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Biomolecular Interactions Sensed with Atomic Force Microscopy

Andreas Ebner*

Johannes Kepler University Linz, Department for Applied Experimental Biophysics, Gruberstrasse 4020 Linz, Austria

Biomolecules are able to specifically recognize and bind their corresponding partner with high efficiency. This process plays a pivotal role in biology, physiology and medicine (e.g., in the immune system, cellular adhesion or inter- and intracellular signaling). From the chemical point of view bio-recognition can be described as a combination of non-covalent weak interactions including electrostatic (ionic), hydrophobic, and van der Waals interactions as well as hydrogen bonding. Furthermore, steric aspects, especially the complementary structure of the two binding partners, are highly relevant for complex formation and stability. Taken together all these aspects determine both, the strength and the characteristic lifetime of the bond. In addition to bulk techniques like nuclear magnetic resonance, surface plasmon resonance, quartz crystal microbalance, and other analytical and biochemical methods single molecule approaches have been established. They allow following transient phenomena like short-lived receptor-ligand intermediate states, rare events, or population heterogeneity, which are otherwise not accessible. For the determination of single receptor ligand interactions, apart from bio-membrane force probe, and optical and magnetic tweezers, atomic force microscopy (AFM) based molecular recognition forces spectroscopy (MRFS) offers the most versatile approach to explore forces during the bio-recognition processes at the molecular level [1].

In MRFS the tip of an AFM cantilever is upgraded to a molecular biosensor [2]. In case of commonly used silicon (or silicon nitride) tip material usually a multistep procedure is performed. This can be done by physisorption or more commonly by covalent coupling protocols: (i) Amino functionalization [3] generates reactive sites on the AFM tip to which (ii) hetero bifunctional distensible cross-linker (e.g., polyethyleneglycols) [2] are bound via their activated carboxylic acid groups. The other end of the linker is used (iii) to couple the ligand of interest. Alternatively, gold coated tips can be functionalized by using thiolated linkers (e.g., SAM components [4] or DNA building blocks like DNA tetrahedral [5]).

The biosensing tip is then repeatedly approached to and withdrawn from a solid support presenting the receptor of interest. During the contact time a receptor-ligand complex can be formed, which is ruptured in the withdrawal phase. The force needed to separate the binding partners is calculated from the cantilever bending in the moment of rupture simply applying Hooke's law for extending a spring. By increasing the pulling velocity the originally thermally driven unbinding process is shifted to a forced dissociation. According to Evans theory [6] the rupture force at a given force loading rate, which can be approximated by multiplication of pulling velocity and effective spring constant of the AFM tip, is related to the rate of dissociation and the width of the energy barrier of this complex. Thus, MRFS allows exploring the energy landscape of a receptor – ligand interaction at the molecular level.

Starting with the pioneering work in the nineties [7-10] force spectroscopy has developed into a key technique to investigate molecular forces on isolated molecules[11], artificial and natural membranes [12-14] as well as on living and fixed cells [15-17]. In a simple recent study [14] MRFS has been used to determine the energy landscape between an artificial membrane embedded anchor proteins for cytochrome c (cyt c) with cyt c as well as with an primary amine ended linker. Cyt c is a water soluble protein playing an important role in physiology as regulator in the electron transport. A special kind of calixarene, the carboxylic acid derivate of calix [6] arenes (namely the oct[6]CH_2COOH calixarene), is expected to mimic the anchoring site for cyt c [18]. To investigate the molecular interaction forces, calix[6] arenes were incorporated into a 1,2-sn-glycerodimyristoylfosfatidylch oline (DMPC) bilayer. Cyt c was coupled covalently to the AFM tip via a PEG linker, and force distance cycles were performed. These MRFS experiments demonstrated specific binding of cyt c to calix[6]arene and yielded the binding parameters k_{off} (1.14 ± 0.59 s-1) and x_β (3.98 ± 0.63Å). MRFS experiments with an amino-ended linker also resulted in highly specific interactions with comparable values for k_{off} (2.74 ± 0.66 s-1) and x_β (5.91 ± 2.55 Å) suggesting that both electrostatic and amino group specific interactions between cyt c and CX cavity exist.

Summed up, molecular recognition force spectroscopy offers a promising tool to investigate molecular interaction between single molecules and natural and artificial particles like viruses or nanotubes with their corresponding receptor immobilized on flat support, but also on biomembranes and cells. It is expected that the high potential of this technique together with technical improvements further increases the number of applications.

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***Corresponding author:** Andreas Ebner, Johannes Kepler University Linz, Department for Applied Experimental Biophysics, Gruberstrasse 4020 Linz, Austria, Tel: +43 732 2468 7637; Fax: +43 732 2468 7633; E-mail: andreas.ebner@jku.at

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