SUPPLEMENTARY MATERIAL

Ultrasensitive lateral flow immunoassay for staphylococcal enterotoxin B using nanosized fluorescent metal-organic frameworks

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Materials. PLGA (lactide: glycolide, 1: 1, mole: mole, Mn 24000), Zirconium (IV) butoxide (80 wt%), benzoic acid, 1-propanol, DMF, hydrochloric acid (HCl), ascorbic acid, potassium carbonate (K_2CO_3), sucrose, Triton X100, Tween-20, Polyvinylpyrrolidone (Mn 40000) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO). Mouse anti-SEB monoclonal antibodies (mAbs), rabbit anti-SEB polyclonal antibodies (pAbs) are in-house made by Nanosong System LLC. Rabbit anti-mouse IgG antibody was obtained from jackson immunoresearch Inc. (West Grove, PA). Nitrocellulose membrane (UniSart® CN140 Membrane) was purchased from Sartorius AG (Göttingen, Germany). Glass fiber, backing cards, and an absorbent pad were purchased from Millipore (Billerica, MA). Ridascreen SET kit was purchased from R-Biopharm AG (Darmstadt, Germany).

Characterizations of NU-901 nanoMOF. TEM samples were prepared by pipetting one drop of water diluted sample suspensions onto carbon-coated electron microscopy grid. After 8 min, the liquid was removed by slowly sucked using filter paper. The grids were dried under room temperature for overnight. TEM measurements were conducted on a 200 kV FEI Tecnai TEM microscope. Powder X-ray diffractions (PXRD) were taken with a Rigaku Ultima IV diffractometer (scanned in $2° < 2\theta < 20°$ range, with a 0.02° step at a 1.2°/min scan rate). Excitation-emission mapping (EEM) and quantum yield were collected by an Edinburgh FS5 spectrofluorometer equipped with 150 mm integrating sphere. The emission lifetimes were described by the time-resolved decay profiles, which were collected with an Edinburgh LifespecII Time-Correlated Single-Photon Counting (TCSPC) spectrophotometer with a picosecond resolution (excitation light source: 403.8 nm, pulsed laser with a pulse width = 60 ps, providing 160 ps IRF).

Fabrication of MOF-LFA. The MOF-LFA comprised a sample pad, a conjugate pad, a nitrocellulose membrane, an absorbent pad and backing. The sample pad (17 mm × 30 cm) was made from glass fiber and saturated with 0.1 M Tris-HCl buffer (pH 8.0, 0.25 wt% Tween-20 and 0.1 M NaCl. Then, the pad was dried at 37°C for 2 h and stored in the desiccator. Test line (stripping rate: 1 μL/cm) and control line (stripping rate: 1 μL/cm) were prepared by dispensing anti-SEB polyclonal antibodies (pAbs) (capture antibody, 1 mg/mL) and rabbit anti-mouse immunoglobulin G (IgG) pAbs (1 mg/mL) at different locations on the nitrocellulose membrane (25 mm × 30 cm) using BioDot BioJet BJQ 3000 dispenser (Irvine, CA, USA). The distance between each line was approximately 5 mm. The nitrocellulose membranes were then dried at 37°C and stored in dry room.

The conjugation pad (8 mm × 30 cm) was made from glass fiber and pretreated with blocking buffer containing 0.1 M of Tris-HCl, 0.5 wt% Tween-20, and 1 wt% PVP40k, pH 8.0. The eluent solution was dispensed on to the conjugated pad using BioDot AirJet BJQ 3000 dispenser (Irvine, CA, USA).

These different pads were assembled on backing (60 mm × 30 cm) with an overlap between them of approximately 1 mm to ensure that the solution could migrate through the LFA. LFA were cut at a width of 3.5 mm using BioDot paper cutter module CM4000 (Irvine, CA, USA).

Accelerated Stability Testing. All aging experiments were performed in the oven. When removed LFA from the dryer room, LFA were stored in a sealed plastic bag filled with silica bead desiccant at either room temperature or 50/60 °C for accelerated aging study. Aging at 50 °C for example, 7.5 weeks is functionally equivalent to room temperature aging for 12 months. In addition, aging at 60 °C for example, 3.8 weeks is functionally equivalent to room

temperature aging for 12 months. Aging conditions were selected following guidelines of ASTM F1980 Standard Guide for Accelerated Aging of Sterile Medical Device Packages and West pack Accelerated Aging Time Calculator. Accelerated aging techniques are based on the assumption that the chemical reaction involved in the deterioration of materials follows the Arrhenius reaction rate function, which states that a 10 °C increase in temperature will cause the reaction rate to double.

Preparation of PLGA NPs.

PLGA (lactide:glycolide, 1:1, mole:mole, Mn 24000) was dissolved at 2 mg/mL in acetonitrile containing different amounts of dye (from 0 to 5 wt% relative to the PLGA). These solutions were added quickly and under stirring (shaking) using a micropipette to a tenfold volume excess of 20 mM phosphate buffer at pH 7.2. The nanoparticle solution was then quickly washed and diluted with the same buffer.

Developing gold colloid (Au)-LFA.

(1) Synthesis of gold colloid

Following Turkevich method (Turkevich et al., 1951), Au colloid of approximately 20 nm diameter were synthesized by citrate reduction of $HAuCl_4$. 50 mL of a 0.01 % (w/v) solution of $HAuCl_4$ were prepared in Milli-Q water. This solution was taken to boiling point and 1.25 mL of 1% (w/v) sodium citrate solution was added under continuous vigorous stirring. The solution was kept boiling for 10 more minutes and then allowed to cool down to room temperature.

(2) Gold colloid conjugation

2 mL of anti-SEB mAbs (200 μg/mL in 1x PBS, pH 7.2) were added to 50 mL of Au colloid solution (pH change to 8.2) and incubated for 5 min at room temperature. BSA solution was then added to the mixture (final 2 wt%), which was incubated for another 5 min and then centrifuged

at 4 °C (13000 g, 40 min). The supernatant was discarded and the pellet was reconstituted in 2 mL of conjugation dilution buffer (pH8.2, 0.1 M Tris-HCl buffer containing 5 wt% sucrose, 0.05 wt% Tween20 and 3 wt% BSA).

(3) Fabrication of Au-LFA

The fabrication steps of Au-LFA are consistent with the synthesis of MOF-LFA.

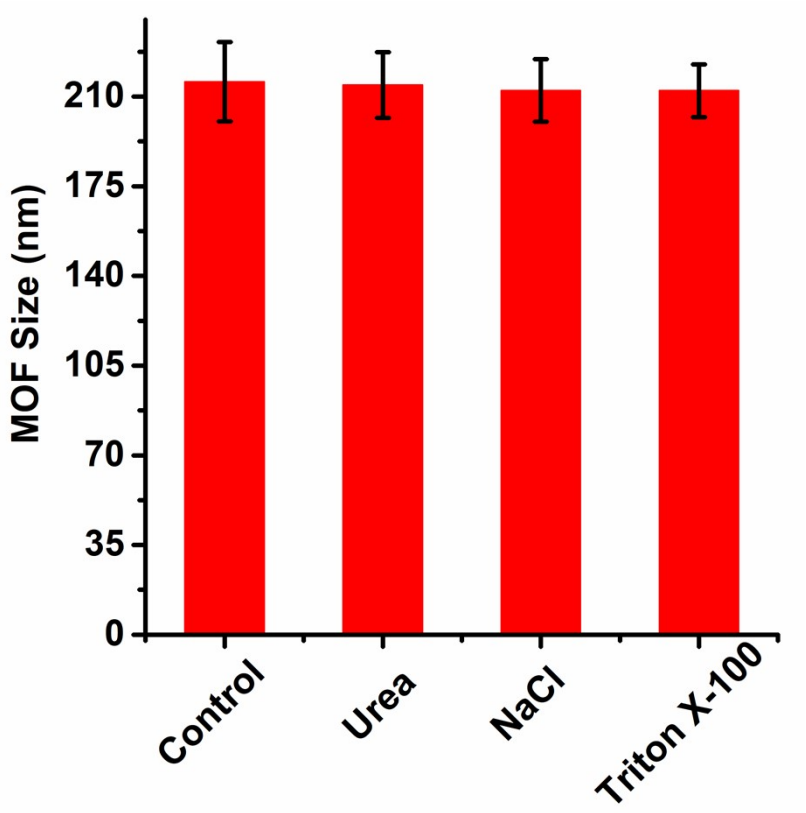

Figure S1. The size changes of nanoMOF after treatment with 100 mM of urea, NaCl and Triton X-100, respectively.

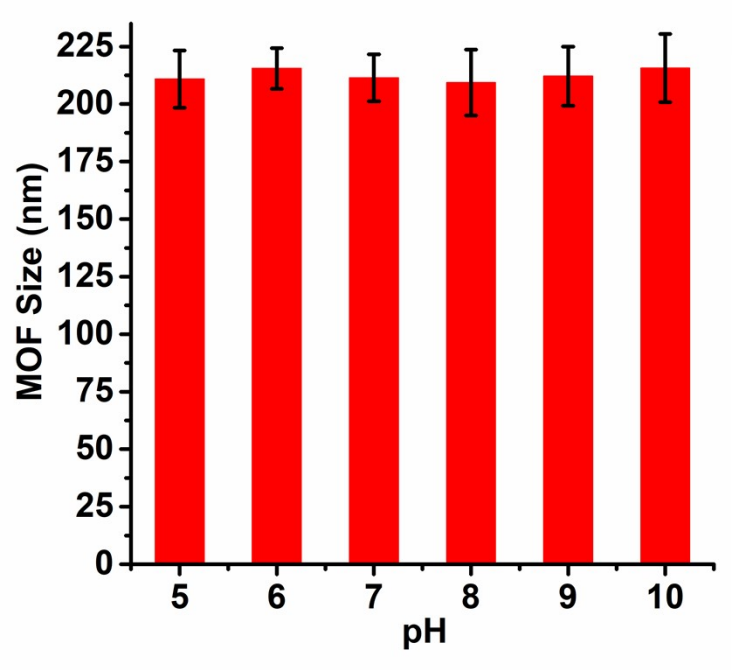

Figure S2. The size changes of nanoMOF in different pH.

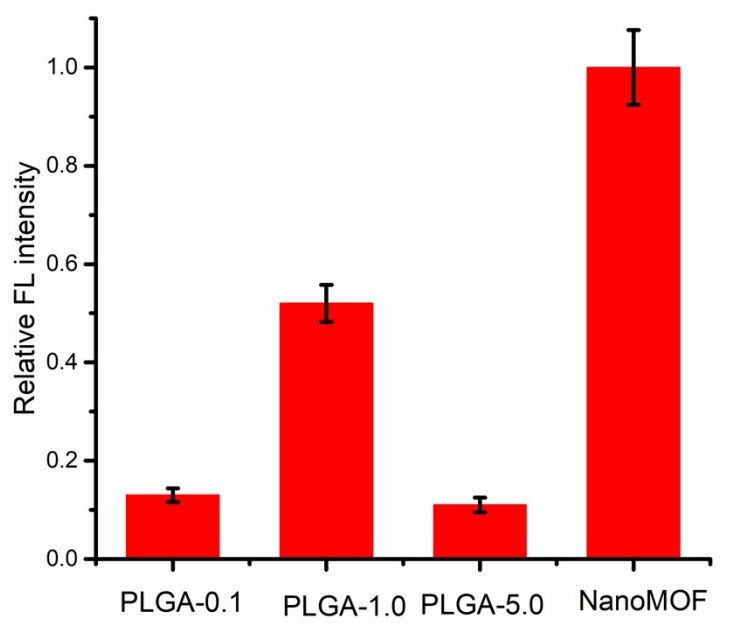

Figure S3. The relative fluorescence intensity of PLGA-0.1, PLGA-1.0, PLGA-5.0 and nanoMOF in aqueous solution.

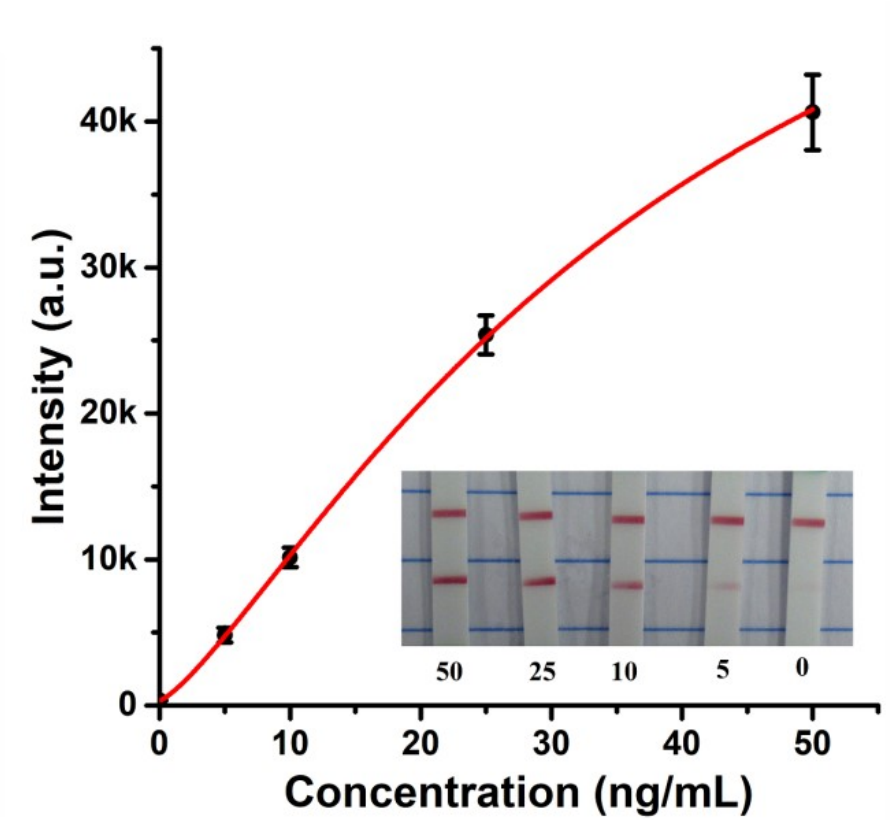

Figure S4. Calibration curve obtained by Au-LFA readers for a series of SEB spiked sample. Concentration of SEB: 0, 5, 10, 25 and 50 ng/mL. Error bars represent standard deviation from six independent experiments. Inset of gold colloid based LFA detection pads. Concentration of SEB: 50 ng/mL, 25 ng/mL, 10 ng/mL, 5 ng/mL and 0.