Supplementary data

Exploring the G-quadruplex binding and unwinding activity of the bacterial FeS helicase DinG

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Table S1. Kinetics and thermodynamic parameters for the interaction of DinG helicase with G4s from *Escherichia coli*.

Name	Sequence	k_{on} ($M^{-1}s^{-1}$) [a]	k_{off} (s^{-1}) [a]	K_D (nM) [b]
EC-6	**5'-GGTGGGGAGGGGTAAGGGG-3'**	$8.2\ 10^3$	$3.0\ 10^{-4}$	36.7
EC-7	**5'- GGGGGAGGAGGACGGGGG -3'**	$3.9\ 10^3$	$2.2\ 10^{-4}$	56.9
EC-9	**5'- GGGGCGGGGTGGGTTGG -3'**	$8.0\ 10^3$	$2.8\ 10^{-4}$	34.9

[a] Errors were within 5%. [b] Errors were within 10%.

Table S2

Substrate/system sequences used for the two-step fluorescence-based helicase assay.

system[a]	Sequence
	5′ – $(A)_{11}$-G4sequence-TATTCCGTTGAGCAGAG-3′- Dabcyl
Oligo FAM	3′-AAGGCAACTCGTCTC-5′-FAM
S-c-*MYC*	AGGGTGGGTAGGGTGGGT
S-c-*KIT*1	AGGGAGGGCGCGCTGGGAGGAGGG
S-T30695	GGGTGGGTGGGTGGGT
S-Tel_{23}	TAGGGTTAGGGTTAGGGTTAGGG
S-*KRAS*	AGGGCGGTGTGGGAATAGGGAA
S-*BCL2*	GGGCGCGGGAGGAATGGGCGGG
S-*Zic1*	GGTGGGGGGGCGGGGGAGGCCGGG
S-EC6	GGTGGGGAGGGGTAAGGGG
S-EC7	GGGGGAGGAGGACGGGGG
S-EC9	GGGGCGGGGTGGGTTGG
Trap	5′-TTCCGTTGAGCAGAG-3′
C-c-*MYC*	5′-CTCTGCTCAACGGAATACCCACCCTACCCACCC-$(T)_{11}$-3′
C-c-*KIT*1	5′-CTCTGCTCAACGGAATACCCTCCTCCCAGCGCCCTCCC-$(T)_{11}$-3′
C-T30695	5′-TCTTGCTCAACGGAATACCCACCCACCCACCC-$(T)_{11}$-3′
C-Tel_{23}	5′-CTCTGCTCAACGGAATACCCTAACCCTAACCCTAACCCTA-$(T)_{11}$-3′
C-*KRAS*	5′-CTCTGCTCAACGGAATACCCCTCTTCCCTCTTCCCACACCGCCC-$(T)_{11}$-3′
C-*BCL2*	5′-CTCTGCTCAACGGAATACCCGCCCATTCCTCCCGCGCCC-$(T)_{11}$-3′
C-*Zic1*	5′-CTCTGCTCAACGGAATACCCGGCCTCCCCCGCCCCCCCACC$(T)_{11}$-3′
C-EC6	5′-CTCTGCTCAACGGAATACCCCTTACCCCTCCCCACC-$(T)_{11}$- 3′
C-EC7	5′-CTCTGCTCAACGGAATACCCCCGTCCTCCTCCCCC$(T)_{11}$-3′
C-EC9	5′-CTCTGCTCAACGGAATACCAACCCACCCCGCCCC$(T)_{11}$-3′

[a] "S" oligonucleotides are the substrates for DinG unwinding; "C" indicates the complementary strands.

Table S3

G-scrambled sequence used for SPR experiments and oligonucleotides used to form the fork DNA, employed in the binding and helicase assays. For the helicase assay a 6-FAM (6-Carboxyfluorescein) label was attached at 5'-end of D1, and a BHQ1 label (Black Hole Quencher 1) was attached at 3'-end of D2.

Name	**Sequence**
G-scrambled	5' GTGTGTGTGTGTGTGTGTGTGTGTGTGT 3'
D1	5'-CTACTACCCCCACCCTCACAACCTTTTTTTTTTTTTT-3'
D2	5'-TTTTTTTTTTTTTTGGTTGTGAGGGTGGGGGTAGTAG-3'
Cap1	5'-CTACTACCCCCACCCTCACAACC-3'

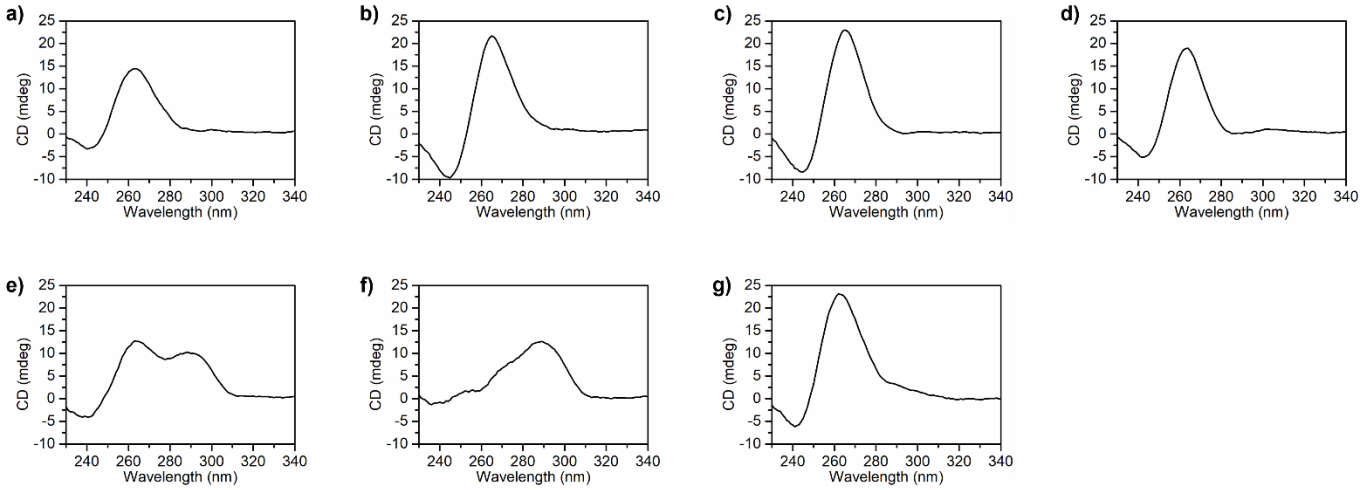


Figure S1. CD spectra of **a)** c-*KIT*1, **b)** c-*MYC*, **c)** *KRAS*, **d)** T30695, **e)** *BCL2*, **f)** Tel_{23}, **g)** *Zic1*

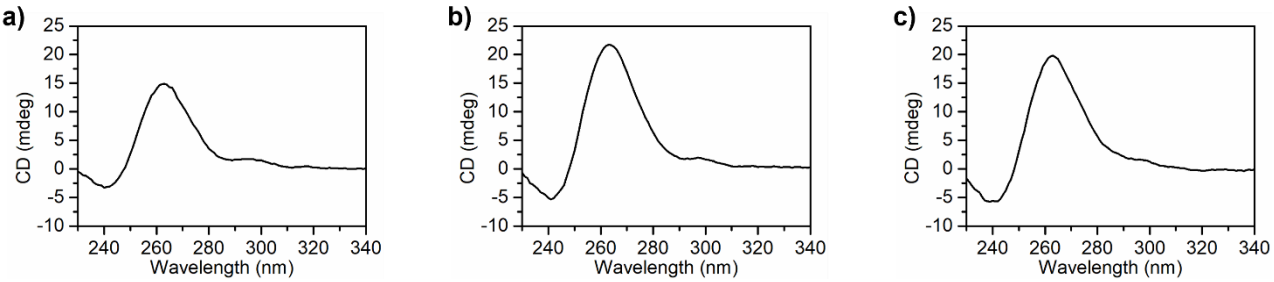


Figure S2. CD spectra of **a)** EC-6, **b)** EC-7, and **c)** EC-9

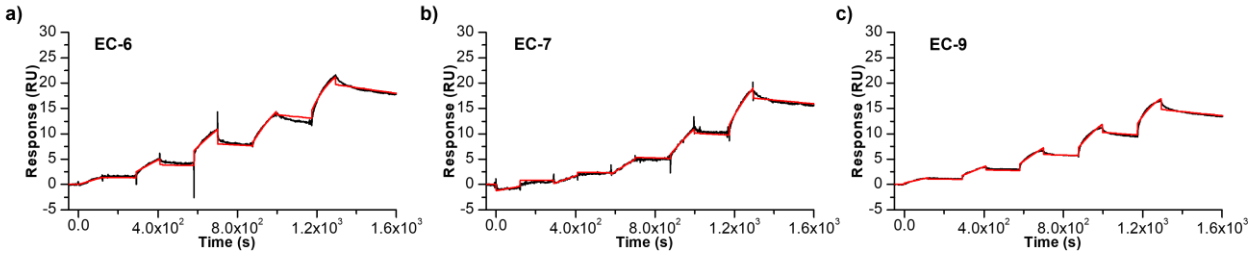
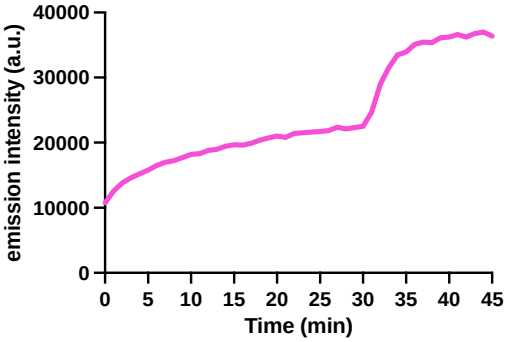
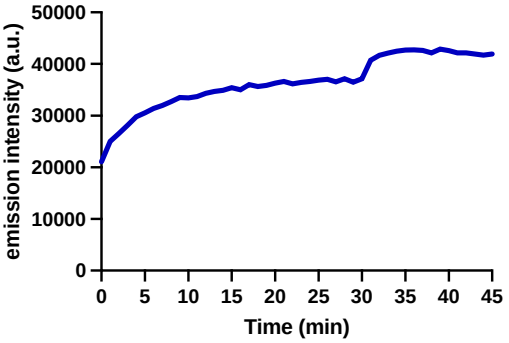


Figure S3. Time evolution SPR sensorgrams obtained at 25 °C by injection of increasing concentrations (from 0.062 to 1 μM) of three bacterial G-quadruplexes **a)** EC-6, **b)** EC-7, **c)** EC-9 on the chip-immobilized DinG helicase.

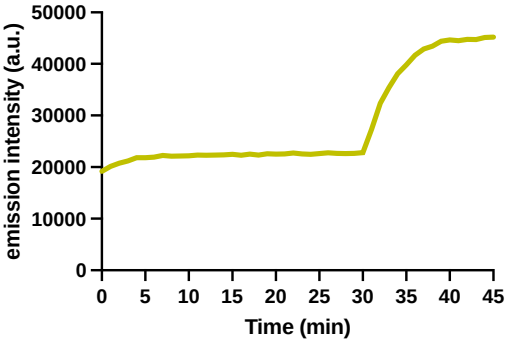
c-*MYC*



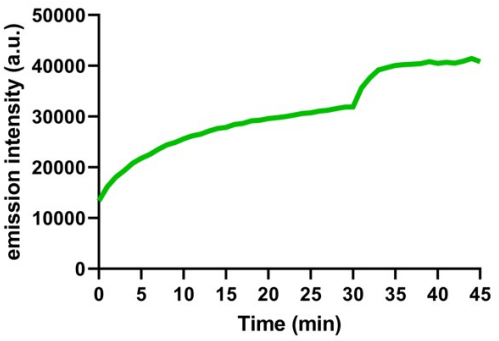
c-*KIT1*



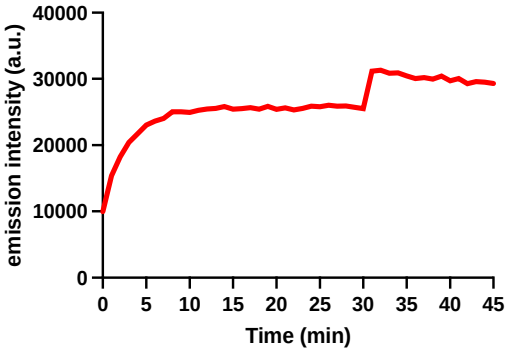
T30695



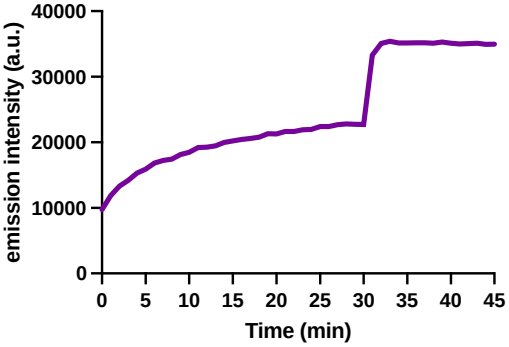
Tel_{23}



KRAS



BCL2



Zic1

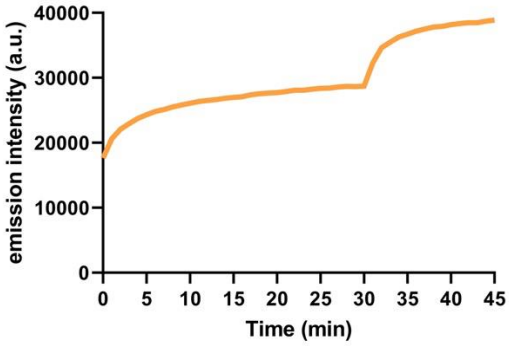


Figure S4. Typical fluorescence emission spectra**.** Two-step unwinding process of selected DNA system (S-c-*MYC*, S-c-*KIT1*, S-*T30695*, S-Tel$_{23}$, S-*KRAS*, S-*BCL2* and S-*Zic1*) after (1) ATP addition (t= 0) and (2) addition of the relative complementary sequence (t= 30 min) (C-c-*MYC*, C-c-*KIT1*, C-*T30695*, C-Tel$_{23}$, C-*KRAS*, C-*BCL2* and C-*Zic1* respectively).

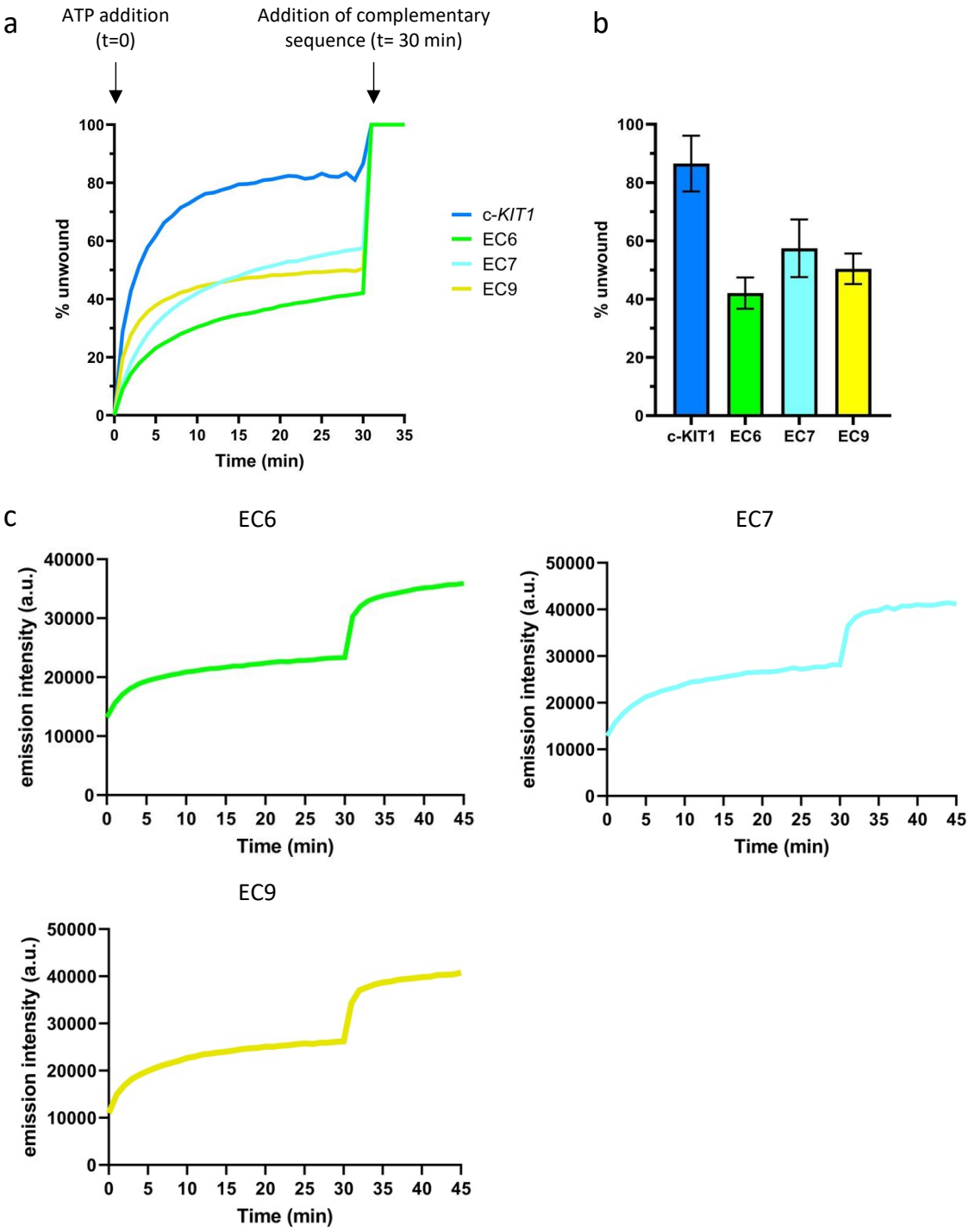

a
ATP addition
(t=0)
Addition of complementary
sequence (t= 30 min)
% unwound
Time (min)
c-KIT1
EC6
EC7
EC9
b
% unwound
c-KIT1
EC6
EC7
EC9
c
EC6
EC7
EC9
emission intensity (a.u.)
Time (min)

Figure S5. a) Representative plots of fluorescence emission vs time for unwinding of selected DNA systems (S-c-*KIT1*, S-EC6, S-EC7 and S-EC9). ATP was added to begin the reaction (t= 0 min); the complemetary strands (C-c-*KIT1*, C-EC6, C-EC7 and C-EC9 respectively) were added once the reactions reached a plateau (t= 30 min). **b)** Quantitation of *E. coli* DinG helicase activity against selected DNA systems; error bars indicate standard deviation of three independent experiments. **c)** Typical fluorescence emission spectra. Two-step unwinding process of selected DNA system (S-EC6, S-EC7 and S-EC9) after (1) ATP addition (t= 0) and (2) addition of the relative complementary sequence (t= 30 min) (C-EC6, C-EC7 and C-EC9 respectively).

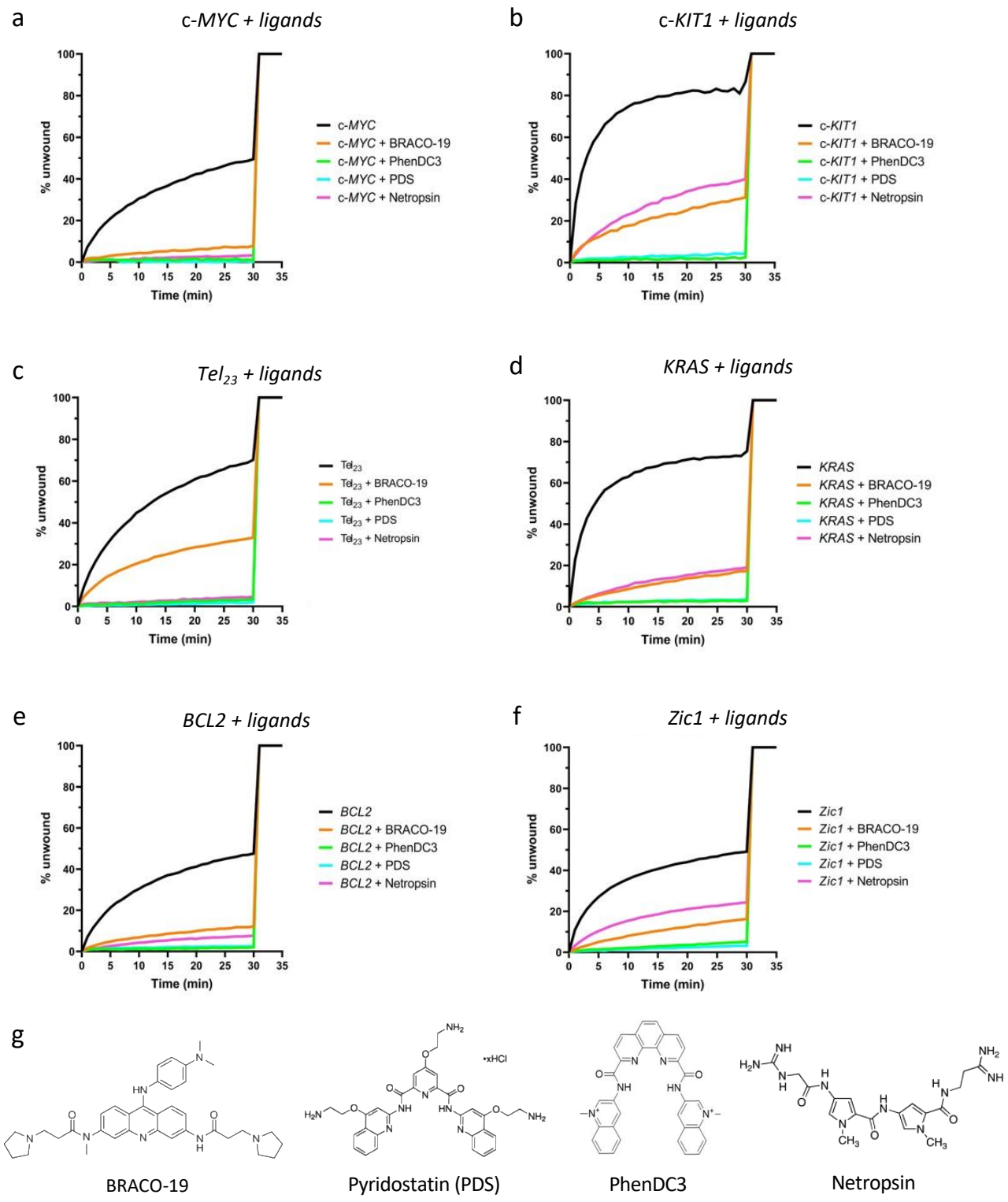


Figure S6. Typical real-time unfolding of **(a)** S-c-*MYC*, **(b)** S-c-*KIT1*, **(c)** S-Tel$_{23}$, **(d)** S-*KRAS*, **(e)** S-*BCL2*, **(f)** S-*Zic1* systems in absence or presence of selected ligands (BRACO-19, PDS, PhenDC3, Netropsin). ATP was added at t= 0; the complementary strand (C-c-*MYC*, C-c-*KIT1*, C-Tel$_{23}$, C-*KRAS,* C-*BCL2* and C-*Zic1* respectively) was added at t= 30 min. **(g)** Structure of selected ligands.

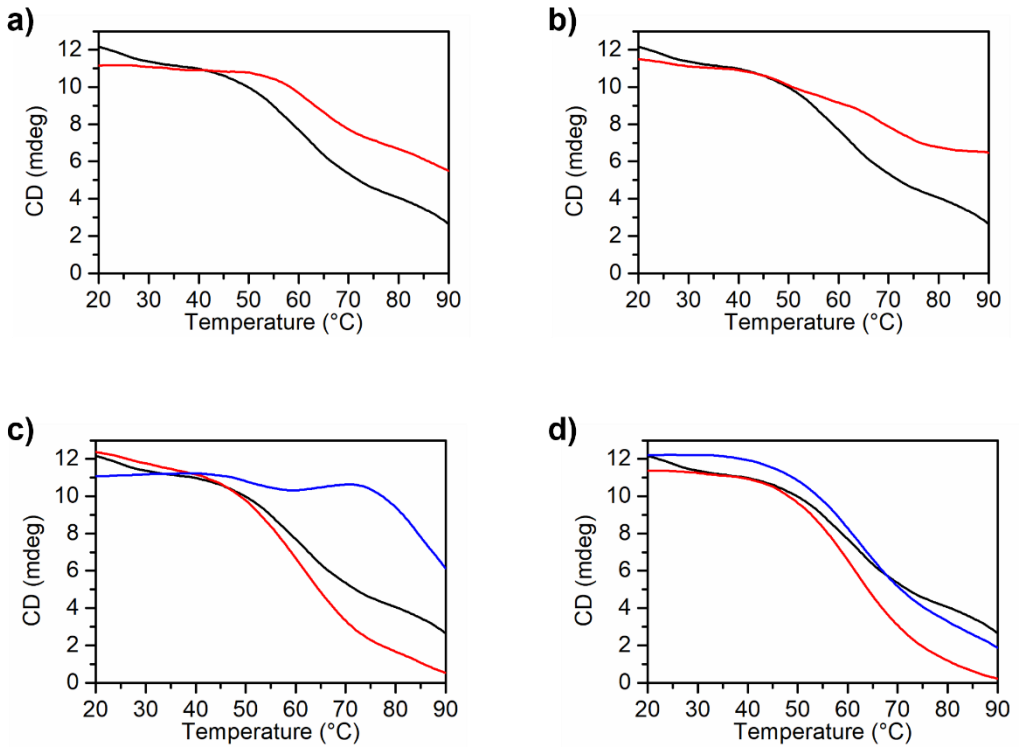


Figure S7. CD melting experiments of c-*KIT1* with 1 mol equiv. (red line) and 2 mol equiv. (blue line) of **a)** PDS, **b)** PhenDC3, **c)** BRACO-19 and **d)** Netropsin.