Supplementary Information

A Novel Alendronate Functionalized Nanoprobe for Simple Colorimetric Detection of Cancer-Associated Hypercalcemia

Abhishek Sahu\textsuperscript{a}, Young Min Hwang\textsuperscript{a}, Cristian Vilos\textsuperscript{b,c}, Jong-Min Lim\textsuperscript{d}, Sunghyun Kim\textsuperscript{e}, Won Il Choi\textsuperscript{e,*}, Giyoong Tae\textsuperscript{a,*}

\textsuperscript{a}School of Materials Science and Engineering, Gwangju Institute of Science and Technology, 123 Cheomdan Gwagi-ro, Oryong dong, Buk-gu, Gwangju 61005, Republic of Korea

\textsuperscript{b}Universidad Andres Bello, Laboratory of Nanomedicine and Targeted Delivery, Center for Integrative Medicine and Innovative Science, Faculty of Medicine, Center for Bioinformatics and Integrative Biology, Faculty of Biological Sciences, Santiago, 8370071, Chile

\textsuperscript{c}Center for the Development of Nanoscience and Nanotechnology, CEDENNA, 9170124, Santiago, Chile

\textsuperscript{d}Department of Chemical Engineering, Soonchunhyang University, 22 Soonchunhyang-ro, Shinchang-myeon, Asan-si, Chungcheongnam-do 31538, Republic of Korea

\textsuperscript{e}Center for Convergence Bioceramic Materials, Convergence R&D Division, Korea Institute of Ceramic Engineering and Technology, 202, Osongsaengmyeong 1-ro, Osong-eup, Heungdeok-gu, Cheongju, Chungbuk 28160, Republic of Korea.

* To whom correspondence should be addressed

E-mail: choi830509@kicet.re.kr / Phone: 82-43-913-1513 / Fax: 82-43-913-1597

E-mail: gytae@gist.ac.kr / Phone: 82-62-715-2305 / Fax: 82-62-715-2304
Supplementary Figure 1. (a) Absorbance spectra of citrate stabilized gold nanoparticles, (b) TEM image of the gold nanoparticles.
Supplementary Figure 2. Ca^{2+} ion induced change in the absorbance spectra of GNPs containing ADP, ATP and ALD.
Supplementary Figure 3. Photos of GNP solutions in the phosphate buffer (10 mM, pH 7.4) in the presence of various salt concentrations. Arrow indicates aggregated state of GNPs at specific salt concentration. ALD chemically conjugated to GNP (Thiolated ALD-GNP) showed better stability against high salt concentration than physically mixed ALD (Non-thiolated ALD-GNP) and non-modified Citrate-GNP.
**Supplementary Figure 4.** Absorbance spectra of GNP-ALD solution in presence of Ca$^{2+}$ ion (0.5 mM) and excess EDTA (5 mM). The chelation of Ca$^{2+}$ ion by EDTA reversed the aggregation of ALD-GNP and changed the red-shifted spectra to the original spectra of GNP-ALD.
Supplementary Figure 5. The absorbance change of GNPs conjugated with thiolated ALD or physically mixed with ALD in the presence of different concentration of Ca^{2+} ion.
Supplementary Figure 6. (a) The absorption spectra ALD-GNP in the presence of metal ion cocktail with and without Ca$^{2+}$ ion. The absorption spectra ALD-GNP without any metal ion was used as a control. (b) The color of the ALD-GNP solution in the presence of metal ion cocktail without and with Ca$^{2+}$ ion. The red-shift in the spectra and change in the color occurred only in the presence of Ca$^{2+}$ ion.
**Supplementary Figure 7.** Ca\(^{2+}\) ion induced color change of the commercial kit and ALD-GNP nanoprobe. The ALD-GNP nanoprobe showed different color with different Ca\(^{2+}\) ion concentration, but the commercial kit showed the same color with different intensity.

<table>
<thead>
<tr>
<th>Ca(^{2+}) ion Concentration (μM)</th>
<th>0</th>
<th>50</th>
<th>100</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial Kit</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALD-GNP Nanoprobe</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>