

MD Simulations – day 2

Part 2a - Analysis



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for Prof. Fuxreiter's course @ University of Padova

May 2024

<https://github.com/giorginolab/MD-Tutorial-Data>

Planning for today

- Part 1 (~11,10-12,30?)
 - MD analysis with the GSN case + h.o.
- Part 2 (~12,45-14,00?)
 - Markov state models + h.o.
- Part 3 (~14,10-15,00?)
 - Docking + h.o.

Part I

Giorgino T, Mattioni D, Hassan A, Milani M, Mastrangelo E, Barbiroli A, et al.
Nanobody interaction unveils structure, dynamics and proteotoxicity of the Finnish-type amyloidogenic gelsolin variant. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*. 2019 Mar 1;1865(3):648–60.

[Journal link.](#)

[Preprint.](#)

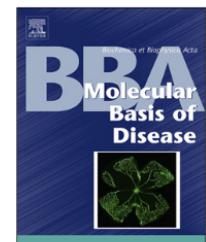
BBA - Molecular Basis of Disease 1865 (2019) 648–660



Contents lists available at ScienceDirect

BBA - Molecular Basis of Disease

journal homepage: www.elsevier.com/locate/bbadis



Nanobody interaction unveils structure, dynamics and proteotoxicity of the Finnish-type amyloidogenic gelsolin variant



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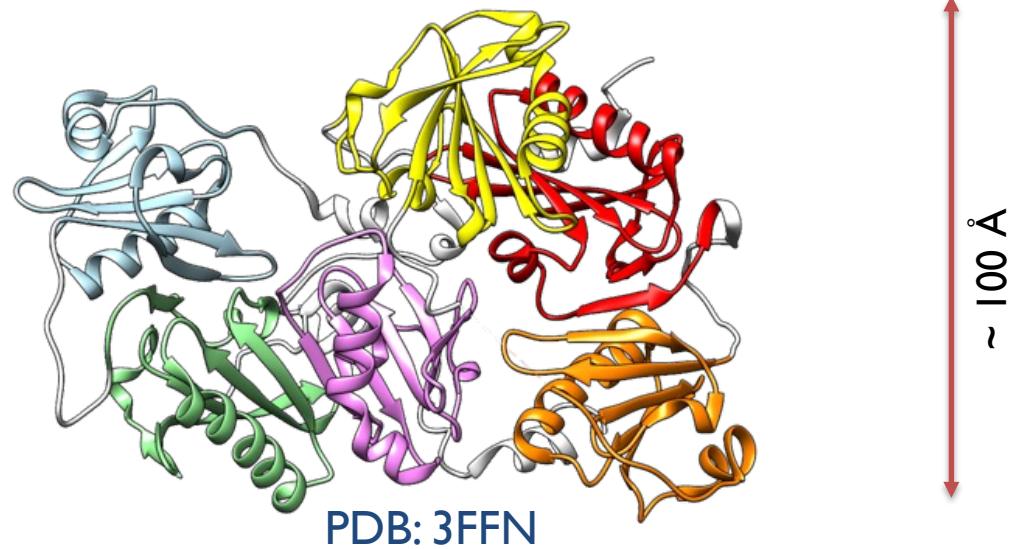
^d Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente, Università degli Studi di Milano, Milano, Italy

^e Department of Molecular Medicine, Department of Molecular and Cellular Neuroscience, Dorris Neuroscience Center, The Scripps Research Institute, La Jolla, CA 92037, USA

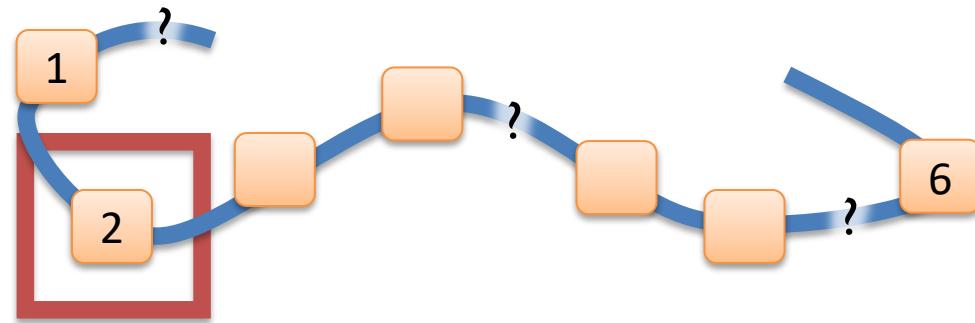
^f Nanobody Lab, Department of Biochemistry, Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium



No Ca^{2+} .
Closed form.
Resolved (2008).



$\sim \text{mM Ca}^{2+}$.
Active form.
Dynamic. Elusive.



However, crystallisation of Ca^{2+} -bound
isolated domains has been successful.

AGel amyloidosis

Also known as...

FAMILIAL Am., FINNISH TYPE

Am., MERETOJA TYPE

Am. DUE TO MUTANT GELSOLIN

& permutations

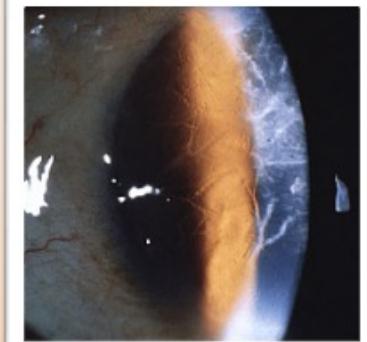
Am. V

Am. CRANIAL NEUROPATHY WITH LATTICE CORNEAL DYSTROPHY
HEREDITARY GELSOLIN Am.

"AGel amyloidosis is a rare, usually systemic amyloidosis characterized by a triad of ophthalmologic, neurologic and dermatologic findings due to the deposition of **gelsolin amyloid fibrils** in these tissues".

- Rare, but endemic in Finland

"... was 1/1,040 among 182,000 inhabitants, whereas in 1860 in the parish Valkeala the prevalence was calculated at 1/155. Today the true prevalence probably lies between these figures." (Meretoja 1973)
- Inherited, autosomal dominant
- Caused by GSN gene mutations
- No cure, just symptomatic treatments



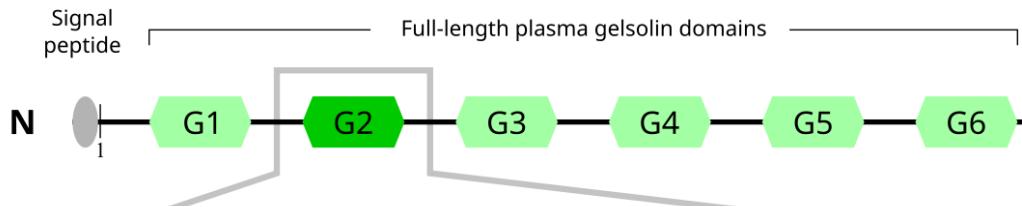
Corneal lattice dystrophy



Cutis laxa

Mutations causing AGel

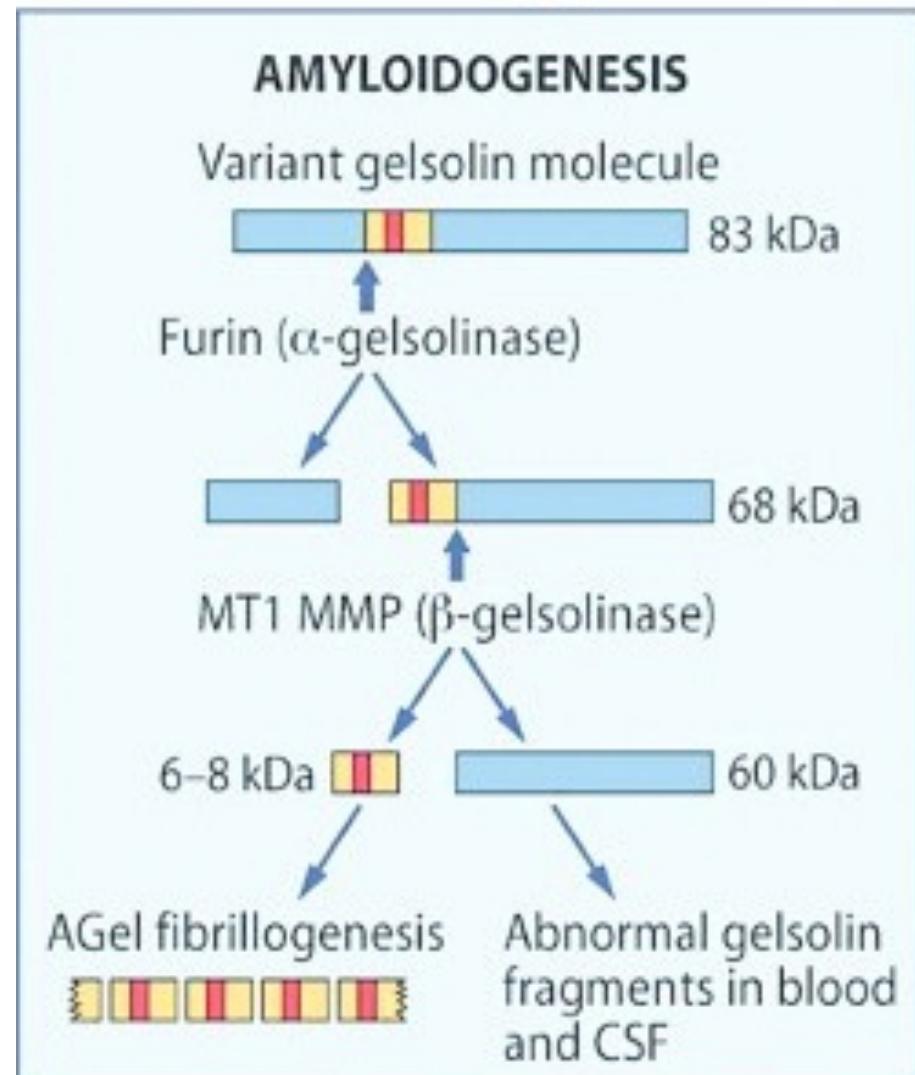
- D187N Systemic, Finnish
- D187Y Systemic, Danish
- G167R Renal
- N184K Renal



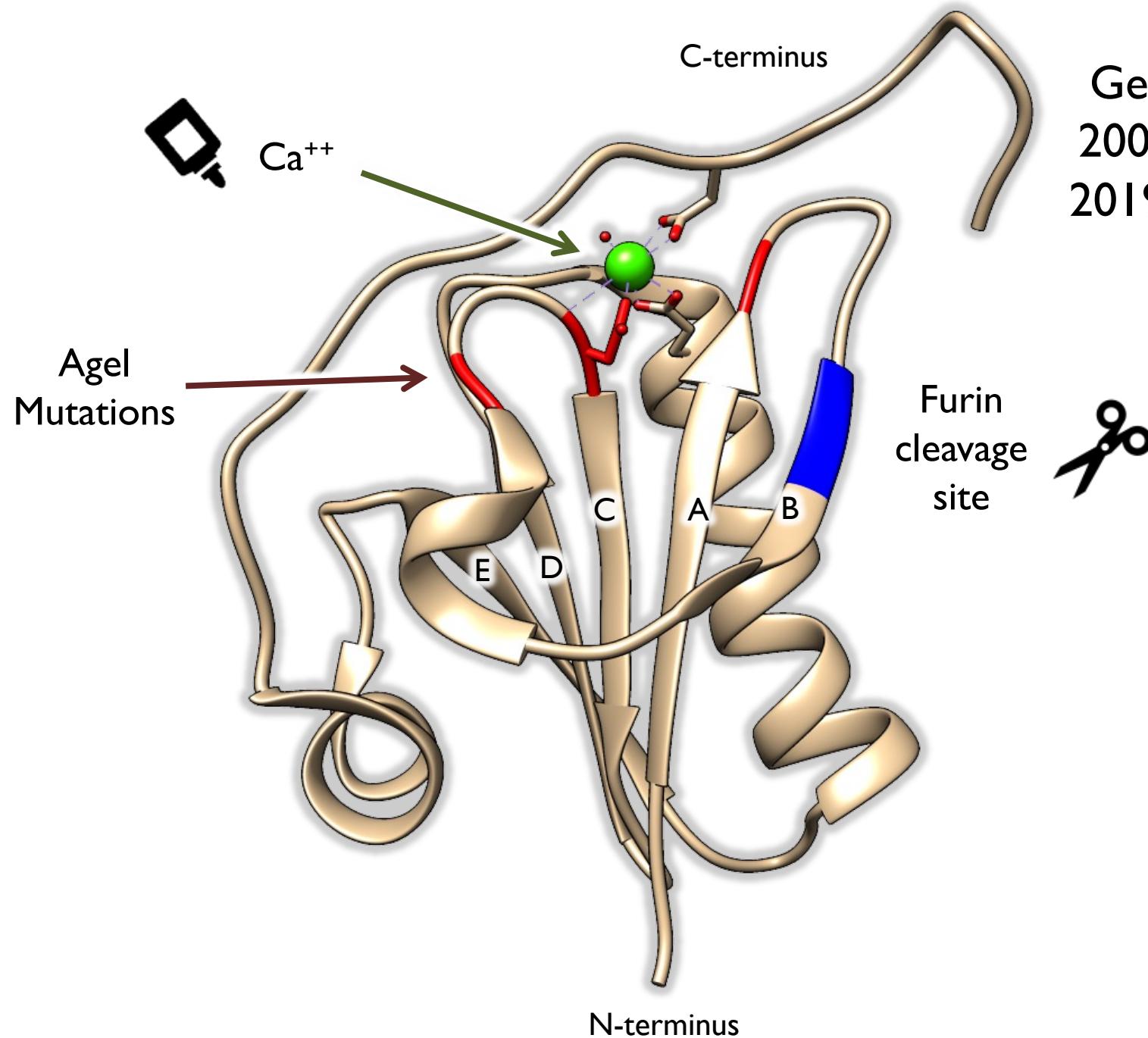
All in G2 domain



- WT plasma GSN encounters furin without consequence since WT is correctly folded.
- Mutant plasma GSN has a structural intermediate state exposing the cleavage site, and undergoes pathological furin processing.



Gelsolin G2 WT
2002 Cd²⁺ IKCQ
2019 Ca²⁺ **6QW3**



Mutant	Structure	Year	Disease form
WT	1KCQ	2002	Sporadic
N184K	5FAF	2015	Renal (rare)
G167R	5O2Z	2017	Renal (rare)
D187N	???		Systemic, Finnish
D187Y	???		Systemic, Danish

Observation: D187X mutants lose Ca^{2+} coordination

Hypothesis: Is this indication of an **order-disorder** shift?

Support for the hypothesis:

- Inability to crystallize
- GSN NMR data *
- Known Ca^{2+} -regulated disorder/order transitions: sortase, a-cyc toxin, etc.
- Thermodynamic stabilities[§]: WT ≠ mutant

* Kazmirska et al. Nat Struct Mol Biol. 2002 Feb;9(2):112

§ Thermal and chemical

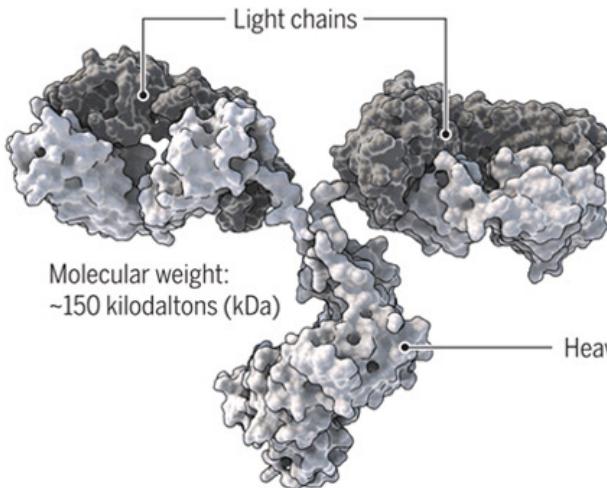


Nanobodies

VHH, variable domain of hcAbs \sim 15 kDa

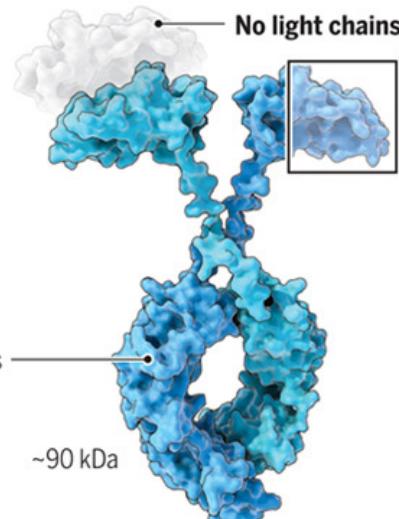
Downsizing antibodies

Human blood teems with conventional antibodies—bulky, Y-shaped proteins that home in on bacteria and viruses. The small antibodies produced by sharks and the camel family differ from those immune molecules not only in size, but also in their structure and binding ability.



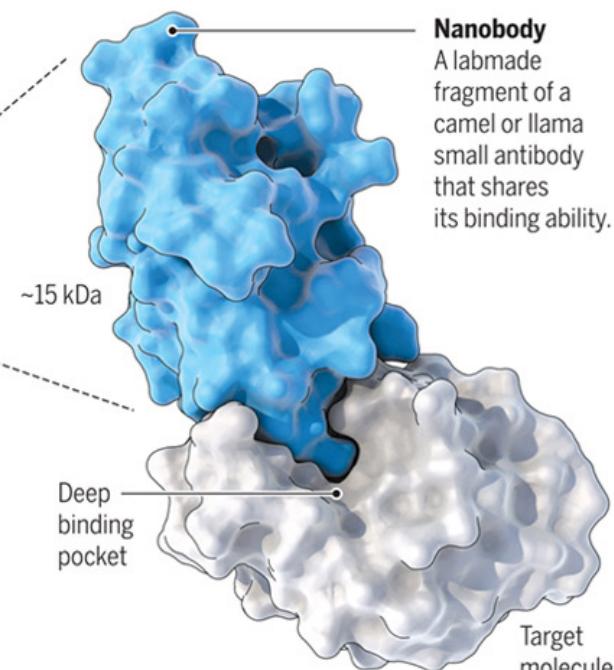
Typical antibody

Two light and two heavy chains intertwine to make a protein that can identify and affix to bits of pathogens or other molecules.



Small antibody

This slimmed-down variety lacks light chains but can still bind to its targets.



Nanobody binding

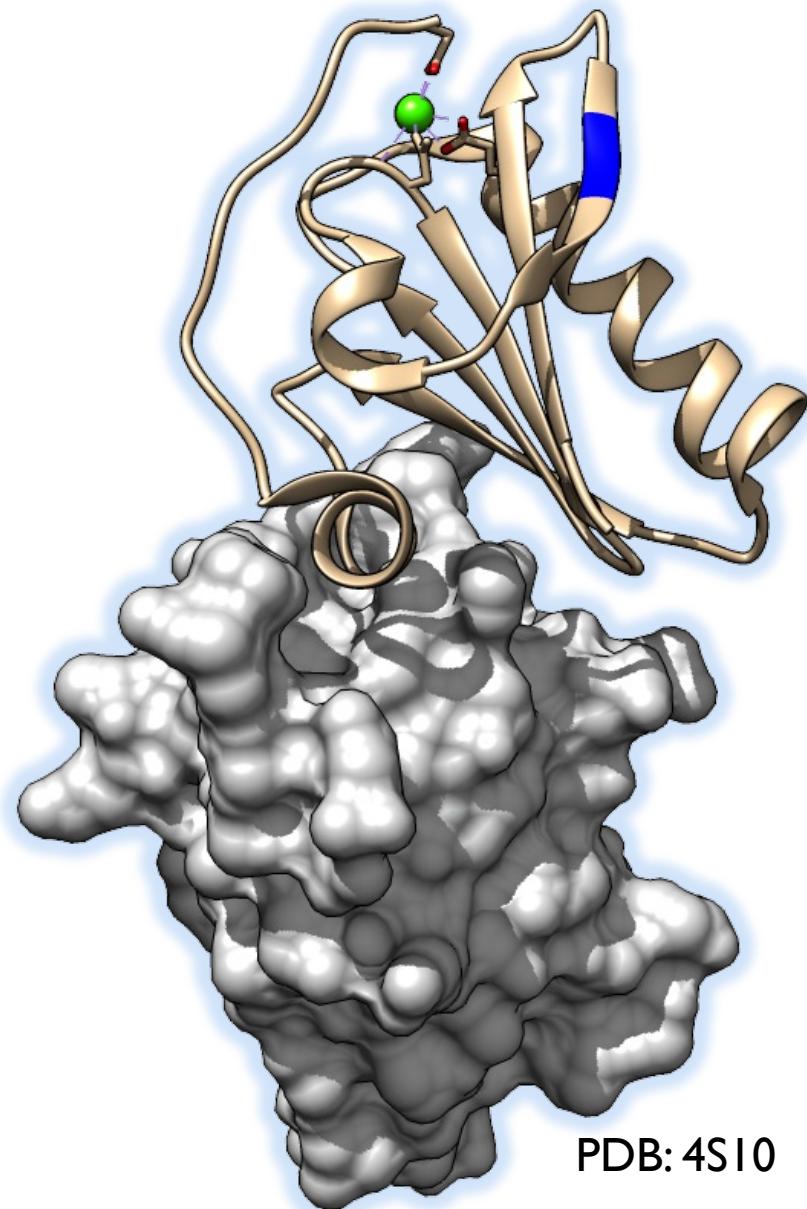
Because of its binding style, a nanobody can fit into crevices on molecules.

2015: Gettemans et al. – G2 Nanobodies

- Inoculate llamas with GSN **WT** G2
- Extract nanobodies (NbII)
- Sequence them
- Obtain WT:Nb structure

Idea

- Can the WT-raised NbII re-stabilize D187N enough to allow crystallization?

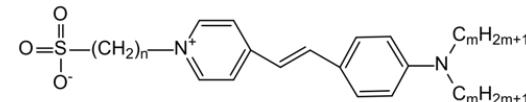
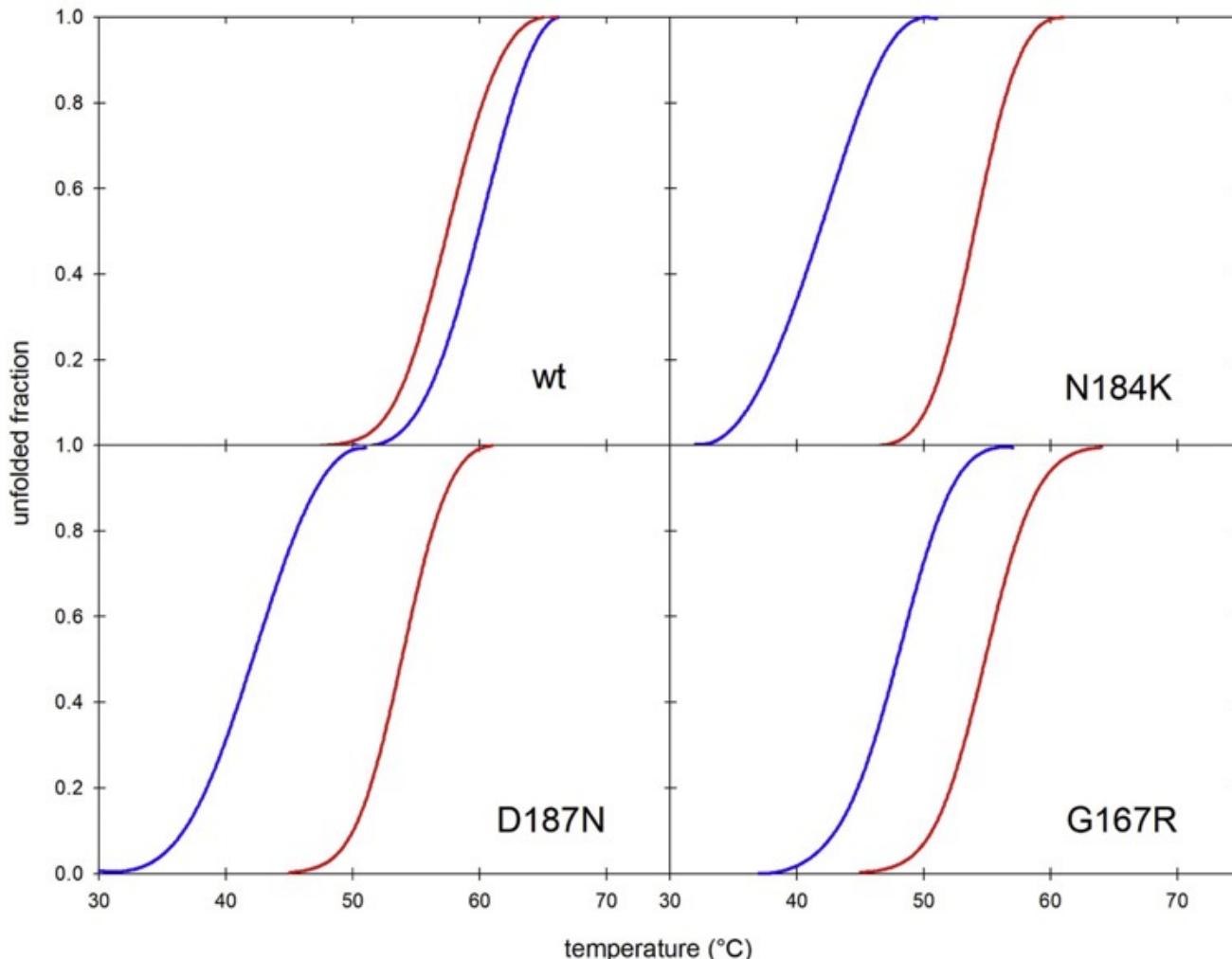


Does NbII increase stability?

→ Thermofluor (differential scanning fluorimetry) experiments: measure the temperature exposing the hydrophobic core.



Mutants are destabilized
NbII-binding re-stabilizes them

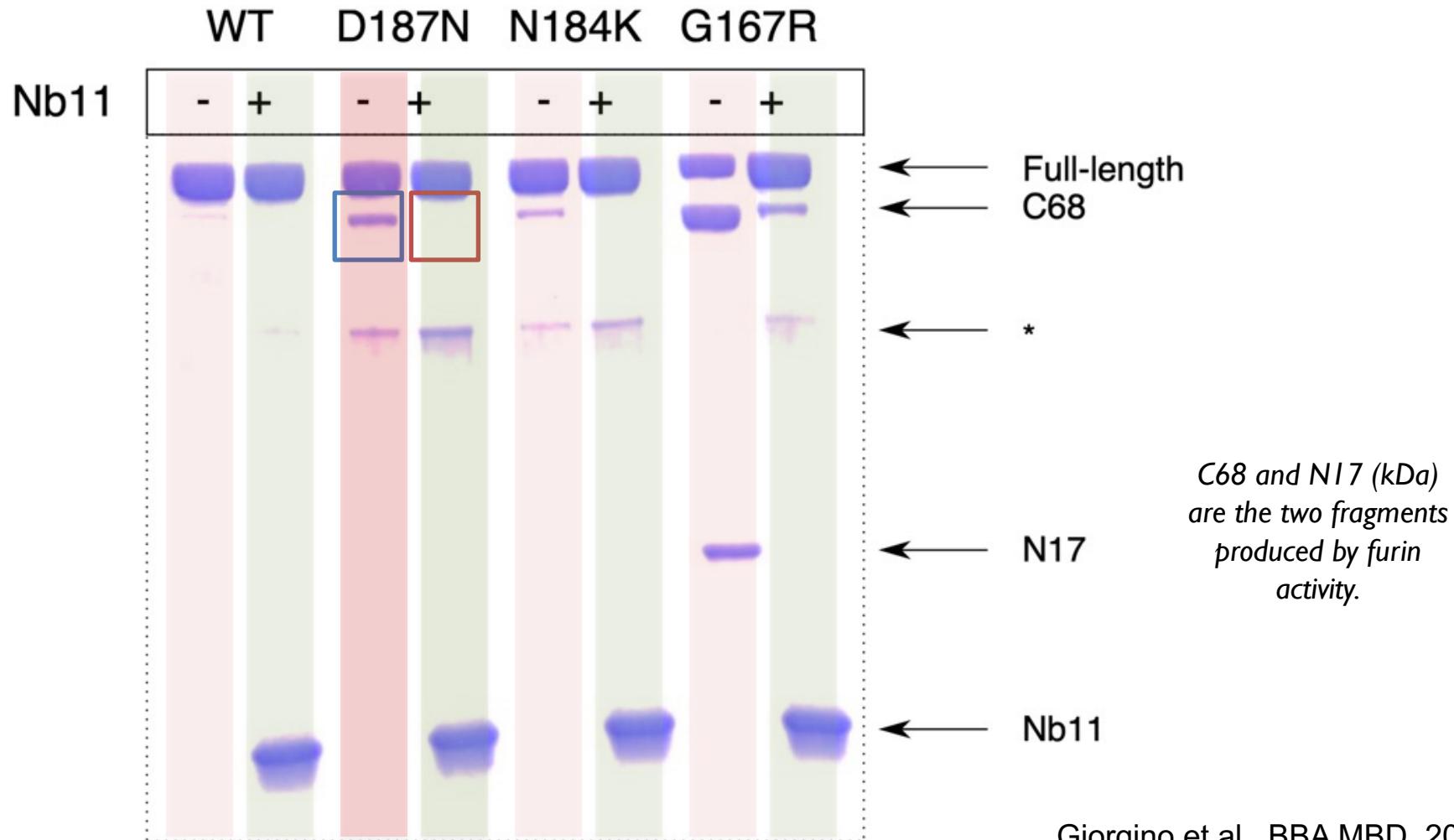


SYPRO Orange binds nonspecifically to hydrophobic surfaces, and water strongly quenches its fluorescence. When the protein unfolds, the exposed hydrophobic surfaces bind the dye, resulting in an increase in fluorescence by excluding water.

Does NbII protect from proteolysis?

→ Furin proteolysis experiments

Mutants are cleaved
NbI I-binding protects them



6H1F: Gelsolin G2+nanobody

[Structure Summary](#)
[3D View](#)
[Annotations](#)
[Experiment](#)
[Sequence](#)
[Genome](#)
[Versions](#)

Biological Assembly 1 



 [3D View: Structure | 1D-3D View](#)

[Electron Density](#) | [Validation Report](#) | [Ligand Interaction](#)

6H1F

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Structure of the nanobody-stabilized gelsolin D187N variant (second domain)

PDB DOI: [10.2211/pdb6H1F/pdb](https://doi.org/10.2211/pdb6H1F/pdb)

Classification: **STRUCTURAL PROTEIN**

Organism(s): *Lama glama*, *Homo sapiens*

Expression System: *Escherichia coli*

Mutation(s): Yes 

Deposited: 2018-07-11 Released: 2019-01-23

Deposition Author(s): [Hassan, A.](#), [Milani, M.](#), [Mastrangelo, E.](#), [de Rosa, M.](#)

Funding Organization(s): Amyloidosis Foundation

Experimental Data Snapshot

Method: X-RAY DIFFRACTION

Resolution: 1.90 Å

R-Value Free: 0.233

R-Value Work: 0.199

R-Value Observed: 0.202

wwPDB Validation

 [3D Report](#)  [Full Report](#)

Metric	Percentile Ranks	Value
Rfree		0.234
Clashscore		6
Ramachandran outliers		0
Sidechain outliers		0
RSRZ outliers		5.2%

Worse  Better 

 Percentile relative to all X-ray structures
 Percentile relative to X-ray structures of similar resolution

This is version 1.0 of the entry. See complete history.

Find Similar Assemblies

Biological assembly 1 assigned by authors and generated by PISA (software)

Biological Assembly Evidence: gel filtration

Macromolecule Content

- Total Structure Weight: 28.49 kDa 
- Atom Count: 1,896 
- Modelled Residue Count: 229 
- Deposited Residue Count: 259 
- Unique protein chains: 2

Literature

 [Download Primary Citation](#)

Nanobody interaction unveils structure, dynamics and proteotoxicity of the Finnish-type amyloidogenic gelsolin variant.

[Giorgino, T.](#), [Matianni, D.](#), [Hassan, A.](#), [Milani, M.](#), [Mastrangelo, E.](#), [Barbiroli, A.](#), [Verhelle, A.](#), [Gettemans, J.](#), [Barzago, M.M.](#), [Diomedede, L.](#), [de Rosa, M.](#)

(2019) *Biochim Biophys Acta Mol Basis Dis* **1865**: 648-660

PubMed: [30625383](https://pubmed.ncbi.nlm.nih.gov/30625383/) [Search on PubMed](#)

DOI: [10.1016/j.bbadiis.2019.01.010](https://doi.org/10.1016/j.bbadiis.2019.01.010)

Primary Citation of Related Structures:

3D Protein Feature View: 6H1F

Help Back

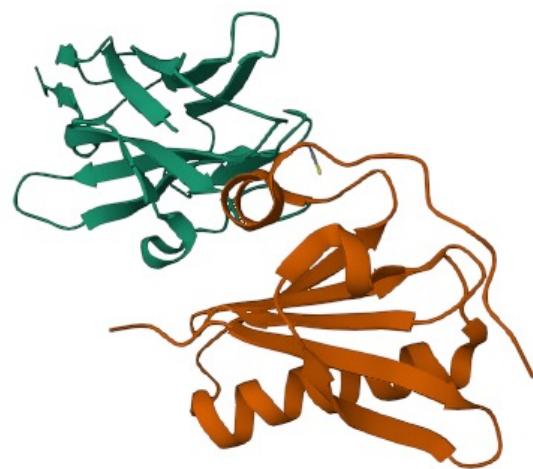
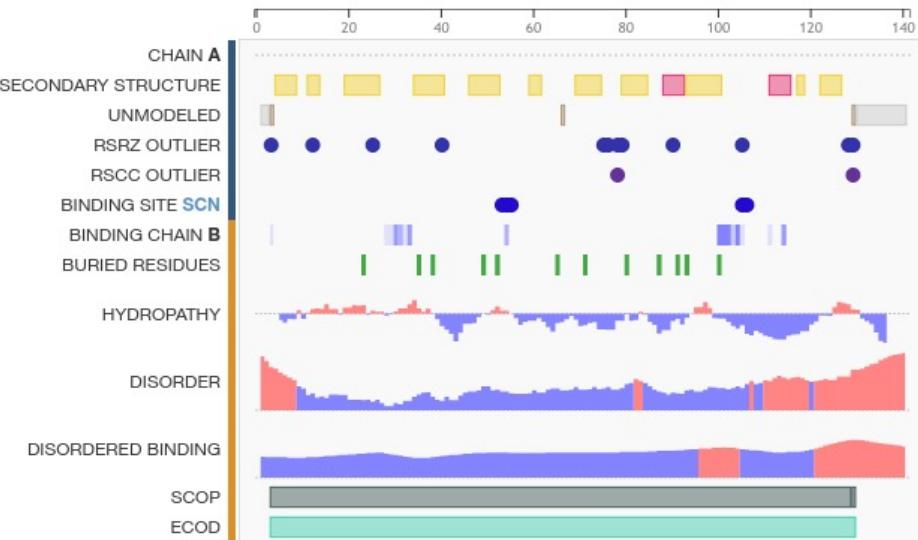
Residue

Structure of the nanobody-stabilized gelsolin D187N variant (second domain)

Chain

A

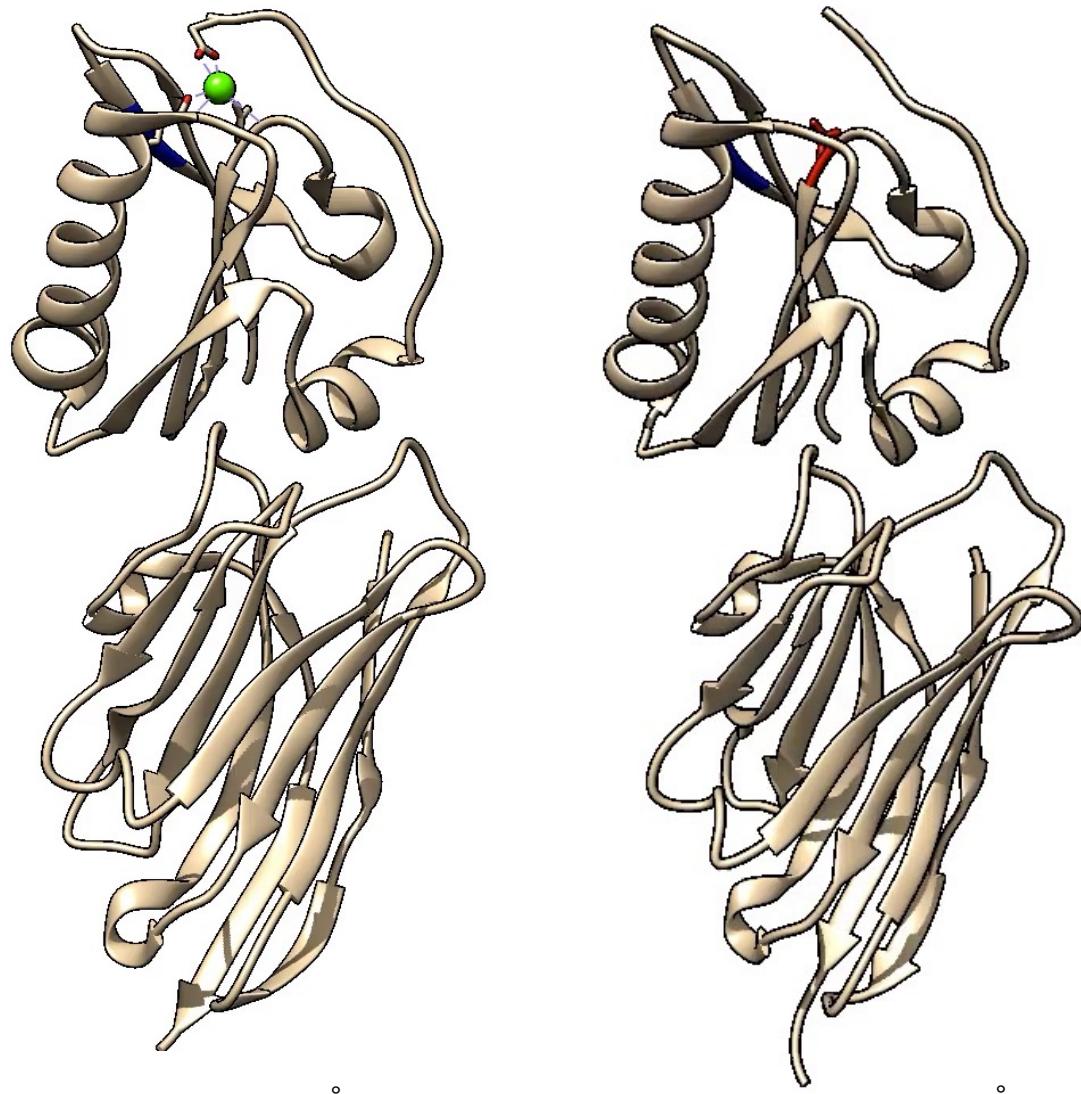
Gelsolin nanobody - Lama glama



Three puzzles!

WT:NbII complex compared to D187N:NbII.

- I. WT and **D187N** are **virtually identical***: same structure, different function
2. NbII binds far from the furin cleavage site...
3. ...and far from the **Ca²⁺** ion



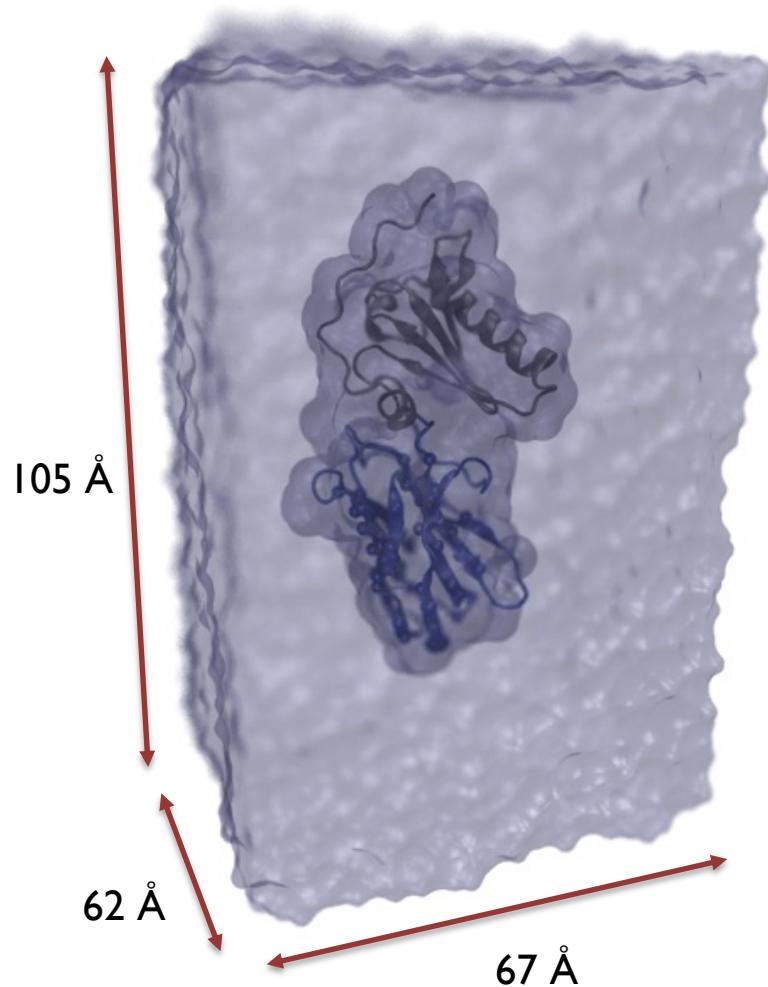
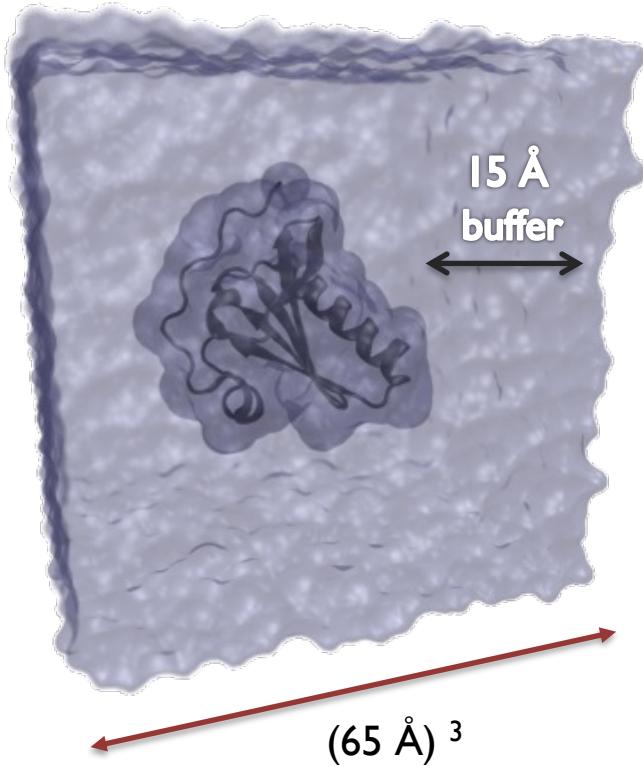
* Except Ca²⁺ binding

GSN \pm NbI MD simulations

- Unbiased sampling @300 °K
- 100 mM NaCl
- Harmonic restraints:
SS NbI @ 0.03 kcal/mol/Å²

CHARMM36

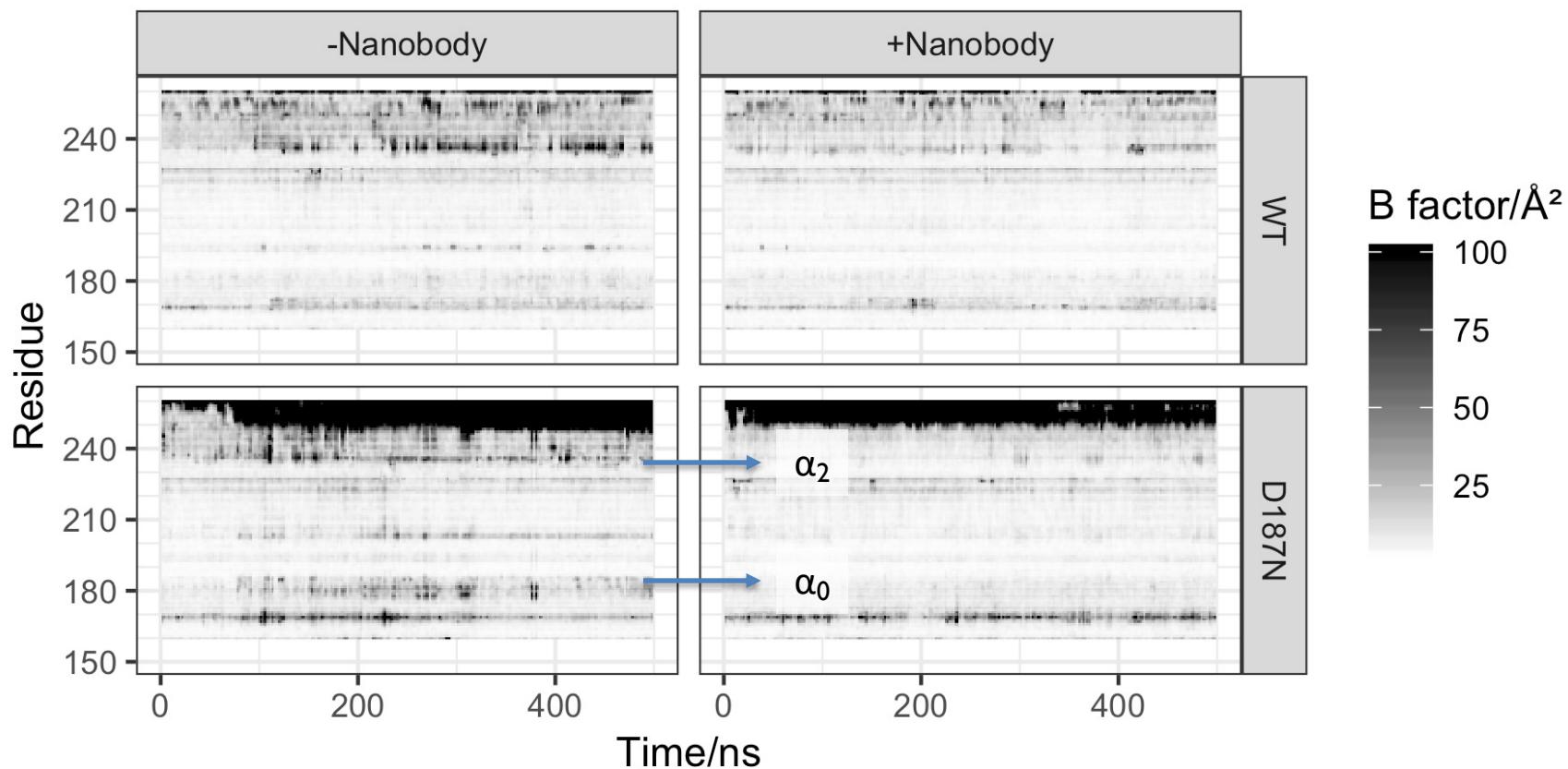
~3 μs tot. ~25k/43k atoms



MD results



Sample	Nb11	Ca ²⁺	Simulated time (ns)	C-terminal disorder onset
WT _{G2}	-	+	800	Not observed
WT _{G2}	+	+	750	Not observed
D187N _{G2}	-	-	748	After 83 ns
D187N _{G2}	+	-	512	After 40 ns

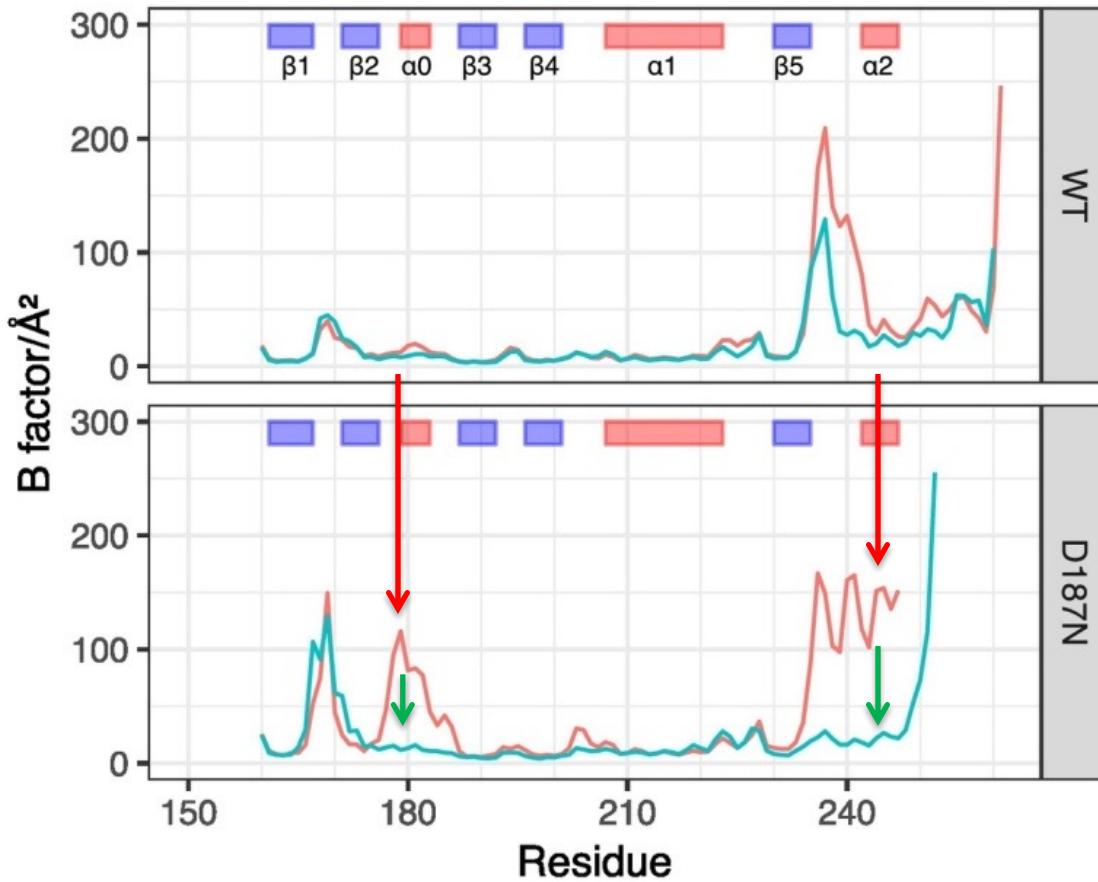


A matter of dynamics?

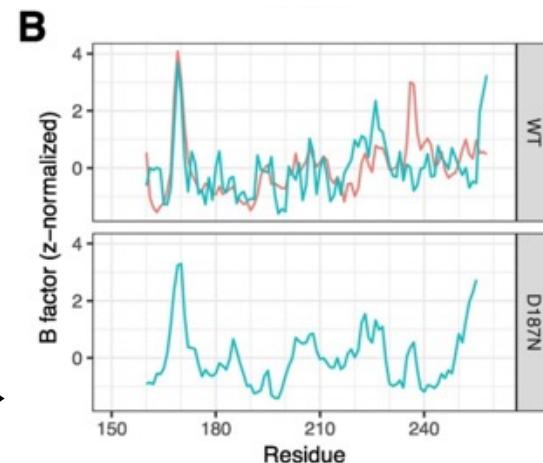
$$B = (8\pi^2/3) \text{ RMSF}^2$$



—Nanobody
—+Nanobody



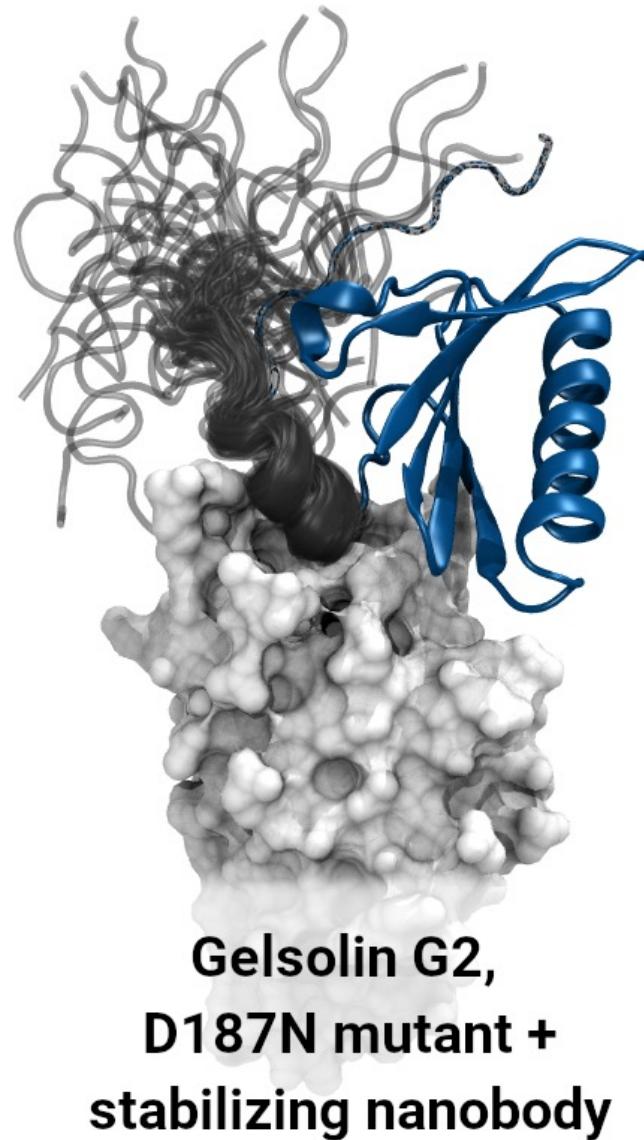
MD vs exp. B-factors →



Reduction of disorder at C-terminus



**Gelsolin G2,
D187N mutant**



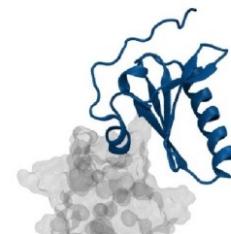
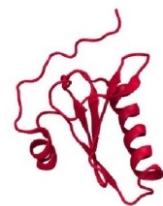
**Gelsolin G2,
D187N mutant +
stabilizing nanobody**

C

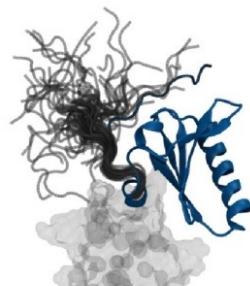
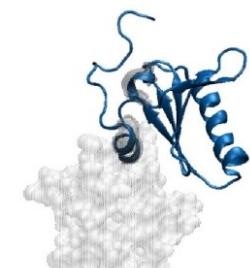
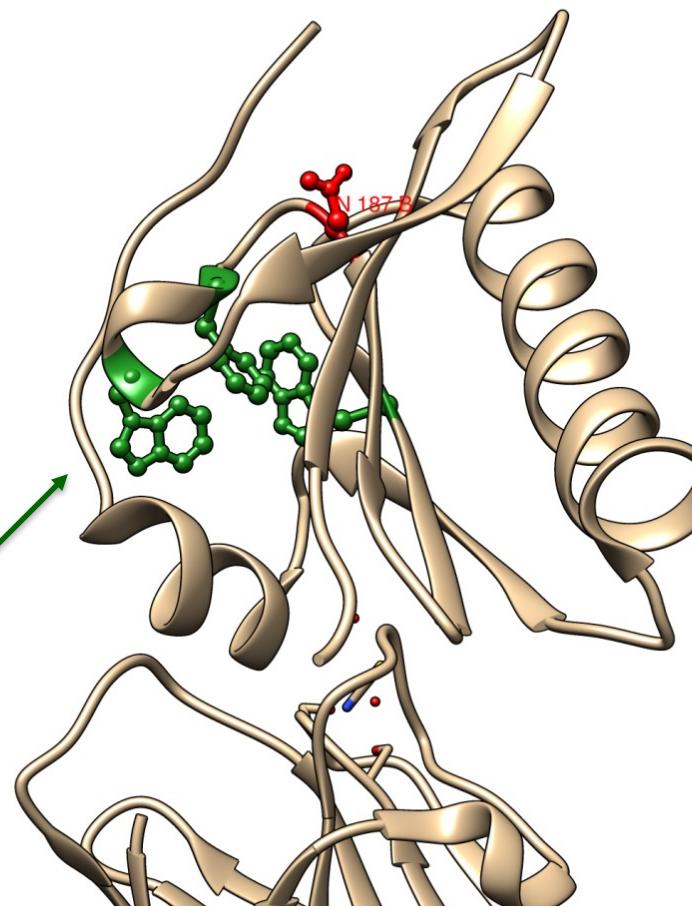
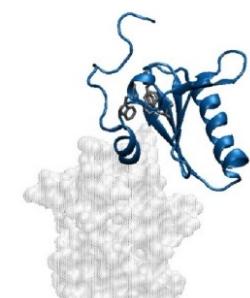
D187N

D187N
+Nb11

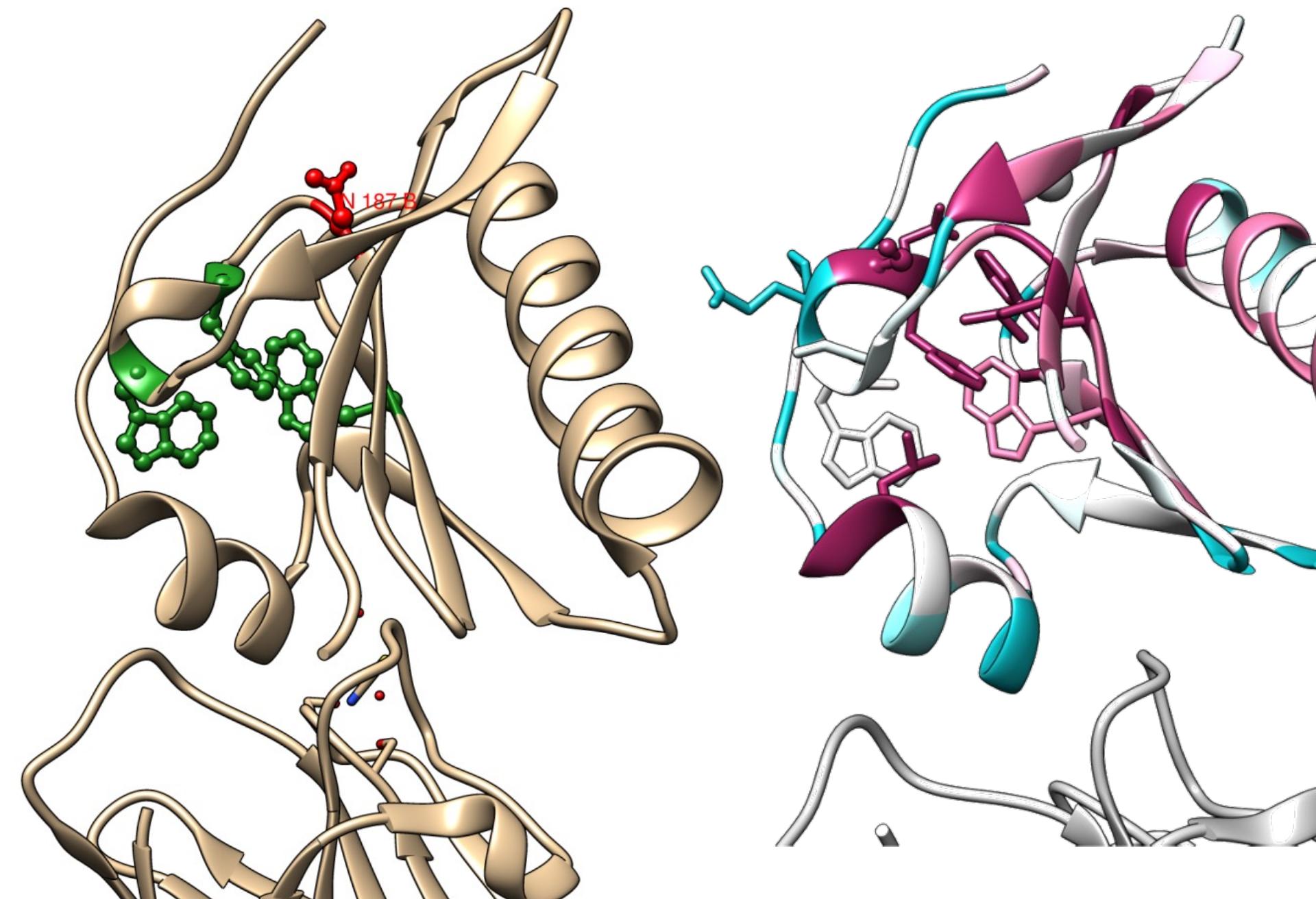
Initial configuration



Reduction of C-terminus entropy

Stabilization of α_0 and α_2 Preservation of the $W_{180}F_{183}W_{200}$ core

W180, F183, W200 form a **conserved** hydrophobic core centered ~ at F183 ($\alpha 0$)



In practice

Using OpenMM on Google Colab

- We'll test OpenMM on Google Colab to run molecular dynamics simulations without the need for installing any software on your local machine.
- **Google Colab** is a free Jupyter environment that allows you to run Python code in the cloud. GPUs runtimes are available.
- To use OpenMM on Google Colab or locally, open the provided notebook (read the comments)



<https://github.com/giorginolab/MD-Tutorial-Data>

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main 1 branch 0 tags Go to file Add file Code

tonigi Created using Colaboratory 6a4dcdf 7 hours ago 5 commits

File/Folder	Commit Message	Time
GSN	import	3 weeks ago
HIVPR	import	3 weeks ago
notebooks	Created using Colaboratory	7 hours ago
README.md	Initial commit	3 weeks ago

README.md

MD-Tutorial-Data

Data for various MD analysis tutorials



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Code

main + 🔎

Go to file t

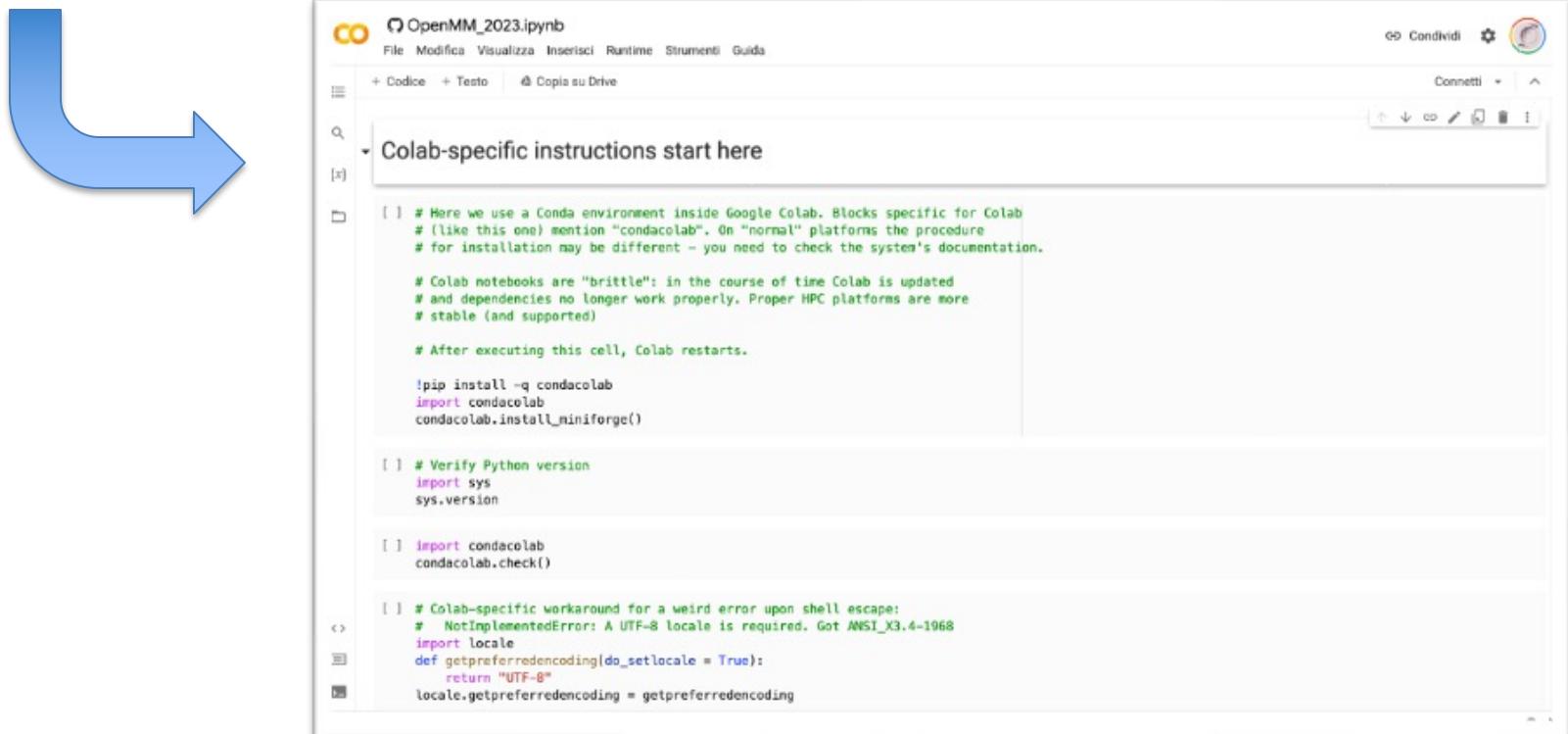
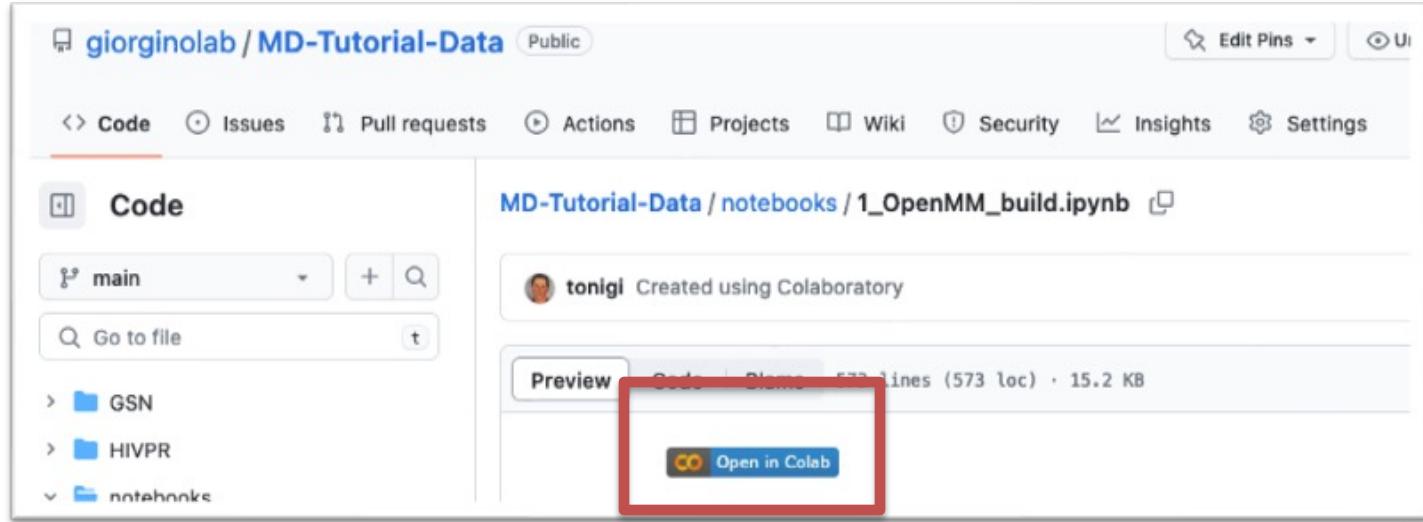
- > GSN
- > HIVPR
- notebooks

MD-Tutorial-Data / notebooks / 1_OpenMM_build.ipynb

tonigi Created using Colaboratory

Preview Code Slides 573 lines (573 loc) · 15.2 KB

Open in Colab



OpenMM_2023.ipynb

File Modifica Visualizza Inserisci Runtime Strumenti Guida

Condividi Connetti

Colab-specific instructions start here

```
[ ] # Here we use a Conda environment inside Google Colab. Blocks specific for Colab  
# (like this one) mention "condacolab". On "normal" platforms the procedure  
# for installation may be different - you need to check the system's documentation.  
  
# Colab notebooks are "brittle": in the course of time Colab is updated  
# and dependencies no longer work properly. Proper HPC platforms are more  
# stable (and supported)  
  
# After executing this cell, Colab restarts.  
  
!pip install -q condacolab  
import condacolab  
condacolab.install_miniforge()  
  
[ ] # Verify Python version  
import sys  
sys.version  
  
[ ] import condacolab  
condacolab.check()  
  
[ ] # Colab-specific workaround for a weird error upon shell escape:  
# NotImplementedError: A UTF-8 locale is required. Got ANSI_X3.4-1968  
import locale  
def getpreferreddencoding(do_setlocale = True):  
    return "UTF-8"  
locale.getpreferreddencoding = getpreferreddencoding
```

...when done...

Visualize

- After you have done the simulation, load the minimized PDB and output.dcd in PyMOL
- What about PBCs? Fix with: `pbc_unwrap ...`



Questions

- How many atoms?
- How many residues?
- Disulfide bridges?
- How many trajectory frames?
- Simulation length in *actual* time?

More questions

- Does density change? Should it?
- What is the box size? Is it appropriate?
- Relaxation time?
- Plot the log file

Markov-state modeling of biomolecular systems

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Consiglio Nazionale
delle Ricerche

<https://github.com/giorginolab/Markov-Tutorial-Data>

Introduction

- The aim of this class is to provide a practical overview of Markov state models in computational structural biology
- General intuition: a Markov model *propagates forward in time the distribution of probabilities of a set of discrete states*

Introduction

- MSM emerging because:
 - reconstruct *kinetic information**[†], including state transition *networks*, from simulated trajectories
 - start from *unbiased simulations* (no *a priori* reaction coordinate hypothesis necessary)
 - microsecond-scale (high-throughput) trajectory data are becoming accessible (e.g., with GPUs)
- Success cases: *ab initio* folding, drug binding, peptide binding, ...

* as well as the corresponding structures

[†] see notes

Motivation

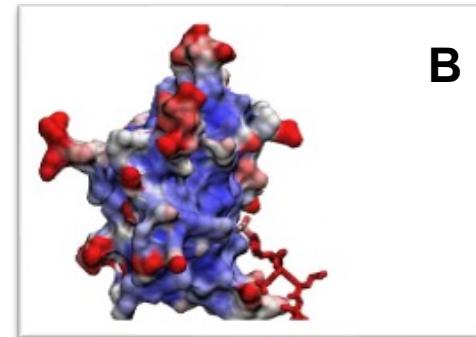
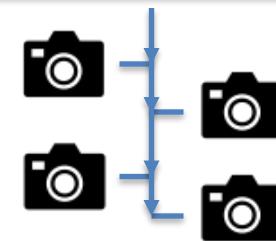
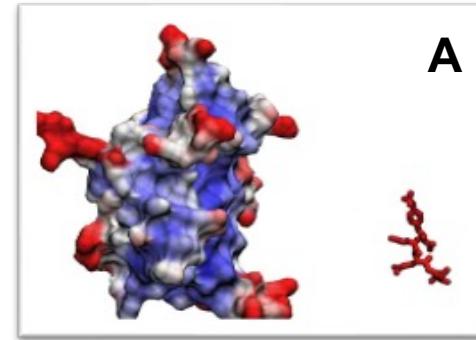
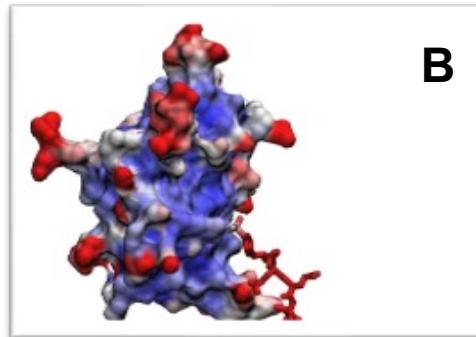
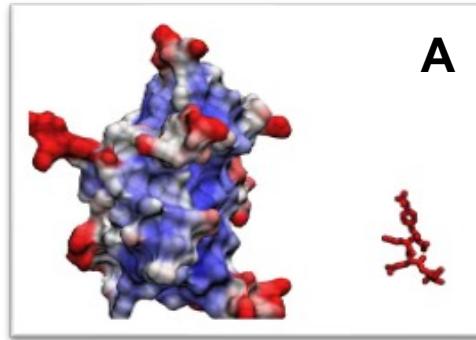
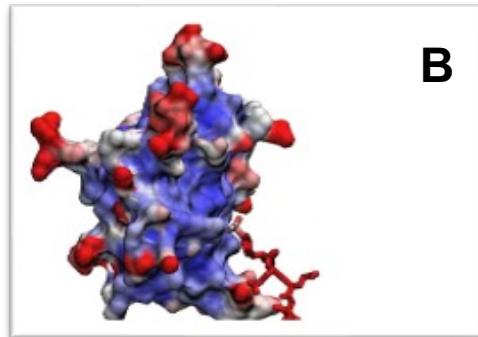
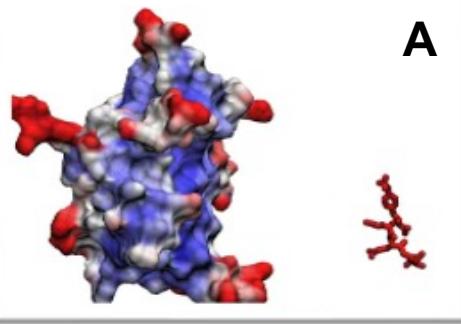
- Can we use an *ensemble* of simulations to estimate dynamic behaviours which happen on timescales longer than each of the observed trajectories?
- In other words, can we leverage several “short-sighted” (non-equilibrium) observations to extrapolate long-time behaviour?

The general idea

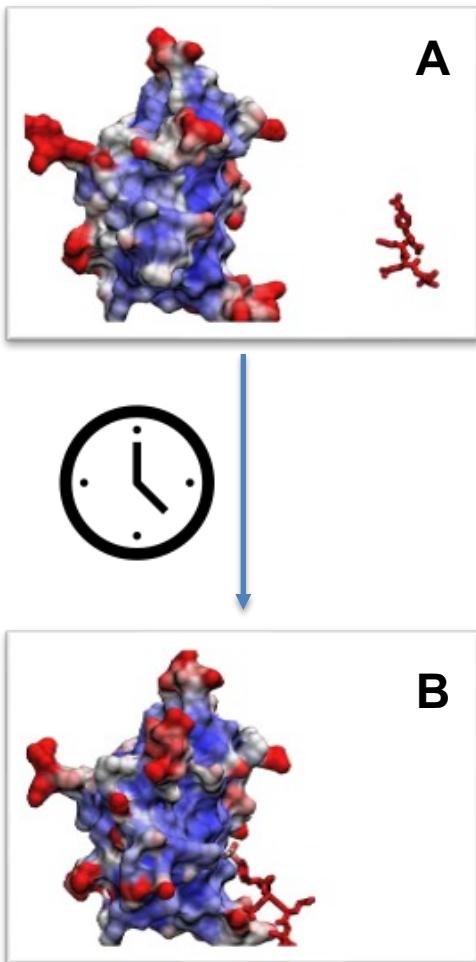
Unbiased sampling

Biased sampling

Markov-state modeling



Unbiased sampling



System evolves in time*.

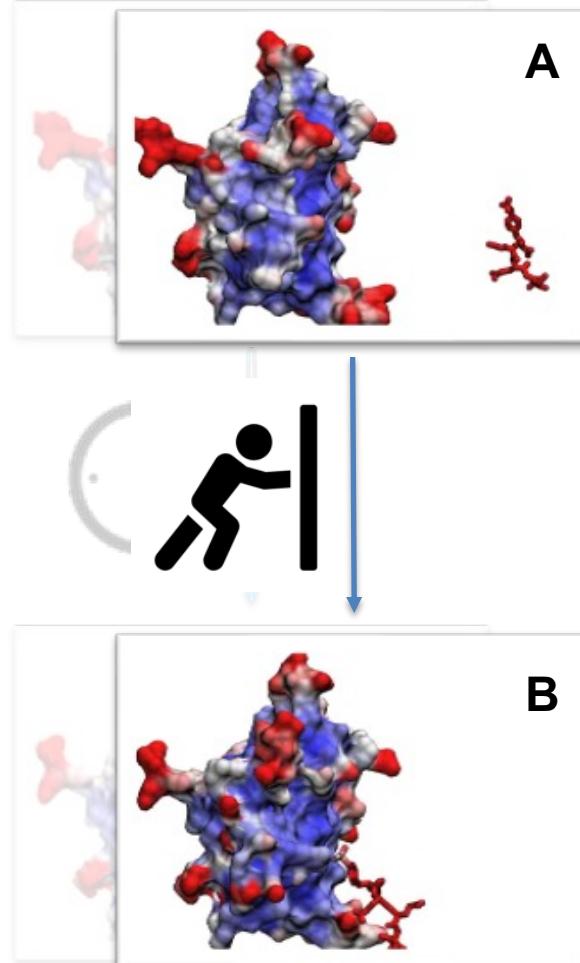
We do not “guide” the system with biases in any particular direction.

Pro: Kinetics are “true”

Con: Slow. Observations
(=sufficient sampling)
may well be unfeasible.

* Under the influence of internal and external forces designed to approximate “real” ones.

Biased sampling



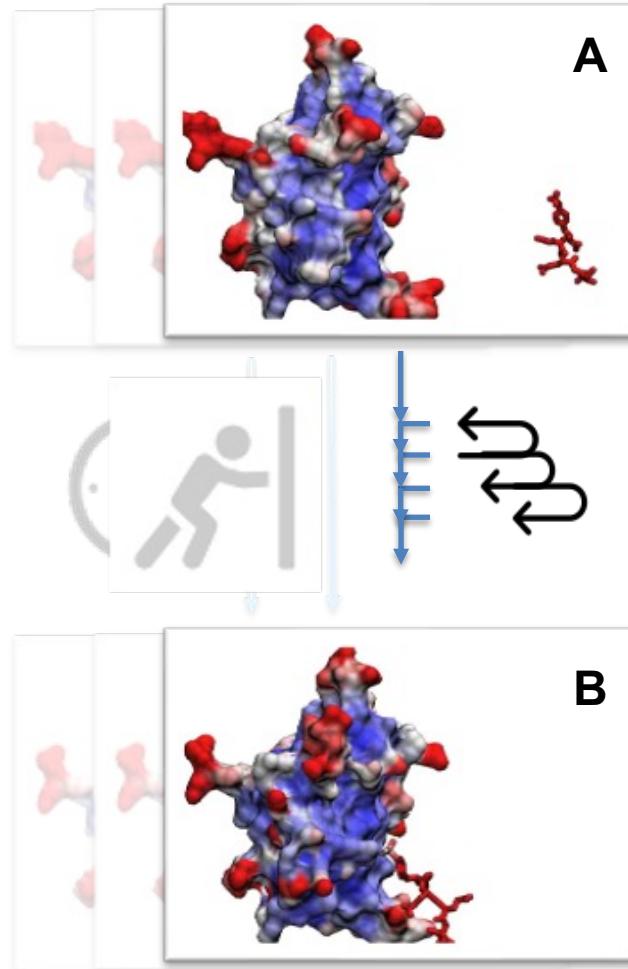
Various strategies* exist to “push” the system *towards* desired states

Pros: Sampling is accelerated..
Can recover ΔG .

Cons: Biasing “direction” must be set, often not trivial.
Kinetics (in general) are altered.

* Examples: metadynamics, steered MD, ...

Markov-state modeling



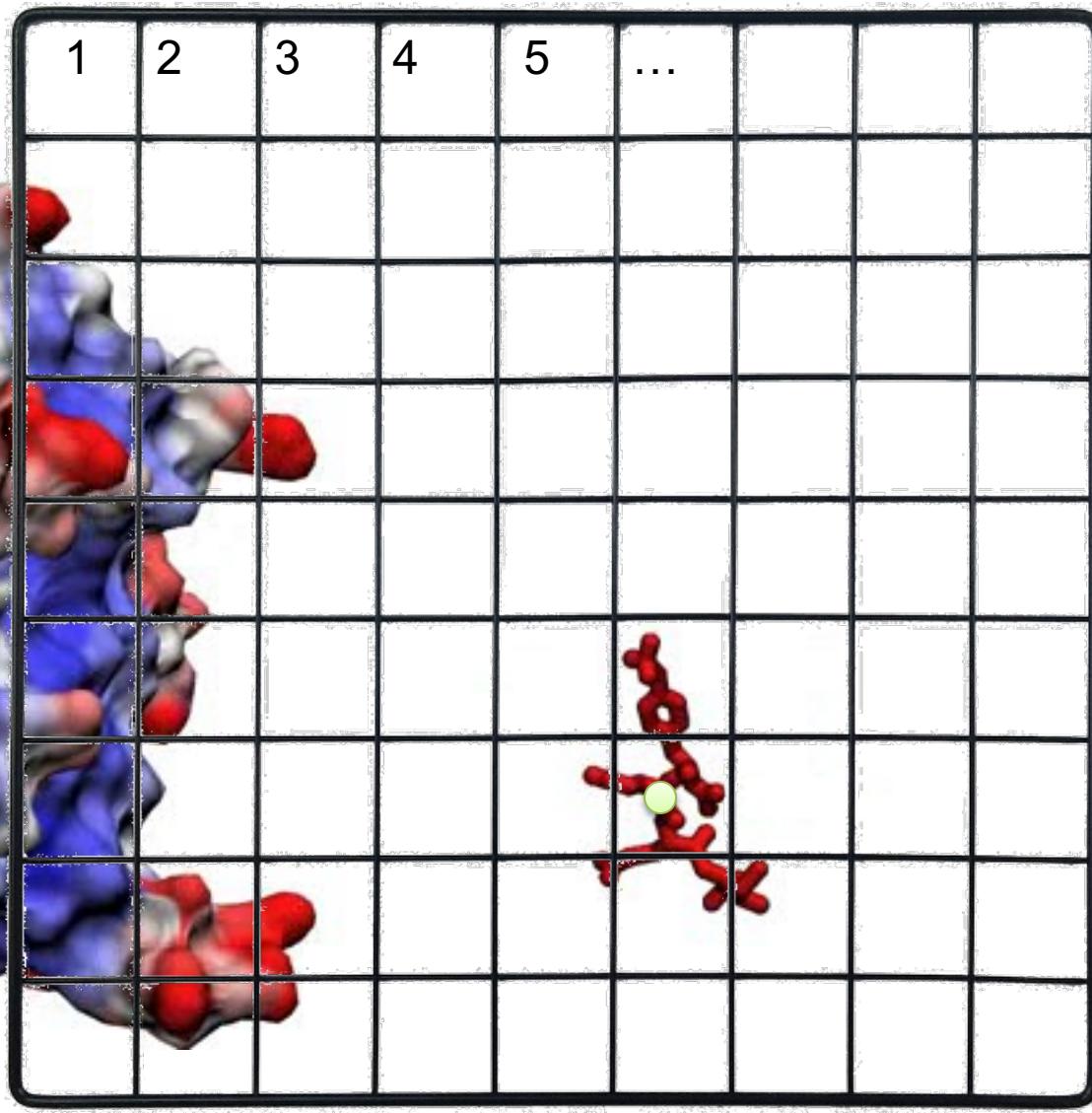
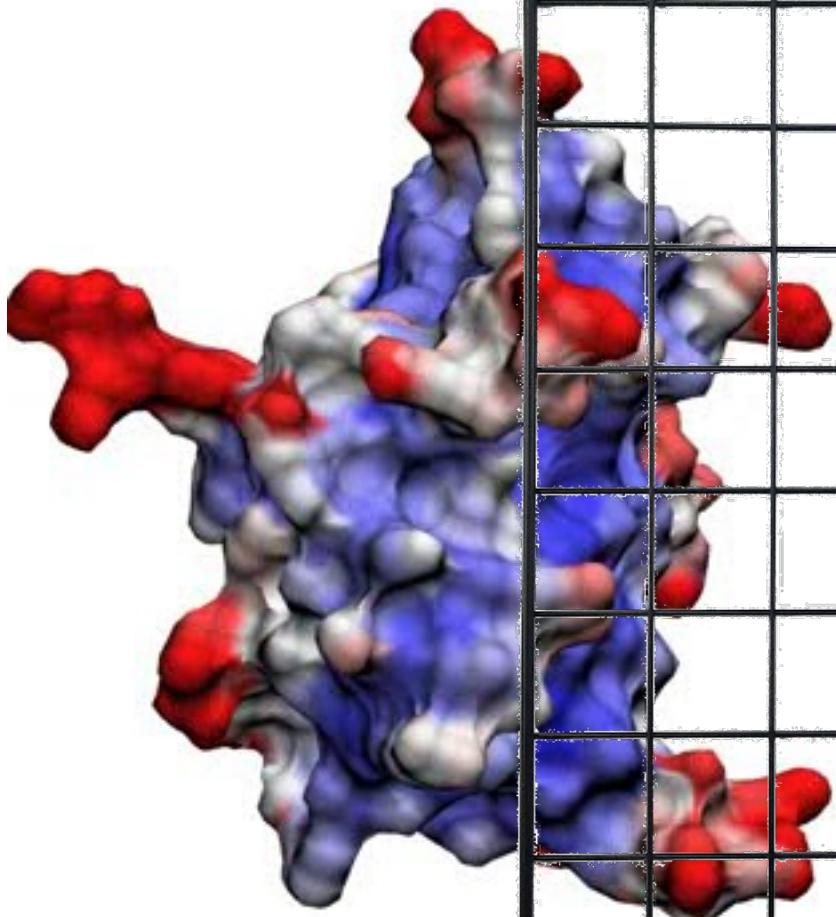
Decompose a system in multiple states. Use shorter trajectories to compose a statistical* picture.

Pros: True kinetics.
No need to pre-set a reaction coordinate.

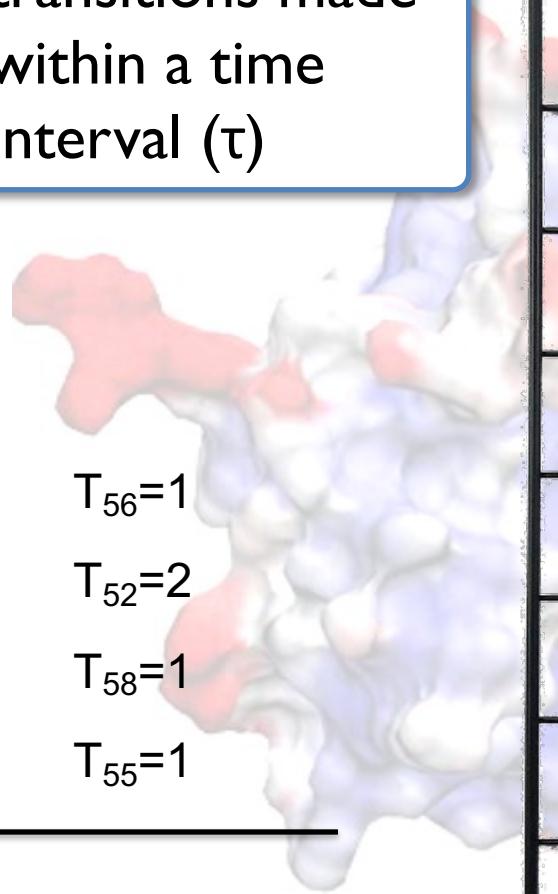
Cons: Non obvious (but software helps).
Sampling still needed for convergence.

* We'll see Markov-based formalism, but others exist, such as *milestoning*

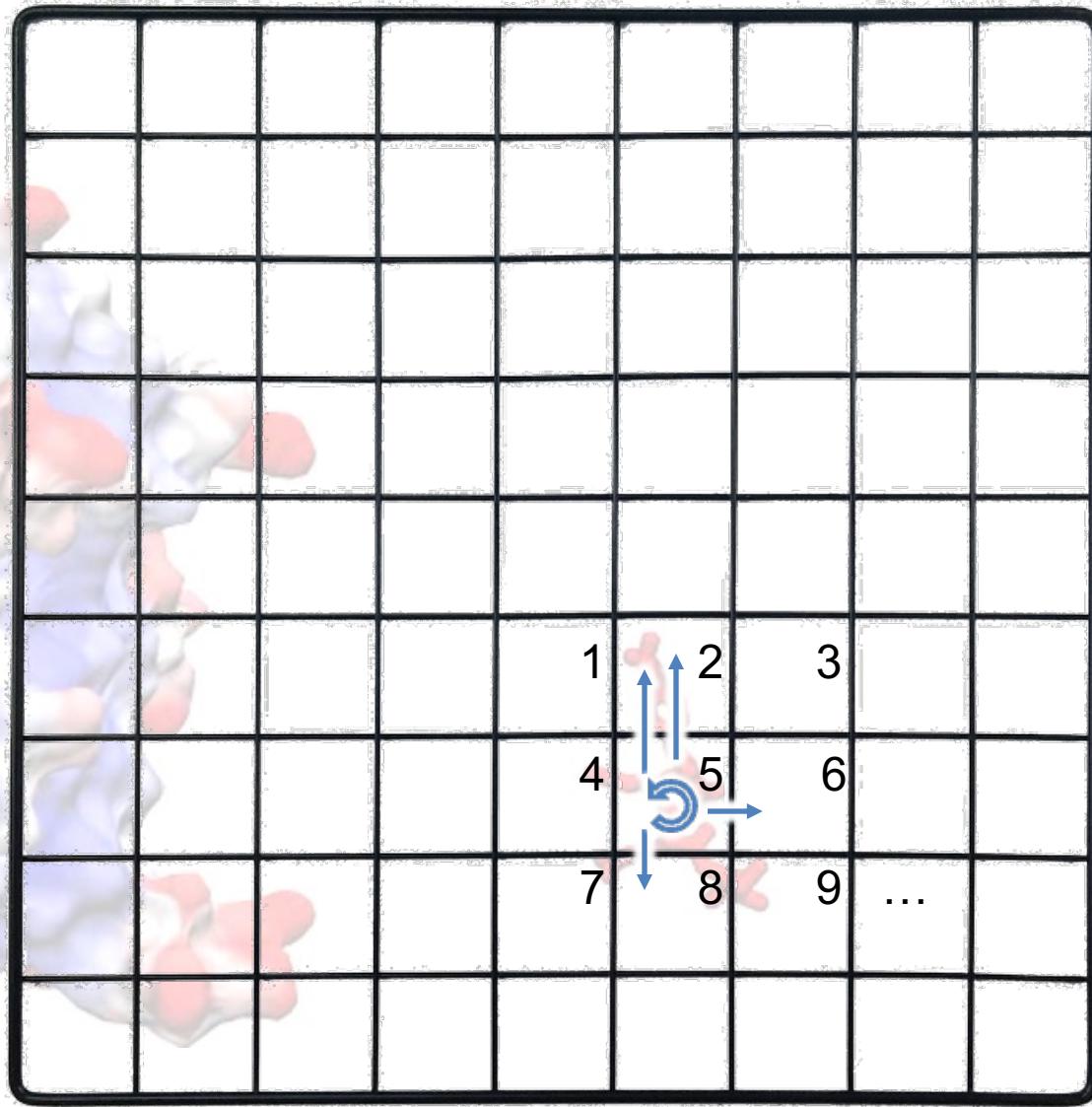
Decomposing the state space



Count the state transitions made within a time interval (τ)



Total: $N_5=5$



$$T_{56}=1$$

$$p_{56} = 1/5 = 0.2$$

$$T_{52}=2$$

$$p_{52} = 2/5 = 0.4$$

$$T_{58}=1$$

$$p_{58} = 1/5 = 0.2$$

$$T_{55}=1$$

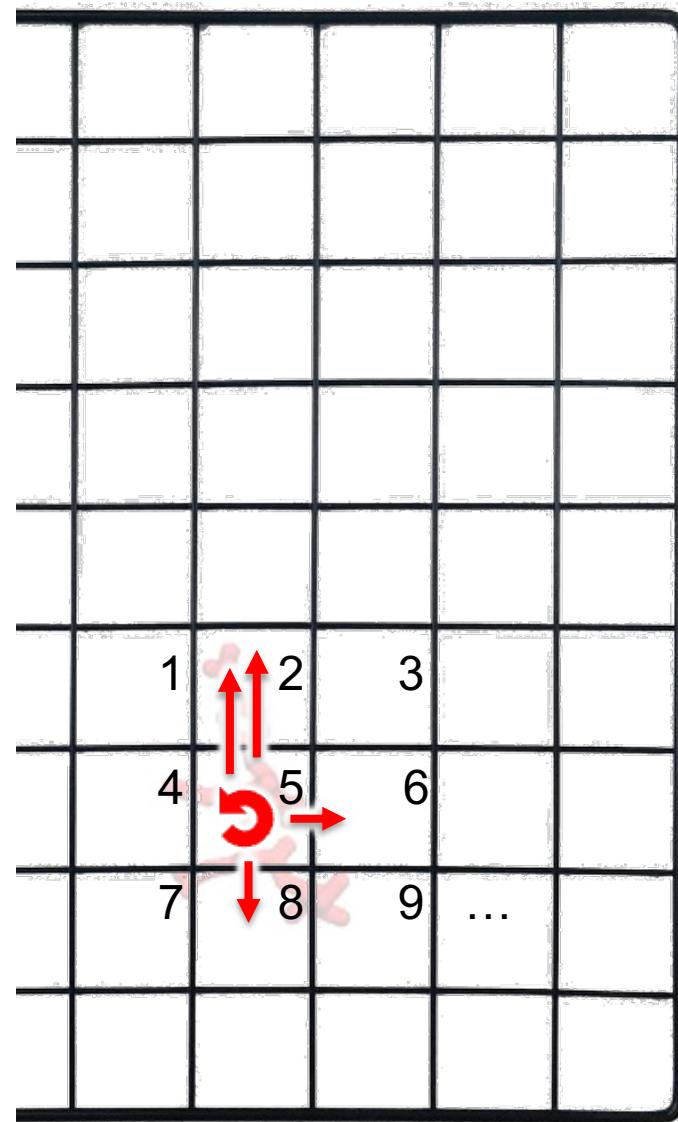
$$p_{55} = 1/5 = 0.2$$

$$N_5=5$$

$$p_{5*} = \sum_i p_{5i} = 1$$

1	2	...	5	6	7	8	...
5		0.4		0.2	0.2		0.2

Row 5 of the
transition probability matrix P

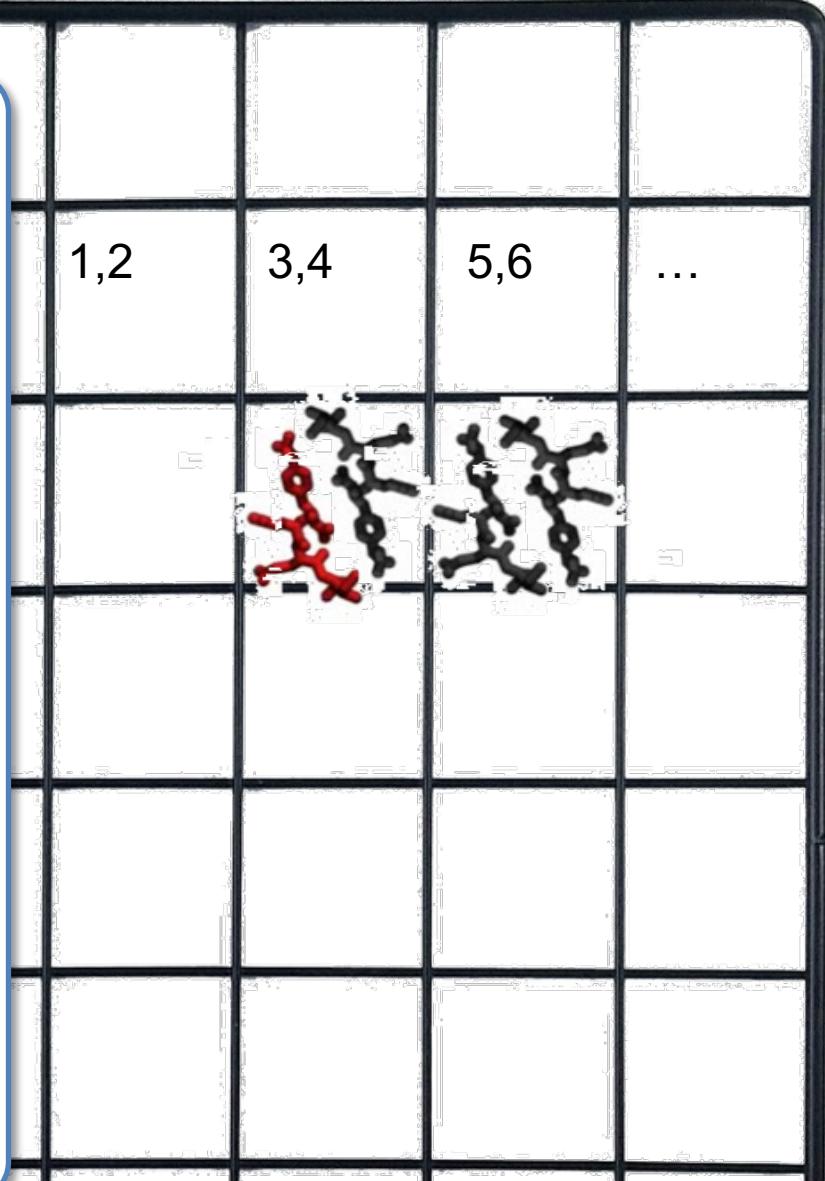


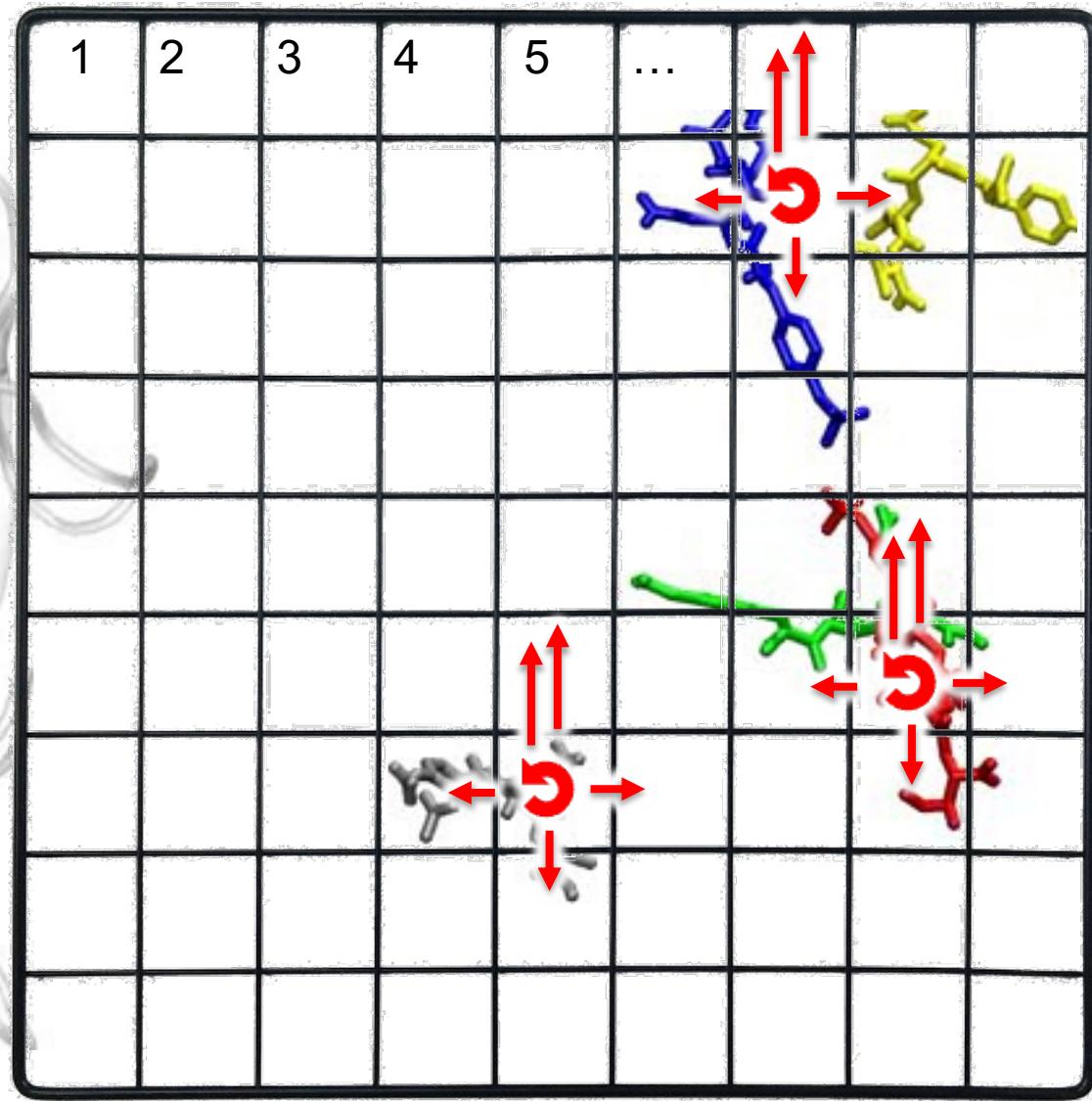
Important

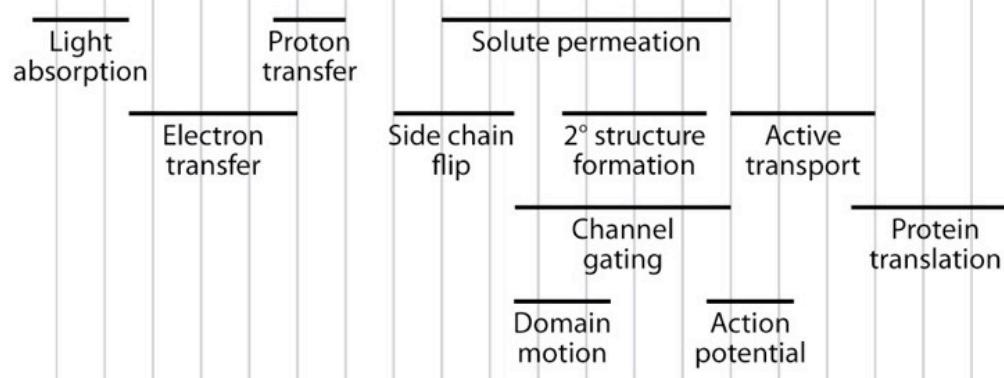
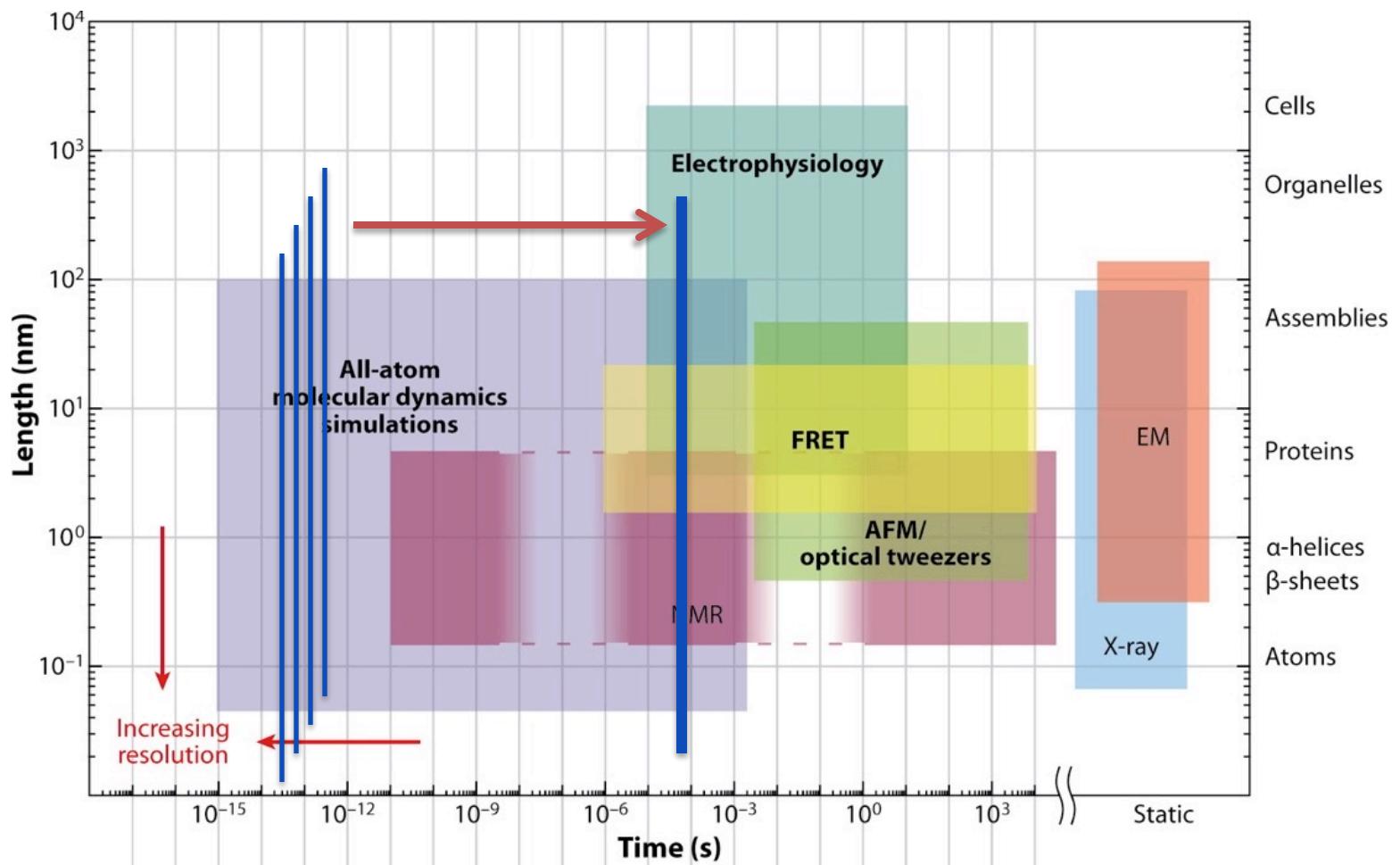
States are more general than a “location in space”.

For example, different conformations of the same structure may count as different states.

The partition (discretization) of the system’s configuration space in states is *up to the user*.

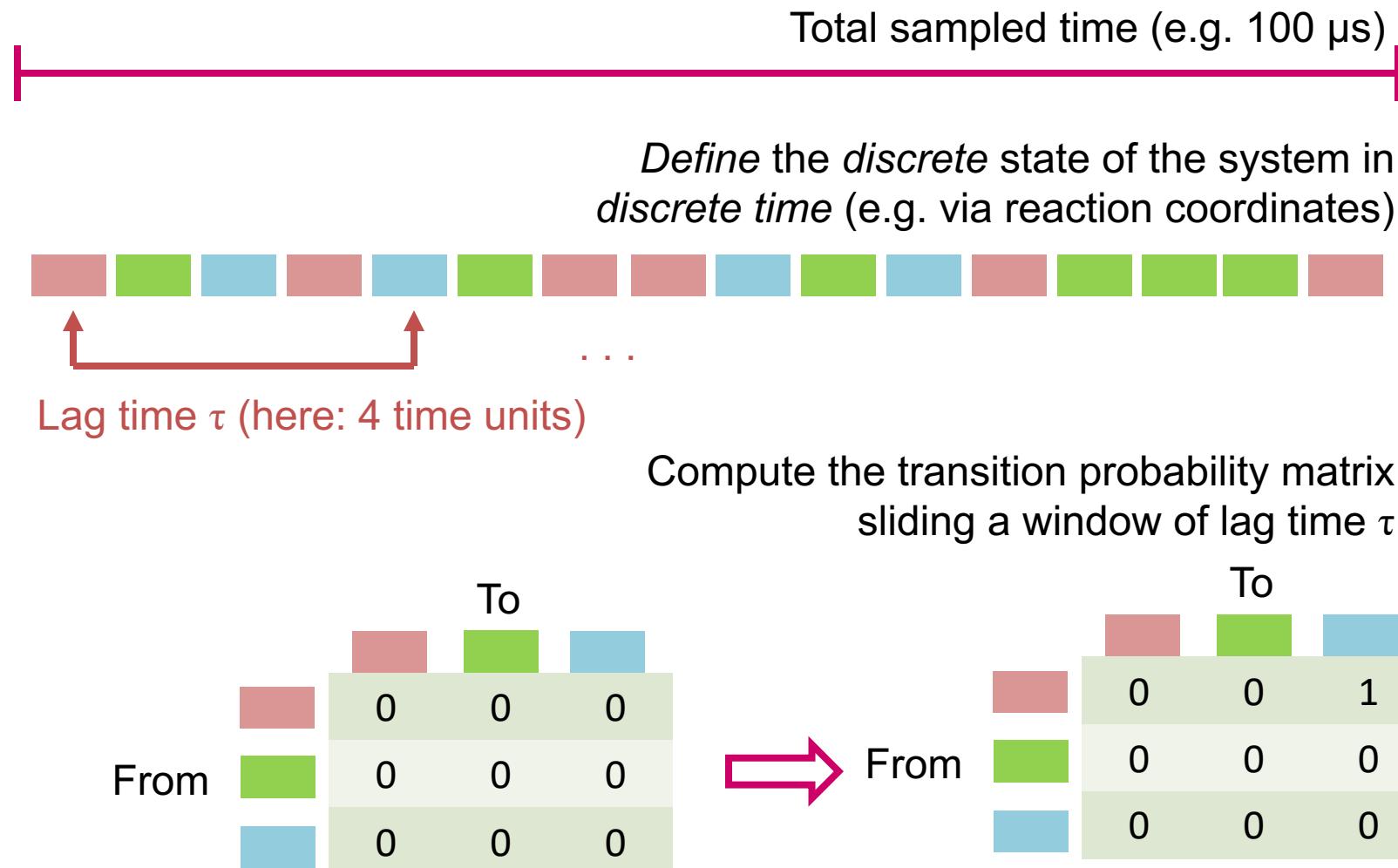




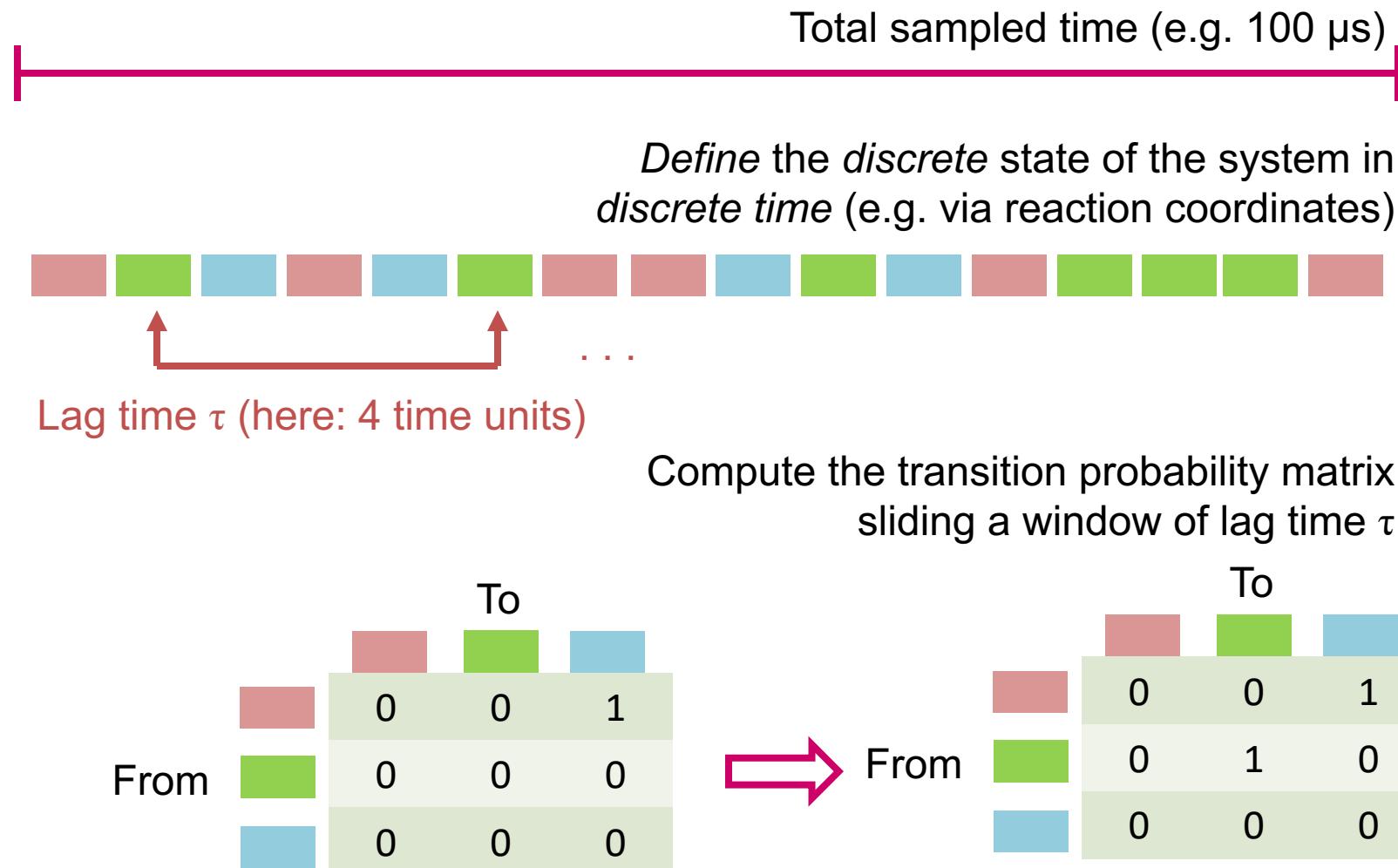


Learning transition matrices from trajectories

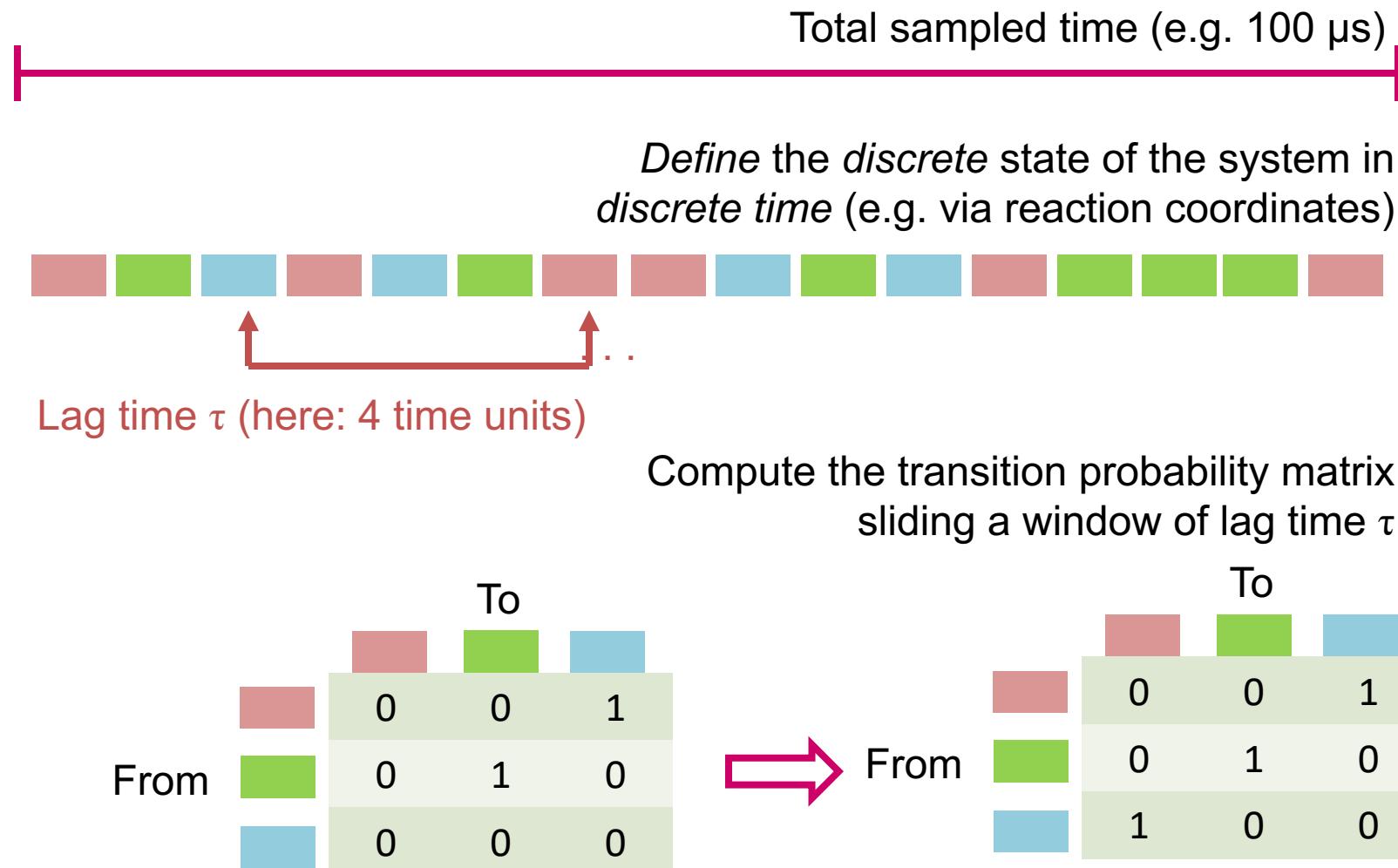
Markov models



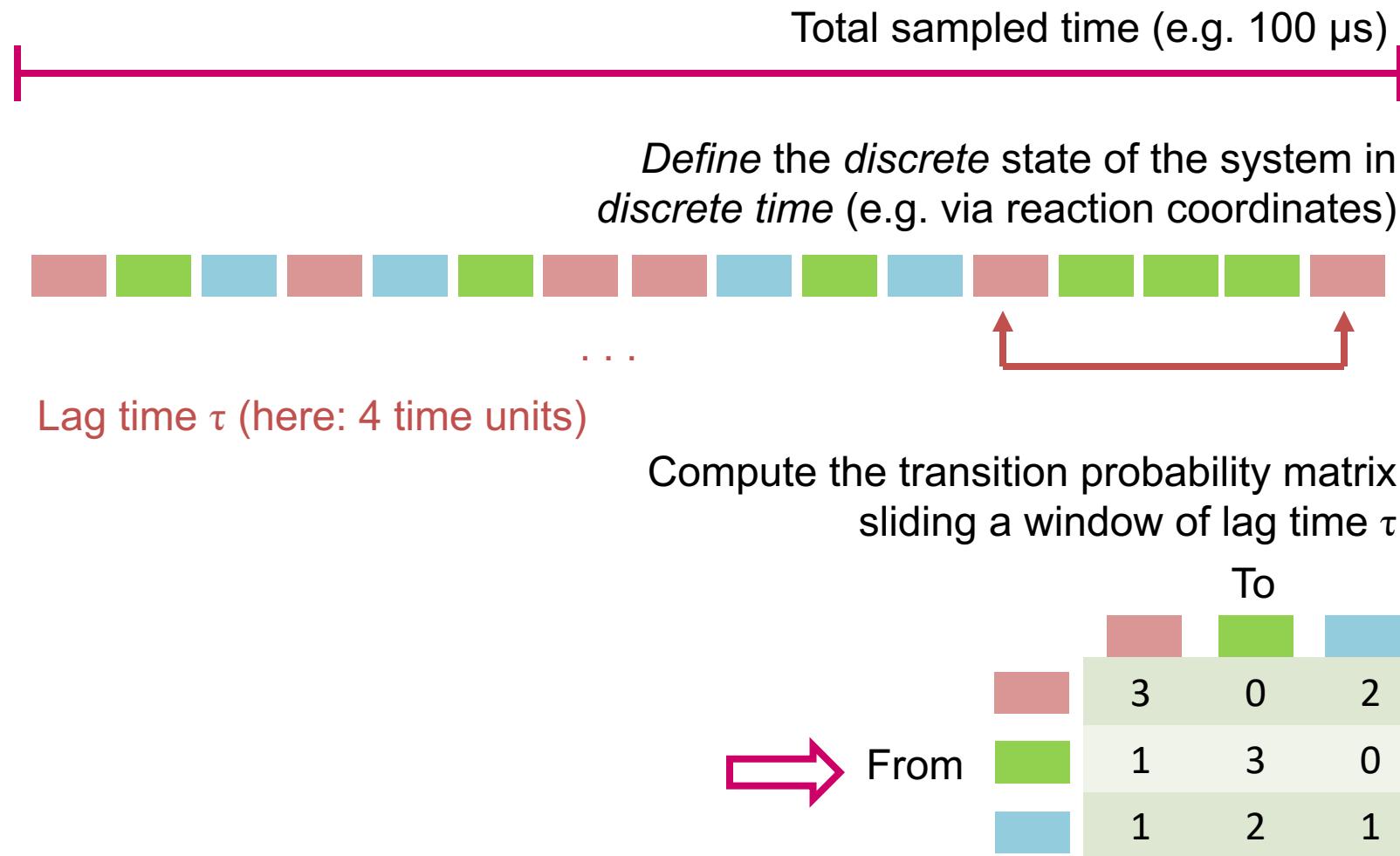
Markov models



Markov models



Markov models



Markov models

Total sampled time (e.g. 100 μs)

Define the discrete state of the system in discrete time (e.g. via reaction coordinates)



Lag time τ (here: 4 time units)

Transition counts

		To	
		3	0
From	Red	3	0
	Green	1	3
Blue	1	2	1

Transition probabilities

		To		\sum_j
		3/5	0	2/5
From	Red	3/5	0	2/5
	Green	$\frac{1}{4}$	$\frac{3}{4}$	0
Blue	$\frac{1}{4}$	$\frac{2}{4}$	$\frac{1}{4}$	1

Normalize by rows

P_{ij}

Repeat until the end of the trajectory.

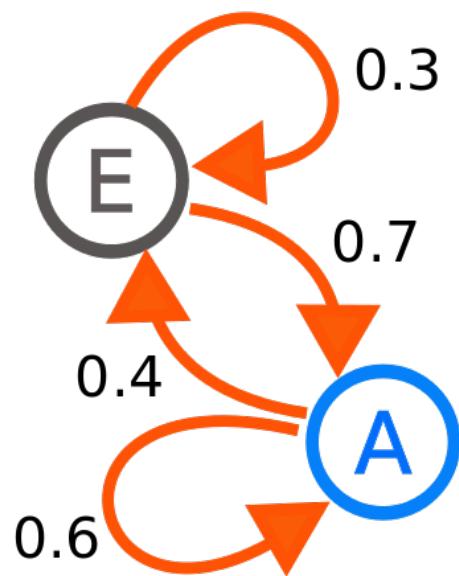
Note the Markovian assumption:
transition probabilities **do not** depend
on history (neither, for us, on time).

(Note how “Early” and “late” events
are squashed in the same matrix.)

Discrete-time Markov Chains



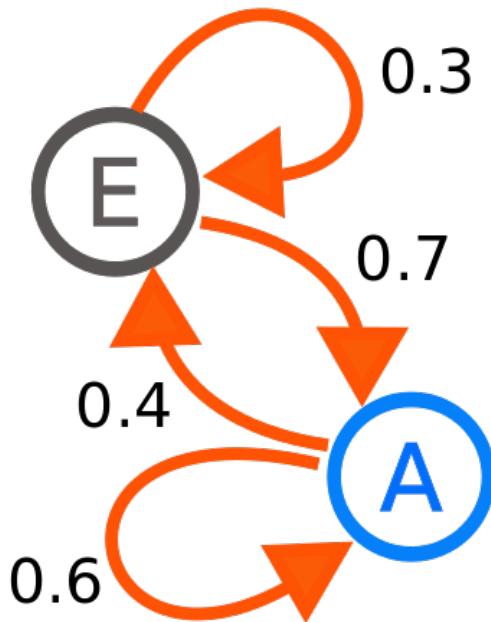
Andrei Markov
1856-1922



Discrete Time Markov Chains

- A **random** process.
- The system's state is a **discrete** variable.
- It undergoes transitions between states at uniformly-spaced (**discrete**) time points.
- Transition probabilities do not depend on the previous history of states (**memorylessness**).

Transition probability matrix

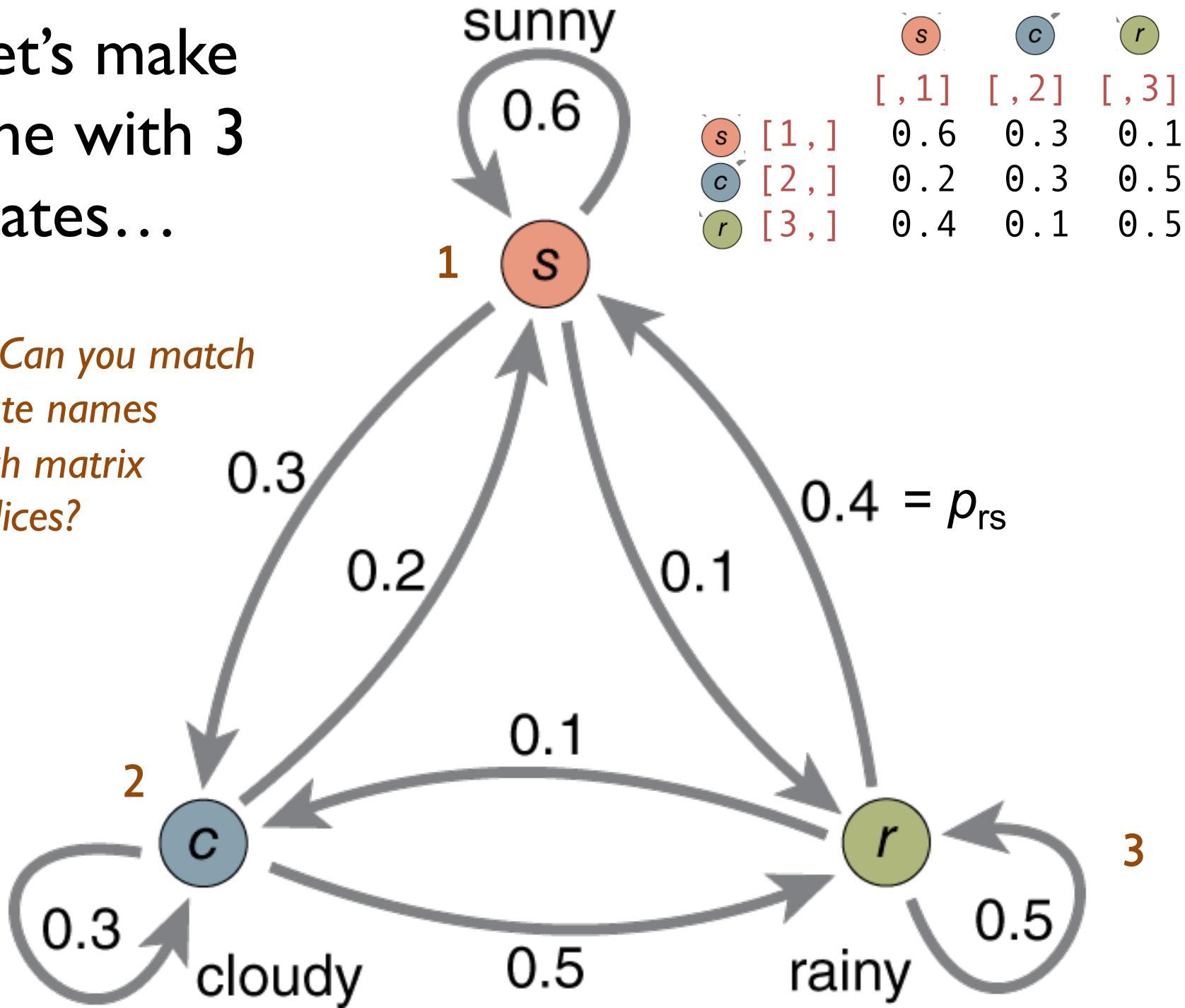


P_{ij} = Probability to change state
from i to j at each time point

$$P_{ij} = P(X_t = j | X_{t-1} = i)$$

		j	
		A	E
i	A	0.6	0.4
	E	0.7	0.3

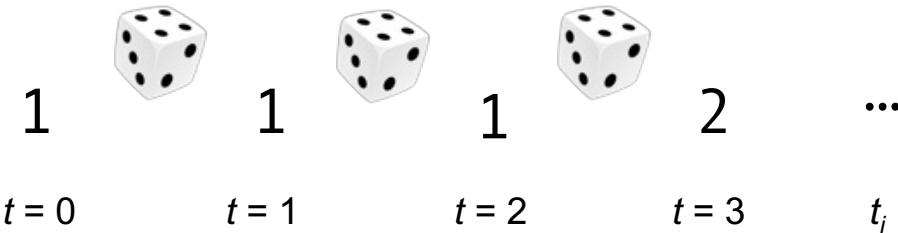
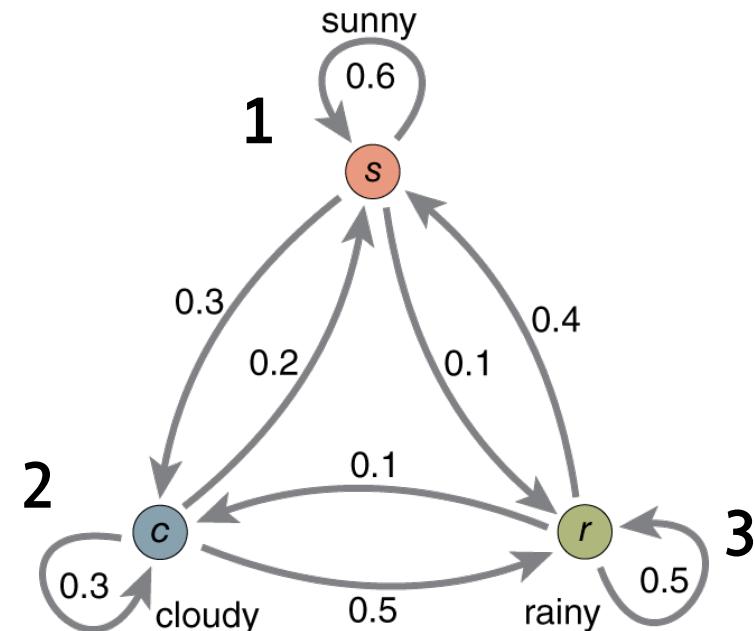
Let's make
one with 3
states...



Let's generate samples...

Start e.g. from state 1
(we'll use numbers from now on).

Play the game and follow the Markov chain for many (discrete) time steps.

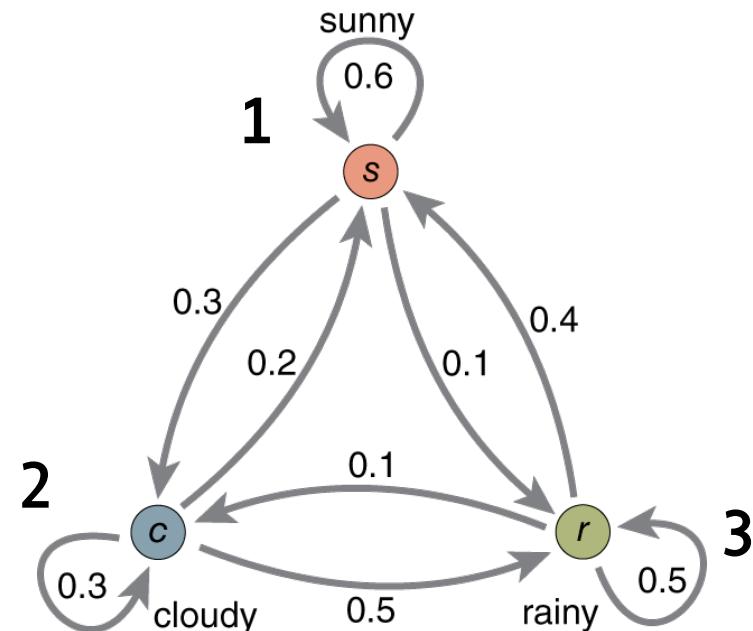


1 1 1 2 2 3 3 1 1 1 2 2 2 2 1 1 1 1 1 3 1 1 1 1 2 3 1 1 3...

Equilibrium probabilities

Question: if we play the game a very long time, what fraction of time would we spend in each state?

- That is the *asymptotic* (equilibrium) distribution.
 - I.e., the probability of finding the system in a given state.



1 1 1 2 2 3 3 1 1 1 2 2 2 2 1 1 1 1 1 1 1 3 1 1 1 1 1 2 3 1 1 3...
(1000 times)

1	2	3
431	220	349



$$\begin{aligned} p_\infty(1) &= 0.431 \\ p_\infty(2) &= 0.220 \\ p_\infty(3) &= 0.349 \end{aligned}$$

$$\sum_i p_\infty(i) = 1$$

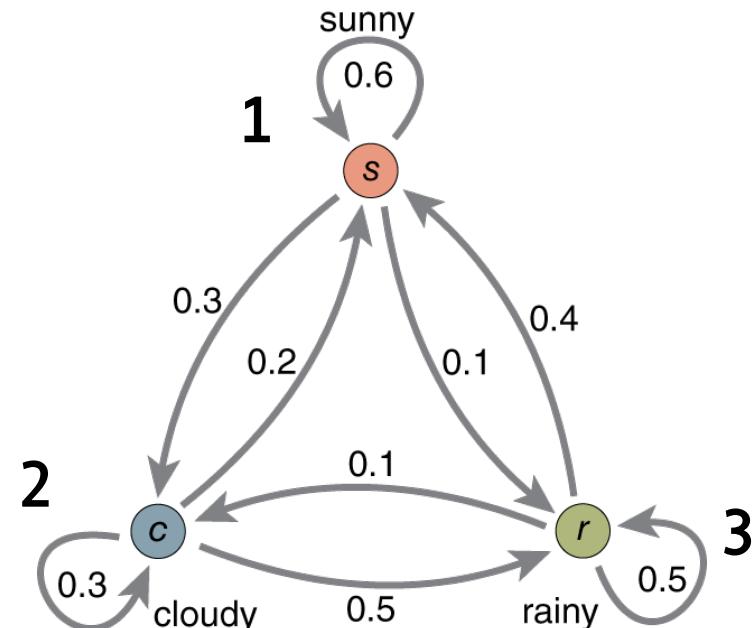
Equilibrium probabilities

Question: if we play the game a very long time, how much time would we spend in each state?

- That is the *asymptotic* (equilibrium) distribution.
 - I.e., the probability of finding the system in a given state.
 - I.e., the free energy (ΔG) of that state!

1 1 1 2 2 3 3 1 1 1 2 2 2 2 1 1 1 1 1 1 1 3 1 1 1 1 1 2 3 1 1 3...
(1000 times)

1 2 3
431 220 349



$$\begin{aligned} p_\infty(1) &= 0.431 \\ p_\infty(2) &= 0.220 \\ p_\infty(3) &= 0.349 \end{aligned}$$

$$\sum_i p_\infty(i) = 1$$

$$G_1 = -k_B T \log(p_1) \simeq 0.50 \text{ kcal/mol}$$

$$G_2 = -k_B T \log(p_2) \simeq 0.91 \text{ kcal/mol}$$

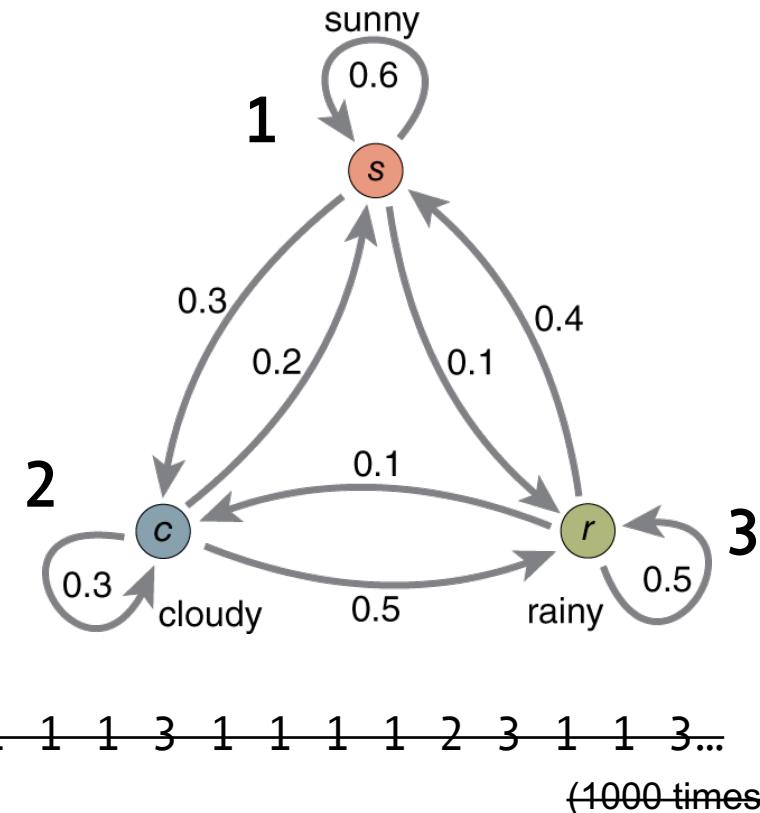
$$G_3 = -k_B T \log(p_3) \simeq 0.63 \text{ kcal/mol}$$

$$\begin{aligned}\Delta G_{11} &\doteq 0 \text{ kcal/mol} \\ \Delta G_{21} &\simeq 0.37 \text{ kcal/mol} \\ \Delta G_{31} &\simeq 0.18 \text{ kcal/mol}\end{aligned}$$

Equilibrium probabilities

Question: if we play the game a very long time, how much time would we spend in each state?

- That is the *asymptotic* (equilibrium) distribution.
 - I.e., the probability of finding the system in a given state.
 - I.e., the free energy (ΔG) of that state!



Sample and count



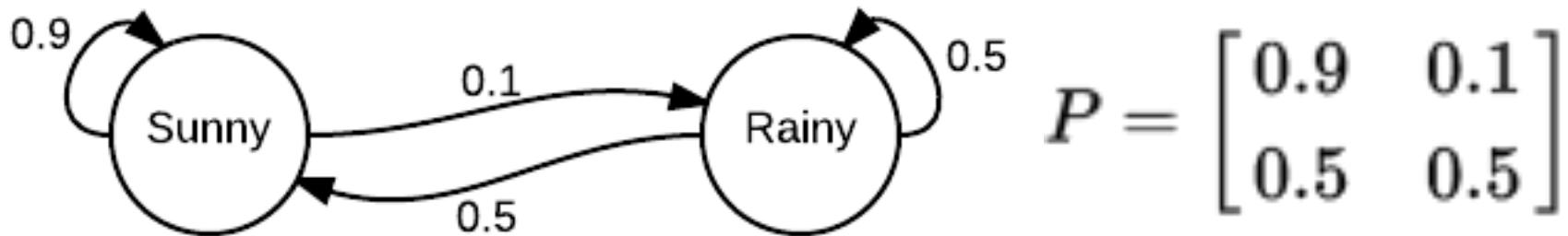
Compute eigenvectors of matrix P

	[, 1]	[, 2]	[, 3]
[1 ,]	0 . 6	0 . 3	0 . 1
[2 ,]	0 . 2	0 . 3	0 . 5
[3 ,]	0 . 4	0 . 1	0 . 5

Compute
eigenvectors:
same result
in one shot

**A slightly more
mathematical point of view**

First example



Assume a deterministic initial condition:

$X_0 = \text{Sunny}$ with certainty; i.e.,

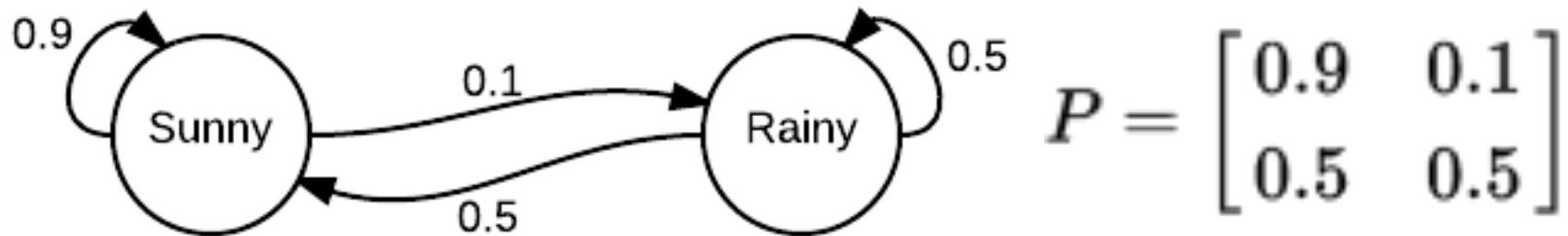
$$p(\text{Sunny} \mid t=0) = 1 \quad \text{and}$$

$$p(\text{Rainy} \mid t=0) = 0 \quad \text{i.e.}$$

$$s_0 = [1, 0]$$

...now, what is s_1 ?

First example



What is s_1 ?

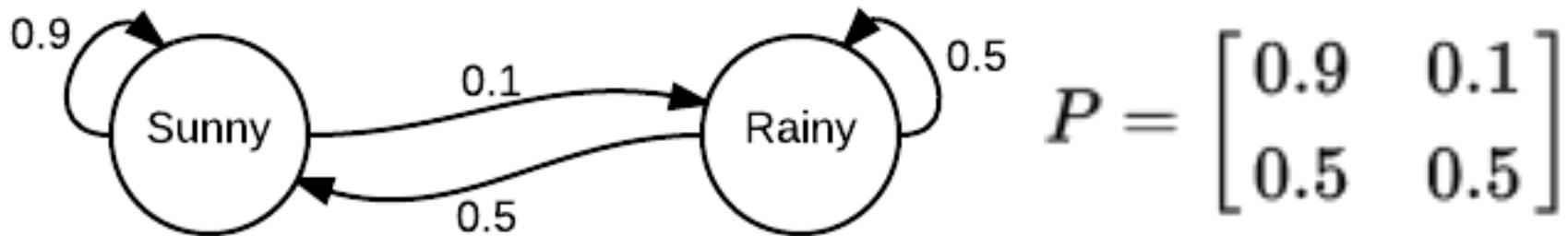
$$s_1 \quad \left\{ \begin{array}{l} p(\text{Sunny} \mid t=1) = 0.9 \\ p(\text{Rain} \mid t=1) = 0.1 \end{array} \right.$$

In matrix form...

$$s_1 = s_0 P$$

...now, what is s_2 ?

First example



What is s_2 ?

$$s_2 \left\{ \begin{array}{l} p(\text{Sunny} | t=2) = 0.9 \quad p(\text{Sunny} | t=1) + 0.5 \quad p(\text{Rainy} | t=1) = 0.86 \\ p(\text{Rainy} | t=2) = 0.1 \quad p(\text{Sunny} | t=1) + 0.5 \quad p(\text{Rainy} | t=1) = 0.14 \end{array} \right.$$

In matrix form...

$$\mathbf{s}_2 = \mathbf{s}_1 P$$

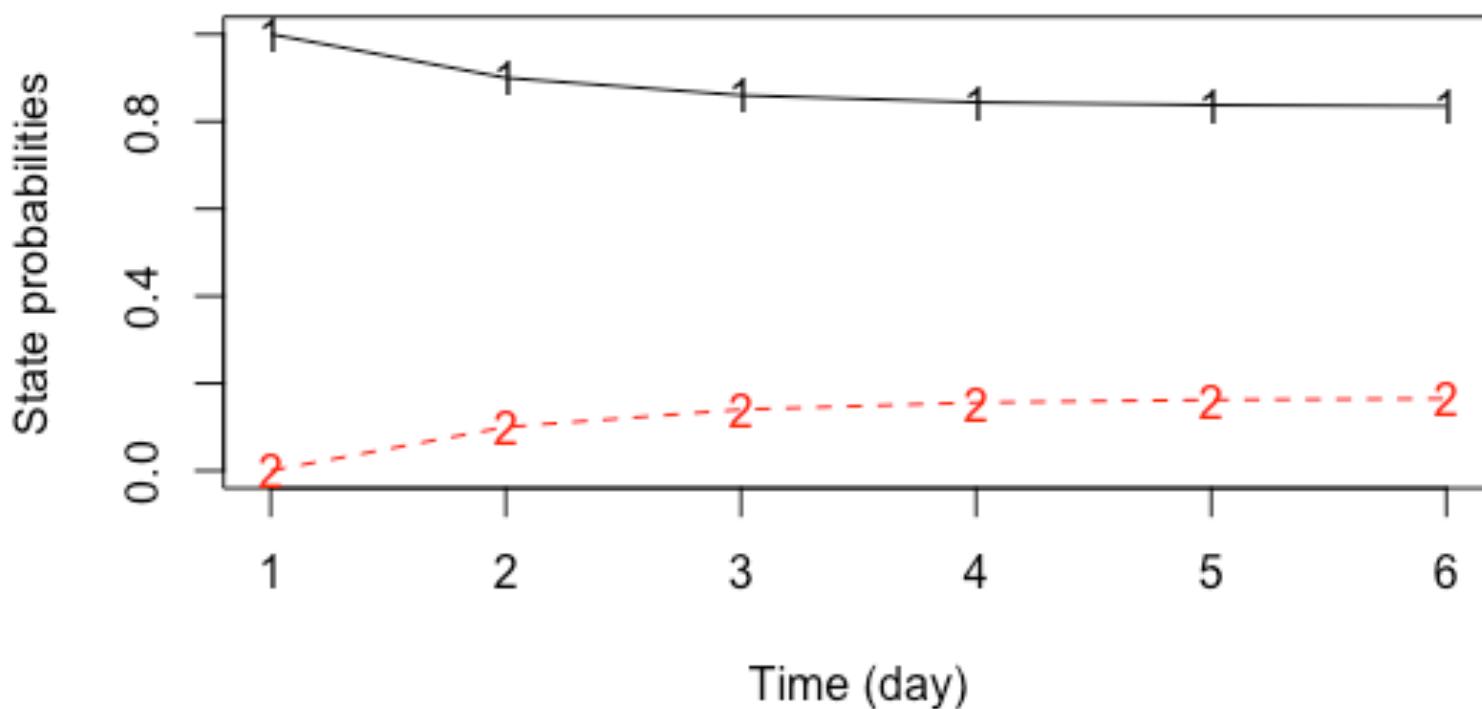
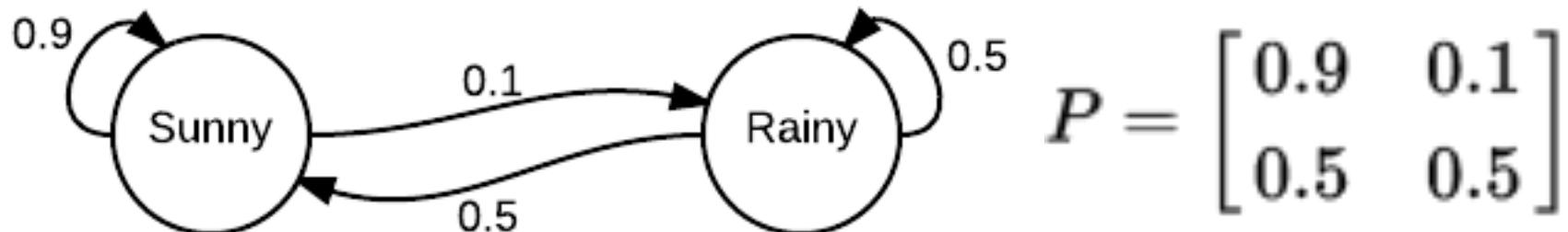
And in general...

$$\mathbf{s}_{t+1} = \mathbf{s}_t P$$

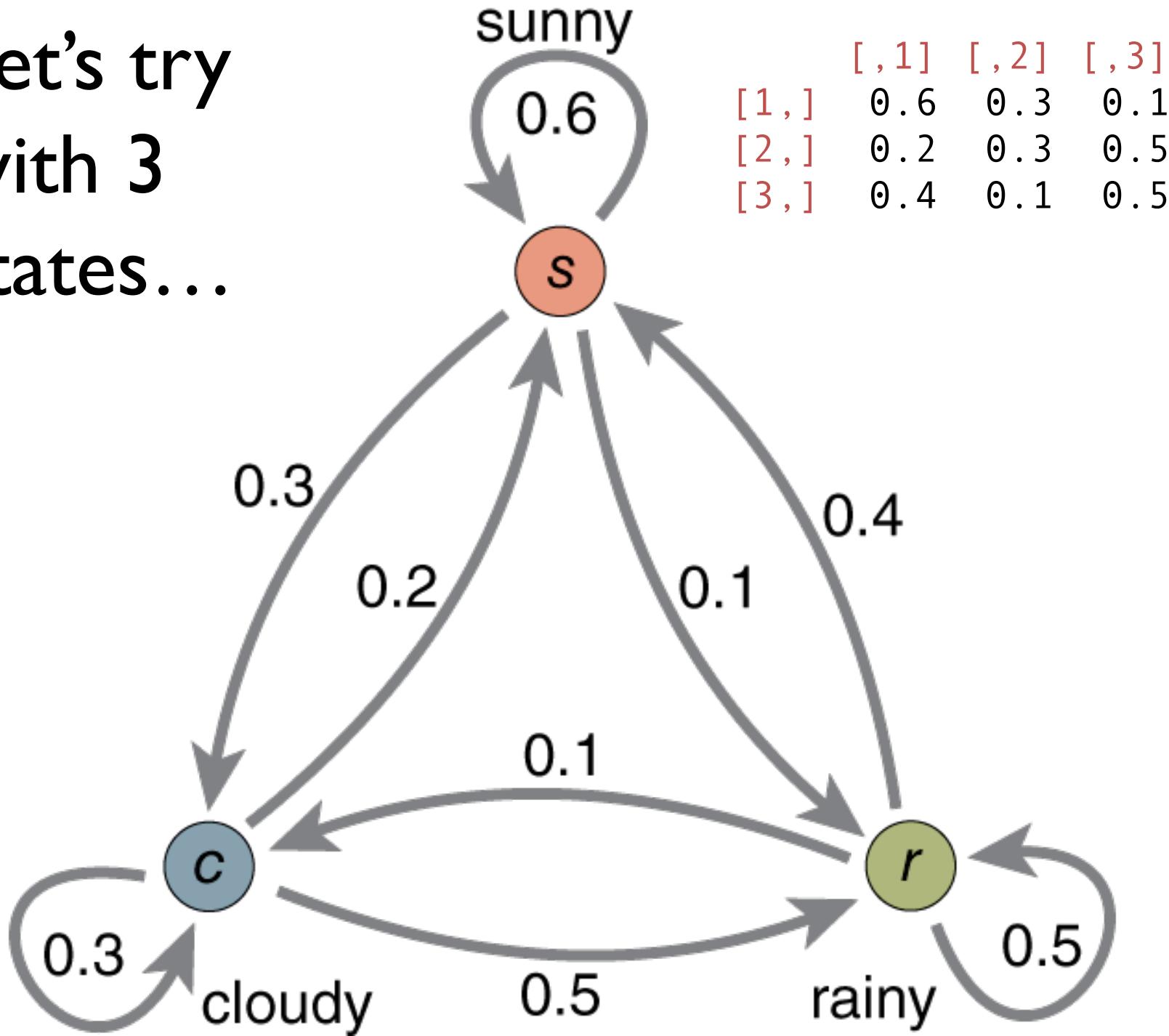
Meaning...

$$\mathbf{s}_t = \mathbf{s}_0 P^t$$

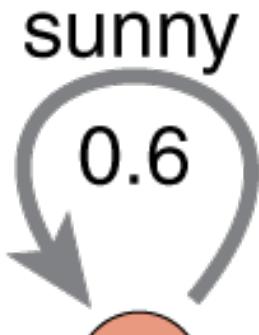
Let's do a numerical test...



Let's try
with 3
states...

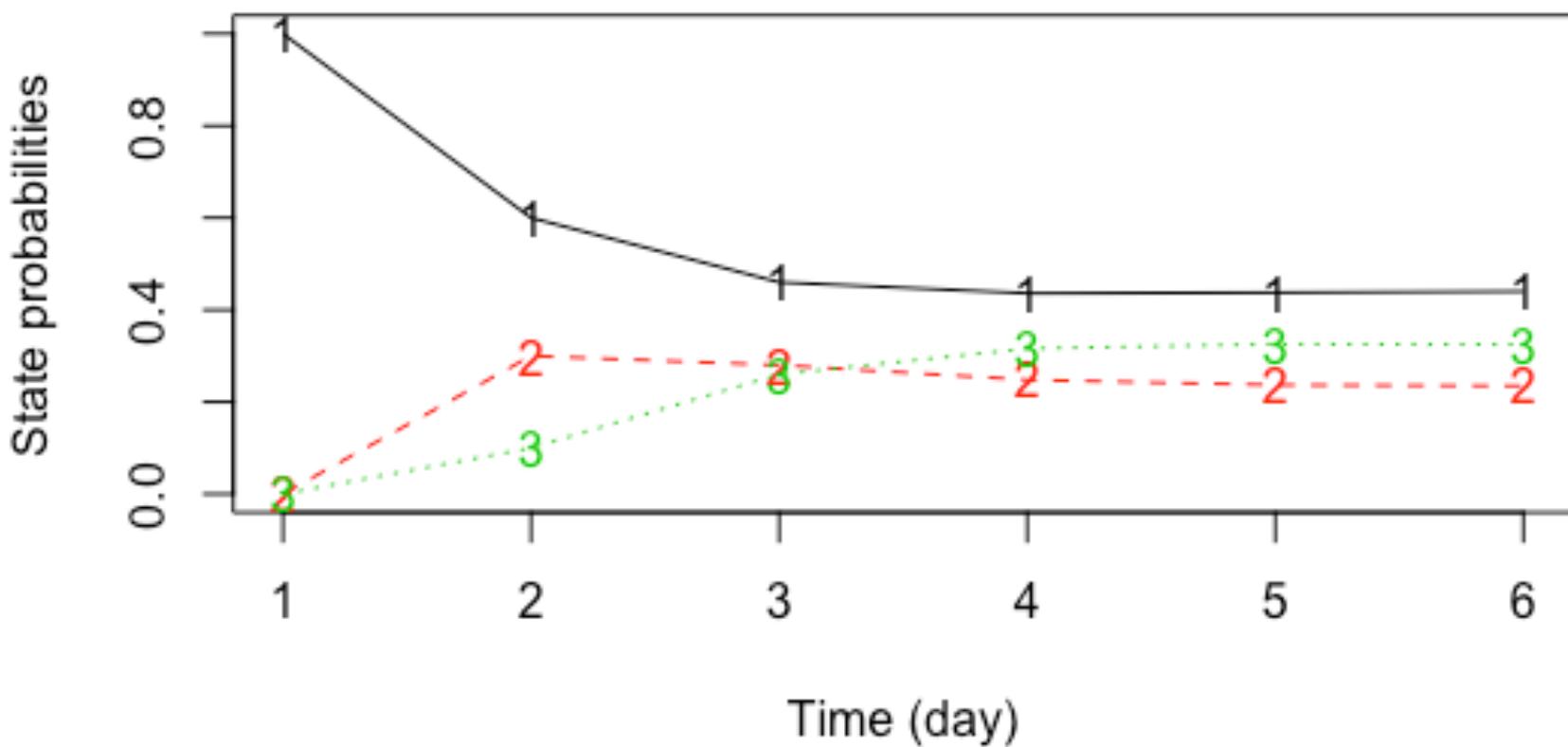


Starting from
Sunny...



	[, 1]	[, 2]	[, 3]
[1 ,]	0 . 6	0 . 3	0 . 1
[2 ,]	0 . 2	0 . 3	0 . 5
[3 ,]	0 . 4	0 . 1	0 . 5

Initial state: $s_0 = [1,0,0]$

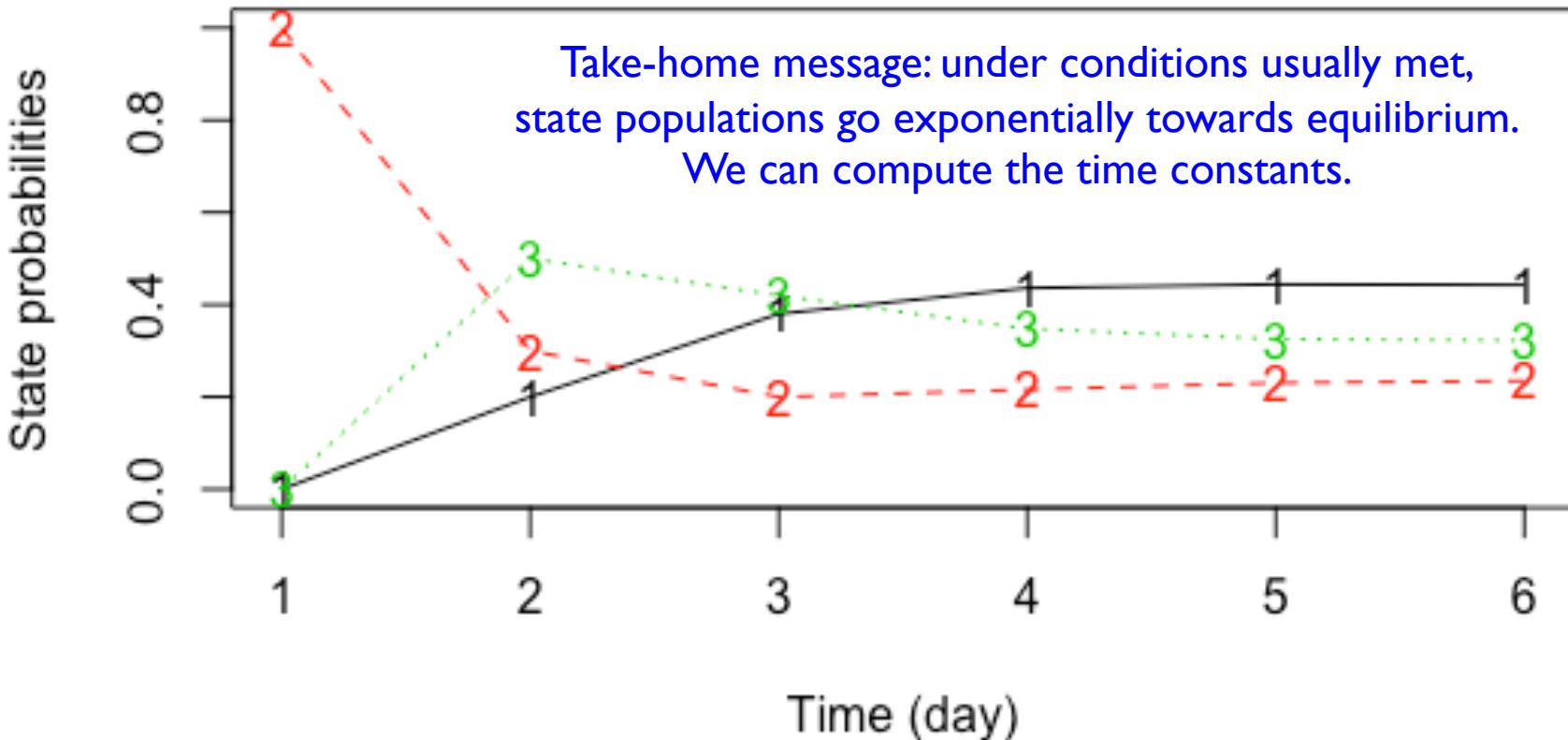


Starting from
Cloudy...



	[, 1]	[, 2]	[, 3]
[1 ,]	0 . 6	0 . 3	0 . 1
[2 ,]	0 . 2	0 . 3	0 . 5
[3 ,]	0 . 4	0 . 1	0 . 5

Initial state: $s_0 = [0,1,0]$



Important quantities we can compute

- Stationary distribution (\rightarrow eq. probabilities)
- Relaxation times
- Mean-first passage times (\rightarrow kinetic rates)
- And others we won't discuss:
 - Commitor probabilities
 - Fluxes
 - ...

The \$25,000,000,000 Eigenvector: The Linear Algebra behind Google*

Kurt Bryan[†]
Tanya Leise[‡]

Abstract. Google's success derives in large part from its PageRank algorithm, which ranks the importance of web pages according to an eigenvector of a weighted link matrix. Analysis of the PageRank formula provides a wonderful applied topic for a linear algebra course. Instructors may assign this article as a project to more advanced students or spend one or two lectures presenting the material with assigned homework from the exercises. This material also complements the discussion of Markov chains in matrix algebra. Maple and *Mathematica* files supporting this material can be found at www.math.hulman.edu/~bryan.

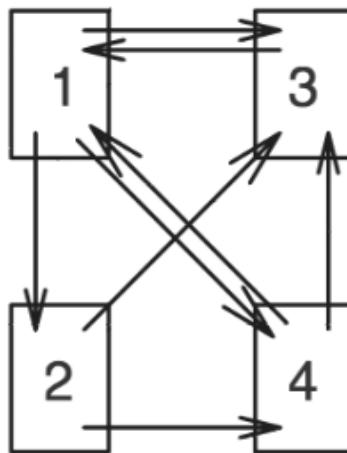
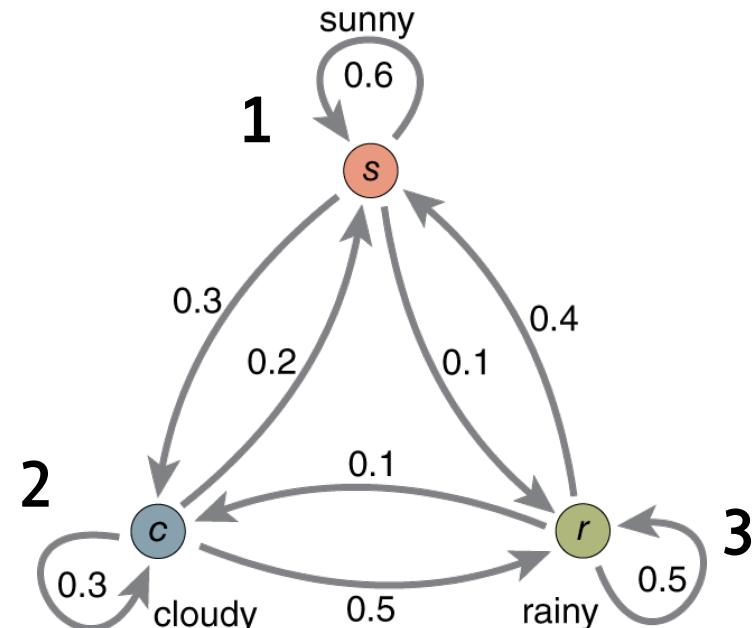


Fig. 1 An example of a web with only four pages. An arrow from page A to page B indicates a link from page A to page B.

Kinetics

Question: if we start from 1, how many steps on average do I wait to reach 2?

- This is the $I \rightarrow 2$ mean first passage time
 - I.e., the (inverse) transition rate ($1/k_{\text{on}}$) between those states!



The diagram shows the sequence starting with 1, 1, 1, 2, 2, 3, 3, 1, 1, 1, 2, 2, 2, 2, 1, 1, 1, 1, 1, 3, 1, 1, 1, 2, 3, ... The first bracket groups the first three terms (1, 1, 1) with an arrow pointing to the number 3. The second bracket groups the next four terms (2, 2, 3, 3) with an arrow pointing to the number 3. The third bracket groups the next eleven terms (1, 1, 1, 2, 2, 2, 2, 1, 1, 1, 1, 1, 3) with an arrow pointing to the number 11. Below these, smaller brackets group the first two terms (1, 1) with an arrow pointing to the number 2, and the next two terms (2, 2) with an arrow pointing to the number 2. The final bracket groups the last term shown (1) with an arrow pointing to the number 1. The text "And so on..." is at the bottom right.

Answer: average all the numbers in red.

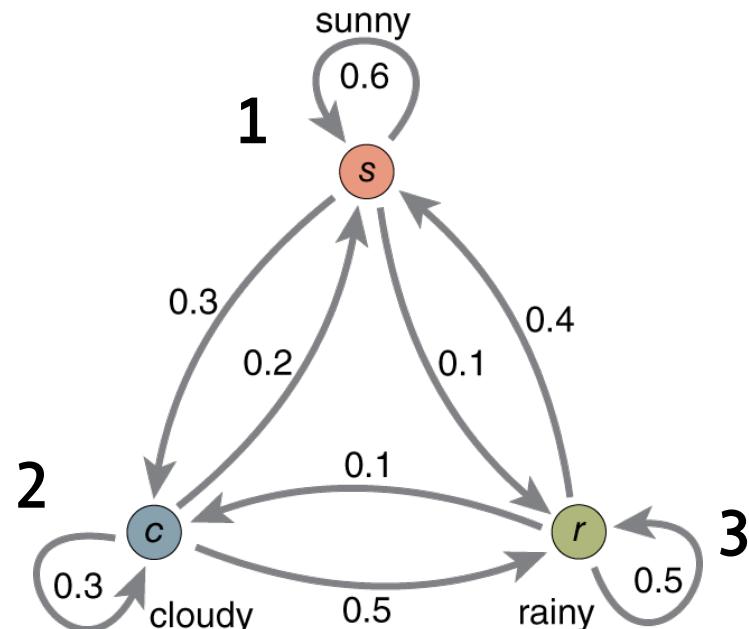
(Actually done mathematically on P_{ij})

- Obviously one can ask the same question about any $i \rightarrow j$
 - Remember that time is steps $\times \tau$

Committor

Question: if we start from 1, what is the probability to pass through 2 w.r.t. 3 first? (regardless of the path)

- This is the $I \rightarrow \{2,3\}$ committer probability
 - States which are equally probable to fall back to the “reactants” or advance to “product” state form the *transition state*.



1 1 1 2 2 3 3 1 1 1 2 2 2 2 1 1 1 1 1 3 1 1 1 1 1 2 3 1 1 3...
 ↓↑ 2 ↓↑ 2 ↓↑ 3
 ↓↑ 2 ↓↑ 2 ↓↑ 3 And so on...

Answer: compute the fraction of end-in-2 vs end-in-3.

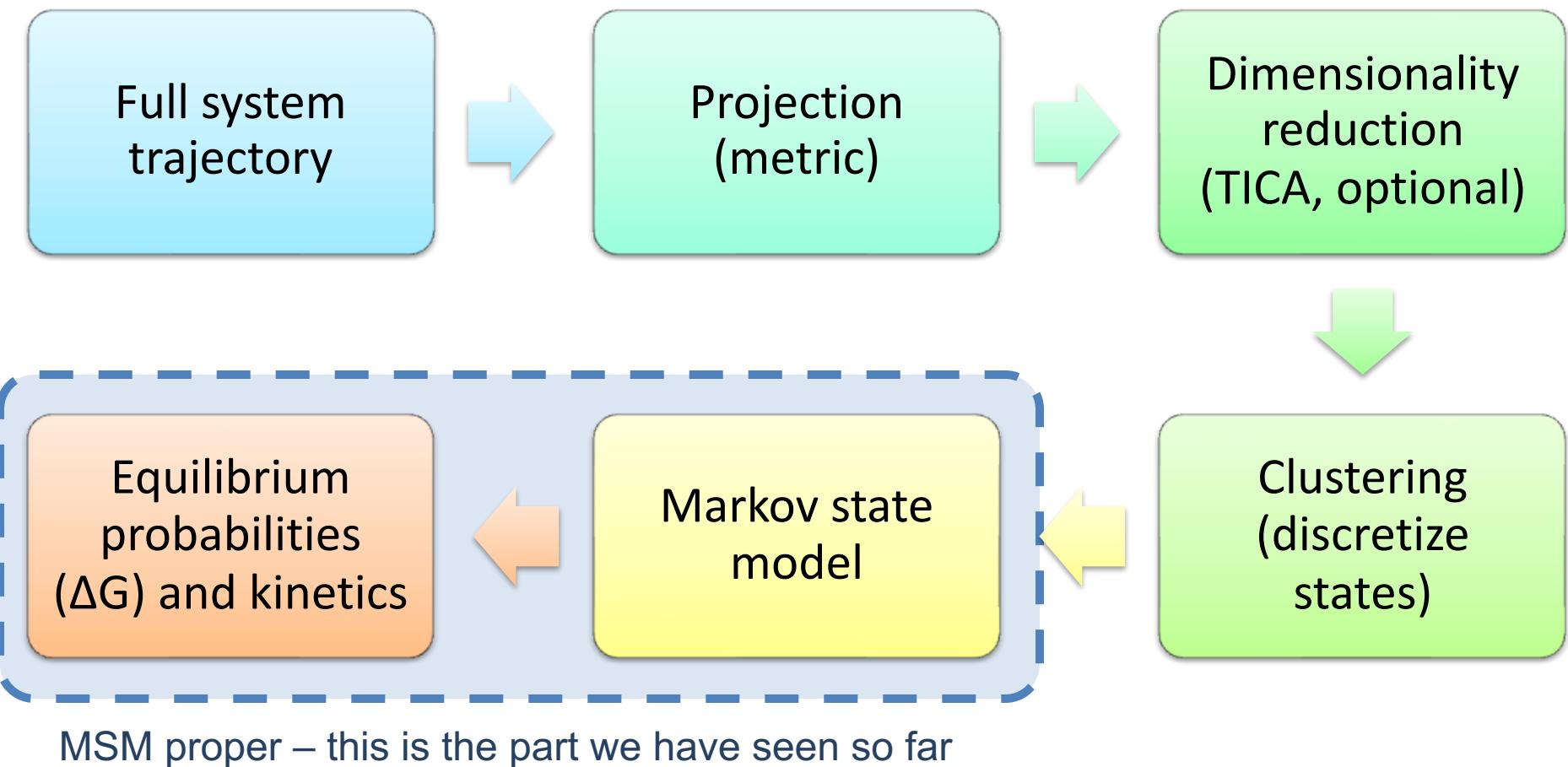
(Actually done mathematically on P_{ij})

Markovianity

- The state transition probabilities only depend on the current state. Examples:
 - Today's weather, not yesterday's
 - Where the ligand is, not how did it got there
- The property may be false at short timescales but true at longer ones: study varying τ
- It does depend on the chosen states: study varying state discretization

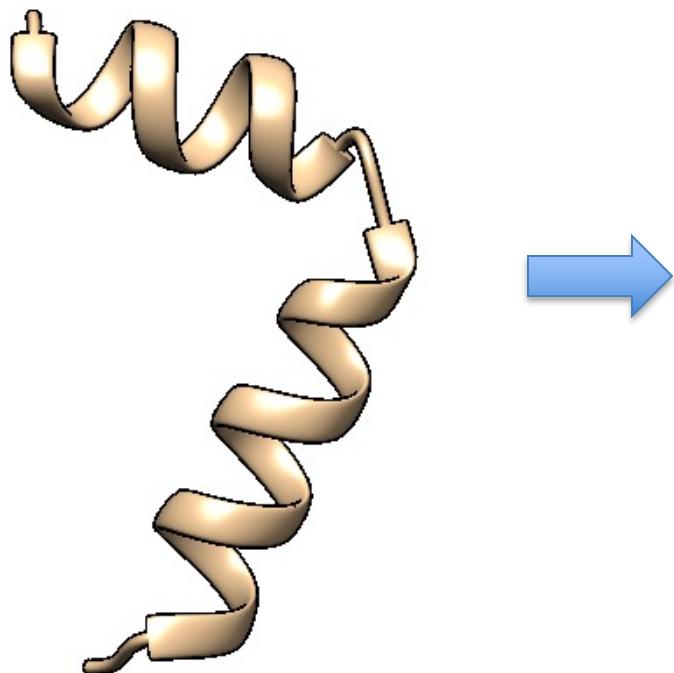
Back to molecules:
>> 1 dimension

MSM-based analysis overview



Metric projection

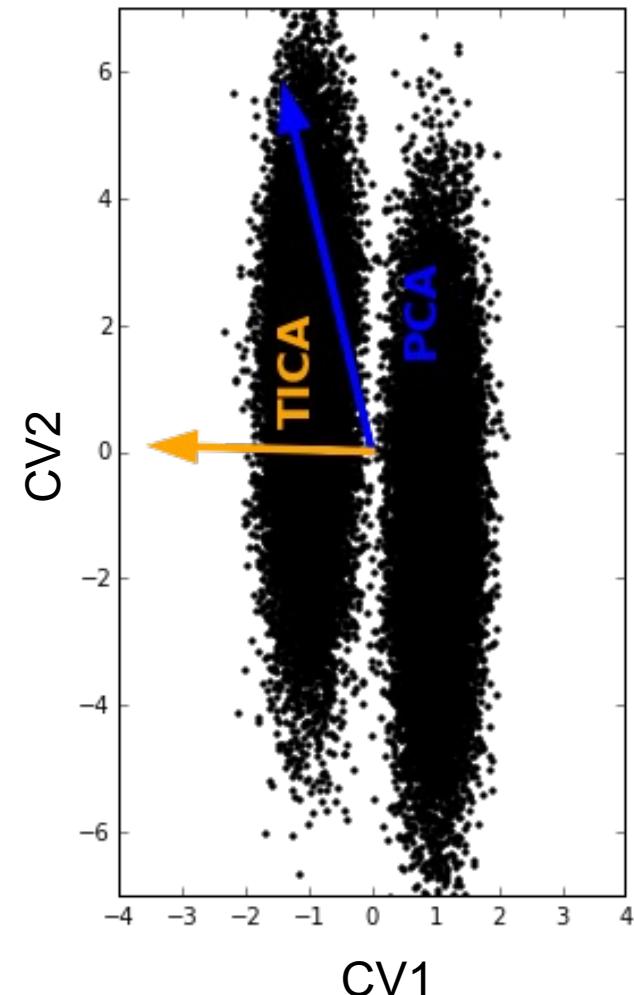
The first step is to project the system state in a lower-dimensional space (“metric”). Many choices are available, e.g.



- Manually chosen distances
- Atom coordinates
- N phi/psi Ramachandran angles
- Distance matrix
- Contact matrix
- ...

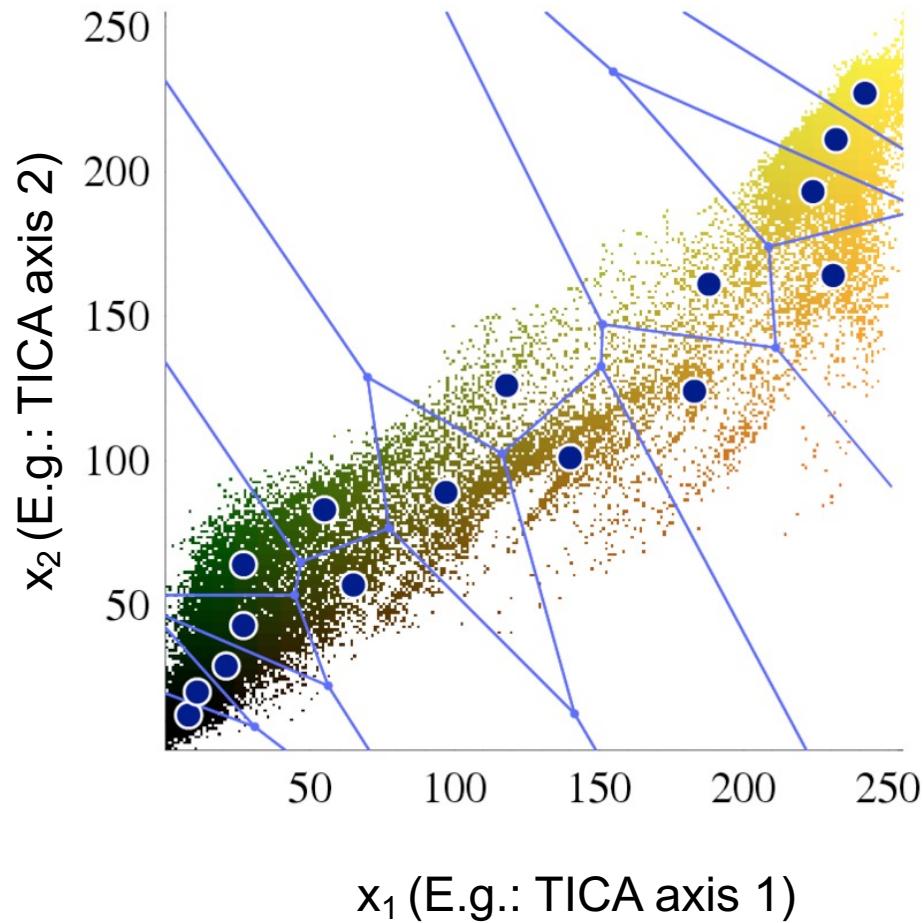
Time-lagged independent component analysis

- The feature space dimensionality may still be too high
- Do a further low-dimensional projection on the “slow” degrees of freedom:TICA
- It is based on lagged autocorrelation
- Contrast with PCA, which fits the “most elongated” ellipsoid, **ignoring time**



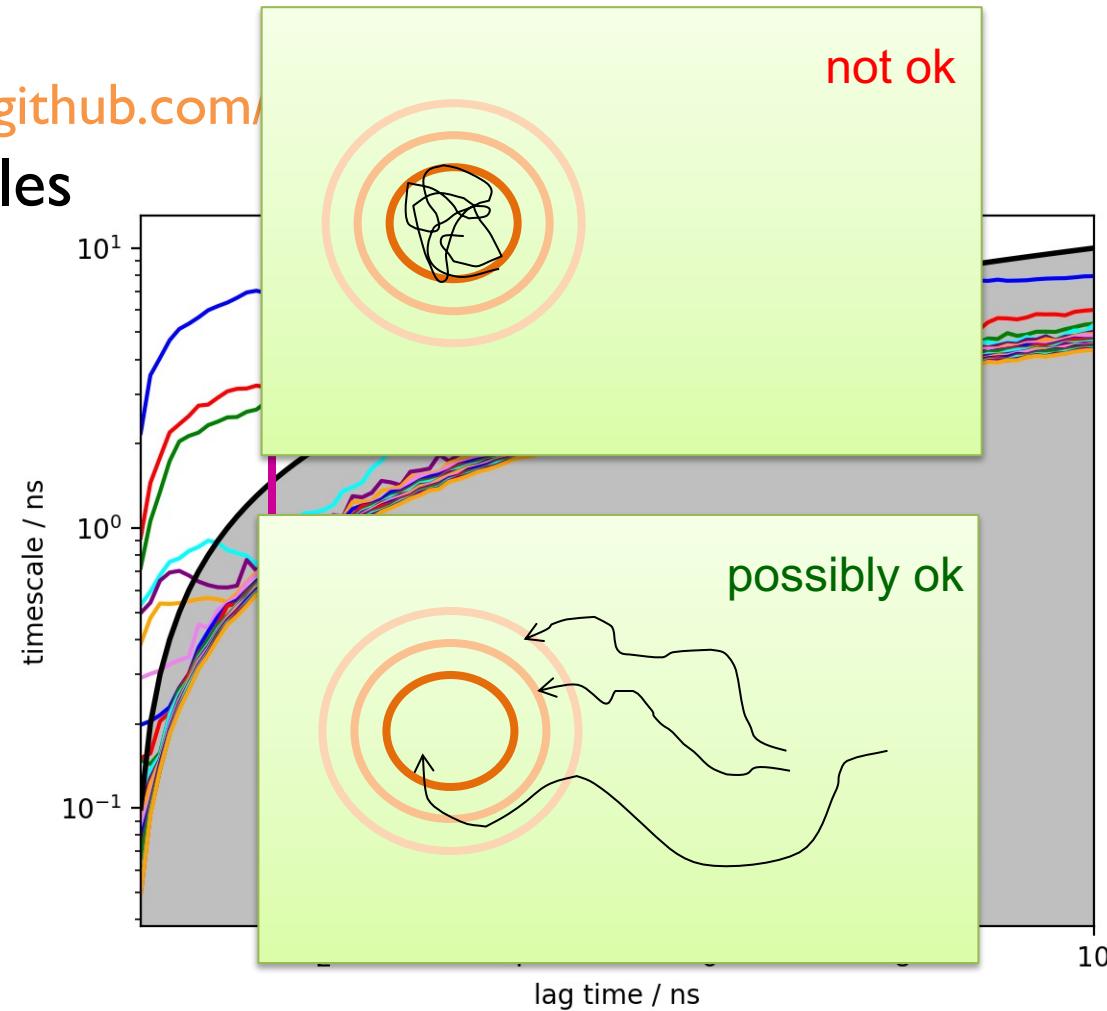
Clustering (1st level)

- Now move to a discrete state space
- Reduction from the low-dimensional space is done by *clustering*
 - Usually called “microstates”
- Several algorithms are implemented in MSM packages (e.g. grid; k-means; etc. We won’t discuss them)



Even when merging multiple trajectories, making efficient use of sampling does not dispense us from sampling the phase space

- Ace-Ala3-Nme
- Part of examples at: github.com/omnisimulations/ace-alanine
- 6 Ramachandran angles
- The system is trapped
- How to «shoot» trajectories:
 - «bathtub» not ok
 - «shower» maybe
 - adaptive spawning
 - or your favourite string-like method



Examples from the literature

Complete reconstruction of an enzyme-inhibitor binding process by molecular dynamics simulations

Ignasi Buch, Toni Giorgino, and Gianni De Fabritiis¹

Computational Biochemistry and Biophysics Laboratory, Universitat Pompeu Fabra, Barcelona Biomedical Research Park, C/Doctor Aiguader 88, 08003 Barcelona, Spain

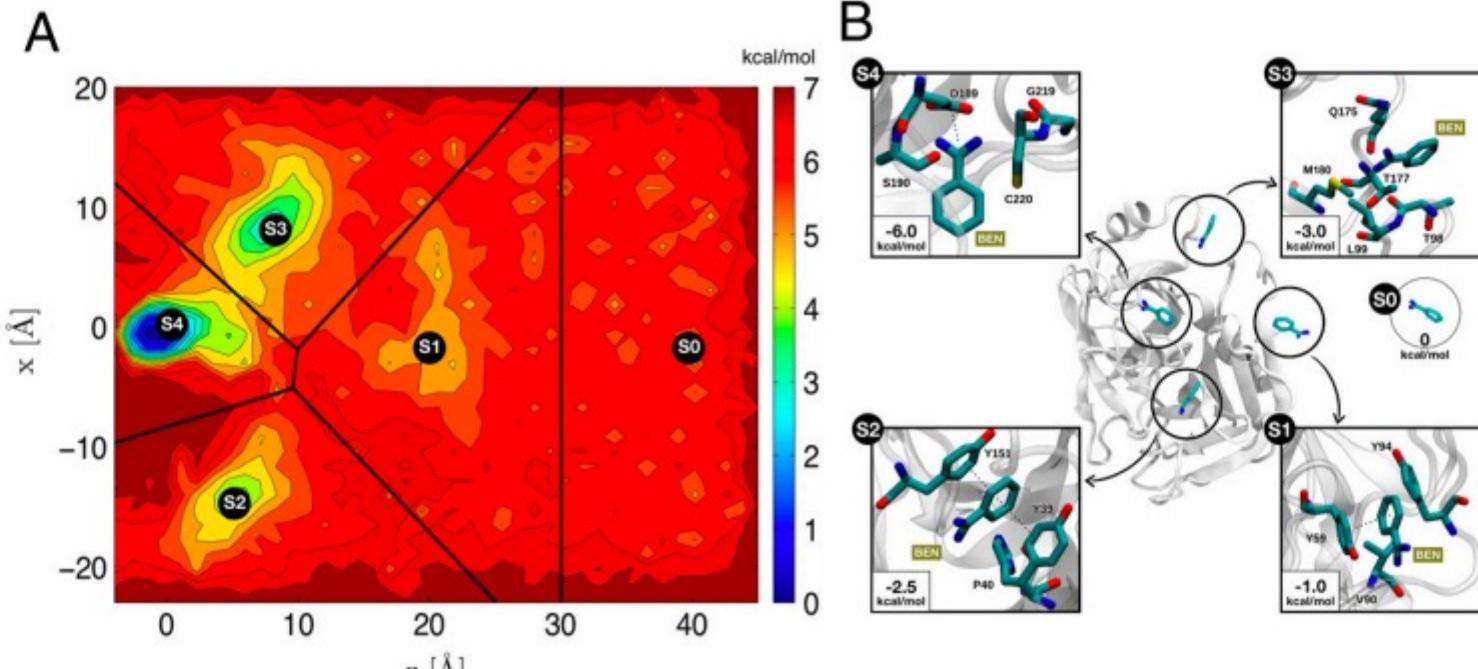
Edited by Arieh Warshel, University of Southern California, Los Angeles, CA, and approved May 11, 2011 (received for review March 4, 2011)

The understanding of protein–ligand binding is of critical importance for biomedical research, yet the process itself has been very difficult to study because of its intrinsically dynamic character. Here, we have been able to quantitatively reconstruct the complete binding process of the enzyme-inhibitor complex trypsin-benzamidine by performing 495 molecular dynamics simulations of the

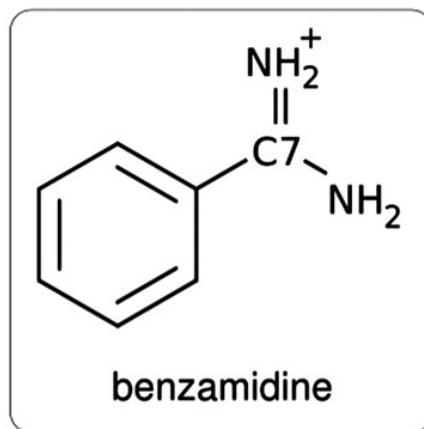
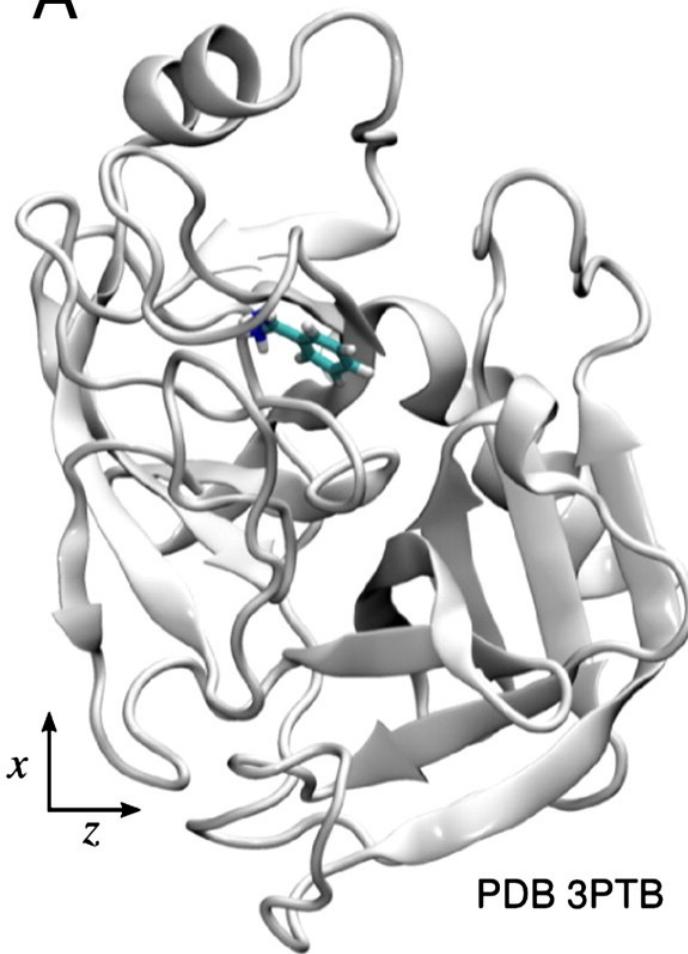
reproduce with atomic resolution the crystallographic mode of binding, but we also provide the kinetically and energetically meaningful transition states of the process.

Free ligand binding has been used in the past to describe computational experiments in which, typically, a ligand is placed at a certain distance from the target protein and first by diffusion and

in the pro-

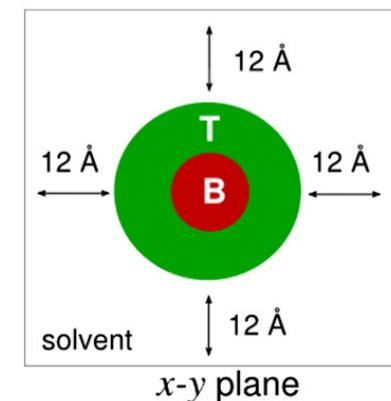
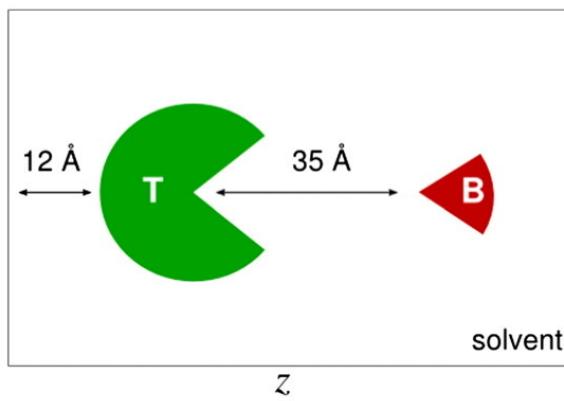


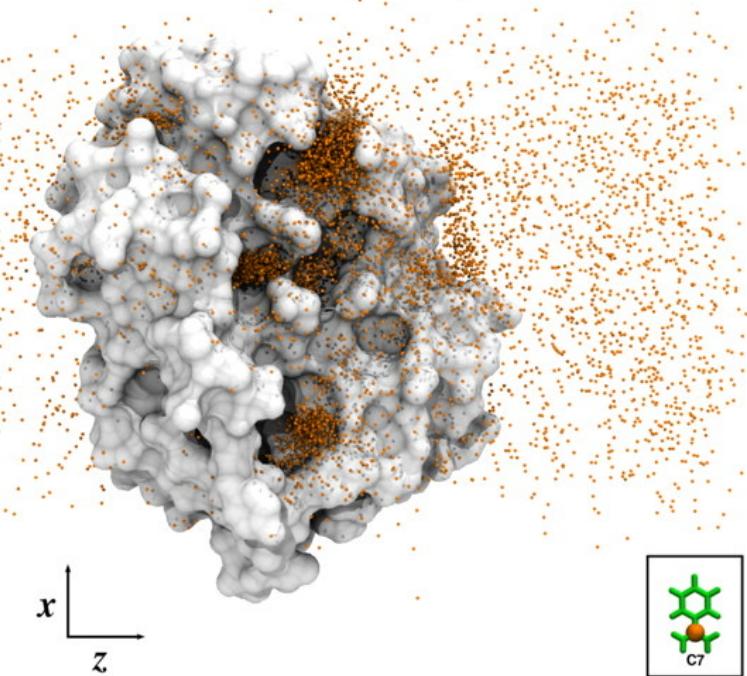
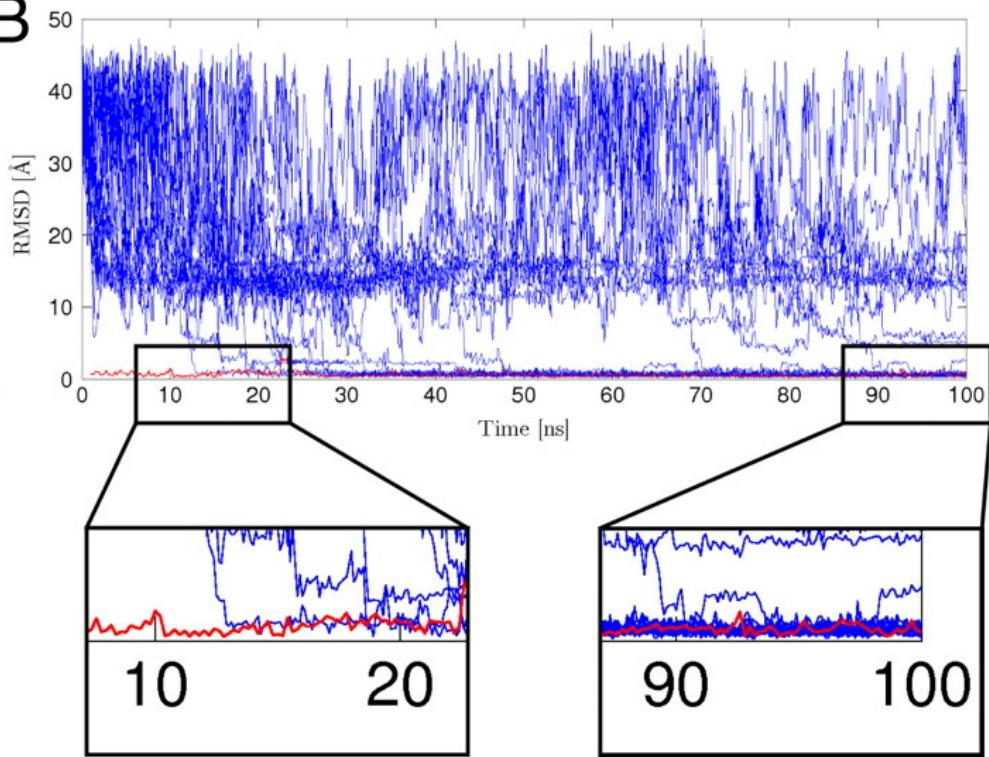
A



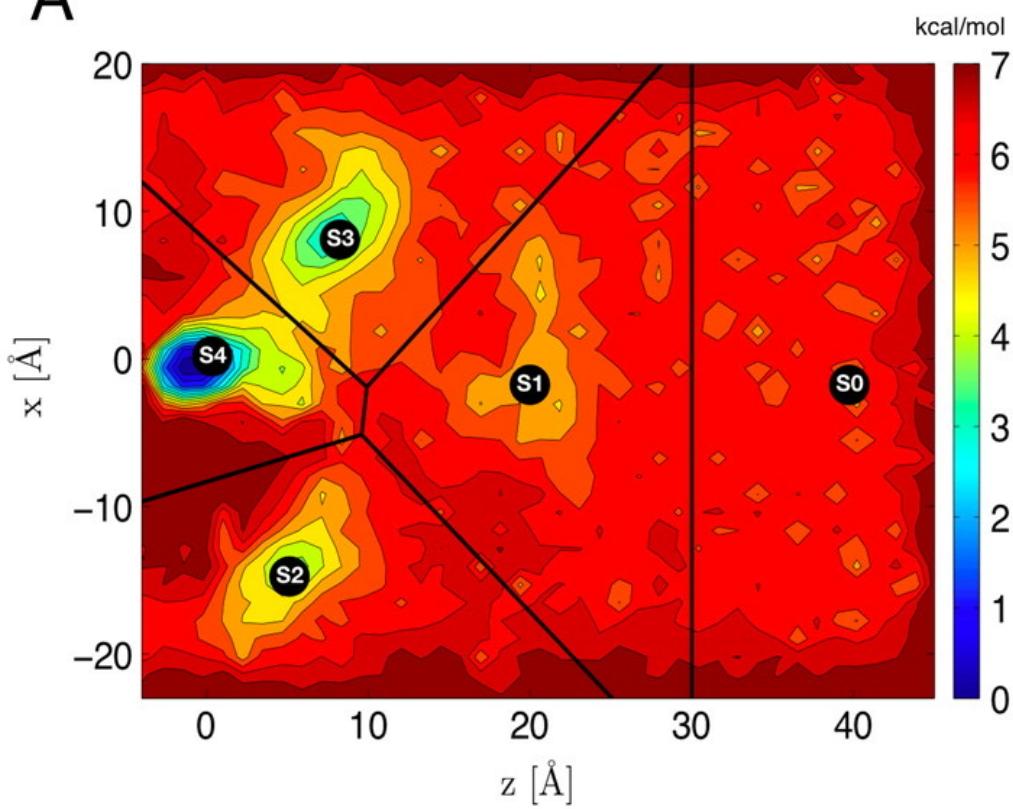
- Rigid ligand: no internal degrees of freedom
- Small ligand: no orientation d.o.f.
- States defined as small cubes according just to the position in 3D space

B

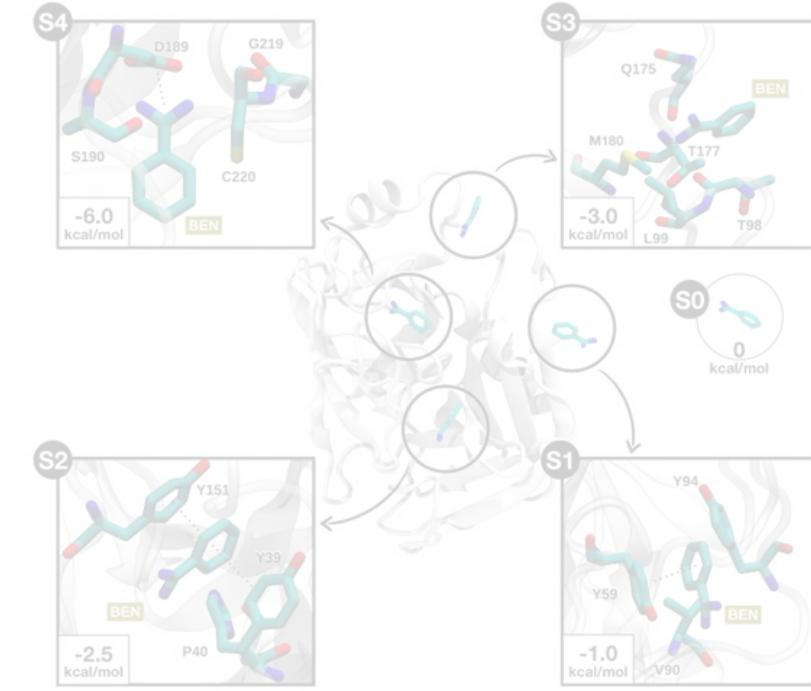


A**B**

A



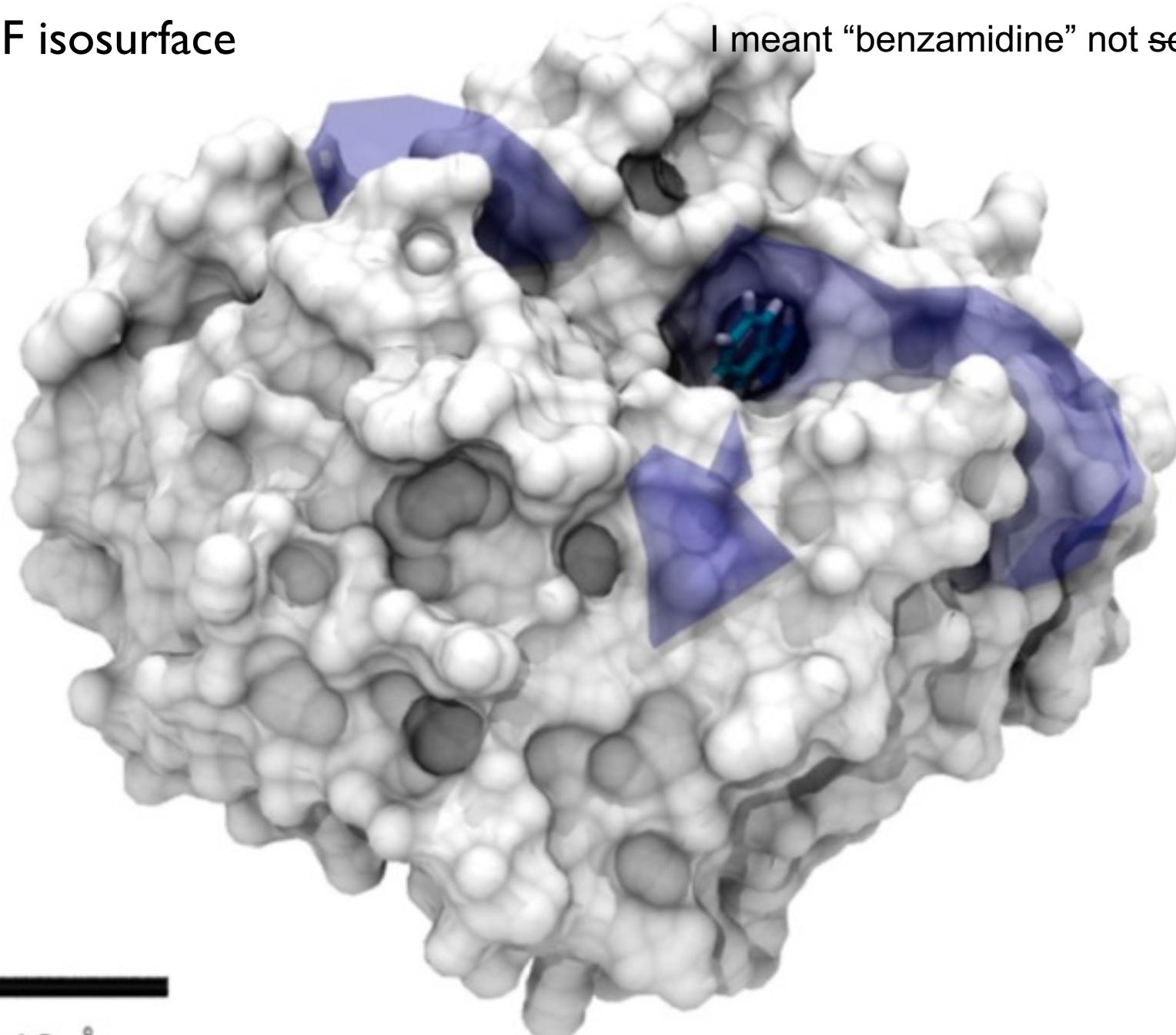
B



Identification of metastable states. (A) PMF in the xz plane. Five different metastable states can be identified from the different free-energy minima (S0 to S4). The relative free energy between the unbound state S0 and the bound state S4 is -6 kcal/mol. The most probable transition to the bound state S4 may be from S3 from the fact that the barrier between the two states is just 1.5 kcal/mol. (B) Structural characterization of metastable states. In states S1 and S2, benzamidine is stabilized by π - π stacking interactions with Y151 and Y39 side chains. In S3, a hydrogen bond may be formed between NH₂ groups of benzamidine (only heavy atoms shown for clarity) and Q175 side chain, or by a cation- π interaction between the Q175 side chain again, and benzamidine's benzene ring.

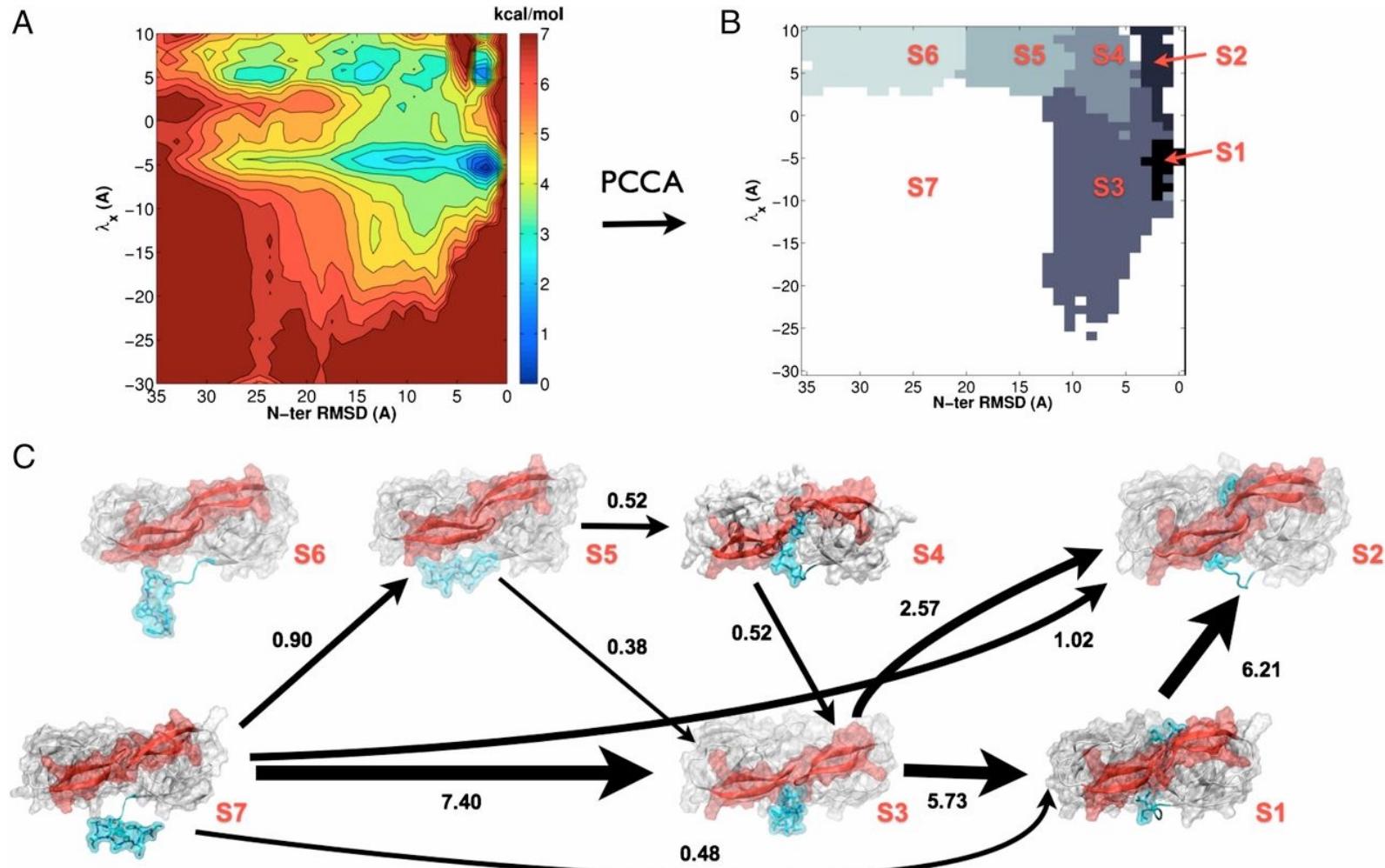
3D PMF isosurface

I meant “benzamidine” not solvent



10 Å

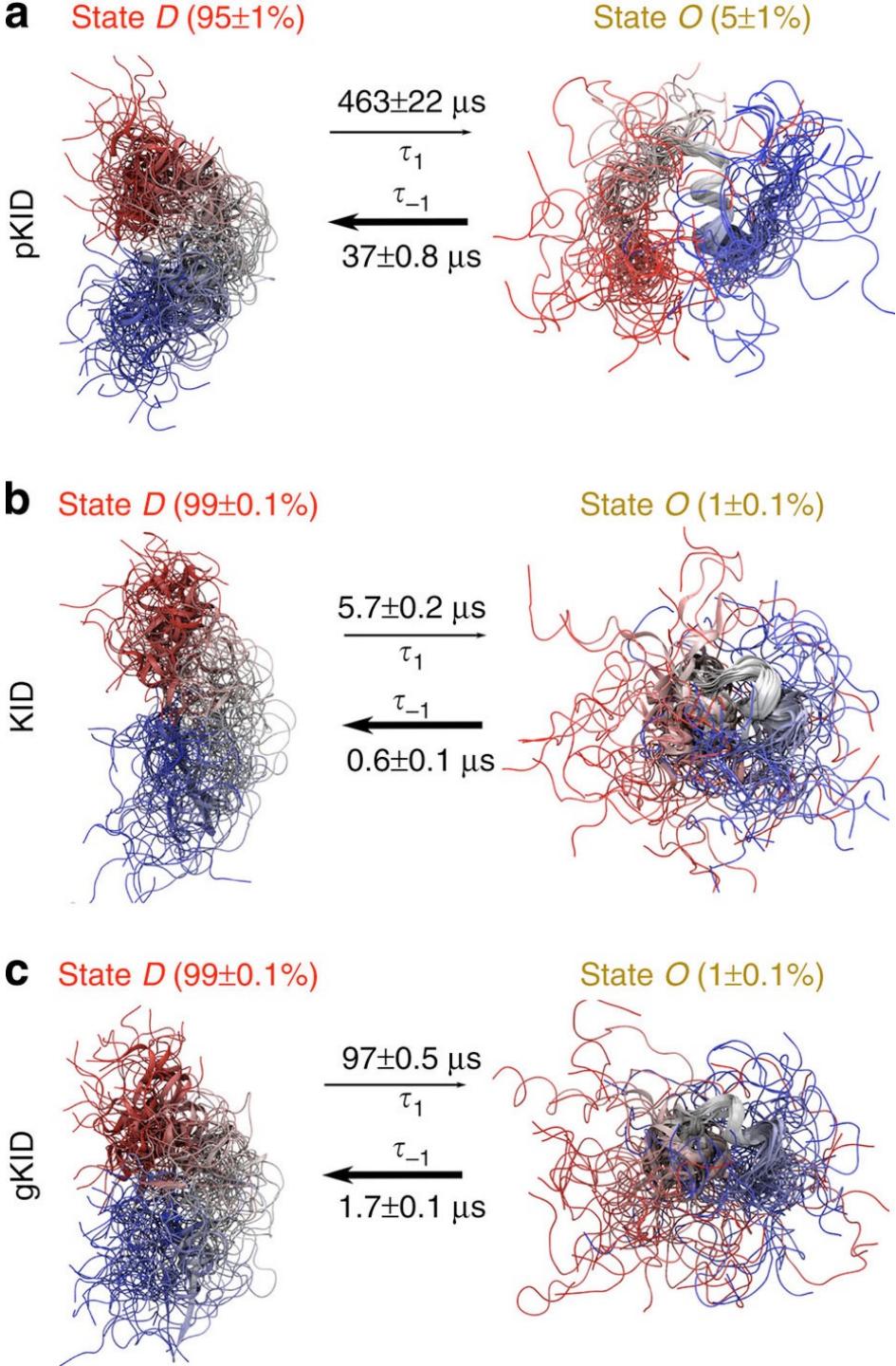
Kinetic characterization of the critical step in HIV-1 protease maturation



Kinetic modulation of a disordered protein domain by phosphorylation

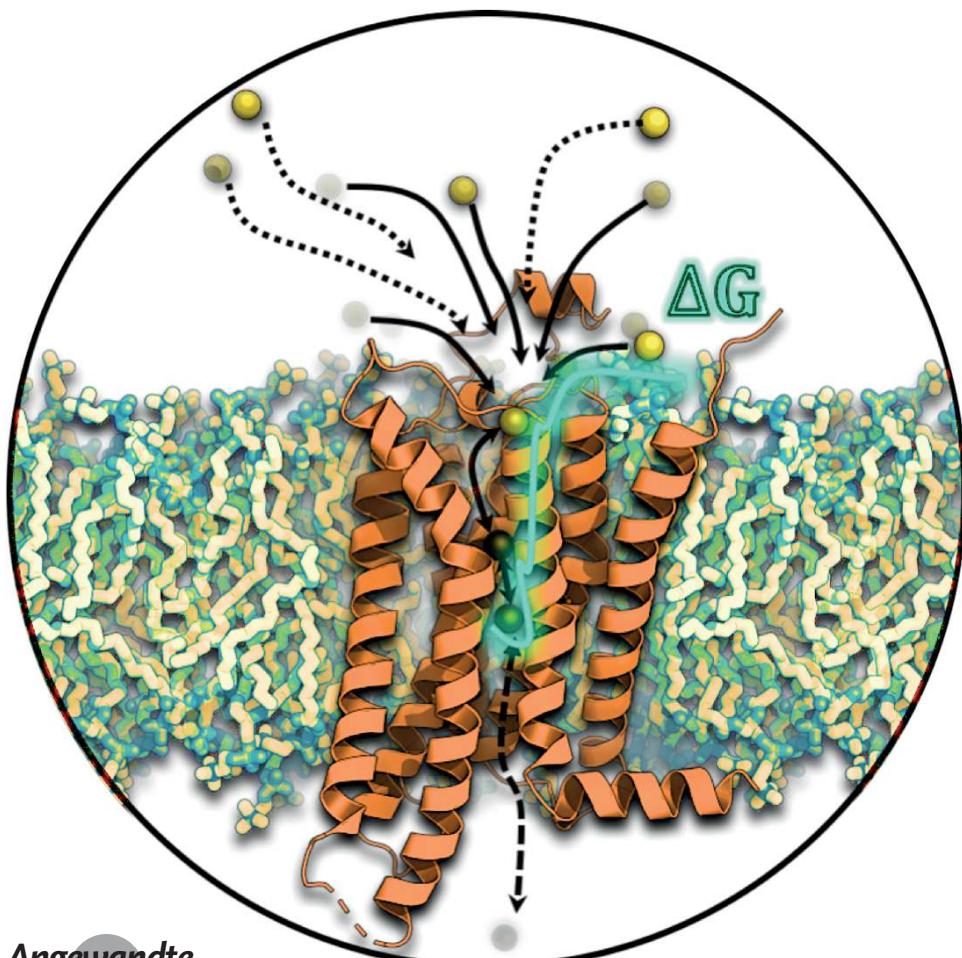
N. Stanley, S. Esteban and G. De Fabritiis, Nat. Commun. 5, 5272 (2014)

doi:10.1038/ncomms6272



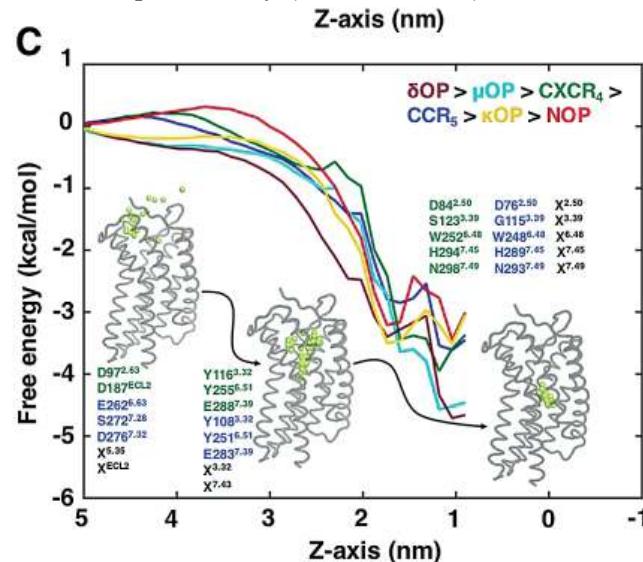
Universality of the Sodium Ion Binding Mechanism in Class A G-Protein-Coupled Receptors

Balaji Selvam, Zahra Shamsi, and Diwakar Shukla*



plays a key role in identifying drug candidates.^[7] Several studies have shown that GPCRs are allosterically modulated by endogenous Na⁺ ions.^[8–11] Selent and co-workers explored the binding mechanism of Na⁺ to the D₂ receptor by molecular dynamics simulations.^[12] Na⁺ binds in the middle of the transmembrane (TM) region to a conserved Asp^{2,50} residue (Ballesteros–Weinstein numbering),^[13] as elucidated by high-resolution crystal structures of the GPCRs.^[14–16] More recent MD studies on μ-, κ-, and δ-opioid (OP) receptors also support Na⁺ ion binding to Asp^{2,50}.^[17,18] Na⁺ binding and a change in the protonation states of titratable residues through the water network have a significant effect on the stabilization of the conformational states of GPCRs.^[19–21]

elusive. Herein, we performed hundreds-of-microsecond long simulations of 18 GPCRs to elucidate their Na⁺ binding mechanism (see the Supporting Information, Tables S1 and S2). We constructed Markov state models (MSMs) to estimate the free energy profiles for Na⁺ binding to each GPCR. Analysis of the Na⁺ binding kinetics revealed key residues that act as major barriers for Na⁺ entry to the intracellular site. We also predicted the average mean first passage time (MFPT) for Na⁺ binding and unbinding events by transition path theory (TPT; Table S3).



Conclusions and Resources

Warning

- Still very active field
- Suggested further steps: worked out real-world examples distributed with software packages*
- For serious work, many more details are in...
 - the theory of Markov state models
 - the discretization, projection, and estimation of models from trajectories

* see the last slides

Conclusions

- MSM methods may be an attractive formalism for medium-sized problems
- Make efficient use of *unbiased* sampling
- Still require *huge* (but achievable) amounts of sampling/simulation for biologically interesting systems
- Strong mathematical foundation, with good software available

Software + Tutorials

- All are Python-based
 - They include clear walkthroughs (highly recommended) with datasets
- DeepTime-ML – deeptime-ml.github.io ←
- HTMD – www.htmd.org
 - Also analysis + system build + adaptive ...
 - Can aggregate large-scale datasets
- MSMBuilder - msmbuilder.org

MD Simulations – day 2

Part 2b - docking



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www.giorginolab.it

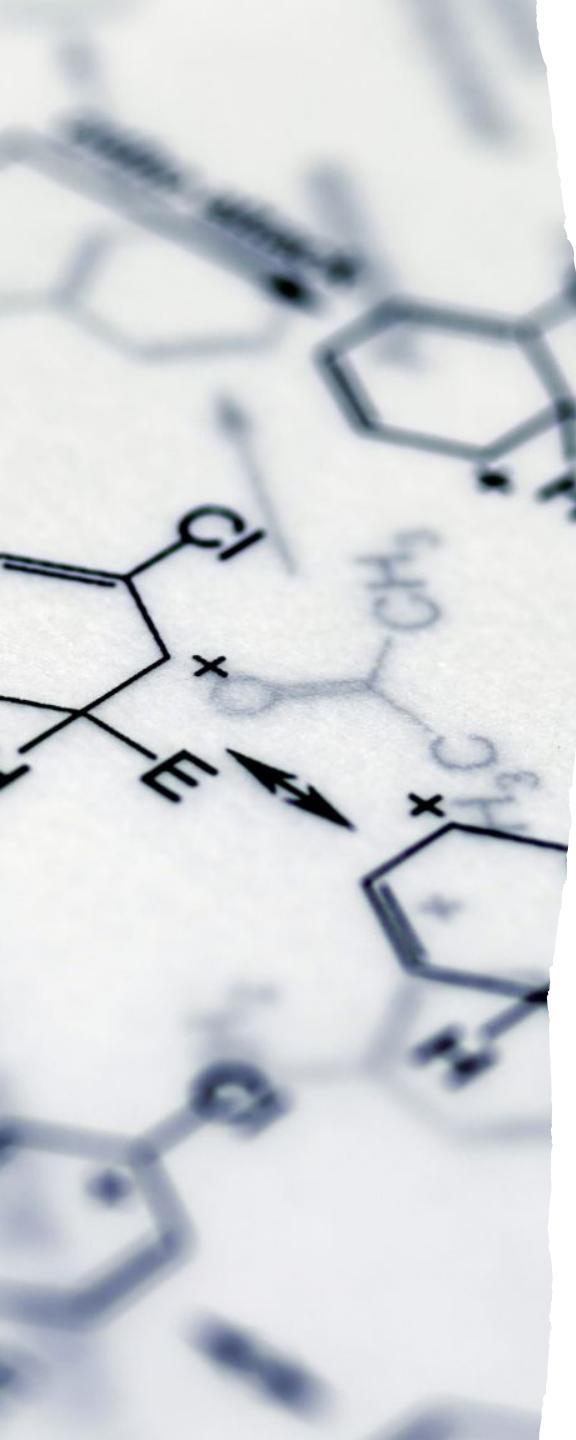


@giorginolab

<https://github.com/giorginolab/MD-Tutorial-Data>

for Prof. Fuxreiter's course @ University of Padova

May 2024



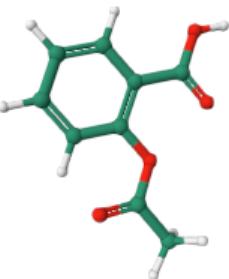
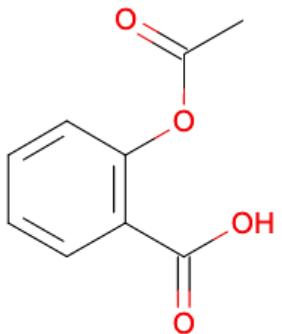
PTGS1* and **PTGS2** are the targets of nonsteroidal anti-inflammatory drugs (NSAIDs) including aspirin and ibuprofen.

Aspirin is able to produce an irreversible inactivation of the enzyme through a serine acetylation.

Inhibition of the PGHSs with NSAIDs acutely reduces inflammation, pain, and fever, and long-term use of these drugs reduces fatal thrombotic events, as well as the development of colon cancer and Alzheimer's disease.

PTGS2 is the principal isozyme responsible for production of inflammatory prostaglandins. New generation PTGSs inhibitors strive to be selective for PTGS2, to avoid side effects such as gastrointestinal complications and ulceration.

* COX1 = Prostaglandin G/H synthase 1
= PGH1_HUMAN = P23219


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AIN

2-(ACETYLOXY)BENZOIC ACID

Find entries where: AIN

is present as a standalone ligand in **8 entries**

[search](#)

Find related ligands:

[Similar Ligands \(Stereospecific\)](#)

[Similar Ligands \(Including Stereoisomers\)](#)

[Similar Ligands \(Quick Screen\)](#)

[Similar Ligands \(Substructure Stereospecific\)](#)

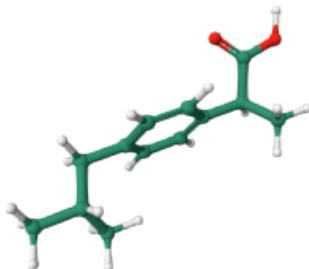
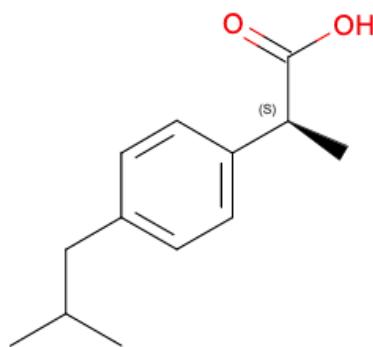
[Similar Ligands \(Substructure Including Stereoisomers\)](#)

Chemical Component Summary

Name	2-(ACETYLOXY)BENZOIC ACID
Synonyms	ACETYLSALICYLIC ACID; ASPIRIN
Identifiers	2-acetoxybenzoic acid
Formula	C ₉ H ₈ O ₄
Molecular Weight	180.157
Type	NON-POLYMER
Isomeric SMILES	CC(=O)Oc1ccccc1C(=O)O
InChI	InChI=1S/C9H8O4/c1-6(10)13-8-5-3-2-4-7(8)9(11)12/h2-5H,1H3,(H,11,12)
InChIKey	BSYNRYMUTXBXSQ-UHFFFAOYSA-N

Chemical Details

Formal Charge	0
Atom Count	21
Chiral Atom Count	0
Bond Count	21
Aromatic Bond Count	6

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IBP

IBUPROFEN

Find entries where: IBP

 is present as a standalone ligand in 14 entries[search](#)

Find related ligands:

[Similar Ligands \(Stereospecific\)](#)[Similar Ligands \(including Stereoisomers\)](#)[Similar Ligands \(Quick Screen\)](#)[Similar Ligands \(Substructure Stereospecific\)](#)[Similar Ligands \(Substructure including Stereoisomers\)](#)

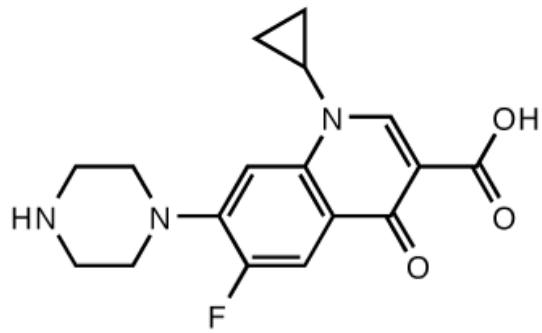
Chemical Component Summary

Name	IBUPROFEN
Synonyms	2-(4-ISOBUTYLPHENYL)PROPIONIC ACID
Identifiers	(2S)-2-[4-(2-methylpropyl)phenyl]propanoic acid
Formula	C ₁₃ H ₁₈ O ₂
Molecular Weight	206.281
Type	NON-POLYMER
Isomeric SMILES	CC(C)Cc1ccc(cc1)[C@H](C)C(=O)O
InChI	InChI=1S/C13H18O2/ c1-9(2)8-11-4-6-12(7-5-11)10(3)13(14)15/h4-7,9-10H, 8H2,1-3H3,(H,14,15)/t10-/m0/s1

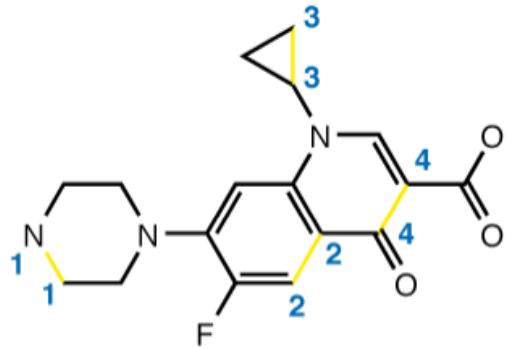
Chemical Details

Formal Charge	0
Atom Count	33
Chiral Atom Count	1
Bond Count	33
Aromatic Bond Count	6

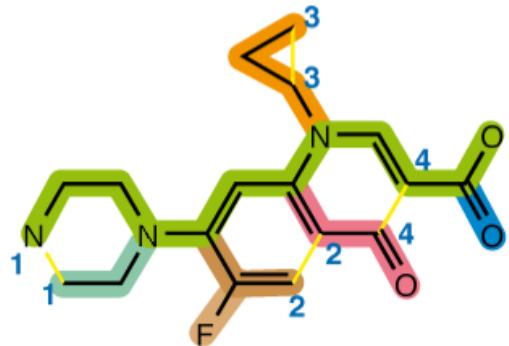
A



B



C



D

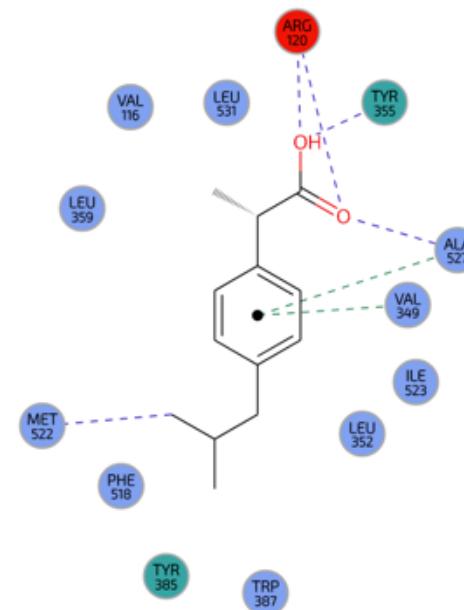
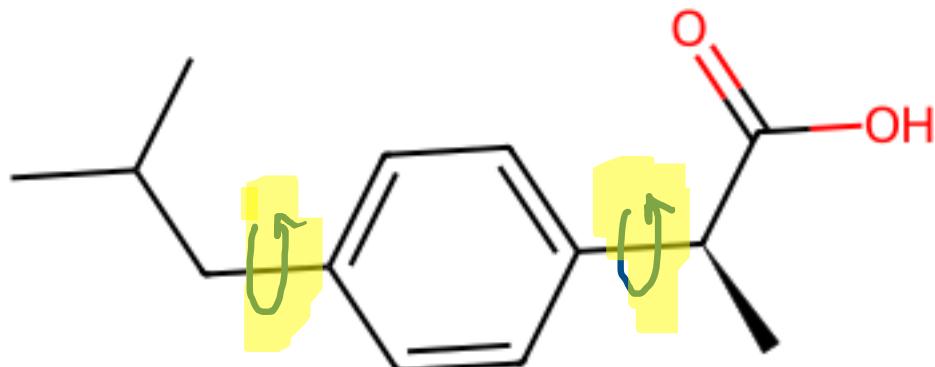
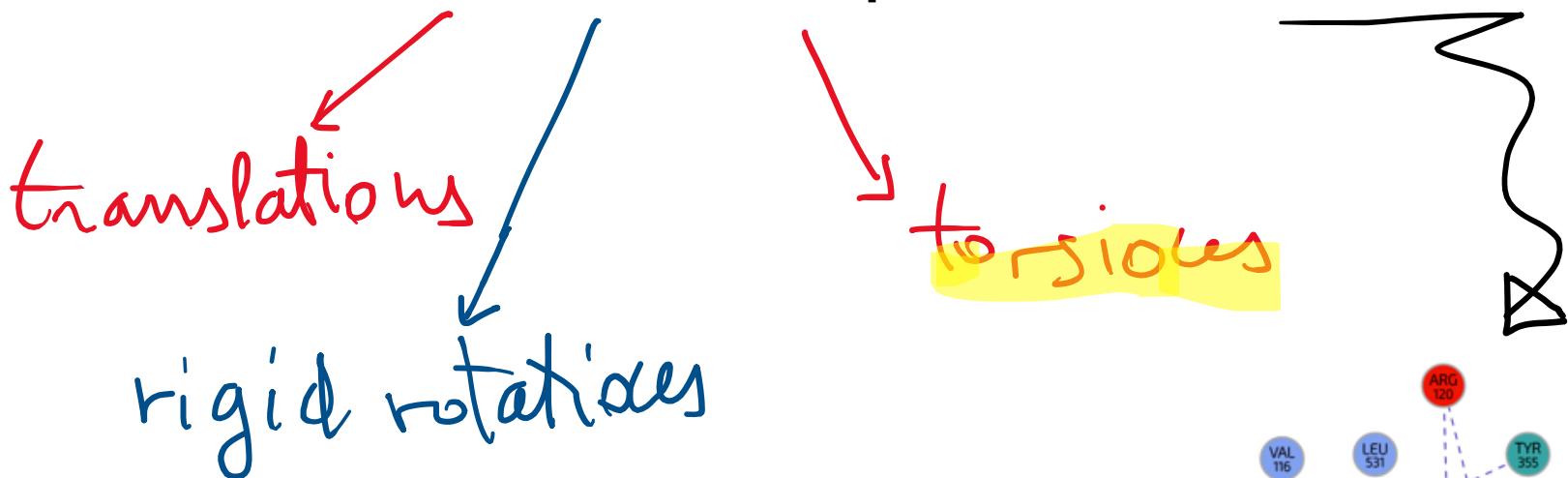
N1CCN(CC1)C(C(F)=C2)=CC(=C2C4=O)N(C3CC3)C=C4C(=O)O



- SMILES is a “ID” representation of the molecular graph.
- However, it **can** encode stereochemistry and cis/trans isomerism.
- It does **not** encode precise 2D or 3D coordinates, which must be generated.
- It is very compact and often molecular databases are distributed as such.

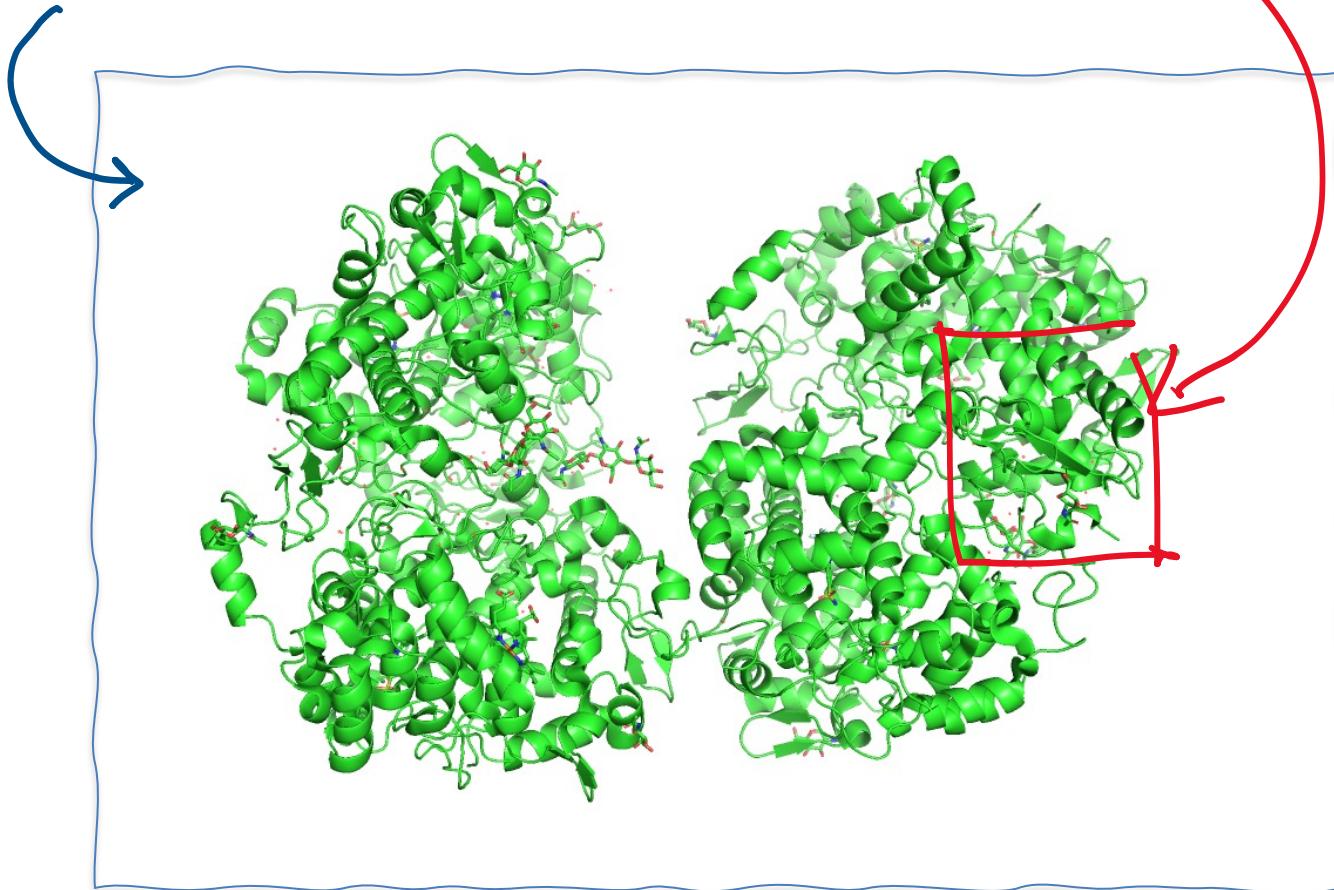
General principle

- Search a $\mathbb{R}^3 \times \mathbb{R}^3 \times \mathbb{R}^m$ space to max. score



Docking types

- Blind docking



- Known-site

Pitfalls

- Protonation states
- Stereoisomerism
- Redocking optimism
- Receptor flexibility
- Receptor protonation states
- Thoroughness of the search
- Non-determinism

Advanced topics

- ML-based docking: diffusion models
 - DiffDock [1]
- Combine MD with docking
 - Ensemble docking [2]
- Enumeration of states
 - gypsum_dl [3]

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- ✓ docking
 - Docking_2024.ipynb
- > etc

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tonigi Creato con Colab

Name
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Docking_2024.ipynb

References

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- Basciu A, Mallochi G, Pietrucci F, Bonvin AMJJ, Vargiu AV. Holo-like and Druggable Protein Conformations from Enhanced Sampling of Binding Pocket Volume and Shape. *J Chem Inf Model.* 2019 Apr 22;59(4):1515–28.
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End

End