Article

Magnetic Nanoclusters Coated with Albumin, Casein, and Gelatin: Size Tuning, Relaxivity, Stability, Protein Corona, and Application in Nuclear Magnetic Resonance Immunoassay

Pavel Khramtsov 1,2,*, Irina Barkina 2, Maria Kropaneva 1, Maria Bochkova 1, Valeria Timaganova 1, Anton Nechaev 3, Ilya Byzov 1,4, Svetlana Zamorina 1,2, Anatoly Yermakov 1,4,5 and Mikhail Rayev 1,2

1 Laboratory of Ecological Immunology, Institute of Ecology and Genetics of Microorganisms of the Ural Branch of the Russian Academy of Sciences, branch of PSRC UB RAS, 13 Golev str., 614081 Perm, Russia; kropanevamasha@gmail.com (M.K.); krasnykh-m@mail.ru (M.B.); timaganovavp@gmail.com (V.T.); ivbyzov@gmail.com (I.B.); mantissa7@mail.ru (S.Z.); yermakov@imp.uran.ru (A.Y.); mraev@iegm.ru (M.R.)
2 Department of Microbiology and Immunology, Biology Faculty, Perm State National Research University, 15 Bukirev str., 614000 Perm, Russia; i_barkina@mail.ru (I.B.)
3 Institute of Technical Chemistry of Ural Branch of the RAS, 3 Academician Korolev str., 614013 Perm, Russia; toxambj@gmail.com (A.N.)
4 M.N. Mikheev Institute of Metal Physics of the Ural Branch of the Russian Academy of Sciences, 18 S. Kovalevskoy str., 620108 Yekaterinburg, Russia
5 Ural Federal University named after the first President of Russia B.N. Yeltsin, 19 Mira str., 620002 Yekaterinburg, Russia

* Correspondence: khramtsovpavel@yandex.ru (P.K.); Tel.: +7-342-280-77-94

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Supporting Information

Experimental section

Synthesis of “small” (100-110 nm) BSA-coated Fe@C-NH2 nanoparticles conjugated with streptavidin

325 μL of 20% BSA in H2O was mixed with 975 μL of the 0.1M acetic buffer with pH 4, then 1300 μL of 10 mg/ml Fe@C-NH2 suspension was added dropwise under vortex stirring. pH was adjusted to 7.25 with 1M NaOH. Resulting suspension (2500 μL) was added dropwise under vortex stirring to an equal volume of 25% glutaraldehyde solution (see Materials section) and incubated on rotating mixer (from now on rotation angle was set at 99 degrees, the rotation rate was 5 rpm) at room temperature for 30 min. Then, 5 ml of nanoparticles activated with glutaraldehyde were passed through the chromatography column (column XK 26/40, medium: Sepharose CL-6B, bed volume: 160 ml, elution speed: 0.65 ml/min, eluent: PBS) to remove the excess of BSA and glutaraldehyde. Fractions containing Fe@C-NH2/BSA nanoparticles were collected and concentrated to approximately 2.5 ml. For concentrations, the suspension was placed in dialysis tubing (10 kDa MWCO, 2 ml per cm) and covered with the layer of 35 kDa PEG. In 2-3 hours the supernatant contained Fe@C-NH2/BSA nanoparticles activated with glutaraldehyde was divided into five equal portions (volume of the portion was approximately 500 μL). Each portion was added to 10 mg/ml streptavidin solution in PBS under vortex stirring; volumes were equalized with PBS. Final streptavidin to Fe@C-NH2/BSA ratios were 10, 20, 40, 80 and 160 μg of streptavidin per 1 mg of nanoparticles. Conjugation of streptavidin was carried out overnight on rotating mixer at +4°C. Unreacted streptavidin was removed by gel-chromatography (column C 10/20, medium: Sepharose CL-6B, bed volume: 10 ml, elution speed: 0.07 ml/min, eluent: PBS). Fractions with the highest concentration of Fe@C-NH2/BSA/Str nanoparticles were combined.

Synthesis of “medium” (170-180 nm) BSA-coated Fe@C-NH2 nanoparticles conjugated with streptavidin
325 μL of 20% BSA in H2O was mixed with 975 μL of 0.1M phosphate buffer pH 8, then 1300 μL of 10 mg/ml Fe@C-NH2 suspension was added dropwise under vortex stirring. pH was adjusted to 7.25 with 1M HCl. Resulting suspension (2500 μL) was added dropwise under vortex stirring to an equal volume of 25% glutaraldehyde solution (see Materials section) and incubated on rotating mixer at room temperature for 30 min. Further procedures were as described for “small” Fe@C-NH2/BSA/Str (see above).

**Synthesis of “large” (210-220 nm) BSA-coated Fe@C-NH2 nanoparticles conjugated with streptavidin**

325 μL of 20% BSA in H2O was mixed with 975 μL of 0.1M phosphate buffer pH 8, then ionic strength of mixture was adjusted to 0.5M with NaCl powder. After that, 1300 μL of 10 mg/ml Fe@C-NH2 suspension was added dropwise under vortex stirring. pH was adjusted to 7.25 with 1M HCl. Resulting suspension (2500 μL) was added dropwise under vortex stirring to an equal volume of 25% glutaraldehyde solution (see Materials section) and incubated on rotating mixer at room temperature for 30 min. Further procedures were as described for “small” Fe@C-NH2/BSA/Str (see above).

**Synthesis of “small” (110-120 nm) casein-coated Fe@C-NH2 nanoparticles conjugated with streptavidin**

738 μL of 8.8% casein in H2O was mixed with 562 μL of 0.1M acetic buffer pH 5, then 1300 μL of 10 mg/ml Fe@C-NH2 suspension was added dropwise under vortex stirring. pH was adjusted to 7.25 with 1M HCl. Resulting suspension (2500 μL) was sonicated for 10 sec (30% amplification, 3 mm probe), added dropwise under vortex stirring to an equal volume of 25% glutaraldehyde solution (see Materials section) and incubated on rotating mixer at room temperature for 30 min. Further procedures were as described for “small” Fe@C-NH2/BSA/Str (see above).

**Synthesis of “medium” (190-210 nm) casein-coated Fe@C-NH2 nanoparticles conjugated with streptavidin**

738 μL of 8.8% casein in H2O was mixed with 562 μL of 0.1M acetic buffer pH 5, then ionic strength of mixture was adjusted to 0.5M with NaCl powder. After that, 1300 μL of 10 mg/ml Fe@C-NH2 suspension was added dropwise under vortex stirring. pH was adjusted to 7.25 with 1M HCl. Resulting suspension (2500 μL) was added dropwise under vortex stirring to an equal volume of 25% glutaraldehyde solution (see Materials section) and incubated on rotating mixer at room temperature for 30 min. Further procedures were as described for “small” Fe@C-NH2/BSA/Str (see above).

**Synthesis of “large” (240-260 nm) casein-coated Fe@C-NH2 nanoparticles conjugated with streptavidin**

738 μL of 8.8% casein in H2O was mixed with 562 μL of 0.1M acetic buffer pH 5, then ionic strength of mixture was adjusted to 1M with NaCl powder. After that, 1300 μL of 10 mg/ml Fe@C-NH2 suspension was added dropwise under vortex stirring. pH was adjusted to 7.25 with 1M HCl. Resulting suspension (2500 μL) was sonicated for 10 sec (30% amplification, 3 mm probe), added dropwise under vortex stirring to an equal volume of 25% glutaraldehyde solution (see Materials section) and incubated on rotating mixer at room temperature for 30 min. All solutions were pre-warmed and kept at +37-40°C to prevent gelation. Further procedures were performed at room temperature as described for “small” Fe@C-NH2/BSA/Str (see above).

**Synthesis of “small” (140-160 nm) gelatin B-coated Fe@C-NH2 nanoparticles conjugated with streptavidin**

650 μL of 10% gelatin B in H2O was mixed with 650 μL of 0.1M acetic buffer pH 4, then 1300 μL of 10 mg/ml Fe@C-NH2 suspension was added dropwise under vortex stirring. pH was adjusted to 7.25 with 1M NaOH. Resulting suspension (2500 μL) was sonicated on a water bath for 5 min (30% amplification, 3 mm probe), added dropwise under vortex stirring to an equal volume of 25% glutaraldehyde solution (see Materials section) and incubated on rotating mixer at +37°C for 30 min. Further procedures were as described for “small” Fe@C-NH2/BSA/Str (see above).

**Synthesis of “medium” (210-240 nm) gelatin B-coated Fe@C-NH2 nanoparticles conjugated with streptavidin**
650 μL of 10% gelatin B in H2O was mixed with 650 μL of 0.1M phosphate buffer pH 8, then ionic strength of mixture was adjusted to 1M with NaCl powder. After that, 1300 μL of 10 mg/ml Fe@C-NH₂ suspension was added dropwise under vortex stirring. Resulting suspension (2500 μL) was sonicated on a water bath for 2 sec (30% amplification, 3 mm probe), added dropwise under vortex stirring to an equal volume of 25% glutaraldehyde solution (see Materials section) and incubated on rotating mixer at +37°C for 30 min. All solutions were pre-warmed and kept at +37-40°C to prevent gelation. Further procedures were performed at room temperature as described for “small” Fe@C-NH₂/BSA/Str (see above).

**Synthesis of “large” (300-320 nm) gelatin B-coated Fe@C-NH₂ nanoparticles conjugated with streptavidin**

1300 μL of 10 mg/ml Fe@C-NH₂ suspension was mixed with 650 μL of 0.1M phosphate buffer pH 8, then 650 μL of 10% gelatin B in H2O was added dropwise under vortex stirring. Resulting suspension (2500 μL) was sonicated for 5 sec (30% amplification, 3 mm probe), centrifuged at 1600g for 5 min to remove large agglomerates, then supernatant was sonicated for another 5 sec and added dropwise under vortex stirring to equal volume of 25% glutaraldehyde solution (see Materials section) and incubated on rotating mixer at +37°C for 30 min. All solutions were pre-warmed and kept at +37-40°C to prevent gelation. Further procedures were performed at room temperature as described for “small” Fe@C-NH₂/BSA/Str (see above).

**Synthesis of BSA-coated Fe@C-NH₂ functionalized with Streptococcal protein G (Fe@C-NH₂/BSA/G)**

2250 μL of 10 mg/ml Fe@C-NH₂ suspension was added dropwise under vortex stirring to 625 μL of 20% BSA in H₂O, then 1595 μL of PBS and 30 μL of 1M NaOH were added to adjust pH to 7.2-7.6. Resulting suspension (4300 μL) was sonicated for 10 s and added dropwise under vortex stirring to an equal volume of 25% glutaraldehyde solution and incubated on rotating mixer at room temperature for 30 min. Then, 8.6 ml of nanoparticles activated with glutaraldehyde were passed through the chromatography column (column XK 26/40, medium: Sepharose CL-6B, bed volume: 160 ml, elution speed: 0.65 ml/min, eluent: PBS) to remove the excess of BSA and glutaraldehyde. Fractions containing Fe@C-NH₂/BSA nanoparticles were collected and concentrated to approximately 3-4 ml. For concentrations, the suspension was placed in dialysis tubing (10 kDa MWCO, 2 ml per cm) and covered with the layer of 35 kDa PEG. After 2-3 hours concentrated suspension was removed from dialysis tubing and centrifuged at 1600g for 5 min. The supernatant contained Fe@C-NH₂/BSA nanoparticles activated with glutaraldehyde was added to 10 mg/ml protein G solution in PBS under vortex stirring. Final protein G to Fe@C-NH₂/BSA ratio was 80 μg of protein G per 1 mg of nanoparticles. Conjugation of protein G was carried out overnight on rotating mixer at +4°C, glycine was added to 6 mM to quench unreacted aldehyde groups and the mixture was incubated at RT for one more hour. Unreacted protein G was removed by gel-chromatography (column XK 26/40, medium: Sepharose CL-6B, bed volume: 100 ml, elution speed: 0.65 ml/min, eluent: PBS). Fractions with the highest concentration of Fe@C-NH₂/BSA/Str nanoparticles were combined. Glycerol, BSA and glycine were added to the final concentrations of 20%, 1%, and 6 mM respectively. Conjugates were stored at +4°C. The concentration of nanoparticles in prepared conjugates were determined by absorbance at 450 nm.

**Synthesis of casein-coated Fe@C-NH₂ functionalized with Streptococcal protein G (Fe@C-NH₂/Casein/G)**

1278 μL of 8.8% casein in H₂O was mixed with 962 μL 0.1M acetic buffer pH 5 and 9.6 μL of 1M NaOH. After that, 2250 μL of 10 mg/ml Fe@C-NH₂ suspension was added dropwise under vortex stirring. Resulting suspension (4300 μL) was sonicated for 10 s and added dropwise under vortex stirring to an equal volume of 25% glutaraldehyde solution and incubated on rotating mixer at room temperature for 30 min. Then, 8.6 ml of nanoparticles activated with glutaraldehyde were passed through the chromatography column (column XK 26/40, medium: Sepharose CL-6B, bed volume: 160 ml, elution speed: 0.65 ml/min, eluent: PBS) to remove the excess of casein and glutaraldehyde. Fractions containing Fe@C-NH₂/Casein nanoparticles were collected and concentrated to approximately 3-4 ml. For concentrations, the suspension was placed in dialysis tubing (10 kDa MWCO, 2 ml per cm) and covered with the layer of 35 kDa PEG. After 2-3 hours concentrated suspension was removed from dialysis tubing and centrifuged at 1600g for 5 min. The supernatant contained Fe@C-NH₂/Casein nanoparticles activated with glutaraldehyde was added
to 10 mg/ml protein G solution in PBS under vortex stirring. Final protein G to Fe@C-NH₂/Casein ratio was 80 μg of protein G per 1 mg of nanoparticles. Conjugation of protein G was carried out overnight on rotating mixer at +4°C, glycine was added to 6 mM to quench unreacted aldehyde groups, and the mixture was incubated at RT for one more hour. Unreacted protein G was removed by gel-chromatography (column XK 26/40, medium: Sepharose CL-6B, bed volume: 100 ml, elution speed: 0.65 ml/min, eluent: PBS). Fractions with the highest concentration of Fe@C-NH₂/BSA/Str nanoparticles were combined. Glycerol, BSA and glycine were added to the final concentrations of 20%, 1%, and 6 mM respectively. Conjugates were stored at +4°C. The concentration of nanoparticles in prepared conjugates was determined by absorbance at 450 nm.

**Synthesis of Gelatin A/B-coated Fe@C-NH₂ functionalized with Streptococcal protein G (Fe@C-NH₂/Gelatin A/G and Fe@C-NH₂/Gelatin B/G)**

1125 μL of 10% gelatin in H₂O was mixed with 1125 μL 0.1M acetic buffer pH 4. After that, 2250 μL of 10 mg/ml Fe@C-NH₂ suspension was added dropwise under vortex stirring; pH was adjusted to 7.25 by 1M NaOH. Resulting suspension (4300 μL) was sonicated for 5 min and added dropwise under vortex stirring to an equal volume of 25% glutaraldehyde solution and incubated on rotating mixer at +37°C for 30 min. All solutions were pre-warmed and kept at +37-40°C to prevent gelation. Then, 8.6 ml of nanoparticles activated with glutaraldehyde were passed through the chromatography column (column XK 26/40, medium: Sepharose CL-6B, bed volume: 160 ml, elution speed: 0.65 ml/min, eluent: PBS) to remove the excess of gelatin and glutaraldehyde. Fractions containing Fe@C-NH₂/Gelatin B nanoparticles were collected and concentrated to approximately 3-4 ml. For concentration, the suspension was placed in dialysis tubing (10 kDa MWCO, 2 ml per cm) and covered with the layer of 35 kDa PEG. After 2-3 hours concentrated suspension was removed from dialysis tubing and centrifuged at 1600g for 5 min. The supernatant contained Fe@C-NH₂/Gelatin B nanoparticles activated with glutaraldehyde was added to 10 mg/ml protein G solution in PBS under vortex stirring. Final protein G to Fe@C-NH₂/Gelatin B ratio was 80 μg of protein G per 1 mg of nanoparticles. Conjugation of protein G was carried out overnight on rotating mixer at +4°C, glycine was added to 6 mM to quench unreacted aldehyde groups, and the mixture was incubated at RT for one more hour. Unreacted protein G was removed by gel-chromatography (column XK 26/40, medium: Sepharose CL-6B, bed volume: 100 ml, elution speed: 0.65 ml/min, eluent: PBS). Fractions with the highest concentration of Fe@C-NH₂/BSA/Str nanoparticles were combined. Glycerol, BSA and glycine were added to the final concentrations of 20%, 1%, and 6 mM respectively. Conjugates were stored at +4°C. The concentration of nanoparticles in prepared conjugates was determined by absorbance at 450 nm.

**Synthesis of BSA-coated Fe@C-NH₂ functionalized with streptavidin (Fe@C-NH₂/BSA/Str)**

2250 μL of 10 mg/ml Fe@C-NH₂ suspension was added dropwise under vortex stirring to 625 μL of 20% BSA in H₂O, then 1595 μL of PBS and 30 μL of 1M NaOH were added to adjust pH to 7.2-7.6. Resulting suspension (4300 μL) was sonicated for 10 s and added dropwise under vortex stirring to an equal volume of 25% glutaraldehyde solution and incubated on rotating mixer at room temperature for 30 min. Then, 8.6 ml of nanoparticles activated with glutaraldehyde were passed through the chromatography column (column XK 26/40, medium: Sepharose CL-6B, bed volume: 100 ml, elution speed: 0.65 ml/min, eluent: PBS) to remove the excess of BSA and glutaraldehyde. Fractions containing Fe@C-NH₂/BSA nanoparticles were collected and concentrated to approximately 3-4 ml. For concentrations, the suspension was placed in dialysis tubing (10 kDa MWCO, 2 ml per cm) and covered with the layer of 35 kDa PEG. After 2-3 hours concentrated suspension was removed from dialysis tubing and centrifuged at 1600g for 5 min. The supernatant contained Fe@C-NH₂/BSA nanoparticles activated with glutaraldehyde was added to 10 mg/ml streptavidin solution in PBS under vortex stirring. Final streptavidin to Fe@C-NH₂/BSA ratio was 80 μg of streptavidin per 1 mg of nanoparticles. Conjugation of streptavidin was carried out overnight on rotating mixer at +4°C, glycine and BSA were added to 6 mM and 1 mg/ml respectively to quench unreacted aldehyde groups, and the mixture was incubated at RT for one more hour. Unreacted streptavidin was removed by gel-chromatography (column XK 26/40, medium: Sepharose CL-6B, bed volume: 100 ml, elution speed: 0.65 ml/min, eluent: PBS). Fractions with the highest concentration of Fe@C-NH₂/BSA/Str
nanoparticles were combined. Conjugates were stored at +4°C in PBS without any stabilizers. The concentration of nanoparticles in prepared conjugates were determined by absorbance at 450 nm.

**Synthesis of casein-coated Fe@C-NH₂ functionalized with streptavidin (Fe@C-NH₂/Casein/Str)**

1278 μL of 8.8% casein in H₂O was mixed with 962 μL 0.1M acetic buffer pH 5 and 9.6 μL of 1M NaOH. After that, 2250 μL of 10 mg/ml Fe@C-NH₂ suspension was added dropwise under vortex stirring. Resulting suspension (4300 μL) was sonicated for 10 s and added dropwise under vortex stirring to an equal volume of 25% glutaraldehyde solution and incubated on rotating mixer at room temperature for 30 min. Then, 8.6 ml of nanoparticles activated with glutaraldehyde were passed through the chromatography column (column XK 26/40, medium: Sepharose CL-6B, bed volume: 160 ml, elution speed: 0.65 ml/min, eluent: PBS) to remove the excess of casein and glutaraldehyde. Fractions containing Fe@C-NH₂/Casein nanoparticles were collected and concentrated to approximately 3-4 ml. For concentrations, the suspension was placed in dialysis tubing (10 kDa MWCO, 2 ml per cm) and covered with the layer of 35 kDa PEG. After 2-3 hours concentrated suspension was removed from dialysis tubing and centrifuged at 1600g for 5 min. The supernatant contained Fe@C-NH₂/Casein nanoparticles activated with glutaraldehyde was added to 10 mg/ml streptavidin solution in PBS under vortex stirring. Final streptavidin to Fe@C-NH₂/Casein ratio was 80 μg of streptavidin per 1 mg of nanoparticles. Conjugation of streptavidin was carried out overnight on rotating mixer at +4°C, glycine was added to 6 mM to quench unreacted aldehyde groups, and the mixture was incubated at RT for one more hour. Unreacted streptavidin was removed by gel-chromatography (column XK 26/40, medium: Sepharose CL-6B, bed volume: 100 ml, elution speed: 0.65 ml/min, eluent: PBS). Fractions with the highest concentration of Fe@C-NH₂/Casein/Str nanoparticles were combined. Conjugates were stored at +4°C in PBS without any stabilizers. The concentration of nanoparticles in prepared conjugates was determined by absorbance at 450 nm.

**Synthesis of Gelatin A/B-coated Fe@C-NH₂ functionalized with streptavidin (Fe@C-NH₂/Gelatin A/Str and Fe@C-NH₂/Gelatin B/Str)**

1125 μL of 10% gelatin in H₂O was mixed with 1125 μL 0.1M acetic buffer pH 4. After that, 2250 μL of 10 mg/ml Fe@C-NH₂ suspension was added dropwise under vortex stirring; pH was adjusted to 7.25 by 1M NaOH. Resulting suspension (4300 μL) was sonicated for 5 min and added dropwise under vortex stirring to an equal volume of 25% glutaraldehyde solution and incubated on rotating mixer at +37°C for 30 min. All solutions were pre-warmed and kept at +37-40°C to prevent gelation. Then, 8.6 ml of nanoparticles activated with glutaraldehyde were passed through the chromatography column (column XK 26/40, medium: Sepharose CL-6B, bed volume: 160 ml, elution speed: 0.65 ml/min, eluent: PBS) to remove the excess of gelatin and glutaraldehyde. Fractions containing Fe@C-NH₂/Gelatin B nanoparticles were collected and concentrated to approximately 3-4 ml. For concentrations, the suspension was placed in dialysis tubing (10 kDa MWCO, 2 ml per cm) and covered with the layer of 35 kDa PEG. After 2-3 hours concentrated suspension was removed from dialysis tubing and centrifuged at 1600g for 5 min. The supernatant contained Fe@C-NH₂/Gelatin B nanoparticles activated with glutaraldehyde was added to 10 mg/ml streptavidin solution in PBS under vortex stirring. Final streptavidin to Fe@C-NH₂/Gelatin B ratio was 80 μg of streptavidin per 1 mg of nanoparticles. Conjugation of streptavidin was carried out overnight on rotating mixer at +4°C, glycine was added to 6 mM to quench unreacted aldehyde groups and the mixture was incubated at RT for one more hour. Unreacted streptavidin was removed by gel-chromatography (column XK 26/40, medium: Sepharose CL-6B, bed volume: 100 ml, elution speed: 0.65 ml/min, eluent: PBS). Fractions with the highest concentration of Fe@C-NH₂/Gelatin B/Str nanoparticles were combined. Conjugates were stored at +4°C in PBS without any stabilizers. The concentration of nanoparticles in prepared conjugates was determined by absorbance at 450 nm.
Figure 1. Casein solution before (right) and after (left) removal of micelles.

Figure 2. Zeta potential of Fe@C-NH$_2$. 
Figure S3. Influence of pH, ionic strength and protein-to-nanoparticle mass ratio on the size (hydrodynamic diameter) and polydispersity index of BSA-coated Fe@C-NH$_2$.

Figure S4. Influence of pH, ionic strength and protein-to-nanoparticle mass ratio on the size (hydrodynamic diameter) and polydispersity index of casein-coated Fe@C-NH$_2$. The absence of bars reveals a severe aggregation of nanoclusters in the corresponding samples.
Figure 5. Influence of pH, ionic strength and protein-to-nanoparticle mass ratio on the size (hydrodynamic diameter) and polydispersity index of gelatin B-coated Fe@C-NH₂. The absence of bars reveals a severe aggregation of nanoclusters in the corresponding samples.

Figure 6. Influence of pH and protein-to-nanoparticle mass ratio on the size (hydrodynamic diameter) and polydispersity index of Gelatin A-coated Fe@C-NH₂. The absence of bars reveals a severe aggregation of nanoclusters in the corresponding samples.
Figure 7. Influence of sonication duration on size and polydispersity of Gelatin B-stabilized Fe@C-NH₂ (pH 8, I=0.5).

Figure 8. Hydrodynamic diameter and polydispersity of “small”, “medium” and “large” Fe@C-NH₂/Protein/Str. Streptavidin to nanoparticles ratio (μg:mg) on the x axis. The absence of bars reveals a severe aggregation of nanoclusters in the corresponding samples.
Figure S9. Zeta potential of “small”, “medium” and “large” Fe@C-NH2/Protein/Str. Conjugates were prepared using various streptavidin to nanoparticles ratio, μg:mg (see legend). The absence of bars reveals a severe aggregation of nanoclusters in the corresponding samples.

Figure S10. Migration of Fe@C-NH2/protein/Str and Fe@C-NH2/protein/G in 0.5% agarose gel (Tris-HCl pH 8, 75V). Photos were taken 5 (left) and 15 (right) minutes after start. Lanes: 1 - Fe@C-NH2; 2 - Fe@C-NH2/Gelatin A/G; 3 - Fe@C-NH2/Gelatin B/G; 4 - Fe@C-NH2/Casein/G; 5 - Fe@C-NH2/BSA/G; 6 - Fe@C-NH2/Gelatin B/Str “Medium”; 7 - Fe@C-NH2/Gelatin B/Str “Large”; 8 - Fe@C-NH2/Gelatin B/Str “Small”; 9 - Fe@C-NH2/Casein/Str “Large”; 10 - Fe@C-NH2/Casein/Str “Medium”; 11 - Fe@C-NH2/Casein/Str “Small”; 12 - Fe@C-NH2/BSA/Str “Large”; 13 - Fe@C-NH2/BSA/Str “Medium”; 14 - Fe@C-NH2/BSA/Str “Small”.

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Figure 11. Principle of NMR-assay of Bi-BSA on nitrocellulose membrane (top). Sample holder with test strip (bottom).
Figure 12. Functional activity of “small”, “medium” and “large” Fe@C-NH₂/Protein/Str in solid phase NMR-assay. Conjugates were prepared using various streptavidin to nanoparticles ratio (μg:mg): 10:1 (red), 20:1 (orange), 40:1 (yellow), 80:1 (green), 160:1 (blue). Bi-BSA - biotinylated BSA.
Figure 13. Day-to-day reproducibility of anti-tetanus toxoid NMR-assay. Three independent assays were performed in different days for each conjugate.

Figure 14. Comparison of ELISA and NMR-assay of IgG against tetanus toxoid.
Figure 15. Storage stability of Fe@C-NH₂/Protein/Str nanoparticles, obtained in the course of size tuning experiments. Streptavidin to nanoparticles ratio (μg:mg) on the x axis. Digit 4 indicates conjugates stored for 4 months. Arrows depict samples with altered size (both increase and decrease).
Figure 16. Protein corona of Fe@C-NH$_2$/Protein/Str after incubation in blood serum and plasma. A: lane 1 - casein, lane 2 - plasma, lane 3 - Fe@C-NH$_2$ (1 hour in plasma), lane 4 - Fe@C-NH$_2$/Gelatin B/Str (1 hour in plasma), lane 5 - Fe@C-NH$_2$/Gelatin A/Str (1 hour in plasma), lane 6 - Fe@C-NH$_2$/Casein/Str (1 hour in plasma), lane 7 - Fe@C-NH$_2$/BSA/Str (1 hour in plasma), lane 8 - protein markers. B: lane 1 - protein marker, lane 2 - BSA, lane 3 - casein, lane 4 - gelatin A, lane 5 - gelatin B, lane 6 - Fe@C-NH$_2$/BSA/Str (1 hour in serum), lane 7 - Fe@C-NH$_2$/Casein/Str (1 hour in serum), lane 8 - Fe@C-NH$_2$/Gelatin A/Str (1 hour in serum), lane 9 - Fe@C-NH$_2$/Gelatin B/Str (1 hour in serum), lane 10 - Fe@C-NH$_2$ (1 hour in serum). C: lane 1 - Fe@C-NH$_2$ (1 hour in PBS), lane 2 - Fe@C-NH$_2$/BSA/Str (1 hour in PBS), lane 3 - Fe@C-NH$_2$/Casein/Str (1 hour in PBS), lane 4 - protein markers, lane 5 - Fe@C-NH$_2$/Gelatin A/Str (1 hour in PBS), lane 6 - Fe@C-NH$_2$/Gelatin B/Str (1 hour in PBS), lane 7 - gelatin A, lane 8 - gelatin B. D: lane 1 - Fe@C-NH$_2$/BSA/Str (1 hour in PBS), lane 2 - Fe@C-NH$_2$/Casein/Str (1 hour in PBS), lane 3 - Fe@C-NH$_2$/Gelatin A/Str (1 hour in PBS), lane 4 - Fe@C-NH$_2$/Gelatin B/Str (1 hour in PBS), lane 5 - protein markers, lane 6 - streptavidin, lane 7 - blood serum, lane 8 - Fe@C-NH$_2$ (1 hour in PBS).
Table 1. Properties of conjugates used in this study.

| Group | Str(protein)/nanoparticle s ratio, µg/mg | Coating | Attached molecule | Dₜ, nm | Pdl | Relaxivity, 1 mM⁻¹ s⁻¹ | Conc., mg/ml | Parent aminated nanoparticles | Were used in following experiments |
|-------|----------------------------------------|---------|------------------|--------|-----|------------------------|-------------|-------------------------------|---------------------------------|
| Small | 10:1 BSA Streptavidin 116 0.207 265.3813 0.3888 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Small | 20:1 BSA Streptavidin 121 0.211 241.8847 0.4779 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Small | 40:1 BSA Streptavidin 114 0.188 236.8949 0.5103 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Small | 80:1 BSA Streptavidin 115 0.208 234.6137 0.5022 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Small | 160:1 BSA Streptavidin 120 0.203 236.5807 0.5427 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Medium | 10:1 BSA Streptavidin 176 0.158 262.0929 0.4536 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Medium | 20:1 BSA Streptavidin 184 0.163 284.4745 0.4293 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Medium | 40:1 BSA Streptavidin 172 0.178 322.8937 0.4212 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Medium | 80:1 BSA Streptavidin 185 0.175 272.6557 0.4941 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Medium | 160:1 BSA Streptavidin 186 0.166 300.4936 0.5022 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Large | 10:1 BSA Streptavidin 221 0.258 186.0697 0.4131 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Large | 20:1 BSA Streptavidin 228 0.246 230.058 0.3726 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Large | 40:1 BSA Streptavidin 233 0.247 214.7827 0.3969 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Large | 80:1 BSA Streptavidin 211 0.25 223.3444 0.3645 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Large | 160:1 BSA Streptavidin 227 0.257 219.0292 0.3564 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Small | 10:1 Casein Streptavidin 114 0.201 310.8042 0.3483 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Small | 20:1 Casein Streptavidin 121 0.186 345.4113 0.3564 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Small | 40:1 Casein Streptavidin 117 0.186 327.2012 0.3807 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Small | 80:1 Casein Streptavidin 120 0.178 344.5608 0.3483 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Small | 160:1 Casein Streptavidin 131 0.196 313.6018 0.3807 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Medium | 10:1 Casein Streptavidin 195 0.169 340.1031 0.3726 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Medium | 20:1 Casein Streptavidin 194 0.132 335.4058 0.3483 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Medium | 40:1 Casein Streptavidin 203 0.167 318.391 0.405 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Medium | 80:1 Casein Streptavidin 225 0.164 337.1553 0.3726 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Medium | 160:1 Casein Streptavidin 198 0.158 321.36 0.2987 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Large | 10:1 Casein Streptavidin 235 0.215 345.0889 0.1863 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Large | 20:1 Casein Streptavidin 274 0.243 330.2673 0.3078 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Large | 40:1 Casein Streptavidin 242 0.202 303.6793 0.3402 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Large | 80:1 Casein Streptavidin 245 0.209 327.5499 0.3483 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Large | 160:1 Casein Streptavidin 242 0.207 309.1843 0.3402 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Small | 10:1 Gelatin B Streptavidin 159 0.246 266.7957 0.26125 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Small | 20:1 Gelatin B Streptavidin 156 0.22 276.309 0.3325 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Small | 40:1 Gelatin B Streptavidin 142 0.211 292.5403 0.30875 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Small | 80:1 Gelatin B Streptavidin 140 0.248 298.0891 0.32456333 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Small | 160:1 Gelatin B Streptavidin 154 0.212 306.3228 0.3325 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Medium | 10:1 Gelatin B Streptavidin aggregated 0.26 377.1929 0.2997 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Medium | 20:1 Gelatin B Streptavidin 229 0.26 377.1929 0.2997 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Medium | 40:1 Gelatin B Streptavidin 243 0.216 324.2472 0.2592 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Medium | 80:1 Gelatin B Streptavidin 212 0.231 296.7454 0.2754 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Medium | 160:1 Gelatin B Streptavidin 236 0.221 312.0377 0.2673 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Large | 10:1 Gelatin B Streptavidin 309 0.257 308.3217 0.2025 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Large | 20:1 Gelatin B Streptavidin 308 0.255 282.6507 0.2106 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Large | 40:1 Gelatin B Streptavidin 279 0.246 301.3985 0.2025 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Large | 80:1 Gelatin B Streptavidin 297 0.227 308.0283 0.1944 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| Large | 160:1 Gelatin B Streptavidin 292 0.244 314.5411 0.1782 AM-SIZE | Size tuning, long-term storage | | | | | | | |
| 80:1 | BSA | Protein G | 105 | 0.163 | 335,7127 | 1,0908 | AM-G |
|------|-----|-----------|-----|--------|----------|--------|------|
|      |     |           |     |        | Long-term storage, IgG NMR-assay, stability in complex media, colloidal stability, thermal stability |
| 80:1 | Casein | Protein G | 132 | 0.161 | 377,0426 | 0.8964 | AM-G |
|      |     |           |     |        | Long-term storage, IgG NMR-assay, stability in complex media, colloidal stability, thermal stability |
| 80:1 | Gelatin B | Protein G | 153 | 0.186 | 368,492 | 0.7668 | AM-G |
|      |     |           |     |        | Long-term storage, IgG NMR-assay, stability in complex media, colloidal stability, thermal stability |
| 80:1 | Gelatin A | Protein G | 167 | 0.18 | 389,485 | 0.5724 | AM-G |
|      |     |           |     |        | Long-term storage, IgG NMR-assay, stability in complex media, colloidal stability, thermal stability |
| 40:1 | BSA | Streptavidin | 111 | 0.161 | 204,8375 | 1,586 | AM-Str |
|      |     |           |     |        | Protein corona, proteolysis, stability in complex media |
| 40:1 | Casein | Streptavidin | 119 | 0.154 | 221,7823 | 1.22 | AM-Str |
|      |     |           |     |        | Protein corona, proteolysis, stability in complex media |
| 40:1 | Gelatin B | Streptavidin | 154 | 0.131 | 232,5224 | 0.712916667 | AM-Str |
|      |     |           |     |        | Protein corona, proteolysis, stability in complex media |
| 40:1 | Gelatin A | Streptavidin | 186 | 0.181 | 232,1508 | 0.737083333 | AM-Str |
|      |     |           |     |        | Protein corona, proteolysis, stability in complex media |
| AM-SIZE | none | NH2 | none | 96.42 | 0.248 | 285,8172 | 8.1 | none |
| AM-G | none | NH2 | none | 110.45 | 0.261 | 335,5421 | 10.8 | none |
| AM-Str | none | NH2 | none | 109.2 | 0.272 | 250,7953 | 12.2 | none |

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