Supplementary Materials:

A phage virus-based electrochemical biosensor for highly sensitive detection of ovomucoid

Jae Hwan Shin[1], Tae Jung Park[2], Moon Seop Hyun[3] and Jong Pil Park[1*]

[1]Basic Research Laboratory, Department of Food Science and Technology, Chung-Ang University, Anseong 17546, Republic of Korea

[2] Department of Chemistry, Institute of Interdisciplinary Convergence Research, Research Institute of Chem-Bio Diagnostic Technology, Chung-Ang University, 84 Heukseok-ro, Dongjak-gu, Seoul 06974, Republic of Korea

[3] National NanoFab Center (NNFC), 291 Daehangno, Daejeon 34141, Republic of Korea

* Corresponding author: Tel.: +82 31 670 4703, Fax: +82 31 675 1381

E-mail address: jppark@cau.ac.kr (J.P. Park)

Table S1. The yield of biopanning

C7C library	Input (PFU/mL)	Output (PFU/mL)	Yield[*]
Round 1	1.0 x 10^{11}	1.1 x 10^{6}	1.1 x 10^{-3}
Round 2	1.0 x 10^{11}	8.0 x 10^{6}	8.0 x 10^{-3}
Round 3	1.0 x 10^{11}	3.5 x 10^{6}	3.5 x 10^{-3}
Round 4	1.0 x 10^{11}	5.0 x 10^{6}	5.0 x 10^{-3}
12mer library	Input (PFU/mL)	Output (PFU/mL)	Yield[*]
Round 1	1.0 x 10^{11}	4.0 x 10^{5}	4.0 x 10^{-4}
Round 2	1.0 x 10^{11}	2.0 x 10^{6}	2.0 x 10^{-3}
Round 3	1.0 x 10^{11}	3.0 x 10^{6}	3.0 x 10^{-3}
Round 4	1.0 x 10^{11}	4.5 x 10^{6}	4.5 x 10^{-3}

[*] Phage display yield was calculated as follows: output / input x 100 (%).

Table S2. Amino acid sequence of peptides selected by biopanning of M13 peptide libraries

Name	Amino acid sequence	Note[*] (hydrophobicity/hydrophilicity)
C7C 2-12	CTDKASSSC	hydrophobic: 11.11%, acidic: 11.11%, basic: 11.11%, neutral: 66.67%
C7C 3-36	CHMYHNATC	hydrophobic: 22.22%, acidic: 0%, basic: 22.22%, neutral: 55.56%
12mer 3-9	LQAYIGPKATWW	hydrophobic: 58.33%, acidic: 0%, basic: 8.33%, neutral: 33.33%
12mer 3-25	HHSRFSTLFNWP	hydrophobic: 41.67%, acidic: 0%, basic: 25%, neutral: 33.33%
12mer 3-30	WWQPYSSAPRWL	hydrophobic: 58.33%, acidic: 0%, basic: 8.33%, neutral: 33.33%

[*] The structural analysis of peptides was performed with Peptide 2.0 program (http://peptide2.com/N_peptide_hydrophobicity_hydrophilicity.php).

Table S3. Calculation of electroactive surface area (*A*) of whole phage-tethered gold electrode with different coupling chemistry approach.

Coupling chemistry		i_p (A)	n	$D^{1/2}$ (cm^2/s)$^{1/2}$	C (moles/cm^3)	$v^{1/2}$ (V/s)$^{1/2}$	electroactive surface area (cm^2)*
Bare gold		2.10×10^{-4}	1	0.03	2.5×10^{-6}	0.23	0.05
MUA-EDC/NHS	C7C	7.16×10^{-5}	1	0.03	2.5×10^{-6}	0.23	0.02
	12mer	7.21×10^{-5}	1	0.03	2.5×10^{-6}	0.23	0.02
Sulfo-LC-SPDP	C7C	1.69×10^{-4}	1	0.03	2.5×10^{-6}	0.23	0.04
	12mer	1.81×10^{-4}	1	0.03	2.5×10^{-6}	0.23	0.04

*Active surface area was calculated as follows: $i_p = (2.69 \times 10^5)\, n^{3/2} A D^{1/2} C v$

Table S4. Comparison of ovomucoid detection performances with the proposed sensor and other methods.

Detection method	Receptor	Detection limit		Dynamic linear range		Reproducibility		Recovery		Reference
		Pure ovomucoid	Sample matrix	Pure ovomucoid	Sample matrix	Pure ovomucoid	Sample matrix	Pure ovomucoid	Sample matrix	
ELISA[†]	Monoclonal antibody	0.041 ng/mL	ND	0.1-6.25 ng/mL	0.2-3.12 ng/mL	5% <	ND	ND	92-104%	(Li et al., 2008)
ELISA[‡]	Polyclonal antibody	ND	0.19-0.23 µg/mL	ND	0-50 µg/mL	ND	20% <	ND	ND	(de Luis et al., 2008)
ELISA[§]	Polyclonal antibody	1 µg/mL	2.5 µg/mL	5-10 µg/mL	2.5-25 µg/mL	ND	ND	ND	62-115%	(Gomaa & Boye, 2015)
Amperometric immunosensor[¶]	Polyclonal antibody	0.1 ng/mL	ND	0.3-25 ng/mL	0-14.3 ng/mL	6%	ND	ND	ND	(Benede et al., 2018)
M13 phage sensor	Affinity peptide	0.12 µg/mL	ND	1.55-12.38 µg/mL	1.55-24.75 µg/mL	4.9%	7.6-9.6%	ND	97.5-108%	This study*

*ND means not described.

[†] The author used egg white samples. [‡] The author used sausage, bread, and pate samples. [§] The author used bread, pasta and cereal samples. [¶] The author used egg white and baked bread samples. * We used egg white samples.

Scheme 1. Schematic illustration of electrochemical phage sensor for ovomucoid detection.

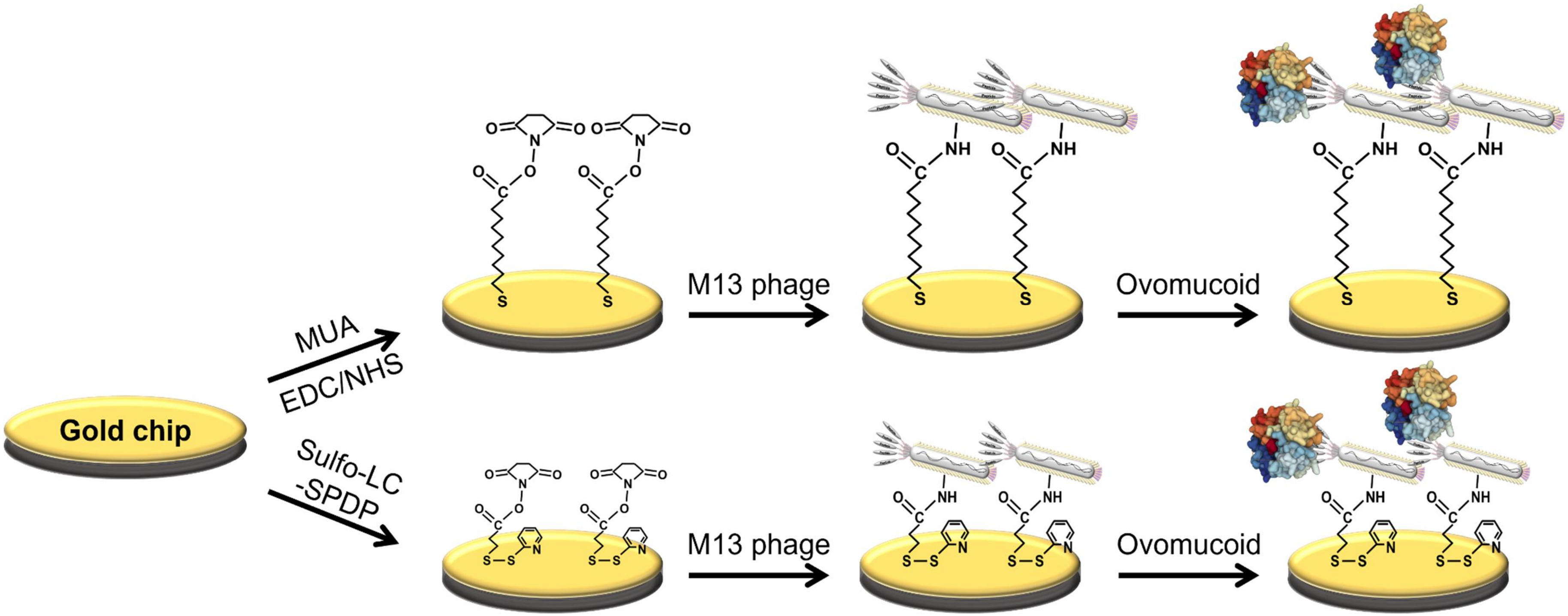


Fig. S1. Characterization of the selected cyclic (C7C) and linear (12mer) phages by ELISA. A) Effect of BSA concentration on C7C 2-12 (10^{12} PFU/mL) binding interaction, and B) Effect of BSA concentration on 12mer 3-30 (10^{12} PFU/mL) binding interaction.

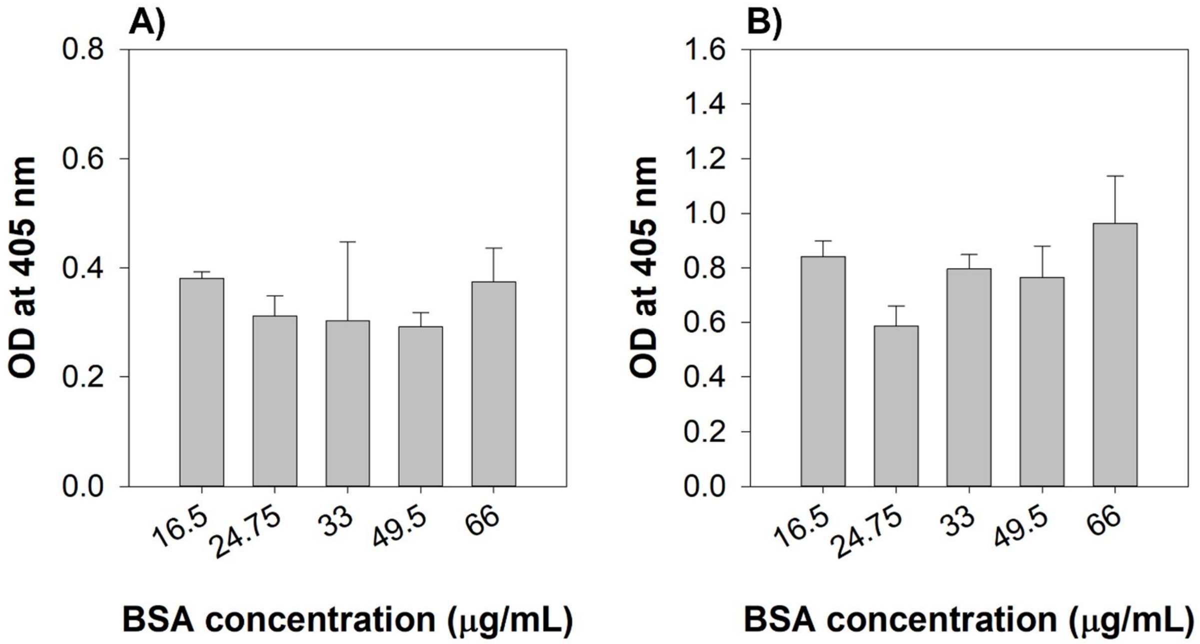


Fig. S2. XPS spectra recorded for bare gold surface, MUA-gold surface, MUA-EDC/NHS-gold surface, MUA-EDC/NHS-12mer 3-30 phage tethered gold surface, MUA-EDC/NHS-C7C 2-12 phage tethered gold surface.

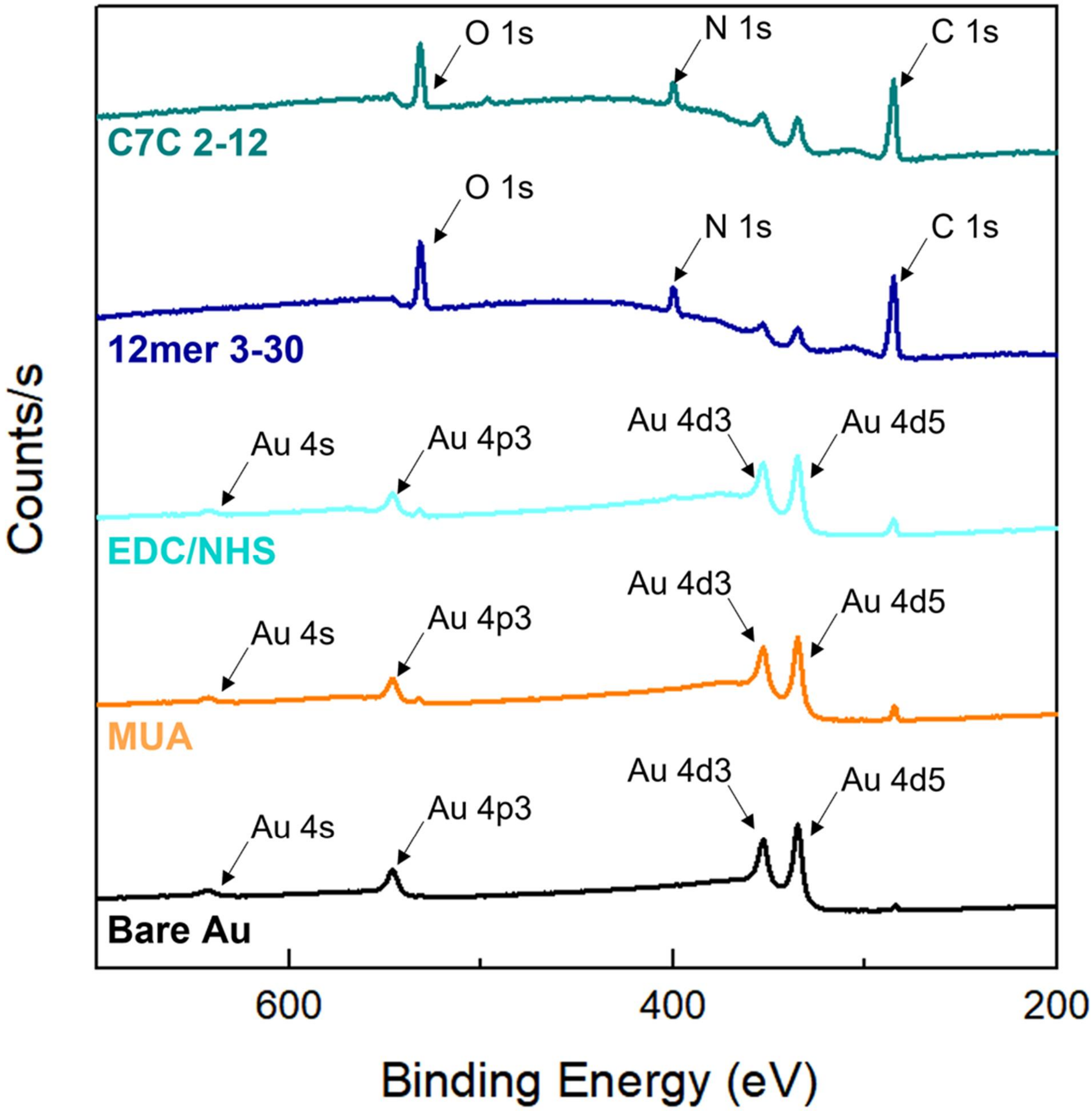


Fig. S3. FT-IR spectra recorded for bare gold surface, MUA-gold surface, MUA-EDC/NHS-gold surface, MUA-EDC/NHS-12mer 3-30 phage tethered gold surface, MUA-EDC/NHS-C7C 2-12 phage tethered gold surface.

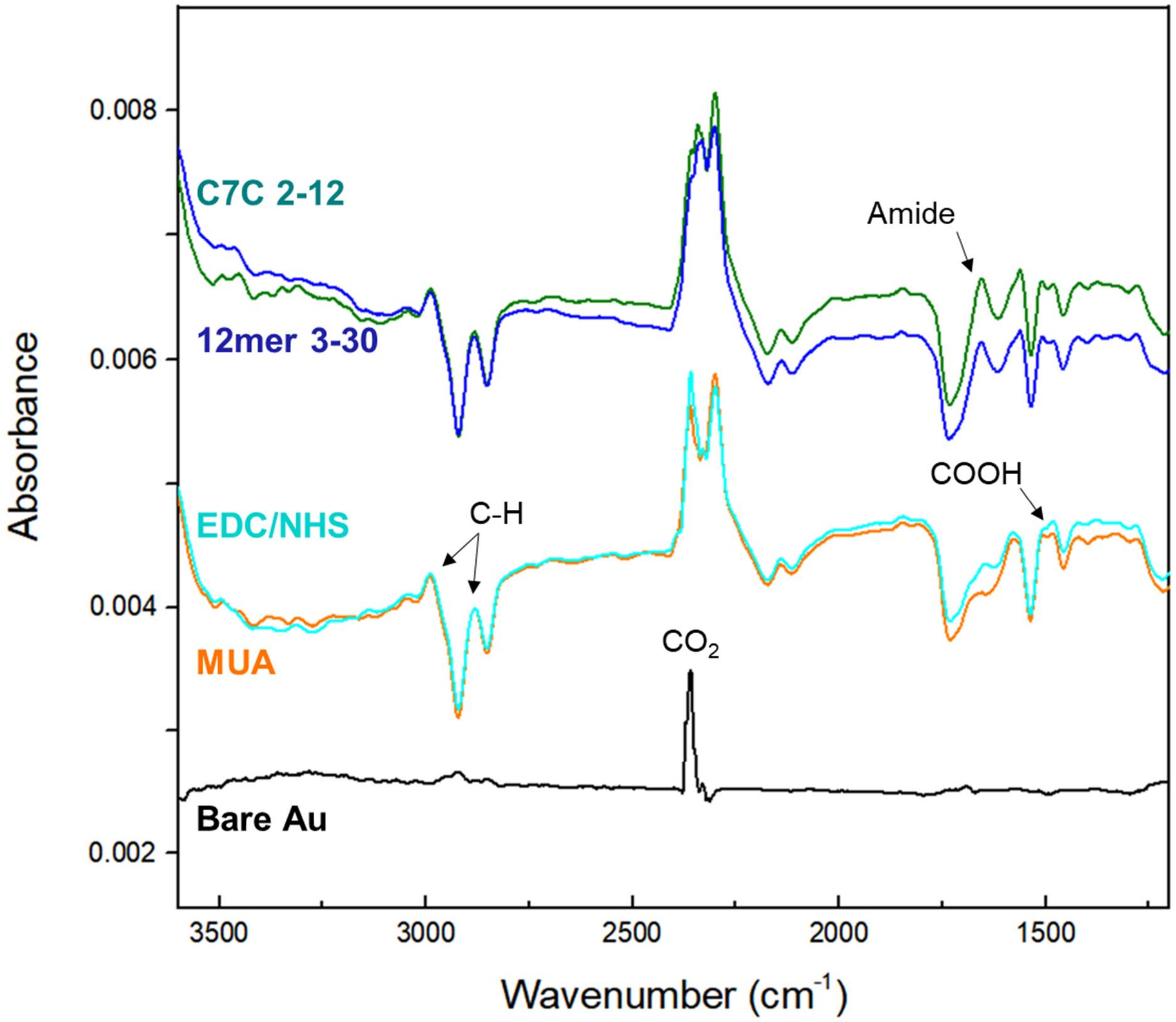


Fig. S4. Optimization of coupling reaction time for functionalization of gold electrode through SPDP chemistry. A) C7C 2-12 phage, b) 12-mer 3-30 phage.

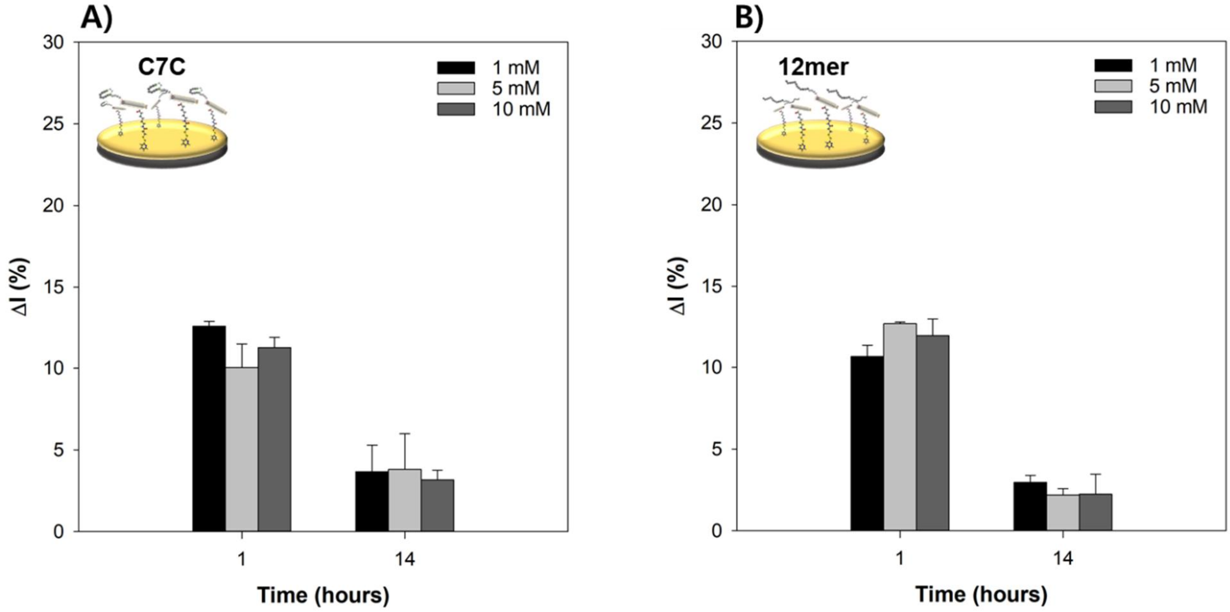


Fig. S5. Cyclic voltammetry curves on electrochemical behavior of chemically modified whole phage electrode (A-D for C7C 2-12, E-G for 12mer 3-3-) for with two coupling chemistry (A, B, E and F for MUA-EDC/NHS and C, D, G and H for SPDP) at different scan rates.

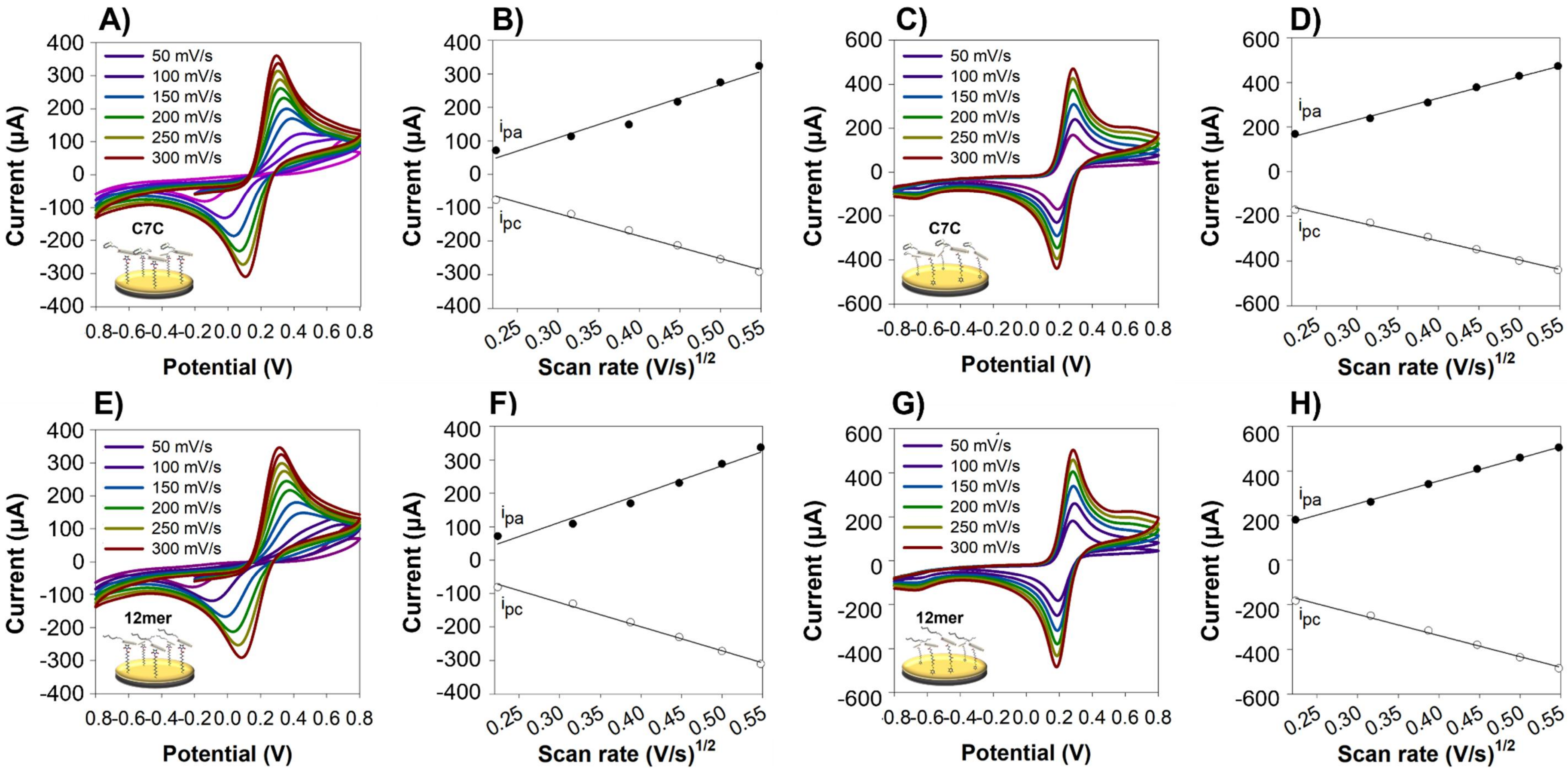


Fig. S6. Electrochemical characterization (A, C, E and F in SWV, B, D, F and H in EIS) of chemically modified whole phage electrode (A-D for C7C 2-12, E-H for 12mer 3-30) with SPDP chemistry.

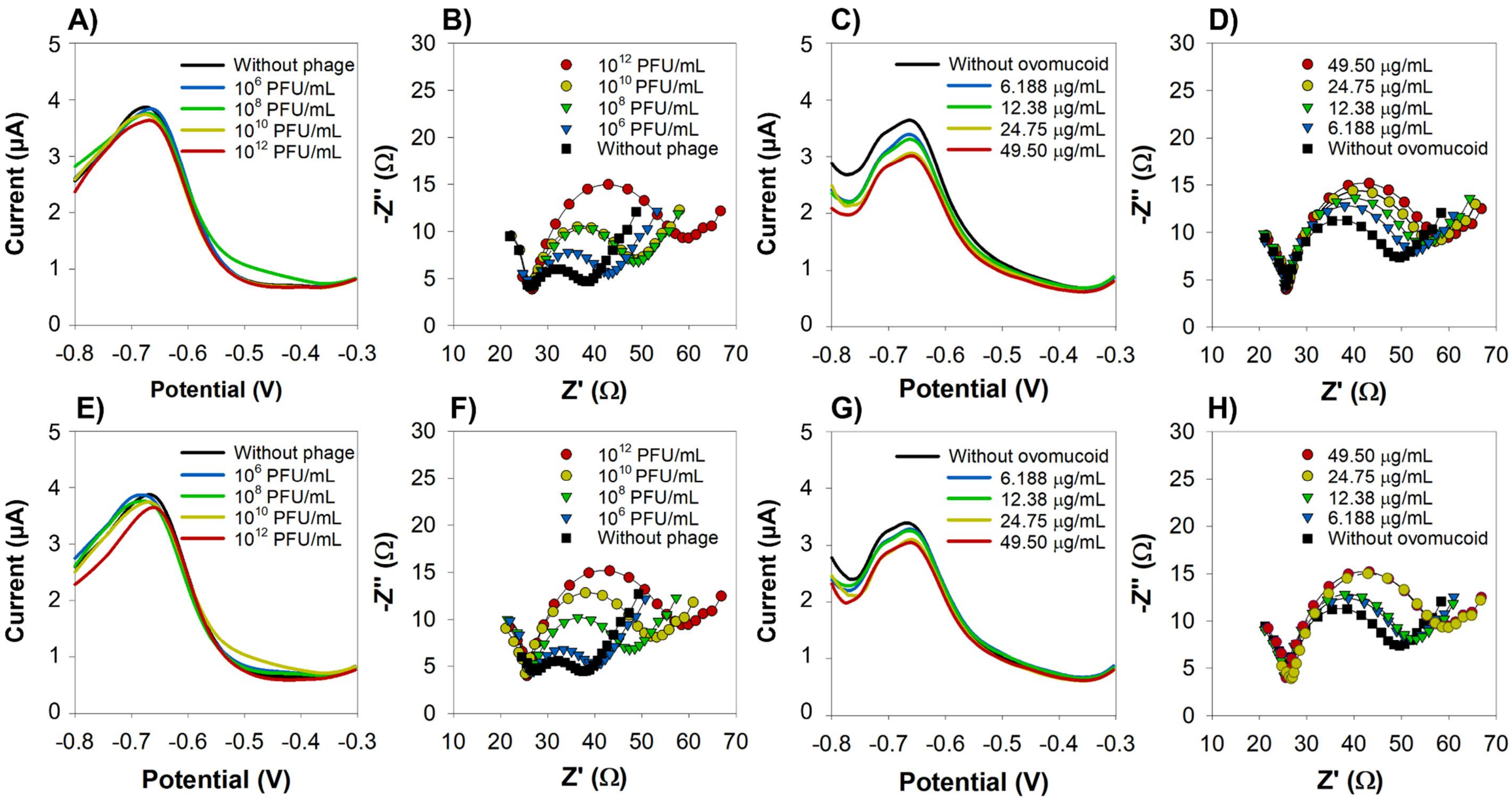


Fig. S7. Comparison of binding constants (K_d) and limit of detection (LOD) of functionalized M13 phage sensor with SPDP chemistry. (A) C7C 2-12, (B) 12mer 3-30. SWV measurements were performed in triplicate at seven different ovomucoid concentrations with each phage particles (10^{12} PFU/mL) in order to estimate the apparent equilibrium dissociation constant.

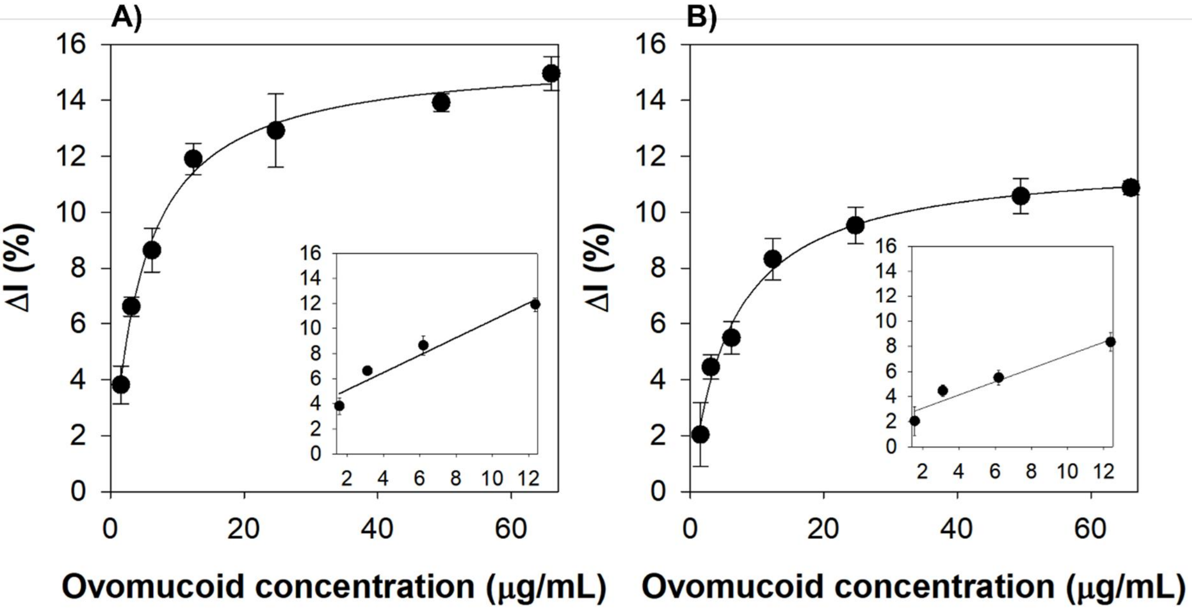


Fig. S8. Specificity test of whole phage-based electrochemical sensor (12mer 3-30). The concentrations of all protein solutions were 24.8 μg/mL. Each run was separately performed using a single electrode with successive tests in ovomucoid or other protein solutions, and the change in current (ΔI%) in response to injections of ovomucoid, ovalbumin, beta-lactoglobulin (BLG), and BSA was measured by SWV. All measurements were performed in triplicate and error bars represent standard deviations.

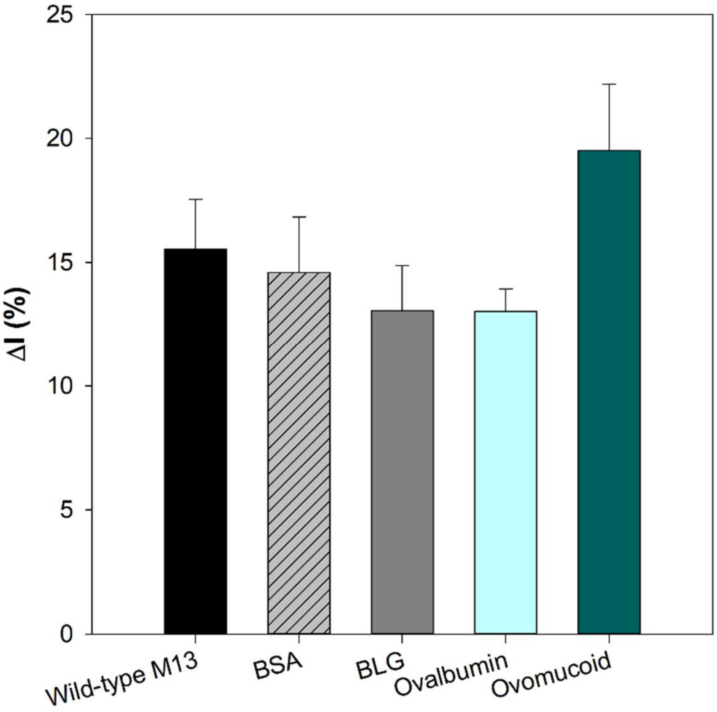


Fig. S9. AFM images of the bare gold electrode (a), C7C 2-12-tethered sensor layer (b) and 12mer 3-30-tethered sensor layer (c).

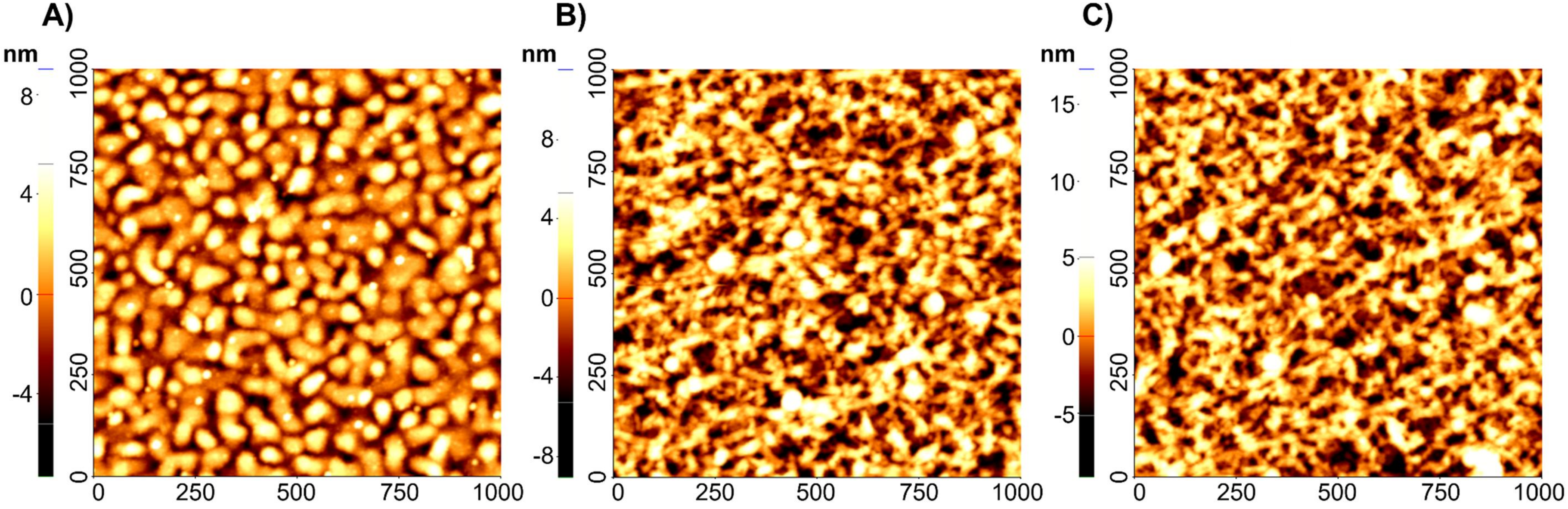


Fig. S10. Reproducibility test of C7C 2-12 phage sensor for ovomucoid detection.

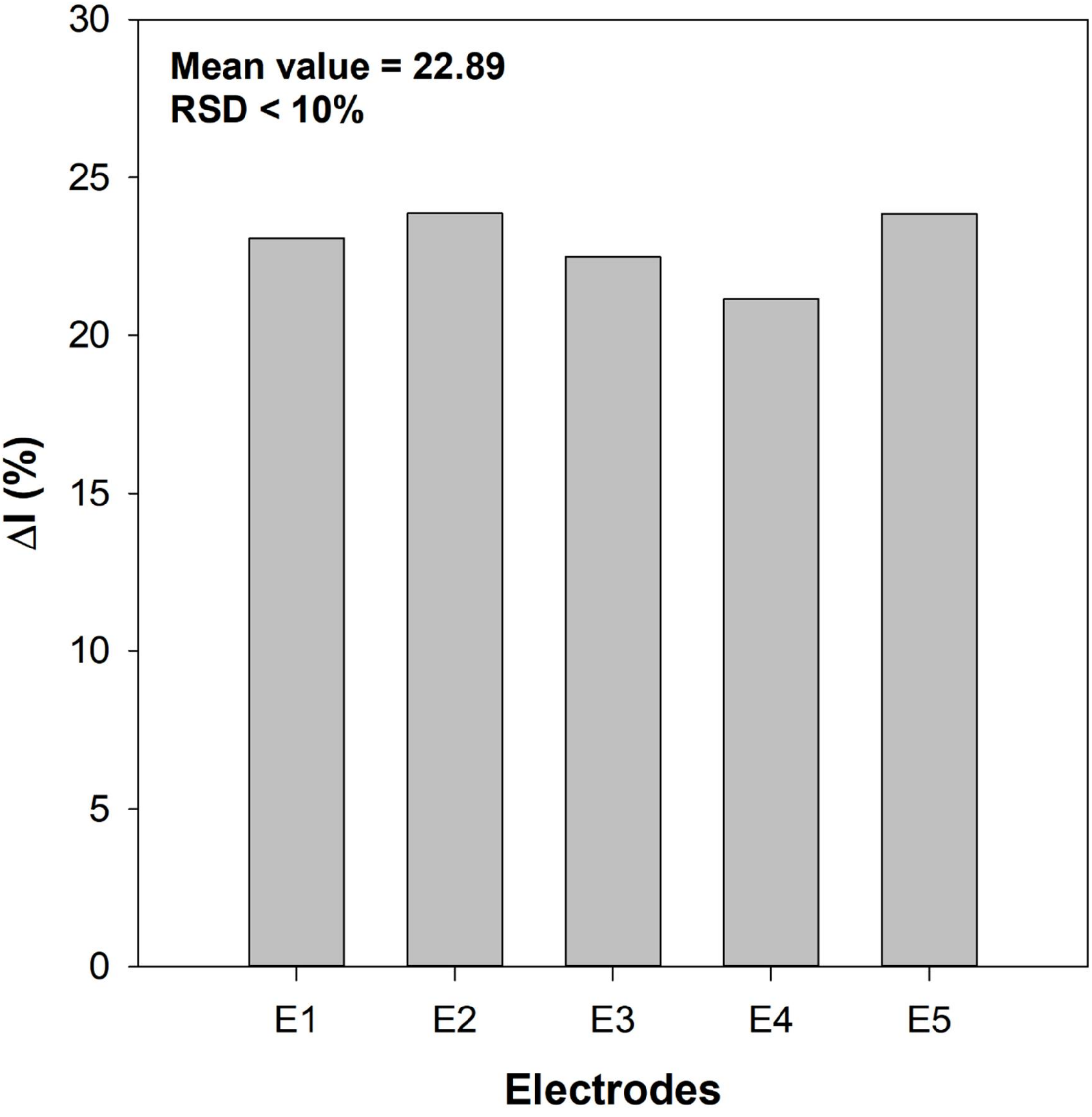
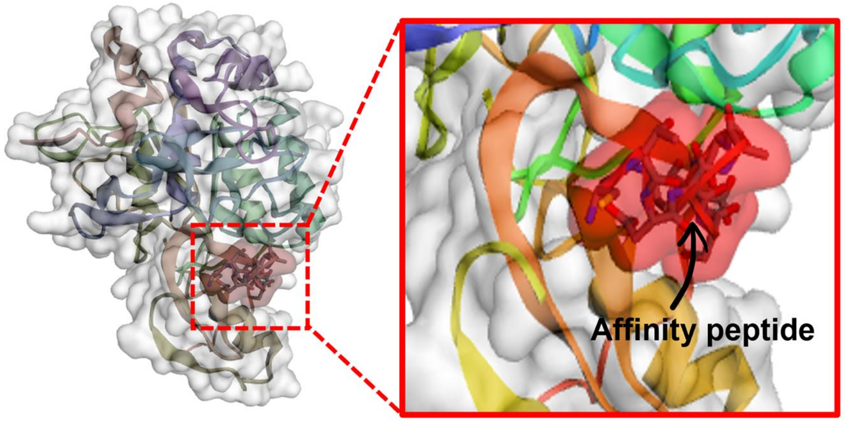


Fig. S11. Computational modeling of specific interaction between affinity peptide (CTDKASSSC) and ovomucoid protein. A) Docking prediction results of affinity peptide and ovomucoid protein, B) Binding pairs of affinity peptide and ovomucoid protein residues closer than 4.5 Å in the selected complex.

A)



- Peptide sequence: CTDKASSSC.
- Peptide secondary structure: CCCCCCCCC.
- Input structure: PDB ID: 1ROR, ovomucoid (protein)
- Average RMSD: 4.65604

B)

Peptide residue	Protein residue	Peptide residue	Protein residue
THR 2	SER 159	ALA 5	GLY 157
THR 2	GLY 160	SER 6	GLY 157
THR 2	ASN 158	SER 6	ILE 165
ASP 3	LYS 29	SER 6	TYR 167
ASP 3	THR 30	SER 7	ASN 36
ASP 3	ASN 158	SER 7	TYR 31
LYS 4	THR 30	SER 8	ALA 40
LYS 4	TYR 31	SER 8	TYR 31
LYS 4	ASN 158	SER 8	GLU 43
LYS 4	SER 156	SER 8	ASN 39
ALA 5	ILE 165	SER 8	ASN 36
ALA 5	ASN 163	SER 8	SER 130
ALA 5	ASN 158		

Additional references

Benede, S., Ruiz-Valdepenas Montiel, V., Povedano, E., Villalba, M., Mata, L., Galan-Malo, P., Torrente-Rodriguez, R. M., Vargas, E., Reviejo, A. J., Campuzano, S., & Pingarron, J. M. (2018). Fast amperometric immunoplatform for ovomucoid traces determination in fresh and baked foods. *Sensors and Actuators B: Chemical*, 265, 421-428. https://doi.org/10.1016/j.snb.2018.03.075.

de Luis, R., Mata, L., Estopanan, G., Lavilla, M., Sanchez, L., & Perez, M. D. (2008). Evaluation of indirect competitive and double antibody sandwich ELISA tests to determine β-lactoglobulin and ovomucoid in model processed foods. *Food and Agricultural Immunology*, 19(4), 339-350. https://doi.org/10.1080/09540100802520755.

Gomaa, A., & Boye, J. (2015). Impact of irradiation and thermal processing on the immunochemical detection of milk and egg allergens in foods. *Food Research International*, 74, 275-283. https://doi.org/10.1016/j.foodres.2015.05.023.

Li, Y., Song, C., Zhang, K., Wang, M., Yang, K., Yang, A., & Jin, B. (2008). Establishment of a highly sensitive sandwich enzyme-linked immunosorbent assay specific for ovomucoid from hen's egg white. *Journal of Agricultural and Food Chemistry*, 56(2), 337-342. https://doi.org/10.1021/jf0724522.