Electronic Supplementary Information for:

Enzyme-Coated Janus Nanoparticles That Selectively Bind Cell Receptors as a Function of the Concentration of Glucose

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EXPERIMENTAL SECION

Fabrication and characterization of Janus particles.

Experiments in Figure 2 were performed with commercially available streptavidin-coated magnetic particles (10 μm, Sigma), whereas the experiments shown in Figures 3 and 4 were performed with streptavidin-coated magnetic nanoparticles synthesized in our laboratory. Unfortunately the same experiments could not be performed with the same type of particle. In Figure 2 the area modified with fluorescent molecules covers a few microns of the large particles, which can be easily imaged with optical microscopy. If the same procedure was applied to the 220 nm nanoparticles it would yield fluorescent areas smaller than 100 nm, which cannot be visualized with optical microscopy. On the other hand, the 8 μm particles sediment quickly when not stirred. This makes it extremely difficult to calculate their diffusion coefficient with dynamic light scattering. Therefore it is not possible to determine whether the addition of glucose triggers the motion of the colloids when using micrometric Janus particles.

The iron oxide nanoparticles were prepared as follows. 1 g of $FeCl_3 \cdot 6H_2O$ and 0.368 g of $FeCl_2 \cdot 4H_2O$ were added to 60 mL of MilliQ water (previously degassed for 20 min with argon gas). After 20 min 7.5 mL of NH_4OH (30 % in H_2O) was added while stirring vigorously for 30 min. Thiolated PEG molecules bearing carboxylate groups (MW 5000, Sigma) were added for 15 min. The nanoparticles were washed with the aid of magnet. The size of the resulting iron oxide nanoparticles was 220 ± 40 nm (calculated by DLS, n =3). The zeta potential of the PEG-covered nanoparticles was 17 ± 8 mV whereas bare nanoparticles had a zeta potential of -36.5 ± 2 mV (n = 3). The nanoparticles were covalently modified with avidin with the following method. 1 mL of nanoparticles was washed 3 times with MES buffer (0.5 M, pH 5.5). 1 mL of freshly prepared EDC (0.1 M) and sulfo-NHS (0.1 M) was added to the nanoparticles. After 30 min the solution was

removed after accumulating the nanoparticles with a magnet, and avidin (1 mL, 1mg/mL) was added for 2 h. Then 0.5 mL of glycine (0.1 M) was added for 30 min in order to block unreacted NHS esters. The resulting avidin-coated nanoparticles were washed 5 times with PBS buffer and stored at 4 degrees until needed.

To prove the successful attachment of avidin to the nanoparticles, biotinylated HRP was added to the 3 samples containing avidin-nanoparticles for 1 h. Control samples were prepared with BSA-covered nanoparticles. After washing away unbound biotinylated molecules with PBS and the aid of a magnet, the presence of HRP in the samples was studied by following the oxidation of the enzyme substrate TMB with a spectrophotometer (TMB kit, Thermo Scientific). In Figure S1 the Absorbance at 652 nm increases when the avidin-nanoparticles were incubated with biotin-HRP. BSA-nanoparticles yield overlapping signals that are very close to zero and that do not increase with time, which demonstrates that the signal registered with the avidin-nanoparticles is originated by biospecific biotin-avidin interactions. These experiments validate the proposed protocol for attaching avidin to the PEG-covered iron oxide nanoparticles.

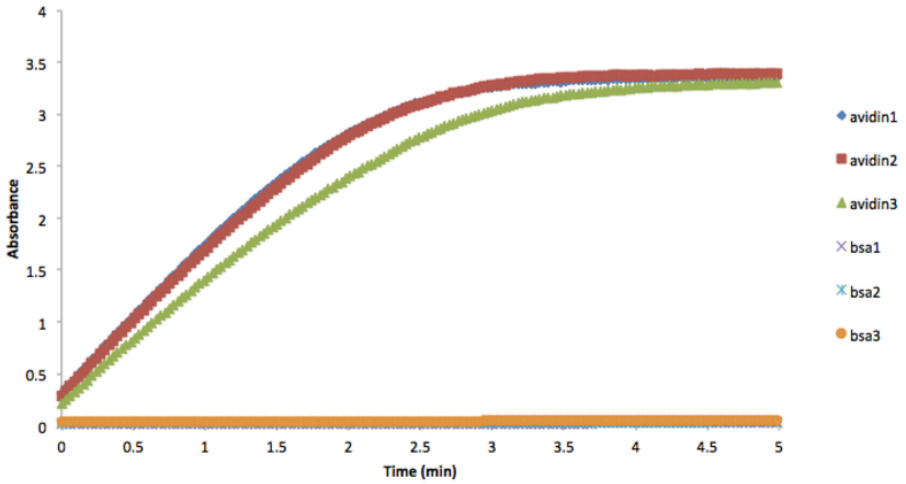

Figure 1. Variation of absorbance with time of avidin-modified nanoparticles or BSA-modified nanoparticles after incubation with bioin-HRP.

To obtain Janus particles, 20-50 μl of streptavidin-coated magnetic colloids was washed 3 times with 0.1 M acetate buffer pH 6 (AB) containing 0.05% Tween-20 (ABT). The colloidal dispersion was then added to a 100 mL beaker and placed on a magnetic plate. 3 mL of ABT was added to the beaker in order to fully cover the colloids with buffer. After 30 minutes, 50 μl of 1 mg mL^{-1} biotinylated glucose oxidase (biotin-GOx) was added to the solution. Biotinylated GOx was obtained using a biotinylation kit (EZ-link NHS-PEO4-Biotinylation kit, Thermo Scientific). Glucose oxidase from Aspergillus Niger type VII, lyophilized powder, ≥100,000 units/g solid was purchased from Sigma. After 1 h the unreacted biotin-GOx was carefully removed and the colloids were washed 3 times with 1 mL ABT with the aid of a magnet. The solution was then concentrated back to the initial volume (20-50 μl) by gathering the colloids with a magnet, removing the solution and adding the desired volume of ABT.

The Janus structure of the colloids was visualized by modifying free streptavidin binding sites with biotinylated fluorescent BSA (Figure 2b in the main text and Figure S2 below). Biotinylated fluorescent BSA was prepared as follows. A solution containing 1 mg mL^{-1} BSA in PBS was prepared. Primary amine groups in BSA were modified with NHS-fluorescein (0.2 mg mL^{-1}, 6-[Fluorescein-5(6)-carboxamido]hexanoic acid N-hydroxysuccinimide ester, Sigma) in PBS for 30 min followed by purification with a PD-10 desalting column. Then carboxylate groups in the BSA were modified with 0.01 M biocytin via addition of 0.01 M EDC (N-(3-Dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride, Sigma) and 0.01 M NHS (N-hydroxysuccinimide, Sigma). The primary amine groups in biocytin reacted with NHS esters on BSA to yield stable amide bonds. After 1 h the resulting biotinylated fluorescent BSA was purified with a P10 desalting column. The protein was kept as single-use aliquots frozen at -20 ^{o}C. To visualize the Janus structure, the colloids containing asymmetrically distributed GOx were incubated

with the biotinylated fluorescent BSA diluted 1:100 in PBS containing 0.05 % Tween-20 (PBST) for 30 min. The colloids were then washed 3 times with PBST followed by washing with PBS. The resulting Janus colloids were imaged with a Nikon Eclipse LV100 with a 20× objective.

Measuring the diffusion coefficient with DLS

DLS experiments were performed with dispersions of Janus nanoparticles in PBS supplemented with glucose at different concentrations. Measurements were performed with a Malvern Zetasizer in triplicate. The diffusion coefficient is related to the size of the nanoparticles calculated by DLS through the Stoke-Einstein equation:

$$D = \frac{RT}{N6\pi\eta r}$$

Where D is the diffusion coefficient, R is the gas constant, T is the temperature, N is the Avogadro number, η is the viscosity and r is the radius of the nanoparticle. The viscosity was corrected to take into account the different concentrations of glucose.[1] Each experiment was repeated 3 times with different samples each time.

Preparation of fluorescent Janus nanoparticles bearing RGDS peptides.

The Janus nanoparticles containing GOx were modified with NHS-fluorescein (0.5 mg/mL in PBS) for 30 minutes followed by 3 washing steps with PBS-tween. This rendered the nanoparticles fluorescent in order to visualize interactions with the cell membrane (Figure 4). Free avidin-binding sites in the resulting fluorescent nanoparticles were then modified with RGDS. The RGDS-biotin peptide was synthesized following solid-phase synthetic methods with Fmoc-protected amino acids. The peptides were dissolved in water and stored at -20^{o}C until needed. 10 μL of RGDS-biotin was added to

Janus colloids containing GOx in PBS for 30 minutes followed by washing 3 times with PBS.

Cell culture and RDS-integrin binding experiments: Chinese hamster ovarian (CHO) cells were kindly gifted from Professor Duncan Graham at the department of Pure and Applied Chemistry, University of Strathclyde, UK. Cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% Fetal Bovine Serum (FBS) and were maintained at 37^{o}C in a 5% CO_2 humidified environment. Two days before the experiments, cells were trypsinized, centrifuged and plated onto 35 mm petri dish in complete cell growth medium. Cell confluence was between 70 and 85% the day of the experiment. To avoid nanoparticle internalization, cells were fixed using a solution of paraformaldehyde 4% in PBS for 45 minutes at 4^{o}C. For imaging experiments, cells were incubated with nanoparticles for 2 h at room temperature (10 µl in 2 ml of PBS supplemented with either 0, 1 or 10 mM glucose). Before imaging, cells were washed three times with the PBS solution to remove unbound nanoparticles. Fluorescence images were obtained with a Nikon Eclipse LV100 with a 20× objective (Nikon CFI LU Plan BD ELWD, NA = 0.55) using the same light intensity and detector (integration time 0.1 s) and a FITC excitation/emission cube set. The fluorescence intensity inside individual cells within unaltered images was estimated by selecting an area inside each cell and measuring the luminosity (pixel density) using the Histogram function of Adobe Photoshop. The average value and standard deviation of 100 individual measurements is shown in Fig. 4d.

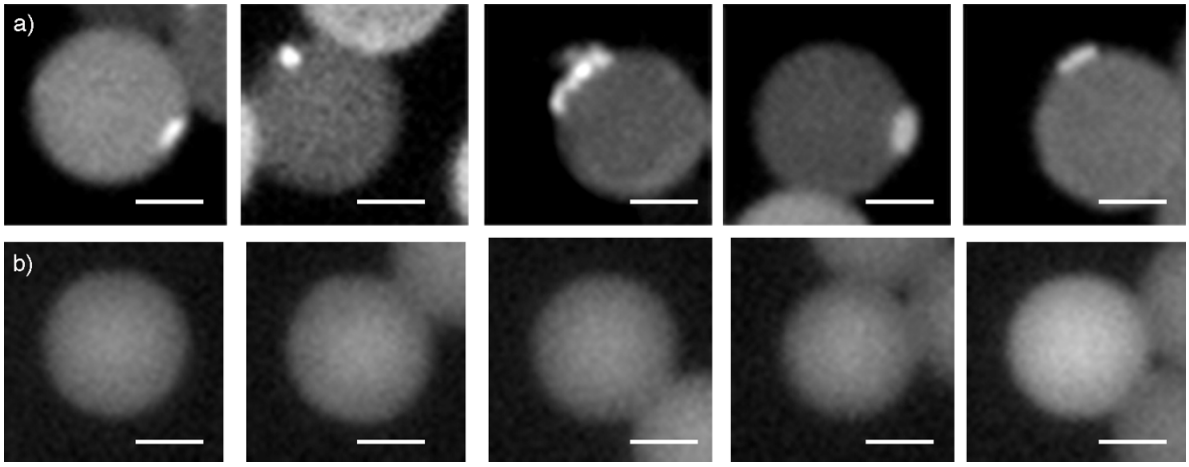

Figure S2. *Additional images of Janus particles containing GOx and fluorescent BSA (a) or only GOx (b). Scale bars: 10 μm.*

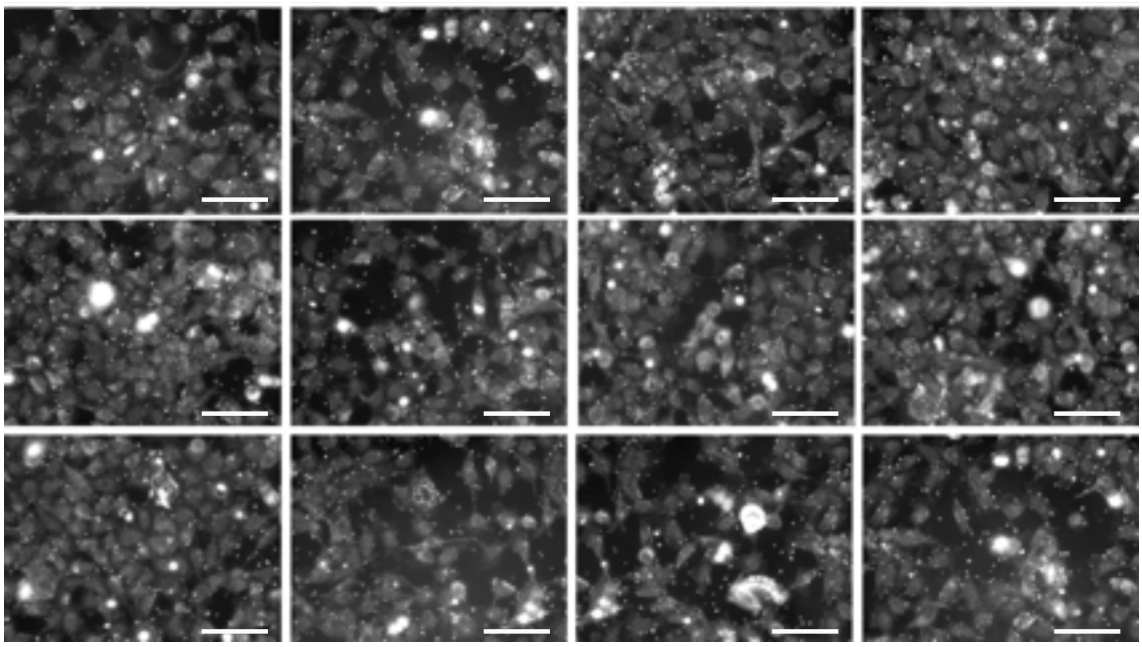

Figure S3. *Fluorescence microscopy images of cells after incubation with fluorescent Janus nanoparticles in the absence of glucose. Scale bar: 100 μm.*

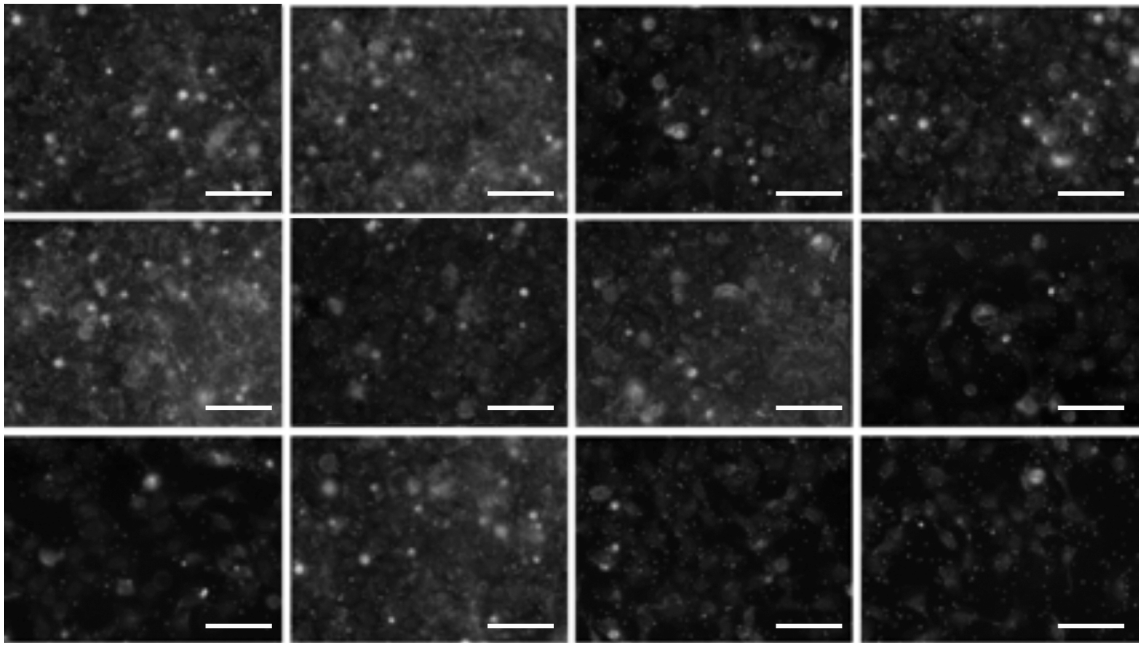

Figure S4. *Fluorescence microscopy images of cells after incubation with fluorescent Janus nanoparticles in the presence of 1 mM glucose. Scale bar: 100 μm.*

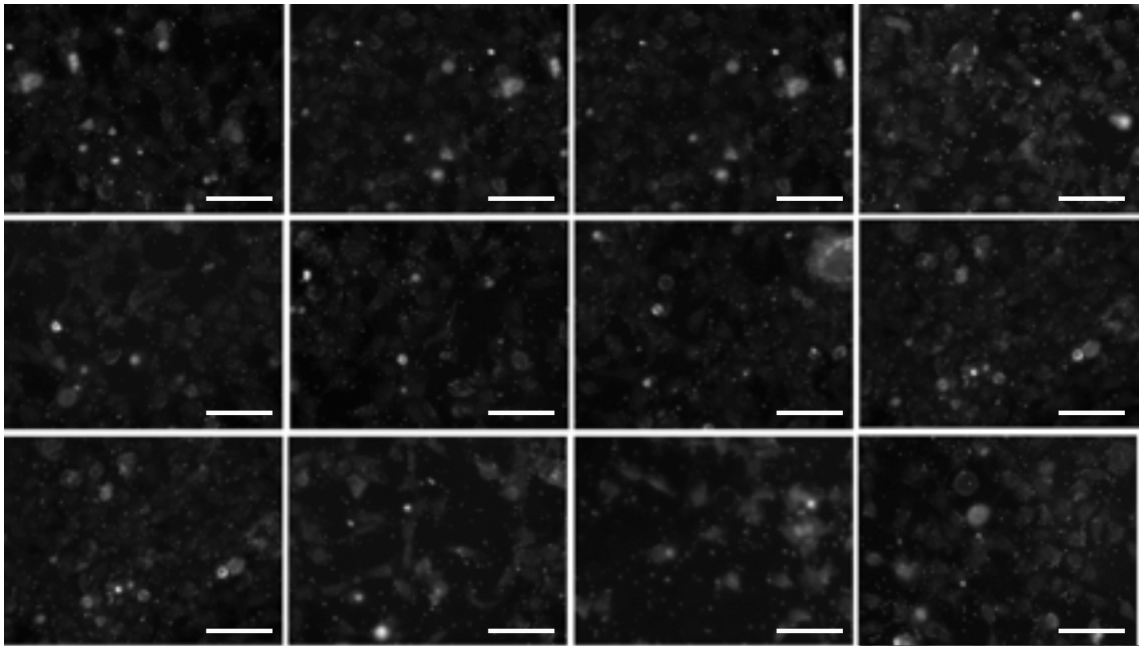

Figure S5. *Fluorescence microscopy images of cells after incubation with fluorescent Janus nanoparticles in the presence of 10 mM glucose. Scale bar: 100 μm.*

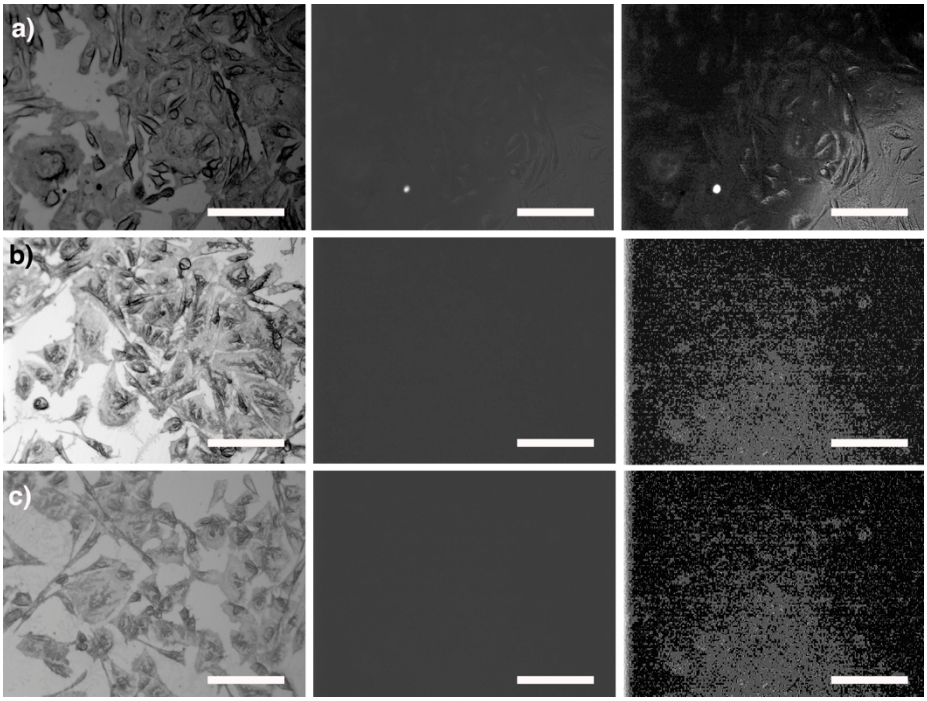

Figure S6. *Bright-field (left) and fluorescence microscopy (middle, right) images of cells incubated with fluorescent Janus nanoparticles that were not modified with RGDS (Scale bar: 100 μm). The membrane of the cells does not become fluorescent after incubation with the nanoparticles, even when the integration time of fluorescence imaging was increased to 1 s (middle) and after increasing the contrast of the images (right). These experiments demonstrate that the nanoparticle-cell interactions observed in Figure 4 and Figs. S3-S5 are specific and originated by interactions between RGDS and the cell membrane.*

References

1 V. R. N. Telis, J. Telis-Romero, H. B. Mazzotti and a. L. Gabas, *Int. J. Food Prop.*, 2007, **10**, 185–195.