Bioinformatics and Biophysics team

Design of stable cyclic peptides for therapeutic applications

Guillaume Postic, PhD

MASIM workshop, Friday, November 17th, 2017
(Méthodes Algorithmiques pour les Structures et Interactions des Macromolécules)

Peptides as drugs

- Small and easily accessible to chemical synthesis
→ Design of novel therapeutics

- Target selectivity and low toxicity
→ Excellent safety, tolerability, and efficacy

- Modifications
→ Cyclizations, D-residues, *N*-methylation, etc.

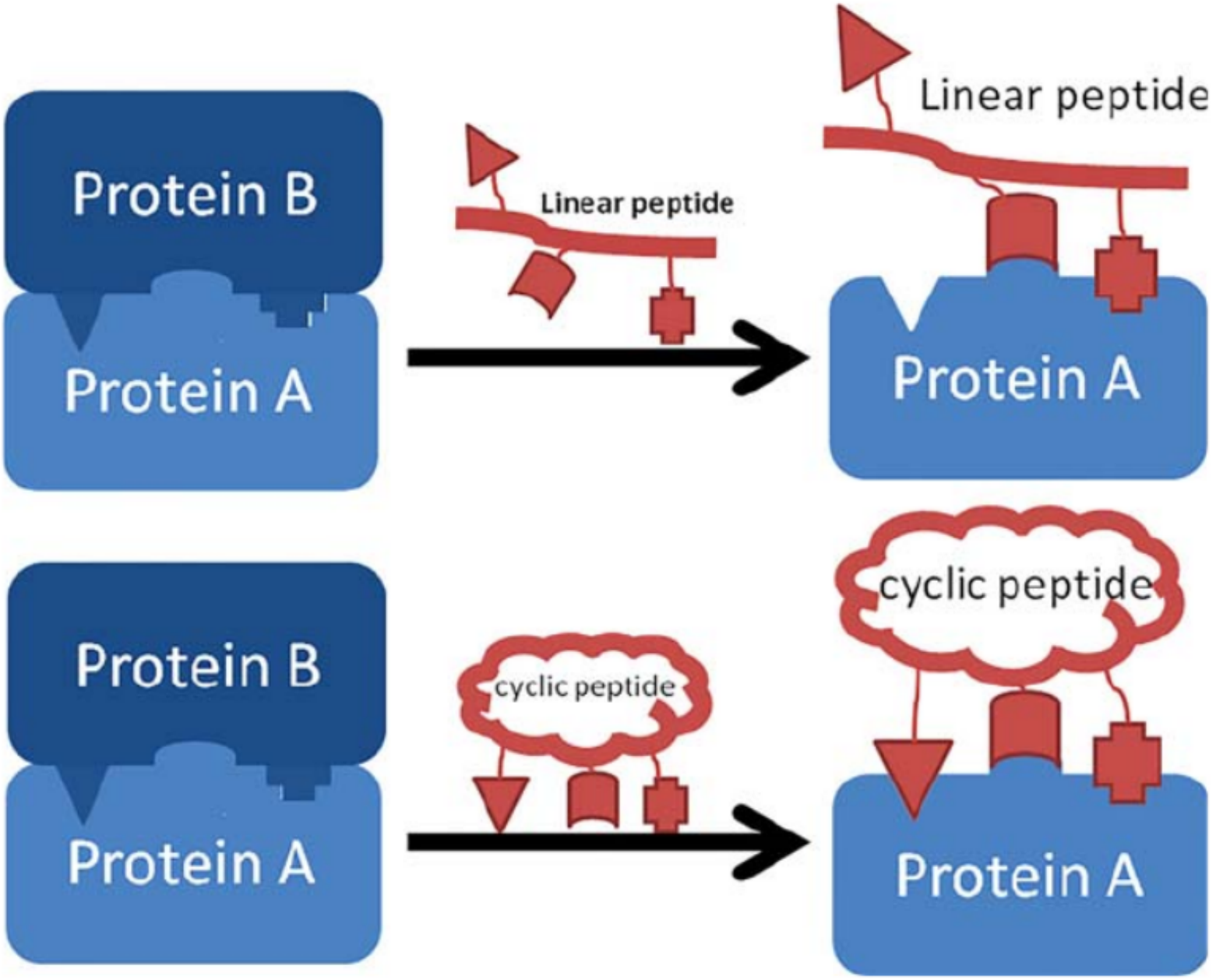
Global sales, examples:
- Lupron™ (Abbott Laboratories) US$2.3 billion in 2011
- Lantus™ (Sanofi) US$7.9 billion in 2013
- Victoza™ (Novo Nordisk) US$2.6 billion in 2013

Cyclic peptides

- Display a large surface area
  → High affinity and selectivity

- Limited conformational flexibility
  → Reduced entropic penalty upon binding
  → Improved binding properties

- Over 40 cyclic peptide drugs are currently in clinical use
  → ~1 new cyclic peptide drug enters the market every year
  → Vast majority are derived from natural products
  e.g. antimicrobials, human peptide hormones



Gao, M., Cheng, K., & Yin, H. (2015). Targeting protein-protein interfaces using macrocyclic peptides. *Peptide Science*, 104(4), 310-316.

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Design of stable cyclic peptides for therapeutic applications

I) Stable cyclic peptides: **Robotics-based approach**

Maud Jusot PhD thesis (2015-2018)

Jacques Chomilier, Dirk Stratmann (IMPMC, UPMC)

Juan Cortés (LAAS)

II) Therapeutic applications: **Caspase inhibitors**

- Caspase-3: Jaysen Sawmynaden PhD thesis (2017-2020)
- Caspase-2: Guillaume Postic/Maxime Louet (postdoc)

Jacques Chomilier, Dirk Stratmann (IMPMC, UPMC)

Fabio Pietrucci (IMPMC, UPMC)

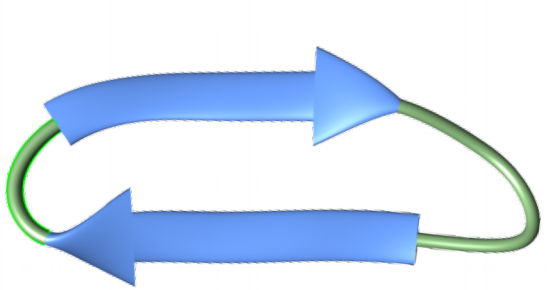
Damien Laage (ENS)

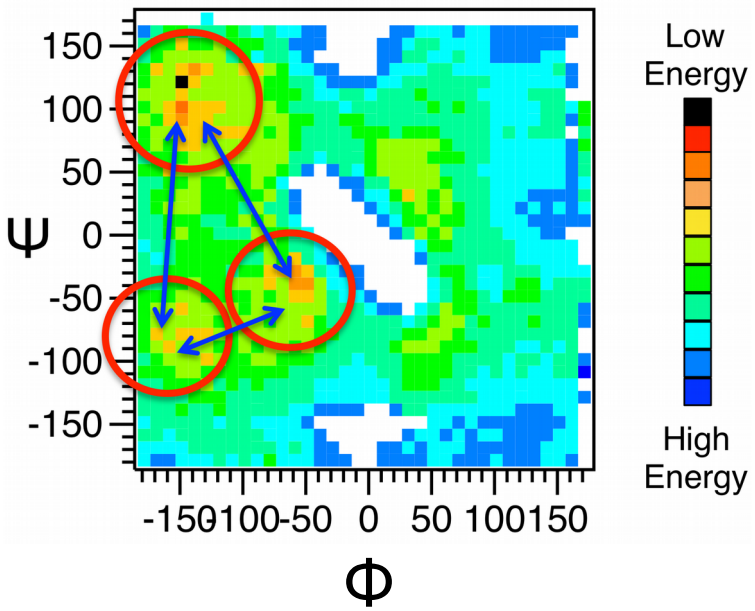
Chahrazade El Amri (IBPS, B2A, UPMC)

Mapping the energy landscape

Good candidates for binding:

- The favorable conformation is a **stable** conformation or is easily **accessible**

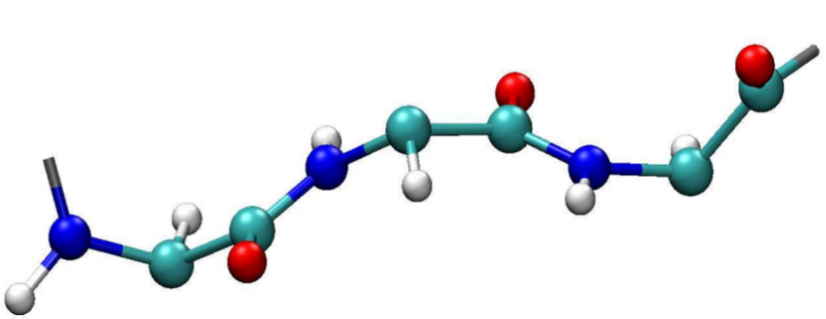


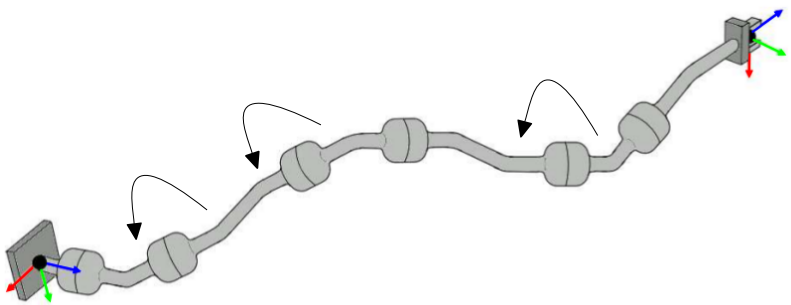


Search for:

- The **local minima**: more stable conformations
- The **transition paths**: conformational changes between minima

Robotics-based representation of the backbone





Dihedral angles ⇔ rotative joints

Fragment of 3 amino acids treated as a kinematic chain similar to a robotic manipulator

→ 6 degrees of freedom

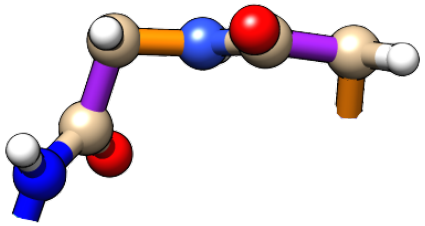
→ Given the terminal positions:

→ **Inverse kinematics (IK)**: 0 to 16 solutions (*i.e.* conformations) that satisfy the terminal positions

J. Cortés, I. Al-Bluwi. *ASME Mechanisms and Robotics Conference*, 2012

Exploration of the conformational space

Cyclic pentapeptide: 10 degrees of freedom = 5 x (Φ,Ψ) angles



Start from a dipeptide: 2 x (Φ,Ψ) angles
→ **Exhaustive** exploration
(grid search)

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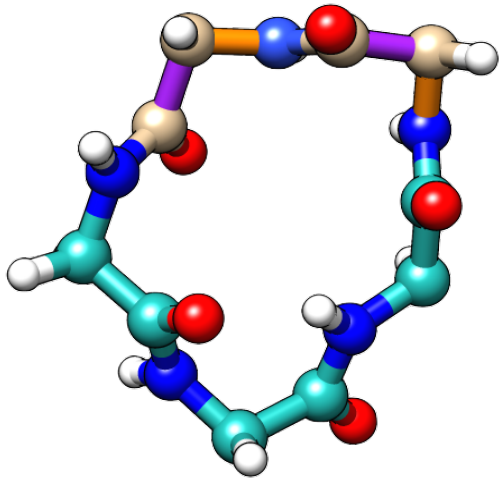

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Add the "robotic" tripeptide
→ Ring closure with **IK** (0 to 16 solutions)

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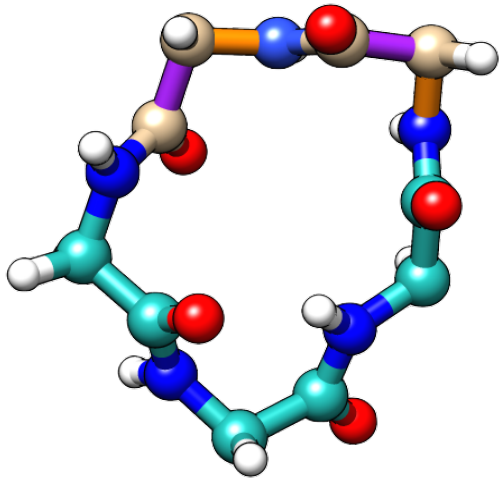

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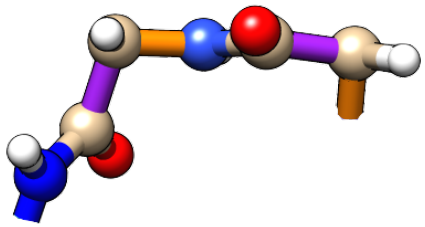
4-dimension exploration
in a 10-dimension space

Exhaustive exploration

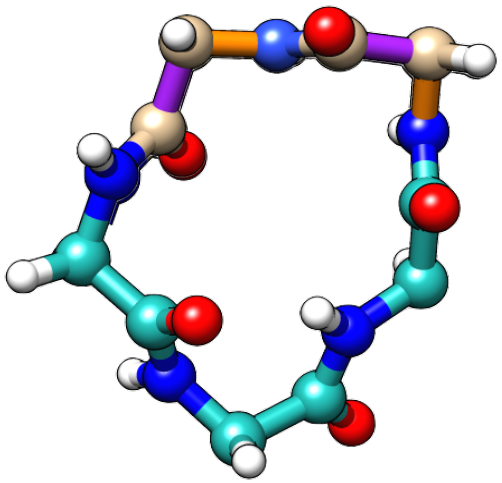
dipeptide

Sampling of Φ_1, Ψ_1, Φ_2, Ψ_2

Grid search with $\Delta\Phi,\Delta\Psi = 10°$



Exhaustive exploration



dipeptide

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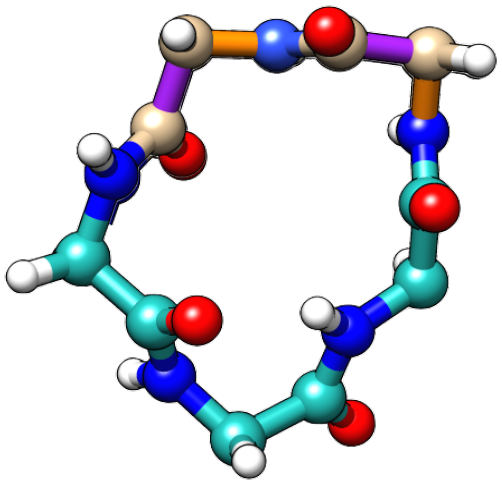
Inverse kinematics:

- 0 solution
- 1 to 16 solutions

Exhaustive exploration



dipeptide

Sampling of Φ_1, Ψ_1, Φ_2, Ψ_2

Grid search with $\Delta\Phi,\Delta\Psi = 10°$

Inverse kinematics:

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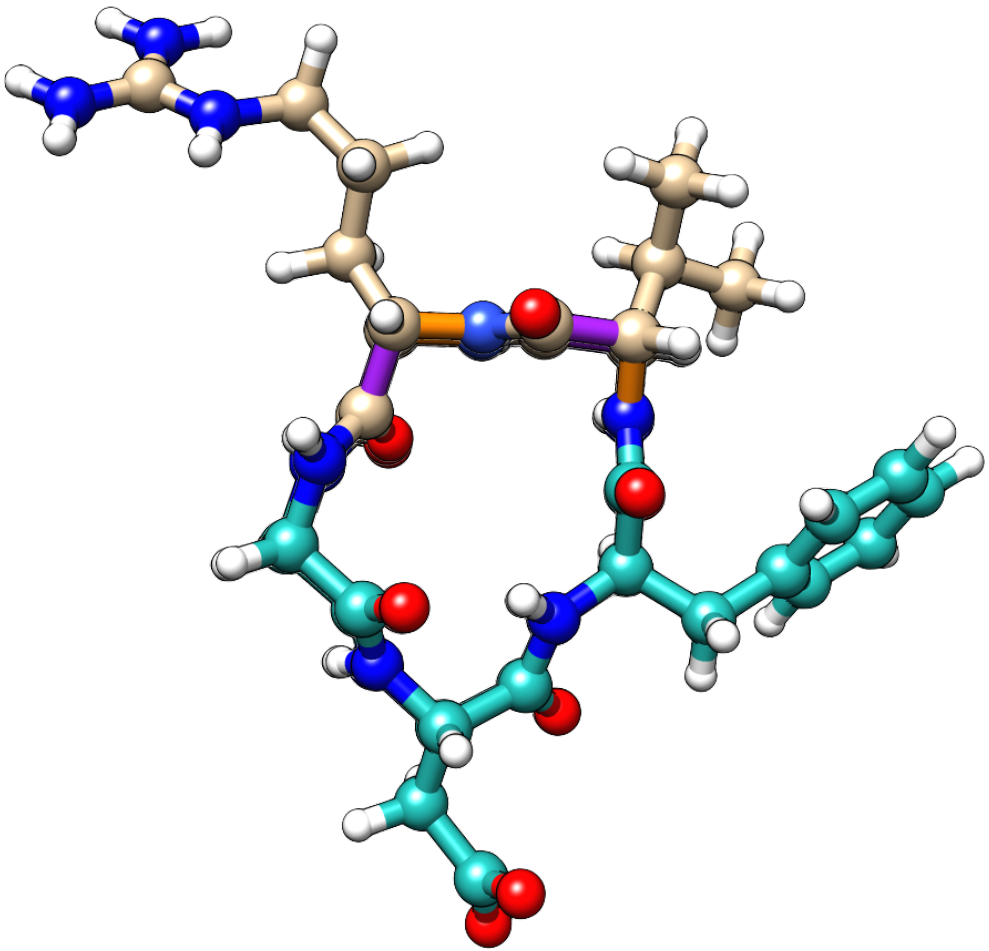
Check collisions

Exhaustive exploration



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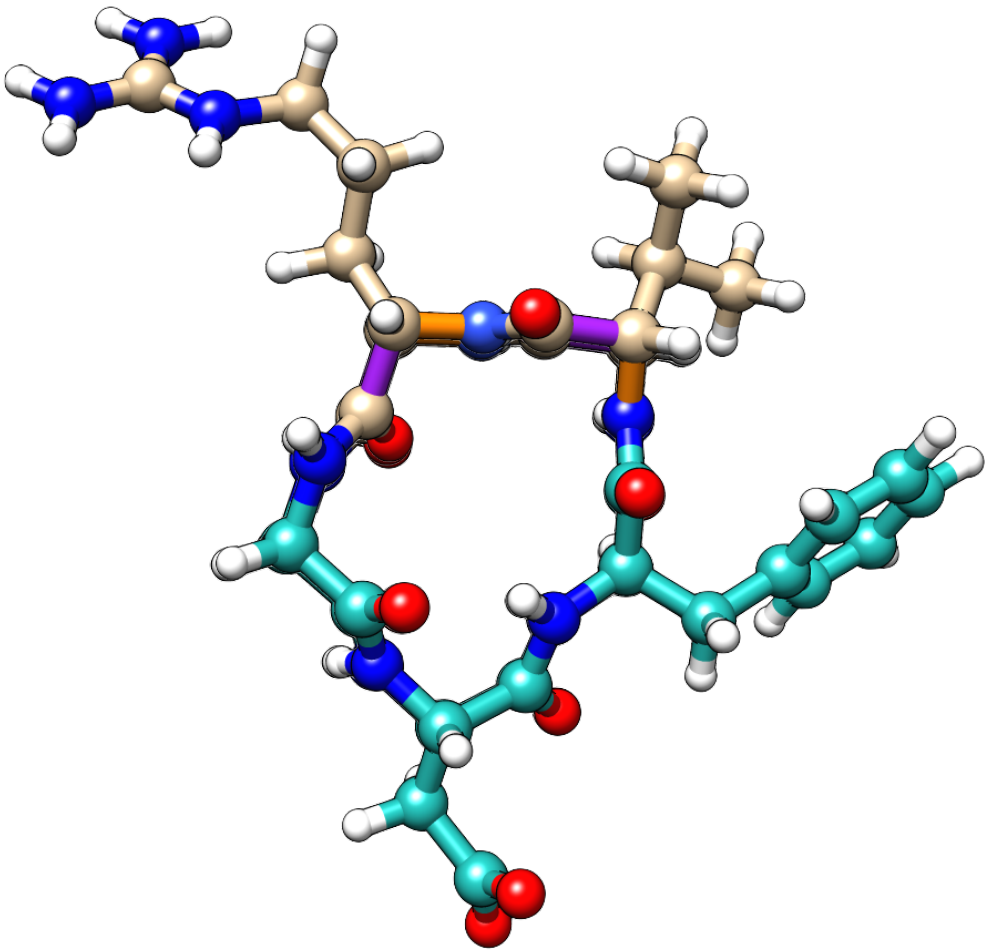
Side chains addition

Exhaustive exploration



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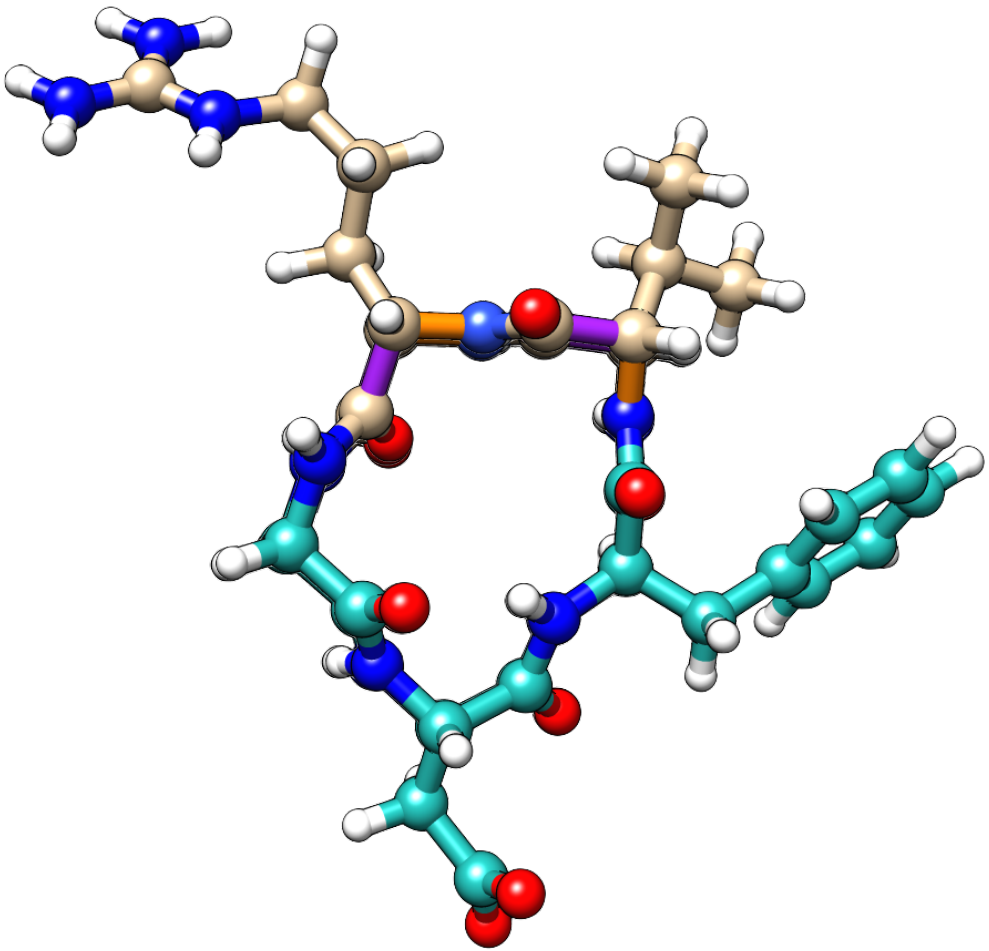
Check collisions

Side chains addition

Relaxation

Exhaustive exploration



dipeptide

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Inverse kinematics:

- 0 solution
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Check collisions

Side chains addition

Relaxation

In theory: $(360/10)^4 \times \{0\text{-}16\}$ = 0 up to 26,873,856 conformations

In practice: ~800,000 conformations

Benchmark: energy landscape of cyclic pentapeptides

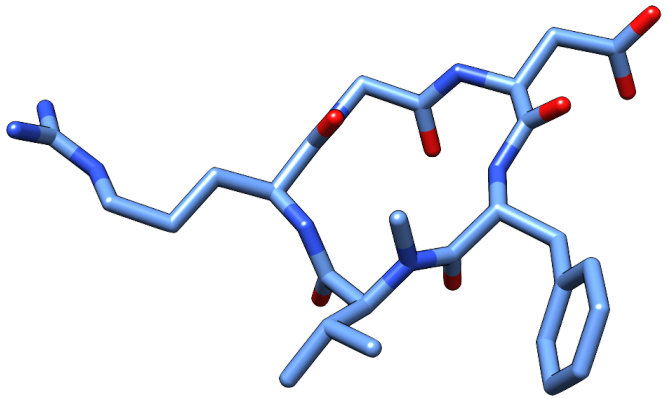
→ Set of 20 cyclic pentapeptides[1]

c(RGDfV)
c(**R**GDfV)
c(R**G**DfV)
c(RG**D**fV)
c(RGD**f**V)
c(RGDf**V**)
c(RG**D**f**V**)
c(**R**GDf**V**)
c(**R**GD**f**V)
c(GGGGG)

c(RGDkV)
c(RGDpV)
c(RGDwV)
c(RGDfK)
c(RGDKv)
c(RGDWv)
c(RGDFV)
c(VfdGr)
c(vfdGR)
c(vfdGr)

lower case: D-form, **N-methyl**

Cilengitide
(cyclo(RGDf-[N -Me]V))[2]



UCSF-Chimera
Tleap (Amber ff96, implicit solvent)
RED Server (*N*-methylated residues)

Energy landscape explored by REMD (Replica-Exchange MD)
Gromacs 5.1.2, 8 replicas, from 300 K to 450 K, 2.4 µs × 8 = 19.2 µs
→ Comparison with our exhaustive exploration

[1]Wakefield AE *et al.*, *J. Chem. Inf. Model* 2015; [2]Mas-Moruno *et al.*, *Angew. Chem.* 2011

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→ Set of 20 cyclic pentapeptides[1]

c(RGDfV) c(RGDkV)
c(RGDfV) c(RGDpV)

Cilengitide
(cyclo(RGDf-[N -Me]V))[2]

Data generated for the benchmark

- 20 structures of cyclic peptides (pdb + topology files)
- ~**500 µs** of simulations
- 2 TB of data

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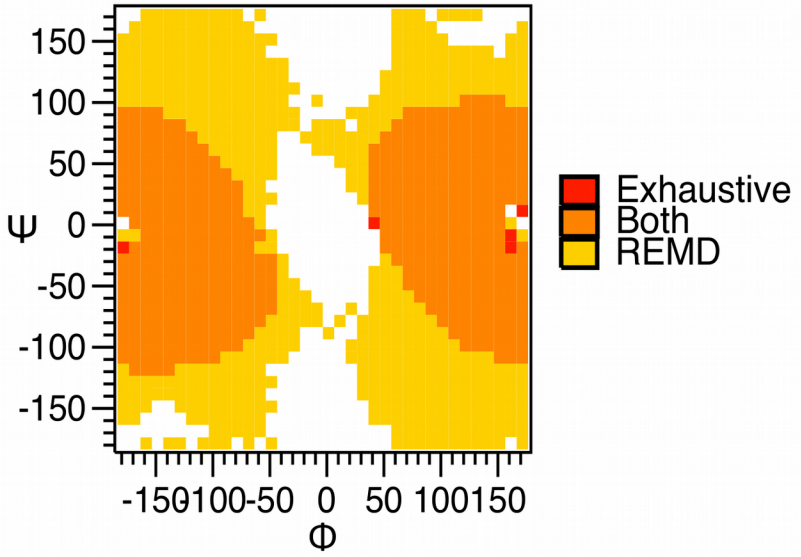
Exhaustive vs REMD

Comparison of the explored areas

Penta-glycine c(GGGGG)

Areas explored
compared to REMD



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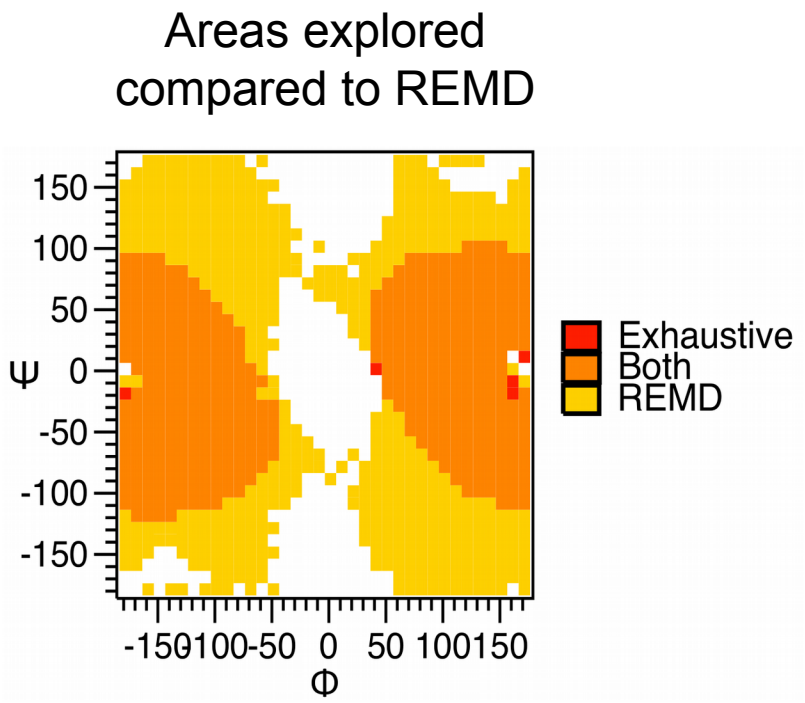
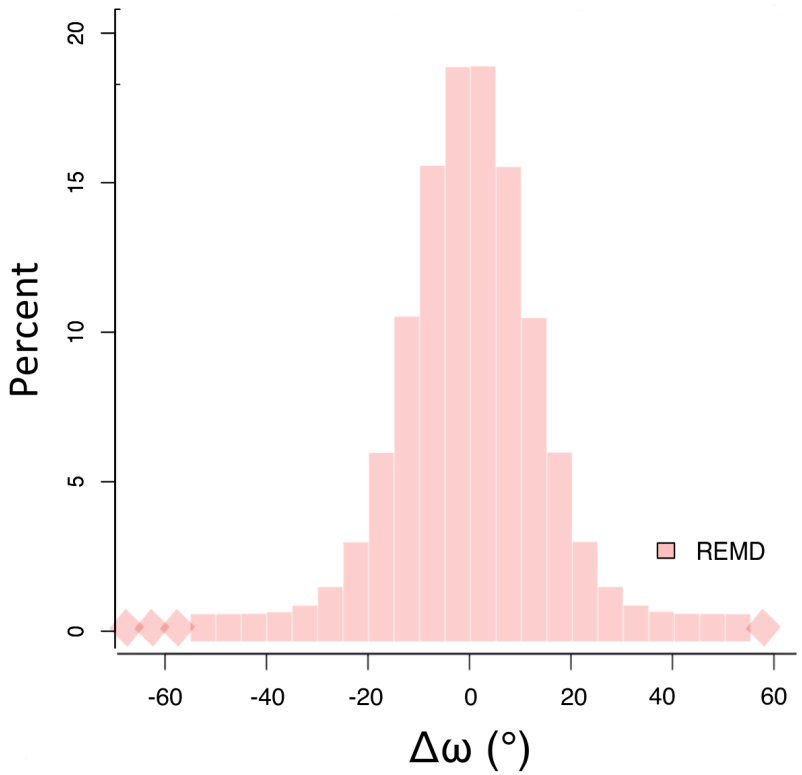
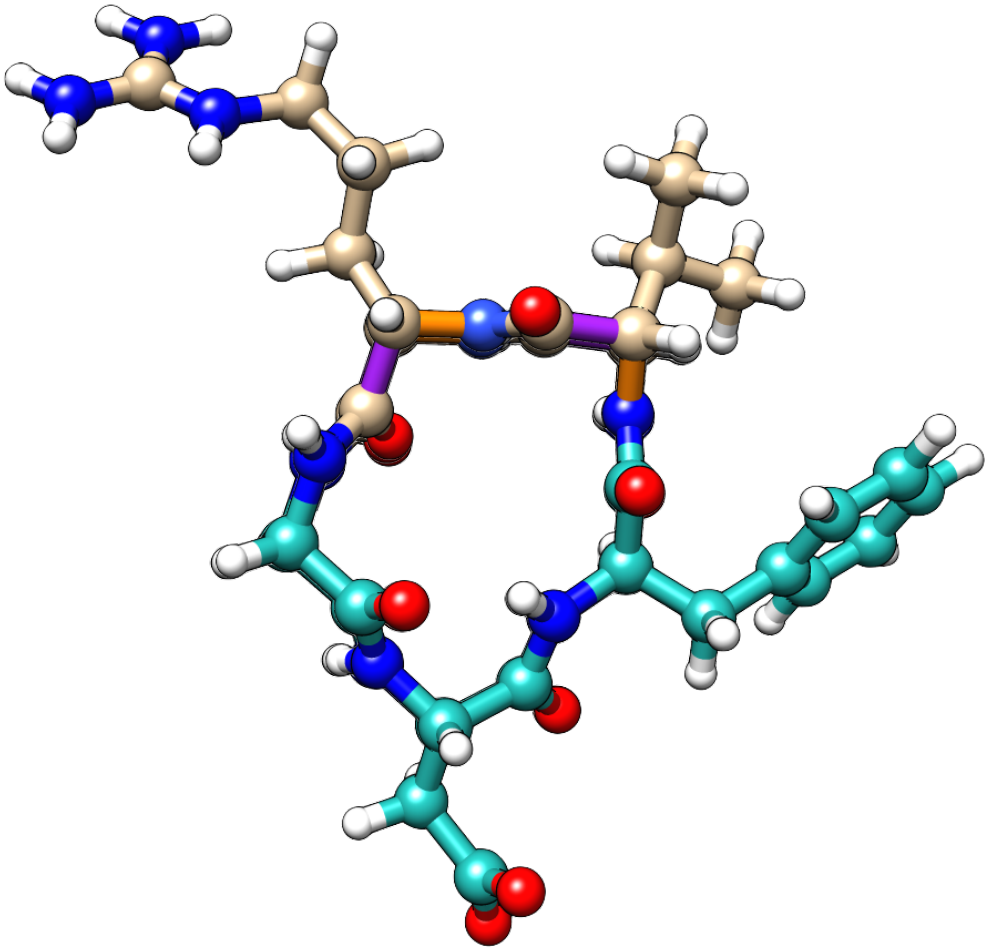




Exhaustive exploration



$\Delta\Phi,\Delta\Psi = 10°$

Sampling of Φ_1, Ψ_1, Φ_2, Ψ_2

Randomly omega sampling

Inverse kinematics x100

- 0 solution
- 1 to 16 solutions

Check collisions

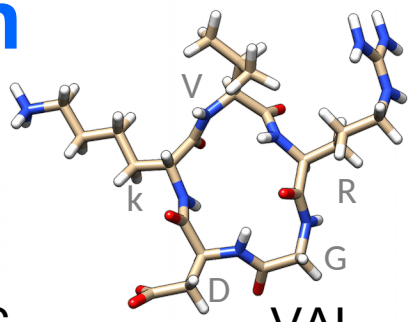
Side chains addition

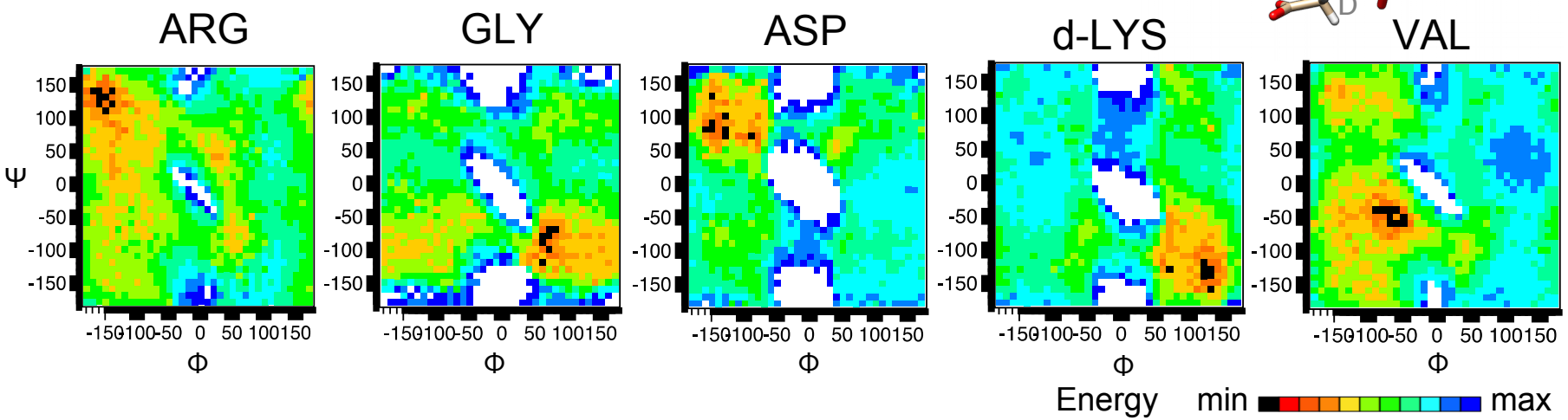
Relaxation

Exhaustive exploration

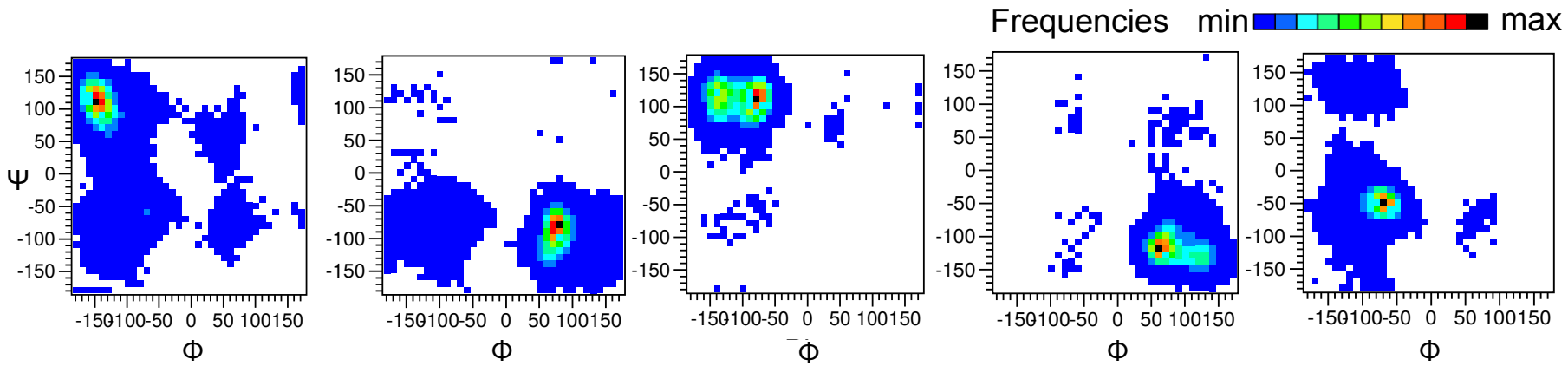
Peptide c(RGDkV)

839,061 conformations found





REMD

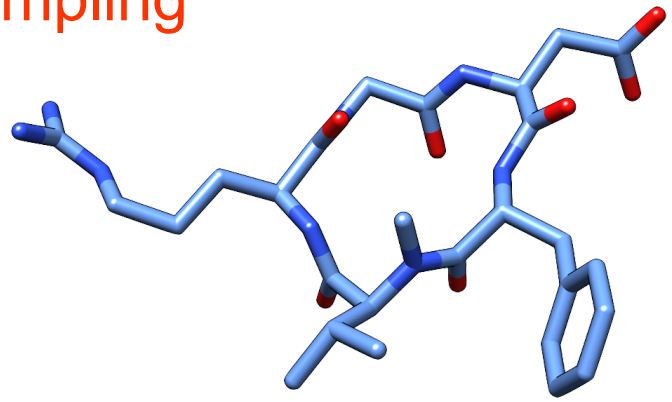


Robotics-based sampling of cyclic peptides: our current method

- Exhaustive exploration of cyclic pentapeptides conformational landscape

- Importance of the ω angles sampling

- Method can treat:
  - Head-to-tail cyclization
  - *N*-methyl residues
  - D-residues



Robotics-based sampling of cyclic peptides: perspectives

To handle longer cyclic peptides:

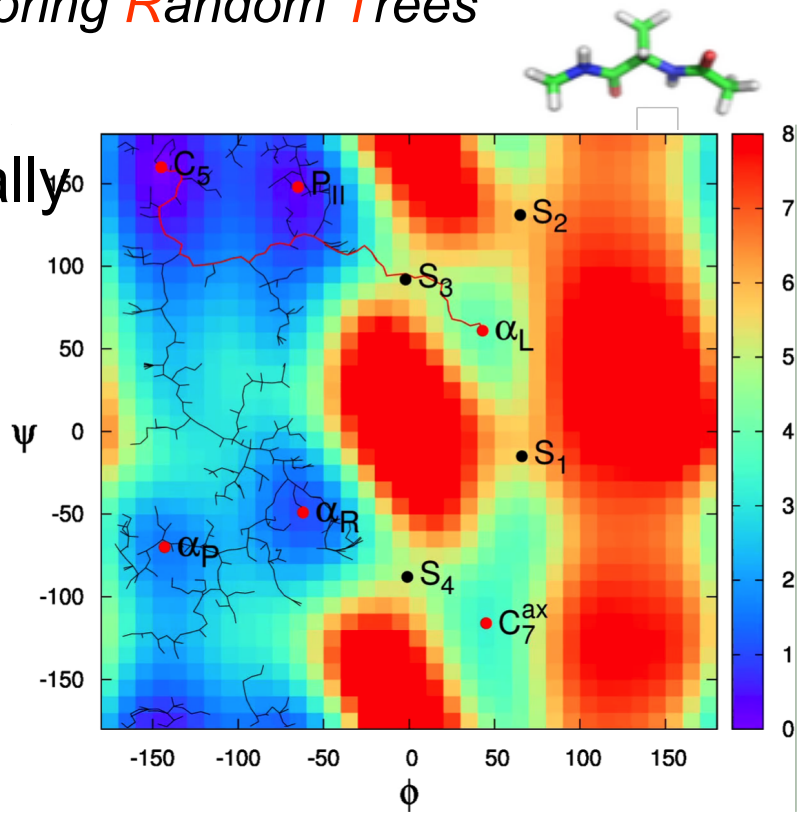
- Basin hopping for minima sampling of the backbone
- T-RRT *Transition-based Rapidly-exploring Random Trees*

for transition path sampling:

→ Explorative method intrinsically biased towards regions:

- unexplored
- energetically favorable

(auto-adaptative temperatures)



Jaillet, *J. Comput. Chem.* 2011

Design of stable cyclic peptides for therapeutic applications

I) Stable cyclic peptides: **Robotics-based approach**

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II) Therapeutic applications: **Caspase inhibitors**

- Caspase-3: Jaysen Sawmynaden PhD thesis (2017-2020)
- Caspase-2: Guillaume Postic/Maxime Louet (postdoc)

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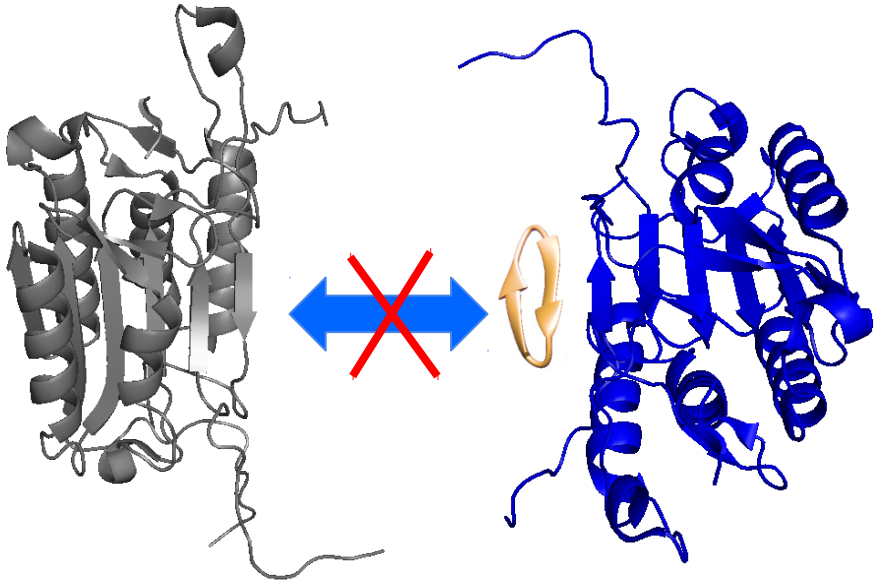
Target proteins: caspases

- Caspases: family of Cysteine-ASPartic proteASES

- Play essential roles in
  - Programmed cell death (apoptosis)
  - Inflammation

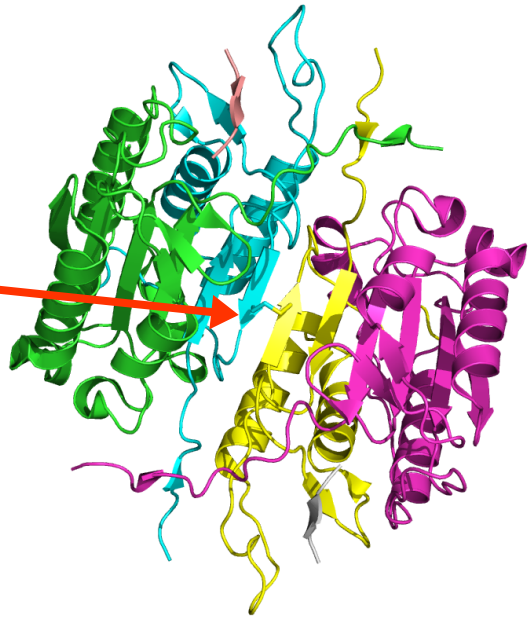
- Caspase-2 and -3
  - → Involved in CNS disorders (Alzheimer)
  - → Active as multimers, with allosteric regulation
  - → No specific inihibitor
    - Caspase active site conserved
    - Multimerization interface specific

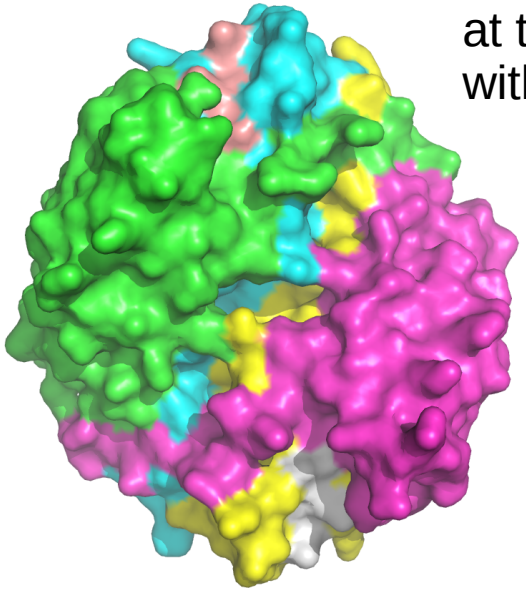
Caspase 3



Peptide (cyclized)
- Large interaction surface
- High affinity

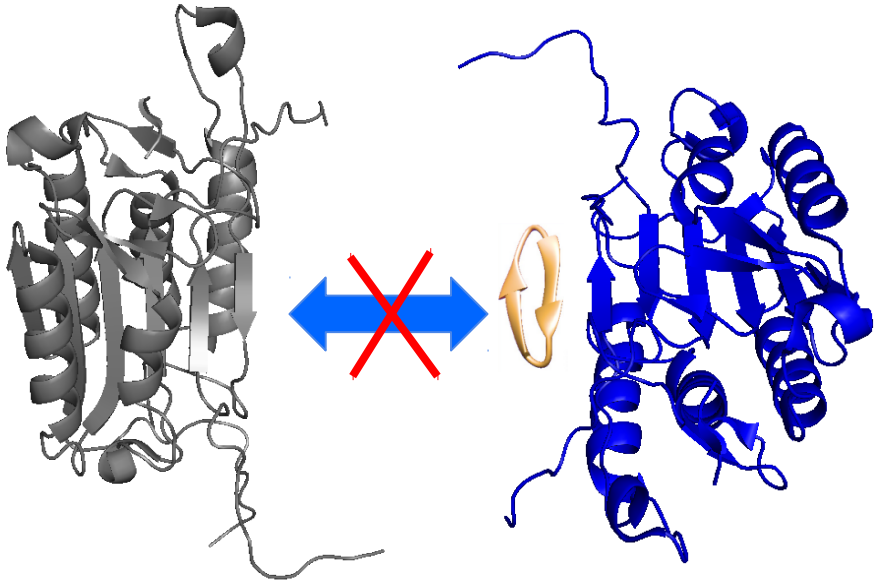
Caspase 2





Target the narrow pocket at the interchain interface with a cyclic pentapeptide

Caspase 3



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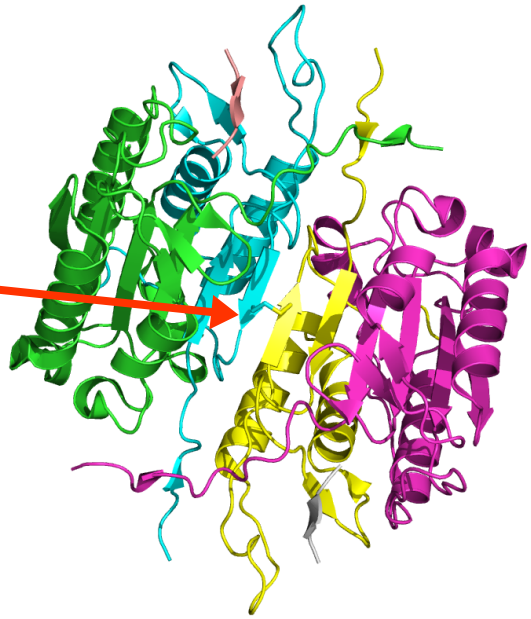
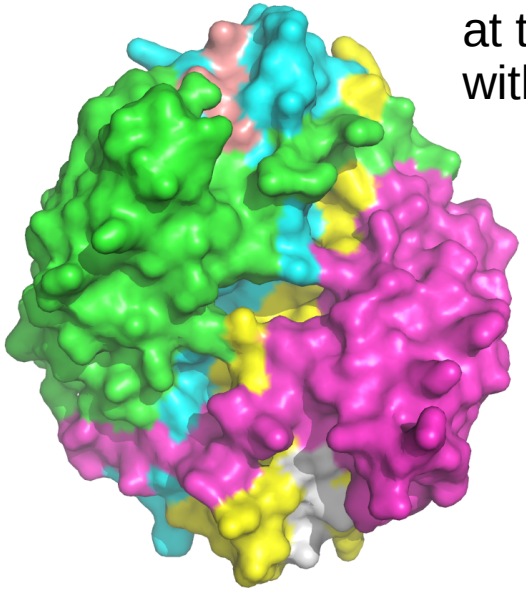
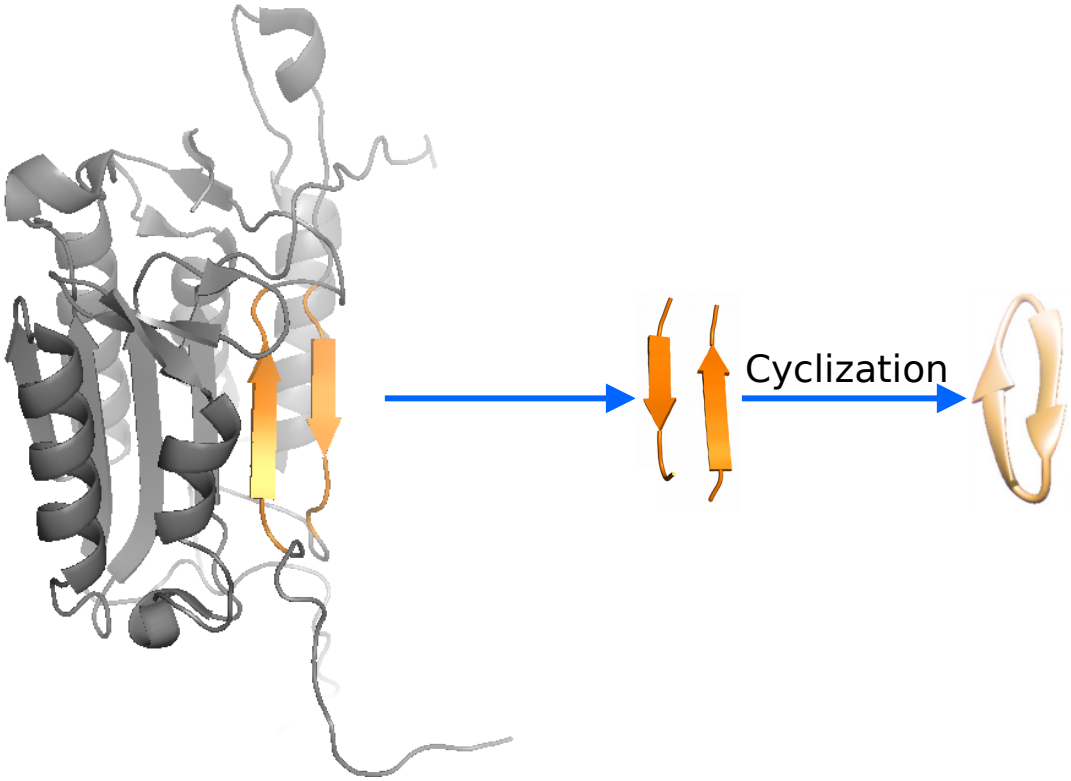


Disulfide bond



Target the narrow pocket at the interchain interface with a cyclic pentapeptide

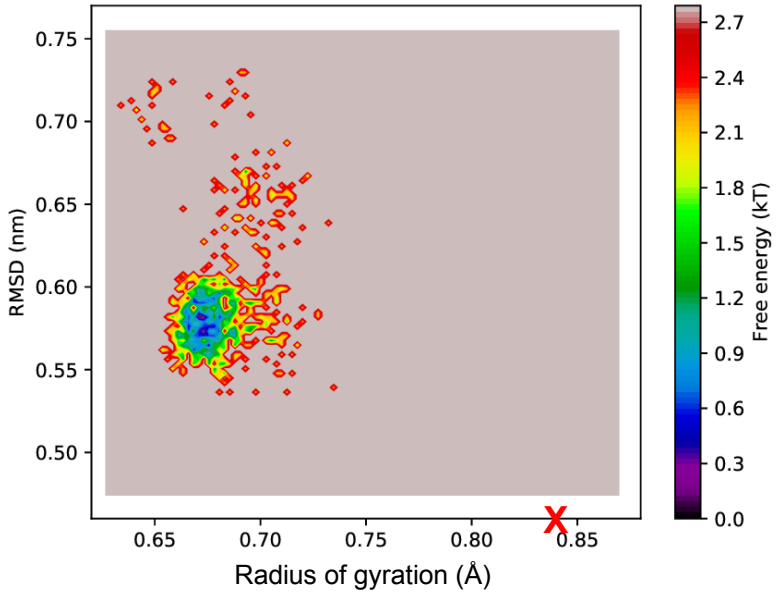
Caspase-3: strategy

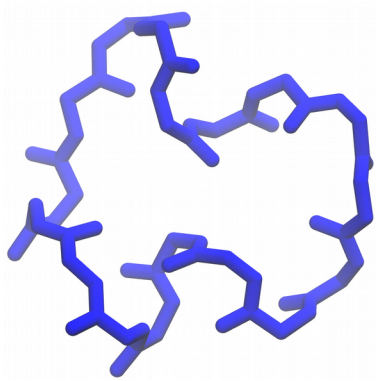


Optimizations:
- Chemical modif cations
- Sequence

Caspase-3: Conformational stability of cyclized peptide in REMD

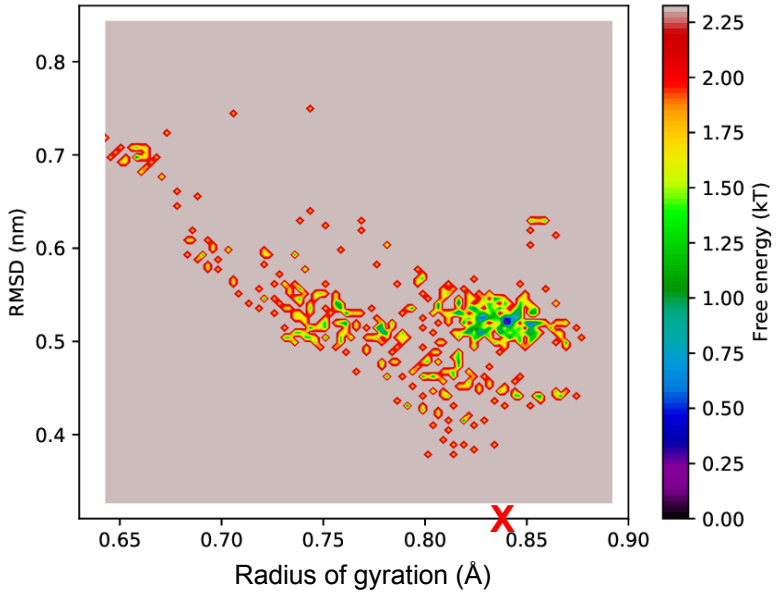
40 ns

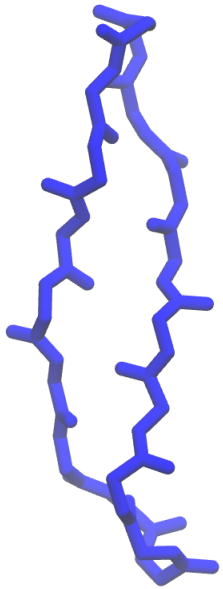




Optimization fail

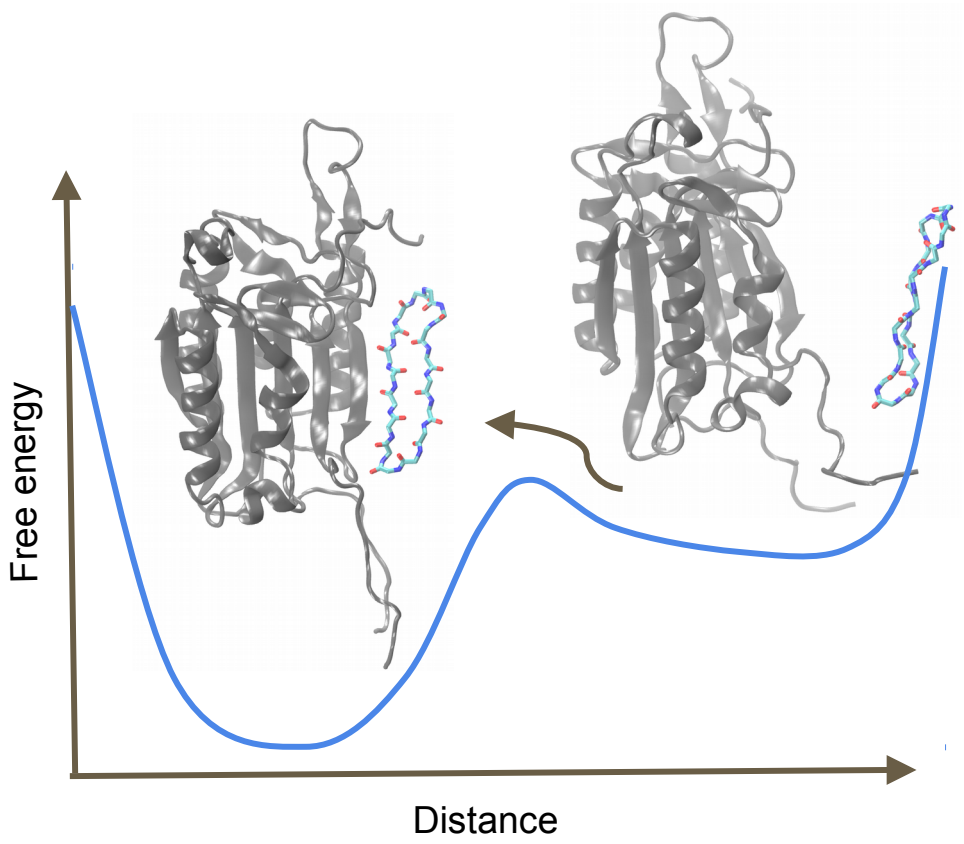
X: initial conformation



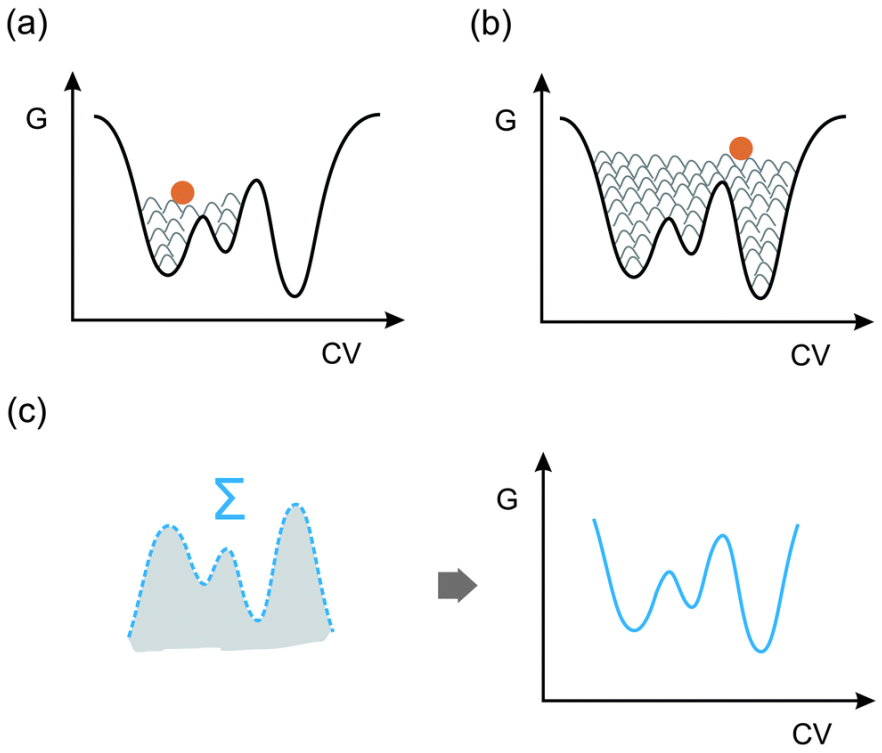


Successful optimization of the conformational stability

Caspase-3: protein-peptide binding with metadynamics



Metadynamics: principles



CV: collective variable
(aka reaction coordinate)

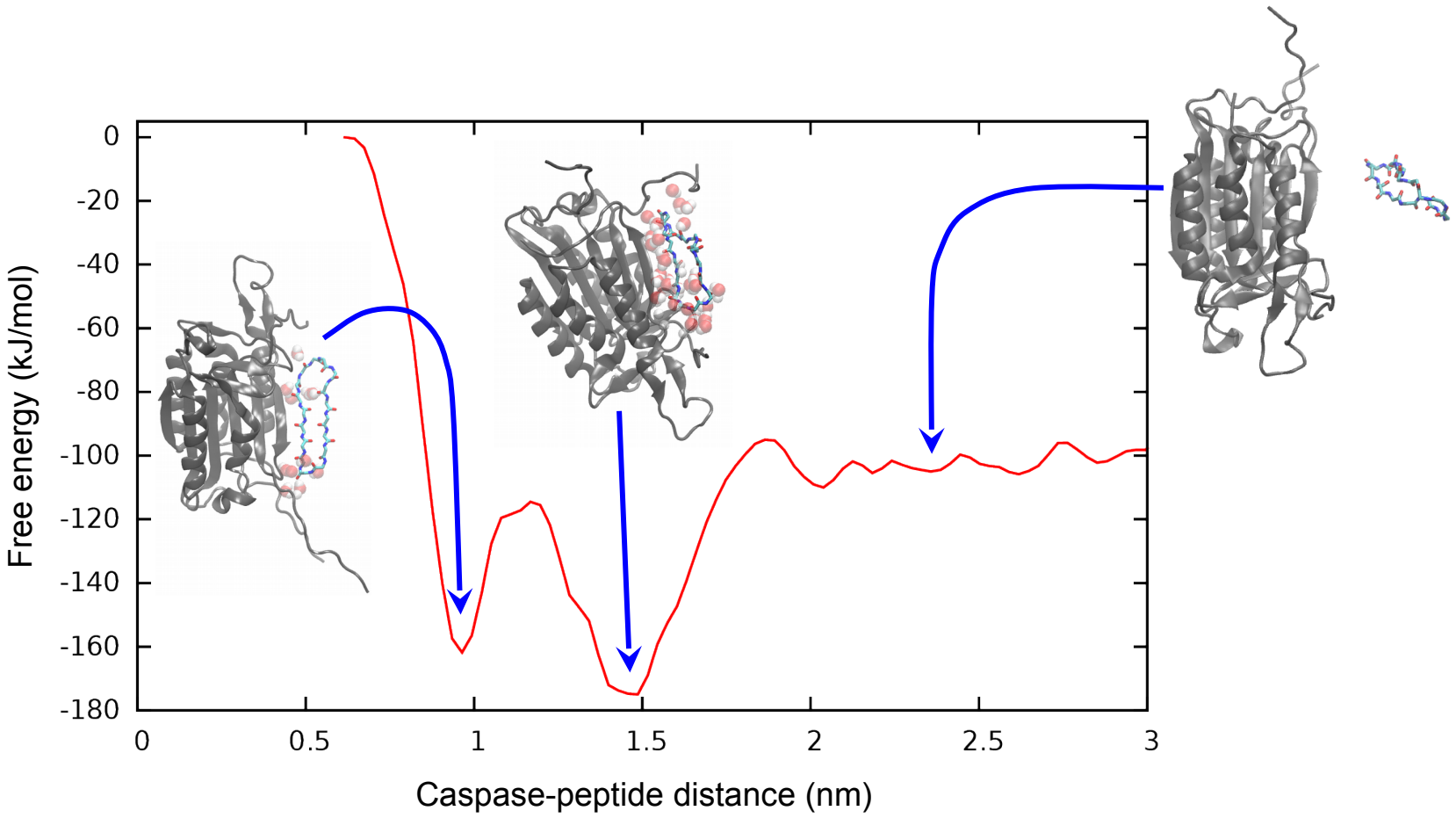
Van Speybroeck *et al.*, *Chem. Soc. Rev.*, 2014

The choice of the biased CV is crucial

- Caspase-peptide distance
- Water molecules at the interface
- Hydrophobic contacts
- Polar contacts

Use all 4 CV simultaneously with bias-exchange metadynamics

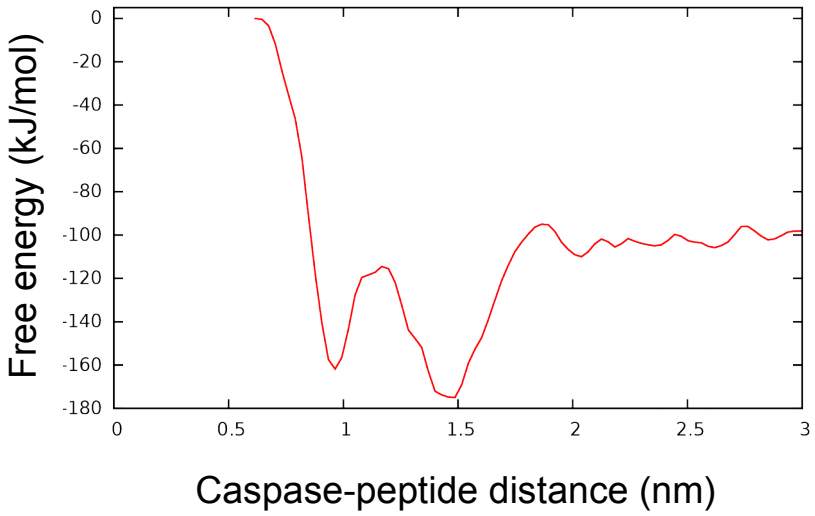
Preliminary results on short trajectories

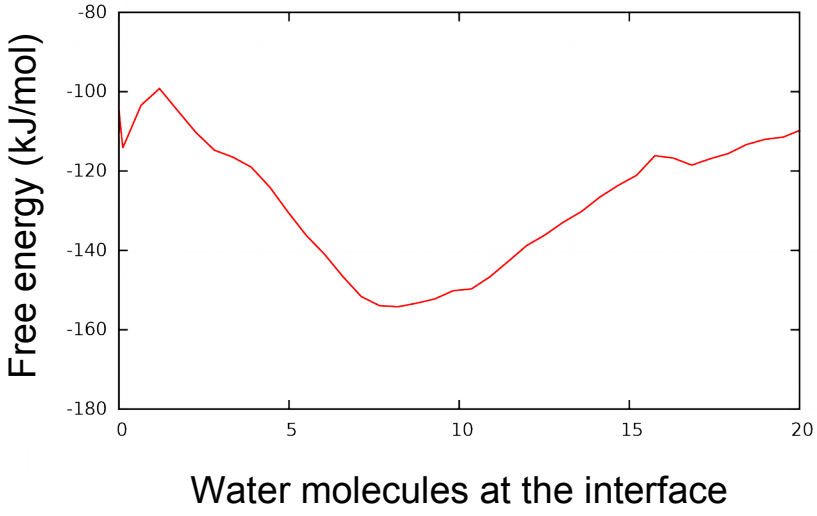


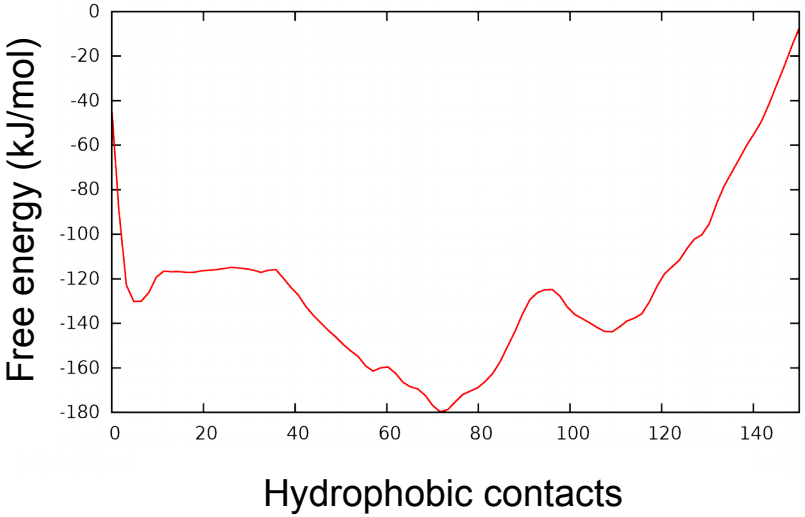
Bias-exchange metadynamics

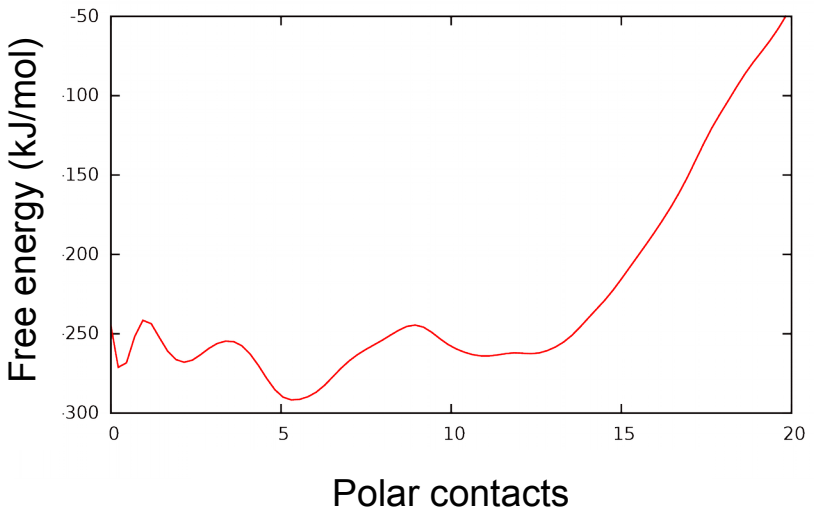
- 4.5 ns simulation; NPT; Amber 96
- 21,117 water molecules (TIP3P)
- 4 replicas (because 4 biased collective variables)

4 biased collective variables







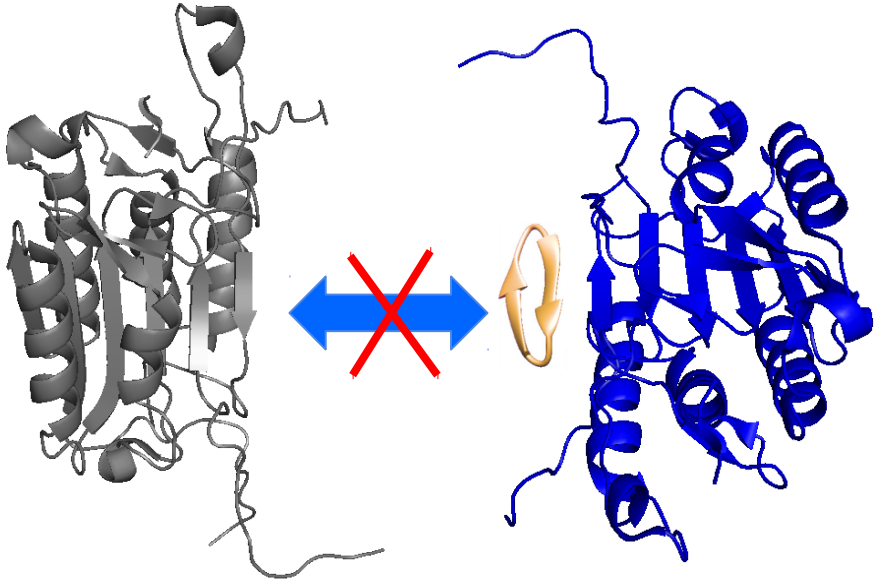


Caspase-3: Perspectives

- Different sets of biased CV
- Longer simulations
- Other peptides/chemical modifications
- Estimate binding affinity and kinetics: rank peptide designs
- Binding to other caspases: specificity to caspase-3

→ **Experimental assays**

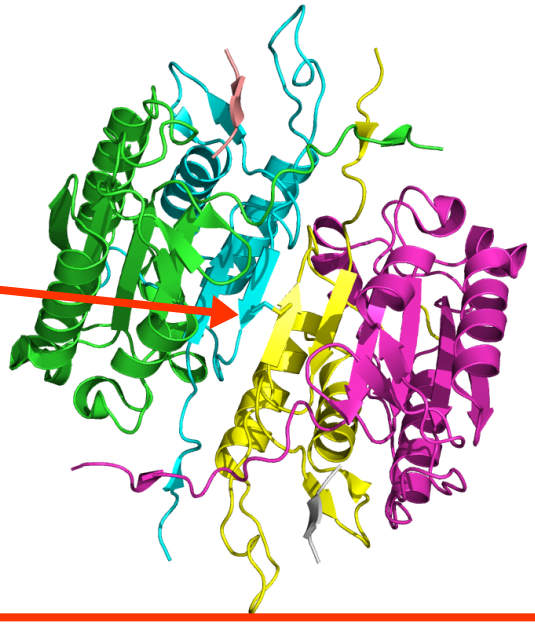
Caspase 3

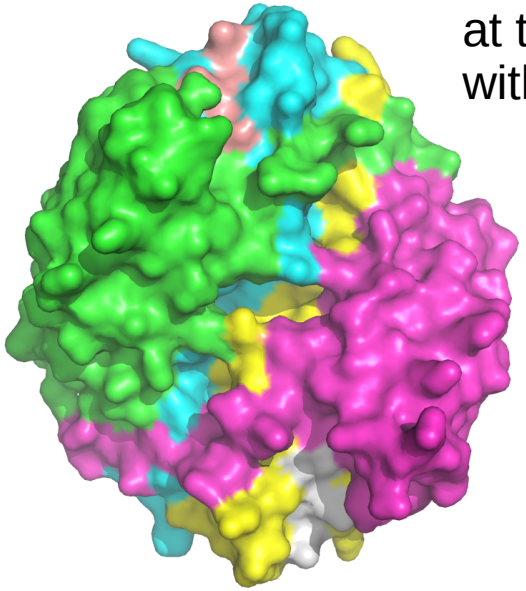


Peptide (cyclized)
- Large interaction surface
- High affinity

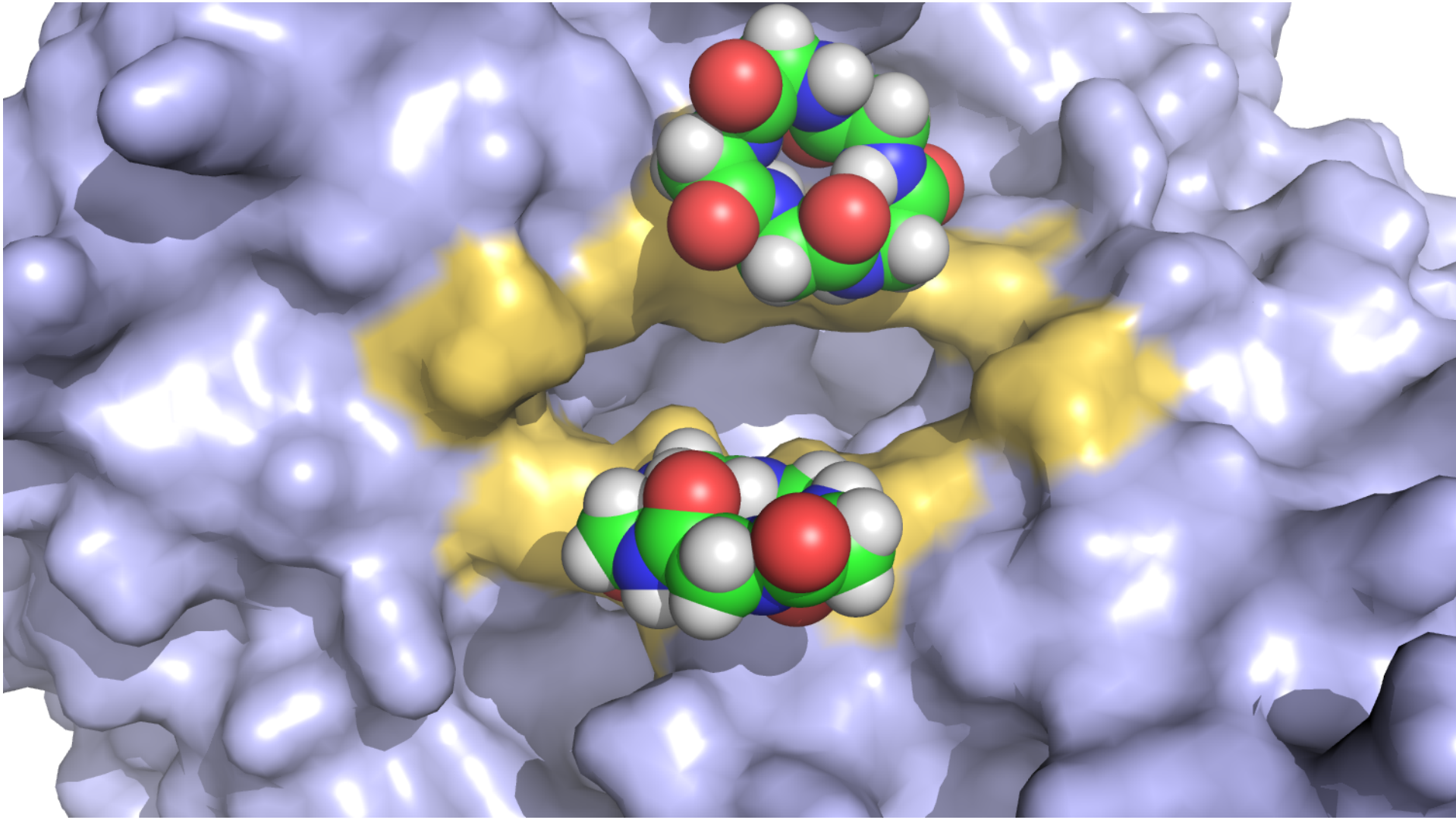
Caspase 2

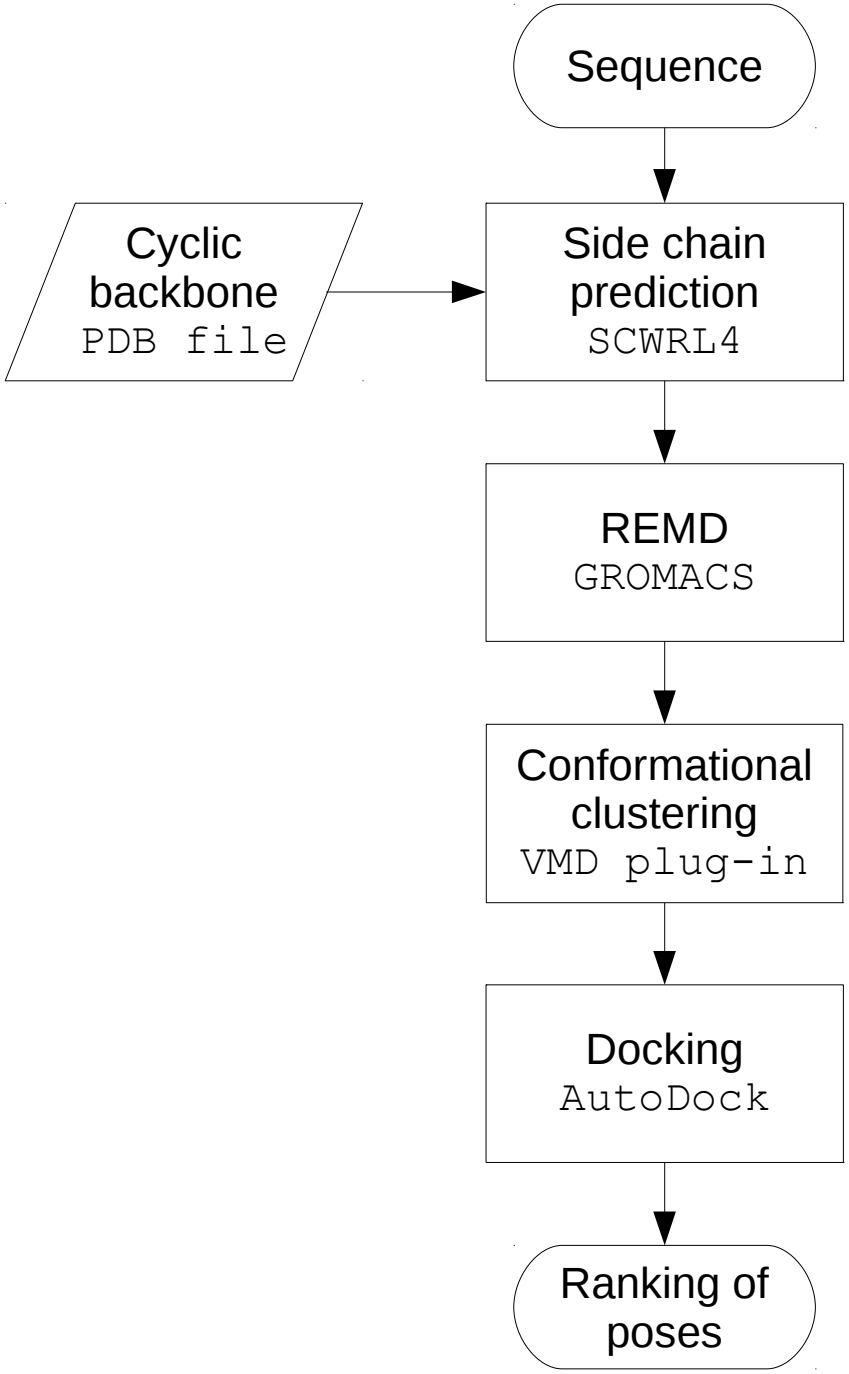
Disulfide bond





Target the narrow pocket at the interchain interface with a cyclic pentapeptide





Sequences:

- GXGXG ($n=20^2$)
- GGGXX ($n=20^2$)
- GXXGX ($n=20^3$)
- GGXXX ($n=20^3$)

X = any residue type

Design of cyclic pentapeptides for the inhibition of caspase-2

- Step 1: Identification of candidates with good affinity for the pocket → Molecular docking

- Step 2: Dynamic study of the binding to the pocket → Metadynamics simulations (`PLUMED 2`)

- Step 3: *in vitro* assays (Prof. C. El Amri, UPMC)

Thank you for your attention

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