Estudi de les estructures *G-quadruplex* e *i-motif* del DNA mitjançant tècniques espectroscòpiques i quimiomètriques

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Acknowledgments

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Many MSc and Graduate Students

Collaborators:

- IQAC: Dr. Ramon Eritja, Dra. Anna Aviñó, Dr. Santi Grijalvo, Dra. Sonia Pérez-Rentero, Dr. Rubén Ferreira …
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- IBMB: Dr. Sébastien Lyonnais, Dra. Maria Solà …
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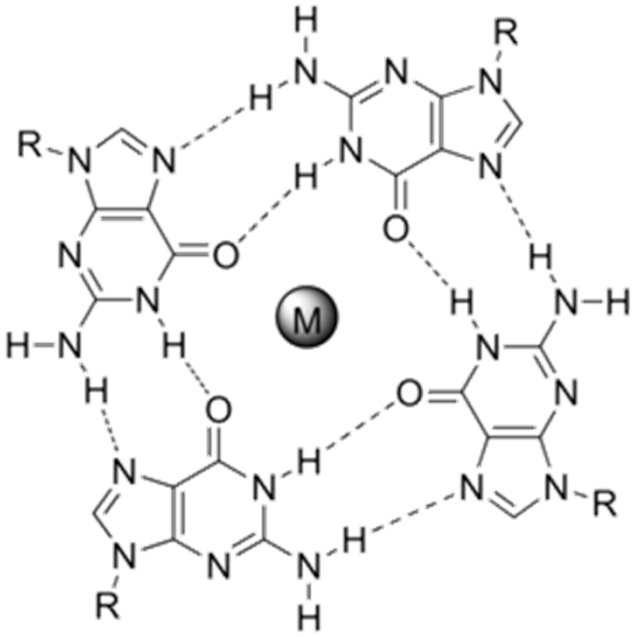
- …

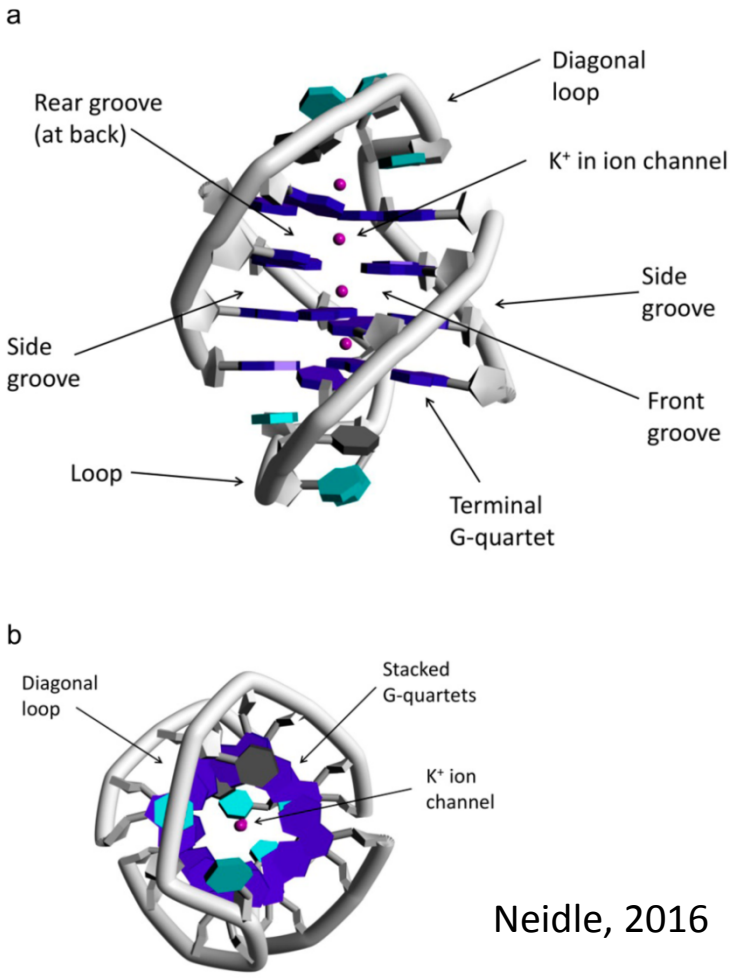
Overview

- Introduction to G-quadruplex and i-motif structures
  - Fundamentals
  - *In vivo*
  - Applications
- Multivariate data analysis methods and examples

G-quadruplex structures. G-tetrad

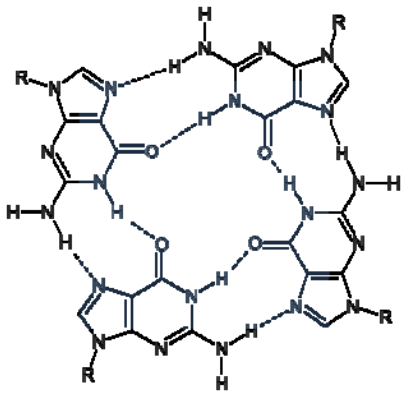


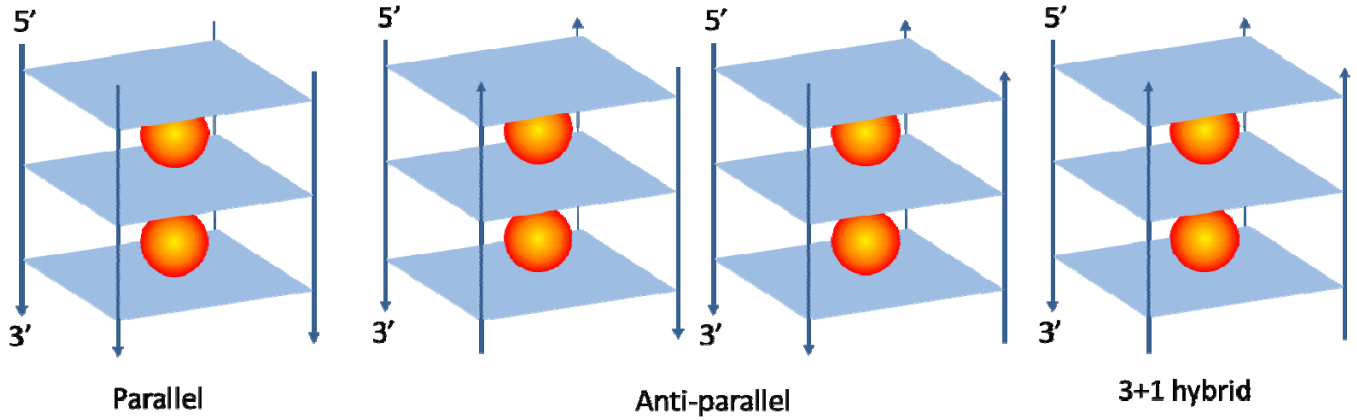
M = K^+, Na^+, NH_4^+, …

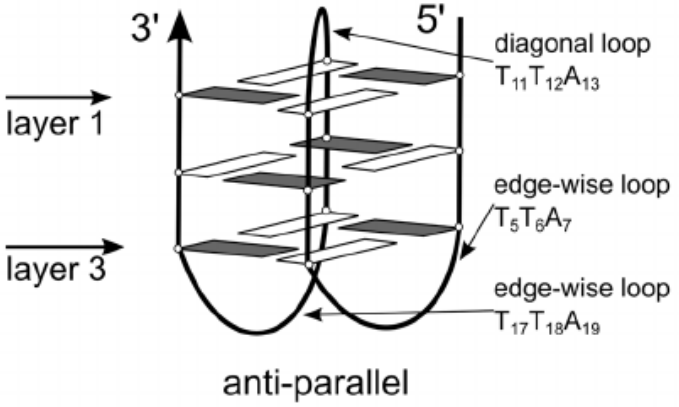


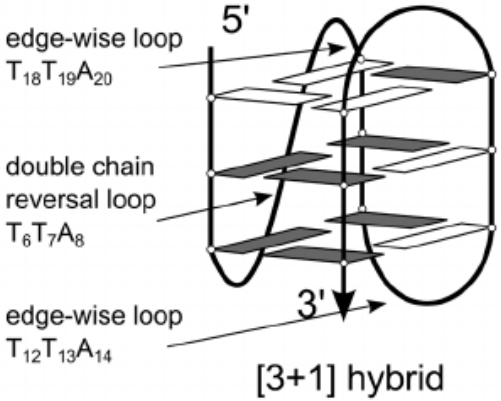
Neidle, 2016

G-quadruplex structures. Topologies and loops



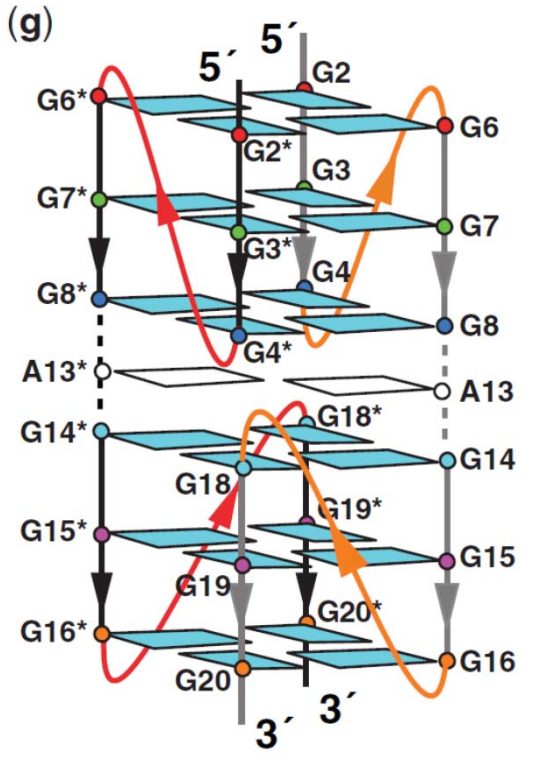






Zhu *et al.* 2013

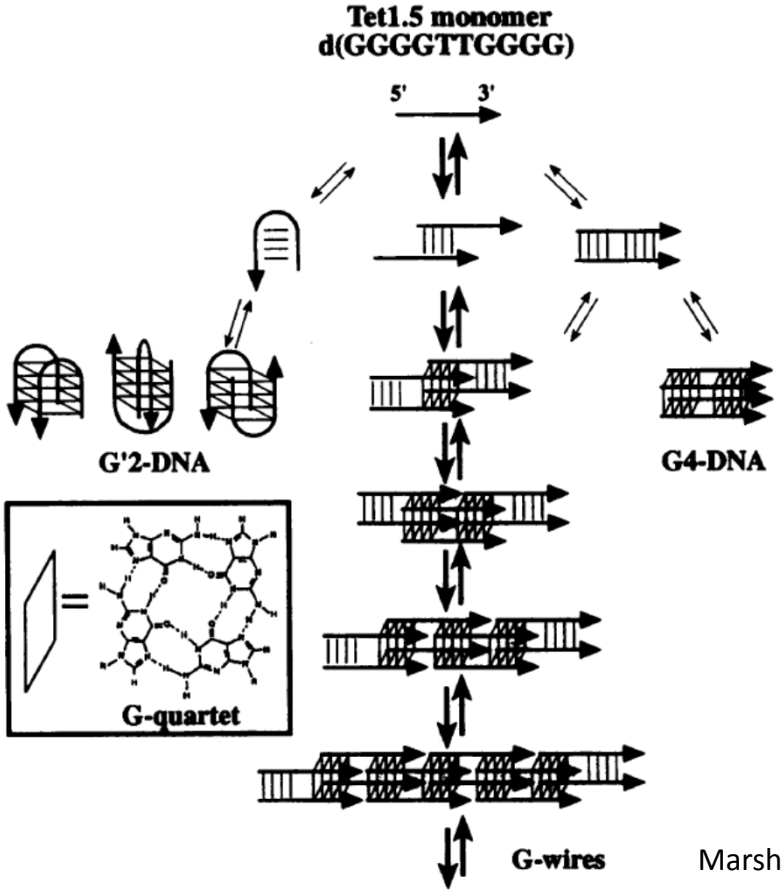
G-quadruplex structures. Polymorphism



Published online 21 June 2010 Nucleic Acids Research, 2010, Vol. 38, No. 19 6757-6773
doi:10.1093/nar/gkq558

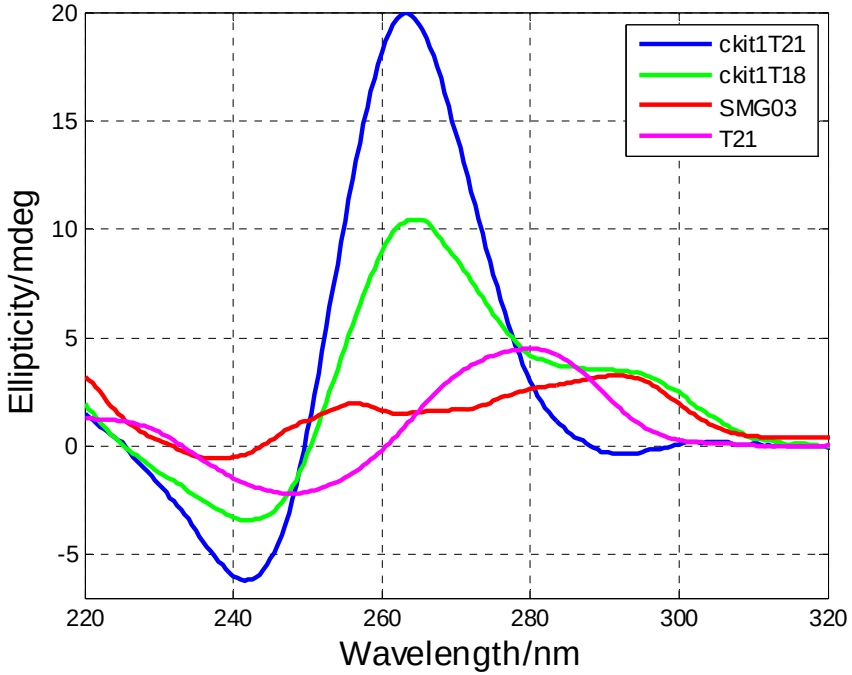
Solution structures of all parallel-stranded monomeric and dimeric G-quadruplex scaffolds of the human *c-kit2* promoter

Vitaly Kuryavyi[1], Anh Tuân Phan[2] and Dinshaw J. Patel[1,*]



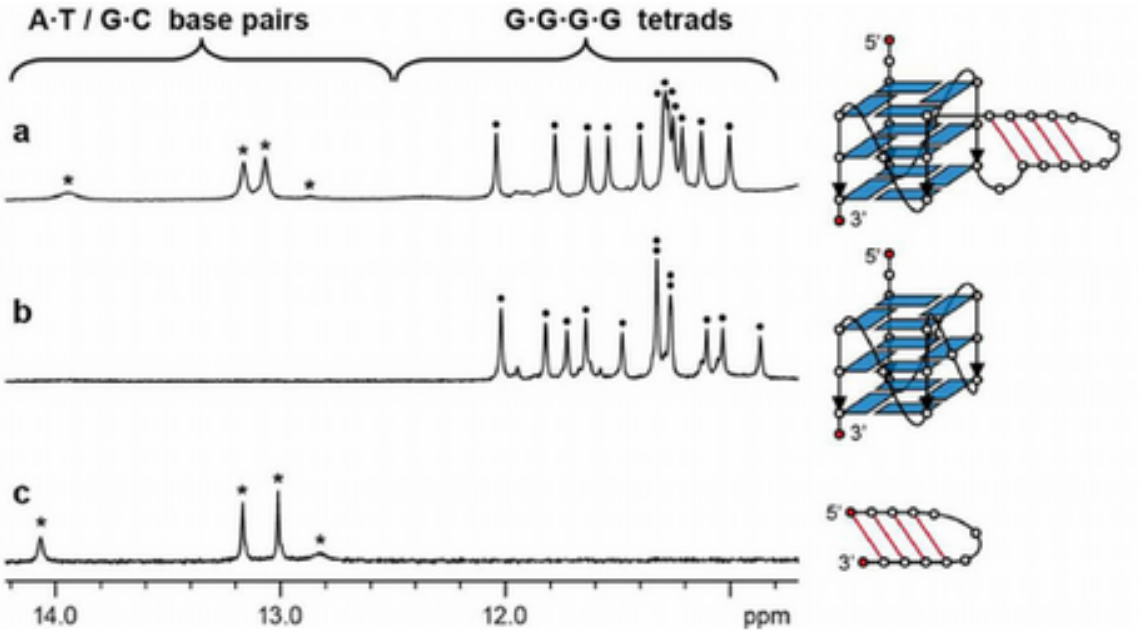
Marsh *et al.*, 1995

Circular Dichroism



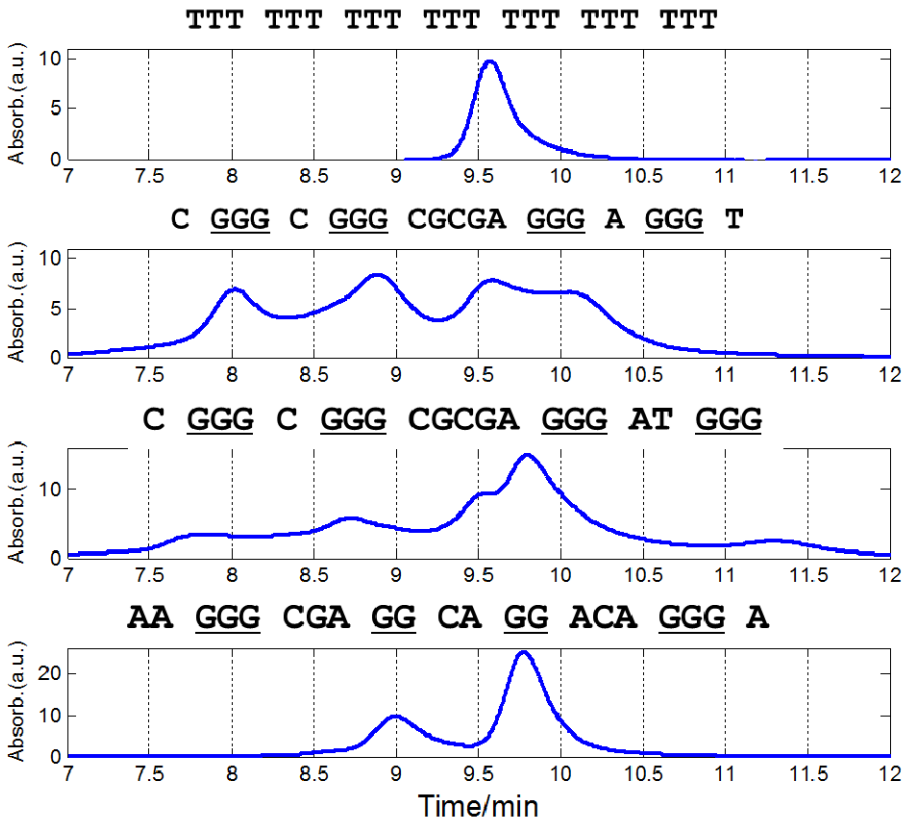
Benito *et al.*, in preparation

NMR

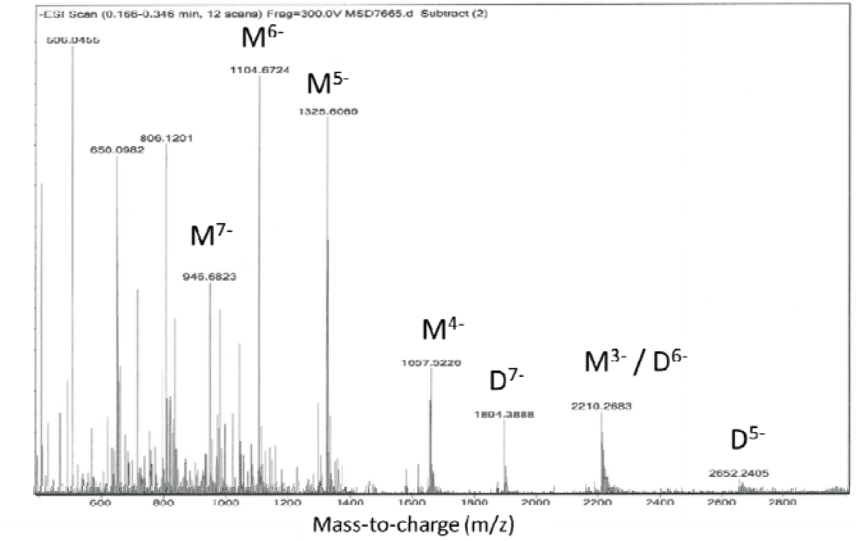


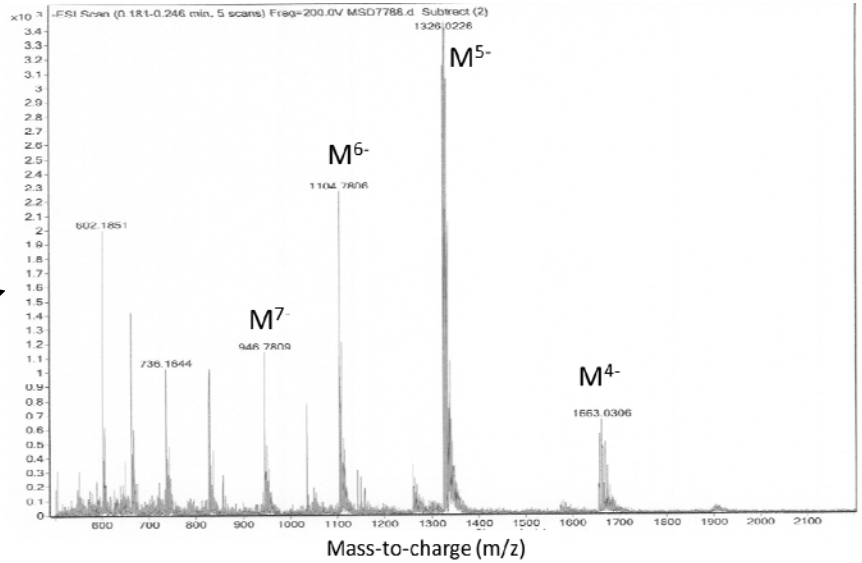
Kah Wai Lim *et al.*, 2015

Size-Exclusion Chromatography



Benito *et al.* In preparation



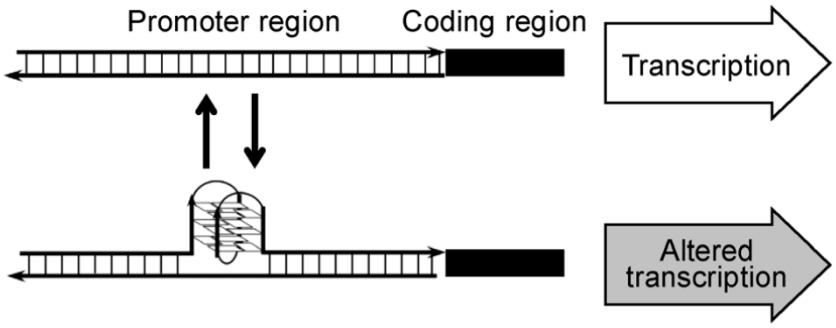


ESI-MS

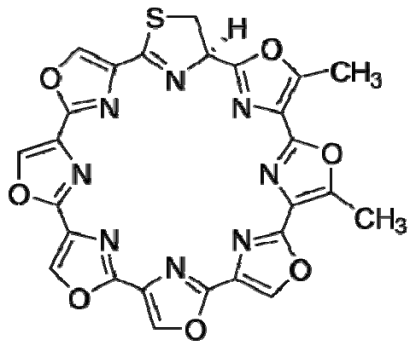
G-quadruplex structures. *In vivo*

Guanine-rich sequences are present in:

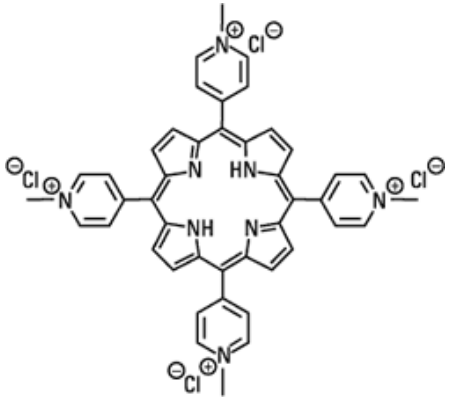
- End of telomeres
  - Human telomere: $(T_2AG_3)_n$
  - Potential role in cellular replication
- Near the promoter regions of several genes (*c-myc*, *c-kit*, *KRAS*, *bcl-2*, ...)
  - They are believed to control gene expression:



Yaku *et al*., 2012

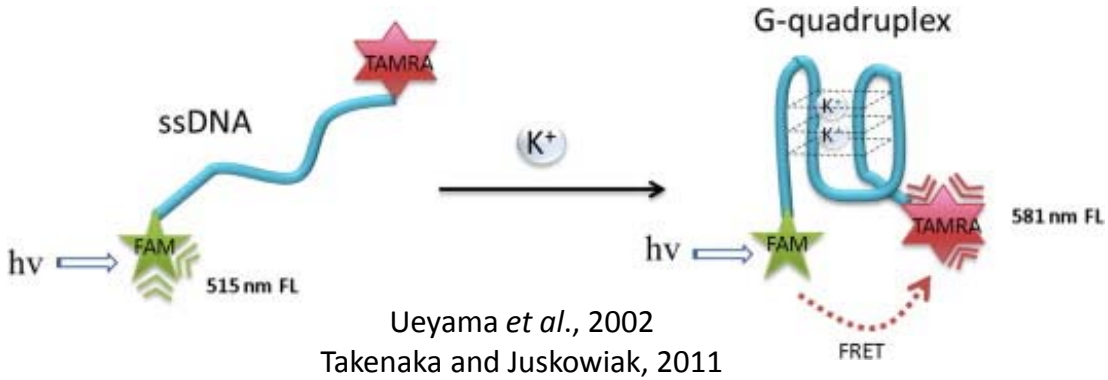


Telomestatin

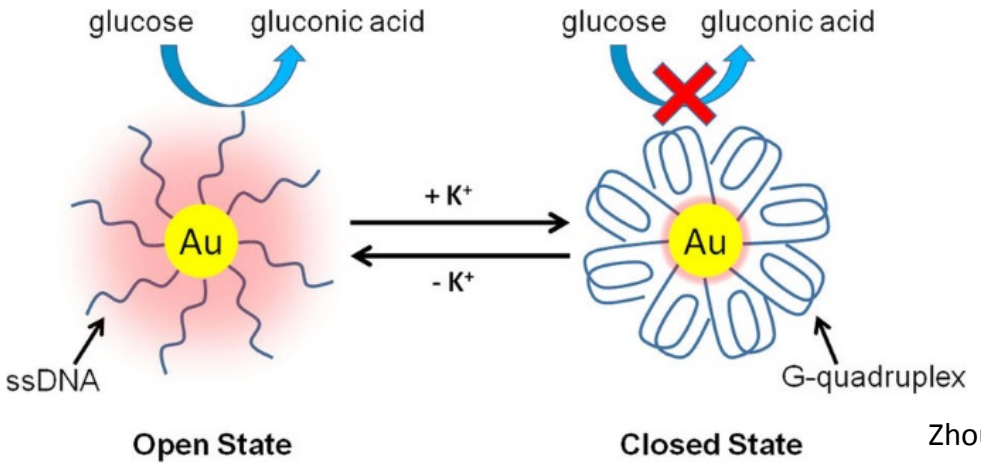


G-quadruplex structures. Applications

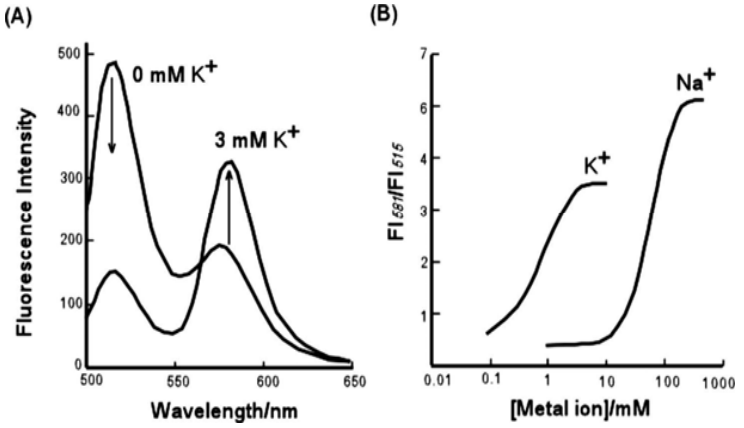
- Sensors for metal ions quantitation
  - Fluorescence-based methods
- Catalysis
- Drug delivery
- …



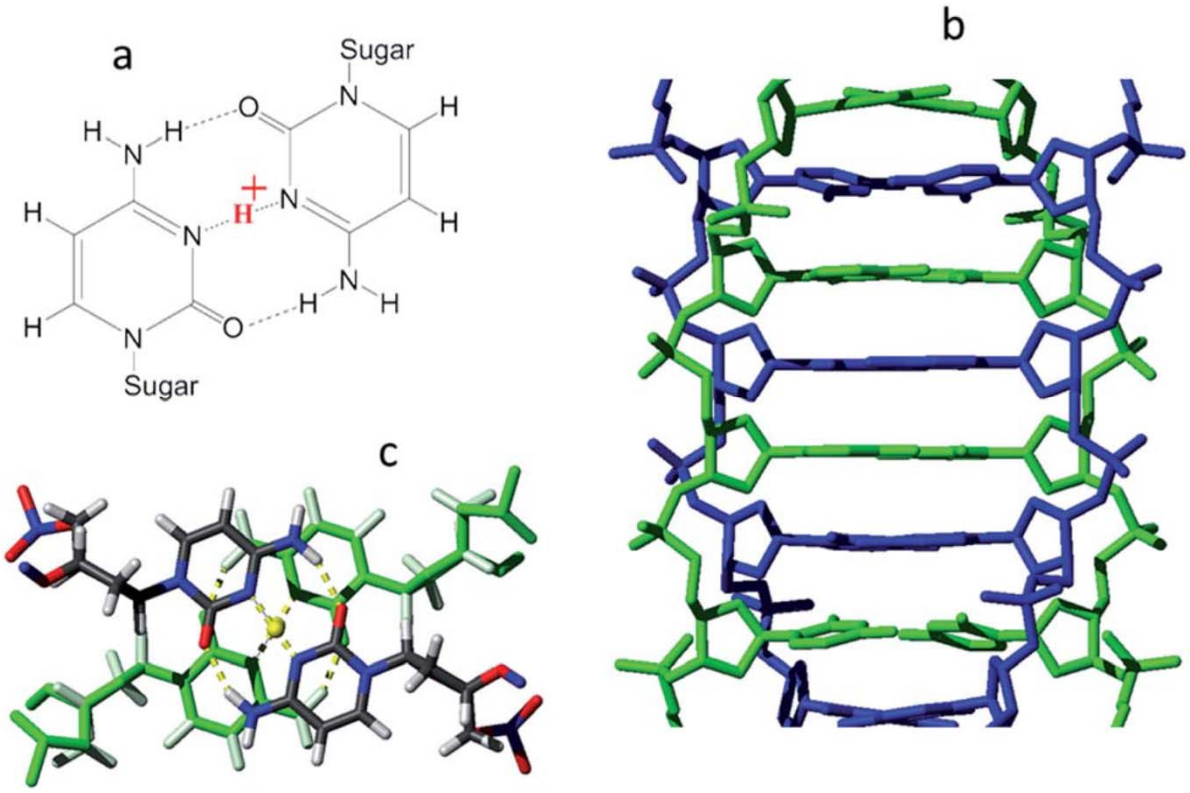
Ueyama *et al*., 2002
Takenaka and Juskowiak, 2011



Zhou *et al*., 2015



i-motif structures. Fundamentals



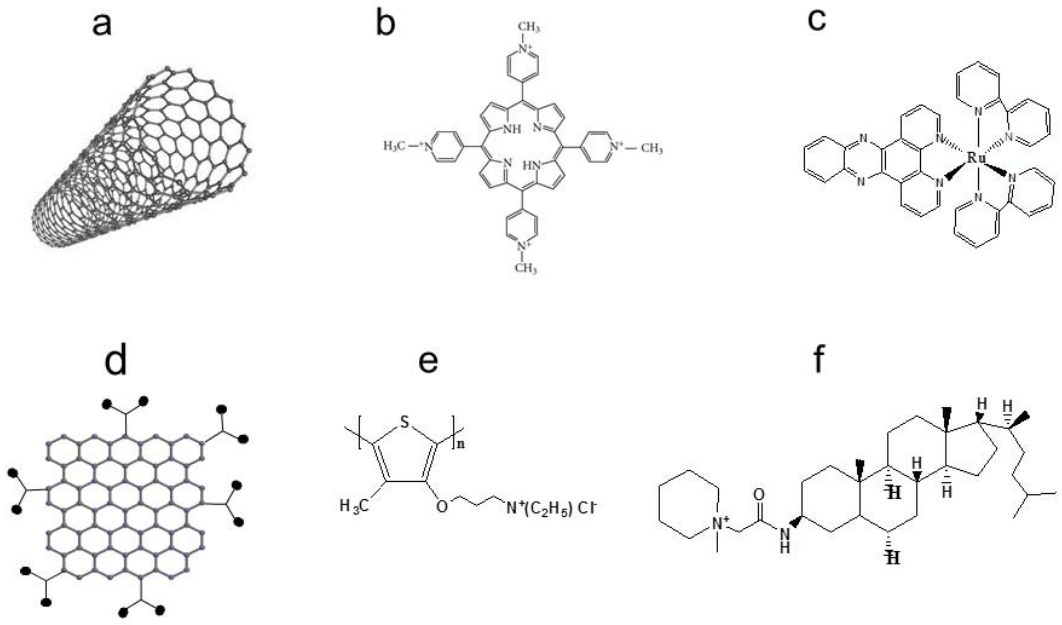
pK_a cytosine ~ 4.5

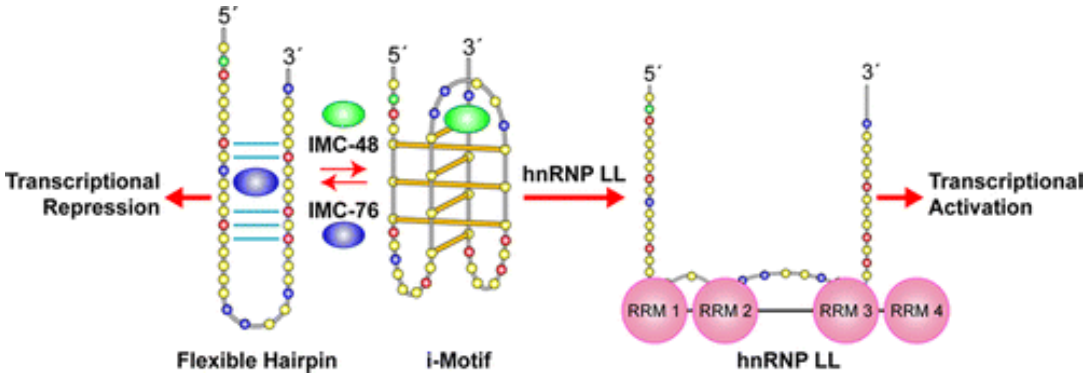
Not very stable at pH 7 and 37°C

As pKa cytosine ~ 4.5, pH control is essential!!

Benabou *et al*. 2014

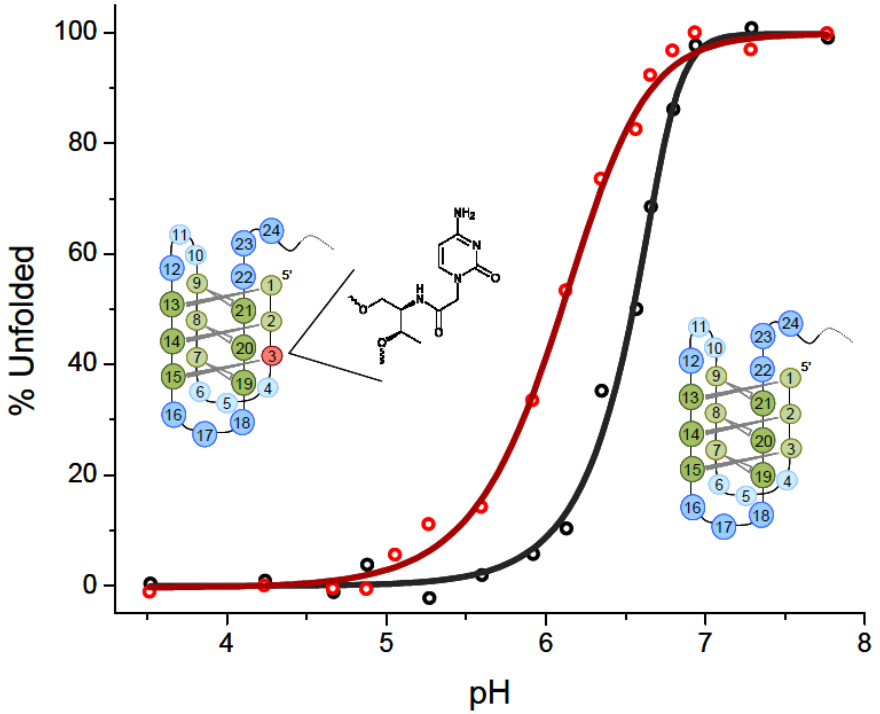
i-motif structures. Ligands



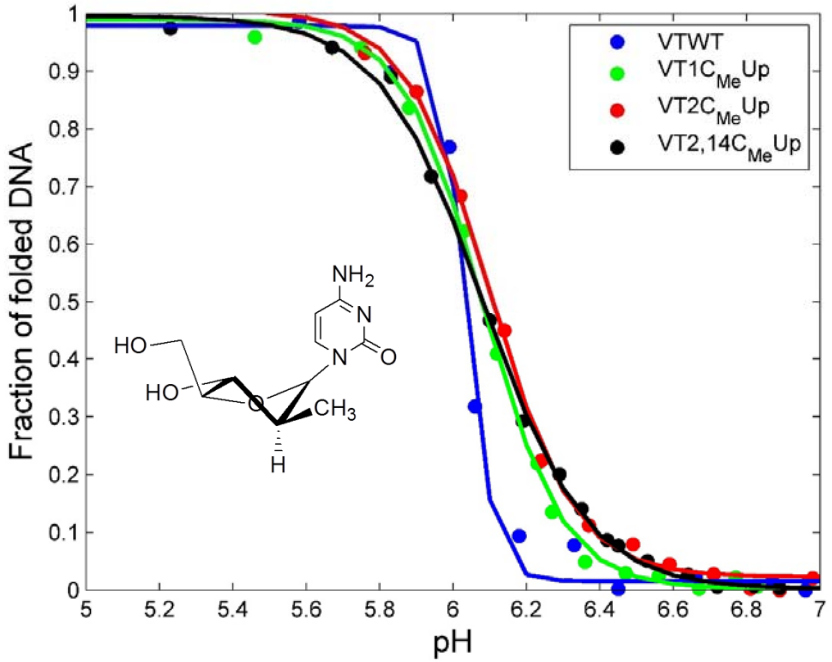


Kang *et al*. 2014

i-motif structures. Chemical modifications



acyclic threoninol cytidine
Pérez-Rentero *et al*. 2015

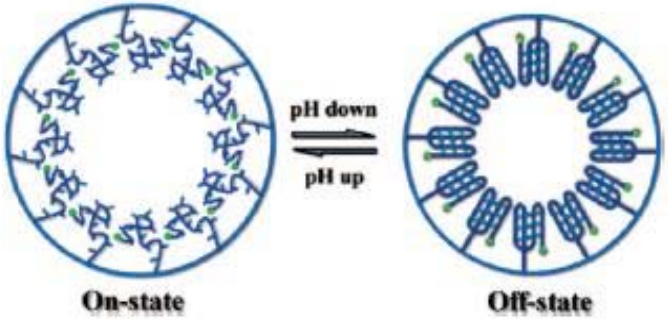


(2'S)-2'-deoxy-2'-C-methyl-cytidine
Aviñó *et al*. 2017

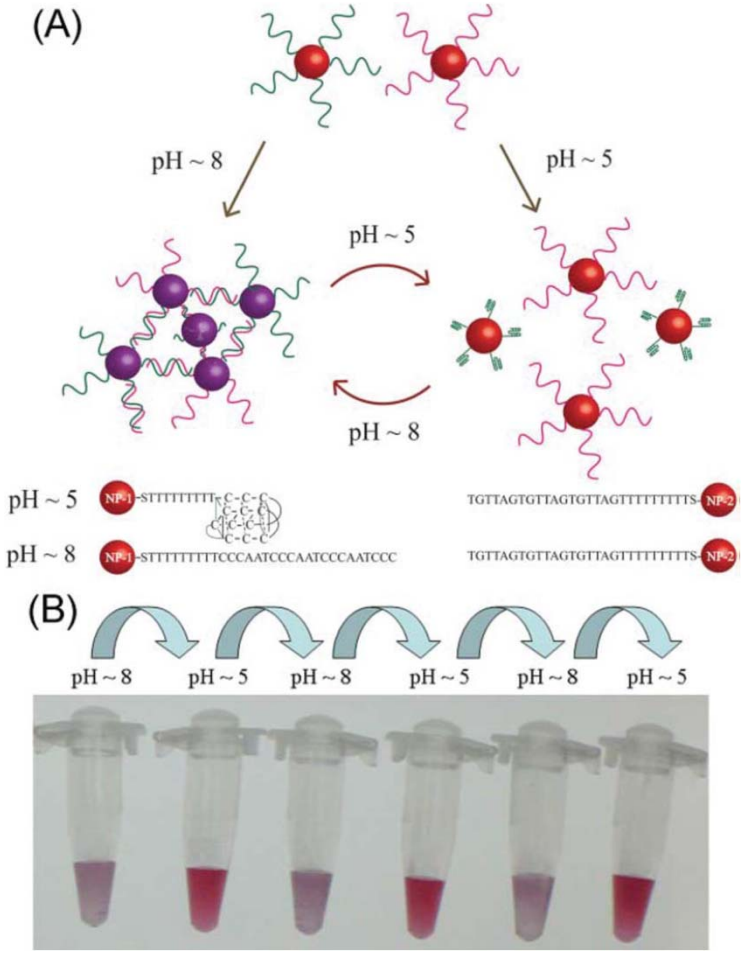
i-motif structures. Applications

Many applications:

- FRET-based pH-sensing devices
- Stabilization of gold and silver nanoclusters and nanoparticles
- pH-fuelled nanomotors



Xia et al. 2008

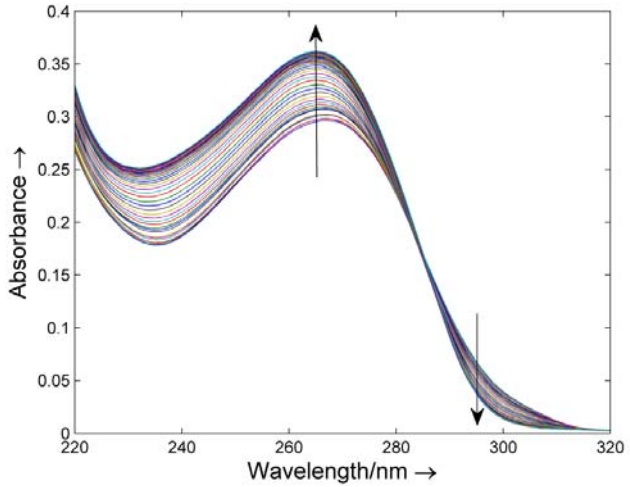


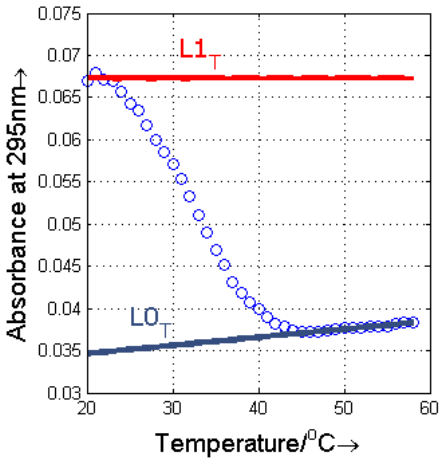
Sharma et al. 2007

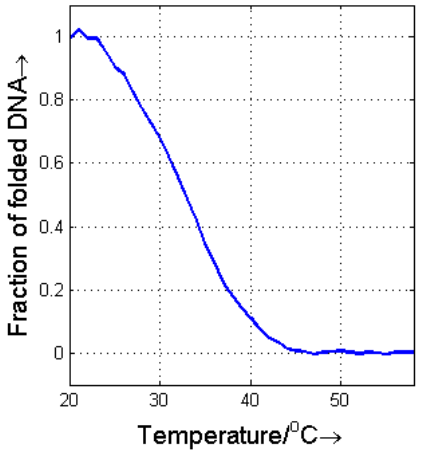
Overview

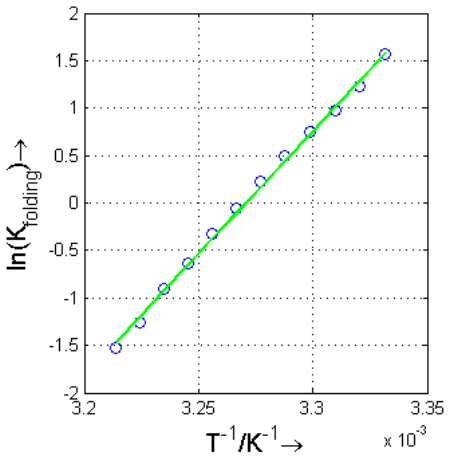
- Introduction to G-quadruplex and i-motif structures
  - Fundamentals
  - In vivo
  - Applications
- Multivariate data analysis methods and examples

Spectroscopically-monitored unfolding of an i-motif



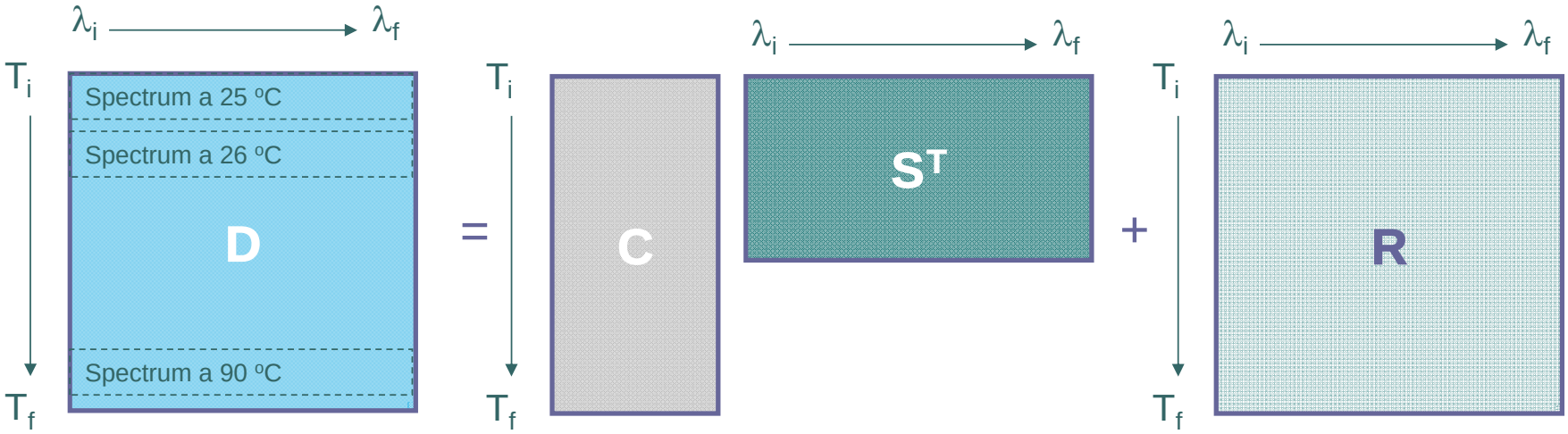






ΔH^0 (kcal/mol) = -52 ± 2 (95%)
ΔS^0 (cal/K·mol) = -169 ± 6 (95%)
T_m (°C) = 32.6

Multivariate analysis. Fundamentals



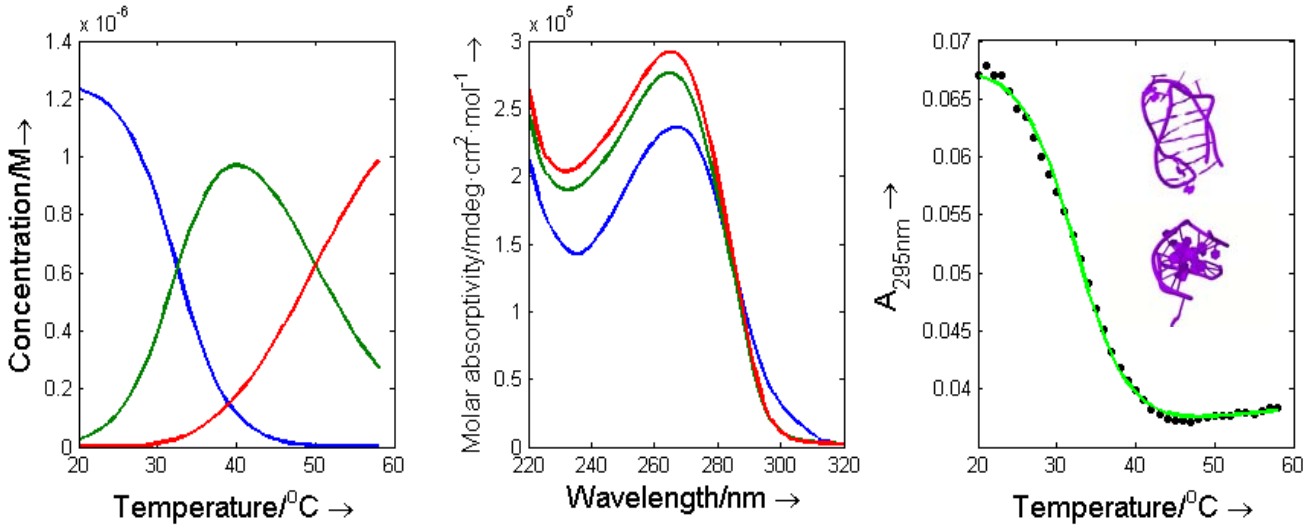
Multivariate analysis. Different approaches

- Multivariate analysis based on a physico-chemical model
  - Acid-base equilibria → stoichiometry and protonation constants
  - Ligand:DNA interaction equilibria → stoichiometry and binding constants
  - Thermally-induced unfolding of a folded structure → thermodynamic variables (ΔH^0, ΔS^0, ΔG)

- Multivariate analysis based without any model
  - Soft-modelling
  - Example: MCR-ALS (Romà Tauler, IDAEA)

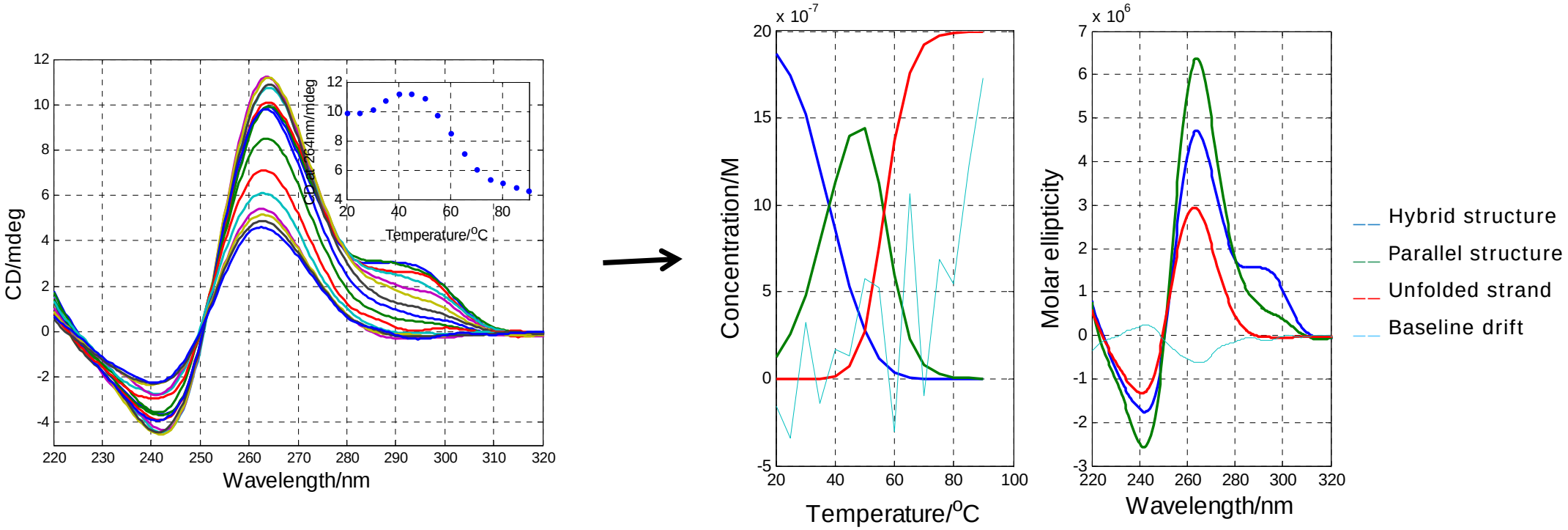
- Hybrid approaches
  - Anna De Juan (UB), Romà Tauler, …

Spectroscopically-monitored unfolding of an i-motif



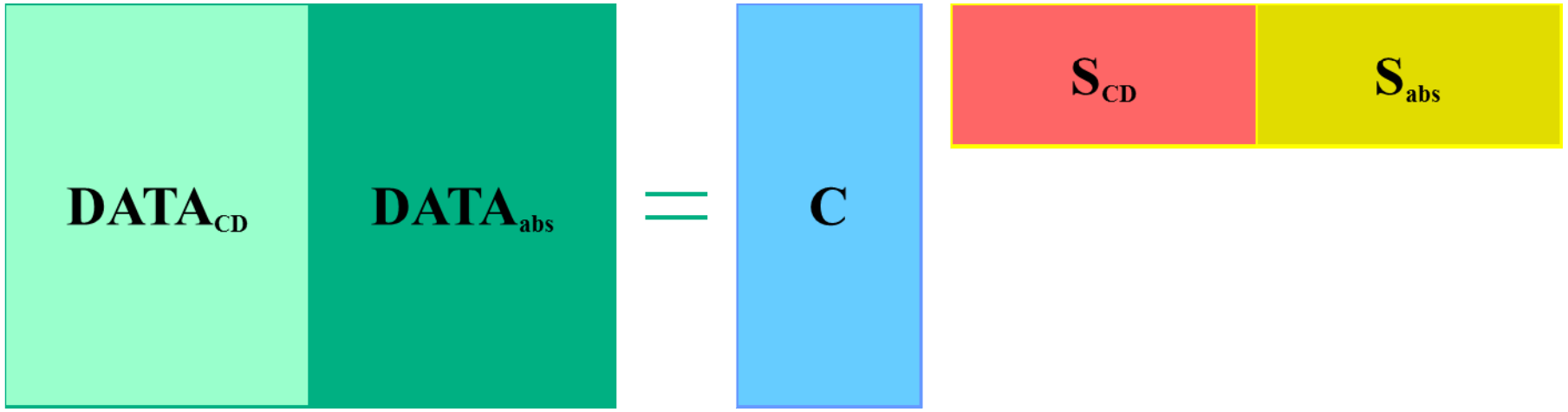
Proposed nature of the transition	ΔH⁰ (kcal / mol)	ΔS⁰ (cal / K·mol)	T_m (°C)
Intramolecular *i*-motif → partially unfolded strand	55	180	32
Partially unfolded strand → completely unfolded strand	34	107	

Spectroscopically-monitored unfolding of a G-quadruplex



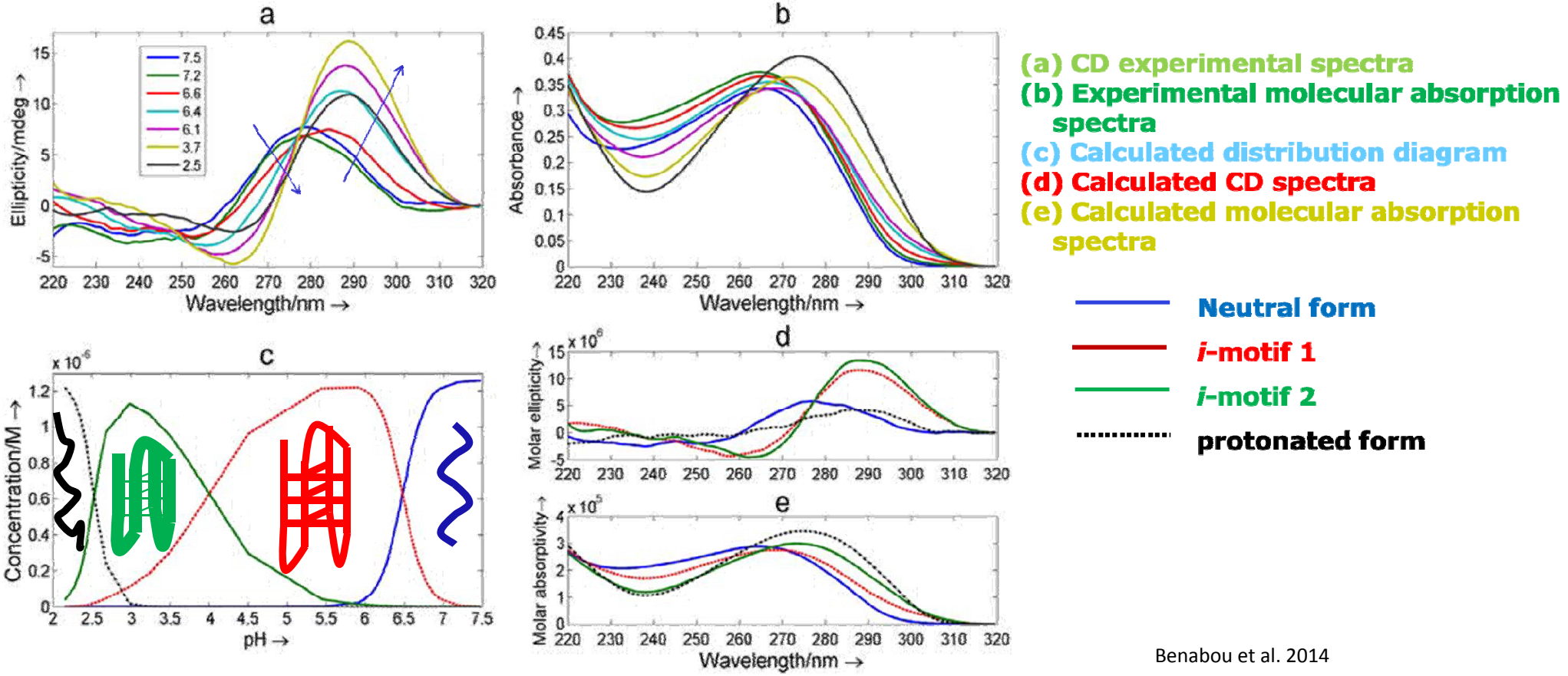
Gargallo, 2014

Acid-base equilibria of i-motif structures



Benabou et al. 2014

Acid-base equilibria of i-motif structures

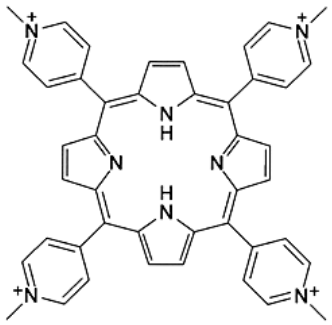


(a) CD experimental spectra
(b) Experimental molecular absorption spectra
(c) Calculated distribution diagram
(d) Calculated CD spectra
(e) Calculated molecular absorption spectra

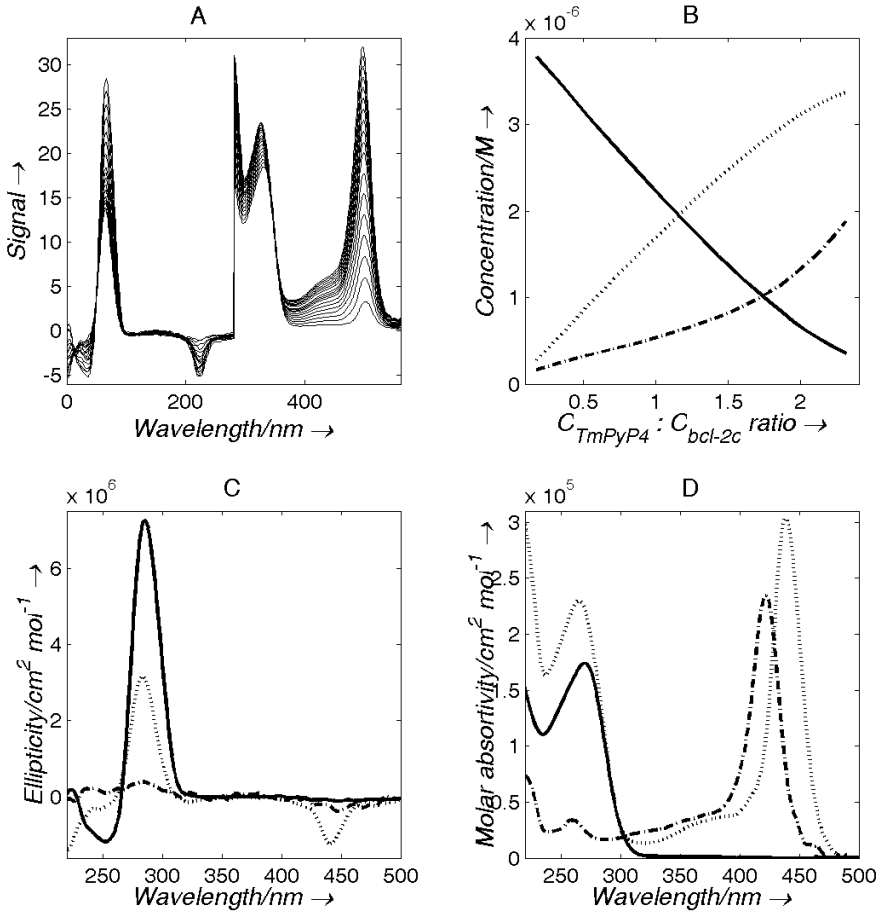
Neutral form
i-motif 1
i-motif 2
protonated form

Benabou et al. 2014

Interaction of an i-motif with a ligand



TMPyP4: model ligand often used in interaction studies



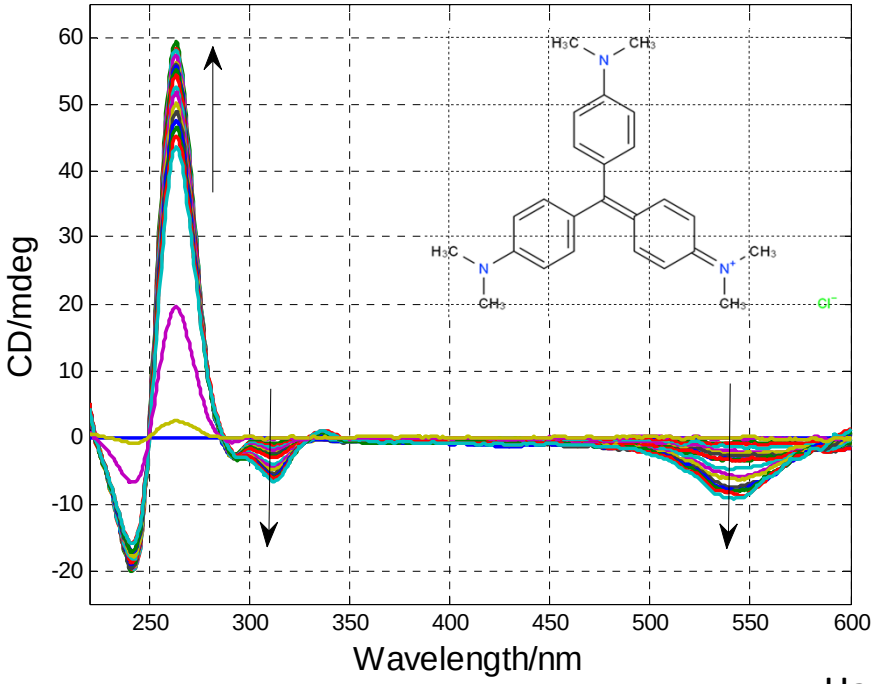
Hard-modelling

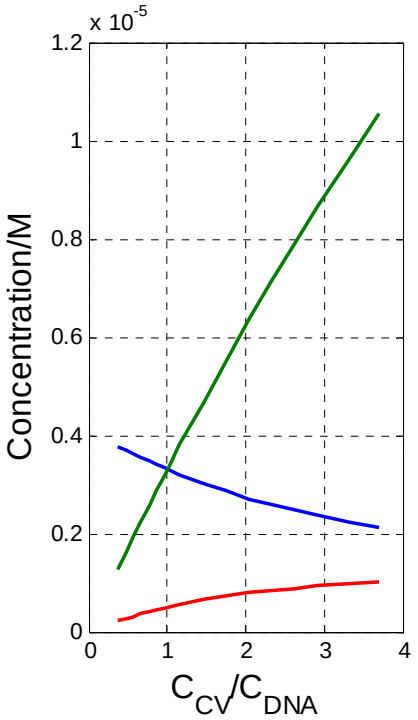
1:2 (DNA:ligand) stoichiometry

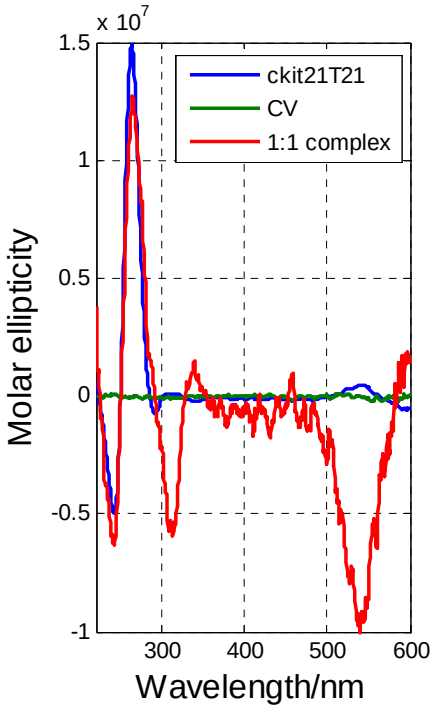
log binding constant = 12.4 ± 0.2

Khan *et al.* 2007

Interaction of a parallel G-quadruplex with a ligand







Hard-modelling

1:1 stoichiometry

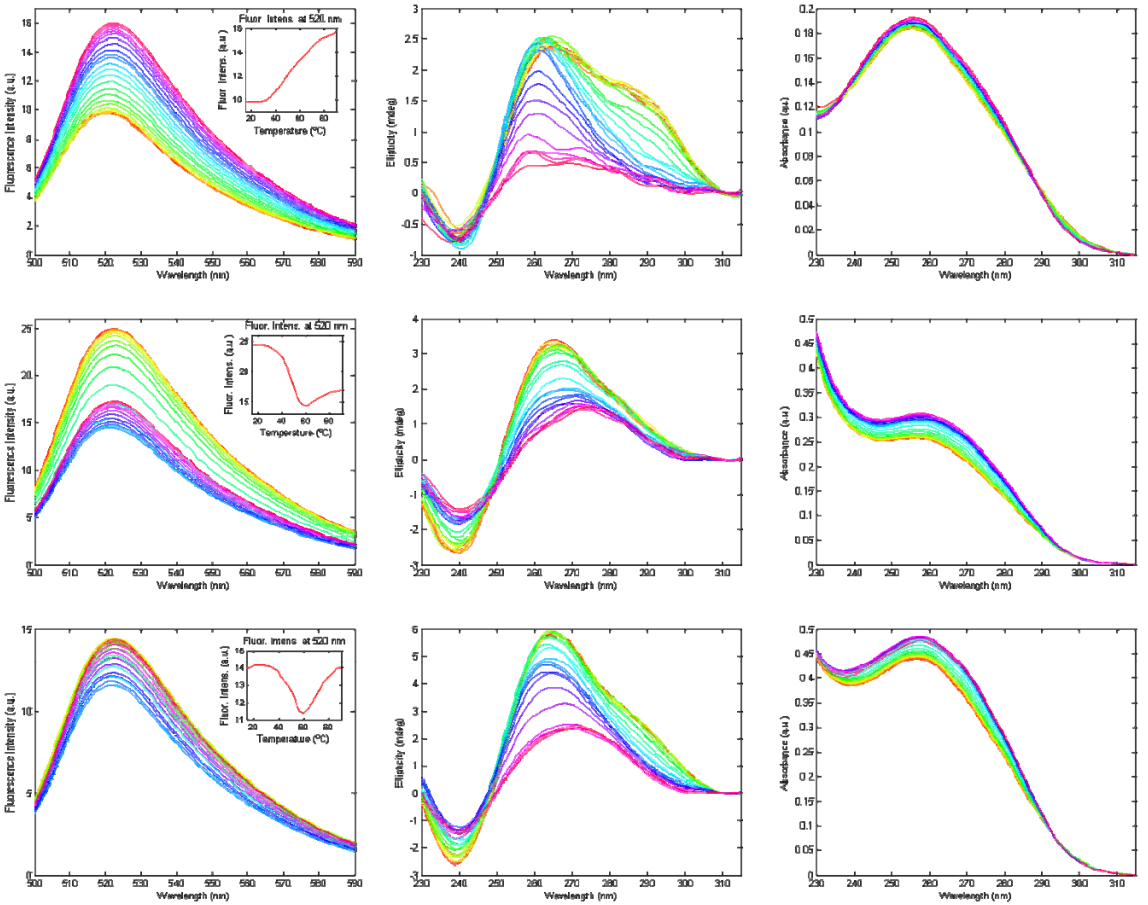
log binding constant = 4.7 ± 0.2

Benito et al. In preparation

Melting of a G-quadruplex and Watson-Crick duplex

Three melting experiments were monitored with three different spectroscopic techniques (molecular fluorescence, circular dichroism and molecular absorption)

1. **Melting of 5’-Fluorescein-TAGGGTTAGGGT-Dabsyl-3’ (SG)**
2. **Melting of the 1:1 mixture of SG and its complementary sequence**
3. **Melting of the 2:1 mixture of SG and its complementary sequence**



Jaumot et al. 2006

Melting of a G-quadruplex and Watson-Crick duplex

1. Melting of 5'-Fluorescein-TAGGGTTAGGGT-Dabsyl-3' (SG)
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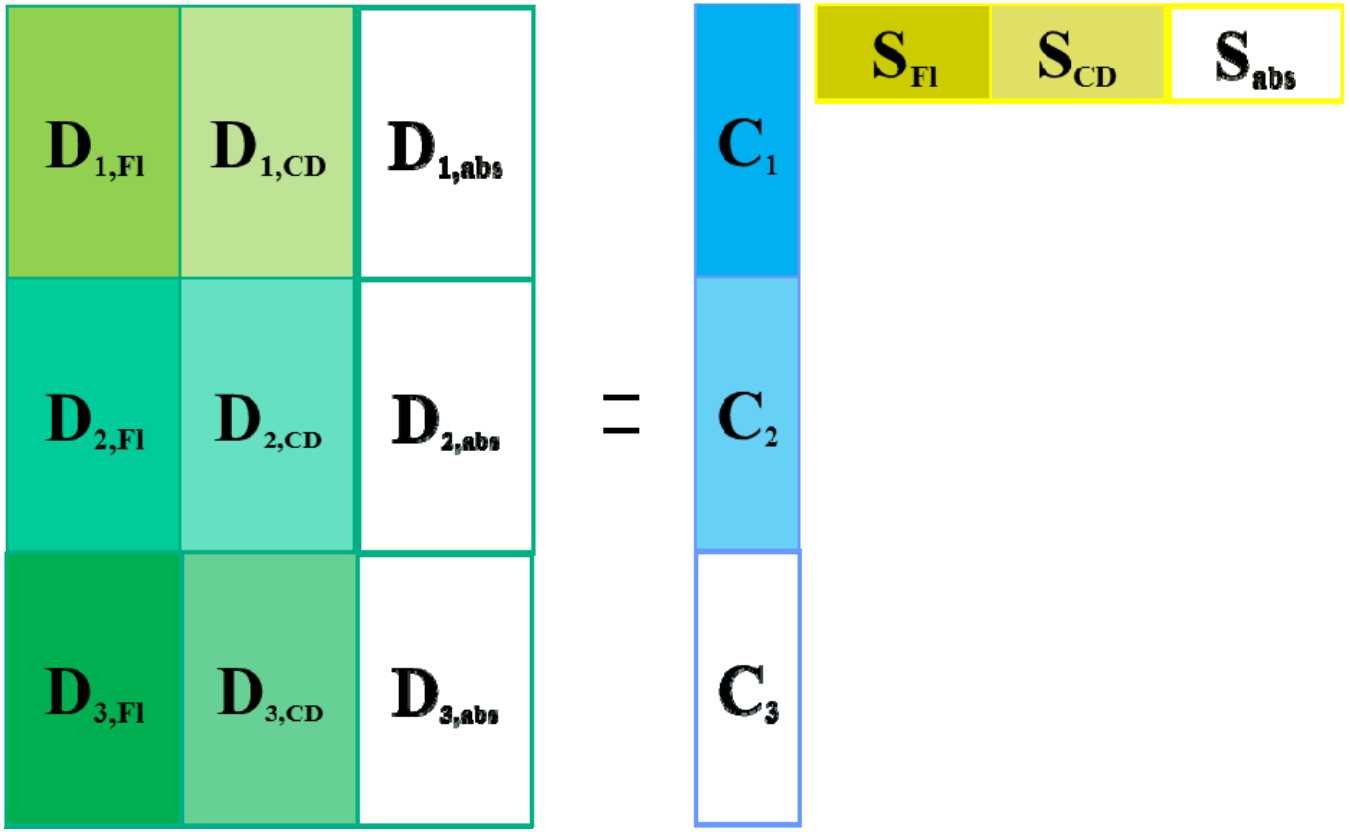

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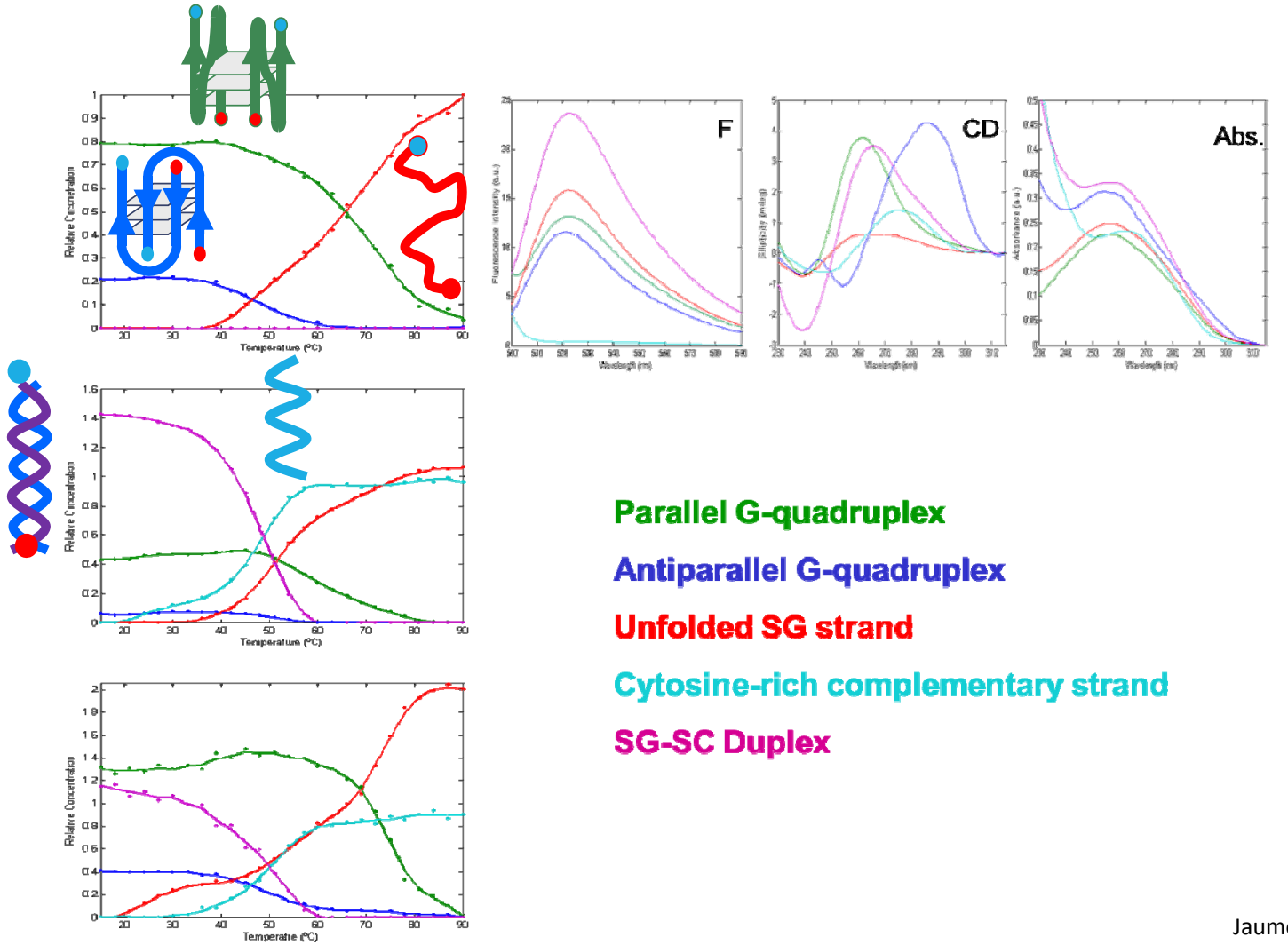


Parallel G-quadruplex

Antiparallel G-quadruplex

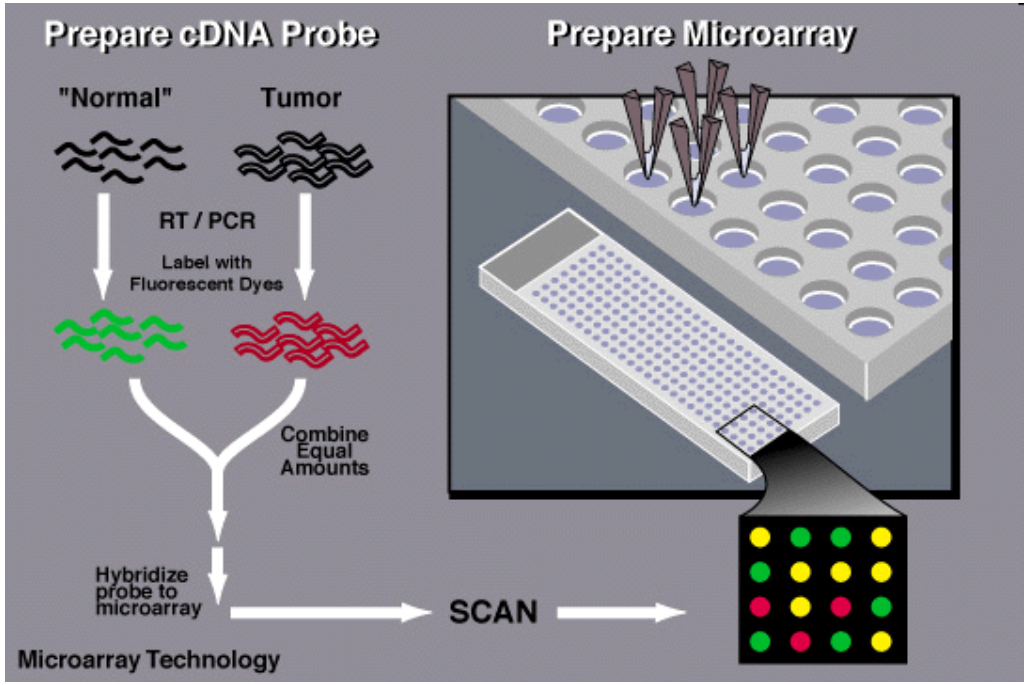
Unfolded SG strand

Cytosine-rich complementary strand

SG-SC Duplex

Jaumot et al. 2006

Other example: analysis of DNA microarrays



www.genome.gov

NCI60 data set (Ross *et al*. Nat. Gen. 2000, 24, 227)

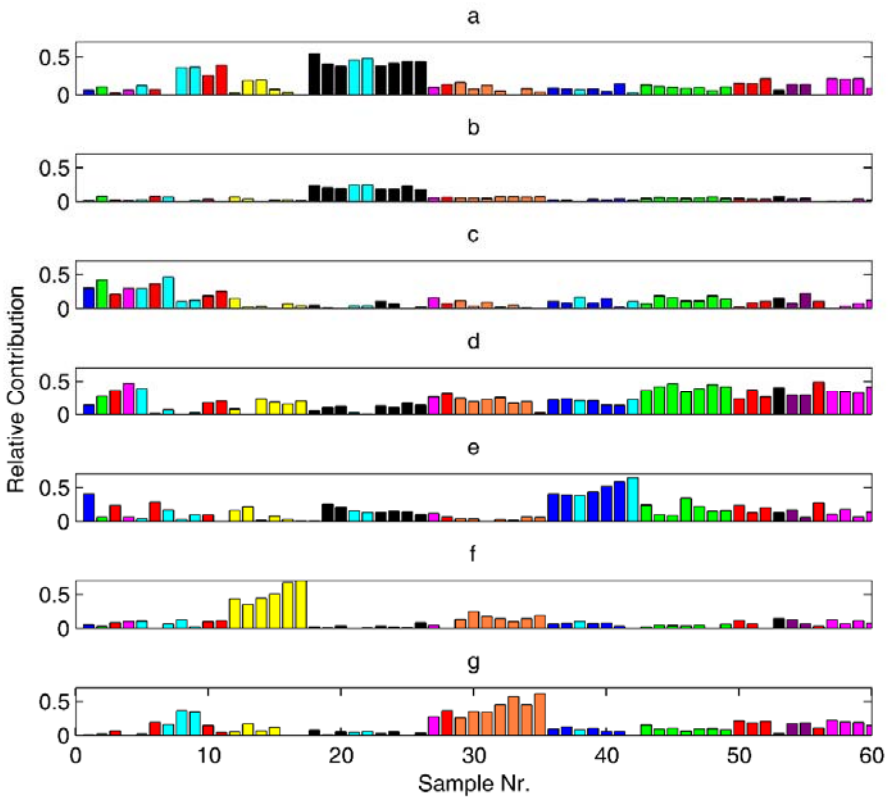
60 samples:

- breast carcinoma (BR)
- central nervous system tumor (CNS)
- colon carcinoma (CO)
- non-small lung cancer (NSLC)
- leukemia (LE)
- melanoma (ME)
- ovarian carcinoma (OV)
- prostate cancer (PR)
- renal carcinoma (RE)

mRNA was extracted and hybridized to cDNA microarrays including 9703 human cDNA clones that represented approximately 8000 different genes.

The analyzed data set contains 60 samples and 1416 variables

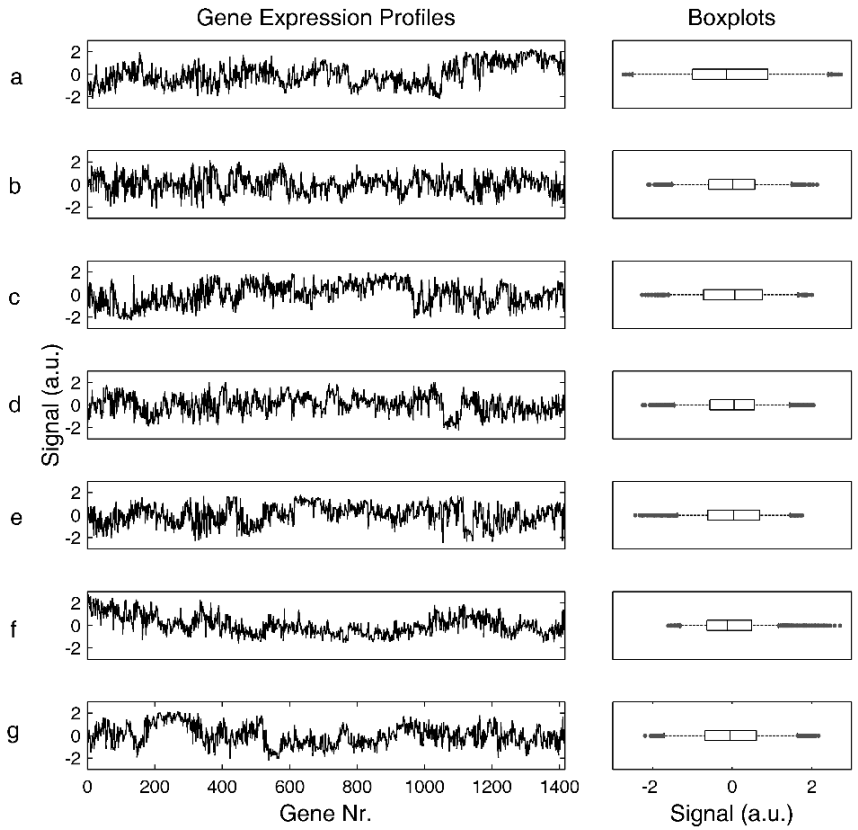
Other example: analysis of DNA microarrays



Component	Cancer
a	melanoma, breast
b	melanoma, breast
c	Not identified
d	
e	Central nervous system
f	leukemia
g	Colon carcinoma

Jaumot et al. 2006

Other example: analysis of DNA microarrays



Jaumot et al. 2006

Acknowledgments

Former PhD students: Dra. Montserrat Vives i Dr. Joaquim Jaumot

PhD Student: Sanae Benabou

Many MSc and Graduate Students

Collaborators:

- IQAC: Dr. Ramon Eritja, Dra. Anna Aviñó, Dr. Santi Grijalvo, Dra. Sonia Pérez-Rentero, Dr. Rubén Ferreira …
- IQFR “Rocasolano”: Dr. Carlos González
- IBMB: Dr. Sébastien Lyonnais, Dra. Maria Solà …
- Masaryk University (Brno, Czech Republic): Dr. Petr Taborsky and many MSc and PhD students
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