Electronic Supplementary Material (ESI) for Analytical Methods.

ESI

Gold Nanoparticle Aggregation Enables Colorimetric Sensing Assays for Enzymatic Decarboxylation

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Contents

1. Materials and Methods

Chemicals were purchased from Sigma-Aldrich (Germany) and used without further purification. L-histidine decarboxylase from Lactobacillus 30a (0.26 units/mg solid) and L-lysine decarboxylase from Bacterium cadaveris (1.6 units/mg solid) were used. UV-Vis measurements were performed on a Varian Cary 4000 UV-Visible spectrophotometer. DLS measurements were performed on a Malvern Zetasizer Nano ZS instrument.

2. Synthesis of Citrate-Capped Gold Nanoparticles

A 250-mL aqueous solution of 1 mM $HAuCl_4$ in a round-bottom flask was boiled under stirring, and then, trisodium citrate (38.8 mM, 25 mL) was added to the solution. The solution was refluxed for 15 minutes. The color changed from pale yellow to deep red. The solution was cooled to room temperature while stirring continuously. The NPs were characterized by using UV-Vis and DLS. The prepared AuNPs showed a maximum absorption at 520 nm and a size of ~15 nm. The concentration of the citrate-NPs was determined according to the reported extinction coefficient (ε = 8.78 ± 0.06 x 10^8 M^{-1} cm^{-1}).[1]

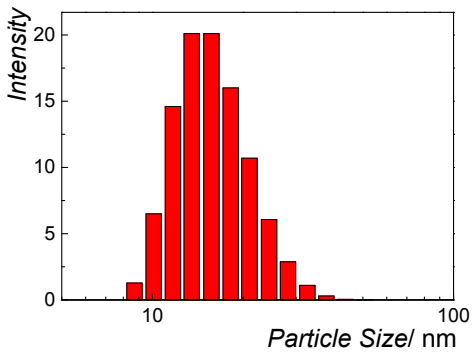

Figure S1. DLS data for the citrate-AuNPs.

3. Monitoring the Enzymatic Assay

The reactions were carried out in sodium phosphate buffer (10 mM, pH 6.5) at 37 °C. The enzyme (100 - 500 μg mL^{-1}) was added to the substrate (0.5 - 1.5 mM), in the presence of 0.1 mM pyridoxal 5'-phosphate (PLP) as a cofactor. For lysine decarboxylase, a 40 μL volume was taken from the reaction mixture at given reaction time (final concentration of 20 μM) and added into 2-ml AuNPs and incubated for 3-5 minutes before UV-Vis absorption measurements were recorded. For histidine decarboxylase, a 4 μL volume was taken from the reaction mixture at given reaction time (final concentration of 2 μM) and added into 2-ml AuNPs and incubated for 3-5 minutes before UV-Vis absorption measurements were recorded. For the inhibition experiment, the enzyme was incubated with 50 μM of DL-alpha-difluoromethylornithine, and then, the substrate was added to the reaction mixture and followed in the same manner as for the uninhibited reaction.

4. Substrate and Product Induce AuNPs Aggregation

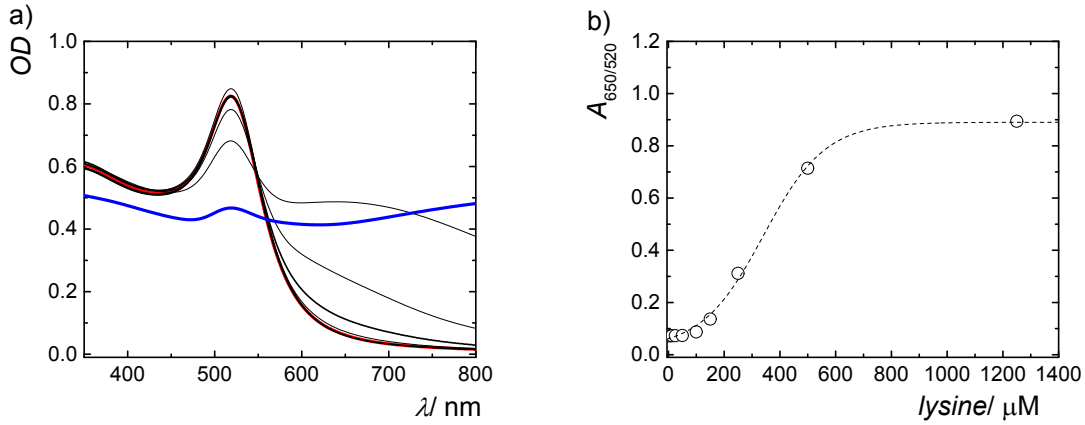

Figure S2. a) Absorption spectral changes of citrate-AuNPs with varying lysine concentrations in 10 mM sodium phosphate buffer (pH 6.5). b) Dependence of the ratio of the absorbance values at 520 and 650 nm on lysine concentration.

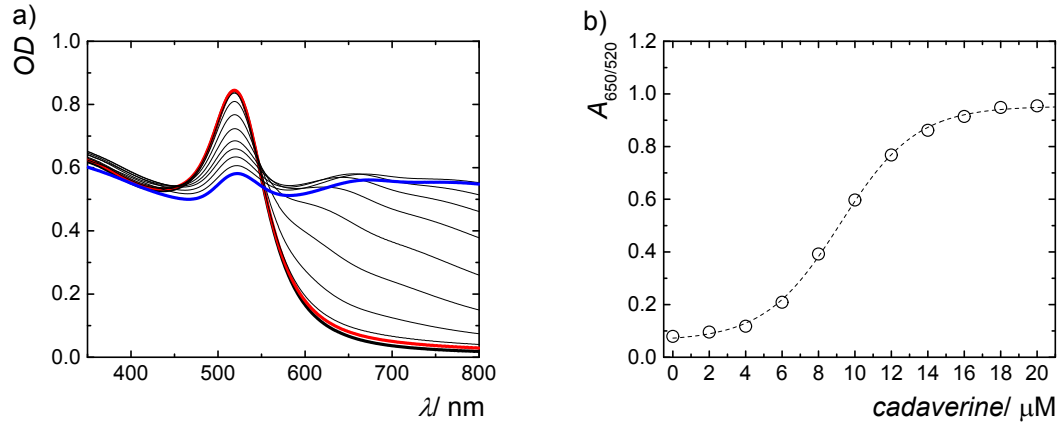

Figure S3. a) Absorption spectral changes of citrate-AuNPs with varying cadaverine concentrations in 10 mM sodium phosphate buffer (pH 6.5). b) Dependence of the ratio of the absorbance values at 520 and 650 nm on cadaverine concentration. Limit of detection (LOD) is determined as 2.5 μM and the linear range spans from 6 to 12 μM.

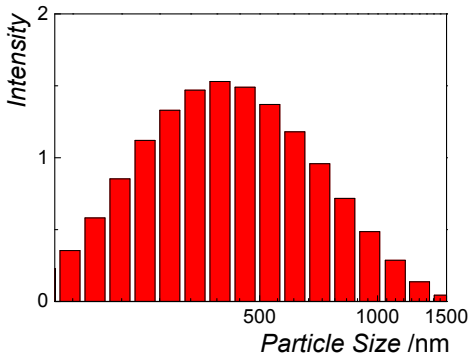

Figure S4. DLS data for the citrate-AuNPs aggregates with cadaverine.

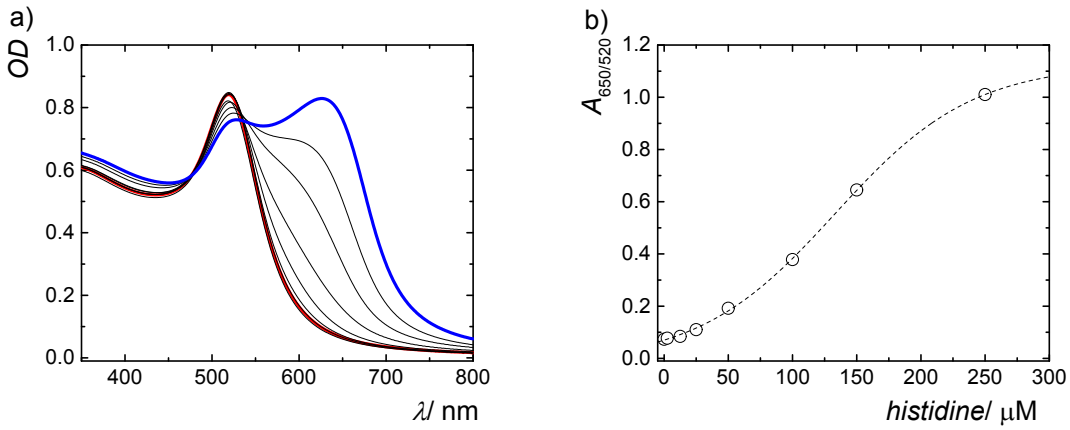

Figure S5. a) Absorption spectral changes of citrate-AuNPs with varying histidine concentrations in 10 mM sodium phosphate buffer (pH 6.5). b) Dependence of the ratio of the absorbance values at 520 and 650 nm on histidine concentration.

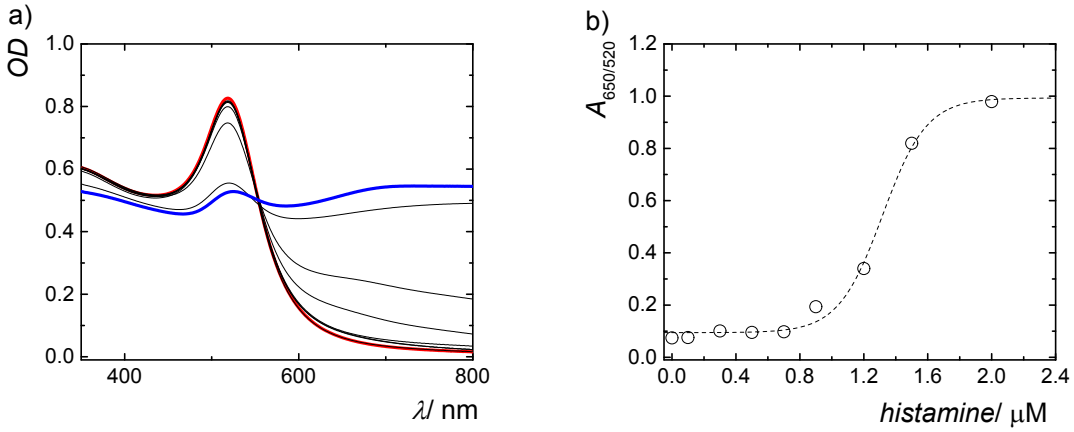

Figure S6. a) Absorption spectral changes of citrate-AuNPs with varying histamine concentrations in 10 mM sodium phosphate buffer (pH 6.5). b) Dependence of the ratio of the absorbance values at 520 and 650 nm on histamine concentration. Limit of detection (LOD) is determined as 0.9 μM and the linear range spans from 1.2 to 1.5 μM.

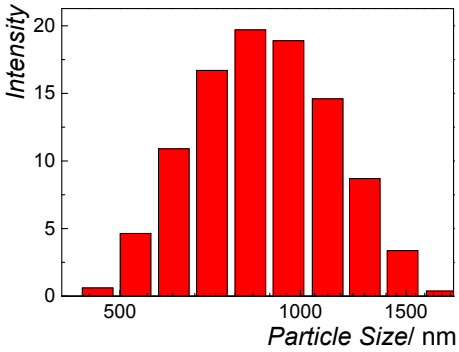

Figure S7. DLS data for the citrate-AuNPs aggregates with histamine.

5. Lysine Decarboxylase (LDC) Activity with Various Substrate Concentrations

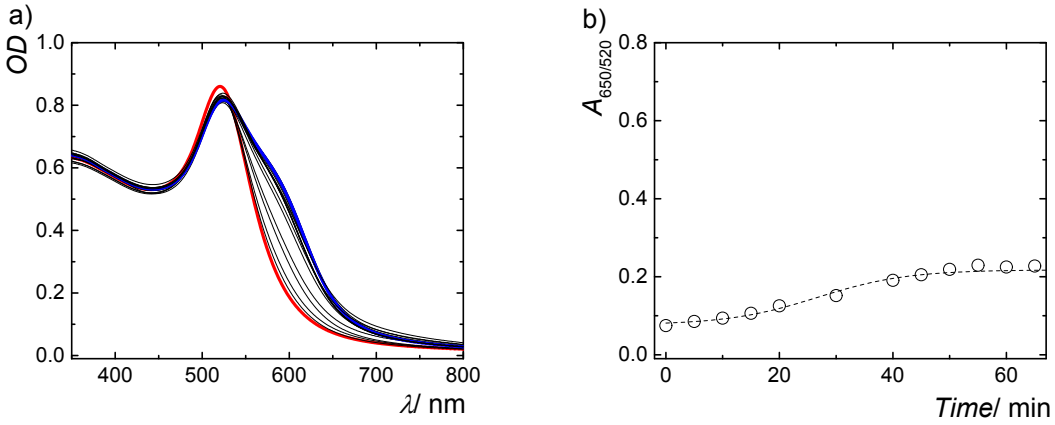

Figure S8. a) Colorimetric enzyme assay for lysine decarboxylase (LDC) using citrate-AuNPs: 10 mM sodium phosphate buffer (pH 6.5), 0.5 mM lysine, 200 μg ml^{-1} LDC, and 0.1 mM PLP. b) Dependence of the ratio of the absorbance values at 520 and 650 nm on reaction time.

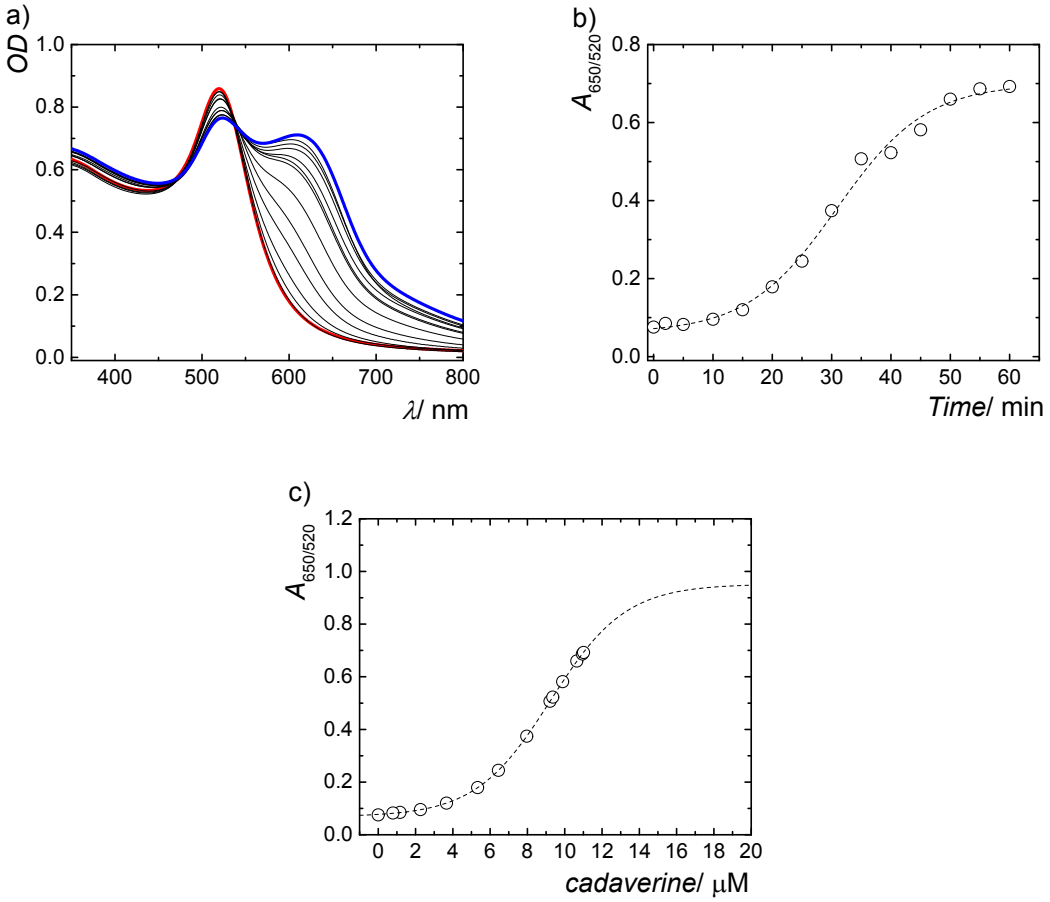

Figure S9. a) Colorimetric enzyme assay for lysine decarboxylase (LDC) using citrate-AuNPs: 10 mM sodium phosphate buffer (pH 6.5), 1.0 mM lysine, 200 μg mL^{-1} LDC, and 0.1 mM PLP. b) Dependence of the ratio of the absorbance values at 520

and 650 nm on reaction time. c) Dependence of the ratio of the absorbance values at 520 and 650 nm on cadaverine concentration from the LDC reaction.

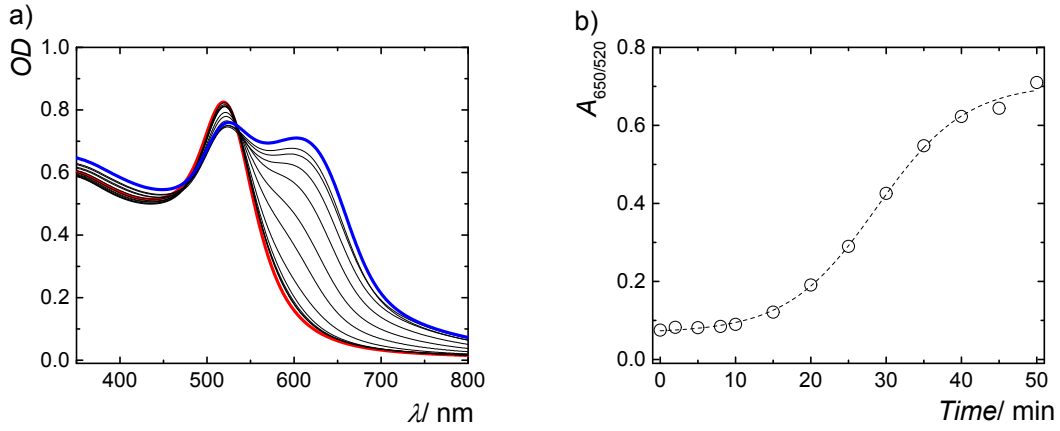

Figure S10. a) Colorimetric enzyme assay for lysine decarboxylase (LDC) using citrate-AuNPs: 10 mM sodium phosphate buffer (pH 6.5), 1.5 mM lysine, 200 μg ml^{-1} LDC, and 0.1 mM PLP. b) Dependence of the ratio of the absorbance values at 520 and 650 nm on reaction time.

6. Histidine Decarboxylase (HDC) Activity with Various Enzyme Concentrations

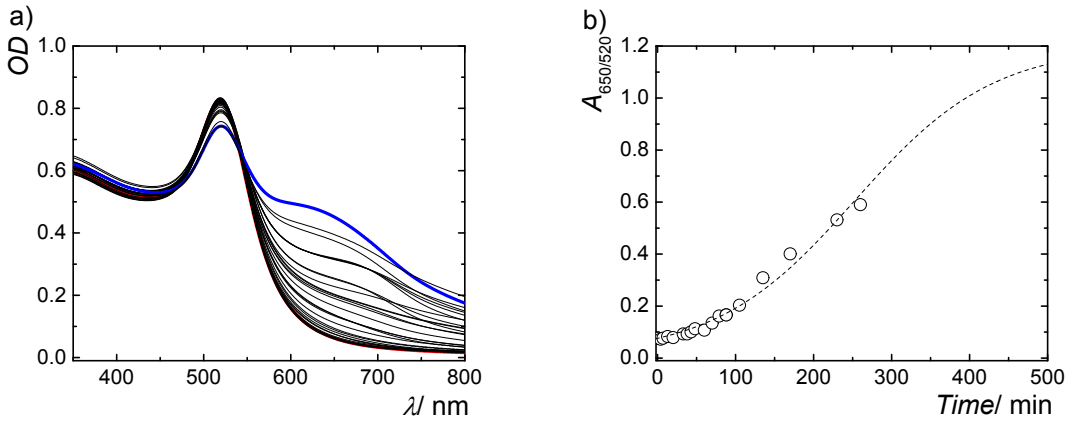

Figure S11. a) Colorimetric enzyme assay for histidine decarboxylase (HDC) using citrate-AuNPs: 10 mM sodium phosphate buffer (pH 6.5), 1 mM histidine, 100 μg mL^{-1} HDC, and 0.1 mM PLP. b) Dependence of the ratio of the absorbance values at 520 and 650 nm on reaction time.

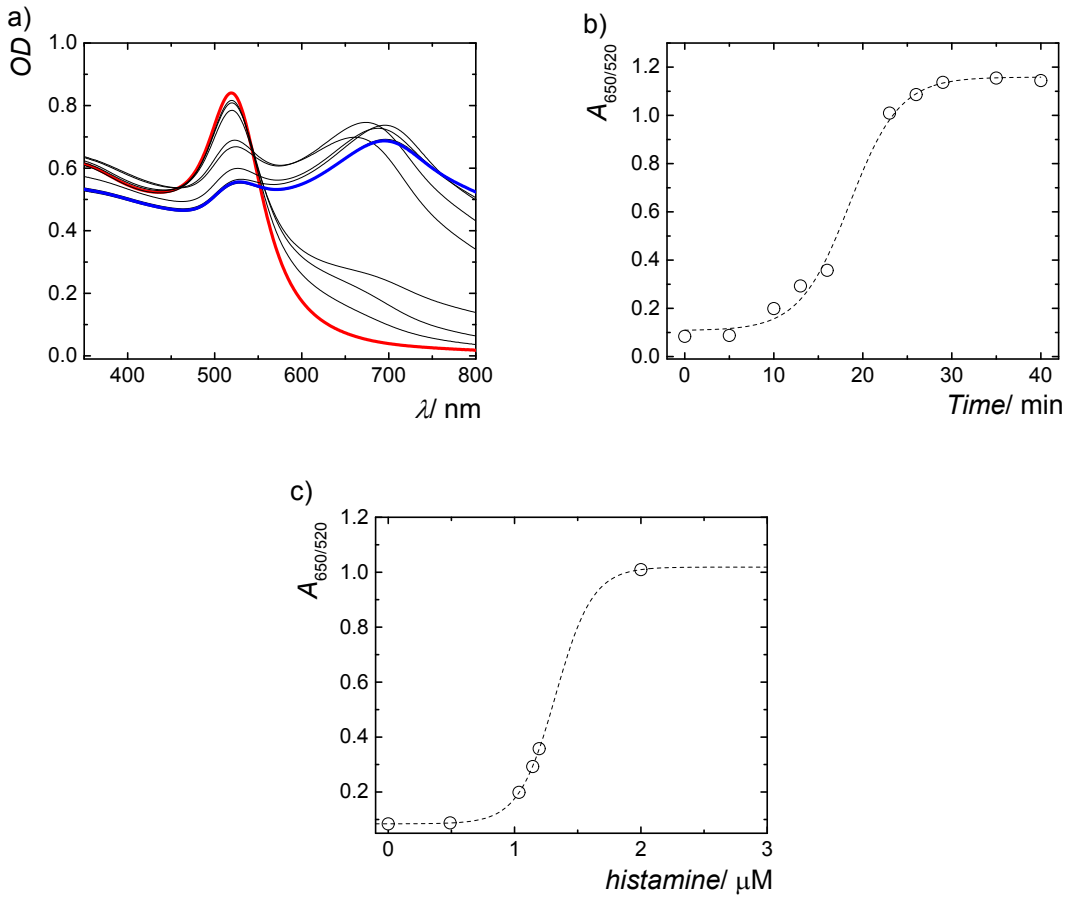

Figure S12. a) Colorimetric enzyme assay for histidine decarboxylase (HDC) using citrate-AuNPs: 10 mM sodium phosphate buffer (pH 6.5), 1 mM histidine, 200 μg mL^{-1} HDC, and 0.1 mM PLP. b) Dependence of the ratio of the absorbance values at 520 and 650 nm on reaction time. c) Dependence of the ratio of the absorbance values at 520 and 650 nm on histamine concentration from the HDC reaction.

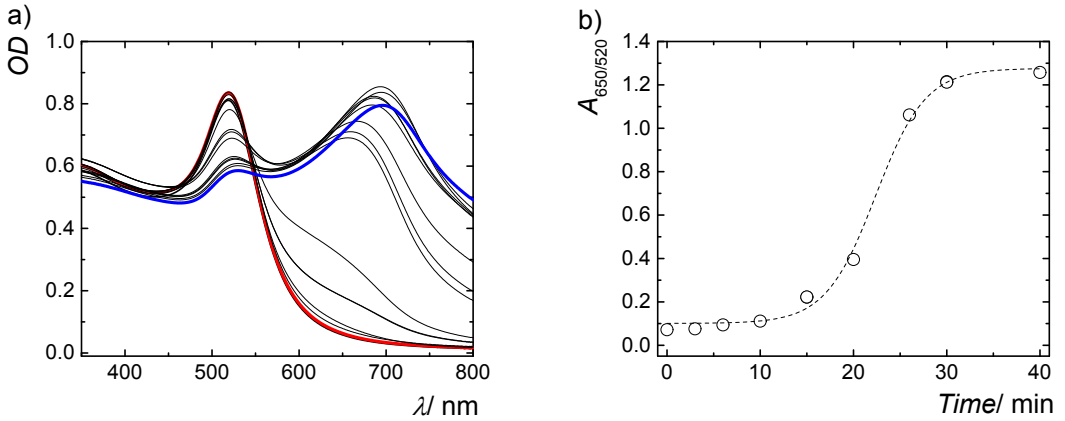

Figure S13. a) Colorimetric enzyme assay for histidine decarboxylase (HDC) using citrate-AuNPs: 10 mM sodium phosphate buffer (pH 6.5), 1 mM histidine, 500 μg ml^{-1} HDC, and 0.1 mM PLP. b) Dependence of the ratio of the absorbance values at 520 and 650 nm on reaction time.

7. References

1) X. Liu, M. Atwater, J. Wang and Q. Huo, *Colloids Surf., B*, 2007, **58**, 3.