Electronic Supplementary Information (ESI)

Graphene Oxide-Peptide Nanoassembly as A General Approach for Monitoring Activity of Histone Deacetylase

Ping Liang, Qing Li*, Zhan Wu, Jian-Hui Jiang*, Ru-Qin Yu

State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, P. R. China

*Corresponding author. E-mail: jianhuijiang@hnu.cn; liqing880523@hnu.edu.cn. Tel: 86-731-88821961; Fax : 86-731-88821961.

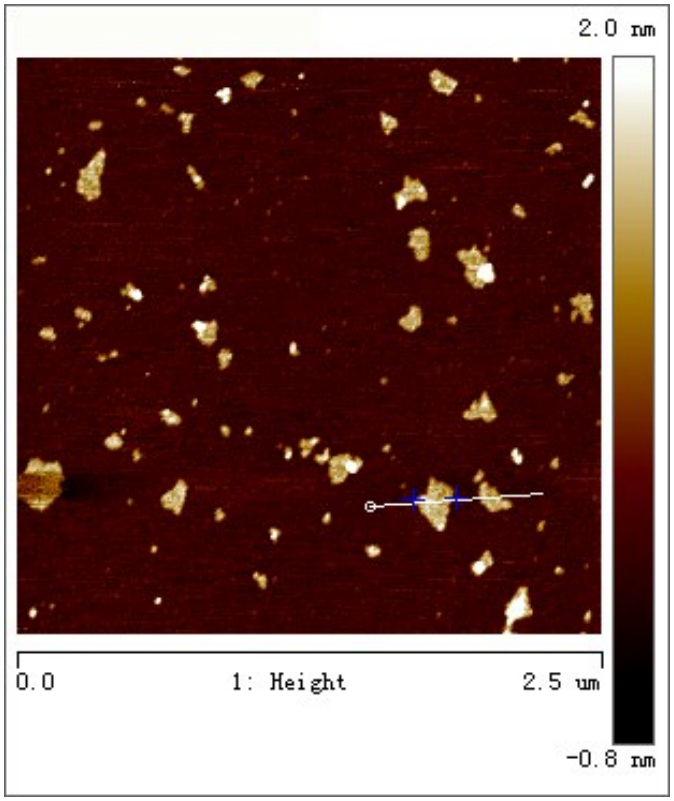
Experimental Section

Reagents and materials.

The peptide probe was synthesized by Beijing SciLight Biotechnology Ltd. Co. Graphene oxide (GO) was synthesized from natural graphite powder by modified Hummers method.1 Histone deacetylase 1 (HDAC 1) and its inhibitor trichostatin A (TSA) were obtained from Cayman Chemical Company (Michigan, USA). Endoproteinase rLys-C was purchased from Promega Corporation (Madison, USA). All other chemicals were of analytical grade and obtained from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). All solutions were prepared using ultrapure water, which was obtained through a Millipore Milli-Q water purification system (Billerica, MA, USA) and had an electric resistance >18.3 MΩ.

Detection of HDAC 1 Activity

In a typical assay, in a 50 μL reaction system containing 200 nM peptide probe was mixed with GO (12 μg/mL) for 5 min in 25 mM Tris-HCl (pH 8.0), 137 mM sodium chloride, 2.7 mM potassium chloride and 1 mM magnesium chloride, then addition of various concentration of HDAC 1 and 1μg/mL rLys-C. The mixture was incubated at 37 ºC for 60 min to allow a complete deacetylation and rLys-C cleavage reaction. The fluorescence spectra were measured at room temperature in a 100 μL quartz cuvette on a Fluorolog-Tau-3 spectrofluorometer (Jobin Yvon Inc., NJ) with the slit set to be 5 nm for both the excitation and the emission. The fluorescence emission spectra were collected from 505 nm to 600 nm at room temperature with a 494 nm excitation wavelength. Time-dependent fluorescence responses of HDAC 1 assay were performed with a time interval of 30 s in a 384-well black microplate on a Tecan Infinite M-1000 microplate reader. The excitation wavelength was 494 nm, and the emission wavelength was 518 nm with both excitation and emission bandwidths of 10 nm.



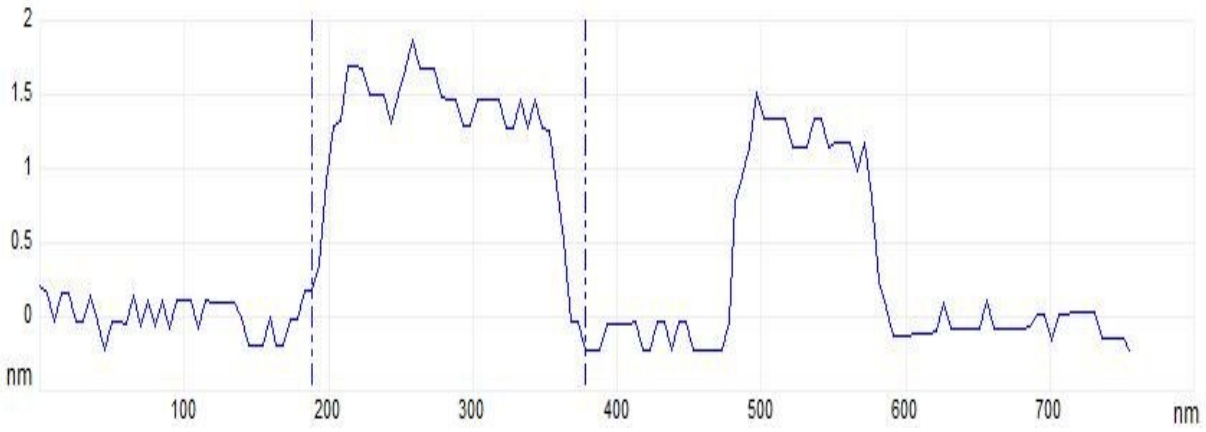


Fig. S1. AFM height image of GO deposited on mica substrates.

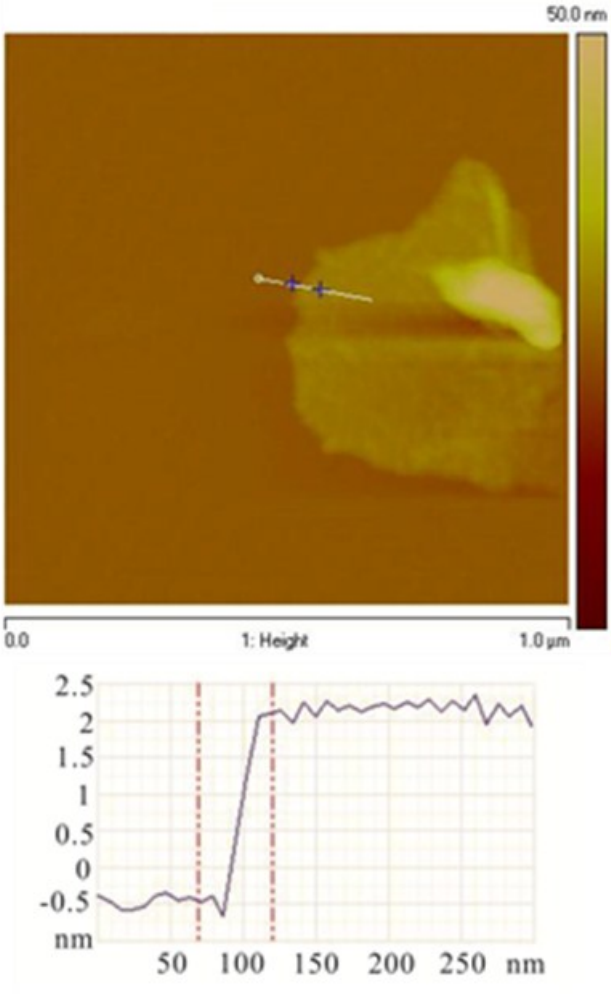


Fig. S2. AFM height image of GO-peptide nanoassembly deposited on mica substrate.

Investigation of peptide probe adsorption onto GO

Prior to the experiments of detection of HDAC activity, the peptide probe adsorption onto GO was studied by evaluating the fluorescence intensity responses to the different concentrations of GO at the fixed concentration of peptide probe. 200 nM peptide probe were added to the increasing concentrations of GO from 0 μg/mL to 28 μg/mL and incubated for 5 min at 37 °C. With the increasing concentration of GO, the fluorescence intensity of the peptide probe decreased and trended to a minimum value at 12 μg/mL (Fig. S3). Thus, 12 μg/mL GO was used in the following experiment.

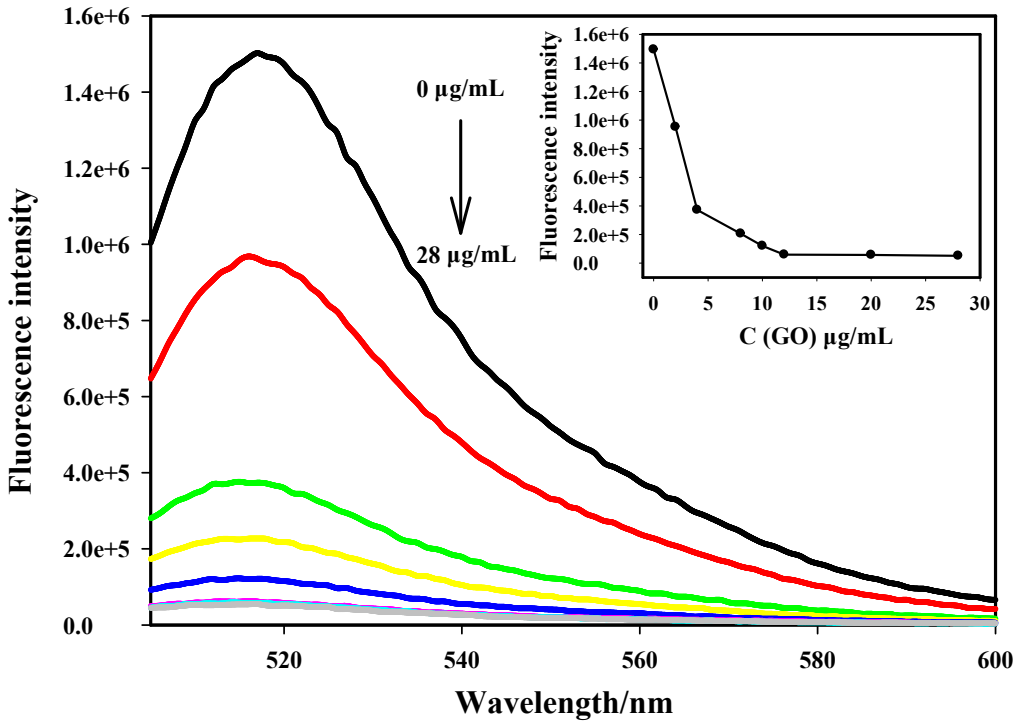


Fig. S3. Fluorescence quenching of 200 nM peptide probe by various concentrations of GO (0, 2, 4, 8, 10, 12, 20, 28 μg/mL). Inset: calibration curve of fluorescence intensity changes at 518 nm as a function of GO concentration.

Investigation of concentration of rLys-C cleavage of peptide probe

The concentration of rLys-C was also study to optimize for full digestion of peptide probe. 200 nM peptide probe cleaved by different concentrations of rLys-C for 1 hour and then mixed with 12 μg/mL GO. The maximum (F/F0) in response to rLys-C is at 1μg/mL. Thus, we choose 1 μg/mL rLys-C in the following experiment.

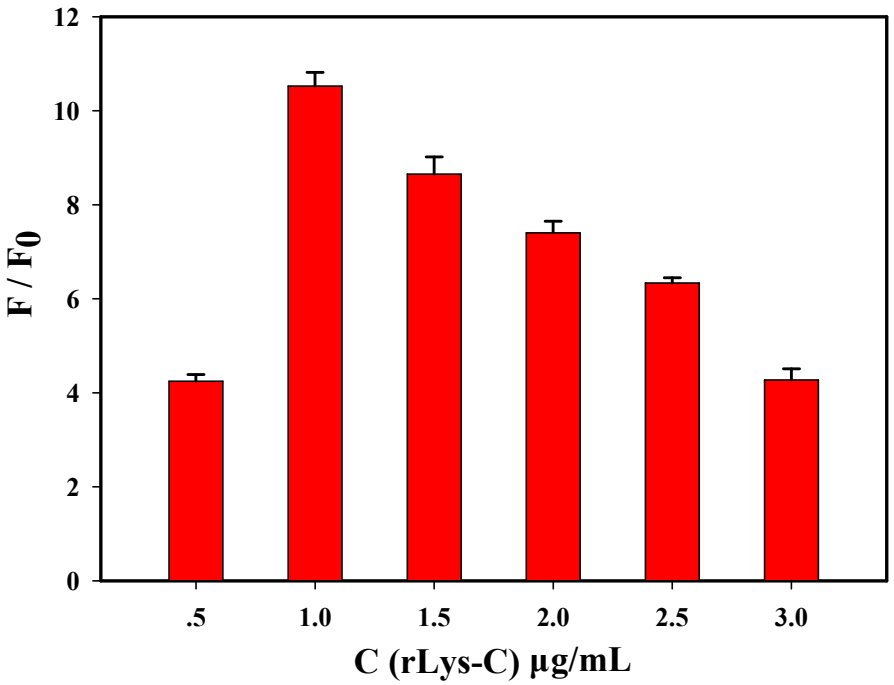


Fig. S4. Fluorescence intensity signal-to-background ratio (F/F0) changes at 518 nm emission in response to various concentrations of rLys-C (0.5, 1, 2, 3, 4, 5 μg/mL). Reactions were performed at 37 ºC for 1 h and peptide 200 nM, GO 12 μg/mL, HDAC1 100 nM were used for all experiments. The error bar represents the standard deviation of three measurements.

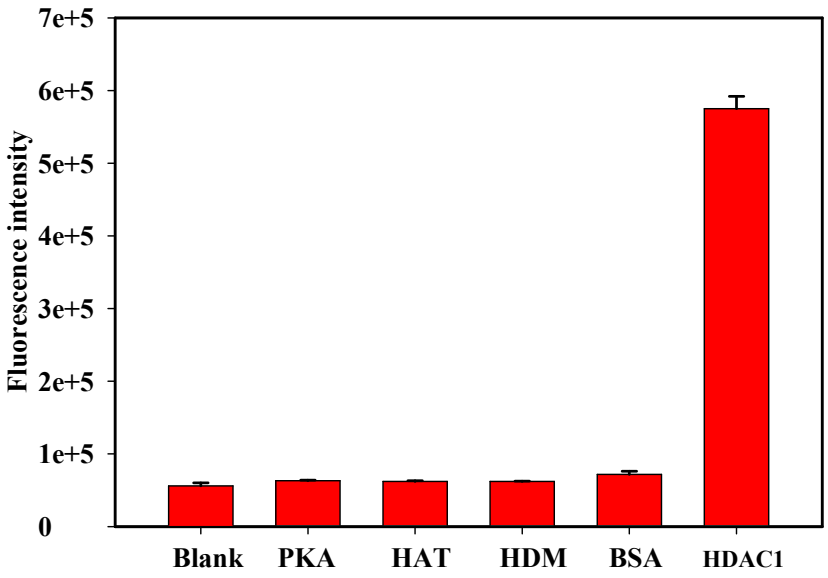
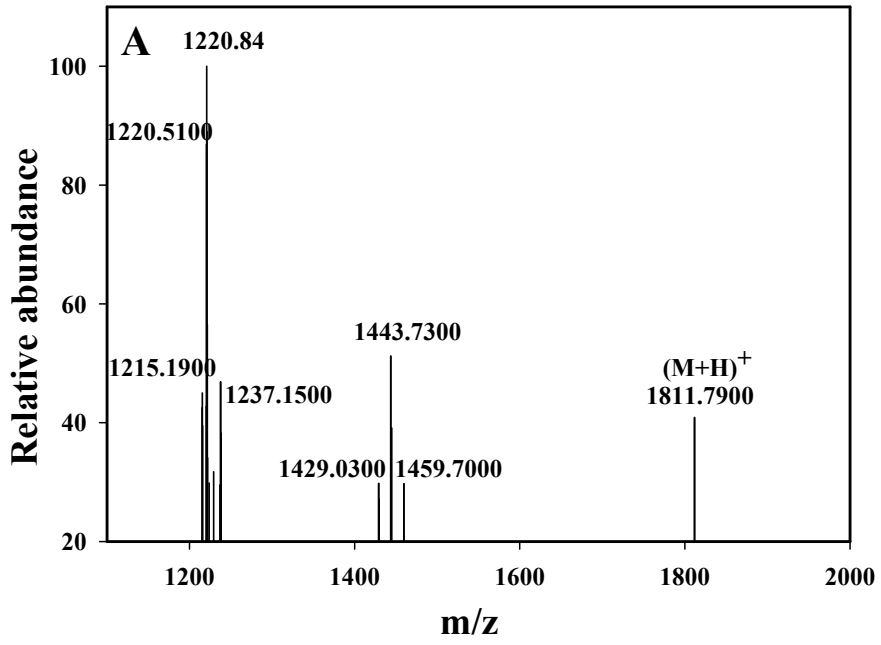
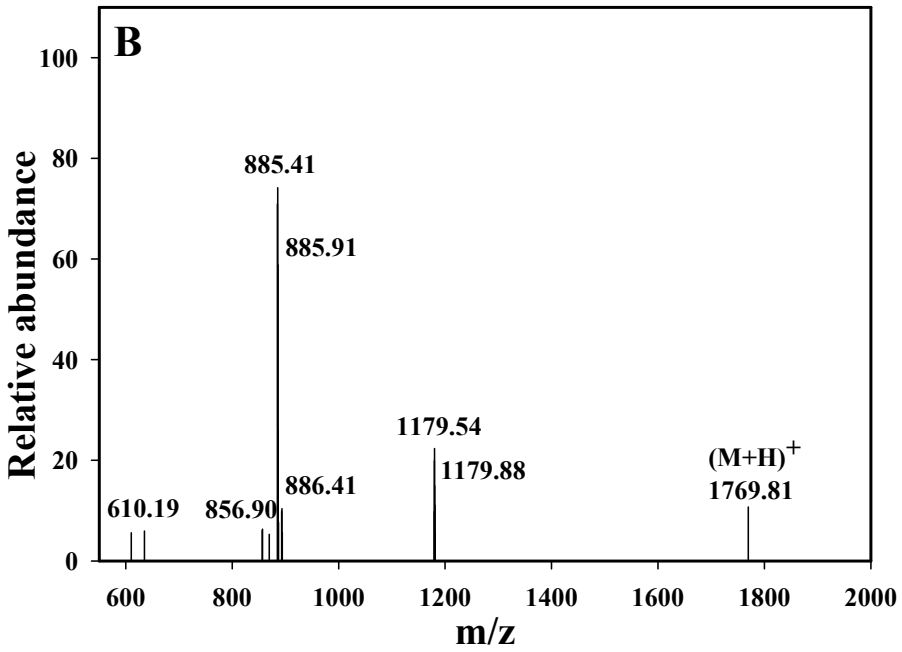


Fig. S5. Selectivity for HDAC1 assay using nanoassembly system. The concentration of each analyte: 250 nM protein kinase A (PKA), 250 nM histone acetyltransferases (HAT), 250 nM histone demethylase (HDM), 10μg/mL BSA, 100 nM HDAC1. Error bars are standard deviation of three repetitive experiments.

Electrospray ionization (ESI) mass spectrometric analysis

ESI-MS was performed on a linear trap quadropole (LTQ) orbitrap Velos mass spectrometry equipped with a nanoelectrospray source (Thermo Fisher Scientific, Bremen, Germany).





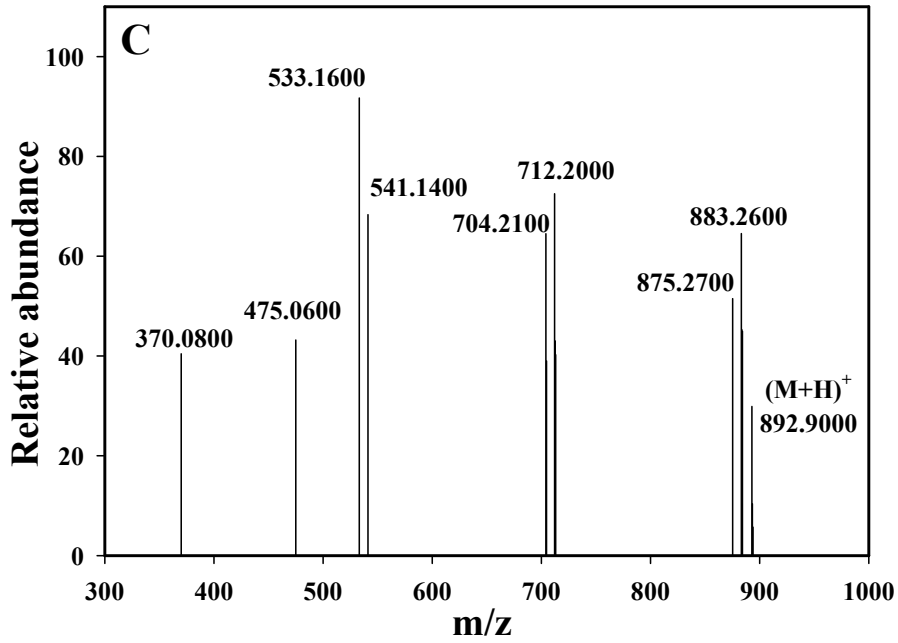


Fig. S6. ESI MS spectra for 100 nM peptide probe (A) treat with HDAC 1 (B) and rLys-C (C). HDAC 1 concentration was 100 nM, rLys-C concentration was 1 μg/mL.

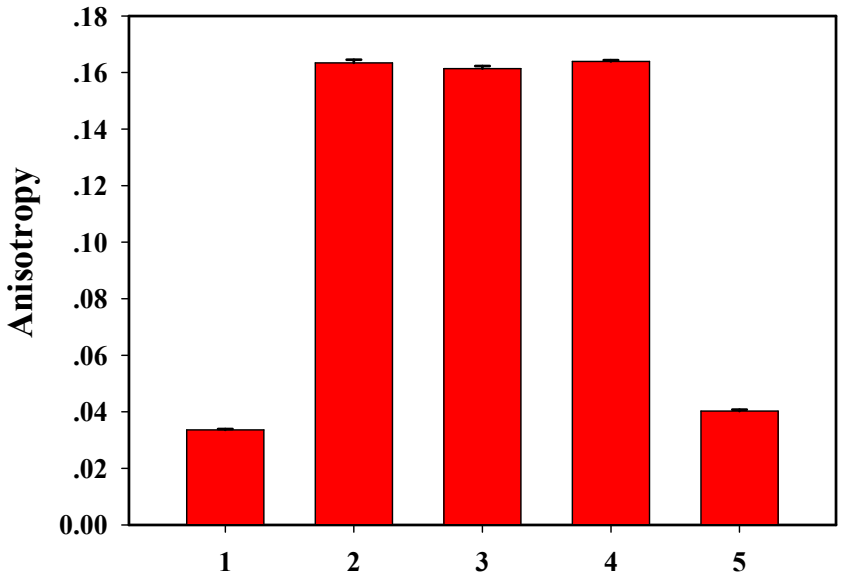


Fig. S7. Fluorescence anisotropy values of (1) peptide probe and (2) GO-peptide nanoassembly, (3) GO-peptide nanoassembly + rLys-C, and (4) GO-peptide nanoassembly + HDAC1, (5) GO-peptide nanoassembly + rLys-C + HDAC1. Reactions were performed at 37 °C for 1 h. 200 nM peptide probe, 12 μg/mL GO, 1μg/mL rLys-C, 100 nM HDAC1, were used for all experiments. Error bars are standard deviation of three repetitive experiments.

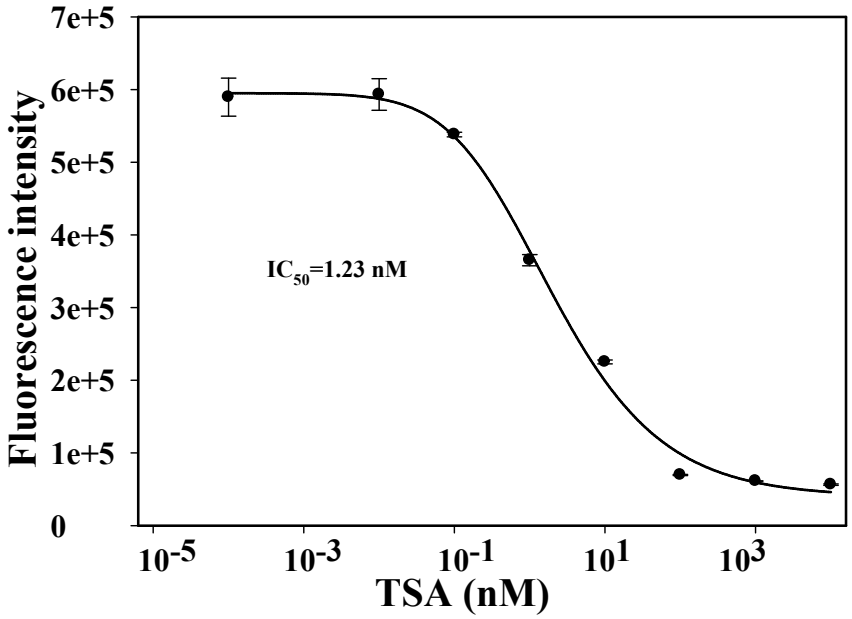


Fig. S8. The inhibition curve obtained with GO-peptide nanoassembly in deacetylated by 100 nM HDAC 1 in the presence of TSA of varying concentrations (10^{-3}, 10^{-1}, 1, 10, 10^{2}, 10^{3}, 10^{4}, 10^{5} nM). Error bars are standard deviation of three repetitive experiments.

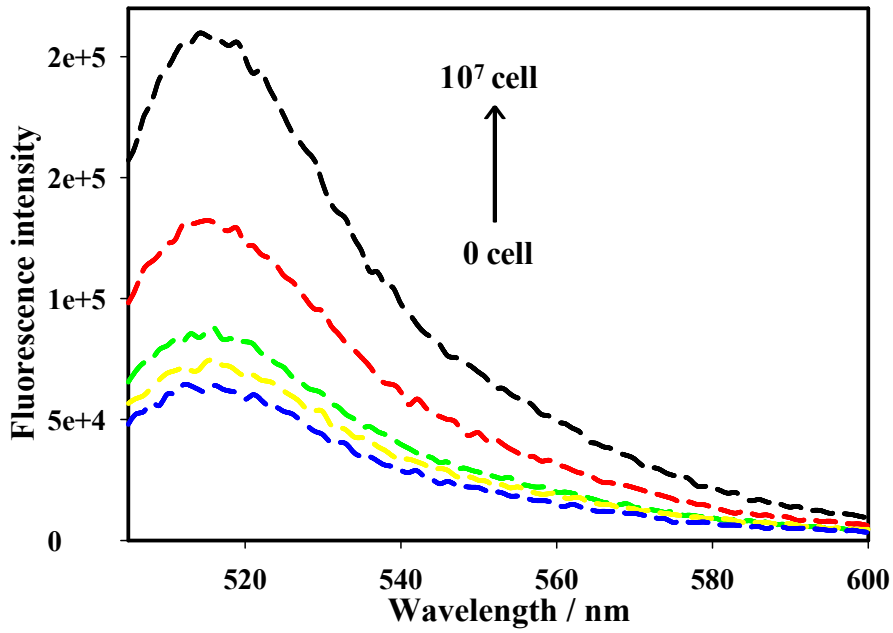


Fig. S9. Fluorescence spectral response of the GO-peptide nanoassembly incubation with nuclear extracts from HeLa cells of different numbers: 10^7 cells, 10^6 cells, 10^5 cells, 10^4 cells. Reactions were performed in a 50 μL system at 37 °C for 1 h with peptide 200 nM, GO 12 μg/mL, rLys-C 1 μg/mL, 5 μL Hela nuclear extract.