What drives DNA folding into G-quadruplex structures and their recognition by ligands?

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Outline

Introduction

- DNA conformations
- Human telomeric (ht) DNA fragment Tel22 = $AGGG(TTAGGG)_3$
- G-quadruplex ligands

Method

- Global thermodynamic analysis of experimental unfolding and binding data

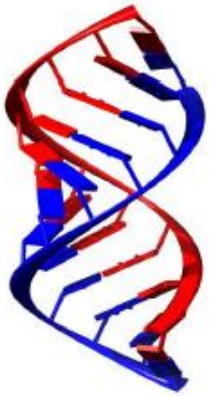
Results

- Driving forces of quadruplex folding and ligand binding

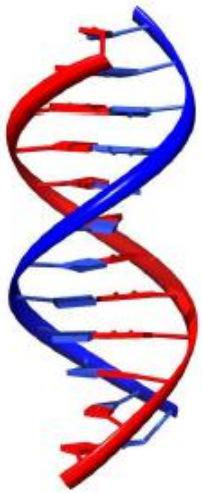
Conclusions

- Meaningful findings

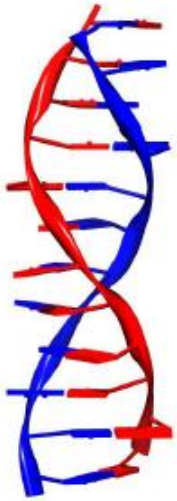
DNA conformations



A-DNA



B-DNA

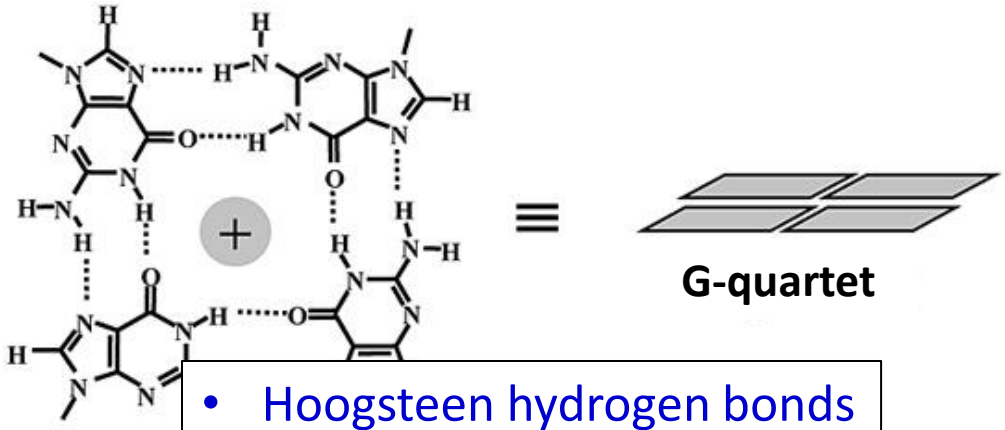


Z-DNA

triplex

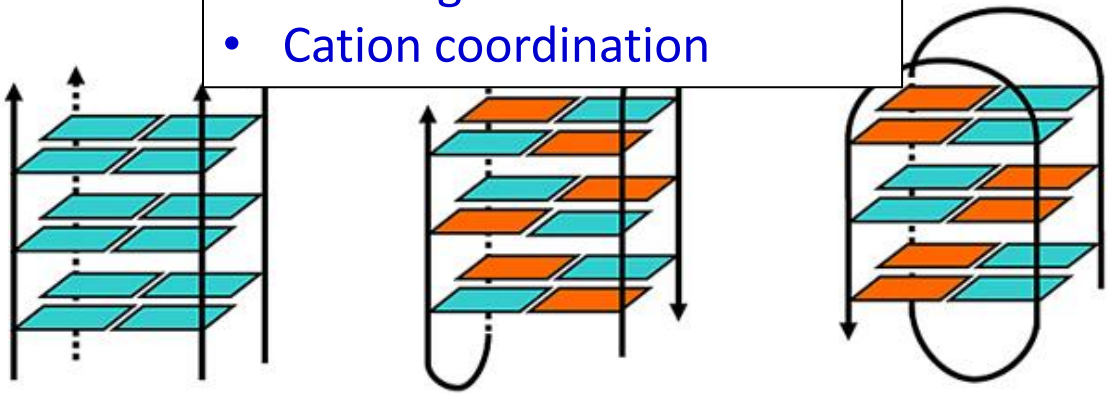
quadruplex

G-quadruplexes



G-quartet

- Hoogsteen hydrogen bonds
- Stacking interactions
- Cation coordination



intermolecular

tetrameric

dimeric

intramolecular

monomeric

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GDCh

International Edition

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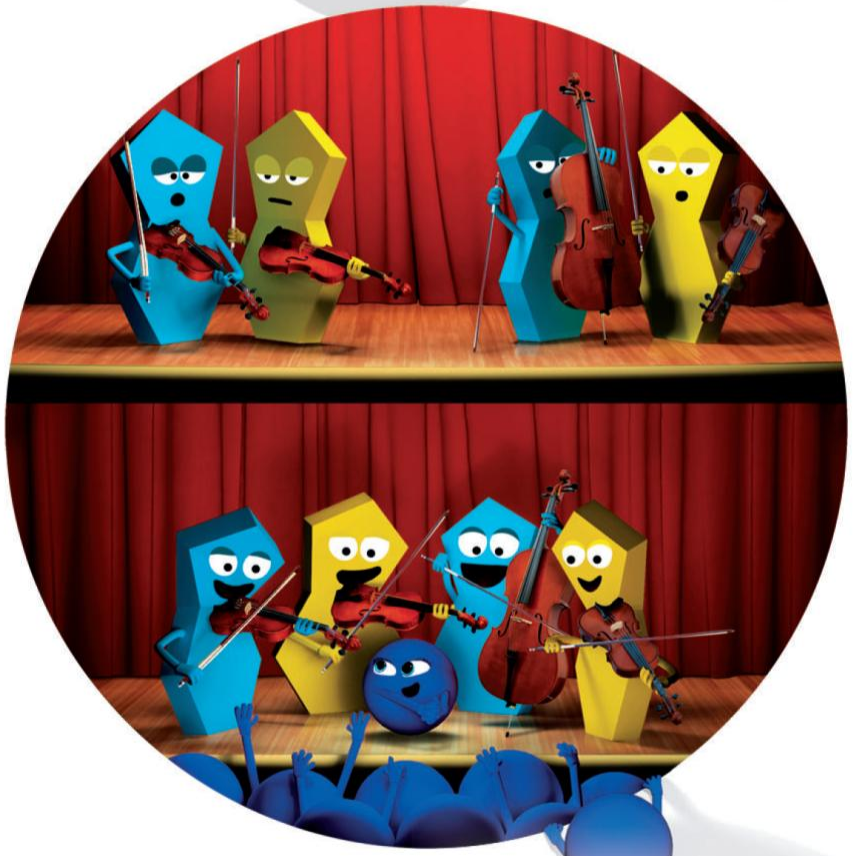
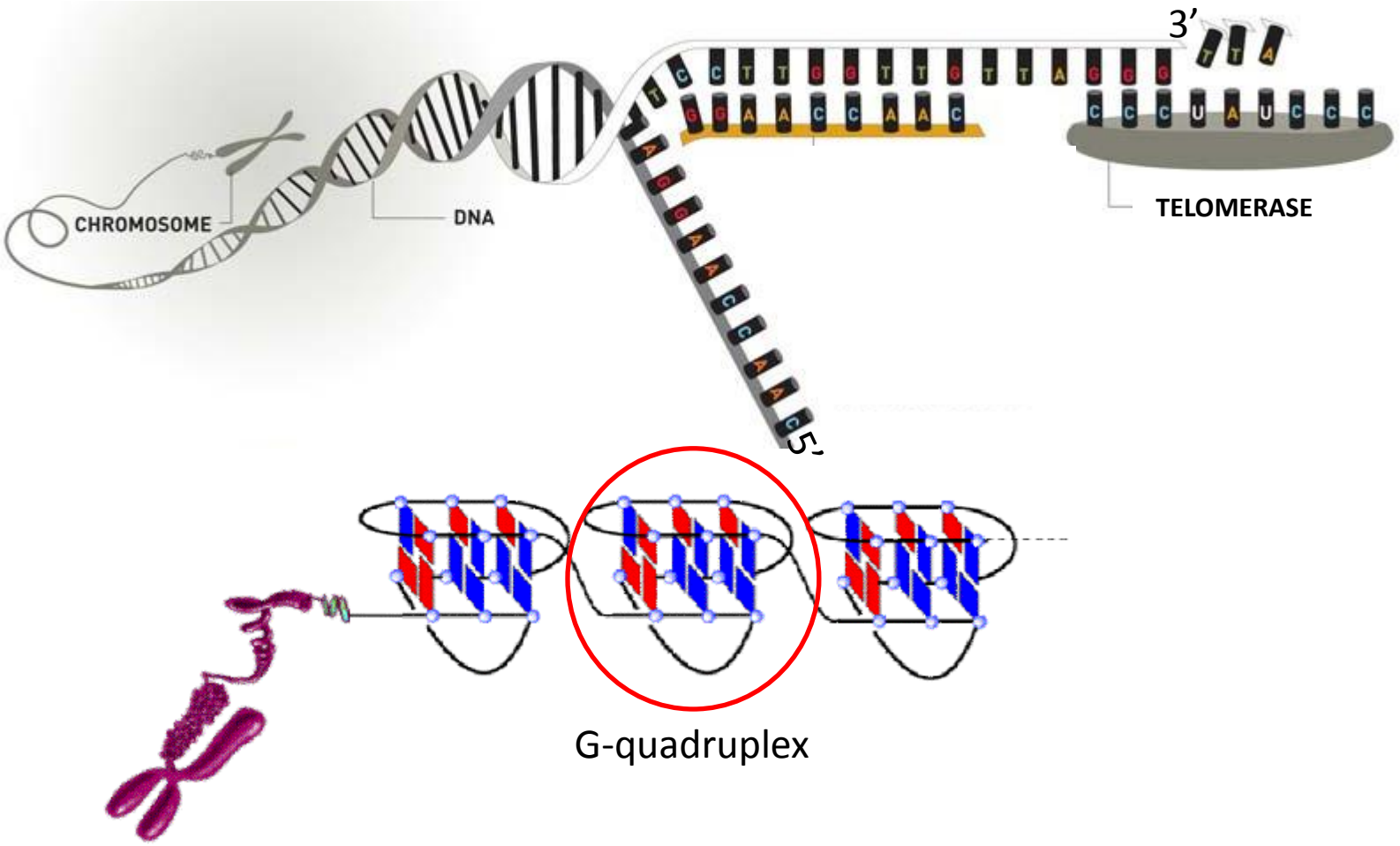


Guanine-rich DNA sequences can self-assemble into specific pre-organized DNA structures, where two by two guanine residues shown as "duets" are involved in GG-N1-carbonyl symmetric base pairs. In their Communication (DOI: 10.1002/anie.201400531), J. Lah, J. Plavec, and co-workers demonstrate the transition of these "duets" into guanine-quartets, presented as a "string quartet" when interacting with cations such as potassium ions. This rearrangement consequently leads to formation of G-quadruplex structures.

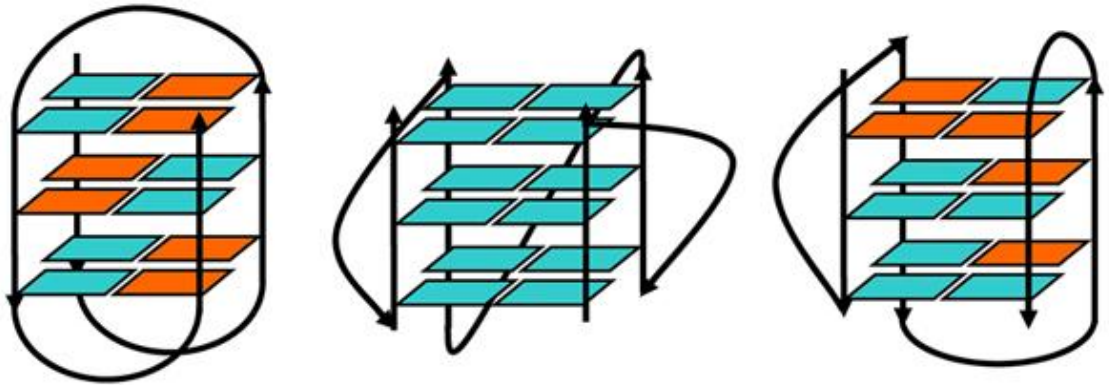
WILEY-VCH

Human telomeric DNA

$(TTAGGG)_N$
3'-overhang; 100-200 bases



Model system: Tel22 = $AGGG(TTAGGG)_3$



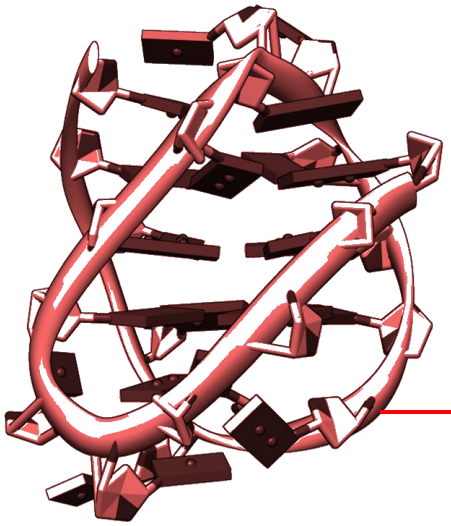
antiparallel
solution (Na^+)

parallel
crystal (K^+)
solution (K^+, n.a. solvents, PEG)

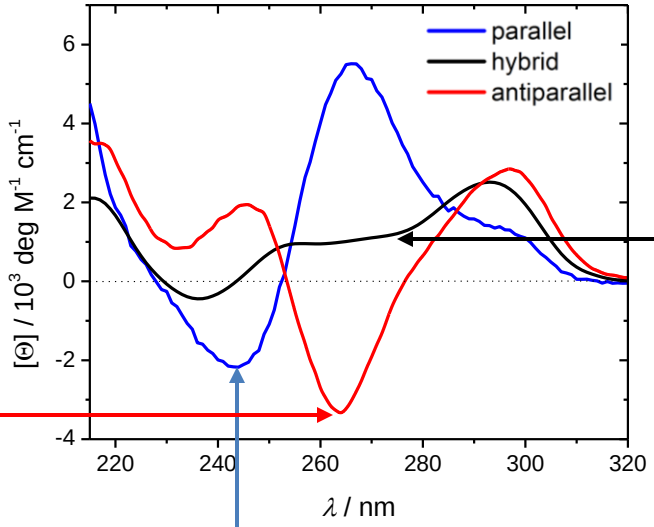
hybrid
solution (K^+)

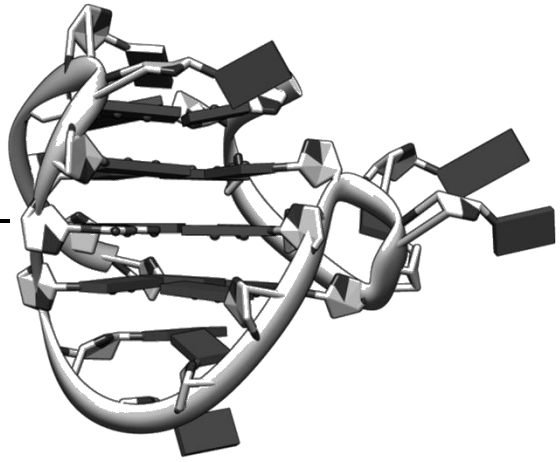
G.N. Parkinson et al. *Nature* 2002, **417**, 876; A. Ambrus et al. *Nucleic Acids Res.* 2006, **34**, 2723.
A.T. Phan et al. *Nucleic Acids Res*. 2007, **35**, 6517; R.D. Gray et al. *Biochemistry* 2010, **49**, 179.
K.N. Luu et al. *J. Am. Chem. Soc*. 2006, **128**, 9963; Y. Wang et al. *Structure* 1993, **1**, 263....

Structural features

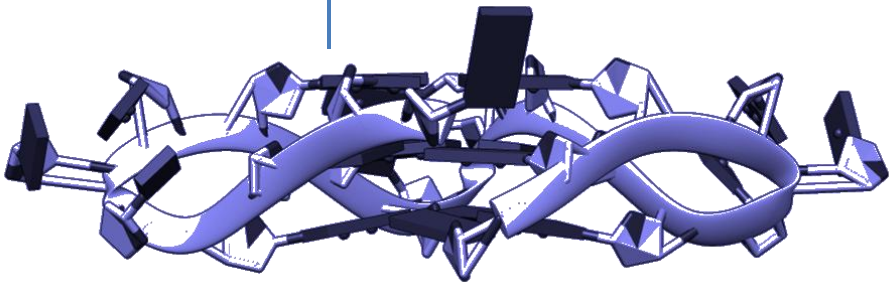


antiparallel (A_{Na+})





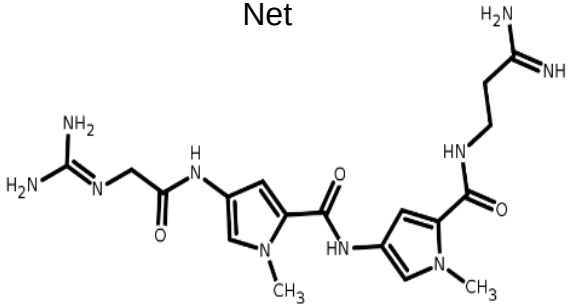
hybrid (H_{K+})



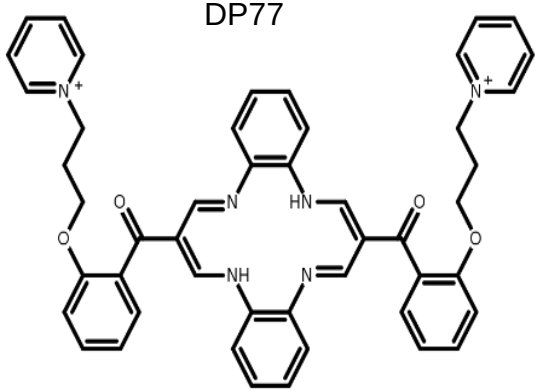
parallel (P_{K+})

Ligands

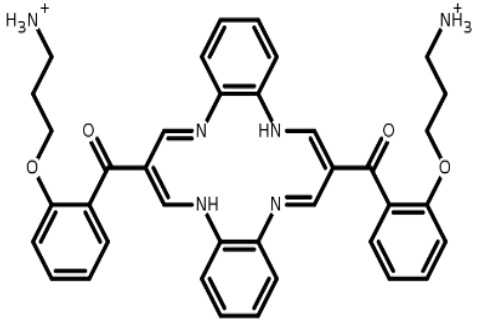
Net

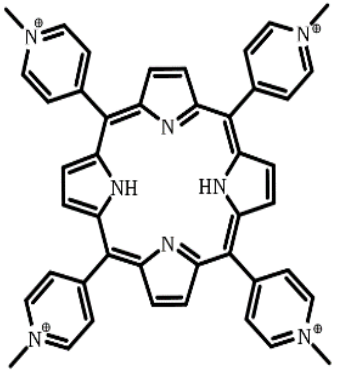


DP77

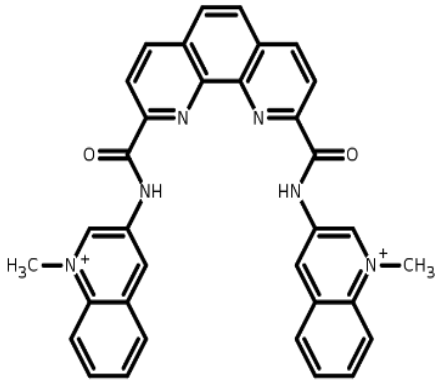


DP78

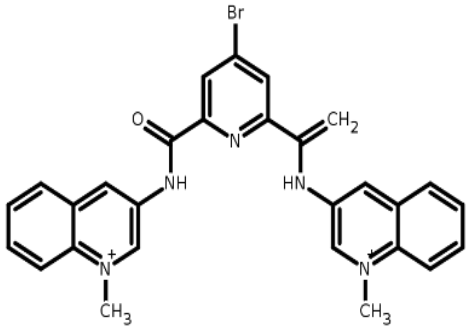




TMPyP4



Phen-DC3



360A-Br

$K_{quadruplex} / K_{duplex} > 50$

Energetic basis of quadruplex folding and ligand binding

Why thermodynamic driving forces of quadruplex folding and ligand binding remain poorly understood?

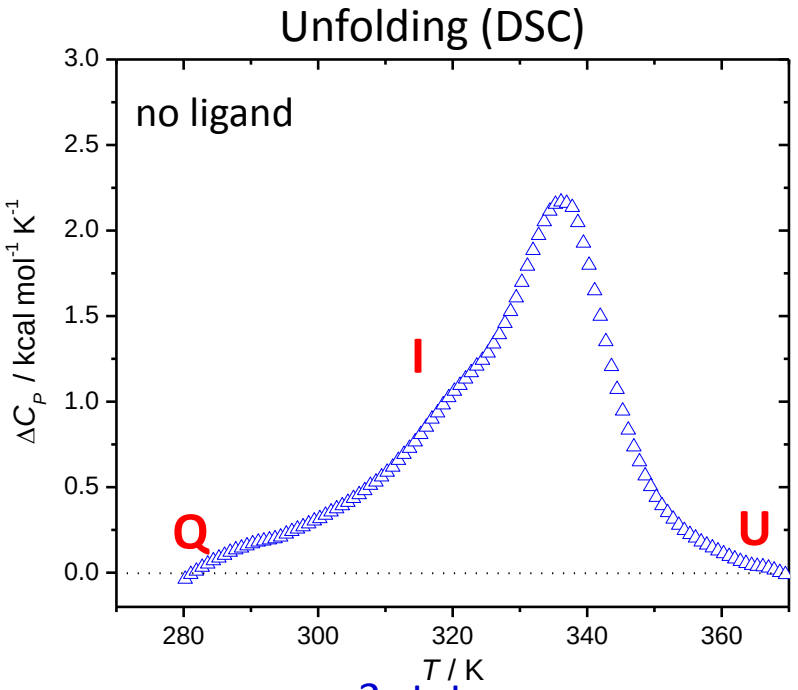
- Quadruplex folding is intrinsically complex: Mechanism?
- Hydration is a major determinant of DNA stability and conformation: $\Delta C_{P,i}$?
- Thermodynamic studies of ligand binding: Folding intermediates?
- "The role of "hydrophobic" forces in DNA folding in general is in fact well known?" (Referee of our manuscript in *2014*)

Energetic basis of quadruplex folding and ligand binding

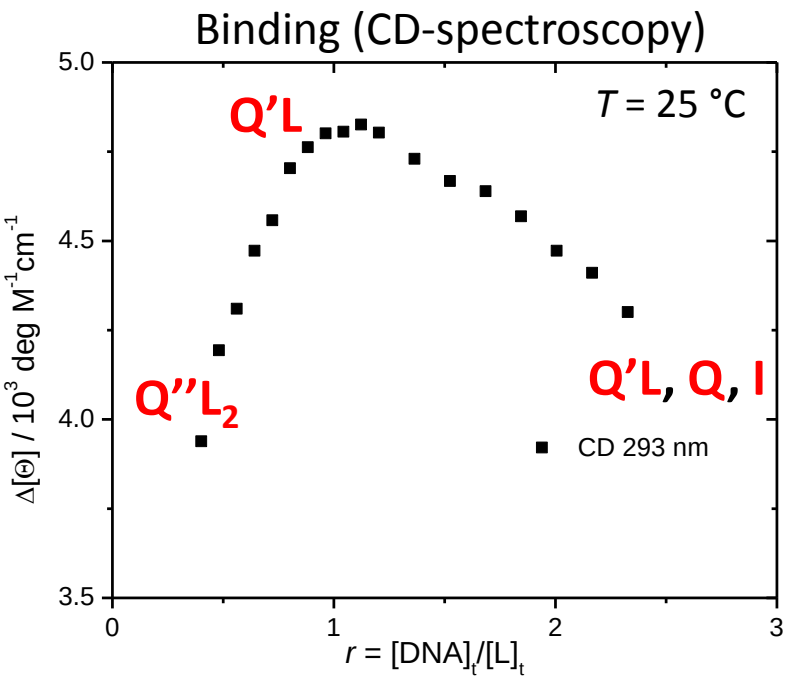
Questions:

- What is the mechanism of folding and binding?
- What drives folding and binding events?
- Are predictions of thermodynamic analysis consistent with structural features?

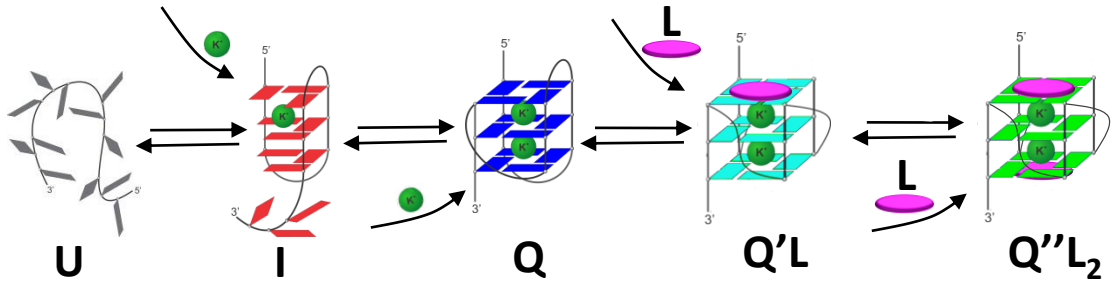
Mechanism (Tel22, ligand = Phen-DC3, K^+ ions)?



3 states

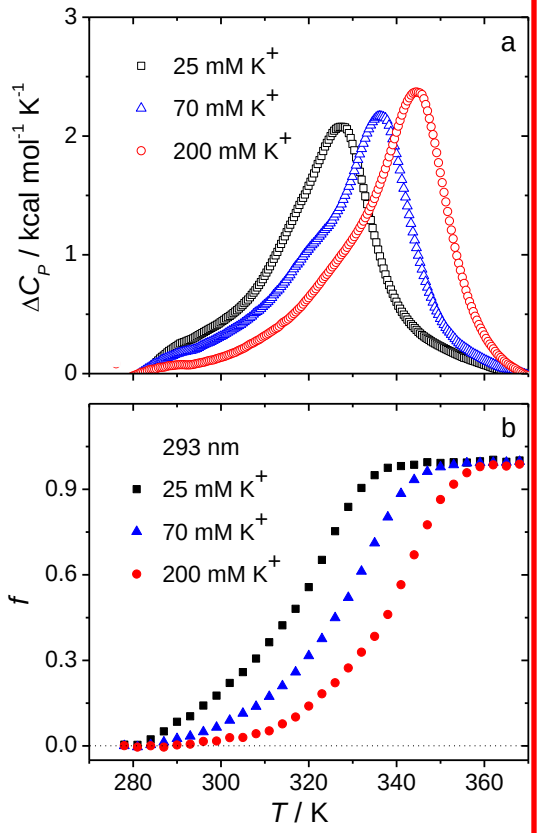


2 complex species with 1 or 2 bound L molecules

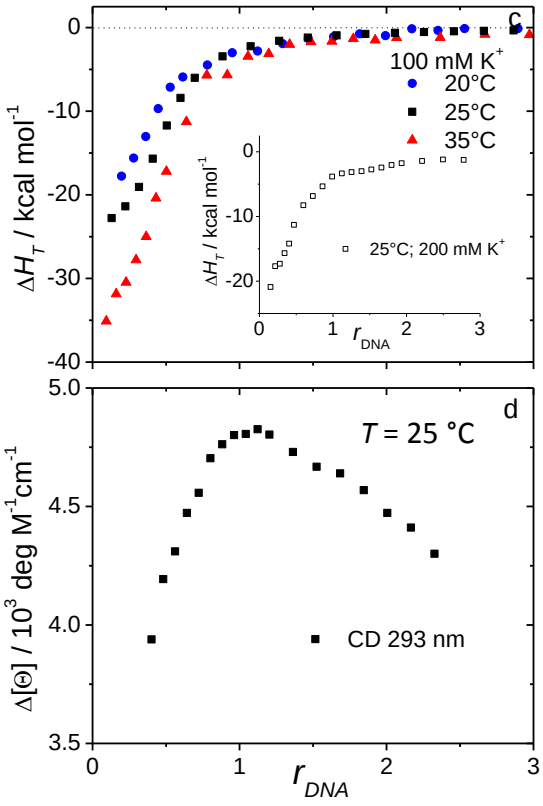


Calorimetry and spectroscopy

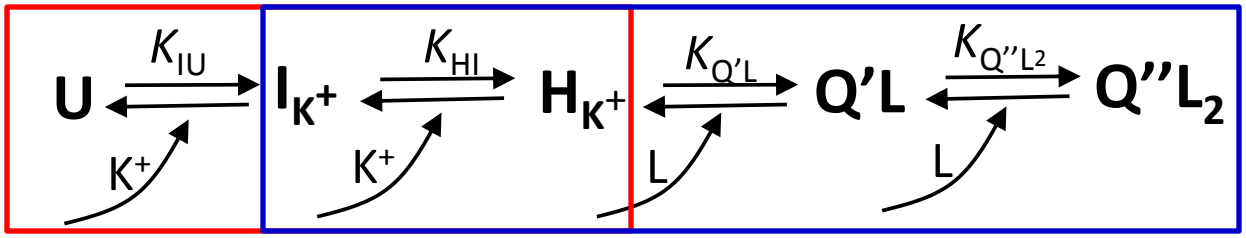
Melting (DSC, CD)



Titrations (ITC, CD)



Modeling folding and binding data



melting

$$\text{DSC: } \Delta C_P = (\partial \alpha_{\mathrm{I}}/\partial T)\Delta H^{\circ}_{\mathrm{FI}(T)} + (\partial \alpha_{\mathrm{U}}/\partial T)\Delta H^{\circ}_{\mathrm{FU}(T)}$$

$$\text{CD: } f = \frac{[\theta] - [\theta]_{\mathrm{F}}}{[\theta] - [\theta]} = \alpha_{\mathrm{I}} f_{\mathrm{FI}} + \alpha_{\mathrm{IU}}$$

How the fractions (α_i) depend on temperature, DNA, salt, and ligand concentrations?

titrations

$$\left((\partial n_{\mathrm{I}}/\partial n_{\mathrm{DNA}})_{n_{\mathrm{L,tot}}} - (\partial n_{\mathrm{I}}/\partial n_{\mathrm{DNA}})_{n_{\mathrm{L}=0}}\right)\Delta H^{\circ}_{\mathrm{QI}(T)}$$

$$\text{CD: } \Delta[\theta] = \Delta[\theta]_{\mathrm{QI}}\alpha_{\mathrm{I}} + \Delta[\theta]_{\mathrm{Q'L}}\alpha_{\mathrm{Q'L}} + (\Delta[\theta]_{\mathrm{Q'L}} + \Delta[\theta]_{\mathrm{Q''L_2}})\,\alpha_{\mathrm{Q''L_2}}$$

Model functions are related to concentrations of species (fractions = α_i) at any temperature, DNA, salt, and ligand concentrations!

Thermodynamics of folding and binding

Adjustable parameters:

$\Delta G^{\circ}_{\mathrm{ij}(T_0)}$ = Δ(Gibbs free energy)

$\Delta H^{\circ}_{\mathrm{ij}(T_0)}$ = Δ(enthalpy)

$\Delta C^{\circ}_{\mathrm{P,ij}}$ = Δ(heat capacity)

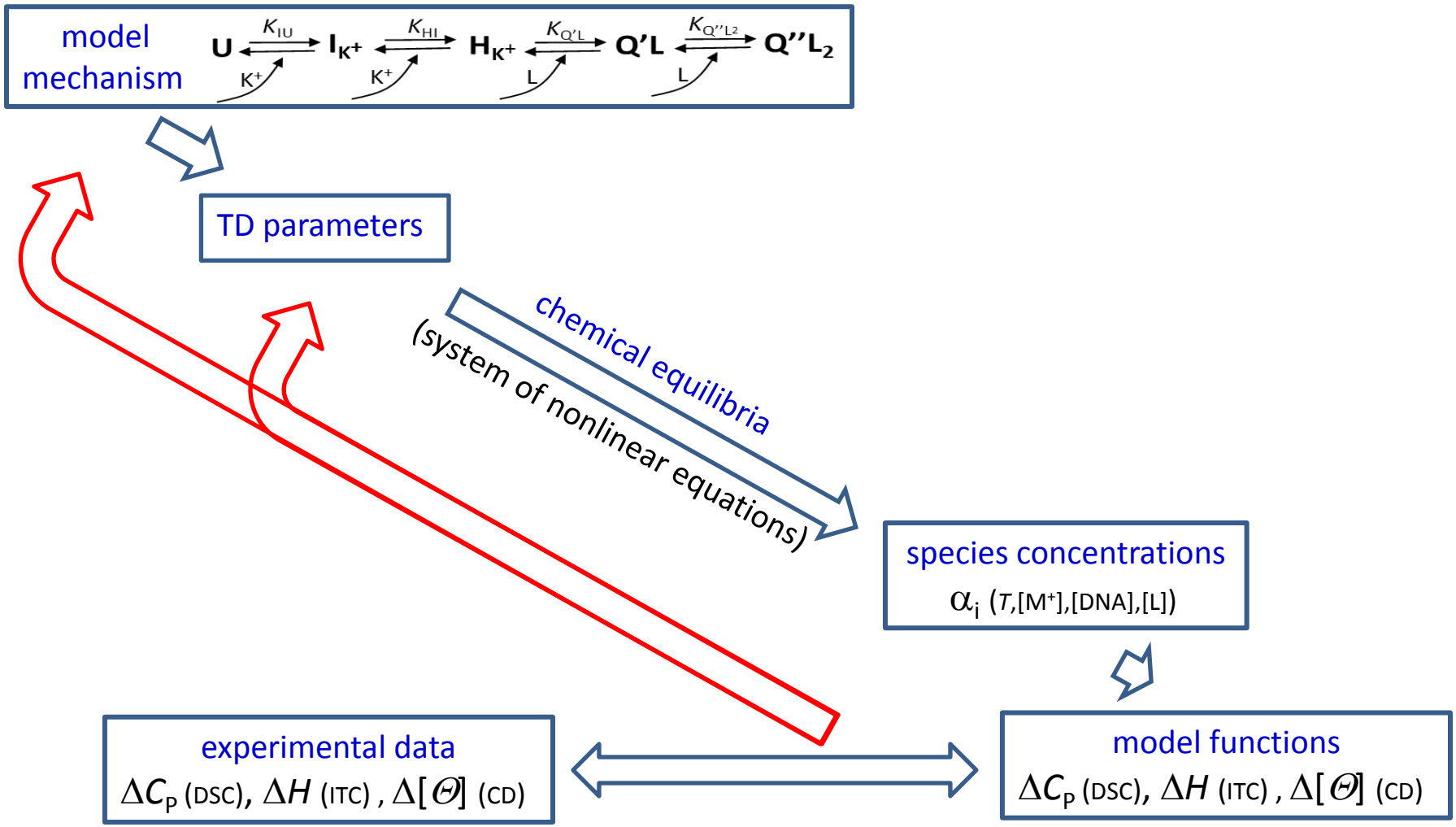
n_{ij} = Δ(no. exchanged cations)

$$\Delta G^{\circ}_{\mathrm{ij}(T,\mathrm{K}^+)} = \Delta G^{\circ}_{\mathrm{ij}(T_0)}\, T/T_0 + \Delta H^{\circ}_{\mathrm{ij}(T_0)}[1 - T/T_0] + \Delta C^{\circ}_{\mathrm{P,ij}}[T - T_0 - T\ln(T/T_0)] + n_{\mathrm{ij}} RT \ln[\mathrm{K}^+]$$

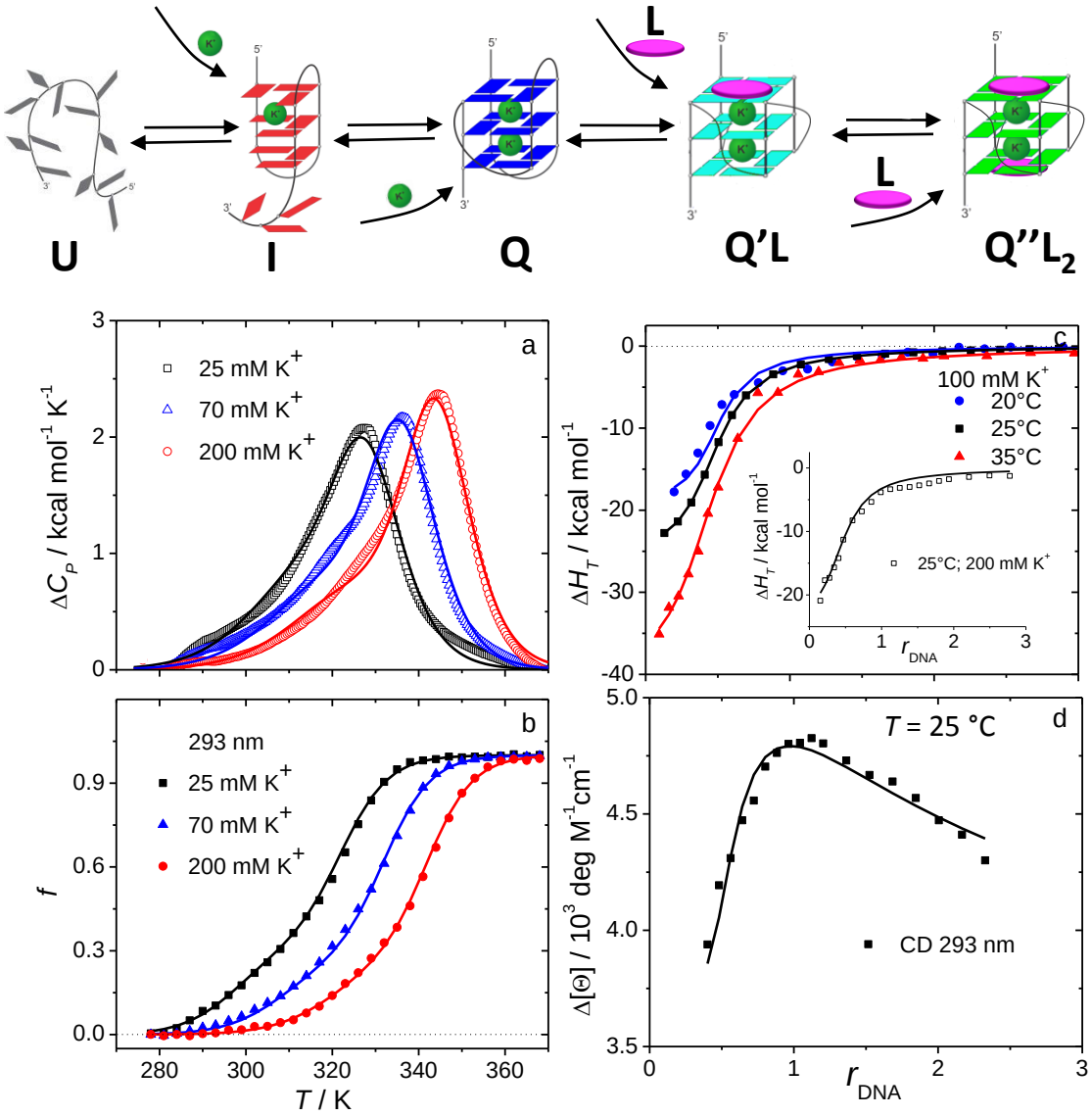
$$K_{\mathrm{ij}(T,\mathrm{K}^+)} = \exp(-\Delta G^{\circ}_{\mathrm{ij}(T,\mathrm{K}^+)}/RT) \Rightarrow \boxed{\alpha_{\mathrm{i}(T,\mathrm{K}^+)} \Rightarrow \frac{\partial \alpha_{\mathrm{i}}}{\partial T}, \frac{\partial \alpha_{\mathrm{i}}}{\partial r}}$$

Model functions are completely defined at any temperature, DNA, salt, and ligand concentrations!

Global thermodynamics analysis



Global thermodynamics analysis



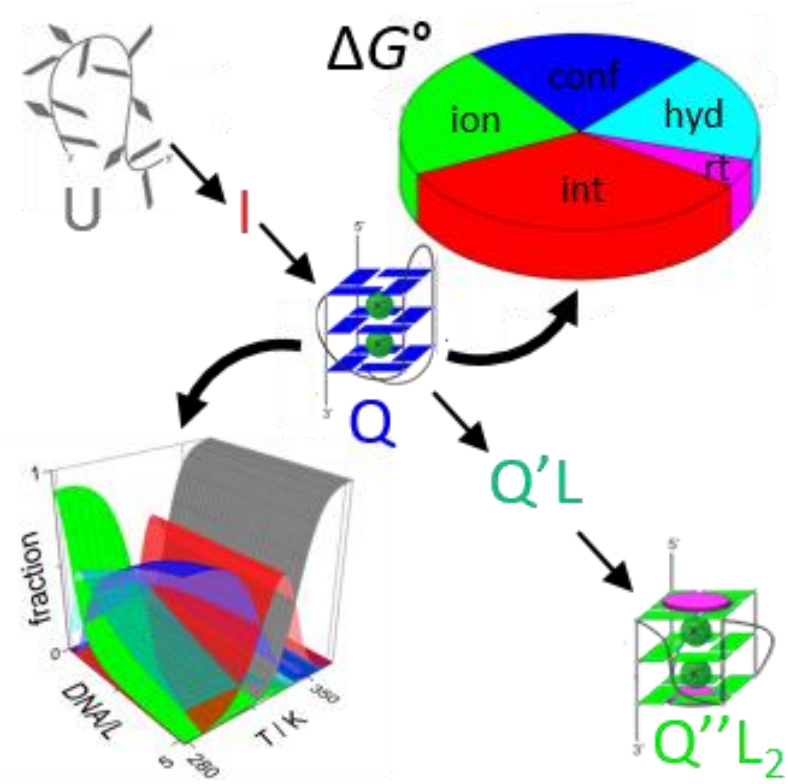
Advantages of global fitting

I. Drobnak et al. (2010). *J. Phys. Chem. B* **114**, 8713.

- More experimental points per fitted parameter
- Greater variety of data analyzed simultaneously
- More accurate parameter values
- **More rigorous test of model appropriateness**

Angewandte Chemie, 2014, 53, 4881; *ChemPhysChem*, 2014, 15, 1827.
J. Am. Chem. Soc., 2013, 135, 1288; *J. Biol. Chem.*, 2012, 287, 8613.
J. Am. Chem. Soc., 2012, 134, 9657; *Nucleic Acids Res.*, 2011, 39, 1933.
J. Agric. Food Chem., 2011, 59, 727; *J. Phys. Chem. B,* 2010, 114, 8713.
J. Phys. Chem. B, 2010, 114, 4313; *J. Biol. Chem.*, 2010, 285, 5606.
J. Biol. Chem., 2009, 284, 20002; *J. Mol. Biol.*, 2009, 392, 63.
J. Am. Chem. Soc., 2008, 130, 14161; *Nucleic Acids Res.*, 2008, 36, 897.
Biophys. J. 2015, in press...

Thermodynamics analysis ($\Delta G° = \Delta H° - T\Delta S°$)



$\Delta G° = \Delta G°_{ion} + \Delta G°_{hyd} + \Delta G°_{int} + \Delta G°_{rt} + \Delta G°_{conf}$

$\Delta G°_{ion}$ … ion desolvation/solvation
(Y.A. Marcus, *Biophys. Chem.*, 1994, **51**, 111)

$\Delta G°_{hyd}$ … DNA and ligand desolvation/solvation
($\Delta G°_{hyd} \approx \Delta C°_p 80(\pm 10)$K at 25 °C)

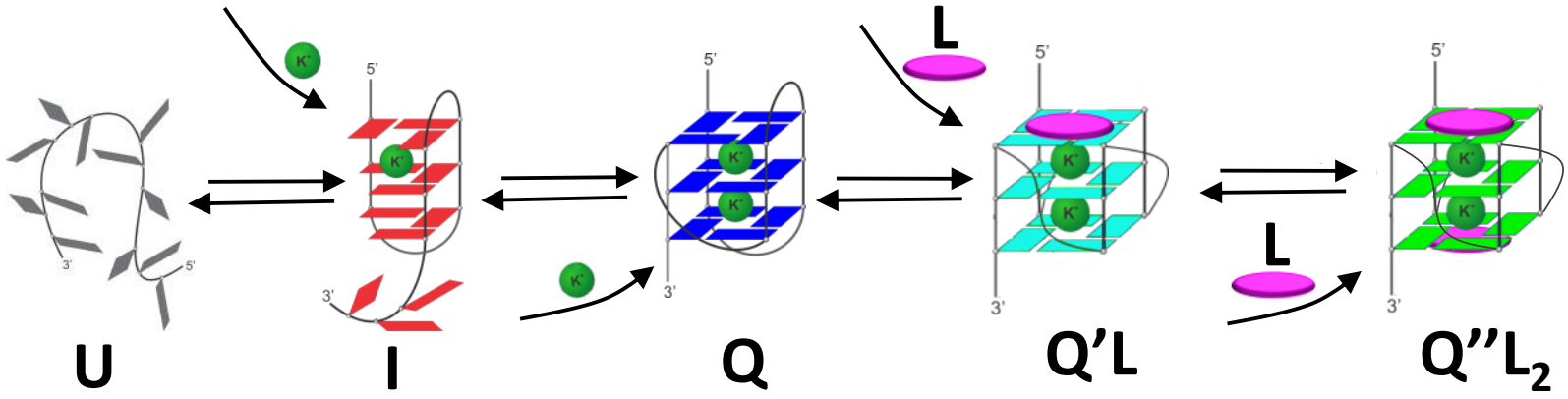
$\Delta G°_{int}$ … stacking, H-bonding, Coulombic interactions, cation coordination ($\Delta G°_{int} \approx \Delta H° - \Delta H°_{ion}$)

$\Delta G°_{rt}$ … rotational and translational freedom of ligand and DNA (R.S. Spolar, M.T. Record, *Science*, 1994, **263**, 777)

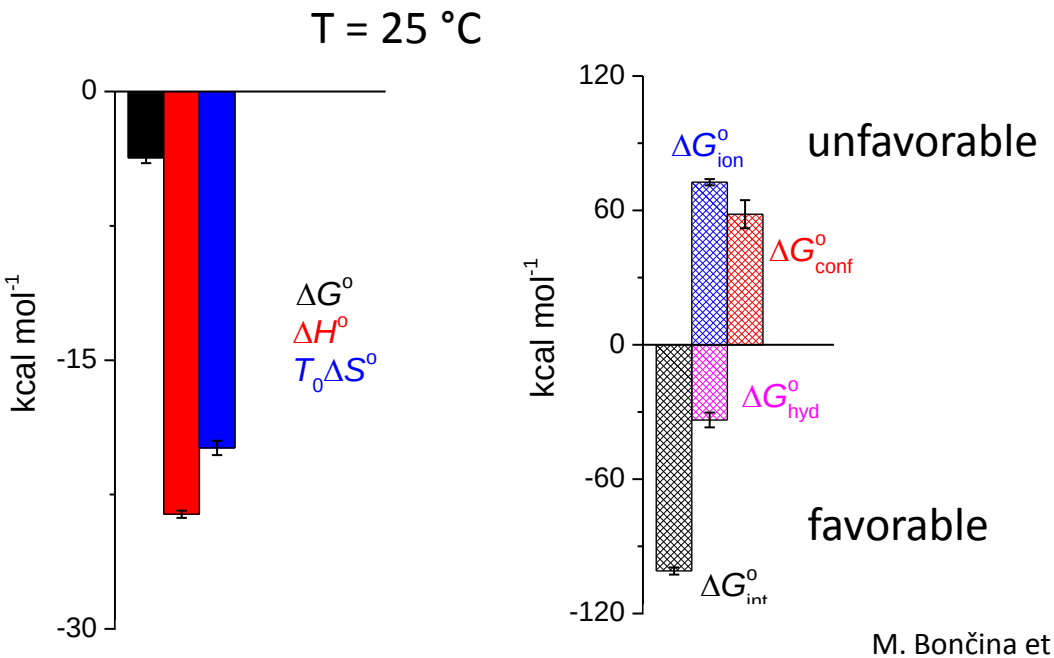
$\Delta G°_{conf}$ … conformation
($\Delta G°_{conf} = \Delta G° - \Delta G°_{solv} - \Delta G°_{int} - \Delta G°_{rt}$)

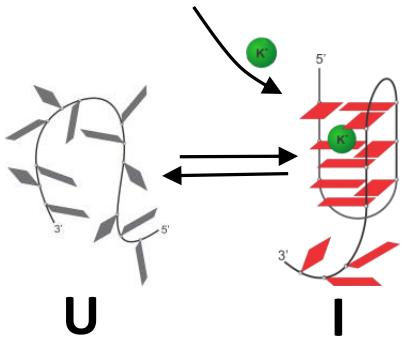
M. Bončina et al. *Biophys. J.,* 2015, in press.

Thermodynamic driving forces



Thermodynamic driving forces



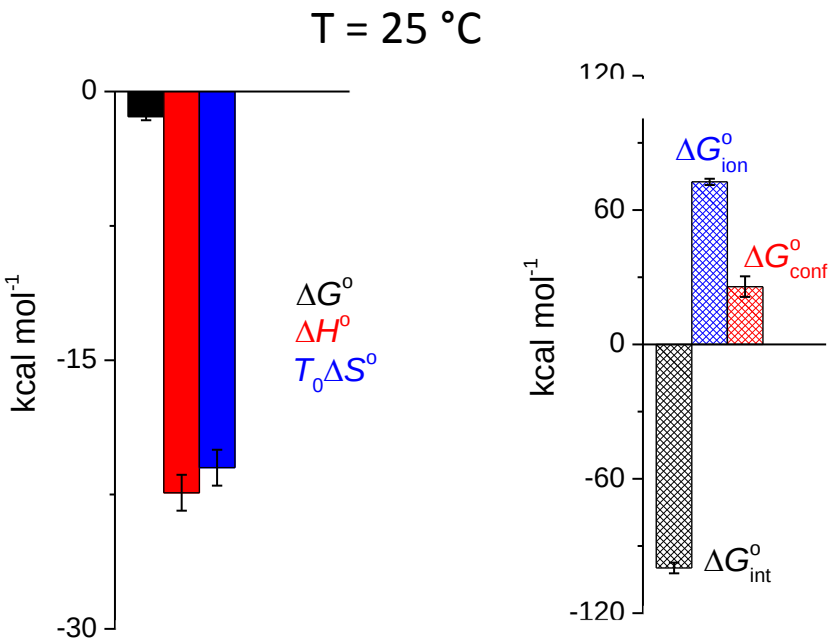


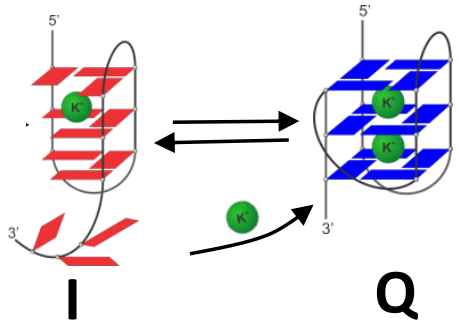
$\Delta C°_P$ = -400 cal mol^{-1} K^{-1}

M. Bončina et al. *Biophys. J.* 2015, in press.
M. Bončina et al. *J. Am. Chem. Soc*. 2012, **134**, 9657.
H. Sugiyama *et al. J. Am. Chem. Soc*. 2010, **132**, 14910.

- Removal of hydrophobic groups (thymines) from water [$\Delta G°_{hyd} < 0$].
- Specific interactions [$\Delta G°_{int} < 0$].

Thermodynamic driving forces





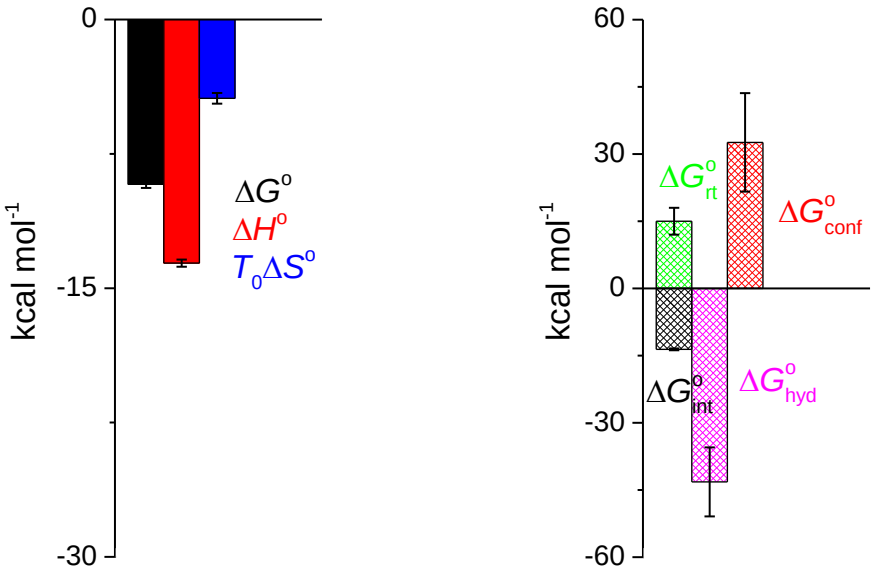
$\Delta C°_P = 0$ cal mol^{-1} K^{-1}

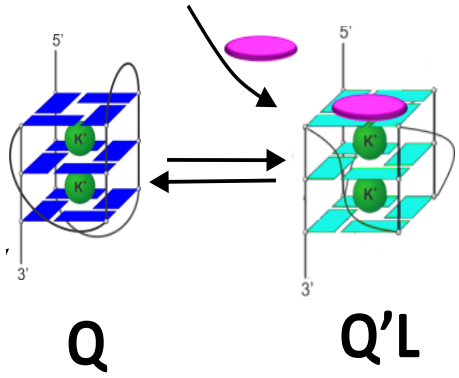
M. Bončina et al. *Biophys. J.* 2015, in press.
M. Bončina et al. *J. Am. Chem. Soc*. 2012, **134**, 9657.
H. Sugiyama *et al. J. Am. Chem. Soc*. 2010, **132**, 14910.

- Specific interactions [$\Delta G°_{int} < 0$; $\Delta G°_{hyd} \approx 0$].
- $\Delta G°_{conf}$ (U → I) ≈ $2\Delta G°_{conf}$ (I → Q) is in accordance with structural properties of I that are closer to Q than to U.

Thermodynamic driving forces

T = 25 °C



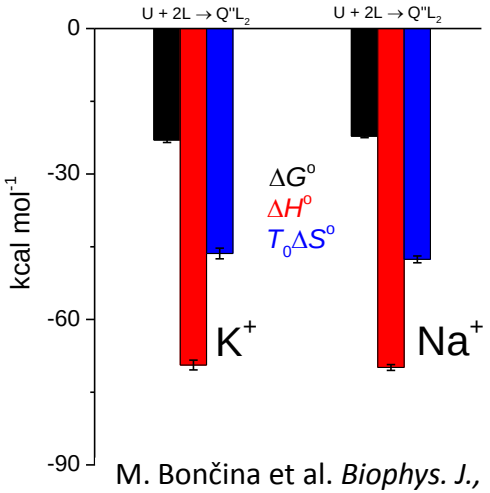


ΔC°_{P} = -540 cal mol^{-1} K^{-1}

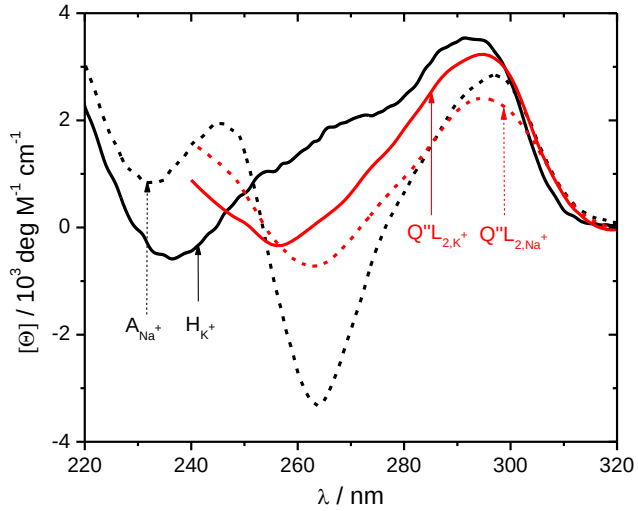
M. Bončina et al. *Biophys. J.* 2015, in press.

- Removal of water from ligand-Tel22 interacting surface [$\Delta G^{\circ}_{hyd} < 0$].
- Specific (π - π stacking) interactions [$\Delta G^{\circ}_{int} < 0$] required for succesful binding.
- Significant DNA conformational entropy loss [$\Delta G^{\circ}_{conf} > 0$].

K+ versus Na+



M. Bončina et al. *Biophys. J.,* 2015, in press.



Thermodynamic analysis

The overall thermodynamics of binding-coupled folding is in the K^+ and Na^+ environment nearly the same.

CD spectroscopy

$Q''L_2(K^+) \approx Q''L_2(Na^+)$ but different to ligand-free H (K^+) or A (Na^+).

Meaningful findings

- Global thermodynamic analysis predicts:

- thermodynamic forces that govern the formation of stable quadruplexes, folding intermediates and ligand-quadruplex complexes.

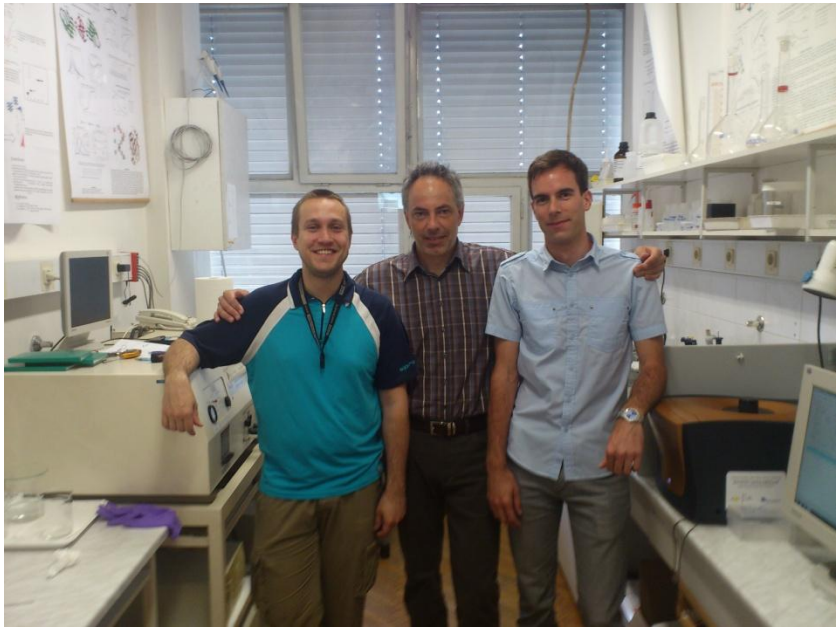
- behavior of DNA fragments in the solution as a function of temperature and concentrations of solutes.

- Predictions are consistent with the observed structural features.

Thanks

Ljubljana (thermodynamics)

- Matjaž Bončina
- Iztok Prislan
- Igor Drobnak
- Gorazd Vesnaver
- Crtomir Podlipnik
- Janez Plavec (NMR)



Paris (ligand synthesis)

- Marie-Paule Teulade-Fichou

Zagreb (ligand synthesis)

- Ivo Piantanida

London (molecular modeling)

- Shozeb Haider

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