THREE-WAY ANALYSIS OF THE CONFORMATIONAL TRANSITIONS OF NUCLEIC ACIDS USING MOLECULAR BEACONS TECHNOLOGY

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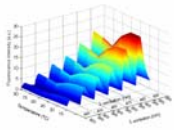
INTRODUCTION

Conformational changes in nucleic acids can be induced by variations of temperature, pH or ionic strength conditions [1]. These equilibria can be monitored by several instrumental techniques like circular dichroism or molecular absorption. Due to the non-fluorescent nature of the natural nitrogenated bases, molecular fluorescence can not be applied to the study of these equilibria. Molecular beacons technology tries to overcome this difficulty.

Molecular beacons are based on the addition of a **fluorophore** and of an appropriate **quencher** in strategic positions of the nucleic acids.

When the nucleic acid is in an ordered conformation, the fluorophore and the quencher are extremely close in the space and the fluorescence signal of the sample is low. When the nucleic acid adopts a random coil or an extended conformation, the fluorophore and the quencher are separated and the fluorescence intensity increases.

Traditionally, molecular beacons technology has been performed by monitoring the process at one single emission wavelength. In this work, equilibria of quadruplex nucleic acids have been monitored by multiple wavelength emission fluorescence, UV-vis molecular absorption and circular dichroism spectroscopies in different experimental conditions. These multiwavelength approaches combined with multivariate resolution methods have demonstrated to be useful tools for the analysis of this kind of data[2].



EXPERIMENTAL

The oligonucleotide 5'> F - TAG GGT TAG GGT – Q <3' (SG) was labelled at the 5' end with **fluorecein** (fluorophore, λ_{exc}=492 nm., λ_{em}=520 nm.) and at the 3' end with **dabsyl** (quencher). Also, the complementary strand 5'> ACC CTA ACC CTA <3' (SC) was synthesized but not labelled with fluorescent or quencher tags.

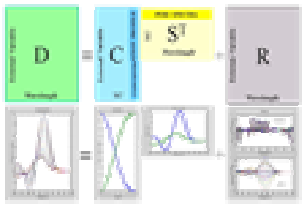
All experiments were carried out with the oligonucleotides in pH 7.0 phosphate buffer. Samples for melting studies were heated at 90°C for 5 min and allowed to renaturalize, cooling slowly until room temperature. Oligonucleotide samples were kept at 4°C until their use.

Melting experiments of SG and mixtures of SG and SC at different concentration ratios were performed. 5 different species can be expected according to the literature [3]:

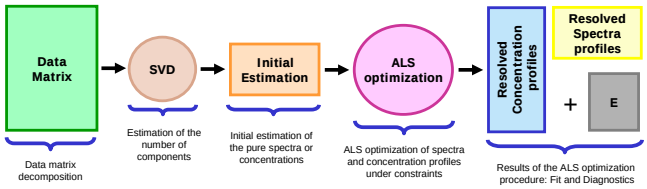
Three spectroscopic techniques have been used to monitor the experiments: Fluorescence, Circular Dichroism and UV-VIS molecular absorption.

MULTIVARIATE CURVE RESOLUTION ALTERNATING LEAST SQUARES

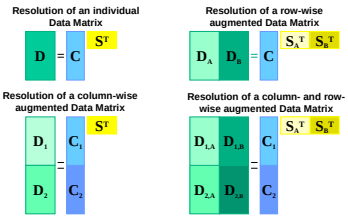
Data decomposition according to the Beer's Law



MCR-ALS flowchart [4]



MCR-ALS analysis options



MCR-ALS RESULTS

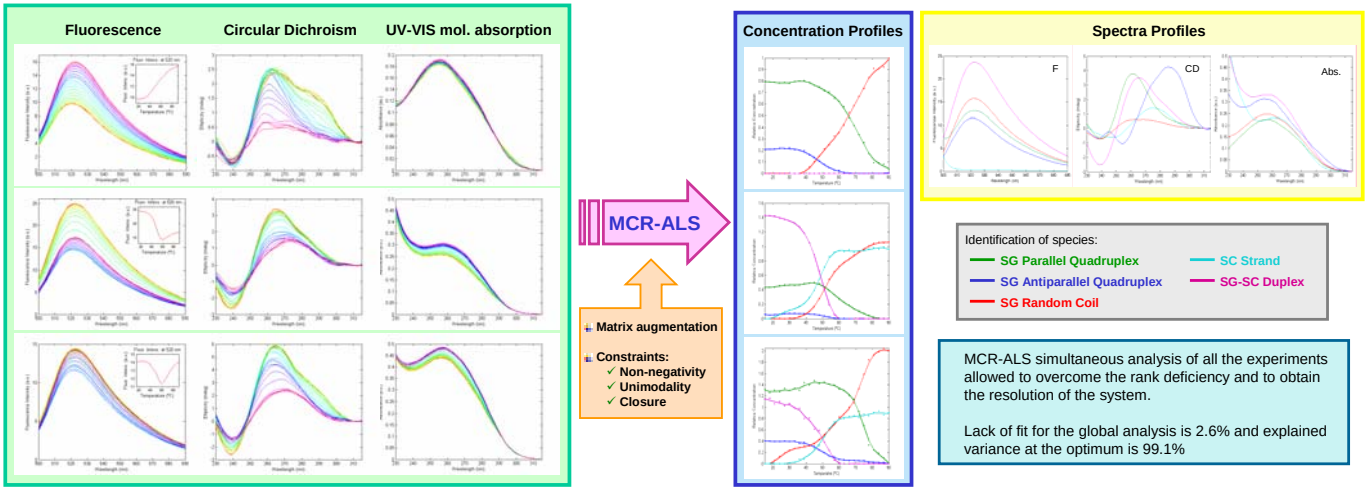
Individual analysis of each one of the experiments does not allow the complete resolution of the system.

WHY ?

RANK DEFICIENCY is present

SOLUTION?

MATRIX AUGMENTATION in row- and column- wise directions



MCR-ALS simultaneous analysis of all the experiments allowed to overcome the rank deficiency and to obtain the resolution of the system.

Lack of fit for the global analysis is 2.6% and explained variance at the optimum is 99.1%

CONCLUSIONS

Simultaneous MCR-ALS analysis of several matrices overcomes the rank deficiency difficulties inherent to this particular system due to the equilibrium of at least two species at the beginning of the experiment.

Complete resolution of the system can only be obtained using data from different spectroscopic techniques due to the extreme overlapping of the signals:

- Concentration profiles describe the equilibrium of the parallel and antiparallel quadruplex structures for SG oligonucleotide. In the experiments where SC is also present, competition between SG-SC duplex and SG quadruplex structures can be observed.
- Spectral profiles provide structural information about the oligonucleotide. Resolution of the circular dichroism spectra of parallel and antiparallel quadruplexes (overlapped in this experimental conditions) is only possible if CD data is analyzed jointly with other spectroscopic data.

REFERENCES

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