

11/13/2024

- Free energy calculations
- Binding free energy calculations
- Work on exercises / meet with teams
- Today's lecture is a key step towards the following learning objective: Explain key concepts related to binding free energy calculations. Compare and contrast molecular docking and binding free energy calculations.

Free energy calculations

Estimating Thermodynamic Properties

- Molecular simulations are used to calculate thermodynamic and kinetic properties
- In general, the thermodynamic properties are
 - expectation values of an observable, including
 - probability of the observable having a certain range of values
 - potential of mean force with respect to the observable
 - free energy differences between thermodynamic states
 - in biomolecular systems, $\Delta G \sim \Delta A$

What is ΔG ?

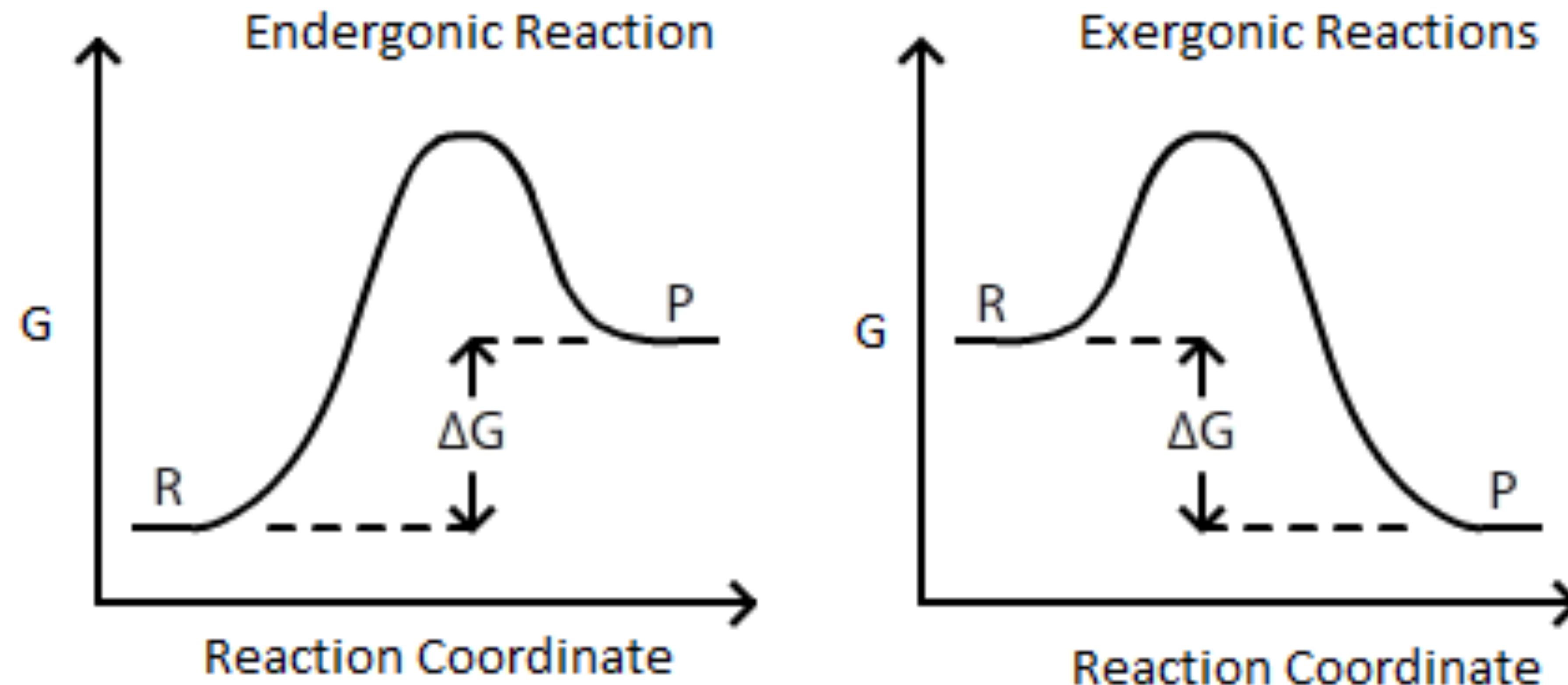
- ΔU is the change in average internal energy.
 - internal energy can be computed for individual structures
 - in biomolecular simulations, internal energy is modeled by the molecular mechanics force field
- $\Delta H = \Delta U + \Delta(pV)$ is the change in enthalpy
 - in biomolecular simulations, change in pV is usually negligible
- ΔG is the Gibbs free energy
 - at constant pressure and temperature, dictates
 - spontaneity and
 - equilibrium constant of process
 - in biomolecular simulation, interest in free energy differences between
 - conformations of a macromolecule
 - thermodynamic states with different temperature, pressure, volume, or other parameters
 - $\Delta G = \Delta H - T\Delta S$, but ΔS is very challenging to compute

What is ΔA ?

- ΔA is the Helmholtz free energy
 - at constant volume and temperature, dictates
 - spontaneity and
 - equilibrium constant of process
 - in biomolecular simulation, ΔA and ΔG are usually assumed to be equal

What is the free energy of a reaction?

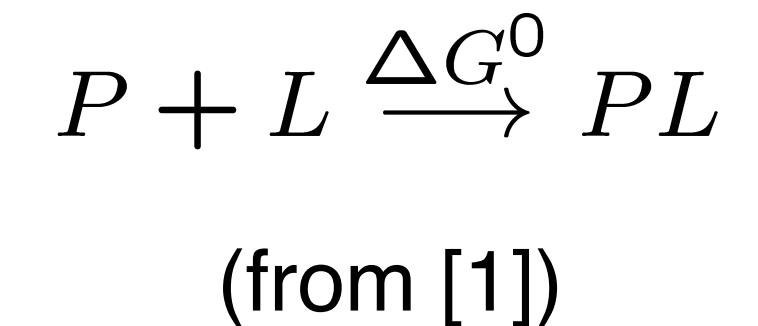
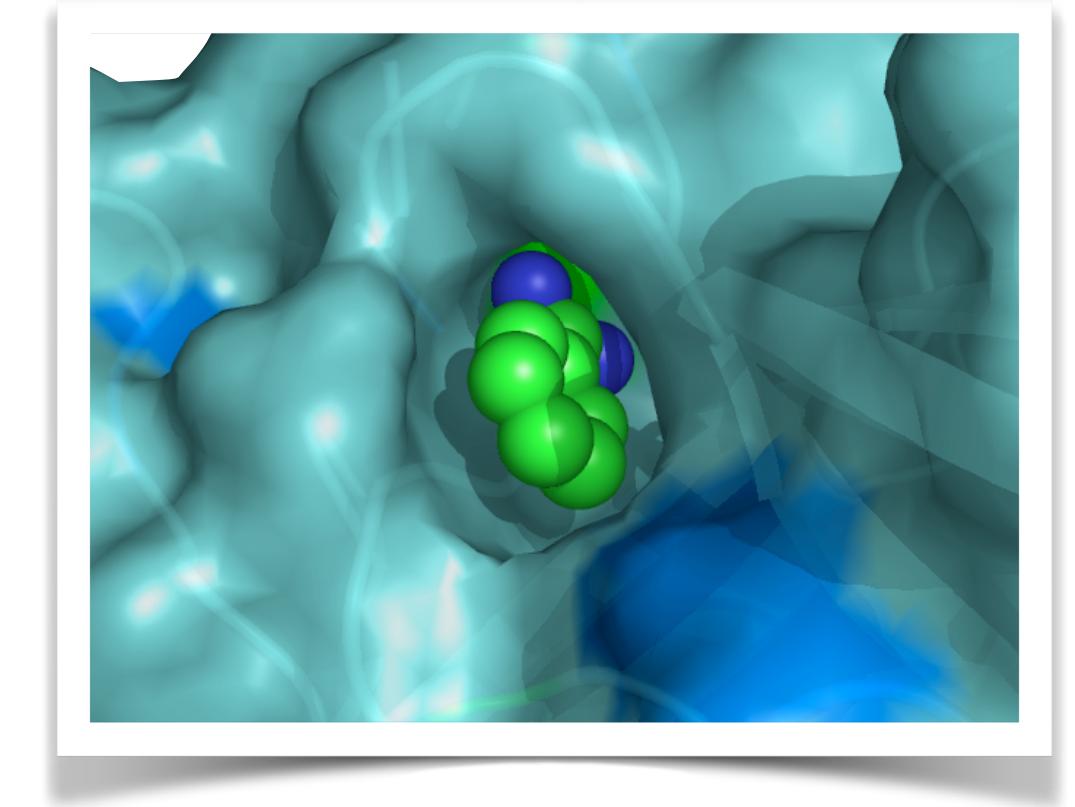
$$\Delta G = \Delta H - T\Delta S = RT \ln K$$



[https://chem.libretexts.org/Bookshelves/Analytical_Chemistry/Supplemental_Modules_\(Analytical_Chemistry\)/Electrochemistry/Electrochemistry_and_Thermodynamics](https://chem.libretexts.org/Bookshelves/Analytical_Chemistry/Supplemental_Modules_(Analytical_Chemistry)/Electrochemistry/Electrochemistry_and_Thermodynamics)

How are free energy calculations useful?

- Noncovalent binding between molecules (see [2])
 - Design molecules to manipulate protein function
 - Recognize toxins
 - Identify enzyme functions
 - Protein design: design binders to target molecule
 - Aid medicinal chemistry, guide synthesis
- Hydration free energies
 - Part of binding free energy & solubility
- Conformational free energies relevant to
 - biological mechanism
 - binding free energy



**How are $\Delta G/\Delta A$ calculated from
molecular simulations?**

Basic Statistical Mechanics

- In the Boltzmann distribution, the probability of a configuration r^N with energy $U_s(r^N)$ is,

$$\pi_s(r^N) \propto \exp[-\beta U_s(r^N)] \text{ (unnormalized)}$$

$$\rho_s(r^N) = \exp[-\beta U_s(r^N)] / Q_s \text{ (normalized)}$$

- A partition function is the normalizing constant of the Boltzmann distribution

$$Q_s = \int \pi_s(r^N) dr^N$$

- The free energy difference is related to a ratio of partition functions

$$\beta(A_1 - A_0) = -\ln \left(\frac{Q_0}{Q_1} \right)$$

The Zwanzig Relation: Derivation

- From before, $\beta(A_1 - A_0) = -\ln \left(\frac{Q_0}{Q_1} \right)$.
- Substituting in partition functions, $\beta(A_1 - A_0) = -\ln \left(\frac{\int e^{-\beta U_1(r^N)} dr^N}{\int e^{-\beta U_0(r^N)} dr^N} \right)$.
- Multiplying by one, $\beta(A_1 - A_0) = -\ln \left(\frac{\int e^{-\beta U_1(r^N) + \beta U_0(r^N) - \beta U_0(r^N)} dr^N}{\int e^{-\beta U_0(r^N)} dr^N} \right)$.
- Defining the potential energy difference $\Delta U(r^N) = U_1(r^N) - U_0(r^N)$,
$$\beta(A_1 - A_0) = -\ln \left(\frac{\int e^{-\beta \Delta U(r^N)} e^{-\beta U_0(r^N)} dr^N}{\int e^{-\beta U_0(r^N)} dr^N} \right)$$

The Zwanzig Relation: In Practice

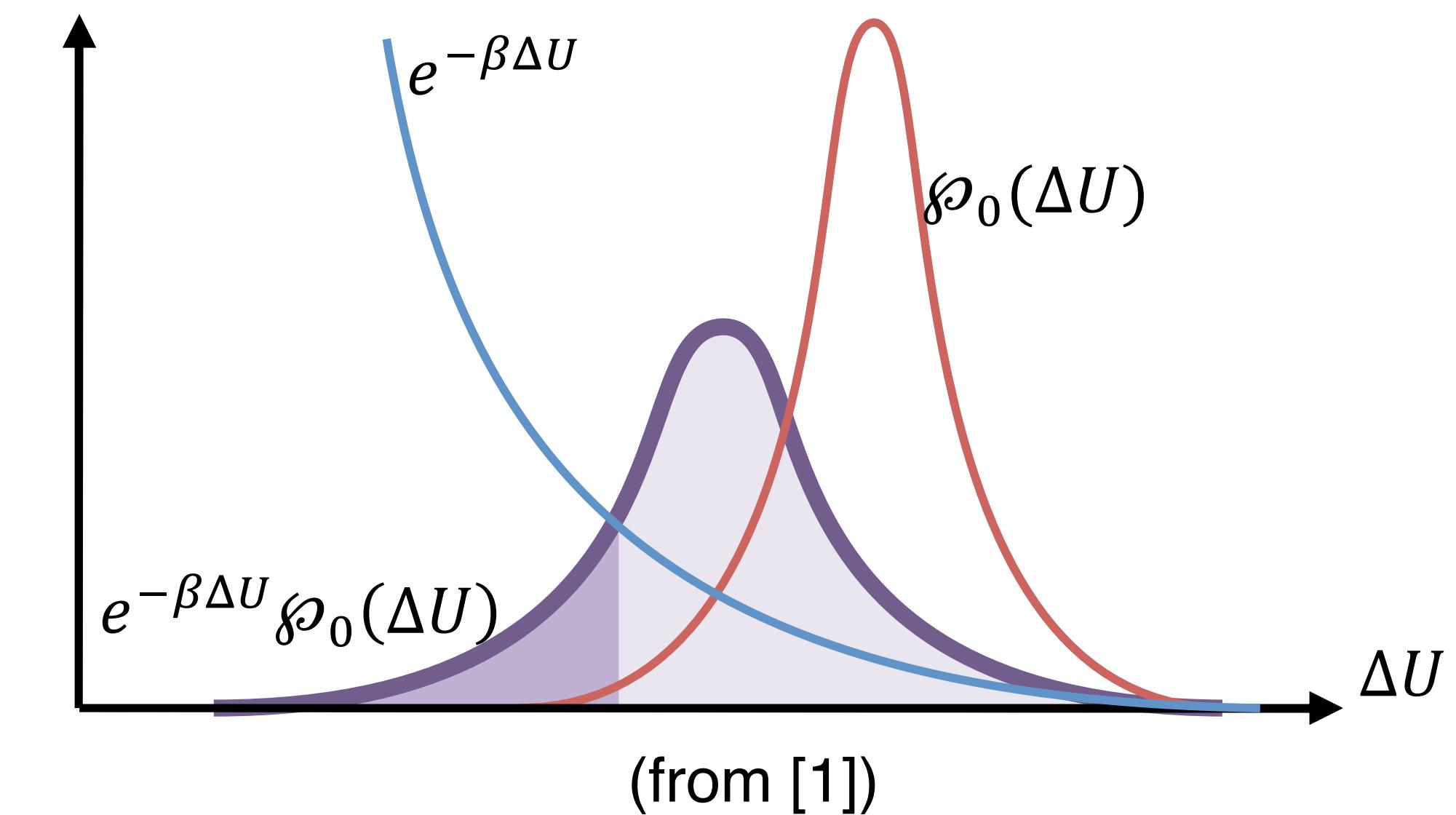
- Using the definition of $\rho_s(r^N)$, $\beta(A_1 - A_0) = - \ln \int \rho_0(r^N) e^{-\beta \Delta U(r^N)} dr^N$.
- The Zwanzig relation [3] is
 - $\beta(A_1 - A_0) = - \ln \langle e^{-\beta \Delta U} \rangle_0$ in a simpler notation.
 - $\beta(A_1 - A_0) = - \ln \langle e^{\beta \Delta U} \rangle_1$ can be derived with similar steps
- This shows us that
 - The free energy difference can be computed based on an average over configurations taken from one of the states of interest
 - We can generate these configurations with MC or MD
 - The free energy comes from evaluating the energies of these configurations in both potentials U_0 and U_1 , and taking an appropriate average of the energy difference

The Zwanzig Relation: Limitations

- In terms of an integral over the distribution of ΔU (instead of over $\rho_o(r^N)$) the Zwanzig relation is,

$$\beta(A_1 - A_0) = - \ln \int e^{-\beta\Delta U} \rho_0(\Delta U) d\Delta U.$$

- Sampling is from the red curve
- Accurate estimation requires the purple curve
- The calculation will not be accurate if U_0 and U_1 are very different!
- Potential energies will be different if the states access different parts of configuration space



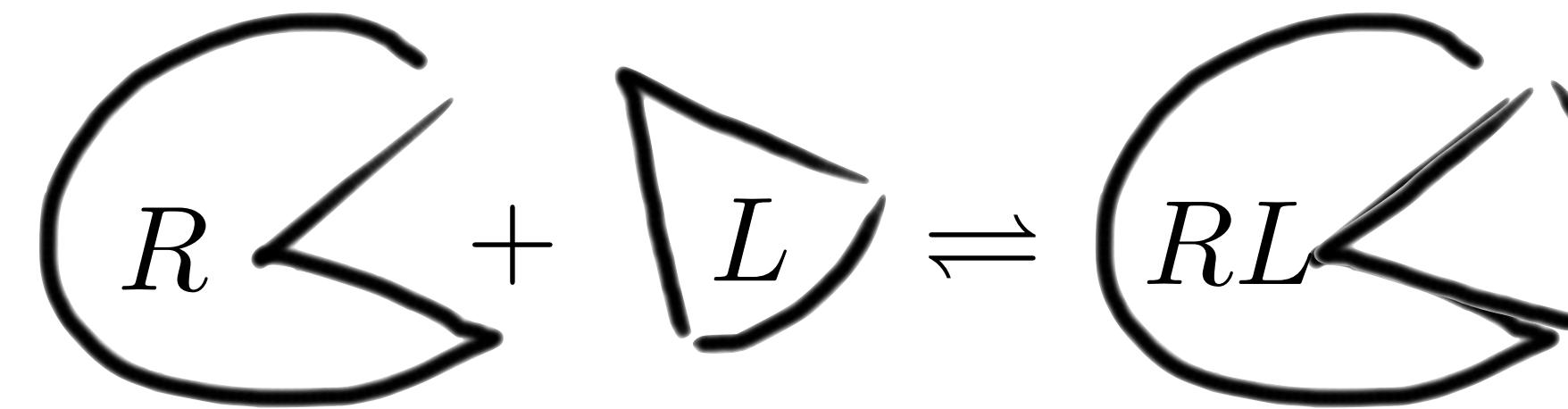
Other ways to calculate ΔG

- Multistate Bennett Acceptance Ratio (MBAR) [5]
 - estimates free energies and thermodynamic expectations from a series of states
 - extension of Bennett Acceptance Ratio (BAR) [4], which uses data from two states
 - Proven to be statistically optimal
- Thermodynamic integration is based on the fundamental theorem of calculus, integrating one the derivative of the free energy with respect to a parameter
- All of the methods require thermodynamic states with configuration space overlap, meaning that
 - similar configurations have similar energies
 - the most relevant configuration space is similar

Binding Free Energy Calculations

- This module will be on the theory of binding free energy calculations
- At the end of this module, you should be able to answer the following questions:
 - How do binding free energy calculations differ from molecular docking?
 - What is a thermodynamic cycle? What types of thermodynamic cycles are used in binding free energy calculations?
 - What is an alchemical transformation?

ΔG° quantifies binding strength



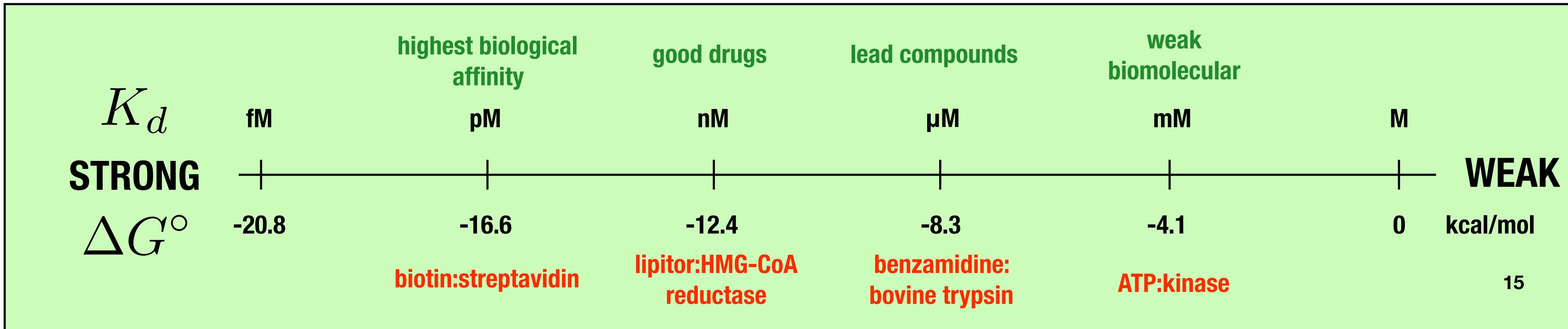
dissociation constant

$$K_d = \frac{C_R C_L}{C_{RL}}$$

binding free energy

$$\Delta G^\circ = \beta^{-1} \ln \left(\frac{K_d}{C^\circ} \right)$$

C_R free receptor concentration
 C_L free ligand concentration
 C_{RL} complex concentration
 C° standard state concentration (1 M)



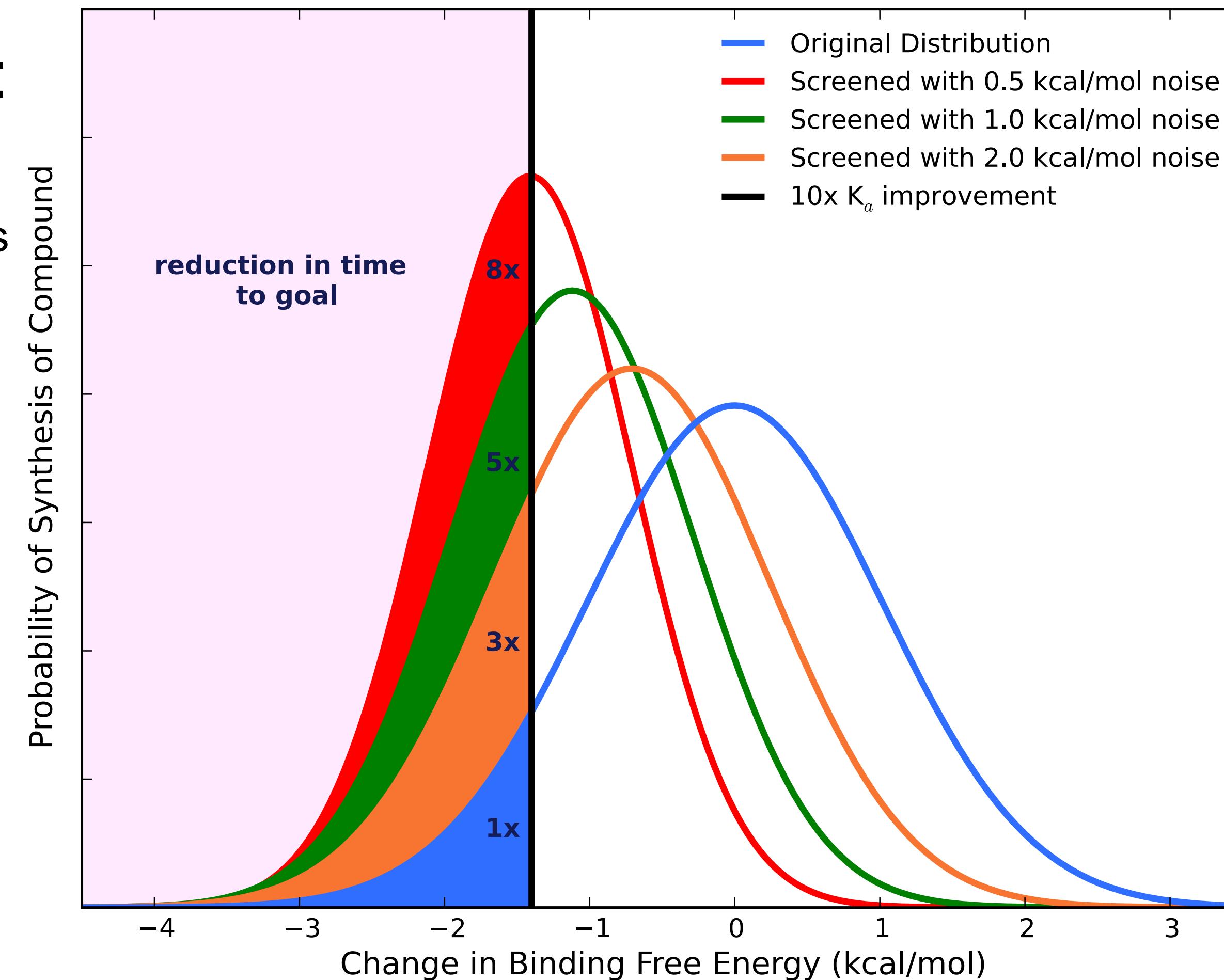
Modestly accurate ΔG° predictions can aid drug discovery

Hypothetical pipeline:

- Medicinal chemist suggests 100 derivatives or compounds per week
- Your job is to pick the top 10 to carry forward

Question: How many molecules do we have to make to gain a factor of 10 in affinity?

- 0.5 kcal/mol noise: Decreases # required by 8x
- 1.0 kcal/mol noise: Decreases by 5x
- 2 kcal/mol noise: Decreases by 3x



Docking scores do not correlate with ΔG°

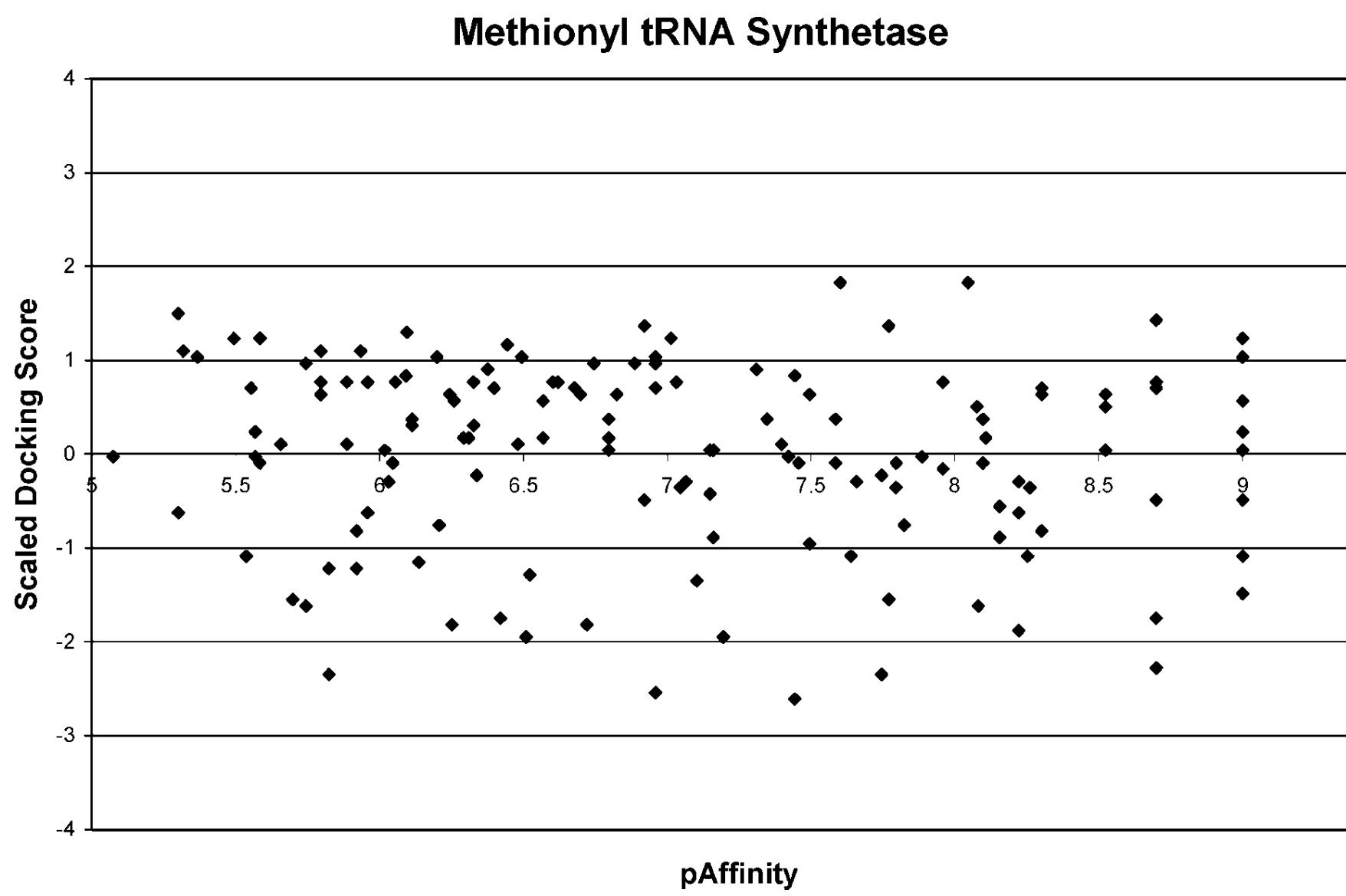
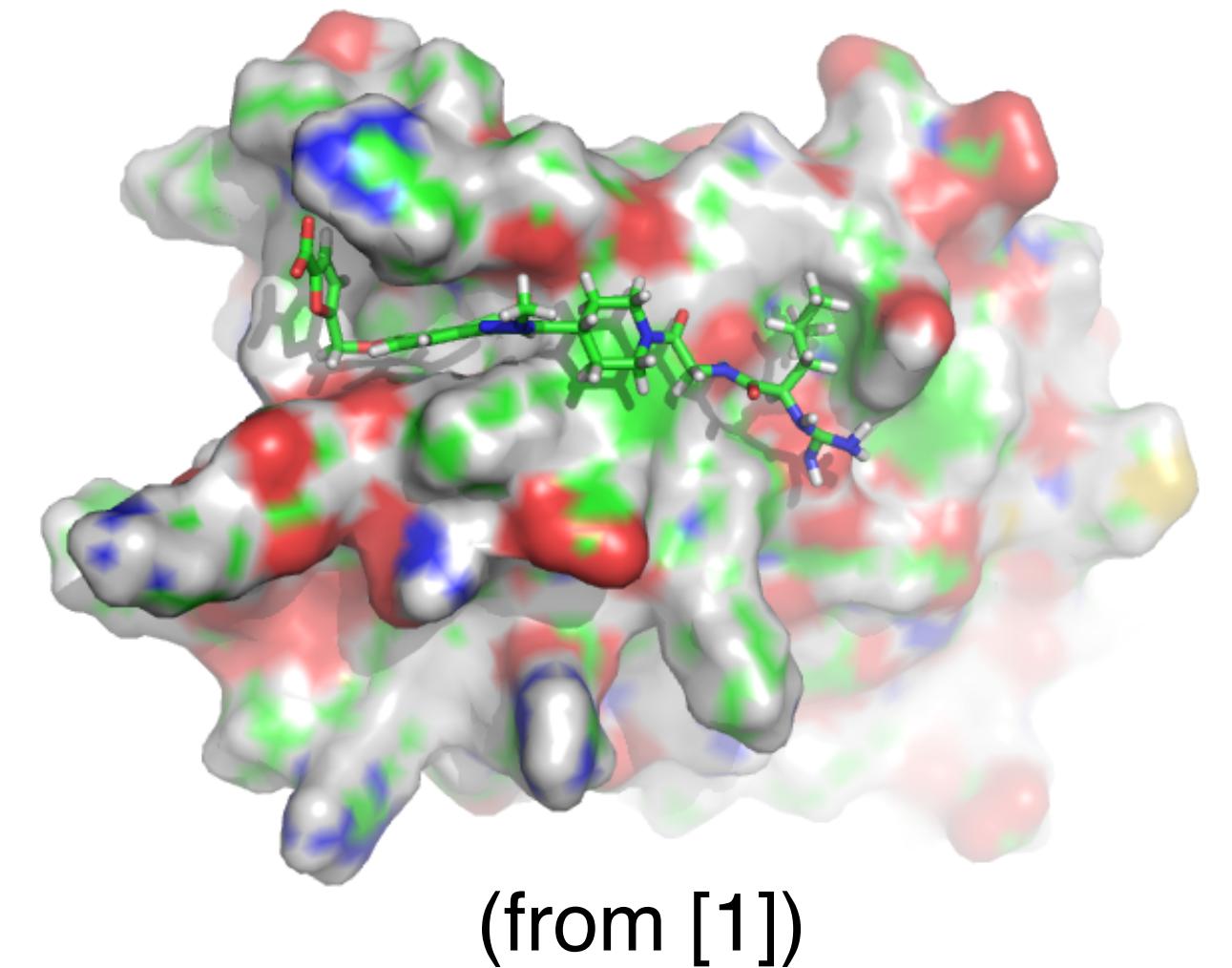


Figure 11. Plot of scaled score vs pAffinity for MRS and PPAR δ . While the calculated correlation coefficient for the data shown for MRS is $r = -0.28$, this plot clearly demonstrates that these values are meaningless. No useful correlation exists between the docking score and compound affinity.

“For prediction of compound affinity, none of the docking programs or scoring functions made a useful prediction of ligand binding affinity.”

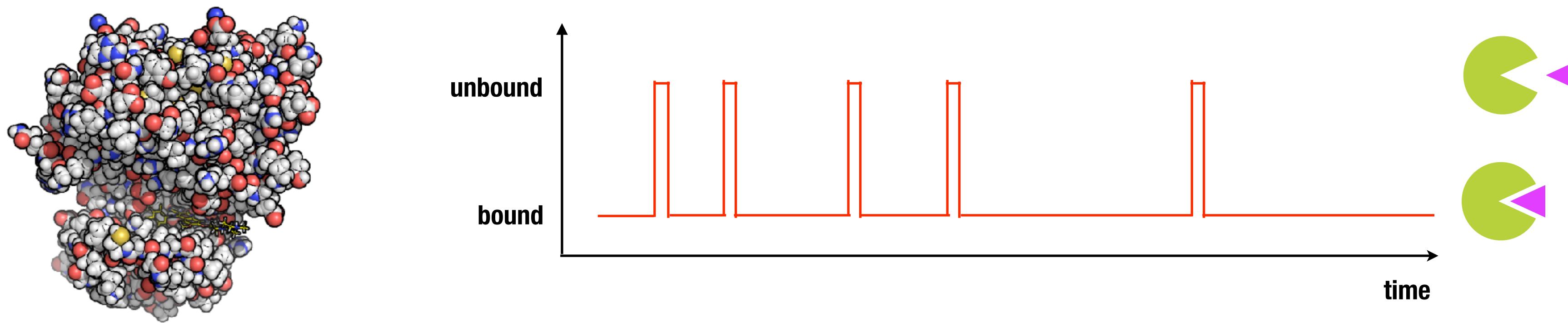
Docking approximates ΔS

- $\Delta G = \Delta H + T\cancel{\Delta S}$
- Docking score $\sim \Delta H$
- It sometimes involves
 - ad hoc ΔS based on the number of rotatable bonds
 - ΔG_{solv}
- Docking is based on “optimal” configurations

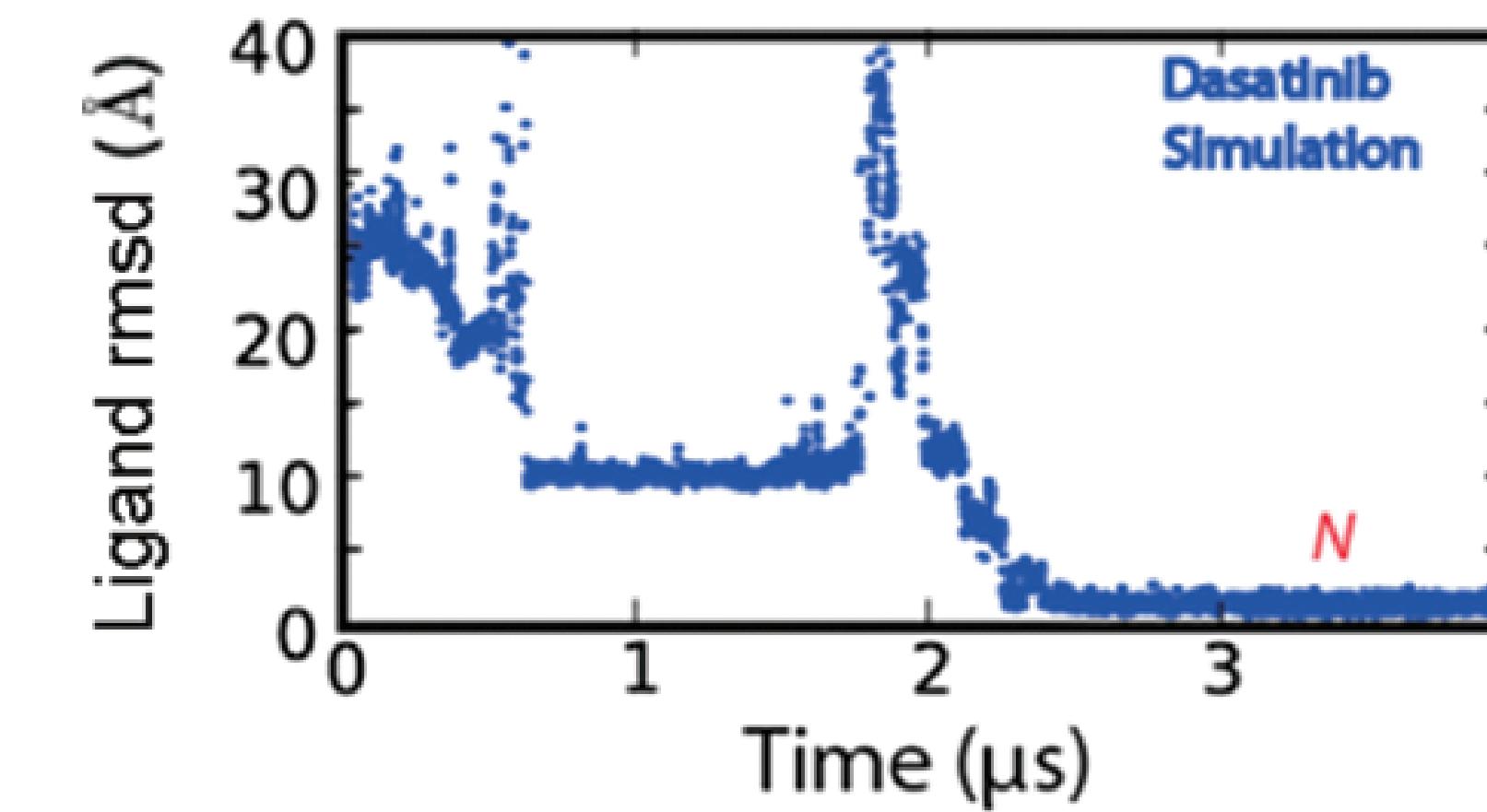
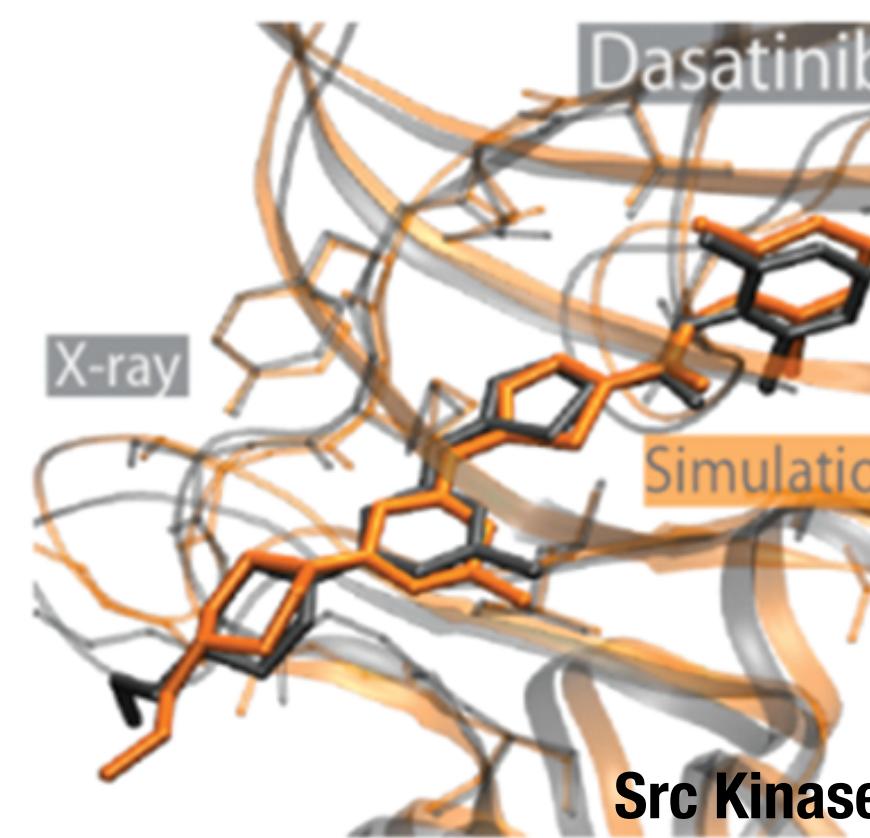


Binding free energies can be determined from long simulations

In principle, we could watch many binding/unbinding events



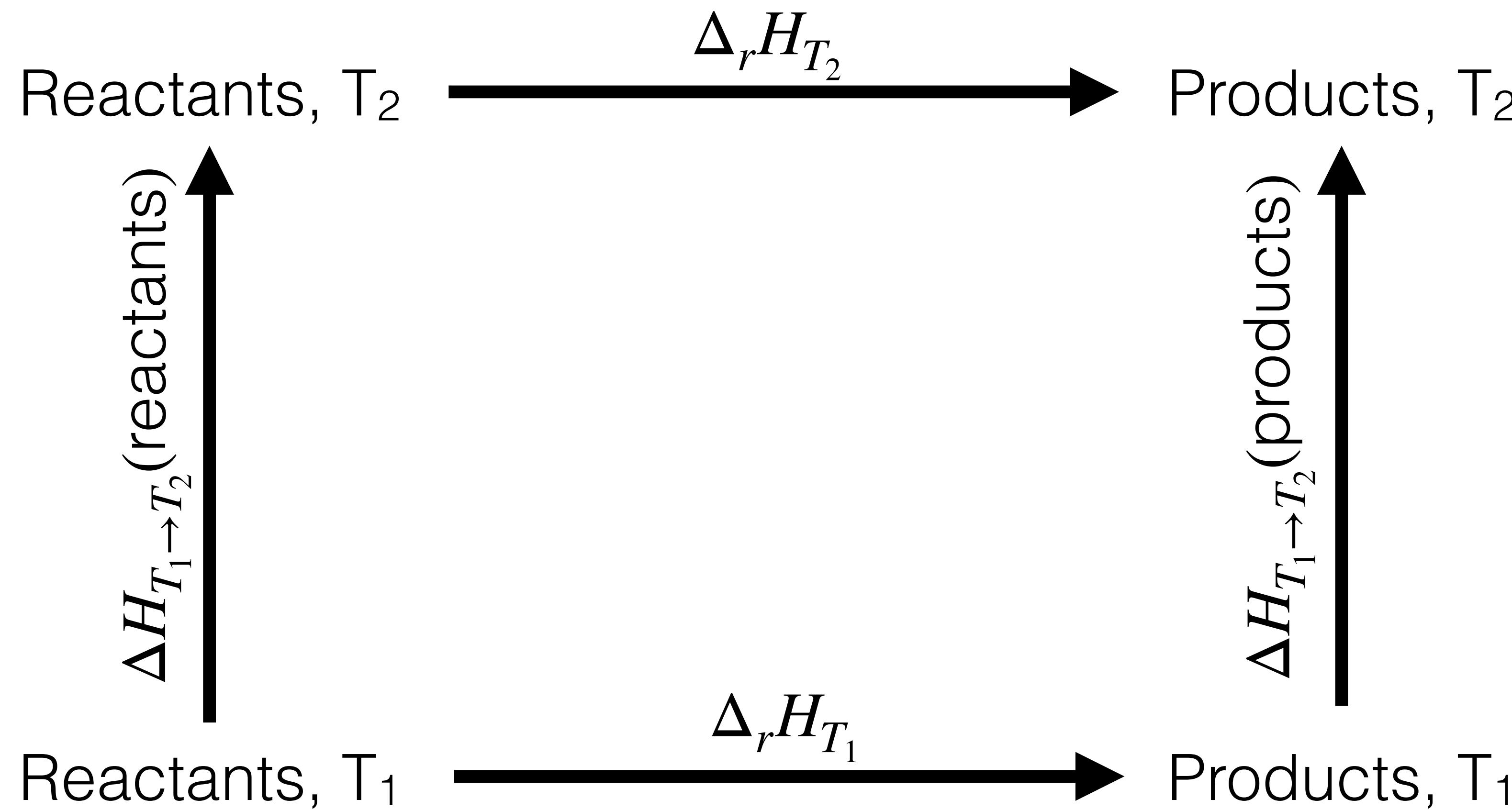
but this is expensive, and only a small number of heroic simulations have been done



Faster ΔG° methods are based on thermodynamic cycles

- Thermodynamic cycles are a series of thermodynamic processes which return to the initial state
 - The total free energy difference around a thermodynamic cycle is zero
 - Differences between free energies (and other thermodynamics state functions) may be determined *indirectly*

Exercise: given this thermodynamic cycle, write an expression for $\Delta_r H_{T_2}$ in terms of $\Delta_r H_{T_1}$, $\Delta_r H_{T_1 \rightarrow T_2(\text{reactants})}$, and $\Delta_r H_{T_1 \rightarrow T_2(\text{products})}$.

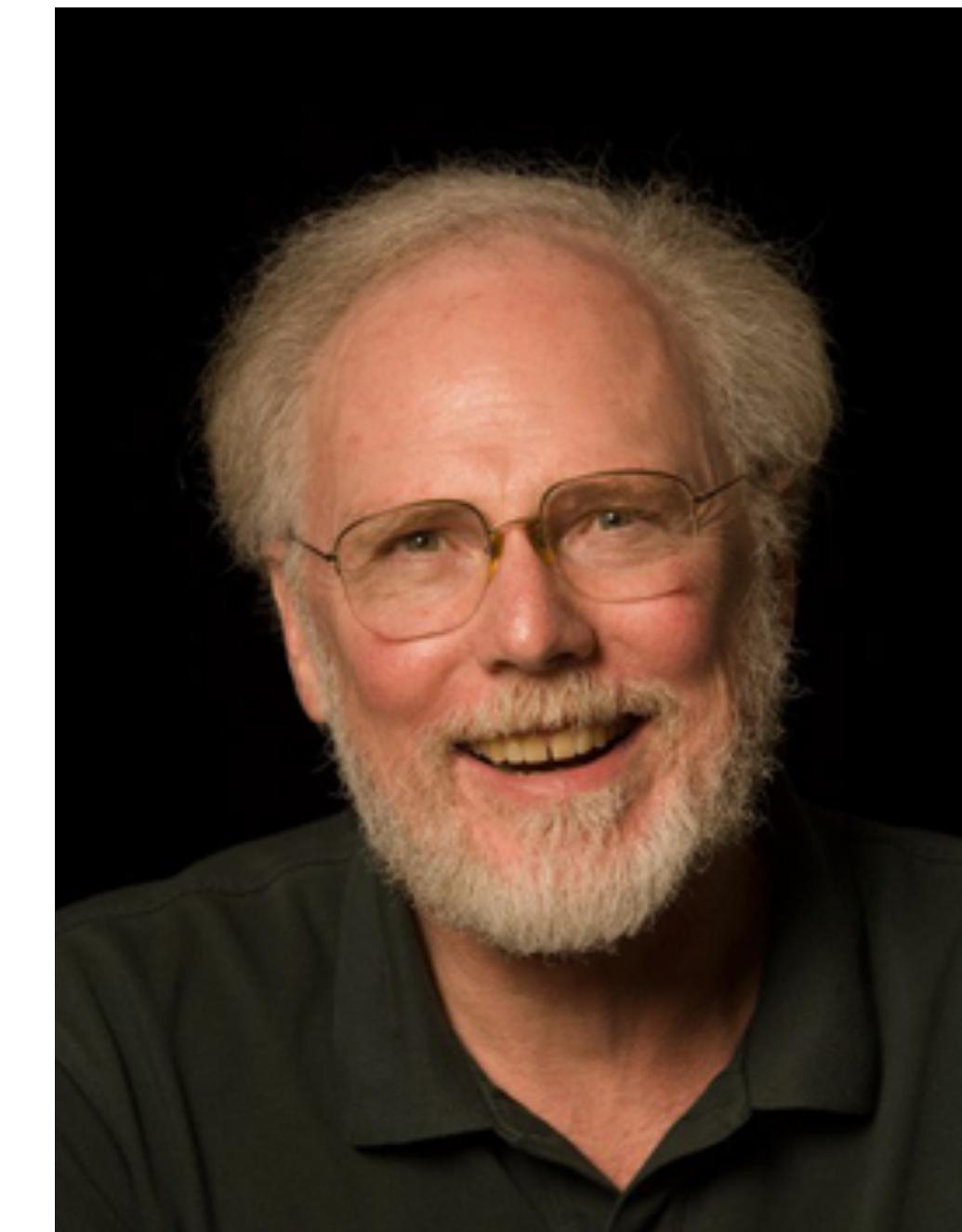


$$\Delta_r H_{T_2} = -\Delta H_{T_1 \rightarrow T_2(\text{reactants})} + \Delta_r H_{T_1} + \Delta H_{T_1 \rightarrow T_2(\text{products})}$$



Autobiography of J. Andrew McCammon

My focus on basic research expanded in the early 1980s when the wife of a close colleague developed cancer. I became very interested in the possible use of molecular dynamics simulations for drug discovery. How could one calculate relevant thermodynamic quantities, such as the free energy of binding an inhibitor to an enzyme? An answer came during another CECAM meeting in Orsay, in July 1983. Herman Berendsen was describing the use of thermodynamic perturbation theory to calculate the change in the free energy of spherical cavities in water with increasing cavity radius. It was a warm afternoon; my mind was wandering a bit. However, suddenly I visualized the use of thermodynamic perturbation theory to calculate the change in free energy of an enzyme–inhibitor complex, when the inhibitor is changed into a slightly different molecule while bound to the enzyme. With a corresponding calculation for the inhibitors in water, one could use thermodynamic cycle arguments to compute the relative binding strengths of the inhibitors—something that should be useful in drug discovery. Bhalu Tembe, a postdoc,



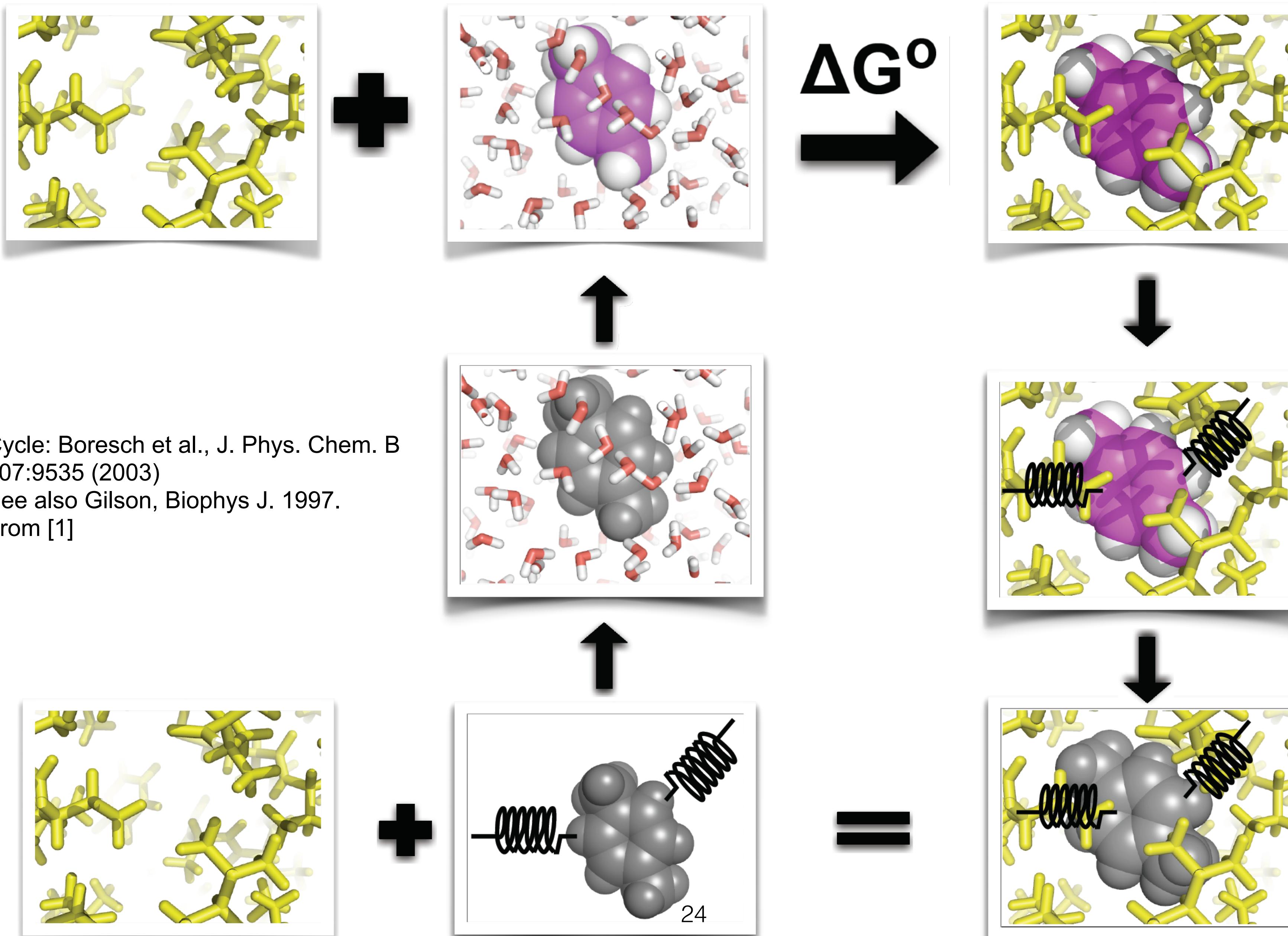
Special Issue: J. Andrew McCammon Festschrift

Published: August 25, 2016

Thermodynamic cycles in ΔG° calculations

- Most binding free energy calculations use thermodynamic cycles that include *alchemical* processes where intermediate states do not necessarily model a physical system, e.g.
 - a drug lead can be morphed into a similar compound (McCammon's idea)
 - harmonic restraints can be added to keep atoms in a certain position
 - states whose energy is a linear interpolation between states 0 and 1 can be defined as, $U_\lambda(r^N) = (1 - \lambda)U_0(r^N) + \lambda U_1(r^N)$
- *Absolute* ΔG° often use
 - restraints to physically separate the receptor and ligand
 - alchemical transformations to decouple a receptor and ligand
- *Relative* binding free energies, $\Delta\Delta G^\circ$, are usually calculated by transforming one small molecule into another

A Thermodynamic Cycle for Absolute ΔG of Binding



Alchemical methods can accurately calculate small $\Delta\Delta G^\circ$

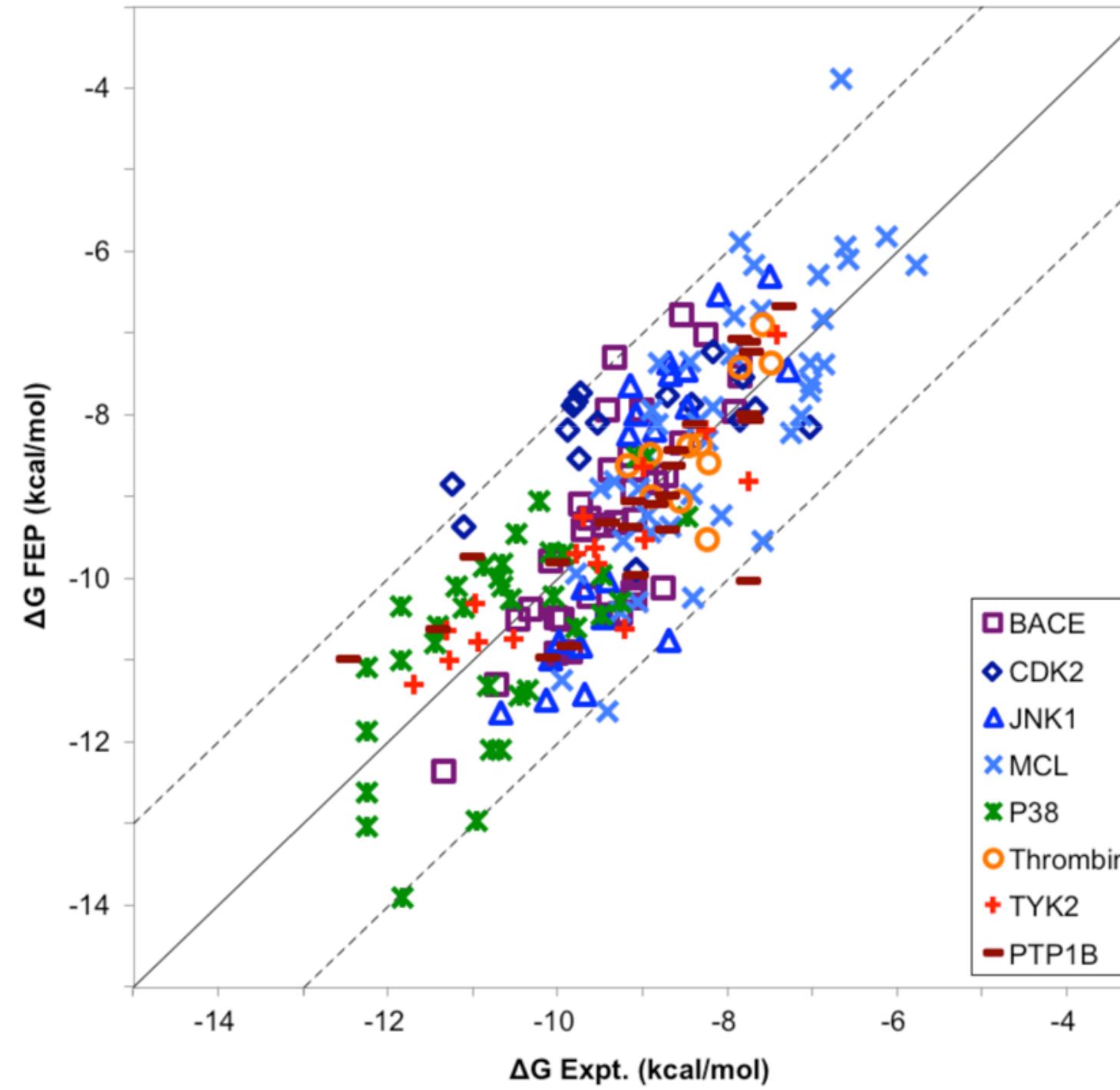


Figure 3. Correlation between FEP-predicted binding free energies and experimental data for all eight systems studied. FEP-predicted binding free energies for most of the ligands are within 1.0 kcal/mol of their experimental values, and only nine of 199 studied ligands deviate from their experimental free energies by more than 2 kcal/mol.

from Wang et al. [Schrodinger], J. Am. Chem. Soc. 137:2695-2703
(2015)

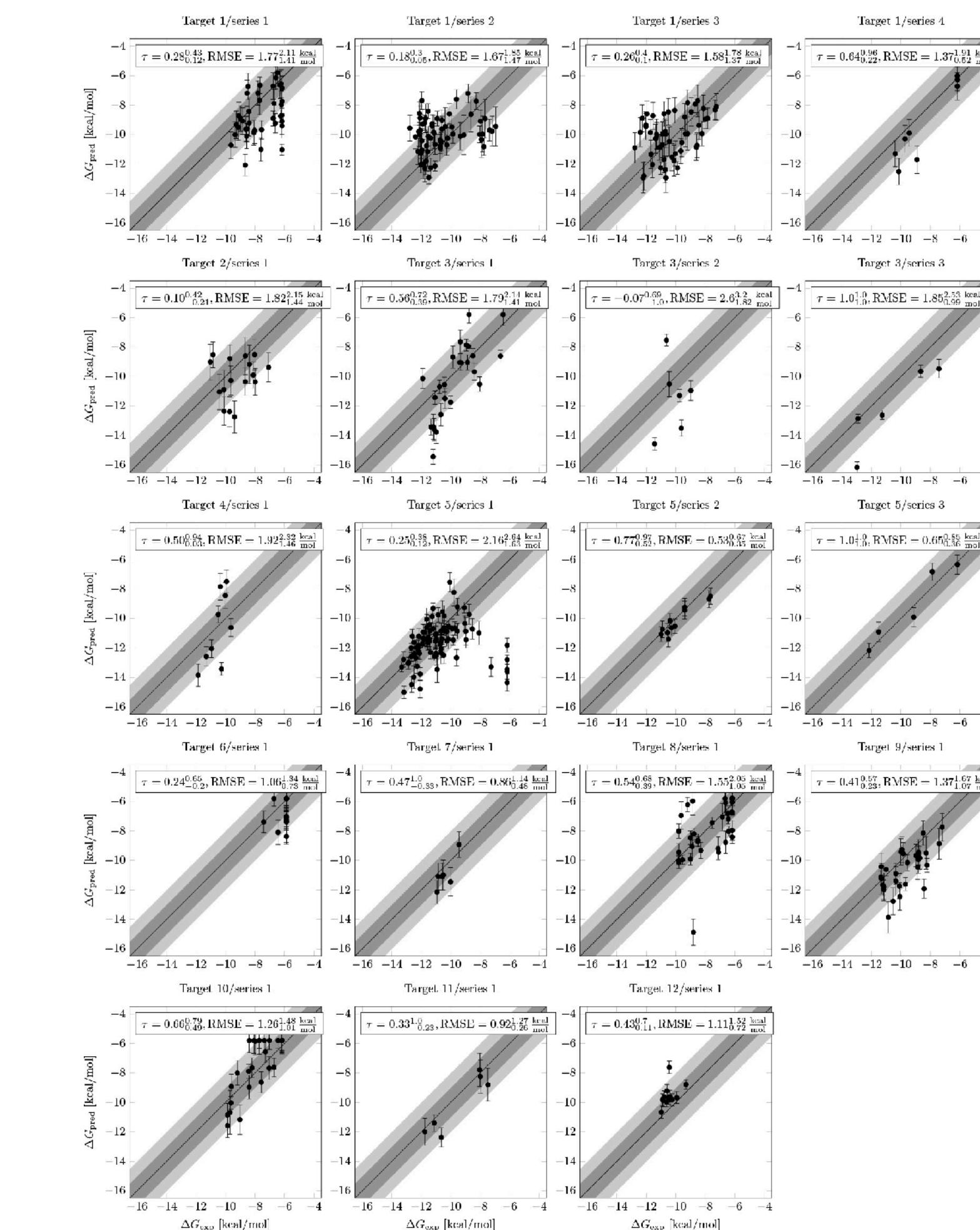


Figure 3. Prospective FEP+ results from 12 targets and 19 chemical series. From Schindler et al. [Merck] J. Chem. Inf. Model. 60:5457-5474 (2020)

Review

- How do binding free energy calculations differ from molecular docking?
- What is a thermodynamic cycle? What types of thermodynamic cycles are used in binding free energy calculations?
- What is an alchemical transformation?

Additional Resources

- Resource for alchemical binding free energy calculations ([http://
www.alchemistry.org/wiki/Main_Page](http://www.alchemistry.org/wiki/Main_Page))