Electronic Supplementary Material (ESI) for Analyst.

Functional Nanoflower Based Lateral Flow Immunoassay for the Rapid and Robust Detection of Pathogen

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Calculation of protein immobilization efficiency in hybrid nanoflowers

According to BCA protein quantitative kit, the standard curve can be obtained: $y=0.7537x+0.00244$ ($R^2=0.999$). The absorbance of the protein sample in the supernatant at 562 nm was determined to be 0.007. According to the above standard curve, and the concentration of free protein in the supernatant was calculated to be 0.00605 mg/mL. The immobilization efficiency of protein in nanoflowers can be calculated by the following formula:

Protein immobilization efficiency = (total amount of protein introduced into the system - total amount of unimmobilized free protein) / total amount of protein × 100%

In this system, the total introduced protein concentration was 0.0467 μg/mL.

According to the formula, the antibody immobilization efficiency = (0.0467 mg/ml-0.00605 mg/ml) / (0.0467mg/ml) × 100%=87.04%

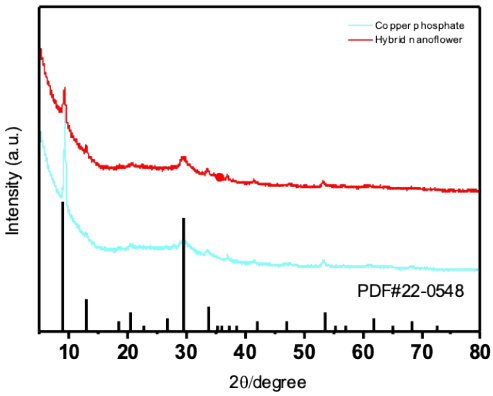

Fig. S1 XRD patterns of hybrid nanoflowers obtained with Mag I and HCG (red line), particles of crystals obtained without protein (blue line), and standard $Cu_3(PO_4)_2 \cdot 3H_2O$ (JPSCD PDF#22-0548) (black line).

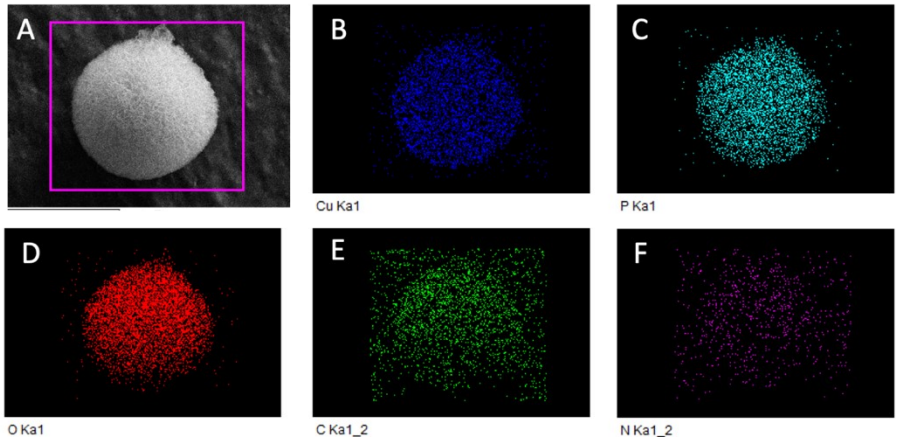

Fig. S2 Element mapping of the Mag I-HCG-$Cu_3(PO_4)_2$ hybrid nanoflowers via EDS: (A) the sample; images (B-F) exhibit the element sensitive maps of copper, phosphorus, oxygen, carbon, and nitrogen.

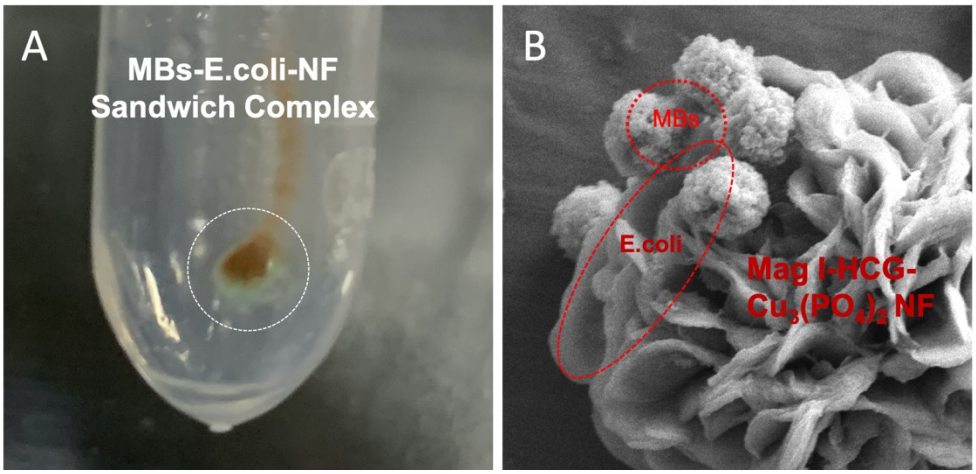

Fig. S3 (A) The visible photo of MBs-E. coli-NF Sandwich Complex; (B) The SEM image of sandwich complex.

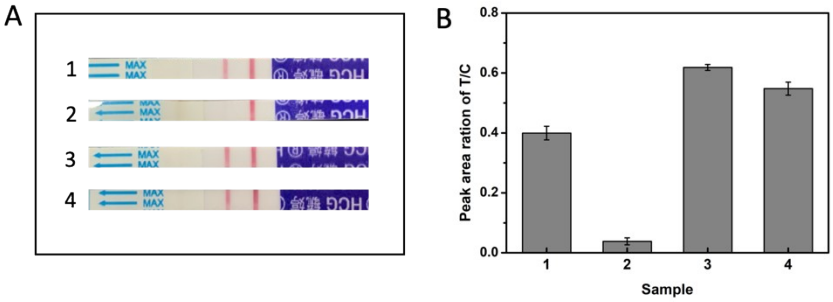

Fig. S4 The photograph of the lateral flow strip assay readout of four different samples (Sample 1 free HCG solution，Sample 2 Mag I- $Cu_3(PO_4)_2$ NF, Sample 3 HCG- $Cu_3(PO_4)_2$ NF, Sample 4 Mag I-HCG- $Cu_3(PO_4)_2$ NF) (B) The Peak area ratio of T/C from this four different samples.

Table S1 Comparison of the reported sensor for *E. coli* and MUC1 detection.

Method	Range of detection	LOD	Reference
Electrochemical Impedance Spectroscopy	10^3-10^7 CFU/mL	10^3 CFU/mL	1
ELISA	10^3-10^7 CFU/mL	10^3 CFU/mL	2
Gold nanoparticle-based Immunoassay	10^2-10^5 CFU/mL	148 CFU/mL	3
Chemiluminescence Immunoassay	4.3×10^3–4.3×10^5 CFU/mL	1.2×10^3 CFU/mL	4
Nanopaper-based Aptasensor	10^2-10^6 CFU/mL	100 CFU/mL	5
This work	10^3-10^7 CFU/mL	10^3 CFU/mL	This work

1. Y. Li, R. Afrasiabi, F. Fathi, N. Wang, C. Xiang, R. Love, Z. She and H.-B. Kraatz, *Biosens.*

Bioelectron., 2014, **58**, 193-199.

2. M. R. Akanda, V. Tamilavan, S. Park, K. Jo, M. H. Hyun and H. Yang, *Anal. Chem.*, 2013, **85**, 1631-1636.
3. A.-R. H. A.-A. Hassan, A. de la Escosura-Muñiz and A. Merkoçi, *Biosens. Bioelectron.*, 2015, **67**, 511-515.
4. Y. Zhang, C. Tan, R. Fei, X. Liu, Y. Zhou, J. Chen, H. Chen, R. Zhou and Y. Hu, *Anal. Chem.*, 2014, **86**, 1115-1122.
5. Y. Liu, G. Mao, W. Wang, S. Tian, X. Ji, M. Liu and Z. He, *Chem. Commun.*, 2019, **55**, 2660-2663.