SUPPORTING INFORMATION

Gold nanoparticle-assisted polymerase chain reaction: effects of surface ligands, nanoparticle shape and material

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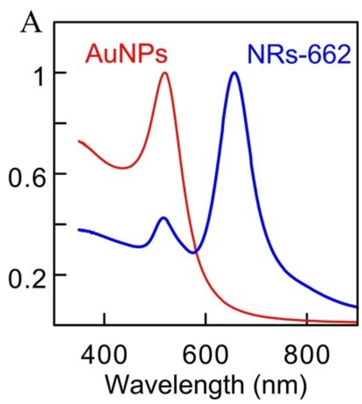

Figure S1. Extinction spectra of as-prepared AuNPs (red curve) and gold nanorods AuNR-662 (blue curve).

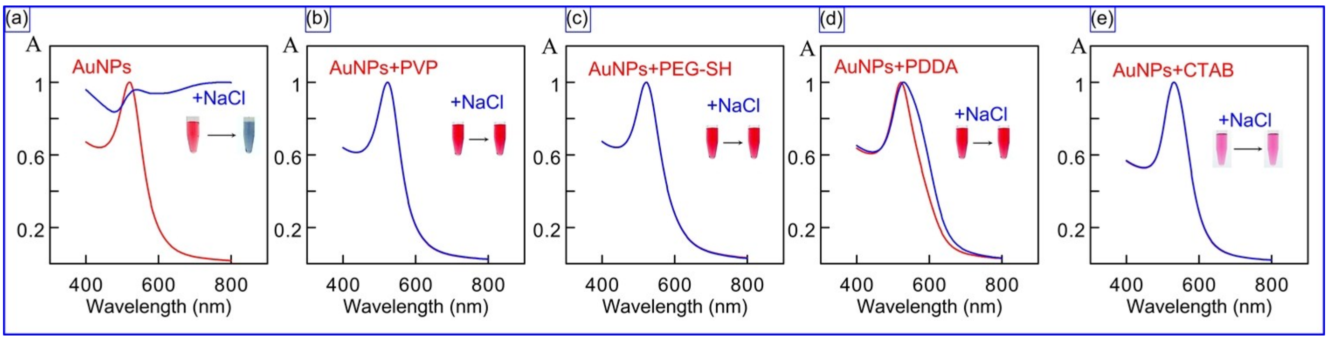

Figure S2. Extinction spectra and photos of bare (a) and functionalized 16-nm NPs with PVP (b), PEG-SH (c), PDDA (d) and 25-nm CTAB (e) before and after addition of NaCl salt. Note that blue spectra in panels (b), (c) and (e) coincide with the corresponding red spectra of as-prepared particles.

S1. Chemical structures and IUPAC names of ligands used for AuNP functionalization

Acronim	IUPAC name (other name)	Chemical structure
PVP	Polyvinylpyrrolidone 1-ethenylpyrrolidin-2-one	$(C_6H_9NO)_n$
PDDA	Polydiallyldimethylammonium chloride	$(C_8H_{16}NCl)_n$
PEG-SH	mPEG-SH (Monofunctional PEG Thiol or Sulfhydryl)	SH $CH_3O\text{-}(CH_2CH_2O)_n$
CTAB	hexadecyl-trimethyl-ammonium bromide	$C_{19}H_{42}BrN$

S2. AuNPs functionalization with surface ligands

Briefly, to obtain mPEG-SH-capped AuNPs[1], the nanoparticle dispersion was adjusted to pH 9 by adding 0.2 M potassium carbonate, and PEG-SH water solution was added to the final concentration 29 mkM thus making 10:1 excess of ligand:AuNPs. After two-hours incubation at room temperature, the unbound PEG-SH molecules were removed by centrifugation at 16 000 g for 60 min and the pellet was re-suspended in initial volume of Milli-Q water. To obtain PVP-capped AuNPs, the nanoparticle solution was mixed with PVP water solution to the final concentration 17 mM thus making 3:1 excess of ligand:AuNPs. After 30 min incubation at room temperature, the unbound PVP molecules were removed by centrifugation at 16 000 g for 60 min and the pellet was re-suspended in initial volume of Milli-Q water. To obtain PDDA-capped AuNPs, the PVP-capped AuNP solution was mixed with PDDA to the final concentration 1% thus making 10:1 excess of ligand:AuNPs. After 30 min incubation at room temperature, the unbound PDDA molecules were removed by centrifugation at 16 000 g for 60 min and the pellet was re-suspended in initial volume of Milli-Q water. To obtain CTAB-capped AuNPs, the as-prepared AuNPs were mixed with 3 mM CTAB at a 2:1 volume thus making 10:1 excess of ligand:AuNPs[2]. Twenty-four hours later, the average hydrodynamic diameters were measured by DLS, the change of the particles charge was confirmed by zeta-potential measurements. The ligand coating was indirectly examined with using DLS data and a salt aggregation test[3] by adding NaCl solution to the final concentration 10 mM to the as-prepared and ligand-capped AuNPs.

S3. Evaluation of nano-PCR Specificity and Efficiency

Here, we adopted an approach suggested by L. Yuan and Y. He (Analyst, **138**, 539, 2013).

First, for each lane L, we used the ImageJ densitometry data to calculate the ratio of band intensities for *target* and *smears*

$$RS^L = I^L_{target} / \left[I^L_{target} + \langle I^L_{smears} \rangle \right], \qquad \text{(S1)}$$

and the ratio of band intensities for *target* and *marker*500bp

$$RE^L = I^L_{target} / I^L_{marker\,500\text{bp}}, \tag{S2}$$

where $\langle I^L_{smears} \rangle$ is the average intensity over all smear intensities as given by ImageJ densitometry histograms. In a sense, parameters RS^L and RE^L can be considered as a measure of band specificity and yield efficiency. Indeed, if no nonspecific band are detected then $RS^L = 1$, whereas for a weak target intensity compared to that of smears, the ratio $RS^L \to 0$. The definition (S2) means that the target intensity is evaluated in terms of the internal standard $I^L_{marker\,500\text{bp}}$. In particular, for an efficient PCR amplification, the target and standard intensities should be comparable thus giving RE^L values about of 1.

The above calculations RS^L and RE^L were performed for lanes without particles and for lanes with different particle concentrations. Then, each value RS^L and RE^L for lanes with nanoparticles was normalized to the corresponding parameters obtained without nanoparticles to yield the band specificity and efficiency

$$Specificity = S^L = RS^L_{with\ NP} / RS_{without\ NP}, \tag{S3}$$

$$Efficiency = E^L = RE^L_{with\ NP} / RE_{without\ NP}. \tag{S4}$$

The normalized quantities S^L and E^L characterize the PCR improvement due to nanoparticle additives. Indeed, if no improvement occurs, then the normalized specificity and efficiency will be equal or less than 1. In the case of improved nano-PCR, the normalized quantities $S^L > 1$ and $E^L > 1$ are expected. These normalized parameters are indicated on the bottom of Figs. 2,3,5 and 6 of the main text and Figs. S2-S5 below. The maximal values of $S_{\max} = \left\{S^L\right\}_{\max}$ and $E_{\max} = \left\{E^L\right\}_{\max}$ among all calculated parameters are summarized in Table 1 together with optimal concentrations for which the maximal specificity and efficiency were obtained.

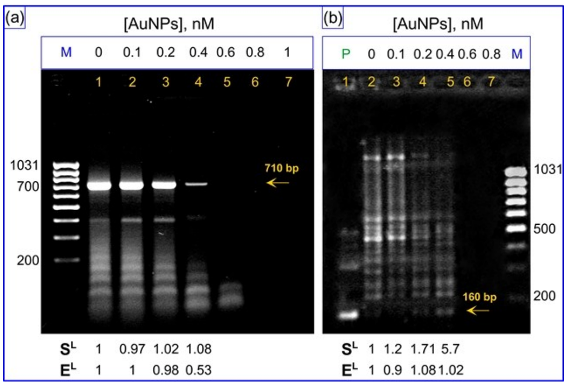

Figure S3. (a) The concentration-dependent effect of citrate-stabilized AuNPs on PCR amplification of 710 bp target from PCR model 1. Lane M stands for DNA markers. The smeared lane 1 was obtained without AuNPs, lanes 2-7 correspond to addition of 0.1-1 nM AuNPs. (b) The enhancing effect of citrate-stabilized AuNPs on PCR amplification of a 160 bp target from PCR model 2. The symbol M stands for DNA markers, the symbol P designates a positive control with a purified DNA of genovar E as a template. The smeared lane 2 was obtained without AuNPs, lanes 3-7 correspond to addition of 0.1-0.8 nM AuNPs. The normalized quantities of PCR specificity (S^L) and efficiency (E^L) for each lane are indicated on the bottom panels.

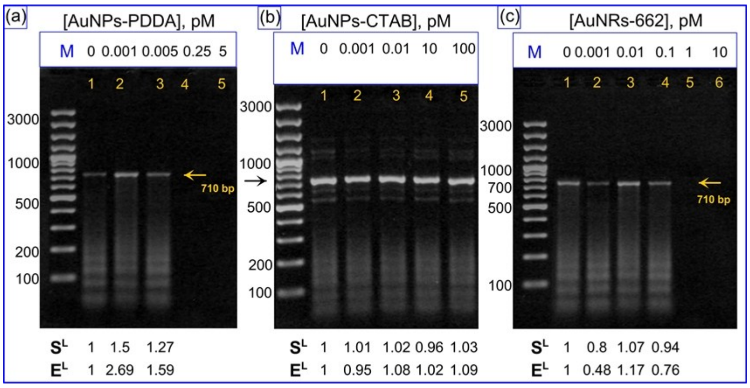

Figure S4. The effect of AuNPs-PDDA (a), AuNPs-CTAB (b) and AuNRs-662 (c) on PCR amplification of 710 bp region of *Nif*D gene from *A. brasilense* Sp7 (model 1). The symbol M stands for DNA markers. Note some amplification of the 710 bp target at a trace 0.001 pM concentration of PDDA-coated AuNPs (the panel (a), lane 2). For CTAB-capped particles (b) and CTAB-coated nanorods (c), no PCR enhancement is observed. The normalized quantities of PCR specificity (S^L) and efficiency (E^L) for each lane are indicated on the bottom panels.

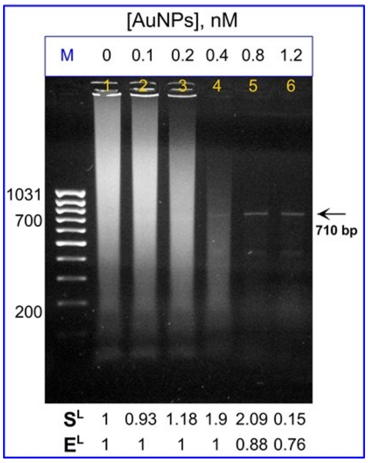

Figure S5. The effect of citrate-stabilized AuNPs on two-round error-prone PCR amplification of 710 bp target from PCR model 1. Lane M stands for DNA markers. The smeared lane 1 was obtained without AuNPs, lanes 2-6 correspond to addition of 0.1-1.2 nM AuNPs. Note that the addition of 0.8 nM AuNPs completely inhibits non-target bands. The normalized quantities of PCR specificity (S^L) and efficiency (E^L) for each lane are indicated on the bottom panels.

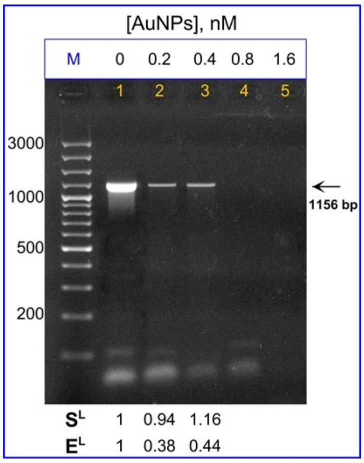

Figure S6. The enhancing effect of citrate-stabilized AuNPs on PCR amplification of a long 1156 bp target from PCR model 3. The symbol M stands for DNA markers. The smeared lane 1 was obtained without AuNPs, lanes 2-5 correspond to addition of 0.2-1.6 nM AuNPs. The normalized quantities of PCR specificity (S^L) and efficiency (E^L) for each lane are indicated on the bottom panels.

References:

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2 T. E. Pylaev, V. A. Khanadeev, B. N. Khlebtsov, L. A. Dykman, V. A. Bogatyrev and N. G. Khlebtsov, *Nanotechnology*, 2011, **22**, 285501.

3 J. De Mey and M. Moeremans. Advanced techniques in biological electron microscopy, Ed. by J.K. Koehler, Springer–Verlag, Berlin, Germany, 1986, **3**, 229–271.