

## Supporting Information:

# **A Bio-orthogonal functionalization strategy for site-specific coupling of antibodies on liposome surfaces after self-assembly**

Meiyu Gai <sup>a,1</sup>, Johanna Simon <sup>a,b,1</sup>, Ingo Lieberwirth <sup>a</sup>, Volker Mailänder <sup>b,a</sup>, Svenja Morsbach <sup>a,\*</sup>  
and Katharina Landfester <sup>a</sup>

<sup>a</sup> Max Planck Institute for Polymer Research, Ackermannweg 10, 55128 Mainz, Germany

<sup>b</sup> Department of Dermatology, University Medical Center of the Johannes Gutenberg-University Mainz, Langenbeckstrasse 1, 55131 Mainz, Germany

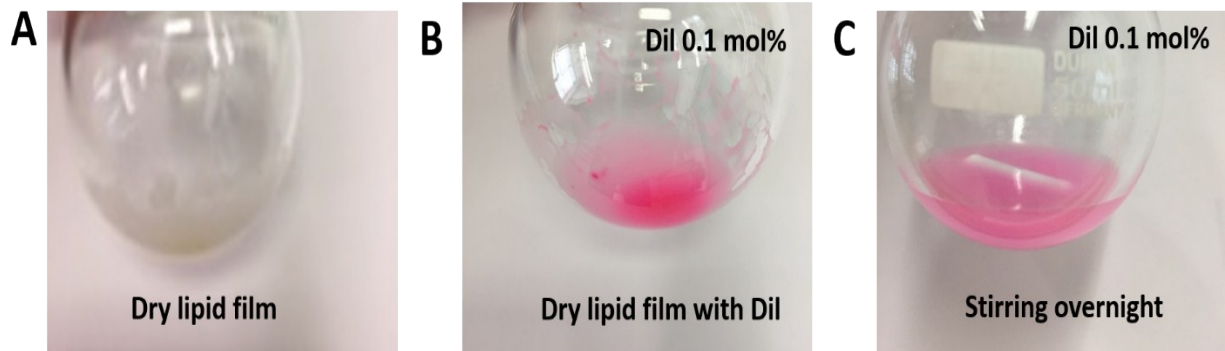
<sup>1</sup> authors contributed equally

Corresponding author E-mail: [morsbachs@mpip-mainz.mpg.de](mailto:morsbachs@mpip-mainz.mpg.de)

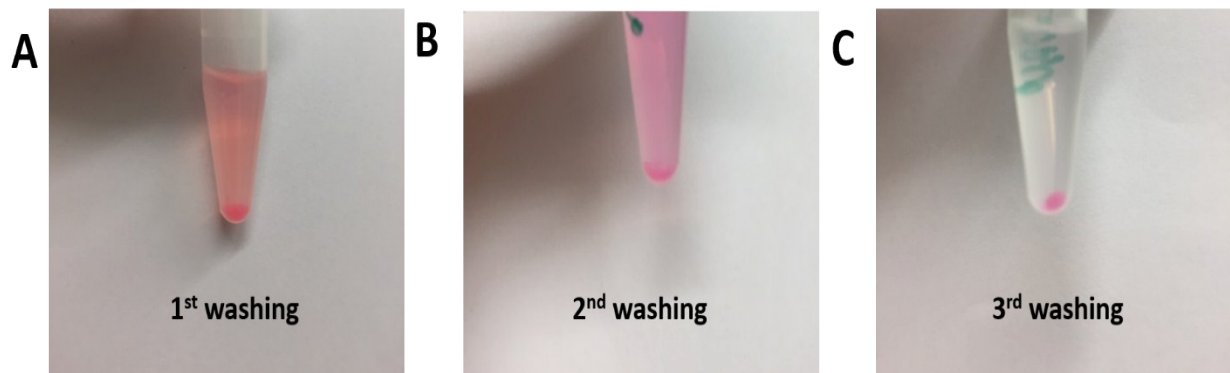
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- **Images of liposome film formation and rehydration**
- **Images of washing procedure**
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- **Zeta potential measurements**
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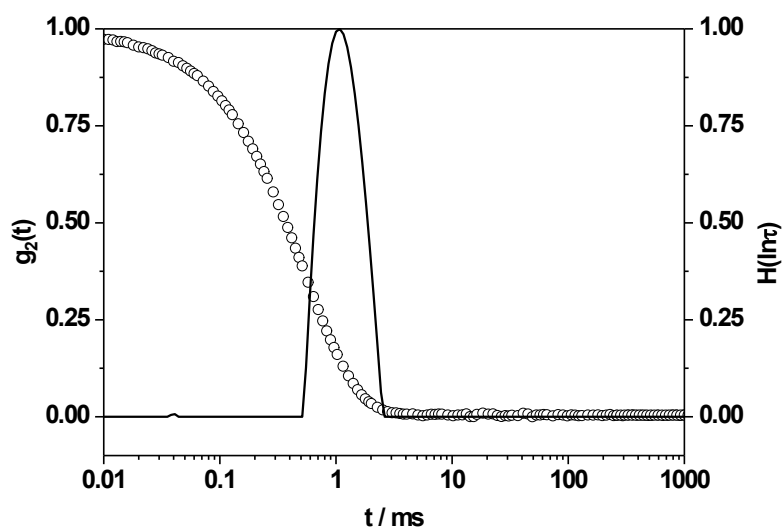
## Additional Figures



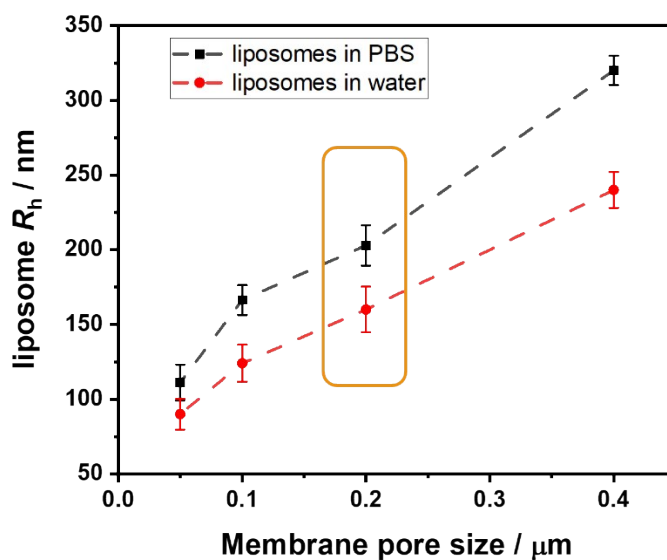
**Figure S1:** Images of liposome synthesis: A) Egg PC:DOPE:Chol = 1:1:1 lipid dry film after the process of rotary evaporation in a round bottom flask; B) same lipid dry film with an addition of 0.1 mol% DiI; C) rehydration and stirring overnight (stirring speed: 700 rpm) in PBS buffer.



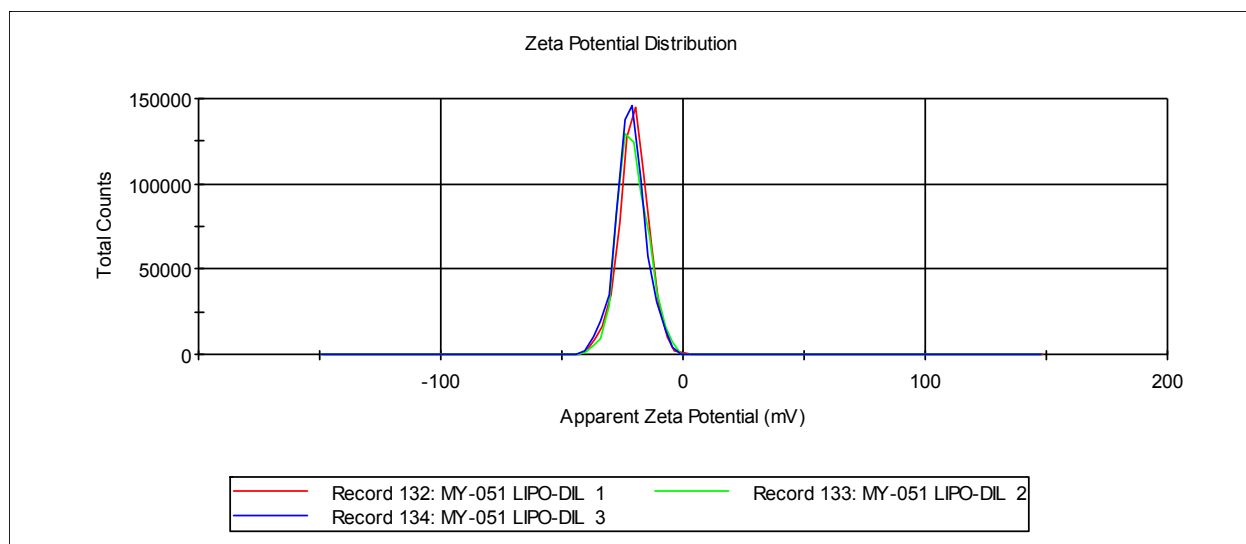
**Figure S2:** Purification of DBCO functionalized liposomes by centrifugation at 20000 g, 1 h and 4 °C and subsequent redispersion in PBS buffer for a total of 3 times.



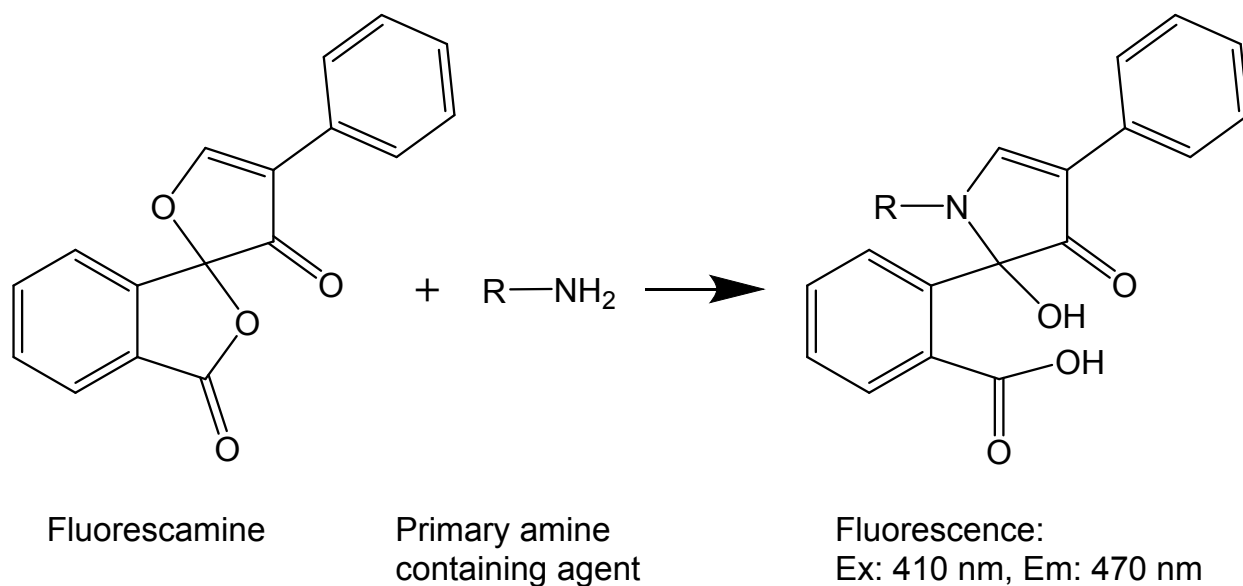
**Figure S3:** Autocorrelation function  $g_2(t)$  of non-functionalized liposomes in PBS buffer for an exemplary scattering angle of  $90^\circ$  together with the distribution of relaxation times  $H(\ln\tau)$  obtained from the CONTIN algorithm.



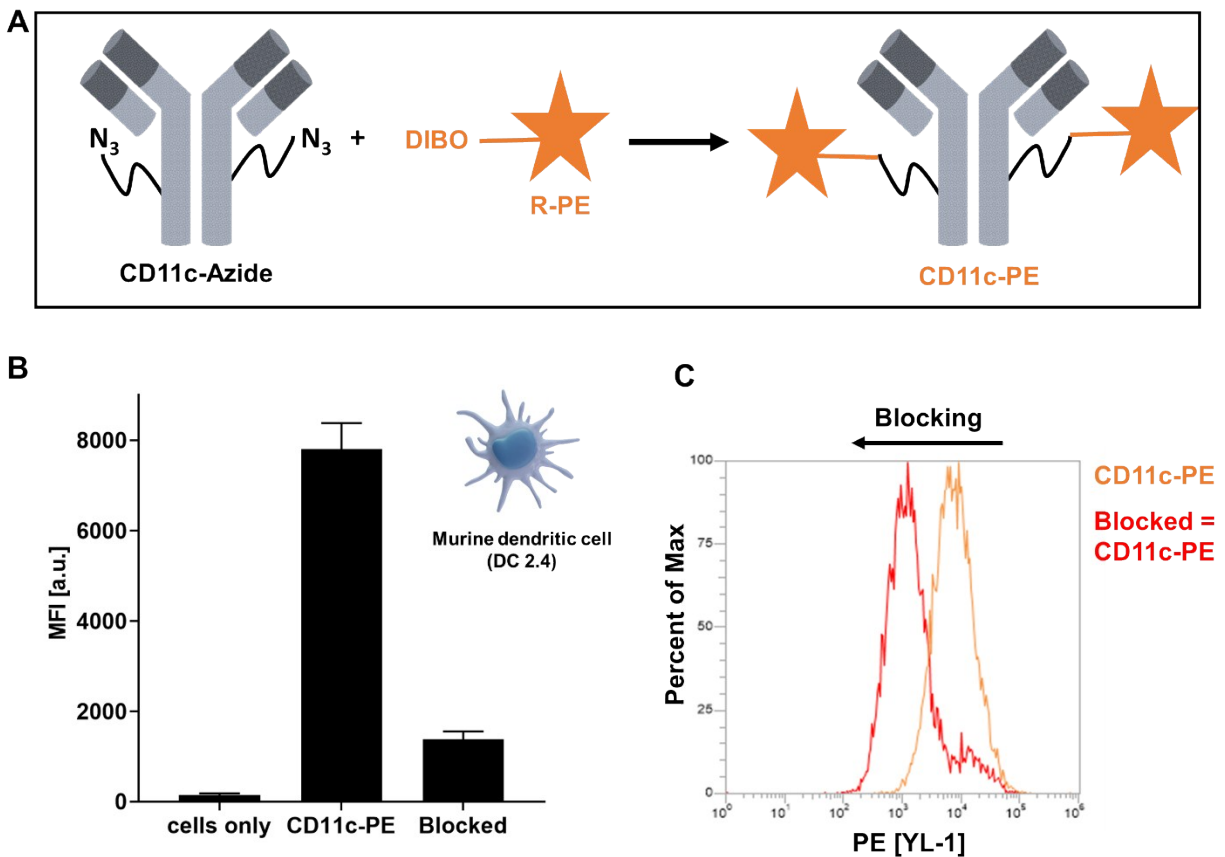
**Figure S4:** DLS measurements of liposomes in PBS buffer or water depending on different membrane pore sizes used for extrusion (dotted lines are a guide to the eye). The membrane pore size of 200 nm (highlighted with a box) was chosen for further synthesis of all liposome samples presented in the manuscript.



**Figure S5:** Zeta potential analysis of liposomes in 0.1 M KCl solution measured in triplicate.



**Figure S6:** Reaction scheme for the fluorescamine assay (FA assay). The fluorescamine can react with primary amine groups (-NH<sub>2</sub>) of liposomes and the fluorescent product can be excited and detected by plate reader ( $\lambda_{Ex} = 410$  nm,  $\lambda_{Em} = 470$  nm). Hexylamine which contains primary amine groups can be used as a reference for making the standard calibration curve.



**Figure S7. Functionalization of azide-modified antibodies with a fluorescent dye.** A) Azide-modified anti-mouse CD11c antibodies ( $0.5 \text{ mg mL}^{-1}$ ,  $15 \text{ }\mu\text{L}$ ) were incubated with DIBO-conjugated R-phycoerythrin (DIBO R-PE,  $11.25 \text{ }\mu\text{L}$ ) overnight in the dark at room temperature. B) and C) Flow cytometry analysis of DC2.4 cells, which were incubated with anti-mouse CD11-PE antibodies ( $0.34 \text{ mg mL}^{-1}$ ,  $1 \text{ }\mu\text{L}$ ). For blocking: Cells were pre-treated with free unlabeled anti-mouse CD11c antibody ( $2 \text{ mg mL}^{-1}$ ,  $5 \text{ }\mu\text{L}$ ) and afterwards anti-mouse CD11-PE antibodies were added. The median fluorescent intensity (MFI) from two independent measurements is shown.

**Table S1.** Concentration and absolute amount of antibodies per liposome

<b>Liposome concentration</b>	1*10 <sup>13</sup> liposomes/mL
<b>Solid content</b>	0.3 wt% (3 mg/mL)
<b>M<sub>w</sub> Antibody</b>	150,000 g/mol

<b>Coupling</b>	
500 µg Liposomes-DBCO	17.5 µg CD11c-Azide
1.67*10 <sup>12</sup> Liposomes-DBCO	7*10 <sup>13</sup> CD11c-Azide
<b>~ 42 Antibodies per Liposome</b>	