Supplementary Material

In situ synthesis of lipopeptides as versatile receptors for the specific binding of nanoparticles and liposomes to solid supported membranes

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Supplementary Methods.

**Abbreviations.** DCC: N,N'-Dicyclohexylcarbodiimide; DIEA: N,N-Diisopropylethyl-amine; DMAP: 4-(Dimethylamino)pyridine; DOPE: 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine; EDC: N-(3-Dimethylaminopropyl)-N’-ethyl-carbodiimide hydrochloride; EDT: Ethanedithiole; HOBT: 1-Hydroxybenzotriazole; MHex-DOPE: N-(6-Maleimidohexanoyl)-1,2-dioleoyl-sn-glycero-3-phosphoethanol-amine; NHS: N-Hydroxysuccinimide; TLC: Thin Layer Chromatography.

**Synthesis of MHex-DOPE.**

**Synthesis of MHex-DOPE using EDC as a coupling reagent (Method A).** 6-Maleimidohexanoic acid (22 mg, 0.1 mmol) and DOPE (75mg, 0.1 mmol) were dissolved in CHCl₃ (15ml) and cooled to 0°C. EDC (23 mg, 0.12 mmol) and DIEA (36µl, 0.2mmol) were added. The clear reaction mixture was stirred over night at room temperature and the reaction was monitored by TLC (mobile phase CHCl₃/MeOH (5:1); KMnO₄ and Ninhydrin staining), revealing that DOPE was transformed into a faster running product with Rₕ = 0.35. After completion of the reaction the reaction mixture was concentrated in a rotavap and purified by flash chromatography on silica gel (mobile phase CHCl₃/MeOH (2:1)). Minor impurities of 6-maleimidohecanoic acid were removed by flash column chromatography on aluminum oxid (activated, basic, Brockmann I; mobile phase CHCl₃/MeOH/H₂O (65:25:4)). Freeze drying from benzene yielded MHex-DOPE as a white solid (63 mg, 67%) with Rₕ = 0.35 (CHCl₃/MeOH 5:1); MALDI-TOF for C₅₁H₈₈N₂NaO₁₁P, MH⁺ at m/z = 940.4 (calcd 940.2);

**Synthesis of MHex-DOPE using DCC as a coupling reagent (Method B).** 6-Maleimidohexanoic acid (32 mg, 0.15 mmol) and DOPE (75mg, 0.1 mmol) were dissolved in CHCl₃ (15ml) and cooled to 0°C. DCC (33 mg, 0.16 mmol) and DMAP (10 mg, 0.08mmol) were added from stock solutions in CHCl₃. Monitoring, general work up and purification are essentially identical to method A with minor changes, i.e. solvent of collected fractions from silica flash chromatography was removed and the precipitate was dissolved in benzene. Insoluble dicyclohexylurea was removed by syringe filtration.
(Millex LCR, 0.45µm) and the product was lyophilized from benzene followed by flash column chromatography on basic aluminum oxid. MHex-DOPE was obtained as a white solid (52 mg, 54%) with R<sub>f</sub> = 0.34 (CHCl<sub>3</sub>/MeOH 5:1); MALDI-TOF for C<sub>51</sub>H<sub>88</sub>N<sub>2</sub>NaO<sub>11</sub>P, MH<sup>+</sup> at m/z = 940.1 (calcd 940.2).

**Synthesis of MHex-DOPE via NHS ester of 6-maleimidohexanoic acid (Method C).** 6-Maleimidohexanoic acid (106 mg, 0.5 mmol) and NHS (63 mg, 0.55 mmol) were dissolved in 10 ml THF and cooled to 0 °C. DCC (114 mg, 0.55 mmol) was added from a stock solution in THF. After 90 min a white precipitation due to insoluble dicyclohexylurea was observed. The turbid suspension was stirred over night at room temperature and the reaction was monitored by TLC (mobile phase CHCl<sub>3</sub>/MeOH (5:1); KMnO<sub>4</sub> staining), revealing that 6-maleimidohexanoic acid was transformed into a faster running product R<sub>f</sub> = 0.71. Insoluble dicyclohexylurea was removed by syringe filtration (Millex LCR, 0.45µm) and the reaction solvent was evaporated in a rotavap. The product was dissolved in 10 ml CHCl<sub>3</sub> and used without further purification. 2.5 ml of the NHS-maleimidohexanoic-ester solution (~0.12 mmol NHS ester) was added to DOPE (75 mg, 0.1 mmol) and diluted to 10 ml with CHCl<sub>3</sub>. The reaction was stirred over night and monitored by TLC. Purification and work up followed method A. MHex-DOPE was received as a white solid (73 mg, 78%) with R<sub>f</sub> = 0.34 (CHCl<sub>3</sub>/MeOH 5:1); MALDI-TOF for C<sub>51</sub>H<sub>88</sub>N<sub>2</sub>NaO<sub>11</sub>P, MH<sup>+</sup> at m/z = 940.2 (calcd 940.2).
Supplementary Figure Legends

**Figure S1.** Two channel confocal laser scanning microscopy images of the H6 lipopeptide mediated binding of Texas Red labeled Ni-NTA vesicles to microstructured lipid bilayers without BSA passivation of the glass substrate. PMT 1 recorded the BODIPY emission and PMT 2 recorded the Texas Red emission. Initially the left control lipid bilayer compartment composed of 98% DOPC/2% BODIPY and the right reactive lipid bilayer compartment composed of 88% DOPC/10% 2/2% BODIPY were exposed to 0.4 µmol H6 for 60 min. After rinsing the sample, it was incubated with unilamellar Texas Red labeled Ni-NTA DOPC vesicles, composed of 89% DOPC/10% Ni-NTA DOGS/1% Texas Red at a final concentration of 0.15 mg/ml for 30 min. The right H6 lipopeptide functionalized lipid bilayer compartment showed exclusively specific binding of Ni-NTA DOPC vesicles, whereas the control bilayer compartment remained non fluorescent in the PMT 2. Strong non-specific adsorption of Texas Red labeled Ni-NTA DOPC vesicles was observed on the glass background due to the lack of BSA passivation.

**Figure S2.** Two channel confocal laser scanning microscopy images of the lipopeptide mediated binding of POPC/POPG vesicles to microstructured lipid bilayers containing K8 lipopeptides. Images were taken by two laser excitation at 488 nm and 543 nm. Photo multiplier tube 1 (PMT 1) recorded the BODIPY-C12-PC (BODIPY) emission between 500 – 530 nm, while PMT 2 detects the rhodamine emission of TAMRA-H6 and the Sulforhodamine 101 DHPE (Texas Red) emission between 580-650 nm. A, B) The right, non-fluorescent lipid bilayer compartments are composed of 90% DOPC and 10% 1 and the left lipid bilayer compartment is composed of 99% DOPC and 1% Sulforhodamine 101 DHPE (Texas Red). C, D) After the addition of 0.1 µmol TAMRA-K8 to the sample, resulting in an overall concentration of 0.03 mM TAMRA-H8 in PBS 6.8 for 60 min and thorough rinsing, the right compartments can be detected by the red light emission of the TAMRA labeled K8 lipopeptides. Prior to the lipopeptide mediated binding of functionalized vesicles, the sample was incubated in BSA.
solution (0.25 mg/ml in PBS 6.8) for 30 min. E, F) BODIPY fluorescence after binding of POPC/POPG vesicles (30%POPC, 68% POPG, 2% BODIPY C$_{12}$-HPC) at a final concentration of 0.1 mg lipid/ml in PBS 6.8 for 30 min.

**Figure S3.** Time course of the $\Delta$ values of the H6 lipopeptide mediated binding of Ni-NTA DOPC vesicles to neat DOPC as a control experiment. Initially, unilamellar vesicles consistent of 100% DOPC were spread. After rinsing with buffer 0.4 µmol H6 in PBS 6.8 were added, yielding in no significant increase in layer thickness. Slight variations in the ellipsometric angle were recorded after the addition of unilamellar Ni-NTA DOPC vesicles (0.5 mg), resulting from non-specific adsorption.

**Figure S4.** Time course of the delta values of the control experiment for the K8 lipopeptide mediated binding of gold nanoparticles. Initially unilamellar vesicles consistent of 100% DOPC were spread. After rinsing 0.4 µmol K8 in PBS 6.8 were added, yielding no significant increase in layer thickness. Constant values in the ellipsometric angle were recorded after the addition of 0.5 nmol gold nanoparticles.

**Figure S5.** AFM image of H6 lipopeptide mediated binding of Ni-NTA functionalized DOPC vesicles. Image was taken from the surrounding scan area of figure 7. Bright areas in the image display intact vesicles which are adsorbed on the H6 lipopeptide functionalized bilayer. Dark areas display the second bilayer, which has formed upon vesicle rupturing.
Figure S1.

PMT 1

PMT 2
Figure S2.

PMT 1  
A  + TAMRA-K8  
C  + BODIPY labeled POPC/POPG vesicles  
E

PMT 2  
B  
D  
F
Figure S3.
Figure S4.
Figure S5.