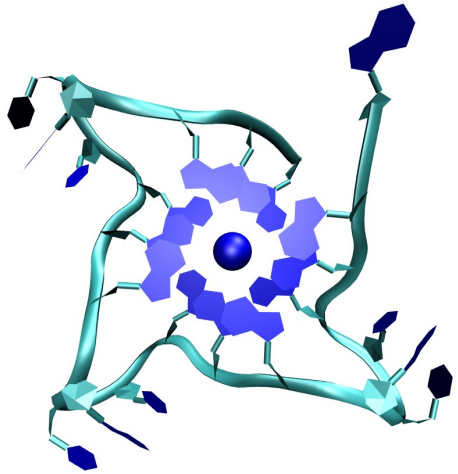
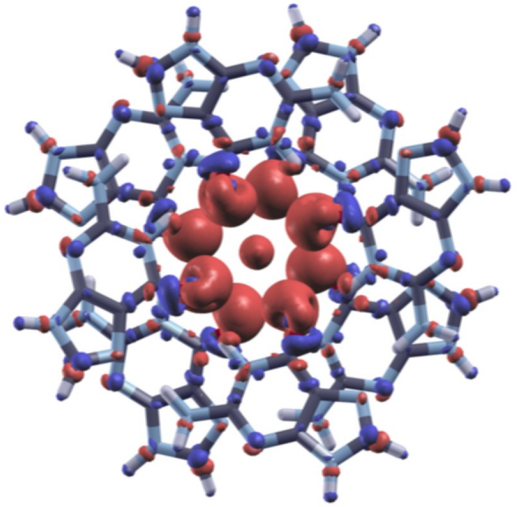
ONETEP–PB/SA: Application to G-Quadruplex DNA Stability





TCM

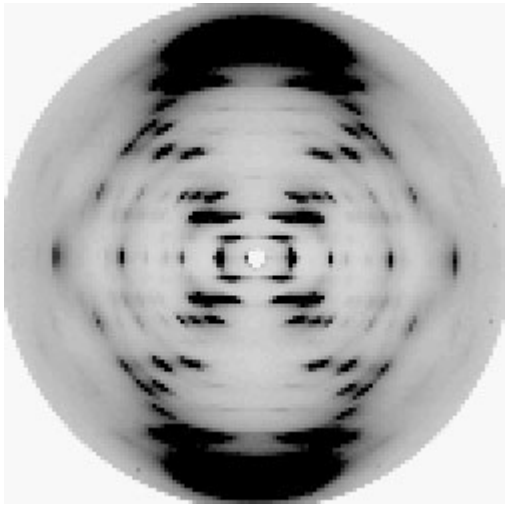
Danny Cole

Introduction

- Historical overview of structure and free energy calculation of complex molecules using molecular mechanics and continuum models.
- MM-PB/SA method for free energy calculation.
- Motivation and method for ONETEP-PB/SA.
- Stability of G-Quadruplex DNA structures in ionic solutions.

MD and Biological Macromolecules

1976-1985 “...all one could do was take a crystallographer's structure of a protein and ruin it.” Showed that proteins have both solid- and liquid-like structures in contrast to the static X-ray structures.

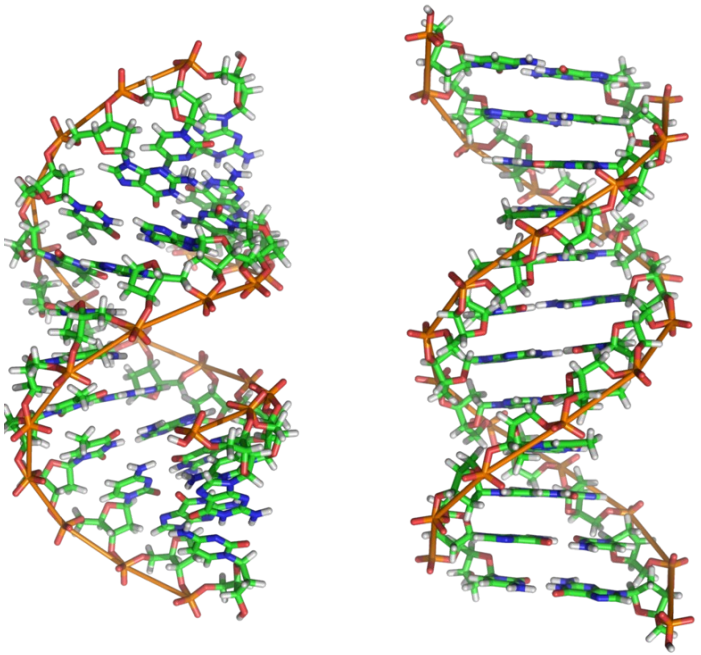


1985-1994 Free energy perturbation approaches. Solvate sphere around the active site and “mutate” one ligand from one form into another → Relative free energies of binding that can be compared with experiment.

Kollman et al., Acc. Chem. Res. 33, 889 (2000)

MD and Biological Macromolecules

Computationally efficient algorithms (PME) and increased computer power allow structural transitions to be studied. e.g. A-DNA → B-DNA.



What if barrier between starting structure and experimental structure is too large to be surmounted in nanosecond simulation time? Can we calculate free energies of different structures and will the native structure be calculated as the lowest in free energy?

MM-PB/SA Approach

Poisson-Boltzmann

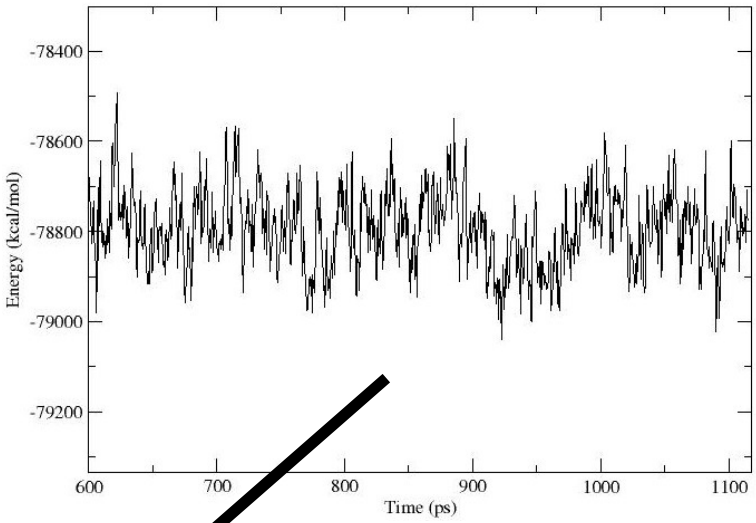
MM-PB/SA

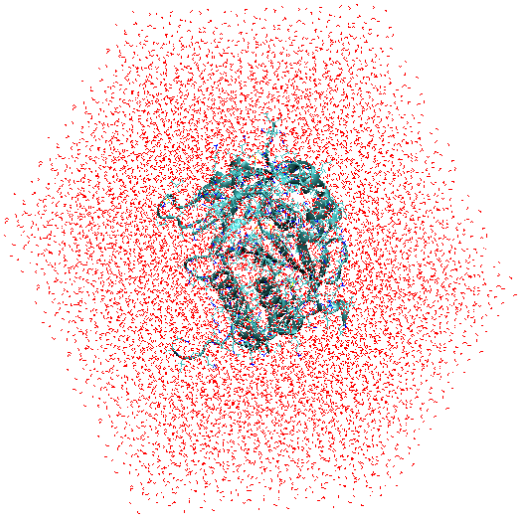
Molecular Mechanics

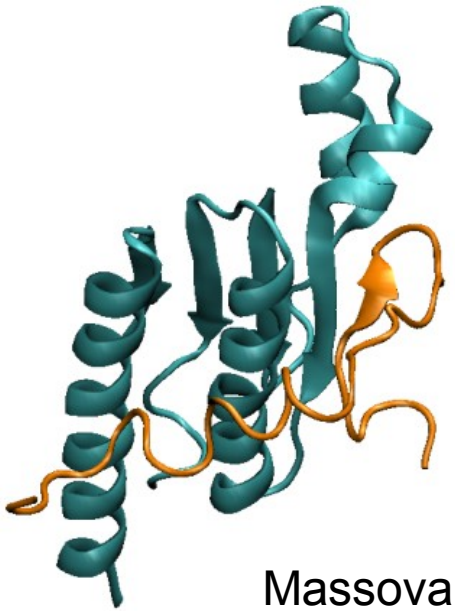
Surface Area

MM-PB/SA Approach

1) Run MD simulation of structure in explicit water with counter-ions using AMBER force field.







2) Sample snapshots of trajectory, removing water molecules and counter-ions.

3) Calculate average free energy of structure in implicit solvent. Do not cut off any non-bonded interactions.

Massova and Kollman, JACS 121, 8133 (1999)

MM-PB/SA Approach

$$G = E_{MM} + G_{PBSA} - TS_{MM}$$

$$E_{MM} = E_{bond} + E_{angle} + E_{dihedral} + E_{vdW} + E_{elec}$$

Grid-based solution of the Poisson-Boltzmann equation assuming high dielectric solvent, low dielectric solute → electrostatic potential due to solute.

$$\nabla \cdot (\epsilon(r) \nabla \phi(r)) = -4\pi \rho(r)$$

$$G_{PBSA} = \frac{1}{2} \sum (q_i \phi_i^{\epsilon=80}(r) - q_i \phi_i^{\epsilon=1}(r)) + (\gamma SA + b)$$

Free energy of solvation is sum of electrostatic and non-polar contributions.

S_{MM} is from normal modes analysis of the entropy arising from solute degrees of freedom.

Advantages of MM-PB/SA

- No need to specify a reaction pathway between two structures.
- Salt effects can be included without recalculation of the trajectory.
- Extendible to computational alanine scanning.
- **Method can be relatively easily adapted to work with linear-scaling DFT codes such as ONETEP…**

ONETEP-PB/SA Approach

$$G = E_{MM} + G_{PBSA} - TS_{MM}$$

$$E_{MM} = E_{bond} + E_{angle} + E_{dihedral} + E_{vdW} + E_{elec}$$

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$$\nabla \cdot (\epsilon(r) \nabla \phi(r)) = -4\pi\rho(r)$$

$$G_{PBSA} = \frac{1}{2} \sum_i (q_i \phi_i^{\epsilon=80}(r) - q_i \phi_i^{\epsilon=1}(r)) + \gamma SA + b)$$

$E_{MM} \rightarrow E_{ab\text{-}initio}$

Free energy of solvation is sum of electrostatic and non-polar contributions.

S_{MM} is from normal modes analysis of the entropy arising from solute degrees of freedom.

ONETEP-PB/SA Approach

High dielectric solvent modifies the Hartree energy calculated within ONETEP

$$E_H[\rho] = \frac{1}{2}\int \rho(\vec{r})\phi[\rho]\, d\vec{r}$$

H. Helal, A. Mostofi, M. Payne

Grid-based solution of the Poisson-Boltzmann equation assuming high dielectric solvent, low dielectric solute → electrostatic potential due to solute.

$$\nabla\cdot(\epsilon(r)\nabla\phi(r)) = -4\pi\rho(r)$$

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Free energy of solvation is sum of electrostatic and non-polar contributions.

S_{MM} is from normal modes analysis of the entropy arising from solute degrees of freedom.

Applications

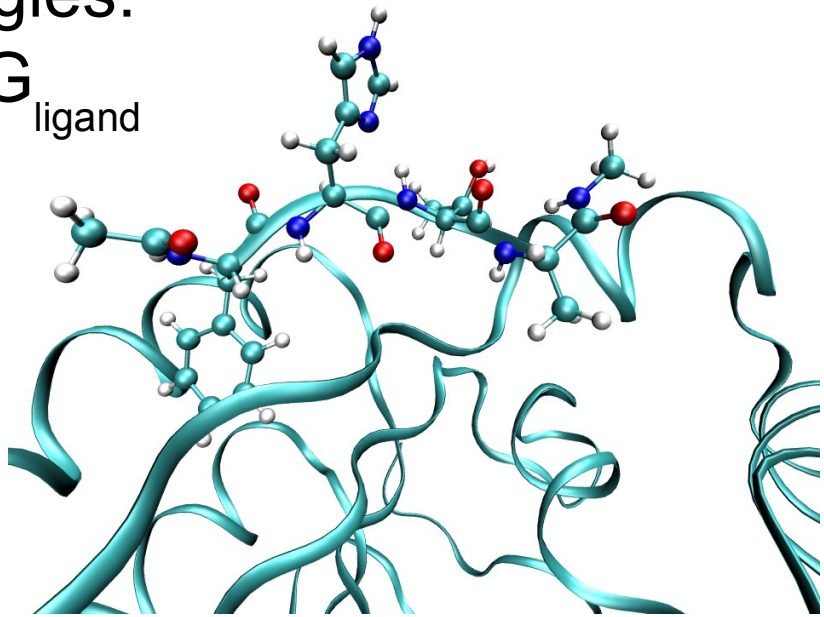
Stabilities of duplex DNA structures:
G(B-DNA) – G(A-DNA) ≈ -20 kcal/mol

Protein folding

Protein-ligand binding free energies:

$$\Delta G = G_{complex} - G_{receptor} - G_{ligand}$$

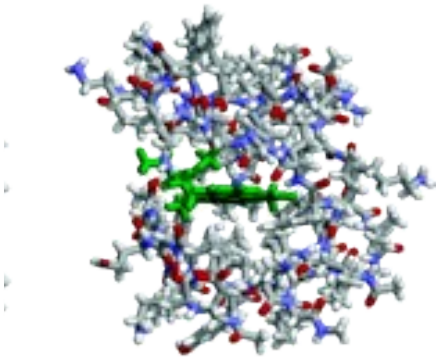
Small molecule targeting of protein-protein interactions in DNA repair pathways.



Applications

Ranking of free energies of binding between protein kinases and their inhibitors:

inhibitor	K_i (μM) exp.	ΔE_g(HB)	ΔE_g(7 Å)	ΔG_{solv}	$\Delta\Delta G$	$\Delta\Delta G$ exp.
1	*(1.2 ± 0.3)E+1*	−20.0	−18.3	19.7	0.0	*0.0*
2	*(1.3 ± 0.2)*	−18.5	−18.4	21.4	+1.6	*−1.4*
5	*(3.1 ± 0.6)E−2*	−14.6	−15.1	19.4	+2.9	*−3.7*
4	*(2.4 ± 0.8)E−2*	−37.1	−43.4	41.4	−3.4	*−3.8*
3	*(6.0 ± 0.5)E−3*	−43.3	−48.2	32.7	−14.1	*−4.7*



inhibitor	$\Delta\Delta G$	$\Delta\Delta G$ exp.	$e^{\Delta\Delta G/k_B T}$	K_i/K_i^{NU2058} exp.
1	0.0	*0.0*	1.0	*1.0*
2	−0.6	*−1.4*	3.8E − 1	*(1.1 ± 3.2)E − 1*
5	−4.9	*−3.7*	3.5E − 4	*(2.6 ± 0.8)E − 3*
4	−3.6	*−3.8*	2.9E − 3	*(2.0 ± 0.8)E − 3*
3	−5.3	*−4.7*	1.8E − 4	*(5.0 ± 1.3)E − 4*

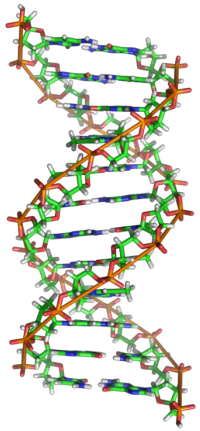
Intermittent H-bonds and polarisation effects reveal need for both dynamics and quantum level of accuracy.

L. Heady et al., J. Med. Chem. 49, 5141 (2006)

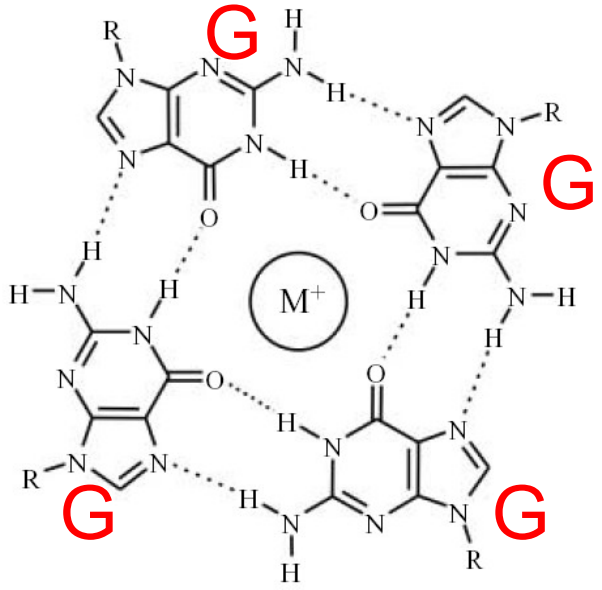
Four-Stranded DNA

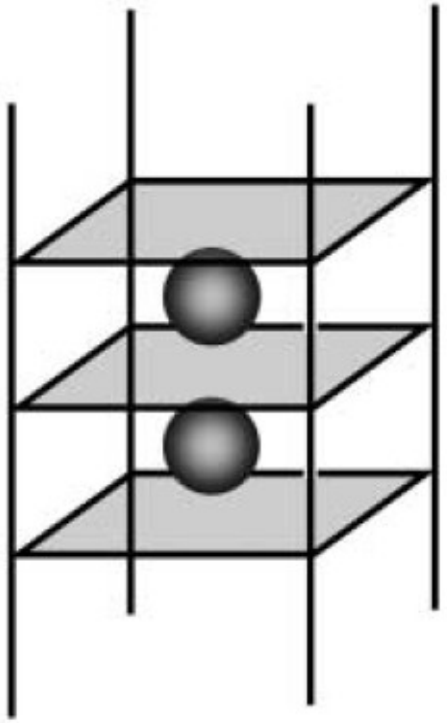
Adenine ↔ Thymine

Cytosine ↔ Guanine



Guanine-rich regions of DNA can form hydrogen-bonded G-quartets, which stack to form G-quadruplexes.





J. Huppert, Phil. Trans. R. Soc. A 365, 2969 (2007)

Telomeres

Human chromosomes end in an overhanging section of DNA called a telomere, which consists of the repeated sequence of nucleobases – d(GGGTTA)n – believed to form G-quadruplexes.

Telomeres distinguish the ends of the chromosome from breaks in the DNA.

Telomeres become shorter with every cell division. The cell dies when the telomere becomes too short.

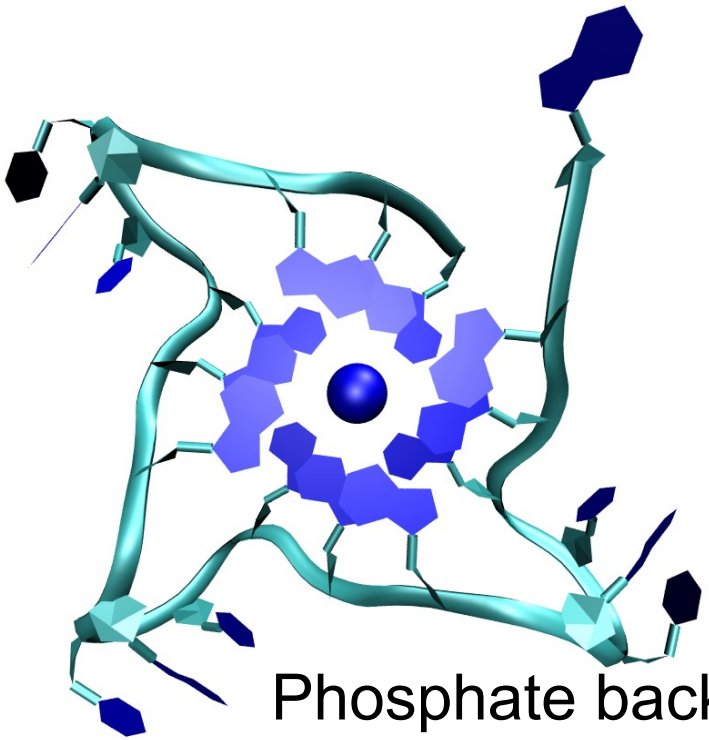
Telomerase is an enzyme, which can lengthen telomeres and keep the cell alive. Telomerase is active abnormally in 85% of cancers.

Drugs are designed to bind to G-quadruplexes and block telomerase activity → A good understanding of the human telomeric G-quadruplex structure is required.

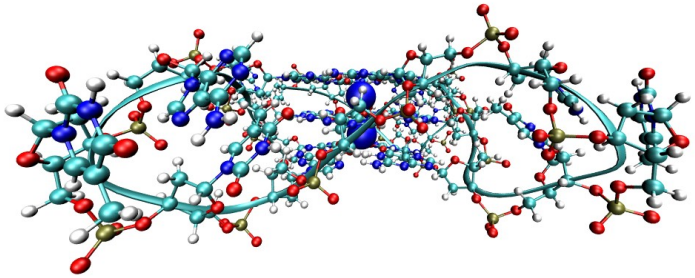
Human Telomeric G-Quadruplex

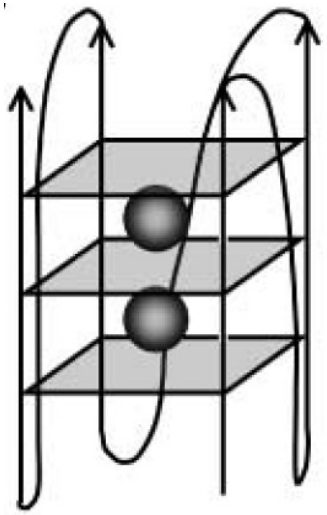
(At least) two different structures have been found using NMR and X-ray crystallography:

Parallel:



Phosphate backbone



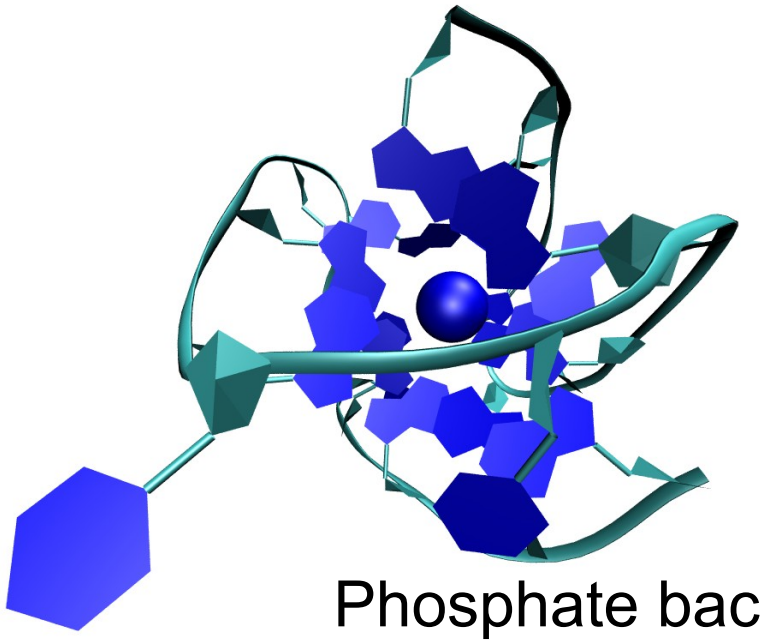


Monovalent metal ions

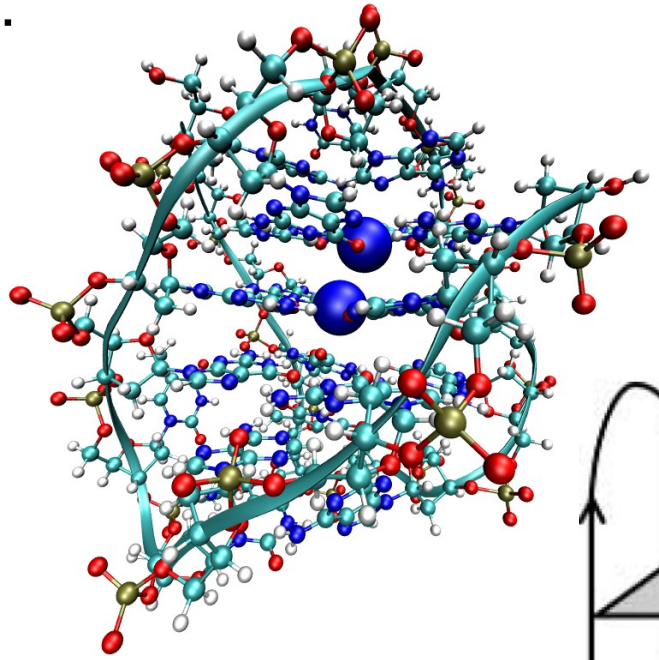
Human Telomeric G-Quadruplex

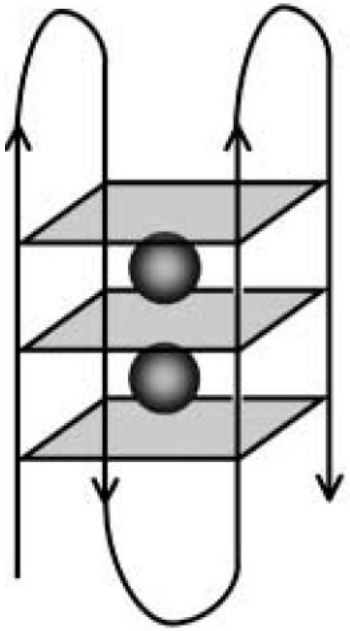
(At least) two different structures have been found using NMR and X-ray crystallography:

Anti-Parallel:



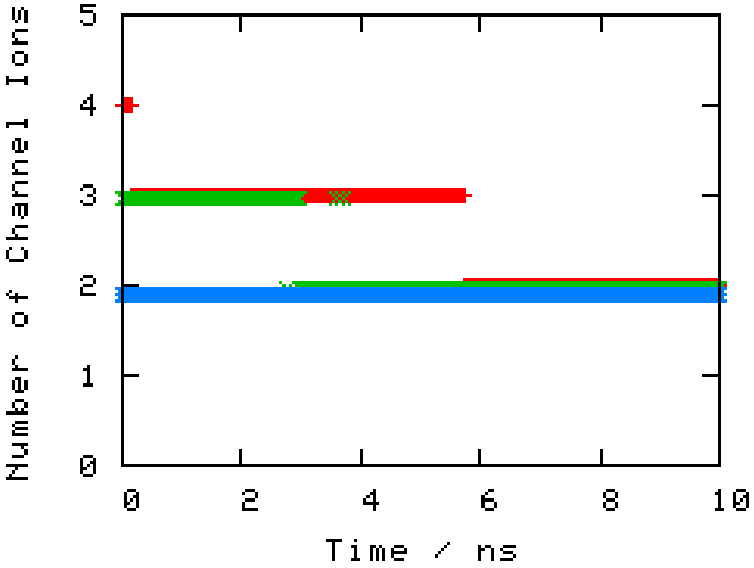
Phosphate backbone

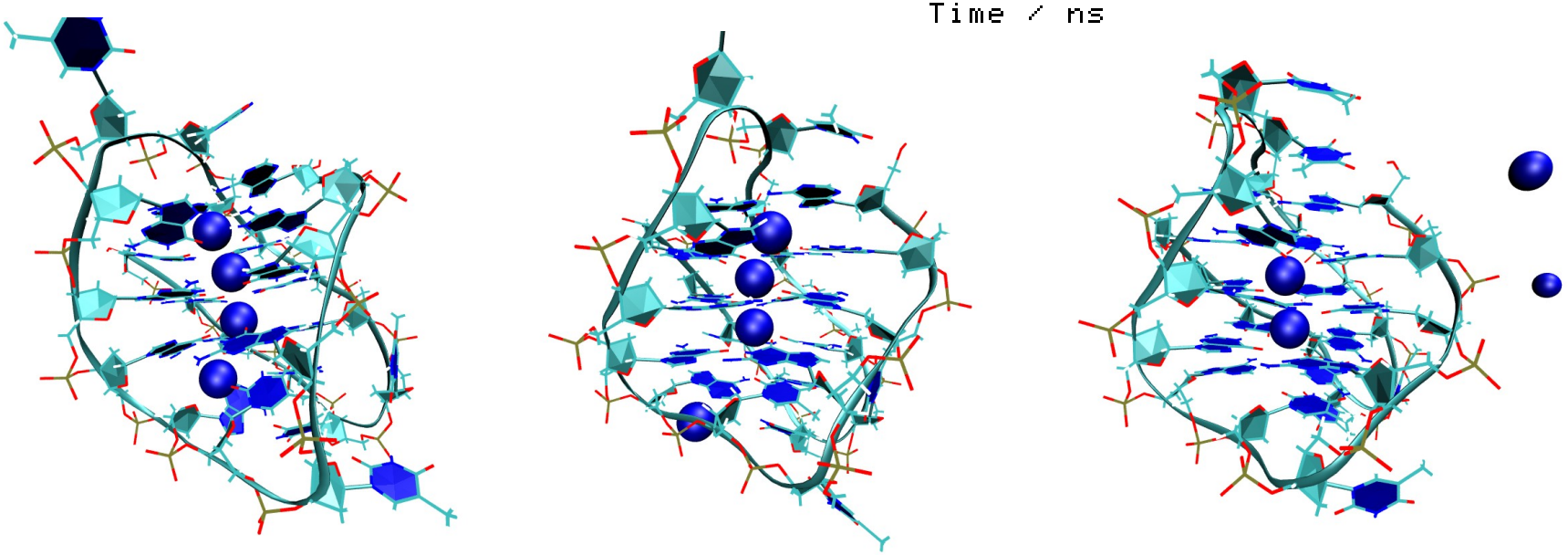




Channel Ions

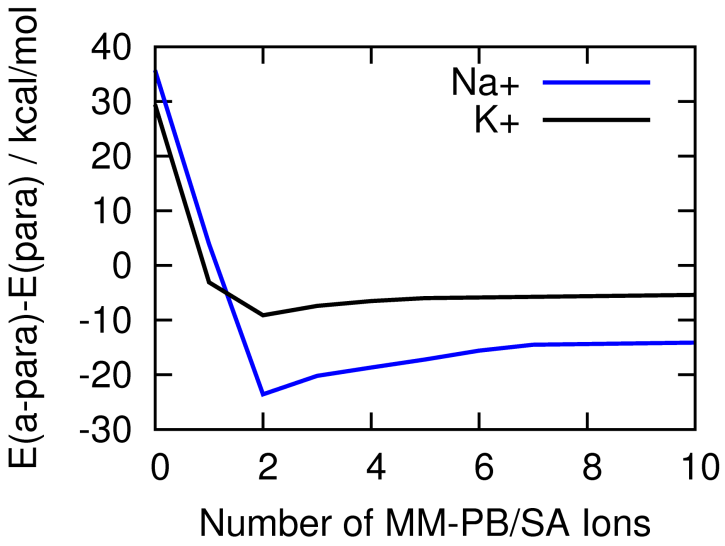
Anti-parallel structure in Na+ solution is stablised by two channel ions.





G-quadruplex structure in Na+

Relative free energies of structures depend on number of ions included in MM-PB/SA analysis
→ converged at ~10 ions

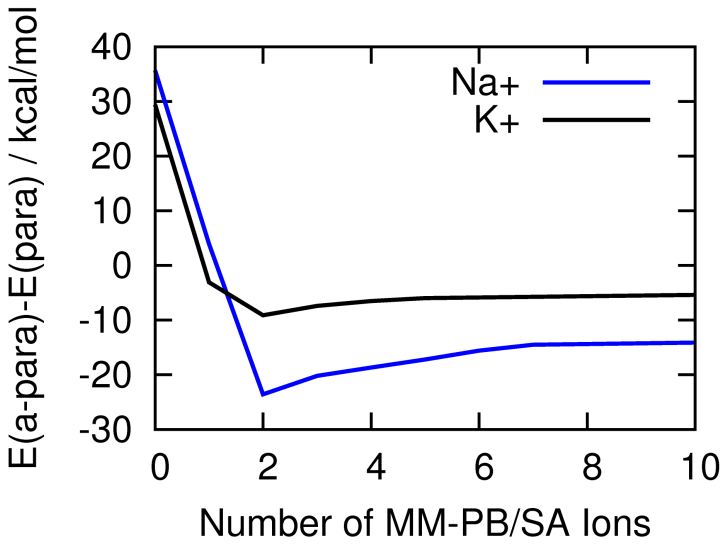


Free energy differences (kcal/mol). The anti-parallel structure is favoured in Na+, in agreement with experiment ($\Delta G < 1$ kcal/mol).

	EMM + GPBSA	-TS	GTOT
parallel	-5110.0	-582.0	-5692.0
anti-parallel	-5124.1	-572.3	-5696.4
a-para - para	-14.1	+9.7	-4.4

G-quadruplex structure in K+

Relative free energies of structures depend on number of ions included in MM-PB/SA analysis
→ converged at ~10 ions



Free energy differences (kcal/mol). The parallel structure is favoured in K+, in agreement with experiment ($\Delta G < 1$ kcal/mol)(?)

	EMM + GPBSA	-TS	GTOT
parallel	-4927.3	-583.0	-5510.3
anti-parallel	-4932.7	-576.7	-5509.4
a-para - para	-5.4	+6.3	+0.9

Metal Ion Selectivity

By taking into account, the free energy of solvation of the metal ions, we can calculate the free energy barrier for the exchange reaction:

Anti-parallel(Na+) + K+ → Parallel(K+) + Na+

ΔG = +1.1 kcal/mol (Na+ structure is favoured)

Experimentally, the K+ structure is favoured in equimolar solution (ΔG = -2.8 kcal/mol), but we have not considered the hybrid structure thought to exist in K+ solution.

MM-PB/SA Convergence

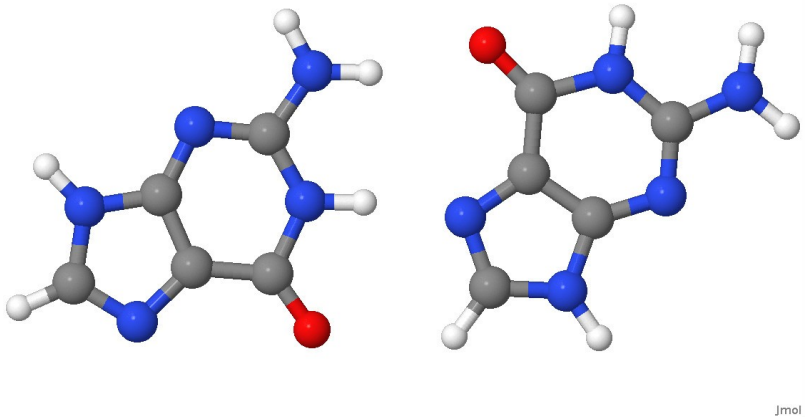
Length of trajectory	G(para)	G(a-para)	ΔG
0-3ns	-5692.0	-5696.4	-4.4
3-6ns	-5686.4	-5696.4	-10.0

Need to investigate change in parallel structure.

Number of snapshots	EMM + GPBSA
25	-4295.7
50	-4298.9
150	-4298.1
500	-4298.6
1500	-4298.6

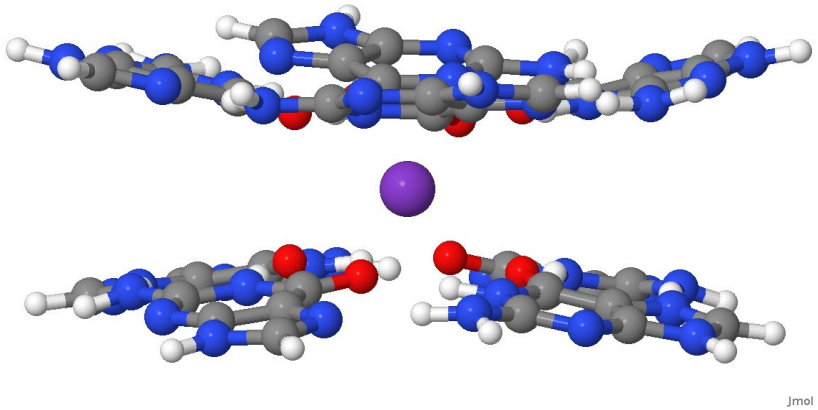
Free energy converged after sampling small number of snapshots
→ encouraging for use of ONETEP

ONETEP comparisons



G-G Dimer
Vacuum binding energy / kcal/mol:

MP2	-18.5
AMBER	-19.4
ONETEP	-20.3



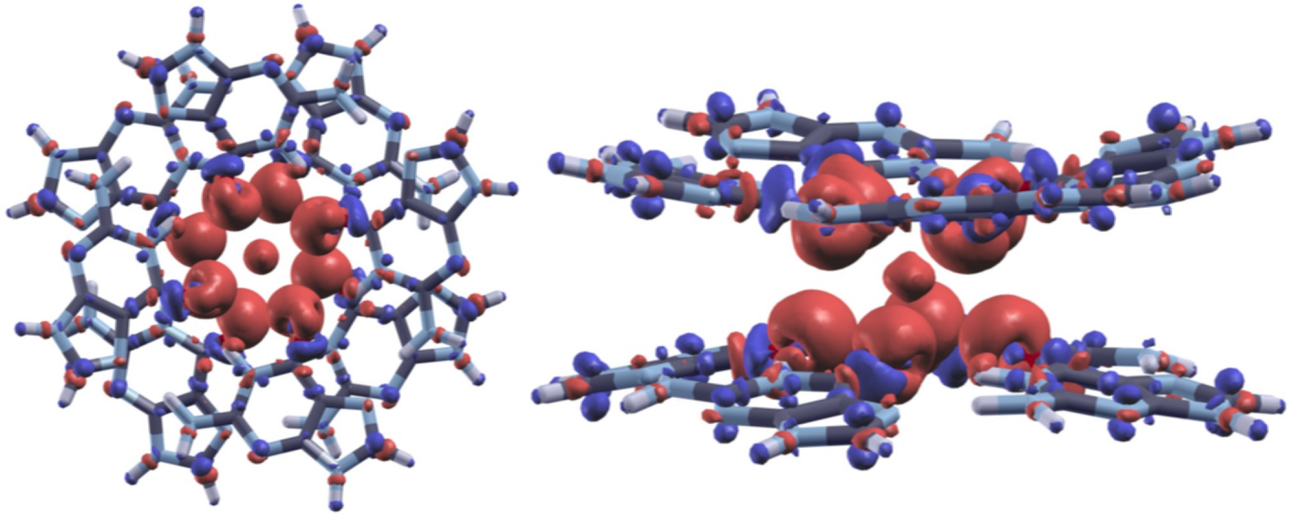
G4 - K+ - G4 Tetrad
Base-ion binding energy / kcal/mol:

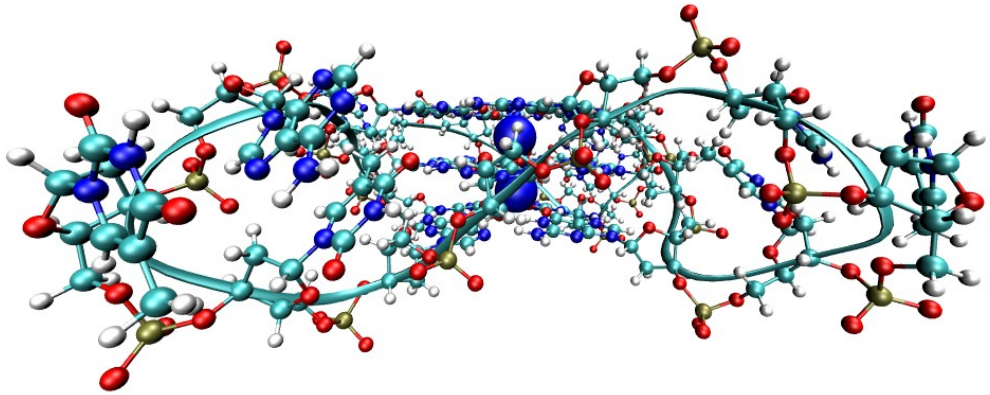
B3LYP-DFT	-121.2
ONETEP	-119.0

But these do not accurately represent the biomolecular structure.

Polarisation Effects

Electron charge density difference on adding K+ ion to stacked G-tetrads





Mulliken population analysis reveals charges ranging from -0.84 to -0.95 e^- on O atoms neighbouring Na+ ion.

Conclusions and Further Work

- G-quadruplexes adopt structures that are very close in free energy. Metal ions may act as conformational switches.
- Mobile ions require dynamic analysis, while polarisation effects require quantum accuracy.
- Compare ONETEP gas phase energies and solvation free energies with classical results.
- Look at binding affinities of ligands to different structures.
- Investigate reasons for particular stability of G-quadruplexes in RNA.

Acknowledgements

Julian Huppert

Chris-Kriton Skylaris

Mike Payne, Michael Rutter,
Mark Robinson

EPSRC and
Cambridge HPCS

ONETEP parameters

~700 atoms

800 eV psinc basis KE cut-off

PBE GGA XC-functional

Dispersion energy contributions included through damped Lennard-Jones potentials.

Hill and Skylaris, Proc. R. Soc. A 465, 669 (2009)