Solution Equilibria of the *i*-motif-forming Region Upstream of the B-Cell Lymphoma-2 P1 Promoter

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Outline

- Introduction
  - The G-quadruplex at the *bcl-2* promoter site
  - The cytosine-rich complementary strand
- Dealing with spectroscopic multivariate data
- Results
  - Acid-base properties
  - Melting behavior
  - A possible structure for the i-motif
  - Interaction with a porphyrin: TmPyP4

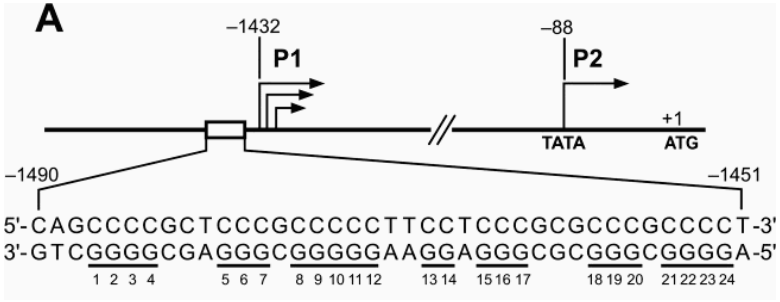
The *bcl-2* oncogene

***Bcl-2* gene** has been classified as a proto-oncogene because of its overexpression in a wide range of human cancers

Bcl-2 gene product is a protein involved in the control of programmed cell death (**apoptosis**)

The oncogenic potential of *bcl-2* is achieved by **reducing the rate of cell death**

Bcl-2 has two promoters:



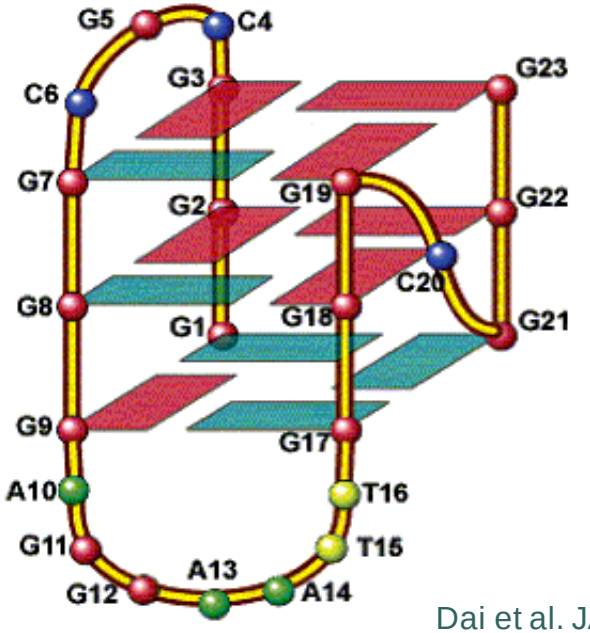
Dexheimer et al. JACS 2006, 128, 5404

The GC-rich region upstream of the P1 promoter has been shown to be critically involved in the **regulation** of *bcl-2* gene expression

The G-quadruplex in *bcl-2*

Dexheimer *et al*. have shown that the guanine-rich strand of the DNA in this region can form three **intramolecular** G-quadruplex structures

The central G-quadruplex, which is the most stable, forms a mixed **parallel/antiparallel** structure:



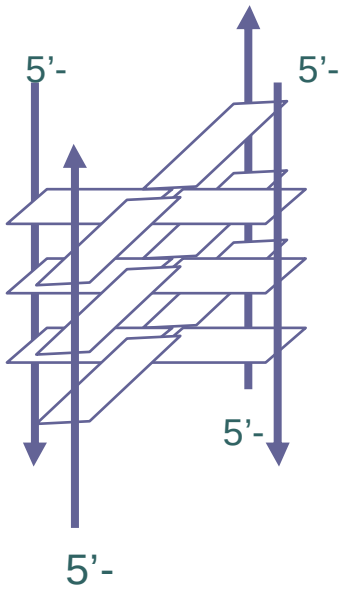
Dai et al. JACS 2006, 128, 1096

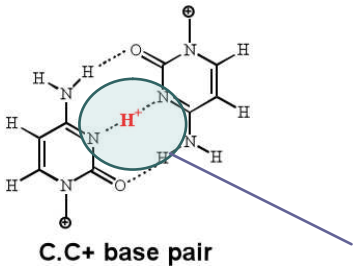
Why the study of the cytosine-rich sequence?

The sequence 5’-GGGCGCGGGAGGAAGGGGGCGGG-3’ has shown to form a G-quadruplex…

… however, this sequence is not isolated *in vivo*, and the **complementary C-rich strand** is also present.

C-rich strands can form stable structures known as **i-motifs**:





Protonation at N3 is required!

Therefore… duplex? formation of G-quadruplex and i-motifs? mixtures?

Objectives of our work

We want to know the solution equilibria of the sequence:

5’CCC GCC CCC TTC CTC CCG CGC CCG-3’

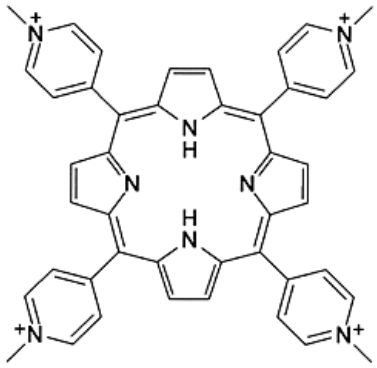
corresponding to the middle region of the *bcl-2* NHE region

“Solution equilibria”:

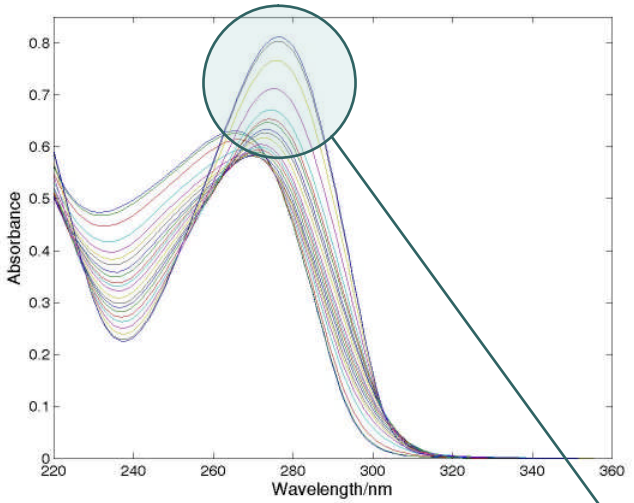
- acid-base properties of this sequence…
  - in which pH range the i-motif is formed?
  - is there more than one i-motif?

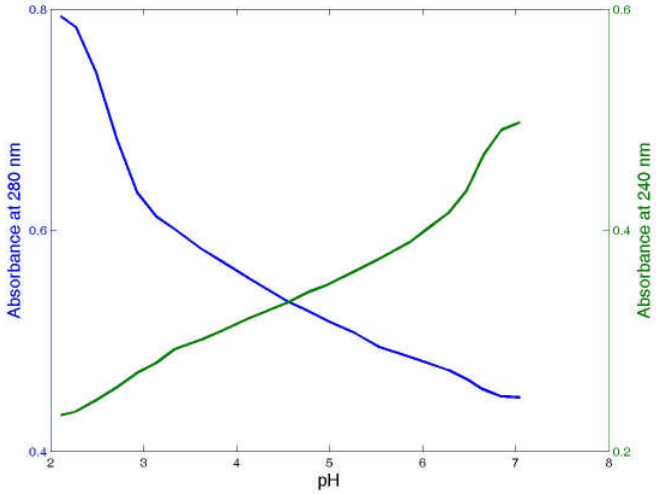
- thermal denaturation

- interaction with a G-quadruplex-binding ligand: TmPyP4



Molecular absorption-monitored acid-base titration



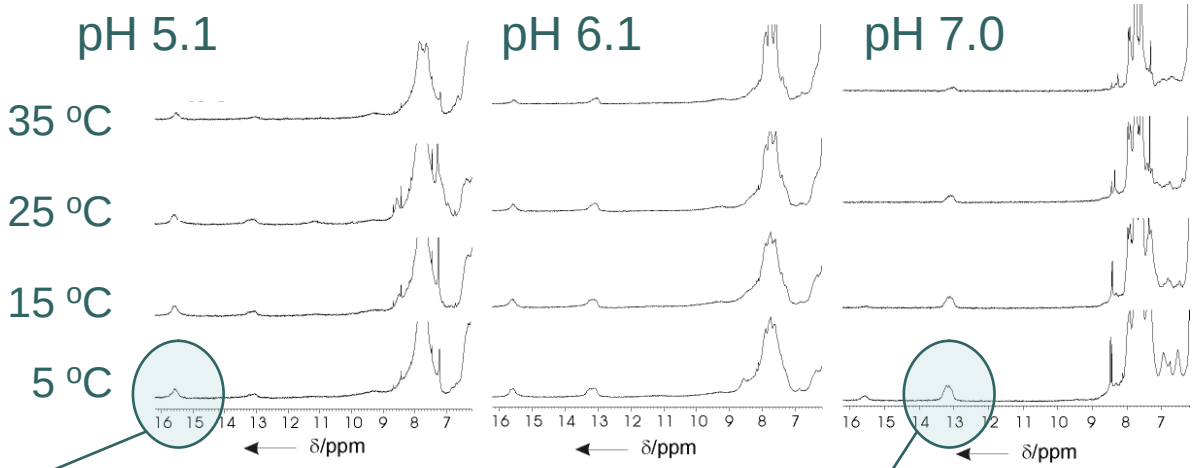


Protonation of cytosines

pKa of cytosines is around 4.5, depending on ionic strength

Therefore, absorbance traces seem to point out to the formation of i-motif at pH < 7

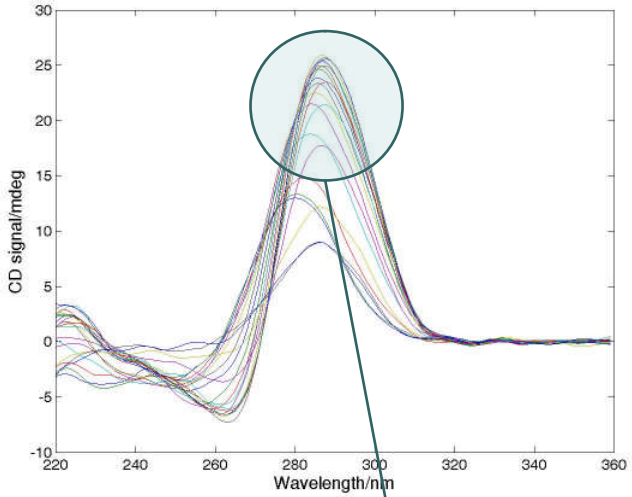
NMR-monitored acid-base titration



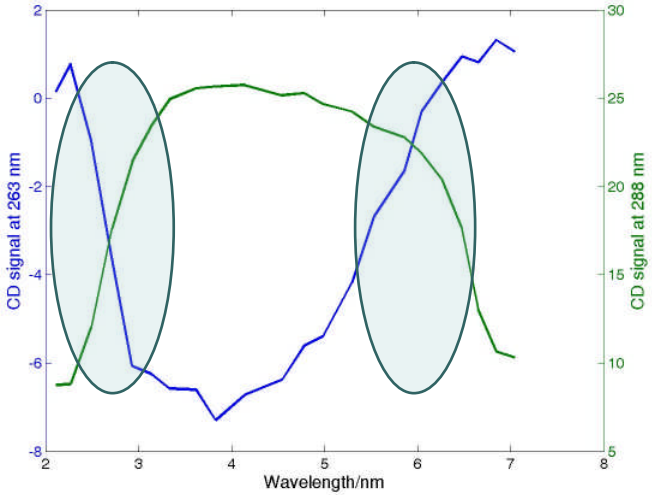
The signal around 15.6 ppm indicates the presence of imino H3C+ protons.

The signal around 13.2 ppm is characteristic of guanine imino protons involved in Watson-Crick base pairs.

CD-monitored acid-base titration



Formation of i-motif at pH < 7
is reflected around 290 nm



At least, two protonation steps
are observed

It is difficult to ascertain the presence of more than one i-motif!

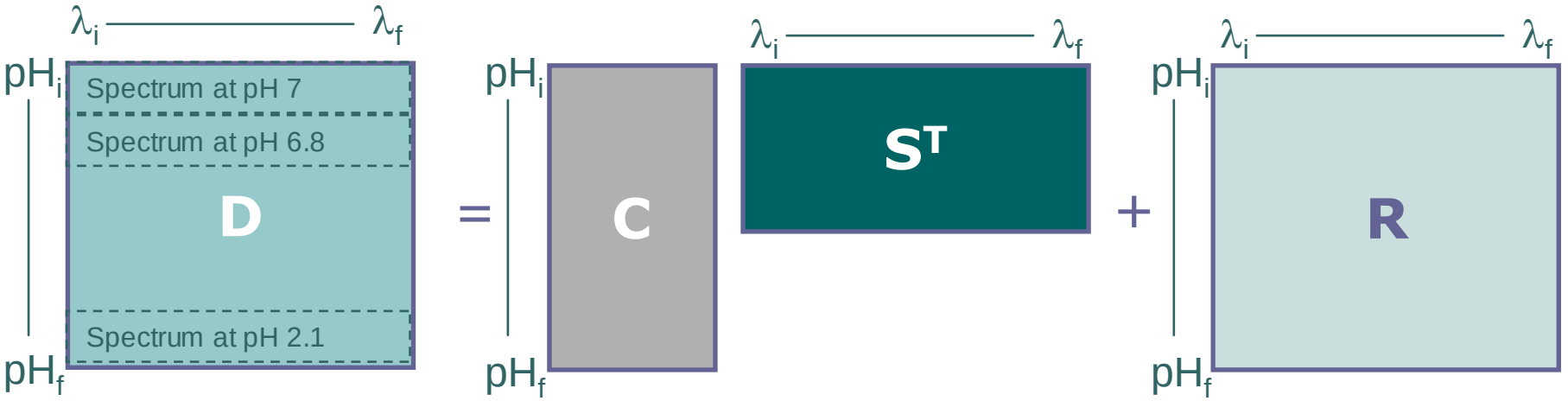
A possible tool: **multivariate data analysis**

Multivariate data analysis

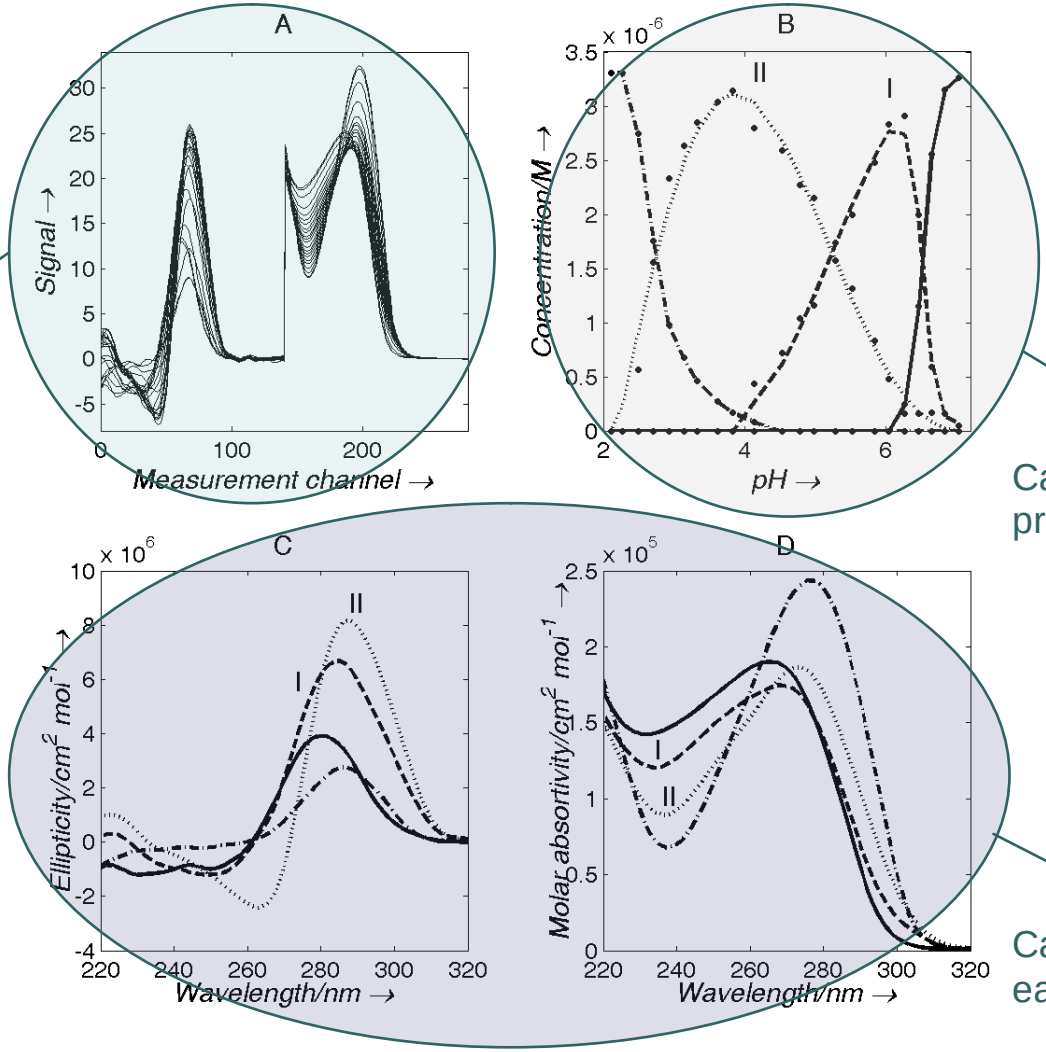
The well-known Beer-Lambert law for a single wavelength... $A_\lambda = c\ \varepsilon_\lambda$

... is now applied to the whole spectrum: $D = CS^T + R$

In a graphical form:



Multivariate analysis: two i-motifs have been detected



The whole dataset was analyzed: **D**

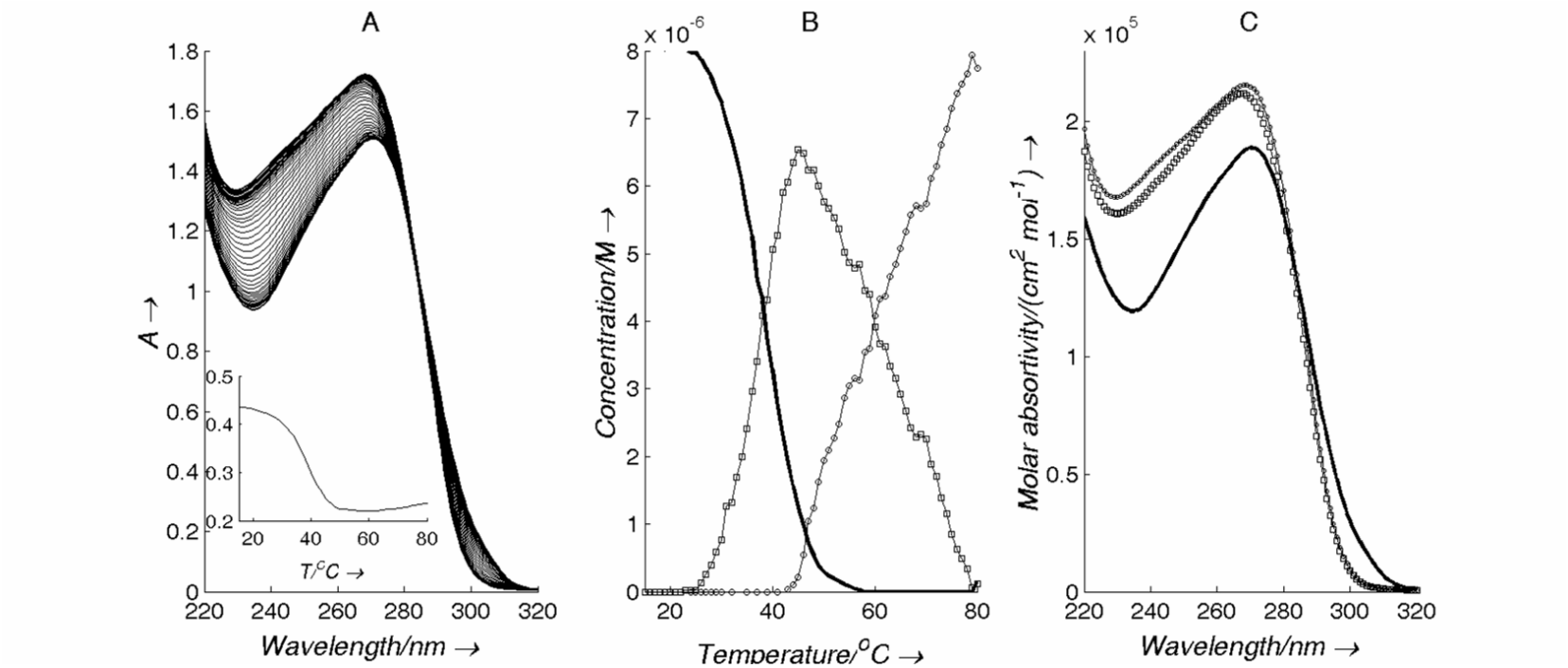
Calculated concentration profiles: **C**

Calculated spectra for each species: **S**

Melting experiments (i)

Melting experiments have been carried out from pH 7 to pH 3

In all cases, multivariate data analysis has been applied



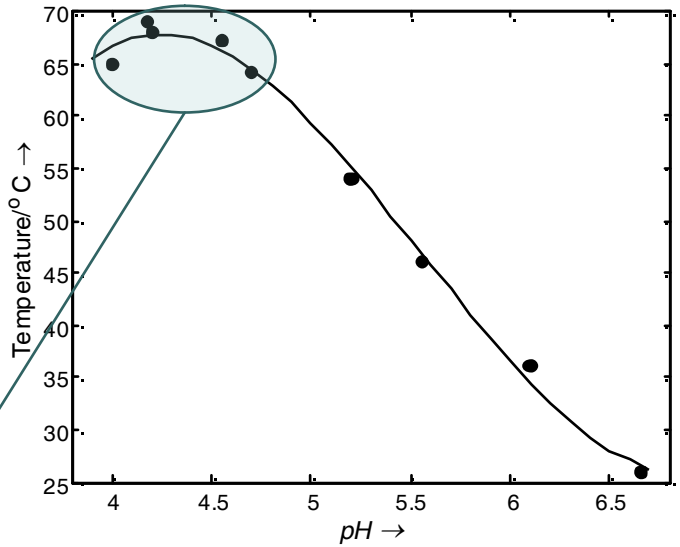
Melting at pH 6.1

Melting experiments (ii)

Results of Van't Hoff analysis:

pH	Tm (°C)	Hyperchromicity at 295 nm (%)	ΔG^0 at 20 °C (kcal mol^{-1})	ΔH^0 (kcal mol^{-1})	ΔS^0 (cal K^{-1} mol^{-1})
4.0	63	+14	-6.6	-52	-153
4.5	68	-8	-7.0	-50	-148
4.7	64	-19	-8.0	-61	-182
5.6	48	-39	-5.5	-63	-196
6.1	36	-41	-2.5	-49	-157
6.8	28	-46	-1.1	-49	-164

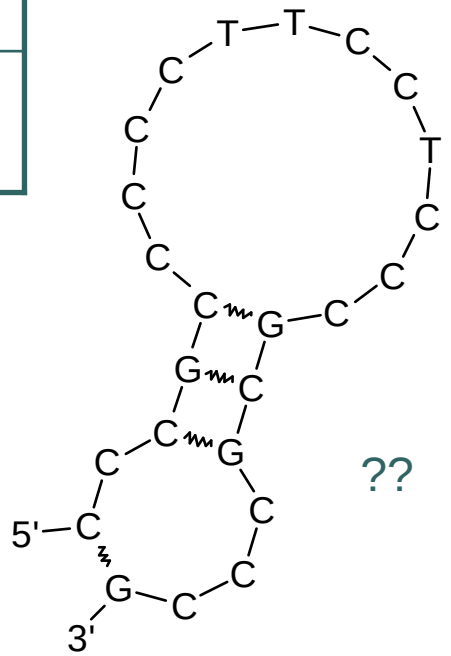
Melting profile agrees with the concentration profile for i-motif II



At pH ~ 4.3, hyperchromicity ~ 0 and T_m reaches a maximum

A proposal for the resolved species

Neutral pH	Deprotonated cytosine involved in Watson-Crick base pairs	Hairpin
pH ~ 6	Cytosines involved in C+·C base pairs	i-motif I
pH ~ 4	Free cytosines not involved in the i-motif core are probably protonated	i-motif II



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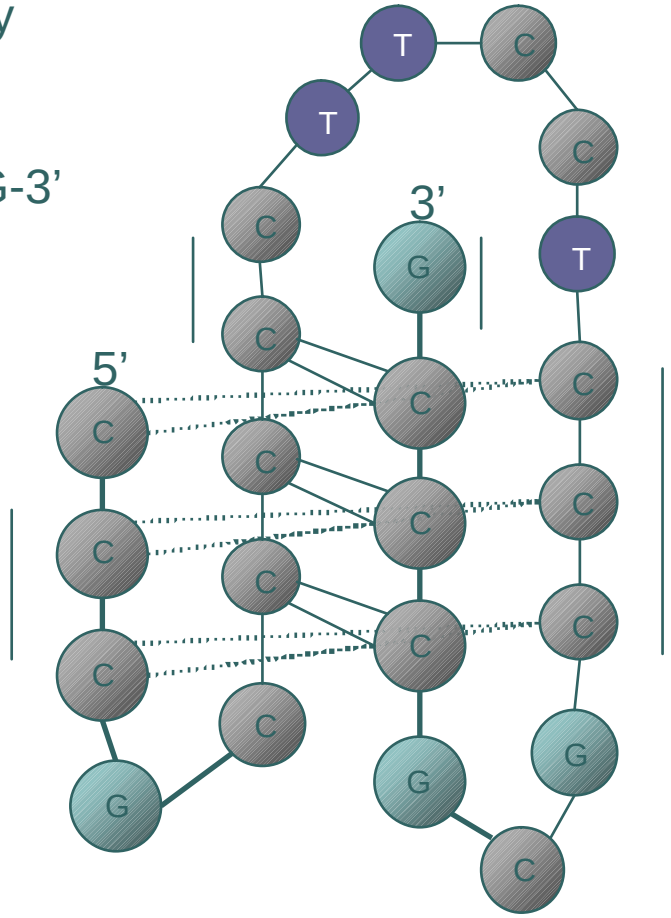
A proposal for the resolved species

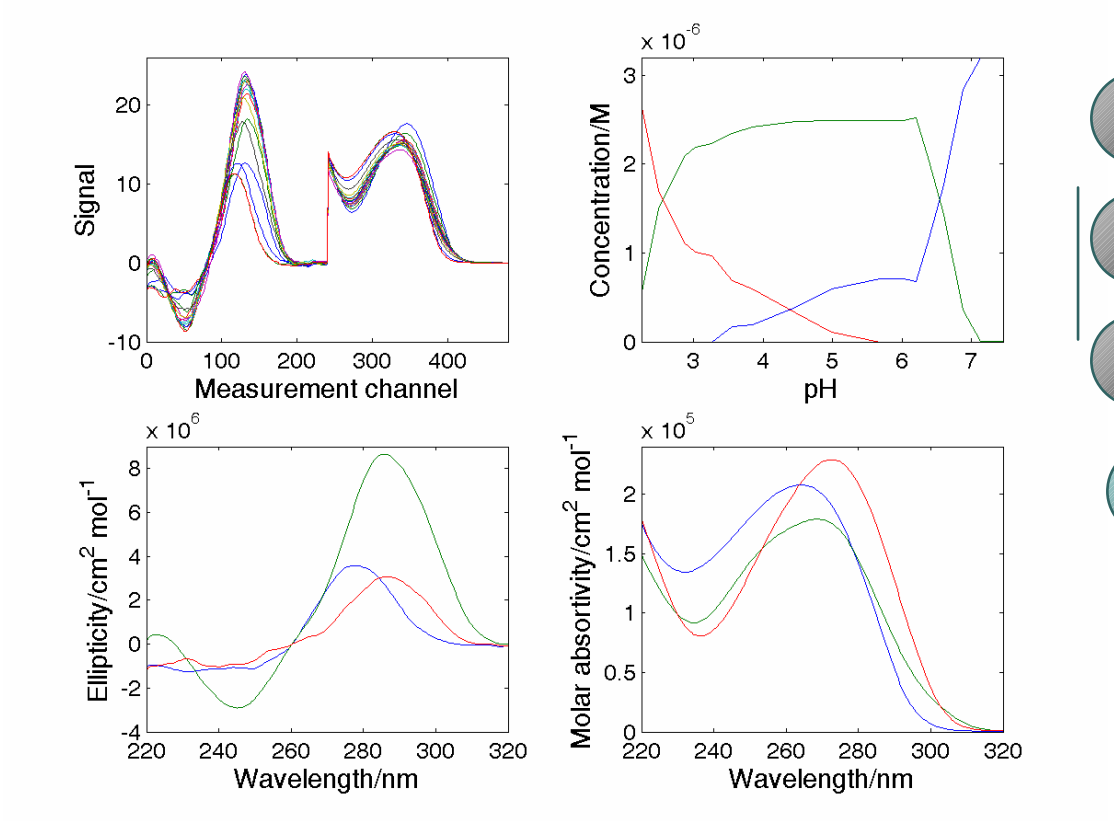
A possible structure for the i-motif I formed by the bcl-2c sequence:

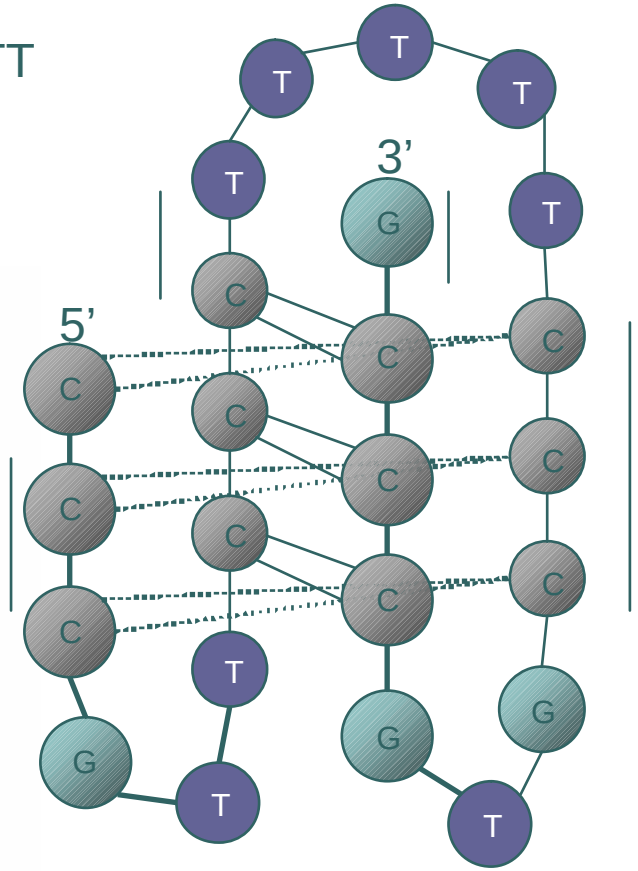
5’CCC GCC CCC TTC CTC CCG CGC CCG-3’



A similar sequence only forms just one i-motif

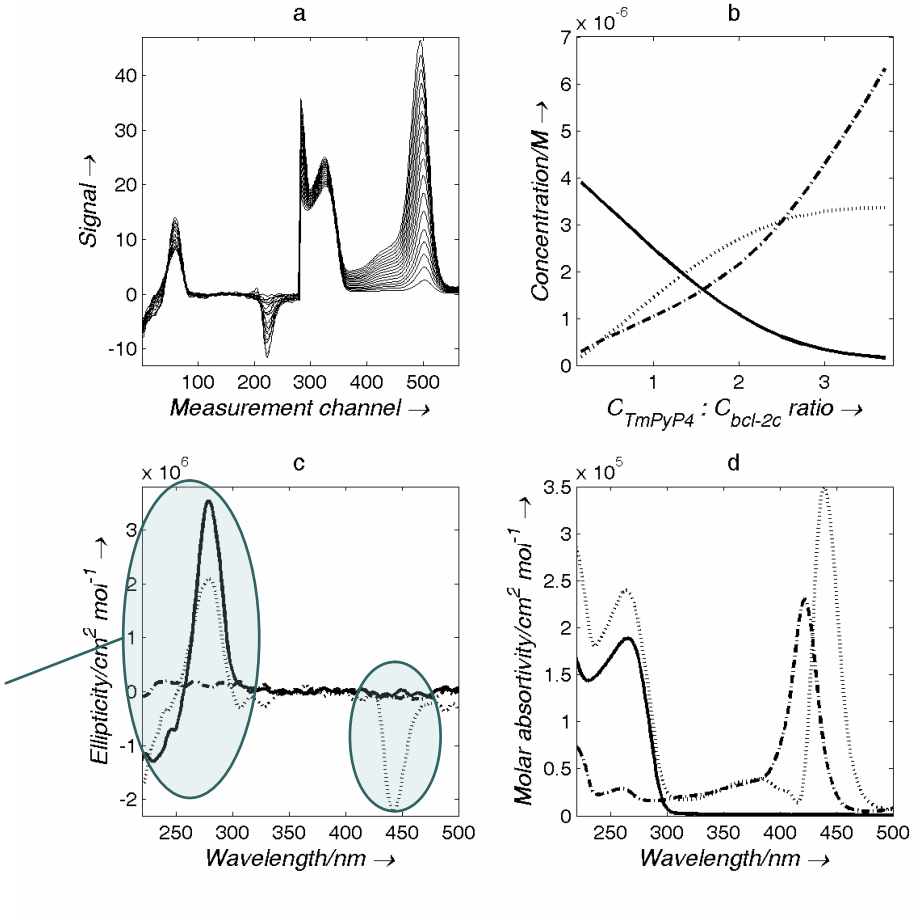
The study of the sequence 5’-CCC GTT CCC TTT TTC CCG TGC CCG -3’ (with any free cytosine) seems to show the formation of an i-motif in the pH range 2 - 7





Interaction with a G-quadruplex ligand: TmPyP4. pH 6.9

At pH 6.9, TmPyP4 interacts with the Watson-Crick hairpin

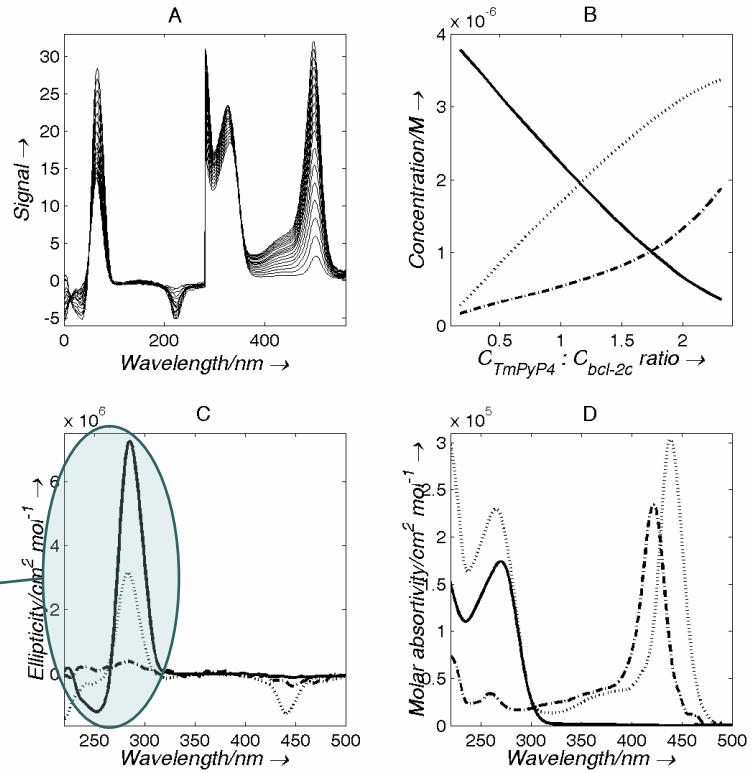


Small variation in bcl-2c structure

A 1:2 (DNA:drug) interaction complex is proposed with log K_{eq} = 11.7 ± 0.1

Interaction with a G-quadruplex ligand: TmPyP4. pH 6.1

At pH 6.1, TmPyP4 interacts with the i-motif I:

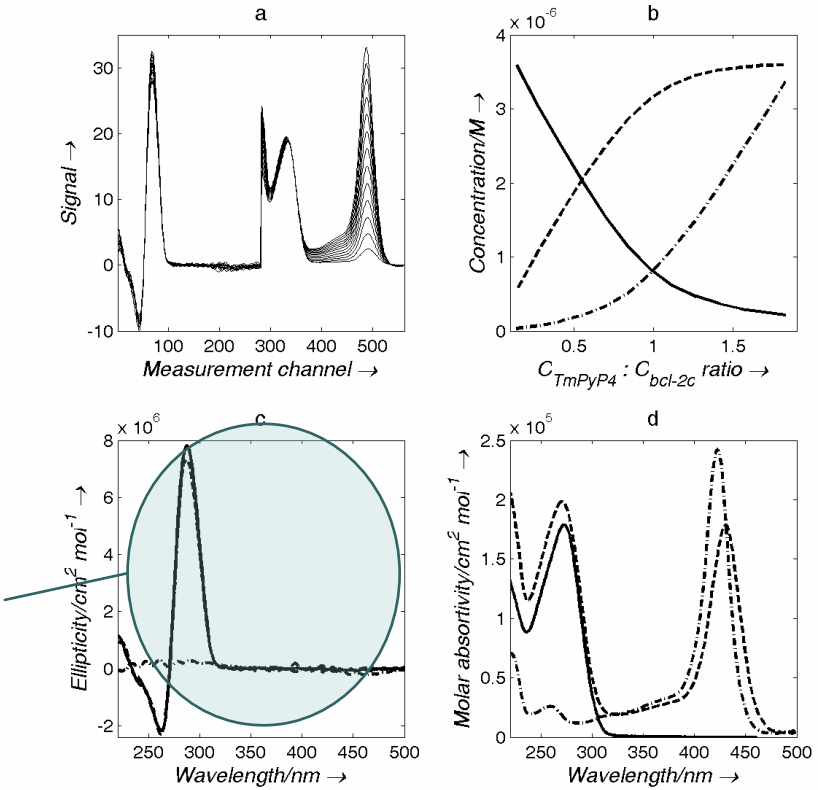


A clear decrease of CD intensity: the i-motif structure is lost?

A 1:2 interaction complex is proposed with log K_{eq} = 12.4 ± 0.2

Interaction with a G-quadruplex ligand: TmPyP4. pH 4.2

At pH 4.2, TmPyP4 interacts with the i-motif II:



The structure is not altered. Intercalation?

A 1:1 interaction complex is proposed with log Keq = 6.7 ± 0.6

Conclusions

- The studied sequence forms two i-motif structures in the pH range 2 – 7
- Stability of the i-motif structures is higher at pH ~ 4.3
- Interaction with TmPyP4 at pH 7 and pH 6 produces a similar product, where TmPyp4 seems to intercalate into DNA
- Interaction with TmPyp4 at pH 4 does not produce any change on i-motif structure.

Acknowledgments

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Thank you!!

More info at: www.ub.es/gesq/dna