

ADVANCED BIOLOGY

Supporting Information

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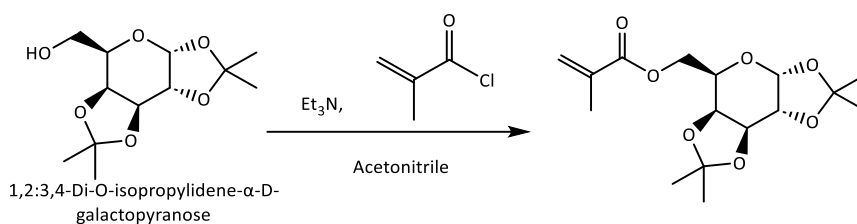
Distinct Network Morphologies from In Situ Polymerization of Microtubules in Giant Polymer-Lipid Hybrid Vesicles

Paula De Dios Andres, Mousumi Akter, Cecilie Ryberg, Brigitte Städler and Allen P. Liu**

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Distinct network morphologies from *in-situ* polymerization of microtubules in giant polymer-lipid hybrid vesicles

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Scheme S1. Polymerization scheme of the 6-O-methacryloyl-1,2:3,4-di-O-isopropylidene- α -galactopyranose monomer (proGalacMA).

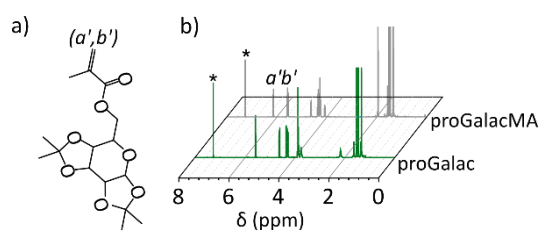
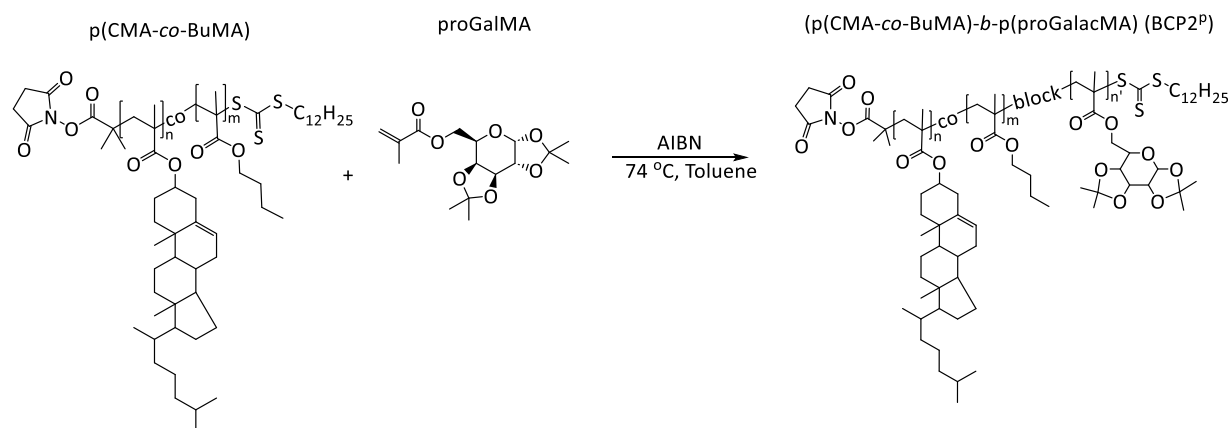
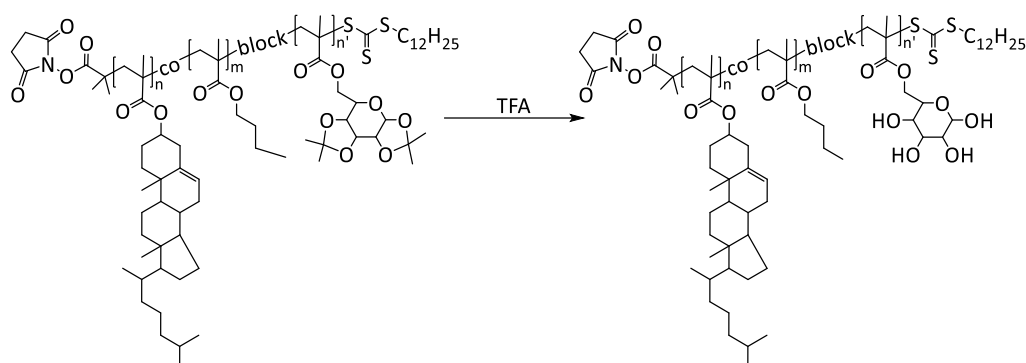
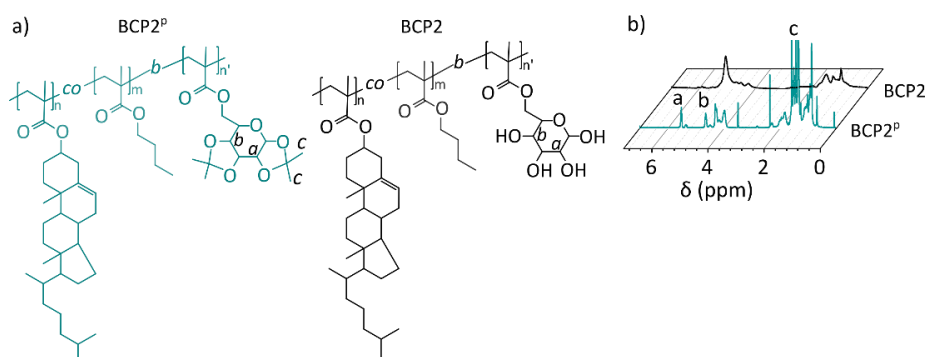


Figure S1. Structure (a) and ¹H NMR (b) of the proGalac and proGalacMA monomers. The ¹H NMR was measured in CDCl₃ (* indicates the solvent peak).



Scheme S2. Polymerization scheme of poly(cholesteryl methacrylate-co-butyl methacrylate)-block-poly(6-O-methacryloyl-D-galactopyranose) (p(CMA-co-BuMA)-b-p(proGalacMA), BCP2P).

p(CMA-co-BuMA)-*b*-p(proGalacMA) (BCP2^p)p(CMA-co-BuMA)-*b*-p(GalacMA) (BCP2)**Scheme S3.** Deprotection of BCP2^p (p(CMA-co-BuMA)-*b*-p(proGalacMA)).**Figure S2.** Structure (a) and ¹H NMR (b) of p(CMA-co-BuMA)-*b*-p(proGalacMA) (BPC2^p, turquoise) in CDCl₃ and p(CMA-co-BuMA)-*b*-p(GalacMA) (BCP2, black) in pyridine *d*-8.**Table S1.** Composition of the different oil/polymer-lipid mixtures.

Sample	DOPC (mg)	Cholesterol (mg)	BCP1 (mg)	BCP2 (mg)	Ratio
GUV ⁰	5	0.069	-	-	100:0
GUV ^{2.5}	5	0.138	-	-	97.5:2.5
GHV1 ^{2.5}	5	-	0.65	-	97.5:2.5
GHV1 ⁵	5	-	1.3	-	95:5
GHV2 ^{2.5}	5	-	-	0.65	97.5:2.5
GHV2 ⁵	5	-	-	1.3	95:5
GHV2 ¹⁰	5	-	-	2.6	90:10

It should be noted that, in all cases, the lipid mixture was mixed with 15 mL silicone oil and 3.8 mL mineral oil.

Table S2. Composition of the encapsulant.

		Premix						
Sample	Tubulin 165 μ M (μ L)	Glucose 231 mM (μ L)	Optiprep (μ L)	GTP 100 mM (μ L)	DTT 40 mM (μ L)	GMPCPP 10 mM (μ L)	ddH ₂ O (μ L)	5x BRB80 buffer (μ L)
0 mM GMPCPP	1.5	-	0.9	-		0	8	2
1 mM GMPCPP	1.5	-	0.9	-		1	5	2
2.5 mM GMPCPP	1.5	-	0.9	-		3	7	2
Empty vesicles	-	18.5	1.5	-		-	-	-
3 μ M tubulin	0.2	-	0.5	1.25	0.5	-	25.75	10
11 μ M tubulin	0.75	-	0.5	1.25	0.5	-	25.75	10
20 μ M tubulin	1.5	-	0.9	1.25	0.5	-	25.75	10
3 μ M tubulin	0.2	-	0.5	-		1	7	2
11 μ M tubulin	0.75	-	0.5	-		1	7	2
20 μ M tubulin	1.5	-	0.9	-		1	7	2

In all cases, 9 μ L of the premix was always used to mix with the indicated volume of tubulin.

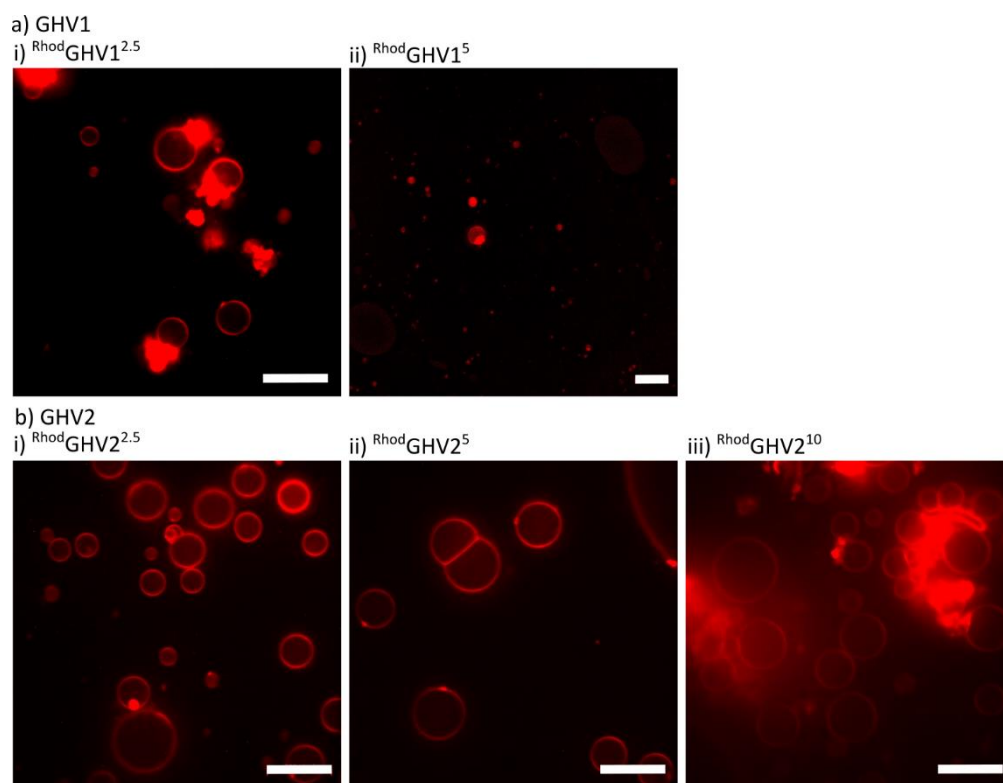


Figure S3. Representative SDCM images of RhodGHV1^n (a) and RhodGHV2^n (b) with 2.5% mol (i), 5% mol (ii) and 10% mol (iii) BCPX (red: RhodPE ; scale bar: 10 μm ; $n=3$).

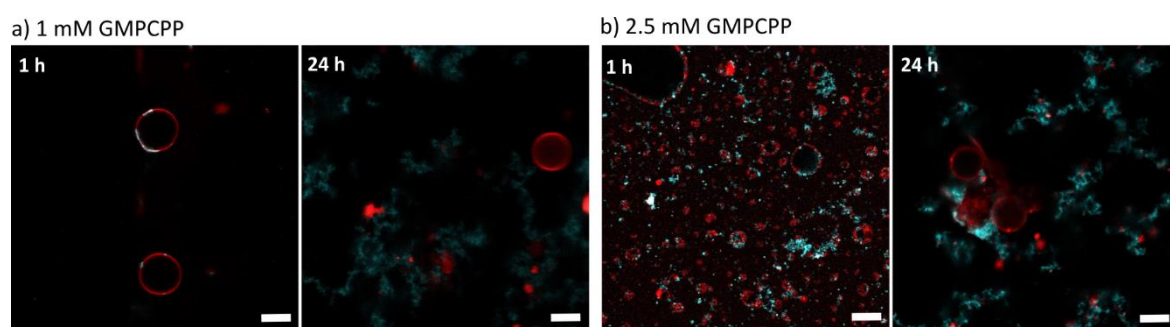


Figure S4. Representative CLSM images of $\text{RhodGUV}^{2.5}$ loaded with either 1 mM (a) or 2.5 mM (b) GMPCPP and tubulin after 1 h and 24 h incubation (red: RhodPE , cyan: tubulin^F; scale bar: 10 μm ; $n = 3$).

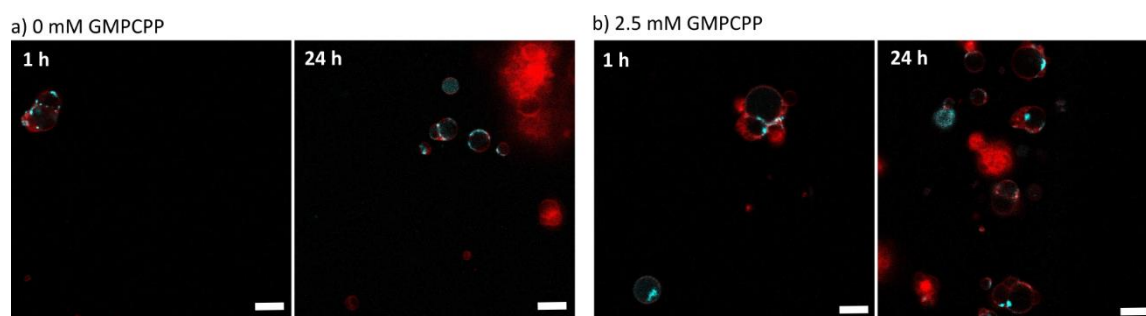


Figure S5. Representative CLSM images of $\text{RhodGHV1}^{2.5}$ loaded with either 0 mM (a) or 2.5 mM (b) GMPCPP and tubulin after 1 h and 24 h incubation (red: RhodPE , cyan: tubulin^F; Scale bar: 10 μm ; $n = 3$).