Supporting Information

Post-Assembly Functionalization of Supramolecular Nanostructures with Bioactive Peptides and Fluorescent Proteins by Native Chemical Ligation

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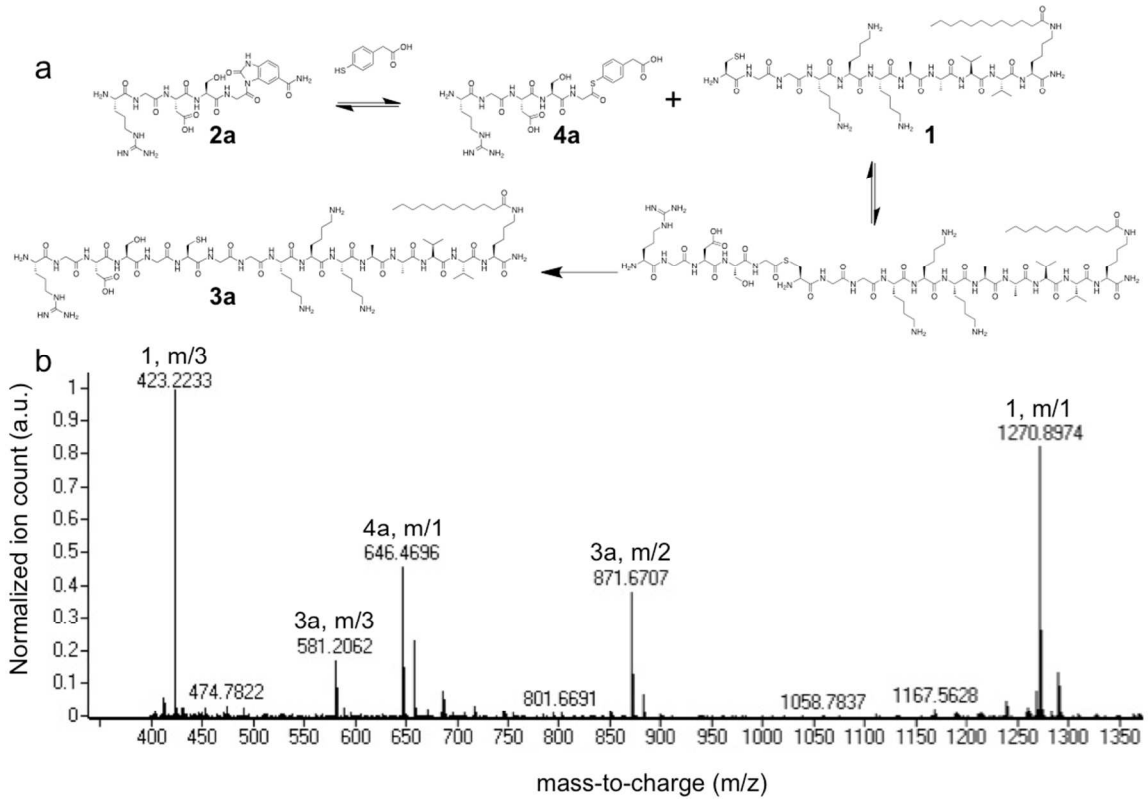
Supplementary Methods

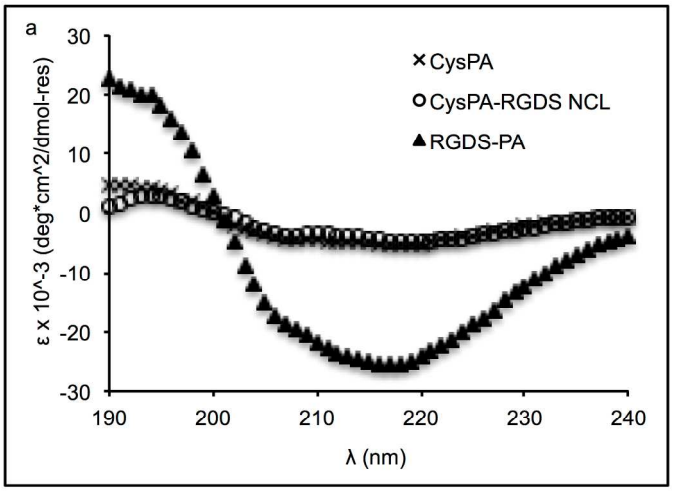
Construction of the Expression Plasmids pTXB1-EYFP and pTXB1-ECFP

All primers were ordered from MWG-Biotech AG. PCR reactions were performed on a Bio-Rad iCycler Thermal Cycler and analyzed on a 1% or 2% agarose gel using a Biorad sub-cell system. *E. coli* Novablue competent cells were purchased from Novagen and made electrocompetent by the supplier's instruction. Transformation by heat shock or electroporation was performed according to the manufacturer's protocol. 100 μg/mL ampicilin (Amp) was used for antibiotic selection. Plasmid DNA from transformed *E. coli* Novablue cells was purified with the QIAprep Spin Miniprep Kit. The concentration of the extract was determined by measuring the absorbance at 260 nm on a Shimadzu Multispec 1501 spectrometer. Plasmid DNA was sent to BaseClear (Leiden, The Netherlands) for sequencing. The IMPACT vector system pTXB1 was purchased from New England Biolabs. The expression vectors pECFP-C1 and pEYFP-N1 (Clontech) were used as source of the EYFP and ECFP genes. The genes encoding EYFP and ECFP were amplified by PCR (94 °C, 1 min, 2 cycles | 94 °C, 30 sec; 68 °C, 3 min; 35 cycles | 68 °C, 3 min, 1 cycle | 4 °C, ∞, 1 cycle) using the forward primer (5'-GGTGGTCATATGGTGAGCAAGGGCGAG-3') and the reversed primer (5'-GGTGGTGAATTCCTTGTACAGCTCGTCCATGC-3'). The forward primer also introduces an Nde1 restriction site at the 5', while the reversed primer introduces an EcoR1 restriction site at the 3'. pTXB1 and the amplified PCR products were double-digested by the restriction endonucleases EcoR1 and Nde1. 1 μg of DNA was digested with 10 units EcoR1 and 10 units Nde1 simultaneously during overnight incubation at 37 °C. The digested vector and PCR products were purified with the QIAquick gel extraction kit (Qiagen). The concentration of the purified products was assessed on a 1% agarose gel using a molecular mass ruler (BioRad). Ligations with 50 ng of the digested pTXB1 vector with the purified PCR products were carried out using T4 DNA ligase from the TA cloning kit (Invitrogen). Total volume of the ligation mixtures was 20 μL with a ratio of 1:3 or 1:6 between the vector and the insert. Ligation was performed overnight at 14 °C. The ligation mixtures were transformed into *E. coli* Novablue competent cells and plated on LB agar plates containing Amp and grown overnight at 37 °C. For colony PCR (95 °C, 15 min, 1 cycle| 94 °C, 45 sec; 54 °C, 1 min; 72 °C, 1 min; 40 cycles| 72 °C, 6 min, 1 cycle | 4 °C, ∞, 1 cycle) single colonies were transferred to a masterplate by a toothpick and boiled in 30 μL milliQ for 10 minutes. 5 μL of the resulting suspension was used in the PCR mixture together with Hotstart DNA polymerase (Stratagene) according to the supplier's protocol. The T7 forward primer (5'-TAATACGACTCACTATAGGG-3') and the reversed primer (5'-GGCACGATGTCGGCGATG-3') were used. Colony PCR was analyzed on a 2% agarose gel and 2 positive clones for each construct were sent for sequencing with the T7 forward primer and the reversed primer. Overnight cultures of the positive colonies were stored in 15% (w/v) glycerol at -80 °C.

Supplementary Figures

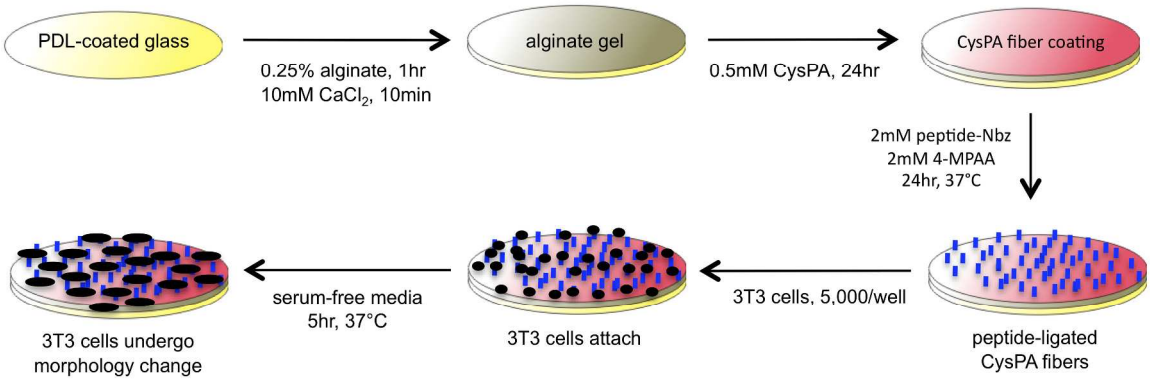


Supplementary Figure 1. Confirmation of NCL reaction mechanism. The NCL reaction mechanism proceeds with initial substitution of the Nbz leaving group with 4-MPAA to form an RGDS-thioester, which is attacked by the thiol on the CysPA, followed by an irreversible intramolecular rearrangement to generate RGDS-PA (a). The RGDS-thioester is observed in the mass spectrum of the NCL reaction mixture at 3 hours (b).

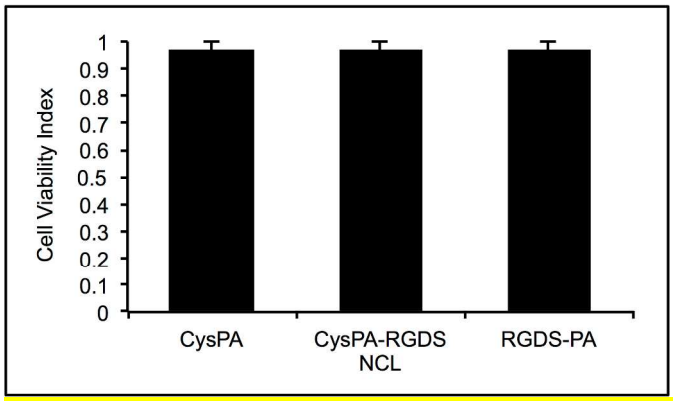


b Condition	α-helix	β-sheet	rnd. coil	R^2
CysPA	0%	57%	43%	0.93
CysPA-RGDS NCL	0%	56%	43%	0.87
RGDS-PA	45%	26%	29%	0.90

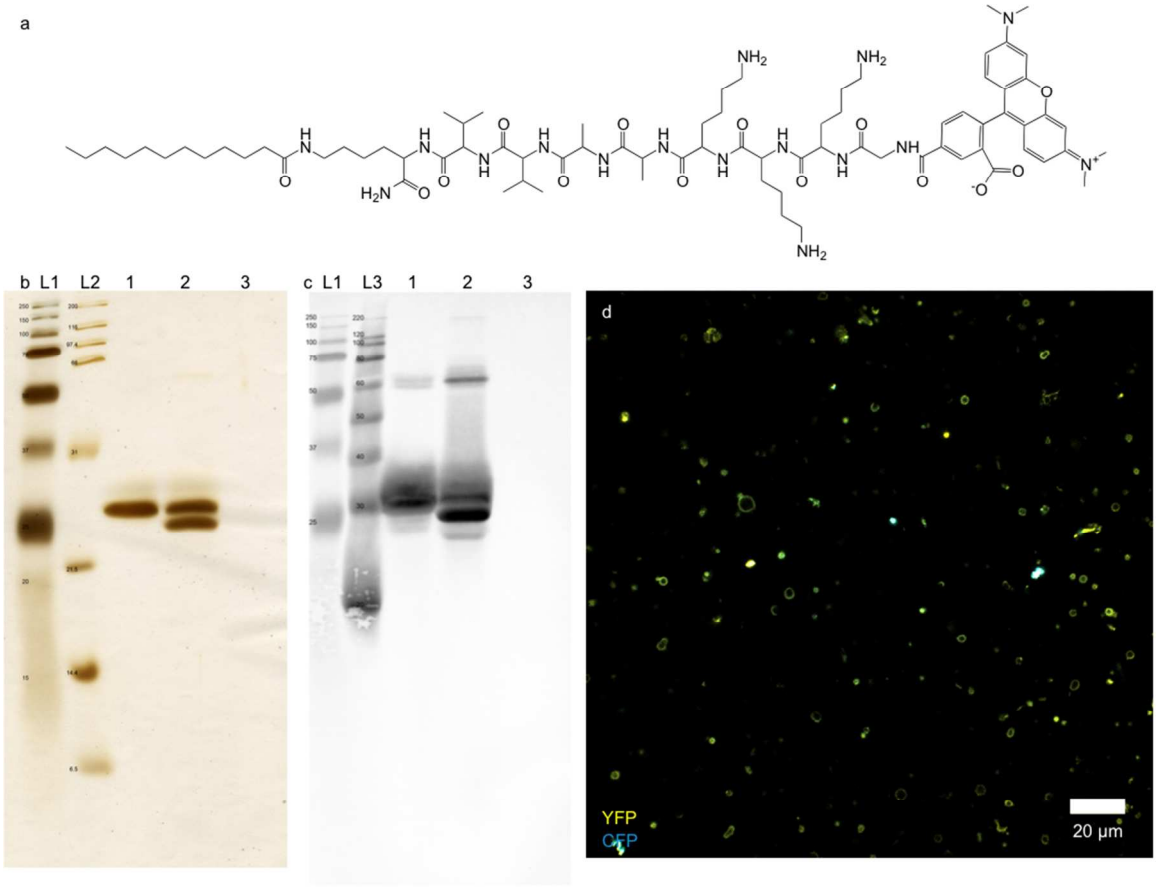
Supplementary Figure 2. (a) CD spectra of the CysPA, CysPA-RGDS NCL reaction mixture, and RGDS-PA in PBS. (b) These spectra were fit to linear combinations of reference spectra using the PEPFIT algorithm.



Supplementary Figure 3. Schematic of PA-coated surface experiments. Poly-D-lysine-coated glass coverslips are coated with a layer of alginate cross-linked with calcium chloride and further coated with CysPA nanofibers. The CysPA nanofibers on the surface are functionalized with peptide via the NCL reaction. 3T3 cells are seeded on the coverslips, and cell morphology is assayed after a 5-hour incubation period.



Supplementary Figure 4. NIH/3T3 fibroblasts cultured for 5 hours on coverslips coated with CysPA, CysPA reacted with RGDS-Nbz via NCL, and RGDS-PA were analyzed for cell viability by staining with calcein-AM and ethidium homodimer-1. The live cell index is calculated as the proportion of calcein-AM cells to total cells. Error bars indicates a 95% confidence interval.



Supplementary Figure 5. (a) The structure of the TAMRA-labeled PA used for FRET. (b-c) SDS-PAGE with visualization by silver stain (b) and Western blot against GFP (c) were used to analyze the NCL reaction with YFP only (lane 1), YFP and CysPA (lane 2), and CysPA only (lane 3) compared to standards (L1 = Precision Plus, L2 = Broad Range, L3 = Magic Mark XP). (d) Confocal micrograph at low magnification of CysPA nanofiber-coated alginate microparticles reacted simultaneously with YFP and CFP.