Research Activity Report - Ph.D

How selectivity drives hybridization and aggregation in random-sequence DNA oligomers

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1 Research Activity

1.1 Monomer aggregation and pretransitional order in DNA liquid crystals

Short DNA duplexes (6-20 base pairs) with end-to-end attractive interactions in aqueous solution show liquid crystal nematic (N) ordering [1]. While DNA nematics have been extensively observed and characterized, studies of pretransitional behaviour are still missing. To this end I investigated, by means of static light scattering technique, the pre-transitional isotropic-nematic (I-N) behaviour of DNA 12-base-long duplexes with stacking interactions as a function of concentration (c = 100 – 300 mg/ml). I performed depolarized scattering experiments in the isotropic phase in a wide range of temperature (T = 70–20 C), spanning from the condition in which there is no aggregation between duplexes (high temperature) to the temperature at which I-N transition occurs. The experimental results show a clear continuous increase of the depolarized scattered intensity (I_{dep}) as the temperature is lowered. Such a I_{dep} growth is not only due by linear aggregation of duplexes (aggregation number M), but also reveals the presence of clusters in which the duplexes are orientationally correlated, making the relationship between M and I_{dep} not trivial. To disentangle the various processes characterizing pre-transitional behavior, the I_{dep} as a function of T and c were analyzed in two steps. Firstly, in order to free the I_{dep} data from T dependencies unrelated to the aggregation process and the development of orientational correlations, we normalized the data by the scattered intensities of a similar DNA duplex with not interacting ends (external mismatches). Secondly, in order to get the information about the orientational correlations as a function of T, it has been performed computer simulation by colleagues that allowed us to quantify how much "orientational drag" is induced on the neighbouring duplexes by aggregation. The simulation results in combination with a theoretical model [[2]] allowed us to interpret the I_{dep} data, obtaining M as a function of T and the stacking energy between the interacting DNA duplexes. The results are compatible with the sharp first-order nature of I-N phase transition and gives an insight of the role on the development of local orientational order both far and in proximity of the I-N phase boundary.
This research activity has been carried out during the third year.

1.2 Monomer Diffusion in Liquid Crystals

The second main topic of my research activity is strictly related to the previous one and regards the dynamics of such short DNA duplexes both approaching and in LC phase. In structured fluids, such a LC, the participation of individual molecules to the structure is typically transient, a condition which enables them to diffuse. The complex interplay of attractive and repulsive interactions among such molecules makes the diffusion description difficult. I investigated and compared the diffusion of repulsive and attractive duplex DNA 12-base-long oligomers in concentrated solution. We studied these systems using a combination of FRAP and static and dynamic, polarized and depolarized light scattering, determining the rotational and translational, self and collective diffusion processes. The main resulti is that both translational and rotational self-diffusion have a strong, nearly exponential and factorizable dependence in c and T of the form of $D(c,T) \approx D_0 e^{A/T} e^{-Bc}$. Moreover it has been understood that the dependence on c follows from electrostatic repulsion while the T dependence reflects – via an Arrhenius law – the interduplex attractive interactions. The comparison between FRAP and Dynamic Light Scattering experiments shows also that: (i) rotational diffusion is more hampered by intermolecular attractive interactions than translational self-diffusion, whereas for repulsive interactions both the self-diffusion are hampered by the same amount; (ii) the translational self-diffusion is unaffected by the onset of the CLC ordering; (iii) collective diffusion is weakly depending on c and T up to the CLC transition, at which viscoelasticity is detected.
This research activity has been carried out mainly during the second year and in first semester of the third year.

1.3 Hybridization and selectivity of random-sequence DNA Oligomers

The remarkably selective hybridization of DNA and RNA oligomers with their complementary sequence is at the basis of many biological processes (e.g. the regulatory mechanism of miRNA) and biological technologies (e.g. PCR). To test and quantify this selectivity I studied the hybridization in context of larger diversity: solutions of random-sequence DNA oligomers(rsDNA) of different length L, in which the number of distinct target sequences is 4^L, i.e. $6 \cdot 10^4$, $16 \cdot 10^6$, 10^{12} for L = 8, 12, 20 respectively. This space of sequences, easily larger than that of the genome, is a simple but rich model system to explore selectivity of interaction in nucleic acids. To this aim I:

- performed at different salt concentrations (c_{NaCl} = [0.15 - 1] M) both standard melting measurements in diluted solutions (c_{DNA}=0.04mg/ml) for rsDNA 12mers and 20mers, and in highly concentrated solution (c_{DNA} = 30 mg/ml) for rsDNA 8mers in a quartz microfluidic cell 10 microns-thick.
- performed Fluorescence Contact Quenching experiments of two complementary DNA sequence A and B at different concentration in rsDNA solution to get a quantitative information about the formation of perrfect duplex AB in rsDNA oligomer solutions.
- developed a simplified statistical theoretical model to predict the distribution of pairing errors in the duplexes formed in these systems at equilibrium.

The model succeeds in describing both the behaviour of the experimental curves as a function of temperature and the melting temperature as a function of salt concentration. Fluorescence Contact Quenching experiments match the theoretical prediction indicating that, even in these super-diverse mixtures, selectivity leads to the onset of a not negligible amount of perfect duplexes in the ocean of rsDNA oligomers.
This research activity has been carried out continuously all over the three years.

1.3.1 Technology Development

To perform melting experiments at high Dna concentration($\approx 30g/l$) I assembled a new experimental setup that it was continuously improved all over the three years. In order to reach such a high concentration it is necessary to reduce the optical path length from standard values of 1 cm for standard couvette to $\approx$ 10 microns. Microfluidic devices makes it possible.
So, during the first year I have realized such a microfluidic device through lithography techniques at the Institut Pierre-Gilles de Gennes pour la Microfluidique in Paris, in my co-supersivor's lab, where I had been worked for two weeks. This microfluidic cell allowed me to perform UV absorbance and melting experiments at high DNA concentration. However it was necessary to improve the sealing of microfluidic cell in order to prevent evaporation at high temperature. Consequently, during the second year it has been bought and used a commercial microfluidic cell, whose noozles were sealed properly with suitable caps, preventing efficiently the evaporation of DNA solution. Moreover, it has been designed and realized an alluminia holder compatible with the spectrophotometer to host and improve the thermalization of the microfluidic cell.
Melting experiments for shorter rsDNA revealed the need to reach lower temperature($\approx 0C$) than the ones alloewed by the peltier of spectrophotometer($\approx 15C$). So, during the third year, I have self-customized the commercial spectrophotmeter. In particular, I have assembled a new peltier that allowed us to reach the desired temperature, and designed through autodesk new alluminia compenents comaptible with the new peltier to host and improve thermalization of both standard couvette and microfluidc cell. The components were realized in a mechanical shop. Morever I progammed with Matlab a new app to make the spectrophotometer comunicate with new peltier system.

2 Attendance to Summer School, Conference, Workshop and Courses

2.1 Summer School

During the third year I have attended the summer School :"Advanced School in Soft Condensed Matter - Solutions in the Summer 2021" from 05/07/2021 to 09/07/2021 online, and I have presented the poster: "Monomer aggregation and pretransitional order in DNA liquid crystals ".

2.2 Conference

I have attended the "Liquid Matter Conferences(LMC)" from 19/07/2021 to 23/07/2021, and I have presented the poster: "Monomer aggregation and pretransitional order in DNA liquid crystals ".

2.3 Workshop

In the first year I have attended the Workshop Biometra on 23/09/2019 at L.I.T.A. (Segrate), and I have presented the poster: "Hybridization and selectivity of random-sequence DNA oligomers".
On 27/09/2021 I will attend the Workshop Biometra at L.I.T.A. (Segrate), and I will present the poster: "Finding your Partner in Superdiverse Environments:the Pairing Statistics of Random DNA Oligomers".

2.4 Courses

During the second semester of the first year I have attended the following courses:

- "Experimental methods for the investigation of systems at the nanoscale": owner prof. Alberto Vailati at Università degli Studi di Milano. Total amount of hours: 30.
- "Soft matter: the structure and rheology of complex fluids": owner Roberto Piazza at Politecnico di Milano. Total amount of hours: 50.
- Comunicazione Efficace: owner Marina Carpineti and Anna Maria Paganoni at Assolombarda.Total amount of hours: 25.

3 Teaching

During the second semester of all the three years I have taught Physics in Fisica Applicata, whose owner is my supervisor prof. Tommaso Bellini. In particular I carried out exercises for a total aount of 28 hours per year. I want to highlight that during the Covid pandemic I spent most of my time in recording and editing lessons for students.

Bibliography

[1] Zanchetta Giuliano et al. "Right-handed double-helix ultrashort DNA yields chiral nematic phases with both right-and left-handed director twist". In: *Proceedings of the National Academy of Sciences* (2010).

[2] De Michele Cristiano et al. "Self-assembly of bifunctional patchy particles with anisotropic shape into polymers chains: Theory, simulations, and experiments". In: *Macromolecules* (2012).

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