AFM STUDIES OF SINGLE MOLECULAR DETECTION AND MOLECULAR RECOGNITION

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Atomic force microscope (AFM) is one of the most widely used tools for imaging surface topologies of a wide variety of materials. More recently, it has been successfully used in so-called force curve mode to measure the intra- and inter-molecular forces to confirm the hidden mechanical properties of proteins and bio-structures at the single-molecule level.
The first target molecule in our study was Green Fluorescent Protein (GFP), a famous marker in biological field due to its natural fluorescence. It holds a unique structure: a rigid 'β-can' constructed of 11 β-sheets wrapped around the central helix. We used circular permutation method to link the natural termini and produce circularly permuted GFP (cpGFP) variants with new termini on surface loops. The mechanical property of two selected cpGFPs were examined by AFM and compared with that of the base one, while the optical properties of two mutants were detected by total internal reflection fluorescent microscopy (TIRFM). We also applied AFM technique to probe the specific molecular recognition, e.g. between proteins and lipid membranes.

i) Mechanical distinguish of the toughness on two cpGFPs (quasi-static measurement)
We have reported, for the first time in the world, that the mechanical toughness of two cpGFP molecules, p-54 and p-32, showed distinct characteristics in their force-extent (F-E) curves. The mechanical unfolding results revealed the different levels of the reduced mechanical stability of cpGFPs and these levels were related to the proximity of the newly introduced termini to β-can [1].

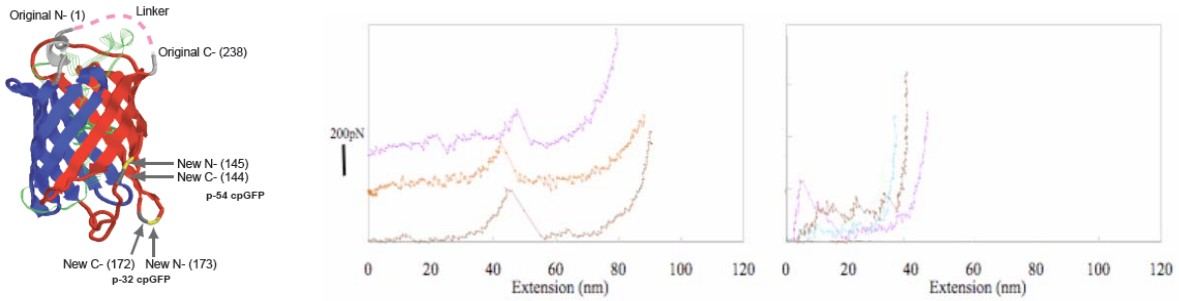

Figure 1. Perspective view of the designed cpGFPs with termini on different loops (left),
F-E curve of p-54cpGFP (middle) and of p-32 cpGFP (right).

ii) Stretch speed-dependence of intra-molecular mechanics
For the 'soft' p-54, the feature of F-E curves were found to be dependant on the stretching speeds. At fast stretching speeds over 100 nm/s, one peak was observed in F-E curves before the final rupture of the extended molecule, which we interpreted as the unfolding of two terminal halves within cpGFPs, while several more force peaks in a saw tooth pattern were detected at a much slower speeds down to 30 nm/s, which we explained the slow stretching speed induced cooperative unfolding of more hidden tiny sub-structures [2]. We also proposed a model to explain the peaks in F-E curves based on the fitting with worm-like chain model.

iii) In-phase response to the sinusoidal input (dynamic measurement)
We found the dynamic relax-stress response of p-54 to an input signal was exactly in-phase, which we conjectured that the elasticity dominates the whole polypeptide chain, so that the unfolding behavior observed on p-54 in our work was determined by the position of each sub-structure, rather than by its mechanical strength [3].

iv) Specific Recognition between Lysenin and Sphingomyelin (SM)
We also applied AFM technique to probe the specific molecular recognition between lysenin and sphingomyelin (SM) in steady state and in live cell. The measurements gave a new understanding of the assembly and functions of lipid rafts, from the mechanical point of view. Furthermore, this method can be extended to investigate several probe-lipid combinations and ligand- receptor interactions on cells. We have established a new method, based on AFM by introducing modified tips with lysenin molecules, for measuring and mapping the specific binding force between the lysenin and SM-rich domain in bilayer membrane or live cells.

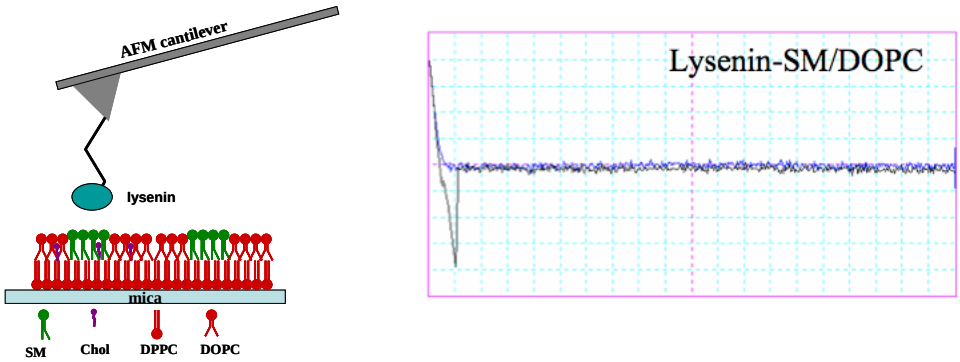

Figure 2. Schematic view of the designed AFM-sample system (left) and F-E curves of lysenin-SM/DOPC (right).

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