Supplementary Information

Cellulose abetted assembly and temporally-decoupled loading of cargo into vesicles synthesized from functionally diverse lamellar phase forming amphiphiles

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Supplementary Figure 1. Direct Red 23 assay for the presence of cellulose in the membranes of PBD$_{46}$PEO$_{30}$ polymersomes prepared through PAPYRUS. The three rows are three different samples. Column (a) shows the transmitted light images, column (b) shows images from the Direct Red 23 channel and column (c) shows an overlay of the transmitted light and Direct Red 23 channels. In the first and third row the brightly fluorescent cylindrical object are cellulose fibers. In the second row the brightly fluorescent object on the upper left is an aggregate of Direct Red 23. The green arrows indicate the location of polymersomes. It is clear
that the fluoresce intensity of Direct Red 23 in the membrane is similar to the background intensity of Direct Red 23 in bulk. We conclude that there is no cellulose present in the membranes. Scale bars 10 µm.
Supplementary Figure 2. Sample images that were used for quantification. (a) Typical image that we obtained from a sample of PBD_{46}PEO_{30} polymersomes that was extracted from the paper. The image size was 850.19 µm x 850.19 µm (1860 x 1860 pixels). The image shows numerous polymersomes and a few cylindrical micelles. (b) The result of the image processing routine of the image shown in (a) showing the identification of the polymersomes (bright multicolored circles) from the background (continuous orange region). (c) Overlay of the detected objects onto the original image, demonstrating that the majority of the polymersomes were detected. The cylindrical micelles are not detected as valid objects to be counted using our image analysis routine. (d) Sketch of the confocal slice thickness and its relation to the size of the objects in the images. The automated image acquisition routine images a volume of dimensions 850.19 µm x 850.19 µm x 13.1 µm. The autofocus routine detects the location of the surface of the glass slide by locating a region that maximally reflects the excitation laser light. The equatorial diameters of objects that rest on the surface of the glass slide of sizes ranging from 1 µm to 30 µm falls within the confocal imaging volume. The diameters of objects that are
larger than 30 µm are underestimated since a section of the sphere below the equatorial diameter is imaged. The maximal diameter $a$, of the section that we image of the sphere is given by $a = 2\sqrt{h(d - h)}$ where $h$ is the thickness of the confocal slice from the surface of the glass slide, $d$ is the diameter of the vesicle. For a vesicle with an actual diameter of 50 µm, the imaging routine will report a diameter of 44 µm. From our images, the majority of the vesicles that form were below 30 µm in diameter (see Figure 2i, Figure 3g, Figure 5e). Scale bar 100 µm.
Supplementary Figure 3. Examples of other amphiphiles that were tested with cellulose paper. a, b) Images of Pluronic L61 polymersomes extracted from cellulose paper 90 minutes after deposition of the amphiphilic triblock copolymer Pluronic L61. Interestingly the Pluronic L61 polymersomes show signs of the coexistence of two phases. The Nile Red dye partitioned into a dye-enriched phase, leaving behind a dye-depleted phase thus forming vesicles with characteristic half-moon shapes. The image in b) is the same image in a) with the image histogram equalized and the contrast enhanced to demonstrate the presence of the dye poor region in the lower right of the image. c) Images of the cellulose fibers 90 minutes after deposition of the non-ionic surfactant Triton X-100. Only small droplets and no vesicles were apparent on the cellulose fibers. d) Images of the cellulose fibers 90 minutes after deposition of the amphiphilic triblock copolymer Pluronic F68. No polymersomes were present on the fibers.
All amphiphile mixtures contained Nile Red for fluorescence visualization. Scale bars a,b) 5 µm, c,d) 20 µm.
Supplementary Figure 4. Confocal fluorescence image of structures extracted from paper with a nominal surface concentration of 2 \( \text{nmol/mm}^2 \) PBD_{46}PEO_{30}. The image shows the presence of numerous cylindrical micelles, nanotubes and bright aggregates coexisting with polymersomes. Growth was performed at 80 °C for 90 minutes. Scale bar 25 µm.
Supplementary Figure 5. Four different locations on the cellulose paper after extracting the PBD$_{46}$PEO$_{30}$ polymersomes. The images reveal that many polymersomes remained on the cellulose paper. The nominal surface concentration of the PBD$_{46}$PEO$_{30}$ was 0.2 nmol/mm$^2$. Scale bar 10 µm.
Supplementary Figure 6. Fluorescence leakage assay of FITC from PBD\textsubscript{46}PEO\textsubscript{30} polymersomes to probe lamellarity. a) A representative confocal multi-channel image of FITC-loaded PBD\textsubscript{46}PEO\textsubscript{30} polymersome prior to exposure to α-hemolysin. The membrane is false colored red, and FITC is false colored green. b) A representative confocal multi-channel image of the sample shown in a) 90 minutes after exposure to 30 µM of α-hemolysin. Most of the polymersomes had FITC intensities similar to the background demonstrating that the FITC had leaked out of the polymersomes upon poration by the α-hemolysin. c) Histogram of the lumen intensity of the FITC-loaded PBD\textsubscript{46}PEO\textsubscript{30} polymersomes before (blue bars) and after (orange bars) exposure to α-hemolysin. The intensity values were normalized by dividing by the mean lumen intensity of the sample of \(n=53410\) FITC-loaded polymersomes. After exposure to α-hemolysin, the encapsulated FITC of unilamellar polymersomes leaked within the course of 90 minutes. > 96% of the polymersomes had lumen intensities < 0.1 after exposure to α-hemolysin demonstrating that > 96% of the polymersomes were unilamellar. Scale bars 20 µm.
Supplementary Figure 7. Growth of PBD$_{46}$PEO$_{30}$ polymersomes at lower temperatures required longer incubation times. (a) Small polymersomes were present on the cellulose fibers 90 minutes after incubation at 37 °C (white arrows). (b) Larger polymersomes were present on the cellulose fibers 90 minutes after incubation at 50 °C but the number of polymersomes was lower when compared to growth at 80 °C (white arrows). (c,d) After a further 10.5 hours, a larger amount of polymersomes were present for the sample incubated at 37 °C, and the paper surface for the sample incubated at 50 °C looked similar to papers obtained when growth was performed at 80 °C. The nominal surface concentration of the PBD$_{46}$PEO$_{30}$ was 0.2 nmol/mm$^2$ for these experiments. Scale bar 10 µm. (e) Boxplot of the extracted polymersomes after 12 hours. The median diameter of the polymersomes (red line) for the sample grown at 50 °C was 7 µm, n=37,151. The median diameter for the sample grown at 50 °C was 11 µm, n=37,151.