

Controversial Role of Transferrin In The Transport of Ruthenium Anticancer Drugs

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Supplementary Material

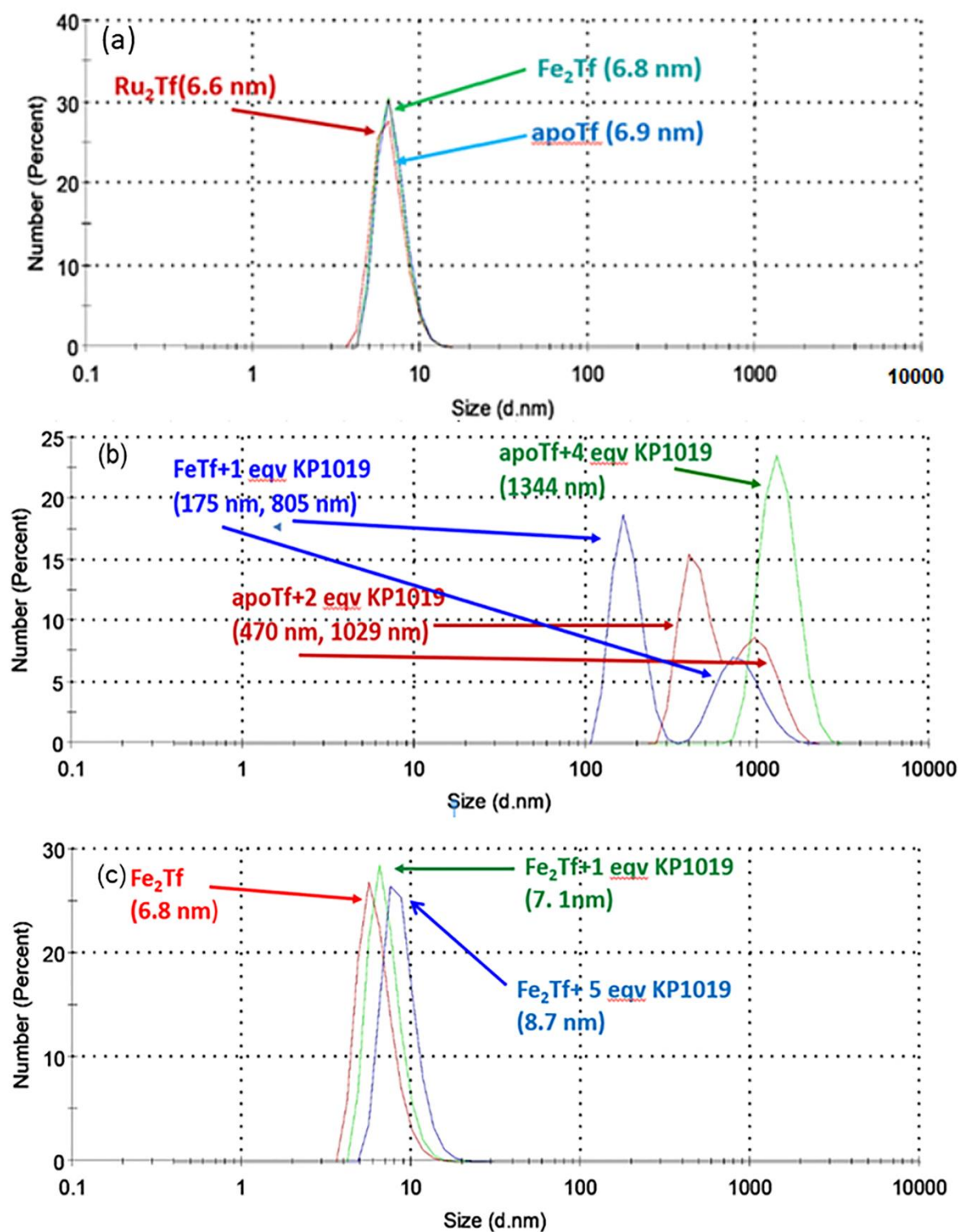


Figure S1. Typical results of particle size distribution measurements by DLS at 298 K. Reaction conditions correspond to those in Tables 1 and 2 (main text): (a) apoTf vs. A1 and A2; (b) A5, A6, and A7; and (c) Fe_2Tf vs. A8 and A9. The reaction mixtures were diluted 10-fold with the binding buffer (20 mM HEPES, 25 mM NaHCO_3 , 140 mM NaCl, pH 7.4) immediately before the experiments.

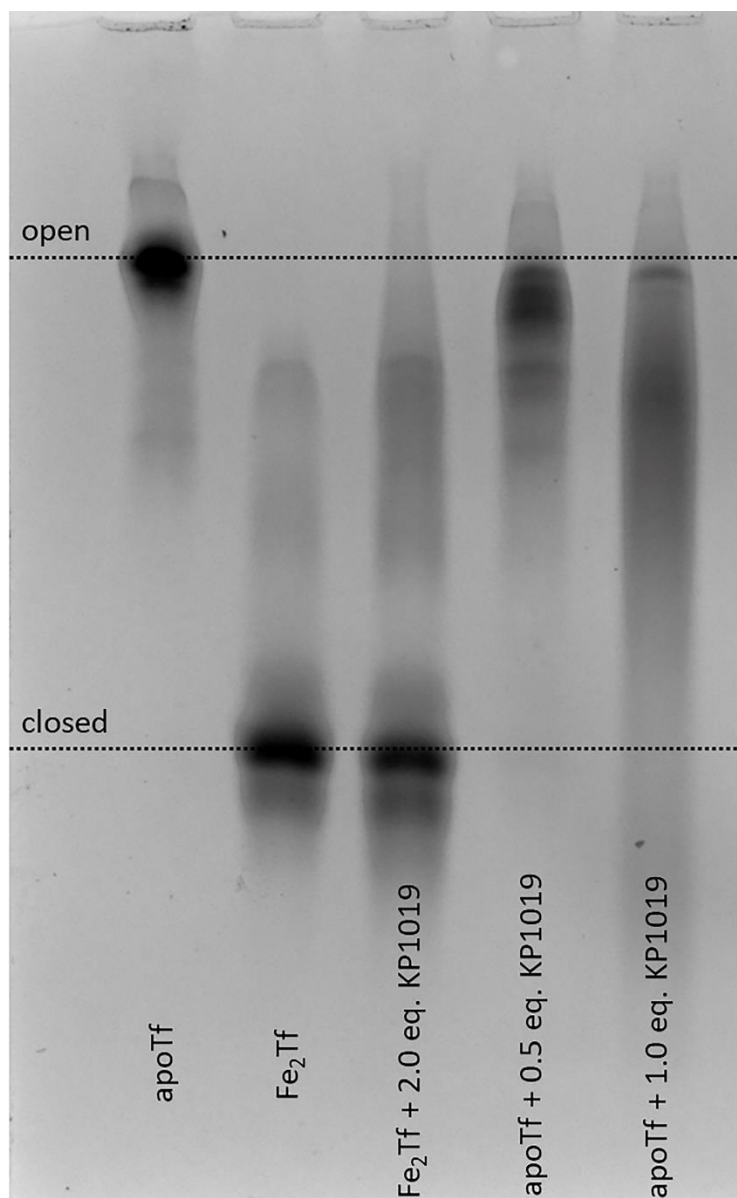


Figure S2. Typical results of urea-PAGE gel electrophoresis of the reaction mixtures containing 30 μM of apoTf or Fe₂Tf in the presence or absence of 15–120 μM of KP1019 in the binding buffer (20 mM HEPES + 25 mM NaHCO₃ + 140 mM NaCl, pH = 7.4) after 24 h reaction at 310 K. All samples were diluted 6-fold with the binding buffer before loading into the gel, and gel electrophoresis was performed as described previously [29].

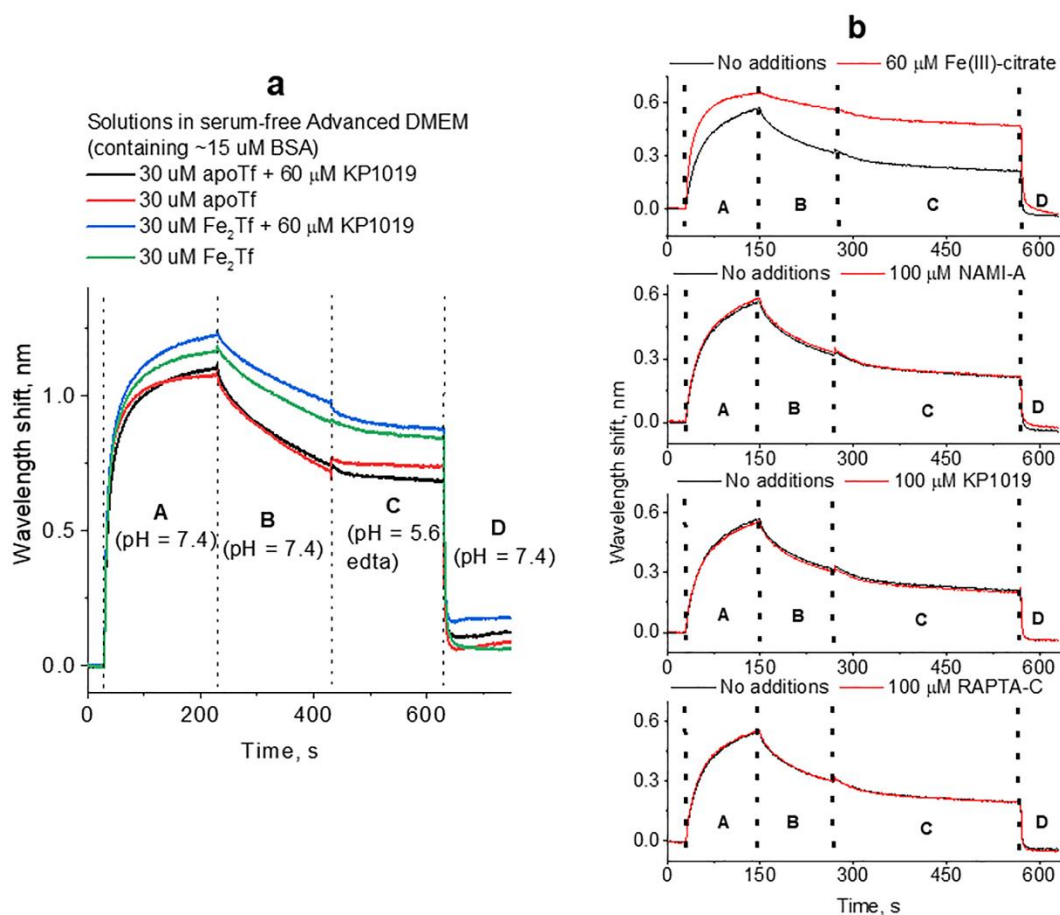


Figure S3.

- (a) BLI response curves for 1.0 μM of human Tf at 295 K in dilute cell culture media after Ru uptake experiments by HepG2 cells (Section 3.2 in the main text). Media (serum-free DMEM, containing 1.0 mg mL^{-1} BSA as a background protein, as well as KP1019 and/or Tf, as shown in the figure legend), were collected after the end of uptake experiments, then were diluted 30-fold with buffer (20 mM HEPES + 25 mM NaHCO_3 + 140 mM NaCl, pH = 7.4) and applied to TfR1-loaded BLI probe. Designations of the steps in Tf cycle (A–D) correspond to those in Figure 1d–f, main text. Calculated K_D values (1:1 binding model, BLItz software) were as follows: 51 nM (apoTf + KP1019); 32 nM (apoTf); 25 nM (Fe_2Tf + KP1019); 22 nM (Fe_2Tf). Lower Fe_2Tf binding affinity to TfR1 compared with that observed in aqueous buffers (Table 2 in the main text) is likely due to the presence of competing Fe(III) binders, such as phosphate, in cell culture medium [27].
- (b) BLI response curves for dilute human serum samples in the presence or absence of metal complexes. Intact commercial human serum (Sigma H4522) was incubated with Fe(III)-citrate (60 μM) or with Ru complexes (100 μM) for 4 h at 310 K, then diluted 200-fold (to [Tf] ~ 150 nM) with the binding buffer (20 mM HEPES, 25 mM NaHCO_3 , 140 mM NaCl, pH = 7.4), and analyzed with the TfR1-loaded BLI probe. Designations of the steps in Tf cycle (A–D) correspond to those in Figure 1d–f, main text. Calculated K_D values (1:1 binding model, BLItz software) were as follows: 8.0 nM (serum + Fe(III) citrate); or 28 ± 2 nM (serum in the presence or absence of Ru complexes). Typical anticancer Ru complexes [1–6], NAMI-A, KP1019 and RAPTA-C, were synthesized and characterized previously in the Lay group [36,37].