Crystal structures and NMR studies of the apo form of the c-MYC:MAX bHLHZip complex reveal a helical basic region in the absence of DNA

Susan Sammak(a)[§]; Najoua Hamdani(a)[§]; Fabrice Gorrec(b); Mark D. Allen(b); Stefan M.V. Freund(b); Mark Bycroft(b); and Giovanna Zinzalla(a)*

(a) Microbiology, Tumor and Cell Biology (MTC), Karolinska Institutet, Solnavägen 9, 171 65 Stockholm, Sweden.
(b) MRC Laboratory of Molecular Biology, Cambridge Biomedical Campus, Francis Crick Ave, Cambridge CB2 0QH, UK.

Table S1. **The eight mixes of additives (44 compounds) integrated in the final formulation of MORPHEUS III**.

Mix name	Solvent	Chemicals
Dipeptides	DW	2 %(w/v) Ala-Ala, 2 %(w/v) Ala-Gln, 2 %(w/v) Gly-Glu, 2 %(w/v) Gly-L-Ala, 2 %(w/v) Gly-L-Asp, 2 %(w/v) Gly-Sar, 2 %(w/v) L-carnosine, 2 %(w/v) Leu-Ala hydrate
Vitamins	DW	3 %(w/v) (+)-sodium L-ascorbate, 3 %(w/v) choline chloride, 3 %(w/v) D-panthenol, 3 %(w/v) pyridoxine HCl, 3 %(w/v) thiamine HCl
Nucleosides	DW	2 %(w/v) cytidine, 2 %(w/v) inosine, 2 %(w/v) ribavirin, 2 %(w/v) thymidine, 2 %(w/v) uridine
Phytochemicals 1	50 %(v/v) EtOH	0.5 %(w/v) menthol, 0.5 %(w/v) caffeic acid, 0.5 %(w/v) D-(−)-quinic acid, 0.5 %(w/v) shikimic acid, 0.5 %(w/v) gallic acid monohydrate, 0.5 %(w/v) N-vanillylnonanamide, 0.5 %(w/v) thymol
Phytochemicals 2	50 %(v/v) EtOH	0.5 %(w/v) D-(−)-salicin, 0.5 %(w/v) esculin hydrate, 0.5 %(w/v) p-arbutin, 0.5 %(w/v) quinine hemisulfate salt monohydrate , 0.5 %(w/v) tryptamin
Antibiotics	DW	1 %(w/v) ampicillin sodium salt, 1 %(w/v) apramycin sulfate, 1 %(w/v) bacitracin, 1 %(w/v) dihydrostreptomycin sesquisulfate, 1 %(w/v) gentamicin sulfate salt, 1 %(w/v) spectinomycin dihydrochloride pentahydrate
Cholic acid derivatives	DW	3 %(w/v) CHAPS hydrate, 3 %(w/v) CHAPSO, 3 %(w/v) sodium glycocholate hydrate, 3 %(w/v) taurocholic acid sodium salt hydrate
Anesthetic alkaloids	DW	2 %(w/v) lidocaine HCl·H2O, 2 %(w/v) procaine HCl, 2 %(w/v) proparacaine HCl, 2 %(w/v) tetracaine HCl

Note some irregularities to the chosen group names, notably ribavirin (a nucleoside derivative) and D-panthenol (provitamin B5).

Table S2. The formulation of the 96 crystallization conditions forming MORPHEUS III (in three sections).

Well	Cryoprotected precipitant mix	Mix of additives	Buffer system
A1	10% w/v PEG 20 000, 20% v/v PEG MME 500	0.2 %(w/v) of each Dipeptide	0.1 M MES/imidazole pH 6.5
A2	10% w/v PEG 8000, 20% v/v ethylene glycol	0.2 %(w/v) of each Dipeptide	0.1 M MES/imidazole pH 6.5
A3	10% w/v PEG 4000, 20% v/v glycerol	0.2 %(w/v) of each Dipeptide	0.1 M MES/imidazole pH 6.5
A4	12.5% w/v PEG 1000, 12.5% w/v PEG 3350, 12.5% v/v MPD	0.2 %(w/v) of each Dipeptide	0.1 M MES/imidazole pH 6.5
A5	10% w/v PEG 20 000, 20% v/v PEG MME 500	0.2 %(w/v) of each Dipeptide	0.1 M MOPS/HEPES-Na pH 7.5
A6	10% w/v PEG 8000, 20% v/v ethylene glycol	0.2 %(w/v) of each Dipeptide	0.1 M MOPS/HEPES-Na pH 7.5
A7	10% w/v PEG 4000, 20% v/v glycerol	0.2 %(w/v) of each Dipeptide	0.1 M MOPS/HEPES-Na pH 7.5
A8	12.5% w/v PEG 1000, 12.5% w/v PEG 3350, 12.5% v/v MPD	0.2 %(w/v) of each Dipeptide	0.1 M MOPS/HEPES-Na pH 7.5
A9	10% w/v PEG 20 000, 20% v/v PEG MME 500	0.2 %(w/v) of each Dipeptide	0.1 M bicine/Trizma base pH 8.5
A10	10% w/v PEG 8000, 20% v/v ethylene glycol	0.2 %(w/v) of each Dipeptide	0.1 M bicine/Trizma base pH 8.5
A11	10% w/v PEG 4000, 20% v/v glycerol	0.2 %(w/v) of each Dipeptide	0.1 M bicine/Trizma base pH 8.5
A12	12.5% w/v PEG 1000, 12.5% w/v PEG 3350, 12.5% v/v MPD	0.2 %(w/v) of each Dipeptide	0.1 M bicine/Trizma base pH 8.5
B1	10% w/v PEG 20 000, 20% v/v PEG MME 500	0.3 %(w/v) of each Vitamin	0.1 M MES/imidazole pH 6.5
B2	10% w/v PEG 8000, 20% v/v ethylene glycol	0.3 %(w/v) of each Vitamin	0.1 M MES/imidazole pH 6.5
B3	10% w/v PEG 4000, 20% v/v glycerol	0.3 %(w/v) of each Vitamin	0.1 M MES/imidazole pH 6.5
B4	12.5% w/v PEG 1000, 12.5% w/v PEG 3350, 12.5% v/v MPD	0.3 %(w/v) of each Vitamin	0.1 M MES/imidazole pH 6.5
B5	10% w/v PEG 20 000, 20% v/v PEG MME 500	0.3 %(w/v) of each Vitamin	0.1 M MOPS/HEPES-Na pH 7.5
B6	10% w/v PEG 8000, 20% v/v ethylene glycol	0.3 %(w/v) of each Vitamin	0.1 M MOPS/HEPES-Na pH 7.5
B7	10% w/v PEG 4000, 20% v/v glycerol	0.3 %(w/v) of each Vitamin	0.1 M MOPS/HEPES-Na pH 7.5
B8	12.5% w/v PEG 1000, 12.5% w/v PEG 3350, 12.5% v/v MPD	0.3 %(w/v) of each Vitamin	0.1 M MOPS/HEPES-Na pH 7.5
B9	10% w/v PEG 20 000, 20% v/v PEG MME 500	0.3 %(w/v) of each Vitamin	0.1 M bicine/Trizma base pH 8.5
B10	10% w/v PEG 8000, 20% v/v ethylene glycol	0.3 %(w/v) of each Vitamin	0.1 M bicine/Trizma base pH 8.5
B11	10% w/v PEG 4000, 20% v/v glycerol	0.3 %(w/v) of each Vitamin	0.1 M bicine/Trizma base pH 8.5
B12	12.5% w/v PEG 1000, 12.5% w/v PEG 3350, 12.5% v/v MPD	0.3 %(w/v) of each Vitamin	0.1 M bicine/Trizma base pH 8.5
C1	10% w/v PEG 20 000, 20% v/v PEG MME 500	0.2 %(w/v) of each Nucleoside	0.1 M MES/imidazole pH 6.5
C2	10% w/v PEG 8000, 20% v/v ethylene glycol	0.2 %(w/v) of each Nucleoside	0.1 M MES/imidazole pH 6.5
C3	10% w/v PEG 4000, 20% v/v glycerol	0.2 %(w/v) of each Nucleoside	0.1 M MES/imidazole pH 6.5
C4	12.5% w/v PEG 1000, 12.5% w/v PEG 3350, 12.5% v/v MPD	0.2 %(w/v) of each Nucleoside	0.1 M MES/imidazole pH 6.5
C5	10% w/v PEG 20 000, 20% v/v PEG MME 500	0.2 %(w/v) of each Nucleoside	0.1 M MOPS/HEPES-Na pH 7.5
C6	10% w/v PEG 8000, 20% v/v ethylene glycol	0.2 %(w/v) of each Nucleoside	0.1 M MOPS/HEPES-Na pH 7.5
C7	10% w/v PEG 4000, 20% v/v glycerol	0.2 %(w/v) of each Nucleoside	0.1 M MOPS/HEPES-Na pH 7.5

Well	Cryoprotected precipitant mix	Mix of additives	Buffer system
C8	12.5% w/v PEG 1000, 12.5% w/v PEG 3350, 12.5% v/v MPD	0.2 %(w/v) of each Nucleoside	0.1 M MOPS/HEPES-Na pH 7.5
C9	10% w/v PEG 20 000, 20% v/v PEG MME 500	0.2 %(w/v) of each Nucleoside	0.1 M bicine/Trizma base pH 8.5
C10	10% w/v PEG 8000, 20% v/v ethylene glycol	0.2 %(w/v) of each Nucleoside	0.1 M bicine/Trizma base pH 8.5
C11	10% w/v PEG 4000, 20% v/v glycerol	0.2 %(w/v) of each Nucleoside	0.1 M bicine/Trizma base pH 8.5
C12	12.5% w/v PEG 1000, 12.5% w/v PEG 3350, 12.5% v/v MPD	0.2 %(w/v) of each Nucleoside	0.1 M bicine/Trizma base pH 8.5
D1	10% w/v PEG 20 000, 20% v/v PEG MME 500	0.05 %(w/v) of each Phytochemical 1	0.1 M MES/imidazole pH 6.5
D2	10% w/v PEG 8000, 20% v/v ethylene glycol	0.05 %(w/v) of each Phytochemical 1	0.1 M MES/imidazole pH 6.5
D3	10% w/v PEG 4000, 20% v/v glycerol	0.05 %(w/v) of each Phytochemical 1	0.1 M MES/imidazole pH 6.5
D4	12.5% w/v PEG 1000, 12.5% w/v PEG 3350, 12.5% v/v MPD	0.05 %(w/v) of each Phytochemical 1	0.1 M MES/imidazole pH 6.5
D5	10% w/v PEG 20 000, 20% v/v PEG MME 500	0.05 %(w/v) of each Phytochemical 1	0.1 M MOPS/HEPES-Na pH 7.5
D6	10% w/v PEG 8000, 20% v/v ethylene glycol	0.05 %(w/v) of each Phytochemical 1	0.1 M MOPS/HEPES-Na pH 7.5
D7	10% w/v PEG 4000, 20% v/v glycerol	0.05 %(w/v) of each Phytochemical 1	0.1 M MOPS/HEPES-Na pH 7.5
D8	12.5% w/v PEG 1000, 12.5% w/v PEG 3350, 12.5% v/v MPD	0.05 %(w/v) of each Phytochemical 1	0.1 M MOPS/HEPES-Na pH 7.5
D9	10% w/v PEG 20 000, 20% v/v PEG MME 500	0.05 %(w/v) of each Phytochemical 1	0.1 M bicine/Trizma base pH 8.5
D10	10% w/v PEG 8000, 20% v/v ethylene glycol	0.05 %(w/v) of each Phytochemical 1	0.1 M bicine/Trizma base pH 8.5
D11	10% w/v PEG 4000, 20% v/v glycerol	0.05 %(w/v) of each Phytochemical 1	0.1 M bicine/Trizma base pH 8.5
D12	12.5% w/v PEG 1000, 12.5% w/v PEG 3350, 12.5% v/v MPD	0.05 %(w/v) of each Phytochemical 1	0.1 M bicine/Trizma base pH 8.5
E1	10% w/v PEG 20 000, 20% v/v PEG MME 500	0.05 %(w/v) of each Phytochemical 2	0.1 M MES/imidazole pH 6.5
E2	10% w/v PEG 8000, 20% v/v ethylene glycol	0.05 %(w/v) of each Phytochemical 2	0.1 M MES/imidazole pH 6.5
E3	10% w/v PEG 4000, 20% v/v glycerol	0.05 %(w/v) of each Phytochemical 2	0.1 M MES/imidazole pH 6.5
E4	12.5% w/v PEG 1000, 12.5% w/v PEG 3350, 12.5% v/v MPD	0.05 %(w/v) of each Phytochemical 2	0.1 M MES/imidazole pH 6.5
E5	10% w/v PEG 20 000, 20% v/v PEG MME 500	0.05 %(w/v) of each Phytochemical 2	0.1 M MOPS/HEPES-Na pH 7.5
E6	10% w/v PEG 8000, 20% v/v ethylene glycol	0.05 %(w/v) of each Phytochemical 2	0.1 M MOPS/HEPES-Na pH 7.5
E7	10% w/v PEG 4000, 20% v/v glycerol	0.05 %(w/v) of each Phytochemical 2	0.1 M MOPS/HEPES-Na pH 7.5
E8	12.5% w/v PEG 1000, 12.5% w/v PEG 3350, 12.5% v/v MPD	0.05 %(w/v) of each Phytochemical 2	0.1 M MOPS/HEPES-Na pH 7.5
E9	10% w/v PEG 20 000, 20% v/v PEG MME 500	0.05 %(w/v) of each Phytochemical 2	0.1 M bicine/Trizma base pH 8.5
E10	10% w/v PEG 8000, 20% v/v ethylene glycol	0.05 %(w/v) of each Phytochemical 2	0.1 M bicine/Trizma base pH 8.5
E11	10% w/v PEG 4000, 20% v/v glycerol	0.05 %(w/v) of each Phytochemical 2	0.1 M bicine/Trizma base pH 8.5
E12	12.5% w/v PEG 1000, 12.5% w/v PEG 3350, 12.5% v/v MPD	0.05 %(w/v) of each Phytochemical 2	0.1 M bicine/Trizma base pH 8.5
F1	10% w/v PEG 20 000, 20% v/v PEG MME 500	0.1 %(w/v) of each Antibiotic	0.1 M MES/imidazole pH 6.5
F2	10% w/v PEG 8000, 20% v/v ethylene glycol	0.1 %(w/v) of each Antibiotic	0.1 M MES/imidazole pH 6.5
F3	10% w/v PEG 4000, 20% v/v glycerol	0.1 %(w/v) of each Antibiotic	0.1 M MES/imidazole pH 6.5
F4	12.5% w/v PEG 1000, 12.5% w/v PEG 3350, 12.5% v/v MPD	0.1 %(w/v) of each Antibiotic	0.1 M MES/imidazole pH 6.5

Well	Cryoprotected precipitant mix	Mix of additives	Buffer system
F5	10% w/v PEG 20 000, 20% v/v PEG MME 500	0.1 %(w/v) of each Antibiotic	0.1 M MOPS/HEPES-Na pH 7.5
F6	10% w/v PEG 8000, 20% v/v ethylene glycol	0.1 %(w/v) of each Antibiotic	0.1 M MOPS/HEPES-Na pH 7.5
F7	10% w/v PEG 4000, 20% v/v glycerol	0.1 %(w/v) of each Antibiotic	0.1 M MOPS/HEPES-Na pH 7.5
F8	12.5% w/v PEG 1000, 12.5% w/v PEG 3350, 12.5% v/v MPD	0.1 %(w/v) of each Antibiotic	0.1 M MOPS/HEPES-Na pH 7.5
F9	10% w/v PEG 20 000, 20% v/v PEG MME 500	0.1 %(w/v) of each Antibiotic	0.1 M bicine/Trizma base pH 8.5
F10	10% w/v PEG 8000, 20% v/v ethylene glycol	0.1 %(w/v) of each Antibiotic	0.1 M bicine/Trizma base pH 8.5
F11	10% w/v PEG 4000, 20% v/v glycerol	0.1 %(w/v) of each Antibiotic	0.1 M bicine/Trizma base pH 8.5
F12	12.5% w/v PEG 1000, 12.5% w/v PEG 3350, 12.5% v/v MPD	0.1 %(w/v) of each Antibiotic	0.1 M bicine/Trizma base pH 8.5
G1	10% w/v PEG 20 000, 20% v/v PEG MME 500	0.3 %(w/v) of each Cholic acid derivatives	0.1 M MES/imidazole pH 6.5
G2	10% w/v PEG 8000, 20% v/v ethylene glycol	0.3 %(w/v) of each Cholic acid derivatives	0.1 M MES/imidazole pH 6.5
G3	10% w/v PEG 4000, 20% v/v glycerol	0.3 %(w/v) of each Cholic acid derivatives	0.1 M MES/imidazole pH 6.5
G4	12.5% w/v PEG 1000, 12.5% w/v PEG 3350, 12.5% v/v MPD	0.3 %(w/v) of each Cholic acid derivatives	0.1 M MES/imidazole pH 6.5
G5	10% w/v PEG 20 000, 20% v/v PEG MME 500	0.3 %(w/v) of each Cholic acid derivatives	0.1 M MOPS/HEPES-Na pH 7.5
G6	10% w/v PEG 8000, 20% v/v ethylene glycol	0.3 %(w/v) of each Cholic acid derivatives	0.1 M MOPS/HEPES-Na pH 7.5
G7	10% w/v PEG 4000, 20% v/v glycerol	0.3 %(w/v) of each Cholic acid derivatives	0.1 M MOPS/HEPES-Na pH 7.5
G8	12.5% w/v PEG 1000, 12.5% w/v PEG 3350, 12.5% v/v MPD	0.3 %(w/v) of each Cholic acid derivatives	0.1 M MOPS/HEPES-Na pH 7.5
G9	10% w/v PEG 20 000, 20% v/v PEG MME 500	0.3 %(w/v) of each Cholic acid derivatives	0.1 M bicine/Trizma base pH 8.5
G10	10% w/v PEG 8000, 20% v/v ethylene glycol	0.3 %(w/v) of each Cholic acid derivatives	0.1 M bicine/Trizma base pH 8.5
G11	10% w/v PEG 4000, 20% v/v glycerol	0.3 %(w/v) of each Cholic acid derivatives	0.1 M bicine/Trizma base pH 8.5
G12	12.5% w/v PEG 1000, 12.5% w/v PEG 3350, 12.5% v/v MPD	0.3 %(w/v) of each Cholic acid derivatives	0.1 M bicine/Trizma base pH 8.5
H1	10% w/v PEG 20 000, 20% v/v PEG MME 500	0.2 %(w/v) of each Anesthetic alkaloids	0.1 M MES/imidazole pH 6.5
H2	10% w/v PEG 8000, 20% v/v ethylene glycol	0.2 %(w/v) of each Anesthetic alkaloids	0.1 M MES/imidazole pH 6.5
H3	10% w/v PEG 4000, 20% v/v glycerol	0.2 %(w/v) of each Anesthetic alkaloids	0.1 M MES/imidazole pH 6.5
H4	12.5% w/v PEG 1000, 12.5% w/v PEG 3350, 12.5% v/v MPD	0.2 %(w/v) of each Anesthetic alkaloids	0.1 M MES/imidazole pH 6.5
H5	10% w/v PEG 20 000, 20% v/v PEG MME 500	0.2 %(w/v) of each Anesthetic alkaloids	0.1 M MOPS/HEPES-Na pH 7.5
H6	10% w/v PEG 8000, 20% v/v ethylene glycol	0.2 %(w/v) of each Anesthetic alkaloids	0.1 M MOPS/HEPES-Na pH 7.5
H7	10% w/v PEG 4000, 20% v/v glycerol	0.2 %(w/v) of each Anesthetic alkaloids	0.1 M MOPS/HEPES-Na pH 7.5
H8	12.5% w/v PEG 1000, 12.5% w/v PEG 3350, 12.5% v/v MPD	0.2 %(w/v) of each Anesthetic alkaloids	0.1 M MOPS/HEPES-Na pH 7.5
H9	10% w/v PEG 20 000, 20% v/v PEG MME 500	0.2 %(w/v) of each Anesthetic alkaloids	0.1 M bicine/Trizma base pH 8.5
H10	10% w/v PEG 8000, 20% v/v ethylene glycol	0.2 %(w/v) of each Anesthetic alkaloids	0.1 M bicine/Trizma base pH 8.5
H11	10% w/v PEG 4000, 20% v/v glycerol	0.2 %(w/v) of each Anesthetic alkaloids	0.1 M bicine/Trizma base pH 8.5
H12	12.5% w/v PEG 1000, 12.5% w/v PEG 3350, 12.5% v/v MPD	0.2 %(w/v) of each Anesthetic alkaloids	0.1 M bicine/Trizma base pH 8.5

Table S3. Data collection and refinement statistics.

Data set	Native (collect5)	Native (collect2)	Native (collect7)
Symmetry	P1	$P2_12_12$	P1
Wavelength	0.9795	0.9763	0.9795
Resolution Range (Å)	38.27-1.35	51.93-2.25	72.54-2.20
Highest resolution shell (Å)	1.42-1.35	2.37–2.25	2.32-2.20
Unique reflections	80704	25627	41701
Completeness (%)[a]	87.9 (81.3)	99.7 (99.7)	80.7 (83.3)
Rmerge[b]	0.062 (0.438)	0.098 (0.545)	0.088 (0.443)
CC(½)[c]	0.989 (0.748)	0.997 (0.875)	0.995 (0.820)
Multiplicity[d]	2.4 (2.3)	4.8 (4.9)	2.3 (2.3)
I/σI[a]	8.7 (2.1)	8.9 (2.8)	6.7 (1.9)
Unit cell parameters	a = 30.40 Å, b = 52.31 Å, c = 73.09 Å, α = 93.89 °, β = 101.92 °, γ = 106.79 °	a = 74.32 Å, b = 145.18 Å, c = 48.50 Å	a = 48.68 Å, b = 74.33 Å, c = 80.06 Å, α = 107.12 °, β = 107.67 °, γ = 90.05 °
Model Refinement			
Resolution Range (Å)	38.27-1.35	40.55-2.25	46.16-2.20
Highest resolution bin (Å)	1.40-1.35	2.34-2.25	2.28-2.20
No. of residues:	A: 89 B: 76 C: 88 D: 76	A: 80 B: 73 C: 78 D: 70	A: 78, B: 68 A: 78, B: 68 E: 78, F: 71 G: 77, H: 67
No. of water, ligands	359, 2 Cl	180, 6 SO_4	480, 14 SO_4
Rwork/Rfree (%)[e]	17.28 / 20.26	20.54 / 23.62	21.49 / 26.81
B average[f] **- protein** **- solvent**	32.07 40.62	44.67 46.42	38.63 44.34
Geometry bond, angles[g]	0.005, 0.756	0.006, 0.677	0.003, 0.510
Molprobity clashscore	8.84	6.17	7.79
Ramachandran[h]	99.38, 0.0	98.29, 0.0	99.13, 0.52
PDB ID[i]	6G6K	6G6J	6G6L

[a] Signal to noise ratio of intensities, highest resolution bin in brackets. [b] R_m : $\sum h \sum i |I/(h,i) - I(h)| / \sum h \sum i / I(h,i)$ where $I(h,i)$ are symmetry-related intensities and $I(h)$ is the mean intensity of the reflection with unique index h . [c] $CC_{1/2}$ is the correlation coefficient of the mean intensities between two random half-datasets. [d] Multiplicity for unique reflections. [e] 5% of reflections were randomly selected for determination of the free R factor, prior to any refinement. [f] Temperature factors averaged for all atoms. [g] RMS deviations from ideal geometry for bond lengths and restraint angles (Engh and Huber). [h] Percentage of residues in the 'most favoured region' of the Ramachandran plot and percentage of outliers (MOLPROBITY). [i] Protein Data Bank identifiers for coordinates.

Crystallography conditions: Collect 5; 10% PEG 8000, 20% ethylene glycol, 5 % EtOH, 0.1 M MOPS/HEPES-Na pH 7.5. 0.075 %(w/v) of each Additive [Additive: 0.75 % menthol, 0.75 % caffeic acid, 0.75 % D-quinic acid, 0.75 % shikimic acid, 0.75 % gallic acid monohydrate, 0.75 % N-vanillylnonanamide]. Collect 2; 20% PEG 3350, 0.2M sodium sulfate decahydrate, pH 7, (20% glycerol was used as cryo-protectant). Collect 7; 15% PEG 8000 15, 0.2M ammonium sulfate, pH 7, (20% glycerol was used as cryo-protectant).

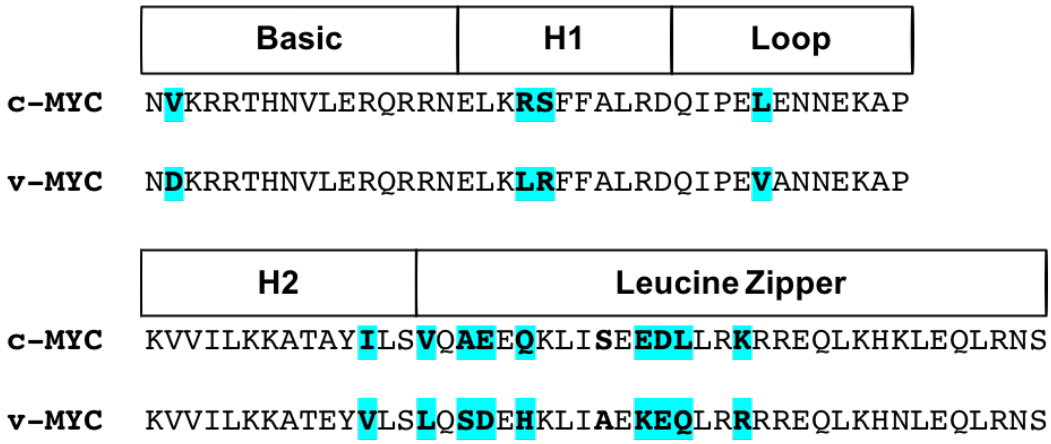

Figure S1. Amino acid sequence alignment for c-MYC and v-MYC bHLHZip proteins.

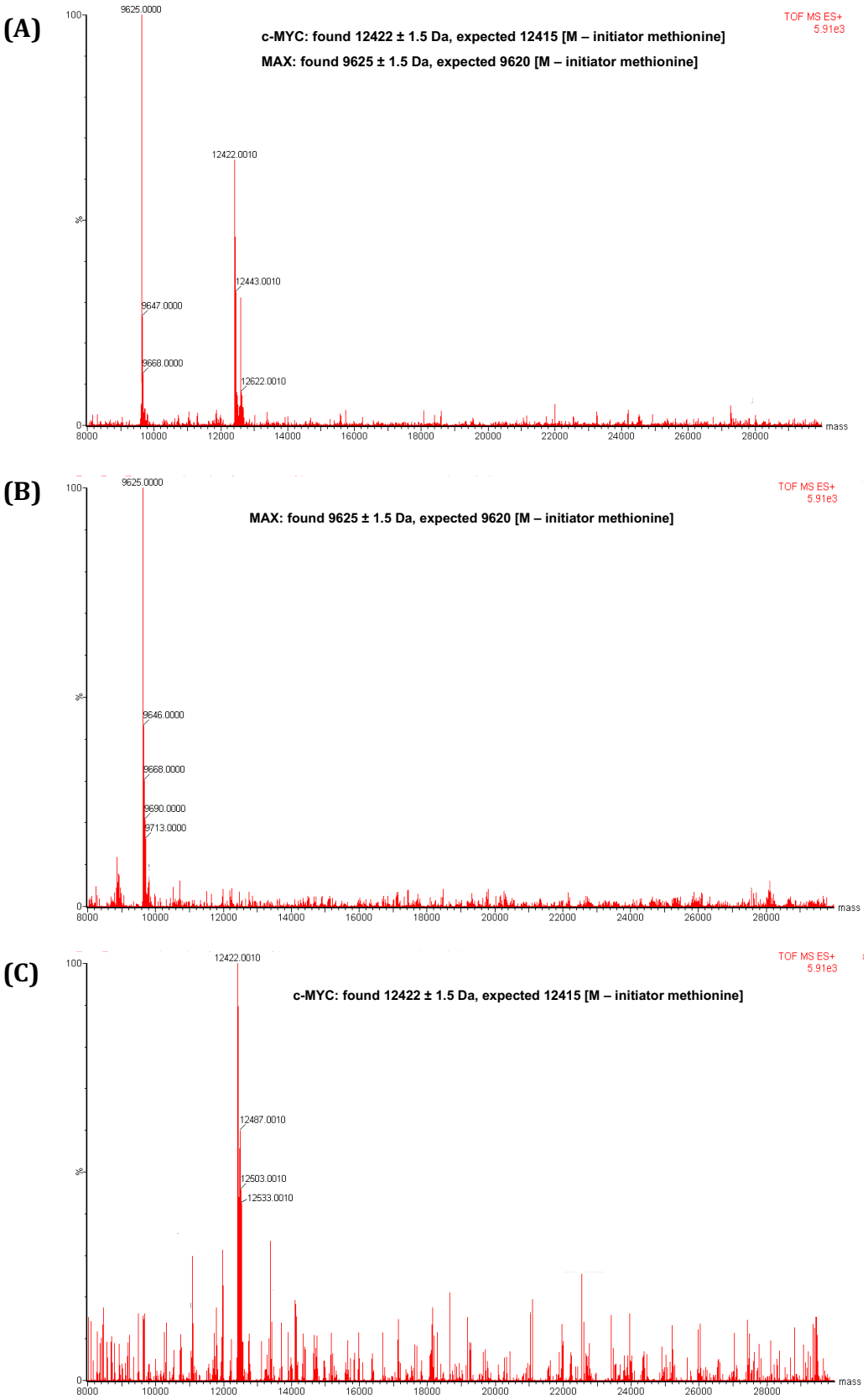

Figure S2. TOF ES+ Mass spectrometry for (A) His-c-MYC:MAX bHLHZip, (B) MAX:MAX bHLHZip, (C) His-c-MYC bHLHZip free protein.

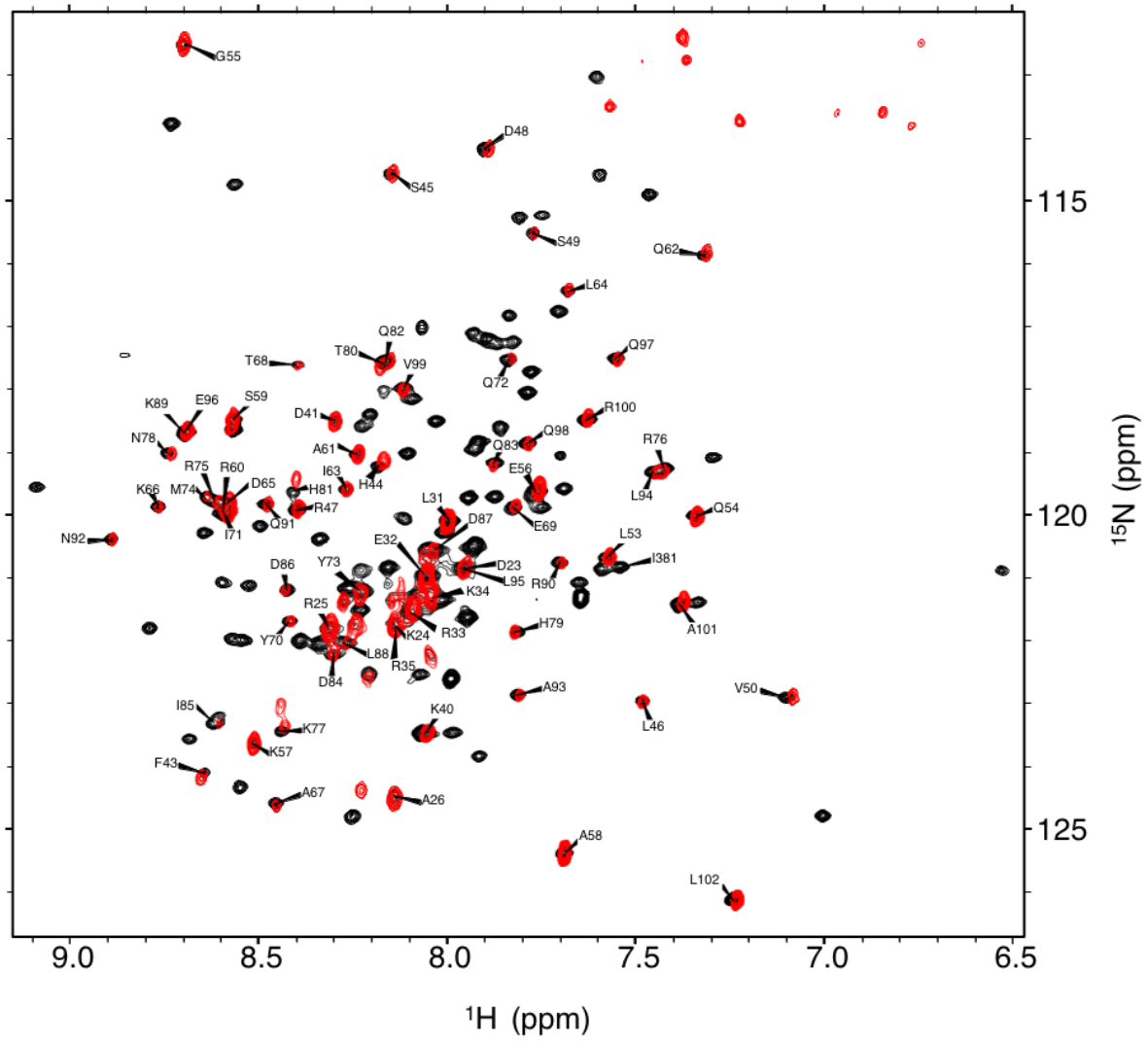

Figure S3. Overlay of ^{1}H/^{15}N HSQC spectra of the ^{1}H,^{15}N-labelled MAX:unlabelled c-MYC bHLHZip complex (in red) and triple labelled c-MYC:MAX bHLHZip complex (in black).

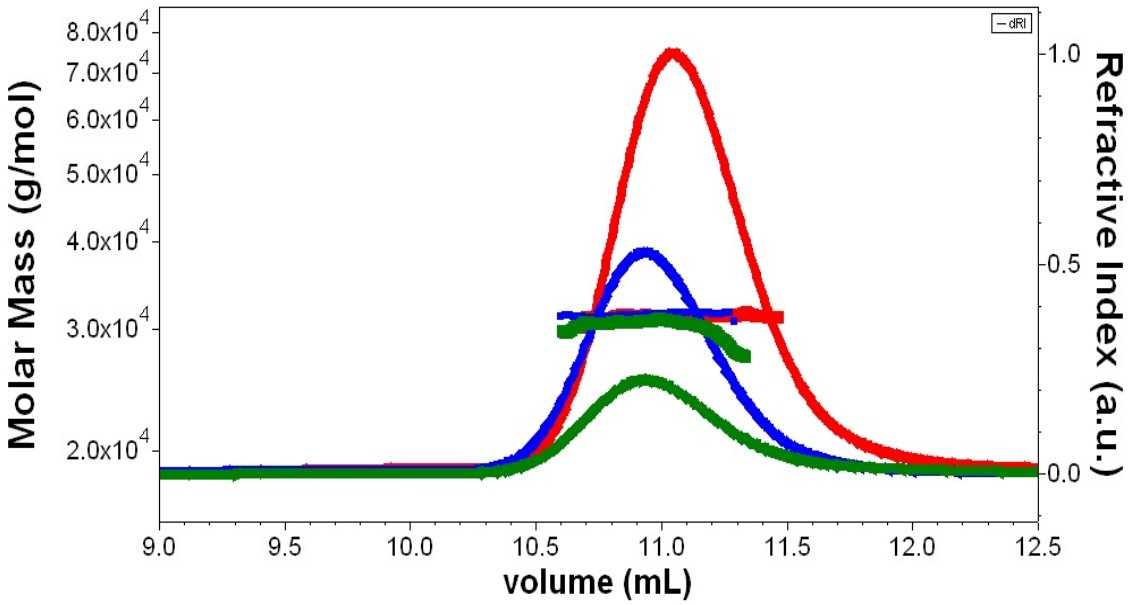

Figure S4. SEC-MALS analysis of of c-MYC:MAX bHLHZip complex bound to DNA.

Evaluated masses for BSA standard (grey) and MM DNA sample at 100 (red), 50 (blue) and 23 uM (green). Masses averaged = 32.2, 32.4 and 31.1 kDa, respectively. Theoretical mass = 31.85 kDa. *The sample of c-MYC:MAX bHLHZip/DNA complex is monodisperse (single mass) at all concentrations.*

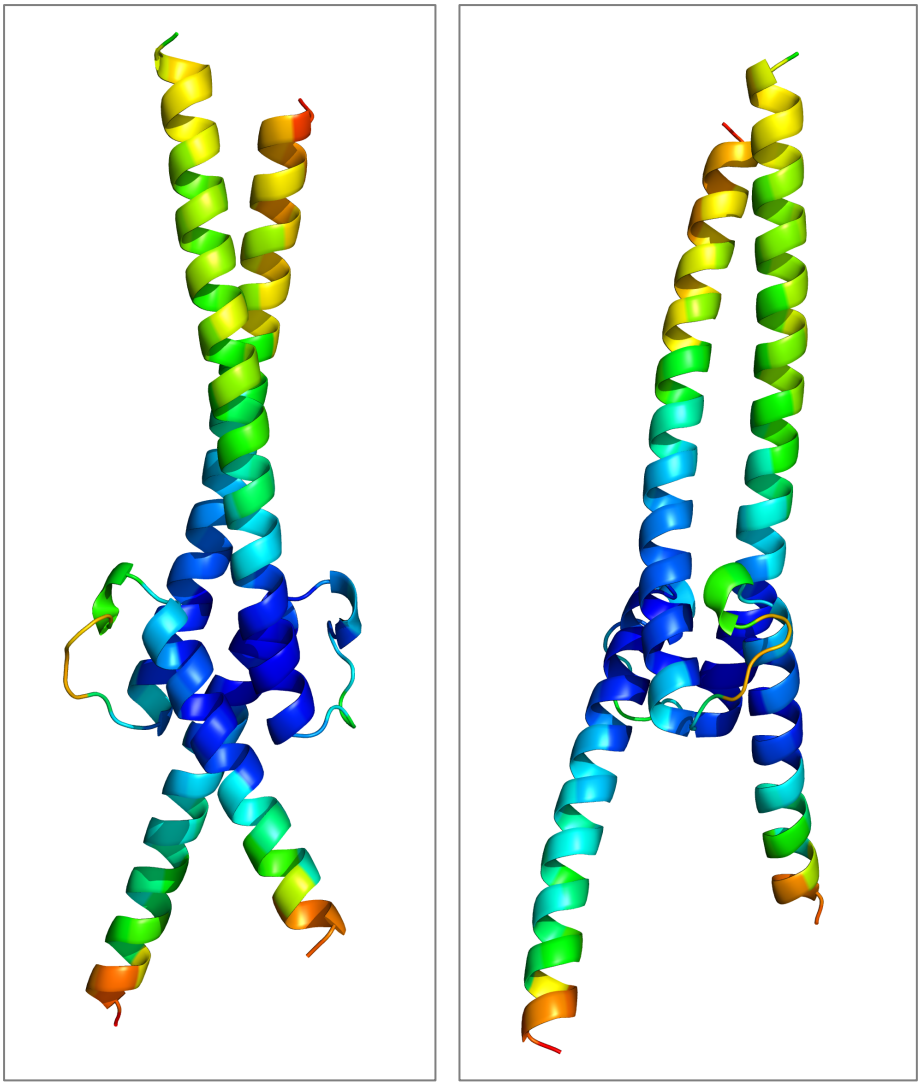

Figure S5. Cartoon representation colour-coded according to B-factor of the c-MYC:MAX apo crystal structure (PDB entry 6G6K).

Left: view of carton representation where blue is representing the lowest B-factor, red the highest. **Right:** the same view rotated by 90° degrees.

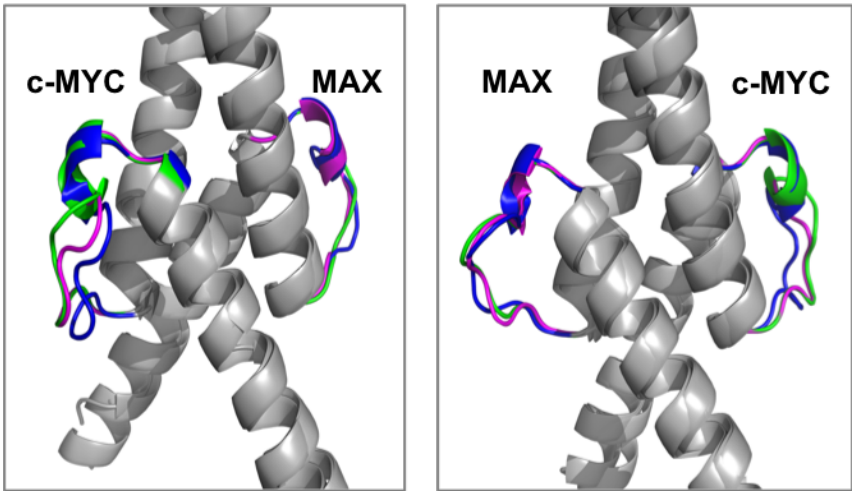

Figure S6. Details of the Loop regions from the overlay of the cartoon representation of three crystal structures of the c-MYC:MAX apo form.

Left: view of the Loop of MYC and MAX of Collect 2/6G6J (magenta), Collect 5/6G6K (blue), and Collect 7/6G6L (green). **Right:** the same view rotated by 180° degrees.