Miniaturized HOXB13 Mimetics Are Sequence-Specific, Methyl-Sensitive DNA Binders

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[1]. INTRODUCTION

Cancer-associated DNA methylation is difficult to target selectively.

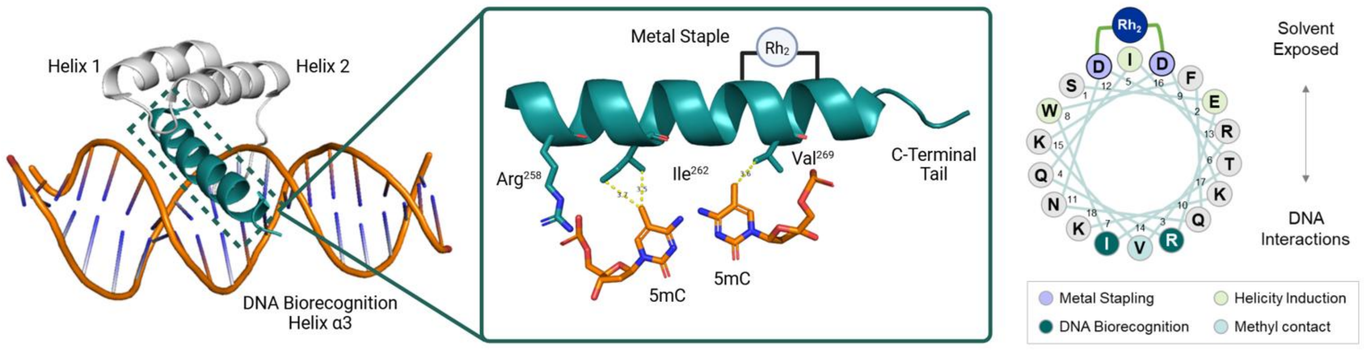
Targeting modification sites is an effective strategy to study these epigenetic changes. The homeobox protein **Hox-B13 (HOXB13)** is an **oncogenic transcription factor** known to bind to regions of high DNA methylation.[1,2]

PROBLEM: HOXB13 recognises specific methylated DNA motifs but is too large to use as a molecular tool.

SOLUTION: Miniaturising transcription factors can be used to create synthetic tools to study transcription factor-DNA interactions and binding preferences.

[2]. MINIATURISATION STRATEGY

HOXB13 Transcription Factor [284 residue] >90% Size Reduction **α3 Stapled Mimetic** [18 residue]



Methylated DNA : 5′-GGACCT**mCG**TAAAACACAA-3′

[3]. METHODS

1. METALLOPEPTIDE SYNTHESIS

[P1] Ac-SERQITIWFQNRRVKEKK-NH_2

[P2] Ac-SERQITIWFQNDRVKDKK-NH_2

$Rh_2(OAc)_2(TFA)_2$
MES buffer, pH 4.5
50^0C

[MP2] Ac-SERQITIWFQNDRVKDKK-NH_2 = Rh_2

P1 (wild type) and **P2 (Asp/Asp modified)** synthesised *via* standard SPPS.

MP2 (metal-stapled) synthesized *via* direct metal chelation.

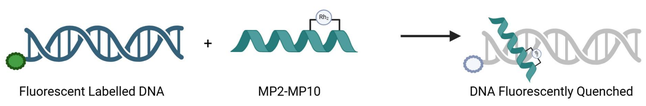
MP2 was systematically modified to investigate influence of **structural rigidity (MP3-5), methyl-recognition (MP6-8) and truncation (MP9-10)** on DNA binding and methyl sensitivity.

Entry	Peptide Sequence	fH	$\Theta_{222}/\theta_{208}$
P1	Ac-SERQITIWFQNRRVKEKK-NH_2	0.07	0.46
MP2	Ac-SERQITIWFQN**D**RVK**D**KK-NH_2	0.15	1.05
MP3	Ac-SERQ**B**TIWFQN**D**RVK**D**KK-NH_2	0.22	1.47
MP4	Ac-S**B**RQITIWFQN**D**RVK**D**KK-NH_2	0.28	1.00
MP5	Ac-SERQIT**B**FQN**D**RVK**D**KK-NH_2	0.24	0.95
MP6	Ac-SERQITIWFQN**D**R**L**K**D**KK-NH_2	0.22	0.97
MP7	Ac-SERQITIWFQN**D**R**T**K**D**KK-NH_2	0.15	1.01
MP8	Ac-SERQITIWFQN**D**R**F**K**D**KK-NH_2	0.20	1.02
MP9	Ac-RQITIWFQN**D**RVK**D**-NH_2	0.26	0.90
MP10	Ac-RQITIWFQN**D**RVK**D**KK-NH_2	0.23	0.83

*B = aminoisobutyric acid (AIB), helical inducing amino acid.

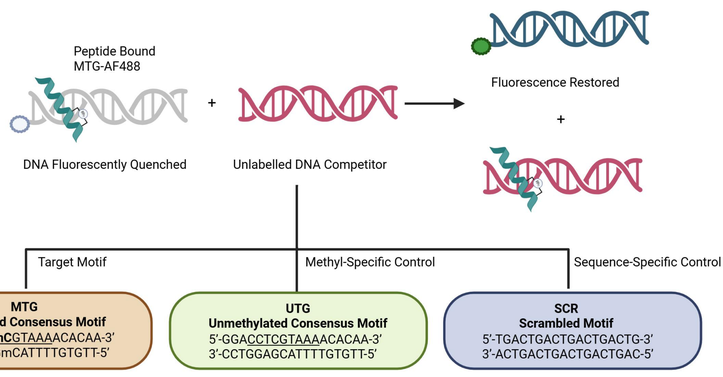
2. DNA BINDING ASSSESSMENT

Direct Peptide-DNA Binding: Fluorescence Quenching Assay



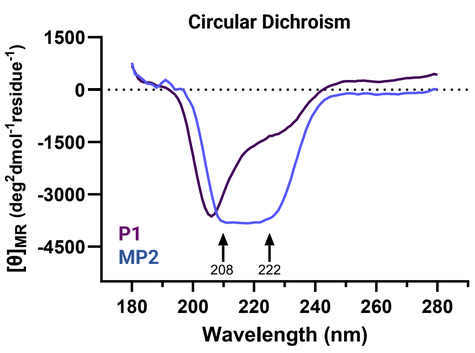
Methylated DNA Tracer **(MTG-AF488)**
5′-GGACCTmCGTAAAACACAA-3′
3′-[AF488]-CCTGGAGmCATTTTGTGTT-5′

Binding Specificity through Competitive Displacement: Fluorescence Restoration Assay

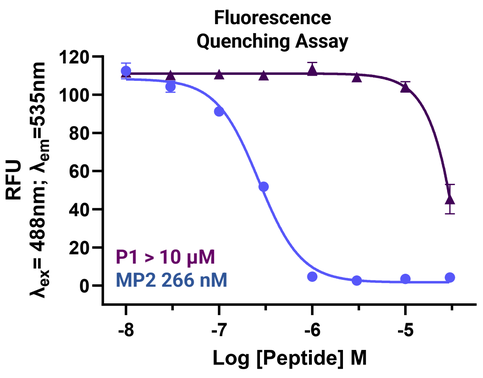


[4]. RESULTS

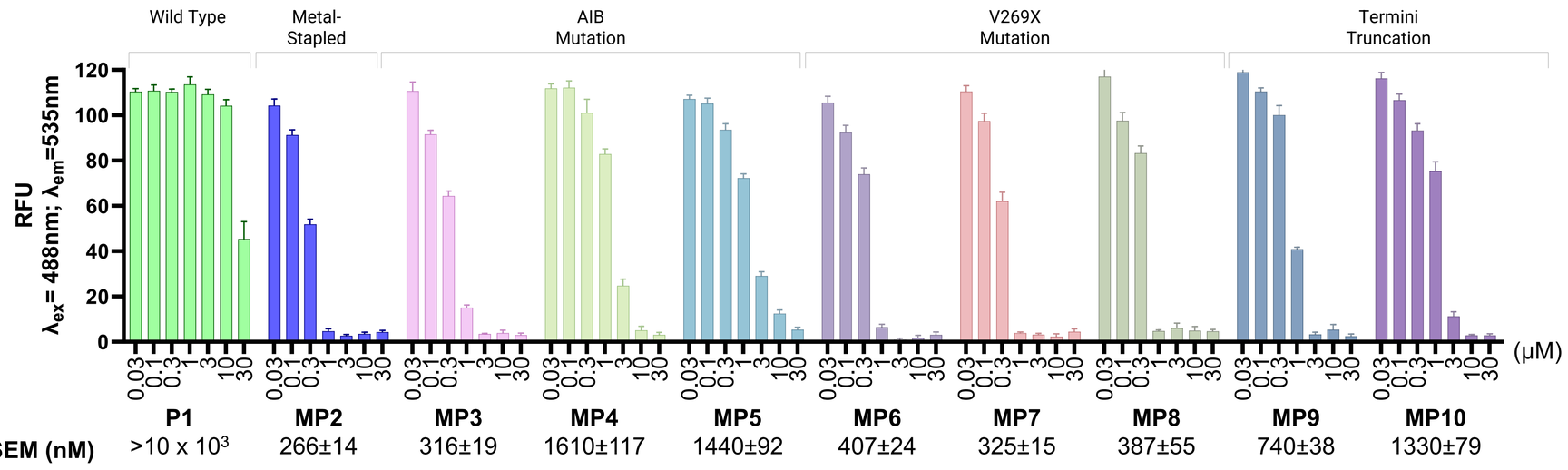
STAPLING INDUCES HELICITY



HELICITY RESTORES DNA BINDING

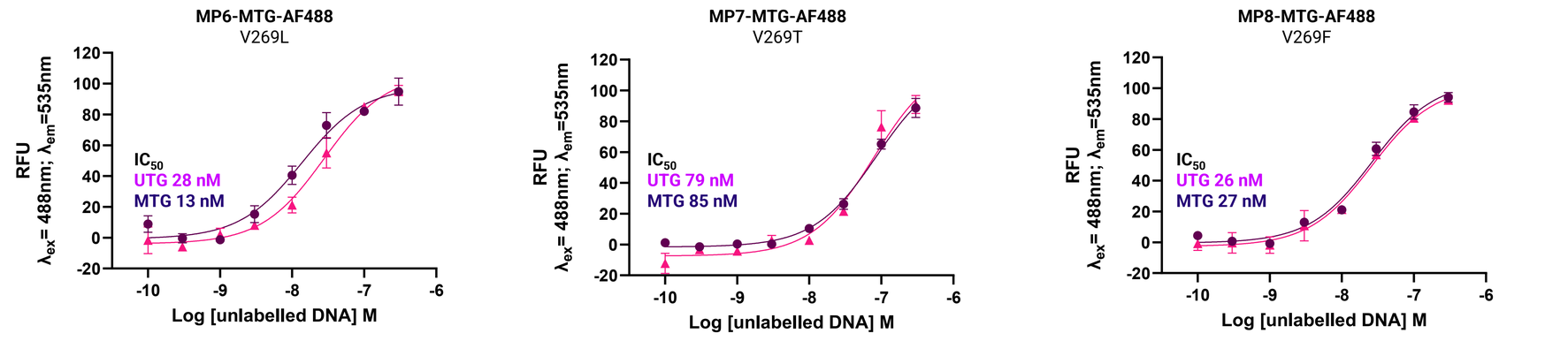


PEPTIDE DESIGN OPTIMISATION

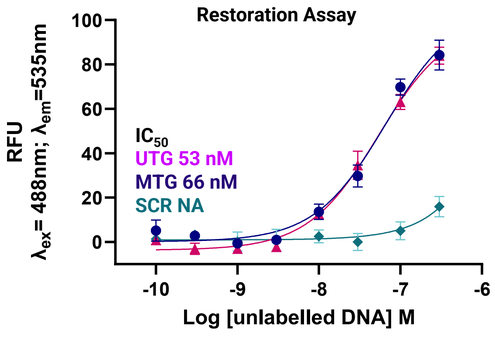


K_D ± SEM (nM)	P1	MP2	MP3	MP4	MP5	MP6	MP7	MP8	MP9	MP10
	>10 x 10^3	266±14	316±19	1610±117	1440±92	407±24	325±15	387±55	740±38	1330±79

ANALYSIS OF METHYL-SENSITIVITY USING V269X MUTANTS



MP2 IS SEQUENCE-SPECIFIC



$IC_{50}^{UTG}/IC_{50}^{MTG}$	2.15	>	0.93	≈	0.96

V269L (MP6) introduces **methyl-sensitivity,** favouring methylated DNA by **2.15-fold. MP7** and **MP8** show no preference.

Metal-stapled HOXB13 mimetics provide compact, sequence-specific tools for interrogating disease-associated DNA methylation.

C. Wong, S. Y. Chow, *J. Am. Chem. Soc.*, 2026, **148**, 30785–30792.

[5]. CONCLUSION

KEY FINDINGS

- ✓ Miniaturisation of HOXB13 DNA binding domain to an 18-residue stapled peptide retains DNA recognition despite >90% size reduction.
- ✓ V269L mutation (MP6) generates a 2.15-fold preference for methylated DNA.
- ✓ MP2 recognises the HOXB13 consensus sequence but not scrambled DNA.

References

1. W. D. Dupont, J. P. Breyer, S. H. Johnson, W. D. Plummer and J. R. Smith, *Scientific Reports 2021 11:1*, 2021, **11**, 1–10.
2. Y. Yin, E. Morgunova, A. Jolma, E. Kaasinen, B. Sahu, S. Khund-Sayeed, P. K. Das, T. Kivioja, K. Dave, F. Zhong, K. R. Nitta, M. Taipale, A. Popov, P. A. Ginno, S. Domcke, J. Yan, D. Schübeler, C. Vinson and J. Taipale, *Science*, 2017, **356**, eaaj2239

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Explore the full story behind our HOXB13 mimetics, recently published in JACs.