

Fig. 1 Excitation (red) and emission (blue and purple) fluorescence spectra of  $\text{RuL}_3$  ( $2 \mu\text{M}$ ) in redistilled water at 298 K. The half-frequency peak of the excitation spectrum is marked.

The aggregation of HA induced by  $\text{RuL}_3$  was investigated by UV-vis spectroscopy following the reported methods.<sup>4,6,12</sup> On the addition of  $\text{RuL}_3$ , a solution of HA displayed an obvious Tyndall effect at a concentration lower than its critical aggregation concentration (CAC). As shown in Fig. 2, the optical transmittance of HA at 650 nm ( $T_{650}\%$ ) gradually decreased with the addition of  $\text{RuL}_3$  (Fig. 2a). An inflection point at  $22 \mu\text{M}$  was observed on a plot of  $T_{650}\%$  versus the concentration of  $\text{RuL}_3$  (Fig. 2b), which referred to a CAC value induced by  $\text{RuL}_3$ . Moreover, the suitable mixing ratio between HA and  $\text{RuL}_3$  was also investigated. On the addition of HA to a  $\text{RuL}_3$  solution ( $[\text{RuL}_3] = 40 \mu\text{M}$ ),  $T_{650}\%$  of the HA- $\text{RuL}_3$  solution first decreased rapidly and then increased gradually. The minimum value was found at  $[\text{HA}] = 0.5 \mu\text{M}$ . Therefore, the solution of the assembly was prepared with such a composition ( $\text{H}_{0.5}\text{R}_{40}:[\text{RuL}_3] = 40 \mu\text{M}$ ,  $[\text{HA}] = 0.5 \mu\text{M}$ ) in the subsequent experiments.

To investigate the structural details of the assembly, transmission electron microscopy (TEM) and dynamic light

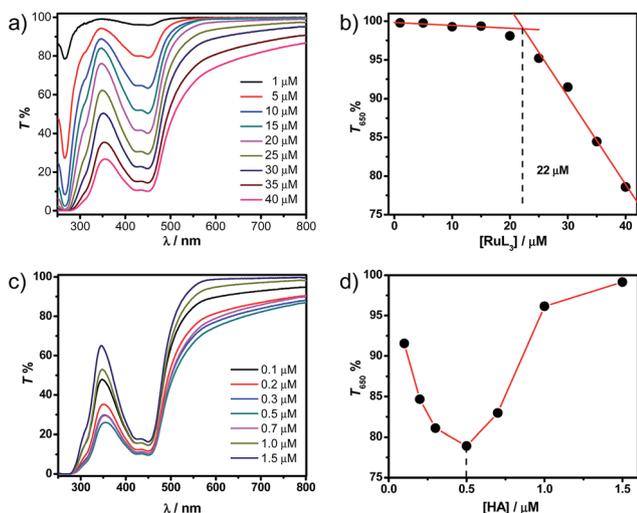


Fig. 2 (a) Optical transmittance of aqueous solutions containing HA ( $0.5 \mu\text{M}$ ) and  $\text{RuL}_3$  ( $1\text{--}40 \mu\text{M}$ ) at  $25^\circ\text{C}$ . (b) Dependence of  $T_{650}\%$  versus the concentration of  $\text{RuL}_3$ . (c) Optical transmittance of aqueous solutions of HA at different concentrations ( $0.1\text{--}1.5 \mu\text{M}$ ) in the presence of  $\text{RuL}_3$  ( $40 \mu\text{M}$ ) at  $25^\circ\text{C}$ . (d) Dependence of  $T_{650}\%$  on the concentration of HA in the presence of  $\text{RuL}_3$  ( $40 \mu\text{M}$ ).

scattering (DLS) were carried out. As shown in Fig. 3, the  $\text{H}_{0.5}\text{R}_{40}$  assembly existed as solid spherical particles with an average diameter of *ca.* 400 nm and the diameter measured by DLS was *ca.* 600 nm. This difference could be due to dryness in preparing the TEM sample. The zeta ( $\zeta$ ) potential of  $\text{H}_{0.5}\text{R}_{40}$  ( $-11.40 \text{ mV}$ ) showed that the surface of the assembly was mainly covered by HA. According to previous reports about assemblies constructed from charged macrocycles and macromolecules,<sup>4,6,12</sup> we proposed that  $\text{H}_{0.5}\text{R}_{40}$  might have a “plum pudding model” structure, where cationic CDs were buried in the chains of HA to form multiple layers.

Before the investigation of the response to HAase of  $\text{H}_{0.5}\text{R}_{40}$ , the stability of  $\text{H}_{0.5}\text{R}_{40}$  was investigated by UV-vis spectroscopy and DLS (Fig. 3c and d). The transmittances and diameters of  $\text{H}_{0.5}\text{R}_{40}$  were nearly unchanged for at least 5 h, which indicates that such assemblies were sufficiently stable in an aqueous solution. The response to HAase was investigated by detecting the transmittance of  $\text{H}_{0.5}\text{R}_{40}$  solution on the addition of the enzyme (see ESI, Fig. S9†). In the presence of HAase, the transmittance of  $\text{H}_{0.5}\text{R}_{40}$  (*e.g.*, at 650 nm) increased gradually, which might be due to the degradation of large assemblies.<sup>4</sup> In addition, a positive correlation between the concentration of HAase and the rate of increase in transmittance was also observed (see Fig. 4).

To obtain more visual information on the formation and degradation of the assembly, a green laser (532 nm) was used to investigate the HAase-induced degradation of the  $\text{H}_{0.5}\text{R}_{40}$  assembly.<sup>13</sup> Samples containing  $\text{H}_{0.5}\text{R}_{40}$ ,  $\text{H}_{0.5}\text{R}_{40}$  + HAase (mixed for 7 h) and  $\text{RuL}_3$  were irradiated with the green laser. As shown in Fig. 5, an obvious color distinction was found under irradiation with the 532 nm laser: that is,  $\text{H}_{0.5}\text{R}_{40}$  was green,  $\text{H}_{0.5}\text{R}_{40}$  + HAase was orange-yellow, and  $\text{RuL}_3$  was red.

To understand this color change, the fluorescence spectra of  $\text{H}_{0.5}\text{R}_{40}$  in the absence/presence of HAase were obtained, and the wavelengths and intensities of the emission peaks were almost the same when the excitation wavelength was set to 532 nm (see ESI, Fig. S10†). This result implied that such a color change was not related to a change in emission intensity.

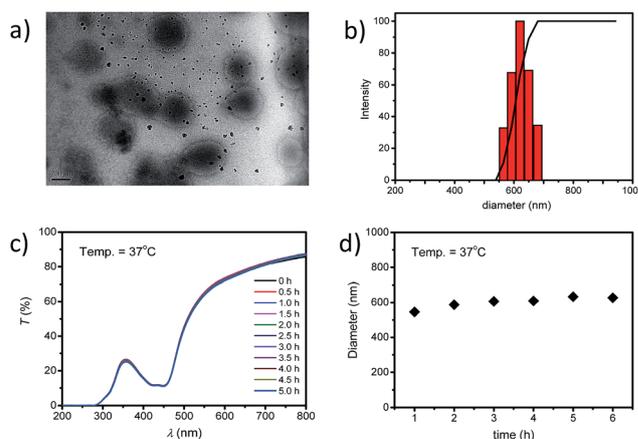


Fig. 3 TEM image (a) and DLS results (b) of  $\text{H}_{0.5}\text{R}_{40}$  and time-dependent UV-vis transmittance (c) and particle diameter from DLS (d). The black bar in (a) represents 200 nm.  $[\text{RuL}_3] = 40 \mu\text{M}$ ,  $[\text{HA}] = 0.5 \mu\text{M}$ .

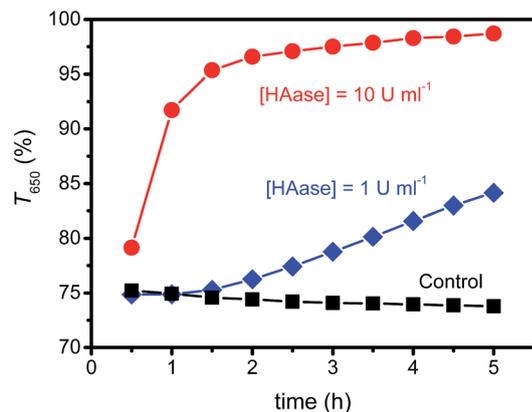


Fig. 4 Time-dependent changes in transmittance of  $H_{0.5}R_{40}$  in the absence (control) and presence of HAase.  $[RuL_3] = 40 \mu M$ ,  $[HA] = 0.5 \mu M$ ,  $T = 37^\circ C$ .

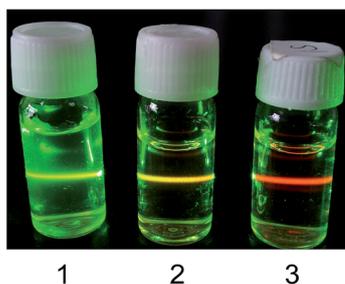


Fig. 5  $H_{0.5}R_{40}$  assemblies irradiated with 532 nm laser. Lane 1:  $H_{0.5}R_{40}$ ; Lane 2:  $H_{0.5}R_{40} + HAase$  (7 h); Lane 3:  $RuL_3$  only. Conditions:  $[RuL_3] = 40 \mu M$ ,  $[HA] = 0.5 \mu M$ ,  $[HAase] = 10 U mL^{-1}$ .

Another possible explanation is a change in the intensity of scattered light. Without HAase,  $H_{0.5}R_{40}$  displayed an intense green light, which was due to the strong Tyndall effect of  $H_{0.5}R_{40}$  nanoparticles. In the presence of HAase, the degradation of HA led to the disassembly of  $H_{0.5}R_{40}$  and weakening of the Tyndall effect. This proposition could be supported by the transmittance change shown in Fig. 4.

To investigate the relationship between the color change and HAase-induced degradation of  $H_{0.5}R_{40}$ , the dependence of the color change on time and HAase concentration was investigated. On placing a  $H_{0.5}R_{40}$  solution with HAase ( $10 U mL^{-1}$ ) in

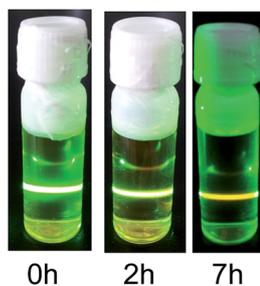


Fig. 6 Time-dependent color changes of  $H_{0.5}R_{40}$  solution with a green laser. Conditions:  $[RuL_3] = 40 \mu M$ ,  $[HA] = 0.5 \mu M$ ,  $[HAase] = 10 U mL^{-1}$ .

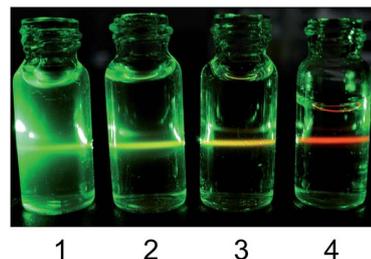


Fig. 7 HAase concentration-dependent color changes of  $H_{0.5}R_{40}$  solution with a green laser. Lane 1:  $[HAase] = 0.1 U mL^{-1}$ ; Lane 2:  $[HAase] = 1 U mL^{-1}$ ; Lane 3:  $[HAase] = 10 U mL^{-1}$ ; Lane 4:  $RuL_3$  only. Conditions:  $[RuL_3] = 40 \mu M$ ,  $[HA] = 0.5 \mu M$ , time = 7 h.

a sealed glass bottle in a  $37^\circ C$  water bath, a clear color change of a light beam was observed. As shown in Fig. 6, the color of the light beam gradually changed from green to orange-yellow. On the other hand, a comparison of  $H_{0.5}R_{40}$  solutions containing HAase at different concentrations ( $0.1$ ,  $1$ , and  $10 U mL^{-1}$ ) showed that a higher concentration of HAase induced a more significant color change under the same conditions (Fig. 7). Therefore, we deduced that HAase activity could be detected by a laser colorimetric assay with a  $H_{0.5}R_{40}$  assembly.

## Conclusions

In summary, a supramolecular assembly that was responsive to HAase was constructed from a cationic  $Ru(II)/CD$  complex and HA. The addition of HAase could induce detectable color changes in the solution of the assembly under irradiation of a green laser, which could be due to the relative intensity of scattered light and fluorescence emission. Therefore, the assembly could be used as a colorimetric sensor of HAase activity. Furthermore, other anionic macromolecules, such as specific peptides and nucleic acids, would also form assemblies with  $RuL_3$ , and the resultant assemblies could be used as colorimetric sensors for specific enzymes.

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