Supporting Information for

Kinase Phosphorylation Monitoring with i-Motif DNA Cross-linked SERS Probes

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Experimental section

Chemicals and agents

Sodium citrate, Tris(2-carboxyethyl)phosphine hydrochloride (TCEP)，$HAuCl_4$, $MgCl_2$, adenosine triphosphate (ATP) and 4-mercaptopyridine (4-MPy) were obtained from Sigma. Tris base is obtained from Promega Corporation. Kinase (Item number 14-529) is obtained from a commercial source (Millipore). The three different oligonucleotide strands used in our experiments were purchased from Integrated DNA Technologies (IDT) (Coralville, IA). The sequences are: CS, HS-TTTTCCTGAGCCACCTATACCAG; PS, ACGGAACCGGTAGTCCACCTAGTTT-SH; i-motif DNA linker: TGCCTTGGC CATCAGGTGGATCAAAGTATCCCTAACCCTAACCCTAACCCCTTGTCCAG G ACTCGGTGGATATGGTC. Peptides were synthesized at a 50 μmol scale with a Protein Technologies Prelude Parallel peptide synthesizer on CLEAR-amide resin (Peptides International), the sequence information of short peptide containing a recognition motif for phosphorylation by Abl kinase (Abl peptide) is: EAIYAAPVAKKKKGGGGC; synthetic phosphorylated Abl peptide, EAIYpAAPVAKKKKGGGGC; Hck, Thz-GGYDTLARPSLPLHPMSSGGC. PC3 cells were provided by Dr. Ratliff's group, those were obtained from the American

Type culture Collection (ATCC); fetal bovine serum (FBS) were purchased from Atlanta Biological, Inc., RPMI medium 1640 from life technology, penicillin, and streptomycin were purchased from Hyclone.

iDCL GNP probes preparation

All glasswares used in the described procedures were washed in a bath of freshly prepared 3:1 HCl: HNO_3 (aqua regia) solution and rinsed thoroughly with water purified by milli-Q system. Gold nanoparticles were synthesized based on the reported method. (G. Frens, *Nature-Phys. Sci.*, 1973, **241**, 20-22.) 1 ml of 1% $HAuCl_4$ is injected into 100 ml H_2O, followed by the quick addition of 1 ml of sodium citrate solution. The solution is kept at a boiling state for 10 min and cooled in room temperature. GNP colloids obtained were stored at 4 ^oC. To prepare iDCL GNP probes, 6 ml of GNP colloid is centrifuged at 6000 rpm two times and redispersed in 1 ml of H_2O, and then mixed with 60 μl of 10 μM 4-MPy and kept overnight. 500 μl of 4-MPy modified GNPs were centrifuged and washed with H_2O once and then redispersed in 490 μl of 0.6 μM CS or PS ssDNA solution. After two days, the obtained GNPs modified with 4-MPy and DNA were centrifuged and washed with H_2O two times and incubated with 8 μl of 10 μM peptide. The peptide modification takes two days to mature. Then 500 μl of GNPs with 4-MPy, CS DNA and peptide is mixed with 500 μl of GNPs modified with 4-MPy, PS DNA and peptide, followed by the addition of 10 μl of 100 μM i-motif DNA linker, and left for two days to complete the hybridization. The cross-linked GNPs conjugated with Abl substrate peptide was then used for Abl kinase detection. The enhancement factor of the prepared iDCL

GNP probes was calculated to be 1.4×10^5 based on the reported work. (H. Z. Yu, N. Xia and Z. F. Liu, *Anal. Chem.*, 1999, **71**, 1354-1358; W. Song, Y. Wang and B. Zhao, *J. Phys. Chem. C*, 2007, **111**, 12786-12791; K. Lee and J. Irudayaraj, *J. Phys. Chem. C*, 2009, **113**, 5980-5983.)

Detection

In a typical detection procedure of the kinase and its phosphorylation, 45 μl of GNP probes is centrifuged and redispersed in 9 μl H_2O, then 1 μl of 0.1 M Tris-HCl buffer (pH=7.4), 1 μl of 10 mM $MgCl_2$ and 1 μl of 1 mM ATP are added in sequence, then 2 μl of kinase in various concentration was injected and the obtained solution was well-mixed. The blank samples were prepared by a similar procedure, where 2 μl of buffer was added instead of Abl Kinase. The resulting sample was then analyzed by Raman spectroscopy and the SERS spectrum was recorded for different incubation times. The intensity of the peak at 1095 cm^{-1} was measured after background subtraction.

PC3 cells were maintained in the RPMI 1640 containing 10% FBS, penicillin–streptomycin, Cells were grown at 37°C with 5% CO2. Prior to SERS detection, cells were plated on 18 mm coverslip in 12 well plates with seeding density of 10000 cells/ml and cultured for 48 hours before detection. For inhibitor treatment, the coverslips with cells were transferred to the culture medium with 2 μM of imatinib for overnight growth, followed by the addition of 50 μl of iDCL GNP probes. After 4 h of culturing, the cells on the coverslip were washed gently with PBS 3 times and immersed in PBS. The SERS spectra of the cells on coverslips were then recorded. To detect live cells, 30 cells from each sample were randomly selected for SERS

measurement and the experiment was repeated three times.

Instrumentation

SERS spectra are recorded with a 20x long WD objective using 785 nm excitation at 100 mW with a SENTERRA confocal Raman system (Bruker Optics, Billerica, MA). UV-vis spectra were obtained with a Jasco V570 UV/Visible/NIR spectrophotometer (Jasco, Inc., Easton, MD). Transmission electron microscopy (TEM) images of the samples were collected with a FEI Tecnai G2 20 at an operation voltage of 100 kV. Scanning electron microscopy images are recorded with a FEI NOVA nanoSEM field emission SEM, (FEI Co., Hillsboro, OR) operating at 5.0 KV.

Simulation

The local surface plasmon resonance electric field of the iDCL GNP probes with i-motif DNA linker contributing to the formation of a linear configuration and a quadruplex-like folding pattern upon phosphorylation was demonstrated by a three dimensional FDTD simulation. Coupling effect of gold nanoparticle with an interparticle distance of 5 nm and 12 nm per the i-motif DNA linker formation was simulated. The refractive index of the background was kept at 1.33. A plane wave incident source with linear polarization was used to calculate the 3D electrical field from 350 to 800 THz. Fast Perfect Boundary Approximation (FPBA) mesh type was used to obtain highly accurate in field results. The Drude model was applied to describe the dispersion of gold used in the design. The local electric field distribution was obtained directly from the simulation.

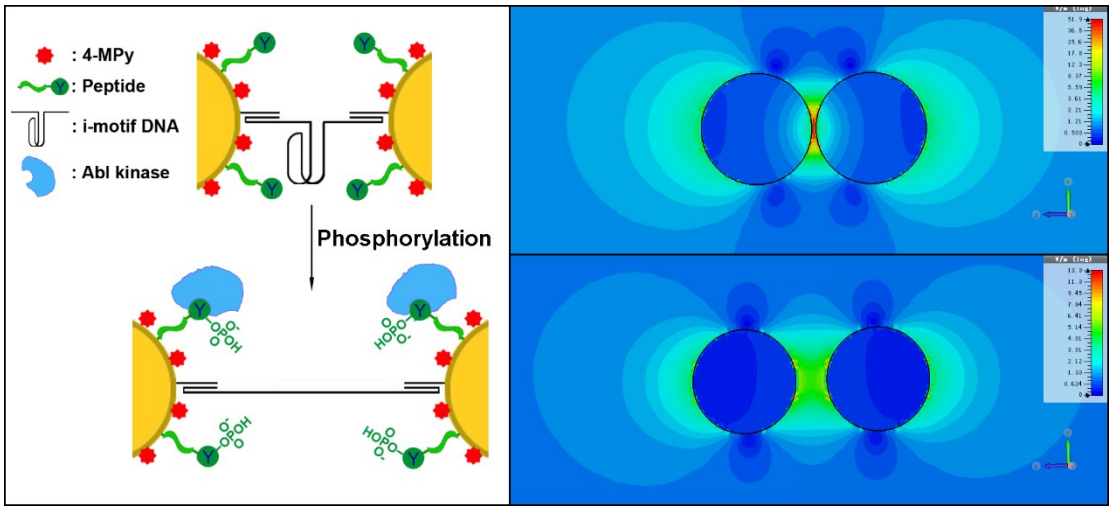

Figure S1. The interparticle distance between modified GNPs in the network of the iDCL GNP probes upon phosphorylation and the corresponding local surface plasmon resonance electric field of the iDCL GNP probe in dimer structure as a typical model upon the conformation change of the i-motif DNA linker in the detection scheme. The simulation based on a dimer model provides a qualitative explanation and of the increase in interparticle distance in the networks of iDCL GNP probes to result in a reduced SERS intensity.

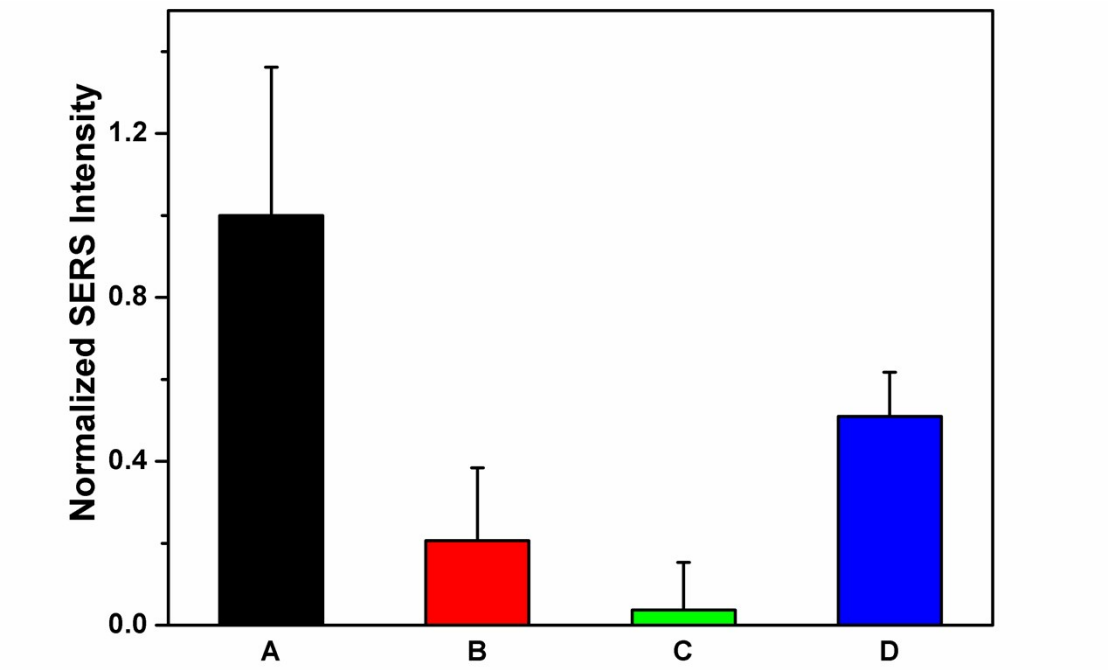

Figure S2. Normalized SERS intensity of the peak at 1095 cm^{-1} from the samples of iDCL GNP probes (A), i-motif DNA cross-linked GNPs modified with the synthetic phosphorylated Abl peptide (B), probes phosphorylated by 1.05 nM of Abl kinase (C) and probes mixed with Abl kinase without ATP (D) with an incubation time of 65 minutes [mean ± SD, n = 3]. Compared with the SERS intensity of probes (A), the SERS reduction of the i-motif DNA cross-linked GNPs modified with the synthetic phosphorylated Abl peptide (B) demonstrates that the transfer of phosphate group will decrease the SERS of probes, while the SERS reduction of the probes mixed with Abl kinase without ATP (D) indicates that the interaction between Abl kinase and Abl peptide will also decrease the SERS of probes. The decrease in SERS from the samples with probes phosphorylated by 1.05 nM of Abl kinase (C) demonstrates that the transfer of phosphate group and interaction between Abl kinase and Abl peptide can affect the SERS signals from the probe simultaneously to result in a weak SERS intensity.

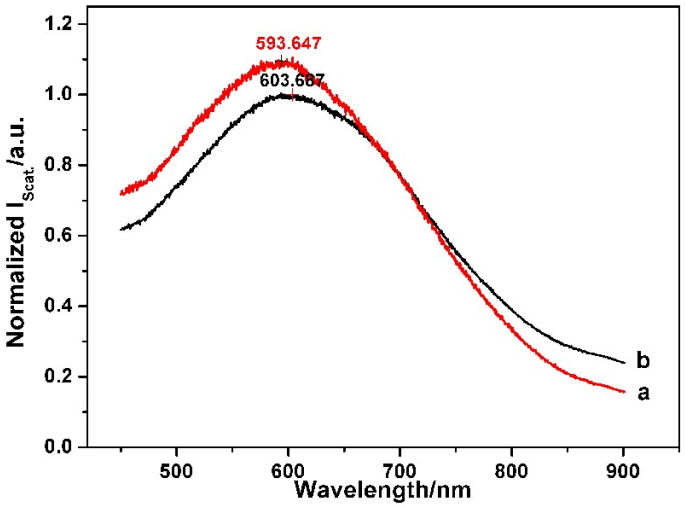

Figure S3. Average scattering spectra of mixture of modified GNPs without i-motif DNA linker (a) and iDCL GNP probes which are networks of modified GNPs linked by i-motif DNA (b). The spectra showed above are the average spectra [n = 5].

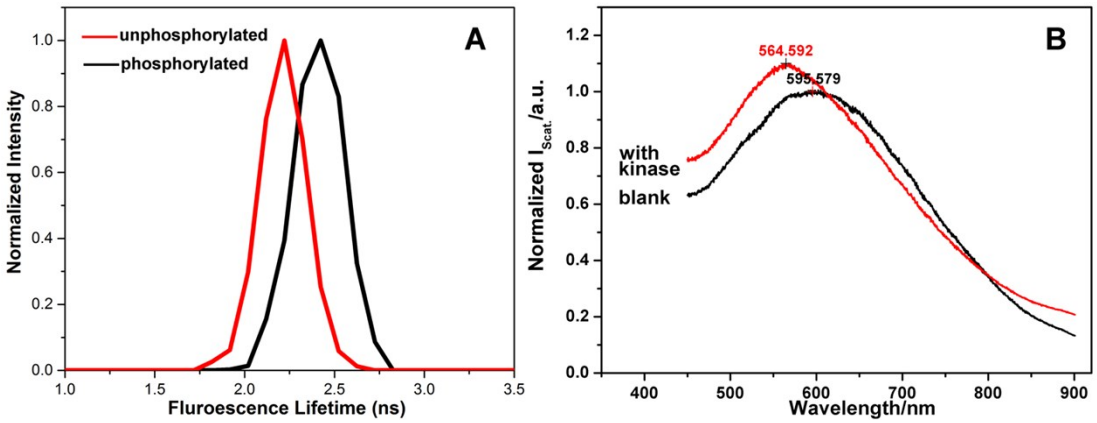

Figure S4. Fluorescence lifetime spectra of peptide sensors before and after phosphorylation (A) and average scattering spectra of iDCL GNP probes with and without Abl kinase (B) [n = 5]. Fluorescence lifetime change signals phosphorylation due to Abl kinase. [N. P. Damayanti, L. L. Parker and J. M. K. Irudayaraj, *Angew. Chem. Int. Ed. Engl.*, 2013, **52**, 3931-3934.] Due to the phosphorylation catalyzed by Abl kinase, per our detection scheme, a blue shift of the peak in the scattering spectra of iDCL GNP probes can be observed, indicating an increased interparticle distance in the iDCL GNP probes upon phosphorylation.

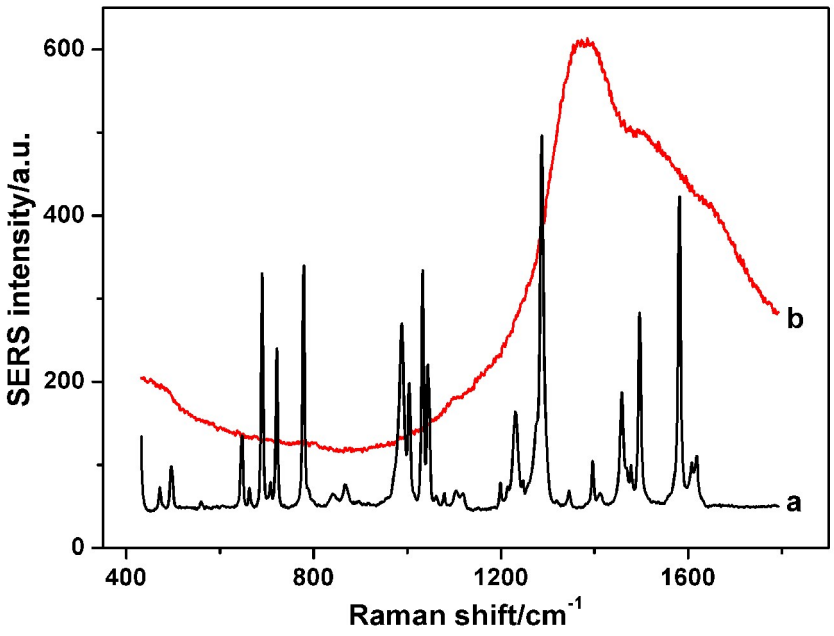

Figure S5. Average spectrum of 0.1 × normal Raman spectrum of 4-MPy solid (a) and background (b) [n=5]. It can be seen that at 1095 cm^{-1} the spectral intensity of the background is weak, suggesting a minor influence of the background during detection.

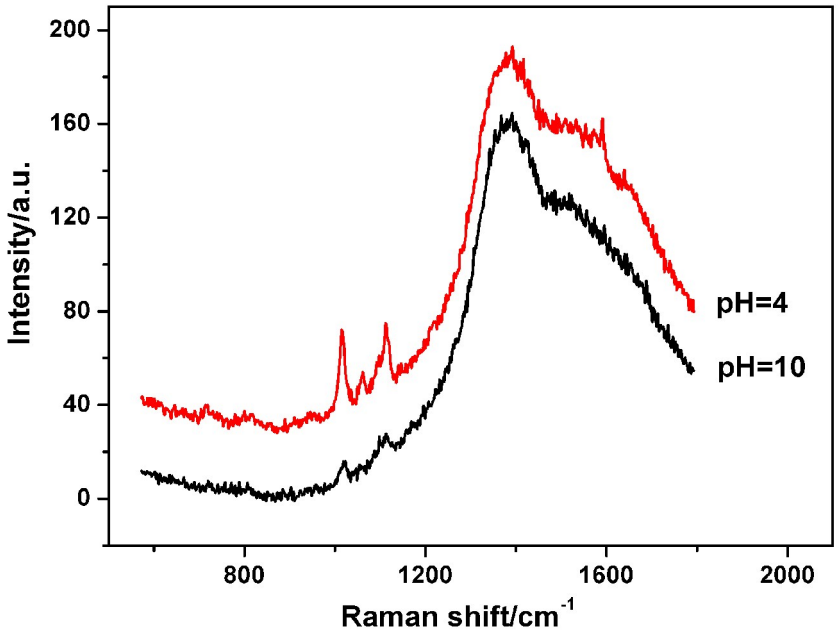

Figure S6. Average SERS spectra of iDCL GNP probes at pH = 4 and pH = 10 [n=3].

During detection the response of iDCL GNP probes will be triggered by the local hydrogen concentration change in the microenvironment near the probe upon phosphorylation, while the pH of the solution is determined by the buffer.

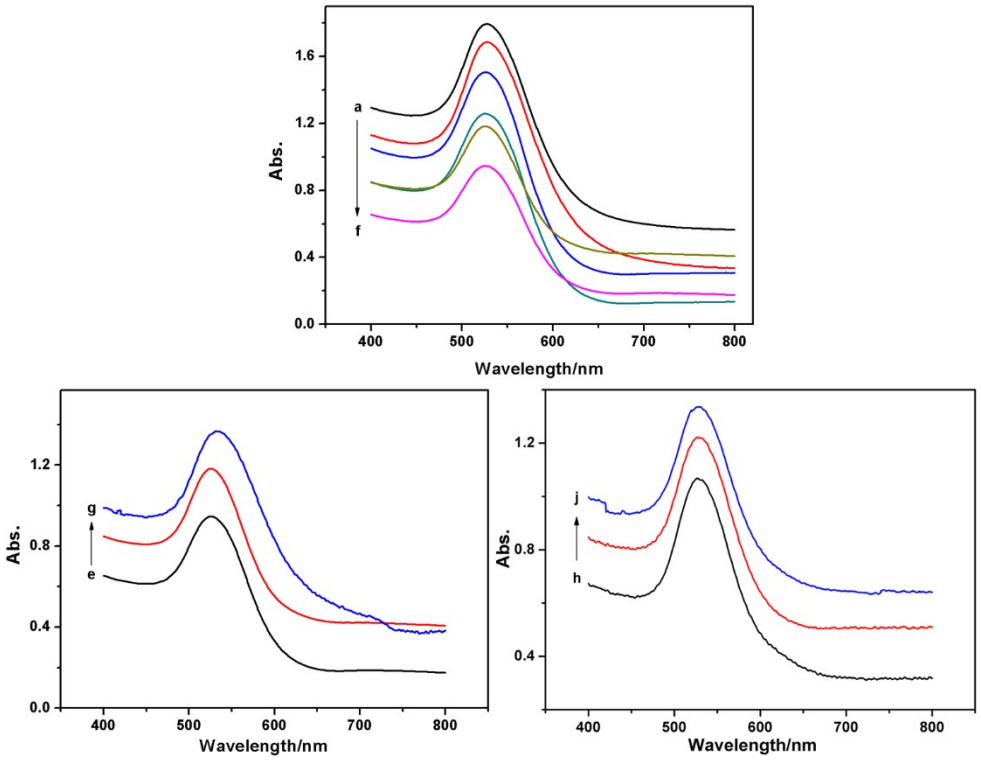

Figure S7. UV spectra of bare GNPs (a), GNPs modified with 4-MPy (b), GNPs modified with 4-MPy and CS DNA strands (c), GNPs modified with 4-MPy and PS DNA strands (d), GNPs modified with 4-MPy and CS DNA strands and unphosphorylated Abl peptide (e), GNPs modified with 4-MPy and PS DNA strands and unphosphorylated Abl peptide (f), iDCL GNP probes (g), GNPs modified with 4-MPy and CS DNA strands and the synthetic phosphorylated Abl peptide (h), GNPs modified with 4-MPy and PS DNA strands and the synthetic phosphorylated Abl peptide (i) and i-motif DNA cross-linked GNPs modified with 4-MPy, CS/PS DNA and the synthetic phosphorylated Abl peptide (j).

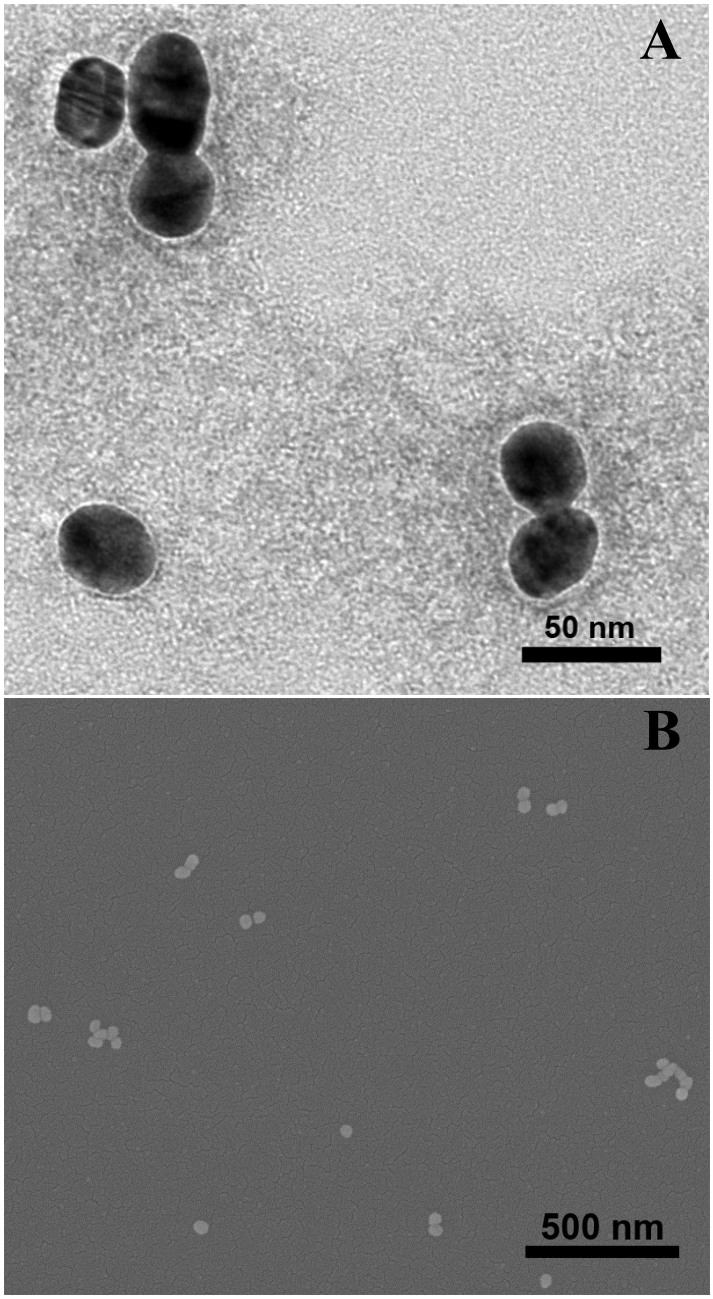

Figure S8. TEM (A) and SEM (B) images of iDCL GNP probes. The obtained images show the formation of the networks of modified GNP probes.

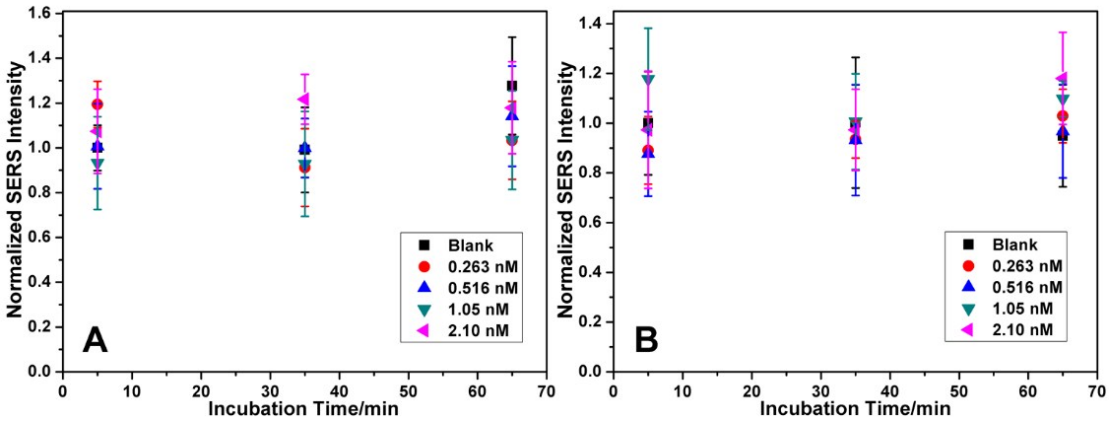

Figure S9. Normalized SERS intensity from i-motif DNA cross-linked GNPs modified without peptide (A), and Hck peptides (B) mixed with various concentration of abl kinase. [mean ± SD, n = 3] It can be seen that with various Abl kinase, there is no obvious difference observed between the blank and samples with Abl kinase. Meanwhile, the results also indicate that i-motif DNA will not specifically interact with Abl kinase in our detection strategy.

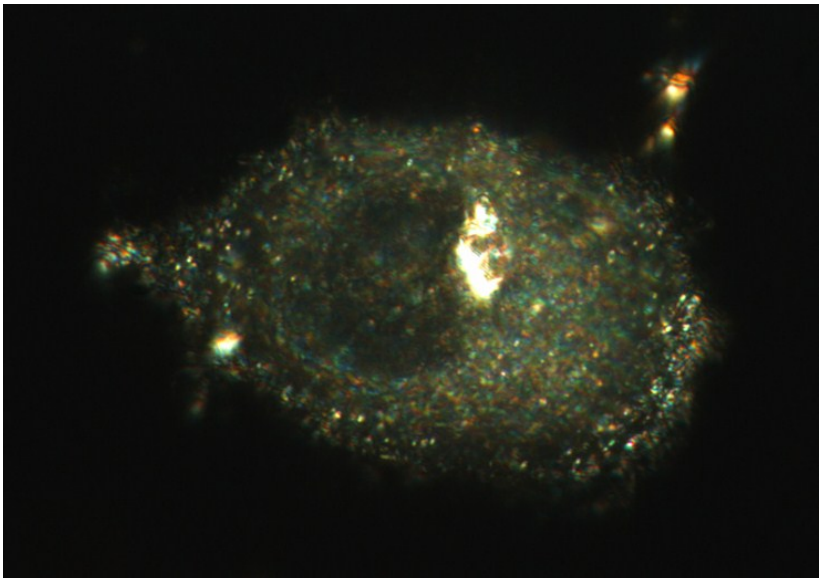

Figure S10. A typical dark field image of live PC3 cell incubated with iDCL GNP probes for 4h.

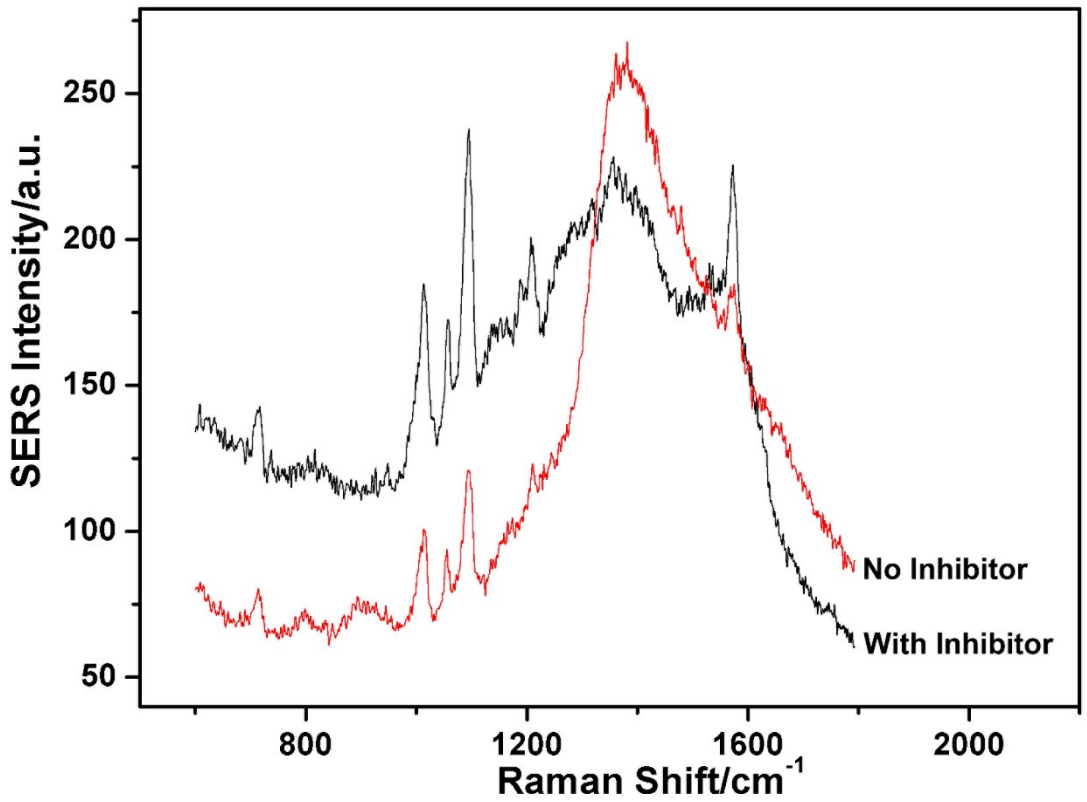

Figure S11. Typical SERS spectra of iDCL GNP probes in live PC3 cells treated with and without the inhibitor. It can be noted that the obtained SERS spectra is mainly assigned to the iDCL GNP probes, while no signal from the bio-molecules in live cells is observed in the SERS spectra, indicating that the bio-molecules in live cells will not influence the detection.

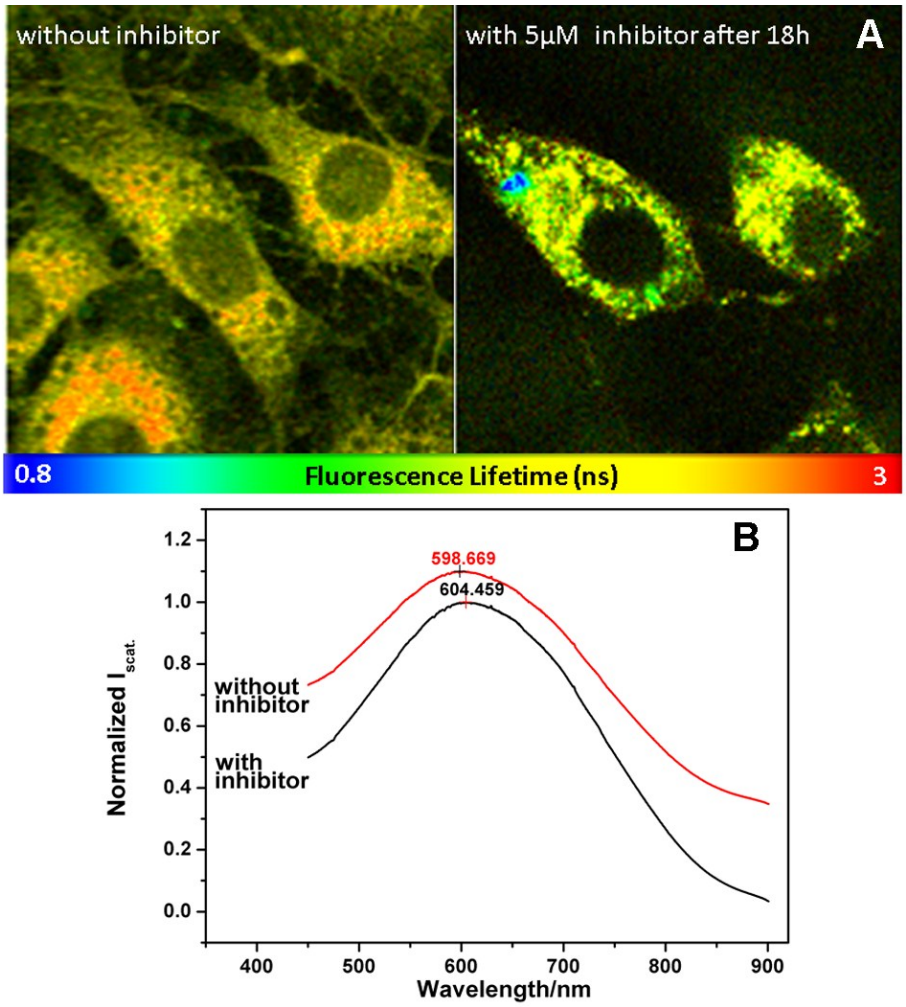

Figure S12. Fluorescence lifetime image of live cells with peptide sensors treated with and without the inhibitor, imatinib (A) and average scattering spectra of iDCL GNP probes in live cells with peptide sensors treated with and without the inhibitor (B) [n=9]. Fluorescence lifetime images show the inhibition of phosphorylation depicted by a decrease in lifetime in live cells with imatinib, consistent with our prior work [N. P. Damayanti, L. L. Parker and J. M. K. Irudayaraj, Angew. Chem. Int. Ed. Engl., 2013, 52, 3931-3934.] With live cells treated with/without inhibitor, the blue shift in the scattering spectra demonstrate the higher response level of iDCL GNP probes to phosphorylation in the cells without inhibitor.