

**Electronic SI for**

**Ultrasensitive Raman sensing of alkaline phosphatase  
activity in the serum based on an enzyme-catalyzed reaction**

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## 1. Materials and instruments

Alkaline phosphatase, fosfosal, salicylic acid and sodium orthovanadate ( $\text{Na}_3\text{VO}_4$ ) were purchased from Aladdin Industrial Corporation (Shanghai, China). Phosphodiesterase (PDE), acid phosphatase (ACP), trypsin and lysozyme were obtained from Sigma-Aldrich. Other chemicals of analytical grade were obtained from Sinopharm Chemical Reagents (Beijing, China). Bovine serum (wt 1%) was purchased from Shenyang Huibai Biotechnology Co., Ltd. Ultraviolet-visible (UV-vis) spectra were acquired on a Shimadzu UV-3600 spectrophotometer with the 1.0 cm path length quartz cell. Raman measurement was carried out by a Horiba J-Y Aramis spectrometer.

## 2. Raman measurements for ALP

The reaction solutions were dropped on the 1 cm×1 cm silicon pellet to form a spot with a diameter of 1.5 mm. Then, Raman spectra of the dried solutions were measured by a confocal Raman system (LabRAM ARAMIS, HORIBA Jobin Yvon, USA) with a 7 mW/633 nm laser as an excitation source and actualized by Synapse Thermoelectric cooled chargecoupled device (CCD) camera (HORIBA Jobin Yvon, USA). Laser excitation and Raman scattering light collection were through a ×50 microscope objective lens (numerical aperture = 0.5, LMLFLN, Olympus, Japan). The outstretched scan spectra with a spectral range from 200 to 1800  $\text{cm}^{-1}$  were obtained with an integration time of 5 s and one accumulation. The 520.7  $\text{cm}^{-1}$  vibrational band of silicon wafer was used as a reference for the wave number calibration. The Raman spectra of the salicylic acid produced from the specific

catalytic reaction between fosfosal and ALP were detected. The signal intensities of salicylic acid at 1326  $\text{cm}^{-1}$  were recorded for different concentrations of ALP to make a working curve.

### 3. Selectivity

We selected other enzymes, such as, phosphodiesterase (PDE), acid phosphatase (ACP), trypsin and lysozyme to investigate the selectivity of fosfosal to ALP. Under the optimal conditions that 7.5 of pH, 35°C of temperature, 10 mM of fosfosal and 30 min of reaction time, the concentrations of the interference enzymes including phosphodiesterase (PDE), acid phosphatase (ACP), trypsin and lysozyme were all 10 U/L. The intensities of the peak at 1326  $\text{cm}^{-1}$  were obtained from the Raman spectra of all the enzymes and they were plotted for comparison.

### 4. Band assignments

Table S1. The Raman vibrational band assignments of fosfosal and SA.

Peak position ( $\text{cm}^{-1}$ ) of fosfosal	Assignment <sup>[1]</sup>	Relative intensity	Peak position ( $\text{cm}^{-1}$ ) of salicylic acid	Assignment <sup>[2]</sup>	Relative intensity
741	$\nu$ P-O	s	566	$\gamma$ O-H	m
773	$\gamma$ C-H	w	773	$\gamma$ C-H	s
930	$\nu$ P-O-C	w	1032	$\delta$ C-H	s
1047	$\delta$ C-H	s	1154	$\nu$ C=O	m
1163	$\nu$ C=O	m	1247	$\nu$ C=C	s
1221	$\nu$ C=C	s	1326	$\nu$ C-H	m
1303	$\nu$ C-H	m	1387	$\delta$ O-H	w
1608	$\nu$ C-C	w	1472	$\delta$ O-H	m

1637	Ar-COOH	w	1582	$\nu$ C-C	w
			1635	Ar-COOH	m

## 5. Optimization of temperature, pH and catalytic reaction time

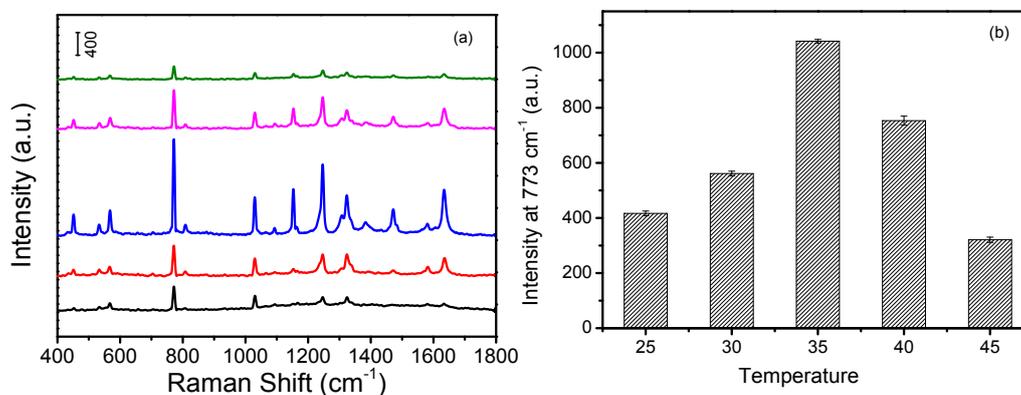


Fig. S1(a) Raman spectrum of mixed solution at different temperature (from bottom to top are 25, 30, 35, 40, 45 °C), while the reaction time kept at 30 min and the pH of mixed solution was set as 7.5 (b) Plot of Raman intensity at 773 cm<sup>-1</sup> with the temperature.

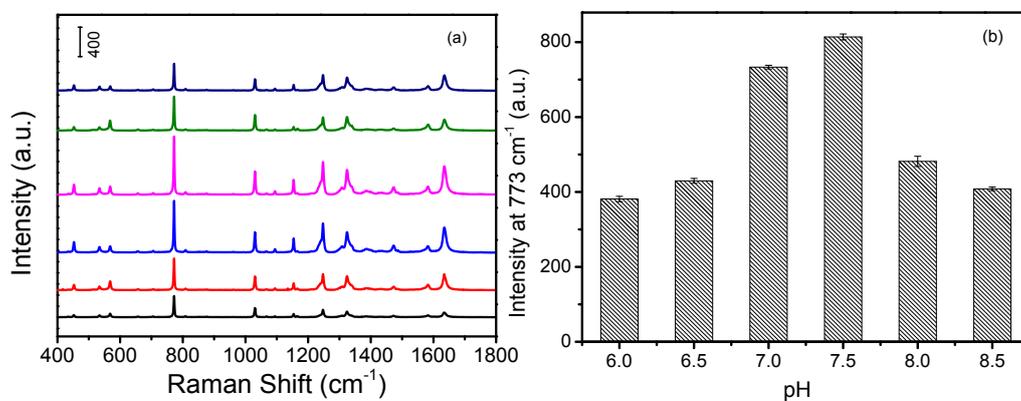


Fig. S2(a) Raman spectrum of mixed solution at different pH (from bottom to top are 6.0, 6.5, 7.0, 7.5, 8.0, and 8.5, respectively) when the reaction time was set as 30 min (b) Plot of Raman intensity at 773 cm<sup>-1</sup> with the pH.

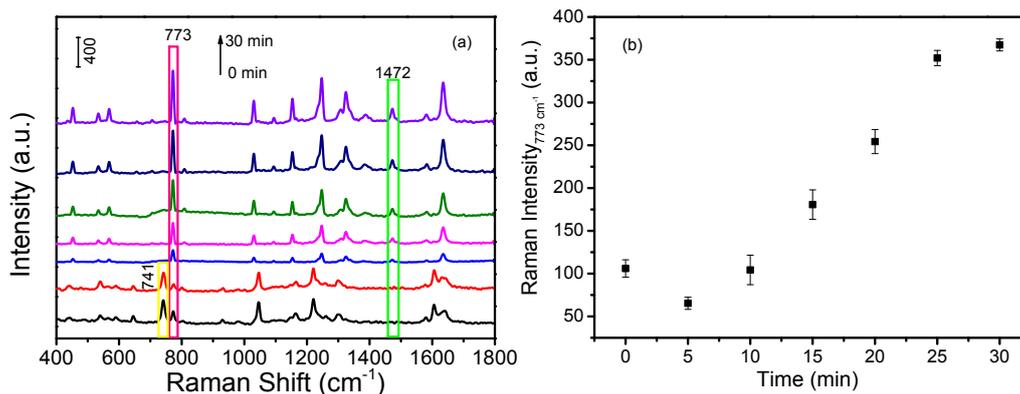


Fig. S3(a) Raman spectrum of mixed solution at different reaction time (from bottom to top are 0, 5, 10, 15, 20, 25 and 30 min, respectively) (b) A plot of the Raman intensity of mixed solution at 773  $\text{cm}^{-1}$  along with reaction time.

## 6. Lowest detectable concentration

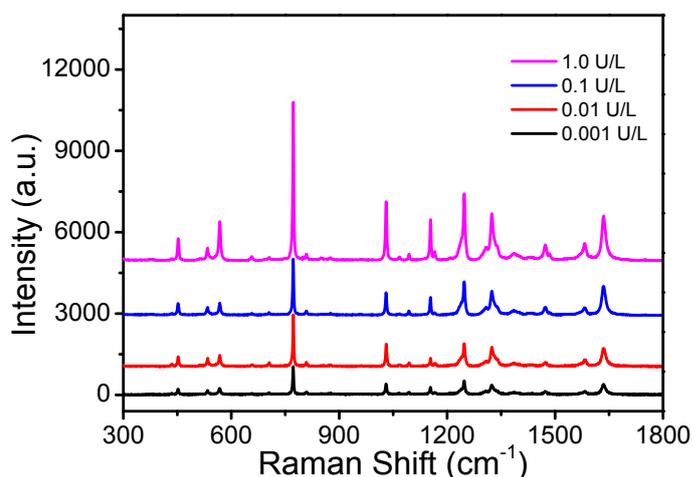


Fig. S4 Raman spectra for sensing different concentrations of ALP solutions. The lowest detectable concentration is 0.001 U/L (signal to noise ratio= 3:1).

## 7. Reproducibility of Raman detection

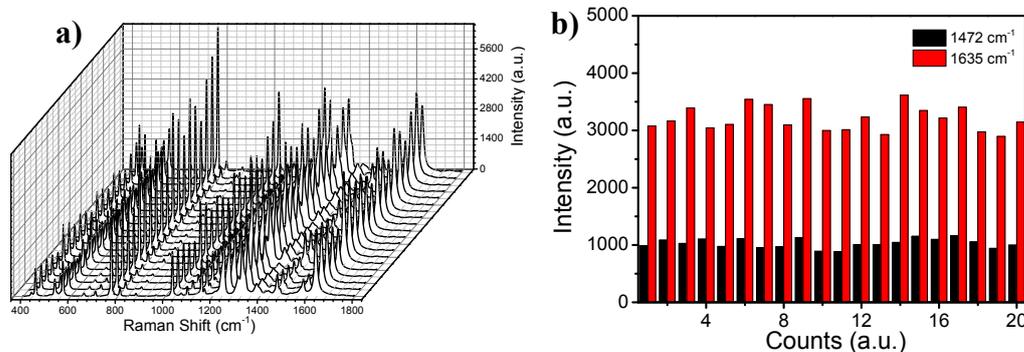


Fig. S5(a) Raman spectra from randomly selected 20 points on the same sample. (b)

The intensities of Raman peaks at 1472 and 1635  $\text{cm}^{-1}$  obtained from data (a).

### References

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- 2 S. S'anchez-Cort'es and J. V. Garc'ia-Ramos, *J. Colloid Interface Sci.*, 2000, **231**, 98–106.