SUPPORTING INFORMATION FOR

Cosolute and Crowding Effects on a Side-By-Side Protein Dimer

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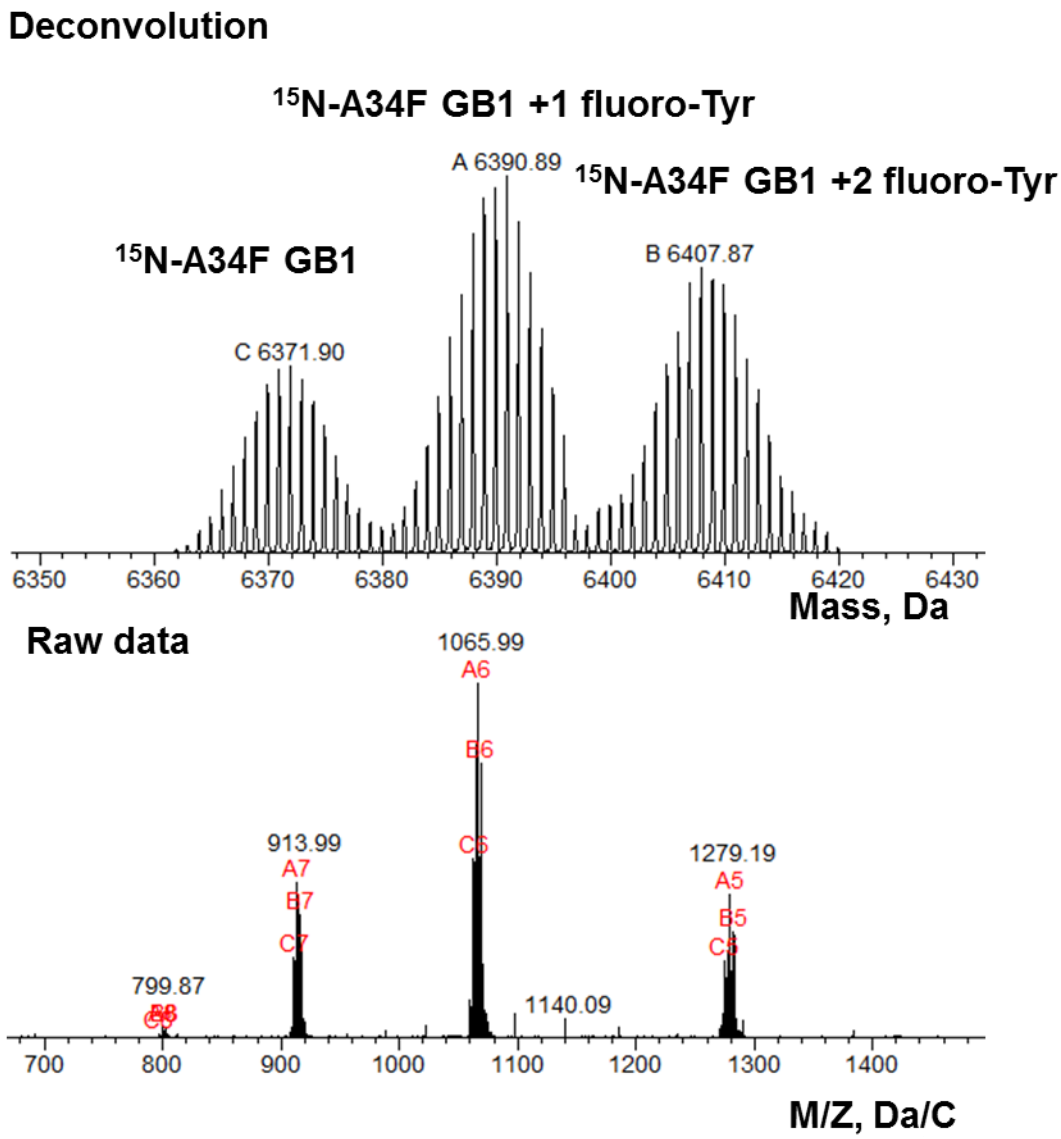

Figure S1: ESI-FT-ICR analysis of fluorinated A34F GB1 reveals 3 populations of fluorinated GB1 containing 0 (6371.90 Da), 1 (6390.89 Da), or 2 (6407.87 Da) fluorotyrosine residues.

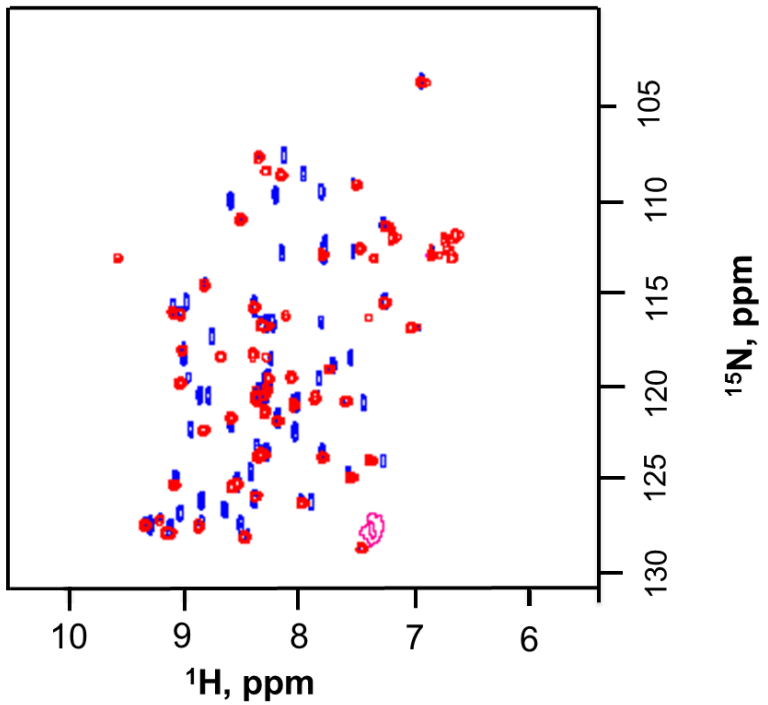

Figure S2: ^{1}H-^{15}N HSQC spectrum of A34F GB1 dimer (3.3 mM, red) and ^{1}H-^{15}N HMQC spectrum of A34F GB1 monomer (10 μM, blue) show chemical shift changes between monomer and dimer states, consistent with published spectra.[1]

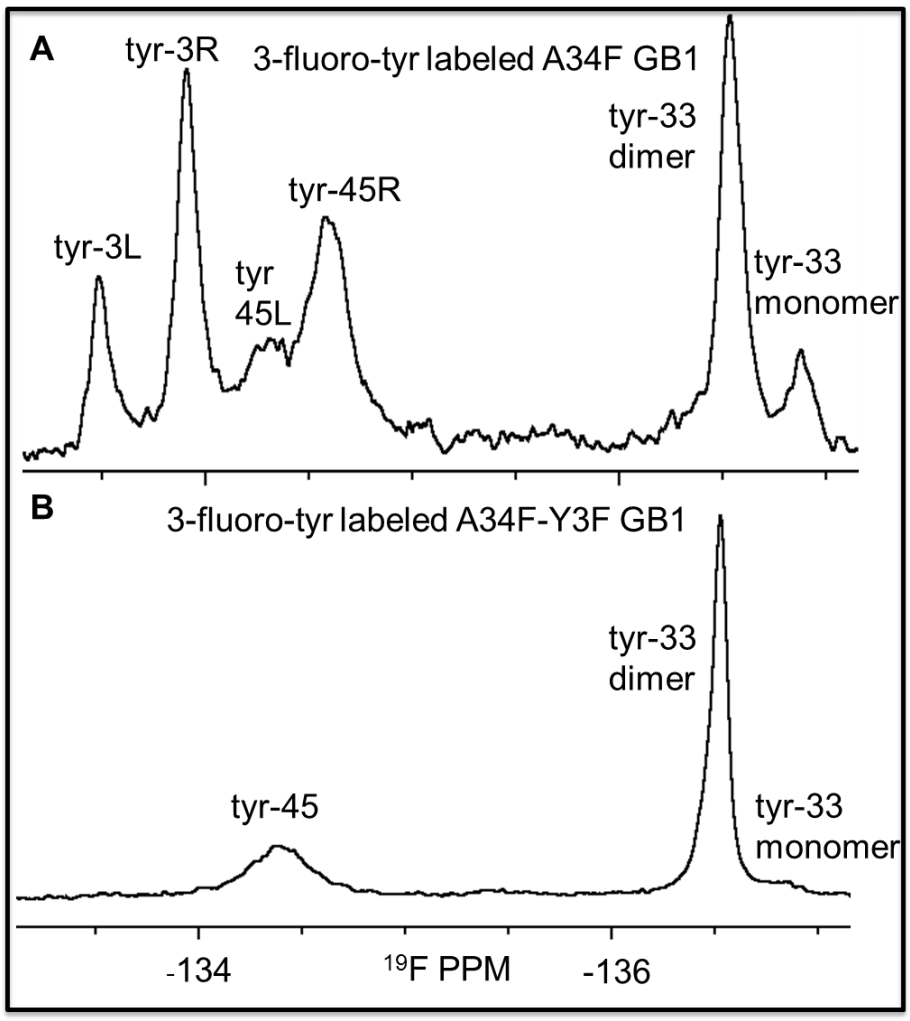

Figure S3: (A)^{19}F NMR spectrum of A34F GB1 shows six peaks, two for Tyr-45 and two Tyr-3, all 4 are in slow exchange, and two resonances near -136.5 ppm that correspond to Tyr-33 in the dimer state (upfield) and monomer state (down field). (B) Peaks were assigned using published data[2] and the A34F Y3F variant data shown here.

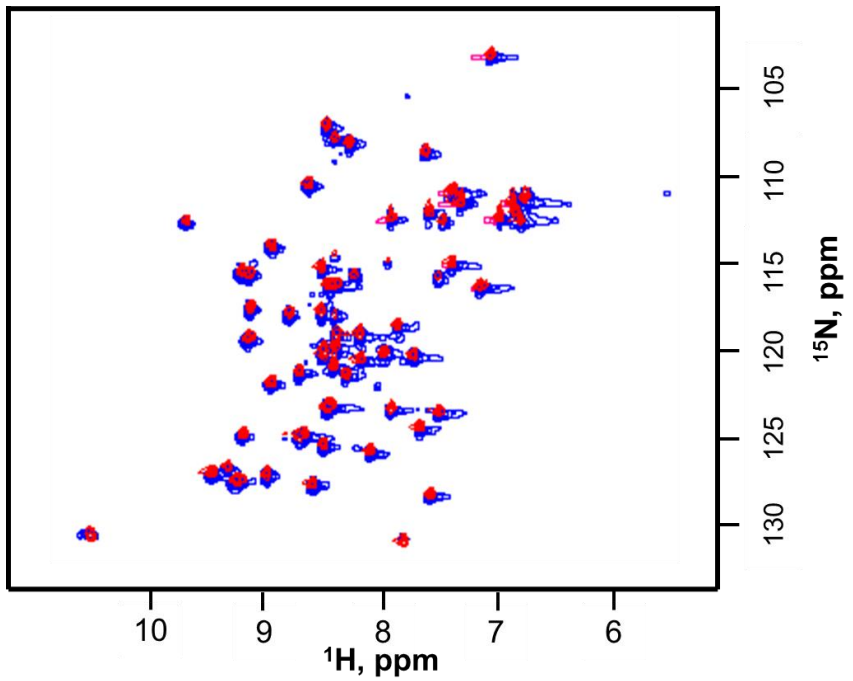

Figure S4: ^{1}H-^{15}N HSQC spectra of ^{19}F-labeled, ^{15}N enriched GB1 (red) and ^{15}N-enriched A34F GB1 (blue) indicate that labeling has a small effect on A34F GB1 structure.

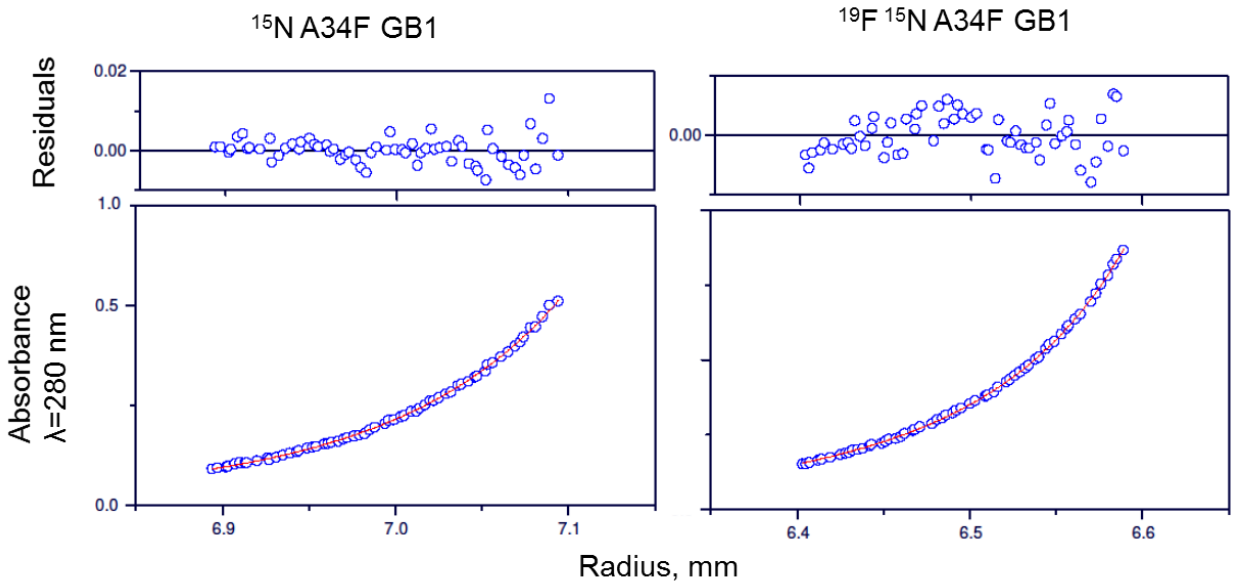

Figure S5: Equilibrium ultracentrifugation data for ^{15}N-enriched A34F GB1 and ^{19}F-labeled ^{15}N-enriched A34F GB1 yield the same dissociation constant and uncertainty (59 ± 10 μM), showing that labeling does not affect dimerization. Samples were spun at 34,000 rpm and 25 ºC for 36 h in a Beckman XL-A ultracentrifuge. Data were fit using Origin 6.03 software (OriginLab Corp.).

(1) Jee, J., Byeon, I.-J. L., Louis, J. M., and Gronenborn, A. M. (2008) The point mutation A34F causes dimerization of GB1. *Proteins 71*, 1420-1431.
(2) Ye, Y., Liu, X., Zhang, Z., Wu, Q., Jiang, B., Jiang, L., Zhang, X., Liu, M., Pielak, G. J., and Li, C. (2013) ^{19}F NMR spectroscopy as a probe of cytoplasmic viscosity and weak protein interactions in living cells. *Chem. -Eur. J. 19*, 12705-12710.