Supporting Information

Plasmonic photothermal activation of an organosilica shielded cold-adapted lipase co-immobilised with gold nanoparticles

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Keywords: gold nanoparticles • silica nanoparticles • enzyme • biocatalysis • surface plasmon resonance

Supporting Figures



Fig. S1. AuNP characterisation. (a) TEM micrographs of the AuNPs produced [scale bars represent 200 nm (main micrograph) and 50 nm (insert)]; (b) AuNP diameter distribution measured on TEM micrographs (measured on 633 AuNPs), polydispersity index (*PDI*) calculated as σ/\bar{d} , where (σ) is the standard deviation and \bar{d} the average diameter of the AuNPs; (c) SEM and (d) AFM micrographs (scale bar in (c) represents 200 nm) of AuNPs-citrate. (e) Absorbance standard curve measured at λ_{520} . (f) absorption spectra of AuNPs-citrate solution before (orange line) and after freeze-drying, in presence of sucrose 5% w/v.



Fig. S2. SP-Au characterisation. Absorption spectra of (a) initial AuNPs-citrate (0.031 mg mL⁻¹) (black line) and of the supernatant after AuNPs immobilisation on SPs (grey line); (b) free AuNPs-citrate (black line), supernatant after glutaraldehyde crosslinking (grey line), SP-Au after glutaraldehyde crosslinking (red line) and SP-Au-CalB (green line).



Figure S3. SP-CalB characterisation. SEM micrographs of (a) bare SPs and (b) SP-CalB_{os}; (c) diameter distribution of SPs (red bars) and SP-CalB_{os} (grey bars). The average diameter of the shielded particles was calculated to be 320 ± 12 nm, corresponding to a layer thickness of 18 ± 4 nm (bare SPs: 284 ± 8 nm). Scale bars represent 200 nm.



Figure S4. Stability of *p***-nitrophenol butyrate (pNPB) and AuNPs catalysis.** Absorbance of *p*NPB measured at 410 nm during 30 min at 10 °C in dark condition (black squares) and upon laser irradiation (grey triangles) at 615 mW laser power (a) omitting AuNPs and (b) in presence of AuNPs.



Figure S5. Immobilisation of AuNPs on the surface of amino-modified SPs. Absorption spectra of the supernatants (after 10-fold dilution) of gold immobilisation on amino-modified SPs with 1.5 (red line), 3.5 (green line), and 15 (grey line) hours of reaction with APTES. Based on the initial concentration of AuNPs (355 μ g/mL) and the acquired calibration curve, the amount of AuNPs immobilised is calculated to be 26, 55, and 98 μ g of AuNPs per mg of SPs, corresponding to immobilisation yields of 24, 49, and 88% for 1.5, 3.5, and 15 hours of APTES reaction durations, respectively.

Supporting Tables

| APTES [mM] | ζ-potential [mV] |
|---------------|---------------------|
| 0 | -55.0 |
| 2.6 | - 46.0 |
| 7.8 | -19.2 |
| 26 | -9.99 |

Table S1. ζ -potential values of SPs functionalised with different concentrations of APTES.

| OD Supernatant | OD Blank | [CalB] initial (µg/mL) | [CalB] supernatant (µg/mL) | [CalB] immobilised (µg/mL) | Average [CalB] immobilised (µg/mL) | Average [CalB] immobilised (μg/mg SPs) |
|-------------------|-------------|------------------------------|----------------------------------|----------------------------------|---|---|
| 0.095 | 0.079 | 96 | 32 | 64 | | |
| 0.095 | 0.079 | 108 | 34 | 74 | 63 | 19.8 |
| 0.097 | 0.080 | 86 | 34 | 52 | | |

Table S2. Protein quantification (BCA assay) carried out on reaction supernatants after immobilisation

 reaction of CalB.

| Sample | d (nm) $\pm \sigma$ | Polydispersity index | N. particles measured |
|--------------|---------------------|----------------------|-----------------------|
| | | (PDI) | |
| AuNPs | 18.1 ± 1.0 | 0.060 | 633 |
| Bare SPs | 283.7 ± 7.9 | 0.027 | 106 |
| SP-CalBos | 319.2 ± 12.0 | 0.037 | 106 |
| SP-Au-CalBos | 324.5 ± 13.6 | 0.042 | 106 |

Table S3. Measured diameters of AuNPs on TEM micrographs, of bare SPs, SP-CalBos and SP-Au- $CalB_{OS}$ on SEM micrographs.