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Chemical Engineering of Transcription Factors Uncovers Cell-Permeable µMax Modulators

Omer Harel,[1] Ferran Nadal-Bufí,[2] Raj V. Nithun,[1] Yumi M. Yao,[3] Ariel Afek,[3] Marc Vendrell,[2] Muhammad Jbara[1]

[1] School of Chemistry, Raymond & Beverly Sackler Faculty of Exact Sciences, Tel Aviv University · [2] Centre for Inflammation Research & IRR Chemistry Hub, The University of Edinburgh · [3] Department of Chemical & Structural Biology, Weizmann Institute of Science · jbaram@tauex.tau.ac.il



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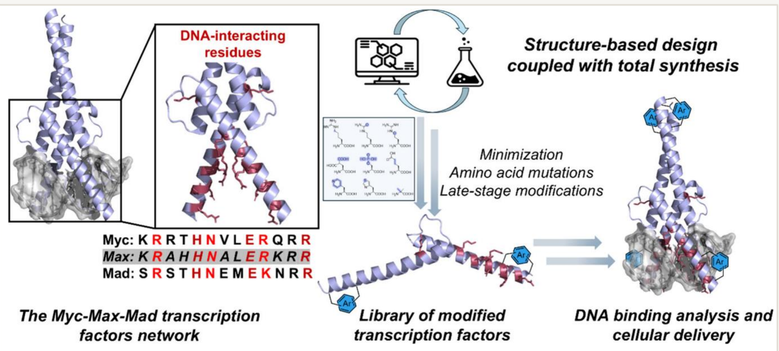
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The Result **Non-natural modifications of Max transcription factor reveal $2S_b$-µMax20 – a stapled, intrinsically cell-permeable, and bioactive engineered transcription factor capable of inhibiting cancer cell proliferation.**

01 Design of Abiotic Transcription Factors

The Myc–Max–Mad network controls ~15% of the human genome and is dysregulated in ~50% of tumors. Synthetic transcription factor (TF) mimetics exist but remain largely constrained to native sequences and structures.

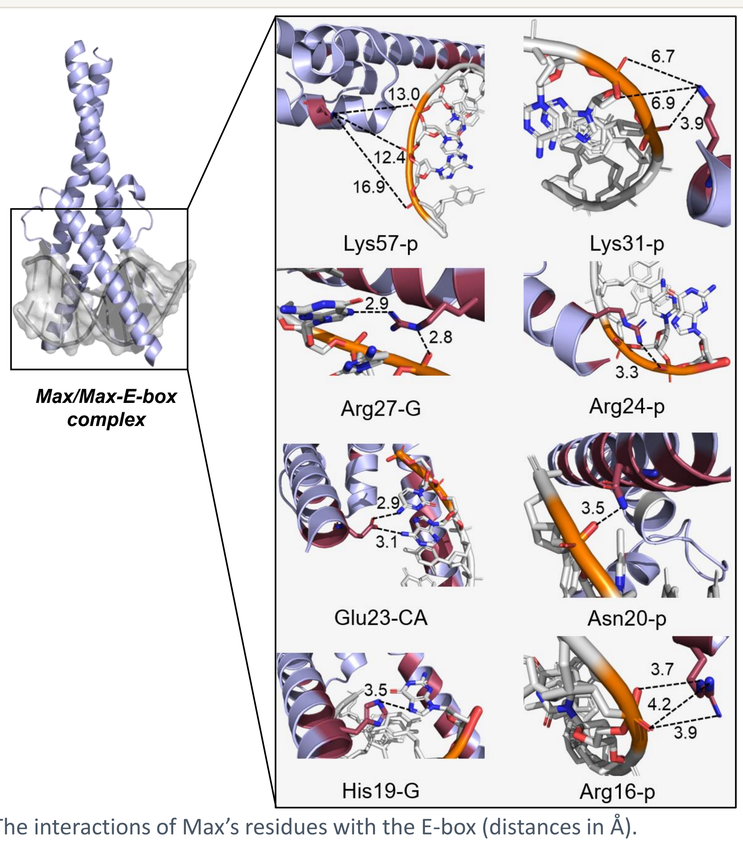
Our approach: Combining rational design with total synthesis to engineer novel abiotic transcription factors with enhanced stability and cell permeability, derived from the DNA-binding domain of Max to suppress Myc-driven gene expression.



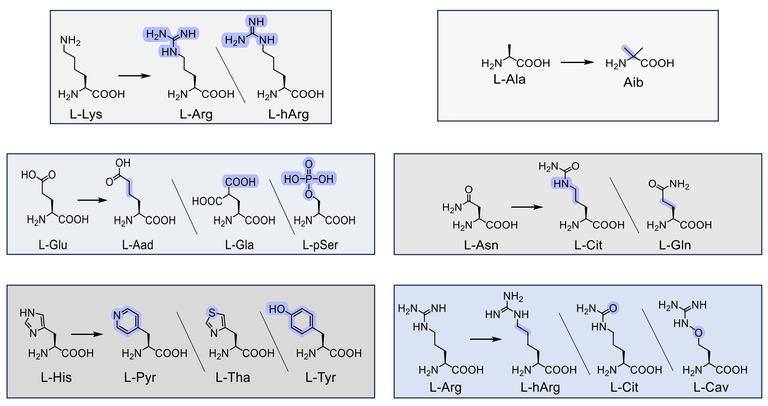
Structure-based design coupled to total synthesis: minimization, strategic mutation, late-stage modification (PDB 1HLO).

02 Engineering of µMax Abiotic Transcription Factors

10 strategic sites, 12 novel residues of which 9 non-proteinogenic. Six residues contact the E-box directly; Lys31 and Lys57 contact the DNA phosphate backbone.



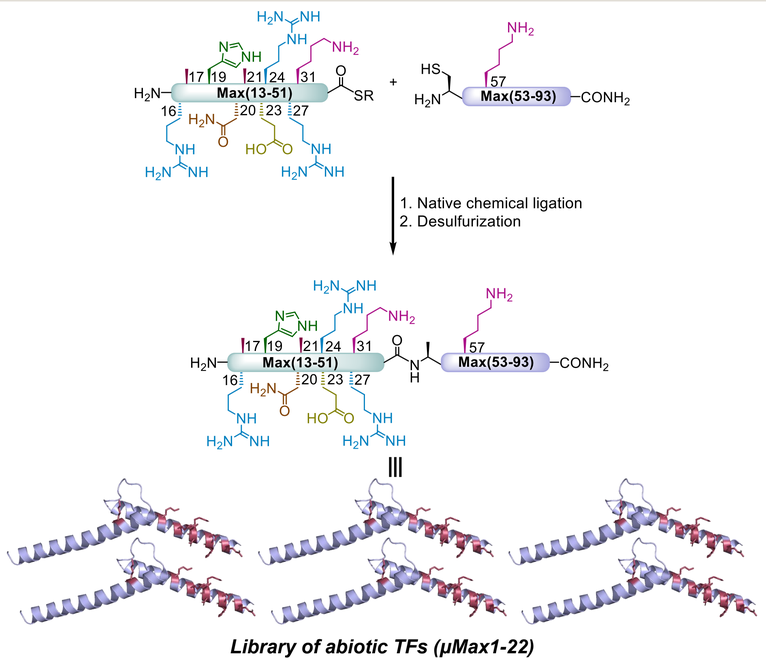
The interactions of Max's residues with the E-box (distances in Å).



The repertoire of canonical and noncanonical amino acids used in this study to replace native amino acids.

03 Chemical Synthesis of µMax Modulators

Two Fmoc-SPPS segments joined by **one-pot native chemical ligation and desulfurization**. Ala52 was temporarily mutated to Cys for ligation.



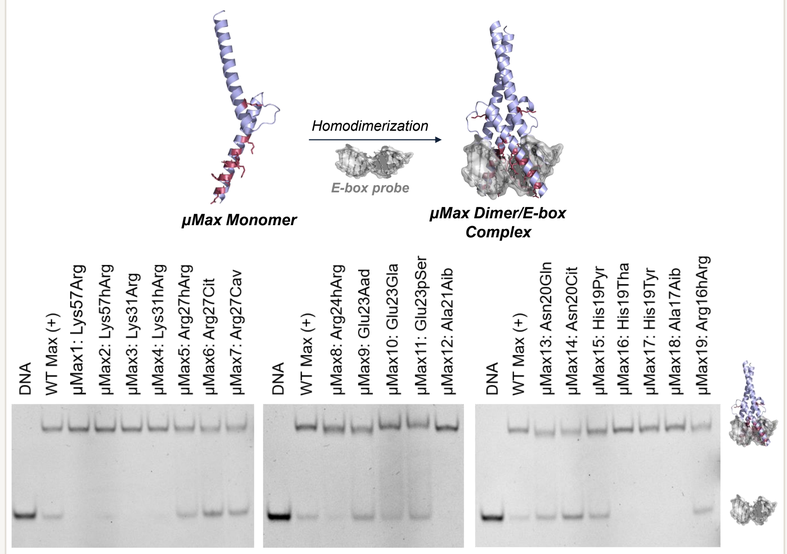
One-pot ligation-desulfurization delivers homogenous, site-specifically modified transcription factors.

30 Abiotic TF analogs

Up to **6** Mutations per chain

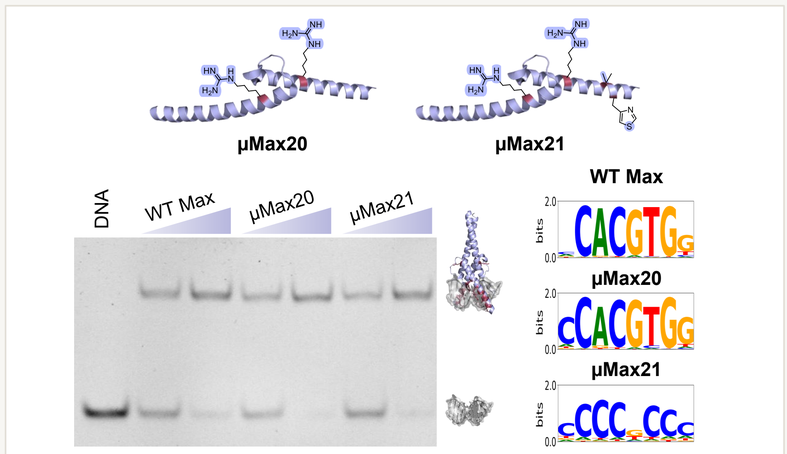
04 Potent DNA-binding Activity and Sequence Specificity

Synthetic **µMax** analogs are functional and capable of binding to the canonical E-box DNA sequence. hArg at Lys31/Lys57 and Aib at Ala17/Ala21 enhance DNA-binding activity.



EMSA analysis of µMax1-19 analogs.

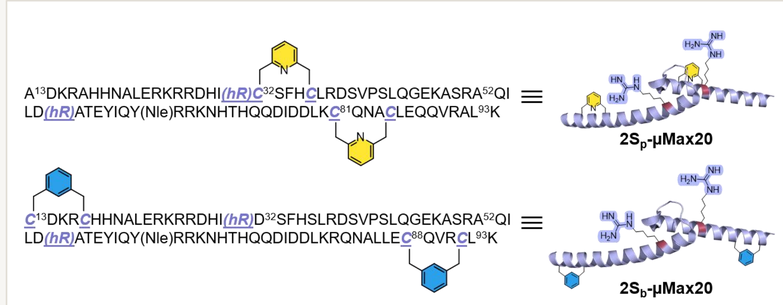
Multi-mutated **µMax** analogs show potent E-box binding: **µMax20** retains wild-type specificity, whereas **µMax21** does not.



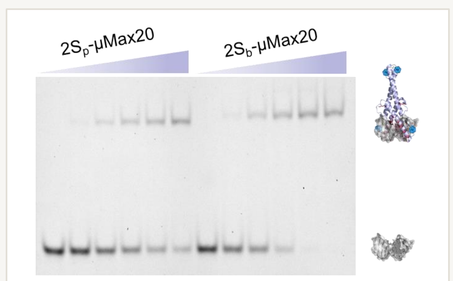
EMSA and PWM analyses of multi-site mutated µMax20-21 analogs.

05 Late-stage editing of µMax Modulators

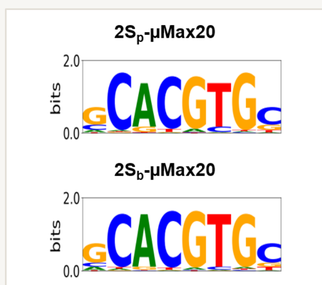
Dual aromatic staples on Cys pairs of **µMax20** give **$2S_p$-** and **$2S_b$-µMax20** yielding analogs with ~2.5-fold higher proteolytic stability, while maintaining E-box binding activity and specificity.



Staples installed by late-stage Cys alkylation with bis(bromomethyl)pyridine /benzene.



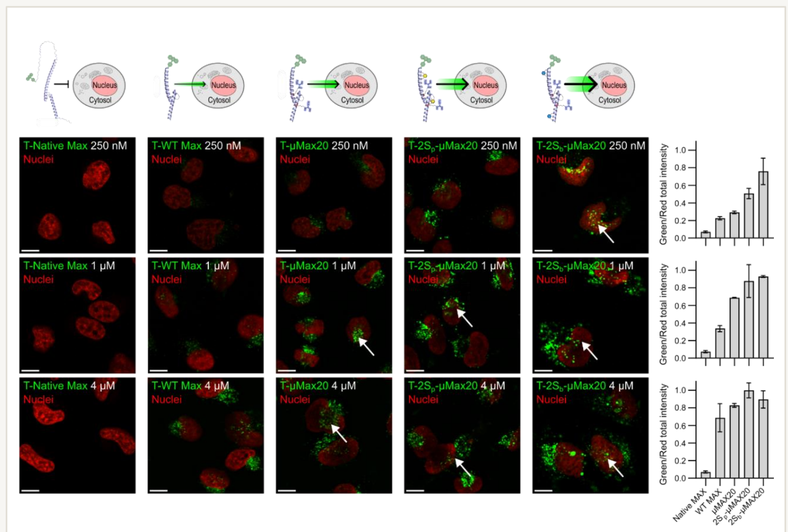
Dose-response EMSA of stapled analogs.



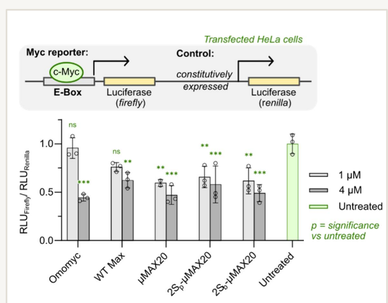
PWM of top-scoring probes.

06 Cell Permeable µMax Inhibits Myc-driven Gene Expression and Cell Proliferation

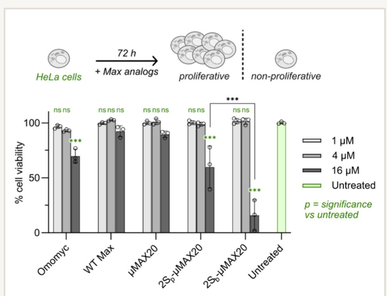
µMax stapled analogs internalize at **250 nM** with nuclear accumulation — without delivery vehicle or cell-penetrating tag.



TAMRA (green) / Hoechst (red) in HeLa; 250 nM, 1 µM, 4 µM. Arrows: nuclear accumulation. Scale bars 10 µm.



Myc reporter suppressed below 1 µM – outperforming clinically trialed Omomyc.



$2S_b$-µMax20 cuts HeLa viability to 16% at 16 µM.

MiTMAB endocytosis inhibitor only partly blocks uptake – the stapled analogs also translocate directly across the membrane.

Conclusions

- Chemical protein synthesis expands the chemical space of transcription factors: 30 abiotic Max analogs with non-proteinogenic residues and aromatic staples capable of binding the DNA target site and inhibit gene expression.
- **µMax20** binds the core E-box with nanomolar affinity while retaining wild-type specificity and α-helical fold.
- **$2S_b$-µMax20** is intrinsically cell-permeable at submicromolar concentration and suppresses Myc-driven transcription and proliferation.

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