Supporting Information

Sensitive fluorescence detection of lysozyme using a tris(bipyridine)ruthenium(II) complex containing multiple cyclodextrins

Fan Zhang, Ying-Ying Zhao, Hong Chen, Xiu-Hua Wang, Qiong Chen and Pin-Gang He*

Department of Chemistry, East China Normal University, Shanghai 200241, P. R. China

Experimental Section:

Apparatus

Fluorescence was recorded on a F-7000 FL instrument (Hitachi High-Technologies Co., Japan).

Chemicals and Materials

Lysozyme, thrombin, bovine serum albumin (BSA) and human immunoglobulin G (IgG) were obtained from Dingguo Biotechnology Inc. (Beijing, China). Adamantane and acetonitrile were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). The sequence of the ssDNA (lysozyme-aptamer), 5′-ATC AGG GCT AAA GAG TGC AGA GTT ACT TAG-3′, was purchased from Sangon Biotech Co., Ltd. (Shanghai, China) and dissolved in deionized water. Adamantane solutions were prepared in acetonitrile. Deionized water (> 18.3 MΩ·cm) was produced from a Millipore Milli-Q water purification system.

Fluorescence performances of metallocyclodextrins with ssDNA

All of the metallocyclodextrin solutions were prepared in deionized water. The fluorescence determination for the impact of ssDNA on metallocyclodextrins was as follows: metallocyclodextrins and equal ssDNA were incubated for 2 h before the measurement. Fluorescence spectra were measured at 25 °C with excitation wavelength of 450 nm and slit width of 10 nm (Unless stated, the parameters of fluorescence spectra are consistent).

Fluorescence performances of the addition of ssDNA into the mixture of metallocyclodextrins and adamantane

Fluorescence spectra of the mixed solution containing metallocyclodextrins and adamantane incubated for 2 h were carried out at 25 °C with an excitation wavelength of 450 nm. An equal amount of ssDNA was added to each of the mixed solutions and incubated at 37 °C for 2 h, and the fluorescence measurement was investigated at the same experimental conditions.

Job-plot for the complex stoichiometry analysis

The total concentration of 6CD-Ru and ssDNA was kept constant during the experiment (1×10^{-5} M) of each compound in different ratios. The mixture was incubated at 37 °C for 2 h before the fluorescence measurement. The solution of 6CD-Ru of the same concentration without ssDNA was also prepared and measured.

Detection of lysozyme

For the quantitative detection, lysozyme at various concentrations was incubated in the binding buffer (0.56 mM Tris-HCl, 4.7 mM NaCl and 0.14 mM KCl, pH=6.5) containing 0.5 μM 6CD-Ru and 0.5 μM lysozyme-aptamer at 37 °C for 2 h. The fluorescence behavior was then monitored.

The selectivity of the aptasensing was investigated by repeating the experiment under the same conditions and using some typical interference proteins (100 nM), including thrombin, BSA and IgG.

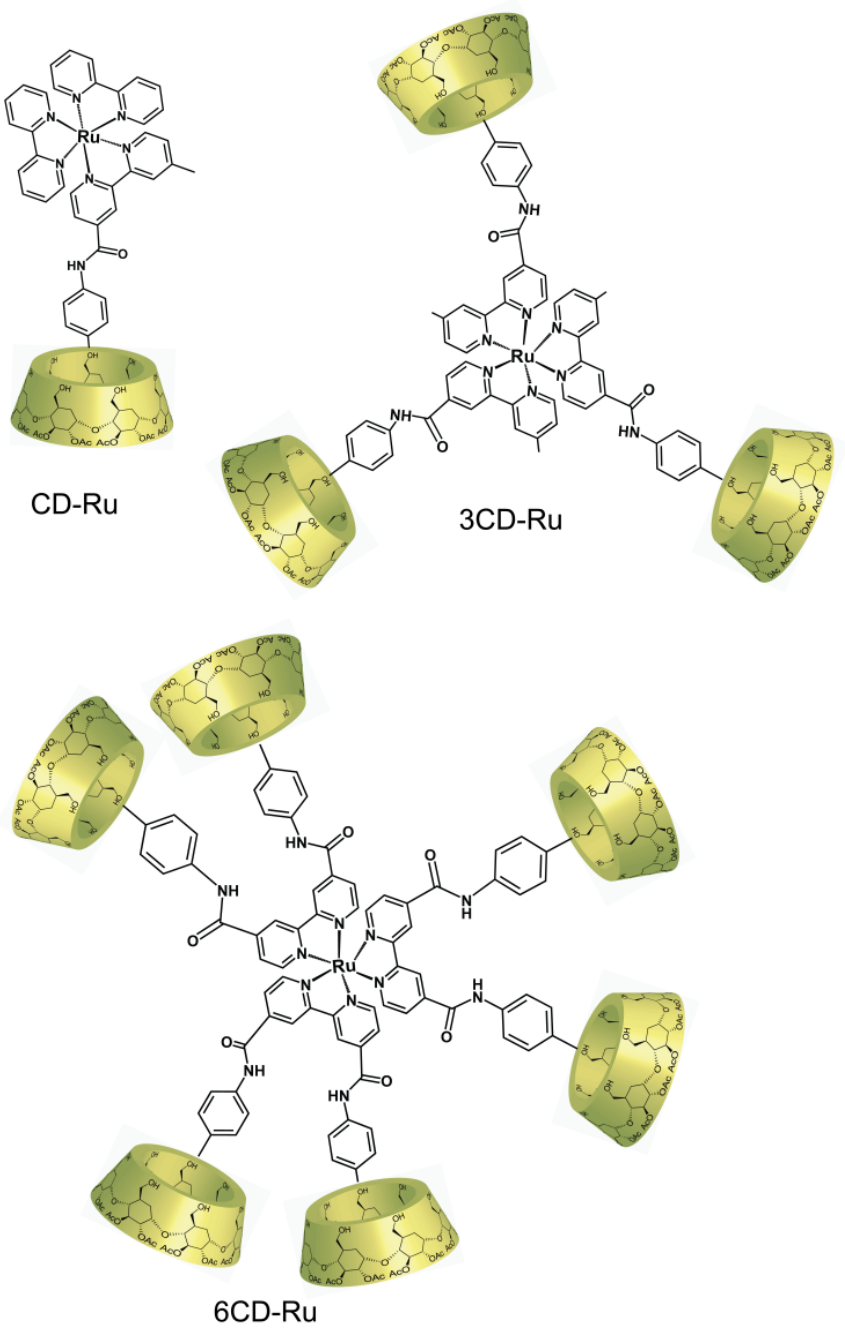

Fig. S1 Structures of the new series of metallocyclodextrins including CD-Ru, 3CD-Ru and 6CD-Ru.[1]

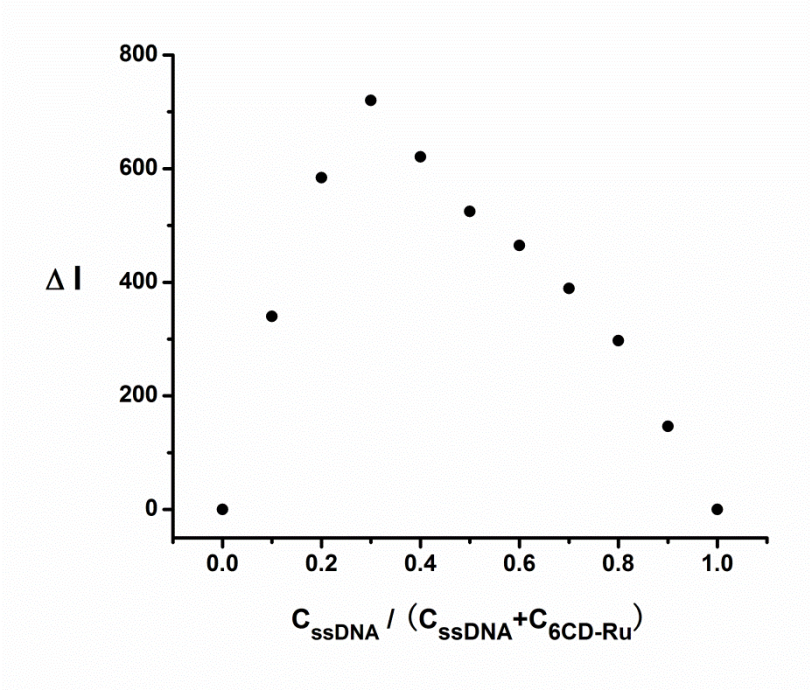

Fig. S2 Job-plot for the binding of ssDNA/aptamer and 6CD-Ru in the aqueous solution. The total concentration of 6CD-Ru and ssDNA/aptamer was kept constant (1×10^{-5} M) during the experiments for each compound in different ratios. ΔI is the difference between the measured emission intensity and the intensity for the solution of 6CD-Ru for the same concentration without ssDNA/aptamer.

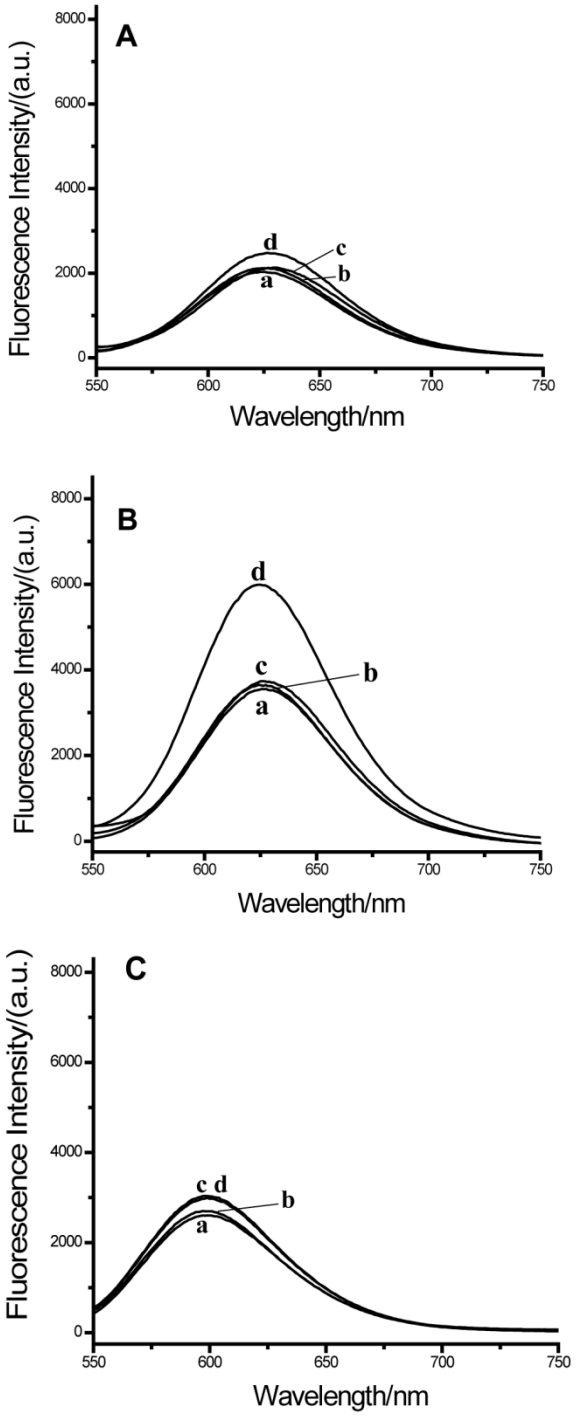

Fig. S3 Fluorescence spectra of (A): (a) 5 μM CD-Ru, (b) 5 μM CD-Ru with 5 μM adamantane, (c) 5 μM CD-Ru with 5 μM adamantane and 5 μM ssDNA, (d) 5 μM CD-Ru with 5 μM ssDNA; (B): (a) 5 μM 3CD-Ru, (b) 5 μM 3CD-Ru with 15 μM adamantane, (c) 5 μM 3CD-Ru with 15 μM adamantane and 5 μM ssDNA, (d) 5 μM 3CD-Ru with 5 μM ssDNA; (C): (a) 5 μM $Ru(bpy)_3^{2+}$, (b) 5 μM $Ru(bpy)_3^{2+}$ with 5 μM adamantane, (c) 5 μM $Ru(bpy)_3^{2+}$ with 5 μM adamantane and 5 μM ssDNA, (d) 5 μM $Ru(bpy)_3^{2+}$ with 5 μM ssDNA

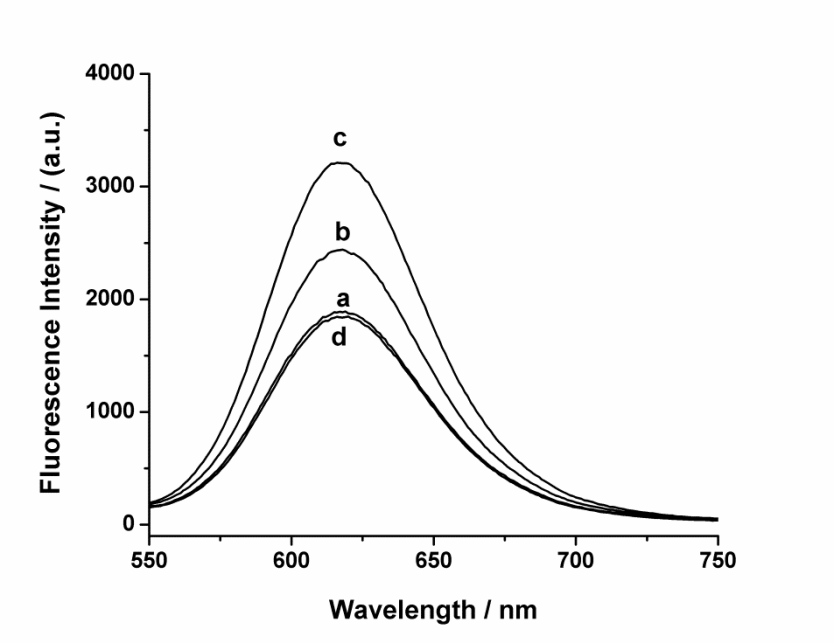

Fig. S4 Fluorescence spectra of (a) 0.5 μM 6CD-Ru; (b) 0.5 μM 6CD-Ru with 0.5 μM lysozyme-aptamer and 50 nM lysozyme; (c) 0.5 μM 6CD-Ru with 0.5 μM lysozyme-aptamer; (d) 0.5 μM 6CD-Ru with 0.5 μM lysozyme.

Reference

1 Y. T. Qi, X. H. Wang, H. Chen, J. Tang, F. Yang and P. G. He, *Supramolecular* Chemistry, 2015, **27**, 44-51.