Supporting Information

Sialyl-Tn Antigen-Imprinted Dual Fluorescent Core-Shell Nanoparticles for Ratiometric Sialyl-Tn Antigen Detection and Dual-Color Labeling of Cancer Cells

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1. Experimental Procedures

1.1 Chemicals and reagents

Thin layer chromatography (TLC) was performed using aluminium plates coated with silica (Kieselgel 60 F254, Merck KGaA, Darmstadt). Spots were detected by A) treatment with 10 % sulfuric acid in ethanol and subsequent heat exposure, B) UV light (254 nm). Pd/C (10 wt. % loading), celite, cation exchange resin (Dowex 50WX8), sodium benzoate (C₆H₅COONa), tetrabutylammonium-hydroxide (TBA-OH), tetraethylammonium-hydroxide (TEA-OH), tetramethylammonium-hydroxide (TMA-OH) and sulfuric acid (98%) were purchased from Sigma Aldrich, sodium perfluorooctanoate (C₇F₁₅COONa) and sodium formate (HCOONa) from Alfa Aesar, dialysis tubes (Micro Float-A-Lyzer 100-500 Da) from Spectra/Por.

1.2 Instruments

High-resolution ESI-spectra were recorded with a LTQ Orbitrap XL mass spectrometer (ThermoFisher). NMR spectra were measured on a Varian Mercury 400 (Agilent) or Ascend 600 (Bruker) instrument. Transmission electron microscopy (TEM) images were registered with a Talos F200S scanning/transmission electron microscope, ThermoFisher. TGA measurements were performed on a Mettler-Toledo TGA/SDTA 851 equipped with a single-arm balance and an auto-sampler, in accordance with ISO 11358:1997. For each case, 5 mg of sample were heated from 25 °C to 1000 °C with 10 °C min⁻¹ in a nitrogen flow of 30 mL min⁻¹. Elemental analysis was performed with a Euro EA-Elementaranalysator. Dynamic light scattering experiments were performed with a Zetasizer Nano ZS (Malvern Instruments). For ζ-potential measurements, 0.1 mg mL⁻¹ particle suspensions were prepared in Milli-Q water and measurements performed using disposable folded capillary cells. The ATR-FTIR spectra were recorded with a Nicolet Nexus 670 FT-IR (ThermoFisherScientific GmbH) in attenuated total reflection (ATR) mode and a "Golden Gate" sample holder. Per measurement 32 scans were taken with a resolution of 4 cm⁻¹. The spectral range was set to 4000 – 600 cm⁻¹ and the spectra were recorded in absorption mode. The software OMNIC was used and the spectra were exported in CSV file format.

1.3 Synthesis of Sialyl-Tn (Neu5Acα2,6-GalNAcα-O-Thr (STn-Thr))

Fmoc-4,7,8,9-tetra-OAc-1-OBn-Neu5NAc-3,4-di-OAc-GalNAc-Thr (200 mg, 170 µmol)¹ was dissolved in dry MeOH (25 mL) and 10% Pd/C was added. The reaction mixture was flushed with Ar and H₂ gas and was then stirred under H₂ atmosphere at RT for 2 d. LC-MS analysis indicated complete removal of the benzyl- and Fmoc-protecting groups. The Pd/C was filtered off over Celite and the filtrate was concentrated under reduced pressure. The residue was taken up into a solution of MeOH/H₂O 1:1 (10 mL). The pH was adjusted to 9.5 with 1 M NaOH. After 3 d of stirring at RT, TLC indicated complete removal of the acetyl groups (TLC: EtOAc/MeOH/AcOH/H₂O 3:3:3:2, R_f = 0.31). The reaction mixture
was neutralized with acidic cation exchange resin. The resin was filtered off and the solvent removed *in vacuo*. The crude product was dialysed to obtain Neu5Ac-GalNAc-Thr (30 mg, 49 µmol) in 29 % yield over two synthetic steps. HR-ESI-MS: m/z: calcd for [M+H]⁺: 614.2403, found: 614.2409. NMR was in accordance with previous published data described from a synthesis of STn-Thr using an enzymatic approach.²

### 1.4 Spectroscopic studies

#### 1.4.1 Interaction studies with molecular components

In the spectroscopic studies of 1 with STn-Thr-Na and the other sodium salts (sodium benzoate, perfluorooctanoate, formate and acetate) in DMF, 15 µL of fluorescent monomer (1, 4 mmol L⁻¹) was mixed with 45 µL of 0 – 1.0 eq. of STn-Thr-Na (or other sodium salts) to obtain a 60 µL solution. Absorption and fluorescence spectra were acquired after pipetting 15 µL of as-prepared solutions to the center of a micro-cell cuvette. In case of the molecularly imprinted polymer (MIP) pre-polymerization mixture, 15 µL of each STn-Thr-Na (or other sodium salts, 5 mmol L⁻¹), 1 (5 mmol L⁻¹), VBA (5 mmol L⁻¹), 2D (40 mmol L⁻¹) and EGDMA (65 mmol L⁻¹) were added to obtain a 75 µL solution. A ca. 5 min sonication was applied to dissolve STn-Thr-Na, sodium salts and monomers in DMF. Absorption and fluorescence spectra were acquired after pipetting 15 µL of as-prepared solution to the center of the micro-cell cuvette. In case of non-imprinted polymer (NIP), 15 µL solvent was added instead of the template solution.

The equilibrium constants (Kₐq) were determined via fitting the plot of the equivalency between 1 and sodium salts vs. ΔA₅₀₀/A₅₀₀_initial=(A₅₀₀ₓ−A₅₀₀_initial)/A₅₀₀_initial, which was calculated for each absorption spectrum (where A₅₀₀ₓ is the absorbance at 500 nm for each spectrum after salt addition, while A₅₀₀_initial is the absorbance at 500 nm before addition of salt) with Bindfit (1:1 model,³,⁴). Calculated parameters of sodium salts were obtained from optimized S₀ ground-state geometries in the gas phase, using the density functional theory (DFT) method employing the hybrid functional B3LYP with a 6-31G basis set and energy minimized as implemented in Gaussian 16W⁵ and linear regressions of KₐqS and the calculated parameters were performed via Microsoft Excel.

#### 1.4.2 Studies with STn-MIPs and NIPs in solution

To perform fluorescence titrations of the STn-MIPs and NIPs with the template as the analyte and potential competitors, 0.5 mg mL⁻¹ suspensions of the STn-MIP and NIP particles were prepared in DMF; a 6.5 mmol L⁻¹ solution of STn-Thr-Na in DMF was also prepared. Increasing volumes of the 6.5 mmol L⁻¹ solution of STn-Thr-Na in DMF was also prepared. Increasing volumes of the 6.5 mmol L⁻¹ solution of STn-Thr-Na in DMF were added to 2 mL suspensions of the STn-MIP and NIP particles, and after 2 min of stirring for each addition step the resultant absorption and fluorescence spectra recorded. ΔF/F₀ = (Fₓ−F₀)/F₀ was calculated for each fluorescence spectrum of the STn-MIP and NIP particles (where Fₓ is the fluorescence intensity at 510 nm for each spectrum after template addition, while F₀ is the fluorescence intensity at 510 nm before addition of STn-Thr-Na).
imprinting factor (IFn) was determined from the MIP:NIP ratio of ΔF/F₀ at the saturation point of the titration. The discrimination factor (DFn) was determined from the ratio of ΔF/F₀ at the saturation point (0.22 mmol L⁻¹) of the STn-MIPs titrated with STn-Thr-Na and with the competitors (NaOAc, NaOBz, Glc, Gal, Man, GlcNAc and GalNAc).

1.5 Cell culture

BxPC-3 cells were cultured in RPMI-1640 medium (Gibco, Cat#32404014) with 10% fetal bovine serum (FBS, HyClone, Cat#SV30160.03) and 2 mmol L⁻¹ L-glutamine (Corning, Cat#25-005-CI). CFPAC-1 cells were cultured in Iscove's Modified Dulbecco's Medium (IMDM, Gibco, Cat#21056-023) with 10% FBS and 2 mM L-glutamine. All experiments were performed with mycoplasma-free cells.

1.6 Immunofluorescence (IF) staining

Cells were seeded on glass coverslips for overnight culture, followed by fixation with 4% paraformaldehyde. The cells were permeabilized in 0.1% Triton X-100 (Sigma-Aldrich, Cat# T8787) for 10 min at RT, which were then blocked in 1% FBS in 1x PBS for 1 h. Afterwards, the cells were stained overnight with primary antibodies (1:50 dilution) at 4 °C. The STn antibody TKH2 and the Tn antibody 5F4 were kindly provided by Henrik Clausen (Copenhagen Center for Glycomics, University of Copenhagen). The cells were then washed three times with PBS and stained with anti-Mouse IgG (Invitrogen, Cat#A-21202) at RT for 1 h. The cell structure and nucleus were, respectively, highlighted by Rhodamine-conjugated phalloidin (Invitrogen, Cat#R415) and DAPI (4',6-diamidino-2-phenylindole, dihydrochloride, PanReac AppliChem, Cat#A1001). The slides were viewed under a ZEISS Apotome microscope (ZEISS). The data were analyzed by the Zen 2.3 Lite software (ZEISS).

1.7 STn-MIPs and NIPs staining for microscopy analysis

Cells were seeded on coverslips for overnight culture, followed by fixation with 4% paraformaldehyde. The cells were then stained in 2 mL STn-MIPs solution (0.02 mg mL⁻¹ STn-MIPs in PBS) in a 6-well plate. The staining was performed on an orbital shaker at 120 rpm under 37 °C for 1 h. After that, the cells were washed three times using 2 mL PBS and stained by DAPI to highlight the nucleus. The slides were viewed under a ZEISS Apotome microscope (ZEISS) in the green channel (65 HE AF488, exc. 493 nm, em. 517 nm, exposure time: 4.65 s) and the red channel (50 Cy5, exc. 650 nm, em. 673 nm, exposure time: 10 s). The data were analyzed by the Zen 2.3 Lite software (ZEISS).

1.8 Cell staining for flow cytometry (fluorescence-assisted cell sorting, FACS) assays

For antibody staining, cells were firstly stained with the primary antibody at 4 °C for 30 min. After three times of washing with PBS, the cells were then stained with anti-mouse IgG (Invitrogen, Cat#A-21202) at 4 °C for 30 min. For lectin staining, SNA (biotinylated Sambucus Nigra Lectin, Vector, Cat#B-1305),
MAL I (biotinylated Maackia Amurensis Lectin I, Vector, Cat#B-1315), or MAL II (biotinylated Maackia Amurensis Lectin II, Vector, Cat#B-1265) were used at a concentration of 20 µg mL\(^{-1}\) at 4 °C for 30 min. After three times of washing with PBS, the cells were stained with FITC-conjugated streptavidin (Invitrogen, Cat#SA1001) at a concentration of 5 µg mL\(^{-1}\) at 4 °C for 30 min. After the final staining with the secondary antibody or streptavidin, the cells were washed three times with PBS and were resuspended in 300 µL of PBS.

For nanoparticle staining, STn-MIPs and NIPs were dissolved and diluted in PBS at a concentration of 0.1 mg mL\(^{-1}\). The cells (0.2 million) were stained in 400 µL of nanoparticle solution at 37 °C with gentle rotation for 30 min.

A flow cytometer LSR II (BD Biosciences) was applied to analyze the antibody/lectin/nanoparticle-stained samples. The data were analyzed with the software FCS Express 6 (DeNovo Software).

1.9 Procedure of the determination of equilibrium constants (\(K_{eq}\)) in solution

1. Measure the absorption spectrum of 1 (1 mmol L\(^{-1}\)) in DMF. The absorbance at 500 nm is set as \(A_{500\text{initial}}\).

2. Measure the absorption spectra of 1 (1 mmol L\(^{-1}\)) with varying equivalents of sodium salts. The absorbance at 500 nm of each spectrum after salt addition is set as \(A_{500x}\).

3. Input the data (\(A_{500\text{initial}}\), \(A_{500x}\) and the concentration of 1 and salts) to BindFit (http://app.supramolecular.org/bindfit/),\(^3,4\) and fit the plot with the UV 1:1 model.

1.10 Procedure of the determination of equilibrium constant (\(K_{eq}\)) of STn-MIPs\(^8,9\)

1. Measure the absorption spectrum of plain silica nanoparticles (ca. 65 nm in diameter, 0.5 mg mL\(^{-1}\)) in DMF. The absorbance at 410 and 500 nm are set as \(A_{410\text{plain}}\) and \(A_{500\text{plain}}\), respectively.

2. Measure the absorption spectrum of STn-MIPs (0.5 mg mL\(^{-1}\)) in DMF. The absorbance at 410 and 500 nm are set as \(A_{410\text{MIP}}\) and \(A_{500\text{MIP}}\), respectively.

3. Calculate the concentration of 1 in STn-MIPs by following equation: \(c_1 = (A_{410\text{MIP}} - A_{410\text{plain}})/\varepsilon_{410} + (A_{500\text{MIP}} - A_{500\text{plain}})/\varepsilon_{500}\). The \(\varepsilon_{410}\) and \(\varepsilon_{500}\) were measured from Figure S1E.

4. Input the data (\(c_1\) and concentration of STn-Thr-Na) to BindFit (http://app.supramolecular.org/bindfit/) and fit the plot with the UV 1:1 model.
1.11 Procedure of the ImageJ based analysis of immunofluorescence images

1. Open image: open ImageJ (National Institutes of Health (NIH), 1.52e), and click File – Open.

2. Select line: chose Straight function, and select an 1000 pixel long line, e.g., from position x=50, y=30 to position x=50, y=1030.
3. Measure: click Analyze – Plot Profile, and save the file.

4. Plot: repeat Step 1 – 3 for other two images (e.g., images measured from red and green channels). Plot three curves in one figure.

5. Analyze whole image: move to next position (x=100, y=30), and repeat Step 1 – 4. Repeat for every 50 pixel until the last position (x=1350, y=30).

6. Counting and calculation: count cells based on the number of peaks of DAPI images’ plots. Count cell-bound nanoparticles when nanoparticle peaks are overlapping or close to (within ca. 10 pixels)
cell peaks. Sum up the number of cells, cell-bound nanoparticles from red and green channels, respectively, and calculate the percentage of positive cells.

1.12 Statistical analyses

For *in vitro* cell experiments, at least two independent experiments were performed. The student t-test was used. The statistical tests were two-sided, and *p* values less than 0.05 were considered to be statistically significant. The mean is the average value of all samples. The standard deviation (SD) is an indication of the variability of all samples.
2. Supplementary Tables and Figures

**Table S1.** Correlations between lowest-energy absorption maxima of 1 in various organic solvents and theoretical as well as empirical solvent polarity scales.

| Solvent | $\lambda_{\text{max}}$ b | $\tilde{\nu}_{\text{max}}$ b | $f(\varepsilon)$ c | $f(\tilde{\varepsilon}, n)$ c | $E_T^{N,d}$ | SPP$^N$ ε | $\pi^*$ f | $\alpha^f$ | $\beta^f$ | SP$^e$ | SdP$^e$ | SA$^e$ | SB$^e$ |
|---------|----------------|----------------|----------------|----------------|-------------|-------------|--------|--------|--------|------|------|------|------|
| CHCl$_3$ | 410.1 | 24384 | 0.359 | 0.210 | 0.254 | 0.259 | 0.786 | 0.58 | – | – | 0.783 | 0.614 | 0.047 | 0.071 |
| MeCN | 412.7 | 24230 | 0.479 | 0.175 | 0.392 | 0.460 | 0.895 | 0.75 | 0.19 | 0.31 | 0.645 | 0.974 | 0.044 | 0.286 |
| Aceton | 413.0 | 24215 | 0.464 | 0.180 | 0.374 | 0.355 | 0.881 | 0.71 | 0.08 | 0.48 | 0.651 | 0.907 | – | 0.475 |
| CHon$^a$ | 415.9 | 24041 | 0.455 | 0.212 | 0.349 | 0.281 | 0.874 | 0.76 | – | 0.53 | 0.766 | 0.745 | – | 0.482 |
| DMF | 423.3 | 23626 | 0.48 | 0.205 | 0.377 | 0.404 | 0.954 | 0.88 | – | 0.69 | 0.759 | 0.977 | 0.031 | 0.613 |
| DMSO | 428.6 | 23332 | 0.484 | 0.221 | 0.374 | 0.444 | 1.000 | 1.000 | – | 0.76 | 0.830 | 1.000 | 0.072 | 0.647 |
| $r^2$ h | 0.203 | 0.016 | 0.153 | 0.108 | 0.804 | **0.911** | – | **0.819** | 0.195 | 0.238 | 0.003 | 0.641 |

$^a$ CHon = cyclohexanone

$^b$ Absorption maxima in wavelengths and wavenumbers, derived from the measured spectra by log-normal band fitting.

$^c$ Classical solvatochromic functions derived by Lippert and Mataga on the basis of dielectric constant $\varepsilon$ and refractive index $n$. The various modifications of the Lippert–Mataga formalism do not lead to significant improvements of the correlations. $^{12-14}$

$^d$ Normalized empirical solvent polarity scale as derived with substituted pyridinium-N-phenol betaines. $^{15}$

$^e$ Normalized empirical solvent polarity scale as derived with donor-acceptor-substituted fluorenes. $^{16}$

$^f$ Linear solvation energy relationships, emphasizing dipolarity/polarizability ($\pi^*$), hydrogen bond donor acidities ($\alpha$) and hydrogen bond acceptor basicities ($\beta$). $^{17}$

$^g$ Refined empirical solvent property scales derived on the basis of the SPP scale employing two different chromophores; SdP – dipolarity scale, SP – polarizability scale, SA – acidity scale, SB – basicity scale. $^{18}$

$^h$ Goodness of the linear regression. Main Text Paragraph.

The correlation of the longest wavelength absorption maxima with various different solvent polarity scales in Table S1 clearly shows that neither theoretical solvation models described the behavior of 1 adequately nor a comparison with empirical solvent polarity scales, the SPP$^N$ scale being the closest. Instead, consideration of dipolarity and polarizability effects as in the $\pi^*$ scale as well as hydrogen bond acceptor basicities as in the $\beta$ scale, both proposed by Kamlet, Taft and co-workers seem to describe the scenario best.
Figure S1. (A) Fluorescence excitation ($\lambda_{em}$ 590 nm) and (B) emission ($\lambda_{ex}$ 500 nm) spectra of 1 (1 mmol L$^{-1}$) with 0–0.7 eq. of NaOAc in DMSO. (C) Fluorescence excitation ($\lambda_{em}$ 590 nm) and (D) emission ($\lambda_{ex}$ 500 nm) spectra of 1 (1 mmol L$^{-1}$) with 0–0.7 eq. of NaOAc in DMF; 0 eq. NaOAc (black), 0.1 eq. NaOAc (red), 0.3 eq. NaOAc (blue), 0.5 eq. NaOAc (green), 0.7 eq. NaOAc (purple); (E) Absorption spectra of 1 (1 mmol L$^{-1}$) in DMF in absence (black) and presence (red) of 1 eq. of tetramethylammonium-hydroxide.
Figure S2. Absorption spectra of 1 (1 mmol L$^{-1}$) titrated with 0–0.7 eq. of (A) STn-Thr-Na, (B) sodium formate (NaOFor), (C) sodium benzoate (NaOBz) and (D) sodium perfluorooctanoate (NaOPFO) in DMF; 0 eq. (black), 0.1 eq. (red), 0.3 eq. (blue), 0.5 eq. (green), 0.7 eq. (purple).
**Table S2.** Linear regression of equilibrium constants ($K_{eq}$) with calculated parameters of sodium salts.\(^a\)

| Sodium formate (HCOONa) | log$K_{eq}$ | av Q(O) (au) \(^b\) | av d(O-Na) (Å) | Q(COO) (au) \(^b\) | Q(COOH) (au) \(^b\) |
|-------------------------|------------|----------------------|----------------|------------------|------------------|
| Acid form               | 3.78       | 0.452                | 0.567          | 2.215            | 0.554            |
| Carboxylate form        |            | 0.598                |                |                  | 0.917            |
| Sodium salt form        |            |                      |                |                  | 0.802            |
| Sodium acetate (CH$_3$COONa) | 3.14       | 0.478                | 0.595          | 2.197            | 0.484            |
| Carboxylate form        |            | 0.619                |                |                  | 0.811            |
| Sodium salt form        |            |                      |                |                  | 0.717            |
| Sodium benzoate (C$_6$H$_5$COONa) | 3.19       | 0.491                | 0.604          | 2.195            | 0.566            |
| Carboxylate form        |            | 0.592                |                |                  | 0.797            |
| Sodium salt form        |            |                      |                |                  | 0.797            |
| Sodium perfluorooctanoate (C$_7$F$_{15}$COONa) | 2.78       | 0.424                | 0.542          | 2.235            | 0.398            |
| Carboxylate form        |            | 0.541                |                |                  | 0.647            |
| Sodium salt form        |            |                      |                |                  | 0.653            |
| | $R^2$ | 0.06 | 0.33 | 0.05 | 0.09 | 0.60 | 0.92 | 0.69 | 0.62 |

| STn-Thr-Na | 2.97 | 0.440 \(^c\) | 0.723 \(^c\) | 0.690 \(^c\) | 0.080 \(^c\) |

\(^a\)As the true nature of the ternary complex or solvated forms thereof, and their relative ratios in a given medium, are unknown and $K_{eq}$ are equilibrium constants, yet no defined pKa values, we refrained from more elaborate theoretical calculations usually performed to approximate pKa values and used this straightforward and practical approach.

\(^b\) absolute value

\(^c\) calculated from fitted equation

NOTE: The deprotonation band at ca. 500 nm increased upon the addition of the sodium salts, resulting in moderate equilibrium constants ($\log K_{eq}$ 2.8–3.8) with 1 in DMF (Figure 4C, S2A-D). Furthermore, the correlation of the $K_{eq}$ with the calculated electronic parameters of the different sodium salts shows a linear regression ($R^2 \approx 0.92$) between $\log K_{eq}$ and the sum of the charges of the carboxylate group, i.e., the charges on the carbon and the two oxygen atoms. In the case of the linear regression between $\log K_{eq}$ and the other calculated parameters (Table S2), $R^2$ values were lower than 0.7, which indicates that 1 mainly interacts with the sodium salts of the carboxylate form in DMF.
Figure S3. Absorption (solid lines) and fluorescence (dashed lines) spectra of 1 (1 mmol L⁻¹) in presence of co-monomers (VBA, 2D) and cross-linker (EGDMA) (black) and with 1 eq. of STn-Thr-Na (purple) in DMF at the chosen polymerization conditions (STn-Thr-Na : 1 : VBA : 2D : EGDMA = 1 : 1 : 1 : 8 : 13).

Figure S4. (A) Absorption (solid) and fluorescence (dashed) spectra (λex 410 nm) of R-CNDs (ca. 1 µg mL⁻¹) and (B) representative TEM image of R-CNDs.
Figure S5. (A) Absorption (bold) and fluorescence (dashed) spectra of plain silica particles (plain SiO$_2$, black) and silica-coated red carbon nanodots (R-CSN, red), (B) ATR-FTIR spectra of plain SiO$_2$ (black) and R-CSNs (red), (C) TEM images of plain SiO$_2$ (left) and R-CSNs (right) and (D) hydrodynamic diameter and zetapotential of plain SiO$_2$ and R-CSNs determined by dynamic light scattering.
Figure S6. (A) TGA plots of the nanoparticles prepared. (B) Amounts of APTES and RAFT agent (CPDB) on nanoparticles (in mmol g\(^{-1}\)) estimated from EA (black bars) and TGA (red bars). (C) Zeta potential and hydrodynamic diameter in nm of R-CNDs (black), R-CSN (red), APTES-R-CSN (blue), RAFT-R-CSN (green), and STn-MIPs (purple) at pH 7.4. The two steps found for CPDB functionalized silica indicate that the RAFT agent is bound in two different ways, presumably physically adsorbed as well as chemically conjugated (in a ratio of ca. 1.3:1), leading to an apparent overall highest weight loss, see \(^{19}\).
**Figure S7.** Representative dot plot for STn antibody TKH2 stained BxPC-3 and CFPAC-1 cell lines.

**Figure S8.** CFPAC-1 cells were stained with DAPI, STn-MIPs and NIPs and analyzed by a fluorescence microscope in the blue, green and red channels.
Figure S9. Plots of selected lines of STn-MIP stained cell images (Figure S7 MIP column). The numbers represent cells (in black), bound nanoparticles from red (in red) and green (in green) channels.
Figure S10. Plots of selected lines of NIP stained cell images (Figure S7 NIP column). The numbers represent cells (in black), bound nanoparticles from red (in red) and green (in green) channels.
Figure S11. Representative dot plot for NIP- and STn-MIPs-stained BxPC-3 and CFPAC-1 cell lines.

Figure S12. Analysis of the expression of Tn on BxPC-3 and CFPAC-1 cell lines after staining with Tn-antibody 5F4 investigated by (A) immunofluorescence staining (scale bar: 100 µm) and (B) flow cytometry, (C) percentage of Tn positive cells from the flow cytometry analysis, (D, E) representative dot plots for each staining.
Figure S13. (A, B) Analysis of the expression of SA on BxPC-3 and CFPAC-1 cell lines by flow cytometry after staining respectively with MAL I, MAL II and SNA and (C) percentage of SA positive cells from the flow cytometry analysis, (D-I) representative dot plot for each staining.
Table S3. MIPs for fluorescence staining of cell surface glycans.

| Entry | Template<sup>a</sup> | Architecture / emitter (position)<sup>b</sup> | Templating interactions<sup>c</sup> | Exploitable fluorescence signal per particle<sup>d</sup> | Cell lines<sup>e</sup> | Prevalent antigens expressed on cell surface<sup>f</sup> | Control experiments<sup>g</sup> | Ref. |
|-------|----------------------|---------------------------------------------|--------------------------------|------------------------------------------|-----------------|-----------------------------------------|---------------------------------|-----|
| 1     | GlcA                 | DD-MIP bMP                                  | Unclear                       | Single                                   | HaCaT           | SNA-positive<sup>20</sup>               | HABP staining for HA           | 21  |
| 2     | SA                   | Pdot bNP                                    | Boronic acid/cis-diol ester   | Single                                   | DU 145, HeLa    | sLe<sup>X</sup>, (DU145 HeLa)<sup>22, 23</sup> | none / not shown               | 24  |
| 3     | SA                   | CS / FP (MIP shell)                         | Urea/carboxylate H bond + boronic acid/cis-diol ester | Single                                   | DU145, PC3      | sLe<sup>X</sup> (DU145, PC-3)<sup>23, 25</sup> | lectin (unspecified) staining  | 26  |
| 4     | SA                   | CS / FP (MIP shell)                         | Urea/carboxylate H bond + boronic acid/cis-diol ester | Single                                   | CLL (HG3, CI, Waosel, AIII) | Unknown                               | lectin (unspecified) staining  | 27  |
| 5     | SA                   | CS / FP (MIP shell)                         | Urea/carboxylate H bond + boronic acid/cis-diol ester | Single                                   | MCF-7, MDAMB231 | SNA-positive (MCF-7, MDAMB231)<sup>28, 29</sup> | SNA, MAL I staining           | 30  |
| 6     | SA                   | CS / FP (MIP shell)                         | Urea/carboxylate H bond + boronic acid/cis-diol ester | Single                                   | A549, A431      | sLe<sup>X</sup>, sLe<sup>A</sup> (A431)<sup>31</sup> | SNA, MAL I staining           | 8   |
| 7     | GlcA                 | CS / CND (core)                             | Unclear                       | Single                                   | HeLa, HaCaT     | sLe<sup>X</sup>, sLe<sup>A</sup> (HeLa)<sup>22</sup> | none / not shown               | 32  |
| 8     | Fuc, Man, SA         | CS / DD-SNP (core)                          | Boronic acid/cis-diol ester   | Single                                   | HepG-2, L-02, MCF-7, MCF-10A | See above                           | SNA, UEA I, LCA staining       | 33  |
| Entry | Template<sup>a</sup> | Architecture / emitter (position)<sup>b</sup> | Templating interactions<sup>c</sup> | Exploitable fluorescence signal per particle<sup>d</sup> | Cell lines<sup>e</sup> | Prevalent antigens expressed on cell surface<sup>f</sup> | Control experiments<sup>g</sup> | Ref. |
|-------|---------------------|-----------------------------------------------|----------------------------------|-----------------------------------------------|------------------|-------------------------------------------------|-----------------|-----|
| 9     | Gal, Fuc, Glc, Man, SA | CS / QD (core) | Boronic acid/cis-diol ester | Single | HepG-2, L-02, MCF-7, MCF-10A, A-431, HaCaT, OS-RC-2, HK-2, SGC-7901, HCT-8, HeLa | sLe<sup>a</sup> (HepG-2, L-02, HeLa, HCT-8, A-431) | none / not shown | 37  |
| 10    | SA                  | CS / DD-SNP (core) | Boronic acid/cis-diol ester | Single | HepG-2 | sLe<sup>a</sup> | none / not shown | 39  |
| 11    | GlcA, SA            | DD-MIP bMP, CS / QD (core) | Unclear | Single (2×) | HaCaT | SNA-positive<sup>20</sup> | HABP staining for HA | 40  |
| 12    | GlcA, SA            | DD-MIP bMP, QD / core | Unclear | Single (2×) | HaCaT | SNA-positive (HaCaT)<sup>20</sup> | HABP staining for HA | 41  |
| 13    | STn                 | CS / CND (core) + FP (MIP shell) | Urea/carboxylate H bond + boronic acid/cis-diol ester | Dual | CFPAC-1, BxPC-3 | STn (CFPAC-1) | SNA, MAL 1, TKH2 staining | This work |

<sup>a</sup> GlcA = glucuronic acid, SA = sialic acid, Gal = galactose, Fuc = fucose, Glc = glucose, Man = mannose, STn = Sialyl-Thomsen-nouveau antigen.

<sup>b</sup> CS = core-shell architecture, CND = carbon nanodot, FP = fluorescent probe, DD = dye-doped, SNP = silica nanoparticle, bMP = bulk microparticle, Pdot = polymer dot, bNP = bulk nanoparticle, QD = quantum dot.

<sup>c</sup> H bond = hydrogen bonding.

<sup>d</sup> (2×) = two single signals in different wavelength ranges, DHC = digital holographic.
HeLa = human cervix adenocarcinoma cells, HaCaT = human keratinocytes, CLL = chronic lymphocytic leukemia cell lines (HG3, CI, Waosel, AIII), HepG-2 = liver cancer cell line, DU 145 = human prostate carcinoma cell line, MCF-7 = human breast cancer cell line, MDAMB231 = human breast cancer cell line, PC3 = human prostate cancer cell line, MCF-10A = normal mammary epithelial cell line, L-02 = normal hepatocyte cell line, A-431 = human epidermal carcinoma cell line, OS-RC-2 = renal carcinoma cell line, HK-2 = normal renal epithelial cell line, SGC-7901 = gastric carcinoma cell line, HCT-8 = colorectal carcinoma cell line, CFPAC-1 = pancreatic adenocarcinoma cell line, BxPC-3 = human pancreatic cancer cell line.

sLeX = sialyl LewisX, sLeA = sialyl LewisA, SNA = Sambucus nigra agglutinin, STn = Sialyl-Thomsen-nouveau.

HABP = hyaluronic acid binding protein, HA = hyaluronic acid or poly(glucuronic acid-β1,4-N-acetyl-D-glucosamine), MAL I = Maackia amurensis lectin I, UEA I = Ulex europaeus agglutinin I, LCA = Lens culinaris agglutinin, TKH2 = mouse anti-sialyl Tn antibody (clone TKH2)
3. Measurement Uncertainties

The overall uncertainty budget of the fluorescence measurements was derived as detailed below. Such an uncertainty budget is always helpful to get an overview of the contributions of the single steps of the protocol to the overall measurement uncertainty. Because of the multiplicative and quotient forms of the respective equations and because correlations between the quantities are assumed to be negligible, a summation of the squares of the relative uncertainties was performed.42

3.1 Preparation of MIP/NIPs and analyte solutions

a) Weighing of ca. 1 mg of MIP/NIPs (balance Mettler Toledo 1 ± 0.01 mg); $u_{ret}^{w1} = 1\%$

b) Dissolving in 2000 µL DMF (Eppendorf Reference pipette ± 0.030 mL); $u_{ret}^{d1} = 1.5\%$

c) Weighing 0.36 mg NaOAc (balance Mettler Toledo 0.36 ± 0.01 mg); $u_{ret}^{wNaOAc} = 2.7\%$

or 0.62 mg NaOBz (balance Mettler Toledo 0.62 ± 0.01 mg); $u_{ret}^{wNaOBz} = 1.6\%$

or 2 mg STn-Thr-Na (balance Mettler Toledo 2 ± 0.01 mg); $u_{ret}^{wSTn} = 0.5\%$

d) Dissolving in 450 µL DMF to prepare the analyte solution (Eppendorf Reference pipette ± 0.005 mL); $u_{ret}^{d2} = 1\%$

3.2 Assay execution and preparation of measurement solutions

a) Transfer of MIP/NIPs DMF suspension into a 10 mm optical path length quartz cell. Since no dilution step is involved, only contribution from cell length (± 0.01 mm); $u_{ret}^{L} = 0.1\%$

b) Addition of 1 µL analyte solution into the MIP/NIPs suspension in the 10 mm optical path length quartz cell (Eppendorf Reference pipette ± 0.000025 mL); $u_{ret}^{d3} = 2.5\%$

Addition of 2 µL analyte solution into the MIP/NIPs suspension in the 10 mm optical path length quartz cell (Eppendorf Reference pipette ± 0.000075 mL); $u_{ret}^{d4} = 1.5\%$

Addition of 7 µL analyte solution into the MIP/NIPs suspension in the 10 mm optical path length quartz cell (Eppendorf Reference pipette ± 0.0001 mL); $u_{ret}^{d5} = 1\%$

Addition of 20 µL analyte solution into the MIP/NIPs suspension in the 10 mm optical path length quartz cell (Eppendorf Reference pipette ± 0.0005 mL); $u_{ret}^{d6} = 3\%$
Addition of 40 µL analyte solution into the MIP/NIPs suspension in the 10 mm optical path length quartz cell (Eppendorf Reference pipette ± 0.0005 mL); $u_{ret}^{d7} = 1\%$

Addition of 70 µL analyte solution into the MIP/NIPs suspension in the 10 mm optical path length quartz cell (Eppendorf Reference pipette ± 0.0005 mL); $u_{ret}^{d8} = 0.7\%$

### 3.3 Fluorescence measurements

a) The relative uncertainty of the emission spectrum across the respective wavelength range: $u_{ret}^{f} \leq 5\%$

b) For the fluorescence intensities at $\lambda_i$, the maximum possible error amounts to; $u_{ret}^{f2} = 0.05\%$

### 3.4 Experimental standard deviation for replicate measurements

$u_{ret}^{s} \leq 5\%$

### 3.5 Relative uncertainty

Relative uncertainty in STn-Thr-Na related measurements: $u_{rel}^{u} = u_{rel}^{w1} + u_{rel}^{d1} + u_{rel}^{wSTn-Thr-Na} + u_{rel}^{d2} + u_{rel}^{L} + u_{rel}^{d3} + u_{rel}^{d4} + u_{rel}^{d5} + u_{rel}^{d6} + u_{rel}^{d7} + u_{rel}^{d8}$

#### Table S4. Calculation of relative errors in the performance assessment of MIP/NIPs (STn-Thr-Na added).

| Measurement          | Relative error of the measurement                                                                                                                                                                                                 | %     |
|----------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|
| MIP/NIPs             | $u_{rel}^{u} = u_{rel}^{w1} + u_{rel}^{d1} + u_{rel}^{wSTn-Thr-Na} + u_{rel}^{d2} + u_{rel}^{L} + u_{rel}^{d3} + u_{rel}^{d4} + u_{rel}^{d5} + u_{rel}^{d6} + u_{rel}^{d7} + u_{rel}^{d8}$                                                                 | 4.1   |
| 1 µL analyte solution| $u_{rel}^{u} = u_{rel}^{w1} + u_{rel}^{d1} + u_{rel}^{wSTn-Thr-Na} + u_{rel}^{d2} + u_{rel}^{L} + u_{rel}^{d3} + u_{rel}^{d4} + u_{rel}^{d5} + u_{rel}^{d6} + u_{rel}^{d7} + u_{rel}^{d8}$                                                                 | 6.6   |
| 3 µL analyte solution| $u_{rel}^{u} = u_{rel}^{w1} + u_{rel}^{d1} + u_{rel}^{wSTn-Thr-Na} + u_{rel}^{d2} + u_{rel}^{L} + u_{rel}^{d3} + u_{rel}^{d4} + u_{rel}^{d5} + u_{rel}^{d6} + u_{rel}^{d7} + u_{rel}^{d8}$                                                                 | 8.1   |
| 10 µL analyte solution| $u_{rel}^{u} = u_{rel}^{w1} + u_{rel}^{d1} + u_{rel}^{wSTn-Thr-Na} + u_{rel}^{d2} + u_{rel}^{L} + u_{rel}^{d3} + u_{rel}^{d4} + u_{rel}^{d5} + u_{rel}^{d6} + u_{rel}^{d7} + u_{rel}^{d8}$                                                                 | 9.1   |
| 30 µL analyte solution| $u_{rel}^{u} = u_{rel}^{w1} + u_{rel}^{d1} + u_{rel}^{wSTn-Thr-Na} + u_{rel}^{d2} + u_{rel}^{L} + u_{rel}^{d3} + u_{rel}^{d4} + u_{rel}^{d5} + u_{rel}^{d6} + u_{rel}^{d7} + u_{rel}^{d8}$                                                                 | 12.1  |
70 µL analyte solution

\[ u_{rel}^2 = u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 \]

Relative uncertainty in NaOAc related measurement: 

\[ u_{rel}^2 = u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 \]

### Table S5. Calculation of relative errors in the performance assessment of MIPs (NaOAc added).

| Measurement | Relative error of the measurement | % |
|-------------|---------------------------------|---|
| MIP/NIPs    | \[ u_{rel}^2 = u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 \] | 6.3 |
| 70 µL analyte solution | \[ u_{rel}^2 = u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 \] | 7.0 |

Relative uncertainty in NaOBz related measurement: 

\[ u_{rel}^2 = u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 \]

### Table S6. Calculation of relative errors in the performance assessment of MIPs (NaOBz added).

| Measurement | Relative error of the measurement | % |
|-------------|---------------------------------|---|
| MIP/NIPs    | \[ u_{rel}^2 = u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 \] | 5.2 |
| 70 µL analyte solution | \[ u_{rel}^2 = u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 + u_{rel}^2 \] | 5.9 |

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