted pockets



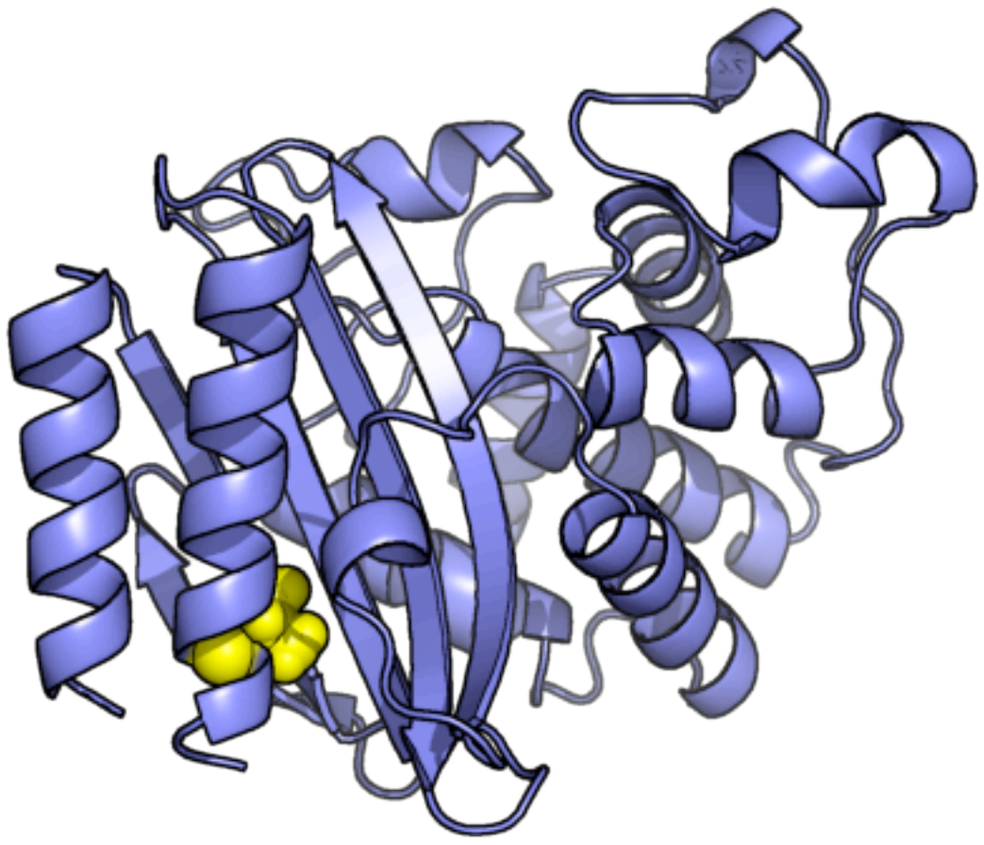
Closed $\rightleftarrows$ Open $\rightarrow$ Labeled

DTNB



Bernstein *et al. PNAS* 2011.

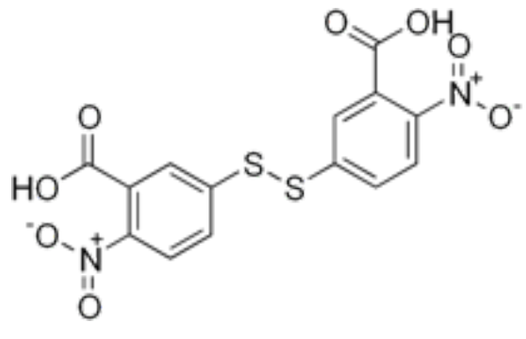
Native state thiol-exchange provides a direct test of my predicted pockets



Closed $\rightleftarrows$ Open $\rightarrow$ Labeled

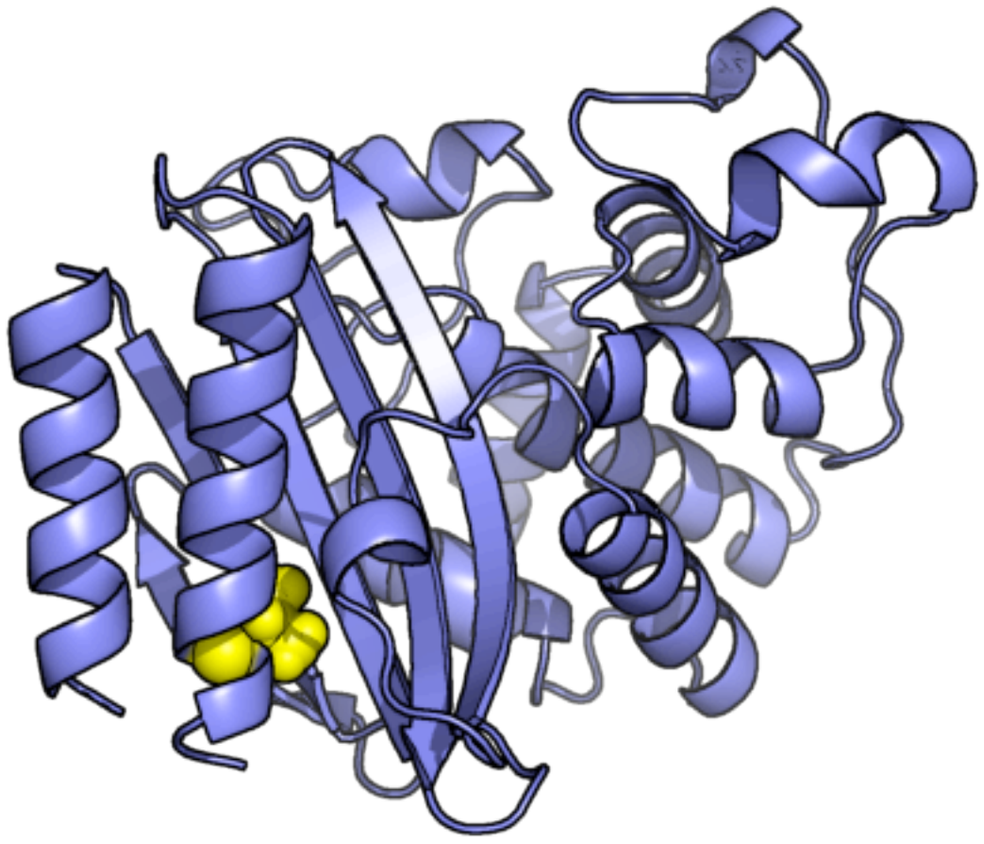
$$k_{obs} = \frac{k_{op} \times k_{int}}{k_{op} + k_{cl} + k_{int}}$$

DTNB



Bernstein *et al. PNAS* 2011.

Native state thiol-exchange provides a direct test of my predicted pockets



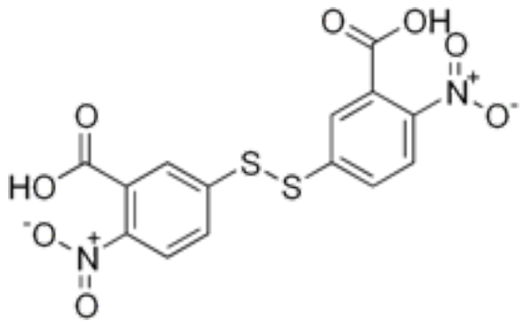
Closed $\rightleftarrows$ Open $\rightarrow$ Labeled

$$k_{obs} = \frac{k_{op} \times k_{int}}{k_{op} + k_{cl} + k_{int}}$$

$$EX1: \; k_{cl} << k_{int}$$

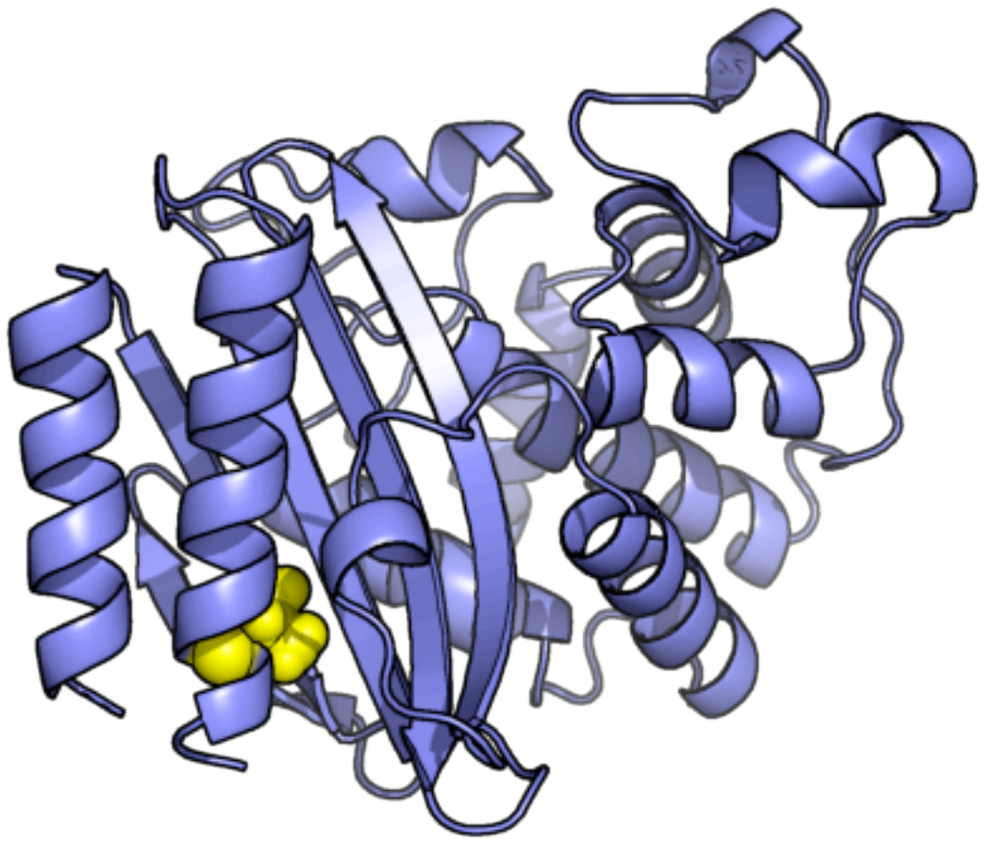
$$k_{obs} = k_{op}$$

DTNB

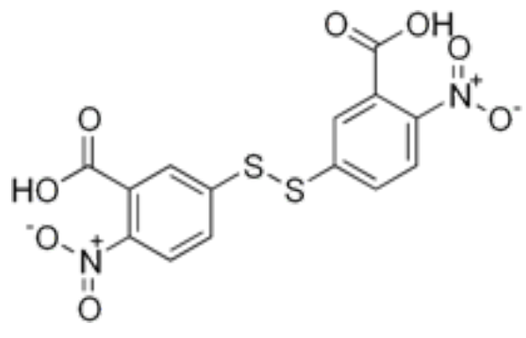


Bernstein *et al. PNAS* 2011.

Native state thiol-exchange provides a direct test of my predicted pockets



DTNB



Closed $\rightleftarrows$ Open $\rightarrow$ Labeled

$$k_{obs} = \frac{k_{op} \times k_{int}}{k_{op} + k_{cl} + k_{int}}$$

$$EX1: \; k_{cl} << k_{int}$$

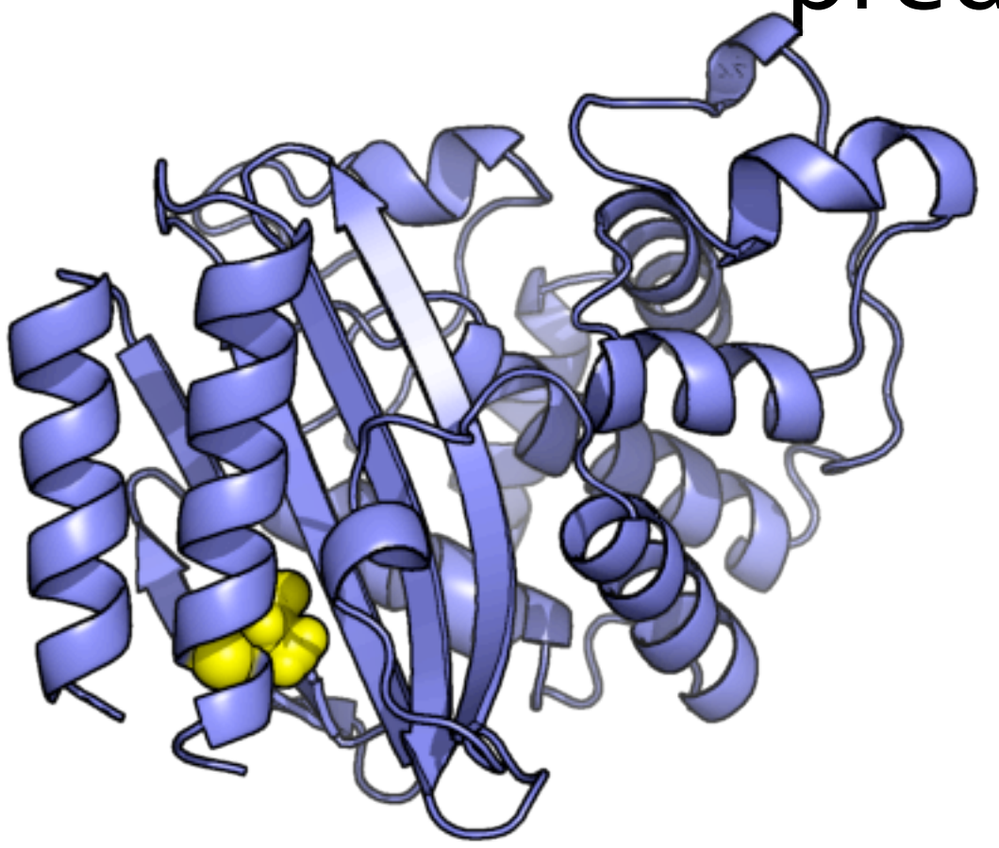
$$k_{obs} = k_{op}$$

$$EX2: \; k_{cl} >> k_{int}$$

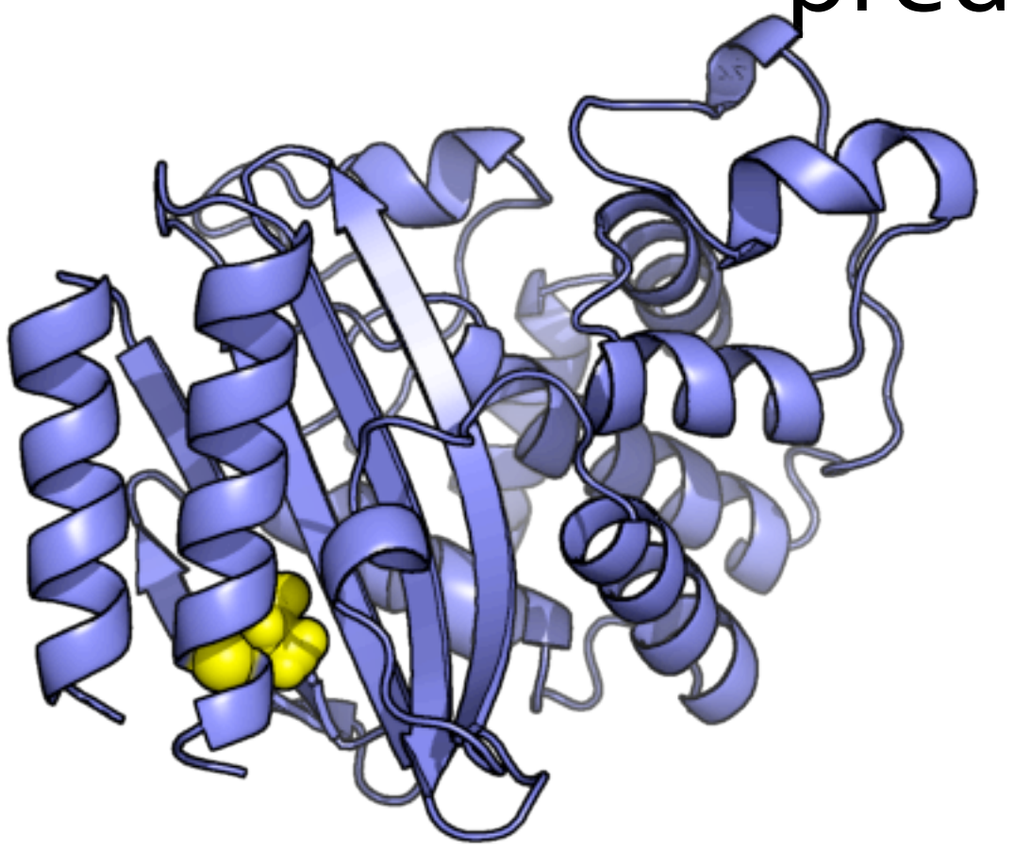
$$k_{obs} = \frac{k_{op} \times k_{int}}{k_{cl}} = K_{op} \times k_{int}$$

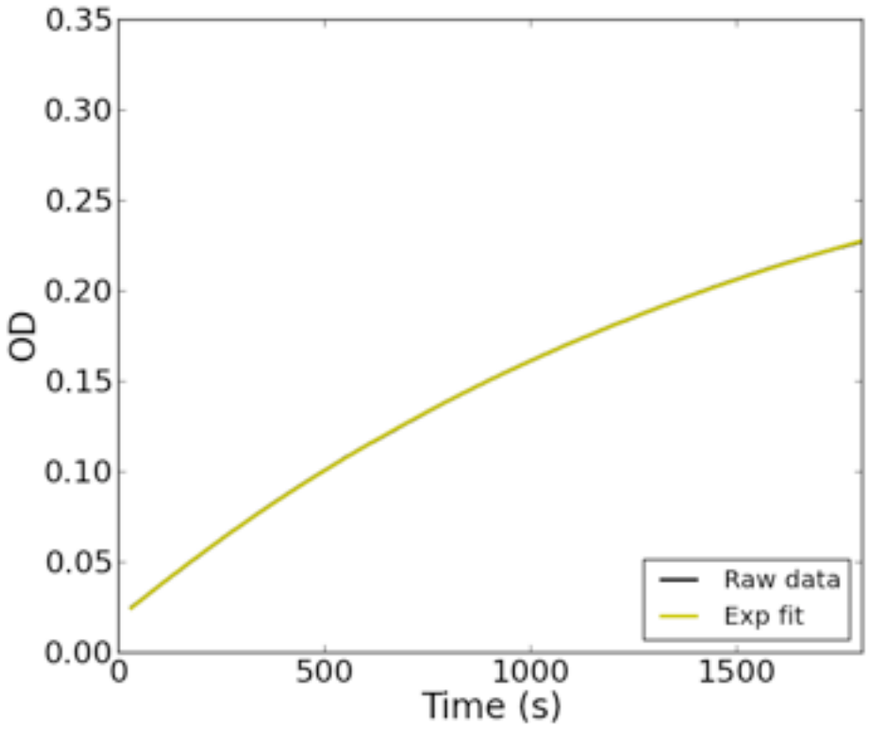
Bernstein *et al. PNAS* 2011.

The known pocket also opens, as predicted

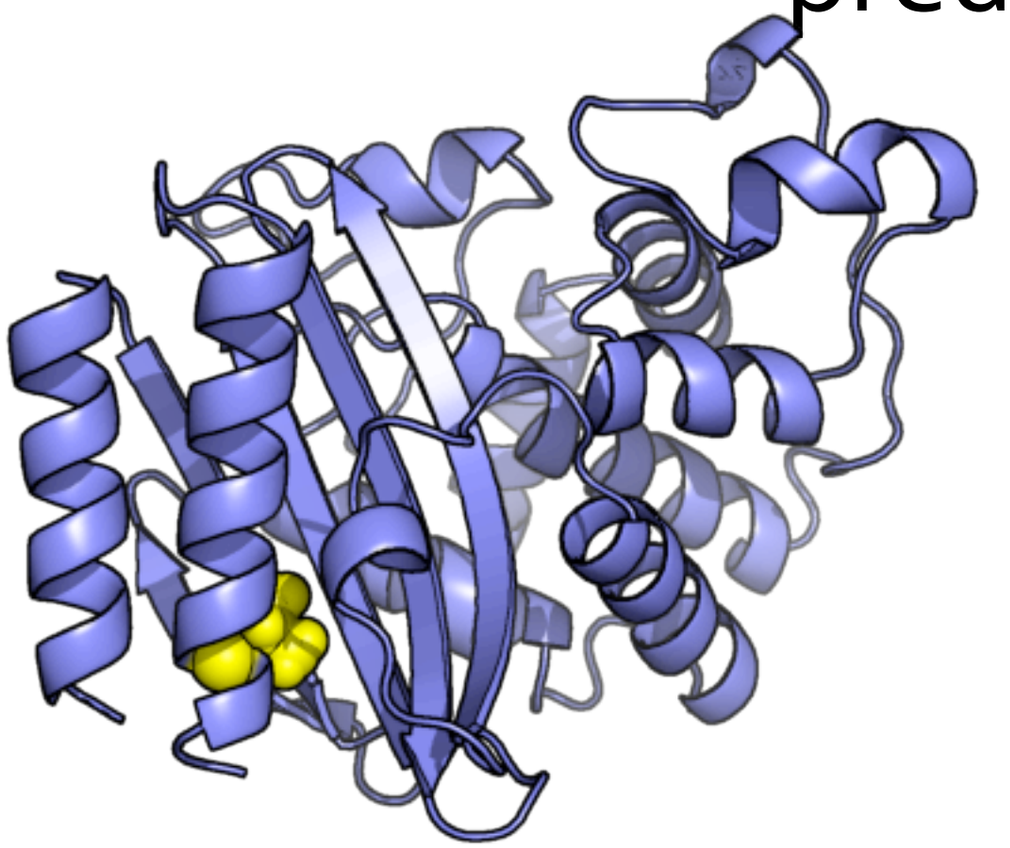


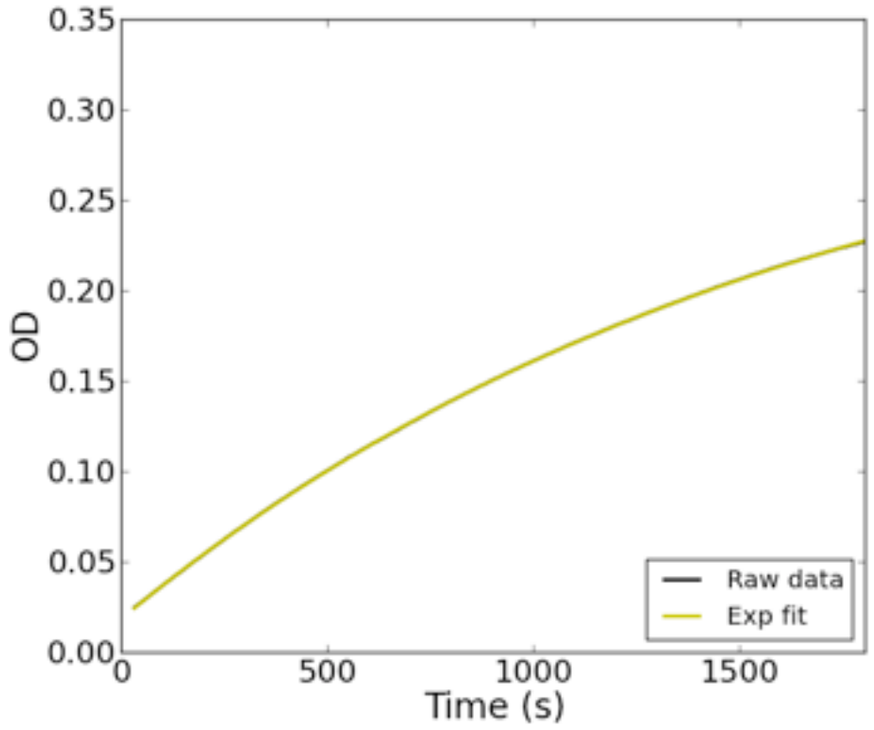
The known pocket also opens, as predicted

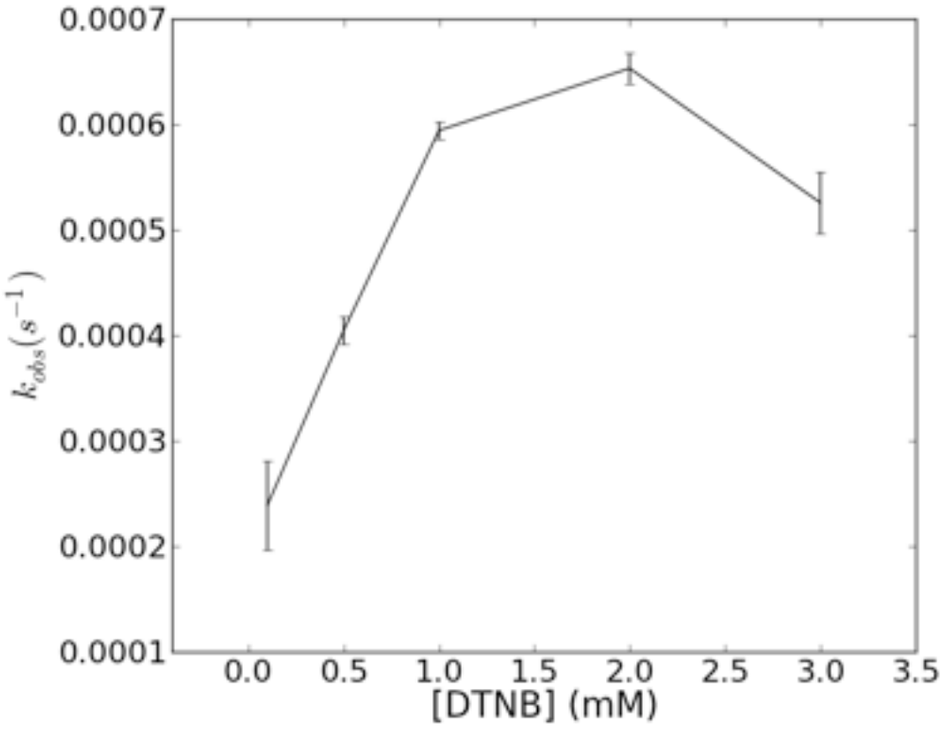




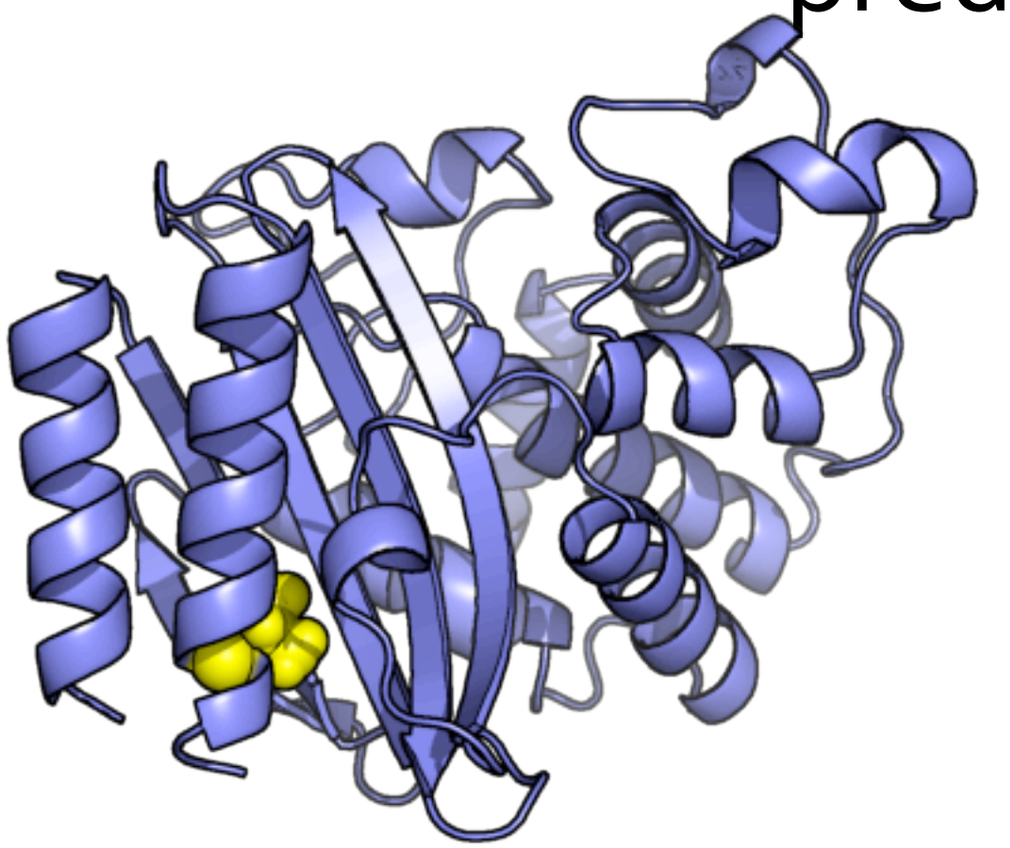
The known pocket also opens, as predicted

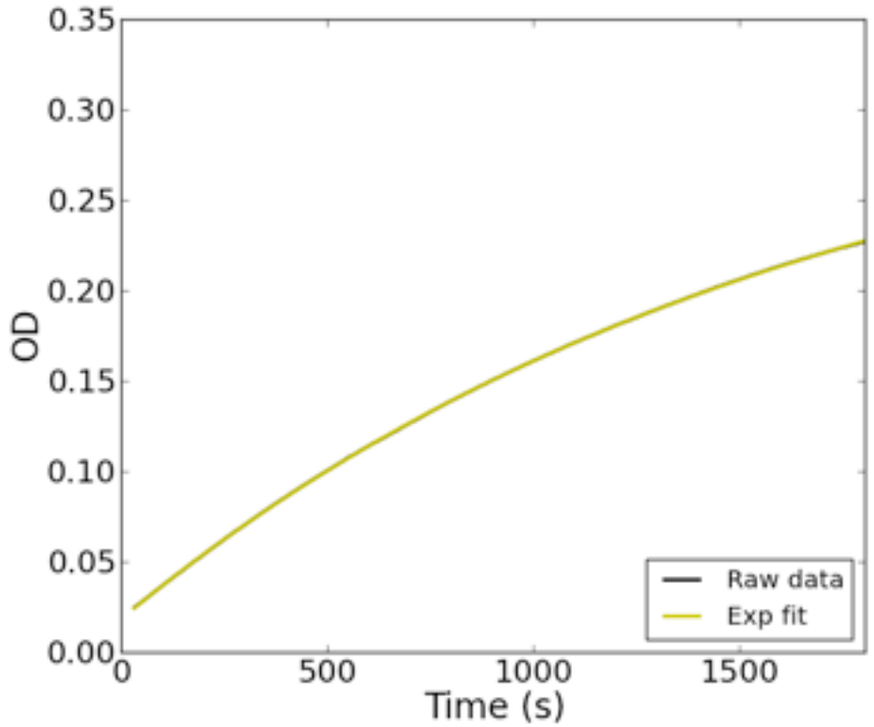




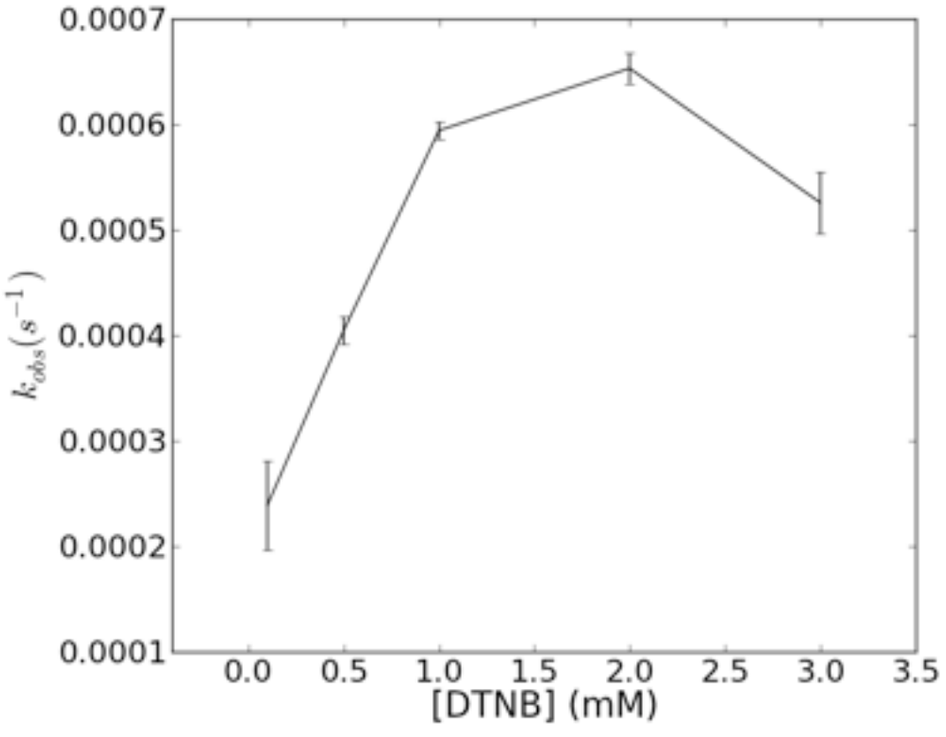


The known pocket also opens, as predicted

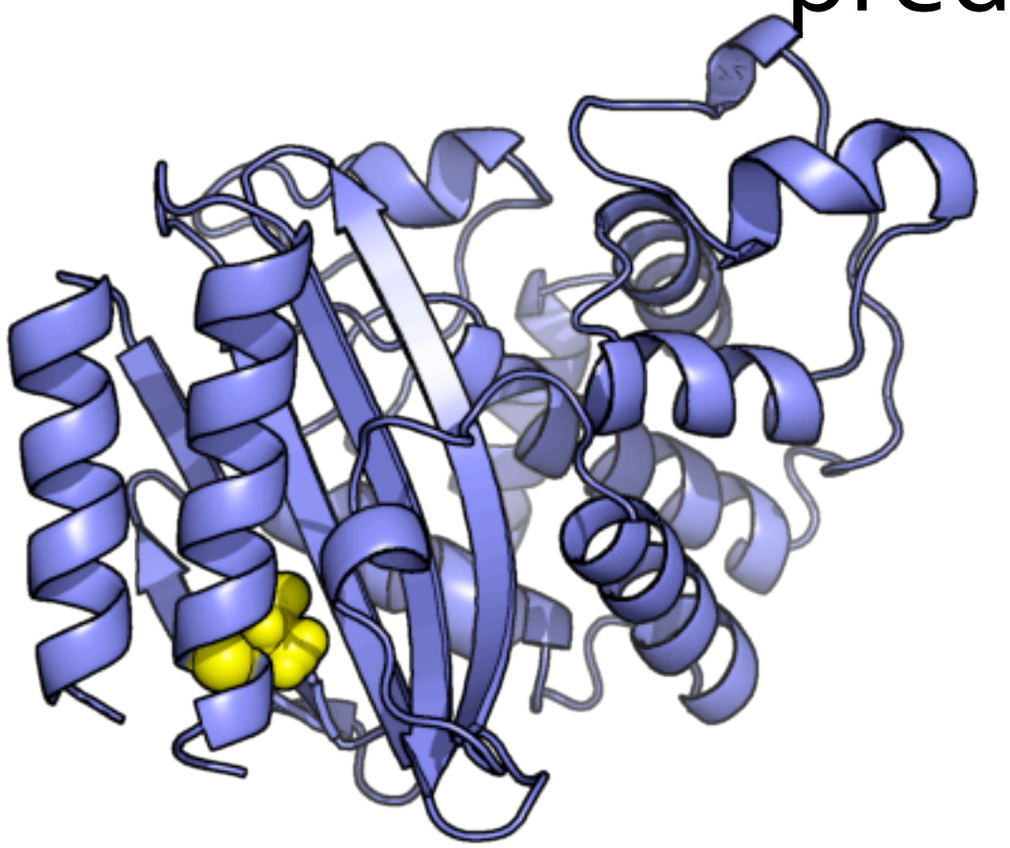


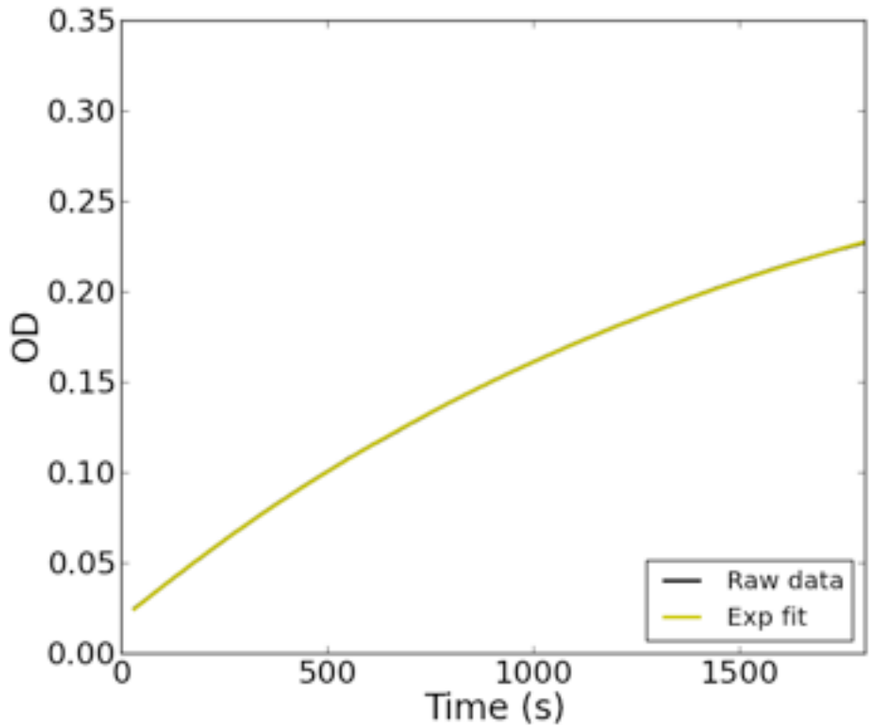


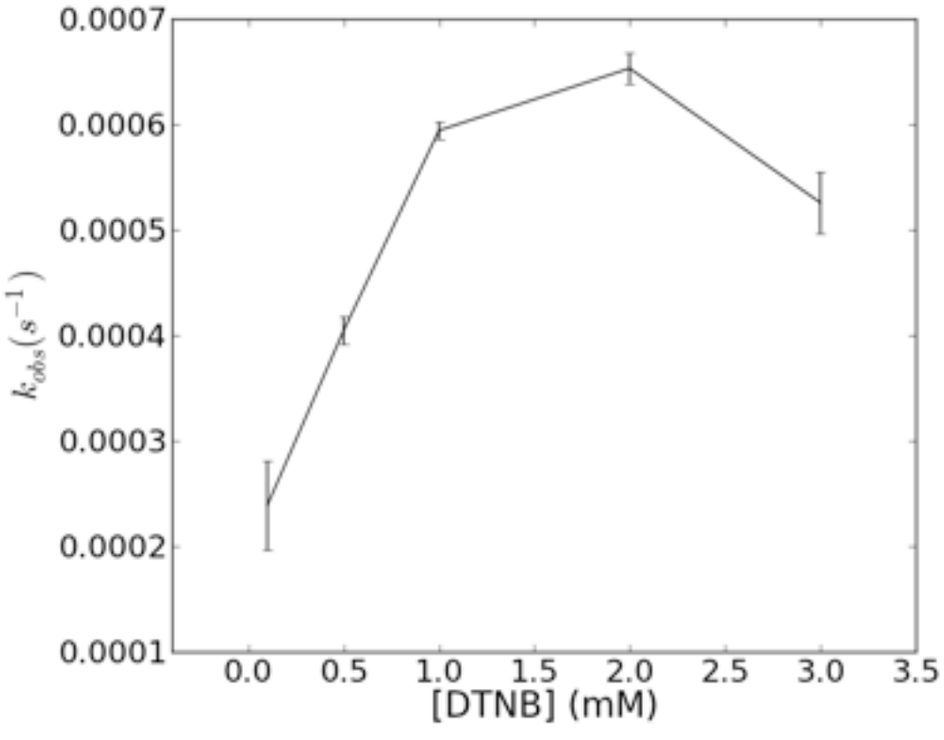
Rate labeling: $6 \times 10^{-4}\ s^{-1}$



The known pocket also opens, as predicted







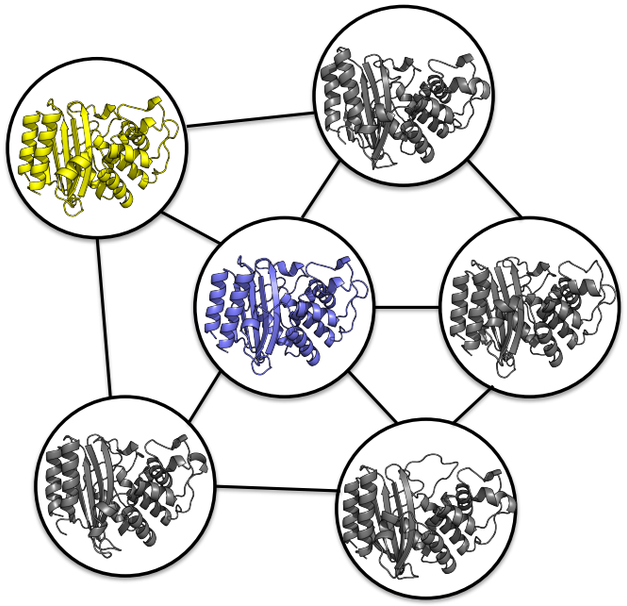
Rate labeling: $6 \times 10^{-4}\ s^{-1}$

Rate unfolding < $2.5 \times 10^{-5}\ s^{-1}$

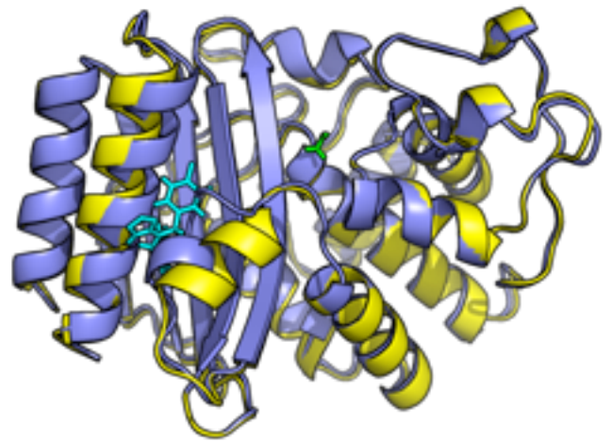
We are also testing a newly predicted pocket

We are also testing a newly predicted pocket

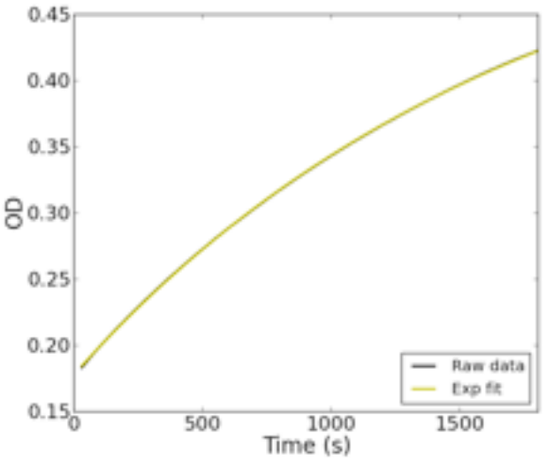
Outline



Computational approach

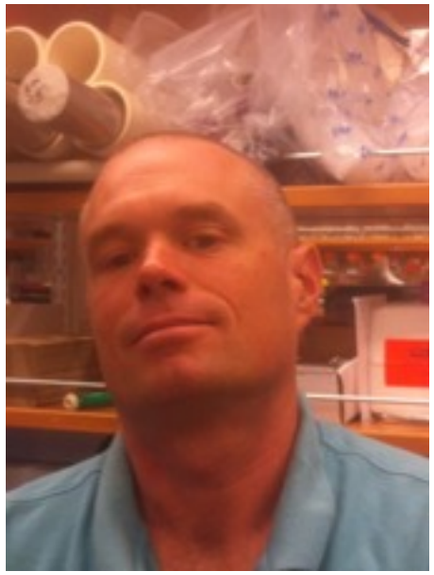


Understanding and predicting allostery

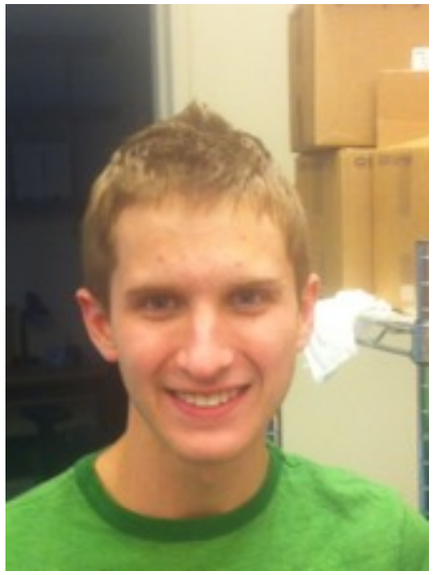


Experimental tests

Thanks!



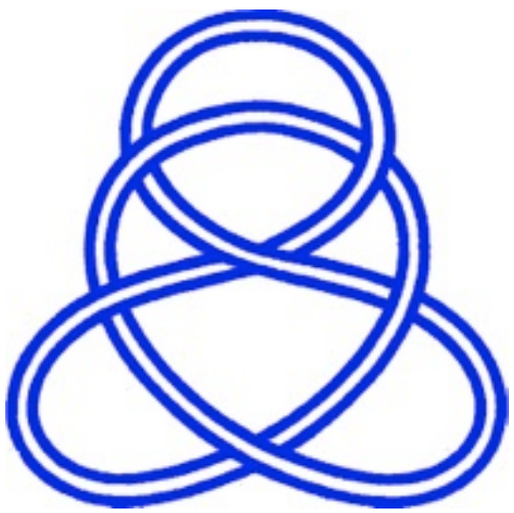
Brendan Maguire



Eric Bolin

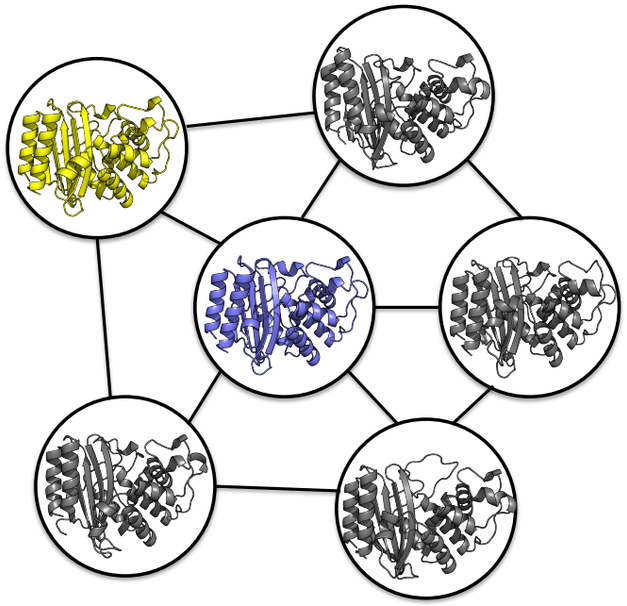
Susan Marqusee

Phillip Geissler

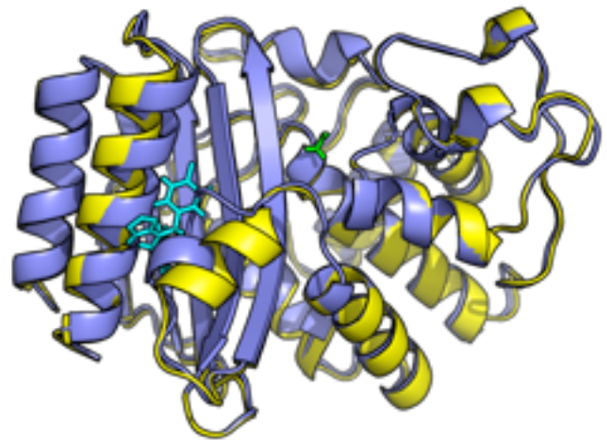


Miller Institute for Basic Research in Science

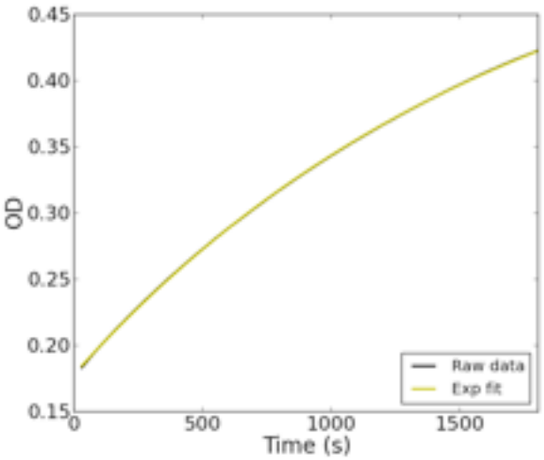
Outline



Computational approach



Understanding and predicting allostery



Experimental tests