Supporting Online Material for

Supramolecular Copolymers with Stimuli-Responsive Sequence Control

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Contents

Materials

Chemicals were purchased from Sigma-Aldrich and used without further purifications. Cy3-NHS and Cy5-NHS esters were purchased from Lumiprobe. DNA oligos with different length were obtained from Eurofins. Ribonuclease A from bovine pancreas (Type XII-A, ≥ 90 %, ~100 Kunitz units / mg) was obrtained from Sigma as lyophilized powder. Dialysis membranes were obtained from Spectrum Laboratories. 1H-NMR and 13C-NMR spectra were recorded on a Varian Mercury Vx 400 MHz NMR spectrometer. Matrix-assisted laser desorption/ionization mass spectra were obtained on a PerSeptive Biosystems Voyager DE-PRO spectrometer or a Bruker autoflex speed spectrometer using α-cyano-4-hydroxycinnamic acid (CHCA) and 2-[(2E)-3-(4-tert-butylphenyl)-2-methylprop-2-enylidene] malononitrile (DCTB) as matrices. Infrared spectra were recorded on a Perkin-Elmer Spectrum One 1600 FT-IR spectrometer or a Perkin-Elmer Spectrum Two FT-IR spectrometer, equipped with a Perkin-Elmer Universal ATR Sampler Accessory. The synthesis of BTA, BTA-Cy3 and BTA-Cy5 have been previously reported[1].

BTA Copolymers Assembly

Stock solutions of BTA, BTA-Cy3 and BTA-Cy5 in methanol were prepared. The dried products obtained after preparative LC-MS purification were dissolved in methanol to an approximate concentration of 1 mM, based on the estimated yields determined by mass. The accurate concentration of labelled BTAs was obtained by absorption spectroscopy. To calibrate the absorption of Cy3 and Cy5 a calibration curve from the commercially available dyes was obtained. Cy3-NHS ester (0.302 mg) was charged to a graduated flask (10.00 mL) and dissolved in 10.00 mL methanol at room temperature, resulting in a 51.17 µM stock solution. Cy5-NHS ester (0.565 mg) was charged to a graduated flask (25.00 mL) and dissolved in 25.00 mL methanol at room temperature, resulting in a 36.67 µM stock solution. For each of these stock solutions, a dilution series was prepared with 10, 20, 40 and 100 times dilution. The absorption spectra for each of the diluted solutions were measured on an absorption spectrophotometer with Peltier temperature controller (JASCO V-650, JASCO, Easton, MD, U.S.A.) at 20°C in quartz cuvettes (Hellma) with a path length of 10.00 mm. The calibration curves obtained are reported in Fig. S.

For BTA polymers assembly procedure stock solution of BTA (10 mM) and labeled BTA-Cy3 and BTA-Cy5 (1 mM) in MeOH were prepared. The organic solvent solutions were mixed at the desired ratio to control the molar ratio of BTA-Cy3/Cy5 on BTA, injected in filtered Milli-Q water (total concentration BTA, 50 µM) and equilibrated for 24 h before experiment. Coassembled stacks were stored in the dark at room temperature. Vials were put on a vortex mixer for 5 seconds before use in spectroscopic measurements to ensure a homogeneous solution.

DNA/RNA recruiters

The sequences and characteristics of the cleavable recruiters used in this chapter are listed below. All nucleic acids were obtained as a powder, dissolved in milliQ water and used without further purification.

Recruiter	Length	Sequence	MW (g/mol)	Tdim (°C)	GC-content
n1	8	AAAGGAAc	2467	< 0°C	37.5%
n2	16	$[AAAGGAAc]_2$	4997	< 0°C	37.5%
n3	24	$[AAAGGAAc]_3$	7526	< 0°C	37.5%
n4	32	$[AAAGGAAc]_4$	10056	< 0°C	37.5%
n6	48	$[AAAGGAAc]_6$	15115	< 0°C	37.5%

Fluorescence spectroscopy

Fluorescence spectra were acquired at room temperature on a Varian CARY Eclipse fluorescence spectrophotometer with multicell holder and temperature controller. Spectra were measured in 400 µL quartz SUPRASIL cuvettes with a path length of 10.00 mm (Type 115-QS, Hellma Analytics). Excitation wavelength was 540 nm with a bandwidth of 10 nm, emission wavelengths were 560-800 nm with a bandwidth of 5 nm. The peaks of Cy3 (570nm) and FRET sensitized emission (670nm) were computed to obtain the FRET ratio (670/570). Alternatively, fluorescence intensities were acquired on a TECAN Safire II plate reader with XFLUOR4SAFIREII v4.62n software. All experiments were done in non-treated transparent polystyrene 96-wells plates (Greiner 650101, Greiner Bio-One GmbH, Frickenhausen, Germany). Spectral peaks were measured at 30°C with a PMT-gain of 150, excitation bandwidth of 10 nm and an emission bandwidth of 5 nm. The number of reads was 25 and the integration time per read was 1000 µs (one read corresponds to one flash of the flash lamp).

BTA clustering experiments

BTA stack with receptor densities of 0.5%, 1.0%, 2.0%, 4.0% and 8.0% were coassembled. For each type of recruiter (MilliQ, 8-mer, 16-mer, 24-mer, 48-mer), 50 µL of coassembled stacks were dispensed into a transparent 96-wells plate in triplo and initial spectral peaks were measured. Clustering was induced by addition of 1 µL of 25 µM recruiter stock solutions for each of the different recruiters. The wells plate was kept in a stove at 30°C for three days to allow for equilibration. Afterwards, spectral peaks were measured to determine the equilibrium FRET-ratios.

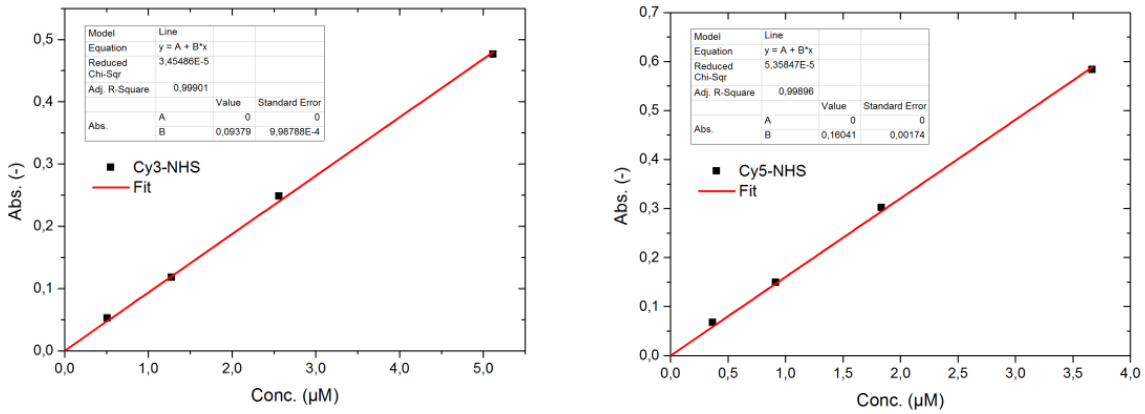

Figure S1: Calibration of BTA-Cy3 and BTA-Cy5 concentration.

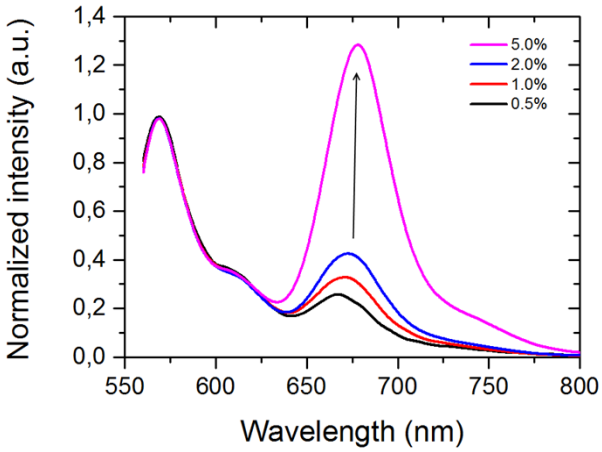

Figure S2: Emission spectra after donor excitation of BTA assemblies at different densities of labeled monomers. Signal is normalized on the Donor emission (570nm).

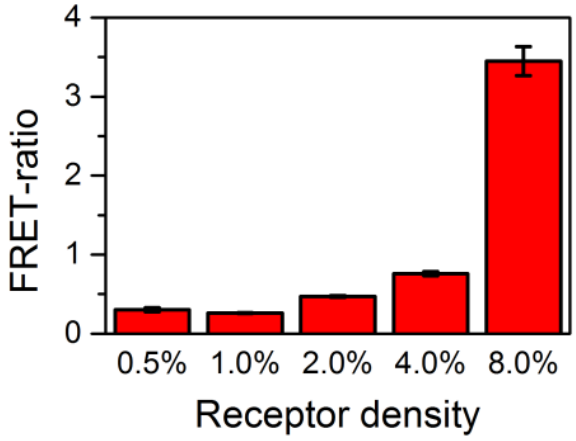

Figure S3: FRET ratio at different densities of labeled monomers. The increase of FRET ratio is consistent with a decrease in the average distance of labeled BTA monomers.

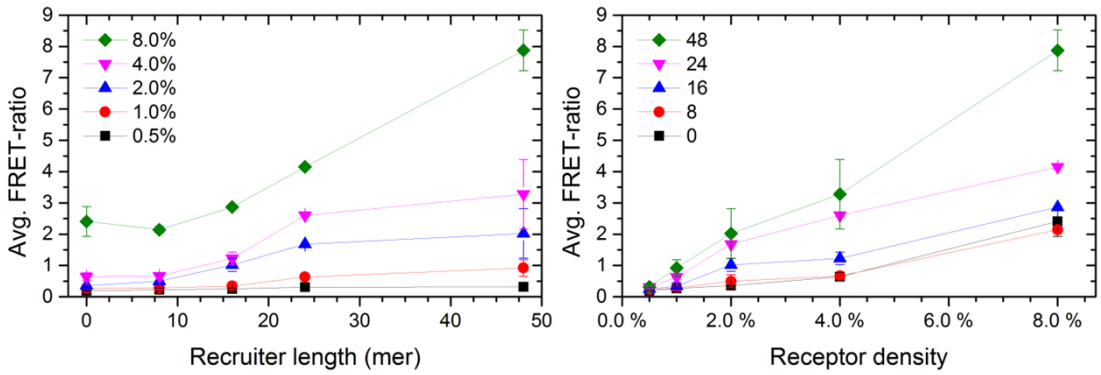

Figure S4: FRET ratio after recruiting with DNA-RNA cleavable strands. Different recruiter lengths and receptor densities are evaluated.

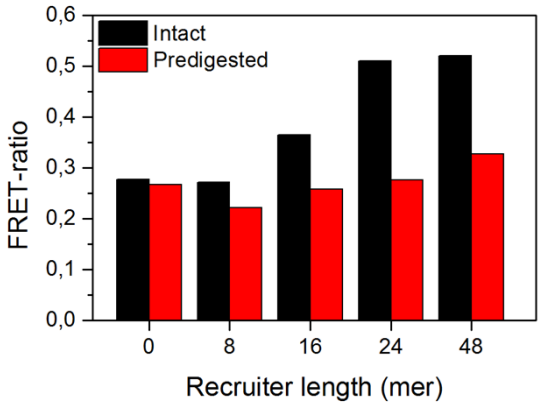

Figure S5: Clustering with intact recruiters (black bars) versus clustering with recruiters that have been predigested with RNAse prior to addition to BTA stacks (red bars).

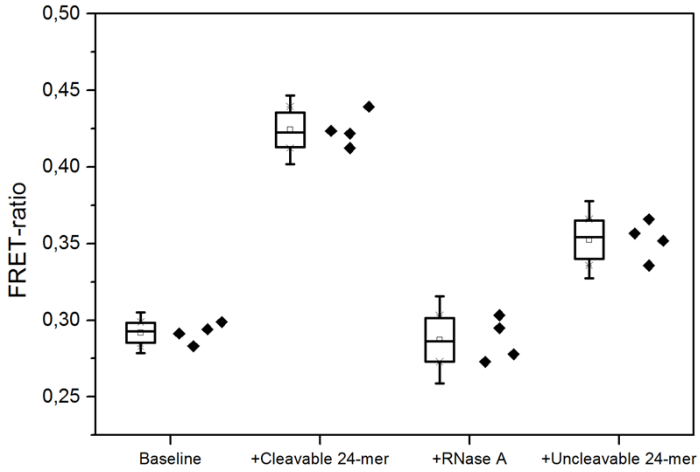

Figure S6: Reclustering experiment (n = 4). FRET-ratios of individual wells at t = 4h (baseline), at t = 24h (with cleavable 24-mer present for 10h), at t = 33h (with RNAse A present for 6h), at t = 48h (with uncleavable 24-mer present for 14h). Boxes represent 1 standard deviation (SD) whiskers represent twice the SD.

1 M. B. Baker, L. Albertazzi, I. K. Voets, C. M. A. Leenders, A. R. A. Palmans, G. M. Pavan and E. W. Meijer, *Nat. Commun.*, 2015, **6**.