CiQUS

Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares

Conferencia:

Real-time structural microscopy of co-transcriptional RNA

Carlos Penedo

School of Physics and Astronomy and Biomedical Science Research Complex

University of St Andrews, U. K.

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Aula de Seminarios do CIQUS

10:00 h

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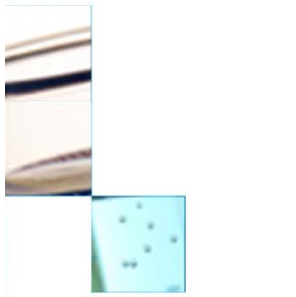

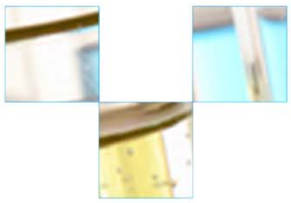

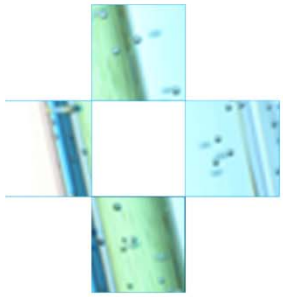

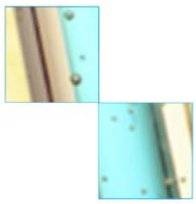

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Carlos Penedo

School of Physics and Astronomy and Biomedical Science Research Complex
University of St Andrews, U. K.
https://www.st-andrews.ac.uk/~singlemol/singlemol.html

Title: Real-time structural microscopy of co-transcriptional RNA

The ability of certain RNA sequences to fold into specific functional structures is crucial for the timely and selective control of many biological processes. Often, these functional structures are transient intermediates, present only for a short time during RNA synthesis. For decades, studying these co-transcriptional transients has remained a challenge, and with few exceptions, our knowledge about RNA structure and folding has been limited to full-length post-transcriptional sequences. Single-molecule Förster Resonance Energy Transfer (smFRET) has been unique to investigate post-transcriptional RNA structures, but its application to co-transcriptional conditions has been impossible since bacterial and eukaryotic RNA polymerases (RNAP) do not accommodate fluorescently-labelled nucleotides. In this seminar, I will introduce a recently developed smFRET method that overcomes these limitations and allows to monitor RNA sequences folding in real time as they emerge from the native transcriptional complex. I will start by describing a new bioconjugation approach combining stepwise transcription and click-chemistry for site specific incorporation of fluorescence probes to RNA sequences within bacterial transcriptional complexes. Using a TPP-sensing regulatory sequence as an example, I will show how the methodology can be used to obtain, for the first time, co-transcriptional data during transcription elongation. It is expected that the technique could be used with various prokaryotic or eukaryotic polymerases and will assist the development of more refined in-silico predictors of co-transcriptional folding.

Research area

Research in my lab lies at the interface between Chemistry, Physics and Biology. The overarching aim is to understand the structural dynamics of biomolecular processes crucial to life at the level of single-molecules or single protein complexes. More recently, we have expanded our research themes to the area of organic semiconductors and plastic electronics. Organic light-emitting materials used for OLED solar cells, and smartphone/TV displays, are conjugated polymeric structures and it is known that the conformation of the polymer impacts the optoelectronic properties of the fabricated device. Therefore, we have developed single-molecule spectroscopy techniques to investigate the conformational dynamics of these polymers in organic solvents and manipulate their end-to-end length using magnetic tweezers (*Dalgarno et al, JACS, 2013; Tenopala-Carmona et al, Sci. Adv. 2018*).

Our interest in the dynamics of biomolecular processes focuses on two main areas: i) mechanisms of DNA repair and ii) Gene regulation by non-coding bacterial RNAs and iii) structural dynamics of mechanosensitive membrane proteins.

The nucleotide excision repair (NER) mechanism deals with UV-induced DNA damage and although it has been extensively studied using conventional biochemical approaches, our understanding of each step along the pathway is far from complete. Using specifically tailored cloning and expression and labelling techniques we have obtained most of the proteins involved in the recognition and DNA-damage

processing steps including XPC, XPA, RPA and the TFIIH multiprotein complex (*Morten et al, NAR 2015; Constantinescu-Aruxandei, NAR, 2016; Craggs et al. NAR 2014*). Our current challenge is to monitor at single molecule level the recruitment of each protein to the damaged DNA, monitor how the initial repair bubble is generated by XPC to expose the lesion and how this is further remodeled by the interaction between XPA, RPA and TFIIH.

In the area of gene regulation by non-coding RNA, we have used smFRET and chemical probing assays to characterize the folding and ligand recognition mechanisms of the adenine-, lysine- and SAM-sensing bacterial riboswitches (*Heppell et al, Nat. Chem. Biol. 2011; Dalgarno et al. NAR 2014; McCluskey et al, PCCP, 2017*). Given that these RNA structures are mostly found in archaeal, bacterial and plants but not higher organisms, they are attracting interest as potential antibiotic targets. In this context, elucidating how they recognize their cognate metabolite is fundamental to rationalize the chemical space in which to develop competitive non-natural metabolites. Importantly, these structures perform their regulatory function only during the transcription process. Thus, a significant challenge was to developed methods that allow to monitor their folding and ligand recognition mechanism whilst they are being synthesized by the RNA polymerase. Using a combination of stepwise transcription and click-chemistry labelling we have developed a protocol that enables to monitor at single-molecule level and in real-time the co-transcriptional folding of any RNA sequence. Currently, we are merging this method with magnetic tweezers for force-based manipulation of the RNA structure during transcription.