Targeting Undruggable Diseases via Intrinsically Disordered Regions

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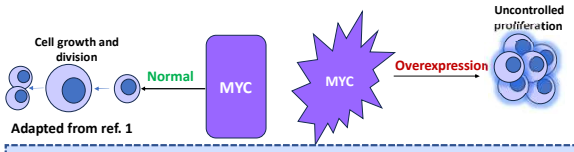
BACKGROUND

Proto-oncogenic Proteins

***MYC** is a **master regulator** involved in regulation of 15% of the genome.*[1]

- Involved in various cellular processes: metabolism, DNA repair, cell cycle, etc.
- MYC dysregulation involved in >70% of human cancers.



Adapted from ref. 1

Basic/Helix-Loop-Helix/Zipper (bHLHZ) Transcription Factors (TF)

Myc/Max/E-box Network

MYC *heterodimerizes* with MAX.

- MYC/MAX dimer binds to enhancer box (**E-box**, 5′-CACGTG).
- Aberrant binding → cancer, respiratory diseases.

MYC/MAX /E-Box network "***undruggable***" by small molecules.[2]
Hard to target → lacks inhibitor binding pockets.

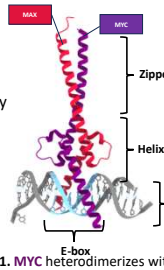


Figure 1. MYC heterodimerizes with MAX to bind **E-box** DNA target (PDB: 1NKP). [3]

PROTEIN THERAPEUTICS

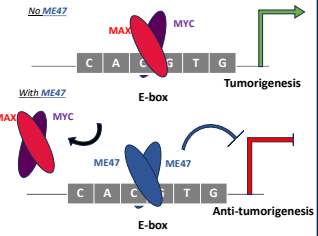
ME47

Combining functional domains:

ME47 = MAX basic region + E47 HLH.

→ ME47 *homodimers* bind to **E-box.**

→ Halts tumor growth in mice.[4]



ME47 homodimer competes for **E-box** binding. Inhibits transcriptional activation by aberrant Myc/Max binding.

Figure 2. ME47, inhibits MYC/MAX binding to **E-box**. [4]

MEF

MEF → ME47 + FosW Leucine Zipper (LZ)

- **LZ** = dimerization domain.
  - Forms *homodimers* via leucine heptad repeats.
- 2-fold stronger binding to **E-box.**
- 4-fold increased specificity for **E-box.**

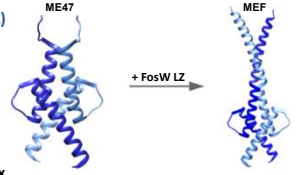


Figure 3. MEF model (PDB: 3U5V [6], 5FV8[7]). [5]

TARGETING SPECIFIC E-BOX SITES

MEFU: Loop Swap

MEF ----LKSDKAQT ← 7 amino acid loop
MEFU DSSMESTKSGQS ← 12 amino acid loop

Short E47 loop replaced by USF1 loop (U-loop)

*Longer loop → reaches **flanking E-box** nucleotides*

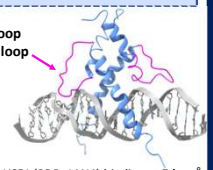


Figure 4. USF1 (PDB: 1AN4) binding to **E-box**.[8]

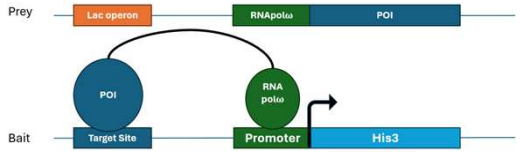
Binding Assays

E-box Target Sites and Flanking Sequences

Nomenclature	DNA Sequence
E-box	5′ CGCCACGTGGCCT
4G	5′ GGACACGTGGGGA
5G	5′ GGACACGTGGGGG

G tracts associated with respiratory diseases, e.g. hereditary asthma.[9]

Bacterial One-Hybrid (B1H)



1. Semi-quantitative
2. Requires US0 cells → lack reporter His3 gene
3. 3-amino-1,2,4-triazole (3-AT) → inhibits cell growth

↑ POI binding
↑ His3 production
↑ Cell growth

Figure 5. B1H. POI is protein-of-interest.[10]

MEFU against 4G/5G

5G preference
MEFU vs. 4G
MEFU vs. 5G

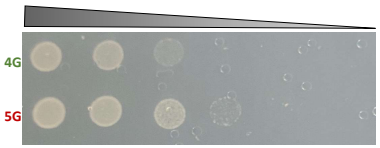


Figure 6. MEFU prefers **5 G-tract.** (25 mM 3-AT)

Electrophoretic Mobility Shift Assay (EMSA)

Quantitative assay → Measures K_d → Binding affinity

Fluorescein-labelled DNA

$$F_B = \frac{I_B}{I_B + I_U} \rightarrow F_B = \frac{[P]}{K_d + [P]}$$

F_B= fraction bound
I_B= intensity bound DNA
I_U = intensity free DNA
P = Protein
K_d= Dissociation constant

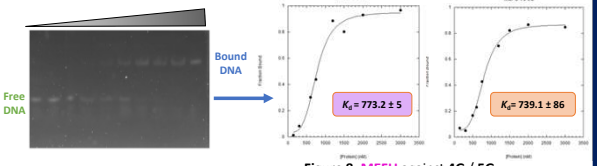


Figure 7. MEFU vs **4G.**

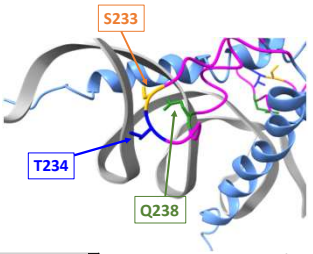
Figure 8. MEFU against **4G** / **5G.** KaleidaGraph.

OPTIMIZING DISORDERED REGIONS

MEFU Arginine (R) loop mutations

→ S233 and T234 contact negatively charged phosphodiester backbone
→ Q238 may contact sugar oxygen of C in G/C pairs flanking **E-box.**[8]

Arginine (R) mutations → enhance Coulombic interactions with negatively charged DNA



Protein	Loop Residues			Loop Amino Acid Length
	233	234	238	
MEFU	S	T	Q	12
MEFU-SR	S	R	Q	12
MEFU-RT	R	T	Q	12
MEFU-RR	R	R	Q	12
MEFU-Q238R	S	T	R	12
MEF2U*	S	T	R	33

Site-directed mutagenesis artifact

Figure 9. USF1 (PDB: 1AN4[8]) binding to **E-box**. S233, T234, Q238 are key residues that the traverse minor groove, contact DNA phosphodiester backbone.

RESULTS

Preliminary Data

Protein	EMSA	B1H	
	4G	4G	5G
	K_d (nM)		
MEFU	773 ± 5	+	++
MEFU-SR	513 ± 72	++	+++
MEFU-RT	515 ± 22	+	++
MEFU-RR	634 ± 62	+	++
MEFU-Q238R	466 ± 53	+	++
MEF2U	459 ±5	+	++

FUTURE DIRECTIONS

1. Conduct circular dichroism to compare α-helicity and stability.
2. Determine binding affinities of 4G vs. 5G E-box sites for all mutants.
3. Compare specificities of mutants against adenine vs. guanine tracts.
4. Explore directed evolution systems to obtain favorable mutations.

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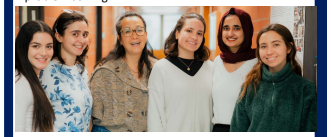

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