Targeted Immobilization of Nanostructures and Biomolecules through Peptide-Based Biolinkers

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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 biosensors, 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.3 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.4 However, particular nanobiotechnological applications require selective and efficient adsorption of receptor molecules onto the desired inorganic solid material substrates preferably via bio-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.4 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, 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.

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 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.

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: