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Supporting Information

# DNA Detection by Flow Cytometry using PNA-Modified Metal–Organic Framework Particles

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## Supplementary results

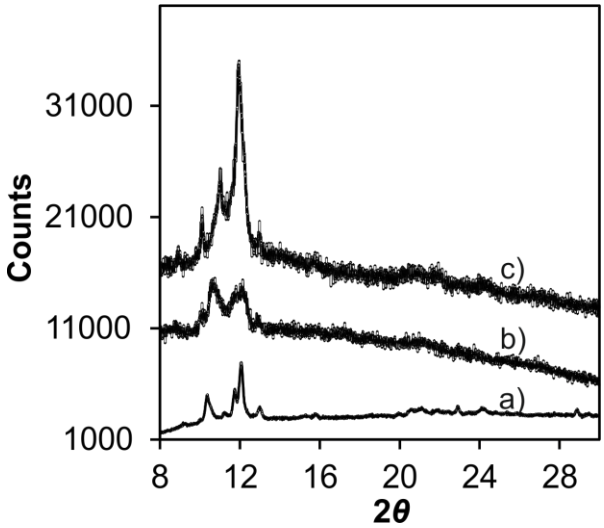


***Figure S1.*** XRD spectra of a) MIL-88A and MIL-88A functionalized with b) 5% 10-undecynoic acid, and c) 1% biotin-COOH capping ligand.

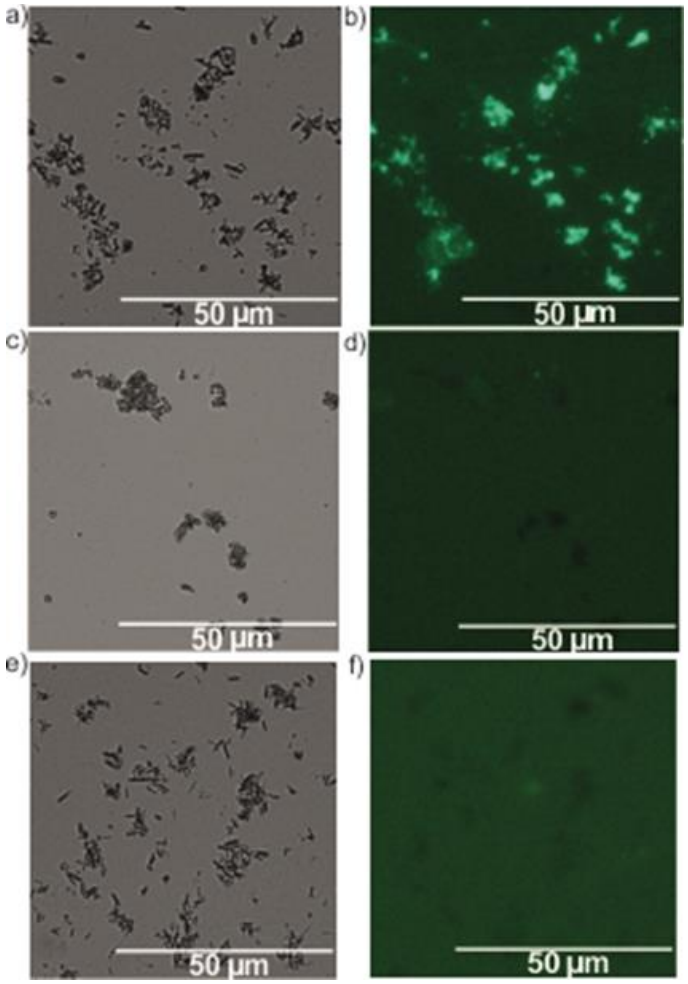


***Figure S2.*** Bright field (a, c, e) and fluorescence (b, d, f) microscopy images of MIL-88A after reaction of alkyne-MIL-88A (0.49 mM of alkyne) with coumarin azide (3.3 mM) (a, b; same as Figure 2), control 1 (c, d; using native MIL-88A instead of alkyne-MIL-88A), and control 2 (e, f; without catalyst).

Particles of biotin-MIL-88A, pre-conjugated with AF488-SAv, were incubated with biotin-PNA at room temperature using a PNA/SAv ratio of 3. Thereafter, hybridization between PNA and the complementary FM target, labeled with Cy5, was performed at 30 °C for 1 h using a PNA/DNA ratio of 1, followed by washing at room temperature (Figure S3) or at 40 °C (Figure 4). The same procedure was followed for the hybridization with the MM and random targets. The samples were characterized using flow cytometry and confocal microscopy. Figure S3a shows the flow cytometry results for FM after rinsing at room temperature, indicating a high intensity of the Cy5 (signal from FM) and AF488 (from SAv-modified MIL-88A). To test whether these interactions were specific (whether biotin-PNA is needed to sense complementary DNA), a control experiment was performed by incubating AF488-SAv-modified biotin-MIL-88A with FM in the absence of biotin-PNA. Flow cytometry showed also a high intensity of Cy5 in this case (Figure S3b), indicating the occurrence of non-specific interactions between FM and the MOF particles when rinsing was performed at room temperature. These interactions are attributed to electrostatic interactions between positively charged MIL-88A and negatively charged DNA. When this sample was rinsed at 40 °C, the flow cytometry results (Figure 4a/b) indicated that non-specific interactions were drastically reduced. Fluorescence imaging (see Figure 4b) confirmed the success of removing nonspecific interactions between FM DNA and non-PNA-modified MIL-88A, showing a strong intensity from AF488-SAv only.

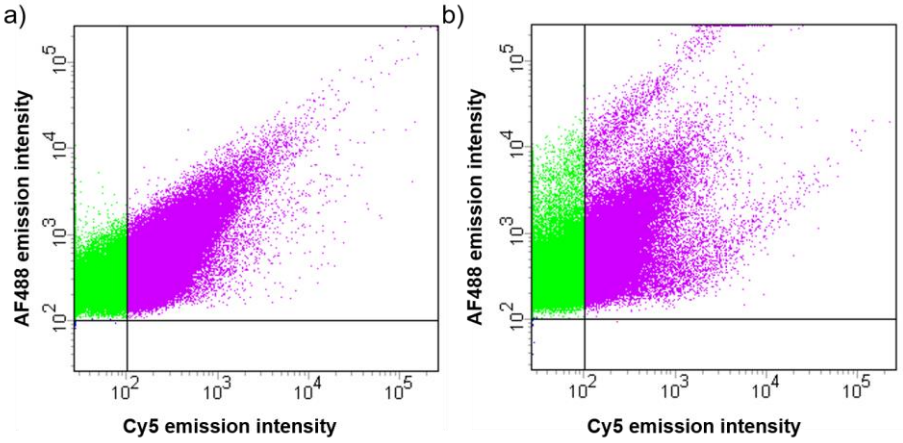


***Figure S3.*** Flow cytometry results for (a) biotin-MIL-88A particles pre-conjugated with AF488-SAv upon incubation with biotin-PNA (room temperature) followed by hybridization with $DNA_{FM}$ (30 °C) and rinsing with water (room temperature), and (b) control of biotin-MIL-88A particles pre-conjugated with AF488-SAv followed by incubation with $DNA_{FM}$ (30 °C) and rinsing with water (room temperature) in the absence of biotin-PNA. Only the fractions of particles are shown for which the AF488 intensity gate intensity is above $10^2$. The green markers indicate particles with a high intensity of AF488 only, and the purple markers represent particles with a high intensity for both AF488-SAv and Cy5.

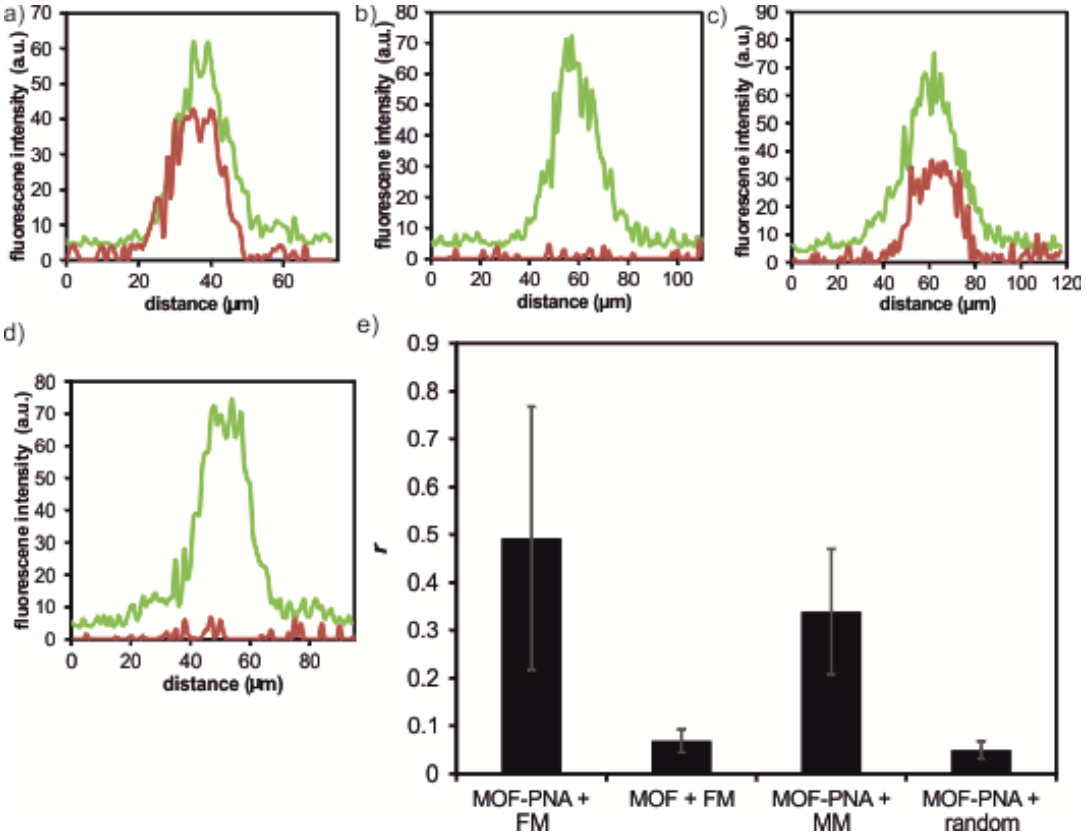


***Figure S4.*** Typical fluorescence intensity profiles from confocal images for a) MOF-PNA + $DNA_{FM}$, b) MOF (no PNA) + $DNA_{FM}$, c) MOF-PNA + $DNA_{MM}$, d) MOF-PNA + $DNA_{rand}$, after washing at 40 °C. Green = AF488 and red = Cy5 fluorescence channels. e) average intensity ratio (*r*) for different confocal images (see Experimental details below).

## Experimental details

### Materials

The following materials have been used as received from the supplier: ferric chloride hexahydrate (Acros, 99%), fumaric acid (Fluka, purum), 10-undecynoic acid (Sigma-Aldrich, 95%), 3-azido-7-hydroxycoumarin (Carbosynth), tris-(benzyltriazolylmethyl)amine (TBTA; Sigma), tetrakis(acetonitrile)copper(I) hexafluorophosphate (Sigma), biotin functionalized with tetra(ethylene oxide) carboxylic acid (Uptima), Alexa Fluor® 488 conjugate with streptavidin (Invitrogen), m-cresol (Fluka), diisopropylethylamine (DIPEA), trifluoroacetic acid (TFA), N,N-dimethylformamide (DMF), acetonitrile, dichloromethane, diethyl ether, Methanol (Sigma), absolute ethanol (Merck, p.a.), O-(benzotriazol-1-yl)-N,N,N,N-tetramethyluronium hexafluorophosphate (HBTU), biotin, acetic anhydride. Fmoc-PNA-monomers were purchased from Link Technologies. Oligonucleotides were purchased from Eurofins and used as received without further purification. Milli-Q water with a resistivity of 18.2 MΩ•cm at 25◦C was used for the synthesis of MOFs and all assembly experiments.

### Synthesis

Synthesis of MIL-88A

MIL-88A was synthesized according to a procedure developed in our group.[1]

Synthesis of MIL-88A functionalized with capping ligands

Alkyne-MIL-88A and biotin-MIL-88A were synthesized in a similar manner as described before. [1]

Synthesis of biotin-PNA: biotin-AEEA-AEEA-AAA CCC TTA ATC CCA-Gly-$NH_2$

The synthesis of biotin-PNA was performed by automated Fmoc-SPPS on a Biotage Syro I synthesizer using a Fmoc-Gly-RinkamideChemMatrix resin (loading 0.23 mmol/g, scale 5 µmol). The monomers were introduced by using the HBTU/DIPEA coupling protocol, the Fmoc group was deprotected by piperidine/DMF (20%). Biotin was introduced by manual coupling at the N-terminus of the last monomer of 2-(2-aminoethoxy)ethoxyacetic acid (AEEA) using HBTU/DIPEA as activating mixture. PNA was cleaved from the resin using a TFA/m-cresol (9:1) solution and precipitated in ether. The purification was carried out by RP-HPLC on a XTerraPrepRP18 column (10 µm, 300 Å, 300x7.8 mm ;flow rate: 4 mL/min and gradient elution from 100% water (0.1% TFA, eluent A) to 50% acetonitrile (0.1% TFA, eluent B) in 35 min. The purified product was characterized (Figure S5) by UPLC-ESI-MS (Acquity UPLC BEH C18, 1.7 µm, 2.1x50 m). The gradient conditions for UPLC and the retention time of the PNA are 0.9 minutes in water (0.2% formic acid, FA), then linear gradient to 50% acetonitrile (with 0.2% FA) in 5.7 minutes at a flow rate of 0.25 mL/min, retention time of biotinylated PNA: 2.92 min. Calcd. MW: 4548.59; calcd. m/z: 1138.1 $[MH4]^{4+}$, 910.7 $[MH5]^{5+}$, 759.1 $[MH6]^{6+}$, 650.8 $[MH7]^{7+}$, 569.6 $[MH8]^{8+}$, 506.4 $[MH9]^{9+}$ found m/z: 1138.4, 910.9, 759.3, 650.9, 569.7, 506.4. After MS analysis, the biotin-PNA was stored at -20°C, solubilized in MilliQ water.

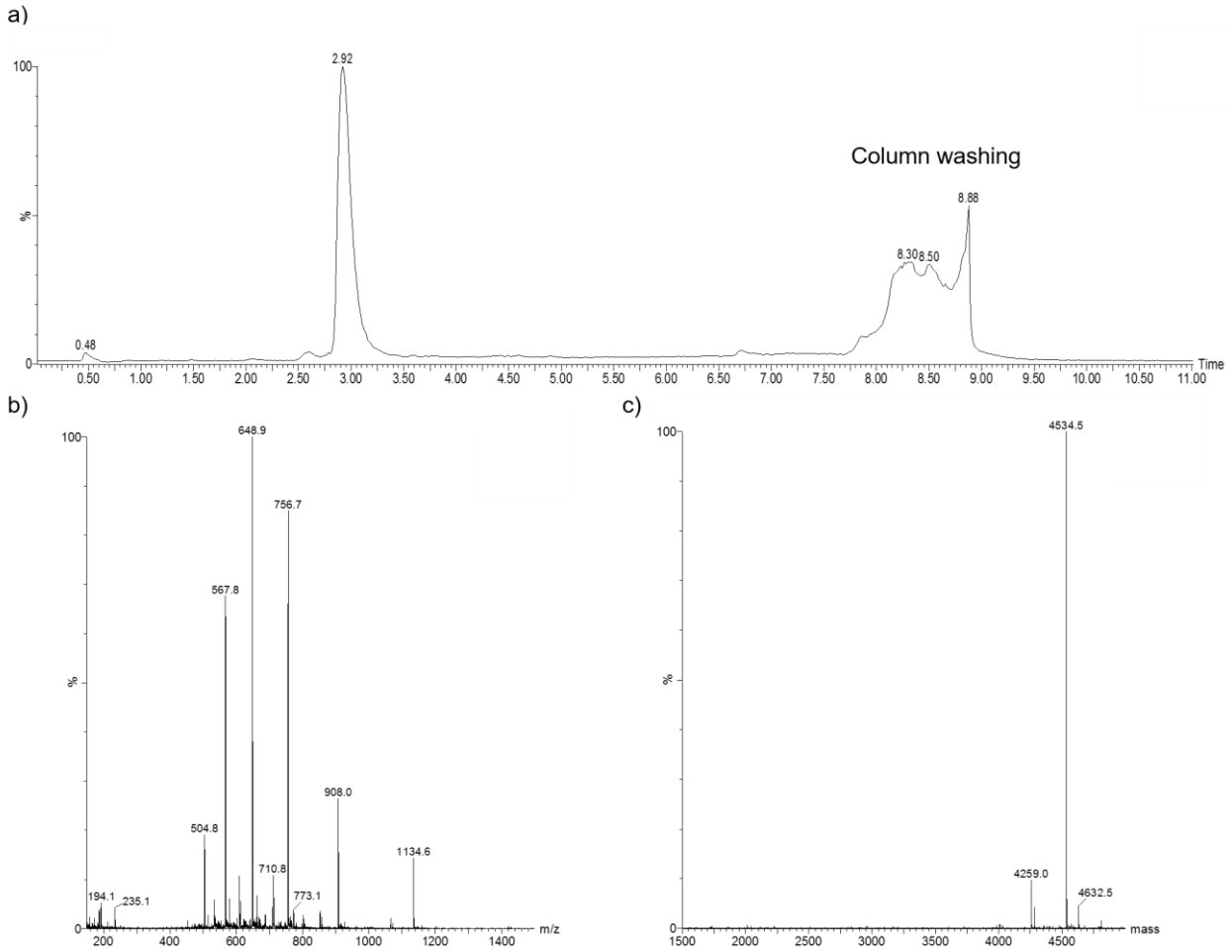


***Figure S5.*** UPLC-MS characterization of the Biotin-PNA; a) UPLC-MS chromatogram, b) ESI-MS spectrum of the 2.99 min peak; c) reconstructed MS spectrum from multicharged pattern of b). Conditions as indicated in the experimental details.

**Methods**

Click synthesis at low concentration

Under nitrogen, alkyne-MIL-88A (3.7 mg, 0.08 µmol alkyne) was dissolved in 162 µL methanol, and coumarin azide (0.54 µmol, 6.75 equiv.), TBTA (0.04 µmol, 0.50 equiv.) and $[Cu^{I}(CH_3CN)_4]PF_6$ (0.41 µmol, 5.13 equiv.) were added to the reaction mixture, which was allowed to react overnight under magnetic stirring at room temperature. For purification, the samples were washed six times using 10 mL of methanol followed by three times washing with water using centrifugation for 10 min at 1000 rpm followed by freeze drying.

Control 1: without alkyne groups at low concentration

Under nitrogen, MIL-88A (5 mg) was dissolved in 219 µL methanol, and coumarin azide (0.73 µmol), TBTA (0.05 µmol) and $Cu^{I}(CH_3CN)_4PF_6$ (0.55 µmol) were added to the reaction mixture, which was allowed to react overnight under magnetic stirring at room temperature. For purification, the samples were washed six times using 10 mL of methanol followed by three times washing with water using centrifugation for 10 min at 1000 rpm followed by freeze drying.

Control 2: without catalyst at low concentration

Under nitrogen, alkyne-MIL-88A (5 mg, 0.11 µmol alkyne) was dissolved in 214 µL methanol, and coumarin azide (0.73 µmol, 6.64 equiv.) and TBTA (0.05 µmol, 0.45 equiv.) were added to the reaction mixture, which was allowed to react overnight under magnetic stirring at room temperature. For purification, the samples were washed six times using 10 mL of methanol followed by three times washing with water using centrifugation for 10 min at 1000 rpm followed by freeze drying.

Click synthesis at high concentration

Under nitrogen, alkyne-MIL-88A (5 mg, 0.11 µmol alkyne) was dissolved in 126 µL methanol, and coumarin azide (0.73 µmol, 6.64 equiv.), TBTA (0.05 µmol, 0.45 equiv.) and $Cu^{I}(CH_3CN)_4PF_6$ (0.55 µmol, 5 equiv.) were added to the reaction mixture, which was allowed to react overnight under magnetic stirring at room temperature. For purification, the samples were washed three times using 10 mL of methanol followed by one time washing with water using centrifugation for 10 min at 1000 rpm followed by freeze drying.

Control 1: without alkyne groups at high concentration

This procedure was similar to the one described for click synthesis, except for replacing alkyne-MIL-88A by MIL-88A.

Control 2: without catalyst at high concentration

This procedure was similar to the one described for click synthesis, except for the absence of the catalyst $Cu^{I}(CH_3CN)_4PF_6$.

Fabrication of MOF (adsorption of AF488-SAv-modified biotin-MIL-88A)

Phosphate-buffered saline (PBS) (0.01 M phosphate; 0.138 M NaCl; 0.0027 M KCl; pH 7.4, at 25 °C) was used for all experiments with streptavidin. PBS was mixed with 0.05% of Tween-20 (PBS-tween). 1 mg of biotin-MIL-88A (0.45 nmol of biotin) was dispersed in 50 mL PBS-tween and mixed with 500 µg of Alexa-Fluor488-streptavidin (AF488-SAv, 1.72 nmol). The suspension was incubated for 1 h followed by centrifugation at 1000 rpm for 30 min. Thereafter, the suspension was washed two times for 1 h using 7.5 mL of PBS-tween followed by centrifugation at 1000 rpm for 30 min each time. The sample was resuspended in 7.5 mL PBS (without tween) to obtain MOF.

Fabrication of MOF-PNA

For this experiment, PBS was used without Tween-20. Here to a 4.5 mL suspension of MOF, 31.2 µL of biotin-PNA (492.53 µM, ~15 nmol) was added to give a PNA/streptavidin molar ratio of 3. The suspension was incubated for 1 h followed by centrifugation at 1000 rpm for 30 min. Then, the suspension was washed two times for 1 h using 4.5 mL of PBS followed by centrifugation at 1000 rpm for 30 min each time. The sample was resuspended in 4.5 mL PBS.

DNA hybridization

For each DNA hybridization, 1.5 mL of MOF-PNA is incubated with three different DNAs: 29.4 µL of $DNA_{FM}$ (170 µM, ~ 5 nmol), 30.1 µL of $DNA_{MM}$ (165 µM, ~ 5 nmol), and 89.1 µL of $DNA_{rand}$ (55.9 µM, ~ 5 nmol) for 1 h at 30 °C. For all these samples the PNA/DNA molar ratio was 1. For the control experiment without biotin-PNA, 1.5 mL of MOF was added to 29.4 µL of $DNA_{FM}$ (170 µM, ~ 5 nmol). All the suspensions were washed four times for 1 h at RT using 1.5 mL of PBS followed by centrifugation at 1000 rpm for 30 min each time. These samples were analyzed using flow cytometry and confocal microscope. Then, all the suspensions were washed one time for 40 min at 40 °C using 1.5 mL of PBS followed by centrifugation at 1000 rpm for 46 min each time and samples were analyzed using flow cytometry and confocal microscopy.

Statistical analysis for Figure S4e and Figure 5

Two t-tests were performed for the results for MOF-PNA + $DNA_{FM}$ vs different cases using the average *r* values from Figure S4e (confocal imaging) using the two-tailed and two-sample t-test with unequal variance (heteroscedastic) and the intensity values from Figure 5 (FACS) using the unpaired t-test[2] using the mean, the standard deviation, and the number of particles.

**Characterization**

X-ray powder diffraction (XRD)

For X-ray diffraction experiments a D2 Phaser (Bruker) using a Cu X-ray source was used.

High resolution scanning electron microscopy (HRSEM)

For HRSEM characterization a Zeiss using ImageJ software. For each sample at least 50 particles were measured.

Confocal fluorescence microscopy

A drop of MOF particles suspended in PBS was put between two glass slides and placed on a Nikon Eclipse Ti confocal laser scanning microscope fitted with a 100x oil objective (NA 1.49, WD 0.12). Differential interference contrast images were taken to examine particle size and morphology. Streptavidin coupled to Alexa-488 was visualized using a 488 nm laser and DNA labeled with Cy5 was visualized using a 633 nm laser.

The intensity ratios, *r*, reported in Figure S4e, were calculated as follows:

$$r = \Delta I_{Cy5}/\Delta I_{AF488} = (q_{90(Cy5)} - q_{20(Cy5)})/(q_{90(AF488)} - q_{20(AF488)})$$

where $q_{x(dye)}$ represents the x-th percentile, i.e., the intensity value below which x (90 or 20) percent of the intensity values in each profile may be found, for both dyes Cy5 and AF488-SAv.

Fluorescence microscopy

Fluorescence microscopy images were taken using an Olympus inverted research microscope IX71 equipped with a mercury burner U-RFL-T as the light source and a digital Olympus DR70 camera for

image acquisition. The filter used for all covalent surface experiments had an excitation of 350 nm and an emission of 460 nm using an Olympus filter cube. All fluorescence images were acquired in air.

Gas sorption

The BET measurements were performed on an Autosorb-1 from Quantachrome. The samples were degassed at 155°C for 3 hours.

Zeta potential (ZP)

ZP measurements were performed on a Zetasizer NanoZS (Malvern Instrument Ltd, Malvern, United Kingdom), with a laser wavelength of 633 nm and a scattering angle of 173°.

Flow cytometry

For the flow cytometry analysis, a BD (Becton Dickinson) FACS Aria II flow cytometer has been used. A 488 nm laser excited the AlexaFluor-488 dye, while the emission was measured using a 530 nm emission filter with a 20 nm bandwidth. The Cy5 dye was excited by a 633 nm laser and the emission was measured using a 660 nm emission filter with a bandwidth of 20 nm. Thresholds of 200 was set for both forward scatter and side scatter in order to focus only on the MOF's particles. For all samples, 45,000-200,000 events were measured at the lowest flow rate of 1 (A.U.) and using a Neutral Density filter of 1.5.

**References**

[1] T. Rijnaarts, R. Mejia-Ariza, R. J. M. Egberink, W. van Roosmalen, J. Huskens, *Chem. Eur. J.* **2015**, *21*, 10296-10301.
[2] http://www.graphpad.com/quickcalcs/ttesFM.cfm?Format=SD