Molecular Occupancy of Nanodot Arrays

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Nanoscale control over the organization of nanomaterials on surfaces enables advances in many areas from nanoelectronics to biological and medical research. An increasingly effective approach is to immobilize the nanomaterial of choice on patterned arrays of metallic nanodots through site-selective chemistry. In particular, biomolecular nanoarrays, using DNA and proteins, have potential applications ranging from biodiagnostics to genomics, proteomics, and cellular biology studies. Many cellular processes are highly sensitive to the geometric arrangement of membrane receptors, which can be controlled by proper design of nanoarrays of their respective binding ligands. For example, nanoarrays of the extracellular matrix (ECM) binding ligands RGD have been used to determine the minimum geometric requirement of integrin mediated binding in adhesion and spreading.[1] Similar nanoarrays of T-cell receptor binding sites were recently used to probe immune response.[2, 3,4] In these experiments, control of the molecular occupancy of each nanodot is crucial because multiple molecules can affect the cellular response.

The molecular occupancy of nanoparticles has recently been measured by counting the labeling NPs with Transmission Electron Microscope (TEM).[5] This is a straightforward method, but it is inefficient and incompatible with surface-based nanoarrays, especially in the context of biological experiments. We have developed an on-chip measurement approach based on fluorescence bleaching analysis. The relationship between the molecular occupancy and factors such as nanodot size and reagent concentration are studied, so that this platform based on metallic nanodots could be optimized to ensure true single-molecule control.

AuPd nanodot arrays (Fig. 1a) were fabricated on glass coverslips using electron beam lithography and self-aligned pattern transfer.[1, 4] An annealing step causes the nanodots to agglomerate into spheres whose size is coded by the lithography (Fig. 1b). A mixture of alkylthiol with biotin/OH end groups was assembled on the nanodots, such that the biotin density could be adjusted to control the streptavidin occupancy. Fig. 1c is a fluorescence image of an array of streptavidin clusters with different size nanodots, indicating that the molecular occupancy increases with nanodot size. The labeling fluorophore on the streptavidin (Alexa555) was bleached after constant exposure. The intensity of a nanodot/cluster decreases with time, as shown in the bleaching curves in Fig. 2a. Discrete steps can be seen in these curves, which represent the bleaching of a single fluorophore. The molecular occupancy can be estimated by either counting the steps in a bleaching curve or simply dividing the initial intensity by the average step size. In Fig. 2b, a histogram and its Gaussian fit of the step sizes reveal that some of them are double steps, which must be taken into consideration to determine the average value. The molecular occupancy is given by Eq. (1):

$$N = \frac{I}{s \cdot r \cdot n} \tag{1}$$

where N is the occupancy, i.e. the number of molecules per nanodot; I is the initial intensity of the nanodot cluster; s is the step size of the bleaching curve; r is the F/P ration of the labeled molecule, i.e. the number of fluorophores per molecule; and n is the number of nanodots per cluster.

By bleaching analysis, streptavidin occupancy for different nanodot size at a given biotin/OH ratio is obtained (Fig. 3a). It is also demonstrated that, for the same nanodot size (5nm here), the molecule occupancy can be adjusted by the mixture ratio (Fig. 3b). This introduces great flexibility, because a single molecule occupancy is no longer limited to a nanodot as small as 4nm.[5] For example, a nanodot size of ~8nm at a biotin/OH ratio of 1:1 will also yield single molecule occupancy.

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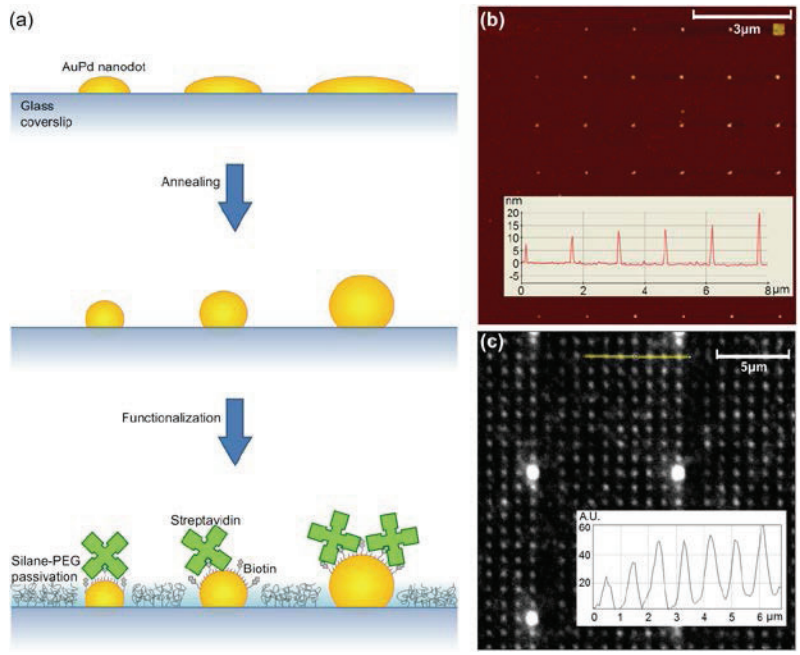

Figure 1. (a) Fabrication and functionalization of the AuPd nanodot array, (b) AFM image of a AuPd nanoarray with various dot size, (c) fluorescence image of a streptavidin nanoarray with different molecule occupancy.

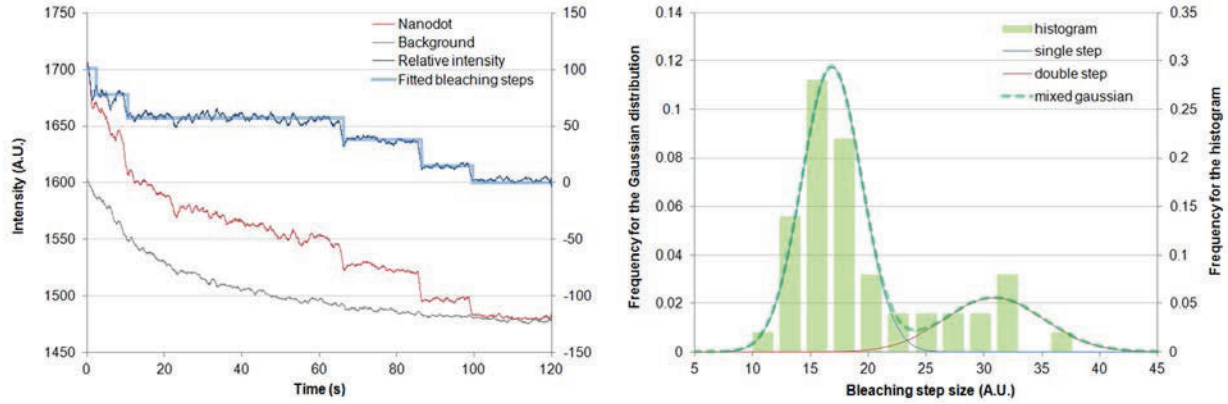

Figure 2. (a) Bleaching curves of streptavidin-Alexa555 on a single nanodot, (b) histogram of the bleaching step size.

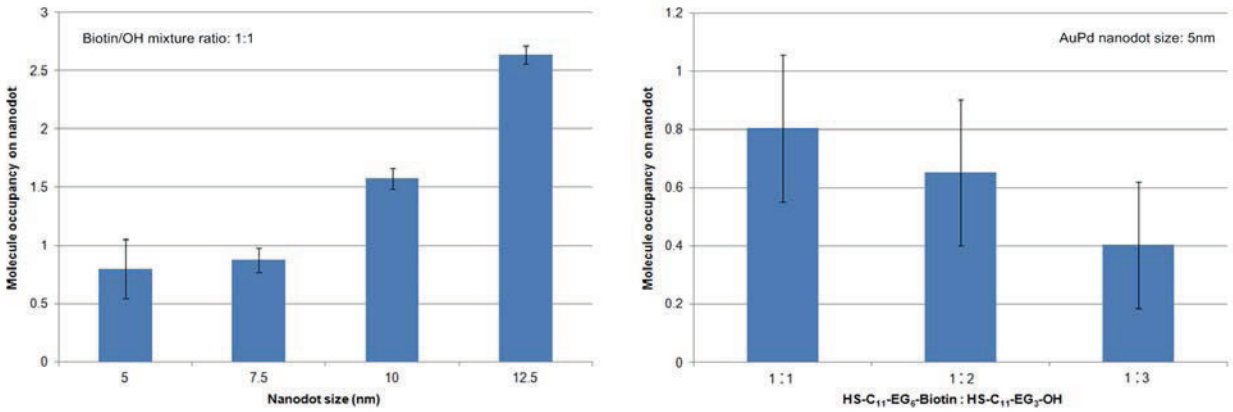

Figure 3. Streptavidin molecule occupancy vs. (a) AuPd nanodot size, (b) biotin/OH mixture ratio.