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Hydrophobic Interactions

Hydrogen Bonds

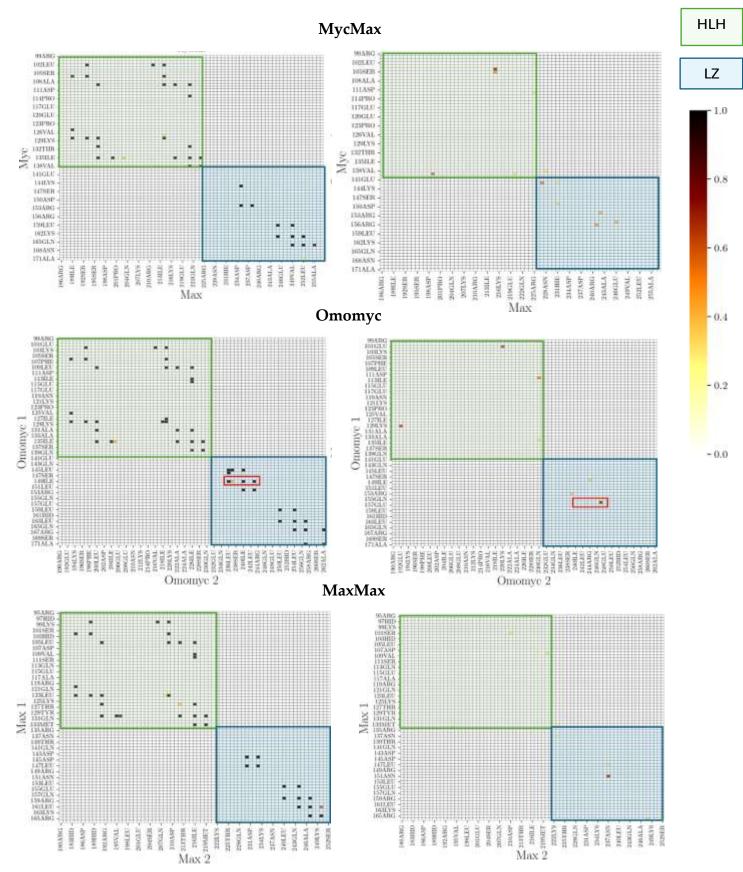


Figure S1: Hydrophobic Interactions and Hydrogen Bonds (HBs) in the HLH and LZ regions of MycMax, Omomyc dimer, and MaxMax. Left panels show a heatmap of the hydrophobic contacts (a contact is recorded when the distance between two hydrophobic residues is within 4.5 Å). Right panel shows a heatmap of the HBs. HLH region is highlighted in green, LZ region in blue. The contacts that were introduced due to the mutation of the 4 residues in Omomyc are highlighted by the red box.

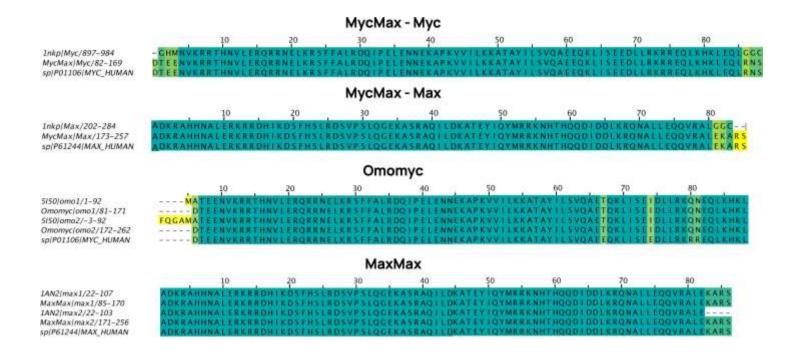


Figure S2: Sequence alignment between the original PDB structures (1NKP for MycMax, 5150 for Omomyc, and 1AN2 for MaxMax), the processed structures used in this study (MycMax, Omomyc, MaxMax), and the UniProt reference sequences (P01106 for Myc and Omomyc, P61244 for Max). The alignment compares individual monomers from the PDB (1NKP/Myc, 1NKP/Max, 5150/Omo1, 5150/Omo2, 1AN2/Max1, and 1AN2/Max2) with their corresponding processed structures (MycMax/Myc, MycMax/Max, Omomyc/Omo1, Omomyc/Omo2, MaxMax/Max1, and MaxMax/Max2) and the reference uniprot sequence (sp/P01106 for Myc and Omomyc, sp/P61244 for Max).

System:Chain	Start Residue	Start Residue in PDB	Start Residue in UNIPROT
МусМах: Мус	D:81	N:900 (DTEE inserted at 896-899)	D:366
MycMax: Max	A:172	D:202 (A inserted at 201)	A:23
Omomyc: Omo1	D:81	D:2	D:366
Omomyc:Omo2	D:172	D:2	D:366
MaxMax:Max1	A:85	A:22	A:23
MaxMax:Max2	A:171	A:22	A:23

Figure S3: Mapping of residue numbering between our processed systems (Start Residue), the original PDB structures (Start Residue in PDB), and the corresponding UniProt residue (Start Residue in UNIPROT) numbers for each chain. Residues that were added to the PDB are shown in parentheses.

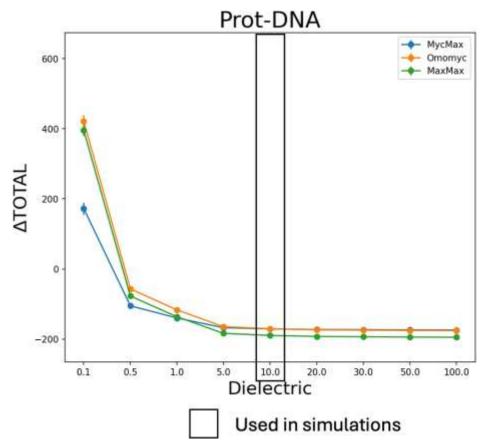


Figure S4: Variation in total MMGBSA binding energy for MycMax (blue), Omomyc (orange), and MaxMax (green) across diZerent dielectric constants. The dielectric constant used in final MMGBSA calculations is highlighted with a box.

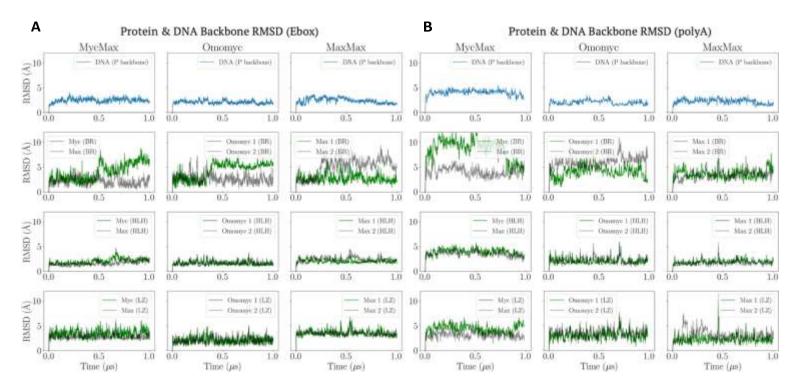


Figure S5: RMSD of protein and DNA backbone for MycMax, Omomyc dimer, and MaxMax complexes bound to E-box (A) and polyA (B). First row is the RMSD of the P atoms of the DNA, second, third and fourth rows are the RMSDs of the heavy atoms of the BR, HLH, and LZ regions for each monomer.

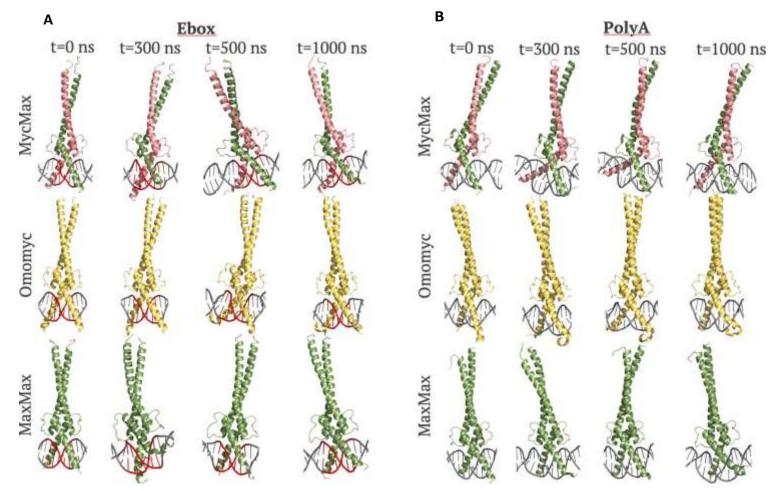


Figure S6. Structural snapshots of MycMax, Omomyc dimer, and MaxMax bound to E-box (A) or polyA (B) at diZerent simulation times (t = 0 ns, 300 ns, 500 ns, 1000 ns). Myc is shown in pink, Max in green, and Omomyc in yellow, with the E-box highlighted in red. Panel (A), previously shown in Figure 2A, is included here for direct comparison with (B), illustrating structural diZerences in TF binding between E-box (A) and polyA (B).

	Angle Deviation (°)	MycMax	Omomyc	MaxMax
200	Ebox	16 ± 9	11±6	12 ± 5
NO.	PolyA	14 ± 3	7±4	5 ± 3

Figure S7: Angular deviation of the HLHLZ domain measured from the simulation. The average and standard deviation of the angle (as shown in the diagram on the left) were measured for MycMax, Omomyc dimer and MaxMax on Ebox and PolyA across the 1-microsecond trajectory.

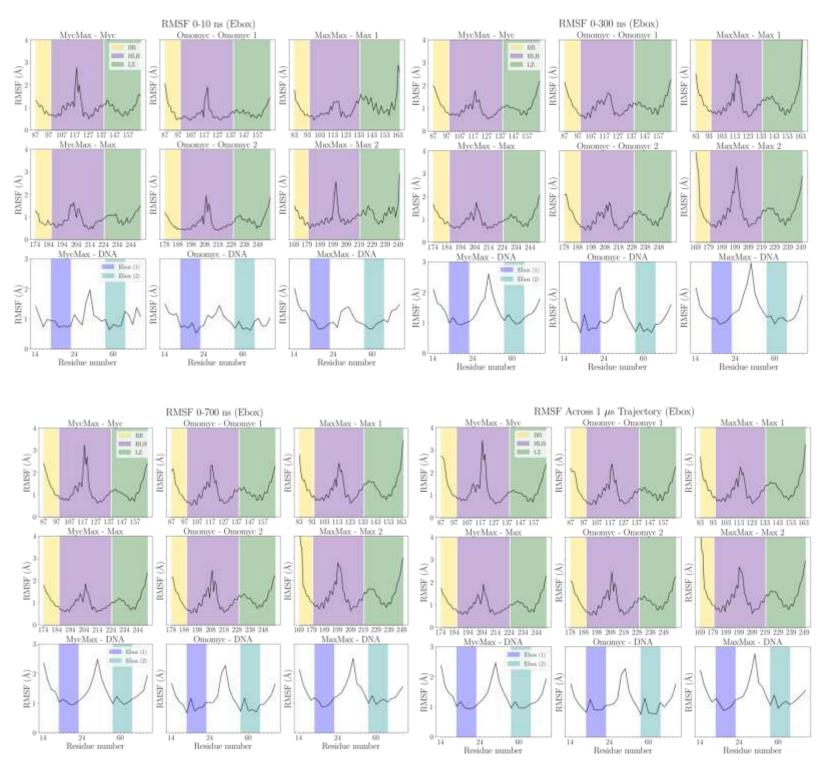


Figure S8: RMSF plots for MycMax, Omomyc dimer, and MaxMax complexes bound to Ebox. RMSF is measured for each monomer (top two rows), as well as for both DNA strands (bottom row, strands delimited by dashed lines), over 10ns (top left), 300ns (top right), 700ns (bottom left), and 1000ns (bottom right) of simulation time. The protein regions are color-coded: BR (yellow), HLH (purple), LZ (green), and the E-box on each DNA strand (blue).

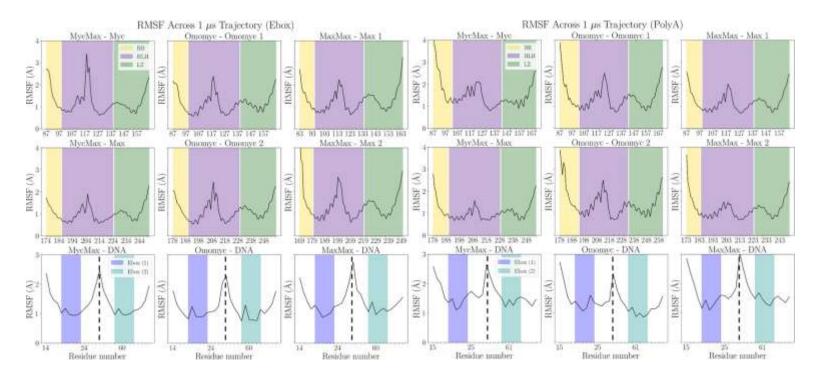


Figure S9: RMSF plots for MycMax, Omomyc dimer, and MaxMax complexes bound to Ebox (left) and polyA (right) over 1000ns of simulation time. RMSF is measured for each monomer (top two rows), as well as for both DNA strands (bottom row, strands delimited by dashed lines. The protein regions are color-coded: BR (yellow), HLH (purple), LZ (green), and the central region for polyA on each DNA strand (blue).

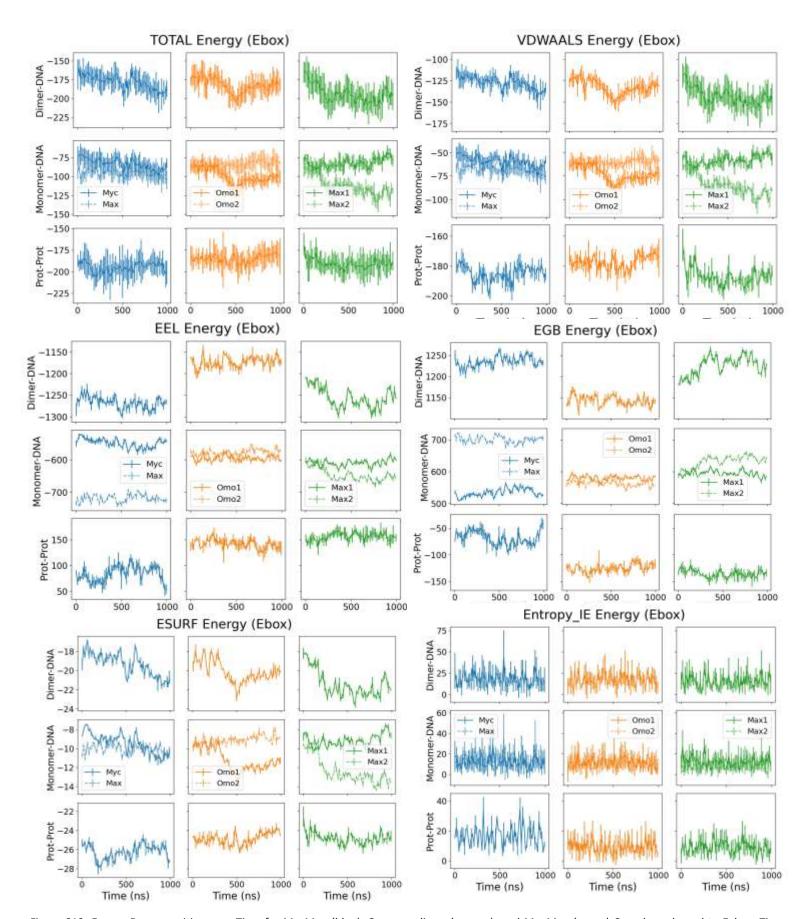


Figure S10: Energy Decomposition over Time for MycMax (blue), Omomyc dimer (orange), and MaxMax (green) Complexes bound to E-box. Time series plots are shown of various regions (entire system in the first row, each monomer interaction with DNA in the second row, and monomer-monomer interactions in the third row) over a 1 µs simulation period. The energy components include TOTAL (Total binding energy), VDWAALS (van der Waals interactions), EEL (Electrostatic energy), EGB (Polar solvation energy), ESURF (non-polar solvation energy) and IE (interaction entropy).

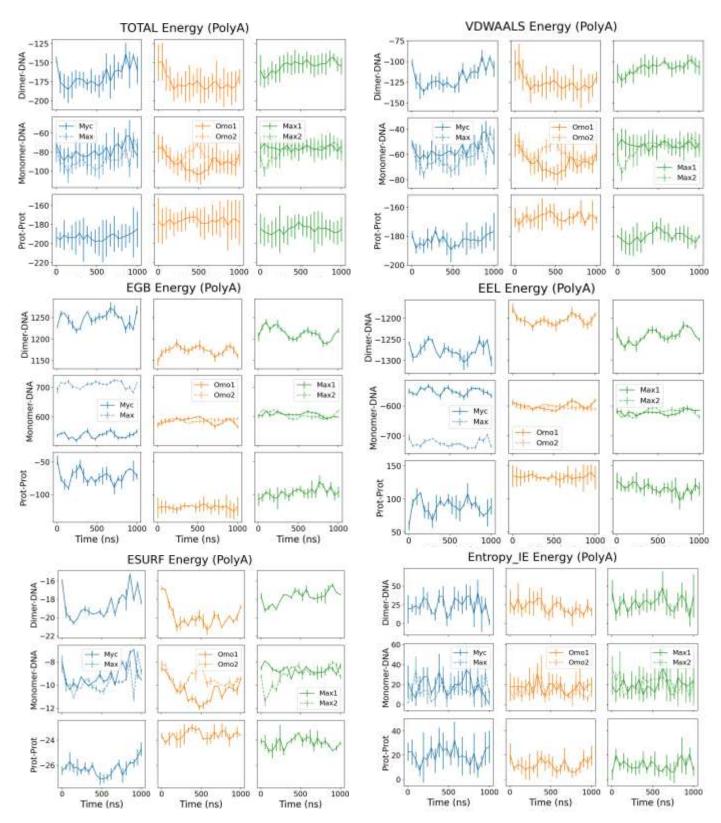


Figure S11: Energy Decomposition over Time for MycMax (blue), Omomyc dimer (orange), and MaxMax (green) Complexes bound to PolyA. Time series plots are shown of various regions (entire system in the first row, each monomer interaction with DNA in the second row, and monomer-monomer interactions in the third row) over a 1 µs simulation period. The energy components include TOTAL (Total binding energy), VDWAALS (van der Waals interactions), EEL (Electrostatic energy), EGB (Polar solvation energy), ESURF (non-polar solvation energy) and IE (interaction entropy).

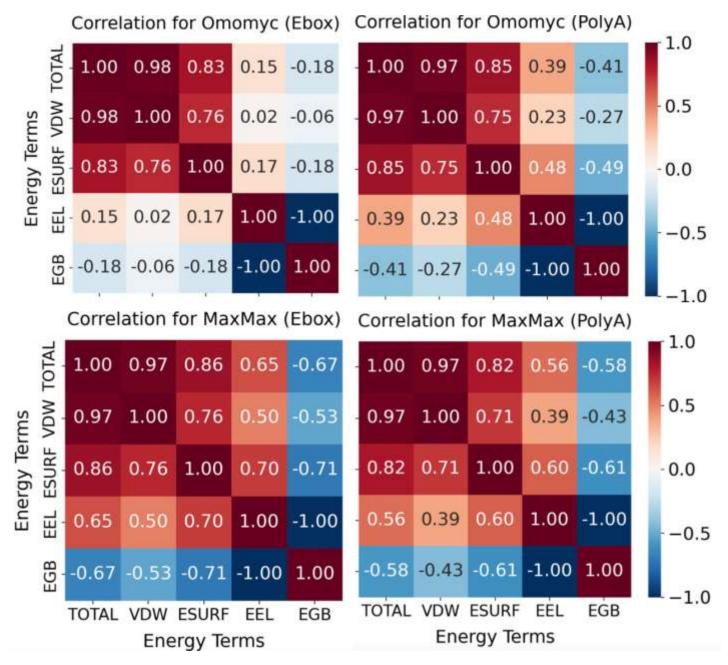


Figure S12: Pearson correlation coeZicient between energy terms TOTAL (Total binding energy), VDW (van der Waals interactions), EEL (Electrostatic energy), EGB (Polar solvation energy), ESURF (non-polar solvation energy) for Ebox (left) and polyA (right) systems. Omomyc terms are shown on the first row, MaxMax on the second.

System	-TΔS (<u>kT</u>)
МусМах	12 (7)
Omomyc	10 (6)
MaxMax	9 (5)
MycMax (polyA)	9 (9)
Omomyc (polyA)	13 (7)
MaxMax (polyA)	16 (9)

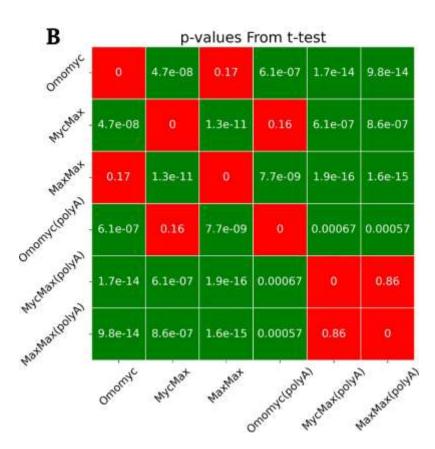


Figure S13. (A) p-values obtained from a two-sample t-test comparing the interaction entropies between pairs of systems. The null hypothesis assumes that the entropic contributions to binding are identical between the two compared systems. A small p-value (<0.05, shown in green) indicates a statistically significant diZerence in entropy between the systems, suggesting distinct entropic contributions, whereas a large p-value (>0.05, shown in red) suggests no significant diZerence. (B) The mean and standard deviation of the entropic contribution to the binding free energy across all systems.

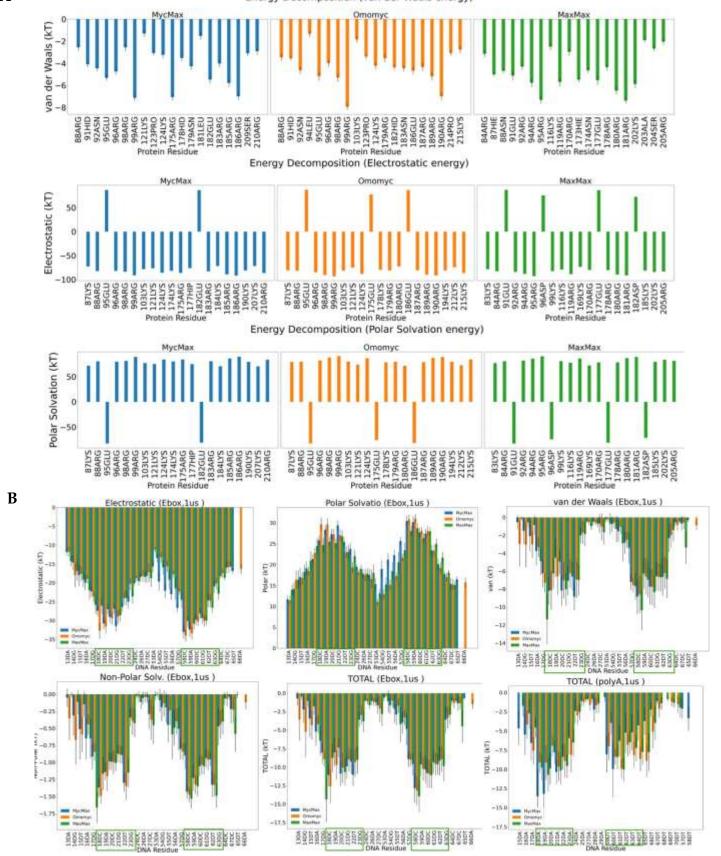


Figure S14: Energy Decomposition Analysis for MycMax, Omomyc dimer, and MaxMax Complexes. (A) Per-residue energy decomposition for protein residues in MycMax, Omomyc, and MaxMax complexes. The plots display the contributions of van der Waals energy, electrostatic energy, and polar solvation energies for the top 20 contributing protein residues. (B) Per-residue energy decomposition for DNA residues in MycMax (blue), Omomyc (orange), and MaxMax (green) complexes bound to polyA (last panel, bottom right) and Ebox. The plots show the contributions of van der Waals, Electrostatic, Polar Solvation, Non-Polar Solvation Energies, and the total energy. The E-box region is highlighted in the green box.

HB Interactions, 0-10 ns

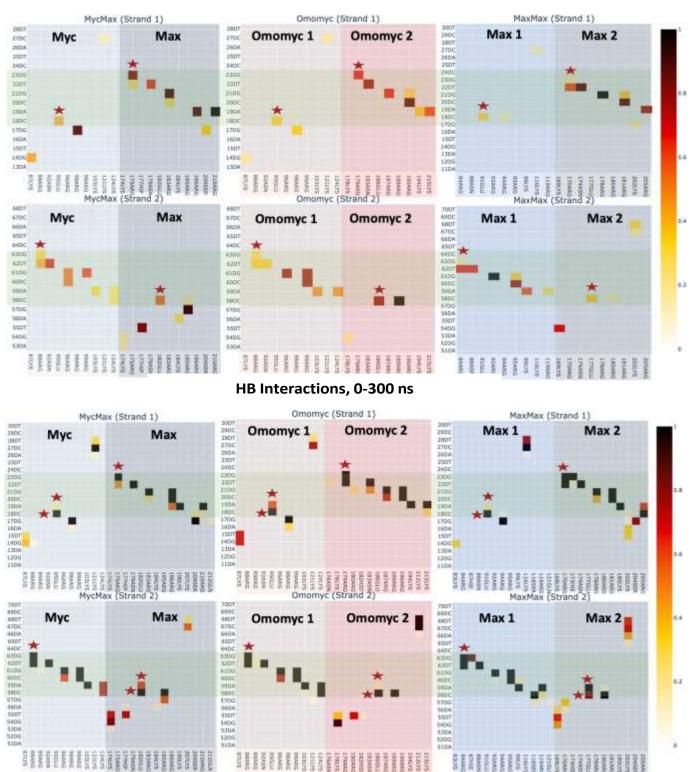


Figure S15: Hydrogen bonding or HB patterns from t=0-10 ns (top) and t=0-300 ns (bottom) for MycMax, Omomyc dimer and MaxMax complexes on E-box DNA. The heatmaps show unique protein-DNA residue contact occupancies for MycMax (left), Omomyc (middle) and MaxMax (right). The top row corresponds to contacts with DNA residues from Strand 1, bottom row corresponds to DNA residues from strand 2. Each contact map has the E-box region highlighted in green shade. Contacts belonging to each monomer are shaded with a diZerent color (light for first monomer, dark for second monomer). Contacts with DNA bases are marked by a star.

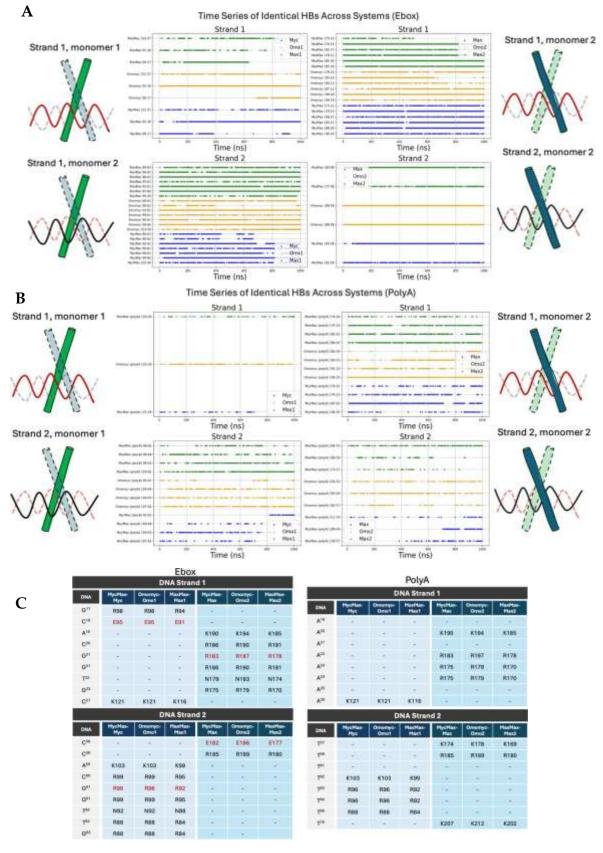


Figure S16: Time series plots showing the persistence of conserved HBs between protein residues and DNA strands in MycMax, Omomyc, and MaxMax bound to Ebox (A) or PolyA (B) over the 1 µs simulation period. Interactions from Myc are colored in blue, Omomyc in orange, and MaxMax in green. The plots are divided into interactions involving Strand 1 (top) and Strand 2 (bottom) with Protein 1 (left) and Protein 2 (right). Schematic representations are shown of the protein-DNA interactions for each strand and protein combination. (C) Tables summarizing the conserved HBs between DNA nucleotides and conserved protein residues for each complex. The tables list the DNA position, the interacting protein residues, and the complexes in which these interactions are observed. The Ebox systems are shown on the left, and the polyA systems on the right.

Count of unique atom pairs making HB contacts:

Ebox polyA

	Ebox	Flank	Total
MycMax	33	13	46
Omomyc	41	8	49
MaxMax	32	13	45

	Center	Flank	Total
MycMax	19	15	34
Omomyc	29	11	40
MaxMax	34	11	45

Figure S17: Count of unique protein-DNA atoms pairs making HB contacts in the Ebox system (left) and polyA system (right) acrossthe 1-microsecond trajectory. Ebox and Center are the 6-bp central binding sites in the Ebox and polyA systems respectively, and Flank are the flanking DNA sites.

III. MycMas Black	800°C	.01H	95/00	16vt.	2HE Omerate (Creat)	1400	COLD	RAYS:	NZ:	Assessment of the Park Committee	W residue D				-	
MycMae Séyo	BRITE.	000	agenc.	165	216 Grunny (Creat)	1907	OPP	8039	NZ.	ME Machine (Mari)	1000	016		94499	1910	List of All HB Contacts
T MycMai Bifytt	6500	DOT	BIANG	1811	204 Grounge (Crost)	THIS	019	36400	1940	88 - Machine (Meet)	KUCT	OSE		80090	100	
MyoMas Shirt	6000	000	9990	1010	207 (Jenseys (Cest))	THE	GIP	MANG	West	10E Muskler (Mart)	1000	OW		BANNO	1010	(Occupancy > 15%)
E Mychiae Silye)	1000	000	SOADN .	1600	200 Overval (Crest)	X0015	OUT	SEATH	9811	96 Mushing (Mect)	600G	947	*	STHE.	WEZ	★ Contacts with bases
M. MycMax Mycs	1800	na •	man	001	#8 Dromp (Droft)	405	OP	98495	7840	ING - Meshfee (Most)	600	OIP		DEADN	HEIR	Contacts with bases
	1800		9990J	069	per Orange (Court)	4007	GIP	90000	mber	T - Markfur (Mari)	HOC	566	*	audin.	DET	Common
H. MysMan (Mys)		010	MANG	WG	888 Decembridation (Creat)	1000	500 W	9000,03	180	df Musikes (Mac1)	8100	- 01F		80040	1012	contacts acro
# MycMae (Myr)	W100				SMF Orango (Omet)	8100	0.0	96460	med	SE Monthley (Vise 1)	entiris	OSF		BONNE	160	1000 March 2000 March
ET MycMan Myrs	most	OM:	95445	100	and Charge (Creek)	4106	OIP	owin	NE	89 Maddachterts	8150	OUR		BOARG	1640	systems
W Nychia Styc	inog	INF.	MARG:	1411	(MR Desiry) (Dear)	21010	001	MARIE	Nert	6 Machine (Mart)	1700	OIF		BANKS	5611	
28 AlyeMae Bilysi	1700	DON	MARG	75	200 Onampi (Omel)	61005	000	90400	(995)	85 Monthlet (Marri)	B00C	1000		BSARG	160	
H MycMax (Mys)	1708	100	MARK	9841	216 Drompt (Drom)	1206	OSP	36400	(AE	84 Manthus (Mant)	BESC	OFF		BOARD	8812	
III MysMax (Mys)	1700	3381	BIANG	1942	SSE Onesia (Creat)	1709	core:	16460	med	se: Number (Marl)	8109	307		MANG	9811	
N MycMan Bhyri	MOC	DOT	MINIS.	15	360 Onesys (Charle	1000	689	SHARE.	HE.	76 Machine (Mart)	990A	2019		MILTS	162	
WysMax Myss	WOOG	0.00	MONAGE.	1947	200 Drump (Dect)	860	001	28450	med	190 Markins (Mari)	amo	OIP		THEFT	162	
94 MycMao Briyst	8100	197 :**	HIMPIO	9817	200 Deserge (Creat)	2109	10' W	96496	1987	BY Manthus (Mart)	2700	CEP		TOLLYS	102	
H. MycMan Edyoj	9904	Diff	mount	146	SNE Desiration (Dear)	91010	GIF	(MAN)	West	SE Markfast (Mast)	2001	OIP		THELYS	162	
B MyMar Mys	E700	D181	strone.	144	BN Drumpt (Deat)	SIDA	Oil	100075	942	60 Markton OttorTi	MOC	DIP		118888	00	
6 NycMai (Myc)	9800	011	1040/5	142	857 Chargo (Charl)	37000	0/P	101019	MA	TT ModNa (Mert)	SHOA	016		TIMMS	1012	
Mi MycMas (Myc)	MICA	CON	104,75	162	EFR Chromic (Creat)	1600	OIF	1940%	NZ.	TR MacNey (Mart)	SHOA	COF		119490	146	
W MycMar Short	5400	CON	THUS	KT	STY Overlap (Overl)	9904	Oth	10439	NZ:	EF Machine March	9800	OFF		HENYS	142	
TE. MycMan Most	9807	009	1740/5	142	### Orango (Omat)	360A	009	1940/5	AZ	36 Machine (March	1001	OIF		170A90	100	
M MycMax Street	2000	C196	TITIMES	1647	861 Owenya (Oreal)	sidora	GOP	179039	NZ	56 Martine March	1001	cor		175496	NC.	
19 MysMas Shud	9801	Corr	171108	NEE	\$46 Onesign (One)D	20517	an	THANK	Mert	at Vertex Med)	1300	ODP		TRIMBI	1040	
M. Thychlac Shart	2001	1000	1794091	1600	248 Onumpt (Ored)	2201	000	THANS	ME	M Marthur (Mart)	2900	06		173465	NEZ	
W MycMas (Mad	MOG	99.0	1835333	-061	856 Orome (Cred)	THEFT	CDP	179495	NAC.	26 Variation (March	2005	QUE		179ADM	NEW	
S MycMai Fried	9904	19.7	1600001	068	SHE Charles (Cred)	200	CODE	Trease.	(Marri)							
ET - NycMar (Mar)	2100	.010	100490	1610	286 Orients (Druct)	2800	CHP	170ANG	100	ME Machine Mmill)	SHOC	166	*	177GL11	010	
M. Myrkles Sileni	704	11287	TRIMIC	100	281 Oranyi (Droft	2827	007	HESAUN	8839	26 MorMor (March	3100	Car		THANG	ME	
M MycMai Phini	2109	COFF	380496	1015	388 Oranje (Ontil)	9600	164 18	HOUSE	061	80 Markler (Vox2)	SADE	520		STRANS	9991	
III. MycMai (Max.	1000	00#	HISANG	Merci	attl. Orange (Small)	800	***	186000	001	\$1. MorNov (Vinc)	2100	1000		THANS:	1010	
M. MycMan Stani	1904	1299*	186AHG.	1912	28 Orway Crist	200	019	(EDMH)	1017	12 MacNex (Vaxi)	SHIC	1085		100000	0445	
MycMai (Med.	3600	-000	180995	140	940 Oranny Christ	2/00:	001	HITARIL	NE.	35 Moder (Med)	SEC	240		181445	NE.	
B Nychlar Phys	3600	00#	HISANG	1611	201 Ommy Const	206	GIF	181445	10011	26 MacNew (Mexil)	900C	4386		ISUNG.	100	
E. Myckles Stant	2504	N7.4	MINNS	1812	278 Orango (Swell	MDC:		180090	1011	27 MarMan (March)	2100	107	*	TRIMIG	2001	
Michael Sheet	1904	0.00	HEOSE	140	200 Orangi Chall	2000	009	THOMPS	100	17 Moder (MacI)	1804	000		1860%	740	
W MycMan (Mari)	6700	018	007LYS	NZ	294 Orango (Onell)					18 MorMax (MaxX)	TROA	009		166079	NE	
B MycNes Steel	1700	0.9	208889	00	238 Charata Charle	2'08	W.	1909091	7840	& MacNas-(Mac2)	1700	088		zione	00	
M MycMar Phot	1904	009	J10MIG	1611	EST Orways Carell	THEM	Oth	THE PER	NET	12 HorNey (Mocil)	1900	0.00		SOSANG	NO	
W NycMan Mari	HODA	COM	210490	1812	200 Orango (Oneil)	THUA	019	2160% 2160%	NE NE							

Figure S18: Unique HBs between protein and Ebox DNA atoms up until t=300ns. Contacts with bases are marked with a star, and the contacts that are common across all 3 systems at the atomic level are highlighted in pink.

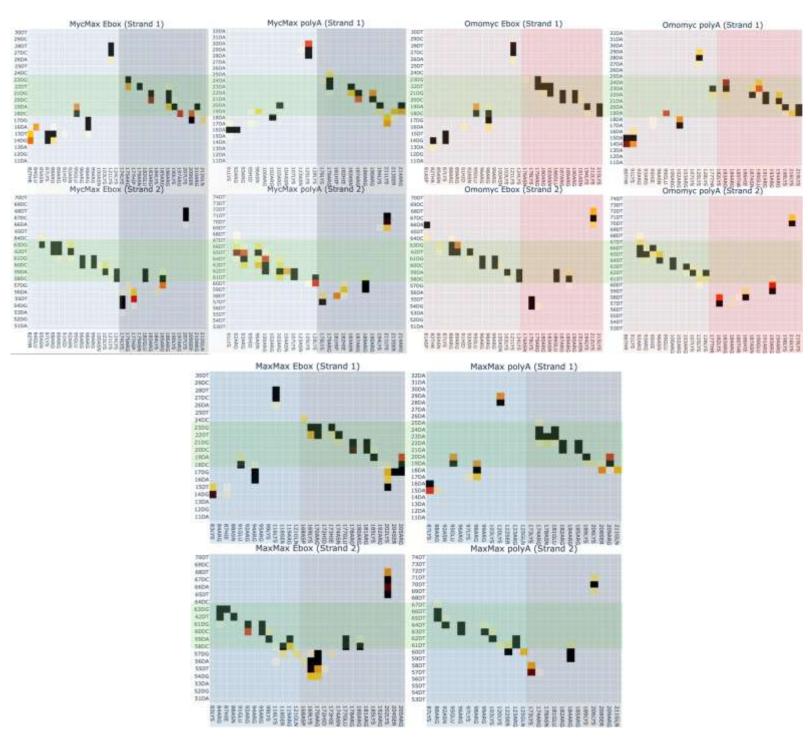


Figure S19: Comparing heatmaps of HB occupancy between Ebox and PolyA for MycMax (top left), Omomyc (top right), and MaxMax (bottom) from individual 1-us MD simulations. E-box region is highlighted in green, and each protein monomer is delimited by a rectangular colored box.

KL Divergence Between Ebox and polyA (Time range: 0-20 ns) Strand 1 3.5 0.2.5 0.2.5 1.0 1.0 MycMax MycMax (Max) Myc) Omomyc (Omo1) Omomyc (Omo2) MaxMax (Max1) MaxMax (Max2 0.0 Strand 2 Strand 2 MycMax (Myc) MycMax (Max) Omomyc (Omo1) Omomyc (Omo2) MaxMax (Max1) MaxMax (Max2) 0.0 -5T -4A 3G-2C 2T 3G 7C 8T -7A 99 -57 16 00 11G 27 37 70 81 KL Divergence Between Ebox and polyA (Time range: 0-300 ns) Strand 1 Strand 1 3.0 2.5 2.0 2.1 1.5 1.0 MycMax (Max) MycMax (Myc) Omi imyc (Omo1) Omomyc (Omo2) MaxMax (M Max Max (Max1) ax2)0.0 Strand 2 Strand 2 3.0 2.5 2.0 1.5 1.0 1.0 MycMax (Myc) MycMax (Max) Omomyc (Omo1) Om myc (Omo2) MaxMax (Max1) MaxMax (Max2) 0.0 00 11G 27 27 57 87 87 KL Divergence Between Ebox and polyA (Time range: 0-1000 ns) Strand 1 Strand 1 KL Divergence 1.5 1.0 0.5 ycMax (Myc) MycMax (Max) momyc (Omo1) Omomyc (Omo2) laxMax (Max1) MaxMax (Max2) 0.0 Strand 2 Strand 2 2.5 1.5 1.0 MycMax (Myc) lycMax (Max) Omomyc (Omo2) Omomyc (Omo1) MaxMax (Max1) taxMax (Max2) ┙0.5 90 11 27 33 34 40 57 87 87

Figure S20: Comparison of MycMax, Omomyc dimer, and MaxMax interactions with E-box and PolyA. (A) KL divergence between E-box and polyA binding modes for MycMax (blue), Omomyc (orange), and MaxMax (green) complexes across both DNA strands over the 20 ns (top), 300 ns (middle), and 1 µs trajectory (bottom). The E-box region is highlighted by the black box.