Characterizing the cell-permeability, stability and toxicity of high affinity foldamer targeting the histone chaperone ASF1

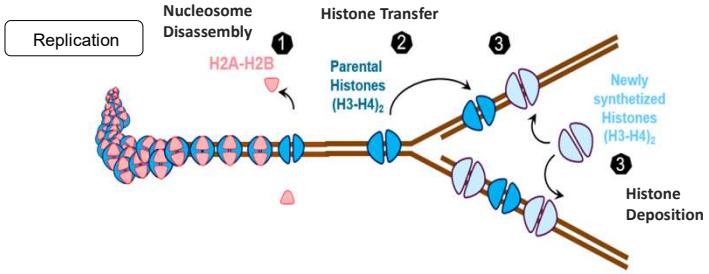
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Nucleosome, the elementary unit of chromatin

In eukaryotic cells, histones are a key component of the chromatin, regulating gene expression and DNA accessibility. During DNA replication, nucleosome undergoes dynamic changes to allow transfer of genetic information. This process can lead to genetic and epigenetic modifications that can cause important diseases such as cancers. Histones buffering, transportation and deposition is facilitated by histones chaperones.

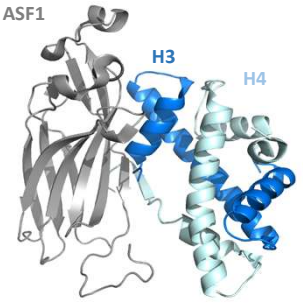


Histone chaperone ASF1 (Anti Silencing Function 1)

ASF1 associate with the histone dimer H3-H4.

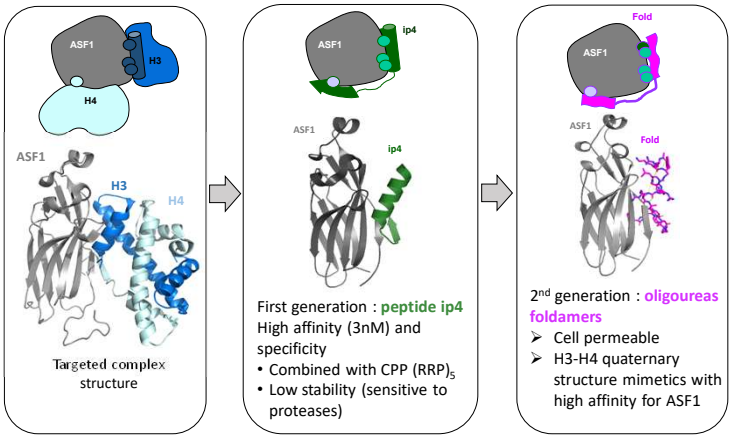
ASF1 plays an essential role in cell growth and proliferation because it is involved in nucleosome assembly/disassembly and regulation of gene expression.

Its overexpression is correlated to tumor proliferation, in particular in triple-negative breast cancer. In contrast, its knockdown makes cancer cells more sensitive to chemo- and radiotherapy, making it an interesting therapeutic target.



X-ray structure of ASF1 in interaction with H3-H4 (1,7A). PDB 2HUE

Inhibitors design



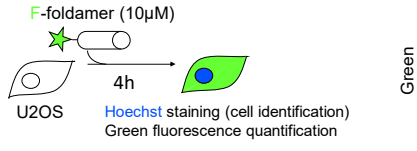
Bakail et al. Cell Chem Biol 2019

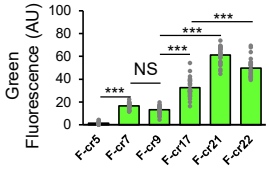
	Optimization	K_D (nM)
cr5	*Formal charge 0*	135 ± 29
cr7	*Formal charge +4*	38 ± 3.1
cr9	L14 + Dap^U + Gly deletion	33 ± 6
cr17	T14 + Dap^U + 3-F + Gly deletion	6 ± 1
cr21	*cr9 analogue + N-methylation*	38 ± 6
cr22	*cr17 analogue + N-methylation*	27 ± 3

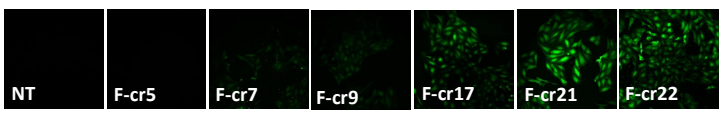
For peptide design and optimization see Poster 331

For foldamers design and synthesis see Poster 136

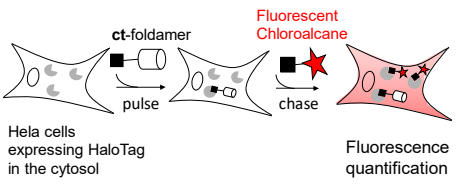
Foldamer cytosolic internalization

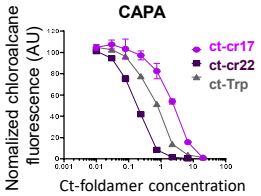






➢ **Positive global charge and N-methylation improves cellular uptake**

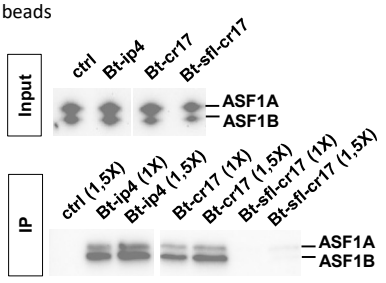


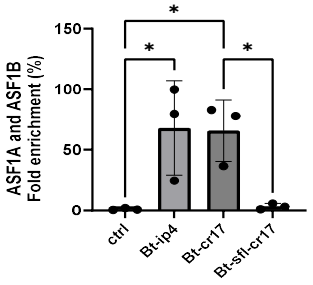


➢ **Foldamers are present in the cytosol of Hela cells**

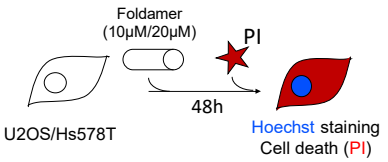
Functional assessment of foldamers

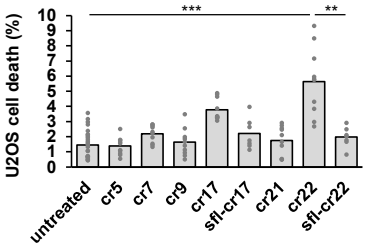
Pull down of biotinylated peptide **Bt-ip4** and foldamers **Bt-cr17** and negative control **Bt-sfl-cr17** from U2OS total cell extracts on streptavidin-functionalized sepharose beads

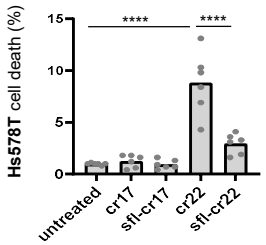




Toxicity of foldamers measurment after 48h incubation in U2OS and Hs578T cell lines.







➢ **Best foldamer is able to bind ASF1 in a cellular context and shows slight toxicity in both U2OS and Hs578T cells, compared to negative control**

Conclusion & Perspectives

Structural analysis enabled the design of a medium-sized, high affinity binder for the histone chaperone ASF1 that recapitulates the quaternary structure of its interaction with H3-H4. These peptide-oligourea foldamers were optimized by formal charge optimization and backbone N-methylation to improve their cellular uptake. Both cr17 and cr22 demonstrate significant cell permeability and cytosolic localization.

Furthermore, cr17 engage ASF1 similarly to the previously designed high-affinity peptide ip4 in a cellular context.

Finally, only cr22 exhibits a significant, although modest cellular toxicity in two cancer cell lines. Ongoing experiments in our laboratory, suggest that this inhibitor and second-generation ASF1 foldamer inhibitors (to be reported) may act synergistically with established anticancer drugs. Preliminary data indicate that, at suboptimal doses, where neither the foldamer nor the cytotoxic agent alone is active, their combination leads to high levels of cell death.

September 2026