Molecular Substrates for Nanobiotechnology

Brad Newsome, Biochemistry, Asbury College

NNIN REU Site: Center for Nanotechnology, University of Washington
Principal Investigator: Mehmet Sarikaya, Materials Science & Engineering, University of Washington
Mentor: Deniz Sahin, Molecular Biology and Genetics, Istanbul Technical University (visiting graduate student)
Contact: bradley.newsome@asbury.edu, sarikaya@u.washington.edu

Abstract:

For nearly two decades, phage display has been used as an extraordinarily powerful tool for many biotechnological and biological applications. It is a very effective tool for isolation of specific peptides for performing specific functions from very high numbers of diverse peptides and proteins. DNA sequences of interest are inserted into the phage genome and the encoded protein is displayed on the surface of the phage as a fusion product to one of the phage coat proteins. This serves as a tool for linking phenotypes of phage displayed peptide or protein with the genotypes encoding that molecule.

Whereas phage display usually involves protein-protein interactions, the focus of this research is using inorganics as a substrate to isolate polypeptides capable of binding inorganic material with high affinity. Single-crystal quartz pieces (001 plane) were used as the substrate for the selection of quartz-specific 12-amino acid peptides from a PhD-12 phage display library. Five biopanning rounds were carried out to obtain DNA sequences and the binding properties of 001 plane quartz were compared to that of 100 plane quartz. The amino acid sequences are compared to search for trends or convergence to a specific sequence.

Introduction:

Molecular Biomimetics is the marriage of materials science engineering and molecular biology for development of functional hybrid systems, composed of inorganics and inorganic-binding proteins [1]. The new approach takes advantage of DNA-based design, recognition, and self-assembly characteristics of biomolecules. (See Figure 1.)

Phage display is a technique developed by George Smith in which proteins or peptides are displayed on the surface of a bacteriophage [2]. Phage display serves as a selection method in which a peptide or protein is expressed as a fusion with a coat protein of a bacteriophage. This results in the display of the fused protein on the surface of the phage while the DNA encoding the fusion resides within the phage.

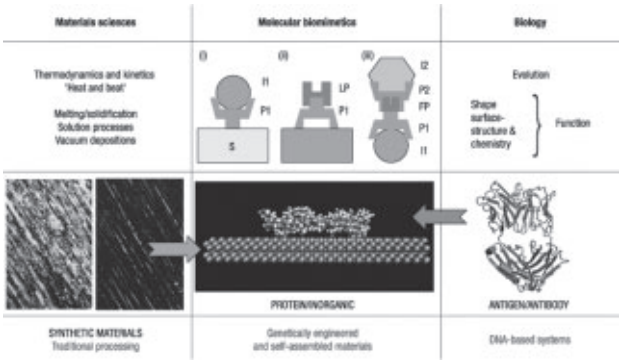

Figure 1: Molecular biomimetics.

A large part of phage display lies in linking the phenotype of a phage displayed peptide with the genotype encoding that molecule, packaged within the same virion. This allows for the selection and amplification of specific clones of phage, as well as the rapid determination of the amino acid sequence of the protein or peptide using DNA sequencing.

Although phage display has typically been used to study protein-protein interactions, this experiment studies protein-inorganic interactions in binding. Because of this, the conditions must be optimized according to that for inorganics. (001) surface plane quartz was used in this experiment to study binding. Comparison with different surface planes of quartz tested previously will provide information about binding characteristics of certain polypeptides on the surface.

The purpose of this research is then to provide the binding characteristics of the different surface planes of quartz for the eventual self-assembly by genetically engineered polypeptides for inorganics (GEPIs).

Procedure:

Substrate Preparation: (001) surface plane quartz was washed with ethanol, water, and buffer using sonication. 0.1% detergent containing PC buffer (pH 7.5) was then applied to the substrate and the phage library was added onto the surface.

Peptide Selection: PhD-12 library was exposed to the single crystal quartz wafer substrate in PC buffer (pH 7.5) containing 0.1% detergent solution to reduce phage-phage interactions on the surface. After a 30 min rotation, the surface was washed with 0.1% detergent-containing PC buffer (pH 7.5) to eliminate the nonspecific bindings. These washing cycles were repeated ten times for each biopanning round, with a total of 5 rounds. The detergent concentration was increased gradually up to 0.5%. The phage were eluted from the surface by addition of elution buffers for 15 minutes, and the eluted phage were transferred to a fresh tube and neutralized with Tris-HCl. The eluted phage after each round were mixed with *Eschericia coli* ER2738 host cell and plated on LB plates containing Xgal and IPTG. Single plaques were picked and ssDNA was isolated from these plates and sequenced.

Sequencing: QIAprep®SpinM13 Kit was then used to isolate single stranded DNA of phage M13 picked up from the XGal/IPTG plates. Then the isolated DNA was isolated by PCR in the presence of dye-labeled terminators. For the purification of the PCR product, Sephadex G-50 was used. DNA samples were then sequenced using automated cycle sequencing. -96 gIII primer, 5`-CCC TCA TAG TTA GCG TAA CG-3`, was used for the amplification of ssDNA.

Results and Conclusions:

The amino acid sequences obtained to date show some convergence, but it is necessary to have more sequences before analysis is beneficial. The similar sequences seen in Figure 2 show that the phage is specifically binding to the quartz surface. At this point, the sequences for the fourth and fifth rounds are pending, as well as more sequences for the first and third rounds.

Although more experimentation is necessary to determine if the peptides are binding specifically and strongly on the surface of the quartz, this research shows with confidence that this process is effective at isolating peptides that bind to the surface of inorganics. This will pave the way for further research in self-assembly by GEPIs.

Future Work:

Sequencing will be continued and the amino acid sequences will be further compared to search for trends or convergence to a specific sequence. Fluorescence microscopy will be used to determine binding affinities of the sequences. Multiple sequence alignment method will be used to analyze the similarities of phage display selected sequences inside and also between the silica binding sequences.

Acknowledgements:

I would like to thank Mehmet Sarikaya, Dong Qin, Ethan Allen, and Deniz Sahin for their leadership roles and education in this field I would also like to thank the NSF for financial support and NNIN for this research opportunity.

References:

[1] Sarikaya, M., Tamerler, C., Jen, A., Schulten, K. & Baneyx, F. Molecular biomimetics: nanotechnology through biology. Nature Materials 2, 577 (2003).

[2] Smith, G. Phage-Display Vectors and Libraries Based on Filamentous Phage Strain fd-tet. Science 228, 1315 (1985).

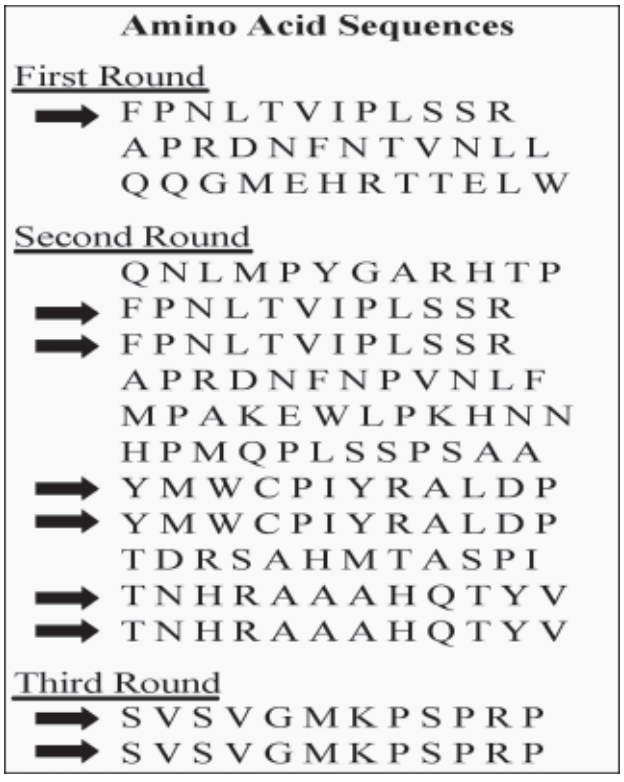

Figure 2: Results.