General information on the functionalization of atomic force microscopy (AFM) tips via long, flexible polyethylene glycol (PEG) chains

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Attachment of a sensor molecule (e.g., an antibody) to the surface of an AFM tip converts it into a specific biosensor which can be used to localize complementary target molecules (e.g., antigens) on the sample surface. Insertion of a long, flexible polyethylene glycol (PEG) chain between the tip surface and the sensor molecule proved highly advantageous, for several reasons: (i) the sensor molecule can reorient rapidly and "palpitate" the sample surface, thus specific binding to cognate target molecules is greatly facilitated; (ii) in force spectroscopy experiments, specific binding is much easier to discriminate from non-specific tip-surface adhesion when using a 6-9 nm long PEG linker between tip and sensor molecule;[1,2,4] (iii) rapid simultaneous scanning of Topography and RECognition sites (TREC) is intrinsically dependent on the use of 6-9 nm long PEG linkers.[3]

The most widely used procedure for AFM tip functionalization via flexible PEG linkers is shown in Figure 2. It employs three steps: (1) Generation of amino group (NH_2) on the tip surface, (2) reaction of these amino groups with one end of the PEG linker only, and (3) attachment of the sensor molecule to the free-tangling end of the PEG chain.

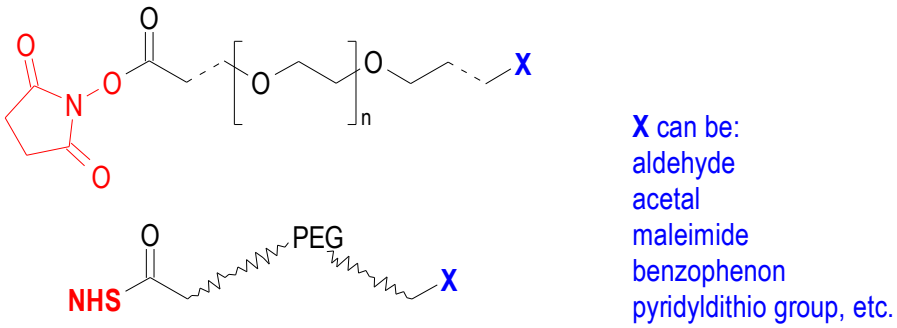

Figure 1: Typical structure of polyethylene glycol (PEG) linkers used for AFM tip functionalization. The linker contains a long PEG chain (n = 18 – 27) and one so-called NHS ester function ("NHS" stands for *N*-hydroxysuccinimide) which reacts with an amino group (NH_2) on the AFM tip to give a stable amide bond (see Figure 2). The other end of the PEG chain carries a different functional group (**X**) which serves to couple a sensor molecule to the AFM tip (see Figure 2). The dashed lines indicate minor variations in the linkages between the PEG chain and the terminal groups.

For step 2 in Figure 2 it is essential that the reactive group **X** on the second end of the PEG linker does not react with the amino groups on the tip surface. This rule is only partially fulfilled with the linker "Aldehyde-PEG-NHS" (see manual *04_AFM_tip_with_aldehyde*).[6] The latter problem was solved by replacing the aldehyde function with an acetal function which can be converted into an aldehyde function after PEG linker attachment to the tip surface (see Figure 3 and the manual *05_AFM_tip_with_acetal*).[8]

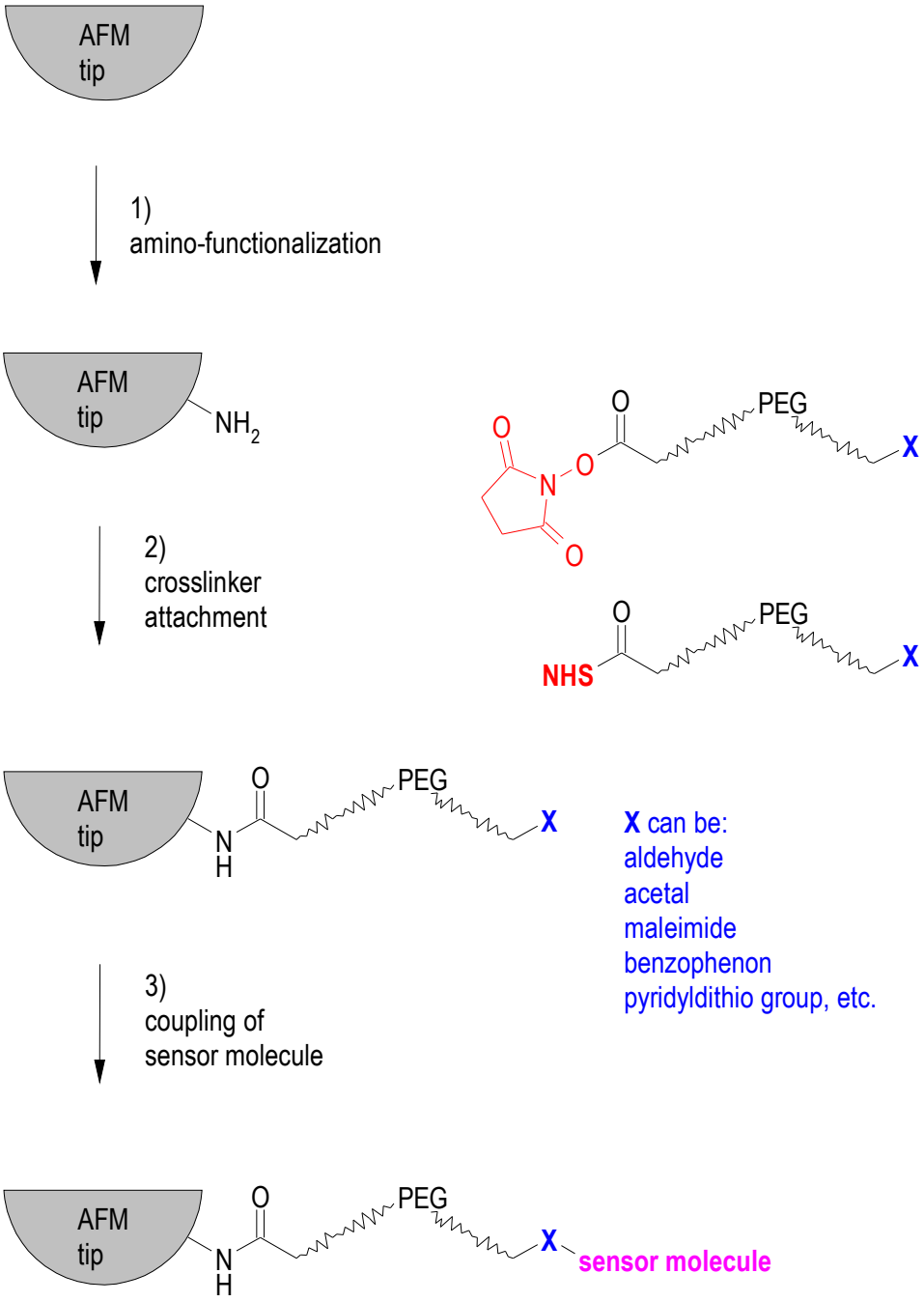

Figure 2: Three-step procedure of AFM tip functionalization with long, flexible PEG linkers: (1) amino-functionalization of the oxidized silicon nitride tip, (2) attachment of one end of the PEG linker by amide bond formation, (3) attachment of the sensor molecule to the free end of the PEG linker.

Figure 3 shows an optimized procedure which is the new "work horse" for coupling of proteins to AFM tips. It is applicable to all commercially available AFM tips (made from silicon or silicon nitride), also to the new generation of MAC levers. The citric acid treatment was not applicable to the old-style MAC levers[8] but this statement in the original publication

is outdated. All commercially available silicon nitride (and silicon) cantilevers can now be functionalized by the acetal procedure in Figure 3.

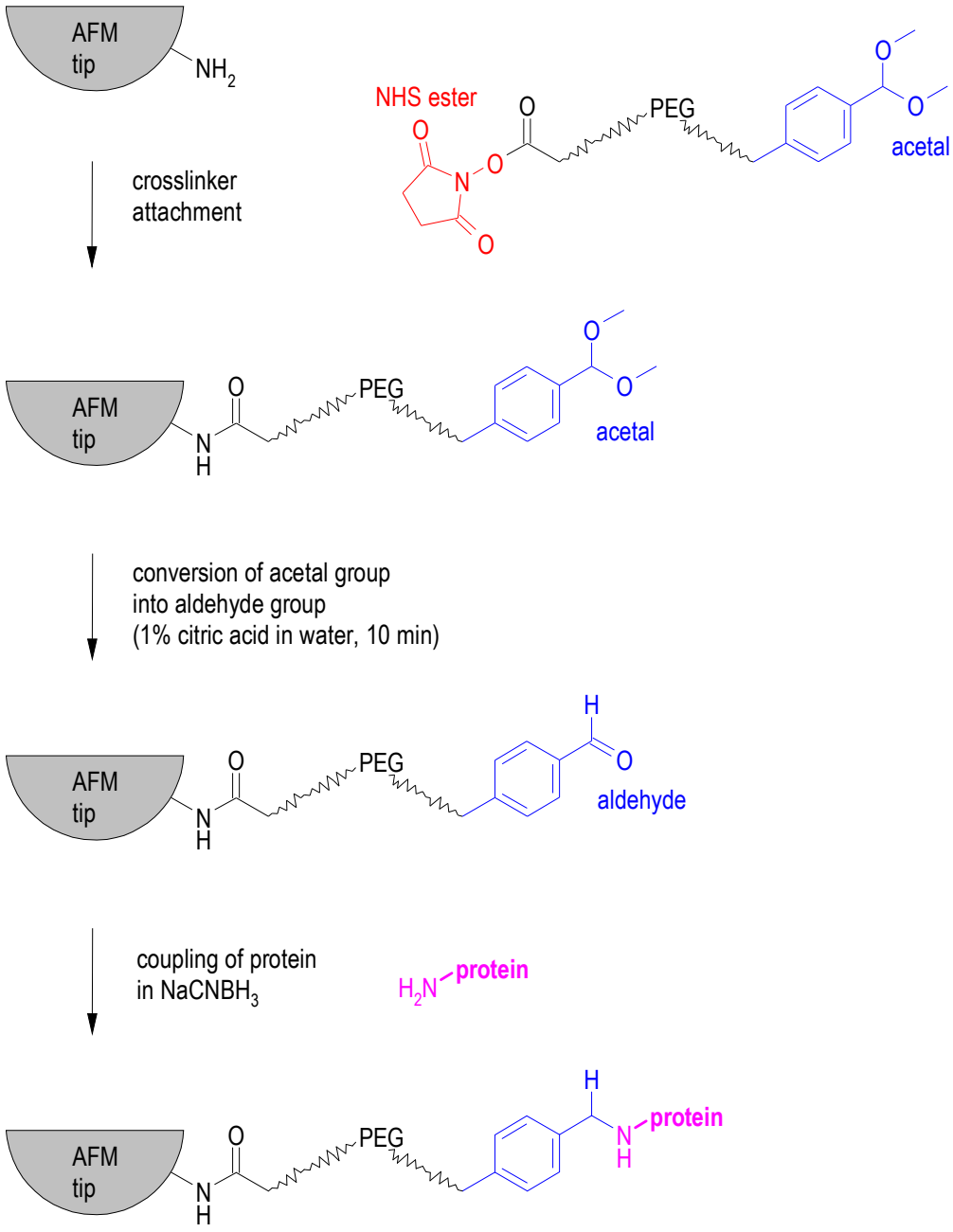

Figure 3: New standard method for protein coupling to AFM tips. The crosslinker molecule has one NHS ester function which is highly reactive towards the amino group (NH_2) on the tip surface, while the **acetal** group on the other end is unreactive. Subsequently, the acetal is converted into an **aldehyde** which couples NH_2 groups, such as the lysine residues of proteins.

Aldehyde functions are particularly useful for coupling of proteins because aldehydes react with amino groups (-NH_2) and most proteins have a high number of lysine residues (NH_2) on their surface (e.g., 80-90 per antibody [Dorner et al. (1967) J. Exp. Med. 125, 823-831]). The disadvantage of aldehyde coupling is that one of the many amino groups is randomly chosen for coupling. In contrast, site-specific coupling is achieved by *06_AFM_tip_with_maleimide* or *07_AFM_tip_with_tris_NTA*.

For the training of beginners (and for testing of new AFM modes, as in reference 3), we have developed a simple test system in which the sensor molecule (**biotin**) is already part of the PEG linker (see Figure 4 and the manual *03_AFM_tip_with_biotin*). The complementary sample surface is prepared by incubating mica in 15 mM NaCl with avidin (0.1 mg/mL, 15 min) which results in a dense monolayer of avidin, as needed in force spectroscopy experiments.[1] Shorter times and lower concentrations of avidin lead to low density, as needed for TREC experiments[3] (see manual *03_AFM_tip_with_biotin*). Force measurements can be performed in 150 mM NaCl, only the adsorption process of avidin must be performed at low NaCl concentration.

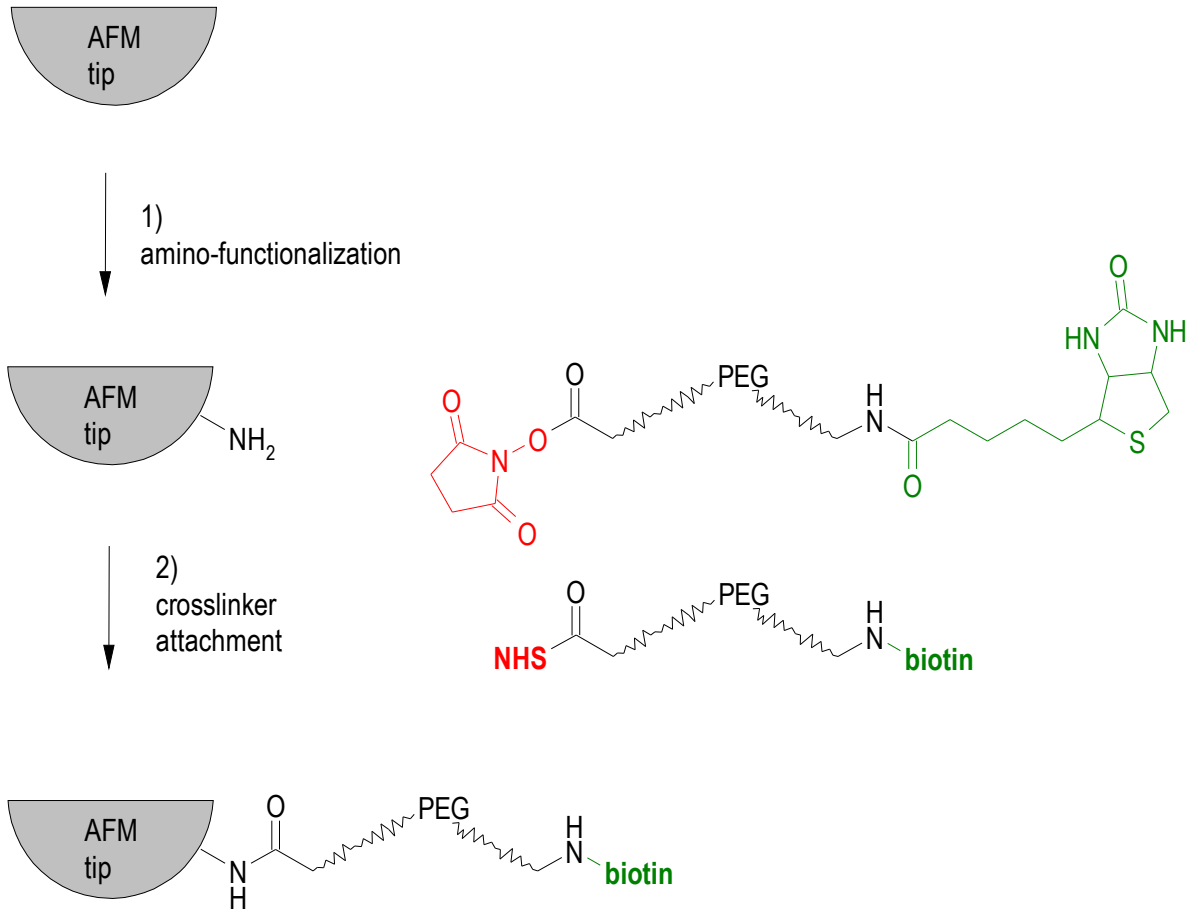

Figure 4: Reaction of amino-functionalized AFM tips with Biotin-PEG-NHS results in a tip which has a high affinity for avidin or streptavidin.

Literature addressing AFM tip functionalization:

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