Supplementary Information

Maghemite nanoparticles stabilize protein corona formed with transferrin presenting different iron-saturation levels

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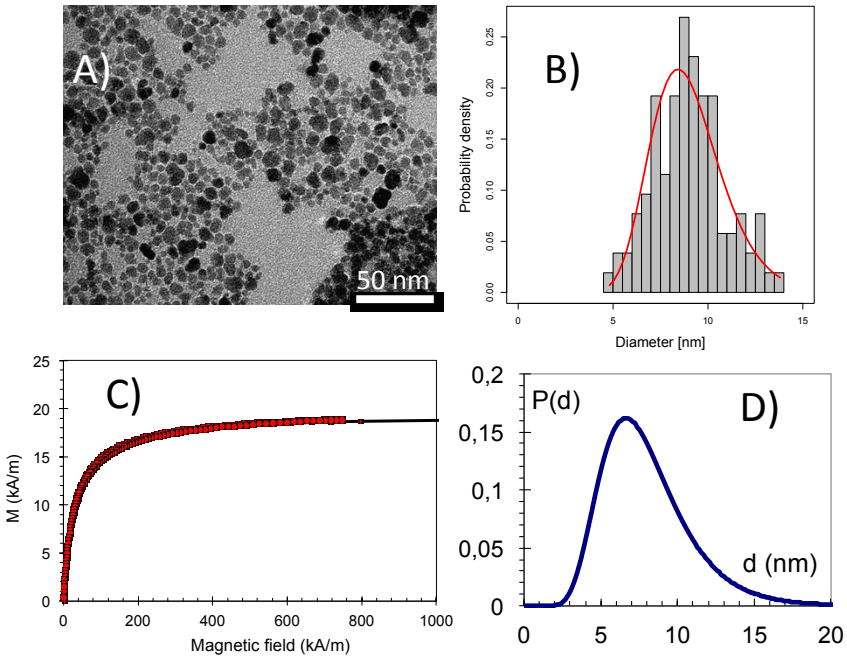

Figure S1. Characterizations of the magnetic NPs: (A) TEM image of magnetic nanoparticles showing their rock-like shape; (B) corresponding size histograms with log-normal fits d_0 = 10.2 nm and = ± 0.11 ; (C) Magnetization curve (dots) of the stable suspension

of ferrofluid used in this study and the corresponding fitting by Langevin law (black line); (D) Fitting log-normal law for the numbers distribution of diameters.

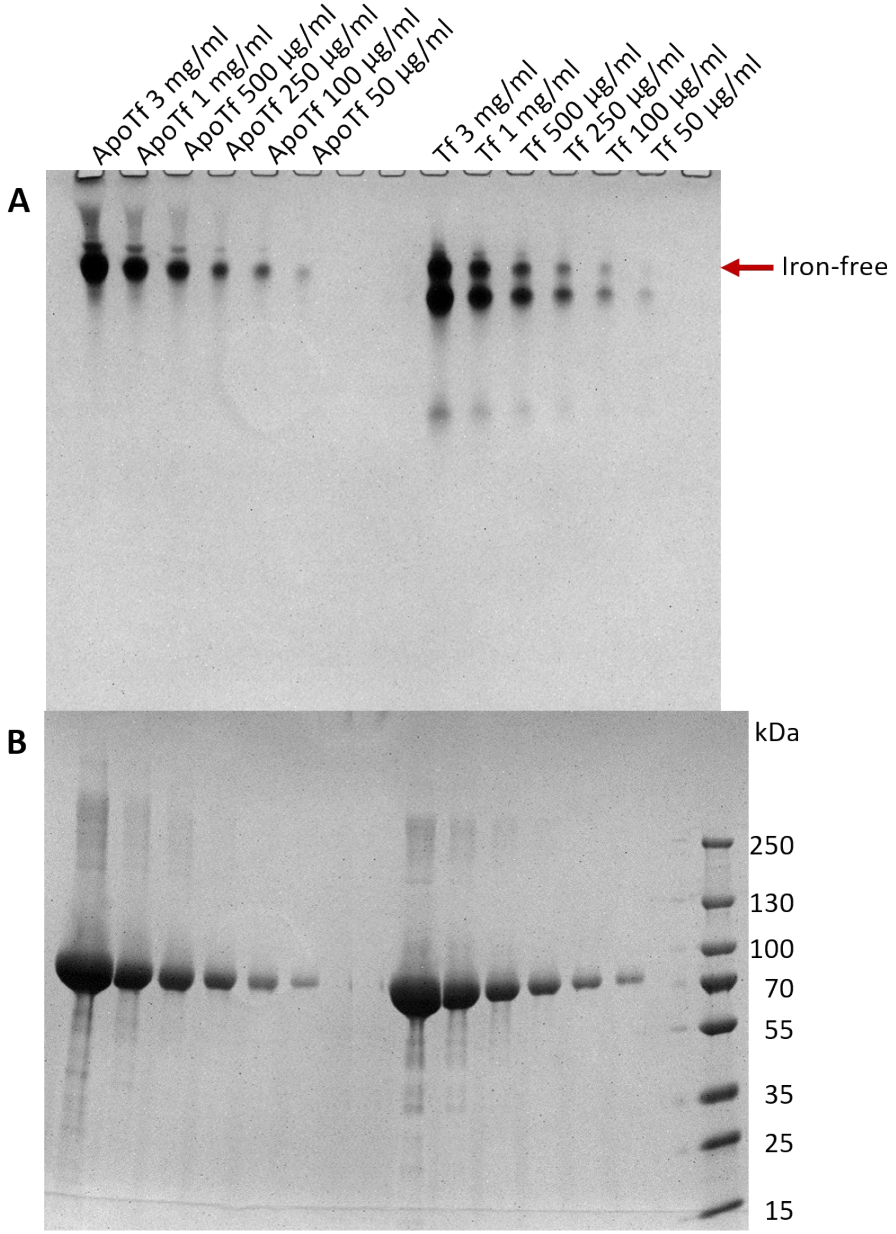

Figure S2. (A) Image of Coomassie-stained urea gel with apotransferrin (ApoTf, left) and transferrin (Tf, right) in different concentrations. For apotransferrin one band is observable on the urea gel, while for transferrin, three single bands appeared. The first band is attributed to the iron-free form of transferrin which is also present for the partly saturated transferrin. The second band corresponds to the monoferric form where one domain consists of Fe atoms and the third bands displays the diferric in which both domains are saturated with Fe. (B) Coomassie-stained SDS-PAGE. Here the same concentrations of apotransferrin and transferrin like in urea gel are used. Both proteins display the same band position at ~80 kDa.

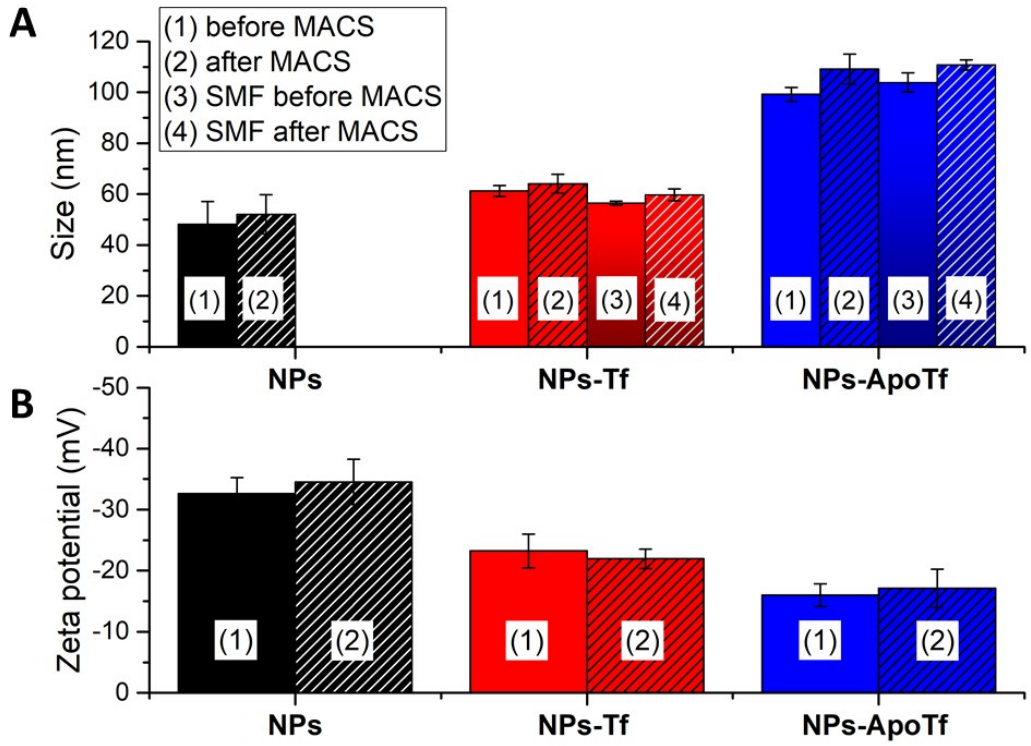

Figure S3. Hydrodynamic diameter (B) and zeta potential (C) of maghemite nanoparticles and their bioconjugates with Tf and ApoTf before and after magnetic separation with MACS columns. The numbers 3 and 4 mark the samples that were incubated with an applied static magnetic field (SMF).

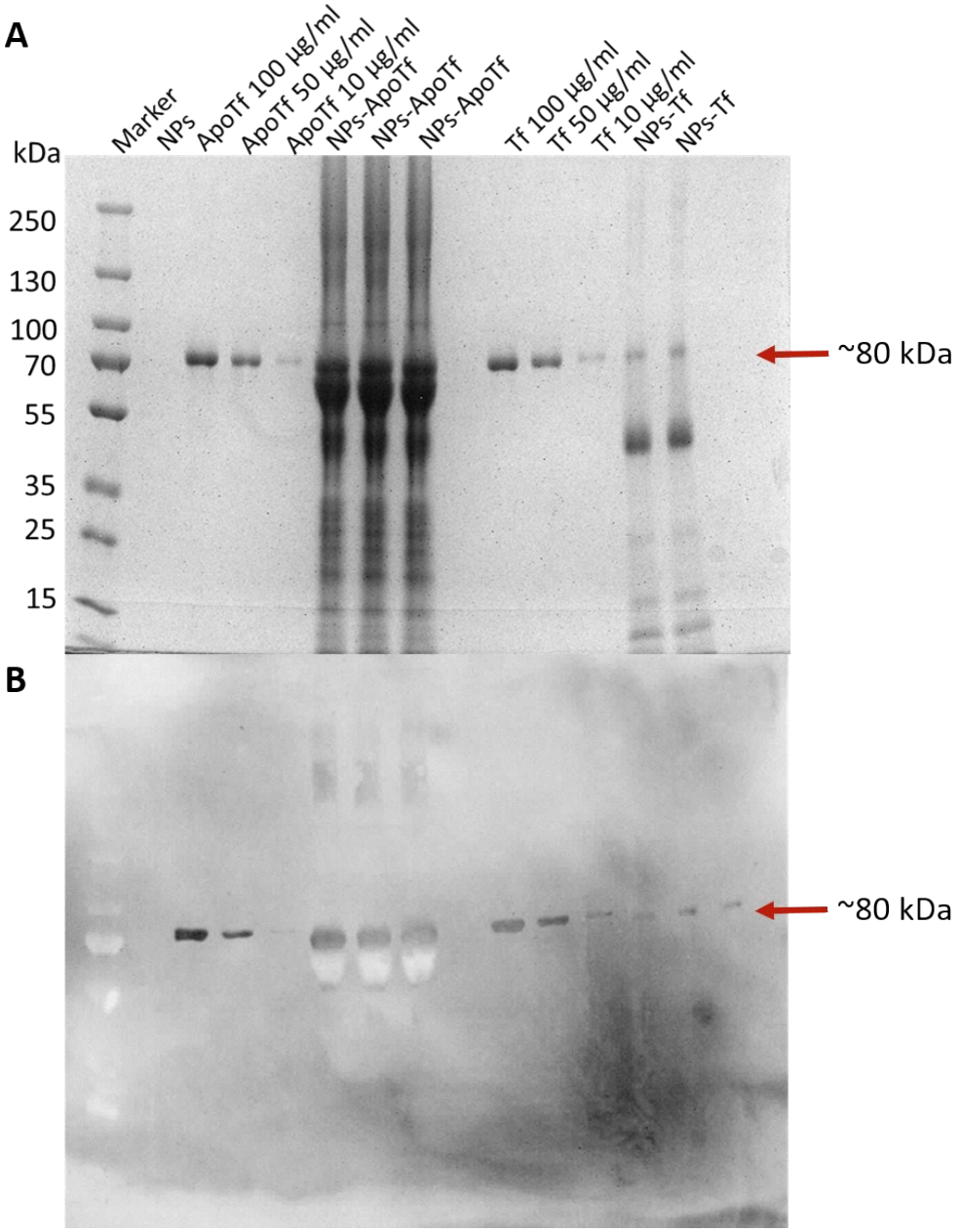

Figure S4. (A) Coomassie-stained SDS gel with bioconjugates which consists of ApoTf and maghemite nanoparticles (on the left) with intensive bands as well as the Tf-NP-conjugates (on the right side). While the proteins, ApoTf and Tf show no differences in the appearance of the bands, the biconjugates display strong variations. In addition to the band at ~80 kDa, also several other bands are observable for the bioconjugates. After incubation with maghemite nanoparticles, ApoTf shows more intensive bands than Tf. (B) Anti-Tf-antibody Western blot analysis with samples similar to the SDS-PAGE displayed in (A). The positive controls with ApoTf and Tf as well as all bioconjugate samples show the transferrin band at 80 kDa.