Electronic Supplementary Material (ESI) for RSC Advances.

Specific Identification of Human Transferrin Conformations by Using Cyanine Dye Supramolecular Assembly

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Abstract: A new method to recognize human transferrin (***Tf***) conformation was developed by cyanine dye supramolecular assembly. We achieved to detect the open conformation of ***Tf*** (***apo-Tf***) in sub-micromolar level against the closed one (***holo-Tf***). It as a protein conformatinal probe can also monitor the transformation between the two conformations of ***Tf***.

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Materials and sample preparation

The cyanine dye (***MTC***, 3, 3' di (3-sulfopropyl) - 4, 5, 4', 5'-dibenzo- 9-methyl - thiacarbocyanine triethylam - monium salt) is the same as the used previously.[1] Human serum transferrin (***hTf***), human apo-transferrin (***apo-Tf***) and human holo-transferrin (***holo-Tf***), collectively referred to as ***Tfs***, were obtained from Sigma (T3309, T2252 and T0665, respectively) as lyophilized powders with a molecular mass of 78~80 kDa and used without further purification. Analytical grade methanol and $FeCl_3$ were purchased from Beijing Chem. Co. Ultrapure water was from a Milli-Q (Millipore) system.

The stock solution of ***MTC*** was prepared by dissolving ***MTC*** in methanol directly. The stock solutions of ***Tfs*** were prepared by dissolving a certain amount of chemicals directly in Tris-HCl buffer solution (10 mM, pH 6.8). $FeCl_3$ was dissolved directly in Tris-HCl buffer. Mixed ***apo-Tf*** and ***holo-Tf*** at different ratios were kept at the total protein concentration of 1 μM. The measured sample was prepared by adding ***Tfs*** solution into ***MTC*** solution at different ratios following by intense stirring for 1-2 s and then diluted to certain volume by Tris-HCl. The samples contained 5% methanol (*v/v*) and then were kept in darkness at 35℃ for more than 2 h prior to measurement.

Experimental section

Given the experimental error in absorbance, all experiments were repeated with at least three independent preparations on different days to ensure the results are repeatable. UV-vis absorption spectra were recorded on UV-1601PC spectrophotometer. The fluorescence spectra were taken on a Hitachi model F-7000 spectrofluorometer for aqueous solutions. Samples were detected at *Ex*=280 nm, Slit widths: 5×5 nm and Scanning speed: 2400 nm/min. All the tests were conducted in 10 mm quartz cells. Circular dichroism spectra were carried out on a Jasco-815 spectrofluorometer. The scanning range is selected at 190-250 nm for secondary structures and 245-650 nm for ***holo-Tf*** characteristic feature, respectively.[2] The spectra were accumulated for three times with a band-width of 2.0 nm and a resolution of 0.5 nm. The cell path-length is 1 mm for the wavelength range 190-250 nm and 10 mm for 245-650 nm.

The absorption spectra of MTC with different conformational Tf and the dectection limit of apo-Tf

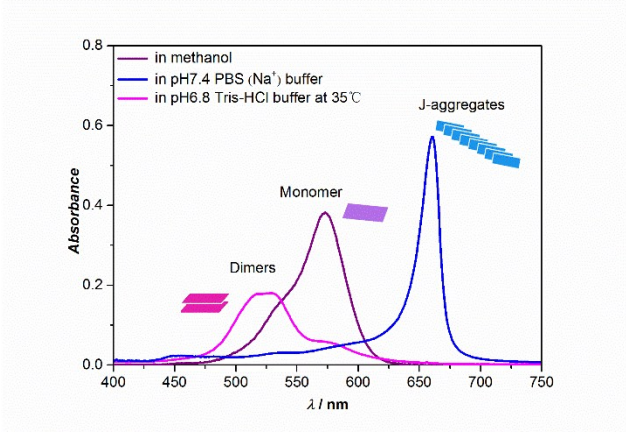

Fig. S1† The absorption spectra of cyanine dye ***MTC*** in different solutions.

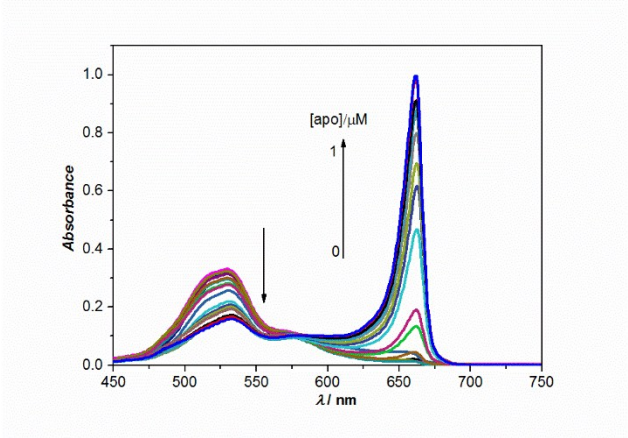

Fig. S2† The absorption spectra of ***MTC*** (5 μM) with ***apo-Tf*** different concentrations: 0, 0.001, 0.002, 0.003, 0.004, 0.005, 0.0075, 0.01, 0.02, 0.04, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.5, 1μM.

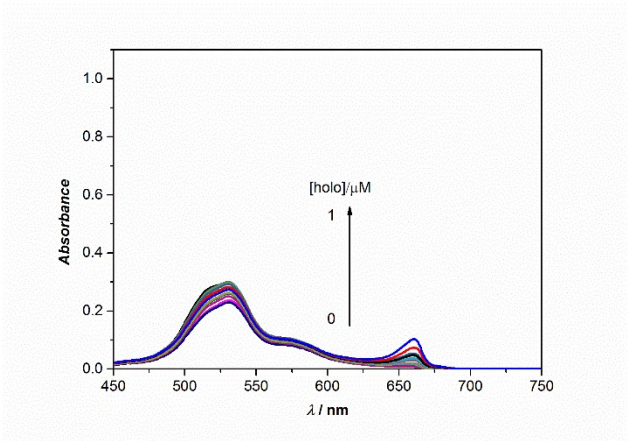

Fig. S3† The absorption spectra of ***MTC*** (5 μM) with ***holo-Tf*** different concentrations: 0, 0.001, 0.002, 0.003, 0.004, 0.0075, 0.01, 0.04, 0.05, 0.1, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 1 μM.

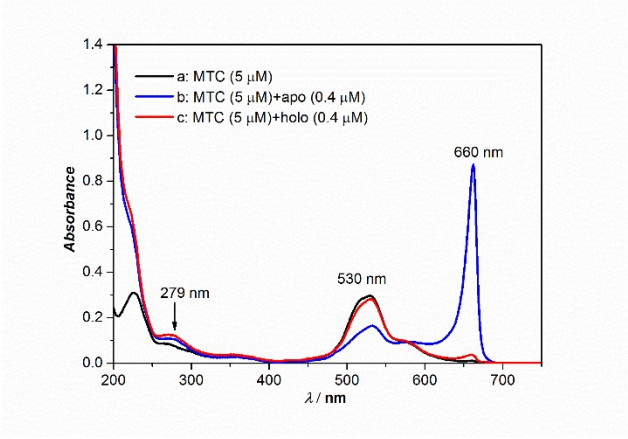

Fig. S4† The complete absorption spectrum of ***MTC*** (5 μM) in the absence and presence of ***apo-Tf*** (0.4 μM) or ***holo-Tf*** (0.4 μM), represented the all titration experiments.

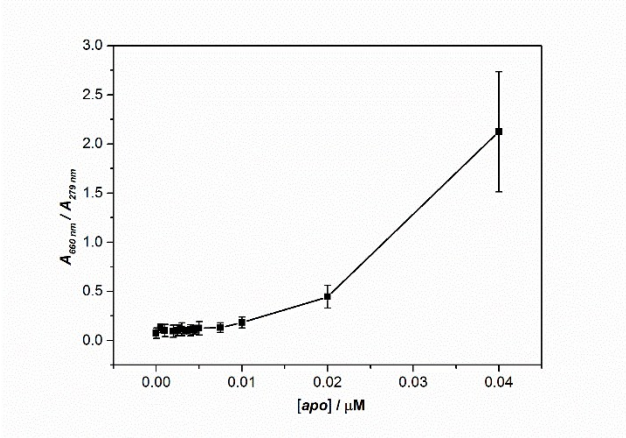

Fig. S5† The detection limit of ***apo-Tf*** by ***MTC*** J-aggregates: plots of absorption change in ***MTC*** J-aggregates (corrected by protein background absorption signal) against ***apo-Tf*** concentrations from 1 to 40 nM; Error bars are plotted as the standard deviation over three replicates.

The circular dichroism spectra and the detection limit of holo-Tf

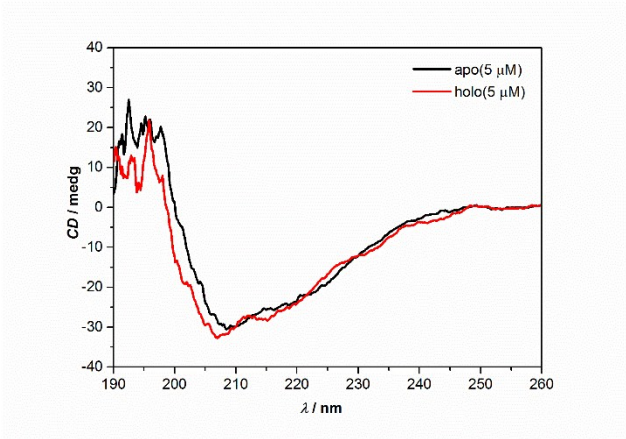

Fig. S6† The ***CD*** spectra of ***apo-Tf*** (5 μM) and ***holo-Tf*** (5 μM) at far ***UV*** region, respectively.

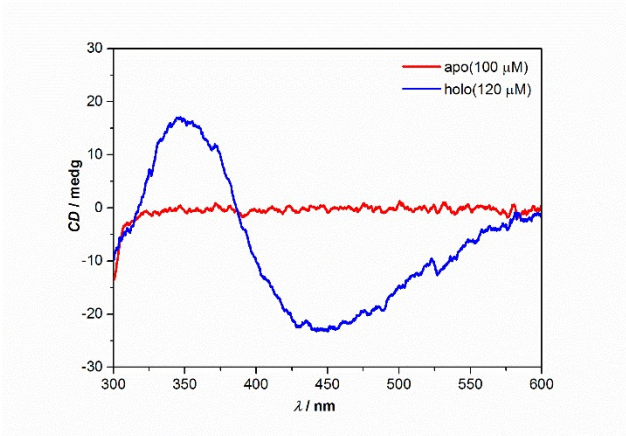

Fig. S7† The ***CD*** spectra of ***apo-Tf*** (100 μM) and ***holo-Tf*** (120 μM) at visible region, respectively.

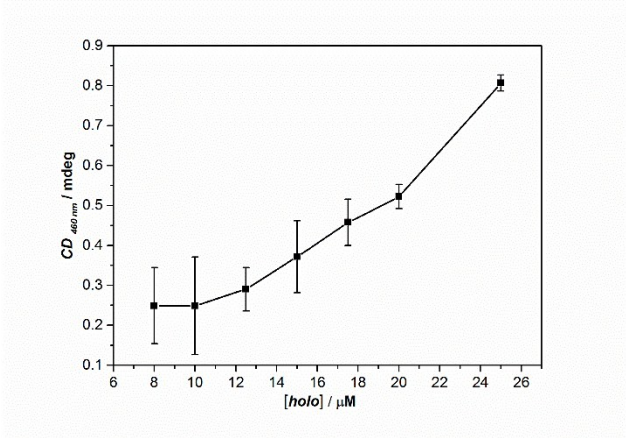

Fig. S8† The detection limit of ***holo-Tf*** at 460 nm by ***CD*** measurement; Error bars are plotted as the standard deviation over three replicates.

The absorption spectra of MTC with Tf conformation mixture and the detection limit of apo-Tf

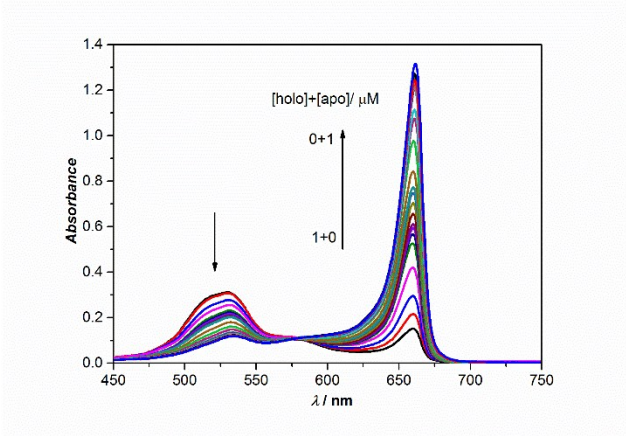

Fig. S9† The absorption spectra of ***MTC*** (5 μM) with mixed ***apo-*** and ***holo-Tf***, where [***apo-Tf***] were from 0 to 1 μM and [***holo-Tf***] on the contrary.

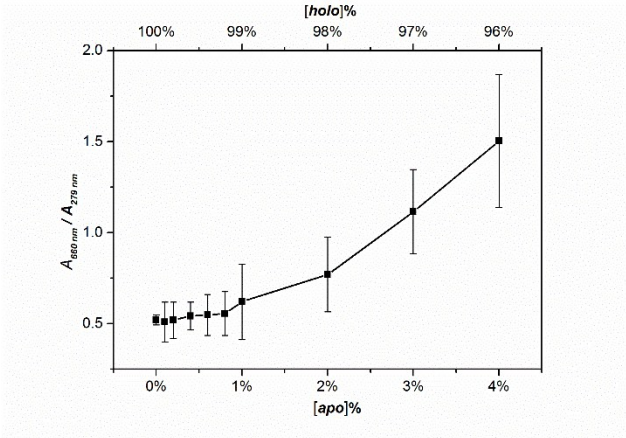

Fig. S10† The detection limit of ***apo-Tf*** by ***MTC*** J-aggregates in the mixed ***apo-*** and ***holo-Tf***: job plots of absorption change in ***MTC*** J-aggregates (corrected by protein background absorption signal) against ***apo-Tf*** percentage from 0% to 4% (***holo-hTf*** from 100% to 96%); Error bars are plotted as the standard deviation over three replicates.

The absorption spectra of MTC with natural hTf

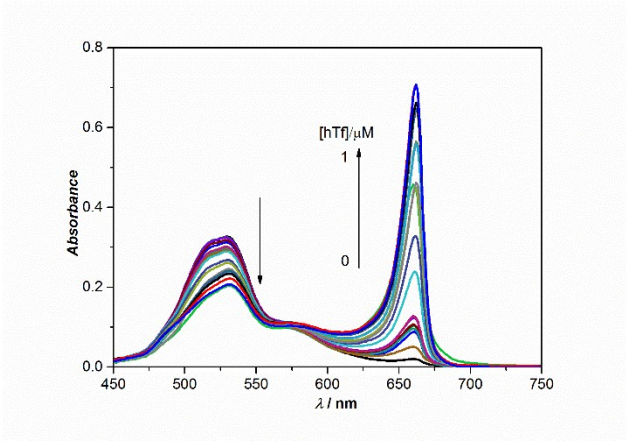

Fig. S11† The absorption spectra of ***MTC*** (5 μM) with ***hTf*** different concentrations: 0, 0.001, 0.002, 0.003, 0.004, 0.0075, 0.01, 0.02, 0.05, 0.1, 0.2, 0.25, 0.3, 0.4, 0.5, 0.75, 1 μM

The fluorescence spectra of apo-Tf with Fe3+ and monitored by MTC assembly

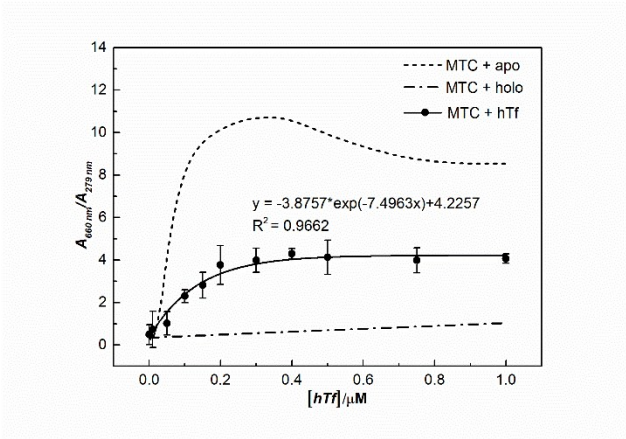

Fig. S12† Job plots of absorption change in ***MTC*** (5 μM) J-aggregates (corrected by the total protein concentration) against ***hTf*** concentrations from 0 to 1 μM, respectively; Error bars are plotted as the standard deviation over three replicates.

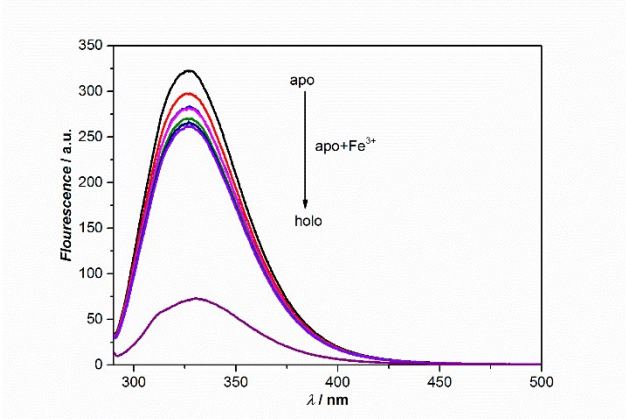

Fig. S13† The fluorescence spectra of ***apo-Tf*** (1 μM) with increasing Fe^{3+} concentrations: 0, 0.5, 1, 2, 4, 6, 8, 10, 12, 15 μM. The purple line is ***holo-Tf*** (1 μM) alone, λ_{ex}=280 nm.

References

[1] a) Y. Z. Zhang, J. F. Xiang, Y. L. Tang, G. Z. Xu and W. P. Yan. *Dyes Pigm*., 2008, **76**, 88; b) X. F. Zhang, L. Chen, Q. F. Yang, Q. Li, X. R. Sun, H. B. Chen, G. Yang and Y. L. Tang. *Colloids Surf., A*., 2015, **469**, 187.

[2] a) S. Tang, R. MacColl and P. J. Parsons. *J. Inorg. Biochem*., 1995, **60**, 175–185; b) H. Y. Du, J. F. Xiang, Y. Z. Zhang, Y. L. Tang and G. Z. Xu. *J. Photoch. Photobio*., 2008, **195**, 127-134; c) H.Y. Du, J. F. Xiang, Y. Z. Zhang and Y. L. Tang. *Bioorg. Med. Chem. Lett*., 2007, **17**, 1701–1704.