Identifying Novel Peptides for Binding to Semiconductor Substrates to Create Nanobiomaterials

Athra Kaviani
Biomedical Engineering, University of Texas at Austin

NNIN REU Site: Nanotech @ UCSB, University of California Santa Barbara
NNIN REU Principal Investigator: Professor Patrick S. Daugherty, Chemical Engineering, University of California Santa Barbara
NNIN REU Mentor: Jeffery J. Rice, Chemical Engineering, University of California Santa Barbara
Contact: a.kaviani@mail.utexas.edu, psd@engineering.ucsb.edu

Abstract:

Nanobiomaterials can potentially be used in a wide range of applications including, but not limited to, the development of biochips, biolabels, drug delivery systems, and bioelectronics. With the purpose of creating nanobiomaterials, we have identified peptide sequences that bind to gold substrates using bacterial surface display.

A constrained peptide library having eleven random positions ($X_2CX_7CX_2$) was presented on the surface of *E. coli* as an insertional fusion within the extracellular loop two of outer membrane protein OmpX. Stringent bacterial selections were performed using both gold particles and gold surfaces, causing bacteria with the ability to bind gold to remain immobilized while non-binding cells are washed away. After four rounds of selection, individual clones were isolated and co-transformed with a plasmid that encodes for a green fluorescent protein. Using the fluorescent cells with displayed peptides and micron sized gold spheres suspended in PBS, it was possible to use flow cytometry to quantify cells bound to gold particles.

Clones were isolated with the ability to bind over half of the gold particles present in solution. The clone exhibiting the strongest binding to gold surfaces displayed the peptide sequence, LVCYWSYSRMCKN. This method can be extrapolated to other material surfaces, increasing our knowledge of protein-material interactions and advancing the field of protein engineering.

Introduction:

Proteins can have specific interactions with inorganic substrates [1]. These recognition properties of proteins can be harnessed to genetically engineer proteins specific for inorganics. Various display techniques have been used to screen peptide libraries for interactions with material surfaces [2]. Cell-surface display is one such biotechnology in which target proteins are stably expressed on the cell surface using transmembrane proteins allowing for peptide libraries to be screened.

Experimental Procedure:

Glass surfaces deposited with gold were used for surface selections and spherical gold powder of less than 10 μm (SigmaAldrich #32658-5) for particle selections. Gold surfaces were regenerated as follows: surfaces were submersed in piranha solution (1:3 hydrogen peroxide, sulfuric acid) to remove organic residues, rinsed with acetone, isopropyl alcohol and Milli-Q water to remove other deposits, and held under a vacuum at 200°C to remove surface oxides.

A 7-mer peptide library ($X_2CX_7CX_2$) was presented on the surface of *E. coli* as an insertional fusion within loop two of OmpX. OmpX was chosen for its long loops, fast expression, and robust display ability [3]. Stringent bacterial selections were performed using both gold particles and gold surfaces. Bacteria libraries were cultured overnight in LB chloramphenicol (Cm) and later sub-cultured. Cell cultures were induced at 0.4 OD_{600} for 45 minutes using arabinose, final concentration 0.02%. Cells were concentrated 40-fold using centrifugation at 5000 rpm and incubated with gold coated surfaces or gold particle suspensions (approximately 5 x 10^7 particles per mL). Samples added to gold particle suspensions were depleted of large cell aggregates using centrifugation at 1000 rpm for one minute. Samples were shaken for 1 hour to allow protein-gold interactions. Samples were then centrifuged at 1000 rpm for 30 seconds to remove gold particles from solution and washed with PBS to remove non-binding cells.

After four rounds of selection, individual clones were isolated and co-transformed with a plasmid that encodes for a green fluorescent protein making it possible to use flow cytometry to detect fluorescent cells bound to gold particles. Samples were sub-cultured into LB Cm carbenicillin media and grown until mid-log phase. Cells were induced for 30 minutes with IPTG (1 mM) and then induced for 1 hour with arabinose (0.04%). Cell aggregate depletions were performed at 1000 rpm for one minute. Bacterial clones were incubated in a 40 μL PBS gold particle solution with approximately 4 x 10^8

cells for one hour to allow for cell-gold interactions. Samples were centrifuged and washed with PBS before being analyzed using cytometry.

Results and Conclusions:

Using bacterial cells displaying a 7-mer constrained peptide library and screening techniques using gold surfaces, we successfully identified clones with a high affinity to gold substrates. After three rounds of gold-plated surface screening, and one round gold particle screening, clones were isolated with the ability to bind over half of the gold particles present in solution. Clone A is shown to bind 10.5% of gold particles in solution as determined using cytometry (Figure 1) and displays the peptide sequence, LVCYWSYSRMCKN. The lower populations in the forward and side scatter plot are bacteria cells, and the upper populations are gold particles. The gated population Gold is shown in the histogram with the gate bound cells indicating a cell/gold particle complex.

The fourth selection (Figure 2) yielded 89.5% of gold particles bound to green fluorescent bacteria cells after an extended incubation time. As seen in Figure 3, each successive round of selection provided a visible increase of the quantity of bound cells. The unselected round indicated 4.3% binding, round 1 indicated 8.3%, round 3 indicated 30.3%, and round 4 indicated 43.5%. The round 2 population exhibited a high degree of cell aggregation (data not shown). After round 2, cell aggregates were depleted as described in the methods section.

The peptide sequences identified using bacterial display through surface and particle selections have been demonstrated to confer high affinity binding to gold. With greater selection stringency, we anticipate clones of even higher affinity.

Future Work:

Our future plans include completing more surface and particle selections. We plan also to extrapolate this method to other inorganic substrates to expand our understanding of protein-inorganic interactions.

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References:

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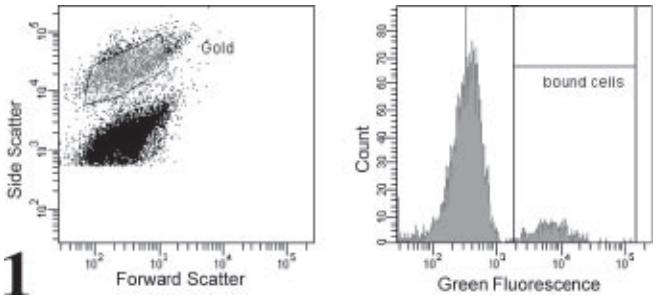

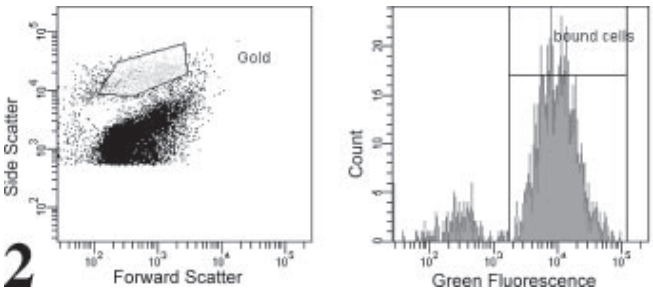

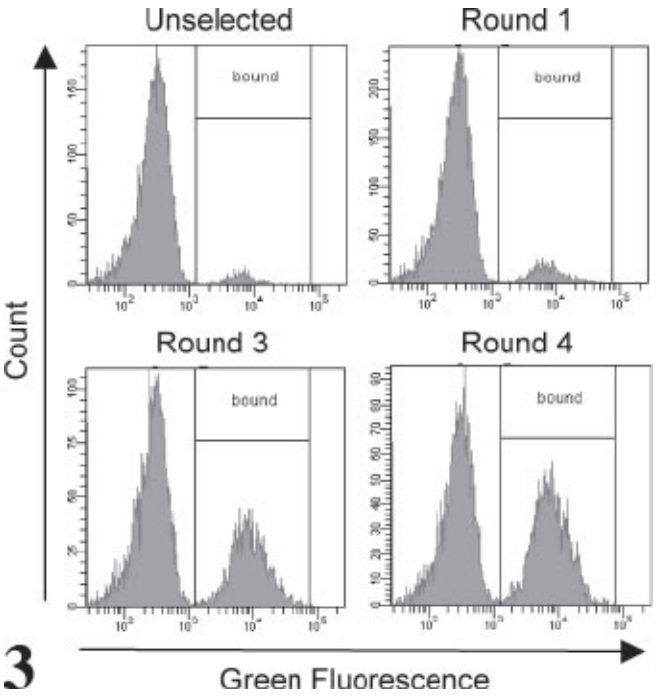

Figure 1, top: Clone A, 10.5% gold particles bound.

Figure 2, middle: Fourth Selection, 89.5% positive.

Figure 3, bottom: Selection rounds 1, 3 and 4.