Supporting Information for Publication

A novel graphene-based label-free fluorescence 'turn-on' nanosensor for selective and sensitive detection of phosphorylated species in biological samples and living cells

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Table of contents **Pages**

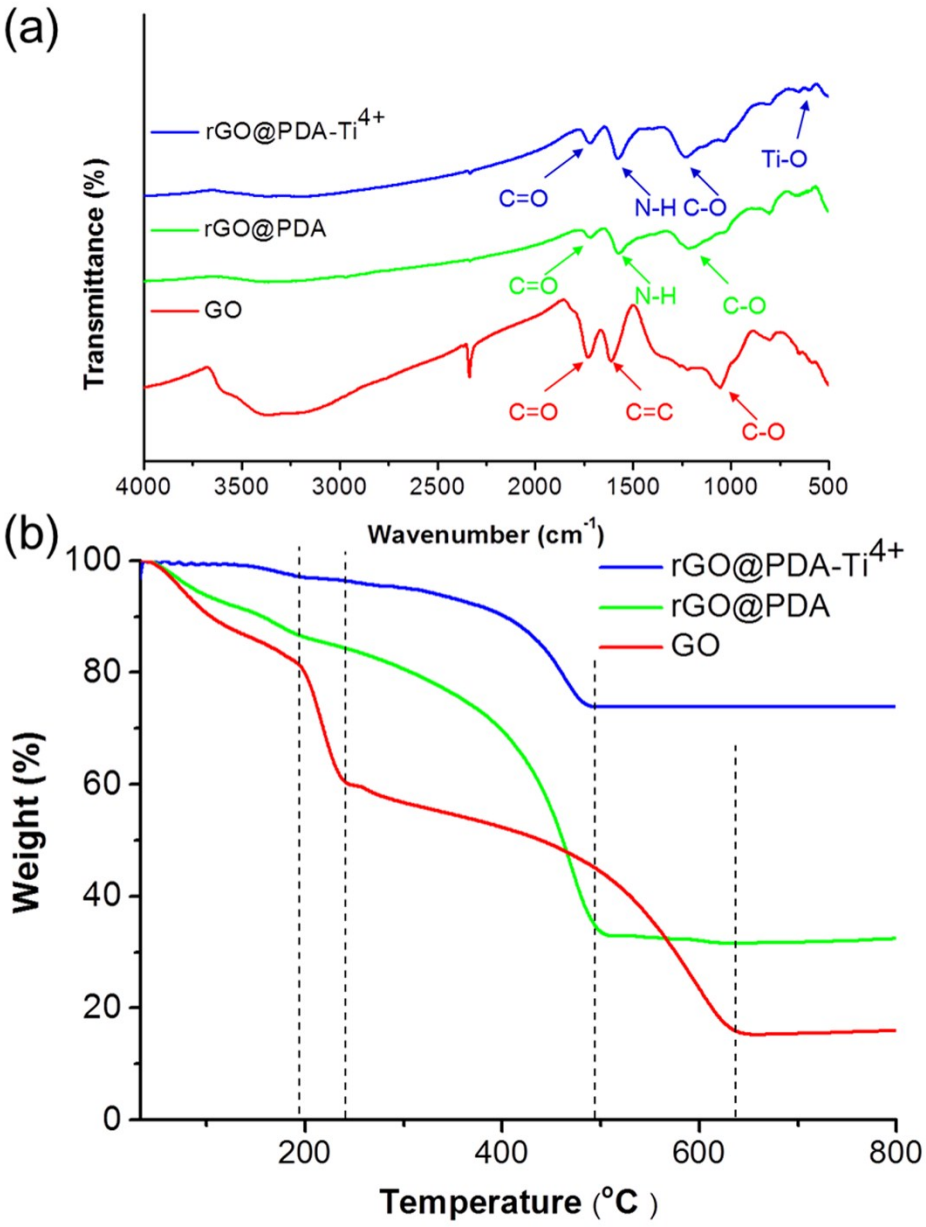

Fig. S1. (a) FTIR spectra of GO, rGO@PDA and rGO@PDA-Ti^{4+} (b) TGA curves of GO, rGO@PDA and rGO@PDA-Ti^{4+}

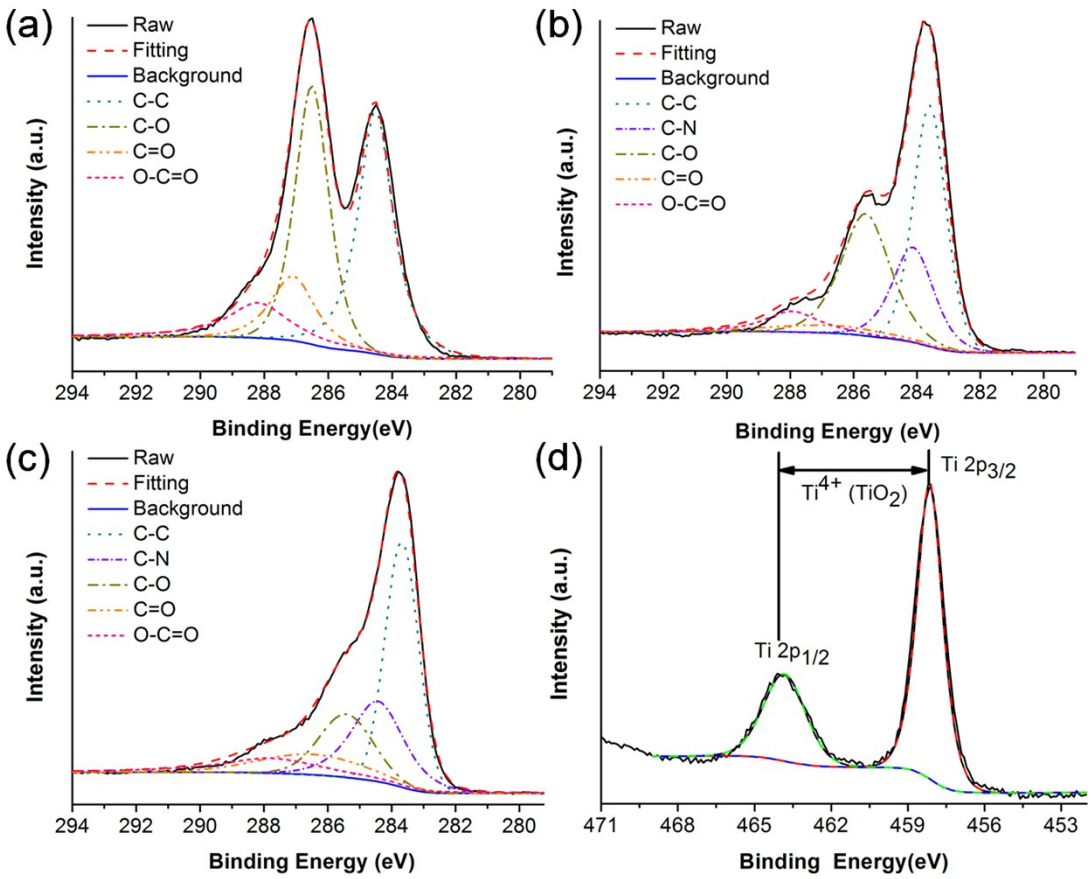

Fig. S2. XPS C1s core-level spectra and fitted curves of (a) GO, (b) rGO@PDA, and (c) rGO@PDA-Ti^{4+}.Ti 2p core-level spectra and fitted curves of (d) rGO@PDA-Ti^{4+}.

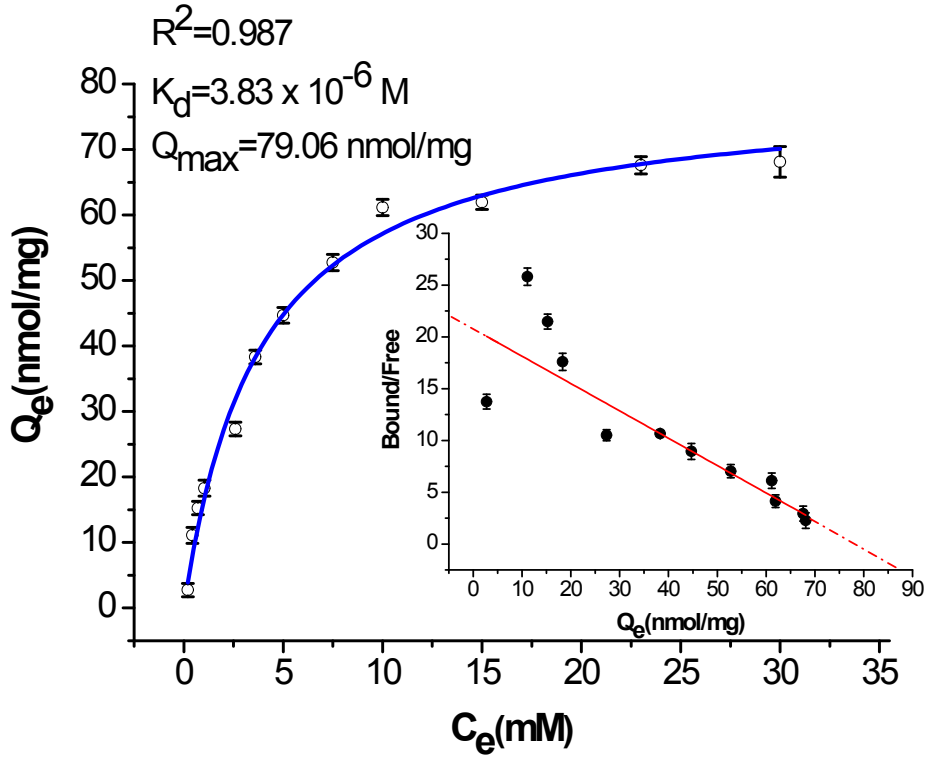

Fig. S3. Equilibrium adsorption isotherm of FMNs binding onto rGO@PDA-Ti^{4+} surface in acidic buffer (pH 4.5). Inset: Scatchard plot for the adsorption of FMNs by rGO@PDA-Ti^{4+}. The mean values of three independent measurements are presented.

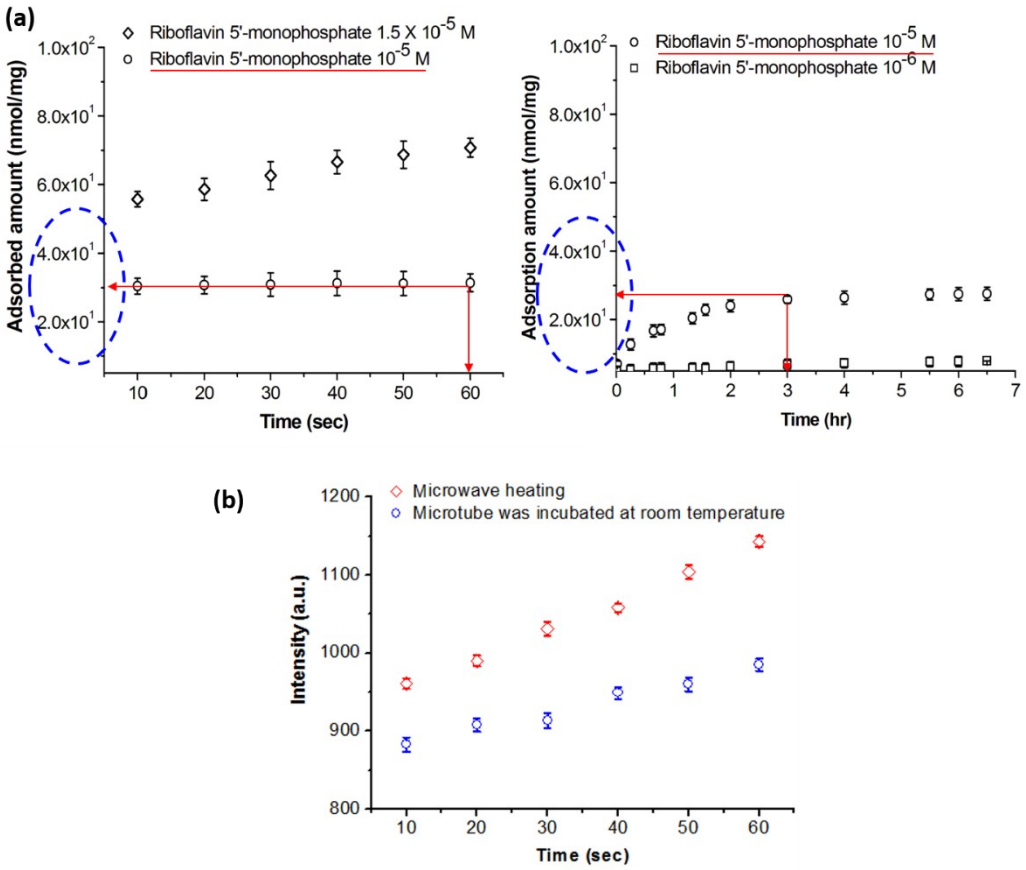

Fig. S4. (a) Left panel: the microwave-assisted adsorption of FMNs (10^{-5} to 1.5×10^{-5} M; marked as diamonds and circles) onto rGO@PDA-Ti^{4+} (10 mg/mL, 5 µL) surface in acidic buffer (pH 4.5) as a function of time; right panel: adsorption of FMNs (10^{-5} to 10^{-6} M; marked as circles and squares) onto rGO@PDA-Ti^{4+} (10 mg/mL, 5 µL) surface in acidic buffer (pH 4.5) under ambient condition as a function of time (b) The changes in the fluorescence intensity (λ_{em} 530 nm, λ_{ex} 450 nm) of rGO@PDA-Ti^{4+}-FMNs (10 mg/mL, 5 µL) after incubation with tryptic digest of α-casein (50 µL, 10^{-5} M) under different conditions as a function of time.

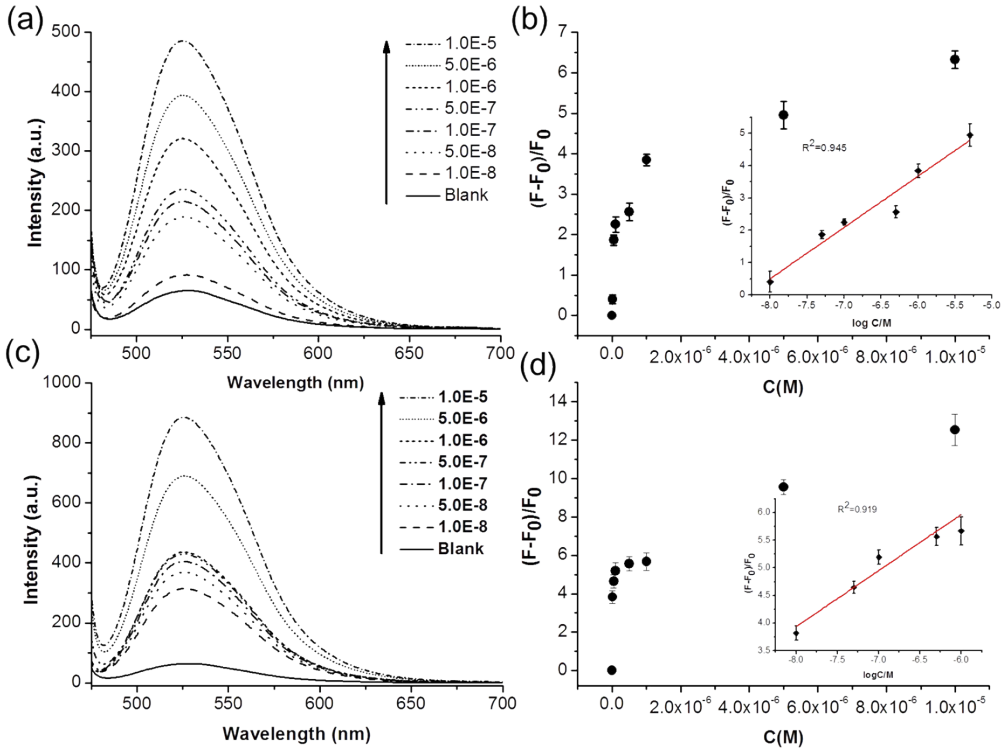

Fig. S5. Fluorescence emission spectral changes (λ_{ex} 450 nm) of rGO@PDA-Ti^{4+}-FMNs (10 mg/mL, 5 μL) at λ 530 nm after incubation with the tryptic digests of (a) β-casein and (c) ovalbumin (0, 0.01, 0.05, 0.1, 0.5, 1.0, 5.0, 10.0 μM each) under microwave heating for 1 min. (b) and (d) corresponding plots of the relative intensity ($(F-F_0)/F_0$) of rGO@PDA-Ti^{4+}-FMNs as a function of the β-casein and ovalbumin concentrations, respectively. Inset: corresponding calibration curves obtained in the linear range (0.05~10 μM and 0.01~1.0 μM) for β-casein and ovalbumin, respectively. Three replicates were conducted for each experiment.

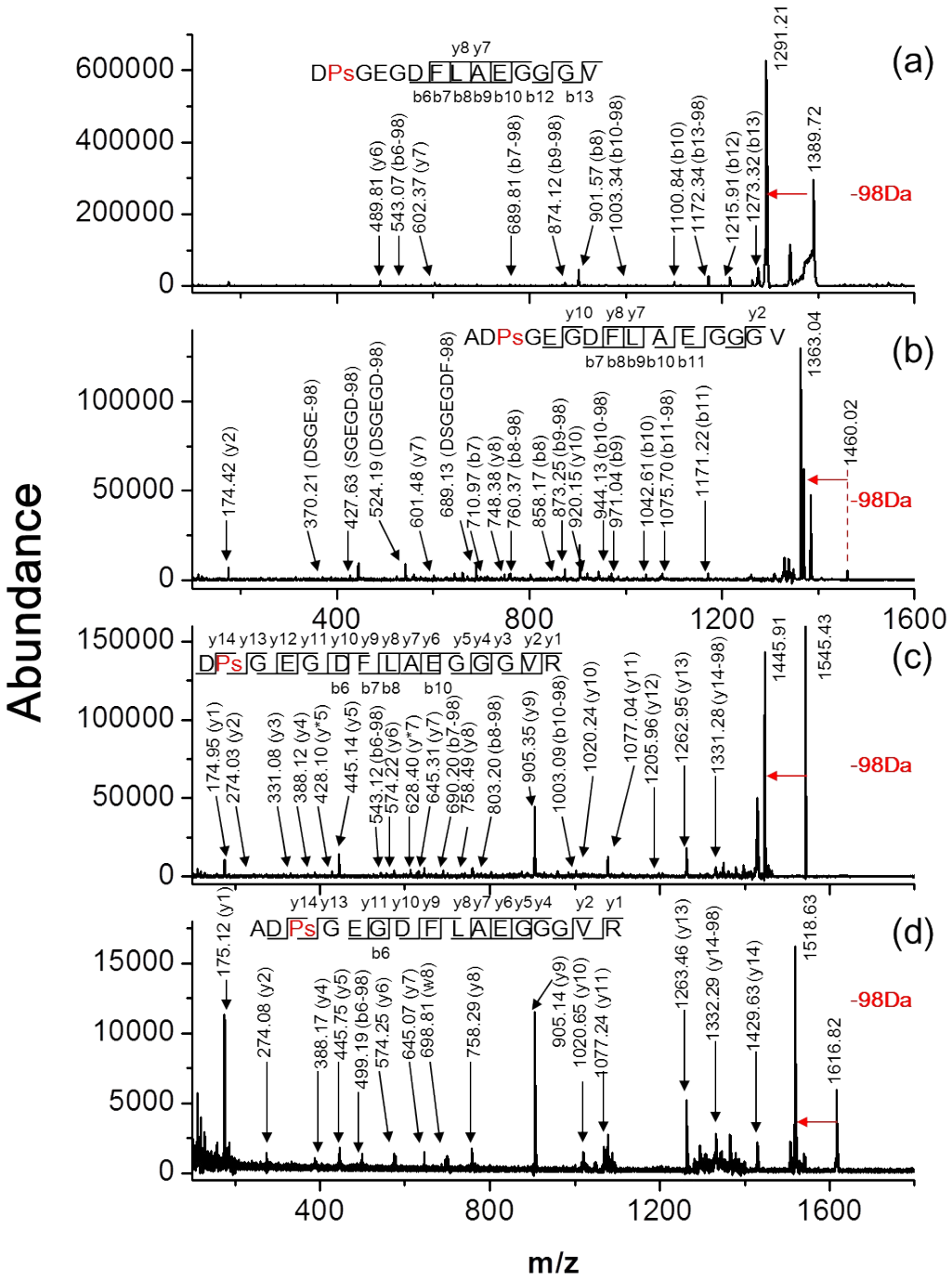

Fig. S6. MALDI-TOF/TOF MS/MS spectra of precursor ions of peptides from human serum at m/z (a) 1389.72, (b) 1460.02, (c) 1545.43, and (d) 1616.82. Each of these precursor ions indicate the presence of the phosphorylated fragment ion adjacent to the parent ion with a mass difference of 98 Da (due to the elimination of Phosphoric acid) in MS/MS spectra.

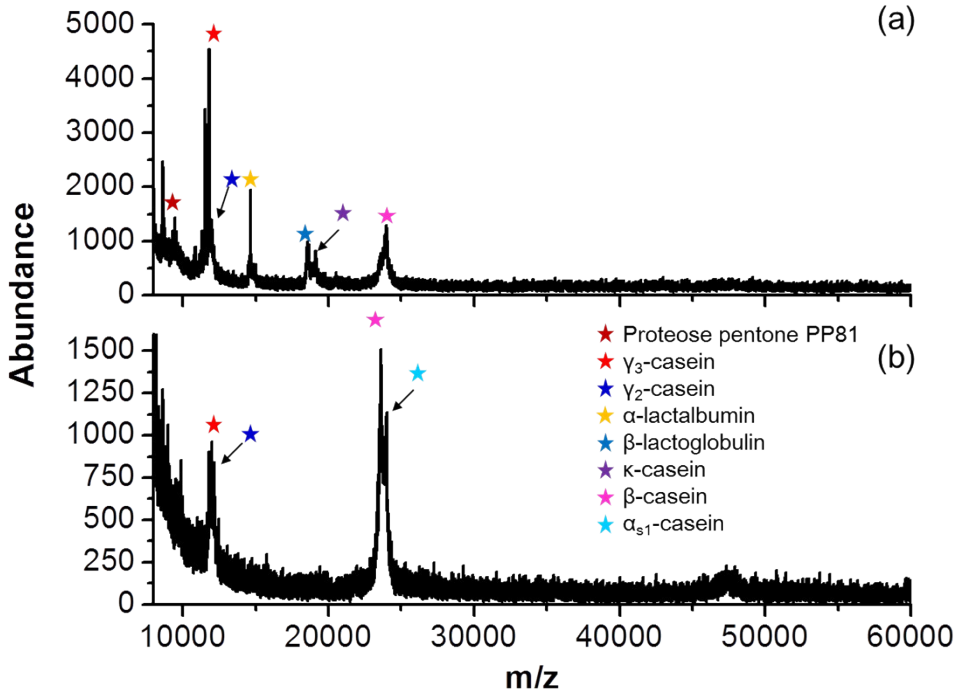

Fig. S7. MALDI-TOF mass spectra of a non-fat milk sample: (a) without any treatment, (b) after trapping the target species (rGO@PDA-Ti^{4+}-Ps) using rGO@PDA-Ti^{4+}-FMNs nanosensor.

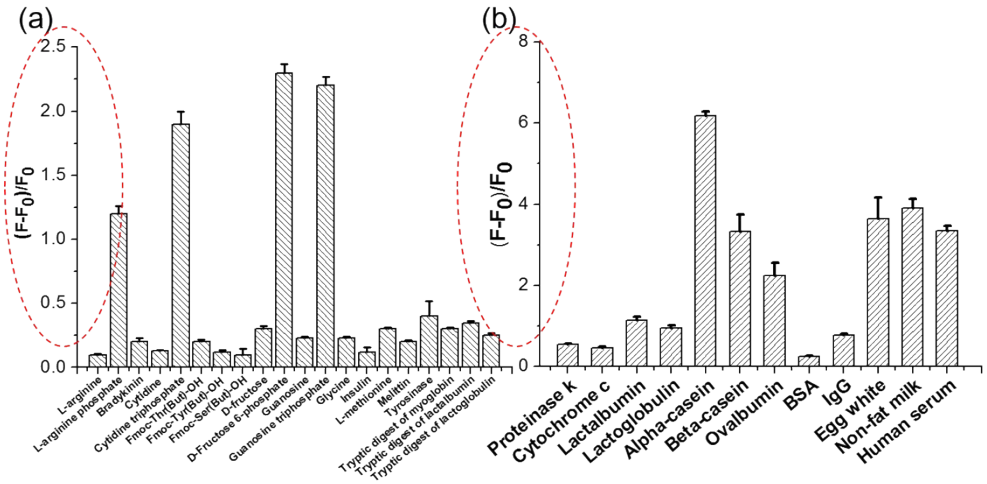

Fig. S8. (a) Bar graph showing the relative fluorescence intensity from supernatants of rGO@PDA-Ti^{4+}-FMNs (10 mg/ml, 5 μL) at λ 530 nm after incubation with a variety of biomolecules (10^{-5} M, 50 μL, containing Ps or without Ps) under microwave heating (1 min) and subsequent centrifugation for 15 min. (b) Bar graph showing the relative fluorescence intensity from supernatants of rGO@PDA-Ti^{4+}-FMNs (10 mg/ml, 5 μL) at λ 530 nm after incubation with a variety of protein (10^{-5} M, 50 μL) and real samples (50 μL) under similar conditions as mentioned above. F stands for the fluorescence intensity of the resulting supernatant, F_0 represents the fluorescence intensity of the blank (only containing rGO@PDA-Ti^{4+}-FMNs). Error bars are based on three consecutive measurements.

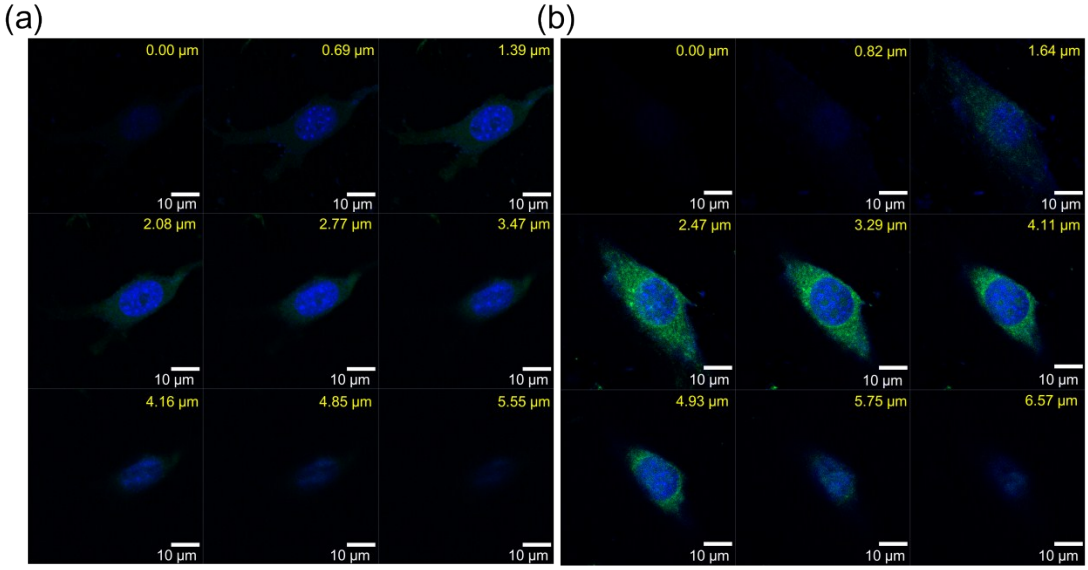

Fig. S9. The optical sections of the Tramp-C1 cells treated with (a) FMNs and (b) rGO@PDA-Ti^{4+}-FMNs. The experiments were conducted on the Carl-Zeiss confocal laser scanning microscopy.

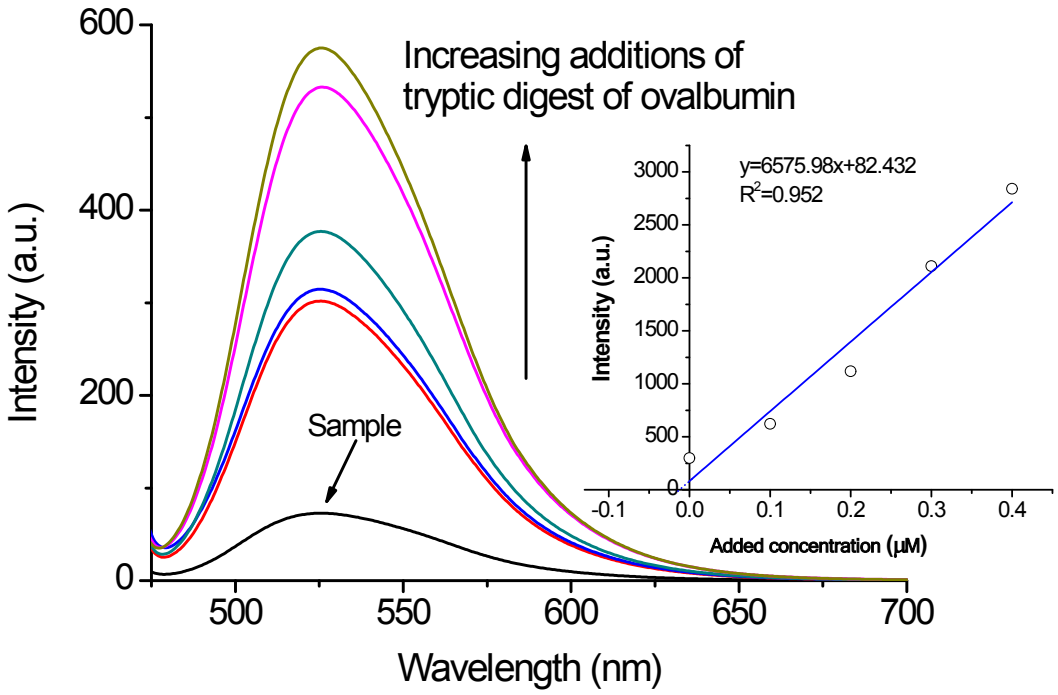

Fig. S10. The standard addition experiment for the tryptic digest of egg white sample.

Table S1. Data for LOD values for different proteins

Proteins	Slope	Intercept	LOD (M)	R^2
α-casein	6.0696	46.0872	1.185×10^{-7}	0.971
β-casein	1.5922	13.2344	2.89×10^{-8}	$R^2 = 0.945$
ovalbumin	1.0154	12.0593	5.48×10^{-8}	$R^2 = 0.919$

Table S2. Detailed information of peptide ion peaks with phosphorylated and dephosphorylated fragments observed in the MALDI-TOFMS spectra from tryptic digest of non-fat milk after trapping by rGO@PDA-Ti^{4+}-FMNs nanosenosr.

Observed $[M+H]^+$	Theoretical $[M+H]^+$	Sequences	Dephosphorylated fragment $[-nHPO_3]$
1253.42	1253.24	TVDMME[Ps]TEVF (α-S2/153-162)	-
1660.83	1660.79	VPQLEIVPN[Ps]AEER (α-S1/106-119)	-
1951.90	1951.95	YKVPQLEIVPN[Ps]AEER (α-S1/104-119)	1872.13
2061.77	2061.83	FQ[Ps]EEQQQTEDELQDK (β/33-52)	-
2618.78	2618.90	NTMEHV[Ps] [Ps] [Ps] EESII[Ps]QETYK (α-S2/2-21)	2538.83, 2458.49, 2378.87
2966.35	2966.16	ELEELNVPGEIVE[Ps]L[Ps] [Ps] [Ps] EESITR (β/2-25)	-
3477.46	3477.85	RELEELNVPGEIVE[Ps]L[Ps] [Ps] [Ps]EESITRINK (β/15-43)	-

Ps respresents the phosphorylated species

Table S3. Detailed information of peptide ion peaks with phosphorylated and dephosphorylated fragments observed in the MALDI mass spectrum from tryptic digest of chicken egg white after trapping by rGO@PDA-Ti^{4+}-FMNs nanosensor.

Observed $[M+H]^+$	Theoretical $[M+H]^+$	Sequences	Dephosphorylated fragment $[-nHPO_3]$
2088.79	2088.91	EVVG[Ps]AEAGVDAASVSEEFR (340-359)	2008.83
2512.17	2512.12	LPGFGD[Ps]IEAQCGTSVNVHSSLR(62-84)	-
2902.78	2902.31	FDKLPGFGD[Ps]IEAQCGTSVNVHSSLR (59-84)	2821.18

Ps respresents the phosphorylated species. C represents the carboxymethyl cysteine

Table S4. Detailed information of peptide ion peaks with phosphorylated and dephosphorylated fragments observed in the MALDI mass spectrum from human serum after trapping by rGO@PDA-Ti^{4+}-FMNs nanosensor.

Observed $[M+H]^+$	Theoretical $[M+H]^+$	Sequences	Dephosphorylated fragment $[-nHPO_3]$
1389.36	1389.51	D[Ps]GEGDFLAEGGGV Fibrinopeptide A (2-15)	1309.54
1460.29	1460.55	AD[Ps]GEGDFLAEGGGV Fibrinopeptide A (1-15)	-
1545.38	1545.61	D[Ps]GEGDFLAEGGGVR Fibrinopeptide A (2-16)	-
1616.41	1616.53	AD[Ps]GEGDFLAEGGGVR Fibrinopeptide A (1-16)	-

Ps respresents the phosphorylated species