Supporting Information

A Charge-Switchable Zwitterionic Peptide for Rapid Detection of SARS-CoV-2 Main Protease

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1. Materials

Bis(*p*-sulfonatophenyl)phenylphosphine dihydrate dipotassium salt (BSPP, 97%), gold(III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$, >99.9%), sodium citrate tribasic dihydrate (>99%), sodium dodecyl sulfate (SDS, >99%) Trizma® base (>99.9%), Trizma® hydrochloride (>90%), DL-dithiothreitol (DTT, >99%), Brij® 35, trifluoroacetic acid (TFA, HPLC grade, >99%), 2,2′-(ethylenedioxy)diethanethiol (EDDET, 95%), and piperidine (ReagentPlus®, 99%) were purchased from Sigma Aldrich (St Louis, MO). Trypsin (Tp), thrombin (Tb), hemoglobin (Hgb), albumin from bovine serum (BSA) were also purchased from Sigma Aldrich. Polyethylene glycol 2,000 (PEG_{2000}) was from Alfa Aesar (Tewksbury, MA). Thioanisole (>99%), N,N-diisopropylethylamine (DIPEA, >99%), and triisopropylsilane (TIPS, >98%) were purchased from Tokyo Chemical Industry Co., Ltd. (TCI). Sodium chloride (certified ACS), sodium phosphate monobasic monohydrate (certified ACS), and sodium phosphate dibasic anhydrous (certified ACS) were purchased from Fisher Scientific International, Inc. (Hampton, NH). Fmoc-protected L/D-amino acids, hexafluorophosphate benzotriazole tetramethyl uronium (HBTU), and Fmoc-Rink amide MBHA resin (0.67 mmol/g, 100-150 mesh) were purchased from AAPPTec, LLC (Louisville, KY). 40-nm Citrate-stabilized AuNPs (5 mL, O.D. =1) was purchased from nanoComposix Inc. (San Diego, CA). The main protease (M^{pro}, no His-tag) of SARS-CoV-2 were given as kind gifts from Dr. Anthony O'Donoghue, UC San Diego, USA. The M^{pro} was stored in 20 mM Tris-HCl (pH 8.0), with 150 mM NaCl, 1 mM DTT, and 5 % glycerol at −80 °C. M^{pro} inhibitor GC376 (507.53 g/mol) was purchased from Selleckchem. The pooled whole human saliva was purchased from Lee Biosolutions, Inc. (Maryland Heights, MO). The exhaled breath condensation (EBC) was collected from a healthy volunteer (COVID-negative) using a lab condensate tube set-up. Organic solvents including N,N-dimethylformamide (DMF, sequencing grade), acetonitrile (ACN, HPLC grade), ethyl ether (certified ACS), methylene chloride (DCM, certified ACS), and dimethyl sulfoxide (DMSO, certified ACS) were also from Fisher Scientific International, Inc. (Hampton, NH). D_2O solvent for ^1H NMR experiments was purchased from Cambridge Isotope Laboratories (Andover, MA). Ultrapure water (18 MΩ·cm) was obtained from a Milli-Q Academic water purification system (Millipore Corp., Billerica, MA). TEM grids (formvar/carbon 300 mesh Cu) were purchased from Ted Pella (Redding, CA). Amicon® ultra-15 centrifugal filter units (M.W. cutoff =100 kDa) and automation compatible syringe filters (hydrophilic PTFE, 0.45 μm) were from MilliporeSigma (St. Louis, MO). The absorbent pad (6613H, treated spunbound

polyester) was obtained from Ahlstrom-Munksjö (Helsinki, Finland). Glassware and stir bars were cleaned with aqua regia (HCl:HNO_3=3:1 by volume) and boiling water before use.

2. Materials Synthesis and Instrumentations

2.1 Peptide Synthesis

Peptides were synthesized using an automated Eclipse™ peptide synthesizer (AAPPTec, Louisville, KY) through standard solid phase Fmoc synthesis on Rink-amide resin (see below). Peptides were lyophilized in a FreeZone Plus 2.5 freeze dry system (Labconco Corp., Kansas, MO).

All peptides were chain assembled by Fmoc-SPPS (solid-phase peptide synthesis) on Rink-amide resin (0.67 mmol/g, 200 mg) using the Fmoc-peptide synthesizer. Amino acid couplings were performed with Fmoc-amino acid (5 equiv.), 0.4 M HBTU in DMF (5 equiv.), and DIPEA (7.5 equiv.). The number of coupling cycles followed the sequence analyzer built in the peptide synthesizer. Finished peptides on the resin were transferred into a syringe filter and washed with five rounds of DCM (~ 5 mL). Then, peptides were cleaved from the resin using a cleavage cocktail (3 mL) that contained TFA (82.5%), EDDET (2.5%), phenol (5%), thioanisole (5%), and H_2O (5%). Resins were treated with the cleavage cocktail for 120 min. After cleavage the resin was filtered and TFA was evaporated using a stream of N_2 over the mixture. Crude peptides were precipitated with cold ether (15 mL), suspended in 50% ACN/H_2O (5 mL), and lyophilized. Peptides were purified by the reversed phase HPLC, characterized by the ESI-MS, aliquoted and stored in dry conditions at −20 °C for use. To quantify the sample concentration, the absorbance at 205 nm of aqueous peptide sample was measured using the nanodrop spectrophotometer.[1] In the standard plot of optical absorbance *vs.* mass concentration, the slope of the linear regression line was indicated as, ε_{205} ~74.6 mL·mg^{-1} cm^{-1} (note that DMSO or acetonitrile contribute absorbance signal at 205 nm; water or alcohol is used).

Peptide purification was carried out using a Shimadzu LC-40 HPLC system equipped with a LC-40D solvent delivery module, photodiode array detector SPD-M40, and degassing unit DGU-403. The sample was dissolved in water and acetonitrile, applied on a Zorbax 300 BS, C18 column (5 μm, 9.4×250 mm) from Agilent, and eluted at 1.5 mL/min[2] with a 40 min gradient from 10% to 95% of acetonitrile in water (with 0.05% TFA). Preparative injections were monitored at 190, 220, and 254 nm. All products were purified by HPLC to reach purity

of >90%.

Peptide synthesis and cleavage was confirmed using electrospray ionization mass spectrometry (ESI-MS) *via* the Micromass Quattro Ultima mass spectrometer in the Molecular MS Facility (MMSF) at Chemistry and Biochemistry Department, UC San Diego. ESI-MS samples were prepared in a 50% MeOH/H_2O mixture. Peptide concentration was determined using a NanoDrop™ One UV-vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA).

2.2 Nanoparticle Synthesis and Characterization

Colloidal Gold Synthesis. The citrate-stabilized AuNPs (~13 nm diameter, TEM) was prepared using Turkevich method by rapidly injecting an aqueous solution of sodium citrate tribasic dihydrate (150 mg, 5 mL) into an aqueous solution of $HAuCl_4 \cdot 3H_2O$ (45 mg, 300 mL) under boiling conditions and vigorous stirring.[3] The reaction mixture was left boiling while stirring for another 15 min, and then cooled down to room temperature. The deep red dispersion was then purified by applying one round of centrifugation at 18,000 *g* for 30 min and the pink supernatant was discarded. The resulting pellet of citrate-AuNPs was redispersed in DI water by sonication and stored at ambient conditions. The ligand exchange was carried out as below. A stock citrate-AuNP dispersion in water (200 mL, 3.4 nM) was vigorously stirred overnight with BSPP (205 mg, 5 mL) in a round bottom flask. The mixture was purified via syringe filtration (hydrophilic PTFE, 0.45 mm) followed by two rounds of centrifugation at 18,000 *g* for 40 min each. The resulting pellet of BSPP-AuNPs was redispersed in DI water at an optical density of 1.5 (c ~3.8 nM, $\varepsilon_{520}=4.0\times10^8$ $M^{-1}cm^{-1}$)[4, 5] and stored at 4 °C for use.

The optical absorption measurements were collected using a hybrid multi-mode microplate reader (Synergy™ H1 model, BioTek Instruments, Inc.) in a clear 96-well plate.

The dynamic light scattering (DLS) and zeta (ζ) potential measurements were carried out using a compact Zetasizer Nano ZS90 (Malvern Panalytical, Inc.).

Transmission electron microscopy (TEM) images of the Au colloids were acquired using a JEOL 1200 EX II operated at 80 kV. The TEM grids were prepared by drop casting samples in water (4 μL) followed by natural drying. A scanning electron microscope (SEM, FEI Apreo) was used to analyze the Au nanoparticles on an absorbent pad. SEM images were taken at an accelerating voltage of 1 kV and a current of 0.10 nA.

Proton nuclear magnetic resonance (^{1}H NMR) was recorded using a Bruker Avance III HD 300 MHz spectrometer equipped with a 5-mm z-gradient Broad Band Observe (BBO) probe (Bruker

SpectroSpin, Billerica, MA), operating at 300 MHz for the ^{1}H frequency. The NMR sample of BSPP-Au colloids were prepared by exchanging the solvent with D_2O using a centrifugal filter unit.

Photographic images were taken by smartphone in a lightbox of white background. A Cricut Explore Air® 2 vinyl cutter (Cricut, Inc.) was used to customize the pieces for assembling the portable sensing strip. The details of sensing strip assembly can be found in a previous report.[6]

3. Cleavage of Peptides

To confirm the enzyme cleavage site, the peptide solution (50 μL, 5 mg/mL) in Tris buffer (20 mM, pH 8.0, with 150 mM NaCl, 1 mM DTT) was incubated with the M^{pro} protease at a molar ratio of 3000:1 (substrate : enzyme) at 37 °C for 3 hours. The mixture was then applied on a Shim-pack GIS, C18, analytical column (5 μm, 4.6×100 mm) from Agilent, and eluted at 1 mL/min[2] with a 40 min gradient from 10% to 95% acetonitrile. Preparative injections were monitored at 190, 220, and 254 nm. All fractions were collected, and the molecular weight was determined using the ESI mass spectrometer.

4. Particle Aggregation Factors

Effects of peptide fragmentation. (i) The *parent* peptide stock: intact peptide was dissolved in the Tris buffer (pH 8.0) with DMSO content < 1% *v/v*. (ii) The *fragments* stock: Enzymatically cleaved product of the parent was obtained by incubating the parent peptide with M^{pro} at a molar ratio of 3000:1 at 37 °C for 3 hours. Next, in a 96-well plate BSPP-AuNPs (100 μL, 3.8 nM, optical density ~1.5) were mixed with the parent/fragment peptides (10 μL) to reach the final concentration of 0, 0.5, 1, 2.5, 5, 10, 30, 60, 120, and 240 μM. At least At least two replicates of each experiment were measured. Absorbance at 600 nm and 520 nm at room temperature was measured every 1 min for 1 h. The ratiometric signal ($\lambda_{600/520}$) at 10 min was extracted for analyses. Tris buffer was used as the blank. In addition, the above mixtures of particle-peptide (at 10 min incubation) were diluted to 800 mL by DI water and their hydrodynamic diameter (D_H) and zeta potential (ζ) were characterized using the Zetasizer Nano ZS90.

Effects of surfactant additives. In a microtube BSPP-AuNPs (100 μL, 3.8 nM) were incubated with the peptide fragment (ZY7) to reach a final $c_{peptide}$ ~10 μM; an instant particle aggregation occurred. The aggregated suspension was then mixed with one of the following aqueous surfactants (60 μL, 1% *w/v*): SDS and CTAB (ionic molecules); PEG_{2000}, Tween-20, Brij 35, and Triton X-100 (nonionic molecules). Next, the particles were spun down at 18,000 *g* for 20 min and redispersed in 100 μL of DI water. These particles were applied to another round of aggregation (by fragments) and dispersion (by surfactants) until no color change was observed. The optical absorbance profiles of the gold particles at each step were recorded.

Effects of pH. (1) Acidic conditions. BSPP-AuNPs (100 μL, 3.8 nM) were spun down at 18,000 *g* for 10 min and resuspended in pH 3 phosphate buffer (adjust by 1 M HCl). Next, in a 96-well plate the pH adjusted BSPP-AuNPs (100 μL, 3.8 nM) were mixed with the ZY7 parent peptide (10 μL) to reach the final concentration of 0, 0.5, 1, 2.5, 5, 10, 30, 60, 120, and 240 μM. Two replicates of each experiment were measured (n = 2). Absorbance at 600 nm and 520 nm at room temperature was measured every 1 min for 1 h. The ratiometric signal ($\lambda_{600/520}$) at 10 min was used for analyses. (2) Basic conditions. The overall procedure was similar to above except that the BSPP-AuNPs were resuspended in pH 13.5 phosphate buffer (adjust using 1 M NaOH) and the ZY7 peptide fragments were used.

Effects of number of Arg residues. The peptides with varying number of arginine on the aggregating site were synthesized and their fragments were prepared *via* standard enzymatic cleavage procedure as mentioned above. Then, in a 96-well plate BSPP-AuNPs (100 μL, 3.8 nM) were mixed with the parent/fragment peptides (10 μL) to reach the final concentration of 0, 0.5, 1, 2.5, 5, 10, 30, 60, 120, and 240 μM. These specific concentrations depended on the solubility of each peptide. At least two replicates of each experiment were measured ($n \geq 2$). The ratiometric signal ($\lambda_{600/520}$) measurements followed the same method, as above.

Effects of particle size. we studied the size and surface ligand effects using the same protocol as above, except that the large BSPP-AuNPs (*i.e.*, 40 nm in diameter, optical density ~1.5) were applied. In principle, the amount of aggregating fragment thus M^{pro} needed for collapsing colloidal suspension is proportional to the number of nanoparticles and surface changes.[7] Within this context, gold nanoparticles of high molar absorption coefficients (ε_A) would benefit the protease detection by presenting more intense color shifts.[5] A simple example illustrates that the colloidal suspension of 40-nm gold contains approximately 15-fold fewer particles

compared to that of 13-nm AuNPs at the same optical density (OD) given their unique molar absorptivity, $\varepsilon_{A\ (40\text{-nm})}$ ~6.1×10^9 *vs.* $\varepsilon_{A\ (13\text{-nm})}$ ~4.0×10^9 $M^{-1}cm^{-1}$.[4] The working range and M^{pro} LoD based on 40-nm BSPP-AuNPs (at the same OD ~0.4) using the ZY7 peptide is determined to be, 0.1−118.1 μM and 7.0 nM, respectively (**Figure S3g,h**).

5. Theoretical Estimation on AuNPs Aggregation

The net charge on the particle surface originates from the terminal groups on the surface ligand. We assume that the surface charge is proportional to the total number of ligands per particle. The density of BSPP ligand on 13-nm AuNP is reported to be ~0.3 BSPP/nm^2, that is, ~150 BSPP/13-nm NP.[8] The measured zeta potential of BSPP-AuNPs is about –25 mV (pK_a of Ph–SO_3^- = –2.8). In order to effectively aggregate the BSPP-AuNPs, **Figure 2d,e** shows that the zeta potential has to increase to more than ~ –10 mV. That is, the minimum amount of fragment added into the solution has to cancel out the charge created by 90 BSPP ligands, which carry −2 charges of each. Considering each ZY7 fragment carries +2 charges (*i.e.*, two arginine) and assuming the stoichiometric ratio is 1:1 for –Arg– and $–SO_3^-$, the molar ratio of [ZY7 fragment] to [BSPP-AuNP] will be 90. Our standard assay used 120 μL of 3.2 nM BSPP-AuNPs, therefore at least 0.3 μM of ZY7 fragment will be required to induce a color change. Experimentally, this value was determined to be at least ~3.2 μM (**Figure 3a**).

6. Limit of Detection (LoD) of M^{pro}

Assays in solution. We describe the use of ZY7 peptide and BSPP-AuNPs to detect M^{pro}, as a representative example. The M^{pro} enzymes of desirable amount were spiked into one of the below media: Tris buffer (pH 8.0, 100%), EBC (100%), and pooled human saliva (50%) to reach a final c_{enzyme} at 0, 0.5, 1, 2, 5, 10, 20, 30, 40, 50, 100, 200 nM (*i.e.*, equiv. to the same amount in a 120 μL solution). Then, the parent ZY7 peptide (2.2 μL, 2.65 mM) was added to the above mixtures in microtubes and the total volume of assay was set to 20 μL by adding Tris buffer (pH 8.0). Next, the new mixtures were tapped, mildly centrifuged, and incubated at 37 °C for 3 hours. At least two replicates of each experiment were made. After that time the assay was transferred into a 96-well plate and incubated with BSPP-AuNPs (100 μL, 3.8 nM).

The absorbance at 600 and 520 nm of above mixtures were readout in the microplate reader at 26 °C every 1 min for 1 h. The ratiometric signal ($\lambda_{600/520}$) at 10 min was extracted for analyses. Control experiments were carried out in Tris buffer (pH 8.0) using the same process with a scrambled peptide sequence. Other combinations of gold particles and peptides for detecting M^{pro} were also carried out following the same protocol, as above. The limit of detection was calculated using a statistical method previously reported in literature (**Figure S5d**):[9]

$$\mathrm{LoD_{int.}} = \mathrm{mean_{blank}} + 1.645 * (\mathrm{SD_{blank}}) + 1.645 * \left(\mathrm{SD_{low\ concentration\ sample}}\right) \quad (S1)$$

Remark: The enzyme detection can also be carried out using a one-pot assay, where enzyme cleavage and particle aggregation occurs *in situ*. A known amount of M^{pro} was spiked into Tris buffer (pH 8.0) to reach a final c_{enzyme} at 0, 5, 10, 20, 30, 40, 50, 100, 200 nM (*i.e.*, equiv. to the same amount in a 120 μL solution). The varying concentrations of M^{pro} were added to a mixture containing the parent ZY7 peptide (2.2 μL, 0.53 mM) and BSPP-AuNPs (100 μL, 3.8 nM) in a 96-well plate. The final volume was brought to 120 μL with Tris buffer (pH 8.0) and at least two replicates of each experiment were made. The ratiometric signal ($\lambda_{600/520}$) measurements were same as above.

Assays on strip. The blister pack was prefilled with 60 μL of BSPP-AuNPs (3.8 nM). The test lane was loaded with 50 μM of the parent ZY7 peptide. The positive control lane was loaded with a freshly synthesized fragment. The test strips were dried overnight at 37 °C. 10 μL of Buffer or EBC spiked with varying concentrations of M^{pro} (c_{enzyme} at 0, 10, 20, 30, 40, 50, 100, 200 nM) was pipetted onto the test lane to simulate sample collection. The M^{pro}-laden testing strips were incubated at 30 °C, 70 − 80% humidity for 3 hours to mimic conditions found in a mask.[10] After incubation, the gold dispersion was clicked and allowed to flow toward the adsorbent pad. Photographs were taken 10 min after gold dispersion.

7. Specificity Tests

Inhibitor assay for M^{pro}. The M^{pro} inhibitor (GC376) of desired amount was pre-incubated with the M^{pro} protease (3.1 μL, 3.8 μM) at molar ratios of 0, 0.05, 0.1, 0.2, 0.4, 0.8, 5, and 10 at room temperature for 10 min. Then, the parent ZY7 peptide (2.2 μL, 2.65 mM) was mixed with the inhibited enzyme and the assay volume was brought up to 20 μL with Tris buffer (pH 8.0). Next, the new mixtures were tapped, mildly centrifuged, and incubated at 37 °C for 3

hours. At least two replicates of each experiment were made. After that time the inhibitor assay was transferred into a 96-well plate, incubated with BSPP-AuNPs (100 μL, 3.8 nM), and readout the absorbance at 600 and 520 nm in the microplate reader at 26 °C every 1 min for 1 h. A linear form of the Morrison equation derived by Henderson was applied to calculate the active enzyme concentration and apparent inhibitory constant, $K_{i\ (app)}$:[11]

$$\frac{[\mathrm{I}]_0}{\left(1-\frac{\mathrm{v_i}}{\mathrm{v_0}}\right)} = [\mathrm{E}]_0 + \mathrm{K_i(app)}\left(\frac{\mathrm{v_0}}{\mathrm{v_i}}\right) \qquad (S2)$$

where $[E]_0$ is the active enzyme concentration in the stock, v_i/v_o is the fractional velocity and is assumed to be proportional to the fractional ratiometric signal in this study. The IC_{50} is calculated using $IC_{50} = [E]_0/2 + K_i(app)$.[12, 13]

Other endogenous mammalian proteins. We tested the responsiveness of ZY7 peptide and BSPP-AuNPs to other protease biomarkers such as including trypsin (Tp), thrombin (Tb), bovine serum albumin (BSA), and hemoglobin (Hb). These proteins of desirable amount were spiked into Tris buffer (pH 8.0) to reach a final *c* at 0, 0.5, 1, 2, 5, 10, 20, 30, 40, 50, 100, 200 nM (*i.e.*, equiv. to the same amount in a 120 μL solution). Then, the parent ZY7 peptide (2.2 μL, 2.65 mM) was added to the above mixtures in microtubes and the total volume of assay was brought up to 20 μL with Tris buffer. Next, the mixtures were tapped, mildly centrifuged, and incubated at 37 °C for 3 hours. At least two replicates of each experiment were made. After that time the assay was transferred into a 96-well plate and incubated with BSPP-AuNPs (100 μL, 3.8 nM). The ratiometric signal measurements were same as above.

Peptide mutated with D-amino acids. We tested the enzymatic activation of DS12 peptide (incorporation of D-Arg/Cys) and BSPP-AuNPs in M^{pro}-spiked saliva (50%), as a representative case. The M^{pro} enzymes of desirable amount were spiked into the pooled human saliva (50%) to reach a final c_{enzyme} at 0, 0.5, 1, 2, 5, 10, 20, 30, 40, 50, 100, 200 nM (*i.e.*, equiv. to the same amount in a 120 μL solution). The DS12 peptide (2.2 μL, 2.65 mM) was added to the above mixtures in microtubes and the total volume of assay was set to 20 μL by adding Tris buffer (pH 8.0). Then, the mixtures were tapped, mildly centrifuged, and incubated at 37 °C for 3 hours. At least two replicates of each experiment were made. After that time the assay was transferred into a 96-well plate and incubated with BSPP-AuNPs (100 μL, 3.8 nM). The ratiometric signal measurements were same as above.

8. COVID-negative Human Test

Human Subjects. This study was approved by the Human Research Protections Program at the University of California, San Diego (project# 202019). Informed written consent from each participant was acquired before testing. The inclusion criteria were as follows: (i) participants had to be 18 y and able to provide consent; and (ii) participants were confirmed to be COVID-19 negative by standard PCR test. Participants (n=10) were recruit for this study.

Solution phase test. The exhaled breath condensation (EBC) was collected from the COVID-negative participants using a lab condensate tube set-up. The work described in this contribution for development of a smart mask was done in preparation for a clinical trial approved by the University of California, San Diego Institutional Review Board. Then, the parent ZY7 peptide (2.2 μL, 2.65 mM) was added to the collected EBC (17.8 μL) in microtubes. The mixtures were tapped, mildly centrifuged, and incubated at 37 °C for 3 hours. At least two replicates of each experiment were made. After that time the assay was transferred into a 96-well plate and incubated with BSPP-AuNPs (100 μL, 3.8 nM). The ratiometric signal measurements were same as above.

Colorimetric Test on Strip. This study was approved by the Human Research Protections Program at the University of California, San Diego (Institutional Review Board, project # 202019). Informed written consent from each participant was acquired before testing. The inclusion criteria were as follows: (i) participants had to be 18 y and able to provide consent; and (ii) participants were confirmed to be COVID-19 negative by standard PCR test. Participants (n=10) were recruit for this study. On a clean absorbent pad, the aqueous ZY7 peptide solution (6 μL, 265 μM) was evenly pipetted onto the test line. The aggregating fragment YF15 (*i.e.*, SGRRGRR) of same amount was loaded on the control line. The absorbent pad was then dried and assembled into the sensing strip with a blister pack prefilled with BSPP-AuNPs (60 μL, 3.8 nM). The peptide-laden testing strip was affixed inside a standard surgical mask near nose. Next, the participants (n=10) wore the surgical masks with strips for 8 h. After that time the strips were collected, and the blister pack was clicked to allow the gold solution to flow toward the adsorbent pad at ambient conditions. The white-light images of the testing strips were taken in a white background at 10 min of testing. Negative and positive controls used a peptide-loaded test strip without wearing and a test strip incubated with M^{pro} (200 nM with respect to 120 μL solution) for 3 h, respectively.

Remark. The intact or fragment peptide can be adhered on the absorbent pad using a tannic acid solution (0.5 mg/mL, **Figure S7**).

9. References

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Table and Figures (Supporting Information)

Table S1. Structural components of the lateral-flow sensing strip.[6]

Piece [a]	Description	Function	Materials	Manufacturer
P1	cover	protective layer, transmission vents	PET carrier film	Drytac Facemount®
P2	absorbent pad	peptide loading	polyester, grade 6613H	Ahlstrom Munskjo
P3	channel spacer	absorbent pad affixing	bi-axially oriented PET film	Clear Grafix Dura-Lar®
P4	reagent storage	color agent loading	clear thermoformed blister pack	Dulcolax®
P6	bottom adhesive	mask affixing, transmission vents	adhesive PET carrier film	Drytac MultiTac™

[a] Pieces are named in order from right to left in **Figure 5a**. The procedure of strip assembly can be found in reference [6].

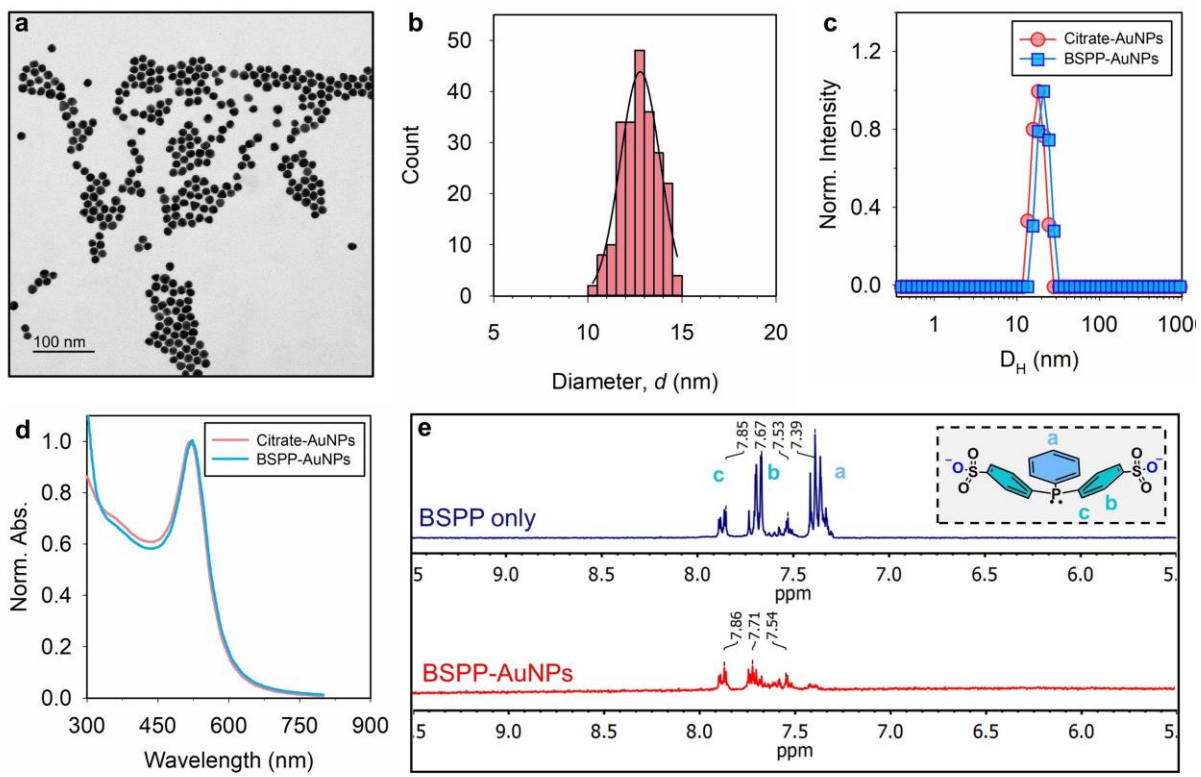

Figure S1. Characterization of gold colloids. **a**, TEM images of BSPP-AuNPs, which is prepared from citrate-coated AuNPs ligand exchanged with BSPP molecules. **b**, A histogram of particle size extracted from the TEM images, with an average diameter of d = 12.74 ± 0.96 nm. Data were analyzed in ImageJ by counting more than 300 particles using a manual size method. **c**, DLS profiles of citrate-capped AuNPs (D_H = 15.9 nm, PDI=0.05, red) and BSPP-coated AuNPs (D_H = 19.1 nm, PDI= 0.10, blue). **d**, Optical absorption profiles of citrate-AuNPs (SPR ~518 nm, red) and BSPP-AuNPs (SPR ~520 nm, blue). A slight increase of the maximum absorbance of the SPR band from 518 to 520 nm is attributed to changes of the dielectric environment around nanoparticles. **e**, ^{1}H NMR spectra collected from free BSPP only (1 mg/mL, 64 NS, top) and BSPP-AuNP (1 μM, 1024 NS, bottom) in D_2O solvent. Note that the ^{1}H NMR spectrum exhibits weak and broadened resonances compared to that of free ligands, which is generally attributed to slower transverse spin relaxations resulting from reduced interproton dipolar interactions for surface-coordinated ligands compared to free ones.[14]

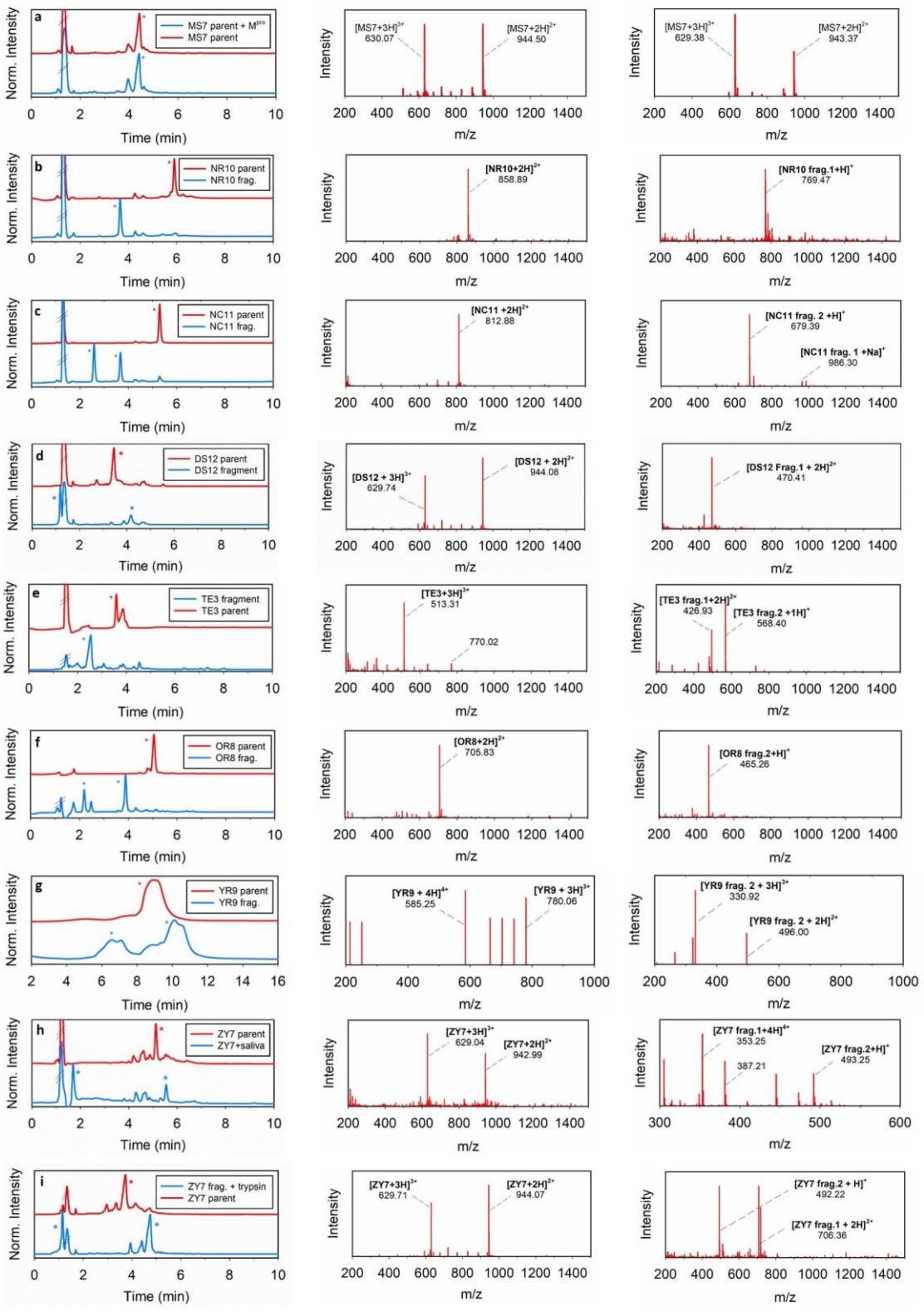

Figure S2. HPLC and ESI-MS data of the synthesized substrates and their fragments cleaved by M^{pro} peptidase. HPLC is shown in the left panel, and MS of parent peptide and the corresponding fragments are shown in the middle and right panel, respectively. **a-g**, Data collected from ZY7, NR10, NC11, DS12, TE3, OR8, and YR9 peptide with M^{pro}; cleavage after Gln (Q). **h**, Data collected from ZY7 peptide with pooled human saliva. **i**, Data collect from ZY7 with trypsin; cleavage after Arg (R). Peaks with "//" are from DMSO solvent molecules.

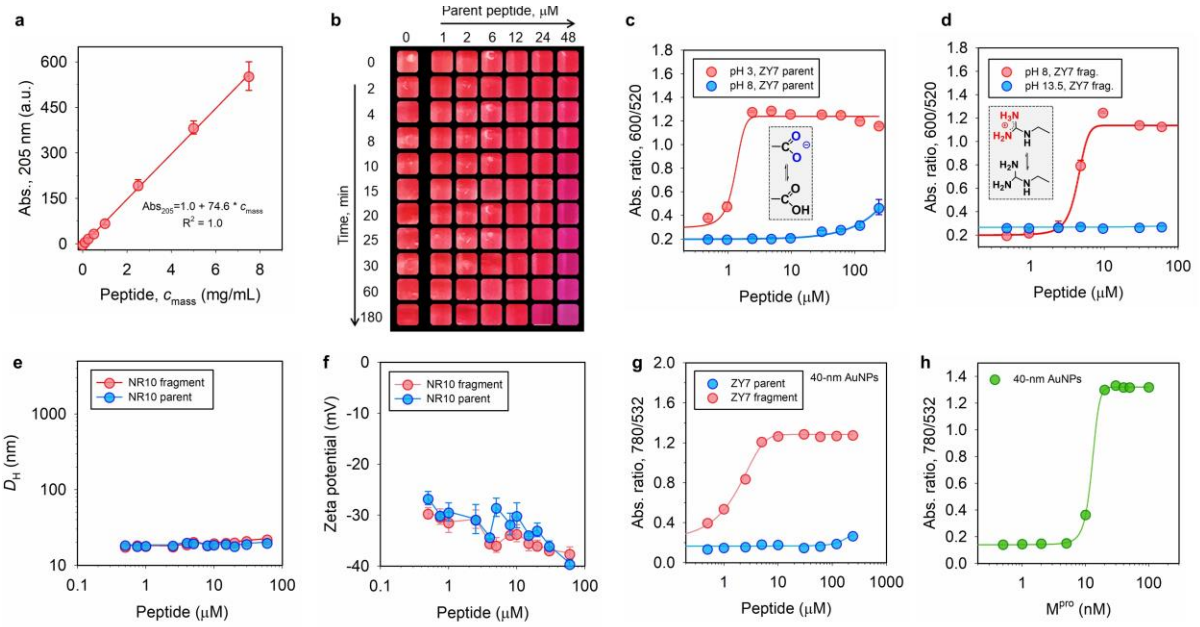

Figure S3. Peptide quantification, control experiments, pH effect, and sensor configuration. **a**, The mass concentration (c_{mass}, mg/mL) of each peptide was determined at the $Abs._{205\ nm}$ using the coefficient of 74.6 $mL \cdot mg^{-1} \cdot cm^{-1}$.[1] Aqueous solutions of ZY7 peptide were used for constructing the standard curve. The standard curve was best fitted with a linear function: $Abs_{205}=1.0+74.6\times c_{mass}$. **b**, The color evolution of BSPP-AuNPs (3.8 nM, 50 μL) in the presence of ZY7 parent peptide (no protease). The particle aggregation kinetic is concentration- and time-dependent, as shown in horizontal and vertical directions. **c**, The threshold of parent ZY7 in clustering BSPP-AuNPs at pH 8 (55.3 μM) and at pH 3 (0.3 μM). The threshold of ZY7 fragments in aggregating BSPP-AuNPs at pH 8 (3.2 μM) and at pH 13.5 (none). **e**, DLS profiles of BSPP-AuNPs (3.8 nM, 100 μL) incubated with increasing concentrations of NR10 parent peptide (blue) and NR10 fragments (red). No change of the hydrodynamic diameter (D_H) for BSPP-AuNPs was observed when incubated with either NR10 peptide or its fragments. **f**, Zeta potential measurements of AuNPs (3.8 nM, 100 μL) incubated with increasing concentrations of NR10 parent peptide (blue) and NR10 fragments (red). Both specimens shifted the surface charges to negative from approx. −28 mV to −38 mV, presumably the negative electrophoretic properties of the NR10 peptide. **g**, The operation window of M^{pro} sensor based on 40-nm BSPP-AuNPs and ZY7 peptide, 0.1−118.1 μM. **h**, The LoD of M^{pro} sensor based on 40-nm BSPP-AuNPs in TB buffer, ~7.0 nM.

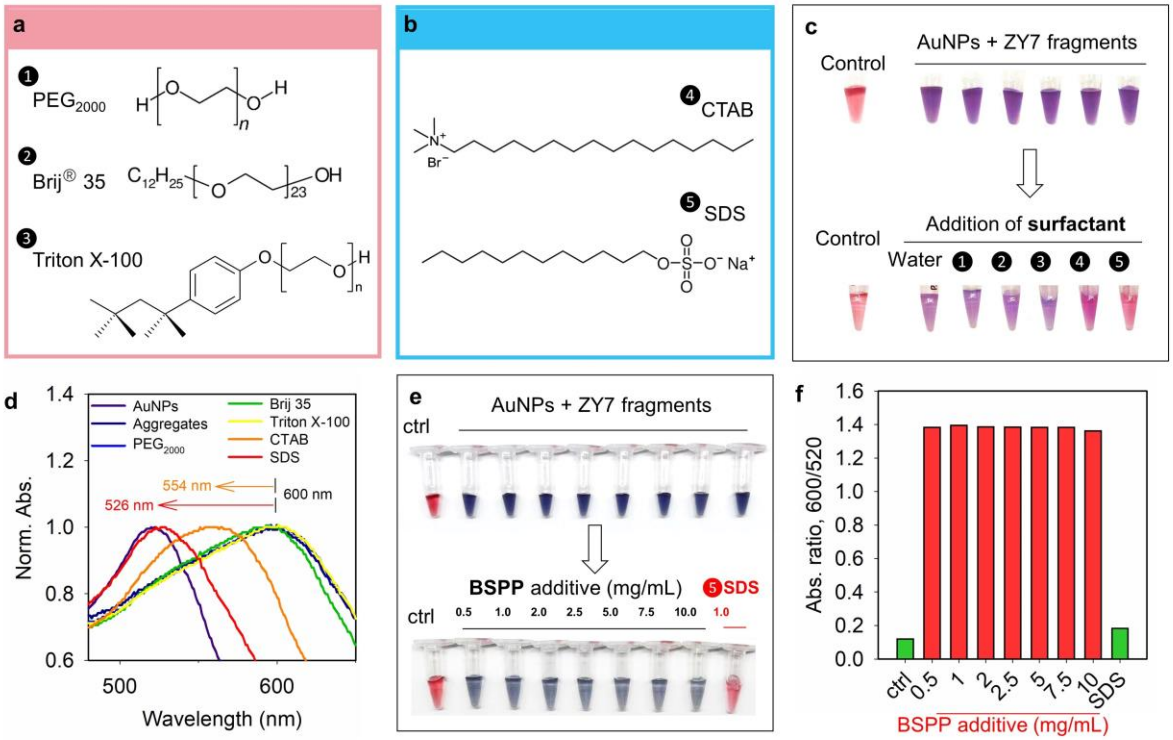

Figure S4. Reversible gold aggregates in the presence of various surfactants. **a**, Nonionic surfactants such as PEG_{2000}, Brij® 35, and Triton X-100 are used to probe the hydrophobic interactions. **b**, Ionic surfactants such as CTAB and SDS are used to break the electrostatic interactions. **c**, Top raw shows the instant aggregation of six replicates of BSPP-AuNPs (3.8 nM, 50 μL) in the presence of ZY7 fragments (c_{final} ~50 μM). To the six gold aggregates, 100 μL of each above surfactants at 1 mg/mL was added. **d**, UV-Vis spectra of pristine BSPP-AuNPs, aggregated AuNPs by ZY7 fragments, and the mixtures of gold aggregates with different surfactants. Nonionic surfactants cannot reverse the aggregation, cationic CTAB partially breaks the aggregation, while the anionic SDS fully recovers the gold nanoparticles from the aggregates. In particular, BSPP-AuNPs could be used for colorimetric assay up to 3 cycles of SDS recovery. (**e**) Top raw shows the instant aggregation of eight replicates of BSPP-AuNPs (3.8 nM, 100 μL) in the presence of ZY7 fragments (c_{final} ~50 μM). To the seven gold aggregates, the BSPP solution (100 μL) was added of increasing concentration from 0.5−10 mg/mL; SDS of 1 mg/mL was added to the last one. (**f**) Ratiometric signal (Abs_{600}/Abs_{520}) shows that the addition of excess BSPP molecule could not disassemble the aggregates formed *via* electrostatic interaction.

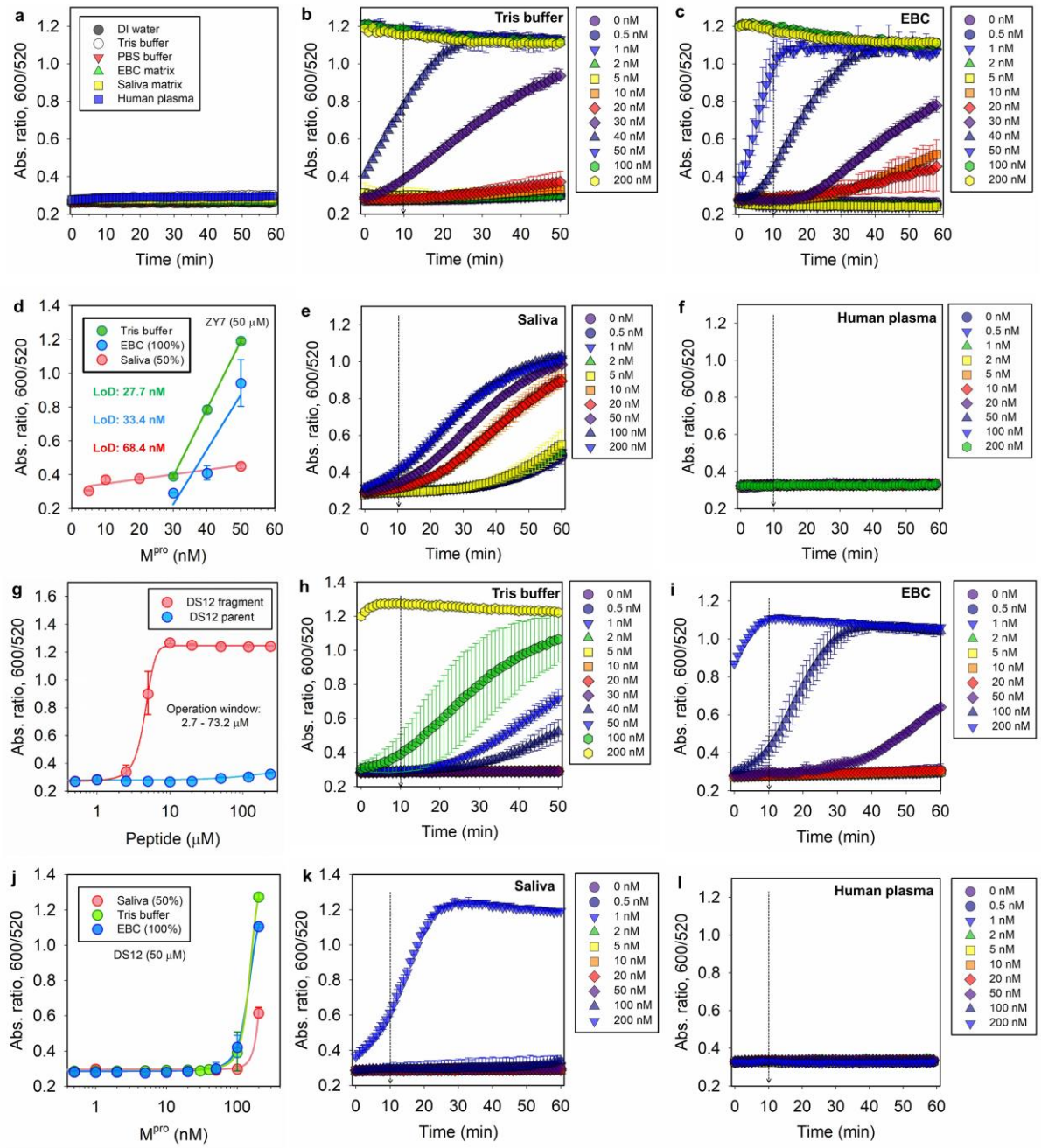

Figure S5. Time progression of the absorbance ratio (Abs600/Abs520) with various M^{pro} concentrations. **a**, Stability test of BSPP-AuNPs in different bio-relevant media for 1 h. No aggregation was observed. Note the AuNPs were also stable with M^{pro} and trypsin enzymes. **b-c, e-f**, Time-dependent absorbance ratio collected from four different enzyme assays for 1 h including in Tris buffer (b), exhaled breath condensate (c), 50% saliva (d), and 50% human plasma (e). Assays started by adding BSPP gold dispersion (c_{final} ~3.2 nM) to the pre-incubated ZY7 substrate (50 μM)/M^{pro} (0−200 nM). Data points at 10 min were indicated as the black vertical line. **d**, The linear regions used to calculate LoDs in different media. Measurements were performed in at least duplicates (error bars = standard deviation). **g**, Ratiometric signal (Abs_{600}/Abs_{520}) collected from BSPP-AuNPs (3.8 nM, 100 μL) incubated with various amount of DS12 parent (blue) and fragments (red). The sensing operation window for DS12 substrate is 2.7−73.2 μM; see **Table 1**. **h-i**, **k-l**, Time-dependent absorbance ratio collected from enzyme assays in four types biological media using DS12 substrate. **j**, Absorbance ratio as a function of M^{pro} concentration, where DS12 substrate (50 μM) is used. The determined LoD for M^{pro} is: 114.4 nM in Tris buffer, 94.2 nM in EBC matrix, and 142.4 nM in 50% saliva.

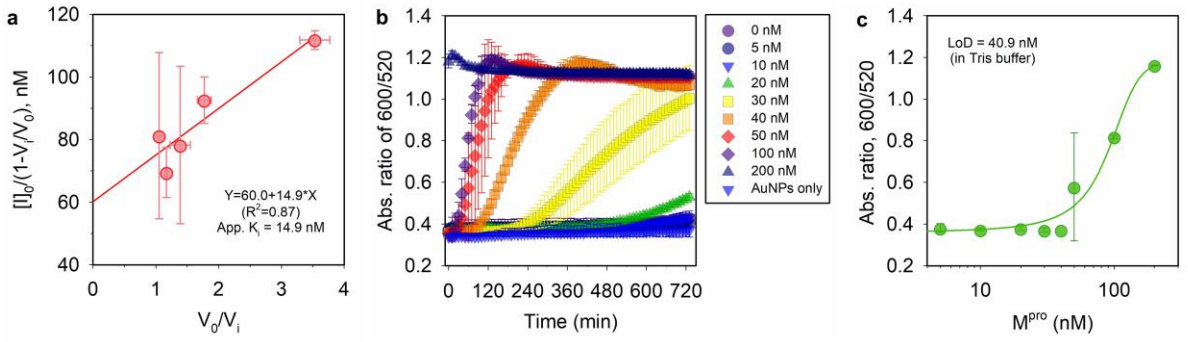

Figure S6. Inhibitor potency and one-pot enzyme assay. **a**, A Henderson equation, $[I]_0/(1-V_i/V_0)=[E]_0+K_{i\,(app)}\times(V_0/V_i)$, was applied to resolve the apparent inhibitor dissociation constant, $K_{i\,(app)}$ =14.9 nM, and active enzyme concentration, $[E]_0$ =60 nM (out of 100 nM stock). The IC_{50} ~45 nM is derived from, $IC_{50} = K_{i\,(app)} + [E]_0/2$.[11, 13] Note that the inverse of fractional velocity, V_0/V_i, is extracted from the absorbance ratio at 15 min in Figure 4a. **b**, One-pot protease assays, where enzyme cleavage and particle aggregation all occur *in situ*. The curves assaying an increasing M^{pro} concentration (c_{final} from 0−200 nM) in the presence of constant BSPP-AuNPs (c_{final} ~3.1 nM) and ZY7 substrate (c_{final} ~10 μM). Data were plotted every 10 min in 12 h. **c**, The M^{pro} LoD of the one-pot assay is 40.9 nM at a readout time ~1 h. Note that M^{pro} enzyme does not affect the dispersity of BSPP-AuNPs.

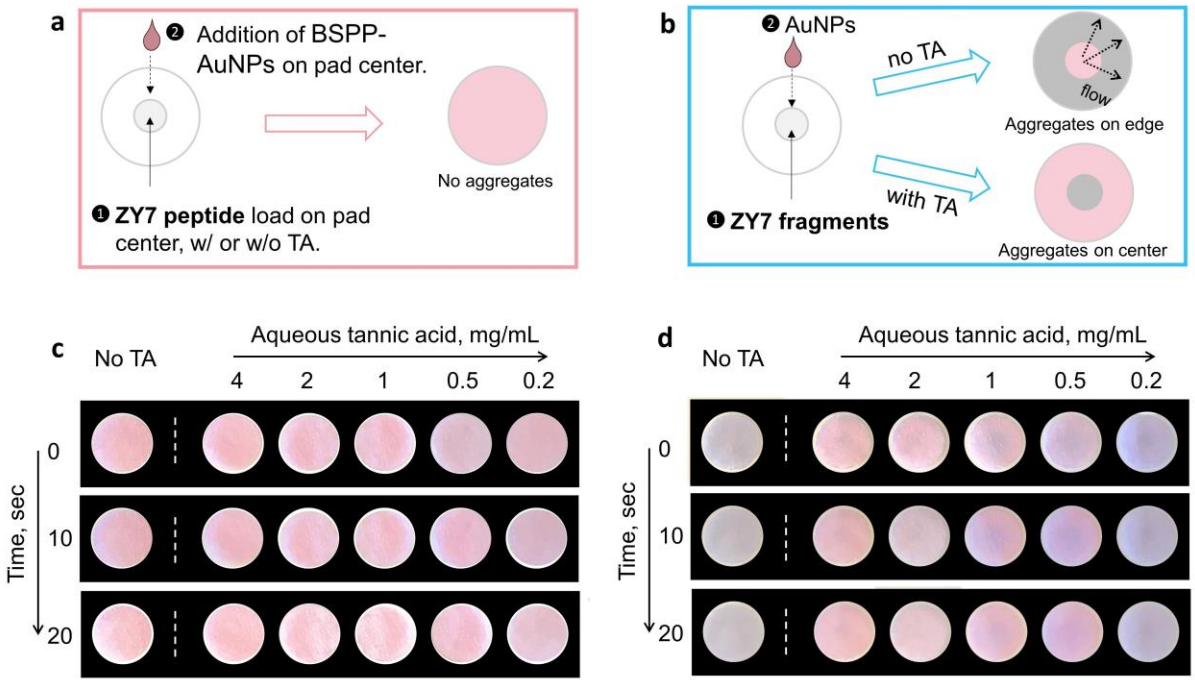

Figure S7. Adhesion of peptides on absorbent pad. A solution of ZY7 peptide/fragments (1 μL, 1 mg/mL) was dried on the center of a spherical polyester pad, followed by addition of BSPP-AuNPs (10 μL, 3.8 nM). **a**, Schematic illustration of ZY7 peptide-loaded pad colored by AuNPs with and without tannic acid (TA) treatment. **b**, Schematic illustration of ZY7 fragments-loaded pad colored by AuNPs with and without tannic acid (TA) treatment. When the pad is not treated with TA, the gold dispersion pushes fragments from the center towards edge leading to a blue-gray color on the edge and pink in the center. When the pad is treated with TA, the fragments were fixed at the center, therefore only the pad center shows gold aggregation while edge is pink-red. **c**, Images of pad treated with various concentrations of TA (0−4 mg/mL) and then loaded with ZY7 parent peptide, followed by addition of AuNPs. **d**, Images show the TA-treated pad concentrates the gray aggregates in the middle with red in edge. While the untreated pad show aggregates on the edge at 0 sec, and the whole pad becomes gray after 20 sec due to the free movements of peptide fragments. TA of 0.5 mg/mL is best use in peptide adhesion. Note TA stabilizes the fragments therefore reducing the degree of gold aggregation.