

Kinetics of DNA hybridization observed on single molecule level using Graphene near-field effects

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In this study we were able to follow DNA unfolding events on the single and few molecule level. Fluorescently labeled target DNA deposited on a densely DNA hairpin functionalized Graphene substrate allows to follow the attachment and associated unfolding of DNA beacons using nearfield effects and fluorescence microscopy techniques [1]. To reach single DNA or few DNA molecule sensitivity, we use a widefield TIRF microscope setup equipped with a high-efficient EM-CCD camera (Andor iXon 897). We detect the fluorescence emission of single Atto488 molecules attached to a target DNA, which is added to a DNA hairpin functionalized Graphene substrate. We combine data analysis strategies originally developed for direct Stochastic Optical Reconstruction Microscopy (dSTORM) microscopy, ThunderSTORM, and extract the sub-pixel positions that match single emitter profiles, via intensity thresholding and spot size selection matching those of the Point spread function (PFS) of our microscope. Then, we extract time traces from image sequences.

In various single spot locations an increase of the intensity can be observed over time, which can be associated to an unfolding of the DNA, which is associated to a movement of the dye away from the quenching Graphene substrate. The unfolding process associated curves could be resolved for several individual DNA molecules and the unfolding times vary from 5 to 25s.

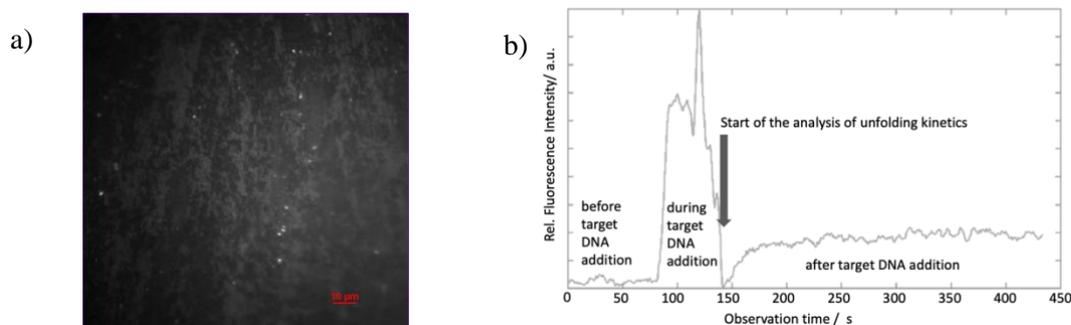


Figure: Widefield / TIRF recorded image and timetrace. a) Overview of an image area where intensity spots are associated to Atto488-labeled target DNA complementary to a DNA hairpin attached to a graphene patch. b) Representative fluorescence intensity time trace, extracted from a sequence of widefield TIRF images, which can be associated to a DNA hairpin unfolding event, after addition of target DNA.

The achieved sensitivity combined with a statistical analysis could be used to study DNA binding kinetics in dependence of relevant parameters, such as the effect of single point mutations in the complementary DNA, as well as the effect of temperature, which indicated the versatility of this technique.

Reference:

[1] RMR Adão, R Campos, E Figueiras, P Alpuim, JB Nieder, "Graphene setting the stage: Tracking DNA hybridization with nanoscale resolution," *2D Mater.* (2019), 6 (4) 45056.