Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary information

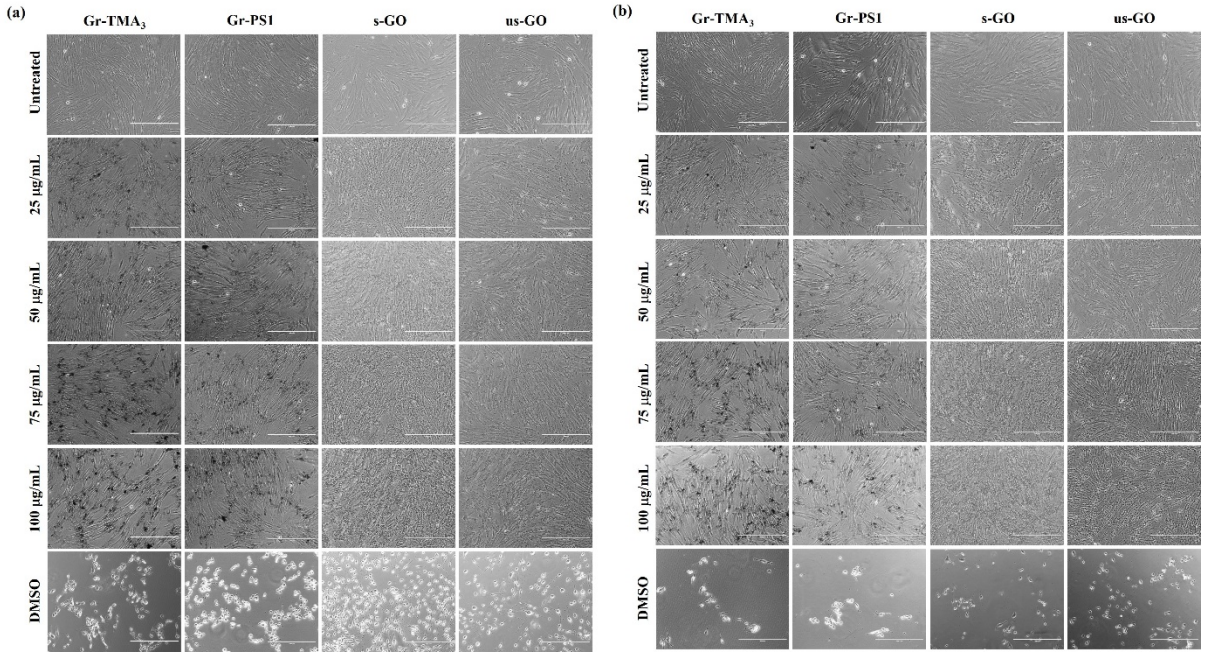


Figure S1: Optical images of HPF cells treated with Gr-TMA_3, Gr-PS1, s-GO and us-GO (25, 50, 75 and 100 µg/mL, w/ FBS, 24 h). Images were taken **(a)** before and **(b)** after washing with PBS (+/+, x 2), 20 % DMSO was used as the positive control. Scale bar = 400 µm.

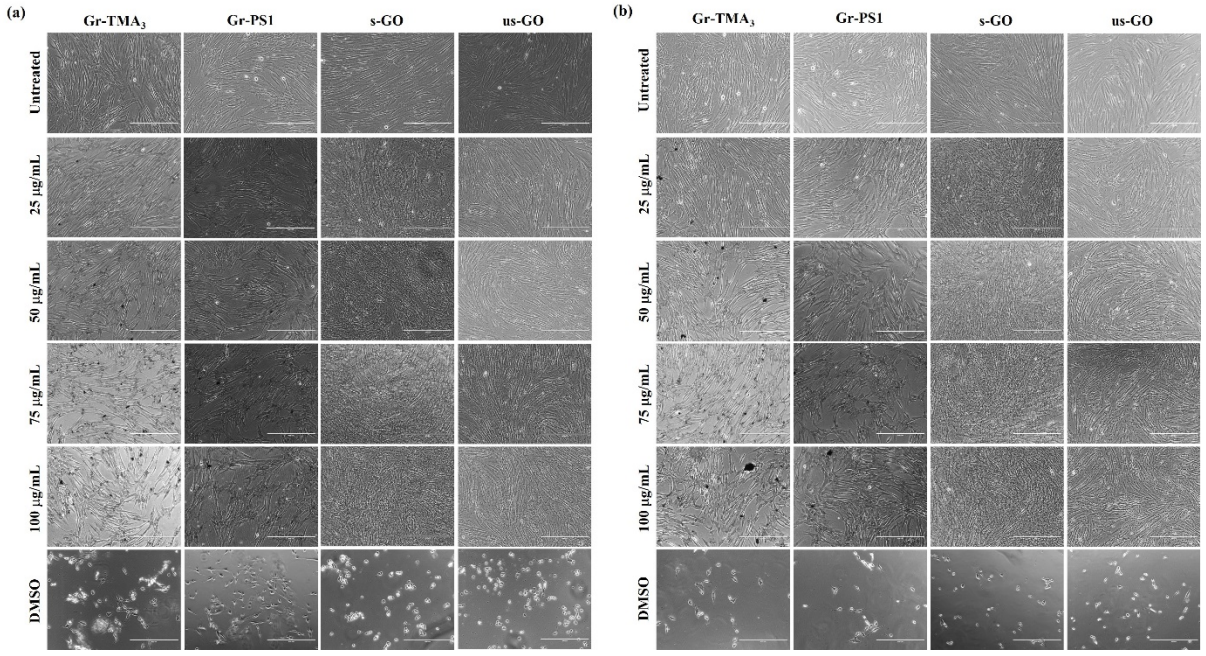


Figure S2: Optical images of MPS VI cells treated with Gr-TMA_3, Gr-PS1, s-GO and us-GO (25, 50, 75 and 100 µg/mL, w/ FBS, 24 h). Images were taken (**a**) before and (**b**) after washing with PBS (+/+, x 2), 20 % DMSO was used as the positive control. Scale bar = 400 µm.

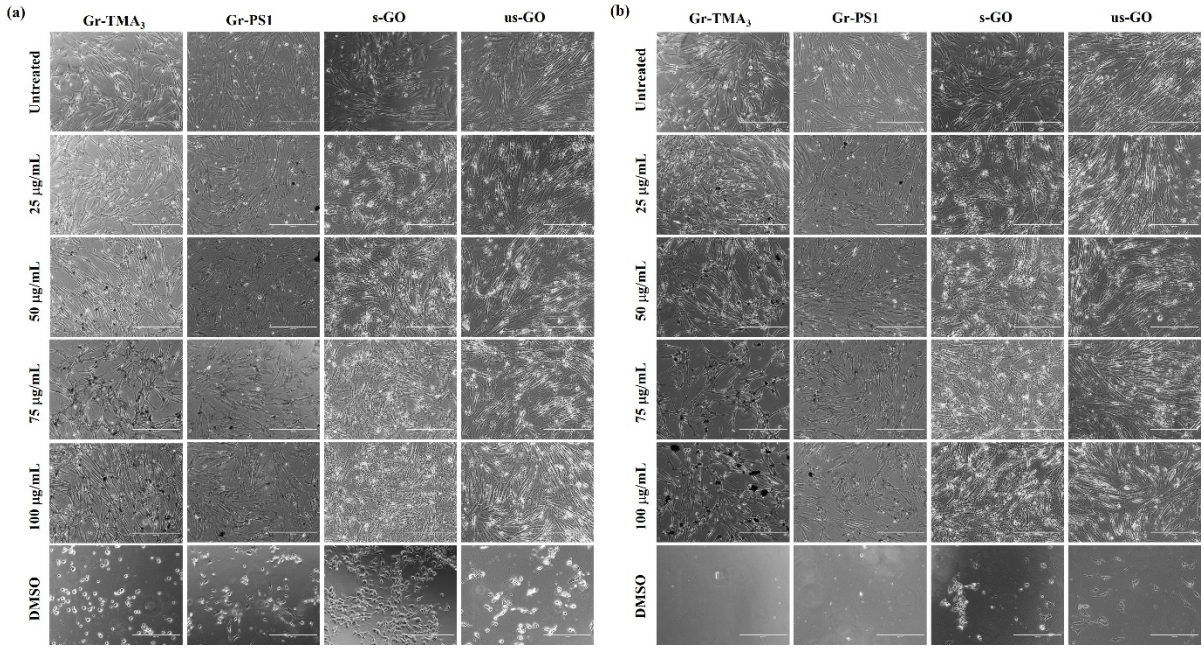


Figure S3: Optical images of Pompe cells treated with Gr-TMA_3, Gr-PS1, s-GO and us-GO (25, 50, 75 and 100 µg/mL, w/ FBS, 24 h). Images were taken (**a**) before and (**b**) after washing with PBS (+/+, x 2), 20 % DMSO was used as the positive control. Scale bar = 400 µm.

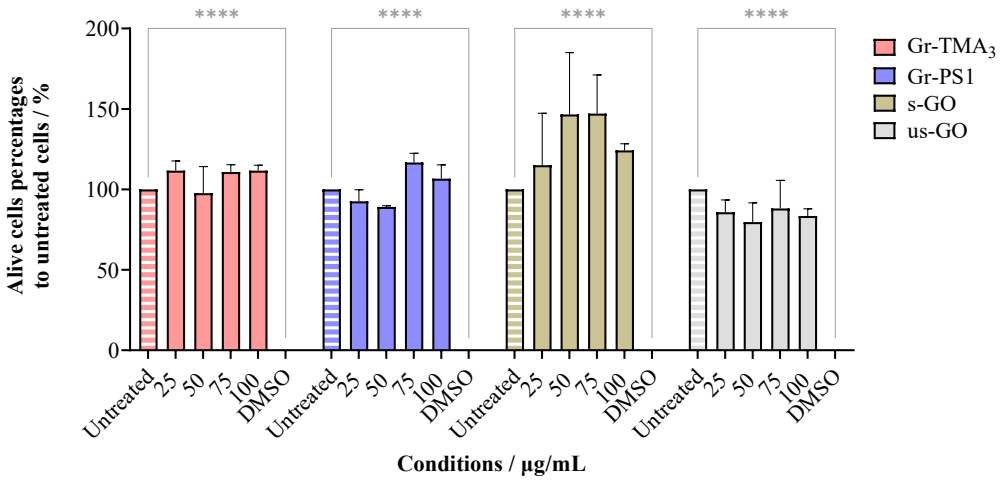


Figure S4: Cytotoxicity assessment of Gr-TMA_3, Gr-PS1, s-GO and us-GO (25, 50, 75 and 100 µg/mL, w/ FBS, 24 h) in Pompe cells by live-cell counting, using trypan blue exclusion dye. The data were statistically analyzed using analysis of variance (two-way ANOVA [main effects only]) with Tukey's

multiple comparisons tests. n = 1 with duplicates. *Statistically different: ****$p < 0.0001$, material-treated cells showed no significant differences from the corresponding untreated cells.

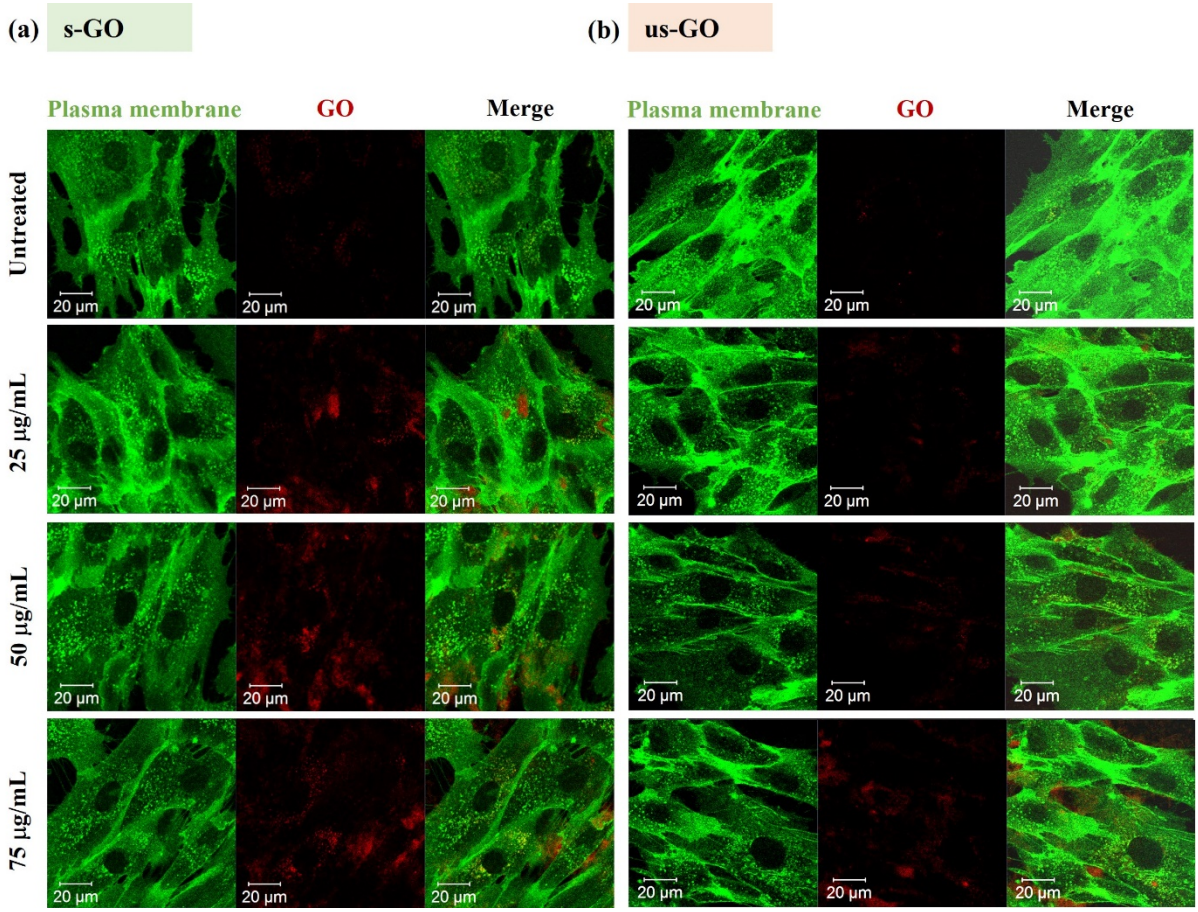


Figure S5: Uptake profile of **(a)** s-GO and **(b)** us-GO in HPF cells (25, 50 and 75 µg/mL, w/ FBS, 24 h). Middle sections of confocal images shown. Green = plasma membrane, red = GO.

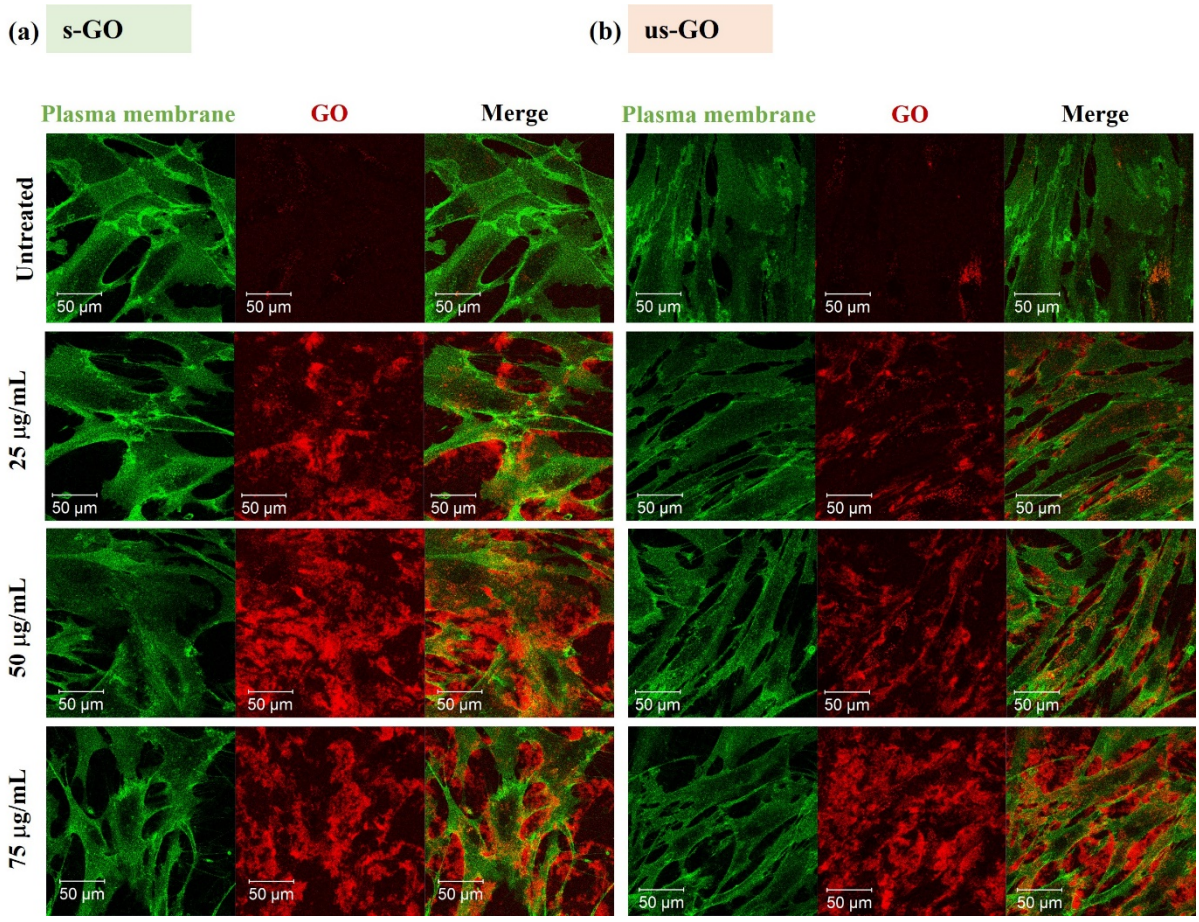


Figure S6: Uptake profile of **(a)** s-GO and **(b)** us-GO in MPS VI cells (25, 50 and 75 µg/mL, w FBS, 24 h). Middle sections of confocal images shown. Green = plasma membrane, red = GO.

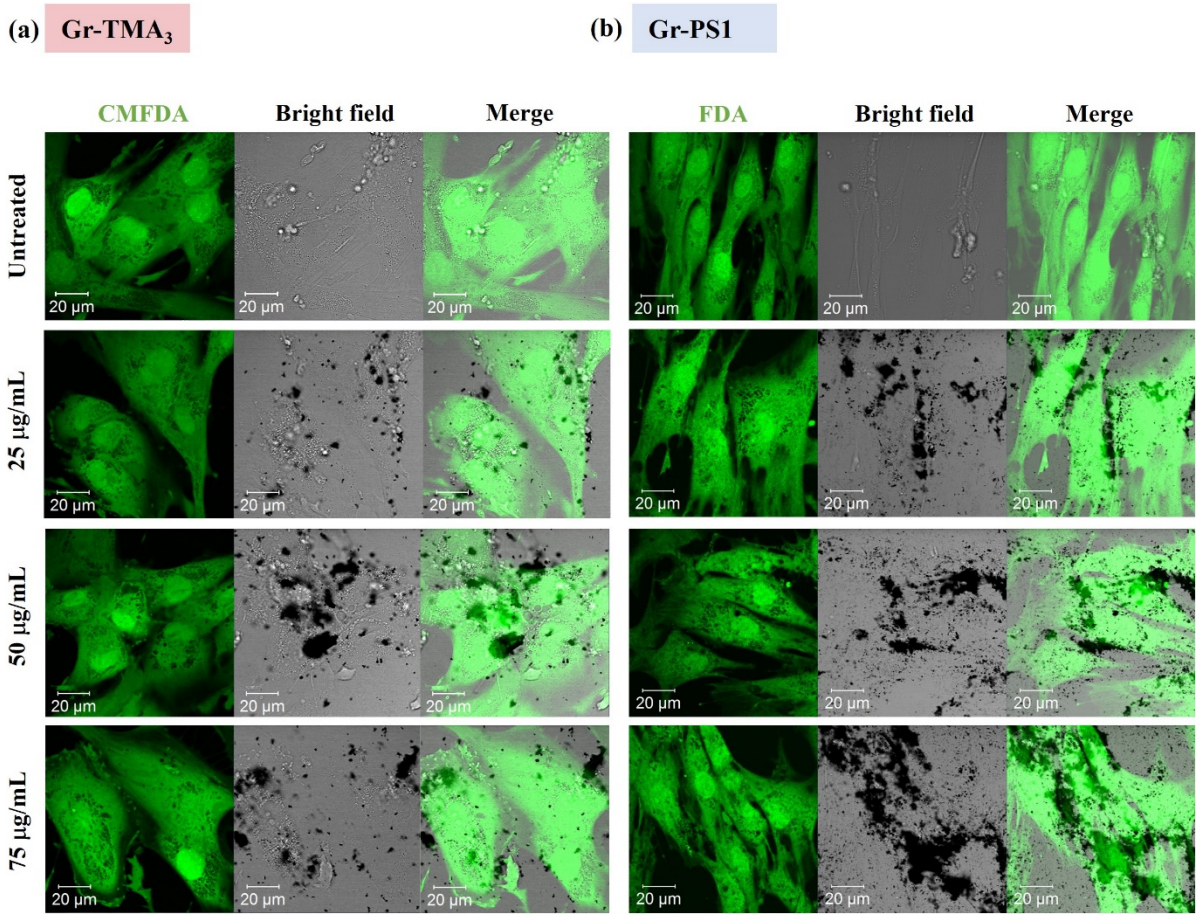


Figure S7: Uptake profile of **(a)** Gr-TMA_3 and **(b)** Gr-PS1 in Pompe cells (25, 50 and 75 µg/mL, w/ FBS, 24 h). Middle sections of confocal images shown. Green = CMFDA dye, black = Gr.

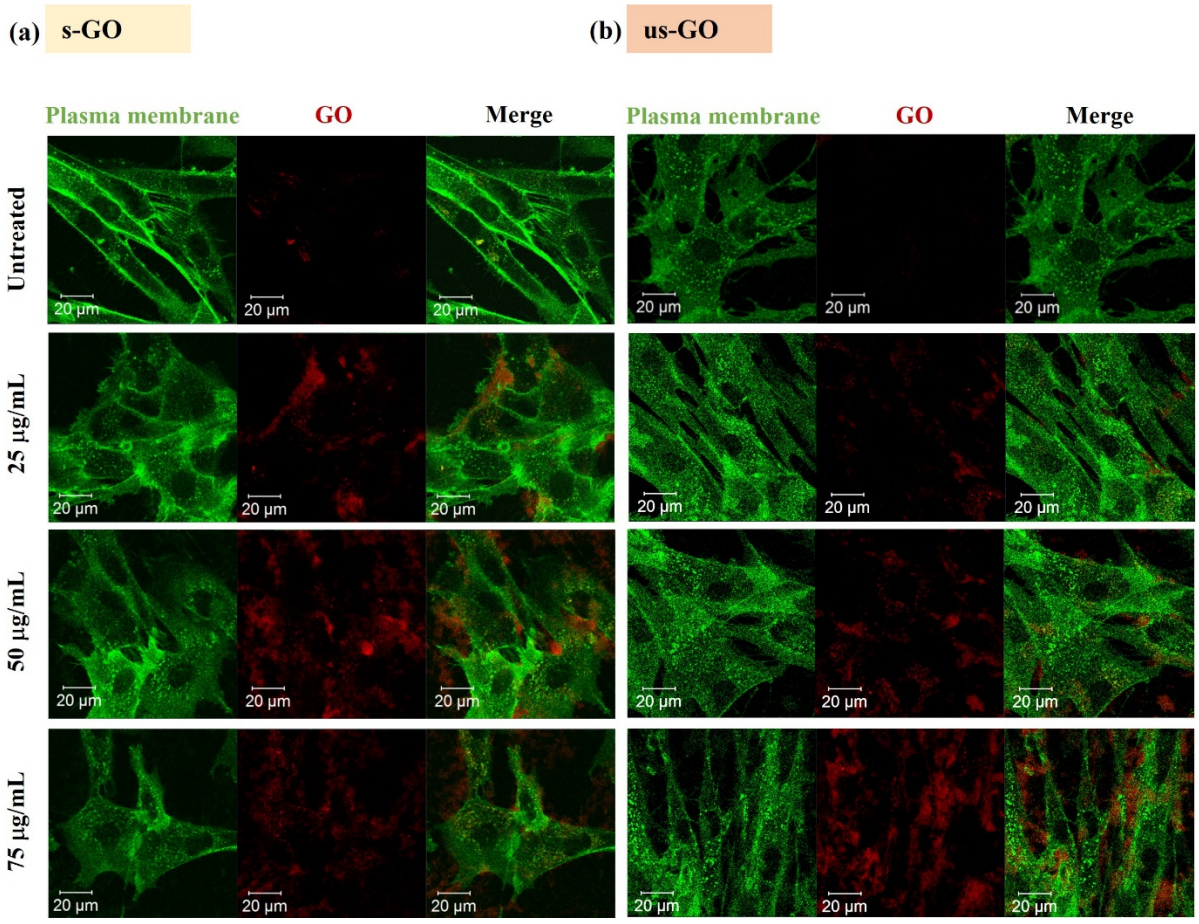


Figure S8: Uptake profile of **(a)** s-GO and **(b)** us-GO (25, 50 and 75 µg/mL, w/ FBS, 24 h) in Pompe cells by confocal imaging. Middle sections of the confocal images shown. Green = plasma membrane, red = GO.

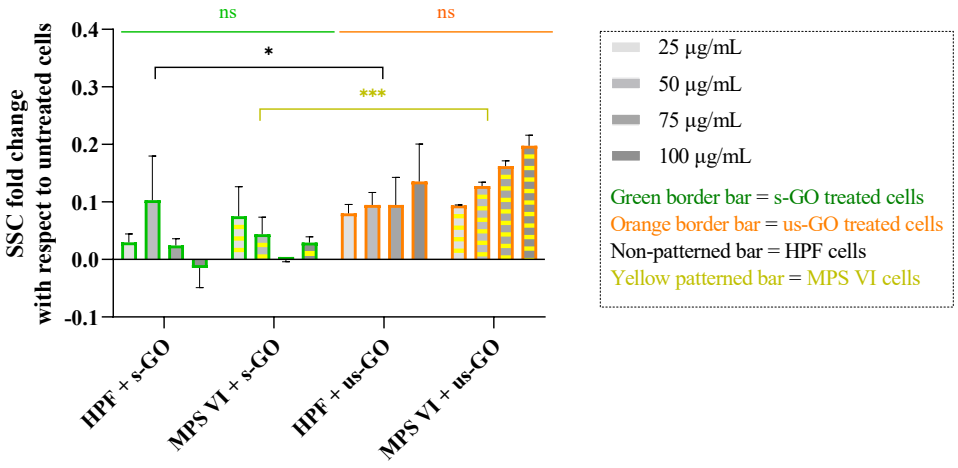


Figure S9: SSC folds change of HPF and MPS VI cells treated with s-GO or us-GO (25, 50, 75, and 100 µg/mL) compared to untreated cells. Flow cytometry data were statistically analysed using analysis of variance (two-way ANOVA [main effects only]) with Tukey's multiple comparisons tests. n = 1 with duplicates. *Statistically different: ns = not significant, $*p < 0.05$, $***p < 0.001$.

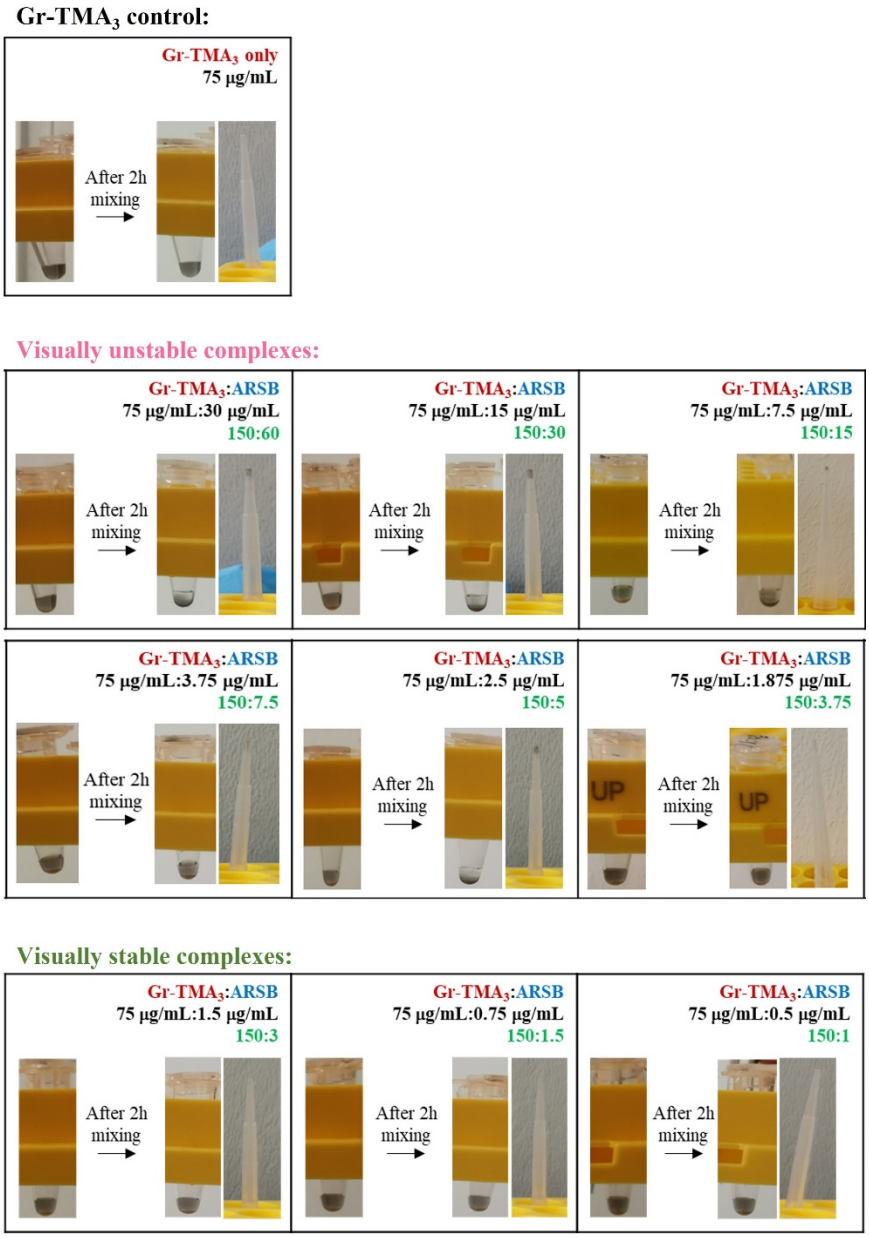


Figure S10: Images of Gr-TMA_3 (75 µg/mL) and Gr-TMA_3:ARSB complexes prepared in different concentration ratios of Gr-TMA_3 and ARSB (150:60, 150:30, 150:15, 150:7.5, 150:5, 150:3.75, 150:3, 150:1.5, and 150:1), taken before and after 2 h of mixing using HC Thermomixer, as well as the image of pipette tip used to re-disperse the solution after mixing. Gr-TMA_3:ARSB complexes in the concentration ratio of 150:60, 150:30, 150:15, 150:7.5, 150:5 and 150:3.75 showed signs of sedimentation and/or residual material on pipette tips after re-mixing. Hence, these complexes were considered visually unstable. On the other hand, Gr-TMA_3:ARSB complexes in the concentration ratio of 150:3, 150:1.5, and 150:1 were visually stable.

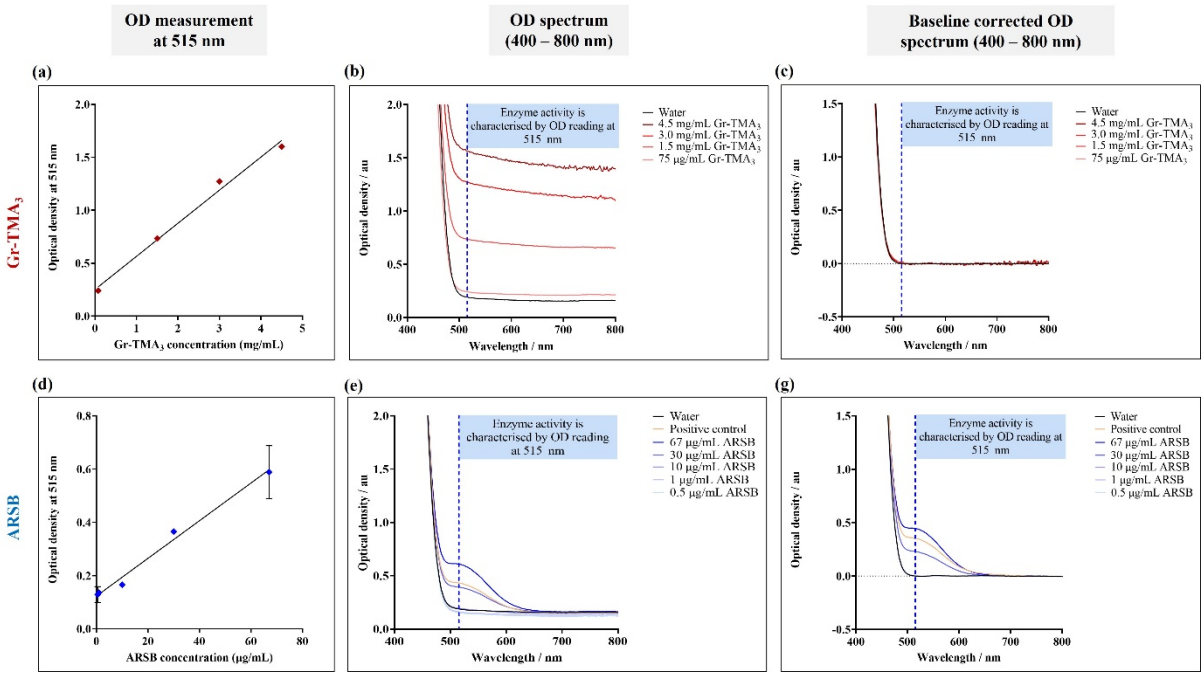
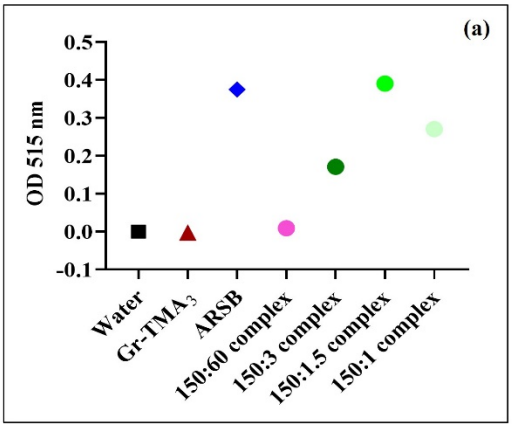
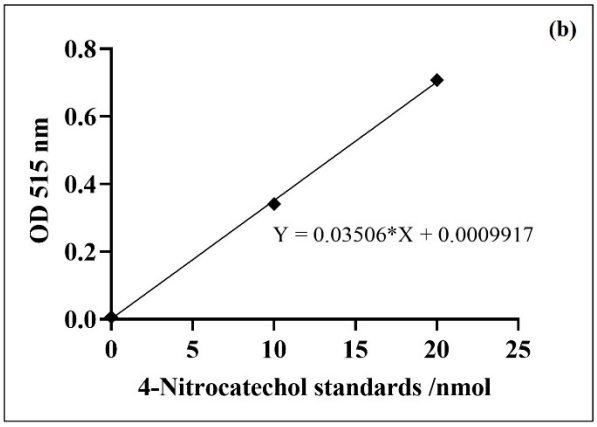


Figure S11: Enzymatic activity of **(a – c)** Gr-TMA_3 (75 µg/mL, 1.5, 3.0, and 4.5 mg/mL) and **(d – e)** ARSB (0.5, 1, 10, 30, and 67 µg/mL) measured using sulfatase activity assay, and compared against the provided positive control in the assay kit. Graphs show the **(a**, **d)** optical density measurement at 515 nm and **(b**, **e)** the corresponding optical density spectrum (400 – 800 nm) for different concentrations of Gr-TMA_3 and ARSB. Also, graphs show the corresponding baseline corrected optical density spectrum (400 – 800 nm) of **(c)** Gr-TMA_3 and **(g)** ARSB using the baseline correction function of OriginPro 2021b.





(c)

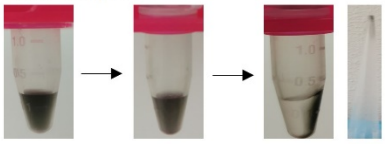
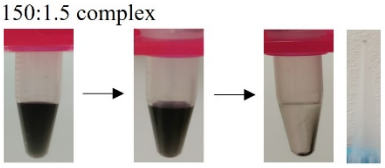
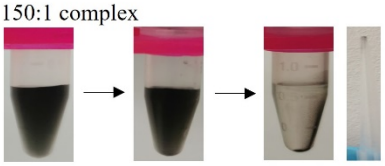
Complex	OD 515 nm (au)	Equivalent amount of 4-Nitrocatechol (nmol)	Protein concentration (mg)	Sulfatase activity (nmol/min/mg)
150:3 complex 	0.17211	4.88073	1.5 ug/mL of ARSB in 200 uL = 1.5 ug/mL* 0.2 mL = 0.3 ug =0.0003 mg	[4.88073/(0.0003 x 30 min)] = 542.303 nmol/mg/min
150:1.5 complex 	0.39919	11.35762	0.75 ug/mL of ARSB in 400 uL = 0.75 ug/mL* 0.4 mL = 0.3 ug =0.0003 mg	[11.35762/(0.0003 x 30 min)] = 1261.958 nmol/mg/min
150:1 complex 	0.27515	7.82182	0.5 ug/mL of ARSB in 600 uL = 0.5 ug/mL* 0.6 mL = 0.3 ug =0.0003 mg	[7.82182/(0.0003 x 30 min)] = 869.091 nmol/mg/min

Figure S12: Enzymatic activity of different Gr-TMA_3:ARSB complexes (in mass ratios of 150:60, 150:3, 150:1.5, and 150:1) compared against the water, Gr-TMA_3 (75 µg/mL) and ARSB (30 µg/mL) controls, using the sulfatase activity assay. Results showed **(a)** optical density measurement at 515 nm. Also, according to the manufacturer's manual, we calculated the **(c)** sulfatase activity of the Gr-TMA_3:ARSB complexes in the ratio of 150:3, 150:1.5, and 150:1 using the **(b)** baseline-corrected 4-nitrocatechol standard curve and the provided equation: [sulfatase activity (nmol/min/mg) = ((equivalent amount of 4-nitrocatechol) / (protein concentration × 30 min)) × dilution factor], no dilution factor employed.

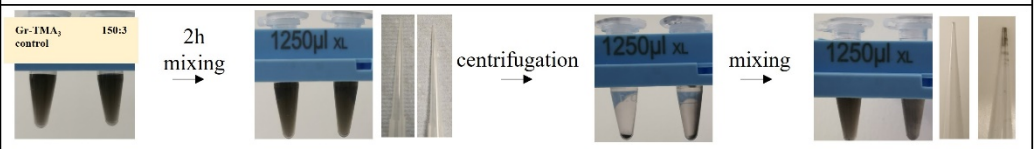
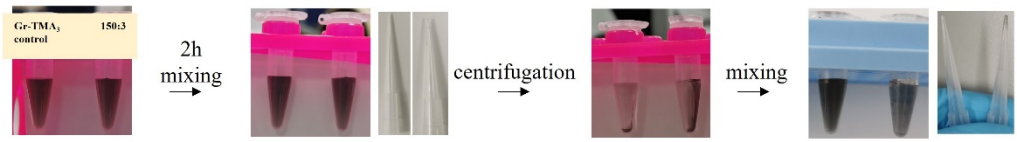
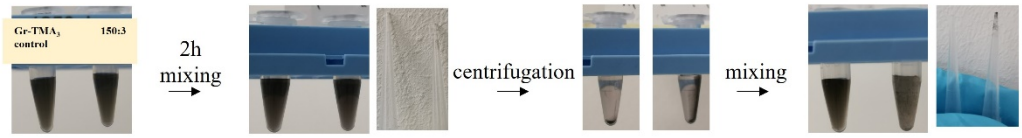
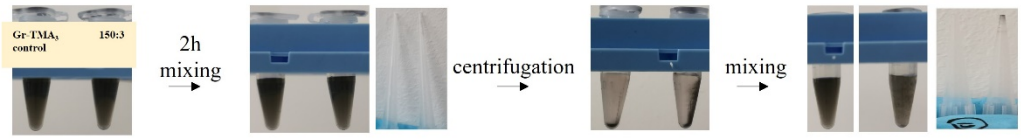
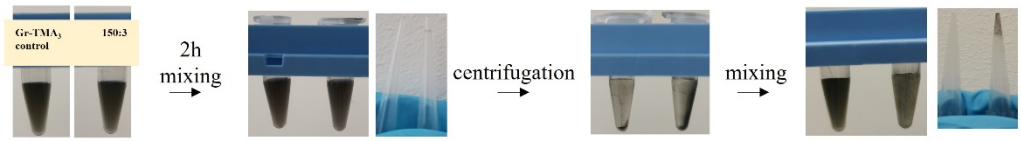
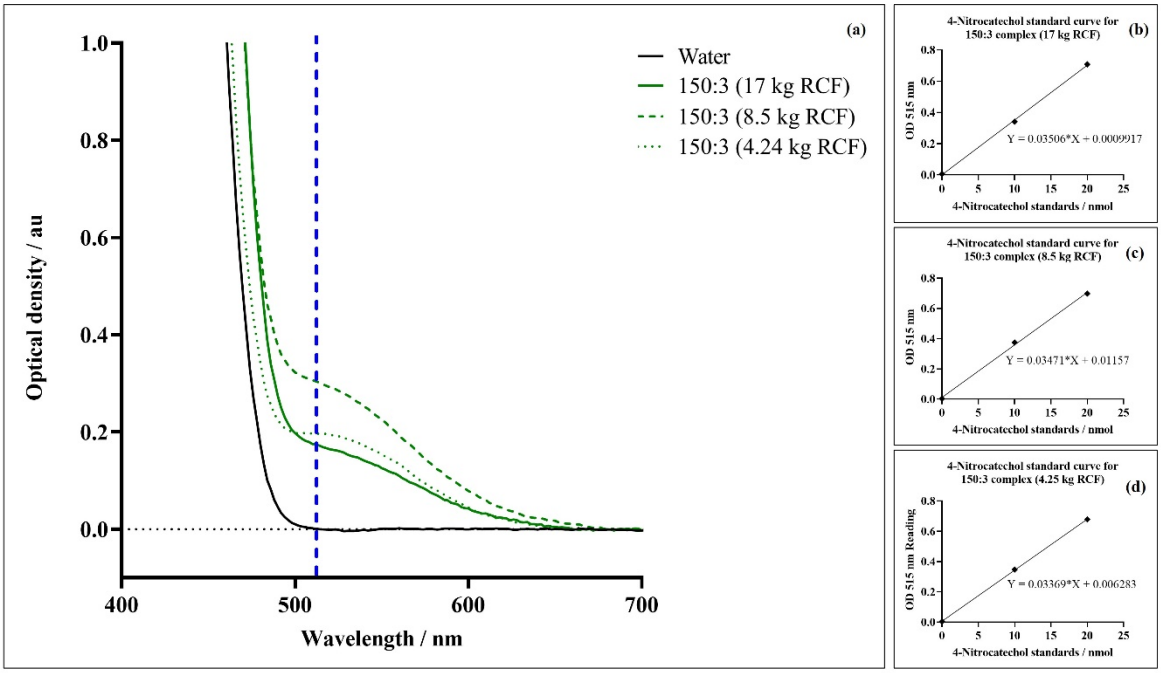
Centrifugation condition	Images
17 kg RCF, 30 min, 19 °C	
8.5 kg RCF, 30 min, 19 °C	
5.68 kg RCF, 30 min, 19 °C	
4.25 kg RCF, 30 min, 19 °C	
3.4 kg RCF, 30 min, 19 °C	

Figure S13: Optimisation of centrifugation conditions for dispersible Gr-TMA_3:ARSB complex (150:3). We showed images of Gr-TMA_3 (75 µg/mL) and Gr-TMA_3:ARSB complex (150:3), taken before and after mixing (2 h), centrifugation, and re-dispersion, as well as the pipette tips used to re-disperse the solution. We selected the initial centrifugation condition with reference to the preparation of graphene flakes[13]. In general, reducing the centrifugation speed can reduce the amount of residual material on tips after re-suspension of the complex. However, none of the conditions enabled the complete dispersion of the complex after centrifugation. Of all the conditions, complexes collected using 8.5 and 4.25 kg RCF showed the least amount of residual material on the tip after centrifugation. Subsequently, we measured the sulfatase activity of the complexes collected using 8.5 and 4.25 kg RCF and compared with those at 17 kg RCF (see **Figure S14**).



(e)

Centrifugation condition	OD 515 nm (au)	4-Nitrocatechol (nmol)	Protein concentration (mg)	Sulfatase activity (nmol/min/mg)
17 kg RCF, 30 min, 19 °C	0.17211	4.88073	1.5 ug/mL of ARSB in 200 uL = 1.5 ug/mL* 0.2 mL = 0.3 ug =0.0003 mg	[4.88073/(0.0003 x 30 min)] = 542.303 nmol/mg/min
8.5 kg RCF, 30 min, 19 °C	0.299855	8.30553	1.5 ug/mL of ARSB in 200 uL = 1.5 ug/mL* 0.2 mL = 0.3 ug =0.0003 mg	[8.30553/(0.0003 x 30 min)] = 922.837 nmol/mg/min
4.25 kg RCF, 30 min, 19 °C	0.19645	5.64461	1.5 ug/mL of ARSB in 200 uL = 1.5 ug/mL* 0.2 mL = 0.3 ug =0.0003 mg	[5.64461/(0.0003 x 30 min)] = 627.179 nmol/mg/min

Figure S14: Enzymatic activity of Gr-TMA_3:ARSB complexes (150:3) collected at different centrifugation conditions (17, 8.5, or 4.25 kg RCF, 30 min, 19 °C) was compared using the sulfatase activity assay. The results showed the **(a)** baseline-corrected optical density spectrum of the complexes and **(b – d)** the corresponding baseline-corrected 4-nitrocatechol standard curve for the complexes. **(e)** The sulfatase activity of the complexes was calculated based on the following equation: [sulfatase activity (nmol/min/mg) = ((e$quvlent\ amount\ of$ 4−$nitrocatechol$)/(p$rotein\ concentration$ ×30 min))×sample dilution factor], with no dilution factor employed. The result showed that the 150:3 complex collected using 8.5 kg RCF displayed the highest sulfatase activity, followed by 4.25 kg RCF and 17 kg RCF. This confirmed that the centrifugation condition could affect the measurement of the sulfatase activity in the complex.

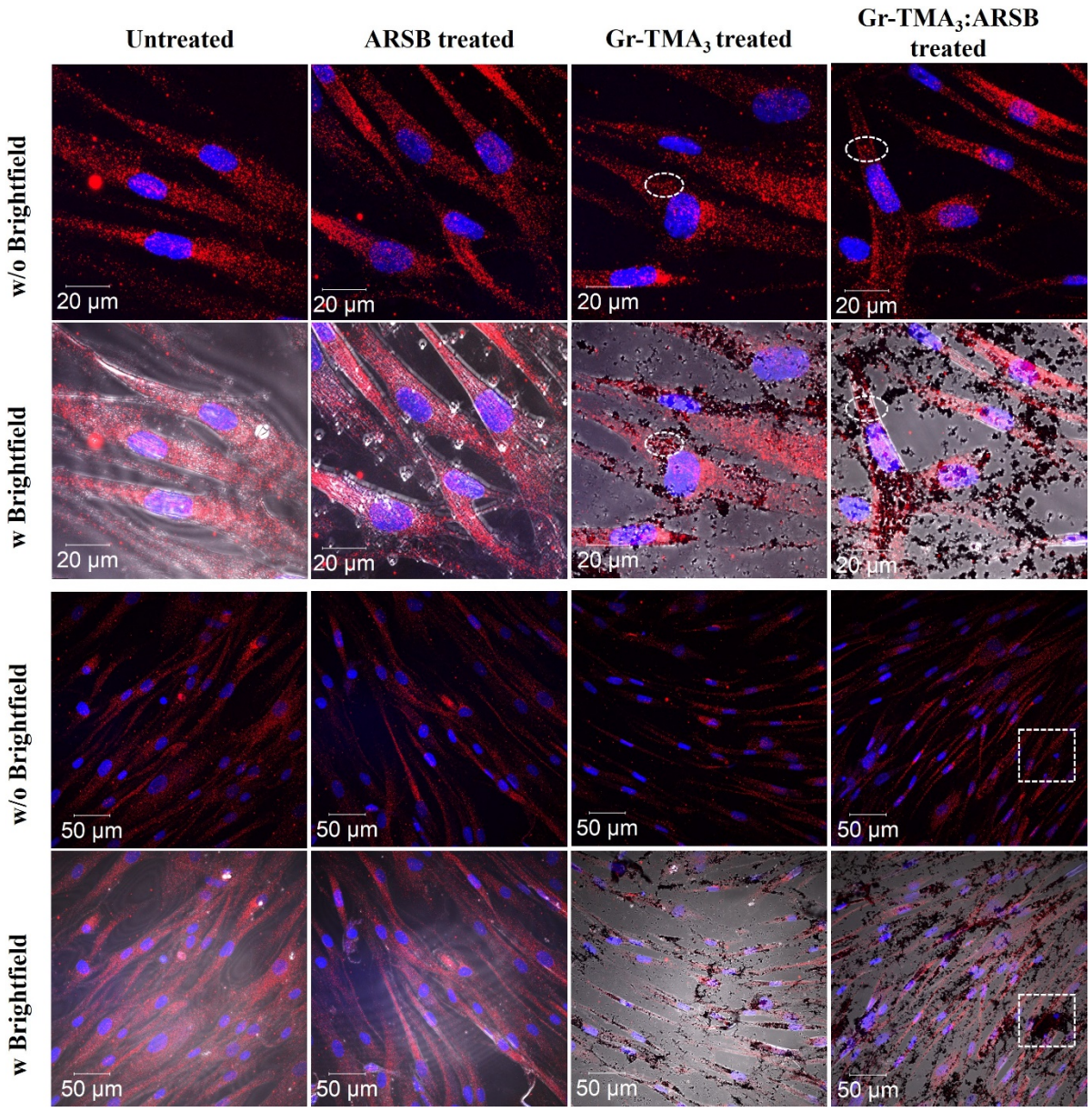


Figure S15: Confocal images (maximum intensity projection) of MPS VI cells treated with ARSB (1.5 µg/mL), Gr-TMA_3 (75 µg/mL), and Gr-TMA_3:ARSB complex in the ratio of 150:3 for 48 h, shown with and without brightfield overlay, at high and low magnifications. White circles show the co-localisation of Gr-TMA_3 or Gr-TMA_3:ARSB with chondroitin sulfate, which indicates that the fluorescent signal is not quenched by the flakes. Also, as shown with white squares, we observed no obvious reduction in the chondroitin sulfate signal, when the material localized extracellularly. This indicates that observed decrease in chondroitin sulfate signal was the result of enzymatic degradation, rather than quenching due to the presence of the flakes. Black = Gr-TMA_3 or Gr-TMA_3:ARSB. Blue = nucleus. Red = chondroitin sulfate.