DOWNSIZING THE HISTONE H3–H4 QUATERNARY STRUCTURE INTO FOLDAMER MIMETICS YIELDS HIGH-AFFINITY AND CELL-PERMEABLE LIGANDS OF ASF1

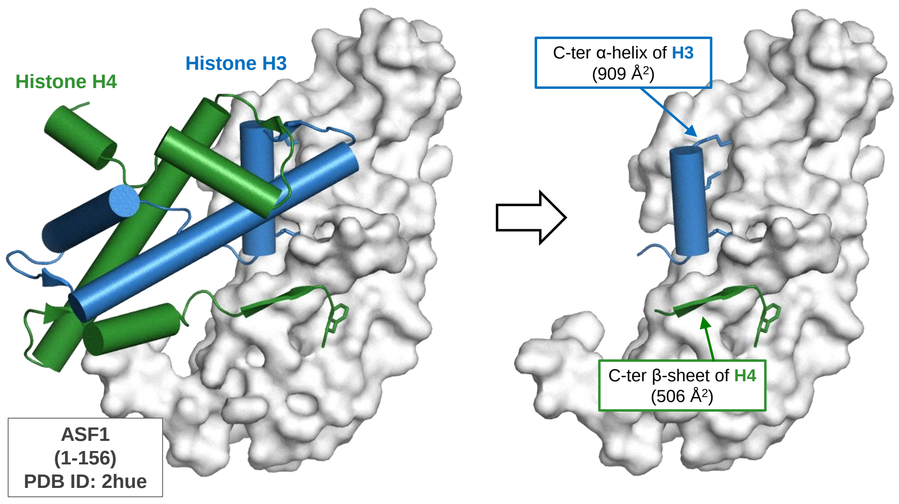
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Introduction

Histone chaperones, such as Anti-Silencing Function 1 (ASF1A and ASF1B) regulate the dynamics of histones within chromatin and contribute to the establishment of numerous epigenetic markers.[1,2] Their dysfunction is associated with a number of pathologies including aging-related diseases, pathogen infections, or cancer.

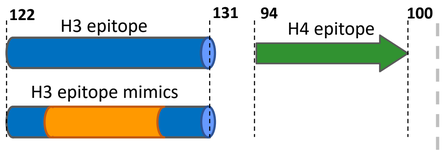


Designing effective ASF1 inhibitors is challenging due to its intracellular localization, a binding affinity for the H3-H4 dimer in the low nM range, and a rather flat and extensive binding interface (1,415 Å²) with two shallow interaction pockets 26 Å apart.[3]

We recently introduced an approach based on **oligourea helical foldamers to reduce the peptide character of PPI inhibitors**,[4] and reported a rationally designed H3 helix mimetic that binds both ASF1A and ASF1B with a K_D in the low µM range and shows improved stability in the human plasma.[5] Capitalizing on this early work, we now report a general workflow that led to the discovery of **cell-permeable, medium-size (M_w < 2.5 kDa) H3-H4 quaternary structure mimetics with high affinity for ASF1.**[6]

[1] F. Mousson et al. *Chromosoma* **2007**, *116*, 79-93
[2] Z. A. Gurard-Levin et al. *Annu. Rev. Biochem.* **2014**, *83*, 487-517
[3] F. Mousson et al. *Proc. Nat. Acad. Sci. U.S.A.* **2005**, *102*, 5975-5980
[4] L. Cussol, et al. *Angew. Chem. Int. Ed.* **2021**, *60*, 2296-2303
[5] J. Mbianda et al., *Science Adv.* **2021**, *7*, eabd9153
[6] B. Li et al. *Angew. Chem. Int. Ed.* **2026**, e4112426

Foldamer Quaternary Structure Mimetics with Optimal Linker Display Enhanced ASF1 Binding Affinity



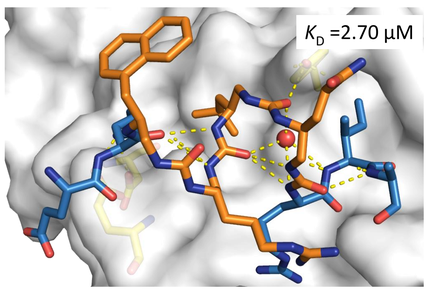
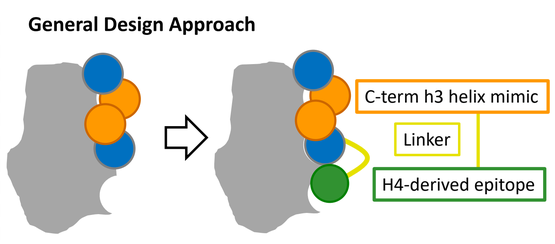


Figure 2: X-ray structure of **C2** in complex with ASF1[5]



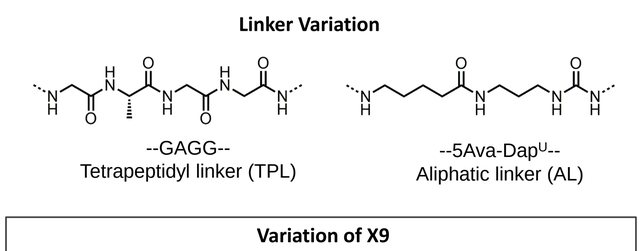
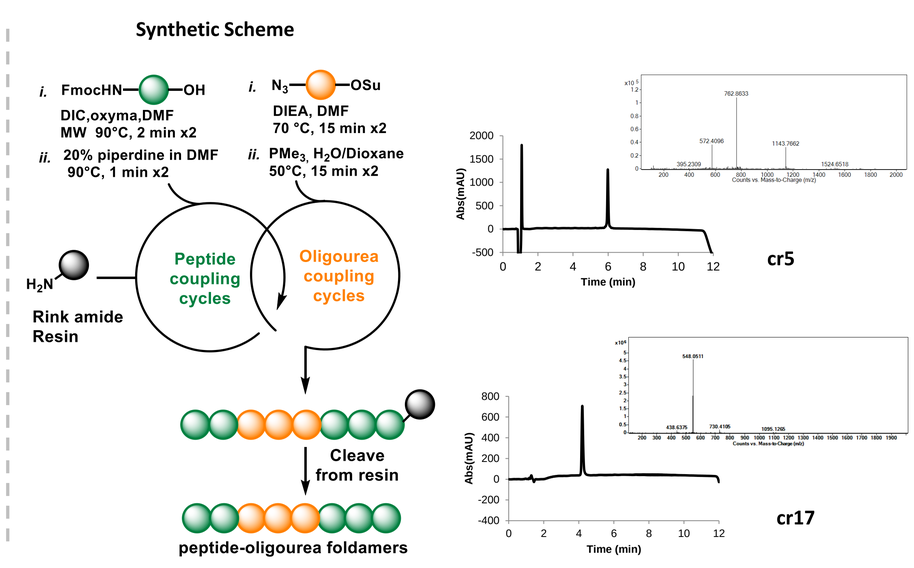
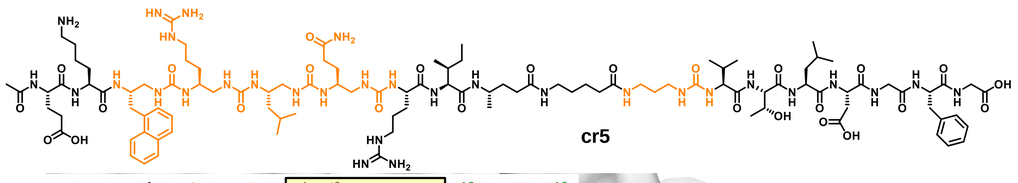


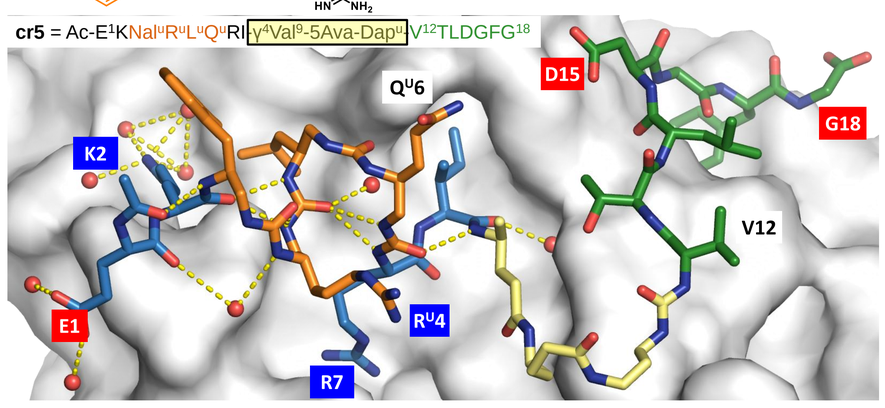
Table 1: Dissociation constants of urea-peptide chimeras as measured by ITC

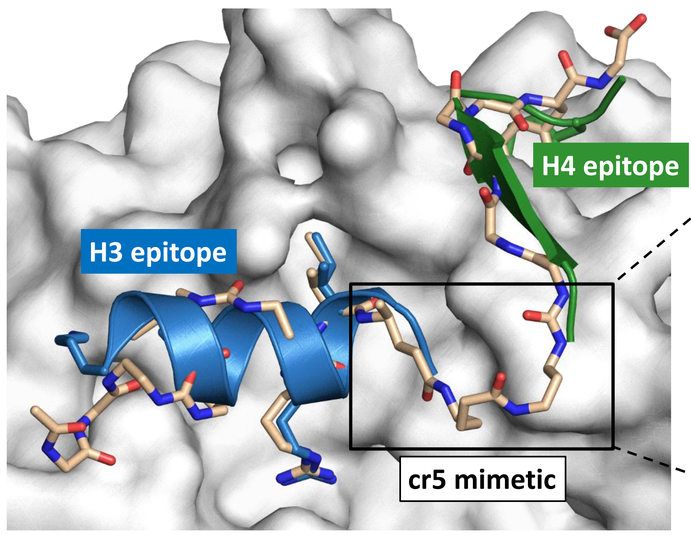
Cmpd	X9+Linker	K_D (µM)
C2		2.70 ± 0.60
cr1	Ala+TPL	1.16 ± 0.06
cr2	Ala+AL	0.69 ± 0.02
cr3	βAla+AL	1.24 ± 0.11
cr4	Ala^U+AL	0.42 ± 0.05
cr5	γ^{4}Ala+AL	**0.14 ± 0.03**
cr6	δ^{5}Ala+AL	0.52 ±0.12



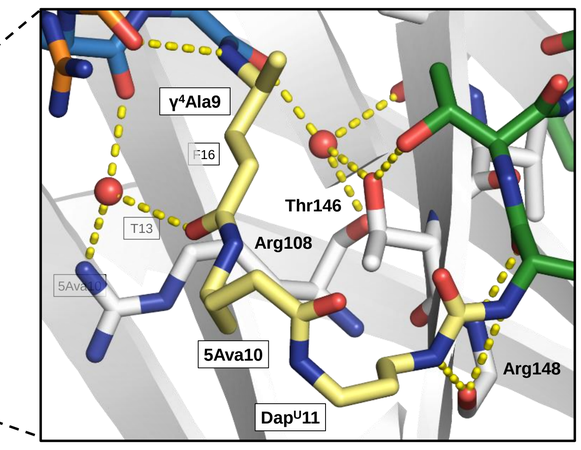
Crystal Structure of the Complex Between ASF1 and cr5







Figures 3 – 5 : X-ray structure of the complex between **cr5** and ASF1. Superimposition with H3 and H4 epitopes at the surface of ASF1 and details of representative H-bond interactions between the linker of **cr5** and ASF1



cr5 Charge Optimization

Table 2: Formal charges and K_D values measured by ITC

		cr5	cr7	cr8
Residue	1	E	R	R
	6	Q^U	K^U	K^U
	11	V	V	R
	C-ter	OH	NH_2	NH_2
Formal charge		0	+4	+5
K_D (nM)		135 ± 29	**38 ± 3.1**	165 ± 10

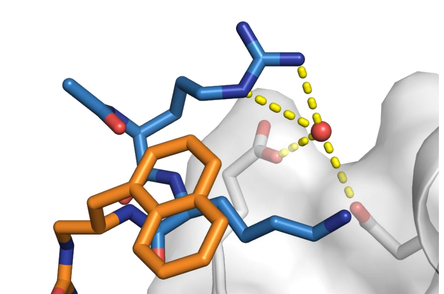
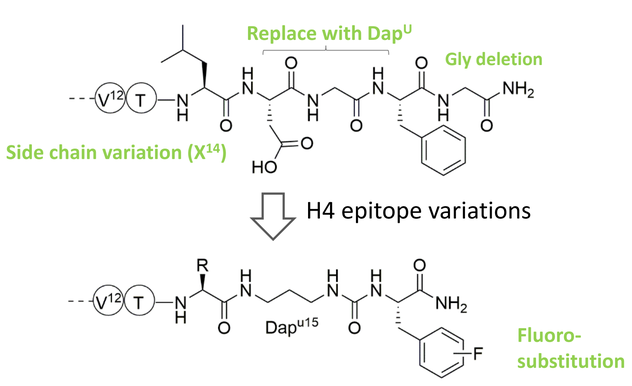
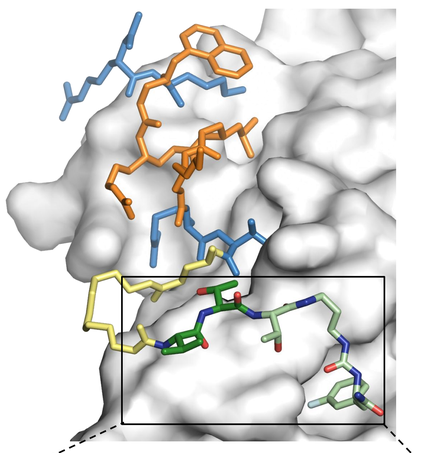


Figure 6 : Crystal structure of **cr7** with ASF1 and details of the H-bond network involving R1

H4 β-strand Mimicry & Backbone *N*-methylation





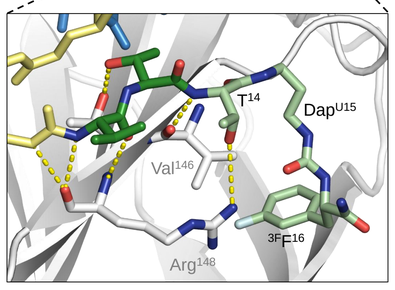


Figure 7: Crystal structure of **cr17** with ASF1 and details of the interactions with H4 mimetic

Table 3 : H4 epitope simplication and optimization strategy with binding affinities as measured by ITC

	X^{14}	Dap^U	Fluorinated Phe	Gly Deletion	K_D (nM)
cr7	L	-	-	-	38 ± 4
cr9	L	yes	-	yes	33 ± 6
cr10	G	yes	-	yes	60 ± 2
cr11	A	yes	-	yes	43 ± 8
cr12	V	yes	-	yes	22 ± 3
cr13	T	yes	-	yes	10 ± 2
cr14	L	-	4-F	-	70 ± 7
cr15	G	yes	4-F	yes	89 ± 3
cr16	L	yes	3-F	yes	22 ± 3
cr17	T	yes	3-F	yes	**6 ± 1**

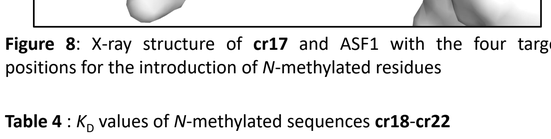


Figure 8: X-ray structure of **cr17** and ASF1 with the four targeted positions for the introduction of *N*-methylated residues

Table 4 : K_D values of *N*-methylated sequences **cr18-cr22**

	N-Methylation	K_D (nM)
cr18[a]	*N*-Me-R1	146 ± 16
cr19[a]	*N*-Me-5Ava10	59 ± 5
cr20[a]	*N*-Me-T13	531 ± 122
cr21[b]	*N*-Me-5Ava10	38 ± 6
cr22[c]	*N*-Me-5Ava10	27 ± 3

[a] **cr18**-**cr20** are **cr10** analogues
[b] **cr21** is a **cr9** analogue
[c] **cr22** is a **cr17** analogue

❑ Conclusions (B. Li et al. *Angew. Chem. Int. Ed.* **2026**, e4112426):

For Biological Results see Poster 76

- **A general workflow to downsize and mimic the quaternary structure of the histone H3-H4 dimer using a combination of structure-guided design, foldamer chemistry, amino acid substitutions for positive charge optimization & backbone *N*-methylation**
- **Discovery of medium-sized (≈ 2 kDa) high-affinity binders for the histone chaperone ASF1**
- **As revealed by X-ray structures, the peptide-oligourea ligands recapitulate the binding mode of the natural protein heterodimer**