Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

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Assessment of Molecular Interactions through Magnetic Relaxation**

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Materials: All reagents were of analytical reagent grade. Iron salts ($Fe_2Cl_3 \bullet 4H_2O$ and $Fe_3Cl_3 \bullet 6H_2O$) were obtained from Fluka. Polyacrylic acid (PAA, MW 1.8 kDa), ammonium hydroxide, hydrochloric acid, N-hydroxysuccinimide (NHS), the CTB pentamer, Avidin, Biotin, Rhein, Glucose, Galactose, Lactose, β-Cyclodextrin and Concanavalin A were purchased from Sigma-Aldrich, whereas Dextran (MW 10 kDa) was received from Pharmacosmos. EDC (1-ethyl-3- [3-dimethylaminopropyl]carbodiimide hydrochloride) and Protein G were obtained from Pierce Biotechnology, and the Tetanus toxin C fragment (TTC) was from Roche Biomedical. Doxorubicin and PBS buffer were purchased from Fisher Scientific. Anti-FR antibody and EpCAM antibody were received from Santa Cruz Biotechnology. MCF-7 and HeLa cell lines were established from ATCC.

Synthesis of protein-carrying bMRs: For the conjugation of proteins to the iron oxide nanoparticles, we utilized EDC/NHS chemistry as previously described[1]. Specifically, poly(acrylic acid)-coated nanoparticles ([Fe] = 0.25 mg/mL) were mixed with 2 mL of MES buffer (pH 6), followed by the dropwise addition of EDC (1 mg, 0.11 mmol) and NHS (0.8 mg, 0.15 mmol). The reaction mixture was incubated for 3 min before the dropwise addition of

Protein G (0.1 mg) or Avidin (0.15 mg) in DI water (0.1 mL). The reaction continued for 30 min at room temperature under continuous mixing, before overnight incubation at 4 °C. To obtain the protein-carrying bMRs, we magnetically separated the reaction mixture through a 1X-PBS-equilibrated LS25 column (Miltenyi). Conjugation of antibodies to Protein G-carrying bMRs (250 μL) was performed as previously reported,[1] by using 0.5 ng of EpCAM Ab (Santa Cruz Biotechnology) or 0.5ng of FR Ab (Santa Cruz Biotechnology), which resulted in low valency bMRs. The nanoparticle valency was evaluated as previously reported[2], through quantification of the nanoparticle's antibody amount using the BCA assay (Pierce Biotechnology).

Preparation of small-molecule-carrying bMRs: Folic acid and doxorubicin- carrying bMRs were prepared as described in the literature[1]. To synthesize Rhein-conjugated bMRs a similar approach was followed. Briefly, propargylated poly(acrylic acid)-coated nanoparticles (3 mg, 2 mg/mL) were added to a low stoichiometric ratio of azide-functionalized Rhein (0.5 μg Rhein−N3, 10 μg/ml DMSO). The reaction was initiated at room temperature in the presence of catalytic amount of CuI (0.01 μg in 500 μL of bicarbonate buffer, pH 8.5), and further incubated for 12 h at room temperature. The final reaction mixture was purified with a magnetic column (LS25, Miltenyi) using DMSO as the elutant. The rhein – nanoparticle preparation was stored at room temperature until further use. Confirmation of the successful conjugation of Rhein to the nanoparticles was achieved through UV-Vis absorption spectroscopy, by recording rhein's absorbance at 443 nm using Cary 300 spectrophotometer.

Dextran-coated Nanoparticle: Dextran-coated bMR nanoparticles were synthesized according to the literature[3].

Assay for the Determination of Dissociation (K_D) Constant via Magnetic Relaxation:

Avidin – Biotin K_D Measurement: For this interaction we utilized Avidin-carrying bMRs (0.015 mg Fe/mL), which had a diameter of 76 nm and a *r*2 relaxivity of 116 $mM^{-1}s^{-1}$. The bMR analyzing solution consisted of 4.5 μL Avidin-carrying bMRs and 2,000 μL of de-ionized water. Samples containing 10 μL of different concentrations of free Avidin (competing ligand, 0.1 fM to 1 pM, in DI water) and 200 μL of the bMR analyzing solution (bMR nanosensor) were prepared followed by the addition of 10 μL Biotin (target small molecule, 205 nM). A negative control sample was prepared in the same fashion, adding 10 μL fresh 1X PBS buffer instead of Biotin (0 M Biotin control sample). Magnetic relaxation measurements were performed after 15 minutes of incubation at room temperature. Dynamic light scattering (DLS) studies were done using a PDDLS/CoolBatch 40T instrument using Precision Deconvolve 32 software. Transverse (T_2) proton relaxation times measurements were done using a Bruker Minispec mq20 NMR analyzer operating at a magnetic field of 0.47 T and at 37 °C.

Protein G – Ig G magnetic-relaxation-based K_D determination: Following a similar approach as the Avidin – Biotin assay, the Protein G – Ig G dissociation constant was measured. An analyzing solution of Protein G-carrying bMRs (0.007 mg Fe/ mL) was incubated with various amounts of free Protein G (0.43 fM – 0.43 pM) in 1X PBS buffer. After the addition of Ig G (50 nM, 1X PBS buffer) in the form of IgG-EpCAM antibody (Santa Cruz Biotechnology) as the target protein a 15-minute incubation period at room temperature was allowed before magnetic relaxation measurements were performed.

Concanavalin A – Dextran K_D determination: Dextran-coated iron oxide nanoparticles (0.012 mg Fe/mL, 115 nm and a *r*2 relaxivity of 175 $mM^{-1}s^{-1}$, in DI water) were used as bMR sensors and introduced into a solution containing various amounts of free dextran (1.25 nM – 62.5 nM). The samples were then incubated with Concanavalin A (50 nM) for 15 minutes at room temperature, followed by magnetic relaxation measurements.\

Folic Acid – Folate receptor expressed on the plasma membrane of HeLa cells: Various samples of 200 μL of an analyzing solution containing folic acid-carrying bMRs (0.01 mg Fe/mL, in 1X PBS) were incubated with varying concentrations of free folic acid (competitor, 1.1 nM – 0.56 μM, 1X PBS). Folate-receptor-expressing HeLa cells in 1X PBS (10 μL , 10,000 cells quantified with a hemocytometer) were added to each sample followed by an incubation period of 30 minutes at room temperature. Magnetic relaxation measurements were then performed.

Anti-folate-receptor Antibody – folate-receptor-expressing HeLa cells: Anti-folate-receptor antibody-carrying bMRs (0.008 mg Fe/mL) were incubated with various concentrations of free anti-folate receptor antibody (competitor, 0.45 pM – 4.5 nM, 1X PBS). The samples were then incubated with FR-expressing HeLa cells (10 μL, 10,000 cells in 1X PBS) for 30 minutes at room temperature before performing magnetic relaxation measurements.

EpCAM Antibody – EpCAM-expressing MCF-7 cells: Samples containing EpCAM antibody-conjugated bMRs (0.008 mg Fe/mL) were incubated with various concentrations of free EpCAM antibody (competing protein, 45 fM – 45 nM, 1X PBS). The samples were then incubated with

EpCAM-expressing MCF-7 cells (10 μL, 8,000 cells in 1X PBS,) for 30 minutes at room temperature followed by magnetic relaxation measurements.

TTC – Doxorubicin: Samples consisting 200 μL of an analyzing solution containing Doxorubicin-conjugated bMR nanosensors (0.01 mg Fe/mL) and increasing amounts of free doxorubicin (0.9 μM – 12 μM, in 1X PBS) were prepared. The target protein TTC (4 nM) was added to each sample followed by a 15- minute incubation at room temperature and magnetic relaxation measurements.

TTC – Rhein: Following the same protocol as with the previous assays, rhein-conjugated bMR were introduced into a solution containing various amounts of free Rhein (0.5 μM – 500 μM, 1X PBS). The samples were then incubated with TTC (4 nM) for 15 minutes at room temperature, before performing magnetic relaxation measurements.

CTB – Carbohydrates: A nanoparticle analyzing solution was made out of 4.5 μL dextran-coated nanoparticles (5 mg Fe/ mL) and 2,000 μL de-ionized water. Samples containing of different concentrations of the carbohydrates of interest (concentration range provided below) and 200 μL of the nanoparticle analyzing solution were prepared, followed by the addition of CTB (10 μL 1.7 μM DI water). The samples were incubated for 15 minutes before performing magnetic relaxation measurements. *Carbohydrates of Interest (DI water)*: Dextran (0.1 μM – 50 μM), Glucose (2.4 μM – 470 μM), Galactose (0.2 μM – 94 μM), Lactose (3.8 μM – 380 μM), β-Cyclodextrin (0.4 μM – 200 μM)

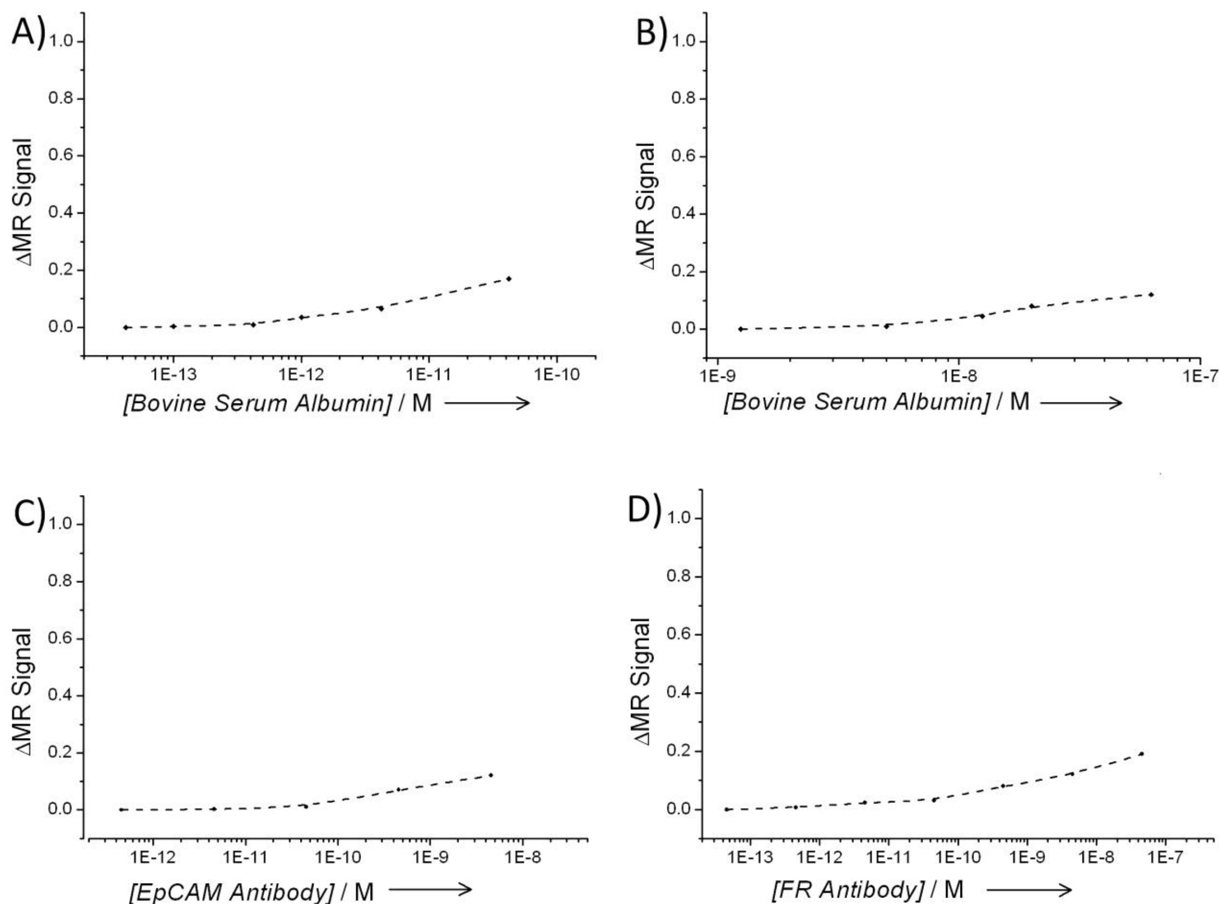

Figure S1. Control experiments using bovine serim albumin (BSA) or a non-related antibody to rule out the effect of non-specific binding. A) Protein G bMR nanosensor targeting IgG with BSA as a competitor. B) Dextran bMR nanosensor targeting Concanavalin A with BSA as a competitor. C) anti-FR antibody bMR targeting FR receptors on HeLa cells with EpCAM antibody as a competitor. D) anti-EpCAM antibody bMR nanosensor targeting EpCAM receptors on MCF-7 cells with FR antibody as a competitor.

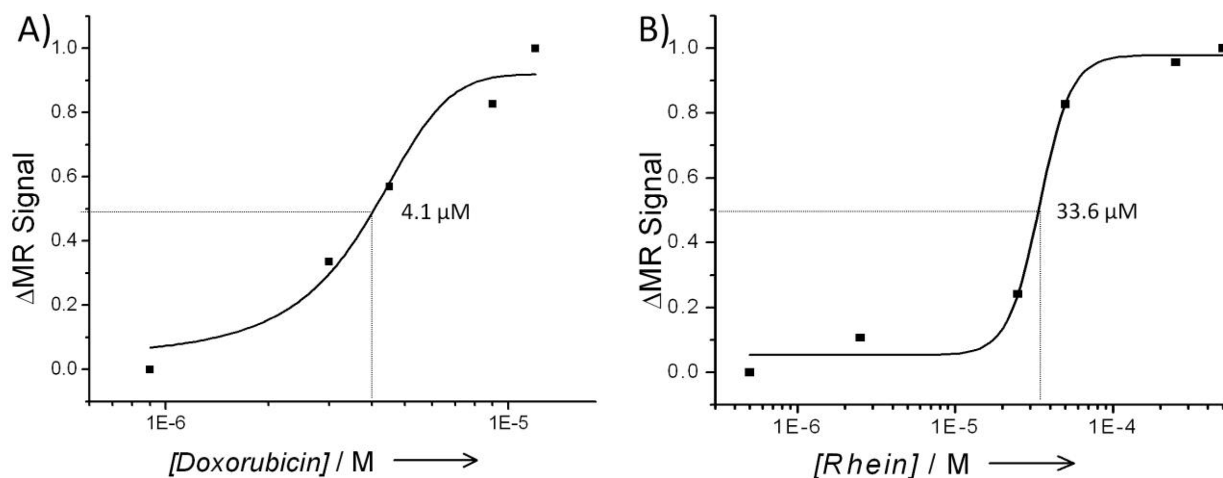

Figure S2. Determination of the dissociation constant of small molecules (doxorubicin and rhein) and TTC, via bMR nanosensors and magnetic relaxation. A) Doxorubicin – TTC. B) Rhein – TTC. (Errors were within 1-2%, which were too small to depict.)

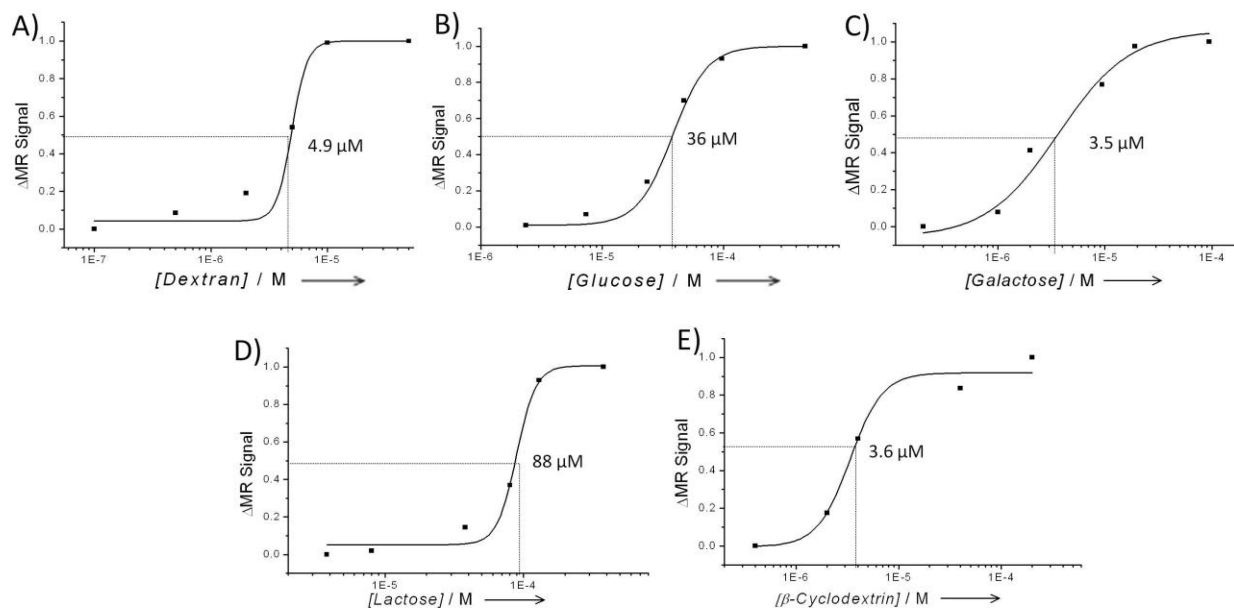

Figure S3. Determination of the dissociation constant of carbohydrates and CTB via magnetic relaxation and bMR nanosensors. A) Dextran – CTB. B) Glucose – CTB. C) Galactose – CTB. D) Lactose – CTB. E) β-Cyclodextrin – CTB. (Errors were within 1-2%, which were too small to depict.)

[1] C. Kaittanis, S. Santra, O. J. Santiesteban, T. J. Henderson, J. M. Perez, *J Am Chem Soc* **2011**, *133*, 3668.

[2] S. Santra, C. Kaittanis, J. Grimm, J. M. Perez, *Small* **2009**, *5*, 1862.

[3] C. Kaittanis, T. Banerjee, S. Santra, O. J. Santiesteban, K. Teter, J. M. Perez, *Bioconjug Chem* **2011**, *22*, 307.