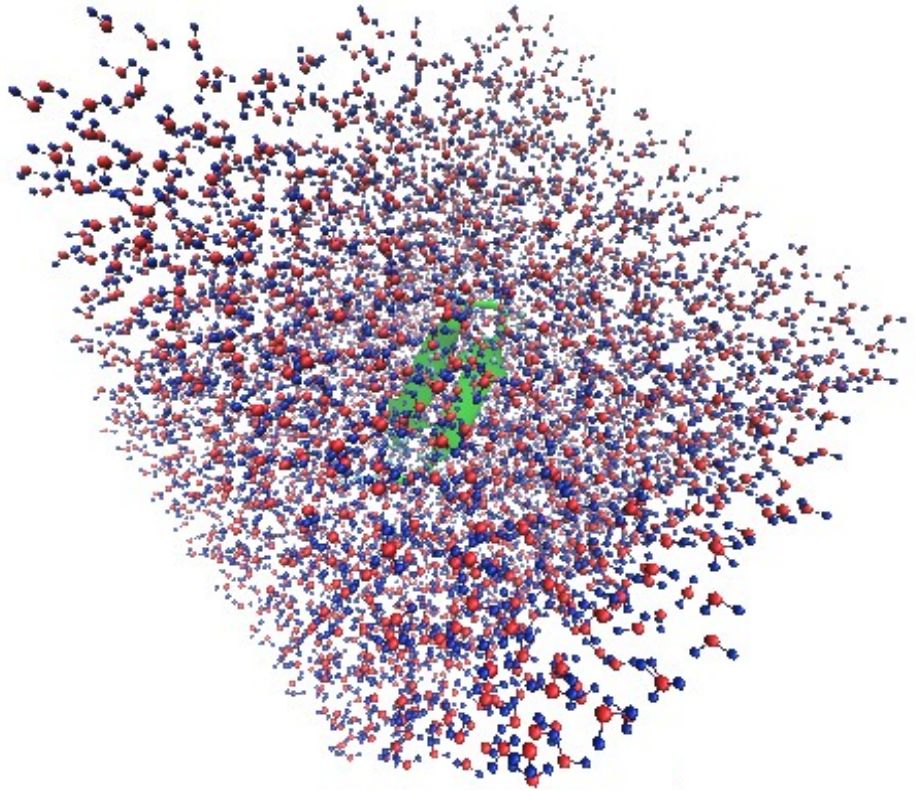
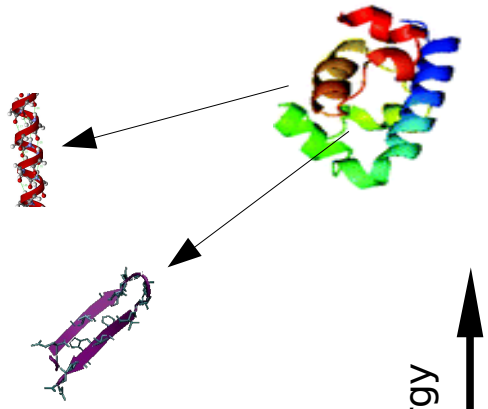
Computational studies of protein elements in different denaturing conditions



Roberto Meloni

Milano
18 November 2014

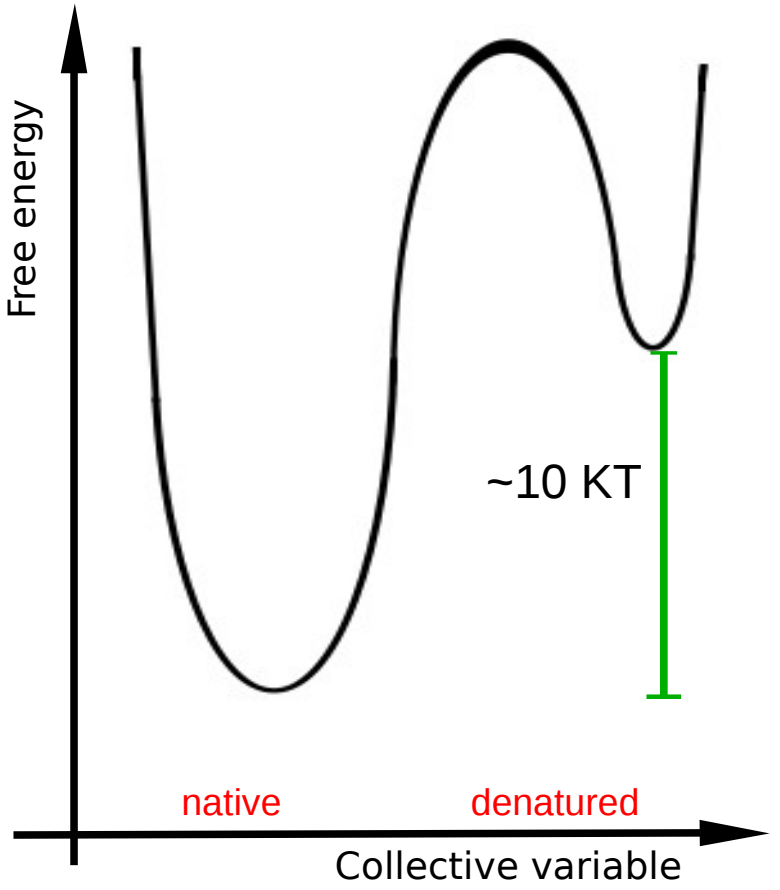
Proteins: two-state systems



$$\tau \sim 10^{-3} - 10\ s$$

NATIVE STATE

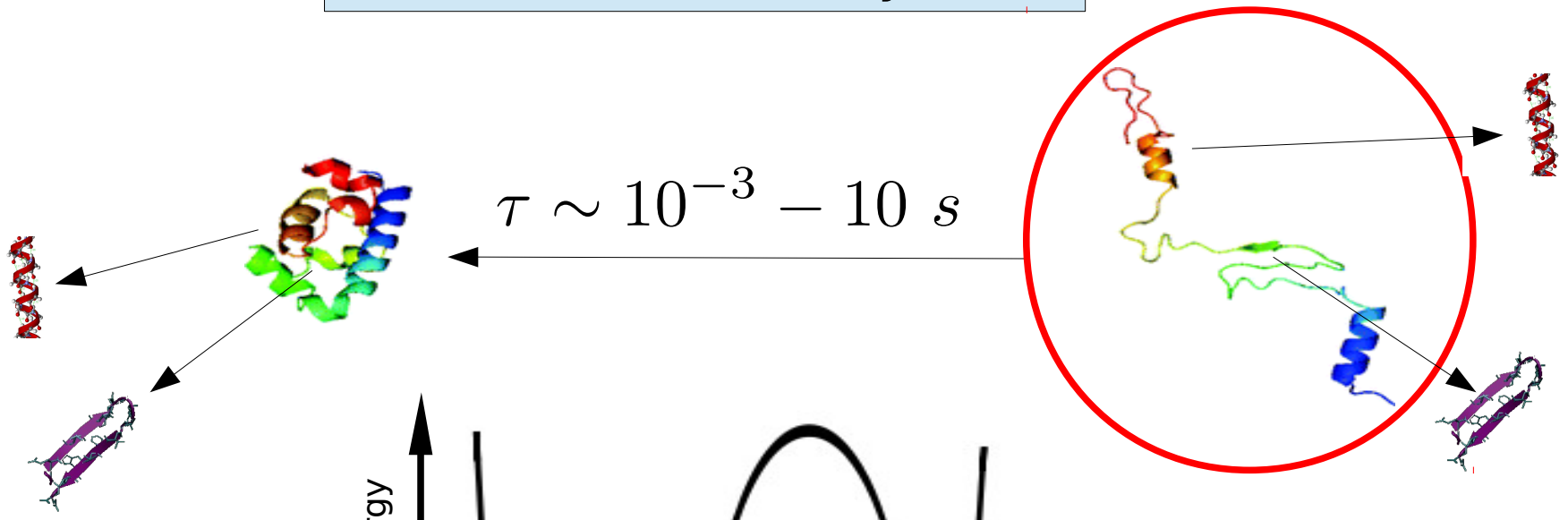
Definite structure
Low entropy
High Probability
Biological function



DENATURED STATE

Disorder
High Entropy
Metastability

Proteins: two-state systems



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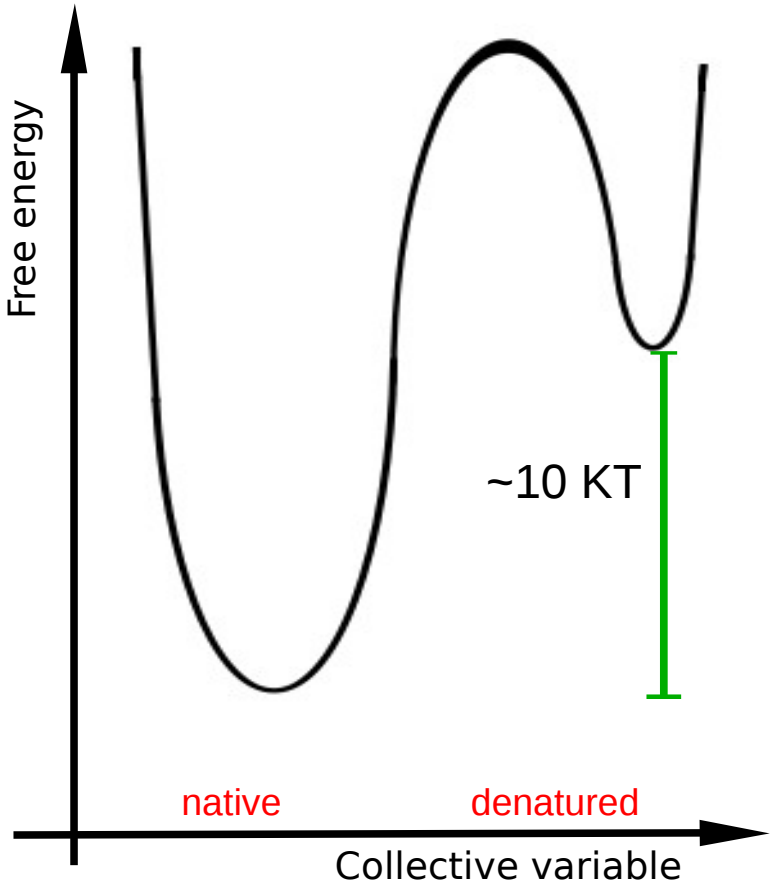

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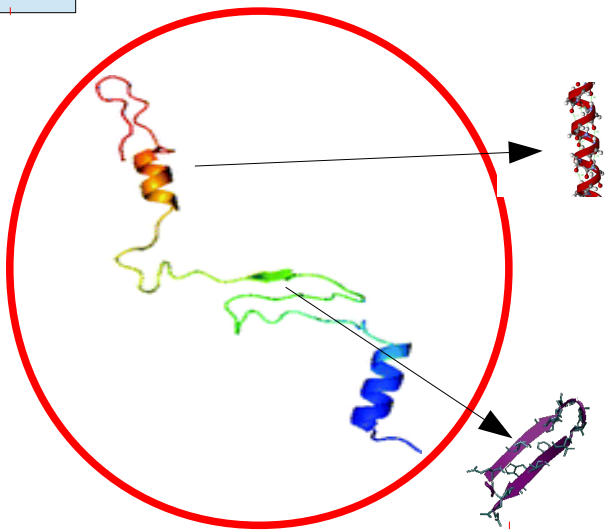
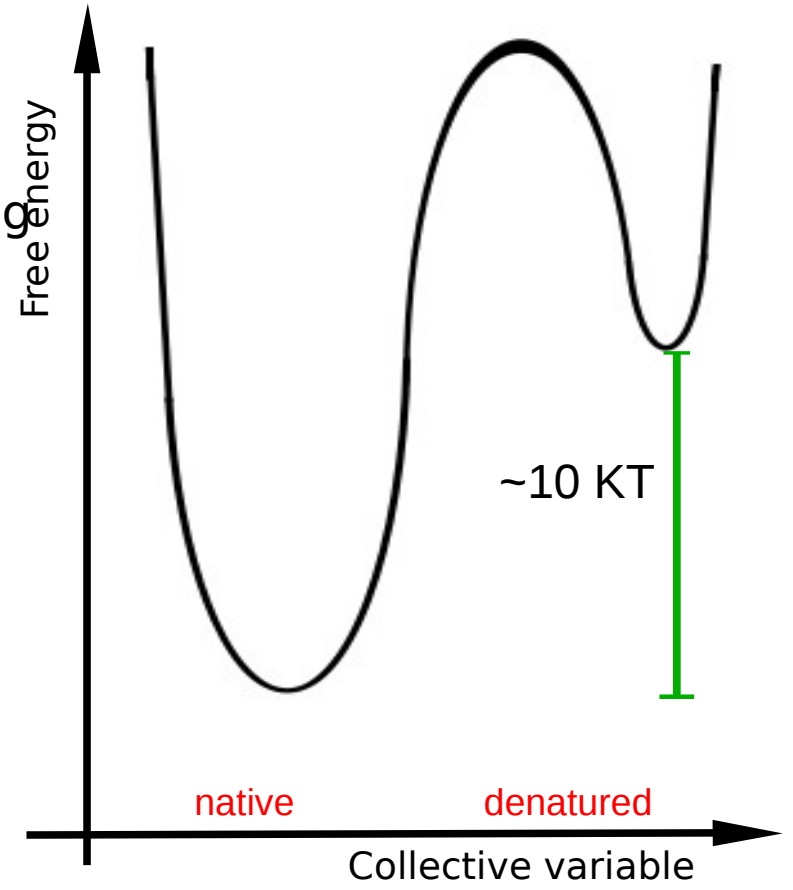


can be achieved by using

- HIGH TEMPERATURE
- CHEMICAL DENATURING AGENTS (urea, guanidine hydrochloride..)



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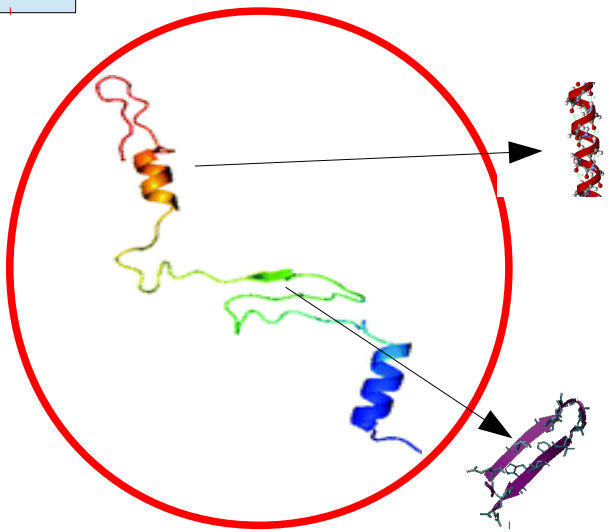

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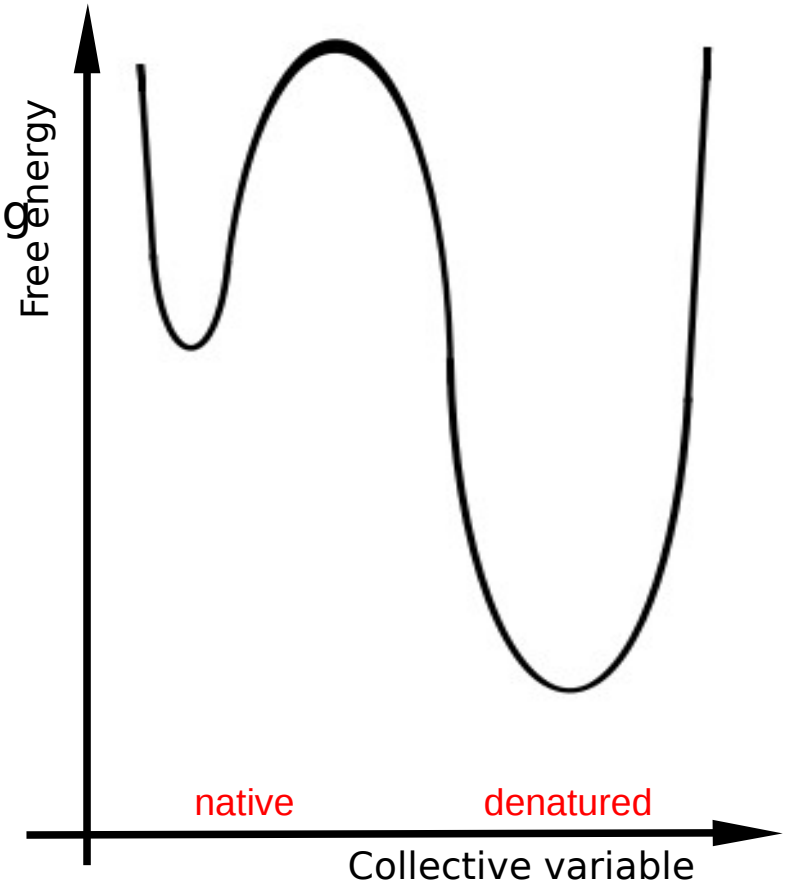

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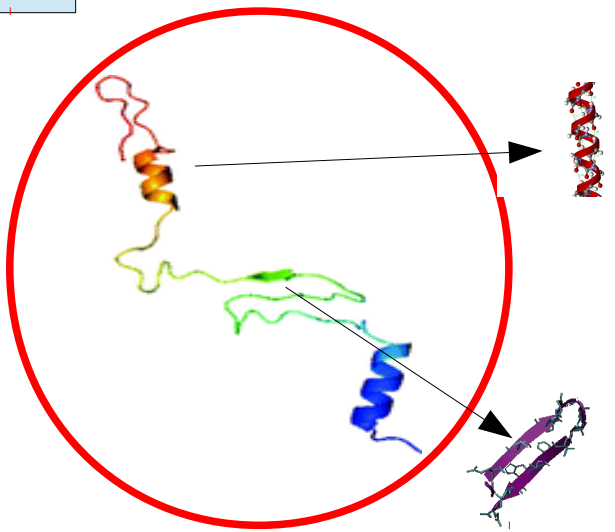

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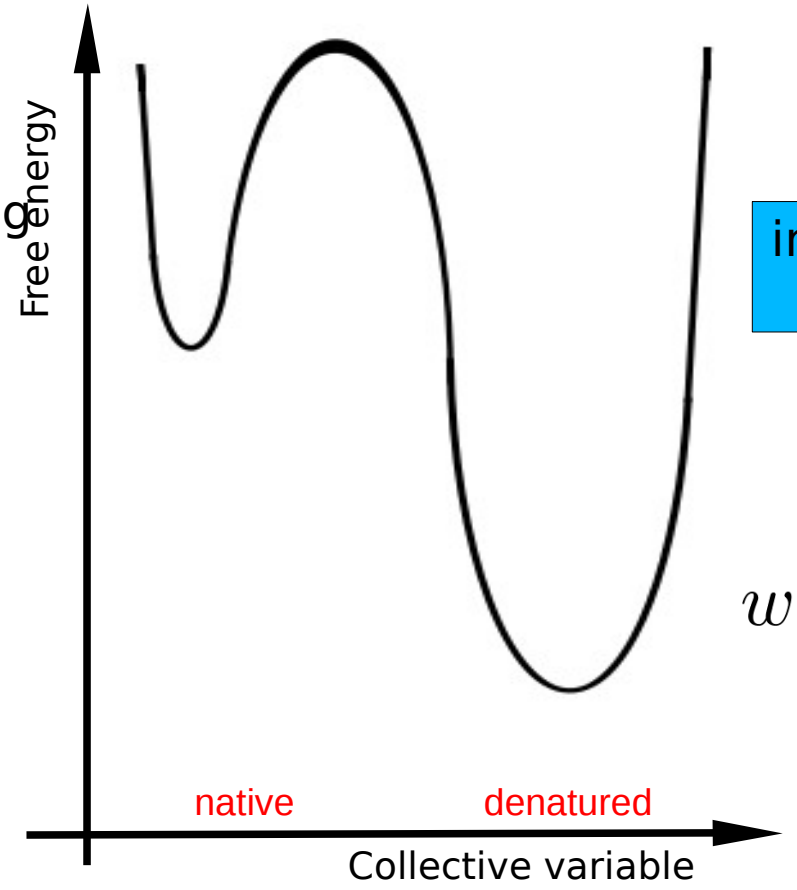


in physiological conditions?
300 K, water

TROUBLE

$$water \neq \begin{matrix} urea \\ guanidine \end{matrix}$$

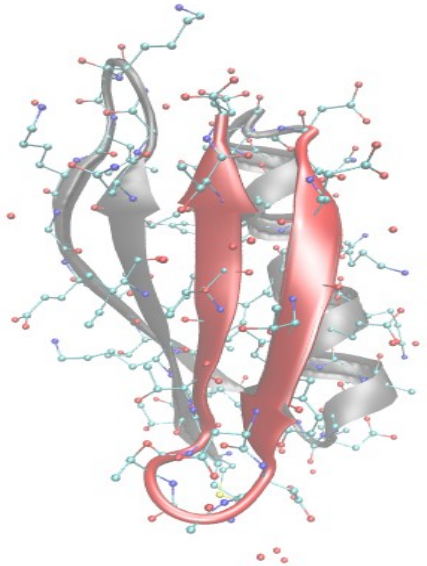
Simplify the question
working on the building blocks

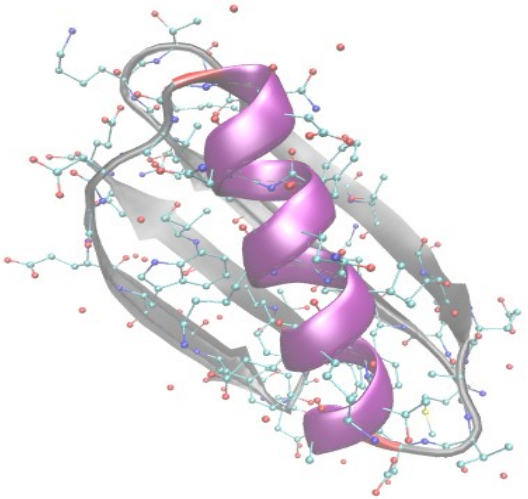
Every different protein has its own thermodynamic properties..

Simplify the question
working on the building blocks

Every different protein has its own thermodynamic properties..

..but quite everyone is made of α-helices and β-sheets.





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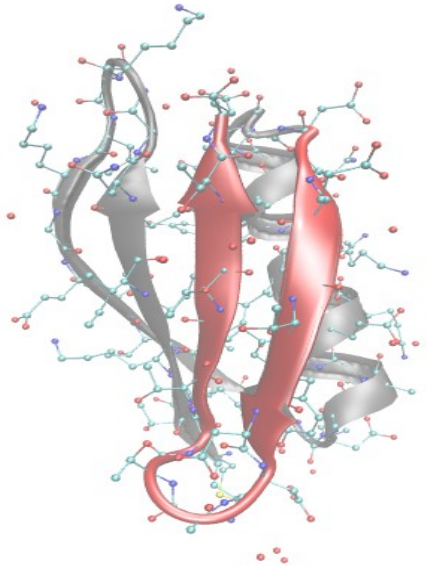
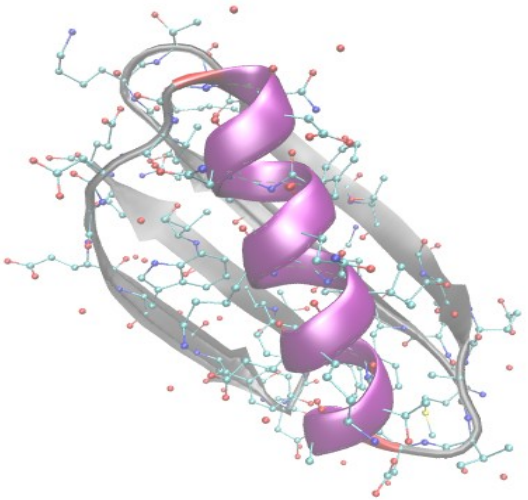




Work on the “building blocks” of proteins and analyse their behaviour in different chemical conditions.

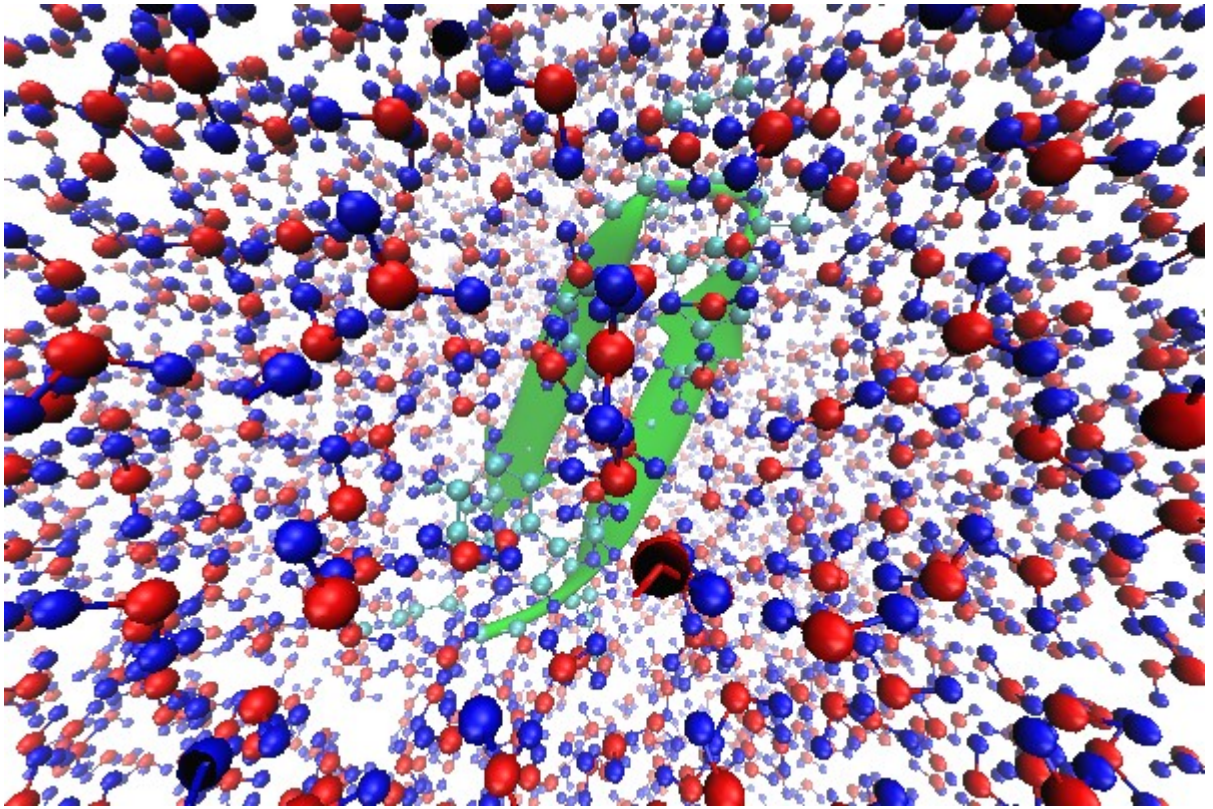
- Water → PHYS. CONDITIONS
- Urea
- Guanidine

} DENATURING AGENTS

Molecular dynamics simulations

NVT ensemble at 300 K
Cubic box, side: ~ 5 nm
~ 11-17k atoms per simulation (explicit solvent)

$$H_0 = T + V_{coul} + V_{vdw} + V_{bonds} + V_{angles} + V_{torsion}$$



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1) overcoming of free energy barriers

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2) diffusion in a wide phase space

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BUT

we want to perform equilibrium averages

EFFICIENT SAMPLING ⟷ EQUILIBRIUM

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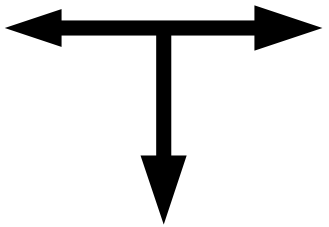


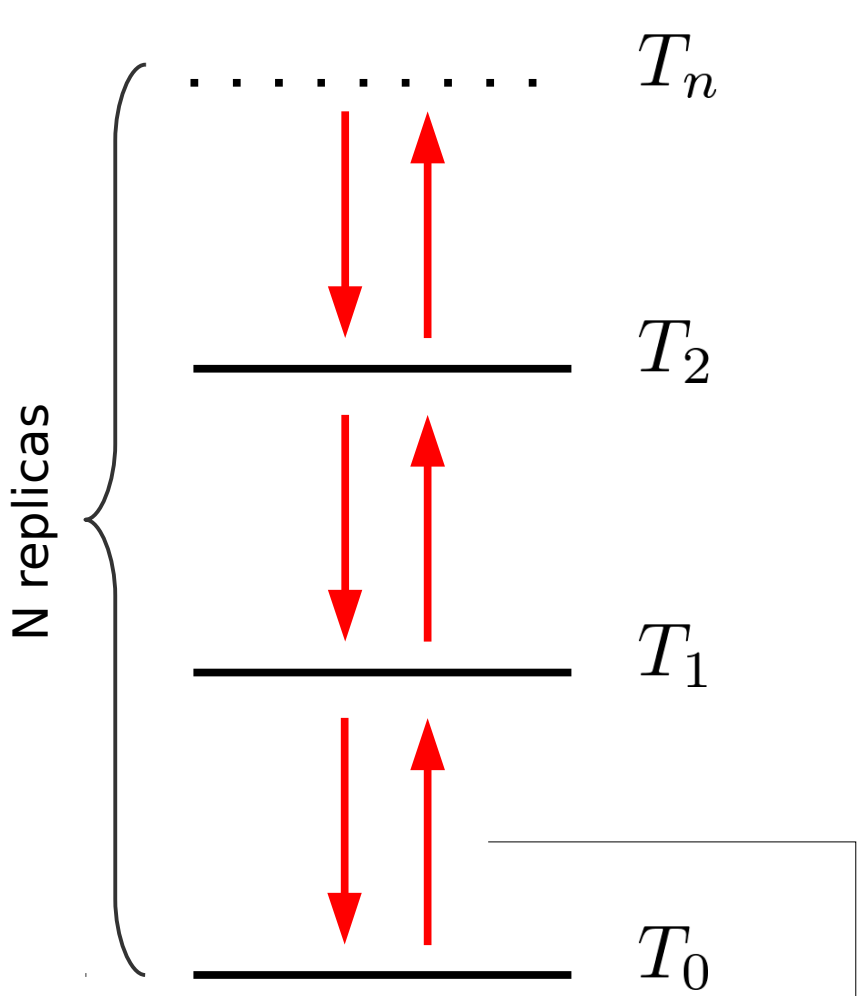
EQUILIBRIUM

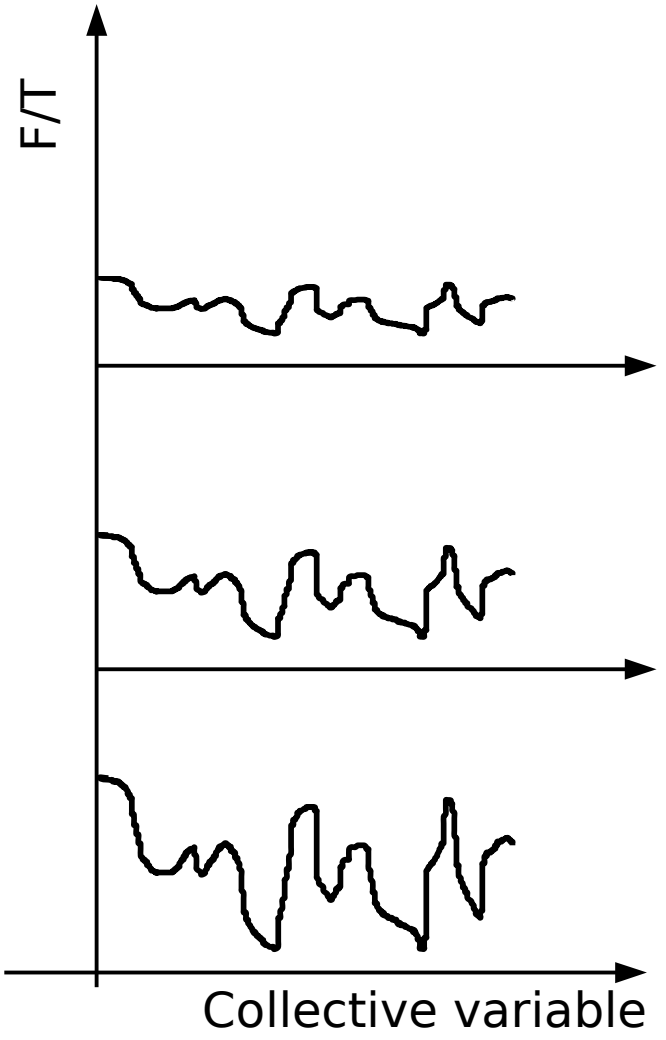
a lot of computational time is needed to achieve these goals

→ must find some trick to speed up the simulation

1

Advanced sampling methods:
usual replica-exchange scheme





Metropolis criterion keeps every replica in its canonical ensemble

Sugita, Y.; Kitao, A.; Okamoto, Y., *J. Chem. Phys. (*2000) **113**, 6042

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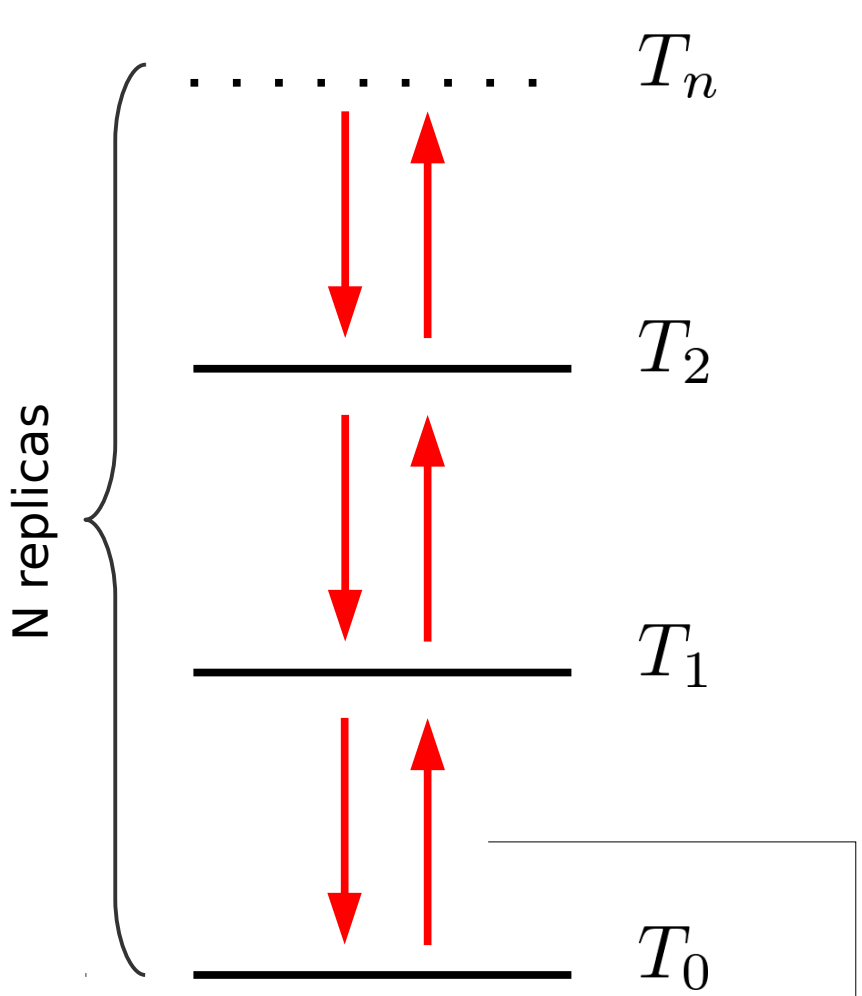

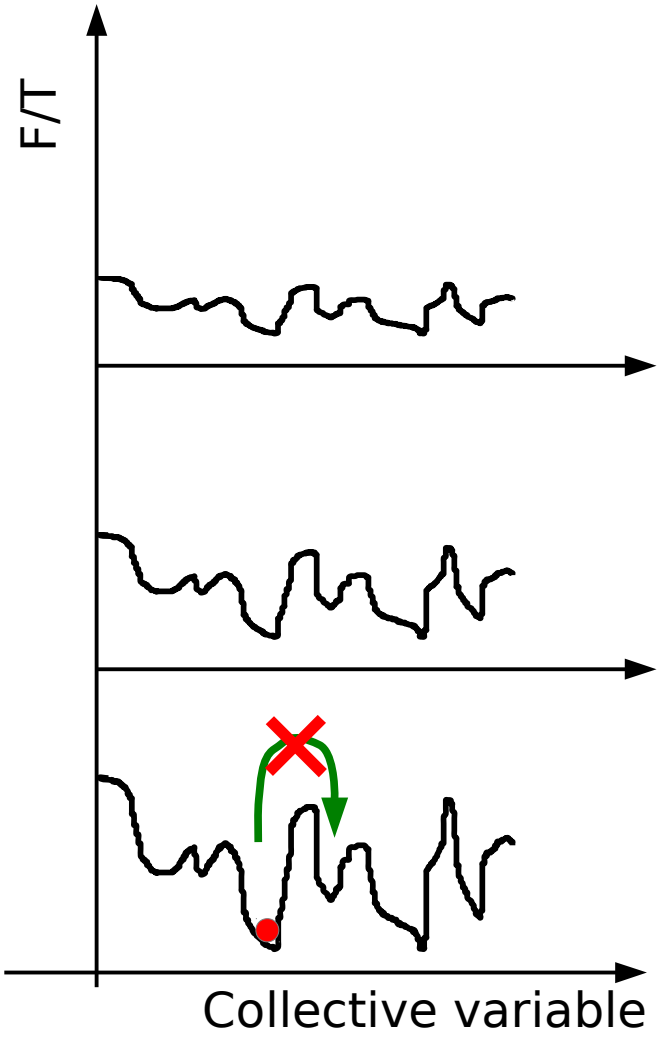

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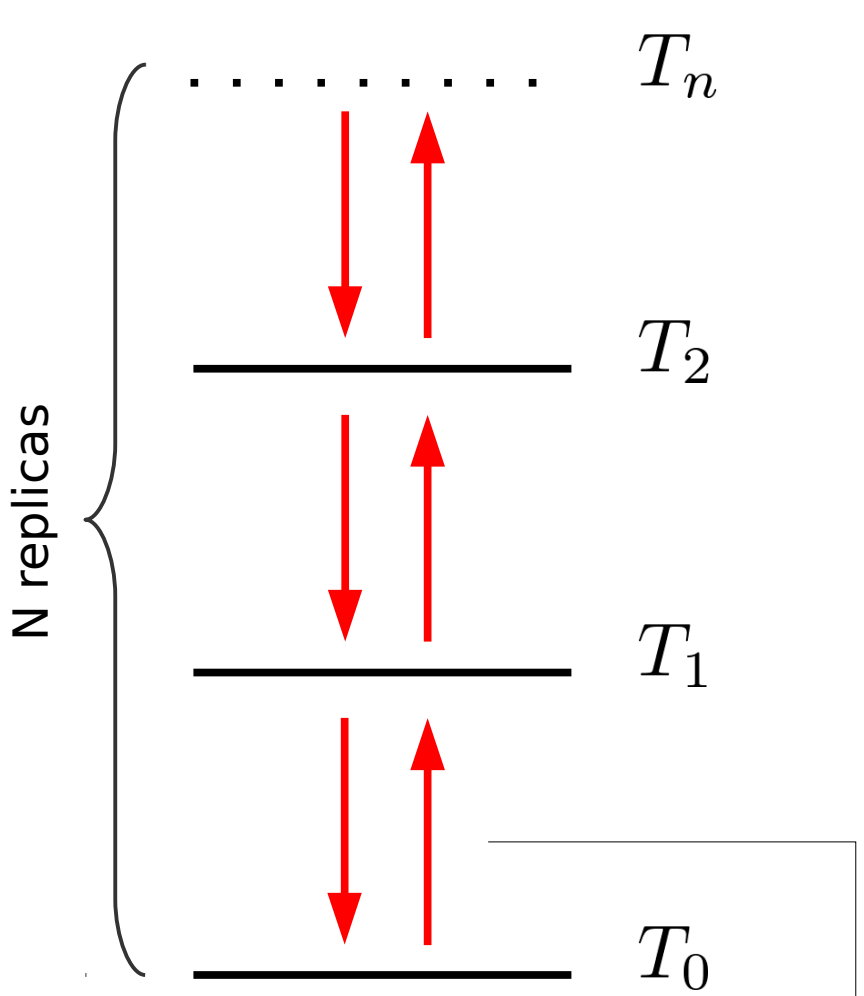

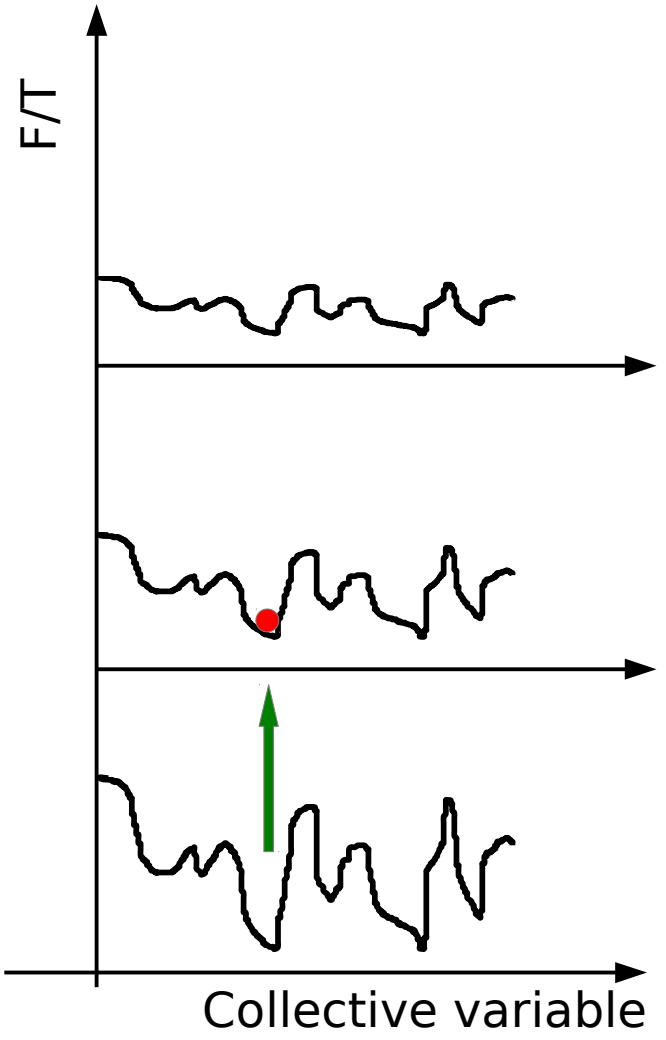

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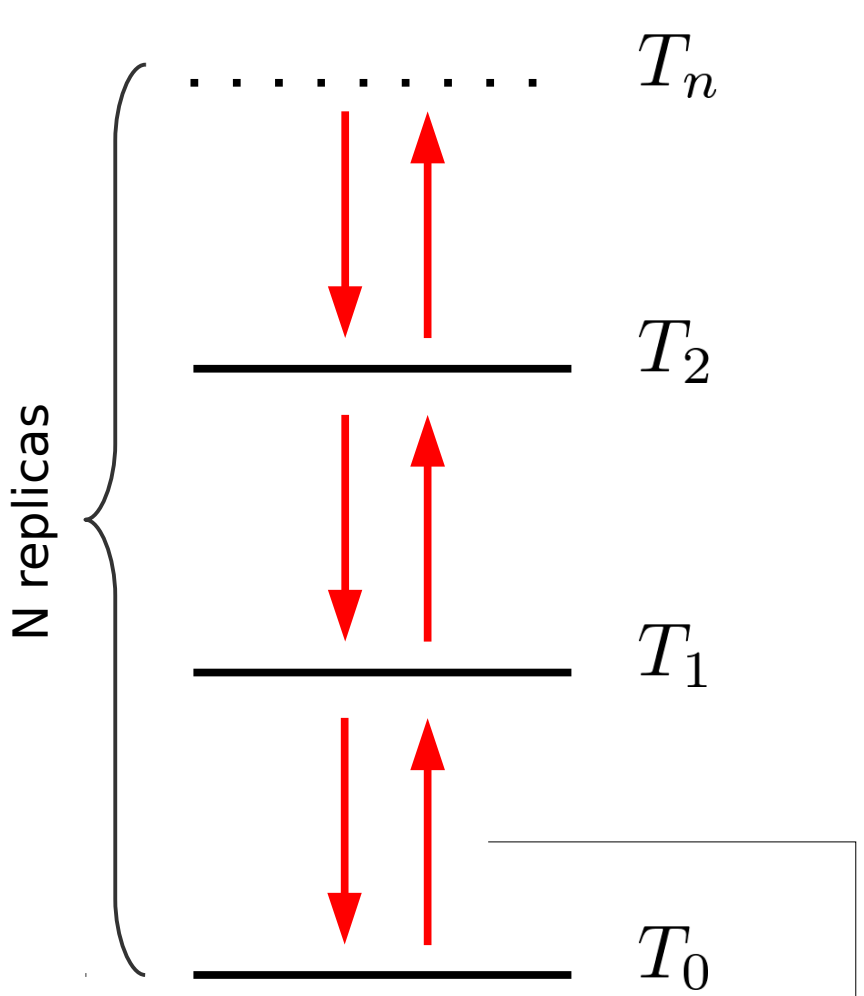

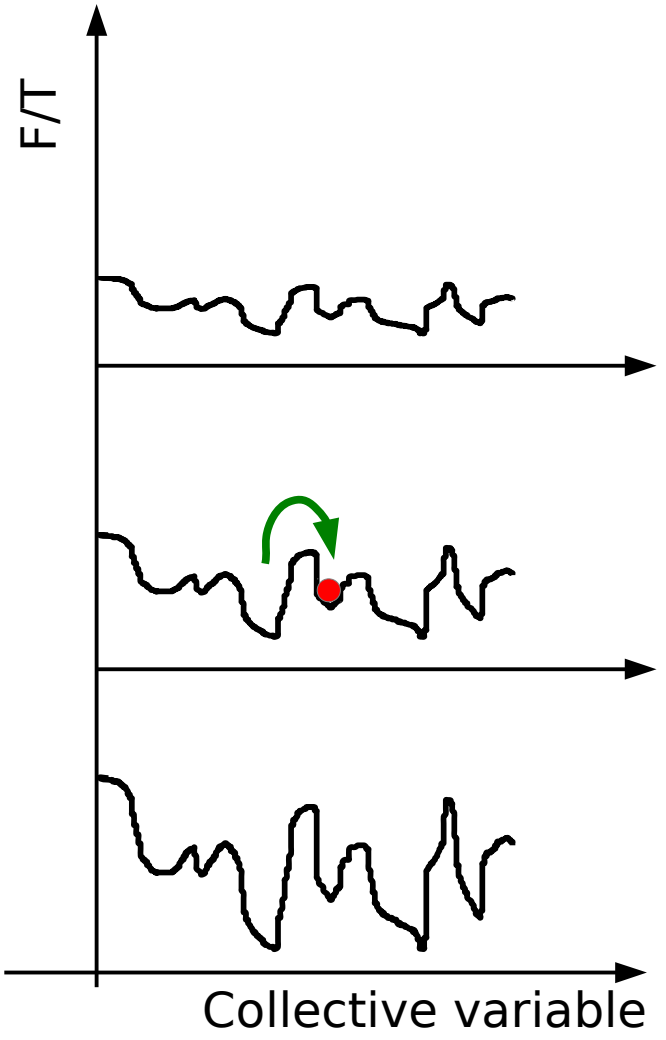

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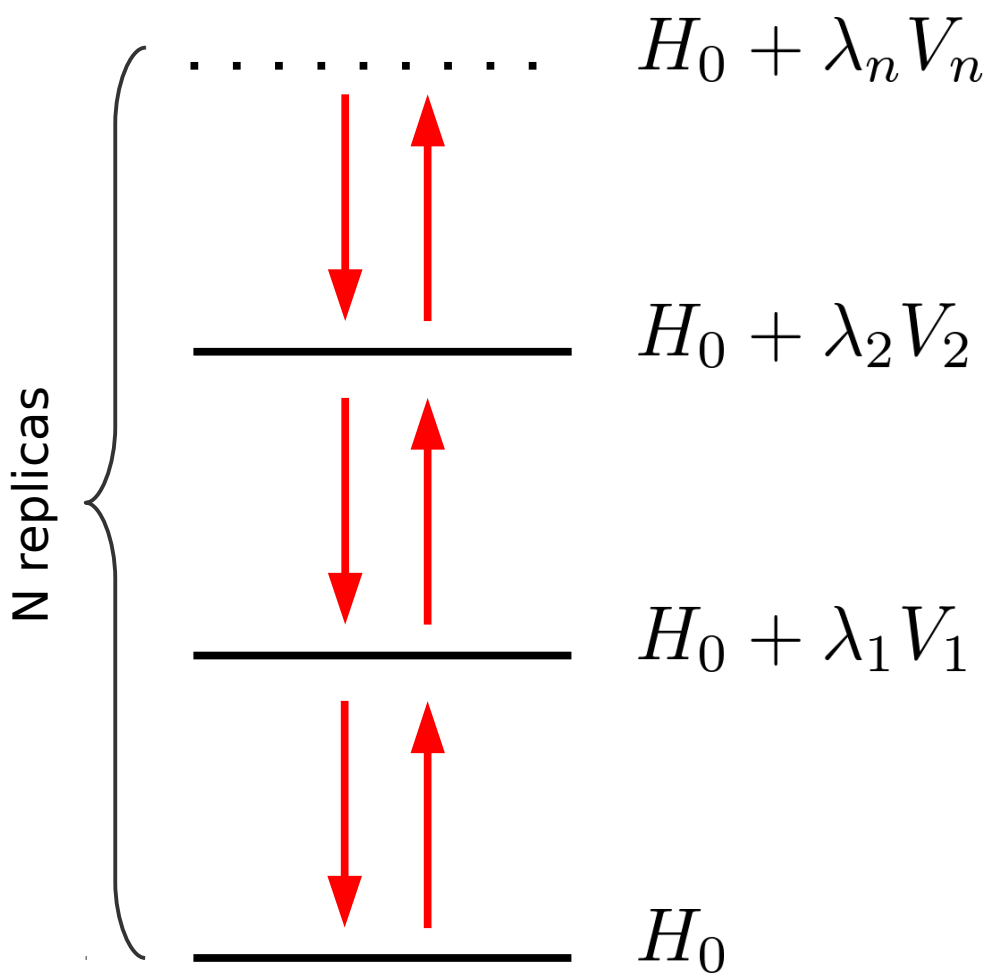
Advanced sampling methods:
hamiltonian replica-exchange



Generally,

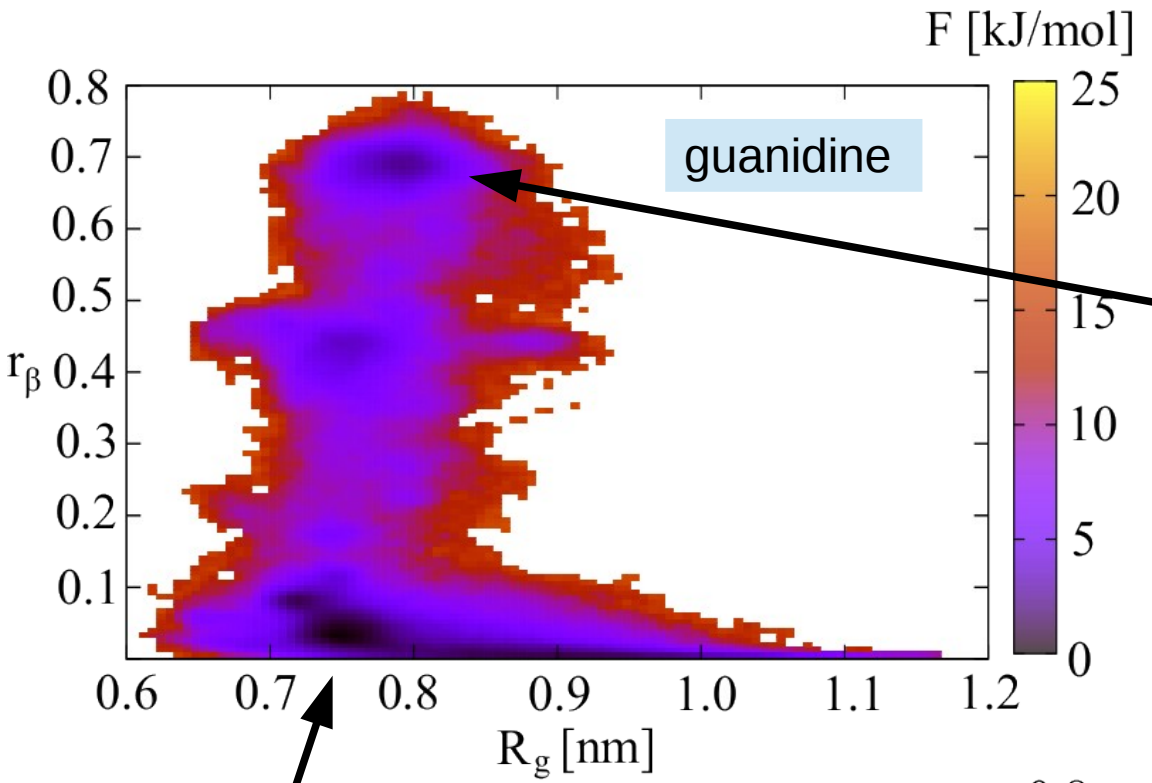
$$H = H_0 + \lambda V$$

where

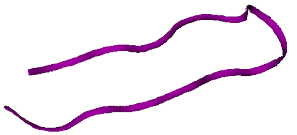
$$V = \frac{1}{2}(\xi - \xi_0)^2$$

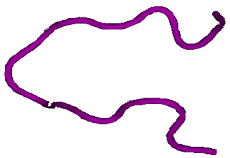
$$\xi = \xi(\mathbf{p}, \mathbf{q})$$

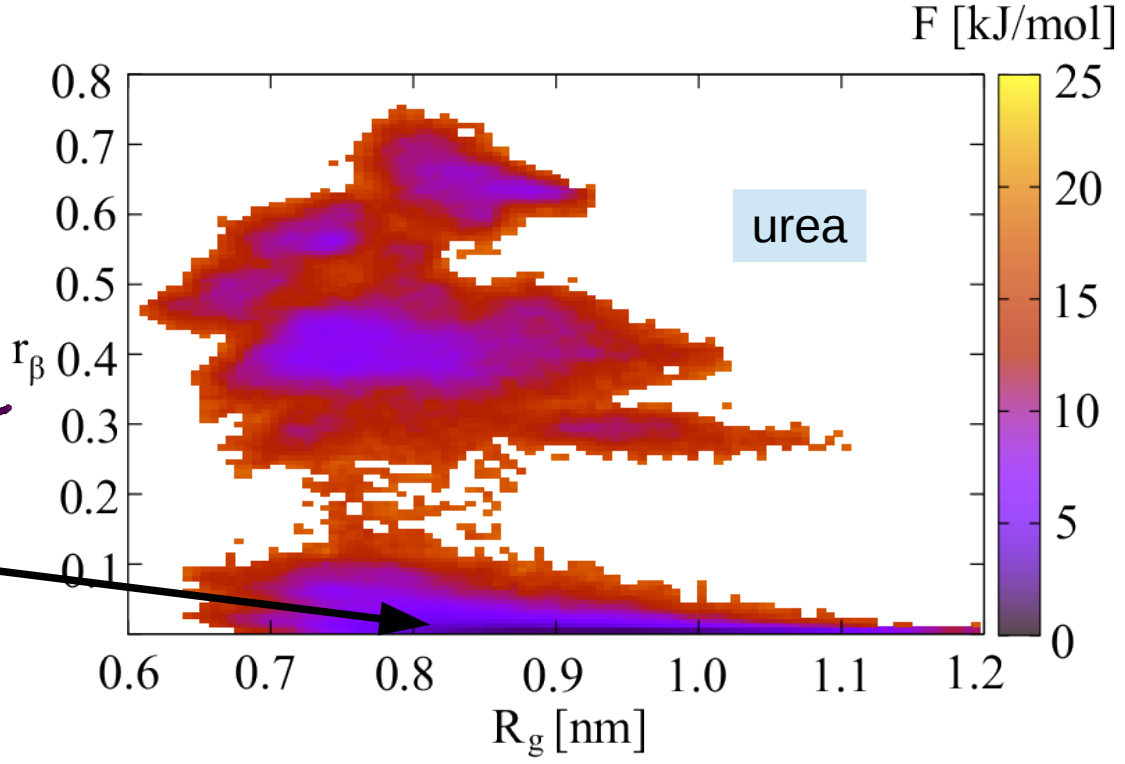
Fukunishi H.; Watanabe O.; Takada S., *J. Chem Phys.* (2002) **116**, 9058



To compare: a completely folded hairpin







Urea is highly disruptive,
while guanidine has a milder effect..
..at least on this hairpin.

Meloni R.; Tiana G.; Camilloni C., *J. Chem Theo. Comp.* (2014) **10**, 846

3

Advanced sampling methods:
metadynamics

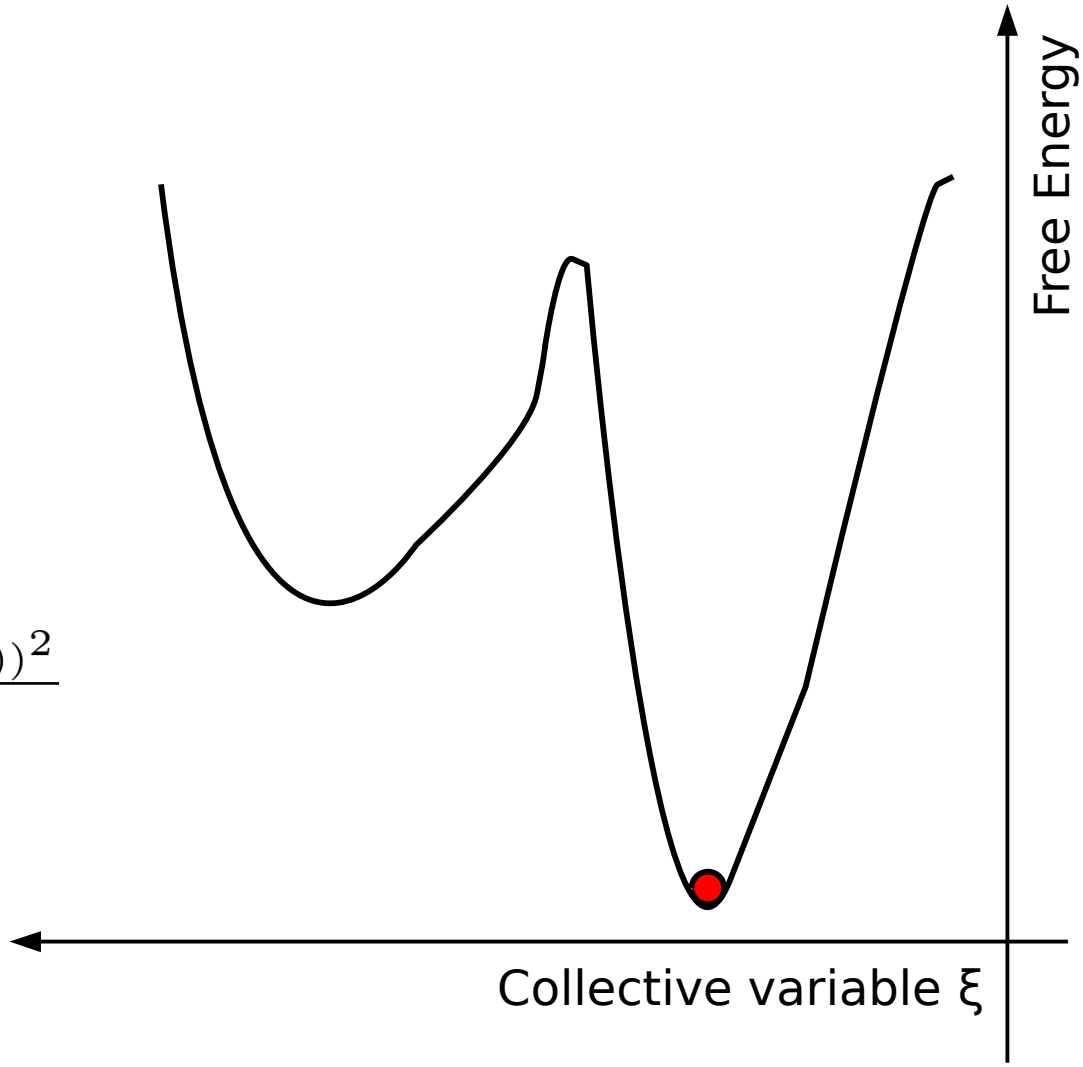
try a new approach:

$$H = H_0 + V(\xi, t)$$

where

$$V(\xi, t) \propto \sum_{t'<t} e^{-\frac{(\xi - \xi(t'))^2}{2\sigma^2}}$$

$$\xi = \xi(\mathbf{p}, \mathbf{q})$$



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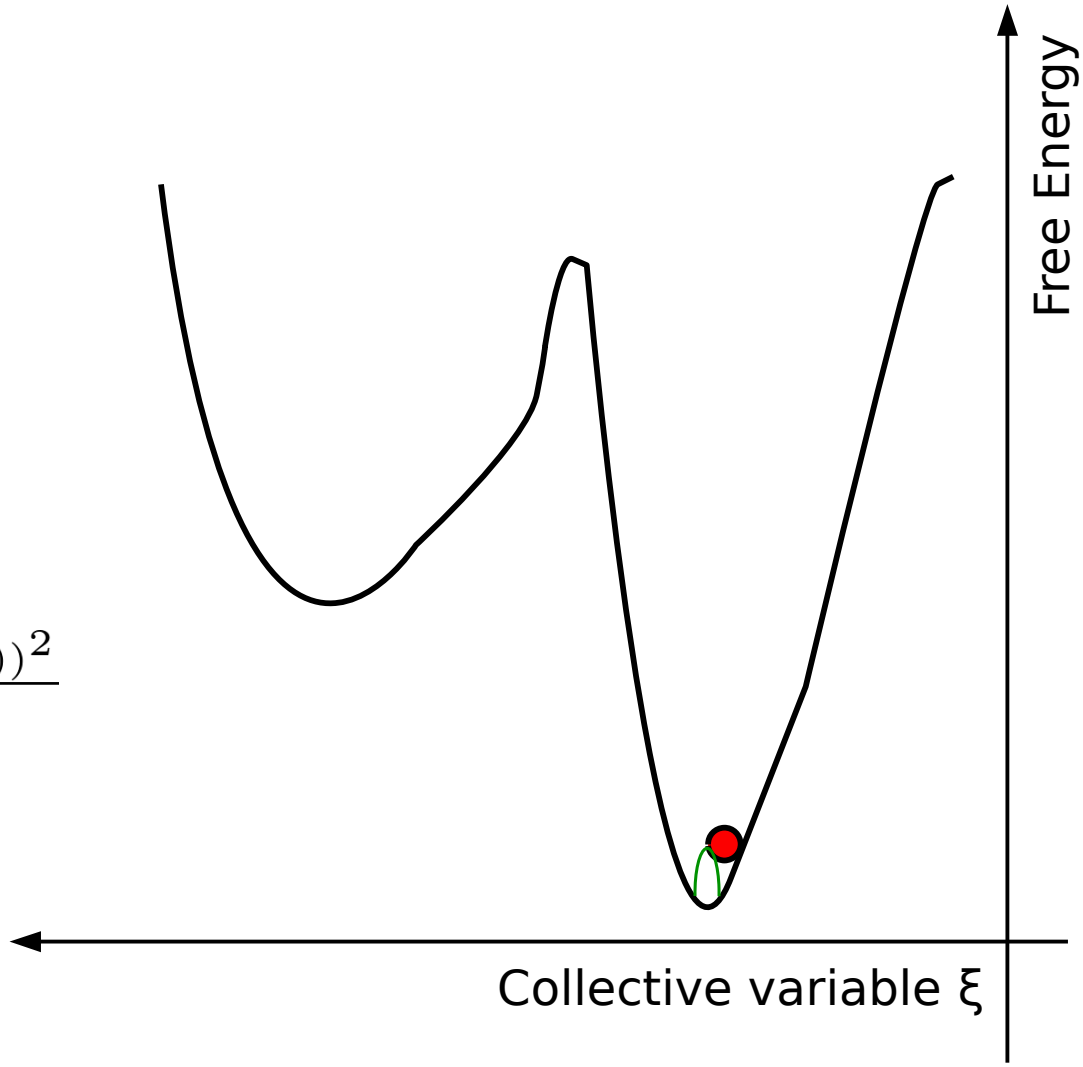

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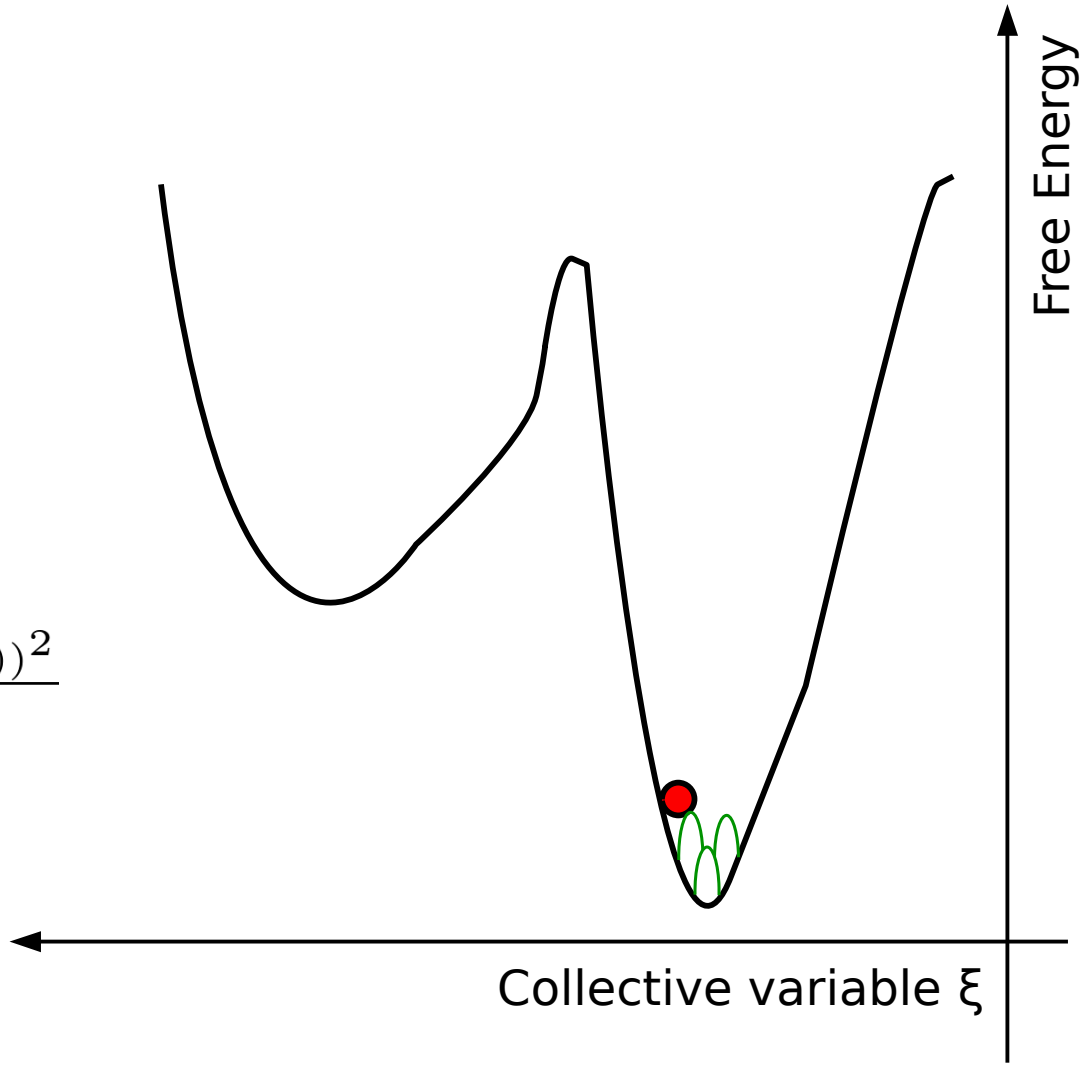

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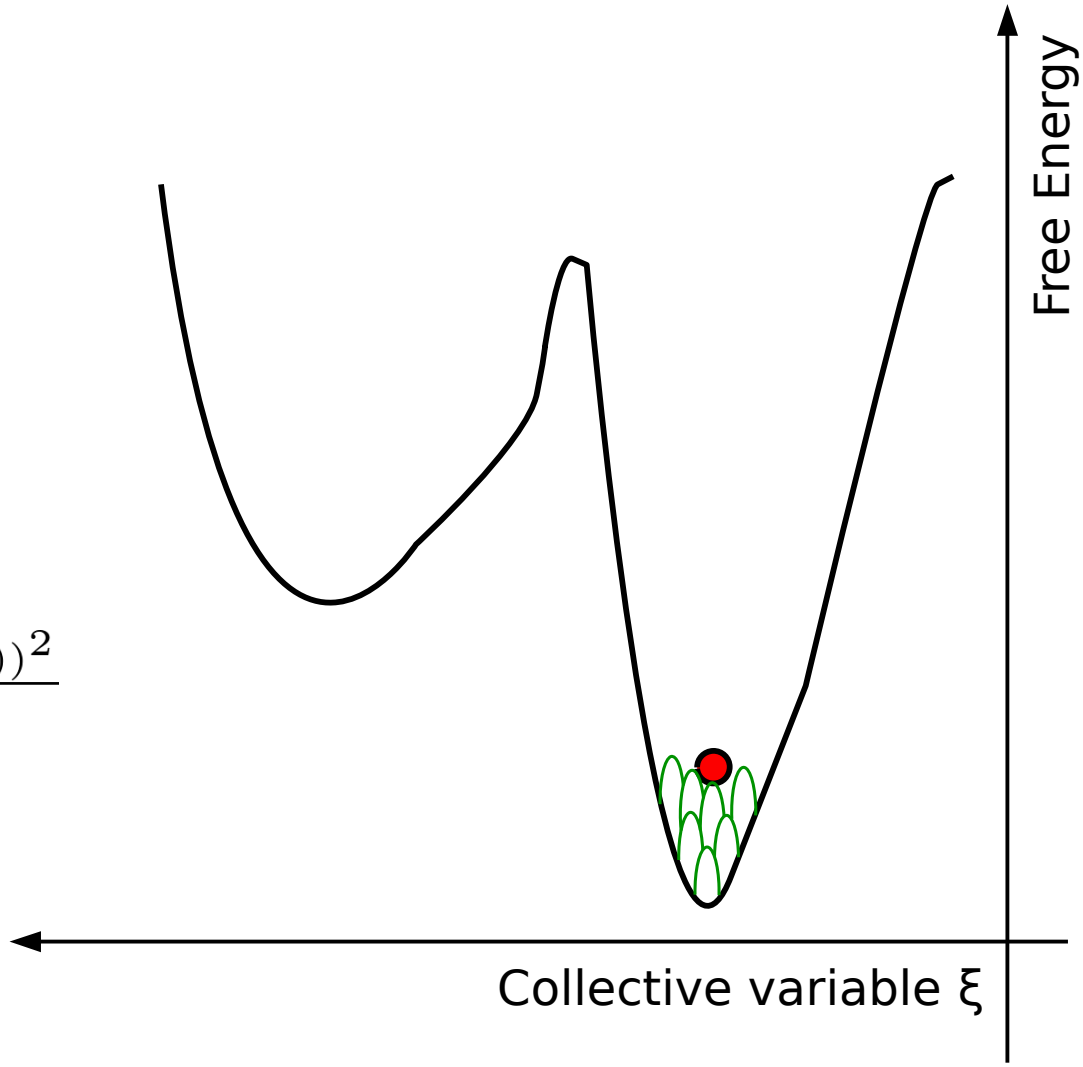

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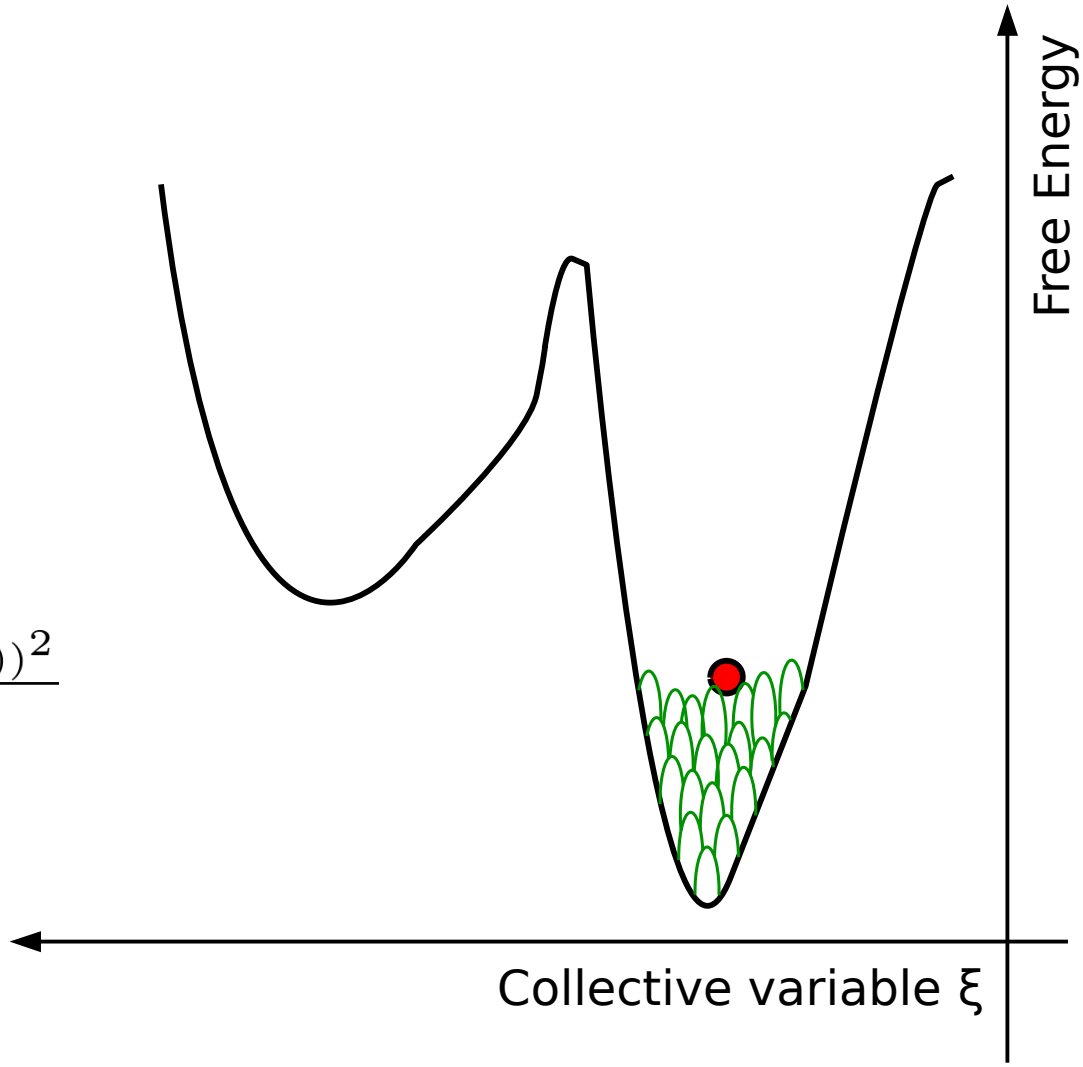

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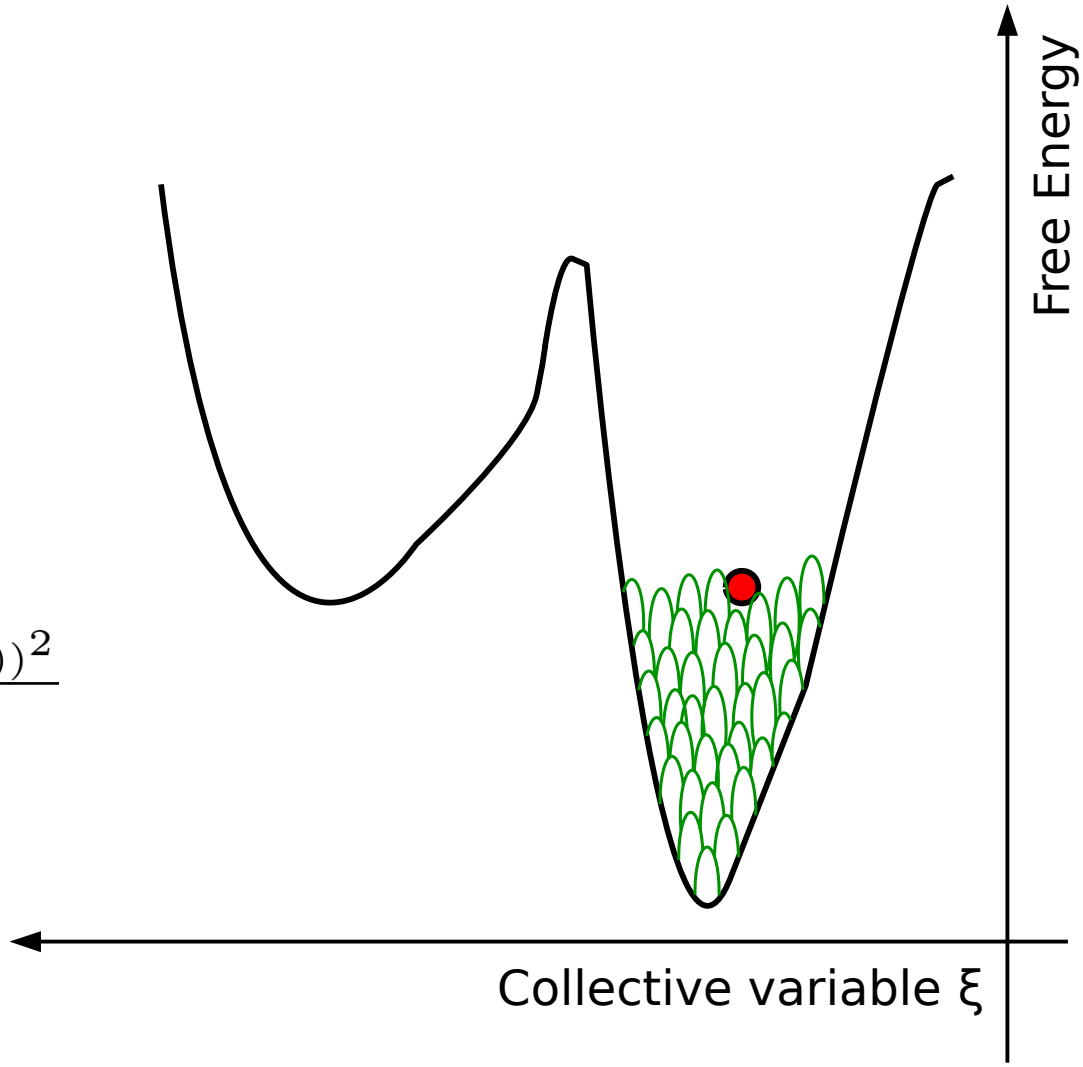

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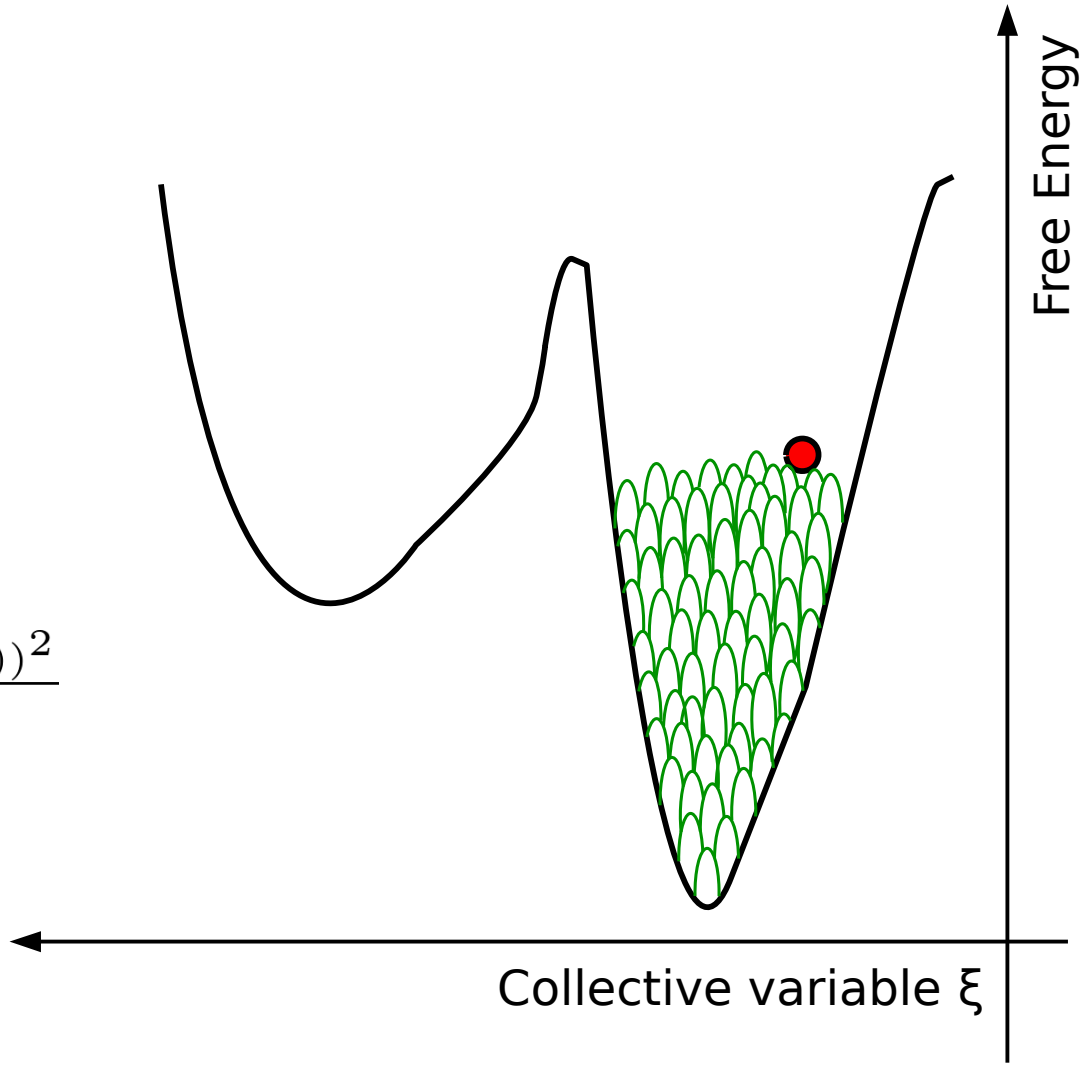

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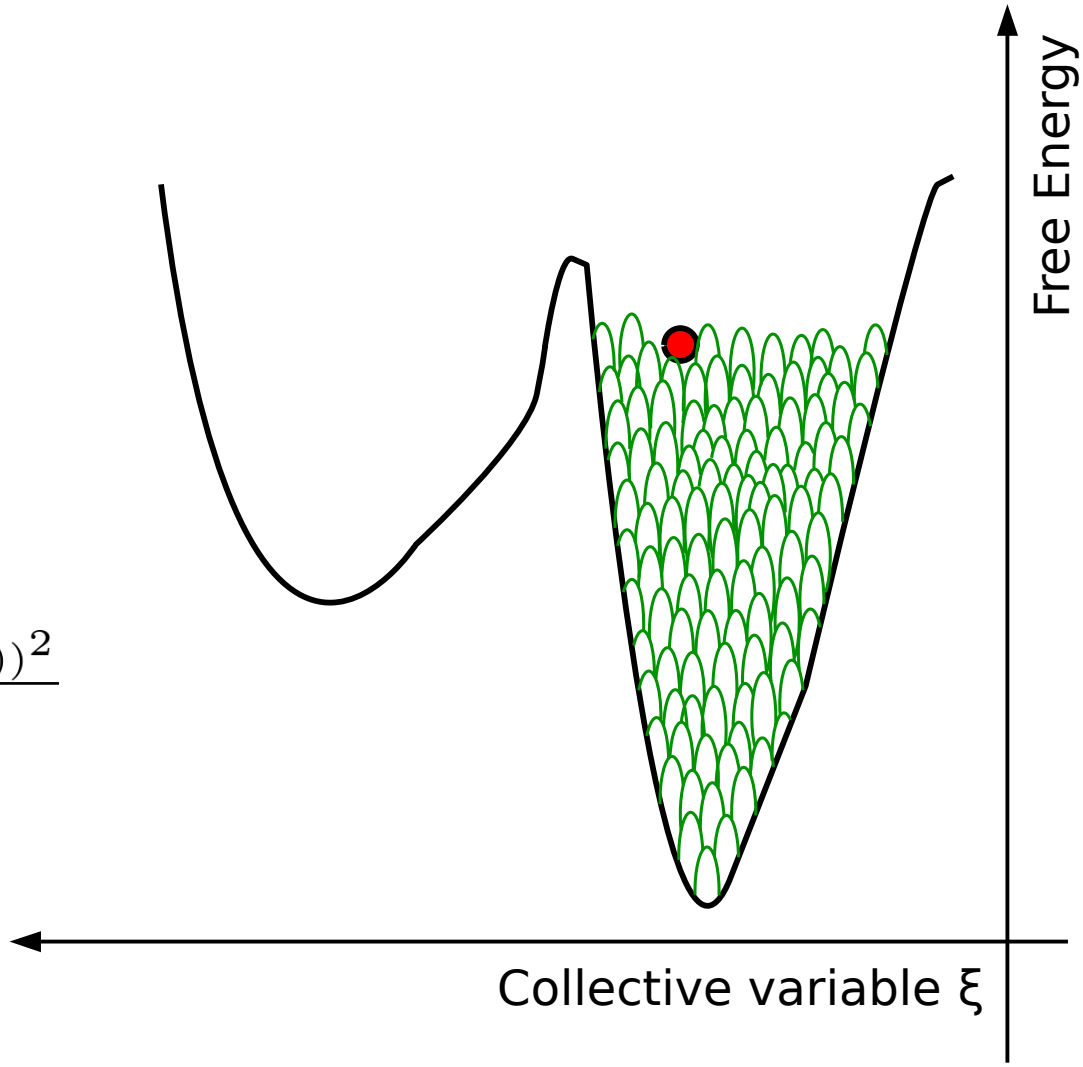

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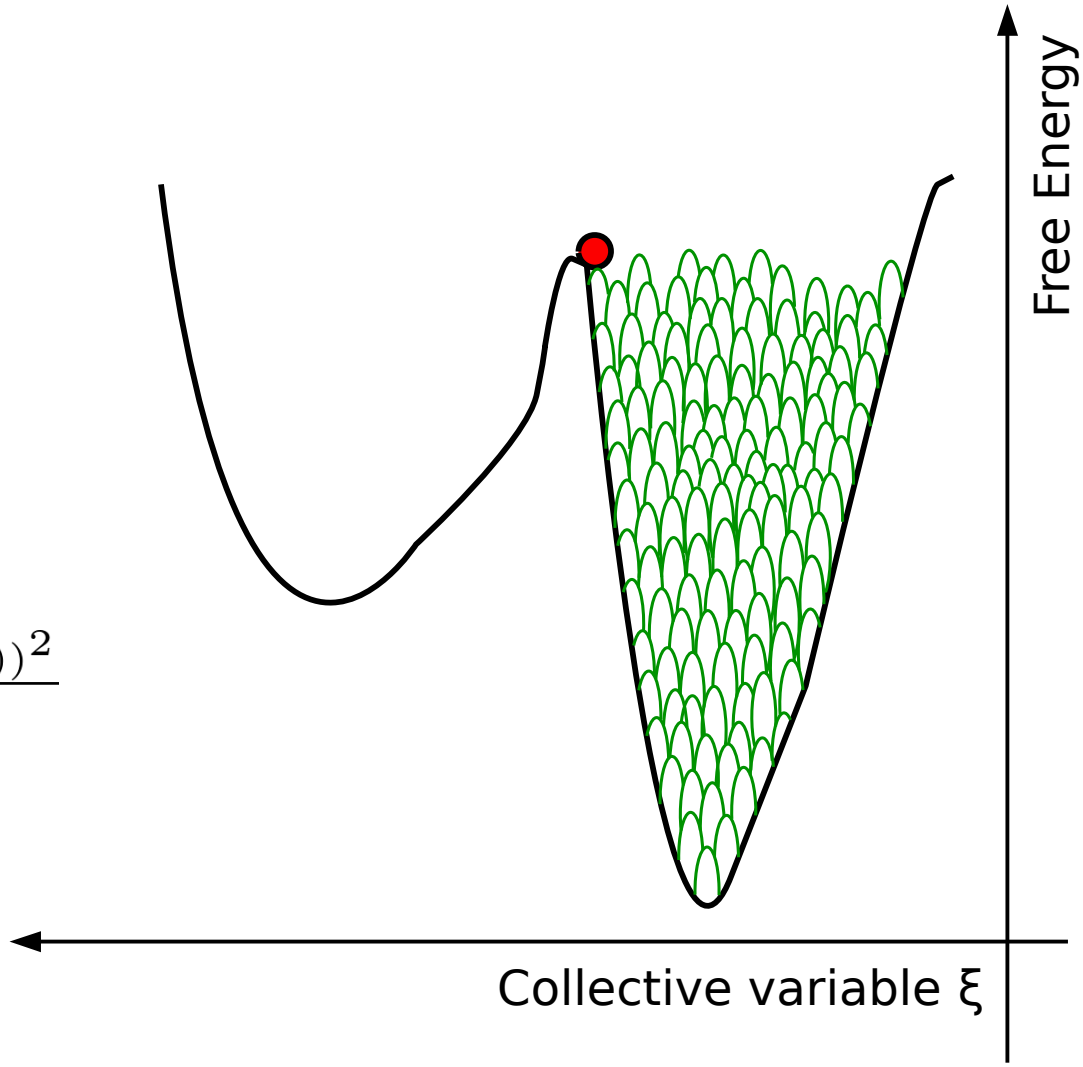

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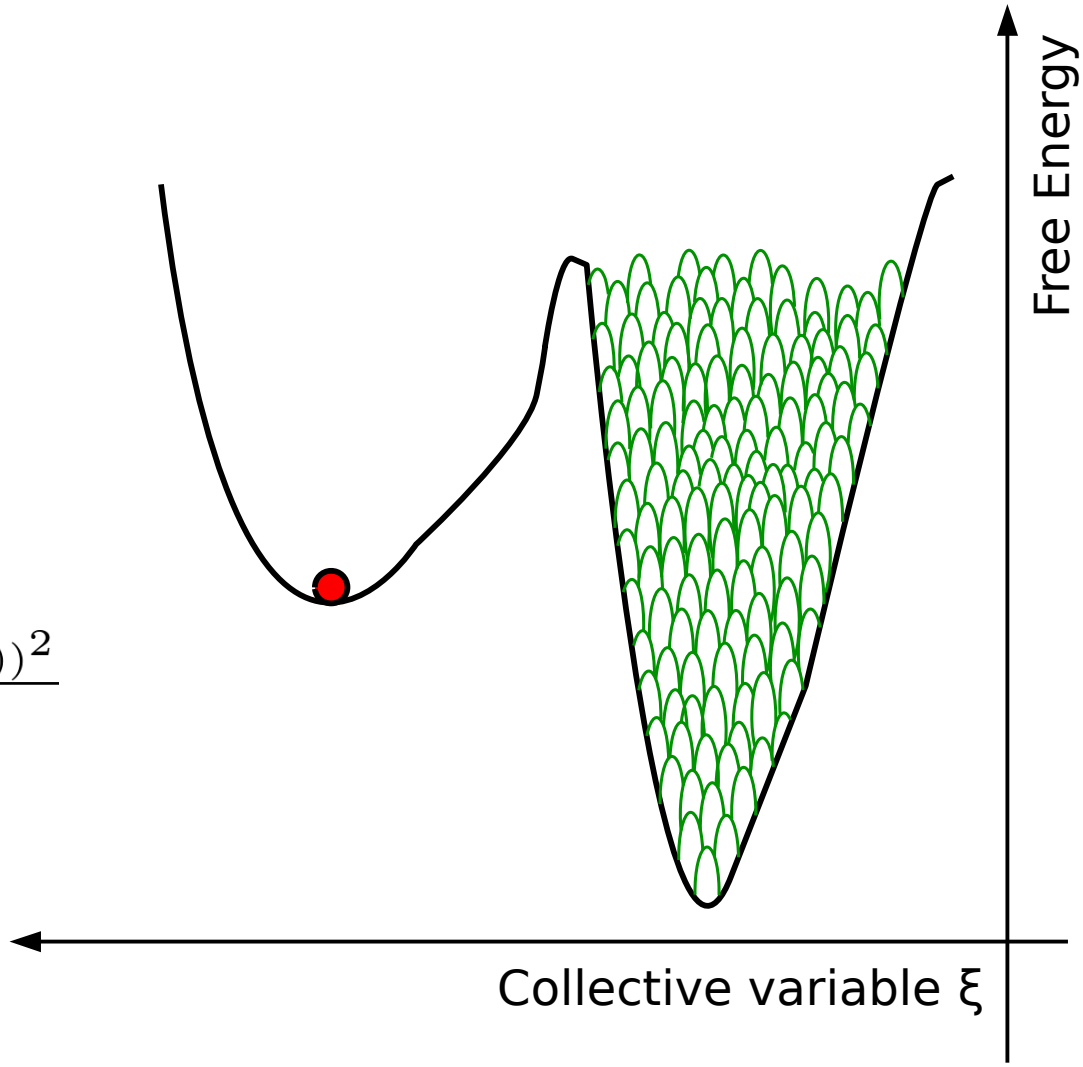
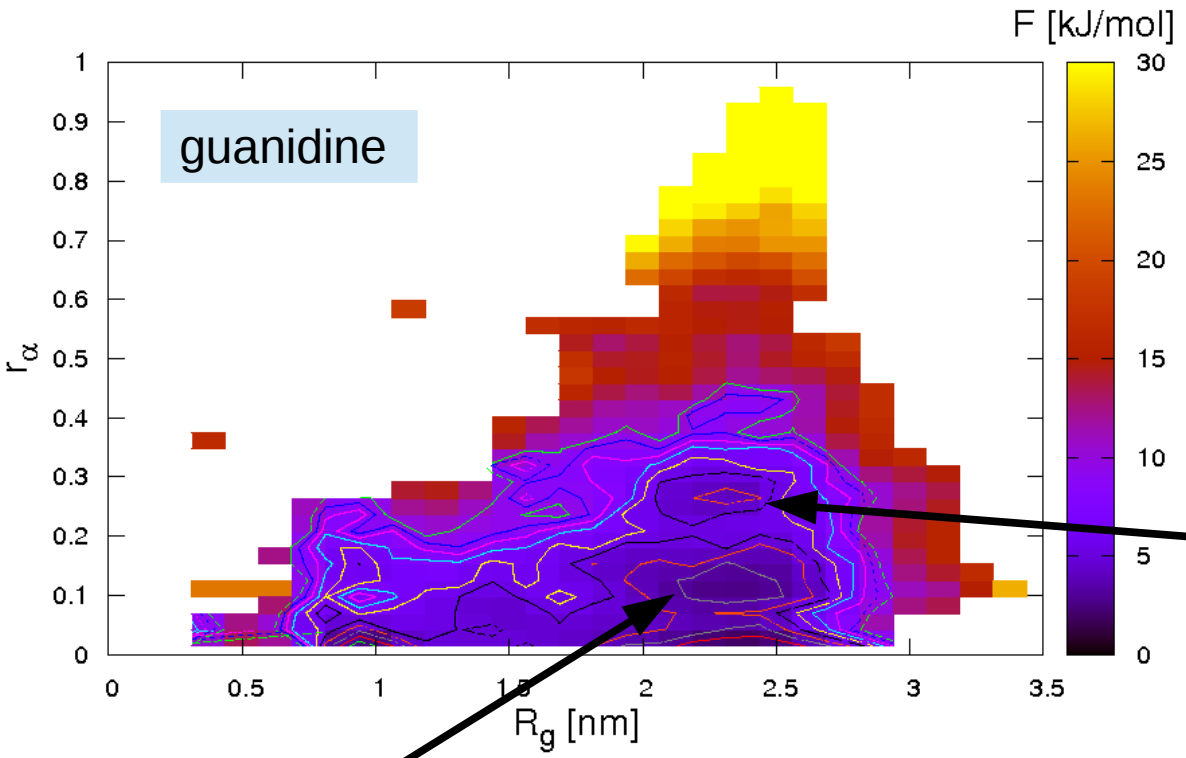
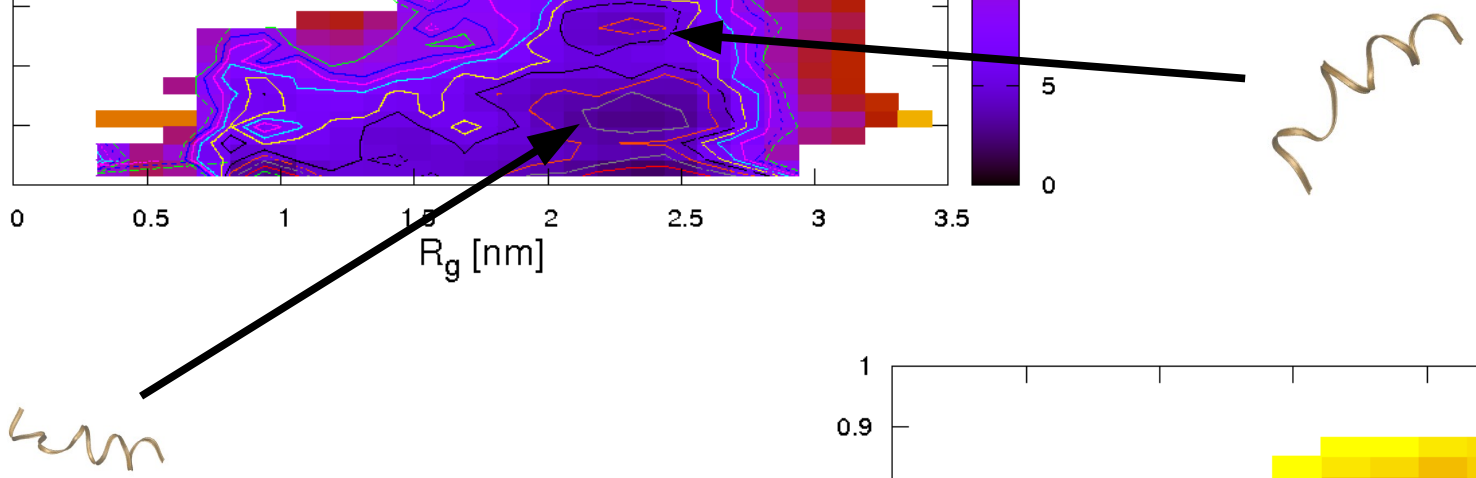
$$\xi = \xi(\mathbf{p}, \mathbf{q})$$



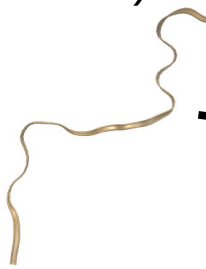
Laio A.; Parrinello M., *Proc. Natl. Acad. Sci.* (2002) **99**, 12562

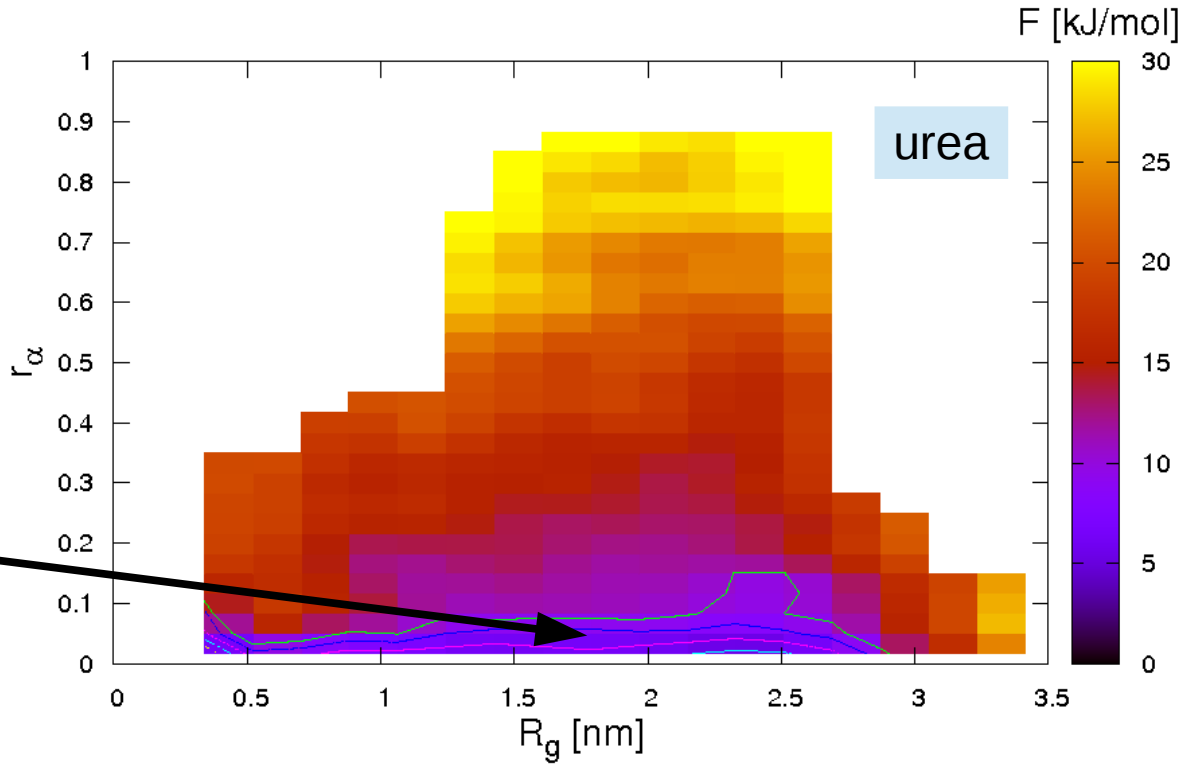


The same effect is observed also on the helix.
Urea is able to destroy both the secondary structures, while some residual helicity can be seen in guanidine.



The denaturing effect of guanidine on the helix is stronger than on the hairpin, as the native state now has a very high energy (~27 kJ/mol)





One has to carefully choose the collective variable(s) to perform metadynamics on

The “good” collective variables should be “slow”:

(time evolution slower than the other degrees of freedom)

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The “good” collective variables should be “slow”:

(time evolution slower than the other degrees of freedom)

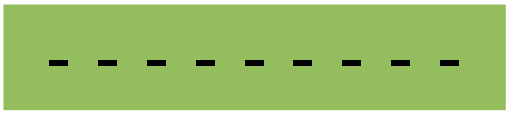
$$\dot{\xi}(t) = h(\xi) + g(\xi)F(t)$$

→ Search for a criterion to test the slowness of collective variables

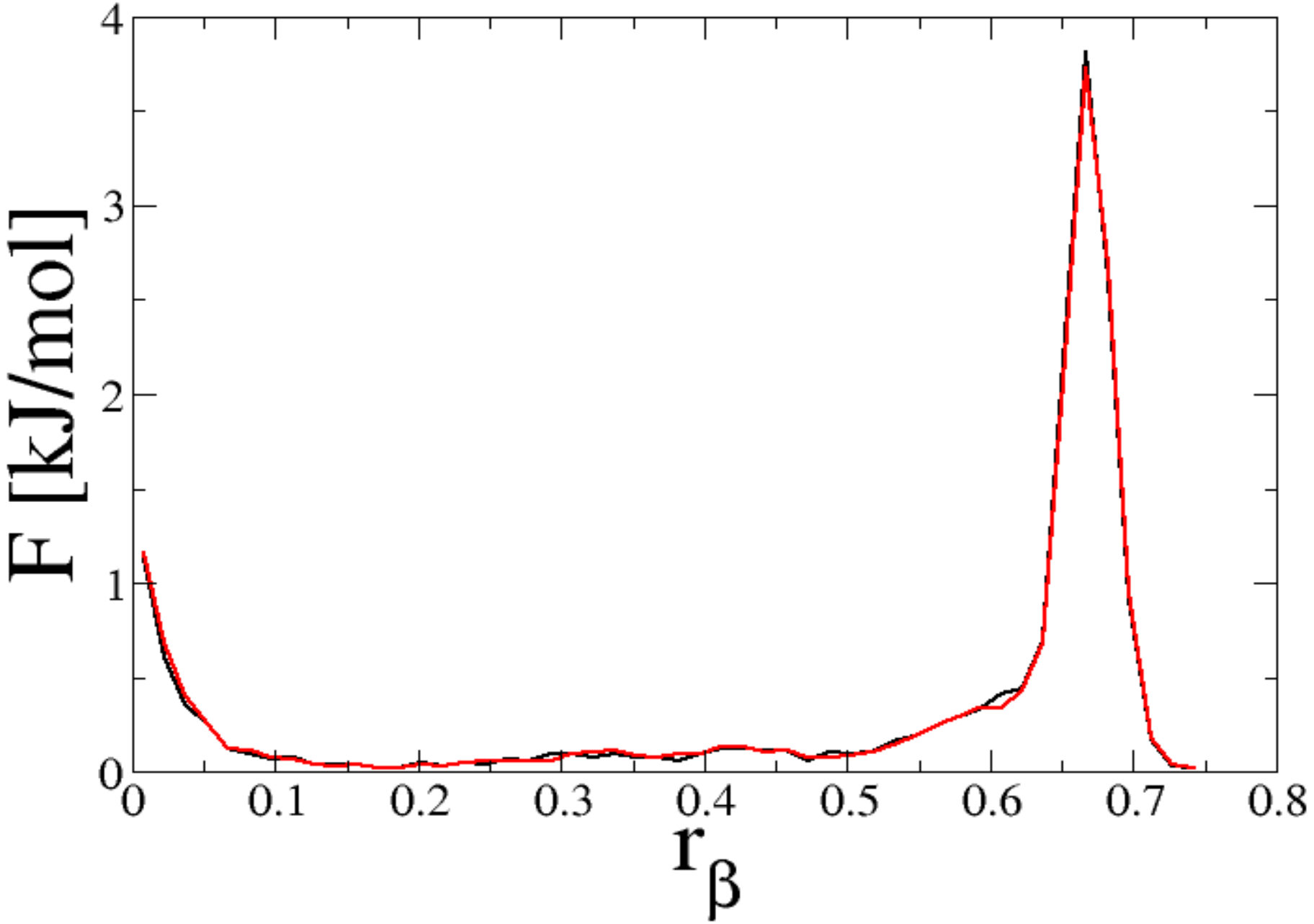
GOAL:

- Test and find good collective variables
- Write a linear combination of them to perform metadynamics
- Optimize their coefficients to better sample the phase space of denatured states

Thanks for your attention



Detail: comparison between 2nd and 3rd third of simulation after 60 ns



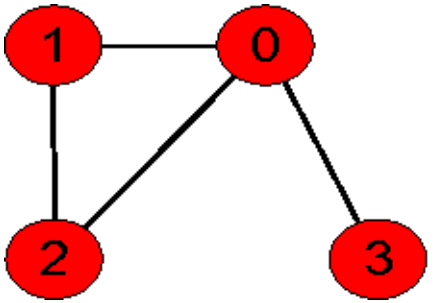
How a standard replica-exchange works

[0 1 2 3 4]

→ [0-1 2-3 4]

→ [0 1-2 3-4]

How our scheme works



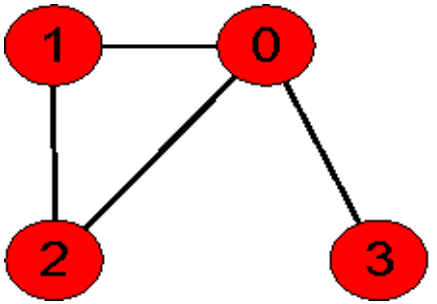
[0 1 2 3]
[0 2 1 3]
[0 3 1 2]

→ [0-1 2-3]
[0-2 1-3]
[0-3 1-2]

→ [0 1-2 3]
[0 2-1 3]
[0 3-1 2]

Generate a neighbour list

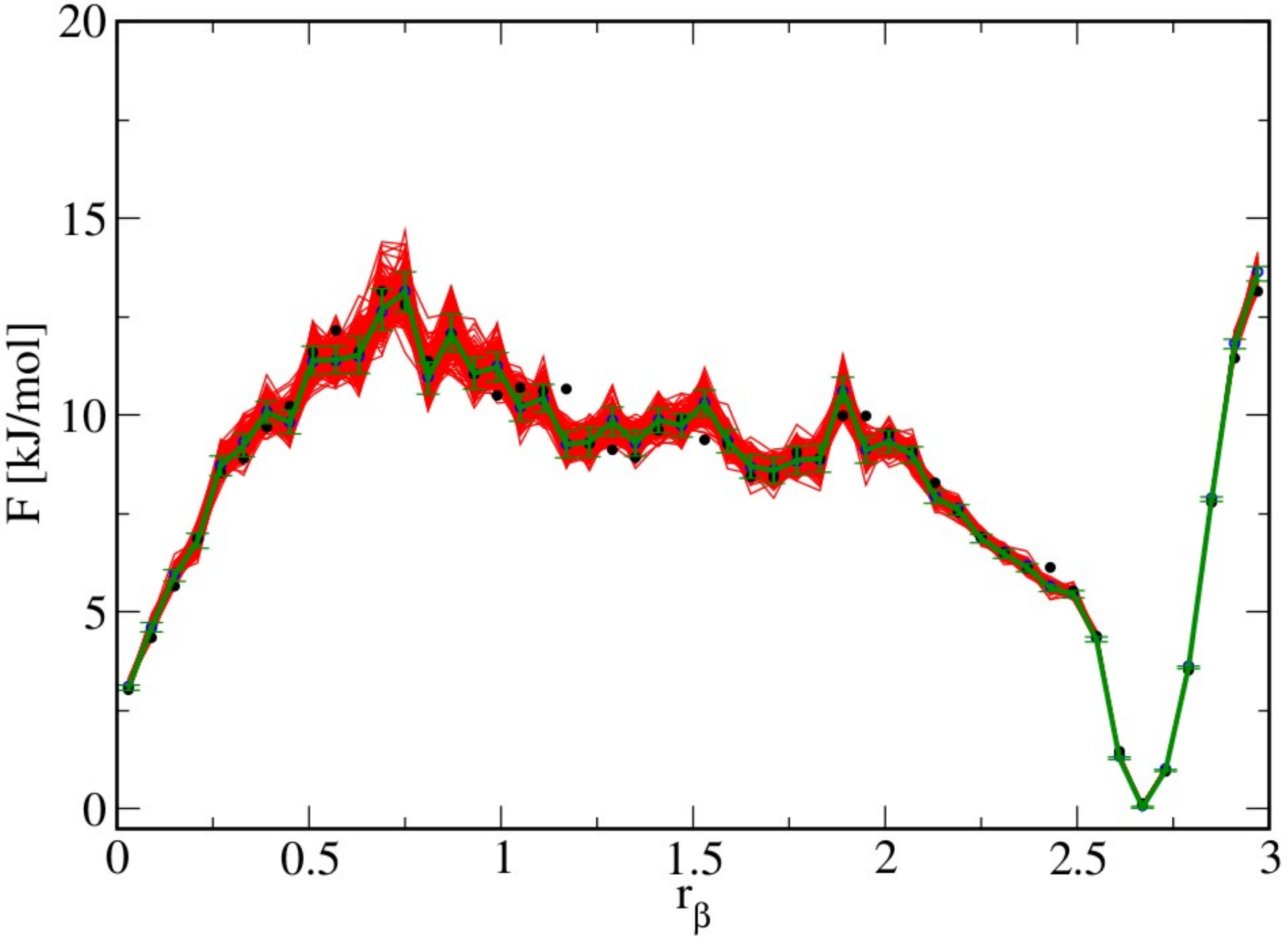
```
#
NREPLICA  4
#
EDGE 0 3
0 1
0 2
0 3
EDGE 1 2
1 0
1 2
EDGE 2 2
2 0
2 1
EDGE 3 1
3 0
```



- First extracted is always 0
- Next is extracted from the neighbour list
- If no replicas are available (because already extracted) the lower between those available is selected

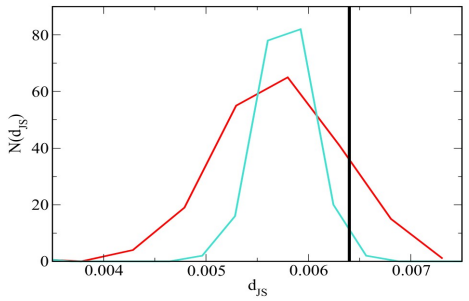
The exchange between two replicas not consecutive in the graph is not wrong

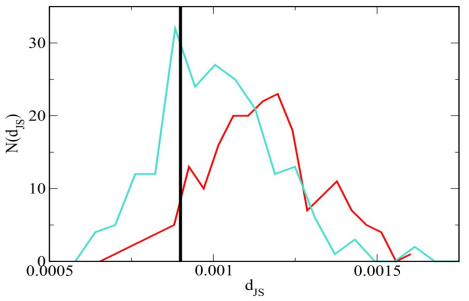
Green line: the original one (e.g. set A).
Red lines: all the bootstraps of A.
Black dots: the set B.

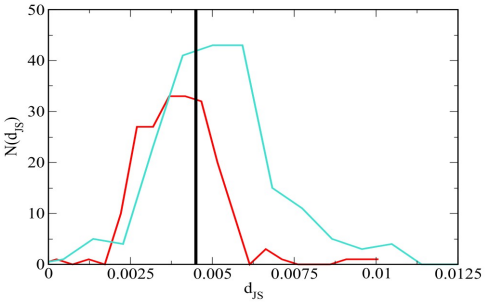


All the JDSs

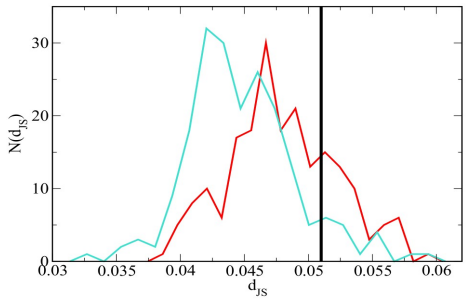
r_α r_β R_g

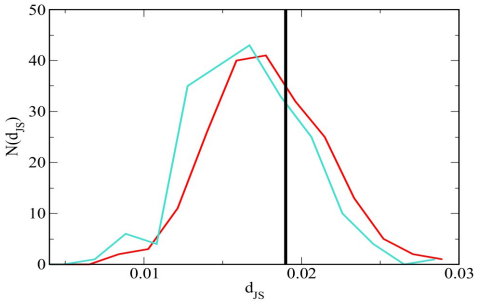


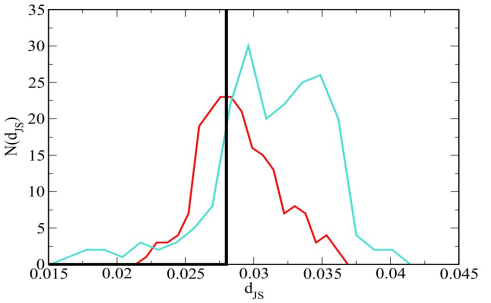




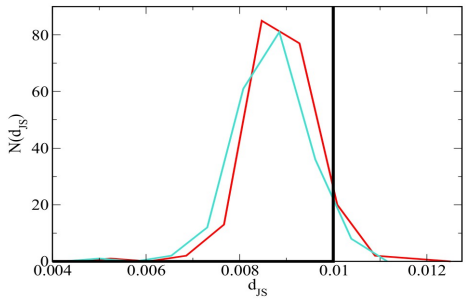
water

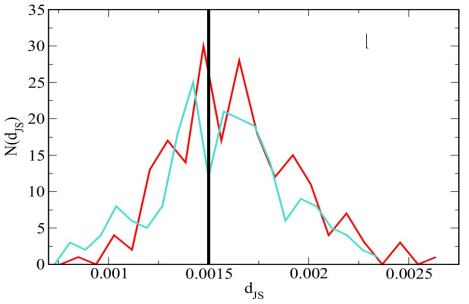


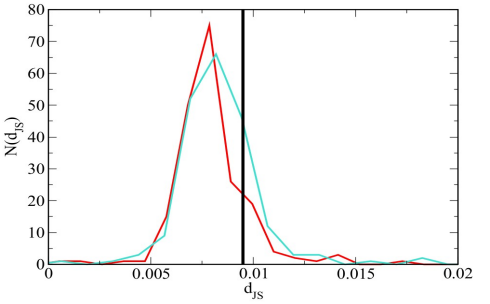




urea







guanidine

Both r_α and r_β have the same functional form:
r_α counts the 6-plets of N-CA-C-CO-CB in α conformation
r_β counts the couples of 3-plets of N-CA-C-CO-CB in β conformation

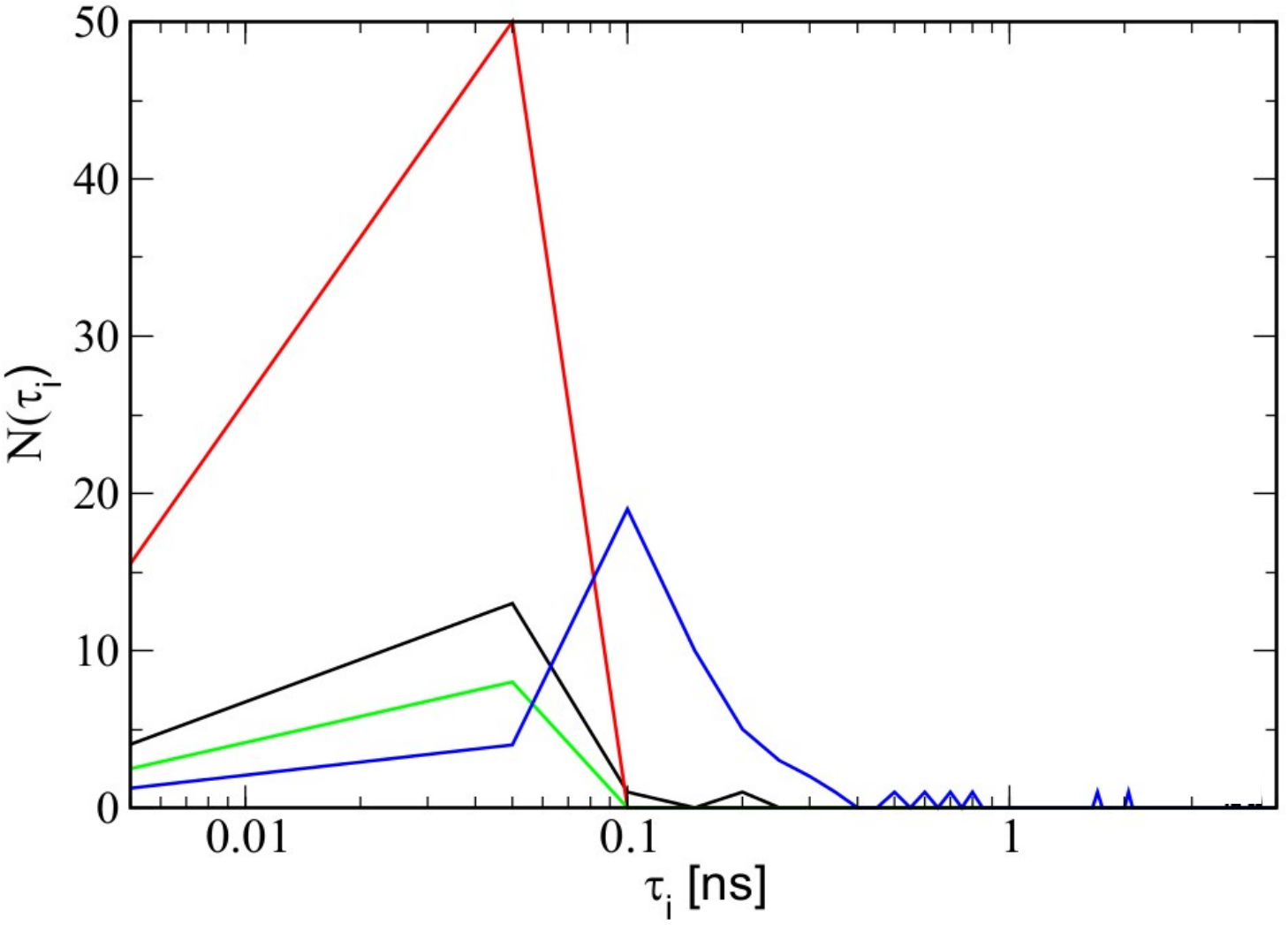
In this work, they have been normalized.

$$s = \sum_i \frac{1 - \left(\frac{r_i - d_0}{r_0}\right)^n}{1 - \left(\frac{r_i - d_0}{r_0}\right)^m}$$

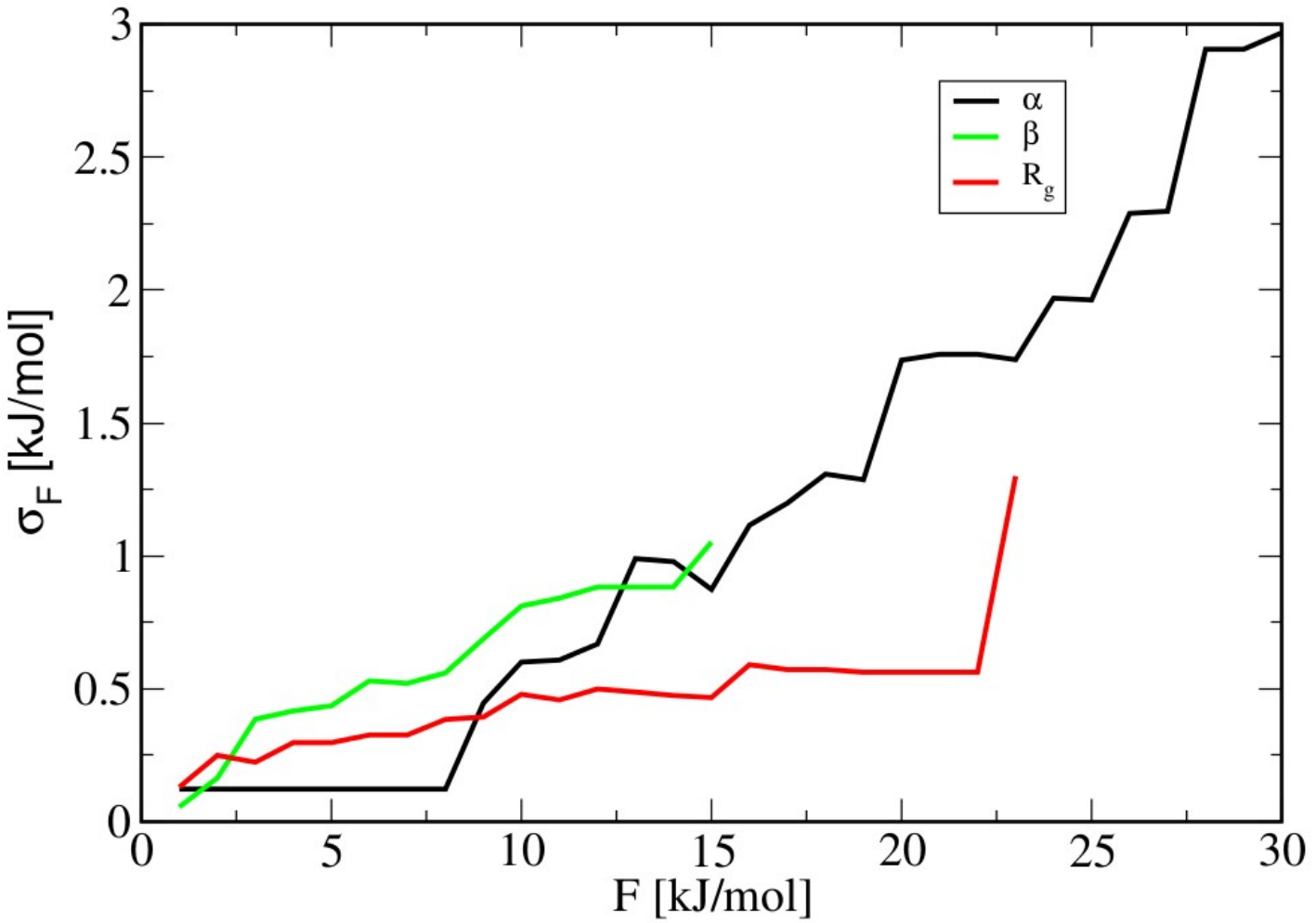
$$n = 8;\ m = 12$$

Pietrucci F.; Laio A., *J. Chem. Theory Comput. (*2009) **5**, 2197

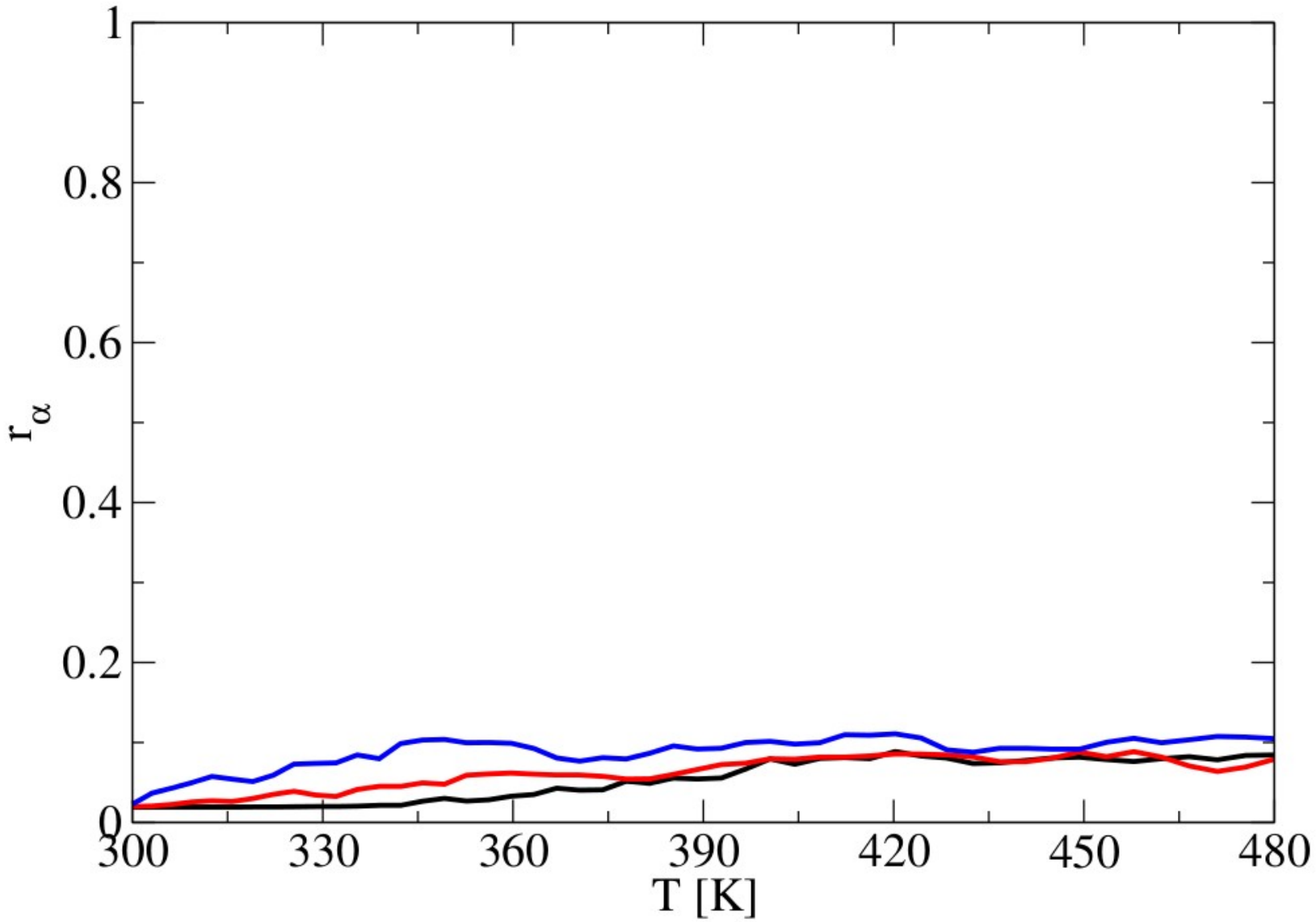
Histogram of autocorrelations of each replica



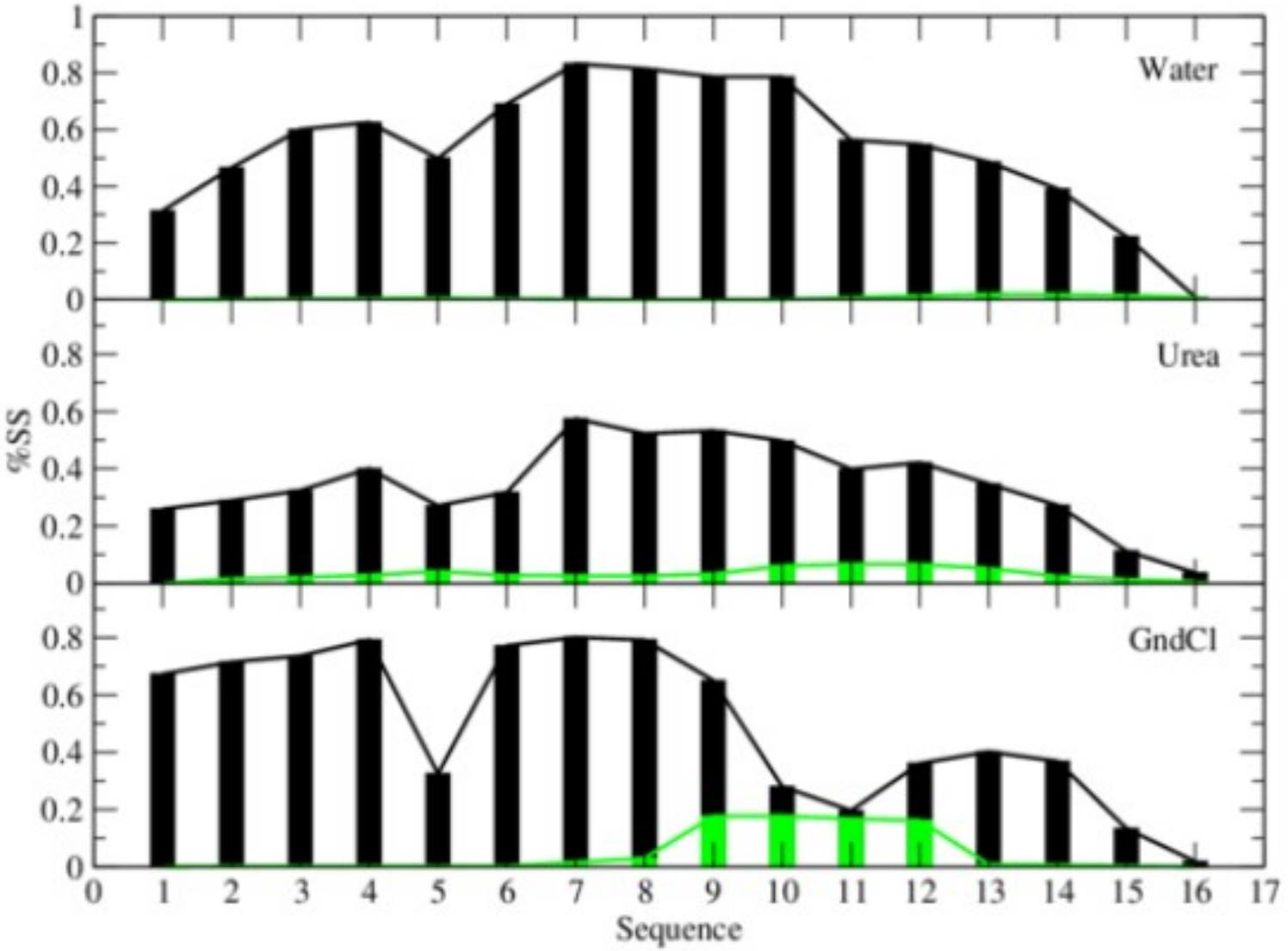
RMSD between 2nd and 3rd third free energy (water) vs Free energy values.
The lower the free energy, the lower the RMSD , that means that , at least in the
re



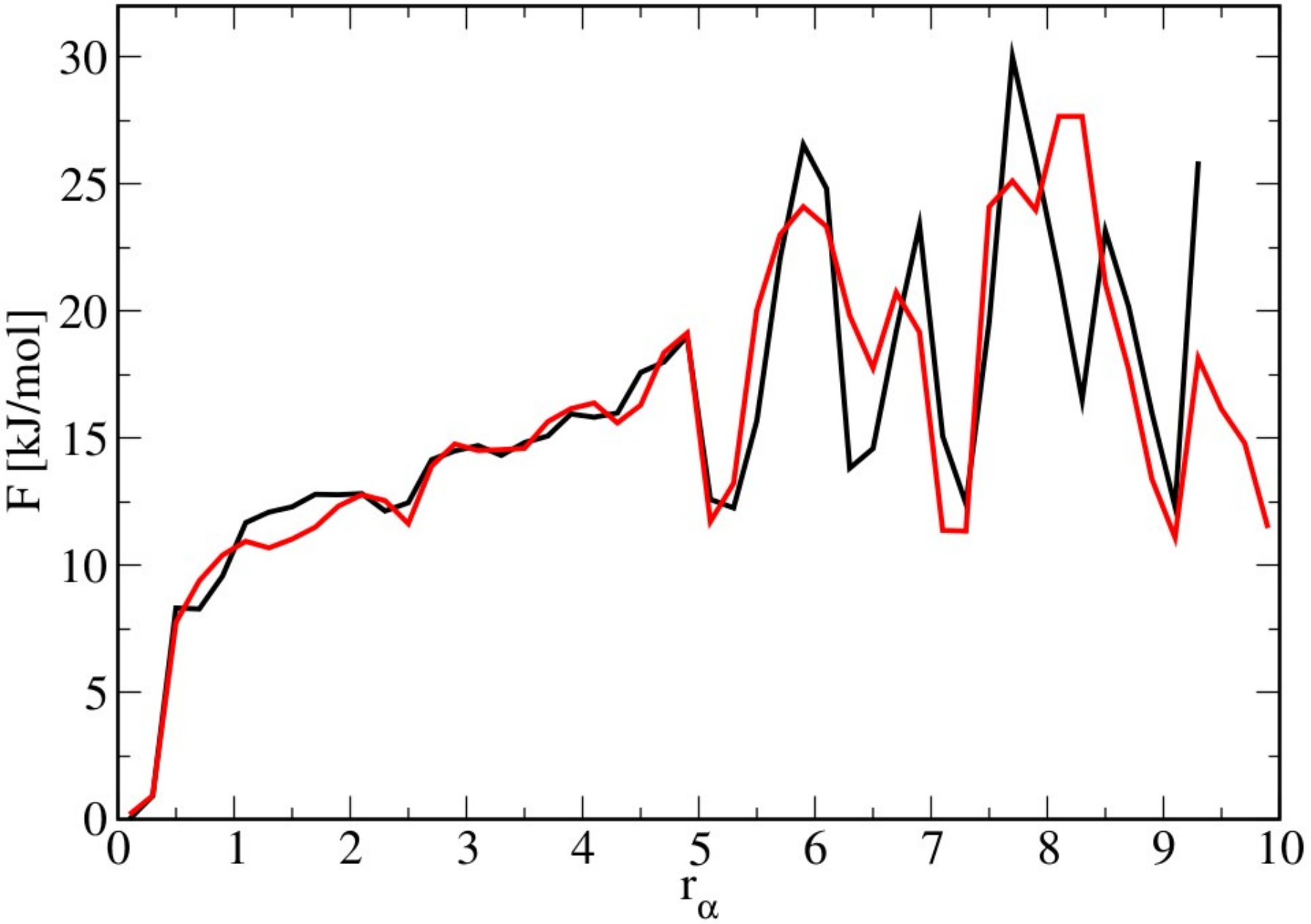
Alpha vs temp

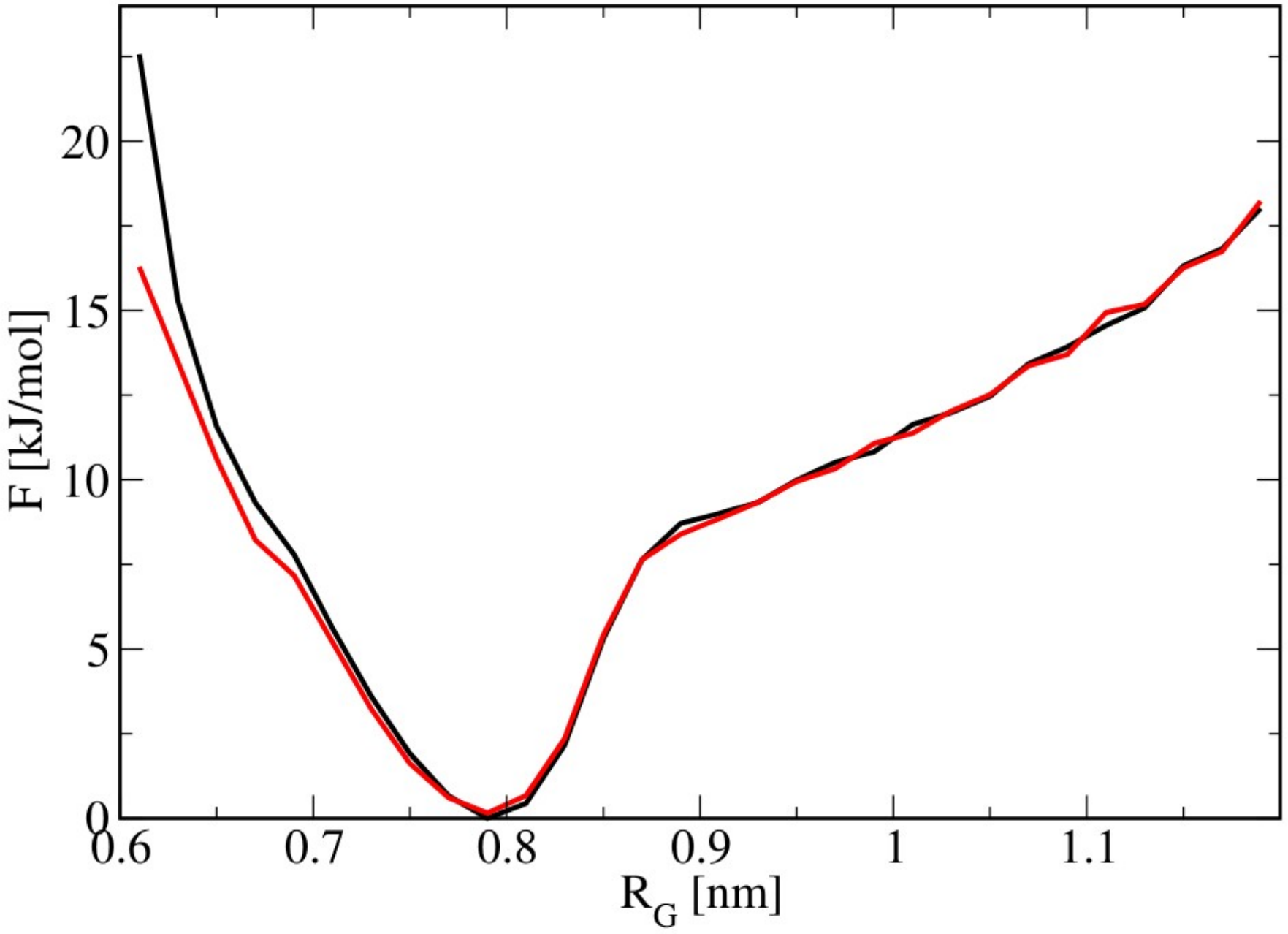


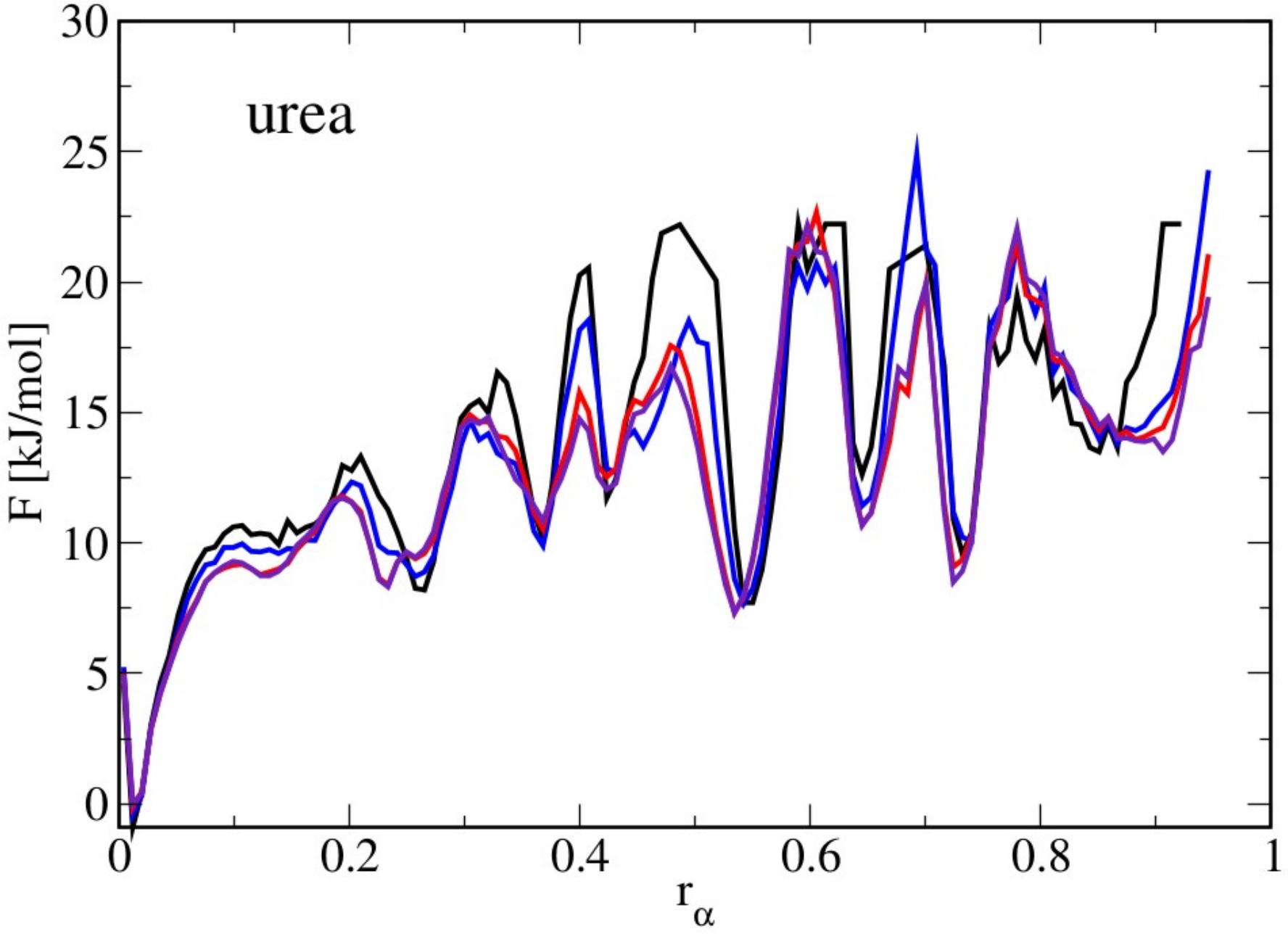
Secondary structure (STRIDE. In green: polyproline)

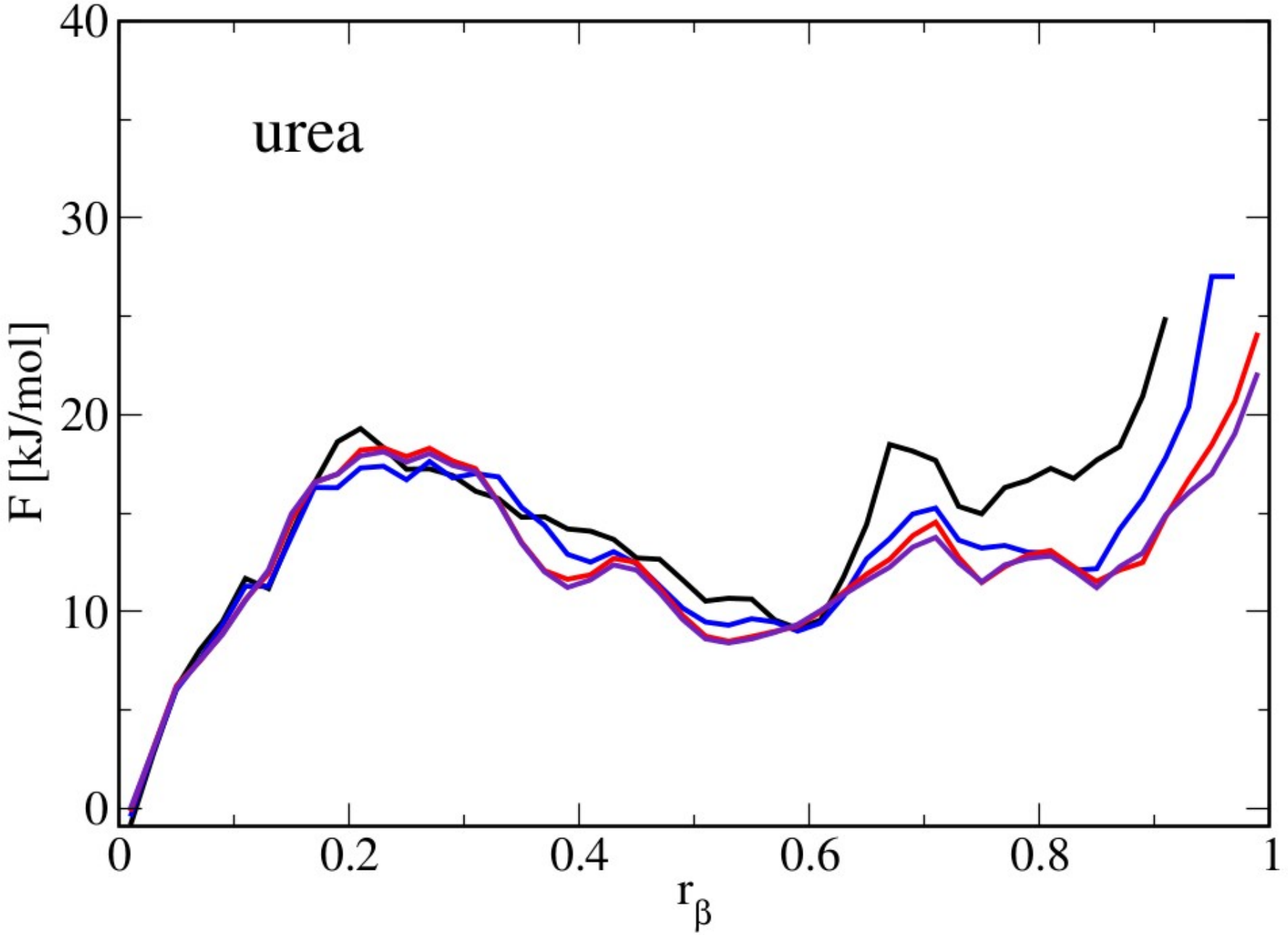


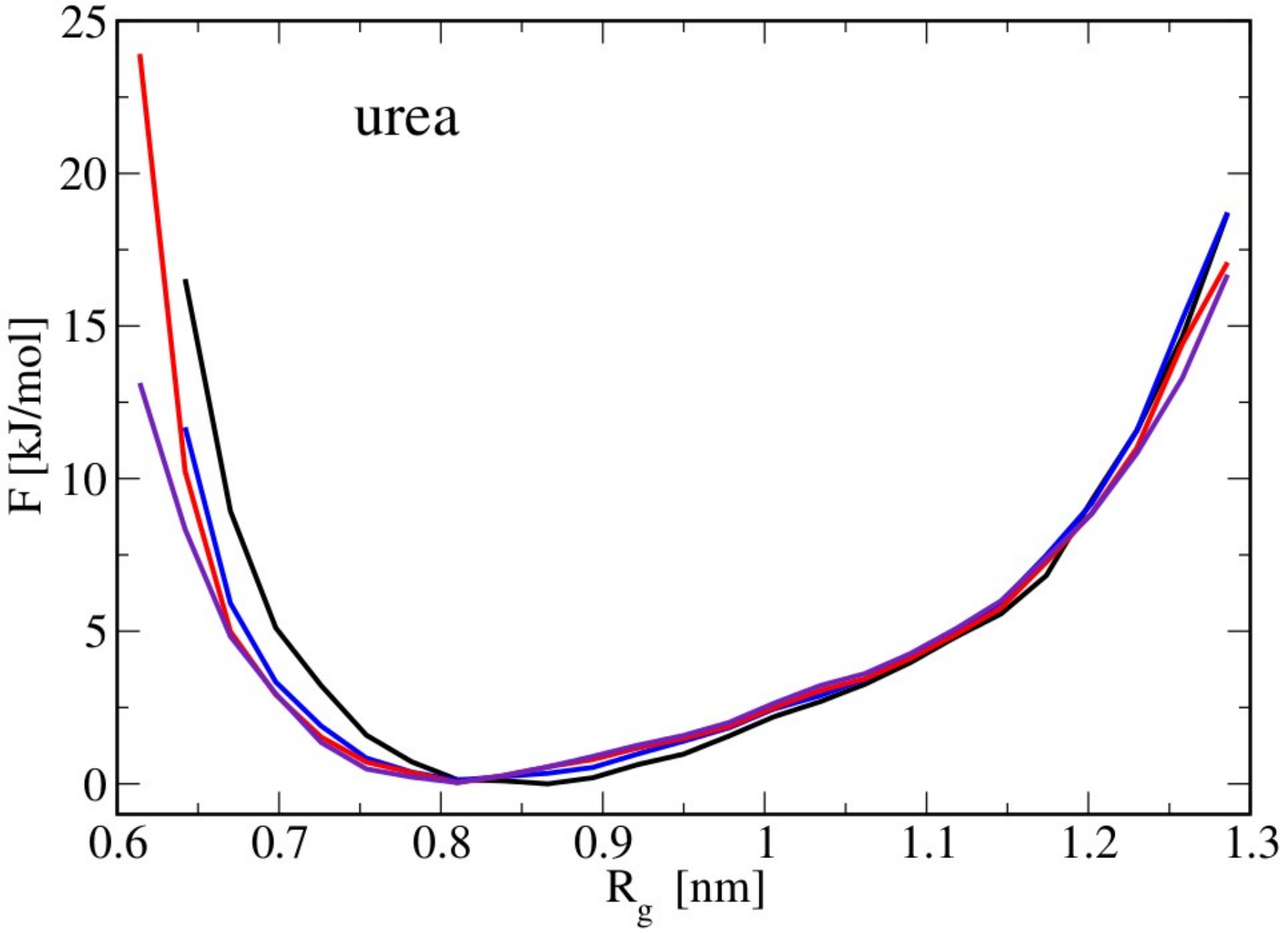
Some other free energy landscapes..

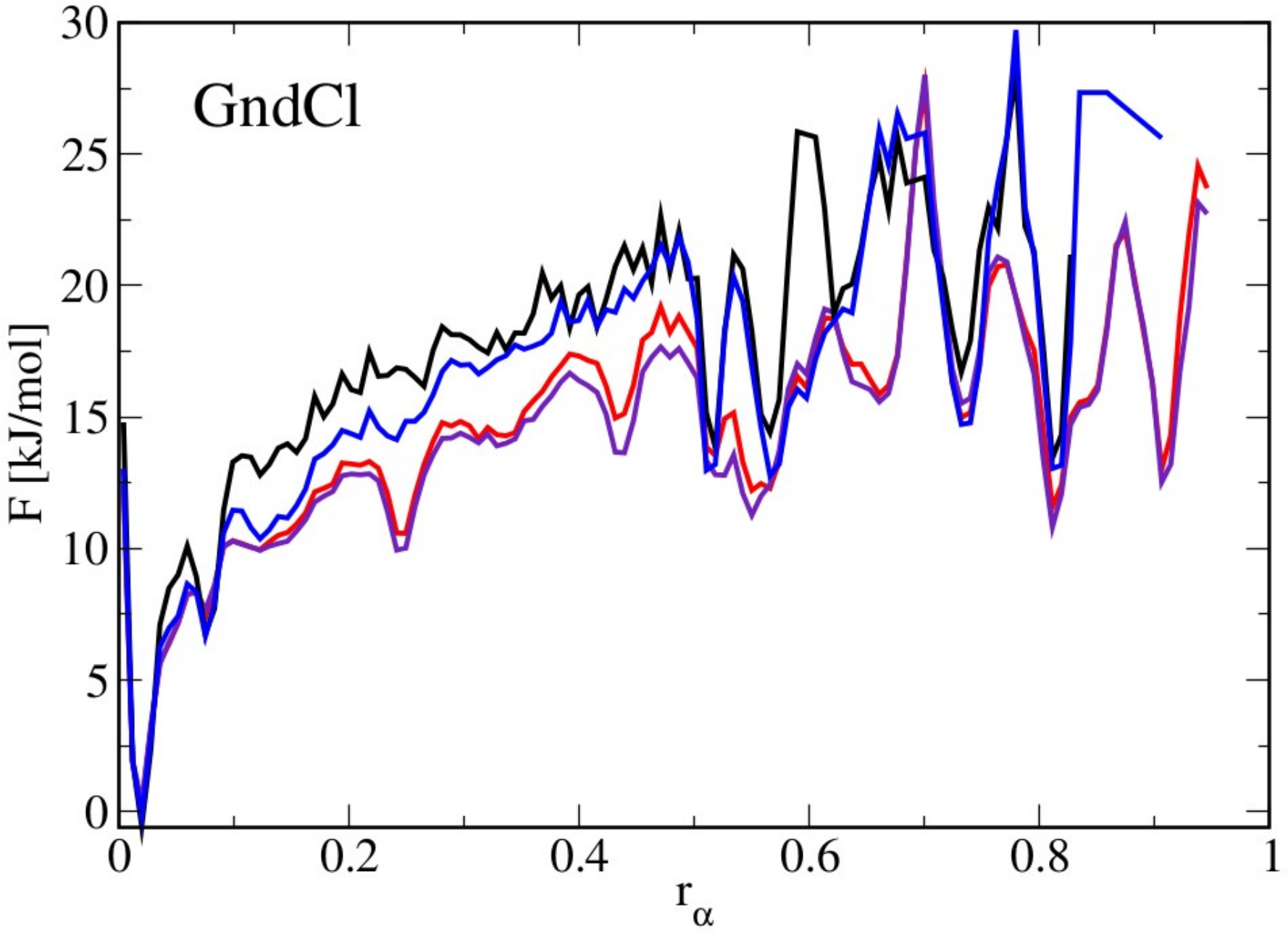


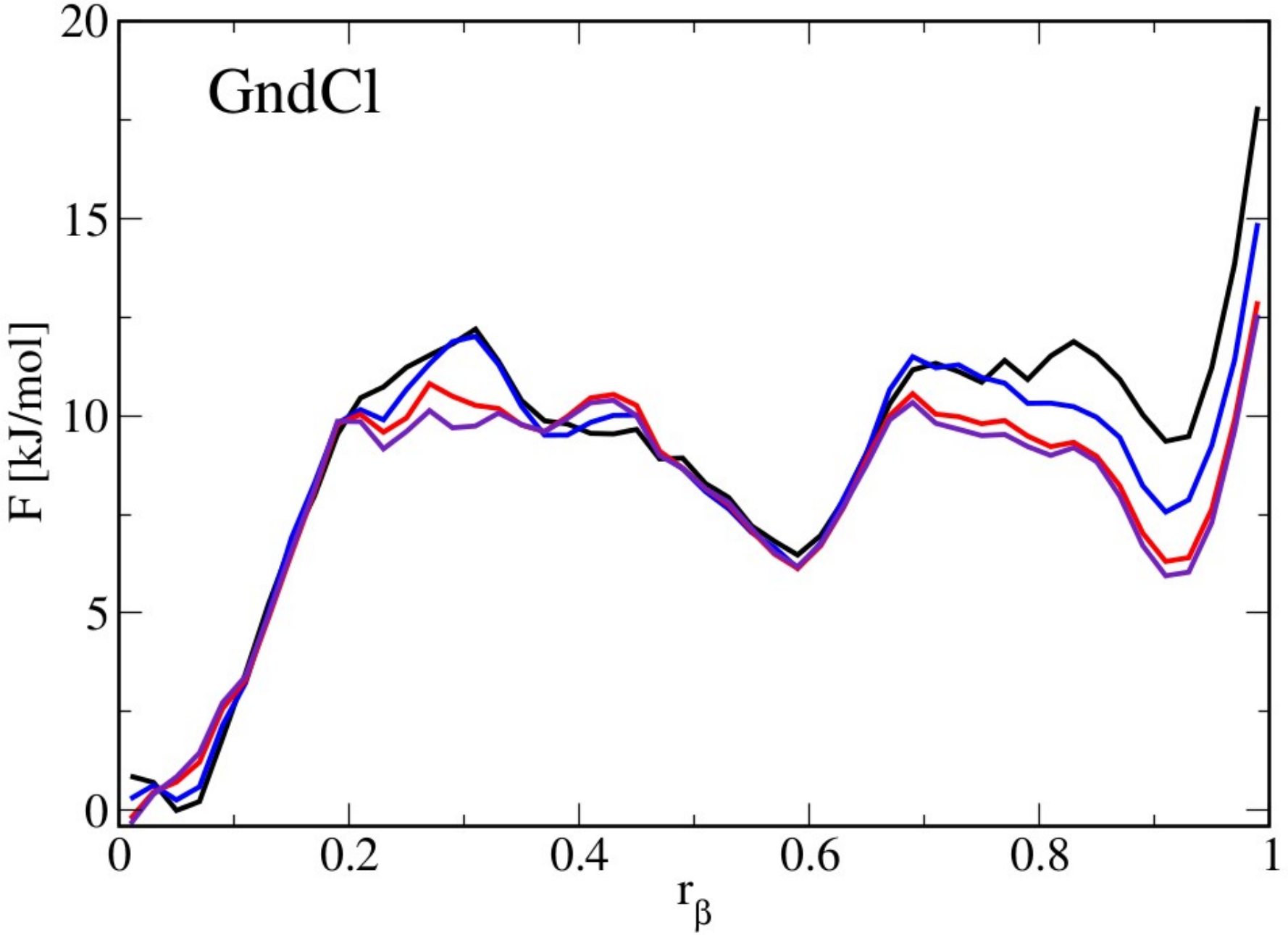

F [kJ/mol]
0
5
10
15
20
0.6
0.7
0.8
0.9
1
1.1
R_G [nm]

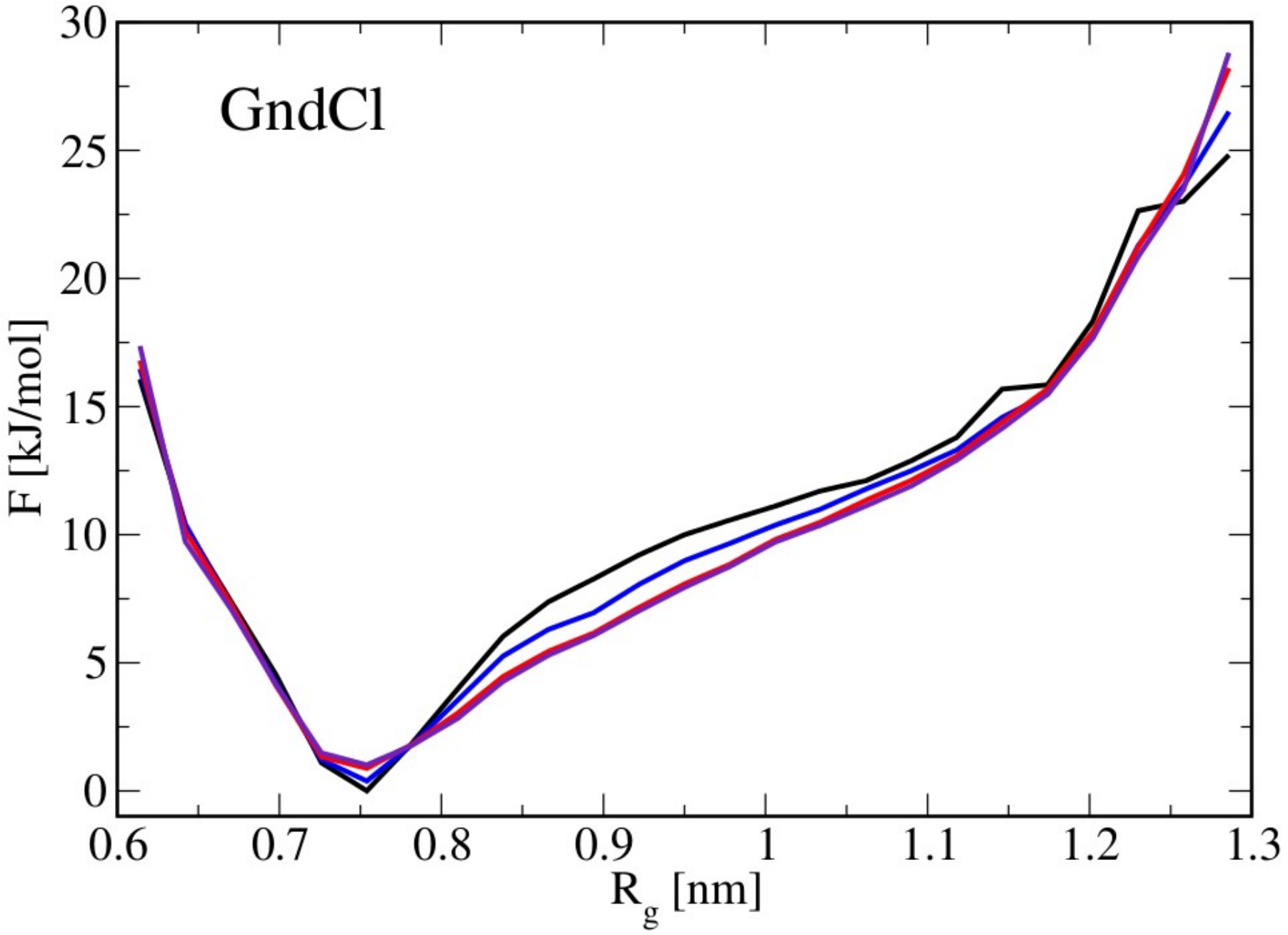

urea
F [kJ/mol]
r_α
0
5
10
15
20
25
30
0
0.2
0.4
0.6
0.8
1


urea
F [kJ/mol]
r_β
0
10
20
30
40
0
0.2
0.4
0.6
0.8
1


urea
F [kJ/mol]
25
20
15
10
5
0
0.6
0.7
0.8
0.9
1
1.1
1.2
1.3
R_g [nm]


GndCl
F [kJ/mol]
r$_\alpha$
0
5
10
15
20
25
30
0
0.2
0.4
0.6
0.8
1


GndCl
F [kJ/mol]
r_β
0
5
10
15
20
0
0.2
0.4
0.6
0.8
1


GndCl
F [kJ/mol]
R_g [nm]
0
5
10
15
20
25
30
0.6
0.7
0.8
0.9
1
1.1
1.2
1.3