Electronic Supplementary Information

For

Impact of Subtle Intermolecular Interactions on Structure and Dynamics of Multicomponent Supramolecular Polymers

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1. General Methods

All materials and chemicals were obtained from commercial sources without further purification. All solvents were of AR quality and purchased from Biosolve. Alkyne-Cy5 was acquired from Lumiprobe.

^{1}H-NMR, and ^{13}C-NMR were recorded on a Bruker ASCEND 400 MHz (400 MHz for ^{1}H-NMR, 100 MHz for ^{13}C-NMR). Proton chemical shifts are reported in ppm (δ) downfield from trimethyl silane (TMS). Peak multiplicities are abbreviated as s: singlet; d: doublet; m: multiplet; t: triplet, p: pentet, dd: double doublet. Carbon chemical shifts are reported in ppm (δ) downfield from TMS. NMR spectra were taken in $CDCl_3$.

Automatic column chromatography was performed on a Grace Reveleris X2 using Büchi prepacked silica columns (4g).

Liquid chromatography-mass spectroscopy (LC-MS) spectra were acquired using a device consisting of multiple components: Shimadzu SCL-10 A VP system controller with Shimadzu LC-10AD VP liquid chromatography pumps (with an Alltima C18 3 u (50 x 2.1 mm) reversed-phase column and gradients of water, a Shimadzu DGU 20A3 prominence degasser, a Thermo Finnigan surveyor autosampler, a Thermo Finnigan surveyor PDA detector, and a Thermo Scientific LCW Fleet. For LC-MS All samples were dissolved in 1:1 ACN:MQ a at a concentration of *ca* 0.1 mg/ml.

Matrix-assisted laser absorption/ionization mass time of flight mass spectroscopy (MALDI-TOF-MS) spectra were obtained on a Bruker Autoflex Speed. α-Cyano-4-hydroxycinnamic acid (CHCA) or *trans*-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene] malononitrile (DCBT) were used as matrix. For MALDI-ToF-MS all samples were dissolved ACN in a *ca.* 1.0 mg/ml concentration.

Ultraviolet-visible (UV-Vis) absorption spectra were recorded on a Jasco V-750 UV-Vis spectrometer equipped with a PAC-743 multi-cuvette holder Measurements were performed using Quartz cuvettes (Hellma) with a path length of 1 cm (3 mL, 50 µM samples). All measurements were performed with a bandwidth of 0.2 nm, a scan speed of 200 nm/min, and a data interval of 0.2 nm, spanning the UV-Vis range from 800 nm to 190 nm.

Structured Illumination Microscopy (SIM) images were taken on a Zeiss Elyra 7 Super Resolution Microscope using lattice SIM mode and an alpha Plan-Apochromat 63x objective.

Fluorescence spectroscopy spectra were recorded on a Varian Cary Eclipse fluorescence spectrometer. A 10x2 mm cuvette (500 µL, Hellma) was used. The samples were excited at the indicated wavelengths and the emission was recorded likewise, at a concentration of 25 or 50 µM.

Time-correlated single photon counting measurements were recorded on a miniTau TCSPC instrument for monitoring short-lived species, which is equipped with three pulsed excitation sources (300, 375, and 450 nm).

Sample preparation:

BTA-3OH and functional monomer co-assemblies were prepared following a standardized heating-cooling procedure. A solution of 50 µM nBTA was prepared in MQ from nBTA stock in MeOH and evaporating the Methanol with a nitrogen flow. Next, the samples were vortexed for 10 seconds and heated to 80 °C for 15 minutes while stirring, to ensure a molecularly dissolved state. The stirrer was subsequently removed and after vortexing, this solution was pipetted into a new another vial containing the appropriate mol% of functionalized monomer (BTABioCy5, BTACy5, or BTACy3) after the methanol was evaporated first to stock solution. This sample was vortexed for 10 seconds and heated to 45 °C for 15 minutes without stirring to not thermally degrade the dyes. The samples were equilibrated in the dark overnight at room temperature and subsequently put in the fridge.

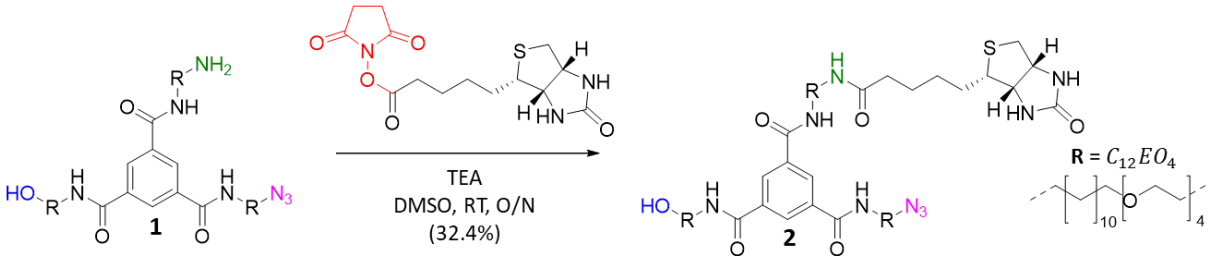
Samples for SIM were prepared at a total BTA concentration of 50 µM with indicated mol% of BTA(Bio)Cy3/5. Samples were aged for *ca. 4-7* days. To image the fibers, 2 µL of solution was injected into an ibidi µ-Slide 18 Well Glass Bottom containing 98 µL 1x PBS yielding 1 µM concentration. After 1 minute incubation samples were washed 3 times with 1x PBS. The adsorbed fibers were then subjected to imaging. Processing was done on Zeiss software by applying SIM deconvolution with medium strength.

Sample of FRET dynamics in solution: To study the dynamics of supramolecular polymers, the acceptor and donor samples (5% in nBTA) were prepared separately following the protocol mentioned above. Subsequently, these donor and acceptor samples were mixed in a 1:1 ratio and monitored the temporal changes in donor and acceptor emissions.

Samples for SIM for study of FRET dynamics: For SIM images the sample was prepared following the same protocol as study of FRET dynamics in solution. For imaging, aliquots were taken out at different time interval and were diluted to a concentration of 1 µM in 1xPBS for imaging. After 1 minute incubation samples were washed 3 times with 1x PBS. The adsorbed fibers were then subjected to imaging. Subsequently, two-channel imaging was conducted to simultaneously visualize Cy3-labeled (green) and Cy5-labeled (red) monomers.

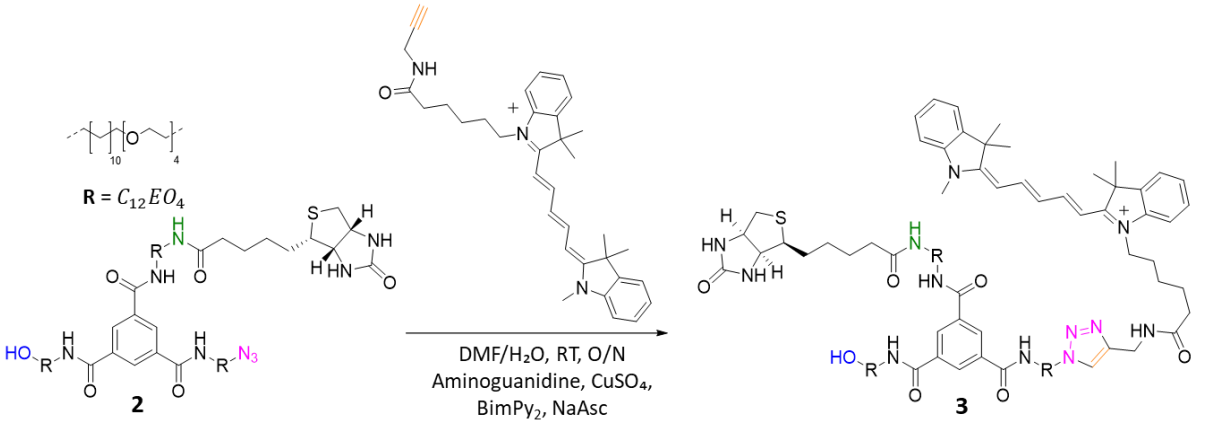
2. Synthetic Schemes and Procedures

BTA,[34] BTACy3/Cy5[35] were synthesised according to previously reported procedures.



Scheme S1: NHS-biotin BTA-amine coupling reaction.

*Synthesis of **2**:* Trifunctional click BTA **(1)** (20 mg, 15.2 µmol, 1 eq), Biotin-NHS (7.8 mg, 15.2 µmol, 1eq), TEA (8.74 µL, 60.4 µmol, 4eq) were dissolved in 2.5 mL DMSO. The reaction was then left to stir overnight at room temperature. Water was added to the crude mixture, followed by lyophilization. Next, the crude solid was dissolved in 0.5 mL DMF and 10 mL cold hexane/diethyl ether 20/80 v/v, was added. The mixture was then placed in the freezer for 10 minutes. After centrifugation at 2000 RPM for 10 minutes, the supernatant was carefully discarded, and the precipitate was dissolved in 50/50 v/v% ACN/MQ and lyophilized yielding a white waxy solid.



Scheme S2: CuAAC reaction on BTA-Biotin-N_3 and alkyne-cy5

*Synthesis of **3**:* Copper sulfate (50 mM) and aminoguanidine (200 mM) were dissolved separately in MQ, BimPy2 in DMF (30 mM), and sodium ascorbate in MQ (200 mM). Then, 100 µL of each cocktail was added successively to a small sample tube, resulting in a staining sequence: blue, green, and brown, respectively. Subsequently, this reaction cocktail was added to a new sample vial containing (**2**) (8.77 mg, 5.7 µmol,1 eq) and alkyne-Cy5 (3.17 mg, 5.7 µmol, 1 eq) in 200 µL of DMF. The mixture was stirred overnight at room temperature. A normal phase column (4 g) was run with MeOH/CHCl3 0à15 v/v% gradient in 10 column volumes. The product fraction was then identified by LC-MS and the solvents were evaporated. The formation of product 3 was then confirmed by MALDI-ToF-MS. Dialysis with a membrane cutoff of 1 kDa was performed, after lyophilization and solvent extractions with cold diethyl ether after which the product **(3)** was collected from the supernatant by rotary evaporation yielding it as a blue waxy solid.

3. Supporting figures:

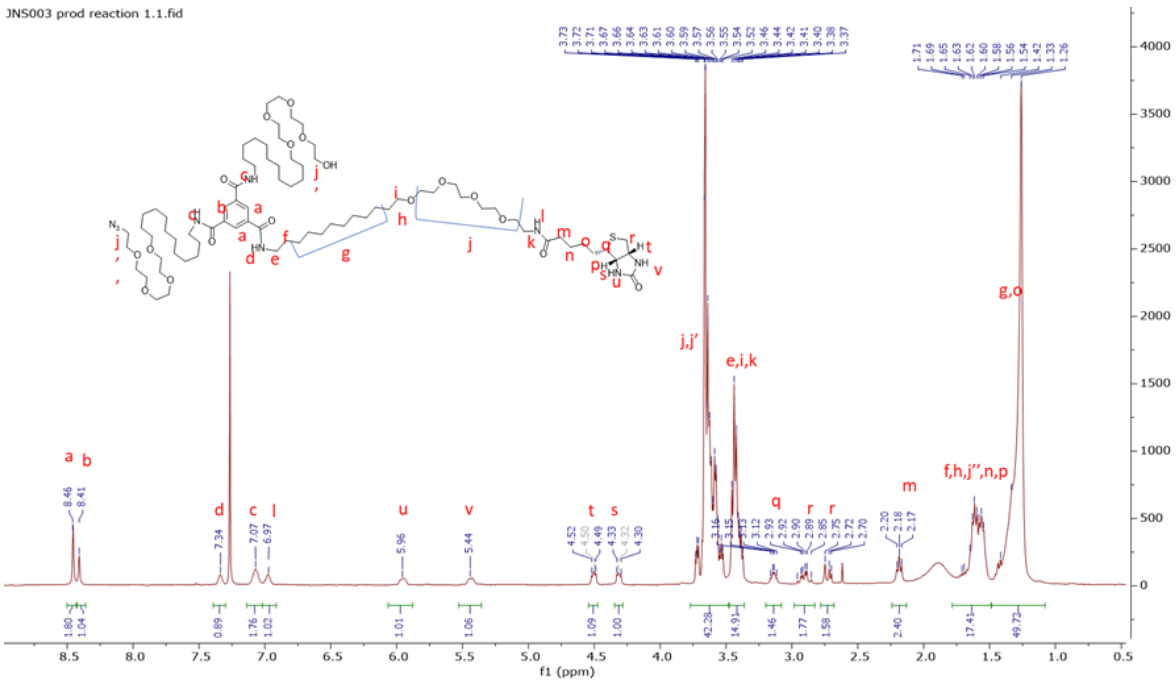


Figure S1. ^{1}H-NMR of **(2)** in $CDCl_3$

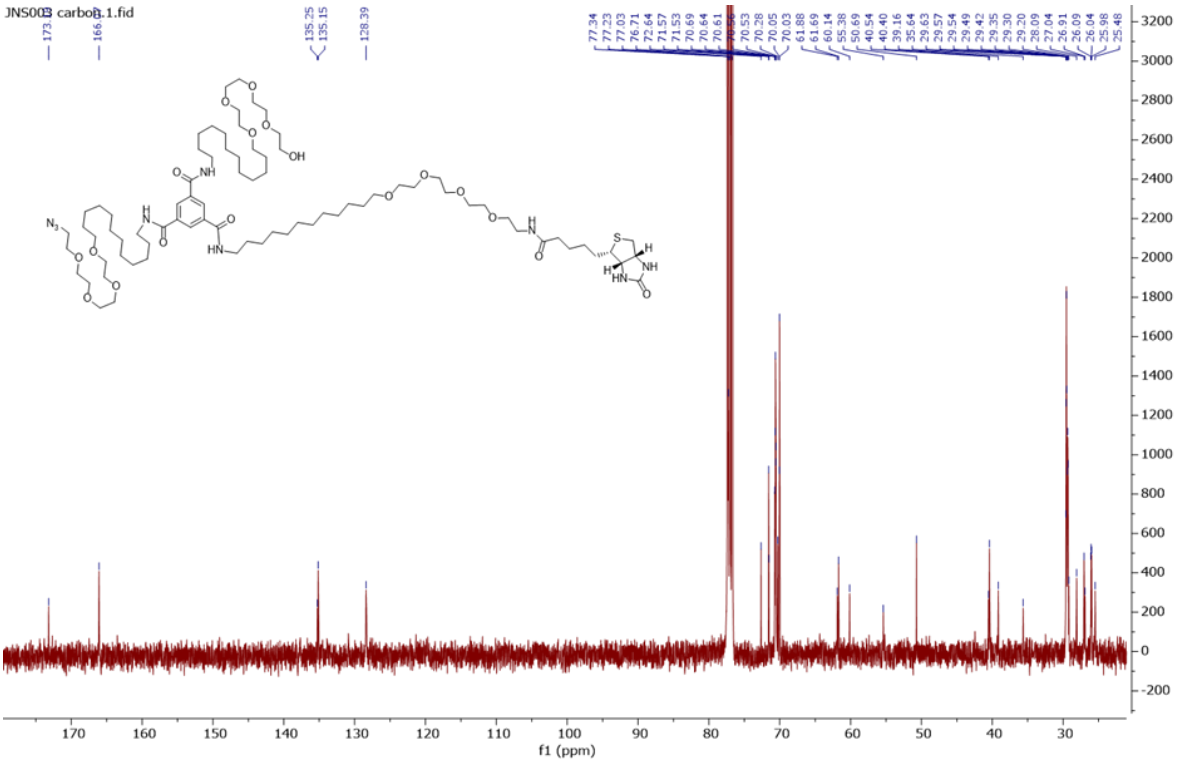


Figure S2. ^{13}C-NMR of **(2)** in $CDCl_3$

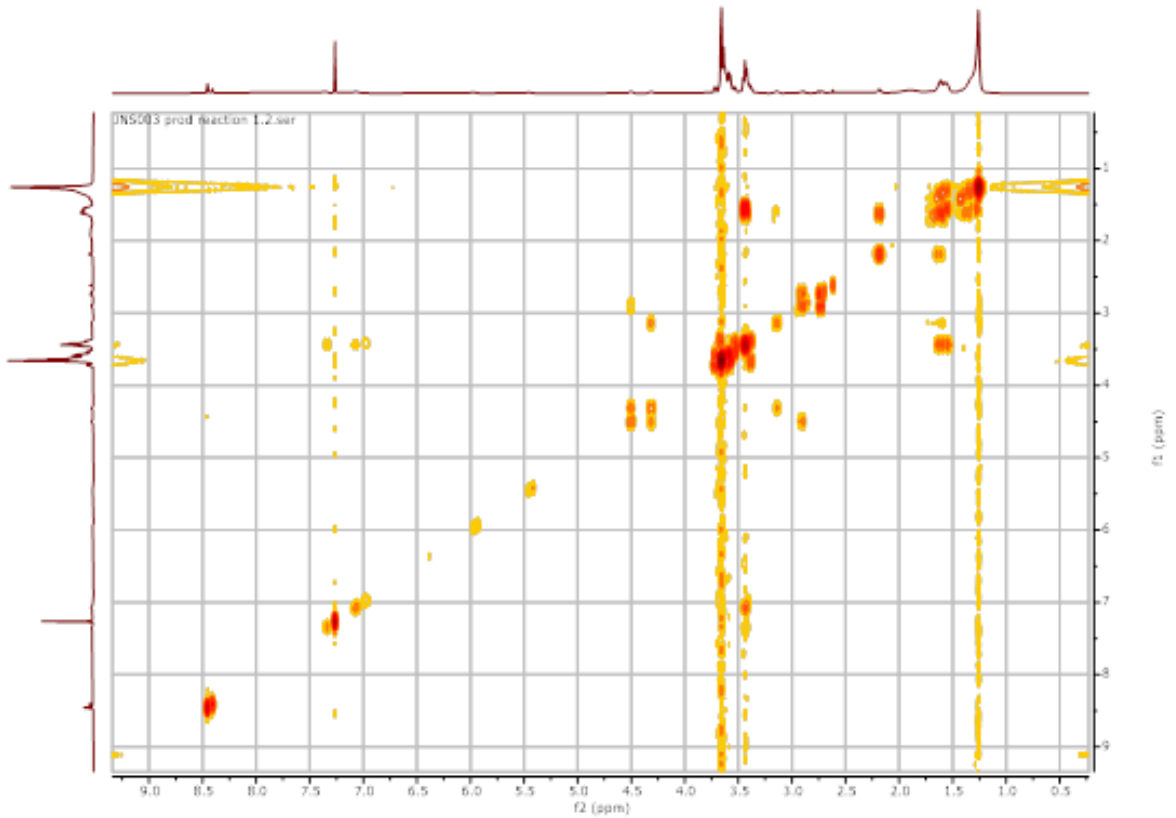


Figure S3. COSY of **(2)** in $CDCl_3$

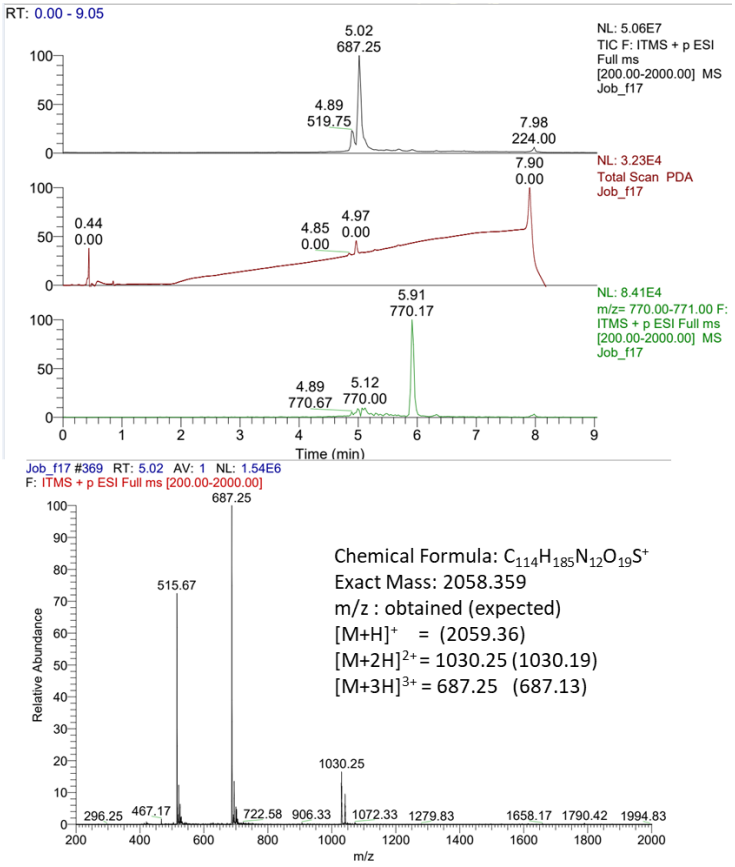


Figure S4. LC-MS to identify product fraction containing **(3)** after column chromatography.

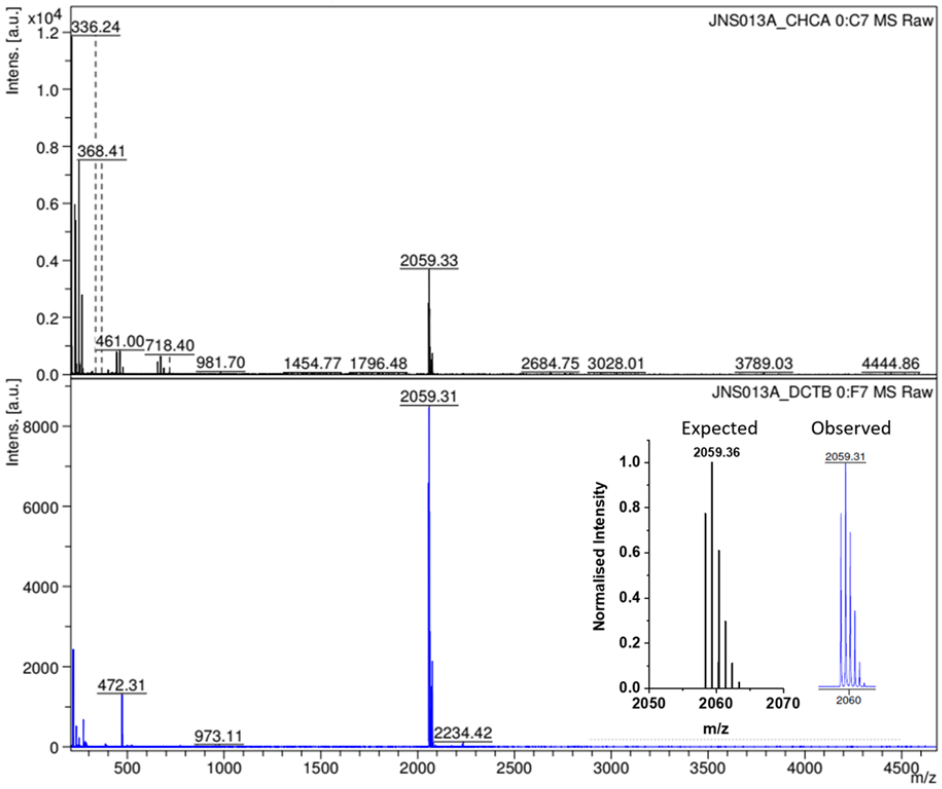


Figure S5. MALDI-ToF MS spectrum of (**3**) with indicated expected and observed isotope pattern.

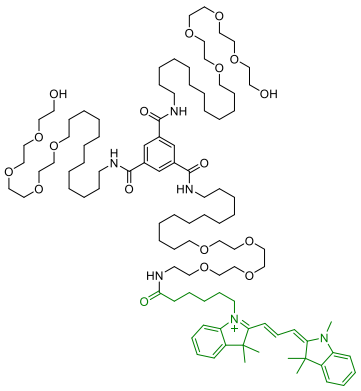


Figure S6. Chemical structure of BTACy3.

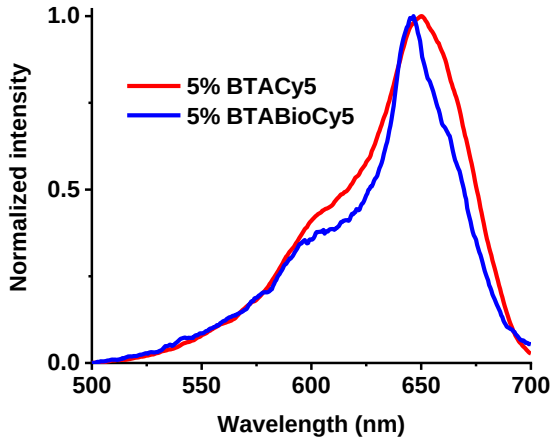


Figure S7. Normalized excitation spectrum of 5% BTACy5 and BTABioCy5 (emission wavelength 725 nm), indicating the presence of an additional absorption should for BTABioCy5.

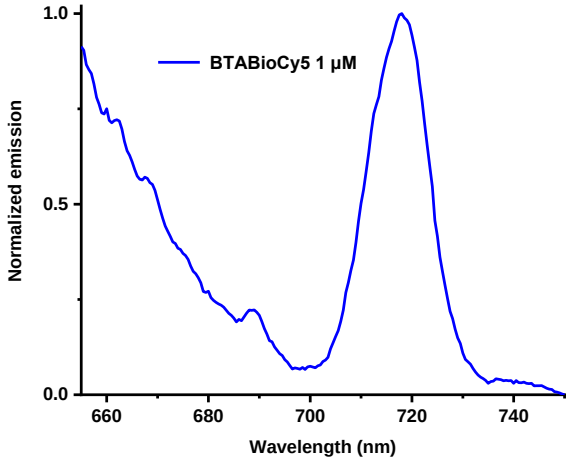


Figure S8. Normalized emission spectrum of 100% BTABioCy5 at 1 µM (excitation wavelength 580 nm), suggesting that origin of the shoulder emission is an aggregated BTABioCy5 species.

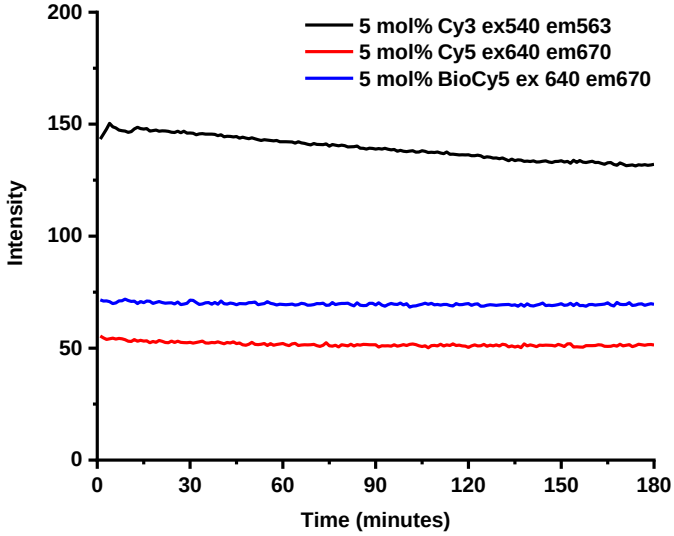


Figure S9. Fluorescence Bleaching of 5% BTACy3, 5% BTACy5 and 5% BTABioCy5 samples at 50 µM.

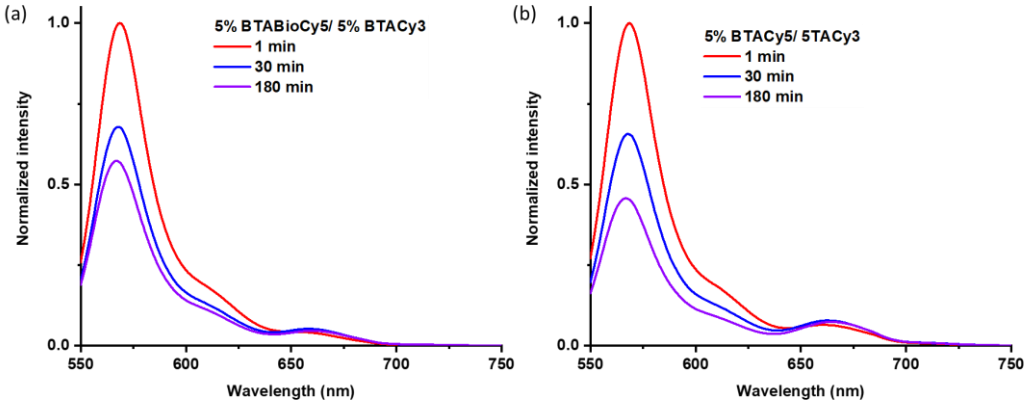


Figure S10. Time-dependent fluorescence spectra for (a) 5% BTABioCy5/ 5% BTACy3 (b) 5% BTACy5/ 5% BTACy3 of 1:1 sample at 50 µM.

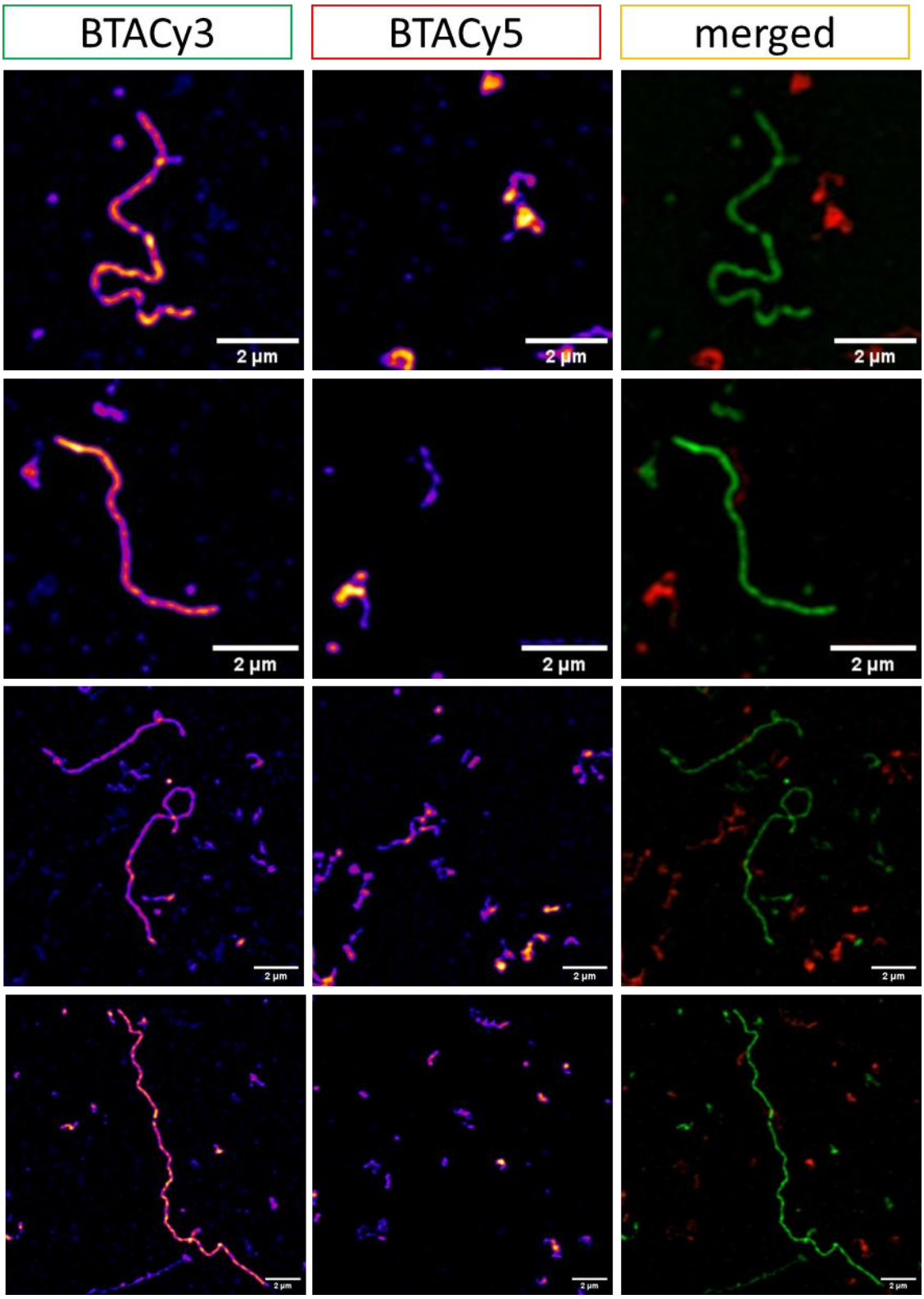


Figure S11. SIM images of 5% BTACy3/BTACy5 fibers mixed at time point = 0 hours at 1 µM in ~1x PBS adhered to the bottom of a microscopy well. Fire LUT is applied using ImageJ in order to show intensity difference.

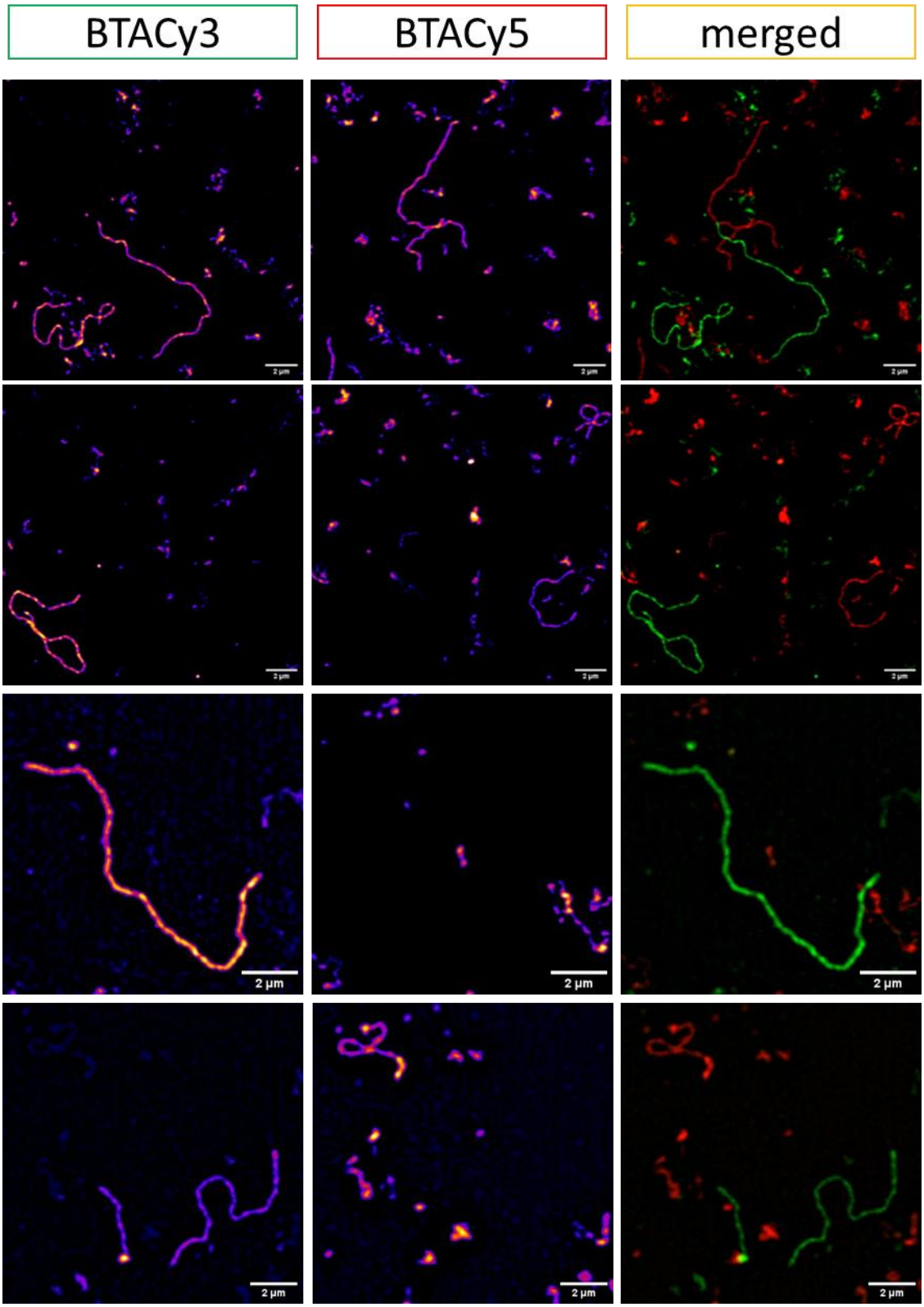


Figure S12. SIM images of 5% BTACy3/BTACy5 fibers mixed at time point = 0 hours at 1 µM in ~1x PBS adhered to the bottom of a microscopy well. Fire LUT is applied using ImageJ in order to show intensity difference.

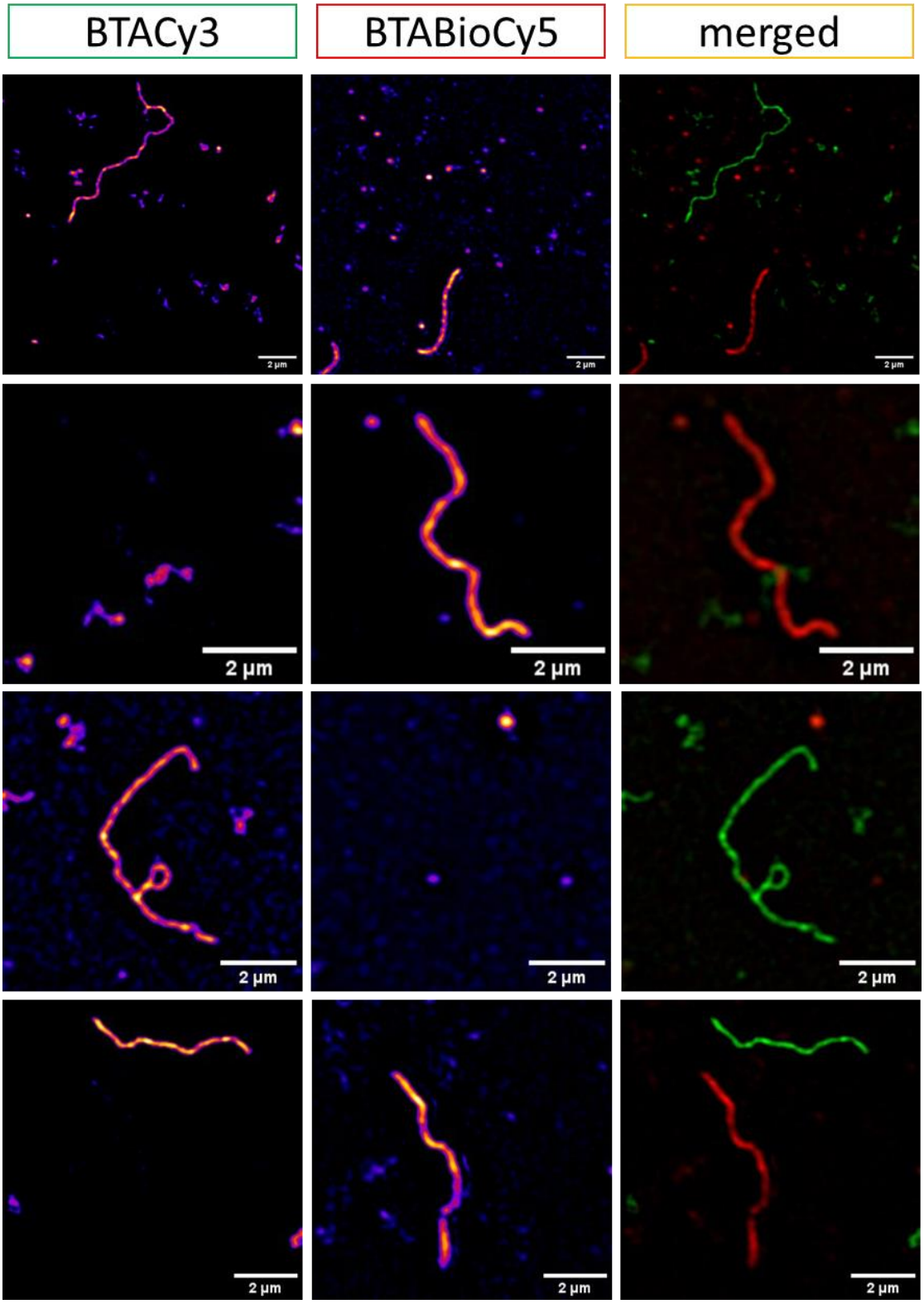


Figure S13. SIM images of 5% BTACy3/BTABioCy5 fibers mixed at time point = 0 hours at 1 µM in ~1x PBS adhered to the bottom of a microscopy well. Fire LUT is applied using ImageJ in order to show intensity difference.

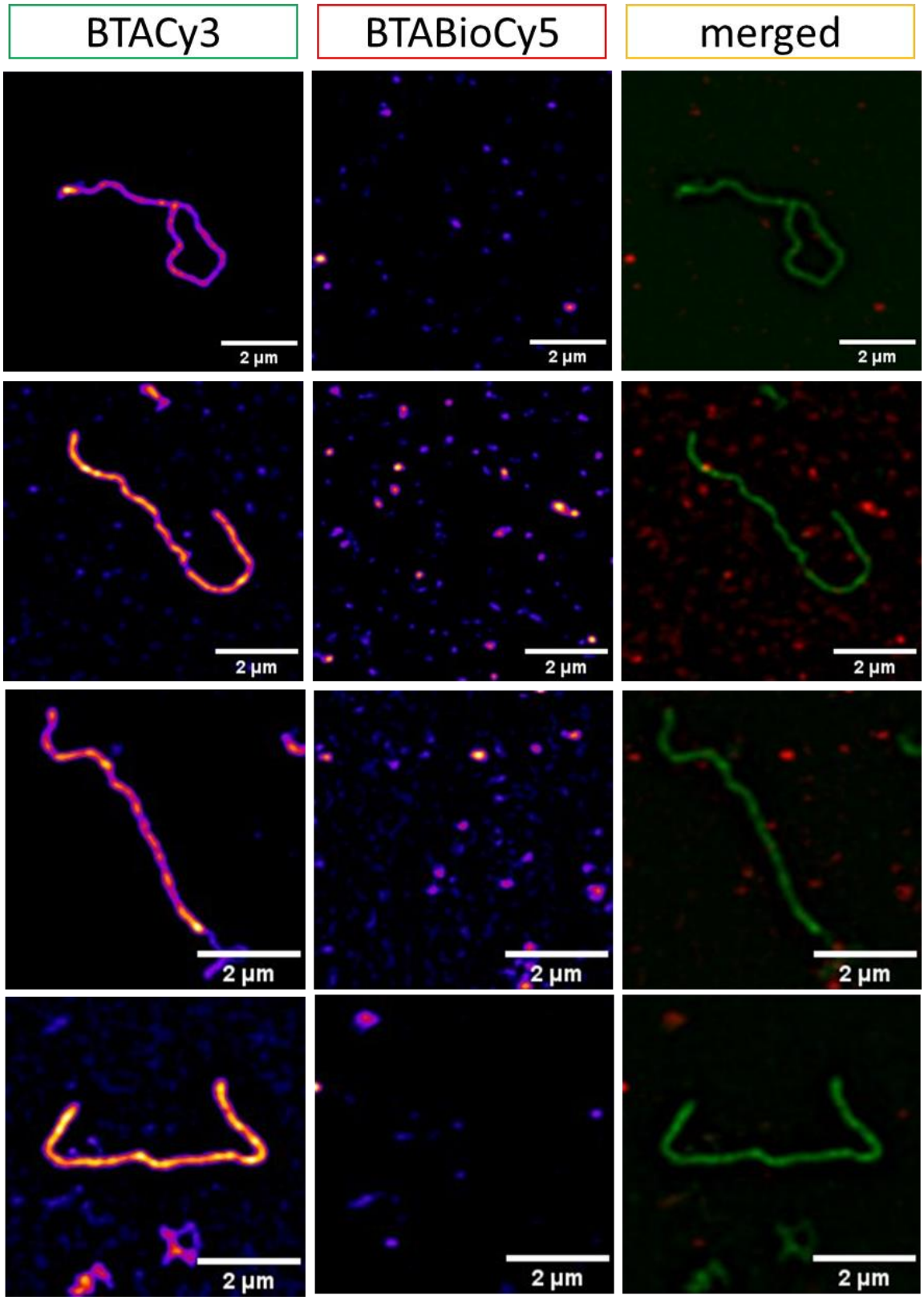


Figure S14. SIM images of 5% BTACy3/BTABioCy5 fibers mixed at time point = 0 hours at 1 µM in ~1x PBS adhered to the bottom of a microscopy well. Fire LUT is applied using ImageJ in order to show intensity difference.

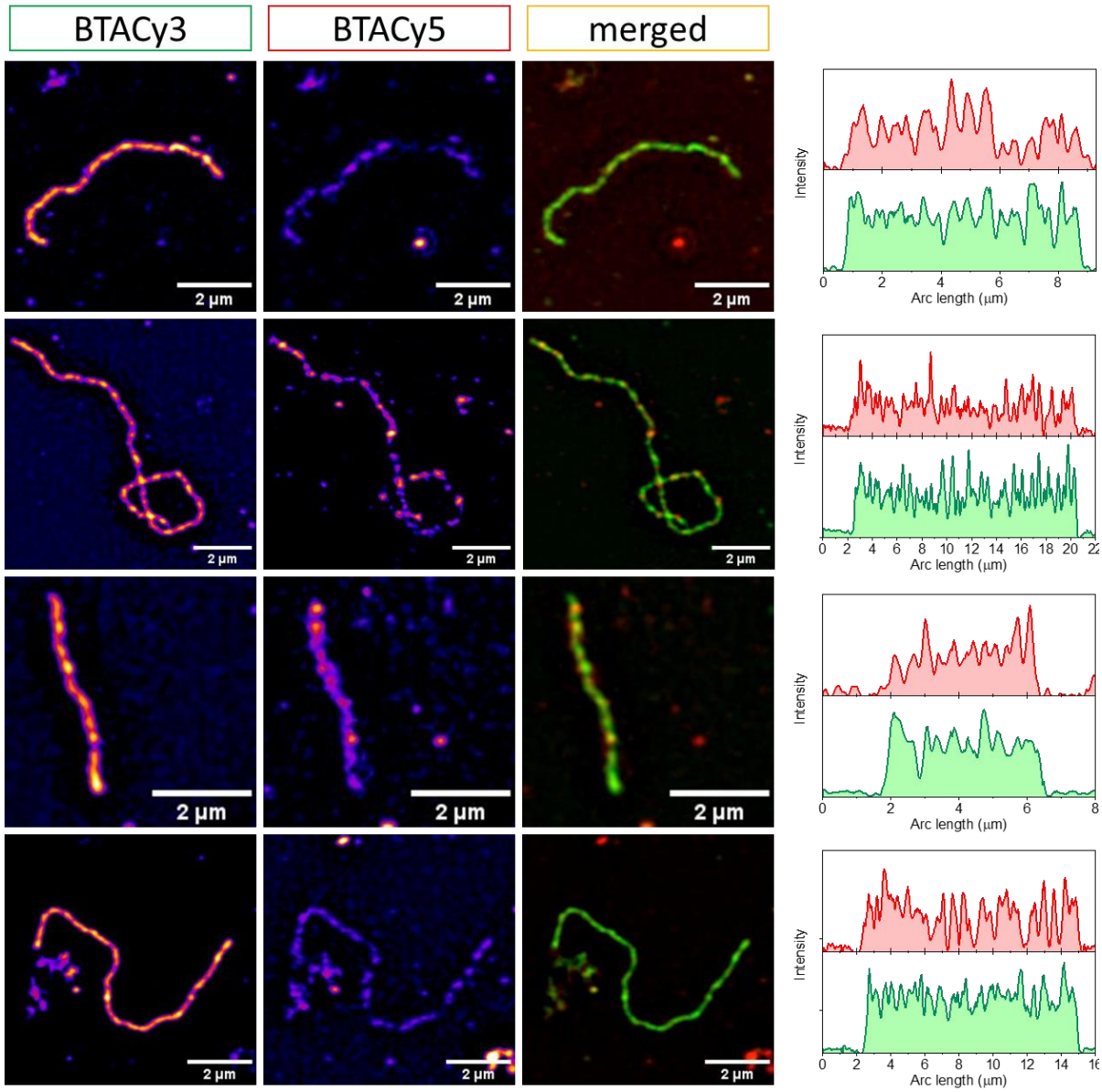


Figure S15. SIM images and intensity profile of the backbone of 5% BTACy3/BTACy5 fibers mixed at time point = 1 hour at 1 µM in ~1x PBS adhered to the bottom of a microscopy well. Fire LUT is applied using ImageJ in order to show intensity difference.

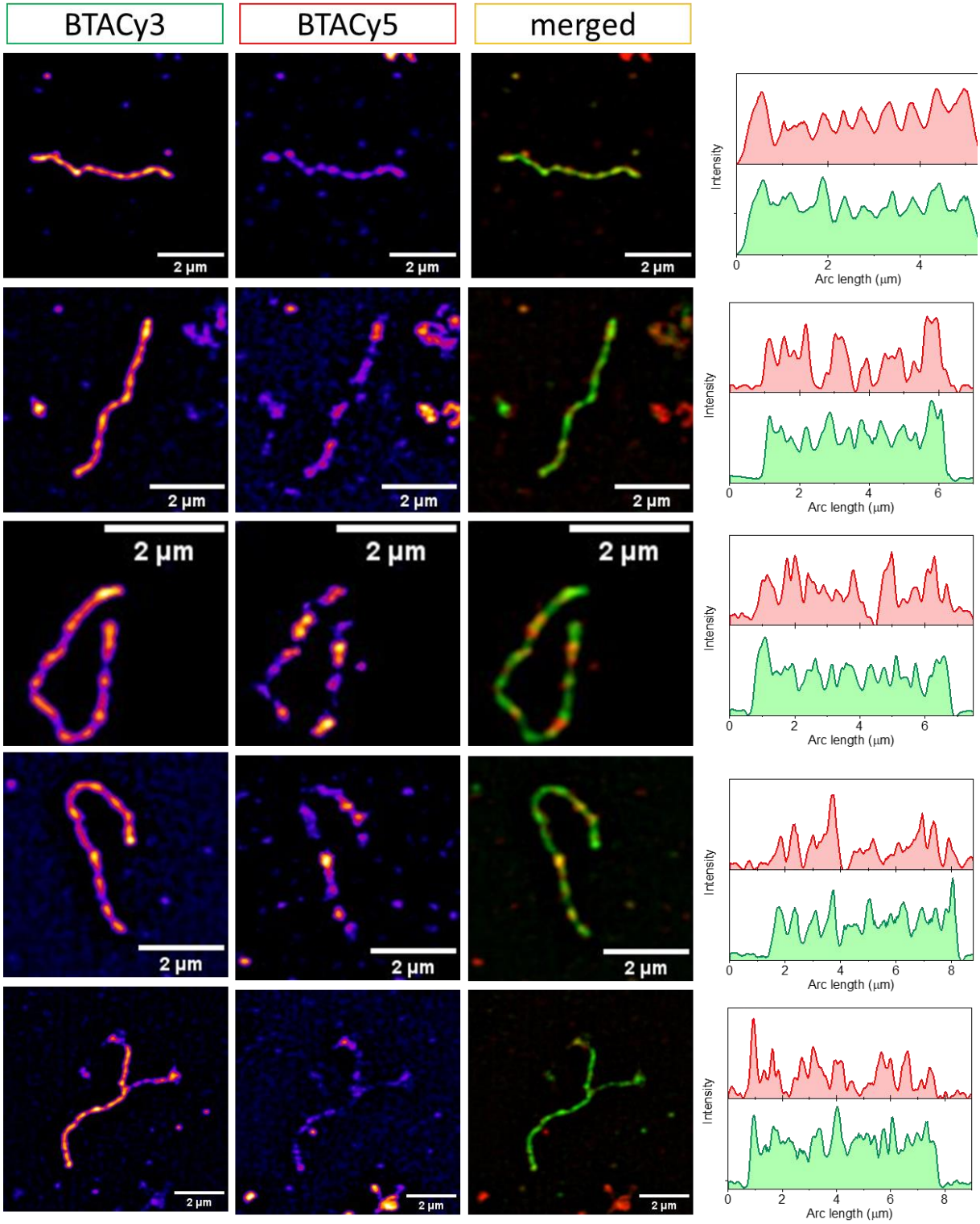


Figure S16. SIM images and intensity profile of the backbone of 5% BTACy3/BTACy5 fibers mixed at time point = 1 hour at 1 µM in ~1x PBS adhered to the bottom of a microscopy well. Fire LUT is applied using ImageJ in order to show intensity difference.

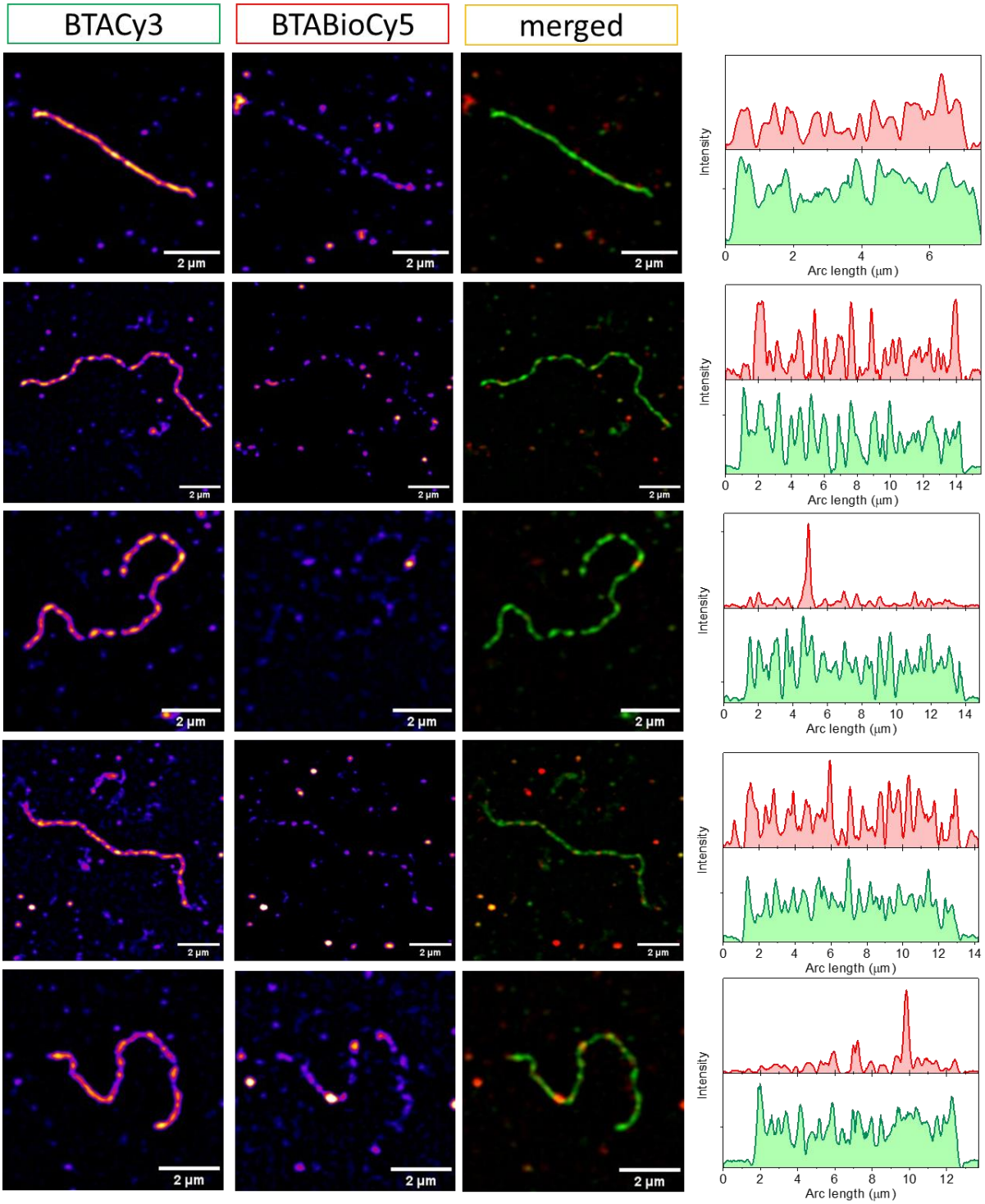


Figure S17. SIM images and intensity profile of the backbone of 5% BTACy3/BTABioCy5 fibers mixed at time point = 1 hour at 1 µM in ~1x PBS adhered to the bottom of a microscopy well. Fire LUT is applied using ImageJ in order to show intensity difference.

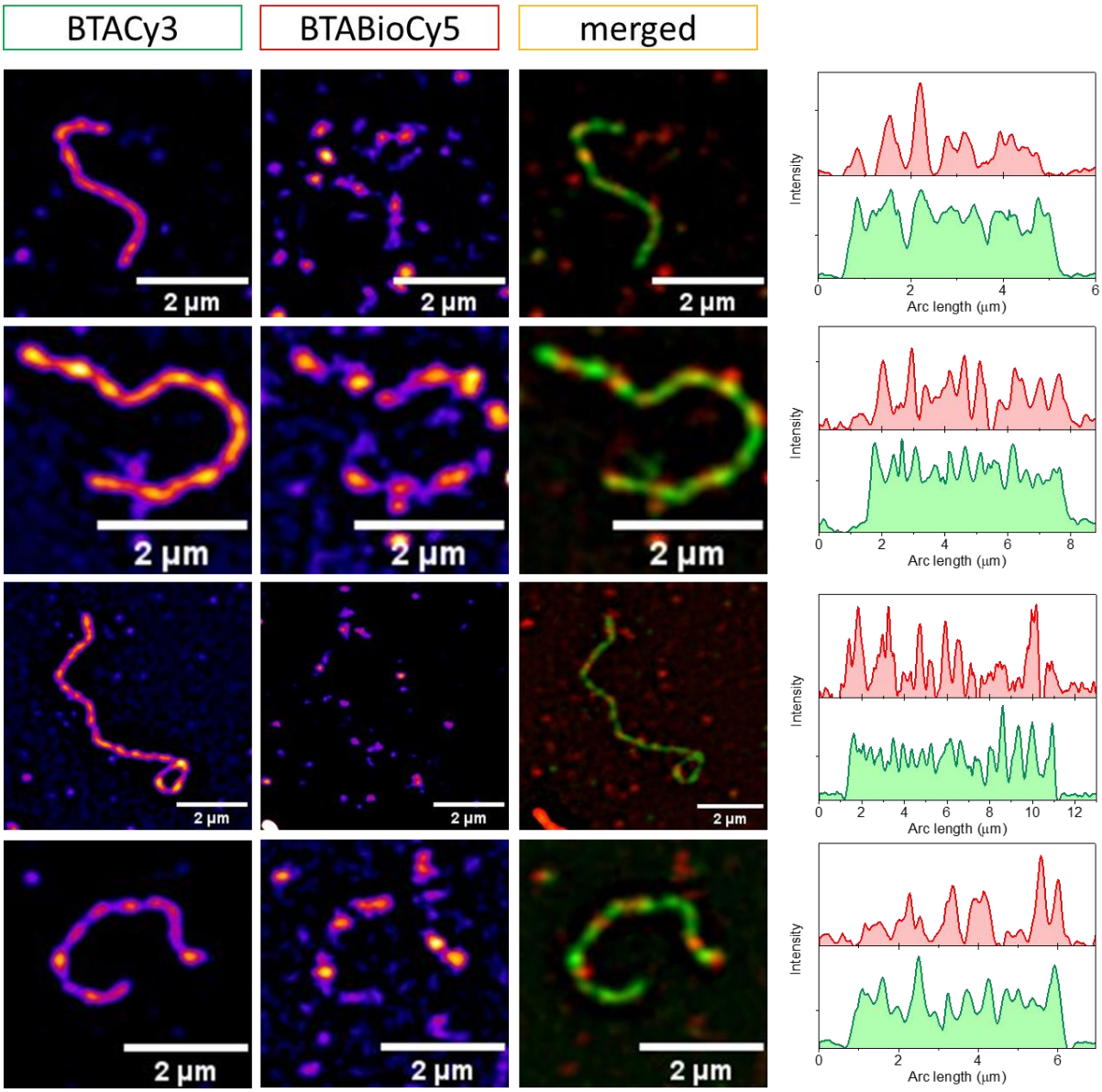


Figure S18. SIM images and intensity profile of the backbone of 5% BTACy3/BTABioCy5 fibers mixed at time point = 1 hour at 1 µM in ~1x PBS adhered to the bottom of a microscopy well. Fire LUT is applied using ImageJ in order to show intensity difference.

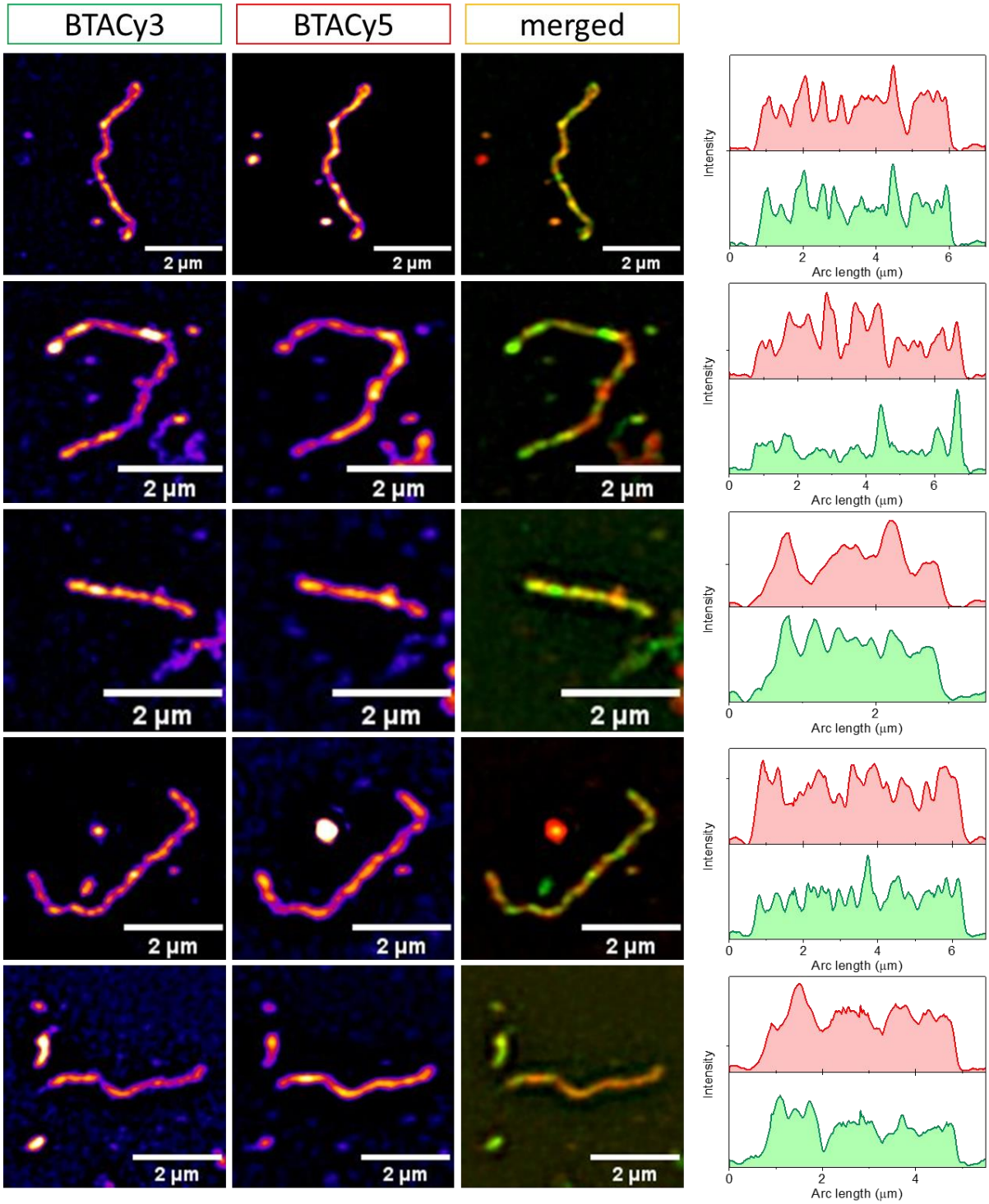


Figure S19. SIM images and intensity profile of the backbone of 5% BTACy3/BTACy5 fibers mixed at time point = 24 hours at 1 µM in ~1x PBS adhered to the bottom of a microscopy well. Fire LUT is applied using ImageJ in order to show intensity difference.

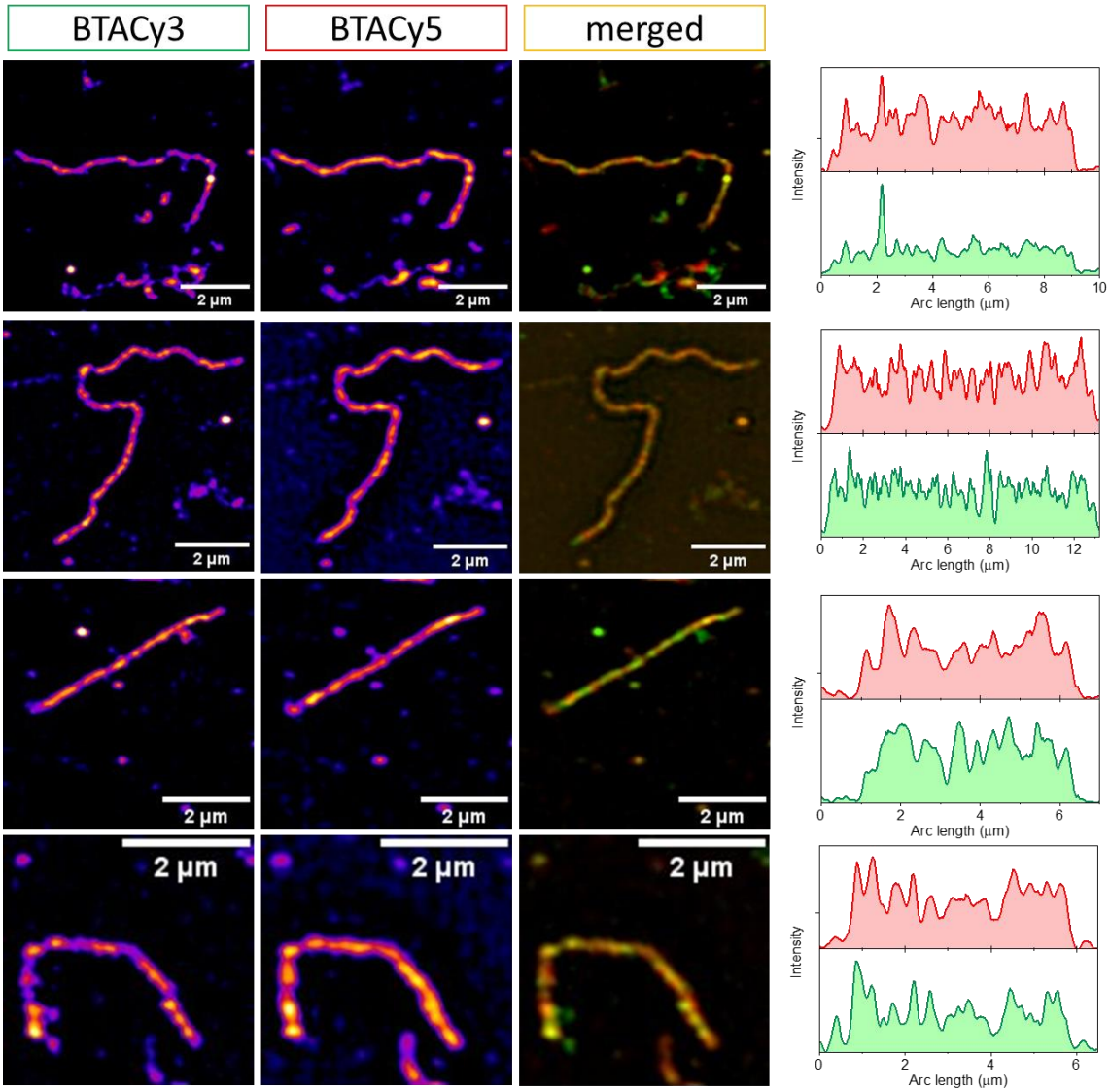


Figure S20. SIM images and intensity profile of the backbone of 5% BTACy3/BTACy5 fibers mixed at time point = 24 hours at 1 µM in ~1xPBS adhered to the bottom of a microscopy well. Fire LUT is applied using ImageJ in order to show intensity difference.

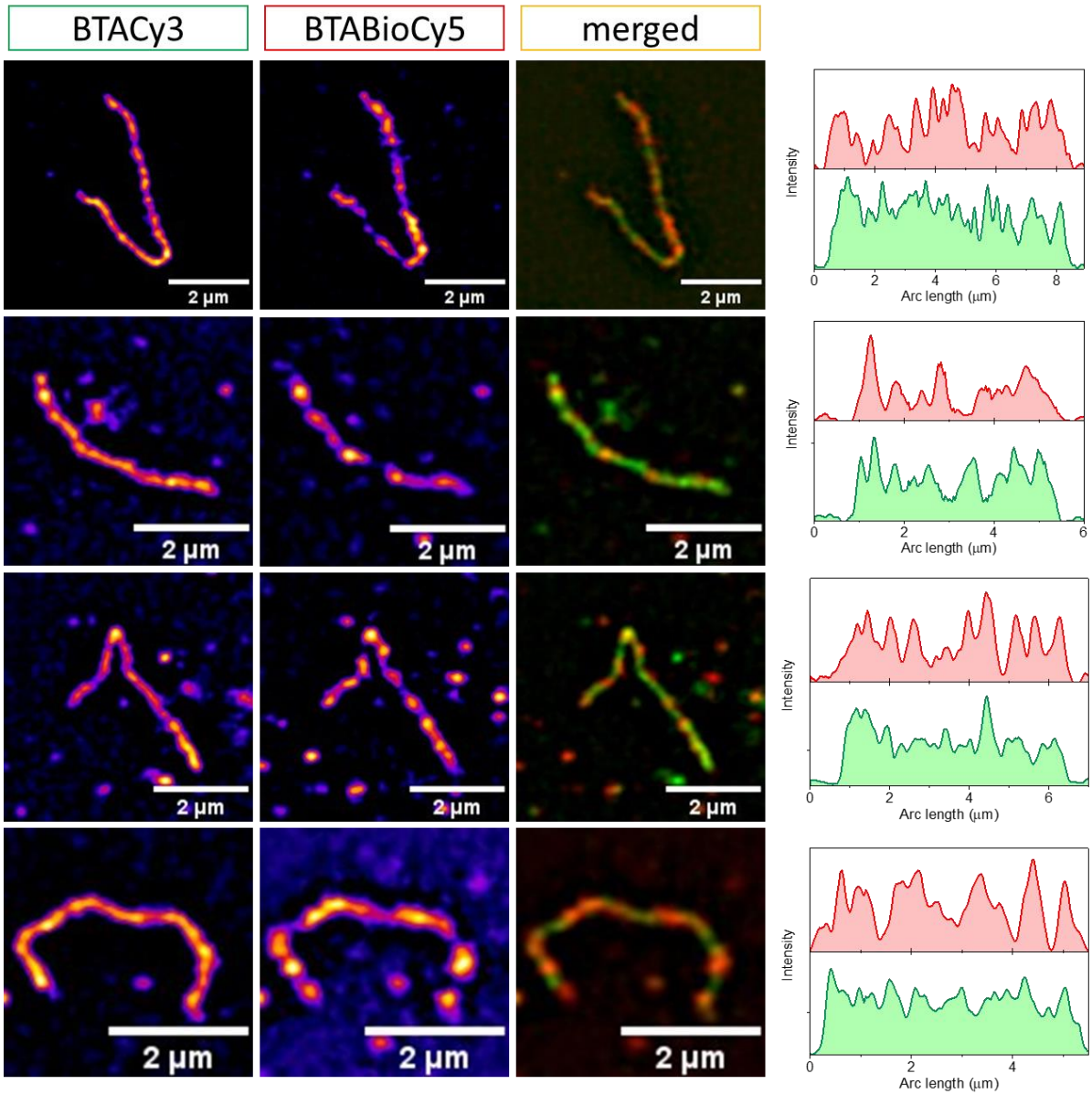


Figure S21. SIM images and intensity profile of the backbone of 5% BTACy3/BTABioCy5 fibers mixed at time point = 24 hours at 1 µM in ~1x PBS adhered to the bottom of a microscopy well. Fire LUT is applied using ImageJ in order to show intensity difference.

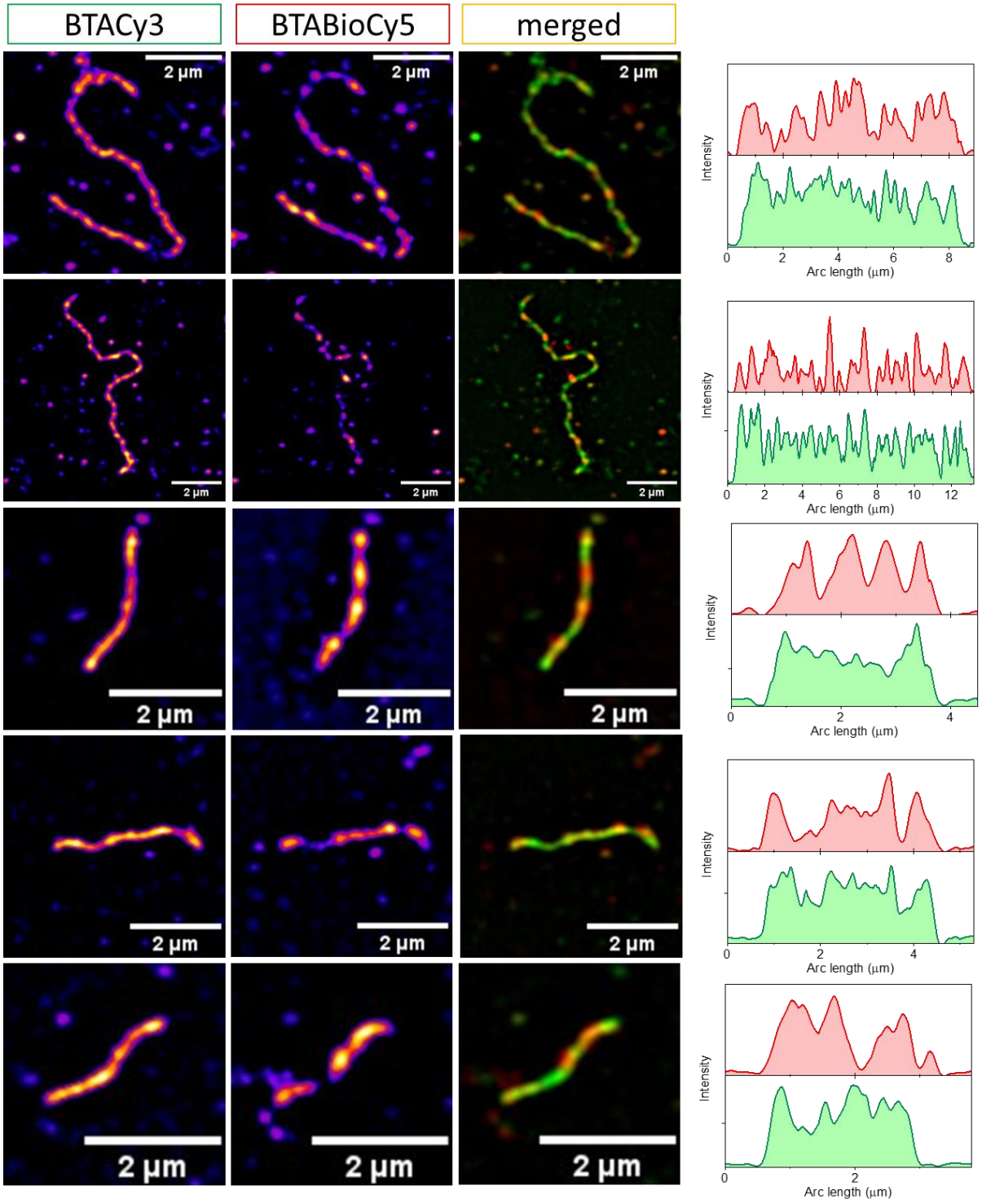


Figure S22. SIM images and intensity profile of the backbone of 5% BTACy3/BTABioCy5 fibers mixed at time point = 24 hours at 1 µM in ~1x PBS adhered to the bottom of a microscopy well. Fire LUT is applied using ImageJ in order to show intensity difference.

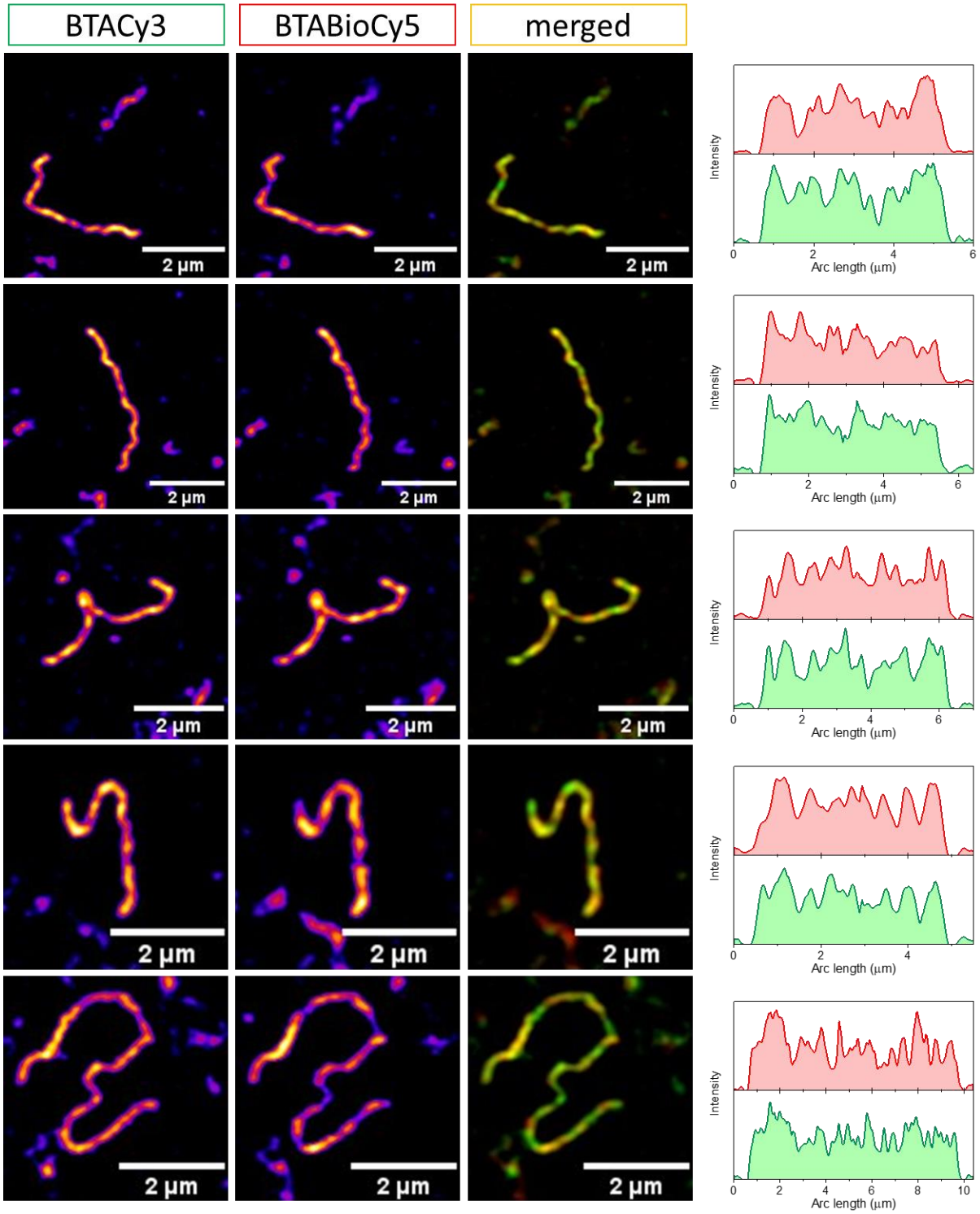


Figure S23. SIM images and intensity profile of the backbone of annealed 2.5% BTACy3 + 2.5 % BTABioCy5 fibers at 1 µM in ~1x PBS adhered to the bottom of a microscopy well. Fire LUT is applied using ImageJ in order to show intensity difference.

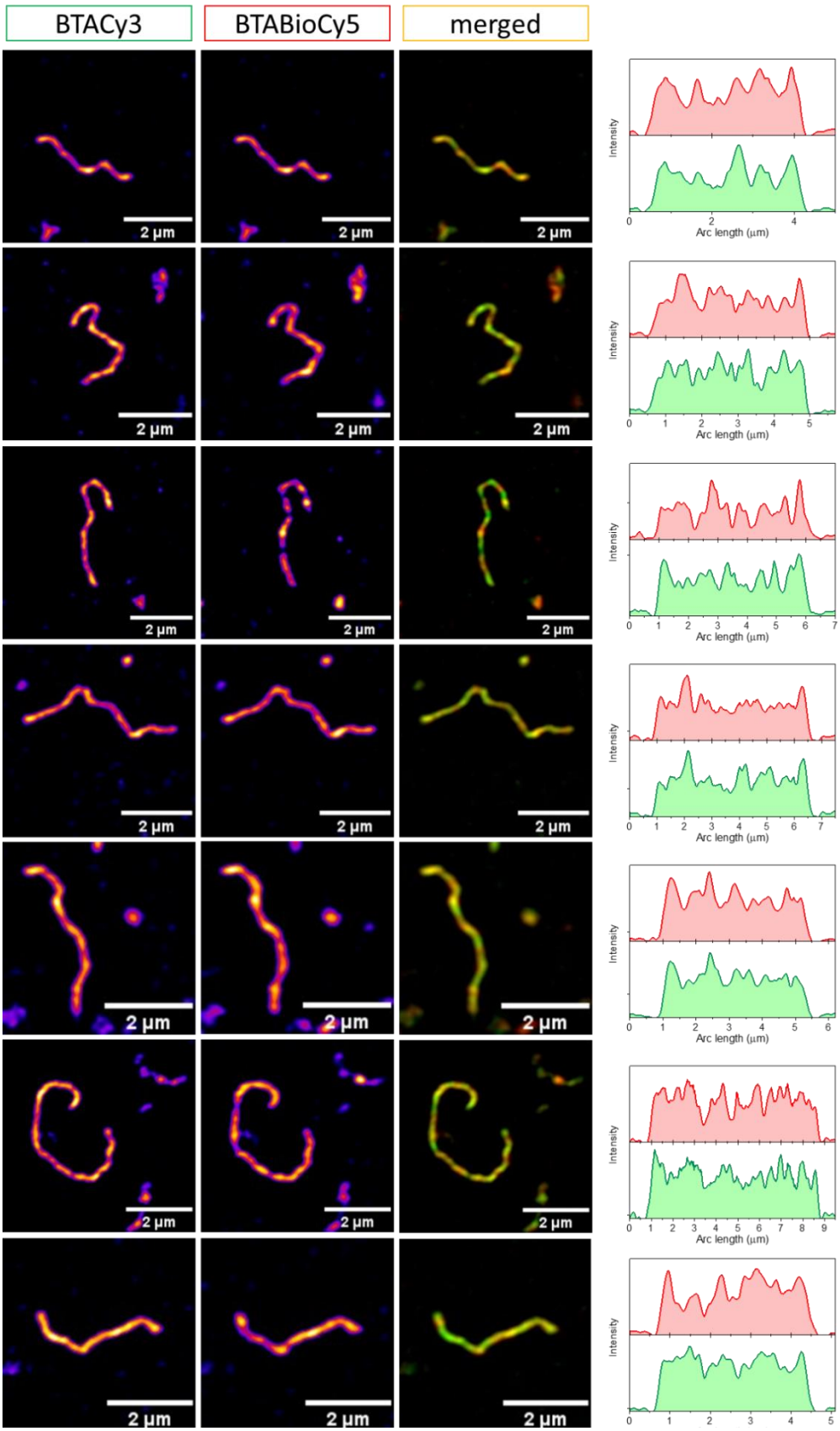


Figure S24. SIM images and intensity profile of the backbone of annealed 2.5% BTACy3 + 2.5% BTABioCy5 fibers at 1 µM in ~1x PBS adhered to the bottom of a microscopy well. Fire LUT is applied using ImageJ in order to show intensity difference.

4. Supplementary Tables

Table S1. FRET rate constants determined by fitting bi or tri exponential fit of the FRET kinetics.

FRET rates	5% BTACy5/ 5% BTACy3	5% BTABioCy5/ 5% BTACy3
k_1 (min^{-1})	2.4	1.7
k_2 (min^{-1})	0.10	0.15
k_3 (min^{-1})	0.01	-

5. References

1. C. M. A. Leenders, L. Albertazzi, T. Mes, M.M.E. Koenigs, A.R.A. Palmans, and E. W. Meijer, *Chem. Commun.* 2013, **49**, 1963–1965.
2. L. Albertazzi, D. van der Zwaag, C.M. Leenders, R. Fitzner, R.W. van der Hofstad, and E.W. Meijer, Science 2014, **344(6183)**, 491-495.