Supporting information

Cellulose acetate/Metal–organic framework composite beads with macroporous adsorption channels as a novel hemoadsorbent for effective virus capture

Yamin Chai[a,b], Wenyan Han[c,b], Yanjia Zhang[b], Yunzheng Du[b], Biao Wang[b], Mengya Chen[b], Nan Li[b], Wei Luo[a], Xiaoyu Zha[a], Lichun Wang[d,b]*, and Lailiang Ou[b]*

[a]General Hospital, Tianjin Medical University, Tianjin, 300052, China.

[b]Key Laboratory of Bioactive Materials, Ministry of Education, College of Life Sciences, Nankai University, Tianjin, 300071, China.

[c]Henan University of Chinese Medicine, Henan, 450046, China.

[d]Hubei Key Laboratory of Multi-media Pollution Cooperative Control in Yangtze Basin, School of Environmental Science & Engineering, Huazhong University of Science and Technology (HUST), Hubei, 430074, China.

[e]Department for Biology, Chemistry, Pharmacy, Free University of Berlin

*Corresponding author

E-mail: wanglichun@hust.edu.cn, ouyll@nankai.edu.cn,

Adsorption of plasma proteins

We further illustrated its practical application potential through adsorption selectivity against proteins, including total protein (TP), albumin (ALB), and globulin (GLOB). 50 mg beads were added to a glass bottle that contained 0.5 mL the HIV lentivirus plasma with a concentration of 3×10^7 $TCID_{50}$ mL^{-1} at 37 ℃ with constant shaking at 160 rpm for 2 h. The levels of proteins before and after the adsorption were evaluated by automatic biochemical analyzer (Vitros, V5600, USA).

Swelling study

50 mg of dry beads to calculate the swelling ratio in this study, and the fabricated adsorbents were swelled in water for 15 min, 30 min, 60 min, 90 min, and 120 min, respectively, and weighed the beads at regular intervals. The swelling ratio is calculated via the following equation:[1, 2]

$$\text{Swelling ratio (\%)} = \frac{W_i - W_t}{W_t} \times 100 \quad (1)$$

where W_t and W_i are the weight of the adsorbents at time and initial weight of the adsorbents, respectively.

Hemolysis assay

A 50 mg adsorbent was prewetted in PBS for 24 h and then transferred to a 1.2 mL of suspension of rabbit red blood cells (serum: PBS = 4:5) at 37 °C and incubated for 1 h. After centrifugation of the samples at 2500 rpm for 5 min, and the supernatants were evaluated at 545 nm by an enzyme-labeling measuring instrument (EnSpire; PerkinElmer, Waltham, MA, USA). The hemolysis rate was calculated using the following equation:

$$\textit{H}\text{emolysis rate (\%)} = \frac{OD_1 - OD_3}{OD_2 - OD_3} \times 100 \quad (2)$$

where, OD_1, OD_2, and OD_3 denote the optical density values of the sample, positive control, and negative control, respectively.

Blood counts

Whole blood was taken from rabbit, and then 200 mg adsorbent was mixed with 2 mL of anticoagulation rabbit blood at 37 °C, and then incubated for 1 h. The red blood cells (RBCs), white blood cells (WBCs), platelets (PLTs) and hemoglobin (HGB) were counted by an automatic hematology analyzer (PE-6100, Mindray, China).

Anticoagulation assay

The anticoagulation assay was performed utilizing a platelet-poor plasma (PPP) test. The PPP was fabricated from fresh blood from rabbits and centrifuged for 15 min at 4000 rpm. Then, 2 mL of PPP was added to 200 mg of adsorbents at 37 °C and incubated for 1 h. After centrifugation of the samples at 4600 g for 5 min, the supernatants were detected by a blood coagulation analyzer (CA-50; Sysmex Co., Lincolnshire, IL, USA) to evaluate the prothrombin coagulative time (PT), activated partial thromboplastin time (APPT), thrombin time (TT), and fibrinogen (FIB).

Cytotoxicity assay

The in vitro cytotoxicity of the adsorbents on HE293T cells was measured with a CCK-8 assay. Calcein-AM and PI (propyl iodide) double fluorescence staining were used to detect the lactase activity and membrane integrity, respectively, to reflect the cell activity and cytotoxicity. The following steps were used. Frist, 2.0 g of sample was immersed in 10 mL DMEM medium (supplemented with 10% fetal bovine serum (FBS) and 1% double antibody) at 37 °C for 24 h, and the extract stock solution was obtained by centrifugation. The HE293T cells were seeded in 96-well plates at a density of 1000 cells/well, cultured at 37 °C for 24 h in a humidified atmosphere with 5% CO_2 and then washed with PBS (pH 7.4, 0.01 M) several times. Subsequently, the HE293T cells was were treated with extract stock solution (100 μL, 1X), then cultured at 37 °C for 96 h. Moreover, the fresh DMEM medium and DMEM medium with 5% DMSO were choose as the negative and positive control, respectively. After the culture solution was removed and 100 μL Calcein AM/PI was added to the cells, the samples were incubated at 37 °C for 30 min in the dark. Finally, fluorescent images were acquired by optical inverted microscope (Axio Imager Z1, ZEISS, German), and the cell viabilities were assessed by a CCK-8 assay.

Table S1† The amount of virus and LV-Enhance.

Volumes of Virus	Volumes of LV-enhance
10 μL	2.2 μL
20 μL	2.4 μL
30 μL	2.6 μL
50 μL	3.0 μL
70 μL	3.4 μL

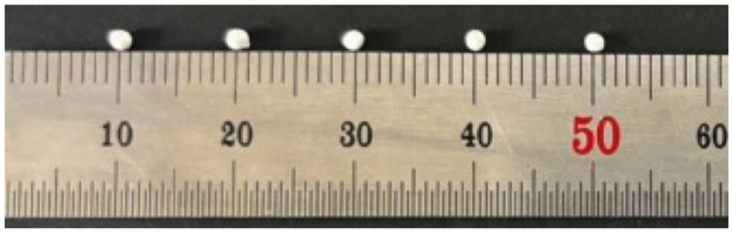

Fig. S1† Corresponding optical images of CNC/MIL-53 composite beads.

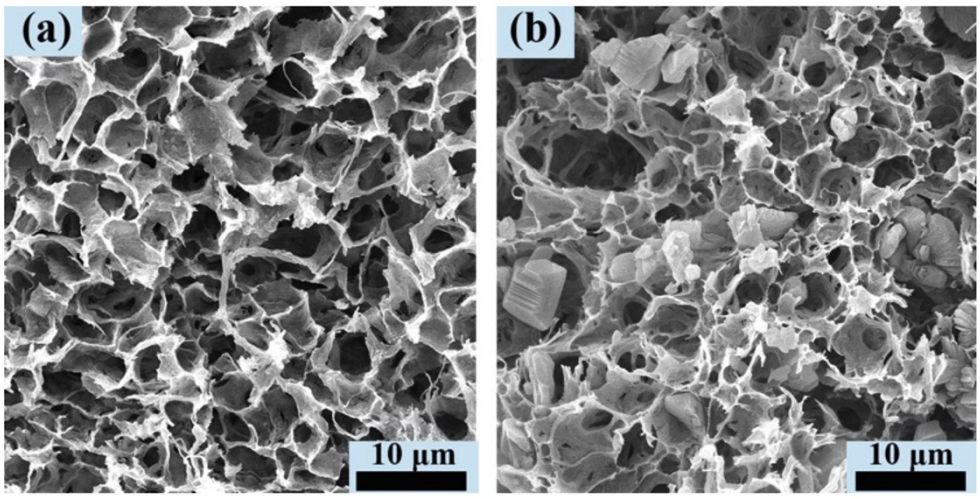

Fig. S2† The cross-section morphologies of the CNC/MIL-53 and CNC with small magnification. (a) CNC, (b) CNC/MIL-53.

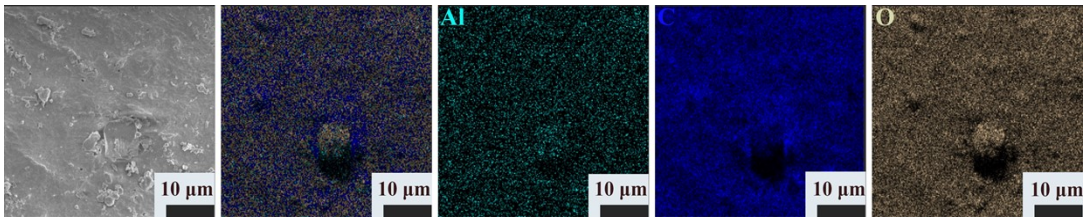

Fig. S3† EDS mappings of CNC/MIL-53 organic-inorganic composite beads for Al, C, and O atoms.

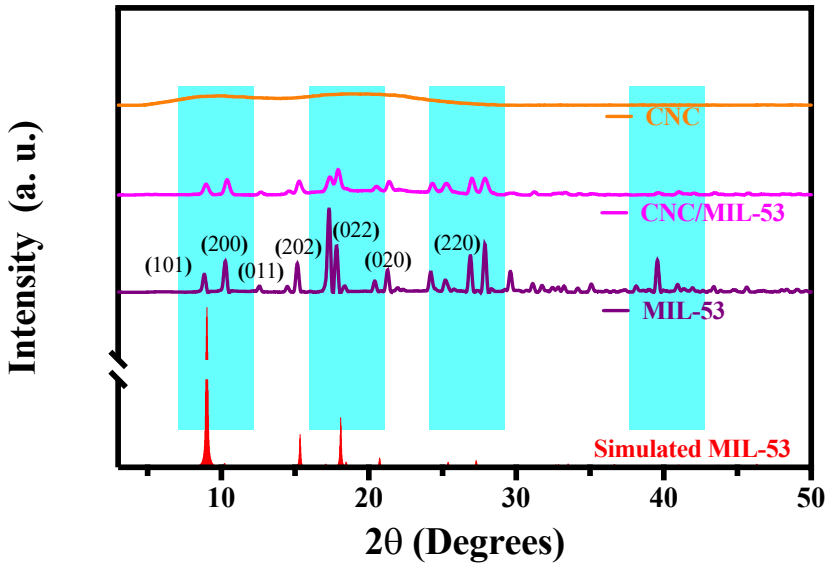

Fig. S4† XRD analysis of simulated MIL-53, MIL-53 particles, CNC/MIL-53 beads, and CNC beads.

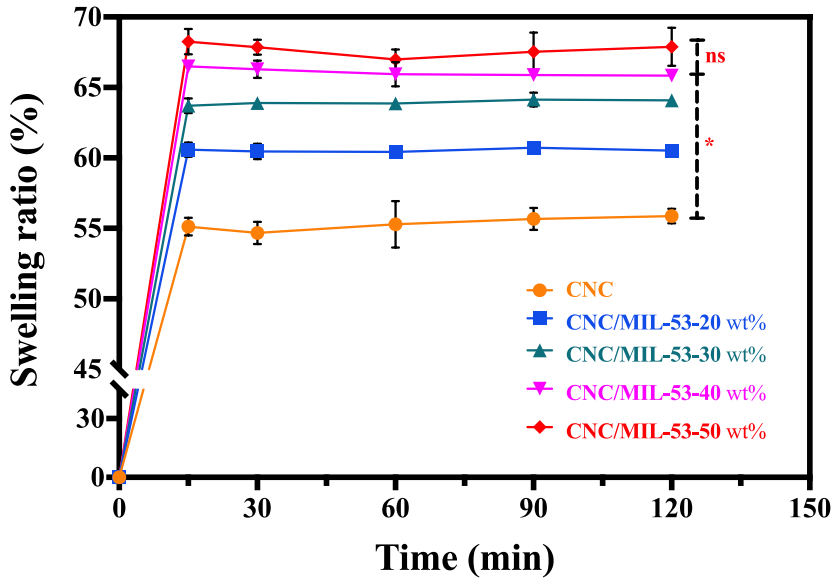

Fig. S5† The swelling tests in water of the dry CNC, CNC/MIL-53-20 wt%, CNC/MIL-53-30 wt%, CNC/MIL-53-40 wt% and CNC/MIL-53-50 wt%. *p < 0.05, ns.: Not significant.

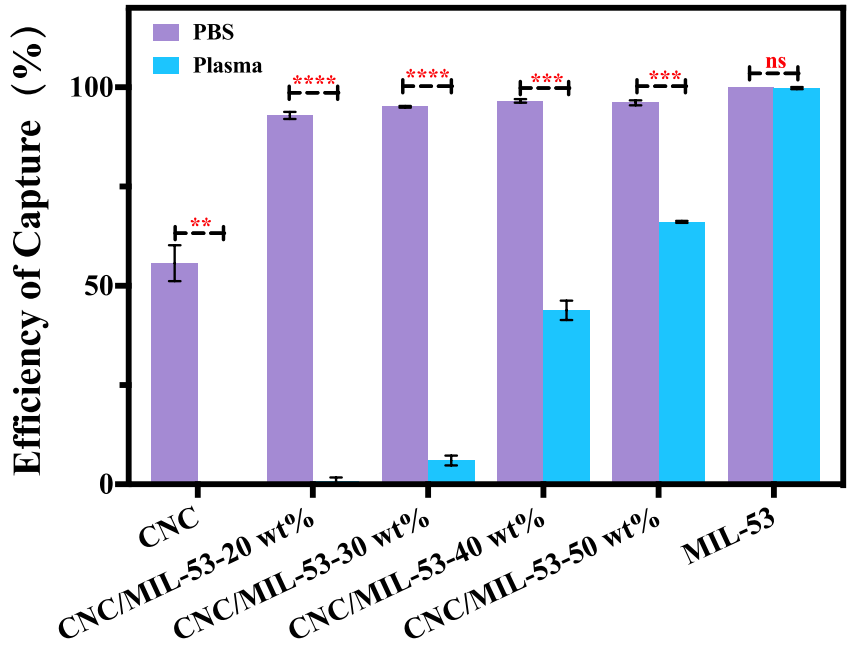

Fig. S6† Capture efficiency of the HIV pseudovirion by the CNC/MIL-53-20 wt%, CNC/MIL-53-30 wt%, CNC/MIL-53-40 wt%, CNC/MIL-53-50 wt%, CNC beads, and MIL-53 particles in PBS and plasma. Adsorbent/adsorbate ratio is 1:50. **p < 0.01, ***p < 0.001, ****p < 0.0001; ns.: Not significant.

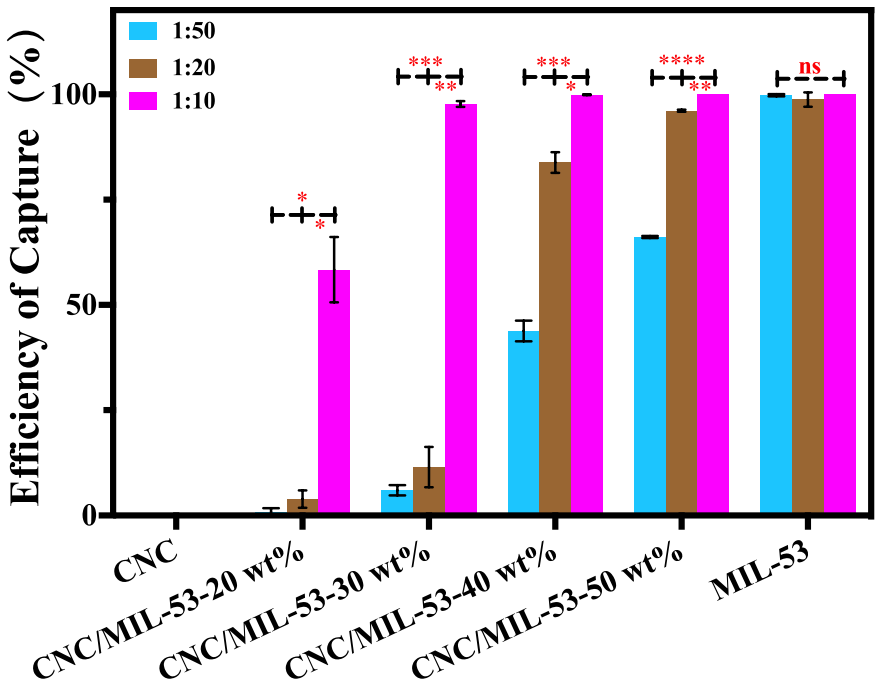

Fig. S7† Capture efficiency of the HIV pseudovirion by the CNC/MIL-53-20 wt%, CNC/MIL-53-30 wt%, CNC/MIL-53-40 wt%, CNC/MIL-53-50 wt%, CNC beads, and MIL-53 particles in plasma

with three adsorbent/adsorbate ratios (1:50, 1:20, 1:10). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001; ns.: Not significant.

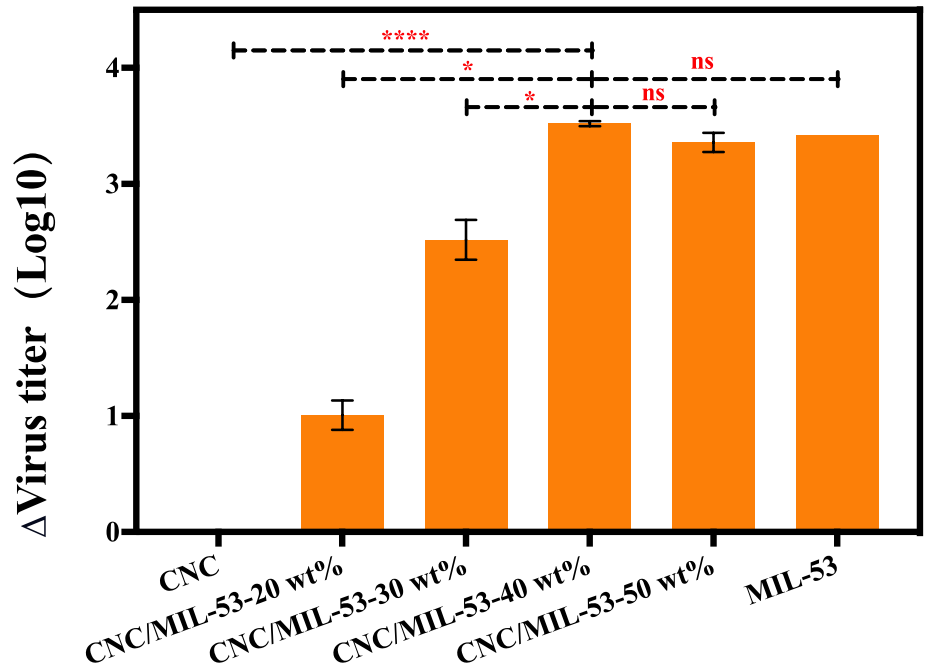

Fig. S8† Virucidal effect of CNC/MIL-53-20 wt%, CNC/MIL-53-30 wt%, CNC/MIL-53-40 wt%, CNC/MIL-53-50 wt%, CNC beads, and MIL-53 particles against HIV pseudovirion, which is expressed in a decrease in the titer of the virus after interaction with samples as compared to the control. *p < 0.05, ****p < 0.0001; ns.: Not significant.

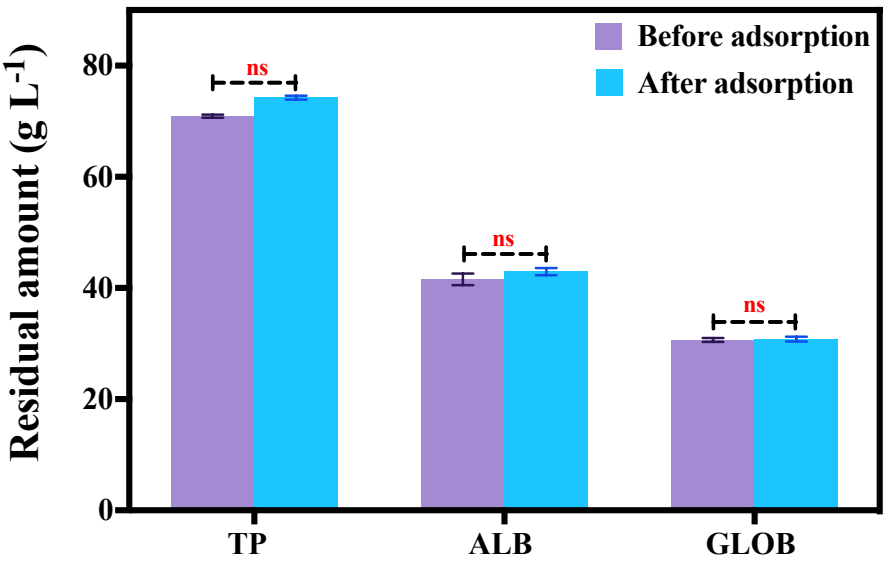

Fig. S9† Relative contents of different proteins in plasma after CNC/MIL-53 adsorption. ns.: Not significant.

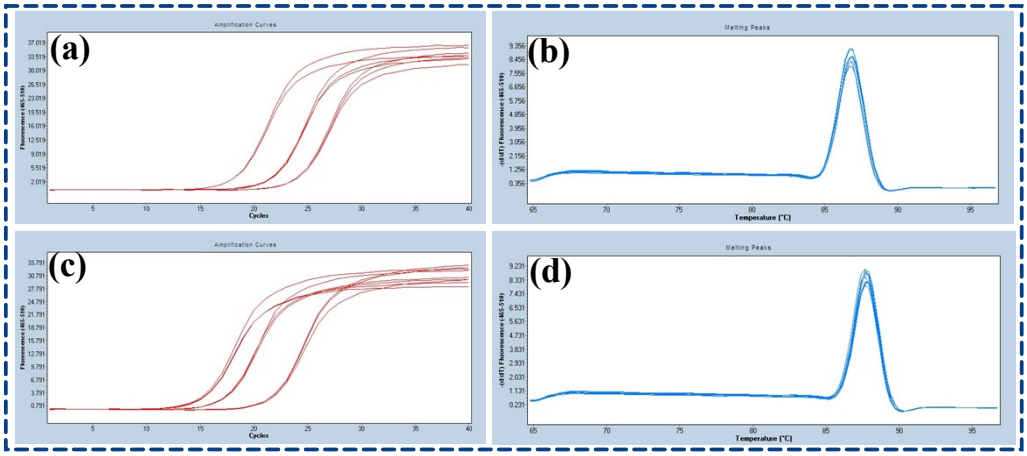

Fig. S10† Amplification curve and dissolution curve of Zsgreen and β-actin.

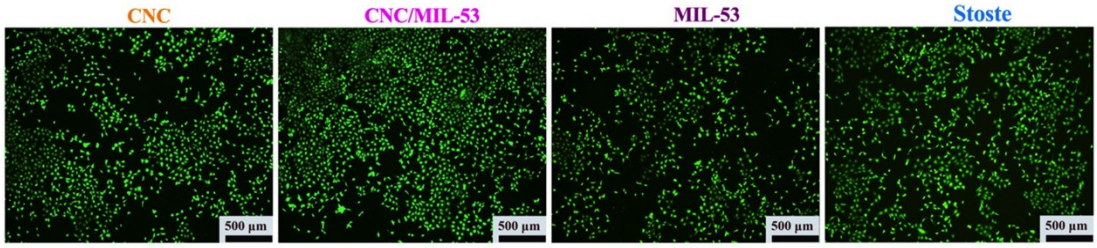

Fig. S11† Live/dead staining fluorescence images of HE293T cultured on cell culture medium soaked with different absorbents by fluorescence microscope.

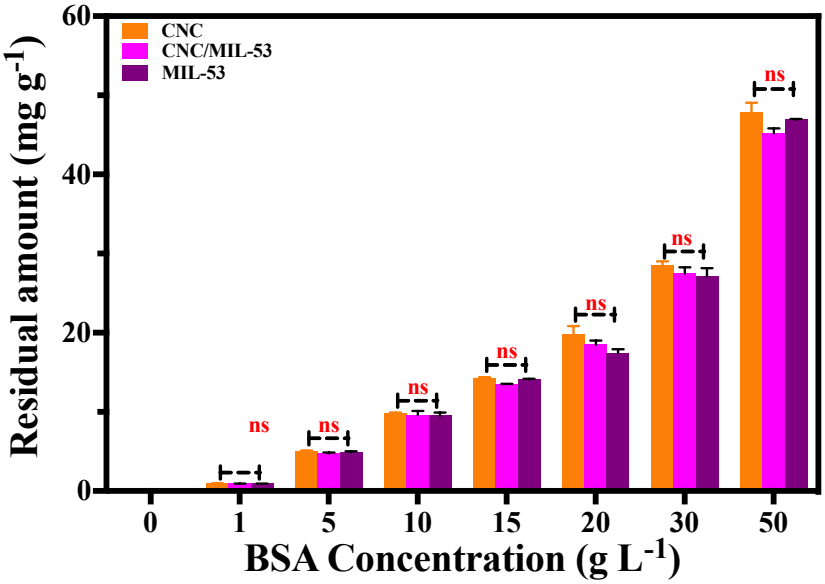

Fig. S12† Residual amount of BSA by different adsorbents in the varying concentration of BSA. ns.: Not significant.

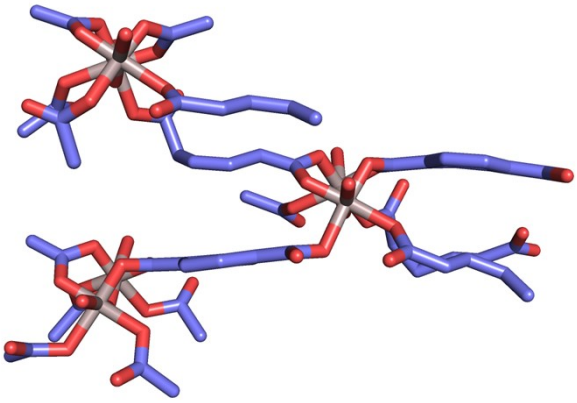

Fig. S13† Optimized geometries of MIL-53(Al) by AutoDock.

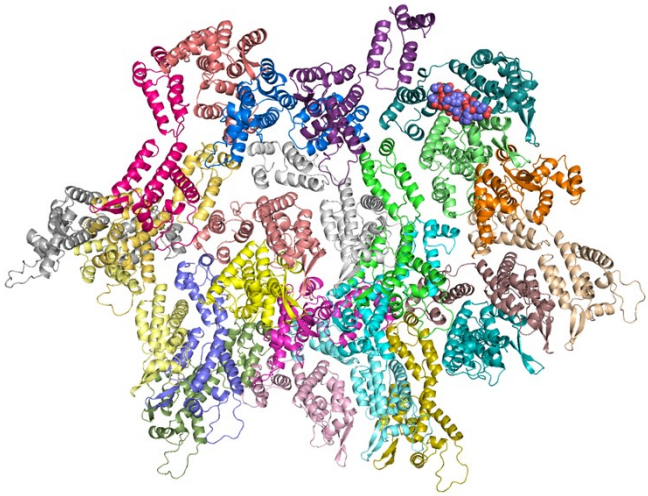

Fig. S14† Optimized geometries of p24 protein by AutoDock.

Notes and References

1. X. Song, K. Wang, C.-Q. Tang, W.-W. Yang, W.-F. Zhao and C.-S. Zhao, *Biomacromolecules*, 2018, **19**, 1966-1978.

2. Z. Ling, C. Jiang, X. Liu, Y. Li, W. Zhao and C. Zhao, *Composites Part B: Engineering*, 2023, **266**, 110994.