Study of chemical equilibria involving G-quadruplex and *i*-motifs by means of spectroscopic techniques and multivariate data analysis methods

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Introduction: multivariate analysis

Chemical equilibria can be monitored efficiently with the aid of spectroscopic techniques. The traditional approach consist on monitoring a process (titration, melting experiment, kinetics experiment…) at one wavelength. Nowadays, modern instruments provide huge collections of data which can be handled by appropriate data analysis methods. These have been probed to be effective and powerful tools to extract information from spectroscopic data [1].

In this work, the application of multivariate data analysis methods to deal with data recorded along the monitoring of processes involving G-quadruplex structures is shown [2]. The studied guanine-rich sequence (**5'-C GGG C GGG CGCGA GGG A GGG G -3'**, ckitG) corresponds to a region 140 base pairs upstream of the **c-kit** transcription initiation site, which three-dimensional structure has been previously studied [3].

The process under study (titration, melting experiment) is monitored with the aid of a spectroscopic technique (circular dichroism and/or molecular absorption). At each discrete point of the experiment (temperature in the case of melting experiment) a complete spectrum is measured. The whole set of spectra recorded throughout the experiment are arranged in a table or data matrix **D**.

Using appropriate methods, it is possible:

1. To determine the number of species of conformations present throughout the experiment,
2. To quantify their relative concentration (distribution diagram, matrix **C**),
3. To recover their circular dichroism and/or molecular absorption spectra (pure spectra, matrix **S**)
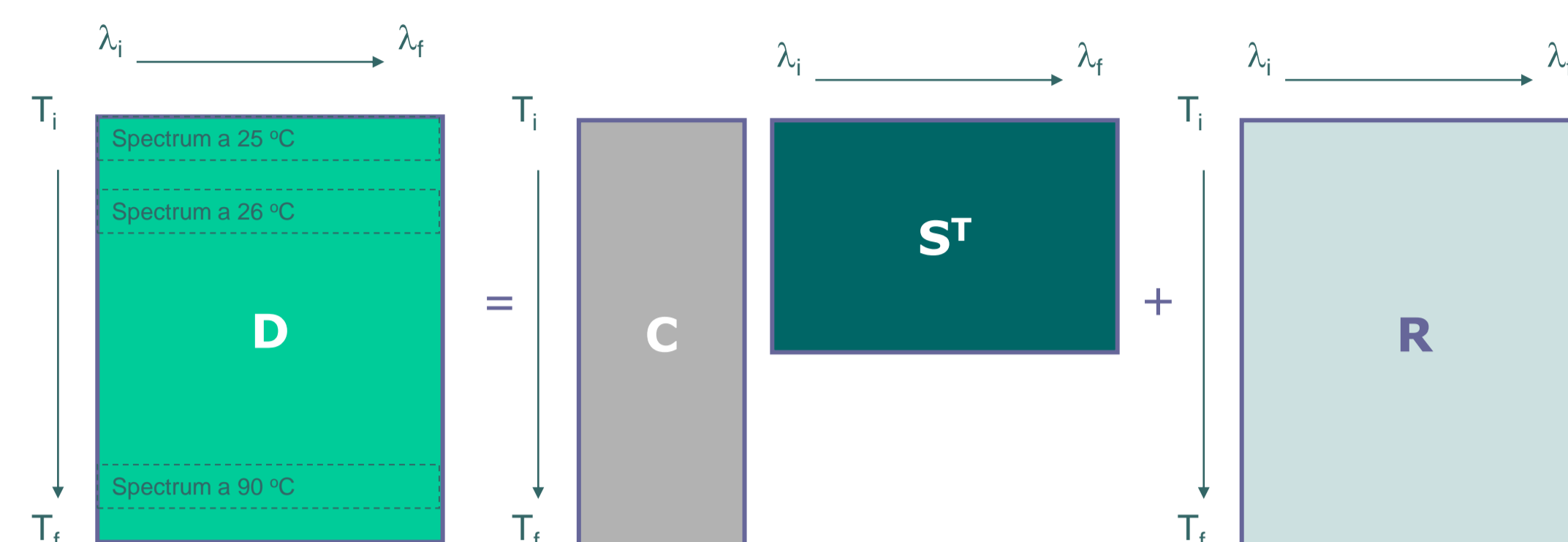
Influence of pH on the solution equilibria of G-quadruplex and *i*-motif

The experimental CD and molecular absorption spectra recorded throughout an acid-base titration of ckitG were analyzed.

In this case, only two acid-base species were needed to fit experimental data. The corresponding CD and molecular absorption spectra were calculated, as well as the distribution diagram (inset).

The G-quadruplex structure was maintained throughout the considered pH range.

The pH transition midpoint was related to protonation of cytosines at the loops.

A similar study was carried out for the complementary cytosine-rich sequence.

In this case, three acid-base species were needed to fit experimental data.

The **i-motif structure** is formed at pH slightly lower than 7 and it reaches its maximal concentration around the pK_a of free cytosine.

The figure shows the calculated distribution diagram for a 1:1 mixture of the guanine-rich and cytosine-rich sequences.

Strand concentrations were 4.1 microM, 25°C, and the ionic strength was 150 mM (KCl).

Yellow line: Watson–Crick 21-bp duplex
Cyan line: i-motif
Red line: protonated form of the cytosine-rich sequence
Blue line: neutral form of the G-quadruplex
Green line: protonated form of the G-quadruplex
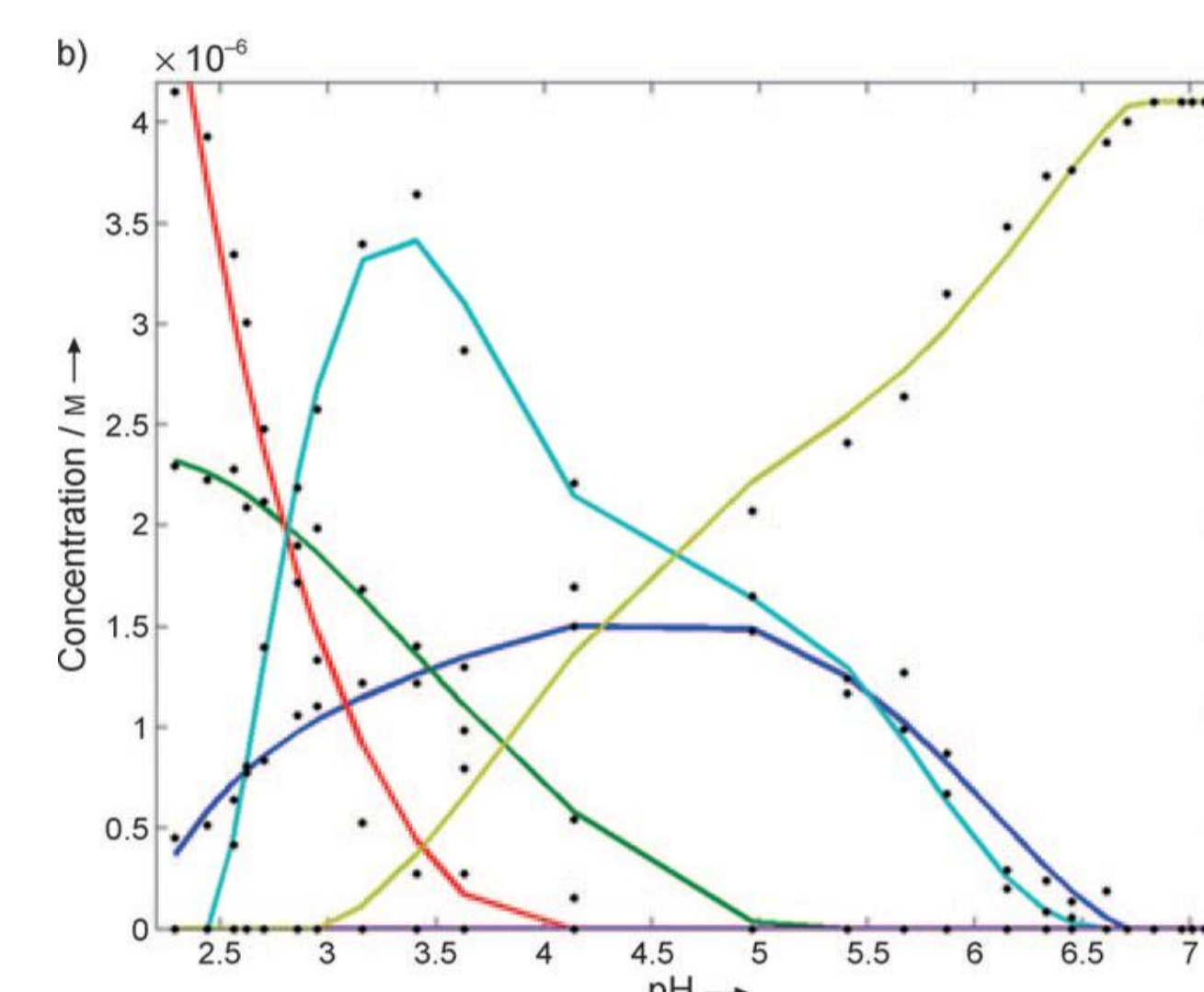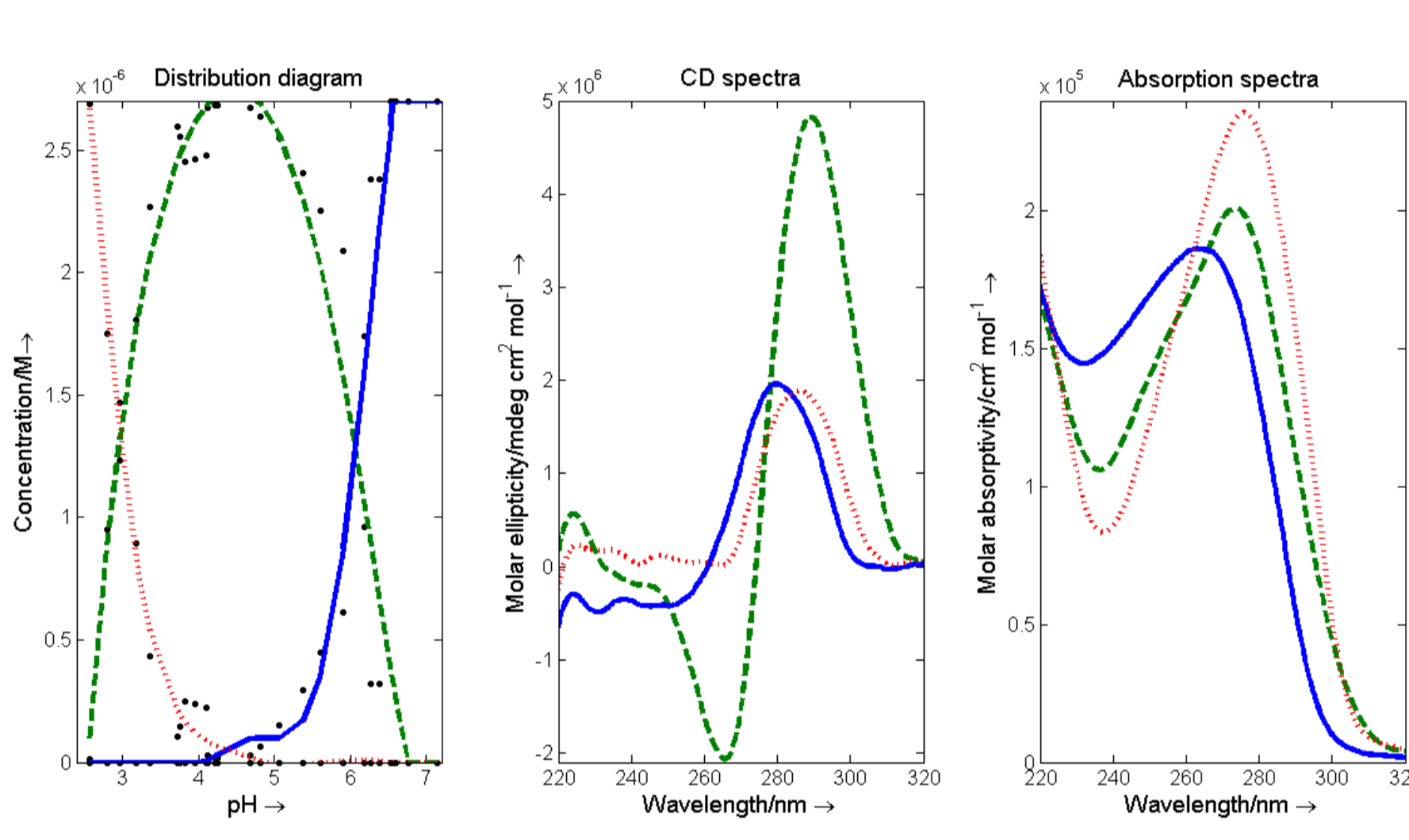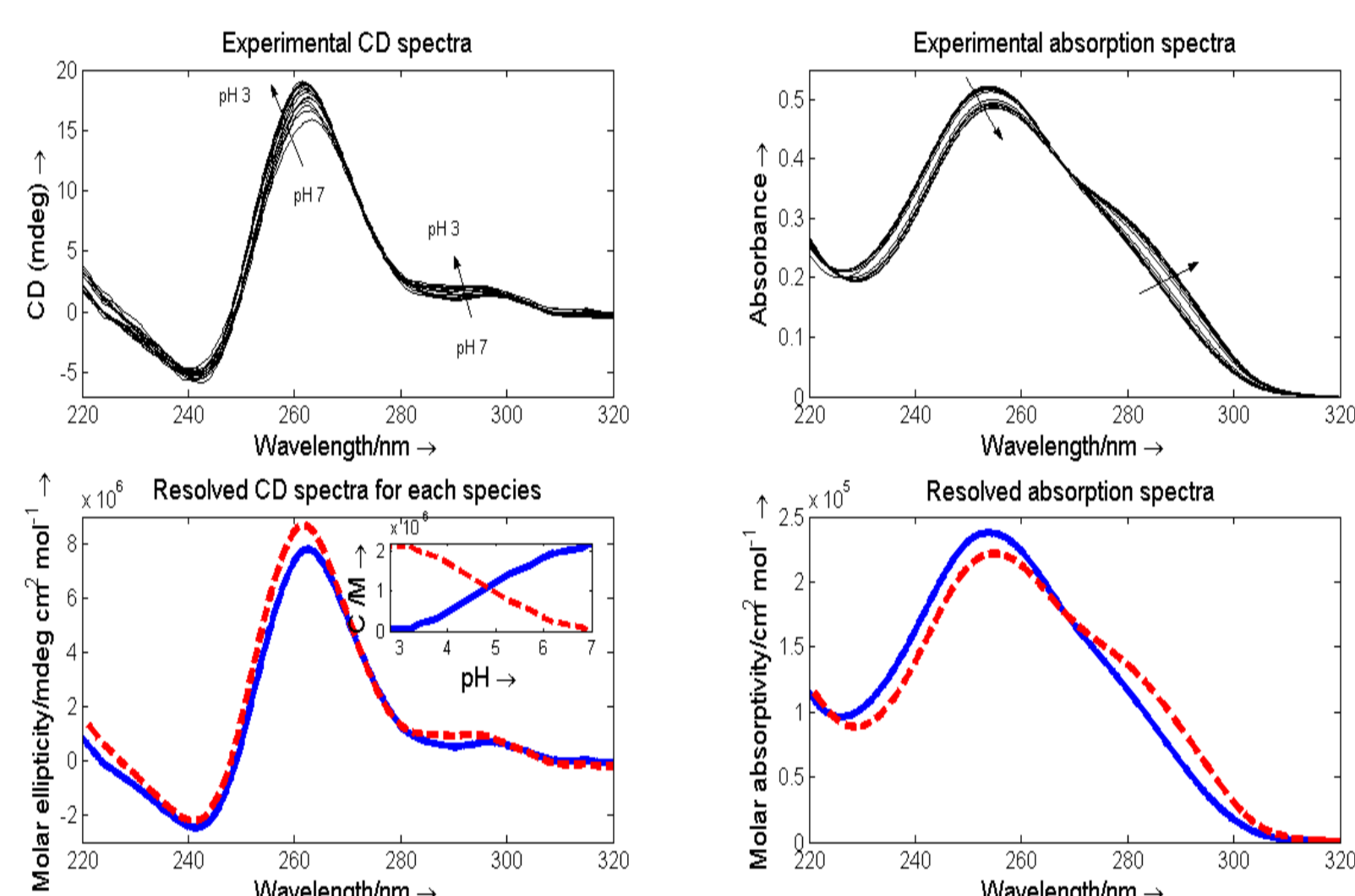
Influence of temperature on the stability of a G-quadruplex and a *i*-motif

The analysis of one of the molecular absorption-monitored meltings of ckitG is shown here.

(a) Experimental spectra recorded from 20 to 90°C.
(b) Absorbance values measured at 260 nm.
(c) Absorbance values measured at 295 nm.
(d) Calculated distribution diagram.
(e) Calculated pure molecular absorption spectra.
(f) Calculated residuals.

Green line: G-quadruplex at low temperatures.
Red line: G-quadruplex at intermediate temperatures.
**Blue line**: random coil.

C_{ckitG}= 3 microM, pH = 5.0, and 150 mM ionic strength (KCl).

The small difference observed between the two species related to the G-quadruplex is just probably due to the baseline drift and / or to small conformational changes at the loops.

The value of T_m (77 ± 1°C) was determined at the crossing point of the concentration profiles for the intermediate conformation and random coil.
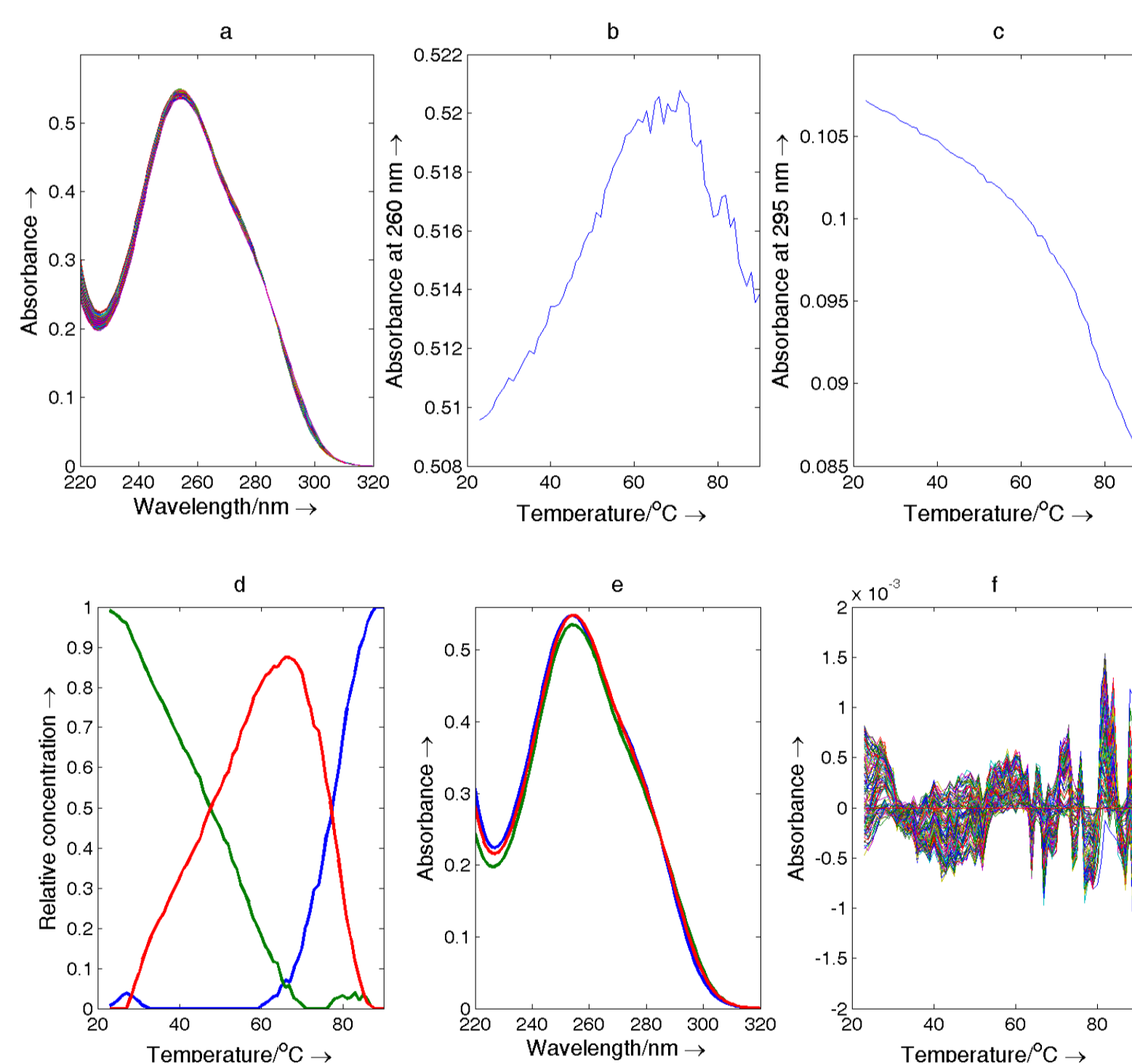
Melting experiments monitored at 295 nm indicated the formation of duplex around pH 7 and of the quadruplex structures at lower pH values.
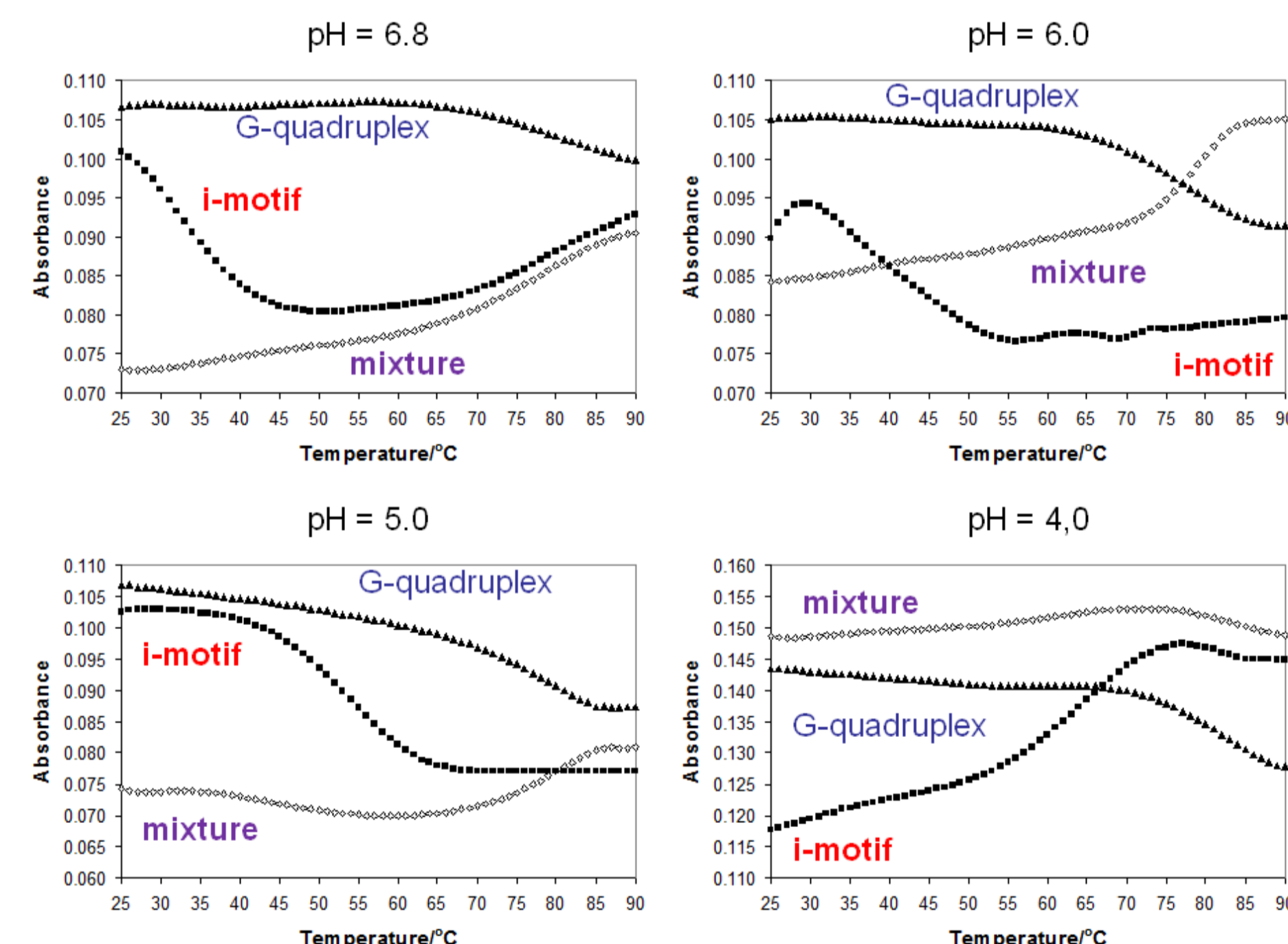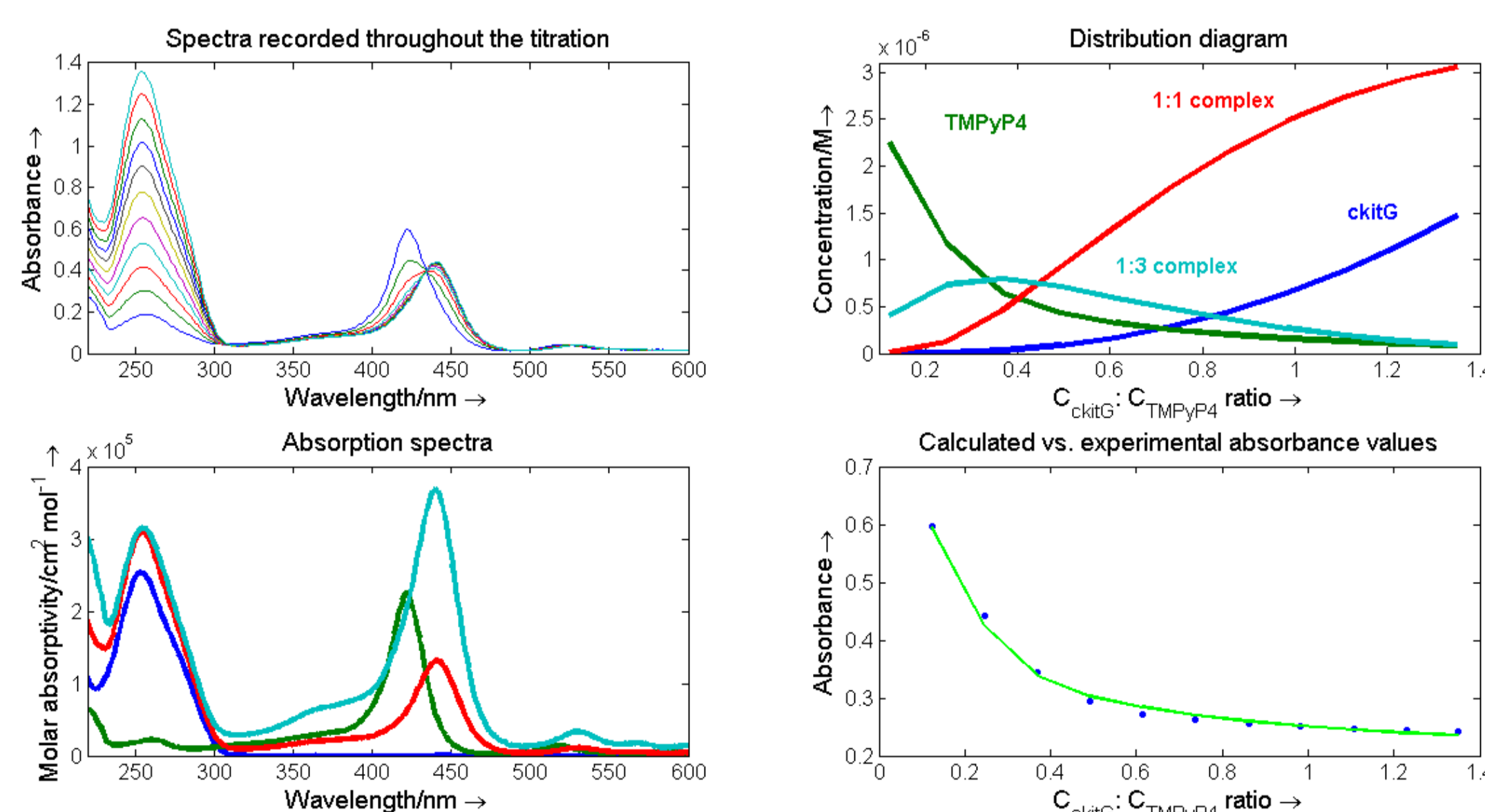

Interaction of a G-quadruplex with TMPyP4

The interaction of the G-quadruplex structure formed by the guanine-rich sequence with TMPyP4 has been studied at pH 7.

Direct and inverse mole-ratio experiments were monitored with CD and/or molecular absorption spectroscopies. In the direct titration, small volumes of a stock solution of **ckitG** were added to a **TMPyP4** solution. In the inverse titration, small volumes of a stock solution of TMPyP4 were added to a ckitG solution.

In both cases, spectroscopic data were fitted well to an interaction model. The spectra of pure drug and pure DNA were added to the calculation to increase the reliability of the results obtained.

Two binding modes were proposed:

log K_a = 7.4 ± 0.5, and stoichiometry 1:1 (TMPyP4:DNA)

log K_a = 6.3 ± 0.7, and stoichiometry 2:1 (TMPyP4:DNA)

According to CD spectra, the interaction with TMPyP4 does not affect apparently the G-quadruplex structure of ckitG.
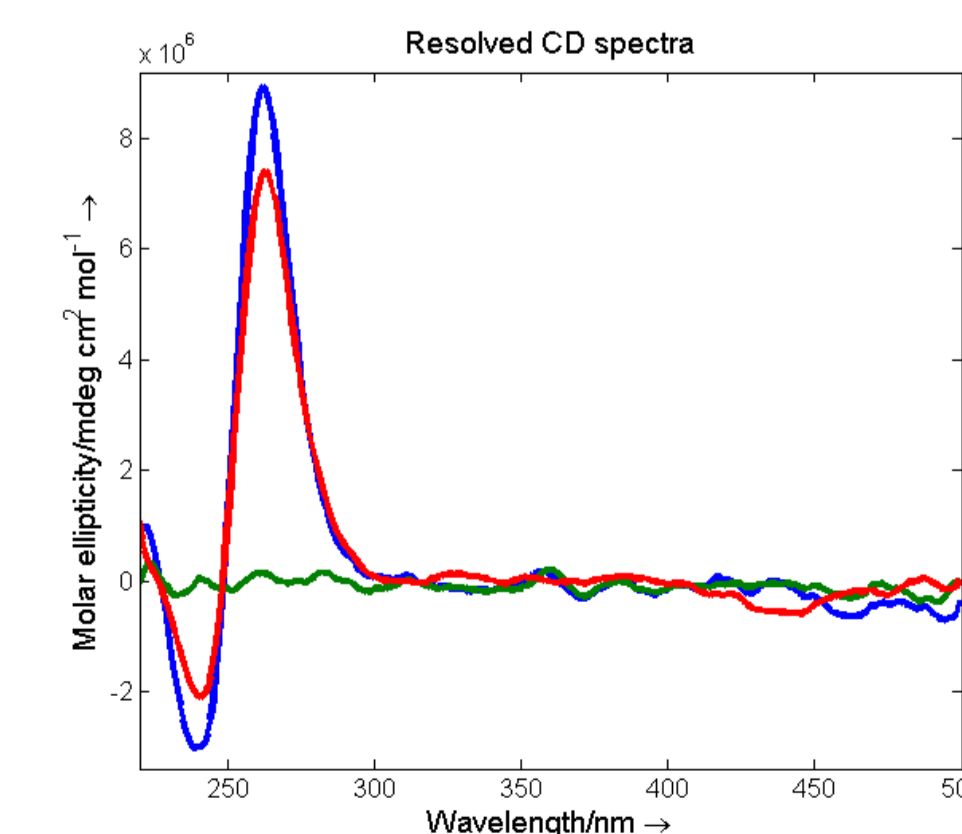
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Acknowledgments