BioChip Journal (2022) 16:334–341
https://doi.org/10.1007/s13206-022-00076-x

ORIGINAL ARTICLE

One-Step Homogeneous Immunoassay for the Detection of Influenza Virus Using Switching Peptide and Graphene Quencher

Hong-Rae Kim[1,2] · Ji-Hong Bong[1] · Tae-Hun Kim[1] · Seung-Shick Shin[3] · Min-Jung Kang[4] · Won-Bo Shim[5] · Do Young Lee[3] · Dong Hee Son[2] · Jae-Chul Pyun[1]

Received: 17 June 2022 / Revised: 15 July 2022 / Accepted: 18 July 2022 / Published online: 27 July 2022

Abstract
One-step homogeneous immunoassay was developed for detecting influenza viruses A and B (Inf-A and Inf-B) using the switching peptide H2. As the fluorescence-labeled switching peptide dissociated from the binding pocket of detection antibodies, the fluorescence signal could be directly generated by the binding of Inf-A and Inf-B without washing (i.e., one-step immunoassay). For the one-step homogeneous immunoassay with detection antibodies in solution, graphene was labeled with the antibodies as a fluorescence quencher. To test the feasibility of the homogeneous one-step immunoassay, the stability of the antibody complex with the switching peptide was evaluated under different pH and salt conditions. The one-step homogeneous immunoassay with switching peptide was conducted using influenza virus antigens in phosphate-buffered saline and real samples with inactivated Inf-A and Inf-B spiked in serum. Finally, the one-step homogeneous immunoassay results were compared with those of commercially available lateral flow immunoassays.

Keywords One-step homogeneous immunoassay · Switching peptide · Influenza-A and influenza-B virus · Lateral flow immunoassay

1 Introduction

The switching peptides were derived from the self-assembly regions of immunoglobulin G (IgG), which are the frame regions (FRs) at the antigen-binding pockets, as shown in Fig. 1a [1]. As the FRs have a conserved amino acid sequence in IgG for different antigens and different source animals, the switching peptides could be generally used for nearly all kinds of IgGs [2–5]. As shown in Fig. 1b, such switching peptides can be effectively used for one-step immunoassays without the need for washing [1, 6–9]. When the antigen binds to the corresponding region of the antigen-binding site of IgG, the switching peptides labeled with fluorescence get dissociated from IgG. In other words, the binding affinity of switching peptides is weaker than that of target antigens against the corresponding antibodies. Usually, the binding constants (K_D) of switching peptides to antibodies range within a few micromoles. Thus, sample mixing with a switching peptide could generate a fluorescence signal in solution when the detection antibodies are bound at a solid phase, such as microplates and microparticles [10, 11].

Homogeneous immunoassays have indicated that the antigen–antibody interaction occurs in solution without immobilization of antibodies. In particular, one-step homogeneous immunoassays can be easily performed in solution by simply mixing the sample and reagent solutions. Such a homogeneous immunoassay has the advantage of detecting antigens far larger than antibodies (on the scale of several

Hong-Rae Kim and Ji-Hong Bong contributed equally to this work.

✉ Jae-Chul Pyun
jcpyun@yonsei.ac.kr

1 Department of Materials Science and Engineering, Yonsei University, 50 Yonsei-Ro, Seodaemun-gu, Seoul 03722, Korea

2 Department of Chemistry, Texas A&M University, College Station, TX 77843, USA

3 OPTOLANE Technologies Inc, 20 Pangyoyeok-ro 241beon-gil, Bundang-gu, Seongnam-si, Gyeonggi-do 13494, Republic of Korea

4 Korea Institute of Science and Technology (KIST), 5 Hwarang-ro 14-gil, Seongbuk-gu, Seoul 02792, Republic of Korea

5 Department of Agricultural Chemistry and Food Science & Technology, Gyeongsang National University, Jinju, Korea

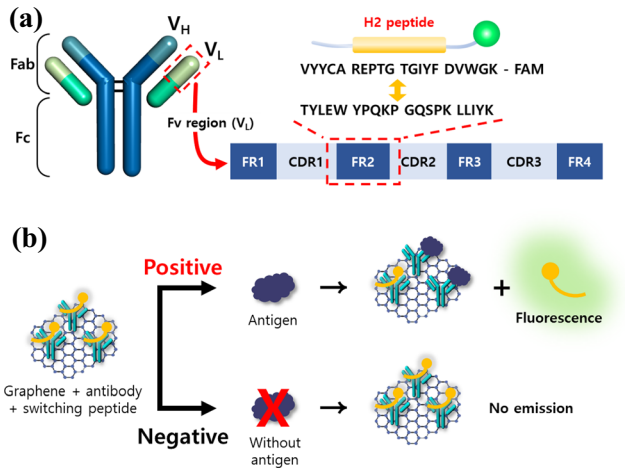

Fig. 1 One-step immunoassay based on switching peptide. **a** Switching peptide. **b** Configuration of one-step immunoassay based on switching peptide

nanometers), such as viruses (on the scale of several hundred micrometers) and bacteria (on the scale of micrometers). As the antibodies show fluorescence after binding to fluorescence-labeled switching peptides, a quencher molecule should be bound to the detection antibodies to absorb the fluorescence from the switching peptides before the binding of antigens (target analytes). Fluorescence quenching effect by graphene is well-known to be occurred by energy transfer from the excited molecule to graphene [12–14]. Then, the excited electron in the valence band of graphene relaxes to the Fermi level non-radiatively. This process is similar to the Foster resonance energy transfer (FRET) occurring between two molecules. The fluorescence quenching of fluorophores can be occurred using graphene as well as metal nanoparticles, and it has been used for the biosensor applications [15, 16]. In this study, a one-step homogeneous immunoassay was presented using switching peptides and graphene as a quencher and one-step detection of influenza A (Inf-A) and influenza B (Inf-B) viruses [17–19].

2 Results and Discussion

2.1 One-Step Homogeneous Immunoassay Using Switching Peptides

Homogeneous immunoassay indicated that antigen–antibody interactions occurred in solution without immobilization of antibodies. One-step homogeneous immunoassays can be conducted in solution by simply mixing the sample and reagent solutions [1]. As the antibodies show fluorescence after binding to fluorescence-labeled switching peptides, a quencher molecule was bound to the detection antibodies to absorb the fluorescence from the switching peptides before the binding of the antigens (target analytes). In this study, the switching peptide H2 (labeled with FAM) was complexed with antibodies against Inf-A and Inf-B, and graphene was labeled as a quencher to absorb the fluorescence from the switching peptide H2 [20, 21]. As graphene could absorb the fluorescence signal in the visible range of 400–700 nm, the fluorescence from the labeled fluorescence dye, FAM (excitation and emission wavelengths of 488 and 514 nm, respectively), could be effectively quenched.

To estimate the quenching efficiency of graphene for FAM, the fluorescence signal was compared for the antibodies with and without graphene after complex formation with the switching peptide H2 [22–24]. As shown in Fig. 2a, the fluorescence signal was measured for the antibody complex with the switching peptide H2 in the emission wavelength range of 500–560 nm. When graphene was labeled to the antibodies, the quenching efficiency was estimated according to the following equation:

$$E = 1 - \left(F/F_i\right) \tag{1}$$

where E represents the quenching efficiency, F represents the donor emission when the quencher is bound, and F_i represents the donor emission when no quencher is bound [25–27]. The quenching efficiency was thus estimated to be 0.71 at the maximum emission wavelength of 514 nm. These results show that the graphene could effectively absorb fluorescence signal from the switching peptide H2 (with a quenching efficiency of 71%).

For the one-step immunoassay, the switching peptide H2 needs to be complexed with the detection antibodies to the greatest extent. The optimal treatment concentration of switching peptide H2 was estimated after incubation of the antibodies with different concentrations of the switching peptide H2. As shown in Fig. 2b, the switching peptide H2 at a concentration range of 1–100 μM was incubated with anti-Inf-A at a fixed concentration of 2 μM, and the fluorescence signal was measured after treatment with the same concentration of antigens. From the comparison of the fluorescence signals, the optimum concentration for the highest fluorescence signal was estimated to be 30 μM for an incubation time of 1 h.

The antibody complex with switching peptide H2 needs to be maintained without dissociation before binding to the target analytes. The stability of the antibody complex was estimated under various pH and salt conditions. As shown in Fig. 2c, the antibody complex was incubated at a pH range of 6.0–8.0, which corresponded to one-step immunoassays. For the anti-Inf-A complex labeled with graphene, the fluorescence signal from the dissociation of switching peptide H2 (labeled with FAM) was estimated to be 0.5×10^3 A.U with a standard deviation of 9% ($n = 3$). The same experiment

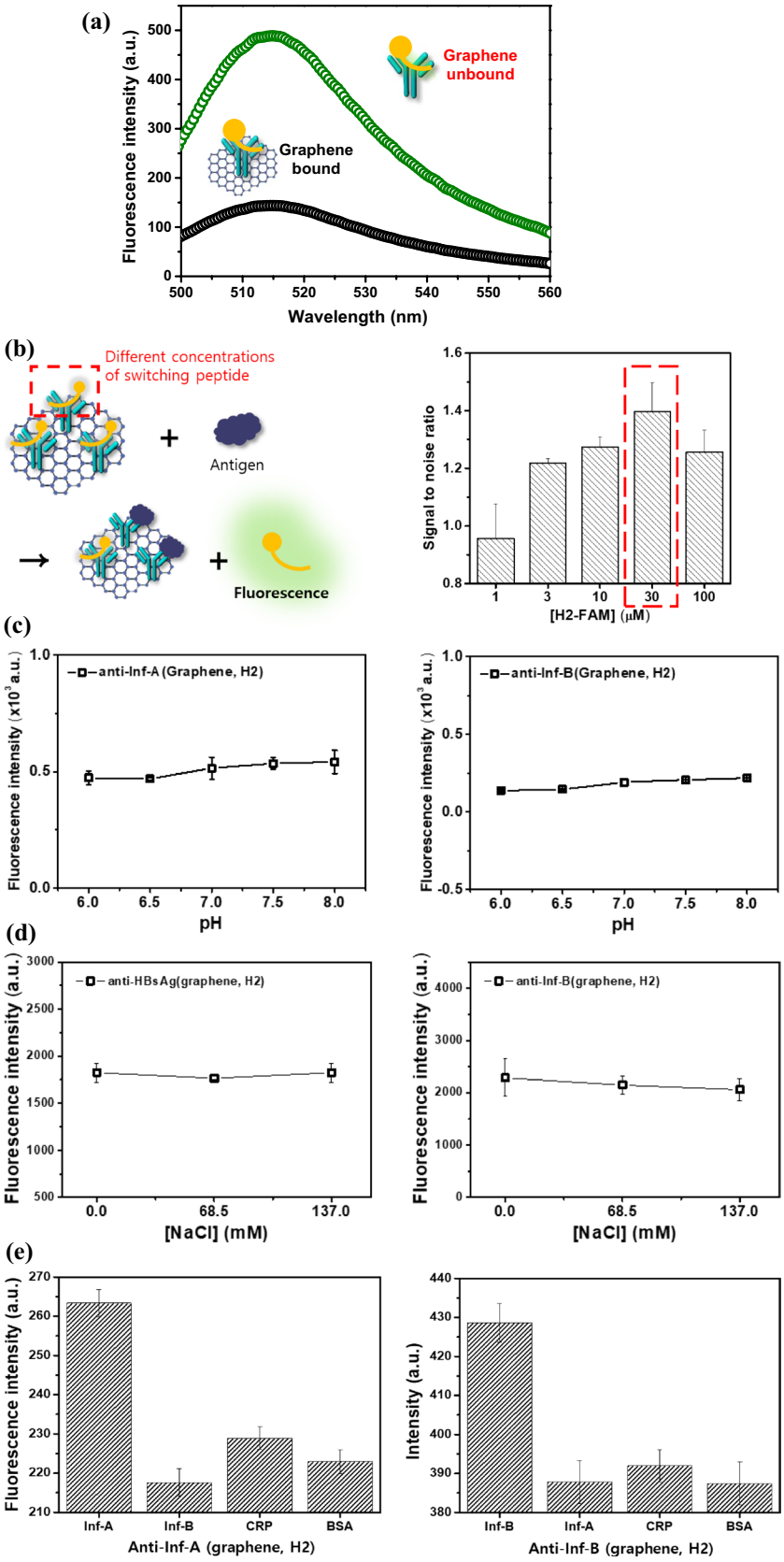

Fig. 2 Optimization of assay conditions. **a** Fluorescence quenching with graphene. **b** Loading concentration of switching peptide. **c** pH stability. **d** Stability in salt. Selectivity of antibodies against **e** Inf-A and Inf-B.

was conducted for the anti-Inf-B complex labeled with graphene, and the fluorescence signal was estimated to be 0.2×10^3 A.U with a standard deviation of 3% ($n = 3$). These results showed that the antibody complex with switching peptide H2 could maintain a stability of more than 91% in pH range of 6.0–8.0. The stability of the antibody complex was estimated under different salt conditions. As shown in Fig. 2d, the antibody complex was incubated at a salt (NaCl) concentration of 0–137 mM, which corresponded to one-step immunoassays with human serum. For the anti-Inf-A complex labeled with graphene, the fluorescence signal from the dissociation of switching peptide H2 (labeled with FAM) was estimated to be 1.8×10^3 A.U with a standard deviation of 5% ($n = 3$). The same experiment was conducted for the anti-Inf-B complex labeled with graphene, and the fluorescence signal was estimated to be 2.1×10^3 A.U with a standard deviation of 10% ($n = 3$). These results showed that the antibody complex with the switching peptide H2 could be maintained at more than 90% stability at salt concentrations between 0 and 137 mM.

The selectivity of the antibodies against Inf-A and Inf-B was estimated using different antigens. For the estimation of selectivity, anti-Inf-A (or Inf-B) was complexed with switching peptide H2 (labeled with FAM), and antigens such as Inf-B (or Inf-A), CRP, and BSA were separately incubated for 1 h. CRP was selected as the infected patients with influenza virus frequently tested CRP levels as an inflammation biomarker, and BSA was chosen as it is a major component of human serum. As shown in Fig. 2e, anti-Inf-A (or Inf-B) were estimated to have a high selectivity against Inf-A (or Inf-B) with a factor of 100% (or 100%) in comparison with the response to Inf-B (or Inf-A). These results showed that the antibodies against Inf-A and Inf-B could be effectively used (1) for the discrimination of other types of influenza viruses and (2) for the one-step immunoassay using human serum without cross-reactivity to other serum proteins.

2.2 One-Step Homogeneous Immunoassay of Inf-A and Inf-B

A one-step homogeneous immunoassay was conducted for Inf-A and Inf-B in PBS and human serum as real samples. The antibody complex with switching peptide H2 was prepared using anti-Inf-A and anti-Inf-B. Standard samples were prepared using influenza virus antigens (Inf-A and Inf-B) in PBS, and real samples were prepared by spiking the influenza viruses (Inf-A and Inf-B) in human serum. For real samples, NATtrol™ Influenza Stock (Inf-A and Inf-B) from ZeptoMetrix (Buffalo, NY, USA) was used by serial dilution to obtain a Ct value in the range of 36.5–30.1 [28, 29]. As shown in Fig. 3a, a homogeneous one-step

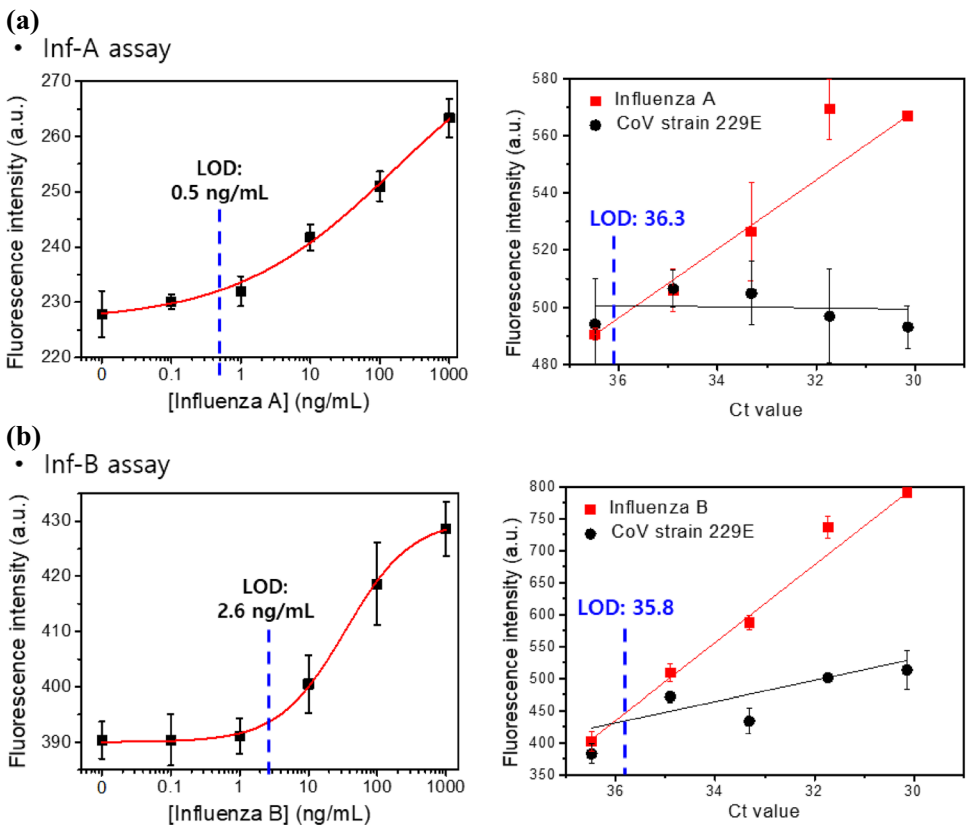

Fig. 3 One-step immunoassay of **a** Influenza-A and **b** Influenza-B in PBS and serum

immunoassay was conducted using the antibody complex with anti-Inf-A and switching peptide H2. When the assay was conducted using Inf-A antigens at a concentration range of 0–1000 ng/mL in PBS, the fluorescence signal increased. The limit of detection (LOD) was calculated to be 0.5 ng/mL from thrice the standard deviation of the blank sample. The same one-step immunoassay configuration with the antibody complex was applied to real samples spiked with Inf-A in human serum, and a coronavirus (NATtrol™ Stock of 229E strain) from ZeptoMetrix (Buffalo, NY, USA) was used as a negative control. The LOD was calculated as a Ct value of 36.3 from thrice the standard deviation of the blank sample ($n=3$).

The same experiment was conducted for the antibody complex with anti-Inf-B and switching peptide H2. As shown in Fig. 3b, the LOD for Inf-B was calculated to be 2.6 ng/mL from thrice the standard deviation of the blank sample. The same one-step immunoassay configuration with the antibody complex was applied to real samples spiked with Inf-B in human serum, and another coronavirus (strain 229E) was used as a negative control. The LOD was calculated as a Ct value of 35.8 from thrice the standard deviation of the blank sample ($n=3$). These results showed that the homogeneous one-step immunoassay was feasible for medical diagnosis of Inf-A with a Ct value of 36.3 and Inf-B with a Ct value of 35.8 in human serum.

The homogeneous one-step immunoassay based on switching peptide H2 was compared with commercially available lateral flow immunoassay kits (SD Biosensor) for Inf-A and Inf-B. Real samples were prepared with the dilution buffer included in the kit using the influenza virus antigen. As shown in Fig. 4a, the one-step immunoassay was conducted in the Inf-A antigen concentration range of 10 pg/mL–1000 ng/mL, and the lateral flow immunoassay was conducted in the concentration range of 1 ng/mL–100 µg/mL. From the standard curves obtained using both methods, the LOD was estimated to be 0.5 ng/mL for the one-step immunoassay and 230 ng/mL for the lateral flow immunoassay using densitometry of the apparent test line. The detection range was estimated to be 0.1–1000 ng/mL for the one-step immunoassay and 1–100 µg/mL for the lateral flow immunoassay ($n=3$). These results showed that a one-step immunoassay based on switching peptides was feasible for a significantly sensitive detection of Inf-A in a far wider detection range than that of the lateral flow immunoassay.

The same experiment was conducted using Inf-B. As shown in Fig. 4b, the one-step immunoassay was conducted in the Inf-B antigen concentration range of 10 pg/mL–1000 ng/mL, and the lateral flow immunoassay was conducted in the concentration range of 1 ng/mL–100 µg/mL. From the standard curves obtained using both methods, the LOD was estimated to be 2.6 ng/mL for the one-step immunoassay and 371 ng/mL for the lateral flow immunoassay using densitometry of the apparent test line. The detection range was estimated to be 1–1000 ng/mL for the one-step immunoassay and 1–100 µg/mL for the lateral-flow immunoassay ($n=3$). Usually, the cutoff value for the medical diagnosis of Inf-A and Inf-B nucleoprotein has been reported to be 7.5 ng/ml–4 µg/ml [30–33]. In comparison with the reported cutoff value, these results showed that the

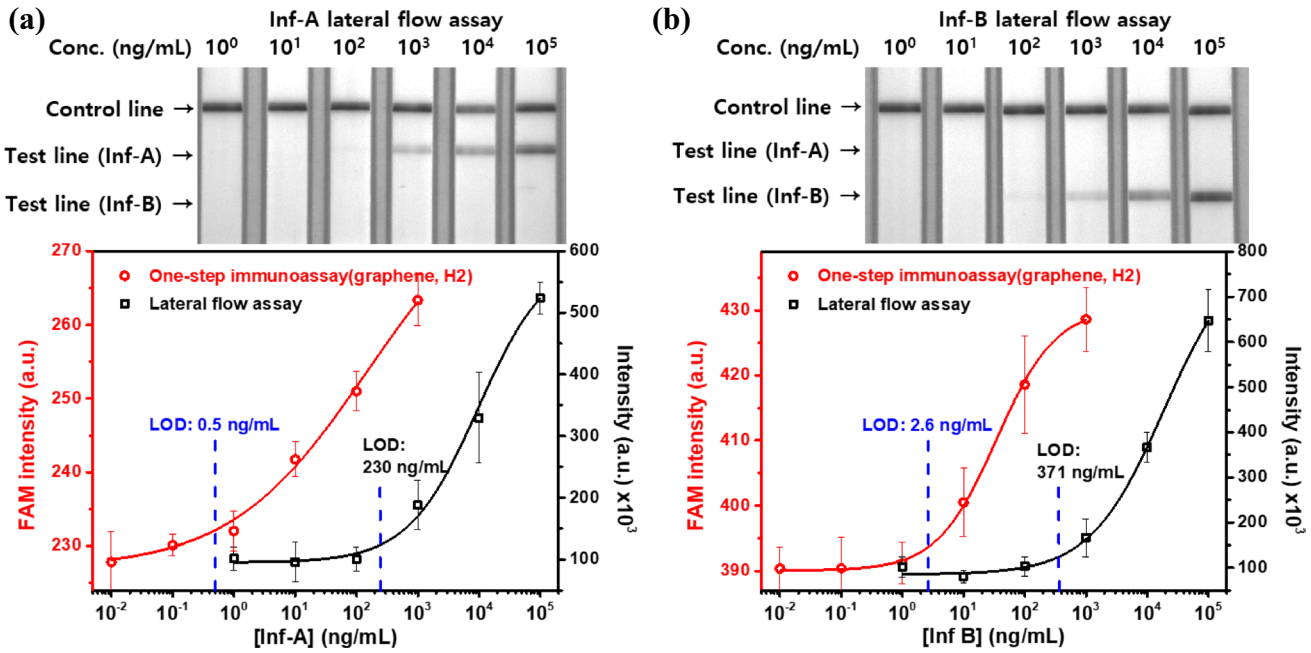

Fig. 4 Comparison of one-step immunoassay of **a** Inf-A and **b** Inf-B with lateral flow immunoassay

homogeneous one-step immunoassay based on switching peptides was feasible for the significantly sensitive detection of both influenza viruses (Inf-A and Inf-B). Additionally, the homogenous one-step immunoassay was determined to have a far wider detection range than that of the lateral flow immunoassay.

3 Conclusions

A one-step homogeneous immunoassay was developed for detecting Inf-A and Inf-B using the switching peptide H2. For the homogeneous one-step immunoassay with the detection antibodies in solution, graphene was labeled to the antibodies as a fluorescence quencher that could absorb the fluorescence from the labeled fluorescent dye FAM (excitation and emission wavelengths of 488 and 514 nm, respectively). The quenching efficiency of graphene for the antibody-bound switching peptide H2 was estimated to be 71%. For the one-step immunoassay, the optimal treatment concentration of the switching peptide H2 was 30 μM for an incubation time of 1 h. Additionally, the antibody complex with switching peptide H2 could be maintained at more than 91% stability between the pH range of 6.0–8.0 and at more than 90% between salt concentrations of 0–137 mM. In this study, the antibodies against Inf-A and Inf-B could be effectively used (1) for the discrimination of other types of influenza viruses and (2) for the one-step immunoassay using human serum without cross-reactivity to other serum proteins. The one-step homogeneous immunoassay with switching peptide was conducted using Inf-A and Inf-B antigens in PBS and real samples with inactivated Inf-A and Inf-B spiked in serum. The one-step homogeneous immunoassay was feasible for the medical diagnosis of Inf-A with a Ct value of 36.3 and Inf-B with a Ct value of 35.8 in human serum. Finally, the one-step homogeneous immunoassay results were compared with those of commercially available lateral flow immunoassays. The one-step homogeneous immunoassay based on switching peptides was feasible for a significantly sensitive detection of both Inf-A and Inf-B in a far wider detection range than that of the lateral flow immunoassay.

4 Experimental

4.1 Materials

Fluorescence-labeled switching peptides were synthesized by Peptron (Daejeon, Korea). Bovine serum albumin (BSA), C-reactive protein (CRP), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), N-hydroxysuccinimide (NHS), and Tween®-20 were purchased from Sigma-Aldrich (Seoul, Korea). The anti-Inf-A and anti-Inf-B monoclonal antibodies were supplied by Prof. Won-bo Shim (Gyeongsang National University, Jinju, Korea). Inf-A and Inf-B viruses (A/New caledonia/20/99 and B/Tokio/53/99, respectively) were purchased from Meridian Bioscience (Cincinnati, OH, USA). NATtrol™ Inf-A and Inf-B virus stocks (A/Singapore/63/04 and B/Florida/02/06, respectively) were purchased from ZeptoMetrix (Buffalo, NY, USA). Graphene nanoplatelets (functionalized with primary amine with an average diameter of approximately 1 μm) were purchased from ENanoTec (Seongnam, Korea).

4.2 Preparation of Switching Peptide and Labeling of Graphene

Anti-influenza antibodies at a concentration of 1 mg/mL (150 μL) were incubated with EDC (400 mM, 200 μL) and NHS (100 mM, 200 μL) in 1450 μL of carbonate buffer for 1 h. The graphene solution at a concentration of 10 mg/mL (500 μL) was mixed with EDC/NHS-activated anti-influenza antibodies for 2 h. The solution was purified by centrifugation at 3000 × *g* for 3 min. The precipitate was dissolved in 500 μL phosphate-buffered saline (PBS). Peptide H2 was switched at a concentration of 30 μM (100 μL) and incubated with the antibody-conjugated graphene solution for 2 h. The solution was purified by centrifugation at 3000 × *g* for 3 min in PBSB (PBS with 1% BSA). The precipitate was resuspended in 500 μL of PBS for a homogeneous one-step immunoassay.

4.3 One-Step Immunoassay for Inf-A and Inf-B

The homogeneous one-step immunoassay for influenza was conducted using inactivated Inf-A and Inf-B (A/New caledonia/20/99 and B/Tokio/53/99, respectively) from Meridian Bioscience and NATtrol™ Influenza stock (A/Singapore/63/04 and B/Florida/02/06) from Zeptometrix. The solutions were prepared for immunoassay (switching peptide-antibody-graphene complex) at a concentration of 100 μg/mL (100 μL) and directly mixed with 100 μL of inactivated viral influenza viruses at concentrations ranging from 0.1 to 1000 ng/mL. After incubation for 1 h, the fluorescence intensity of the mixture was measured using a fluorometer (QFX fluorometer, Denovix, Wilmington, DE, USA).

For the real sample, chemically modified influenza viruses (NATtrol™ Influenza AH1 virus stock and NATtrol™ Influenza B virus stock) from Zeptometrix were used [28, 29]. These reagents were developed as standards for molecular diagnostic testing and can be used as independent quality control materials. The stock contained inactivated microorganisms and materials of human and animal origin. The matrix of these samples was manufactured from human serum albumin, which has been tested and found

to be non-reactive at the donor level for antibodies against HIV-1/HIV-2, HBsAg, and HCV using FDA-licensed donor screening test methods. NATtrol™ Influenza stocks at a concentration of 36–28 ct were mixed with antibodies bound to the quencher and switching peptides. After incubation for 1 h, the fluorescence intensity of the mixture was measured using a fluorometer (QFX fluorometer).

The results of the homogeneous one-step immunoassay were compared with those of a commercially available Inf-A/B test kit from SD Biosensor (Suwon, Korea). According to the manufacturer's instructions, the test kit was certified to have 97% sensitivity and 100% specificity for the patient's samples. For Inf-A/B detection, 200 µL of inactivated viral influenza virus (A/New caledonia/20/99 and B/Tokio/53/99) was added onto the Inf-A/B detection strip at concentrations ranging from 1 ng/mL to 100 µg/mL. After 12 min, the detection results displayed on the test line were analyzed.

Acknowledgements This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI19C1344).

Declarations

Conflict of interests The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Bong, J.-H., Kim, H.-R., Jung, J., Park, J.-H., Sung, J.S., Lee, C.K., Choi, K.-H., Shin, S.-S., Kang, M.-J., Kim, H.O.: Switching-peptides for one-step immunoassay and its application to the diagnosis of human hepatitis B. Biosens. Bioelectron. **178**, 112996 (2021)
2. Toughiri, R., Wu, X., Ruiz, D., Huang, F., Crissman, J.W., Dickey, M., Froning, K., Conner, E.M., Cujec, T.P., Demarest, S.J.: Comparing domain interactions within antibody Fabs with kappa and lambda light chains. MAbs **8**, 1276–1285 (2016)
3. Vargas-Madrazo, E., Paz-García, E.: An improved model of association for VH–VL immunoglobulin domains: asymmetries between VH and VL in the packing of some interface residues. J. Mol. Recognit. **16**, 113–120 (2003)
4. Makabe, K., Nakanishi, T., Tsumoto, K., Tanaka, Y., Kondo, H., Umetsu, M., Sone, Y., Asano, R., Kumagai, I.: Thermodynamic consequences of mutations in vernier zone residues of a humanized anti-human epidermal growth factor receptor murine antibody, 528. J. Biol. Chem. **283**, 1156–1166 (2008)
5. Akiba, H., Tsumoto, K.: Thermodynamics of antibody–antigen interaction revealed by mutation analysis of antibody variable regions. J. Biochem. **158**, 1–13 (2015)
6. Sung, J.S., Bong, J.-H., Lee, S.J., Jung, J., Kang, M.-J., Lee, M., Shim, W.-B., Jose, J., Pyun, J.-C.: One-step immunoassay for food allergens based on screened mimotopes from autodisplayed FV-antibody library. Biosens. Bioelectron. **202**, 113976 (2022)
7. Jung, J., Bong, J.-H., Kim, H.-R., Park, J.-H., Lee, C.K., Kang, M.-J., Kim, H.O., Pyun, J.-C.: Anti-SARS-CoV-2 nucleoprotein antibodies derived from pig serum with a controlled specificity. Biochip J. **15**, 195–203 (2021)
8. Bong, J.-H., Kim, T.-H., Jung, J., Lee, S.J., Sung, J.S., Lee, C.K., Kang, M.-J., Kim, H.O., Pyun, J.-C.: Pig Sera-derived Anti-SARS-CoV-2 antibodies in surface plasmon resonance biosensors. Biochip J. **14**, 358–368 (2020)
9. Bong, J.-H., Kim, T.-H., Jung, J., Lee, S.J., Sung, J.S., Lee, C.K., Kang, M.-J., Kim, H.O., Pyun, J.-C.: Competitive immunoassay of SARS-CoV-2 using pig sera-derived anti-SARS-CoV-2 antibodies. Biochip J. **15**, 100–108 (2021)
10. Matson, R.S.: Microarray methods and protocols. CRC Press, Baco Raton (2009)
11. Li, L.-L., Ge, P., Selvin, P.R., Lu, Y.: Direct detection of adenosine in undiluted serum using a luminescent aptamer sensor attached to a terbium complex. Anal. Chem. **84**, 7852–7856 (2012)
12. Cortijo-Campos, S., Ramírez-Jiménez, R., De Andrés, A.: Raman and fluorescence enhancement approaches in graphene-based platforms for optical sensing and imaging. Nanomaterials **11**, 644 (2021)
13. Xie, L., Ling, X., Fang, Y., Zhang, J., Liu, Z.: Graphene as a substrate to suppress fluorescence in resonance Raman spectroscopy. J. Am. Chem. Soc. **131**, 9890–9891 (2009)
14. Swathi, R., Sebastian, K.: Resonance energy transfer from a dye molecule to graphene. J. Chem. Phys. **129**, 054703 (2008)
15. Kato, N., Caruso, F.: Homogeneous, competitive fluorescence quenching immunoassay based on gold nanoparticle/polyelectrolyte coated latex particles. J. Phys. Chem. B **109**, 19604–19612 (2005)
16. Mayilo, S., Kloster, M.A., Wunderlich, M., Lutich, A., Klar, T.A., Nichtl, A., Kürzinger, K., Stefani, F.D., Feldmann, J.: Long-range fluorescence quenching by gold nanoparticles in a sandwich immunoassay for cardiac troponin T. Nano Lett. **9**, 4558–4563 (2009)
17. Oh, W.-C., Areerob, Y.: Modeling dye-sensitized solar cells with graphene based on nanocomposites in the Brillouin zone and density functional theory. J. Korean Ceram. Soc. **58**, 50–61 (2021)
18. Hasani, A., Do, H.H., Tekalgne, M., Hong, S.H., Jang, H.W., Kim, S.Y.: Recent progress of two-dimensional materials and metal–organic framework-based taste sensors. J. Korean Ceram. Soc. **57**, 353–367 (2020)
19. Moghaddas, S., Salehi, M., Bagheri-Kazemabad, S.: Preparation, characterization, and photocatalytic degradation of methylene blue of SnO2/RGO nanocomposite produced by facile hydrothermal process. J. Korean Ceram. Soc. (2022). https://doi.org/10.1007/s43207-022-00210-3
20. Zheng, Y., Chen, J., Li, Y., Xu, Y., Chen, L., Chen, W., Liu, A., Lin, X., Weng, S.: Dual-probe fluorescent biosensor based on T7 exonuclease-assisted target recycling amplification for simultaneous sensitive detection of microRNA-21 and microRNA-155. Anal. Bioanal. Chem. **413**, 1605–1614 (2021)
21. Lan, L., Chen, D., Yao, Y., Peng, X., Wu, J., Li, Y., Ping, J., Ying, Y.: Phase-dependent fluorescence quenching efficiency of MoS2 nanosheets and their applications in multiplex target biosensing. ACS Appl. Mater. Interfaces **10**, 42009–42017 (2018)
22. Liu, Z., Ciais, P., Deng, Z., Lei, R., Davis, S.J., Feng, S., Zheng, B., Cui, D., Dou, X., Zhu, B.: Near-real-time monitoring of global CO2 emissions reveals the effects of the COVID-19 pandemic. Nat. Commun. **11**, 1–12 (2020)
23. Tits, J., Walther, C., Stumpf, T., Macé, N., Wieland, E.: A luminescence line-narrowing spectroscopic study of the uranium (VI) interaction with cementitious materials and titanium dioxide. DTr **44**, 966–976 (2015)
24. Khrenova, M., Topol, I., Collins, J., Nemukhin, A.: Estimating orientation factors in the FRET theory of fluorescent proteins: the TagRFP-KFP pair and beyond. Biophys. J. **108**, 126–132 (2015)

25. Gautier, V., Boumeester, A.J., Lössl, P., Heck, A.J.: Lysine conjugation properties in human IgGs studied by integrating high-resolution native mass spectrometry and bottom-up proteomics. Proteomics **15**, 2756–2765 (2015)
26. Veerapathiran, S., Wohland, T.: Fluorescence techniques in developmental biology. J. Biosci. **43**, 541–553 (2018)
27. Goryashchenko, A.S., Khrenova, M.G., Savitsky, A.P.: Detection of protease activity by fluorescent protein FRET sensors: from computer simulation to live cells. Methods Appl. Fluoresc. **6**, 022001 (2018)
28. Pickering, S., Batra, R., Merrick, B., Snell, L.B., Nebbia, G., Douthwaite, S., Reid, F., Patel, A., Ik, M.T.K., Patel, B.: Comparative performance of SARS-CoV-2 lateral flow antigen tests and association with detection of infectious virus in clinical specimens: a single-centre laboratory evaluation study. Lancet Microbe **2**, e461–e471 (2021)
29. Parker, C.W., Singh, N., Tighe, S., Blachowicz, A., Wood, J.M., Seuylemezian, A., Vaishampayan, P., Urbaniak, C., Hendrickson, R., Laaguiby, P.: End-to-end protocol for the detection of SARS-CoV-2 from built environments. Msystems **5**, e00771-e820 (2020)
30. Wu, K., Klein, T., Krishna, V.D., Su, D., Perez, A.M., Wang, J.-P.: Portable GMR handheld platform for the detection of influenza a virus. ACS Sens. **2**, 1594–1601 (2017)
31. Wu, K., Liu, J., Saha, R., Su, D., Krishna, V.D., Cheeran, M.C.-J., Wang, J.-P.: Magnetic particle spectroscopy for detection of influenza a virus subtype H1N1. ACS Appl. Mater. Interfaces **12**, 13686–13697 (2020)
32. Maneeprakorn, W., Bamrungsap, S., Apiwat, C., Wiriyachaiporn, N.: Surface-enhanced Raman scattering based lateral flow immunochromatographic assay for sensitive influenza detection. RSC Adv. **6**, 112079–112085 (2016)
33. Wiriyachaiporn, N., Sirikaew, S., Chitchai, N., Janchompoo, P., Maneeprakorn, W., Bamrungsap, S., Pasomsub, E., Japrung, D.: Pre-clinically evaluated visual lateral flow platform using influenza A and B nucleoprotein as a model and its potential applications. RSC adv. **11**, 18597–18604 (2021)