Supporting Information

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SI Text

Biological Activity. The biological activity of deposited biotin-BSA was investigated as depicted in Fig. S1 and described in the article. After biotin-BSA patterning, the substrates were immersed in solutions of streptavidin, followed by biotin-IgG. A regular increase in the lateral dimension of the patterned dots was observed (see Fig. 4), which is consistent with a monolayer of streptavidin and biotin-IgG surrounding the features and laying flat on the substrate (Fig. S1*D*). We hypothesize that the IgG lays flat upon drying of the substrate, which is likely a favorable conformation because of the electrostatic attraction between the positively charged IgG and negatively charged MHA. This hypothesis is supported by an observed height increase in the biotin-BSA/streptavidin/biotin-IgG features of ≈10 nm when imaged while wet (submerged in NanoPure water) with tapping-mode AFM (Fig. S2) compared with the dry features. A height increase of this size suggests that the biotin is attached to the IgG at its 4-nm dimension. This would result in ≈10-nm height increase when the streptavidin/biotin-IgG complex is in its native state and a much larger diameter increase (between ≈18 and 40 nm) after drying of the substrates. Thus, after drying, we believe the proteins undergo a reconformation from an upright configuration to a laying-sideways configuration. Note, substrates imaged while hydrated were covered with a droplet of NanoPure water and imaged in tapping mode by using a Veeco Dimension 3100 AFM with a passive liquid cell.

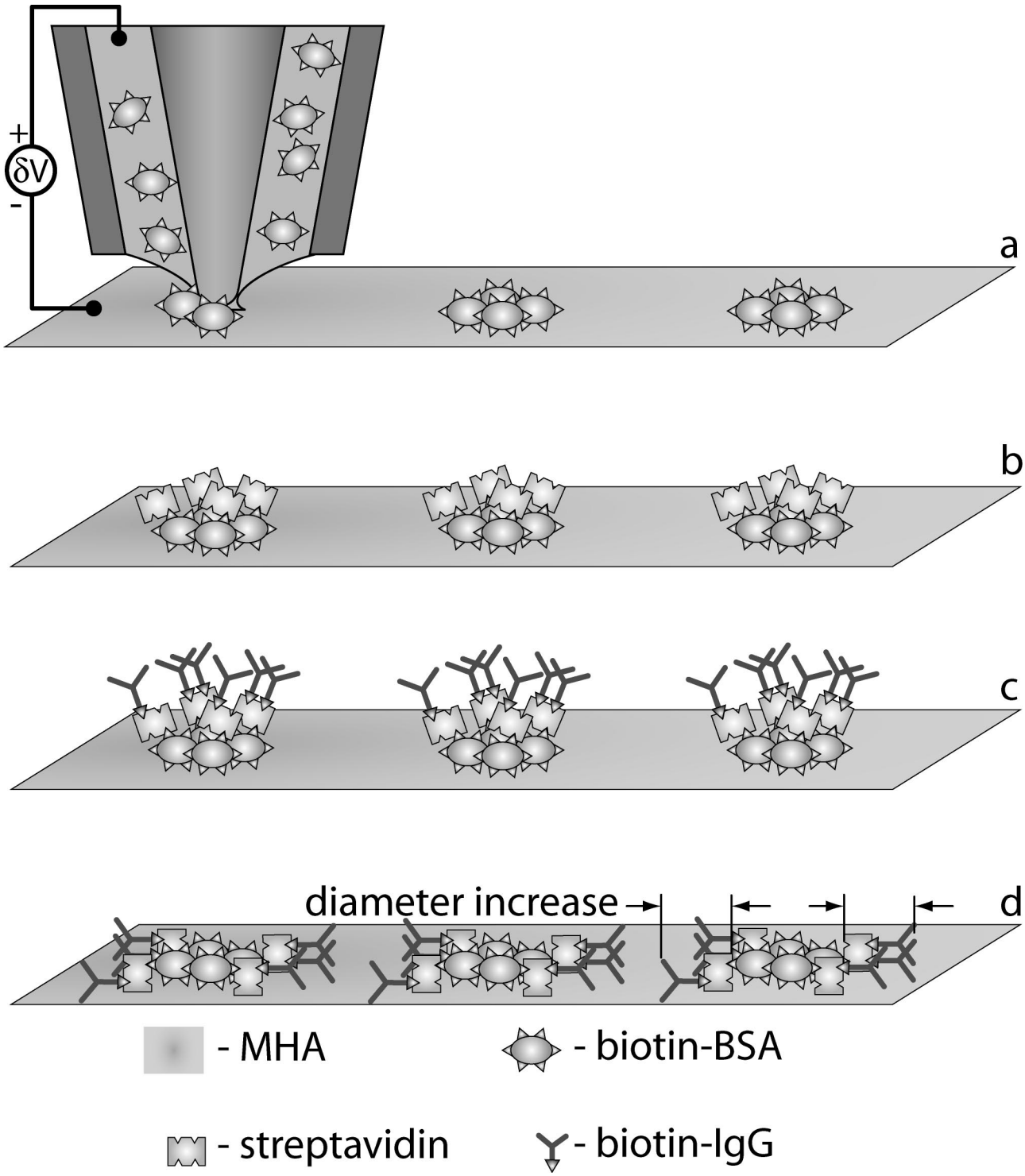

Fig. S1. Schematic representation of the test for biological activity of patterned biotin-BSA proteins. (*a*) Biotin-BSA was deposited on an MHA substrate then rinsed. (*b–d*) The substrate was then immersed in streptavidin solution (*b*), rinsed, immersed in biotin-IgG solution (*c*), and rinsed again. (*d*) Upon drying, it is believed that the biotin-IgG lays flat on the substrate because of its electrostatic attraction to the MHA, resulting in a perceived increase in diameter of the patterned features.

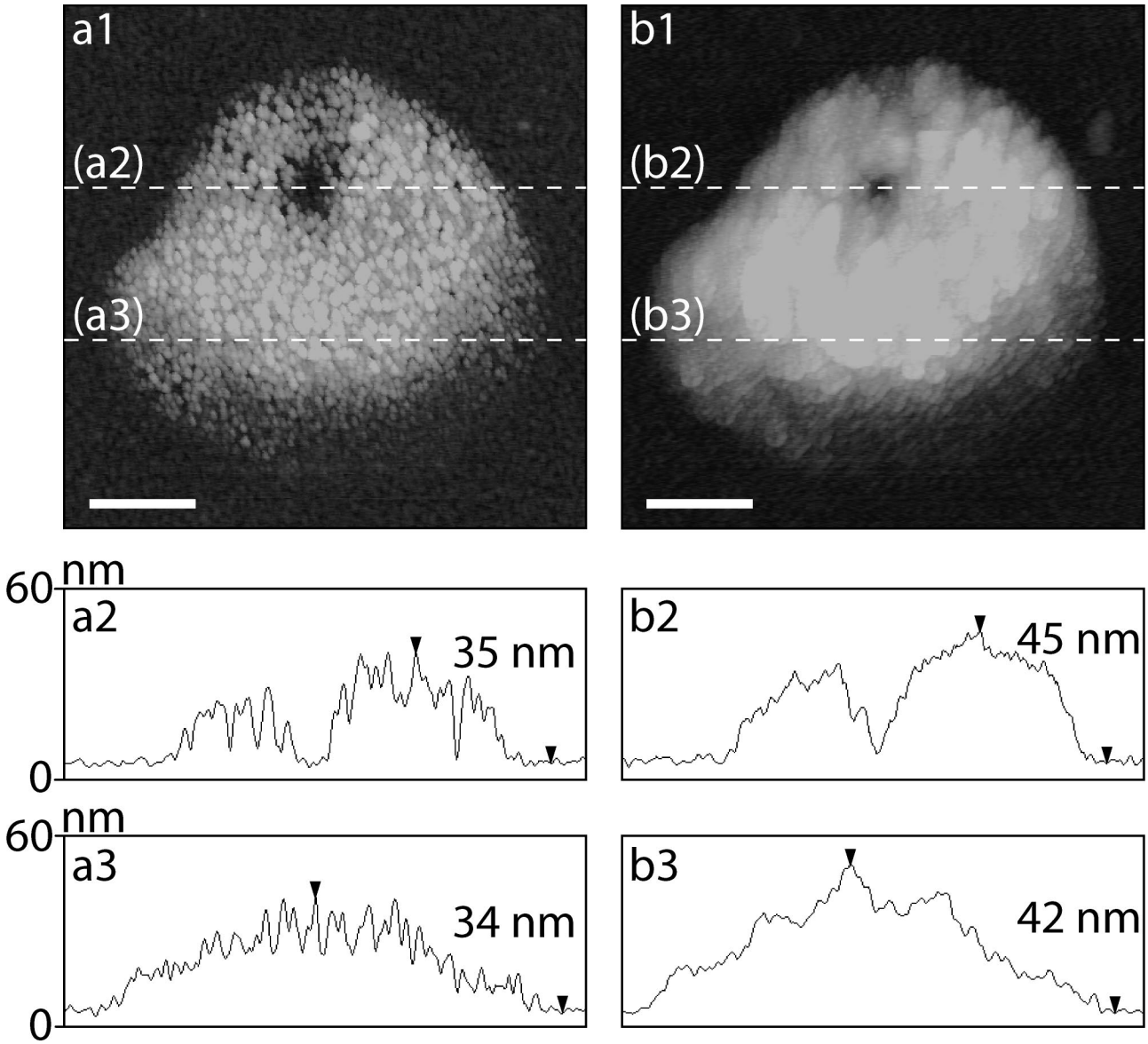

Fig. S2. Tapping-mode AFM images and height profiles of the same dot feature of biotin-BSA after immersion in streptavidin then biotin-IgG (*a*) in air (dry), and submerged in NanoPure water (*b*). (Scale bars: 500 nm). Height scale is 50 nm. There is a height increase of 8–10 nm when wet.