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Abstract

Recent Progress in Molecular Recognition Imaging of Protein Systems at the Nanoscale Level †

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Abstract: Identification of proteins has received considerable attention in recent years due to the increasing interest to resolve individual biomolecules under physiological relevant conditions. In this framework, atomic force microscopy (AFM), has shown its great potential to acquire a variety of biomolecular physico-chemical properties at the single molecule level [1]. Particularly, force spectroscopy based on AFM (AFM-FS) allows to determine the intermolecular interactions between two biomolecules, requiring being one covalently immobilized on a flat surface and the other linked onto the AFM tip. Previous work was developed in this field by simultaneous topography and recognition imaging (TREC) [2] and tuning-fork-based transverse dynamic force microscopy (TDFM) [3] albeit both methods lack of providing quantitive information. To overcome the aforementioned limitations force-volume (F-V) [4] emerged as promising alternative but the extremely large data acquisition times can lead drifting effects during the image record. Here, we present the intermittent jumping force mode (JM) as suitable approach to gather quantitative high-resolution force maps at local areas of the scanned sample with fast-acquisition times. Using this mode applying very low forces under respulsive regime conditions simultaneous maps of topography and specific rupture forces corresponding to the unbinding of the protein:ligand complexes are obtained. Two different protein systems are employed to illustrate the capabilities of the built up methodological improvements. First, the flavoenzyme system formed between flavodoxin NADP$^+$ reductase (FNR) and its redox partners, ferredoxin and flavodoxin [5, 6] and second, the strongest non-covalent complexes observed in nature between avidin and streptavidin and biotin [7]. In the first case, the results were optimized when an oriented immobilization procedure was designed. In the second work, discrimination between avidin and streptavidin molecules in a hybrid sample was achieved with an unique sensor ligand. The most relevant scientific outcomes can serve as proof-of-principle stage to design diagnostic devices with ultra-sensitivity detection signal for drug screening applications.

Keywords: molecular recognition imaging; atomic force microscopy; functionalized tips; adhesion maps; protein-protein interactions; single molecule level; jumping mode

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