## **Supporting Information**

## Binding Modes and Pathway of RHPS4 to Telomeric G-Quadruplex and Duplex DNA Probed by All-Atom Molecular Dynamics Simulations with Explicit Solvent

## by Kelly Mulholland, Farzana Siddiquei and Chun Wu

**Table S1**. Summary of RHPS4 use in treating various cancers

Cell line	Target	Target Disease	
U251MG, U87MG, T67 and T70	Telomeric G-quadruplex	Astrocytoma	1
DAOY	Telomeric G-quadruplex	Medulloblastoma	2
PFSK-1 CNS PNET	Telomeric G-quadruplex	CNS primitive neuroectodermal	2
U87, KNS42	Telomeric G-quadruplex	Glioblastoma	2
Res196	Telomeric G-quadruplex	Ependymoma	2
C6	Telomeric G-quadruplex	Glioma	2
HCT116	Telomeric G-quadruplex	Colorectal	3

1. Berardinelli, F.; Siteni, S.; Tanzarella, C.; Stevens, M. F.; Sgura, A.; Antoccia, A., The Gquadruplex-stabilising agent RHPS4 induces telomeric dysfunction and enhances radiosensitivity in glioblastoma cells. *DNA Repair* **2015**, 25, 104-115

 Lagah, S.; Tan, I. L.; Radhakrishnan, P.; Hirst, R. A.; Ward, J. H.; O'Callaghan, C.; Smith, S. J.; Stevens, M. F. G.; Grundy, R. G.; Rahman, R., RHPS4 G-Quadruplex Ligand Induces Anti-Proliferative Effects in Brain Tumor Cells. *Plos One* 2014, 9

**3**. Johnson, L. A.; Byrne, H. M.; Willis, A. E.; Laughton, C. A., An integrative biological approach to the analysis of tissue culture data: application to the antitumour agent RHPS4. *Integrative Biology* **2011**, 3, 843-849

**Figure S2.** Initial structures of the simulation systems with unbound RHPS4: duplex DNA (A), parallel quadruplex DNA (B, pdb 1KF1), antiparallel DNA quadruplex (C, pdb 143D) and hybrid DNA quadruplex (D, 2HY9). 5' and 3' are indicated by a red and blue ball, respectively.



**Figure S3.** RMSD plot of each system: 1NZM (with complex, ligand alone and receptor alone in red, green and blue, respectively) (A), duplex DNA (B), parallel quadruplex DNA (C), anti-parallel quadruplex DNA (D), and hybrid quadruplex DNA (E).



		0 20	50 40	Time (ns)	0 800	1000
	2.5 1.5 0.5	And the second	influences-undersedund	NewWorldshandshandha	al-loadhroomlanalhoomlad-ann	Run 10
	0.5					Run 9
	4.5 3.5 2.5	E Edunaritzarihiallarantuari	hypologically and the strange	white the second	nonhamandalipulariswamalilla	-hundrantullautrality
	2.5 1.5 0.5		 	ار میں میں اور میں میں ان ا		Run 8
	0.5 4.5 3.5	. Munmun Mundul	under where where the fill	Wm Why have we where	a horacian and an hadren and an an an	willingtown much
	3.5 2.5 1.5	En Inville remaindulement	Whaten Hungell holder and halfed	www.	all and the superior of the second	
	1.5 0.5 4.5	E E El Mal di Multur (Mu) wik. N	i Leonard Industry Constants Leonard Industry	an meraharah na kanan kanan An	ייא אעראייר אין אין איין איין איין איין איין א	Run 6
R	4.5 3.5 2.5	And the stand of t	กล่างไม่ไม่ไม่ไหล should มอมเปล่มสมบบได้	when the white the second here the	all Lange and the strend second	all wanter a maille
<b>ASD</b>	3.5 2.5 1.5	EnlyWhenthewardwydla	HHILmonth HAVI Under White	nthemersen with the second stand	N/h/whilehourseller	
Ð	1.5 0.5 4.5	– – МаМсћи Мајања спостађа М	i Ing. Ad de	i Hindi Arasimwadalkasala uk. a	Alexandrat New York And the shell	ייאיאיµאיµאאיזאיא Run 4-
	0.5 4.5 3.5	East the one with holes, Mills in	ر. منه والدرايين أن واست الدوارية الم	+ hand dd, han I, myddian y diaeddolau	in anna aileadh ann a' se an An Ann, an	Run 3
	3.5 2.5 1.5	perautolulu-anti-the-worked	hour and the second second	un musical and the second line	MANNAMAN	11.mlandadhadhalandadha
	1.5 0.5 4.5	елүгий 		l		Run 2
	4.5 3.5 2.5	L. W.M. W. W. W. W. W. W.	have been and the stand	and the property and the	-lucharcanon phaloper and	when we have been a faith of the second
	3.5 2.5 1.5 0.5	Hach I and A way and A	~MWWWWWWWWWWWWWWWW	Varveladnad alfrand meridete e e e e e e e e e e e e e e e e e e	al addrochdiae cumollulu izmete ik niedzaj	Run 1
	4.5	المارية الم	أساله مباراته	A hilles and a public herder	Mar Lasher Mar art die a Hak I had	العارية بالملقة العرارية

Figure S4. RMSD of duplex DNA system and RHPS4 of each trajectory



Figure S5. RMSD of parallel DNA quadruplex system and RHPS4 of each trajectory



Figure S6. RMSD of antiparallel DNA quadruplex system and RHPS4 of each trajectory



Figure S7. RMSD of hybrid DNA quadruplex system and RHPS4 of each trajectory



Figure S8. Contact number between duplex DNA system and RHPS4 of each trajectory



**Figure S9.** Contact number between parallel DNA quadruplex system and RHPS4 of each trajectory



**Figure S10.** Contact number between antiparallel DNA quadruplex system and RHPS4 of each trajectory



**Figure S11.** Contact number between hybrid DNA quadruplex system and RHPS4 of each trajectory

**Figure S12.** Representative structures of the most populated complex structure families (population  $\ge 1$  %) of human telomeric duplex DNA from the clustering analysis of the combined binding trajectories. 5' and 3' are indicated by a red and blue ball, respectively.

Binding Mode	Intercalation Binding			
Cluster ID	A1	A2	A3	
Representative Structure (Front View)				
Representative Structure (Top View)				
Population	23%	22%	20%	
Cluster ID	A4	A5		
Representative Structure (Front View)				
Representative Structure (Top View)				
Population	19%	1%		

Binding Mode	Groove Binding
Cluster ID	B1
Representative Structure (Front View)	
Representative Structure (Top View)	
Population	5%

	•	
Binding Mode Bottom-Stacking		Top-Stacking
Cluster ID	D1	C1
Representative Structure (Front View)		
Representative Structure (Top View)		
Population	5%	3%



**Figure S13**. Last snapshot of ten parallel G-quadruplex-RHPS4 simulations. 5', 3' and K+ are indicated by a red, blue and yellow ball, respectively.

**Figure S14.** Representative structures of the most populated complex structure families (population  $\geq 1$  %) of human telomeric parallel quadruplex DNA from the clustering analysis of the combined binding trajectories. 5' and 3' are indicated by a red and blue ball, respectively.

Binding Mode	Top-Stacking			
Cluster ID	A1	A2	A3	
Representative Structure (Front View)				
Representative Structure (Top View)				
Population	52%	17%	4%	

Binding Mode	Bottom-Stacking				
Cluster ID	B1	B2	B3	B4	
Representative Structure (Front View)					
Representative Structure (Top View)					

Population	13%	5%	4%	4%
Binding Mode	Side-Loop Bi	nding		
Cluster ID	C1			
Representative Structure (Front View)				
Representative Structure (Top View)		-		
Population	5%			



**Figure S15**. Last snapshot of ten anti-parallel G-quadruplex-RHPS4 simulations. 5', 3' and Na+ are indicated by a red, blue and yellow ball, respectively.

**Figure S16.** Representative structures of the most populated complex structure families (population  $\ge 1$  %) of human telomeric antiparallel quadruplex DNA from the clustering analysis of the combined binding trajectories. 5' and 3' are indicated by a red and blue ball, respectively.

Binding Mode	Top Intercalation/Insertion			
Cluster ID	A1	A2	A3	
Representative Structure (Front View)				
Representative Structure (Top View)				
Population	19%	17%	11%	

Binding Mode	Bottom Insertion		
Cluster ID	B1	B2	
Representative Structure (Front View)			
Representative Structure (Top View)			
Population 35%		6%	

Binding Mode	Side-Groove Binding
Cluster ID	C1
Representative Structure (Front View)	
Representative Structure (Top View)	
Population	5%



**Figure S17**. Last snapshot of ten hybrid G-quadruplex-RHPS4 simulations. 5', 3' and K+ are indicated by a red, blue and yellow ball, respectively.

Binding Mode	Top-Stacking			
Cluster ID	A1	A2	A3	
Representative Structure (Front View)				
Representative Structure (Top View)				
Population	62%	4%	2%	

**Figure S18.** Representative structures of the most populated complex structure families (population  $\geq 1$  %) of human telomeric hybrid quadruplex DNA from the clustering analysis of the combined binding trajectories. 5' and 3' are indicated by a red and blue ball, respectively.

Binding Mode	Bottom-Stacking
Cluster ID	B1
Representative Structure (Front View)	
Representative Structure (Top View)	
Population	16%

Binding Mode	Side-Loop Binding			
Cluster ID	C1	C2	C3	
Representative Structure (Front View)				
Representative Structure (Top View)				
Population	8%	3%	2%	

**Figure S19.** A trajectory of RHPS4 intercalating to human telomeric duplex DNA with a broken base pair. The order parameters include hydrogen bonds in first (red), second (blue) and third (yellow) of duplex base paring layer (H-Bond), center-to-center distance (R), the drug-base dihedral angle, ligand RMSD and MM-GBSA binding energy ( $\Delta E$ ). 5' and 3' of the DNA chain are indicated by a red and blue ball, respectively.





**Figure S20.** A trajectory of RHPS4 intercalating to human telomeric duplex DNA with a broken base pair. The order parameters include hydrogen bonds in first (red), second (blue) and third (yellow) of duplex base paring layer (H-Bond), center-to-center distance (R), the drug-base dihedral angle, ligand RMSD and MM-GBSA binding energy ( $\Delta E$ ). 5' and 3' of the DNA chain are indicated by a red and blue ball, respectively.





**Figure S21.** A representative trajectory of RHPS4 binding to the top of human telomeric parallel quadruplex DNA. The order parameter include hydrogen bonds present in first (red), second (blue) and third (yellow) of G-tetrad layer of quadruplex (H-Bond), center-to-center distance (R), the drug-base dihedral angle, ligand RMSD and MM-GBSA binding energy ( $\Delta E$ ). 5' and 3' of the DNA chain are indicated by a red and blue ball, respectively.



**Figure S22.** A representative trajectory of RHPS4 binding to the side loop of human telomeric parallel DNA quadruplex. The order parameter include hydrogen bonds present in first (red), second (blue) and third (yellow) of G-tetrad layer of quadruplex (H-Bond), center-to-center distance (R), the drug-base dihedral angle, ligand RMSD and MM-GBSA binding energy ( $\Delta E$ ). 5' and 3' of the DNA chain are indicated by a red and blue ball, respectively.



**Figure S23.** A representative trajectory of RHPS4 binding to the bottom of human telomeric antiparallel DNA quadruplex. The order parameter include hydrogen bonds present in first (red), second (blue) and third (yellow) of G-tetrad layer of quadruplex (H-Bond), center-to-center distance (R), the drug-base dihedral angle, ligand RMSD and MM-GBSA binding energy ( $\Delta E$ ). 5' and 3' of the DNA chain are indicated by a red and blue ball, respectively.



**Figure S24.** A representative trajectory of RHPS4 binding to the bottom of human telomeric antiparallel DNA quadruplex. The order parameter include hydrogen bonds present in first (red), second (blue) and third (yellow) of G-tetrad layer of quadruplex (H-Bond), center-to-center distance (R), the drug-base dihedral angle, ligand RMSD and MM-GBSA binding energy ( $\Delta E$ ). 5' and 3' of the DNA chain are indicated by a red and blue ball, respectively.



**Figure S25.** A representative trajectory of RHPS4 binding to the side loop of human telomeric antiparallel DNA quadruplex. The order parameter include hydrogen bonds present in first (red), second (blue) and third (yellow) of G-tetrad layer of quadruplex (H-Bond), center-to-center distance (R), the drug-base dihedral angle, ligand RMSD and MM-GBSA binding energy ( $\Delta E$ ). 5' and 3' of the DNA chain are indicated by a red and blue ball, respectively.



**Figure S26.** A representative trajectory of RHPS4 stacking to the top of human telomeric hybrid DNA quadruplex. The order parameter include hydrogen bonds present in first (red), second (blue) and third (yellow) of G-tetrad layer of quadruplex (H-Bond), center-to-center distance (R), the drug-base dihedral angle, ligand RMSD and MM-GBSA binding energy ( $\Delta E$ ). 5' and 3' of the DNA chain are indicated by a red and blue ball, respectively.





Time (ns)

**Figure S27.** A representative trajectory of RHPS4 binding to the side-groove of human telomeric hybrid DNA quadruplex. Center-to-center distance (R), the drug-base dihedral angle, ligand RMSD and MM-GBSA binding energy ( $\Delta E$ ). 5' and 3' of the DNA chain are indicated by a red and blue ball, respectively.



Figure S28. AMBER GAFF2 force field of RHPS4 (+1) in Mol2 format.

@<TRIPOS>MOLECULE LG1 43 47 1 0 1 **SMALL** USER CHARGES @<TRIPOS>ATOM -0.039648 1 C05 -4.633283 -0.879556 -0.410733 C 1 LG1 2 H09 -5.685850 -0.984618 -0.594163 H 1 LG1 0.169889 3 C06 -4.088818 0.338063 -0.120959 C 1 LG1 -0.069123 4 H10 -4.730669 1.193743 -0.103335 H 1 LG1 0.107580 5 C01 -2.700480 0.085709 C 1 LG1 0.479750 -0.012257 6 N14 -2.142871 1.723490 0.280836 N 1 LG1 -0.003253 7 C13 -0.798672 1.909236 1 LG1 0.094583 C -0.002912 8 C18 -0.252244 3.188724 -0.025402 C 1 LG1 -0.058652 9 H26 -0.890076 4.047234 -0.054356 H 1 LG1 0.098625 10 C17 1.110085 3.370543 -0.145221 C 1 LG1 -0.055059 11 C16 2.267576 -0.134051 C 1 LG1 1.970963 -0.058247 12 H28 2.443512 -0.200435 H 1 LG1 3.025862 0.116197 13 C15 1.475559 0.991668 -0.033717 C 1 LG1 -0.019249 14 C19 2.317536 -0.205373 -0.032496 C 1 LG1 -0.025508 15 C20 3.688499 -0.152691 -0.309714 C 1 LG1 -0.028871 4.175684 0.765505 -0.566568 H 16 H29 1 LG1 0.140390 4.436576 -1.293739 -0.268996 C 1 LG1 17 C21 -0.003493 5.727948 -1.230270 -0.533712 F 18 F30 1 LG1 -0.084337 19 C22 3.873787 -2.523126 0.035187 C 1 LG1 -0.042024 4.487805 -3.403137 1 LG1 20 H31 0.046013 H 0.153671 21 C23 2.528662 -2.589890 0.299952 C 1 LG1 -0.072281 22 H32 2.097756 -3.547475 0.508451 H 1 LG1 0.103667 23 C24 1.738465 -1.435703 0.268823 C 1 LG1 -0.019030 24 NP5 0.348207 -1.533479 0.506008 N 1 LG1 0.023646 25 C11 -0.462110 -0.506249 0.228254 C 1 LG1 0.073884 26 C02 -1.890072 -0.663637 0.044008 C 1 LG1 0.011322 27 C03 -2.470218 -1.904999 -0.308057 C 1 LG1 -0.019154 28 H07 -1.872254 -2.778425 -0.457763 H 1 LG1 0.117318 29 C04 -3.804487 -1.996499 -0.519540 C 1 LG1 -0.012023 30 F08 -4.340081 -3.155268 -0.860380 F 1 LG1 -0.088658 31 C12 0.071532 0.791445 0.064029 C 1 LG1 0.046444 32 C34 -0.110828 -2.699161 1.283618 C 1 LG1 -0.000787

33 H35	0.594764	-2.858288	2.084924 H	1 LG1	0.070118
34 H36	-1.072647	-2.489904	1.716164 H	1 LG1	0.070118
35 H37	-0.171204	-3.592102	0.678483 H	1 LG1	0.070118
36 C27	1.688665	4.754603	-0.292167 C	1 LG1	-0.022544
37 H38	2.401816	4.951947	0.501035 H	1 LG1	0.053165
38 H39	0.921182	5.516146	-0.256793 H	1 LG1	0.053165
39 H40	2.214743	4.844969	-1.236584 H	1 LG1	0.053165
40 C33	-2.991755	2.859435	0.646868 C	1 LG1	-0.008669
41 H41	-3.357953	3.385684	-0.226369 H	1 LG1	0.071099
42 H42	-3.823513	2.506508	1.233227 H	1 LG1	0.071099
43 H43	-2.427791	3.535943	1.267122 Н	1 LG1	0.071099
_					

@<TRIPOS>BOND

1	1	21
2	1	31
3	1	29 1
4	3	41
5	3	51
6	5	61
7	5	26 1
8	6	71
9	6	40 1
10	7	81
11	7	31 1
12	8	91
13	8	10 1
14	10	11 1
15	10	36 1
16	11	12 1
17	11	13 1
18	13	14 1
19	13	31 1
20	14	15 1
21	14	23 1
22	15	16 1
23	15	171
24	17	181
25	17	191
26	19	20 1
27	19	21 1
28	21	22 1

29	21	23 1	
30	23	24 1	
31	24	25 1	
32	24	32 1	
33	25	26 1	
34	25	31 1	
35	26	27 1	
36	27	28 1	
37	27	29 1	
38	29	30 1	
39	32	33 1	
40	32	34 1	
41	32	35 1	
42	36	37 1	
43	36	38 1	
44	36	39 1	
45	40	41 1	
46	40	42 1	
47	40	43 1	
@ <t]< td=""><td>RIPO</td><td>S&gt;SUBSTRUCTURE</td><td></td></t]<>	RIPO	S>SUBSTRUCTURE	
1	LG1	1 0	