Beyond Affinity:

Predicting Enzyme Kinetics from Computational Models

Maximilian Faissner

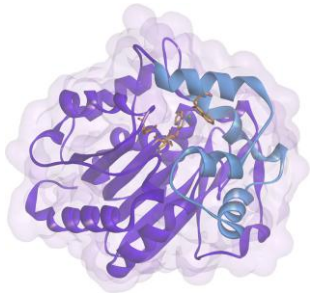
Main Supervisor: **Christoph Flamm** (Institute for Theoretical Chemistry)

In collaboration with:
Shelley Copley University of Colorado, Boulder
Walter Fontana Harvard Medical School

Project Summary

Prediction Input:

- 3d Structures of enzymes



DccA from *Caulobacter crescentus* (5ESR)

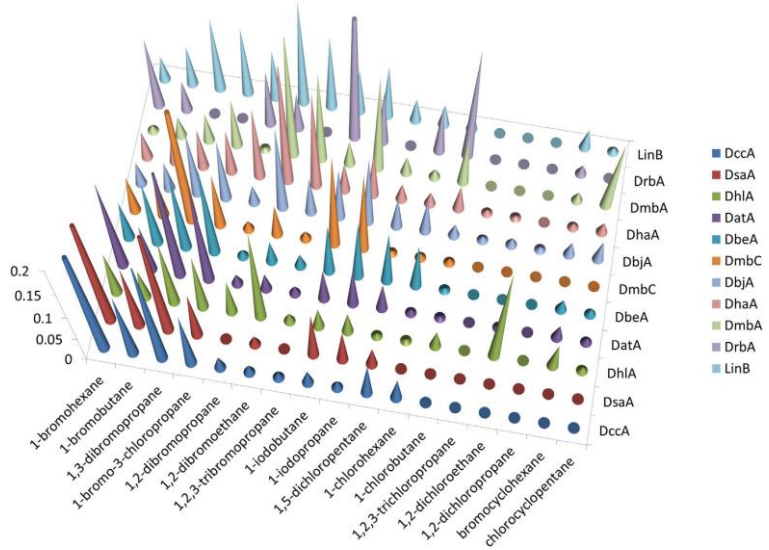
- Reaction Scheme

$$R\text{–}CH_2\text{–}X + H_2O \rightarrow R\text{–}CH_2\text{–}OH + X^-$$

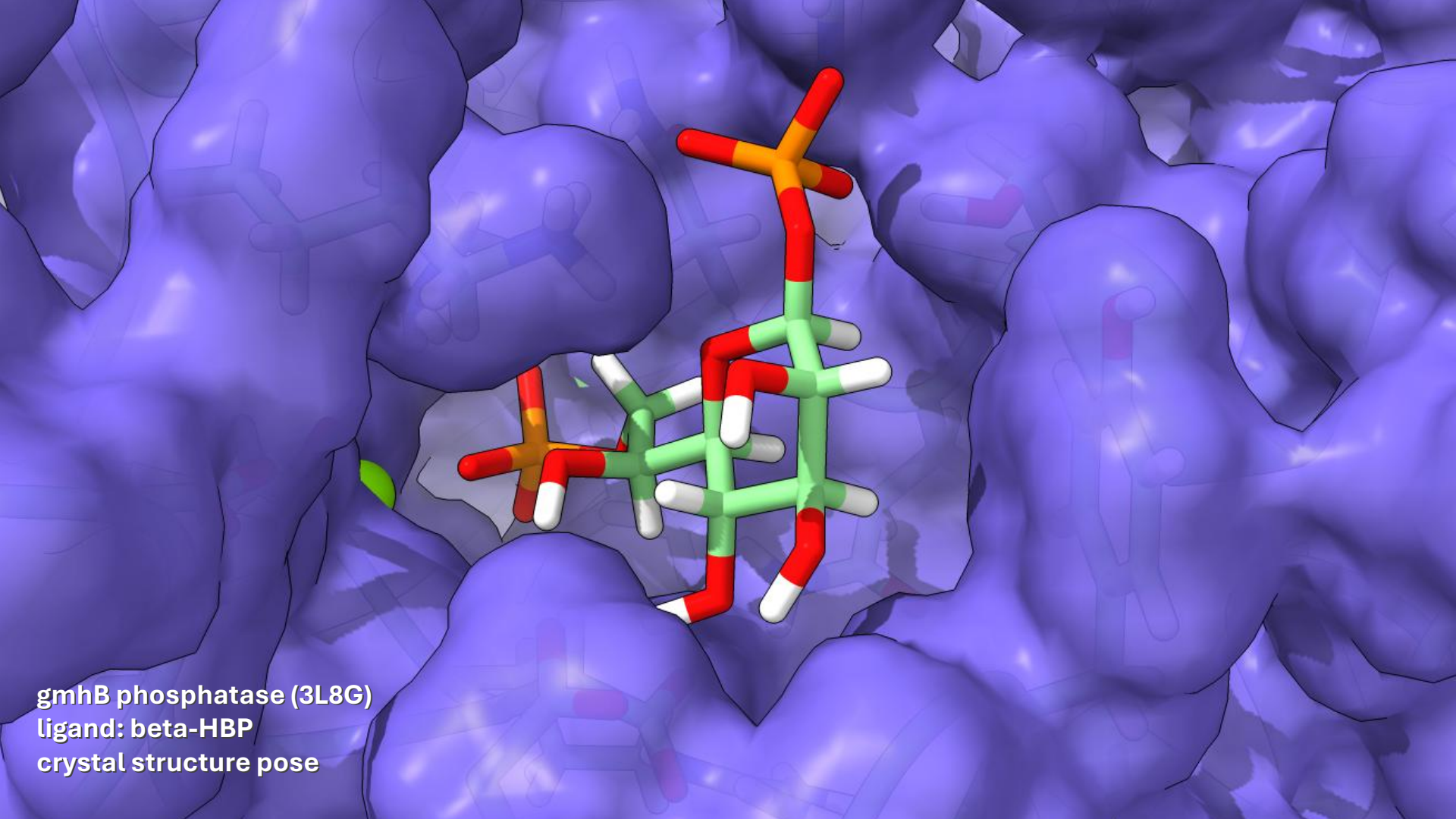
- Substrates of interest (SMILES, 3d-conformers)

Output:

- Kinetic Parameters **enzyme+substrate**
- Specificity Profile (ex: Haloalkane Dehalogenases)



L . Carlucci et al. (2015)


gmhB phosphatase (3L8G)
ligand: beta-HBP
crystal structure pose

Thermodynamic Context of Enzyme Catalysis

Fundamental Concept

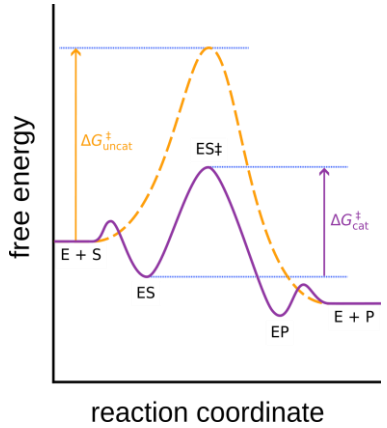
- Enzymes Lower Activation Energy
- Reaction Speed up by transition state stabilization

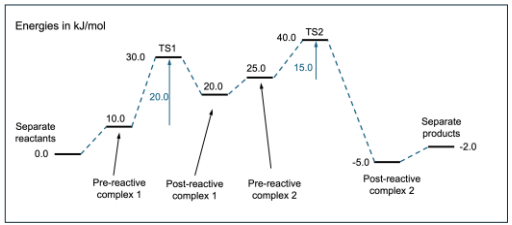
Multiple Energy Barriers

- Substrate Binding
- Catalysis
- Product Release
- Extensions: multi-step reactions, cofactors, …

How do we quantify enzyme efficiency?

Expectation vs. Reality





chemistry.stackexchange.com/…

Enzyme Kinetics - how quickly enzymes process substrates

$k_{cat}/\ K_m$ Catalytic Efficiency

Equilibrium Constants $[M]$:

- $\boldsymbol{K_m}\ (M)$ Substrate concentration at half-max. velocity
- $\boldsymbol{K_d}\ (M)$ Eq. binding affinity

Rate Constants $[s^{-1}]$, $[M^{-1}s^{-1}]$:

- $\boldsymbol{k_{cat}}\ (s^{-1})$ overall turnover
- $\boldsymbol{k_1}$ second-order
- $\boldsymbol{k_2}$ to $\boldsymbol{k_5}$ first-order

$$E + S \rightleftharpoons E_{b,1} \rightleftharpoons E_{b,2} \rightleftharpoons \ldots \rightleftharpoons E_{b,n} \xrightarrow{k_n} E + P$$

$$E + S \underset{k_2\ (k_{\text{off}})}{\overset{k_1\ (k_{\text{on}})}{\rightleftharpoons}} ES \underset{k_4}{\overset{k_3}{\rightleftharpoons}} EP \xrightarrow{k_5} E + P$$

$$K_M = \frac{k_{\text{off}}(k_2) + k_{\text{cat}}}{k_{\text{on}}(k_1)} \qquad k_{\text{cat}} = \frac{k_3 \cdot k_5}{k_3 + k_4 + k_5}$$

K_M (Michaelis constant) vs. K_D

Common interpretation: an approximate measure of enzyme–substrate **affinity**

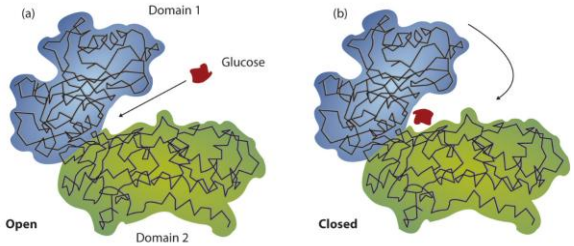
$$K_M = \frac{k_{\text{off}} + k_{\text{cat}}}{k_{\text{on}}} \qquad K_D = \frac{k_{\text{off}}}{k_{\text{on}}}$$

valid only under the minimal one-substrate Michaelis–Menten scheme:

$$\text{If } k_{\text{cat}} \ll k_{\text{off}}, \text{ then } K_M \approx K_D$$

Even with slow turnover:

- conformational gating (domain-level movements)
- partially reversible product-formation steps



Affinity Predictions K_D

Setup

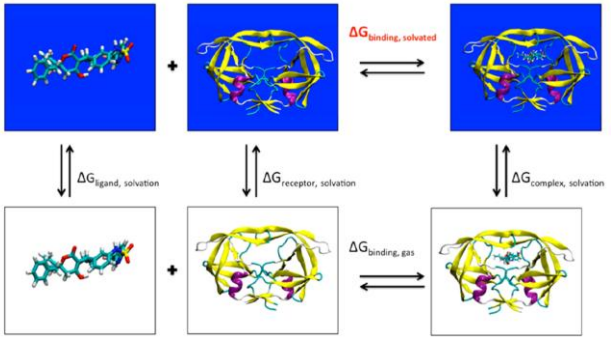
- All-atom force field (e.g. Amber/GAFF) for enzyme + substrate
- ns-scale MD simulations in explicit solvent
- Compute snapshots from equilibrated trajectories

Common Option: ΔG via MM-PBSA/GBSA:

- Formula:

$$\Delta G_{\text{bind,solv}} = G_{\text{complex,solv}} - G_{\text{protein,solv}} - G_{\text{ligand}}$$

$$G = E_{\text{bnd}} + E_{\text{el}} + E_{\text{vdW}} + \Delta G_{\text{pol}} + \Delta G_{\text{np}} - TS_{\text{solute}}$$



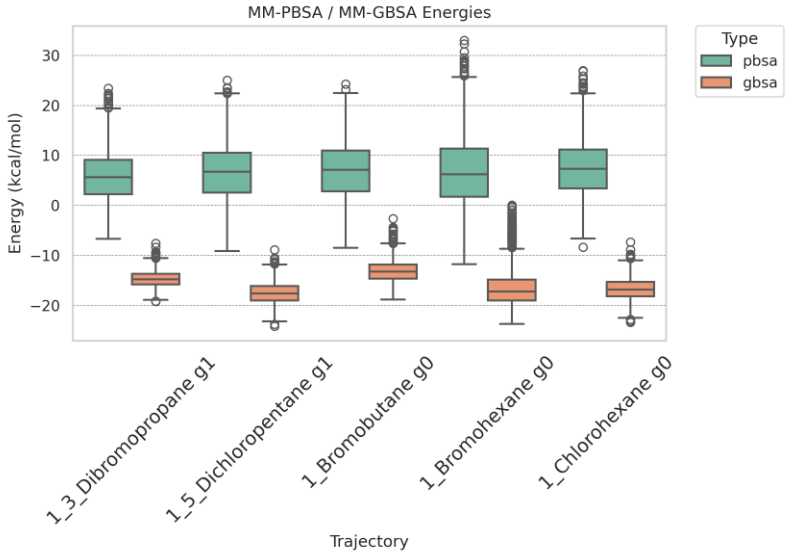
Caveats / Limits:

- Representative conformations required
- Short Timescale (domain level movements!)
- ns-scale MD simulations in explicit solvent

Affinity Predictions K_D - Results (DccA)

Example: DccA (Haloalkane Dehalogenase)

$$R\text{–}CH_2\text{–}X + H_2O \rightarrow R\text{–}CH_2\text{–}OH + X^-$$



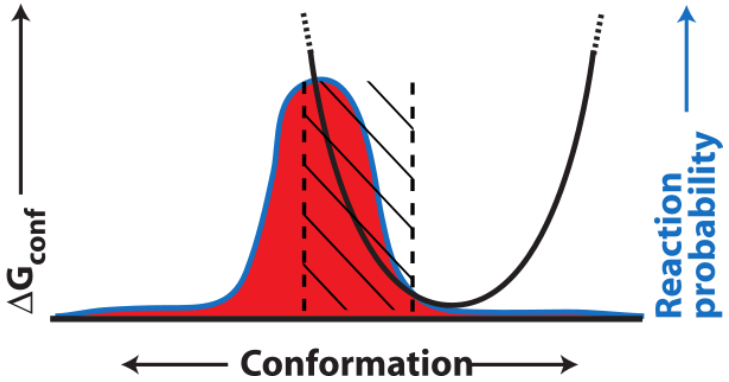
Substrate	k_{cat} (s^{-1})	K_m (mM)	k_{cat}/K_m ($M^{-1}s^{-1}$)
1-bromohexane	23.6 ± 0.8	1.1 ± 0.1	$21{,}000 \pm 2000$
1-bromobutane	13.0 ± 0.4	6.6 ± 0.6	2000 ± 200
1,3-dibromopropane	29 ± 1	7.2 ± 0.8	4000 ± 500
1-chlorohexane	3.1 ± 0.1	1.2 ± 0.1	2600 ± 200
1,5-dichloropentane	5.0 ± 0.1	1.2 ± 0.1	4200 ± 400

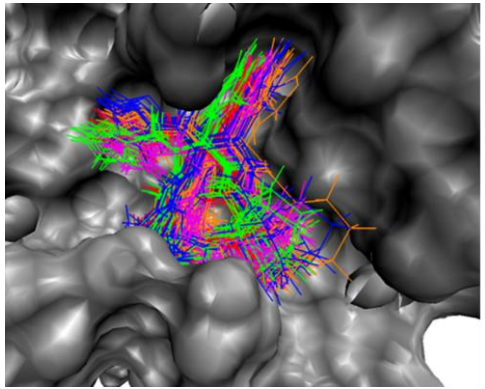
Bridge between Structure and Energetics

(a) Ground-state conformation is unlikely to be most reactive (if at all?)

(b) higher-energy “rare” states often dominate catalysis
→ better alignment with the transition state

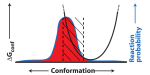
Established computational methods: QM/MM simulations + sampling to estimate energy landscape + transition states





Kraut D. A. et al. (2003)

How can we predict k_{cat}?



$$k_{\text{cat}} \sim \underbrace{P(\text{reactive conformation})}_{\text{fraction of time}} \times \underbrace{k_{\text{chem}}}_{\substack{\text{Catalytic step rate} \\ \text{(once in the reactive geometry)}}}$$

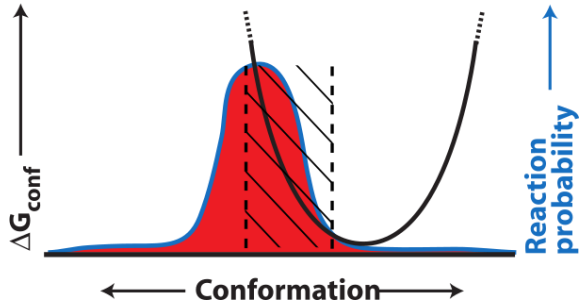
When can you ignore the k_{chem} differences?

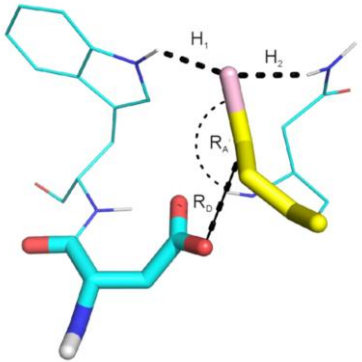
Practical Example:

Can we predict if k_{cat} of … is lower/higher than 23.6 by only considering P?

1-Bromohexane	1-Bromobutane	1,3-Dibromopropane	1-Chlorohexane
			
$k_{cat} = 23.6\ /s$	?	?	?

P(reactive conformation) estimation





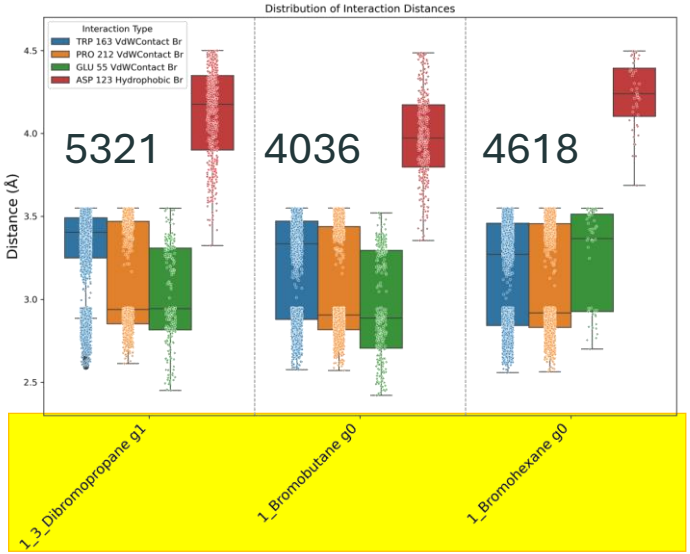
How to estimate the reactive fraction (red part)?

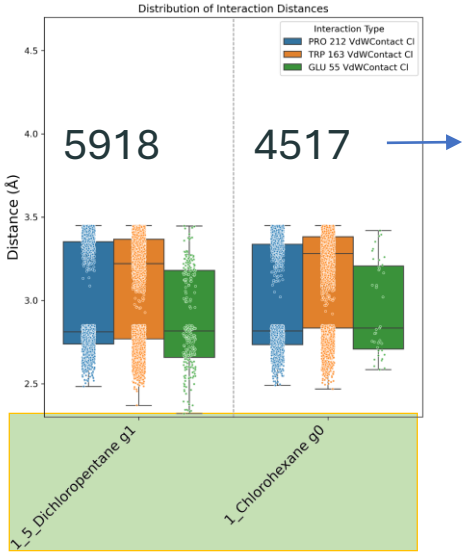
- Define close/relevant amino acid residues (~ catalytic triad)
- Define reactive substrate atoms
- Generate interaction fingerprints along MD trajectories
- Compute “Reactive Uptime”

MD Setup

- Same setup as affinity predictions
  - All-atom force field
  - explicit solvent

Molecular Interaction Footprint Results





total interaction frames (out of 100k)

Cleaving a C–Cl bond requires more energy!

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Project Outlook

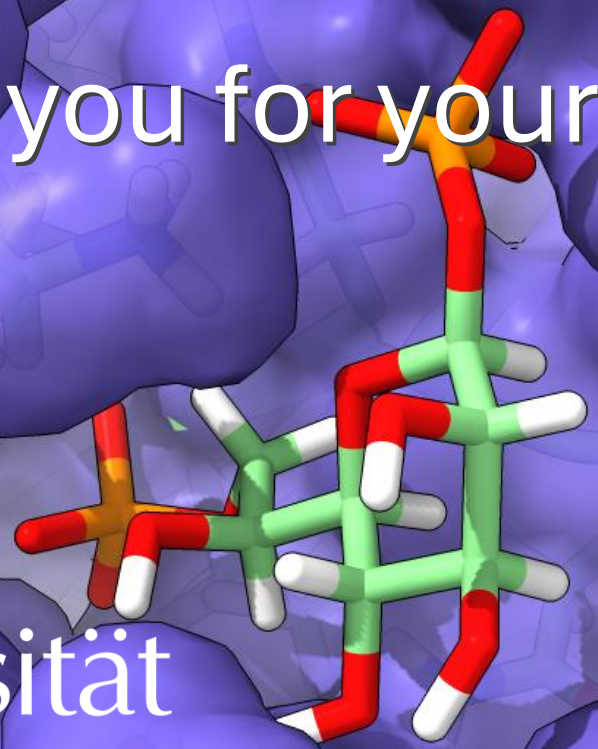
Short Term Goals

- Extend simulations to additional enzyme classes
    - dehydrogenases, kinases
- Accelerated MD
    - Solution to time scale limitations?
    - Better energy landscape exploration?

Long Term Goals

- QM/MM?

Thank you for your attention!



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RCSB ID: 1RMT
AphA phosphatase
complexed with adenosine, Mg^{2+}