RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

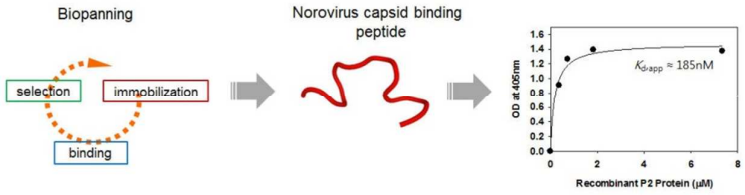
Graphic Abstract

Identification of high affinity peptides for capturing Norovirus capsid proteins

H. J. Hwang, M.Y. Ryu and J. P. Park*

Department of Pharmaceutical Engineering, Daegu Haany University, 290 Yugok-dong, Gyeongsan, Gyeongbuk 712-715, Republic of Korea

The best peptide specific for recombinant Norovirus capsid proteins was found to have nanomolar affinity.



RSC Advances

COMMUNICATION

Identification of high affinity peptides for capturing Norovirus capsid proteins

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

H. J. Hwang,[a] M. Y. Ryu,[a] and J. P. Park[a]*

Here, we present a platform where novel short and linear peptide motifs that enable binding to Norovirus capsid proteins are selected by M13 phage display. The best peptide which recognizes recombinant Norovirus 6H-P2 proteins has a sequence of 'QHIMHLPHINTL' and the apparent dissociation binding constants ($K_{d,app}$) of the selected peptides was found to be 185 nM of affinity.

The Noroviruses (Norwalk-like virus) are one of the major causative factors of viral gastroenteritis in humans.[1, 2] It contains a single stranded RNA consisting of three open reading frames (ORFs). In fact, ORF1 encodes non-structural proteins, while ORF2 encodes the major (VP1) and minor (VP2) capsid proteins. Among ORF2, VP1 has two major domains containing the shell (S) and the protruding (P) domain. The P domain is structurally categorized into P1 and P2 sub-domain. In some of the structure-to-function studies with crystallographic and mutational approaches,[2-4] P1 subdomain was connected with S domain inside, while P2 subdomain was positioned on the outmost with exposed shape and involved in Norovirus binding to their host cells with immunogenic activity. Even though several studies have been reported for efficient identification and biosensing of Norovirus,[3, 5, 6] it still has some bottlenecks including no reliable cell culture with laborious preparation steps for ready-to-use in a real test.

In an effort to solve these limitations, an efficient diagnostic method has been developed for the early detection of Norovirus from various resources including polluted water, food and other types of samples.[6-8] For example, antibody-based immunoassay, reverse transcription (RT)-PCR and other methods are currently being studied.[6, 7, 9] However, only one immunoassay has been clinically approved and extensively used for Norovirus detection.[7] It relies mainly on antibodies as molecular binders and requires multi-step processing for sample preparation and labeling of the samples in some cases.[10] Polyclonal antibody are relatively cheap to produce by immunizing animals, but are heterogeneous to binding properties, while monoclonal antibody are expensive to produce, but homogeneous. Both antibodies easily lose their binding properties under unfavorable environmental conditions such as high temperature, pH and other harsh circumstances.[10, 11] Several efforts have been made to address these bottlenecks on the molecular sensing including the use of aptamers[12] and peptides.[7, 11] Compared to antibody-based immunoassay, unique and short linear peptides have proven to be effective method for biosensing platform technology.[7, 10, 11]: a) one of major benefits is the small size of the binding peptides which can be synthesized easily and cost-effectively; b) peptides are more stable and amenable than antibodies to manipulate at the molecular scale and this can facilitate the creation of a novel diagnostic biosensor, c) peptides have lower immunogenicity and higher surface density and it can thus be employed for novel functional biomaterials. Therefore, improving current diagnostic technology is needed for simple, sensitive and accurate detection with high sensitivity and specificity.

Biopanning of M13 phage displayed peptide libraries is a powerful technology that enables the identification of novel peptide motifs that bind to almost limitless targets of interest.[10, 13] There have been some reports where M13 phages are employed as the sensing probes on clinical application[7], imaging probes on cancer therapy[14], novel scaffold materials for tissue engineering[15] and energy generation.[16] Thus, small and recognizing peptides can be used in a wider variety of sensing material for high-throughput capturing and detection of biomarkers.

In this study, we utilized M13 phage display library to identify novel peptide motifs that bind to recombinant Norovirus capsid VP1 proteins. We selected the P1 and P2 subdomain from P domain as target antigens for the use of Norovirus detection. Therefore, we constructed the plasmid, pET22b-6H-P1 (amino acids 406-520) and pET22b-6H-P2 (amino acids 279-405) fusion protein expression vectors. The recombinant 6H-P1 fusion proteins were expressed as insoluble fraction in *E. coli* BL21(DE3) and therefore it was denatured with 8M urea and refolded by dialysis. Unlikely, the recombinant 6H-P2 proteins were successfully purified as soluble form with Ni-NTA column chromatography (Fig.

[a] *Department of Pharmaceutical Engineering, Daegu Haany University, Gyeongsan 712-715, Republic of Korea. Email: jppark@dhu.ac.kr; Fax: +82-53-819-1406*
Electronic Supplementary Information (ESI) available: Experimental details including construction of plasmid, expression, purification and biopanning of M13 phages. See DOI: 10.1039/x0xx00000x

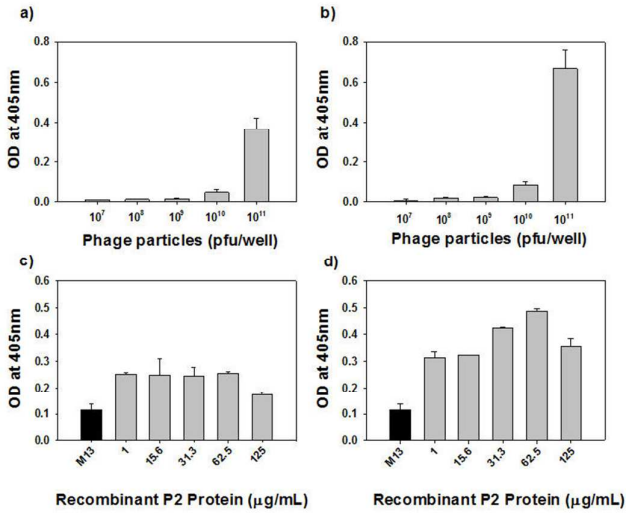


Fig. 1 ELISA assays of the selected phage clones. (a) and (c): 5R7A2, (b) and (d): 5R7A10

S1, S2 and S3). Finally purified two fusion proteins were used as target proteins for further studies. At this point, we first verified the reactivity of two recombinant proteins, 6H-P1 and 6H-P2 with interacting anti-Norovirus capsid VP1 antibody (Fig. S4). It was shown that recombinant 6H-P2 proteins play an important role in antibody binding compared to recombinant 6H-P1 proteins. Although we have no obvious clue in these results, it is probably due to the most exposed region of the P2 domain and thus the epitope of this polyclonal antibody may be close to P2 domain.

To identify linear peptide motifs that specifically bind to recombinant 6H-P1 or 6H-P2 fusion proteins, we biopanned an M13 phage library against both two proteins. The enrichment after each round is listed in Table S1 and S2. As expected, the yields of eluted phage clones increased during biopanning experiments in both cases. For the recombinant 6H-P1 proteins, the percent yields increased from 1.48×10^{-5} after the first round to 6.86×10^{-1} after the fifth round. When biopanning against recombinant 6H-P2 proteins, the percent yield increased from 3.68×10^{-6} after the first round to 2.28×10^{-1} after the fifth round.

Samples of the eluted phages from the fourth and fifth rounds of biopanning were used to purify plaques of individual phage clones and to analyze DNA sequencing. In the case of the recombinant 6H-P1 proteins, 20 clones (9 from the 5th round and 11 from the 4th round) were used to prepare genomic DNA for sequencing analysis (Table S3). The sequencing results showed that 10 of the 20 sequences obtained were found to be identical (5R5A12 with amino acid sequence IRPHRMRMLIQM). In the recombinant 6H-P2 proteins, 65 clones were prepared for DNA sequencing and from these 15 readable sequences were obtained. The sequences showed up 7 within the 15 clones (5R7A2 with amino acid sequence LSITSLRIMRLQ and 5R7A10 with amino acid sequence QHIMHLPHINTL). Since these are the dominant sequence to recombinant 6H-P2 proteins, only two clones were used for further characterization.

ELISA was performed to determine the binding affinity of the 5 phage clones for their target proteins. The results showed that

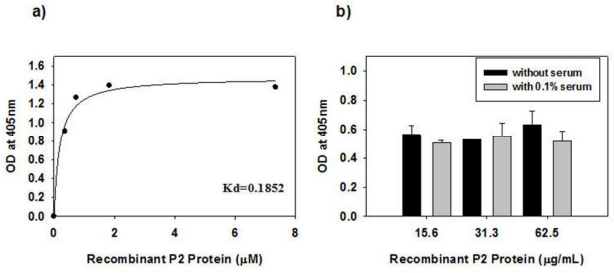


Fig. 2 (a) Apparent binding constants (Kd,app) of the best peptides following biopanning, (b) Effects of serum for binding to target proteins

two phages (5R7A2 and 5R7A10) appeared to bind with higher affinity to recombinant 6H-P2 proteins, while the affinities of phages (4R5A9, 5R5A9 and 5R5A12) against recombinant 6H-P1 proteins were less (Fig. 1a and 1b). Among the 5 positive phage clones, 5R7A2 and 5R7A10 clones were found to be potential peptide candidates for targets.

To study the binding affinity of the selected phage clones at different protein concentrations, 5R7A2 and 5R7A10 clones were tested by ELISA. As shown in Fig. 1c and 1d, only the 5R7A10 clones appeared to be better than the 5R7A2 clones, indicating that the relative binding affinity of 5R7A2 clones was much less to the same target proteins. In detail, the binding affinity of 5R7A10 clones increased with the increase in recombinant 6H-P2 protein concentrations. At the recombinant protein concentration of 62.5 μg/mL, the interaction of phage clones displaying peptides with proteins was almost completed. The sequences have a several basic amino acids like His and they are rich in hydrophobic amino acids like Ile, Met, Leu and Pro, suggesting that hydrophobic interactions is exploited in the binding of the recombinant Norovirus capsid proteins. And, the sequence displaying on 5R7A10 clones contains a single Pro which may stabilize peptide conformation or flexibility.

To measure the apparent dissociation binding constants ($K_{d,app}$) of the 5R7A10 clones, we performed in-direct ELISA protocol, as described previously.[10] As shown in Fig. 2a, the $K_{d,app}$ values of 5R7A10 clones were determined to be 185 nM of binding affinity for their targets. We also investigated the effects of serum for binding against target proteins. Interestingly, binding affinity of the best peptides, 5R7A10 were still comparable after incubation with 0.1% serum, even though binding affinity was slightly decreased in the presence of serum (Fig. 2b). Therefore, we concluded that the best peptide (5R7A10) identified by biopanning of M13 phages are found to be potential molecular binders for detection of Norovirus in contaminated food or water.

Conclusions

In this study, novel and linear peptide motifs which bind to Norovirus capsid VP1 proteins were identified and characterized. The best peptide recognizing recombinant 6H-P2 proteins had a sequence of QHIMHLPHINTL. Based on the kinetic ELISA assay, the apparent binding constants of the best

peptides was found to be nanomolar affinity for their target. These results suggest that evolved or manipulated peptide derivatives from this study may be useful for the detection of Norovirus in a miniaturized peptide biosensor with high sensitivity and selectivity. In this regard, the characterization of synthetic peptides away from the phage particles both in solution and upon immobilization by using QCM as well as SERS is now under consideration.

Acknowledgements

This study was supported by the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2011-0010312) and by the Korea government (MSIP) (No. NRF-2014R1A2A2A01005612).

Notes and references

1 M. Tan, P. Fang, T. Chachiyo, M. Xia, P. Huang, Z. Fang, W. Jiang and X. Jiang, *Virology*, 2008, **382**, 115-123.
2 W. Bu, A. Mamedova, M. Tan, M. Xia, X. Jiang and R. S. Hegde, *Journal of Virology*, 2008, **82**, 5340-5347.
3 M. Tan and X. Jiang, *Journal of Virology*, 2005, **79**, 14017-14030.
4 T. Yoda, Y. Terano, A. Shimada, Y. Suzuki, K. Yamazaki, N. Sakon, I. Oishi, E. T. Utagawa, Y. Okuno and T. Shibata, *Journal of Medical Virology*, 2000, **60**, 475-481.
5 G. S. Hansman, D. W. Taylor, J. S. McLellan, T. J. Smith, I. Georgiev, J. R. H. Tame, S.-Y. Park, M. Yamazaki, F. Gondaira, M. Miki, K. Katayama, K. Murata and P. D. Kwong, *Journal of Virology*, 2012, **86**, 3635-3646.
6 S. Ishida, S. Yoshizumi, T. Ikeda, M. Miyoshi, M. Okano and T. Okui, *Journal of Medical Virology*, 2008, **80**, 913-920.
7 J. D. Rogers, N. J. Ajami, B. G. Fryszczyn, M. K. Estes, R. L. Atmar and T. Palzkill, *Journal of Clinical Microbiology*, 2013, **51**, 1803-1808.
8 D. Sano, T. Matsuo and T. Omura, *Applied and Environmental Microbiology*, 2004, **70**, 3434-3442.
9 A. Giamberardino, M. Labib, E. M. Hassan, J. A. Tetro, S. Springthorpe, S. A. Sattar, M. V. Berezovski and M. C. DeRosa, *PLoS One*, 2013, **8**, e79087.
10 J. P. Park, D. M. Cropek and S. Banta, *Biotechnology and Bioengineering*, 2010, **105**, 678-686.
11 J. Wu, J. P. Park, K. Dooley, D. M. Cropek, A. C. West and S. Banta, *PLoS One*, 2011, **6**, e24948.
12 R. Beier, C. Pahlke, P. Quenzel, A. Henseleit, E. Boschke, G. Cuniberti and D. Labudde, *FEMS Microbiol Lett*, 2014, **351**, 162-169.
13 S. Swaminathan and Y. Cui, *RSC Advances*, 2012, **2**, 12724-12727.
14 J. W. Kehoe and B. K. Kay, *Chem Rev*, 2005, **105**, 4056-4072.
15 S. Y. Yoo, J. W. Oh and S. W. Lee, *Langmuir*, 2011, **28**, 2166-2172.
16 B. Y. Lee, J. Zhang, C. Zueger, W. J. Chung, S. Y. Yoo, E. Wang, J. Meyer, R. Ramesh and S. W. Lee, *Nat Nanotechnol*, 2012, **7**, 351-356.

Identification of high affinity peptides for capturing Norovirus capsid proteins

H. J. Hwang, M.Y. Ryu and J. P. Park*

Department of Pharmaceutical Engineering, Daegu Haany University, 290 Yugok-dong, Gyeongsan, Gyeongbuk 712-715, Republic of Korea

Materials and Methods

Chemicals

Horseradish peroxidase (HRP) conjugated anti-M13 monoclonal antibody was purchased from GE Healthcare (Piscataway, NJ). Tween 20 and ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt were purchased from Sigma (St. Louis, MO). Unless otherwise stated, all of chemicals were of reagent grade.

E. coli strains and bacteriophages

Escherichia coli strain ER2738 [F'*proA*$^{+}$*B*$^{+}$ *lacI*^{q} Δ(*lacZ*)*M15 zzf::Tn*10(Tet^{R})/*fhuA2 glnV* Δ*(lac-proAB) thi-1* Δ(*hsdSmcrB)5*] as host for M13 phage infection and M13 random phage displayed library (Ph.D.-12) which expresses random and linear 12-mer peptides were obtained from New England Biolabs (Ipswich, MA). In brief, this peptide library is fused to pIII protein which causes five copies of a particular peptide to be displayed on the surface of phage and the sequence of each peptide is encoded in the phage genome.

Cloning of the norovirus capsid P1 or P2 domain

The Norwalk virus capsid gene (VP1) sequences were obtained from GenBank (accession number no. Q83884). The gene encoding Norwalk virus envelope protein of 124 or 126 amino acids was located in the base pairs from 5358 to 6950. The P1 domain consists of amino acid residues 225 to 278 and 406 to 520. The P2 domain consists of amino acids 279 to 405 as an insertion into the P1 domain. To improve the expression of P1 proteins containing 406-520 amino acids or P2 proteins containing 279-405 amino acids fused with six histidines at N-terminal, the Norwalk virus capsid gene coding sequence was altered to reflect the optimized codon-usage pattern of *E. coli* with high-frequency codons. Based on this optimized sequence, recombinant gene was synthesized by using HT-oligoTM synthesizer (Bioneer, Daejeon, Korea).

Notably, the synthesized genes have the same amino acid sequence as the native protein sequence in Norwalk virus. For the cloning and expression of pET22-6H-P1 and pET22-6H-P2 fused genes in *E. coli*, PCR experiments were carried out using the following two primers. The forward primer 5'-CCCAGAACTCATATGCACCACCACCACCACCACGCCCCTTCTGTATACCCC CCTGGT TTCGGAGAG GTA for P1 domain was designed to contain an *Nde*I site (underlined). The backward primer, 5'-ACAAACCTACTCGAGTCATCGGCGCAGACCAAGCCTACCTCTTGCCGAGCT GGCAGT for P1 domain was designed to contain an *Xho*I (underlined). The forward primer 5'-CCCAGAACTCATATGCACCACCACCACCACCACACCACCCCAGTTTCATTGTCACAT GTTGCCAAGATA for P2 domain was designed to contain an *Nde*I (underlined). The backward primer 5'-

ACAAACCTACTCGAGTCATAGATGTGTTGCCTCCGTAATACTTGACCCATAATT for P2 domain was designed to contain an *Xho*I (underlined).

PCR experiments were performed with a PCR thermal Cycler T1 (Biometra Co., Goettingen, Germany) using the High Fidelity PCR machine (Boehringer Mannheim, Mannheim, Germany). All restriction enzymes use in this study were purchased from New England Biolabs (Beverly, MA). The DNA sequences of all genes were confirmed by sequencing with the automatic DNA sequencer (ABI Prism model 377, Perkin Elmer Co., Downer's Grove, IL). The amplified fragments were digested with restriction enzymes *Nde*I and *Xho*I (sites underlined) and ligated into the same sites of pET22b(+) (Novagen, Darmstadt, Germany) to make pET22-6H-P1 and pET22-6H-P2.

Production of 6H-P1 and 6H-P2 fusion proteins

The 6H-P1 and 6H-P2 fusion proteins were produced by cultivation of *E. coli* BL21(DE3) harboring pET22-6H-P1 and pET22-6H-P2, respectively. For the expression, cells were cultivated in 250 mL flask containing 100 mL of LB medium (tryptone 10 g/L, yeast extract 5 g/L, NaCl 5 g/L) supplemented with ampicillin (50 μg/mL) in as shaking incubator at 37℃ and 200 rpm. At the OD_{600}=0.6, isopropyl-β-D-thiogalactopyranoside (IPTG, Sigma, St. Louis, MO) was added to a final concentration of 1 mM. Cells were further cultivated for 5 h and were harvested by centrifugation at 6,000 rpm for 10 min at 4℃ and washed two times with buffer (50 mM Tris-HCl, 1 mM EDTA, pH 8.0). And then, cells were disrupted by sonication for 1 min at

40% ouput. After centrifugation at 12,000 rpm for 30 min at 4℃, the supernatant was used for the purification by using Ni-NTA column chromatography (Qiagen, Valencia, CA).

Purification of fusion proteins

As two fusion proteins have a six histidine tag at N-terminus, they could be simply purified using Ni-NTA resin. The soluble 6H-P2 recombinant proteins expressed from *E. coli* BL21(DE3) cells were purified under native conditions with a Bio-Rad HR system (Bio-Rad). Briefly, the cell lysates were loaded onto a column containing IDA Excellose resin (Bioprogen Co., Daejeon, Korea) and the samples of flow-through containing unbound proteins were collected for subsequent analysis. The insoluble 6H-P1 recombinant protein fractions of *E. coli* cells as inclusion bodies were re-suspened in 50 mM Tris-HCl, 1 mM EDTA, 2M Urea (pH 8.0) and denatured under denaturing conditions (50 mM Tris-HCl (pH8.0), 0.2 M NaCl, 8 M Urea, 10 mM DTT, 10% Glycerol. After denaturation, the samples were incubated with IDA Excellose resin at 4 °C for 1 h with shaking for effective binding of the 6His-tagged proteins to the resin. Then, the fusion proteins were refolded by slowly removing urea with dialysis. Finally, the eluted proteins were dialyzed against following buffers (refolding method; step dilution from 8 M to 2 M (50 mM Tris-HCl, 0.2 M NaCl, 1mM DTT, 10% Glycerol, pH 8.0).

SDS-PAGE electrophoresis

The recombinant proteins from total cell extracts, soluble and insoluble proteins were separately by SDS-PAGE. For total cell extracts and insoluble fractions after cell disruption, cell

pellets were directly re-suspended in an appropriate sample buffer and analyzed in 15% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) gels with Coomassie blue staining. The protein concentration was determined using Bradford protein assays.

General M13 methods and DNA sequencing analysis

Phage particle purification, concentration and DNA isolation were carried out according to the instructions of the manufacturer. Clones of interest were sequenced using the -96 pIII sequencing primer (New England Biolabs, 5'-GCCCTCATAGTTAGCGTAACG-3').

Biopanning phage-displayed peptide library

Highly purified two recombinant proteins encoding pET22-6H-P1 or pET22-6H-P2 were dissolved in 50 mM Tris-HCl (pH 7), 50 mM NaCl buffer and transferred to the wells of the 96-microwell plates. After overnight incubation at 4°C, coated wells were filled with blocking buffer (0.1 M $NaHCO_3$ (pH 8.6), 5 mg/mL BSA, 0.02 % NaN_3) and incubated at 4°C for 1 h. After removing the blocking solution and unbound reagents, wells were washed six times with TBST (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 0.1% Tween-20). The Ph.D.-12 phage displayed random peptide library (1.5×10^{11} pfu) in 100 μL of TBS buffer (50 mM Tris–HCl (pH 7.5), 150 mM NaCl) was added to each well containing immobilized recombinant proteins, and the plate was shaken gently at 4°C for overnight. To remove the unbound phage, the plate was washed 10 times with TBST. Five rounds of biopanning were performed for each recombinant proteins. After washing, the bound phages were eluted using 100 μL of 0.2 M glycine–HCl (pH

2.2). The eluent was immediately neutralized with 15 μL Tris–HCl (pH 9.1) to prevent the destruction of the phage.

The eluted phages were amplified using *E. coli* ER2738 strains to make sufficient copies for subsequent rounds of biopanning. The amplified phages were harvested by NaCl/polyethylene glycol precipitation (20% (v/v) polyethylene glycol-8000 with 2.5 M NaCl). After every round of biopanning, the recovered phages were titered by plating aliquots of the infected *E. coli* ER2738 prior to amplification on Luria–Bertani (LB) agar containing isopropyl β-D-thiogalactopyranoside (IPTG) and X-gal. The plates were incubated overnight at 37°C and the blue colonies were counted. The enrichment of bound phages was calculated as follows: output phage/input phage × 100. The blue plaques were randomly picked and amplified for DNA sequencing.

Sequence analysis and peptide synthesis

Single-stranded DNAs of positive phage clones were sequenced by Genotech (Daejeon, Korea) using the -96 pIII sequencing primer: 5′-CCC TCA TAG TTA GCG TAA CG-3′. According to the phage pIII gene-derived reading frame in the coding strand, we derived the amino acid sequence of the exogenous protein fusion to the M13 coat protein pIII. BLAST searches were performed using the SWISSPROT database to determine sequence similarity with previously identified peptides or proteins. The Clustal Omega program (http://www.ebi.ac.uk/Tools/msa/clustalo/) and ExPASy Tools (web.expasy.org/translate) translate were used to analyze the sequences identified earlier.

ELISA for peptide binding to recombinant proteins

To test whether the selected phage clones could specifically bind to recombinant proteins (6H-P1 or 6H-P2), ELISA assays were performed. Plates were coated with recombinant proteins for overnight at 4°C, blocked with blocking buffer (0.1 M $NaHCO_3$, pH 8.6, 1 % non-fat dry milk, 0.02 % NaN_3), and washed six times with TBS solution. One hundred microliters of amplified phages (10^{11} pfu) were then added and incubated for 1 h at room temperature. After washing six times with the same buffer to remove unbound phages, HRP-conjugated anti-M13 monoclonal antibody (diluted 1:10,000 in blocking buffer) was added and incubated at room temperature for 1 h. The antibody solution was removed and the plate was washed again with TBS. Freshly prepared HRP substrate (36 µL of 30% H_2O_2 added to 21 mL of ABTS stock: 22 mg of 2,2'-Azino-bis (3-ethylbenzthiazoline)-6-sulfonic acid in 100 mL of 50 mM sodium citrate, pH 4.0) was added and measured at 405 nm with a microplate spectrophotometer (Multiskan FC, Thermo Scientific, CA, USA). Polystyrene wells coated with 1 mg/mL of BSA, and wells incubated with 1 % of milk in TBS were used as negative controls.

Measurement of the apparent dissociation constants

The measurement of apparent dissociation constants ($K_{d,\mathrm{app}}$) for the selected peptides was performed according to the method of Friguet *et al.* (1985) with minor modifications. Briefly, recombinant P2 proteins at various concentrations were first incubated with the desired phage clones in TBS buffer containing 1% BSA at a fixed concentration (10^{11} pfu/mL). After 24 h of incubation at 4°C, 100 µl of each mixtures were transferred into well plates pre-coated with each recombinant P2 proteins and re-incubated for 1 h at room temperature with mild shaking. After

washing twice with TBS buffer, the bound phages were detected by adding the HRP-conjugated anti-M13 antibody and ABTS, and the color changes in the wells were measured at 405 nm. The apparent $K_{d,\mathrm{app}}$ values were estimated from the slopes of the regression curves obtained by plotting the fraction of bound antibody versus the molar concentration of recombinant P2 protein. Student's t-test was used to compare the $K_{\mathrm{d,app}}$ values.

Fig. S1. Construction of plasmids for the expression of recombinant 6H-P1 (a) or 6H-P2 protein (b).

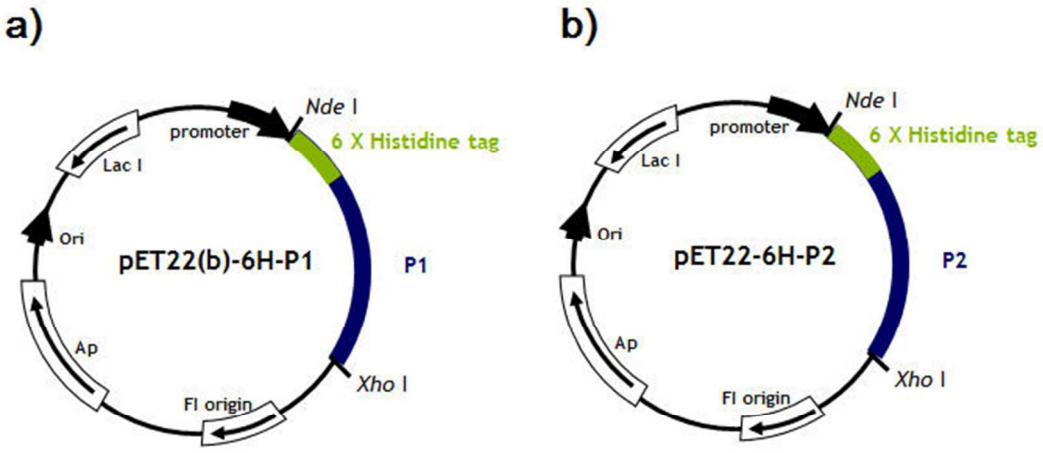


Fig. S2. SDS-PGAE analysis of recombinant fusion proteins. For 6H-P1 fusion proteins expression, *E. coli* BL21(DE3) cells harboring pET22-6H-P1 (a) or pET22-6H-P2 (c)were cultivated in 1,500 mL containing 100 mL of LB medium supplemented with ampicillin (50 μg/mL) in shaking incubator. Cells were further cultivated for 5 h after induction with IPTG were harvested by centrifugation. And then, cells were disrupted by sonication and recombinant proteins from total cell extracts, soluble and insoluble proteins were separately by SDS-PAGE. Finally, the recombinant 6H-P1 (c) or 6H-P2 (d) fusion protein was successfully purified by using Ni-NTA resins.

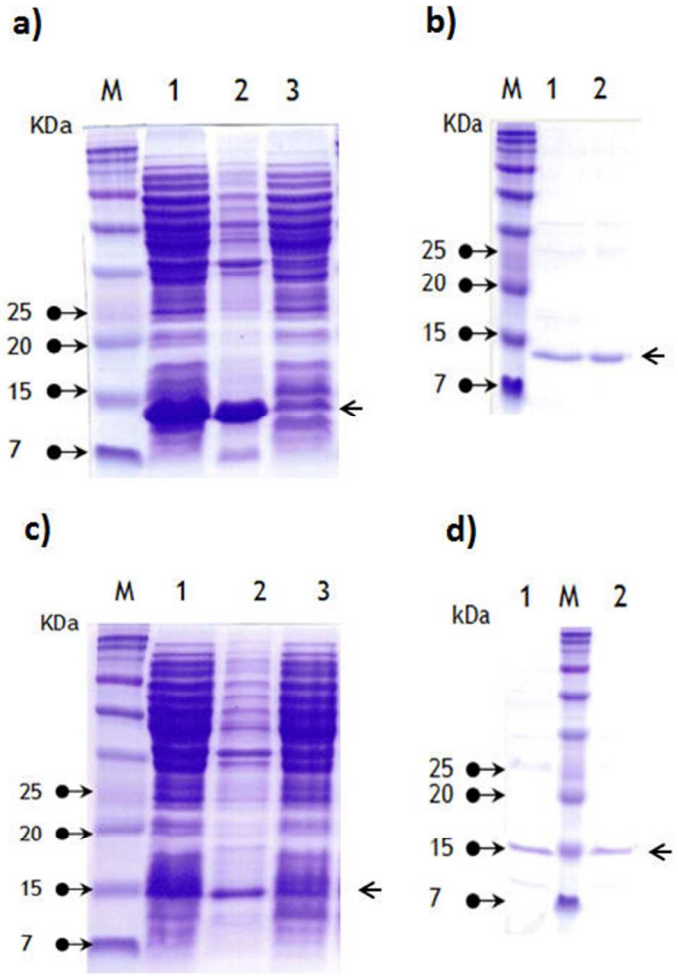


Fig. S3. SDS-PAGE analysis of recombinant 6H-P1 proteins after refolding step.

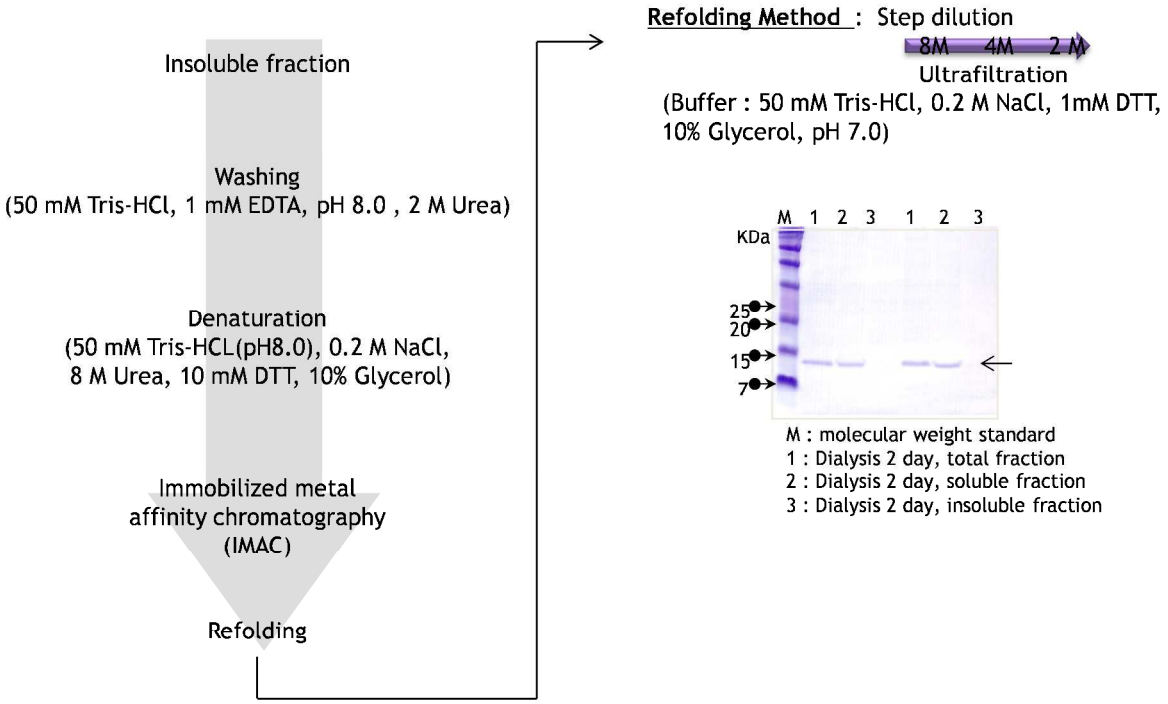


Fig. S4. Reactivity test of recombinant 6H-P1 and 6h-P2 proteins. For the antigenicity analysis, purified 6H-P1 or 6H-P2 fusion proteins was immobilized on the 96-well microplate for overnight at 4°C, blocked with blocking buffer (0.1 M $NaHCO_3$, pH 8.6, 1 % non-fat dry milk, 0.02 % NaN_3), and washed six times with TBS solution. And then, anti-Norovirus capsid protein VP1 antibody (ab92976, abcam, MA, USA) (diluted 1:5,000 in blocking buffer) was added and incubated at room temperature for 1 h. The antibody solution was removed and the plate was washed again with TBS. Freshly prepared HRP conjugated anti-IgG antibody (diluted 1:1,000 in blocking buffer) was added into the wells and incubated for 1 hr. After washing with buffer, ABTS (17 µL of H_2O_2 added to ABTS stock: 2.2 mg of ABTS in 10 mL of 50 mM of sodium citrate buffer, pH 4.0) was added and measured at 405 nm with a microplate spectrophotometer (Multiskan FC, Thermo Scientific, CA, USA).

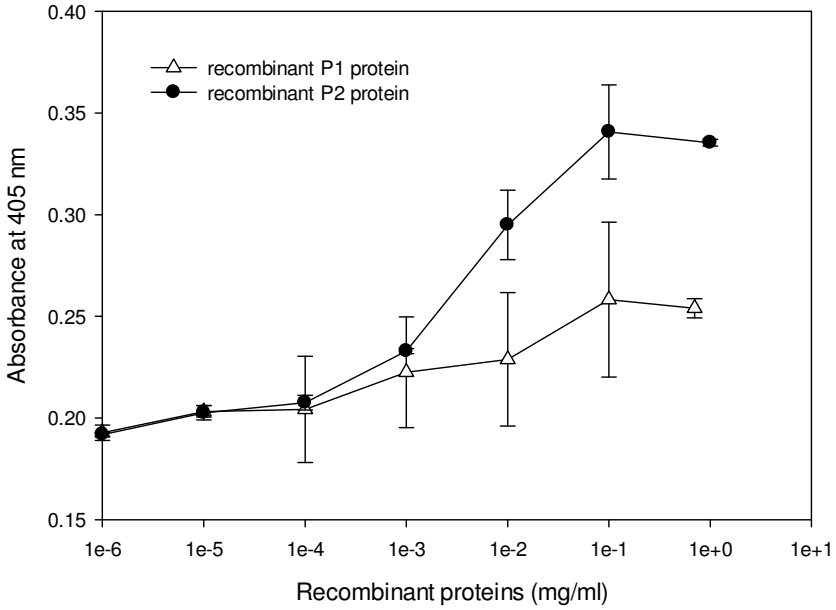


Table S1. Enrichments obtained during biopanning with the phage-displayed peptide library against Norovirus capsid recombinant protein

Biopanning round	Titer (pfu/mL)		Yield [a] (%)	
	Recombinant 6H-P1 protein	Recombinant 6H-P2 protein	Recombinant 6H-P1 protein	Recombinant 6H-P1 protein
1st				
Input	9.5×10^{12}	9.5×10^{12}	1.48×10^{-5}	3.68×10^{-6}
Output	1.41×10^{6}	3.5×10^{5}		
2nd				
Input	1.94×10^{12}	1.0×10^{12}	1.49×10^{-5}	2.5×10^{-6}
Output	2.9×10^{5}	2.5×10^{4}		
3rd				
Input	5.2×10^{11}	1.14×10^{12}	7.11×10^{-2}	2.63×10^{-3}
Output	3.7×10^{8}	3.0×10^{8}		
4th				
Input	1.2×10^{11}	3.6×10^{11}	8.0×10^{-3}	8.33×10^{-4}
Output	9.6×10^{6}	3.0×10^{7}		
5th				
Input	2.3×10^{11}	2.1×10^{11}	6.86×10^{-1}	2.28×10^{-1}
Output	1.58×10^{9}	4.8×10^{8}		

[a] Percent yield was calculated as follows: output/input phage × 100

Table S2. Sequences of the selected phage clones following the fourth and fifth rounds of biopanning against two recombinant proteins

Name	Amino acid	Frequency	Notes
For recombinant 6H-P1 proteins			
4R5A9	INRRQRPRTRHI	2/20	Selected after 4th round
5R5A9	HKRQLRHTIMRT	2/20	Selected after 5th round
5R5A12	IRPHRMRMLIQM	10/20	Selected after 5th round
For recombinant 6H-P2 proteins			
5R7A2	LSITSLRIMRLQ	7/15	Selected after 5th round
5R7A10	QHIMHLPHINTL	7/15	Selected after 5th round Strong binder by ELISA Chosen for characterization

Table S3. Analysis of the hydrophobicity/hydrophilicity of the selected peptides

Clone name	Negative charged amino acid	Positive charged amino acid	Total net charge	Total hydrophobic ratio (%)
5R7A2	no	Arg at positions 7 and 10	+2	50
5R7A10	no	His at positions 2, 5 and 8	0	41

Sequence analysis was performed with secondary structure prediction program (http://aps.unmc.edu/AP/prediction/prediction_main.php)

References

1. Sidhu SS, Fairbrother WJ, Deshayes K. 2003. Exploring protein-protein interactions with phage display. hembiochem 4:14 –25.

2. Samoylova TI, Norris MD, Samoylov AM, Cochran AM, Wolfe KG, Petrenko VA, Cox NR. 2012. Infective and inactivated filamentous phage as carriers for immunogenic peptides. J. Virol. Methods 183:63– 68.

3. Rakonjac J, Bennett NJ, Spagnuolo J, Gagic D, Russel M. 2011. Filamentous bacteriophage: biology, phage display and nanotechnology applications. Curr. Issues Mol. Biol. 13:51–76.

4. Paschke M. 2006. Phage display systems and their applications. Appl. Microbiol. Biotechnol. 70:2–11.

5. Monjezi R, Tan SW, Tey BT, Sieo CC, Tan WS. 2013. Detection of hepatitis B virus core antigen by phage display mediated TaqMan realtime immuno-PCR. J. Virol. Methods 187:121–126.

6. Schofield DA, Sharp NJ, Westwater C. 2012. Phage-based platforms for the clinical detection of human bacterial pathogens. Bacteriophage 2:105–283.

7. Yoo, M.K.; Kang, S.K.; Choi, J.H.; Park, I.K.; Na, H.S.; Lee, H.C.; Kim, E.B.; Lee, N.K.; Nah, J.W.; Choi, Y.J.; Cho, C.S. Targeted delivery of chitosan nanoparticles to Peyer's patch using M cell-homing peptide selected by phage display technique. *Biomaterials* **2010,** *31*, 7738-7747.

8. Larocca, D.; Burg, M.A.; Jensen-Pergakes, K.; Ravey, E.P.; Gonzalez, A.M.; Baird, A. Evolving phage vectors for cell targeted gene delivery. *Curr. Pharm. Biotechnol.* **2002,** *3*, 45-57.

9. Friguet B, Chaffotte AF, Djavadi-Ohaniance L, Goldberg ME. Measurements of the true affinity constant in solution of antigen-antibody complexes by enzyme-linked immunosorbent assay. J. Immunol. Methods. **1985**, 77, 305-319.