

Electronic supplementary information

Generic Phosphatase Activity Detection using Zinc Mediated Aggregation Modulation of Polypeptide-Modified Gold Nanoparticles

Robert Selegård, Karin Enander, Daniel Aili*

Division of Molecular Physics, Department of Physics, Chemistry and Biology, Linköping University, SE-583 36 Linköping, Sweden
E-mail: daniel.aili@liu.se

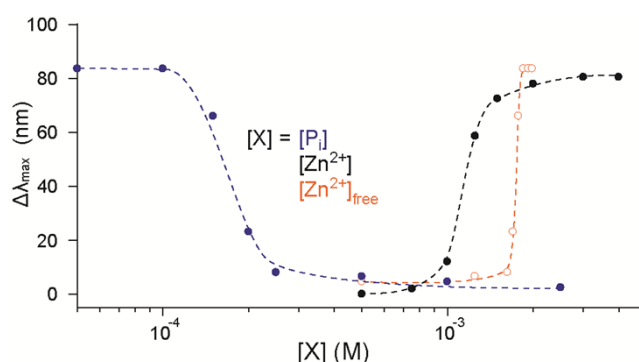


Figure S1. Change in LSPR peak position of JR2EC-AuNPs as a function of $[\text{Zn}^{2+}]$ (black), $[\text{P}_i]$ and 2 mM Zn^{2+} (blue) and concentration of non-complexed Zn^{2+} ($[\text{Zn}^{2+}]_{\text{free}}$) after addition of P_i (orange). $[\text{Zn}^{2+}]_{\text{free}}$ was calculated assuming 100% complex formation and a $\text{Zn}^{2+}:\text{P}_i$ ratio of 3:2. The relative shift of this curve (orange) compared to the Zn^{2+} response in the absence of P_i (black) indicates formation of ternary complexes between Zn^{2+} , JR2EC and P_i that interfere with polypeptide dimerization and prevent nanoparticle aggregation, in addition to the reduction in $[\text{Zn}^{2+}]$ because of formation of Zn_3PO_4 .^[1]

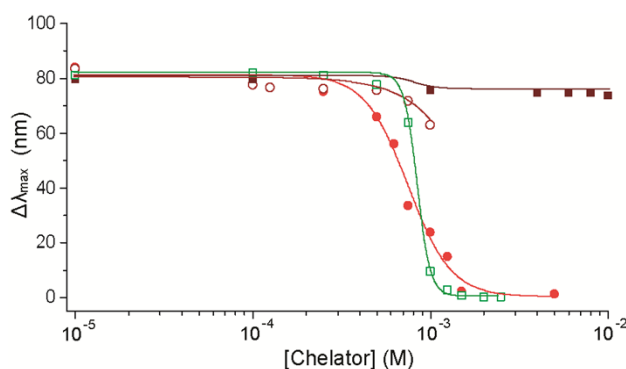


Figure S2. Change in LSPR peak position of JR2EC-AuNPs as a function of $[\text{ADP}]$ (●), $[\text{AMP}]$ (○), $[\text{adenosine}]$ (■) and $[\text{GMP}]$ (□) in a 30 mM Bis-Tris buffer (pH 7) containing 2 mM Zn^{2+} . Lines were obtained by fitting data to a Hill equation.

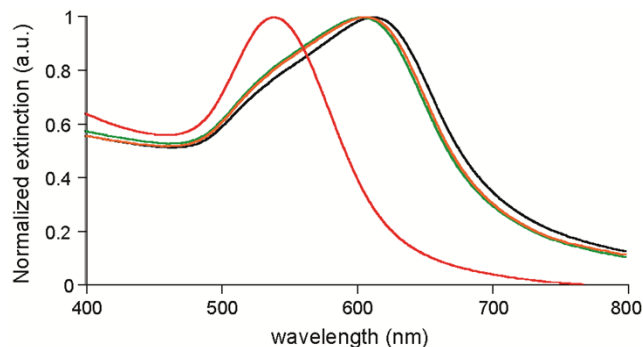


Figure S3. UV-vis spectra of JR2EC-AuNPs exposed to 2 mM Zn^{2+} (black), 2 mM Zn^{2+} and 100 μM ATP (green), 2 mM Zn^{2+} and 2 μM ALP (orange) and 2 mM Zn^{2+} , 100 μM ATP and 10 nM ALP incubated for 30 min at 37 °C (red). All samples were treated with 1 mM Mg^{2+} in Tris buffer (50 mM, pH 9) prior to dilution with Zn^{2+} and JR2EC-AuNPs giving a final concentration of 50 μM Mg^{2+} .

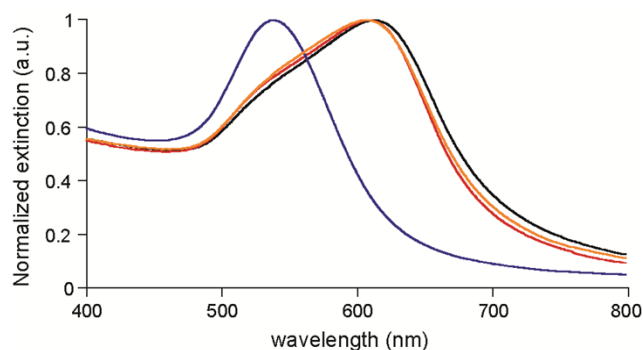


Figure S4. UV-vis spectra of JR2EC-AuNPs exposed to 2 mM Zn^{2+} (black), 2 mM Zn^{2+} and 175 μM PP_i (red), 2 mM Zn^{2+} and 56 nM PPase (orange) and 2 mM Zn^{2+} , 175 μM PP_i and 56 nM PPase incubated for 10 min at 30 °C (blue). All samples were treated with 1 mM Mg^{2+} in Tris buffer (50 mM, pH 7.2) prior to dilution with Zn^{2+} and JR2EC-AuNPs giving a final concentration of 50 μM Mg^{2+} .

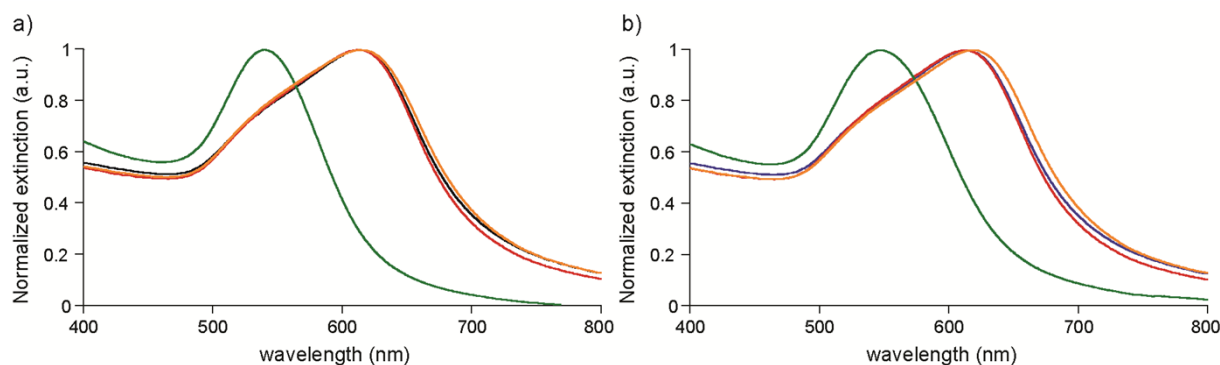


Figure S5. a) UV-vis spectra of JR2EC-AuNPs exposed to 2 mM Zn^{2+} (black), 2 mM Zn^{2+} and 250 μM ATP (orange), 2 mM Zn^{2+} and 68 nM apyrase (red) and 2 mM Zn^{2+} , 250 μM ATP and 68 nM Apyrase incubated for 2.5 h at 30 °C (green). All samples were treated with 5 mM Ca^{2+} in Mes buffer (50 mM, pH 6) prior to dilution with Zn^{2+} and JR2EC-AuNPs giving a final concentration of 250 μM Ca^{2+} . b) UV-vis spectra of JR2EC-AuNPs exposed to 2 mM Zn^{2+} (black), 2 mM Zn^{2+} and 250 μM GTP (orange), 2 mM Zn^{2+} and 68 nM apyrase (red) and 2 mM Zn^{2+} , 250 μM GTP and 68 nM apyrase incubated for 2.5 h at 30 °C (green). All samples were treated with 5 mM Ca^{2+} in Mes buffer (50mM, pH 6) prior to dilution with Zn^{2+} and JR2EC-AuNPs giving a final concentration of 250 μM Ca^{2+} .

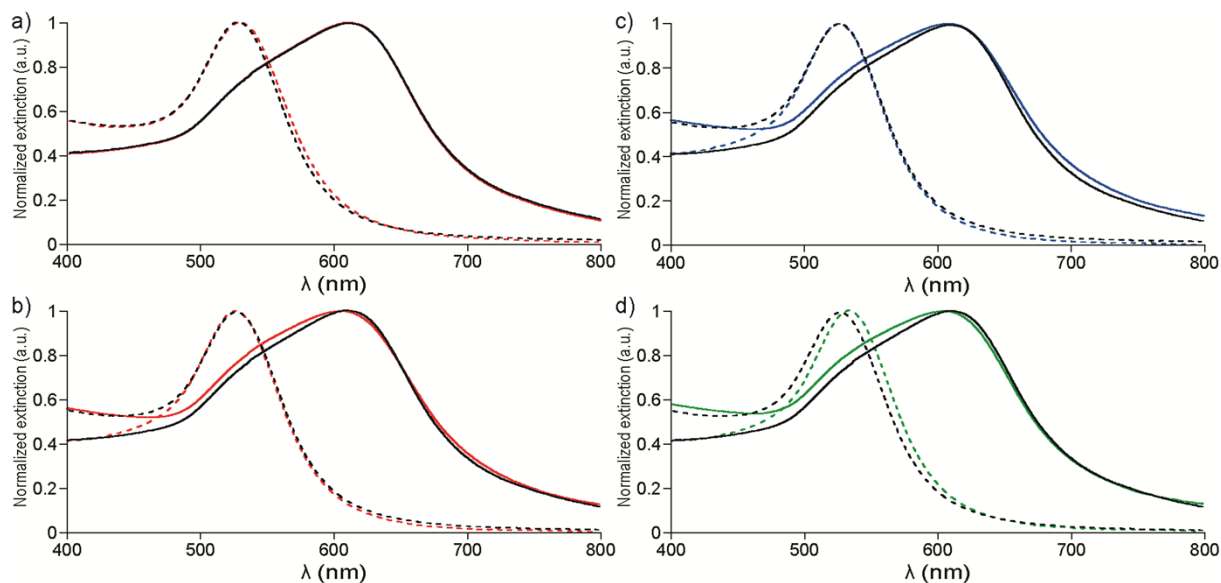


Figure S6. UV-vis spectra of JR2EC-AuNPs with (solid line) and without (dashed line) 2 mM Zn^{2+} , both in the presence of the highest concentration inhibitors used (colored) and in its absence (black). a) Na_3VO_4 (50 μM), b) theophylline (50 μM), c) NaF (50 μM) and d) GdCl_3 (50 μM). All inhibitors (1mM) were dissolved in the reaction buffer for each phosphatase including cofactors (Mg^{2+} or Ca^{2+}) before dilution with a Zn^{2+} -containing Bis-Tris buffer giving a final concentration of 50 μM .

Data fitting: The data were fitted using a monophasic Hill equation (Eq S1), where max and min are the largest and smallest LSPR shifts or % inhibition, respectively, x is either time or the concentration of chelant or inhibitor, n is the Hill coefficient and XX_{50} is $t_{1/2}$, CA_{50} or IC_{50} . Eq. 1 was used for fitting data in Figure 2a) and 2d), Figure 4 a)-b), Figure 5 a)-b), Figure S1 and Figure S2.

$$y = \min + \frac{(\max - \min)}{1 + \left(\frac{x}{XX_{50}}\right)^n}$$

(Eq. S1)

References

- 1 M. Mak, R. Selegård, M. Garbrecht and D. Aili, *Part. Part. Syst. Charact*, 2014, In press.