USI Lugano

PLUMED Masterclass 2022

31 January 2022

Ligand Binding Free-Energy Calculations with Funnel-Metadynamics

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di
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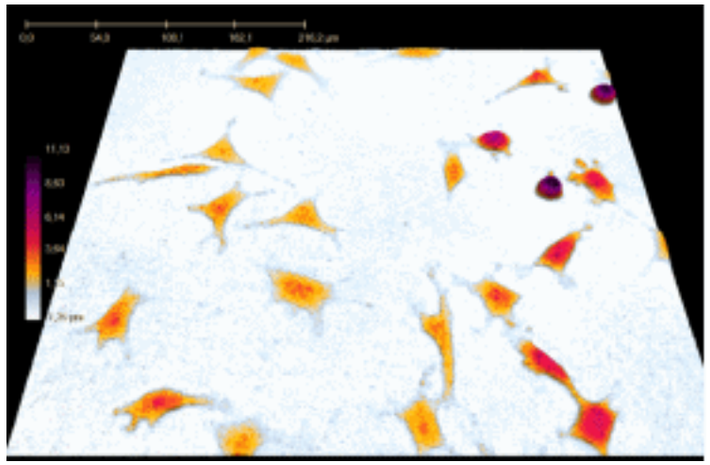
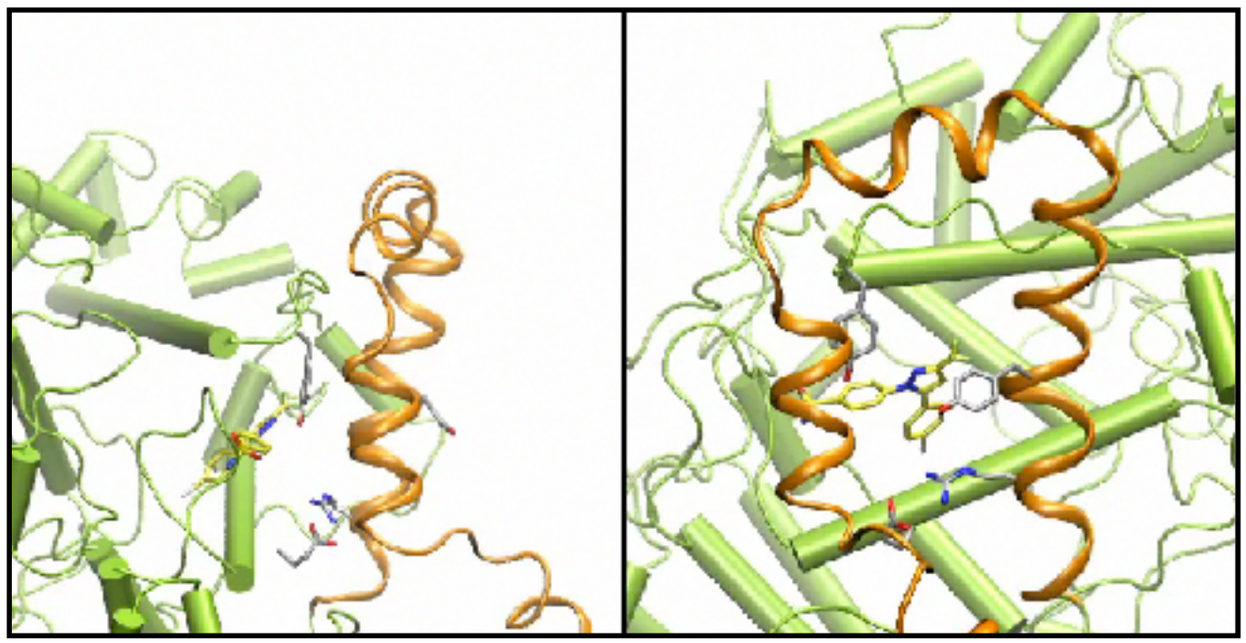
Life is Dynamics



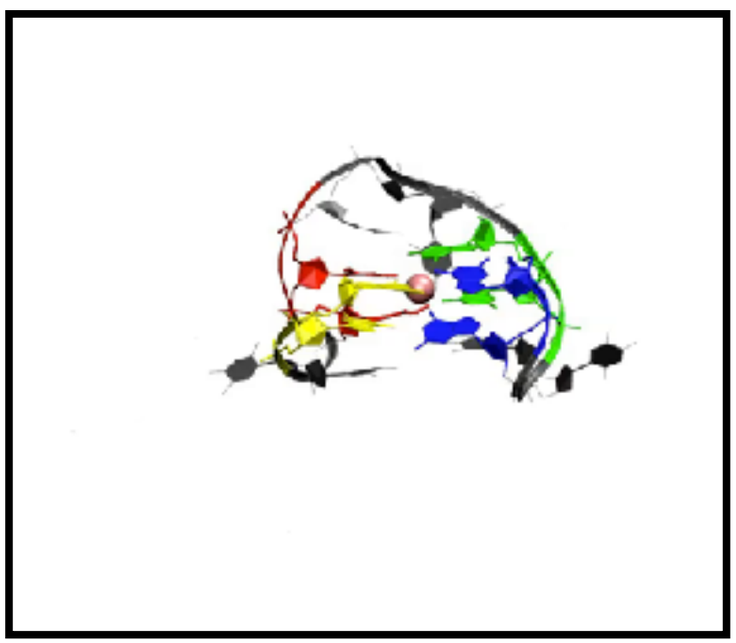
Source: wikimedia commons

…including proteins and DNA

Limongelli et al., *PNAS* (2010)



Limongelli et al., *Angew. Chem. Int. Ed.* (2013)

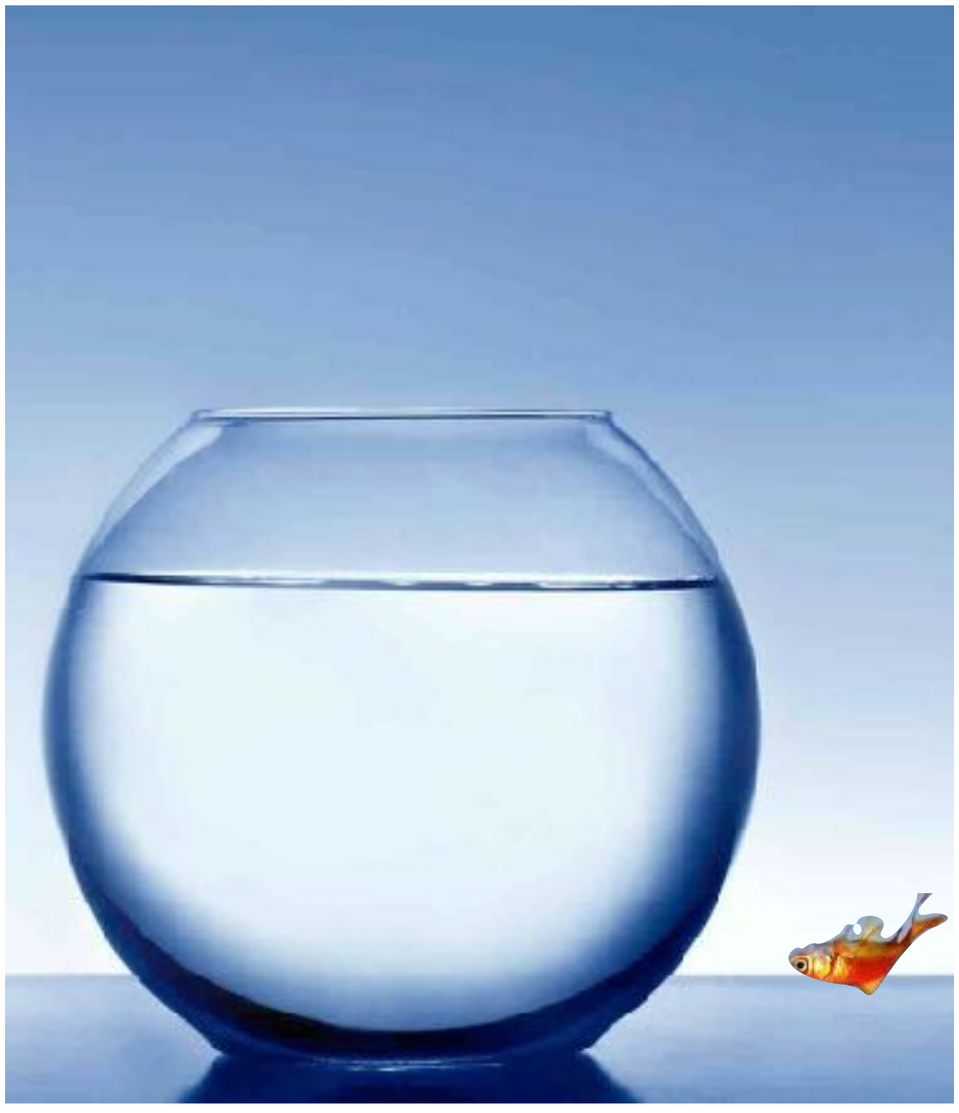


Life is Dynamics

What if we neglect dynamics?

:)

Life is Dynamics

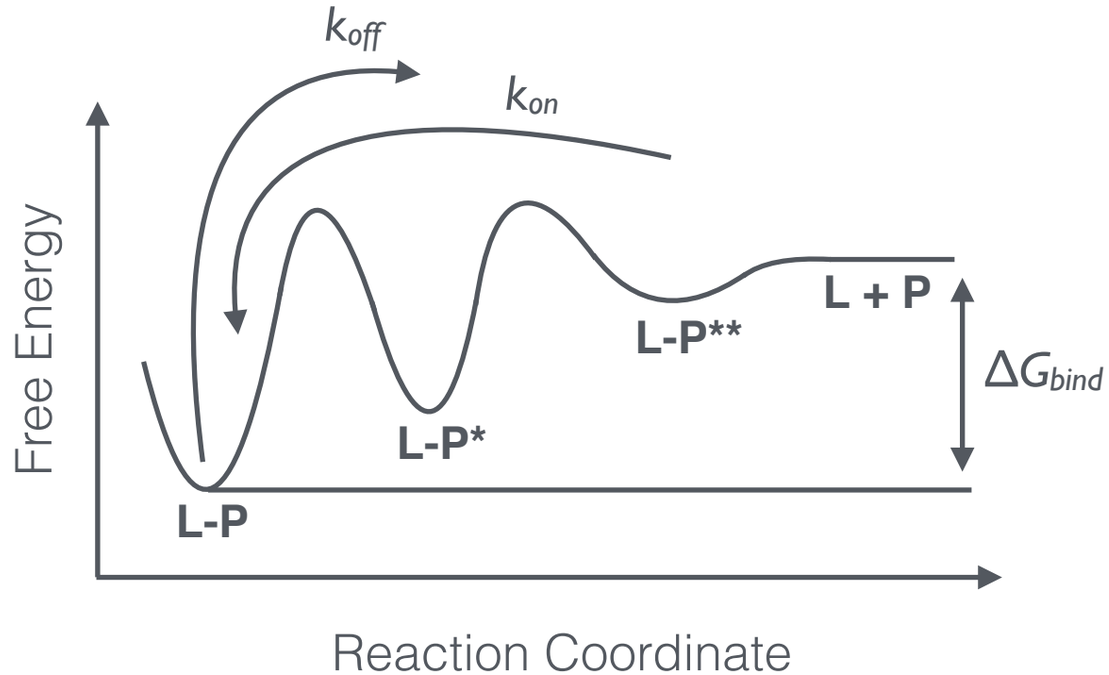


:(

Molecular Binding

$$[L] + [P] \underset{k_{on}}{\overset{k_{off}}{\rightleftharpoons}} [LP]$$

$$K_b = \frac{[LP]}{[L]\,[P]} \qquad K_b = \frac{k_{on}}{k_{off}}$$



✓ K_b can be measured by experiments

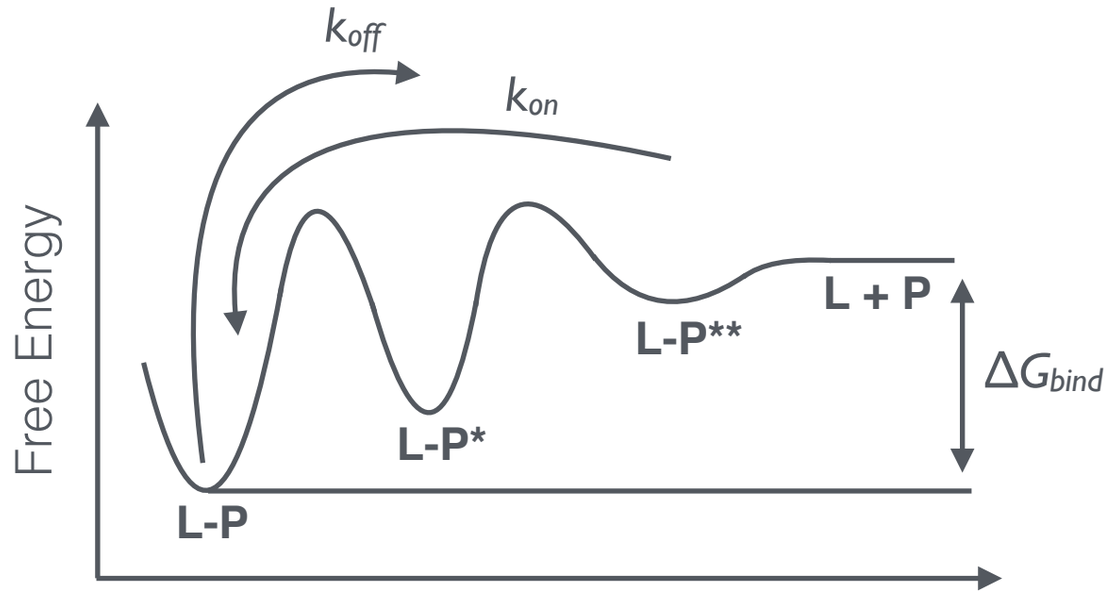
✓ K_b is correlated to ΔG_{bind} as follows:

$$^{*}\Delta G_{bind} = G_{[LP]} - G_{[L][P]} = -k_b T \ln K_b$$

* in case of competitive inhibitors

Limongelli *WIREs Comput. Mol. Sci.* (2020)
Gilson, Zhou. *Annu Rev Biophys Biomol Struct* (2007)

Molecular Binding



$$\Delta G_{bind} = G_{[LP]} - G_{[L][P]} = -k_b T \ln K_b$$

$$\Delta G^0 = \mu_{LP} - \mu_L - \mu_P = -k_b T ln \left(\frac{C^0}{8\pi^2} \frac{\int e^{-(U_{(rLP)} + S_{(rLP)}/k_b T)} dr_{(LP)}}{(\int e^{-(U_{(rL)} + S_{(rL)}/k_b T)} dr_{(L)})(\int e^{-(U_{(rP)} + S_{(rP)}/k_b T)} dr_{(P)})} \right)$$

Limongelli *WIREs Comput. Mol. Sci.* (2020)
Gilson, Zhou. *Annu Rev Biophys Biomol Struct* (2007)

Computational Methods

General Rule

*The accuracy is **always** traded for the speed*

Fast Methods → Molecular Docking

Accurate Methods → Molecular Dynamics, Monte Carlo

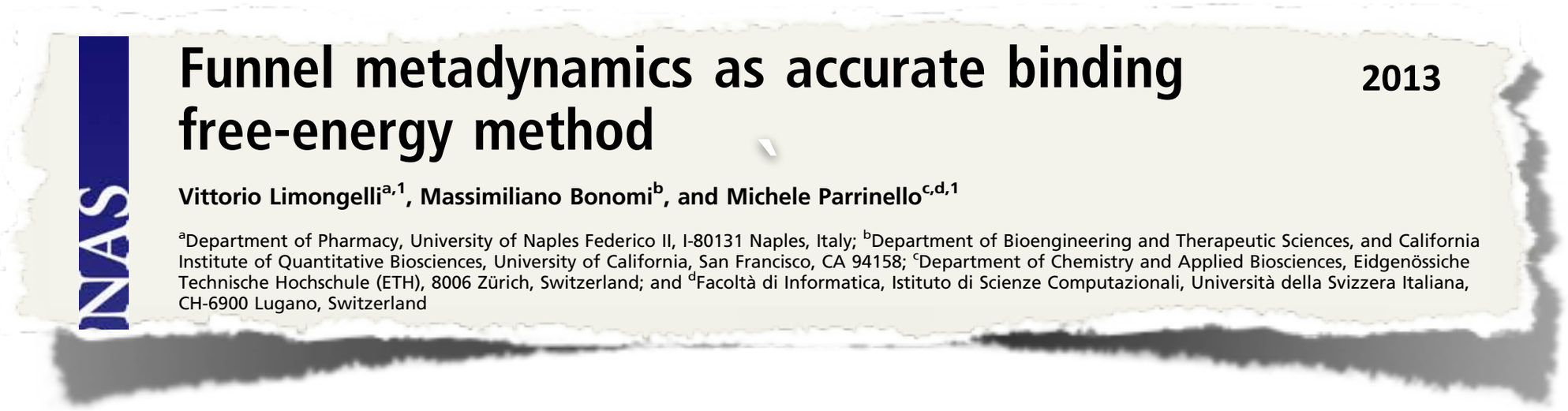
- End Point Methods: LIE - MMPBSA
- Pathway Free Energy Methods: FEP - TI - Umbrella Sampling - ***Funnel-Metadynamics (FM)***

Limongelli *WIREs Comput. Mol. Sci.* (2020)
Gilson, Zhou. *Annu Rev Biophys Biomol Struct* (2007)

Outline of the Class

✓Funnel-Metadynamics (FM)

- Ligand/Protein binding free-energy
- Ligand/Protein binding kinetics
- Funnel-Metadynamics Automated Protocol (FMAP)



PNAS

Funnel metadynamics as accurate binding free-energy method 2013

Vittorio Limongelli[a,1], Massimiliano Bonomi[b], and Michele Parrinello[c,d,1]

[a]Department of Pharmacy, University of Naples Federico II, I-80131 Naples, Italy; [b]Department of Bioengineering and Therapeutic Sciences, and California Institute of Quantitative Biosciences, University of California, San Francisco, CA 94158; [c]Department of Chemistry and Applied Biosciences, Eidgenössiche Technische Hochschule (ETH), 8006 Zürich, Switzerland; and [d]Facoltà di Informatica, Istituto di Scienze Computazionali, Università della Svizzera Italiana, CH-6900 Lugano, Switzerland

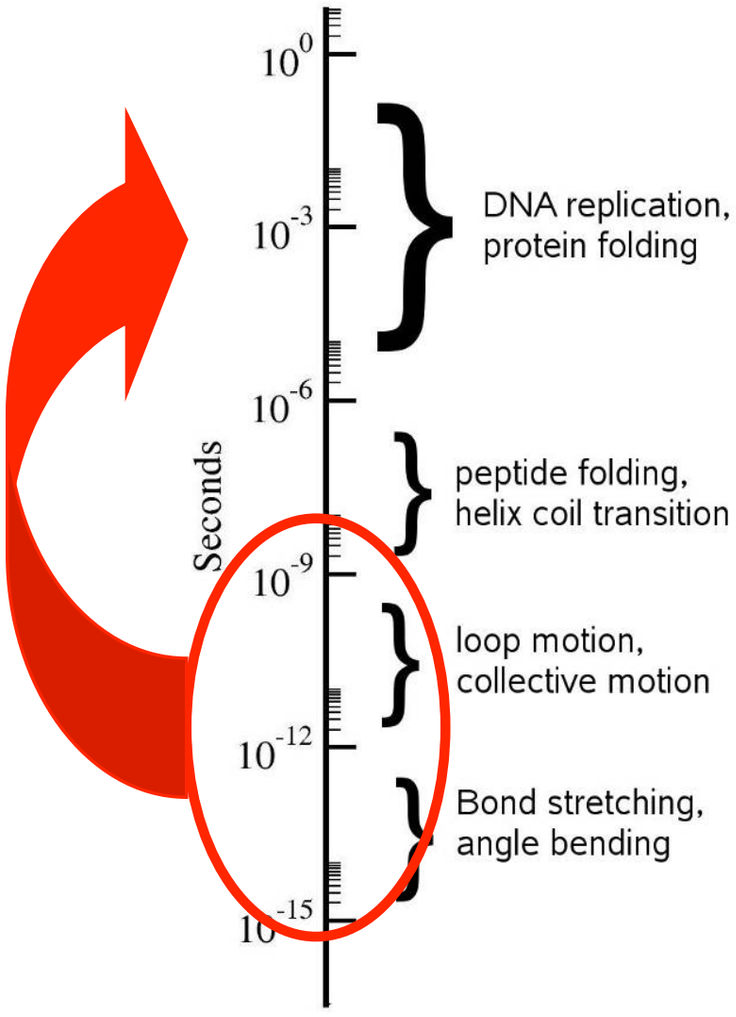
Further Reading:
Raniolo & **Limongelli** *Nature Protocols* (2020)
Limongelli and co. *PNAS* (2017)

Limongelli et al. *PNAS* (2010)
Limongelli et al. *PNAS* (2012)
Grazioso, **Limongelli** et al. *JACS* (2012)

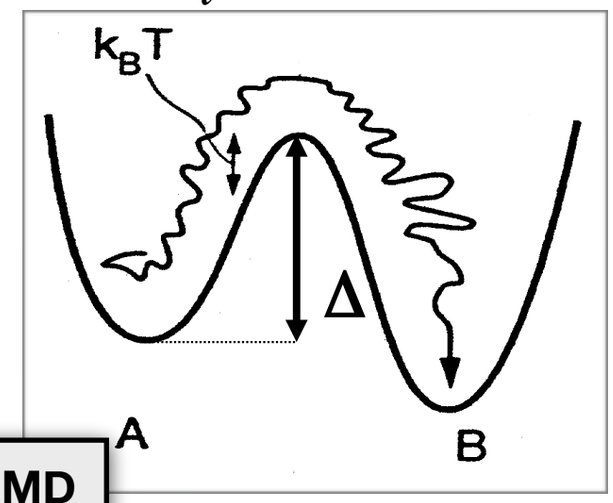
Theory in the Class

- Metadynamics and Well-Tempered Metadynamics
- Collective Variables (CVs)
- Variational Approach to Conformational dynamics (VAC) Metadynamics
- Funnel-Metadynamics (FM)
- Infrequent Metadynamics (IM)
- Path Collective Variables (PCV)
- Multiple Walker Simulations
- Reweigthing the Boltzmann distribution in not-biased CV space

The Time Scale Issue



$$\vec{F} = m\vec{a} \qquad F_i = \frac{\partial E}{\partial x_i}$$



unbound

bound

Classical MD

- MD timestep ~ 1 fs (bond-stretching, bending,...)
- Biologically relevant processes (ligand binding, protein/DNA folding...) > 10^{1} μs

Dimensional Reduction

Many microscopic variables
q [atomic coordinates]

fine-grained description
potential energy:
U(q) Newton dynamics $\vec{F} = m\vec{a}$

Zwanzig *Phys Rev* (1961)

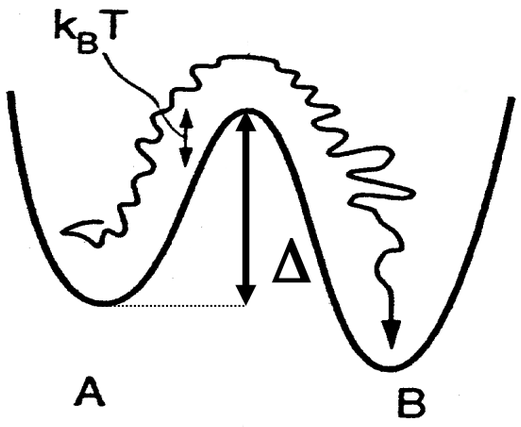
A few collective variables (CVs)
s [distances, angles, ...]

coarse-grained description
free energy:
$F(s)=-k_B T \log \int \exp(-\beta U)\delta(s-s(q))dq$
Langevin dynamics

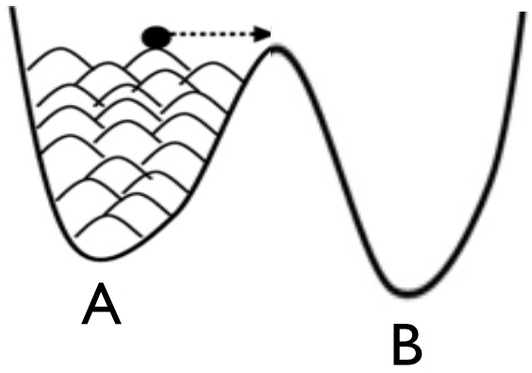
Major advantage:
Analysis of q-space trajectories by means of modeling dynamics in s-space

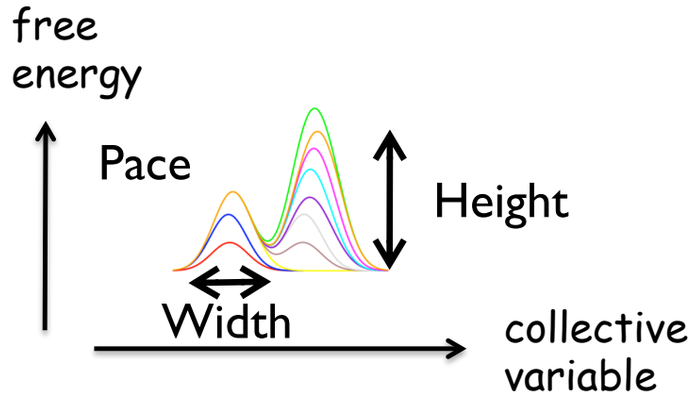
Metadynamics

Classical MD



Metadynamics





Laio and Parrinello, *PNAS* (2002)

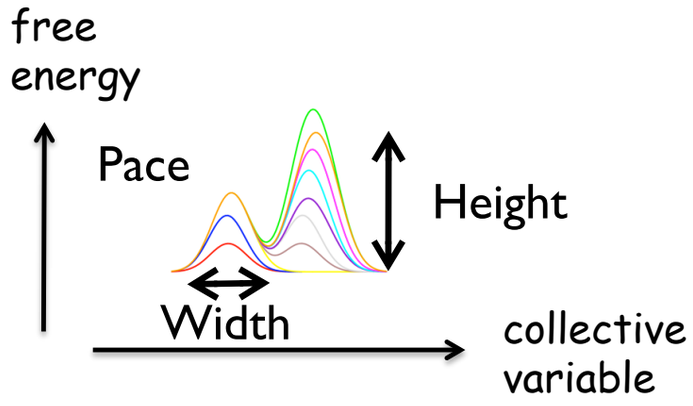
Metadynamics

$$V_G(\boldsymbol{S},t) = \int_0^t dt'\, \omega\, exp\left(-\sum_{i=1}^{d} \frac{(S_i(\boldsymbol{R}) - S_i(\boldsymbol{R}(t')))^2}{2\sigma_i^2}\right)$$

bias rate $\omega = \frac{W}{\tau_G}$ — W: Gaussian height; τ_G: pace

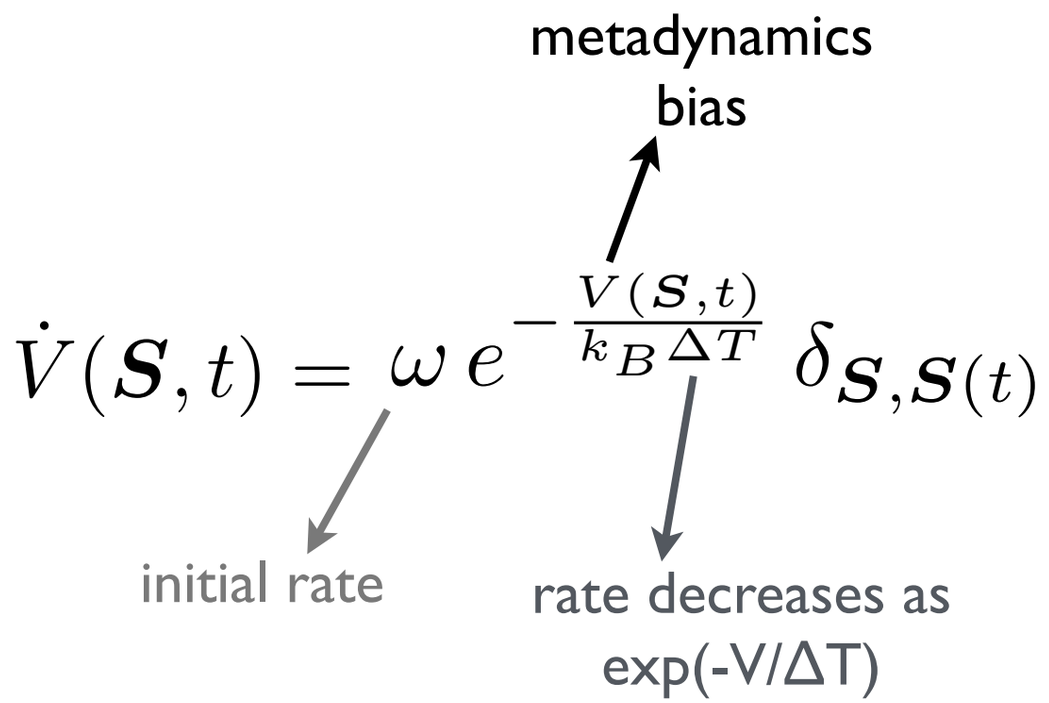
σ_i: Gaussian width

$$V_G(\boldsymbol{S}, t \to \infty) = -F(\boldsymbol{S}) + C$$



Laio and Parrinello *PNAS* (2002)
Bussi, Laio, Parrinello *PRL* (2006)

Well-Tempered Metadynamics



$$F(s,t) = -\frac{T+\Delta T}{\Delta T}\, V(s,t)$$

Barducci, Bussi, Parrinello *PRL* (2008)
Dama, Parrinello, Voth *PRL* (2014)

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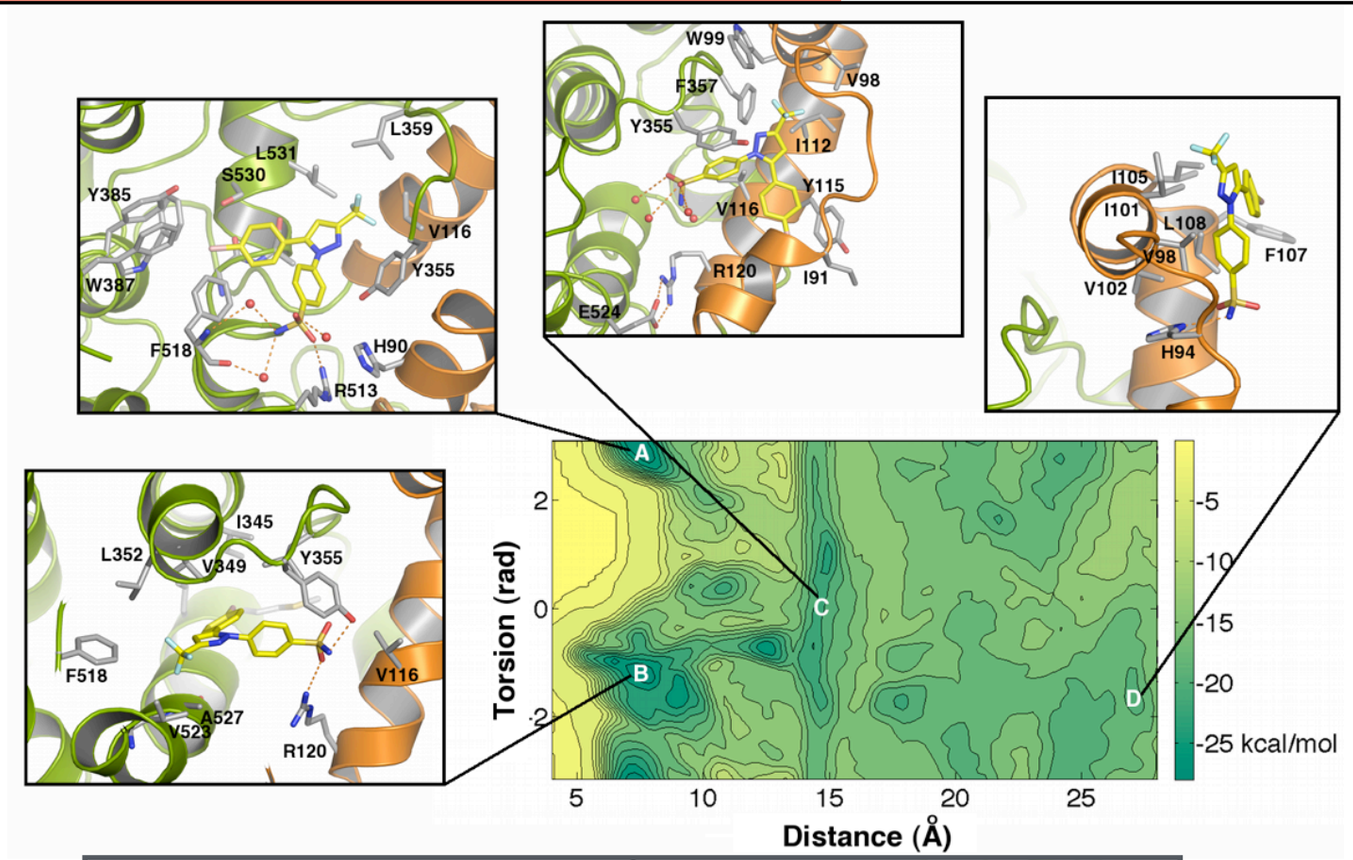
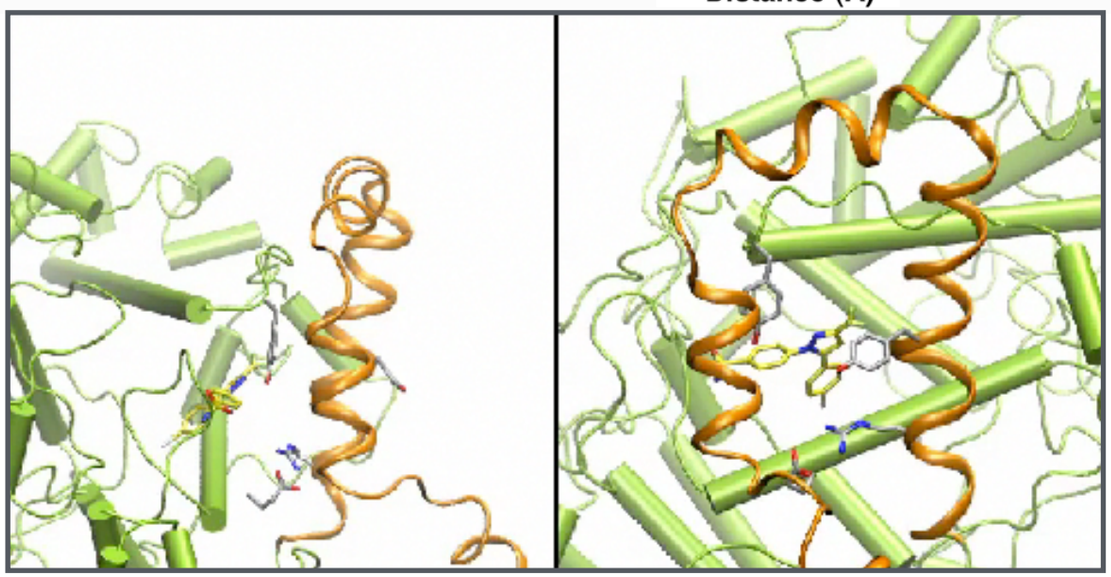
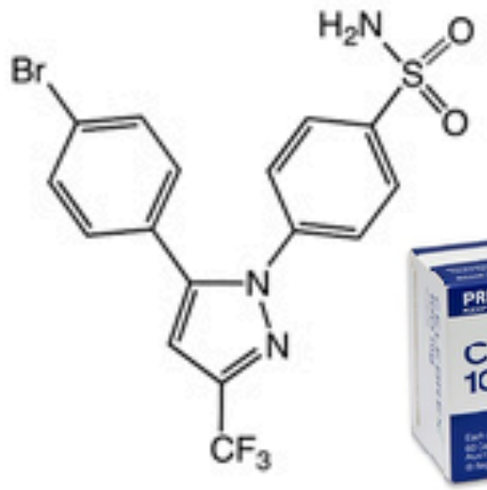
NSAIDs Mechanism of Action







SC-558 activities:

IC_{50}(COX-1) = 17.7 μM

IC_{50}(COX-2) = 9.3 nM

Model for COX-2 Diarylheterocycle Binding

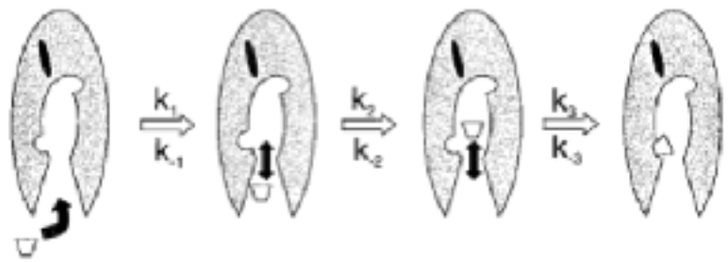
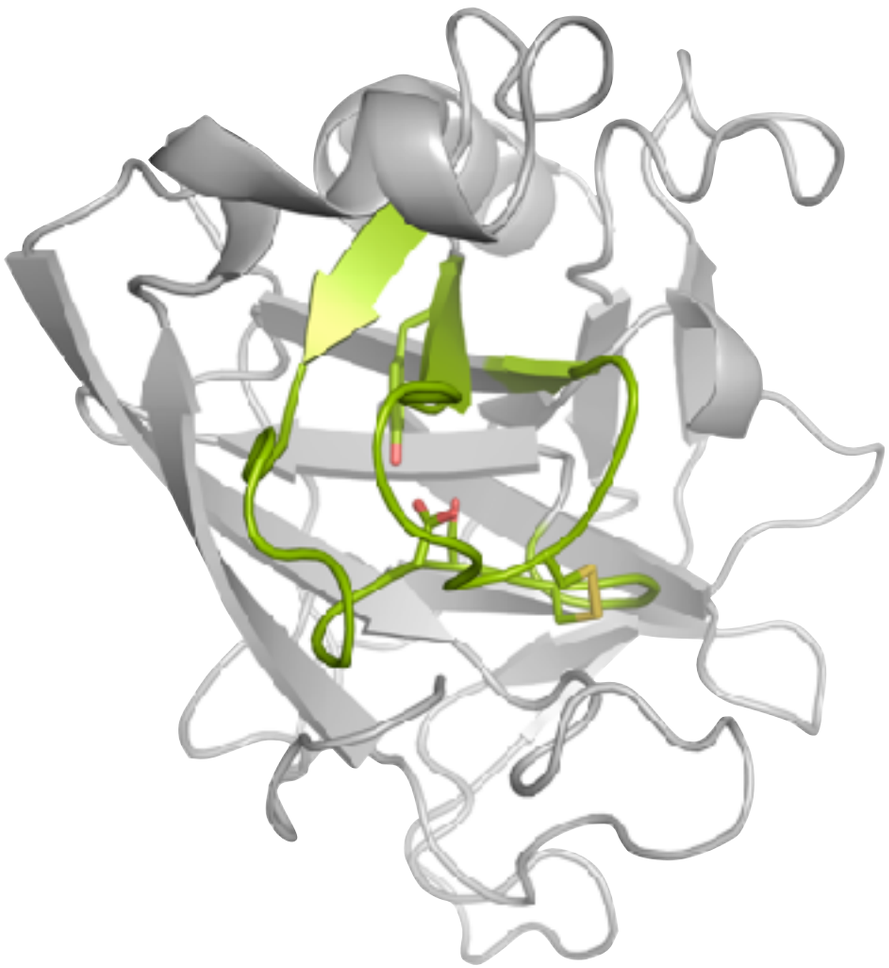


FIGURE 7: Three-step model of SC299 binding to COX-2. SC299 binds in the lobby region in the first step, moves past the constriction comprised of Arg120, Tyr355, and Glu524 in the second step, and then inserts into the side pocket bordered by Val523 in the third step. Insertion into the side pocket accounts for the stability of SC299 binding and the stability of inhibition.

Lanzo et al., Biochemistry (2000)

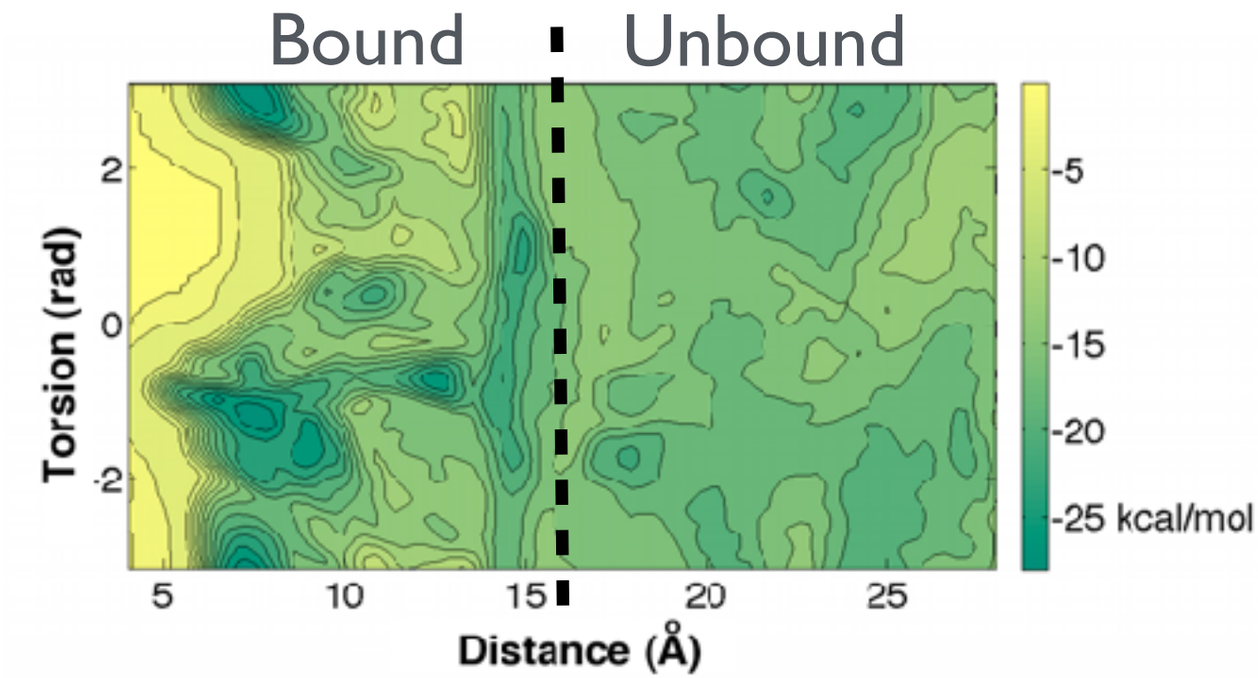
Limongelli et al. *PNAS* (2010)

Protein/Ligand Binding



$$\Delta G_b^0 = -\frac{1}{\beta} ln(C^0 \ K_b)$$

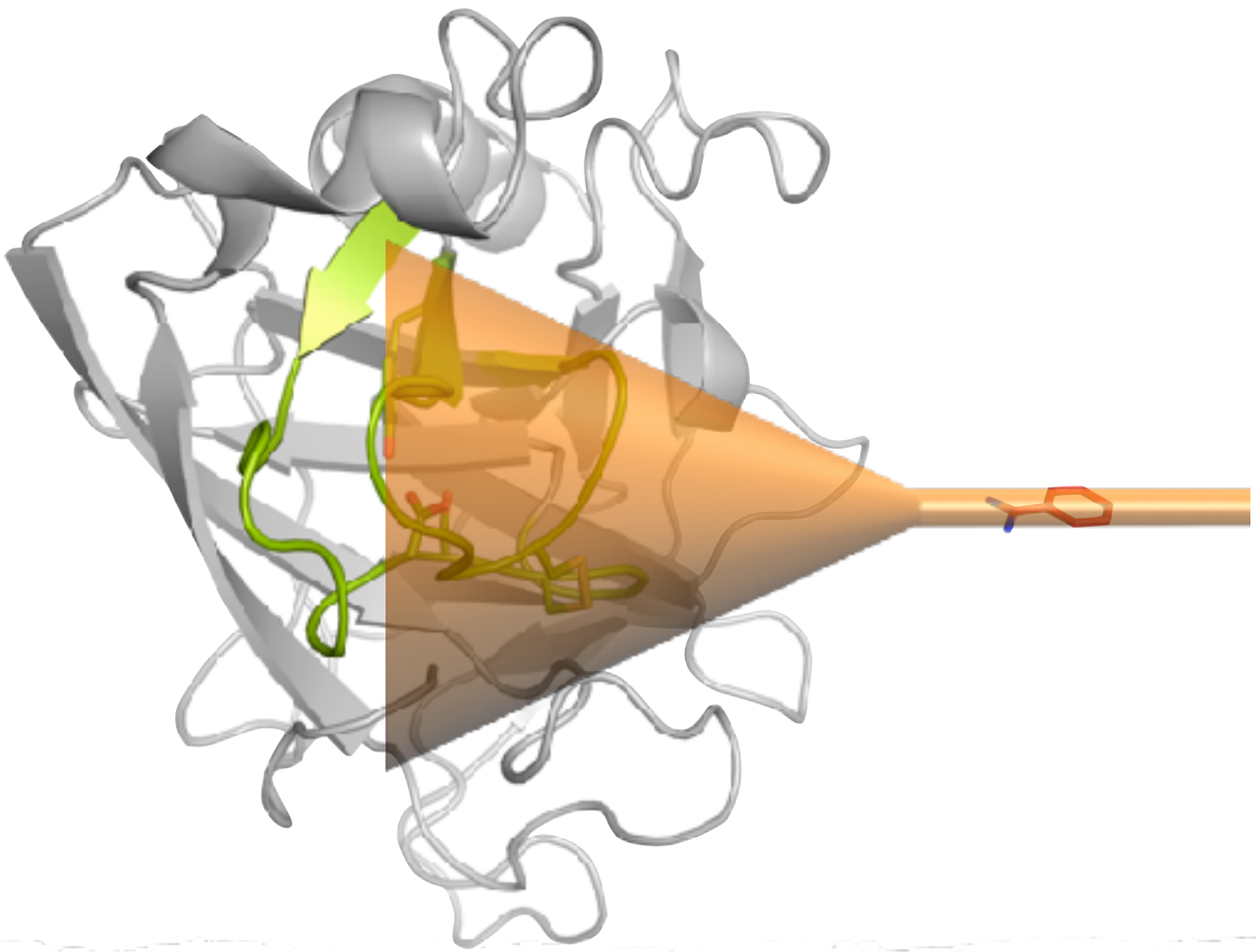
C^0 = Standard Concentration (constant)

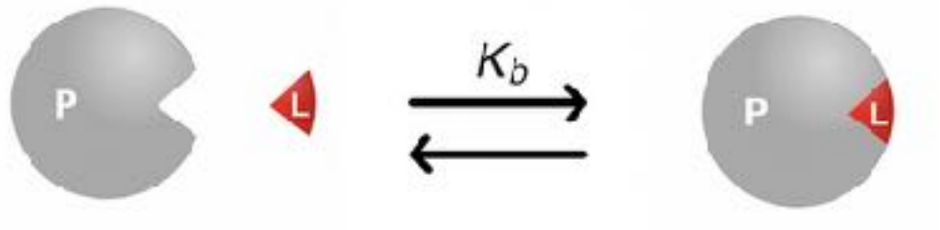


Problems in converging the free energy in the unbound region

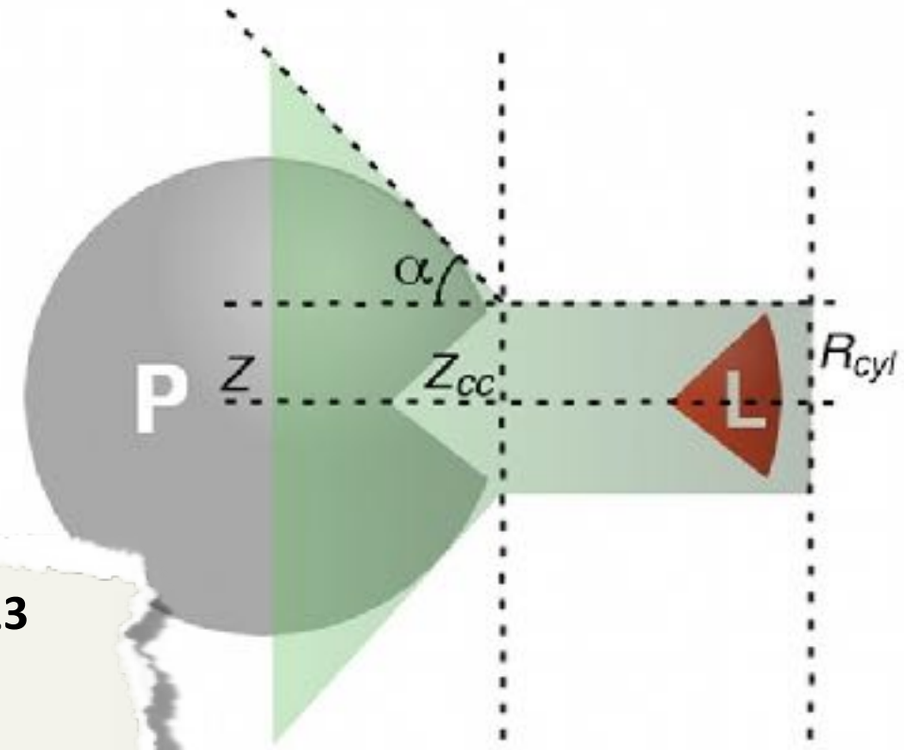
Limongelli et al. *PNAS* (2010)

Protein/Ligand Binding





$$\Delta G_b^0 = -\frac{1}{\beta} ln(C^0 \ K_b)$$

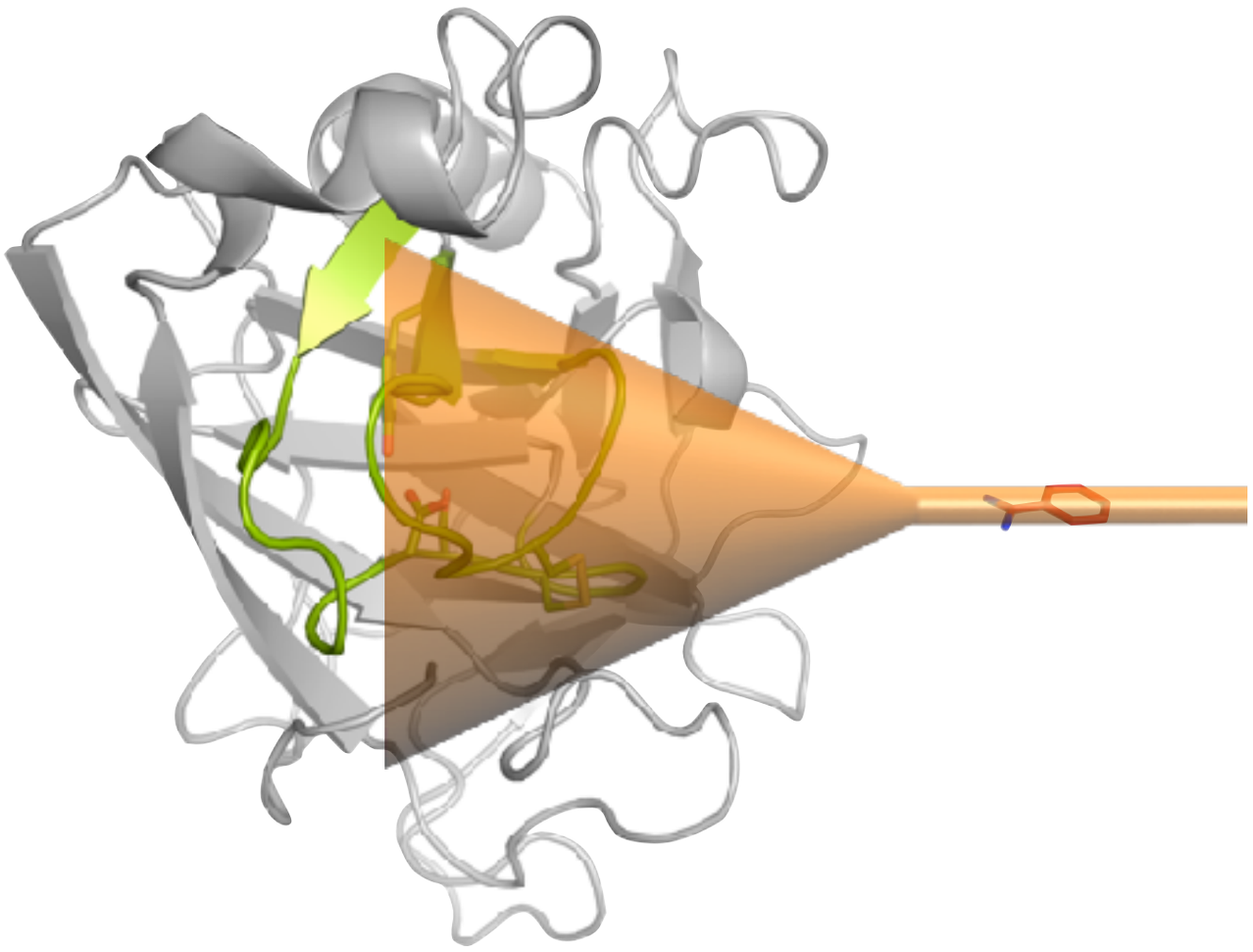


Funnel metadynamics as accurate binding free-energy method **2013**

Vittorio Limongelli[a,1], Massimiliano Bonomi[b], and Michele Parrinello[c,d,1]

[a]Department of Pharmacy, University of Naples Federico II, I-80131 Naples, Italy; [b]Department of Bioengineering and Therapeutic Sciences, and California Institute of Quantitative Biosciences, University of California, San Francisco, CA 94158; [c]Department of Chemistry and Applied Biosciences, Eidgenössiche Technische Hochschule (ETH), 8006 Zürich, Switzerland; and [d]Facoltà di Informatica, Istituto di Scienze Computazionali, Università della Svizzera Italiana, CH-6900 Lugano, Switzerland

Funnel-Metadynamics



$$\Delta G_b^0 = -\frac{1}{\beta} ln(C^0 \ K_b)$$

$$^*K_b = e^{\beta \Delta G_{site}} \int_{site} dz \ e^{-\beta[W(z) - W_{ref}]} \ S_u$$

ΔG_{site} is equal to 0

potential of the unbound state

potential of the bound state

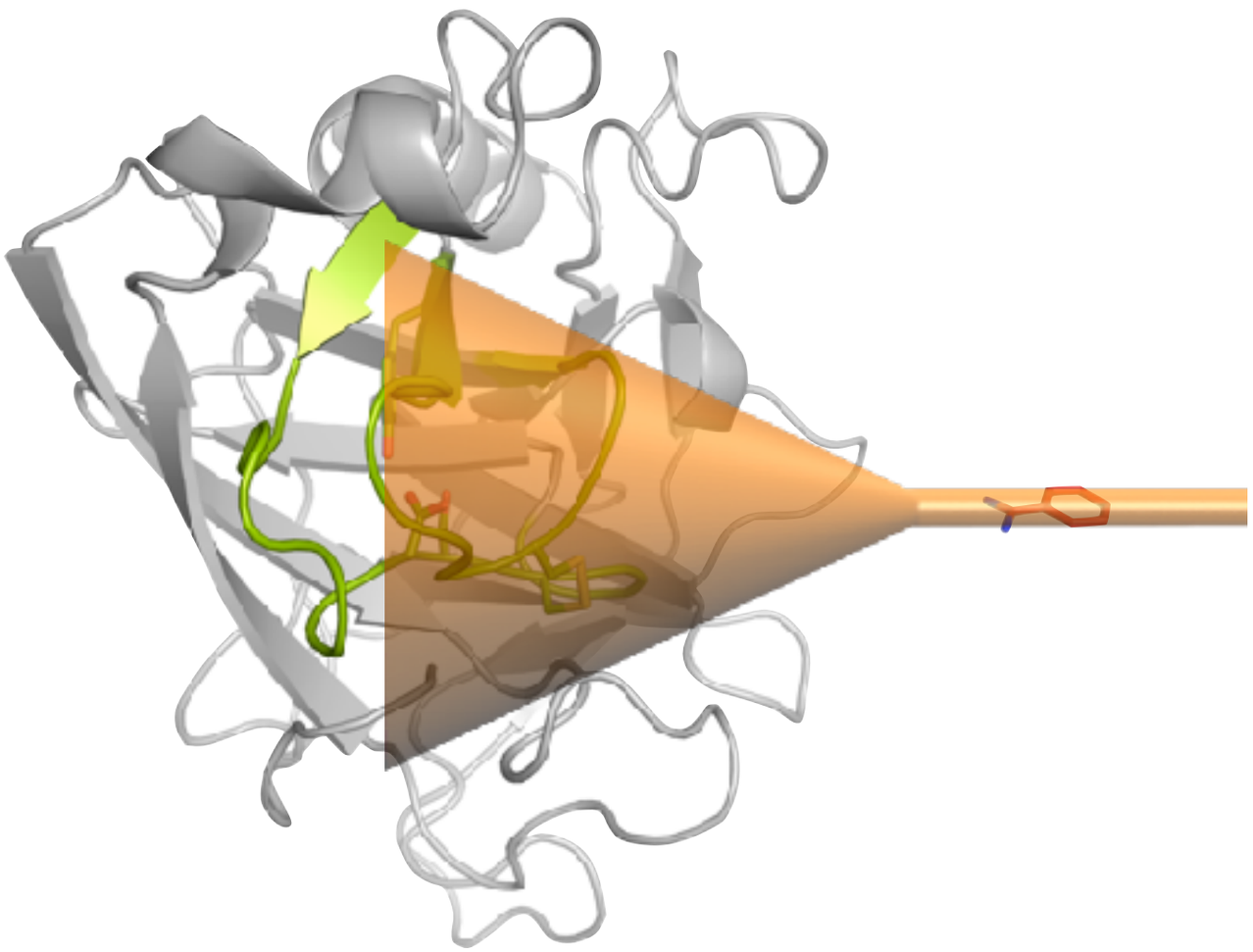
Su is equal to πR_{cyl}^2

References:

* Allen et al., *PNAS* (2004); Roux et al., *J. Chem. Phys.* (2008)

** **Limongelli**, Bonomi & Parrinello, *PNAS* (2013)

Funnel-Metadynamics (FM)



$$\Delta G_b^0 = -\frac{1}{\beta} ln(C^0 \ K_b)$$

ΔG_{site} is equal to 0

potential of the unbound state

$$^{*}K_b = e^{\beta \Delta G_{site}} \int_{site} dz \ e^{-\beta[W(z) - W_{ref}]} \ S_u$$

potential of the bound state

Su is equal to πR_{cyl}^2

$$^{**}\Delta G_b^0 = \Delta G - \frac{1}{\beta} ln(\pi R_{cyl}^2 C^0)$$

Free Energy difference between the bound and unbound state

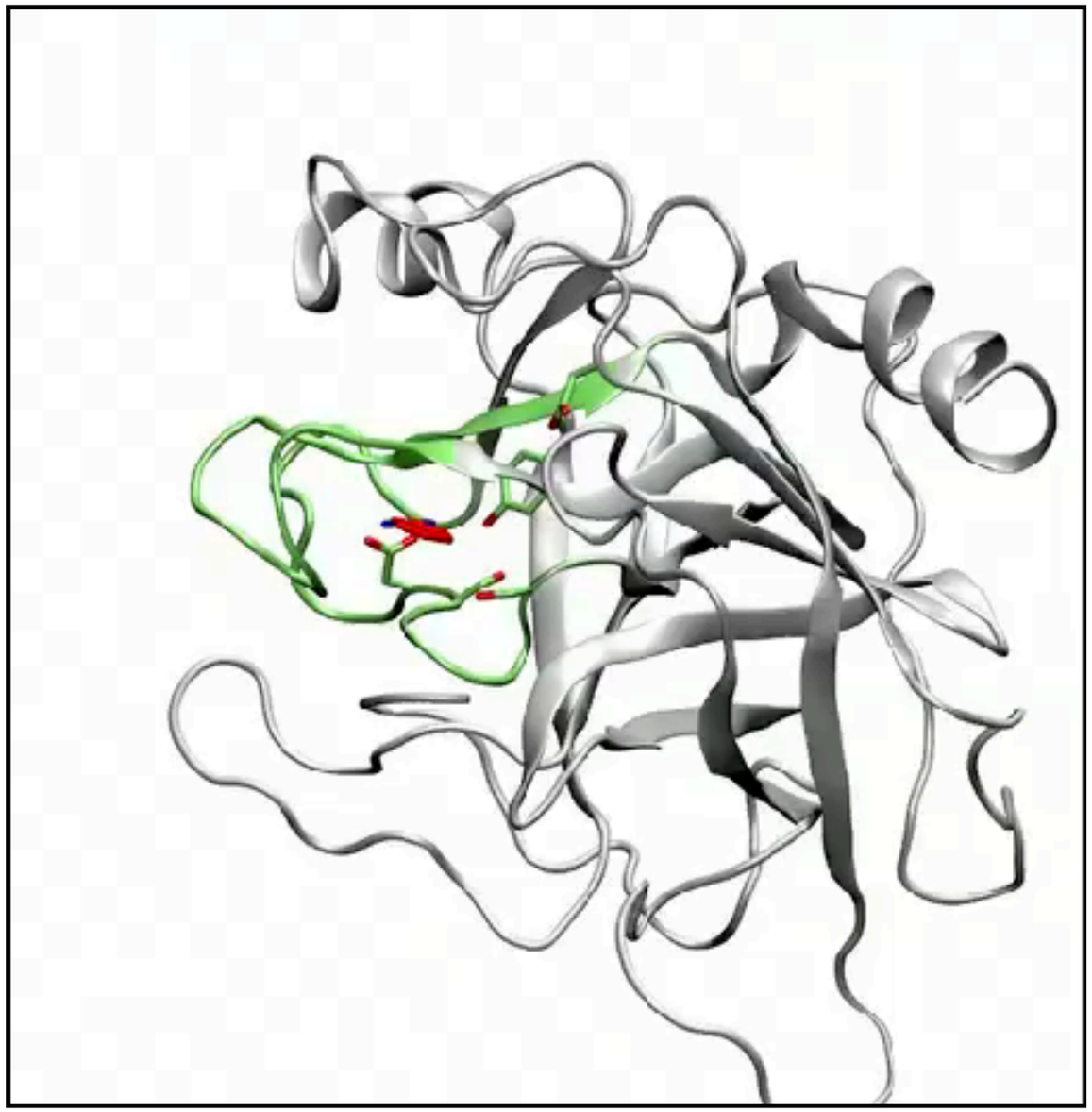
Analytical correction

References:

* Allen et al., *PNAS* (2004); Roux et al., *J. Chem. Phys.* (2008)

** **Limongelli**, Bonomi & Parrinello, *PNAS* (2013)

Trypsin/Benzamidine Binding with FM



Funnel-Metadynamics code available on my website and GitHub:
https://sites.google.com/view/vittoriolimongelli/software?authuser=0

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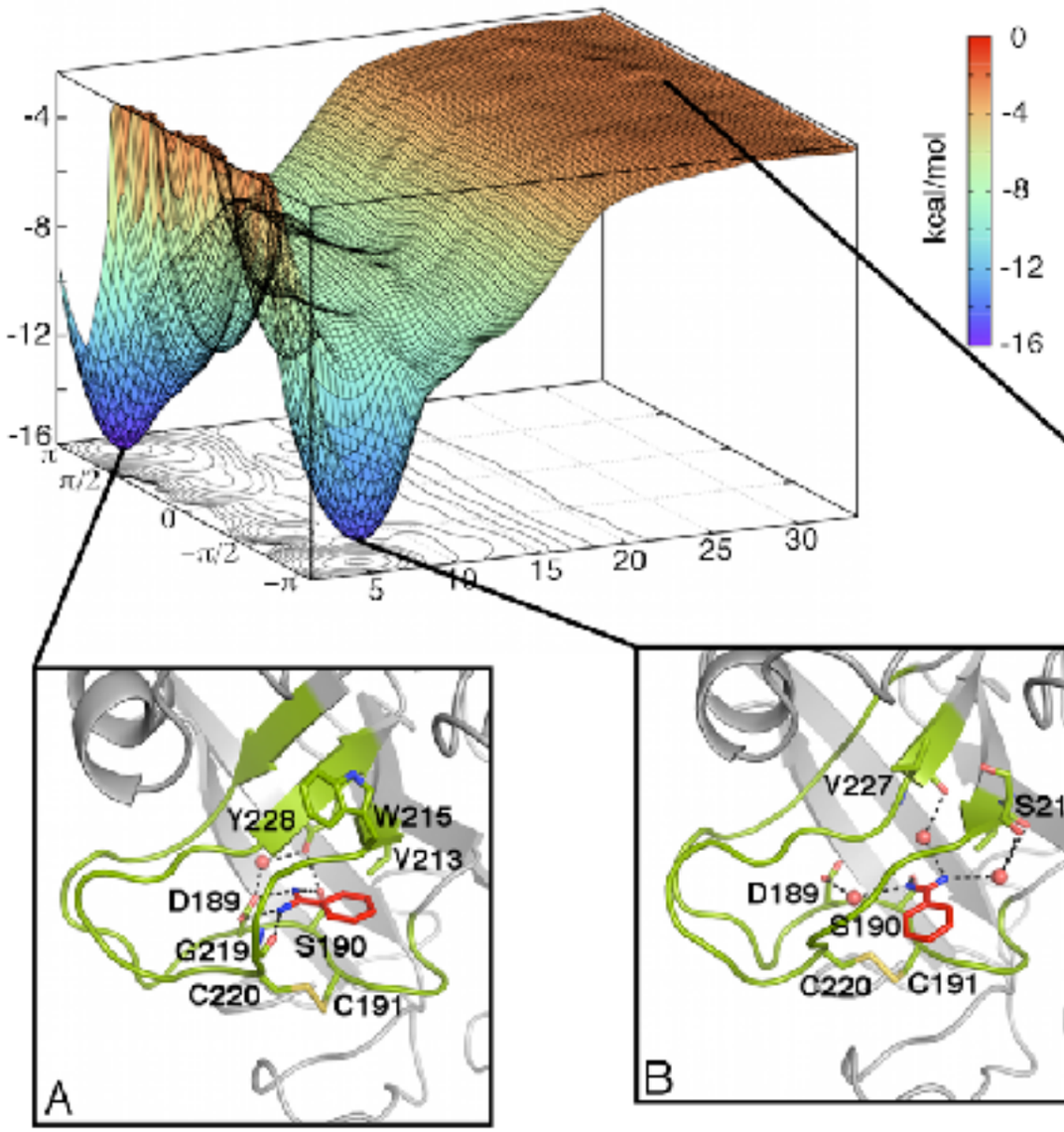
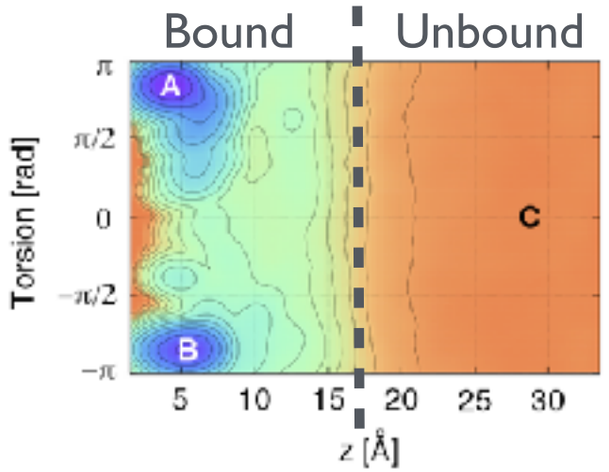
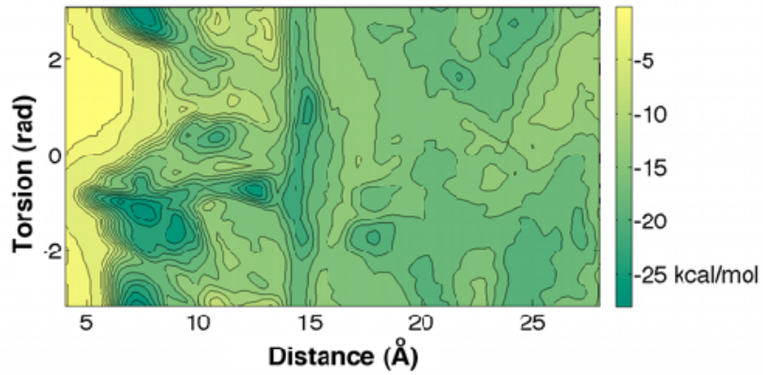
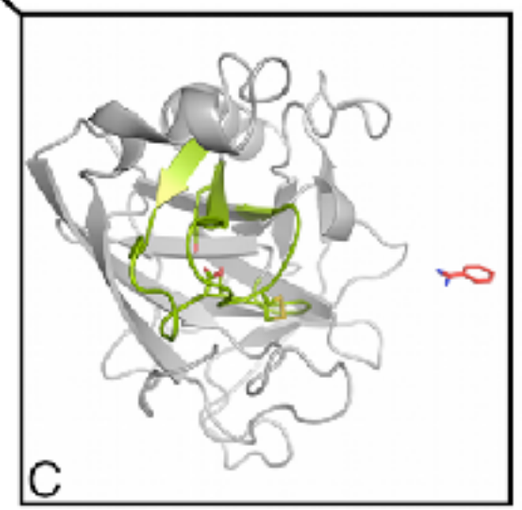
Ligand Binding Free Energy









$G_{(A)}$-$G_{(C)}$ = - 12.3 kcal/mol

3.8 kcal/mol

$$\Delta G_b^0 = \Delta G - \frac{1}{\beta} ln(\pi R_{cyl}^2 C^0)$$

ΔG_b^0 = −8.5 ± 0.7 kcal/mol

Previous estimates −5.5 to −9.0 kcal/mol*

*Talhout et al., *Eur. J. Bochem.* (2001)
Katz et al., *J. Mol. Biol.* (2001)
Doudou et al., *JCTC* (2009)

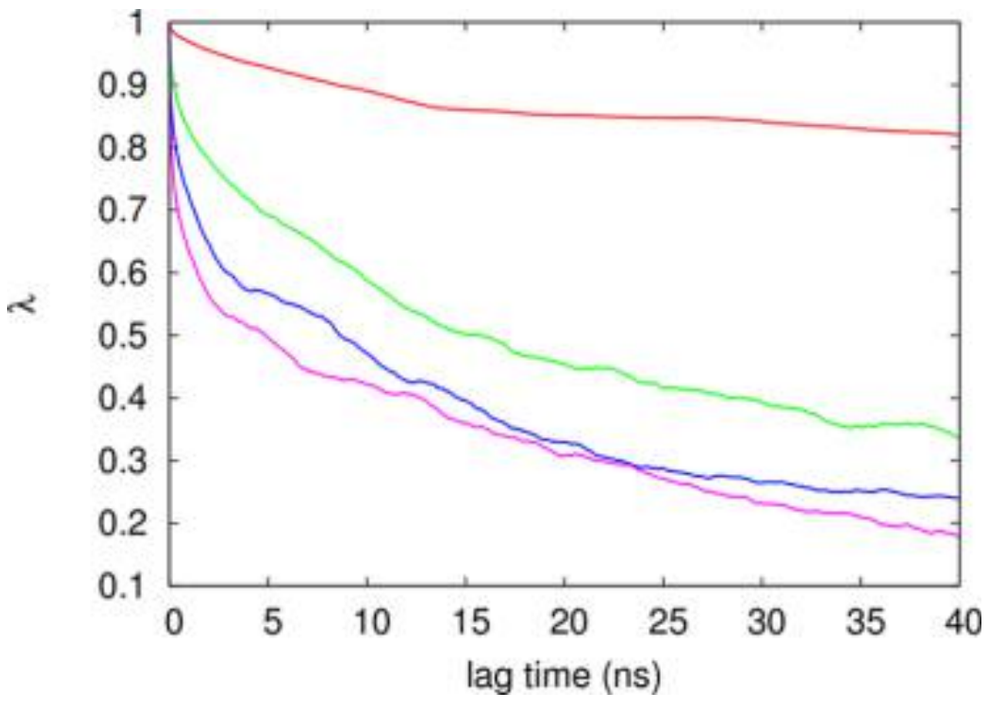
Limongelli, Bonomi & Parrinello, *PNAS* (2013)

VAC-FM

$$s_i(\mathbf{R}) = \sum_k^N b_{ik} d_k(\mathbf{R})$$

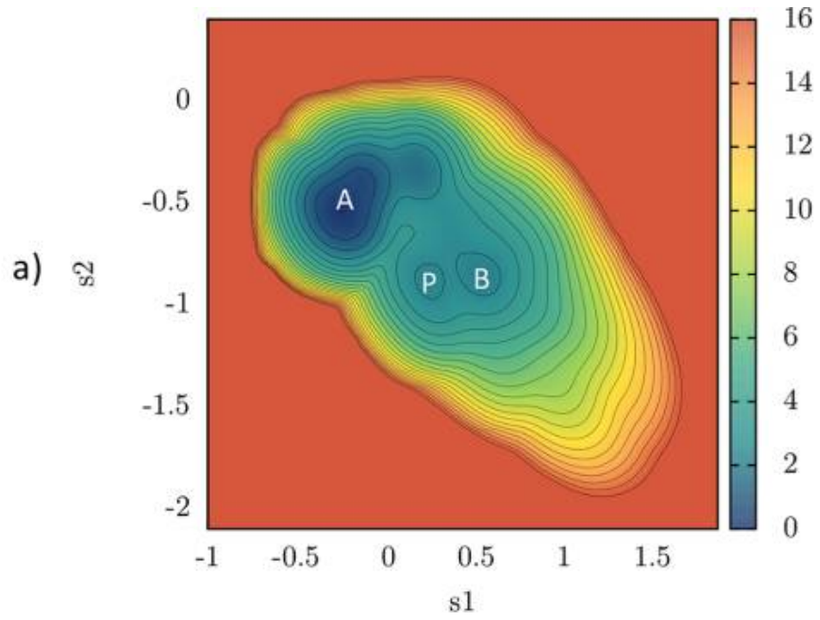
$$d_k(\mathbf{R}) = N_{\text{pr/lig}}, W_{\text{pock}}, \alpha\beta(\theta), W_{\text{lig}}$$

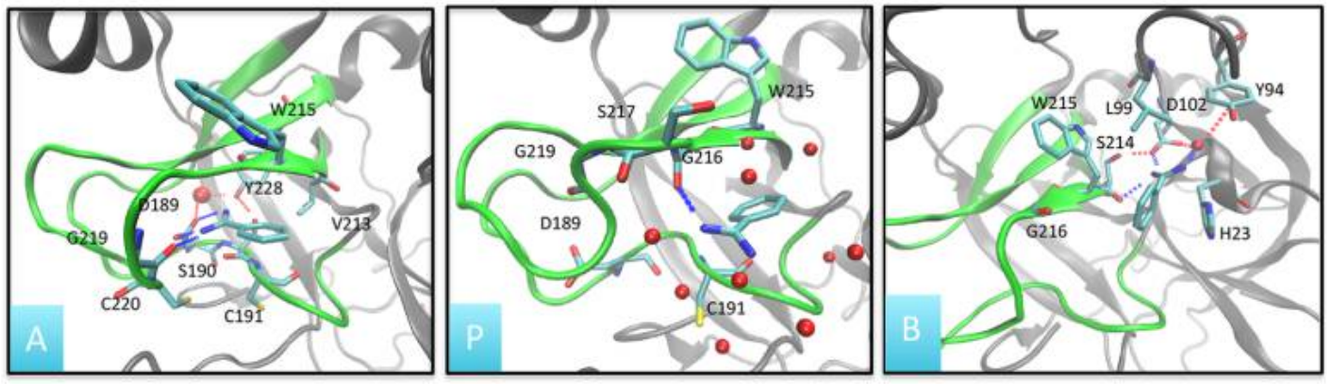
$$\overline{\mathbf{C}(\tau)}\mathbf{b}_i = \overline{\mathbf{C}(0)}\lambda_i\mathbf{b}_i$$



VAC-MetaD*:
Variant of TICA** adapted for biased simulations

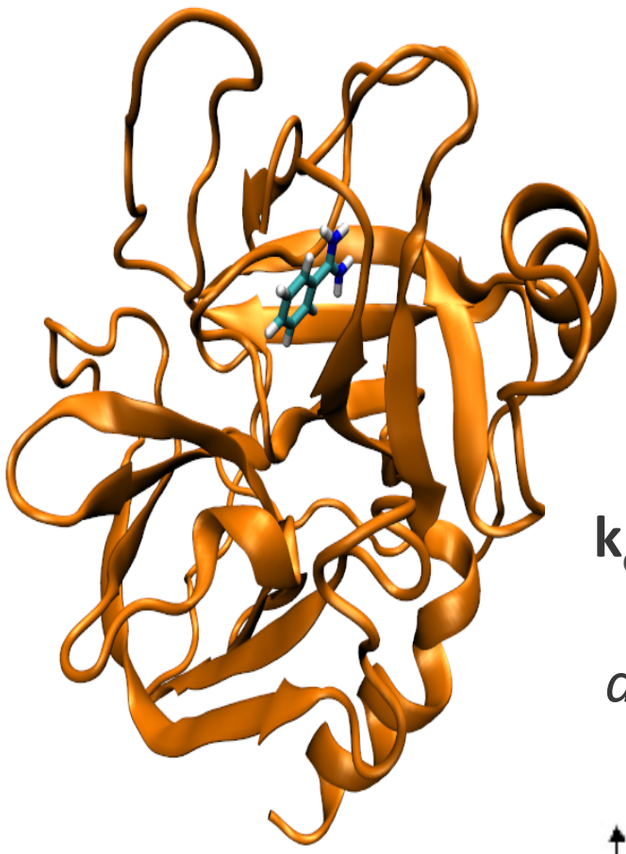
* Mccarty & Parrinello *JCP* (2017)
**Perez-Hernandez et al. *JCP* (2013)
Noé et al. *Multiscale Model. Simul.* (2013)





Brotzakis, **Limongelli** & Parrinello *JCTC* (2019)

What about Ligand Kinetics?

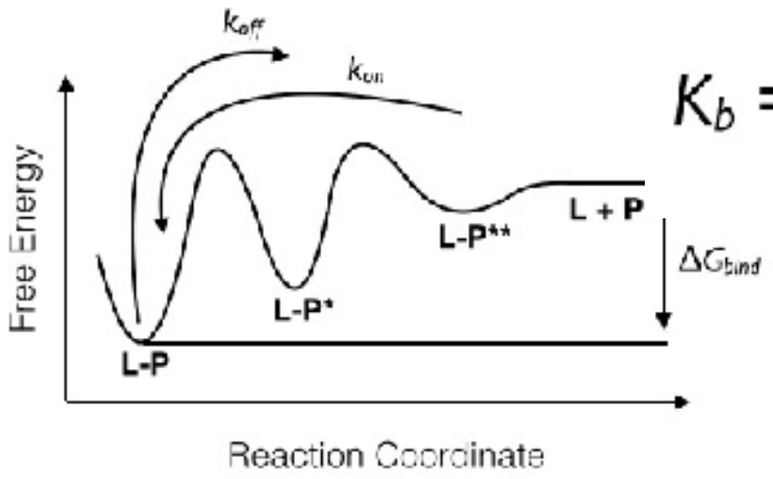


Ligand bound with protein

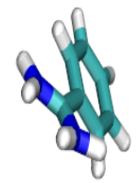
k_{off} = 1/<residence time>
(≈ milliseconds to hours)

k_{off} important for lead optimization in drug design:

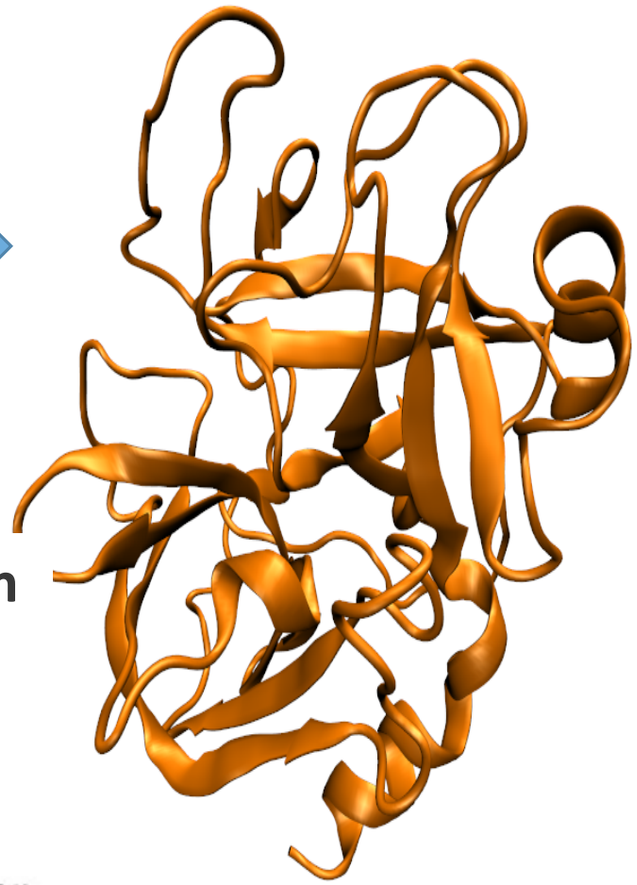
drug works only as long it is bound



$$K_b = \frac{k_{on}}{k_{off}}$$



Ligand unbound from protein



Kinetics of protein–ligand unbinding: Predicting pathways, rates, and rate-limiting steps

2015

Pratyush Tiwary[a,b], Vittorio Limongelli[b,c], Matteo Salvalaglio[b,d], and Michele Parrinello[a,b,1]

[a]Department of Chemistry and Applied Biosciences, Eidgenössische Technische Hochschule Zürich, 8006 Zurich, Switzerland; [b]Università della Svizzera Italiana, Faculty of Informatics, Institute of Computational Science, CH-6900 Lugano, Switzerland; [c]Department of Pharmacy, University of Naples Federico II, I-80131 Naples, Italy; and [d]Institute of Process Engineering, Eidgenössische Technische Hochschule Zürich, 8006 Zurich, Switzerland

What about Ligand Kinetics?

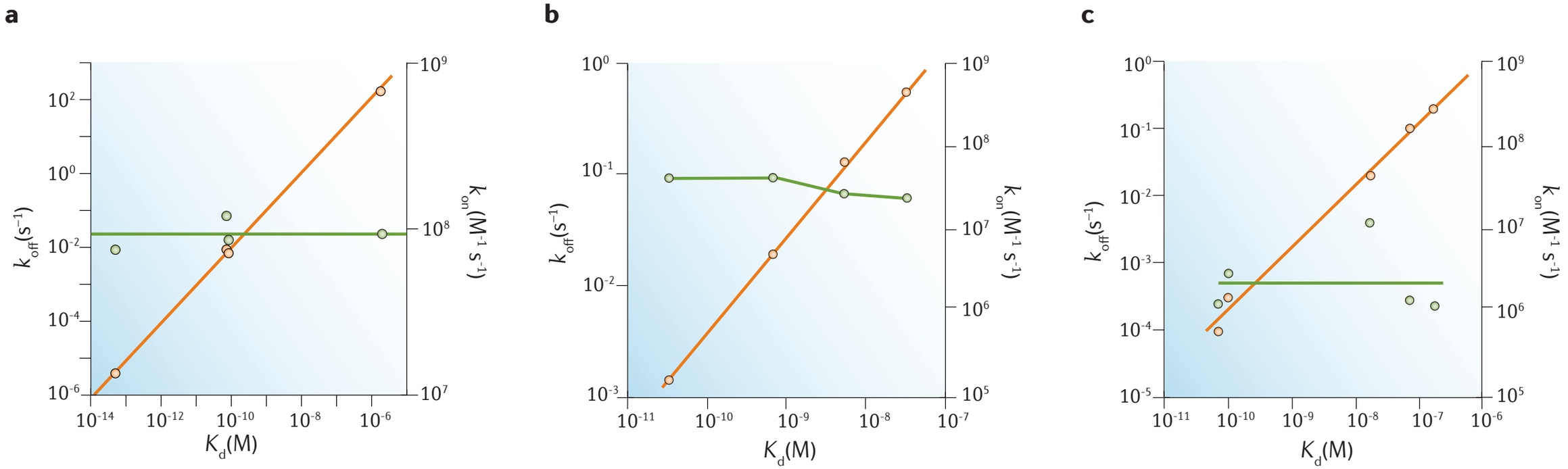
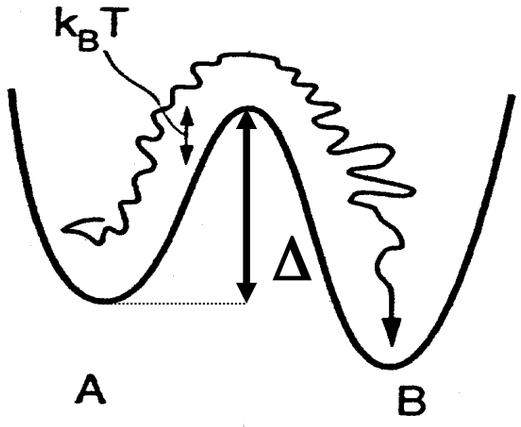


Figure 2 | **Drug affinity (target potency) is often driven by drug–target residence time.** Correlation between the dissociation rate constant (k_{off}; orange circles) or association rate constant (k_{on}; green circles) with the equilibrium dissociation constant (K_d) for biotin binding to wild-type and mutant forms of streptavidin[16] (part **a**), saquinavir binding to wild-type and resistant mutants of HIV protease[17] (part **b**), and a series of aminonucleoside inhibitors binding to the protein methyltransferase DOT1L[18] (part **c**).

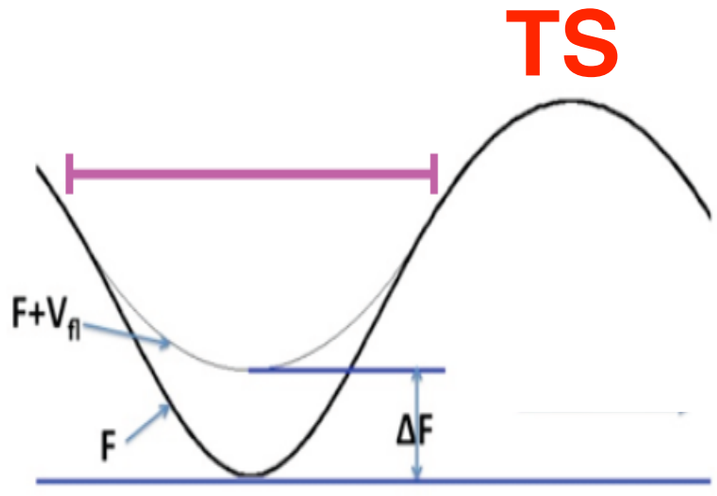
Copeland *Nature Review Drug Discovery* (2016)

Infrequent Metadynamics



$$k_{\beta\to\alpha} \propto \kappa \frac{Z_{TS}}{Z_\beta}$$ without bias

$$k^*_{\beta\to\alpha} \propto \kappa \frac{Z_{TS}}{Z^*_\beta}$$ with bias



$$\frac{k^*_{\beta\to\alpha}}{k_{\beta\to\alpha}} = \frac{Z_\beta}{Z^*_\beta} = \left\langle e^{\beta V_{fl}} \right\rangle^*$$

$$\alpha = \frac{Z_\beta}{Z^*_\beta} = \left\langle e^{\beta V_{fl}} \right\rangle^*$$

$$\alpha = \frac{\tau}{\tau_M}$$

Acceleration factor

References:
Tiwary & Parrinello, *PRL* 2013
Salvalaglio, Tiwary & Parrinello, *JCTC* 2014
Grubmueller, *PRE* 1995; Voter, *JCP* 1997

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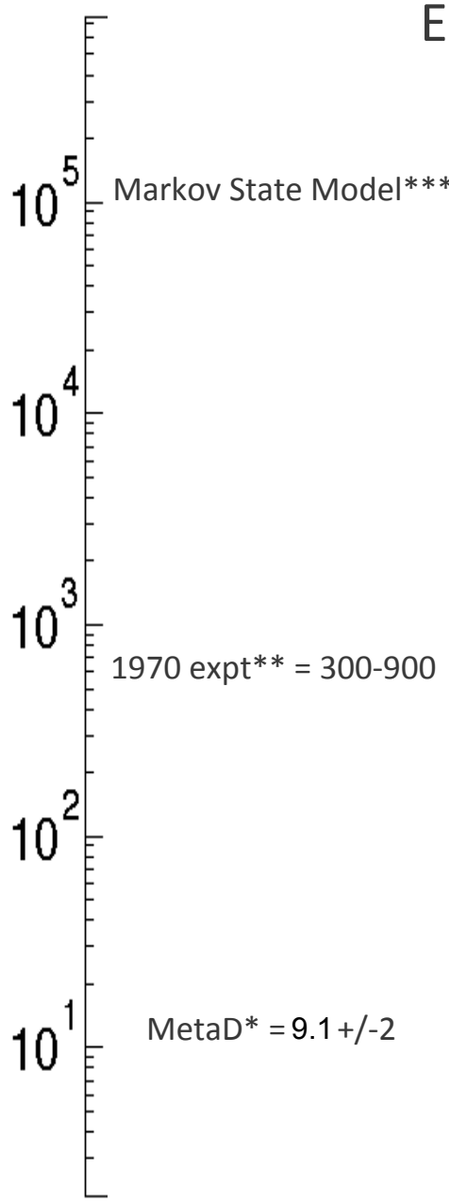
k_{off} from Unbinding Trajectories

n (unbinding events)/ total time = k_{off} = **9.1/sec** *

Experiment**= 600(+/- 300)/sec

MSM***=10^5/sec



$\Delta G_{binding}$ (kcal/mol)

-6.4 to -7.3 (experiments)

-8.5 +/- 0.7 (MetaD)

-5.2 +/- 0.4 (MSM)

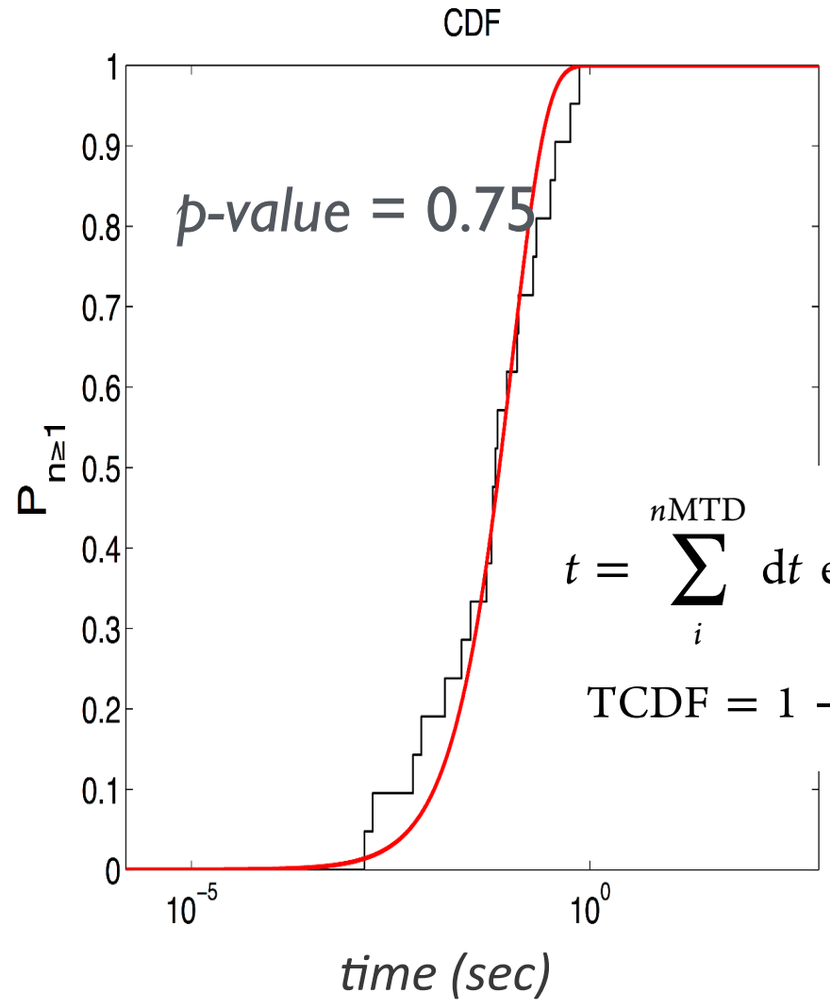
Binding rates

$k_{on} = 1/C_0\, k_{off} e^{-\beta\Delta G}$ ($M^{-1}s^{-1}$)

3 x 10^7 (experiments)

1.2 +/-1 x 10^7 (MetaD – ***indirect***)

15 +/- 2 x 10^7 (MSM – ***direct***)



$$t = \sum_{i}^{n\text{MTD}} \mathrm{d}t\ \mathrm{e}^{\beta V(s(t_{i\text{MTD}}),t_{i\text{MTD}})}$$

$$\text{TCDF} = 1 - \exp\left(-\frac{t}{\tau}\right)$$

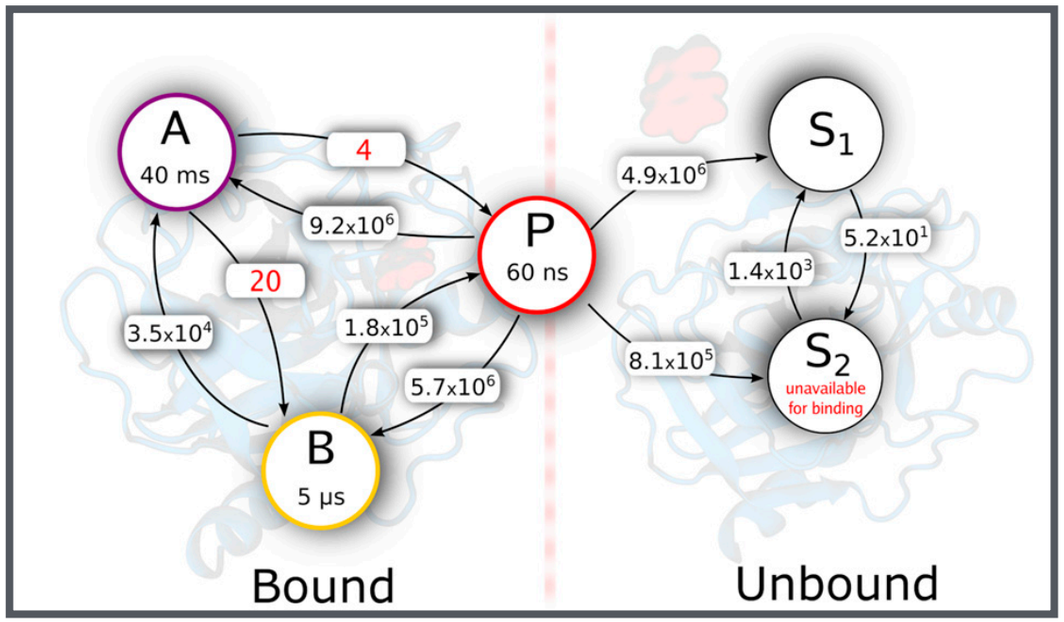
* Tiwary, **Limongelli**, Salvalaglio & Parrinello, *PNAS* (2015)

* **Tiwary, Limongelli, Salvalaglio and Parrinello, *in preparation***

** **Guillain, Thusius JACS 1970**

*** **Indirect calculation via k_{on}, Buch *et al* PNAS 2011**

k_{off} and Rate-Determining Step

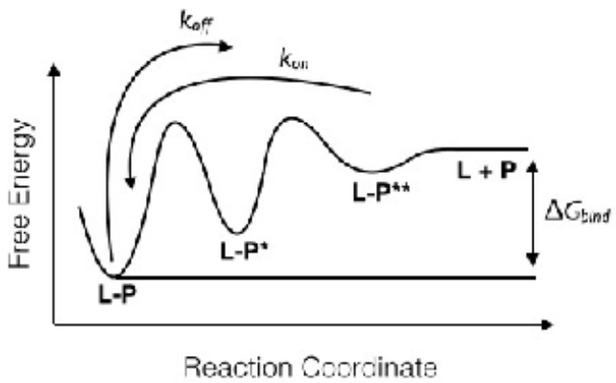


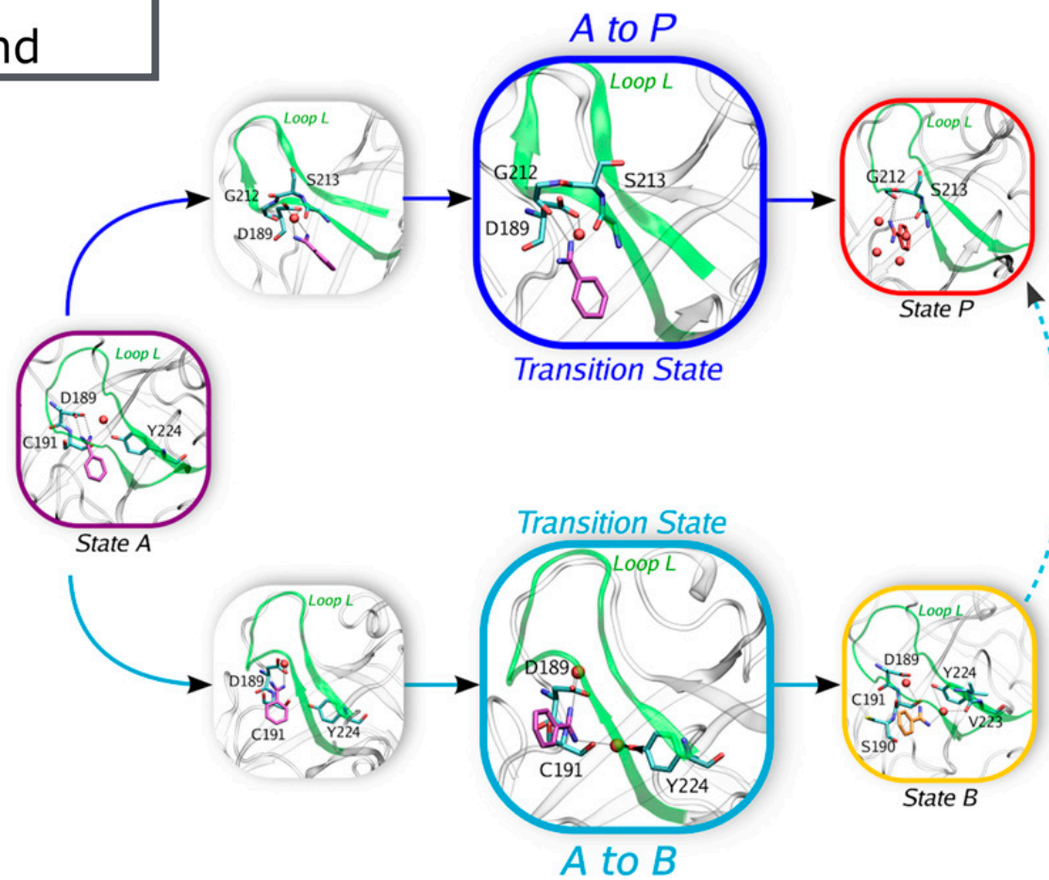
$$\alpha = \frac{\tau}{\tau_M} \qquad \alpha = \frac{Z_\beta}{Z_\beta^*} = \left\langle e^{\beta V_{fl}} \right\rangle^*$$

Markov state model K_{off} = 7.3 /sec

Estimate from full unbinding runs = 9.1 ± 2.5 /sec

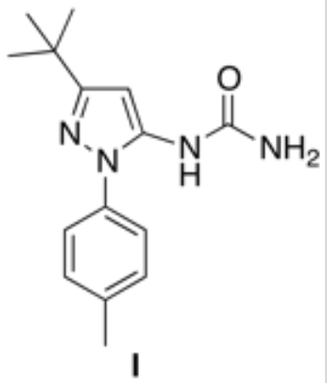
$$K_b = \frac{k_{on}}{k_{off}}$$





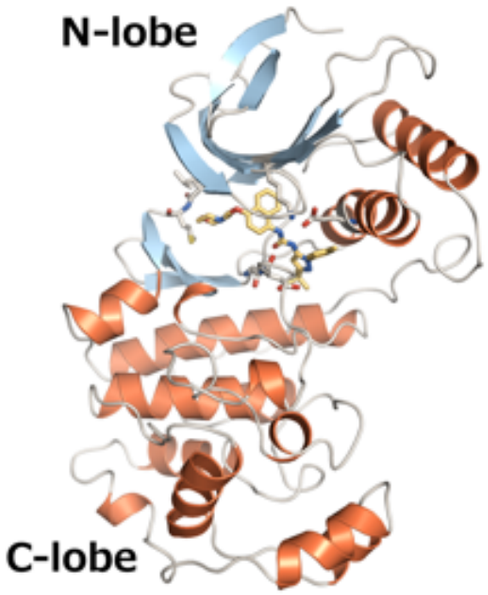
Tiwary, **Limongelli**, Salvalaglio, Parrinello *PNAS* (2015)

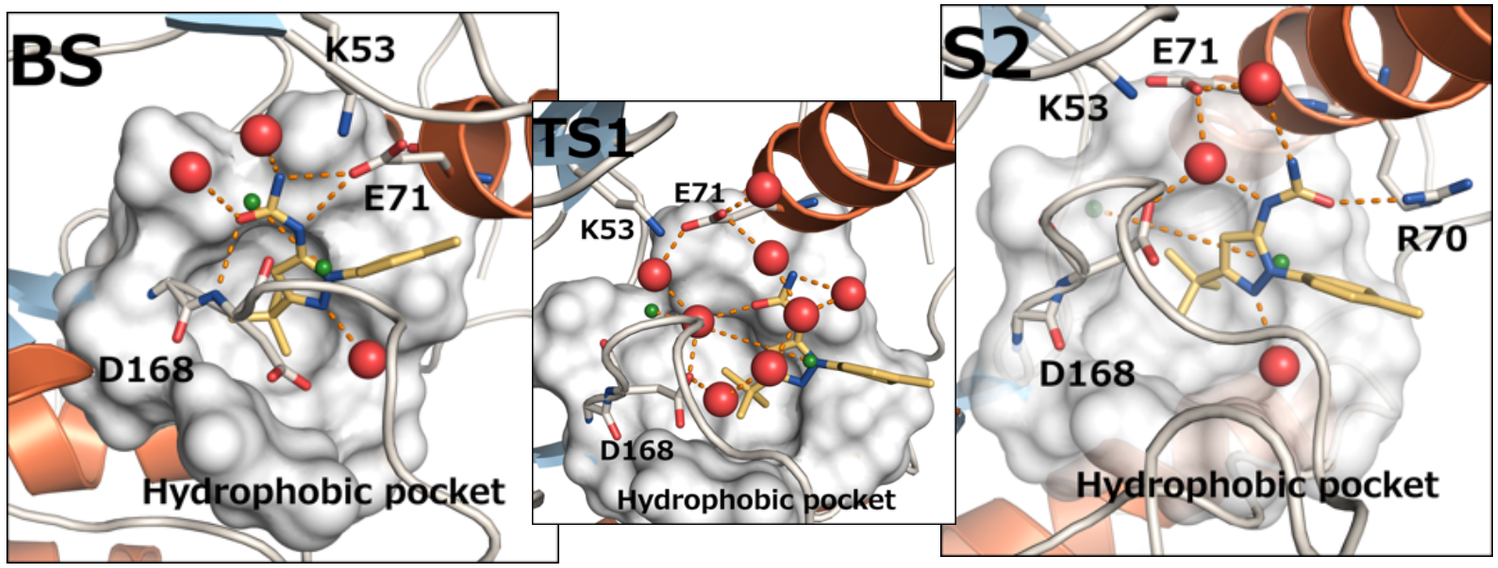
Ligand Binding Kinetics to Kinase

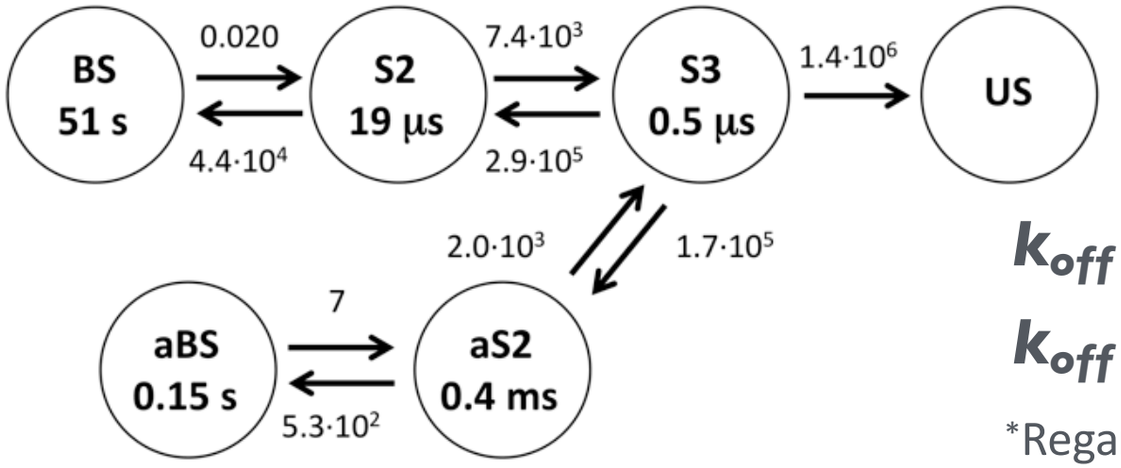


I

k_{on} ($M^{-1}s^{-1}$) $1.18 \cdot 10^5$
k_{off} (s^{-1}) $1.4 \cdot 10^{-1}$







$k_{off\ (calc)} = 0.02 \pm 0.01$ /sec

$k_{off\ (exp)} = 0.14$ /sec*

*Regan et al. *BMCL* (2003)

JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Article
pubs.acs.org/JACS
2017

Unbinding Kinetics of a p38 MAP Kinase Type II Inhibitor from Metadynamics Simulations

Rodrigo Casasnovas,[†] Vittorio Limongelli,[#,‡,§] Pratyush Tiwary,[#,∥] Paolo Carloni,[*,†] and Michele Parrinello[*,⊥]

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PRX V Inhibitors

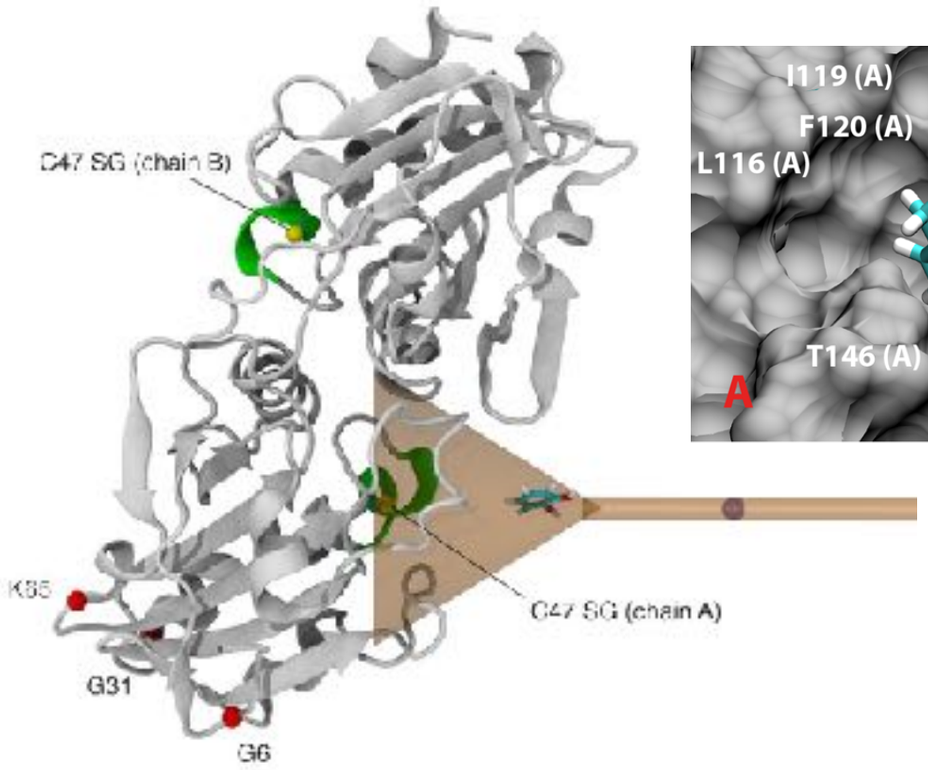
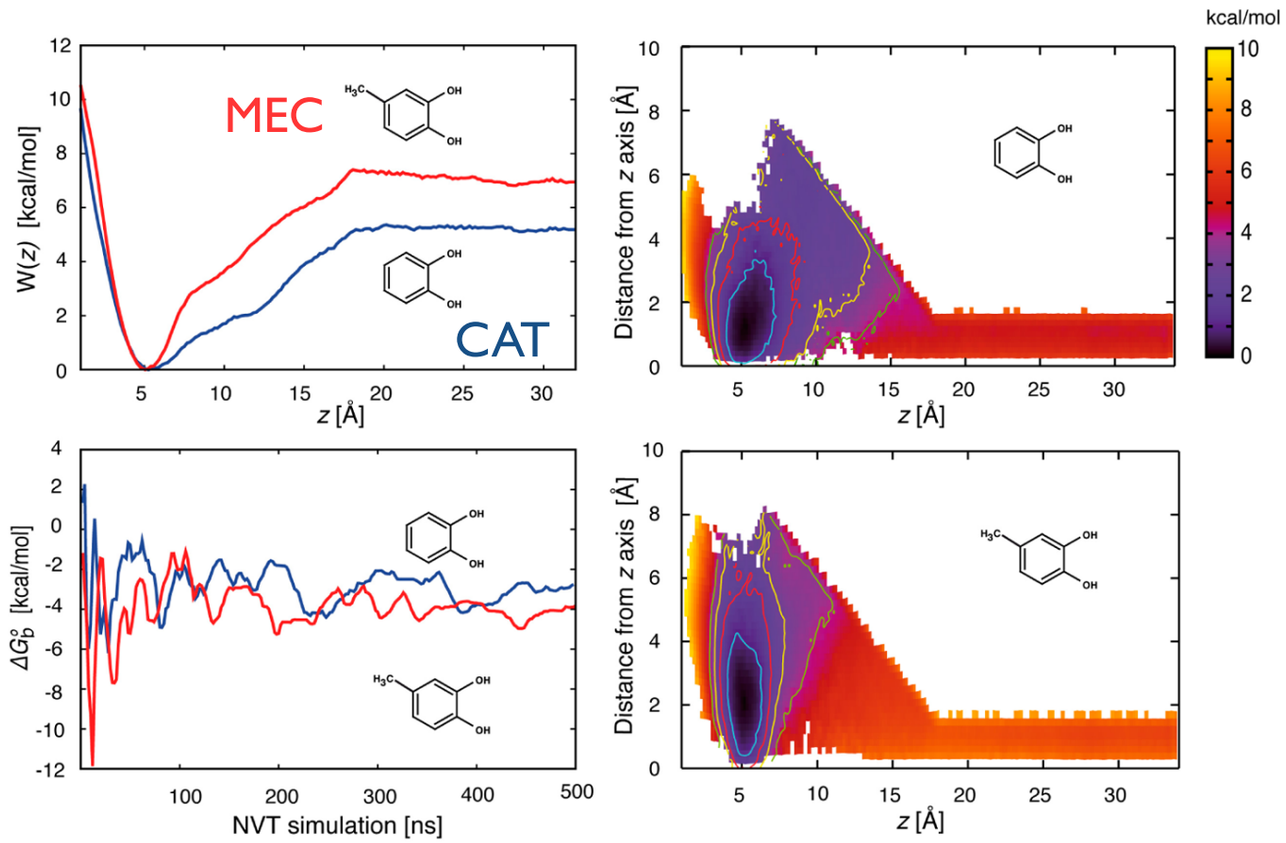


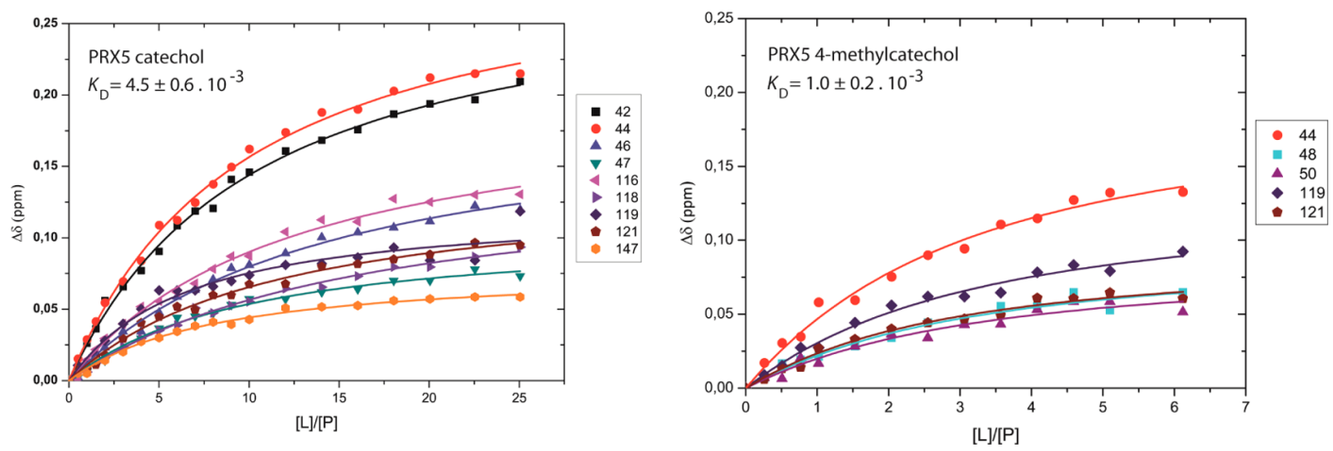
Table 1. Comparison of Calculated and Experimental Dissociation Constants K_D and Absolute Binding Free Energy ΔG_b^0 of Catechol and 4-Methylcatechol for Human PRX5

	catechol	4-methylcatechol
$\Delta G_b^{0\,a}$		
FM[b]	−3.0 ± 0.2	−4.2 ± 0.3
NMR	−3.2 ± 0.1	−4.1 ± 0.1
K_D (10^{-3})		
FM[b]	6.9 ± 2.1	0.9 ± 0.4
NMR	4.5 ± 0.6	1.0 ± 0.2

[a]kcal/mol. [b]Mean value over the last 100 ns of 500 ns FM.

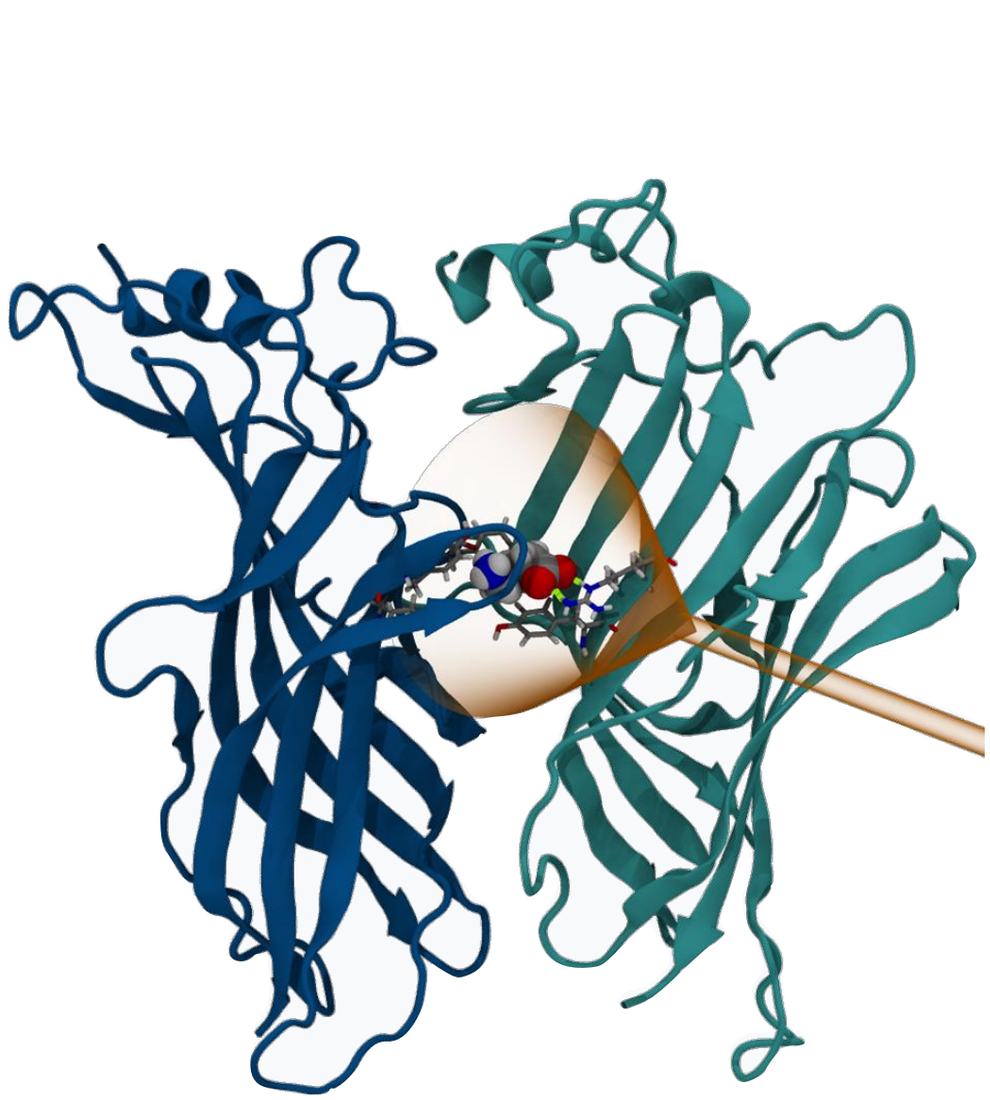


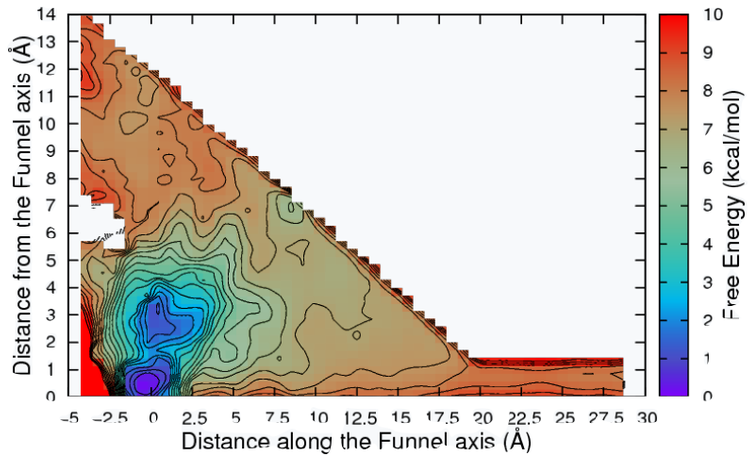
NMR-based K_D



Troussicot, Guillere, **Limongelli**, Walker, Lancelin *JACS* (2015)

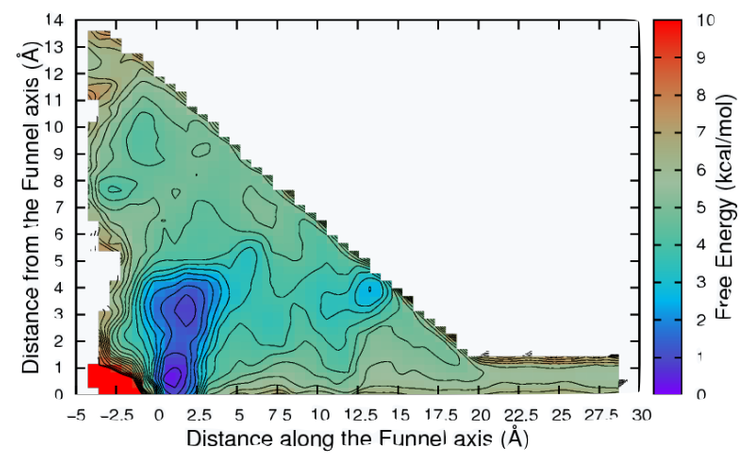
Fruit Fly Resistance to Dieldrin PLGIC





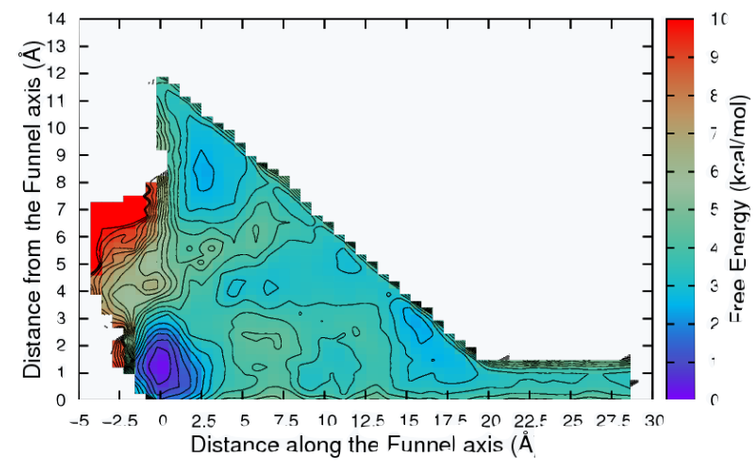
Wild type

$\Delta G_b^0 = -9.3 \pm 0.2$ kcal/mol



E204A mutant

$\Delta G_b^0 = -6.3 \pm 0.1$ kcal/mol



R111A mutant

$\Delta G_b^0 = -3.4 \pm 0.3$ kcal/mol

Comitani, **Limongelli**, Molteni, *JCTC* (2016)

Ligand/Protein Binding

What if we have large protein conformational changes? (configurational entropy)

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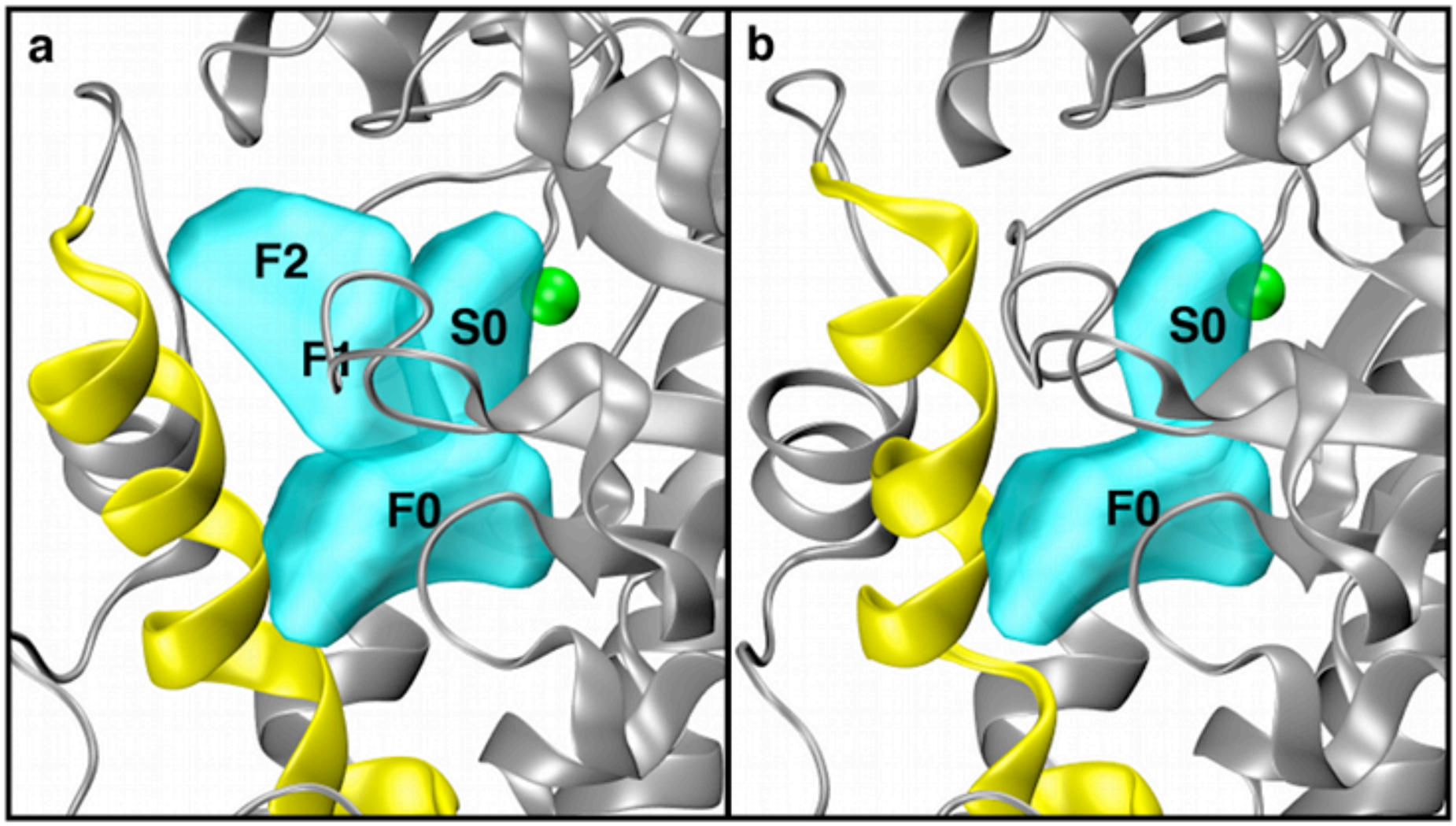
Adenosine DeAminase (ADA)

Helix displacement in the apo and bound X-ray structure



PNAS

Sampling protein motion and solvent effect during ligand binding **2012**

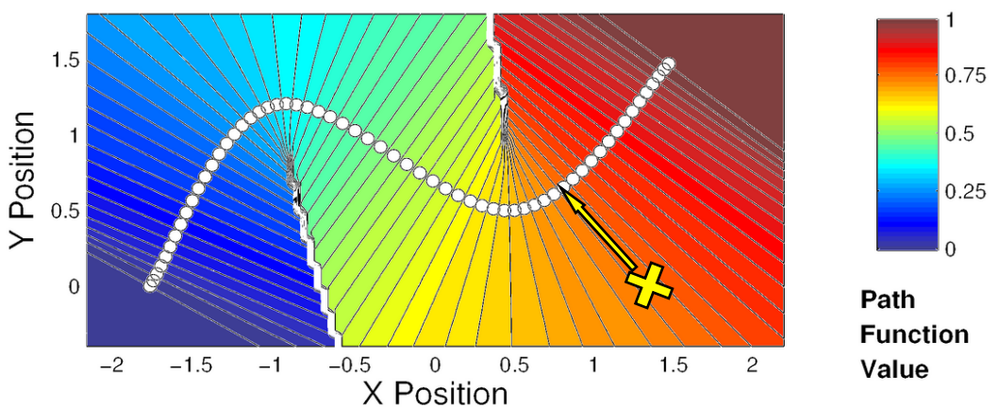
Vittorio Limongelli[a,b,c,1], Luciana Marinelli[c], Sandro Cosconati[d], Concettina La Motta[e], Stefania Sartini[e], Laura Mugnaini[e], Federico Da Settimo[e], Ettore Novellino[c], and Michele Parrinello[a,b,1]

[a]Department of Chemistry and Applied Biosciences, Computational Science, Eidgenössiche Technische Hochschule (ETH), Zürich, Switzerland; [b]Institute of Computational Science (ICS), Università della Svizzera Italiana, Via Giuseppe Buffi 13, CH-6900 Lugano, Switzerland; [c]Dipartimento di Chimica Farmaceutica e Tossicologica, Università di Napoli "Federico II", Via D. Montesano, 49, I-80131 Naples, Italy; [d]Dipartimento di Scienze Ambientali, Seconda Università di Napoli, Via Vivaldi 43, I-81100 Caserta, Italy; and [e]Dipartimento di Scienze Farmaceutiche, Università di Pisa, Via Bonanno, 6, I-56126 Pisa, Italy

Path Collective Variables

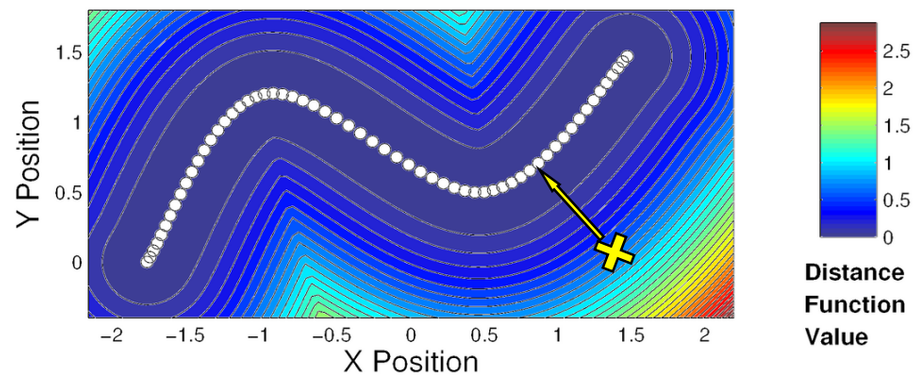
•s(R)* allows the system to go from state A (closed) to state B (open)**

$$s(\mathbf{R}) = \lim_{\lambda \to \infty} \frac{\int_0^1 t e^{-\lambda(\mathbf{R} - \mathbf{R}(t))^2} dt}{\int_0^1 e^{-\lambda(\mathbf{R} - \mathbf{R}(t))^2} dt}$$



•z(R)* allows exploring states far from the guess path

$$z(\mathbf{R}) = \lim_{\lambda \to \infty} -\frac{1}{\lambda} \ln \int_0^1 e^{-\lambda(\mathbf{R} - \mathbf{R}(t))^2} dt.$$



* Branduardi et al. *JCP* (2007); Bonomi et al. *JACS* (2008); Ren et al. *JCP* (2005)
** Closed and open state defined by the available X-ray ADA structures

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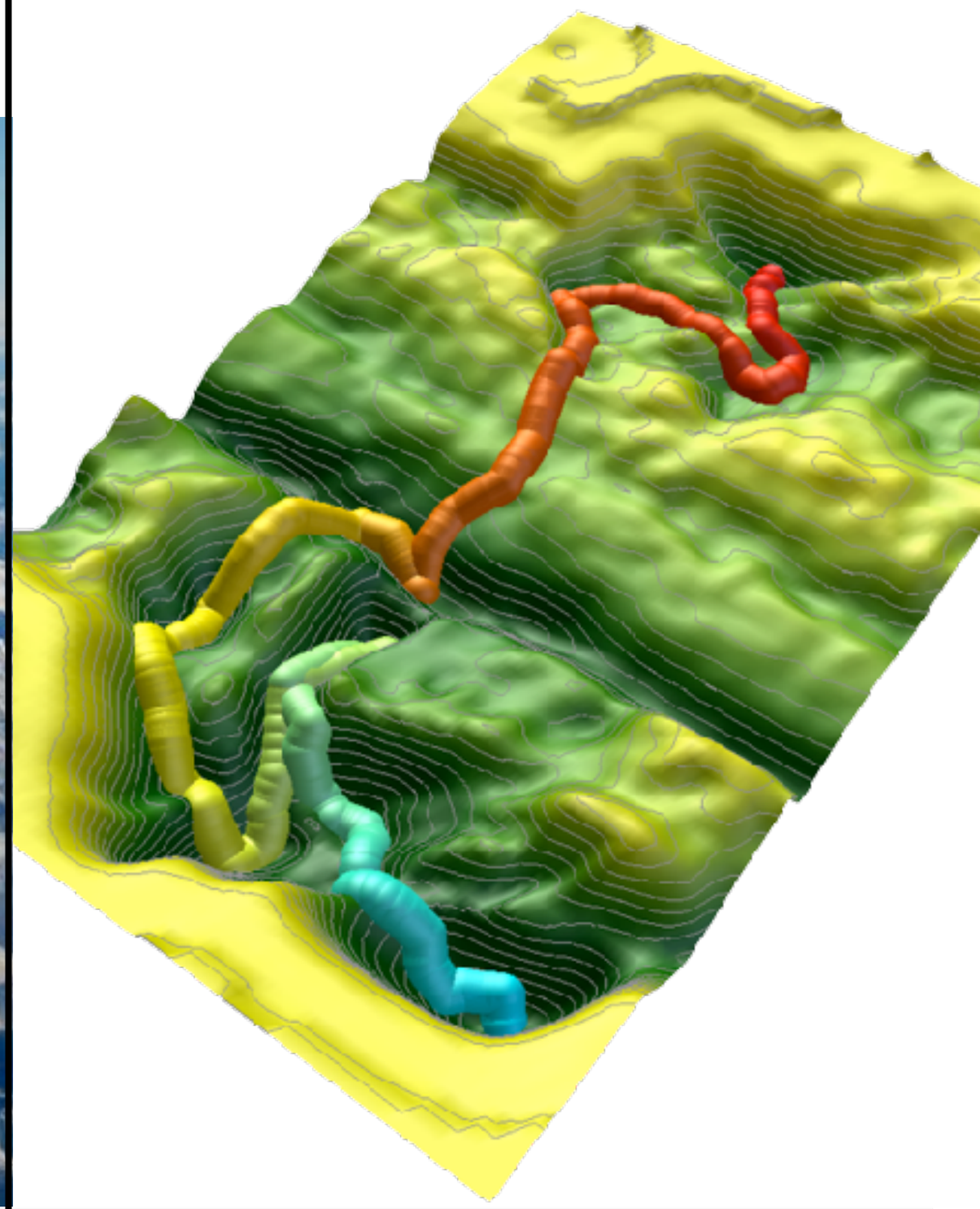
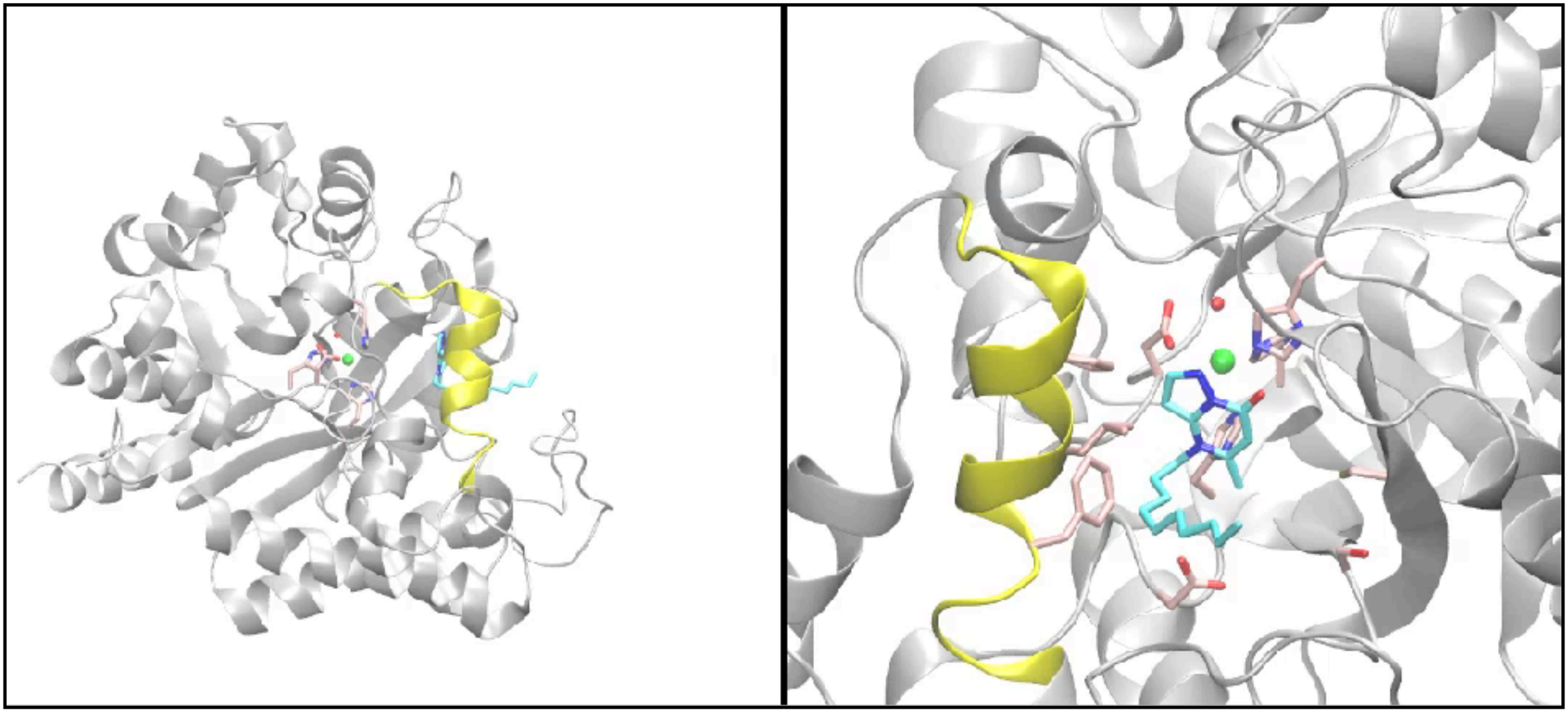
Path Collective Variables



Limongelli et al. *PNAS* (2010)

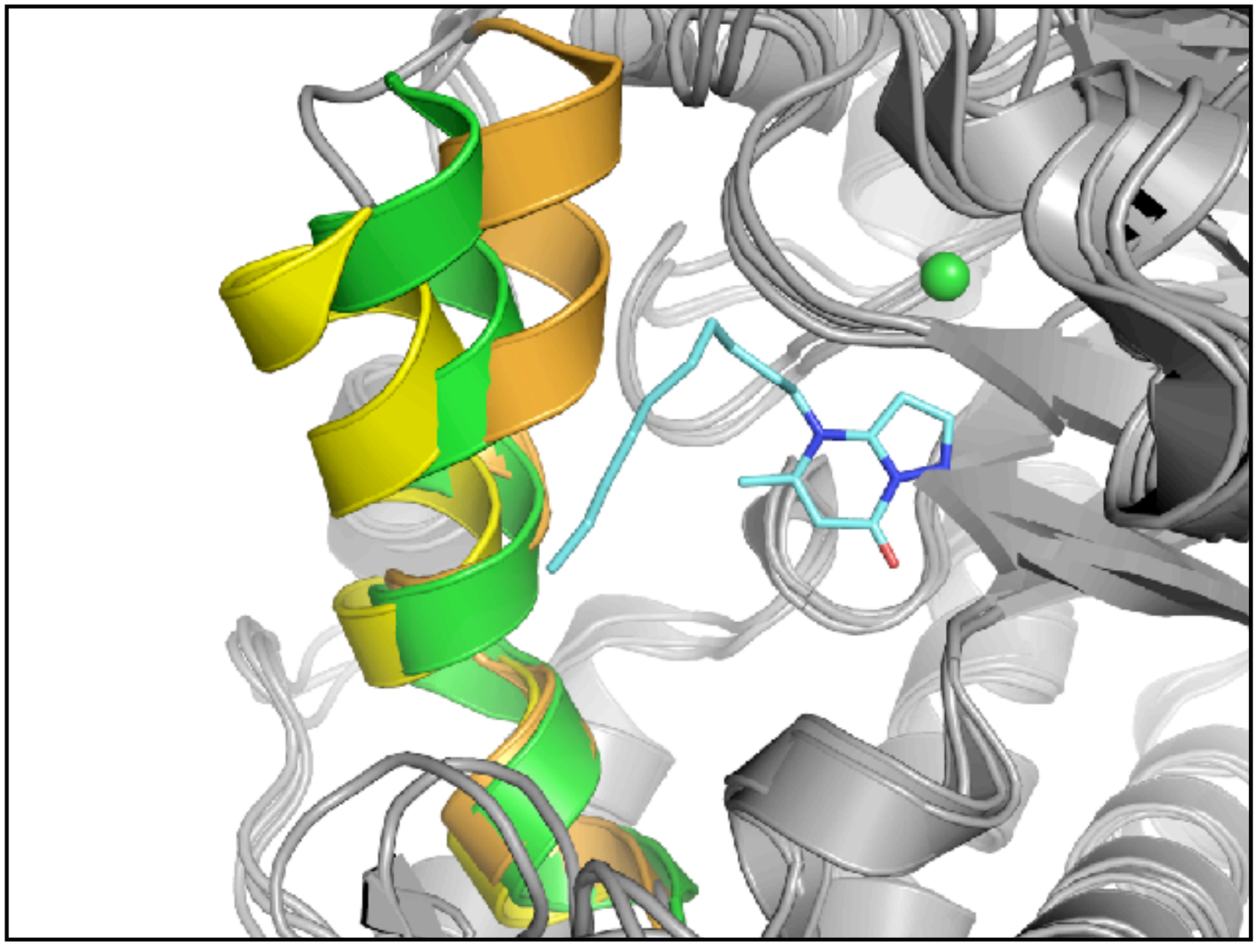
The ADA Movie



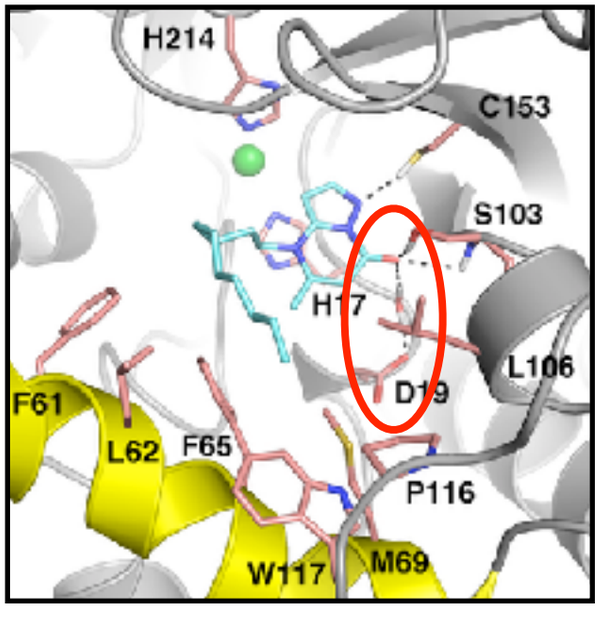
Limongelli et al. *PNAS* (2012)

The Binding Mode

The ligand binds to ADA in the open conformation



- closed X-ray
- open X-ray
- open metadynamics



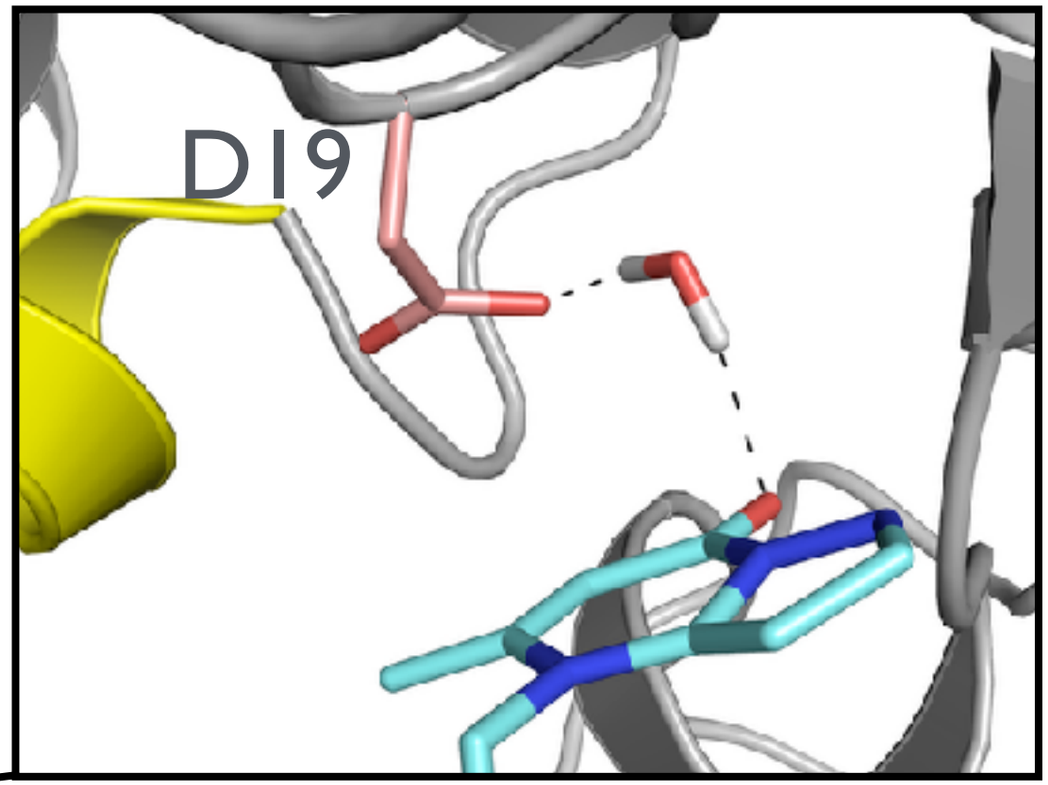
Limongelli et al. *PNAS* (2012)

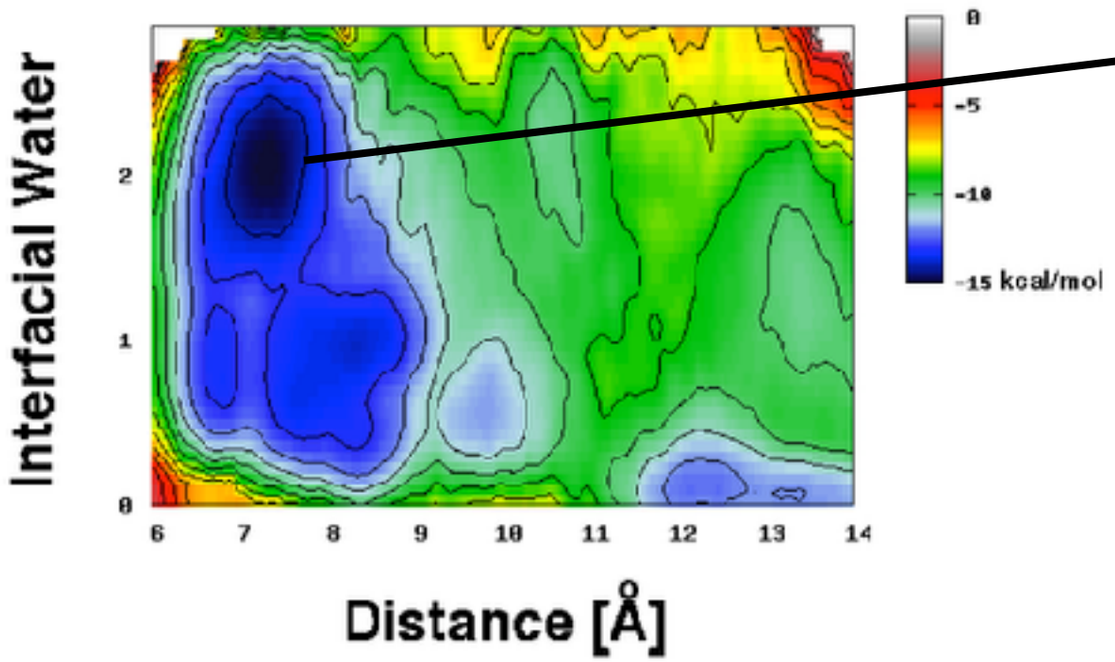
Reweighting the FES

The Boltzmann probability distribution for different CVs can be computed as:

$$P_B(\boldsymbol{R}) \propto e^{+\beta V_G(\boldsymbol{S}(\boldsymbol{R}),t)}\, P(\boldsymbol{R},t)$$

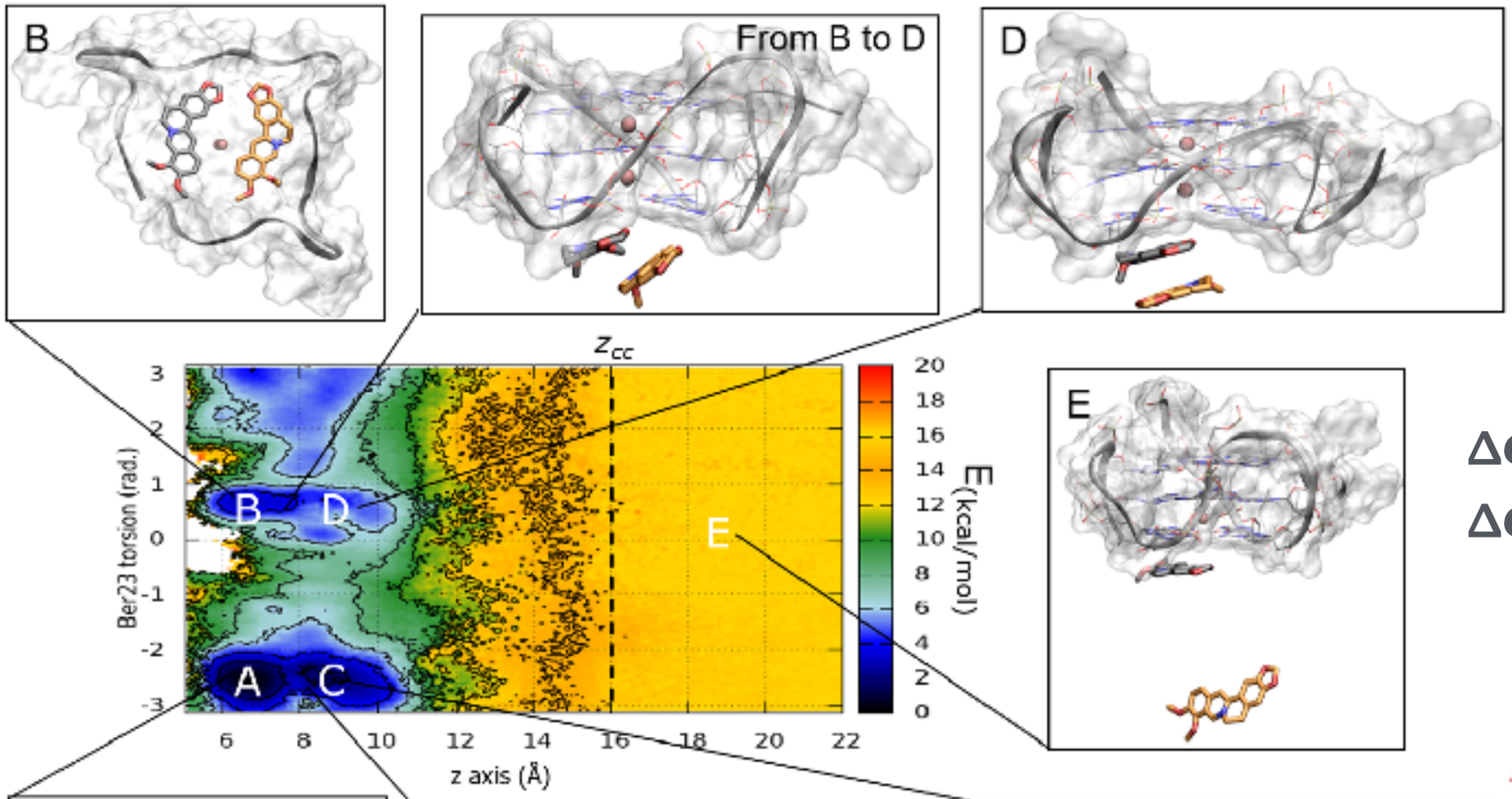
Bonomi et al. *JCC* (2009)

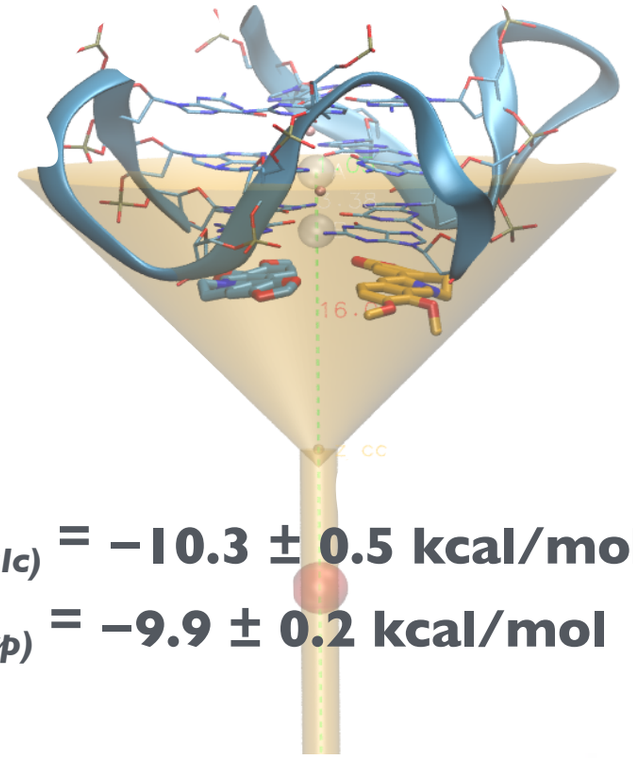




$$\mathrm{IntWat} = \sum_{i}^{n_0} \left(\frac{1 - \left(\frac{|\mathbf{r}_i - \mathbf{r}_1|}{r_0}\right)^n}{1 - \left(\frac{|\mathbf{r}_i - \mathbf{r}_1|}{r_0}\right)^m} \right) \left(\frac{1 - \left(\frac{|\mathbf{r}_i - \mathbf{r}_2|}{r_0}\right)^n}{1 - \left(\frac{|\mathbf{r}_i - \mathbf{r}_2|}{r_0}\right)^m} \right)$$

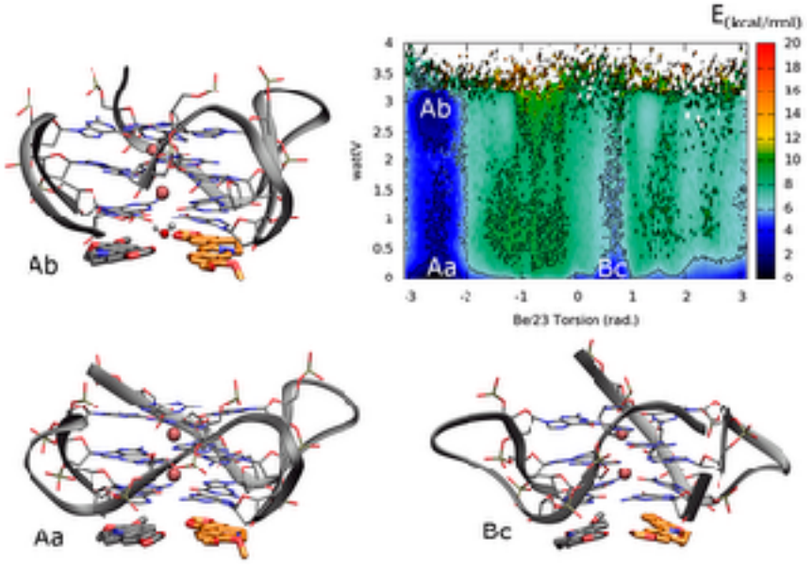
Ligand/G-quadruplex DNA





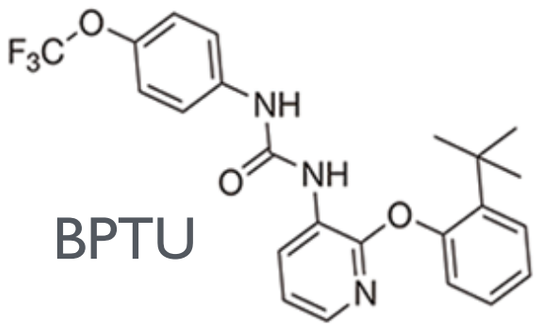
$\Delta G_b^{0}{}_{(calc)} = -10.3 \pm 0.5$ kcal/mol

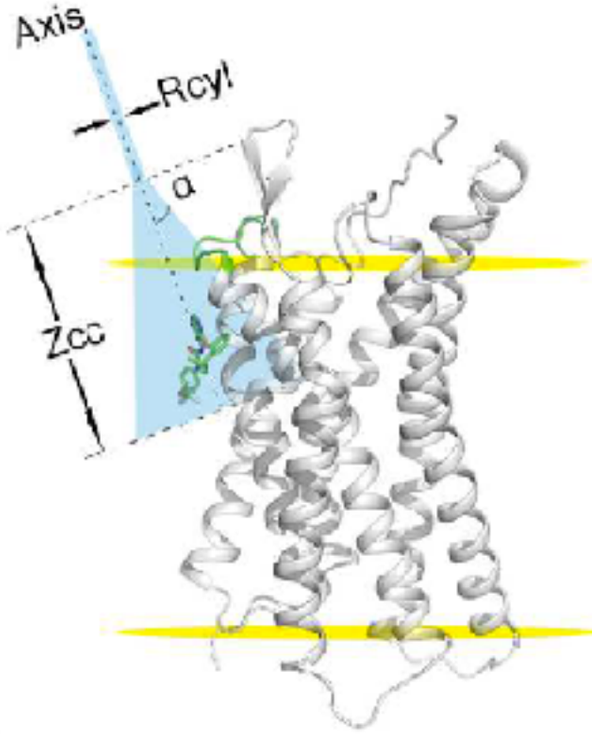
$\Delta G_b^{0}{}_{(exp)} = -9.9 \pm 0.2$ kcal/mol

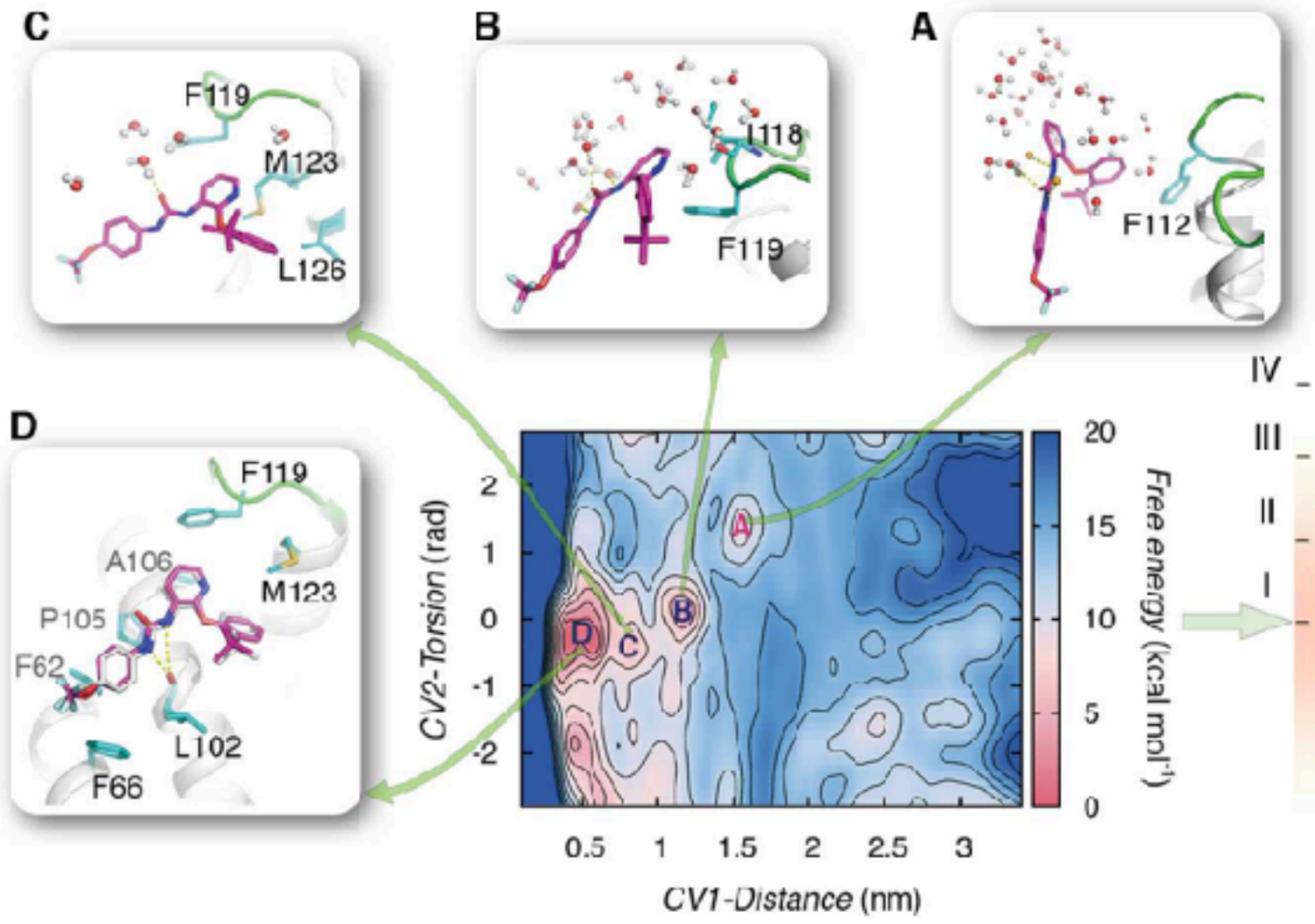


Limongelli and co. *PNAS* (2017)

Allostery in the $P2Y_1$ GPCR

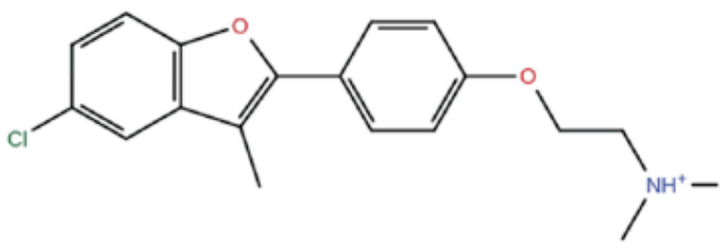


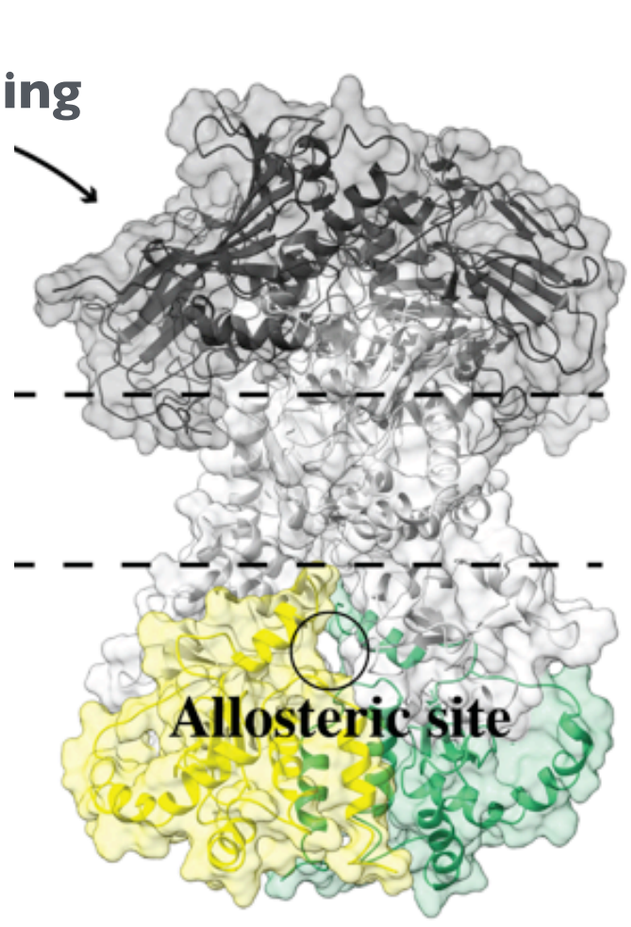


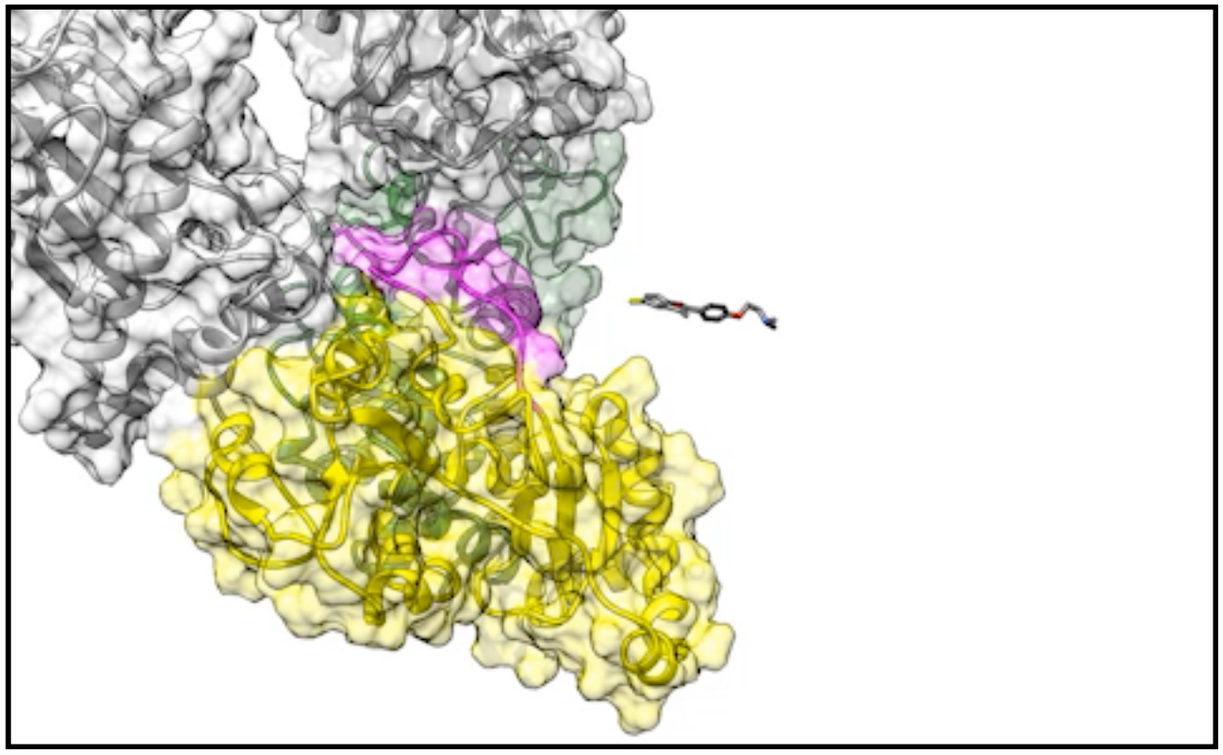


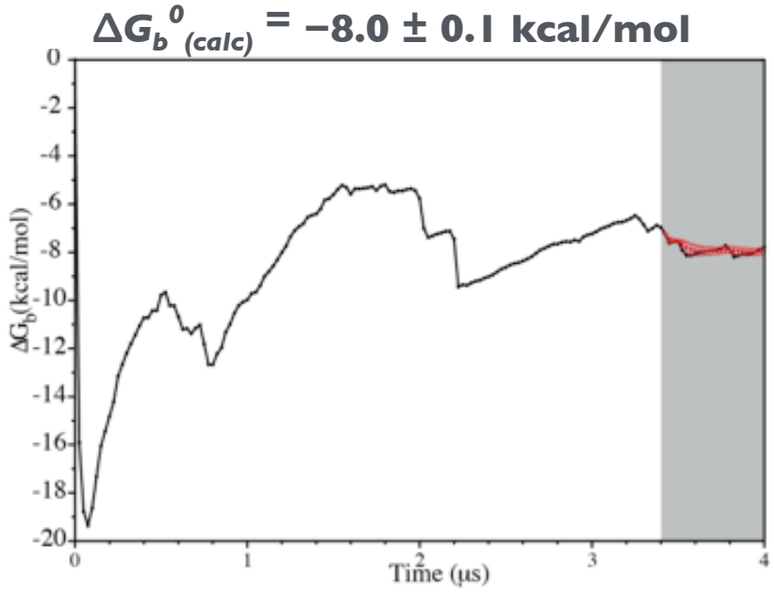
Yuan, Raniolo, **Limongelli**, Xu, *JCTC* (2018)

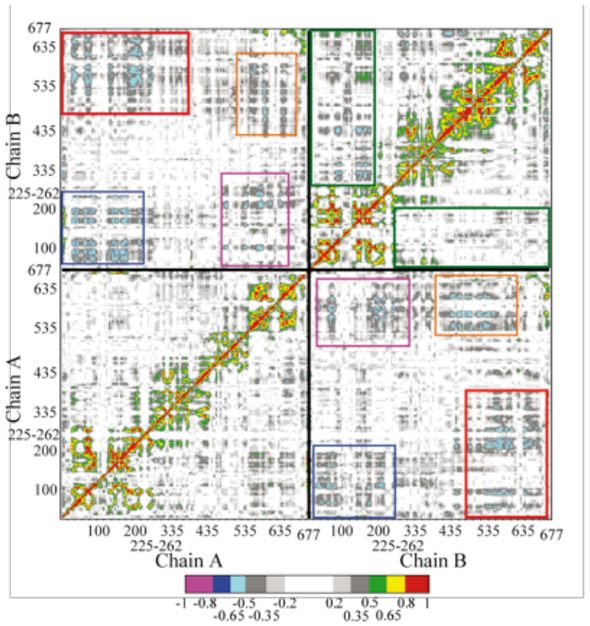
Allostery in HSP90











D'Annessa, Raniolo, **Limongelli**, Di Marino, Colombo *JCTC* (2019)

Funnel-Metadynamics Advanced Protocol (FMAP)

nature protocols 2020 PROTOCOL
https://doi.org/10.1038/s41596-020-0342-4

Check for updates

Ligand binding free-energy calculations with funnel metadynamics

Stefano Raniolo[1] and Vittorio Limongelli[1,2]✉

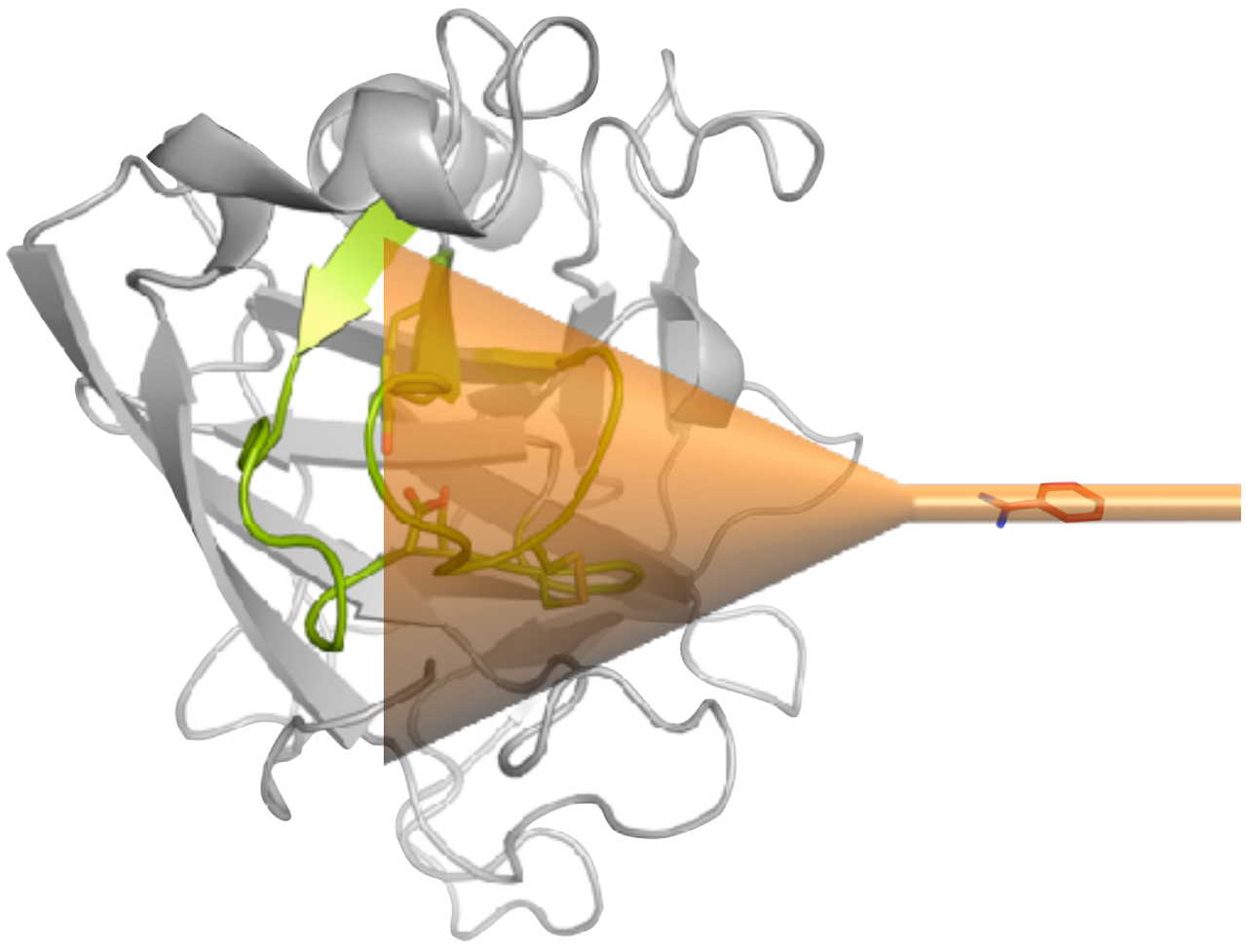
1. Pre-processing

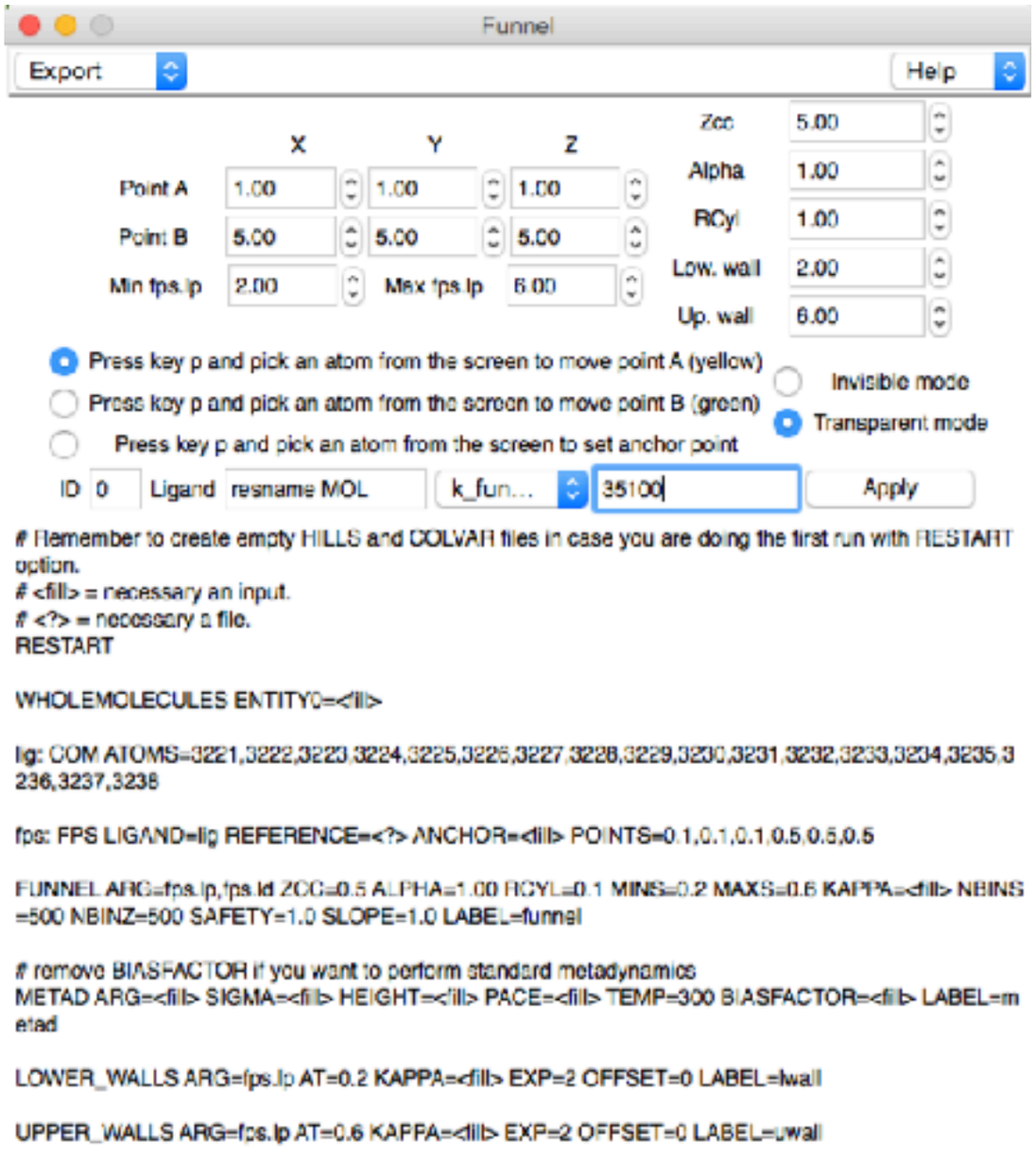
2. FM Simulation

3. Post-processing

Funnel-Metadynamics Advanced Protocol (FMAP)

1. Pre-processing





Raniolo & **Limongelli** *Nature Protocols* (2020)

```
[Info)    Atoms: 3238
[Info)    Bonds: 3275
[Info)    Angles: 0  Dihedrals: 0  Impropers: 0  Cross-terms: 0
[Info)    Bondtypes: 0  Angletypes: 0  Dihedraltypes: 0  Impropertypes: 0
[Info)    Residues: 224
[Info)    Waters: 0
[Info)    Segments: 1
[Info)    Fragments: 2   Proteins: 1   Nucleics: 0
[Info) Finished with coordinate file /Users/Sannian/video/start.pdb.
ERROR) BaseMolecule: attempt to init atoms while structure building in progress!
ERROR) Invalid number of atoms in file: 104905
[Info) Using plugin xtc for coordinates from file /users/Sannian/video/temp_1.xtc
ERROR) Incorrect number of atoms (104906) in
ERROR) coordinate file /Users/Sannian/video/temp_1.xtc
ERROR) Mismatch between existing molecule or structure file atom count and coord
inate or trajectory file atom count.
[Info) Finished with coordinate file /Users/Sannian/video/temp_1.xtc.
[Info) Using plugin trr for coordinates from file /Users/Sannian/video/cut.trr
[Info) Finished with coordinate file /Users/Sannian/video/cut.trr.
[Info) In any publication of scientific results based in part or
[Info) completely on the use of the program STRIDE, please reference:
[Info)  Frishman,D & Argos,P. (1995) Knowledge-based secondary structure
[Info)  assignment. Proteins: structure, function and genetics, 23, 566-579.
```

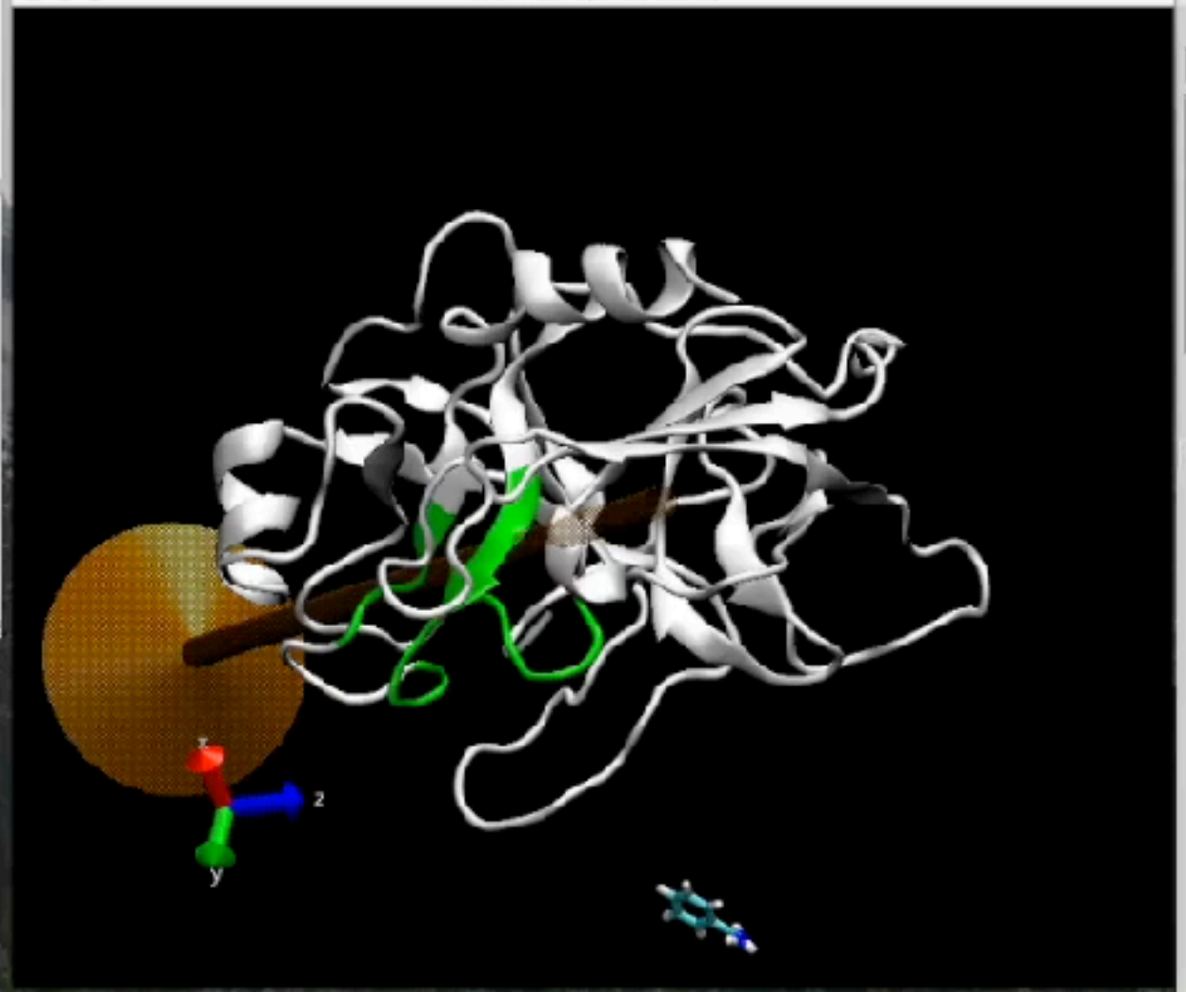
VMD Main

File Molecule Graphics Display Mouse Extensions Help

ID	T	A	D	F	Molecule	Atoms	Frames	Vol
0	T	A	D	F	start.pdb	3238	10	0

zoom | Loop | step | speed

VMD 1.5.3 OpenGL Display



Graphical Representations

Selected Molecule

0. start.pdb

Create Rep | Delete Rep

Style	Color	Selection
NewCartoon	ColorID 8	all
Licorice	Name	resname MO
NewCartoon	ColorID 7	same resid

Selected Atoms

all

Draw style | Selections | Trajectory | Periodic

Coloring Method | Material

ColorID | 8 | Opaque

Drawing Method

NewCartoon | Default

Spline Style: Catmull-Rom

Aspect Ratio: 4.10

Thickness: 0.30

Resolution: 10

Apply Changes Automatically | Apply

VMD TkConsole

Tunnel

Export | Help

	X	Y	Z
Point A	1.00	1.00	1.00
Point B	5.00	5.00	5.00
Min fps.lp	2.00	Max fps.lp	6.00

Zoc 6.00
Alpha 1.00
nCyl 1.00
Low. wall 2.00
Up. wall 6.00

Press key p and pick an atom from the screen to move point A (yellow)
Press key p and pick an atom from the screen to move point B (green)
Press key p and pick an atom from the screen to set anchor point

Invisible mode
Transparent mode

ID 0 Ligand | Apply

Facoltà
di
scienze
biomediche

Funnel-Metadynamics Advanced Protocol (FMAP)

nature protocols 2020 PROTOCOL
https://doi.org/10.1038/s41596-020-0342-4

Check for updates

Ligand binding free-energy calculations with funnel metadynamics

Stefano Raniolo[1] and Vittorio Limongelli[1,2]✉

1. Pre-processing

2. FM Simulation

3. Post-processing

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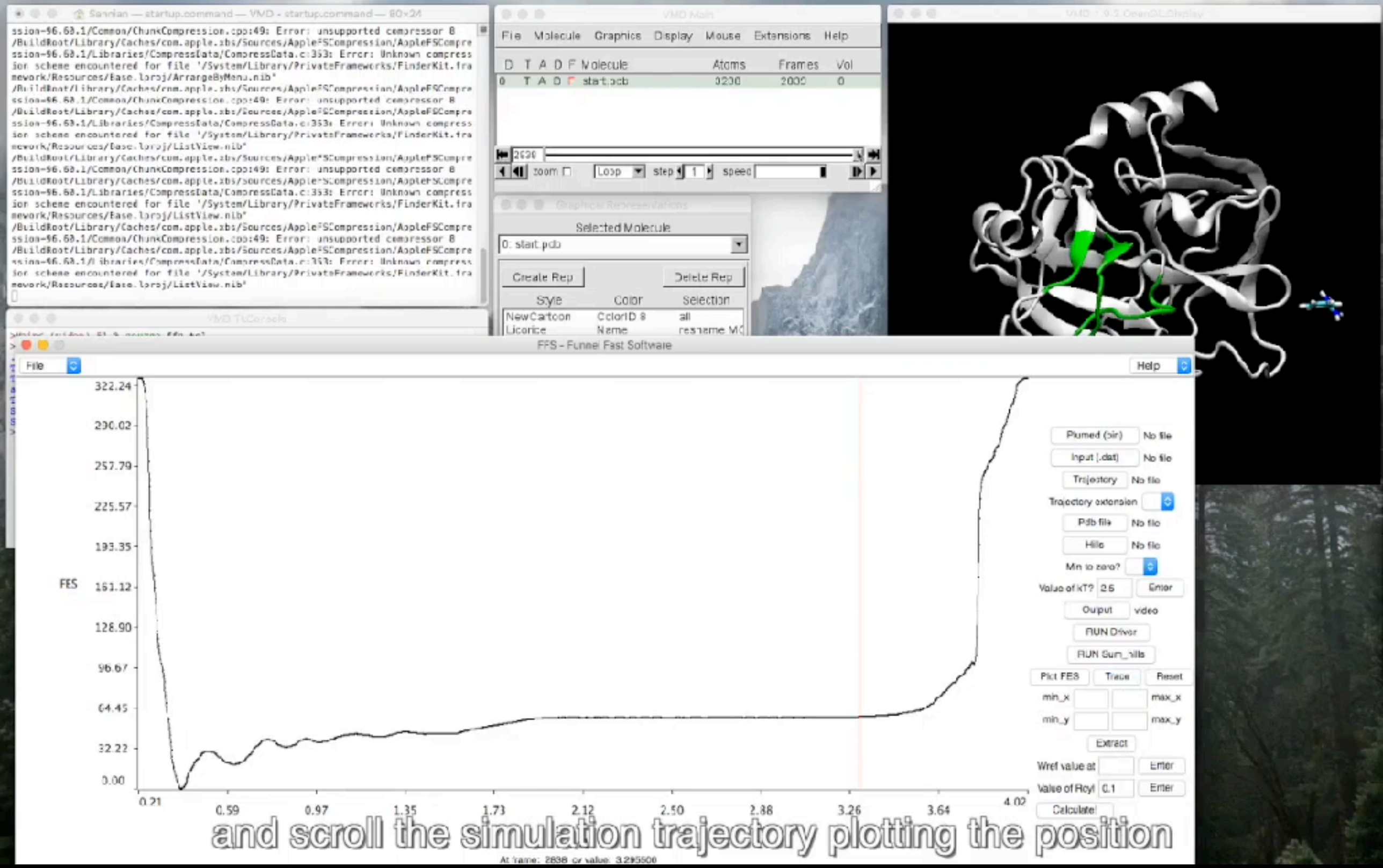
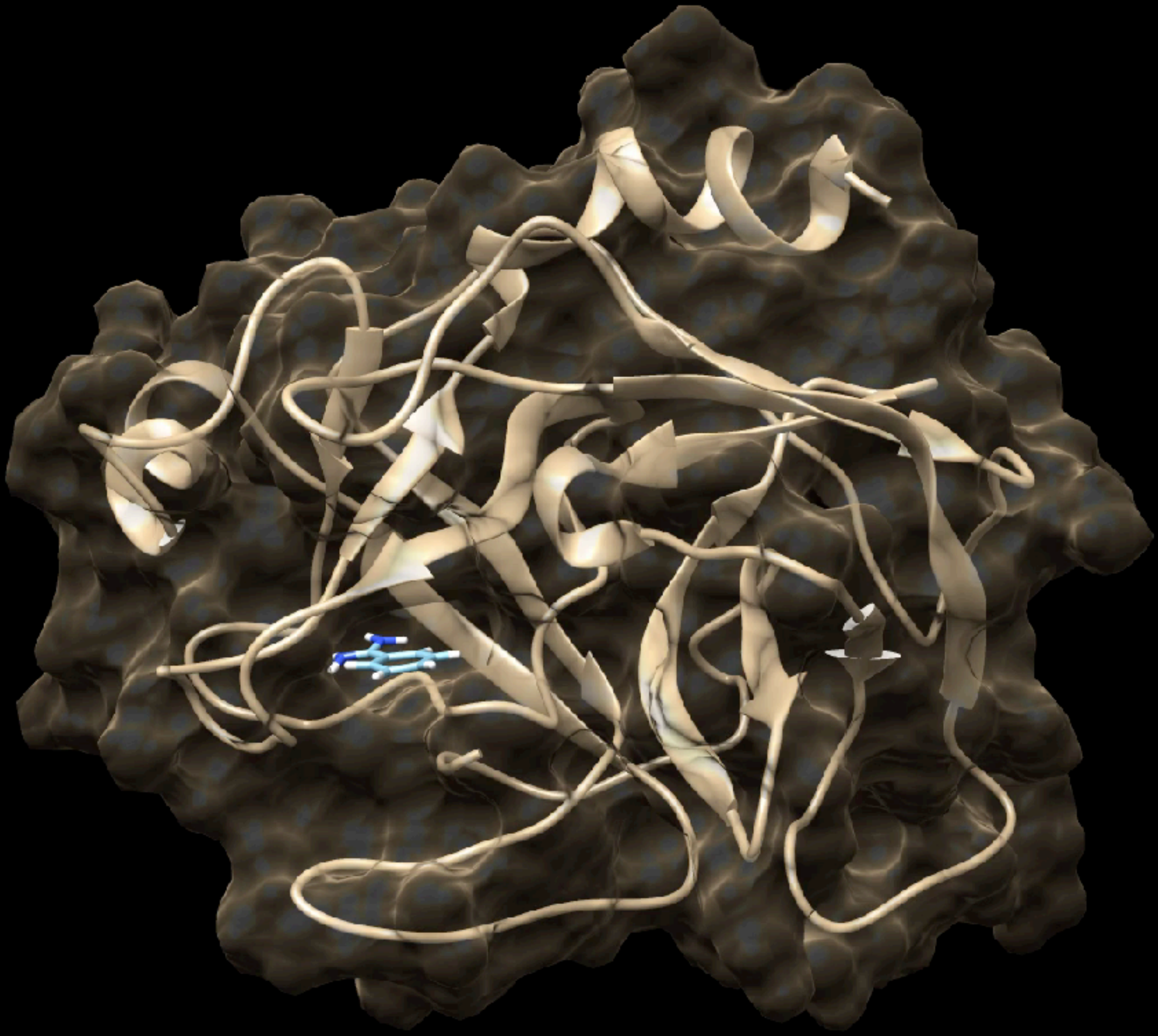

FFS - Funnel Fast Software
File
Help
322.24
290.02
257.79
225.57
193.35
161.12
128.90
96.67
64.45
32.22
0.00
FES
0.21
0.59
0.97
1.35
1.73
2.12
2.50
2.88
3.26
3.64
4.02
Plumed (dir)
No file
Input [.dat]
No file
Trajectory
No file
Trajectory extension
Pdb file
No file
Hills
No file
Min to zero?
Value of kT?
2.5
Enter
Output
video
RUN Driver
RUN Sum_hills
Plot FES
Trace
Reset
min_x
max_x
min_y
max_y
Extract
Wref value at
Enter
Value of Rcyl
0.1
Enter
Calculate!
and scroll the simulation trajectory plotting the position



Funnel-Metadynamics (FM) + FMAP

https://sites.google.com/view/vittoriolimongelli/software?authuser=0

Limongelli, Bonomi, Parrinello *PNAS* (2013)
Raniolo, **Limongelli** *Nat. Protoc.* (2020)

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www.plumed-nest.org

Bonomi, Bussi, Camilloni, Tribello…**Limongelli** et al. *Nature Methods* (2019)