Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supplementary Information

Adamantylglycine as a High-Affinity Peptide Label for Membrane Transport Monitoring and Regulation

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Materials and Instrumentation

Materials

Reagents for buffer preparation and analytical measurements were from Fluka or Fisher Scientific and of highest purity available. Phospholipids were from Avanti Polar Lipids (Alabaster, AL, USA). Deuterated solvents were from Deutero GmbH (Kastellaun, Germany). Cucurbit[7]uril (CB7)[1] and *p*-sulfonatocalix[4]arene tetrapentylether (CX4-C5)[2] were synthesized according to literature methods. (*S*)-adamantylglycine was obtained from Ark Pharm Inc. (IL, USA), converted into the Fmoc-derivative as described in the Experimental Section, and then incorporated into the Ada-R7 peptide by commercial solid-phase peptide synthesis (Biosyntan, Berlin, Germany). Heptaarginine was from Bachem (Bubendorf, Swizerland) and Phe-R7 was from custom-synthesized by GL Biochem (Shanghai, China). All commercial and custom-synthesized peptides had a purity >95% as confirmed by HPLC analysis; the identity of the peptides was confirmed by MS by the supplier. Millipore water was prepared by a Merck Millipore Simplicity UV water purification system providing ultrapure water Type 1 with ultra-low TOC levels (≥18.2 MΩ cm, ≤5 ppb TOC). Size exclusion chromatography (SEC) was performed with Cytiva Illustra NAP-25 columns.

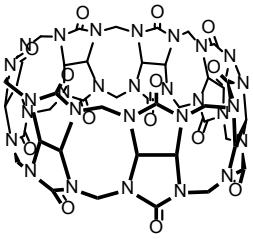
Instrumentation

^{1}H and ^{13}C NMR spectra were measured on a JEOL ECX 400 spectrometer or on a Bruker AMX 500 MHz NMR. Dynamic light scattering (DLS) was performed on a Malvern Zetasizer Nano-S (Kassel, Germany). Isothermal titration calorimetry (ITC) experiments were carried out with a MicroCal VP-ITC (Northhampton, MA, USA). Fluorescence measurements were performed on a Jasco FP-8300 spectrofluorimeter and UV/VIS absorption measurements were performed on a Jasco V-750 spectrophotometer (Pfungstadt, Germany); both instruments were equipped with a Peltier temperature controller and magnetic stirrer. pH measurements were performed with a WTW pH 526 pH meter with a WTW SenTix Mic electrode.

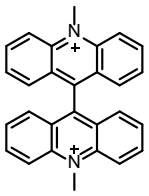
Abbreviations

Ada: (*S*)-2-(adamantan-1-yl)-2-aminoacetic acid (adamantylglycine); CB7: cucurbit[7]uril; CF: 5(6)-carboxyfluorescein; CX4: *p*-sulfonatocalix[4]arene; CX4-C5: *p*-sulfonatocalix[4]arene tetrapentylether; DLS: dynamic light scattering; ITC: isothermal titration calorimetry; LCG: lucigenin; NMR: nuclear magnetic resonance spectroscopy; Phe: L-phenylalanine; PXD: *p*-xylylenediamine; SEC: size exclusion chromatography; TOC: total organic carbon; TX-100: triton X-100

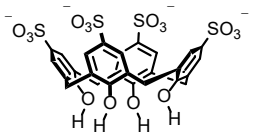
Structure Overview



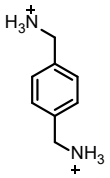
cucurbit[7]uril
(CB7)



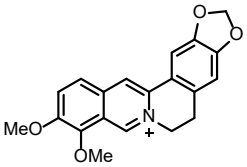
lucigenin
(LCG)



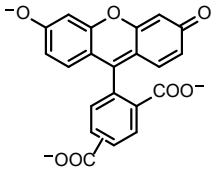
p-sulfonatocalix[4]arene
(CX4)



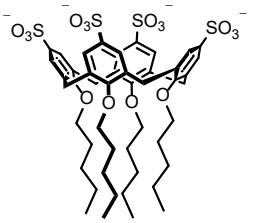
p-xylelene diamine
(PXD)



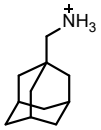
berberin (BE)



5(6)-carboxyfluorescein
(CF)



p-sulfonatocalix[4]arene
pentylether (CX4-C5)



aminomethyladamantane
(AMADA)

Chart S1.

Experimental Section

Synthesis

(*S*)-adamantylglycine (Ada)[3] was converted into its Fmoc derivative by reaction with Fmoc-OSu. Ada-R7 as well as the other peptides were synthesized by Fmoc-based solid phase peptide synthesis.

Fmoc-Ada-OH: ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.76 (d, J = 7.4 Hz, 2H, Ar-H), 7.60 (d, J = 7.4 Hz, 2H, Ar-H), 7.46-7.28 (m, 4H, Ar-H), 4.62-4.33 (m, 2H, CH_2), 4.23 (t, 1H, CH), 4.09 (d, 1H, CH-N). 1.99 (s, 1H, Ad-CH), 1.83-1.49 (m, 12H, Ad-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 175.7, 156.4, 144.0, 141.5, 127.9, 127.2, 125.2, 120.1, 67.1, 62.9, 47.4, 38.7, 36.7, 28.4.

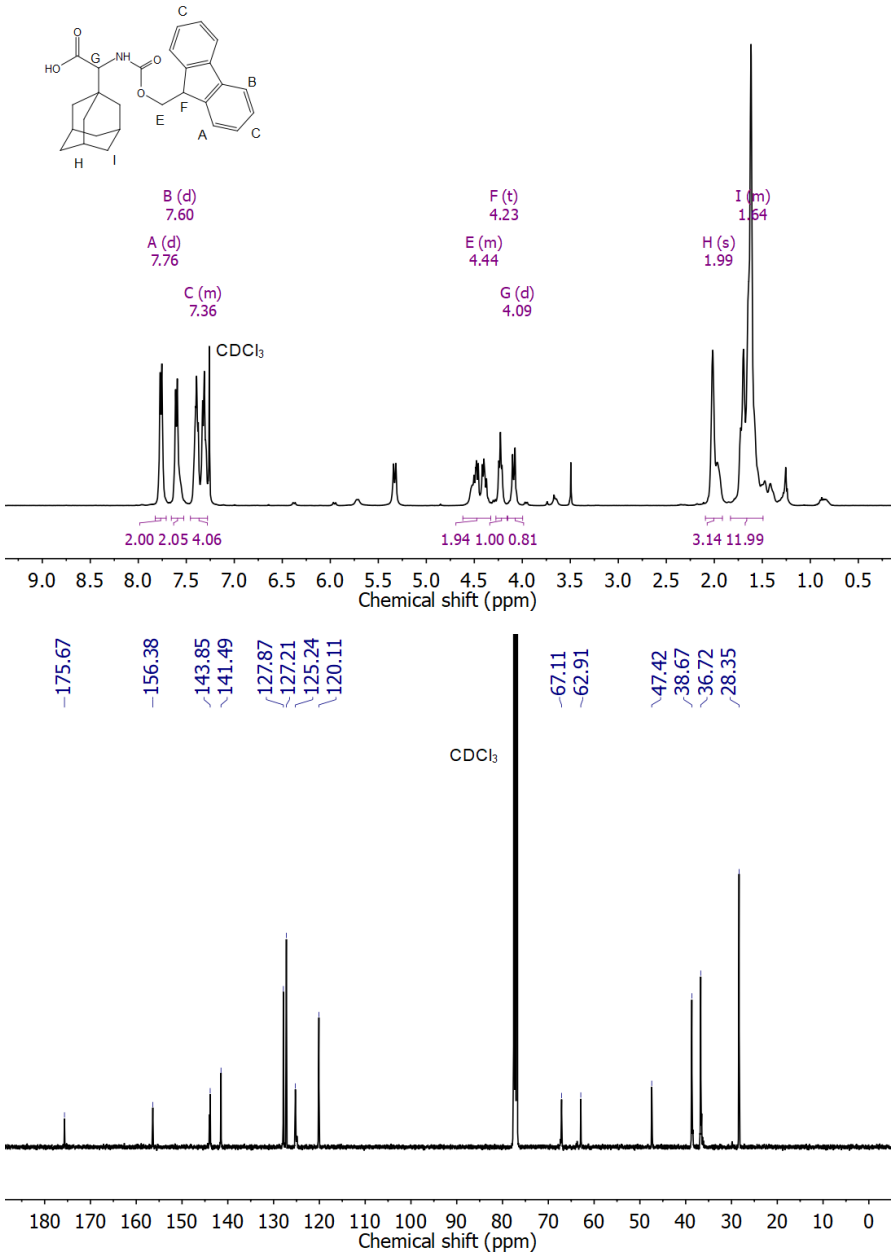


Figure S1. ^{1}H and ^{13}C NMR of Fmoc-Ada-OH.

Preparation of Stock Solutions

The buffer for all transport experiments and fluorescence titrations was 10 mM NaH_2PO_4, pH 7.2, which was prepared by dissolving the required amount of NaH_2PO_4 in Millipore water and adjustment of the pH to 7.20±0.01 by addition of NaOH(aq).

Peptide stock solutions of Ada-R7, Phe-R7, and R7 were prepared in Millipore water at ca. 1 mM and the concentrations of the peptide stock solutions were determined by measuring the absorbance at 214 nm and considering their respective extinction coefficient (Table S1).

CB7 concentrations in commercial and self-synthesized samples were determined by ^{1}H NMR using maleic acid as a standard in D_2O, pD 7.4 or by titration with cobaltocenium hexafluorophosphate.[4]

For CX4-C5, a stock solution of 2 mM was prepared in DMSO and subsequently diluted to 80 μM in the respective buffer, which was then added to the cuvette during the transport experiment (total dilution factor = 2500). The final DMSO concentration (0.4%) had no influence on the transport experiments.

Table S1. Molar extinction coefficient of peptides.[a)]

Peptide	ε_{214} (M^{-1} cm^{-1})				
	peptide bonds	Arg side chains	Phe side chain	Ada side chain	Σ
R7	6 × 923	7 × 102			6252
Phe-R7	7 × 923	7 × 102	1 × 5200		12375
Ada-R7	7 × 923	7 × 102		1 × 45 [b)]	7220

a) Theoretical values according to ref. [5]. b) Published value for leucine and isoleucine.

Preparation of Large Unilamellar Vesicles (LUVs)

POPC/POPS(9:1)⊃CX4/LCG-LUVs.

A thin lipid film was prepared by mixing 100 µl of 25 mg/ml POPC in $CHCl_3$ and 33 µl of 10 mg/ml POPS in $CHCl_3$ and purging with nitrogen. The lipid film was subsequently dried overnight in high vacuum and then rehydrated with 1 ml 10 mM NaH_2PO_4, 700 µM CX4, 500 µM LCG, pH 7.2 by gentle agitation for 30 min with a rotary evaporator. Then, 15 freeze-thaw cycles (freezing in liquid N_2 and thawing in a water bath at 40 °C) were performed to down-size the multilamellar vesicles. Extravesicular components were removed by size exclusion chromatography using a NAP-25 column and elution with 10 mM NaH_2PO_4, pH 7.2.

POPC/POPS(9:1) ⊃CB7/BE-LUVs

A thin lipid film was prepared by mixing 100 µL of 25 mg/ml POPC in $CHCl_3$ and 33 µL of 10 mg/ml POPS in $CHCl_3$ and purging with nitrogen. The lipid film was subsequently dried overnight in high vacuum and then rehydrated with 1 ml 10 mM NaH_2PO_4, 300 µM CB7, 500 µM BE, pH 7.2 by gentle agitation for 30 min with a rotary evaporator. Then, 20 freeze-thaw cycles (freezing in liquid N_2 and thawing in a water bath at 40 °C) were performed to down-size the multilamellar vesicles. Extravesicular components were removed by size exclusion chromatography using a NAP-25 column and elution with 10 mM NaH_2PO_4, pH 7.2.

POPC/POPS(9:1)⊃CF LUVs

A thin lipid film was prepared by mixing 100 µL of 25 mg/ml POPC in $CHCl_3$ and 33 µL of 10 mg/ml POPS in $CHCl_3$ and purging with nitrogen. The lipid film was subsequently dried overnight in high vacuum and then rehydrated with 1 ml of 10 mM NaH_2PO_4, 10 mM KCl, 50 mM CF, pH 7.2 by gentle agitation for 30 min with a rotary evaporator. Then, 5 freeze-thaw cycles (freezing in liquid N_2 and thawing in a water bath at 40 °C) were performed and the solution was 15 times extruded through a polycarbonate membrane (100 nm pore size) to down-size the multilamellar vesicles. Extravesicular components were removed by size exclusion chromatography using a NAP-25 column and elution with 10 mM NaH_2PO_4, 107 mM KCl, pH 7.2.

Quality Control of LUV Preparations

Vesicle Size

The size distribution of the liposomes was determined by dynamic light scattering (DLS).

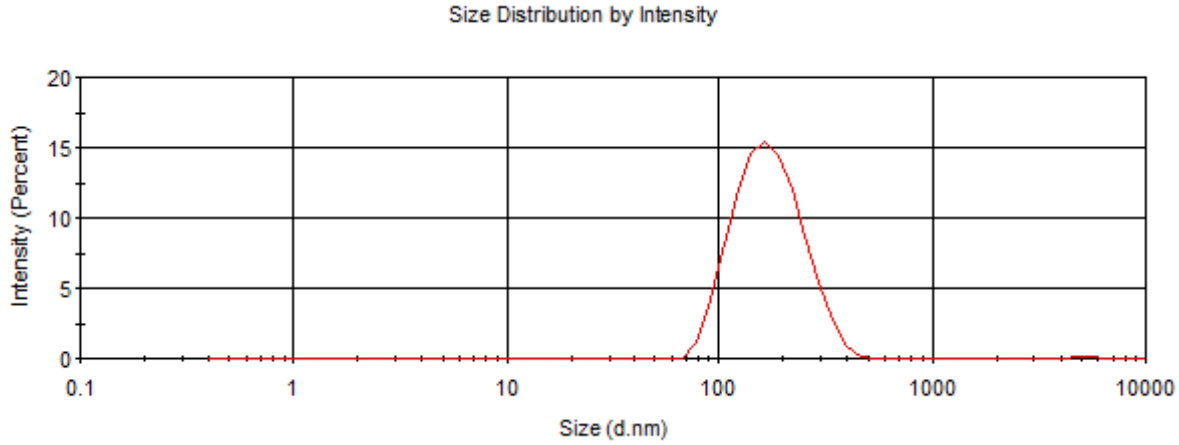


Figure S2. DLS analysis of CB7/BE⊃POPC/POPS-LUVs with an average diameter of 162 nm in 10 mM sodium phosphate buffer, pH 7.2 at 25 °C.

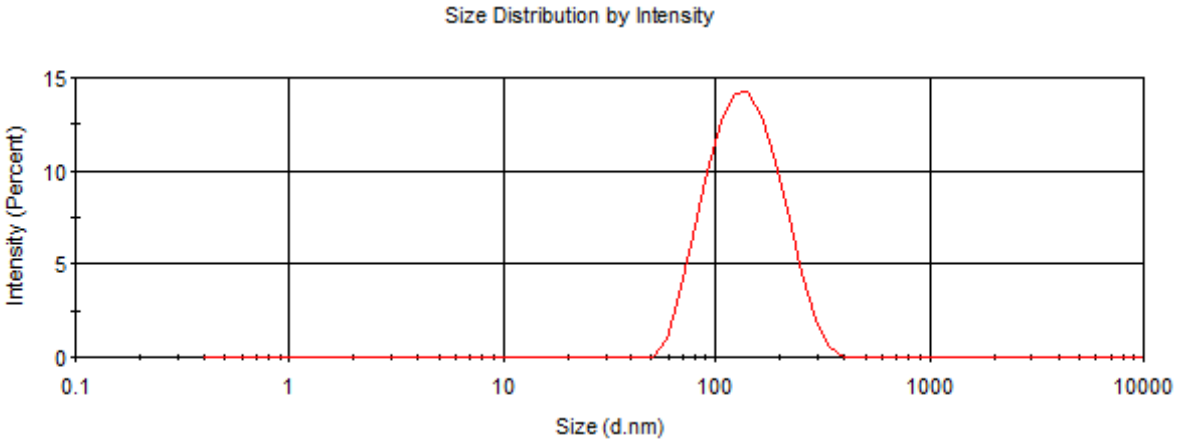


Figure S3. DLS analysis of CX4/LCG⊃POPC/POPS-LUVs with an average diameter of 128 nm in 10 mM sodium phosphate buffer, pH 7.2 at 25 °C.

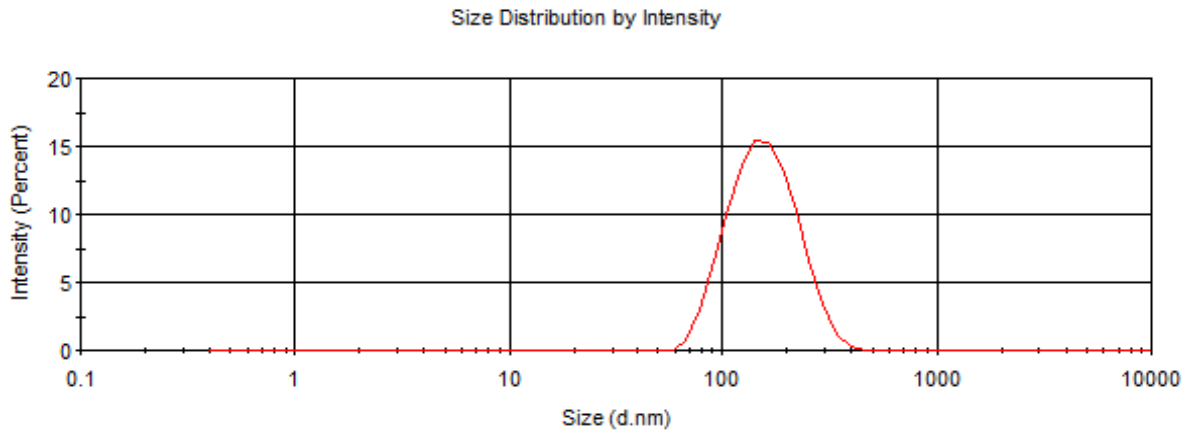


Figure S4. DLS analysis of CF⊃POPC/POPS-LUVs with an average diameter of 146 nm in 10 mM sodium phosphate buffer, pH 7.2 at 25 °C.

Absence of Extravesicular Reporter Pair

To ensure absence of extravesicular reporter pairs, 10 µM R7 (for the CX4/LCG) or 10 µM cadaverine (for the CB7/BE assay) were added, which are established non-permeable guests in absence of counterion activators.[6]

Phospholipid Concentrations

Phospholipid concentrations of the liposome suspensions were determined by ^{1}H NMR.[7] In brief, 50 µL of the liposome stock solution were mixed with 430 µL CD_3OD, 100 µL $CDCl_3$, and 10 µL of a 5 mM stock solution of 3-(trimethylsilyl)propionic-2,2,3,3-d4 acid (TMSP) in D_2O. The solution was transferred into an NMR tube and a ^{1}H NMR spectrum was recorded (64 scans, 500 MHz). Subsequently, the liposome peak at 0.88 ppm was integrated, which is assigned to the terminal methyl groups on the fatty acid side chains of the phospholipids. The phospholipid concentration of the liposome stock solution, c_{PL}, was then calculated by

$$c_{PL} = \frac{3\, c_{st} V_{st} I_{PL}}{2\, V_{PL} I_{st}}$$

where c_{st} is the concentration of the standard in the stock solution (here: 5 mM), V_{st} is the volume of the standard (here: 10 µL), V_{PL} is the volume of the liposome stock solution (here: 50 µL), and I_{st} and I_{PL} are the integrated peak areas of the standard at 0 ppm and the phospholipids at 0.88 ppm, respectively. Phospholipid stock concentrations were in the range of 1.8 to 3.1 mM and phospholipid concentrations during the measurements were ca. 20 µM.

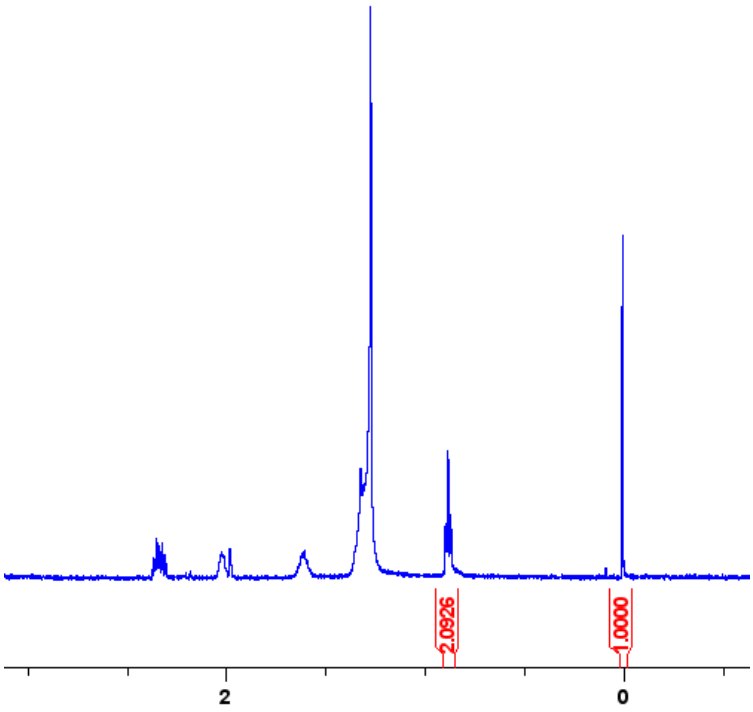


Figure S5. ^{1}H NMR of 50 µL liposomes mixed with CD_3OD (430 µL), $CDCl_3$ (100 µL) and TMSP (reference, 10 µL of 5 mM stock in D_2O), measured at 500 MHz, with 64 scans and water suppression.

Determination of Binding Constants

Fluorescence Titrations

Binding titrations with host-dye pairs and competitive binding titrations were performed in 10×10 mm quartz glass cuvettes at sufficiently low dye concentrations to avoid the inner-filter effect. Here, 1 µM BE and 0.75 µM CB7 or 0.5 µM LCG and 1 µM CX4 in 10 mM NaH_2PO_4, pH 7.2, 25 °C were used for displacement titrations. The data was analysed using Origin (OriginLab Corporation, Northhampton, MA) as previously described.[8]

ITC

ITC titrations were performed as competitive titrations at 25 °C in 10 mM *p*-xylelenediamine, 0.25 mM CB7 in H_2O, adjusted to pH 7.0 by addition of HCl. As titrant, a peptide stock solution in H_2O, adjusted to pH 7.0 was used. The solutions were degassed and thermostatted by a ThermoVac accessory for ITC experiments. Fitting was performed using the "one-site binding model" in the Origin software provided by the instrument manufacturer.

NMR

For competitive ^1H NMR titrations, Ada-R7, PXD, and CB7 stock solutions were prepared in D_2O and the pD was adjusted to pD 7.4 by addition of NaOD or DCl and pD was also controlled after combining stock solutions for measurements. For conversion of the pH value of the pH meter to pD values, the known[9] relation (pD = pH + 0.4) was used. NMR spectra were subsequently analysed and integrated as described previously using Bruker Topspin V4.30.[10]

Transport Assays

An appropriate volume of the liposome stock solutions was diluted to 2000 µL with 10 mM NaH_2PO_4, pH 7.2 in a 10×10 mm quartz glass cuvette. The cuvette was placed into the fluorimeter sample holder and gently stirred at 300 rpm at (25.0±0.1) °C. Subsequently, the time-dependent change in fluorescence intensity (CB7/BE: $\lambda_{exc/em}$ = 425/495 nm; CX4/LCG: $\lambda_{exc/em}$ = 369/502 nm; CF: $\lambda_{exc/em}$ = 492/517 nm) was recorded during addition of 20 µL 80 µM CX4-C5 in buffer with 4% DMSO at 60 s (final concentration: 0.8 µM and 0.4% DMSO) and 20 µL of varying concentrations of peptide in H_2O at 120 s. At the end of each experiment, 20 µL of a calibration cocktail was added for calibration to afford 1.2% triton X-100 (TX-100) and 5.4 µM protamine at 800 s in the CX4/LCG assay, 25 µM adamantylamine in the CB7/BE assay and 1.2% TX-100 at 540 s in the CF assay.

Time-dependent fluorescence intensity traces, $I(t)$, were subsequently normalized to fractional intensities, $I_f(t)$, using equation (1) for the CF and CX4/LCG assay and equation (2) for the CB7/BE assay. Therein, I_0 is the intensity just before addition of peptide and I_∞ is the constant intensity after addition of the calibration cocktail.

CF and CX4/LCG assay: $$I_f(t) = \frac{I(t) - I_0}{I_\infty - I_0} \quad (1)$$

CB7/BE assay: $$I_f(t) = \frac{I(t) - I_\infty}{I_0 - I_\infty} \quad (2)$$

The concentration-dependent fractional intensities just before the addition of the calibration cocktail, I_f, were then normalized to fractional membrane activities, Y, using equation (3) for the CF and CX4/LCG assay and to the fractional displacement (in percent), $\%D$, using equation (4) for the CB7/BE assay. Therein, $I_{f,0}$ is the fractional intensity just before addition of the calibration cocktail in absence of peptide and $I_{f,\infty}$ is the constant fractional intensity after addition of the calibration cocktail.

CF and CX4/LCG assay: $$Y = \frac{I_f - I_{f,0}}{I_{f,\infty} - I_{f,0}} \quad (3)$$

CB7/BE assay: $$\%D = \frac{I_f - I_{f,0}}{I_{f,\infty} - I_{f,0}} \times 100 \quad (4)$$

The fractional membrane activities, Y, or fractional displacement, $\%D$, were subsequently plotted against the peptide concentration, c, and analysed using the Hill equations (5) and (6) to obtain the effective molar concentration, EC_{50}, and the Hill coefficient, n. Y_0 (or D_0) and Y_{max} (or D_{max}) are the fractional membrane activities or displacements in absence of peptide and at maximal peptide concentrations.

CF and CX4/LCG assay: $$Y = Y_0 + \frac{Y_{max} - Y_0}{1 + \left(\frac{c}{EC_{50}}\right)^n} \quad (5)$$

CB7/BE assay: $$\%D = D_0 + \frac{D_{max} - D_0}{1 + \left(\frac{c}{EC_{50}}\right)^n} \quad (6)$$

Determination of Binding Constants

Host-Dye Binding Constants

CX4/LCG

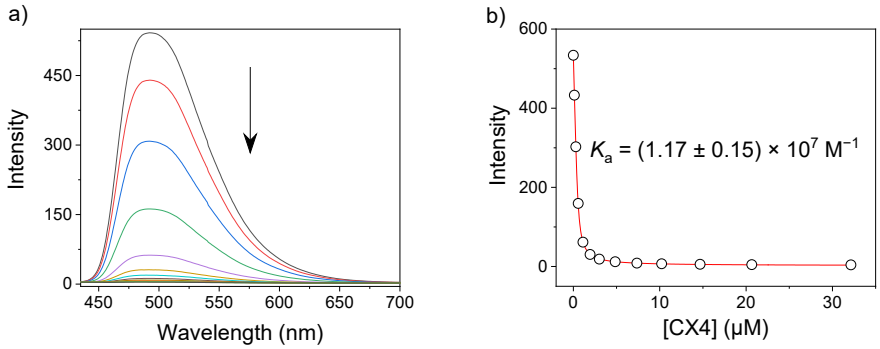


Figure S6. Binding titration of 0.5 μM LCG with CX4 in 10 mM NaH_2PO_4, pH 7.2. a) Fluorescence spectral changes (λ_{exc} = 369 nm). b) Respective titration curve (λ_{em} = 502 nm) with fitted line according to a 1:1 host-guest binding isotherm.

CB7/BE

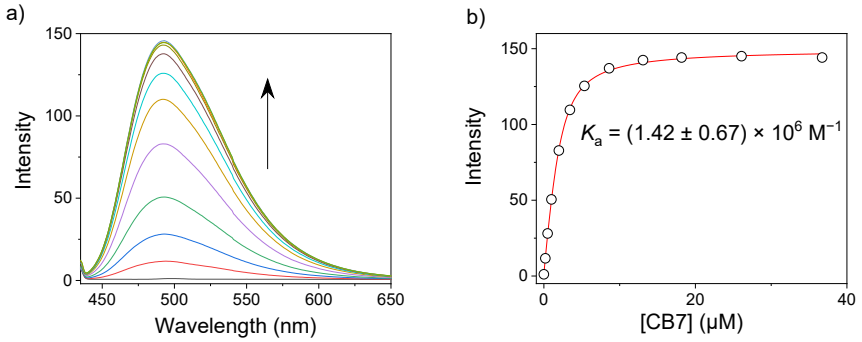


Figure S7. Binding titration of 2 μM BE with CB7 in 10 mM NaH_2PO_4, pH 7.2. a) Fluorescence spectral changes (λ_{exc} = 425 nm). b) Respective titration curve (λ_{em} = 495 nm) with fitted line according to a 1:1 host-guest binding isotherm.

CB7/PLM

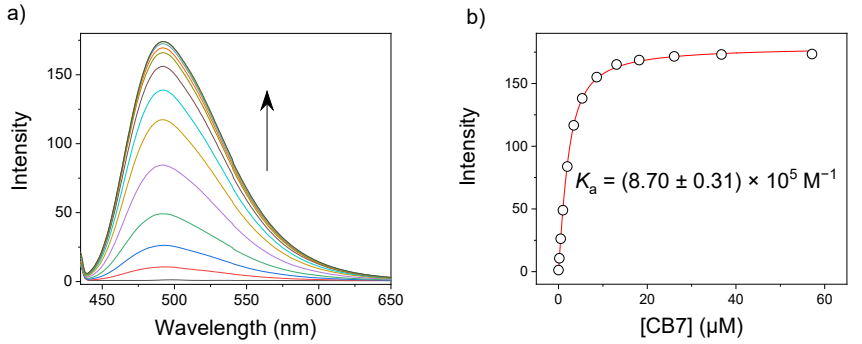


Figure S8. Binding titration of 2 μM PLM with CB7 in 10 mM NaH_2PO_4, pH 7.2. a) Fluorescence spectral changes (λ_{exc} = 425 nm). b) Respective titration curve (λ_{em} = 495 nm) with fitted line according to a 1:1 host-guest binding isotherm.

Ada-(Arg)$_7$-OH (Ada-R7)

Ada-R7 with CB7

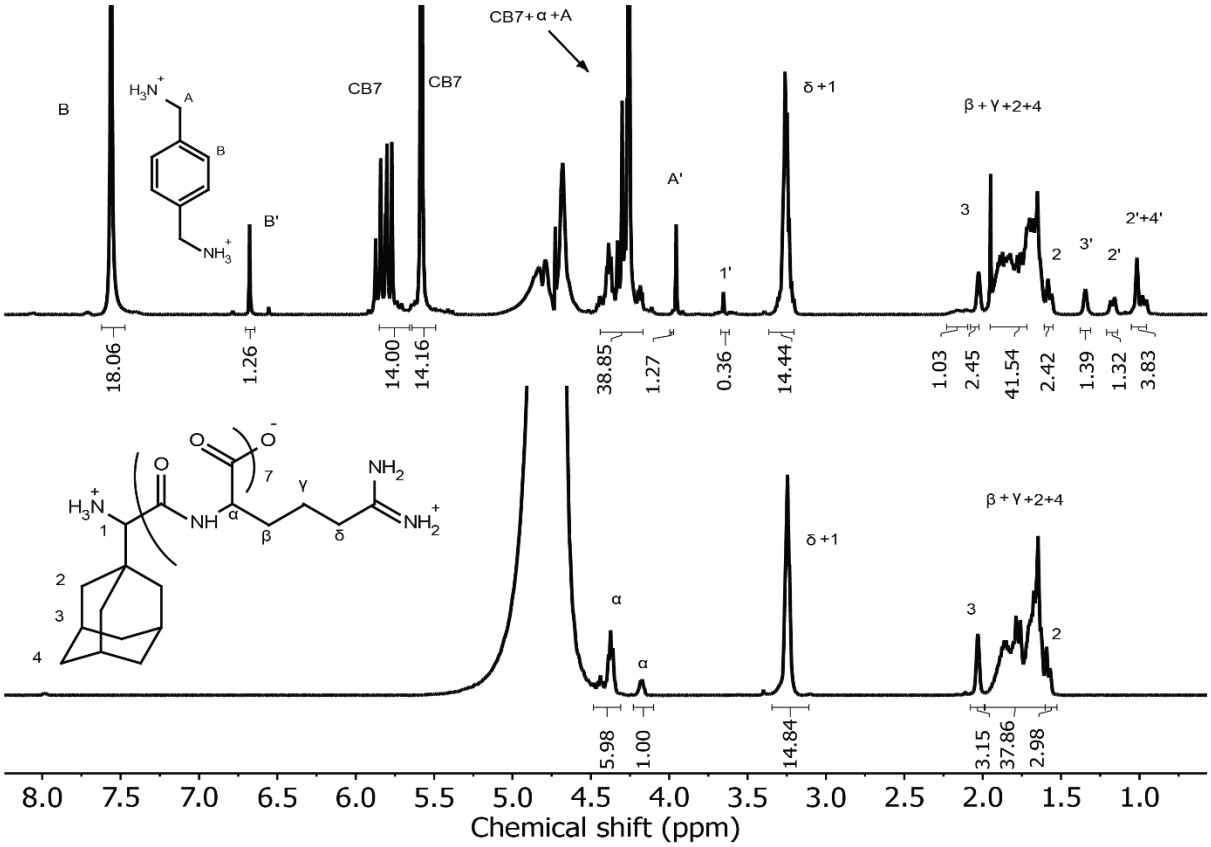


Figure S9. a) Ada-R7 (0.56 mM) in presence of CB7 (0.5 mM) and PXD (2.5 mM) after 10 days and b) ^{1}H NMR spectrum of Ada-R7 (1.06 mM). Both spectra were measured in D_2O, pD 7.4 at 25 °C.

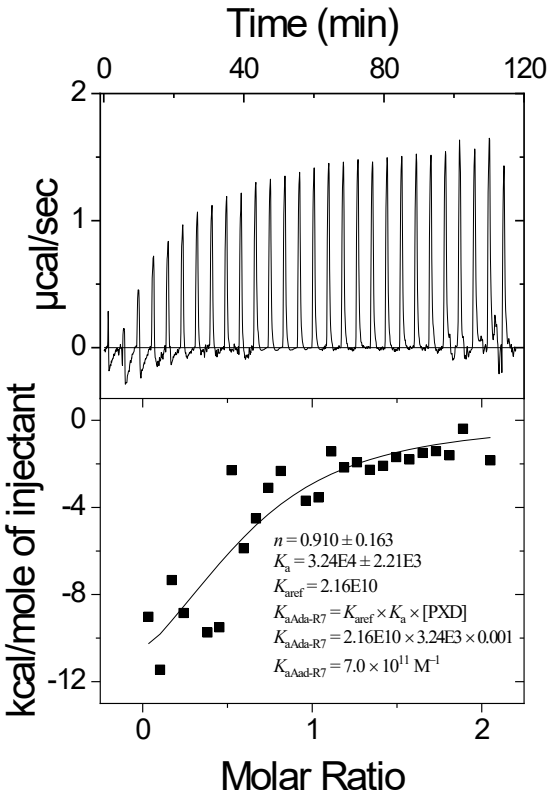


Figure S10. ITC competitive titration of CB7 (0.1 mM) with varying concentration of Ada-R7 in presence of 1 mM PXD in Millipore water, pH 7.0 at 25 °C. The binding constant (K_a) in the lower panel was fitted after subtraction of the heat absorbed by dilution of Ada-R7 into water.

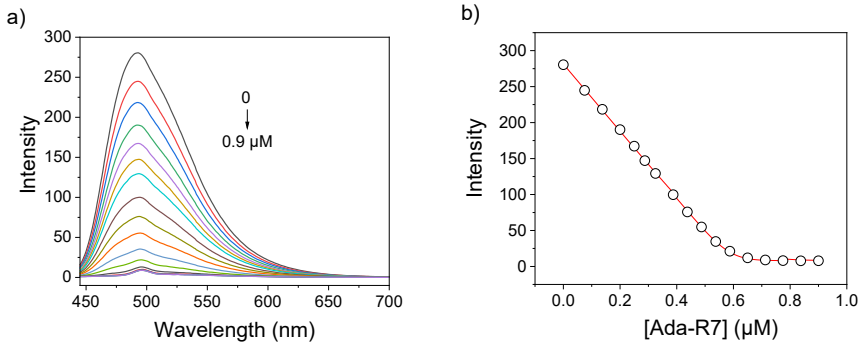


Figure S11. Competitive fluorescence titration of Ada-R7 with CB7/BE in 10 mM NaH_2PO_4, pH 7.2. a) Fluorescence spectra (λ_{exc} = 425 nm) of BE (1 µM) in presence of 0.6 µM CB7 and varying concentrations of Ada-R7. b) Change in fluorescence intensity of BE (λ_{em} = 495 nm) with varying concentrations of Ada-R7.

Ada-R7 with CX4

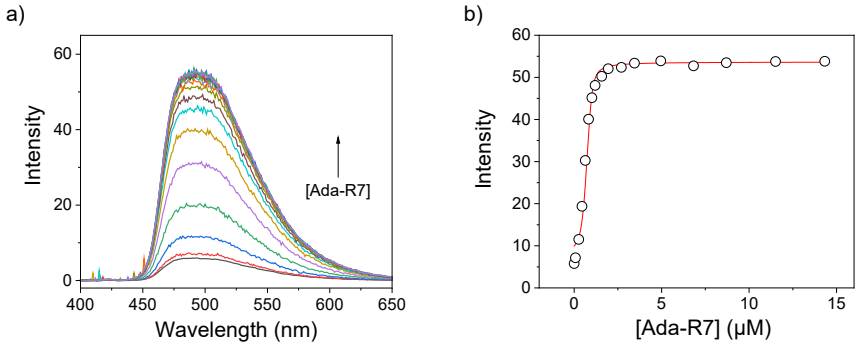


Figure S12. a) Fluorescence spectra of LCG (0.5 μM) in presence of CX4 (1 μM) and varying concentration of Ada-R7. b) Change in fluorescence intensity of LCG at 502 nm with varying concentration of Ada-R7 (Excitation at 369 nm and in 10 mM sodium phosphate buffer of pH 7.2).

Heptaarginine (R7)

R7 with CB7

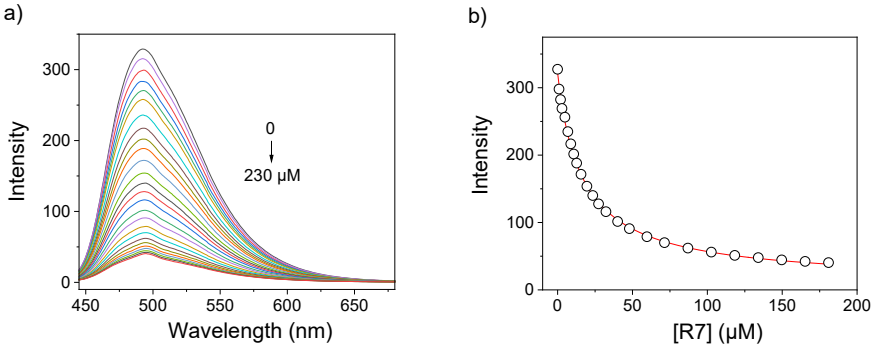


Figure S13. Competitive fluorescence titration of R7 with CB7/BE in 10 mM NaH_2PO_4, pH 7.2. a) Fluorescence spectra (λ_{exc} = 425 nm) of BE (1 μM) in presence of CB7 (0.75 μM) and varying concentrations of R7. b) Change in fluorescence intensity of BE (λ_{em} = 495 nm) with varying concentration of R7.

R7 with CX4

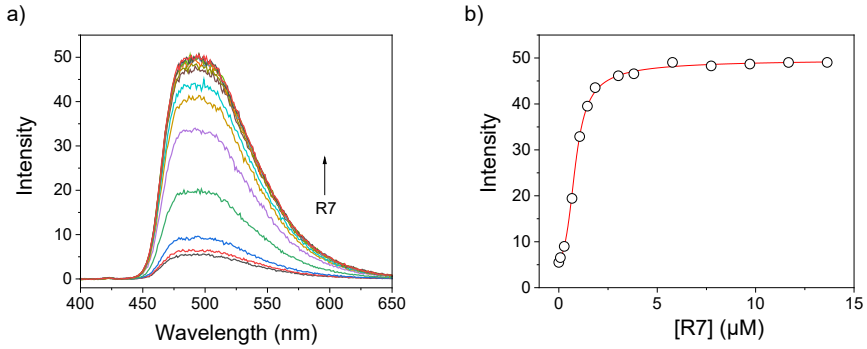


Figure S14. Competitive fluorescence titration of R7 with CX4/LCG in 10 mM NaH_2PO_4, pH 7.2. a) Fluorescence spectra (λ_{exc} = 369 nm) of LCG (0.5 μM) in presence of CX4 (1 μM) and varying concentrations of R7. b) Change in fluorescence intensity of LCG (λ_{em} = 502 nm) with varying concentration of R7.

Phe-(Arg)$_7$-OH (Phe-R7)

Phe-R7 with CB7

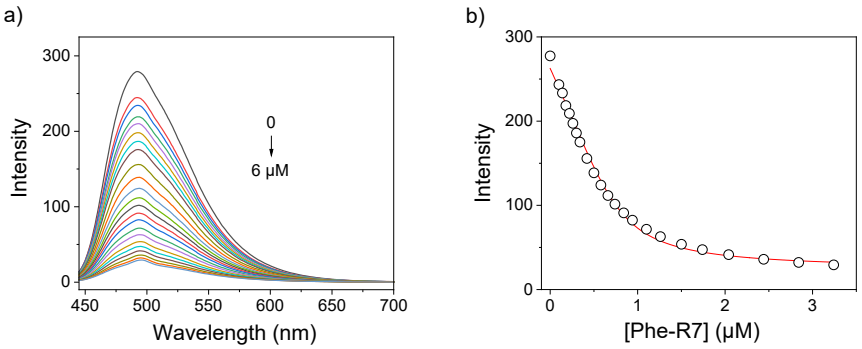


Figure S15. Competitive fluorescence titration of Phe-R7 with CB7/BE in 10 mM NaH_2PO_4, pH 7.2. a) Fluorescence spectra (λ_{exc} = 425 nm) of BE (1 μM) in presence of CB7 (0.75 μM) and varying concentrations of Phe-R7. b) Change in fluorescence intensity of BE (λ_{em} = 495 nm) with varying concentration of Phe-R7.

Phe-R7 with CX4

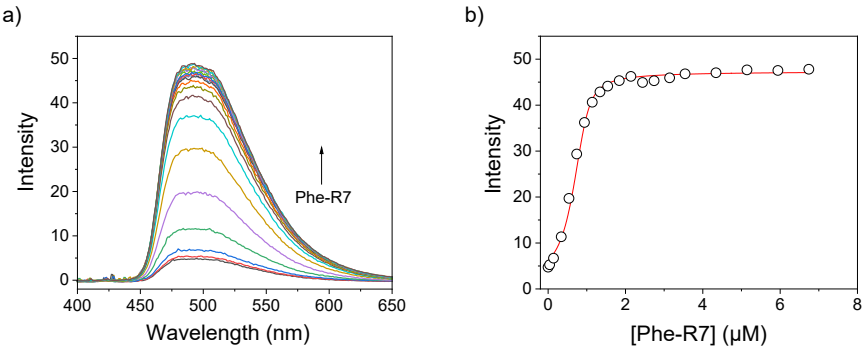


Figure S16. Competitive fluorescence titration of Phe-R7 with CX4/LCG in 10 mM NaH_2PO_4, pH 7.2. a) Fluorescence spectra (λ_{exc} = 369 nm) of LCG (0.5 μM) in presence of CX4 (1 μM) and varying concentrations of Phe-R7. b) Change in fluorescence intensity of LCG (λ_{em} = 502 nm) with varying concentration of Phe-R7.

Transport Assays

CX4/LCG Assay

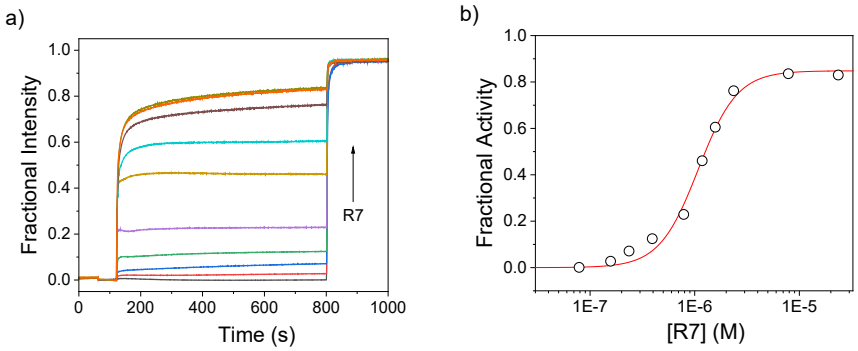


Figure S17. Membrane transport experiment with R7 and CX4/LCG⊃POPC/POPS(9:1)-LUVs in 10 mM NaH_2PO_4, pH 7.2. a) Fractional emission intensity (λ_{exc} = 369 nm, λ_{em} = 502 nm) of LCG during addition of 0.8 µM CX4-C5 at 60 s, varying concentrations of R7 at 120 s, and protamine (5.4 µM) in 1.2% TX-100 at 800 s. b) Respective Hill plot of fractional membrane activity against R7 concentration.

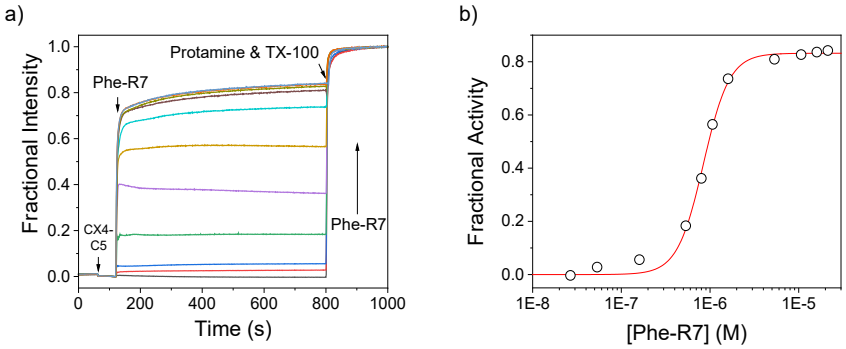


Figure S18. Membrane transport experiment with Phe-R7 and CX4/LCG⊃POPC/POPS(9:1)-LUVs in 10 mM NaH_2PO_4, pH 7.2. a) Fractional emission intensity (λ_{exc} = 369 nm, λ_{em} = 502 nm) of LCG during addition of 0.8 µM CX4-C5 at 60 s, varying concentrations of Phe-R7 at 120 s, and protamine (5.4 µM) in 1.2% TX-100 at 800 s. b) Respective Hill plot of fractional membrane activity against Phe-R7 concentration.

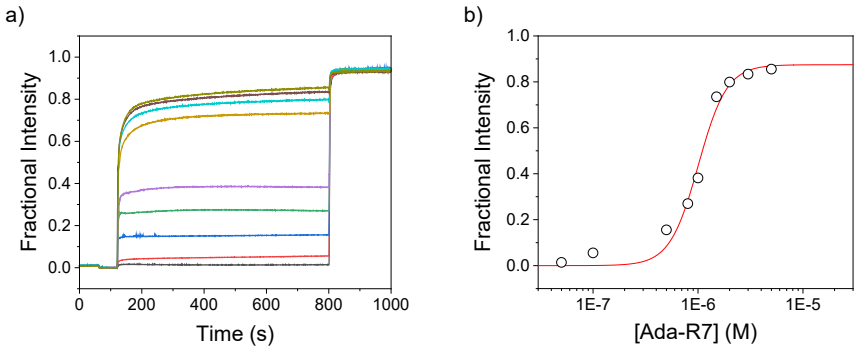


Figure S19. Membrane transport experiment with Ada-R7 and CX4/LCG⊃POPC/POPS(9:1)-LUVs in 10 mM NaH_2PO_4, pH 7.2. a) Fractional emission intensity (λ_{exc} = 369 nm, λ_{em} = 502 nm) of LCG during addition of 0.8 µM CX4-C5 at 60 s, varying concentrations of Ada-R7 at 120 s, and protamine (5.4 µM) in 1.2% TX-100 at 800 s. b) Respective Hill plot of fractional membrane activity against Ada-R7 concentration.

CB7/BE Assay

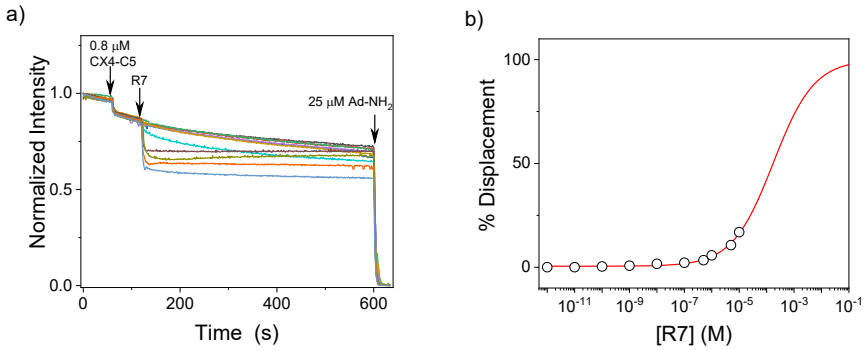


Figure S20. Membrane transport experiment with R7 and CB7/BE⊃POPC/POPS(9:1)-LUVs in 10 mM NaH_2PO_4, pH 7.2. a) Fractional emission intensity (λ_{exc} = 425 nm, λ_{em} = 495 nm) of BE during addition of CX4-C5 (0.8 µM) at 60 s, varying concentrations of R7 at 120 s, and 25 µM Ada-NH_2 at 800 s. b) Respective Hill plot of fractional displacement against R7 concentration.

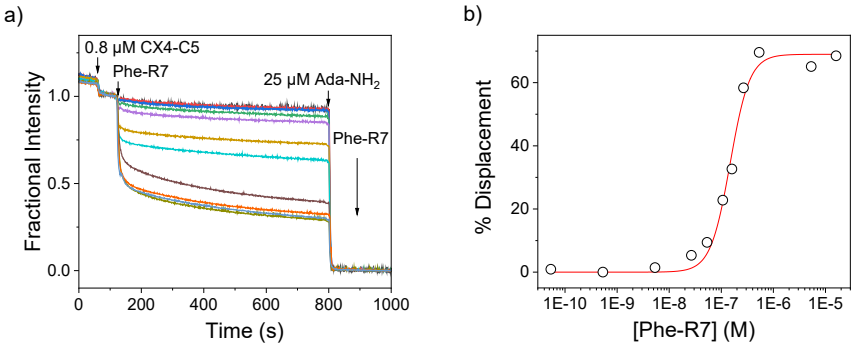


Figure S21. Membrane transport experiment with Phe-R7 and CB7/BE⊃POPC/POPS(9:1)-LUVs in 10 mM NaH_2PO_4, pH 7.2. a) Fractional emission intensity (λ_{exc} = 425 nm, λ_{em} = 495 nm) of BE during addition of CX4-C5 (0.8 µM) at 60 s, varying concentrations of Phe-R7 at 120 s, and 25 µM Ada-NH_2 at 800 s. b) Respective Hill plot of fractional displacement against Phe-R7 concentration.

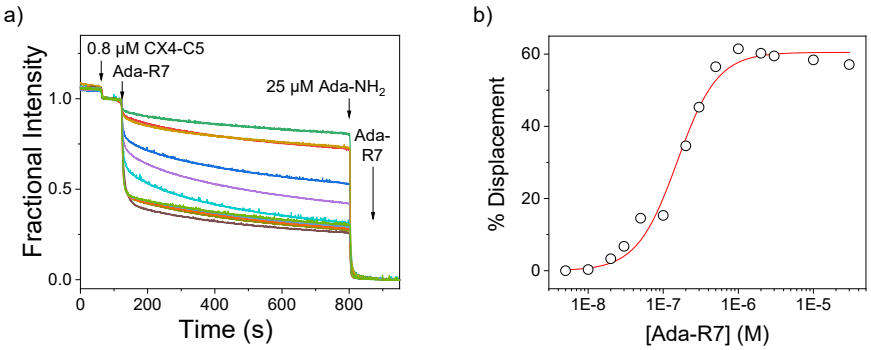


Figure S22. Membrane transport experiment with Ada-R7 and CB7/BE⊃POPC/POPS(9:1)-LUVs in 10 mM NaH_2PO_4, pH 7.2. a) Fractional emission intensity of BE (λ_{exc} = 425 nm, λ_{em} = 495 nm) during addition of CX4-C5 (0.8 µM) at 60 s, varying concentrations of Ada-R7 at 120 s, and 25 µM Ada-NH_2 at 800 s. b) Respective Hill plot of fractional displacement against Ada-R7 concentration.

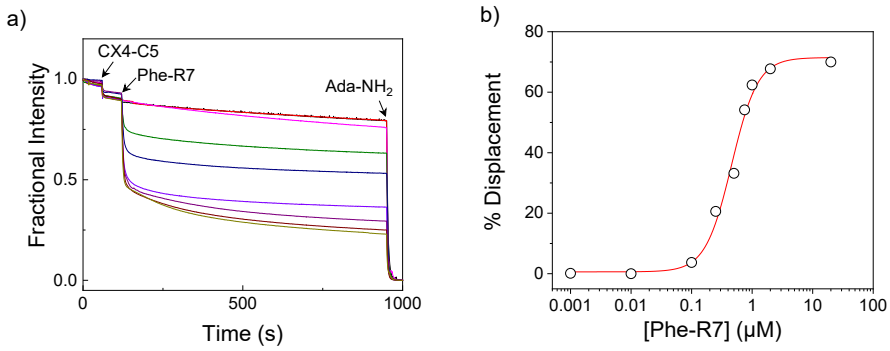


Figure S23. Membrane transport experiment with Phe-R7 and CB7/PLM⊃POPC/POPS(9:1)-LUVs in 10 mM NaH_2PO_4, pH 7.2. a) Fractional emission intensity (λ_{exc} = 425 nm, λ_{em} = 495 nm) of PLM during addition of CX4-C5 (0.8 μM) at 60 s, varying concentrations of Phe-R7 at 120 s, and 25 μM Ada-NH_2 at 800 s. b) Respective Hill plot of fractional displacement against Phe-R7 concentration.

CF Assay

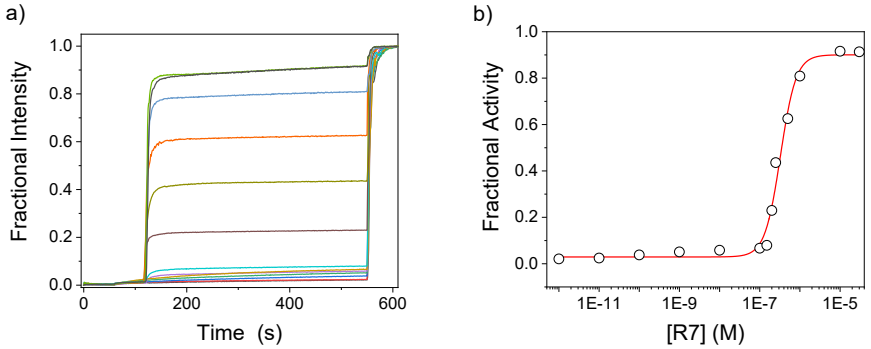


Figure S24. CF assay of R7 in CF⊃POPC/POPS-LUVs in 10 mM NaH_2PO_4, pH 7.2. a) Fractional emission intensity (λ_{exc} = 492 nm, λ_{em} = 517 nm) of CF during addition of CX4-C5 (0.8 μM) at 60 s, varying concentration of R7 at 120 s, and 1.2% TX-100 at 550 s. b) Respective Hill plot of fractional membrane activity against R7 concentration.

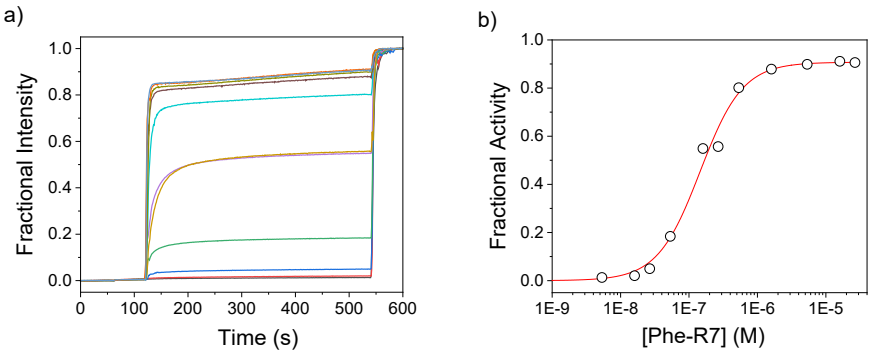


Figure S25. CF assay of Phe-R7 in CF⊃POPC/POPS-LUVs in 10 mM NaH_2PO_4, pH 7.2. a) Fractional emission intensity (λ_{exc} = 492 nm, λ_{em} = 517 nm) of CF during addition of CX4-C5 (0.8 μM) at 60 s, varying concentration of Phe-R7 at 120 s, and 1.2% TX-100 at 550 s. b) Respective Hill plot of fractional membrane activity against Phe-R7 concentration.

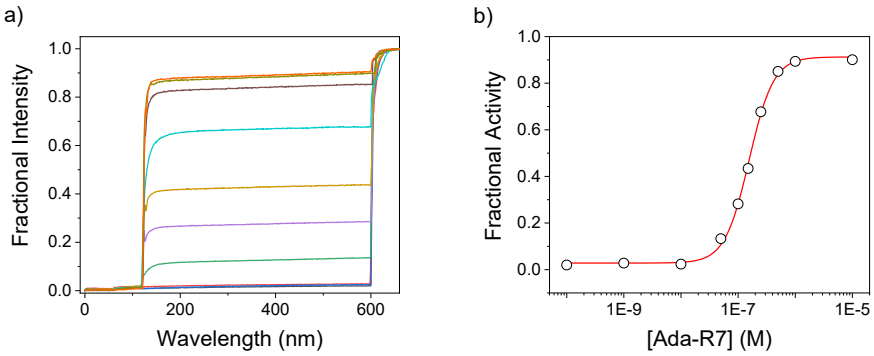


Figure S26. CF assay of Ada-R7 in CF⊃POPC/POPS-LUVs in 10 mM NaH_2PO_4, pH 7.2: a) Fractional emission intensity (λ_{exc} = 492 nm, λ_{em} = 517 nm) of CF during addition of CX4-C5 (0.8 µM) at 60 s, varying concentration of Ada-R7 at 120 s, and 1.2% TX-100 at 550 s. b) Respective Hill plot of fractional membrane activity against Ada-R7 concentration.

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