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Materials

Unless otherwise stated, all chemicals were purchased from Sigma (St. Louis, MO) and were of the highest purity available. Oligonucleotides were purified by polyacrylamide gel electrophoresis (PAGE) and obtained from Midland Certified Reagent Company, Inc (Midland, TX, USA). The following are the sequences of oligonucleotides and their respective molar extinction coefficients (ε, x10^3 $M^{-1}cm^{-1}$): triplex R2, 5′-CCCCTCCC TTTTT GGGAGGGG CGCTTAT GGGGAGGG-3′ (366.0); triplex GT, 5′-GGGG TTTT GGGG TTTT GGGG AAAA GGGG TTTT CCCC TTTT CCCC-3′ (446.4); hairpin duplex R2D, 5′-CCCCTCCC TTTTT GGGAGGGG-3′ (201.1); linear duplex DS12, 5′-CTT GAG CTC AAG-3′ (129.0); and G4-DNA G4Htel, 5′-TTA GGG TTA GGG TTA GGG TTA GGG TTA-3′ (303.4).

We determined that the full-length oligonucleotides migrated as single bands by using denaturing PAGE [7 M urea, 89 mM Tris-borate, 2 mM EDTA (TBE)] on 20% acrylamide gels stained with SYBR Gold (Thermo Fisher, Waltham, MA). Concentrations of oligonucleotide stock solutions (prepared in nuclease-free water) were calculated using their respective molar extinction coefficients, and their absorbances were measured at 260 nm using a NanoDrop 2000 Spectrophotometer (Thermo Scientific, Wilmington, DE). Oligonucleotides were kept at -20°C until resuspended. Oligonucleotide solutions were re-suspended in assay buffer and annealed by heating to 95 °C for 5 min, followed by slow cooling to room temperature.

Fluorescence assays were performed in a Corning black 384-well plate obtained from Thermo Scientific (Waltham, MA, catalog #: 3821).

Procedures

Development of the coralyne-based, ‘light-up’ intramolecular triplex assay

To probe the dose-dependent RFU change of coralyne, we titrated varying concentrations of triplex-forming R2 with 1 μM coralyne in 1x assay buffer composed of 20 mM, Tris, pH 7.4, and 0.01% Tween 20 in a 20 μL assay volume. In detail, 10 μL of various concentrations of R2 (0 –

800 nM) was mixed with 10 μL of a 1 μM coralyne solution and the fluorescence was observed after a 2 h incubation at room temperature. In investigating the binding affinity of coralyne with different structure-forming sequences as substrates, we assayed equal volumes of 100 nM of the DNA substrate with 1 μM coralyne. The assay buffer used depended on the DNA structure: for R2, GT and R2D - 20 mM, Tris, pH 7.4, 0.01% Tween 20; for G4-DNA - 20 mM sodium cacodylate pH 7.0, 0.1 mM EDTA, 110 mM KCl, 0.01% Tween 20; for DS12 - 20 mM sodium cacodylate pH 7.0, 100 mM NaCl, 0.1 mM EDTA, 10 mM $MgCl_2$, 0.01% Tween 20. The fluorescence signal of coralyne was read on a black 384-well plate with excitation and emission at 424/9.0 and 494/9.0 nm, respectively, using a Synergy H4 plate reader (Biotek, Winooski, VT). An unpaired t-test was utilized to compare mean % RFU change between R2 and G4Htel substrates.

The BePI and doxorubicin competition assay was conducted by assaying 10 μL of varying concentrations of BePI (or doxorubicin) dissolved in water, with an equal volume of 2x coralyne: R2 complex solution in 2x buffer (40 mM Tris pH 7.4, 0.02% Tween 20). The final concentrations of coralyne and R2 were 500 and 50 nM, respectively. Solutions were incubated for 2 h at room temperature before reading.

Coralyne affinity and % RFU change experiments in the presence of BePI and Dox using another triplex-forming sequence, the GT triplex, were conducted following the procedures described above.

UV thermal denaturation experiments

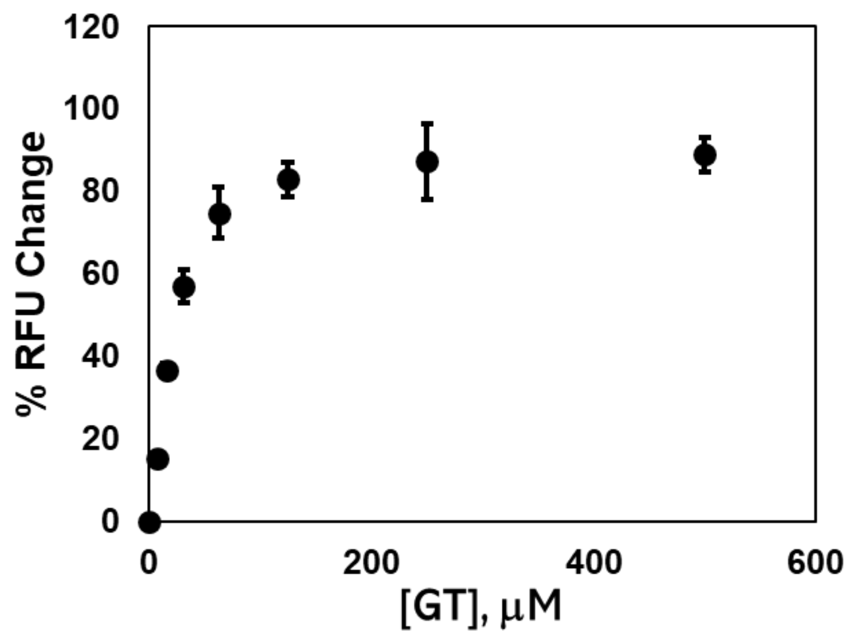
To determine the thermal melting temperature of R2 (0.5 μM) in the absence or presence of BePI (0.5 μM) or doxorubicin (0.5 μM), DNA solutions were annealed using Tris buffer (20 mM, Tris, pH 7.4). Thermal denaturation of the DNA solutions in 0.4 cm quartz cuvettes was monitored from 30–100 °C at 260 nm with a ramp rate of 0.4 °C/min using a Cary 4000 UV-Vis equipped with a Peltier temperature controller (Santa Clara, CA). The end point of the melting curve was

normalized to Abs = 1.0, and the thermal melting temperature (T_m) calculated as the maximum value of the first derivative of the absorbance versus temperature, using the thermal application of Cary WinUV Ver 6.2 software (Santa Clara, CA).

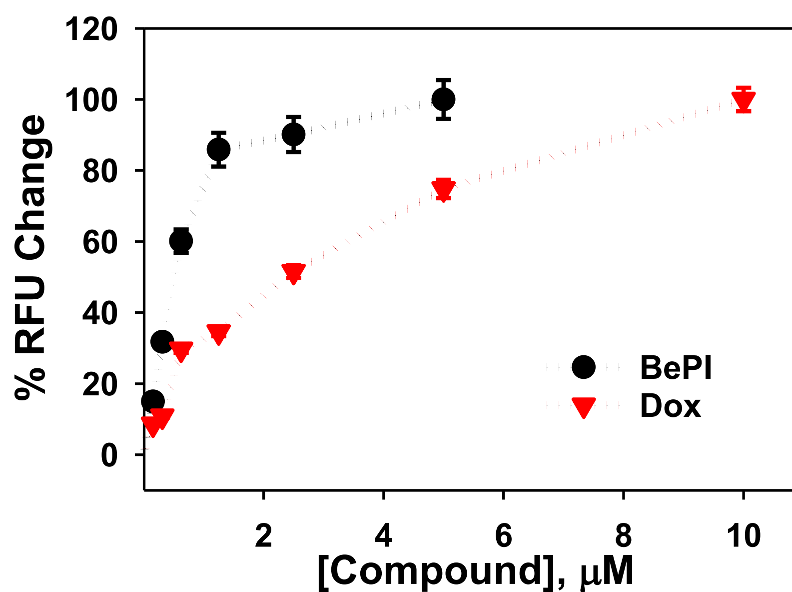
FID assay performance validation

Master mixtures of both positive and negative controls were prepared as follows: the negative control contained 500 nM coralyne and 0.1% DMSO in 1x assay buffer (20 mM, Tris, pH 7.4, 0.01% Tween 20) and the positive control contained 50 nM R2, 500 nM coralyne, and 0.1% DMSO in 1x assay buffer (20 mM, Tris, pH 7.4, 0.01% Tween 20). To a Corning 384-well plate, 5 μL of each control solution was dispensed using an electronic pipettor as illustrated in ESI-Figure 3. Columns 11, 22, 23, and 24 were left empty to monitor instrument stability over the time. Fluorescence signals were monitored at different time points (1, 2, 3, and 24 h) to evaluate room temperature stability.

Supplementary data



ESI-Figure 1. Dose-dependent % RFU change of coralyne in the presence of the GT triplex. $[C_{50}] \approx 30$ nM.



ESI-Figure 2. Dose-dependent % RFU change of coralyne in the competitive displacement assay using the GT triplex. $[C_{50}]_{BePI} \approx 0.5$ µM, $[C_{50}]_{Dox} \approx 2.5$ µM.

Negative controls

Positive controls

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	8841	7452	7164	7148	7748	7375	7556	7381	7300	7071	889	1693	1649	1686	1662	1650	1657	1650	1676	1662	1627	856	953	926
B	7227	7339	7058	7060	6465	7675	7089	7505	7089	7351	806	1781	1598	1590	1628	1562	1715	1832	1690	1658	1551	835	933	940
C	8343	7468	7428	7152	7438	7513	7625	7563	7353	7169	844	1623	1641	1647	1665	1726	1693	1770	1761	1667	1743	937	984	974
D	7523	7542	7066	7076	6363	7600	7062	7467	7003	7647	835	1756	1612	1640	1726	1646	1751	1717	1677	1636	1588	861	948	925
E	8235	7459	7373	7419	7476	7668	7451	7595	7465	7194	870	1628	1714	1691	1663	1666	1817	1695	1627	1593	1795	854	876	1022
F	7301	7306	6939	7171	6442	7395	7219	7260	7303	7451	896	1709	1679	1635	1707	1674	1640	1590	1662	1781	1528	893	902	950
G	8573	7398	7453	7209	7419	7437	7538	7460	7492	7292	871	1637	1778	1784	1764	1733	1720	1593	1856	1562	1714	894	993	941
H	7678	7386	7280	7200	6741	7454	7128	7524	7363	7373	847	1803	1765	1617	1719	1736	1801	1824	1545	1827	1534	843	932	896
I	7770	7320	7403	7440	7559	7405	7295	7527	7433	7368	823	1753	1692	1744	1702	1706	1611	1779	1801	1582	1708	840	891	880
J	7483	7267	7102	7086	6490	7595	7289	7522	5983	7386	874	1762	1767	1703	1643	1668	1563	1832	1584	1564	1794	855	920	967
K	8020	7560	7372	7129	7569	7760	7560	7519	7246	7558	868	1713	1684	1750	1635	1638	1742	1601	1758	1781	1717	884	919	902
L	7214	7386	7057	6214	6692	7678	7066	7487	7277	7657	1040	1629	1747	1747	1628	1680	1611	1629	1622	1717	1641	873	915	877
M	8123	7401	7355	7510	7372	7947	7326	7541	7229	7106	898	1639	1682	1698	1716	1623	1794	1698	1766	1715	1724	858	873	933
N	7330	7294	7126	7059	6977	7050	7334	7417	6457	8128	875	1819	1728	1794	1694	1669	1702	1669	1593	1674	1659	872	959	899
O	8195	7256	7427	6507	7157	7696	6596	7139	6436	7765	904	1820	1738	1738	1563	1708	1808	1819	1870	1692	1716	875	888	954
P	7199	7246	6948	7094	6268	7152	7765	6510	6637	6235	931	1761	1563	1927	1868	1559	1585	1716	1624	1719	1703	887	953	870

ESI-Figure 3. Plate layout and typical readouts of the FID assay shown in a 384-well plate format.