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Characterization of helices and their arrays in biological and synthetic macromolecules by time-resolved fluorescence

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In 1967, Stryer and Haugland demonstrated that fluorescence resonance energy transfer (FRET) between a naphthyl donor and a dansyl acceptor could be applied to probe the distance between the two ends of a poly(L-proline) type II trans helix that had been end-labeled with these dyes. This study defined FRET as a spectroscopic ruler which explains the supremacy of FRET to this date among other fluorescence techniques when seeking structural information about macromolecules. Yet time-resolved fluorescence anisotropy (TRFA) and pyrene excimer fluorescence (PEF) are becoming increasingly used to characterize the helical nature of well and poorly defined macromolecules. This presentation will illustrate the applicability of these methods based on TRFA and PEF. In

particular, the ability of TRFA to determine the hydrodynamic volume of rigid macromolecular objects was applied to demonstrate that well-defined oligoquinoline foldamers labeled at one end with an oligo(phenylene vinylene) dye retained a helical conformation in chloroform. By contrast, PEF can be applied to probe the helical nature of poorly defined macromolecules, i.e. polydisperse macromolecules of different lengths but of same chemical composition. PEF does this by determining the dimension of the restricted volume where an excimer can be formed from the encounter between an excited and a ground-state pyrene covalently attached to a macromolecule. Analysis of the fluorescence decays acquired with pyrene-labeled macromolecules (PyLMs) yields the dimension of the restricted volume, also referred to as a blob from the knowledge of the pyrene content of the PyLM. Molecular Mechanics Optimizations (MMOs) can then be employed to assess where the size of a blob are compatible with a helical conformation. This

feature has been applied to demonstrate that poly(L-glutamic acid) in DMF_{1,2} and amylose in DMSO₃ both adopt helical conformations. An additional feature of handling PEF with the FBM is that it provide information about interhelical distances between helices constituting the building blocks of a macromolecule. This was used to determine the interhelical distance between the strands of oligo(L-glutamic acid)₄ and oligosaccharide helices constituting a poly(L-glutamic acid) arborescent polymer and amylopectin.

Biography

Jean has been trained as a chemical engineer in France at the Ecole Nationale Supérieure des Industries Chimiques, Jean Duhamel obtained in PhD in 1989 under the supervision of Dr. André at the Institut National Polytechnique de Lorraine (France). Following a first post-doc at the U. of Toronto (Canada) with Prof. Winnik and a second one at the U. of Pennsylvania (USA) with Prof. Lu, he joined the U. of Waterloo in 1996. Over the past 20+ years, work from his research group has aimed to establish the versatility of pyrene excimer fluorescence or PEF to probe the dynamics, conformation, and interactions of any macromolecule in solution. He has published over 100 refereed papers to date with close to half of them having been published in *Macromolecules*, the top scientific journal for macromolecular science.

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