Electronic supplementary information (ESI)

Sensitive colorimetric immunoassay of *Vibrio parahaemolyticus* based on specific nonapeptide probe screening from phage display library conjugated with MnO_2 nanosheets with peroxidase-like activity

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Contents:

1. Supplementary Experimental Details

Selection of V. parahemolyticus-binding phages with f8/9 landscape phage display library.

Procedures of biopanning against bacterial cells, preparation of starved cells, titering and amplification of landscape phages have been already described in preceding publications[1-6]. Briefly, 50 μL of concentrated freshly grown target bacterial cells (~2×10^7 cells·mL^{-1}) were put into a 96-well plate and incubated at 37 ℃ overnight to dryness, and blocked with 1 % (w/v) BSA for 1 h at room temperature (RT). After washing with TBST (TBS containing 0.5 % Tween 20) for eight times, about 10^{11} phage virions from f8/9 landscape phage library in 50 μL library diluents (TBST and 1 mg·mL^{-1} BSA) were added to the wells as the input for the first round of biopanning. After incubation for 1 h at RT, the wells were washed ten times with TBST to remove unbound phage virions. The bound phages were eluted by adding elution buffer (0.2 M glycine–HCl, pH 2.2, containing 1 mg·mL^{-1} BSA) to each well and incubated for 10 min at RT. The eluate was neutralized with 19 μL of 1 M Tris–HCl (pH 9.1) and eluted phages were propagated and purified for the next round of selection. The results of each round of selection were presented as the ratio of output to input phages in percent. After three rounds of selection, individual phage clones were picked randomly. Their genome region encoding guest-peptides were PCR amplified, detected by 1 % agarose gel electrophoresis and sequenced to determine the corresponding foreign nonapeptide sequences.

Phage capture assay

We used phage capture assay to determine the specificity and affinity of selected phages toward different pathogenic bacteria. Briefly, 50 μL of concentrated freshly grown *V. parahaemolyticus, V. anguillarum* cells *S. aureus, E. tarda, E. coli, B. cereus* and *P. vulgaris* (~2×10^7 cells·mL^{-1}) were added into different wells of a 96-well plate and incubated at 37 ℃ overnight to dryness. Then wells were covered with blocking buffer (10 mg·mL^{-1} BSA) for 1 h at RT and washed 8 times with TBST. *V. parahaemolyticus*-binding phages (1×10^6 CFU) were added to the wells with different bacteria and incubated for 1 h at RT. The following steps were the same with

the above selection protocol, and the titers of eluates from each well were determined and the phage recovery was calculated to compare the captured phages by different targets.

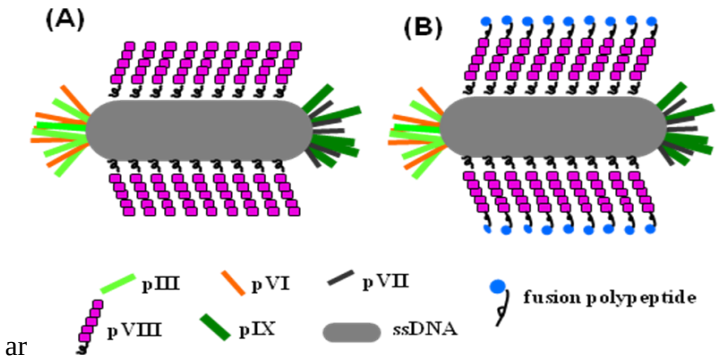
2. Mechanism study of MnO_2 nanosheets catalyzing TMB and H_2O_2

To understand the kinetics mechanism, the catalytic reaction rate of the MnO_2 NWs was measured with the different concentrations of H_2O_2 (1, 3 and 6 mM) and TMB (0.2, 0.5 and 1 mM). Subsequently, the obtained data were adjusted to the Lineaweaver-Burk double reciprocal plot. As shown in Fig. S6, the lines were parallel with each other, which was characteristic of Ping-pong BiBi mechanism. For Ping-pong BiBi mechanism, the catalyst interacted with the first substrate and released the first product before reacted with the second substrate. Based the above results and previous work[7], we proposed an electron transfer mechanism, where an electron to MnO_2 NWs and was oxidized into chromogenic derivative, and subsequently MnO_2 NWs transfer the electron to H_2O_2 and H_2O_2 was reduced into H_2O.

References

1. Brigati, J., Williams, D.D., Sorokulova, I.B., Nanduri, V., Chen, I.H., Turnbough, C.L., Petrenko, V.A. *Clin. Chem*. 2004, 50 (10), 1899-1906.
2. Brigati, J.R., Samoylova, T.I., Jayanna, P.K., Petrenko, V.A. Phage Display for Generating Peptide Reagents. Current Protocols in Protein Science, 2001, John Wiley & Sons, Inc.
3. Lang, Q.L., Wang, F., Yin, L., Liu, M.J., Petrenko, V.A., Liu, A.H. *Anal. Chem.* 2014, 86 (5), 2767-2774.
4. Liu, P., Han, L., Wang, F., Petrenko, V.A., Liu, A.H. *Biosens. Bioelectron*. 2016, 82, 195-203.
5. Sorokulova, I.B., Olsen, E.V., Chen, I.H., Fiebor, B., Barbaree, J.M., Vodyanoy, V.J., Chin, B.A., Petrenko, V.A. *J. Microbiol. Methods* 2005, 63 (1), 55-72.
6. Wang, F., Liu, P., Sun, L., Li, C., Petrenko, V.A., Liu, A. *Sci. Rep*. 2014, 4, 6808.
7. L. Han, J. Shi, A. Liu, *Sensors Actuators B: Chem*., 2017, 252, 919-926.

3. Supplementary Scheme



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Scheme S1. Phage display. (A) Phage vector f8-6 (Petrenko and Smith 2005) is composed of ~4,000 copies of the pVIII major coat protein encapsulating a ss DNA genome, and minor coat proteins pIII, pVI, pIX, and pVII. (B) pVIII phage display library f8/9. Random 9-mer peptide is fused to every copy of pVIII proteins.

4. Supplementary Table

Table S1. Amino acid sequences of selected phages

phage	nucleic acid sequence	peptide sequence
F1	GTGCAGACGGTTCAGATTGGTTCGGAT	VQTVQIGSD（3/5）
F2	GTTTCGTCTGTTAGTACGGGTGAGTCG	VSSVSTGES（1/5）
F3	GTTTCGACGTTGGAGTCTAATCAGGAT	VSTLESNQD（1/5）

5. Supplementary Figures

fd	**AEGDDPAKAAFDSLQASATEYIGYAWAMVVVIVGATIGIKLFKKFTSKAS-50**
f8/8:	**AXXXXXXXXDPAKAAFDSLQASATEYIGYAWAMVVVIVGATIGIKLFKKFTSKAS-55**
f8/9:	**AXXXXXXXXXPAKAAFDSLQASATEYIGYAWAMVVVIVGATIGIKLFKKFTSKAS-55**

Fig.S1 Major coat protein in phage fd and polyvalent f8-type fusion-peptide phage-displayed libraries (landscape libraries). See description in the text. X-random amino acid.

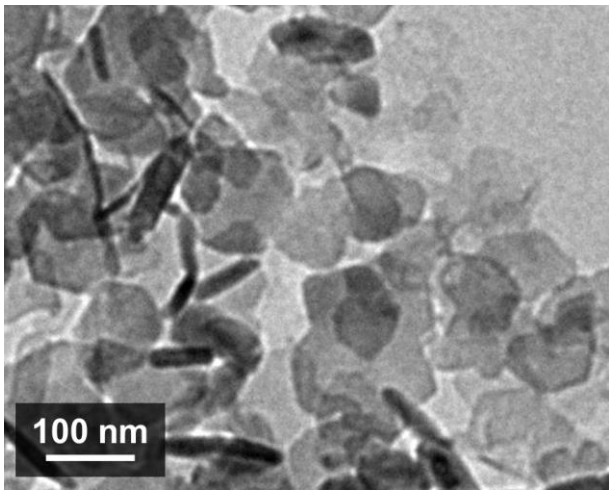

Fig. S2 TEM images of pVIII-templated MnO_2 NSs

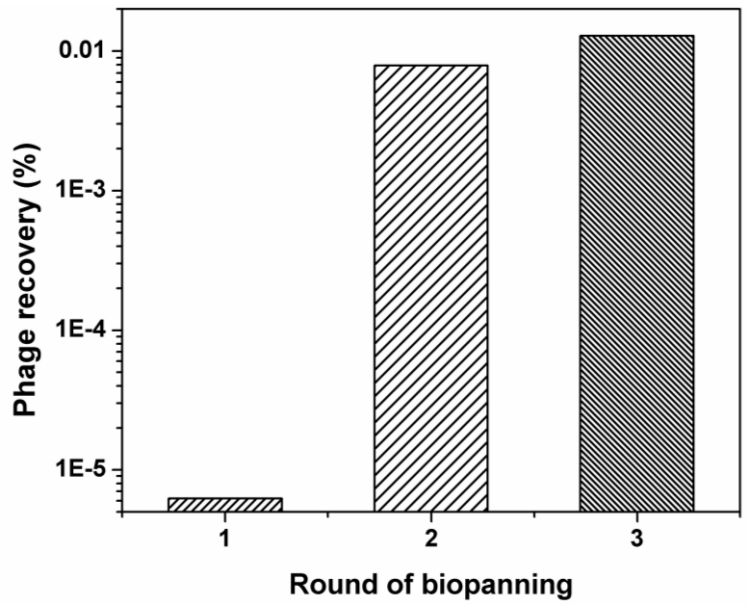

Fig.S3 Phage recovery in three rounds of biopanning. The recovery rate was calculated as a percentile of the ratio of output phages to input phages.

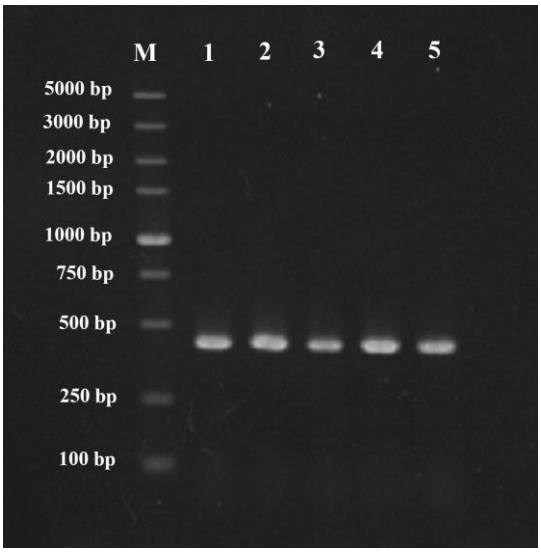

Fig. S4 The identification of PCR products by electrophoresis. Lane M, DNA marker; Lanes 1-5, the selected phages.

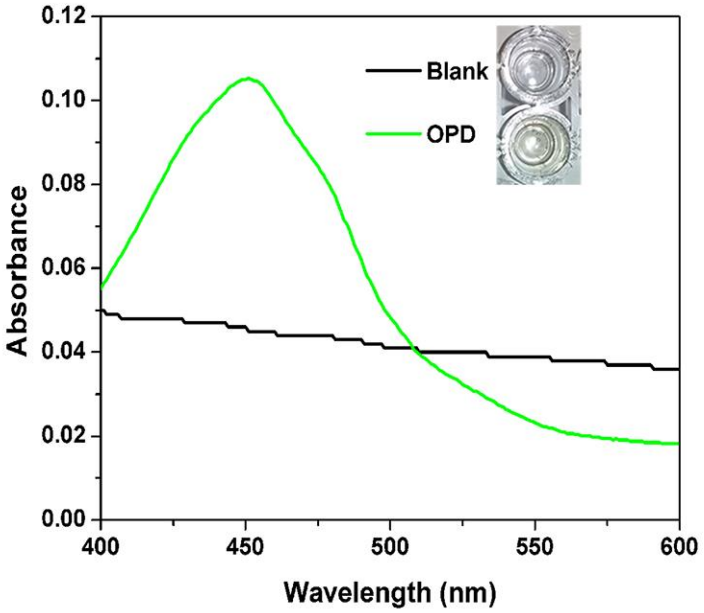

Fig.S5 The peptide-MnO_2 NSs catalyze oxidation of peroxidase substrate (OPD) in the absence (black) and presence (green) of H_2O_2. Inset, the corresponding picture.

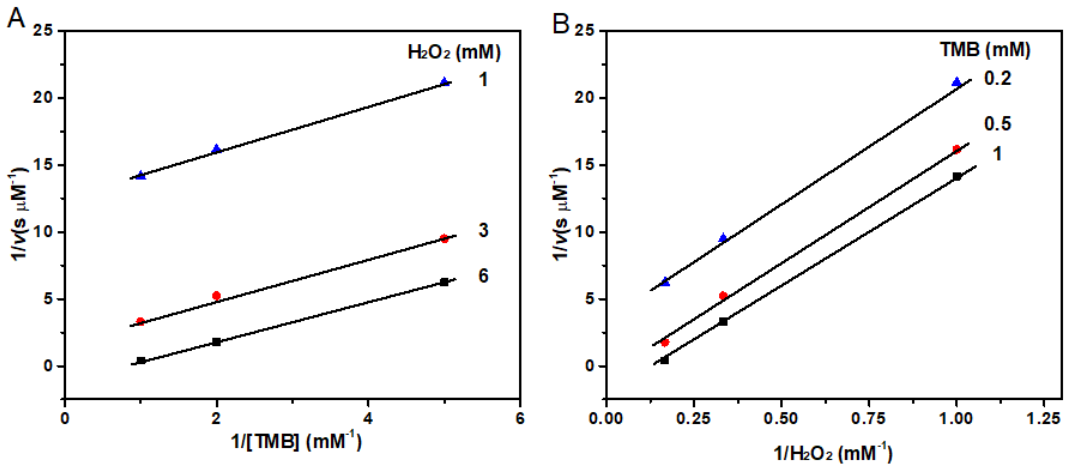

Figure S6. The Lineaweaver-Burk (double-reciprocal) plots from activity data of the concentrations of H_2O_2 (1, 3 and 6 mM) (A) and TMB (0.2, 0.5 and 1 mM) which were varied one at a time (B).

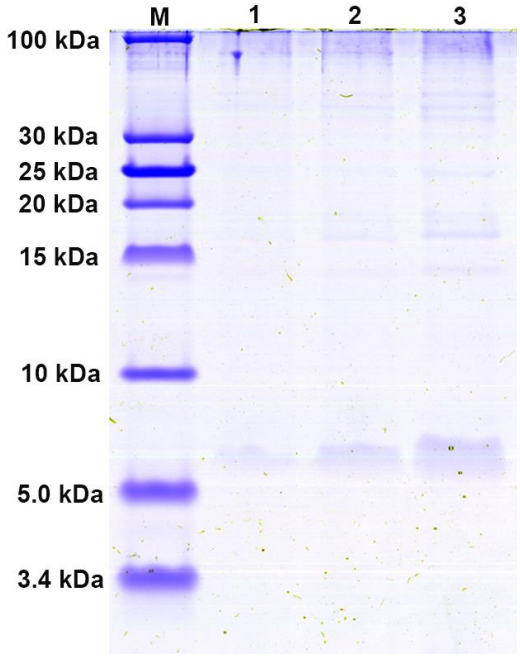

Fig. S7 16 % Tricine-SDS-PAGE of purified coat proteins of F1 phage, of which lane M was protein marker and lanes 1, 2, and 3 were loaded 0.25, 0.5, and 1 μg of purified protein, respectively.

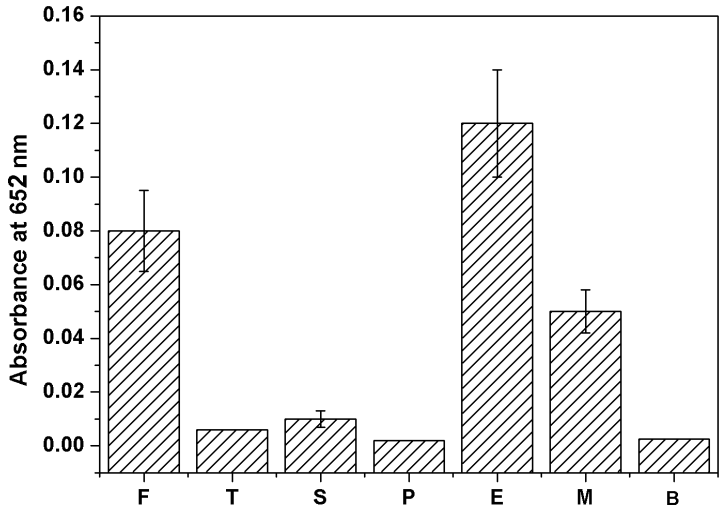

Fig. S8 Selectivity test of MnO_2 NSs@control-pVIII fusion. The corresponding absorbance at 650 nm of the reaction solutions. F, *V. parahaemolyticus*; M, *V. anguillarum*; S, *S. aureus*; E, *E. tarda*; T, *E. coli*; P, *P. vulgaris*; B, *B. cereus*. Error bars represent the standard deviation of three replicates.