PRACE Scientific Conference 2013

Leipzig, Sunday, Jun 16th

A New DNA Structural Motif: the G-triplex

Vittorio Limongelli

Department of Pharmacy

University of Naples "Federico II" (ITA)

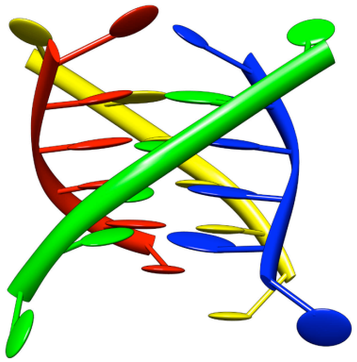
vittoriolimongelli@gmail.com

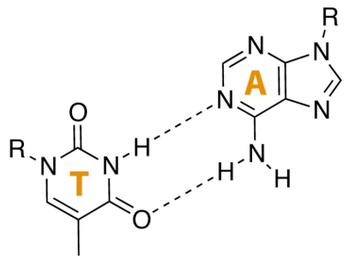
Chemical Background

Duplex

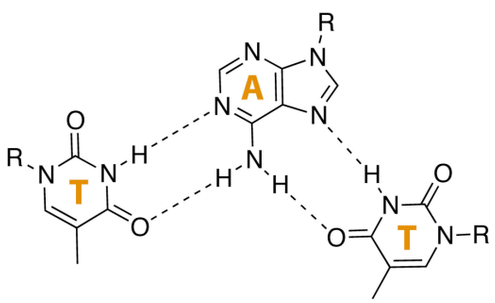
Triplex

G-Quadruplex

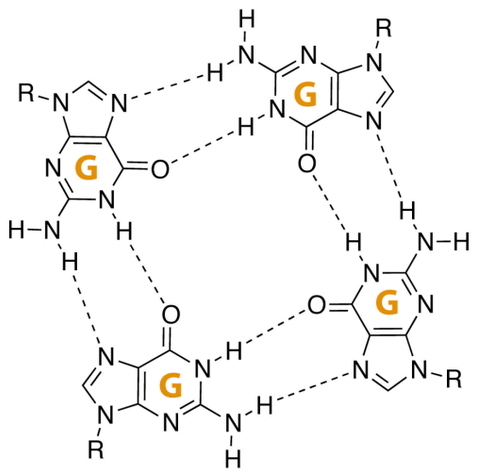




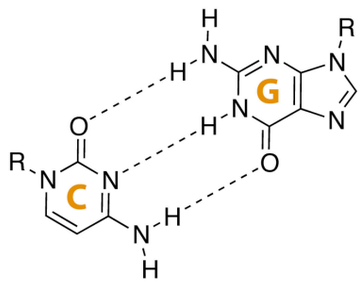
T-A base pair



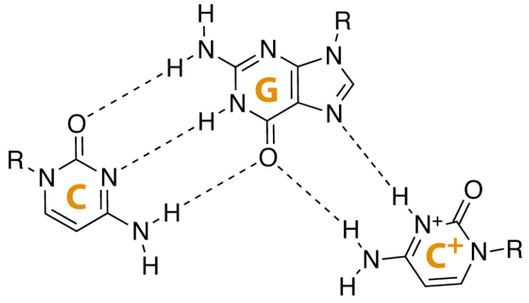
T-A:T base pair



G-tetrad

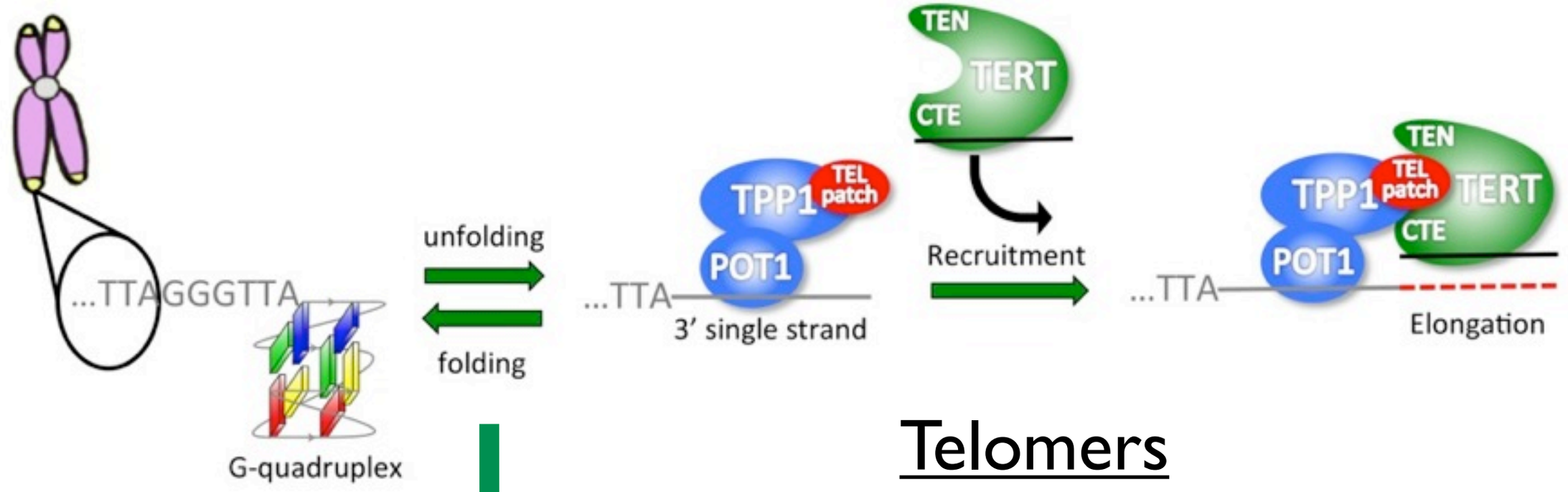


C-G base pair



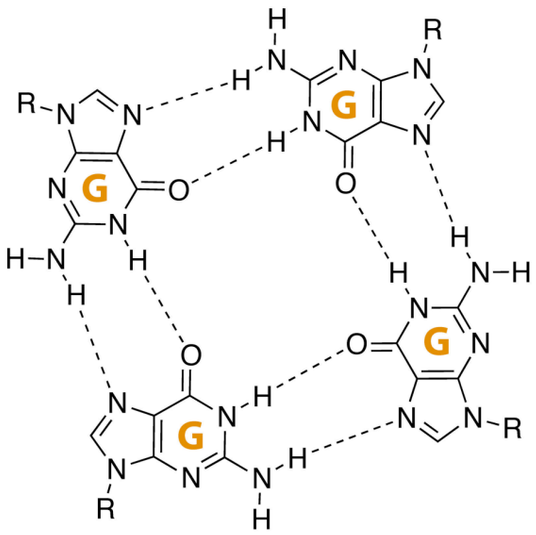
C-G:C⁺ base pair

Biological Background

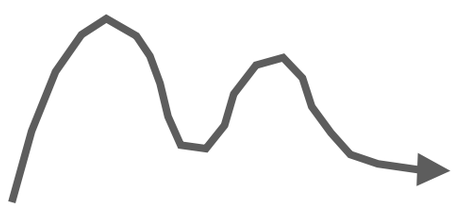


Telomers

Neidle et al., *Curr. Opin. Struct. Biol.* (2003)
Kim et al., *Science* (2004)
Wang et al., *Nature* (2007)
Biffi et al., *Nat. Chem.* (2013)



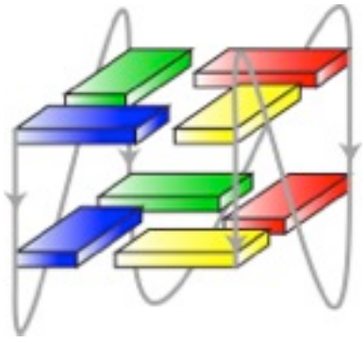
G-tetrad



DNA single strand

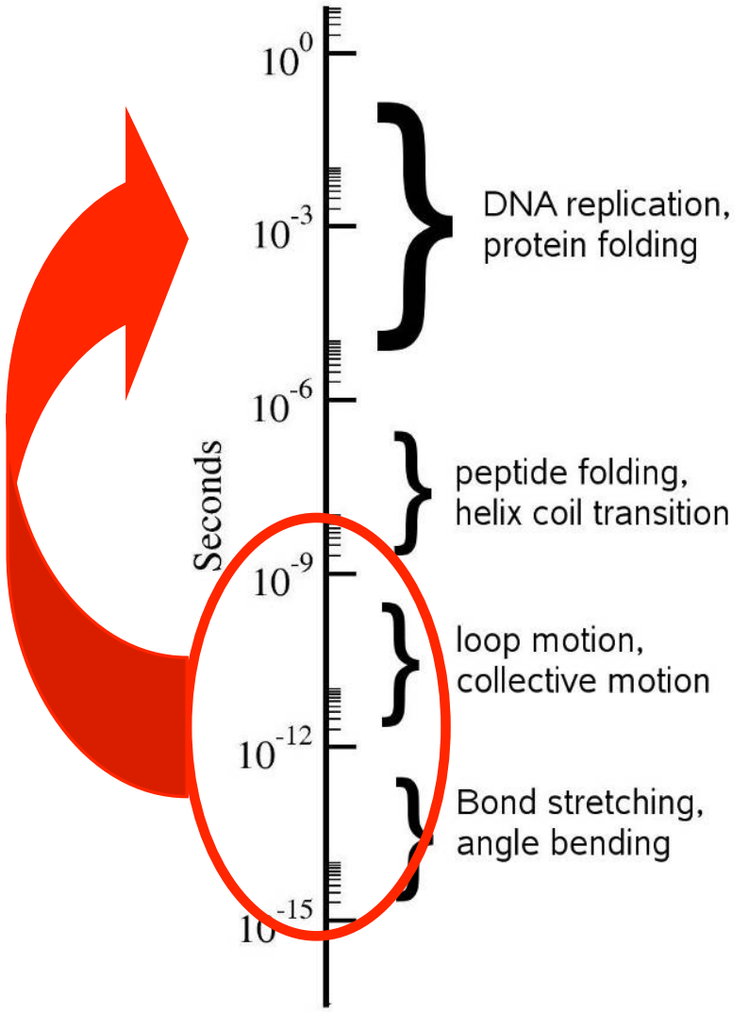
???

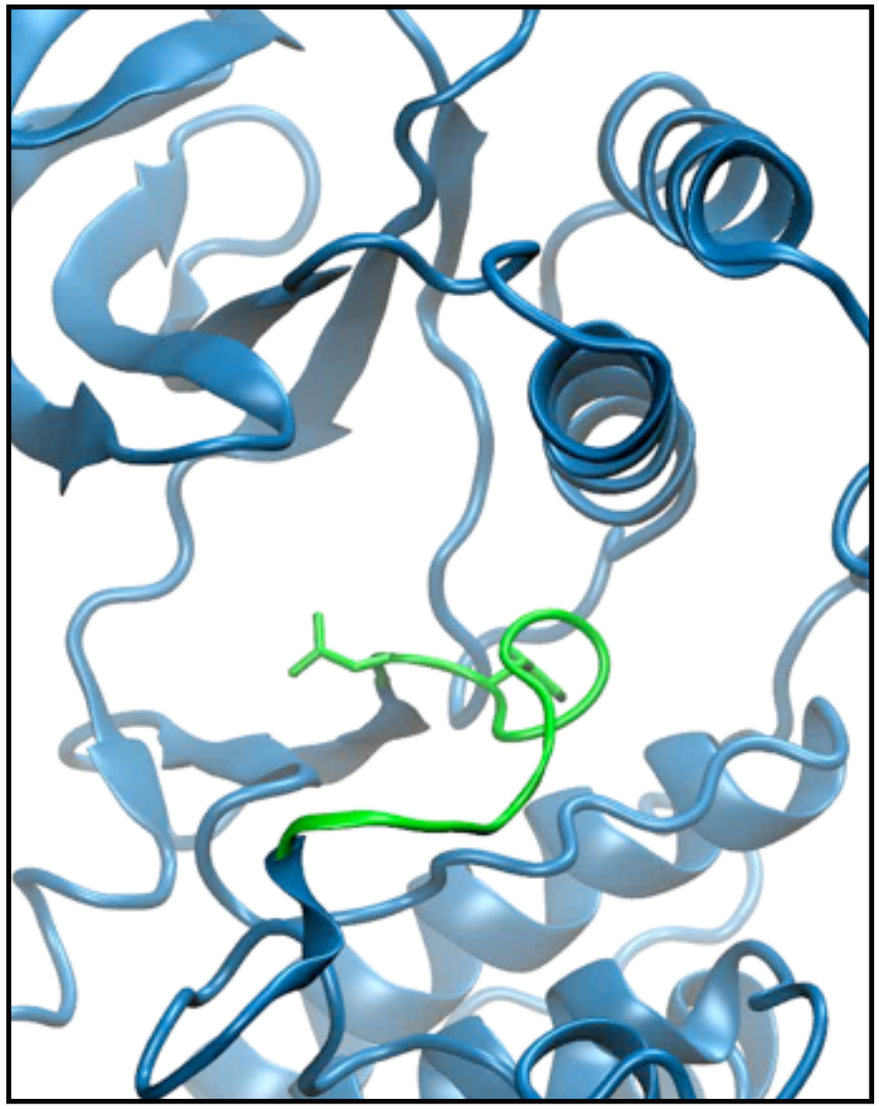
Intermediate States



G-quadruplex

Time Scale Problems



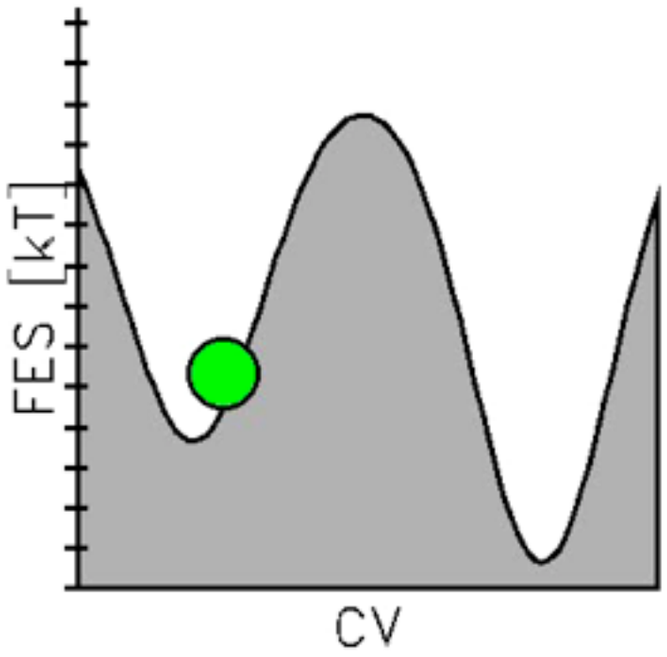


DFG-IN/OUT transition in a MAP Kinase

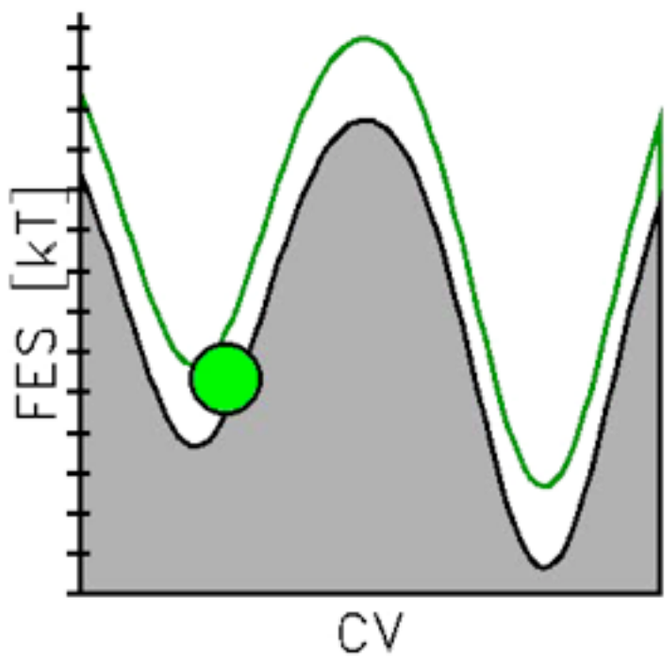
movie by Anna Berteotti

- Molecular Dynamics timestep ~ 1 fs (bond-stretching, bending,...)
- Most biologically relevant processes (docking/undocking process, protein/DNA folding, phase transitions,...) >~ 1μs

MD vs. Metadynamics



movies by G. Bussi



- *V(s;t)* disfavours the visited states (in the CVs space)
- *V(s;t)* grows logarithmically with histogram *N(s;t)*
- The error is progressively damped
- V(s) converges to -ΔT/(T+ΔT)F(s)

Metadynamics Potential

$$\dot{V}(s;t) = \omega e^{-\frac{V(s;t)}{\Delta T}} \delta_{s,s(t)}$$

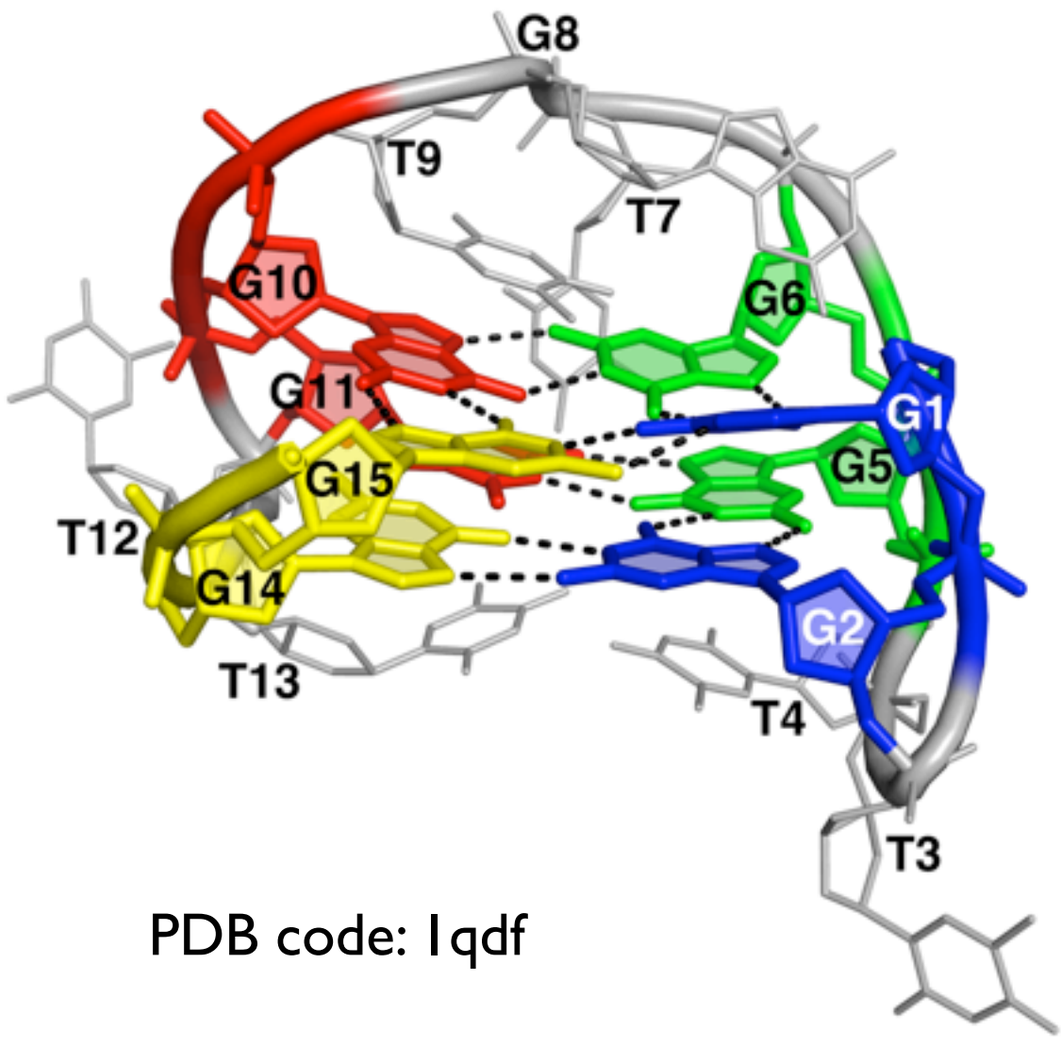
initial rate

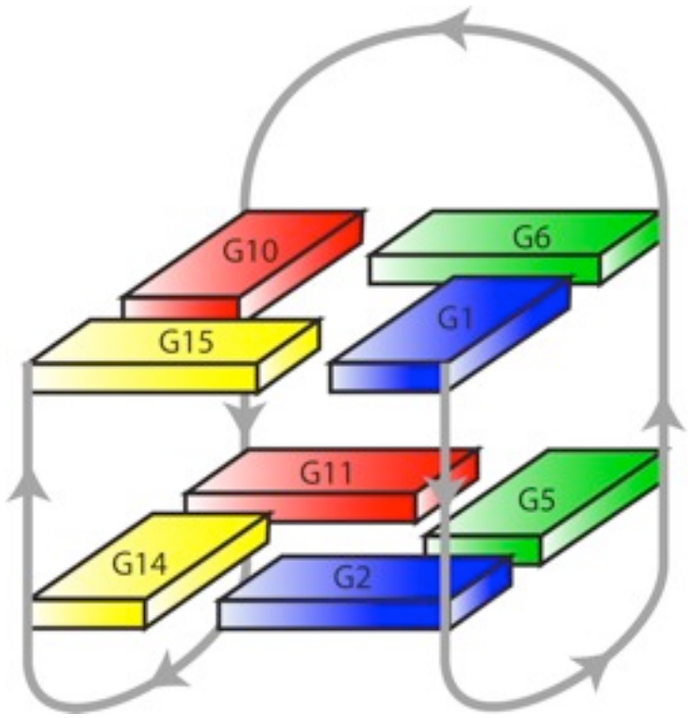
rate decreases as exp(-V/ΔT)

Laio and Parrinello, *PNAS* (2002)
Barducci, Bussi, Parrinello, *PRL* (2008)
http://www.multimedia.ethz.ch/speakers/cscs/cscsonscscs/?doi=10.3930/ETHZ/AV-f9761e89-c38e-484b-9378-163dfe9a0efb&autostart=false

Thrombin Binding Aptamer (TBA)

TBA is a simple example of DNA G-quadruplex (15-mer)





PDB code: 1qdf

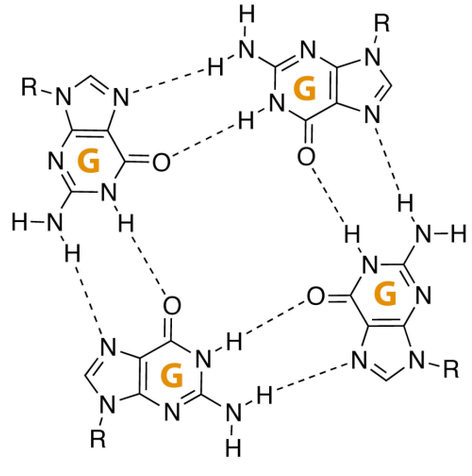
5'-GGTTGGTGTGGTTGG-3'

Methods

CVs Setting:

1.Radius of Gyration (Guanines O6) $$R_{core} = \left(\frac{\sum_i^n |r_i - r_{com}|^2}{\sum_i^n m_i} \right)^{1/2}$$

2.Number of H-bonds between Guanines $$H_{core} = \sum_{ij} \frac{1 - (\frac{d_{ij}}{r_0})^n}{1 - (\frac{d_{ij}}{r_0})^m}$$



G-tetrad

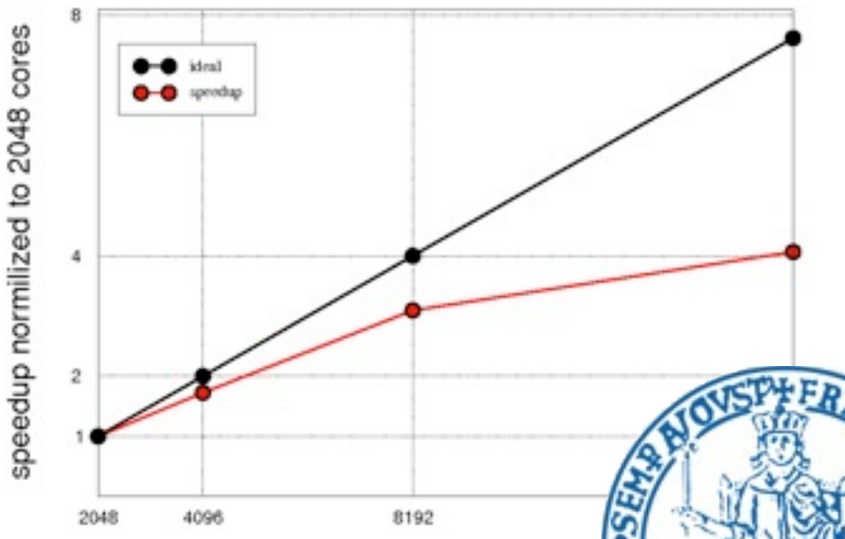
Metadynamics and Parallel Tempering-MetaD Simulations

PT-MetaD Setting:

✓GROMACS4.5.3 + PLUMED + parmbsc0 FF*

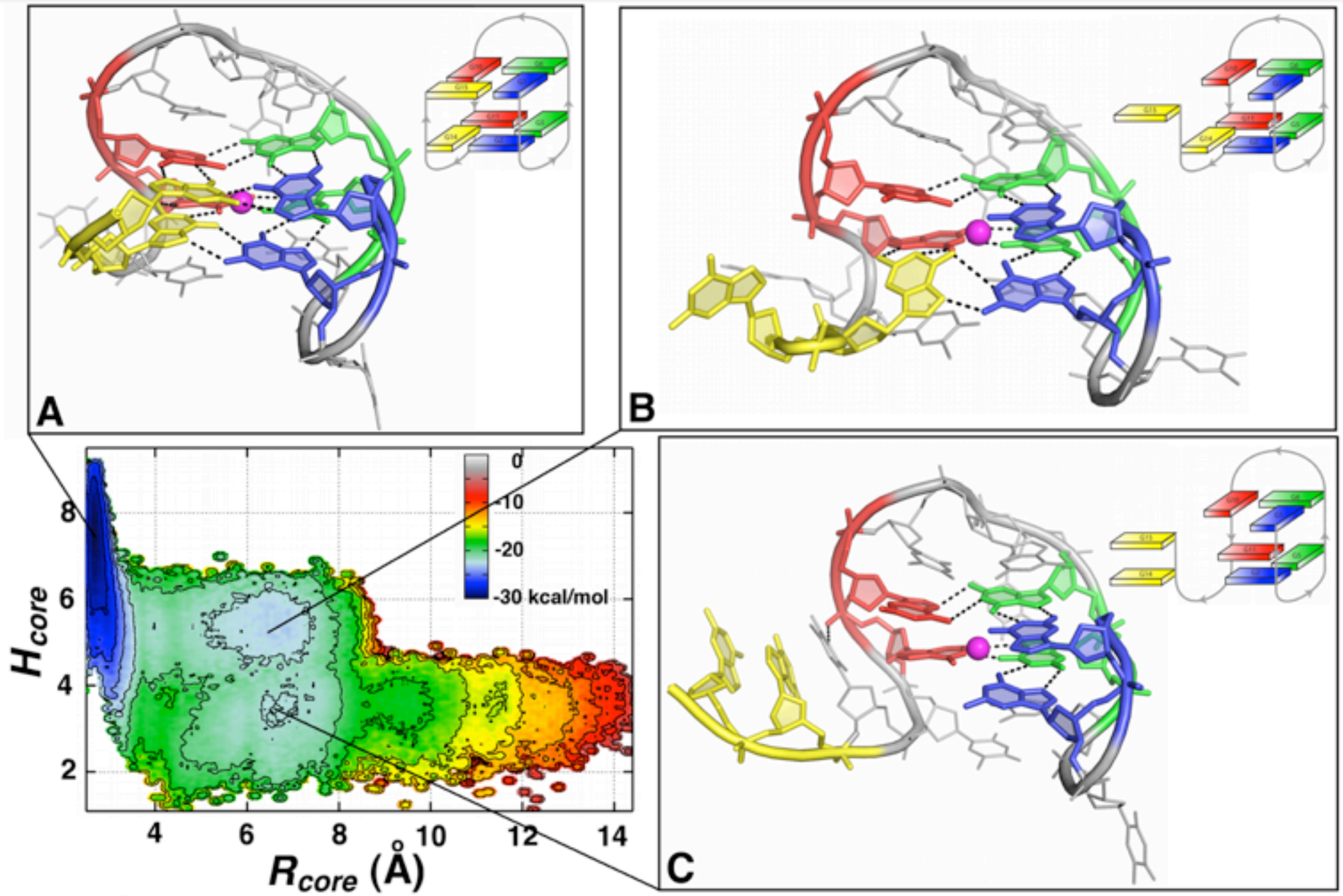
✓256 replica = 4096 cores (T range 280-600 K)

✓100 ns per replica (~10000 waters)



* Perez et al., *Biophys. J.* (2007)

3’ end folding/unfolding



Angewandte Chemie

DNA Structures

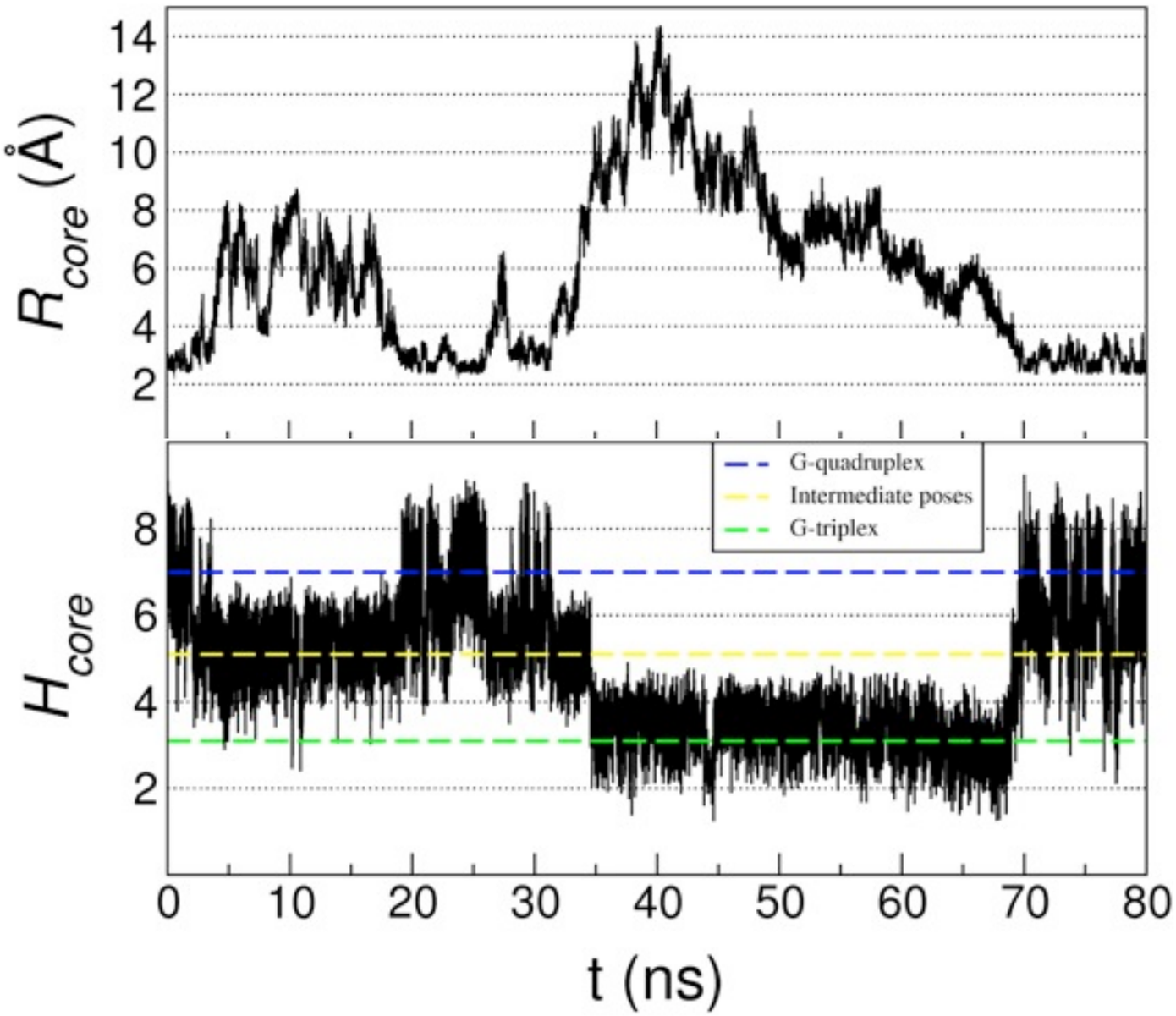
DOI: 10.1002/ange.201206522

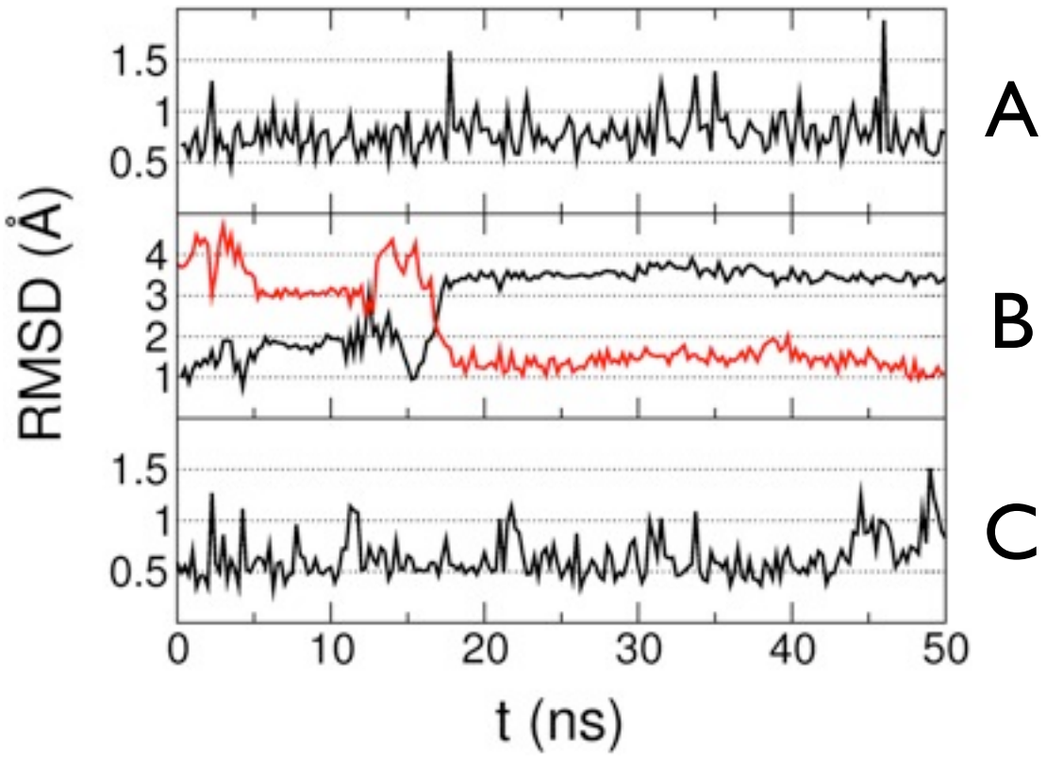
The G-Triplex DNA**

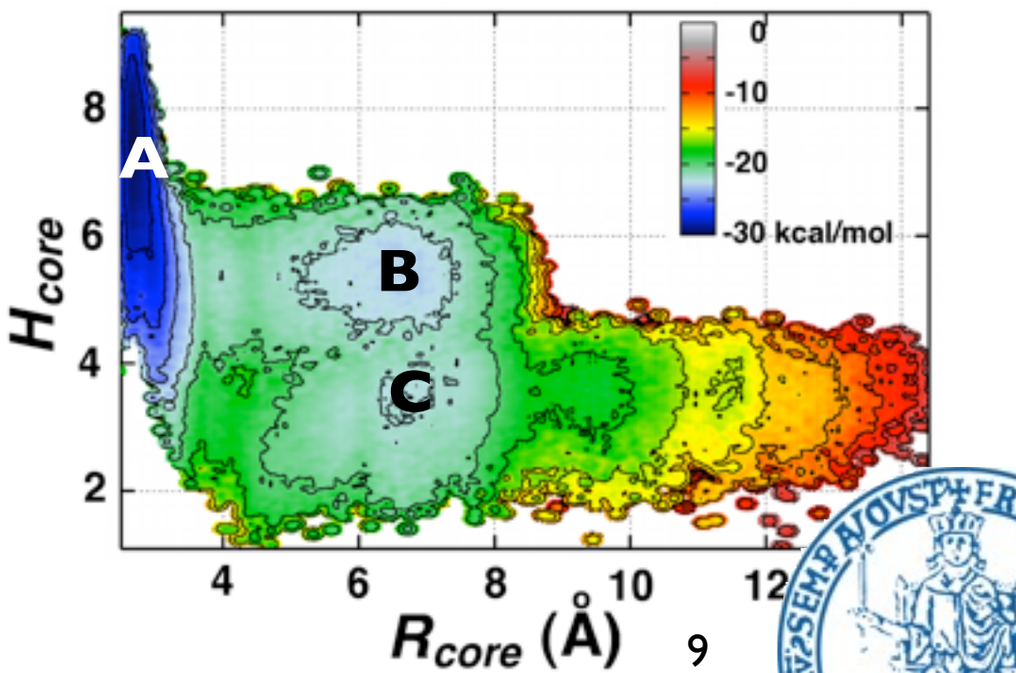
Vittorio Limongelli, Stefano De Tito, Linda Cerofolini, Marco Fragai, Bruno Pagano, Roberta Trotta, Sandro Cosconati, Luciana Marinelli, Ettore Novellino, Ivano Bertini, Antonio Randazzo, Claudio Luchinat,* and Michele Parrinello**

In memory of Ivano Bertini

3’ end folding/unfolding

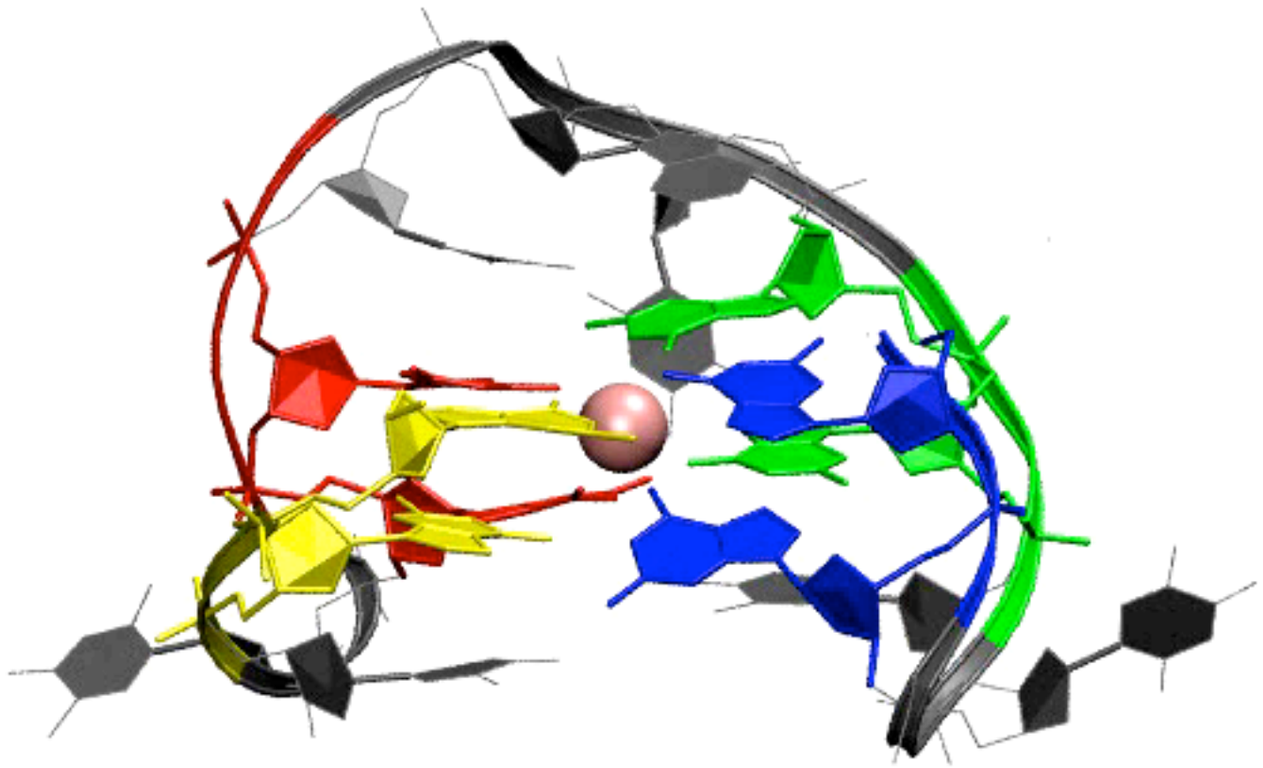






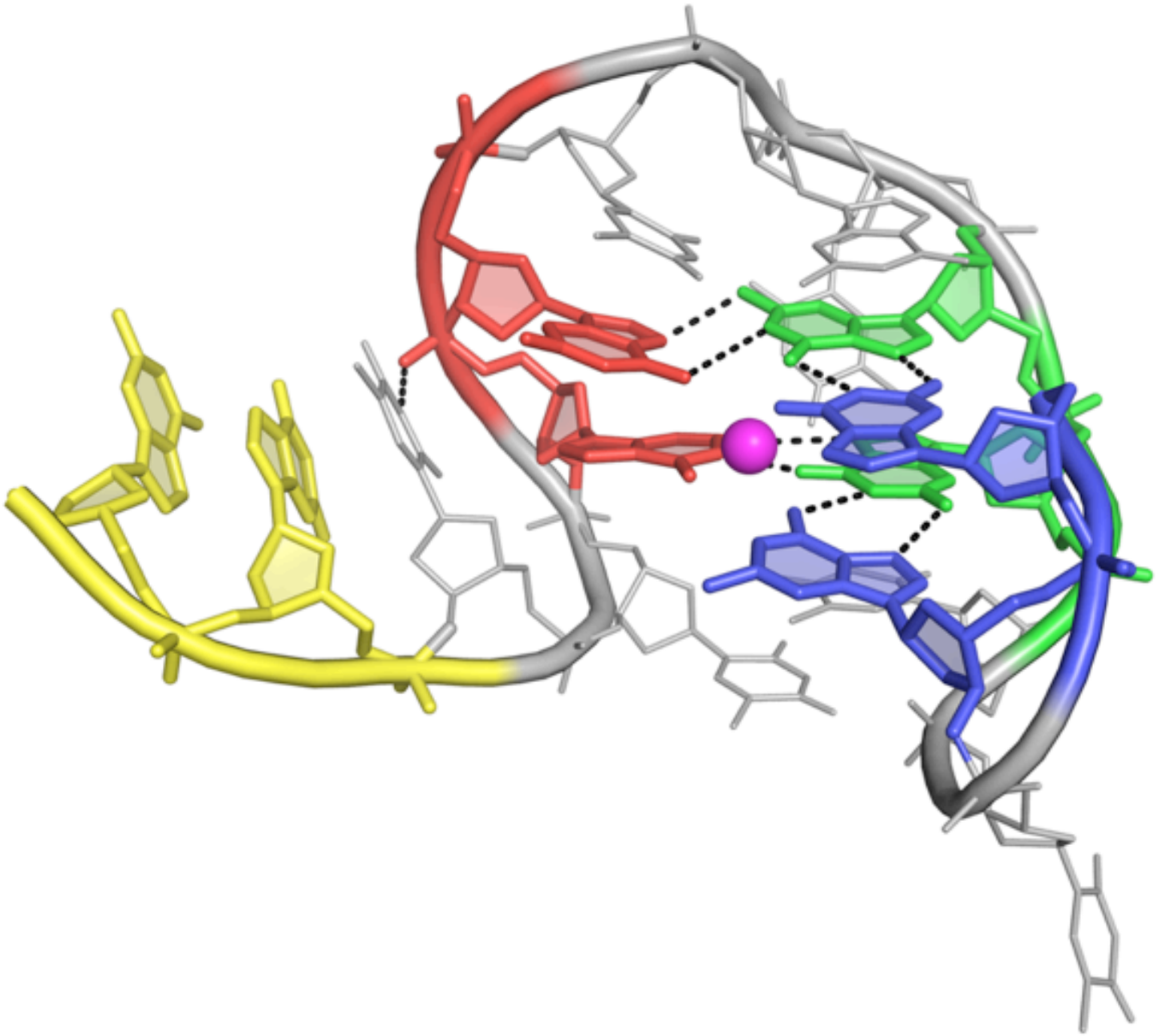
Limongelli et al. *Angew. Chem. Int. Ed.* (2013)

The movie



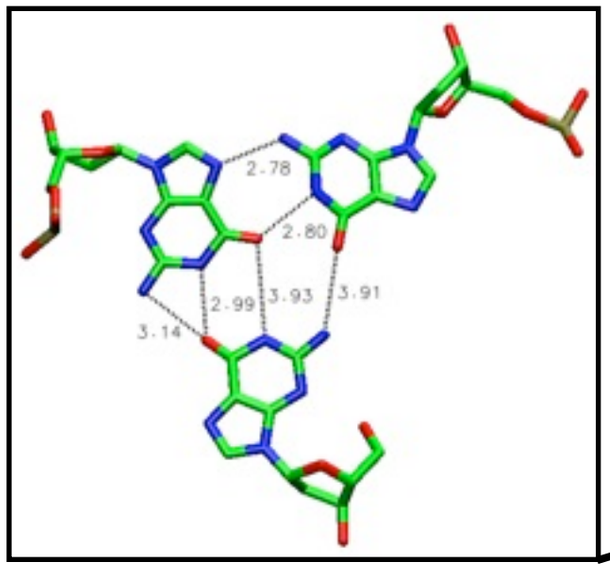
TBA Truncation

15-mer → 11-mer

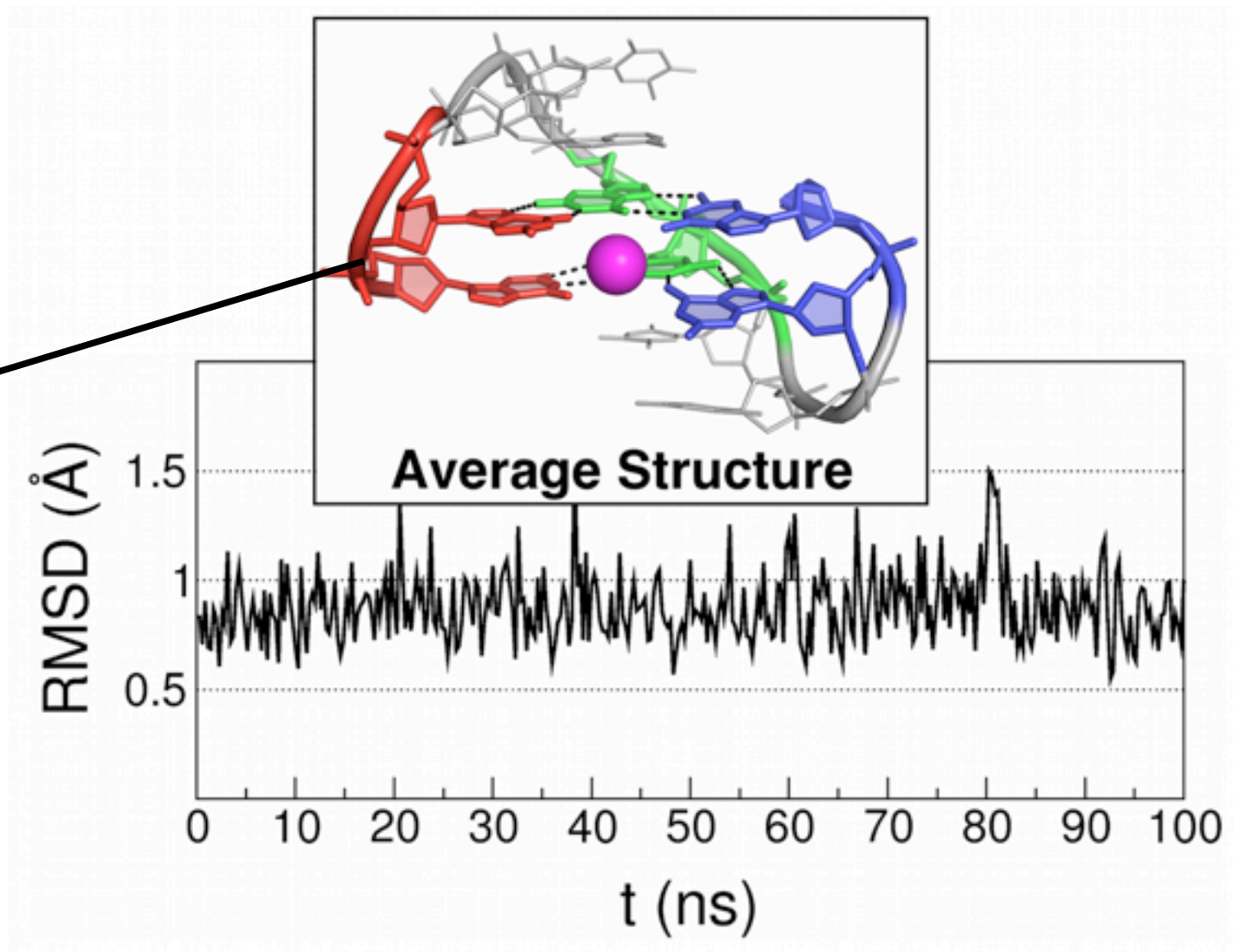


MD on 11 mer

70 mM KCl = 26 K^+, 12 Cl^-



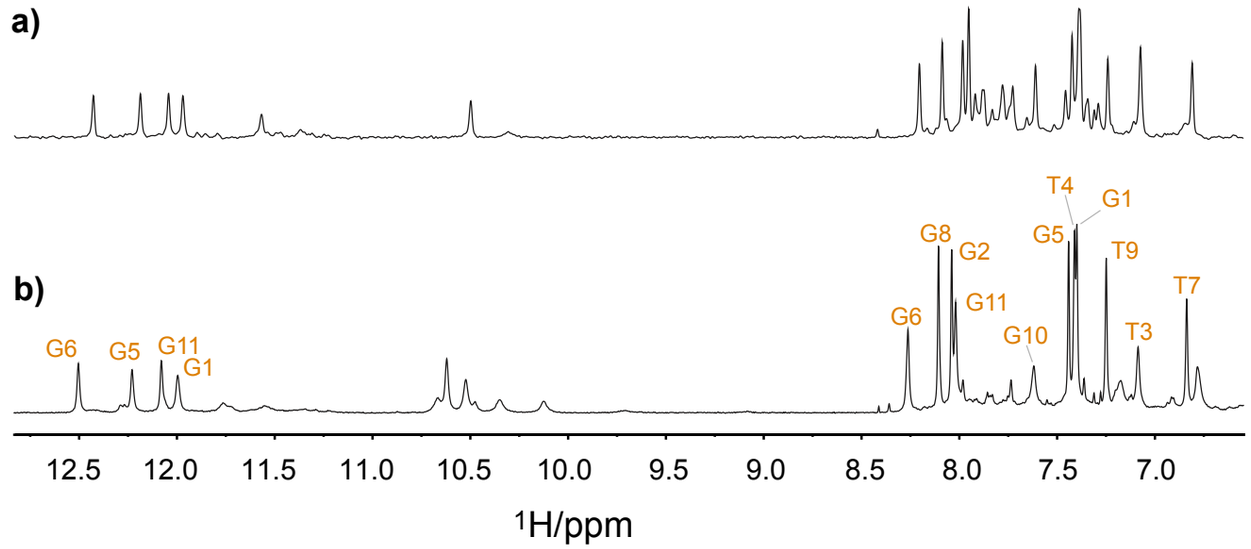
G-Triad

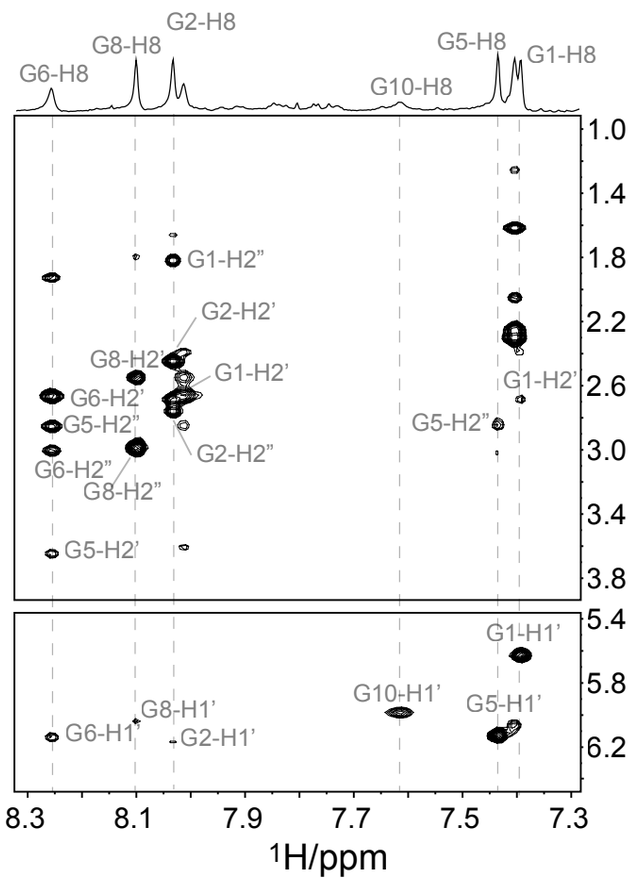


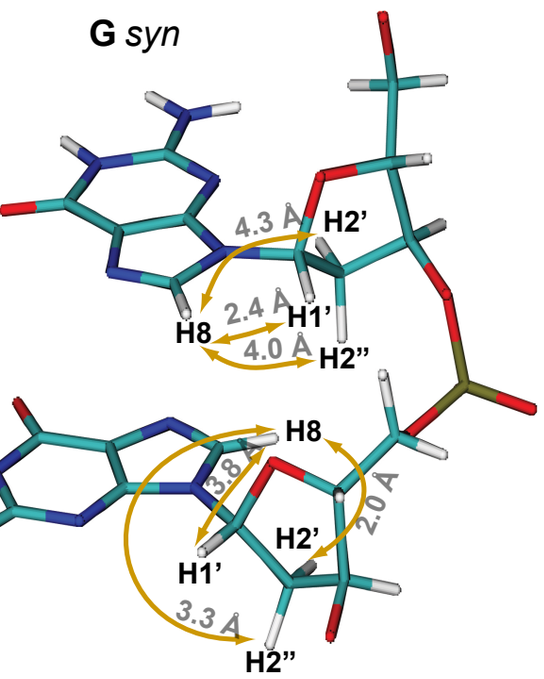
Experimental Validation

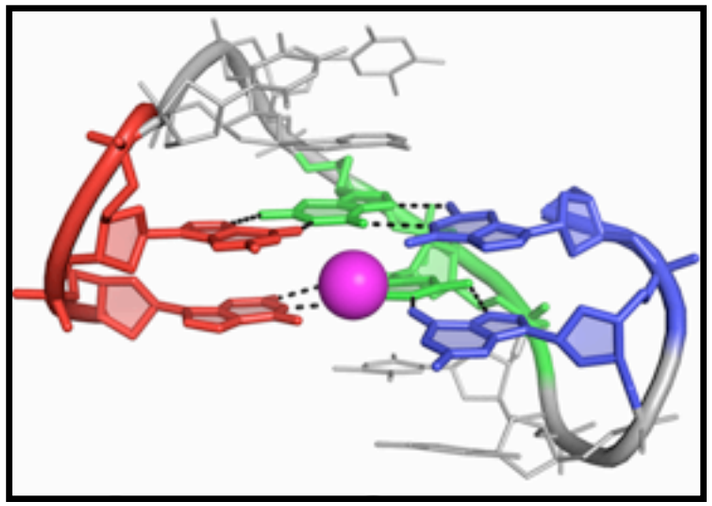
T=298 K

T=274 K





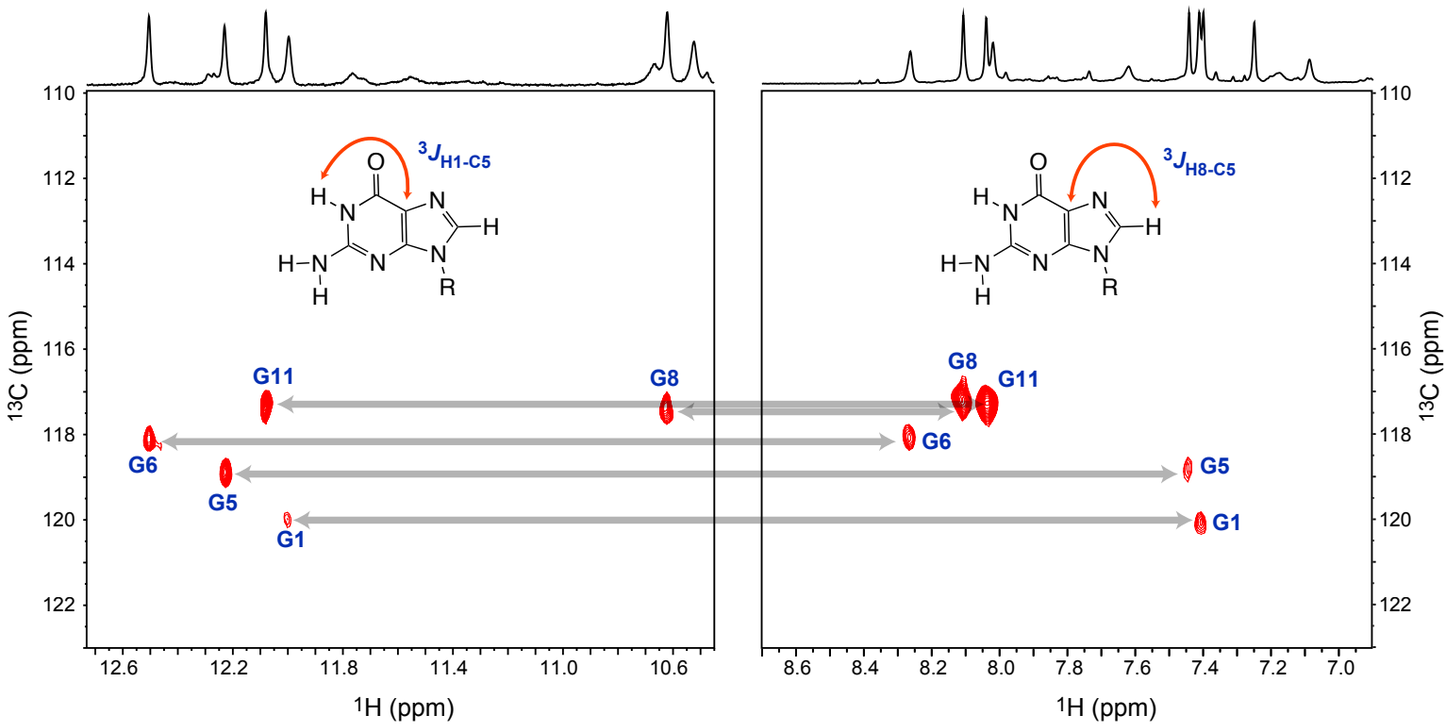


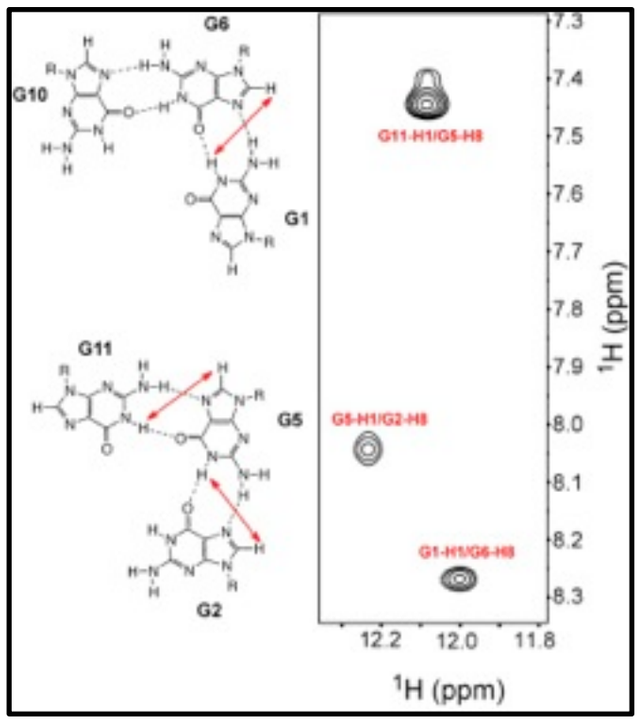


G-triplex

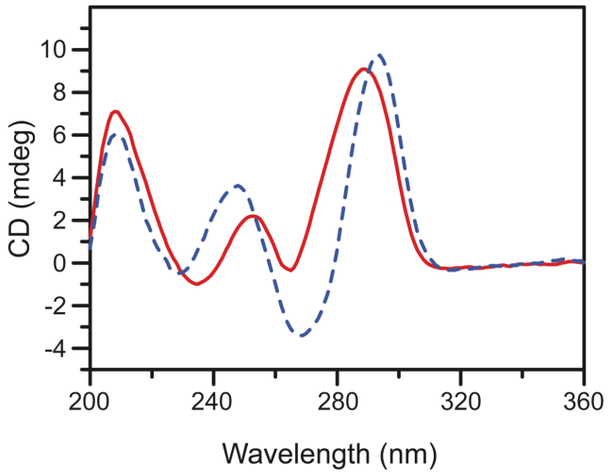
Limongelli et al. *Angew. Chem. Int. Ed.* (2013)

Experimental Validation

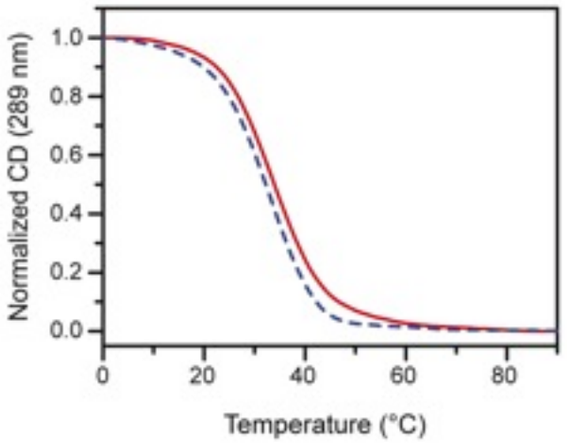




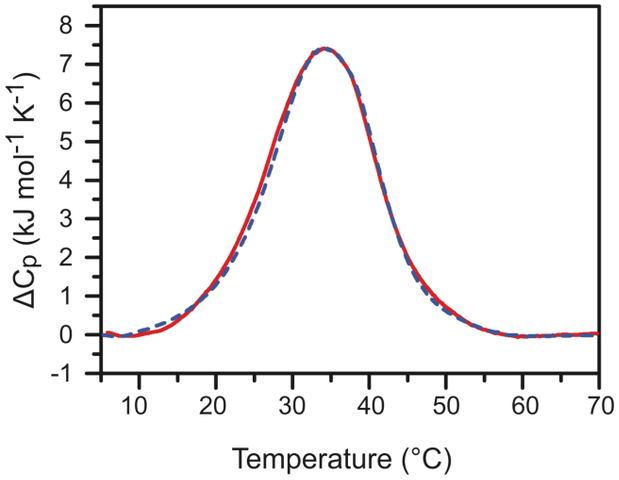
NOESY NMR



CD spectrum



CD melting



G-quadruplex

G-triplex

DSC

Tm=307 K

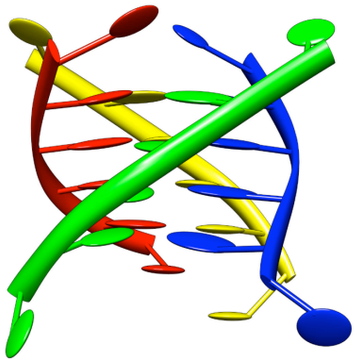
Limongelli et al. *Angew. Chem. Int. Ed.* (2013)

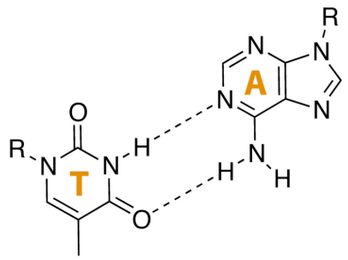
DNA Structures

Duplex

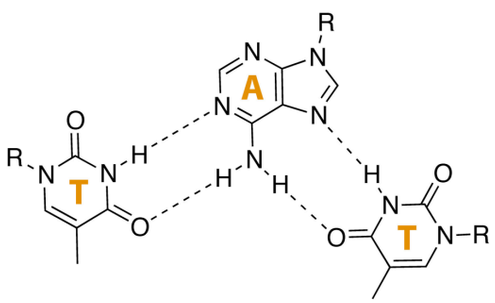
Triplex

G-Quadruplex

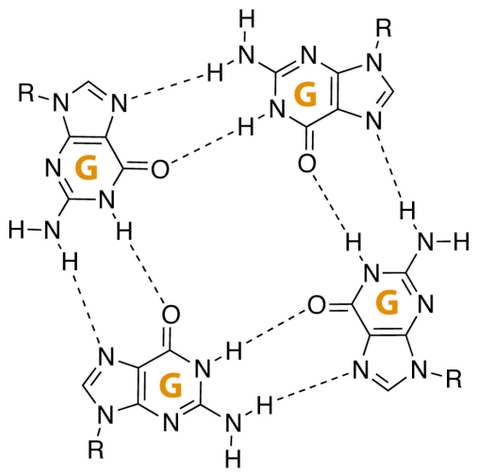




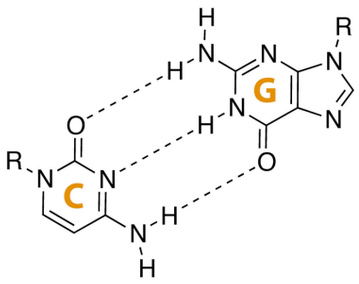
T-A base pair



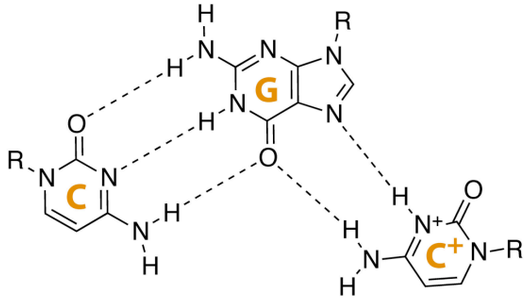
T-A:T base pair



G-tetrad



C-G base pair



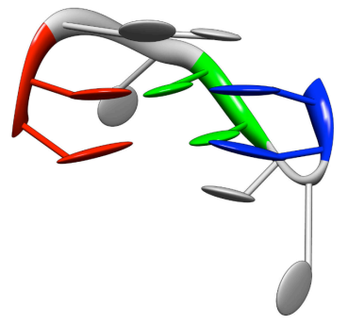
C-G:C$^+$ base pair

DNA Structures

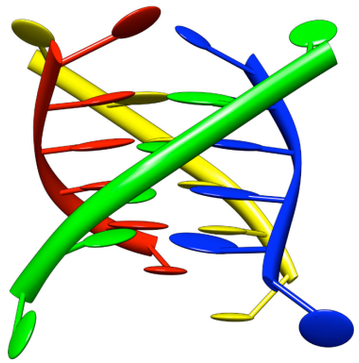
Duplex

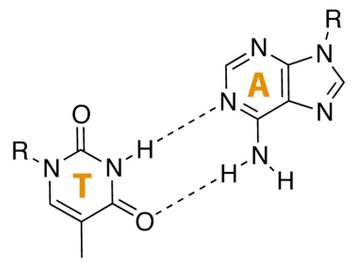
Triplex

G-Triplex

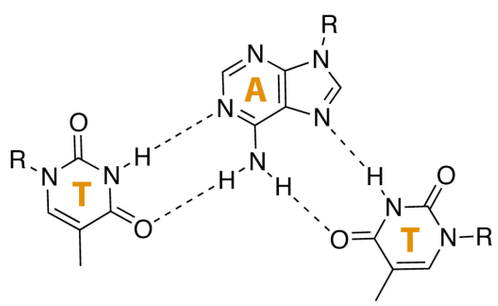


G-Quadruplex

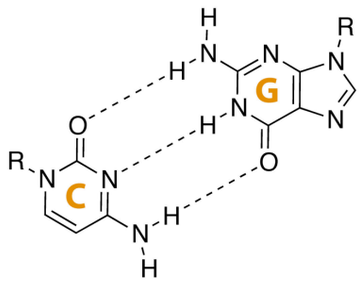




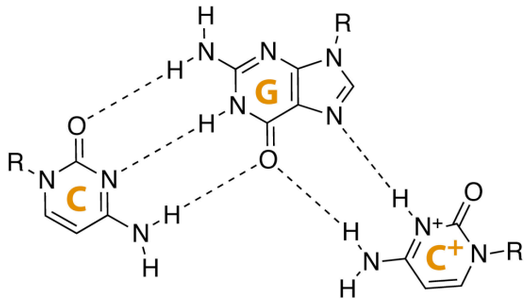
T-A base pair



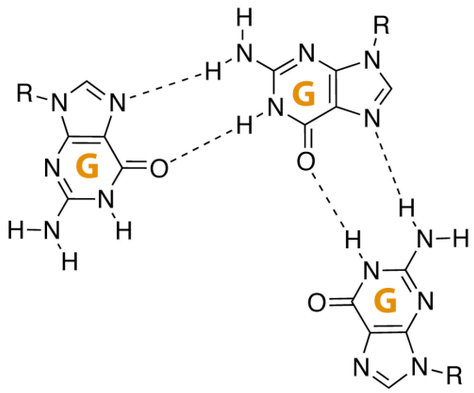
T-A:T base pair



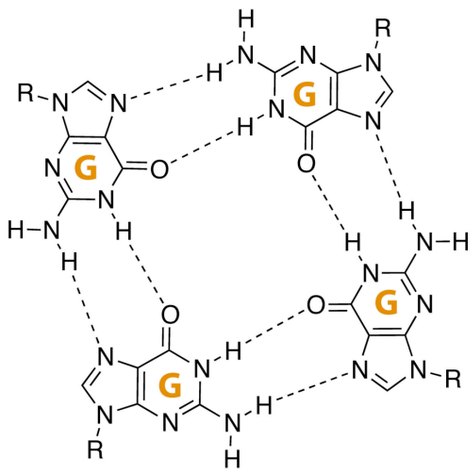
C-G base pair



C-G:C$^+$ base pair



G-triad



G-tetrad

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

Conclusions & Perspectives

✓ Sampling large time-scale biological events (e. g. DNA folding) requires the use of non-standard techniques and HPC resources

✓ Identification and characterization of a new DNA structure: the G-triplex

✓ Has the G-triplex a biological role?

✓ Are G-triplex structures present in the folding process of other DNA or RNA structures?

✓ Are G-triplex structures useful for the design of new aptamers?

✓ Is the G-triplex druggable? (VS campaign)

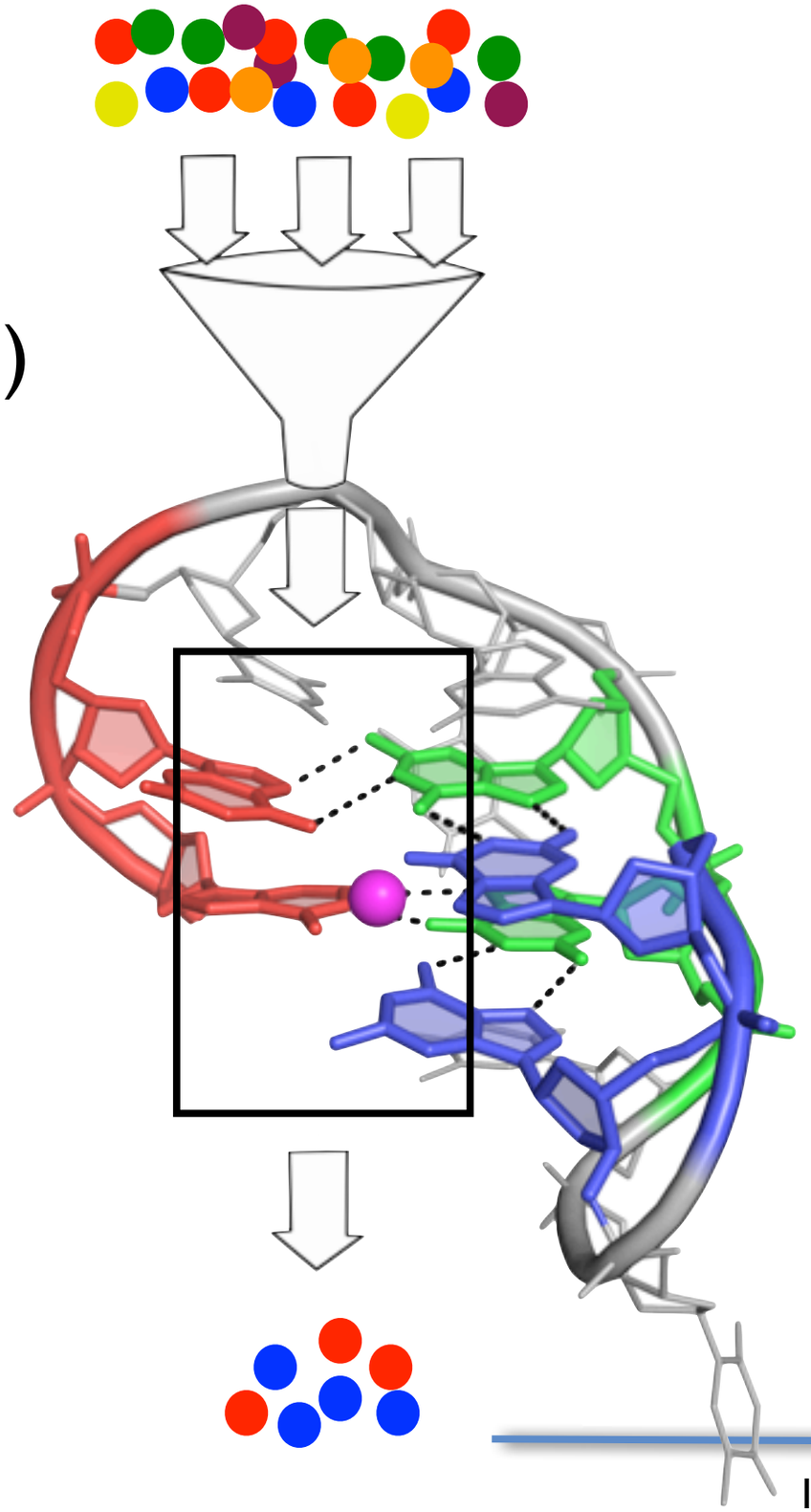
VS on G-Triplex

1. Chemical Libraries Selection

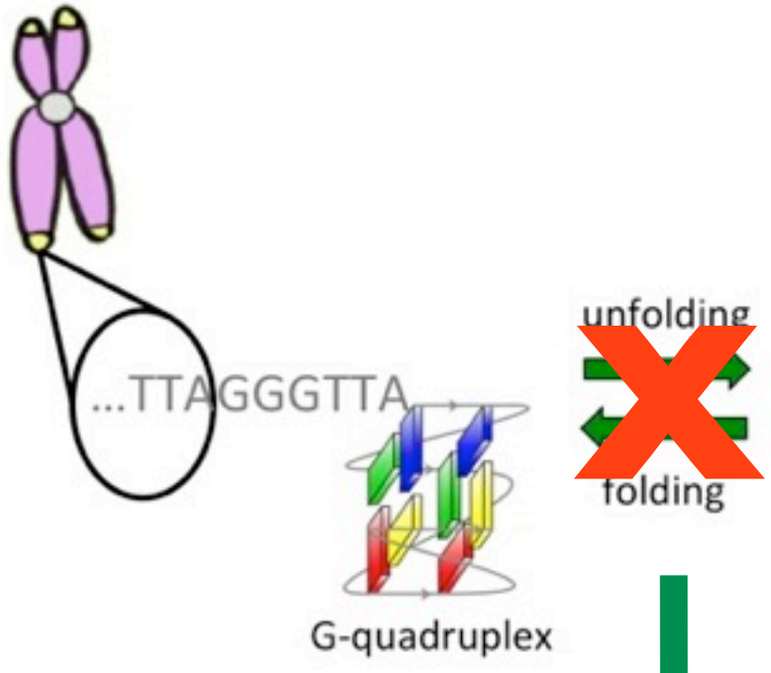
2. Filtering (Lipinski's role, ADME etc.)

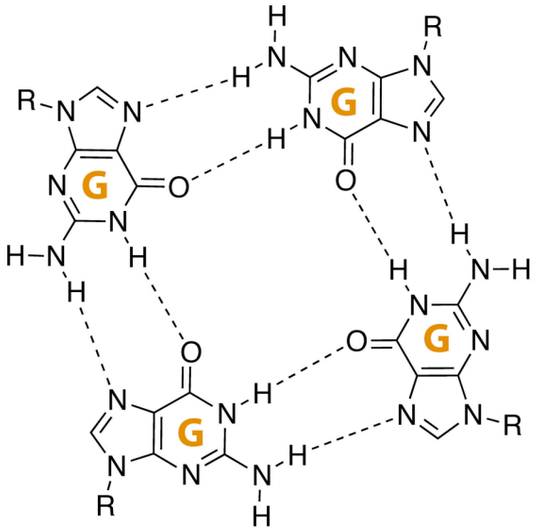
3. Docking in the specific groove of the G-triplex

4. Identification of lead compounds able to bind the G-triplex



Biological Background



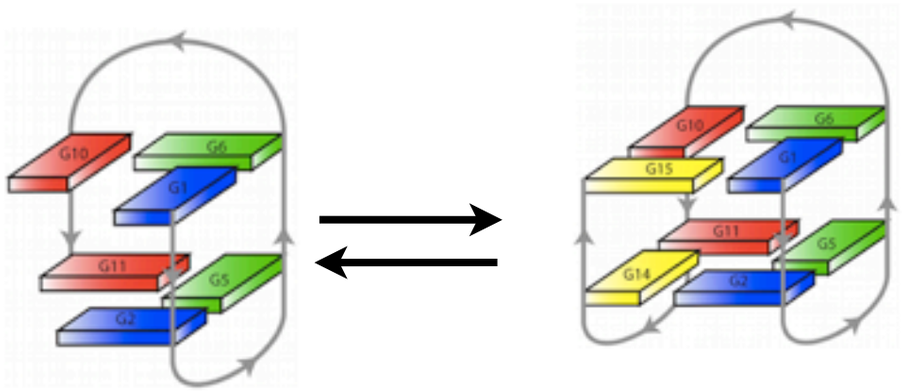


G-tetrad

DNA single strand

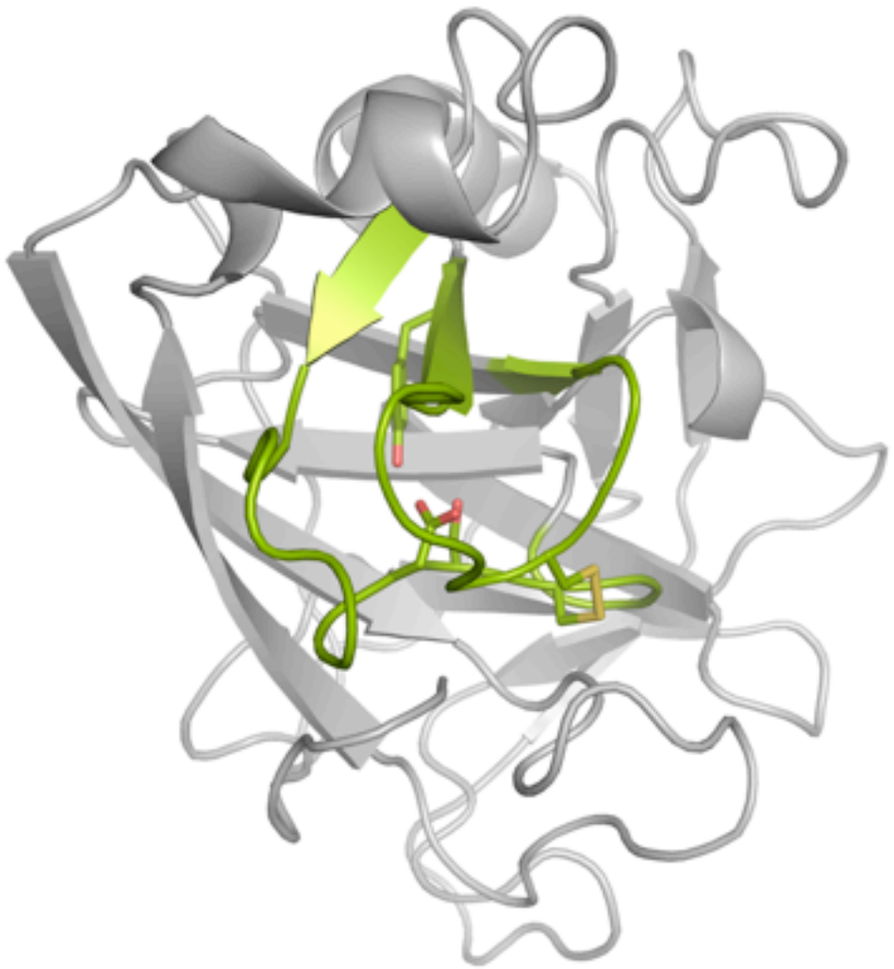
Telomers

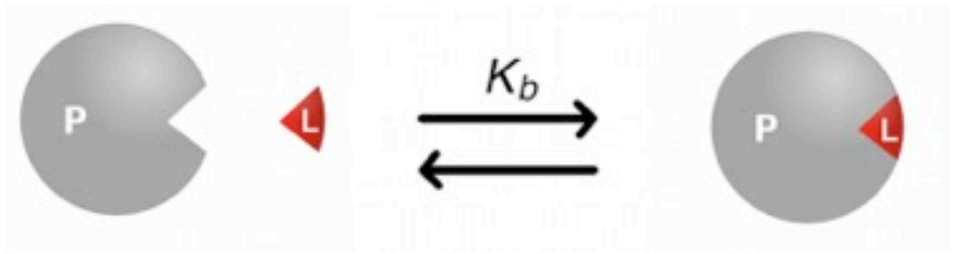
Neidle et al., *Curr. Opin. Struct. Biol.* (2003)
Kim et al., *Science* (2004)
Wang et al., *Nature* (2007)
Biffi et al., *Nat. Chem.* (2013)



G-triplex G-quadruplex

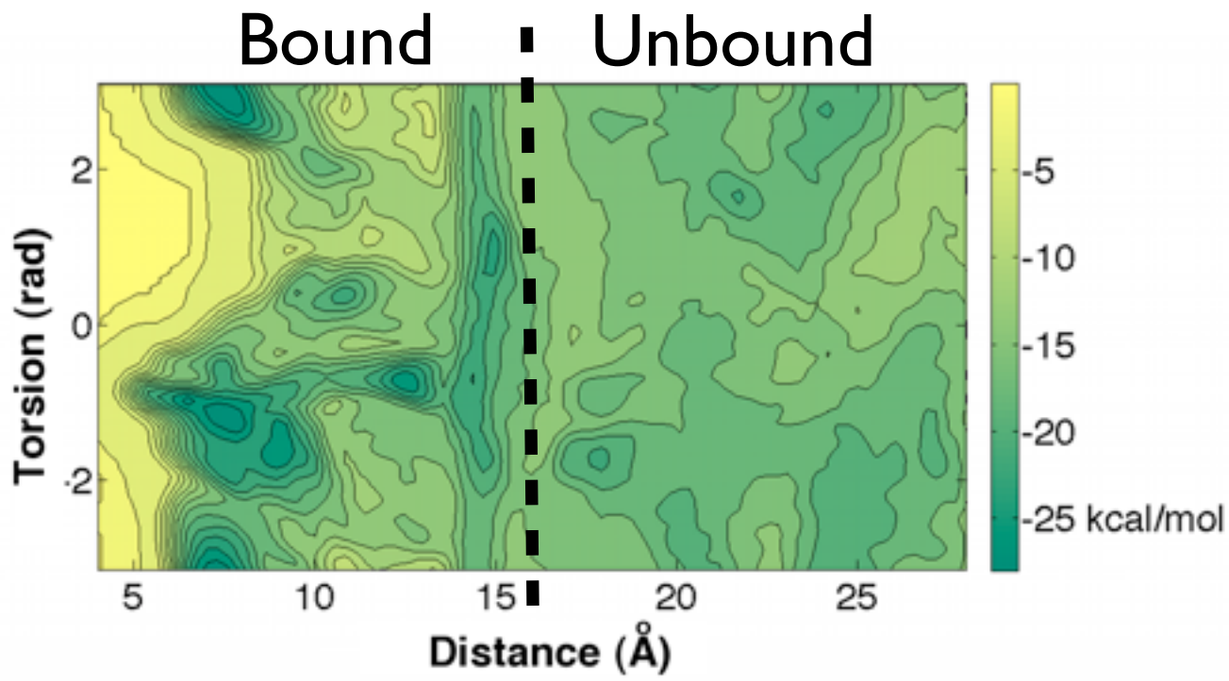
Protein/Ligand Binding Free Energy





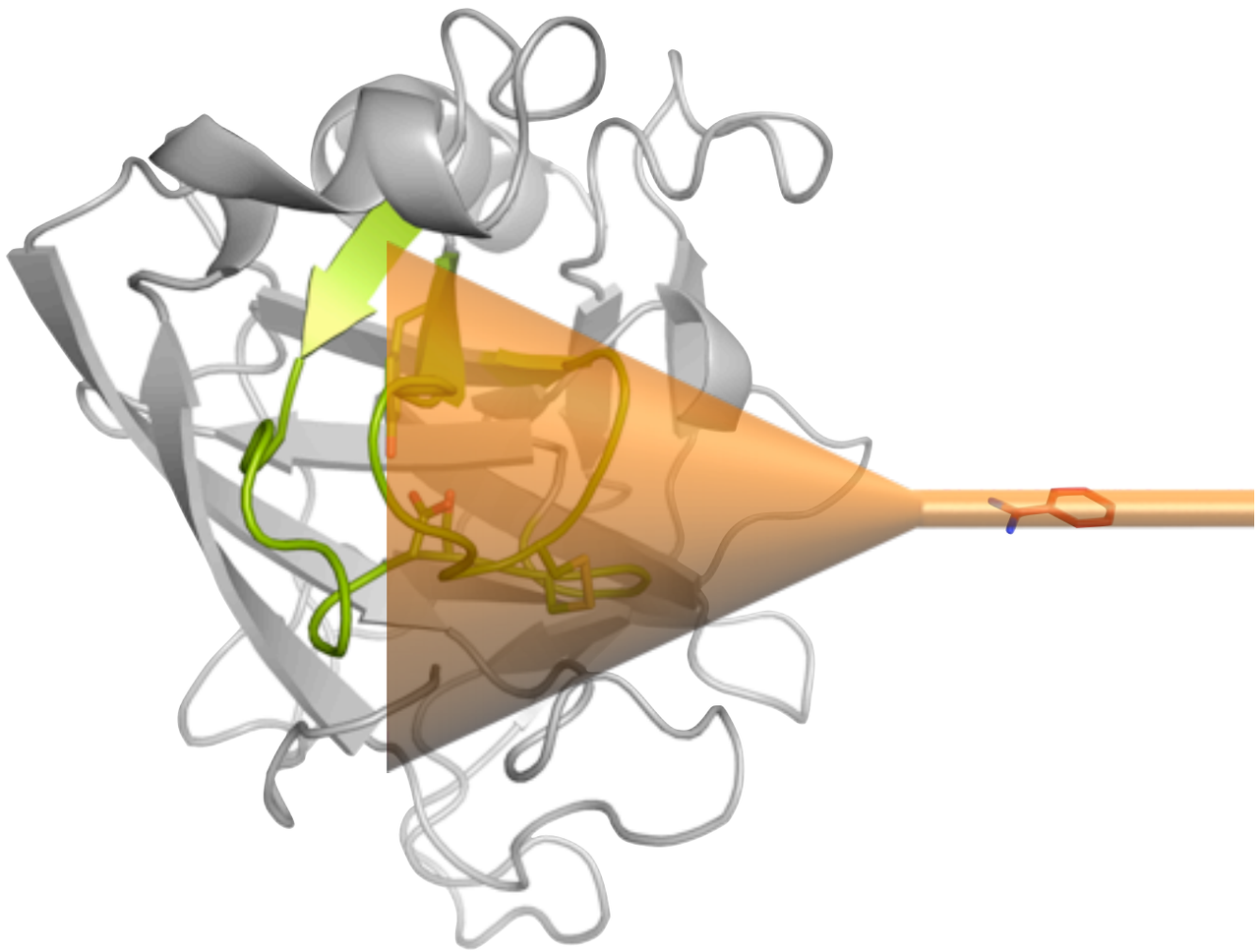
$$\Delta G_b^0 = -\frac{1}{\beta} ln(C^0 \ K_b)$$

C^0 = Standard Concentration (constant)



Poor convergence of the FES in the ligand unbound state

The Idea



Driving the ligand out of the protein using a funnel restraint potential

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

ΔG_{site} is equal to 0

potential of the unbound state

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

potential of the bound state

Su is equal to πR_{cyl}^2

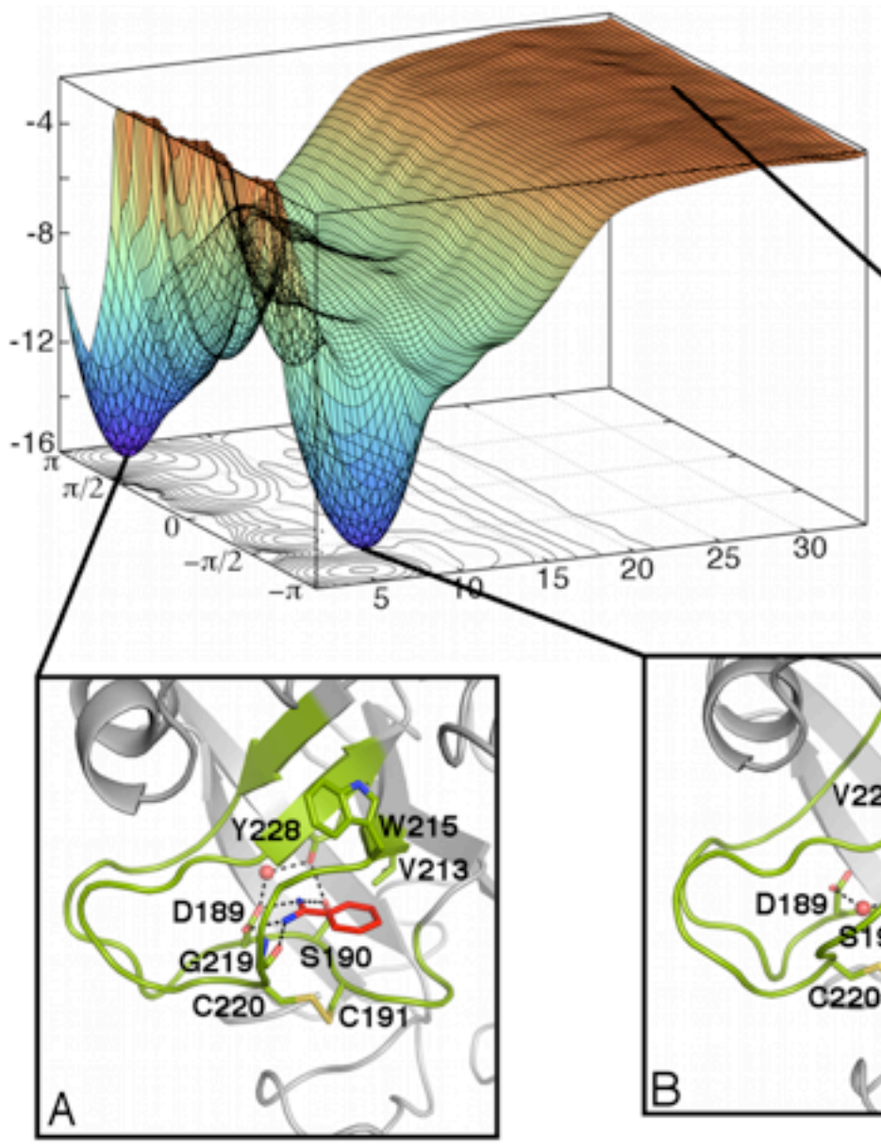
$$^{**}\Delta G_b^0 = \Delta G - \frac{1}{\beta} ln(\pi R_{cyl}^2 C^0)$$

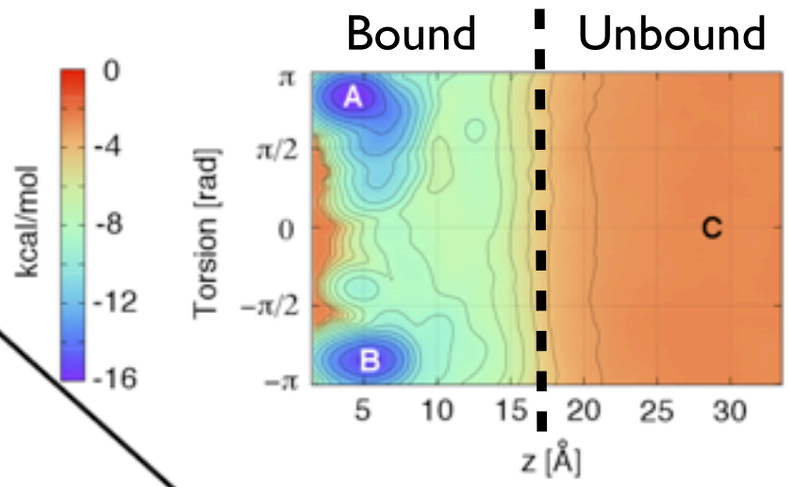
Free Energy difference between the bound and unbound state

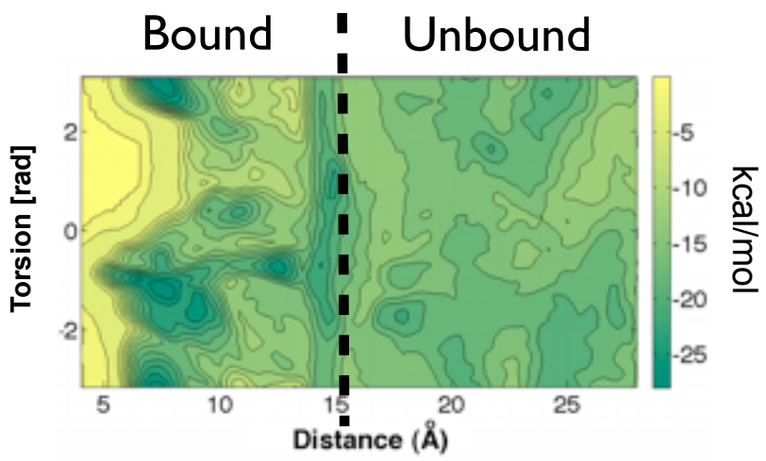
* Allen et al., *PNAS* (2004); Roux et al., *J. Chem. Phys.* (2008)

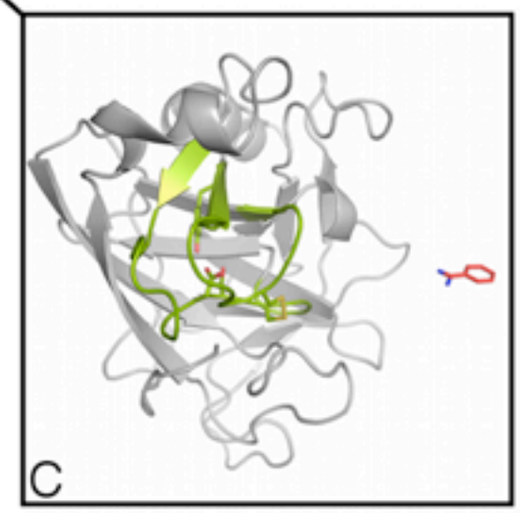
** Limongelli, Bonomi and Parrinello, *PNAS* (2013)

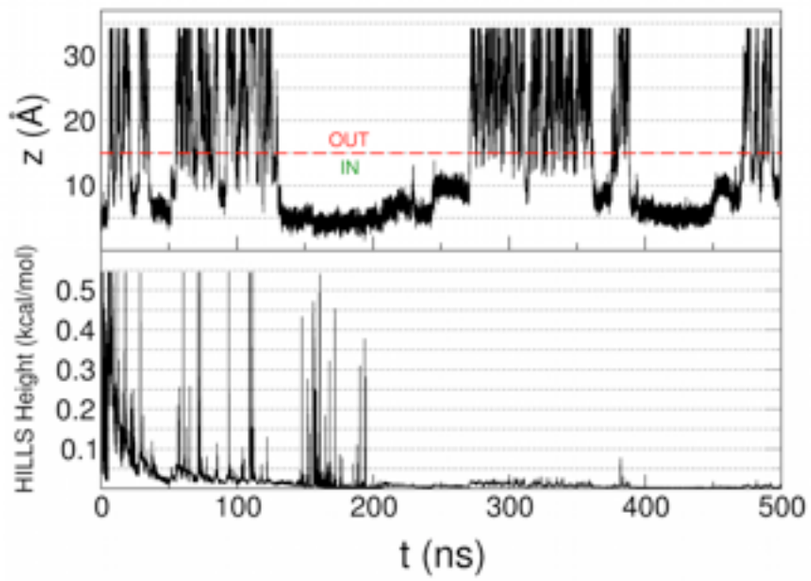
Funnel-Metadynamics (FM)











$\Delta G_b^0 = -8.5 \pm 0.7$ kcal/mol (previous calculations −5.5 to −9.0 kcal/mol)*

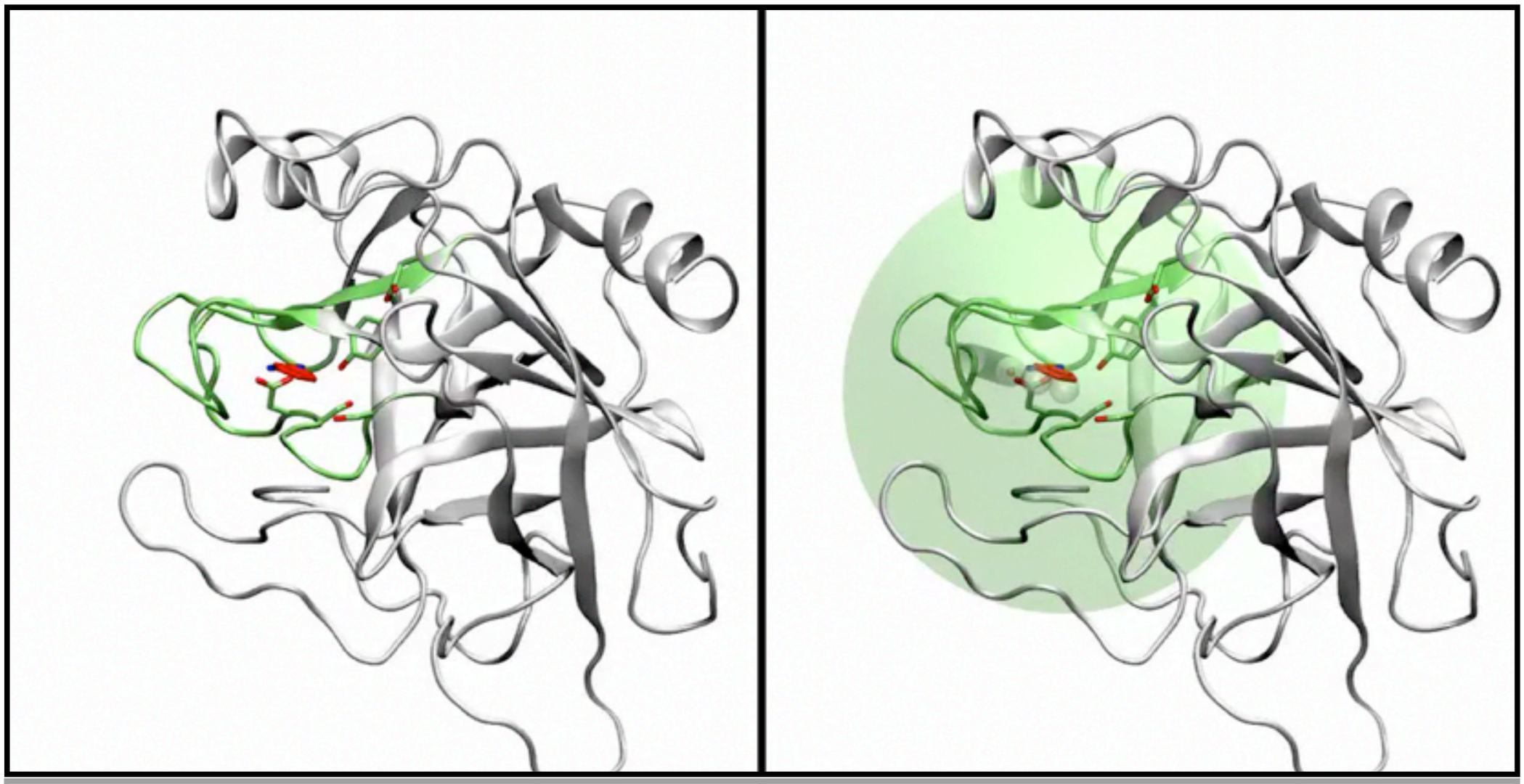
*Doudou et al., J. Chem. Theory Comput. (2009)

Funnel metadynamics as accurate binding free-energy method

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

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

FM Movie



Acknowledgements

University of Naples

Ettore Novellino

Antonio Randazzo

Stefano De Tito

Bruno Pagano

Università della Svizzera italiana

ETH Eidgenössische Technische Hochschule Zürich Swiss Federal Institute of Technology Zurich

Michele Parrinello

CERM Firenze

Ivano Bertini

UCSF University of California San Francisco

Massimiliano Bonomi

Computational Resources

PRACE

CSCS Centro Svizzero di Calcolo Scientifico Swiss National Supercomputing Centre

PhD and PostDoc positions available

vittoriolimongelli@gmail.com

Thank You

Experimental Validation

TBA: 5′-GGTTGGTGTGGTTGG-3′

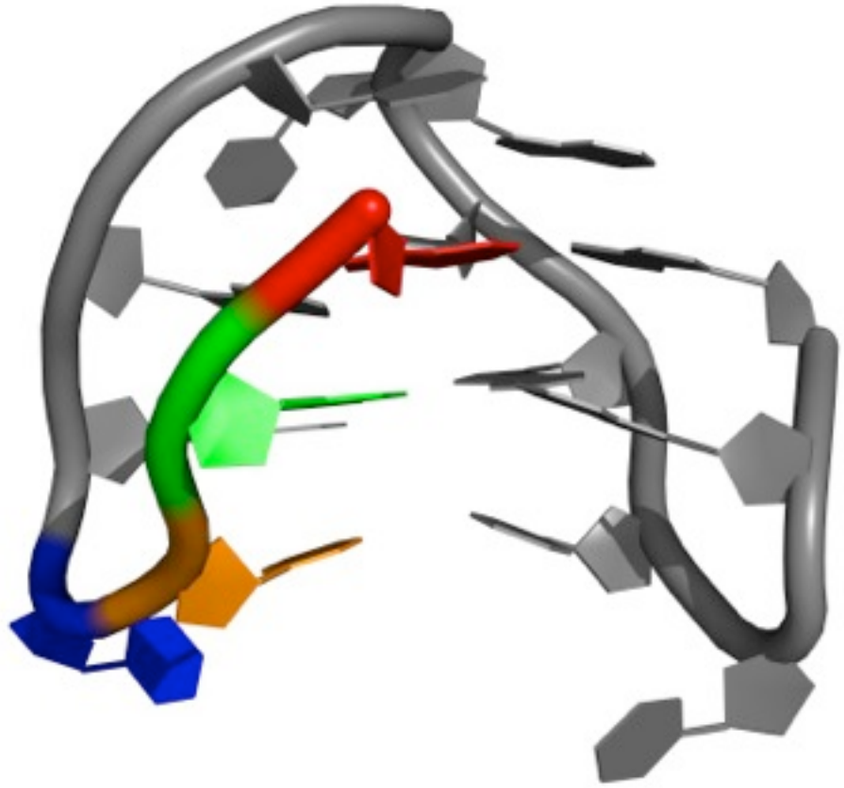
1) 5′-GGTTGGTGTGGTTG-3′

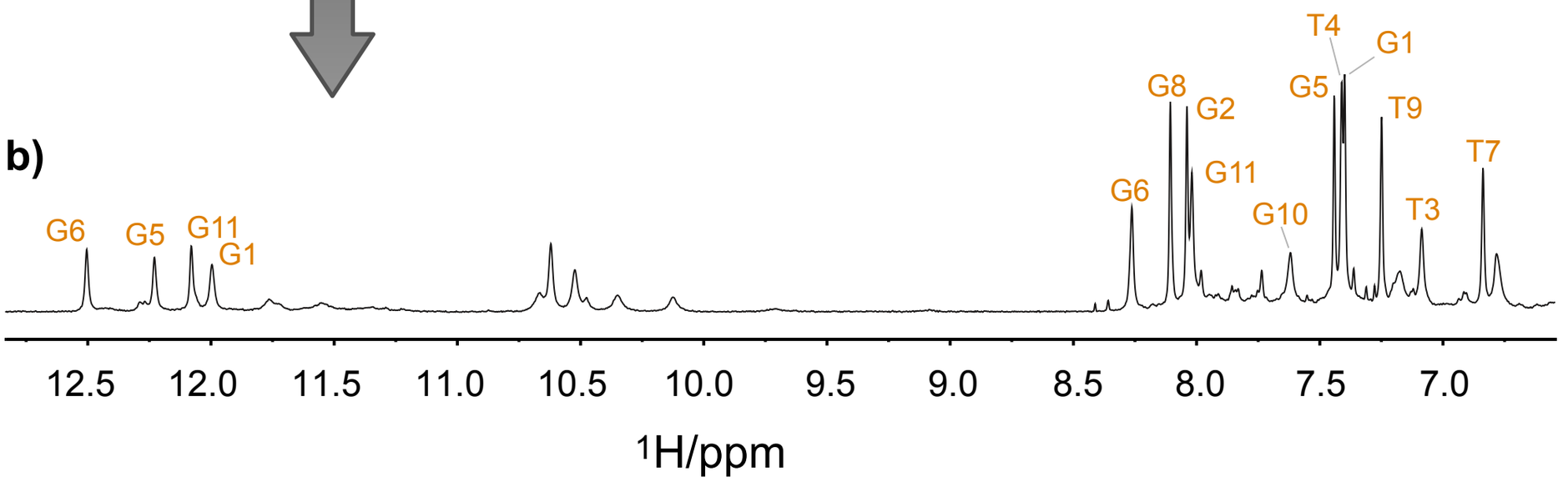
2) 5′-GGTTGGTGTGGTT-3′

3) 5′-GGTTGGTGTGGT-3′

4) 5′-GGTTGGTGTGG-3′

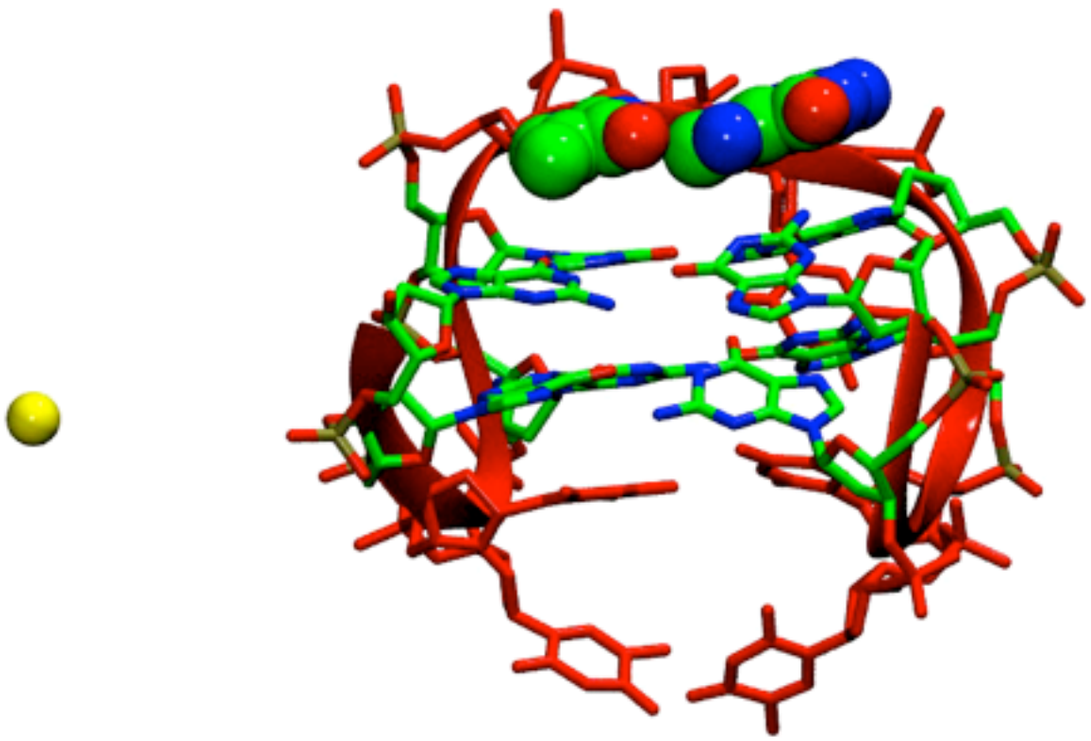
Conformational heterogeneity



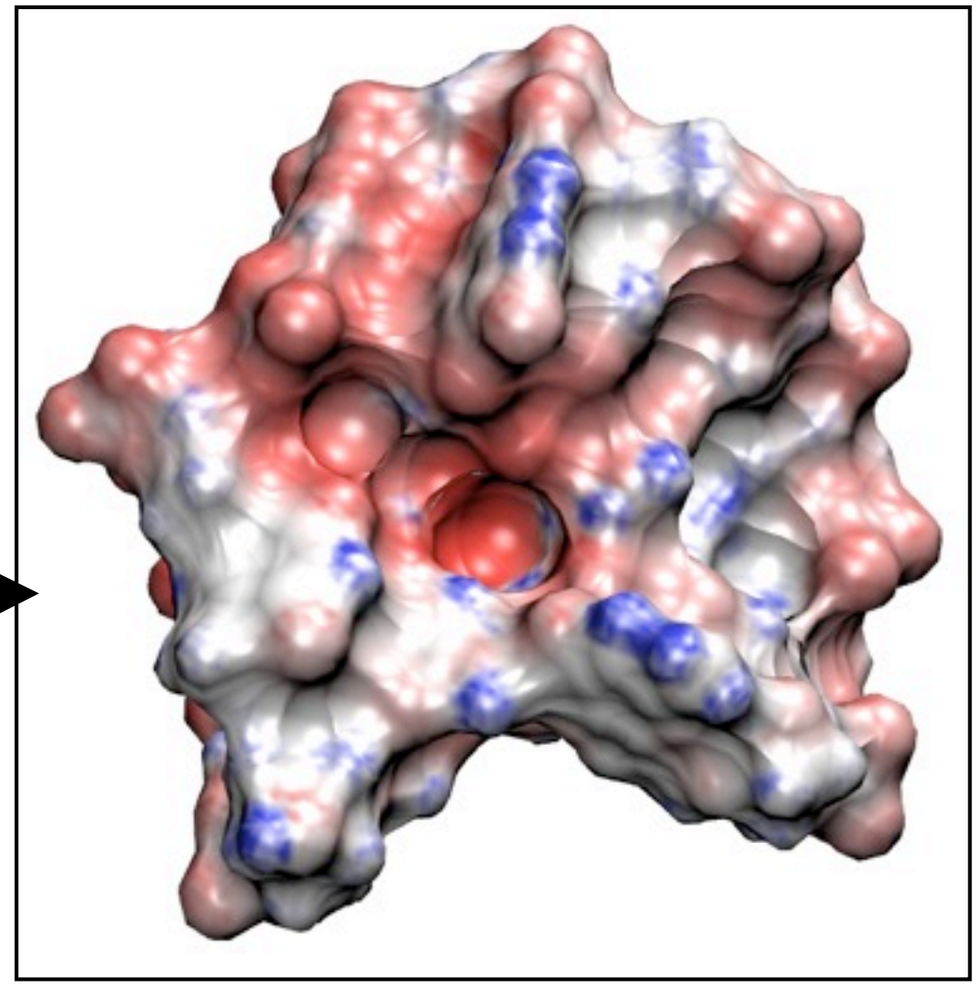


Ion Binding

70 mM KCl = 26 K^+, 12 Cl^-



90°



-10 k_b T e_c^{-1} — 10 k_b T e_c^{-1}

How to compute $\Delta G_{binding}$

$$[L] + [P] \leftrightharpoons [LP] \qquad K_{eq} = \frac{[LP]}{[L]\,[P]}$$

ΔG_{site} is equal to 0

potential when the ligand is bound

$$^{*}K_{eq} = e^{\beta \Delta G_{site}} \int_{site} dz\, e^{-\beta[w(z)-w(z\to\infty)]}\, S_u$$

Su is equal to πr^2

potential when the ligand is unbound

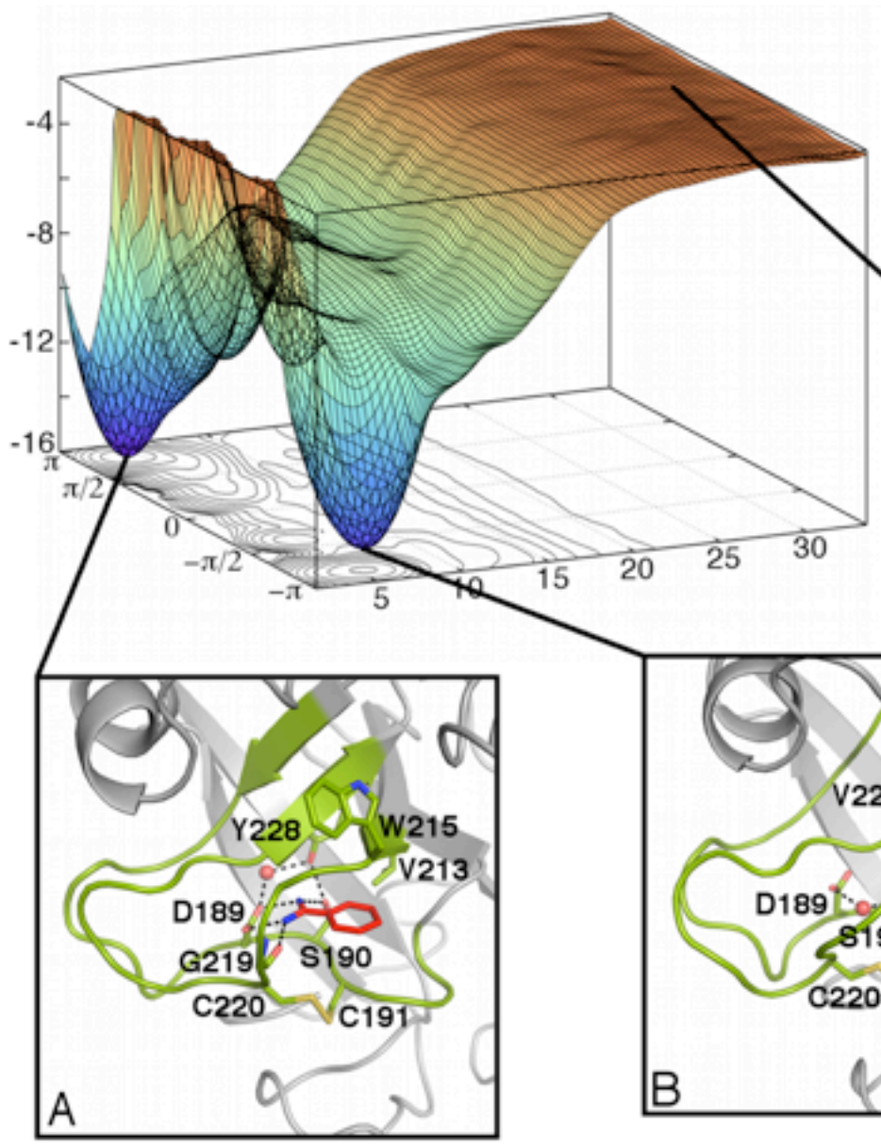
Using a cylindric restrain

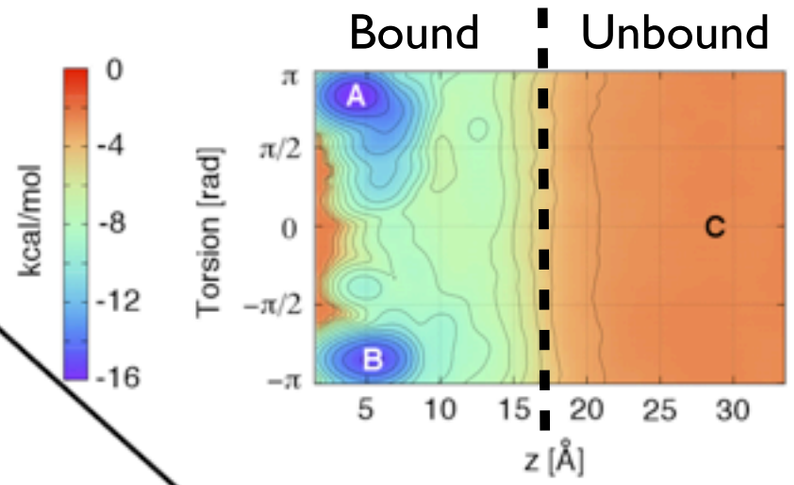
lated for K^+, in good accord with experiment.[3] The development above leading to Eq. (4) makes it clear why S_u is equal to πR^2 in Ref. 3; because R was purposely chosen to be much larger than the lateral fluctuations of the bound ion in the site, the restraining potential u is not felt by the ion in the binding site, which means that $\Delta G_{\text{site}}=0$ identically. Bastug

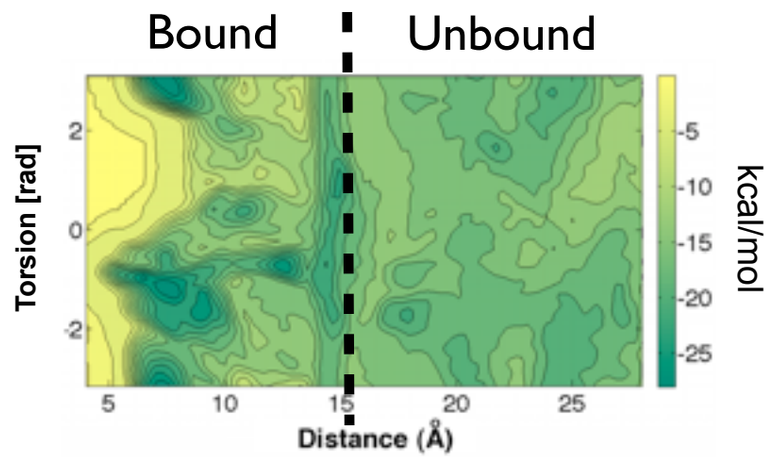
$$\Delta G_{binding} = -K_b T \ln (K_{eq}\, C_0)$$ C_0 = Standard Concentration (constant)

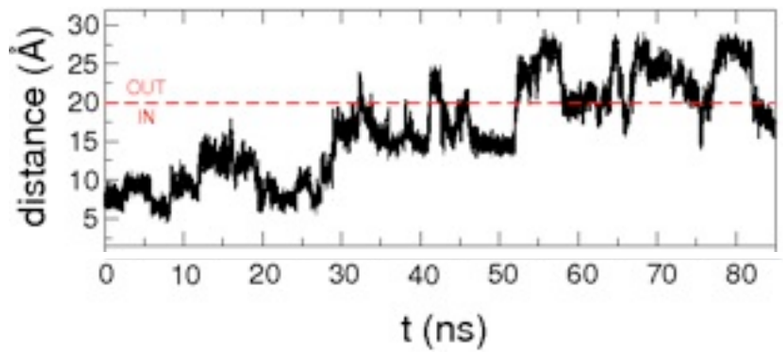
Allen et al., *PNAS* (2004)
Roux et al., *J. Chem. Phys.* (2008)

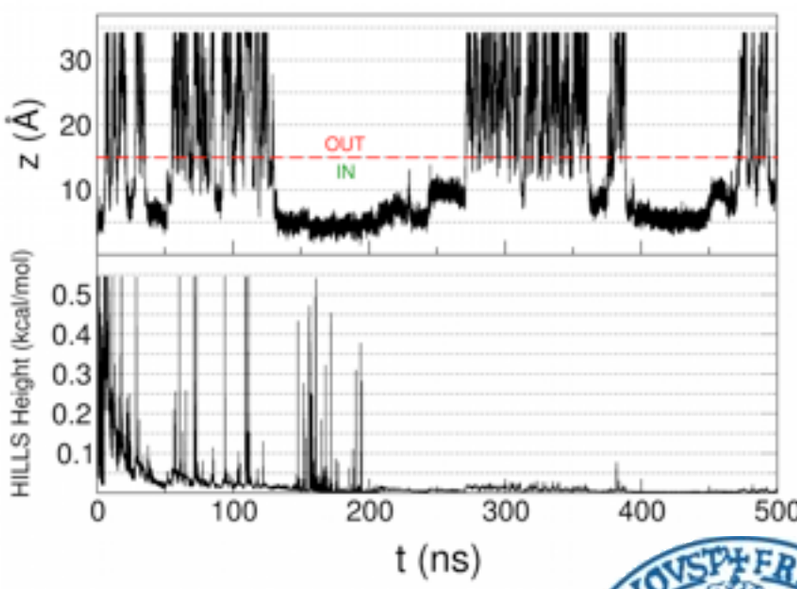
Funnel-Metadynamics (FM)











$\Delta G_b^0 = -8.5 \pm 0.7$ kcal/mol (previous calculations −5.5 to −9.0 kcal/mol)

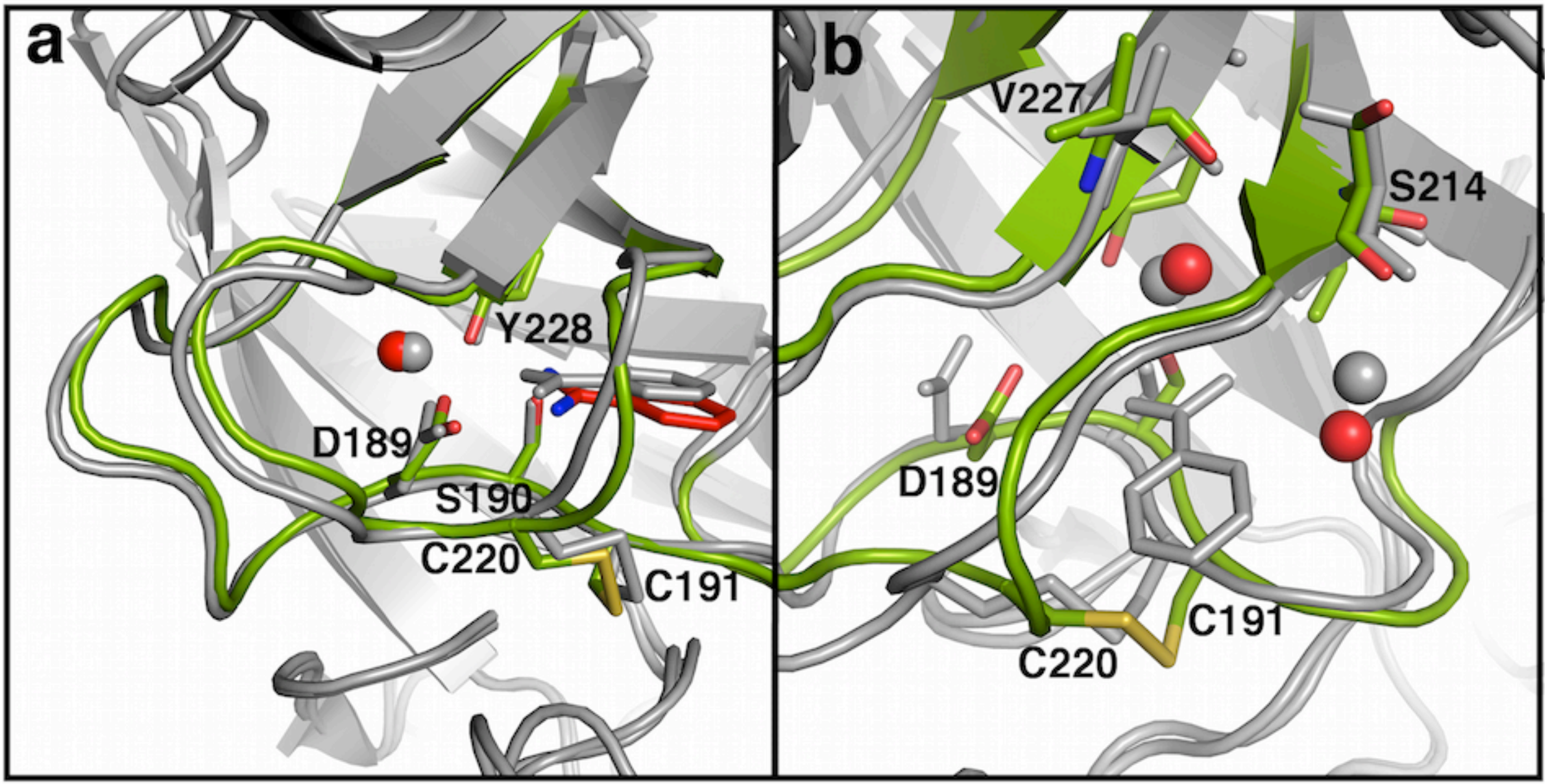
PNAS

Funnel metadynamics as accurate binding free-energy method

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

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

Waters Role



Convergence FM