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Summary

Elasticity of Biomolecules: probing, pushing and pulling using atomic force microscopy

The elasticity of biomolecules from DNA, proteins, colloids to viruses were studied using the combined imaging and force spectroscopy capabilities of atomic force microscopy (AFM). The AFM is similar to a blind man's stick. It creates a topographical picture of the surface by mechanical *probing* using a flexible cantilever (as the stick) with a very sharp tip. Using high-precision piezo-electric scanners, it can image biomolecules with sub-nanometer resolution (1/1000 000 000 of a meter). As a contact microscope, it is also used to apply forces by *pushing* and *pulling* on the sample and measure forces by monitoring the amount of cantilever bending. Using very flexible cantilevers, the AFM can apply and detect forces with a few piconewton resolution (1/1000 000 000 000 of a newton).

As an imaging technique, I used the AFM to investigate how the compaction of DNA by a protein called TFAM affects the replication/transcription processes in the mitochondria, how radioactive and fluorescent labeling affects the size and uptake of nanocolloidal albumins in detecting tumors and how capsid proteins of Norovirus spontaneously disassemble and reassemble into virus-like particles.

From the conformation images of DNA molecules at different TFAM concentrations, it is found that the physiological concentration of TFAM is a narrow window in which both nearly-naked and compacted DNA molecules exist, suggesting that TFAM acts as a regulation factor in controlling the number of mitochondrial DNA molecules available for replication and transcription. The AFM images show that the labeling of nanocolloidal albumin with ^{99m}Tc, ^{89}Zr and IRDye 800CW dye does not affect the size and uptake of these particles by cancer cells. The confirmation of these factors opens up new techniques such as PET scan and fluorescence microscopy for tumor imaging and diagnostics. AFM imaging of Norovirus-like particles show that they reversibly disassemble and reassemble into 38-nm particles with T=3 icosa-

hedral symmetry at acidic conditions (2<pH<8), into 24-nm T=1 particles upon alkaline treatment (8<pH<10). These results suggest that the ability of the virus to reversibly disassemble/reassemble under a wide range of pH conditions is beneficial for it to withstand the fluctuating pH during its oral-to-fecal route of infection. This finding may be useful in vaccine development against viral gastroenteritis or stomach flu, and also to the development of new drug delivery systems.

As a force spectroscope, I investigated the elastic property and structural strength of Norovirus by *pushing* on them, and studied the folding-driven secretion mechanism of the E. coli autotransporter protein Hbp, albeit in reverse, through its unfolding pathways by *pulling* the surface-bound proteins with the AFM tip.

The nanoindentation results on the Norovirus-like particles showed that the protruding domain of its capsid protein introduces a prestress which makes the wild type viral capsid stiffer and brittle as compared to the mutant (with no protruding domain). This result indicates that the virus is in prestress condition which enhances stability during its infection cycle, and that prestress might have facilitated the genome release during the attachment of the virus at the host cell's surface.

The unfolding experiments on Hbp show that there are specific parts in the β-helical structure of the protein which are resistant to unfolding. These are the β-helices close to the C-terminal where most of the buried aromatic residues are stacked on top of each other. This indicates that these β-helices, create a mechanically strong and stable conformation in the protein that provides the anchor for folding which initiates the translocation of the protein. This finding supports the folding-driven secretion model of Hbp, where the attractive interaction between the stacked aromatic residues drives the sequential and processive folding of the protein at the surface of the outer membrane, letting the protein pull itself in the process. The results may shed light on this proton gradient- and ATP-independent translocation process, and may be useful in the development of treatments that target toxins produced by Gram-negative bacteria.