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High-speed optical tweezers for the study of protein-DNA interaction

We developed a constant-force laser trap that allows us to investigate molecular interactions and sub-nanometer conformational changes occurring on a time scale of few tens of microseconds. The method is effective in studying the sequence-dependent affinity of DNA-binding proteins along a single DNA molecule. The improvement in time resolution provides important means of investigation on the long-puzzled mechanism of target search on DNA. In fact, one poorly understood issue in the field of protein-DNA interaction is how proteins weakly interact with non-cognate DNA sequences and how they efficiently find the sequence of interest among an extremely large amount of non-specific sequences. Using our technique, we could discriminate sequence and conformational dependent interactions of a single Lac repressor protein (LacI) on DNA at physiological salt concentrations. The lac operon is a well-known example of gene expression regulation, based on the specific interaction of LacI with its cognate DNA sequence (operator). We observed LacI switching between different interaction modalities on DNA (weak, strong, sliding), depending on the molecule conformation and DNA sequence. We provide a method for measuring 1D-diffusion constants of DNA-binding proteins along DNA with a spatial resolution of about 30 base pairs, observing a broad distribution of 1D-diffusion constants of LacI and sequence-dependent diffusion constants. Our measurements provide a model of target-search and molecular switching mechanism of Lac repressor.

Biography

Marco Capitanio is Senior Researcher at the Department of Physics of the University of Florence, Italy, and Group Leader at the European Laboratory for Non-linear Spectroscopy (LENS). He then obtained his PhD in Physiology. He then joined LENS, a research institute which is part of a European network of laser and spectroscopy facilities. His research interests lie across Physics and Biology. On one hand, his research is focused on the physics of biological systems and on the development of techniques for the study of biology at the molecular scale, with a particular interest on optical methods. On the other hand, he is particularly interested in the molecular mechanisms underlying mechanical regulation of biological systems and the conversion of mechanical signals into changes in gene expression and cell fate.

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