

# **Supporting Information: Single-Site Mutations in a Hyperthermophilic Variant of the B1 Domain of Protein G Result in Self-Assembled Oligomers**

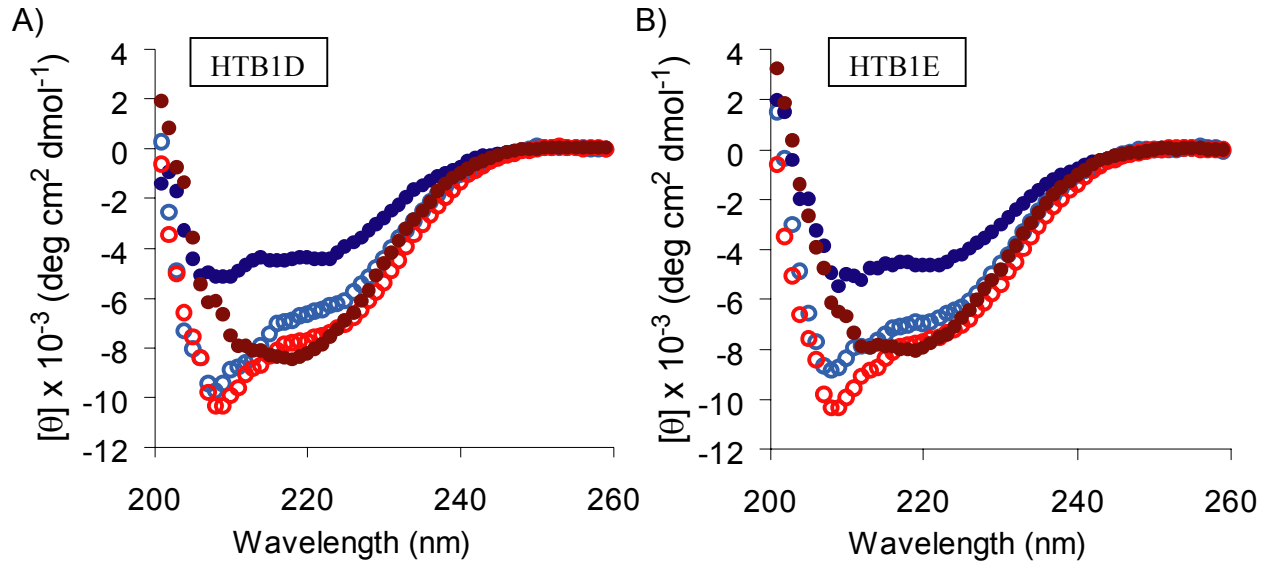
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1) *Circular Dichroism Spectroscopy with 0.1% SDS (SDS PAGE conditions)*



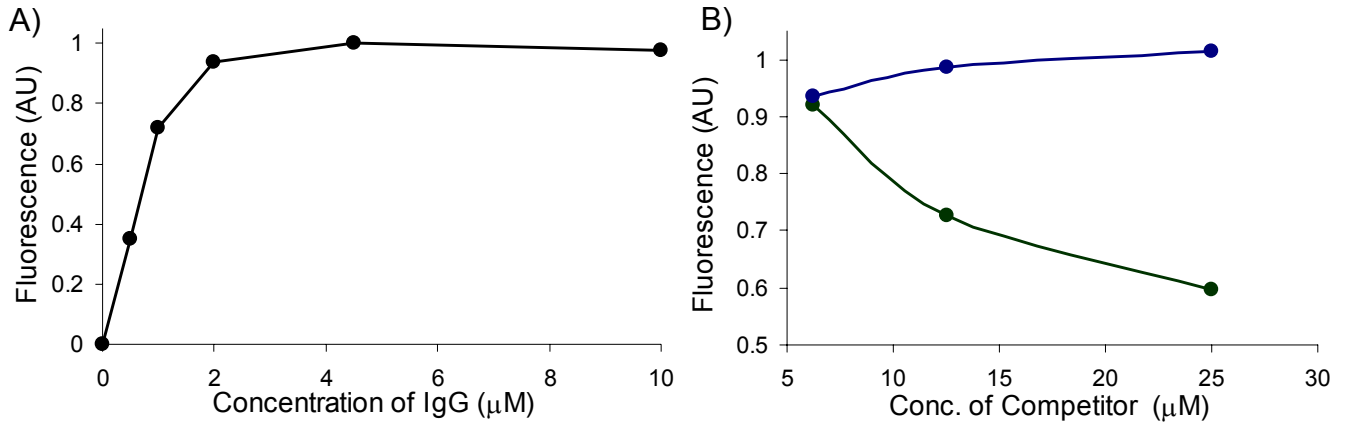
Circular dichroism data of HTB1D and HTB1E peaks in SDS. To determine whether or not the anomalous migration in the SDS-PAGE gels was due to an extended monomer conformation, or a non-covalent dimer, SDS was added to samples of the separated peaks for HTB1D and HTB1E, which were then heated to 95°C for 10 minutes to simulate SDS-PAGE sample preparation conditions. CD spectra taken on the same samples before SDS denaturation are also shown for comparison.

(A) HTB1Dp2 with (light blue open) and without (dark blue solid) 0.1 % SDS and HTB1Dp1 with (red open) and without (maroon solid) 0.1 % SDS. (B) HTB1Ep2 with (light blue open) and without (dark blue solid) 0.1 % SDS and HTB1Ep1 with (red open) and without (maroon solid) 0.1 % SDS.

## **2) *Binding Competition Assay Between Labeled HTB1 and the Mutants.***

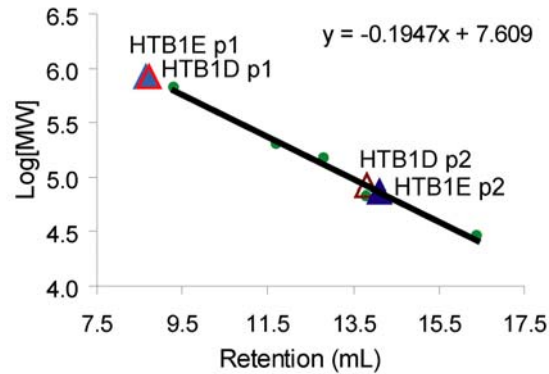
The extent to which the mutants could bind HTB1's native target, IgG, was measured via an assay first developed by Hellinga. A Q33C mutant of HTB1 (FHTB1) was labeled with acylodan in the following manner: 4.7 mg (21 $\mu$ mol) acylodan was dissolved in 1mL DMSO (dimethylsulfoxide). This solution was then added to 1.05 $\mu$ mol of the Q33C mutant in 15 mL of PBS buffer (70  $\mu$ M final concentration). The reaction vessel was shaken for 2 hours, at which point the reaction was quenched with 20 $\mu$ L of BME ( $\beta$ -mercaptoethanol). The labeled protein, FHTB1, was first purified on a nickel-agarose affinity column, and then on a NAP-10 column (Amersham Pharmacia). The structure was confirmed via mass spectrometry (expected mass: 8571 Da; found: 8569 Da) as well as UV visualized and coomassie blue stained SDS-PAGE.

The competition assay was run on a SpectraMax Gemini Fluorometer (Molecular Devices) in 20 mM Tris-HCl at pH = 7.4 with an excitation wavelength of 392 nm. Fluorescence emission was monitored at 490nm in all cases. Human IgG (immunoglobulin G) was added in increasing concentrations to solutions of FHTB1 (3 $\mu$ M final) and readings were taken after a 20 minute incubation time. In the competition assay, increasing amounts of the competitor (HTB1, HTB1E, or HTB1D) were pre-mixed with FHTB1 and added to IgG (3 $\mu$ M FHTB1 and 3 $\mu$ M IgG final concentrations). Fluorescence was measured after a 20 minute incubation of FHTB1 and the competitor with IgG.



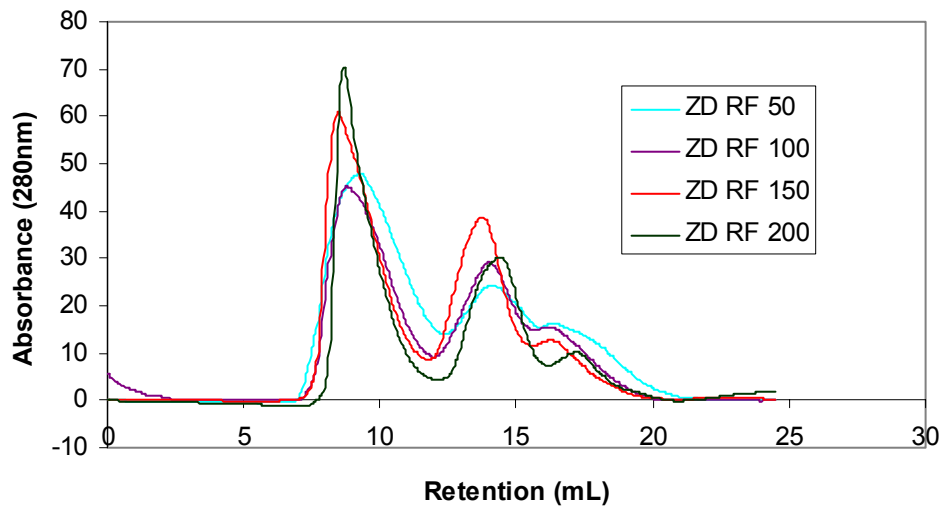
A) Fluorescently labeled HTB1 (FHTB1) at  $3\mu\text{M}$  was titrated with full human immunoglobulin G, showing an increase in fluorescence. B) Upon addition of non-labeled HTB1 (green), the fluorescence of FHTB1 decreases, but no such decrease is seen with increasing concentrations of HTB1E (blue).

### 3) Determination of Molecular Weights via Gel Filtration



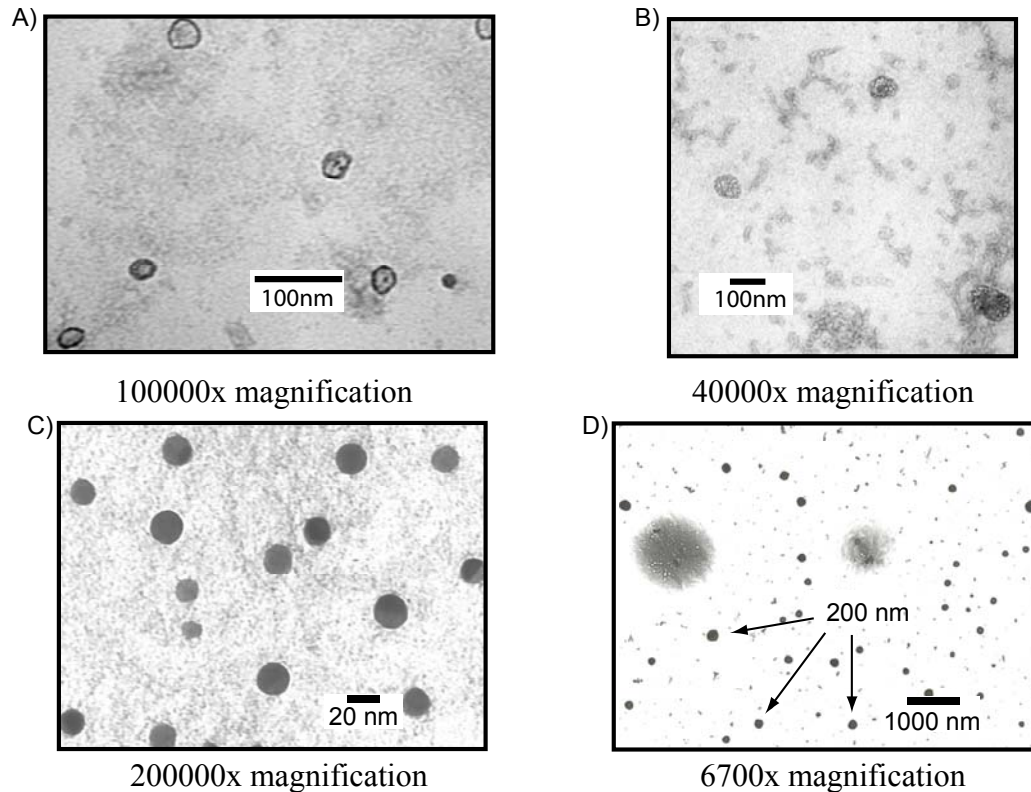
Molecular weight determination from gel filtration chromatography. Thyroglobulin (670 kDa), beta-amylase (200 kDa), alcohol dehydrogenase (150 kDa), BSA (66 kDa), and carbonic anhydrase (29 kDa) were used as protein standards. HTB1Dp1 and HTB1Dp2 (open red triangles) and HTB1Ep1 and HTB1Ep2 (closed blue triangles) with their elution volumes are projected on a linear fit of the standard curve. All samples were run on a Superdex HR 200 10/30 gel filtration column in 20 mM Tris-HCl and 150mM NaCl at pH = 7.4 and 4°C.

#### 4) Gel Filtration of Refolded Mutants at Varying Concentrations



After refolding at different concentrations (50 –200  $\mu$ M) of HTB1D from 6M guanidine-HCl in 20 mM Tris-HCl at pH = 7.4, the mixture of oligomers were rerun on the Superdex 200 HR 10/30 gel filtration column to ascertain distribution of oligomers.

5) *Lack of Fibrillar Species by Transmission Electron Microscopy.*



TEM was carried out to demonstrate that no fibrillar species was present in the samples of HTB1E and HTB1D.

HTB1, HTB1E, or HTB1D was diluted to a final concentration of 25  $\mu\text{M}$  in 10 mM Tris-HCl, 75 mM NaCl (pH = 7.4) and 1% phospho tungstic acid for 1-10 minutes to allow for staining. The stained solution was adsorbed onto the surface of a carbon coated Piloform plastic (60-90nm plastic, approximately 5nm carbon) and placed on 200 mesh grid. Excess fluid was withdrawn and the samples were allowed to dry. Samples were observed and photographed on a JEOL 100 CX II TEM at 80 kV with various magnifications. The TEM micrographs of HTB1E and HTB1D were almost indistinguishable under all conditions tested and most notably did not show any fibers.