Electronic Supplementary Material (ESI) for Analytical Methods.
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1 **Supporting information for *Analytical Methods***

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3 Development of a Monoclonal Antibody-Based Lateral Flow 4 Immunoassay for the Detection of Benzoic Acid in Liquid Food

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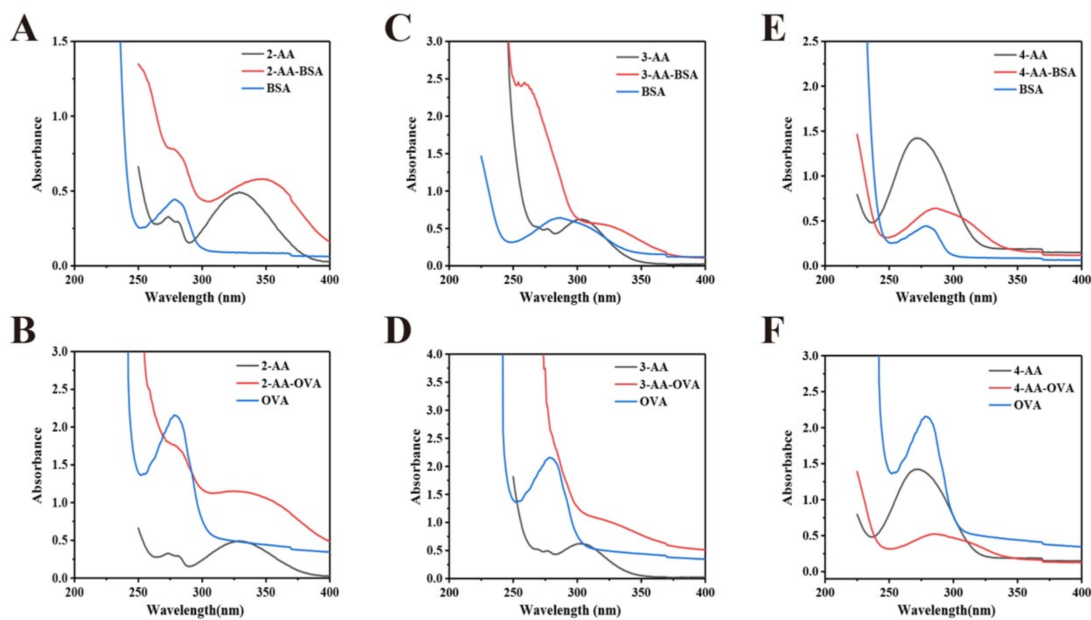
29 # 1. Characterization of haptens, carrier protein and conjugates



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31 **Figure S1.** UV-Vis spectroscopy of haptens, carrier protein and conjugates; A, C, E: confimation

32 of immunogens (2-AA-BSA, 3-AA-BSA and 4-AA-BSA); B, D, F confirmation of coating antigens

33 (2-AA-OVA, 3-AA-OVA and 4-AA-OVA).

34

2. ELISA procedure

The procedure of ELISA was as follows: the coating antigen was diluted with carbonate buffer (pH 9.6), and the dropped into a 96-well plate at 100 μL/well. The plate was coated at 4 °C for 12 h, and discarded the coating solution. Subsequently, the plate was washed with PBST (10 mM PBS containing 0.5% tween-20) for three times, and 150 μL/well of 3% skim milk was added for another 1 h at 37 °C. Then discarded the blocking buffer, 50 μL/well 10 mM PBS and 50 μL/well antibody working solution were added in sequence into the well, and incubated at 37 °C for 30 min. After the washing procedure, 100 μL/well of peroxidase-conjugated goat anti-mouse IgG (diluted 5000-folds) was added and incubated at 37 °C for 30 min. The plates were washed as above again, 100 μL/well of substrate solution was added into the wells. Finally, the reaction was terminated by 50 μL/well of 2M H_2SO_4, and measured at 450 nm by using a microplate spectrophotometer.

For the icELISA procedure, the 50 μL 10 mM PBS was replaced by 50 μL series concentration of BA standard solution.

3. Cloning of scFv-6D8 from hybridoma cells

The mAb 6D8 was IgG2a with kappa-type light chains characterized by a monoclonal antibody typing kit. The variable region of mAb 6D8 was sequenced according to our previous procedure.[1] As shown in Fig. S2 and S3, the total length of the scFv-6D8 gene was about 850 bp with the $(Gly_4Ser)_4$ linker. The amino acid sequence of scFv-6D8 is shown in Tables S1.

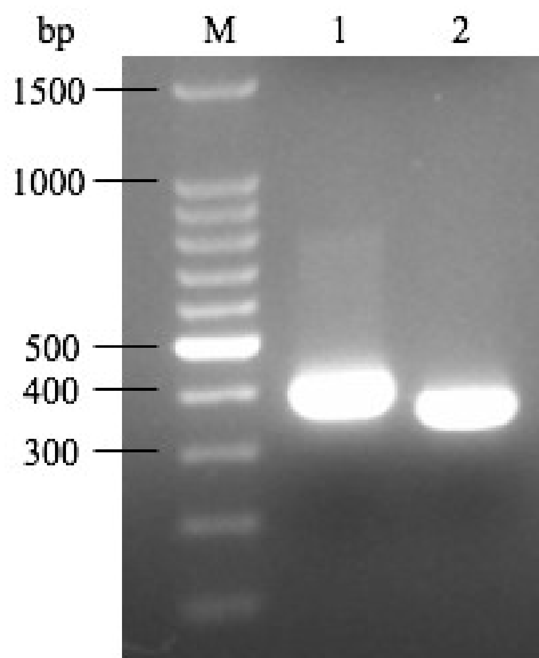
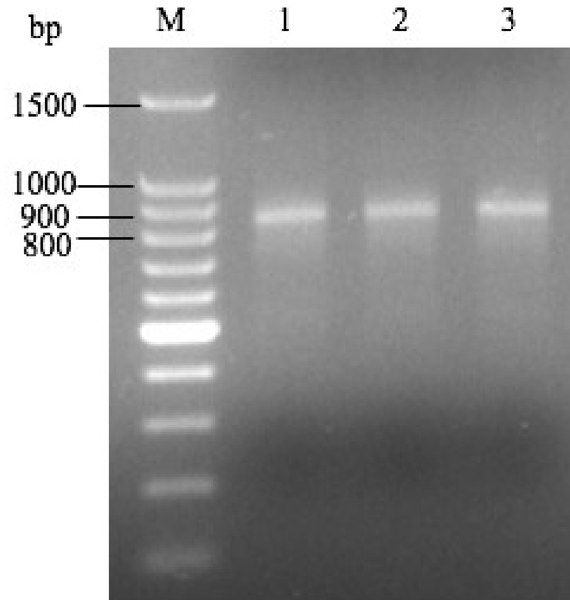


Figure S2. VH and VL genes of mAb 6D8. Lanes 1: VH gene; Lanes 2: VL gene; Lane M: 100bp ladder DNA marker.



59

60 **Figure S3.** The scFv gene of mAb 6D8. Lanes 1–3: scFv gene of 1; Lane M: 100bp

61 ladder DNA marker.

62

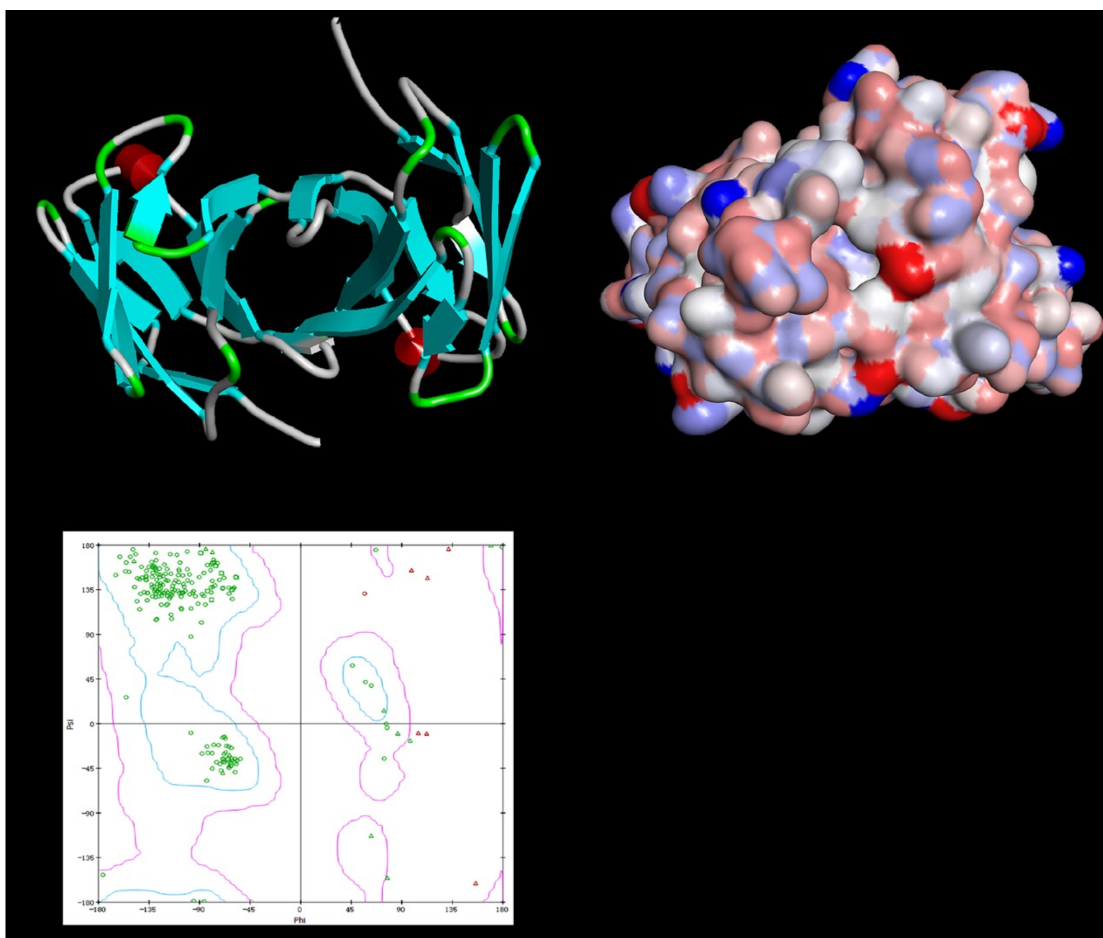
63 **Table S1.** The amino acid sequence of scFv-6D8.

CDR-H1
QVQLKESGPGLVQPSQSLSITCTVS**AFSLTNYA**IHWVRQTPGKGLE
CDR-H2
WLGV**IWSGGST**DYNTAFMSRLSISKDNSKSQVFFKMNSLQADDTA
CDR-H3 Linker
IYYC**ARTLRPY YVMDY**WGQGTSVTVSS**GGGGSGGGGSGGGGSG**
CDR-L1
GGGSDVLMTQTPLSLPVSLGDQASISCRSS**QSLLHSNGNTN**LHWYL
CDR-L2
QKPGQSPKLLIY**KVS**SRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGV
CDR-L3
YFC**SQSTHVPRT**FGGGTKLEIK

64

65 **4. Homology modeling of scFv-6D8**

66 The three-dimensional structure of scFv-6D8 was predicted using MODELER
67 according to the standard antibody modeling cascade (Discovery Studio 19.1).[2] The
68 framework templates were identified at the first step by using 1MRD L for the variable
69 light chain (VL) with 100.0% similarity, 1T2Q H (97.7% similarity) for the variable
70 heavy chain (VH), and 4M7J LH for the VH/VL interface with 98.2% similarity to the
71 mAb 6D8 sequence. Next, CDR conformations were built and refined based on the
72 following homology templates: 1MRD, 1T66, 5MYK, 1A2Y, 4M93, 6C5K for CDR
73 L1 (98.8% similarity), L2 (100.0% similarity), L3 (86.8% similarity), H1 (84.7%
74 similarity), H2 (83.9%similarity) and H3 (76.0% similarity), respectively. Finally, a
75 top-ranked model for the whole scFv-6D8 (Fig. S4A and S4B) was selected with PDF
76 total energy of 2632.1, PDF physical energy of 201.2, and DOPE score of −25698.3.
77 According to the Ramachandran plot figure (Figure S4C), 92.2% of residues were in
78 the favored region, 4.8% of residues in the allowed region and 3% of residues in the
79 outlier region. In addition, 92.2% of the residues had an averaged 3D-1D score of no
80 less than 0.2 using the Verify 3D program (Figure S4D). These results indicated that
81 the quality of the modeled 3D structure could be used as a model for molecular
82 recognition in subsequent experiments.

83 

84 **Figure S4.** Structure prediction of scFv-6D8 (A and B). Validation by Ramachandran

85 plot (C) and 3D verify score (D) of scFv-6D8.

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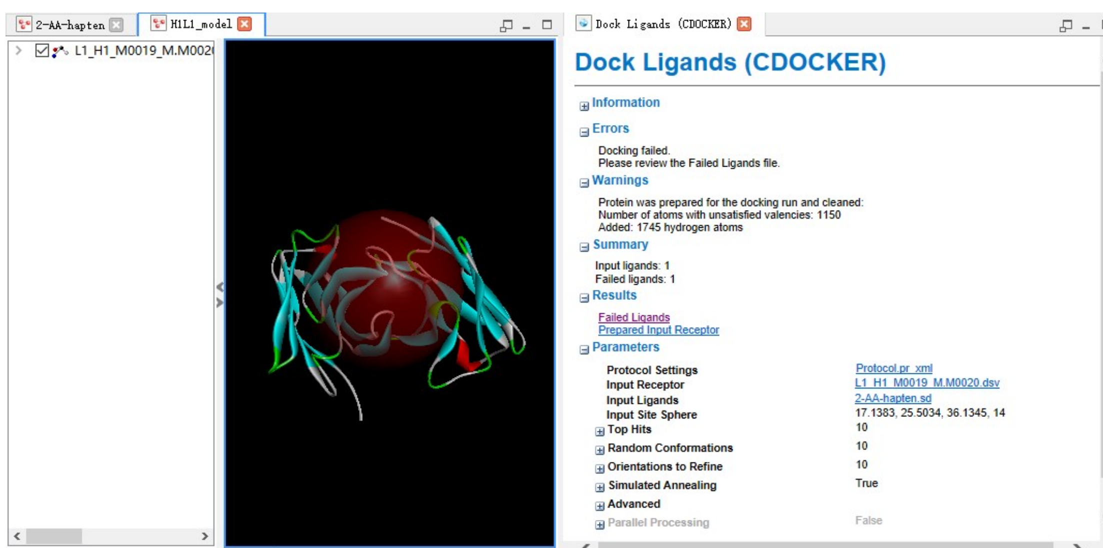
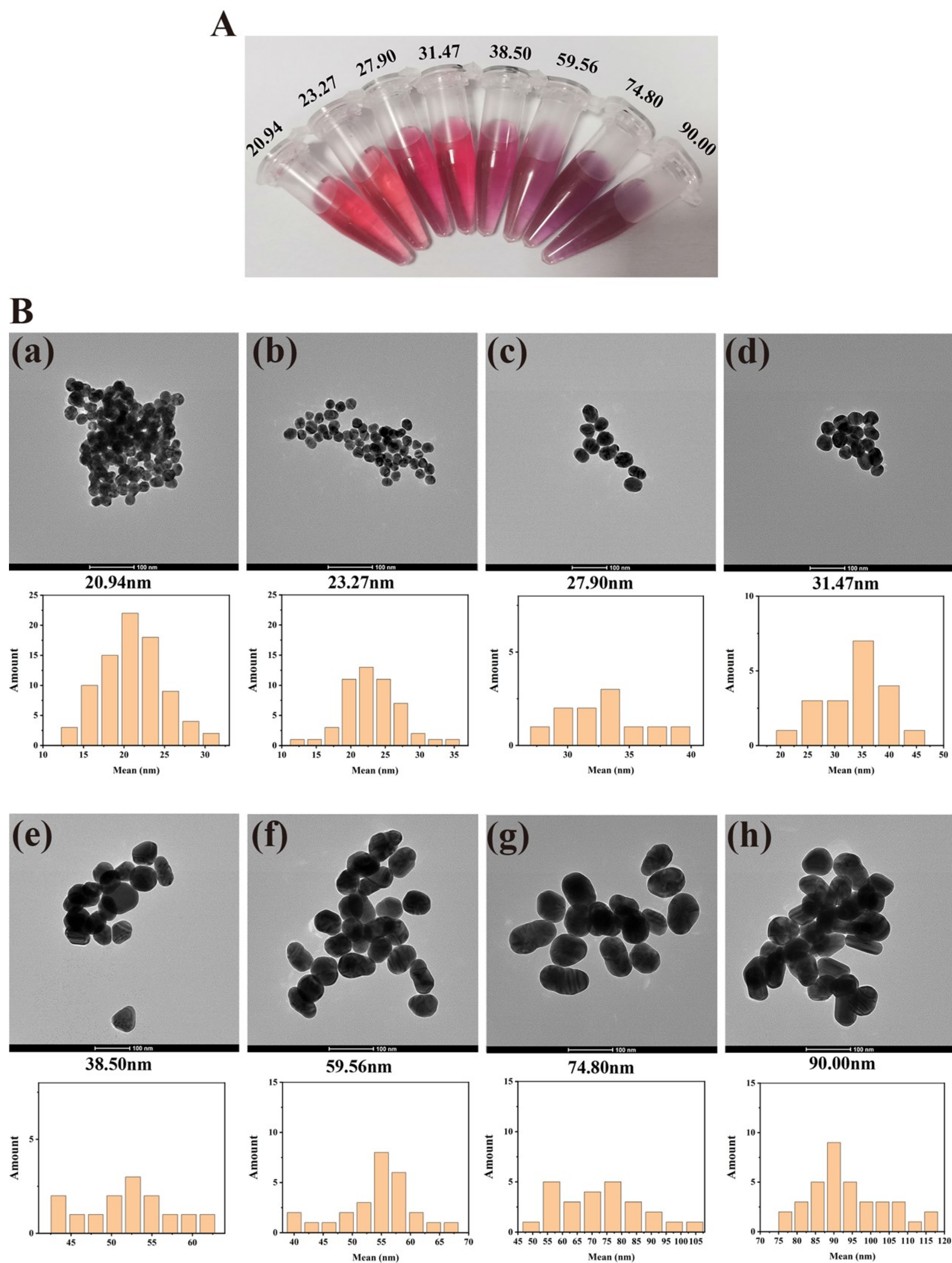
87 

88 **Figure S5**. The results of the ligand of 2-AA docking with scFv-6D8.

89 ## 5. Characterization of eight sizes of the CGNPs



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91 **Figure S6.** (A) Color of the CGNPs solution. (B) Different sizes of the CGNPs

92 charactered by TEM, and the corresponding particle size distribution.

6. pH adjustment

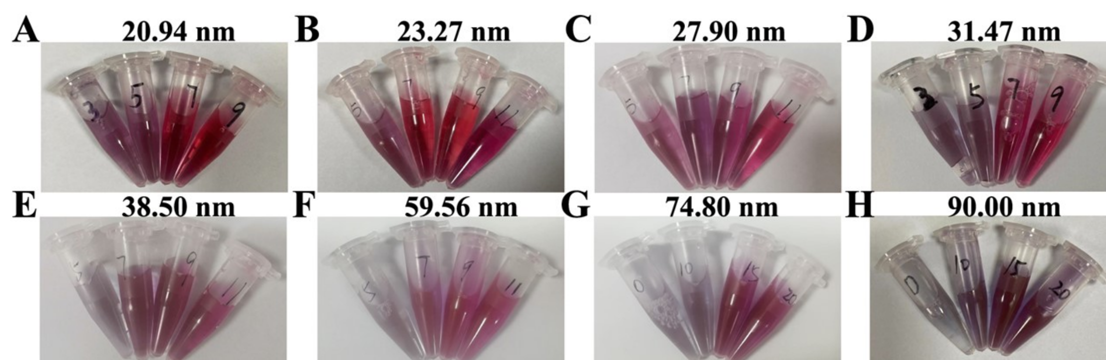
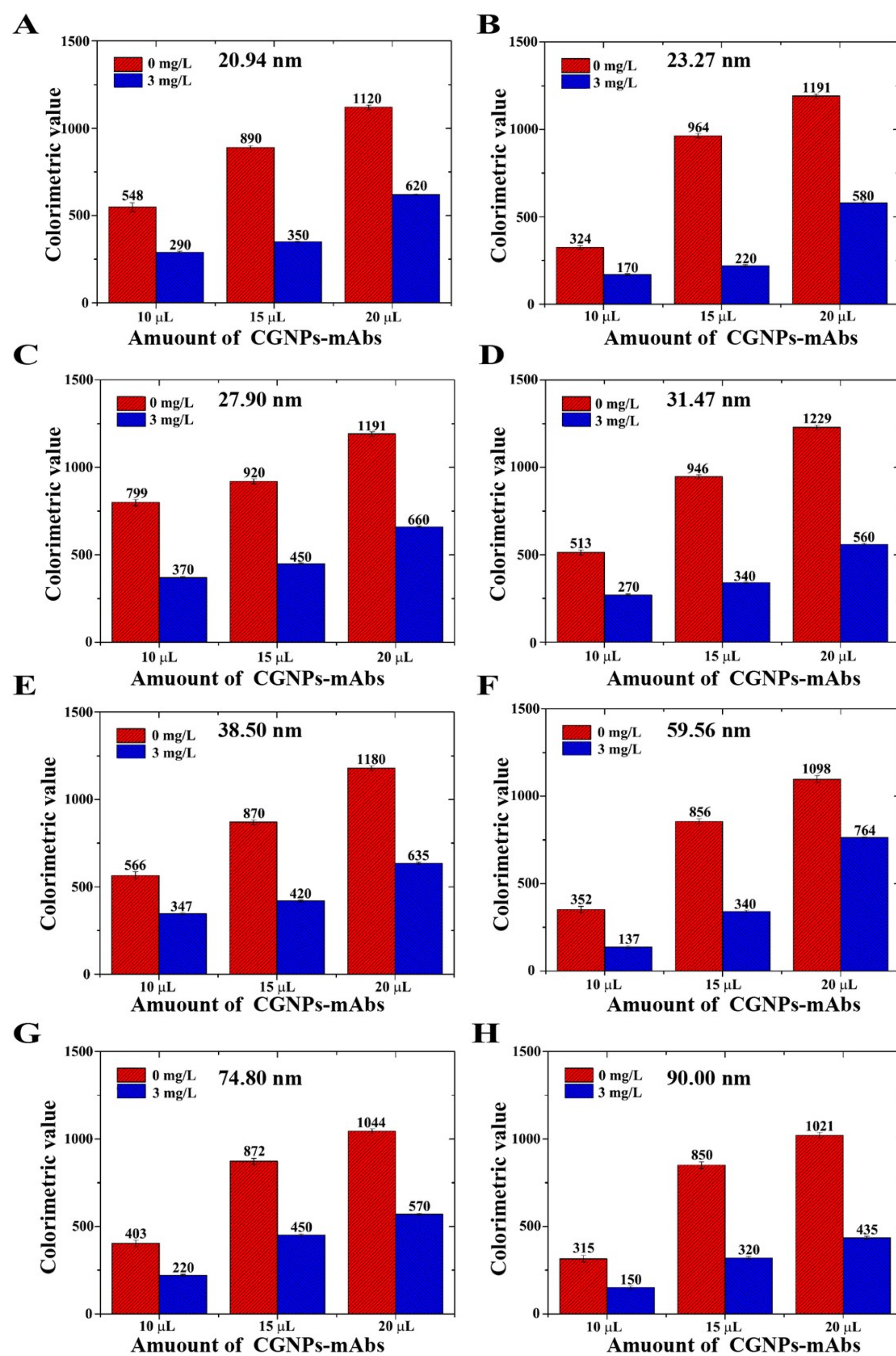


Figure S7. Optimized the pH adjust by 0.1 M K_2CO_3. 3 μL, 5 μL, 7 μL, 9 μL for 20.94 nm CGNPs (A) and 31.47 nm CGNPs (D). 5 μL, 7 μL, 9 μL, and 11 μL for 23.27 nm (B), 27.9 nm (C), 38.5 nm, and 59.56 nm CGNPs (F). 0 μL, 10 μL, 15 μL and 20 μL for 74.8 nm (G) and 90 nm CGNPs (H).

99 # 7. Usage of CGNPs-mAbs optimization



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101 **Figure S8.** Optimized the usage of CGNPs-mAbs.

102 # 8. Conditions and procedures for UV and HPLC analysis.

103 **8.1 Sample preparation for UV spectroscopy detection**

104 50 mL of the liquid samples were first ultrasounded at 50 °C for 20 min. Then, 10
105 mL of the samples was added into a 50 mL separatory funnel. Subsequently, 2 mL 3 M
106 HCl was added, and the solution was extracted with 20 mL ether for four times. The
107 ether were combined, and was further extracted by 20 mL 5% Na_2HPO_4 for four times.
108 Combined the aqueous phase into a 100 mL beaker, and placed in a 60 °C water solution
109 to remove the ether. Finally, the aqueous phase into a 100 mL volumetric bottle, and
110 set volume to 100 mL for the detection of UV spectroscopy detection.

111 **8.2 Sample preparation for HPLC-UV detection**

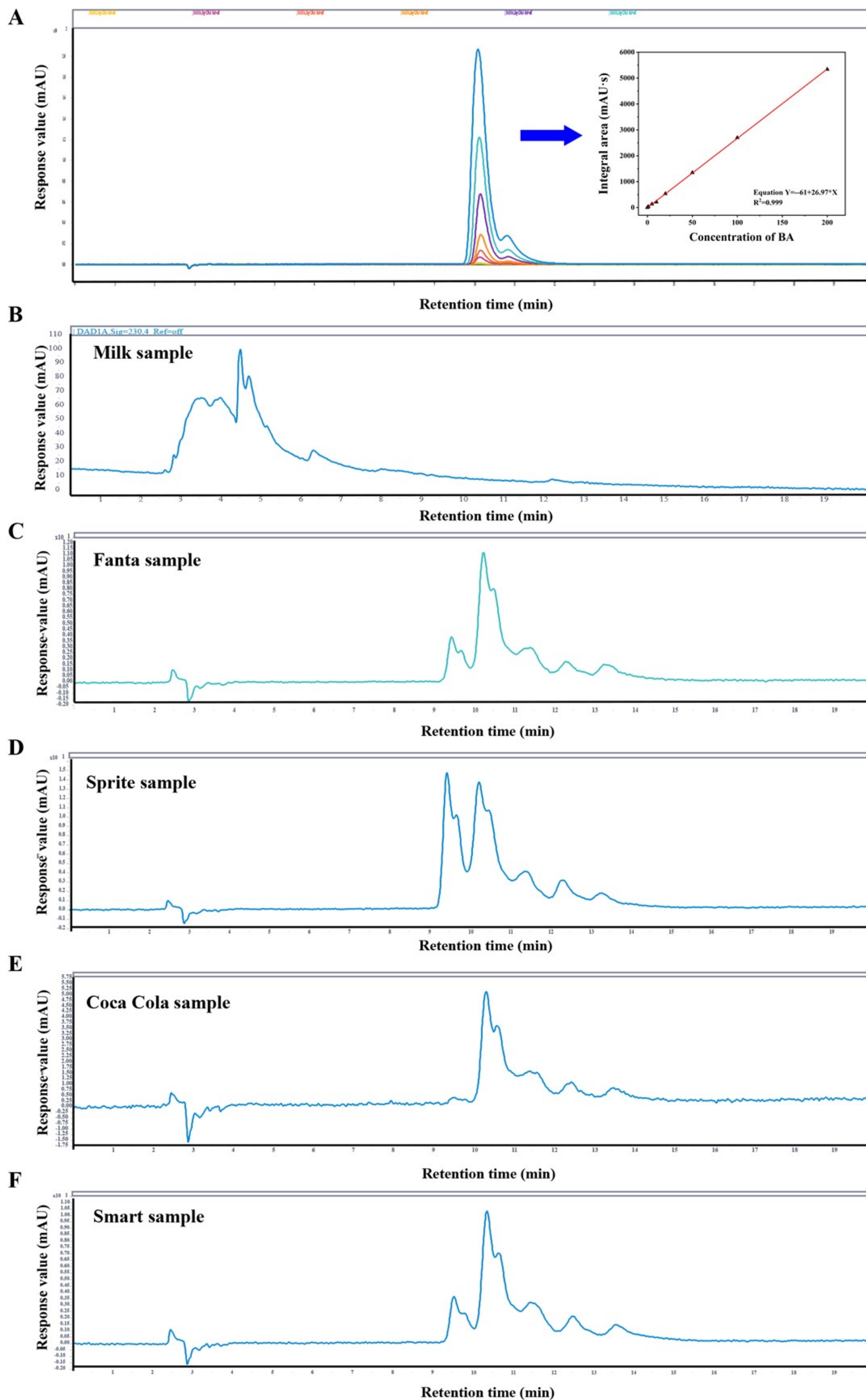
112 10 mL of milk was added into a 50 mL separatory funnel. Subsequently, 2 mL 3
113 M HCl was added, and the solution was extracted with 20 mL ether for two times. The
114 ether was combined and dried under N_2. Then the residue was dissolved with 2 mL of
115 methanol. Finally, the solution was taken through membrane (0.22 μm) to be
116 determined by HPLC-UV.

117 10 mL of the Fanta, Sprite, Coca Cola, or Smart samples were first ultrasounded
118 at 50 °C for 20 min. Subsequently, 2 mL of the liquid samples were diluted 10-fold by
119 deionized water, and were taken through membrane (0.22 μm) to be determined by
120 HPLC-UV.

121 **8.3 Conditions of the HPLC**

122 Column used was a C18 (4.6 mm × 150 mm × 5 um), mobile phase is ammonium
123 acetate containing 5% methyl alcohol, flow rate was 1 mL/min, injection volume is 10

124 μL, and the measure wavelength is 230 nm. The limited of detection (LOD) and limit
125 of quantification (LOQ) of BA were 0.10 mg/kg and 0.20 mg/kg, respectively.
126 Moreover, the result indicated that the concentration of BA in milk sample was below
127 0.10 mg/kg, which was no effect on our developed LFA.



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129 **Figure S9.** The HPLC chromatogram of standard solution of BA (concentration with
130 0, 0.5, 1, 5, 25, 50, 100 and 200 mg/L, respectively) (A); The HPLC analysis results for
131 milk, Fanta, Sprite, Coca cola and Smart sample, respectively. (B-F)

132
9. References

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