Supplementary Material (ESI) for Analytical Methods

Supporting Information:

Screening Kinase Inhibitors with Microarray-Based Raman Spectroscopic Assay

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Supporting Figures S1-S7

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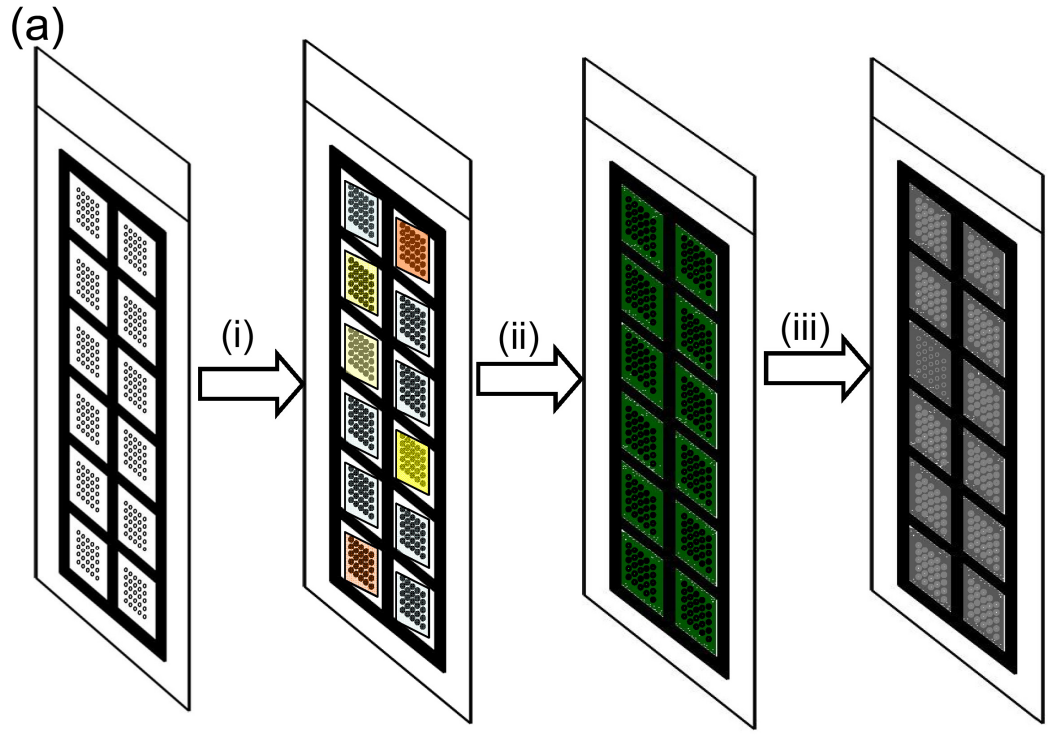

(b)

negative -control	A11	B2	B4	C7	C8
D9	F9	F5	H11	A1	positive-control

Figure S1. (a) Schematic representation of the assay used to screen kinase inhibitors. Each slide was divided into 12 independent 5 × 5 subarrays by a PTFE masker, which includes negative-control reactions without added compounds and positive-control reactions without kinase to set high and low boundaries for SRES signals, respectively. A primary screen was carried out in the subarray at a single concentration (10 μM) of each compound to identify inhibitors which induce kinase inactivity.

Reaction steps: (i) subjected reaction mixtures (ATP, kinase + ATP or kinase +ATP+ compound) to the subarray; (ii) recognized by biotinylated antibody, labeled by

avidin-fluorescein; and (iii) attached by peptide-stabilised gold nanoparticles, applied by silver enhance and obtained SERS signal by Raman spectrophotometer.

(b) The inhibitions of kinase PKA with selected 10 compounds from the EMD library are shown as an example.

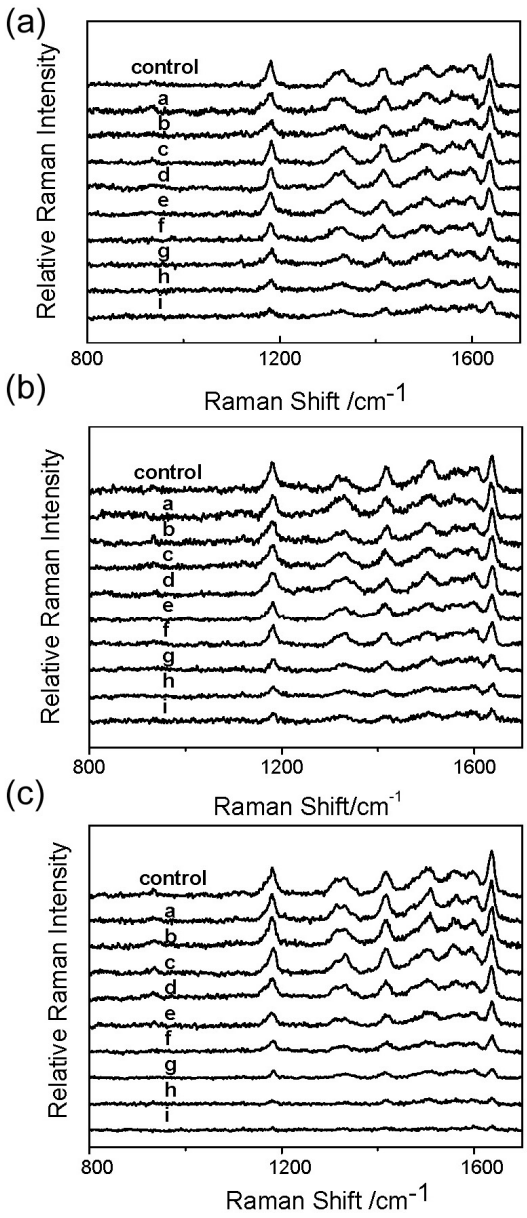

Figure S2 Corresponding SERS on the effect of various concentrations of inhibitors (a) C7, (b) F9 and H11 in the PKA solution. The concentrations of inhibitors are increased from curve a to i.

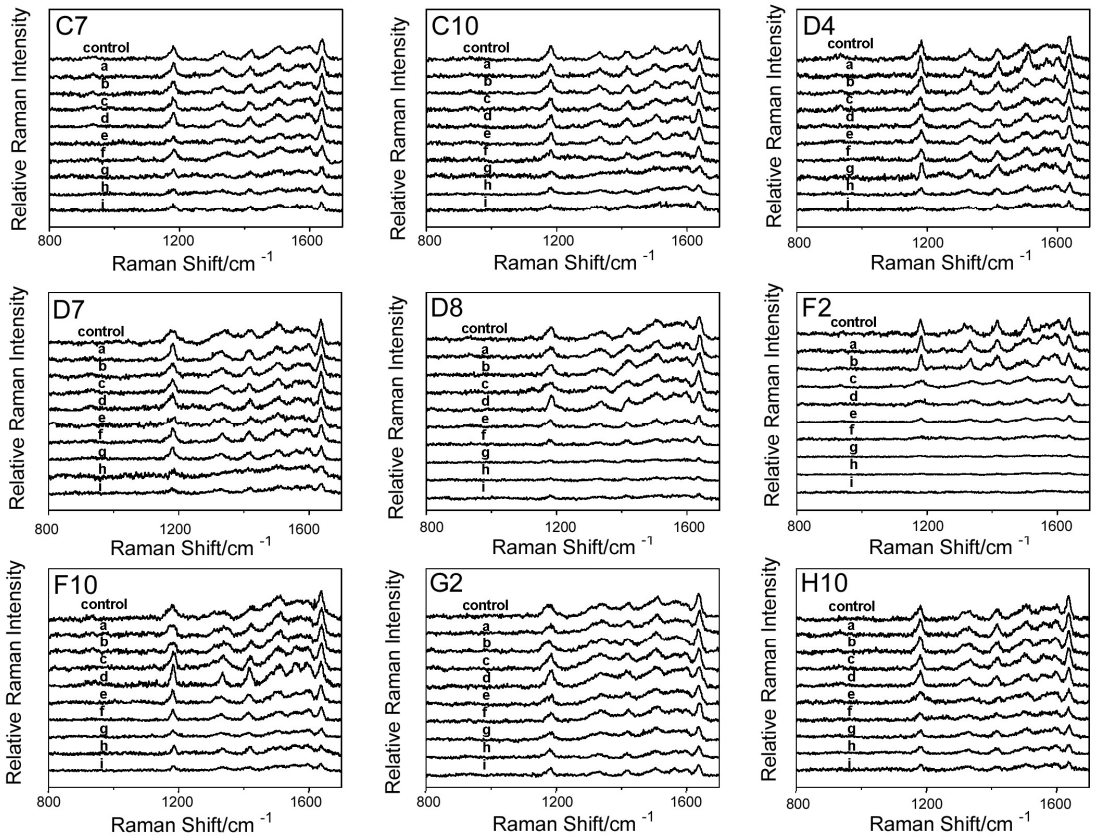

Figure S3 Corresponding SERS on the effect of various concentrations of inhibitors (as shown in the figure) in the LCK solution. The concentrations of inhibitors are increased from curve a to i.

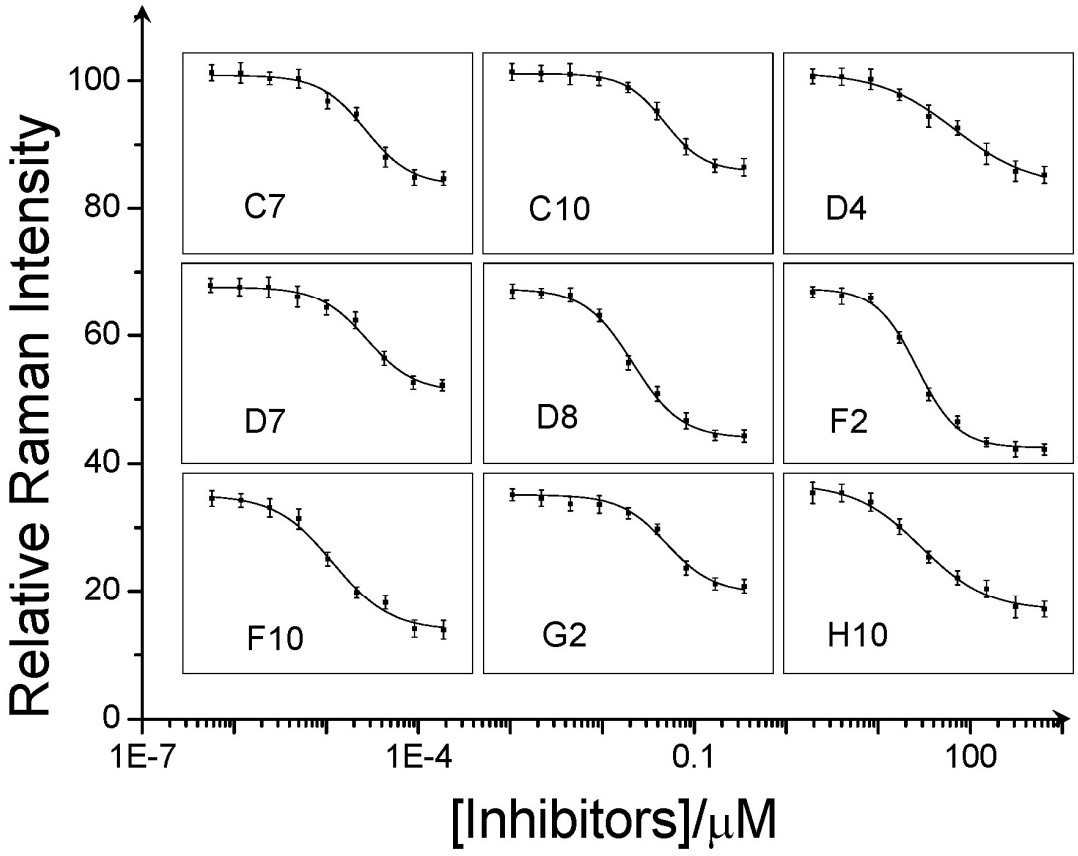

Figure S4 The IC_{50} curves for three LCK inhibitors. The signals have been corrected for background noise (positive control) and normalized to the average SERS intensity at 1638 cm^{-1} obtained in the absence of inhibitors (negative control). All of IC_{50} curves have similar scales as shown in the graph.

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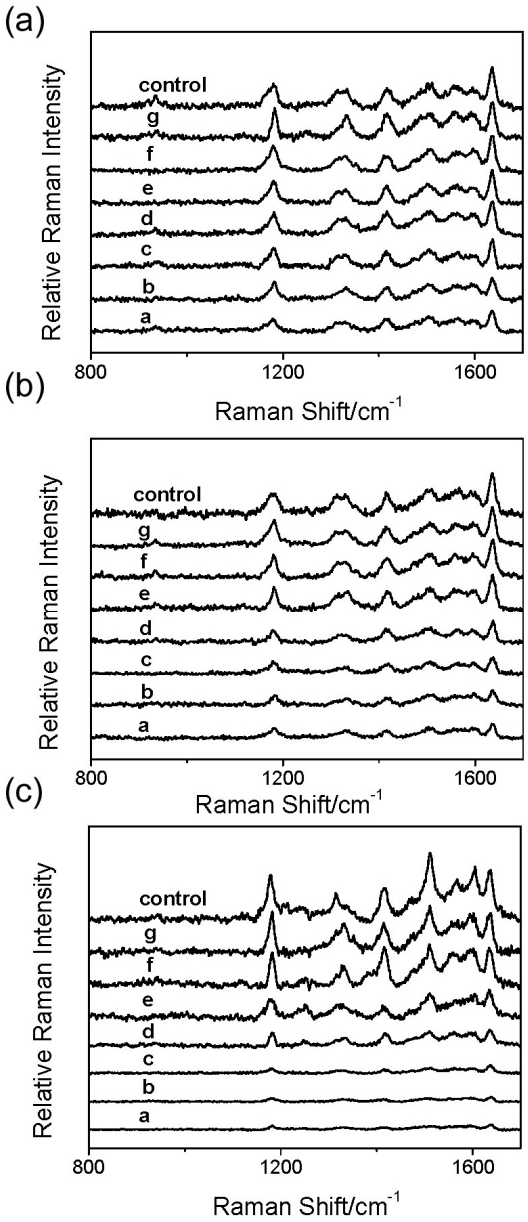

Figure S5 The effect of various concentrations of ATP in the PKA solution on SERS spectra of the peptide microarrays. The PKA solution contains 10 μM inhibitors ((a) C7, (b) F9 and H11), respectively. The concentrations of ATP are increased from curve a to g.

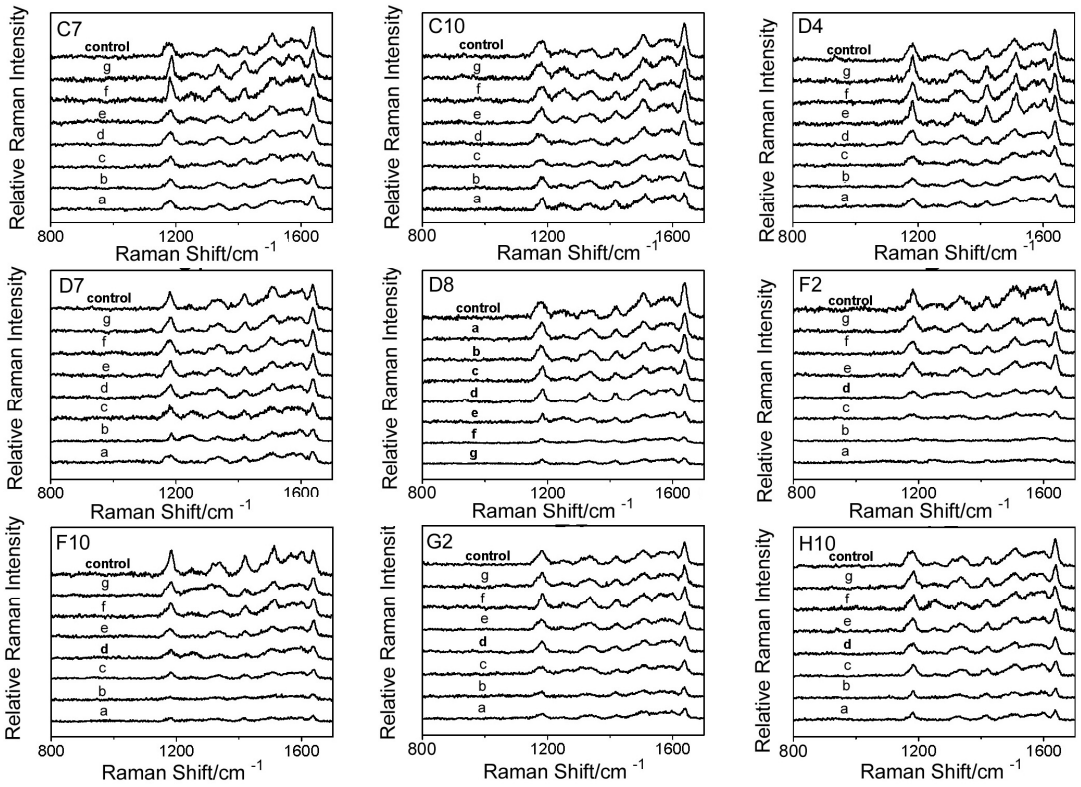

Figure S6 The effect of various concentrations of ATP in the LCK solution on SERS spectra of the peptide microarrays. The LCK solution contains 10 μM inhibitors (as shown in the figure), respectively. The concentrations of ATP are increased from curve a to g.

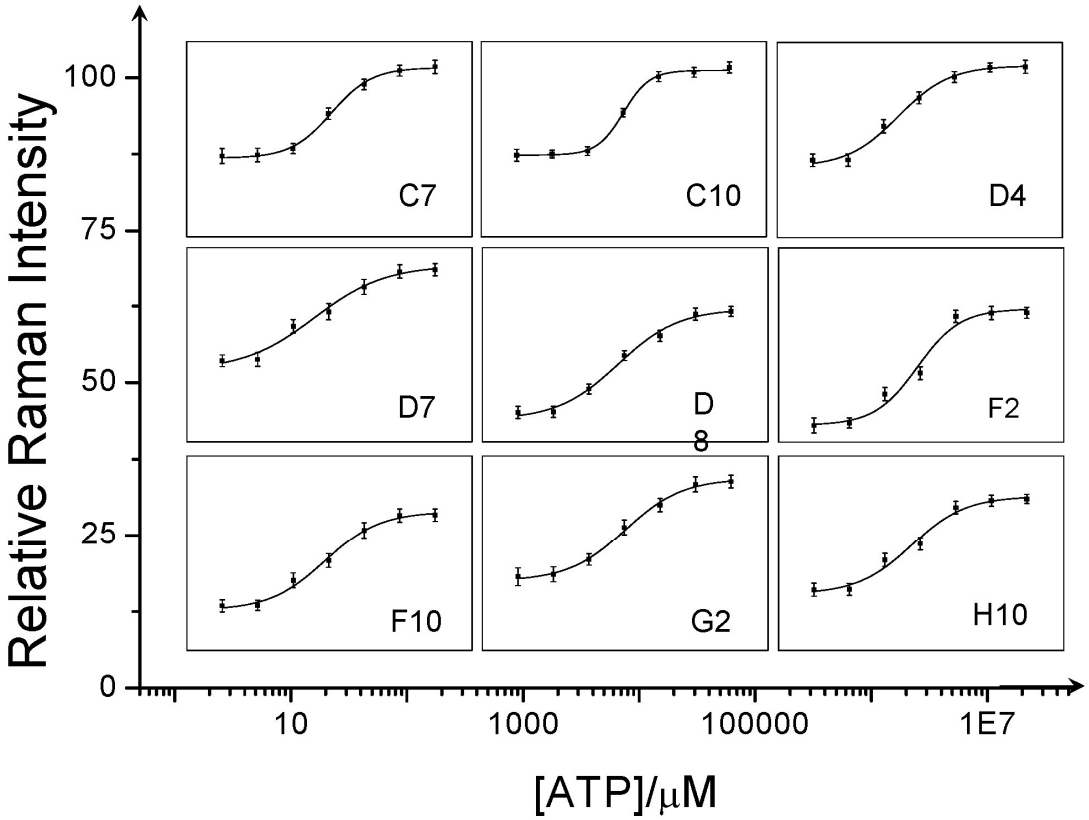

Figure S7 The effect of various concentrations of ATP in the LCK solution. The LCK solution contains 10 μM inhibitors (as shown in the figure), respectively. The signals have been corrected for background noise (positive control) and normalized to the average SERS intensity at 1638 cm^{-1} obtained in the absence of inhibitors (negative control). All of curves have similar scales as shown in the graph.