Targeted Immobilization of Nanostructures and Biomolecules through Peptide-Based Biolinkers

Marketa Hnilova[1], Christopher So[1], Ersin Emre Oren[1], Turgay Kacar[1,2], Candan Tamerler[1,2] and Mehmet Sarikaya[1]

[1] GEMSEC, Department of Materials Science and Engineering, University of Washington, Seattle, WA 98195, USA,
[2] MOBGAM, Department of Molecular Biology and Genetics, Istanbul Technical University, Istanbul, 34469 Turkey.

Statement of Purpose: Hybrid nanostructures composed of various inorganic materials coupled with functional biomolecules, such as antibodies, enzymes, or DNA, are of considerable interest in the development of new research strategies for potential practical applications in biotechnological and biomedical fields, including bio-sensors, delivery and targeting platforms.[1,2] Current approaches for coupling inorganic surfaces with biomolecules predominantly require multiple surface functionalization steps *via* self assembled monolayers (SAMs), e.g., thiols or silanes on either metal or metal oxide substrates, respectively. Although frequently used in traditional approaches, SAM systems have certain limitations associated with the complex chemical processes that may potentially result in low coupling efficiencies and limited stability of biomolecules.[1] The other main disadvantage of SAM systems is their nonspecific linker/substrate interactions, e.g., thiol molecules are known to bind to a variety of noble metals with similar affinities without any metal-specific characteristics and, similarly, silane molecules bind to several different oxides indiscriminately.[3] However, particular nanobiotechnological applications require selective and efficient adsorption of receptor molecules onto the desired inorganic solid material substrates preferably via biology-friendly linkers that are both versatile under biological conditions and amenable to genetic manipulation. A novel alternative to current chemical coupling may be the combinatorial selected inorganic-binding peptides.[1] In addition to specific recognition of inorganic surfaces e.g., metal versus oxide surface, these short amino acid sequences are robust building blocks that can be genetically engineered or chemically modified to tailor their functionalities such as binding, linking and assembling.

Methods: We selected gold-binding peptides from FliTrx bacterial surface library displaying randomized dodecapeptides inserted into FLITRX chimera bacterial surface flagellin protein in a standard biopanning experiment. For the initial binding characterization of isolated clones we used fluorescent microscopy technique, previously adapted for the inorganic-binding characterization and described in our earlier studies.[1] Combinatorially selected gold-binding sequences were synthesized using solid phase Fmoc peptide synthesis. Following peptide syntheses, we then modified the peptide sequences at the N-terminus with biotin using a Sulfo-NHS-LC-biotin reagent (Pierce, USA) to create bifunctional peptide-biotin molecules can direct streptavidin-modified nanostructures, e.g. streptavidin-functionalized CdSe-ZnS QDots nanostructures (Molecular Probes, USA), onto various surfaces through combination of peptide material-selectivity characteristics and bio-SA interactions.

Results: The targeted assembly of various organic and inorganic entities on multimaterial surfaces *via* specific peptide linkers was verified using fluorescence microscopy, atomic force microscopy and surface plasmon resonance spectroscopy. As evidenced by our results, the selected gold-binding peptide exhibited both the desired multi-functionality and stability and thus enabled the direct immobilization of functional molecules and nanoparticles onto solid surfaces. In summary as demonstrated here the solid-binding peptides, offer unique features that are potentially significant in practical applications: i. The peptides are robust and can be genetically-engineered or chemically-modified for tailored multifunctionality; ii. Engineered peptides can selectively bind to inorganic targets (metals, oxides, or semiconductors); iii. The nanomaterial assembly processes can be carried out under ambient conditions, i.e., room temperature and neutral pH in aqueous solutions, providing biology-friendly environment, desirable for most bionanotechnology applications.

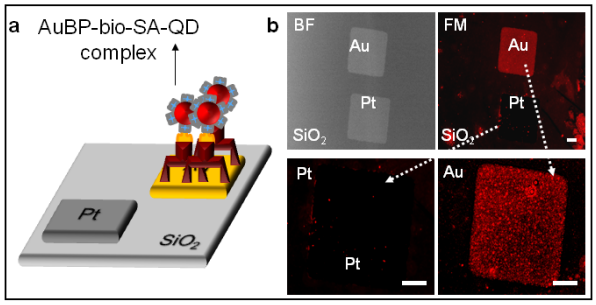

Figure 1. Targeted immobilization of SA-QDots *via* material specific peptide-based linkers: (a) schematic of specific immobilization of peptide-QDots complexes on multimaterial surfaces, (b) Bright field and fluorescence images of QDots immobilized on Au surface via peptide-based linkers. The size bars correspond to 20μm.

Conclusions: The described simple assembly process using material-specific peptides could be of unique utility in the targeted assembly of multiple nano- and micro-entities such as nanoparticles, quantum dots, functional synthetic or biological molecules (e.g., enzymes), and organisms (viruses or cells) onto spatially distributed specific locations on complex multimaterial substrates for a wide-range of applications such as surface functionalization of implant materials, tissue restoration, proteomics, single cell studies, and biosensors. *The project supported by an NSF-MRSEC at the UW.*

References:

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