**Supporting information**

# Sequence-defined antibody-recruiting macromolecules

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## Materials

Deuterated solvents, DMSO-$d_6$ (≥ 99.8 %) and $CDCl_3$-d (≥ 99.8 %) were purchased from Euriso-top. D,L-Homocysteine thiolactone hydrochloride (99 %) was purchased from Haihang Industry (Jinan City, China). Magnesium sulphate (dried ≥ 99 %), sodium hydrogen carbonate (≥ 99 %) and sodium chloride (≥ 99 %) were purchased from Carl Roth. Fmoc-protected rink-amide resin (100–200 mesh, 1% DVB, 0.4 mmol g−1) was purchased from Iris Biotech GmbH. Anhydrous dichloromethane (99.8 %), N,N'-dimethylformamide (99.8 %) and tetrahydrofuran (99.5 %), extra dried over molecular sieves, 1,4-dioxane (99 %+), pyridine (99 %+), piperidine (99 %), and Tin(II) 2-ethylhexanoate were purchased from Acros Organics. 2-Hydroxyethyl acrylate (96%), N-hydroxyethyl acrylamide (97%), methyl acrylate (99%, acrylic acid (99%), ethyl vinyl ether (99%), ethanolamine (>98%), 1-chloro-2,4-dinitrobenzene (97%, DNP-Cl), Biotin (>99%), trifluoroacetic acid (TFA , 99%), 4-dicyclohexylcarbodiimide (99 %), N,N'-diisopropylcarbodiimide and phenothiazine (>98%) were purchased from Sigma Aldrich and used as received. Acetonitrile (99.9 %, gradient grade), chloroform and methanol, HPLC grade, were also purchased from Sigma Aldrich. 4-Dimethylaminopyridine (DMAP) and triphosgene, (98.0 %) were purchased from Tokyo Chemical Industry. Diethyl ether, analytical reagent grade and N,N'-dimethylformamide (99.8 %) laboratory reagent grade were purchased from Fischer Scientific. Acetone, HPLC grade HiPerSolv CHROMANORM was purchased from VWR. When anhydrous chloroform was required, HPLC grade chloroform was dried over calcium hydride.

## Characterization

### Liquid chromatography mass spectrometry (LC-MS)

LC-MS spectra were recorded on an Agilent technologies 1100 series LC/MSD system equipped with a diode array detector and single quad MS detector (VL) with an electrospray source (ESI-MS) for classic reversed phase LC-MS and MS analysis. All results were recorded in positive mode unless otherwise stated. Analytic reversed phase HPLC (high-performance liquid chromatography) was performed with a Phenomenex C18 (2) column (5 µm, 250 × 4.6 mm) using a solvent gradient (0 → 100 % acetonitrile in $H_2O$ in 10 min) and the eluting compounds were detected via UV-detection (λ = 214 nm). The purities were determined by LC-MS from the UV absorbance at 214 nm and 360 nm by comparing areas under the peaks of compounds with a molecular weight of >400 g/mol to exclude contamination as a result of improper washing or drying.

### Nuclear magnetic resonance (NMR) spectroscopy

$^1H$ spectra were recorded on a Bruker Avance 300 (300 MHz) and a Bruker Avance 400 (400 MHz). $CDCl_3$ or DMSO-$d_6$ were used as solvents. Chemical shifts are presented in parts per million (δ) and calibrated to the characteristic residual solvent signal at 2.50 ppm ($^1H$, DMSO-$d_6$) or 7.24 ppm ($^1H$, $CDCl_3$).

### Matrix-assisted laser desorption/ionization – Time of flight (MALDI-ToF)

The MALDI-ToF measurements were conducted on an Applied Biosystems Sciex 4800+ MALDITOF/TOF analyser, controlled by a 4000 Series Explorer software (Applied Biosystems, Germany). Stock solutions of the matrix, trans-2-[3-(4-tert-butylphenyl)-2-methyl-2propenylidene]malonitrile (DCTB, 30 mg/mL) and sodium trifluoroacetate (NaTFA, 10 mg/mL) were solubilized in DMF. Oligomeric samples were

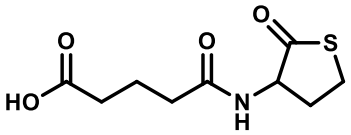
prepared also in tetrahydrofuran (10 mg/mL). To spot the MALDI plate, 45 µL of the matrix stock solution was mixed with 15 µL of the stock solutions of each, the salt and the sample. The spots were dried and then the plate was loaded into the instrument. The measurements were performed in reflective positive ion mode.

### Biolayer Interferometry

Before starting the experiment, biosensors were hydrated in PBS (+ Ca2+ and Mg2+) for at least 10 minutes. A dilution series of the biotinylated oligomers was prepared in PBS (+ Ca2+ and Mg2+). A 500 nM stock solution of anti-DNP antibody was prepared in PBS (+ Ca2+ and Mg2+) containing 0.05 % Tween. The assay was performed in a black flat bottom 96-well plate using an Octet RED96 system (Pall Fortébio). First, a baseline was recorded in PBS (+ Ca2+ and Mg2+) for 60 seconds. A dilution series of biotinylated oligomers was loaded onto the sensors for the next 300 seconds followed by a washing step for 30 seconds in the same buffer as the baseline. Before recording the association of the anti-DNP antibody for 600 seconds, a second baseline was performed for 120 seconds in PBS (+ Ca2+ and Mg2+) containing 0.05 % Tween. Finally, dissociation was recorded for 600 seconds in the same buffer as the second baseline. The concentration of biotinylated oligomer that resulted in a binding response of anti-DNP antibody between 0.5 and 1 nm was selected for the use in further experiments. For the latter, sensors were loaded with the selected concentration of biotinylated oligomers. All sensors were then incubated with different concentrations of anti-DNP antibody as described in detail above. Curve fitting and calculation of KD values was performed by the FortéBio software package using an Octet RED96 model.

## Synthesis and characterization of the building blocks

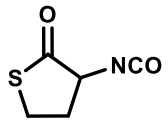
**Acid-functionalised thiolactone (TLa-COOH) used for the first coupling to the solid-support**




TL-COOH was synthesised following a previously published procedure.[1] Typically, DL-homocysteine thiolactone hydrochloride (TLa $NH_2$.HCl) (30.0 g, 0.20 mol, 1 eq.) was dissolved in 450 mL $H_2O$/1,4-dioxane mixture (1:1) and cooled with an ice bath. Sodium bicarbonate (82.0 g, 0.98 mol, 5 eq.) was added portion wise. The reaction mixture was stirred for 30 min. Then glutaric anhydride (44.6 g, 0.39 mol, 2 eq.) was slowly added. The mixture was allowed to warm up to room temperature and stirred overnight. The pH of the solution was adjusted to 1 by the addition of a concentrated aqueous hydrochloric acid solution. The white precipitate was filtered off and dried. The solution was extracted with ethyl acetate (3 x 200 mL). The combined organic phases were dried using magnesium sulphate, filtered and the solvent was removed under reduced pressure. The remaining solid was recrystallised in acetone. TLa-COOH was obtained as white crystals after filtration. Yield: 41.7 g, 0.18 mol, 93%.

**$^1$H-NMR (300 MHz, DMSO- $d_6$):** δ (ppm) = 12.03 (s, 1H, C-N*H*-CH), 8.17-8.15 (d, 1H, *H*O-C-), 4.64-4.54 (m, 1H, -N-C*H*-(C-)CH2-), 3.43-3.24 (m, 2H, -$CH_2$-C$H_2$-S-), 2.45-2.36 (m, 1H, -CH-C(*H)*H-$CH_2$-), 2.25-1.99 (m, 5H, -C-C$H_2$-$CH_2$-, -$CH_2$-C$H_2$-C, -CH-C(*H)*H-$CH_2$-), 1.76-1.66 (m, 2H, -$CH_2$-C$H_2$-$CH_2$-).

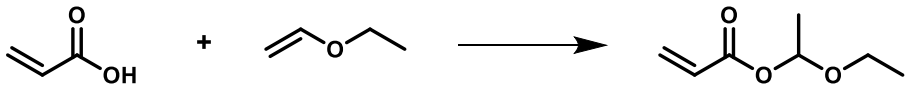
## *α*-Thiolactone-*γ*-isocyanate (TLa-NCO)




The synthesis was carried out according to a previously reported procedure.[1] Triphosgene (50 g, 168 mmol, 0.33 equiv.) was dissolved in ice-cooled $CH_2Cl_2$ (900 mL) in a two-neck flask, placed under argon atmosphere and stirred for 15 minutes. Subsequently, DL-homocysteine thiolactone hydrochloride (74 g, 482 mmol, 1 eq.) was added. Next, dry pyridine (120 mL, 1.5 mol, 3.11 equiv.) was added dropwise over a time span of 20 minutes using an addition funnel. After 1 hour, the ice-bath was removed and the mixture was stirred for an additional 4 hours. The aqueous work-up of the crude mixture was performed fast to avoid degradation. The crude mixture was directly filtered into a separation funnel to remove the pyridinium hydrochloride salt and was rinsed with cold $CH_2Cl_2$. The organic phase was washed with an ice-cooled 2 M HCl solution (500 mL), ice water (500 mL) and brine (500 mL). The water phase was extracted an additional time with 300 mL $CH_2Cl_2$. All the organic phases were collected and dried with magnesium sulphate and subsequently filtered over a large Büchner filter and concentrated in vacuo. A brown liquid was obtained that was further purified by vacuum distillation, the fraction between 83–95 °C (0.08 mbar) was collected, yielding a transparent liquid (61.04 g, 88.5%).

**$^1$H-NMR** (300 MHz, $CDCl_3$, *δ*): 4.23 (dd, 1H, J=12.6, 6.8 Hz), 3.30 (m, 2H), 2.64 (m), 2.11 (m). **$^{13}$C-NMR** (125 MHz, $CDCl_3$, *δ*): 203.1 (C), 127.6 (C), 62.6 (CH), 32.1 (CH2), 27.0 (CH2).

## 1-ethoxy ethyl acrylate (1-EA)

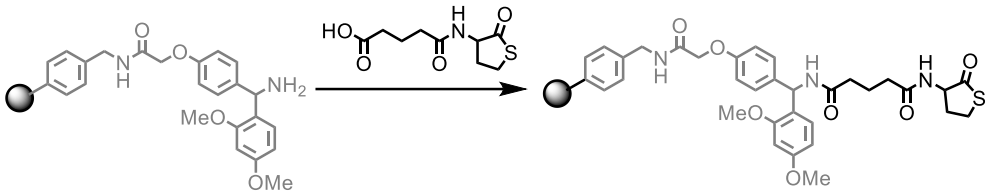



1-EA was synthesised by the acid-catalysed addition reaction of acrylic acid and ethyl vinyl ether as previously reported.[2] In a nitrogen flushed 1 L round bottomed flask, 114.9 mL of ethyl vinyl ether (1.2 mol) and 200 mg of phosphoric acid as a catalyst were placed and cooled down while stirring to 0°C in an ice bath for 15 minutes. Next, 85.2 mL of acrylic acid (1.0 mol) was slowly added to the mixture and the reaction sealed. After 1 hour, the ice bath was removed and the reaction allowed to stir for an additional 48 hours at room temperature. Next, the excess catalyst was removed by passing the mixture over a column of pre-washed $Mg_6Al_2(OH)_{16}$-$CO_3$·$4H_2O$ and the excess vinyl ether removed under reduced pressure. The product was distilled using phenothiazine as inhibitor and collected as a clear solution (90%).

**$^1$H NMR (300 MHz,** $CDCl_3$, *δ*, ppm**):** 1.07 (3H, t, -$OCH_2CH_3$), 1.31 [3H, d, -COOCH($CH_3$)], 3.40-3.66 (2H, m, -$OCH_2$-), 5.70 (1H, d, $CH_2$=C*H*-), 5.92 (1H, q, -COOC*H*($CH_3$)), 6.03 (1H, dd, $CH_2$=C*H*-), 6.28 (1H, d, $CH_2$=CH-). $^{13}$C NMR ($CDCl_3$, δ, ppm): 14.8 (-$OCH_2CH_3$), 20.8 (-COOCH(*C*H3)), 64.8 (-O*C*H2-), 96.2 (-COO*C*H(CH3)), 128.3 (*C*H2=CH-), 130.5 (CH2=*C*H-), 165.9 (-*C*OO-).

## Synthesis of the sequences

### 1. Loading of the resin with TLa-COOH



Fmoc-protected resin (2.5 g, 1.0 equiv.) was swollen for 5 min in 20 mL anhydrous $CH_2Cl_2$. The solvent was then filtered off and the resin was washed twice with $CH_2Cl_2$. A 20 % piperidine in $CH_2Cl_2$ solution (20 mL) was added and the reaction mixture was shaken vigorously at room temperature for 4 h to remove the Fmoc protecting group. The reaction was then filtered and the resin washed with DMF (3 x 30 mL) and $CH_2Cl_2$ (3 x 30 mL). The resin was again swollen for 5 minutes in anhydrous $CH_2Cl_2$ (16 mL). The thiolactone-carboxylic acid molecule (2.08 g, 5.0 equiv.), N,N'-dicyclohexylcarbodiimide (DCC) (1.86 g, 5.0 equiv.), 4-dimethylaminopyridine (DMAP) (23 mg, 0.1 equiv.) and anhydrous DMF (4 mL) were added. The reaction was shaken vigorously at room temperature for 36 hours. The resin was then filtered and washed with $CH_2Cl_2$ (3 x 30 mL), DMF (3 x 30 mL) and diethyl ether (3 x 30 mL). The resin was then dried under vacuum at room temperature for 2 hours.

### 2. Chain growth

The macromolecules were synthesised by repeating the 1st and 2nd step described below until the targeted dimers or heptamers were formed. Afterwards, biotin was added at the chain end and the final macromolecules were subsequently cleaved from the support:

1st step (i) or (ii) in Scheme 1: One pot aminolysis of the thiolactone ring and nucleophilic aromatic substitution with DNP-Cl (Scheme 1(i)) or thiol-Michael reaction using either methyl- or 1-ethoxyethyl-acrylate (Scheme 1(ii)).

- #### General procedure for the aminolysis of the thiolactone and incorporation of DNP in the side chain

DNP-Cl (200 mg, 20 eq.) was dissolved in 1 mL of chloroform and added to the freshly washed resin. Under gentle shaking, ethanolamine (60 µL, 20 eq.) was added and the reactor immediately sealed and allowed to react for 30 minutes. To ensure full functionalisation, the above steps were repeated twice, after which the resin was washed using chloroform (3x), DMF (3x) and chloroform (3x).

- #### General procedure for the aminolysis of the thiolactone and incorporation of 1-ethoxy ethyl acrylate (1-EA) in the side chain

The freshly washed resin was swollen in 1 mL of chloroform for 10 minutes. Next, 144.17 µL of 1-EA (20 eq.) was added and the reactor gently shaken to fully homogenise the monomer in solution. Then, 30 µL of ethanolamine (10 eq.), the reactor was sealed and allowed to react for 30 min. To ensure full functionalisation, the above steps were repeated twice after the resin was washed using chloroform (3x), DMF (3x) and chloroform (3x).

2nd step (Scheme 1 (iii)): Chain extension with TLa-NCO.

- General procedure for the addition of TLa-NCO

Anhydrous chloroform (1 mL) was added to a 5 mL reactor containing 100 mg of the loaded OH-functionalized resin (obtained after the previous step, see Scheme 1) for the swelling step that lasted 10 minutes. Next, TLa-NCO (20 eq.) and Tin(II) 2-ethylhexanoate (20 µL) were added to the mixture, the reactor sealed and the reaction allowed to proceed. After 15 minutes shaking, the resin was washed using copious amounts of DMF (3x) and chloroform (3x), and the above described steps repeated for a second time, to ensure 100% functionalisation. After the reaction was completed, the resin was washed using DMF (3x), MeOH (3x), chloroform (3x) and diethylether (3x) to continue with the aminolysis steps.

### 3. End-functionalisation using Biotin-COOH

OH terminal macromolecules were functionalised with Biotin-COOH using a simple DIC/DMAP coupling. For this purpose, Biotin (122 mg, 10 eq.) was dissolved in 2 mL of DMF under stirring and gentle heating. In parallel, the resin was pre-washed with 5 mL of DMF, to avoid any precipitation. Next, the Biotin solution was transferred to the resin-containing reactor and gently shaken to disperse the resin in solution. Then, DIC (73 µL, 9 eq.) was added and the reactor shaken once again. Finally, 6 mg of DMAP (0.5 eq.) was added and the reactor quickly sealed and the reaction allowed to proceed for 1 h. The above steps were repeated for a second and third time to ensure full functionalisation after washing the resin with copious amounts of DMF, MeOH, chloroform, DMF in-between reactions.

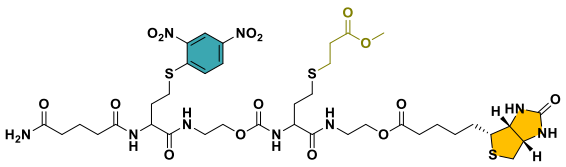
### 4. Cleavage

All macromolecules were washed a final time using DMF (3x), MeOH (3x), Chloroform (3x) and $Et_2O$ (3x).

Cleavage of the product prior to LC-MS/SEC analysis was achieved through the treatment of the resin (2 mg for LC-MS, 5 mg for SEC) with a 10% (v/v) trifluoroacetic acid (TFA) solution in $CH_2Cl_2$ (1 mL) for 15 minutes. The immediate formation of a red colour is related to the formation of on-resin cations, and their intensity allows a qualitative determination of the cleavage process. Next, the solid support was removed through filtration, and the sample was concentrated in vacuo. Finally, the obtained sample was dissolved in acetonitrile or THF (1 mL) for LC-MS (measured for M1 and M2) or MALDI-ToF MS analysis (measured for M3-M5).

## Characterization of the sequences

### Sequence M1:



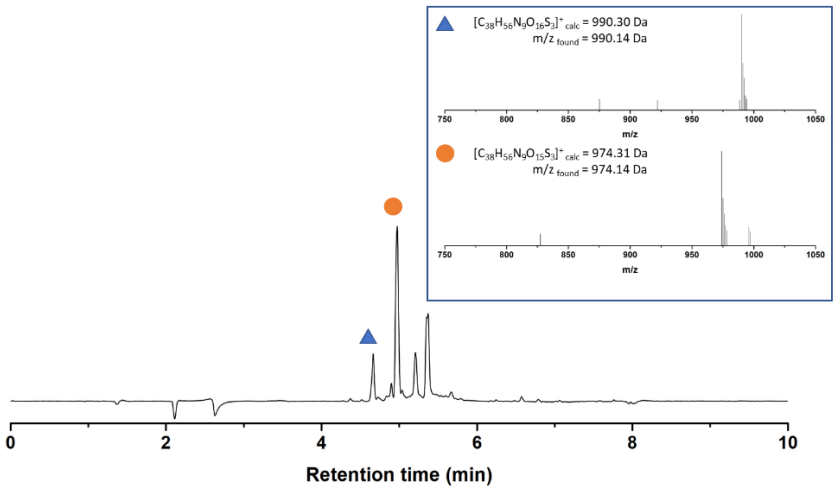



**Figure S1.** LC-MS spectra of **M1** (λ = 360 nm). The blue triangle corresponds to the thioether oxidation of the desired product. The two peaks on the right cannot be identified. They do not correspond to truncated structures and are ascribed to a contamination of the LCMS samples.

Sequence M2:

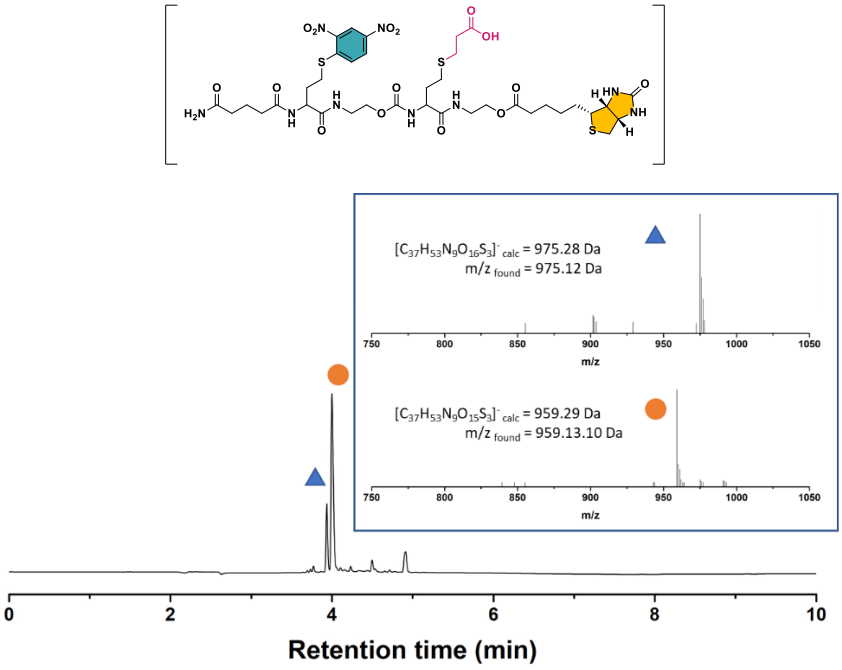



**Figure S2.** LC-MS spectra of **M2** (λ = 214 nm). Blue triangle: Thioether oxidation of the desired product.

Sequences M3:

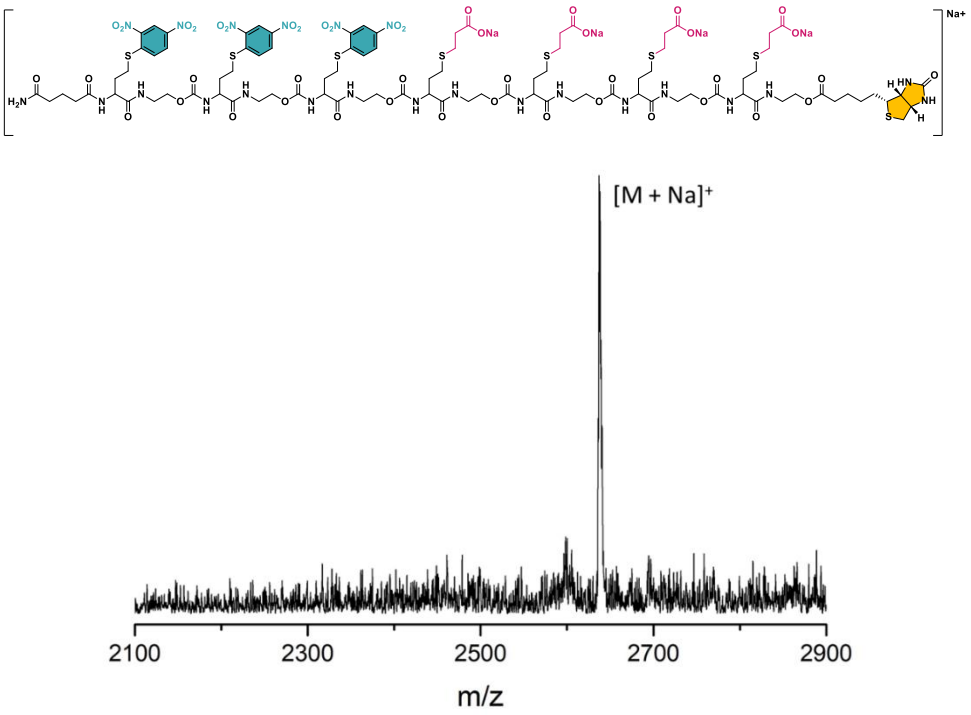



**Figure S3**. MALDI-ToF spectrum of **M3**. $[M+Na]^+_{calc}$ = 2638.55, $[M+Na]^+_{found}$ = 2637.17]

Sequence M4:

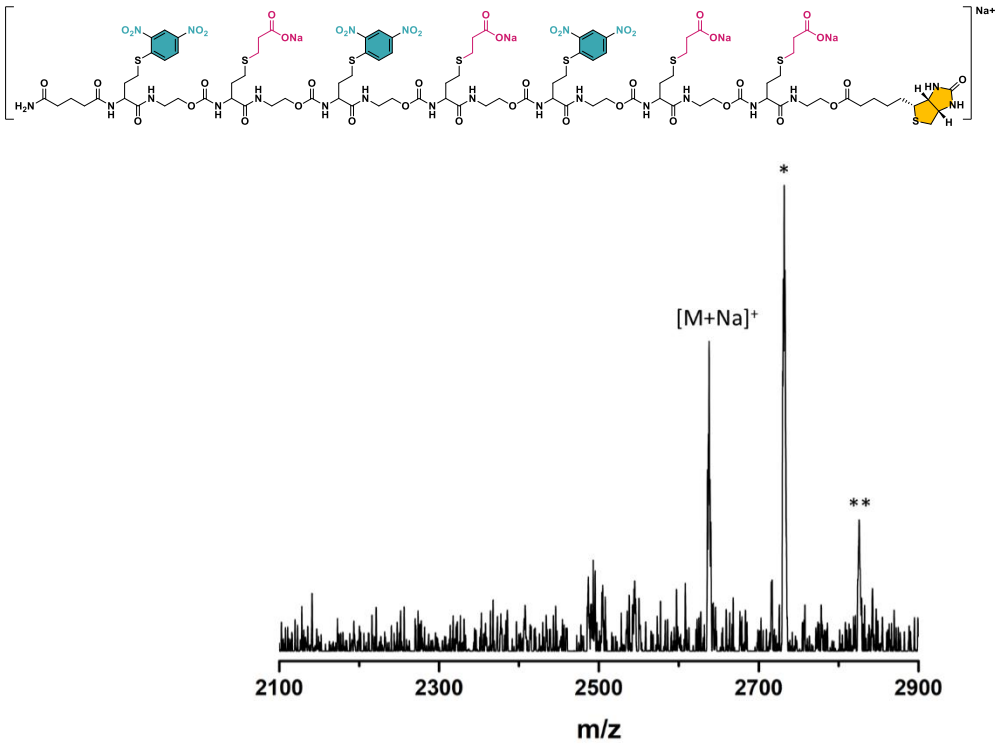
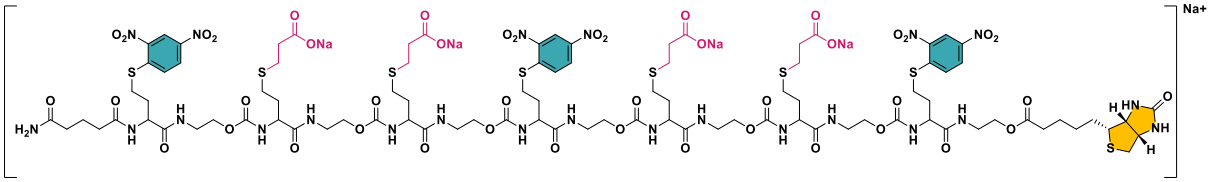



**Figure S4**. MALDI-ToF spectrum of **M4**. $[M+Na]^+_{calc}$ = 2638.55 , $[M+Na]^+_{found}$ = 2638.07. * Corresponds to $[M+TFA+Na]^+$ and ** to $[M+2TFA+Na]^+$.

Sequence M5:



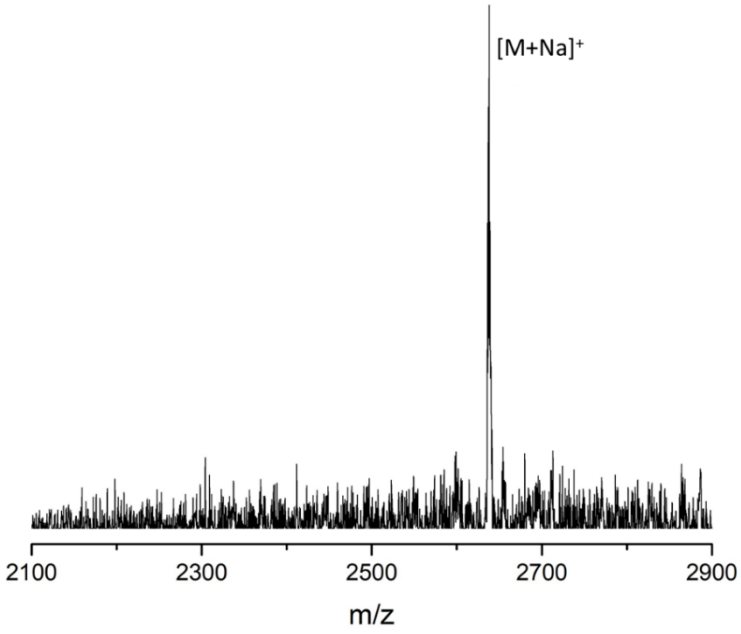




**Figure S5**. MALDI-ToF spectrum of **M5**. $[M+Na]^+_{calc}$ = 2638.55 , $[M+Na]^+_{found}$ = 2638.12

## Molecular dynamics (MD)

### Protocol

Carboxylates, DNP and biotin residues were built using Discovery Studio 2019.[3] The charges were calculated within the antechamber module from AMBER16[4] using AM1-BCC charge model.[5] The General AMBER Force Field (GAFF2)[6] was used for the other atomic parameters. The residues were bound together with the LEaP program implemented in AMBER16. The same program was used to add water molecules (TIP3P model) in a truncated octahedron box with edge no closer than 10.0 Å from the oligomer. Sodium ions were added to neutralize the system. The geometry of the water molecules and ions were optimized by fixing the oligomer with positional restraints and a force constant of 25.0 kcal.mol$^{-1}$ Å$^{-2}$ with a first minimization consisting of 1,000 steps of steepest descent followed by 9,000 steps of conjugate gradient algorithms. The entire system was then minimised using the same procedure but without restraints on the oligomers. A heating from 0 to 300 K was performed in *N,V,T* ensemble with periodic boundaries conditions (PBC) during 1 ns with a 2 fs time step, followed by a 1 ns equilibration process. The 2 fs time step was used as we constrained the length of bonds involving hydrogen atoms with the SHAKE algorithm.[7] During this 2 ns dynamic, the oligomer was restrained with a force constant of 10 kcal.mol$^{-1}$ Å$^{-2}$. A 755 ns MD was then performed in PBC in the *N,P,T* ensemble at 1 bar with Berendsen barostat. The temperature was maintained at 300 K using the Langevin thermostat with a 1.0 ps$^{-1}$ collision frequency. Electrostatic energy was calculated with the

Particle Mesh Ewald (PME) method with a cutoff of 10.0 Å. Positional restraints were applied on biotin residues with a restraint force constant of 25.0 kcal.mol$^{-1}$ Å$^{-2}$ during the whole MD. Production MD was considered after equilibration of the system (380 ns), and the last 375 ns of MD were used to perform the analysis. Snapshots were recorded every 0.5 ns resulting in a set of 750 structures for analysis. All the analysis were performed using the cpptraj module[8] implemented in AMBER. The area of the triangle ABC formed by the DNPs was measured by considering the geometric center of the DNP aromatic cycle as vertex and was calculated with the formula: $A = \frac{1}{2}\left|\overrightarrow{AB} \times \overrightarrow{AC}\right|$. The Solvent-Accessible Surface Area (SASA)[9] of the DNP fragments was calculated with the Linear Combinations of Pairwise Overlaps (LCPO) method.[10] The MD were visualized with VMD 1.9.3[11] and the snapshots were rendered with PyMOL 2.3.0.[12]

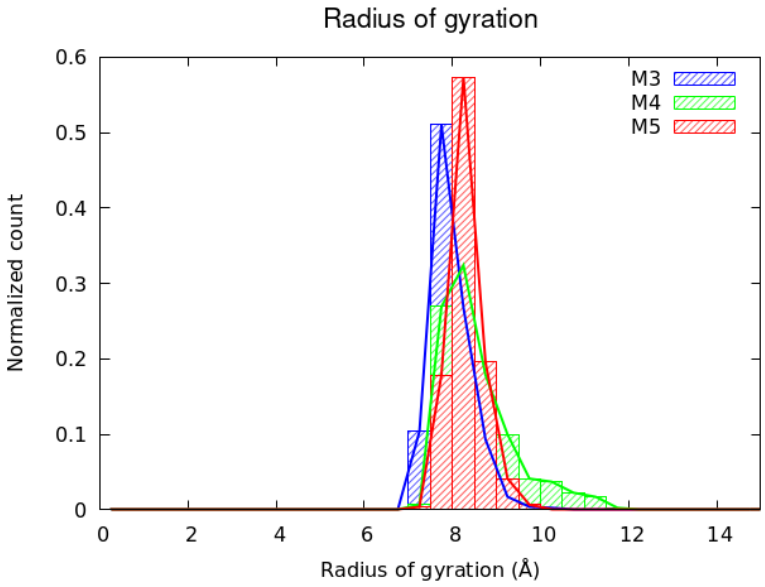



**Figure S6.** Distribution plot of the radii of gyration for molecules M3, M4 and M5.

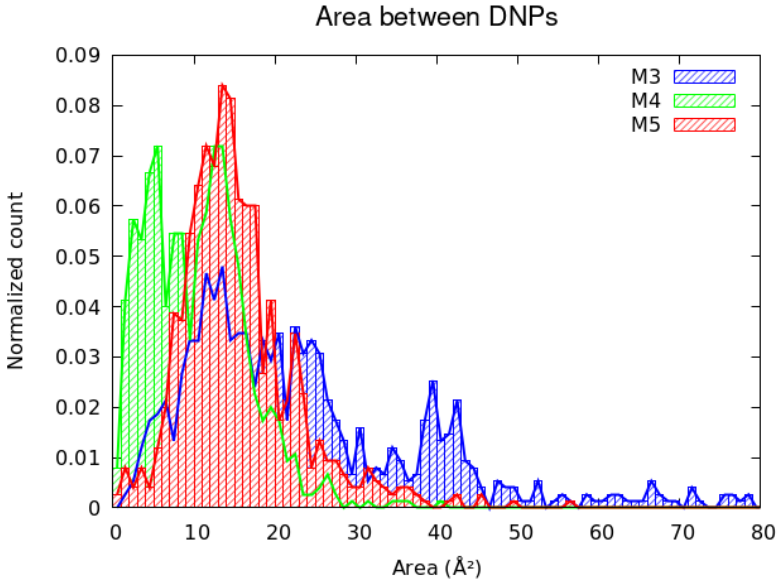



**Figure S7.** Distribution plot of the areas between DNPs for molecules M3, M4 and M5.

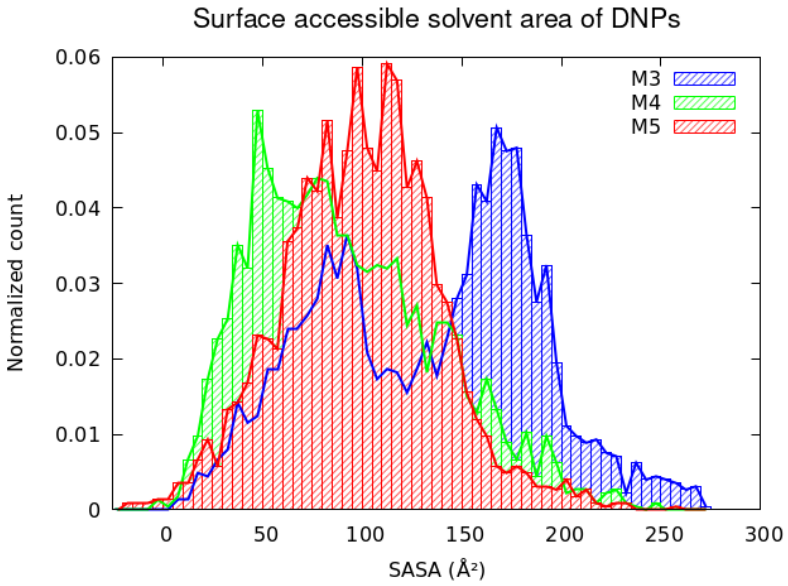



**Figure S8.** Distribution plot of DNPs SASA for molecules M3, M4 and M5.

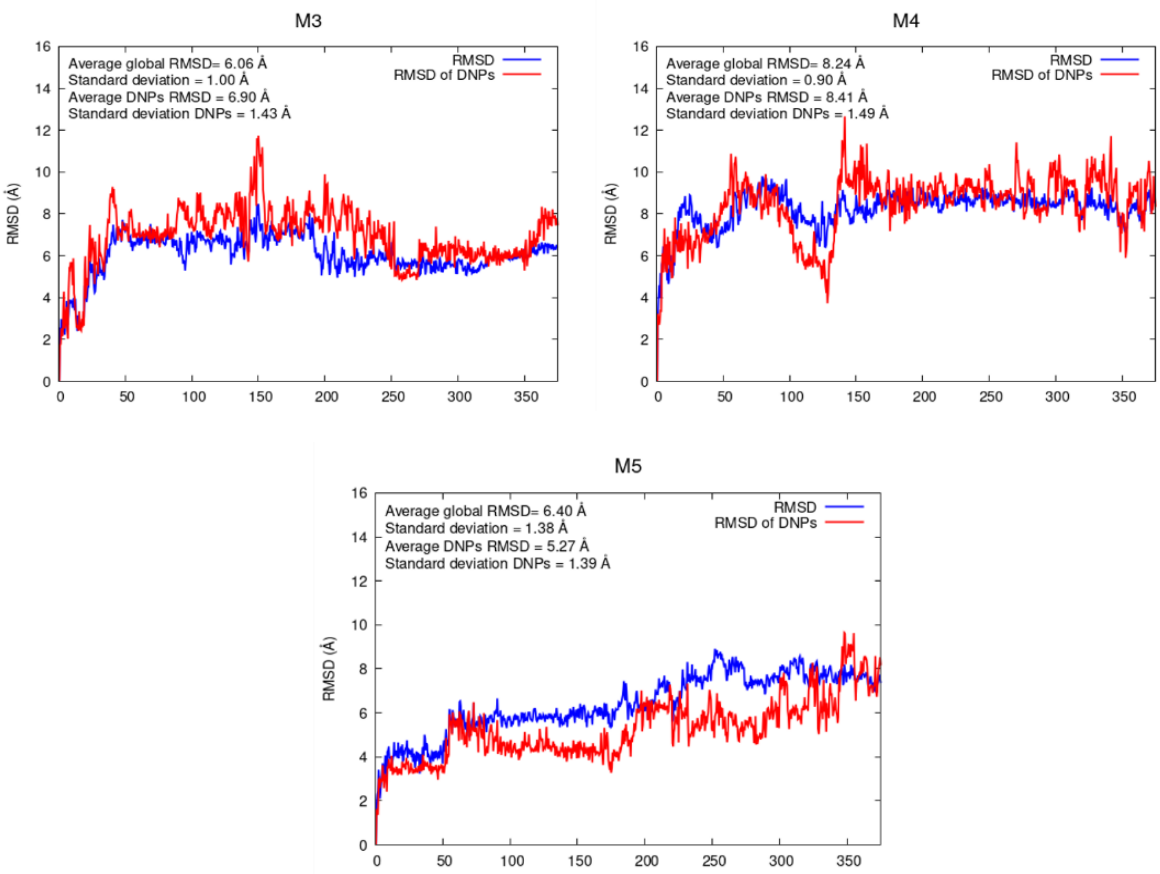



**Figure S9.** RMSD graphs for heavy atoms of molecules M3, M4 and M5. The blue line represents the global RMSD of the molecule and the red line represents the RMSD of the DNPs.

**Table S1.** Average SASA values for individual DNP residues. The DNPs are indexed by their position in the sequence, starting from the terminal amide to the biotin.

| Molecule | DNP | Average SASA (Å$^2$) |
|---|---|---|
| **M3** | 1 | 128.2 |
| | 2 | 109.6 |
| | 3 | 161.9 |
| **M4** | 1 | 94.1 |
| | 2 | 77.4 |
| | 3 | 104.0 |
| **M5** | 1 | 91.3 |
| | 2 | 107.8 |
| | 3 | 99.6 |

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