Supporting Information

The phosphatase-like activity of zirconium oxide nanoparticle and its application in near-infrared intracellular imaging

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Fig. S1 The TEM images of ZrO$_2$ NPs under different magnification. Scale bars: (A) 100 nm; (B) 50 nm; (C) 20 nm; (D) 5 nm.
Fig. S2 The apparent zeta potential distribution of the ZrO$_2$ NPs.
Fig. S3 (A) XPS survey spectrum of ZrO$_2$ NPs. (B) Zr 3d XPS spectra of the ZrO$_2$ NPs.
Fig. S4 The fluorescent kinetic curves of 5 µM 4-MUP in the presence of different concentrations of ZrO$_2$ NPs. From a to f: 0 µg/mL, 15 µg/mL, 30 µg/mL, 50 µg/mL, 80 µg/mL, and 100 µg/mL ZrO$_2$ NPs. The fluorescence emission at 455 nm were recorded under excitation at 360 nm.

Fig. S5 The kinetic curves of 5 µM 4-MUP in the presence of 20 mM HEPES buffer (a), 100 µg/mL ZrO$_2$ NPs (b), 100 µg/mL ZrOCl$_2$ (c), 100 µg/mL Zr(OH)$_4$ (d), and the supernatant of 100 µg/mL ZrO$_2$ NPs after centrifugation (e).
Fig. S6 (A) The Steady-state kinetic assays of ZrO$_2$ NPs at pH 8.5, the concentration of ZrO$_2$ NPs was 100 µg/mL. (B) The Lineweaver-Burk plots of the double reciprocal of the Michaelis-Menten equations, which was obtained from the kinetic data of ZrO$_2$ NPs.
Table S1 Comparison of the kinetic parameters of ZrO$_2$ NPs with that of CeO$_2$ NPs and ALP (pH 8.5). \( K_m \) is the Michaelis-Menten constant, \( V_{max} \) is the maximal reaction rate, \( K_{cat} \) is the catalytic constant, \( K_{cat} = V_{max}/[E] \), and \([E]\) is the concentration of catalyst. The kinetic parameters of CeO$_2$ NPs and ALP were obtained from our lab, which were calculated using the same protocol with ZrO$_2$ NPs. The concentration of ZrO$_2$ NPs was 1.94 nM (100 µg/mL), while the concentration of ALP was 0.18 nM (0.01 µg/mL) and the concentration of CeO$_2$ NPs was 0.34 µM (100 µg/mL).

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Substrate</th>
<th>( K_m / \mu M )</th>
<th>( V_{max} / M \cdot s^{-1} )</th>
<th>( K_{cat} / s^{-1} )</th>
<th>( K_{cat}/K_m [s^{-1} \mu M^{-1}] )</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZrO$_2$</td>
<td>4-MUP</td>
<td>14.7</td>
<td>5.31×10$^{-9}$</td>
<td>2.74</td>
<td>0.19</td>
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<tr>
<td>CeO$_2$</td>
<td>4-MUP</td>
<td>16.5</td>
<td>2.76×10$^{-8}$</td>
<td>0.08</td>
<td>0.005</td>
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<tr>
<td>ALP</td>
<td>4-MUP</td>
<td>1.13</td>
<td>1.87×10$^{-9}$</td>
<td>10.39</td>
<td>9.19</td>
</tr>
</tbody>
</table>

Fig. S7 The time-dependent absorbance changes of 20 mM HEPES buffer (pH 8.5) (curve a), 500 µM \( p \)-NPP (curve b), and 500 µM \( p \)-NPP in the presence of 100 µg/mL ZrO$_2$ NPs (curve c). The absorbance were measured at a wavelength of 405 nm. Inset: the corresponding images captured by a mobile phone.
Fig. S8 The absorbance values of the malachite green assay in the presence of 20 mM HEPES buffer (curve a), 25 µM 4-MUP (curve b), 25 µM ATP (curve c), 25 µM o-phospho-L-tyrosine (curve d), and 25 µM DNA strand S1 (curve e). The malachite green solution was prepared according to the kit instructions, and the absorbance values at 620 nm represents the formation of free phosphate in the solutions.
Fig. S9 The gel electrophoresis analysis of the cleavage of DNA by ZrO$_2$ NPs and CeO$_2$ NPs. Lane 1: DNA strand S$_2$ after incubated with 100 µg/mL ZrO$_2$ NPs at 25 °C; Lane 2: DNA strand S$_2$ after incubated with 100 µg/mL CeO$_2$ NPs at 25 °C; Lane 3: DNA strand S$_2$ after incubated in buffer at 25 °C; Lane 4: DNA strand S$_2$ after incubated with 100 µg/mL ZrO$_2$ NPs at 60 °C; Lane 5: DNA strand S$_2$ after incubated with 100 µg/mL CeO$_2$ NPs at 60 °C; Lane 6: DNA strand S$_2$ after incubated in buffer at 60 °C.

Fig. S10 (A) The fluorescent spectra of 5 µM FDP in the absence (curve a) and presence (curve b) of 100 µg/mL ZrO$_2$ NPs. The fluorescent spectra were performed by using a excitation wavelength of 490 nm. (B) The fluorescent spectra of 5 µM SunRed phosphate in the absence (curve a) and presence (curve b) of 100 µg/mL ZrO$_2$ NPs. The fluorescent spectra were carried out by using a excitation wavelength of 620 nm.
Fig. S11 Cytotoxicity of different concentrations of ZrO$_2$ NPs after incubation with HeLa cells for 24 h. The error bars represent the standard deviation of three measurements.