Protein Kinase Assay on Peptide-Conjugated Gold Nanoparticles by Using Secondary Ion Mass Spectrometric Imaging

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Experimental Procedures

Reagents

Abl tyrosine kinase and cAMP-dependent protein kinase (*PKA*) were purchased from New England Biolab. Monoclonal anti-phosphotyrosine antibody (anti-pY, mouse IgG2b isotype from PY20) and mouse immunoglobulin G (mIgG) were from Sigma. Two kinase inhibitors, stautosporin for *Abl* and 4-cyano-3-methylisoquinoline (CMIQ) for *PKA*, were purchased from Sigma and Calbiochem, respectively. Amine-functionalized glass (Nexterion™ Slide A+) was obtained from Schott (Germany), and poly (ethylene glycol)-succinimidyl α-methylbutanoate (*m*PEG-SMB, MW 2000) was purchased from Nektar Inc (USA). Three kinds of peptide substrates used in this study were synthesized from Peptron Inc. (Korea): Ac-IYAAPKKC, Ac-IYAAPKK$(G)_4$C and Ac-LRRASL$(G)_4$C. Hydrogenetrachloroaurate(III) trihydrate (99.9% $HAuCl_4 \cdot 3H_2O$), sodium citrate dihydrate (99.9%, 2-hydroxy-1,2,3-propanetricarboxylic acid trisodium salt, $C_6H_5Na_3O_7 \cdot 2H_2O$) and sodium borohydride (99%, $NaBH_4$) were purchased from Sigma-Aldrich. A chambered silicon coverslip (50 wells, 3mm × 1mm, sterile) was also from Sigma-Aldrich.

Determination of phosphorylation efficiency and IC_{50}

Phosphorylation efficiency by kinase reaction was calculated using the following equation;

$$E_{\mathrm{P}} = \frac{A_{\mathrm{MP}}}{A_{\mathrm{M}} + A_{\mathrm{MP}}} \times 100$$

where E_{P} is phosphorylation efficiency (%), A_{M} and A_{MP} the peak areas of $[\mathrm{MH{-}SH{-}COOH}]^+$ and $[\mathrm{MH{-}SH{-}COOH}+HPO_3]^+$, respectively. Peak area was calculated within the limits of the full width half-maximum (FWHM).

For IC_{50}, phosphorylation efficiency was plotted as a function of inhibitor concentration, and then fitted to a 4-parameter logistic equation (dose-response model for ligand binding) by the non-linear regression procedure of Sigmaplot (ver 10.0, SYSTAT Software) using the following equation:

$$E_{\mathrm{P}} = \frac{E_{\mathrm{Pmax}} - E_{\mathrm{Pmin}}}{1 + (\mathrm{C}/\mathrm{C}_0)^{\mathrm{n}}} + E_{\mathrm{Pmin}}$$

where E_{Pmax} and E_{Pmin} ; the maximum and minimum phosporylation efficiencies, respectively. C_0 ; the median inhibitory concentration. C; the inhibitor concentration. N ; the slope factor. IC_{50} value is equal to the inflection point (C_0) of the best-fit curve.

Fourier transform-infrared (FT-IR) spectroscopy

FT-IR spectra were obtained in a single electron mode using a FT-IR spectrophotometer (dry N_2-purged Thermo Nicolet Nexus FT-IR, Thermo Electron Corp.) equipped with the SAGA (Smart Apertured Grazing Angle) accessory. We averaged

about 2,000 scans to yield the spectrum at a resolution of 2 cm^{-1}, and spectra of the surface of peptide-conjugated AuNPs and its PEG-coating surface were displayed in the absorption mode.

Surface plasmon resonance (SPR) spectroscopy

The modification of a gold chip followed the procedures in order of AuNPs adsorption, peptide attachment, and PEG coating as described above. To check a specific binding capacity, anti-pY antibody (10 μg mL^{-1}in PBST) was subsequently injected over the phosphorylated and non-phosphorylated surfaces for 30min. Mouse IgG (10 μg mL^{-1}in PBST) was also injected under the same condition as a control.

Microfluidic patterning for multiple SIMS imaging

For multiple SIMS imaging, microfluidic patterning was performed using a BIAcore-3000 instrument (BIAcore, Sweden) on a SPR gold sensor. The gold sensor chip was first modified with SAMs of 11-mercaptoundecylamine (MUAM, Dojindo). AuNPs were attached onto the amine-terminated SAMs, and the resulting surface was conjugated with a mixture of two peptides (Ac-IYAAPKK$(G)_4$C and Ac-LRRASL$(G)_4$C) followed by treatment with mPEG-SMB and washing. Phosphorylation was carried out by flowing respective kinase at each channel (*Abl* for channel 1, and *PKA* for channel 2) at a flow rate of 2 μL min^{-1} at 30 °C. The chip surface was finally washed with 0.5% diammonium citrate dibasic for 10 min. For inhibition assay, kinase reaction was performed in the presence of 10 μM inhibitor using the remaining channels (channel 3 and 4). For TOF-SIMS imaging, the Bi_1^+ beam (25 kV) was randomly rastered over a surface area of 300 × 300 μm^2 with a primary ion influence of 4×10^{12} ions cm^{-2} in positive SIMS mode at 256 × 256 pixels.

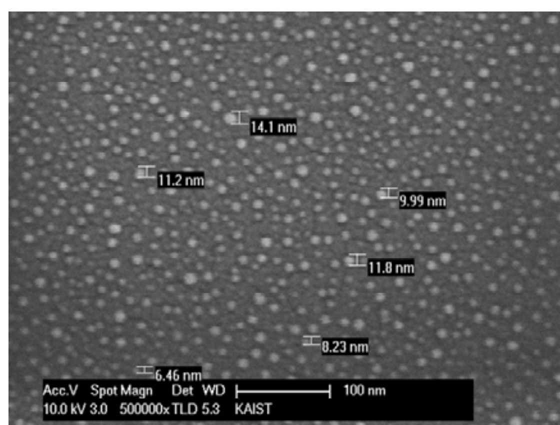

Figure S1. FE-SEM image of AuNPs attached on an amine-functionalized glass surface. The average size of AuNPs was estimated to be 10.7 ± 2.7 nm.

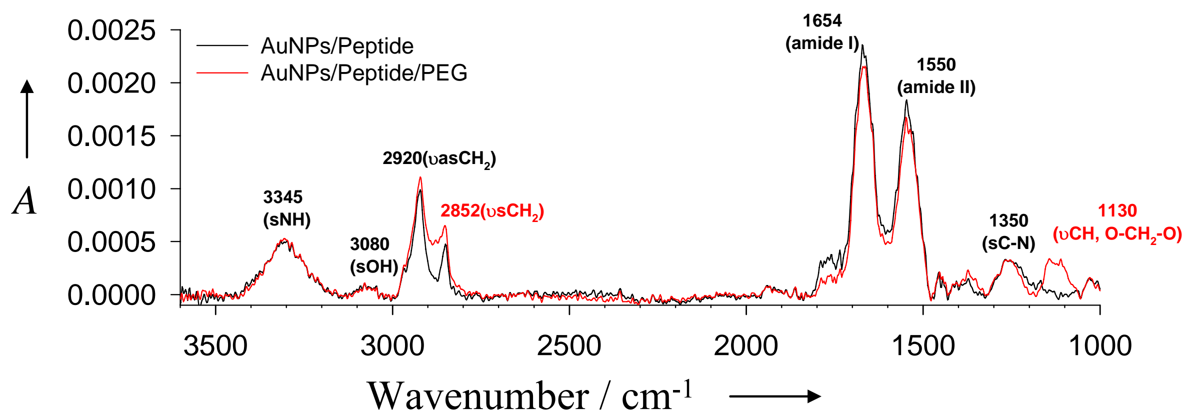

Figure S2. FT-IR spectra from the surface of peptide-conjugated AuNPs in the absence (black line) and presence (red line) of *m*PEG-SMB treatment. No signal suppression of peptide-specific peaks was observed for the N-H stretching peak (~ 3345 cm^{-1}), amide I (~ 1654 cm^{-1}), and amide II (~ 1550 cm^{-1}). On the other hand, absorbance of C-H stretching mode (~ 2920 $cm^{-1}$and 2852 cm^{-1}) and C-H vibration mode (~1130 cm^{-1}) were increased by addition of *m*PEG-SMB.

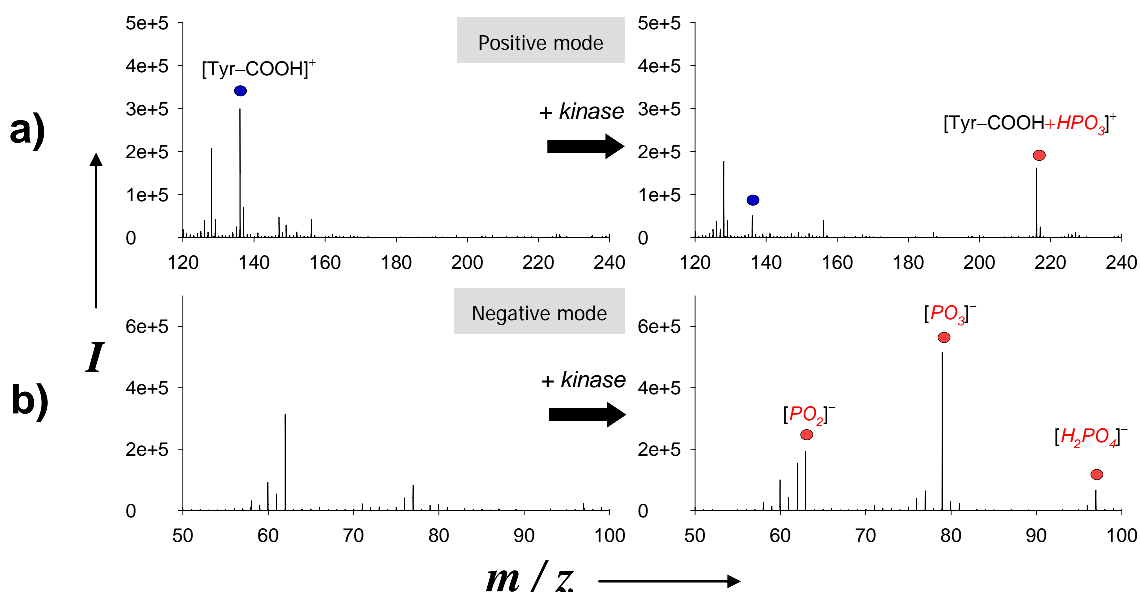

Figure S3. a) Positive and b) negative TOF-SIMS spectra from P_{Abl} (Ac-IYAAPKK(G)$_4$C; M_r=1162.58) before and after kinase reaction. Phosphorylated peaks were observed at the *m/z* values indicated by red circles.

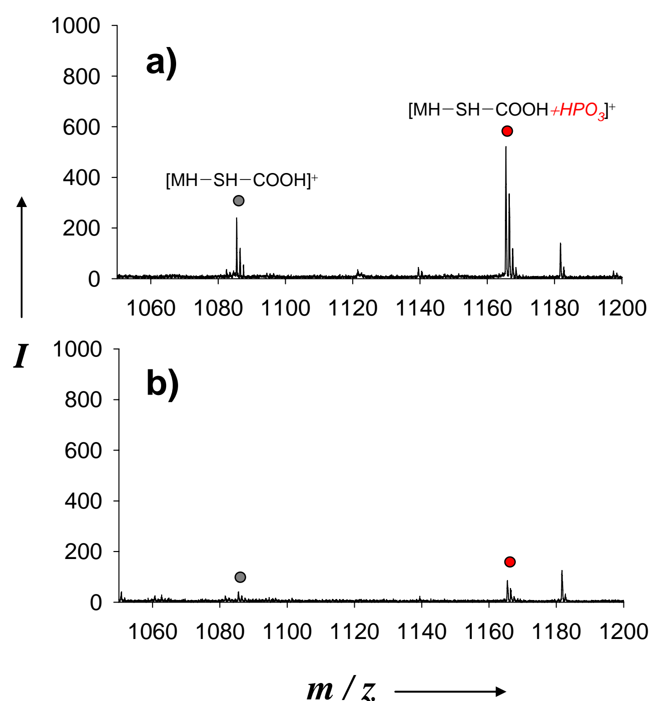

Figure S4. Positive TOF-SIMS spectra from P_{Abl} (Ac-IYAAPKK(G)$_4$C; M_r=1162.58) after kinase reaction a) with and b) without PEG-blocking of the AuNPs/peptide surface. Significant reduction in signal intensity was observed for the surface without PEG treatment as can be seen in b).

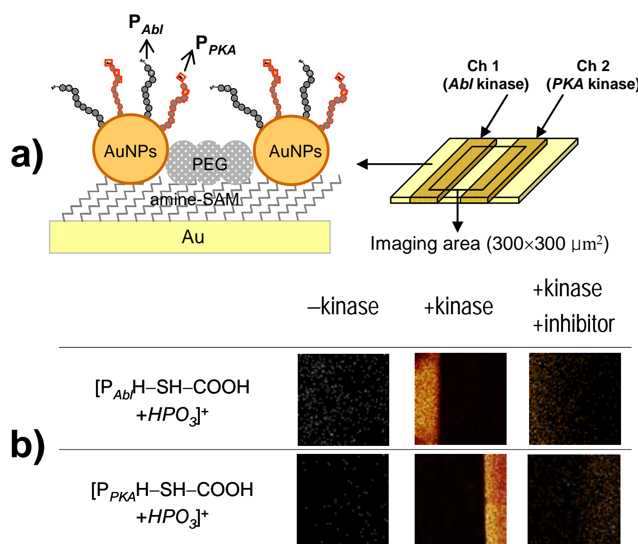

Figure S5. a) Construction of the AuNP surface containing a mixture of two peptides (P_{Abl} and P_{PKA}) on the SPR gold sensor chip and scheme of microfluidic channels for assay of a respective kinase. b) Multiplexing SIMS images on the sensor chip after kinase reaction. Kinase (*Abl* or *PKA*) was flowed over the AuNP surface in the absence and presence of their inhibitor. Concentration of inhibitor was 10 μM.

Respective kinase solution (*Abl* or *PKA*) was flowed over the AuNP surface containing a mixture of two peptides through microfluidic channels (Figure S5a). As shown in Figure S5b, patterned surface by kinase reaction in the absence and presence of inhibitor was distinctly revealed by SIMS chemical imaging. Kinase-flowed channels yielded strong ion mass images (*i.e.* $[P_{Abl}H\text{–}HS\text{–}COOH+HPO_3]^+$ and $[P_{PKA}H\text{–}SH\text{–}COOH+HPO_3]^+$), and addition of inhibitor dramatically reduced the intensity of ion mass images. No cross-images of kinases against opposite peptides were observed.