Supporting information

Stable Anchoring of Bacteria-based Protein Nanoparticles for Surface Enhanced Cell Guidance

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S1. Size and Surface Charge Determination of IB-like pNPs

IB-like pNPs were resuspended in ultrapure water and sonicated for 10 min. The resulting suspensions of IB-like pNPs (20 µg/ml) were dispensed into disposable plastic cuvettes before analysis in a dynamic light scattering (DLS) equipment Zetasizer Nanoseries Nano-ZS (Malvern Instruments, UK). Size distribution and Z-potential were measured for both *E. coli* and *L. lactis* produced samples. The size measurement through DLS of *E. coli* and *L. lactis* produced nanoparticles yielded average values of 320 and 1080 nm, while the Z-potential measurements yielded values of -36.9 and -36.8 mV, respectively.

Figure S1 shows representative images of isolated pNPs by High Resolution Scanning Electron Microscopy (HRSEM) showing round morphologies and average sizes of 350 and 520 nm for *E. coli* and *L. lactis* produced pNPs, N(particles) = 24 and N(particles) = 125, respectively.

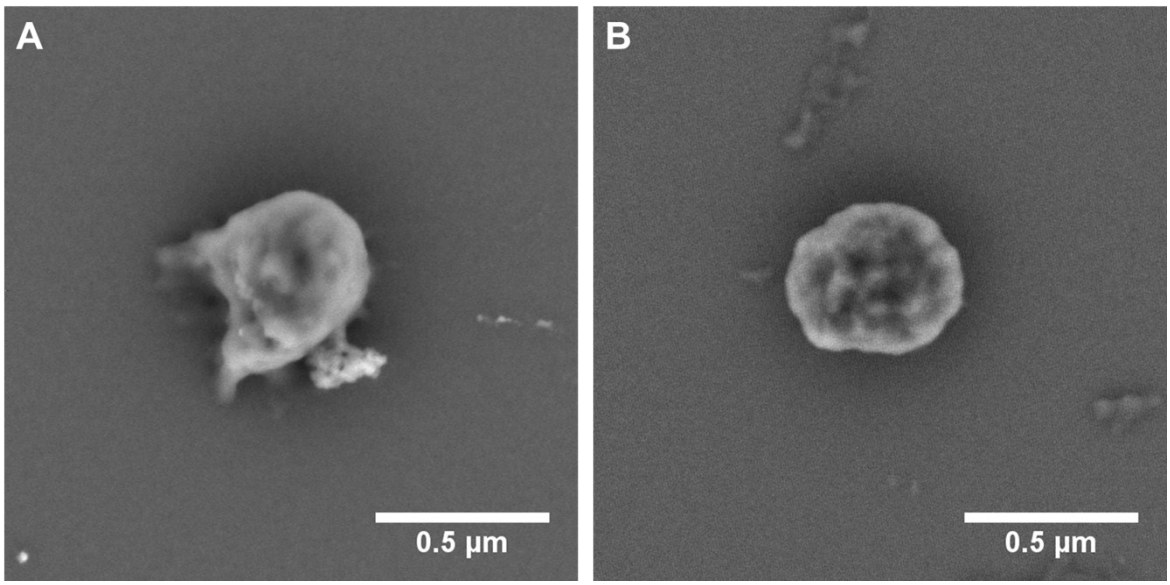

Figure S1. HRSEM images of **a)** E. coli-produced IB-like pNPs and **b)** L. lactis-produced IB-like pNPs. Images were acquired operating at 2 kV with a vCD back-scattered electron detector.

S2. Contact Angle Measurement of Deposited IB-like pNPs

Upon deposition for 2 h on HO-terminated and maleimide-terminated SAMs, *E. coli* and *L. lactis* produced pNPs showed differential increase of wettability of the surface. Contact angle measurements revealed that deposition of *L. lactis* produced pNPs increased the contact angle by 4° respect to *E. coli* produced pNPs, **(Figure S2)** thus indicating that the deposited material in the first case is denser and more hydrophobic than in the second one.

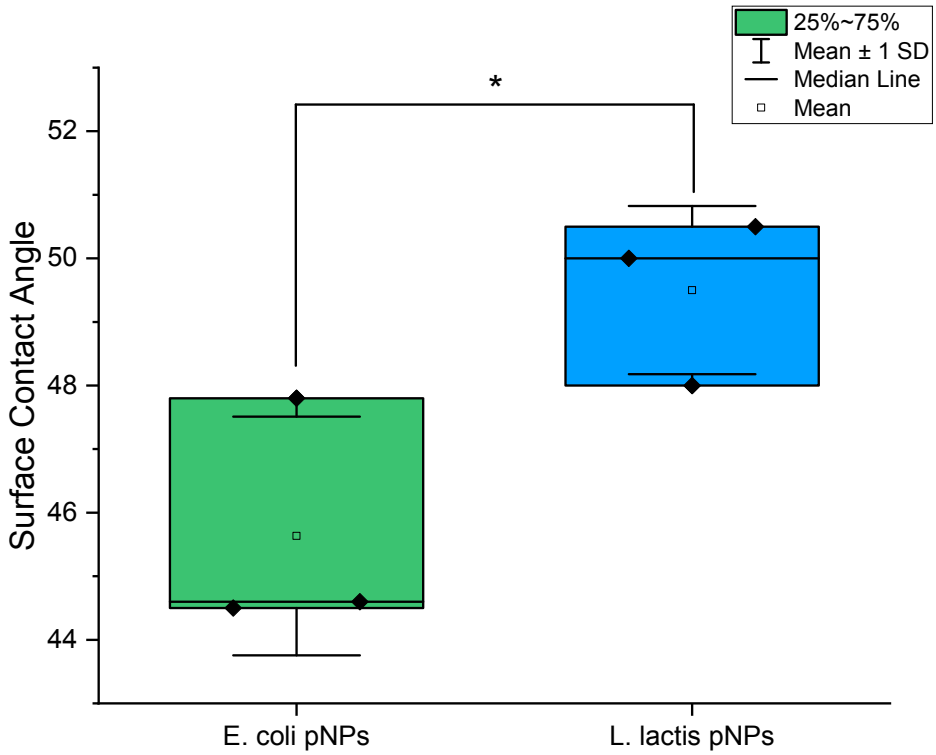

Figure S2. Surface contact angle measurements with H_2O of surfaces with deposited Coli pNPs and Lactis pNPs.

S3. Comparative Colorimetric Determination of Accessible Sulfhydryl Groups on pNPs

The quantity of accessible thiol groups of pNPs was assessed by the 4,4'-dithiopyridine (DTDP) method. After dilution of suspensions of pNPs in 1 ml of PBS and 0.2 ml of a buffer at pH 6.8, DTDP was added and the absorbance at 324 nm was measured for 10 min to monitor the reaction. The difference of absorbance after 10 min showed a higher increase of absorbance in Lactis-produced pNPs in comparison to Coli-produced pNPs, **(Figure S3)** hinting that LAB-produced pNPs feature a higher quantity of accessible

thiol groups on their surface, and thus being able to be covalently anchored to maleimide-functionalized surfaces through the thiol-maleimide reaction.

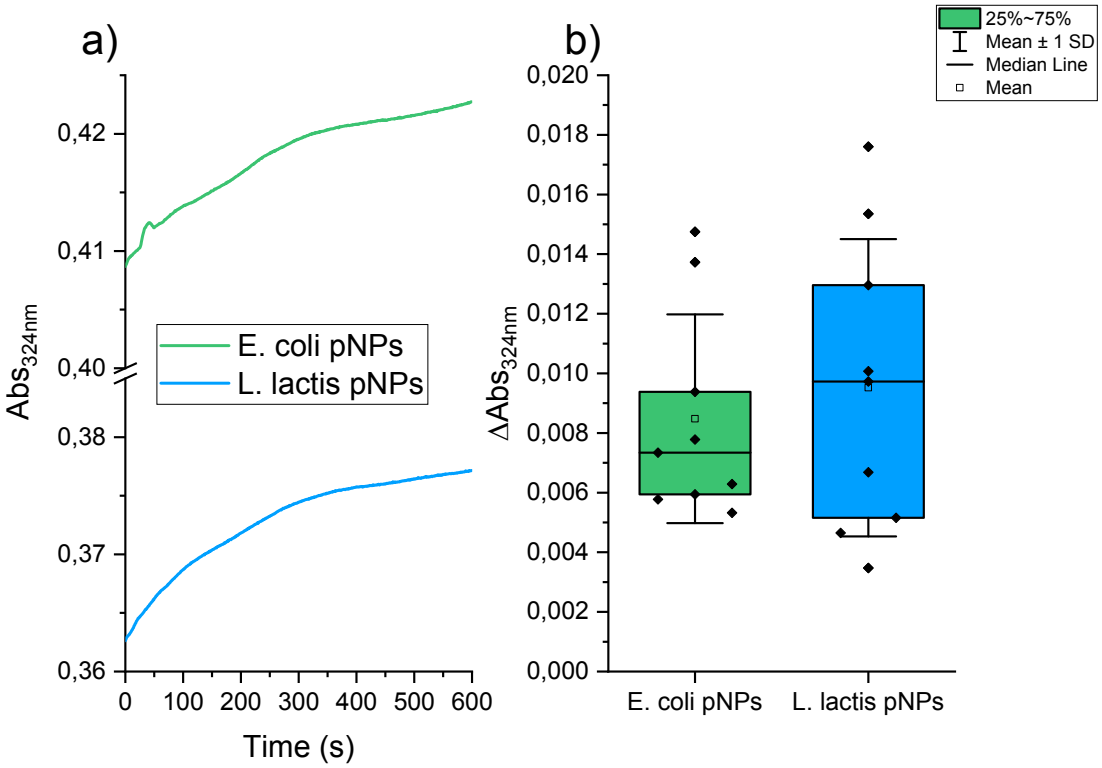

Figure S3. a) Time-course of the absorption at 324 nm during 10 min of Coli pNPs and Lactis pNPs suspensions after addition of DTDP. Average of 9 measurements. **b)** Difference of absorption at 324 nm after 10 min of both Coli- and Lactis-pNPs after addition of DTDP. N(measurements) = 9.

S4. Specific Fluorescence of GFP IB-like pNPs

The specific fluorescence of *E. coli* produced and *L. lactis* produced IB-like pNPs was measured in order to be able to be able to better understand the retention and coverage analysis. *E. coli* produced IB-like pNPs (12.30± 0.95 UE/µg) featured a higher specific fluorescence than its *L. lactis* produced counterparts (6.32 ± 0.05 UE/µg). This result is remarkable when taking into account that the fluorescence intensity in previous experiments was higher for *L. lactis*, corroborating that indeed a higher amount of material was present in our samples in comparison to *E. coli* produced pNPs.