Supplementary Materials

Fluorochrome-Functionalized Nanoparticles for Imaging DNA in Biological Systems

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Purification of fluorochrome functionalized NP's: The separation of fluorochrome functionalized NP's from low molecular fluorochromes on by G-25 Sephadex is shown in Figure S1.

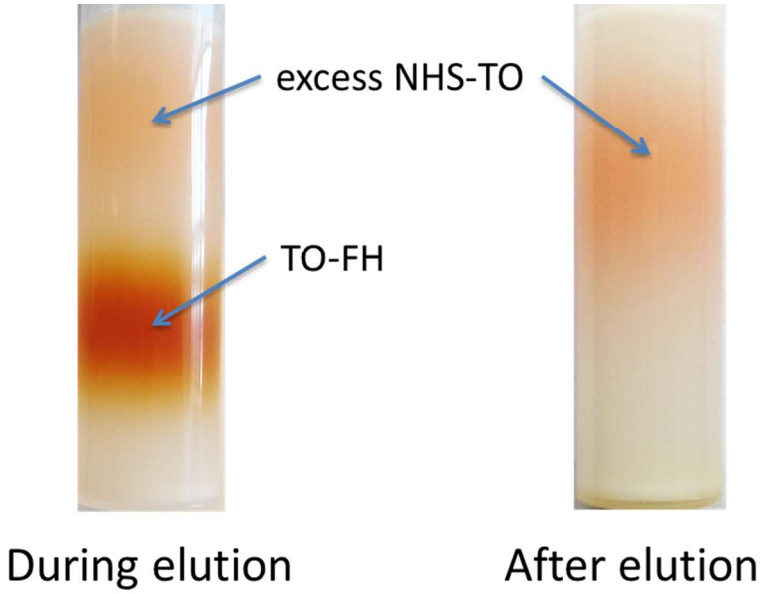

Figure S1. Separation of TO-PRO 1 from FH on Sephadex G-25. TO-PRO 1 adheres to Sephadex while TO-FH flows through. TO-FH free of TO-PRO is obtained.

Comparison of the concentrations of sites for fluorochrome intercalation on DNA with the concentrations of fluorochromes from fluorochrome functionalized NP's: When microaggregates of TO-FH and DNA form (at the EC50's from T2 or light scattering measurements), there are between 3 to 10 times more fluorochromes on TO-FH NP's than sites on DNA available for their intercalation. Hence the model shown in Figure 3d is supported.

An intercalation site consists of a set of two base pairs and has a molecular weight of 1320 daltons (one base pair = 660 Da).[1, 2] In Table S1, the concentration of intercalating sites at an EC50 is expressed as μg DNA/mL (as it was in Table 2) or nM. The concentration of intercalating sites can then be compared with the fluorochrome equivalent concentrations from TO-FH present for a DNA assay curve.

Table S1. Reaction of Fluorochrome Functionalized NP's With DNA by Fluorescence, T2 changes, and light scattering.

Concentrations of Fluorochrome Equivalents and DNA Intercalating Sites at the EC50 are compared.

Com-pound	Fig.	n	NP, nM	Fluor. Equiv., nM	Max. ΔFluor., a.u	EC50, µg, DNA/mL	Intercal Sites @ EC50, nM	nM Intercal. Sites/ nM Fluor. Equiv
Thiaz. Or.	2b	1	Not app	50.0	979	1.52	*1152*	23.03
TO-PRO3	2b	1	Not app	50.0	2750	2.27	*1722*	34.44
TO-TO	2b	2	Not app	50.0	3240	0.17	*132*	2.64
TO-PRO1	2b	1	Not app	50.0	1920	0.23	*174*	3.48
TO-FH	2b	3.6	13.89	50.0	2090	0.22	*164*	3.28
TO-FH	2b	8.2	6.10	50.0	2710	0.23	*175*	3.50
TO-FH	2b	17	2.94	50.0	4400	0.36	*271*	5.42
TO-FH	2b	20	2.50	50.0	5770	0.47	*356*	7.12

Com-pound	Fig.	n	NP, nM	Fluor. Equiv., nM	Max. ΔFluor., a.u	EC50, µg DNA/mL	Intercal Sites @ EC50, nM	nM Intercal. Sites/ nM Fluor. Equiv
TO-FH	3c	3.6	15.2	54.7	1996.4	0.382	*289*	5.29
TO-FH	3c	8.2	15.2	124.6	5731.6	0.736	*558*	4.47
TO-FH	3c	17	15.2	258.4	9430	1.105	*837*	3.24
Com-pound	Fig.	n	NP, nM	Fluor. Equiv., nM	Max. ΔFluor., a.u	EC50, µg DNA/mL	Intercal Sites@ EC50, nM	nM Intercal Sites/ nM Fluor. Equiv
TO-FH	3c	3.6	15.2	54.7	409.3	0.018	*13*	0.24
TO-FH	3c	8.2	15.2	124.6	535.9	0.055	*42*	0.33
TO-FH	3c	17	15.2	258.4	590.1	0.071	*54*	0.21
Com-pound	Fig.	n	NP, nM	Fluor.Equiv., nM	Max. ΔFluor., a.u	EC50, µg DNA/mL	Intercal Sites @ EC50	nM Intercal Sites/ nM Fluor. Equiv
TO-FH	3c	3.6	15.2	54.7	166.6	0.013	*10*	0.18
TO-FH	3c	8.2	15.2	124.6	166	0.027	*20*	0.16
TO-FH	3c	17	15.2	258.4	179	0.032	*24*	0.09

Stability of TO-FH in mouse serum:

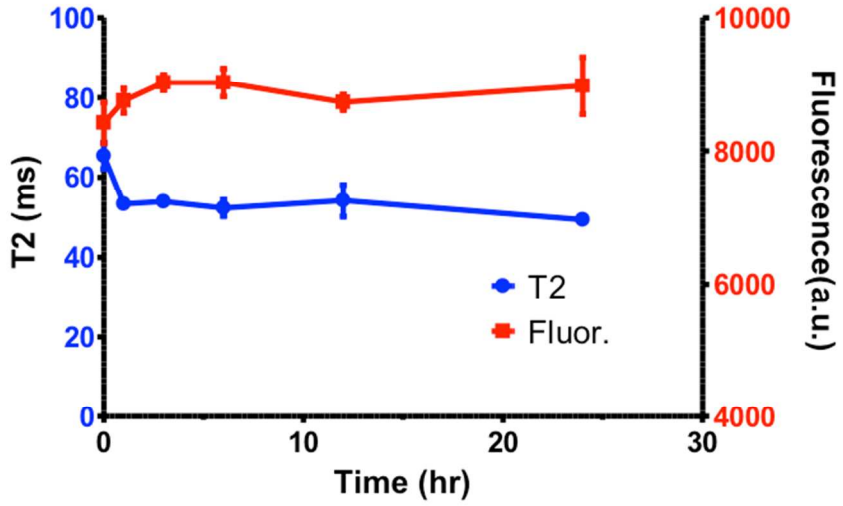

Figure S2. Stability of TO-FH in mouse serum by fluorescence and relaxation effects.

Determination of NP blood half-lives: For blood half-life measurements, anesthetized mice were injected with 10 mg Fe/kg by tail vein and 50 µL blood samples collected by tail vein. Blood half-life was determined from the T2 measurements and the clearance equation below, with data analyzed by Graphpad Prism software. Similar methods are used to determine NP blood half-life clinically.[3, 4]

$$1/Tt = \mathrm{A}e^{-kt} + 1/T_0$$

where $T_{1/2} = ln2/k$

$1/T_0 = 1/T_2$ preinjection baseline value

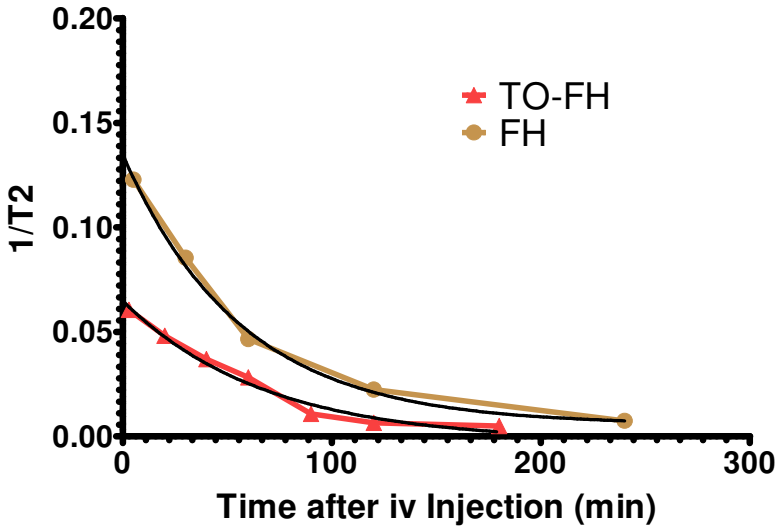

Figure S3. Determination of blood half-lives from 1/T2 values. 1/T2 versus time curves yielding blood half-lives for FH and TO-FH after intravenous injection in mice. Blood half-life was calculated using the clearance equation. Black lines are fits using the clearance equation. The half-life of FH is 39.1 min and TO-FH is 47.1 min.

Cell death kinetics of HT-29 cells exposed to 5-FU and oxaliplatin (Figure 6a): Figure S3 shows the dot plots used to obtain survival fractions (lower right, quadrant 3) as a function of time of treatment. Cells were stained with Anx-Cy and the TO-FH or low molecular reference vital fluorochromes, TO-PRO 1 or Sytox Green. Annexin V positive or vital fluorochrome positive cells are scored as dying (apoptotic) or dead (necrotic).

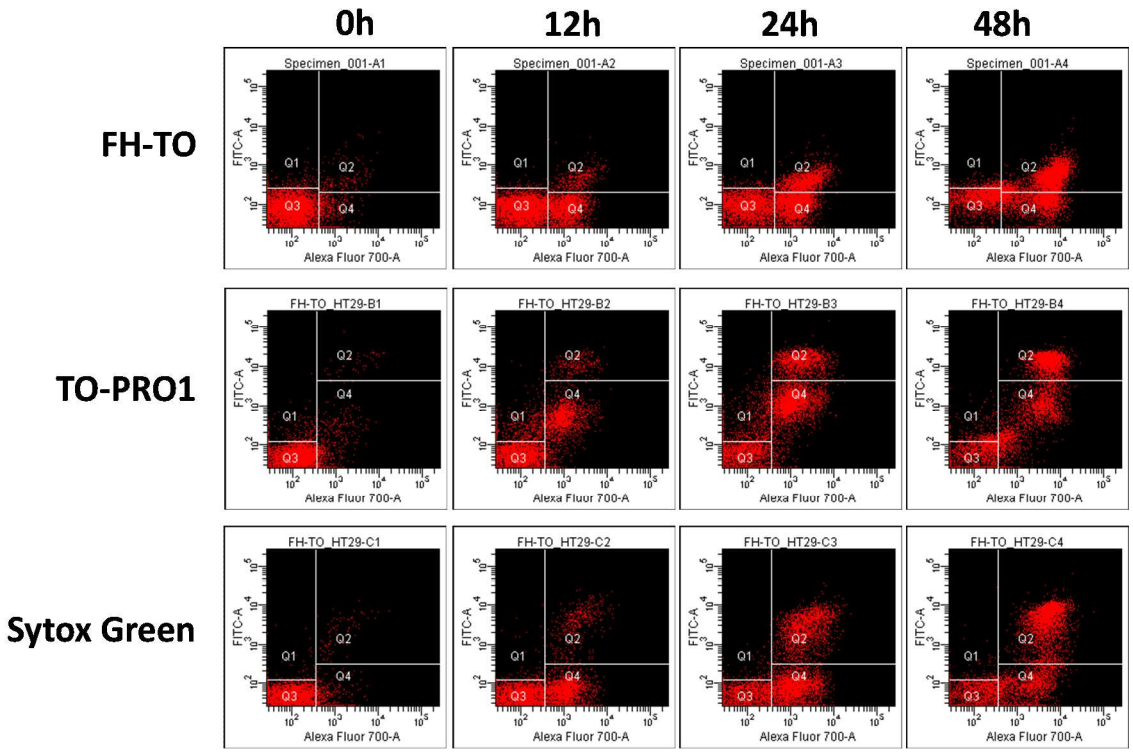

Figure S4. Detection of necrotic HT-29 cells with TO-FH by flow cytometry. HT-29 cells were treated with 5FU and oxaliplatin for the indicated times. Cells were stained with the indicated fluorochrome (TO-FH, TO-PRO1 or Sytox Green) and Anx-Cy.

References

1. Benvin, A. L.; Creeger, Y.; Fisher, G. W.; Ballou, B.; Waggoner, A. S.; Armitage, B. A., Fluorescent DNA nanotags: supramolecular fluorescent labels based on intercalating dye arrays assembled on nanostructured DNA templates. *J Am Chem Soc* **2007,** *129*, 2025-34.

2. Persil, O.; Hud, N. V., Harnessing DNA intercalation. *Trends in biotechnology* **2007,** *25*, 433-6.

3. Hamm, B.; Staks, T.; Taupitz, M.; Maibauer, R.; Speidel, A.; Huppertz, A.; Frenzel, T.; Lawaczeck, R.; Wolf, K. J.; Lange, L., Contrast-enhanced MR imaging of liver and spleen: first experience in humans with a new superparamagnetic iron oxide. *J Magn Reson Imaging* **1994,** *4*, 659-68.

4. McLachlan, S. J.; Morris, M. R.; Lucas, M. A.; Fisco, R. A.; Eakins, M. N.; Fowler, D. R.; Scheetz, R. B.; Olukotun, A. Y., Phase I clinical evaluation of a new iron oxide MR contrast agent. *J Magn Reson Imaging* **1994,** *4*, 301-7.