Understanding Aggregation-based Assays: Nature of Protein Corona and Number of Epitopes on Antigen Matters

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Preparation of DIII-His protein

N-terminal hexa-histidine-tagged, recombinant DIII (DIII-His) protein from the envelope glycoprotein of Dengue virus serotype 2 was expressed and purified as described previously[1, 2]. Briefly, DIII-His protein was isolated from *E. coli* inclusion bodies through solubilization with 8 M urea. The protein was purified using 1 mL of nickel-nitrilotriacetic acid (Ni-NTA) resin (Bio-Rad Laboratories, USA) via gravity flow. Washes were performed using 50 mL of 20 mM imidazole while elution was performed using 500 mM imidazole. Purified DIII-His protein was refolded through dialysis to Tris buffer before monomeric DIII-His protein was isolated via size exclusion chromatography (SEC).

Electrophoretic analysis of each wash and eluate fraction from the first-step Ni-NTA affinity purification revealed that majority of bacterial contaminating proteins was removed during the first wash (W1) (Figure 1a). The second wash (W2) further removed residual contaminating proteins, which led to Eluate E1 having a relatively clean protein profile. DIII-His protein elution reached a maximum at Eluate E5.

Eluate E5 to E11 containing DIII-His protein were pooled together and refolded before the protein was subjected to SEC using a fast protein liquid chromatography (FPLC) system. Electrophoretic analysis showed that multimeric DIII-His protein was eluted in Peak 1 of the UV chromatogram while its monomeric counterpart was eluted in Peak 2 (Figures 1b and 1c). All subsequent experiments were performed using monomeric DIII-His protein isolated from Peak 2.

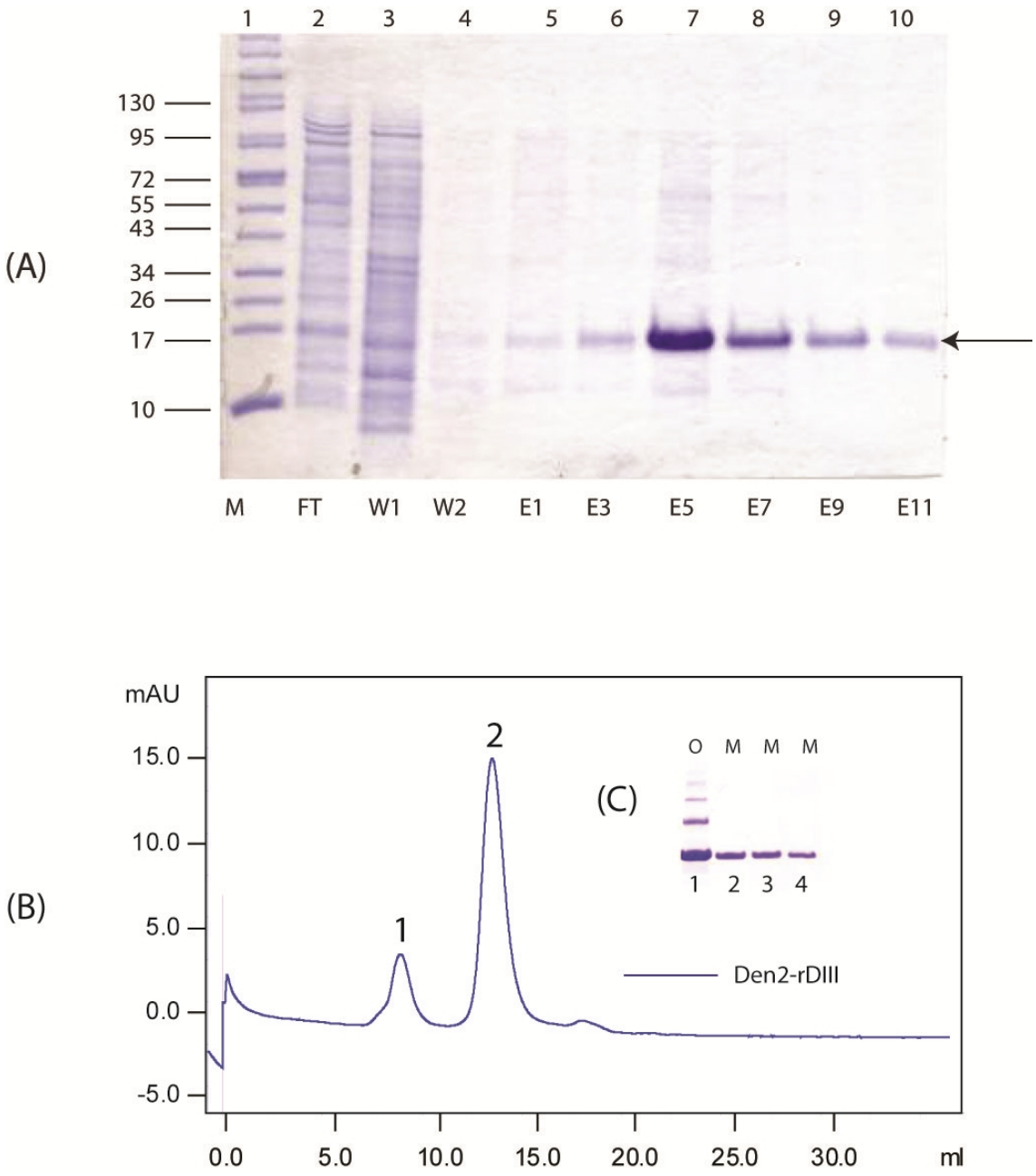

Figure S1. Two-step purification of recombinant envelope Domain III with hexa-histidine tag (DIII-His) protein of Dengue virus serotype 2. (a) Electrophoretic analysis of protein profiles of flow-through (FT; Lane 2), first wash (W1; Lane 3), second wash (W2; Lane 4) and eluate fractions (E1 to E11; Lanes 5 to 10) obtained from the first-step Ni-NTA affinity purification. The black arrow indicates recombinant DIII-His protein. M (Lane 1): protein ladder. (b) Chromatogram of UV absorbance at 280 nm generated during the second-step size exclusion chromatography (SEC) of recombinant DIII-His protein showing two absorbance peaks (Peak 1 and 2). (c) Electrophoretic analysis of protein profiles of eluates collected at Peak 1 (Lane 1) and the three fractions collected across Peak 2 (Lanes 2 to 4) from SEC. Oligomeric (O) and monomeric (M) DIII-His proteins were eluted in Peaks 1 and 2, respectively.

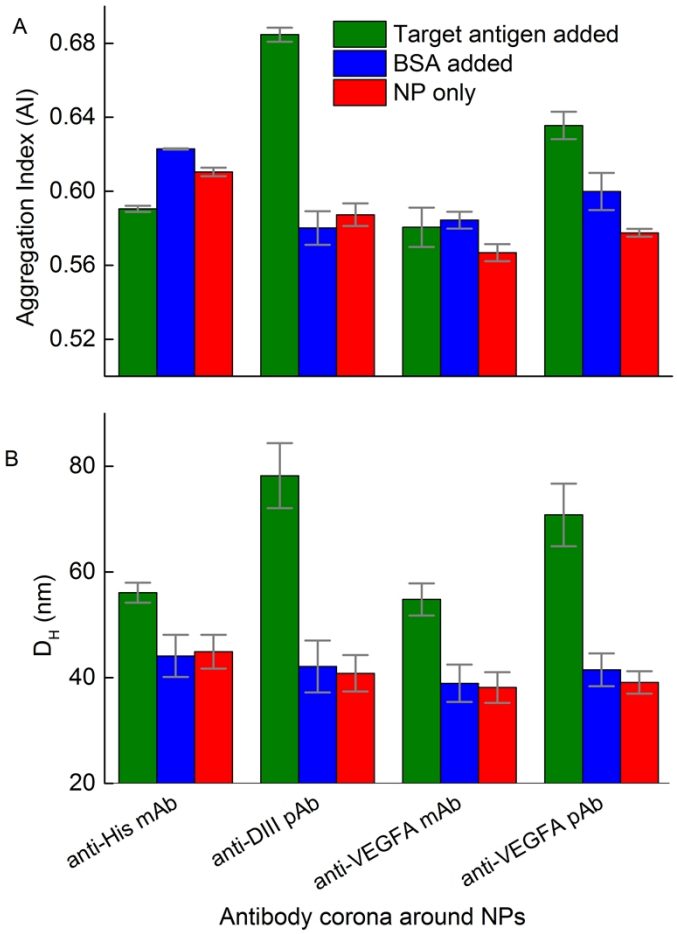

Figure S2. (a) Aggregation index and (b) hydrodynamic diameter, D_H of NPs with the four different antibody coronas after addition of target antigen (green bars) or BSA as a control (blue bars) to the respective NP-antibody corona at an antigen:antibody molar ratio of 5:1 for 1 h. NP-antibody (red bars) with buffer added are included for further comparison.

References

1. L. C. Tan, A. J. Chua, L. S. Goh, S. M. Pua, Y. K. Cheong and M. L. Ng, *Protein expression and purification*, 2010, **74**, 129-137.
2. P. Krupakar, Ngo, A. M.-L. and Ng, M.-L, in *Protein Purification*, ed. M. a. A. Benitez, V., Nova Science Publishers, New York2012, pp. 147-169.