Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Supersensitive detection of single-histamine molecule on nanoplates by turn-on small molecule fluorescence sandwich immunoassay

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Contents

Materials and methods

Regents

Dithiobis(succinimidyl propionate) (DSP, A35393) and recombinant protein A/G (21186) were purchased from Pierce (Rockford, IL, USA). Dimethyl sulfoxide (DMSO, 276855), glycine (G8898), phosphate-buffered saline (PBS, P3813), and human AB serum (H4522) were obtained from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Tris(hydroxymethyl)-aminomethane was purchased from Daejung Chemical and Metals Co., Ltd. (Siheung, Korea). StabilGuard™ Immunoassay Stabilizer (BSA-free) solution was purchased from Surmodics (Eden Prairie, MN, USA). A HIS competitive ELISA kit was purchased from Abcam (ab213975, Cambridge, UK). The general HIS antibody pair kit (MBS2111473, for capture antibody (rabbit polyclonal antibody) and standard antigen and biotin recombinant protein A/G conjugated antibody (MBS2003749) were purchased from MyBioSource (San Diego, CA, USA). Streptavidin, Alexa Fluor™ 488 conjugate (SA-Flu, S11223), and quantum dot (QD) 525 streptavidin conjugate (SA-QD, Q10141MP) were purchased from Invitrogen (Rockford, IL, USA). Rabbit triiodothyronine (T3: 650.9735 Da, $C_{15}H_{12}I_3NO_4$) polyclonal antibody (MBS2001953), rabbit T3 polyclonal antibody (biotin-conjugated antibody, MBS2007851), rabbit thyroxine (T4: 776.87 Da), polyclonal antibody (MBS2032233), and rabbit T4 polyclonal antibody (biotin-conjugated antibody, MBS2092023) were purchased from MyBioSource (San Diego, CA, USA). T3 (125-300) and T4 (225-300) antigens were purchased from Monobind Inc. (Lake Forest, CA, USA).

Real sample preparation

The HIS in the cheese sample (Dongwon, Haegang-ro, Korea) was extracted, as reported by Shan-Shan et al [1]. Briefly, 5.00 g of the crushed cheese was placed in 10-mL methanol, followed by vortex vibration for 1 min, sonication for 30 min, and centrifugation (10000 ×g) for 10 min at 4°C. The supernatant was collected and dissolved in 1 mL of ultra-pure water. The red wine sample (The seventh-generation red wine–Merlot; Vivino, CA, USA) was extracted using a previously reported method [2]. 0.50 g of polyvinylpyrrolidone as bleach was added to a 10-mL red grape solution. The mixture was shaken for 15 min on an Orbital SH30 shaker (FINEPCR, Gyeonggi-do, Korea) and then filtered through a 0.25-μm filter. In the case of serum, HIS standard was spiked into human AB serum diluted 100-fold with PBS so that the final concentration was 4.49 nM. For the ELISA and turn-on smFSIA experiments, each mixture was diluted 10^3-fold and 10^6-fold before the immunoreaction, respectively.

Fluorescence sandwich immunoassay on gold nanoplates

A 5 × 5 gold nanoplate (diameter of 300 nm) as a substrate for the sandwich immunoreaction was fabricated on a 1-cm glass (Boro-33, thickness 500 μm) using an electron beam evaporator (Korea Advanced Nano-Fab Center, Suwon, Korea) according to a previously reported method [3]. In a sandwich noncompetitive assay, immunoreactions were conducted at 25°C according to a previously reported procedure (Fig. 1, upper) [3]. The gold nanoplate chip was reacted with 4 mg/mL of fresh DSP in DMSO in a stepwise manner for 30 min and then rinsed with DMSO and PBS. Next, 0.1 mg/mL of protein A/G was added to facilitate Fc binding for 1 h. Unreacted succinimide was blocked with 10-mM Tris (pH 7.5) and 1-M glycine for 30 min. In order to

effectively preserve the conformation and activity of dried proteins and at the same time block the surface to reduce nonspecific binding, it was immersed in a StabilGuard solution for 30 min, followed by reaction with 20 μL of 20 μg/mL HIS capture antibody for 1 h. Once the antibody reaction was completed, the HIS (5 zM–50 pM) and real samples (i.e., human serum, wine, and cheese) were loaded on the nanoplate chip and reacted for 1 h. Biotin-conjugated antibody with 20 μL of 20 μg/mL was reacted for 1 h. Streptavidin-Alexa fluor488 (SA-Flu) with 20 μL of 20 μg/mL was reacted for 1 h. The products from all subsequent steps were washed with PBS buffer. After the abovementioned steps, the nanoplate chip was placed on a dove prism and observed with a detection system by TIR fluorescence/plasmonic microscopy in the laboratory.

Competitive ELISA in microplates

The concentrations of the HIS standard and real samples were quantified using a quantitative competitive ELISA immunoassay to validate our developed immunoassay (Fig. 1, bottom). In a competitive assay, HIS standards (270 pM−225 nM) and real samples are added to a goat anti-rabbit IgG antibody-coated microplate. A polyclonal antibody to HIS and a solution of biotinylated HIS antigen (tracer) are both added to the microplate wells. To detect a measurable signal that directly corresponds to the amount of HIS in the sample, a solution containing SA–HRP was added to react with the biotinylated HIS tracer. All standards and reagents were prepared, as described in the manual provided by the company. The observation was performed at λ_{abs} = 450 nm (HRP) using the multi-mode microplate reader (Synergy HT, BioTek Instruments, VT, USA).

Supersensitive TIR fluorescence/plasmonic microscopy

The schematic representation and physical layout of TIR fluorescence/plasmonic microscopy were represented with modifications from a previously published system [3]. Briefly, the lab-based TIR fluorescence/plasmonic microscopy was performed with an upright Olympus BX53 microscope (Olympus Co., Ltd., Tokyo, Japan). The TIR microscopy consists of a 473-nm laser (200 mW, SL-473 nm TEMoo, Korea Lasertronix, Inc., Gangnam-gu, Seoul, Korea), a mechanical shutter (model LS3Z2, Uniblitz, Rochester, NY, USA), an objective lens (UPlanFLN, ×100, Olympus Co., Ltd., Tokyo, Japan), and two bandpass filters (Semrock, Rochester, NY, USA) with central wavelengths of 473/10 nm for the plasmonic scattering signal of the gold nanoplate and 520/15 nm for the fluorescence signal of the detection probe (SA-Flu), respectively. The gold nanoplate chip after the immunoreaction is mounted on a transmitting dove-type prism (BK7, 15 × 63 × 15 mm, Korea Electro-Optics Co., Ltd., Incheon, Korea) and imaged using an electron-multiplying cooled charge-coupled device camera (QuantEM 512SC, Photometrics, Tucson, AZ, USA). Fluorescence intensity was calculated using the difference between the intensities of the selected signal regions and the intensities of the background regions with the same CCD image area using the MetaMorph software (Version 7.8.6, Universal Imaging, Sunnyvale, CA, USA).

References

1 S.-S. Yang, Y.-N. Yang, X.-L. LI and Y. Zhang, *Chin. J. Anal. Chem.*, 2016, **44**, 396–402.

2 H. T. T. Nguyen, S. Lee, J. Lee, J. H. Ha and S. H. Kang, *Sens. Actuator B-Chem.*, 2021, **345**, 130354.

3 S. Lee, J. Lee, I. Batjikh, H. Yu and S. H. Kang, *ACS Sens.*, 2022, **7**, 1372–1380.

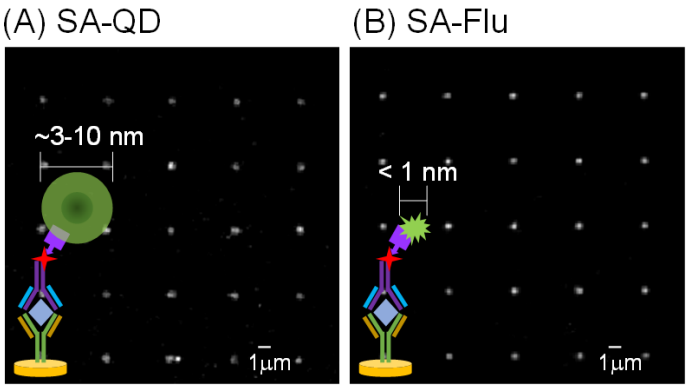

Fig. S1 Fluorescence images by immunoscreening using TIR system after small molecule immunoreaction using two types of nanoprobes. The concentration of HIS was 5 pM. SA–QD, quantum dot 525 streptavidin conjugate; SA-Flu, Alexa Fluor™ 488 streptavidin conjugate.

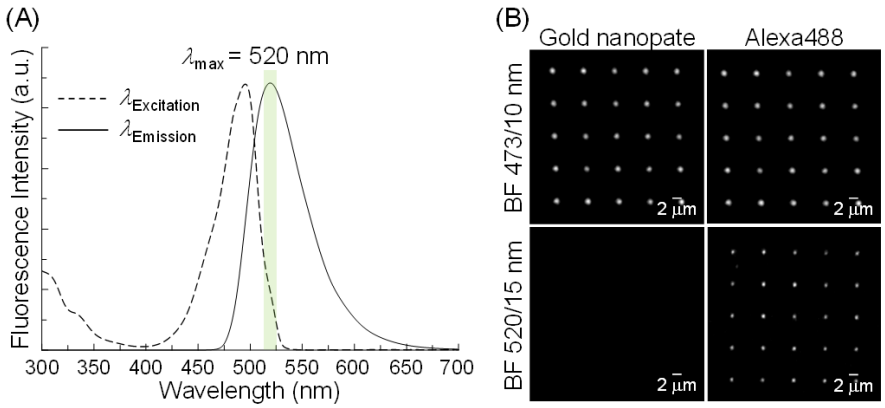

Fig. S2 (A) Fluorescence spectra of Alexa488 fluorescent dye (λ_{ex} = 495 nm and λ_{em} = 520 nm). The green box means the full width at half-maximum of the bandpass filter for 520/15 nm. (B) Images of the scattering signal of gold nanoplate and the fluorescence signal of Alexa488 through two bandpass filters (i.e., BF 473/10 nm and BF 520/15 nm).

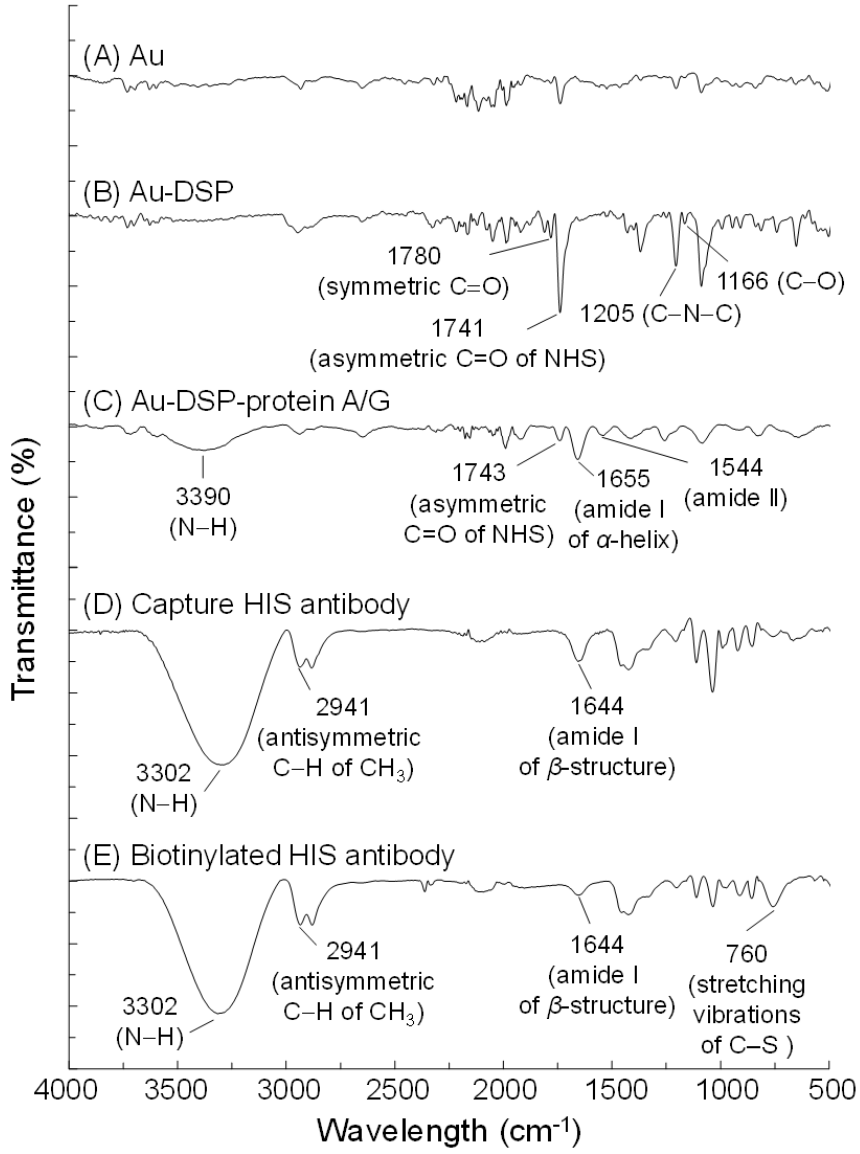

Fig. S3 Fourier transform infrared spectra of (A) a bare gold (Au), (B) dithiobis(succinimidyl propionate) (DSP) on a gold surface, (C) protein A/G-DSP on a gold surface, (D) HIS antibody-protein A/G-DSP on a gold surface, and (E) biotinylated HIS antibody-HIS antigen-HIS antibody-protein A/G-DSP on gold surface.

Table S1 Comparison of turn-on smFSIA with other methods for HIS detection

Detection methods	Linear dynamic range[a]	LOD[a]	Reference
UPLC-MS/MS	0.18–9.00 nM	0.03 nM	[1]
Sandwich ELISA	0.11–13.50 nM	0.05 nM	[2]
ic-ELISA	–	0.80 nM	[3]
ic-CLEIA	–	0.66 nM	[3]
UV-Vis	0.001–10 μM	0.87 nM	[4]
Fluorescence spectrum assay	0.01–1.0 μM	2.04 nM	[4]
Electrochemical biosensor	0.45–1.05 mM	48.7 μM	[5]
Capillary electrophoresis	–	17.99 mM	[6]
Colorimetric method	0.18–1.08 mM	0.18 mM	[7]
Turn-on smFSIA	5 zM–50 pM	5 zM	This work

Indication: UPLC, ultrahigh performance liquid chromatography; ic-ELISA, indirect competitive enzyme-linked immunosorbent assay; ic-CLEIA, indirect competitive chemiluminescence enzyme immunoassay; turn-on smFSIA, turn-on small molecule fluorescence sandwich immunoassay; LOD, limit of detection. [a]The conversion of HIS concentration was calculated based on the molecular weight of HIS (111.148 Da).

References

1 Q. H. Tran, T. T. Nguyen and K. P. Pham, *Int. J Anal. Chem*., 2020, **2020**, 2187646 (1–9).

2 F. Yang, L. Xu, A. C. P. Dias and X. Zhang, *Food Chem.*, 2021, **350**, 129196.

3 L. Xu, J. Zhou, S. Eremin, A. C. P. Dias and X. Zhang, *Anal. Bioanal. Chem*., 2020, **412**, 4739–4747.

4 J. Bi, C. Tian, G.-L. Zhang, H. Hao and H.-M. Hou, *Foods*, 2020, **9**, 316.

5 M. B. Gumpu, N. Nesakumar, S. Sethuraman, U. M. Krishnan and J. B. B. Rayappan, *Sens. Actuator B-Chem*., 2014, **199**, 330–338.

6 E. Numanoglu, I. H. Boyaci and A. Topcu, *J. Food Drug Anal*., 2008, **16**, 74–80.

7 R. Lv, X. Huang, C. Dai, W. Ye and X. Tian, *J. Food Process Eng*., 2019, **42**, 13099.