The 7th International Electronic Conference on Medicinal Chemistry (ECMC 2021)
01–30 NOVEMBER 2021 | ONLINE

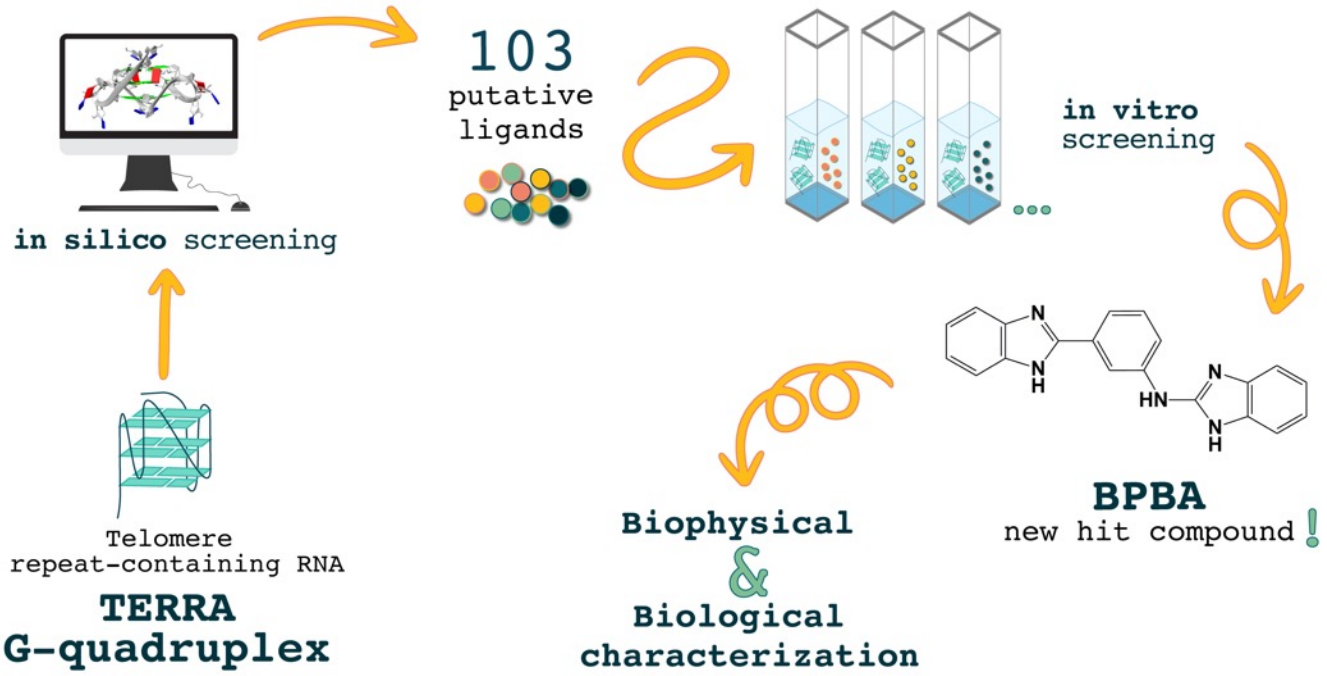
Targeting of telomeric repeat-containing RNA G-quadruplexes: From screening to biophysical and biological characterization of a new hit compound

Jussara Amato [1],* Simona Marzano [1], Eleonora Vertecchi [4], Bruno Pagano [1], Nunzia Iaccarino [1], Anna Di Porzio [1], Stefano De Tito [2,3], Erica Salvati [4] and Antonio Randazzo [1]

[1] Department of Pharmacy, University of Naples Federico II, Via D. Montesano 49, 80131 Naples, Italy;
[2] Molecular Cell Biology of Autophagy, The Francis Crick Institute, 1 Midland Road, London NW1 1AT, UK;
[3] Institute of Experimental Endocrinology and Oncology, National Research Council, 80131 Naples, Italy;
[4] c/o Department of Biology and Biotechnology "C. Darwin", Institute of Molecular Biology and Pathology, National Research Council, Sapienza University of Rome, Via degli Apuli 4, 00185 Rome, Italy

* Corresponding author: jussara.amato@unina.it

Targeting of telomeric repeat-containing RNA G-quadruplexes: From screening to biophysical and biological characterization of a new hit compound



International Journal of
Molecular Sciences

MDPI

Article

Targeting of Telomeric Repeat-Containing RNA G-Quadruplexes: From Screening to Biophysical and Biological Characterization of a New Hit Compound

Simona Marzano [1], Bruno Pagano [1], Nunzia Iaccarino [1], Anna Di Porzio [1], Stefano De Tito [2,3], Eleonora Vertecchi [4], Erica Salvati [4], Antonio Randazzo [1] and Jussara Amato [1,*]

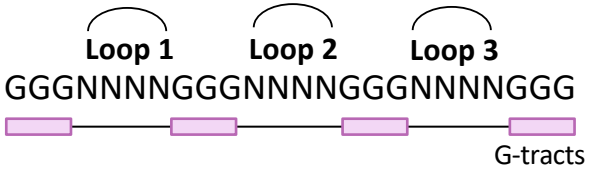
Abstract: DNA G-quadruplex (G4) structures, either within gene promoter sequences or at telomeres, have been extensively investigated as potential small-molecule therapeutic targets. However, although G4s forming at the telomeric DNA have been extensively investigated as anticancer targets, few studies focus on the telomeric repeat-containing RNA (TERRA), transcribed from telomeres, as potential pharmacological targets. Here, a virtual screening approach to identify a library of drug-like putative TERRA G4 binders, in tandem with circular dichroism melting assay to study their TERRA G4-stabilizing properties, led to the identification of a new hit compound. The affinity of this compound for TERRA RNA and some DNA G4s was analyzed through several biophysical techniques and its biological activity investigated in terms of antiproliferative effect, DNA damage response (DDR) activation, and TERRA RNA expression in high vs. low TERRA-expressing human cancer cells. The selected hit showed good affinity for TERRA G4 and no binding to double-stranded DNA. In addition, biological assays showed that this compound is endowed with a preferential cytotoxic effect on high TERRA-expressing cells, where it induces a DDR at telomeres, probably by displacing TERRA from telomeres. Our studies demonstrate that the identification of TERRA G4-targeting drugs with potential pharmacological effects is achievable, shedding light on new perspectives aimed at discovering new anticancer agents targeting these G4 structures.

Keywords: TERRA G-quadruplex; drug discovery; biophysical characterization; conformation-selective ligand; in vitro biological assays

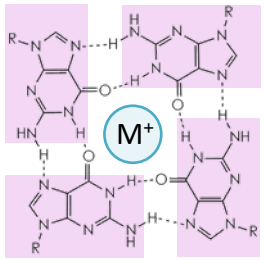
https://www.mdpi.com/1422-0067/22/19/10315/pdf

G-quadruplex structures

G-quadruplexes (G4s) are four stranded nucleic acid structures formed by guanine rich DNA or RNA sequences with a consensus motif $G_{\geq 3}N_{1\text{-}7}G_{\geq 3}N_{1\text{-}7}G_{\geq 3}N_{1\text{-}7}G_{\geq 3}$. The core scaffold of a G4 is the G-tetrad. Two or more G-tetrads can self-associate into vertical stacks giving rise to G4s, which are extremely stable structures. G4s can adopt a wide variety of topologies, depending on the number and orientation of the strands, and size of the loops which connect the G-rich repeats.

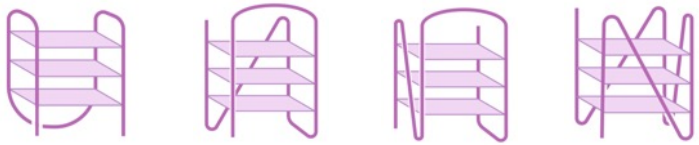


G-tetrad



M$^+$ = $K^+ > Na^+ > NH_4^+ > Li^+$

G-quadruplexes



intra-molecular

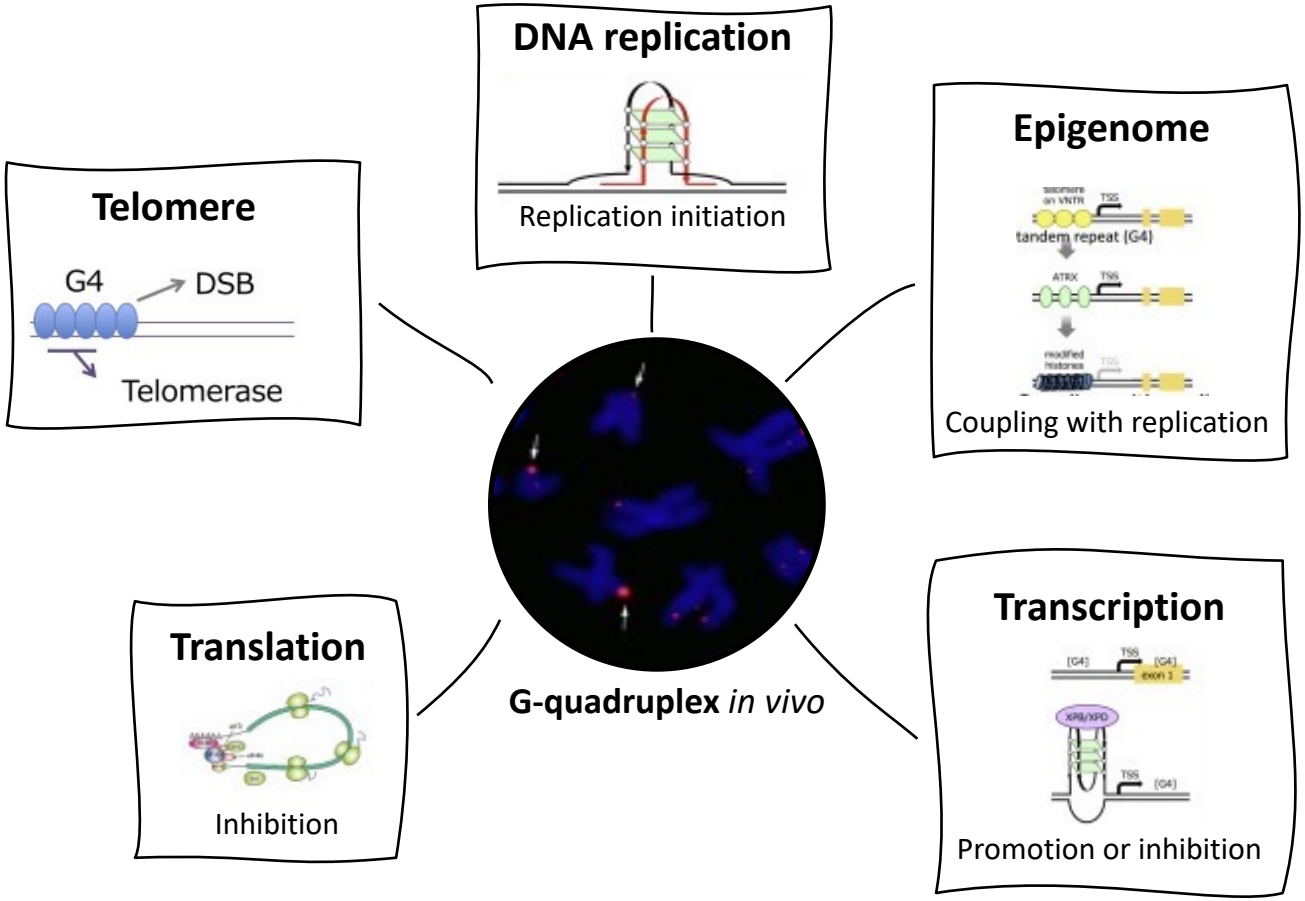
...

inter-molecular

Burge, S. et al., *Nucleic Acids Res.* **2006**, 34, 5402–5415.

G-quadruplex biological relevance

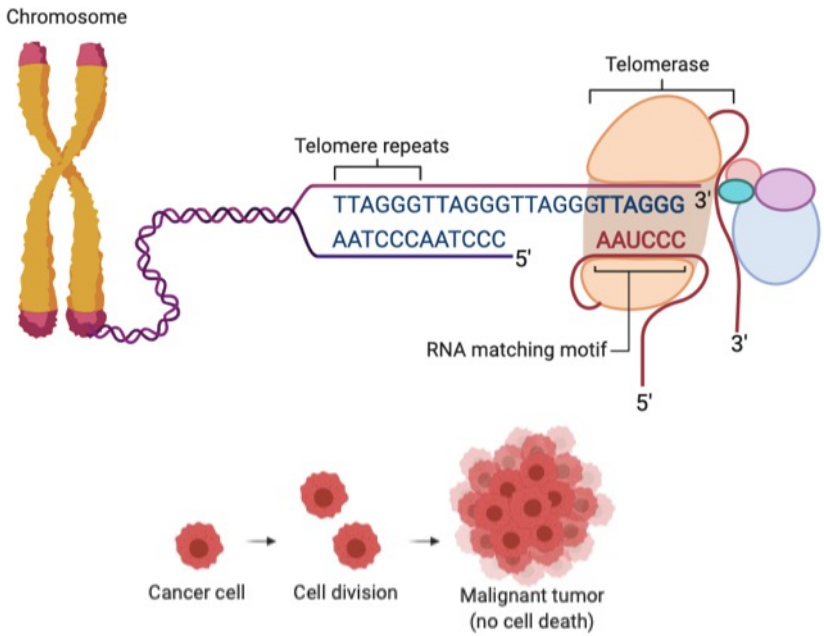
Accumulating evidence shows that G4 structures are formed in living cells, and play pivotal roles in regulating cell processes

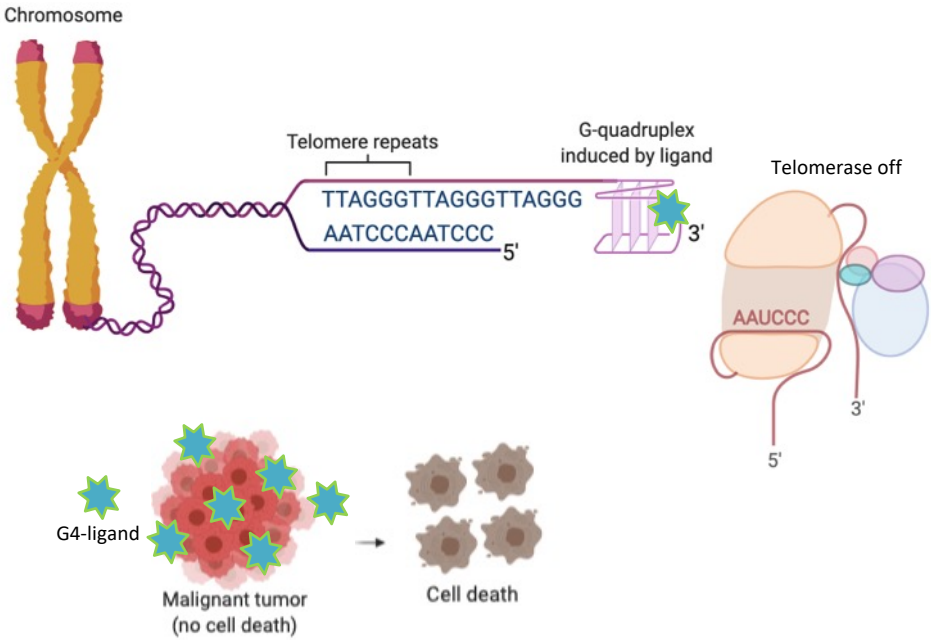


Telomeres and telomerase

Telomeres are specialized DNA-proteins complexes located at the ends of chromosomes. In human, the DNA part of telomeres comprises double-stranded «TTAGGG» repeats together with a 3’ single-stranded overhang of 50−500 nucleotides, which can adopt G4 structures.

Small molecules able to stabilize G4 formation at telomeric level can inhibit the **telomerase**, an enzyme which is overexpressed in cancer cells with the role of inducing uncontrolled cell division, and thus induce **cell death.**

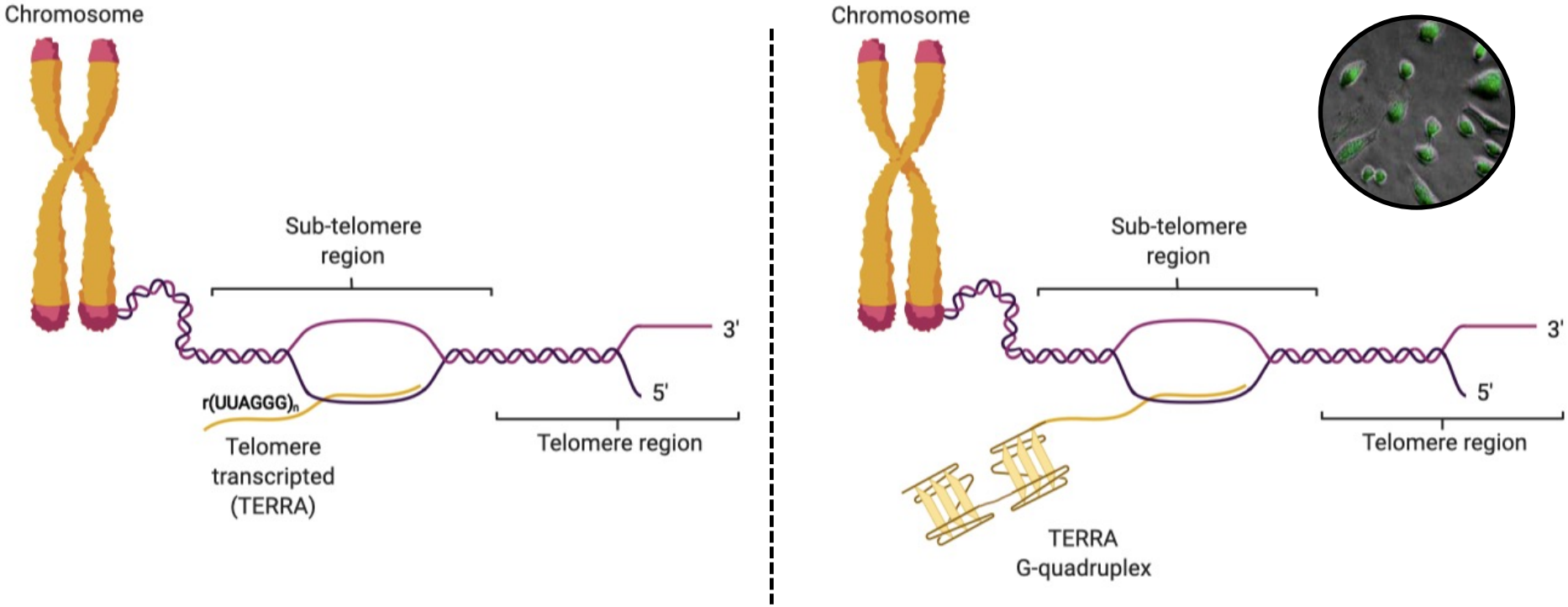




Bryan, T.M., *Molecules* **2020**, 25, 3686.

Telomeric repeat-containing RNA G-quadruplexes

The sub-telomere region is transcribed into a long noncoding RNA, called **TERRA**, which has a canonical G-rich motif of r(UUAGGG) sequence. As such, TERRA can fold into G4 structures as well.

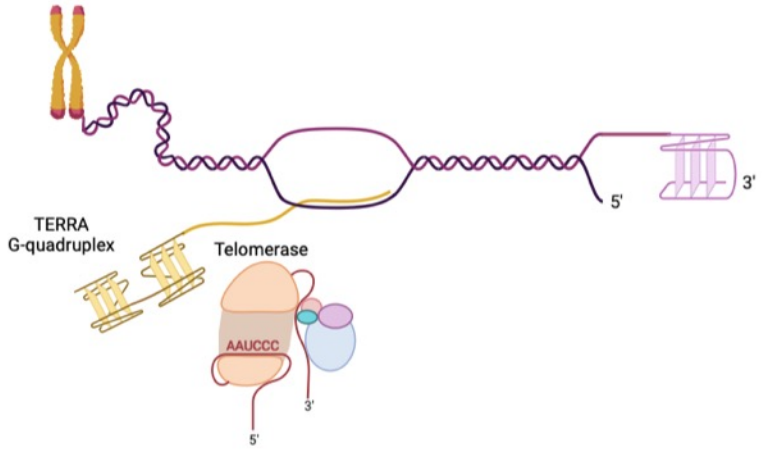


Xu, Y., *Telomere Telomerase* **2016,** 3: e1455.

Why targeting TERRA G4?

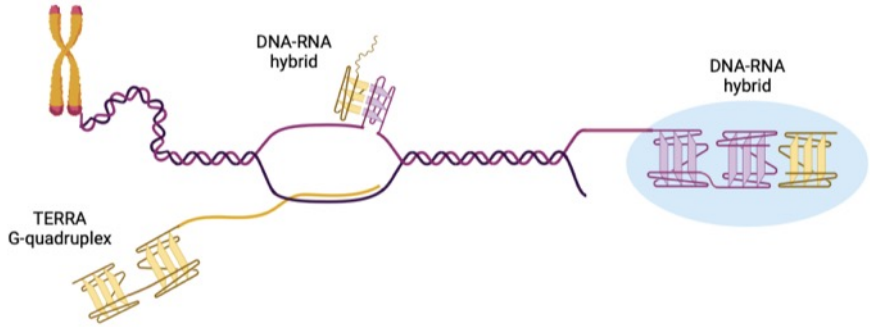
1 **Telomerase-positive** cancer cells:

TERRA G4 may bind and sequester telomerase and prevent its access to the telomere



2 **Telomerase-negative** cancer cells**,** which activate a mechanism of **Alternative Lengthening of Telomeres** (**ALT**):

TERRA could physically interact with telomeric chromatin by forming RNA-DNA hybrids that are required for telomere homeostasis.



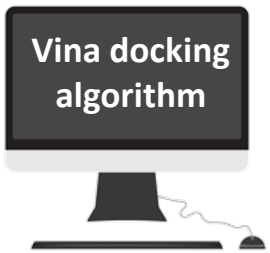
Moye, A., *Nat Commun* **2015**, 6, 7643.
Bryan, T.M., *Molecules* **2020**, 25, 3686.
Xu, Y., *Telomere Telomerase* **2016,** 3: e1455.

Ligand-induced persistent *TERRA G4* formation and accumulation, is supposed to displace TERRA from telomeres and induce telomere dysfunction, leading to cell death.

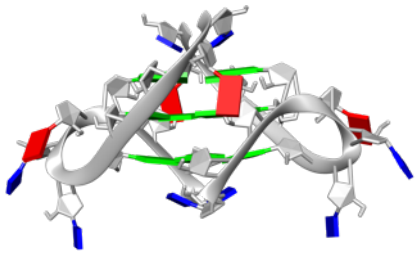
TERRA should be a valuable **target** for **anticancer drugs** directed against **telomeres**!

… even more than its DNA counterpart!

High-throughput *in silico* screening



1. A receptor-based virtual-screening approach was used to identify druggable RNA **hot spots.**



r(UAGGGUUAGGGU)

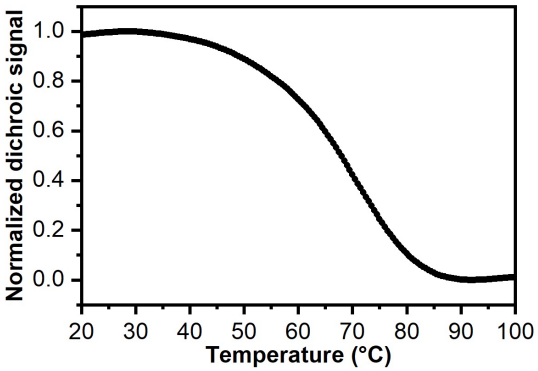
2. Docking calculations, restricted to the identified binding sites, were performed by using a diverse set of **58870** commercially available compounds as a screening library.

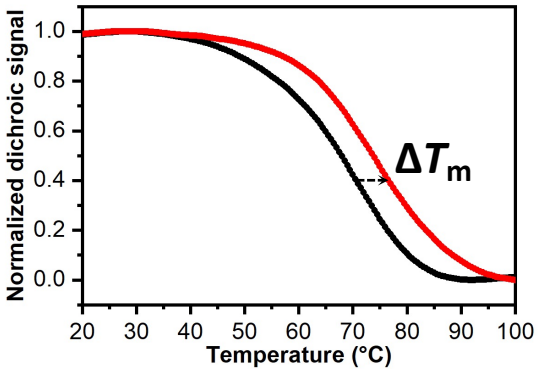
mcule
·MORE·THAN·MOLECULES·

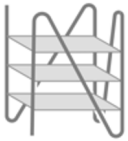
Identification of
103
Putative ligands

Experimental validation of the putative hits

The **G4 stabilizing properties** of the 103 putative hits were evaluated by performing CD melting assays, measuring the ligand-induced change in the apparent melting temperature (ΔT_m) of the G4 structure.







G-quadruplex + **putative ligand** = **G-quadruplex-Ligand complex!**

T_m **(G4) = x**

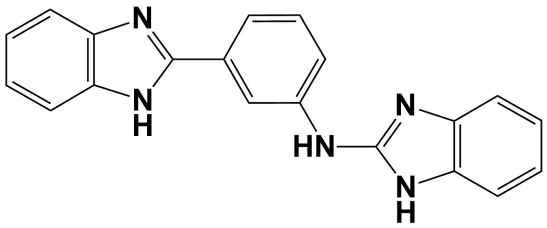
T_m **(G4-L) > x**

$$\Delta T_m = T_m \text{(G4-L)} - T_m \text{(G4)} > 0$$

Screening by circular dichroism (CD) melting experiments

103

Putative ligands



N-[3-1H-1,3-benzodiazol-2-yl)phenyl]-1H-1,3-benzodiazol-2-amine

BPBA

Melting temperature variations for *TERRA G4* upon addition of 10 molar equiv of ligands determined by CD melting experiments.

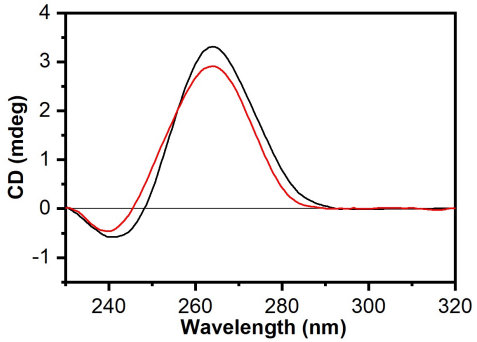
ΔT_m (°C) = T_m (TERRA + 10 molar equiv of **ligand**) (°C)− T_m (TERRA) (°C)

$\Delta T_m \leq 0$; $0 < \Delta T_m < 2.0$ °C	$2.0\ °C \leq \Delta T_m < 4.0$ °C	$\Delta T_m \geq 4.0$ °C

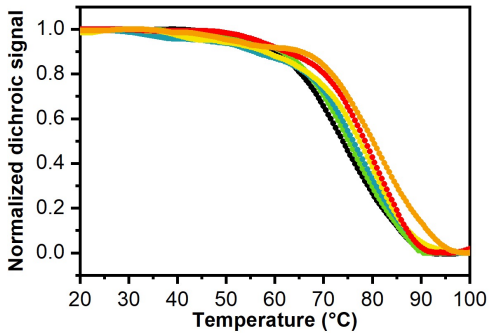
1	2	3	4	5	6	7	8	9	10	11	12	13
14	15	16	17	18	19	20	21	22	23	24	25	26
27	28	29	30	31	32	33	34	35	36	37	38	39
40	41	42	43	44	45	46	47	48	49	50	51	52
53	54	55	56	57	58	59	60	61	62	63	64	65
66	67	68	69	70	71	72	73	74	75	76	77	78
79	80	81	82	83	84	85	86	87	88	89	90	91
92	93	94	95	96	97	98	99	100	101	102	103	

Biophysical investigation of BPBA binding to TERRA G4

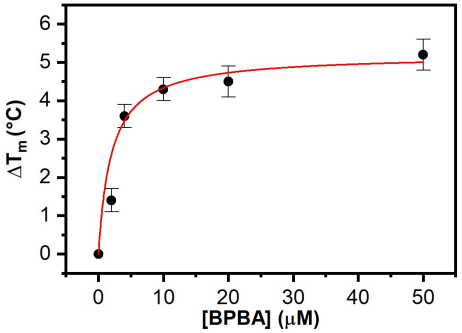
CD spectra of *TERRA G4* in the absence (black) and presence (red) of 10 molar equiv of **BPBA** recorded at 20 °C.



The parallel *TERRA G4* conformation is preserved in the presence of **BPBA**!



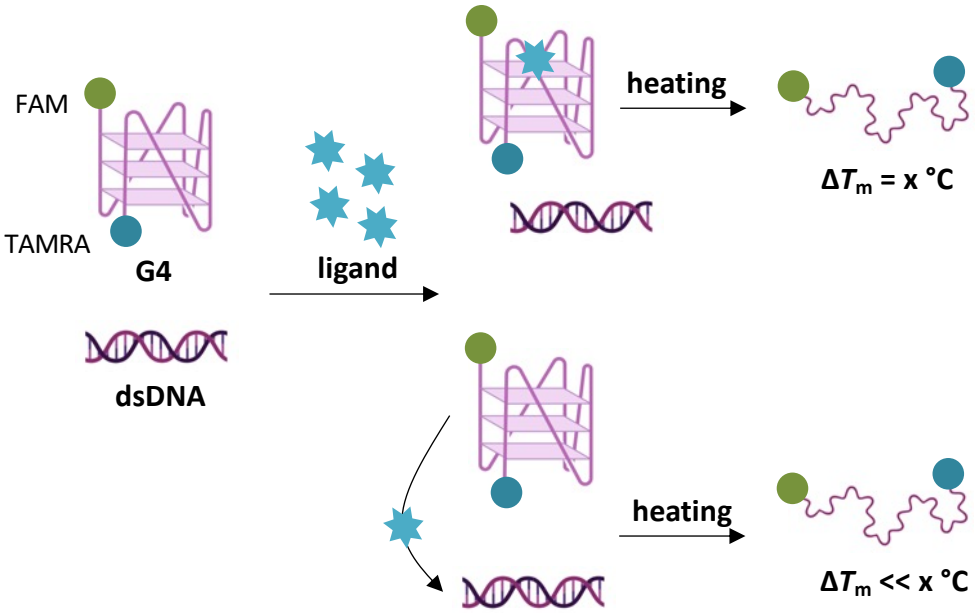
Normalized CD melting profiles of *TERRA G4* in the absence (black) and presence (colored) of increasing amounts of **BPBA**.

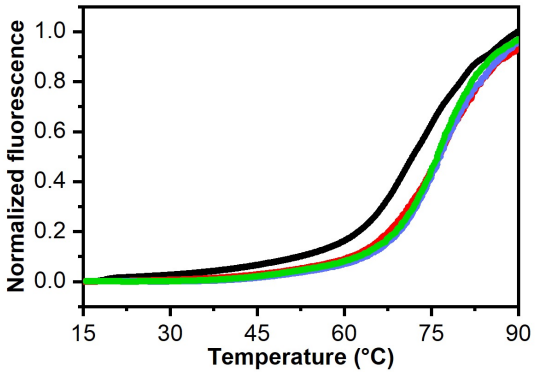


CD stabilization curve for *TERRA G4* with **BPBA**.

The interaction of **BPBA** with *TERRA G4* is specific!

Biophysical investigation of BPBA binding to TERRA G4



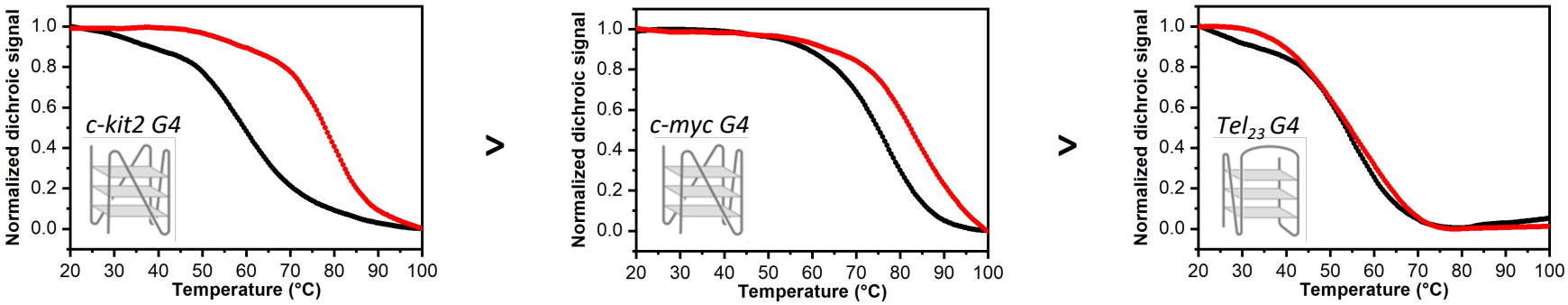


Competition FRET melting experiments for *F-TERRA-T* (0.2 μM) in the absence and presence of **BPBA** (2.0 μM) and Hrp_{27} duplex (6.0 μM and 20.0 μM).

BPBA selectively stabilizes *TERRA G4* over duplex DNA!

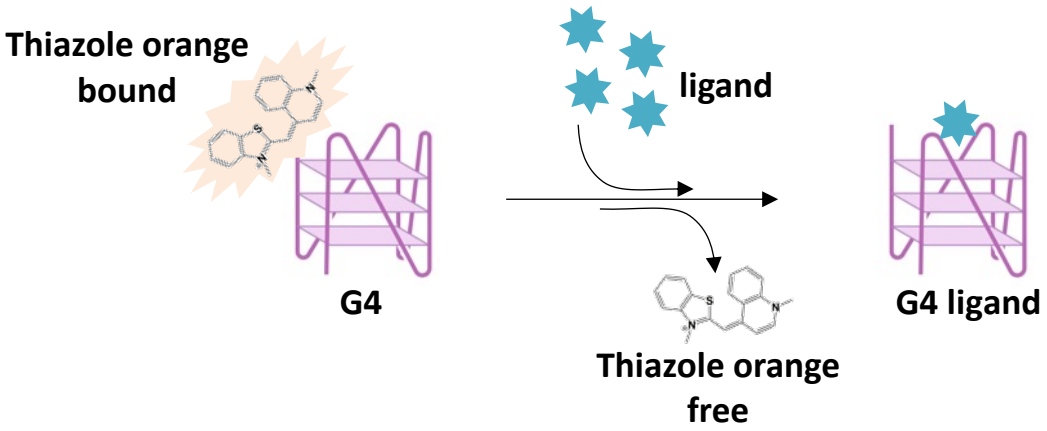
BPBA exhibits preference for parallel G4 conformations

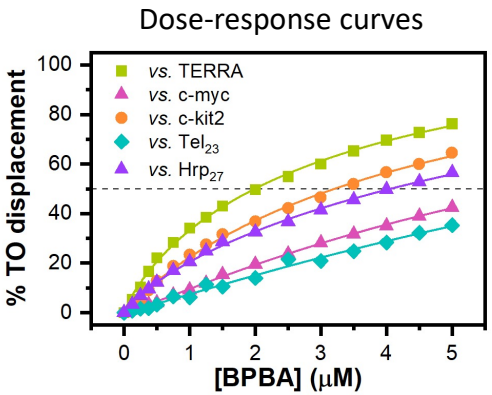
Normalized CD melting profiles of *G4s* in the absence and presence of **BPBA**

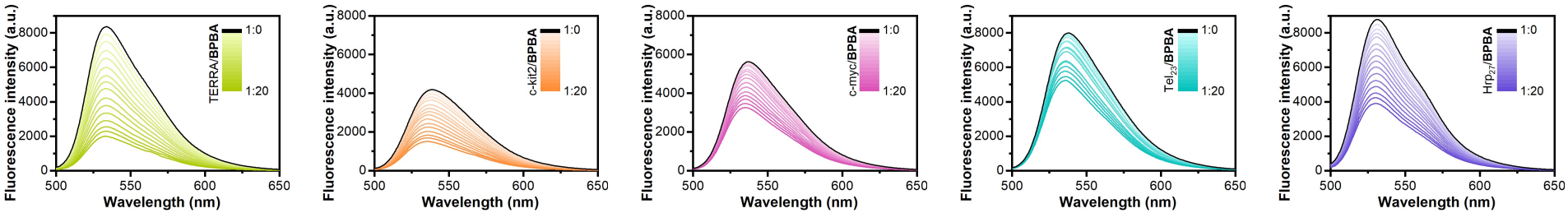


ΔT_m (°C)	Circular dichroism (CD) melting				
	TERRA G4	*c-kit2 G4*	*c-myc G4*	*Tel*$_{23}$ *G4*	*Hrp*$_{20}$
	4.5 (±0.4)	18.7 (±0.3)	9.4 (±0.3)	0.7 (±0.2)	1.0 (±0.5)

BPBA affinity evaluation by fluorescent intercalator displacement (FID) assay



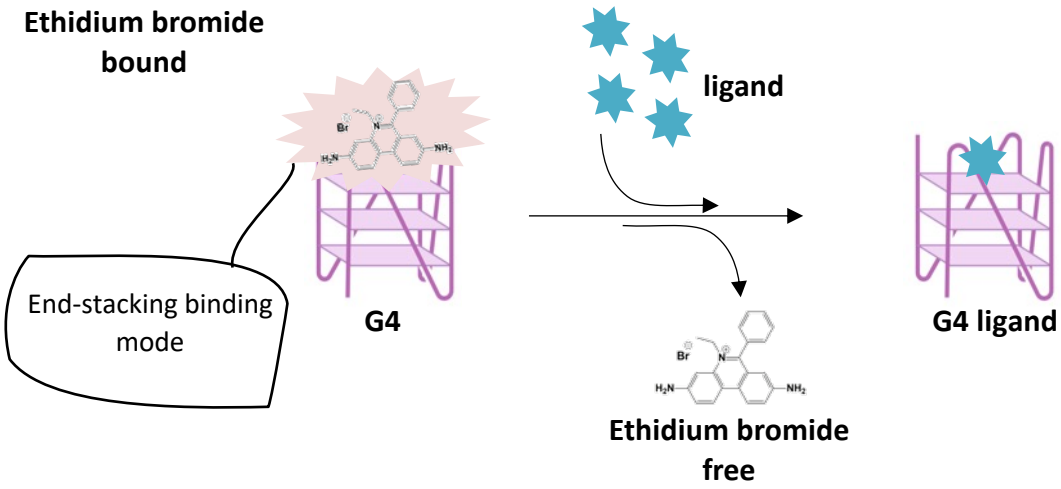


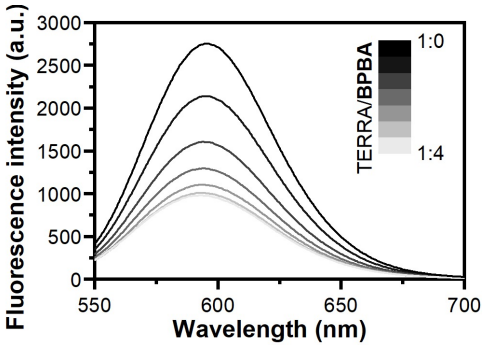


TO-displacement titrations for RNA and DNA G4 and duplex upon addition of increasing amounts of **BPBA**

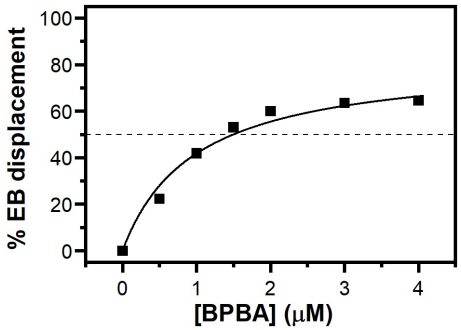
	Fluorescent intercalator displacement (FID) – Thiazole orange (TO)				
DC_{50} (μM)	*TERRA G4*	*c-kit2 G4*	*c-myc G4*	*Tel_{23} G4*	*Hrp_{27}*
	2.4 (±0.4)	3.8 (±0.6)	n.d.	n.d.	4.1 (±0.5)

BPBA interacts with TERRA G4 by end-stacking





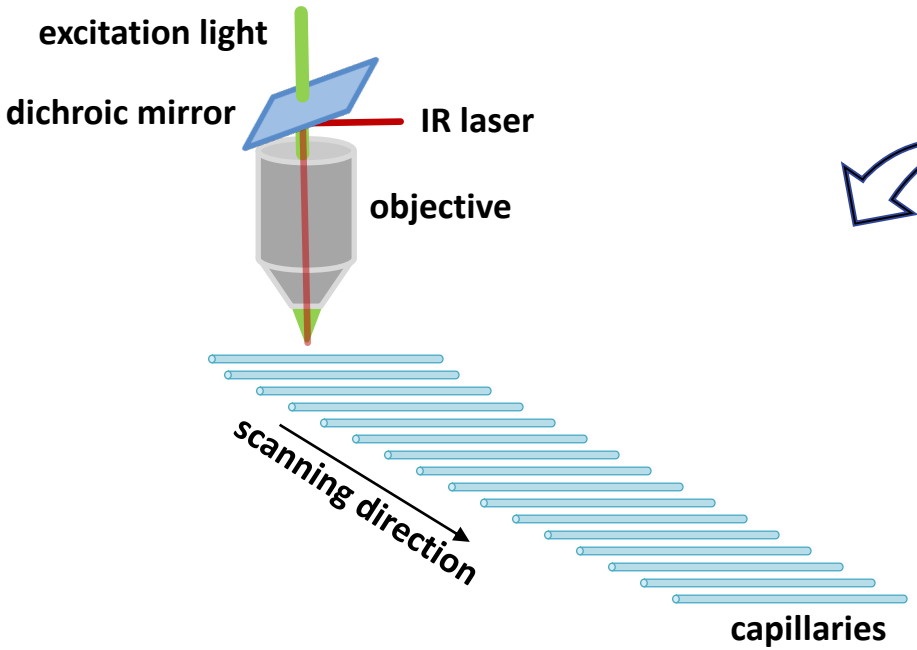
EB-displacement titrations for *TERRA G4* upon addition of increasing amounts of **BPBA.**

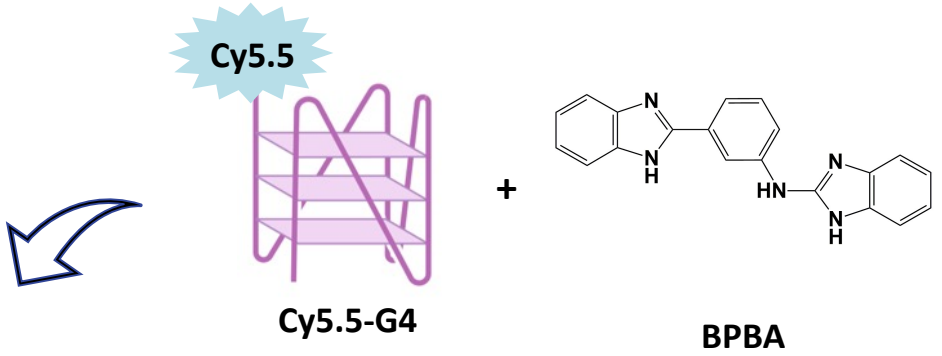


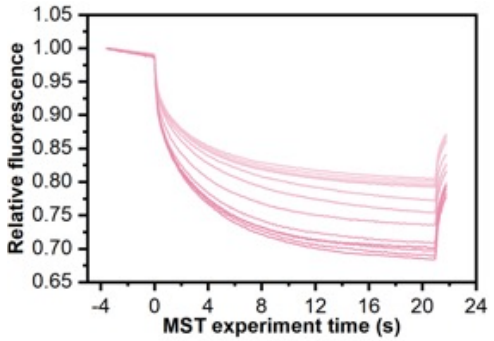
Dose-response curve.

DC_{50} = 1.3 (±0.4) μM

BPBA affinity evaluation by MicroScale Thermophoresis (MST)

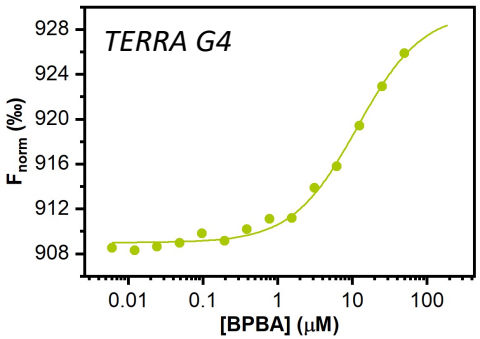


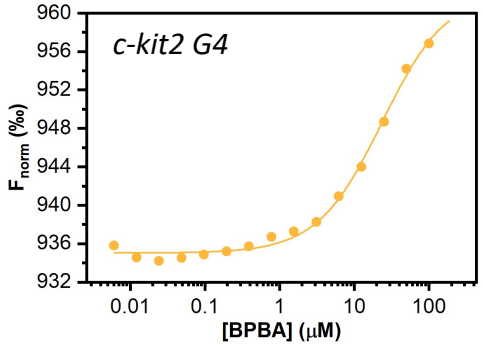


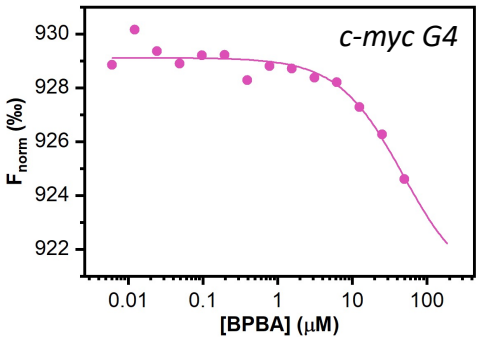


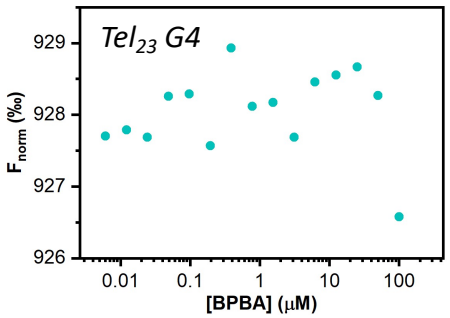
BPBA has better affinity for TERRA G4

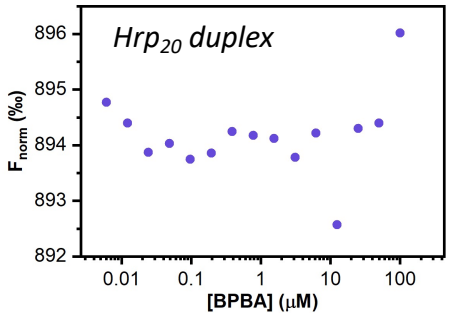
Binding curves obtained from MST measurements for the interaction of **BPBA** with:





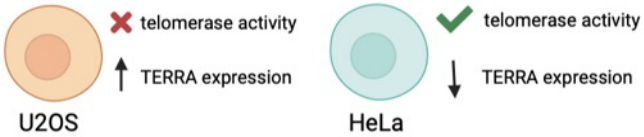


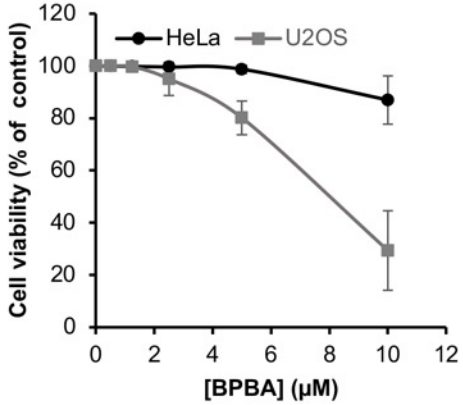




	Microscale thermophoresis (MST)				
K_d (μM)	*TERRA G4*	*c-kit2 G4*	*c-myc G4*	*Tel_{23} G4*	*Hrp_{20}*
	9.6 (±0.6)	23.5 (±0.6)	47.5 (±0.4)	n.d.	n.d.

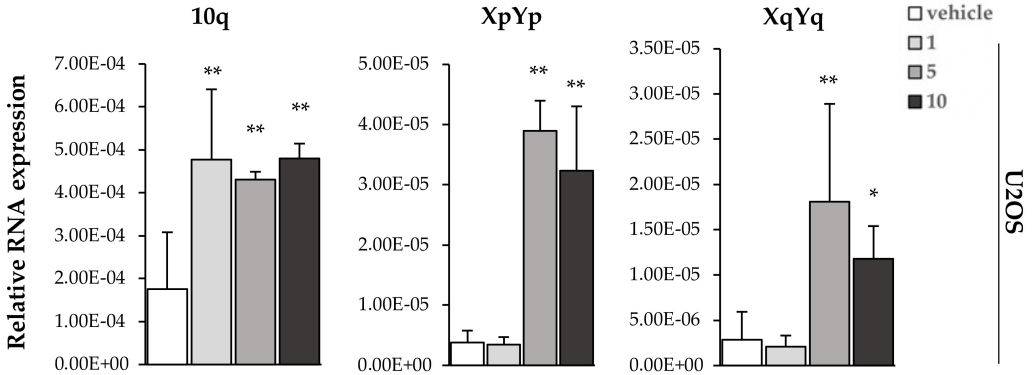
Biological activity of BPBA



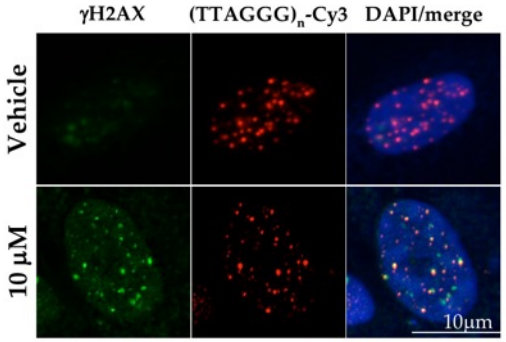


in U2OS IC_{50} = 8.1 (±1.0) μM
in HeLa IC_{50} >> 10 μM

U2OS cells were significantly more sensitive to **BPBA** than **HeLa** cells



BPBA led to an accumulation of the RNA within the U2OS cell



BPBA was able to induce DNA damage response, coincident with telomere loci, in a significant fraction of U2OS cells

Summary

- The application a virtual screening approach in tandem with an experimental screening via CD melting assay succeeded in the identification of a new hit compound (BPBA) as binder of *TERRA G4*.
- Results of biophysical assays clearly showed that **BPBA** features high selectivity toward G4s, with enhanced binding affinity toward *TERRA G4* vs. other G4-forming DNA sequences, along with an high selectivity for G4 over duplex DNA.
- The biological characterization demonstrated that **BPBA** can bind and stabilize TERRA *in-cellulo*.
- The highest cytotoxic effect exerted by **BPBA** in U2OS cells (low telomerase activity, high TERRA expression) well correlates with its ability to induce DNA damage response and telomere dysfunction.

Take-home message

Targeting TERRA with **G4-Ligands** could represent an effective pharmacological strategy to **hit ALT-positive tumors**, which are generally associated with worst prognosis.

Thank You!

University of Naples Federico II

CNR

Erica Salvati

Simona Marzano
Bruno Pagano
Nunzia Iaccarino
Anna Di Porzio
Antonio Randazzo

Eleonora Vertecchi

Stefano De Tito