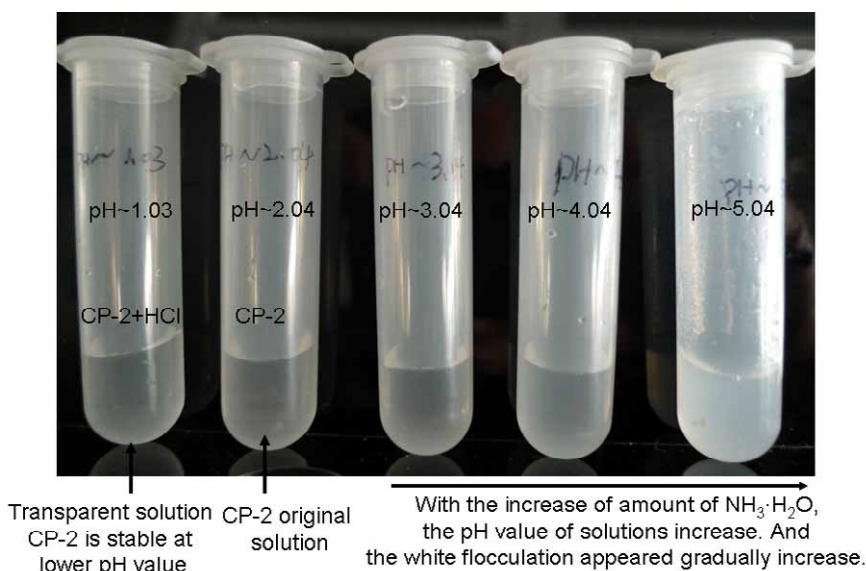


## An investigation of zirconium(IV)-glycine(CP-2) hybrid complex in bovine serum albumin protein matrix under varying conditions

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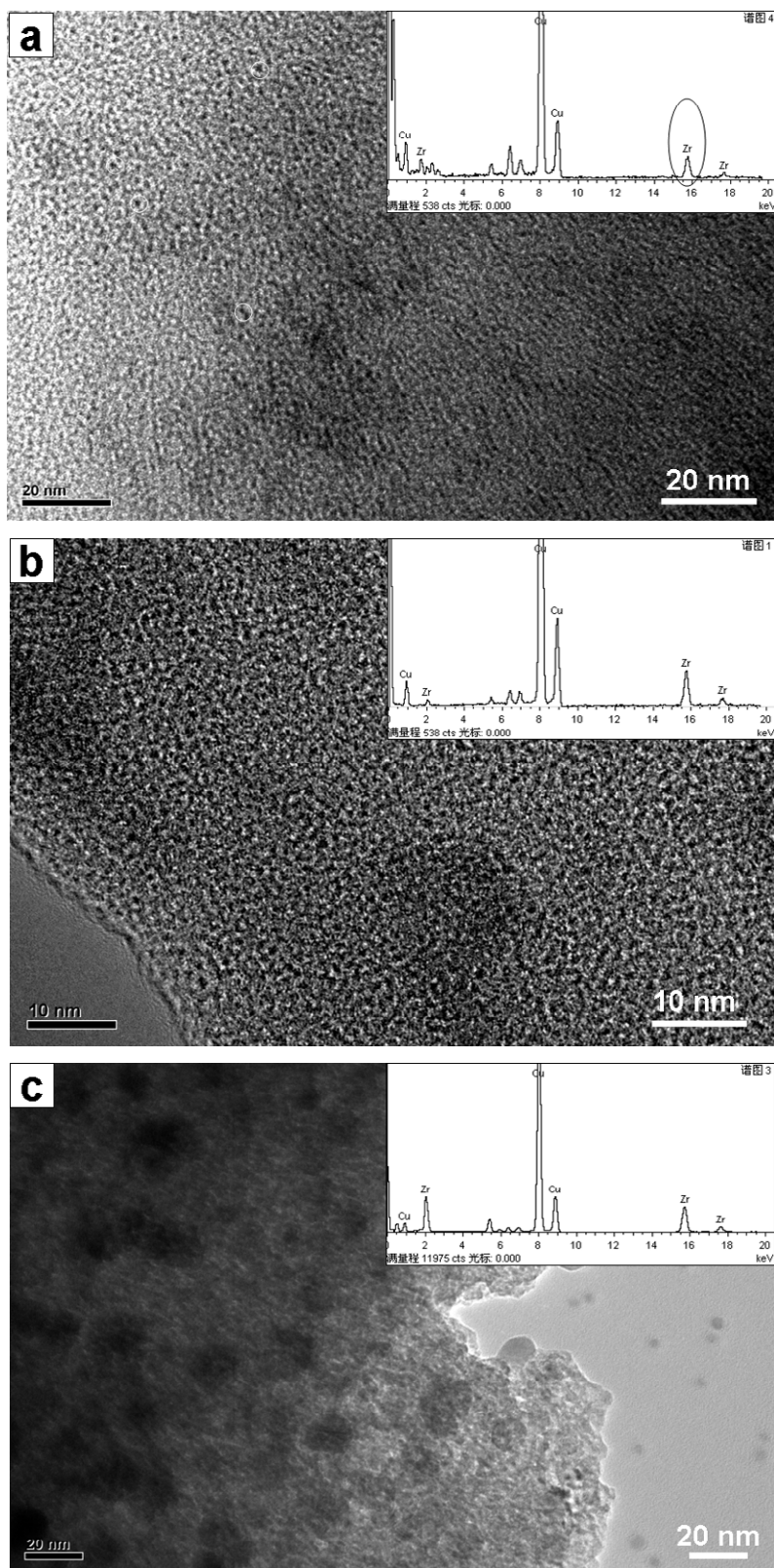
### *Characterization of CP-2 clusters solutions with different pH value*

The stability and morphologies variation of CP-2 clusters in aqueous solutions with different pH value were studied. Fig. S1 shows that the original CP-2 aqueous solution and the solution with lower pH value are transparent which indicates that the CP-2 clusters are highly soluble in water and stable in solution of low pH value. With increase of pH value in solutions by adding the ammonia water, the white floccules appeared and gradually increased indicating a phase transformation of CP-2 clusters triggered by alkali.



**Fig. S1** Photographs of CP-2 cluster aqueous solutions with different pH value. At the lower pH value and original pH value the solutions are transparent and stable. With the increase of pH value the white floccules appeared in solutions and gradually increased.

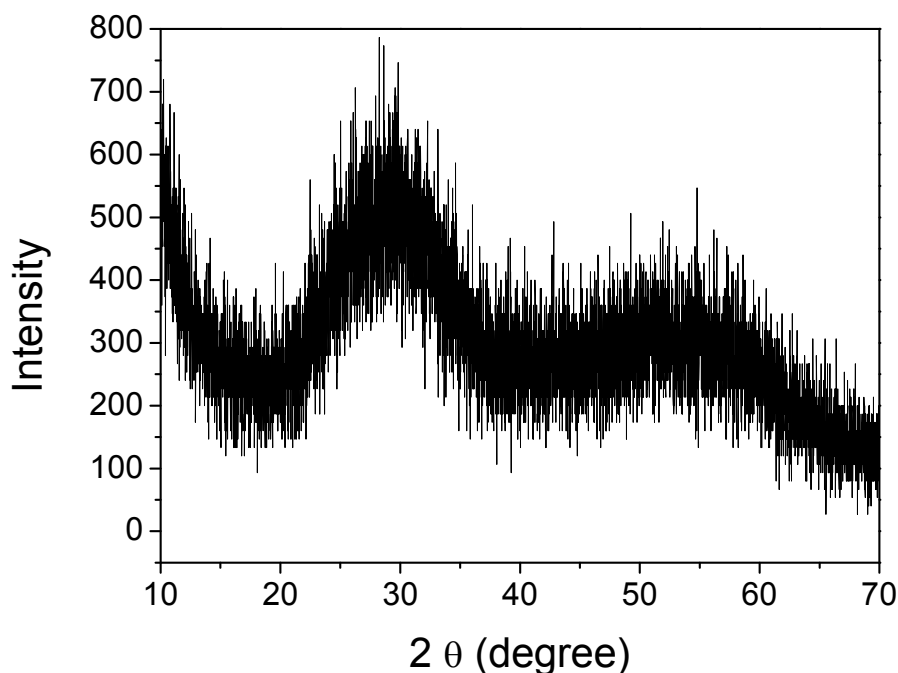
The morphologies and structures of CP-clusters in solutions with different pH value were characterized by TEM. As shown in Fig. S2a, the morphologies of CP-2 clusters in solution of pH ~ 1.0 were highly dispersed, similar to the original CP-2 cluster solution. This indicates that CP-2 clusters are mono-dispersed and stable in solution with lower pH value. The EDX analysis (inset in Fig. S2a) confirmed that those dispersed nanoparticles contain Zr element. With increase pH value of CP-2 solution to ~ 3 by adding ammonia water, the small particles began to aggregate with each other. As shown in Fig. S2b, the nanoparticles were closely packed together to form the bulk film. However, the bulk film was only aggregation of small particles not single crystal. It means that ammonia water promoted the random aggregation of small particles but can not induce the periodic arrange of small particles or further growth of CP-2 clusters. The EDX analysis (inset in Fig. S2b) showed that those aggregated nanoparticles contain Zr element indicating that the amorphous aggregates were formed initially from CP-2 clusters. When pH value of CP-2 cluster solution increased to ~ 5, the obvious white floccules were formed and the whole solution became opaque. The TEM image (Fig. S2c) shows that the bigger aggregation was generated by adding more ammonia water into CP-2 cluster solution but the aggregation was still amorphous. The EDX analysis (Fig. S2c) also confirmed that the aggregates were generated from CP-2 clusters. The HRTEM results indicated that CP-2 clusters are stable and mono-dispersed in solutions with lower pH value and trend to aggregate in solutions with higher pH value. The higher pH value induced the serious aggregation. All aggregates were amorphous.



**Fig. S2** (a), (b) and (c) HRTEM images of CP-2 clusters in solution of pH value ~1, 3, and 5 respectively. Insets are corresponding EDX results.

The white floccules generated via the interaction of CP-2 clusters in ammonia solution were

isolated by centrifugation for further characterization. The obtained floccules were washed by DIW for three times and dried at 50 °C in vacuum oven. The Powder X-ray diffraction (PXRD) pattern (Fig. S3) shows that the obtained white floccules are amorphous, which is consistent with the results by high resolution TEM observation.



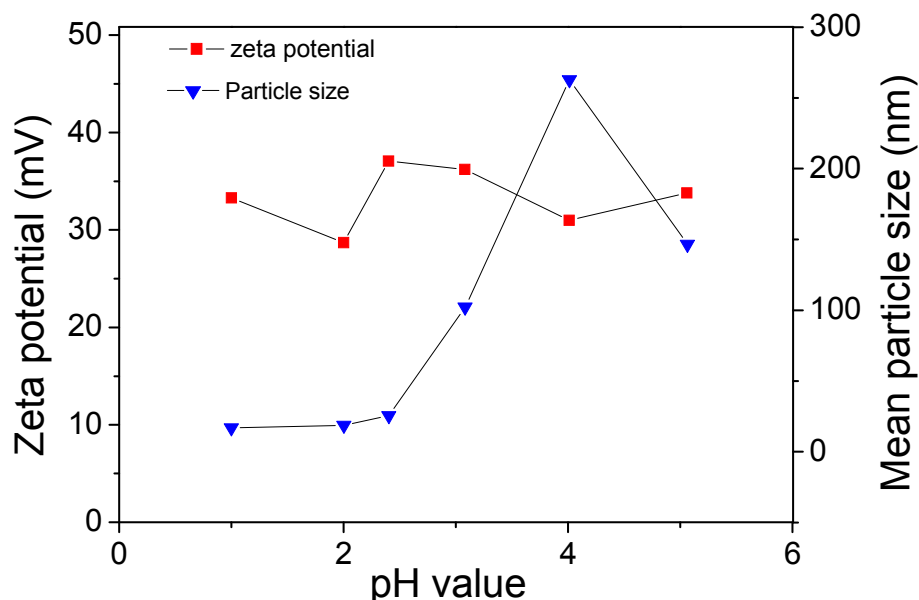
**Fig. S3** PXRD pattern of the white floccules yielded from interaction of CP-2 clusters in ammonia solution.

Elemental analysis (EA) result of the white floccules was shown Table S1. The content of elements in white floccules changed very much compared with that of original CP-2 clusters. In particular, the content of N and C decreased to a very low limit, indicating that the glycine ligands surrounded the hexanuclear zirconium (IV) cores was disconnected. Furthermore, the hexanuclear zirconium (IV) core collapsed as well. Instead, the OH<sup>-</sup> coordinated with Zr to form amorphous zirconium hydroxide complexes. Thus, the elemental content of the white floccules is similar to that of Zr(OH)<sub>4</sub>. Scheme S1 illustrates the transformation process from CP-2 to amorphous zirconium hydroxide complexes. It is considered that the OH<sup>-</sup> ligands attacked the

hexanuclear zirconium (IV) core and expelled the Gly<sup>-</sup> leaving the core. Meanwhile, the hexanuclear zirconium (IV) core was also destroyed by the OH<sup>-</sup> ligands by forming amorphous zirconium hydroxide complexes.

**Table S1** The comparison of elemental analysis of the white floccules, CP-2, and Zr(OH)<sub>4</sub>.

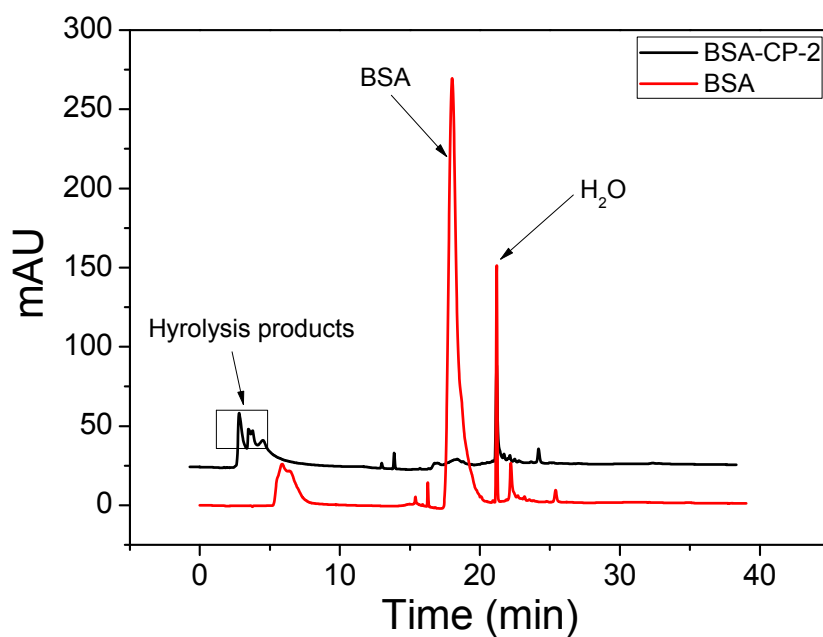
Elements	White floccules (Expt. wt. %)	CP-2 (Calcd. wt. %)	Zr(OH) <sub>4</sub> (Calcd. wt. %)
N	0.35	6.69	0
C	2.26	11.4	0
H	3.38	4.57	2.53
Zr	52.4	32.7	57.2



**Fig. S4** Influence of pH value on the zeta potential and mean particle size of BSA-CP-2 hybrid system. Left: The zeta potential of BSA-CP-2 hybrid system varied little with change of pH value. Right: Mean particle size of BSA-CP-2 hybrid system increased to 262.9 nm with increasing pH value to 4 and then decreased to 146.5 nm when the pH value was adjusted to 5.

High performance liquid chromatography (HPLC) analysis was carried out to investigate the hydrolysis of BSA assisted by CP-2. The testing samples are 10 mg/ml of pure BSA and 10 mg/ml of BSA-CP-2 hybrid which are both stored under ambient conditions for 30 days. Before

test, the 0.1M of EDTA was added into the BSA-CP-2 hybrid solution to chelated the Zr(IV). The HPLC results were plotted in Fig. S5 showing that the in the solution of BSA-CP-2 hybrid a little BSA was retained and the assisted role of CP-2 in the hydrolysis of BSA.



**Fig. S5** HPCL chromatograms of BSA and BSA-CP-2.