Preparation of Luminescent Chemosensors by Post-Functionalization of Vesicle Surfaces

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Electronic Supplementary Information

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1. General Methods and Material

General

Commercially available solvents of standard quality were used. Starting materials and analytes (sodium pyrophosphate, D-fructose 1,6-bisphosphate, ampicillin, benzylpenicillin, amoxicillin) were purchased from either Acros or Sigma-Aldrich and used without any further purification. Phospholipids were purchased from Avanti Polar Lipids Inc. The hexapeptide **Pep-P-His** was obtained from GL Biochem Ltd. (Shanghai) and the thiolated aptamer **Apt-Amp** was synthesized by IBA GmbH (Göttingen).

Thin layer chromatography (TLC) analyses were performed on pre-coated silica gel 60 F-254 with a 0.2 mm layer thickness (Pre-coated TLC-sheets ALUGRAM Xtra SIL G/UV_{254} from Macherey-Nagel). The detection was done *via* UV light at 254 or 366 nm or by staining with $KMnO_4$. Flash column chromatography was performed on silica gel 60 (70–230 mesh) from Macherey-Nagel.

Melting Points

Melting points were determined on a Stanford Research Systems OptiMelt MPA100 with a heating rate of 1–2 °C/min.

NMR Spectra

For NMR spectroscopy, a Bruker Avance 300 (^{1}H: 300.1 MHz, ^{13}C: 75.5 MHz, T = 293 K) or Bruker Avance 400 (^{1}H: 400.1 MHz, ^{13}C: 100.6 MHz, T = 293 K) was used. All chemical shifts are reported in δ [ppm] (multiplicity, coupling constant *J*, number of protons, assignment) relative to the solvent residual peak as the internal standard ($CDCl_3$: ^{1}H: δ = 7.26 ppm, ^{13}C: δ = 77.16 ppm; CD_3OD: ^{1}H: δ = 3.31 ppm, ^{13}C: δ = 49.00 ppm; CD_3CN: ^{1}H: δ = 1.94 ppm, ^{13}C: δ = 1.32 ppm). The coupling constants are given in Hertz [Hz]. Abbreviations used for signal multiplicity: ^{1}H-NMR: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Assignments of the ^{13}C-NMR signals were made using DEPT technique (pulse angle: 135 °) and given as (+) for CH_3 or CH, (–) for CH_2 and (C_q) for a quaternary carbon.

Mass Spectra

Mass spectra were measured on a ThermoQuest Finnigan TSQ 7000 or an Agilent Q-TOF 6540 UHD mass spectrometer.

Fluorescence Measurements

Fluorescence spectroscopy was carried out on a Varian Cary Eclipse or a HORIBA FluoroMax-4 fluorescence spectrophotometer with temperature control using 10 × 4 mm Hellma quartz cuvettes at 21 °C. Vesicles containing **Dans-C_{12}** were excited at 280 nm, those with embedded **CF-C_{12}** at 495 nm. For better comparison of the different measurements, the relative fluorescence changes (ΔI_{max} / $I_{0,max}$) were calculated.

Dynamic Light Scattering (DLS)

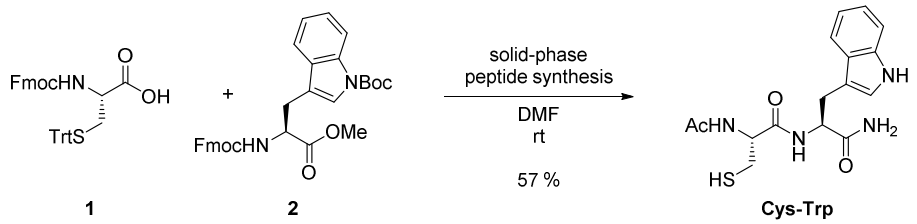
Dynamic light scattering was performed on a Malvern Zetasizer Nano ZS at 25 °C using 1 cm disposable polystyrene cuvettes. Data analysis was performed using the Malvern Zetasizer software.

2. Synthesis

The amphiphiles **Mal-C_{16}**,[1] **Dans-C_{12}**,[2], **CF-C_{12}**[3] were prepared according to previously reported procedures, the syntheses of **Cys-Trp** and of **Zn-Cyclen$_2$** is described below.

2.1 Synthesis of *N*-acetyl-L-cysteinyl-L-tryptophanamide (Cys-Trp)

The synthesis of dipeptide **Cys-Trp** was carried out following a standard protocol for Fmoc-based solid-phase peptide synthesis (Scheme S1).[4] As solid phase, Fmoc-Rink-Amide-MBHA resin (200 mg, 0.106 mmol) was used. After precipitation in cold Et_2O, the peptide **Cys-Trp** was obtained as white solid (21 mg, 0.060 mmol, 57 %).



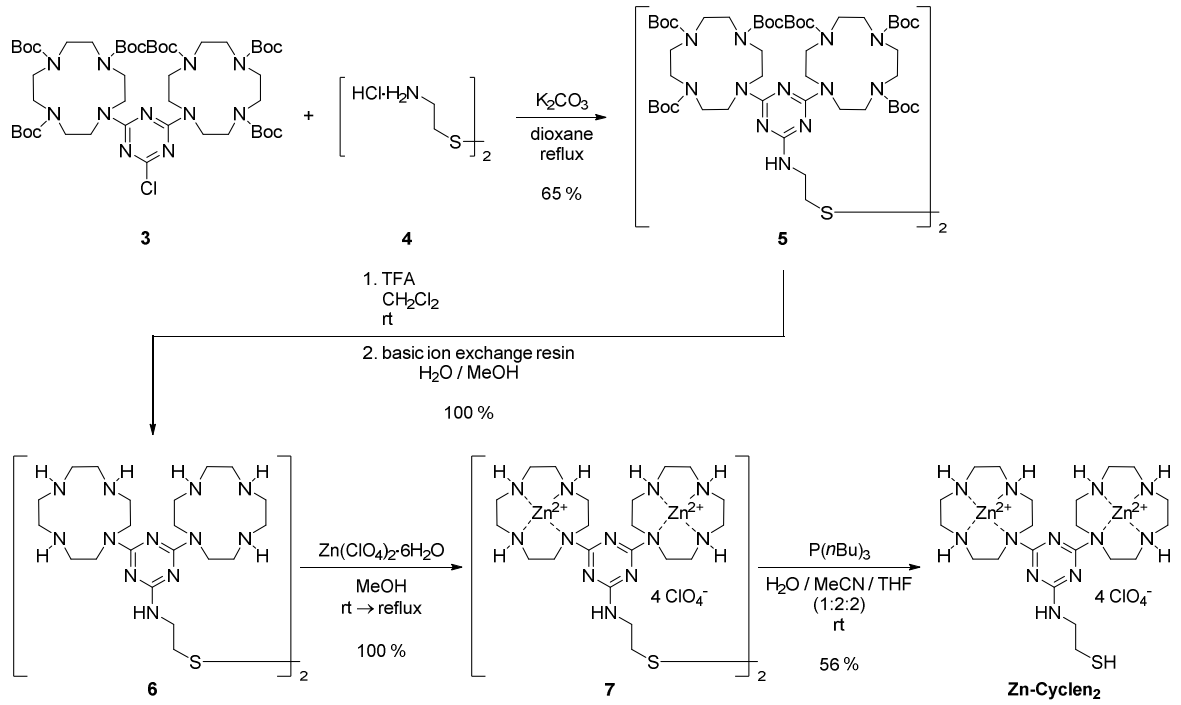
Scheme S1: Synthesis of dipeptide **Cys-Trp**.

R_f = 0.17 (MeOH/CH_2Cl_2 10:90). — ^{1}H-NMR (CD_3OD, 300 MHz): δ = 1.89 (s, 3H, Me), 2.66–2.82 (m, 2H, CH_2), 3.17–3.22 (m, 1H, C*H*H), 3.28–3.37 (m, 1H, CH*H*), 4.34–4.42 (m,

1H, CH), 4.63–4.72 (m, 1H, CH), 6.98–7.05 (m, 1H, CH, indole), 7.05–7.10 (m, 1H, CH, indole), 7.11 (s, 1H, CH, indole), 7.32 (d, J = 7.7 Hz, 1H, CH, indole), 7.62 (d, J = 7.7 Hz, 1H, CH, indole). — ^{13}C-NMR (CD_3OD, 75 MHz): δ = 22.37 (+, CH_3), 26.43 (–, CH_2), 28.66 (–, CH_2), 55.26 (+, CH), 57.44 (+, CH), 110.77 (C_q, indole), 112.32 (+, CH, indole), 119.40 (+, CH, indole), 119.94 (+, CH, indole), 122.49 (+, CH, indole), 124.70 (+, CH, indole), 128.81 (C_q, indole), 138.08 (C_q, indole), 172.22 (C_q=O), 173.85 (C_q=O), 176.57 (C_q=O). — HRMS (ESI$^+$): m/z = $[C_{16}H_{20}N_4O_3S + H^+]^+$ calculated 349.1329; found 349.1329.

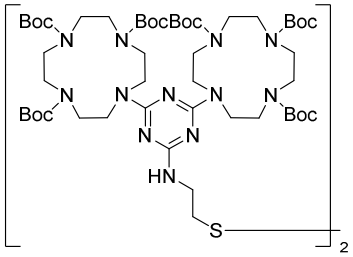
2.2 Synthesis of Zn-Cyclen$_2$

Starting from Boc-protected bis-cyclen **3**[5] (Scheme S2), dimer **5** was prepared by nucleophilic aromatic substitution with diamine **4**. Deprotection of **5** and coordination of **6** with Zn^{2+} yielded complex **7**. Due to its sensitivity towards oxidation, the thiol function was generated in the very last step of the synthesis by reduction of the disulfide bond providing **Zn-Cyclen$_2$**.



Scheme S2: Synthesis of thiolated bis-Zn^{2+}-cyclen complex **Zn-Cyclen$_2$**.

2.2.1 N,N′-*(2,2′-Disulfanediylbis(ethane-2,1-diyl))bis(4,6-di(1,4,7,10-tetraazacyclododecan-1,4,7-tricarboxylic acid tri-tert-butyl ester-1-yl)-[1,3,5]-triazine-2-amine) (**5**)*

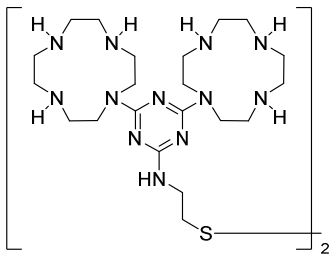


5

To a solution of bis-cyclen **3**[5] (300 mg, 0.284 mmol, 2.00 eq) and cystamine dihydrochloride (**4**, 32.0 mg, 0.142 mmol, 1.00 eq) in 1,4-dioxane (5.0 mL), potassium carbonate (193 mg, 1.42 mmol, 10.0 eq) was added. The reaction mixture was stirred at 120 °C for 14 h resulting in the formation of a fine white precipitate. Subsequently, the solvent was evaporated under reduced pressure and the obtained white residue was purified by column chromatography (SiO_2, EtOAc/PE 1:1) to yield disulfide **5** as white solid (203 mg, 0.093 mmol, 65 %).

R_f = 0.43 (EtOAc/PE 1:1). — MP: 126–132 °C. — ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 1.35 (br s, 36H, 4 × Boc), 1.37 (s, 72H, 8 × Boc), 2.74 (t, J = 5.5 Hz, 4H, 2 × CH_2S), 3.03–3.73 (br m, 68H, 32 × CH_2, cyclen, 2 × NHCH_2), 4.91–5.40 (m, 2H, 2 × NH). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 28.44 (+, Me, Boc), 28.47 (+, Me, Boc), 38.05 (–, $NHCH_2CH_2S$), 39.27 (–, $NHCH_2CH_2S$), 50.28 (–, br, CH_2, cyclen), 79.74 (C_qMe_3, Boc), 79.87 (C_qMe_3, Boc), 156.30 (br, C_q=O, Boc), 165.60 (C_q, triazine). — MS (ESI$^+$, CH_2Cl_2/MeOH + 10 mM NH_4OAc): *m/z* (%) = 1097.3 (100) $[M + 2H^+]^{2+}$, 2193.0 (11) $[M + H^+]^+$.

2.2.2 N,N′-*(2,2′-Disulfanediylbis(ethane-2,1-diyl))bis(4,6-di(1,4,7,10-tetraazacyclododecan-1-yl)-[1,3,5]-triazine-2-amine) (**6**)*



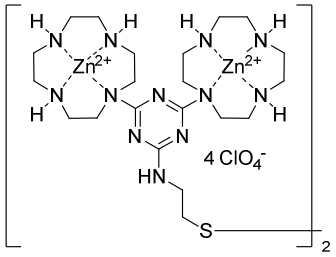
6

To a solution of disulfide **5** (190 mg, 0.087 mmol, 1.00 eq) in CH_2Cl_2 (6.0 mL), TFA (565 mg, 0.38 mL, 4.96 mmol, 57.0 eq) was added. The reaction mixture was stirred at rt (21 °C)

for 17 h. The solvent was then removed under reduced pressure providing the TFA salt of **6** as yellowish oil. A strong basic ion exchange resin was swollen in H_2O/MeOH (1:1) for 15 min and washed neutral with H_2O. A column was charged with the swollen resin (2.10 mL, 1.89 mmol hydroxy equivalents at a given capacity of 0.9 mmol/mL, 22.0 eq). Subsequently, a solution of the TFA salt of **6** in H_2O/MeOH (10:1, 10 mL) was given onto the column and eluated with additional H_2O/MeOH (10:1, 10 mL). The eluate was lyophilized to yield tetra-cyclen **6** as light brown solid (86 mg, 0.087 mmol, 100 %).

MP: 120–125 °C. — ^{1}H-NMR (CD_3OD, 300 MHz): δ = 2.62–2.71 (m, 16H, 8 × CH_2, cyclen), 2.71–2.79 (m, 16H, 8 × CH_2, cyclen), 2.85–3.07 (m, 20H, 8 × CH_2, cyclen, 2 × CH_2S), 3.65 (t, J = 6.9 Hz, 4H, 2 × NHCH_2CH_2S), 3.69–3.81 (m, 16H, 8 × CH_2, cyclen). ^{13}C-NMR (CD_3OD, 75 MHz): δ = 39.55 (–, $NHCH_2CH_2S$), 41.08 (–, $NHCH_2CH_2S$), 46.55 (–, br, CH_2, cyclen), 47.08 (–, br, CH_2, cyclen), 49.26 (–, br, CH_2, cyclen), 49.33 (–, br, CH_2, cyclen), 166.98 (br, C_q, triazine), 167.98 (br, C_q, triazine). — MS (ESI$^+$): *m/z* (%) = 248.68 (100) $[M + 4H^+]^{4+}$, 331.24 (33) $[M + 3H^+]^{3+}$, 496.35 (6) $[M + 2H^+]^{2+}$.

2.2.3 N,N′-*(2,2´-Disulfanediylbis(ethane-2,1-diyl))bis(4,6-di(1,4,7,10-tetraazacyclodo-decan-1-yl)-[1,3,5]-triazine-2-amine)-tetra-zinc(II)-octa-perchlorate (**7**)*



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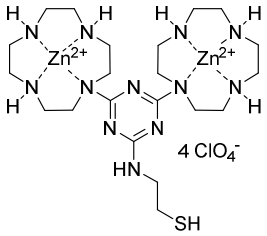
A solution of zinc(II) perchlorate hexahydrate (97 mg, 0.260 mmol, 4.20 eq) in MeOH (2.0 mL) was slowly added to a solution of tetra-cyclen **6** (61 mg, 0.062 mmol, 1.00 eq) in MeOH (3.5 mL) resulting in the formation of a white precipitate. The reaction mixture was stirred at rt (21 °C) for 20 h and then heated to 80 °C for further 17 h. The solvent was removed *in vacuo* providing compound **7** as yellowish residue (127 mg, 0.062 mmol, 100 %).

^{1}H-NMR (CD_3CN, 400 MHz): δ = 2.70–3.83 (br m, 72H, 32 × CH_2, cyclen, 2 × NHCH_2CH_2S), 3.94–4.10 (m, 4H, 4 × NH, cyclen), 4.23–4.42 (m, 8H, 8 × NH, cyclen), 6.52 (t, J = 5.7 Hz, 2H, 2 × NHCH_2CH_2S). ^{13}C-NMR (CD_3CN, 101 MHz): δ = 38.35 (–,

$NHCH_2CH_2S$), 40.74 (–, $NHCH_2CH_2S$), 44.62 (–, br, CH_2, cyclen), 46.14 (–, br, CH_2, cyclen), 46.97 (–, br, CH_2, cyclen), 48.85 (–, br, CH_2, cyclen), 166.79 (C_q, triazine), 171.26 and 171.63 (C_q, triazine, tautomers). — MS (ESI^+, H_2O/MeCN): *m/z* (%) = 372.2 (34) $[M^{8+} + 4AcO^-]^{4+}$, 515.9 (100) $[M^{8+} + 5AcO^-]^{3+}$, 529.1 (85) $[M^{8+} + 4AcO^- + ClO_4^-]^{3+}$.

*2.2.4 2-(4,6-Di(1,4,7,10-tetraazacyclododecan-1-yl)-1,3,5-triazin-2-ylamino)ethanethiol-di-zinc(II)-tetra-perchlorate (**Zn-Cyclen$_2$**)*



Zn-Cyclen$_2$

Disulfide **7** (51 mg, 0.025 mmol, 1.00 eq) was dissolved in a mixture of H_2O/MeCN/THF (1:2:2, 1.0 mL). The solution was degassed by bubbling nitrogen through it for 5 min. Tri-*n*-butylphosphine (18 mg, 0.088 mmol, 3.50 eq) was slowly added and the reaction mixture was stirred at rt (21 °C) for 20 min. After evaporation of the solvents, the resulting residue was redissolved in MeCN (2.0 mL), H_2O (10 mL) was added and the aqueous mixture was extracted with CH_2Cl_2 (5 × 10 mL). Lyophilization of the aqueous phase yielded free thiol **Zn-Cyclen$_2$** as pale yellow solid (29 mg, 0.028 mmol, 56 %).

MP: > 150 °C (Decomposition). — ^{1}H-NMR (CD_3CN, 400 MHz): δ = 2.60–3.87 (br m, 36H, 16 × CH_2-cyclen, $NHC\mathit{H}_2C\mathit{H}_2SH$), 3.89–5.05 (br m, 6H, 6 × NH, cyclen), 5.70–6.30 (br m, 1H, $N\mathit{H}CH_2CH_2SH$). ^{13}C-NMR (CD_3CN, 101 MHz): δ = 42.99 (–, $NHCH_2CH_2SH$), 44.65 (–, $NHCH_2CH_2SH$), 46.18 (–, br, CH_2, cyclen), 46.52 (–, br, CH_2, cyclen), 46.76 (–, br, CH_2, cyclen), 47.09 (–, br, CH_2, cyclen), 166.92 (C_q, triazine), 171.26 and 172.25 (C_q, triazine, tautomers). — MS (ESI^+, H_2O/MeOH + 10 mM NH_4OAc): *m/z* (%) = 343.2 (18) $[M^{4+} - H^+ + AcO^-]^{2+}$, 373.1 (9) $[M^{4+} + 2AcO^-]^{2+}$.

3. General Methods for Vesicle Preparation and Characterization

3.1 Preparation of Functionalizable Luminescent Vesicles

The surface-reactive luminescent vesicles were prepared analogously to formerly established protocols.[1,3,6]

In a small glass vessel, stock solutions of DSPC (2.00 mM in $CHCl_3$, 900 μL), amphiphilic fluorophor **Dans-C_{12}** or **CF-C_{12}** (1.00 mM in $CHCl_3$, 100 μL) and **Mal-C_{16}** (1.00 mM in $CHCl_3$, 100 μL) were mixed to yield a molar ratio of DSPC / fluorophor / **Mal-C_{16}** = 90:5:5. The organic solvent was evaporated under a gentle stream of nitrogen and the remaining film of amphiphiles was dried in high vacuum. Aqueous HEPES buffer solution (25 mM, pH 7.4, 1.00 mL) was added to obtain a total amphiphile concentration of 2.00 mM. The sample was sonicated for 5 min at room temperature resulting in a slightly turbid multilamellar vesicle suspension. A dispersion of unilamellar vesicles was obtained by extrusion through 100 nm pore size polycarbonate membranes with a LiposoFast liposome extruder from Avestin at 60 °C.

3.2 Post-Functionalization of Vesicle Surfaces with Cys-Trp, ETG or Zn-Cyclen$_2$

DSPC vesicles **V-Dans** or **V-CF** containing maleimide **Mal-C_{16}** (5.0 mol-%) and one of the amphiphilic fluorescent dyes **Dans-C_{12}** or **CF-C_{12}** (5.0 mol-% in each case) were prepared. 50–100 μL of the non-functionalized vesicle solution were diluted 5–10-fold with HEPES buffer (25 mM, pH 7.4, 1.00 mL) in a fluorescence cuvette. An appropriate volume of stock solutions of thiols **Cys-Trp** (1.00 mM), **ETG** (5.00 mM) or **Zn-Cyclen$_2$** (1.00 mM) in HEPES buffer was added yielding a thiol concentration of 2.0–7.5 mol-% with respect to the total concentration of the amphiphiles. The reaction mixture was left at room temperature and agitated from time to time. In order to monitor the functionalization process, fluorescence spectra of the vesicle solution were measured after certain time intervals. The surface functionalization reaction was completed after 0.5–2 h.

3.3 Imprinting of Vesicle Surfaces

For imprinting experiments, a stock solution of **Zn-Cyclen$_2$** (1.00 mM, 1.0–2.0 μL, equates to 1.0–2.0 mol-%) in HEPES buffer was added to a stock solution of the template **Pep-P-His** (1.00 mM, 0.5–1.0 μL, corresponds to 0.5 equivalents with respect to the receptor concentration). This mixture was then incubated with the vesicle dispersion **V-CF** (50 μL, diluted 10-fold with 450 μL of HEPES buffer) as described above. After completion of the functionalization reaction, the sample was subjected to size-exclusion chromatography (SEC, see below) in order to remove the template molecules. For control experiments with non-imprinted vesicles, the peptide **Pep-P-His** (same amount as above) was added to the vesicle solution after surface functionalization with **Zn-Cyclen$_2$** and likewise removed by SEC.

3.4 Size-Exclusion Chromatography (SEC) of Vesicles

Sephadex LH-20 gel was swollen in HEPES buffer and placed in a small syringe (1.25 mL gel volume). Excessive buffer solution was removed by centrifugation at 4400 rpm for 30 s. The vesicle solution (500 μL) was then placed on top of the gel bed. The mini column was centrifuged at 2000 rpm for 2 min and the eluate was collected in an Eppendorf tube.

3.5 Aptamer-Functionalization of Vesicle Surfaces

A stock solution of the thiolated aptamer **Apt-Amp** (0.10 mM, 2.5–10 μL, equates to 0.5–2.0 mol-%) was mixed with an aqueous solution of the disulfide reducing agent tris(2-carboxyethyl)phosphine hydrochloride[7] (TCEP, 1.00 mM, 1.25–5.0 μL, corresponds to 5.0 equivalents with respect to the amount of **Apt-Amp**) and left at room temperature for 20 min. This mixture was then added to 25 μL of the freshly prepared vesicle solution **V-CF**, diluted 20-fold with HEPES buffer yielding a total volume of 500 μL, in a fluorescence cuvette. The reaction mixture was left at room temperature and agitated from time to time. In order to monitor the functionalization process, fluorescence spectra of the vesicle solution were measured after certain time intervals. The surface functionalization reaction was completed after approximately 2 h. For determination of the fluorescence response in presence of the target, a stock solution of ampicillin or of control analytes (0.10 mM, 2.0 μL) in HEPES

buffer was added and time-dependent fluorescence spectra were recorded until a stabilization of the emission signal was observed.

3.6 Calculation of Concentration of Surface-Accessible Mal-C_{16}

With the assumption that the membrane-embedded maleimides **Mal-C_{16}** distribute equally between the outer and the inner surface of the vesicles with a radius of *r* nm and a bilayer thickness of 5 nm,[8] the concentration of surface-accessible maleimide was estimated according to the following equation:

$$[M]_{out} = \frac{r^2}{r^2 + (r-5)^2}[M]_{total}$$

3.7 Determination of Reaction Kinetics

Based on the integrated form for the second-order rate law and the assumption of a linear relationship between the concentration of the formed addition product *P* and the change of the fluorescence signal

$$\Delta I_{\max} = \varepsilon[P]_t = \varepsilon([T]_0 - [T]_t),$$

the apparent second-order rate constants k_{obs} were obtained by non-linear curve fitting of the time-dependent fluorescence data according to the following equation:

$$\Delta I_{\max} = \varepsilon \frac{[T]_0[M]_0\left(e^{([T]_0-[M]_0)k_{obs}t} - 1\right)}{[T]_0 e^{([T]_0-[M]_0)k_{obs}t} - [M]_0}$$

where *t*: reaction time

$[T]_0$: initial concentration of thiol

$[M]_0$: initial concentration of surface-accessible **Mal-C_{16}**

3.8 Determination of Binding Constants

A freshly prepared solution of receptor-functionalized vesicles **V-CF-Zn** (500–1000 μL) was filled into a fluorescence cuvette. Aliquots (0.5–5.0 μL) of the analyte stock solutions (0.20–1.00 mM) were added and the fluorescence spectrum was measured after each addition. All fluorescence spectra were corrected for dilution. Binding constants were obtained by non-linear curve fitting (ΔI_{max} *versus* total concentration of added analyte) using the mathematical expressions below.

3.8.1 Vesicular Sensor for Phosphates

On basis of the law of mass action and the assumption of a linear relationship between the concentration of the formed receptor–analyte complex and the change of the fluorescence signal ΔI_{max}, the apparent affinity constants K_a were obtained by non-linear curve fitting of the fluorescence titration data according to the following expression:[9]

$$\Delta I_{max} = \varepsilon \frac{[A]_0 + [R]_0 + 1/K_a - \sqrt{([A]_0 + [R]_0 + 1/K_a)^2 - 4[A]_0[R]_0}}{2}$$

where $[A]_0$: total concentration of analyte

$[R]_0$: total concentration of receptor

3.8.2 Imprinting Studies

For the estimation of the apparent binding constants K_a of the bivalent hexapeptide **Pep-P-His** by non-linear curve fitting, the following Hill equation was used:

$$\Delta I_{max} = \frac{B_{max}[A]_0^n}{1/K_a + [A]_0^n}$$

4. Dynamic Light Scattering (DLS) of Vesicles

Vesicle size distributions were determined by dynamic light scattering. Figure S1 shows the typical size distribution of freshly extruded vesicles.

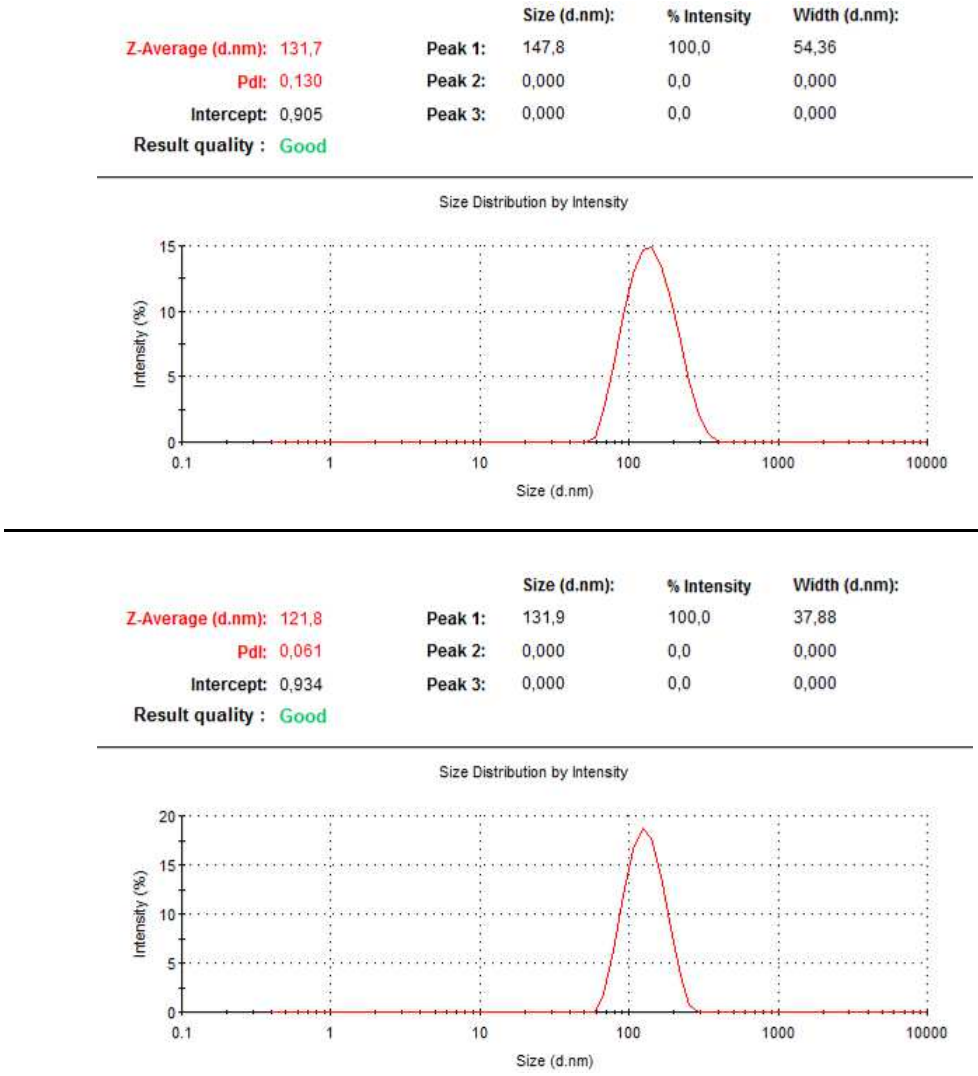


Figure S1: Typical size distribution of freshly prepared vesicles **V-Dans** (top) and **V-CF** (bottom).

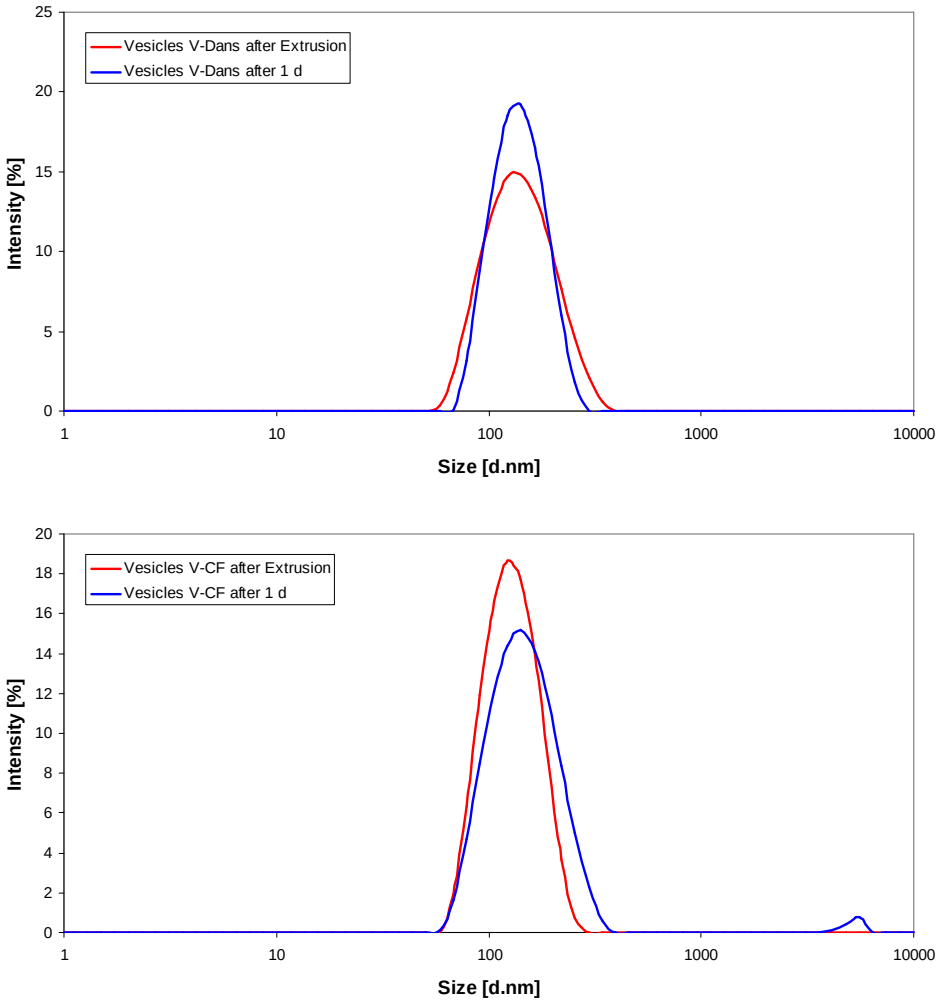


Figure S2: Comparison of size distribution of vesicles **V-Dans** (top) and **V-CF** (bottom) directly after extrusion (red curves) and after one day (blue curves).

5. Surface Functionalization of Vesicles with Cys-Trp or ETG

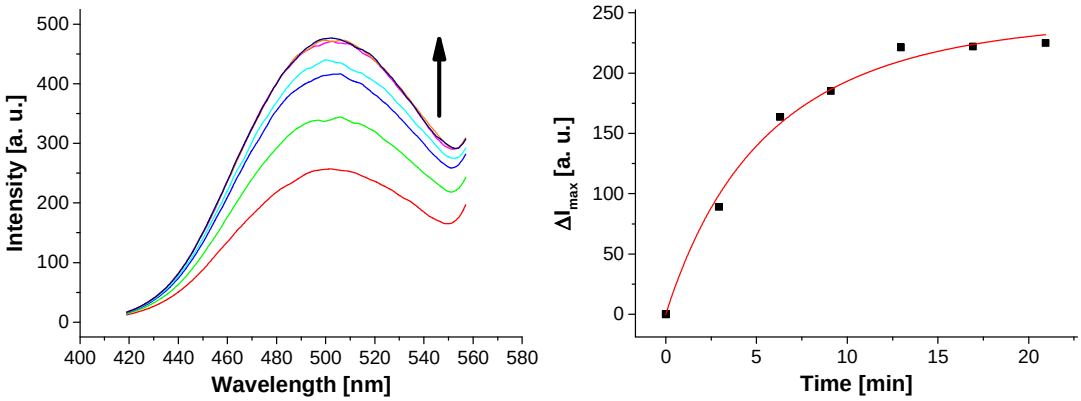


Figure S3: Time-dependent fluorescence spectra of vesicles **V-Dans** after addition of 7.5 mol-% of **ETG**; λ_{ex} = 280 nm.

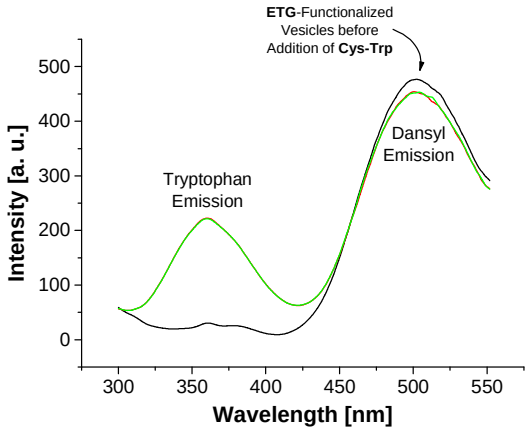


Figure S4: Time-dependent fluorescence spectra (0–7 min) after addition of 5.0 mol-% of peptide **Cys-Trp** to **ETG**-functionalized vesicles **V-Dans** from Figure S3; λ_{ex} = 280 nm.

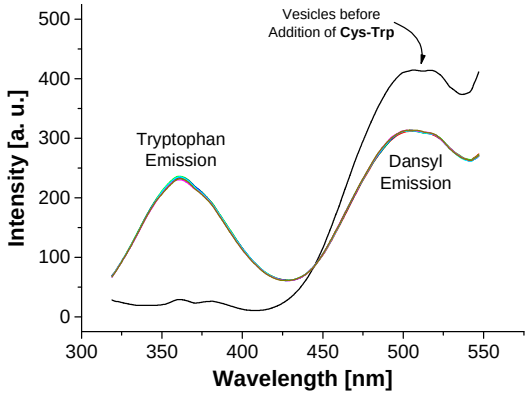


Figure S5: Time-dependent fluorescence spectra (0–17 min) of vesicles **V-Dans** lacking the maleimide **Mal-C_{16}** after addition of 7.5 mol-% of peptide **Cys-Trp**; λ_{ex} = 280 nm.

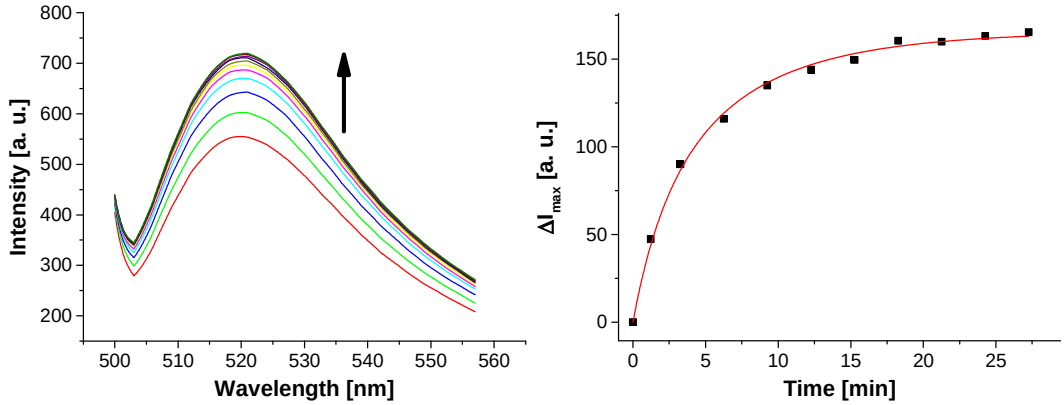


Figure S6: Time-dependent fluorescence spectra of vesicles **V-CF** after addition of 5.0 mol-% of **ETG**; λ_{ex} = 495 nm.

6. Post-Functionalization of Vesicles V-CF with Zn-Cyclen$_2$

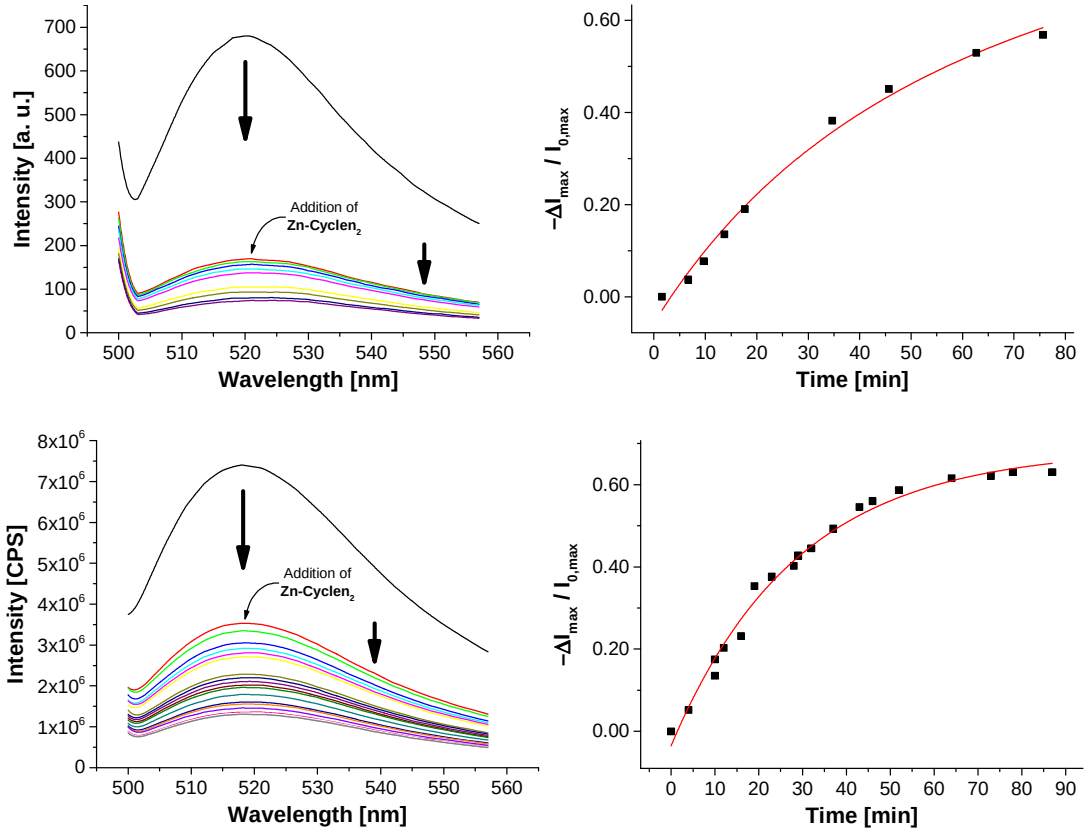


Figure S7: Functionalization progress of vesicles **V-CF** with **Zn-Cyclen$_2$** (top: 5.0 mol-%, bottom: 2.5 mol-%) in absence of PP$_i$; λ_{ex} = 495 nm.

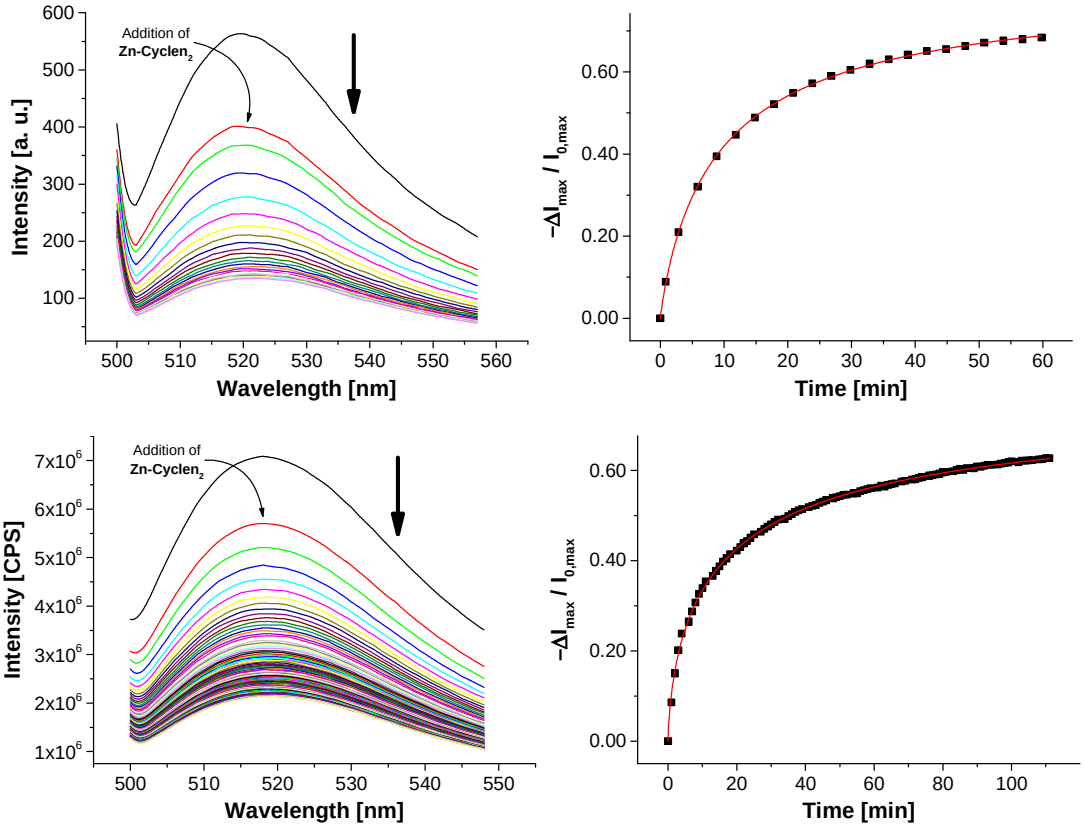


Figure S8: Functionalization progress of vesicles **V-CF** with **Zn-Cyclen$_2$** (top: 5.0 mol-%, bottom: 2.5 mol-%) in presence of PP_i (2.0 equivalents in each case with respect to **Zn-Cyclen$_2$**); λ_{ex} = 495 nm.

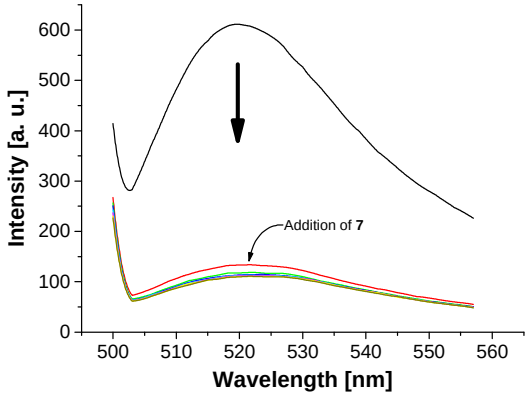


Figure S9: Time-dependent fluorescence response (0–15 min) of **V-CF** after addition of bis-Zn^{2+}-cyclen disulfide **7** (Scheme S2, 2.5 mol-%); λ_{ex} = 495 nm.

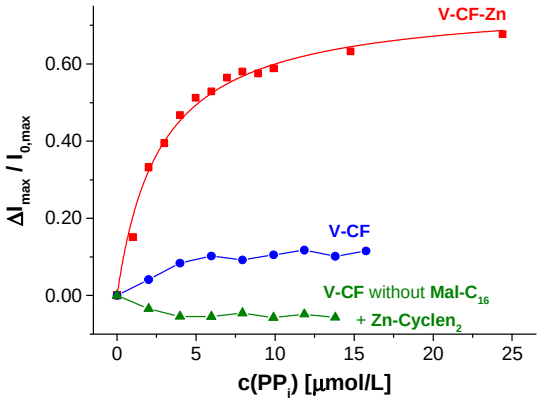


Figure S10: Fluorescence responses of functionalized vesicles **V-CF-Zn** (red squares), non-functionalized vesicles **V-CF** (blue dots) and **Mal-C$_{16}$**-lacking vesicles **V-CF** incubated with 1.5 mol-% of **Zn-Cyclen$_2$** (green triangles) upon titration with PP_i; λ_{ex} = 495 nm.

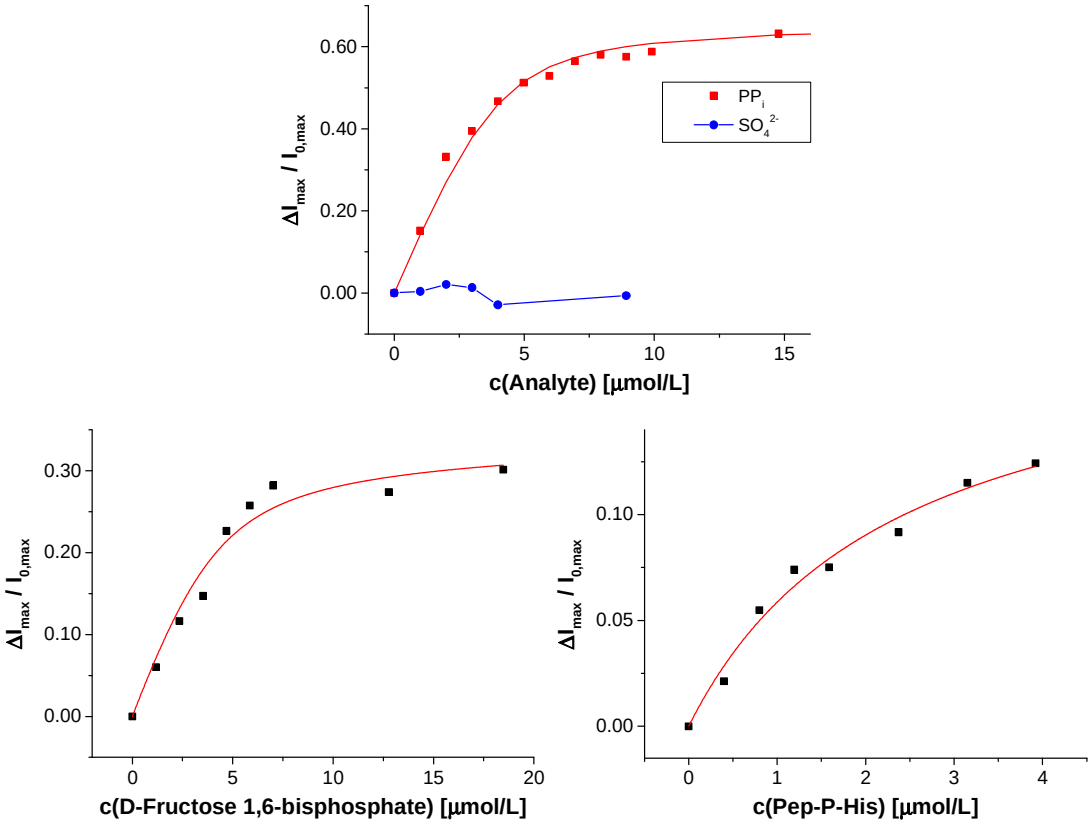


Figure S11: Binding isotherms of **V-CF-Zn** upon addition of different phosphates or Na_2SO_4; λ_{ex} = 495 nm.

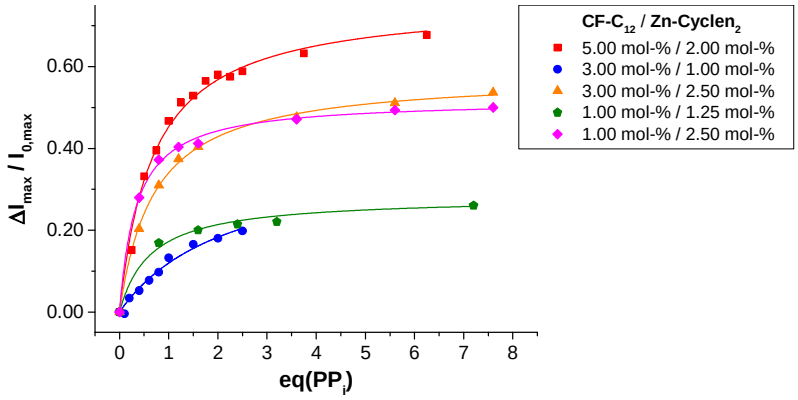


Figure S12: Dependency of the fluorescence response of **V-CF-Zn** after addition of PP_i on the dye and receptor concentrations on the vesicle surface; λ_{ex} = 495 nm.

7. Imprinting of Vesicle Surfaces with Bivalent Peptide Pep-P-His

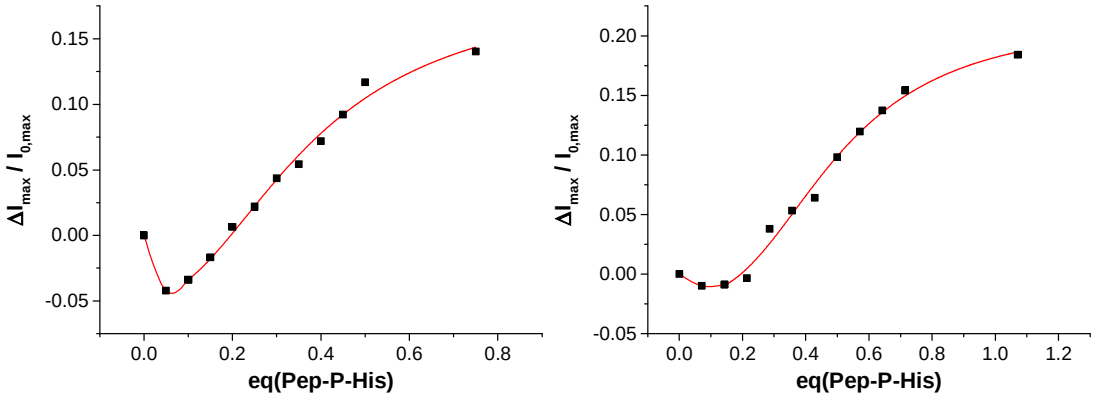


Figure S13: Rebinding isotherms of vesicles **V-CF** functionalized with 0.8 equivalents of **Zn-Cyclen$_2$** receptor (with respect to surface-accessible **Mal-C$_{16}$**) in presence (left) and absence (right) of the template **Pep-P-His**; λ_{ex} = 495 nm.

8. Vesicle Functionalization with an Aptamer for Ampicillin

8.1 Optimization of Buffer System

For the investigation of the stability of non-functionalized vesicles **V-CF**, we prepared vesicle solutions in different buffer systems and determined their size distribution by DLS directly after extrusion (Table S1).

Table S1: DLS measurement results of vesicles **V-CF** in different buffer systems.

Entry	Buffer	Added Salts	Z-Average [nm]	PDI	Peak 1 [nm]	Peak 2 [nm]
1	TRIS (20 mM, pH 8.0)	KCl (5 mM), $MgCl_2$ (5 mM)	208.4	0.274	257.3 (98.0 %)	4946 (2.0 %)
2	TRIS (20 mM, pH 8.0)	NaCl (50 mM), KCl (5 mM), $MgCl_2$ (5 mM)	208.3	0.655	128.9 (54.5 %)	1797 (45.5 %)
3	HEPES (25 mM, pH 7.4)	–	121.8	0.061	131.9 (100 %)	–
4	HEPES (25 mM, pH 7.4)	$MgCl_2$ (5 mM)	271.2	0.562	133.2 (41.7 %)	825.1 (58.3 %)
5	HEPES (25 mM, pH 7.4)	KCl (5 mM), $MgCl_2$ (5 mM)	185.6	0.212	213.9 (97.9 %)	4829 (2.1 %)
6	HEPES (25 mM, pH 7.4)	NaCl (50 mM), KCl (5 mM), $MgCl_2$ (5 mM)	755.3	0.861	505.6 (95.7 %)	5560 (4.3 %)

The best stability of vesicles was observed in HEPES buffer without added salts. Under those conditions however, no fluorescence response was observed after addition of ampicillin to aptamer-functionalized vesicles **V-CF-Amp** (Table S2). This result shows that the presence of certain cations is essential for the ability of the aptamer to bind to ampicillin. In buffer systems which resulted in significant aggregation of the vesicles, also non-specific fluorescence responses of aptamer-functionalized vesicles upon addition of the control analyte benzylpenicillin was obtained.

Table S2: Relative fluorescence response and specificity of **V-CF-Amp** after addition of ampicillin or benzylpenicillin (0.40 μM in each case).

Entry	Buffer	Added Salts	Ampicillin	Benzyl-penicillin
1	TRIS (20 mM, pH 8.0)	KCl (5 mM), $MgCl_2$ (5 mM)	3 %	n. d.
2	TRIS (20 mM, pH 8.0)	NaCl (50 mM), KCl (5 mM), $MgCl_2$ (5 mM)	17 %	6 %
3	HEPES (25 mM, pH 7.4)	–	3 %	n. d.
4	HEPES (25 mM, pH 7.4)	$MgCl_2$ (5 mM)	9 %	5 %
5	HEPES (25 mM, pH 7.4)	KCl (5 mM), $MgCl_2$ (5 mM)	50 %	–2 %
6	HEPES (25 mM, pH 7.4)	NaCl (50 mM), KCl (5 mM), $MgCl_2$ (5 mM)	no response	n. d.

n. d.: not determined

8.2 Variation of Aptamer Concentration on the Surface

For comparison to vesicles **V-CF** functionalized with 2.0 mol-% of the aptamer **Apt-Amp**, we also prepared vesicles with 0.5 mol-% of **Apt-Amp** on the surface and investigated their fluorescence response after addition of ampicillin (Figure S14).

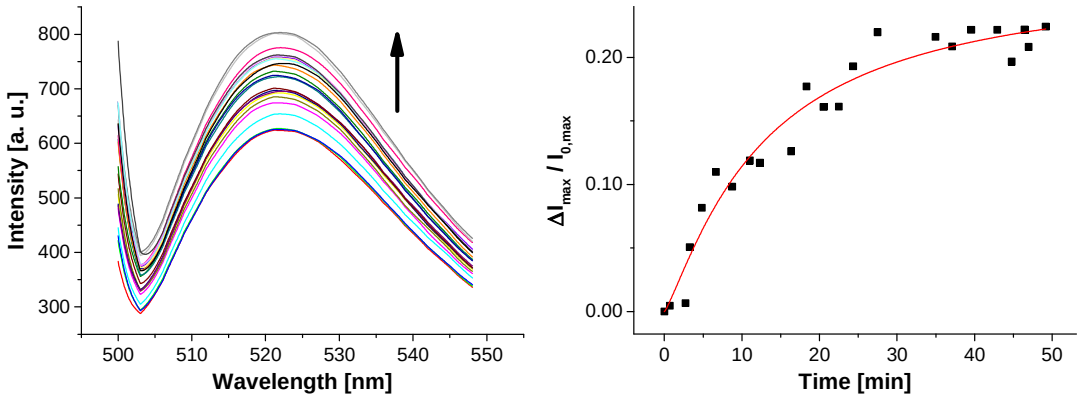


Figure S14: Time-dependent change of fluorescence emission of aptamer-functionalized vesicles **V-CF-Amp** (0.5 mol-% of **Apt-Amp**) after addition of ampicillin (0.40 μM); λ_{ex} = 495 nm.

8.3 Vesicle Size Distribution after Aptamer-Functionalization and Addition of Ampicillin

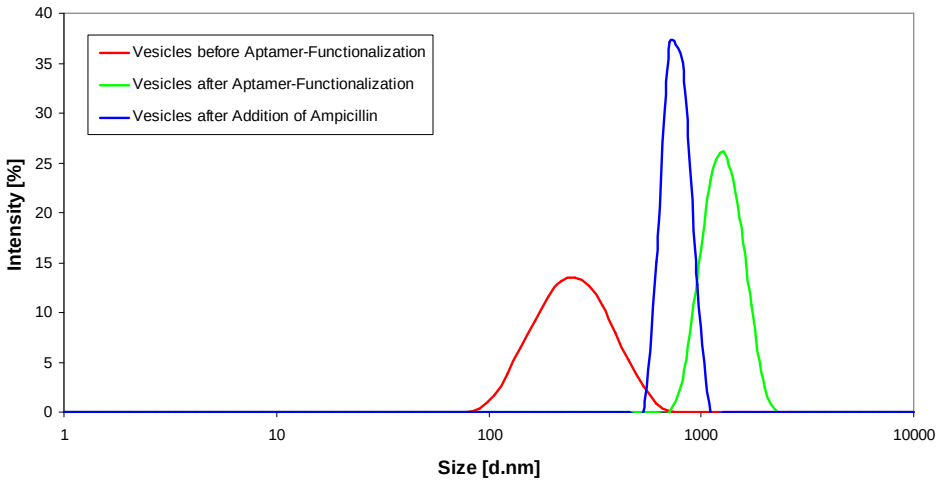


Figure S15: Comparison of vesicle size distribution before aptamer-functionalization of **V-CF** (red curve), after functionalization with **Apt-Amp** (green curve) and after addition of ampicillin to functionalized liposomes **V-CF-Amp** (blue curve).

9. References

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