Supporting Information for

Immunoassay of SARS-CoV-2 Nucleocapsid Proteins Using Novel Red Emission Enhanced Carbon Dots-Based Silica Spheres

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Supporting Information Content:

Experimental Section

Materials

Citric acid, amidinothiourea, N, N dimethylformamide (DMF), sodium hydroxide (NaOH), ethanol, hydrochloric acid (HCl), N-β-(aminoethyl)-γ-aminopropyltrimethoxysilane (AEAPTMS), (3-Aminopropyl)-trimethoxysilane (APTES), cetyltrimethylammonium bromide (CTAB), sodium salicylate, tetraethoxysilane (TEOS), triethanolamine (TEA), succinic anhydride, ammonia aqueous solution (28%), Tirton X-100 and Sodium chloride (NaCl) were purchased from were purchased from Sinopharm Chemical Reagent Co., Ltd. Bovine serum albumin (BSA) was obtained from Sangon Biotech Co., Ltd. N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) was purchased from Energy Chemical Co., Ltd. Phosphate buffer solution (PBS, 10 mM, pH 7.4) was freshly prepared before use. Goat antimouse IgG antibody, sample pads, nitrocellulose membranes, absorbent pads, and black plastic adhesive cards were purchased from Shanghai JieYi Biotechnology Co. Ltd. (Shanghai, China). Severe acute respiratory syndrome coronavirus 2 nucleocapsid proteins (SARS-CoV-2 NP) and the corresponding labeled antibody and coated antibody were provided by Huadong medical institute of biotechniques.

Characterizations

Transmission electron microscopy (TEM) measurements were carried out under a Tecnai G2 20 transmission electron microscope (FEI.USA) and a field-emission high resolution transmission electron microscopy Talos F200X (FEI, USA). The fluorescent spectra were performed on a FluoroMax-4 spectrofluorometer (Horiba, USA). Zeta potentials were acquired with a Marlvern Zetasizer Instrument (Nano ZS, England). The UV-vis absorption spectra were recorded by a Shimadzu UV-2450 spectrophotometer (Japan). The absolute photoluminescence quantum yields were tested using a quantum yield accessory, including an integrating sphere which was attached to FluoroLog-3 modular spectrofluorometer (Horiba Jobin Yvon Inc.). Fourier transform infrared (FR-IR) spectrum was collected from a Nicolet 5700

(Thermo Nicolet Corporation, USA) IR spectrometer. The model of fluorescence microscope we use to observe the fluorescence signal on the test line and control line is Olympus IX73.

Synthesis of CDs

Amidinothiourea (6 g) and citric acid (2 g) were dissolved in DMF (20 mL), followed by high temperature solvothermal reaction (160°C, 6 h). After cooling to room temperature, the raw CDs were obtained. The solution was mixed with NaOH ethanol solution (50 mg mL^{-1}, 40 mL) and the precipitate obtained by centrifugation was washed several times with water and ethanol. Finally, the obtained product was dispersed in 5% HCl, and then the precipitate obtained by high-speed centrifugation was washed with ethanol several times to neutrality to obtain CDs. Dried the CDs slightly to set aside.

Synthesis of dendritic SiO_2 nanospheres

TEA (68 mg) was added to H_2O (25 mL) and mixed (80°C, 30 min), then CTAB (380 mg) and sodium salicylate (168 mg) were added and stirring continued (1 h). Then TEOS (4 mL) was quickly injected into the solution and gently stirred at 80°C for 2 h. The mixture was then diluted with ethanol and the product was collected by centrifugation. After washing the product with ethanol several times, it was dispersed in a HCl/ethanol mixture and stirred at 60°C for 24 h. After centrifugation, it was washed several times with ethanol and H_2O, and dispersed in ethanol.

Synthesis of red emissive CDs-based silica (RCS) nanospheres

Firstly, the CDs and AEAPTMS solution were mixed and shaken (6 h). After high-speed centrifugation, the precipitate was discarded and the red solution was retained. Then, the above solution (1 mL) was mixed with SiO_2 spheres (20 mg) in ethanol solution (9 mL), and ammonia (200 μL) was added for continuous stirring (12 h), the precipitate was recovered by centrifugation, and washed with ethanol several times. Subsequently, the obtained precipitate was dispersed in ethanol (10 mL), and after adding APTES (30 μL) and ammonia (200 μL) and stirring for (12 h), the product was collected by centrifugation, washed with ethanol several times and dispersed in DMF (10 mL).

Finally, after adding succinic anhydride (0.1 g) and stirring for 6 h, the RCS anospheres with carboxyl ends was obtained by centrifugation and washing, which was dispersed in H_2O for further use.

Preparation of immune-RCS spheres

The carboxyl-rich *RCS* spheres were conjugated with labeled antibody of SARS-CoV-2 NP though classical carbodiimide coupling reaction. The specific steps were as follows: the aqueous solution RCS spheres (0.5 mL) was mixed with of newly prepared EDC aqueous solution (10 mg mL^{-1}, 70 μL), then added labeled antibody of SARS-CoV-2 NP solution (100 μg mL^{-1}, 1 mL). After shaking the mixture at room temperature for 3 h, the precipitate was collected by centrifugation and redispersed in 1 mL phosphate buffer solution (PBS, 0.01 M, pH=7.4, 1% BSA) to obtain the immune-RCS spheres storage solution and stored in a refrigerator at 4°C for later use.

Fabrication of the RCS-LFI Test Strip

The sample pads were treated with 10 mM PBS (pH = 7.4) containing 1% (v/v) Tirton X-100 and 2% NaCl and dried at 37°C. The coated antibody against SARS-CoV-2 NP (2 mg mL^{-1}) and goat anti-mouse IgG antibody (2 mg mL^{-1}) were dispersed to the test and control lines on the NC membrane, respectively, using a double-headed marker with a minimal pen tip (SJ002, 1.97 × 34 mm, Shenzhen, Stationery Store), followed by drying at 37°C for 2 h. The absorbent pad, NC membrane, and pretreated sample pad were adhered to a black plastic backing, and then the assembled strips were cut into 3 mm wide pieces. The prepared test strips were sealed and stored at 4°C.

Detection of SARS-CoV-2 NP Using RCS-LFI Test Strip

The test strips were placed on the platform, and the mixture of 40 μL SARS-CoV-2 NP standard solution and 30 μL iSCS spheres storage solution was dropped on the sample pad. After 20 min, the test results were observed under a 365 nm UV lamp / Olympus IX73 fluorescence microscope. The results of observation under UV lamp were taken by Huawei mobile phone. The results of observation under the fluorescence microscope were taken by the equipped camera, and the result were processed by ImageJ software for quantitative analysis.

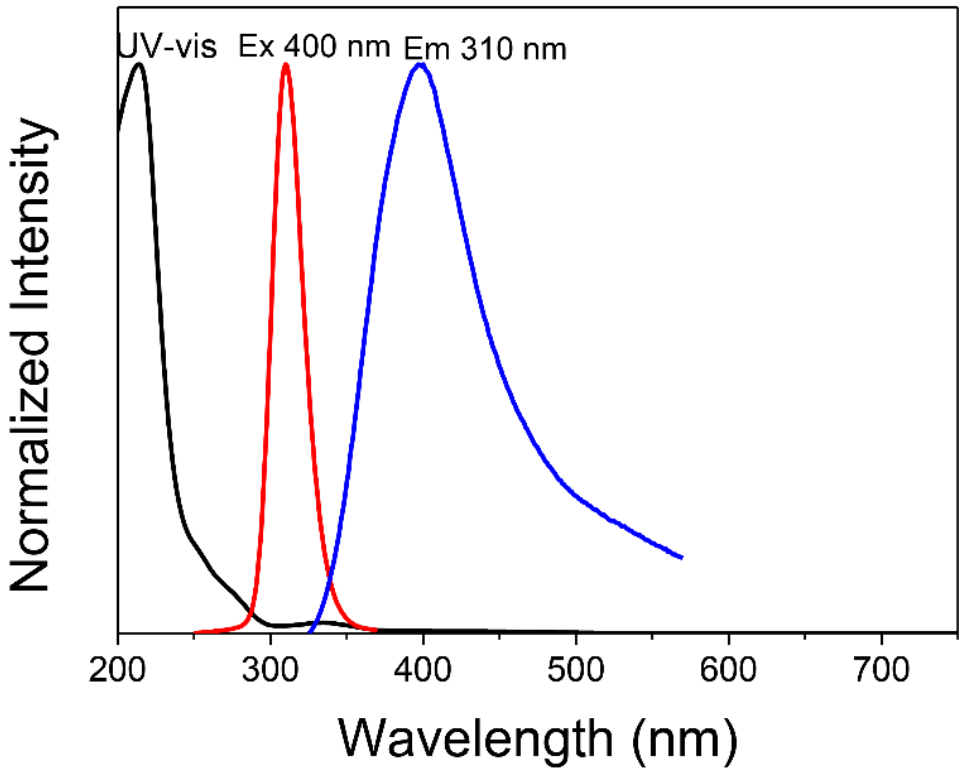

Figure S1. UV-vis absorption spectum (black line), excitation spectrum (red line) and emission spectra (blue line) of raw CDs.

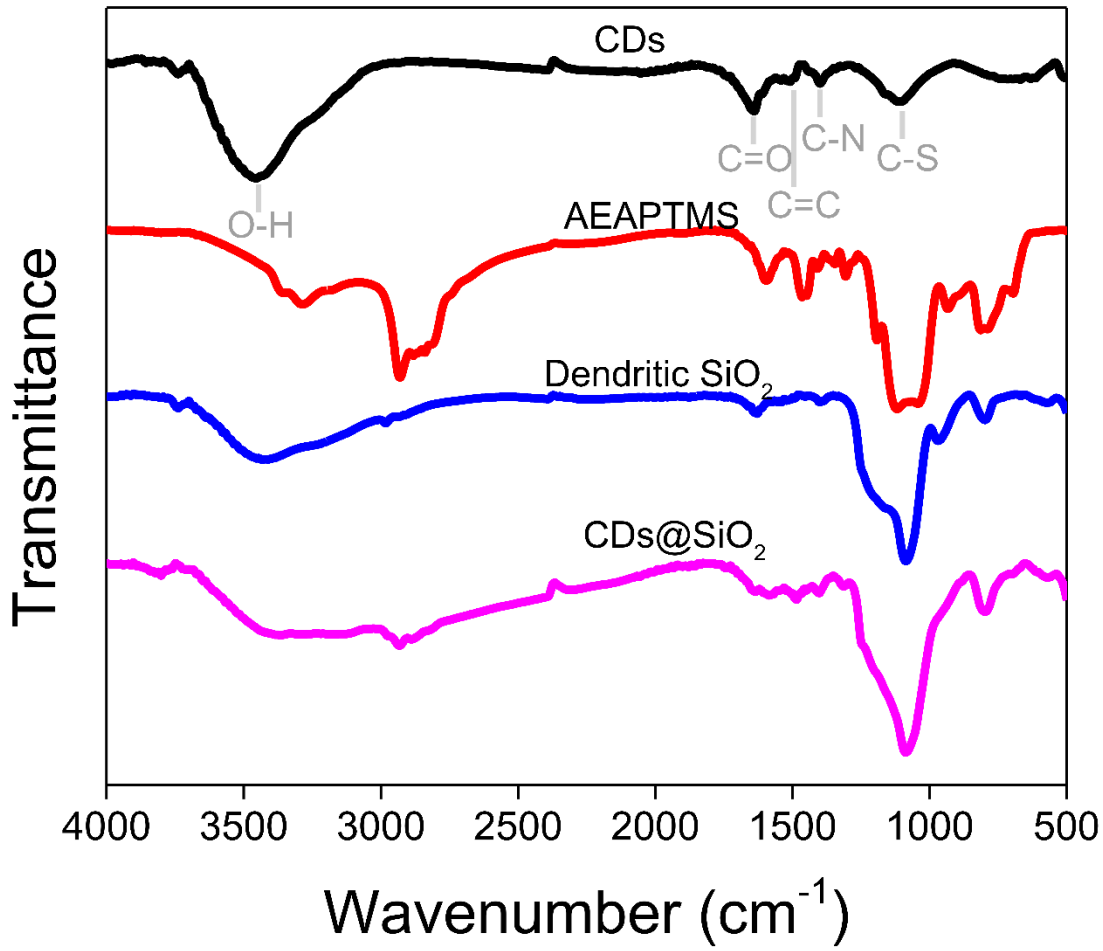

Figure S2. FT-IR spectra of CDs, AEAPTMS, Dendritic SiO_2 and CDs@SiO_2 spheres.

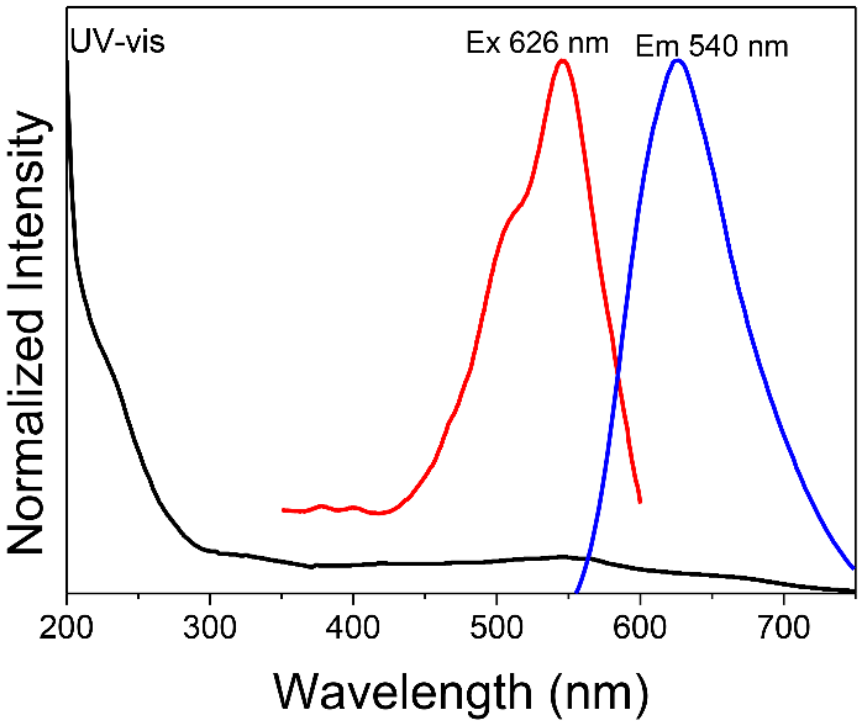

Figure S3. UV-vis absorption spectum (black line), excitation spectrum (red line) and emission spectra (blue line) of CDs aqueous solution.

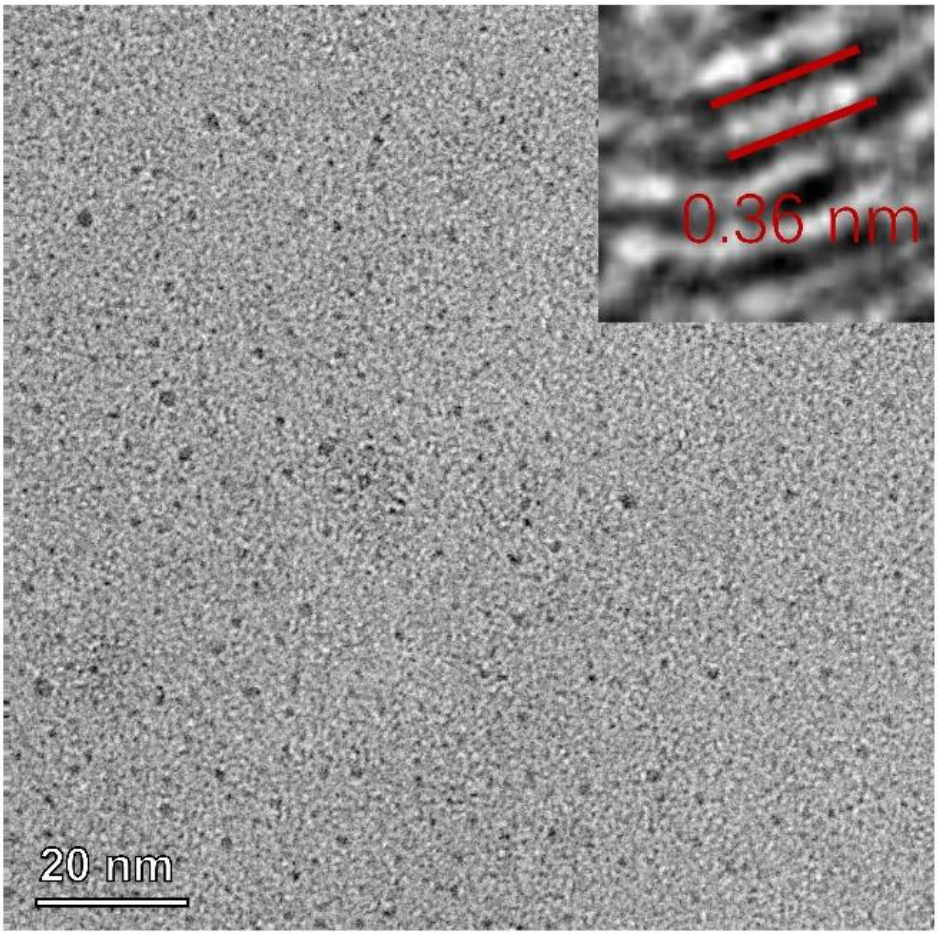

Figure S4. TEM/HRTEM images of CDs.

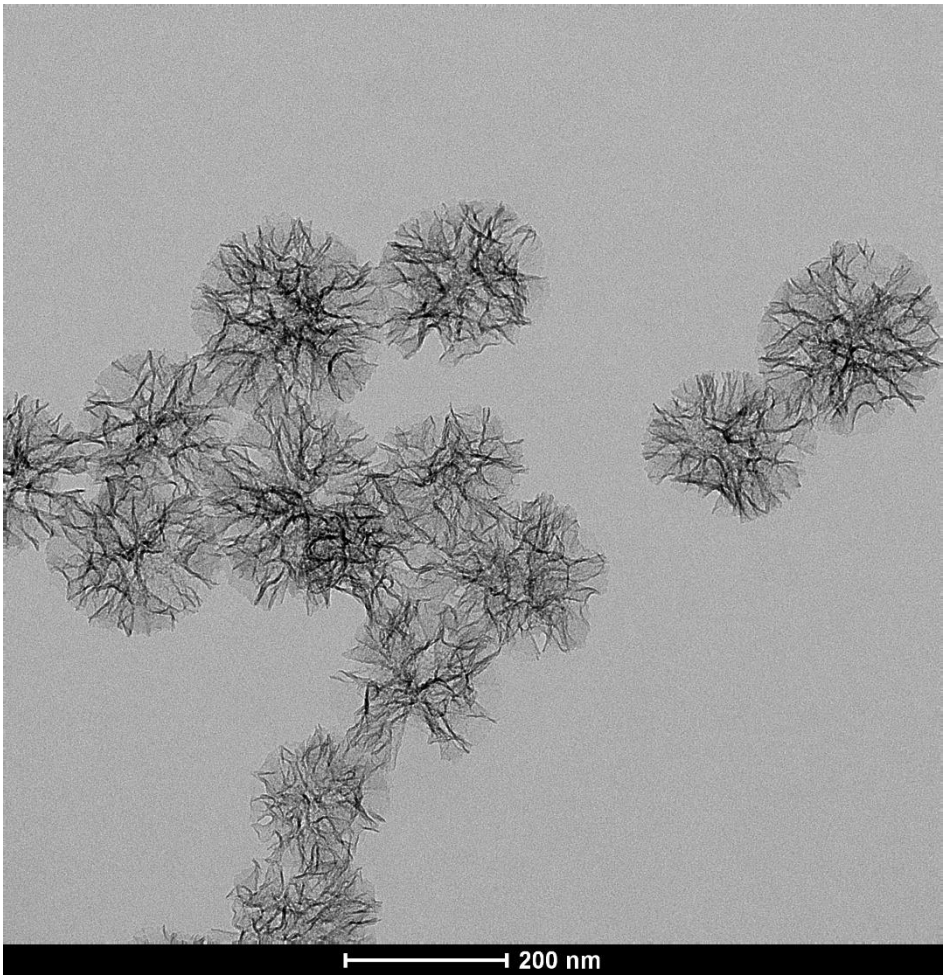

Figure S5. TEM image of dendritic SiO_2 nanospheres.

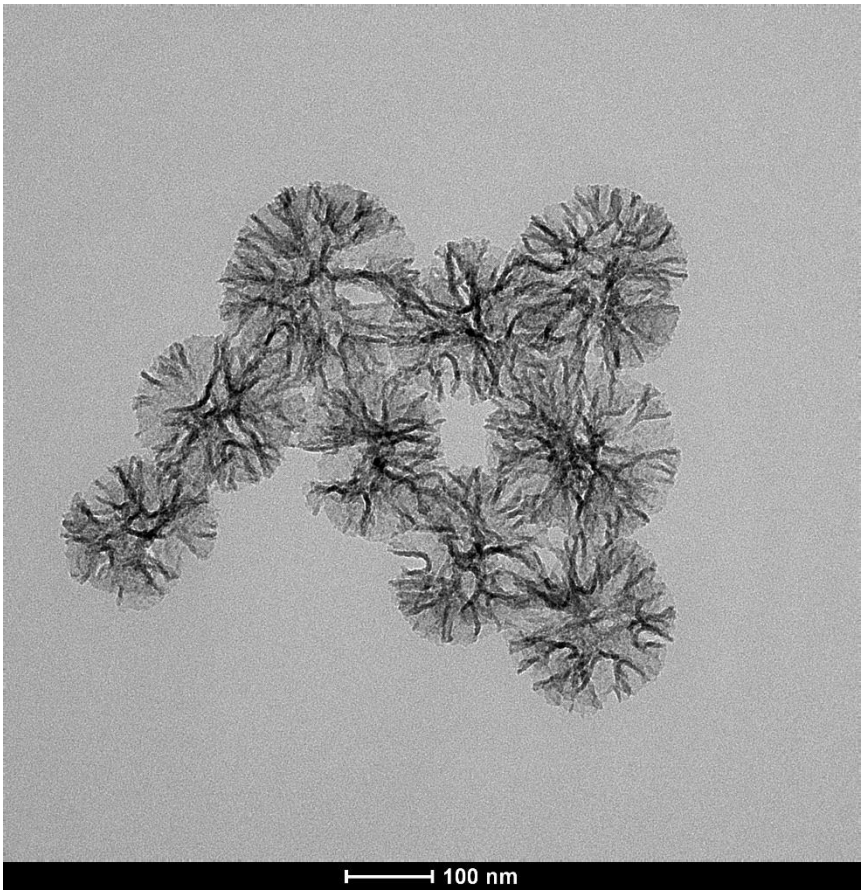

Figure S6. TEM image of RCS nanospheres.

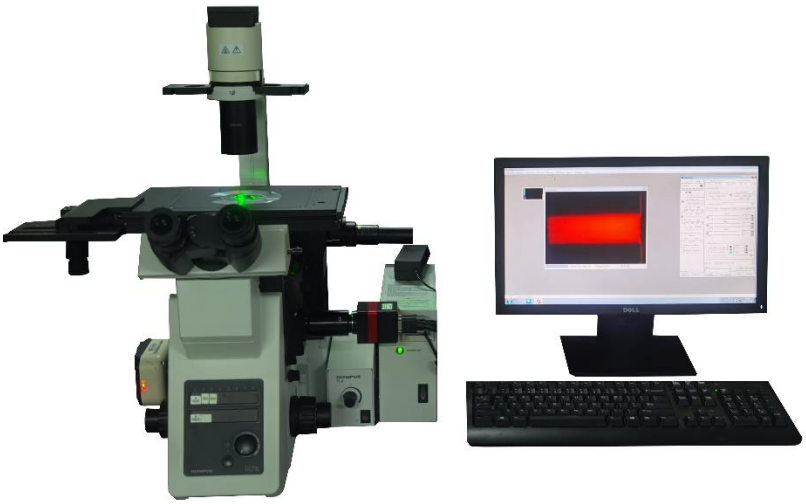

Figure S7. Olympus IX73 inverted fluorescence microscope used in RCS-LFI technology.

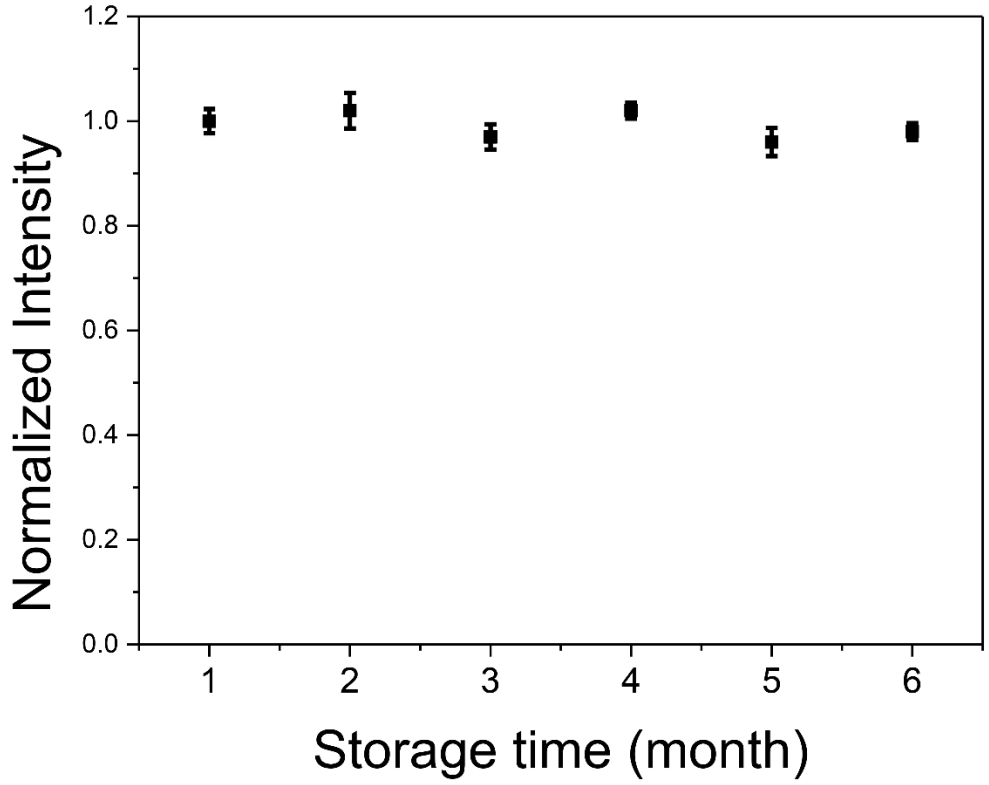

Figure S8. Stability test of the RCS-antibody conjugates at different storage times.