Electronic Supporting Information

Simple, rapid detection of influenza A (H1N1) viruses using a high sensitive peptide-based molecular beacon

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Materials

Oligomers were synthesized with a Cy3 donor conjugated to its 9-terminus (Cy3-TTTTGGGGG-SH) and a black hole quencher 2 (BHQ2)-coupled oligomers to its 9-terminus as an acceptor (BHQ2-AAAACCCCC-SH), which were obtained from Bioneer Inc. (Daejeon, Korea). Peptide substrate were conjugated from PEPTRON (Daejeon, Korea). DTT solution, DMSO and piperidine were obtained from Sigma-Aldrich Company. Sulfo-SMCC were purchased from Thermo Scientific. All other chemicals and reagents were of analytical grade.

Preparation and characterization of the peptide-based molecular beacon (PEP-MB)

PEP-MB molecules for virus detection were synthesized with an H1N1 virus-targeting peptide and two oligomers: a donor-conjugated oligomer with Cy3 and an acceptor-conjugated oligomer with BHQ2. The peptide and oligomers were purchased from Peptron (Daejeon, Korea) and Bioneer, Inc. (Daejeon, Korea), respectively. PEP-MB was created using the following sequences: Fmoc-ARLSPTMVHPNGAQP-NH_2, 5'-SH-CCCCCAAAA-BHQ2-3' (Oligo A), and 5'-Cy3-TTTTGGGGG-SH-3' (Oligo B). Fmoc-CDDYYYGFGCNKFCRPR-NH_2 was used as a control peptide. All peptides were synthesized using standard solid-phase Fmoc peptide chemistry.[1, 2]

Each oligomer was suspended in duplex buffer (100 mM potassium acetate; 30 mM HEPES, pH 7.5; available from IDT) at a concentration of 100 pmol. Before modification, oligomers were first treated with 1 M DTT solution for 15 min at 25°C to activate the protected thiol groups and then desalted using a Sephadex NAP-5 column (GE Healthcare, UK). To obtain PEP-MB, the amine (-NH2) group of the peptide and the thiol (-SH) group of Oligo A were conjugated using sulfo-SMCC (4.8 mg/mL) as a cross-linker and purified using a NAP-5 column. Subsequently, 20% piperidine in DMSO was used to remove the Fmoc group from Pep-Oligo A before this peptide was conjugated with Oligo B. Oligo B was then conjugated with Pep-Oligo A using Sulfo-SMCC, and the product was purified using a column. The synthesized PEP-MB was annealed at 95°C for 2 min and

then slowly cooled to 25°C to allow the hairpin structure to form. PEP-MB fluorescence intensity was measured using an Infinite M200 Pro (Tecan, Australia) instrument at 530 nm (excitation) and 560 nm (emission) to confirm the efficacy of fluorescence quenching.[3] We synthesized Control MB using the control peptide sequence and measured its efficacy in the same manner.

Viruses

We used H1N1 (Human/Korea/2009), H3N2 (Canine/Korea/01/2007), H6N5 (Bird/Korea/CN5/2009) and H5N2 (Bird/Korea/CN2-MA/2009) 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.

Measurement of peptide-based molecular beacon binding affinity on viruses

We measured the binding affinity between peptide-based molecular beacon (PEP-MB) and viruses using a biolayer interferometry-based biosensor BLItz system (FortéBio). First, biotinylated PEP-MB were synthesized by using same peptide sequence, 5'-SH-CCCCCAAAAA-Biotin-3' and 5'-TTTTTGGGGG-SH-3' under same conditions with that of PEP-MB. Streptavidin biosensors were loaded with biotinylated PEP-MB, equilibrated in PBS for 2 min to establish a stable baseline, and then dipped into 4uL of virus samples of same concentrations (10^4) to obtain the association curve for 360s. By soaking in 250uL of sample diluent, the dissociation curve was obtained for 360s. Afterwards, Binding affinities were calculated by fitting the curves using BLItz software.[4, 5]

Influenza A (H1N1) virus detection using the peptide-based molecular beacon

PEP-MB was tested against various types of viruses (H1N1, H3N2, H5N2, H6N5 and corona viruses) to determine the specificity of H1N1 virus detection. These viruses were added to a 96-well plate and treated with PEP-MB (35 pmol in 100 μL). The fluorescence of the plate was measured with a microplate reader at various time intervals at a temperature of 25°C, using wavelengths of 530 nm (excitation) and 560 nm (emission).

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5. J. Dong, T. Kojima, H. Ohashi and H. Ueda, *J. Biosci.Bioeng.* **2015**, doi:10.1016/j.jbiosc.2015.03.014.

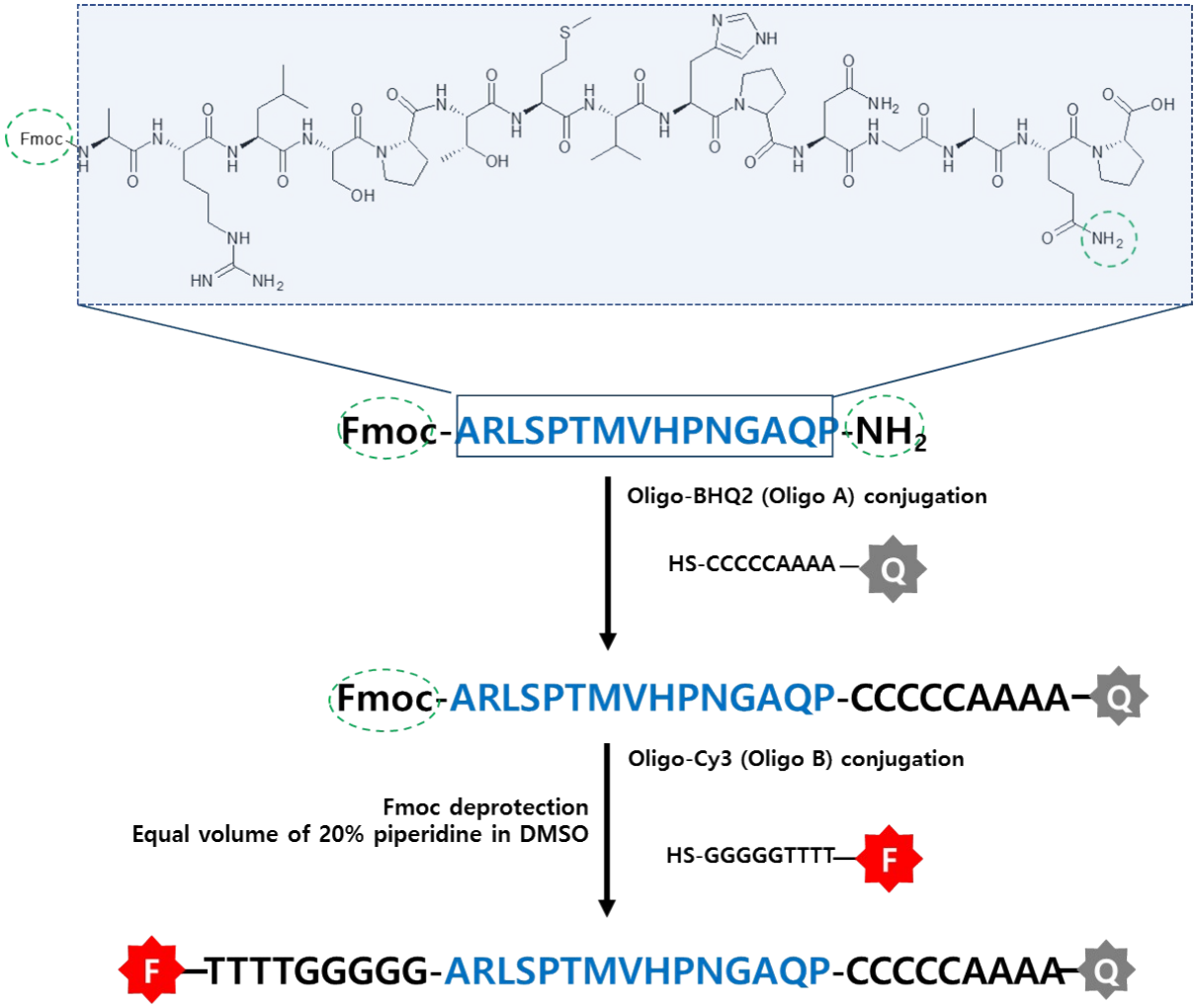


Fig. S1. Synthetic scheme for the peptide-based molecular beacon (PEP-MB) [Cy3-TTTTGGGGG-ARLSPTMVHPNGAQP-CCCCCAAAA-BHQ2].

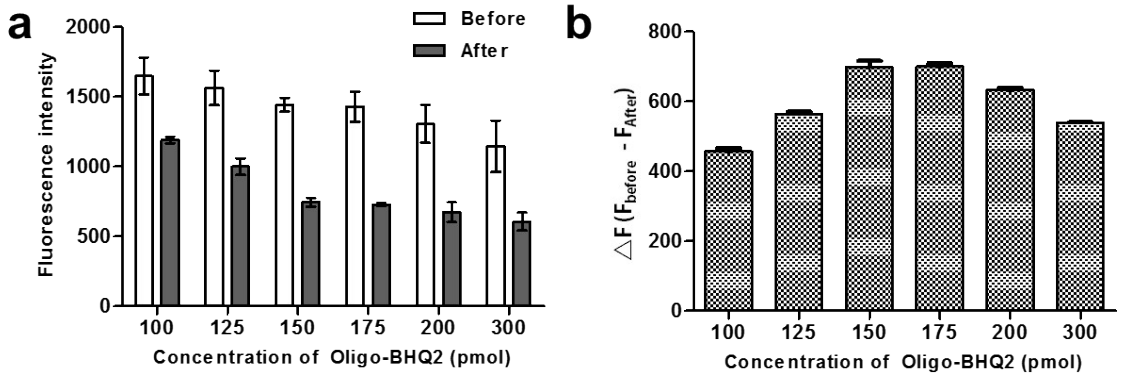
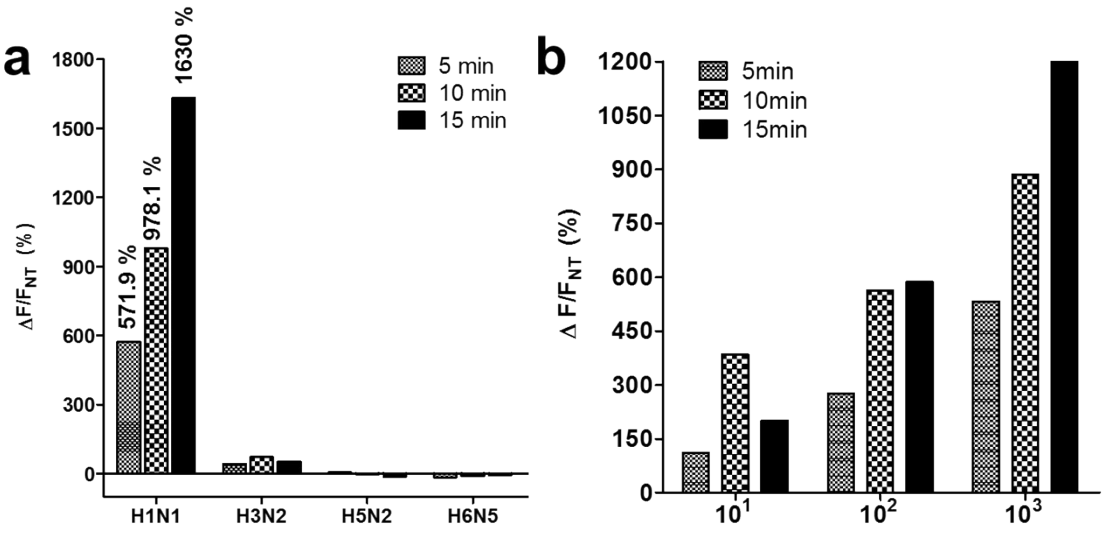


Fig. S2. Fluorescence intensities (F) of complementary oligonucleotides at a fixed concentration of Oligo-Cy3 with increasing concentrations of Oligo-BHQ2. (a) Fluorescence intensities of oligonucleotides before and after annealing. (b) Changes in oligonucleotide fluorescence intensity.



5 min	111.8 %	276.5 %	532.4 %
10 min	385.7 %	562.9 %	885.7 %
15 min	200.0 %	885.7 %	1265.8 %

Fig. S3. Relative fluorescence intensities (F) of (a) all viruses and (b) H1N1 virus under various concentrations (0, 10^1, 10^2 and 10^3 copies/well) after PEP-MB incubation with different times (5, 10 and 15 min) compared with the corresponding fluorescence intensities of non-treatment (NT) ($\Delta F = F - F_{NT}$).