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Supplementary Information for

The intracellular environment affects protein-protein interactions

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Table S1. Equilibrium Dissociation Parameters at 298 K for 6-fluorotryptophan- and 288 K for 3-fluorotyrosine- labeled proteins.

Condition	$K_{D\rightarrow M}(\mu M)$	$\Delta G°'_{D\rightarrow M}$ (kcal/mol)	$\Delta\Delta G°'_{D\rightarrow M}$[a] (kcal/mol)
A34F; D40N[-3,b], pH 7.6, 6-fluorotryptophan labeled			
20 mM NaP[c]	9 ± 1[d]	6.88 ± 0.04	0.84 ± 0.07[d]
E. coli	only monomer	< 6	< 0.7[e]
pH 7.4, 3-fluorotyrosine labeled			
20 mM NaP	22 ± 1	6.14 ± 0.03	0.34 ± 0.04
Oocytes	only dimer	> 7[f]	> 2[f]
A34F[-4], pH 7.6, 6-fluorotryptophan labeled			
20 mM NaP	37 ± 4	6.04 ± 0.06	0
E. coli	11 ± 4	6.8 ± 0.2	0.7 ± 0.2
pH 7.4, 3-fluorotyrosine labeled			
20 mM NaP	40 ± 2	5.80 ± 0.03	0
Oocytes	6.5 ± 0.7	6.83 ± 0.06	1.03 ± 0.07
A34F; K10N[-5], pH 7.6, 6-fluorotryptophan labeled			
20 mM NaP	58 ± 3	5.78 ± 0.06	-0.26 ± 0.08
E. coli	only dimer	> 7[e]	> 0.7[e]
pH 7.4, 3-fluorotyrosine labeled			
20 mM NaP	132 ± 6	5.11 ± 0.03	-0.69 ± 0.04
100 g/L Lysate	48 ± 3	5.69 ± 0.03	-0.11 ± 0.04
Oocytes	16 ± 2	6.31 ± 0.05	0.51 ± 0.06
A34F; N37D[-5], pH 7.6, 6-fluorotryptophan labeled			
20 mM NaP	16 ± 1	6.54 ± 0.06	0.50 ± 0.08
E. coli	only dimer	> 7[e]	> 0.7[e]
pH 7.4, 3-fluorotyrosine labeled			
20 mM NaP	21.9 ± 0.6	6.14 ± 0.02	0.34 ± 0.04
Oocytes	1.7 ± 0.1	7.60 ± 0.03	1.80 ± 0.04

Footnotes

[a] $\Delta\Delta G°'_{D\rightarrow M} = \Delta G°'_{D\rightarrow M,var} - \Delta G°'_{D\rightarrow M,A34F,buffer}$

[b] Superscripts denote net charge at neutral pH

[c] NaP, sodium phosphate buffer

[d] Uncertainties are the standard deviation of the mean from triplicate measurements

[e] Stabilities greater than or less than the detection limit

[f] Not determined because monomer and dimer have similar shifts in oocytes, but lysate data suggest the dimer is more stable in oocytes.

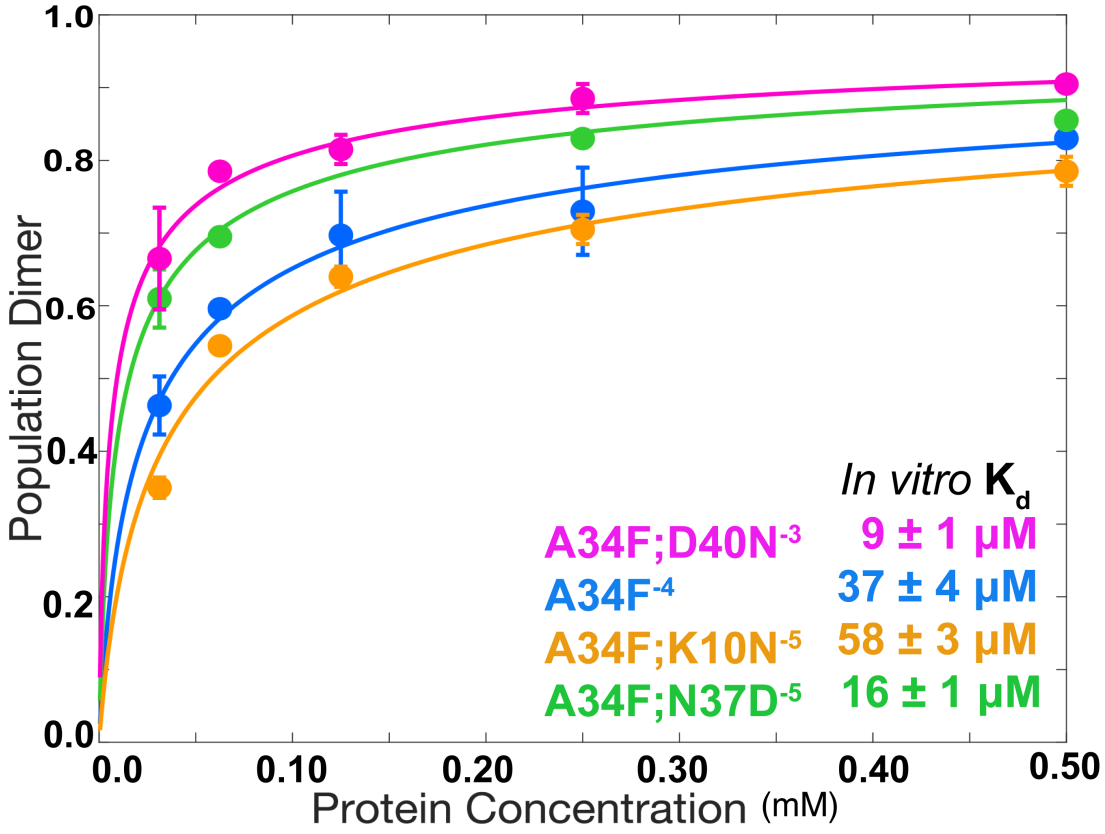

Fig. S1: Quantifying dissociation of 3-fluorotryptophan labeled A34F^{-4}, A34F;N37D^{-5}, A34F;D40N^{-3}, and A34F;K10N^{-5} GB1 in buffer. Uncertainties are the standard deviation of the mean from triplicate experiments. Superscripts denote net charge at pH 7.5.

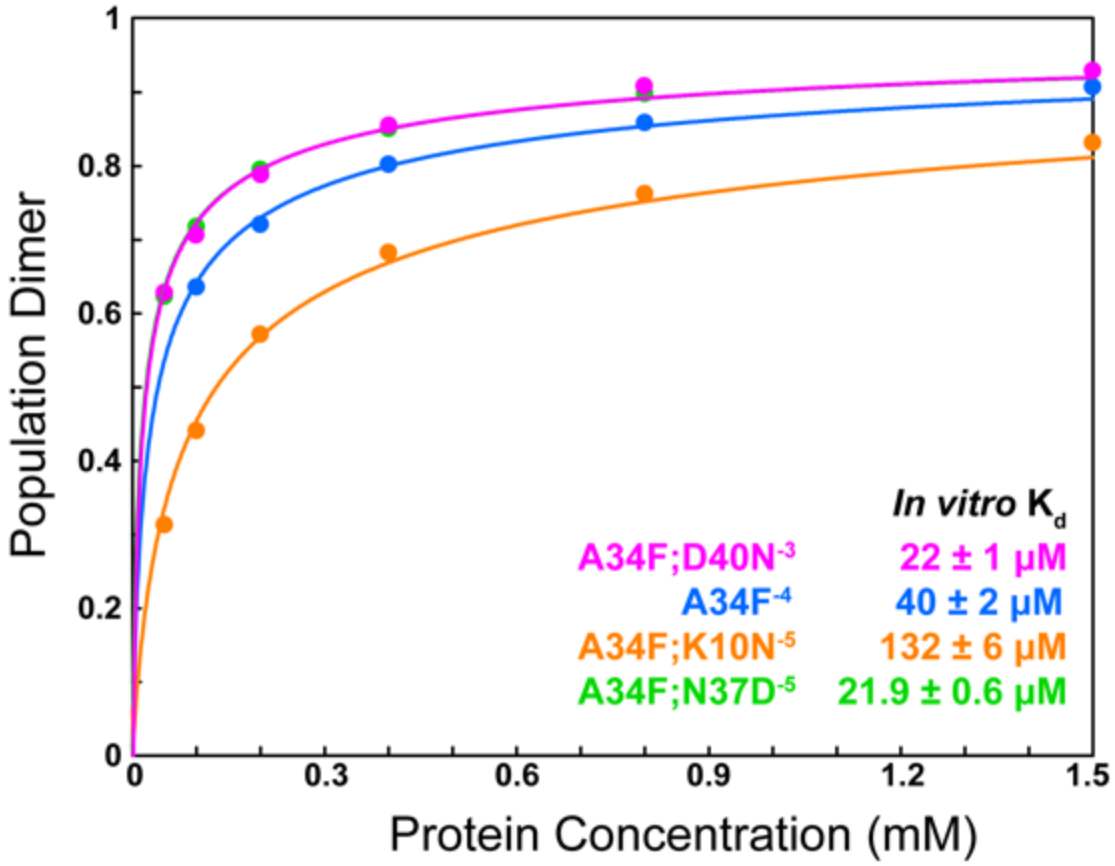

Fig. S2: Quantifying dissociation of 3-fluorotyrosine labeled A34F^{-4}, A34F;N37D^{-5}, A34F;D40N^{-3}, and A34F;K10N^{-5} GB1 in buffer. Superscripts denote net charge at pH 7.5. Uncertainties are derived from least squares fitting.

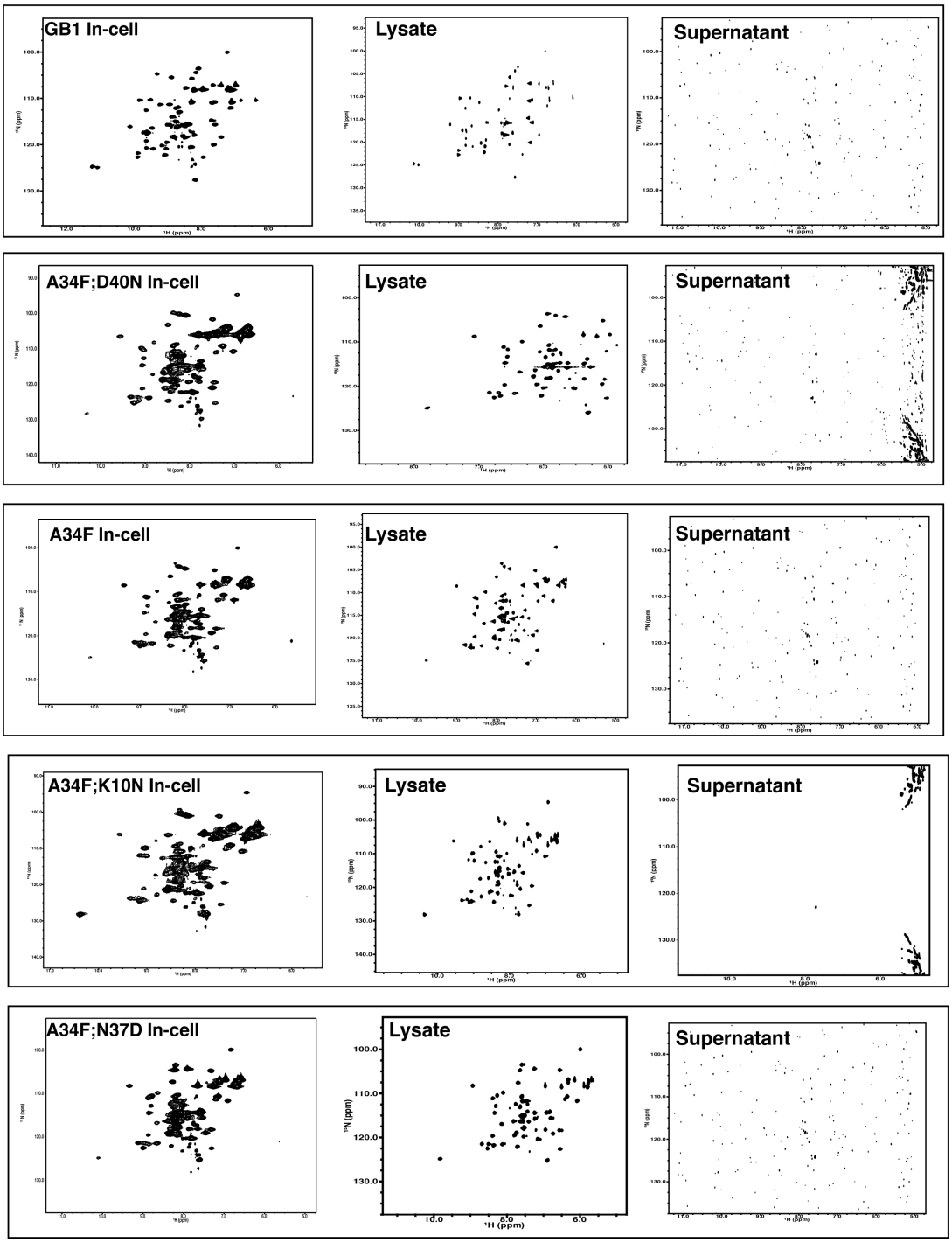

Fig. S3: ^{15}N-^{1}H HSQC spectra of ^{19}F-Trp labeled GB1^{-4}, A34F;D40N^{-3} GB1, A34F^{-4} GB1, A34F;K10N^{-5} GB1, and A34F;N37D^{-5} GB1 in *E. coli* at 298 K, pH 7.5. Lysate and supernatant controls verify that the protein of interest is inside cells. Supernatant spectra are zoomed-in to show there is no protein leakage.

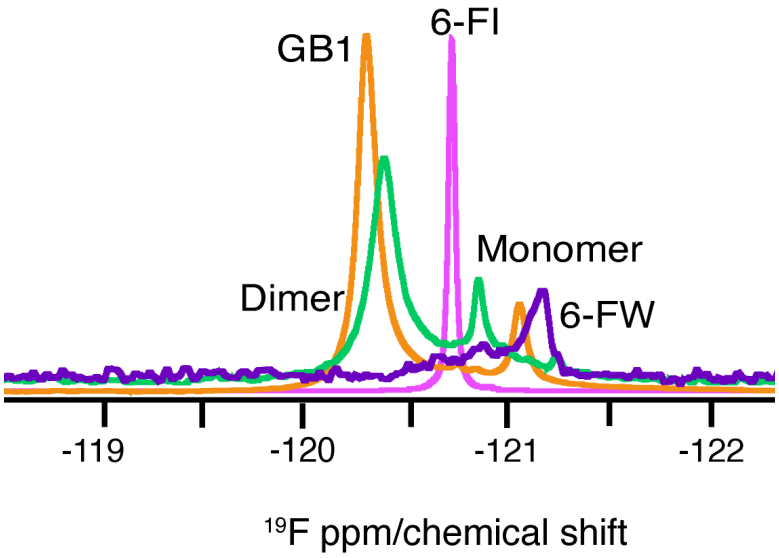

Fig. S4: ^{19}F spectra of GB1 (orange) and A34F GB1induced in *E. coli* (green) confirm that there is no overlap of monomer and 6-FW in cells. The spectrum of A34F GB1without inducer confirms the second resonance in the GB1 spectrum is 6-FW (purple). Control spectrum of 6-FI in buffer (magenta) confirms there is no overlap with the monomer or dimer in cells.

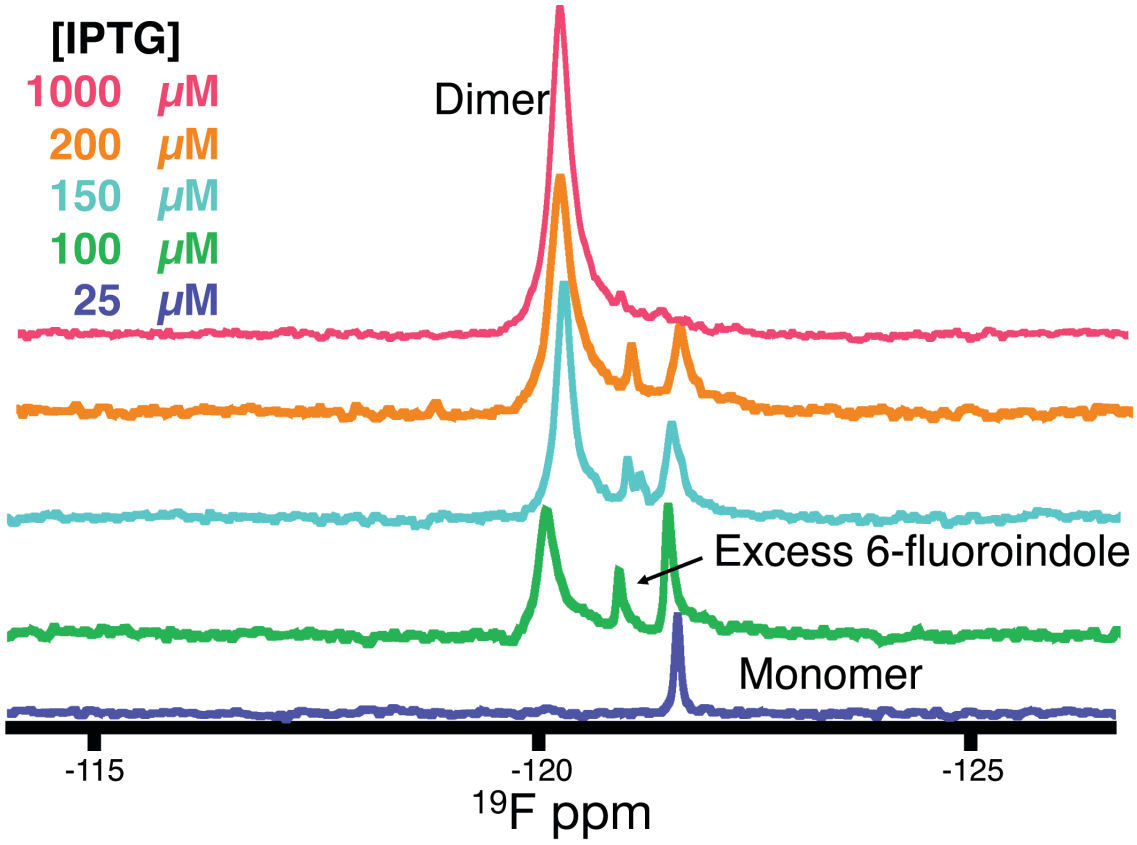

Fig. S5: ^{19}F NMR Spectra of 6-fluoroindole-labeled A34F GB1 in *E. coli* Tuner cells as a function of inducer (IPTG) concentration.

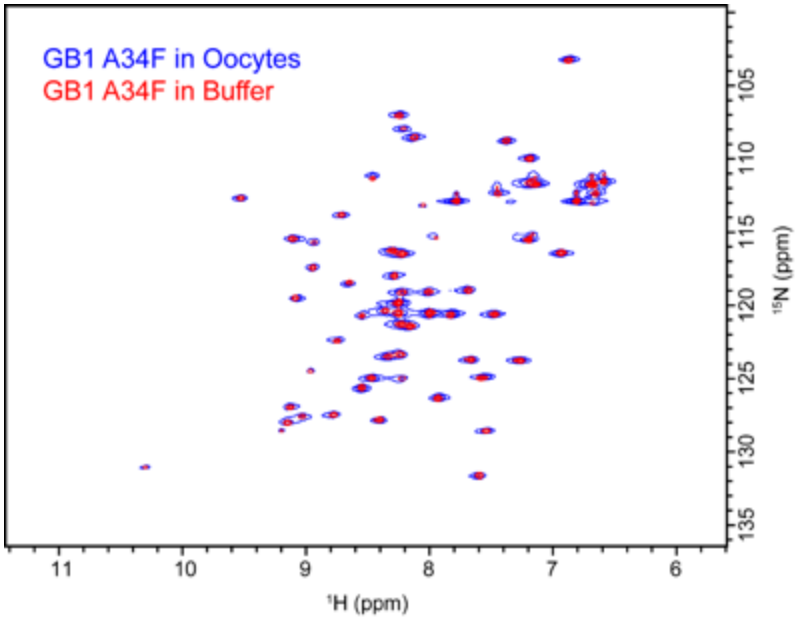

Fig. S6: ^{1}H-^{15}N HSQC spectra in oocytes (blue) and in buffer (red, 20 mM phosphate buffer, pH 7.4) of ^{15}N-enriched, 3FY-labeled A34F GB1.

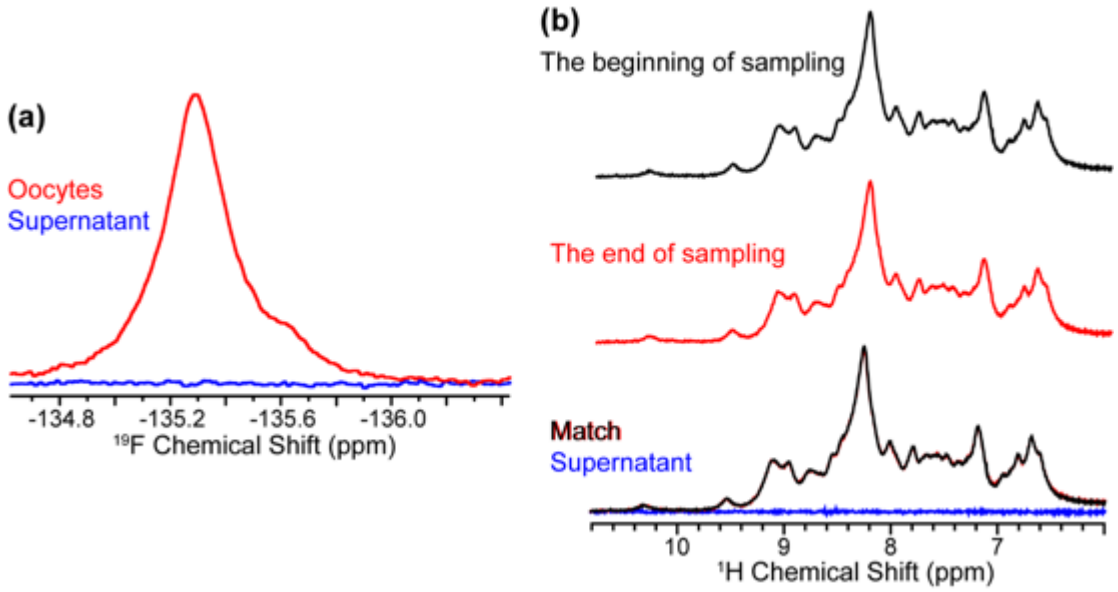

Fig. S7: A34F GB1 is stable in oocytes for the duration of the NMR experiment, and there is no leakage. (a) ^{19}F NMR spectra of A34F GB1 in oocytes (red) and supernatant (blue). (b) 1D ^{1}H-^{15}N HSQC spectra of A34F GB1 in oocytes before (black) and after (red) acquisition of the ^{19}F NMR spectrum.

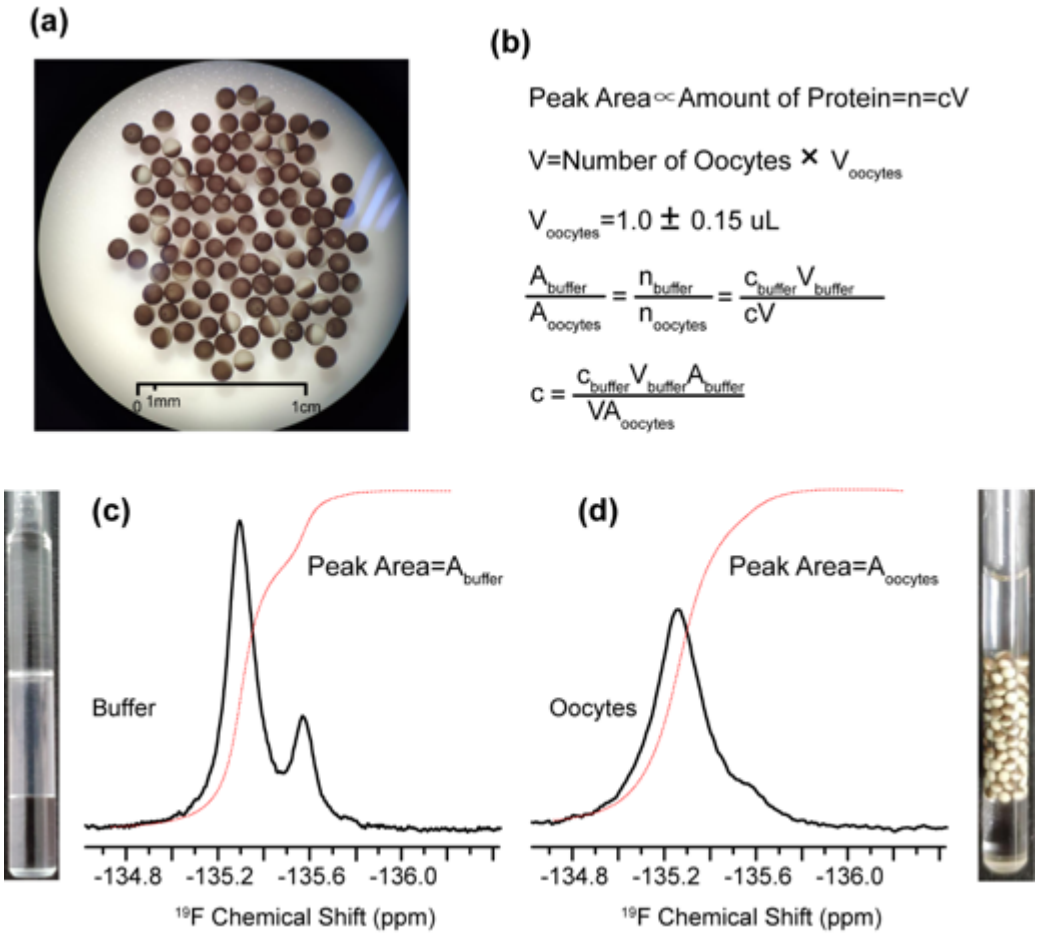

Fig. S8: Concentration of A34F GB1 in oocytes. (a) Oocytes for in-cell NMR experiments. (b) Equation to determine intraoocyte A34F concentration [A_{buffer}, $A_{oocytes}$, areas of ^{19}F NMR resonance in buffer; n_{buffer}, $n_{oocytes}$, total moles of proteins; c_{buffer}, $c_{oocytes}$, concentration of proteins in buffer, oocytes; V_{buffer}, $V_{oocytes}$, volumes of buffer]. (c and d) Buffer and oocytes containing A34F GB1 in Shigemi micro-NMR tubes and their integrated ^{19}F NMR spectra.

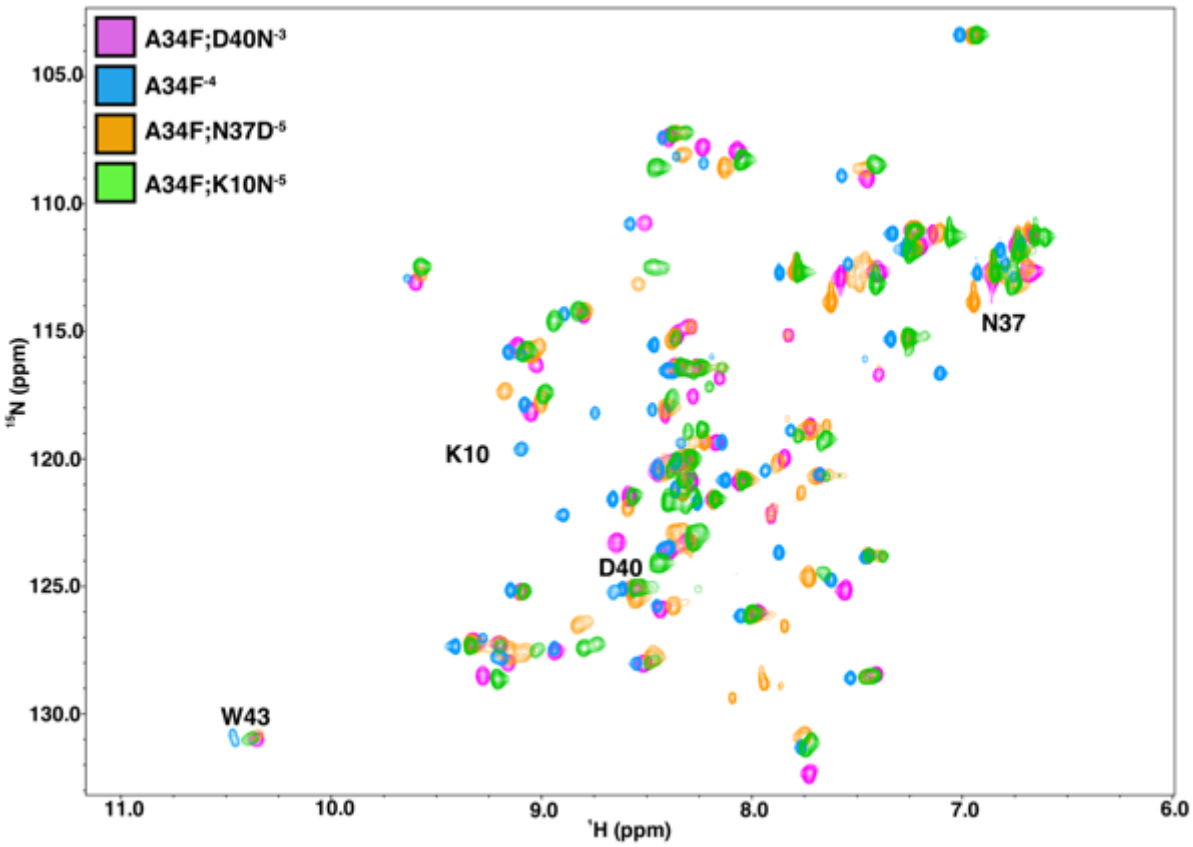

Fig. S9: ^{15}N-^{1}H HSQC spectra of 6FI-labeled proteins in buffer alone. Superscripts denote net charge at neutral pH.

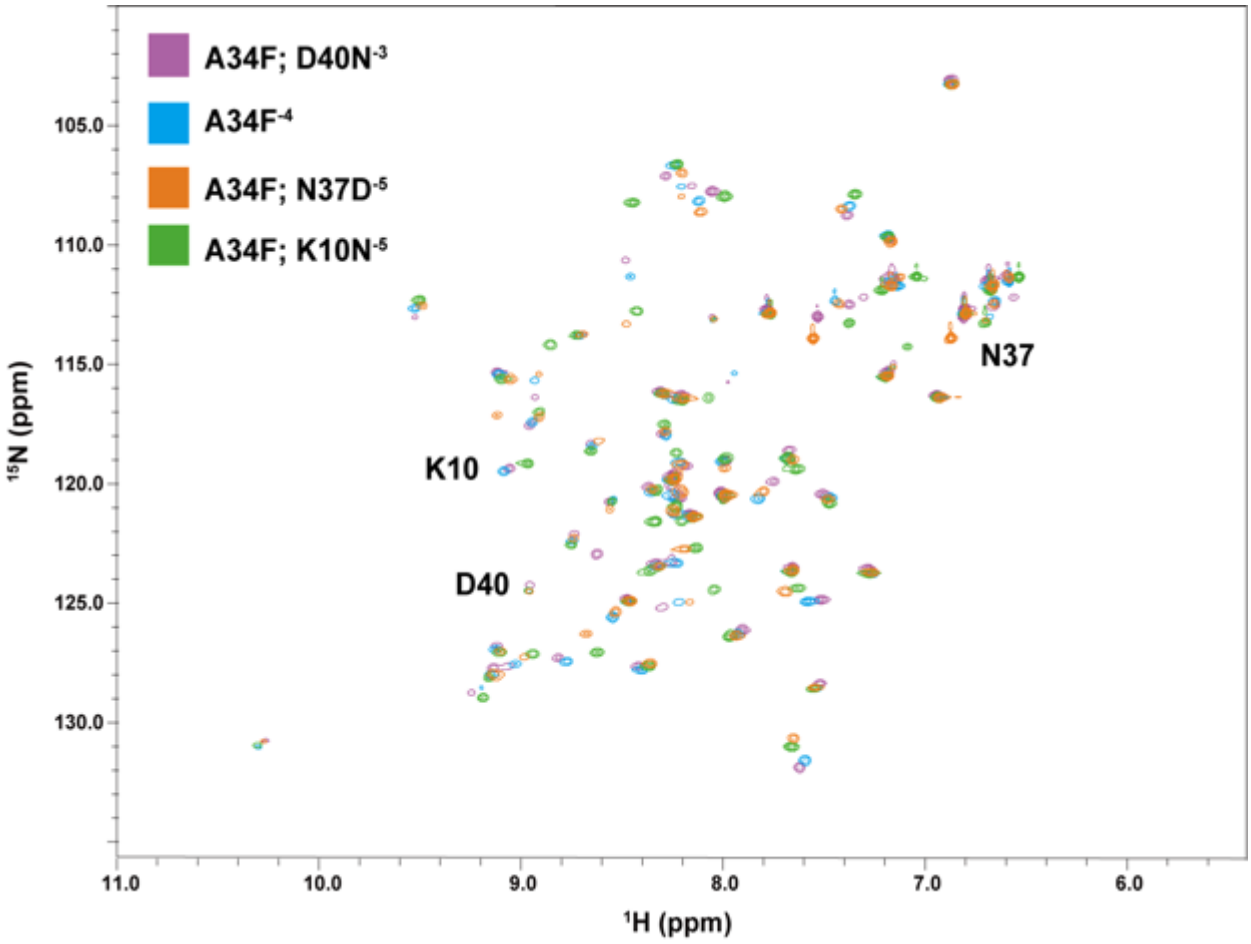

Fig. S10: ^{15}N-^{1}H HSQC spectra of 3FY-labeled proteins in buffer alone. Superscripts denote net charge at neutral pH.

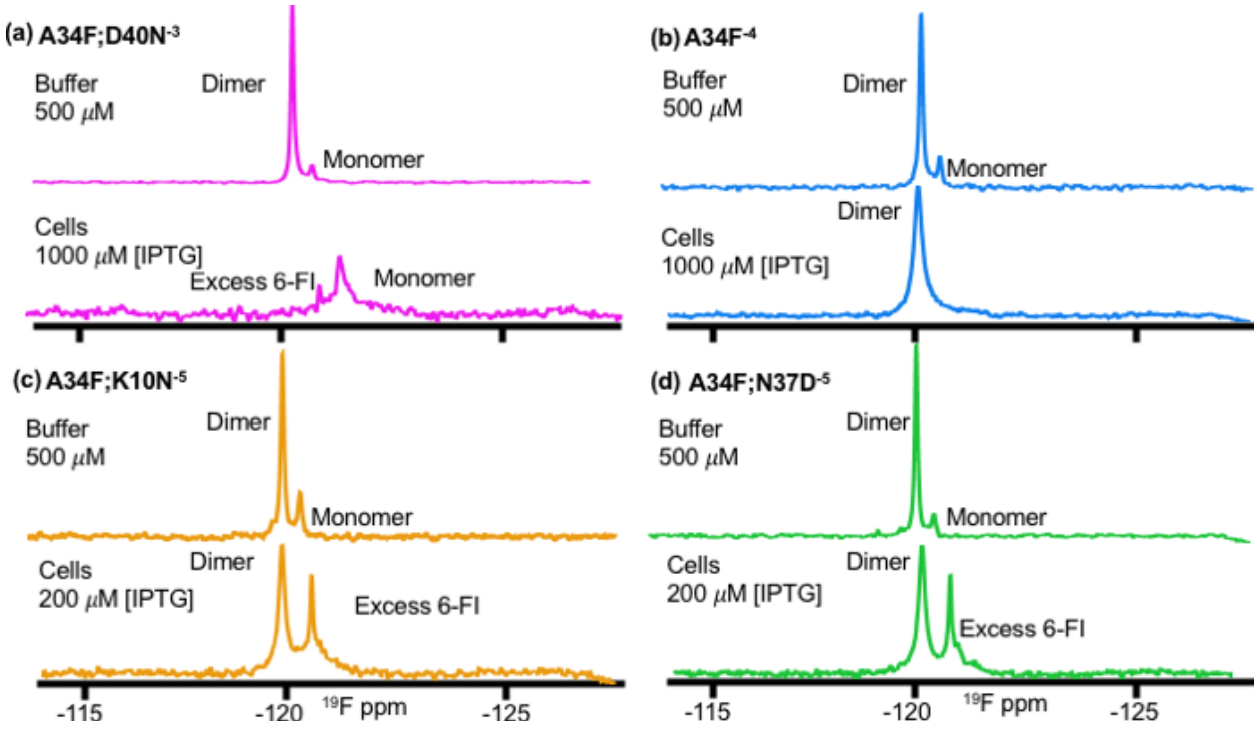

Fig. S11: ^{19}F NMR spectra in buffer and *E. coli* of 6-fluorotryptophan (6-Fl)-labeled variants that show only monomer or dimer in cells: A34F; D40N^{-3} (a), A34F^{-4} (b), A34F; K10N^{-5}, (c) A34F; N37D^{-5}. Superscripts denote net charge at pH 7.5.

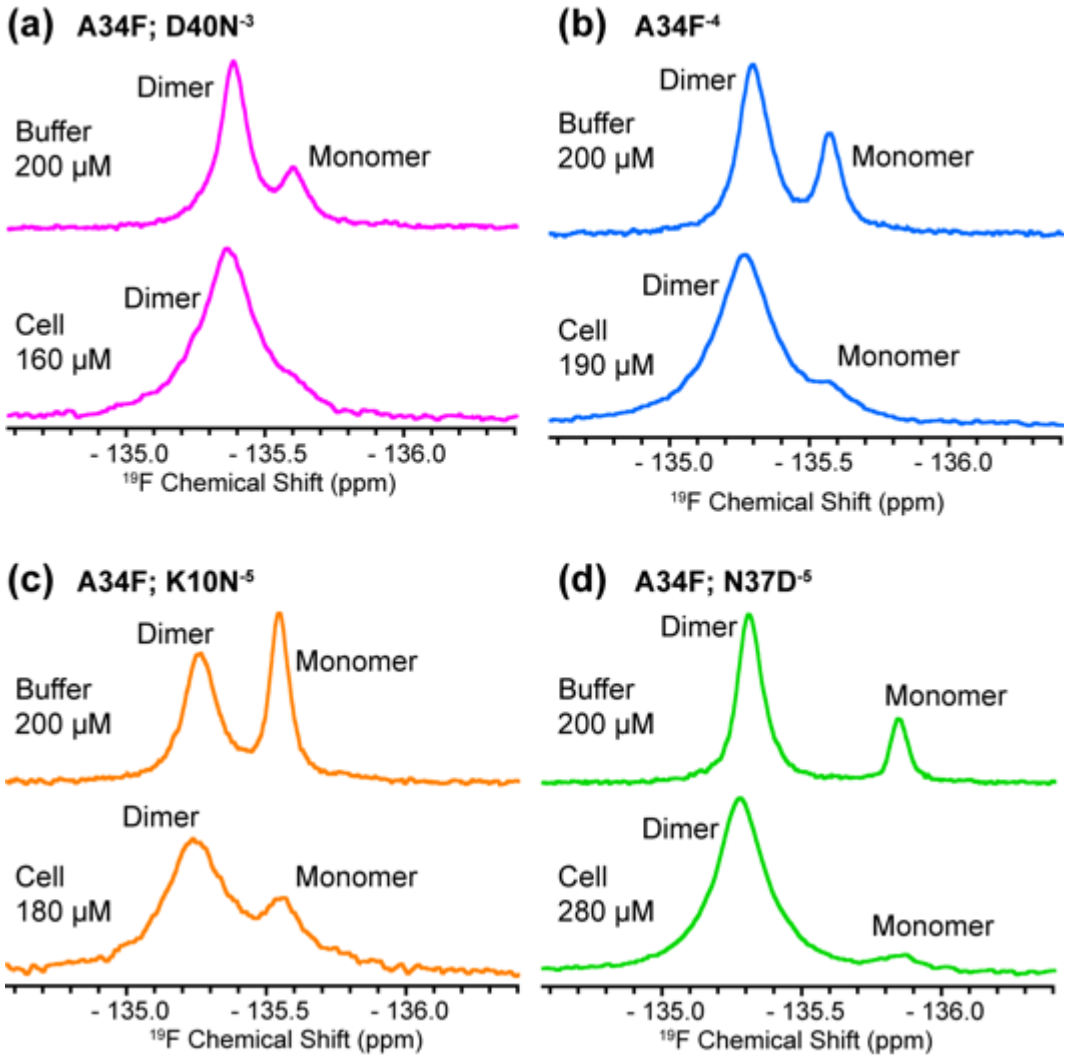

Fig. S12: ^{19}F spectra of 3-fluorotyrosine labeled A34F;D40N^{-3} GB1 (a) ,A34F^{-4} GB1 (b) , A34F;K10N^{-5} GB1 (c) and A34F;N37D^{-5} GB1 (d) in buffer (red) and oocytes (blue). Superscripts denote net charge at pH 7.5.