Supplementary Information

Specific and sensitive detection of Influenza A virus using a biotin-coated nanoparticle enhanced immunomagnetic assay

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Biotin-nanoparticle release from the CPG support

After DNA synthesis, the ratio of DNA per NP was estimated by UV-visible measurement (Varian Cary 100 Bio UV-visible spectrophotometer (Agilent Technologies)) using a quartz cuvette of 1cm path length.

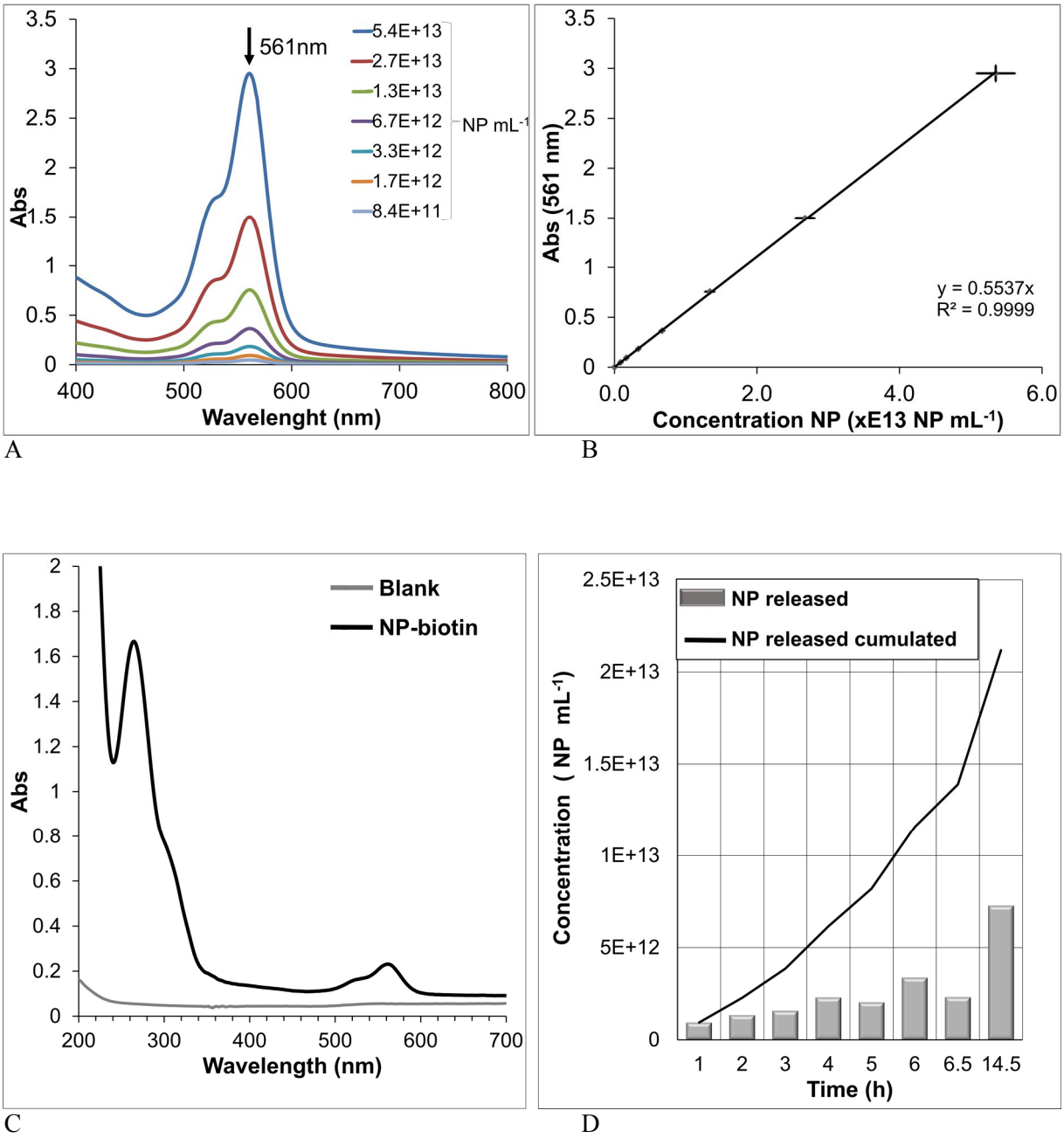


Fig. S1 A) UV spectra obtained with Varian Cary 100 Bio UV-visible spectrophotometer B) calibration curve of biotin-NP at 561nm C) UV- spectra obtained after NP release D) NP release kinetics, each sample or cumulated curves

Size characterization

Dynamic Light Scattering (DLS) measurements were carried out with a Zetasizer nanoZS (Malvern) in water. After functionalization, the hydrodynamic diameter was superior due to the spacer arm DNA synthetized on the surface.

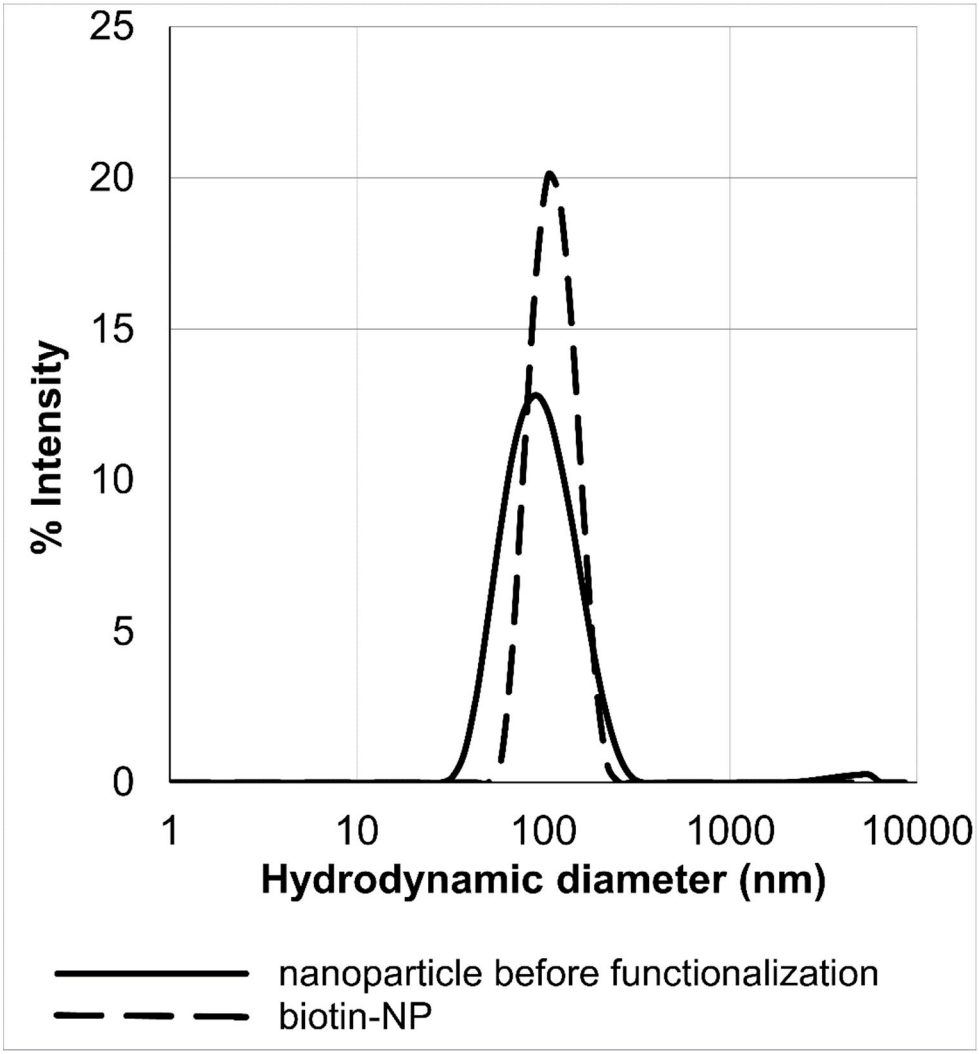


Fig. S2 DLS of biotin-NPs before and after functionalization

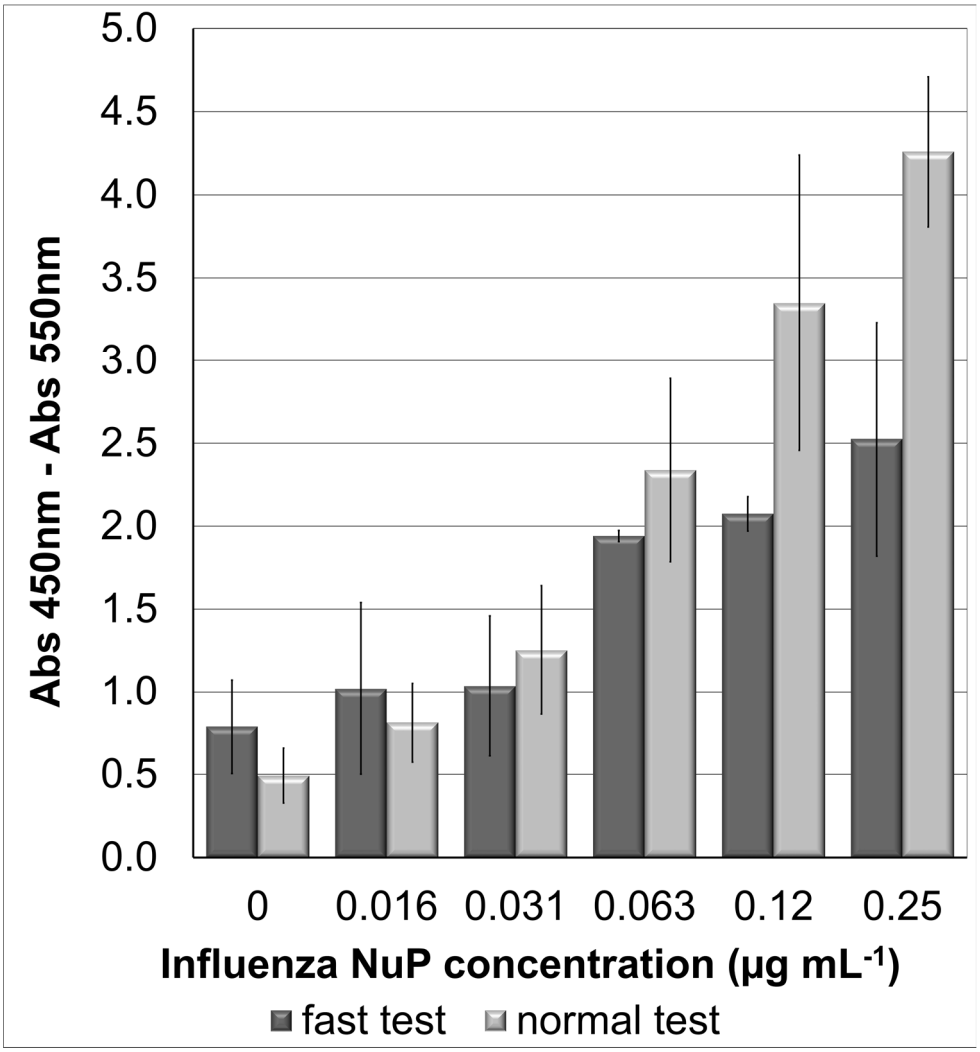


Fig. S3 Optimization of MELISA assay for NuP detection. Two different incubation times (30 and 60 min) were tested with different NuP concentrations

Biotin labeled nanoparticle synthesis : Nano-On-Micro (NOM) support characterization

Scanning electron microscopy (SEM) was performed to characterize Controlled Pore Glass-NP assemblies, at an acceleration voltage of 10kV using a VEGA TESCAN SEM. Before analysis, samples were metallized with 5 nm thickness of gold.

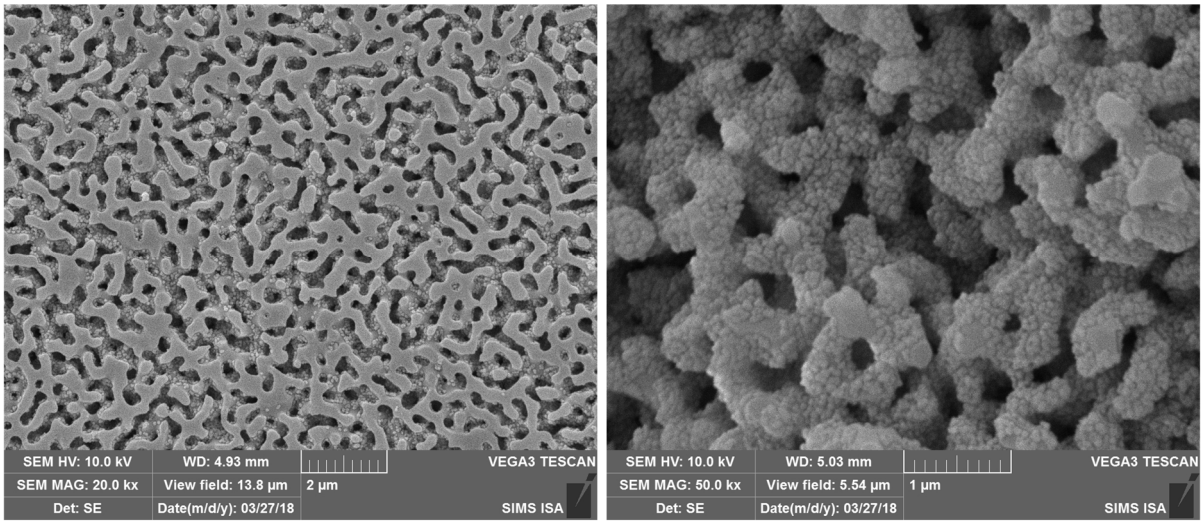


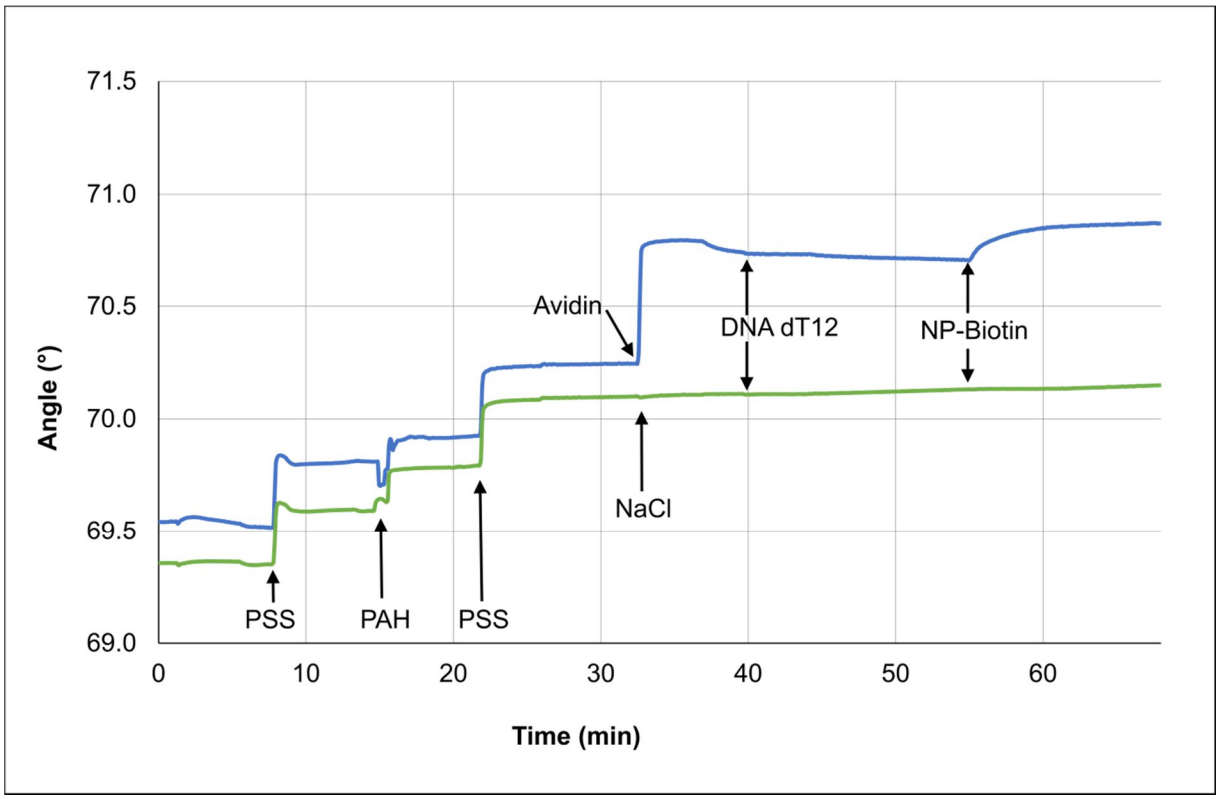
Fig. S4 SEM images of the CPG functionalized with NPs (nano-on-micro (NOM) support)

SPR characterization of biotin-NPs

Surface Plasmon Resonance was recorded with a SPR Navi 200 (Bionavis, Finland). The coated gold chip was positioned in the SPR cell and primed with a circulating NaCl 0.15 M solution at 50 µL min^{-1}. A multilayer of polylectrolyte was assembled on the surface by successive additions of opposite charge polyelectolytes (PEI (poly(ethyleneimine)); PSS (poly(styrenesulfonic acid); PAH (poly(allylamine)) 0.1 mg mL^{-1} in NaCl 0.15 M. Then in one channel, avidin (1 µg mL^{-1} in NaCl 0.15 M) was added, and in the other channel, NaCl 0.15 M was injected. After a passivation step with DNA dT12 (1 nM in NaCl 0.15 M), biotin-NP suspension was added. The biotin-NP were specifically captured on the avidin-coated chip surface as proven by the increase of the SPR angle at 55 min (Fig. S5A).

The chip was then removed from the SPR and directly observed with a fluorescence optical microscope. We observe the immobilization of the fluorescent biotin-NP (with rhodamine in the NP core) on the gold surface used for SPR (Fig. S5B).

A



B

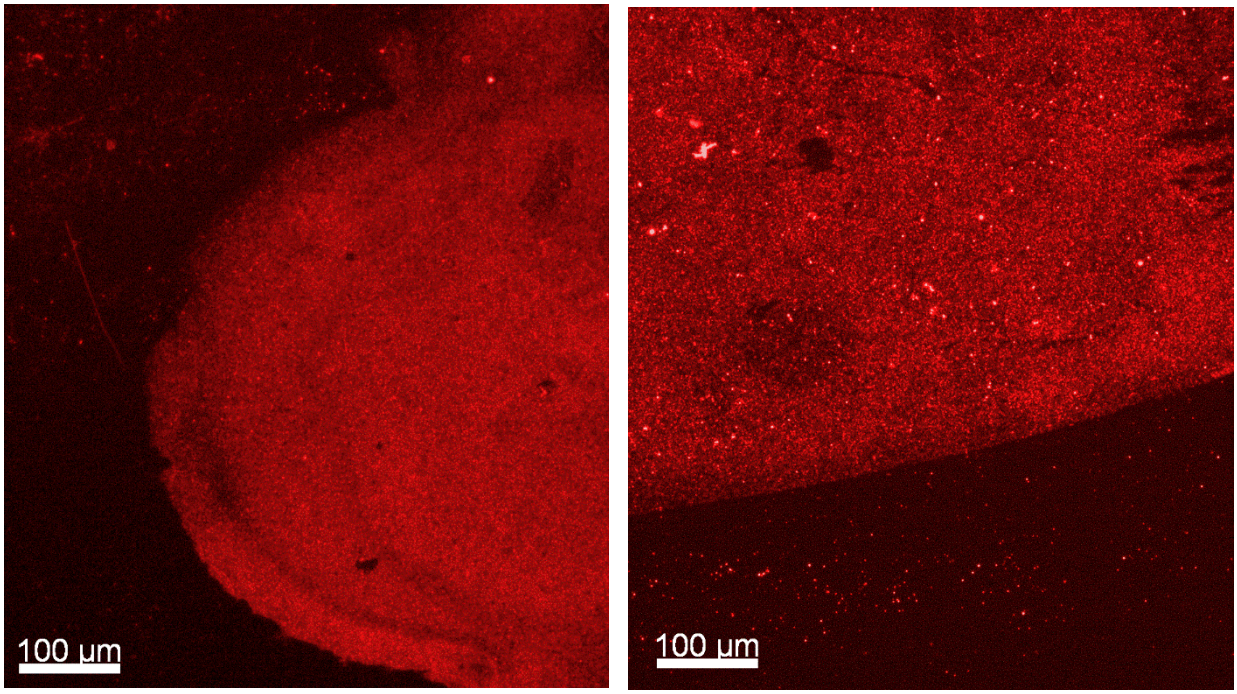


Fig. S5 A) SPR characterization of the biotin-NP specific capture on the avidin-functionalized surface B) Photos by fluorescence microscopy of the gold surface after SPR