Lithographically patterned nanostructures for geometric control of coiled-coil protein placement and alignment

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Nanoscale localization of individual proteins, or clusters thereof on nanopatterned surfaces is important for a number of applications such as high sensitivity medical diagnostics and regenerative medicine. While block copolymer micelle nanolithography and scanning probe methods have been used for this purpose,[1-4] they are limited to achieving only spherically shaped nanoparticles for randomly oriented protein binding. In this work, we used electron beam lithography and block copolymer (BCP) lithography to create arrays of designed nanoscale feature geometries and pattern topologies down to the 10-nm scale. Patterns are transferred to gold by either e-beam evaporation and lift-off, or by a templated dewetting process. In templated thin film dewetting, the formation of high density self-ordered arrays of nanoparticles is guided by the BCP pattern. Surface treatment by attaching a monolayer of methoxy polyethylene glycol (PEG) silane to the native silicon dioxide on silicon substrates prevented non-specific binding of proteins on the nonpatterned areas of the substrate. Purified coiled-coil proteins, cortexillin and tropomyosin were then selectively immobilized on the surface. Atomic force microscopy (AFM) was used to assess the protein structure and location, and scanning electron microscopy (SEM) was also used to characterize the surface, after tagging with Au nanoprobes. Combining the versatility of e-beam lithography and BCP lithography and the specificity of bottom-up protein binding makes this approach attractive for scalable production of nanobiointerfaces for applications leveraging the capability of high resolution control on individual biomolecule location and direction in a designed pattern.

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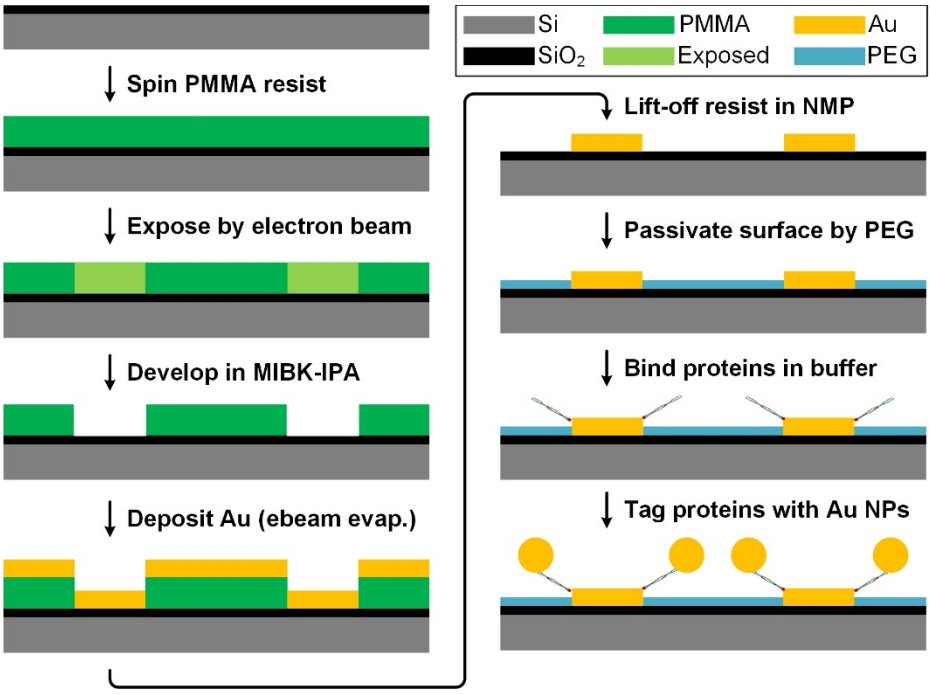

Figure 1: Process schematic showing the fabrication of gold nanostructures by electron beam lithography (EBL) in poly(methyl methacrylate) (PMMA) resist followed by developing in in a mixture of methyl isobutyl ketone (MIBK) and isopropyl alcohol (IPA) (3:1 IPA:MIBK by volume). After gold deposition and lift-off using N-Methyl-2-pyrrolidone (NMP), nonspecific binding of proteins is prevented by attaching methoxy polyethylene glycol (PEG) silane to the surface. Samples are then incubated with proteins in buffer solution (Tris-HCl), followed by incubation in a solution containing gold nanoprobes for protein tagging.

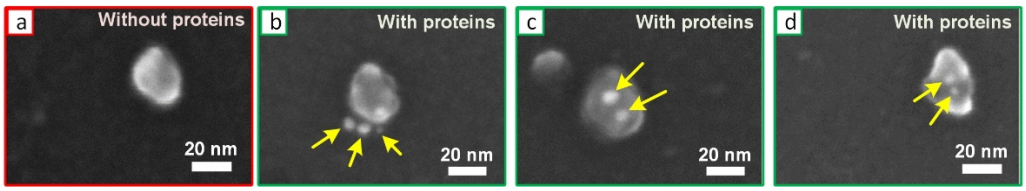

Figure 2: Scanning electron microscopy (SEM) images showing initial results for protein localization on 20 nm gold posts created by electron beam lithography. (a) gold nanopost without proteins. (b-d) gold nanoposts with proteins, showing the nanoparticle tags.