Supporting information

Preparation of dyed polymer microspheres by a physical-chemical dual-binding method and their application in lateral flow immunoassay

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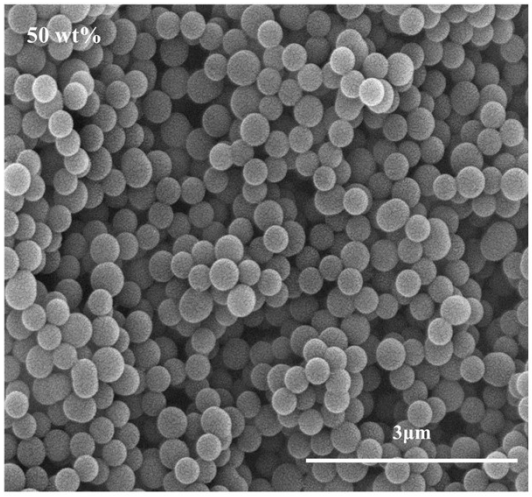

Figure. S1. SEM images of PSVMNa with 50 wt % VBC.

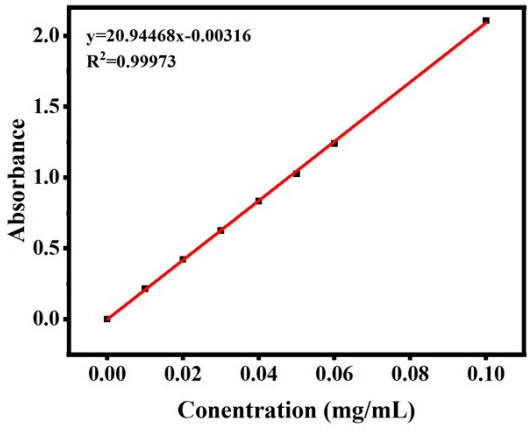

Figure. S2. Standard curve of dye concentration versus UV absorption.

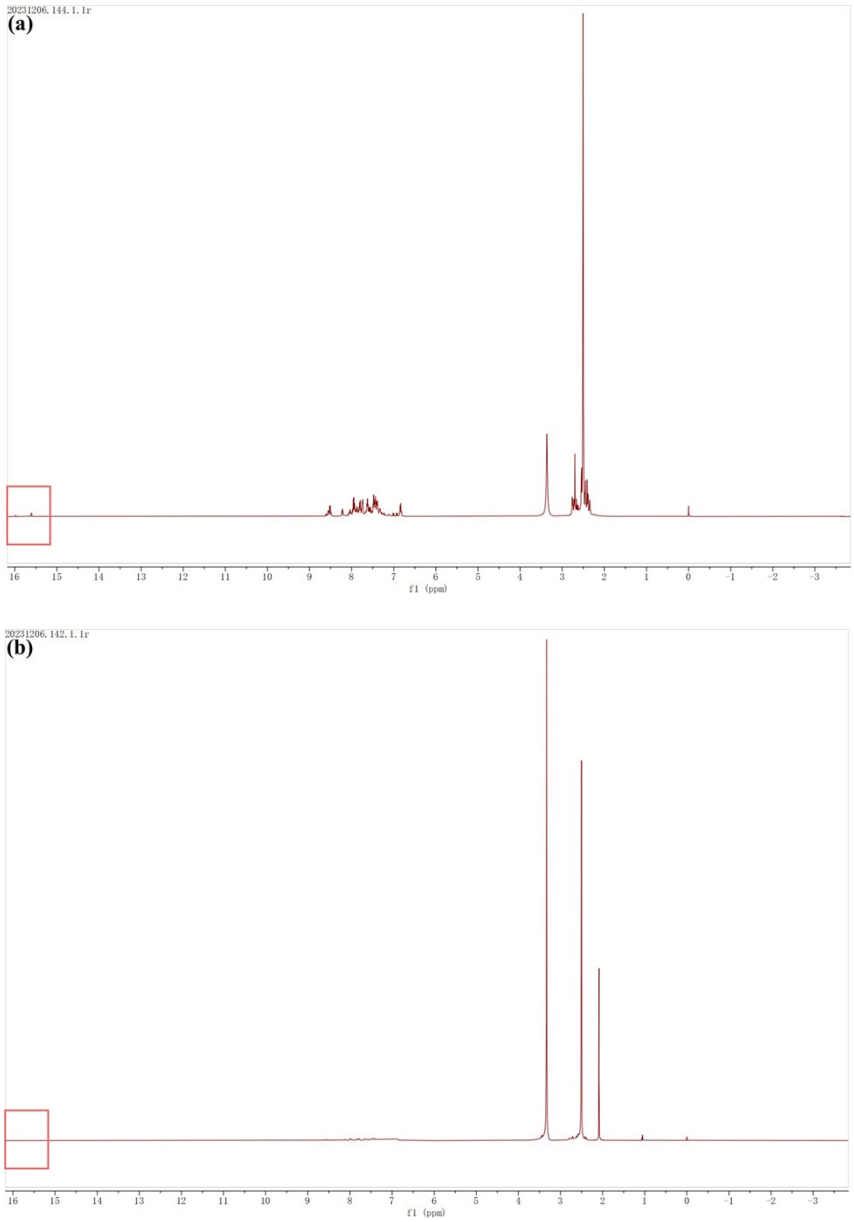

Figure. S3. ^{1}H NMR of 1-[[4-[(dimethyl phenyl)azo]dimethyl phenyl]azo]-2-naphthol (a) and dyed microsphere(b).

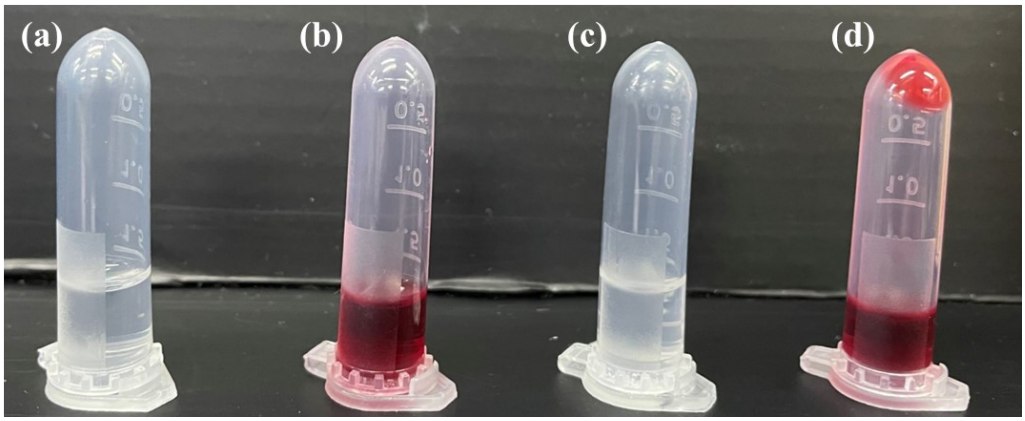

Figure. S4. Image of dissolved in tetrahydrofuran after centrifugation: (a) PSMNa; (b) dyed PSMNa; (c) PSVMNa; (d) dyed PSVMNa.

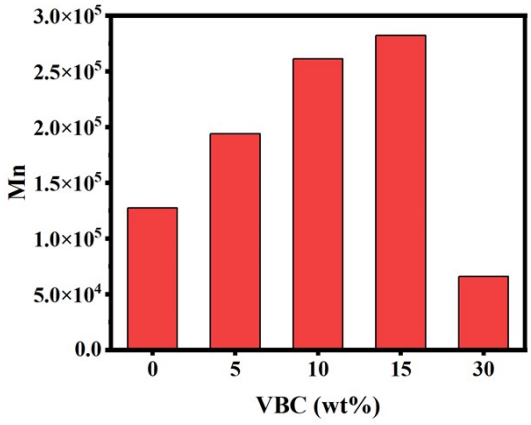

Figure. S5. Molecular weight contrast of microspheres at different addition amount of VBC.

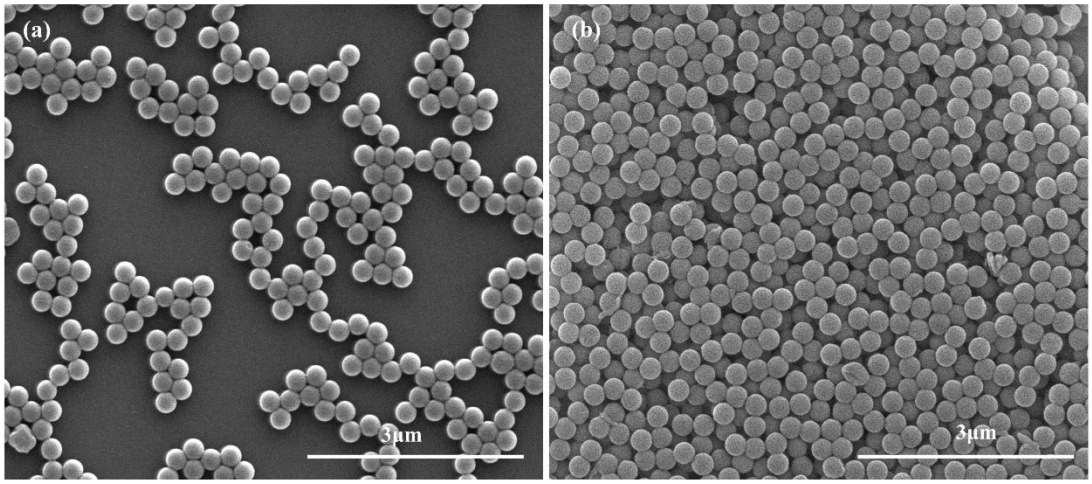

Figure. S6. SEM images of polymer microsphere before (a) and after (b) swelling and grafting procedure.

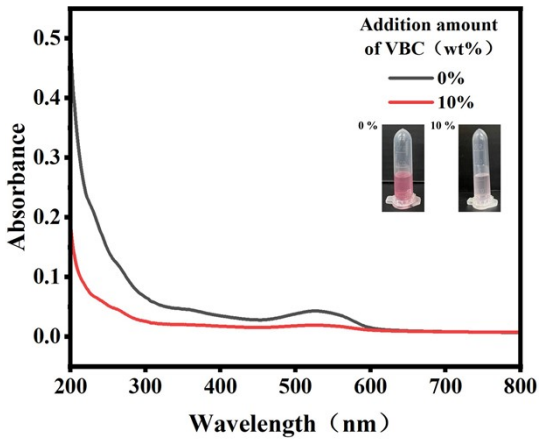

Figure. S7. UV absorption spectra of the supernatant of dyed microspheres after centrifugation by sonication for 10 min. The inset shows photographic images of the supernatant.

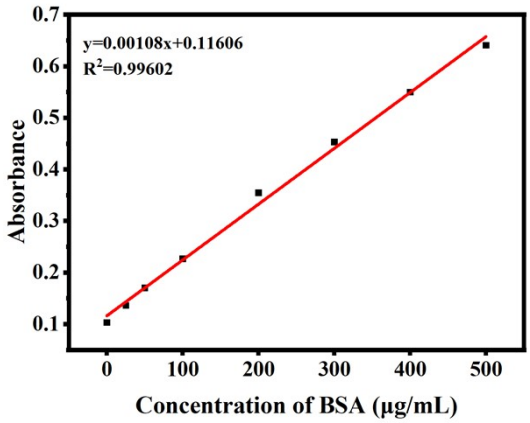

Figure. S8. Standard curve of BSA.

Optimization of antibody labeling

According to the steps of experimental part 2.5., the dyed microspheres were activated for 30 min, centrifuged and washed twice, and 1ML of PBS buffer (10 mM, pH =7) was added to obtain the activated microsphere solution. Then 0 mg,0.01 mg,0.05 mg,0.1mg, 0.15 mg, 0.2 mg, 0.25 mg,0.3 mg and 0.4 mg of antibodies were added to each of them, and the coupling reaction was incubated at 37 ℃ with slow rotation at 37 °C for 2 h. After the reaction, the mixture was centrifuged at 1200 r/min for 12 min. The absorbance of the supernatant was recorded to reflect the antibody coupling on the microspheres, and the optimal antibody amount was finally determined.

As shown in the Figure. S9, the amount of antibody coupled on the microspheres increased with an increasing of antibody and then tended to be constant. Therefore, 0.25 mg is the most appropriate amount of antibody.

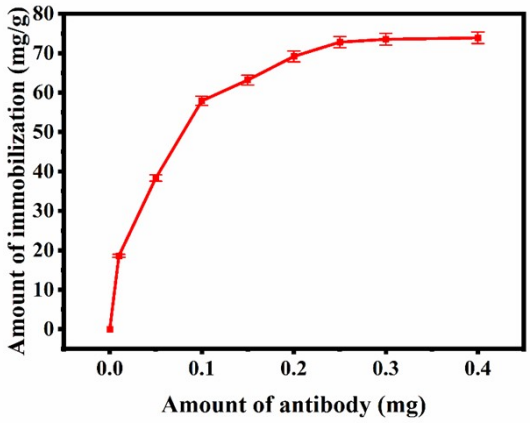

Figure. S9. Antibody coupling curves of dyed microspheres under different antibody dosages.

Table. S1. Test results of different content of COVID-19 Virus N Protein.

COVID-19 Virus N Protein (COI)	Mid concentration	High concentration
Number of tests	10	10
Average (COI)	20.91	87.84
CV (%)	4.0	1.8

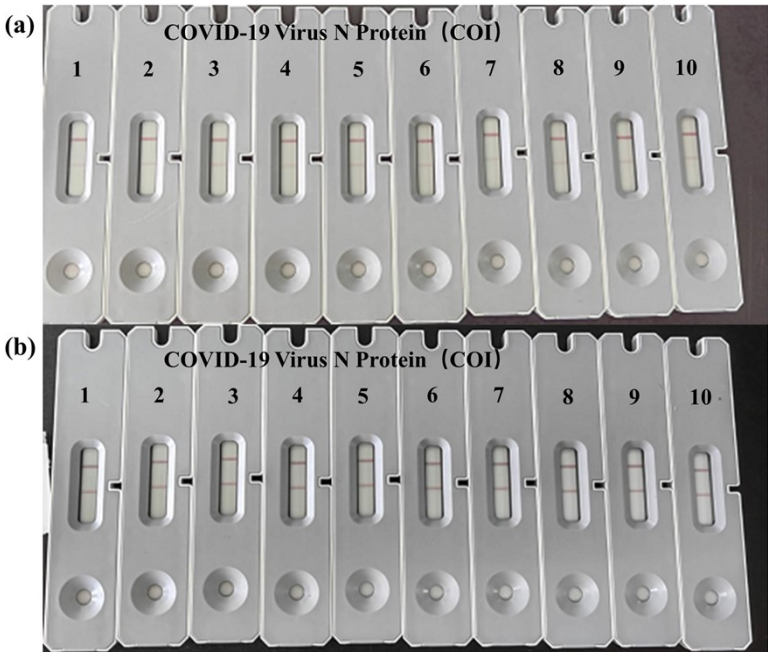

Figure. S10. Photographs of repeatability of the LFIA evaluated using two different concentrations ((a) 20.91 COI and (b) 87.84 COI) of COVID-19 Virus N Protein.