Supporting Information

Redox Sensitive Protein Droplets from Recombinant Oleosin

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Table S1. Oleo30G and Oleo30G-cys mutants' protein sequences.

	Sequence
Oleo30G	G S T T T Y D R H H V T T T Q P Q Y R H D Q H T G D R L T H P Q R Q Q Q G P S T G K L A L G A T P L F G V I G F S P V I V P A M G I A I G L A G V T G F Q R D Y V K G K L Q D V G E Y T G Q K T K D L G Q K I Q H T A H E M G D Q G Q G Q G Q G G G K E G R K E G G K L E H H H H H H
Oleo30G_S2C	G **C** T T T Y D R H H V T T T Q P Q Y R H D Q H T G D R L T H P Q R Q A M G I A I G L A G V T G F Q R D Y V K G K L Q D V G E Y T G Q K T K E G R K E G G K L E H H H H H H
Oleo30G_T3C	G S **C** T T Y D R H H V T T T Q P Q Y R H D Q H T G D R L T H P Q R Q Q Q G P S T G K L A L G A T P L F G V I G F S P V I V P A M G I A I G L A G V T G F Q R D Y V K G K L Q D V G E Y T G Q K T K D L G Q K I Q H T A H E M G D Q G Q G Q G Q G G G K E G R K E G G K L E H H H H H H
Oleo30G_T4C	G S T **C** T Y D R H H V T T T Q P Q Y R H D Q H T G D R L T H P Q R Q Q Q G P S T G K L A L G A T P L F G V I G F S P V I V P A M G I A I G L A G V T G F Q R D Y V K G K L Q D V G E Y T G Q K T K D L G Q K I Q H T A H E M G D Q G Q G Q G Q G G G K E G R K E G G K L E H H H H H H
Oleo30G_T5C	G S T T **C** Y D R H H V T T T Q P Q Y R H D Q H T G D R L T H P Q R Q Q Q G P S T G K L A L G A T P L F G V I G F S P V I V P A M G I A I G L A G V T G F Q R D Y V K G K L Q D V G E Y T G Q K T K D L G Q K I Q H T A H E M G D Q G Q G Q G Q G G G K E G R K E G G K L E H H H H H H
Oleo30G_T12C	G S T T T Y D R H H V **C** T T Q P Q Y R H D Q H T G D R L T H P Q R Q Q Q G P S T G K L A L G A T P L F G V I G F S P V I V P A M G I A I G L A G V T G F Q R D Y V K G K L Q D V G E Y T G Q K T K D L G Q K I Q H T A H E M G D Q G Q G Q G Q G G G K E G R K E G G K L E H H H H H H
Oleo30G_T24C	G S T T T Y D R H H V T T T Q P Q Y R H D Q H **C** G D R L T H P Q R Q Q Q G P S T G K L A L G A T P L F G V I G F S P V I V P A M G I A I G L A G V T G F Q R D Y V K G K L Q D V G E Y T G Q K T K D L G Q K I Q H T A H E M G D Q G Q G Q G Q G G G K E G R K E G G K L E H H H H H H
Oleo30G_S39C	G S T T T Y D R H H V T T T Q P Q Y R H D Q H T G D R L T H P Q R Q Q Q G P **C** T G K L A L G A T P L F G V I G F S P V I V P A M G I A I G L A G V T G F Q R D Y V K G K L Q D V G E Y T G Q K T K D L G Q K I Q H T A H E M G D Q G Q G Q G Q G G G K E G R K E G G K L E H H H H H H

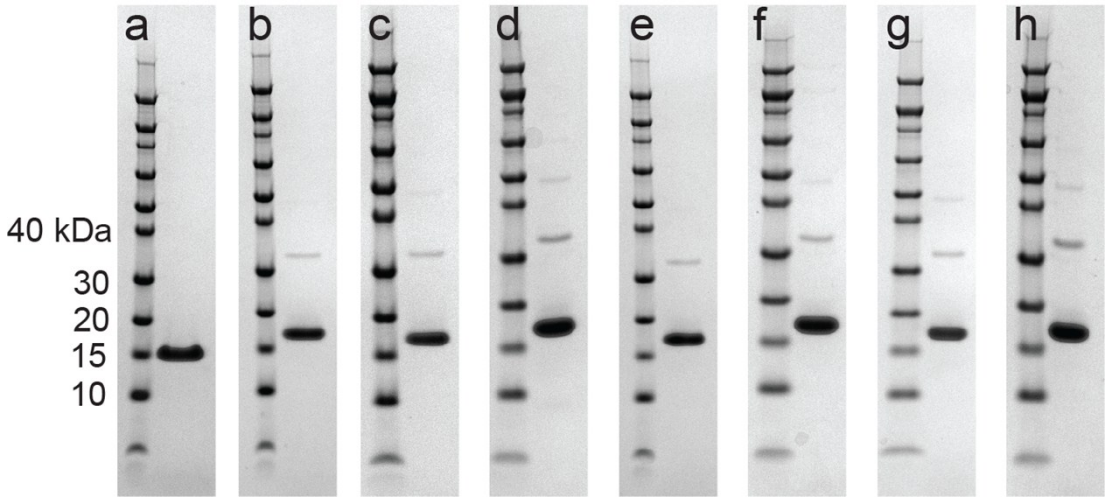

Figure S1. SDS-PAGE gels of (A) Oleo30G, (B) Oleo30G_S2C, (C) Oleo30G_T3C, (D) Oleo30G_T4C, (E) Oleo30G_T5C, (F) Oleo30G_T12C, (G) Oleo30G_T24C, (H) Oleo30G_S39C. Gels Indicate that proteins were pure and at the expected molecular weight after IMAC purification. Faint bands can be seen at double the expected molecular weight due to formation of a disulfide bond while running on the gel.

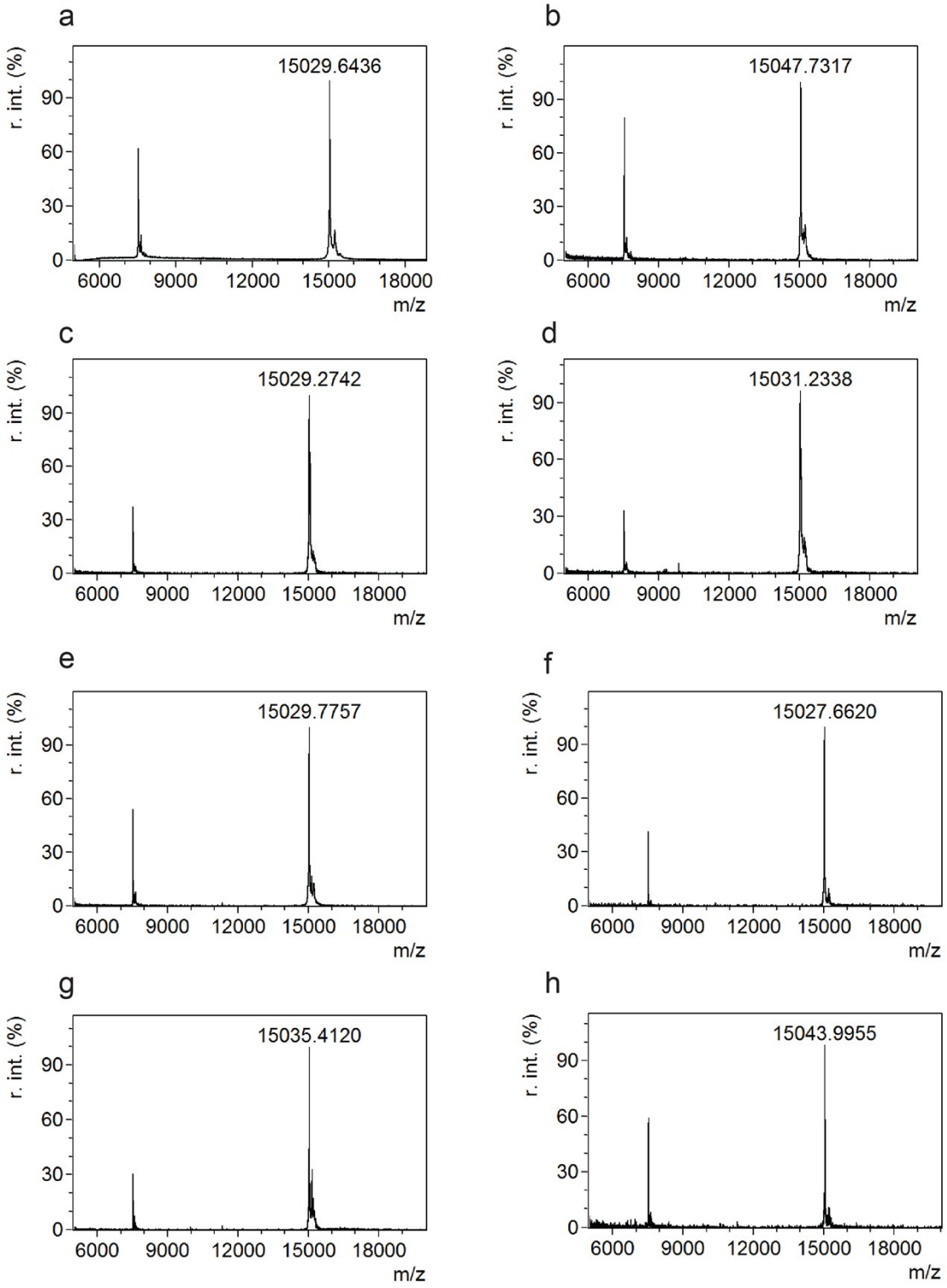

Figure S2. MALDI mass spectra of (A) Oleo30G, (B) Oleo30G_S2C, (C) Oleo30G_T3C, (D) Oleo30G_T4C, (E) Oleo30G_T5C, (F) Oleo30G_T12C, (G) Oleo30G_T24C, (H) Oleo30G_S39C. Mass/charge ratio of the peak corresponding to singly charged protein is shown on plots. The second peak at half of the mass/charge ratio corresponds to the doubly charged protein.

Table S2. Oleo30G and Oleo30G-cys mutants' expected molecular weights and molecular weights measured by MALDI-TOF-MS.

	Expected MW	**Measured MW**	**Difference**
Oleo30G	15,026.63	15,029.64	3.01
Oleo30G_S2C	15,042.69	15,047.73	5.04
Oleo30G_T3C	15,028.66	15,029.27	0.61
Oleo30G_T4C	15,028.66	15,031.23	2.57
Oleo30G_T5C	15,028.66	15,029.78	1.12
Oleo30G_T12C	15,028.66	15,027.66	1.00
Oleo30G_T24C	15,028.66	15,035.41	6.75
Oleo30G_S39C	15,042.69	15,044.00	1.31

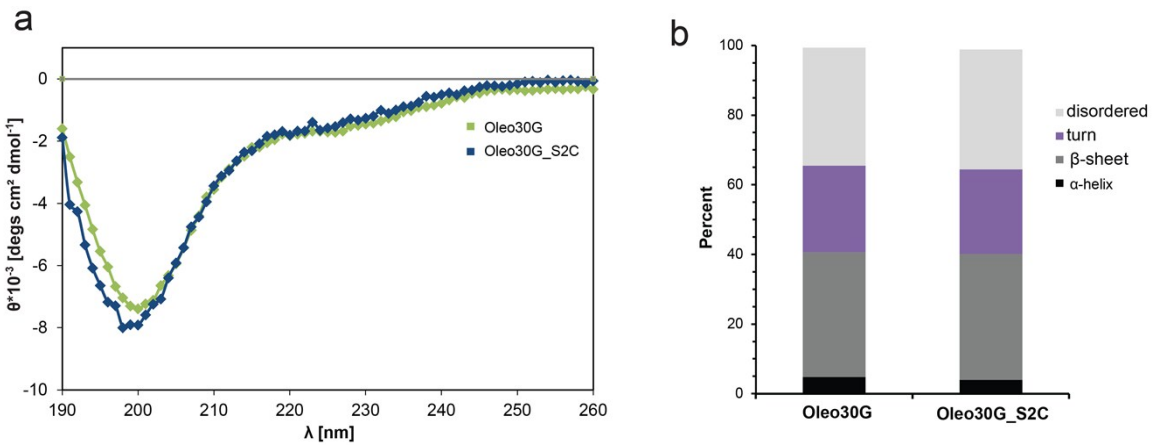

Figure S3. (A) Circular dichroism spectra of Oleo30G (green) and Oleo30G_S2C (blue). (B) Circular dichroism analysis of Oleo30G and Oleo30G_S2C. No meaningful difference in CD spectra was observed between the two proteins. Both proteins were predicted to be about 30% disordered. There were also a substantial percentage turns and β-strands predicted. Only a small percentage (<10%) of the proteins are predicted to be α-helical likely due to the elimination of hydrophobic α-helical sections from the WT oleosin to form Oleo30G.

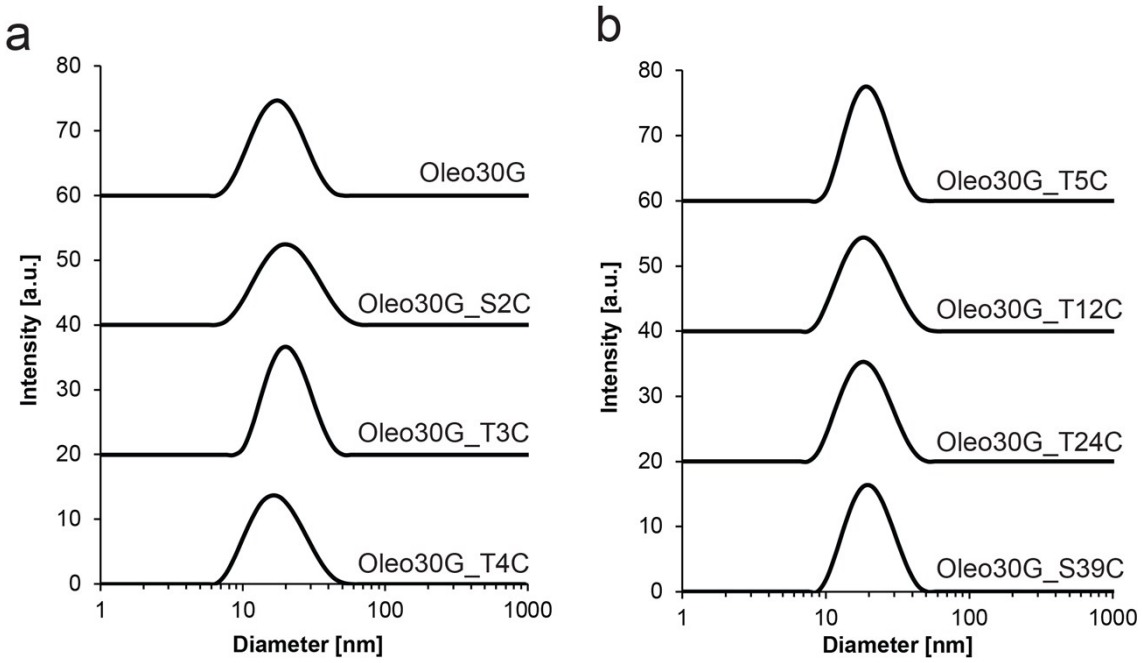

Figure S4. DLS traces of Oleo30G and Oleo30G-cys mutants. Curves are offset for clarity. Oleo30G and Oleo30G-cys mutants all showed a single peak around 20 nm. This indicates that these molecules likely form spherical micelles with a hydrodynamic diameter of about 20 nm.

Table S3. Oleo30G and Oleo30G-cys mutants' hydrodynamic diameter and polydispersity index as measured by DLS.

	d [nm]	**PDI**
Oleo30G	18.62	0.193
Oleo30G_S2C	22.27	0.202
Oleo30G_T3C	21.54	0.142
Oleo30G_T4C	21.47	0.140
Oleo30G_T5C	20.56	0.118
Oleo30G_T12C	20.44	0.172
Oleo30G_T24C	19.86	0.151
Oleo30G_S39C	21.07	0.097

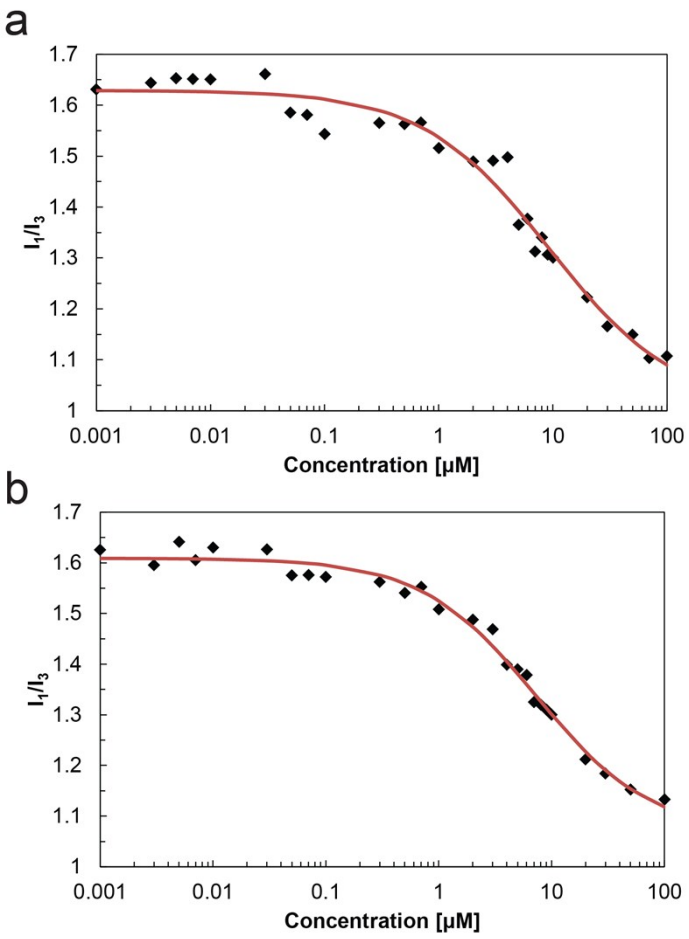

Figure S5. Pyrene fluorescence assay of (A) Oleo30G and (B) Oleo30G_S2C. The ratio of the intensities of the of the first and third peak of the emission spectrum is plotted against the protein concentration. The red line is a sigmodal curve fit to the data. The critical micelle concentration (cmc) was taken as the inflection point of the sigmodal curve. Oleo30G had a cmc of 9.36 μM and Oleo30G_S2C had a cmc of 7.30 μM.

Table S4. Size and number density of Oleo30G and Oleo30G-cys droplets. Protein solutions were at a concentration of 80 μM protein in DPBS with 1 mM DTT. Samples were chilled on ice for 10 minutes before transferring to chambered coverglass coated with pluronic F-127. Images were taken at the coverglass. Droplet sizes were determined using imageJ. Averages were taken of multiple experiments for six total fields of view (228 x 228 μm per field of view). Droplet sizes are ± standard deviation of all droplets measured. Droplet number density are ± standard deviation of six fields of view.

	Droplet Size (μm)	**Droplets per 100 μm^2**
Oleo30G_S2C	3.33 ± 1.32	1.48 ± 0.21
Oleo30G_T3C	3.07 ± 1.08	0.31 ± 0.06
Oleo30G_T4C	3.24 ± 1.72	0.57 ± 0.20
Oleo30G_T5C	2.98 ± 1.66	0.55 ± 0.30
Oleo30G_T12C	2.63 ± 1.20	0.44 ± 0.05
Oleo30G_T24C	2.56 ± 1.40	0.42 ± 0.18
Oleo30G_S39C	1.81 ±1.19	0.24 ± 0.05
Oleo30G	1.56 ± 1.13	0.17 ± 0.07

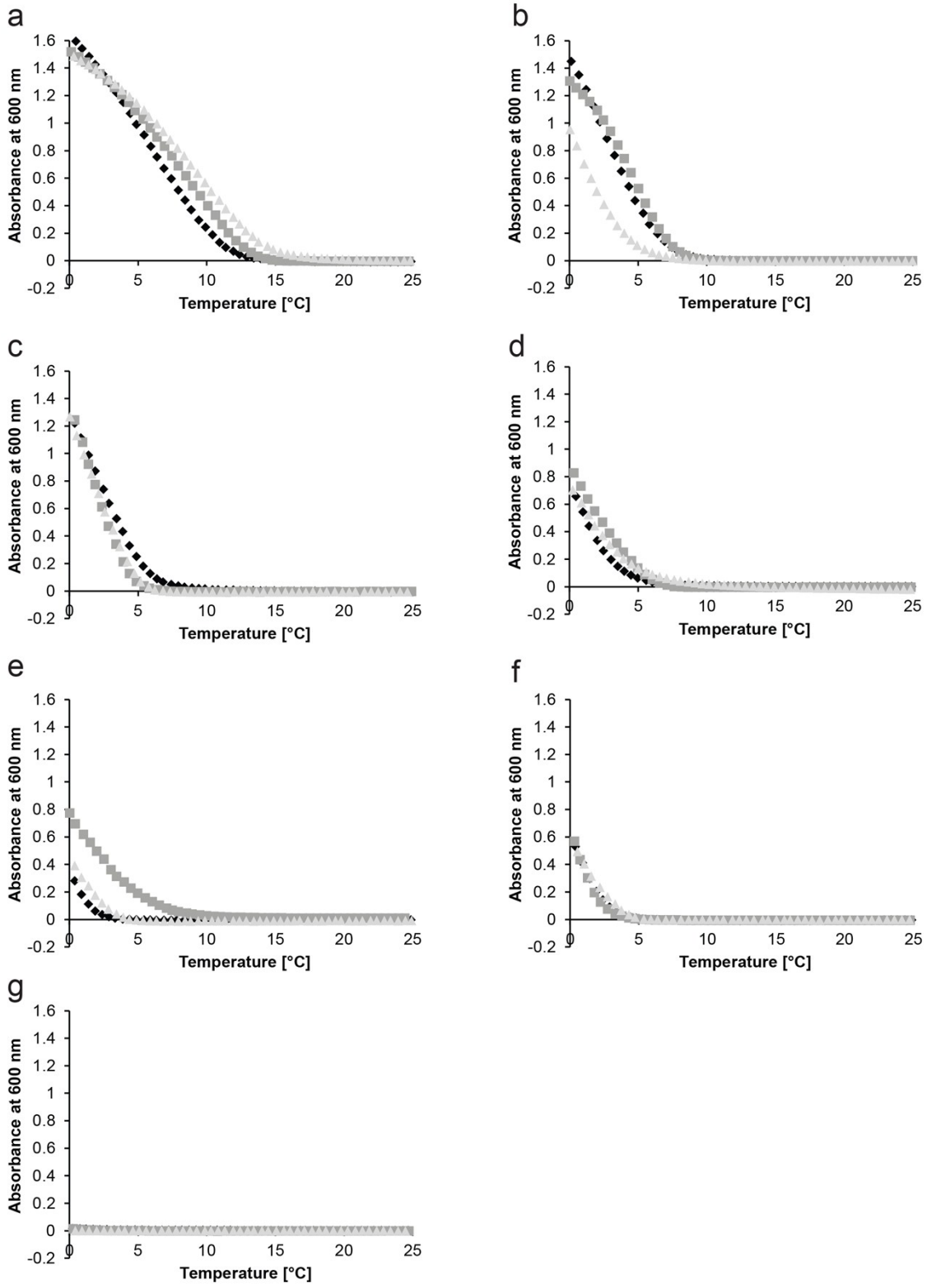

Figure S6. UV-vis spectroscopy traces of (A) Oleo30G_S2C, (B) Oleo30G_T3C, (C) Oleo30G_T4C, (D) Oleo30G_T5C, (E) Oleo30G_T12C, (F) Oleo30G_T24C, (G) Oleo30G_S39C, (H) Oleo30G. Protein solutions were at a concentration of 80 μM protein in DPBS with 1 mM DTT. Measurements were taken starting at 37 °C and cooling at a rate of 1 °C per minute. Measurements were taken in increments if 0.5 °C. For clarity, data is shown only for 25 °C and below. Triplicates are shown for each Oleo30G-cys mutant.

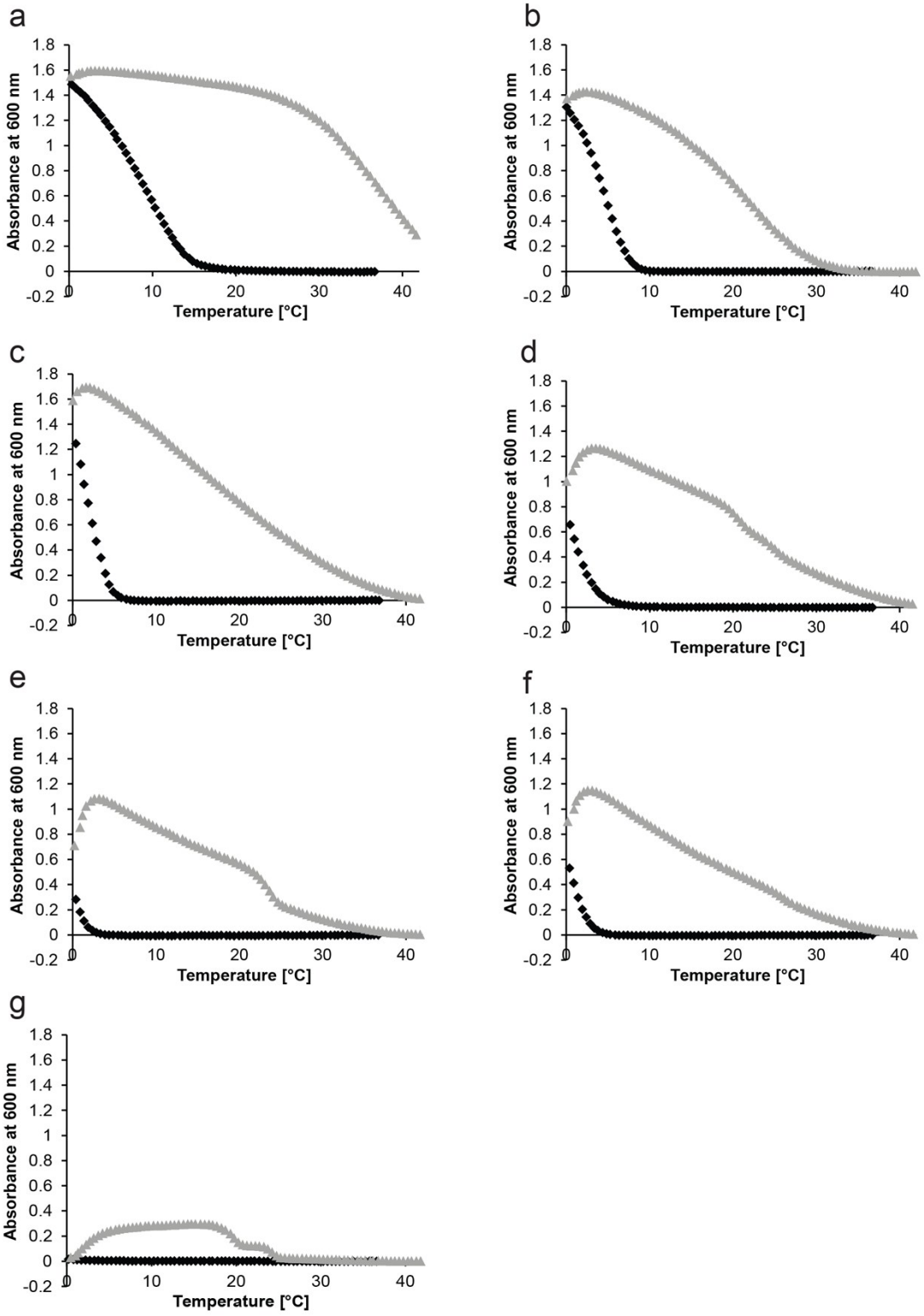

Figure S7. UV-vis spectroscopy traces of (A) Oleo30G_S2C, (B) Oleo30G_T3C, (C) Oleo30G_T4C, (D) Oleo30G_T5C, (E) Oleo30G_T12C, (F) Oleo30G_T24C, (G) Oleo30G_S39C. Solutions were at a concentration of 80 μM protein in DPBS with 1 mM DTT. Measurements were taken starting at 37 °C and cooling at a rate of 1 °C per minute to a final temperature of 0 °C (black curves) then warming at 1 °C per minute to a final temperature of 42 °C (grey curves). Measurements were taken in increments if 0.5 °C.

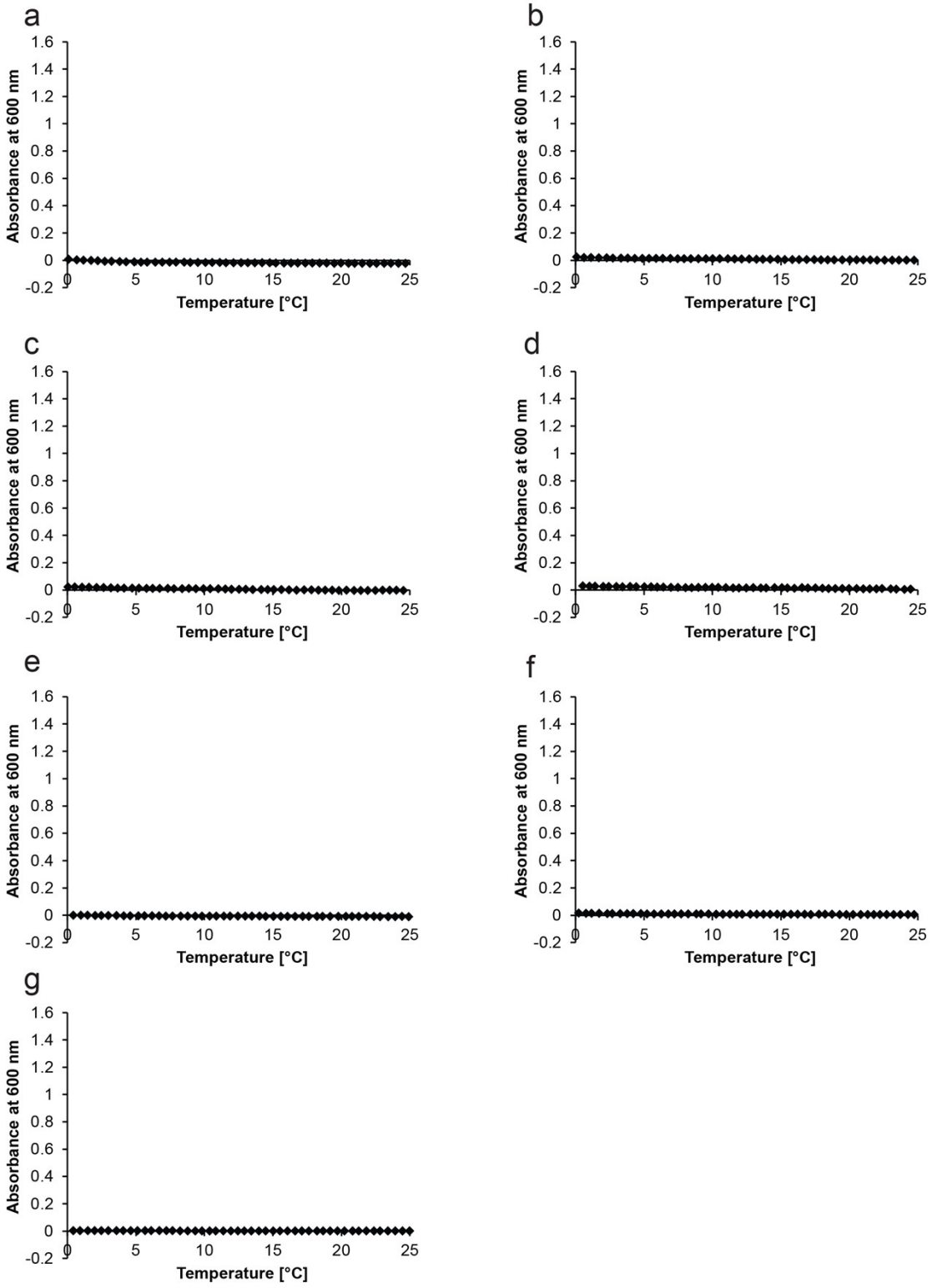

Figure S8. UV-vis spectroscopy traces of (A) Oleo30G_S2C, (B) Oleo30G_T3C, (C) Oleo30G_T4C, (D) Oleo30G_T5C, (E) Oleo30G_T12C, (F) Oleo30G_T24C, (G) Oleo30G_S39C. Solutions were at a concentration of 80 μM protein in DPBS with 1 mM DTT. βME was added to the protein solution to a final concentration of 80 mM. Measurements were taken cooling at a rate of 1 °C per minute to a final temperature of 0 °C. Measurements were taken in increments if 0.5 °C.

Table S5. Size and number density of Oleo30G, Oleo30G_S2C and blend droplets. Protein solutions were at a concentration of 80 μM protein in DPBS with 1 mM DTT. Samples were chilled on ice for 10 minutes before transferring to chambered coverglass coated with pluronic F-127. Images were taken at the coverglass. Droplet sizes were determined using imageJ. Averages were taken of multiple experiments for six total fields of view (228 x 228 μm per field of view). Droplet sizes are ± standard deviation of all droplets measured. Droplet number density are ± standard deviation of six fields of view.

	Droplet Size (μm)	**Droplets per 100 μm^2**
Oleo30G_S2C	3.33 ± 1.32	1.48 ± 0.21
75:25 Oleo30G_S2C:Oleo30G	3.38 ± 1.89	0.61 ± 0.07
50:50 Oleo30G_S2C:Oleo30G	2.19 ± 1.47	0.68 ± 0.14
25:75 Oleo30G_S2C:Oleo30G	2.09 ± 1.45	0.37 ± 0.07
Oleo30G	1.56 ± 1.13	0.17 ± 0.07