AFM: A POWERFUL TOOL FOR NANOBIOTECHNOLOGY

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In the past 15 years, atomic force microscopy (AFM) has emerged as a powerful tool for exploring biological systems at the nanoscale and has opened exciting perspectives in biomedicine and nanobiotechnology. While AFM imaging makes it possible to visualize surface structure under physiological conditions and at high resolution, force spectroscopy provides information on biomolecular interactions and physical properties [1,2]. In this contribution, I will discuss some of these capabilities using recent examples from my group, going from single biomolecules to lipid membranes and living cells.

The remarkable force sensitivity of AFM, which falls within the piconewton range, makes it possible to probe the interaction **forces between single biomolecules**. Using this approach, we recently measured the antigen binding forces of individual Fv fragments of anti-lysozyme antibodies (Fv) [3]. To detect single molecular recognition events, genetically engineered histidine-tagged Fv fragments were coupled onto AFM tips modified with mixed self-assembled monolayers of nitrilotriacetic acid- and tri(ethylene glycol)-terminated alkanethiols while lysozyme (Lyso) was covalently immobilized onto solid substrates. AFM force-distance curves recorded at a loading rate of 5,000 pN/s between Fv and Lyso modified surfaces yielded a distribution of unbinding forces composed of integer multiples of an elementary force quantum of about 50 pN that we attribute to the rupture of a single antibody-antigen pair. Force measurements performed at various loading rates revealed two distinct linear regimes with ascending slopes, suggesting multiple barriers were present in the energy landscape. The detection of individual molecular recognition events, as discussed here, is an important step towards developing new nanobioanalytical devices.

AFM imaging is very useful for **observing biomolecules and biomembranes** in real-time. For instance, this capability was used to visualize the interaction of the antibiotic azithromycin with lipid domains in model membranes [4; Fig. 1]. Various supported lipid bilayers were prepared by fusion of unilamellar vesicles on mica and imaged in buffer solution. Phase-separation was observed in the form of domains made of dipalmitoylphosphatidylcholine (DPPC), sphingomyelin (SM), or SM/cholesterol (SM/Chl) surrounded by a fluid matrix of dioleoylphosphatidylcholine (DOPC). Time-lapse images collected following addition of azithromycin revealed progressive erosion and disappearance of DPPC gel domains within 60 min, an effect attributed to the disruption of the molecular packing of the lipids by the drug. By contrast, SM and SM-Chl domains were not modified by azithromycin, presumably due to stronger intermolecular interactions between the SM molecules. This type of nanoscale investigations may lead to new applications in pharmacology.

Recently, AFM has also opened novel possibilities for **probing the surface of microbial cells** (fungal spores, yeasts and bacterial cells) in their native environment: observing the surface ultrastructure in real-time, mapping surface charge and hydrophobicity, probing cell wall elasticity and molecular elasticity and measuring receptor-ligand interactions [5]. After a brief overview, I will discuss a recent study [6] dealing with *Mycobacterium tuberculosis*, which adheres to epithelial cells via the heparin-binding haemagglutinin adhesin (HBHA). We first measured the adhesion forces between HBHA and its receptor heparin at the single molecule level. Both the adhesion frequency and adhesion force increased with contact time, indicating

that the HBHA-heparin complex is formed via multiple intermolecular bridges. We then mapped the distribution of single HBHA molecules on the surface of living mycobacteria and found that the adhesin is not randomly distributed over the mycobacterial surface, but concentrated into nanodomains. In the future, these studies may contribute to the development of new antimicrobial strategies, i.e. drugs capable to block the adhesin interaction.

References

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Figure 1. Real-time imaging of supported lipid bilayers interacting with drugs. AFM height images (7.5 µm x 7.5 µm; z-scale : 10 nm) of a mixed dioleoyl-phosphatidylcholine (DOPC)/dipalmitoyl-phosphatidylcholine (DPPC) bilayer recorded in solution at increasing incubation times with the antibiotic azithromycin.