Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supplementary Information

Hierarchical supramolecular hydrogels: Self-assembly by peptides and photo-controlled release *via* host-guest interaction

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Materials and general methods

Chemicals and solvents

All chemicals were purchased from the companies listed below and used as received:

- Acros Organics (*Thermo Fischer Scientific Inc.*, Waltham, Massachusetts, USA)
- Aldrich (*Sigma-Aldrich Corp.*, St. Louis, Missouri, USA)
- Alfa Aesar (*Alfa Aesar*, Ward Hill, Massachusetts, USA)
- Carbolution Chemicals (*Carbolution Chemicals GmbH*, Saarbrücken, Germany)
- Fluka (*Sigma-Aldrich Corp.*, St. Louis, Missouri, USA)
- Iris Biotech GmbH (*Iris Biotech GmbH*, Marktredwitz, Germany)
- Merck (*Merck KGaA*, Darmstadt, Germany)
- TCI (*TCI Co., Ltd.*, Tokyo, Japan)

The inert reaction condition was performed by standard Schlenk technique with argon flow. When the dry condition was required, the solvent was prepared by standard drying method, DCM over calcium hydride, THF over sodium, methanol and acetonitrile over 3Å molecular sieve, and DMF over 4Å molecular sieve.

Preparative HPLC

Purification of peptides was done by using a Knauer preparative HPLC setup consisting of a Knauer pump manager 5050, Knauer pump 1000, Knauer UV-detecor 2600 and Knauer dynamic mixing chamber controlled by the software of *Chromgate*® (*v.3.3.2*). Separation was performed on an *Atlantis Prep dc18 OBD* (19 × 250 mm, 100Å pore size, 10 µm particle size) preparative HPLC column. Samples were dissolved under starting condition and injected over a 2 mL loop. The fractions were collected by an automatic *Foxy R1 Teledyn ISCO*. The flow rate was 7 mL/min and the UV absorption was monitored at 210 nm.

Preparation of CDV and NBD-cholesterol embedded CDV

Amphiphilic *β*-cyclodextrin was synthesized as reported.[S1] Several milligrams of amphiphilic *β*-CD dissolved in a minimum amount of $CHCl_3$ were slowly dried either by rotatory evaporation or flowing with argon stream. The residual solvent was removed under high vacuum and a thin film was obtained. The thin film of *β*-CD was then suspended in different amount of ddH_2O and stirred overnight to yield desired concentration of vesicle solution. To obtain unilamellar vesicles, the suspension was repeatedly extruded through a polycarbonate membrane (diameter: 100 nm) for at least 13 times.

In the case of NBD-cholesterol embedded CDV, a stock solution of NBD-cholesterol in $CHCl_3$ was prepared (1 mg/mL). As described above, 0.5 wt% of NBD-cholesterol was

mixed with several milligrams of amphiphilic *β*-CD while forming a thin film. The thin film was suspended in different amount of ddH_2O and stirred overnight. An extrusion was also applied to obtain unilamellar vesicles.

Preparation of hydrogels

Fmoc-RGDS hydrogels were prepared as previous report.[S2] Several milligram (5 mg for 2.5% sample; 10 mg for 5% sample) of Fmoc-RGDS peptide was put into a 1 mL vial. After 200 µL of ddH_2O was added, the vial was gently heated at 45°C for 5 min and left for gelation overnight. A hydrogel formation was confirmed by reverse-vial test.

For supramolecular cross-linked hydrogels, 5% of Fmoc-RGDS peptide, 20 mg/mL of Fmoc-RGDS-AAP and various concentration of CDV were freshly prepared. In a 1 mL vial, 100 µL of 5% Fmoc-RDGS peptide was added followed by 25 µL of Fmoc-RGDS-AAP. Sodium hydroxide solution (0.5 M) was added to adjust the pH before 50 µL of CDV solution (200 µM) was added. Additional ddH_2O was added to obtain a 200 µL of hydrogel sample and gently heated before left for gelation.

Dye release experiment

A solution of 20 mg/mL of Fmoc-RGDS-AAP peptide in ddH_2O was firstly prepared. 100 µL of 5% Fmoc-RGDS peptide solution was put into a 1 mL disposable PMMA cuvette and 25 µL of Fmoc-RGDS-AAP stock solution was added. Sodium hydroxide solution (0.5 M, 15 µL) was added to adjust the pH before 50 µL of CDV solution (200 µM) was added. The solution was gently mixed after 10 µL of fluorescent dye (0.2 mg/ mL for FITC-Isomer I; 10 mg/mL for FITC-Dex4000) was added. The cuvette was warmed up at 45°C for 2-3 min and left overnight for gelation.

For CDV release experiment, 200 µM of NBD-cholesterol embedded CDV was used instead. Otherwise, the procedure was as described above. As a result, hydrogels in presence of 2.5% Fmoc-RGDS, 0.25% Fmoc-RGDS-AAP and 50 µM of CDV were placed at the bottom of PMMA cuvette.

Full release of dyes or CDV is recorded by fluorescent intensity of fully dissolved hydrogel after each measurement. The percentage of release was calculated by dividing the intensity of full release sample at different time point.

Dynamic Light Scattering (DLS)

Particle sized distribution of CD vesicles and Rh-CDV was determined on a *Zetasizer Nano ZS* (*Malvern Instruments Ltd*., Herrenberg, Germany) at 25°C. Samples were prepared in disposable PMMA cuvettes with a path length of 1 cm and sample volume of 1 mL.

Isothermal titration calorimetry (ITC)

ITC was carried out from a *TA Instruments Nano ITC Low Volume* (*Waters Corp*., Milford, MA) with a cell volume of 170 µL using *ITCRun version 2.1.7.0 Firmware*

version 1.31 (*TA Instruments*, *Waters Corp*., Milford, MA). All titrations were performed by using a 50 µL syringe and 20 injections of 2.5 µL at a temperature of 25 °C with a stirring rate of 350 rpm, while titrating cyclodextrin to AAP-peptide. The sample was dissolved in distilled deionized water (ddH_2O) and degassed for 10 min before use. A blank titration of CD into ddH2O was subtracted from obtained data for correction. The data were analyzed by using *NanoAnalyse Data Analysis version 2.36* (*TA Instruments*, *Waters Corp*., Milford, MA) and *OriginPro* (OriginPro 2017, *OriginLab Corporation*, Northampton, USA).

UV/Vis spectroscopy

UV/Vis spectra were recorded on a *JASCO V-650* double-beam spectrophotometer (*JASCO Germany GmbH*, Gross-Umstadt, Germany). Sample dissolved in ddH_2O was measured in a 1 mL low-volume disposable PMMA cuvette (*Brand GmbH* & *Co*. *KG*, Wertheim, Germany) at 25°C. Spectra analysis was carried out on *Spectra Manager Version 2* (Spectra Analysis version 2.07.01, *JASCO Germany GmbH*, Gross-Umstadt, Germany) and *OriginPro* (OriginPro 2017, *OriginLab Corporation*, Northampton, USA).

For photoswitching experiment, two different light sources were applied. A LSC-G HighPower-LED emitting at 365 nm was used for *trans*- to *cis*-isomerisation and emitting at 520 nm was utilized for reverse isomerisation. Irradiation time for both isomerisation is 15 min under dark environment. Photoswitching experiment was performed with 3 cycles of isomerisation.

Scanning electron microscope (SEM)

Hydrogel samples were applied on silicon wafer chips and dried overnight under ambient environment. The chips were fixed on an aluminum sample holder with a diameter of 12 mm by using conductive carbon adhesive tape. The electric conductivity of sample surface was enhanced by gold coating, which was performed by a Q150T sputter coater from *Quorum* under argon atmosphere. Each sample was sputtering for 30 seconds at a current of 45 mA in order to obtain a very thin layer of gold.

An *AURIGA CrossBeam Workstation* from *Zeiss* (*Carl Zeiss AG*, Oberkochen, Germany) equipped with a field emission gun (Schottky type) was used for SEM imaging. The acceleration voltage of 1.5 kV was applied at a working distance of 2 mm. Image processing was done by using *ImageJ* v1.50i.

Rheology measurement

Rheology measurements were performed by using a shear rheometer (MCR 101, Anton-Paar) equipped with a CP25-2 cone plate (radius = 12.5 mm, cone angle = 2°, sample volume = 0.16 mL). The measurement temperature was maintained at 25°C. Strain sweep measurements were performed at a frequency of 10 rad/s and frequency sweep measurements were carried out at a 0.5% strain amplitude.

For irradiation experiment, 1 mL of hydrogel sample was prepared. The frequency sweep measurement for the first sample was performed, while the other was irradiated with UV light for 1 h. After UV irradiation, the second sample was measured and the

last sample was irradiated by green light (λ = 520 nm) for 1 h. The recovered hydrogel after irradiation was measured to accomplish as one switching cycle.

Fluorescence spectroscopy

Fluorescence spectra were recorded on a *FP-6500* spectrofluorimeter (*JASCO Germany GmbH*, Gross-Umstadt, Germany). Sample dissolved in ddH_2O was measured in a 1 mL low-volume disposable PMMA cuvette (*Brand GmbH* & *Co*. *KG*, Wertheim, Germany) at 25°C. Spectra analysis was carried out on *Spectra Manager Version 2* (Spectra Analysis version 2.07.01, *JASCO Germany GmbH*, Gross-Umstadt, Germany) and *OriginPro* (OriginPro 2017, *OriginLab Corporation*, Northampton, USA). Emission spectra for three cases of release were excited at 450 nm and recorded from 475 nm to 700 nm.

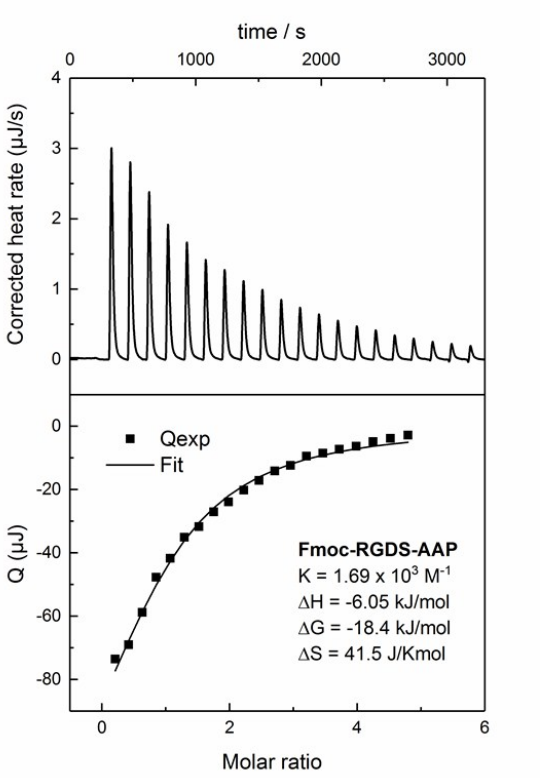


Fig. **S1** Isothermal titration for Fmoc-RGDS-AAP and *β*-CD.

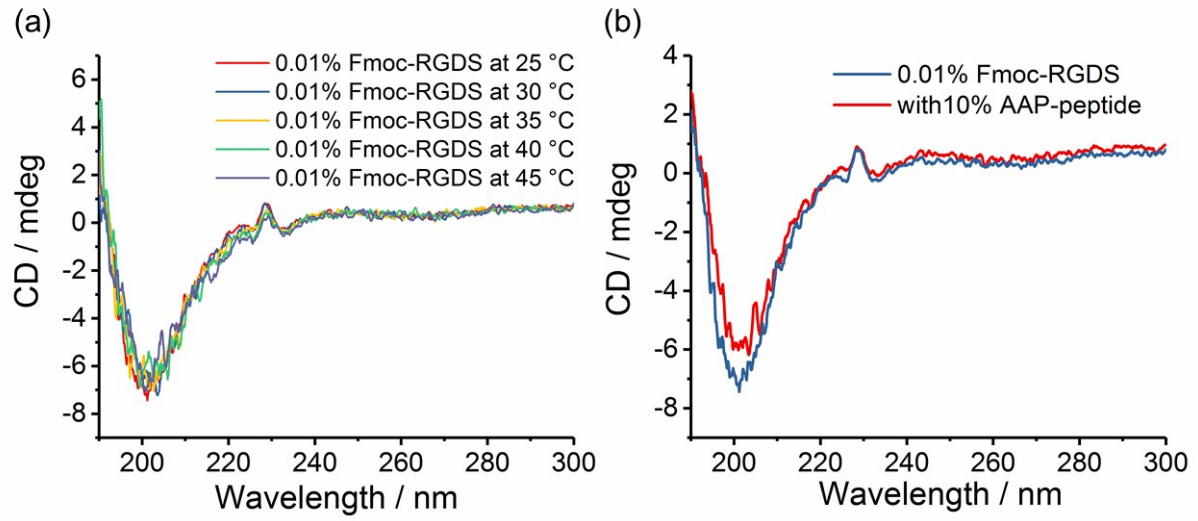


Fig. **S2** CD spectra of (a) 0.01% Fmoc-RGDS at different temperature and (b) the effect of insertion of Fmoc-RGDS-AAP.

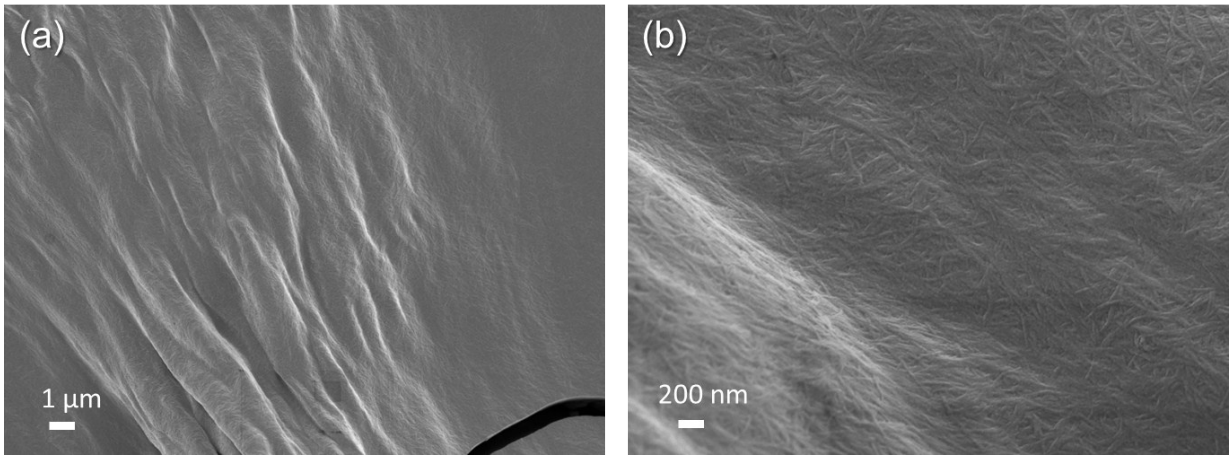


Fig. **S3** SEM images of 2.5 wt% Fmoc-RGDS peptide at (a) 10kx and (b) 25kx magnification.

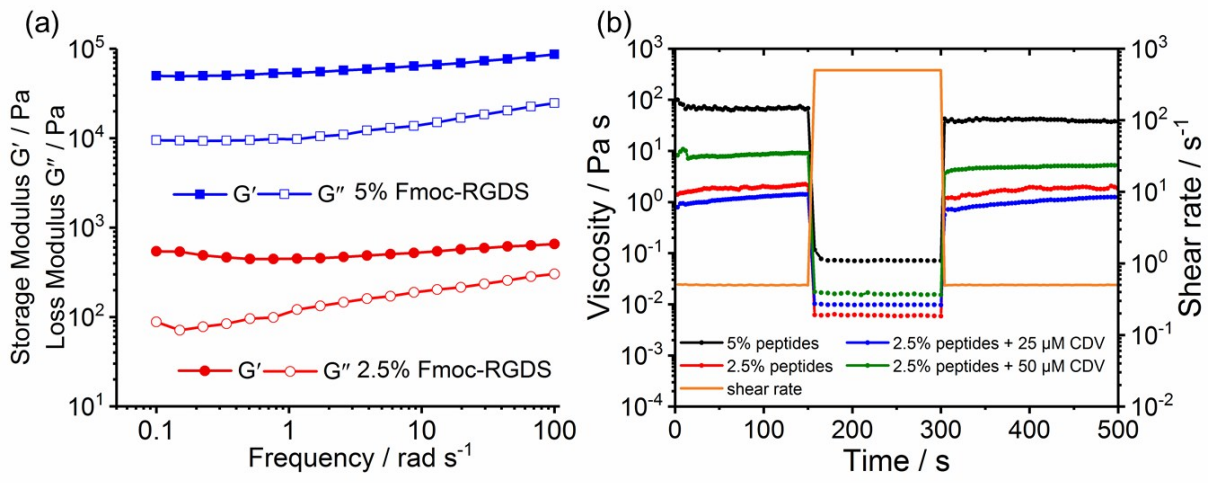


Fig. **S4** a) Frequency-sweep of oscillatory rheological measurements performed at 0.5% strain for 2.5 and 5 wt% of peptide. b) Step-rate measurements of 5 wt% Fmoc-RGDS and 2.5 wt% Fmoc-RGDS with 0-0.25 wt% Fmoc-RGDS-AAP and 0-200 µM CDV.

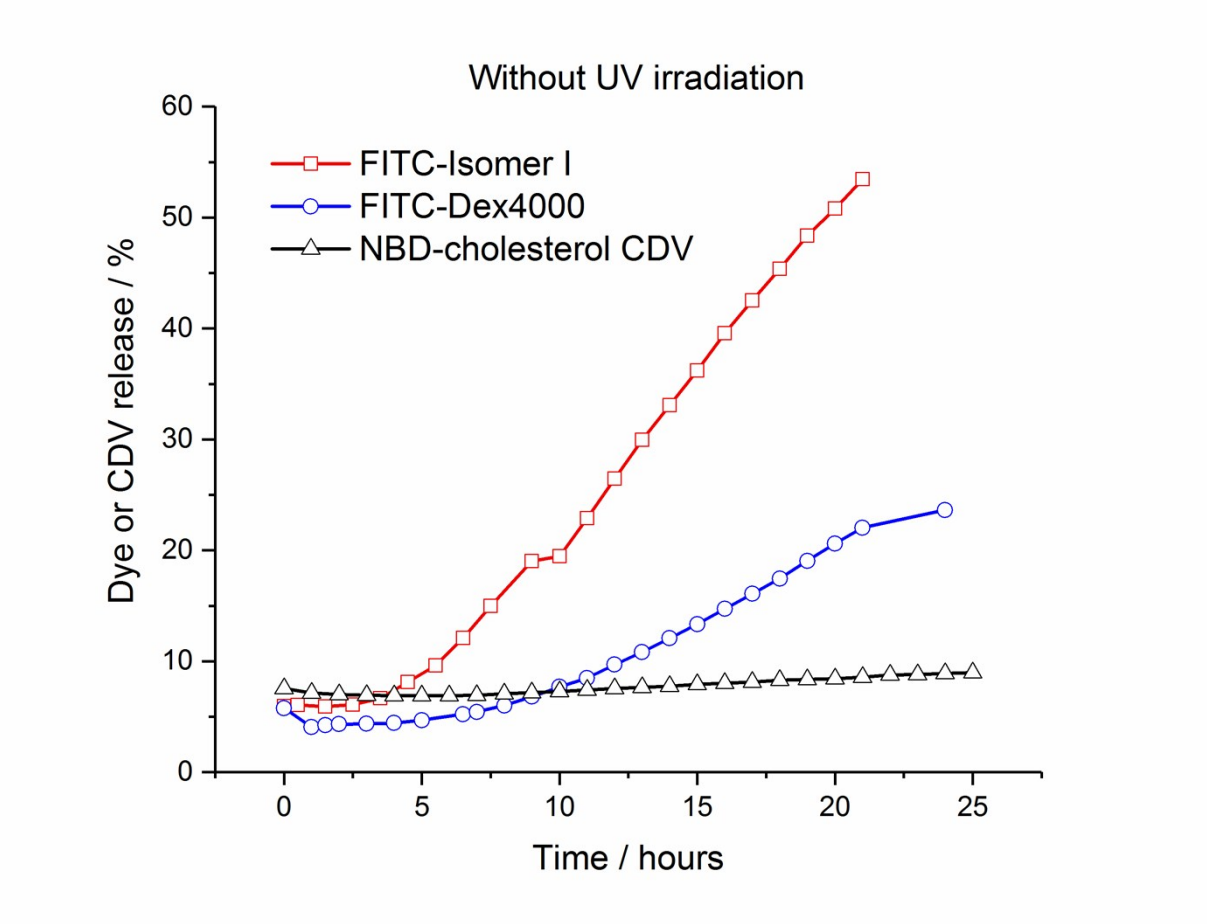


Fig. **S5** Release of dyes or CDV without UV irradiation as control experiments.

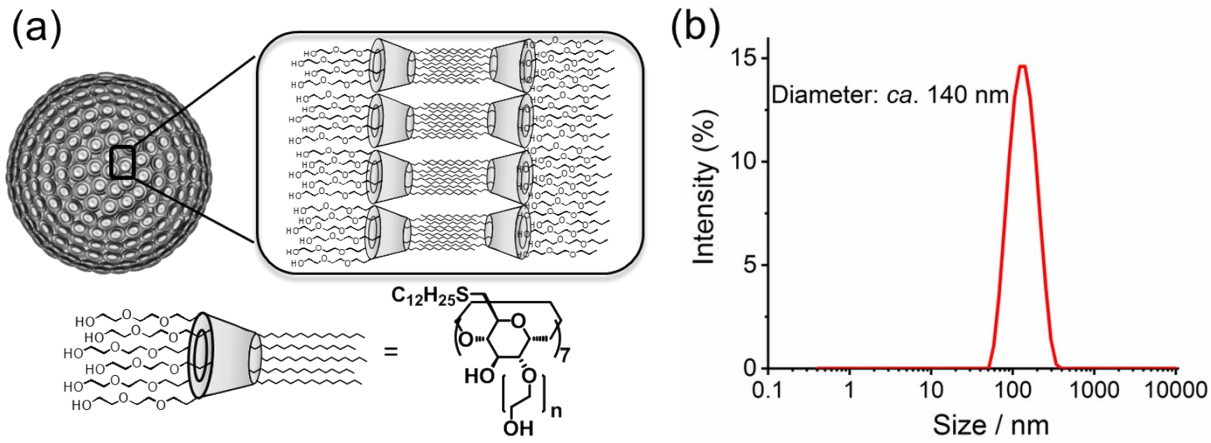


Fig. **S6** a) Molecular structure of amphiphilic β-cyclodextrin and schematic structure of CDV; b) Size distribution of CDV determined by DLS.

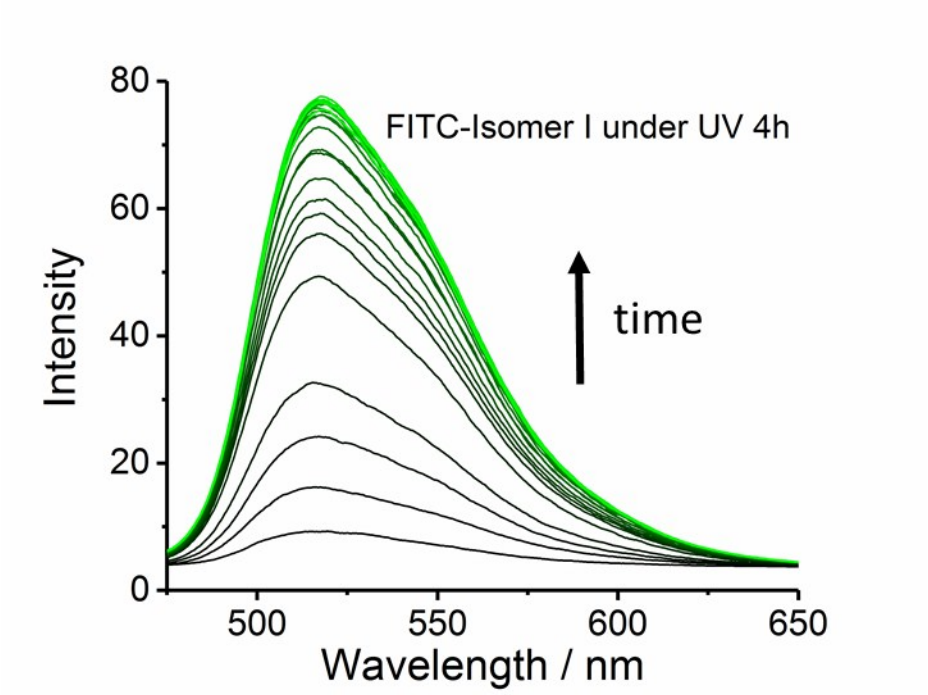


Fig. **S7** Fluorescence spectra for release of FITC-Isomer I under UV irradiation.

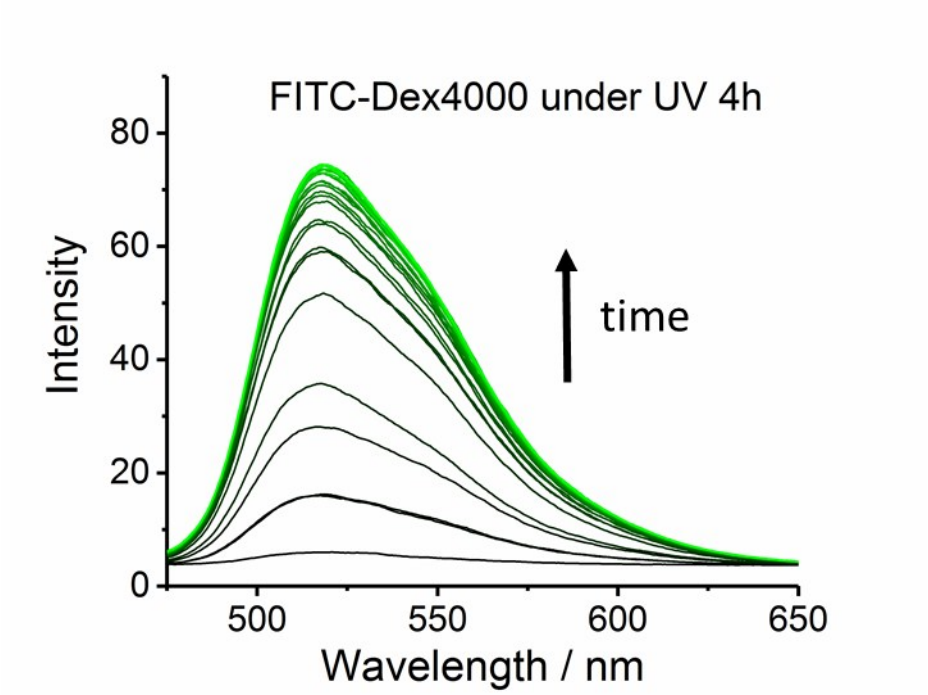


Fig. **S8** Fluorescence spectra for release of FITC-Dex4000 under UV irradiation.

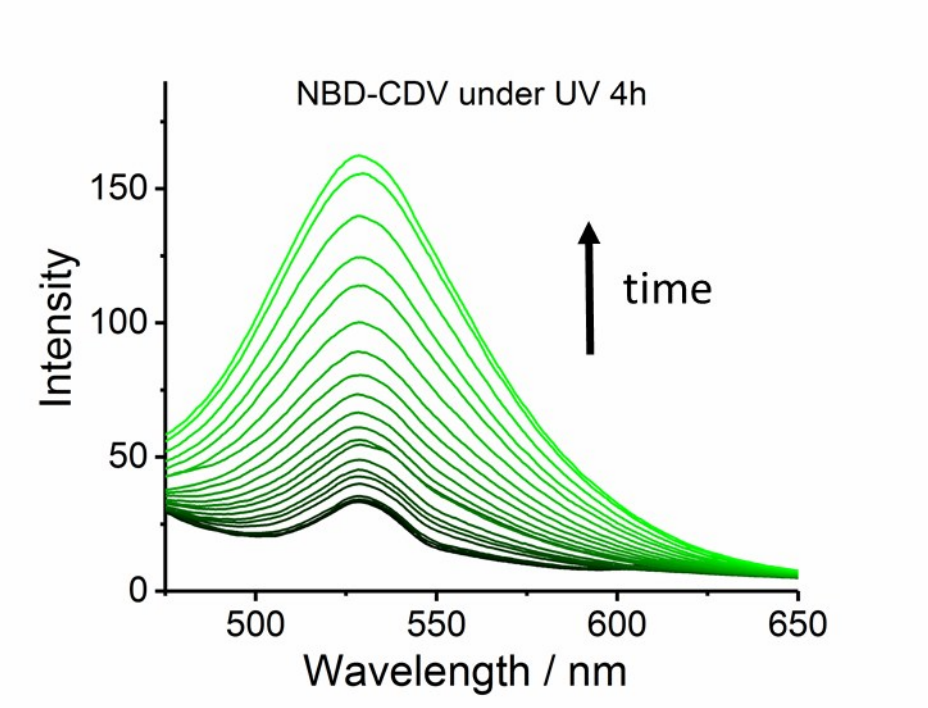
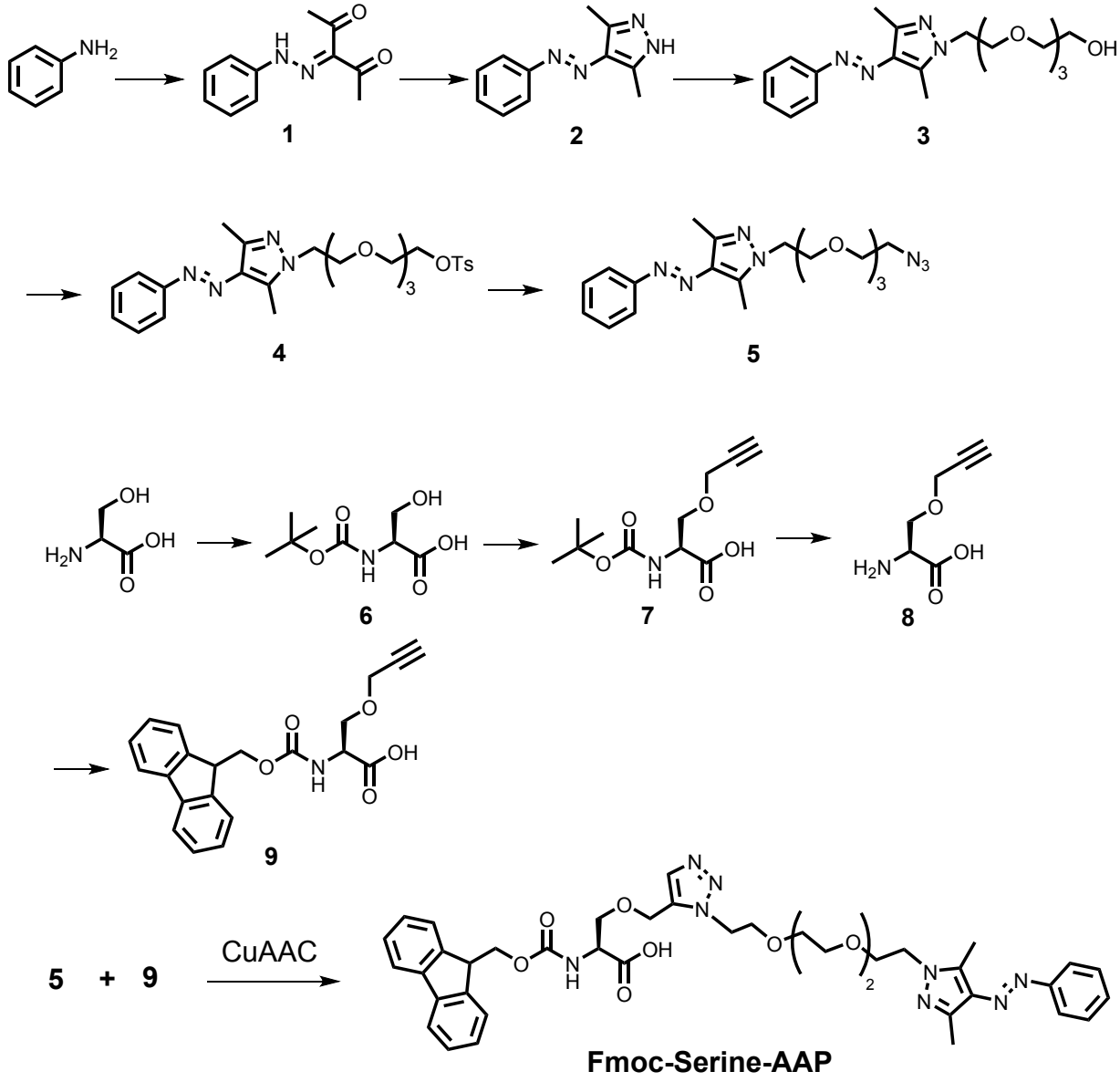


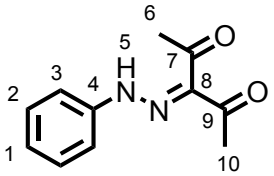
Fig. **S9** Fluorescence spectra for release of NBD-CDV under UV irradiation.

Synthesis of AAP-peptide precursor



Scheme S1. Synthesis route of Fmoc-serine-AAP.

Synthesis of **1**, 3-(2-Phenylhydrazono)pentane-2,4-dione[S3]



To a solution of aniline (0.931 g, 10 mmol, 1 eq.) and HCl (12 M, 2.3 mL) in acetic acid (15 mL), $NaNO_2$ (0.827 g, 12 mmol, 1.2 eq.) dissolved in minimum water was added dropwise at 0°C. After stirred for 45 min at this temperature, the reaction mixture was transferred to a suspension of pentane-2,4-dione (1.3 g, 13 mmol, 1.3 eq.) and sodium

acetate (2.46 g, 30 mmol, 3 eq.) in ethanol/water mixture (10 mL/6 mL). The mixture was stirred for 1 h and the yellow precipitate was collected by filtration. The solid was washed with water (50 mL) and ethanol/water (1:1, 50 mL) and dried under vacuum. The product was obtained as yellow solid (1.9 g, 9.3 mmol, 93% yield).

Molecular formula: $C_{11}H_{12}N_2O_2$

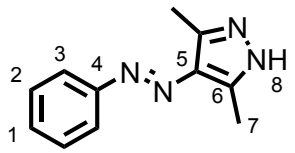
^{1}H (300 MHz, $CDCl_3$, 298 K): δ = 14.74 (s, 1H, 5-H), 7.43-7.38 (m, 4H, 2- and 3-H), 7.23-7.17 (m, 1H, 1-H), 2.60 (s, 3H, 6-H), 2.49 (s, 3H, 10-H).

^{13}C (75 MHz, $CDCl_3$, 298 K): δ = 198.07 (C_q, 7-C), 197.25 (C_q, 9-C), 141.65 (C_q, 4-C), 133.33 (C_q, 8-C), 129.79 (2CH, 3-C), 126.03 (2CH, 2-C), 116.39 (CH, 1-C), 31.82 (CH_3, 6-C), 26.78 (CH_3, 10-C).

ESI-MS (*m*/*z*): calculated for $[C_{11}H_{12}N_2O_2Na]^+$: 227.0796, found: 227.0805.

These analysis data is consistent with our previous report.[S3]

Synthesis of **2**, (*E*)-3,5-Dimethyl-4-(phenyldiazenyl)-1*H*-pyrazole (**AAP**)[S3]



Hydrazine x hydrate (283 mg, 8.82 mmol, 1 eq.) and 3-(2-phenylhydrazono)pentane-2,4-dione (1.8 g, 8.82 mmol, 1 eq.) were dissolved in ethanol (90 mL) and refluxed for 3 h. The orange solution was concentrated under reduced pressure and the product was obtained as orange solid after dryness (1.76 g, quantitative).

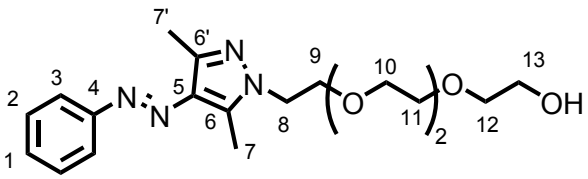
Molecular formula: $C_{11}H_{12}N_4$

^{1}H (300 MHz, $CDCl_3$, 298 K): δ = 7.82-7.78 (m, 2H, 3-H), 7.50-7.44 (m, 2H, 2-H), 7.43-7.36 (m, 1H, 1-H), 2.62 (s, 6H, 7-H).

ESI-MS (*m*/*z*): calculated for $[C_{11}H_{12}N_4H]^+$: 201.1135, found: 201.1127.

These analysis data is consistent with our previous report.[S3]

Synthesis of **3**, (*E*)-2-(2-(2-(2-(3,5-dimethyl-4-(phenyldiazenyl)-1*H*-pyrazol-1-yl) ethoxy) ethoxy)ethoxy) ethan-1-ol (**AAP-TEG**)



To a solution of AAP (1.83 g, 9.4 mmol, 1 eq.), K_2CO_3 (6.5 g, 47 mmol, 5 eq.) and LiBr (catalytical amount, 50 mg) in acetonitrile (50 mL), mono-tosylated tetraethylene glycol (TEG-Tos)[S4] (5.67 g, 11.28 mmol, 1.2 eq.) dissolved in 5 mL of acetonitrile was added.

The reaction mixture was refluxed for 2 days before evaporation under reduced pressure. The residue was dissolved in DCM (100 mL) and washed with water (3 × 100 mL) and brine (3 × 100 mL). The organic phase was dried over $MgSO_4$ and concentrated. The crude product was further purified by column chromatography (DCM/MeOH = 97:3) and a red oil was obtained as title product (2.18 g, 5.8 mmol, 62% yield).

Molecular formula: $C_{19}H_{28}N_4O_4$

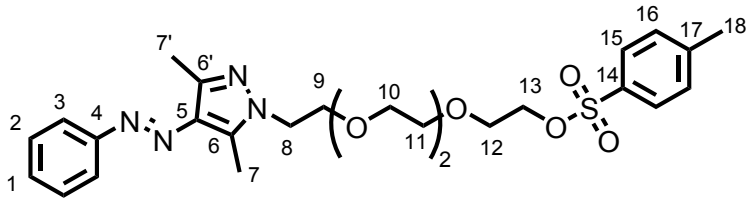
^{1}H (300 MHz, $CDCl_3$, 298 K): δ = 7.80-7.74 (m, 2H, 3-H), 7.48-7.42 (m, 2H, 2-H), 7.38-7.33 (m, 1H, 1-H), 4.21 (t, 2H, 8-H), 3.86 (t, 2H, 9-H), 3.72-3.52 (m, 12H, 10-13-H), 2.61 (s, 3H, 7-H), 2.49 (s, 3H, 7'-H).

^{13}C (75 MHz, $CDCl_3$, 298 K): δ = 153.71 (C_q, 4-C), 142.64 (C_q, 5-C), 140.48 (C_q, 6'-C), 135.07 (C_q, 6-C), 129.38 (CH, 1-C), 128.99 (2CH, 2-C), 121.84 (2CH, 3-C), 72.60, 70.81, 70.69, 70.62, 70.41, 69.99 ($6CH_2$, 9-12-C), 61.77 (CH_2, 13-C), 49.12 (CH_2, 8-C), 14.21, 10.02 ($2CH_3$, 7- and 7'-C).

ESI-MS (*m*/*z*): calculated for $[C_{19}H_{28}N_4O_4Na]^+$: 399.2003, found: 399.2010.

These analysis data is consistent with our previous report.[S4]

Synthesis of **4**, (*E*)-2-(2-(2-(2-(3,5-dimethyl-4-(phenyldiazenyl)-1*H*-pyrazol-1-yl) ethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (**AAP-TEG-Tos**)



To a solution of AAP-TEG (2.54 g, 6.8 mmol, 1 eq.), triethylamine (0.759 g, 7.5 mmol, 1.1 eq.) and DMAP (catalytical amount, 50 mg) in DCM (50 mL) at 0°C, 4-toluenesulfonyl chloride (1.42 g, 7.5 mmol, 1.1 eq.) dissolved in 50 mL of DCM was added over 1 h. The reaction mixture was then stirred at room temperature overnight. The mixture was washed with water (3 × 100 mL) and brine (3 × 100 mL). The organic phase was dried over $MgSO_4$ and concentrated. The crude product was further purified by column chromatography (pure ethyl acetate) and a orange oil was obtained as title product (2.52 g, 4.76 mmol, 79% yield).

Molecular formula: $C_{26}H_{34}N_4O_6S$

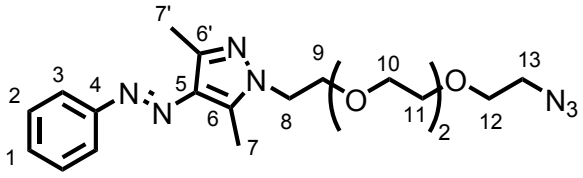
^{1}H (300 MHz, $CDCl_3$, 298 K): δ = 7.82-7.72 (m, 4H, 3- and 15-H), 7.50-7.41 (m, 2H, 2-H), 7.40-7.35 (m, 1H, 1-H), 7.34-7.28 (m, 2H, 16-H), 4.21 (t, 2H, 8-H), 4.11 (t, 2H, 13-H), 3.86 (t, 2H, 9-H), 3.63 (t, 2H, 12-H), 3.59-3.47 (m, 8H, 10- and 11-H), 2.61 (s, 3H, 7-H), 2.49 (s, 3H, 7'-H), 2.43 (s, 3H, 18-H).

^{13}C (75 MHz, $CDCl_3$, 298 K): δ = 153.73 (C_q, 4-C), 144.91 (C_q, 17-C), 142.64 (C_q, 5-C), 140.57 (C_q, 6'-C), 135.09 (C_q, 6-C), 133.10 (C_q, 14-C), 129.93 (2CH, 16-C), 129.40 (CH, 1-C), 129.02 (2CH, 2-C2), 128.09 (CH, 15-C), 121.85 (2CH, 3-C), 70.86, 70.65,

70.61, 70.04, 69.31, 69.78 (7CH_2, 9-13-C), 49.18 (CH_2, 8-C), 21.77 (CH_3, 18-C), 14.27, 10.05 (2CH_3, 7- and 7'-C).

ESI-MS (*m*/*z*): calculated for $[C_{26}H_{34}N_4O_6SNa]^+$: 553.2091, found: 553.2100.

Synthesis of **5**, (*E*)-1-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)-3,5-dimethyl-4-(phenyldiazenyl)-1*H*-pyrazole (**AAP-TEG-Azide**)



To a solution of AAP-TEG-Tos (1.67 g, 3.15 mmol, 1 eq.) in DMF (35 mL) at 0°C, sodium azide (1.02 g, 15.8 mmol, 5 eq.) was added carefully. The reaction mixture was stirred at 80°C overnight before evaporation the solvent. The residue was redissolved in DCM (100 mL) and washed with water (3 × 100 mL) and brine (3 × 100 mL). The organic phase was dried over $MgSO_4$ and concentrated. The product was obtained as orange oil (1.12 g, 2.8 mmol, 89% yield).

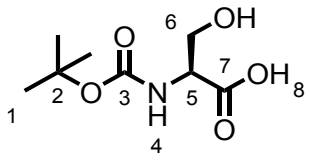
Molecular formula: $C_{19}H_{27}N_7O_3$

^{1}H (300 MHz, $CDCl_3$, 298 K): δ = 7.80-7.76 (m, 2H, 3-H), 7.49-7.43 (m, 2H, 2-H), 7.40-7.35 (m, 1H, 1-H), 4.22 (t, 2H, 8-H), 3.86 (t, 2H, 9-H), 3.66-3.55 (m, 8H, 10-12-H), 3.33 (t, 2H, 13-H), 2.62 (s, 3H, 7-H), 2.50 (s, 3H, 7'-H).

^{13}C (75 MHz, $CDCl_3$, 298 K): δ = 153.71 (C_q, 4-C), 142.58 (C_q, 5-C), 140.59 (C_q, 6'-C), 135.07 (C_q, 6-C), 129.37 (CH, 1-C), 128.99 (2CH, 2-C), 121.83 (2CH, 3-C), 70.91, 70.78, 70.71, 70.68, 70.11, 70.04 (6CH_2, 9-12-C), 50.71 (CH_2, 13-C), 49.19 (CH_2, 8-C), 14.27, 10.04 (2CH_3, 7- and 7'-C).

ESI-MS (*m*/*z*): calculated for $[C_{19}H_{27}N_7O_3Na]^+$: 424.2068, found: 424.2073.

Synthesis of **6**, *N*-Boc-L-Serine



To a solution of 50% $NaHCO_3$ (140 mL), Na_2CO_3 was added and stirred at 0°C. A solution of L-serine (10.5 g, 100 mmol, 1 eq.) in dioxane (25 mL) was added dropwise to above solution. After addition of Boc_2O in one portion, the reaction mixture was allowed to warm up to room temperature and stirred overnight. Two thirds of the solvent was removed under reduced pressure and the residue was treated with concentrated HCl at 0 °C until pH = 2-3. The suspension was extracted with ethyl acetate (50 mL × 6) and the organic phase was combined and dried over $MgSO_4$. The product was

obtained as colorless oil after evaporation under reduced pressure (20.9 g, quantitative).

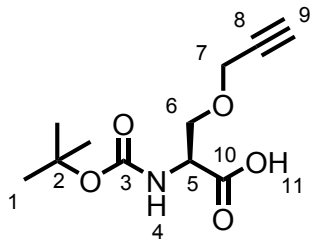
Molecular formula: $C_8H_{15}NO_5$

^{1}H (300 MHz, $CDCl_3$, 298 K): δ = 5.81 (d, 1H, 4-H), 4.37 (m, 1H, 5-H), 4.03 (dd, 1H, 6-H_a), 3.82 (dd, 1H, 6-H_b), 1.45 (s, 9H, 1-H).

^{13}C (75 MHz, $CDCl_3$, 298 K): δ = 174.09 (C_q, 7-C), 156.36 (C_q, 3-C), 80.82 (C_q, 2-C), 63.14 (CH_2, 6-C), 55.57 (CH, 5-C), 28.45 (3 CH_3, 1-C).

ESI-MS (*m*/*z*): calculated for $[C_8H_{15}NO_5Na]^+$: 228.0848, found: 228.0850.

Synthesis of **7**, *N*-Boc-*O*-Propargyl-L-serine



N-Boc-L-serine (4.197 g, 20.5 mmol, 1eq.) was dissolved in anhydrous DMF (160 mL) at 0°C under argon atmosphere. Sodium hydride (60% dispersion in mineral oil, 1.64 g, 2 eq., 41 mmol) was added portionwise into the above solution. The reaction mixture was stirred for 30 min until no bubbles were generated. Propargyl bromide solution (80% in toluene, 2.2 mL, 1 eq., 20.5 mmol) was added dropwise over 15 min. The reaction mixture was then stirred at 0°C for 2 h until full conversion (monitored by TLC analysis, DCM : MeOH : AcOH = 95 : 3 : 1). The reaction was quenched by water (50 mL) and the solvent was removed by reduced pressure. The crude product was re-dissolved in water (100 mL) and washed with diethyl ether (50 mL × 5). The aqueous solution was acidified by $KHSO_4$ (0.5 M) until pH = 2-3 and extracted with DCM (100 mL × 3). The organic phase was washed with $KHSO_4$ solution (50 mL × 3) and dried over $MgSO_4$. A brownish oil was obtained after evaporation of solvent under *vacuo* (4.86 g, 98% yield).

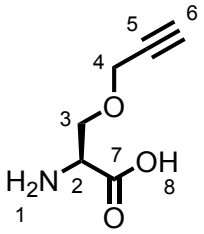
Molecular formula: $C_{11}H_{17}NO_5$

^{1}H (300 MHz, $CDCl_3$, 298 K): δ = 9.26 (br, 1H, 11-H), 5.41 (d, 1H, 4-H), 4.45 (m, 1H, 5-H), 4.15 (d, 2H, 7-H), 3.96 (dd, 1H, 6-H_a), 3.80 (dd, 1H, 6-H_b), 2.43 (t, 1H, 9-H), 1.43 (s, 9H, 1-H).

^{13}C (75 MHz, $CDCl_3$, 298 K): δ = 173.25 (C_q, 10-C), 155.70 (C_q, 3-C), 80.10 (C_q, 2-C), 79.01 (C_q, 8-C), 75.19 (CH, 9-C), 69.82 (CH_2, 6-C), 58.71 (CH_2, 7-C), 53.74 (CH, 5-C), 28.40 (3 CH_3, 1-C).

ESI-MS (*m*/*z*): calculated for $[C_{11}H_{17}NO_5Na]^+$: 266.1010, found: 266.1000.

Synthesis of **8**, *O*-Propargyl-L-serine hydrochloride salt



N-Boc-*O*-Propargyl-L-serine (4.82 g, 20.6 mmol, 1 eq.) was dissolved in ethyl acetate (10 mL) and HCl (12 M, 12 mL) was added by syringe. The above solution was stirred at room temperature for 10 min before evaporation under reduced pressure. The residue was redissolved in water (20 mL) and washed with diethyl ether (30 mL × 3) and DCM (30 mL × 3) respectively. A brownish solid was obtained after evaporation of the solvent (2.9 g, quantitative.).

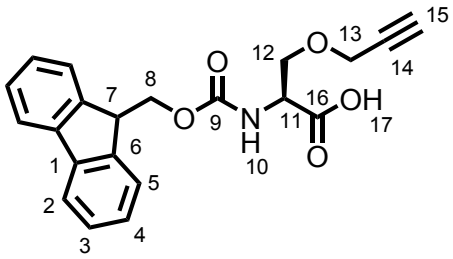
Molecular formula: $C_6H_9NO_3$

^{1}H (300 MHz, D_2O, 298 K): δ = 4.29 (dd, 1H, 2-H), 4.23 (d, 2H, 4-H), 4.07 (dd, 1H, 3-H_a), 3.95 (dd, 1H, 3-H_b), 2.87 (t, 1H, 6-H).

^{13}C (75 MHz, $CDCl_3$, 298 K): δ = 169.72 (C_q, 7-C), 78.62 (C_q, 5-C), 76.35 (CH, 6-C), 66.49 (CH_2, 3-C), 58.34 (CH_2, 4-C), 52.91 (CH, 2-C).

ESI-MS (*m*/*z*): calculated for $[C_{11}H_{17}NO_5Na]^+$: 144.0610, found: 144.0660.

Synthesis of **9**, Fmoc-*O*-Propargyl-L-serine



O-Propargyl-L-serine hydrochloride salt (2.127 g, 14.9 mmol, 1 eq.) was dissolved in dioxane/water mixture (2:1, 75 mL) and Na_2CO_3 was added. A solution of Fmoc-OSu in dioxane (25 mL) was added dropwise to the above suspension and stirred at 0°C for 3 h. After full conversion (monitored by TLC analysis, DCM : MeOH = 95 : 3), the reaction mixture was acidified by 1 N HCl until pH = 2 and extracted with ethyl acetate (150 mL). The organic phase was the washed with HCl (1 M, 150 mL), water (200 mL) and brine (200 mL). After drying with $MgSO_4$ and evaporation under reduced pressure, the product was obtained as white solid (5.48 g, quantitative).

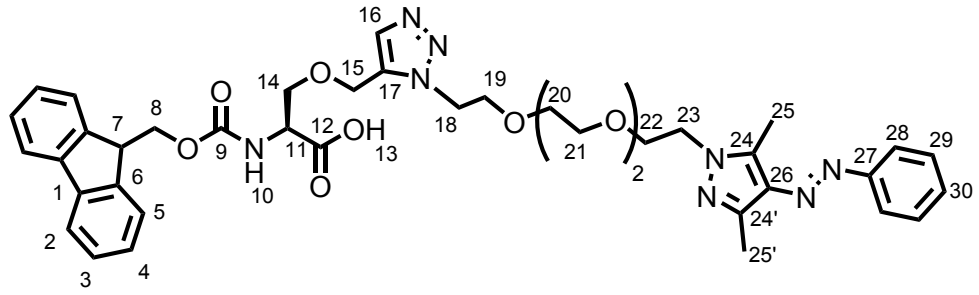
Molecular formula: $C_{21}H_{19}NO_5$

^{1}H (300 MHz, DMSO-d_6, 298 K): δ = 12.86 (br, 1H, 17-H), 7.89 (d, 2H, 2-H), 7.75 (d, 2H, 5-H), 7.70 (d, 1H, 10-H), 7.42 (t, 2H, 3-H), 7.33 (t, 2H, 4-H), 4.30-4.19 (m, 4H, 7-, 8- and 11-H), 4.17 (d, 2H, 13-H), 3.72 (d, 2H, 12-H), 3.48 (t, 1H, 15-H).

^{13}C (75 MHz, $CDCl_3$, 298 K): δ = 171.48 (C_q, 16-C), 156.05 (C_q, 9-C), 143.81 (C_q, 6-C), 140.71 (C_q, 1-C), 127.65 (CH, 3-C), 127.09 (CH, 4-C), 125.37 (CH, 5-C), 120.11 (CH, 2-C), 79.88 (C_q, 14-C), 77.57 (CH, 15-C), 68.68 (CH_2, 12-C), 65.80 (CH_2, 8-C), 57.69 (CH_2, 13-C), 53.97 (CH, 11-C), 46.60 (CH, 7-C).

ESI-MS (*m*/*z*): calculated for $[C_{21}H_{19}NO_5Na]^+$: 388.1155, found: 388.1133.

Synthesis of **Fmoc-L-Serine-TEG-AAP**



To a solution of AAP-TEG-Azide (1 g, 2.5 mmol, 1.5 eq.) in DMF (15 mL), Fmoc-*O*-propargyl-L-serine (0.6 g, 1.66 mmol, 1 eq.) dissolved in DMF (10 mL) was added. Copper (II) sulfate pentahydrate (2.5 g, 10 mmol, 6 eq.) was added to above mixture. A suspension of sodium ascorbate (1.31 g, 6.64 mmol, 4 eq.) in DMF (10 mL) was added. The reaction mixture was stirred at room temperature for 2 days before evaporation of solvent. The residue was redissolved in DCM (100 mL) and washed with HCl (1 M, 100 mL × 2) and brine (100 mL × 2). The organic phase was dried over $MgSO_4$ and concentrated. The crude product was further purified by column chromatography (DCM/MeOH = 90:10) and the title product was obtained as orange oil (775 mg, 1.01 mmol, 61% yield).

Molecular formula: $C_{40}H_{46}N_8O_8$

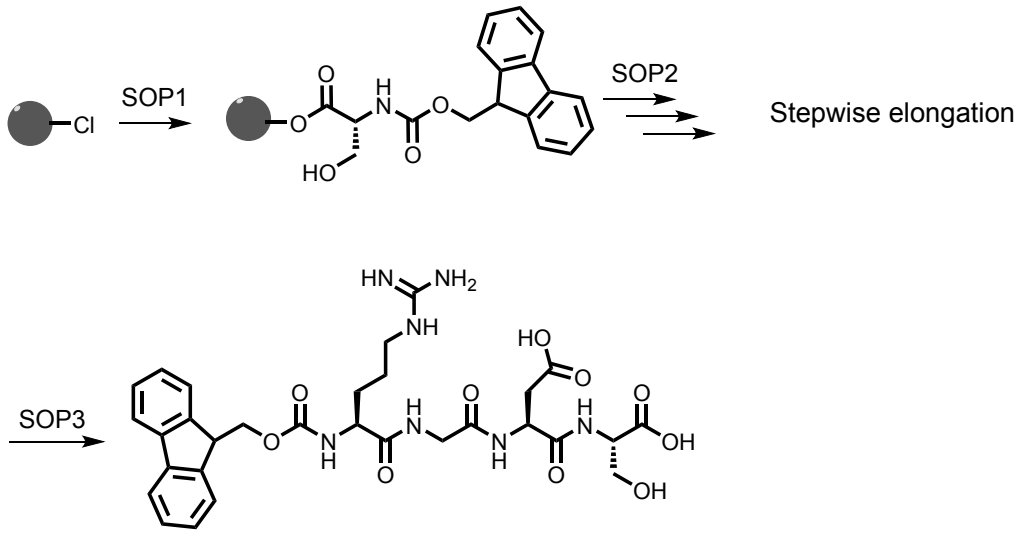
^{1}H (300 MHz, DMSO-d_6, 298 K): δ = 7.96 (s, 1H, 16-H), 7.90-7.84 (m, 2H, 2-H), 7.75-7.65 (m, 4H, 5- and 28-H), 7.53-7.46 (m, 2H, 29-H), 7.44-7.26 (m, 5H, 3-, 4- and 30-H), 6.94 (br, 1H, 10-H), 4.49 (s, 2H, 15-H), 4.43 (t, 2H, 23-H), 4.29-4.20 (m, 3H, 7- and 8-H), 4.05-3.94 (m, 1H, 11-H), 3.77-3.70 (m, 4H, 14- and 18-H), 3.47-3.35 (m, 10H, 19-21H), 2.55 (s, 3H, 25-H), 2.36 (s, 3H, 25'-H).

^{13}C (75 MHz, DMSO-d_6, 298 K): δ = 162.32 (C_q, 12-C), 155.66 (C_q, 9-C), 152.99 (C_q, 27-C), 143.89 ($2C_q$, 6-C), 140.83 (C_q, 26-C), 140.70 ($2C_q$, 24- and 17-C), 140.60 ($2C_q$, 1-C), 134.26 (C_q, 24'-C), 129.45 (CH, 16-C), 129.19 (CH, 30-C), 127.59 (2CH, 29-C), 127.08 (2CH, 3-C), 125.28 (2CH, 4-C), 124.14 (2CH, 5-C), 121.36 (2CH, 28-C), 120.08 (2CH, 2-C), 70.53, 69.84, 69.64, 69.57, 69.50, 69.04, 68.65 ($8CH_2$, 8-, 14-, and 19-22-C) 65.54 (CH_2, 15-C), 63.62 (CH, 11-C), 49.25 (CH_2, 23-C), 48.56 (CH_2, 18-C), 46.69 (CH, 7-C), 14.04, 9.42 ($2CH_3$, 7- and 7'-C) .

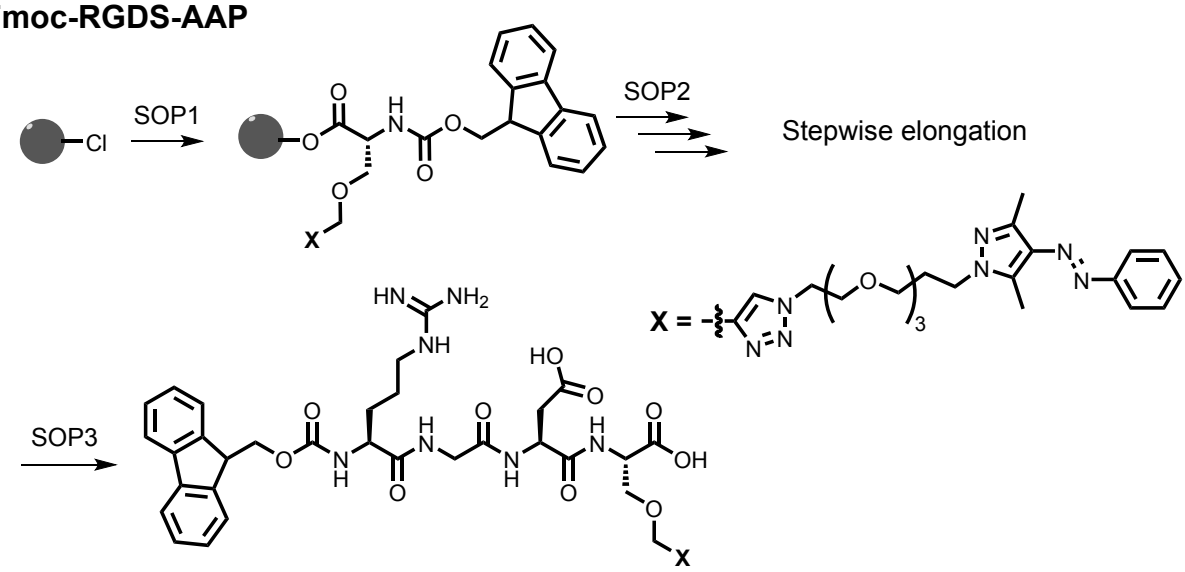
MALDI-MS (*m*/*z*): calculated for $[C_{40}H_{46}N_8O_8H]^+$: 767.35, found: 767.37.

Solid-phase peptide synthesis (SPPS)

Fmoc-RGDS



Fmoc-RGDS-AAP



Scheme S2. Solid-phase peptide synthesis of Fmoc-RGDS and Fmoc-RGDS-AAP.

Standard operation procedures (SOPs) for SPPS

Loading of the resin (SOP1)

Loading of the resin was performed according to reported literatures.[S5] The first amino acid (Fmoc-L-Serine or Fmoc-L-Serine-TEG-AAP, 1.5 eq. relative to resin loading) was dissolved in dry DCM and little amount of DMF. The above solution was added to 2-chlorotrityl-resin (1.6 mmol/g) under argon atmosphere. The first portion of DIPEA (2 eq. relative to resin loading) was added while agitating by the argon stream through the reaction mixture. After 5 min, another portion of DIPEA (3 eq. relative to resin loading) was added. The reaction mixture was then agitated by argon stream for 1.5 h. The solvent was removed and the unreacted resin was quenched by MeOH p.a. (1 mL/g resin) for 15 min. After filtration, the resin was washed with DCM p.a. (20 mL ×

2), DMF p.a. (20 mL × 3), DCM p.a. (20 mL × 3) and MeOH p.a. (20 mL × 3) respectively. The resin was dried overnight under vacuum. The loading ratio can be calculated by the increase of weight.

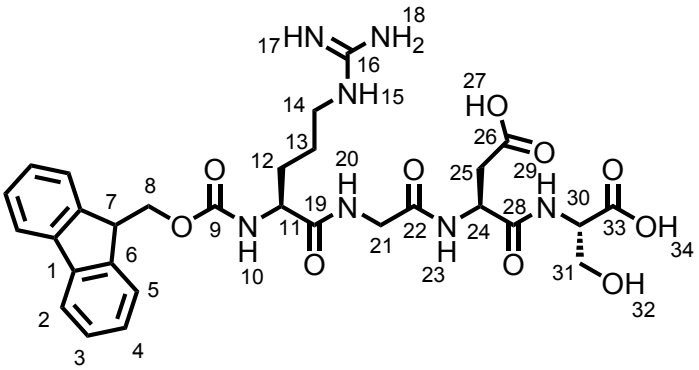
Stepwise elongation by SPPS (SOP2)

Stepwise elongation was based on literature.[S6] The dry bead was immobilised in DMF (20 mL) and swollen by shaking the reaction vessel for 1 h. After the DMF was sucked off, the Fmoc-protective group was cleaved by 20% piperidine in DMF for 20 min. The resin was washed with DMF p.a. (4 times) and alternatively with DCM p.a. (2 times) and isopropanol p.a. (2 times). A Kaisser test was applied to confirm the success of cleavage. A few beads were suspended in solution composed of equal amount of 5% ninhydrin in EtOH, 80% phenol in EtOH and 0.001 M KCN in pyridine and heated at 80°C for 1 min. A deep blue color was obtained indicating free amino groups on the resin. The next Fmoc-protective amino acid (3 eq. relative to loading resin) and Oxyma pure® (4 eq. relative to loading resin) were dissolved in SPPS grade DMF and mixed with resin. DIPCDI (4 eq. relative to loading resin) was added and the reaction vessel was shaking for 2 h. After the DMF was sucked off, the resin was washed with SPPS grade DMF (4 times). The success of coupling was confirmed by Kaiser test which showing no color change. The deprotection and coupling steps were repeated until a desired peptide was obtained. The Fmoc-protectiing group of the last amino acid should not be cleaved.

Cleavage of peptide from resin and removal of protecting groups (SOP3)

The cleavage from the resin and removal of protecting groups are based on reported literature.[S7] The resin was suspended in a solution of TFA:H_2O:TIS = 95:2.5:2.5 and stirred at room temperature overnight. The resin was washed by TFA (5 mL × 5) and the filtrate was collected and concentrated. The precipitation of peptide was achieved by addition of cold ether and the suspension was stored at the fridge overnight. The precipitate was collected by filtration.

Synthesis of **Fmoc-RGDS**[S2]



The peptide was synthesised as described in SOP1, SOP2 and SOP3 starting with Fmoc-Ser(tBu)-OH as first amino acid. In SOP2, Fmoc-Asp(OtBu)-OH, Fmoc-Gly-OH and Fmoc-Arg(Pbf)-OH were added respectively while chain elongation. The crude peptide obtained in SOP3 was further purified by preparative HPLC using acidic

condition (A: ddH_2O, 0.05% TFA; B: ACN/ddH_2O = 80/20 (v/v), 0.05% TFA) and lyophilisation as white powder.

HPLC gradient:

Time / min	0	50	60	65
Ratio A:B	95:5	20:80	95:5	95:5

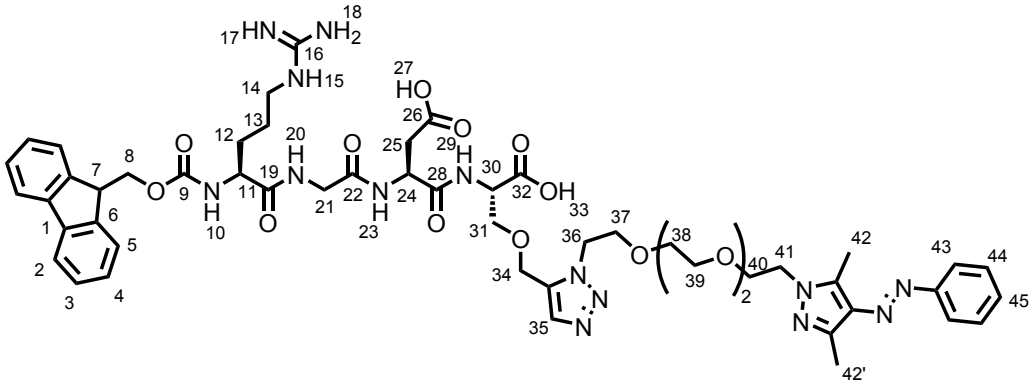
Molecular formula: $C_{30}H_{37}N_7O_{10}$

^1H (300 MHz, DMSO-d_6, 298 K): δ = 8.19, 8.17, 7.93 (m, 3H, amide bond, 20-, 23- and 29-H), 7.89 (d, 2H, 2-H), 7.73 (dd, 2H, 5-H), 7.6 (d, 1H, 10-H), 7.55 (t, 1H, 15-H), 7.42 (t, 2H, 3-H), 7.33 (t, 2H, 4-H), 4.72-4.63 (m, 1H, 24-H), 4.33-4.19 (m, 4H, 7-, 8- and 11-H), 4.06-3.98 (m, 1H, 30-H), 3.78-3.59 (m, 4H, 21- and 31-H), 3.13-3.04 (m, 2H, 14-H), 2.67-2.56 (m, 2H, 25-H), 1.8-1.65 (m, 1H, 12-H_a), 1.58-1.39 (m, 3H, 12-H_b and 13-H).

HR-MS (*m*/*z*): calculated for $[C_{30}H_{37}N_7O_{10}H]^+$: 656.26747, found: 656.26748.

These analysis data is consistent with reported literature.[S2]

Synthesis of **Fmoc-RGDS-AAP**



The peptide was synthesised as described in SOP1, SOP2 and SOP3 starting with Fmoc-Serine-TEG-AAP as first amino acid. In SOP2, Fmoc-Asp(OtBu)-OH, Fmoc-Gly-OH and Fmoc-Arg(Pbf)-OH were added respectively while chain elongation. The crude peptide obtained in SOP3 was further purified by preparative HPLC using basic condition (A: ddH_2O, 0.05% NH_3; B: ACN/ddH_2O = 80/20 (v/v), 0.05% NH_3) and lyophilisation as yellow powder.

HPLC gradient:

Time / min	0	50	60	65
Ratio A:B	95:5	20:80	95:5	95:5

Molecular formula: $C_{52}H_{66}N_{14}O_{13}$

^1H (500 MHz, DMSO-d_6, 298 K): δ = 8.22-8.11 (m, 3H, amide bond, 20-, 23- and 29-H), 8.00 (s, 1H, 35-H), 7.89 (d, 2H, 2-H), 7.77-7.68 (m, 4H, 5- and 43-H), 7.61 (d, 1H,

10-H), 7.54-7.27 (m, 9H, 3-, 4-, 5-, 44- and 45-H), 4.74-4.63 (m, 1H, 24-H), 4.52 (s, 2H, 34-H), 4.45 (t, 2H, 41-H), 4.32-4.15 (m, 4H, 7-, 8- and 11-H), 4.07-3.97 (m, 1H, 30-H), 3.79-3.67 (m, 7H, 17-, 21-, 31-, 36-H), 3.66-3.60 (m, 2H, 40-H), 3.49-3.38 (m, 10H, 37-39-H), 3.14-3.01 (m, 2H, 14-H), 2.76-2.63 (m, 2H, 25-H), 2.56, 2.37 (s, 6H, 42- and 42'-H), 1.75-1.60 (m, 1H, 12-H_a), 1.59-1.42 (m, 3H, 12-H_b and 13-H).

HR-MS (*m*/*z*): calculated for $[C_{52}H_{66}N_{14}O_{13}H]^+$: 1095.50065, found: 1095.50082.

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