Electronic Supplementary Material (ESI) for Molecular BioSystems

Supporting Information

Densely surface functionalization using peptides that recognize differences in organized structures of self-assembling nanomaterials

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1. Characterization of the nanofiber: Structures of Y9 and bY9C peptide nanofibers were observed by TEM (Figure S1 a, b). These nanofibers have similar structures. Surface modification of bY9C nanofibers were performed by using AuNPs conjugated anti-biotin antibodies. Because bound AuNPs were rarely observed, biotin groups were not exist at surfaces of the nanofibers.

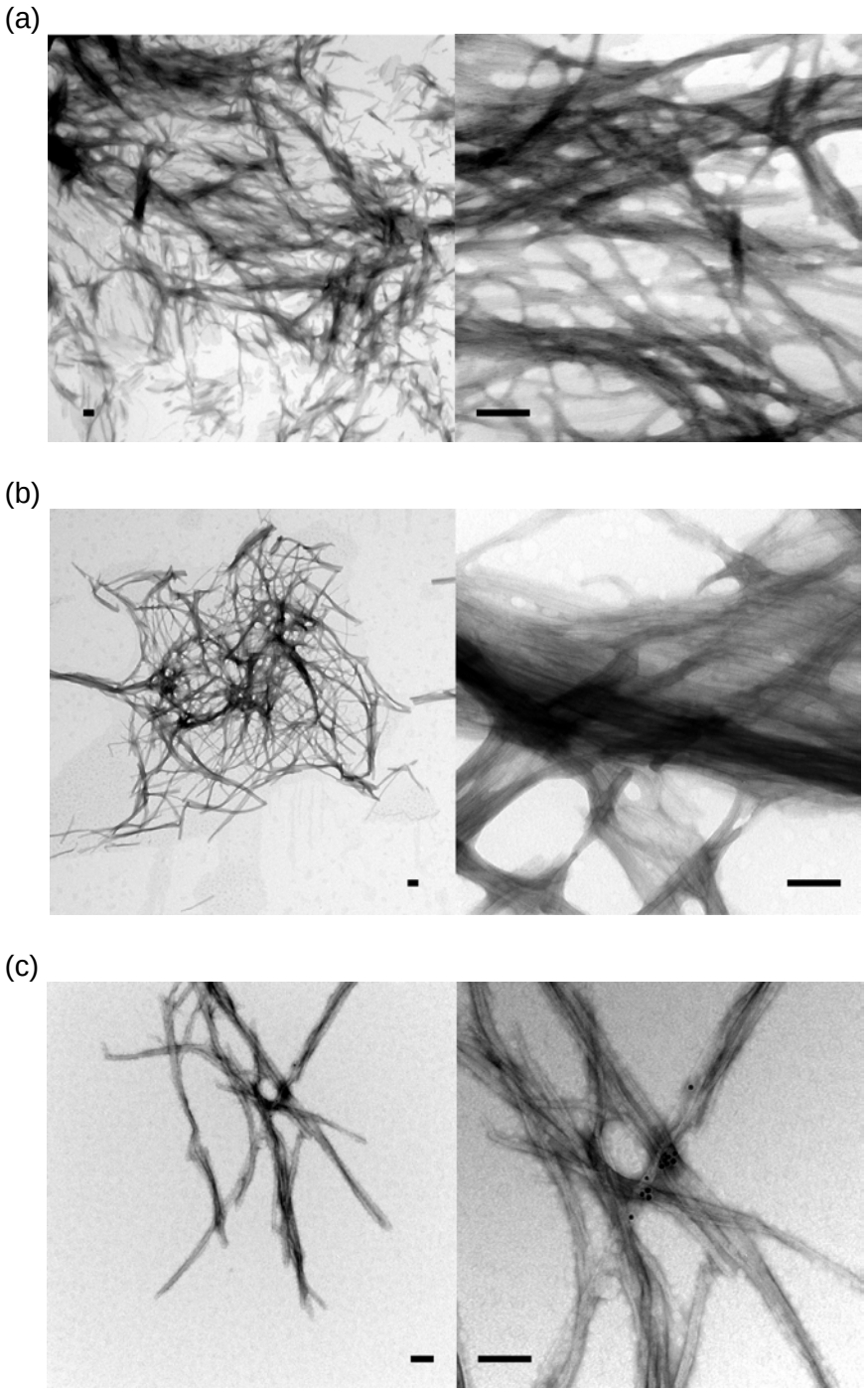

Figure S1. TEM images of (a) Y9 and (b) bY9C peptide nanofibers, and (c) surface-functionalized bY9C nanofibers by AuNPs conjugated anti-biotin antibodies. All scale bars represent 100 nm.

2. Binding affinity of phage pools against nanofibers:

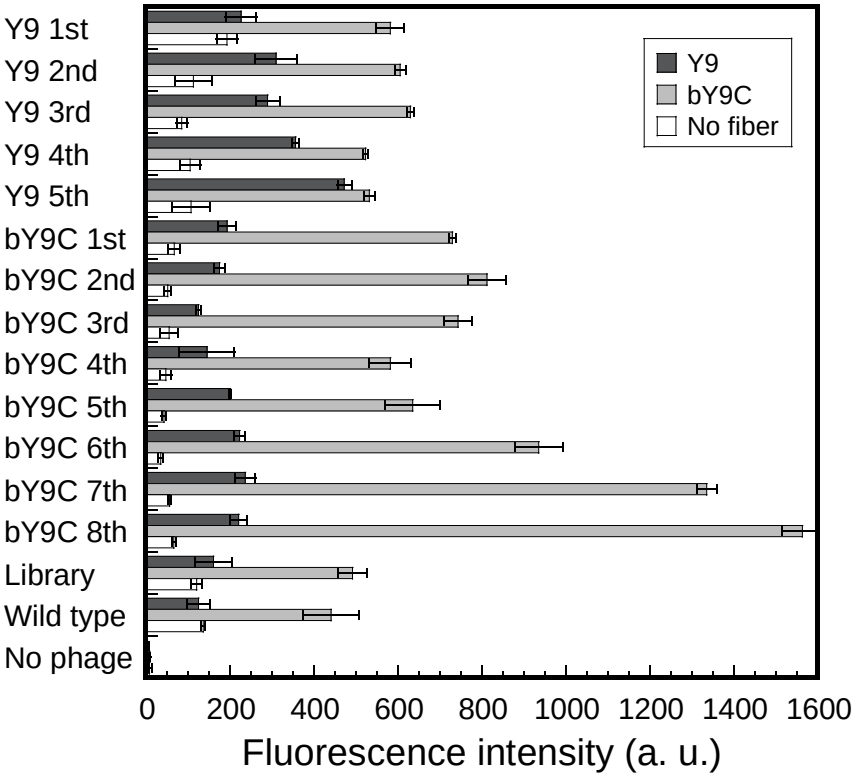

Figure S2. The binding amounts of the phage pools at each round of biopanning. The black, gray, and white bars indicate the amounts against Y9, bY9C, and microtiter plate, respectively. For all samples $n = 3$. Error bars represent the standard deviation.

3. Adsorbed amounts of nanofibers against microtiter plate: Adsorbed amounts of nanofibers were determined by fluorescamine assay. A standard curve was prepared using each peptides in solution. The fluorescence intensity values for each known peptide concentration in solution was measured and a linear plot (data not shown) of known concentration versus fluorescence intensity was generated. This plot was used to determine the actual adsorbed nanofiber on microtiter plate. Baseline fluorescence intensity was subtracted from each sample. To calculate the actual adsorbed amounts of nanofibers, the un-adsorbed nanofiber concentration was subtracted from the known input peptide concentration. The fluorescence intensity and calculated amounts were shown in Figure S3.

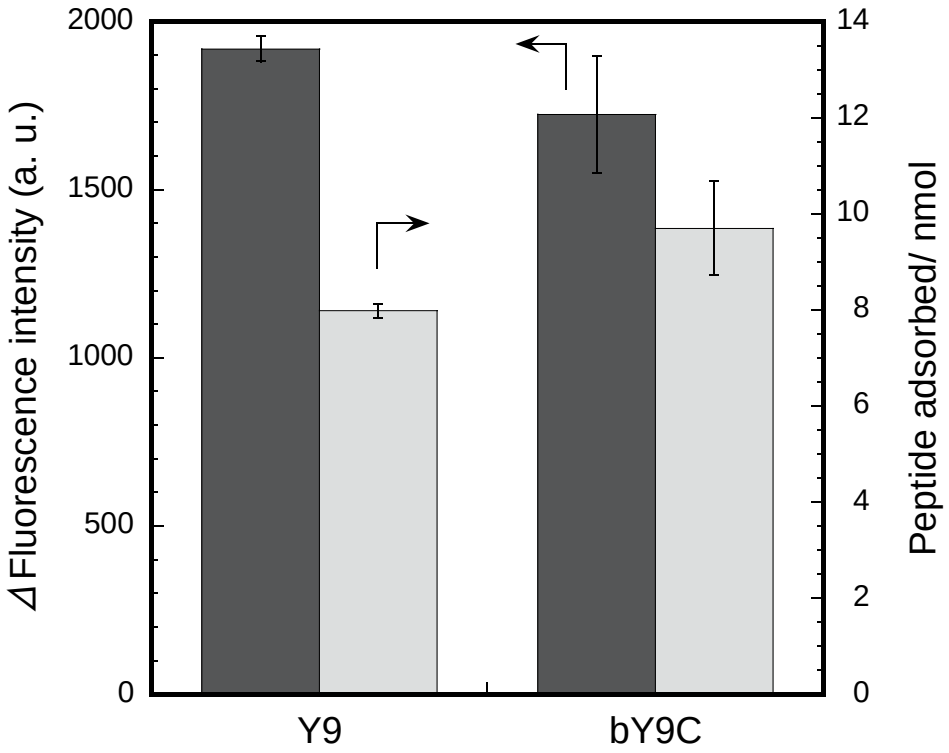

Figure S3. The adsorbed amounts of nanofibers at 500 μM. The black and gray bars indicate fluorescent intensity and adsorbed amount calculated by each standard curve, respectevily. For all samples n = 3. Error bars represent the standard deviation.

4. Amino acid appearance used in screened peptides: Percent appearance of amino acid used in screened peptides was compared with that of phage library described in the instruction manual of PH.D.-7 phage display kit (NEB) to understand about interaction between screened peptides and the nanofibers.

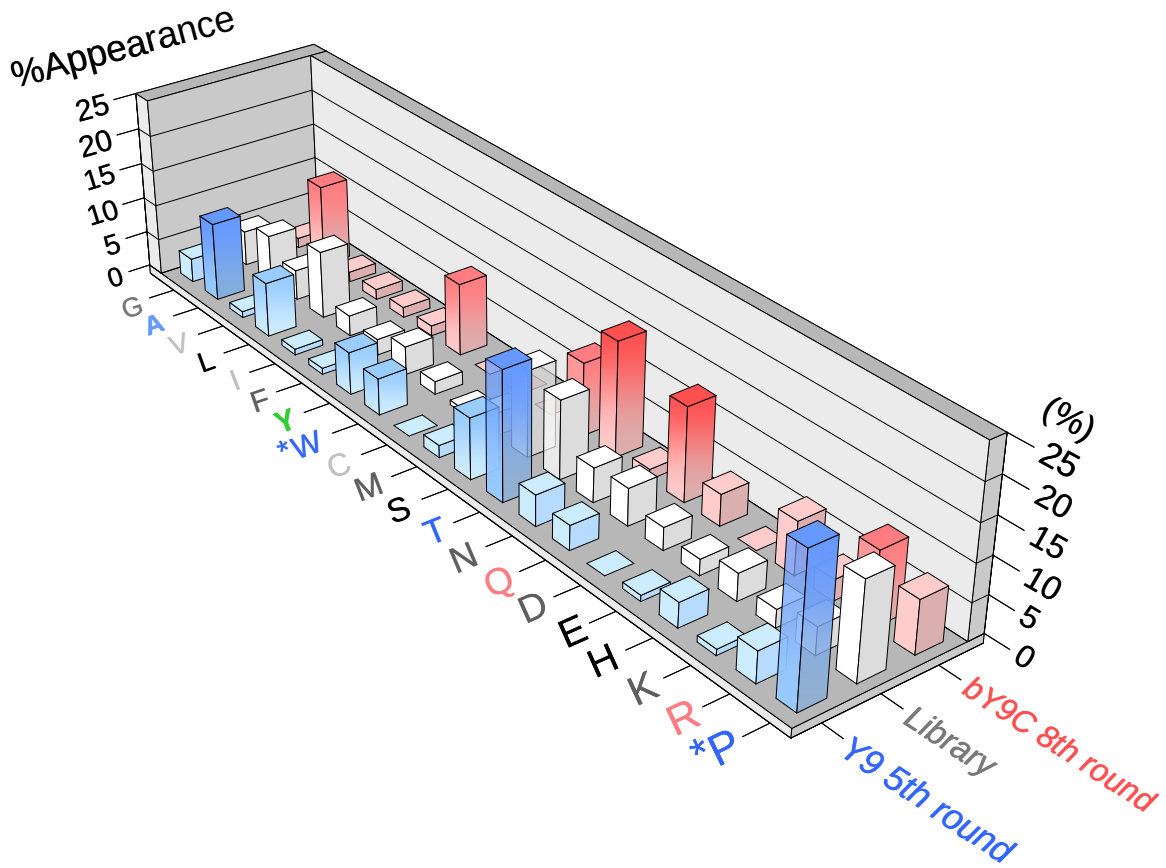

Figure S4. Percent appearance of amino acids in peptide sequences for Y9 and bY9C nanofibers. Blue, red, and white bars represent appearances of amino acids used for Y9 clones (5th), bY9C clones (8th), or the library phages, respectively. Blue, red, green, and gray letters represent an amino acid which increased over twice in Y9 or bY9C clones, both clones, or decreased in both clones. Letters with an asterisk represent an amino acid that increased in Y9 clones but decreased in bY9C clones.

5. Dependence of phage concentrations against relative binding amount: Typical examples of the dependence of the phage concentrations against fluorescence intensity values (mean relative amounts) are shown in Figure S5. Assuming a Langmuirian adsorption, the plots were fitted to the following equation:

$$\frac{[phage]}{RA} = \frac{1}{RA_{max}}[phage] + \frac{1}{RA_{max} K_{app}}$$

where RA is the relative amount, RA_{max} is the maximum RA, and K_{app} is the apparent affinity constant. The fitted curves were also shown in Figure S5.

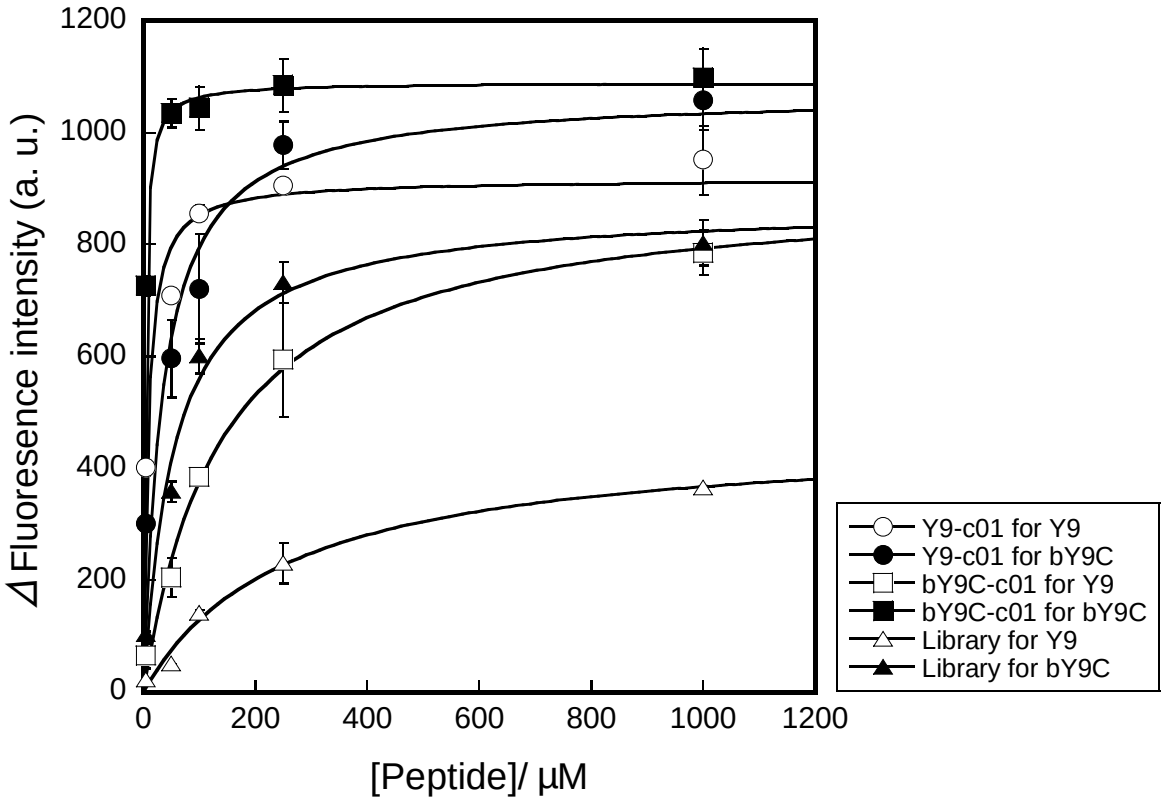

Figure S5. Typical examples of the dependence of the phage concentrations against the relative binding amount. The circle, square, and triangle indicate the affinities of Y9-c01, bY9C-c01, and library phages, respectively. White and black symbols represents affinities against Y9 and bY9C nanofibers, respectively. For all samples n = 3. Error bars represent the standard deviation.

6. Dependence of peptide concentrations against relative binding amount: Typical examples of the dependence of the peptide concentrations against fluorescence intensity values (mean relative amounts) are shown in Figure S6. Assuming a Langmuirian adsorption, the plots were fitted to the equation:

$$\frac{[peptide]}{RA} = \frac{1}{RA_{max}}[peptide] + \frac{1}{RA_{max} K_a}$$

where RA is the relative amount, RA_{max} is the maximum RA, and K_a is the binding constant. The fitted curves were shown in Figure S6.

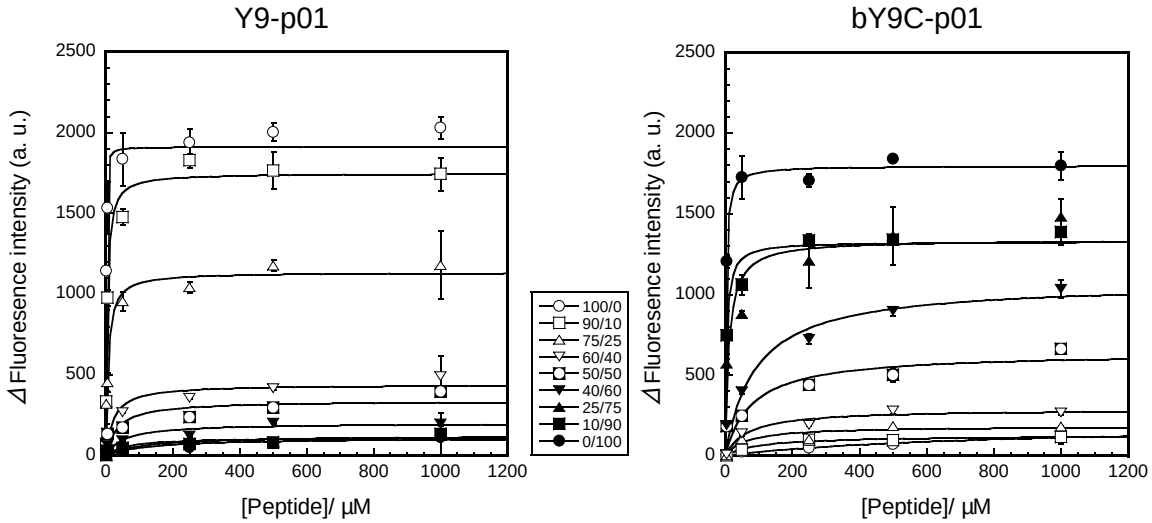

Figure S6. Typical examples (left: Y9-p01, right: bY9C-p01) of the dependence of the biotinylated peptide concentrations against the relative binding amount. The indications of the symbols were displayed in the Figure. For all samples n = 3. Error bars represent the standard deviation.

7. TEM observations of nanofibers:. Surface modifications of peptide nanofibers by Au-Ab *via* TAMRA conjugated peptides were observed by TEM.

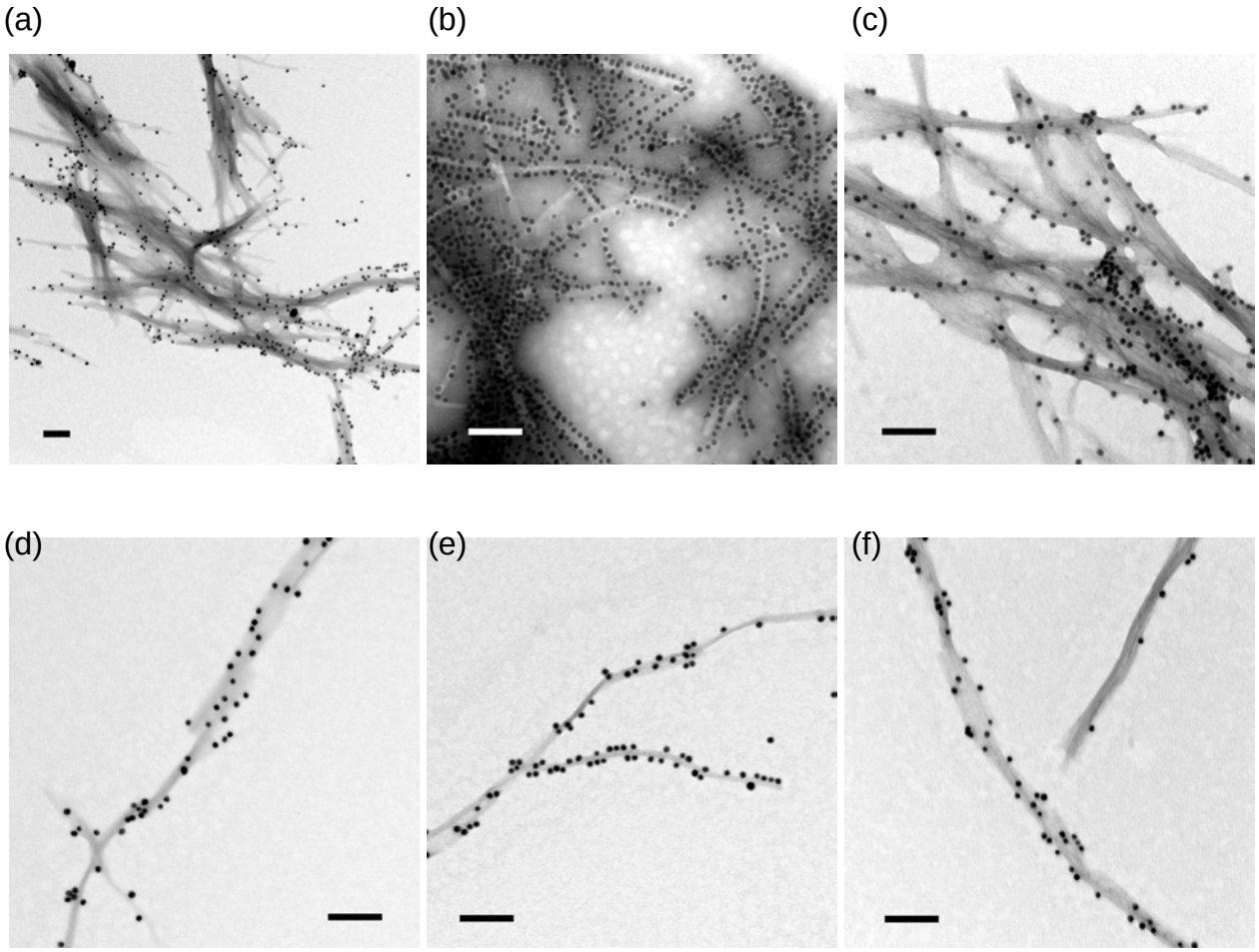

Figure S7. Surfaces modifications of the nanofibers by Au-Ab. A TEM image of Au-Ab on (a) Y9 nanofibers *via* TAMRA-Y9-p04 and that on bY9C nanofibers *via* (b) TAMRA-bY9C-p01 and (c) TAMRA-bY9C- p08. Surface modification of the lower networked nanofibers. Used peptides and nanofibers of (d), (e), and (f) are same as (a), (b), and (c), respectively. All samples were negatively stained by 2% uranyl acetate for 5 sec. All scale bars represent 100 nm.

8. Condition of biopanning:

Table S1. Conditions for screening phages against Y9 and bY9C peptide nanofibers.

round	affinity / min		wash / min × time		negative selection[a] / min	
	Y9	bY9C	Y9	bY9C	Y9	bY9C
1st	10	30	n.d.[b]	n.d.	n.d.	n.d.
2nd	10	30	1 × 1	3 × 1	1	1
3rd	5	20	3 × 1	10 × 1	5	10
4th	3	10	10 × 1	10 × 3	5	15
5th	1	10	10 × 1	10 × 3	5	15
6th	–	5	–	10 × 3	–	15
7th	–	3	–	10 × 5	–	15
8th	–	1	–	10 × 10	–	15

[a] Phages that nonspecifically bound to glass slides were removed before interactions with the target nanofibers.