Electronic Supplementary Information (ESI) for Lab on a Chip

Rapid screening swine foot-and-mouth disease virus using micro-ELISA system

Supplementary Information

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1. Materials

Phosphate buffered saline in dry powder form(Cat. No. P3563), Tris Buffered Saline in dry powder (Cat. No.T6664) form, 4-Morpholineethanesulfonic acid (MES, CAT. NO. M3671-50G), Ethylenediaminetetraacetic acid disodium salt dehydrate (EDTA, Cat. No.E5134), Tween 20(CAT. NO. 93372), 6-amino-2-(bis(carboxymethyl)amino) hexanoic acid (ABHA, Product No. 14580), ProClin (R) 300 (Product No. 48912-U), horseradish peroxidase (HRP) labeled anti-pig IgG (whole molecule) antibody produced in rabbit (Product No.A5670), were purchased from Sigma-Aldrich. Sodium chloride (Cat. No.37144-02) was from Kanto Chemical Co., Ltd. Puraterm Nickel sulfate hexahydrate (Product No. 93-2856) was from Strem Chemicals, Inc. Ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDAC, Product No. 527-31131) was purchased from Thermo-fisher Scientific.

Carboxylated polystyrene microbeads(Lot No. PC07N/9233) was from BangsLabs. pTriEx-4 Neo plasmid, E. coli. Rosetta™ (DE3)pLacI Competent Cells were from Merck-Novagen. ProBond™ protein purification kit(Cat. No. K85001) was from Invitrogen. Slide-A-Lyzer® protein dialysis cassettes (Cat. No. 66807) was from Thermo-fisher Scientific. Vivaspin® for protein concentration (Cat. No. VS1501) was from Sartorius Stedim Biotech.

Bovine Serum Albumin (Cat. No.017-21273) was from Wako Pure Chemical Industries, Ltd. SureBlue™ TMB peroxidase substrate (Cat. No. 52-00-01) was purchased from Kirkegaard & Perry Laboratories.

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Figures were charted with San Diego Supercomputer Center Sirius ™ (Ver. 1.2) and Microcal™ Origin (Ver. 6.1), respectively.

2. Methods

2.1 Expression and purification of recombinant 3ABC protein. Based on previously reported work [7,8], the complete 3ABC coding region of FMDV O/China/99 was inserted into a pTriEx-4 Neo plasmid using Bgl II and Sal I restriction sites. The recombinant plasmids were then transformed into *E. coli* Rosetta (DE3) pLacI host and protein expression induced with 1 mM IPTG. Stimulation of the expression of fusion 3ABC protein was carried out according to the pTriEx™ System Manual The recombinant protein with an N-terminal 6 His-tag and S-tag was purified using the following procedure. The concentrated cell pellets with inclusion body were first lysised in pH 7.8, 6 M Guanidinium buffer Hydrochloride, 20 mM Sodium Phosphate, 500 mM NaCl. The supernatant containing the solubilized protein was collected for subsequent metal affinity chromatographic binding to ProBond™ Ni-NTA His binding resin according to the manufacturer's instructions. Three consecutive Slide-A-Lyzer® dialysis using 10 mM Tris, pH 8.0, 0.1% Triton X-100 overnight at 4℃ were performed then, followed by further purification to remove small impurities in the protein with Vivaspin® using membrane of cutoff molecular weight value of 10,000, such purified/concentrated recombinant 3ABC proteins of 1 mg/ ml approximately after analysis with SDS-PAGE (Fig. S4) was stored at -70℃ in small aliquots for use as the coating antigen for final microbeads immobilization and subsequent immunoassay.

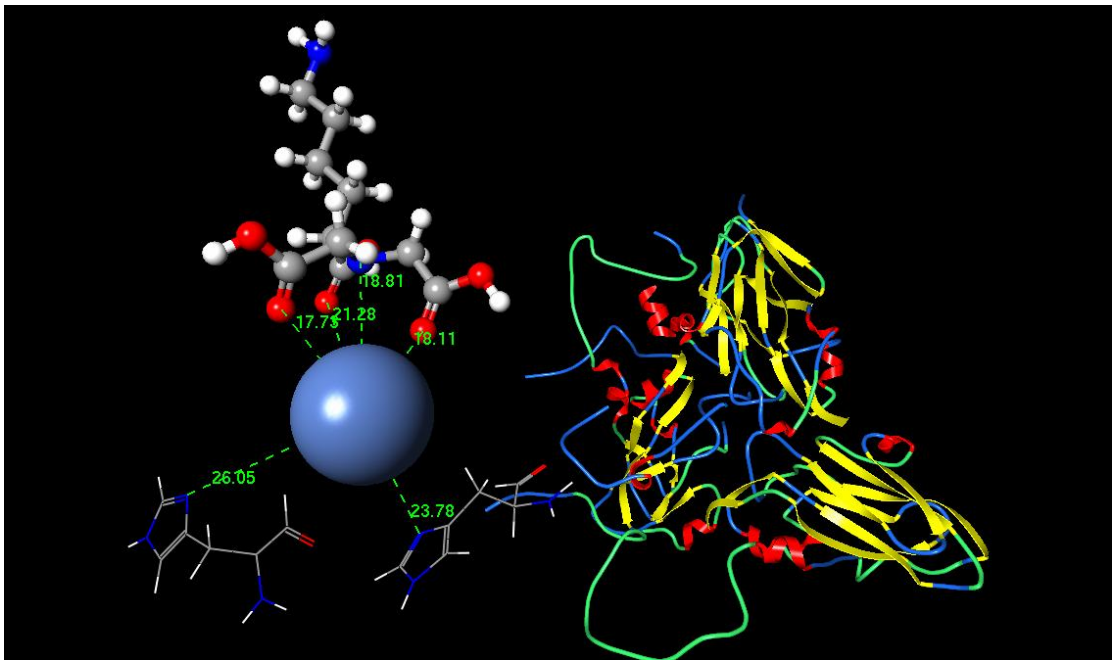
2.2 Immobilization of microbeads A nickel(II) chelating chemistry was used to immobilize the recombinant 6-His-tagged protein to polystyrene microbeads for successive microELISA immunoassay. We first pipetted 0.17 ml of microbeads and pelleted the microbeads via centrifugation for 10 minutes at 800xG, resuspended the pellet in 0.4mL of coupling buffer (50mM MES, pH 5.2; 0.05% Proclin® 300), pelleted, centrifuged and suspended the pellet again in 0.17 ml coupling buffer, then added 20µl 200 mg/ ml of the 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDAC) solution freshly prepared in coupling buffer to the suspension and mixed gently end-over-end for 15 minutes at room temperature. Secondly, we added 0.4 ml coupling buffer to wash out extra EDAC, added 0.1ml 10mM 6-amino-2-(bis(carboxymethyl)amino) hexanoic acid (ABHA) in coupling buffer and incubate microbeads overnight at room temperature, then we washed the microbeads with 0.05% Tween, 50 mM Tris HCl pH 7.5, 500 mM imidazole, 0.05% Tween and 100 mM EDTA, pH 8.0, successively. Thirdly, we incubated the microbeads with 10 mM $NiSO_4$ for 20 min at room temperature, and washed the pellet with 0.05% Tween and 50 mM Tris HCl pH 7.5, respectively. Lastly, we added 6-His tagged protein equivalent to 100 µg approximately to incubate with processed microbeads for 30-60 minutes at room temperature under gentle end-over-end mixing, centrifuged mixture for 10 minutes at 800 x G, then wash with 50 mM Tris HCl, pH 7.5, 0.05% Tween and stored the microbeads at 2-8℃ in 0.5 ml PBS containing 0.05% Tween prior to use.

2.3 MicroELISA protocol Micro-beads based microfluidic thermal lens immuno-microscopy for FMDV screening was performed automatically on microELISA platform (Fig. 2), the protocol was depicted as follows: the microchannel, the nozzle of the injector, and the PEEK tubing were firstly rinsed with 350 µl carrier solution of pH 7.4 PBS with 1% BSA and 0.05% Tween thoroughly, then 8 µl microbeads was introduced into the microchip, stopped and stacked

immediately at the upstream of the inner-dam structure, and was washed with 52μl carrier solution afterwards. 10 μl swine serum sample was introduced subsequently to the microchip at a speed of 1.0 μl/min and reacted with 6-His-tagged protein immobilized on the microbeads, then residual serum sample in the microchip was flushed out using 10 μl carrier solution followed by several post-wash steps for both micro-channel and microbeads. Finally, 10 μl horseradish peroxidase (HRP) labeled rabbit anti-pig detection antibody of 0.1μg/ml at a speed of 5 μl/min was introduced to capture the swine sera 3ABC FMDV antibody, 2μl Sureblue® TMB solution as the enzymatic reaction substrate was sucked into micro-channel at a speed of 10 μl/min, reacted for another 3 min under stop flow condition, the chromophoric substance catalyzed by HRP was flushed out by the introduction of 5μl TMB solution at a speed of 10 μl/min and was detected by the thermal lens microscopic probe integrated on the microchip.

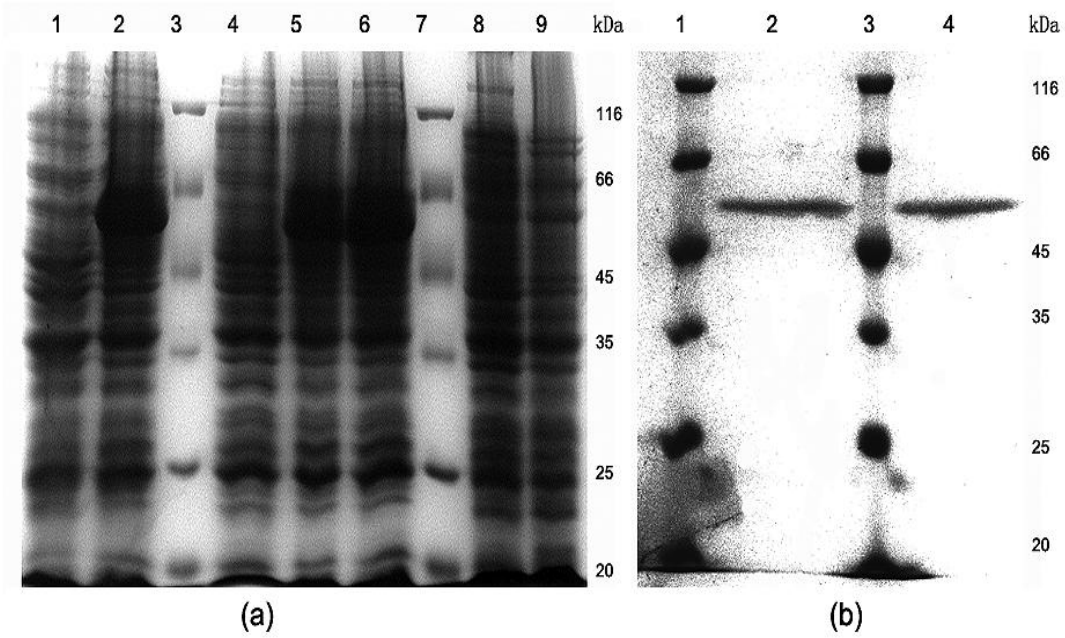
Iterated tests were automatically fulfilled by renewing microbeads for each assay, microbeads were flushed out by a counterflow of carrier solution from port ***e*** to port ***b*** when port ***a*** was synchronously closed with 3-way valve 2 checked and injector tip held with connector tightly.

3. Fig. S1: Nickel (II) chelating immobilization scheme



The central blue sphere denotes the nickel (II). The upper left is ABHA molecular in ball–and-stick style, which has four sites coordinated with nickel. The rest is the 6-His-tagged recombinant protein with two His tag coordinated with nickel to form a hexadentate.

4. Fig. S2: SDS-PAGE characterization of the expressed 3ABC protein



(a) SDS-PAGE detection the cell lysate of E. coli for expressing 3ABC protein. Lanes 1,4,8 and 9 are the cell lysates of Escherichia coli before induction while lanes 2, 5, 6 contain the cell lysate of E. coli after 4 h of induction at 37℃. Lane 3 and lane 7 contain the protein marker. **(b)** His-binding resin purification of 3ABC fusion protein. Lane 1 and lane 3 contain the protein marker. Lanes 2 and 4 are fluid eluted by pH 4.5 elution buffer. Products were separated on a 12% SDS-PAGE by Coomassie blue staining.