How the Assignment of Protonation States in Distal Residues May Alter Protein-Ligand Binding in Molecular Dynamics Simulations

Helena Girame[1], Marc Garcia-Borràs[1], Ferran Feixas[1]

[1]Institut de Química Computacional i Catàlisi (IQCC), Universitat de Girona (UdG), 17003 Girona, Spain

helena.girame@udg.edu

3/05/2022

Student Webinar: BioExcel Spring School 2022

INTRODUCTION

- On the starting point of molecular dynamics
- Object of study
- The His57 hypothesis

On the Starting Point of Molecular Dynamics

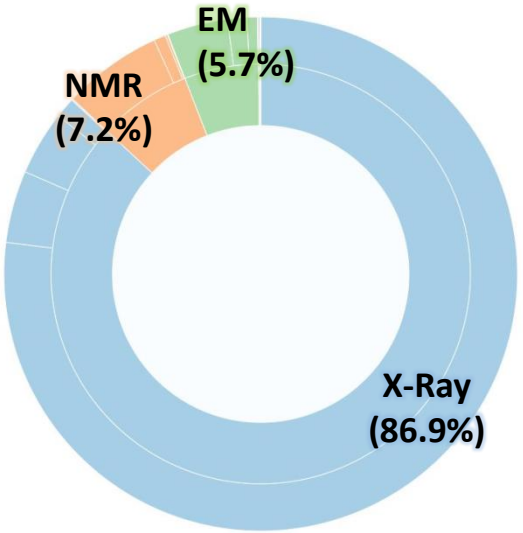
X-ray as starting point

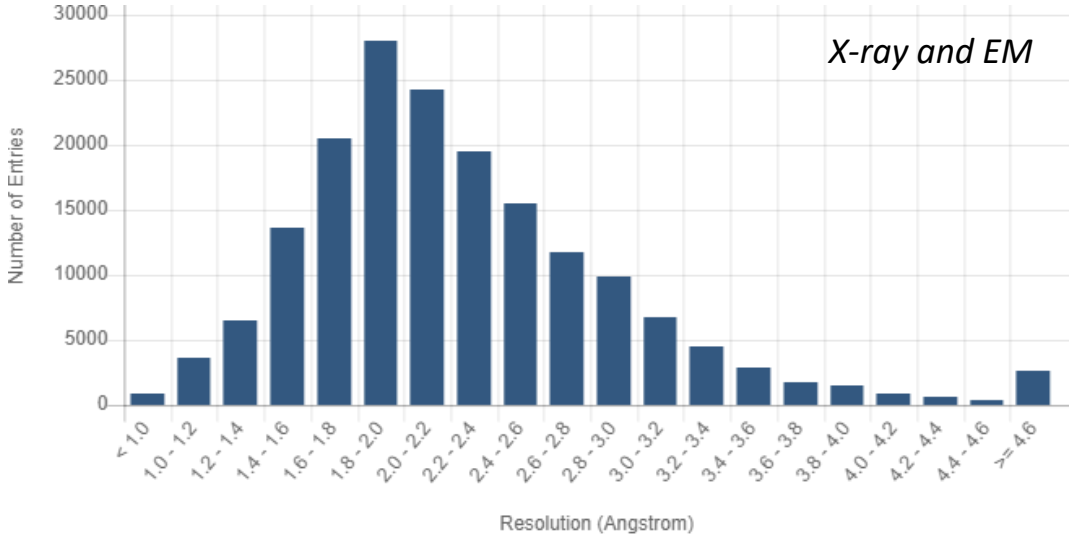
↓

No information on H atoms

↓

Ambiguity in protonation states





From: *https://www.rcsb.org/stats/*

On the Starting Point of Molecular Dynamics

X-ray as starting point

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No information on H atoms

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Ambiguity in protonation states

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Protonation tools

Protonation Tools:

- MD modeling software
- H++
- PROPKA

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X-ray as starting point

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No information on H atoms

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Ambiguity in protonation states

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Protonation tools

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Not infallible

Protonation Tools:

- MD modeling software
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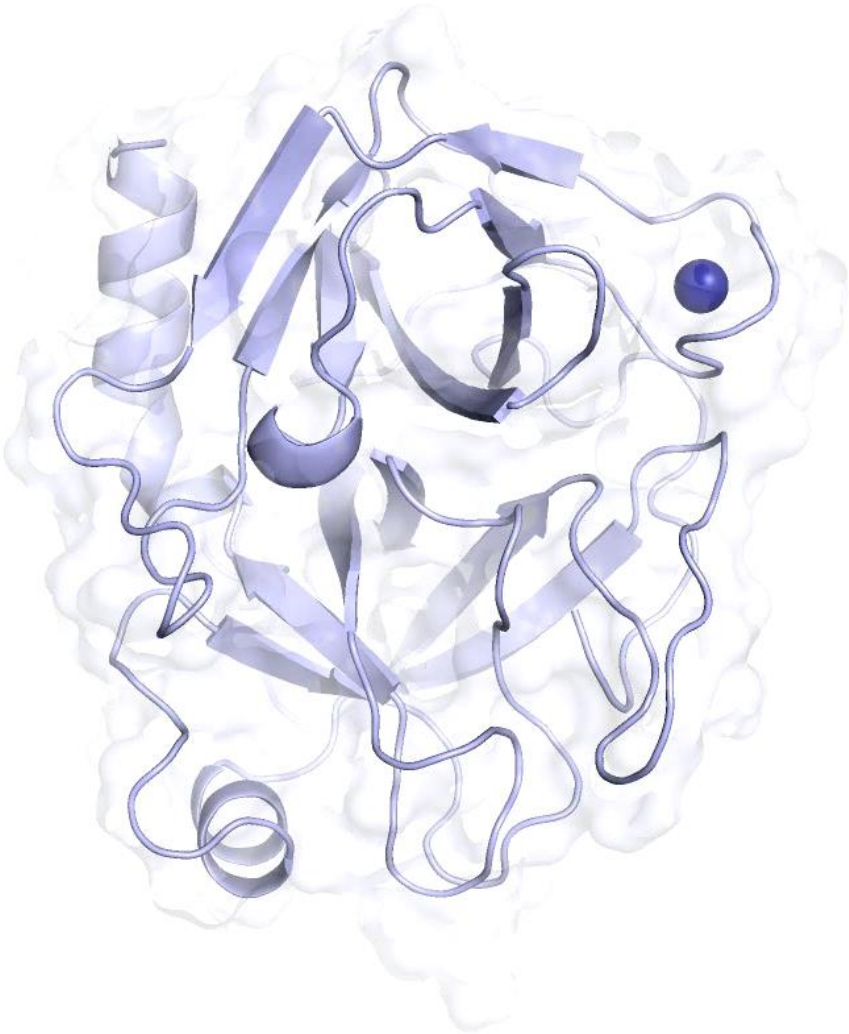
On the Starting Point of Molecular Dynamics

- X-ray as starting point
- No information on H atoms
- Ambiguity in protonation states
- Protonation tools
- Not infallible
- User revision at the region of interest



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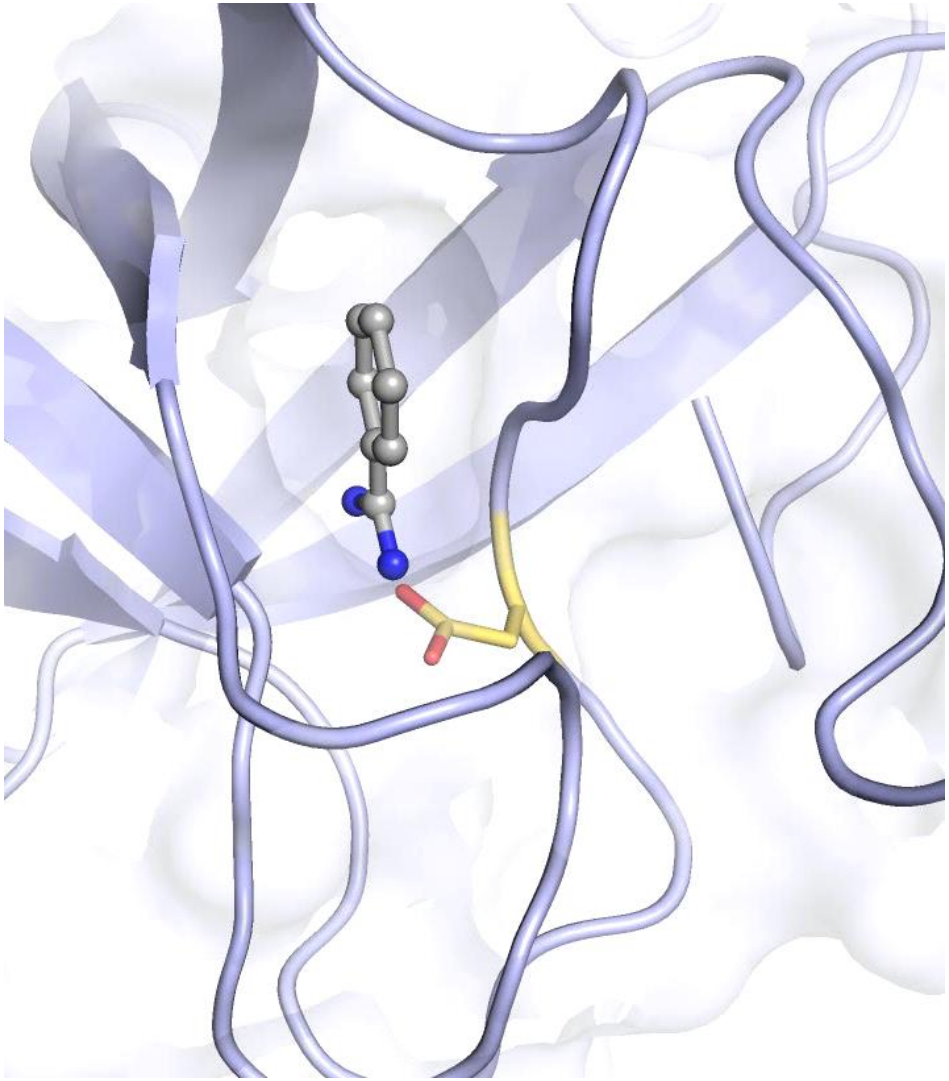

On the Starting Point of Molecular Dynamics

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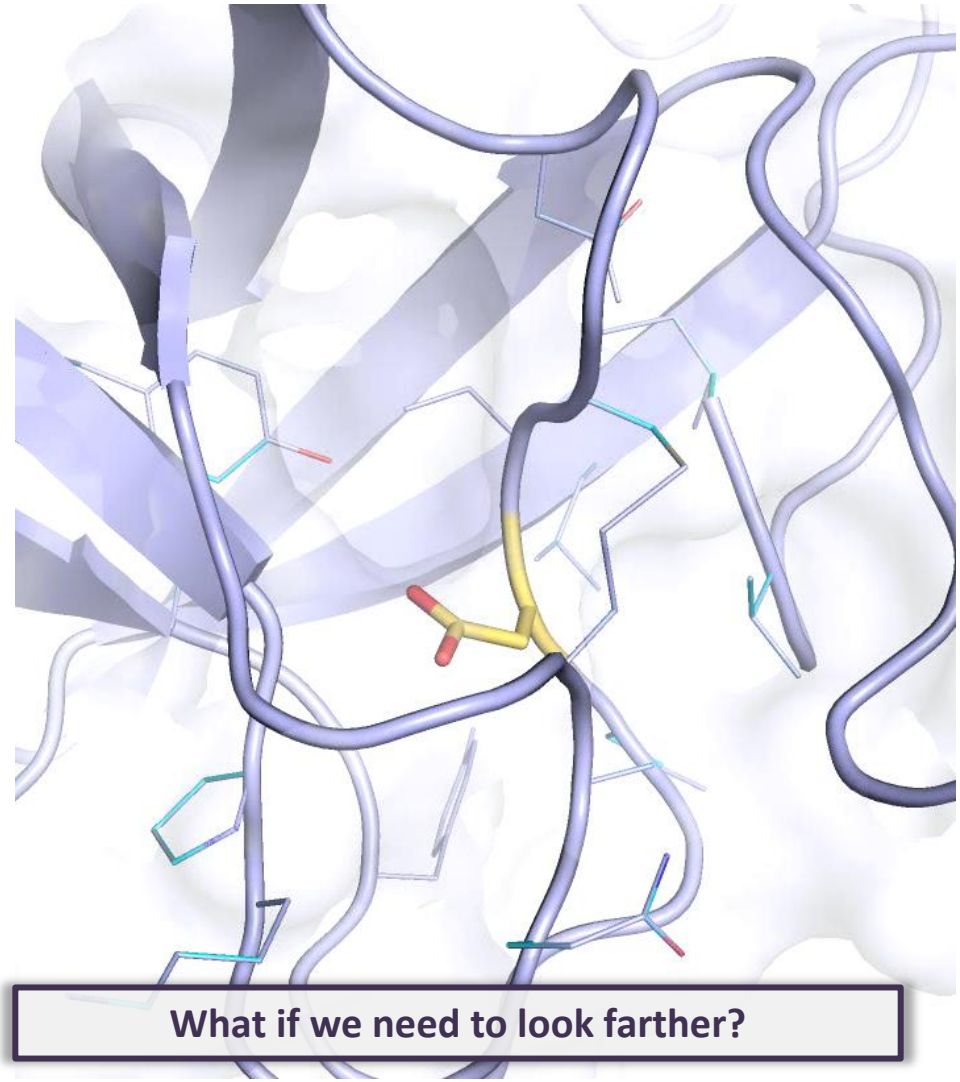
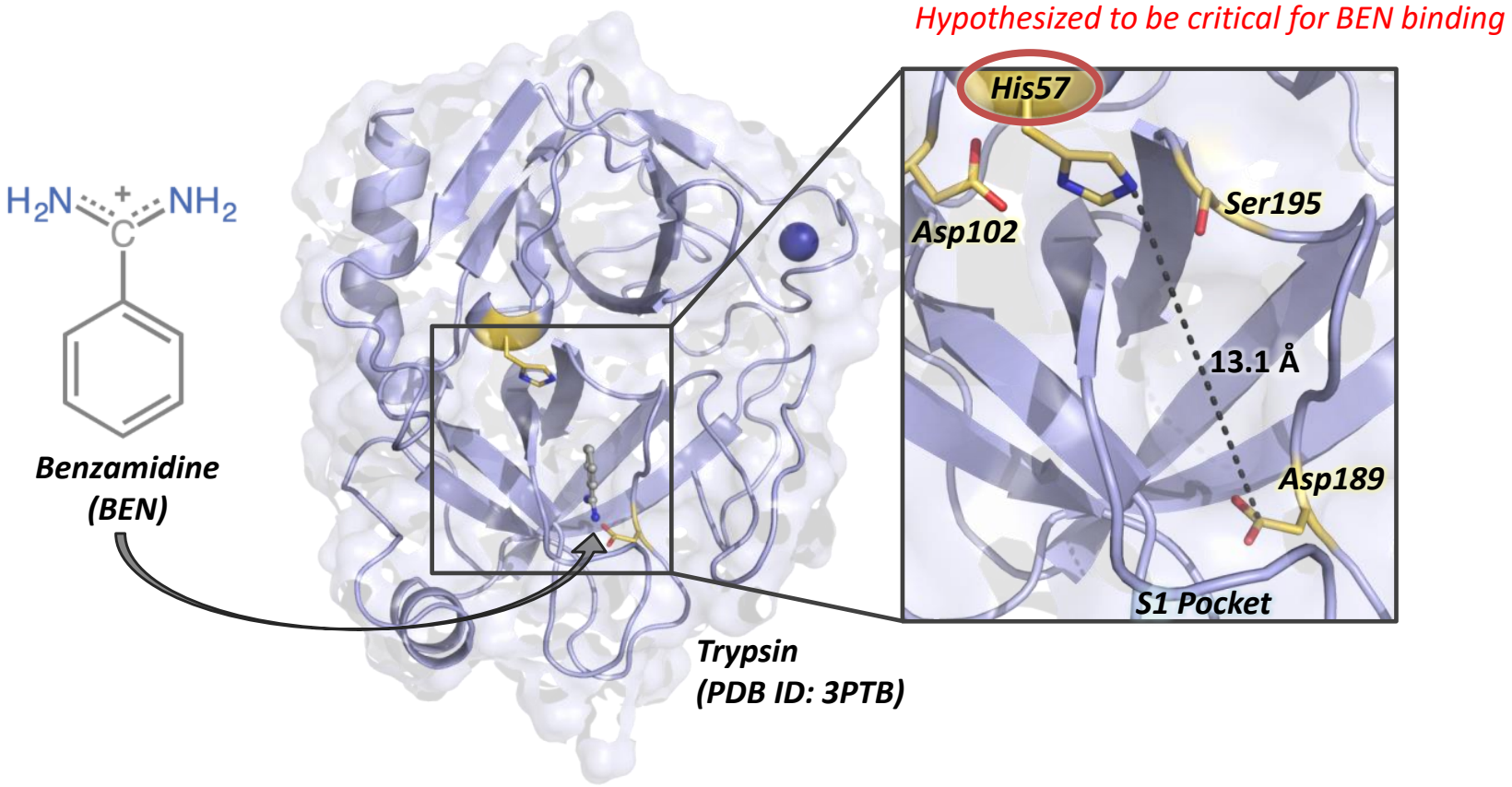
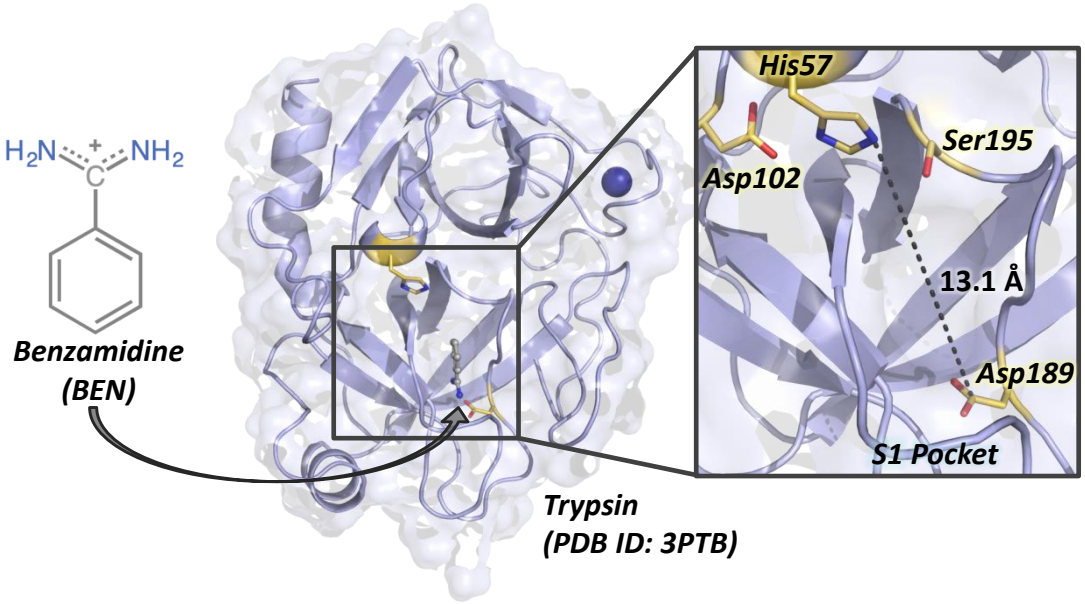


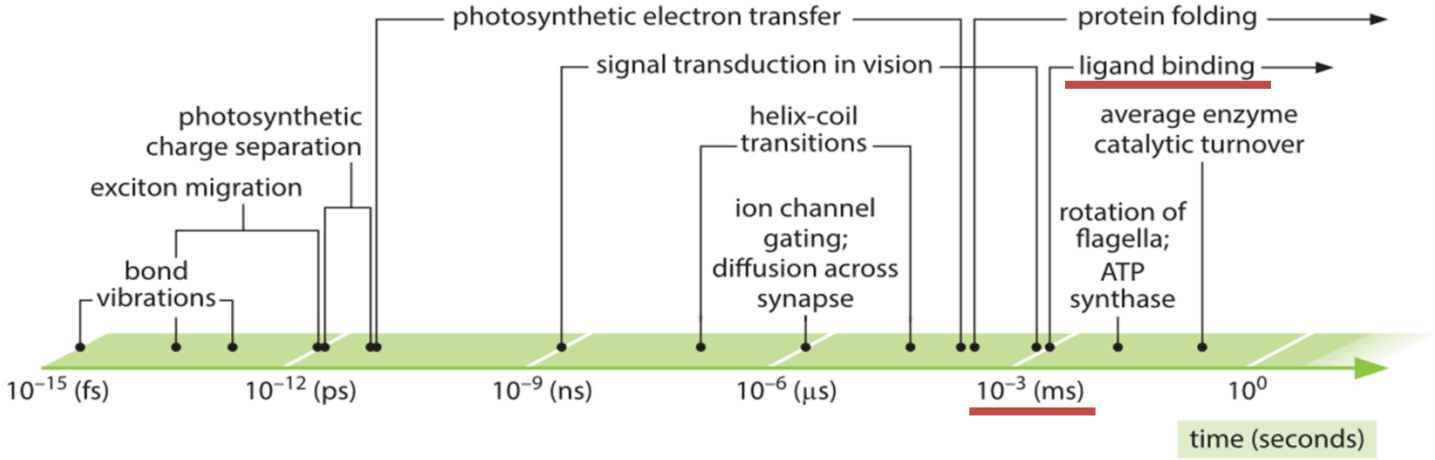
Object of Study



Object of Study

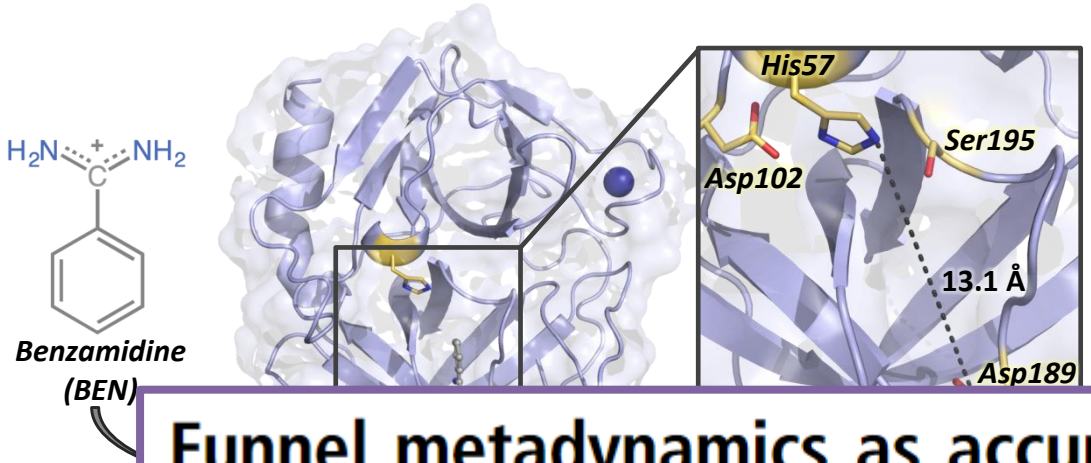


- Standard protein-ligand model

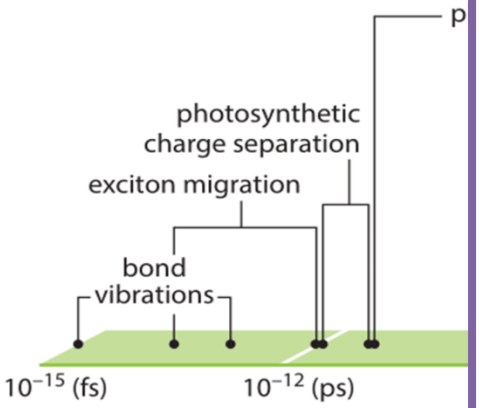


From: Milo, R. and Phillips, R. (2015). *Cell Biology by the Numbers*

Object of Study



- Standard protein-lig



From: Milo, R. and Phillips,

Funnel metadynamics as accurate binding free-energy method

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

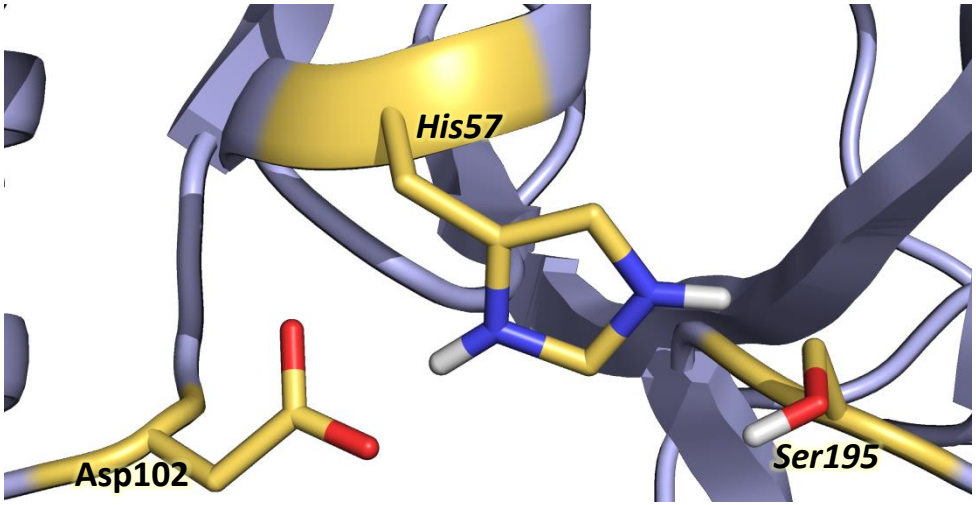
Protein conformational plasticity and complex ligand-binding kinetics explored by atomistic simulations and Markov models

Nuria Plattner[1] & Frank Noé[1]

Ligand Gaussian Accelerated Molecular Dynamics (LiGaMD): Characterization of Ligand Binding Thermodynamics and Kinetics

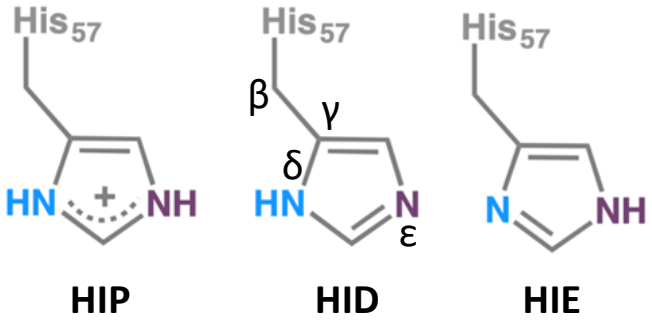
Yinglong Miao,* Apurba Bhattarai, and Jinan Wang

The His57 Hypothesis



HIP is the suggested one

Hydrogen-bond analysis - HID is also a possibility



Tool	pH 7.0	pH 8.0
H++ (3.0)	HIP	HIP
PROPKA3	HIP	HID

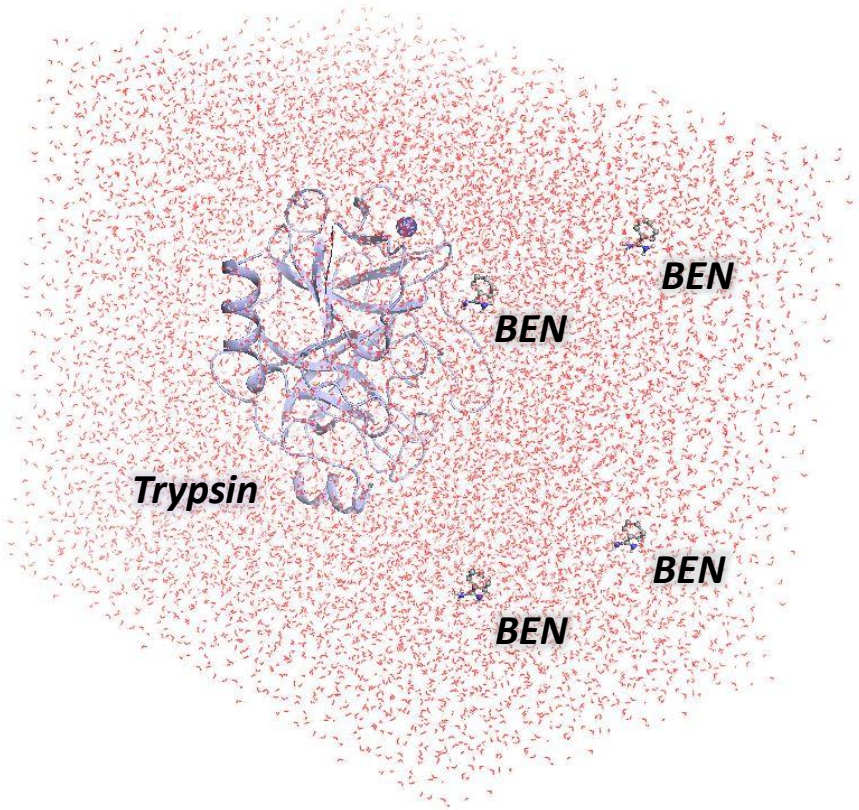
pH of activation [7-9]

METHODOLOGY

- Spontaneous molecular dynamics
- Spontaneous constant-pH molecular dynamics

Spontaneous Molecular Dynamics

- Spontaneous MD simulations in explicit solvent.



3 sets of { **HIP** / **HID** / **HIE** } x **50** replicas x 200 ns

AMBER18

- AMBER-ff14SB - Trypsin | GAFF – BEN
- Cubic box with 12Å of TIP3P water molecules
- 300K | time step of 2 fs

Spontaneous Constant-pH Molecular Dynamics

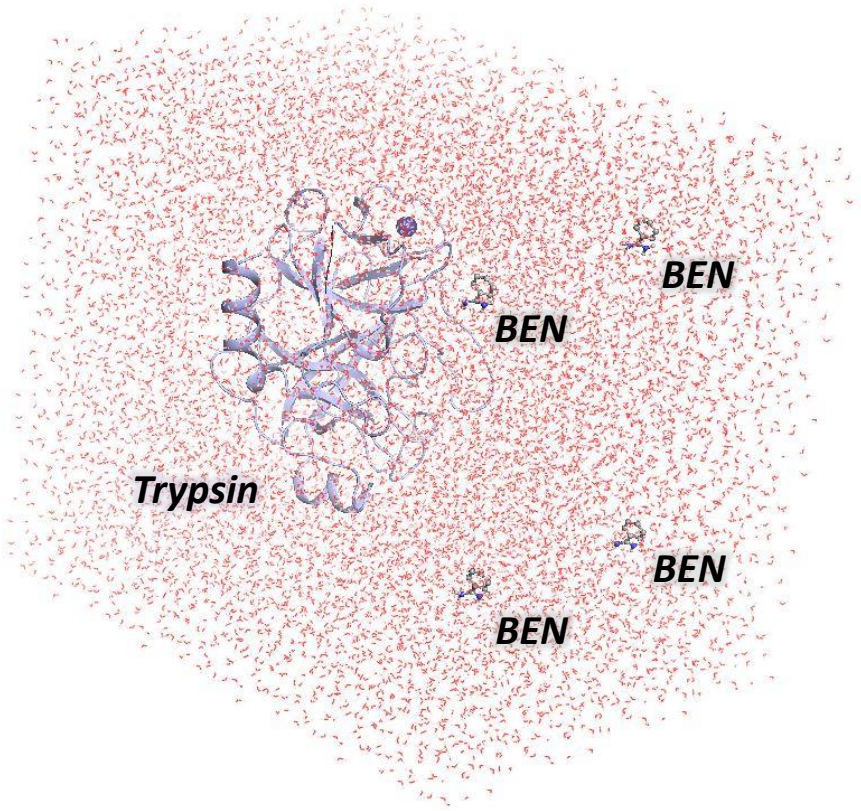
- **Discrete Constant-pH** in explicit solvent:
  - User selects which residues are allowed to titrate
  - After a set of MD steps, these residues may **change** their **protonation state** via **Metropolis Monte Carlo attempts**
- Accounts for the equilibrium

2 sets	of	**pH 7** / **pH 8**	x	**30** replicas	x	200 ns

pH of activation [7-9]

AMBER18, constant-pH parameters:

- All His were allowed to titrate
- 50 MD steps before protonation change attempt
- 100 solvent relaxation steps
- GB parameters CpHMD was parametrized for



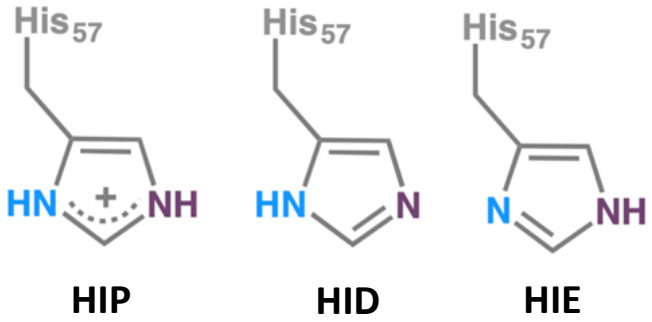
RESULTS AND DISCUSSION

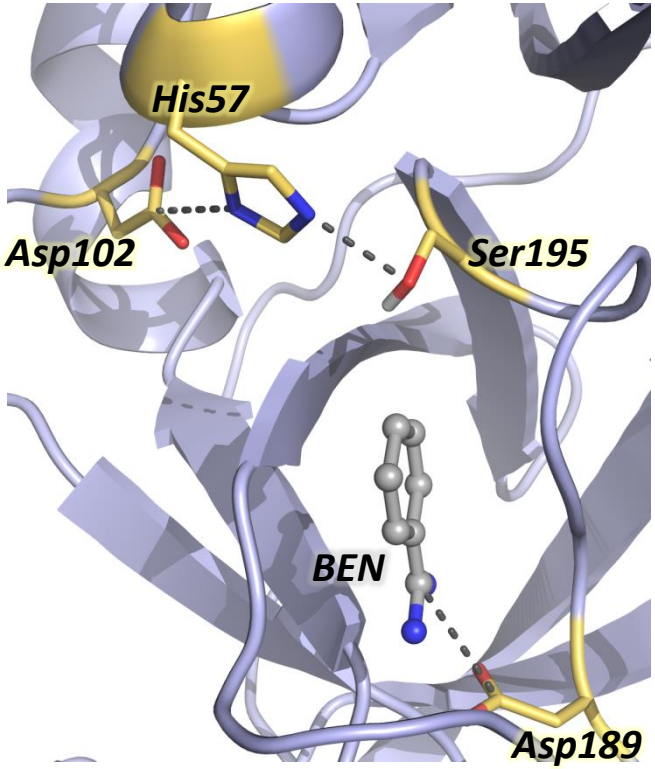
- Conventional MD
- Free-energy landscape
- Binding Pathway
- Constant-pH
- Binding Pathway is Altered by His57

Conventional MD

$$Binding\ Events[\%] = \frac{\#\ replicas\ w/binding}{\#total\ replicas}$$

	HIP	HID	HIE
Binding Events [%]	10	48	44



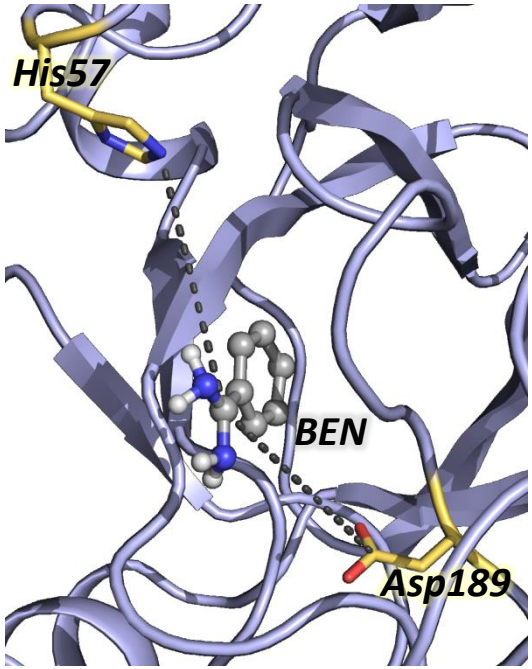


- His57 alters the number of encountered binding events

	HIP	HID	HIE	X-Ray
d(*His57-Asp102*) [Å]	3.3 ± 0.1	3.4 ± 0.1	6.7 ± 0.5	3.5
d(*His57-Ser195*) [Å]	3.1 ± 0.5	3.9 ± 0.4	4.6 ± 0.9	3.0
d(*BEN-Asp189*) [Å]	3.8 ± 0.1	3.8 ± 0.1	3.8 ± 0.1	3.9

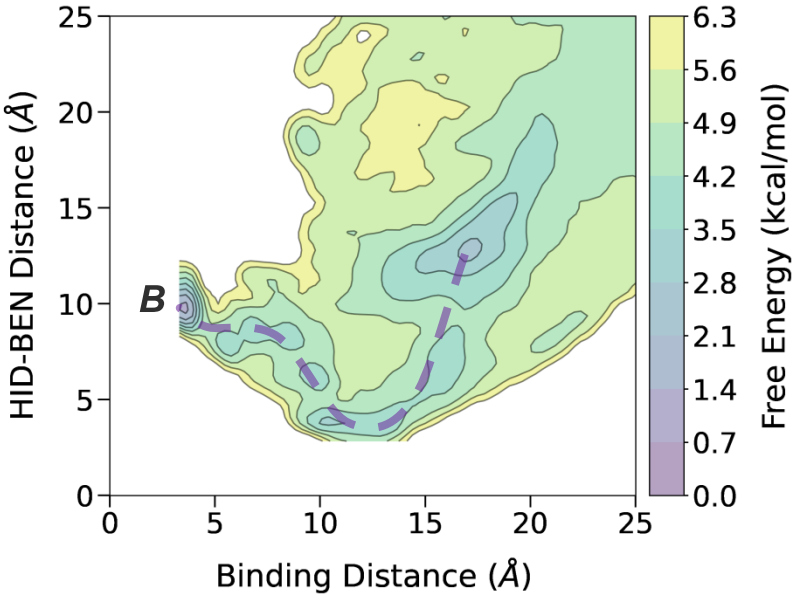
- HIE diverges too much from the crystal structure

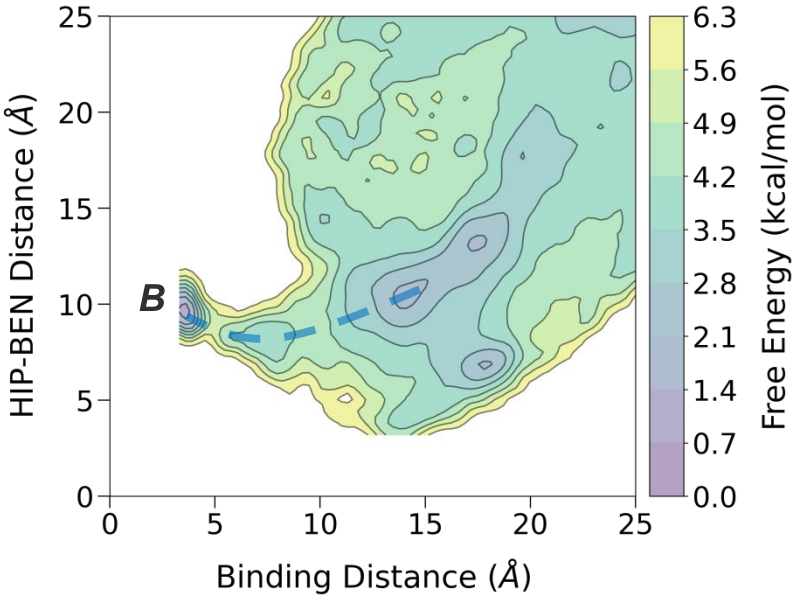
Free Energy Landscape



2D-Histogram

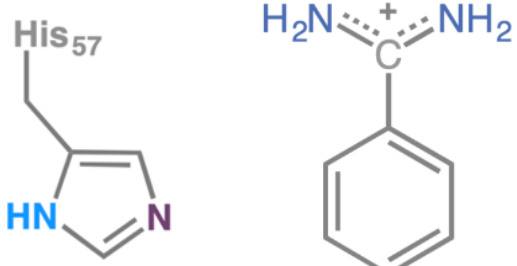
$$\Delta G = -RTln(P)$$





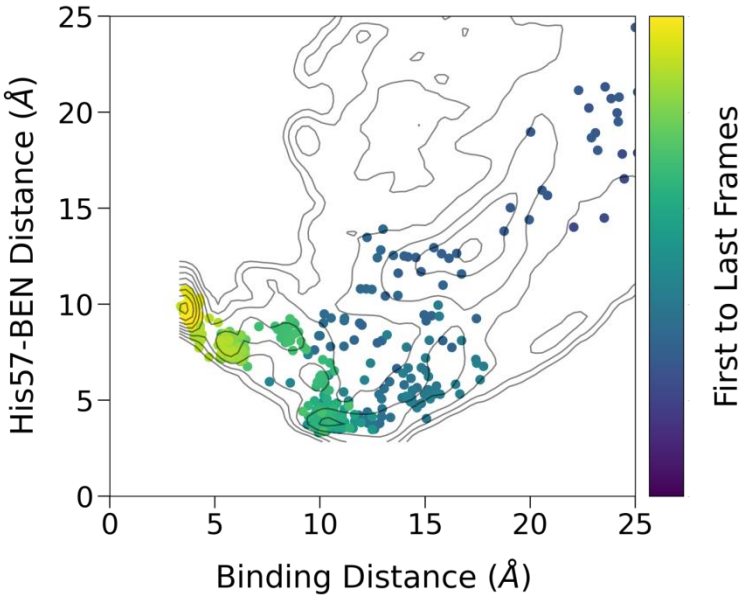
- Two different binding pathways

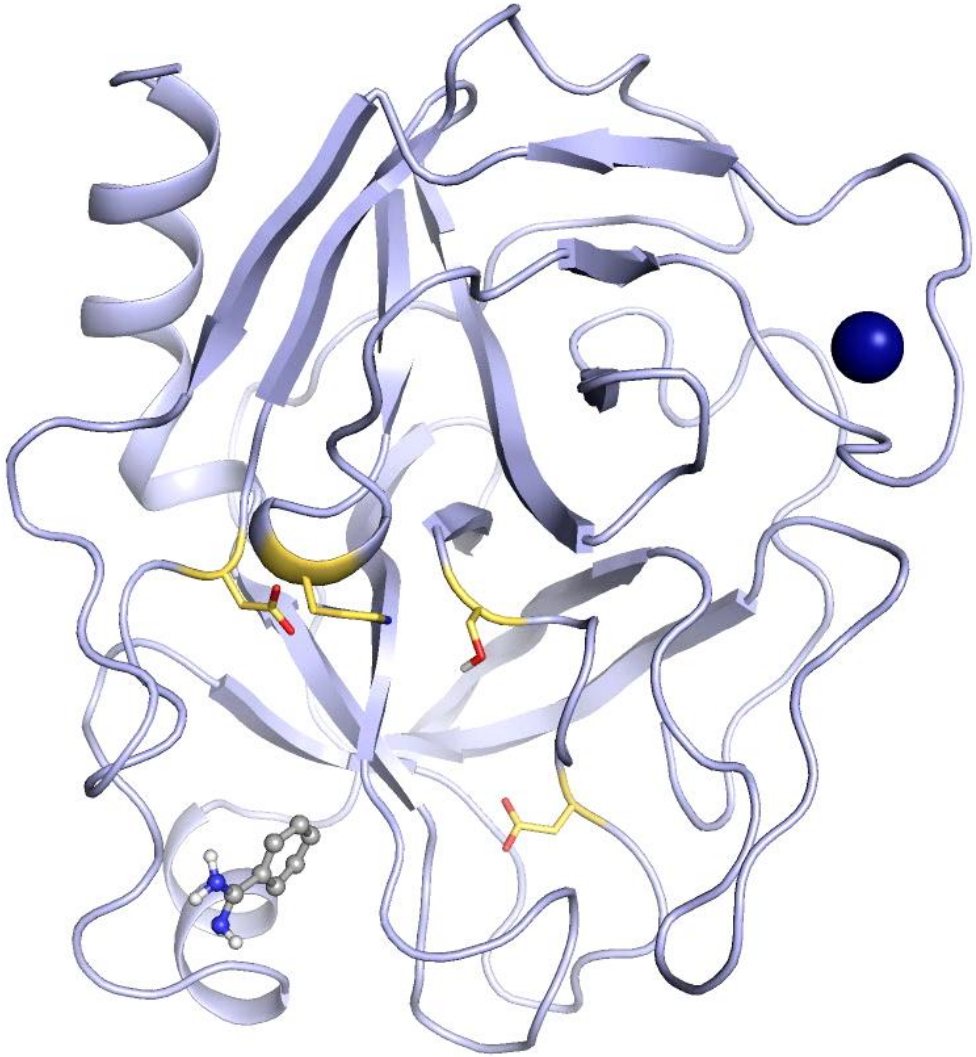
Binding Pathway



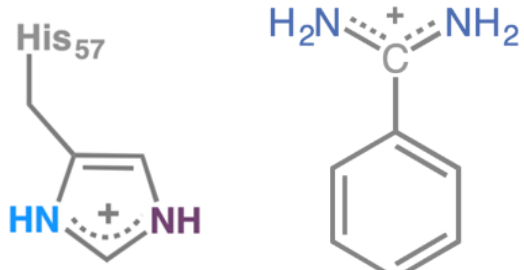
HID

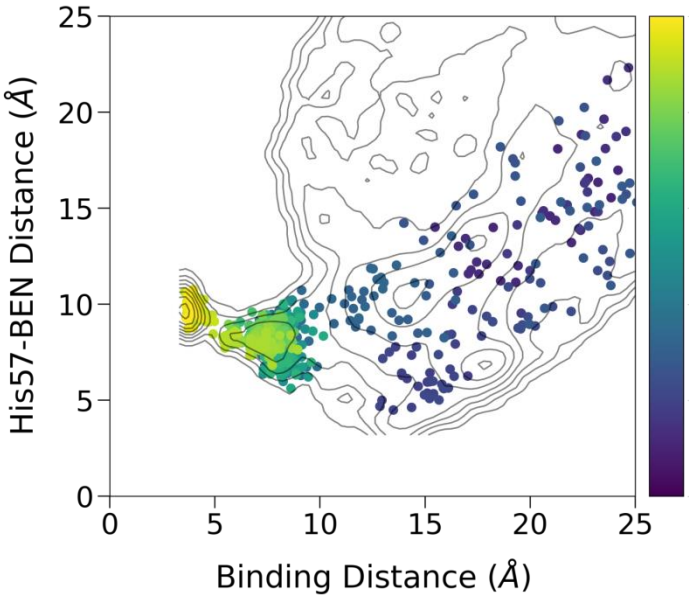
BEN

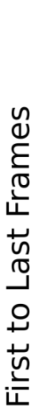


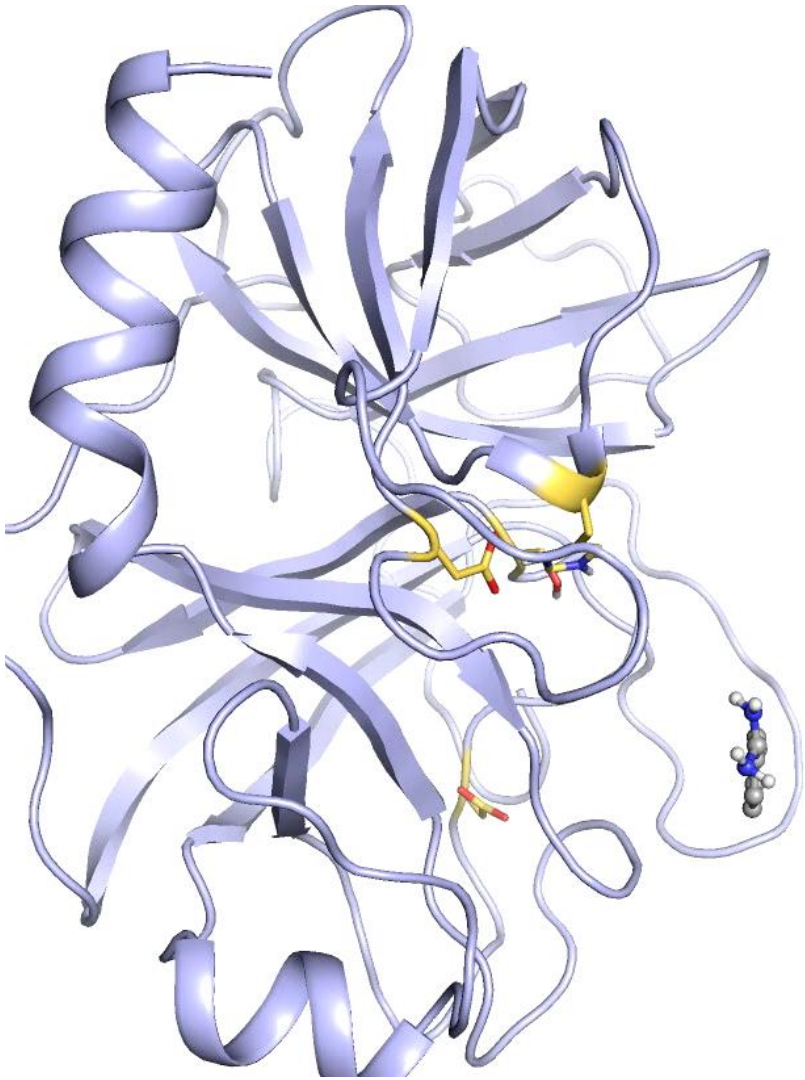


Binding Pathway









Constant-pH

	pH 7.0	pH 8.0
Binding Events [%]	23	27
Populations [%] HIP – HID – HIE	74-26-0	42-57-0

- **Discrete Constant-pH** in explicit solvent:
  - User selects which residues are allowed to titrate
  - After a set of MD steps, these residues may **change** their **protonation state** via **Metropolis Monte Carlo attempts**

$$Binding\ Events[\%] = \frac{\#\ replicas\ w/binding}{\#total\ replicas}$$

	HIP	HID	HIE
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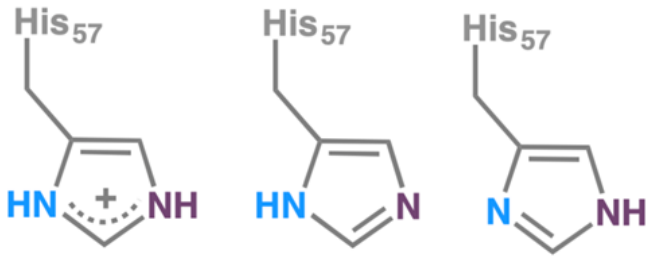
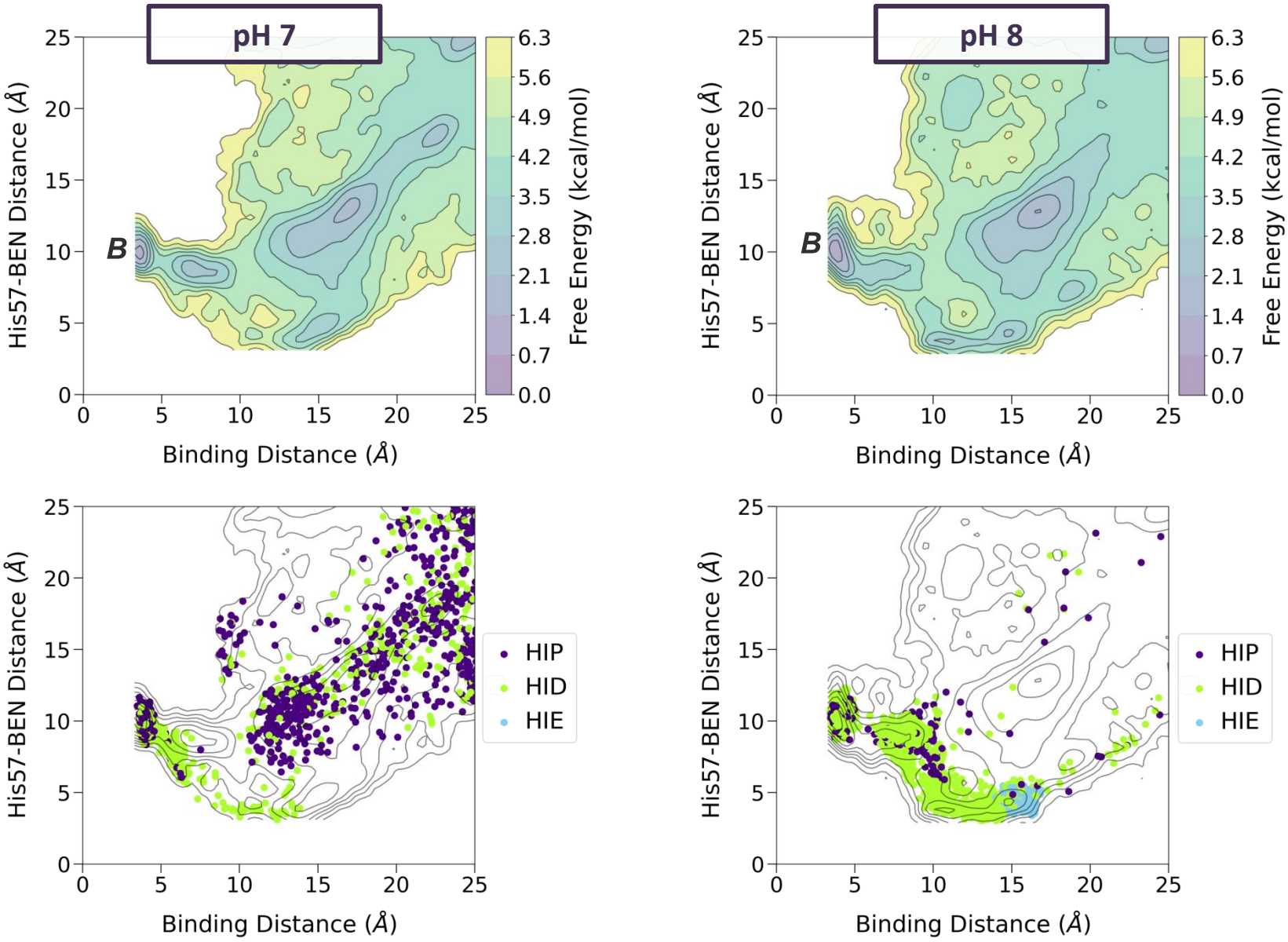


HIP **HID** **HIE**

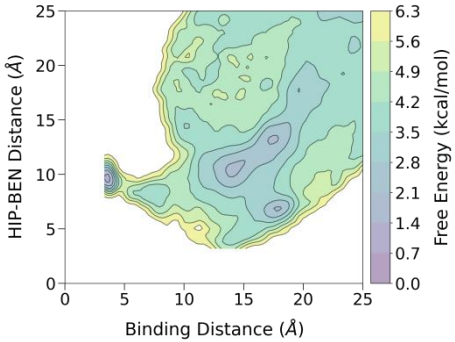
Constant-pH

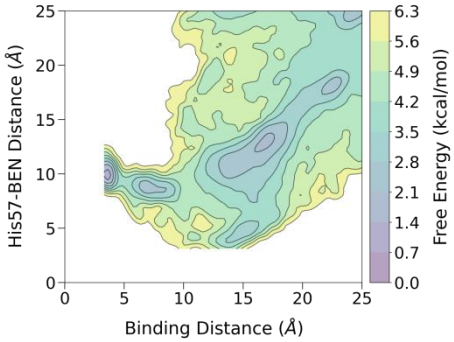

pH 7
pH 8
B
B
His57-BEN Distance (Å)
Binding Distance (Å)
Free Energy (kcal/mol)
6.3
5.6
4.9
4.2
3.5
2.8
2.1
1.4
0.7
0.0
HIP
HID
HIE

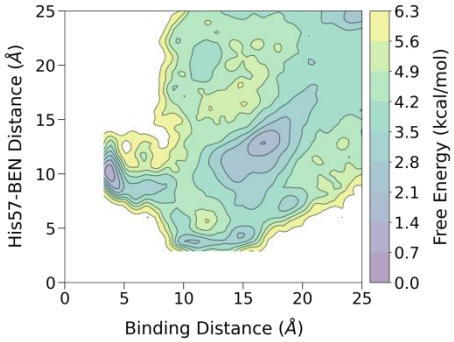
Binding Pathway is Altered by His57

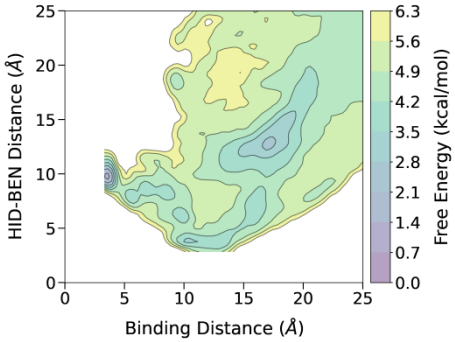
Binding Events; Populations [HIP-HID-HIE]

HIP	**pH 7**	**pH 8**	**HID**
10 %	23 %	27 %	48 %
100-0-0 %	74-26-0 %	42-57-0 %	0-100-0 %









- With increasing HID population, another binding pathway close to His57 appears
- This binding pathway is responsible for the increase in the amount of binding events

CONCLUSIONS

- Concluding remarks

Concluding Remarks

- Assignment of protonation states distal to the binding site can influence the computational characterization of protein-ligand binding.

- In spontaneous binding simulations and other path-relevant studies, **the impact of protonation states in residues found along the pathway should always be considered**; especially when modeling difficult or not sufficiently-known systems.

- Simulating at pH 7 and with fixed protonation states is a common procedure in MD simulations. **These assumptions cannot always be made**:
  - Over 60% of binding events involve protonation states.
  - Enzymes are sensitive to pH changes and catalytic residues commonly change their protonation states at different stages of the catalytic cycle.

  Consider the system's relevant pH, and whether to employ constant-pH methods.

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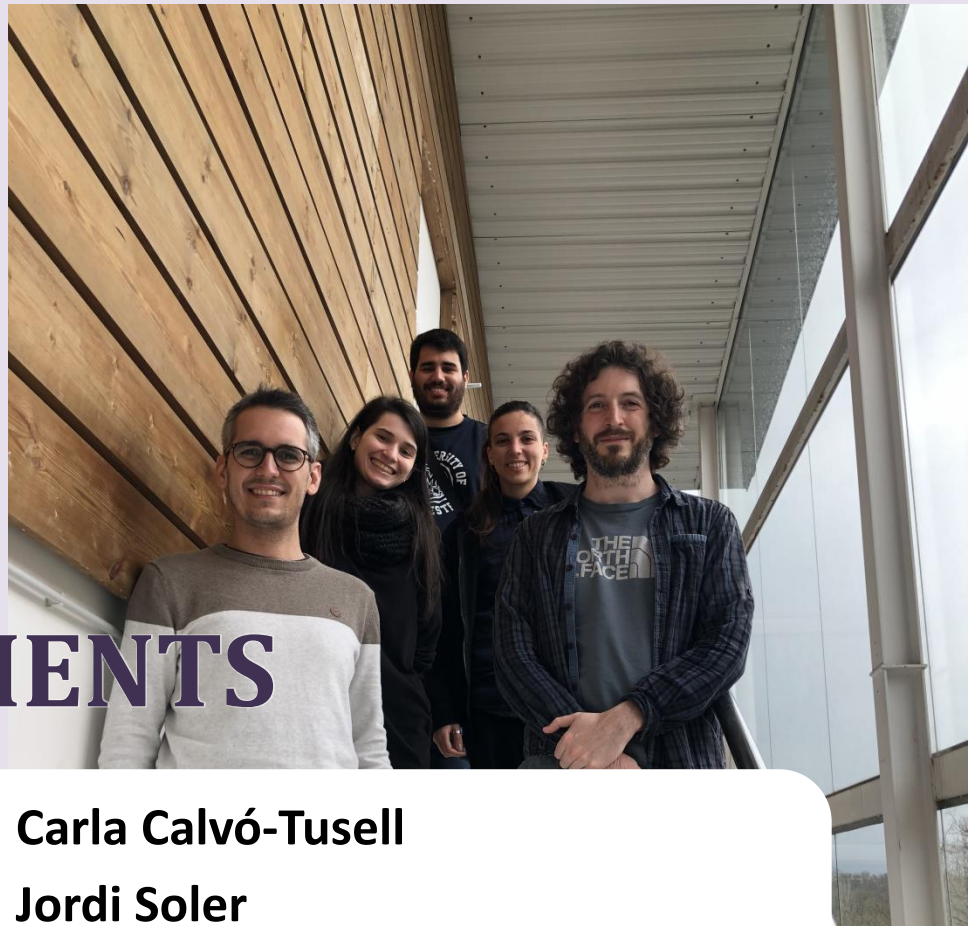

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iQCC Institut de Química Computacional i Catàlisi
Universitat de Girona

THANK YOU
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ATTENTION!

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How the Assignment of Protonation States in Distal Residues May Alter Protein-Ligand Binding in Molecular Dynamics Simulations

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helena.girame@udg.edu

3/05/2022

Student Webinar: BioExcel Spring School 2022

Constant-pH

- Accounts for the equilibrium of protonation states.
  - **Discrete Constant-pH** – Metropolis Monte Carlo
  - Continuous Constant-pH

AMBER18

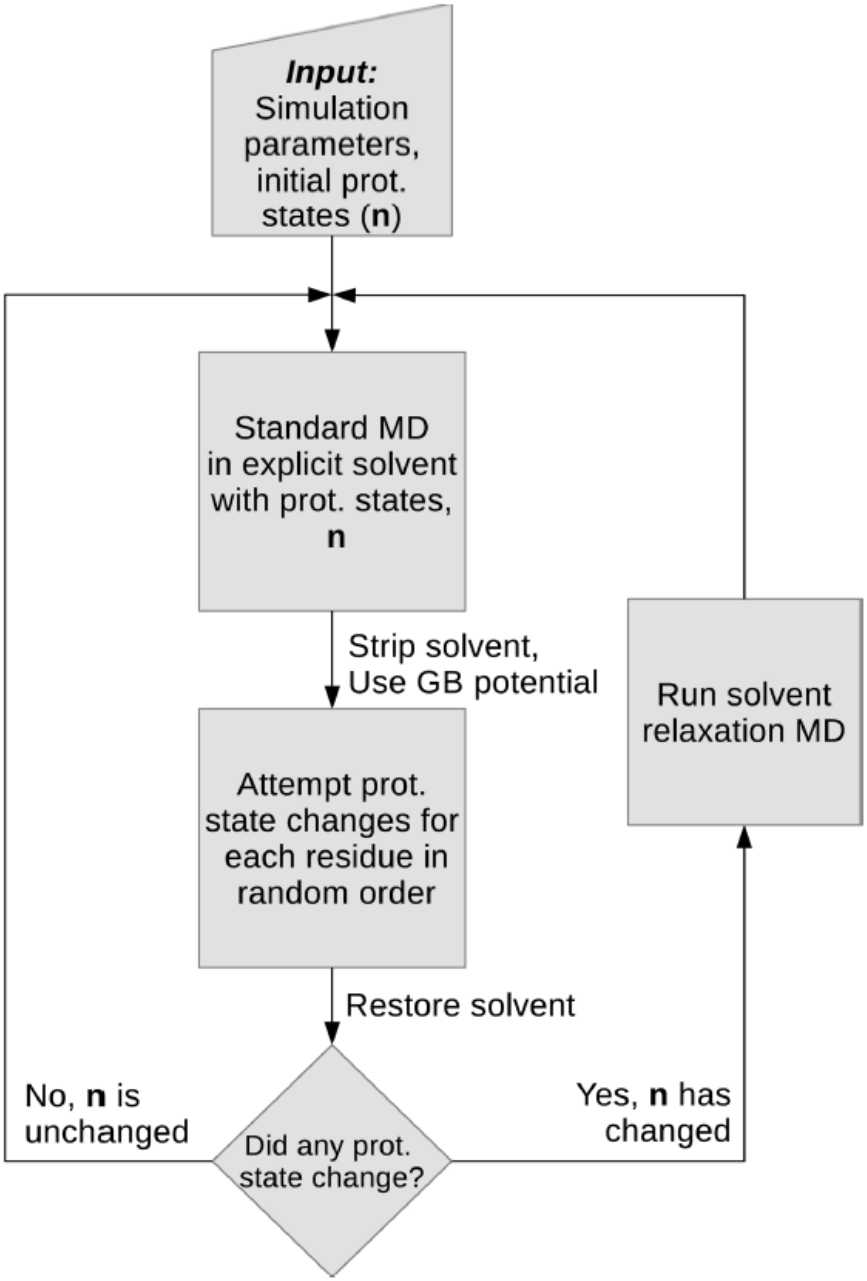
All His were allowed to titrate

50 MD steps before protonation change attempt

100 solvent relaxation steps

GB parameters CpHMD was parametrized for

2 sets of { **pH 7** / **pH 8** } x **30** replicas x 200 ns



From: *J. Chem. Theory Comput.*, **2014**, *10,* 1341-1352