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Talking molecules: the networks that shape the living world
March 19-20, 2018, Warsaw, Poland

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EUROPEAN COOPERATION
IN SCIENCE & TECHNOLOGY

FOLDING AND BINDING LANDSCAPES OF G-QUADRUPLEX (G4) DNA STRUCTURES

and

How not to run a biophysics core facility…

Jonathan *B.* Chaires
James Graham Brown Cancer Center
University of Louisville
Louisville, Kentucky USA

Supported by:
NIH Grants GM077422 & CA35635
NIH/NCRR COBRE P20RR018733
The James Graham Brown Foundation

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James Graham Brown Cancer Center
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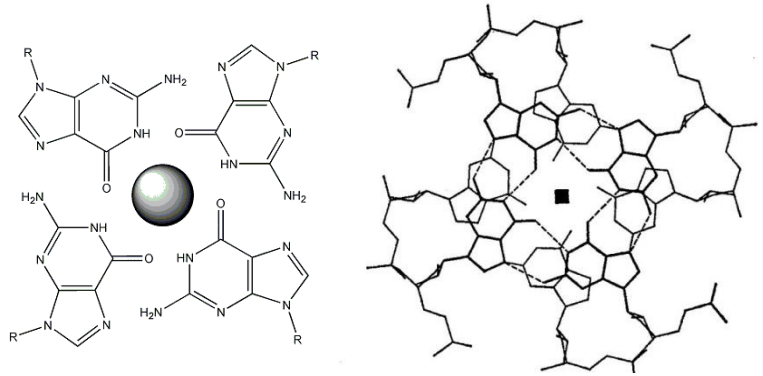
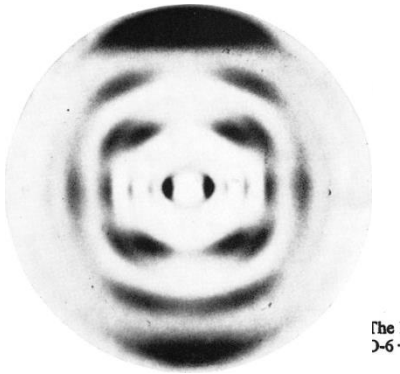
A Brief History of Quadruplex DNA…

Ivar Bang



David Davies



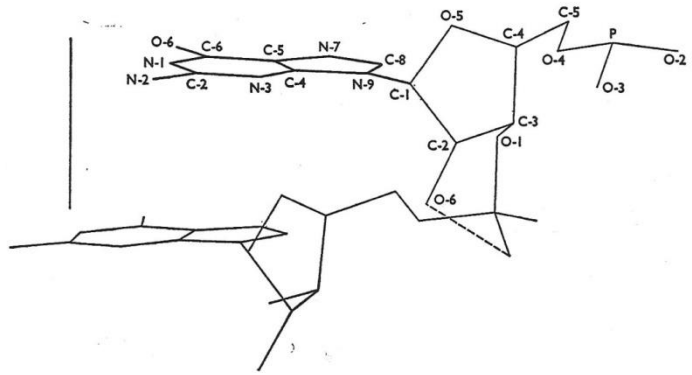
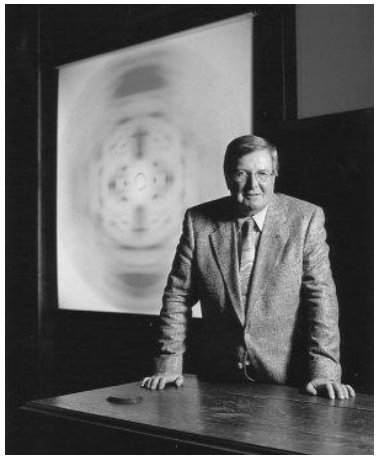
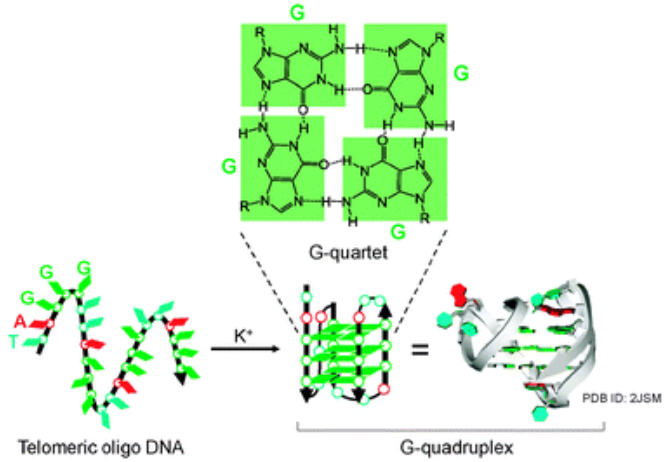


Fig. 4. *Side view of successive residues in a single* (23_2) *chain of the four-stranded structure of poly(I) or poly (G)* The helix axis is represented by the vertical line. Atoms in a nucleotide are labelled. The intramolecular hydrogen bond O-6 · · · · O-3 is shown by a dashed line.



Struther Arnott

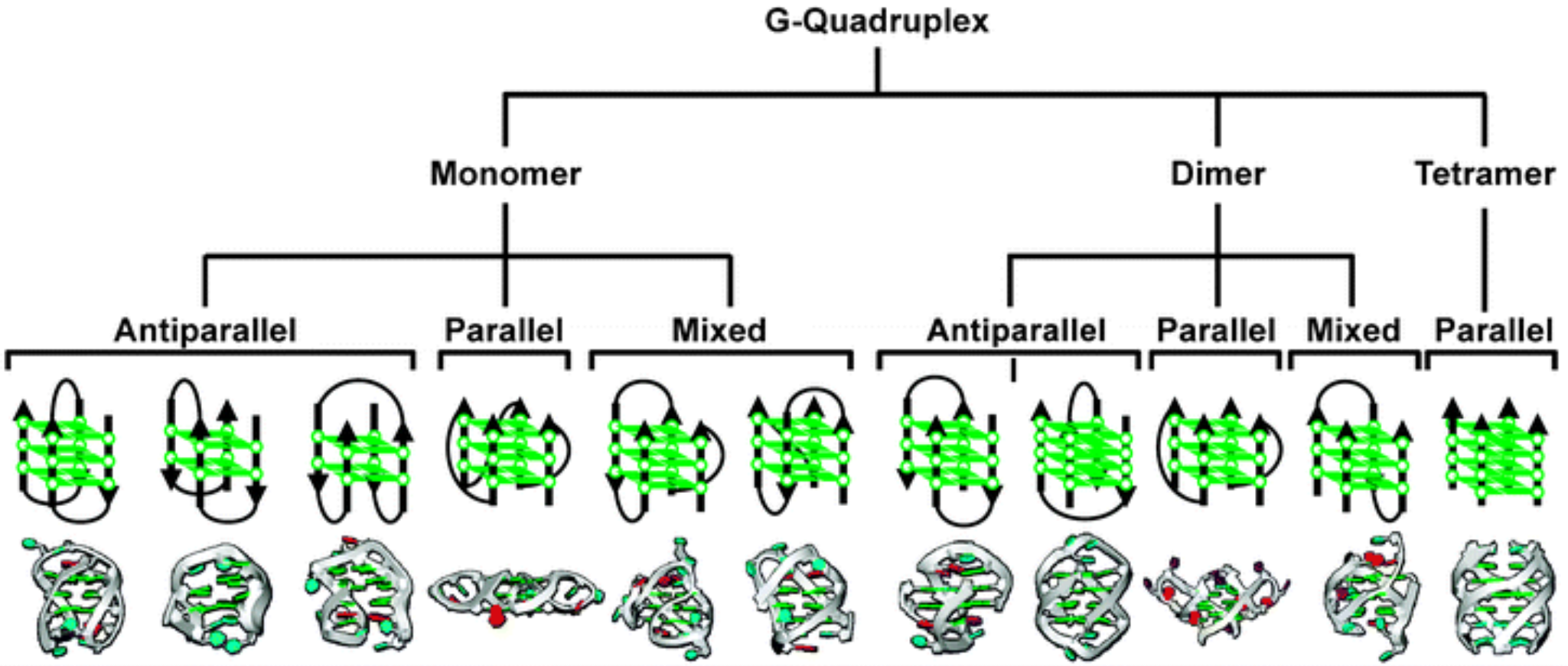


Bang, I. (1910). Untersuchungen über die Guanylsäure. *Biochemische Zeitschrift*, **26**, 293–311.

Gellert, M., Lipsett, M. N., & **Davies, D. R**. (1962). Helix formation by guanylic acid. *Proc. Nat. Acad. Sci. USA*, **48**, 2013–2018.

Struther Arnott, R. Chandrasekaran and C. M. Martilla (1974) Structures for Polyinosinic Acid and Polyguanylic Acid. *Biochem. J*. **141**: 537-543

Diversity of G-quadruplex structures

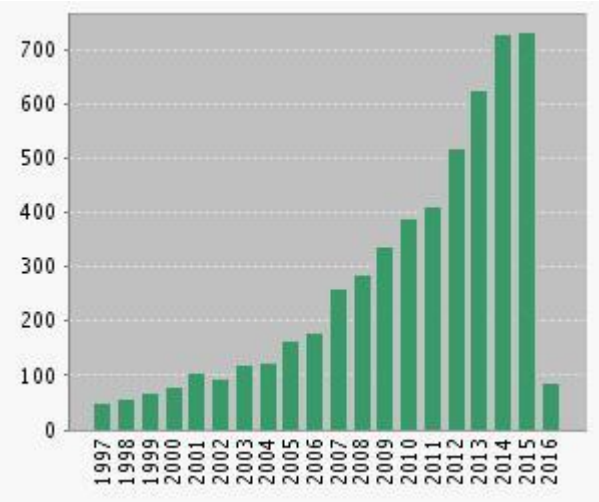


Sequences	$d[AG_3(T_2AG_3)_3]$	$d(G_2T_2G_2TGTG_2T_2G_2)$	$d[(G_3T_2A)_3G_3T]$	$d[AG_3(T_2AG_3)_3]$	$d[(T_2AG_3)_4T_2]$	$d[(G_3T_2A)_3G_3T]$	$d[G_3T_2CAG_2]$	$d[G_4T_4G_4]$	$r[UAG_3U_2AG_3U]$	$d[(GIG(T_2AG_3)_2T]$ $d[TAG_3U]$	$d[T_2G_4T]$
Metal ions	Na^+	K^+	K^+	K^+	K^+	K^+	Na^+	K^+	K^+	K^+	K^+
PDB ID	143D	148D	2KF7	1KF1	2JPZ	2KF8	1F3S	1JPQ	2KBP	2AQY	139D

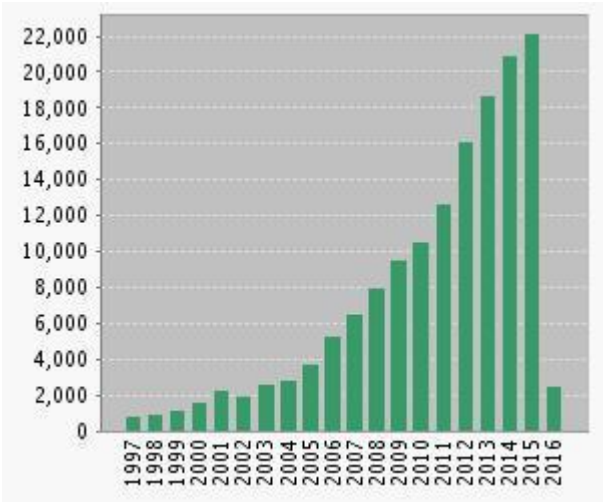
Hidenobu Yaku, Takeshi Fujimoto, Takashi Murashima, Daisuke Miyoshi and Naoki Sugimoto *Chem. Commun*., 2012, **48**, 6203-6216

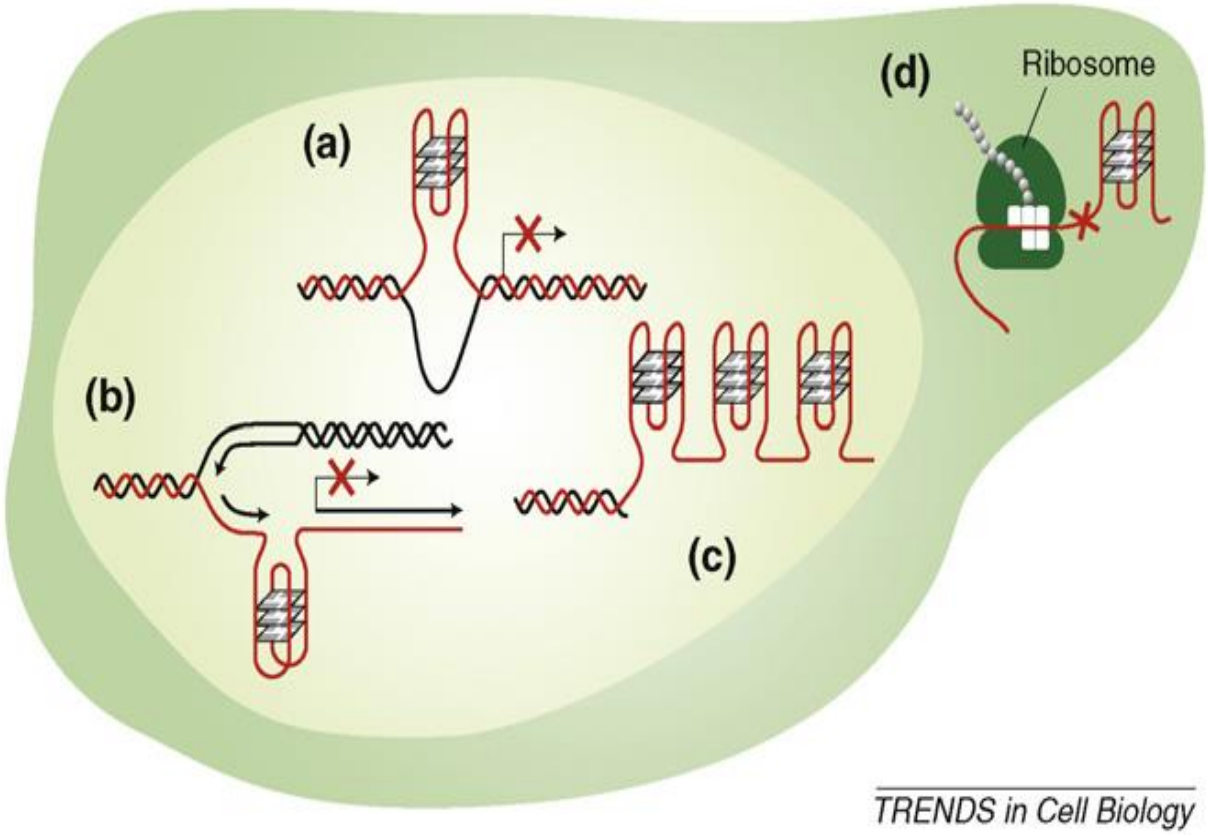
Growing interest in quadruplexes…

Published Items in Each Year



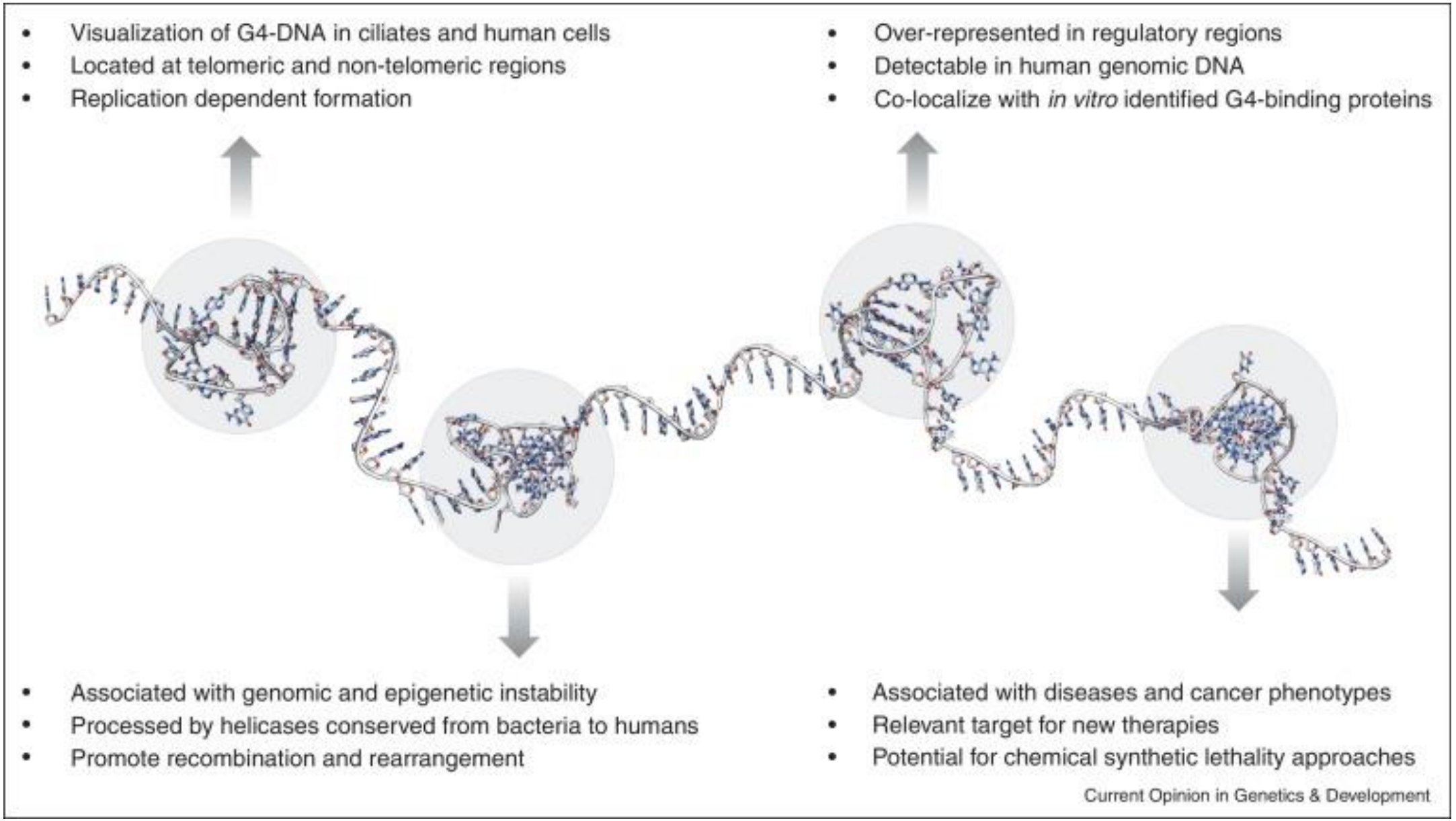
Citations in Each Year





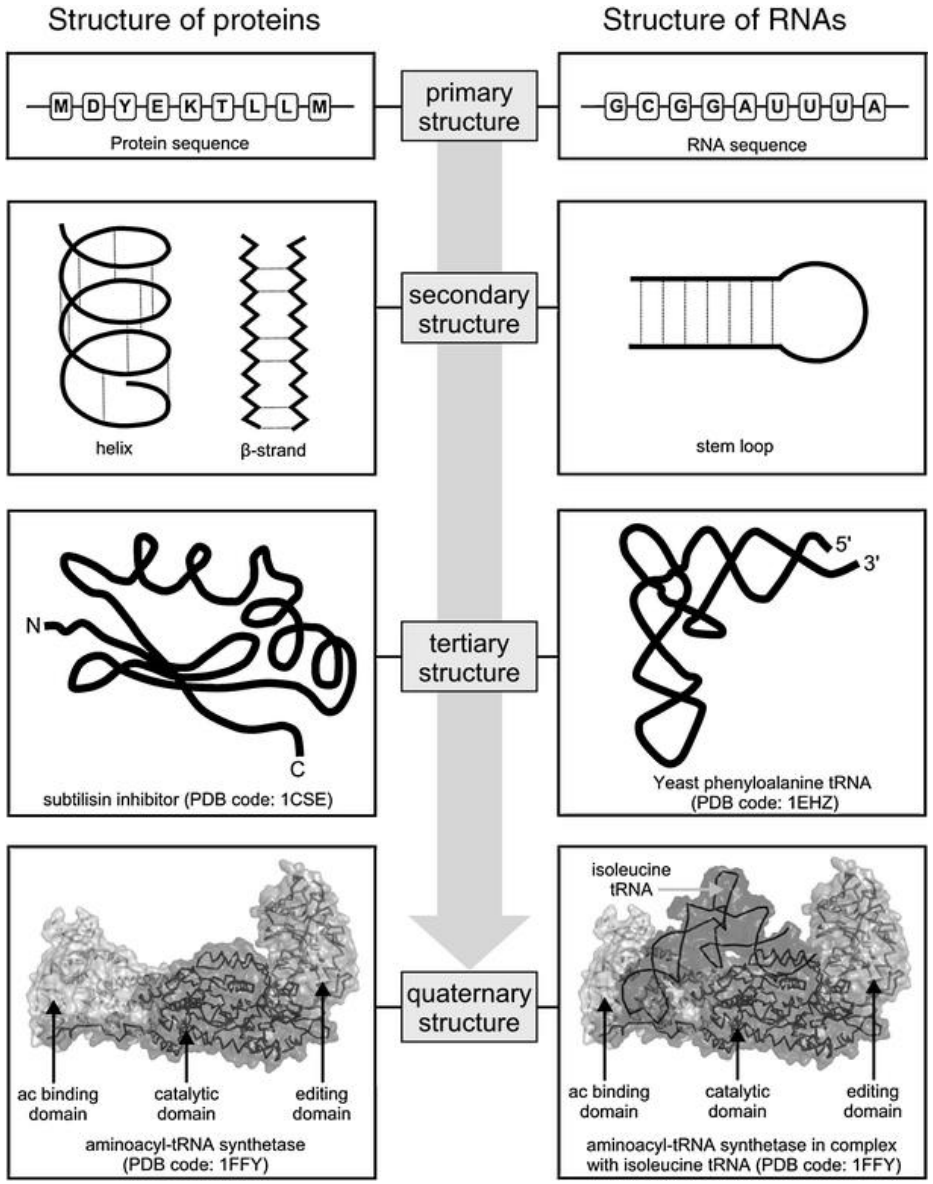
Quadruplex-forming sequences are highly conserved and non-randomly distributed.

Transient or permanent single-strands can fold into functional non-duplex structures

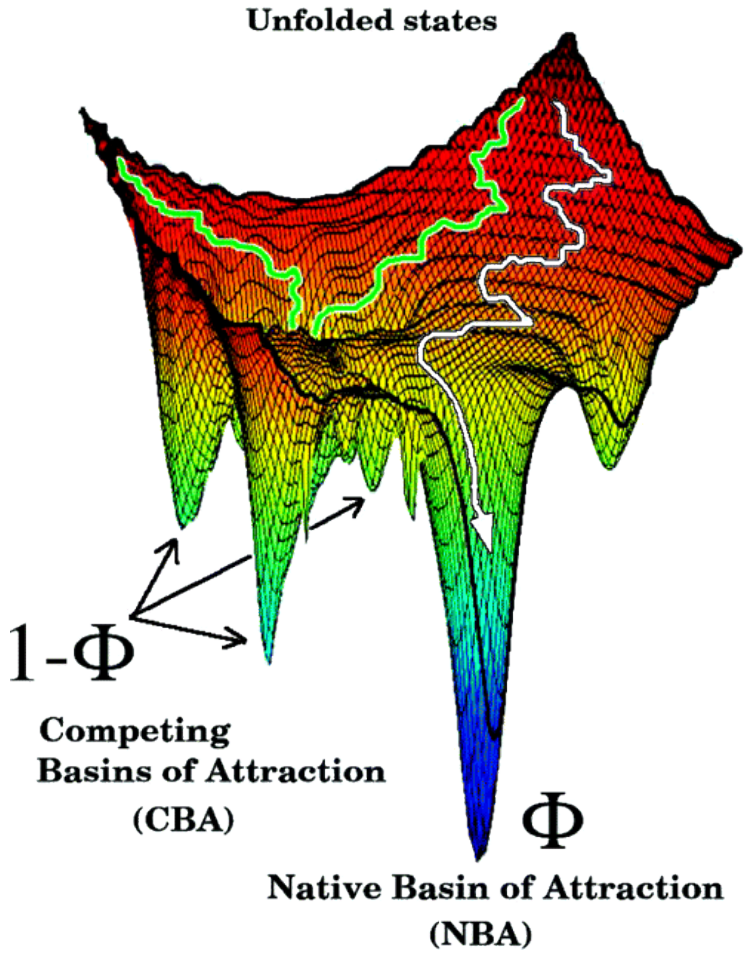


Pierre Murat, Shankar Balasubramanian *Existence and consequences of G-quadruplex structures in DNA. Current Opinion in Genetics & Development*, 2014, **25**:22–29

How to biomolecules fold? G4 is DNA acting like proteins and RNA



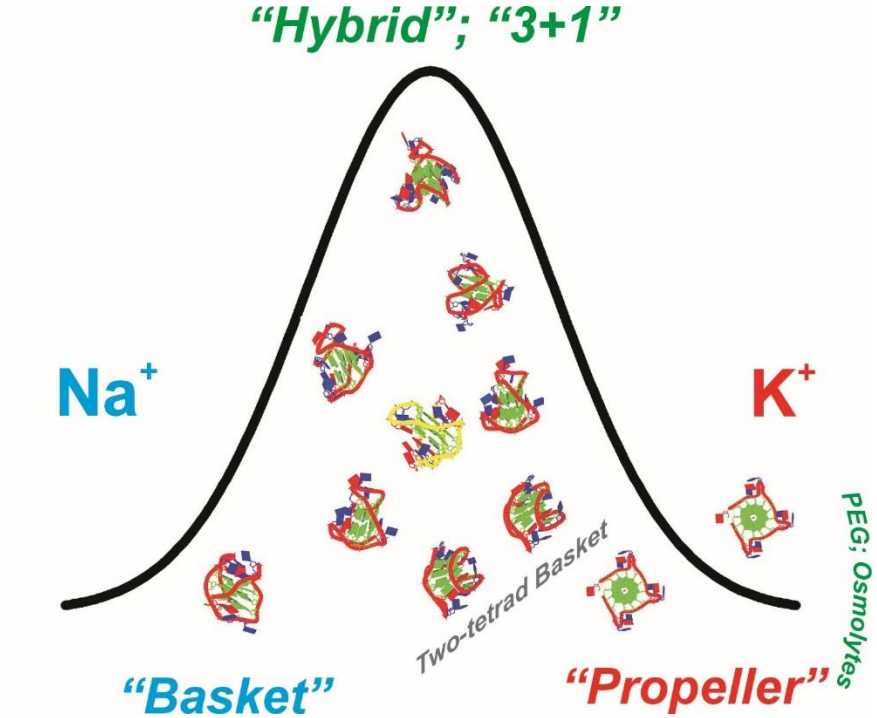
Rother, K., Rother, M., Boniecki, M. et al. J Mol Model (2011) 17: 2325. https://doi.org/10.1007/s00894-010-0951-x

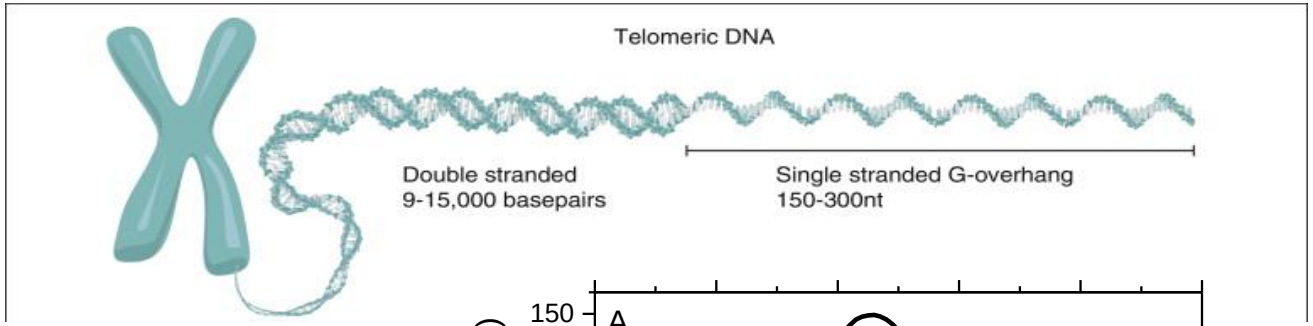


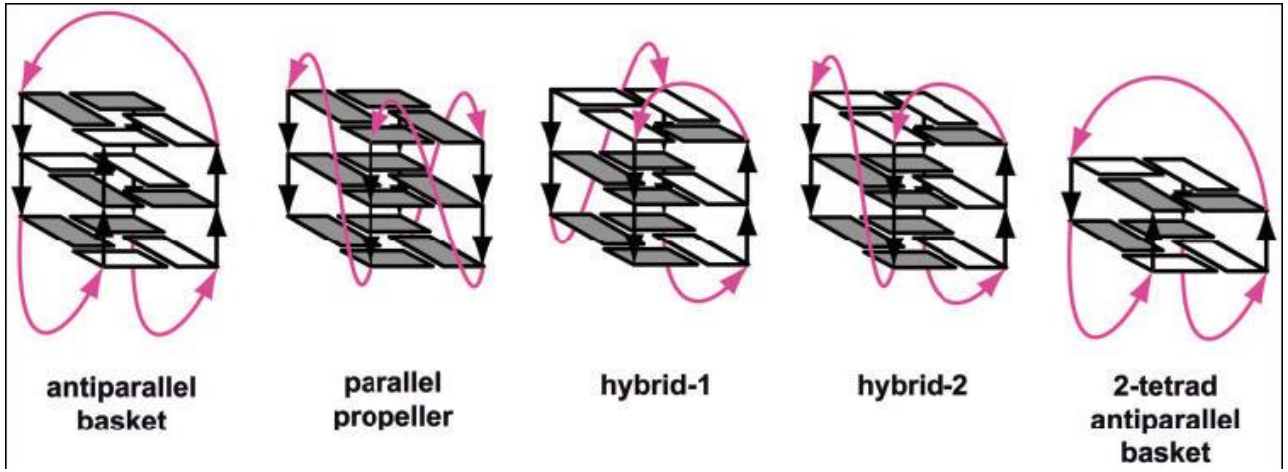
D. Thirumalai and and Changbong Hyeon Biochemistry 2005 44 (13), 4957-4970
DOI: 10.1021/bi047314+

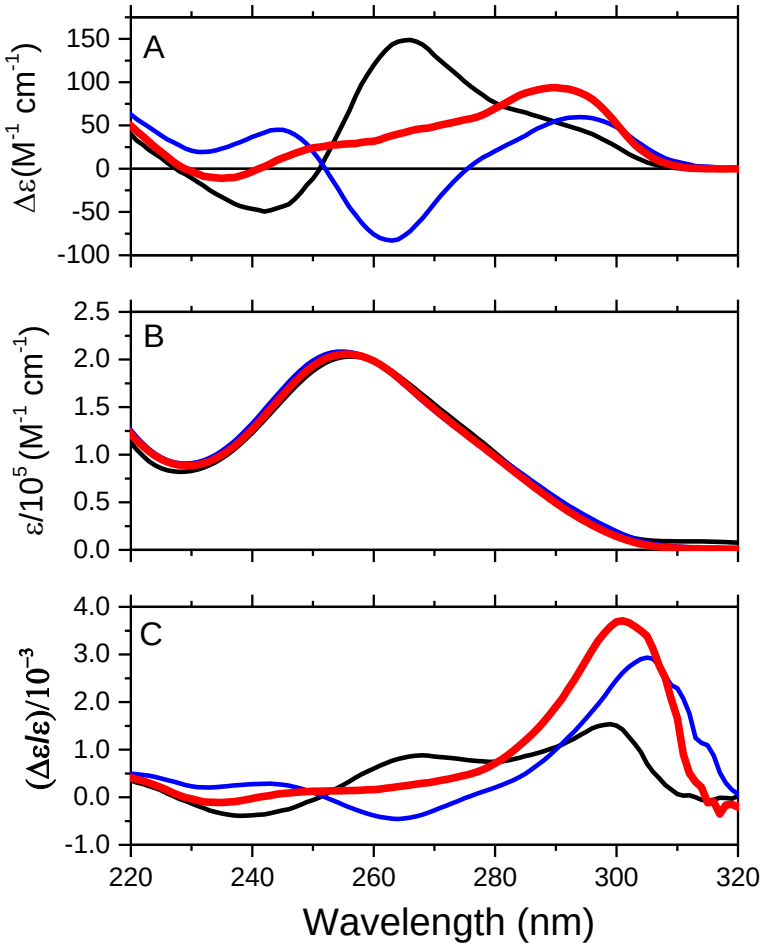
D. Thirumalai* and and S. A. Woodson Accounts of Chemical Research 1996 29 (9), 433-439

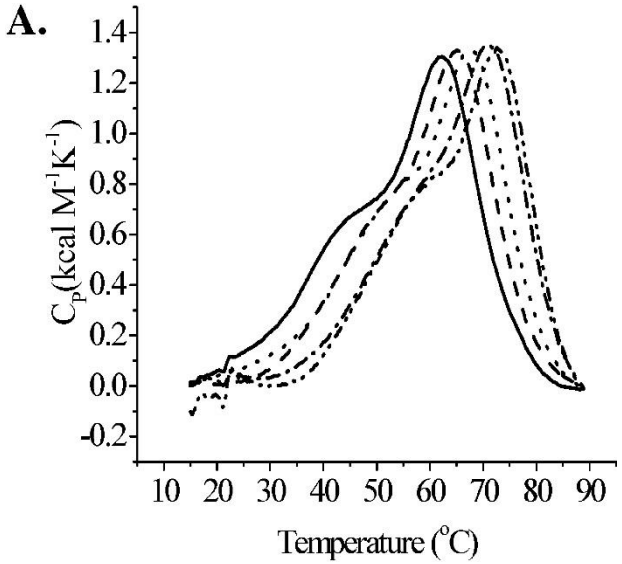
Human telomere G-quadruplex: 5’-XGGG(TTAGG)$_3$Y-3’

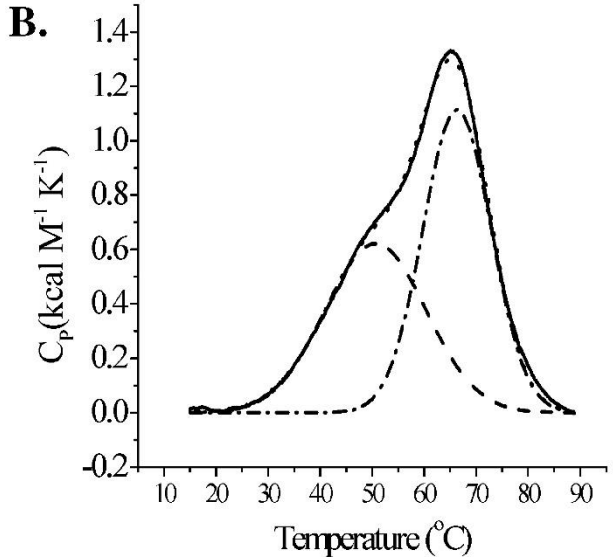


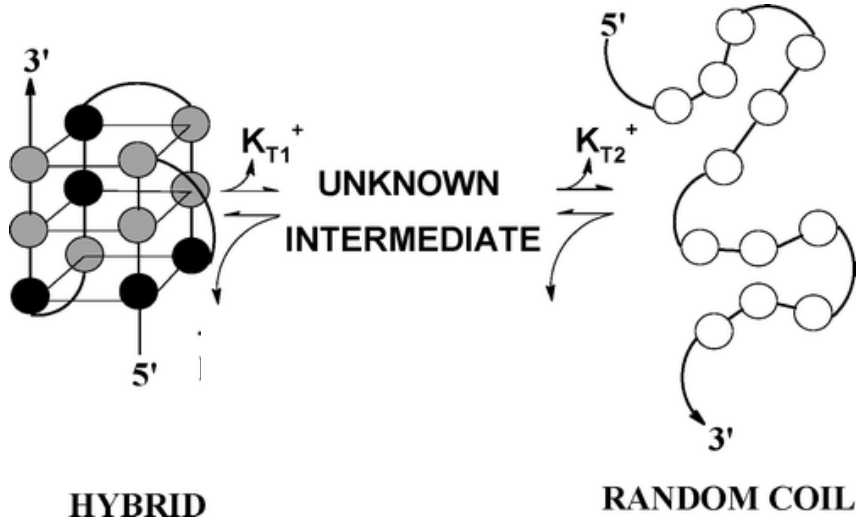


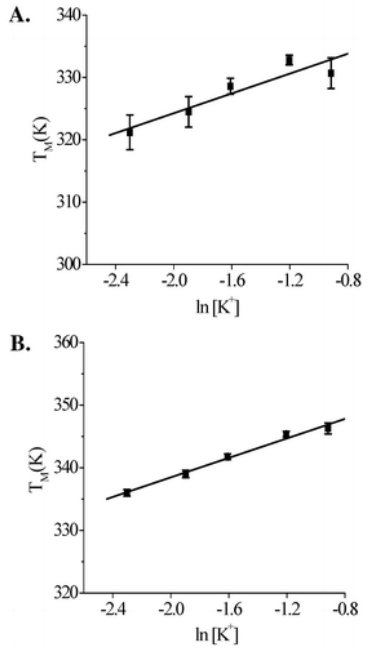




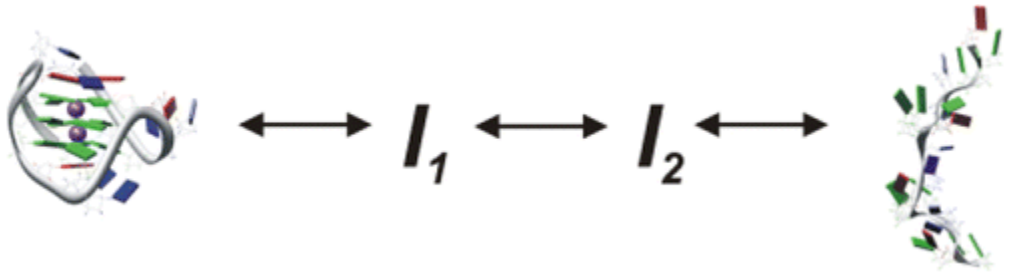


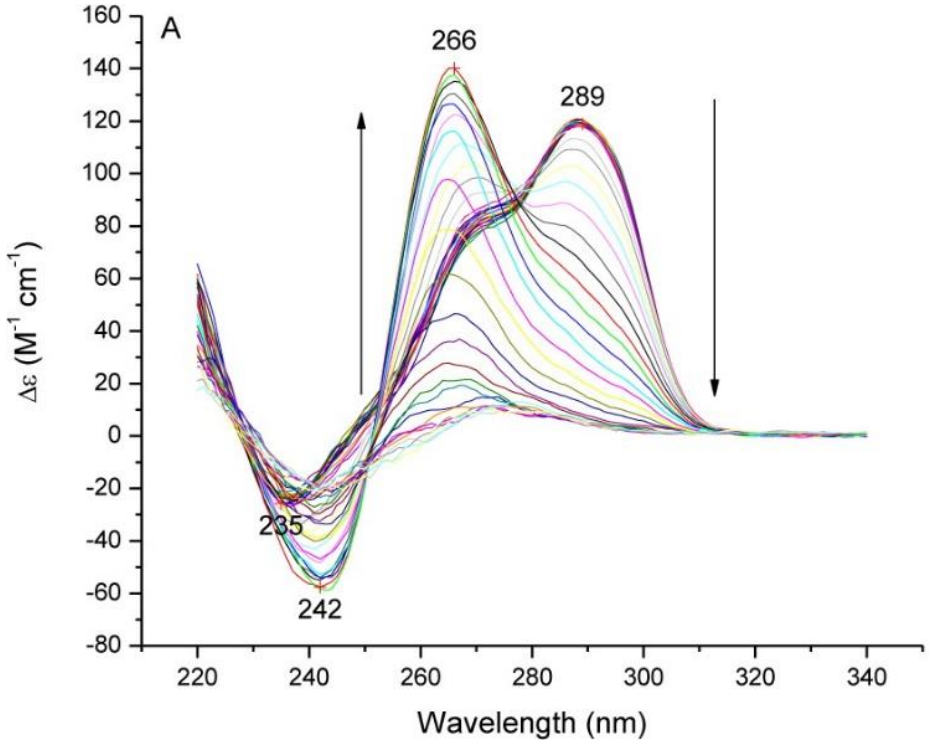


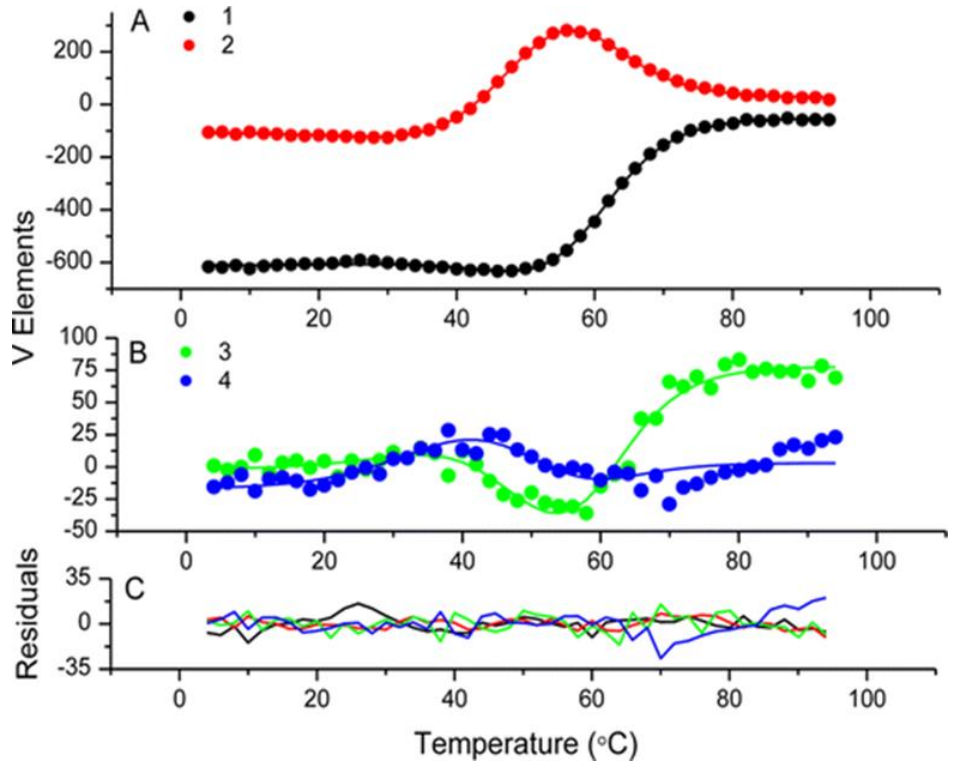




Published in: Cosimo Antonacci; Jonathan B. Chaires; Richard D. Sheardy; *Biochemistry* **2007,** 46, 4654-4660.
DOI: 10.1021/bi602511p







Singular **V**alue **D**ecomposition

Gray RD, Chaires JB Analysis of multidimensional G-quadruplex melting curves. *Curr Protoc Nucleic Acid Chem.* 2011 Jun;Chapter 17:Unit17.4. doi: 10.1002/0471142700.nc1704s45.

Published in: Robert D. Gray; Robert Buscaglia; Jonathan B. Chaires; *J. Am. Chem. Soc.* **2012,** 134, 16834-16844.
DOI: 10.1021/ja307543z

1) $F \rightleftharpoons U$

2) $F_1 \rightleftharpoons U \rightleftharpoons F_2$

3) $F \rightleftharpoons I \rightleftharpoons U$

4) $F \rightleftharpoons I_1 \rightleftharpoons I_2 \rightleftharpoons U$

5) $F_1 \rightleftharpoons I \rightleftharpoons U$
$\updownarrow$
F_2

F = folded state(s)
I = intermediate state(s)
U = unfolded ensemble

Table 3. Model-dependent fitting statistics for thermal unfolding of Tel22 in 25 mM KCl monitored by CD

Model	Model	χ^2	AIC Value
Single Transition	1	720265.5	1544.1
Two Transitions	2	15235.4	846.6
Parallel Transitions	3	15235.4	846.6
Three Transitions	4	7759.5	734.5
Parallel Intermediate Transitions	5	7757.3	734.4

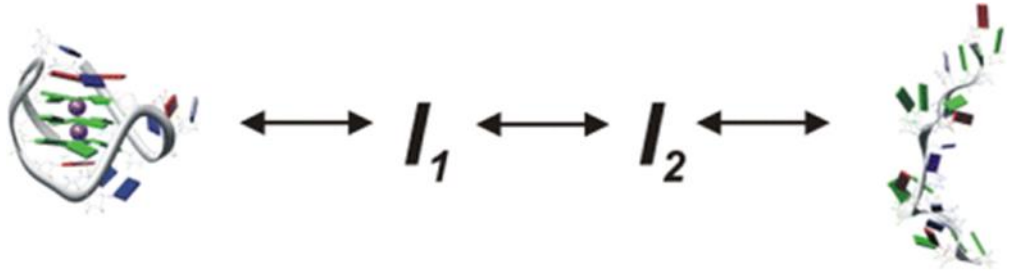
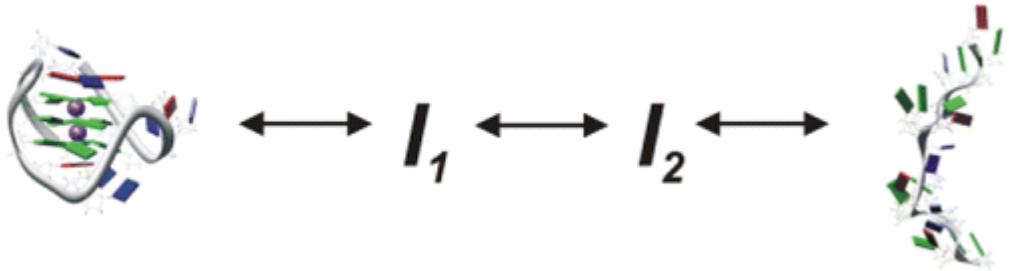


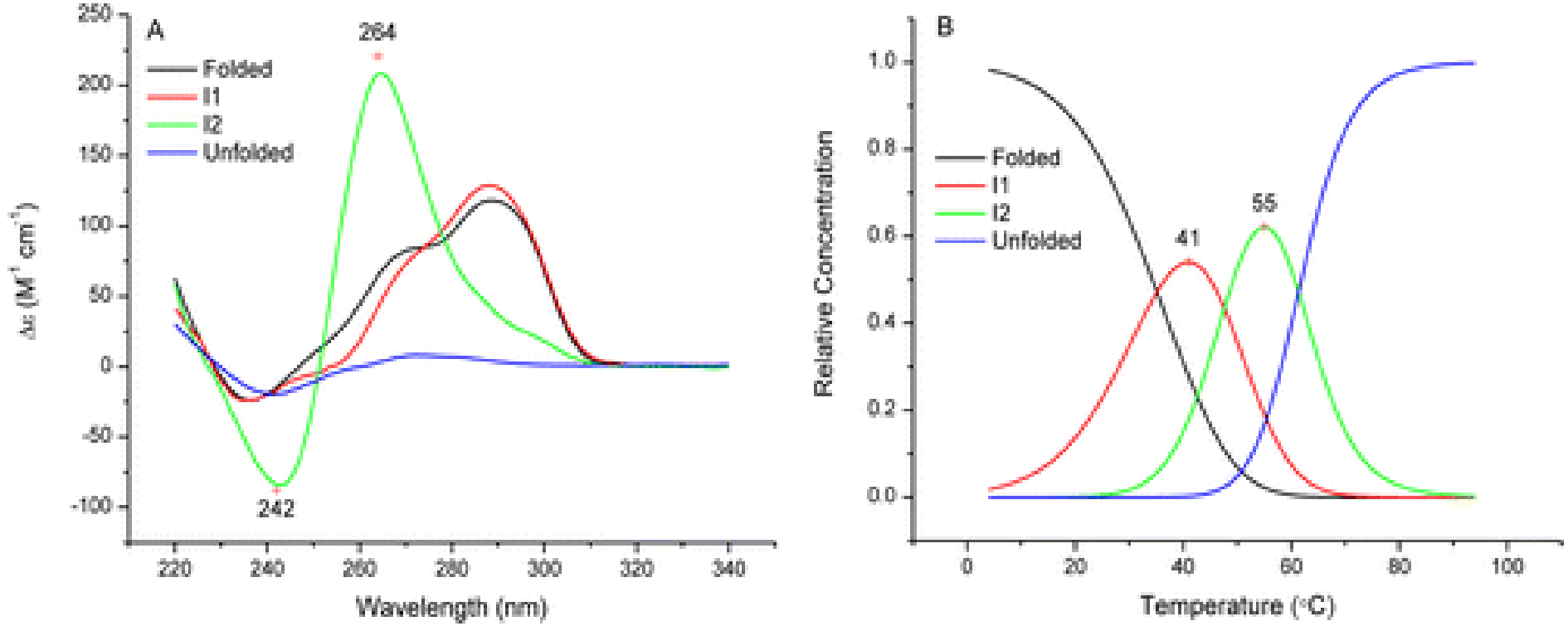
Table 4. Optimal values of thermodynamic parameters for folding Tel22 in 25 mM KCl[a]

	Model 4	Model 5
ΔH_1	-21.9 ± 4.7[b]	-25.3
T_{m1}	35.2 ± 5.3	48.4
ΔH_2	-33.4 ± 4.2	-54.6
T_{m2}	47.6 ± 1.6	42.8
ΔH_3	-45.3 ± 4.2	-43.6
T_{m3}	61.4 ± 1.0	60.7
ΔG_1	-1.1 ±0.4	-2.2
ΔG_2	-2.9 ± 0.4	-3.9
ΔG_3	-5.6 ± 0.6	-5.3
ΔG_{Total}	-9.5 ± 0.7	-11.5

Greenfield, N. J. (2007) Using circular dichroism collected as a function of temperature to determine the thermodynamics of protein unfolding and binding interactions. *Nat. Protocols 1*, 2527-2535.

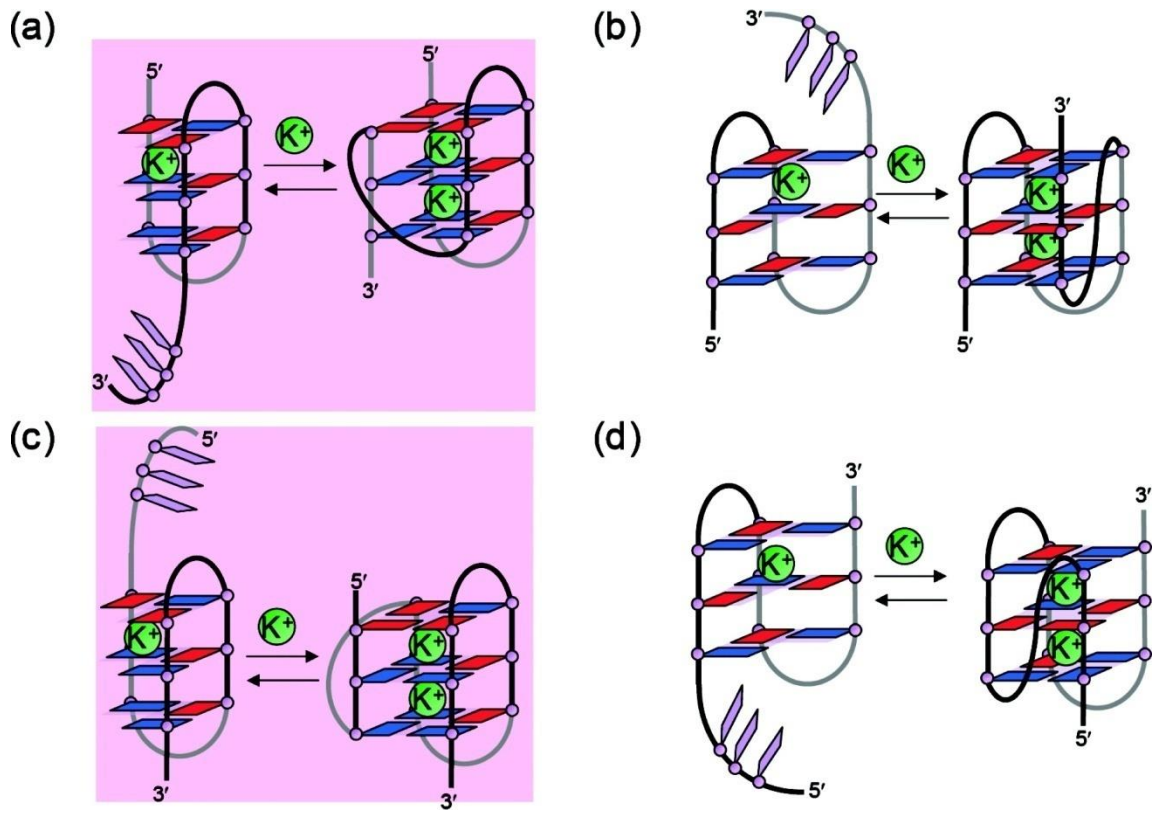
Published in: Robert D. Gray; Robert Buscaglia; Jonathan B. Chaires; *J. Am. Chem. Soc.* **2012,** 134, 16834-16844.
DOI: 10.1021/ja307543z





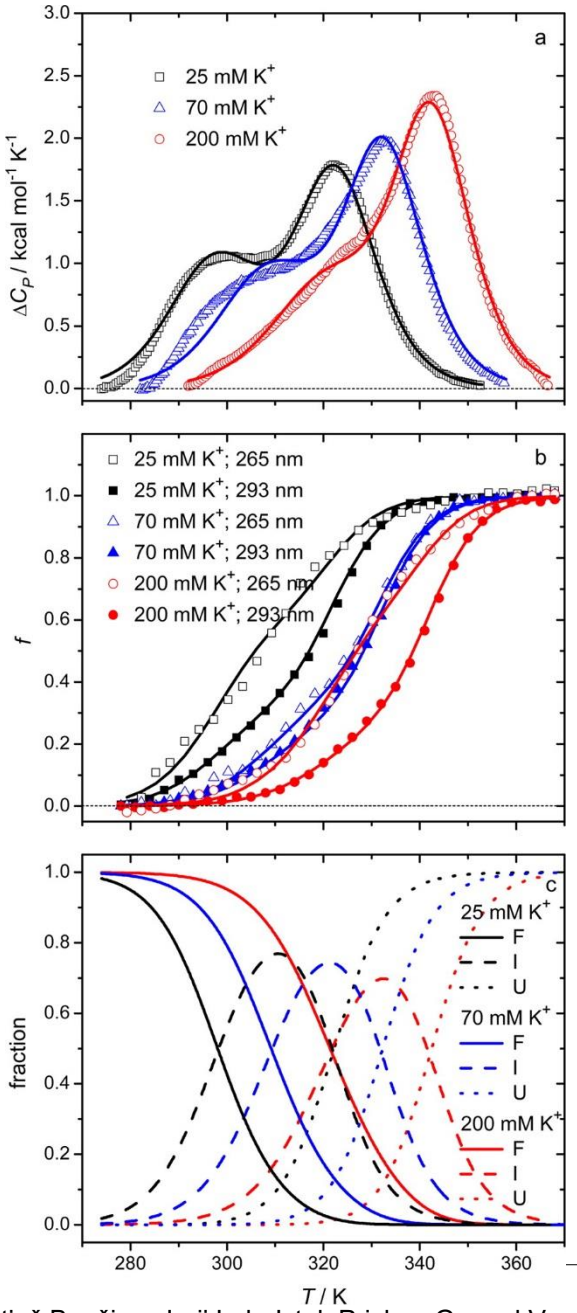
Published in: Robert D. Gray; Robert Buscaglia; Jonathan B. Chaires; *J. Am. Chem. Soc.* **2012,** 134, 16834-16844.
DOI: 10.1021/ja307543z

What is it?



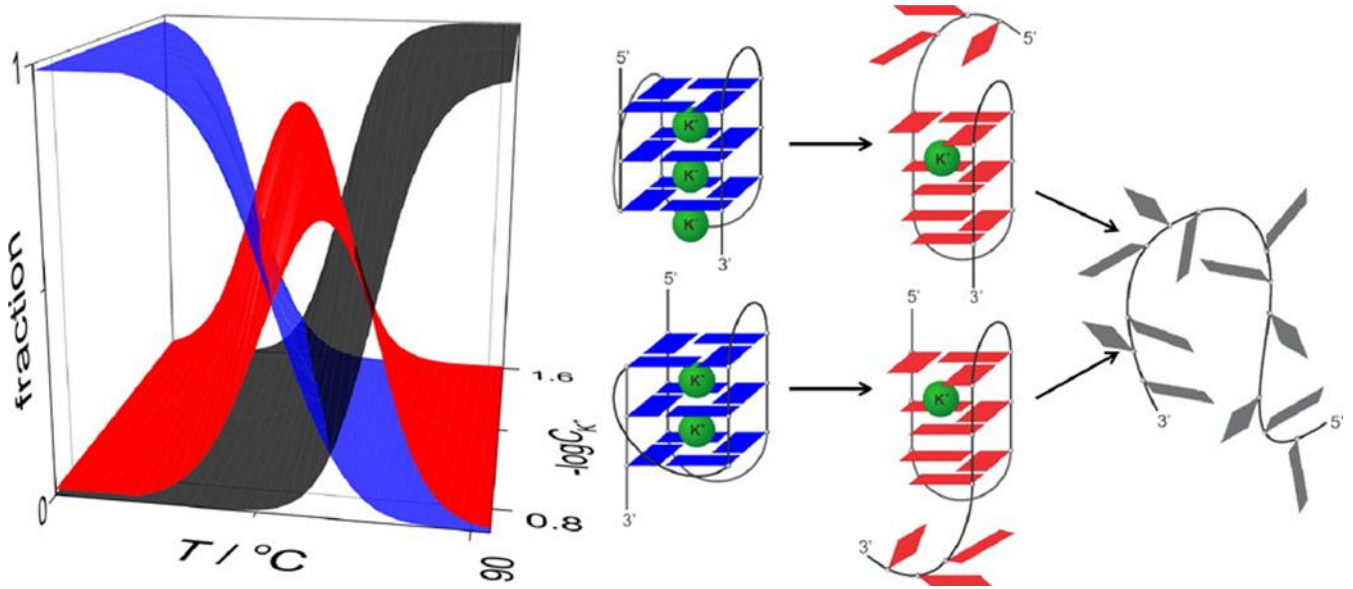
Models of the predicted stable triplexes with four syn conformation of deoxyguanosine and their quadruplex folding. Triplex (a) was formed from hairpin1−2. This structure is the same as triplex (d) in Figure 6. In the same way, triplexes (b) and (c) were formed from hairpin2−3. These structures are the same as triplexes (e) and (l) in Figure 6, respectively. Triplex (d) was formed from hairpin3−4. This structure is the same as triplex (m) in Figure 6. The pink background indicates the stable G-quadruplex structures.

Published in: Tomoko Mashimo; Hirotaka Yagi; Yuta Sannohe; Arivazhagan Rajendran; Hiroshi Sugiyama; *J. Am. Chem. Soc.* **2010,** 132, 14910-14918.
DOI: 10.1021/ja105806u



Jurij Lah

Univerza *v Ljubljani*



Published in: Matjaž Bončina; Jurij Lah; Iztok Prislan; Gorazd Vesnaver; *J. Am. Chem. Soc.* **2012,** 134, 9657-9663.
DOI: 10.1021/ja300605n

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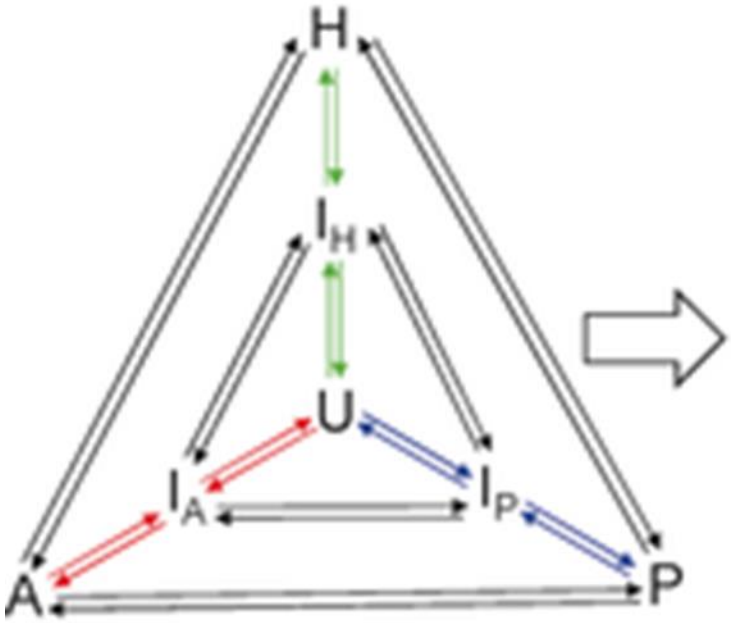
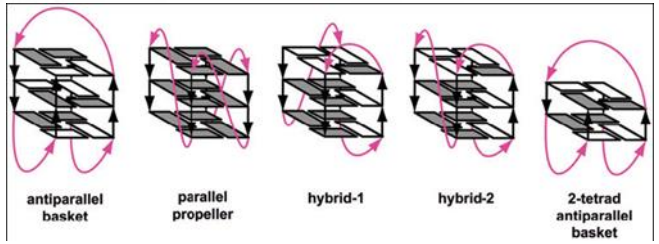
Unravelling the Thermodynamics of the Folding and Interconversion of Human Telomere G-Quadruplexes

$$\Delta G_{(T,\mathrm{K},\mathrm{Na},\mathrm{PEG})} = \Delta G_{(T)} - RT\left(n_{\mathrm{K}}\ln[\mathrm{K}^+] + n_{\mathrm{Na}}\ln[\mathrm{Na}^+] + m_1[\mathrm{PEG}] + m_2[\mathrm{PEG}]^2\right)$$





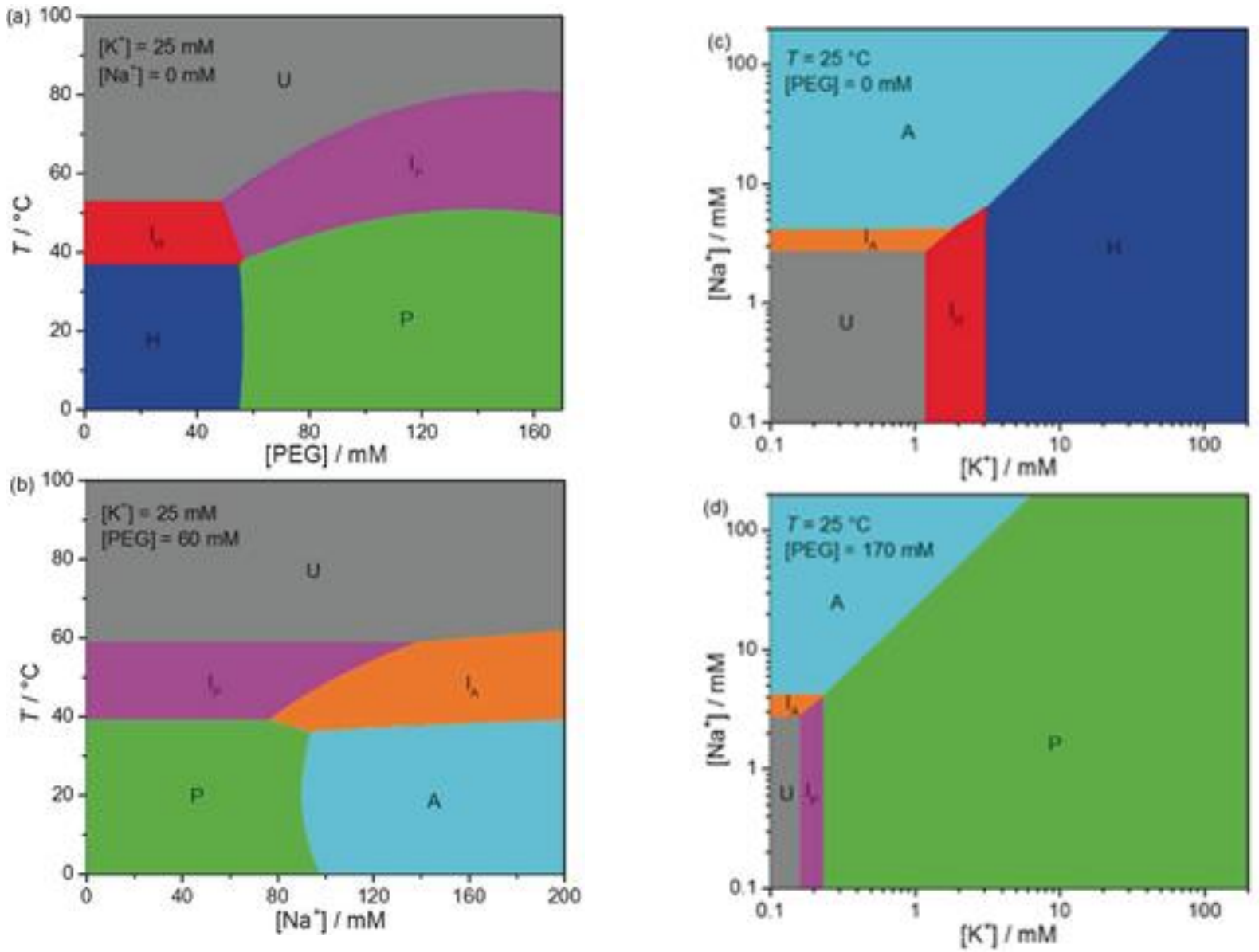
	U⟶P	U⟶H[a]	U⟶A[a]	U⟶I_P	U⟶I_H[a]	U⟶I_A[a]
$\Delta G_{(T_0)}$ / kcal mol^{-1}	–3.5±0.1	–8.0±0.1	–6.2±0.1	–2.6±0.1	–5.6±0.1	–4.9±0.1
$\Delta H_{(T_0)}$ / kcal mol^{-1}	–45.0±0.3	–46.0±0.6	–45.5±1.0	–19.3±0.3	–23.6±0.6	–25.4±1.0
$T\Delta S_{(T_0)}$ / kcal mol^{-1}	–41.5±0.3	–38.0±0.6	–39.3±1.0	–16.7±0.3	–18.0±0.6	–20.5±1.0
ΔC_P / cal mol^{-1} K^{-1}	–280±20	–420±30	–390±30	–280±20	–420±20	–390±30
n_{K}	–2.1±0.2[a]	–2.1±0.2	0	–1.4±0.1[a]	–1.4±0.1	0
n_{Na}	0	0	–1.8±0.1	0	0	–1.4±0.1
m_1 / L mol^{-1}	–166±4	/	/	–101±2	/	/
m_2/ L^2 mol^{-2}	554±10	/	/	322±5	/	/
25 mM K$^+$ and/or Na$^+$[b]						
$\Delta G_{(T_0,\mathrm{X})}$ / kcal mol^{-1}	1.1±0.1	–3.4±0.1	–2.4±0.1	0.5±0.1	–2.5±0.1	–2.0±0.1
$T\Delta S_{(T_0,\mathrm{X})}$ / kcal mol^{-1}	–46.1±0.3	–42.6±0.6	–43.1±1.0	–19.8±0.3	–21.1±0.6	–23.4±1.0

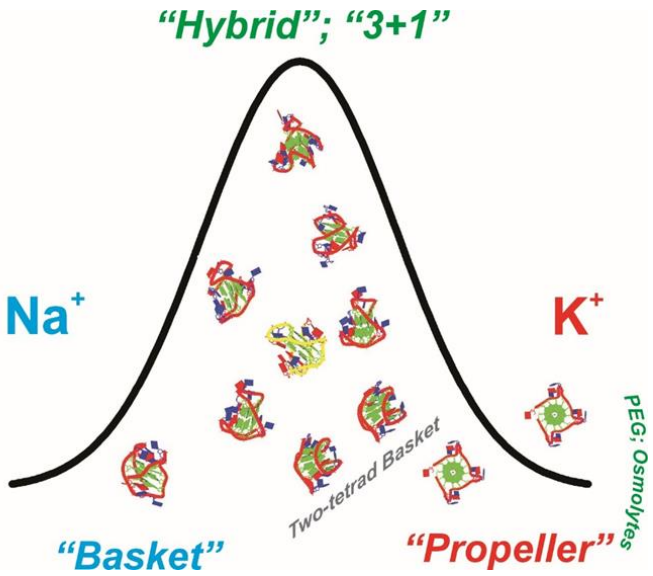
Matjaž Bončina, Gorazd Vesnaver, Jonathan Brad Chaires, Jurij Lah

Angewandte Chemie International Edition
Volume 55, Issue 35 pages 10340-10344, 3 AUG 2016 DOI: 10.1002/anie.201605350
http://onlinelibrary.wiley.com/doi/10.1002/anie.201605350/full#anie201605350-fig-0003

Angewandte International Edition Chemie

Unravelling the Thermodynamics of the Folding and Interconversion of Human Telomere G-Quadruplexes





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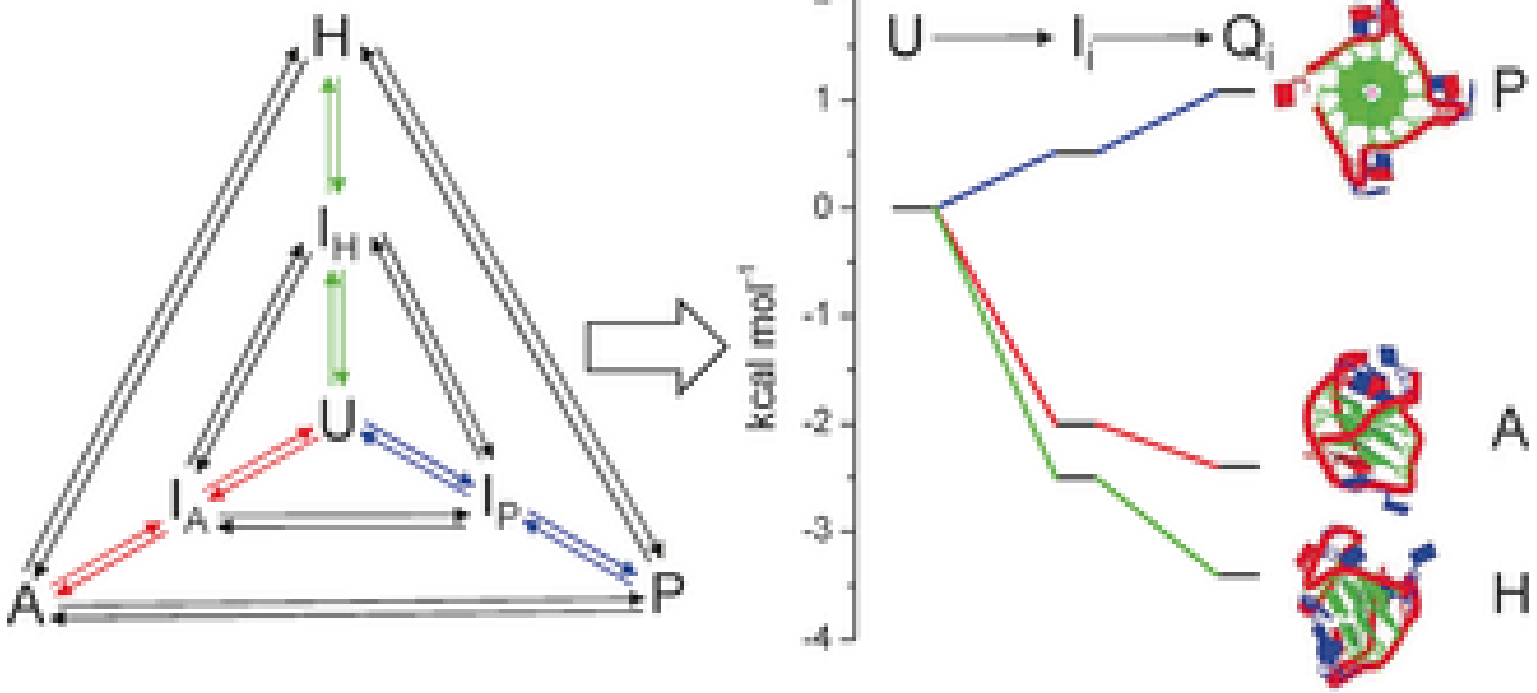
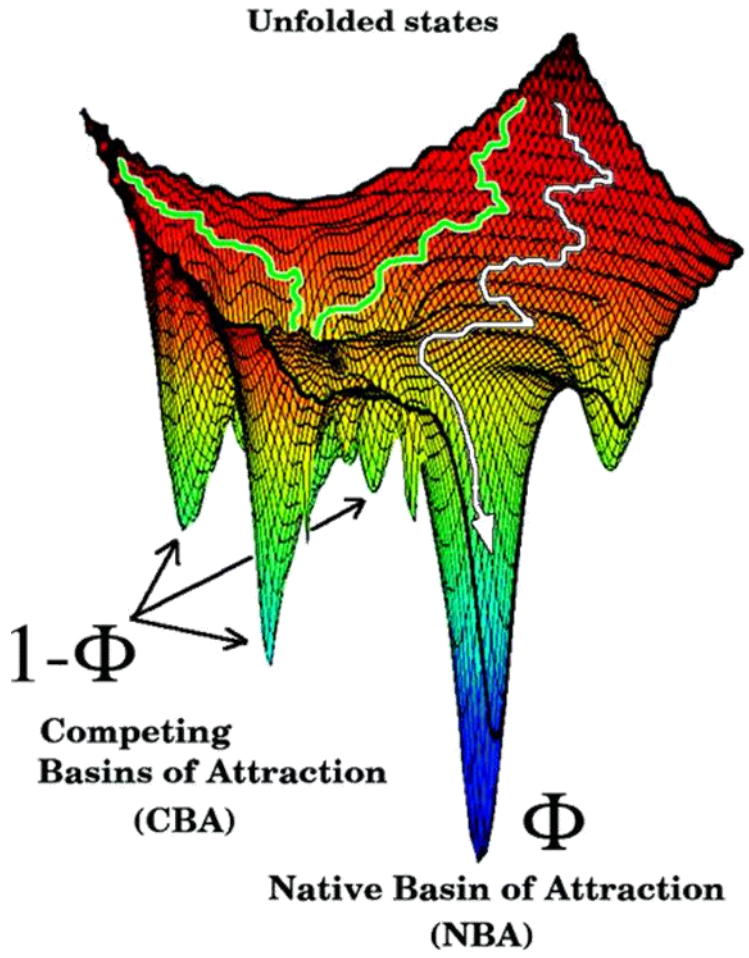
Volume 55, Issue 35, pages 10340-10344, 3 AUG 2016 DOI: 10.1002/anie.201605350
http://onlinelibrary.wiley.com/doi/10.1002/anie.201605350/full#anie201605350-fig-0003

Unraveling the Thermodynamics of the Folding and Interconversion of Human Telomere G-Quadruplexes





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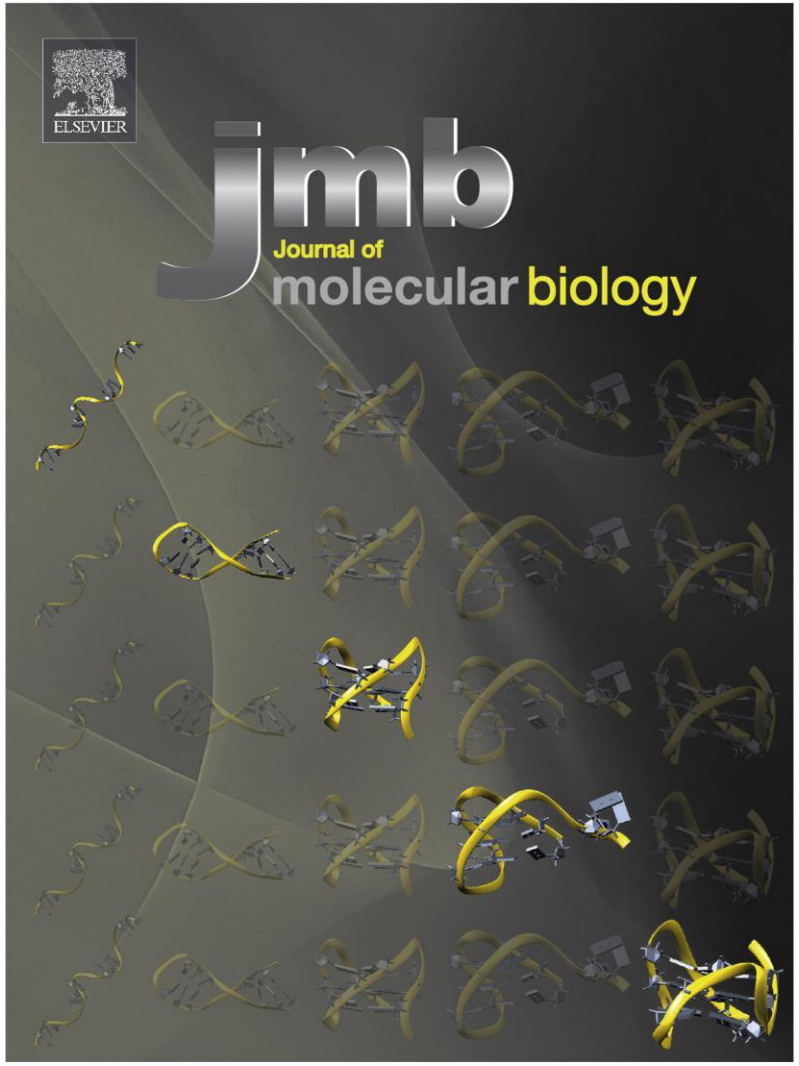
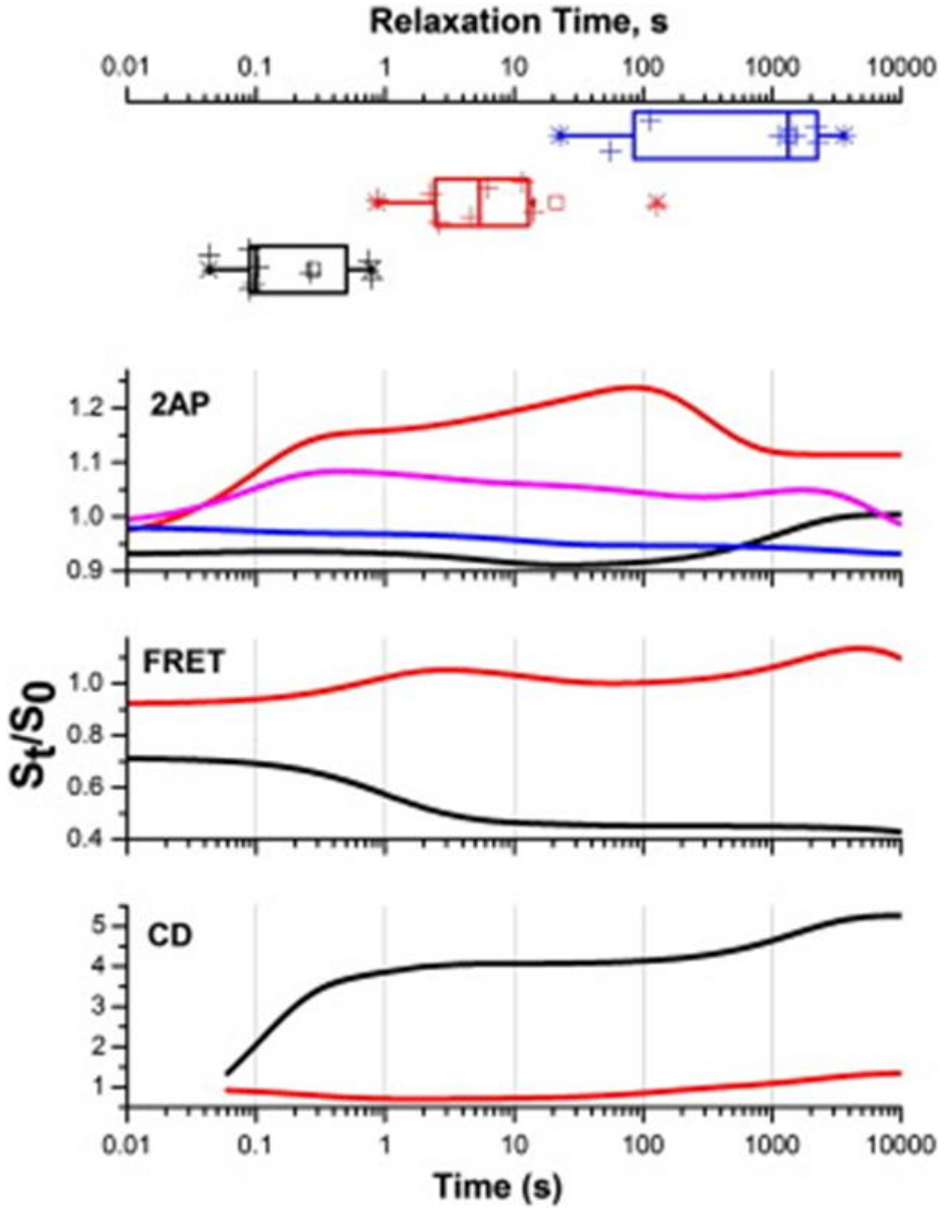
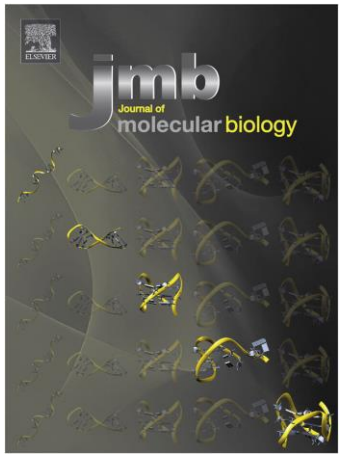
http://onlinelibrary.wiley.com/doi/10.1002/anie.201605350/full#anie201605350-fig-0003

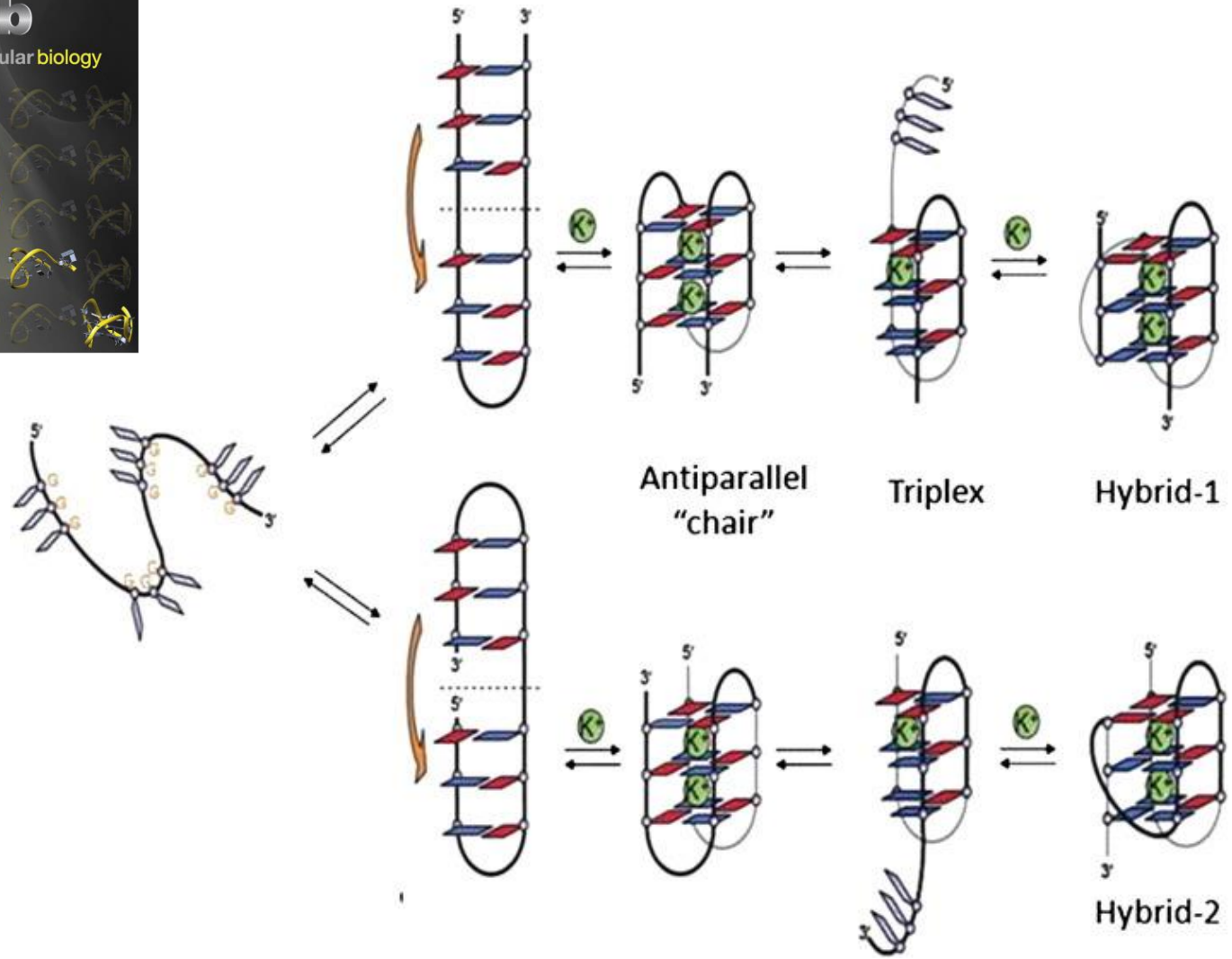


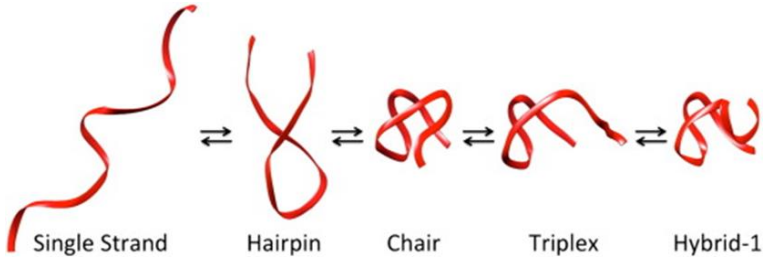


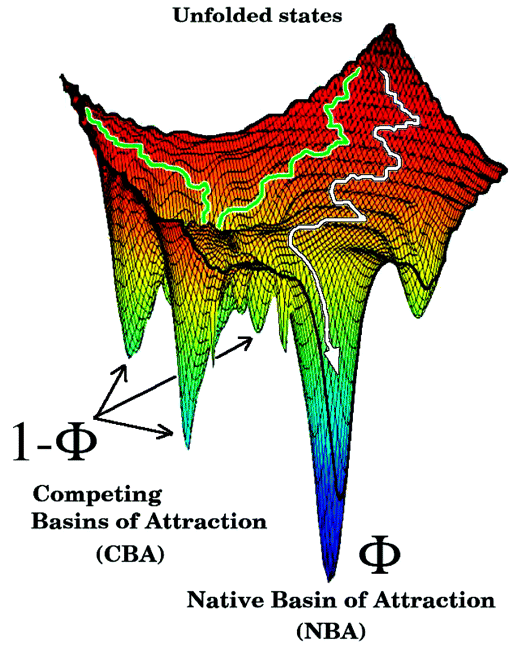
[Stopped-flow KCl jump experiments]

RD Gray, JO Trent & JB Chaires Journal of Molecular Biology Volume 426, Issue 8, 17 April 2014, Pages 1629-1650







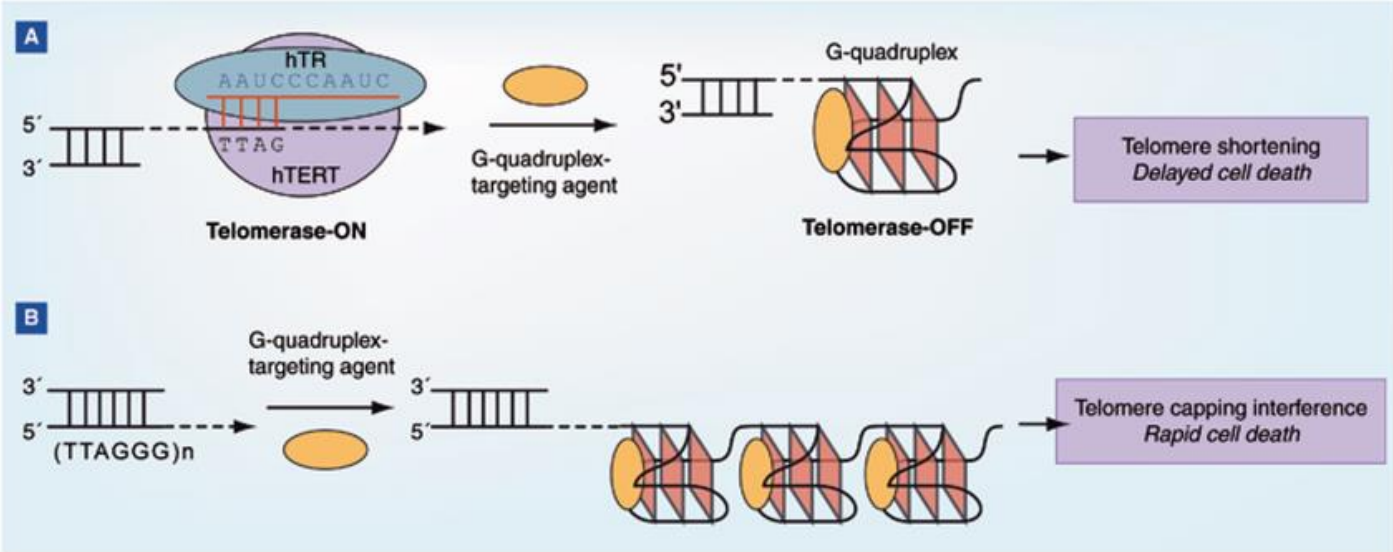


(Movies available online a JMB website)

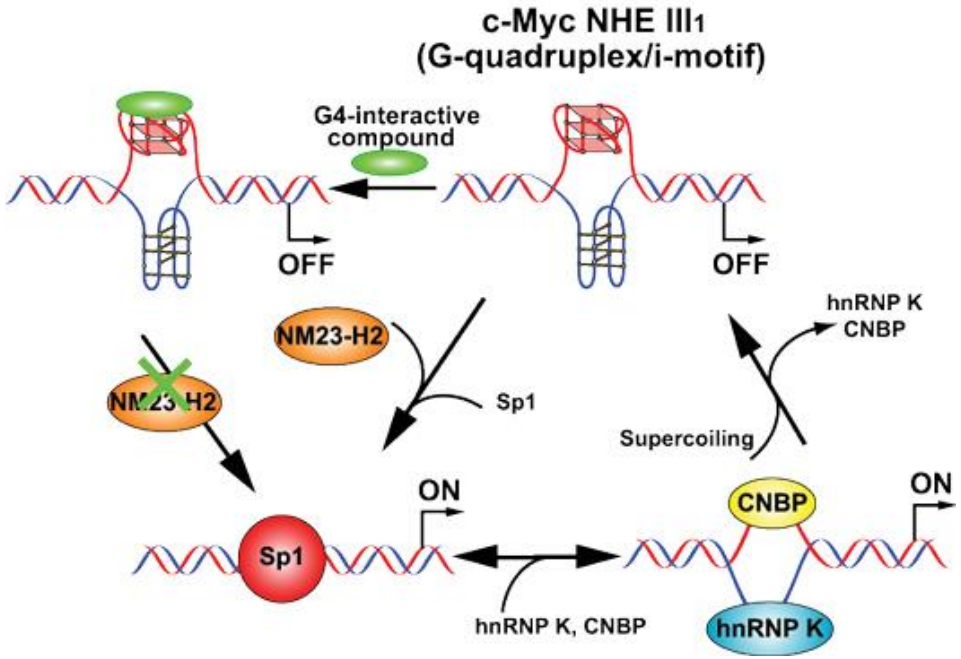
RD Gray, JO Trent & JB Chaires Journal of Molecular Biology Volume 426, Issue 8, 17 April 2014, Pages 1629-1650

G-quadruplexes as drug targets

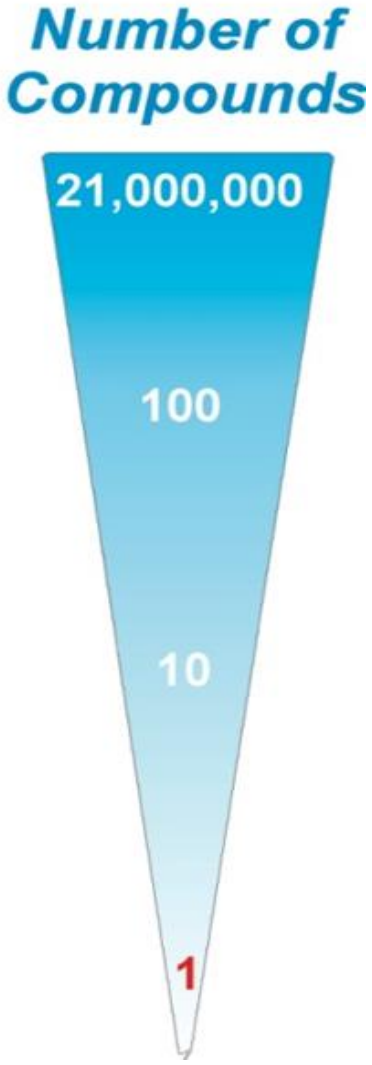
At telomeres:

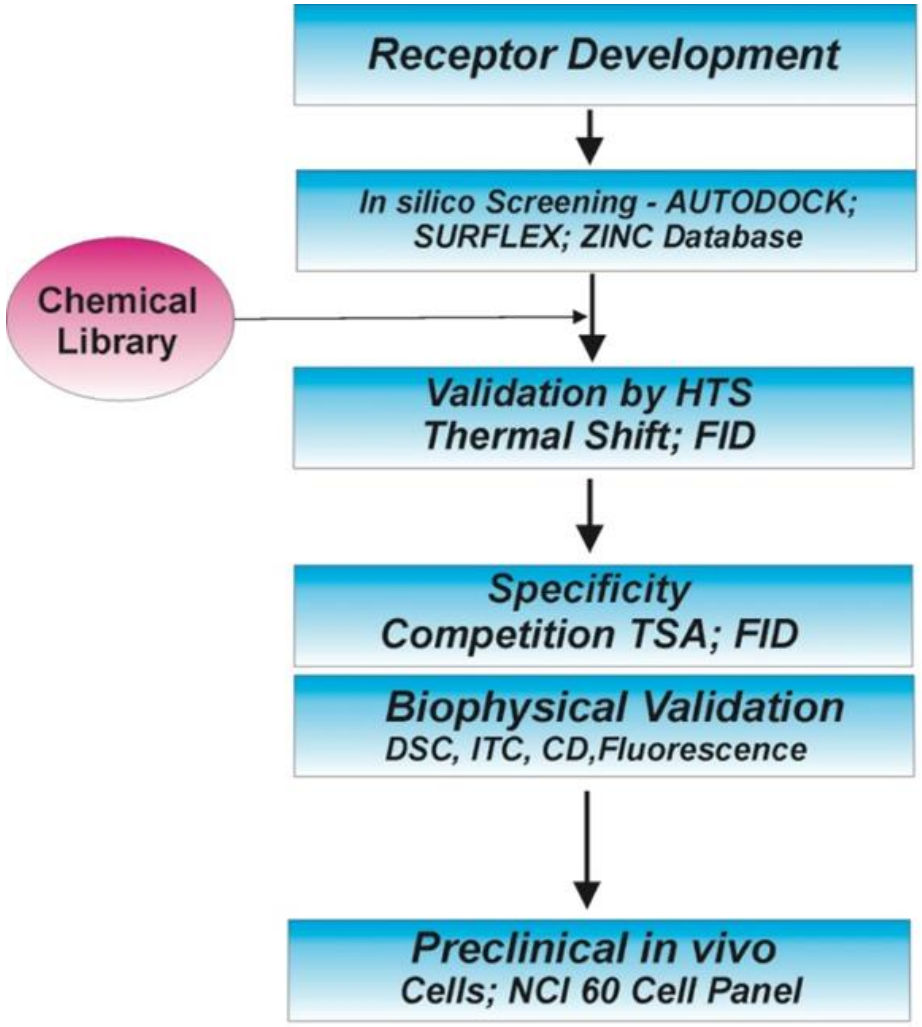


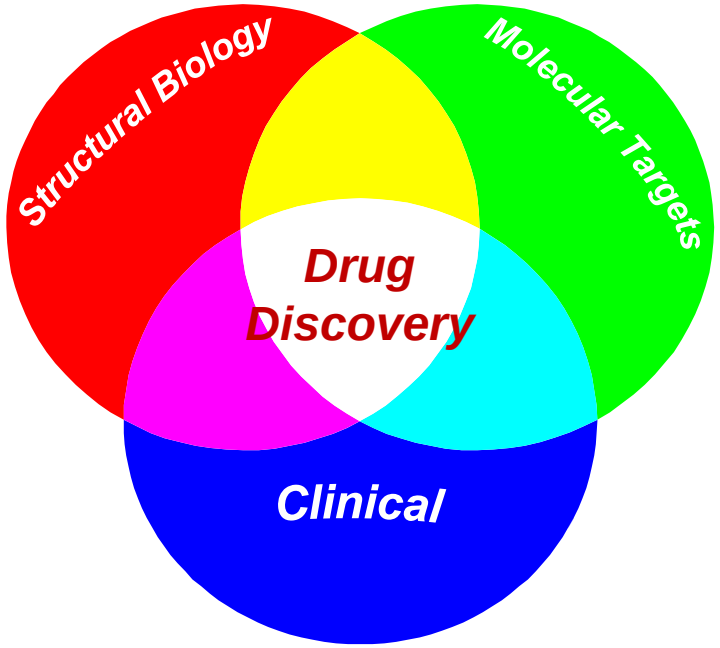
In gene promoters:



Drug Discovery at the JGBCC





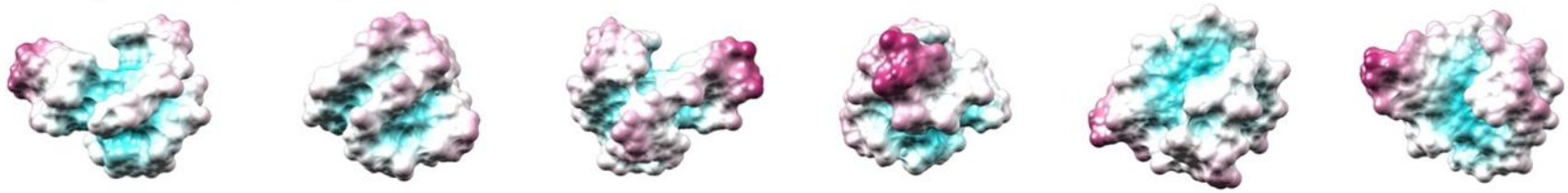


Calorimetry and thermodynamics in drug discovery
Annu. Rev. Biophys. 2008. 37:135–51

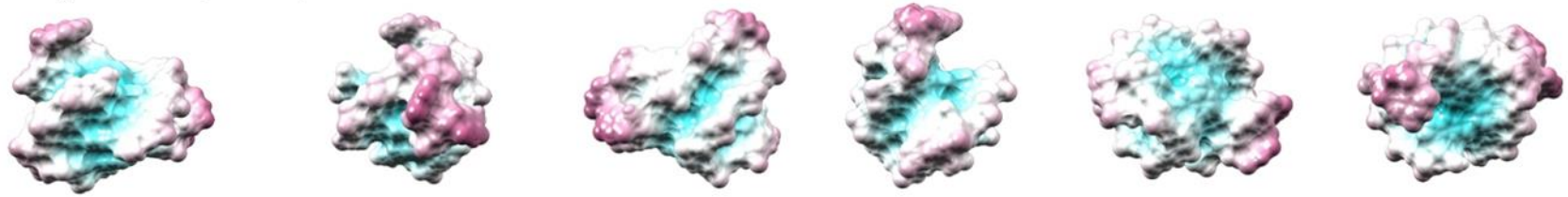
CHAPTER 4
Thermal Denaturation of Drug–DNA Complexes
JONATHAN B. CHAIRES
Chemical Biology No. 7
DNA-targeting Molecules as Therapeutic Agents
Edited by Michael J. Waring
The Royal Society of Chemistry 2018
Published by the Royal Society of Chemistry
www.rsc.org

Dock to unique G4 binding sites…

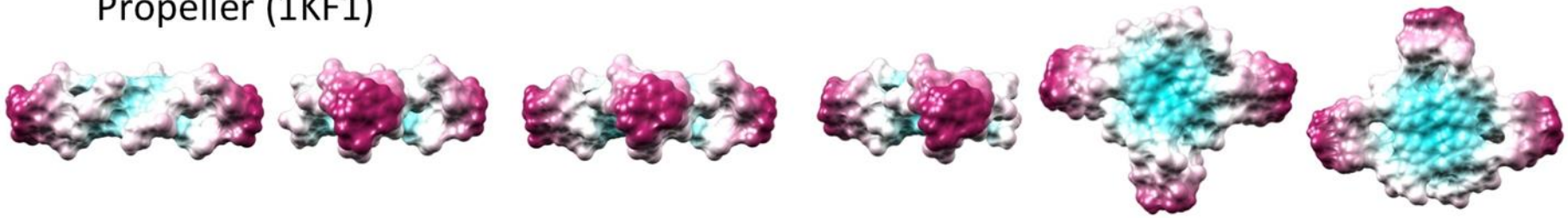
Hybrid 1 (2HY9)



Hybrid 2 (2JPZ)



Propeller (1KF1)



ThermoFluor®:

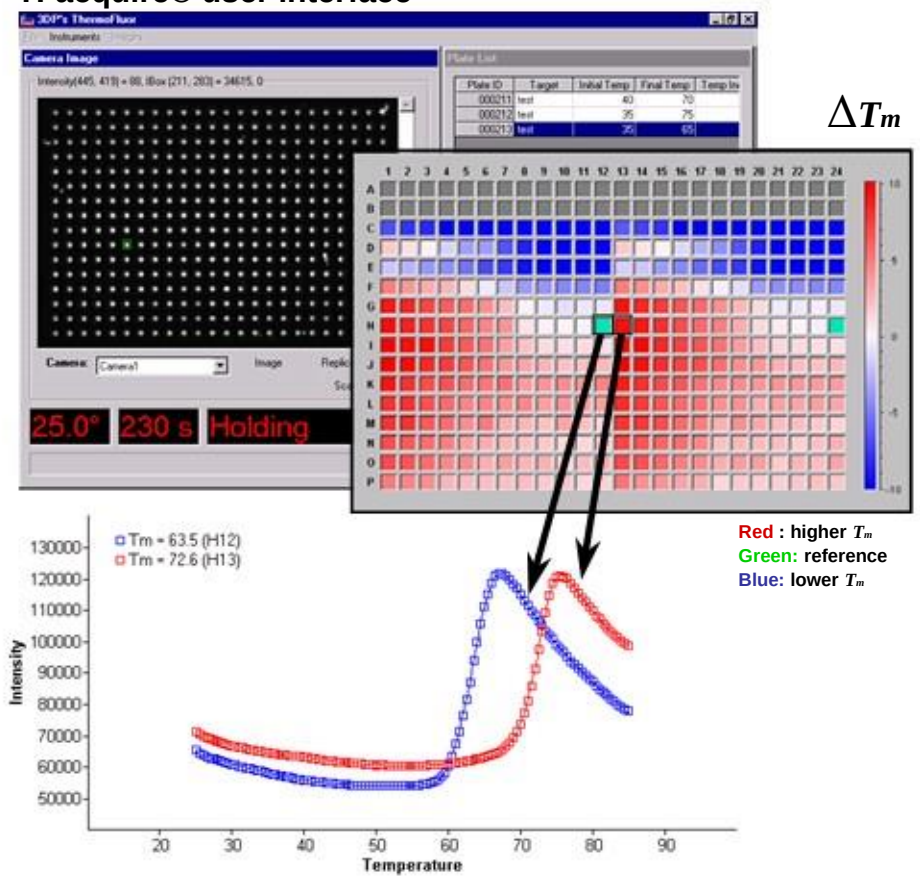
High Throughput Thermodynamic Assay

Plate-based Protein Unfolding

- § 384-well assay plate; high throughput characterization and screening of proteins.
- § Low volume, 3 µl, small-scale reactions, ~1 µM protein; typically < 200 ng well.
- § Each well comprises an individual protein unfolding assay.
- § Compound binding free energy adds to protein stability - shifts stability curve to higher temperature.

Optimization for HTS is an optimization of protein stability and signal intensity.

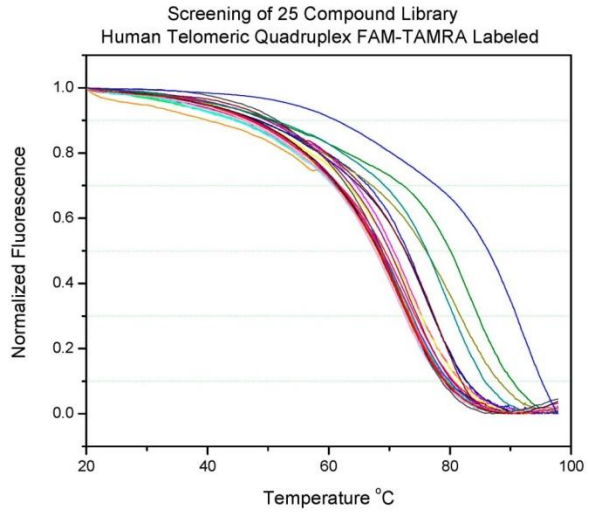
TFacquire® user interface

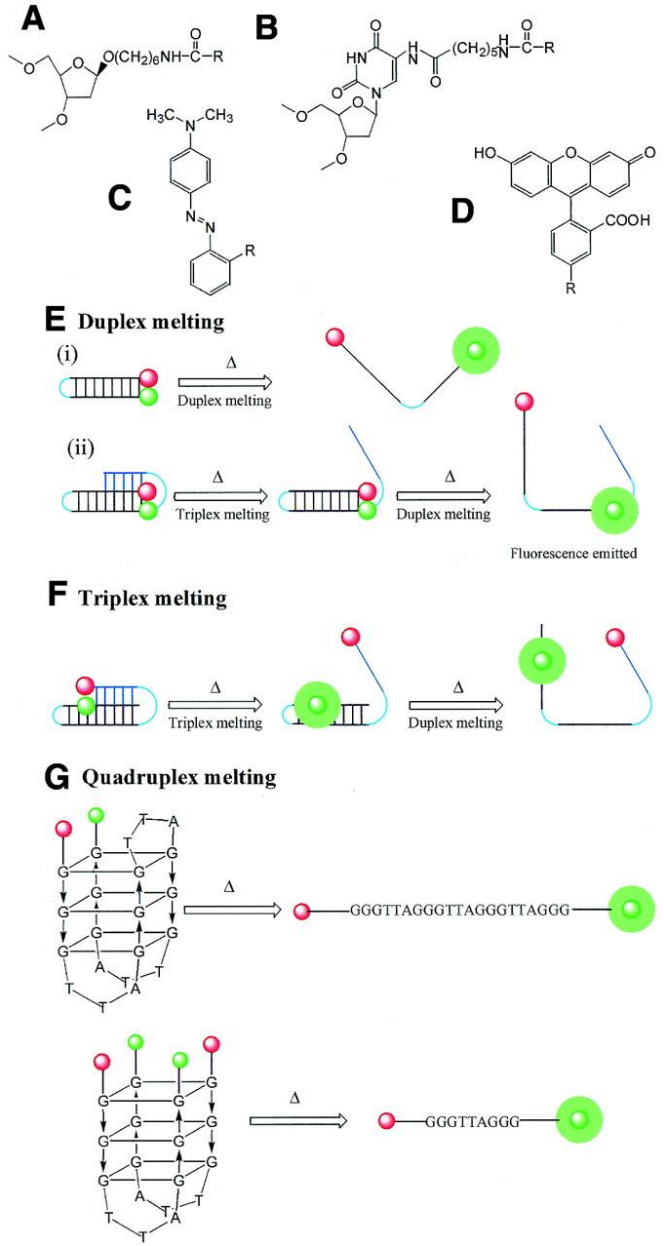


M. W. Pantoliano *et al.* (2001) *J. Biomol. Screen.* **6**: 429

M. J. Todd & F. R. Salemme (2003) *Gen. Eng. News* **23**

D. Matulis *et al*. (2005) *Biochemistry* **44**: 5258





Mergny, JL; Maurizot, JC Fluorescence resonance energy transfer as a probe for G-quartet formation by a telomeric repeat. CHEMBIOCHEM Volume: 2 Issue: 2 Pages: 124-132 DOI: 10.1002/1439-7633(20010202)2:2<124

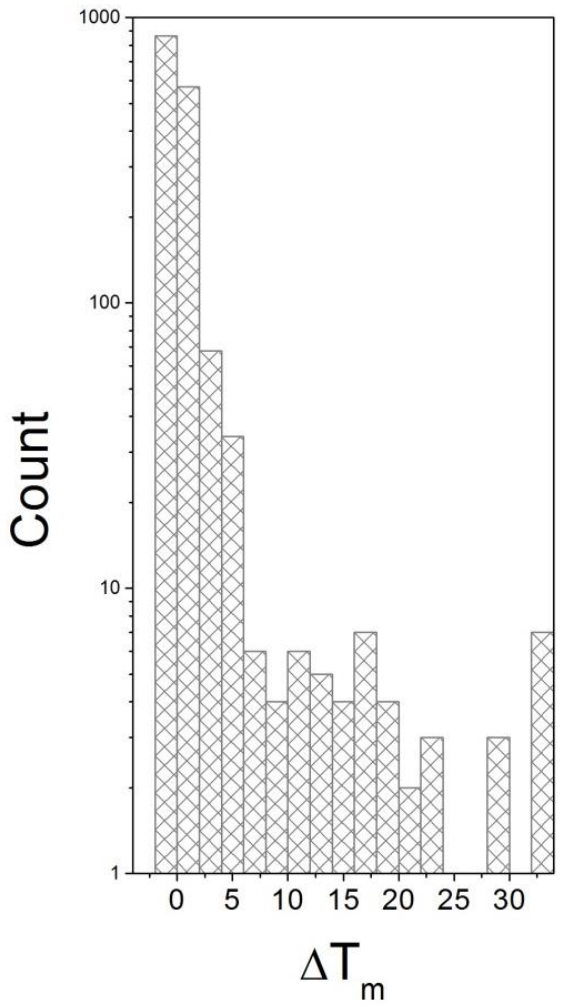
Richard A. J. Darby, Matthieu Sollogoub, Catherine McKeen, Lynda Brown, Antonina Risitano, Nicholas Brown, Christopher Barton, Tom Brown, and Keith R. Fox High throughput measurement of duplex, triplex and quadruplex melting curves using molecular beacons and a LightCycler Nucl. Acids Res. (2002)

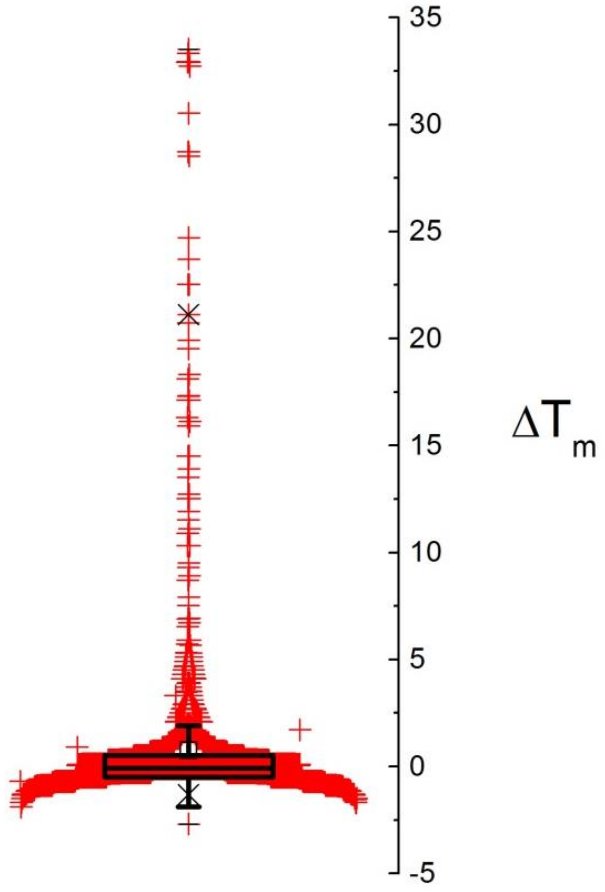
De Cian, A; Guittat, L Kaiser, M ; Sacca, B ; Amrane, S; Bourdoncle, A; Alberti, P; Teulade-Fichou, MP; Lacroix, L ; Mergny, JL Fluorescence-based melting assays for studying quadruplex ligands. METHODS Volume: 42 Issue: 2 Pages: 183-195 DOI: 10.1016/j.ymeth.2006.10.004 Published: JUN 2007

Darby R A J et al. Nucl. Acids Res. 2002;30:e39-e39

Results from HTS of NCI Diversity Set III

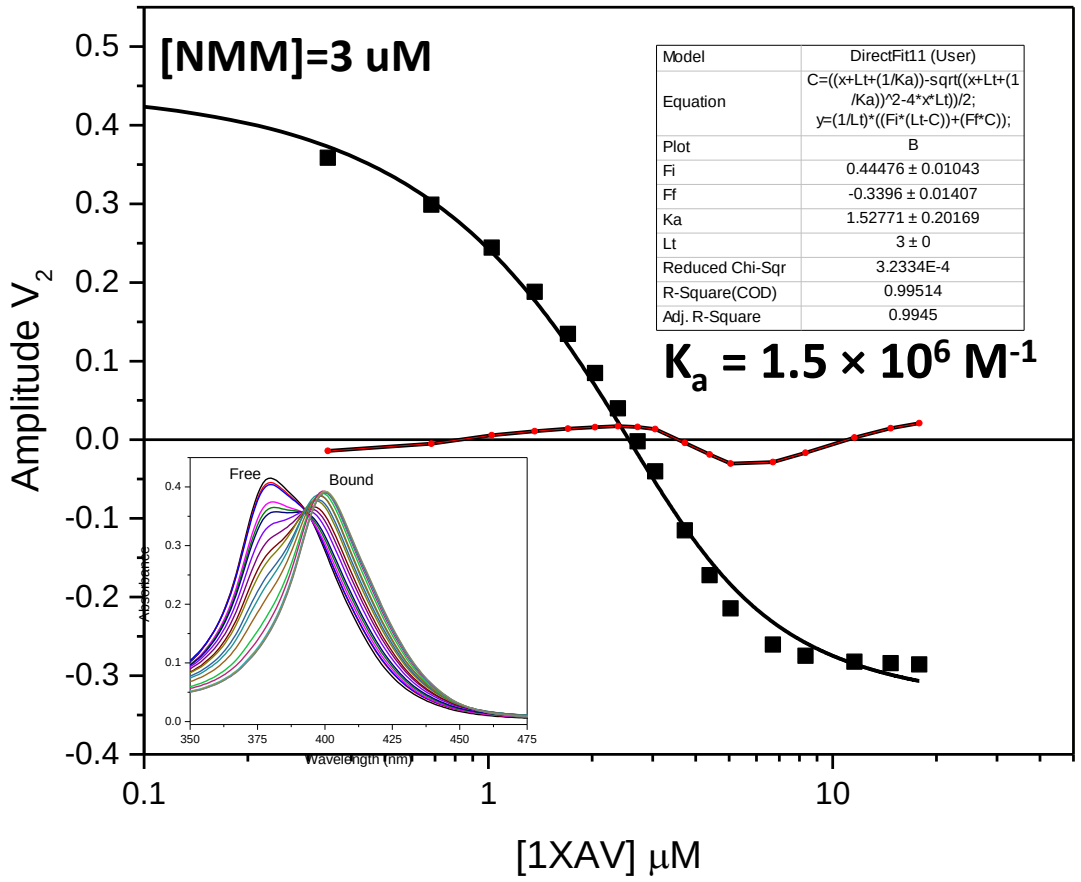
The NCI Diversity Set III of 1596 compounds is available on Greiner 650201 96-well PP U-bottom plates. Compounds are arrayed at 20 uL /10 mM in DMSO





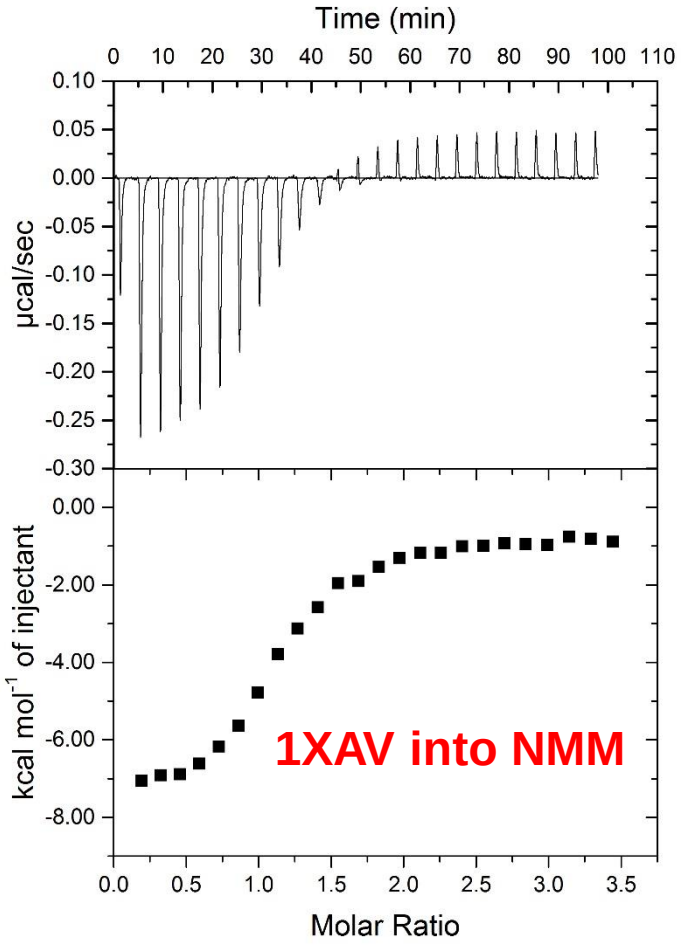
Binding to human telomere quadruplex in K^+

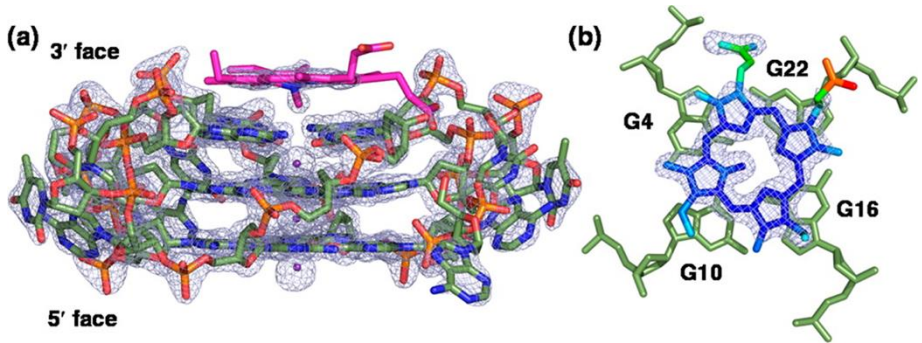
Binding measured by Isothermal Titration Calorimetry



Model	DirectFit11 (User)
Equation	C=((x+Lt+(1/Ka))-sqrt((x+Lt+(1/Ka))^2-4*x*Lt))/2; y=(1/Lt)*((Fi*(Lt-C))+(Ff*C));
Plot	B
Fi	0.44476 ± 0.01043
Ff	-0.3396 ± 0.01407
Ka	1.52771 ± 0.20169
Lt	3 ± 0
Reduced Chi-Sqr	3.2334E-4
R-Square(COD)	0.99514
Adj. R-Square	0.9945

$K_a = 1.5 \times 10^6\ M^{-1}$

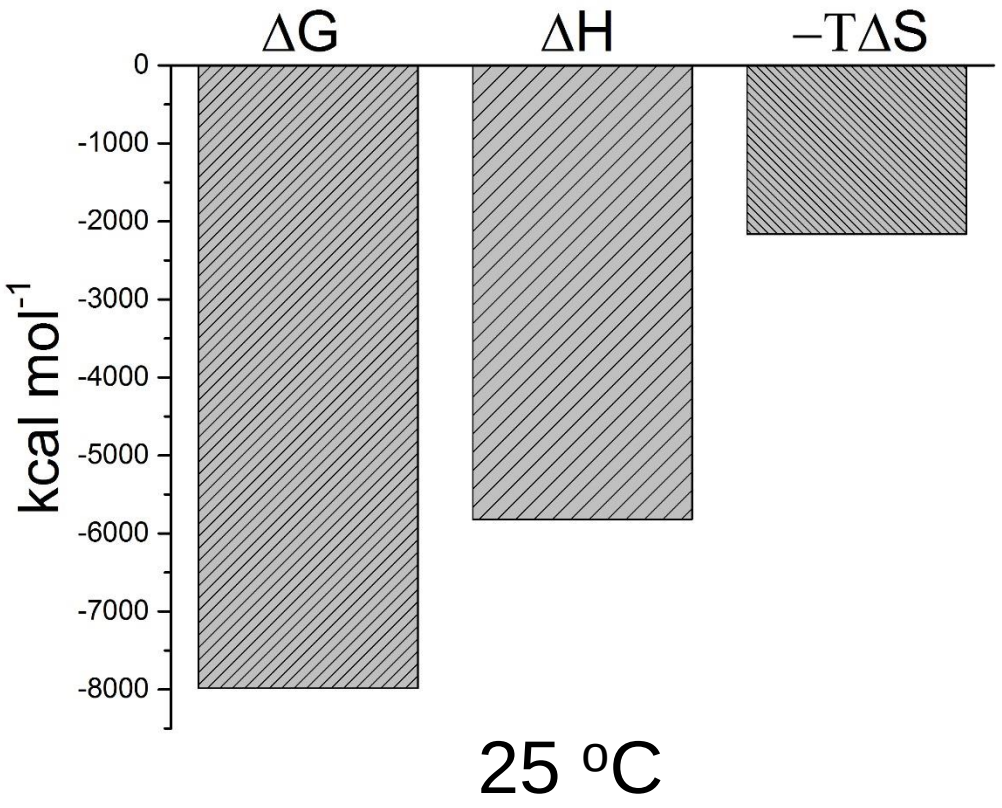


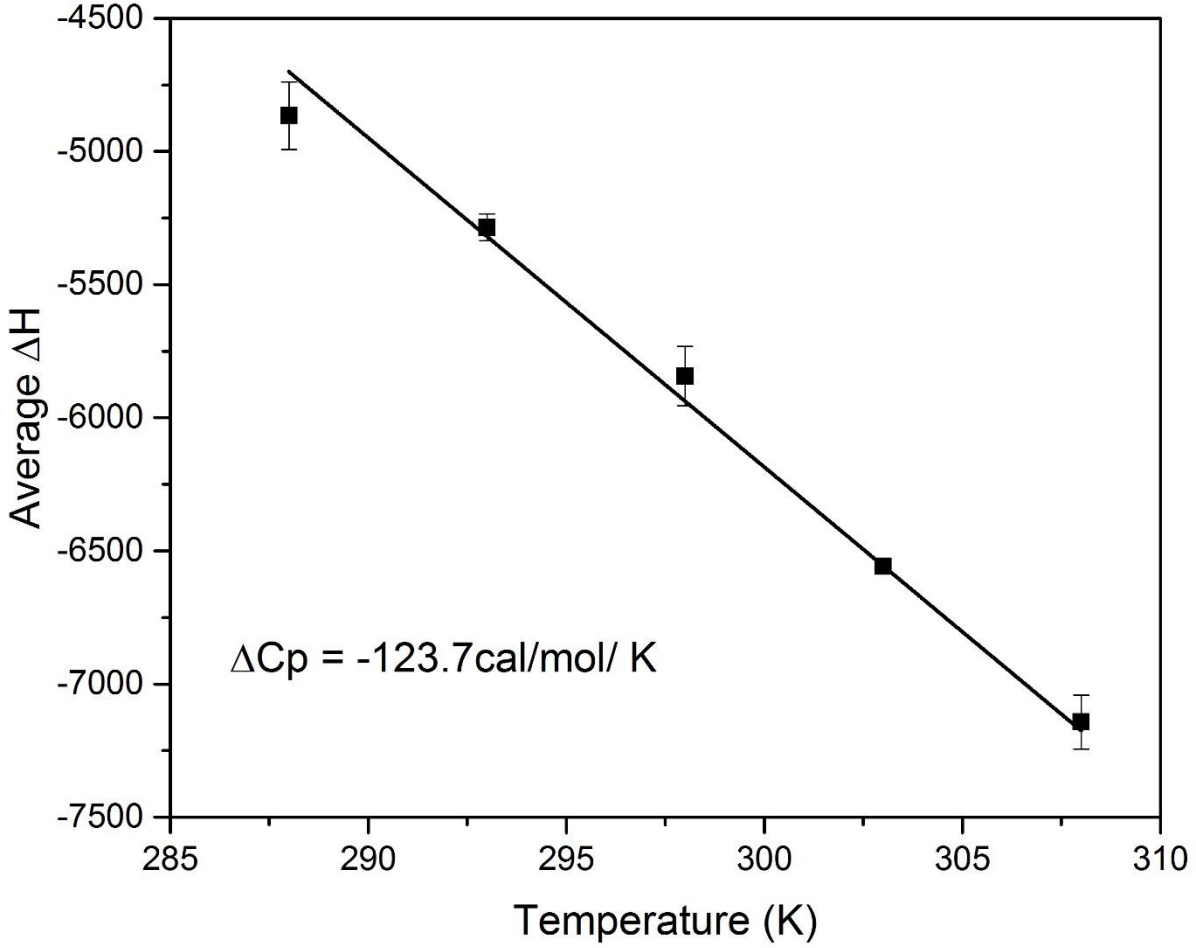


n	1.1
Ka	$2.1 \times 10^6\ M^{-1}$
ΔH	-5.7 kcal mol^{-1}
ΔH_{dil}	+0.8 kcal mol^{-1}

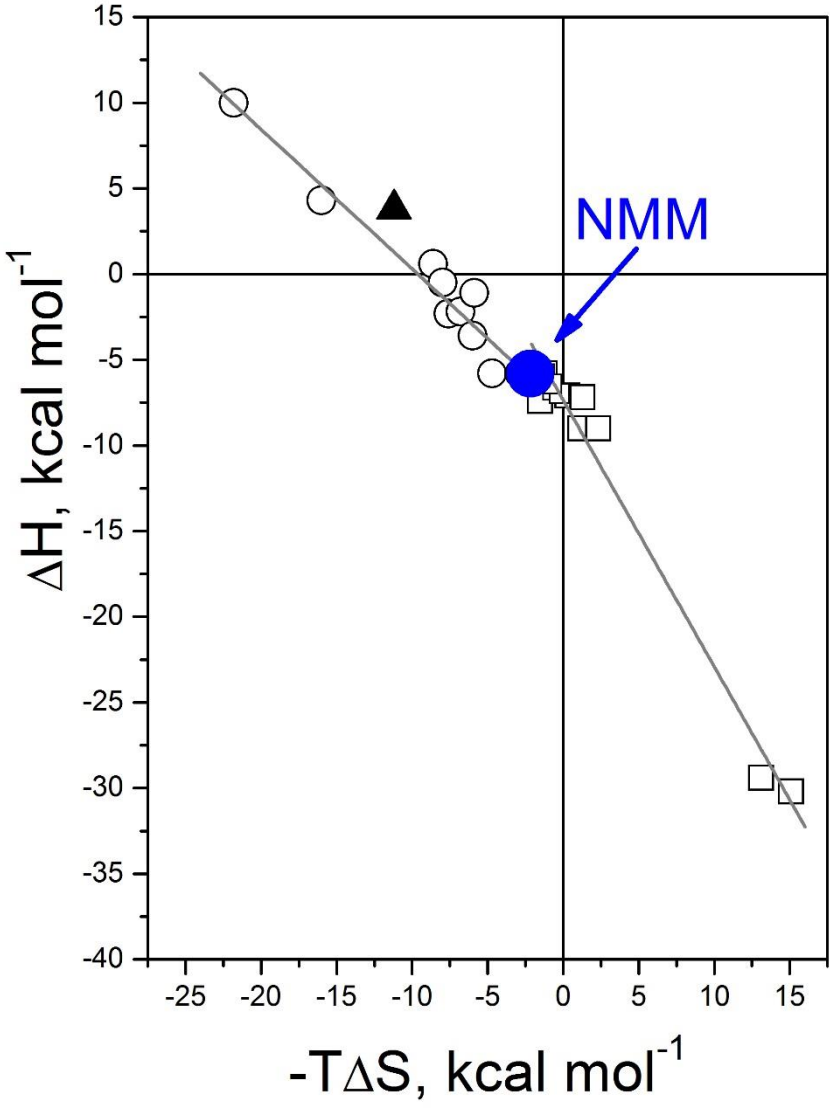
Thermodynamic Profile for NMM Binding to 1XAV – Enthalpy Driven

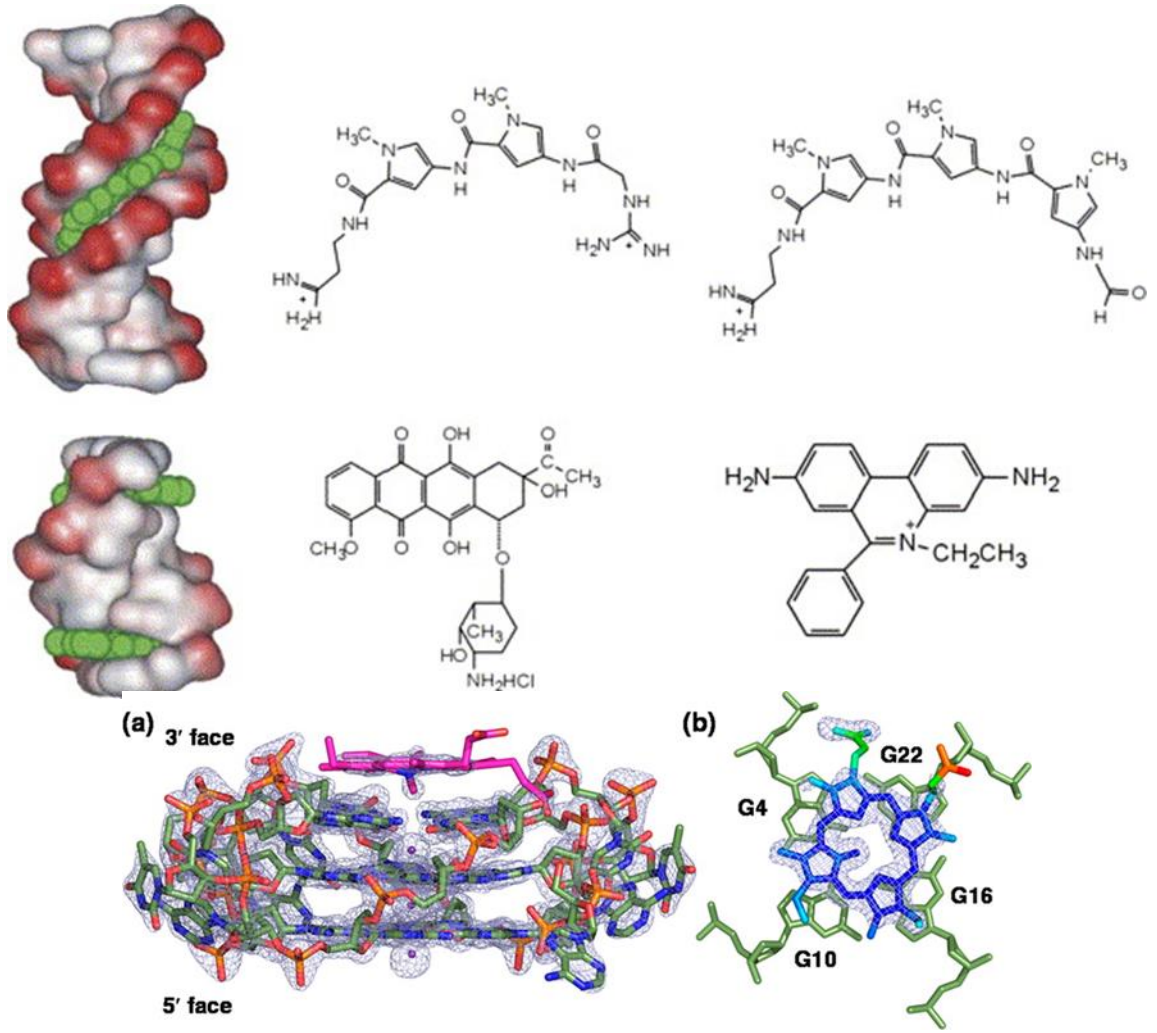
$\Delta G = \Delta H - T\Delta S$





The NMM binding profile is at the interface between intercalators and groove-binders!





Jonathan B. Chaires **A thermodynamic signature for drug–DNA binding mode** *Archives of Biochemistry and Biophysics* Volume 453, Issue 1, 2006, 26–31

A Biophysical Core Facility for the James Graham Brown Cancer Center
University of Louisville Health Science Center

Dr. J. Brad Chaires, Director
Dr. Nichola Garbett, Assistant Director & Manager

$1 million+ invested in 2004

Operates as a fee-for-service facility providing the tools necessary for detailed thermodynamic and kinetic studies for:

- drug-macromolecule interactions
- macromolecular association reactions
- macromolecule stability
- long-term collaborative interactions
- preliminary studies for grant proposals

Will do experiments using samples provided, or will train personnel to use instruments

Available to University community

Will provide services to nonuniversity companies or Academics

Expected to be at least 50% self-sufficient; subsidized by Departments, Centers, training and program project grants

Nonprofit

Techniques Available

Calorimetry:

- ❖ **Microcal VP DSC (with Pressure Perturbation)**
- ❖ **Microcal VP ITC**
- ❖ **Microcal Capillary VP DSC ("High-throughput")**

Spectroscopy:

- ➢ **Jasco J-800 CD**
- ➢ **Jasco Fluorescence**
- ➢ **UV/Vis**
- ➢ **Biacore SPR**
- ➢ **OLIS Rapid Scan Stopped-flow**

Analytical Ultracentrifuge

What I learned…

- **Don’t set up a biophysics core facility in a medical school – not enough users**
- **“Nonprofit” is the operative word – it is hard to even break even**
- **Deciding appropriate user fees is almost impossible – administrators want “more” but “more” drives users away**
- **The cost of service contracts will kill you**
- **Promised subsidies often never appear**
- **Start up companies sometimes don’t pay their bills**
- **Trained users don’t care about instruments as much as you do. Their incompetence can be costly.**
- **Users rarely listen to good advice, especially about sample purity and quantity**
- **Don’t provide too many techniques. With limited personnel it is hard to provide necessary expertise across too many platforms**
- **The best, most likely to succeed facility, would probably be focused on a single method (ie AUC or ITC or DSC).**