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CO_2-switchable response of protein microtubule: behaviour and mechanism

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Preparation and characterization

Isothermal titration calorimetry (ITC) data were taken on a MicroCal VP-ITC at 20.00±0.01°C. Dynamic light scattering (DLS) was performed on Zetasizer Nano ZS90 of Malvern Instruments (UK). Circular dichroism (CD) spectra were taken by Chirascan instrument from Applied Photophysics Ltd with 1 mm cuvette. Ultraviolet–visible (UV-Vis) spectra were taken under a Shimadzu UV-2550 spectrophotometer with 1 mm cuvette. Size exclusion chromatography (SEC) was performed on superdex G-75 10/300. Transmission electron microscopy (TEM) images were taken on a Tecnai G2 20 TWIN (FEI) at 200 kV. Cryogenic transmission electron (Cryo-TEM) images were taken on a Tecnai G2 F20 at 200 kV.

TEM samples were prepared by dropping 4 mL microtubule solution on the carbon film. After about 30 seconds, the excess liquid was removed by filter paper and subsequently, the samples on carbon film were stained by 1 wt% uranyl acetate. Cryo-TEM samples were prepared by dropping 4 mL solution on the carbon film. After about 30 seconds, the excess liquid was removed by filter paper and subsequently the samples were quickly immersed into liquid ethane and then transferred into liquid nitrogen. The cryogenic samples were

kept at liquid nitrogen temperature during imaging. All TEM grids were glow-discharged before use in order to increase hydrophilicity.

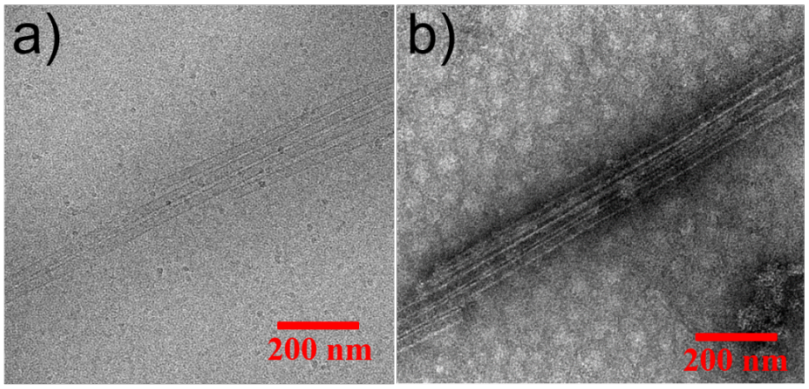

Fig. S1 (a), (b) Images of Cryo-TEM and TEM with sample negative staining of SBA/R3GN (pH=7.2, [SBA]: 0.1 mM, [R3GN]: 0.2 mM and 4°C), respectively.

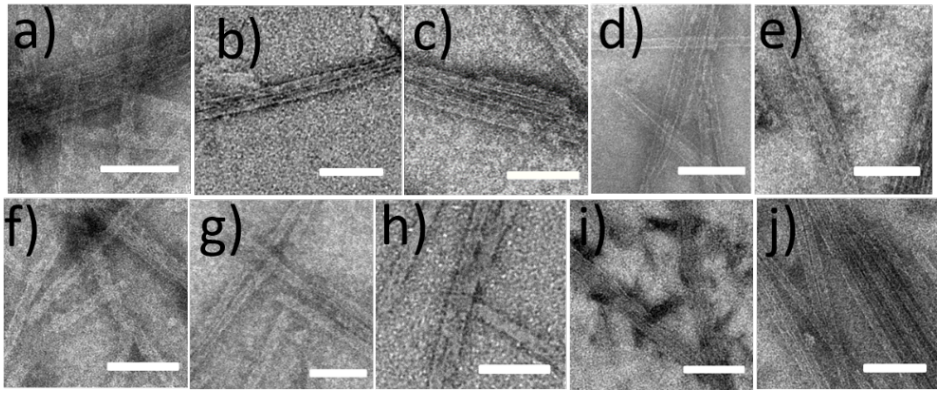

Fig. S2 TEM images of SBA/R3GN stained by uranyl acetate at different pH from 6.2 to 7.1 (from a to j) with 0.1 interval. Scale bar: 200 nm.

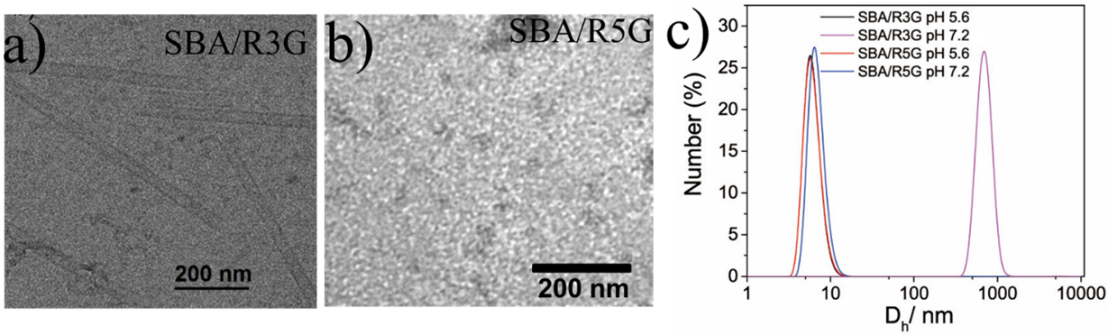

Fig. S3 (a) Cryo-TEM image of SBA/R3G after incubating 24h at 4°C (pH=7.2, [SBA]: 0.1 mM and [R3G]: 0.2 mM). (b) TEM image of SBA/R5G at the same conditions of (a). (c) DLS results of SBA/R3G and SBA/R5G at different pH.

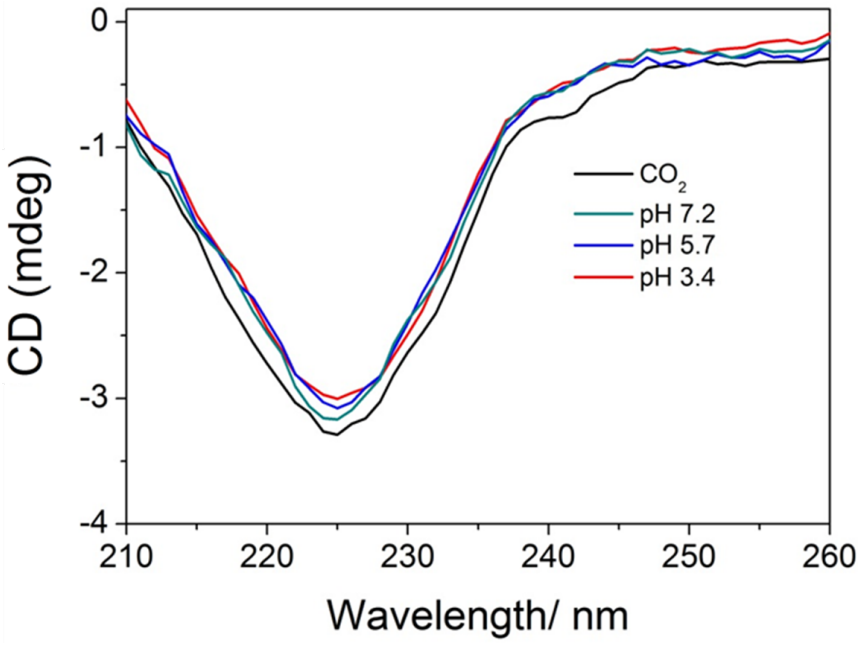

Fig. S4 CD spectra of SBA after passing CO_2 for 10 min at 4°C (black), pH=7.2 (green), pH=5.7 (blue) and pH=3.4 (red).

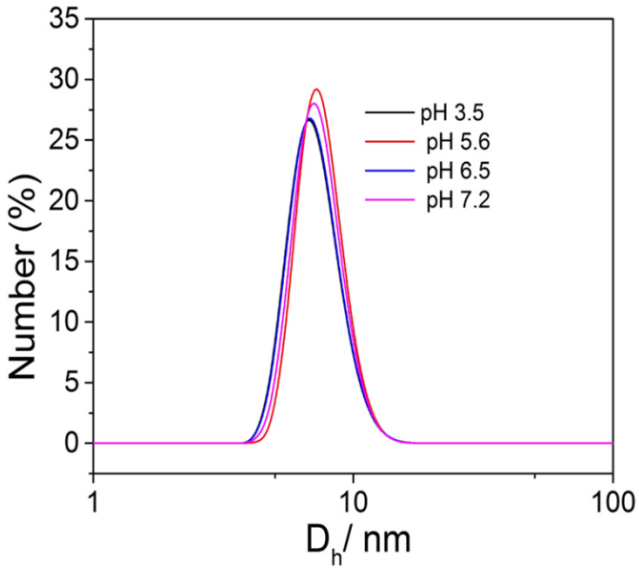

Fig. S5 DLS results of SBA at different pH.

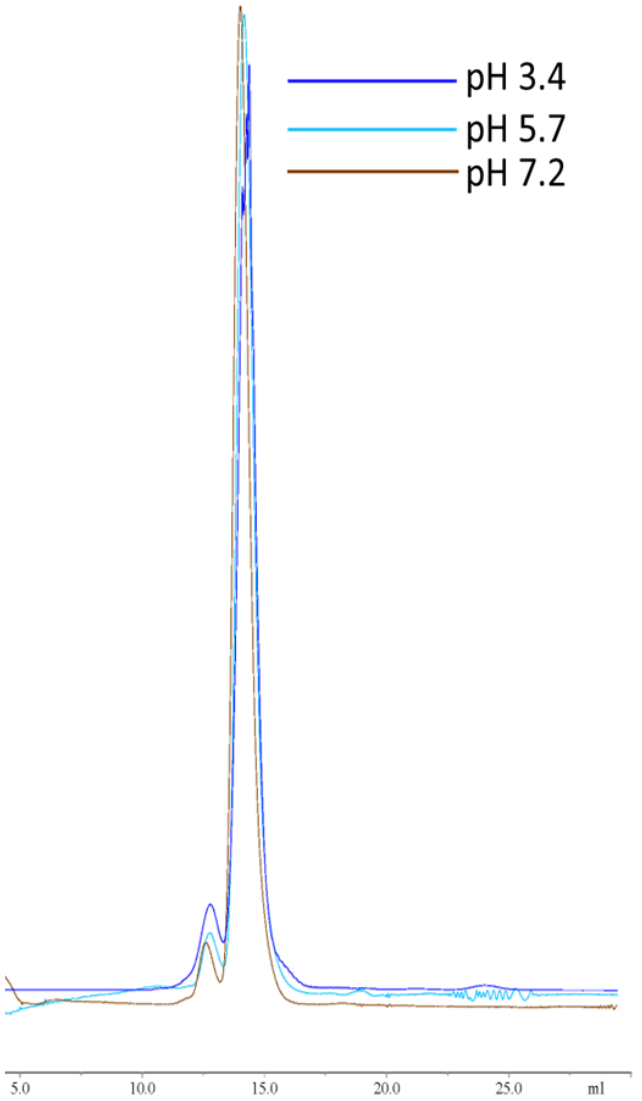

Fig. S6 SEC results of SBA at different pH.

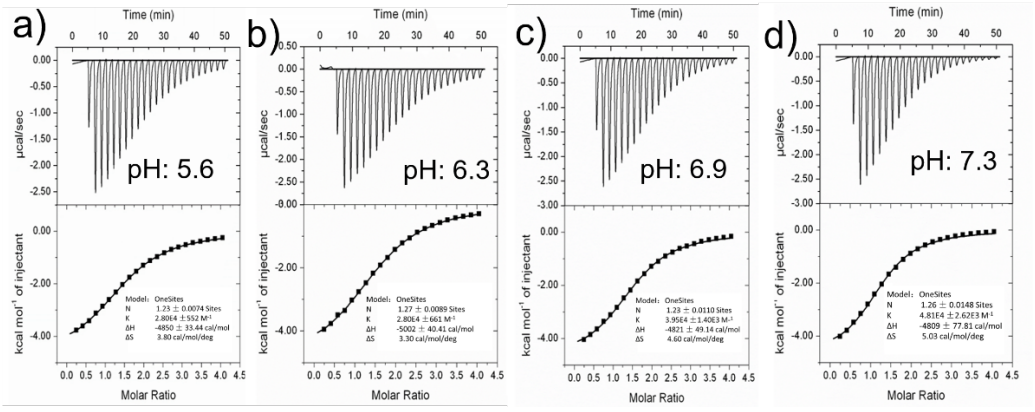

Fig. S7 ITC results of R3GN (1.5 mM) to SBA (0.1 mM) at 4°C with different pH. a) pH=5.6, b) pH=6.3, c) pH=6.9 and d) pH=7.3

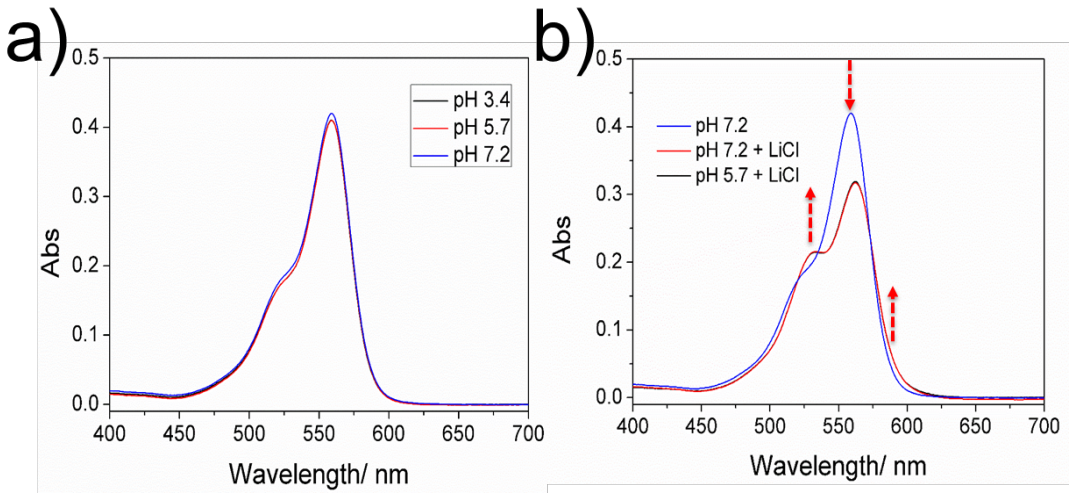

Fig. S8 (a) Ultraviolet–visible absorption spectra of a 0.075 mM R3GN at different pH (3.4 black, 5.7 red and 7.2 blue). (b) Ultraviolet–visible absorption spectra of 0.075 mM R3GN with/without LiCl (10 M) at different pH value (pH=5.7 and [LiCl]=10 M (black), pH=7.2 and [LiCl]=0 M (blue), and pH=7.2 and [LiCl]=10 M (red)).

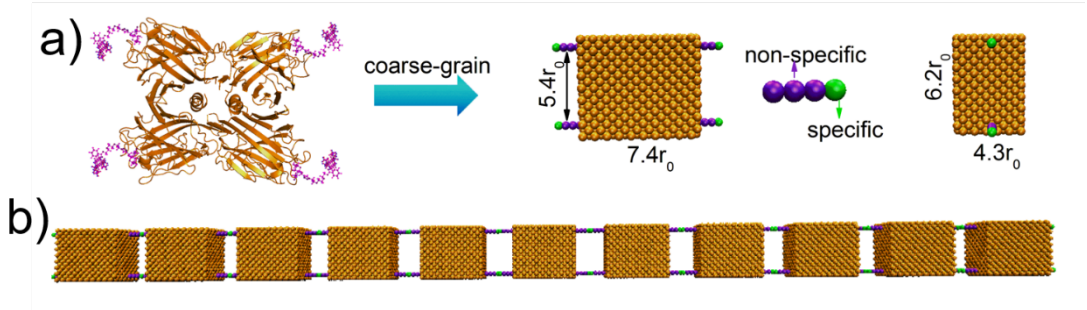

Fig. S9 (a) Coarse-grained mapping of SBA/RnG (n=3 or 5) in Brownian dynamics (BD) simulations, where the cuboid particle with the length of 7.4 r_0, width of 6.2 r_0, height of 4.3 r_0 was modeled as the SBA, and the oligomer with the length of 4 and 6 is modeled as R3G and R5G respectively, where the last bead of oligomer is specific and can form bond with each other. The right panel showed the cross-sections of the cuboid particle from different views. Besides, the coating site of the oligomers was about 0.4 r_0 away from the boundary, i.e., the distance of the two coating sites in the same face was about 5.4 r_0. (b) Initial conformation of the protein chains in the BD simulations: the particles were put closely along their long axis.

In order to compare the stability of the protein chains in different cases, the Brownian dynamics (BD) simulations[1] were used. For the sake of simplicity, the cuboid particles (by arranging the coarse-grained beads on an fcc lattice with lattice constant 0.39 r_o) with similar size of SBA were used as proteins;[2,3]

the oligomers decorated on the cuboid particle surface were treated as the R3G or R5G, and the coating sites of the oligomers were also approximately the same as that in all-atom simulations (see Fig. S9a). For R3G, the length of coating oligomers was four, where three beads were non-specific and the last one was specific (for R5G, the length of coating polymers was six, where five beads were non-specific and the last one was specific). The harmonic spring interaction $U_s = k_s(l_{i,i+1} - l_0)^2$ was applied between connected beads in the polymers and the binding sites of particle beads, where k_s=*1700* k_BT/r_0^2, l_0=*0.35* r_0. A shifted Lennard-Jones (LJ) potential,[4] cut off at $2^{1/6}\sigma$, was used to model the repulsive interaction among unconnected non-specific bead, specific bead and particle bead, where ϵ is chosen as ϵ/kT_0 = 1.0 and σ = 0.35 r_0. Additionally, a standard Lennard-Jones potential (cut off at 2σ) was used to model the attractive interaction between the specific beads, where ϵ is chosen as ϵ/kT_0 = 1.0 and σ = 0.35 r_0. Besides, the same potential (i.e., standard LJ potential) was also used to model the attractive interaction between the non-specific bead of polymers and the particle bead in cases R3G/ATT and R5G/ATT (Fig. 4c). In particular, we adopted the dynamic bond $(U_d = k_d(l_{i,i+1} - l_0)^2, k_d = 170k_BT/r_0^2)$ to generate the reversible bonding/nonbonding process between the specific bead of different polymers by the following rule:[5] when the bond length between the specific beads of the two polymers was shorter than the range of their pairwise interaction (i.e., the cutoff of LJ potential 0.70 r_0), the bond formed with a probability P_{on}; when the length was longer than 0.70 r_0, the bond was broken with a probability being P_{off}. Here, for the sake of simplicity, P_{on}, P_{off} were both set as 1.0.

Eleven cuboid particles were initially put closely with each other, thus the neighboring polymers can easily form the dynamic bonds (see Fig. S9b). Then we investigated the conformational change and equilibrium property of the proteins chains. All BD simulations were performed in the NVT ensemble by using the LAMMPS package (15 May2015).[6] During the simulation, the temperature was coupled at 1.0 T_0 using the Langevin thermostat. The time

step was 0.008 τ, and the data were collected every 80 τ, with the total simulation time lager than 800000 τ.

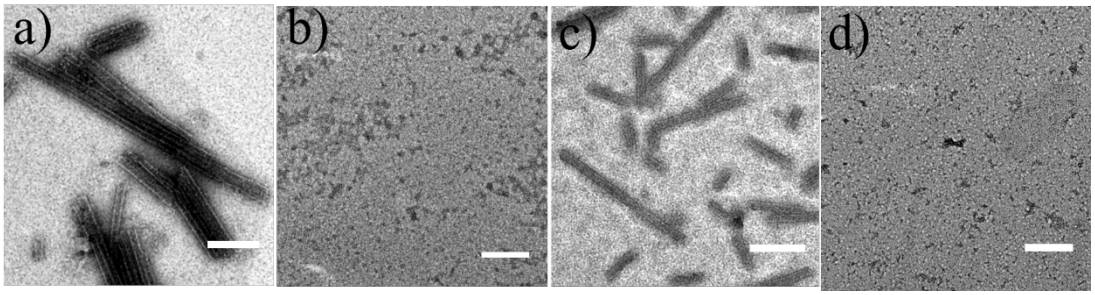

Fig. S10 TEM images of SBA/R3GN at 4°C: (a) after incubation 1 day with 100 mM $Ca(NO_3)_2$, (b) 1 day with 300 mM $Ca(NO_3)_2$, (c) 4 days with 300 mM $Ca(NO_3)_2$ and (d) 4 days with 500 mM $Ca(NO_3)_2$. Scale bar: 200 nm.

R3GN，**R3G** and **R5G** were synthesized and chracterized in our previous report. [2,7]

Reference

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