Supporting Information for

A rhodium-cyanine fluorescent probe: detection and signaling of mismatches in DNA

Adela Nano, Adam N. Boynton, Jacqueline K. Barton*

Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California, 91125

Table of Contents

1. Materials **3**

2. Synthesis **5**

2.1. Synthesis of Cy3-COOH **6**

2.2. Synthesis of DPA-NH_2 ..... **9**

2.3. Synthesis of RhCy3 conjugate **11**

2.4. Synthesis of the model compounds **13**

3. Photocleavage experiments **15**

4. Photophysical measurements **16**

4.1 Steady-state fluorescence **16**

4.2. Fluorescence lifetimes **17**

5. Cell cultures **17**

6. Genomic DNA extraction and purification procedure **17**

7. Figures **19**

8. Quantification of mismatches in genomic DNA **29**

9. References **31**

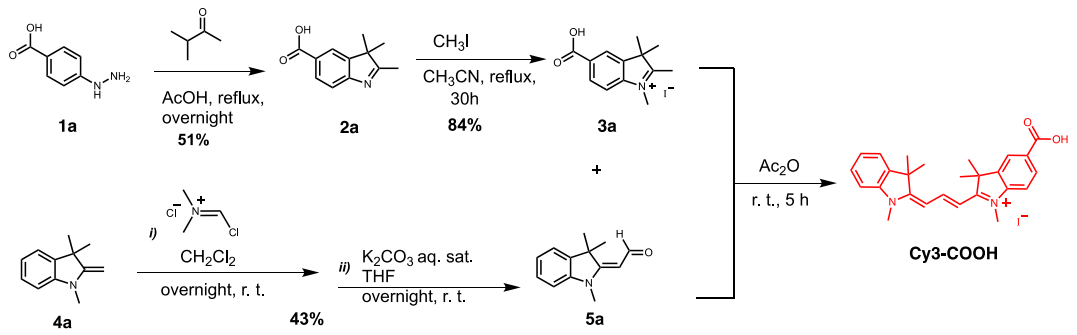
Table of Figures

Figure S1. Absorption spectra of RhCy3 and its spectroscopic models. 19
Figure S2. Steady-state emission spectra of Cy3-linker. 20
Figure S3. Full TOF-MS ES+ spectrum of RhCy3. 21
Figure S4. HPLC trace of RhCy3. 22
Figure S5. ^{1}H NMR spectrum of 3b. 23
Figure S6. ^{13}C NMR spectrum of 3b. 23
Figure S7. ^{1}H NMR spectrum of DPA-NH_2 24
Figure S8. ^{13}C NMR spectrum of DPA-NH_2 24
Figure S9. ^{1}H NMR spectrum of DPA-Cy3 25
Figure S10. ^{13}C NMR spectrum of DPA-Cy3 25
Figure S11. ^{1}H NMR spectrum of RhCy3. 26
Figure S12. ^{1}H NMR spectrum of Cy3-linker. 27
Figure S13. ^{13}C NMR spectrum of Cy3-linker. 27
Figure S14. Fluorescence lifetime decay curve for RhCy3 free in solution. 28
Figure S15. Fluorescence lifetime decay curve for RhCy3 with WM DNA. 28
Figure S16. Fluorescence lifetime decay curve for RhCy3 with WM DNA 29

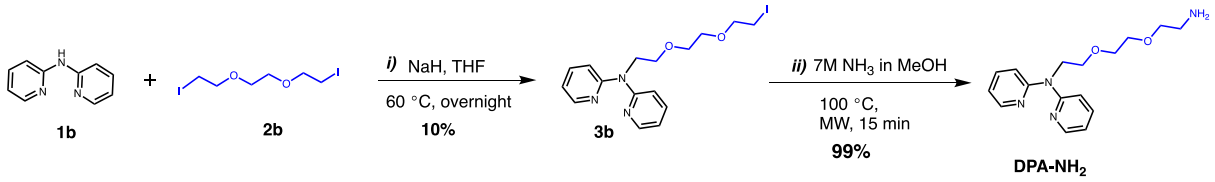
1. Materials

All reactions except when mentioned were performed under a dry atmosphere of argon. $RhCl_3$ was purchased from Pressure Chemical, Inc. 2,2'-dipyridylamine (HDPA), 1,2-bis(2-iodoethoxy)ethane, anhydrous $^{i}Pr_2NH$, anhydrous DMF, HBTU and Sephadex ion exchange resin were obtained from Sigma-Aldrich and used as purchased. Sep-Pak C18 solid phase extraction cartridges were purchased from Waters Chemical Co. ^{1}H, ^{13}C NMR spectra were recorded on a Bruker Spectrometers (400 or 300 MHz). ^{1}H, ^{13}C chemical shifts were reported to the delta scale in ppm relative to the residual peak of the deuterated used solvent as internal standards: chloroform-d_1 (^{1}H: δ = 7.26 ppm; ^{13}C: δ = 77.16 ppm), methanol-d_4 (^{1}H: δ = 3.31 ppm; ^{13}C: δ = 49.00 ppm), dichloromethane-d_2 (^{1}H: δ = 5.32 ppm; ^{13}C: δ = 54.00 ppm), acetonitrile-d_3 (^{1}H: δ = 1.94 ppm; ^{13}C: δ = 118.26 ppm). Chromatographic purifications were performed using 40-63 μm silica gel or Sep-Pak C18 cartridges. HPLC purifications were performed on an HP1100 high-pressure liquid chromatography system equipped with a diode array detector using a Varian DynaMax C18 semi-preparative column. Mass spectrometry was performed at Caltech mass spectrometry facility. UV-vis absorption spectra were recorded on a Cary 100 Bio spectrophotometer.

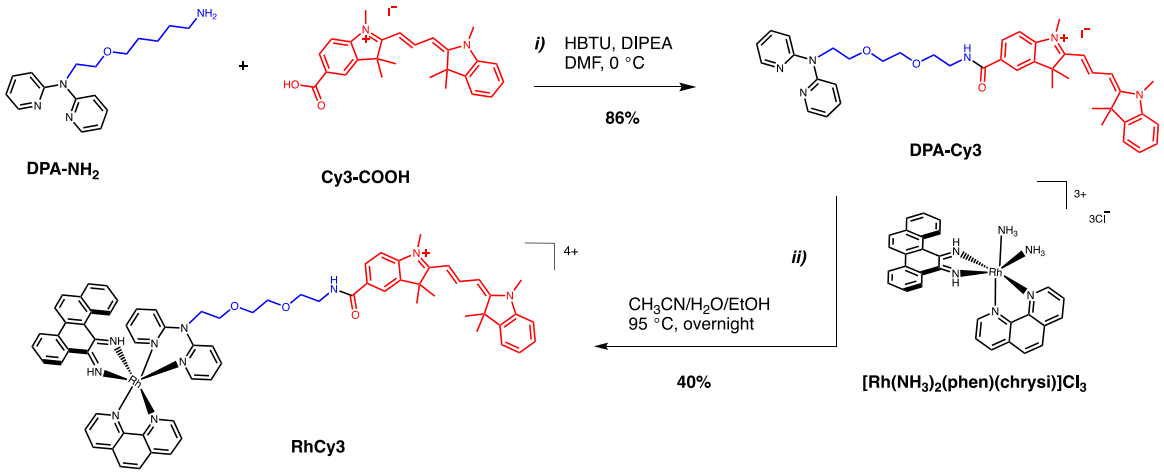
2. Synthesis



Scheme S1. Synthesis of **Cy3-COOH**.

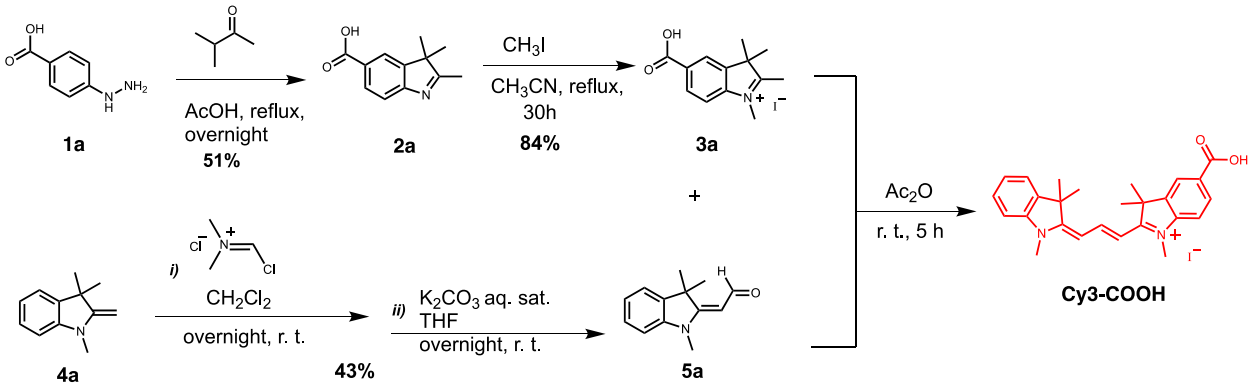


Scheme S2. Synthesis of **DPA-NH_2**.



Scheme S3. Synthesis of **RhCy3**.

2.1. Synthesis of Cy3-COOH



Scheme S1. Synthesis pathway for **Cy3-COOH** and its precursors.

Compound 2a.[1] 4-Hydrazinobenzoic acid (1.5 g, 9.85 mmol) and 3-methyl-2-butanone (1.27 g, 14.79 mmol) were refluxed in glacial acetic acid (20 ml) solution for about 16 h. Afterwards, the reaction was cooled down to room temperature and quenched with water. The crude was extracted with dichloromethane (2x) and washed with water (3x) followed by drying the organic phase with anhydrous Na_2SO_4. Dichloromethane was evaporated under vacuum and the crude was purified over a silica gel column chromatography, eluting with acetonitrile/dichloromethane (2/8). The product was obtained as yellowish solids in 51% yield (1.014 g). **^{1}H NMR** (400 MHz, Methanol-d_4) δ 8.38 (d, J = 1.5 Hz, 1H), 8.32 (dd, J = 8.4, 1.6 Hz, 1H), 7.97 (d, J = 8.4 Hz, 1H), 4.12 (s, 3H), 1.68 (s, 6H). **^{13}C NMR** (101 MHz, MeOD) δ 199.26, 166.68, 145.28, 141.93, 132.28, 130.81, 124.10, 114.96, 54.65, 34.43, 21.01. MW = 203.24 Da, chemical formula: $C_{12}H_{13}NO_2$. **ESI-MS** (positive) *m/z* (% intensity): calculated for $[M]^+$ 203.09, found 203.13

***Compound 3a*:**[2] A solution of compound **2a** (1, 014g, 4.99 mmol) and iodomethane (4.247g, 29.93 mmol) in acetonitrile (20.0 ml) was stirred at 90 °C under argon during 30 h. The reaction was cooled down to room temperature and the precipitate was filtered off and washed with acetonitrile and hexane. The indolium salt **3a** was obtained as white solids which turn slowly yellowish under normal atmosphere (1.440g, 84%). MW = 345.18 Da, chemical formula: $C_{13}H_{16}INO_2$. **ESI-MS** (positive) *m/z* (% intensity): calculated for $[M - I]^+$ 218.12 (100), found 218.14 (100).

***Compound 5a: Method I*.**[3] DMF (25 ml) was introduced in a flame-dried Schlenk flask under vacuum which was then filled with argon. $POCl_3$ (1.2 eq, 2.12g, 0.013 mol) was added dropwise followed by the addition of 2-methylene-1,3,3-trimethylindoline **4a** (2g, 0.011 mol). The final mixture was stirred at 50 °C during 45 min. After cooling it down to room temperature, the reaction was neutralized with a saturated solution of sodium acetate and extracted with dichloromethane. Organic phase was dried with anhydrous Na_2SO_4 and concentrated under vacuum. The crude was purified using a silica gel column chromatography eluted with DCM/MeOH 0.6%. This method afforded **5a** as a yellowish oil in 60%.

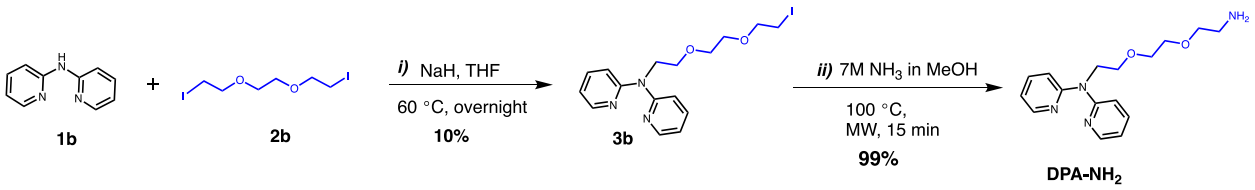
***Method II*.**[4] (Chloromethylene)dimethylammonium chloride (8.86 g, 0.069 mmol) was dissolved in anhydrous dichloromethane (40.0 ml) and stirred at room temperature during 30 minutes. A solution of the Fischer base, 2-methylene-1,3,3-trimethylindoline (2.0 g, 0.011 moles), in dichloromethane (15.0 ml) was added dropwise and the mixture was stirred at room temperature overnight. Afterwards, the solvent was evaporated under vacuum and the resulting crude was dissolved in THF (30.0 ml) and a saturated aqueous solution of K_2CO_3 (30.0 ml) which was added solely. Following the addition of K_2CO_3 solution, the color of the mixture turns from deep

red to orange. After stirring at room temperature for about 12 h, THF was removed under vacuum and the aqueous phase extracted with dichloromethane. The organic phase was washed 2x with water and dried with anhydrous Na_2SO_4, filtered and concentrated under vacuum. The crude was purified over a silica gel column chromatography, eluted with DCM/MeOH 0.6%. The aldehyde-derivative was collected as yellowish oil (43%, 954 mg). **^{1}H NMR** (400 MHz, Chloroform-d) δ 10.03 (d, J = 8.9 Hz, 1H), 7.34 – 7.22 (m, 2H), 7.08 (td, J = 7.5, 0.9 Hz, 1H), 6.87 (d, J = 7.9 Hz, 1H), 5.41 (d, J = 8.9 Hz, 1H), 3.26 (s, 3H), 1.68 (s, 6H). **^{13}C NMR** (101 MHz, CDCl3) δ 186.54, 173.64, 143.45, 139.39, 128.04, 122.45, 121.82, 107.98, 99.01, 47.43, 29.62, 29.58. MW = 201.27 Da, chemical formula: $C_{13}H_{15}NO$. **ESI-MS** (positive) *m*/z (% intensity): calculated for $[M + H]^+$ 202.12 (100), found 202.15 (100).

Cy3-COOH: Indolium salt **3a** (343 mg, 0.99 mmol) and the aldehyde-Fischer base **5b** (200 mg, 0.99 mmol) were combined together in a solution of glacial acetic anhydride (10 ml). The solution was stirred at room temperature for 2.5 h. The cyanine dye precipitated during the formation, therefore it was filtered off and washed with diethyl ether. To obtain the final product as highly pure, silica gel column chromatography was performed eluting with dichloromethane/methanol 5% to 8%. The cyanine dye was obtained as dark green solids which in solution were bordeaux red in 61% yield after column chromatography. MW = 528.43 Da, chemical formula: $C_{26}H_{29}IN_2O_2$.

^{1}H NMR (400 MHz, Methanol-d4) δ 8.59 (t, J = 13.5 Hz, 1H), 8.16 – 8.08 (m, 2H), 7.60 (dd, J = 7.4, 1.5 Hz, 1H), 7.54 – 7.33 (m, 3H), 6.55 (d, J = 13.7 Hz, 1H), 6.46 (d, J = 13.2 Hz, 1H), 5.51 (s, 1H), 3.76 (s, 3H), 3.69 (s, 3H), 3.37 (s, 4H), 1.81 (d, J = 2.7 Hz, 12H). **ESI-MS** (positive) *m*/z (% intensity): calculated for $[M-I]^+$ 401.22 (100), found 401.42 (100).

2.2. Synthesis of DPA-NH_2



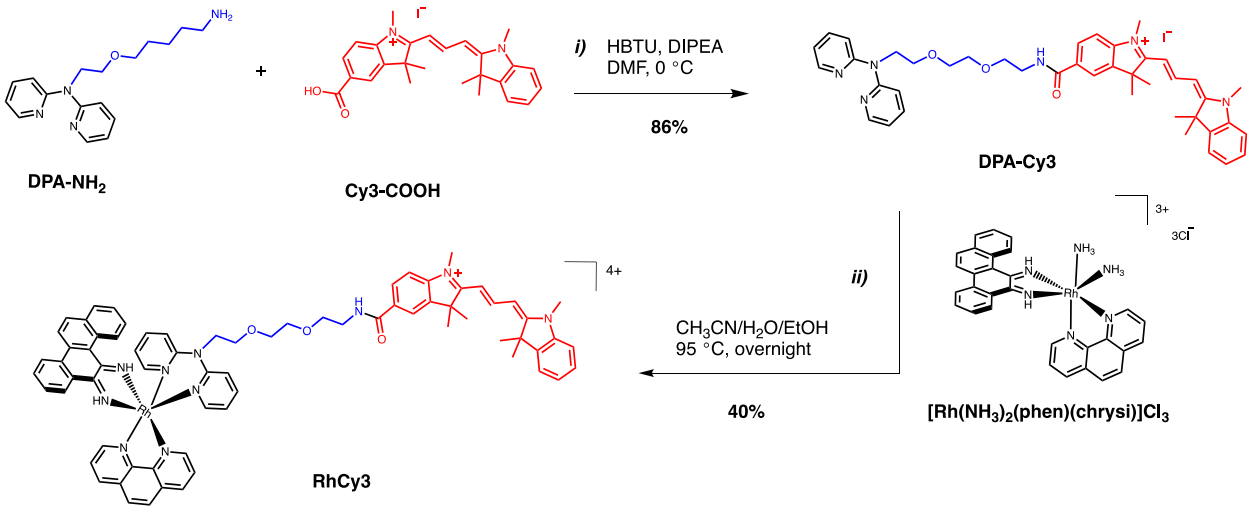
Scheme S2. Synthesis pathway for **DPA-NH_2**.

***Compound 3b*:** 2,2'-dipyridylamine **1b** (1.0 g, 5.84 mmol) was placed in a flame-dried Schlenk flask under vacuum and then filled with argon. THF (15 ml) was added followed by the slow addition of NaH (182 mg, 7.93 mmol) under argon. The solution was stirred at room temperature during 1.5 h prior to the addition of 1,2-bis(2-iodoethoxy)ethane (5.0 g, 13.51 mmol). The final mixture was protected from light and was stirred at 70 °C during 20 h. After cooling down to room temperature, the reaction mixture was quenched with water and extracted with dichloromethane. The organic phase was washed 2x with water, dried with anhydrous Na_2SO_4 and concentrated *in vacuo.* Most of the starting material **1b** was precipitated (or recrystallized) from a solution of the crude in dichloromethane by adding diethyl ether. Afterwards, the filtrate was concentrated under vacuum and purified over a silica gel column chromatography. The mobile phase was dichloromethane/ethyl acetate 5% to 20 %. The desired product was obtained as a yellowish oil in about 10 % yield after column chromatography purification. Many fractions were collected as a mixture with the starting material **1b**. **^{1}H NMR** (400 MHz, Chloroform-*d*) δ 8.34 (ddd, *J* = 4.9, 2.0, 0.9 Hz, 2H), 7.54 (ddd, *J* = 8.4, 7.2, 2.0 Hz, 2H), 7.19 (dt, *J* = 8.4, 0.9 Hz,

2H), 6.88 (ddd, *J* = 7.2, 5.0, 1.0 Hz, 2H), 4.42 (t, *J* = 6.1 Hz, 2H), 3.83 (t, *J* = 6.1 Hz, 2H), 3.69 (dd, *J* = 7.4, 6.5 Hz, 2H), 3.64 – 3.54 (m, 4H), 3.21 (dd, *J* = 7.4, 6.5 Hz, 2H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 157.40, 148.12, 137.17, 117.05, 114.90, 77.34, 77.02, 76.70, 71.94, 70.36, 70.18, 69.46, 47.95, 2.96 (*C*H_2-I). MW = 413.26 Da, chemical formula: $C_{16}H_{20}IN_3O_2$. **ESI-MS** (positive) *m/z* (% intensity): calculated for $[M + H]^+$ 414.07 (100), found 414.04 (100).

***Compound DPA-NH$_2$*:**[5] A solution of 7 M ammonia in methanol (5 ml) was added to **3b** (130 mg, 0.314 mmol) in a 10 ml microwave vial. The reaction was stirred at 100 °C during 15 minutes in an auto-sampling microwave. The final product (which is soluble preferably in water) was extracted with water and washed with ethyl acetate to eliminate any residual of starting material. The water was evaporated and the product was obtained pure in 95 % yield and used as obtained in the next step (90 mg). **^{1}H NMR** (400 MHz, Acetonitrile-d_3) δ 8.34 (ddd, *J* = 5.0, 2.0, 0.9 Hz, 2H), 7.65 (ddd, *J* = 8.4, 7.2, 2.0 Hz, 2H), 7.17 – 7.10 (m, 2H), 6.98 (ddd, *J* = 7.3, 4.9, 0.9 Hz, 2H), 4.33 (t, *J* = 5.8 Hz, 2H), 3.74 (t, *J* = 5.8 Hz, 2H), 3.72 – 3.66 (m, 2H), 3.52 (S, 4H), 3.13 – 3.05 (m, 2H). **^{13}C NMR** (101 MHz, CD_3CN) δ 157.75, 148.38, 138.37, 118.01, 117.79, 115.58, 70.34, 70.08, 69.10, 66.20, 48.18, 39.93 (*C*H_2-NH_2). **^{15}N{^{1}H} NMR** (41 MHz, CD_3CN) δ 244.55 (s). MW = 302.38 Da, chemical formula: $C_{16}H_{22}N_4O_2$. **ESI-MS** (positive) *m/z* (% intensity): calculated for $[M + H]^+$ 303.18 (100), found 303.19 (100).

2.3. Synthesis of RhCy3 conjugate



Scheme S3. Synthesis pathway for **RhCy3**.

[Rh(phen)(chrysi)$(NH_3)_2$]Cl_3 was synthesized from [Rh(phen)$(NH_3)_4$]OTf_3 and 5,6-chrysenequinone as previously reported.[6,7]

***Compound DPA-Cy3*: Cy3-COOH** (86 mg, 0.163 mmol) was introduced in a Schlenk flask and dried under vacuum for 1 h before addition of DMF (6 ml) under argon. The solution was kept at 0 °C during the addition of the coupling reagent HBTU (2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) (62 mg, 0.163 mmol) and DIPEA (27 mg, 0.209 mmol). **DPA-NH_2** (90 mg, 0.209 mmol), which was also dried under vacuum during 1 h, was dissolved in 3 ml of DMF and added to the solution of **Cy3-COOH**. Additional 0.209 mmol of DIPEA were added to the final solution that was stirred during 2 h while keeping the temperature at 0 °C. Afterwards, 100 ml of water were added to the reaction mixture and the product was extracted with dichloromethane (100 ml). The organic phase was washed 2x with water, dried

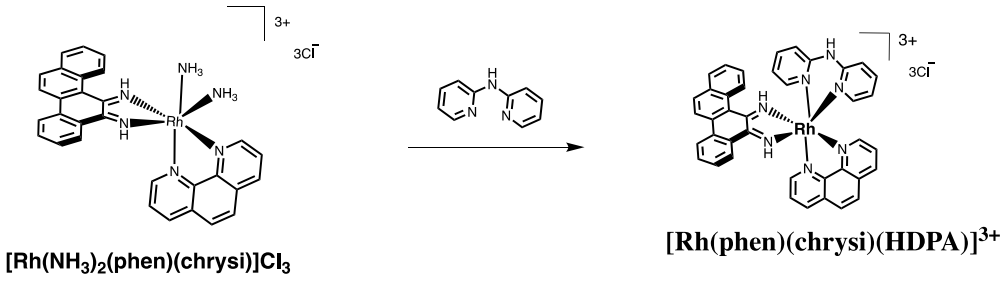
with anhydrous Na_2SO_4 and concentrated *in vacuo*. The coupled product **DPA-Cy3** was obtained pure after extraction with dichloromethane, therefore no further purifications were employed. **DPA-Cy3** was obtained as dark solids, bordeaux red in solution, in 86% yield (118 mg). **^{1}H NMR** (400 MHz, Acetonitrile-d_3) δ 8.49 (t, *J* = 13.6 Hz, 1H), 8.39 (ddd, *J* = 5.5, 1.9, 0.8 Hz, 2H), 8.08 (ddd, *J* = 9.1, 7.4, 1.9 Hz, 2H), 7.92 (d, *J* = 1.6 Hz, 1H), 7.85 (dd, *J* = 8.3, 1.8 Hz, 1H), 7.61 – 7.45 (m, 4H), 7.42 – 7.31 (m, 2H), 7.35 – 7.25 (m, 2H), 7.21-7.24 (m, 1H), 6.40 (d, *J* = 13.7 Hz, 1H), 6.30 (d, *J* = 13.3 Hz, 1H), 4.36 (t, *J* = 5.1 Hz, 2H), 3.88 (t, *J* = 5.1 Hz, 2H), 3.66 – 3.69 (m, 2H), 3.65 (s, 1H), 3.61 – 3.63 (m, 2H), 3.58 – 3.60 (m, 2H), 3.58 (s, 1H), 3.51 (q, *J* = 6.4, 5.9 Hz, 2H), 1.75 (d, *J* = 2.0 Hz, 12H). **^{13}C NMR** (101 MHz, CD_3CN) δ 176.85, 175.33, 166.89 (NH*C*O), 160.11, 159.74, 154.43, 151.23, 146.08, 143.33, 143.13, 142.63, 141.66, 141.24, 131.54, 129.35, 128.70, 126.55, 122.88, 121.74, 119.63, 117.90, 116.46, 115.17, 112.19, 110.92, 104.22, 102.85, 71.22, 70.22, 69.78, 68.77, 50.22, 50.21, 49.40, 39.97, 32.05, 31.68, 27.67, 27.41, 1.56, 1.52, 1.36, 1.31, 1.25, 1.15, 1.11, 1.05, 0.95, 0.90, 0.84, 0.81, 0.69, 0.60, 0.49, 0.39, 0.28. MW = 812.80 Da, chemical formula: $C_{42}H_{49}IN_6O_3$. **HRMS** (ES+) *m/z* (% intensity): found for $[M - I]^+$ = 685.3871 (100) calculated 685.3861.

RhCy3: **DPA-Cy3** (60 mg, 0.073 mmol) and **$[Rh(phen)(chrysi)(NH_3)_2]Cl_3$** (88 mg, 0.086 mmol) were added in a 100 ml flask and dissolved in a H_2O/EtOH/CH_3CN (6/6/6 ml) mixture. The reaction mixture was refluxed overnight at 95 °C. After cooling down to room temperature, the solvents were evaporated and the crude was dissolved in a minimum volume of acetonitrile. Addition of dichloromethane provokes precipitation of unreacted rhodium precursor. The filtrate was collected, concentrated under vacuum and re-dissolved in water. Extraction of the aqueous phase with dichloromethane allowed to eliminated small amount of unreacted **DPA-Cy3** ligand.

The aqueous phase was concentrated under vacuum and the crude was purified on a HPLC eluting with H_2O (0.1 % TFA)/acetonitrile and Sep-Pak C_{18} cartridges eluting with acetonitrile. The chloride salt was obtained by loading an acetonitrile solution of the conjugate on a Sephadex QAE anion exchange column equilibrated with 0.1 M $MgCl_2$. The reaction yielded the product at about 70% yield after the first purification. 40 mg were obtained highly pure after several purifications (40% yield) as dark solids, deep bordeaux red in solution. **^{1}H NMR** (400 MHz, Methanol-d_4) δ 9.24 (dd, J = 13.3, 8.0 Hz, 1H), 9.16 – 8.94 (m, 4H), 8.80 (dd, J = 82.2, 6.4 Hz, 2H), 8.64 – 8.14 (m, 11H), 8.10 – 7.33 (m, 17H), 7.26 (d, J = 8.3 Hz, 1H), 7.00 (d, J = 17.7 Hz, 2H), 6.58 (dd, J = 13.6, 2.3 Hz, 1H), 6.45 (dd, J = 13.2, 4.1 Hz, 1H), 4.72 (d, J = 6.7 Hz, 1H), 4.29 (d, J = 30.2 Hz, 1H), 3.93 (s, 1H), 3.78 (s, 3H), 3.71 – 3.35 (m, 7H), 3.29 – 2.89 (m, 4H), 1.86 – 1.70 (m, 12H).

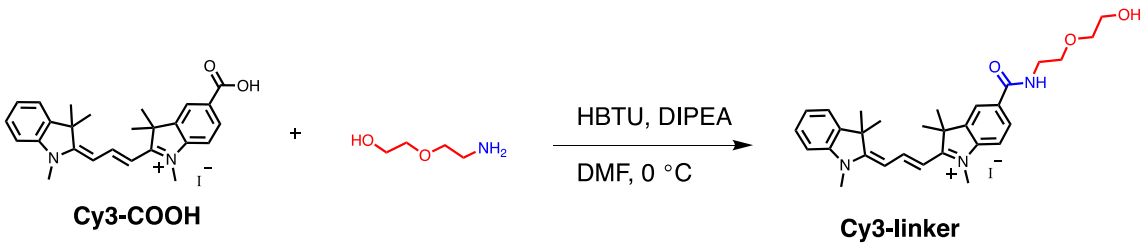
UV-Vis in Tris buffer (5 mM Tris, 200 mM NaCl, pH = 7.4) at room temperature: λ_{abs} 553 nm (ε = 72,500 M^{-1} cm^{-1}), 515 nm (ε = 41,500 M^{-1} cm^{-1}), 400 nm (ε = 4,800 M^{-1} cm^{-1}), 268 nm (ε = 51,000 M^{-1} cm^{-1}); **HRMS** (ES+) *m/z* (% intensity): found for $[M]^{+2}$ = 611.7226 (100) calculated 611.7238; found for $[M+H]^{+3}$ = 408.1502 calculated 408.1531.

2.4. Synthesis of the model compounds



Scheme S4. Synthesis of **[Rh(phen)(chrysi)(HDPA)]Cl_3**.

[Rh(phen)(chrysi)(HDPA)]Cl_3 was synthesized from $[Rh(NH_3)_2(phen)(chrysi)]Cl_3$ and 2,2'-dipyridylamine as previously reported.[8] UV-Vis in Tris buffer (5mM Tris, 200 mM NaCl, pH = 7.4) at room temperature: λ_{abs} 400 nm (ε = 2,880 M^{-1} cm^{-1}), 300 nm (ε = 12,000 M^{-1} cm^{-1}), 264 nm (ε = 22,700 M^{-1} cm^{-1}). **ESI-MS** (positive) *m/z*: calculated for $[M]^+$ 708.14, found 708.25; for $[M]^{+2}$ calc. 354.57 found 354.67.



Scheme S5. Synthesis of the spectroscopic model **Cy3-linker**.

Cy3-linker: A Schlenk flask was flame-dried under vacuum and filled with argon before adding **Cy3-COOH** (50.0 mg, 0.095 mmol) and the DMF (10 ml). HBTU (36.0 mg, 0.095 mmol) and DIPEA (12.2 mg, 0.095 mmol) were added at 0 °C and the mixture was stirred for 10-15 minutes at 0 °C before proceeding with the addition of 2-(2-aminoethoxy)ethanol (10.0 mg, 9.5 µl, 0.114 mmol) and 1 additional equivalent of DIPEA. The final mixture was kept at 0 °C during 1 h. Afterwards, the reaction was extracted with dichloromethane and the organic phase was washed with water (2x), dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude was purified over a silica gel column chromatography, eluting with DCM/EtOH 1% to 7%. **^{1}H NMR** (400 MHz, Methanol-d_4) δ 8.59 (t, *J* = 13.5 Hz, 1H), 8.08 – 7.90 (m, 2H), 7.60 (dd, *J* = 7.6, 1.1 Hz, 1H), 7.50 (ddd, *J* = 8.4, 7.3, 1.2 Hz, 1H), 7.46 – 7.35 (m, 3H), 6.55 (d, *J* = 13.7 Hz, 1H),

6.44 (d, *J* = 13.3 Hz, 1H), 5.51 (s, 1H), 3.75 (s, 3H), 3.72 (m, *J* = 5.6, 3.8 Hz, 4H), 3.68 (s, 3H), 3.66 – 3.59 (m, 4H), 1.82 (s, 6H), 1.80 (s, 6H). **^{13}C NMR** (101 MHz, MeOD) δ 176.44, 174.79, 167.99, 151.05, 145.52, 142.53, 141.00, 140.53, 130.74, 128.62, 128.37, 125.93, 122.04, 121.15, 111.37, 110.14, 103.80, 102.40, 72.02, 69.23, 60.80, 49.61, 48.73, 39.72, 30.64, 26.84, 26.57. MW = 615.56, chemical formula: $C_{30}H_{38}IN_3O$. **HRMS** (ES+) *m/z* (% intensity): calculated for $[M - I]^+$ 488.2913, found 488.2907 (100). **UV-Vis** in Tris buffer (5mM Tris, 200 mM NaCl, pH = 7.4) at room temperature: λ_{abs} 546 nm (ε = 103,600 M^{-1} cm^{-1}), 515 nm (ε = 62,000 M^{-1} cm^{-1}).

3. Photocleavage experiments

The oligonucleotide was ^{32}P-labeled at the 5'-end by incubating with ^{32}P-ATP (MP Biomedicals) and polynucleotide kinase (PNK) at 37 °C for about 2 h. A small amount of labeled DNA (about 1% of the total amount of DNA) was added to 2 μM of non-labeled DNA in 100 mM NaCl, 20 mM NaP_i, pH 7.1 buffer. The DNA was annealed by heating at 90 °C for 15 min and cooling slowly to room temperature over a period of 2 h. Annealed 2 μM DNA and 10 μM compound (RhCy3, Cy3-linker or $[Rh(phen)(chrysi)(HDPA)]^{3+}$) were mixed in a microcentrifuge tube and incubated at 37 °C for 10 min. Dark controls (samples were protected from light) for each sample were prepared. The samples were irradiated using an Oriel Instruments 1000 W, Hg/Xe solar simulator (320−440 nm) during 20 min. The samples (irradiated and dark controls) were dried on a speedvac and electrophoresed in 20% denaturing polyacrylamide gel PAGE (SequaGel, National Diagnostics) with formamide loading dye. Further, the gel was exposed to a phosphor screen and developed using a Typhoon FLA 9000 instrument (a high-resolution biomolecular imager) and, subsequently visualized with ImageQuant software.

4. Photophysical measurements

4.1 Steady-state fluorescence

The fluorescence spectra were recorded on an ISS-K2 spectrofluorometer at 25 °C. Temperature was controlled using a water circulation system. Excitation wavelength was λ_{Ex} = 520 nm and emission was recorded from 545 - 750 nm. The **RhCy3** fluorescence titrations with increasing amounts of a 27-mer DNA duplex containing a CC mismatch was used to determine the binding affinity of the conjugate with the mismatched DNA duplex. [DNA] was considered as concentration on full fragments, *i.e.* the 27-mer oligonucleotide. The plotted data were fit to a one-site specific binding equation curve with GraphPad Prism 7.0. The binding constant calculated for the mismatched dsDNA was K_d = 0.32 ± 0.033 µM and the binding affininty K_B (CC) = 3.1 x 10^6 M^{-1}. **RhCy3** fluorescence titrations were performed also in the presence of increasing amount of fully well-matched dsDNA, but very little variation in fluorescence intensity was observed. The plotted data were fit to two-sites non-specific binding equation curve with GraphPad Prism 7.0. The calculated dissociation constant was K_d = 122 µM and the binding affinity for the well matched dsDNA was K_B (WM) = 8.0 x 10^3 M^{-1}.

For the fluorescence titrations with genomic DNA (gDNA), [gDNA] was considered as concentration on base pairs (BP) of DNA.

Stock solutions of the conjugate **RhCy3** and the model compounds were prepared by dissolving weighed amounts of solids in a Tris buffer solution (5 mM Tris, 200 mM NaCl, pH = 7.4). The working solutions were obtained by dilution of stock solutions. The 27-mer oligonucleotides were synthesized using standard phosphoramidite chemistry at Integrated DNA Technologies (Coralville, IA) and purified by HPLC using a reverse-phase column (Varian, Inc.) on a Hewlett-Packard 1100 HPLC. Genomic DNA was extracted from seeded HCT116O, HCT116N, SKOV3

and DU145 cell lines and purified using PureLink® Genomic DNA Kits purchased from Invitrogen.

4.2. Fluorescence lifetimes

Time-resolved spectroscopic measurements were carried out at the Beckman Institute Laser Resource Center. Samples were prepared in 1x1 cm quartz cuvettes and excited with ~15 ps pulses from the second harmonic (532 nm) of a regeneratively amplified mode-locked Nd:YAG laser (Spectra Physics Vanguard 2000-HM532, Continuum RGA60 Regenerative Amplifier) operating at 10 Hz. Magic-angle-polarized luminescence was collected using reflective optics, spectrally filtered through a long-pass colored glass filter and a spectrograph (Acton Research Corporation SpectraPro 275), then directed through an optical fiber array onto the entrance slit of a picosecond streak camera (Hamamatsu C5680) operating in photon-counting mode. Data were collected on a 1 ns sweep range with 10,000 exposures.

5. Cell cultures

HCT116N and HCT116O cells were grown in RPMI medium 1640 supplemented with: 10% FBS; 2 mM L-glutamine; 0.1 mM nonessential amino acids; 1 mM sodium pyruvate; 100 μg/mL penicillin; 100 μg/mL streptomycin; and 400 μg/mL geneticin (G418). DU145 and SKOV3 cells were grown in DMEM (1X) medium without L-glutamine supplemented with: 10% FBS; 100 μg/mL penicillin; 100 μg/mL streptomycin. All cell lines were grown in tissue culture flasks (Corning Costar, Acton, MA) at 37 °C under 5% CO and humidified atmosphere.

6. Genomic DNA extraction and purification procedure

The genomic DNA was extracted and purified using PureLink® Genomic DNA Kits purchased from Life Technologies Corporation-Invitrogen (Carlsbad, CA) following the manufacturer's

protocol. Prior to DNA extraction, HCT116N, HCT116O, SKOV3 and DU145 cells were seeded and grown in RPMI or DMEM medium at <5 x 10^6 cells/ml. The lysates were prepared by removing the growth medium from the culture plate and cells were harvested by trypsinization then re-suspended in 200 µL PBS. ProteinaseK (20 µL) and RNase (20 µL) were added to the sample, mixed by vortexing and incubated at room temperature for 2 minutes. 200 µL of PureLink® Genomic Lysis/Binding Buffer were added, mixed and vortexed to obtain a homogenous solution. The samples were incubated at 55 °C for 10 minutes to promote digestion then 200 µL of 96-100% ethanol was added to the lysate which was further mixed by vortexing for 5 seconds. The DNA was washed by adding 500 µL of Wash Buffer 1 then Wash Buffer 2 provided by the kit, followed by DNA eluting process using the spin columns. The spin columns were eluted with sterile MilliQ water (200µl) two times to recover a maximum of genomic DNA. The samples were lyophilized and the dry DNA was solubilized in Tris buffer solution (5 mM Tris, 50 mM NaCl, pH = 8.0) in order to obtain a highly concentrated solution. The concentration of gDNA solutions was determined on a NanoDrop 2000 Spectrophotometer (Thermo Scientific) by pippeting 2 µL of the sample solution. The sample purity was determined by calculating the absorbance ratios A260/A280 nm and A230/A260 nm. For HCT116N: A260/A280 = 1.99, A230/A260 = 2.14; HCT116O: A260/A280 = 1.98, A230/A260 = 2.20; SKOV3: 260/280 = 2.00, 230/260 = 1.78; and DU145: 260/280 = 1.99, 230/260 = 1.90. The gDNA concentration of the final solutions used for fluorescence titration measurements was adjusted at 3800 ng/µl (5.7 mM base pairs DNA).

7. Figures

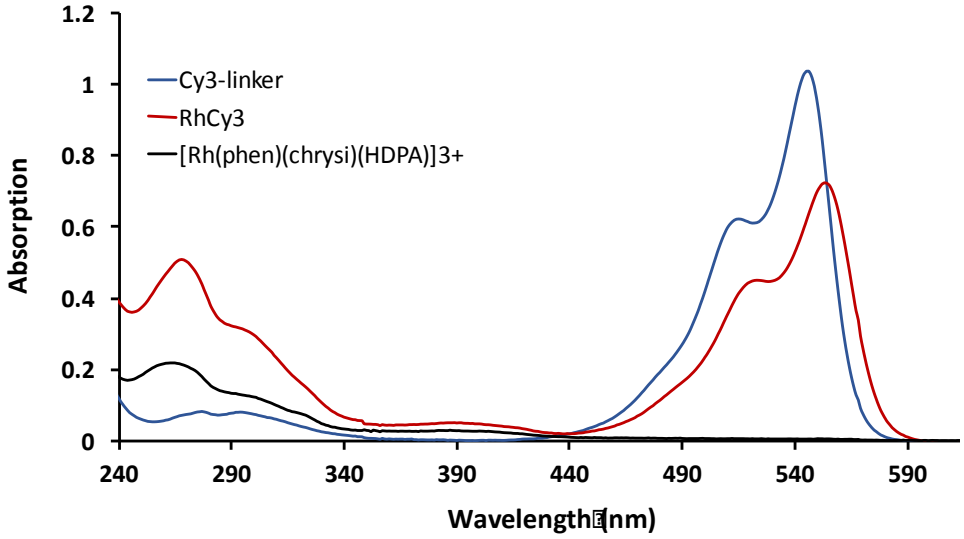


Figure S1. Absorption spectra of **RhCy3** and its spectroscopic models.

The measurements were performed in Tris buffer (5 mM Tris, 200 mM NaCl, pH = 7.4) at room temperature at a concentration C =10 x 10^{-6} M. Absorption wavelengths for **RhCy3**: 553 nm (ε = 72,500 M^{-1} cm^{-1}), 515 nm (ε = 41,500 M^{-1} cm^{-1}), 400 nm (ε = 4,800 M^{-1} cm^{-1}), 268 nm (ε = 51,000 M^{-1} cm^{-1}); for **Cy3-linker**: 546 nm (ε = 103,600 M^{-1} cm^{-1}), 515 nm (ε = 62,000 M^{-1} cm^{-1}), 280 nm (ε = 8,000 M^{-1} cm^{-1}); for **[Rh(phen)(chrysi)(HDPA)]$^{3+}$**: 400 nm (ε = 2,880 M^{-1} cm^{-1}), 300 nm (ε = 12,000 M^{-1} cm^{-1}), 264 nm (ε = 22,700 M^{-1} cm^{-1}).

Table S1. Photophysical properties of **RhCy3** and its model compounds.

Compound	λ_{max} Abs/nm (ε /x 10^3 M^{-1} cm^{-1})	λ_{max} Em/nm	τ/ns
RhCy3	553 (73), 515 (42), 400 (5), 268 (51)	570	0.111
Cy3-linker	546 (ε = 104), 515 (62), 280 nm (8)	563	0.105
Rh(HDPA)*	400 (3), 300 (12), 264 (23)	n.d.*	n.d.

Electronic absorption (λ_{max} abs) and emission maxima (λ_{max} em), molar absortivities (ε) and fluorescence lifetime (τ) for the conjugate and its spectroscopic references were measured in Tris buffer (200 mM NaCl, 5 mM Tris) at pH = 7.4 at 25 °C. * n.d. = not determined, Rh(HDPA) = $[Rh(phen)(chrysi)(HDPA)]^{3+}$.

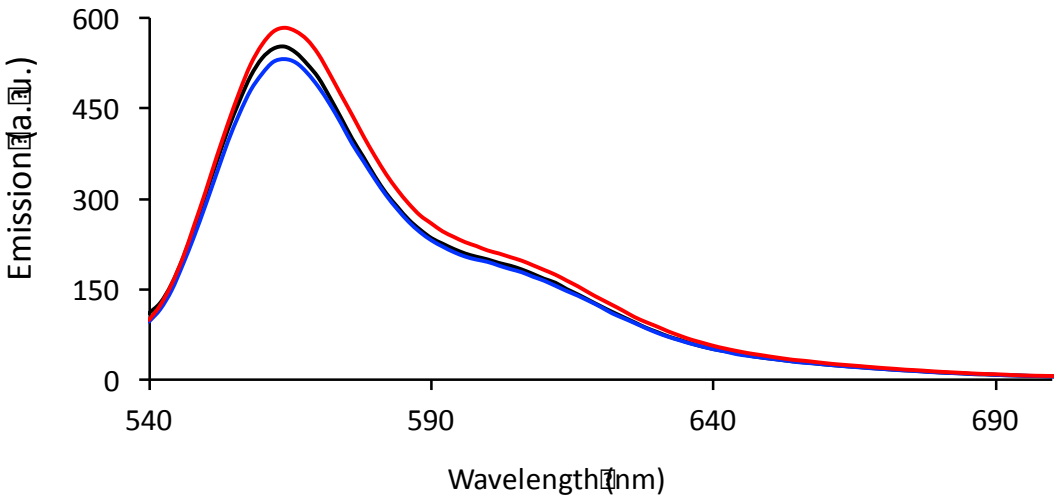


Figure S2. Steady-state emission spectra of **Cy3-linker**.

Emission of **Cy3-linker** (1 μM) free in solution (black), or in the presence of 1 μM DNA duplex oligomer: WM DNA (blue) or MM DNA (red). The DNA duplex used was a 27-mer oligonucleotide with complement: 5'-GAC CAG CTT ATC A***C***C CCT AGA TAA GCG-3' where

the MM strand contains a (***C***) at the mismatched site vs. (***G)***. In all cases fluorescence was measured in Tris buffer (200 mM NaCl, 5 mM Tris, pH 7.4) at 25 °C, λ_{Ex} = 520 nm.

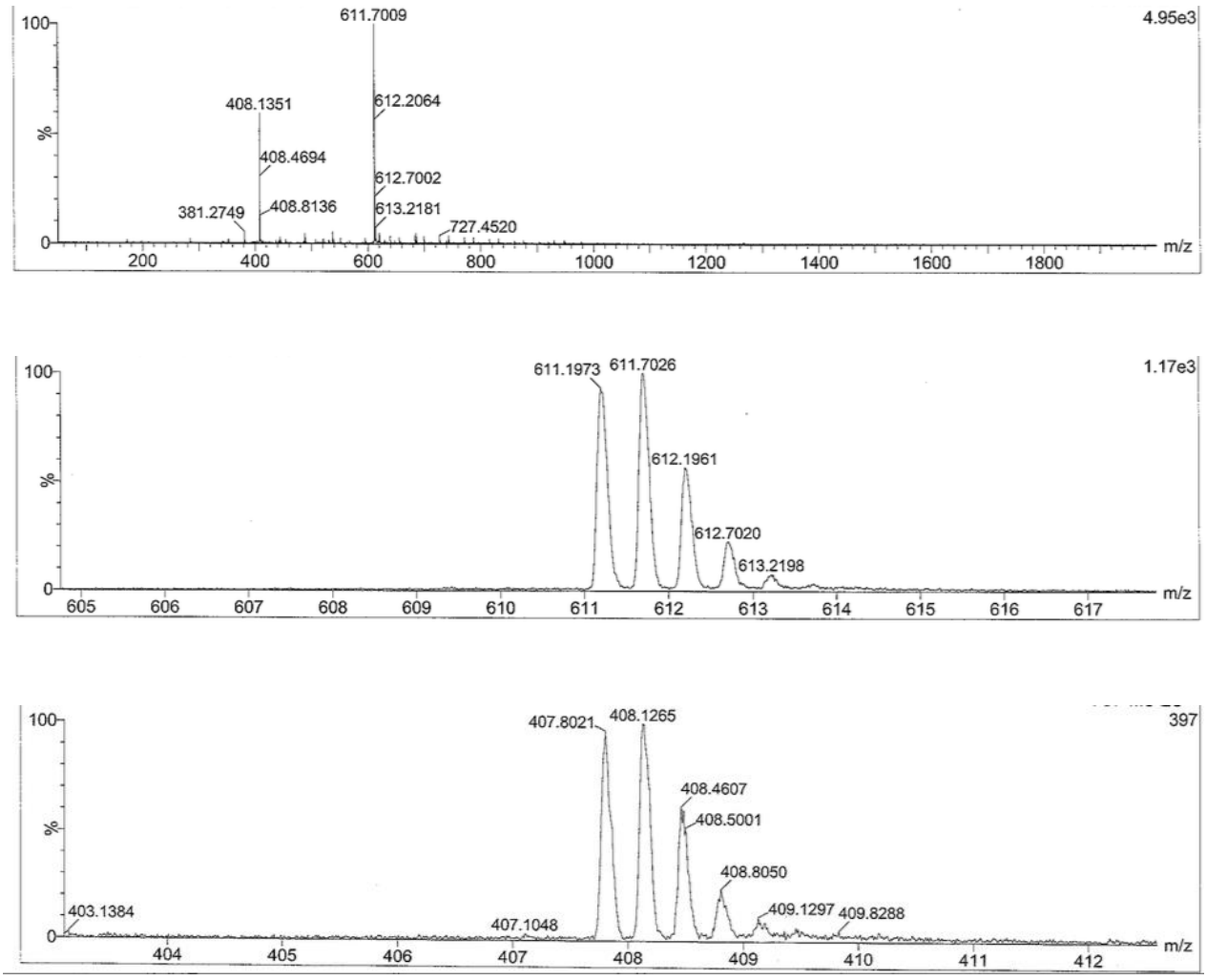


Figure S3. Full TOF-MS ES+ spectrum of **RhCy3**. (Top figure) full spectrum; (middle figure) isotopic profile of ion 2+; (bottom figure) isotopic profile of ion 3+.

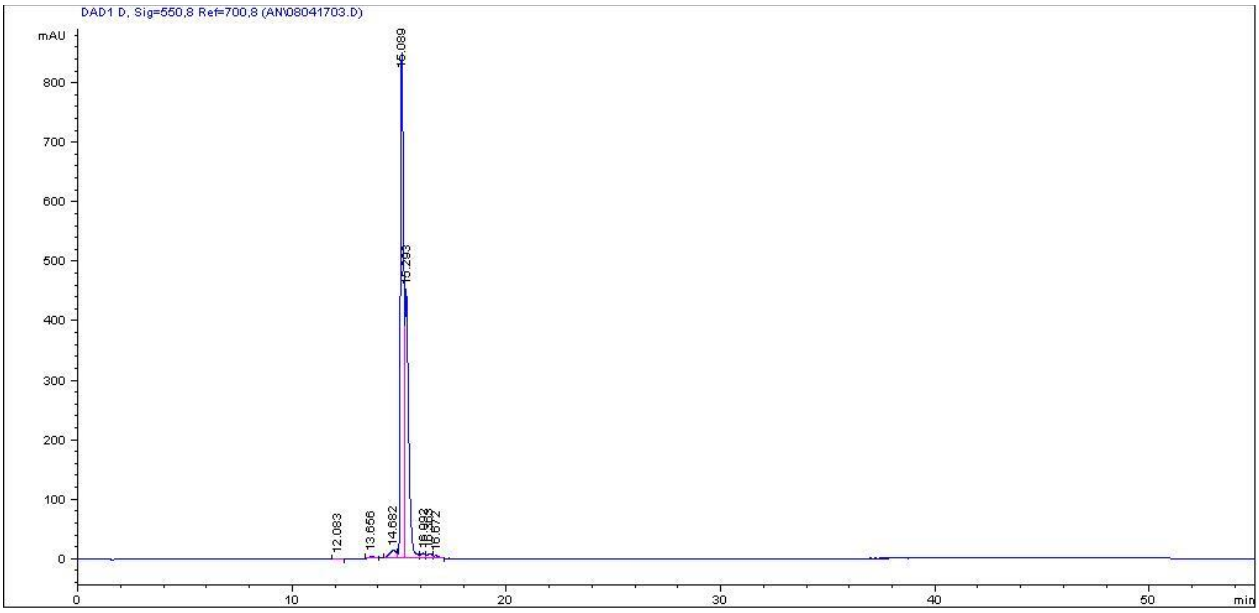


Figure S4. HPLC trace of **RhCy3**. The column was eluted with a gradient system CH_3CN/H_2O (0.1% TFA) over 60 min.

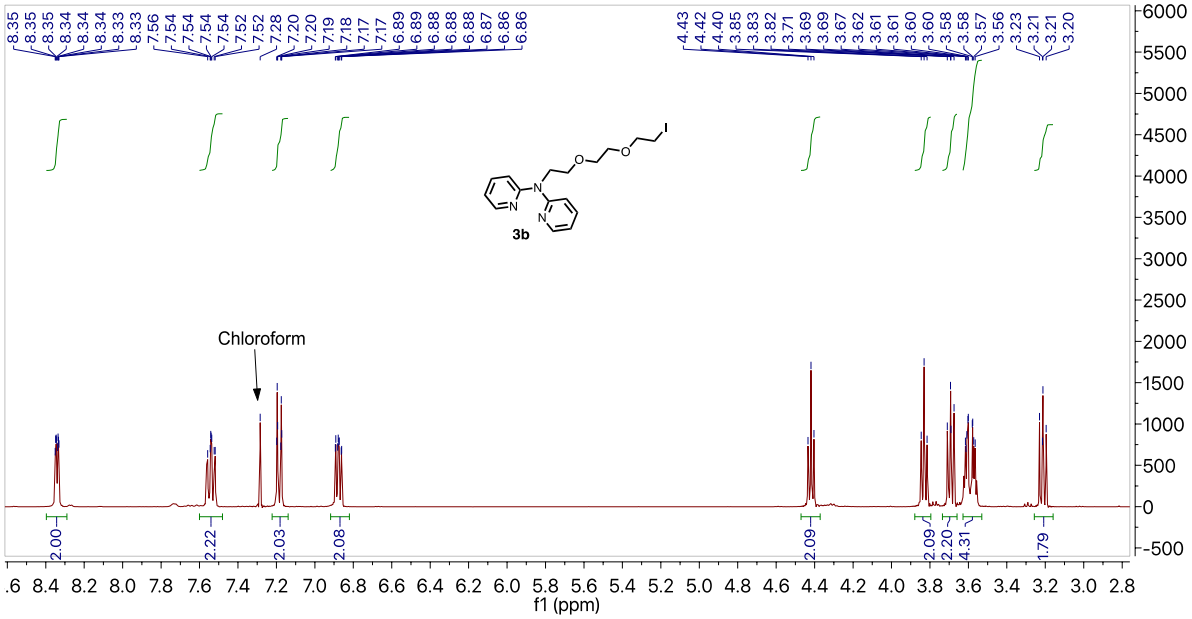


Figure S5. ^{1}**H** NMR spectrum of **3b**. The spectrum was recorded in $CDCl_3$ at room temperature.

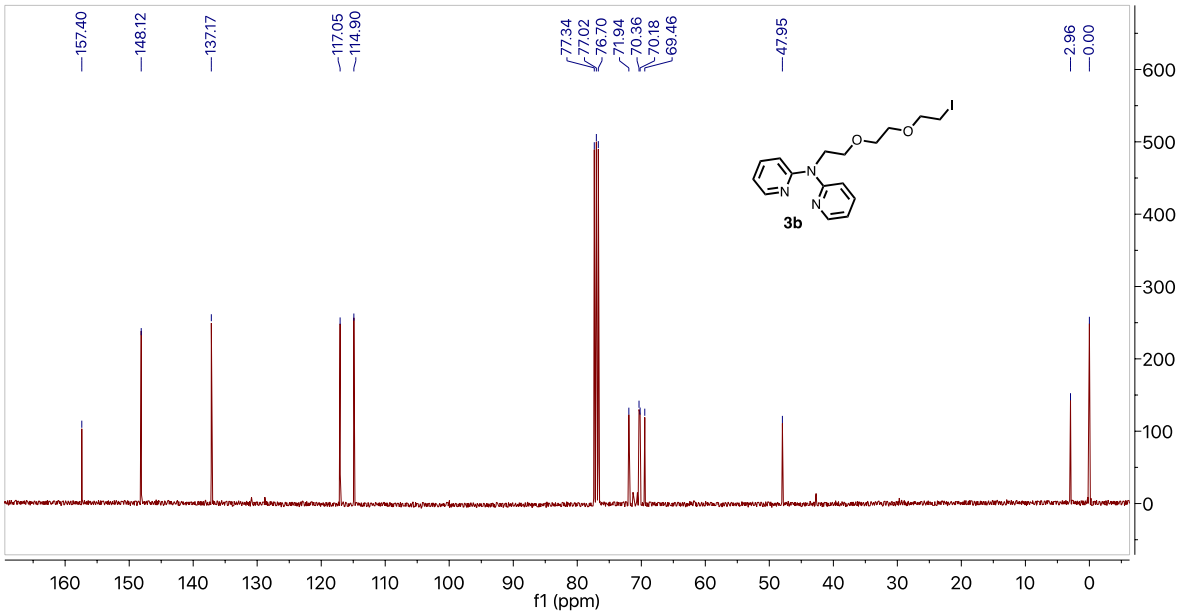


Figure S6. ^{13}C NMR spectrum of **3b**. Spectrum recorded in $CDCl_3$ at room temperature.

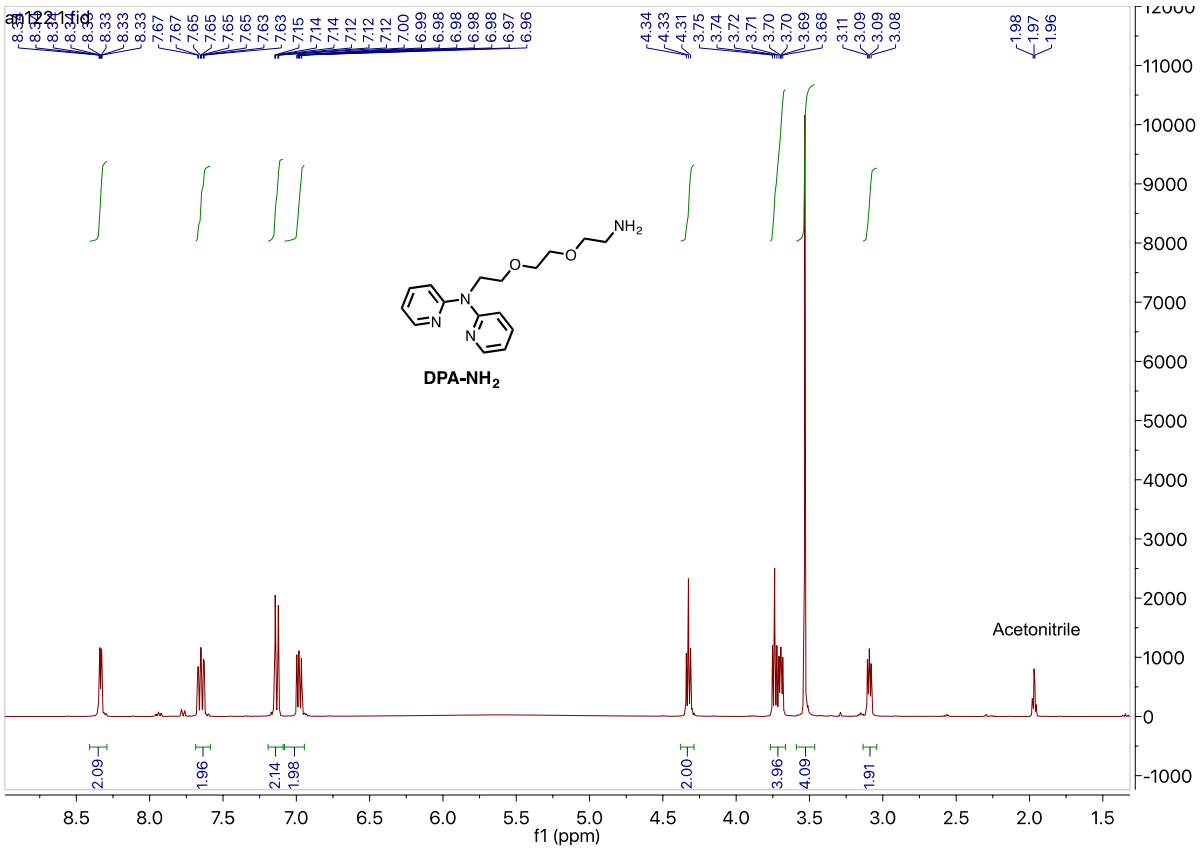


Figure S7. ^{1}H NMR spectrum of **DPA-NH$_2$**. Recorded in $CDCl_3$ at room temperature.

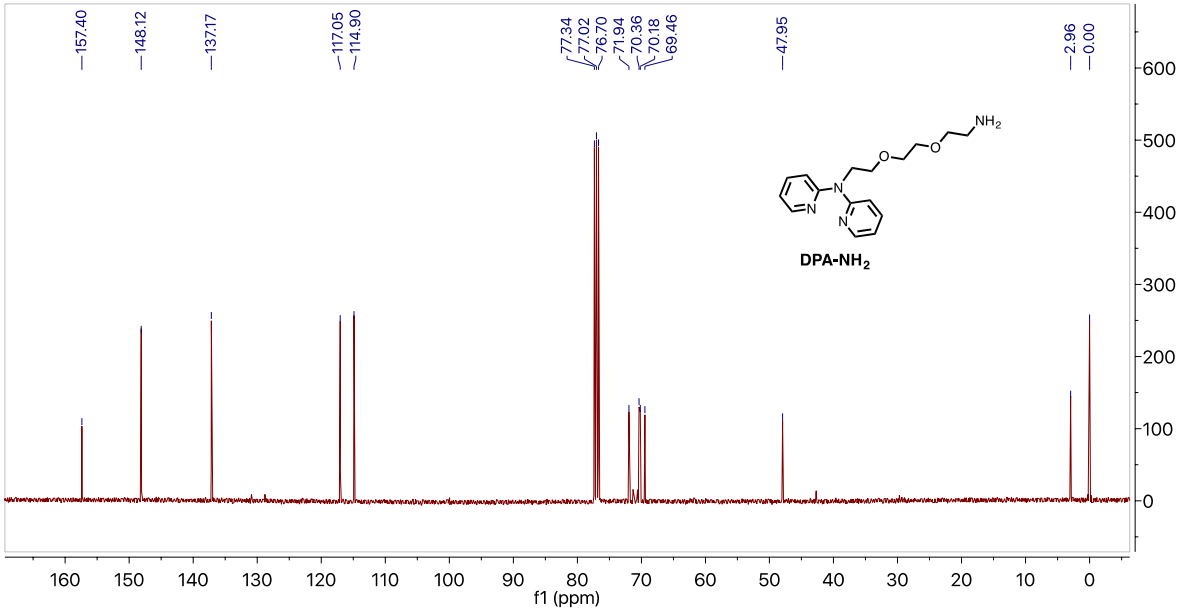


Figure S8. ^{13}C NMR spectrum of **DPA-NH$_2$**. Recorded in $CDCl_3$ at room temperature.

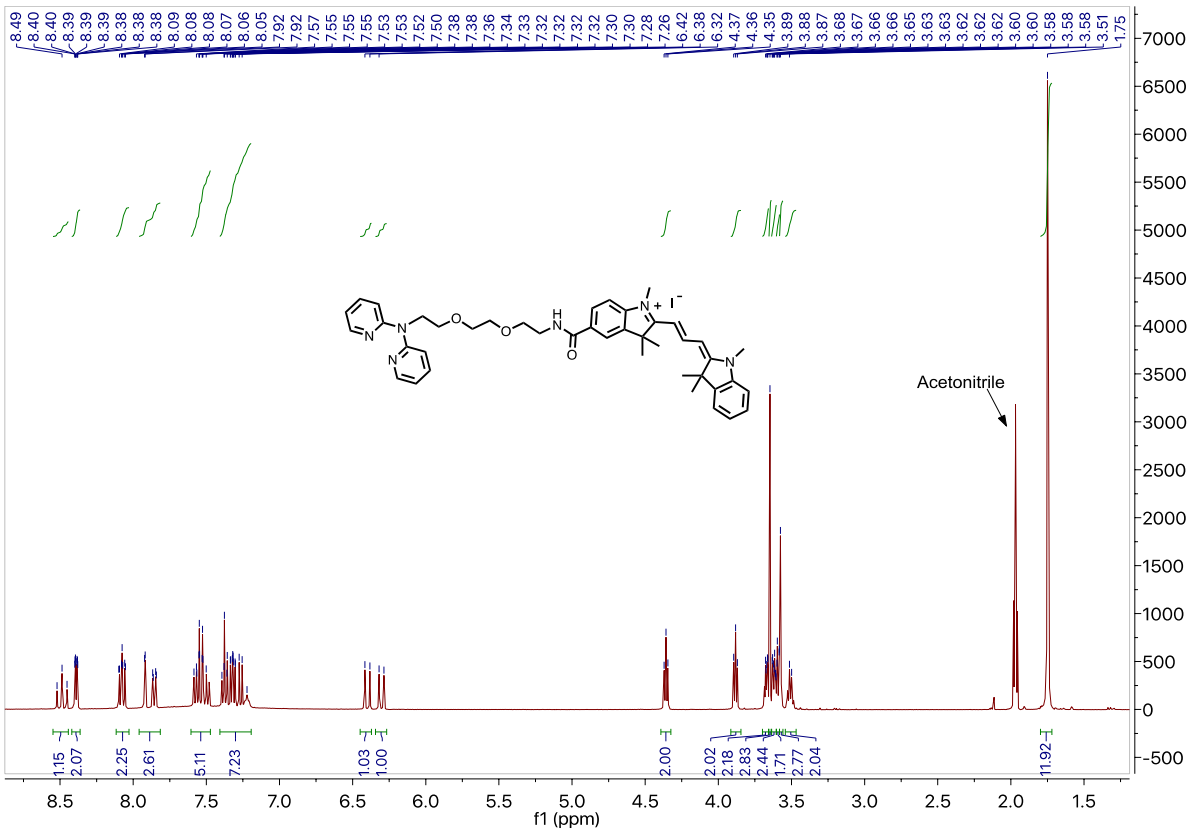


Figure S9. ^{1}H NMR spectrum of **DPA-Cy3**. Recorded in CD_3CN at room temperature.

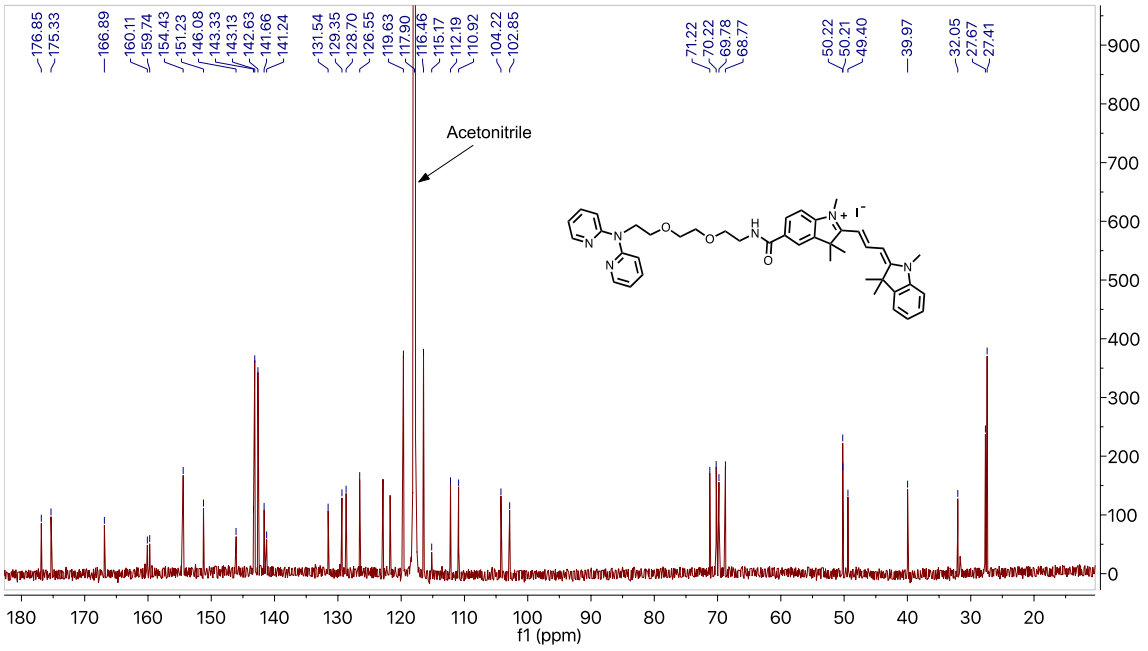


Figure S10. ^{13}C NMR spectrum of **DPA-Cy3**. Recorded in CD_3CN at room temperature.

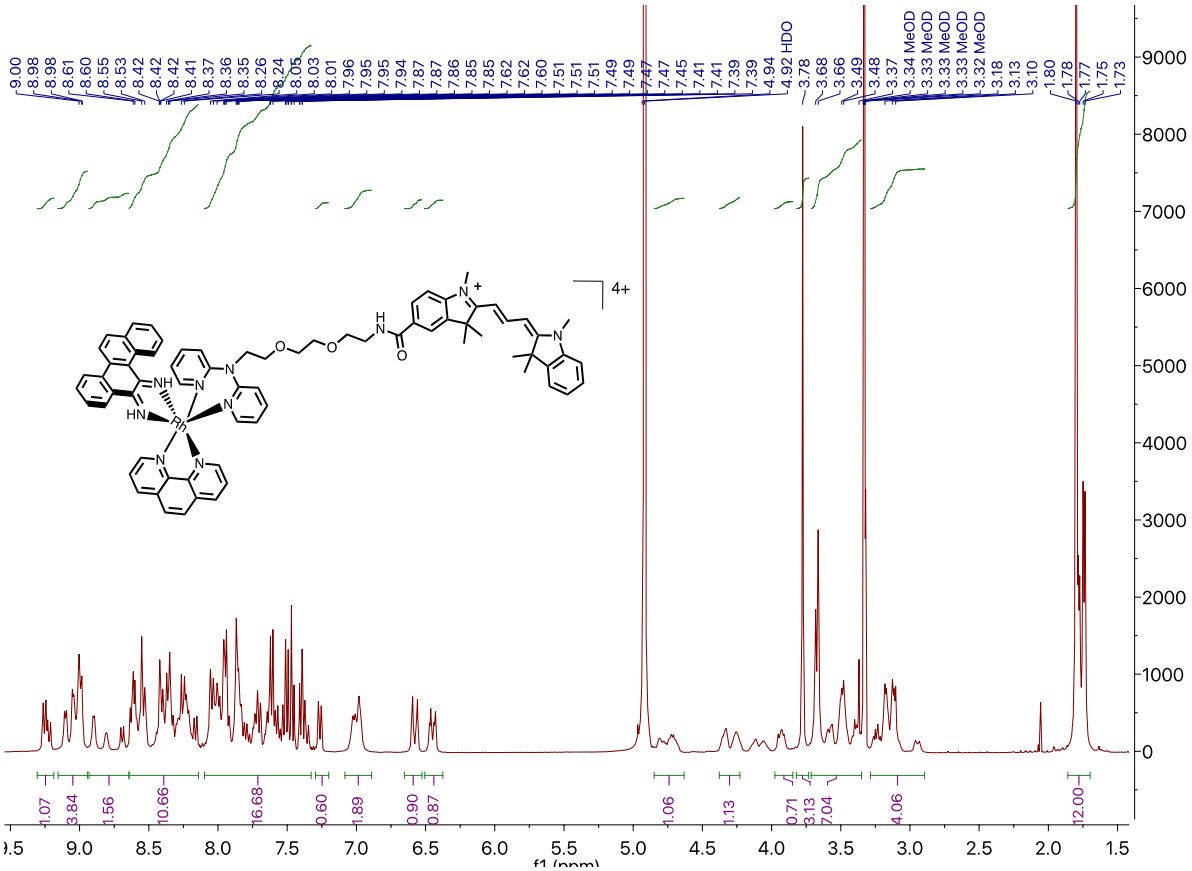


Figure S11. ^{1}H NMR spectrum of **RhCy3**. Recorded in CD_3OD at room temperature.

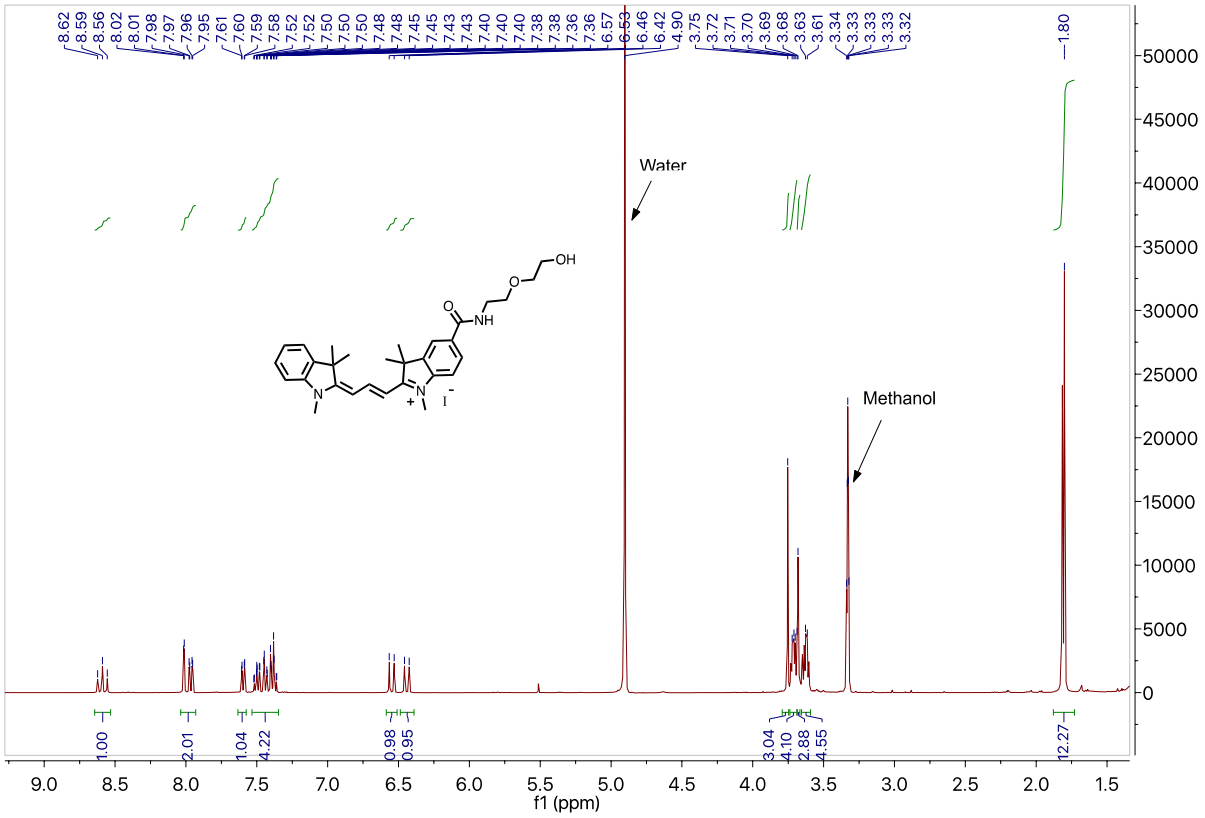


Figure S12. ^{1}H NMR spectrum of **Cy3-linker**. Recorded in CD_3OD at room temperature.

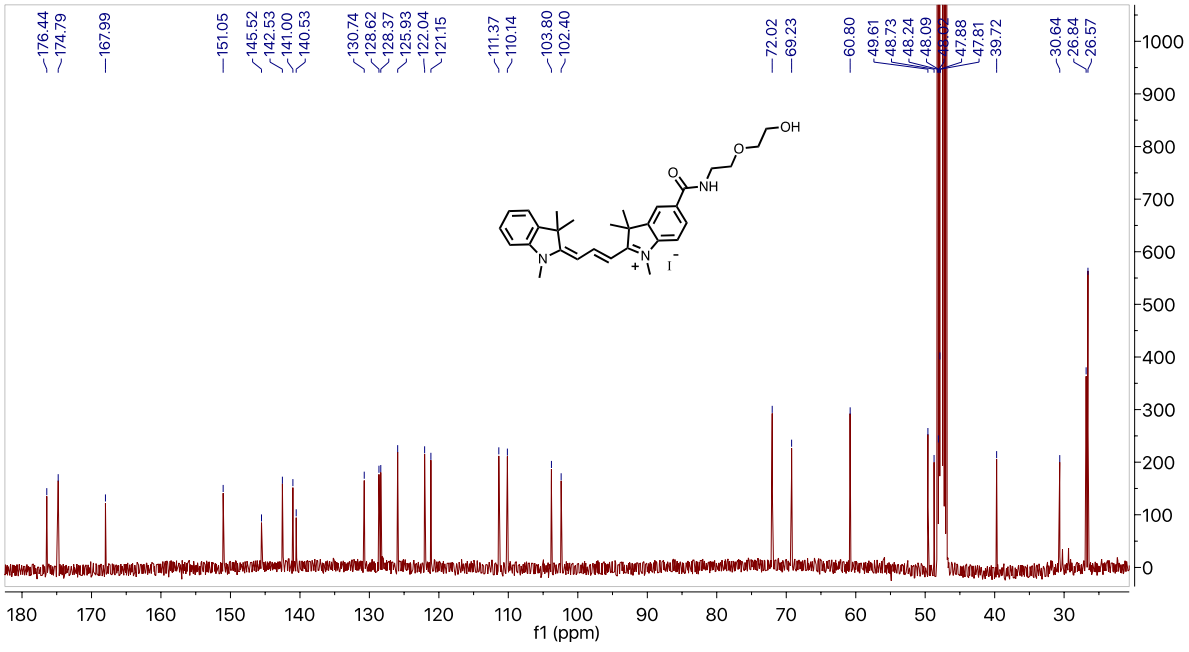


Figure S13. ^{13}C NMR spectrum of **Cy3-linker**. Recorded in CD_3OD at room temperature.

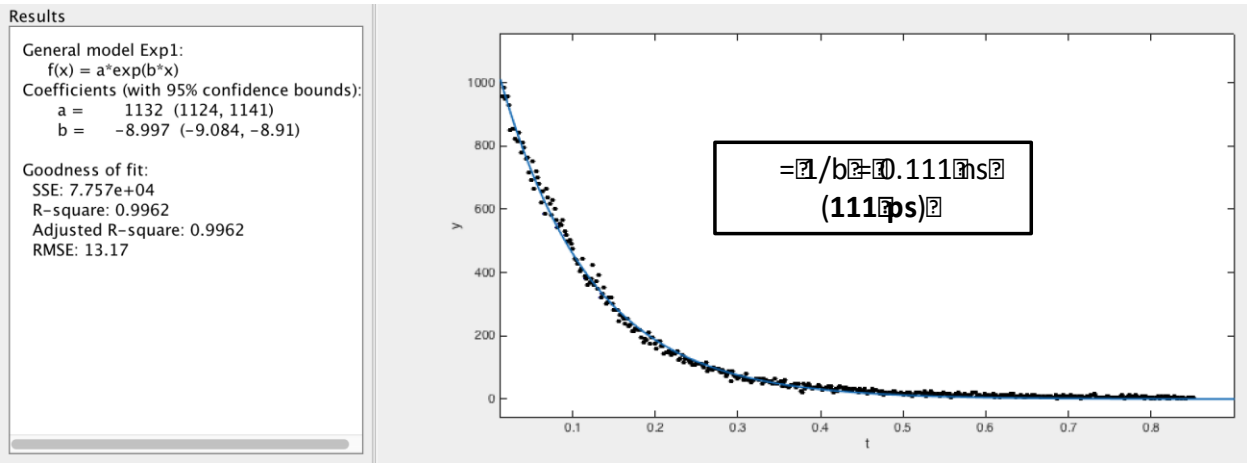


Figure S14. Fluorescence lifetime decay curve for **RhCy3** free in solution. **[RhCy3]** = 1μM in Tris buffer (5 mM Tris, 200 mM NaCl, pH = 7.4) at room temperature.

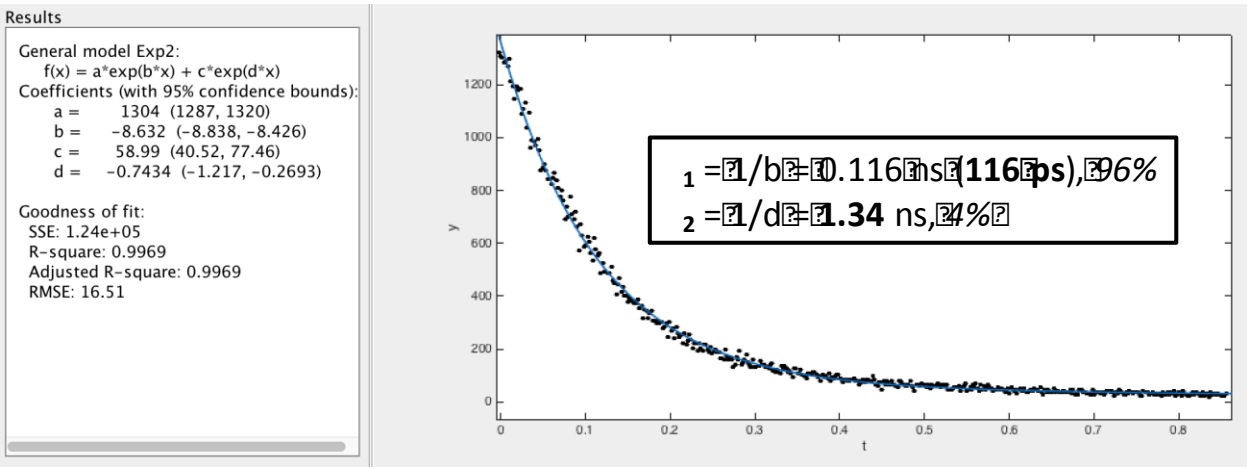


Figure S15. Fluorescence lifetime decay curve for **RhCy3** with WM DNA. **RhCy3** and WM DNA were mixed in a 1:1 ratio at 1 μM conc. in Tris buffer (5 mM Tris, 200 mM NaCl, pH = 7.4) at room temperature.

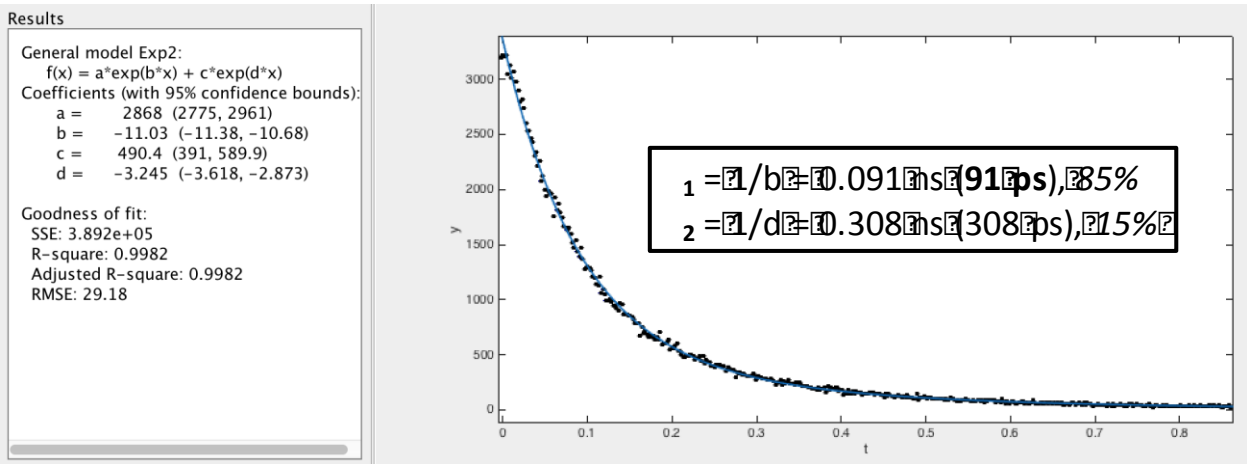


Figure S16. Fluorescence lifetime decay curve for **RhCy3** with WM DNA. **RhCy3** and WM DNA were mixed in a 1:1 ratio at 1 µM conc. in Tris buffer (5 mM Tris, 200 mM NaCl, pH = 7.4) at room temperature.

8. Quantification of mismatches in genomic DNA

From the fluorescence titrations with increasing amount of 27-mer dsDNA (WM or MM) we could extract the binding constant of **RhCy3** for the DNA. Also, based on the **RhCy3** fluorescence titrations with addition of 27-mer dsDNA containing a central CC mismatch we can extract the number of mismatches (N_{mm}) that are present in the solution and associate it to a fluorescence intensity (E_{max}):

At concentration C (DNA) = 1 µM -> E_{max} = 235 (a. u.) for a sample volume V = 400 µl

$$C = \frac{n}{V}$$

$$n = C \times V = 4 \times 10^{-10} \text{moles}$$

The moles can be converted in number of mismatches N_{mm}:

$$N_{mm} = n(N_A) = 4 \times 10^{-10} \times (6.02 \times 10^{23}) = 2408 \times 10^{11} \text{mismatches}$$

where N_A is the Avogadro's constant.

From the genomic DNA fluorescence titrations:

A concentration C = 958 µM [BP gDNA] for a sample volume V = 700 µl corresponds to approximately 133 x 10^6 genomes calculated as follow:

$$n = C \times V = (958 \times 700)10^{-12} = 6.7 \times 10^{-7} \text{moles BP}$$

Moles are converted in number of total base pairs (BP) of gDNA, BP_{tot} [gDNA]:

$$n(6.02 \times 10^{23}) = 6.7 \times 10^{-7} \times (6.02 \times 10^{23}) = 4 \times 10^{17} \text{ base pairs of DNA}$$

Knowing that the number of base pairs in the human genome is $N_{bp} = 3 \times 10^9$, we can estimate the number of genomic DNA that is associated to an emission value 235 a. u. (arbitrary unit):

$$\frac{BP_{tot}\,[gDNA]}{N_{bp}} = \frac{4 \times 10^{17}}{3 \times 10^9} = 133 \times 10^6 \text{genomes}$$

For HCT116N:

235 = 2408 x 10^{11} mutations

210 = X

$$X = \frac{210(2408 \times 10^{11})}{235} = 2151 \times 10^{11} \text{mutations}$$

Therefore, the number of mismatches estimated for HCT116N cells:

$$k_B(\text{WM})\frac{2151 \times 10^{11}}{N_g} = 0.008\frac{\mathbf{2151 \times 10^{11}}}{\mathbf{133 \times 10^6}} = 0.008(26 \times 10^5) \approx 1.3 \times 10^4 \text{/genome}$$

where k_B(WM) is the binding constant of **RhCy(3)** for the well matched 27-mer DNA; N_g is the estimated number of genomes in 958 µM of BP gDNA solution.

For HCT116O:

235 = 2408 x 10^{11} mutations

$$268 = X$$

$$X = \frac{268(2408\times10^{11})}{235} = 2746 \times 10^{11}\text{mutations}$$

The number of mismatches for HCT116O cells:

$$k_B(\text{MM})\frac{2748\times10^{11}}{\text{N}_\text{g}} = 3.1\frac{\mathbf{2746\times10^{11}}}{\mathbf{133\times10^{6}}} = 3.1(26\times10^{5}) \approx 6.4 \times 10^{6} \text{ / genome}$$

where k_B(MM) is the binding constant of **RhCy3** for the mismatched 27-mer DNA.

From these calculations it is estimated that the number of mismatches in HCT116O (MMR-deficient) is ~500-fold higher than HCT116N (MMR-proficient).

9. References

[1] Terpetsching, E.; Szmacinski, H.; Ozinskas, A.; Lakowicz, J. R. *Analytical Biochem*. **1994**, *217*, 197-204.

[2] Tomasulo, M; Kaanumal, S. L.; Sortino, S.; Raymo, F. M. *J. Org. Chem.* **2007**, *72*, 595-605.

[3] Wang, J.; Cao, W.-F.; Su, J.-H.; Tian, H.; Huang, Y.-H.; Sun, Z.-R. *Dyes Pigm*, **2003**, *57*, 171-179.

[4] Meguellati, K.; Fallah-Araghi, A.; Baret, J.-C.; El Harrak, A.; Mangeat, T.; Marques, C. M.; Griffiths, A. D.; Ladame, S. *Chem. Commun.* **2013**, *49*, 11332-11334.

[5] Saulnier, M. G.; Zimmermann, K.; Struzynski, C. P.; Sang, X.; Velaparthi, U.; Wittman, M.; Frennesson, D. B. *Tetrahedron lett.* **2004**, *45*, 397-399.

[6] Muerner, H.; Jacson, B. A.; Barton, J. K. *Inorg. Chem.* **1998**, *37*, 3007-3012.

[7] Komor, A. C.; Barton, J. K. *J. Am. Chem. Soc.* **2014**, *136*, 14160-14172.

[8] Komor, A. C.; Schneider, C. J.; Weidman, A. G.; Barton, J. K. *J. Am. Chem. Soc.* **2012**, *134*, 19223-19233.