Electronic Supplementary Material (ESI) for Nanoscale Advances.

Supporting Information

A dual-color plasmonic immunosensor for salivary cortisol measurement

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Gold nanostar characterization

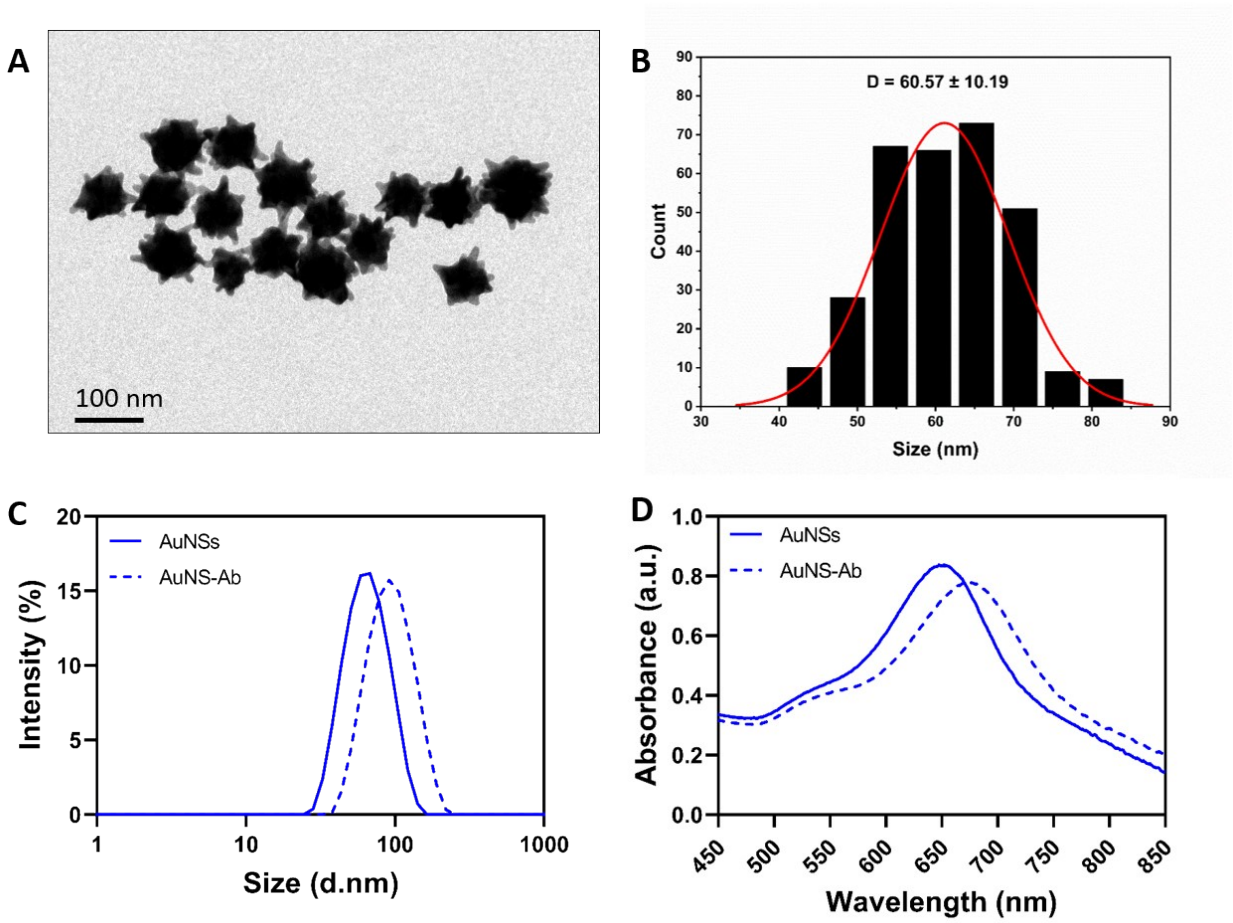

Figure S1. Characterization of the as-synthetized nanoparticles and AuNS-Ab conjugates. (A) Representative TEM image of 60 nm gold nanostars (AuNSs) and (B) relative size distribution. (C) DLS analysis of AuNSs (solid line) and AuNS-Ab conjugates (dashed line). The conjugate maintains good monodispersity with a peak shift of ca. 20 nm, consistent with a hydrodynamic radius given by the protein corona on the surface of AuNSs. (D) UV-vis absorption spectra of AuNSs (solid line) and AuNS-Ab conjugates (dashed line). The peak of the conjugate is shifted of ca. 20 nm with respect to pristine particles, in agreement with the presence of stable complexes and the formation of a protein corona around the AuNSs.

Gold nanosphere characterization

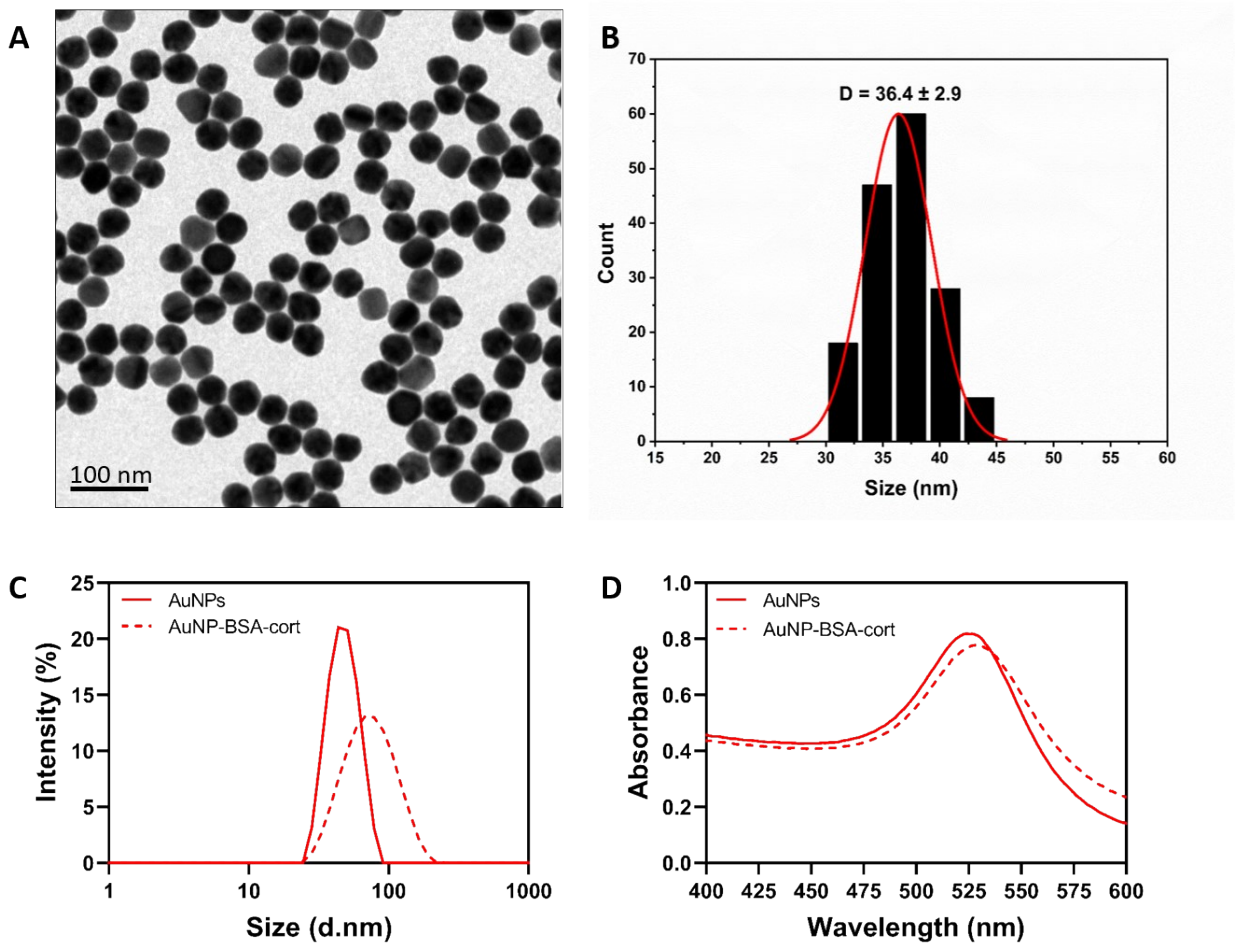

Figure S2. Characterization of the as-synthetized nanoparticles and AuNP-BSA-cortisol conjugates. (A) Representative TEM image of 35 nm gold nanospheres (AuNPs) and (B) relative size distribution. (C) DLS analysis of AuNPs (solid line) and AuNP-BSA-cortisol conjugate (dashed line). The conjugate shows a peak shift of ca. 30 nm, consistent with the formation of a protein corona on the surface of AuNPs. (D) UV-vis absorption spectra of AuNPs (solid line) and AuNP-BSA-cortisol conjugate (dashed line). Also in this case, a red-shift of the plasmon the peak was observed, in line with DLS data.

Single-color LFIA

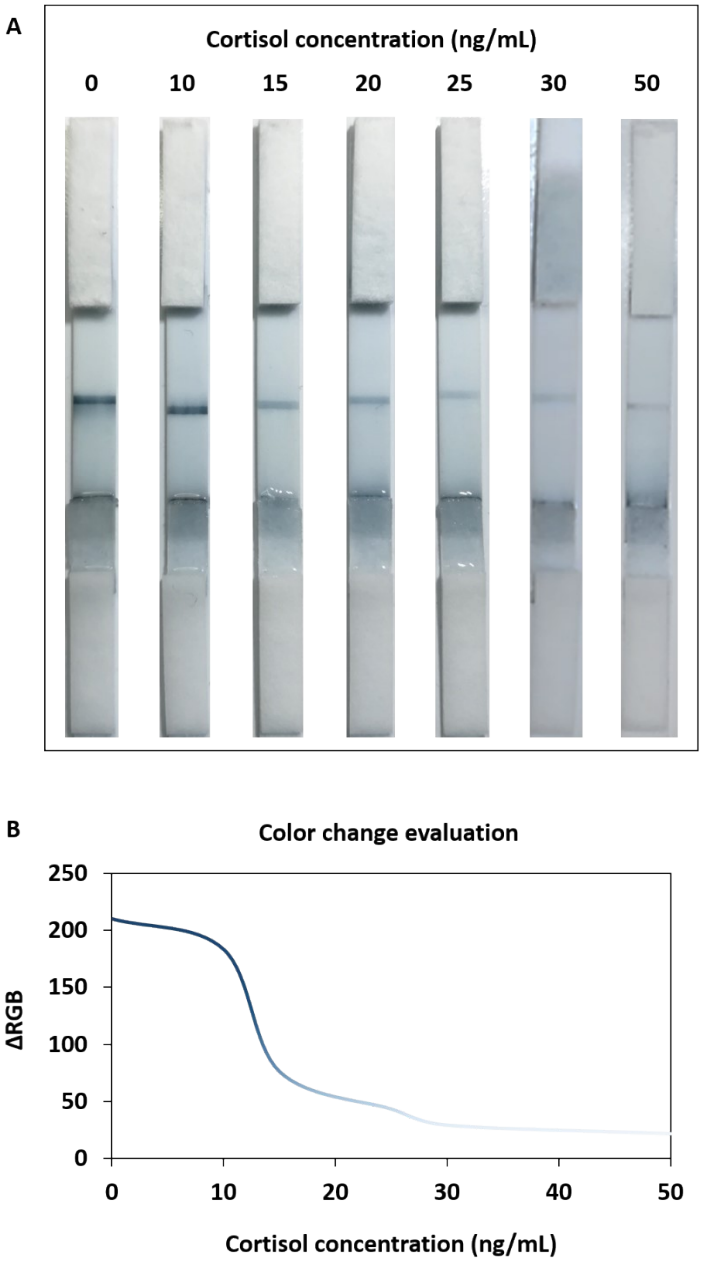

Figure S3. Cortisol level evaluation in a saliva sample by a single-color LFIA exploiting AuNSs. (A) Representative pictures obtained probing the LFIA with a cortisol concentration range from 0 to 50 ng/mL. At physiological concentrations of cortisol (up to 10 ng/mL), the test line appears blue. Increasing the level of the hormone (15-25 ng/mL), the competition for the detection bioreceptor takes place and the blue color starts to fade. In presence of pathological levels of cortisol (30-50 ng/mL), the blue color is almost disappeared. In this case, the naked-eye evaluation of the cortisol levels in the different concentration ranges is not straightforward, requiring reference standards and/or RGB analysis (B).

Prototype of the final device

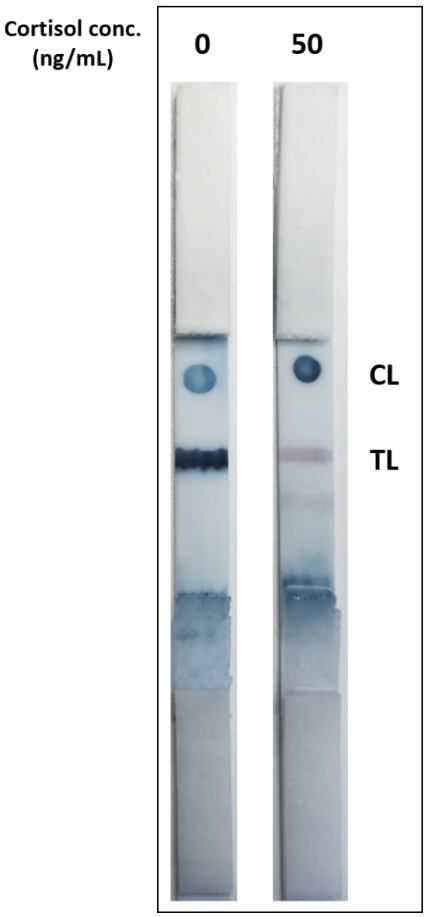

Figure S4. Representative pictures of two LFDs tested at 0 and 50 ng/mL of cortisol in a prototype device including both the test line (TL) and the control line (CL). The presence of the CL avoids possible misinterpretations of the results (e.g., false positives), ensuring a clear distinction between excess cortisol conditions and no sample/not properly working device. The experiment was performed employing a real saliva sample.