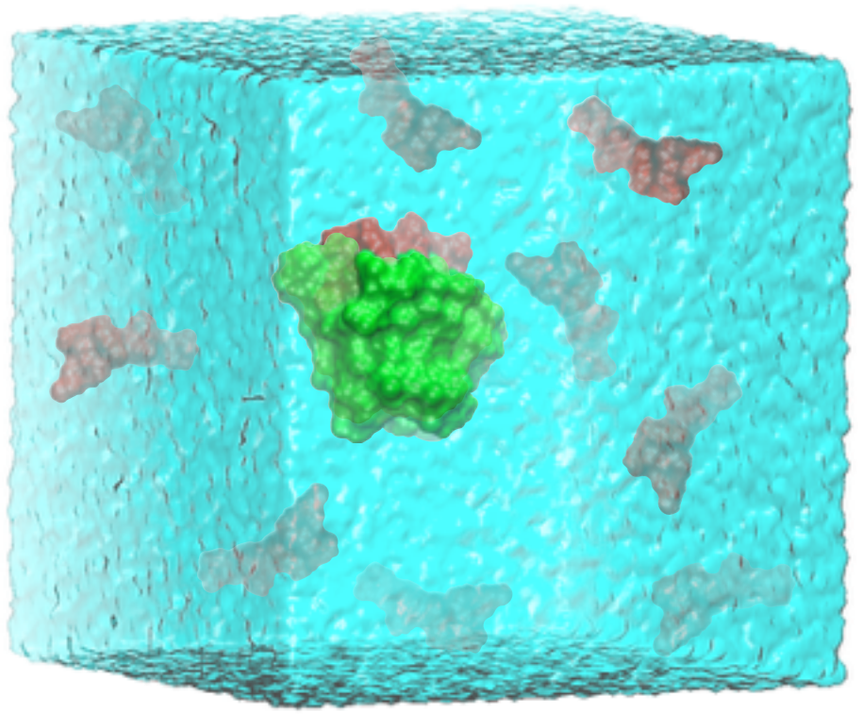
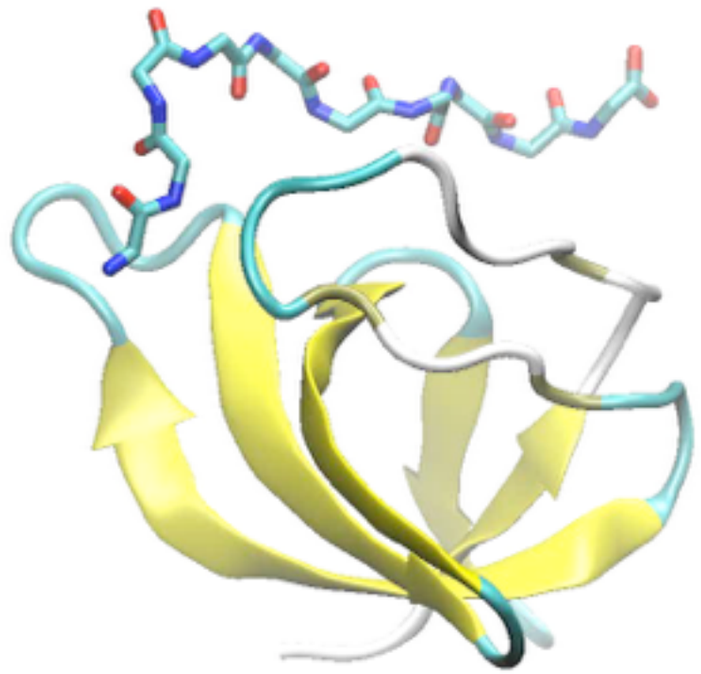
Accurate calculation of

ligand binding energy





James C. (JC) Gumbart

Georgia Institute of Technology, Atlanta

Chris Chipot

U. Illinois, Urbana & CNRS, U. Lorraine France

Computational Biophysics Workshop | Georgia Tech | Nov. 16 2016

Outline

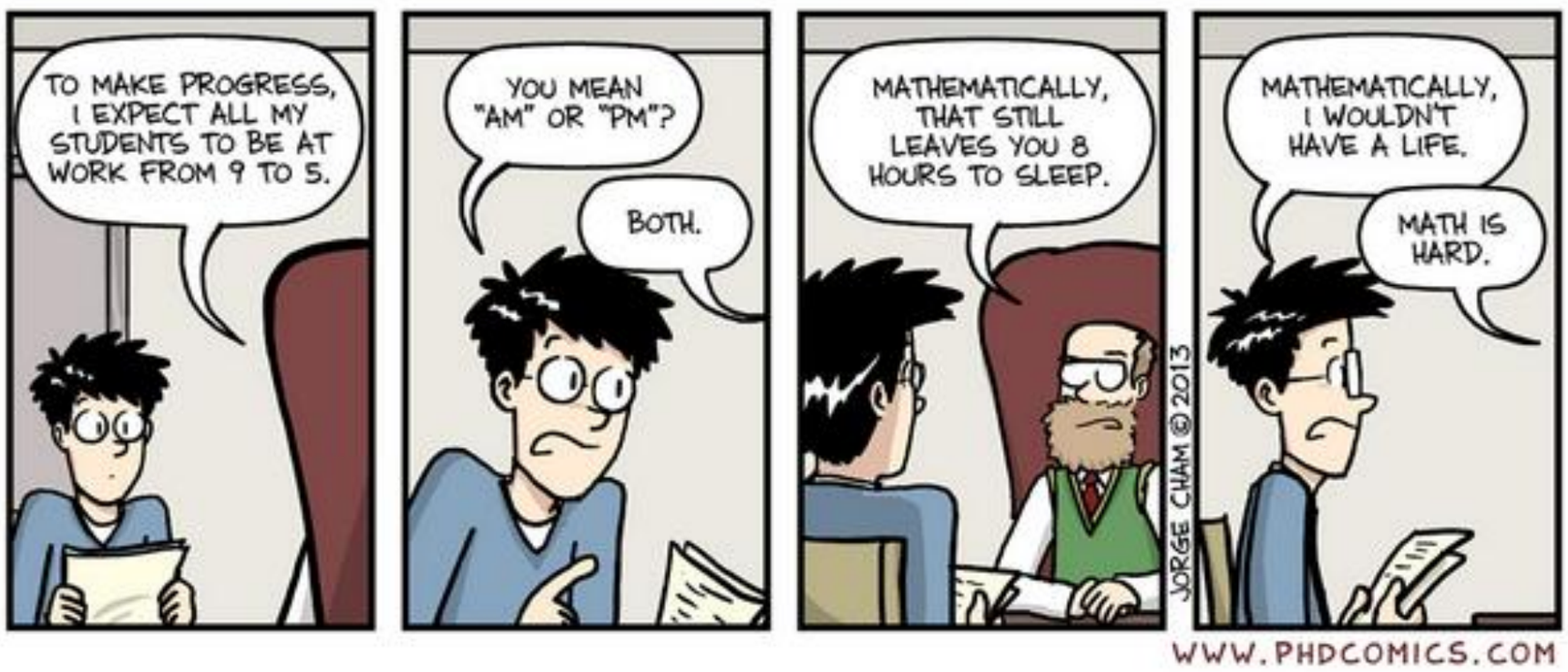
I. *What is an **absolute** binding energy?*

II. *Using restraints to reduce the sampling problem*

III. *Calculating the requisite PMFs*

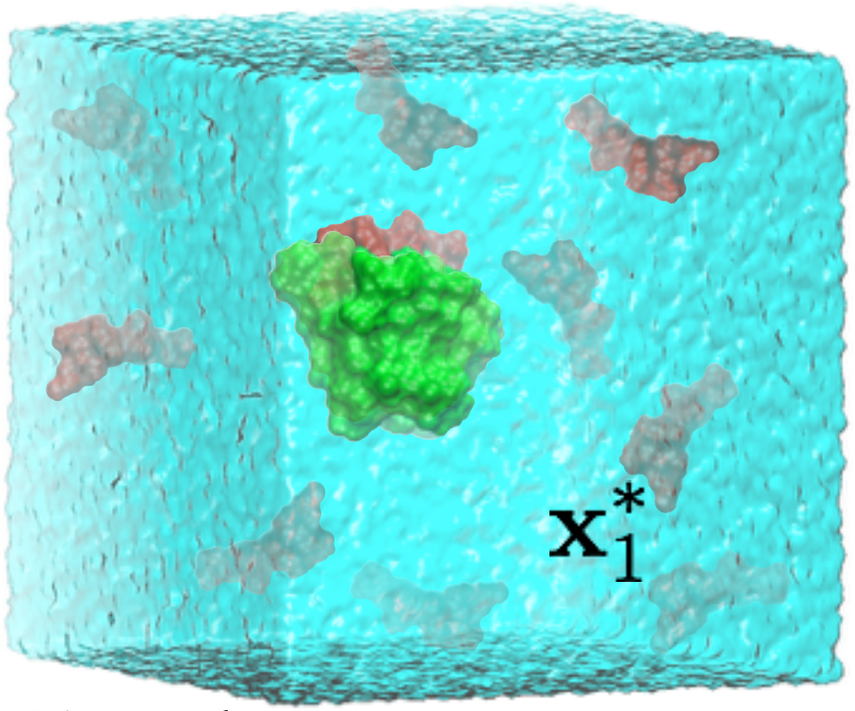
IV. *Comparing geometric with alchemical approach*

V. *Illustration with barstar-barnase binding*



http://phdcomics.com/

Challenge: Absolute binding free energies



$$\text{protein} + \text{ligand} \underset{}{\overset{K_{eq}}{\rightleftharpoons}} \text{protein : ligand}$$

$$K_{eq} = \frac{[\text{protein : ligand}]}{[\text{protein}][\text{ligand}]} = \frac{p_1[\text{protein}]_{tot}}{[\text{ligand}]\ p_0\ [\text{protein}]_{tot}} = \frac{1}{[\text{ligand}]}\ \frac{p_1}{p_0}$$

N ligands

$$K_{eq} = \frac{1}{[\text{ligand}]} \frac{N \int_{site} \mathbf{d1} \int_{bulk} \mathbf{d2} \ldots \int_{bulk} \mathbf{dN} \int \mathbf{dx}\ e^{-\beta U}}{\int_{bulk} \mathbf{d1} \int_{bulk} \mathbf{d2} \ldots \int_{bulk} \mathbf{dN} \int \mathbf{dx}\ e^{-\beta U}} = \frac{1}{[\text{ligand}]} \frac{N \int_{site} \mathbf{d1} \int \mathbf{dx}\ e^{-\beta U}}{V_{bulk} \int_{bulk} \mathbf{d1}\ \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int \mathbf{dx}\ e^{-\beta U}}$$

$p_i \propto Z_i$

*1, 2,…,**N** refer to each ligand*
1 is bound (site) in num.,
unbound (bulk) in denom.

*Energy does not depend on position of ligand when unbound (**bulk is isotropic**), so can pick out a specific point **x**1* and hold it there*

$$[\text{ligand}] = N/V_{bulk} \longrightarrow K_{eq} = \frac{\int_{site} \mathbf{d1} \int \mathbf{dx}\ e^{-\beta U}}{\int_{bulk} \mathbf{d1}\ \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int \mathbf{dx}\ e^{-\beta U}}$$

$$\Delta G^0 = -kT \ln(K_{eq} C^\circ) \qquad C^\circ = 1/1661 \text{Å}^3$$

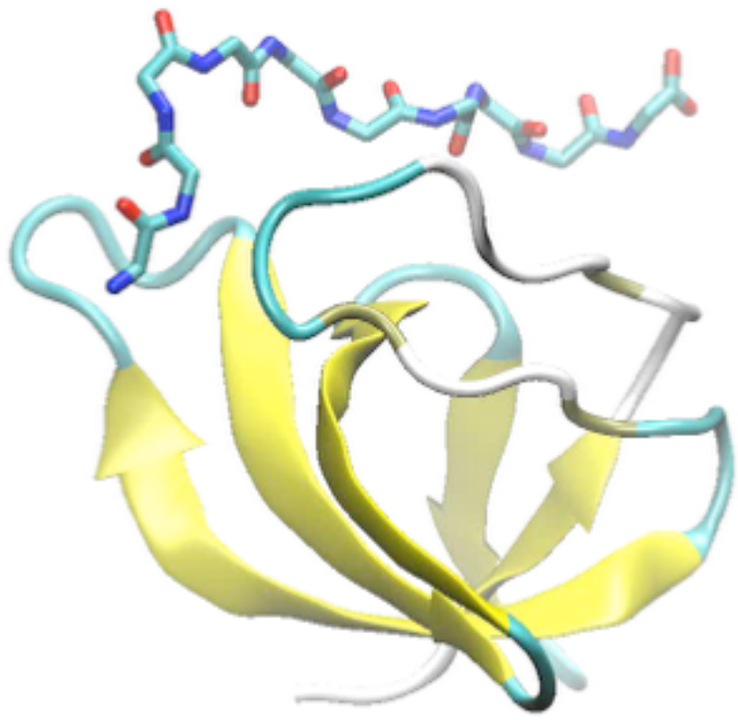
C° is the standard concentration of 1 M → **binding free energies are concentration dependent!**

Illustration using Abl SH3 domain

A well known and conserved domain of Abl kinase

Chosen ligand: APSYSPPPPP (flexible!)

designed to bind with high affinity peptide, so doesn't require novel parametrization



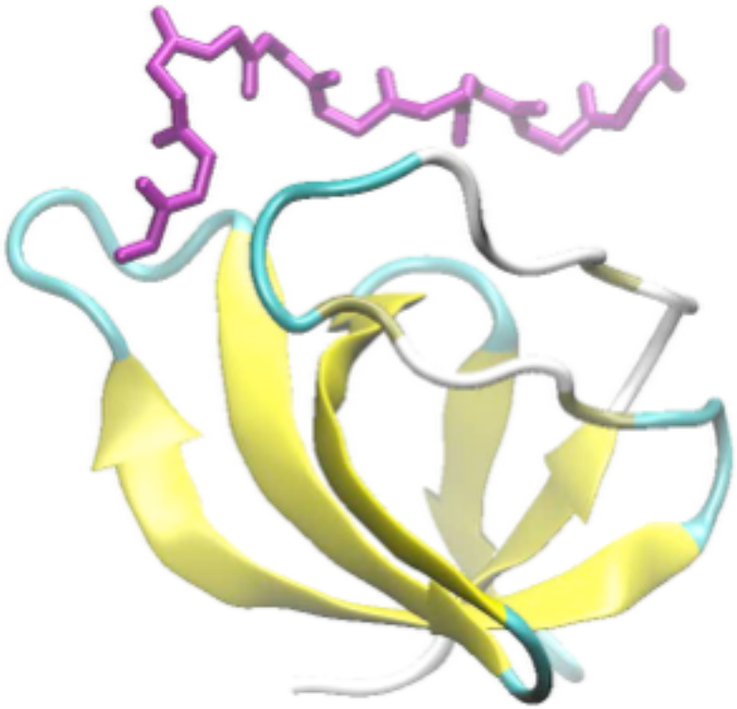
ΔG^0 = **-7.94 kcal/mol (exp)**

MM/PBSA estimate: **-2.6 kcal/mol** !

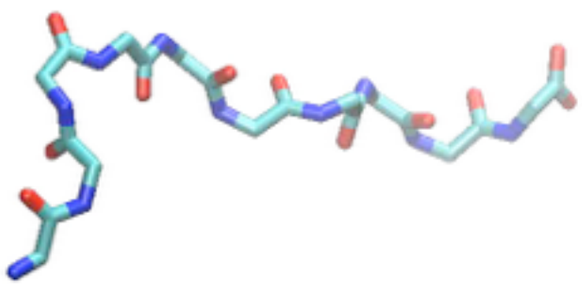
Pisabarro, M. T.; Serrano, L. *Biochemistry* **1996**, 35, 10634-10640
Hou, T. et al. *PLoS Comput. Biol*. **2006**, 2, 0046-0055

Inefficient sampling dominates

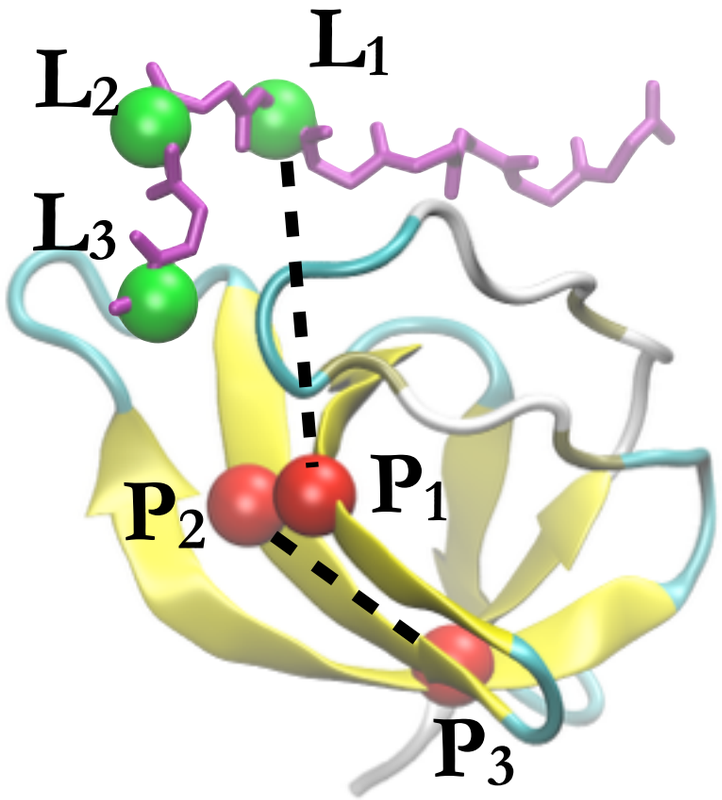
Overcoming sampling issues with restraints



Bound state RMSD restrained



Free state RMSD restrained



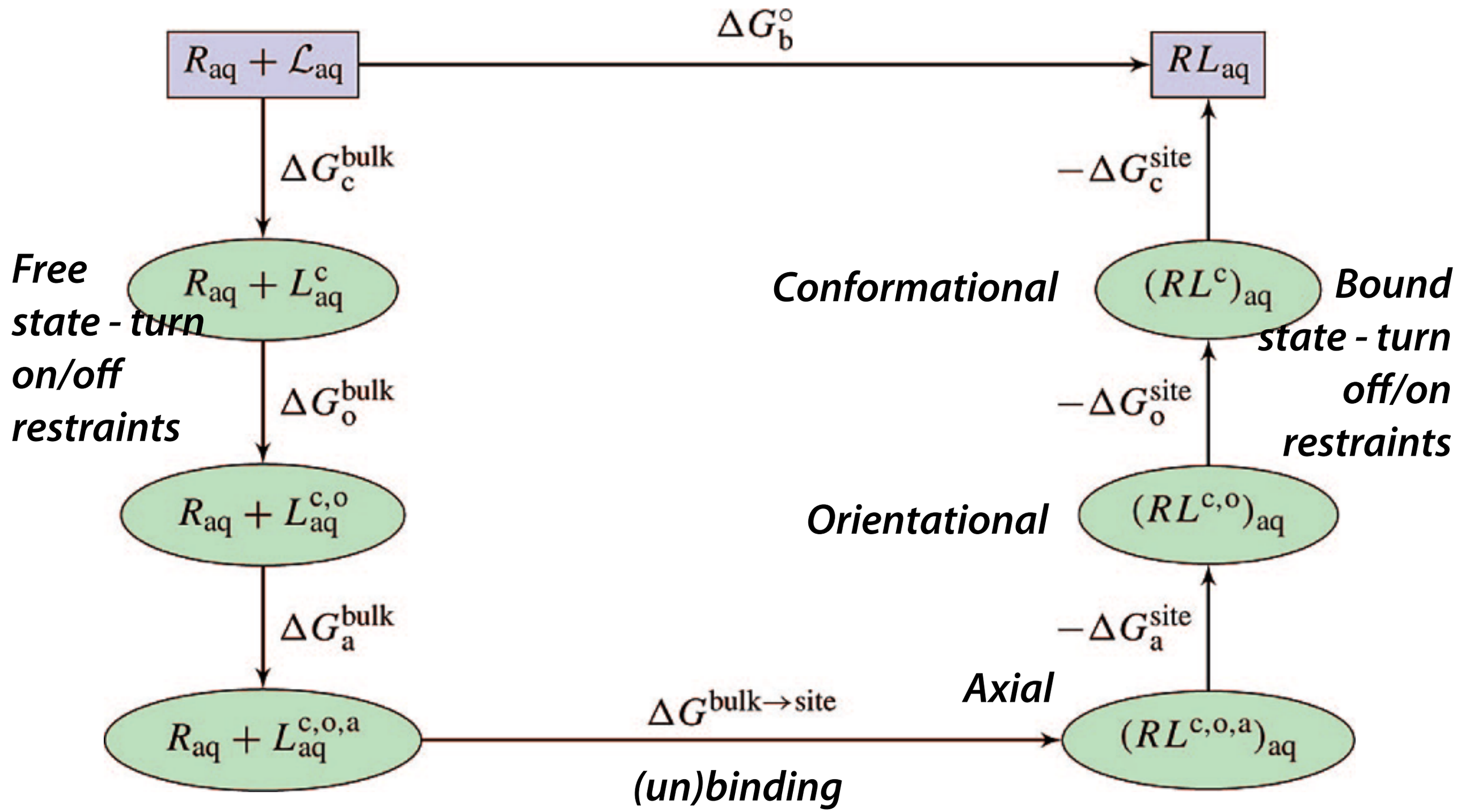
Assorted spatial/rotational restraints

-Design set of restraints to reduce conformational space needed to be sampled

-Contributions of each restraint to free energy need to be rigorously computed

Remember! Biasing is okay as long as we can unbias

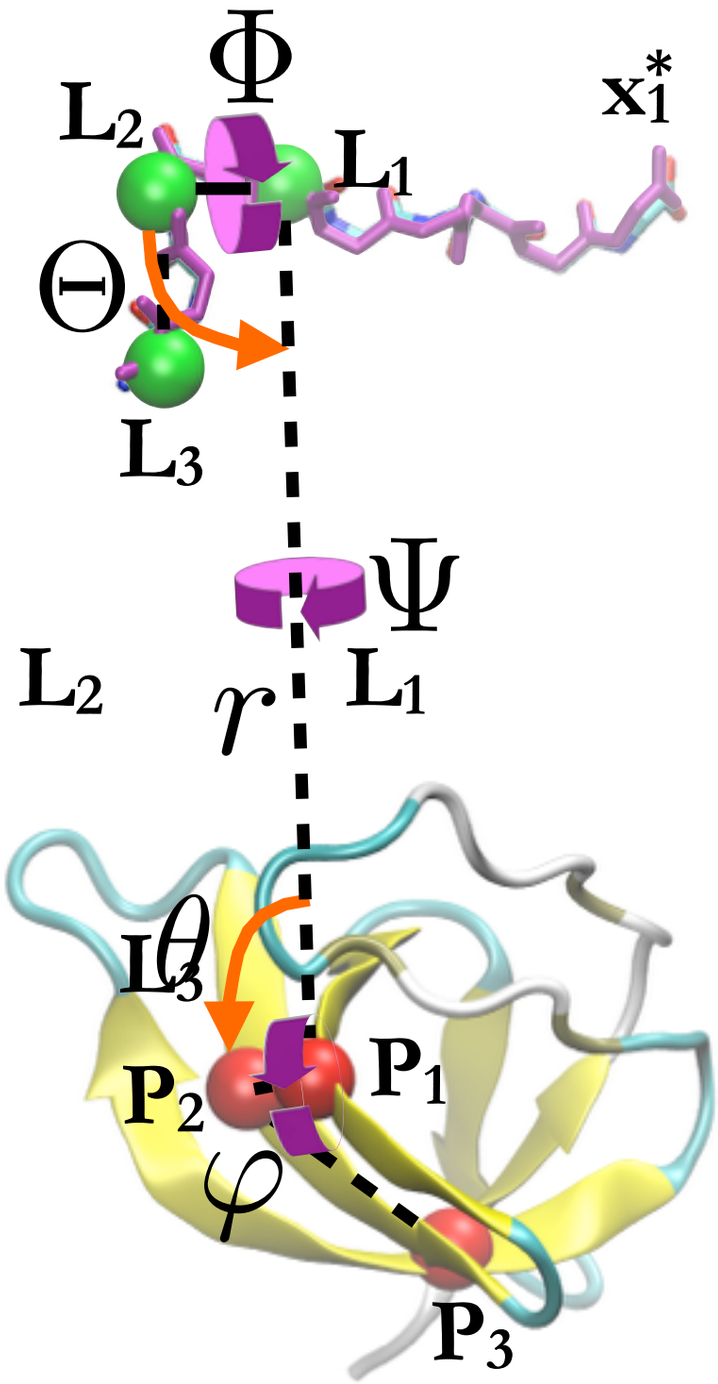
Overcoming sampling issues with restraints



Schematic of process

From: Deng and Roux. (2009)
J. Phys. Chem. **113**: 2234-2246.

Binding free energy from PMFs



$$K_{\mathrm{eq}} = \frac{\int_{\mathrm{site}} \mathrm{d1} \int \mathrm{dx}\ \mathrm{e}^{-\beta U}}{\int_{\mathrm{site}} \mathrm{d1} \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c)}}$$

$$\times \frac{\int_{\mathrm{site}} \mathrm{d1} \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c)}}{\int_{\mathrm{site}} \mathrm{d1} \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c+u_\Theta)}}$$

$$\times \frac{\int_{\mathrm{site}} \mathrm{d1} \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c+u_\Theta)}}{\int_{\mathrm{site}} \mathrm{d1} \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c+u_\Theta+u_\Phi)}}$$

$$\times \frac{\int_{\mathrm{site}} \mathrm{d1} \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c+u_\Theta+u_\Phi)}}{\int_{\mathrm{site}} \mathrm{d1} \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c+u_\Theta+u_\Phi+u_\Psi)}}$$

$$\times \frac{\int_{\mathrm{site}} \mathrm{d1} \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c+u_o)}}{\int_{\mathrm{site}} \mathrm{d1} \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c+u_o+u_\theta)}}$$

$$\times \frac{\int_{\mathrm{site}} \mathrm{d1} \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c+u_o+u_\theta)}}{\int_{\mathrm{site}} \mathrm{d1} \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c+u_o+u_\theta+u_\varphi)}}$$

$$\times \frac{\int_{\mathrm{site}} \mathrm{d1} \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c+u_o+u_p)}}{\int_{\mathrm{bulk}} \mathrm{d1}\ \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c+u_o)}}$$

$$\times \frac{\int_{\mathrm{bulk}} \mathrm{d1}\ \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c+u_\Theta+u_\Phi+u_\Psi)}}{\int_{\mathrm{bulk}} \mathrm{d1}\ \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c+u_\Theta+u_\Phi)}}$$

$$\times \frac{\int_{\mathrm{bulk}} \mathrm{d1}\ \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c+u_\Theta+u_\Phi)}}{\int_{\mathrm{bulk}} \mathrm{d1}\ \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c+u_\Theta)}}$$

$$\times \frac{\int_{\mathrm{bulk}} \mathrm{d1}\ \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c+u_\Theta)}}{\int_{\mathrm{bulk}} \mathrm{d1}\ \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c)}}$$

$$\times \frac{\int_{\mathrm{bulk}} \mathrm{d1}\ \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c)}}{\int_{\mathrm{bulk}} \mathrm{d1}\ \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int \mathrm{dx}\ \mathrm{e}^{-\beta U}}$$

Yu, Y. B. et al. (2001) *Biophys. J.*, **81**:1632-1642.

Woo, H. J.; Roux, B. (2005) *Proc. Natl. Acad. Sci. USA*, **102**:6825-6830.

Maffeo, C., Luan, B., Aksimentiev, A. (2012) *Nucl. Acids Res.* **40**:3812-3821.

How to evaluate all of these integrals?

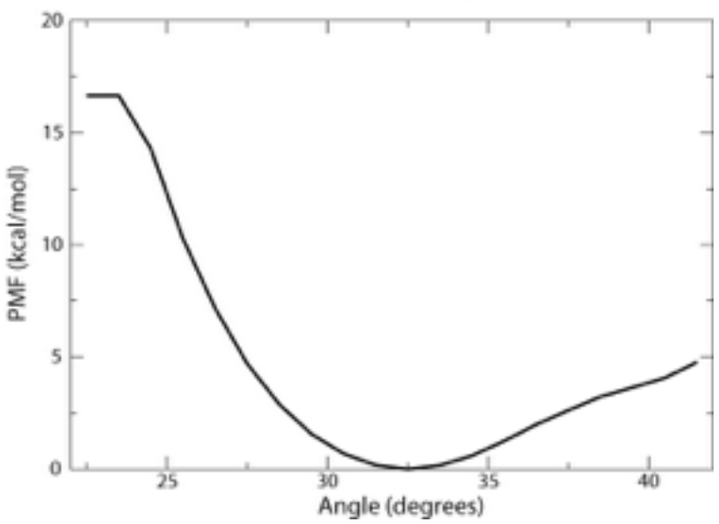
$$\frac{\int_{\text{site}} \mathrm{d}\mathbf{1} \int \mathrm{d}\mathbf{x}\ \mathrm{e}^{-\beta(U+u_c+u_\Theta+u_\Phi)}}{\int_{\text{site}} \mathrm{d}\mathbf{1} \int \mathrm{d}\mathbf{x}\ \mathrm{e}^{-\beta(U+u_c+u_\Theta+u_\Phi+u_\Psi)}} =$$

ratio of integrals can be related to a free energy

$$\frac{\int_{\text{site}} \mathrm{d}\mathbf{1} \int \mathrm{d}\mathbf{x}\ \mathrm{e}^{-\beta(U+u_c+u_\Theta+u_\Phi)}}{\int_{\text{site}} \mathrm{d}\mathbf{1} \int \mathrm{d}\mathbf{x}\ (e^{-\beta u_\Psi})\mathrm{e}^{-\beta(U+u_c+u_\Theta+u_\Phi)}} = \frac{1}{\mathrm{e}^{-\beta\Delta G_\Psi^{\text{site}}}} = \mathrm{e}^{+\beta\Delta G_\Psi^{\text{site}}}$$

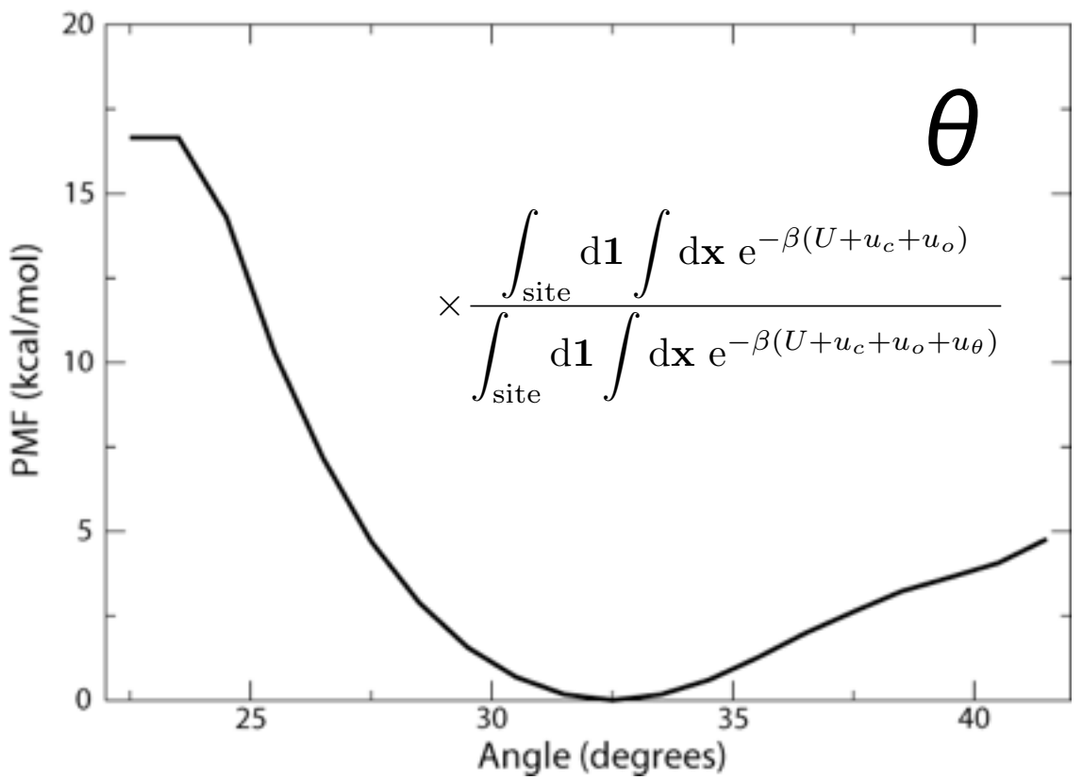
$$\mathrm{e}^{+\beta\Delta G_\Psi^{\text{site}}} = \frac{\int \mathrm{d}\Psi \mathrm{e}^{-\beta[w_{\text{site}}(\Psi)]}}{\int \mathrm{d}\Psi \mathrm{e}^{-\beta[w_{\text{site}}(\Psi)+u_\Psi]}}$$

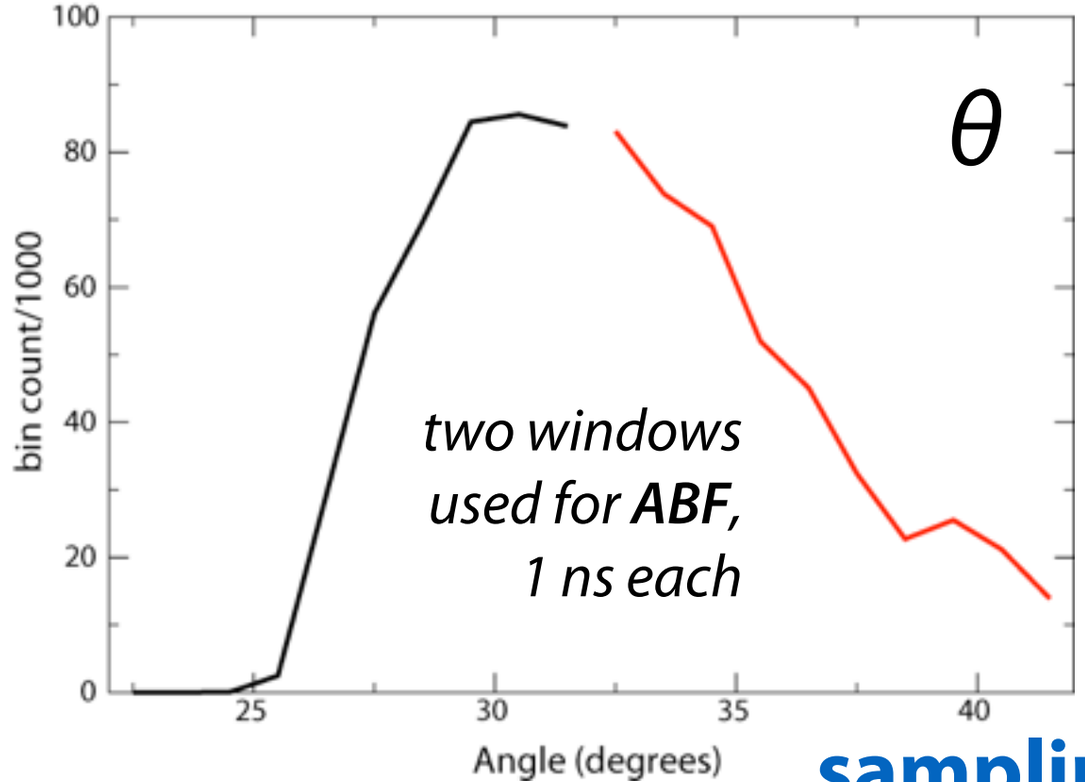
Potential of mean force, $w_{\text{site}}(\Psi)$, encapsulates all degrees of freedom



In practice, one determines the PMFs successively and then integrates them as prescribed above

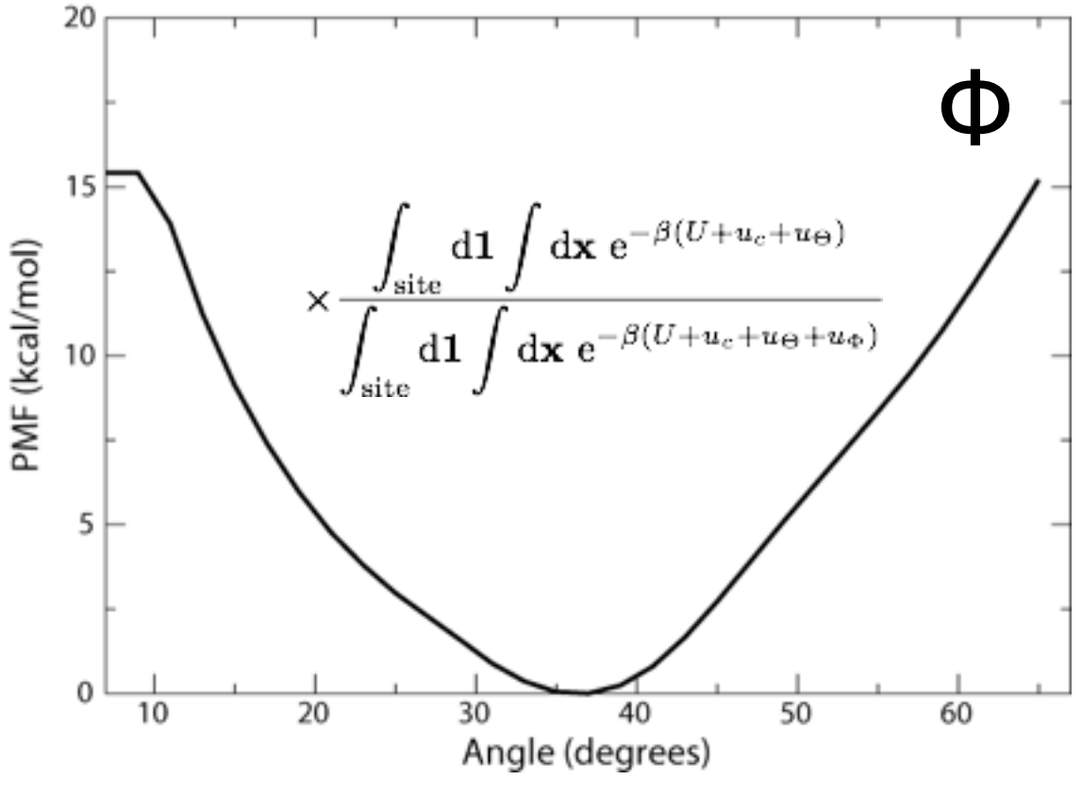
Many PMFs are very straightforward

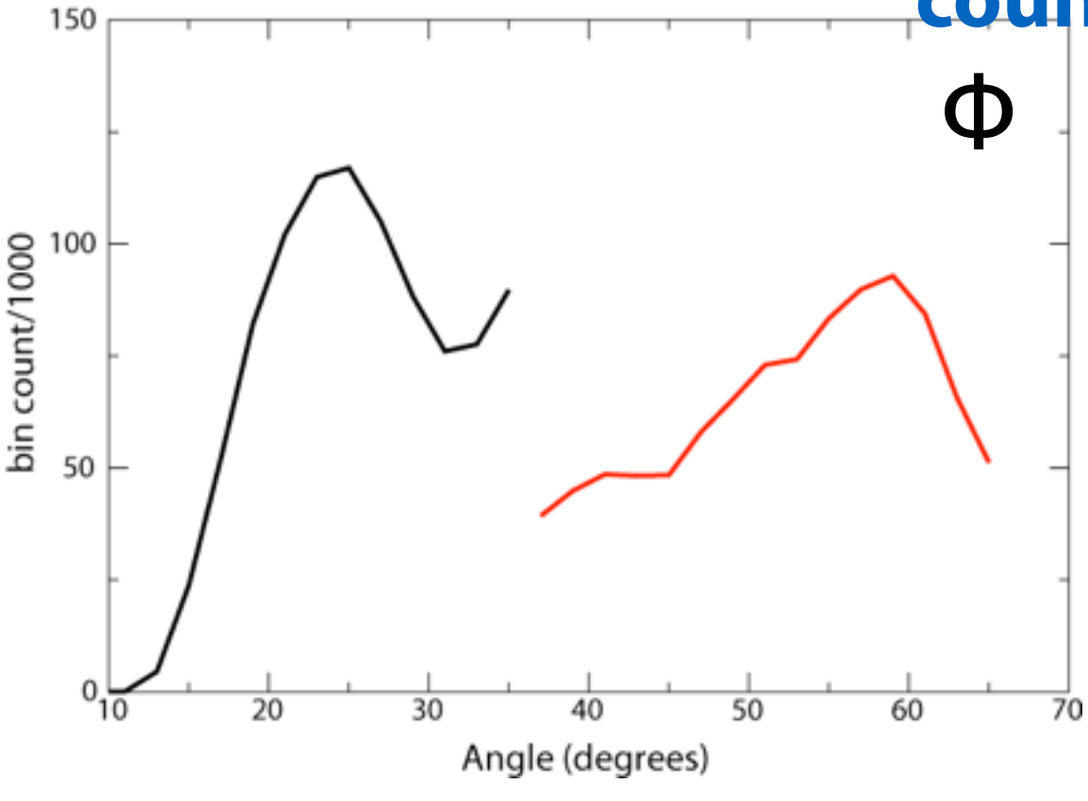




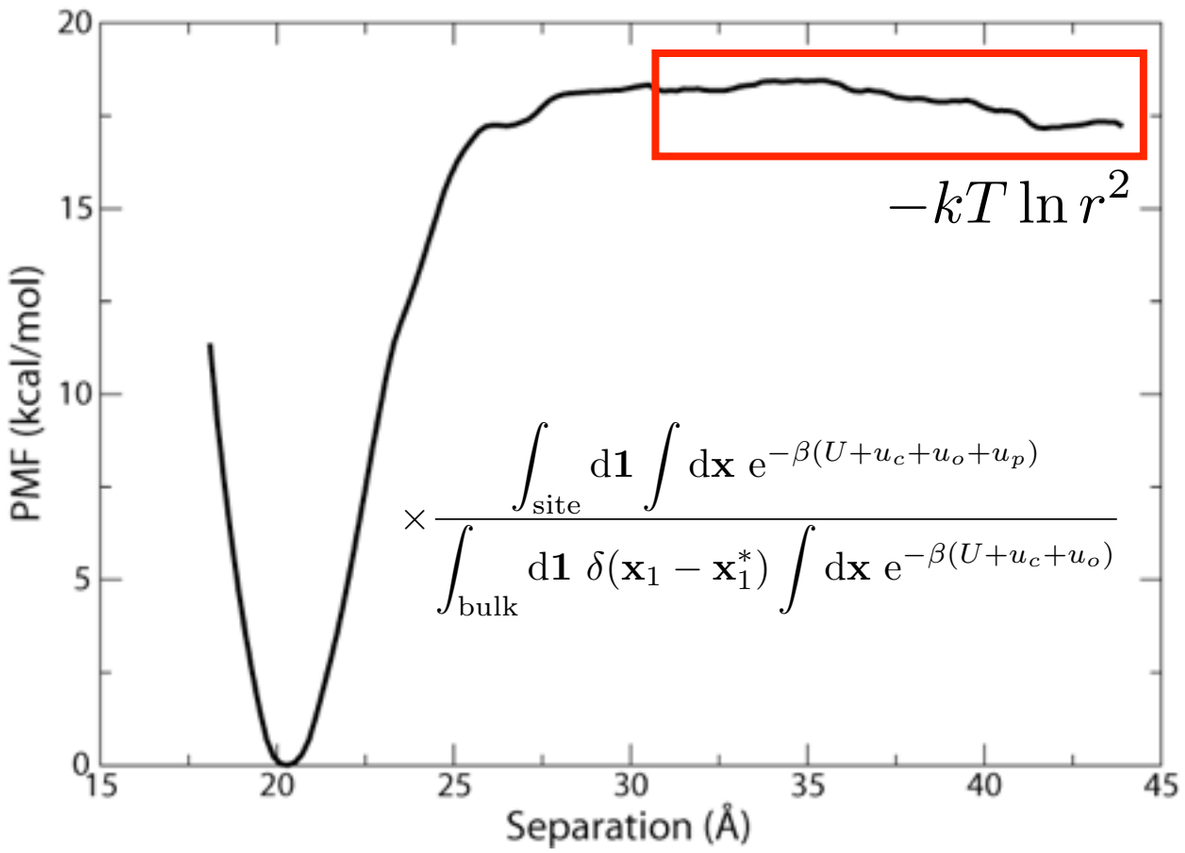
PMFs

sampling counts

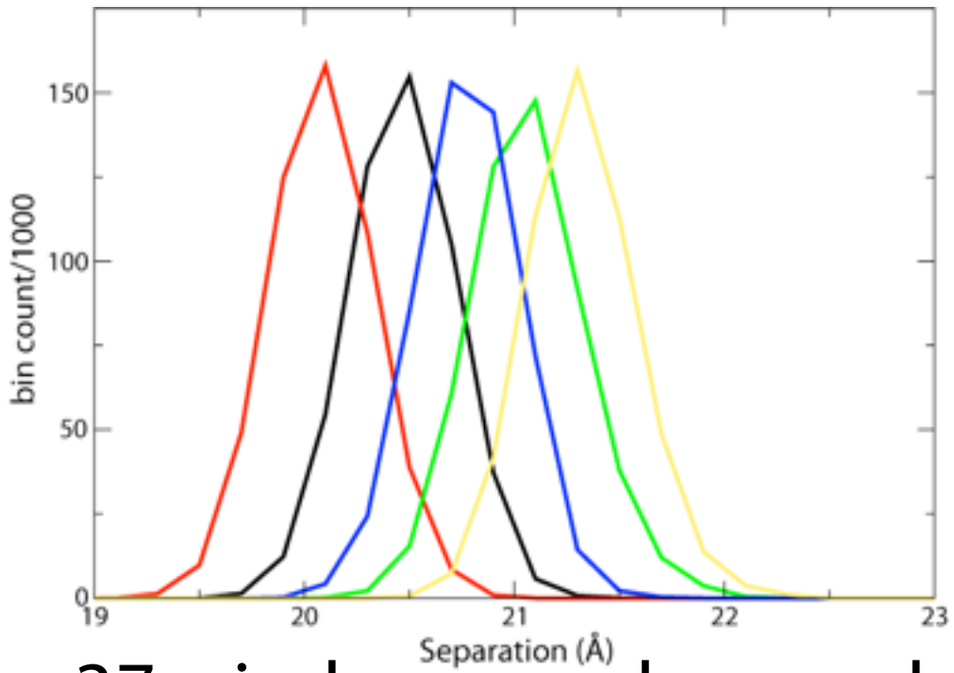




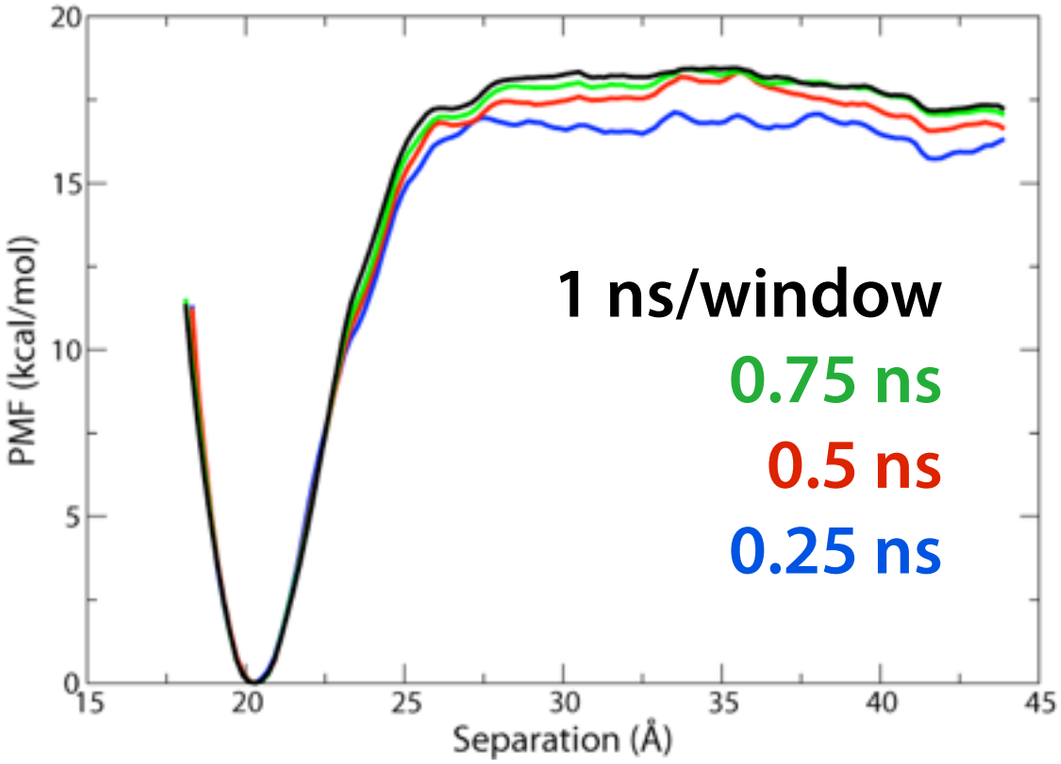
Separation PMF from umbrella sampling



entropic decay despite no interactions

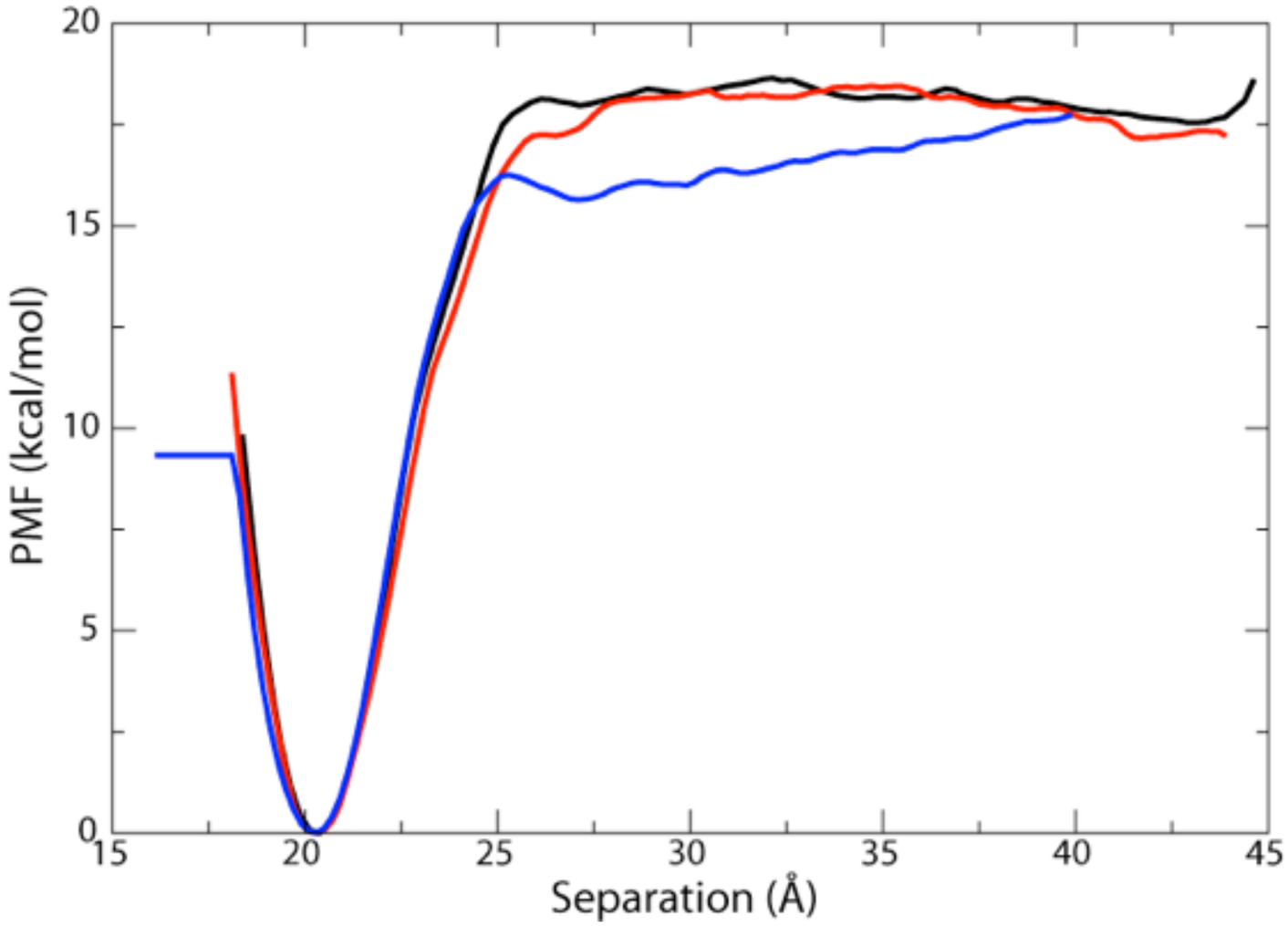


37 windows used, spaced
0.5 - 1 Å apart
-histograms are overlapping



PMF was already converged
within ~20 ns

Replica-exchange umbrella sampling



REMD-US - 37 ns

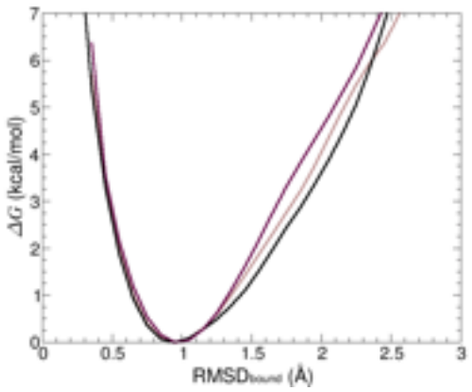
US - 37 ns

ABF - 70 ns

-for this problem, **REMD-US** does not converge notably faster than standard **umbrella sampling**

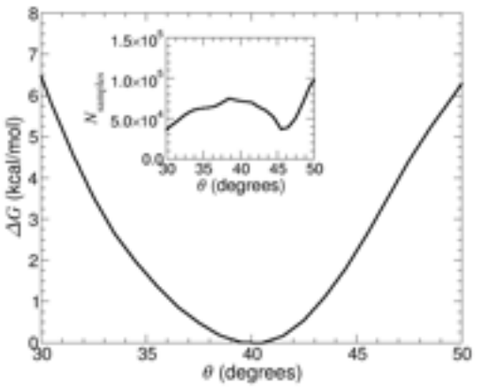
-however, both fare significantly better than **ABF**

What you get in the end (a big mess!)



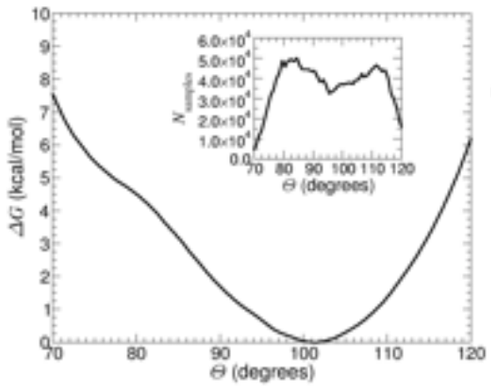
$$e^{-\beta\Delta G_c^{site}} = \frac{\int_{site} d\mathbf{l} \int d\mathbf{x}\, e^{-\beta(U+u_c)}}{\int_{site} d\mathbf{l} \int d\mathbf{x}\, e^{-\beta U}} = \langle e^{-\beta u_c} \rangle_{site,U}$$

$$\Delta G_c^{site} = +3.5 \text{ kcal/mol}$$



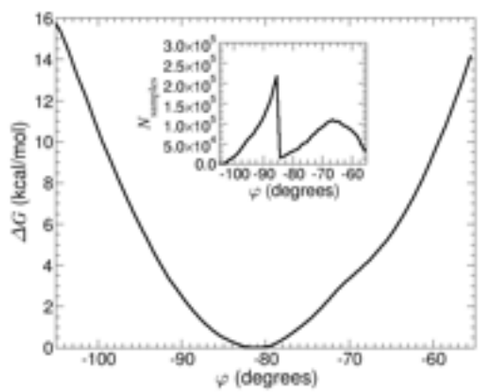
$$e^{-\beta\Delta G_\theta^{site}} = \frac{\int_{site} d\mathbf{l} \int d\mathbf{x}\, e^{-\beta(U+u_c+u_o+u_\theta)}}{\int_{site} d\mathbf{l} \int d\mathbf{x}\, e^{-\beta(U+u_c+u_o)}} = \langle e^{-\beta u_\theta} \rangle_{site,U+u_c+u_o}$$

$$\Delta G_\theta^{site} = +0.1 \text{ kcal/mol}$$



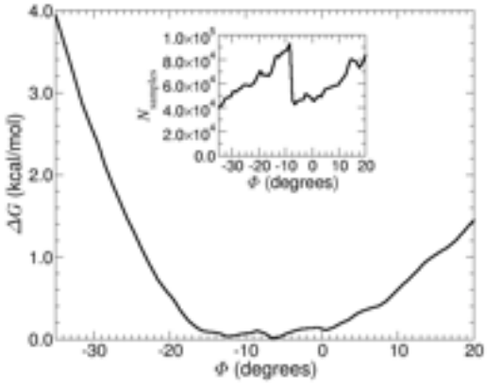
$$e^{-\beta\Delta G_\Theta^{site}} = \frac{\int_{site} d\mathbf{l} \int d\mathbf{x}\, e^{-\beta(U+u_c+u_\Theta)}}{\int_{site} d\mathbf{l} \int d\mathbf{x}\, e^{-\beta(U+u_c)}} = \langle e^{-\beta u_\Theta} \rangle_{site,U+u_c}$$

$$\Delta G_\Theta^{site} = +0.1 \text{ kcal/mol}$$



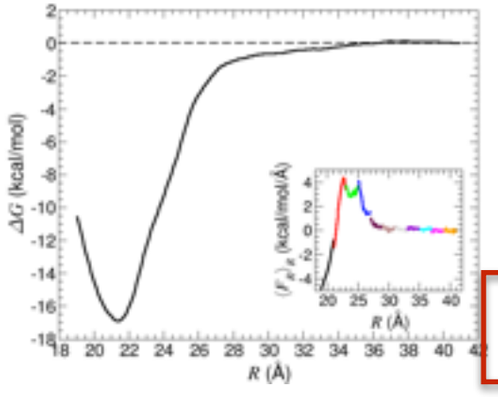
$$e^{-\beta\Delta G_\varphi^{site}} = \frac{\int_{site} d\mathbf{l} \int d\mathbf{x}\, e^{-\beta(U+u_c+u_o+u_\theta+u_\varphi)}}{\int_{site} d\mathbf{l} \int d\mathbf{x}\, e^{-\beta(U+u_c+u_o+u_\theta)}} = \langle e^{-\beta u_\varphi} \rangle_{site,U+u_c+u_o+u_\theta}$$

$$\Delta G_\varphi^{site} = +0.2 \text{ kcal/mol}$$



$$e^{-\beta\Delta G_\Phi^{site}} = \frac{\int_{site} d\mathbf{l} \int d\mathbf{x}\, e^{-\beta(U+u_c+u_\Theta+u_\Phi)}}{\int_{site} d\mathbf{l} \int d\mathbf{x}\, e^{-\beta(U+u_c+u_\Theta)}} = \langle e^{-\beta u_\Phi} \rangle_{site,U+u_c+u_\Theta}$$

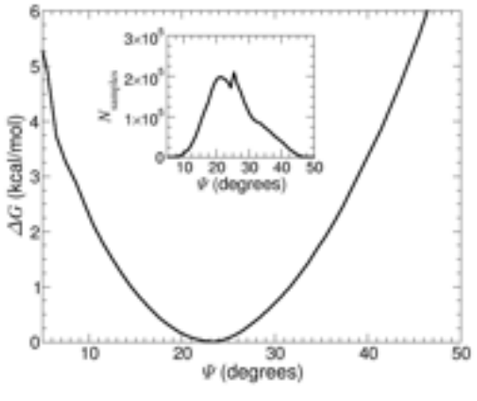
$$\Delta G_\Phi^{site} = +0.1 \text{ kcal/mol}$$



$$S^* = r^{*2} \int_0^\pi d\theta_1 \sin\theta_1 \int_0^{2\pi} d\phi_1\, e^{-\beta u_p} = +12.6 \text{ Å}^2$$

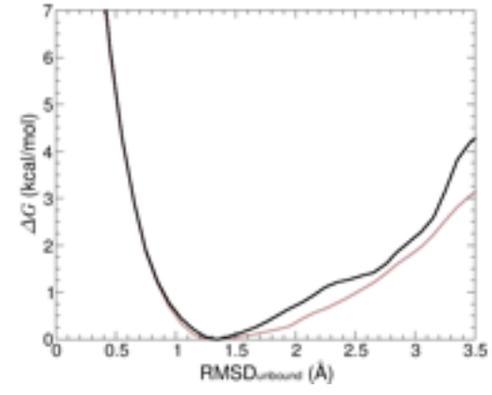
$$I^* = \int_{site} d\mathbf{x}_1\, e^{-\beta[w(\mathbf{x}_1)-w(\mathbf{x}_1^*)]} = +6.1 \times 10^{12} \text{ Å}$$

$$\Delta G_r = -\frac{1}{\beta} \ln(S^* I^*) = -14.6 \text{ kcal/mol}$$



$$e^{-\beta\Delta G_\Psi^{site}} = \frac{\int_{site} d\mathbf{l} \int d\mathbf{x}\, e^{-\beta(U+u_c+u_\Theta+u_\Phi+u_\Psi)}}{\int_{site} d\mathbf{l} \int d\mathbf{x}\, e^{-\beta(U+u_c+u_\Theta+u_\Phi)}} = \langle e^{-\beta u_\Phi} \rangle_{site,U+u_c+u_\Theta+u_\Phi}$$

$$\Delta G_\Psi^{site} = +0.5 \text{ kcal/mol}$$



$$e^{-\beta\Delta G_c^{bulk}} = \frac{\int_{bulk} d\mathbf{l}\ \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int d\mathbf{x}\, e^{-\beta(U+u_c)}}{\int_{bulk} d\mathbf{l}\ \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int d\mathbf{x}\, e^{-\beta U}} = \langle e^{-\beta u_c} \rangle_{bulk,U}$$

$$\Delta G_c^{bulk} = +5.4 \text{ kcal/mol}$$

Back to the Abl kinase story...

$$K_{\text{eq}} = \frac{\int_{\text{site}} \mathrm{d}\mathbf{1} \int \mathrm{dx}\ \mathrm{e}^{-\beta U}}{\int_{\text{site}} \mathrm{d}\mathbf{1} \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c)}}$$

$$\times \frac{\int_{\text{site}} \mathrm{d}\mathbf{1} \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c)}}{\int_{\text{site}} \mathrm{d}\mathbf{1} \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c+u_\Theta)}}$$

$$\times \frac{\int_{\text{site}} \mathrm{d}\mathbf{1} \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c+u_\Theta)}}{\int_{\text{site}} \mathrm{d}\mathbf{1} \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c+u_\Theta+u_\Phi)}}$$

$$\times \frac{\int_{\text{site}} \mathrm{d}\mathbf{1} \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c+u_\Theta+u_\Phi)}}{\int_{\text{site}} \mathrm{d}\mathbf{1} \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c+u_\Theta+u_\Phi+u_\Psi)}}$$

$$\times \frac{\int_{\text{site}} \mathrm{d}\mathbf{1} \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c+u_o)}}{\int_{\text{site}} \mathrm{d}\mathbf{1} \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c+u_o+u_\theta)}}$$

$$\times \frac{\int_{\text{site}} \mathrm{d}\mathbf{1} \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c+u_o+u_\theta)}}{\int_{\text{site}} \mathrm{d}\mathbf{1} \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c+u_o+u_\theta+u_\varphi)}}$$

$$\times \frac{\int_{\text{site}} \mathrm{d}\mathbf{1} \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c+u_o+u_p)}}{\int_{\text{bulk}} \mathrm{d}\mathbf{1}\ \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c+u_o)}}$$

$$\times \frac{\int_{\text{bulk}} \mathrm{d}\mathbf{1}\ \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c+u_\Theta+u_\Phi+u_\Psi)}}{\int_{\text{bulk}} \mathrm{d}\mathbf{1}\ \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c+u_\Theta+u_\Phi)}}$$

$$\times \frac{\int_{\text{bulk}} \mathrm{d}\mathbf{1}\ \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c+u_\Theta+u_\Phi)}}{\int_{\text{bulk}} \mathrm{d}\mathbf{1}\ \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c+u_\Theta)}}$$

$$\times \frac{\int_{\text{bulk}} \mathrm{d}\mathbf{1}\ \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c+u_\Theta)}}{\int_{\text{bulk}} \mathrm{d}\mathbf{1}\ \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c)}}$$

$$\times \frac{\int_{\text{bulk}} \mathrm{d}\mathbf{1}\ \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int \mathrm{dx}\ \mathrm{e}^{-\beta(U+u_c)}}{\int_{\text{bulk}} \mathrm{d}\mathbf{1}\ \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int \mathrm{dx}\ \mathrm{e}^{-\beta U}}$$

~30 ns $\Delta G_c^{\text{site}} = 3.52$ kcal/mol

6 ns $\Delta G_o^{\text{site}} = 0.71$ kcal/mol

4 ns $\Delta G_a^{\text{site}} = 0.20$ kcal/mol

37 ns $\Delta G_r^{\text{sep}} = -14.47$ kcal/mol

(analytical) $\Delta G_o^{\text{bulk}} = 5.77$ kcal/mol

30 ns $\Delta G_c^{\text{bulk}} = 5.43$ kcal/mol

$$\Delta G^o = (\Delta G_c^{\text{bulk}} - \Delta G_c^{\text{site}}) + (\Delta G_o^{\text{bulk}} - \Delta G_o^{\text{site}})$$

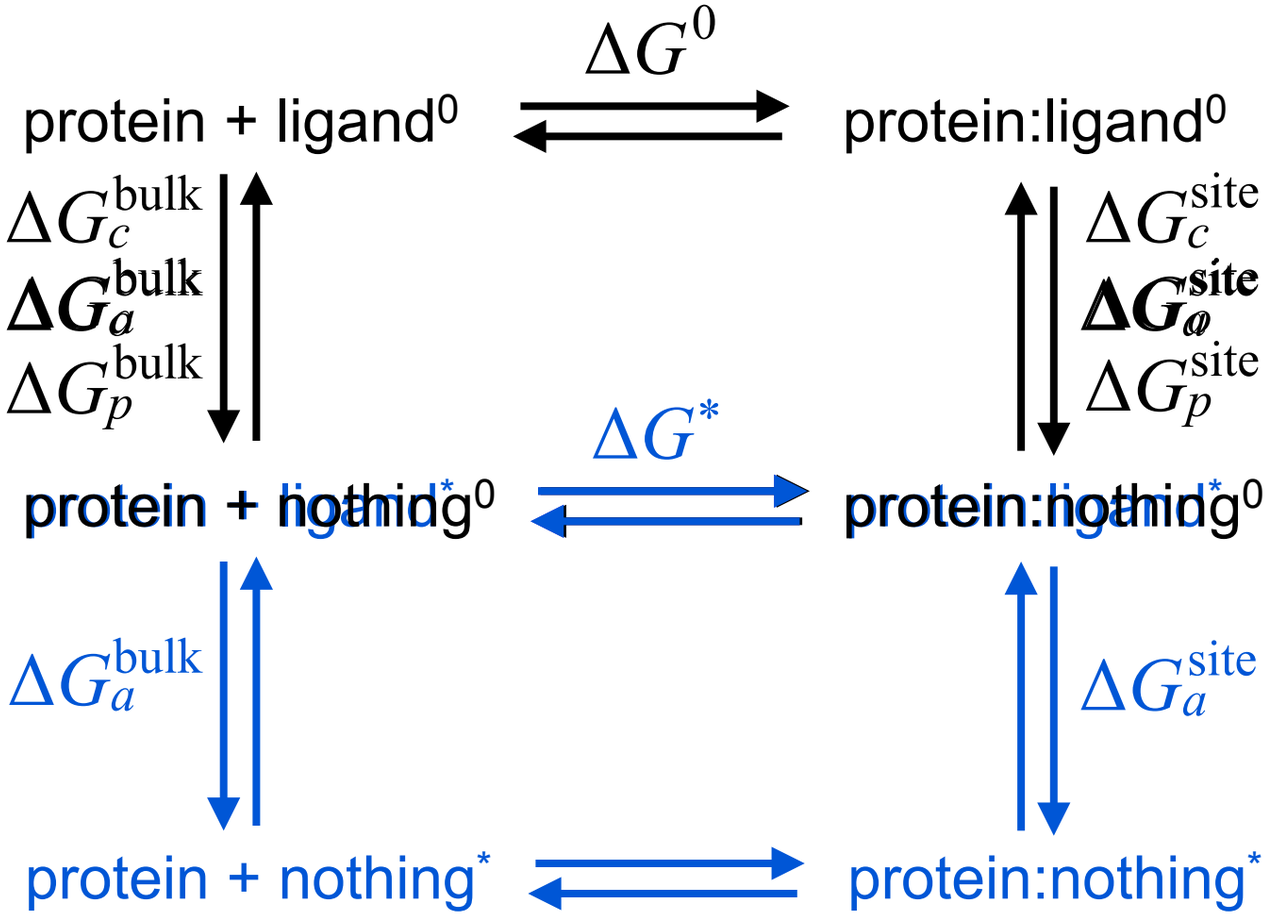
$$+\Delta G_r^{\text{sep}} - \Delta G_a^{\text{site}} = \boxed{-7.7 \text{ kcal/mol}}$$

~ 100 ns required

ΔG^0 = -7.94 kcal/mol (**exp**) **Agreement within 0.25 kcal/mol!**

There's more than one way to…

Can use **FEP** to (de)couple the ligand to the binding site of the protein



"**Floating ligand**" problem

Avoided through definition of a set of **restraints**

Follow a formalism akin to the reaction-coordinate (geometric) route

- Alchemical transformations performed bidirectionally using **FEP**
- Bennett acceptance ratio (BAR) estimator
- Free-energy contributions due to restraints measured using TI
- Most appropriate for buried ligands (no extraction pathway)

Bennett, C. H. *J. Comp. Phys.* , **1976**, *22*, 245-268 Gilson, M. K. et al. *Biophys. J.*, **1997**, *72*, 1047-1069

Comparison of alchemical and geometric routes

$$K_{\mathrm{eq}}^{\mathrm{PMF}} = \frac{\int_{\mathrm{site}} \mathrm{d1} \int \mathrm{dx}\, \mathrm{e}^{-\beta U}}{\int_{\mathrm{site}} \mathrm{d1} \int \mathrm{dx}\, \mathrm{e}^{-\beta(U+u_c)}} \times \frac{\int_{\mathrm{site}} \mathrm{d1} \int \mathrm{dx}\, \mathrm{e}^{-\beta(U+u_c)}}{\int_{\mathrm{site}} \mathrm{d1} \int \mathrm{dx}\, \mathrm{e}^{-\beta(U+u_c+u_o)}} \times \frac{\int_{\mathrm{site}} \mathrm{d1} \int \mathrm{dx}\, \mathrm{e}^{-\beta(U+u_c+u_o)}}{\int_{\mathrm{site}} \mathrm{d1} \int \mathrm{dx}\, \mathrm{e}^{-\beta(U+u_c+u_o+u_p)}} \times \frac{\int_{\mathrm{site}} \mathrm{d1} \int \mathrm{dx}\, \mathrm{e}^{-\beta(U+u_c+u_o+u_p)}}{\int_{\mathrm{bulk}} \mathrm{d1}\, \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int \mathrm{dx}\, \mathrm{e}^{-\beta(U+u_c+u_o)}} \times \frac{\int_{\mathrm{bulk}} \mathrm{d1}\, \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int \mathrm{dx}\, \mathrm{e}^{-\beta(U+u_c+u_o)}}{\int_{\mathrm{bulk}} \mathrm{d1}\, \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int \mathrm{dx}\, \mathrm{e}^{-\beta(U+u_c)}} \times \frac{\int_{\mathrm{bulk}} \mathrm{d1}\, \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int \mathrm{dx}\, \mathrm{e}^{-\beta(U+u_c)}}{\int_{\mathrm{bulk}} \mathrm{d1}\, \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int \mathrm{dx}\, \mathrm{e}^{-\beta U}}$$

$\Delta G_c^{\mathrm{site}} = +3.5$ kcal/mol

$\Delta G_o^{\mathrm{site}} = +0.7$ kcal/mol

$\Delta G_p^{\mathrm{site}} = +0.3$ kcal/mol

$\Delta G_r = -14.6$ kcal/mol

$\Delta G_o^{\mathrm{bulk}} = +5.8$ kcal/mol

$\Delta G_c^{\mathrm{bulk}} = +5.4$ kcal/mol

$\Delta G^0 = -7.8$ kcal/mol

$$K_{\mathrm{eq}}^{\mathrm{FEP}} = \frac{\int_{\mathrm{site}} \mathrm{d1} \int \mathrm{dx}\, \mathrm{e}^{-\beta U_1}}{\int_{\mathrm{site}} \mathrm{d1} \int \mathrm{dx}\, \mathrm{e}^{-\beta(U_1+u_c)}} \times \frac{\int_{\mathrm{site}} \mathrm{d1} \int \mathrm{dx}\, \mathrm{e}^{-\beta(U_1+u_c)}}{\int_{\mathrm{site}} \mathrm{d1} \int \mathrm{dx}\, \mathrm{e}^{-\beta(U_1+u_c+u_o)}} \times \frac{\int_{\mathrm{site}} \mathrm{d1} \int \mathrm{dx}\, \mathrm{e}^{-\beta(U_1+u_c+u_o)}}{\int_{\mathrm{site}} \mathrm{d1} \int \mathrm{dx}\, \mathrm{e}^{-\beta(U_1+u_c+u_o+u_p)}} \times \frac{\int_{\mathrm{site}} \mathrm{d1} \int \mathrm{dx}\, \mathrm{e}^{-\beta(U_1+u_c+u_o+u_p)}}{\int_{\mathrm{site}} \mathrm{d1} \int \mathrm{dx}\, \mathrm{e}^{-\beta(U_1+u_c+u_o+u_p+u_r)}} \times \frac{\int_{\mathrm{site}} \mathrm{d1} \int \mathrm{dx}\, \mathrm{e}^{-\beta(U_1+u_c+u_o+u_p+u_r)}}{\int_{\mathrm{site}} \mathrm{d1} \int \mathrm{dx}\, \mathrm{e}^{-\beta(U_0+u_c+u_o+u_p+u_r)}} \times \frac{\int_{\mathrm{bulk}} \mathrm{d1} \int \mathrm{dx}\, \mathrm{e}^{-\beta(U_0+u_c+u_o+u_p+u_r)}}{\int_{\mathrm{bulk}} \mathrm{d1}\, \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int \mathrm{dx}\, \mathrm{e}^{-\beta(U_0+u_c+u_o)}} \times \frac{\int_{\mathrm{bulk}} \mathrm{d1}\, \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int \mathrm{dx}\, \mathrm{e}^{-\beta(U+u_c+u_o)}}{\int_{\mathrm{bulk}} \mathrm{d1}\, \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int \mathrm{dx}\, \mathrm{e}^{-\beta(U+u_c)}} \times \frac{\int_{\mathrm{bulk}} \mathrm{d1}\, \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int \mathrm{dx}\, \mathrm{e}^{-\beta(U_0+u_c)}}{\int_{\mathrm{bulk}} \mathrm{d1}\, \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int \mathrm{dx}\, \mathrm{e}^{-\beta(U_1+u_c)}} \times \frac{\int_{\mathrm{bulk}} \mathrm{d1}\, \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int \mathrm{dx}\, \mathrm{e}^{-\beta(U_1+u_c)}}{\int_{\mathrm{bulk}} \mathrm{d1}\, \delta(\mathbf{x}_1 - \mathbf{x}_1^*) \int \mathrm{dx}\, \mathrm{e}^{-\beta U_1}}$$

$\Delta G_c^{\mathrm{site}} = +4.2$ kcal/mol

$\Delta G_o^{\mathrm{site}} = +1.7$ kcal/mol

$\Delta G_p^{\mathrm{site}} = +0.6$ kcal/mol

$\Delta G_r^{\mathrm{site}} = +0.4$ kcal/mol

$\Delta G_a^{\mathrm{site}} = -35.9$ kcal/mol

$\Delta G_t^{\mathrm{bulk}} = +4.0$ kcal/mol

$\Delta G_o^{\mathrm{bulk}} = +6.6$ kcal/mol

$\Delta G_a^{\mathrm{bulk}} = -53.3$ kcal/mol

$\Delta G_c^{\mathrm{bulk}} = +6.1$ kcal/mol

$\Delta G^0 = -7.7$ kcal/mol

Error analysis

Reaction-coordinate route

$$\Delta A \simeq -\Delta\xi \sum_{i=1}^{p} \langle F_\xi \rangle_{\xi_i}$$

$$\begin{cases} \mu &= \langle F_\xi \rangle_\xi = \dfrac{1}{p} \sum_{i=1}^{p} \langle F_\xi \rangle_{\xi_i} = \dfrac{1}{p} \sum_{i=1}^{p} \mu_i \\ \sigma^2 &= \dfrac{1}{p} \sum_{i=1}^{p} \sigma_i^2 + (\mu - \mu_i)^2 \end{cases}$$

$$\langle |\Delta A - \overline{\Delta A}|^2 \rangle = (\Delta\xi)^2 \sum_{i,j=1}^{p} \sum_{k=1}^{n_i} \sum_{l=1}^{n_j} \frac{1}{n_i n_j} \langle (F^i_{\xi,l} - \mu)(F^i_{\xi,k} - \mu) \rangle$$

$$\simeq (\Delta\xi)^2 \left\{ \frac{p}{N} \sum_{i=1}^{p} [\sigma_i^2 + (\mu - \mu_i)^2] + 2\sigma^2 \frac{p^2}{N} \kappa \right\}$$

$$\sigma_{\Delta A} \simeq \Delta\xi \, \frac{\sigma}{N^{1/2}} (1 + 2\kappa)^{1/2}$$

RMSD	±0.5 kcal/mol
$\Theta, \Phi, \Psi, \theta, \phi$	±0.2 kcal/mol
r	±0.4 kcal/mol
	±0.9 kcal/mol

Alchemical route

$$\sigma^2_{\Delta\widehat{A}} = \frac{(1+2\kappa_0)}{n_0\beta^2} \left(\frac{\langle f^2(x) \rangle_0}{\langle f(x) \rangle_0^2} - 1 \right) + \frac{(1+2\kappa_1)}{n_1\beta^2} \left(\frac{\langle f^2(-x) \rangle_1}{\langle f(-x) \rangle_1^2} - 1 \right)$$

$$f(x) = 1/(1+e^x) \quad \text{with} \quad x = \beta(\Delta U - C)$$

- Low statistical errors
- Estimates burdened by systematic error

RMSD	±0.4 kcal/mol
$\Theta, \Phi, \Psi, \theta, \phi$	±0.0 kcal/mol
r	±0.0 kcal/mol
alchemy	±0.7 kcal/mol
	±1.0 kcal/mol

often very tedious but you should still do it!

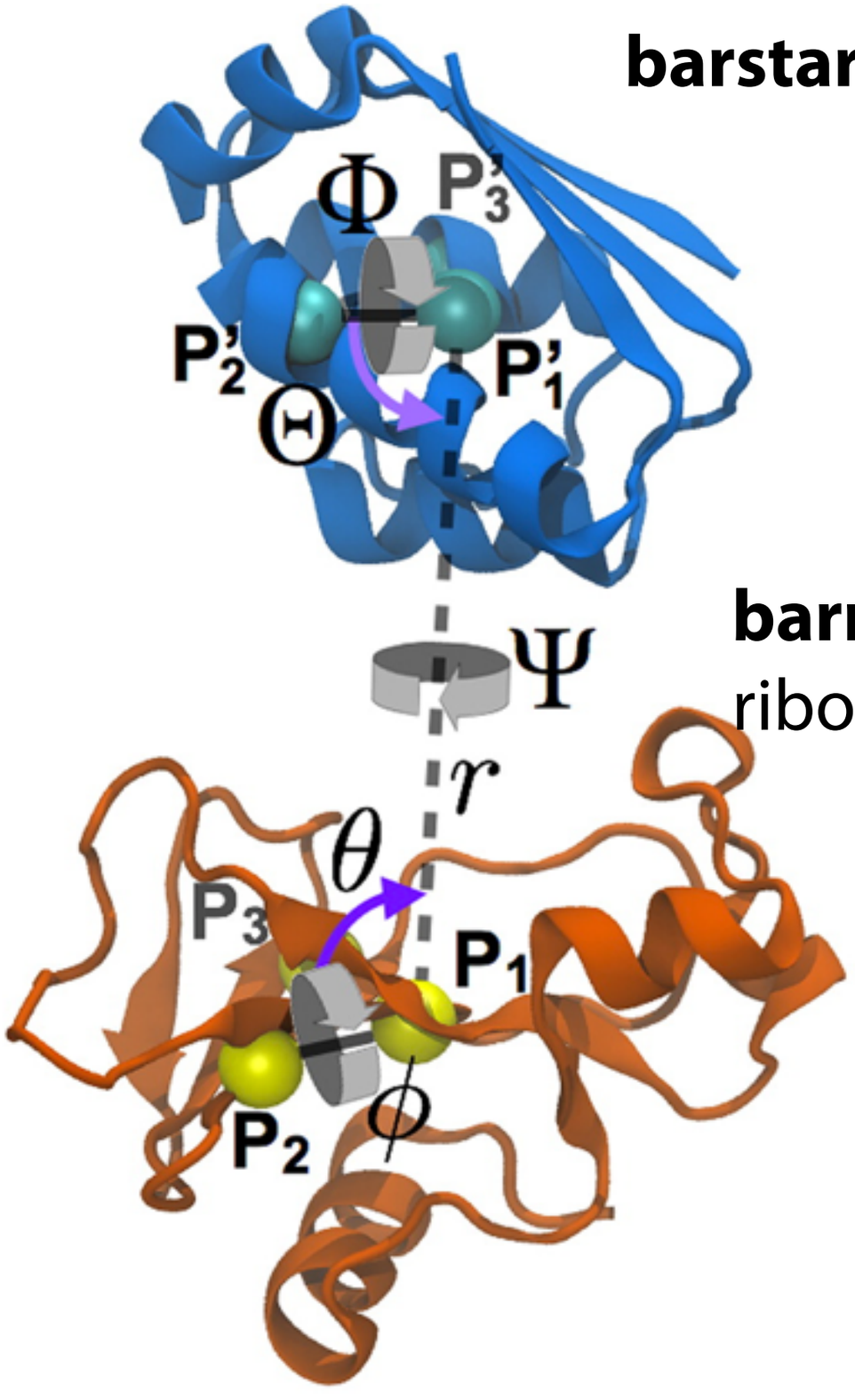
Hahn, A. M.; Then, H. Phys. Rev. E Stat. Nonlin. Soft Matter Phys. **2009**, 80, 031111

Pohorille, A. et al. J. Phys. Chem. B, **2010**, 114, 10235-10253

Rodriguez-Gomez, D. et al. J. Chem. Phys., **2004**, 120, 3563-3578

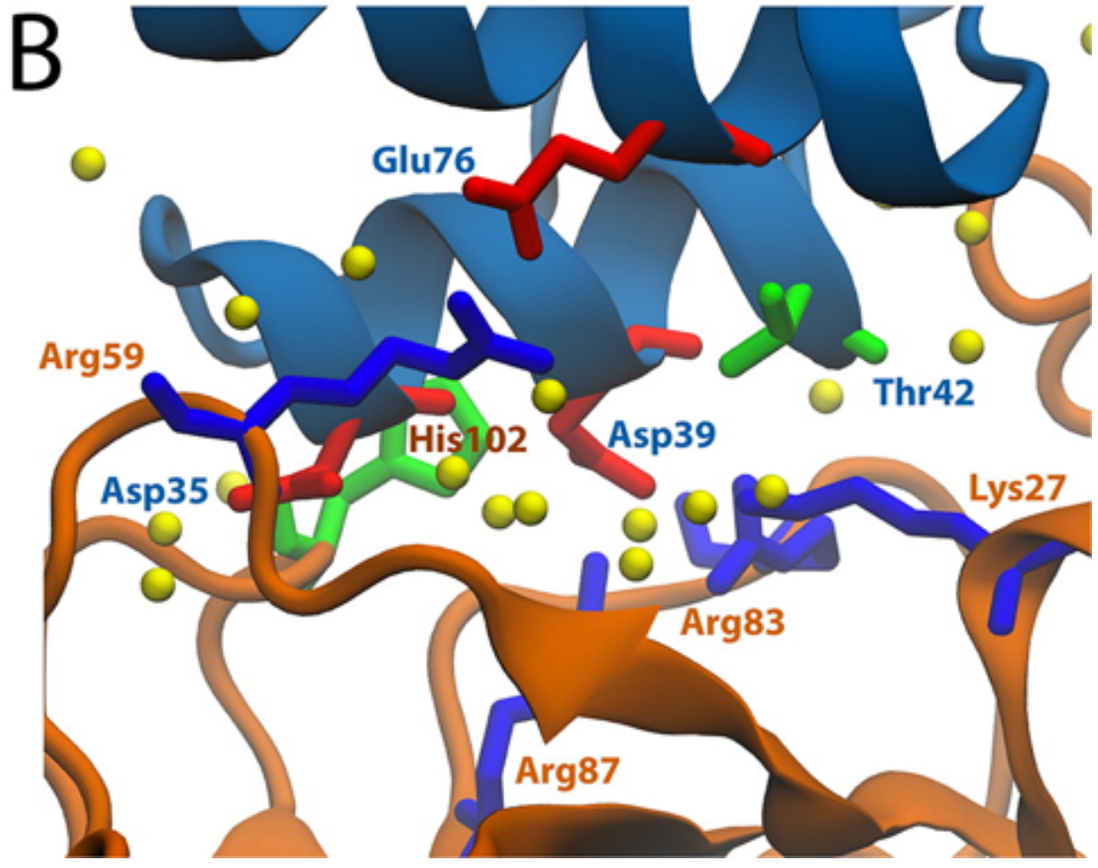
Hénin, J.; Chipot, C. *J. Chem. Phys.* **2004**, *121*, 2904-2914

Protein-protein binding free energy



barstar - an inhibitor

barnase - a ribonuclease



interface is highly solvated

ΔG^0 = -19.0 kcal/mol (exp)

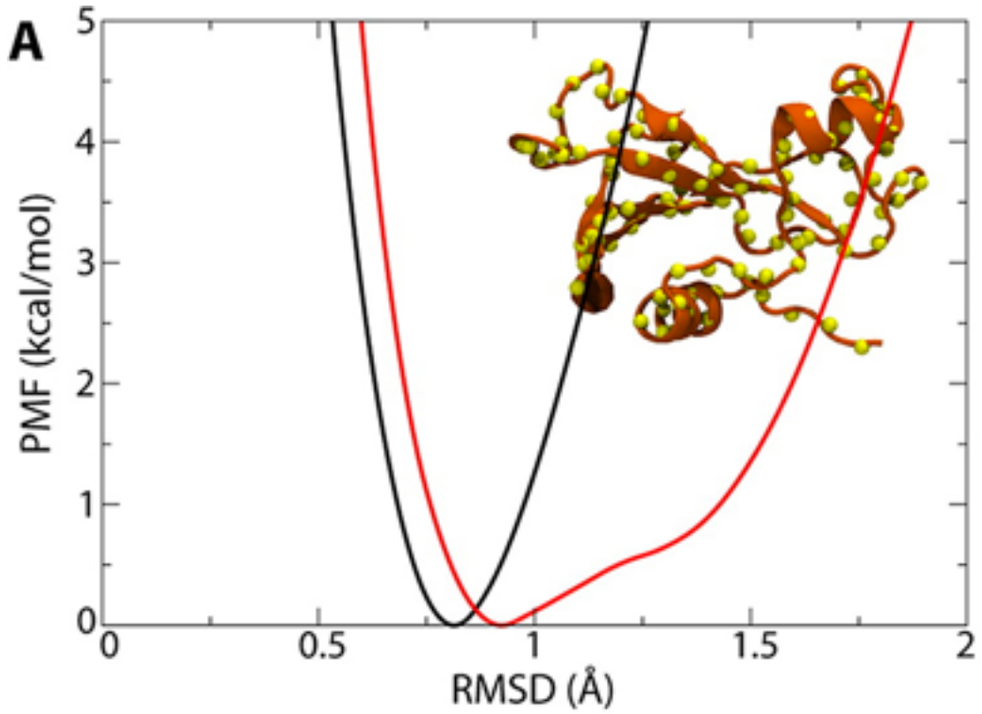
Schreiber & Fersht. *JMB*, **248**:478-486. 1995.

Gumbart, Roux, Chipot. Efficient Determination of Protein–Protein Standard Binding Free Energies from First Principles. *JCTC* **9**:3789-3798. 2013.

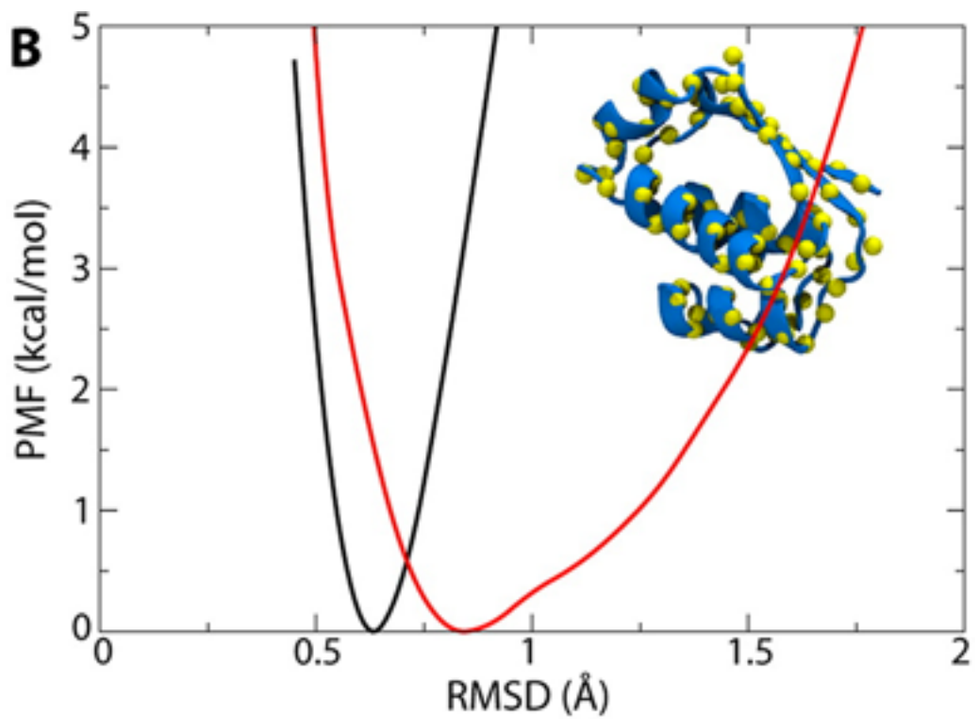
Numerous restraints needed

Gumbart, Roux, Chipot. *JCTC* **9**:3789-3798. 2013.

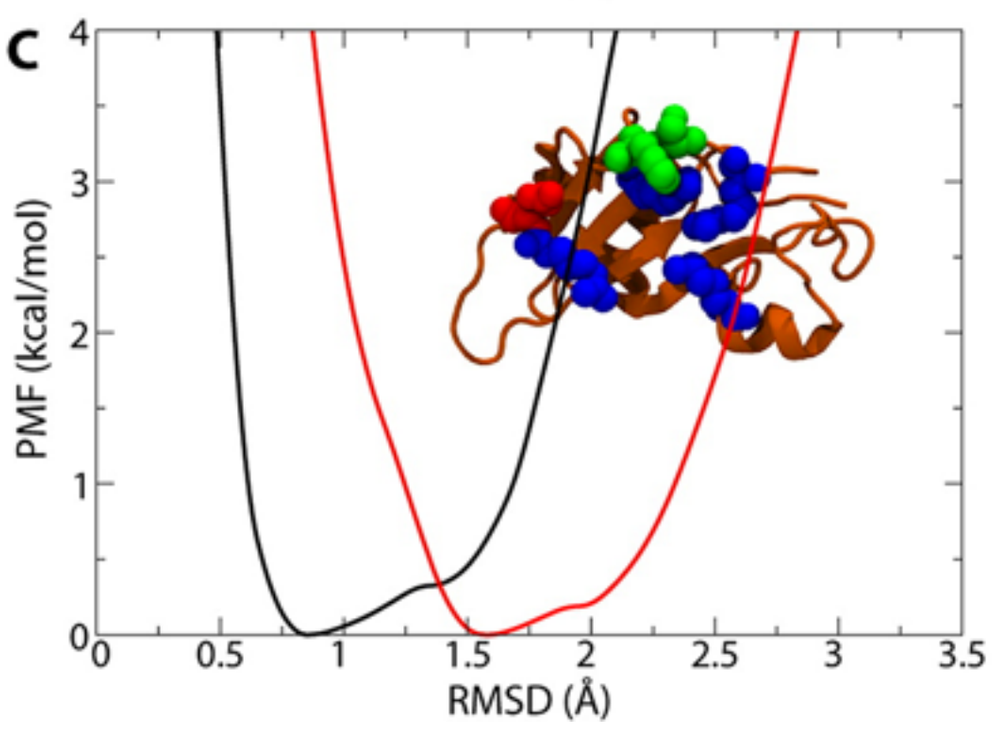
RMSD on **barnase backbone**



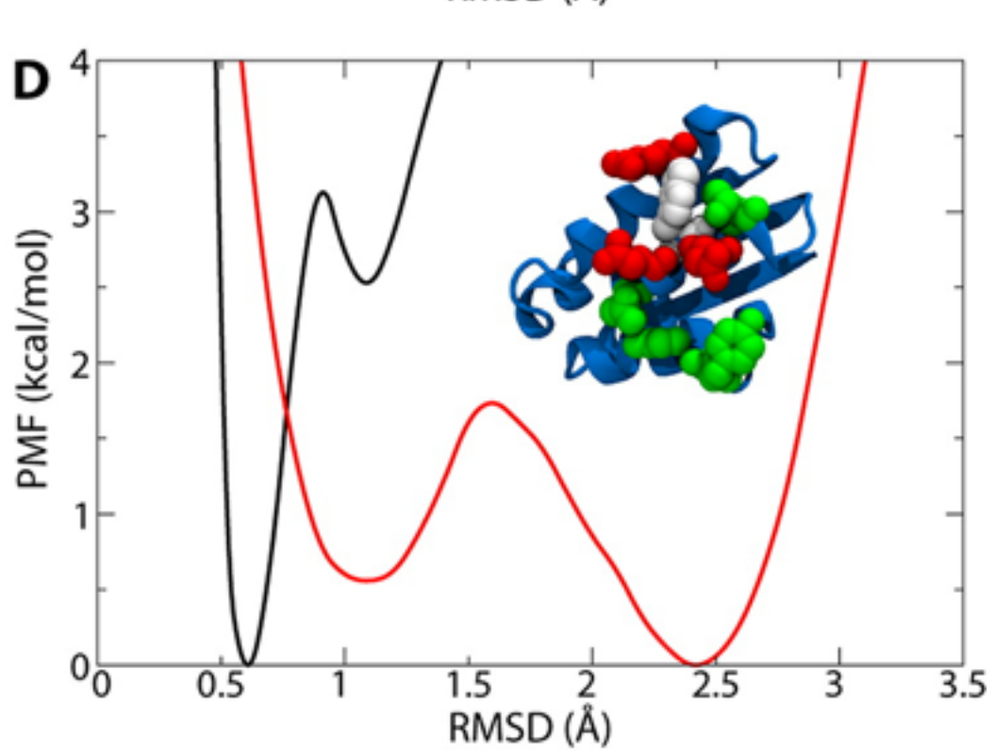
RMSD on **barstar backbone**



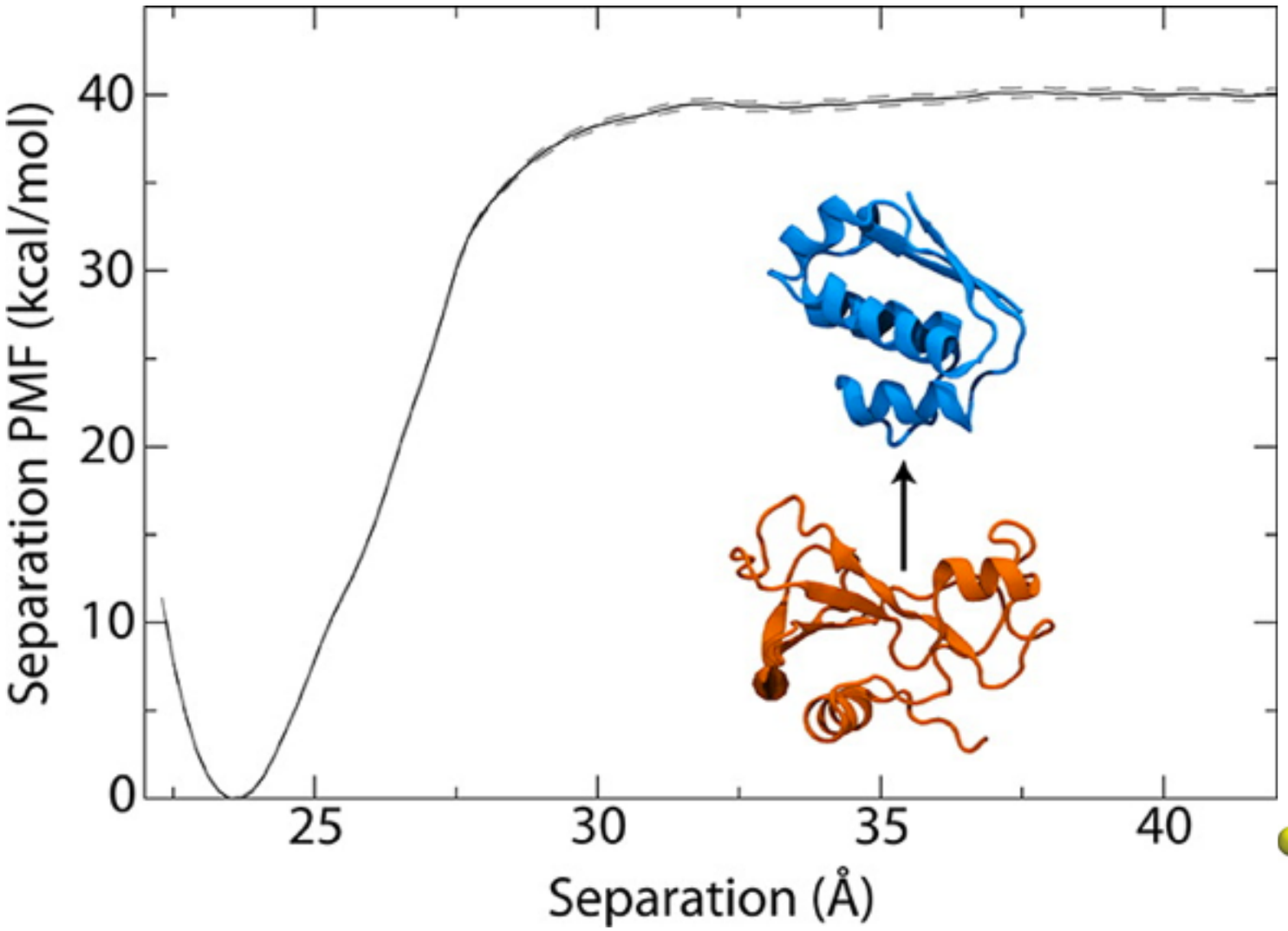
RMSD on **barnase side chains**



RMSD on **barstar side chains**



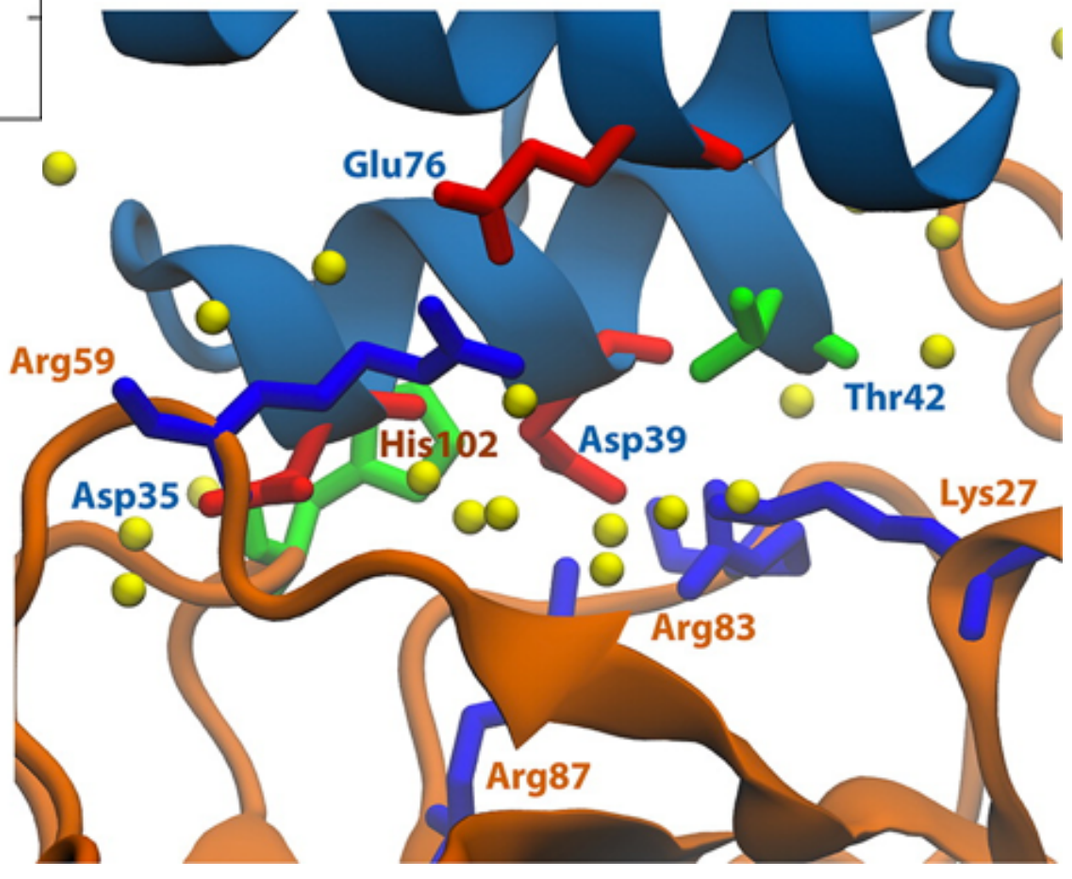
Separating the proteins



PMF took over 50 windows spaced by 0.5 Å and ~200 ns to fully converge

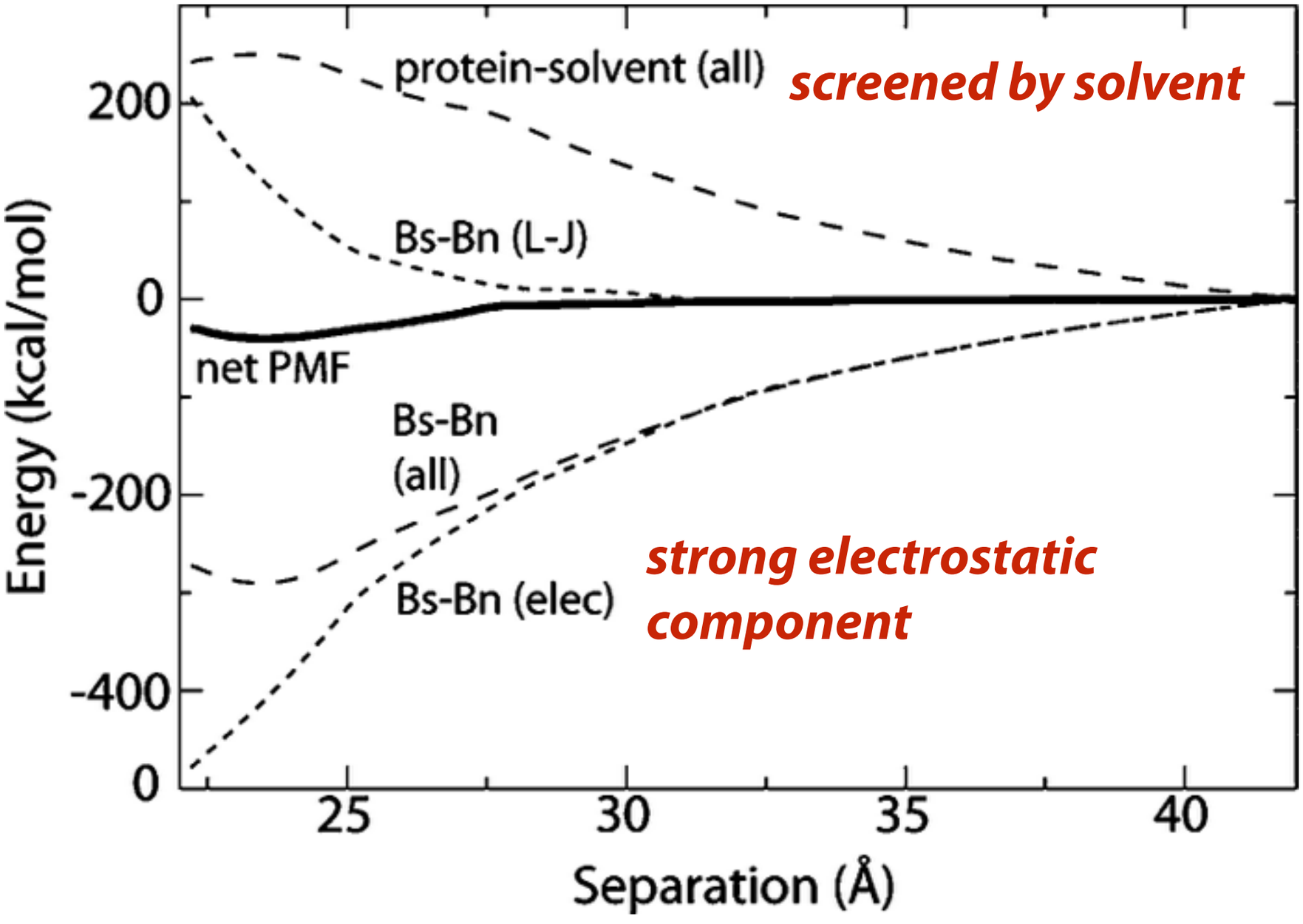
without side-chain restraints, PMF did not converge ***even in 400 ns***

the appropriate choice of restraints is problem dependent!!!



Gumbart, Roux, Chipot. *JCTC* **9**:3789-3798. 2013.

Decomposing the PMF



Force decomposition reveals key contributions to the PMF

Gumbart, Roux, Chipot. *JCTC* **9**:3789-3798. 2013.

And fourteen separate calculations later…

contribution	PMF (kcal/mol)	time (ns)
$\Delta G^{site}_{Bs,c}$	-1.98 ± 0.31	6
$\Delta G^{site}_{Bn,c}$	-3.13 ± 0.06	12
$\Delta G^{site}_{Bs,res}$	-1.87 ± 0.75	12
$\Delta G^{site}_{Bn,res}$	-3.45 ± 0.63	24
ΔG^{site}_{Θ}	-0.09 ± 0.42	8
ΔG^{site}_{Φ}	-0.35 ± 0.08	4
ΔG^{site}_{ψ}	-0.24 ± 0.09	8
ΔG^{site}_{θ}	-0.13 ± 0.33	4
ΔG^{site}_{ϕ}	-0.05 ± 0.12	4
$-(1/\beta)\ln(S^*I^*C^\circ)$	-37.10 ± 0.29	212
ΔG^{bulk}_{o}	+6.61	
$\Delta G^{bulk}_{Bn,res}$	$+8.05 \pm 0.31$	21
$\Delta G^{bulk}_{Bs,res}$	$+5.15 \pm 0.42$	15
$\Delta G^{bulk}_{Bn,c}$	$+4.15 \pm 0.53$	18
$\Delta G^{bulk}_{Bs,c}$	$+3.39 \pm 0.17$	24
ΔG°_{bind}	-21.04 ± 1.43	372

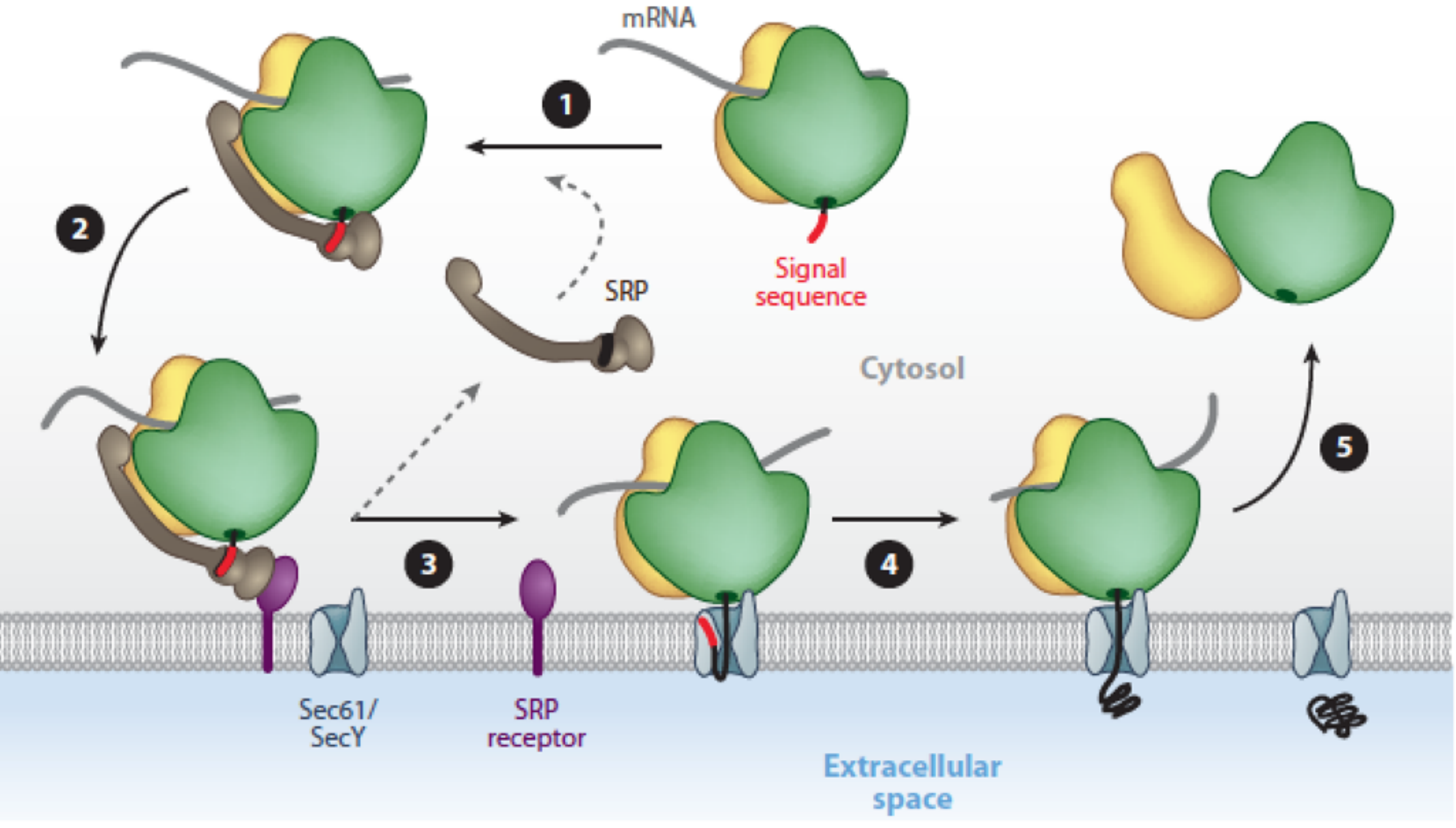
ΔG^0 = -19.0 kcal/mol (exp)

Within 2 kcal/mol!!!

Gumbart, Roux, Chipot. *JCTC* **9**:3789-3798. 2013.

any questions?

Bonus example! Protein synthesis and translocation

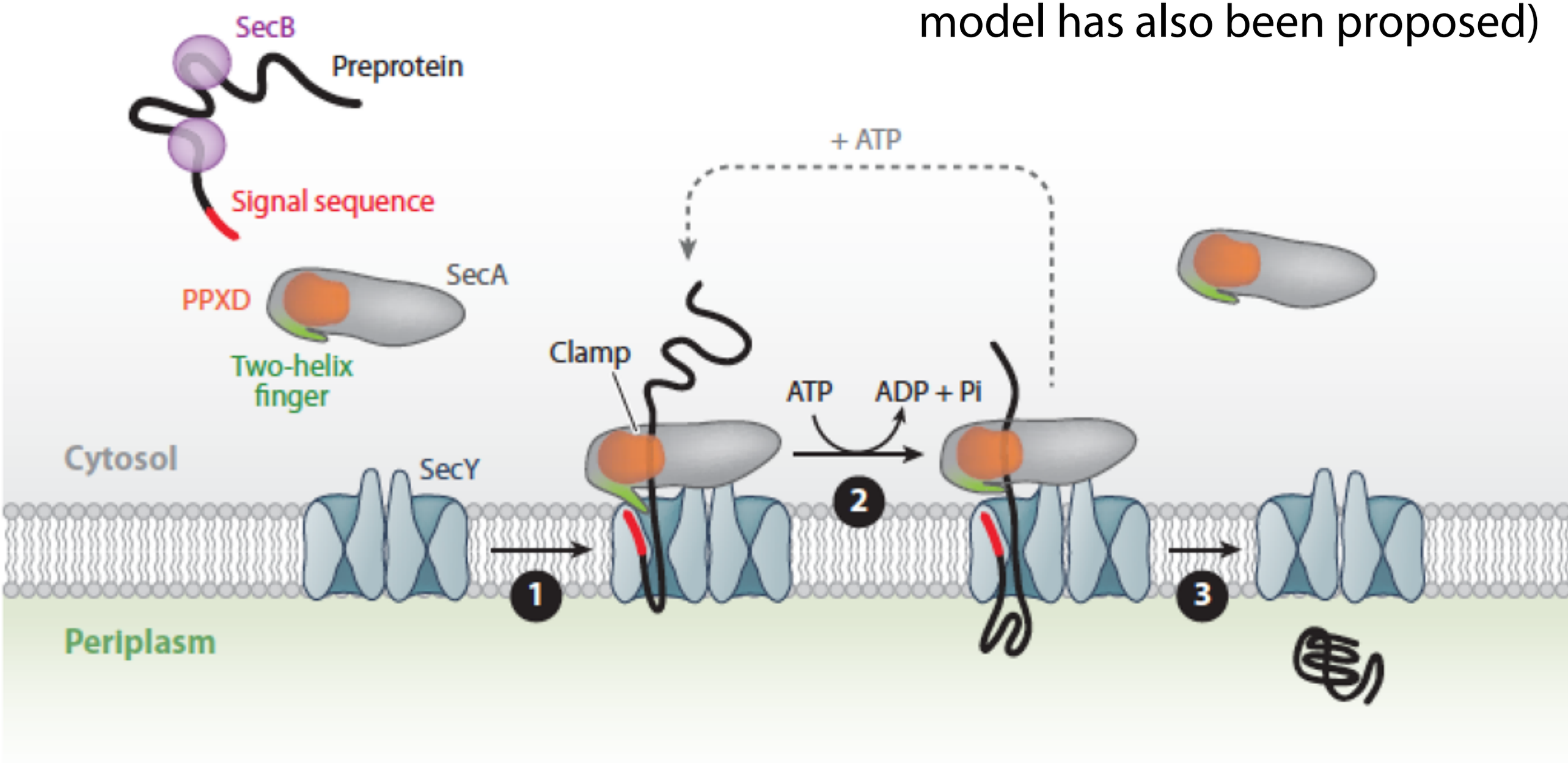


ribosome interacts directly with SecY channel to translocate nascent protein across membrane

Park and Rapoport. *Annu. Rev. Biophys.* 41:21–40. (2012)

Role of SecA in bacteria

monomeric SecA model (transient dimer model has also been proposed)



SecA helps those proteins not immediately targeted to cross the membrane *post-translationally*

Park and Rapoport. ***Annu. Rev. Biophys.*** 41:21–40. (2012)

SecA structure is complex

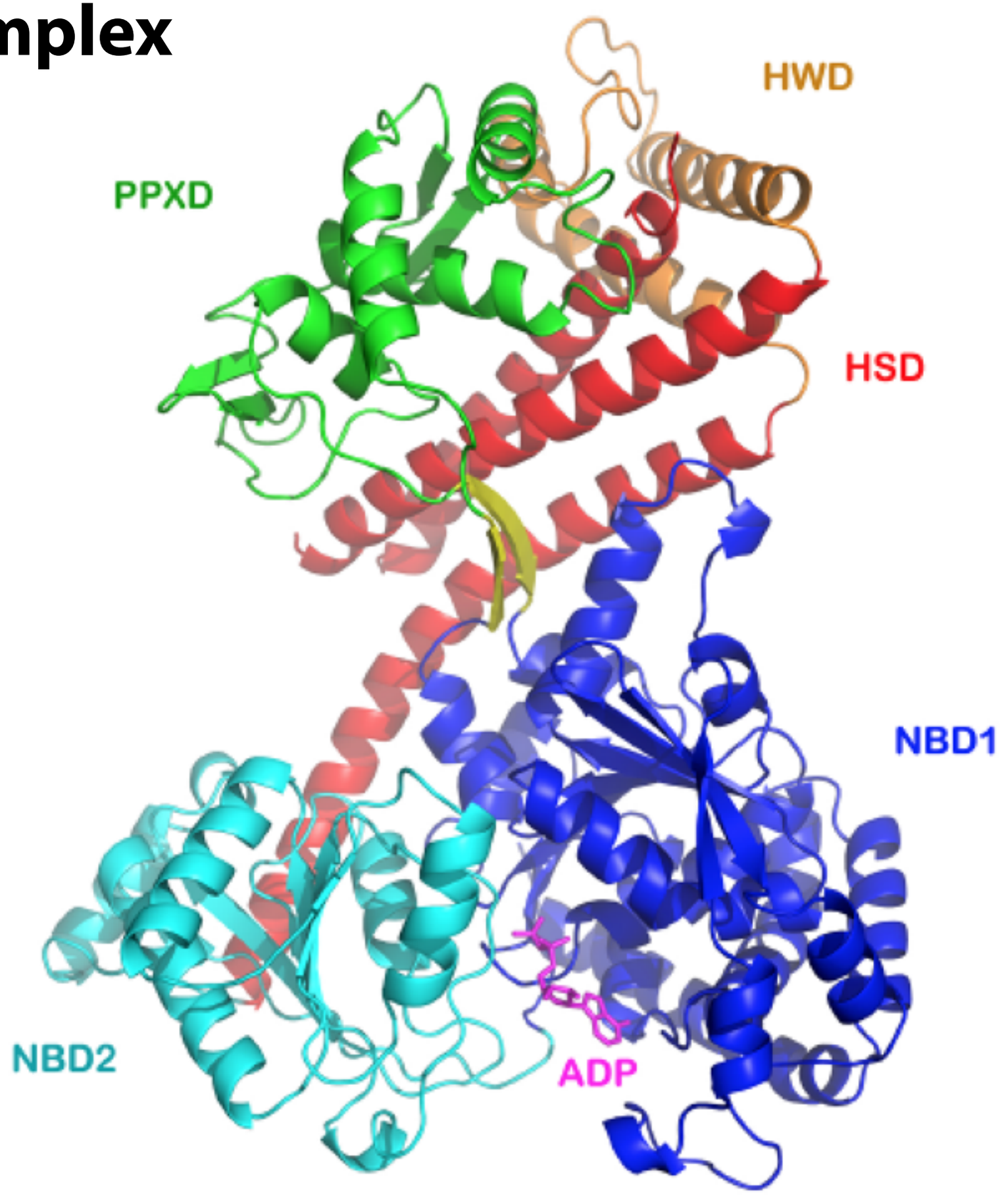
SecA is over 800 residues and is composed of multiple functional domains:

-Nucleotide Binding Domains 1 and 2 (**NBD1** and **NBD2**)

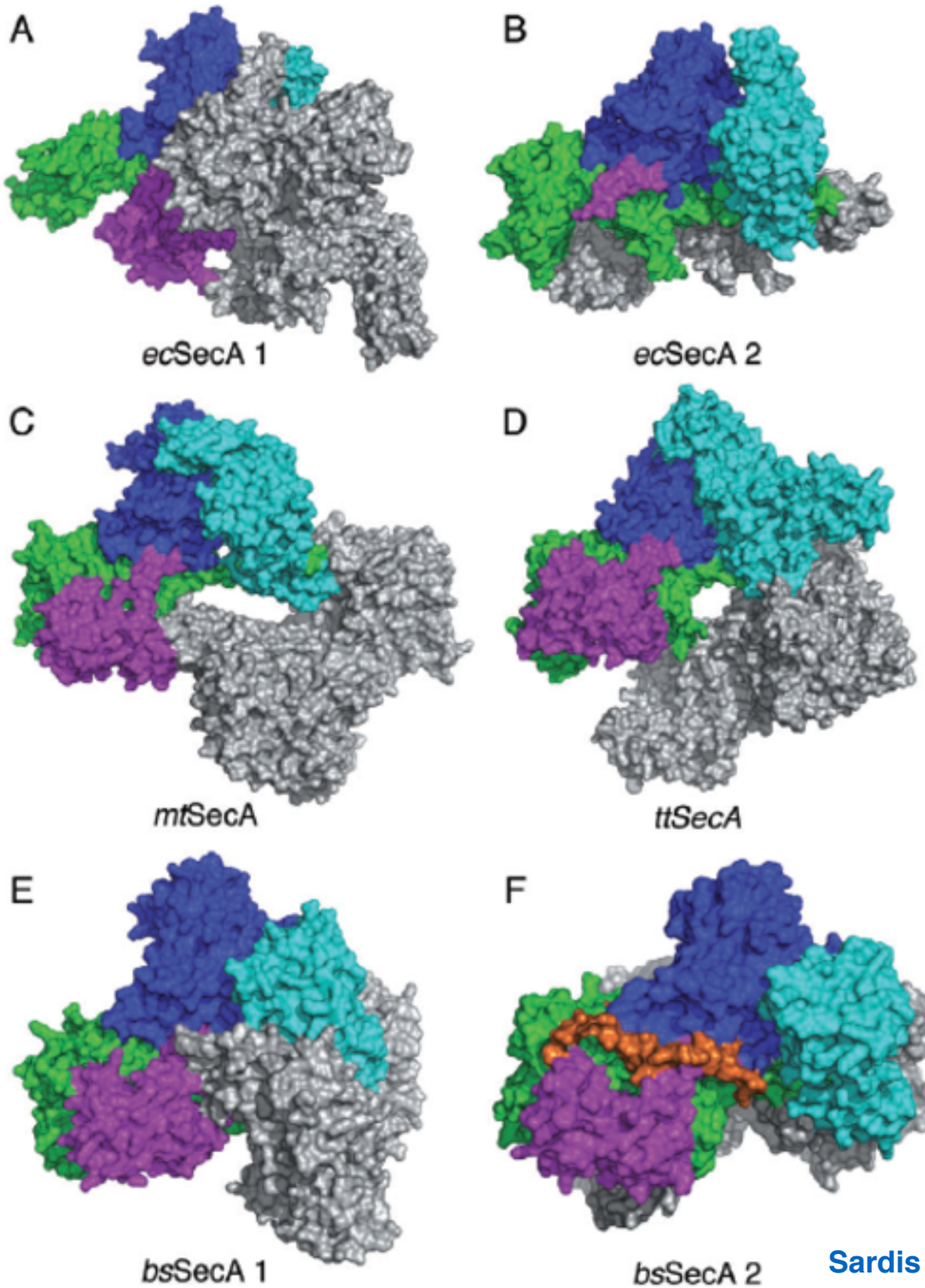
-Polypeptide-binding domain (**PPXD**)

-Helical Scaffold Domain (**HSD**)

-Helical Wing Domain (**HWD**)



dimer structures of SecA in disagreement



numerous structures have been solved showing apparent dimers, with all conflicting with one another

likely a result of crystal packing, also shows **PPXD** and other domains in a variety of positions (**flexible**!)

which conformations are functional? which are artifacts?

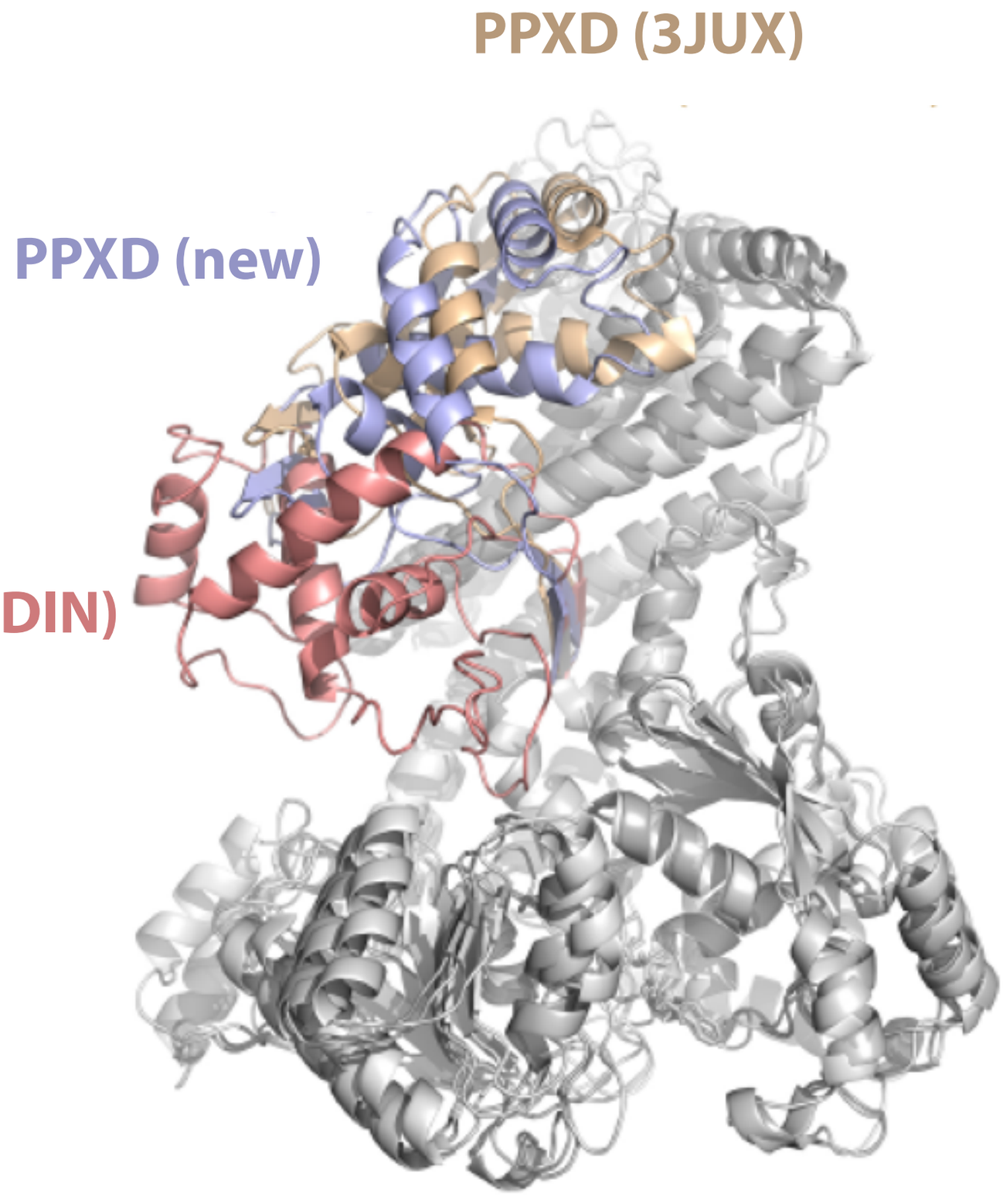
Sardis and Economou. *Mol. Microbiol.* 76:1070-81. (2010)

A new structure appears to reveal little

three structures from the same species are nearly identical except for **PPXD**

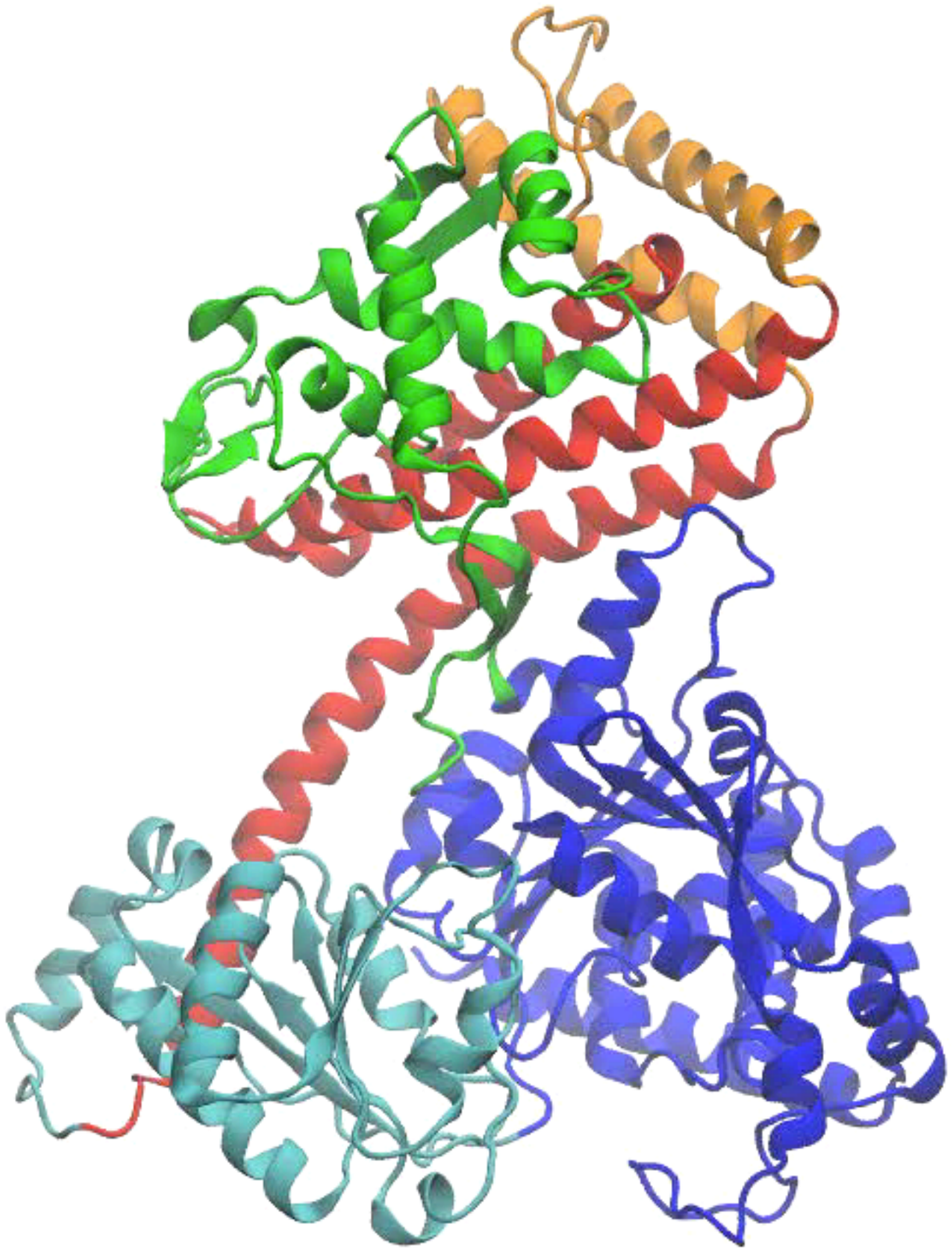
One was in complex with channel SecY (**3DIN**), one without (**3JUX**)

new structure from lab of Tom Rapoport (Harvard U.) is in a nearly identical conformation as **3JUX** - ***what does it tell us?***



Chen, Bauer, Rapoport, Gumbart. Conformational Changes of the Clamp of the Protein Translocation ATPase SecA. *JMB* 427:2348–59 (2015)

dynamics isn't very helpful



Ran ~**1 μs** of equilibration of each structure (3DIN, 3JUX, new) at 353 K (optimal for *T. maritima*)

PPXD fluctuates a bit
but never separates from **HWD**
nor does it approach **NBD2**

Chen, Bauer, Rapoport, Gumbart. Conformational Changes of the Clamp of the Protein Translocation ATPase SecA. *JMB* 427:2348–59 (2015)

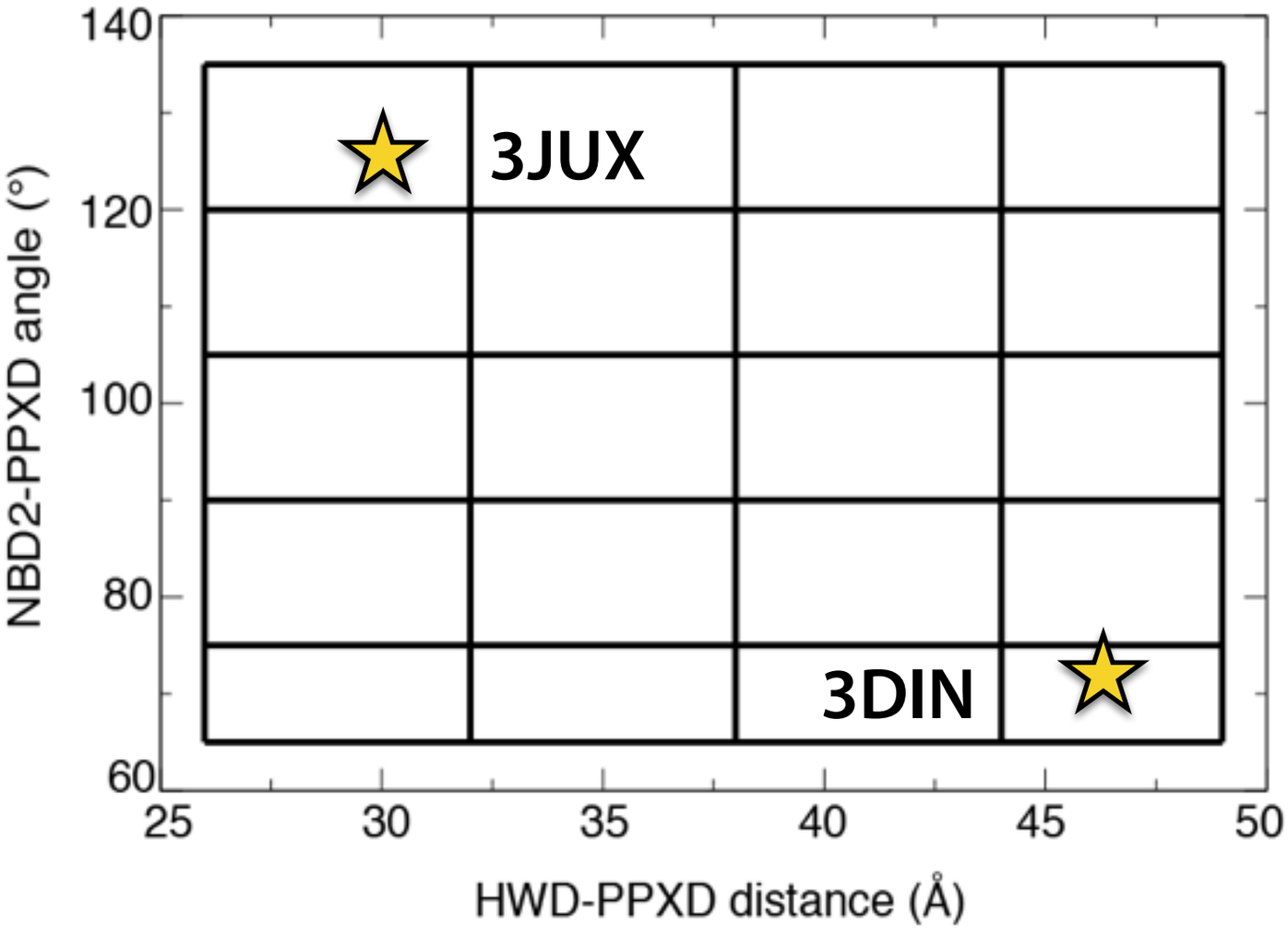
turning to free energy calculations

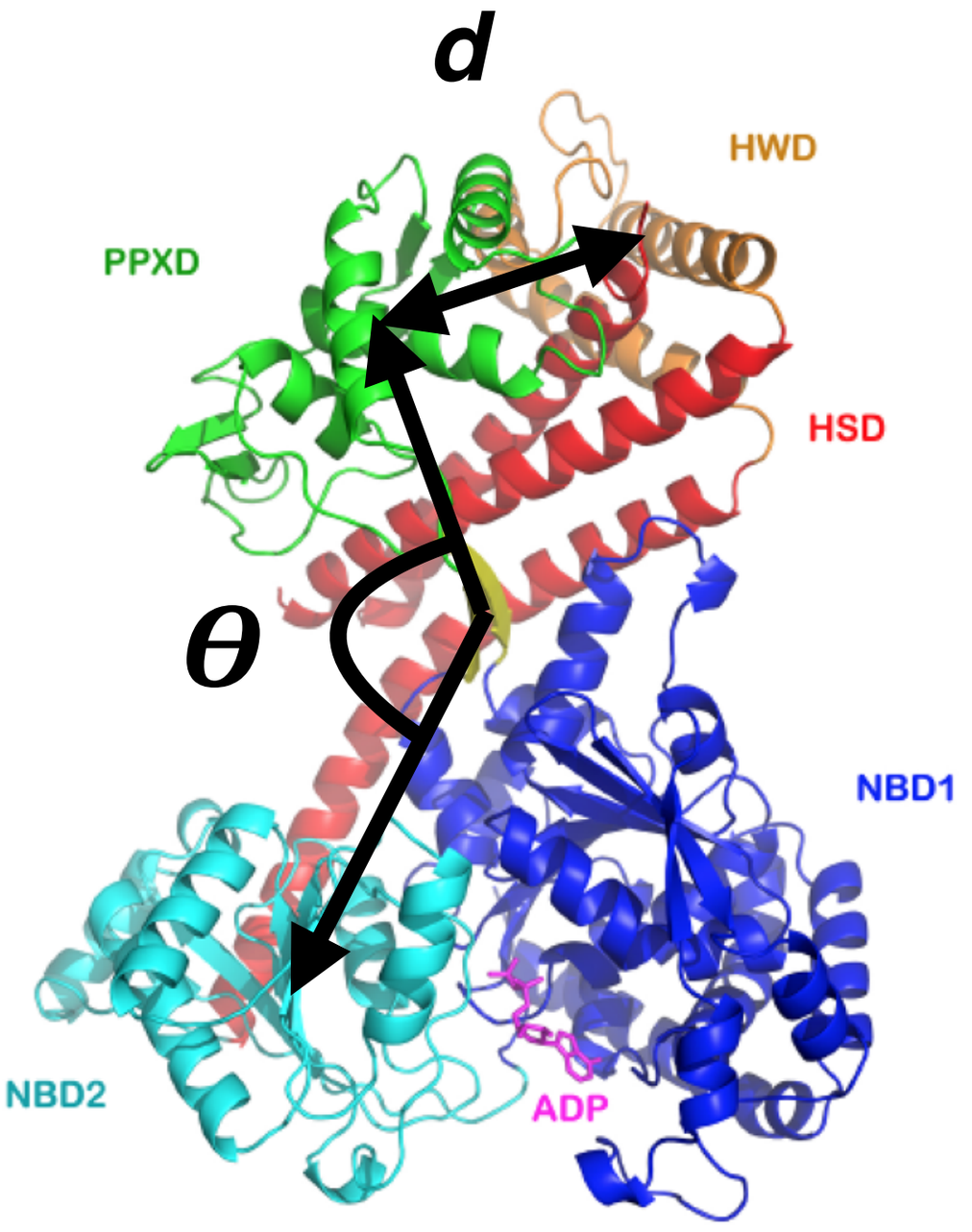
Chen, Bauer, Rapoport, Gumbart.
JMB 427:2348–59 (2015)

based on structures, identify two relevant reaction coordinates

-angle between **PPXD** and **NBD2** (the so-called "clamp")

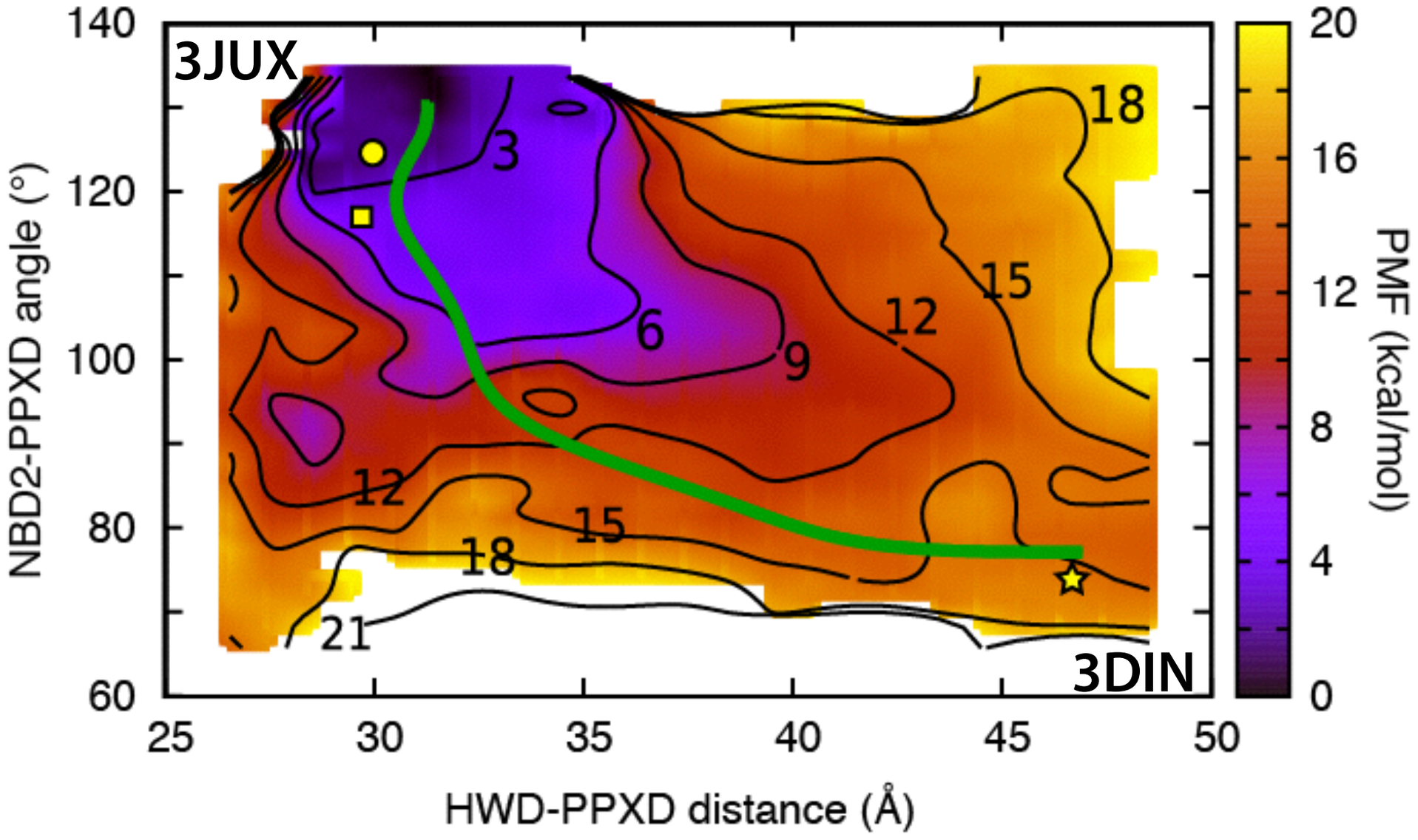
-distance between **HWD** and **PPXD**





two structures define range of RCs, subdivided in 24 windows for **ABF**

Results from 600 ns of ABF simulations



new crystal structure lies on least-free-energy path connecting **3JUX** (open clamp) and **3DIN** (closed clamp)

going from open to closed requires first concerted motion of **PPXD** and **HWD** before separation and continuation of **PPXD**

Chen, Bauer, Rapoport, Gumbart. *JMB* 427:2348–59 (2015)

Comparison with equilibrium sims

Chen, Bauer, Rapoport, Gumbart.
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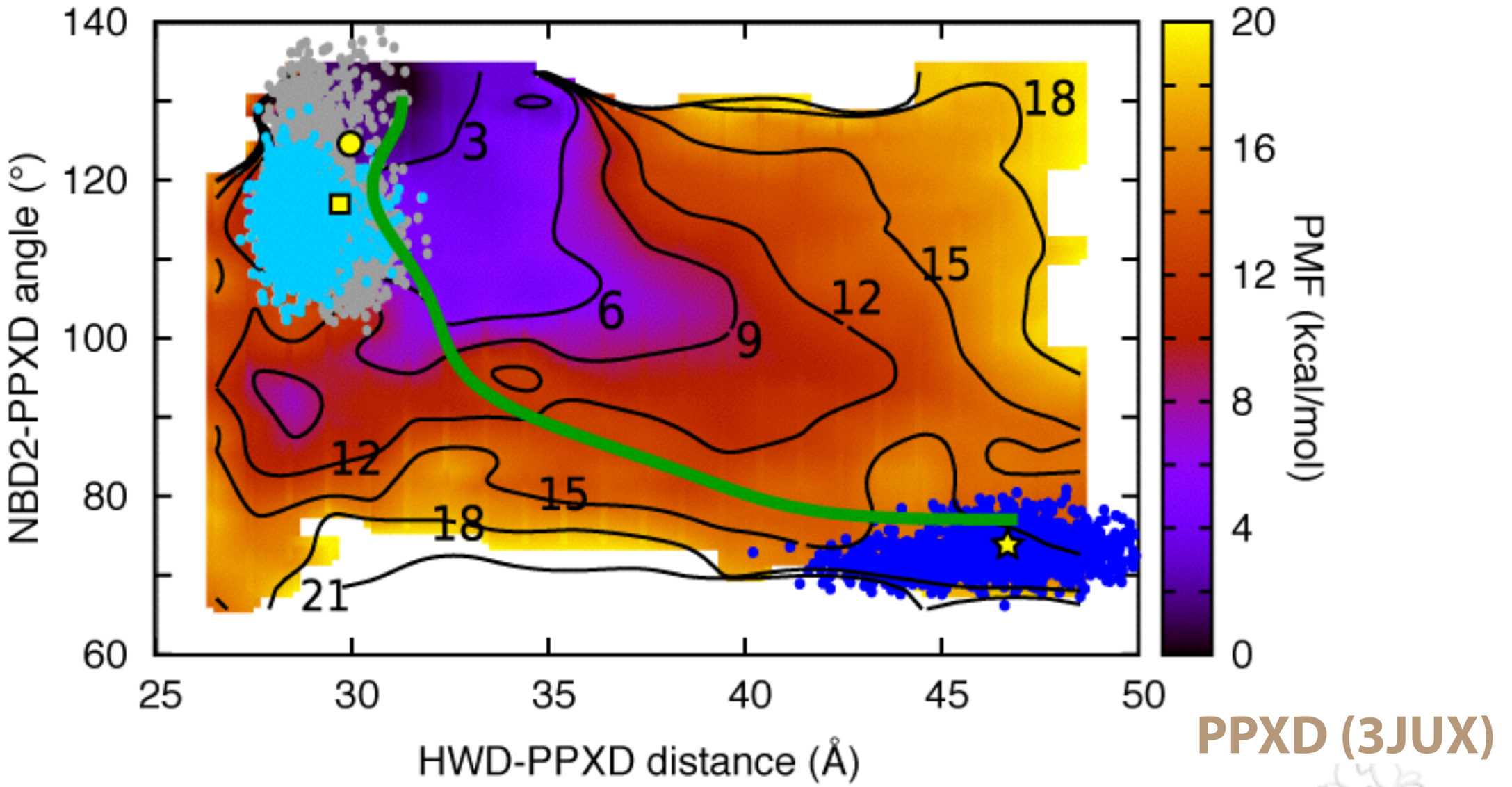


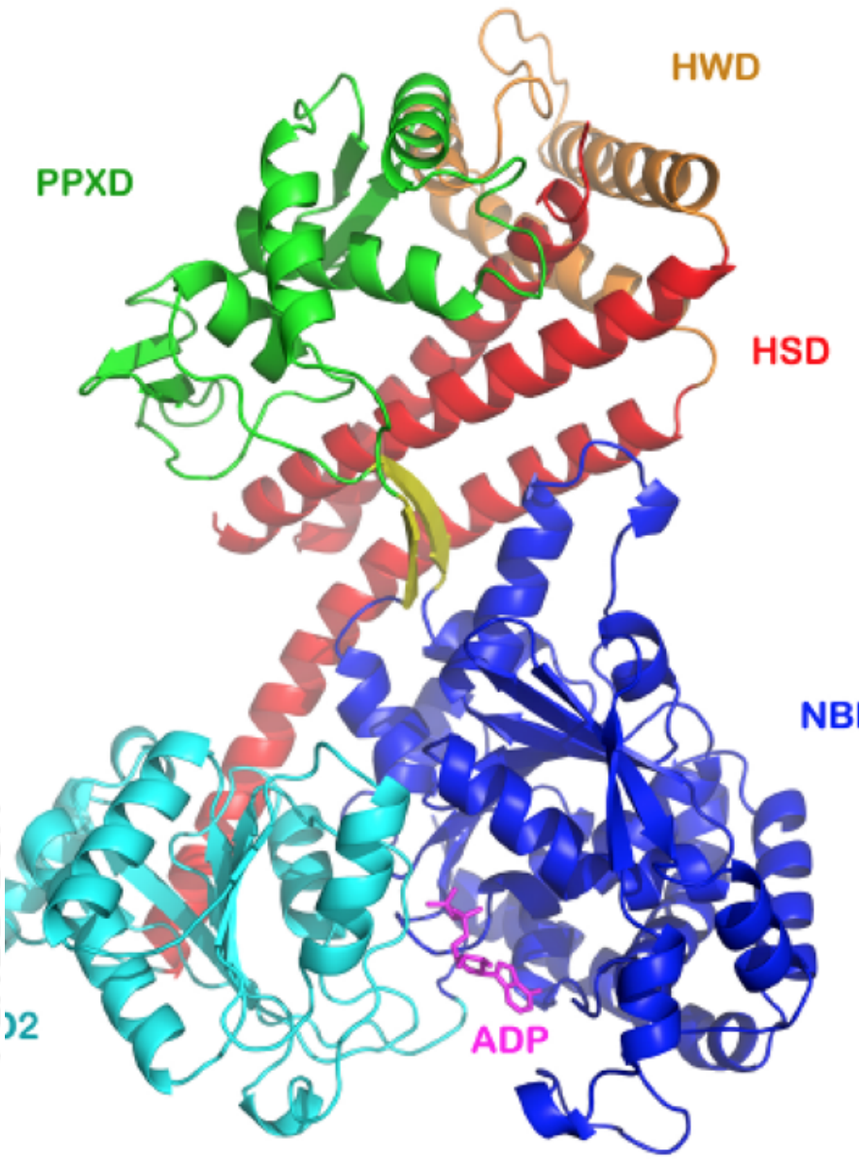
Systems sample only a small range of conformational space in 1 μs

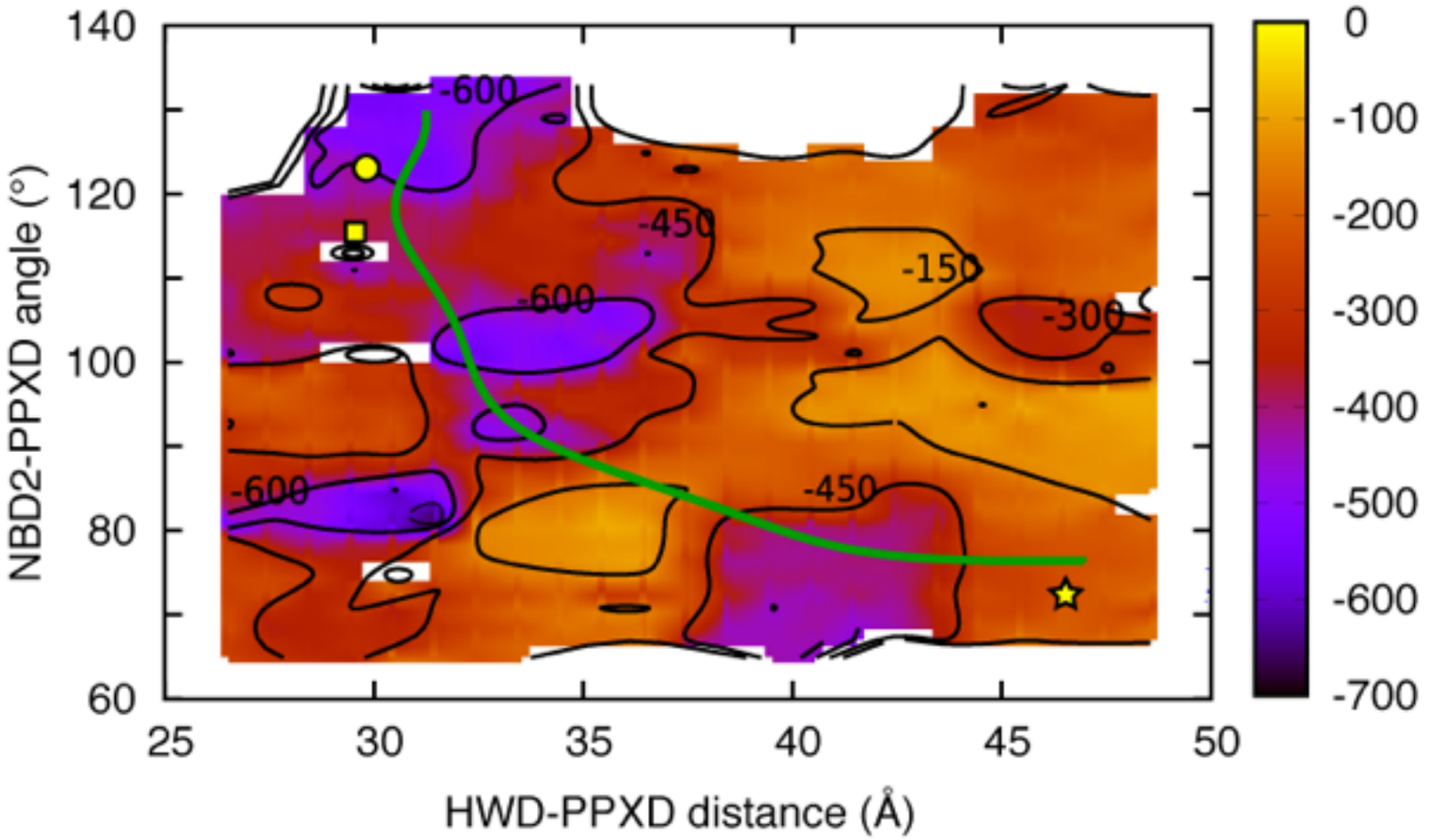
the new crystal structure (**cyan**) explores a range similar to 3JUX (**grey**)

Model for initiation of protein translocation

largest jump in **free** energy occurs upon separation of **PPXD** from **HWD**

interaction energy (below) between **PPXD** and **HSD** drops the most at the separation point

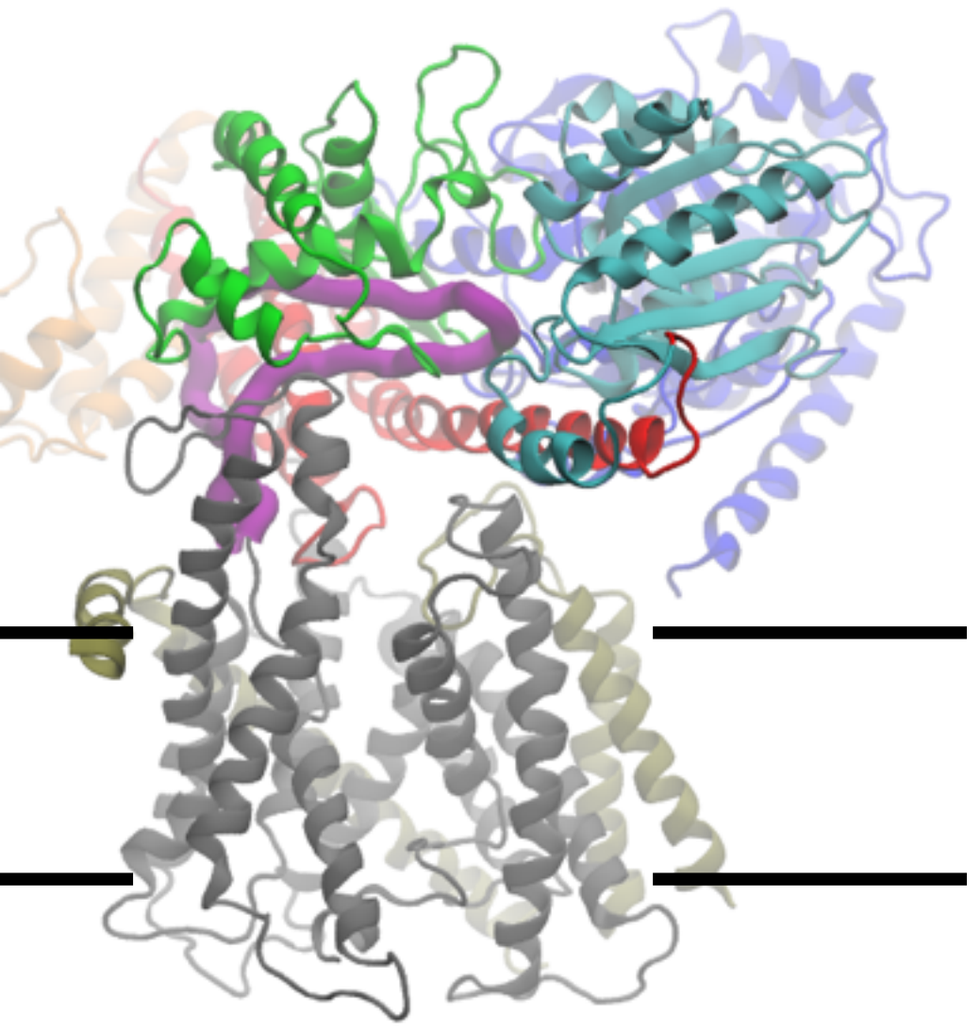


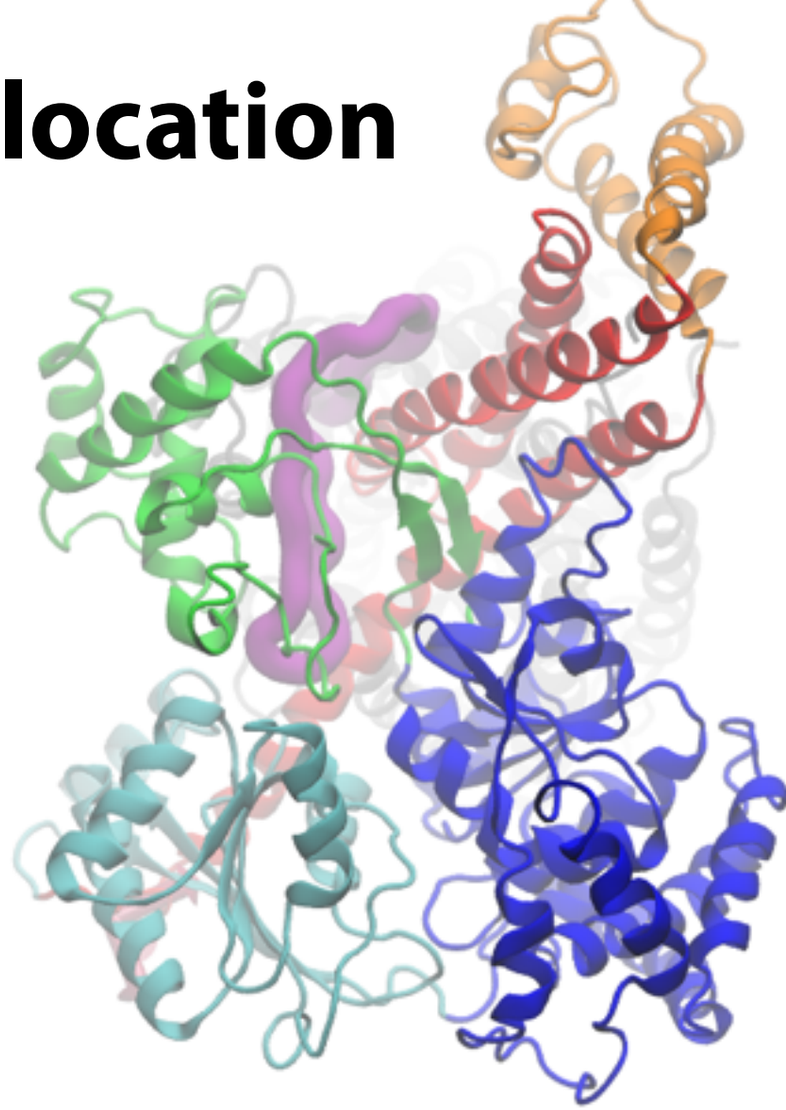


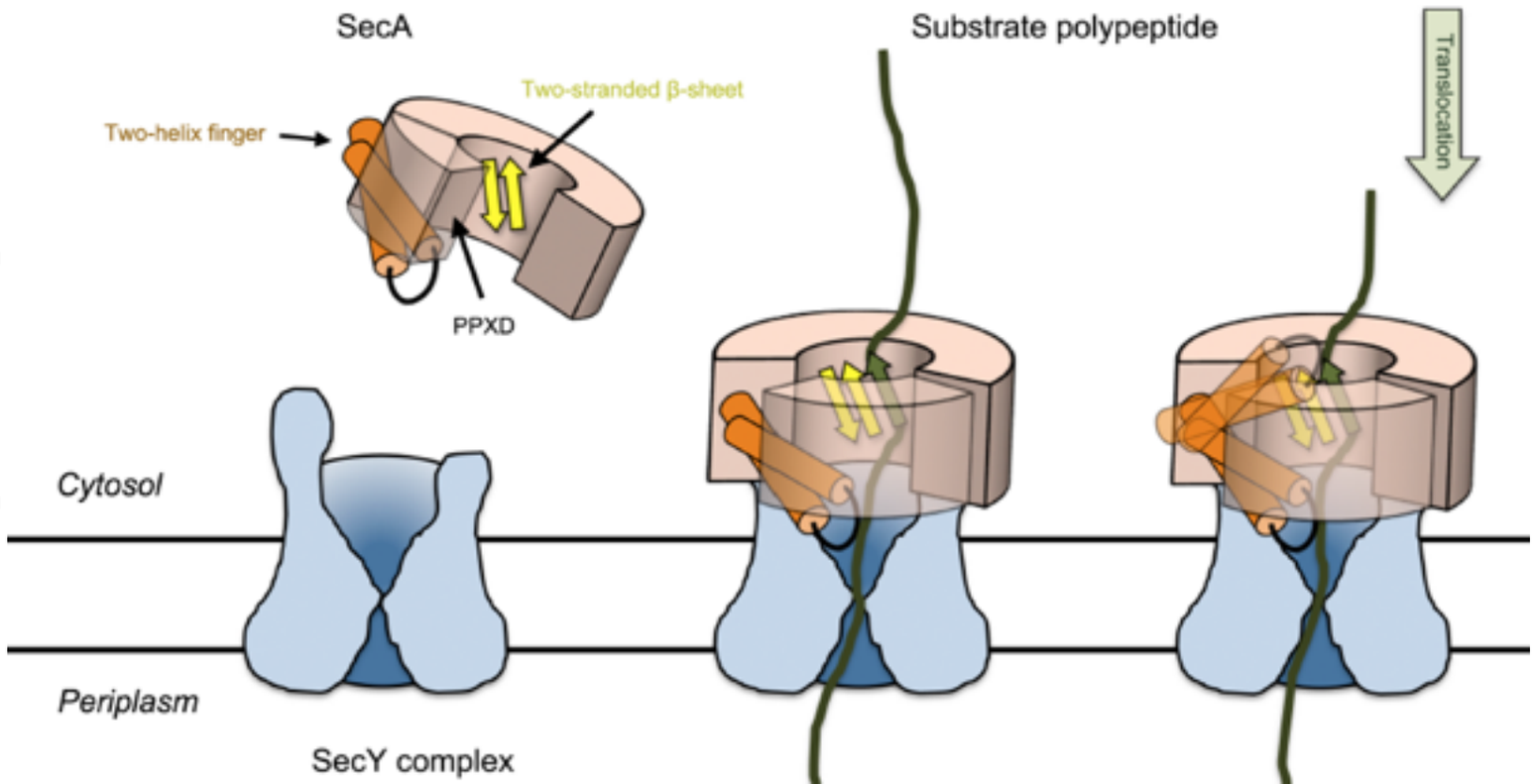
Chen, Bauer, Rapoport, Gumbart. *JMB* 427:2348–59 (2015)

Model for initiation of protein translocation

energetically unfavorable separation of **PPXD** from **HSD** can be induced by binding to **SecY**, which inserts between the two domains







Chen, Bauer, Rapoport, Gumbart.
JMB 427:2348–59 (2015)