Supplementary information

Effects of mutants in bHLH region on structure stability and protein-DNA binding energy in DECs

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Figure S1 Analysis of DEC2 protein globularity. (A) The x-axis represents the sequence residues, while the sum of disorder propensities and protein binding propensities are on the y-axis. (B) The protein sequence is colored according to the Russel/Linding disorder definition, where the red residues were disordered.

Figure S2. Radius of gyration for each monomer. The left column represents the DEC1 homodimer while the middle and right columns represent the DEC1/HES-1 heterodimer and DEC2 homo- and heterodimers, respectively.

Figure S3. Solvent accessible surface area ratio between hydrophobic and hydrophilic atoms. Ratio = $SASA_{\text{hydrophobic}}/SASA_{\text{hydrophilic}}$

The left column represents the DEC1 homodimer while the middle and right columns represent the DEC1/HES-1 heterodimer and DEC2 homo- and heterodimers, respectively.

Figure S4. Hydrogen bond numbers between protein and water.

Figure S5. Geometrical property of high flexible region in dimers.

Figure S6. Contribution of residues to the binding energy.

Supplementary Table S1. Final configuration for molecular dynamics simulation.

Molecules	DEC1_A/DEC1_B	DEC1_A/DEC1_B	DEC1_A/DEC1_B	DEC1_A/DEC1_B	DEC1/HES-1	DEC1/HES-1	DEC1/HES-1	DEC1/HES-1
	WT	H57A	R65A	LL7879AA	WT	H57A	R65A	LL7879AA
Number of atoms (protein)	2108	2094	2080	2072	2254	2247	2240	2241
Number of H ₂ O molecules	41256	46740	43088	47860	52428	51564	48440	52536
Box dimensions (Å ³)	70x70x70	70x70x70	70x70x70	70x70x70	70x70x70	70x70x70	70x70x70	70x70x70
Residual charge neutralization (Cl ⁻)	10	10	8	10	16	16	15	16
Simulation time	50 ns	50 ns	50 ns	50 ns	50 ns	50 ns	50 ns	50 ns
Molecules	DEC2_A/DEC2_B	DEC1_A/DEC1_B	DEC1_A/DEC1_B	DEC1_A/DEC1_B				
	WT	H57A	R65A	LL7879AA				
Number of atoms (protein)	2232	2196	2316	2298				
Number of H ₂ O molecules	41508	52492	52484	52464				
Box dimensions (Å ³)	70x70x70	70x70x70	70x70x70	70x70x70				
Residual charge neutralization (Cl ⁻)	10	10	16	16				
Simulation time	50 ns	50 ns	50 ns	50 ns				

Cl⁻ chloride ions. Å-angstrom; wt-wild-type.

Supplementary Table S2. Available templates with similar structure with the DEC1/2 sequence. *Mm* - *Mus musculus*;

PDB	ID	Species	E-Value	Identity	Length	Bits(score)	Resolution	Method
2MH3 (A, B)	HES1/HES1+DNA	<i>Hs</i>	2.0e-6	42% - 27/65(A,B)	70	114	-	NMR
4H10 (A, B)	ARNTL1/CLOCK	<i>Hs</i>	0.022	27% - 19/51(A)	73	36.6	2.4 Å	X-ray
4F3L (B)	BMAL1/CLOCK	<i>Ms</i>	0.044	25% - 15/59(B)	71	29.3	2.4 Å	X-ray
1AM9 (A, B, C, D)	SREBP-1A/SREBP-1A	<i>Hs</i>	0.096	27% - 21/77 (A,B,C,D)	82	34.7	2.3 Å	X-ray
1AN4 (A, B)	USF/USF	<i>Hs</i>	0.16	28% -16/57 (A, B)	65	33.9	2.9 Å	X-ray
1R05 (A, B)	Max/Max	<i>Hs</i>	1.9	29% - 23/79 (A, B)	87	30.8	-	NMR
2EFL (A)	FBP17/FBP17	<i>Hs</i>	6.1	26 - 23/87 (A)	305	31.2	2.9 Å	X-ray

Hs - *Homo sapiens*; NMR - nuclear magnetic resonance; Å - angstrom (10^{-10} m).

Supplement table 2. Available templates with similar structure with the DEC1/2 sequence.

Table S3. Average values of the geometrical property statistics during the last 30 ns of MD simulations

Å – angstrom (10⁻¹⁰ m); wt - wild-type; Rg - radius of gyration; RMSD – root-mean-square-deviation, taking as a reference their respective starting structures. mon1 is the monomer

Model		Δ SASA(A ²)	RMSD (A)		Rg(A)									
HOMODIMER (DEC1_A/DEC1_B)			Total		Basic		Helix I		Loop		Helix II		Basic	
			Mon1	Mon2	Mon1	Mon2	Mon1	Mon2	Mon1	Mon2	Mon1	Mon2	Mon1	Mon2
	wt	-27.4	0.39	0.23	1.06	0.46	0.29	0.18	0.35	0.25	0.34	0.20	0.58	0.62
	H57A	-27.9	0.34	0.20	0.99	0.32	0.30	0.16	0.27	0.28	0.24	0.20	0.61	0.61
	R65A	-28.5	0.33	0.17	1.01	0.28	0.28	0.15	0.27	0.21	0.20	0.15	0.59	0.62
	LL7879AA	-23.2	0.37	0.36	1.02	0.97	0.28	0.31	0.33	0.29	0.30	0.25	0.62	0.60
HOMODIMER (DEC2_A/DEC2_B)														
	wt	-28.3	0.38	0.21	0.88	0.31	0.36	0.21	0.30	0.19	0.30	0.17	0.57	0.46
	LL7071AA	-37.3	0.37	0.18	0.71	0.30	0.29	0.19	0.34	0.15	0.25	0.15	0.59	0.47
HETERODIMER (DEC1/HES-1)														
	wt	-31.2	0.26	0.19	0.54	0.45	0.21	0.17	0.27	0.22	0.22	0.18	0.59	0.57
	H57A	-28.8	0.27	0.22	0.74	0.61	0.22	0.23	0.29	0.25	0.20	0.21	0.63	0.59
	R65A	-23.7	0.28	0.30	0.74	0.64	0.20	0.30	0.27	0.23	0.25	0.22	0.63	0.60
	LL7879AA	-25.6	0.31	0.27	0.6	0.49	0.28	0.22	0.33	0.28	0.24	0.24	0.61	0.59
HETERODIMER (DEC2/HES-1)														
	wt	-34.4	0.23	0.20	0.41	0.44	0.23	0.18	0.18	0.15	0.18	0.18	0.58	0.40
	LL7071AA	-32.4	0.60	0.21	1.39	0.30	0.48	0.20	0.41	0.19	0.57	0.17	0.58	0.41

DEC1_A/DEC2_A in the homodimer and DEC1/DEC2 in the heterodimer; mon2 is the monomer DEC1_B/DEC2_B in the homodimer and monomer HES-1 in the heterodimer