Electronic Supporting Information

Colorimetric detection of influenza A (H1N1) virus based on peptide functionalized polydiacetylene (PEP-PDA) nanosensor

Sinae Song, Kab Ha, Kyeonghye Guk, Seulki Hwang, Jong Min Choi, Taejoon Kang, Panki Bae, Juyeon Jung* and Eun-Kyung Lim*

1H NMR (600 MHz, CDCl3) δ (ppm): 0.88 (t, 3H), 1.22-1.45 (m, 26H), 1.45-1.52 (m, 4H), 1.80 (m, 2H), 2.22 (t, 3H), 2.42 (t, 3H), 2.78 (t, 2H).

Materials

10,12-pentacosadiynoic Acid (PCDA), n-Hydroxysuccinimide (NHS), anhydrous dichloromethane (DCM), magnesium sulfate (MgSO4), and acetone were obtained from Sigma-Aldrich Company. (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride) (EDC-HCl) was purchased from Thermo Scientific. Peptide for pandemic H1N1 virus specific interaction (Fmoc-ARLSPTMVHPNGAQP-NH2) and control peptide (Fmoc-CDDYYYGFGCNKFCRPR-NH2) substrate were conjugated from PEPTRON (Daejeon, Korea). All other chemicals and reagents were of analytical grade.

Preparation and characterization of the PCDA-NHS

To a solution of 10,12-pentacosadiynoic acid (1.00 g, 2.7 mmol) in anhydrous dichloromethane (DCM, 27 mL, 0.1 M), N-hydroxysuccinimide (NHS) (0.35 g, 3.0 mmol) and EDC-HCl (0.57 g, 3.1 mmol) were added. The solution was stirred at room temperature for two hours. The reaction mixture was extracted with dichloromethane and water for three times and washed with brine solution. The organic layer was dried over MgSO4, filtered, and the organic solvent was removed by rotary evaporation to give white solid PCDA-NHS, yield 95% (1.30 g). The characteristic bands of PCDA-NHS were confirmed using Fourier-transform infrared spectroscopy (FT-IR) (Alpha FTIR, Bruker Optics) and 1H-NMR (600 MHz) (Inova 600NB, Varian) with CDCl3-d2 as a solvent. 1H NMR (600 MHz, CDCl3) δ (ppm): 0.88 (t, 3H), 1.22-1.45 (m, 26H), 1.45-1.52 (m, 4H), 1.80 (m, 2H), 2.22 (t, 3H), 2.42 (t, 3H), 2.78 (t, 2H).
**Preparation and characterization of the peptide functionalized polydiacetylene nanosensor (PEP-PDA nanosensor)**

First, polydiacetylene (PDA) nanoparticles were prepared following the nanoprecipitation method. Briefly, a mixture of PCDA (11.23 mg) and PCDA-NHS (1.12 mg) (mole ratio, 9:1) was dissolved in 4 mL of acetone to make a total lipid concentration of 1 mM. This mixture was rapidly injected into 20 mL of DI water under magnetic stirring and constantly stirred at room temperature for 8 hours to totally evaporate acetone. This formed nanoparticles were stored at 4°C for overnight. Also, PDA nanoparticles should be stored in cool (4°C) to protect conformational change by external conditions before use. Photo-induced polymerization of PDA nanoparticles were carried out under UV irradiation with 256 nm for 20 minutes to obtain a deep-blue colored PDA nanosensors. The size distribution and morphology of PDA nanosensor were confirmed by using dynamic light scattering (ELS-Z, Otsuka Electronics) and high-resolution transmission electron microscope (HR-TEM) (TECNAI G2 F30), respectively. Negative staining was done with a 1% solution of phosphotungstic acid during TEM analysis. Its optical property (absorbance) was measured using a UV-Vis spectrophotometer (DU®800 Spectrometer). The unique optical properties of PDA nanosensors by chaining their confirmational change under various temperature and pH conditions were determined by UV-Vis spectrophotometer. We subjected to heating at temperatures of 40, 50, 60 and 70°C for 10 min by using Digital Heating Block and adjusted pH conditions with 1M HCl or 1M NaOH, respectively. Subsequently, 250 μL of peptide (PEP) (1 mg/mL) was conjugated with 1mL of PDA nanosensor for PEP-PDA nanosensor for 5 hours at room temperature. We synthesized control nanosensor using the control peptide sequence in the same manner.

**Viruses**

We used pandemic H1N1 (Influenza A/CA/07/2009 (pH1N1) viruses provided by the BioNano Health Guard Research Center (H-GUARD). All virus titers were determined by real-time PCR, with a One-Step RT-PCR kit (Promega) used in accordance with the manufacturer’s instructions.

**Colorimetric detection of pH1N1 using PEP-PDA nanosensor**

PEP-PDA nanosensor was tested against pH1N1 to determine the colorimetric detection of pH1N1. pH1N1 were added to a 96-well plate and treated with PEP-PDA nanosensor. After 5 minutes, their colorimetric change were observed and measured with a multi detection micro-plate reader (SpectraMax M2e, Molecular devices).
Fig. S1. Synthesis of PCDA-NHS.
Fig. S2. FT-IR spectra of PCDA (red) and PCDA-NHS (blue).
Fig. S3. $^1$H-NMR spectrum of PCDA.
Fig. S4. $^1$H-NMR spectrum of PCDA-NHS.