

Supplementary Information

Not the Usual Suspects: Alternative Surfactants for Biopharmaceuticals

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Index	Name	Category	Screening drop out stage	PubChem SID	CAS No.	MW (Da)	HLB	CMC (w/v%)
1	PS20	3	N/A	472407007	9005-64-5	1228	16.7	0.007
2	PS80	3	N/A	472385962	9005-65-6	1310	15.0	0.002
3	PLX188	3	N/A	472405058	9003-11-6	8400	16.0	0.400
4	Labrafil M2125 CS (Linoleoyl Macrogl-6 glycerides)	1	1	135278950	61789-25-1	-	-	-
5	Labrafil M1944 CS	1	1	472217119	69071-70-1	-	-	-
6	Poly(ethylene glycol) monooleate	1	2	441152743	9004-96-0	860	13.6	0.006
7	Lansurf CDE-G	1	1	363902551	68603-42-9	-	-	-
8	Lansurf CDE	1	1	363902551	68603-42-9	-	-	-
9	Lansurf SMO	1	1	363898660	1338-43-8	-	-	-
10	Lansurf STO85	1	1	363898653	9005-70-3	-	-	-
11	Lansurf STO	1	2	341113964	26266-58-0	957	1.8	-
12	Lansurf CAPO	1	1	48419813	68155-09-9	-	-	-
13	Suppocire a pellets	1	1	135356513	85665-33-4	-	-	-
14	Gelucire 44/14 (Lauroyl Macrogl-32 glycerides)	1	1	441153334	57107-95-6	-	-	-
15	Gelucire 48/16 pellets (Polyoxyl-32 stearate)	1	2	441152744	9004-99-3	622	16	0.008
16	Tefose 63 (PEG-6 stearate and ethylene glycol palmitostearate and PEG-32 stearate)	1	1	441152744	91031-31-1	-	-	-
17	Labrafil M 2130 CS (Lauroyl Macrogl-6 Glyceride)	1	1	441153334	57107-95-6	-	-	-
18	Tefose 1500 (PEG-6 Stearate(and)PEG-32 Stearate)	1	1	441152744	9004-99-3	-	-	-
19	Gelucire 50/13 Pellets (Stearoyl Macrogl-32 Glyceride)	1	1	472404499	9011-21-6	-	-	-
20	Gelucire 43/01 Pellets	1	1	470740159	85665-33-4	-	-	-
21	TCA Sodium Taurocholate duretgul-M-007	1	1	254774705	345909-26-4	-	-	-
22	TCA Sodium Taurocholate 90% duretgul-M3-013	1	1	254774705	345909-26-4	-	-	-
23	TCDCA Taurochenodeoxycholate GOSSEBR1-SSS011	1	1	254774705	345909-26-4	-	-	-
24	TCA Sodium Taurocholate 90% duretgul-M3-003-006	1	1	254774705	345909-26-4	-	-	-
25	TCA Sodium Taurocholate	1	1	254774705	345909-26-4	-	-	-
26	GLCA Glycolithocholic acid GOSSEBR1-SSS015A	1	1	375925049	474-74-8	-	-	-
27	TCA Sodium Taurocholate 98% duretgul-M3-013	1	1	254774705	345909-26-4	-	-	-
28	TDCA Taurodeoxycholic acid M03-bigorf11-003	1	1	363898662	516-50-7	-	-	-
29	TLCA Taurolithocholic acid M03-bigorf11-002	1	1	363898662	516-50-7	-	-	-
30	KoolAid Surfactant	1	2	434274298	2306441-11-0	-	-	-
31	Tallowamine polyethoxylated	1	2	387021720	61791-26-2	1500	5.0	0.013
32	Cholic glycine	1	1	341125635	475-31-0	-	-	-

33	Kolliphor ELP	1	2	470667819	61791-12-6	2500	13.0	0.006
34	SPGS 550M	1	3	329767804	81125-67-9	1057	10.7	0.002
35	Brij30	1	1	254787116	9002-92-0	-	-	-
36	PQS-750	1	2	470636525	521313-35-9	1588	14.0	0.020
37	PS-750-M (FI-750-M)	1	2	434214546	2135447-74-2		16.4	0.050
38	VEDS	2	4	-	-	1652	13.0	0.011
39	VEDG-2.2	2	4	-	-	1666	12.9	0.040
40	Brij58S	2	4	-	-	1447	14.9	0.008

Table S1. Details of all the surfactants investigated in this study. **Index** a sequential index, shared across all tables in this paper. **Name** name of the surfactant investigated. **Category** numerical value indicating the origin of the surfactant (1 = available before this study, 2 = prepared *ad hoc* for this study, 3 = benchmark surfactant). **Screening drop out stage** Best screening gate reached by the surfactant in the selection phase (1 = gate 1 (red shading), 2 = gate 2 (yellow shading), 3 = gate 3 (light green shading), 4 = final candidates (dark green shading)). **PubChem SID** substance identifier (SID) in the PubChem database of chemical molecules. **CAS No** numeric identifier assigned to a substance registered in the CAS REGISTRY database. **MW** theoretical molecular weight expressed in Da. **HLB** theoretical hydrophilic-lipophilic balance value. **CMC** critical micelle concentration, provided by the supplier (category 1/3 surfactants) or measured in 10mM His pH 5.0 (category 2 surfactants). MW, HLB and CMC are provided only for surfactants reaching at least screening gate 2.

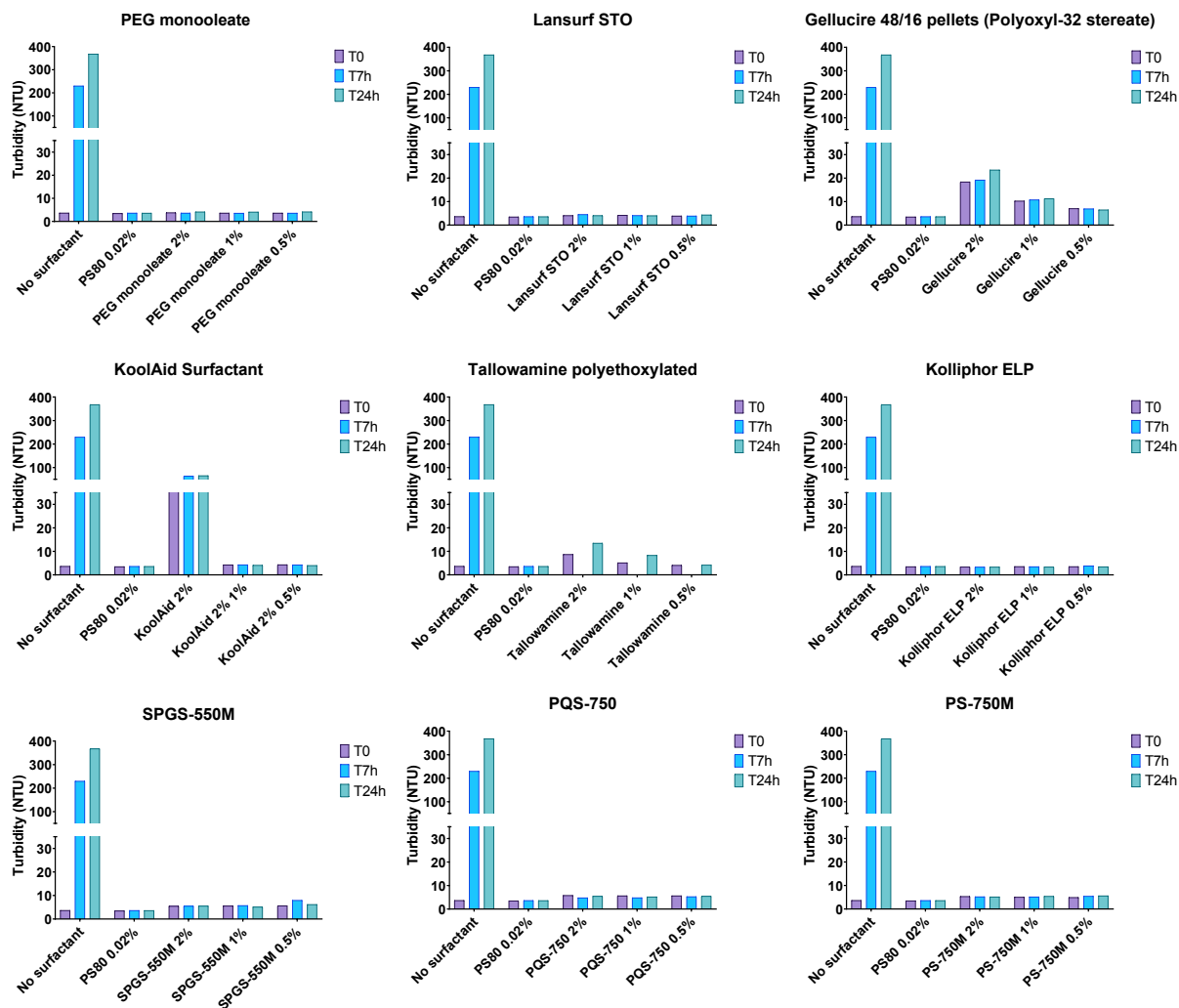


Figure S1. Turbidity data of the top 9 candidates of category one, selected during screening gate one. Selected candidates were tested at three concentrations: 2, 1 or 0.5% (w/v). The turbidity (NTU) is indicated for the selected candidate, as well as for a negative control in absence of any surfactant and a benchmark including PS80 0.02%, after 0 (purple), 7 (cyan) or 24 hours (green).

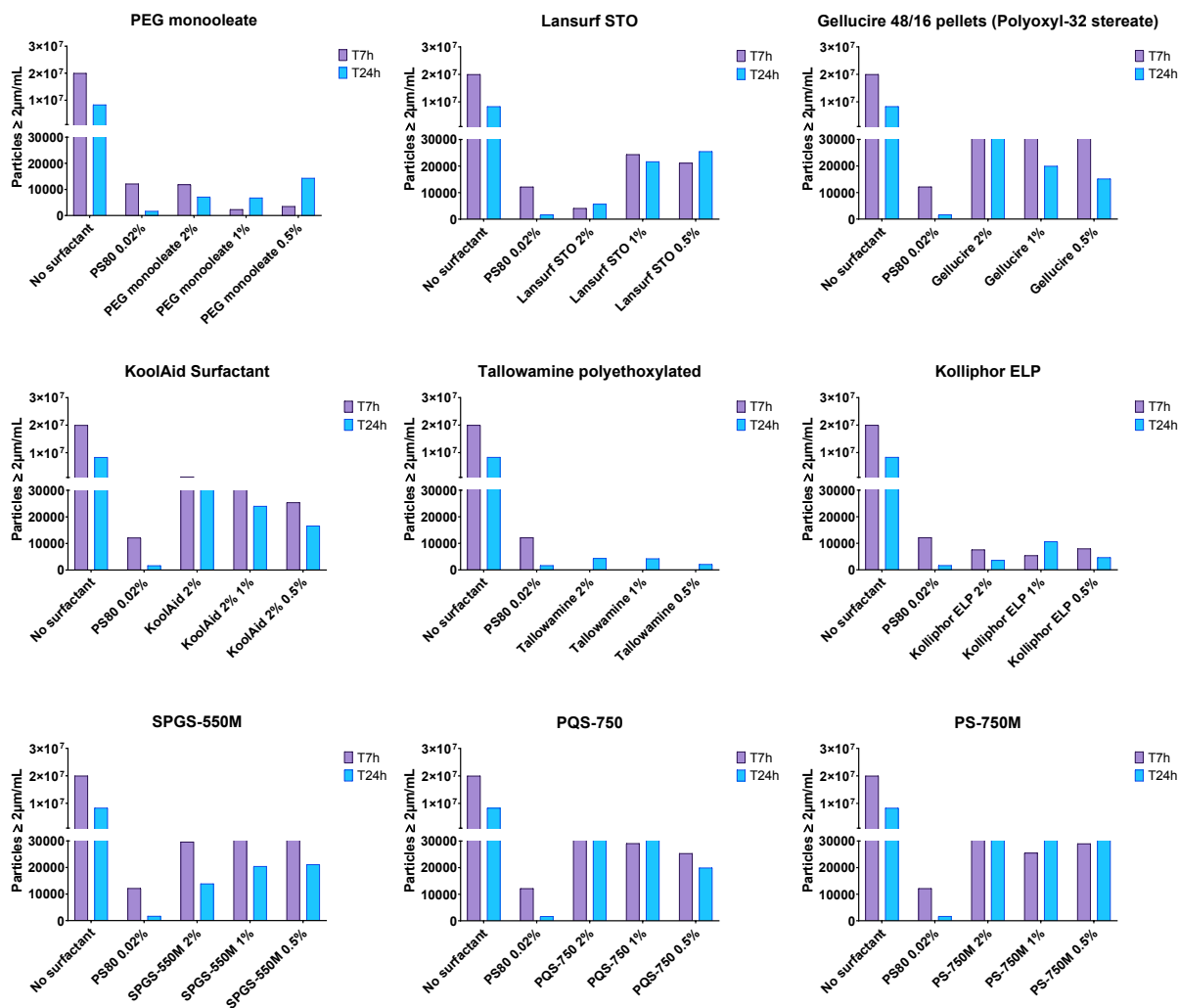


Figure S2. Particle counts obtained from MFI data of the top 9 candidates of category one, selected during screening gate one. Selected candidates were tested at three concentrations: 2, 1 or 0.5% (w/v). The particles count ($>2\mu\text{m/mL}$) is indicated for the selected candidate, as well as for a negative control in absence of any surfactant and a benchmark including PS80 0.02%, after 7 (purple) or 24 hours (cyan).

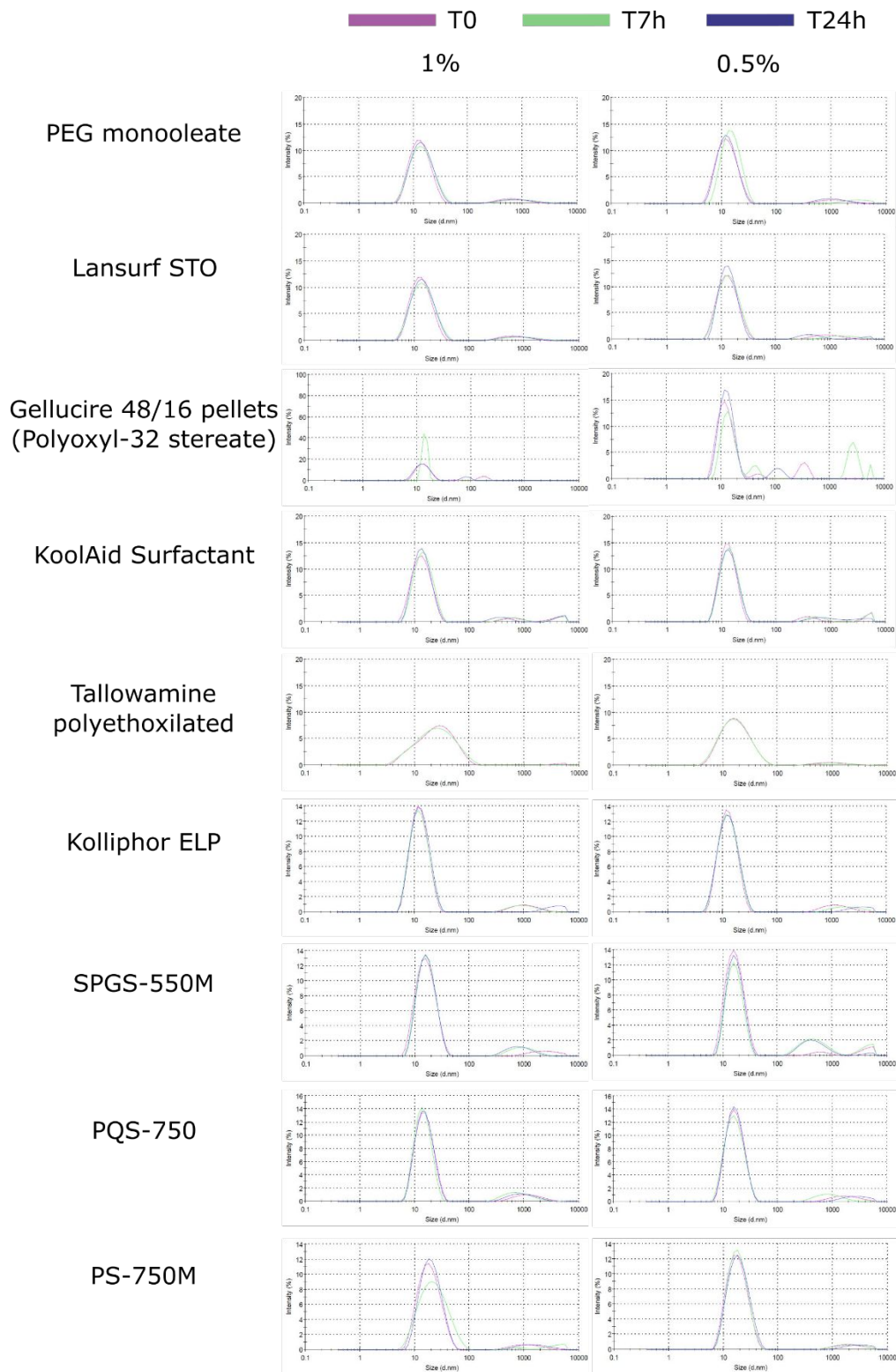


Figure S3. Size distribution data obtained from DLS of the top 9 candidates of category one, selected during screening gate one. Selected candidates are shown at two concentrations: 1% (left panels) or 0.5% w/v

(right panel). The size distribution by intensity is indicated for the selected candidate after 0 (purple), 7 (green) or 24 hours (blue).

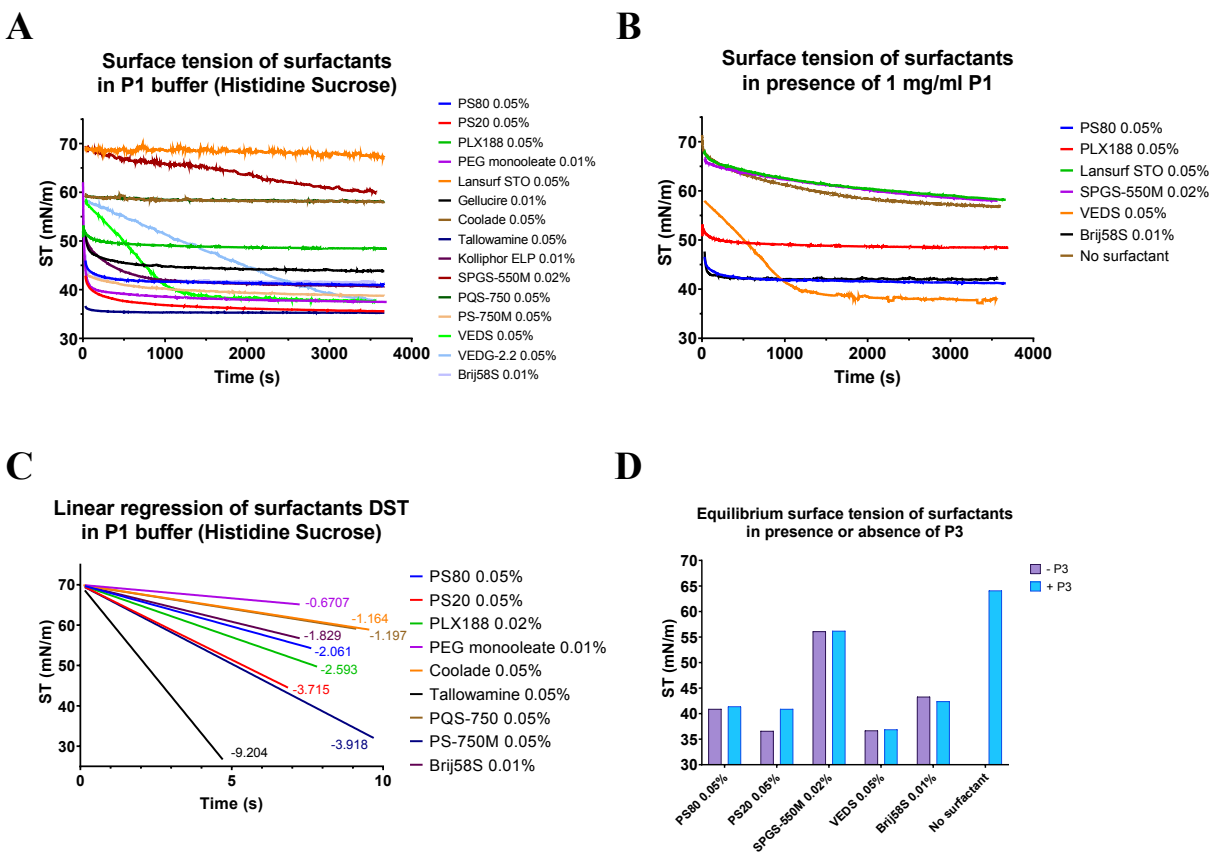


Figure S4. Additional details of surface tension characterization. **A** DST of the entire surfactant dataset after screening gate one through the pendant drop method. Surfactants were measured at the concentration (w/v) indicated in P1 buffer. **B** DST of selected surfactant candidates through the pendant drop method. Surfactants were measured at the concentration (w/v) indicated in P1 buffer in presence of 1 mg/mL P1. **C** Linear regression of DST of surfactant candidates measured through the maximum bubble pressure method (**Figure 2B**). For each surfactant, datapoints between 0.1s and 10s were used to generate a linear regression. The y-intercept was forced at 70 mN/m in order to enhance the visual comparison between candidates. Slopes are indicated next to the corresponding curve. **D** EST of selected candidates. Surfactants were measured at the concentration (w/v) indicated in P3 buffer in absence (purple) or presence (cyan) of 10 mg/mL P3, ensuring that the system was in equilibrium.

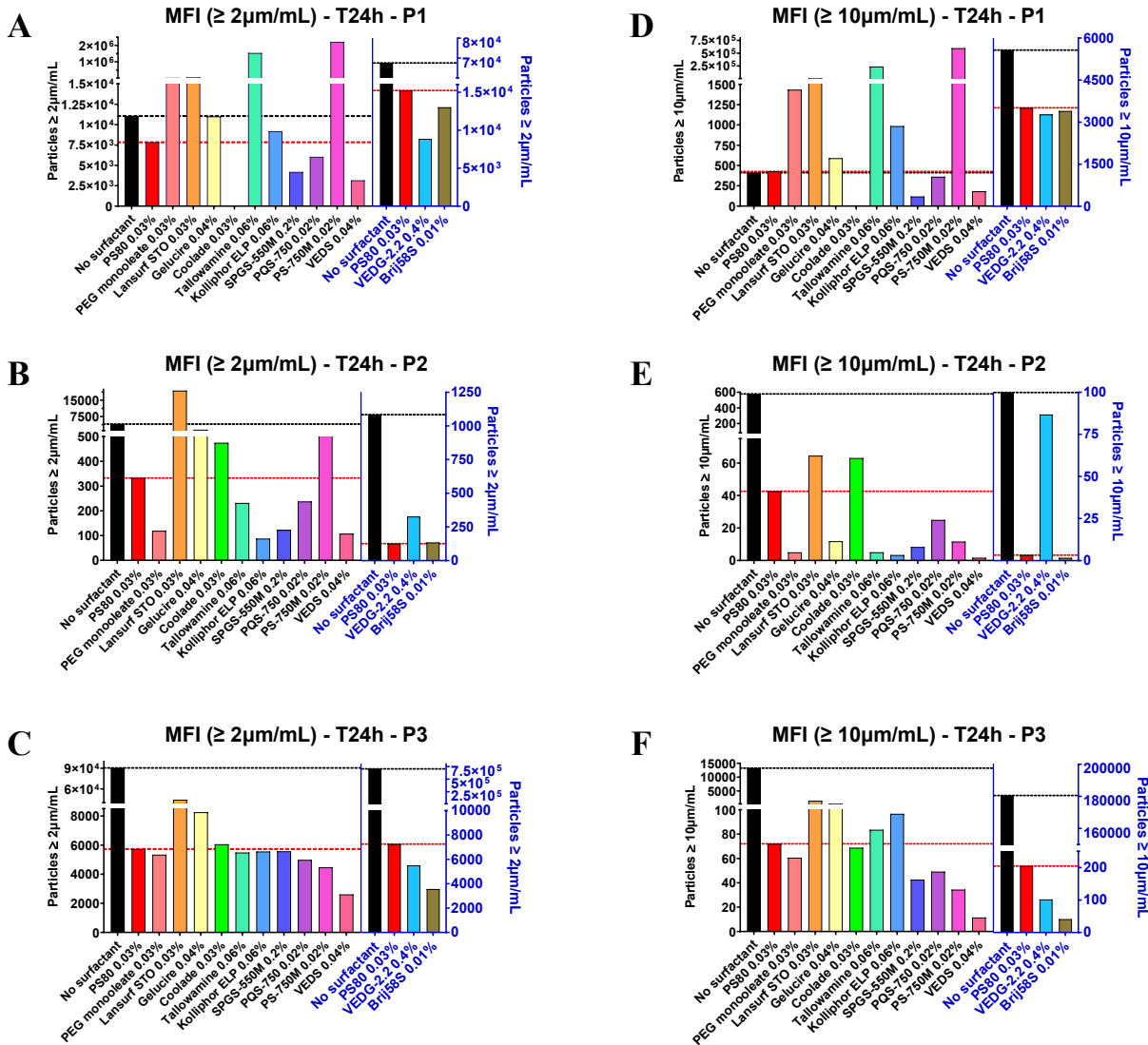


Figure S5. Agitation-induced aggregation assays particles counts ($\geq 2\mu\text{m/mL}$ and $\geq 10\mu\text{m/mL}$) determined by MFI after 24 hours. The bars are surfactant color-coded to ease comparison between figures, panels and graphs. Each individual panel is composed by two adjacent bar graphs separated by a vertical blue line, one on the left (black axis) and one on the right (blue axis), indicating different runs with distinct scales that should therefore be compared separately. The no surfactant and PS80 0.03% samples are repeated in each run as benchmark surfactants and their levels are indicated by a black and red dashed line, respectively. **A** Particles $\geq 2\mu\text{m/mL}$ in P1. **B** Particles $\geq 2\mu\text{m/mL}$ in P2. **C** Particles $\geq 2\mu\text{m/mL}$ in P3. **D** Particles $\geq 10\mu\text{m/mL}$ in P1. **E** Particles $\geq 10\mu\text{m/mL}$ in P2. **F** Particles $\geq 10\mu\text{m/mL}$ in P3.

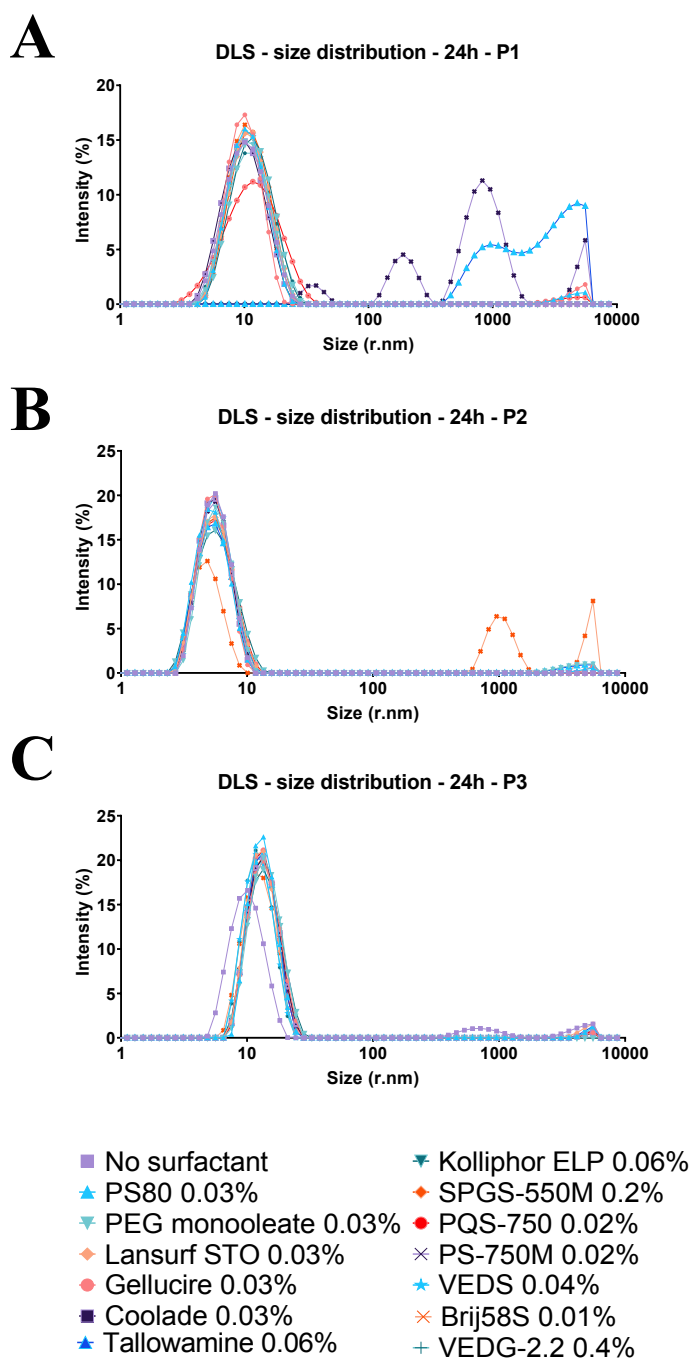


Figure S6. Agitation-induced aggregation assays particles size distribution determined by DLS after 24h. **A** protein 1 (P1). **B** Protein 2 (P2). **C** Protein 3 (P3)

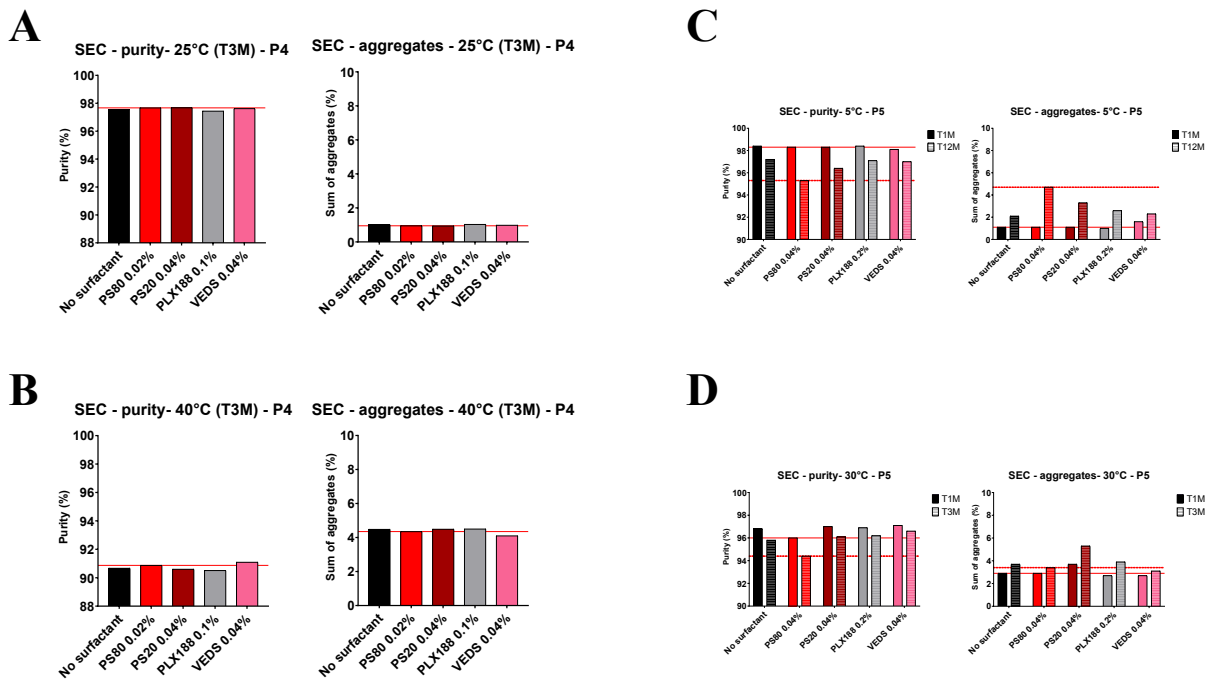


Figure S7. Additional data on protein’s purity (left) and aggregates (right) determined by SEC-UPLC in quiescence stability studies. Datapoints measured in the first and last timepoint, where present, are indicated by full and striped bars, respectively. The PS80 0.03% benchmark surfactants level at the first and last timepoint, where present, is indicated by a full and dashed red line, respectively. The bars are surfactant color-coded to ease comparison between figures, panels and graphs. **A** Protein 4 (P4) formulations measured at 25°C at 3 months. **B** Protein 4 (P4) formulations measured at 40°C at 3 months. **C** Protein 5 (P5) formulations measured at 5°C at 1 and 12 months. **D** Protein 5 (P5) formulations measured at 30°C at 1 and 3 months.

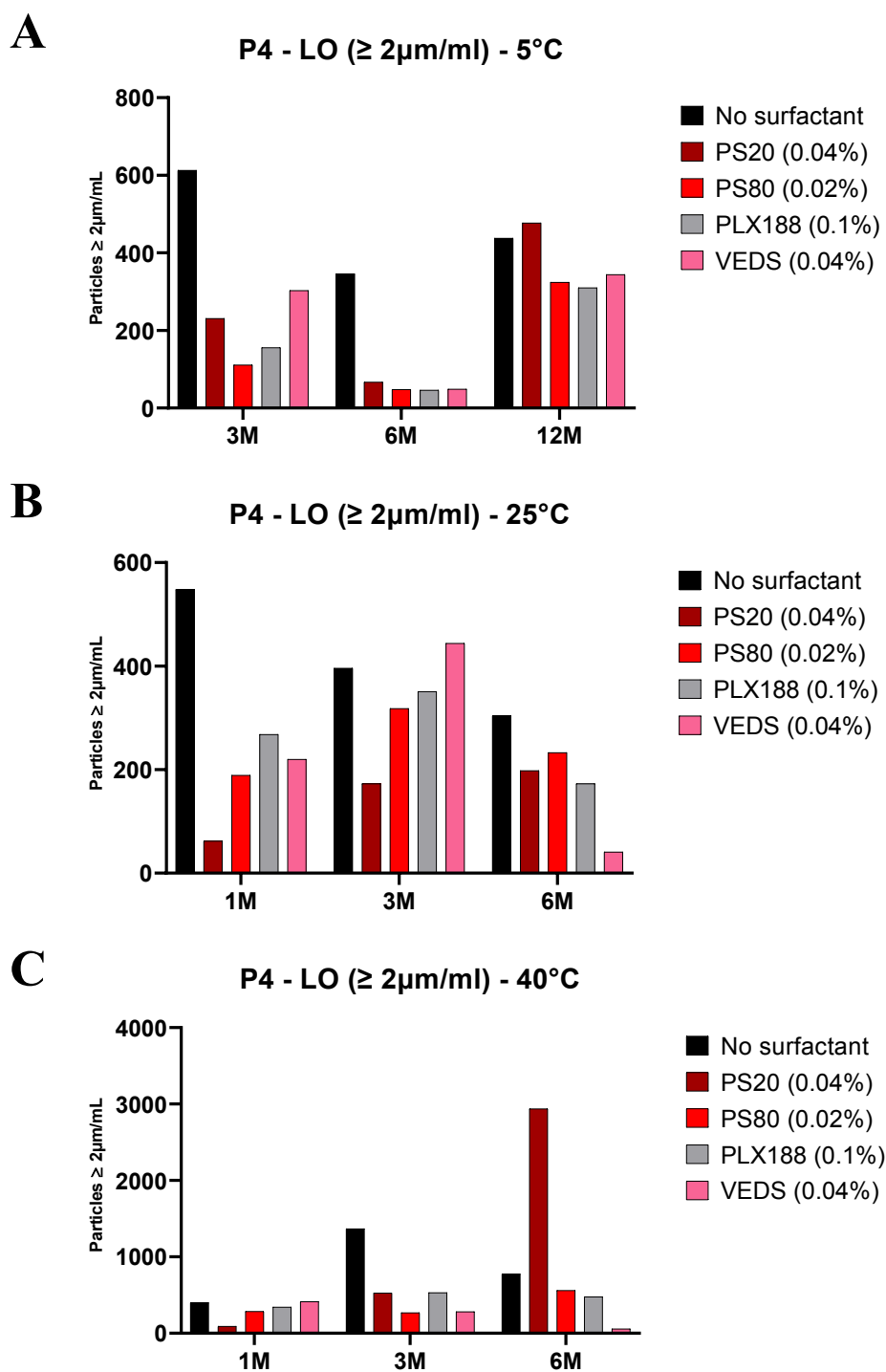


Figure S8. Particles counts ($\geq 2\mu\text{m/mL}$) determined by LO in quiescence stability studies in protein 4 (P4) formulations. The bars are surfactant color-coded to ease comparison between figures, panels and graphs. **A** Formulations at 5°C. **B** Formulations at 25°C. **C** Formulations at 40°C.

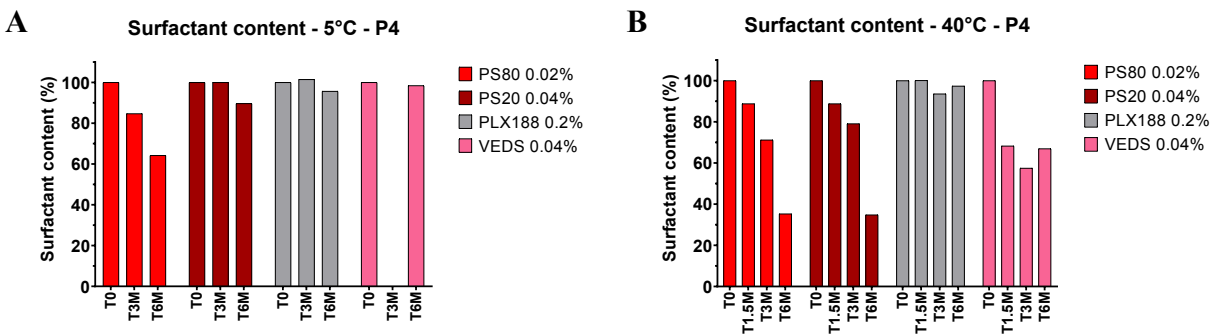


Figure S9. Additional surfactant content in P4 quiescence stability studies measured by HPLC. Each surfactant content is normalized to its first timepoint. The bars are surfactant color-coded to ease comparison between figures, panels and graphs. **A** 5°C. **B** 40°C.