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Generating short peptidic ligands for silver nanowires from phage display random libraries

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Abstract—We report the generation of peptide ligands for silver nanowires using a linear 12-mer peptide phage display random library technique. Phage clones that specifically bind the silver nanowires are sequenced after three rounds of biopanning, and obtained DNA sequences suggest that there are a few conserved amino acid residues which may be critical for binding. A selected binding peptide, together with two mutant peptide sequences, were subsequently synthesized on Tentagel resins to examine the importance of both the identities and positions of the conserved amino acid residues.

Phage display random library is an established and useful tool to rapidly generate binding peptide ligands against a wide variety of targets in biomedical, physical, and chemical research.[1–7] A particularly exciting field is the application of the phage library against nano- and microparticles. In specific, there have been a series of elegant work by Belcher and co-workers regarding the generation of peptide sequences for the self-assembly of semi-conducting nanoparticles.[8–11] In addition, Stone and co-workers have reported the usage of phage display library against acid-etched AgNP for in vitro biosynthesis of AgNP through Ag-binding peptides.[12,13] As our long-term interest lies in the usage of biomolecules for patterning and/or self-assembly of metallic nanowires, we seek to address the 'universality' of peptide sequences against Ag structures with different morphologies, for example, spherical particles versus wires.

AgNW are obtained from Nanoplex Technologies Inc. (Mountain View, CA), in which their dimensions are 250 nm in diameter and 6 μm in length. The AgNW are supplied in H_2O, with a concentration of $\sim 10^9$ nanowires per milliliter. The linear 12-mer phage display peptides library kit is obtained from New England Biolabs (Medford, MA). The library is generated from filamentous *Escherichia coli* phage M13, with the random peptide portion being fused to N-terminal of the minor coat proteins pIII. The complexity of the library is reported to contain $\sim 1.28 \times 10^9$ independent sequences. The library was maintained and amplified with *E. coli* strain ER2738, which was provided along with the phage display library kit.

As for the biopanning process, $\sim 10^3$ AgNW are being placed in a microfuge tube, washed twice, and resuspended in 100 μL of binding buffer (100 μL TBS–0.1% Tween 20). Subsequently, 10–20 μL of the library stock solution ($\sim 2 \times 10^{12}$ phage) in 1 mL of binding buffer was added, and incubated, with mixing, for 1 h at room temperature. The microfuge tube was then centrifuged at 5000 rpm for 1 min to pellet the AgNW, and the unbound phage was carefully removed along with the supernatant. The AgNW-phage pellet was then washed five times by repeated resuspension and centrifugation of AgNW in 500 μL of washing buffer (TBS–0.1% Tween 20). Finally, the bound phage was eluted by adding 100 μL of 0.2 M glycine–HCl, pH 2.2, containing 0.1% BSA. After 10 min of incubation at room temperature, the mixture was neutralized with 15 μL of 1 M Tris–HCl, pH 9.1. The neutralized mixture containing

Abbreviations: AgNW, silver nanowires; AgNP, silver nanoparticles; X-Gal, 5-bromo-4-chloro-3-indolyl-β-D-galactoside; IPTG, isopropyl-β-D-thiogalactoside.

Keywords: Phage display random library; Nanowires; Amino acid residues, Tentagel resins; In vitro biosynthesis.

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the bound phage was then titered and amplified. Two more additional rounds of biopanning were performed with procedures as described above, except with the washing buffer being changed to TBS containing 0.5% Tween 20. After the third round of biopanning, the final eluted phage was infected with *E. coli* ER2537 host cells and plated on Luria Broth plates containing X-Gal (Invitrogen, Carlsbad, CA) and IPTG (Invitrogen, Carlsbad, CA). Approximately 35 independent blue plaques phage clones were randomly selected for sequencing.

To monitor the enrichment of the AgNW biopanning process, the input and output phage of each biopanning rounds were titered. The titering process was performed according to the manufacturer's protocol without any modifications. With the above information, the recovery percentage of each round can be derived via dividing the quantity of eluted phage by the quantity of input phage. The recovery % was observed to improve from 3.6×10^{-7} to 9.5×10^{-3}, from rounds 1 to 3, which is ~3700-fold (see Table 1).

Upon obtaining the DNA sequences, the identities of the binding peptide sequences can be elucidated. After aligning the obtained AgNW-binding peptide sequences, it was realized that there are a few repeating conserved amino acid residues. In specific, it was observed for eight of the obtained peptide sequences that serine residues are either singly, or contiguously, present at the 3rd to 6th positions. In addition, the proline residues are also either singly, or contiguously, present at both the 8th and 9th positions. Sequences from four representative peptides are shown in Figure 1. Also, it is important to note that nearly ~77% of peptide sequences, that is, 27 sequences do not demonstrate any homology to the above-mentioned conserved residues. Nonetheless, upon comparing our obtained sequences with the results previously obtained by Stone and co-workers against AgNP via regular panning approach, the sequences showed high similarity to a few of their obtained amino acid residues.[12,13] However, the positions of the identified amino acid residues do differ slightly. Upon calculation of the isoelectric pH (pI) for the four representative AgNW binding peptides, it was realized that the pIs for the peptides are all within the range 5.50–8.88 (**JT4** = 8.88, **JT21** = 8.88, **JT12** = 6.25, and **JT17** = 5.55). These values seem to also fall in the range for the AgNP-binding peptides obtained by Stone and co-workers.[12,13]

Table 1. Enrichment of the phage library from rounds 1 to 3 expressed in recovery percentage (%)

Rounds	Input phage (pfu)	Eluted phage (pfu)	Recovery %
1	2.01×10^{12}	7.21×10^{3}	3.58×10^{-7}
2	8.27×10^{11}	8.44×10^{3}	1.02×10^{-6}
3	1.04×10^{12}	9.83×10^{7}	9.45×10^{-3}

a Four representative AgNP binding peptide sequences:

1 *5* *10*

JT4: NH_2-Gly-Pro-Gly-Val-Ser-Ser-Ala-Pro-Pro-Phe-Ser-Lys-CO_2H

JT21: NH_2-Leu-Lys-Ser-Ser-Gly-Ser-Ala-Pro-Pro-Gly-Pro- Phe-CO_2H

JT12: NH_2-Ala-Gly-Lys- Ala-Ser-Lys-Ile-Pro-Asp-Pro-Gly-Phe-CO_2H

JT17: NH_2-Val-Pro-Leu-Ser-Ala-Gly-Ala-Pro-Pro-Leu-Met-Ala-CO_2H

b AgNP mutant binding peptide sequences:

1 *5* *10*

JT4M1: NH_2-Gly-Pro-Gly-Val-*Ala-Ala*-Ala-*Ala-Ala*-Phe-Ser-Lys-CO_2H

JT4M2: NH_2-Gly-Pro-*Ser-Ser*-Gly-Val-Ala-Pro-Pro-Phe-Ser-Lys-CO_2H

Figure 1. (a) Selected AgNP peptide sequences showing consensus amino acid residues (underlined) after three rounds of biopanning. (b) Two mutant peptide sequences without the hypothesized (underlined) consensus residues.

Encouraged by these findings, we proceed to ensure that the selected peptides do indeed bind the AgNW. Among the peptides that showed certain degree of homology in their binding sequences, we decide to pursue **JT4** as it contained the highest degree of conserved residues (Fig. 1). Two other mutant peptide sequences were also designed to probe the factors required for Ag nanowire binding. First, the four identified conserved residues, namely serines at 5th and 6th position, and prolines at 8th and 9th positions, were all rendered non-functional by conversion to an alanine (peptide **JT4M1**). Second, locations of the serines at 5th and 6th position were shifted to the 3th and 4th position, respectively (peptide **JT4M2**). This was designed to understand if the location of the serine residues is important in their AgNW-binding capacity. Furthermore, both the mutant peptides were also calculated to have pIs at 8.88, which is consistent to **JT4**.

All the peptides, namely **JT4**, **JT4M1** and **JT4M2**, were synthesized on Tentagel S–NH_2 solid resins (Peptides International Inc., Louisville, KY), which are polyethylene glycol-grafted copolymers consisting of a low crosslinked polystyrene matrix. The Tentagel resins utilized have a diameter of ~90 μm and a loading capacity of ~0.2 to 0.3 mmol/g. The resins have previously been reported to be widely used for solid-phase 'one-bead one-compound' peptide library synthesis. Accordingly, the detailed synthetic procedures of the three peptides on Tentagel resins were accomplished according to previously published procedures.[14,15] Briefly, Fmoc-protected amino acids were coupled in a sequential basis onto overnight DMF-swollen Tentagel beads. The progress of the peptide coupling steps was monitored via the ninhydrin test. Upon completion of the 12-mer peptide synthesis, sidechains of the incorporated residues were subsequently deprotected with 95% TFA. The peptide-conjugated Tentagel resins were then washed and stored in PBS. The exact peptide sequences were confirmed via peptide sequencing.

For the binding studies, we first perform a control study to see if underivatized Tentagel resins will bind to the Ag nanowires. In a microfuge tube, ~10 to 20 underivatized binding-buffer equilibrated Tentagel resins were incubated with ~10^3 AgNW for 30 min at room temperature. The microfuge tube was then

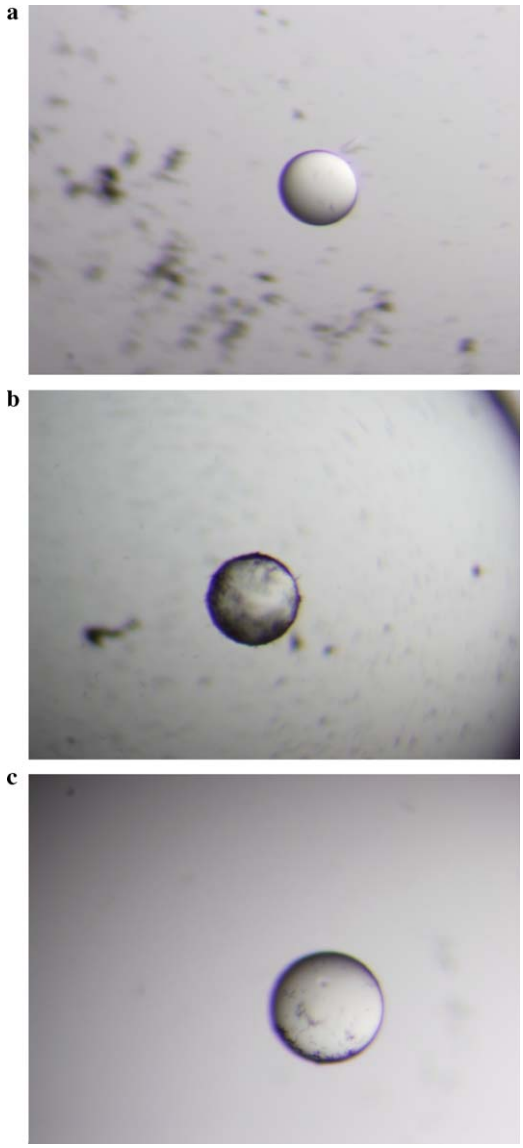

Figure 2. Micrographs of (a) underivatized 90 μm Tentagel S–NH_2 resins, which do not bind to the AgNW; (b) **JT4**-conjugated Tentagel resins, which binds and is coated with AgNW; (c) **JT4M2**-conjugated Tentagel resins, which also binds to the AgNW; but to a lesser extent.

centrifuged (7000 rpm) for 1 min to pellet both the resins and nanowires. After removal of the supernatant, the pellet was thoroughly washed 3× with 500 μL PBS, and finally resuspended in 200 μL of PBS. The suspension was then placed in a glass Petri-dish and imaged using an Olympus SZX12 stereomicroscope at 144× magnification. As shown in Figure 2a, the obtained micrograph showed that the underivatized Tentagel resins do not exhibit any binding to the AgNP. Using the same approach, after incubating ~10 to 20 **JT4**-conjugated Tentagel resins with ~10^3 AgNW, we observed that there is a significant increase in the amount of coating of the **JT4**-conjugated Tentagel resins (Fig. 2b). This observation lends strong evidence that the peptide **JT4** is indeed binding to the AgNW.

Last, we also incubated ~10^3 of both **JT4M1**- and **JT4M2**-conjugated Tentagel resins with ~10 to 20 AgNW. After 30 min of incubation, it was observed that while there is no binding of **JT4M1** to the AgNW (micrographs not shown), **JT4M2** does exhibit binding to the AgNW, albeit to a lesser quantity compared to **JT4** (Fig. 2c). These results indicate that serines at 5th and 6th position while important for binding, can be shifted to the 3rd and 4th position without drastically affecting their AgNW-binding capabilities. However, upon the replacement of the serines to alanine, binding is abolished. Furthermore, we speculate that while prolines at both 8th and 9th positions do not participate directly in binding to the AgNW, the residues may be important to ensure a robust structural conformation for optimized AgNW binding.

Finally, we also investigated if our selected peptide has the ability to synthesize AgNP according to the previously published procedure by Stone and co-workers.[12,13] Again, we chose to study both peptides **JT4** and **JT4M2** as they were observed to demonstrate AgNW-binding properties. Additionally, we also included **JT4M1** to function as a negative control. A fourth vial, which does not contain any peptide, was also included in the experiment to function as a negative control. Approximately 5 mg of the above three peptides (obtained commercially form Anaspec, San Jose, CA) was incubated in a 0.1 M HEPES buffer, pH 7.5, supplemented with 0.2 mM silver nitrate, at rt for 48 h. After the incubation period, we did not observe any visible color changes and/or differences between the four vials. This result indicates that the all the studied peptides, namely **JT4**, **JT4M1** and **JT4M2**, do not possess any AgNP-synthesizing properties.

In conclusion, we have employed the phage display technique to successfully enable novel peptidic ligands against AgNW. The phage display random library technique is both rapid and efficient, in which the entire biopanning, cloning, and sequencing processes can be accomplished in <4 weeks. Our generated binding peptide sequences indicate that both serine and proline residues are important for binding to AgNW, and the obtained sequences bear strong resemblance to the sequences previously obtained against AgNP. Taken together, these observations suggest that amino acid residues may be binding to the Ag atoms, and is indiscriminate of their structural morphologies. Finally, it was of interest to note that the peptide **JT4** generated in our experiment, although able to bind to AgNW, fails to serve as possible agent for controlled nucleation and growth of AgNP. The mechanism and sites for peptide binding on the AgNW are currently being investigated and will be reported in due time.

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