Application of Multivariate Curve Resolution for the study of folding processes of DNA monitored by Fluorescence Resonance Energy Transfer

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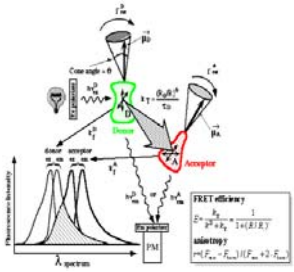
Abstract

In the present study, Fluorescence Resonance Energy Transfer (FRET) has been used to monitor the folding of a 31-mer cytosine rich DNA segment, from the promoter region of the human *c-myc* oncogene. Spectroscopic FRET data recorded along experiments carried out at different pH and ionic strength conditions were individual and simultaneously analyzed by Multivariate Curve Resolution (MCR) (1). The simultaneous analysis of several data matrices allowed the resolution of the system, removing most of the ambiguities related to factor analysis. From the results obtained, we report the evidence of the formation of a highly ordered structure (a quadruplex known as *i*-motif) in acidic and neutral pH values.

FRET

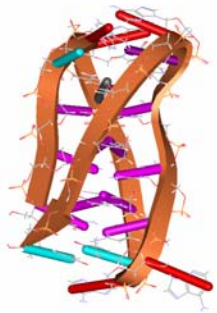
Fluorescence excitation energy transfer (FRET) is a dipole-dipole resonance interaction between two "close" molecules, where one molecule, called the "donor", transfers, its excitation energy to the other, called the "acceptor" (2).



FRET has provided valuable information about the structure of various biologically relevant molecules, including nucleic acids, because of its distance and orientation dependence.

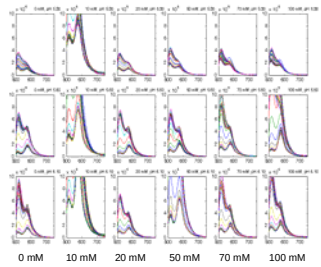
The i-motif

Non B-DNA structures such as cytosine-rich tetraplexes have been reported to play functional roles as promoter elements in gene expression and as repeating units in telomeres and centromeres (3). These pyrimidine-rich strands can form unusual secondary structures, primarily due to nucleotide stacking, known as C-tetraplex (i-motif). Presence of tetraplex sequence motifs have been shown in the regulatory region of the *c-myc* gene. Altered fine tuning of regulation, as a result of secondary structure formation, has been implicated in tumor progression, diabetes and neurological disorders like mental retardation and dystrophy.



Raw data

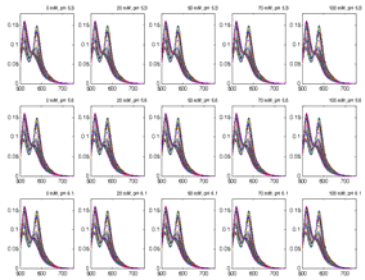
- High variability among data matrices
- Need of a data pretreatment before MCR
- Data sets at 10 mM were removed



Normalized data

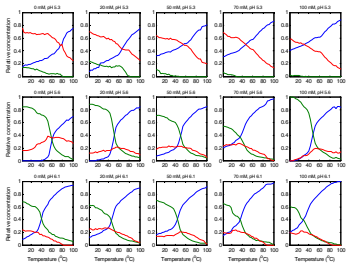
Each spectrum is divided by the norm:

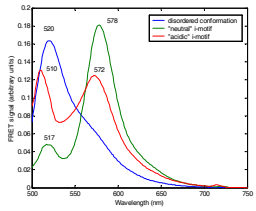
$$A_n = \sqrt{\sum_{j=1}^{J} x_j^2}$$



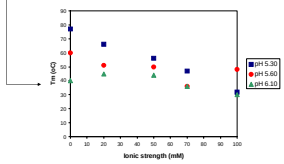
MCR-ALS

- Simultaneous analysis
- Non-negativity for concentration and spectral profiles
- Closure





- Emission around 580 nm indicates the formation of ordered structures, where acceptor and donor are close in space, yielding FRET.
- High emission around 515 nm indicates the absence of FRET.



- Tm values were determined at the crossing point of the MCR resolved concentration profiles of major conformations.
- At pH 5.30, Tm is dependent on ionic strength, which could reflect a loose of the stability for the structured form due to the competition of sodium ions.

Conclusions

1. MCR has been shown to be an useful tool for the analysis of FRET data recorded along independent melting experiments.
2. An ordered structure at acidic pH values, in addition to the i-motif present at neutral pH values, has been observed.

References

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