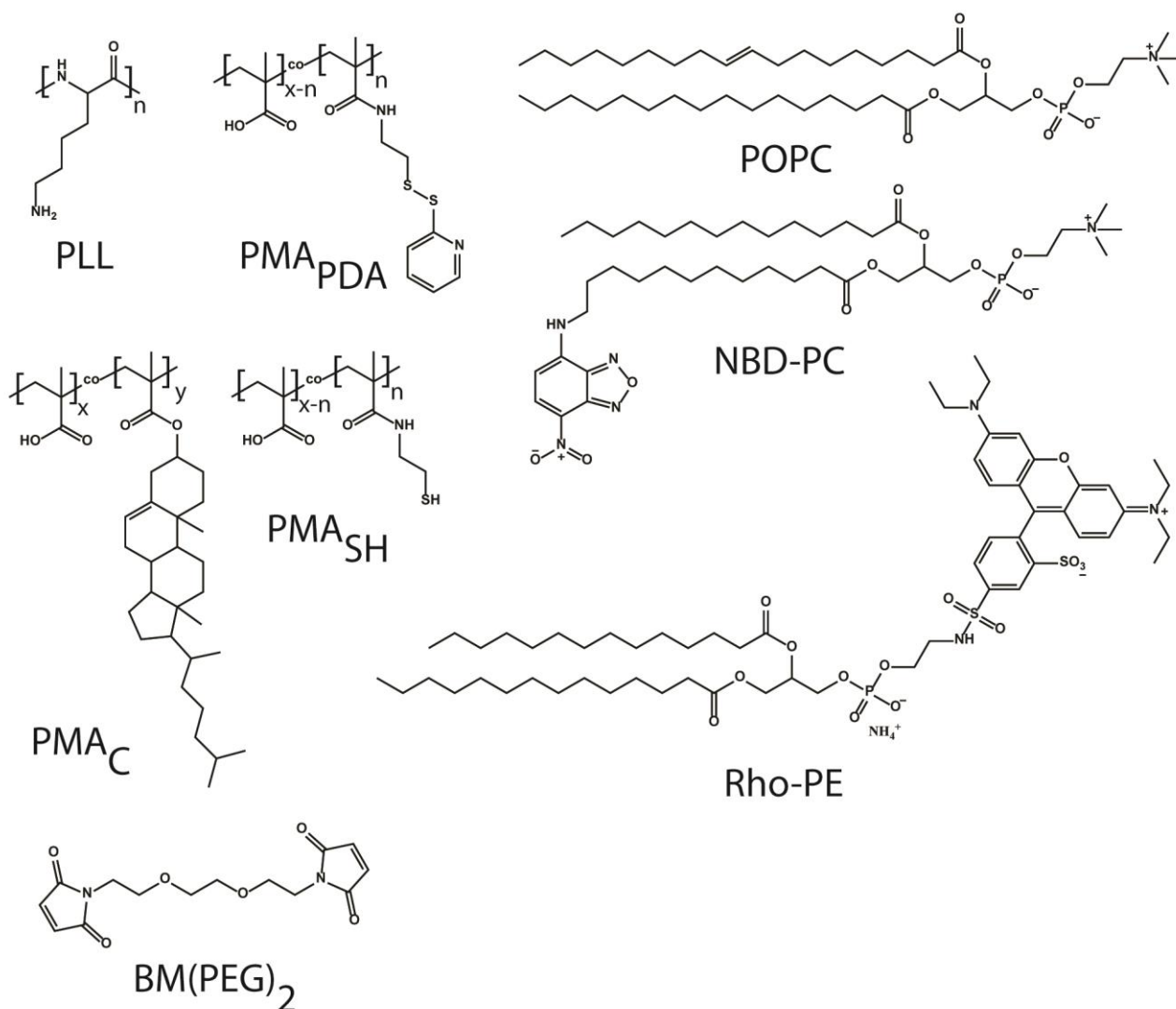


## Janus Subcompartmentalized Microreactors

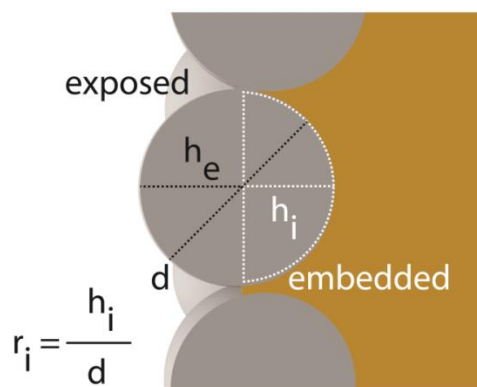
Philipp Schattling,<sup>a</sup> Cindy Dreier<sup>a</sup> and Brigitte Städler<sup>a\*</sup>

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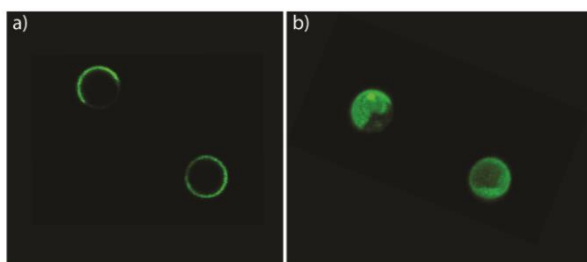
\*E-mail: [bstadler@inano.au.dk](mailto:bstadler@inano.au.dk)



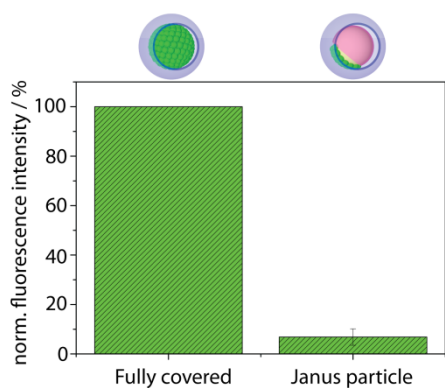
**Fig. S1** Chemical structures of the most important compounds used in this report.



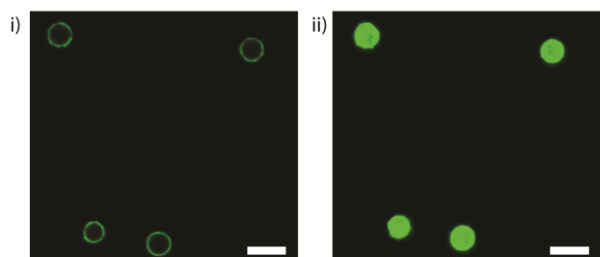
**Fig. S2** The penetration depth of the silica particle into the wax, was estimated by a simplified geometric calculation: the diameter of the silica particle ( $d$ ) was interrelated to the fraction of the particle exposed ( $h_e$ ) and embedded ( $h_i$ ) into the wax.



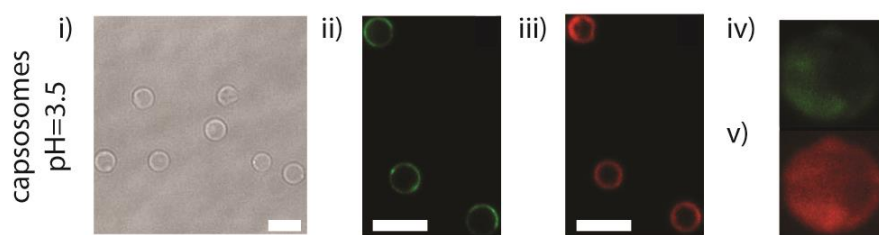
**Fig. S3** CLSM images demonstrating the difference of 2D and 3D (or z-stacked) images. In a) the right particle suggested a complete coverage. b) The screening through different planes of this particle revealed the existence of the non-fluorescent patch.



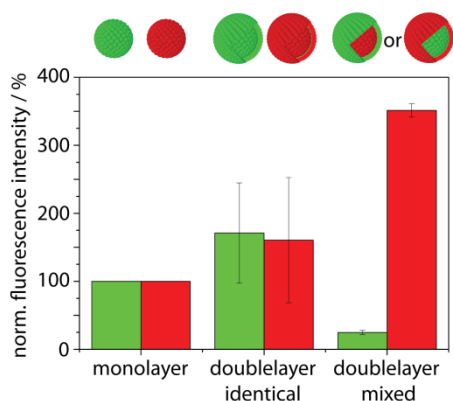
**Fig. S4** Normalized fluorescence of silica particles fully covered with  $L_g$  and Janus particle covered with  $L_g$  on one hemisphere.



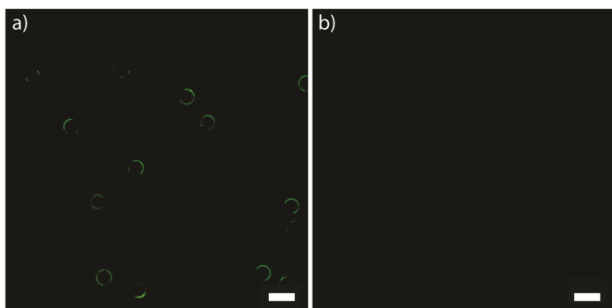
**Fig. S5** PLL coated silica particles after the deposition of NBD-labeled liposomes and one further layer of  $PMA_C$ . i) CLSM image and ii) the corresponding z-stack image of these particles. The scale bars are 5  $\mu\text{m}$ .



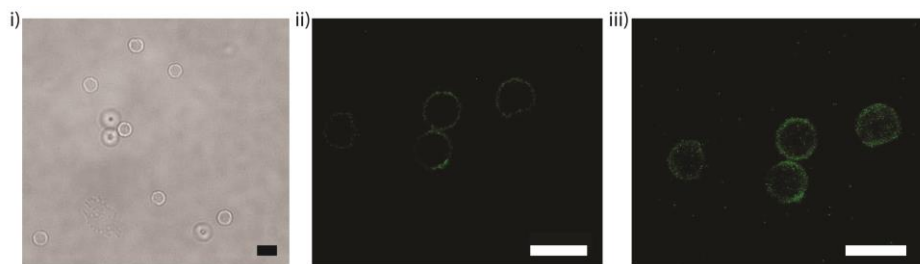
**Fig. S6** Janus capsosomes at pH 3.5: i) Bright field image of Janus capsosomes after removal of the silica core. 2D CLSM images visualizing  $L_g$  (ii) and the enclosing AF633-labeled hydrogel shell (iii). Z-stacked images of one exemplary capsosome visualizing  $L_g$  (iv) and the enclosing AF633-labeled hydrogel shell (v).



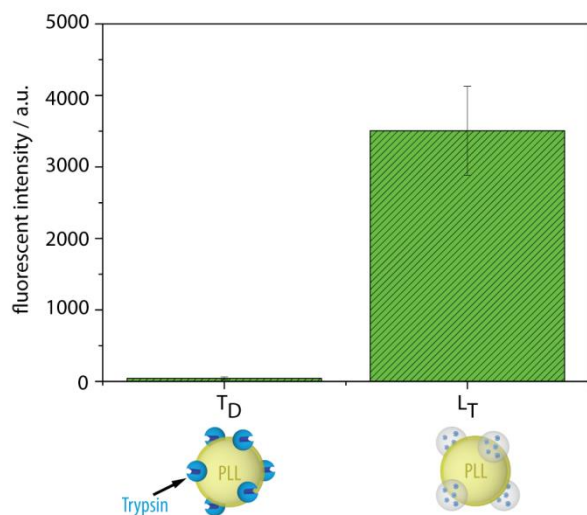
**Fig. S7** Normalized fluorescence intensity of the PLL pre-coated particles upon adsorption of NBD-labeled liposomes ( $L_g$ ) or RhoB-labeled ( $L_r$ ) liposomes measured by flow cytometry. The fluorescent intensity (FI-1 channel is depicted by green bars, FI-2 channel is depicted by red bars) monitored after the first liposome deposition step was put to 100% and the subsequent values were normalized to these values (left columns). The deposition of an additional layer of the same set of liposomes used for the first layer to PLL/ $L_g$  or  $r$ /PLL pre-coated particles (i.e.,  $L_g$  to PLL/ $L_g$ /PLL or  $L_r$  to PLL/ $L_r$ /PLL) showed the expected results of nearly doubling the measured fluorescent intensities (middle columns). On the other hand, the deposition of  $L_g$  and  $L_r$  to PLL/ $L_r$  or  $g$ /PLL pre-coated particles, yielded mixed double layer assemblies, which led to an decrease in the FI-1 channel and increase in the FI-2, respectively (right columns). This result was expected due to unfavorable cross talk between the two fluorescent channels in the flow cytometer. While through the FI-1 filter (533 +/- 30 nm) the fluorescence of the NBD dye was detected, a significant contribution of the NBD emission was recognized through the FI-2 filter (585 +/- 40 nm) as well. In addition, the emission and absorption spectra of NBD and RhoB, respectively, exhibited a spectral overlap, yielding in a significant decrease of the fluorescence of the green channel, after deposition of  $L_r$ . As a consequence, the evaluation regarding  $L_r$  coverage of the hierarchical organized capsosomes could not be quantified satisfactory.



**Fig. S8** CLSM image of Janus core-shell particles containing  $L_g$ , visualized in the green (a) and red (b) channel, demonstrating that the green (NBD) fluorescence was not observed in the red channel with the used settings for imaging.



**Fig. S9** Bright field image of  $(PVP-PLL/L_T/PMA_c)/(PVP/PMA_{SH})_3$  Janus microreactors with removed core (i) and the corresponding CLSM image (ii) and z-stack image (iii) after incubation with the substrate BA-Rho-110. Scale bars are 5  $\mu\text{m}$ .



**Fig. S10** Fluorescent intensity of the supernatant of a solution with either PLL/ $T_D$  or PLL/ $L_T$  coated silica particles after incubation with the substrate BA-Rho-110 for 30 min.