Supporting Information

Small

Semi-Synthetic Biogenic Magnetosome Nanoparticles for the Detection of Proteins and Nucleic Acids**

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Supplement Materials

Experimental Procedures

General

Biogenic MPs **1** were produced in the magnetotactic bacteria *Magnetospirillum gryphiswaldense* [1]. Various batches of MPs were used for stoichiometric determination experiments. TEM-analyses of the MPs revealed particle diameters of 36 ± 2 nm. The concentrations of MPs in HEPES buffer (10 mM HEPES, 1 mM EDTA, pH 7.3, containing 0.04 % NaN_3) were calculated using TEM-, AAS- and UV/Vis-spectroscopy-measurements. All oligonucleotides used in this work were purchased from Thermo Electron, Germany. The sequences are listed in Table 1.

Biofunctionalization of Magnetosomes

For the synthesis of MP-conjugates, the MPs were biotinylated in the first step either by 1,2-Dipalmitoyl-sn-Glycero-3-Phosphoethanolamine-N-(Biotinyl) (Sodium Salt) (biotin-DPPE **2**, Avanti polar lipids) or sulfo-N-hydroxy-succinimide ester sodium salt (NHS-biotin **3**, Sigma).In typical synthesis, 8 ml of a 20 nM solution of MP **1** (in HEPES buffer) were mixed with 1 ml of **2** (1 mg/ml in HEPES) and incubated overnight. For the covalent attachement of biotin molecules to proteins of the magnetosome membrane, 1.5 mg of NHS-biotin **3** were dissolved in 60 µl DMF and subsequently filled up to 1 ml with PBS buffer (0.1 M phosphate, 0.15 M NaCl, pH 7.3). The solution was added to 8 ml of a 20 nM solution of MP **1** (in PBS) and incubated for 2 hours. After incubation the MPs were purified by repeated magnetic separation steps using an external magnetic field and the particles were redispersed in TE buffer (20 mM Tris-HCl, 5mM EDTA, pH 7.35). The resulting biotinylated MPs **4** were quantified by UV/Vis-measurements at 450 nm (extinction coeficient of 1.93×10^7, as determined by TEM and AAS). The attachment of STV **5** to biotinylated MPs **4** was carried out by mixing 3000 molar equivalents of STV **5** with 1 eq of either **4a** or **4b** (in TE, 15 nM each). Samples were incubated for 30 min and the resulting STV-coated MPs **6a** and **6b** were purified in TE buffer, similarly as described above. Again, the concentration was measured UV/Vis-quantification at 450 nm.

Functionalization of STV-coated MPs **6a** and **6b** with biotinylated oligonucleotides or antibodies to yield monofunctional MPs, e.g. **MP-10**, **MP-11** or **MP-13**, was carried out by incubation, similarly as described above. For example, monofunctional MP conjugates **MP-10** were prepared by mixing a solution of biotinylated oligonucleotide **7** with a solution of **6**, such that the final oligonucleotide concentration was 10 µM. Similarly, **6** was functionalized with biotinylated anti-

mouse antibody **9** (Sigma Aldrich). For the preparation of difunctional DNA–nanoparticle conjugates **MP-12**, a mixture comprised of an equimolar ratio of the two biotinylated oligonucleotides **7** and **8**, was added to a solution of STV-coated MPs, e.g. **6a**. The final oligonucleotide concentration was 10 µM. For the preparation of the difunctional DNA/antibody conjugate **MP-14**, a mixture, containing equimolar ratios of biotinylated oligonucleotide **8** and biotinylated anti-mouse antibody **9** (3 µM each in TE buffer) were added to the STV-coated MP solution (2 nM in TE buffer). All samples were incubated for 45 min at room temperature and purified by magnetic separation. For example, a solution, containing monofunctional MPs **10** was purified by repeated magnetic separation and redispersion steps in fresh TE buffer before the pellet was finally redispersed in TBS buffer (0.15 M NaCl, 20 mM Tris-HCl, pH 7.35). The resulting conjugates were quantified by UV/Vis-spectroscopy. All final MP-conjugates **10** - **14** were quenched first with D-biotin (81 µM in TBS) during the purification steps before they were used in immobilization assays on STV-coated microplates or in the hybridization with DNA-STV-AP conjugates **19**.

Quantification of surface coverages

Fluorescence assays were performed to determine the surface coverage of MPs with biotin groups, biotinylated oligonucleotides and for the determination of hybridization efficiencies of DNA-MP conjugates. First, the amount of biotin molecules per MP was determined by coupling **4a** and **4b** with STV-Cy5 (Pharmacia). In a typical experiment, aliquots of biotinylated MPs (30 µl, 4 nM, corresponding to 120 fmol), were mixed with 30 µl of varying amounts of STV-Cy5 in the range from 40 pmol to 640 pmol. The samples were incubated for 45 min and then purified by magnetic separation to remove unbound STV. The fluorescence of the samples was measured at 670 nm in a fluorimeter (Varian) and the resulting signals were then converted into molar concentrations of STV-Cy5 by interpolation from a standard linear calibration curve, obtained from known amounts of STV-Cy5. Finally, the average numbers of biotin groups per MP were obtained by dividing the measured STV-Cy5 molar concentration by the original particle concentration.

The surface coverages of MPs with biotinylated oligonucleotides were determined by the addition of varying amounts of biotinylated and Cy5-labeled oligonucleotides **7** (5’–biotin-TCC TGT GTG AAA TTG TTA TCC GCT-Cy5-3’) in the range from 25 pmol to 300 pmol to aliquots of STV-modified **6** (30 µl, 4 nM, corresponding to 120 fmol). A standard linear calibration curve of known amounts of Cy5-labeled oligonucleotides **7** at 670 nm was recorded to convert the resulting fluorescence signals of the samples into molar

concentrations and finally, the average number of oligonucleotides per MP was calculated by dividing the measured Cy5-labeled **7** molar concentration by the original particle concentration. The hybridization efficiency of the MP-conjugates was measured by hybridization of **MP-10** (30 µl, 4 nM) with various amounts of a complementary Cy5-labeled oligonucleotide (5'–Cy5-AGC GGA TAA CAA TTT CAC ACA GGA-3') in the range from 25 pmol to 300 pmol. The hybridization efficiency of **MP-10** conjugates, e.g. **MP-10a**, was then calculated by determining the number of hybridized complementary Cy5-modified oligonucleotides per MP. To this end, a standard linear calibration curve of known amounts of Cy5-labeled oligonucleotides at 670 nm was recorded to convert the resulting fluorescence signals of the samples into molar concentrations and finally, the average number of hybridized oligonucleotides per MP-conjugate was calculated as described above for calculation the number of Cy5-labeled **7** molar concentration per MP.

Crosslinking with DNA-modified gold nanoparticles

The preparation of DNA-modified AuNP-conjugates was carried out as previously described. [2] In brief, 5'-thiol-labeled oligonucleotides **15** (DNA sequence in table 1) were used for preparation of monofunctional DNA-AuNPs **16** with particle-sizes of 11 ± 1 nm ($AuNP_{11}$) and 24 ± 5 nm ($AuNP_{24}$) in diameter.

For DNA-directed aggregation experiments, a mixture, containing 1 molar eq of **MP-11a** or **MP-11b** (3.15 nM each), 10 molar eq of DNA-$AuNP_{11}$ conjugate **16** and 20 molar eq of complementary DNA linker **17** was prepared in TBS buffer. The sample was incubated for 24 h at room temperature. For TEM-analyses, the precipitated binary DNA-AuNP-MP particle-aggregate was purified by magnetic separation to remove unbound AuNPs from the solution and resuspended in TBS buffer. Subsequently, 5 µl of the aggregate solution were placed on a carbon grid (Plano) for 5 min. The grid was rinsed with water and immediately dried with Nitrogen. In control experiments, a mixture of the two different conjugates was incubated without linker **17** before the immobilization on a carbon grid. For the aggregation of **MP-10a** with DNA-$AuNP_{24}$ conjugate **16**, a mixture, containing 1 molar eq of **MP-10a** (2.3 nM), 1 molar eq of DNA-$AuNP_{24}$ conjugate **16** and 32 molar eq of complementary DNA linker **17** was prepared in TBS buffer and purified as described above.

Microplate binding assays

The DNA-directed formation of particle double-layers was performed by using streptavidin-coated microplates,[3] which

were functionalized with biotinylated capture oligonucleotides (5′–biotin-AGC GGA TAA CAA TTT CAC ACA GGA-3′), complementary to oligonucleotide sequence **7**. For positive controls, wells of a microplate were functionalized with biotinylated capture oligonucleotide with DNA-sequence **8**. For the immobilization of the first particle layer of magnetosomes, 50 µl of **MP-12** (ca. 3 nM) were added to the wells and incubated for 1.5 h. For the formation of the second layer, 50 µl of DNA-$AuNP_{24}$ conjugate **16** (0.4 nM), containing 32 molar eq of linker **17,** were added to the wells containing the immobilized **MP-12** conjugates and incubated for 2 hours. Subsequent to hybridization, a silver amplification step was carried out using silver-enhancer-kits by ICN. Quantification of immobilized particles was conducted photometrically by absorbance measurement at 490 nm in a microplate reader (Synergy). As a positive control, 50 µl of a mixture of DNA-$AuNP_{24}$ conjugate **16** (0.4 nM) and 32 equivalents of linker **17** were added into the wells of the microplate, previously functionalized with capture-oligonucleotide **8**.

Covalent DNA-STV conjugates **18** were prepared in 20 % yield from 5´-thiol modified oligonucleotides (5′–SH-AGC GGA TAA CAA TTT CAC ACA GGA-3′) and recombinant STV (Roche) using the heterobispecific cross-linker sSMCC (Pierce), similar as previously described.[4] To prepare the DNA-STV alkaline phosphatase conjugate **19**, 170 µl of **18** (200 nM) in TBSE buffer (0.15 M NaCl, 20 mM Tris-HCl, pH 7.35 containing 5mM EDTA) was mixed with 170 µl of a solution of biotinylated alkaline phosphatase (200 nM, Sigma) in TBSE buffer and the mixture was incubated for 15 min at room temperature. After incubation, the mixture was quenched with d-biotin (81 µM in TBS).

To couple alkaline phosphotase conjugate **19** with surface-bound oligomer **7** of **MP-14a**, 240 µl of **MP-14a** (3.2 nM) was mixed with 32 molar eq of **19**. Similarly, for the preparation of negative control samples, 130 µl (3.2 nM) of **MP-11a** or **MP-13a**, respectively, were mixed with the same amount of **19**. The mixtures were incubated for 5 min, before they were used in the sandwich immunoassay.

Sandwich immunoassays were carried out using goat anti-mouse IgG (Sigma, 50 µl, 20 nM)-coated microplates. 50 µl of a solution containing (50 µl, 20 nM) of the mouse IgG antigen (Sigma) were added to the microplate wells and incubated for 45 min at room temperature. After unbound antigens were removed by washing with TBS, 50 µl of the various mixtures, all containing alkaline phosphotase conjugate **19** and either conjugate **MP-11**, **MP-13** or **MP-14,** respectively, were added to the wells and incubated for 2 hours. After removal of unbound conjugates by repeated washing with TBS, 50 µl of Attophos

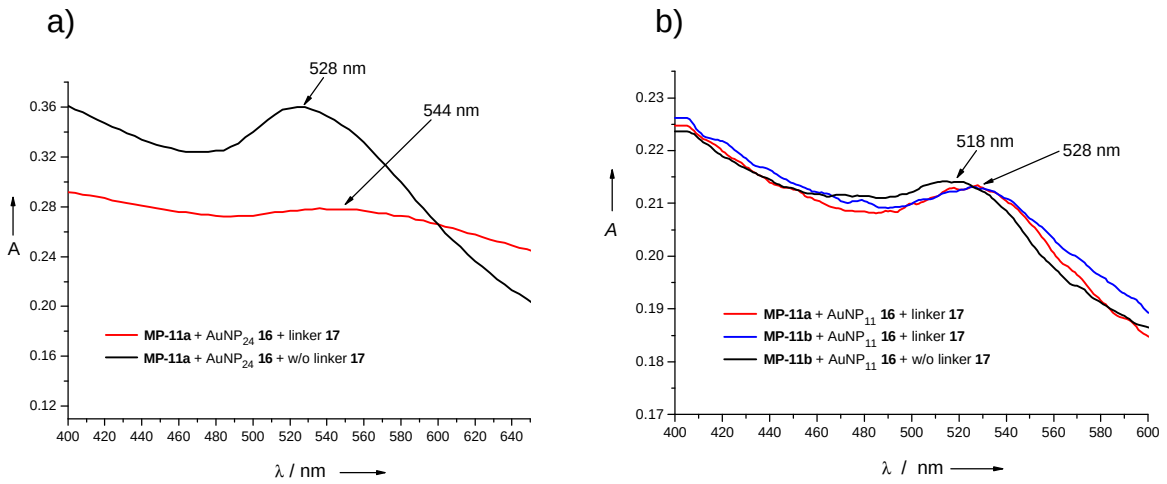
(Roche) was added into each well for fluorescence signal detection at 560 nm, using a microplate reader (Synergy).

	STV / MP **6**		Oligomer / MP **10**		Hybridized oligomers / MP **10**	
	NHS-Biotin **6b**	Biot.-DPPE **6a**	NHS-Biotin **10b**	Biot.-DPPE **10a**	NHS-Biotin **10b**	Biot.-DPPE **10a**
MPs from wildtype						
Batch 1	34	70	n.d.	n.d.	23	54
Batch 2	55	31	135	87	33	33
Batch 3	37	230	49	451	20	151
Batch 4	24	160	33	340	19	146
Average	38 ± 11	122 ± 78	54 ± 50	220 ± 183	24 ± 6	96 ± 53
MPs from MSR-1K						
Batch 1	25	71	79	88	38	38
Batch 2	14	70	4	35	3	14
Batch 3	10	45	17	25	10	23
Average	16 ± 6	62 ± 12	33 ± 32	49 ± 28	17 ± 15	25± 10

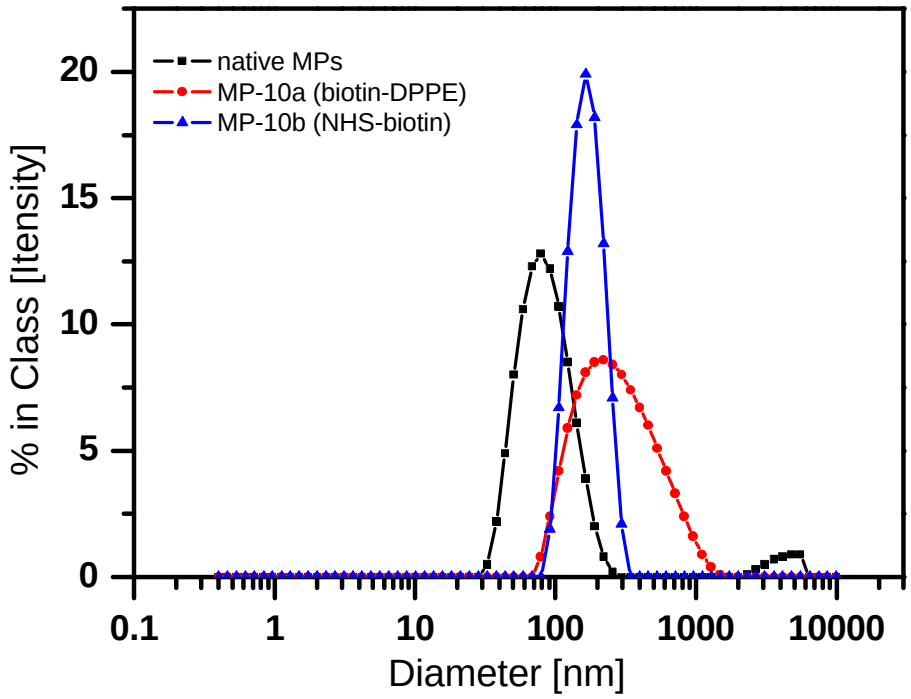
Supplement Table S1: Surface coverages of the intermediate synthesis steps and the hybridization efficiencies of DNA-functionalized MPs, derived from various batches of particles, which were isolated from wildtype strains (36 ± 2 nm in diameter) and or from mutant strains MSR-1K (29 ± 1 nm) of magnetotactic bacteria. The MPs from the wildtype strain showed higher amounts of surface coverages than the MPs of MSR-1K, due to their larger particle diameter. Note that MPs from wildtype as well as from mutant strain showed on average an about 3-4 fold higher biotinylation rate in the case of biotin-DPPE modification in comparison to those obtained with NHS-Biotin. The higher biotinylation rate with biotin-DPPE caused a greater coupling efficiency of STV (i.e., MPs **6**) and biotinylated oligonucleotides. The hybrization efficiencies of the DNA-functionalized MPs with complementary Cy5-labeled oligomer probes were in the range of about 50%, and were independent from the biotinylation method. The errors for the surface coverages of MPs in Table S1 are possibly explained by the different aggregation tendencies of the various magnetosome samples. These might stem from the biological variations of the magnetosome materials and might be associated to membrane infringement during magnetosome preparation and handling. However, the

data of the various experiments with magnetosomes from the same batch showed significantly smaller errors of about 10%.

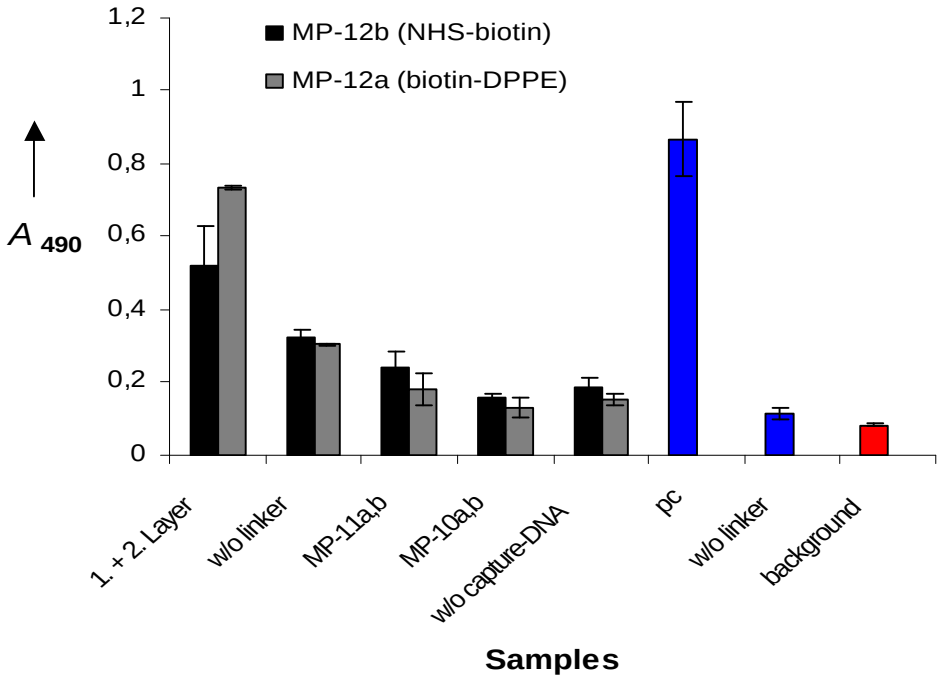
Ceyhan et al. Supplement Fig. S2



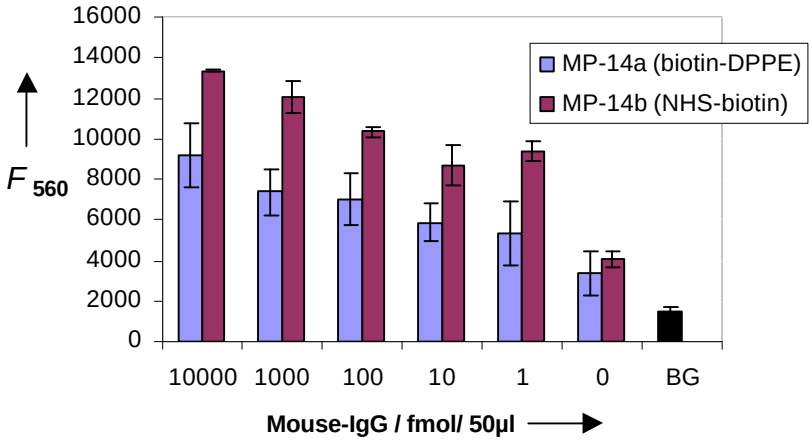
Supplement Fig. S2: Spectroscopic analysis of the DNA-directed aggregation of MP- and AuNP-conjugates; a) hybridization of **11a** with 24 ± 5 nm ($AuNP_{24}$) DNA-modified gold nanoparticles **16** via the complementary linker-oligomer **17** showed the characteristic shift of the gold nanoparticle's plasmon absorption band (red curve) in comparison to the sample, in which the linker-oligomer **17** was not present in the reaction mixture (black curve), thus confirming that the oligomer moities of **11a** were capable of binding complementary targets. b) Comparison of the absorbance shifts of the DNA-directed aggregation by using **11a** or **11b** for the aggregation of 11 ± 1 nm ($AuNP_{11}$) DNA-modified gold nanoparticles **16** via linker-oligomer **17**. The spectra indicate the similar aggregation efficiency regardless of whether **11a** or **11b** were used. Note that the aggregation of larger $AuNP_{24}$ leads to higher shifts of the absorption band than those obtained by aggregation of $AuNP_{11}$ with the smaller particle-diameter.



Supplement Fig. S3: Dynamic light scattering measurements of DNA-modified MPs (**MP-10**) were carried out to determine the hydrodynamic diameter of MPs after functionalization the magnetosomes with DNA-oligomers. Peak signals show that the hydrodynamic diameter of native MPs of ca. 80nm (black peak) were increased after DNA-modification. Here the **MP-10a** conjugate, which was obtained by biotinylation of the MPs with biotin-DPPE in the first step (see Figure 1) show a broader size distribution and higher shift (red peak, peak maximum at 210 nm) as compared to **MP-10b** conjugates, which were prepared by biotinylation the MPs with NHS-biotin (blue peak, peak maximum at 160 nm). These results suggest that partially a cohesion of membranes of the MPs occur during the conjugate synthesis, thus leading to the formation of larger particle aggregates.



Supplement Fig. S4: DNA-directed formation of a surface-bound double layer of MPs and Au-nanoparticles in a microplate by using difunctional MPs from mutant strain MSR-1K (see Table S1). The difunctional **MP-12a,b** conjugates used for the formation of the first layer were prepared either by biotinylation of the MPs with biotin-DPPE or with NHS-biotin. The formation of the double-layer and subsequent signal detection was carried out as described in the manuscript (Fig. 3, and corresponding text). The highest signals were obtained only when difunctional conjugates **MP-12** were used in the immobilization assay. Negative control samples, in which DNA-modified Au-nanoparticles **15** were incubated in the absence of linker-oligomer **16** or by using monofunctional **MP-10a,b** or **MP-11a,b** showed significantly lower signals, indicating the specificity of the signals of the double-layer formation obtained by using difunctional **MP-12**. Note that the signal of the double-layer formation obtained by using NHS-biotin funtionalized **MP-12b** conjugates is slightly lower than the signal obtained by biotin-DPPE funtionalized **MP-12a**. As a positive control (pc) DNA-modified Au-nanoparticles **15** were immobilized directly at the microplate through hybridization via linker oligomer **16** using biotinyated capture-oligomer **7**.



Supplement Fig. S5: Detection of various amounts of the model antigen mouse-IgG in a sandwich immunoassay. MPs from mutant strain MSR-1K were used in this assay and **MP-14a,b** was prepared either by biotinylation of the MPs with biotin-DPPE or with NHS-biotin. The immunoassay and the signal detection were carried similar as described in the manuscript (Fig. 4, and corresponding text). The signals show that down to 1 fmol of the antigen can be detected by means of **MP-14**. Note that the signals obtained with particles by the NHS-biotin route (**MP-14b**) are higher than the signals obtained by using biotin-DPPE functionalized **MP-14a**.

References

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