Electronic supplementary information

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

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Figure S1. Change in LSPR peak position of JR2EC-AuNPs as a function of [Zn$^{2+}$] (black), [P$_i$] and 2 mM Zn$^{2+}$ (blue) and concentration of non-complexed Zn$^{2+}$ ([Zn$^{2+}$]$_{\text{free}}$) after addition of P$_i$ (orange). [Zn$^{2+}$]$_{\text{free}}$ was calculated assuming 100% complex formation and a Zn$^{2+}$: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 [Zn$^{2+}$] because of formation of Zn$_3$PO$_4$.\textsuperscript{[1]}

Figure S2. Change in LSPR peak position of JR2EC-AuNPs as a function of [ADP] (●), [AMP] (○), [adenosine] (●) and [GMP] (□) in a 30 mM Bis-Tris buffer (pH 7) containing 2 mM Zn$^{2+}$. Lines were where 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$_3$VO$_4$ (50 µM), b) theophylline (50 µM), c) NaF (50 µM) and d) GdCl$_3$ (50 µM). All inhibitors (1mM) where 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**