Supporting information

RGD-decorated conjugated polymer particles as fluorescent bio-medical probes prepared by Sonogashira dispersion polymerization

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Materials:
All chemicals, solvents and materials were obtained from Sigma Aldrich apart from cRGD, which was obtained from Bachem Holding AG (Switzerland). All chemicals were used without further purification.

General synthetic protocol for fluorene diacetylene phenylene *co*-polymer particles prepared by Sonogashira dispersion polymerization
Equal moles of 9,9-dioctyl-2,7-diiodofluorene and 1,3-diethynylbezene, Triton X-45 (800 mg) and PVPA (720 mg) were dissolved in 6,6 mL of 1-propanol. The resulting mixture was filtered into a 25 mL flask with a syringe filter (0.45 mm, PTFE). After degassing for 15 min by bubbling with argon, bis(triphenylphosphine) palladium(II)dichloride (1.5 mg) and CuI (3 mg) were added. The reaction flask was then charged with a stirrer bar, sealed with a rubber septum and degassed by using argon for another 10 min. The nozzle of a tip sonicator was inserted in the flask through a hole in the septum so that the tip was ca. 2 mm below the surface of the reaction mixture. The reaction mixture was heated to 70 °C with an oil bath and stirred (at 1300 rpm), while the tip sonicator was running consistently at 12 W. After all the catalyst had dissolved, 1.6 mL of degassed solution of triethylamine (15% in 1-propanol) were injected to start the polymerization. The reaction mixture turned turbid after ca. 30 min. The reaction mixture was left to completion for 2 h. The particles were purified by repeated centrifugation and exchange of the supernatant (at least three times exchanged each with 1 %wt Triton X-45 in 1-propanol, 1-propanol and water). The yield was varied between 30 to 50% depending on the purification procedure.

Functionalization of fluorene diacetylene phenylene co-polymer particles with cRGD
A 10 mL glass vial with rubber septum was charged with a mixture of 12 mg of fluorene diacetylene phenylene co-polymer particles, 2,2-dimethoxy-2-phenylacetophenone (30 mg) and 3 mL of dimethyl sulfoxide. The reaction mixture was degassed for at least 15 min by bubbling with argon. Cyclo(-Arg-Gly-Asp-D-Phe-Cys) solution (1.5 mg in 1mL DMSO) was injected to the reaction mixture and degassed again for 10 min. The reaction mixture was irradiated with a UV lamp with an intensity maximum at 365 nm at room temperature under magnetic stirring overnight. The particles were purified by washing with distilled water several times.

Functionalization of fluorene diacetylene phenylene co-polymer with PEG-thiol
A 10 mL glass vial with rubber septum was charged with a mixture of 12 mg of fluorene diacetylene phenylene co-polymer particles, 2,2-dimethoxy-2-phenylacetophenone (30 mg) and 3 mL of dimethyl sulfoxide. The reaction mixture was degassed for at least 15 min by bubbling with argon. PEG-thiol (2.5 mg) was injected to reaction mixture and degassed again for 10 min. The reaction mixture was irradiated using a UV lamp with an intensity maximum at 365 nm at room temperature under magnetic stirring. The particles were purified by washing with distilled water several times.

Cell culture
Human umbilical vein endothelial cells (HUVEC, Promocell, Germany) were cultured using Vasculife basal medium (Lifeline, Germany) supplemented with 2% growth supplements, 3% fetal calf serum and 1% Pen/Strep (10,000 U/ml penicillin; 10,000 µg/ml streptomycin, Invitrogen, Germany). Cells were cultured in T75 cell culture flasks (Cell star, Greiner, Germany) and incubated at 37°C, 5% CO_2 and 95% relative humidity.

Cell viability assay
The toxicity of the particles on HUVEC was investigated by trypan blue staining. 3 x 10^5 cells were seeded in 6-well plates (Greiner, Germany) and cultured for 24h. Medium was removed and each well was washed with 2ml of phosphate buffered saline (PBS, Invitrogen, Germany). Cells incubated with cell culture medium served as negative controls. Non-functionalized and functionalized particles were diluted in cell culture medium to a concentration of 250 µg/ml. 3 ml of cell culture medium or medium containing particles was added to the cells and the cells were incubated for 24h. Three samples per condition were analyzed. After incubation, medium was removed and transferred to 15 ml reaction tubes (Greiner, Germany). Cells were washed once with PBS and subsequently trypsinized with 0.25 % Trypsin/ 0.05 % EDTA (Invitrogen, Germany). The cell suspension was centrifuged at 1000 rpm (Multifuge, Thermo scientific, Germany) for 5 min and the supernatant was removed. The cell pellet was dissolved in 0.5 ml of cell culture medium. 50µl of the cell suspension were mixed with 50 µl of the Trypan blue staining solution (Sigma-Aldrich, Germany). Trypan blue positive cells (dead cells) were counted using the Cedex XS cell counter (Innovates AG, Germany). The percentage of positive cells as a function of the total cell number was calculated.

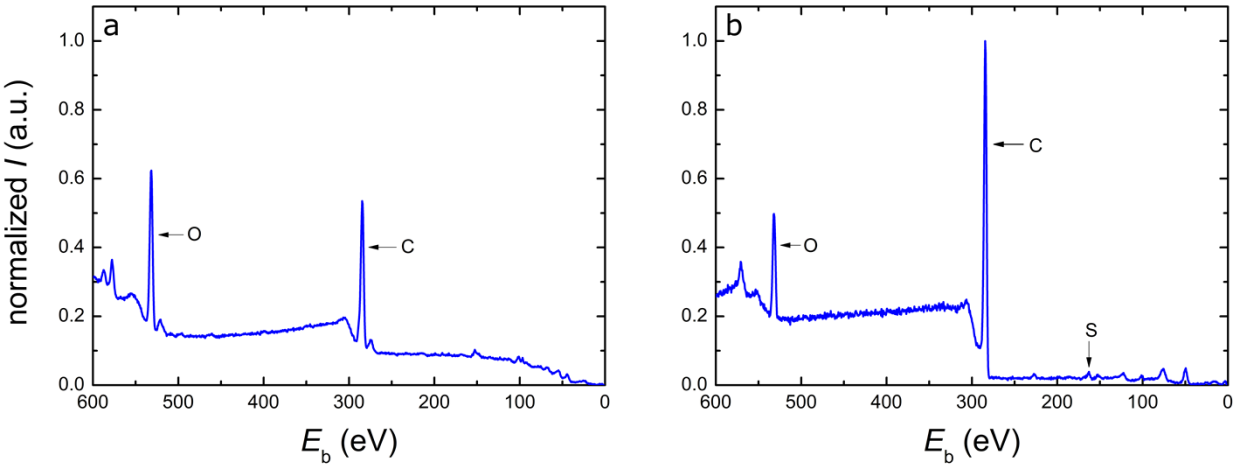
Cellular uptake of non-functionalized and functionalized particles in-vitro
Cellular uptake of nanoparticles by HUVEC was tested using fluorescence microscopy (Axio Imager Z2, Carl Zeiss, Germany). 3 x 10^5 cells were seeded in 6-well plates (Greiner, Germany) and cultured for 24h. Four hours prior incubation with

nanoparticles, cells were stimulated to express a higher amount of αvβ3-integrin using 4 ng/ml of human recombinant tumor necrosis factor-alpha (TNF-α) (ProSpec-Tany TechnoGene Ltd, USA). Medium was removed and each well was washed with 2 ml of phosphate buffered saline (PBS, Invitrogen, Germany). HUVEC incubated with cell culture medium served as negative controls. Non-functionalized and functionalized particles were diluted with cell culture medium to a concentration of 250 µg/ml. 3 ml of cell culture medium or medium containing particles was added to the cells and incubated for 6 h. 3 samples per condition were analyzed. After incubation, medium was removed and cell nuclei were stained with DAPI (4',6-diamidino-2-phenylindole, Vector Laboratories, Germany). Cells were washed with PBS and subsequently trypsinized as described above. Cells were transferred to glass slides (Langenbrinck, Germany) and mounted with Mowiol mounting medium (Sigma-Aldrich, Germany). Fluorescent micrographs were captured using an epifluorescence microscope (Axio Imager.M2, Carl Zeiss, Germany) equipped with a high resolution camera (AxioCam MRm Rev.3; Zeiss, Germany). Quantification was performed by counting the amount of intracellular nanoparticles within five randomly selected micrographs for each condition.

***In vitro* binding study (flow chamber experiments)**

Binding of nanoparticles on the surface of HUVEC was evaluated using flow chamber experiments. 3 x 10^5 cells were seeded in 35 mm Petri dishes (Greiner, Germany) and cultured for 24 h. Four hours prior incubation with nanoparticles, cells were stimulated to express a higher amount of αvβ3-integrin using 4 ng/ml of TNF-α. One hour prior incubation, cell membranes were stained with 5 µg/ml of wheat germ agglutinin Alexa Fluor 594 conjugate (Life technologies, Germany). Four hours after the addition of TNF-α, the 35 mm Petri dishes were mounted to a customized flow chamber. Subsequently, a suspension of either 250 µg/ml of non-functionalized or functionalized nanoparticles in cell culture medium, or a combination of 250 µg/ml functionalized particles together with free cRGD in excess (10,000 fold, for competition) was injected into the tube connecting both ends of the flow chamber. A peristaltic pump (Gilson Inc, USA) allowed circulation of the nanoparticles for 10 minutes at a flow rate of 0.25 ml/min. After incubation, the loop was opened and unbound nanoparticles were washed out with cell culture medium for 5 minutes using the same flow rate. Nuclei were stained with DAPI. Fluorescence microscope images were acquired using the Axio Imager.M2 (Carl Zeiss, Germany). For each condition (non-functionalized and functionalized nanoparticles, and competition with free cRGD), n=6 flow chambers were used. The mean number of bound nanoparticles per cell was determined by manually counting all nanoparticles and cells in the images.

Figure S1: a) XPS of non-functionalized particles. The **O**xygen and **C**arbon signals arise from residual Triton X-45 surfactant. Note that there is no **S**ulfur signal present. b) XPS spectrum of PEG functionalized particles. Note the S signal, which arises from the thiol-yne click reaction binding the PEG to the particle surface.



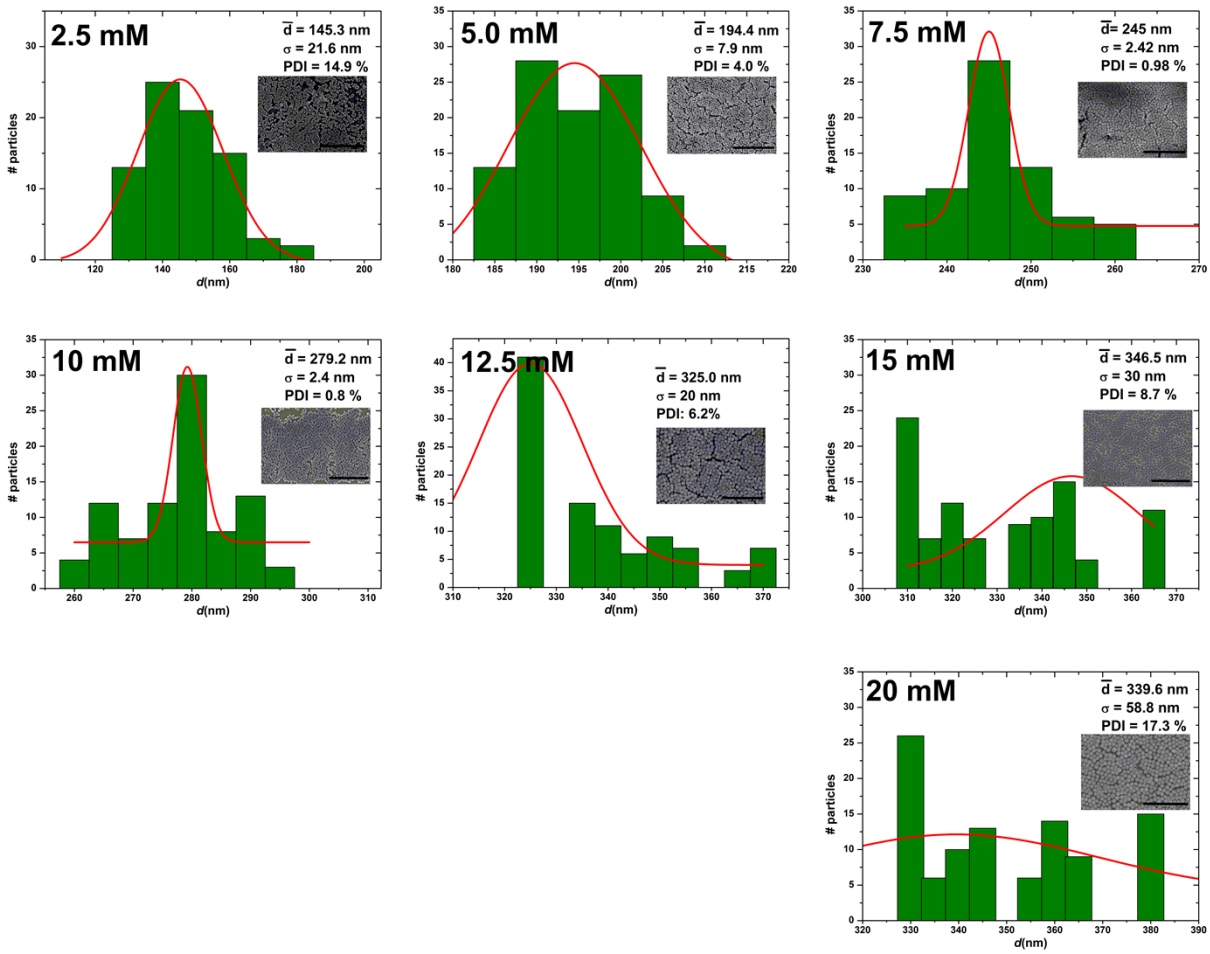


Figure S2: Size distribution histograms obtained from image analysis. The insets show SEM images of the respective particles. Scale bars represent 4 µm except 10 mM, here the scale bar represents 5 µm.

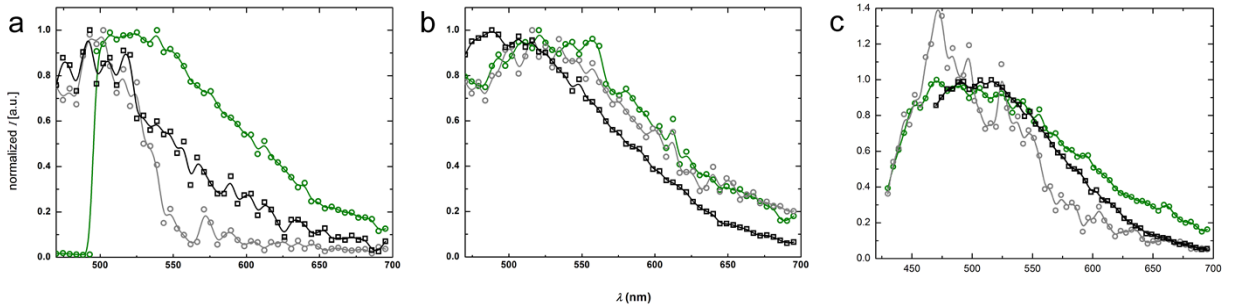


Figure S3: Fluorescence spectra (λ_{ex} = 405 nm) of particles in a) deionized water, b) PBS buffer and c) cell culture medium. The green data represents particles which are fleshly prepared, the gray data after exposure to 10 minutes of exposure to 370 mW laser radiation at 405 nm and black squares represents data taken after 10 days of storage in the different media.

Table S1: DLS measurement of the particle sizes in different media.

Fluorene-co-diacetylene phenylene particles in media:	**diameter**	**standard deviation**
pH = 1	262.5 nm	2.3 nm
pH = 3	269.6 nm	2.9 nm
pH = 5	271.2 nm	7.5 nm
deionized water (pH ~ 7)	245.1 nm	3.7 nm
PBS buffer (pH = 7.4)	246.0 nm	5.8 nm
pH = 9	251.9 nm	5.7 nm
pH = 11	261.3 nm	4.5 nm

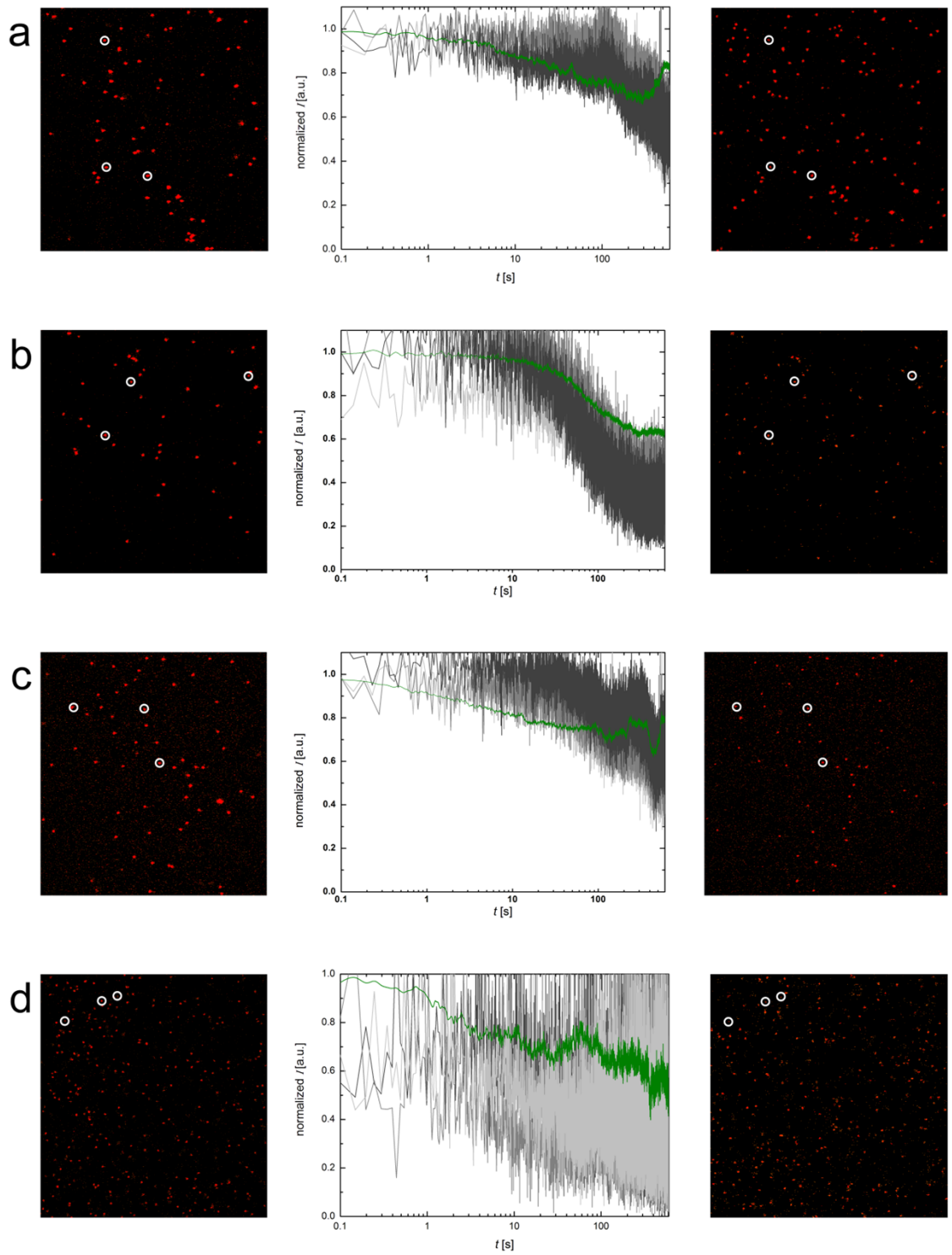


Figure S4: Bleaching study. 10 minutes of exposure to a 730 mW, 405 nm laser. The left side confocal images depict the sample before and the confocal images on the right after the bleaching experiment. The green data represents an average over the entire frame. The three gray lines represent single particles bleaching traces as indicated by the white circles in the confocal microscopy images. a) Particles in deionized water, b) in PBS buffer, c) cell culture medium. Data in d) represent PS particles dyed with PM 546 in deionized water for comparison. (Some particles attach and detach from the substrate over the course of 10 minutes. As a result the averaged data (green) can also rise over time as non-exposed particles enter the field of view. The three marked particles in each experiment are stationary at the substrate over the entire experiment.)