Co-Surfactant-Free Bioactive Protein Nanosheets for the Stabilisation of Bioemulsions Enabling Adherent Cell Expansion

Supplementary Information

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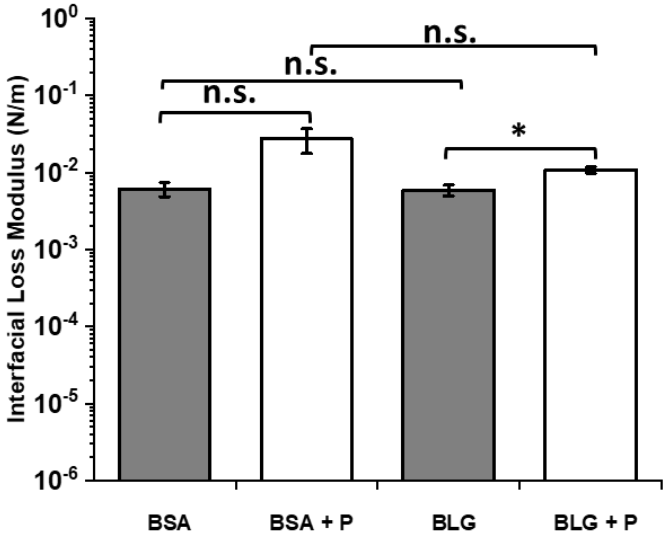
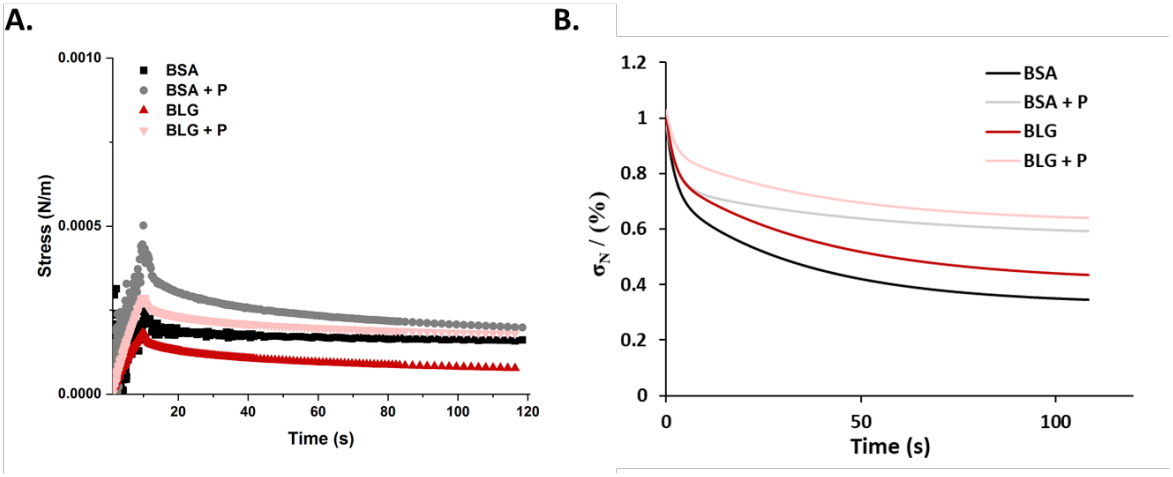
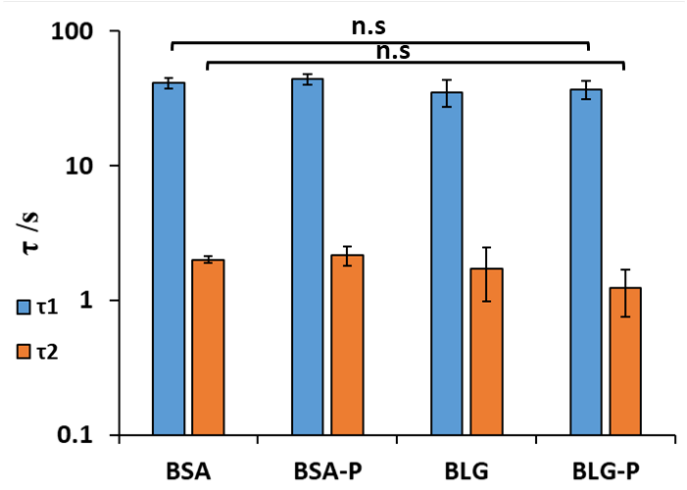


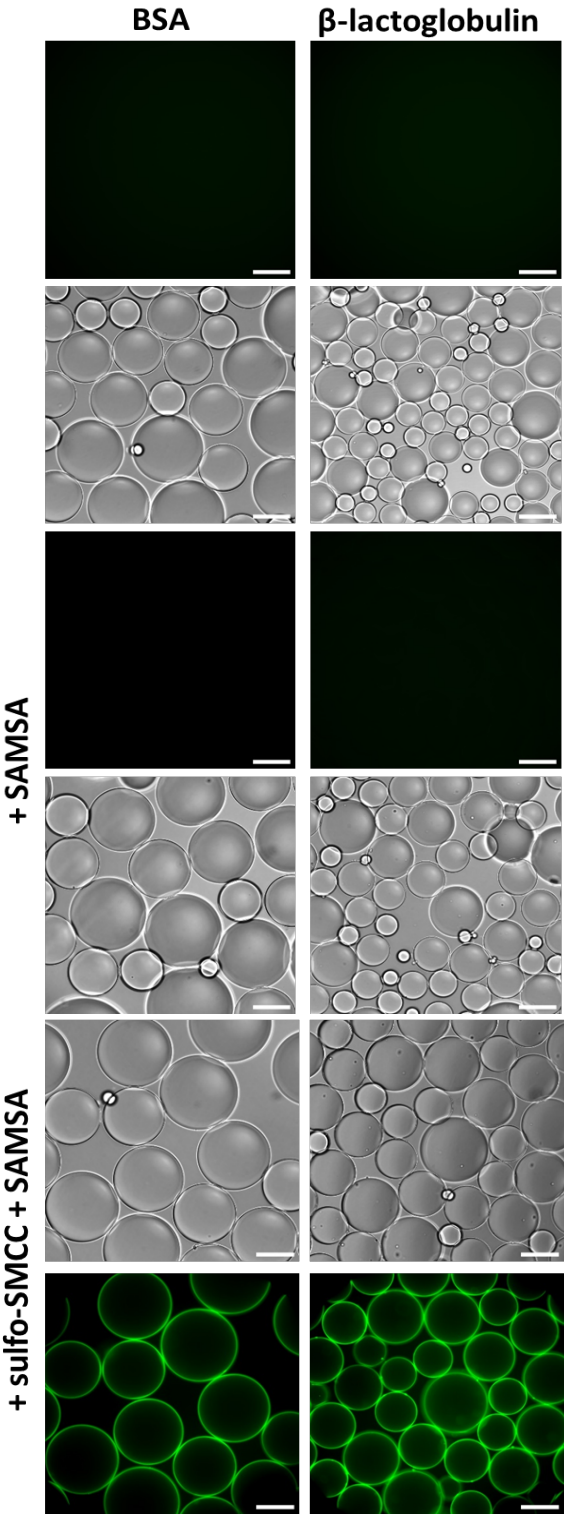
Supplementary Figure S1. Interfacial shear loss modulus of BSA, BSA + PFBC, BLG, BLG+PFBC, casein and casein + PFBC at oscillating amplitude 10^{-4} rad and frequency of 1 Hz. Error bars are s.e.m; n=3.



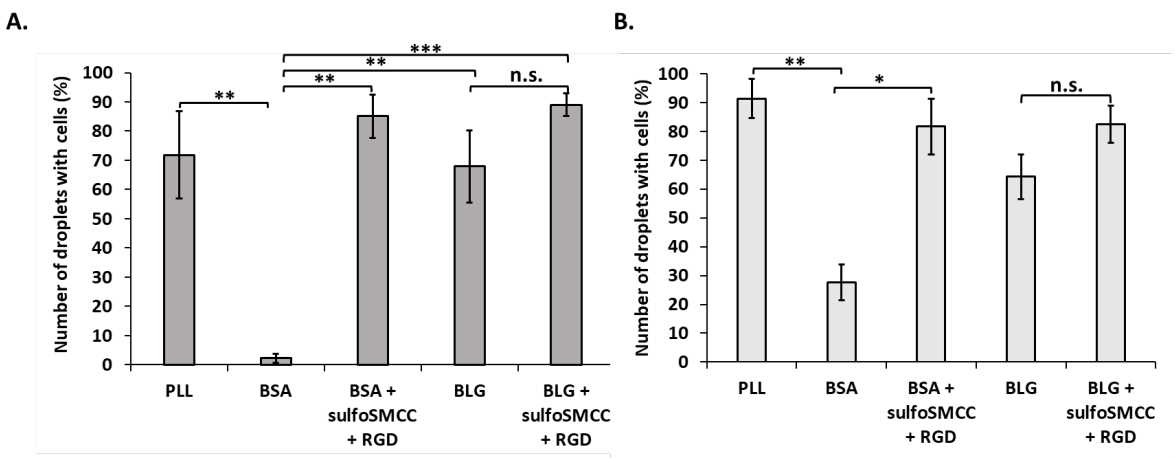
Supplementary Figure S2. Stress relaxation experiments carried out on protein nanosheets formed at liquid-liquid interfaces with and without PFBC (10 μg/mL). Data is shown as normalised stress (σ_N) extracted from stress relaxation experiments at a strain 0.5%.



Supplementary Figure S3. Characterisation of the stress relaxation profiles associated with protein nanosheets studied. Data were extracted from stress relaxation at a strain of 0.5%. (A) Comparing BSA, BLG and casein (all at 1 mg/mL) with and without PFBC. Error bars are s.e.m; n=3.



Supplementary Figure S4. Epifluorescence microscopy images were SAMSA fluorescein (green, AB F3648) conjugated. Scale bars, 200 μm.



Supplementary Figure S4. Percentage number of droplets that were covered with (A) MSCs and (B) HEK293T.

Supplementary Tables

Supplementary Table S1 Summary of statistical analysis of storage modulus data obtained by frequency sweep after the proteins were adsorbed at fluorinated-PBS interfaces with and without co-surfactant (PFBC) at 10 μg/ mL .

	MeanDiff	Prob	
BSA + PFBC BSA	0.11869	0.14564	**n.s**
BLG + PFBC BLG	0.04136	0.00209	****
Casein + PFBC Casein	0.10352	0.03876	*
BLG BSA	-0.00383	0.88433	**n.s**
Casein BSA	-0.0356	0.0104	*
Casein BLG	-0.03177	0.01747	*
BLG + PFBC BSA + PFBC	-0.08115	0.42242	**n.s**
Casein + PFBC BSA + PFBC	-0.05077	0.69215	**n.s**
Casein + PFBC BLG + PFBC	0.03038	0.8718	**n.s**

Supplementary Table S2 Summary of statistical analysis of storage modulus data obtained by frequency sweep after the proteins were adsorbed at fluorinated-PBS interfaces with and without co-surfactant (PFBC) at 10 μg/ mL.

	MeanDiff	Prob	
BSA + PFBC BSA	0.0211	0.09442	**n.s**
BLG + PFBC BLG	0.00488	0.02317	*
Casein + PFBC Casein	0.03089	0.01316	*
BLG BSA	-2.20E-04	0.9853	**n.s**
Casein BSA	-0.00564	0.01322	*
Casein BLG	-0.00542	0.01583	*
BLG + PFBC BSA + PFBC	-0.01644	0.29054	**n.s**
Casein + PFBC BSA + PFBC	0.00415	0.90799	**n.s**
Casein + PFBC BLG + PFBC	0.02059	0.1718	**n.s**

Supplementary Table S3 Summary of statistical analysis of data obtained from stress relaxation experiments at a strain of 0.5 % .

	MeanDiff	Prob	
BSA-P BSA	22.55267	0.09127	**n.s**
BLG-P BLG	11.965	0.06436	**n.s**
BLG-P BSA-P	-2.98533	0.9436	**n.s**
Casein-P BSA-P	-26.076	0.06569	**n.s**

Casein-P BLG-P	-23.0907	0.00896	****
BLG BSA	7.60233	0.01709	*
BLG BSA-P	-14.9503	0.21667	**n.s**

Supplementary Table S4 Summary of statistical analysis of data of τ_1 values obtained from stress relaxation experiments at a strain of 0.5 %.

	MeanDiff	**Prob**	
t1 BLG t1 BSA	1.48429	0.99375	n.s
t1 BSA-P t1 BSA	5.88717	0.74876	n.s
t1 BSA-P t1 BLG	4.40288	0.87215	n.s
t1 BLG-P t1 BSA	8.50963	0.50131	n.s
t1 BLG-P t1 BLG	7.02534	0.64101	n.s
t1 BLG-P t1 BSA-P	2.62246	0.96783	n.s

Supplementary Table S5 Summary of statistical analysis of data of τ_2 values obtained from stress relaxation experiments at a strain of 0.5 %.

	MeanDiff	**Prob**	
t2 BLG t2 BSA	-0.49612	0.84427	n.s
t2 BSA-P t2 BSA	0.76719	0.60681	n.s
t2 BSA-P t2 BLG	1.26331	0.23655	n.s
t2 BLG-P t2 BSA	0.92303	0.46832	n.s
t2 BLG-P t2 BLG	1.41916	0.16735	n.s
t2 BLG-P t2 BSA-P	0.15585	0.99356	n.s

Supplementary Table S6 Summary of statistical analysis of data obtained from the SPR data for the protein binding (1 mg/mL) at the perfluorodecanethiol pre-treated chips.

	MeanDiff	**Prob**	
β-lactoglobulin BSA	461.2667	0.45494	**n.s**
β-casein BSA	1184.367	0.03828	*
β-casein β-lactoglobulin	723.1	0.19123	**n.s**

Supplementary Table S7 Summary of statistical analysis of data obtained from the SPR data for the sulfo-SMCC (2 mg/mL) at the surface of protein layers (BSA, BLG and casein).

	MeanDiff	**Prob**	
β-lactoglobulin BSA	105.0667	0.93711	**n.s**
β-casein BSA	-80.7333	0.9622	**n.s**
β-casein β-lactoglobulin	-185.8	0.81995	**n.s**

Supplementary Table S8 Summary of statistical analysis of data obtained from the SPR data for the RGD (1.6 mg/mL) at the surface of the sulfo-SMCC layer.

	MeanDiff	Prob	
β-lactoglobulin BSA	26.06667	0.88869	**n.s**
β-casein BSA	47.4	0.68847	**n.s**
β-casein β-lactoglobulin	21.33333	0.92352	**n.s**

Supplementary Table S9 Summary of statistical analysis of data obtained from the epifluorescence images for the SAMSA-flurorescein binding on BSA, BLG and casein emulsion droplets in the presence and absence of sulfo-SMCC.

	MeanDiff	Prob	
BLG BSA	-23.5313	0.93412	**n.s**
Casein BSA	-37.1652	0.8461	**n.s**
Casein BLG	-13.6339	0.97721	**n.s**
BLG BSA	-23.5313	0.99966	**n.s**
Casein BSA	-37.1652	0.997	**n.s**
Casein BLG	-13.6339	0.99998	**n.s**
BSA + SAMSA BSA	127.9757	0.63783	**n.s**
BSA + SAMSA BLG	151.507	0.47613	**n.s**
BSA + SAMSA Casein	165.1409	0.39065	**n.s**
BLG + SAMSA BSA	6.54822	1	**n.s**
BLG + SAMSA BLG	30.07956	0.9989	**n.s**
BLG + SAMSA Casein	43.71344	0.99363	**n.s**
BLG + SAMSA BSA + SAMSA	-121.427	0.68331	**n.s**
Casein+ SAMSA BSA	40.45822	0.99554	**n.s**
Casein+ SAMSA BLG	63.98956	0.96608	**n.s**
Casein+ SAMSA Casein	77.62344	0.9267	**n.s**
Casein+ SAMSA BSA + SAMSA	-87.5174	0.88595	**n.s**
Casein+ SAMSA BLG + SAMSA	33.91	0.99805	**n.s**

BLG + sulfo-SMCC + SAMSA BSA + sulfo-SMCC + SAMSA	-649.481	0.53284	**n.s**
Casein+ sulfo-SMCC + SAMSA BSA + sulfo-SMCC + SAMSA	96.22956	0.98474	**n.s**
Casein+ sulfo-SMCC + SAMSA BLG + sulfo-SMCC + SAMSA	745.7107	0.44729	**n.s**
BSA + sulfo-SMCC + SAMSA BSA	4146.55	8.79E-04	*******
BLG + sulfo-SMCC + SAMSA BLG	3520.6	2.10E-04	*******
Casein+ sulfo-SMCC + SAMSA Casein	4279.945	7.43E-04	*******

Supplementary Table S10 Summary of statistical analysis of data obtained from cell (MSCs) on emulsion droplets after seven days in culture.

	MeanDiff	**Prob**	
PLL TCP	-17544.4	0.36446	n.s
BSA TCP	-75266.7	1.52E-05	***
BSA PLL	-57722.2	2.16E-04	***
BLG TCP	-30788.9	0.03204	***
BLG PLL	-13244.4	0.63719	n.s
BLG BSA	44477.78	0.00222	**
BSA +sulfo-SMCC + RGD TCP	-29829.6	0.03876	*
BSA +sulfo-SMCC + RGD PLL	-12285.2	0.70135	n.s
BSA +sulfo-SMCC + RGD BSA	45437.04	0.00186	**
BSA +sulfo-SMCC + RGD BLG	959.2593	1	n.s
BLG +sulfo-SMCC + RGD TCP	-15144.4	0.50989	n.s
BLG +sulfo-SMCC + RGD PLL	2400	0.99969	n.s
BLG +sulfo-SMCC + RGD BSA	60122.22	1.46E-04	***
BLG +sulfo-SMCC + RGD BLG	15644.44	0.47765	n.s
BLG +sulfo-SMCC + RGD BSA +sulfo-SMCC + RGD	14685.19	0.54015	n.s

Supplementary Table S11 Summary of statistical analysis of data obtained from cell (HEK293T) on emulsion droplets after seven days in culture.

	MeanDiff	**Prob**	
BSA +sulfo-SMCC + RGD BSA	284000	0.0242	*****
BLG +sulfo-SMCC + RGD BLG	297944.4	0.02759	*****
BSA +sulfo-SMCC + RGD TCP	-146056	0.25486	**n.s**
BLG +sulfo-SMCC + RGD TCP	-148444	0.24588	**n.s**
BLG +sulfo-SMCC + RGD BSA +sulfo-SMCC + RGD	-2388.89	0.99953	**n.s**

Supplementary Table S12 Summary of statistical analysis of data obtained from the number of droplets covered with cells (MSCs) on emulsion droplets after seven days in culture.

	MeanDiff	Prob	
BSA PLL	-69.5612	0.00242	**
BSA + sulfoSMCC + RGD PLL	6.0635	0.98904	**n.s**
BSA + sulfoSMCC + RGD BSA	75.62472	0.00129	**
BLG PLL	-3.88011	0.99801	**n.s**
BLG BSA	65.68111	0.00367	**
BLG BSA + sulfoSMCC + RGD	-9.94361	0.93631	**n.s**
BLG + sulfoSMCC + RGD PLL	17.19101	0.689	**n.s**
BLG + sulfoSMCC + RGD BSA	86.75223	4.32E-04	***
BLG + sulfoSMCC + RGD BSA + sulfoSMCC + RGD	11.12751	0.90814	**n.s**
BLG + sulfoSMCC + RGD BLG	21.07113	0.52247	**n.s**

Supplementary Table S13 Summary of statistical analysis of data obtained from the number of droplets covered with cells (MSCs) on emulsion droplets after seven days in culture.

	MeanDiff	Prob	
BSA PLL	-63.759	0.00328	**
BSA + sulfoSMCC + RGD PLL	-9.69143	0.932	**n.s**
BSA + sulfoSMCC + RGD BSA	54.06757	0.01017	*
BLG PLL	-27.1195	0.26481	**n.s**
BLG BSA	36.63949	0.08696	**n.s**
BLG BSA + sulfoSMCC + RGD	-17.4281	0.64397	**n.s**
BLG + sulfoSMCC + RGD PLL	-8.80211	0.95079	**n.s**
BLG + sulfoSMCC + RGD BSA	54.9569	0.00914	**
BLG + sulfoSMCC + RGD BSA + sulfoSMCC + RGD	0.88933	0.99999	**n.s**
BLG + sulfoSMCC + RGD BLG	18.31741	0.60368	**n.s**