pH-modulated Watson-Crick duplex - quadruplex equilibria of guanine-rich and cytosine-rich DNA sequences upstream of the *c-kit* transcription initiation site

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Outline

- Introduction
  - G-quadruplex structures at the *c-kit* promoter site
  - The cytosine-rich complementary strand
- Dealing with spectroscopic multivariate data
- Results
  - Solution equilibria of the guanine-rich region
    - Acid-base properties
    - Thermal stability
  - Solution equilibria of the cytosine-rich region
    - Acid-base properties
    - Thermal stability
  - Competition Watson-Crick duplex – secondary structures

G-quadruplex structures at the *c-kit* oncogene

The *c-kit* gene encodes a receptor tyrosine kinase involved in cell proliferation

c-kit activity is elevated in gastrointestinal stromal tumors, and its therapeutic inhibition by small molecules such as imatinib, is clinically validated

Two G-quadruplex-forming regions near the transcription initiation site:

5'-AGG GAG GGC GCT GGG AGG AGG G-3', 87 nucleotides upstream of the transcription start site

It has been deeply studied and its structure resolved [Todd, 2007; Phan, 2005; Rankin, 2005]

5'-CGG GCG GGC GCG AGG GAG GGG-3', 140 nucleotides upstream of the transcription activation site

Fernando et al. *Biochem*. **2006**, 45, 7854-7860:

predominant parallel G-quadruplex structure

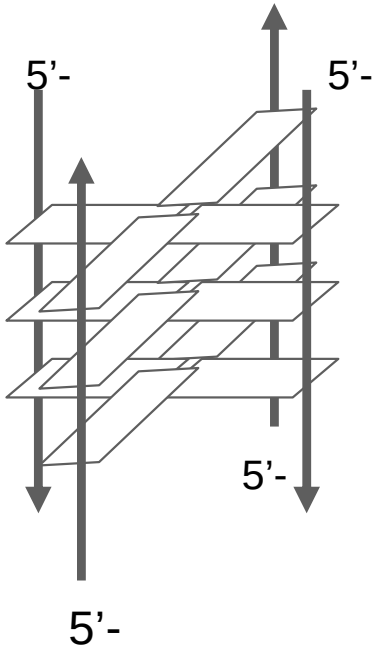
The cytosine-rich complementary strand

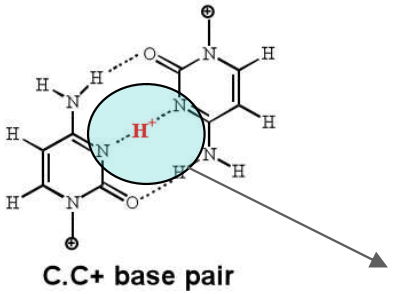
The sequence 5’-CGG GCG GGC GCG AGG GAG GGG-3’ has shown to form a G-quadruplex…

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

ckitC1 5’-CCC CTC CCT CGC GCC CGC CCG-3’

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





Protonation at N3 is required!

Objective

We will study the competition equilibria between these two sequences:

ckitG1 5’-CGG GCG GGC GCG AGG GAG GGG-3’
ckitC1 5’-CCC CTC CCT CGC GCC CGC CCG-3’

duplex? formation of G-quadruplex and i-motif? a mixture?

“Solution equilibria”:

- how does pH affect to the structures formed by these sequences?
- how do these structures behave in front of a temperature increase?
- can we quantify each species or conformation in a mixture?

Tools:

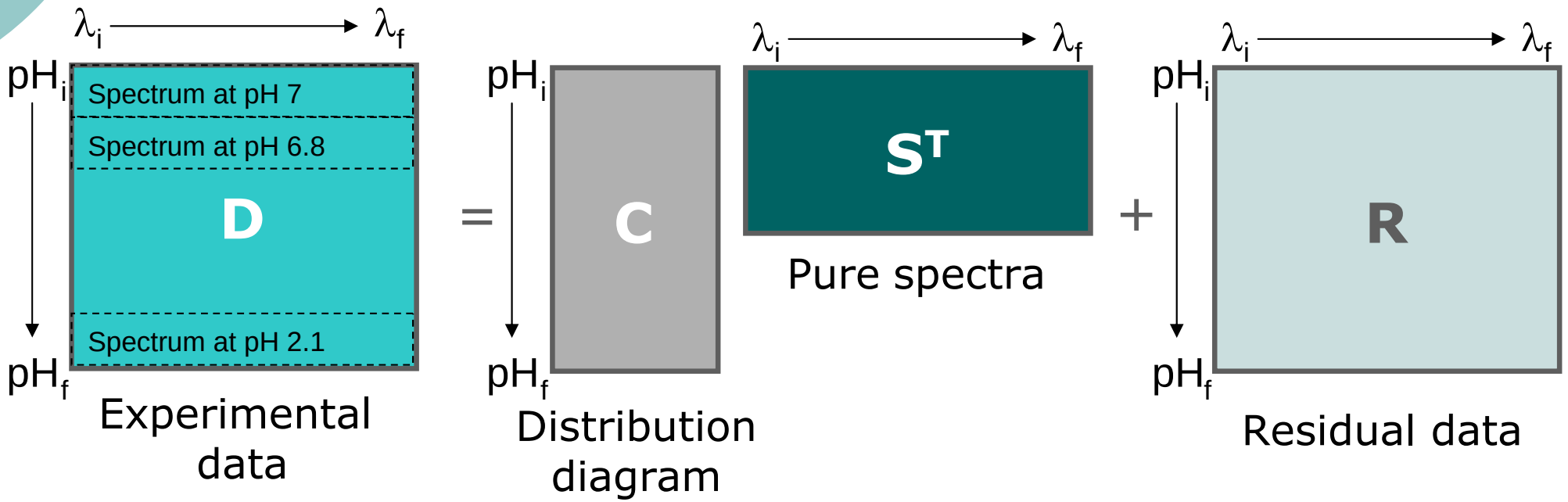
- experimental procedures: pH titrations, melting experiments, mole-ratio studies
- spectroscopic techniques: CD and molecular absorption
- multivariate data analysis methods

Multivariate data analysis: theory

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

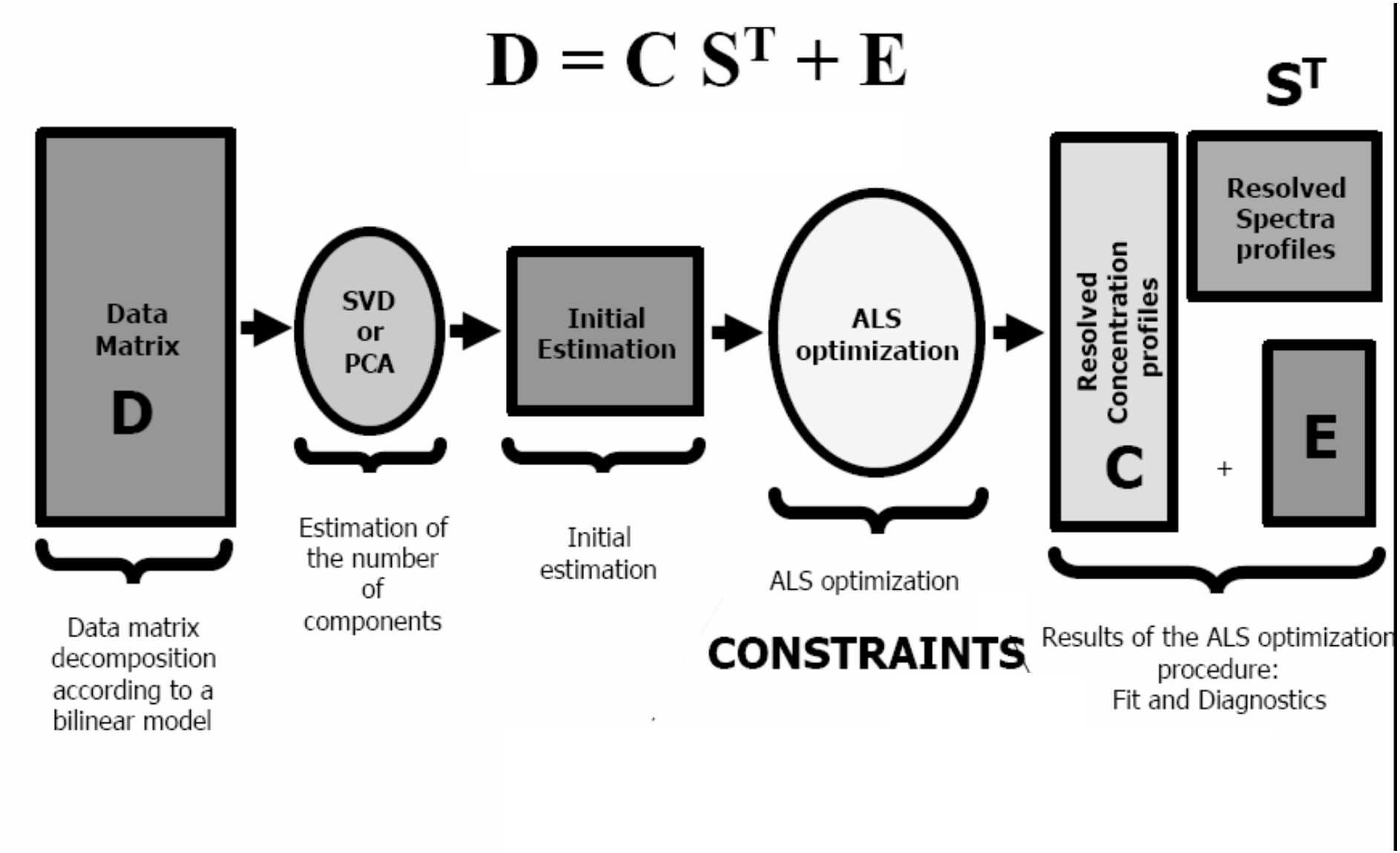
... is now applied to the whole spectrum: $D = CS^T + R$

In a graphical form:



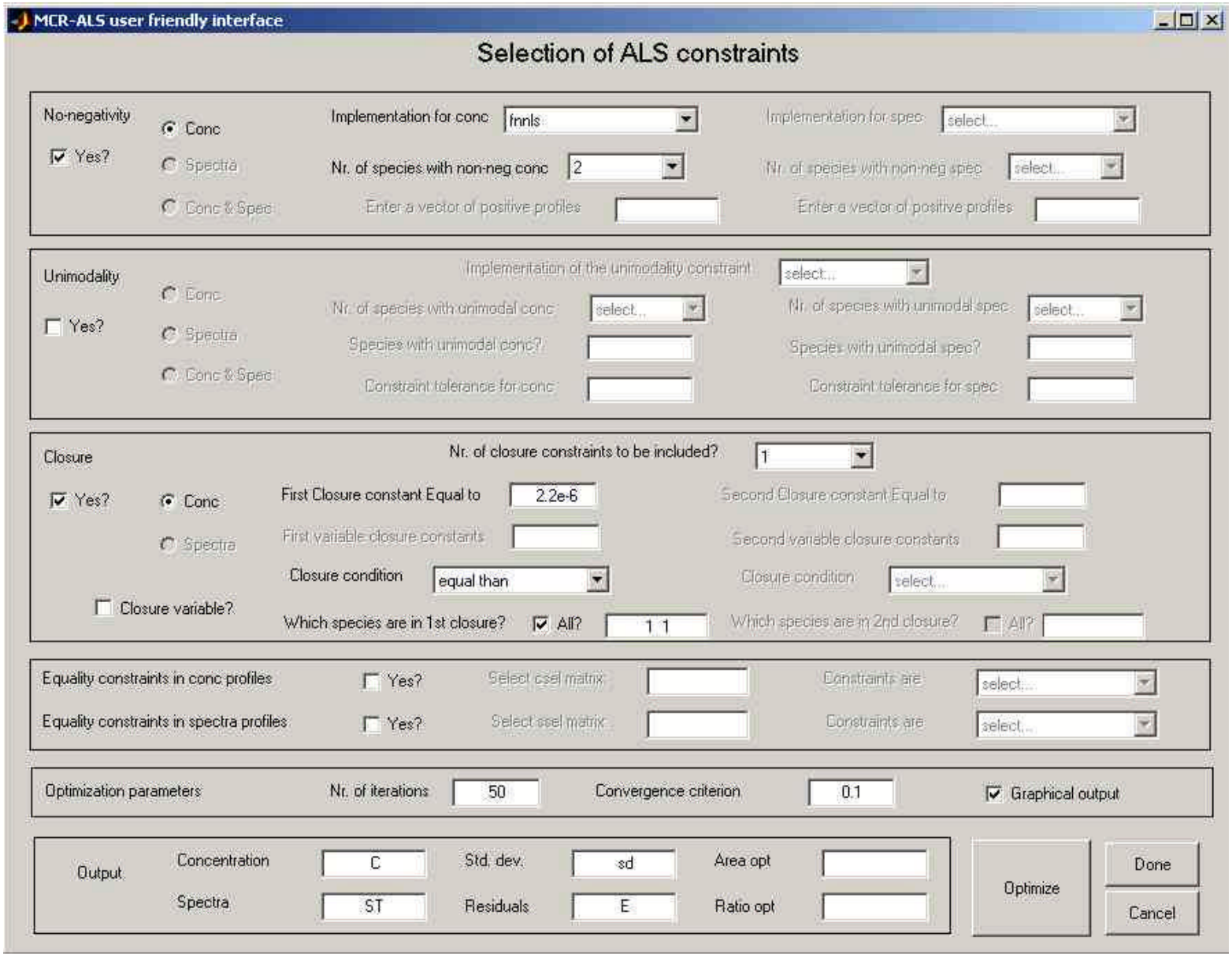
Available software and tutorials at: www.ub.es/mcr

Multivariate data analysis: theory



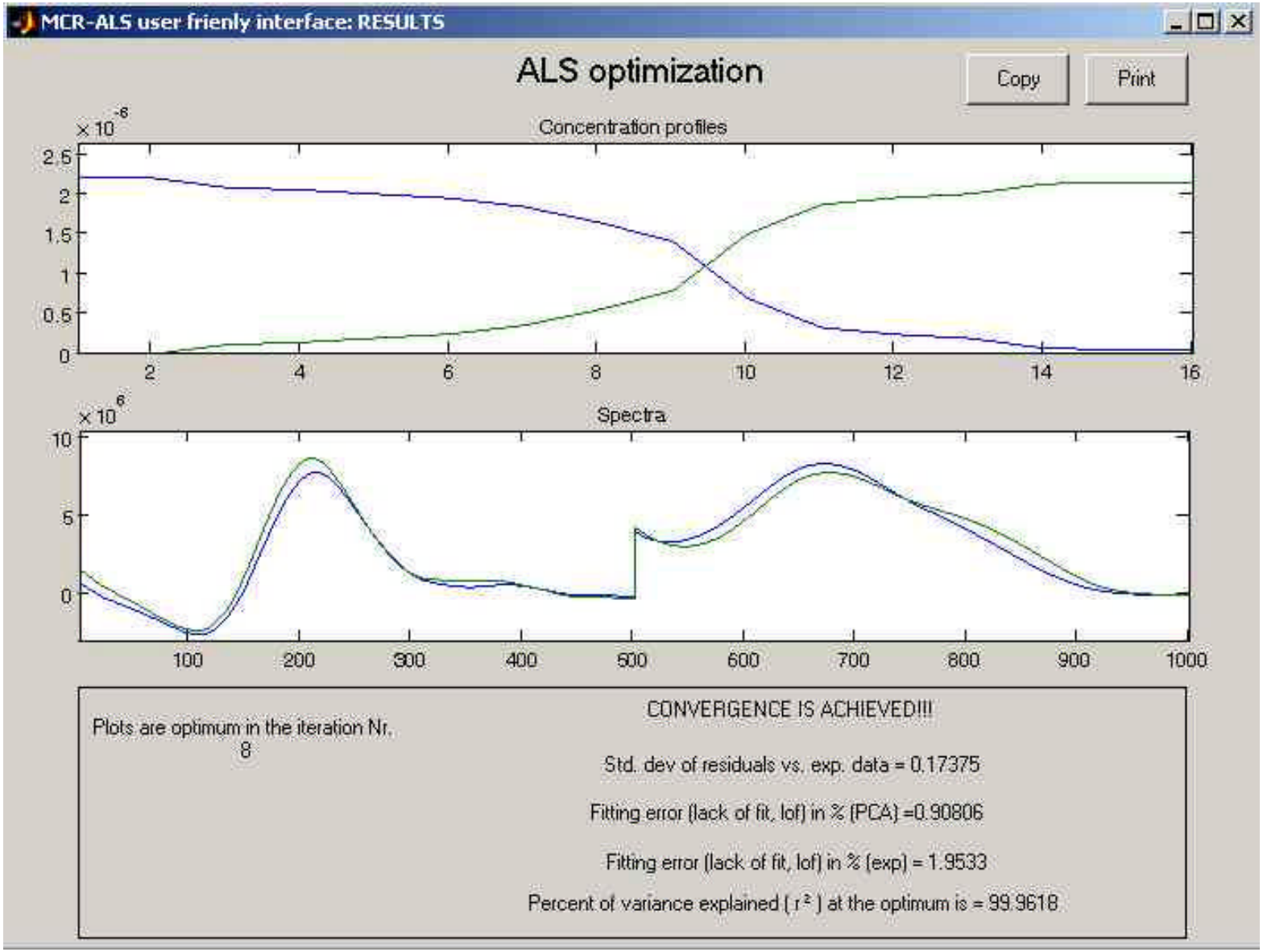
Available software and tutorials at: www.ub.es/mcr

MCR-ALS in practice (1)



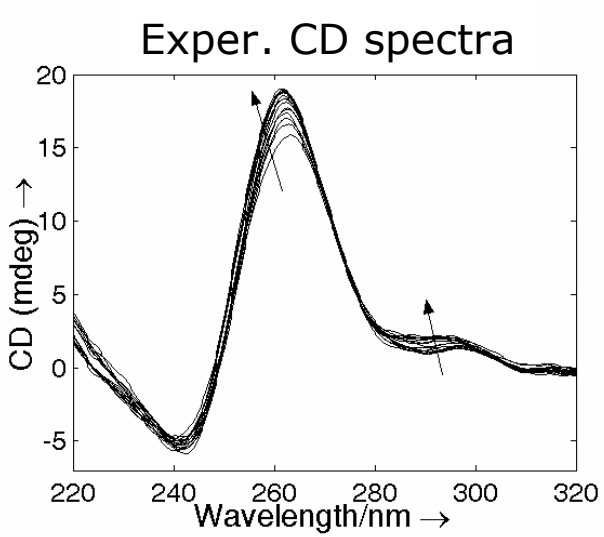
Available software and tutorials at: www.ub.es/mcr

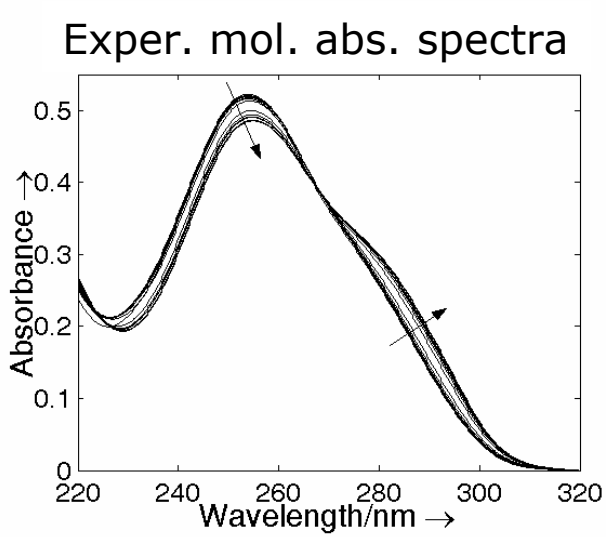
MCR-ALS in practice (2)

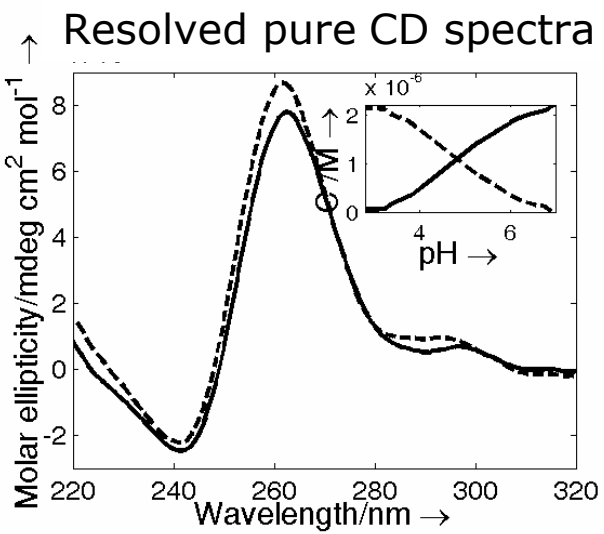


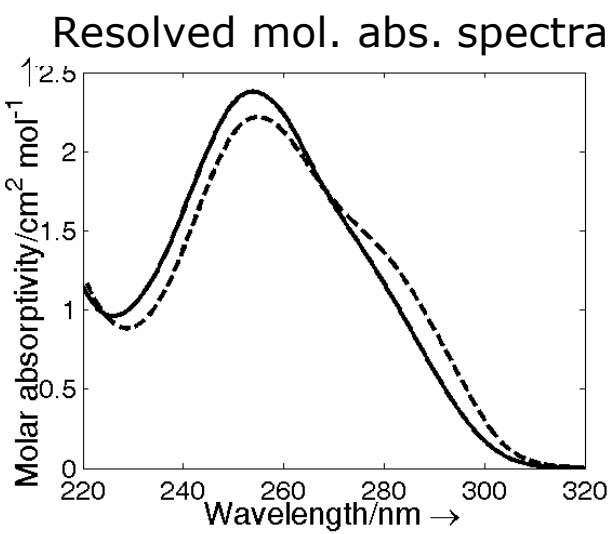
Available software and tutorials at: www.ub.es/mcr

ckitG1: acid-base properties





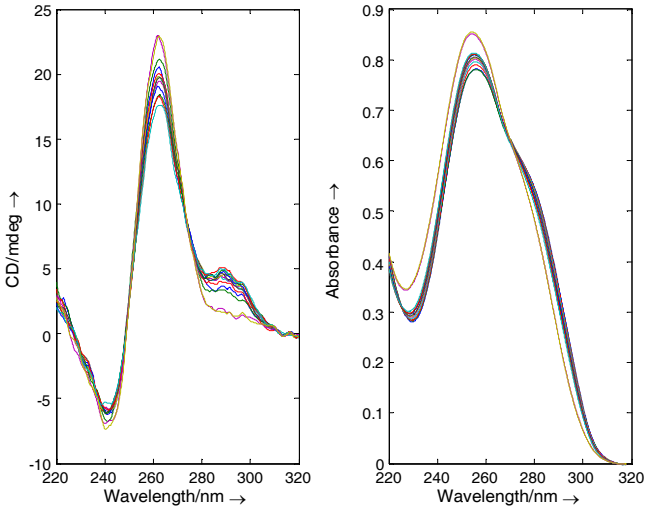




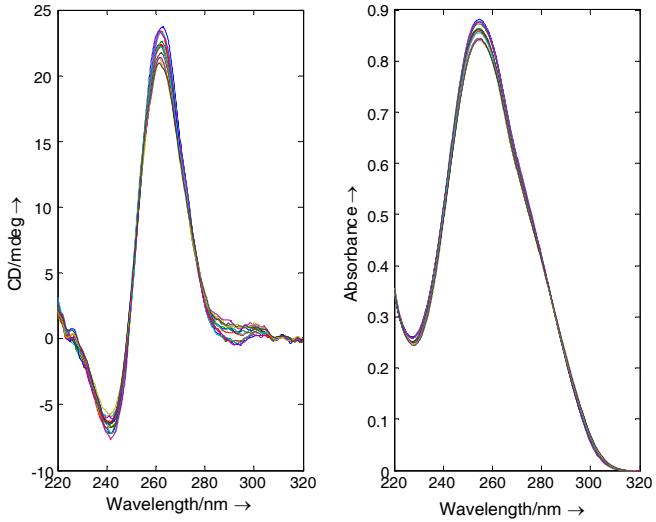
Titration of a ckitG1 sample with HCl, 1 mM Mg^{2+}, 150 mM ionic strength with KCl

ckitG1 5′-CGG GCG GGC GCG AGG GAG GGG-3′

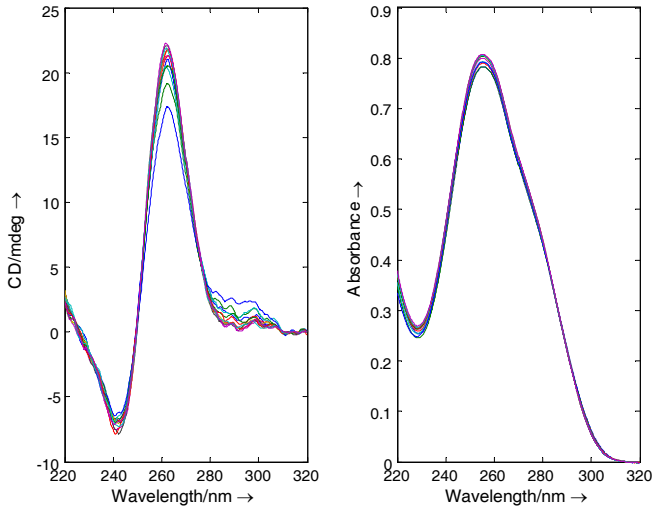
ckitG1-related sequences: acid-base properties



5′-CGG GCG GGC GCG TGG GTG GGG-3′

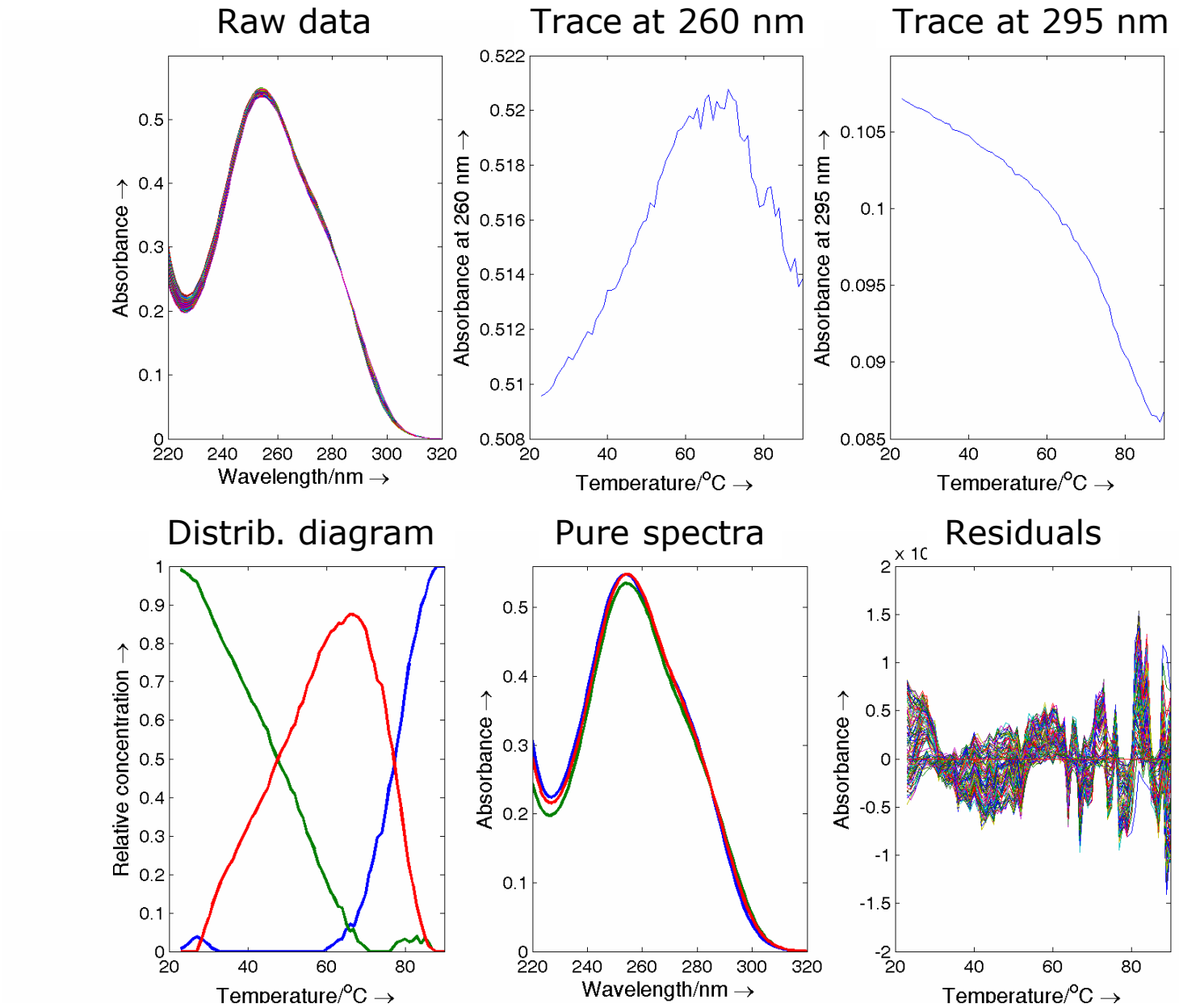


5′-TGG GTG GGT GTG TGG GTG GGG-3′



5′-TGG GTG GGT GTG AGG GAG GGG-3′

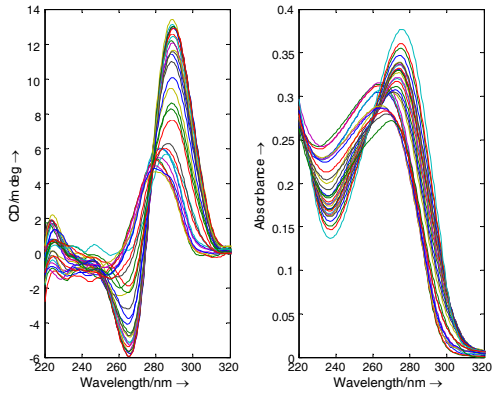
ckitG1: melting properties



Melting of a ckitG1 sample, 1 mM Mg^{2+}, pH 6.0, 150 mM ionic strength with KCl

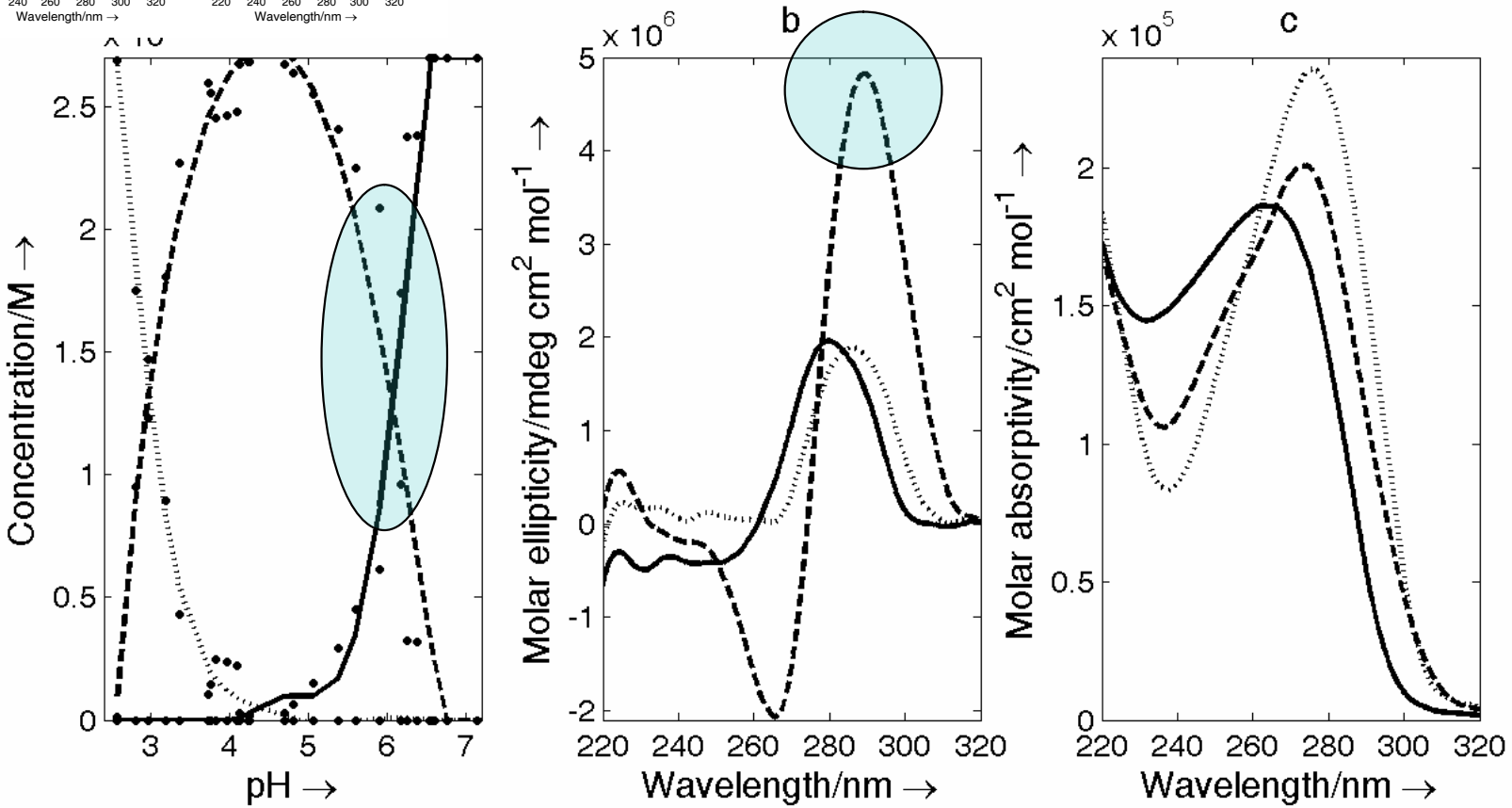
ckitG1 5′-CGG GCG GGC GCG AGG GAG GGG-3′

c-kitC1: acid-base properties



Titration of a ckitC1 sample with HCl, 1 mM Mg^{2+}, 150 mM ionic strength with KCl

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



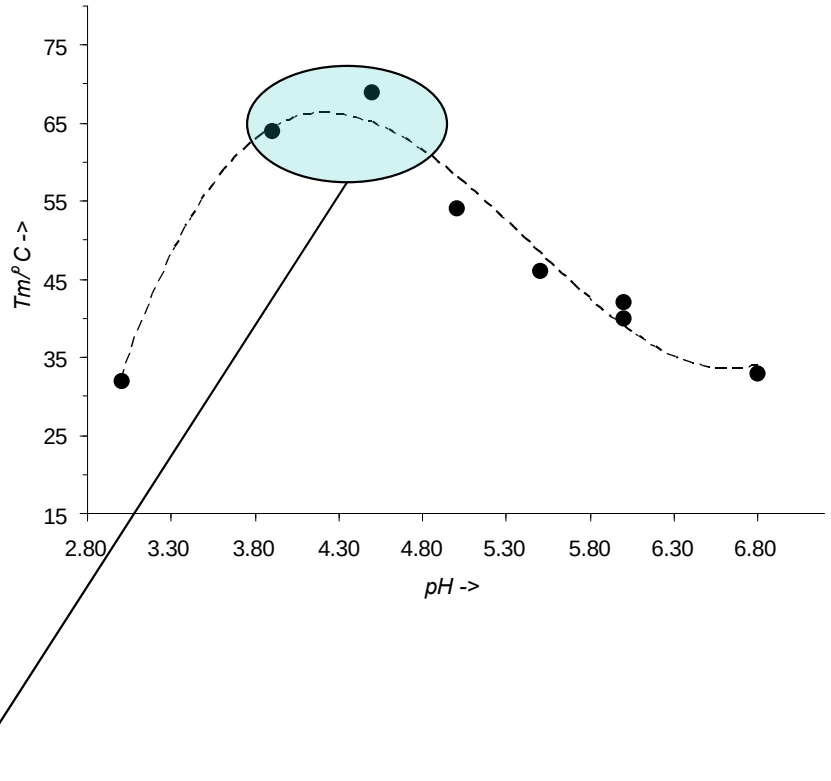
ckitC1 5′-CCC CTC CCT CGC GCC CGC CCG-3′

ckitC1: melting studies

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

Results of Van't Hoff analysis:

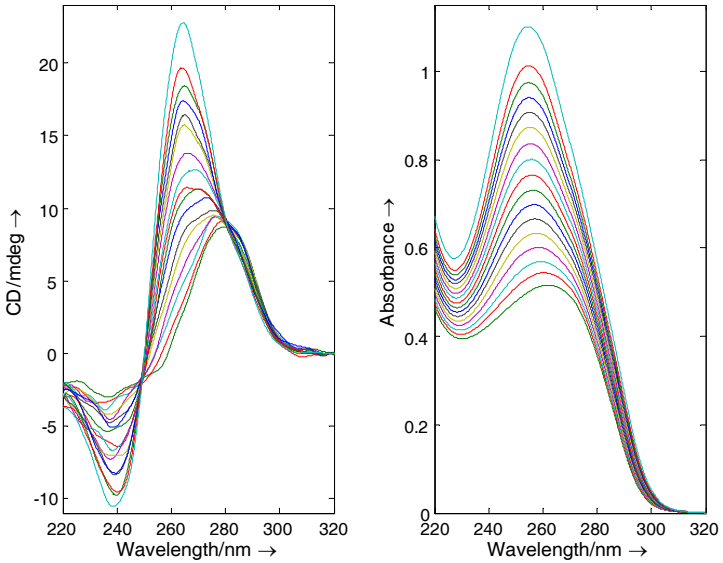
pH	Tm (°C)	ΔG^0 at 20 °C (kcal mol^{-1})	ΔH^0 (kcal mol^{-1})	ΔS^0 (cal K^{-1} mol^{-1})
4.0	64	-5.5	-49	-146
4.5	69	-7.7	-54	-156
5.0	54	-3.9	-45	-139
5.5	47	-3.5	-47	-147
6.0	41	-2.7	-52	-167
6.8	33	-1.4	-47	-152



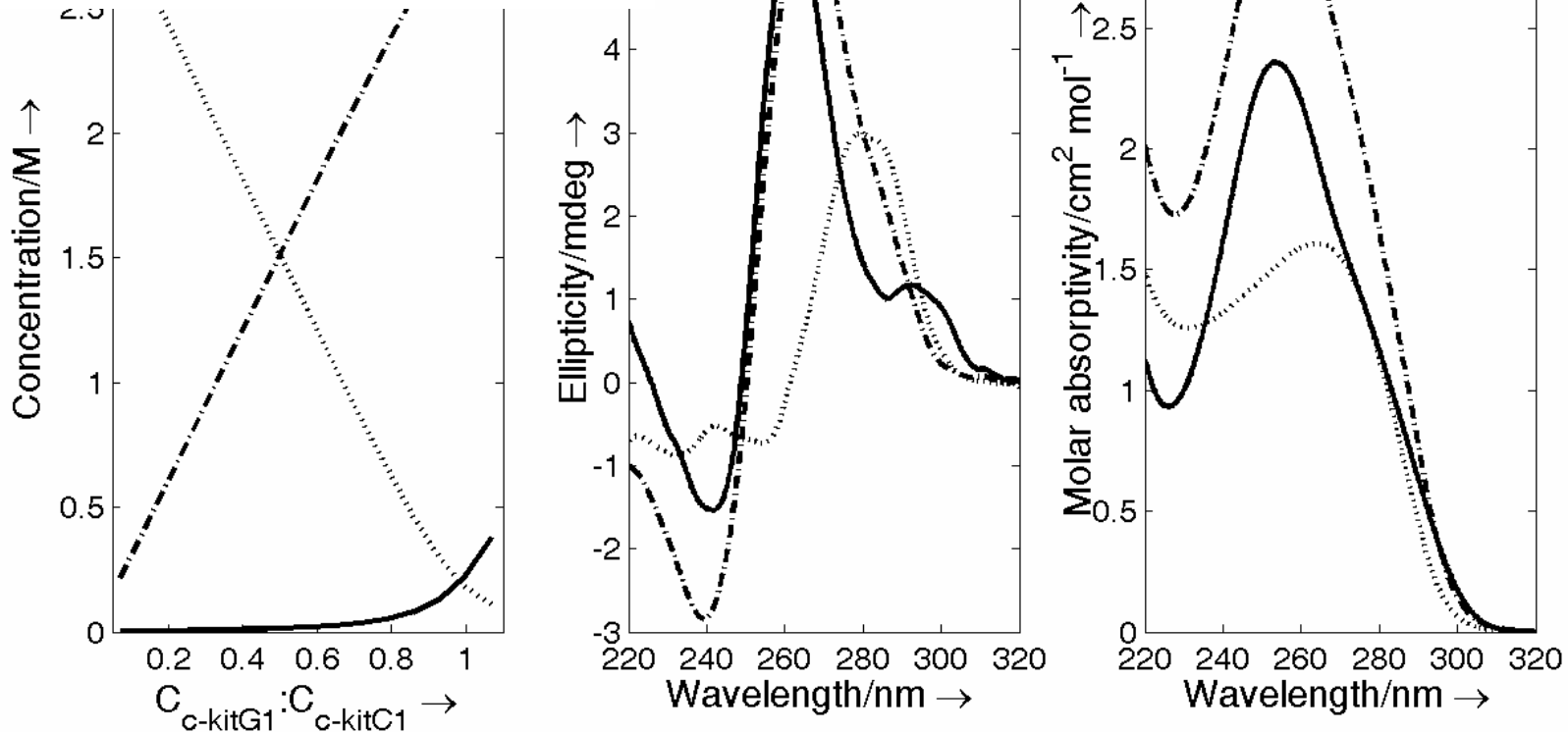
At pH ~ 4.3, T_m reaches a maximum

ckitC1 5′-CCC CTC CCT CGC GCC CGC CCG-3′

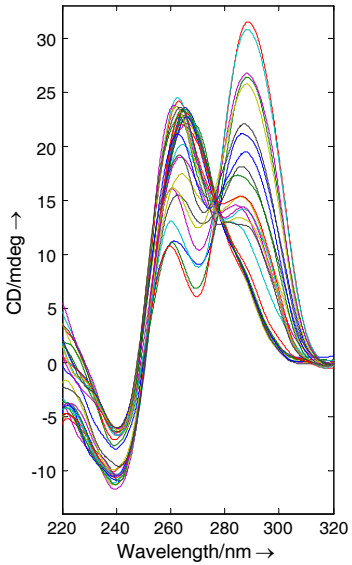
Competition: mole-ratio study

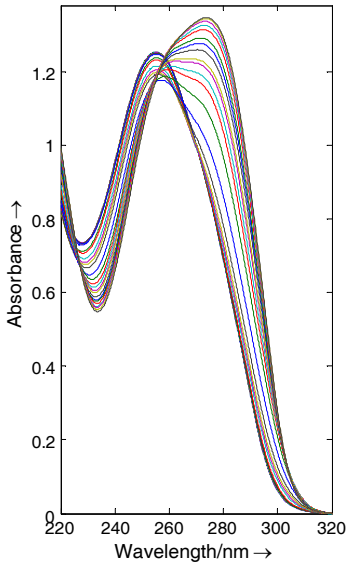


Titration of a ckitC1 mixture with a ckitG1 stock solution, pH 7.1, 1 mM Mg^{2+}, 150 mM ionic strength with KCl

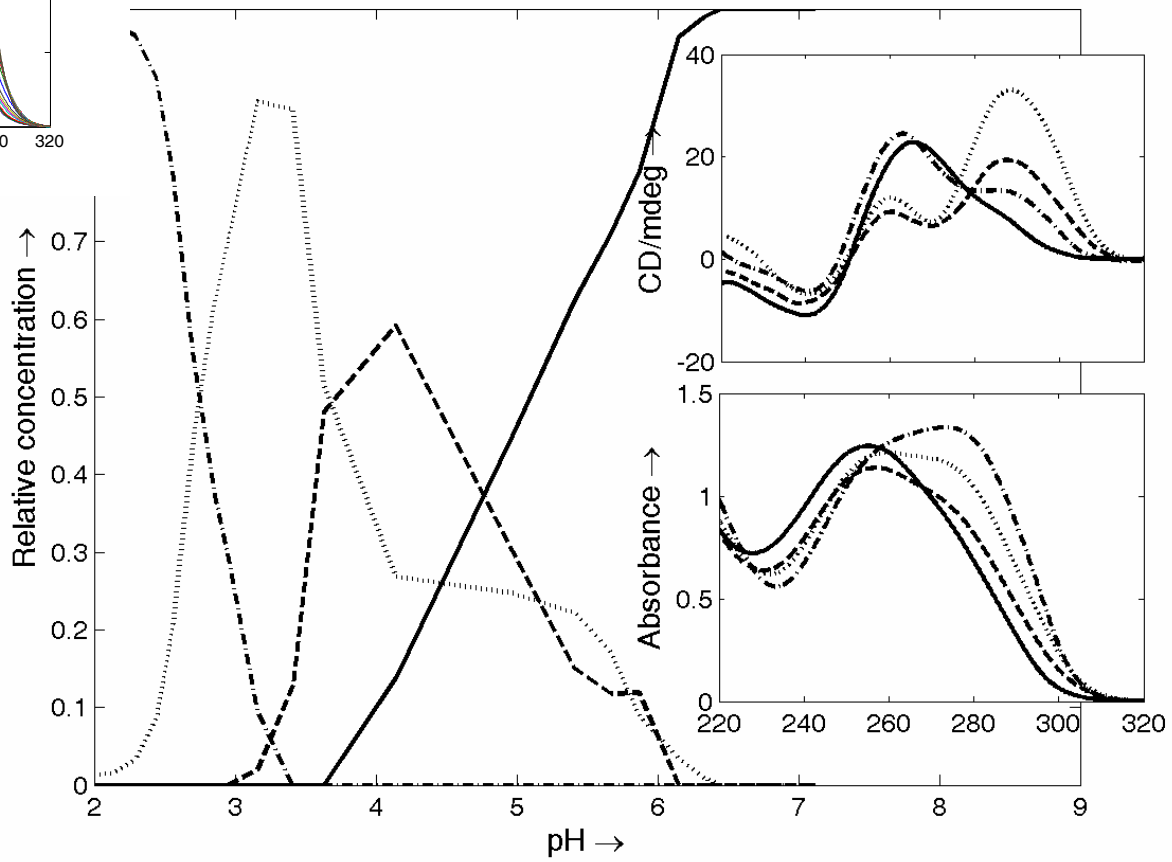


Competition: acid-base study





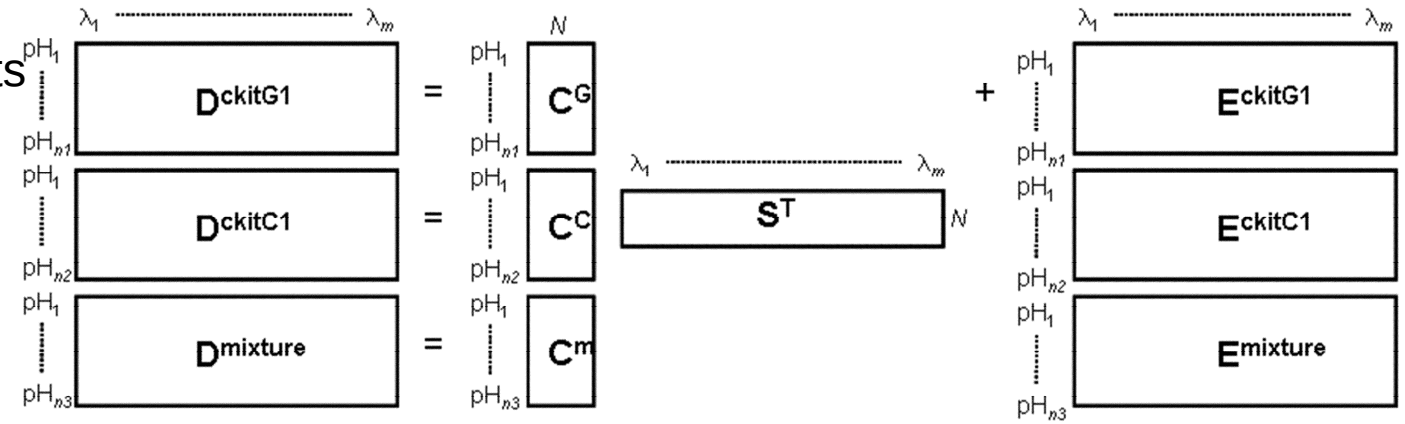
Here, only spectra recorded along the acid-base titration of a 1:1 mixture have been analyzed



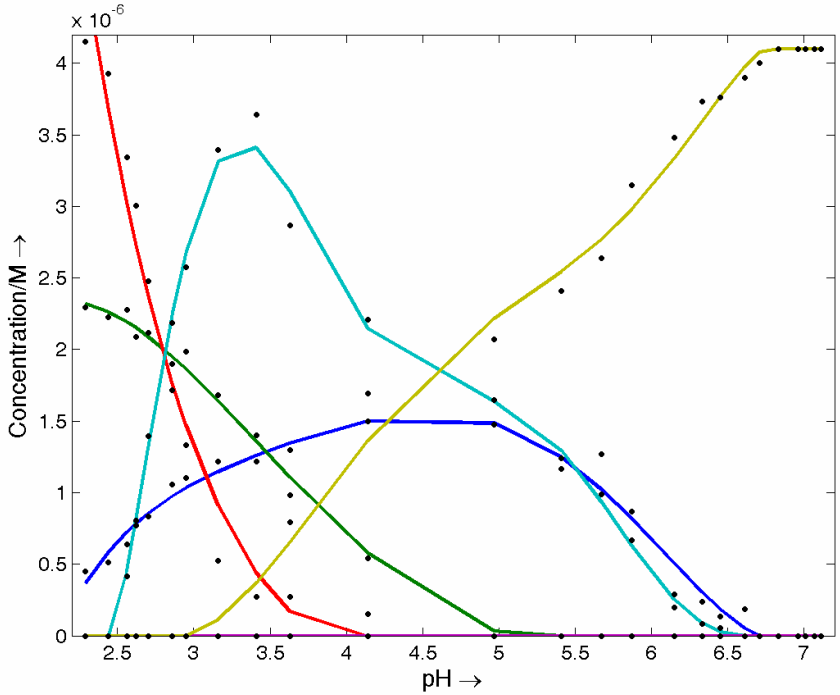
4 species, which are not easy to be explained!

Competition: acid-base study

Now, three data sets have been simultaneously analyzed:



And the results can be explained in a easiest way:



Legend:

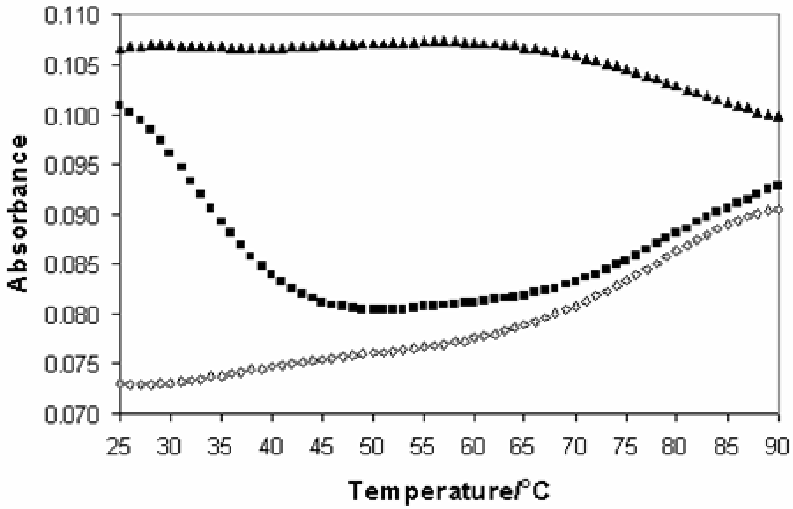
Watson-Crick duplex

ckitC1 i-motif
protonated ckitC1

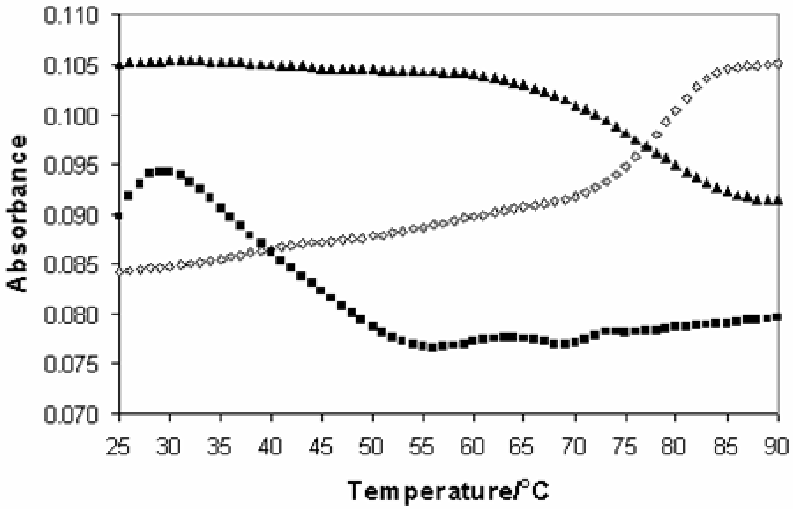
neutral ckitG1 quadr.
protonated ckitG1 quad.

Competition: melting studies

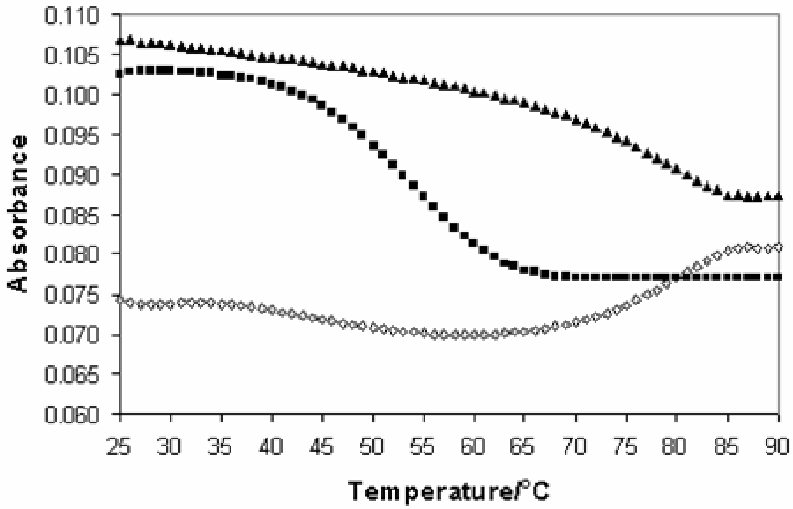
pH 7.0



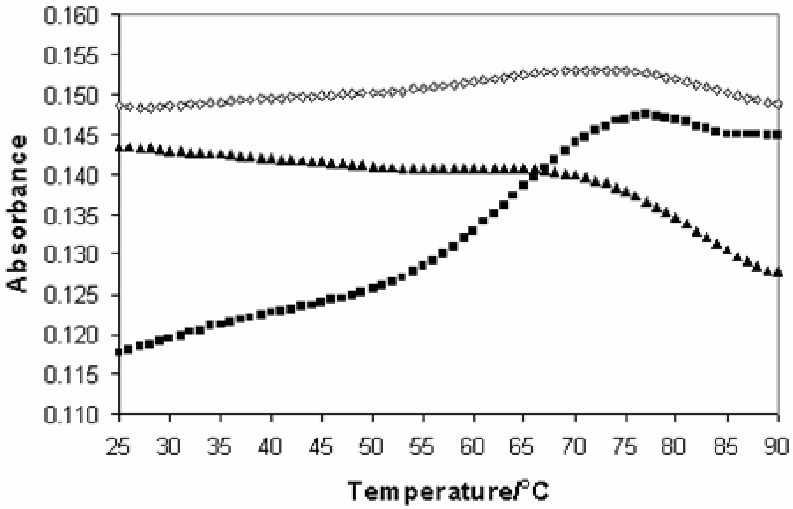
pH 6.0



pH 5.0



pH 4.0



▲: ckitG1; ■: ckitC1; □: ckitG1:ckitC1 1:1 mixture

Conclusions and future work

- ckitG1 sequence forms a G-quadruplex structure throughout the pH range 3 – 7
- An increase of stability is observed at low pH which has been associated to the reduction of repulsive interaction of phosphates
- ckitC1 sequence forms just one *i*-motif structure in the pH range 2- 7
- Stability of the *i*-motif structure is higher at pH ~ 4.3
- Watson-Crick duplex is the predominant structure at pH higher than 5, being practically the only one at pH 7.0
  - Effect of Mg^{2+} ions?
  - Effect of polyamines?
  - Crowding conditions?

Acknowledgments

- Funded projects BFU2004-02048/BMC and CTQ2006-15052-C02-01/BQU from the Ministerio de Educación y Ciencia
- Scientific-Technical Services UB
- Nicolas Roucou (IUT, Lannion, France)

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Thank you!!

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