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From Peptides to Bioactive Lactam-constrained Foldamers

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Introduction

α-amino-γ-lactam-amino acids (Agl-AA), so-called Freidinger's lactams, are bridged dipeptides that stabilize type II' β-turn conformation.[1] Widely used in modified peptides, they successfully lead to bioactive molecules.[2] Our group developed a straightforward synthesis to generate Agl-AA oligomers from peptide sequences and investigated their structural and physicochemical properties. We demonstrated that Agl-αAA oligomers adopt stable ribbon-like structures[3] and exhibit higher cellular uptake and proteolytic resistance than the well-known cell-penetrating peptides (CPPs), making them suitable for drug delivery.[4]

Further investigating the α-amino-γ-lactam motif in peptide sequences, we focused on Agl oligomers with alternating β-aminoacids (Agl-βAA). Structural studies using nuclear magnetic resonance (NMR), X-ray diffraction (XRD) and circular dichroism (CD) revealed that this new family of oligomers adopts an original 12-Helix with an elliptical cross-section, stabilized by a zigzag hydrogen bond network. Surprisingly, these oligomers display high water solubility despite exclusively containing hydrophobic side-chains such as Trp, Phe and Ile.

Synthesis of Agl oligomers

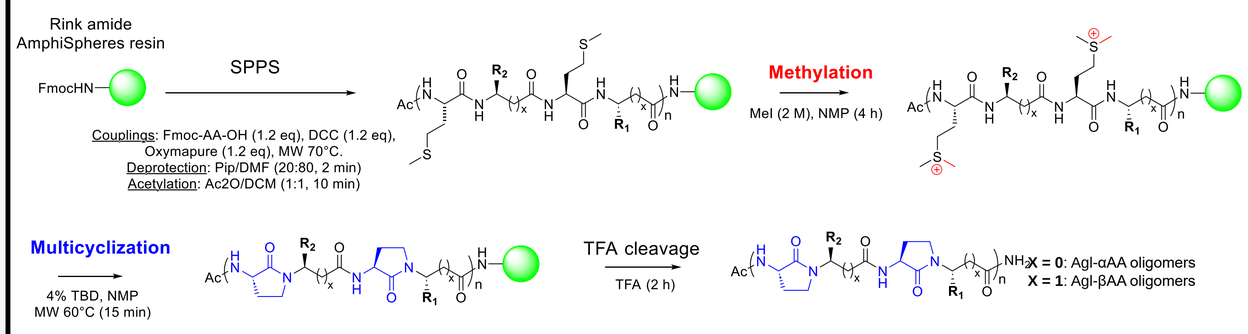


Figure 1: Agl oligomers synthesis pathway. Oligomers are generated on solid support directly from peptide sequences allowing a wide range of side chain diversity.[3]

Agl-αAA oligomers

Physicochemical properties of Agl-AA oligomers can be easily modulated by the peptide sequence and the location of side chains on each face of the ribbon. We identified cell-penetrating Agl-AA oligomers, by alternating Trp and Arg residues along the sequence that displays higher cellular uptake and proteolytic resistance than the well-known R6W3 (RRWWRRWRR) cell-penetrating peptide (Figure 3 and 4). [4]

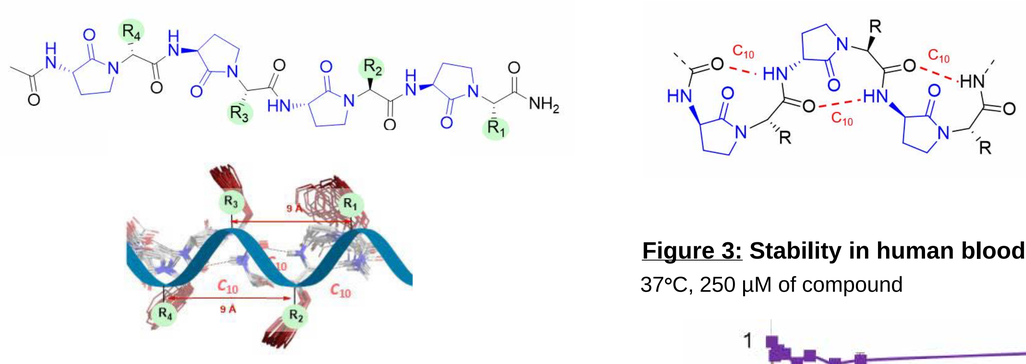


Figure 2: Schematic representations and NMR structure of Agl-αAA oligomers.

Agl-αAA oligomers adopt ribbon-like structures orchestrated by a repetition of β-II' turns which are stabilized by hydrogen bonds, forming 10-membered pseudocycles (C_{10}). The ribbon displays two faces, with side chains aligned and separated by a distance of 9 Å.

Figure 3: Stability in human blood serum.
37°C, 250 µM of compound

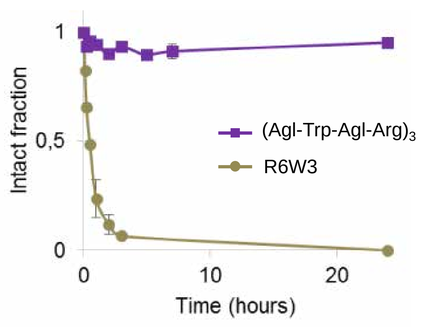


Figure 4: Cellular uptake of Agl-αAA-based foldamers.

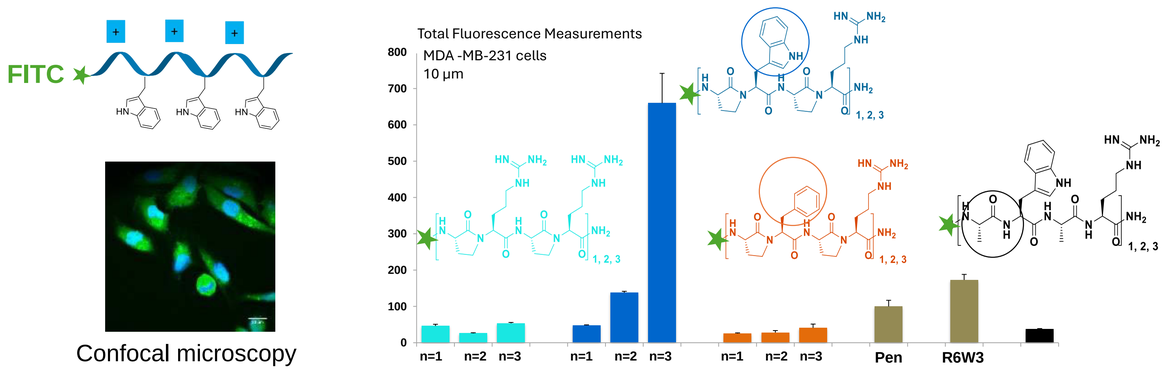
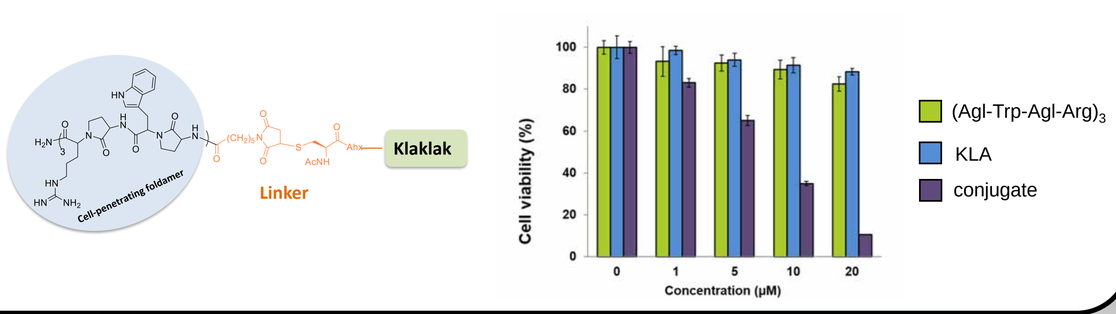
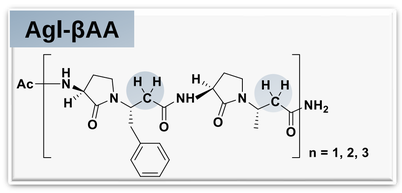


Figure 5: Vectorization potency of $(Agl\text{-}Trp\text{-}Agl\text{-}Arg)_3$ oligomer. Vectorization of the KLA proapoptotic peptide. Conjugate displayed cytotoxicity against MDA-MB-231 cancer cell line compared to KLA and vector alone.



Agl-βAA oligomers

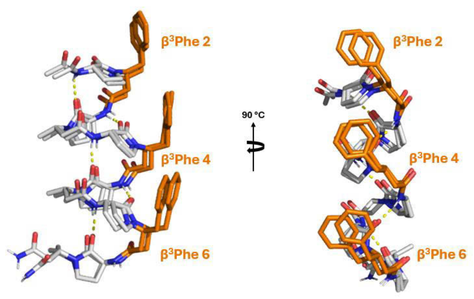
To investigate the impact of Agl motif in different peptide sequences, a new series of oligomers was designed by alternating Agl with β-amino acids and synthesized in the same way as the Agl-αAA oligomers by substituting α-amino acids with β-amino acids. This design strategy was implemented to explore how the additional methylene group in β-amino acids would locally disrupt the Agl-AA β-turn conformation and potentially lead to novel structures. The oligomers' structures were investigated by NMR in various solvents. The solid-state structure of compound **2a** was obtained through slow evaporation in MeOH. These two studies allowed for a comparison between the solution and solid-state structures. The CD signatures of this family of molecules were also reported.



Model Agl-βAA oligomers of different sizes

1a: Ac-**Agl**-β³F-**Agl**-β³A-NH_2
2a: Ac-**Agl**-β³F-**Agl**-β³A-**Agl**-β³F-**Agl**-β³A-NH_2
3a: Ac-**Agl**-β³F-**Agl**-β³A-**Agl**-β³F-**Agl**-β³A-**Agl**-β³F-**Agl**-β³A-NH_2
4a: Ac-**Agl**-β³F-**Agl**-β³A-**Agl**-β³F-**Agl**-β³A-**Agl**-β³F-**Agl**-β³A-**Agl**-β³F-**Agl**-β³A-NH_2

➢ DRX



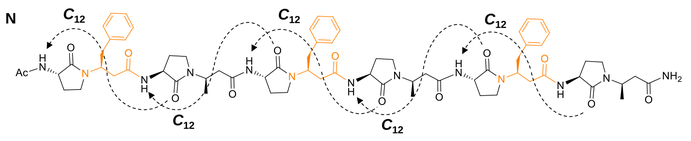


Figure 7: X-ray crystal structure of 3a. Hydrogen bonds are shown in yellow dashed lines. β³-Phe residues are colored in orange.

The 12-Helix has an elliptical cross-section and is stabilized by zigzag hydrogen bonds network with a macrodipole directed from C-ter to N-ter (Figure 7).

➢ NMR & CD

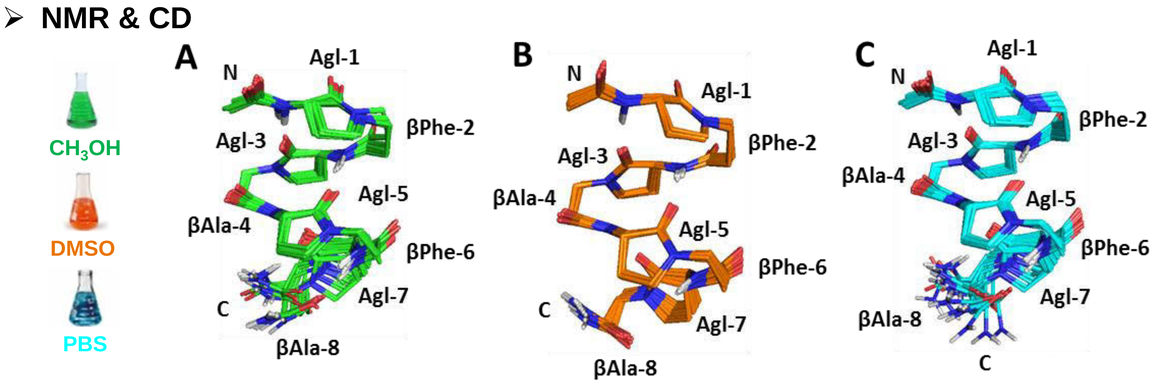


Figure 8: NMR solution structures of 2a in MeOH-d_3, DMSO-d_6 and PBS (10% D_2O, pH 6.5). Agl-βAA oligomers are highly stable and adopt a 12-Helix in chaotropic and aqueous media (Figure 8).

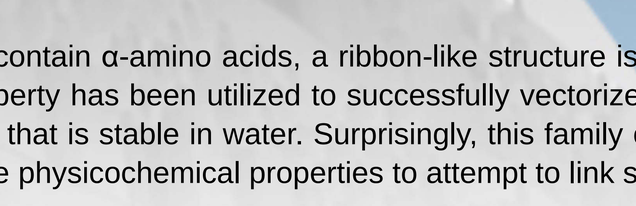


Figure 9: CD spectra at 20 °C of 4-mer 1a, 8-mer 2a and 12-mer 3a in methanol and PBS (pH 7.4).

The 12-Helix CD signature is characterized by negative maxima near 204 and 215 nm. Signal intensities show that longer oligomers further stabilized the 12-Helix and the latter is more stable in methanol than in PBS (Figure 9).

➢ Solubility

Oligomers with exclusive hydrophobic side chains such as Ile and Trp were synthetized to evaluate the solubility of Agl-βAA oligomers (Figure 10).

Sequence	n	s (µM)	LogP
Ac-Agl-βPhe-Agl-βAla-NH_2	1	>200	-0.94
Ac-(Agl-βPhe-Agl-βAla)$_2$-NH_2	2	>200	-0.60
Ac-(Agl-βPhe-Agl-βAla)$_3$-NH_2	3	>200	-0.42
Ac-Agl-βIle-Agl-βAla-NH_2	1	>200	-1.01
Ac-(Agl-βIle-Agl-βAla)$_2$-NH_2	2	>200	-0.82
Ac-(Agl-βIle-Agl-βAla)$_3$-NH_2	3	>200	-0.45
Ac-Agl-βIle-Agl-βIle-NH_2	1	>200	0.15
Ac-(Agl-βIle-Agl-βIle)$_2$-NH_2	2	173±5	1.00
Ac-(Agl-βIle-Agl-βIle)$_3$-NH_2	3	172±2	2.13
Ac-Agl-βTrp-Agl-βAla-NH_2	1	>200	-0.72
Ac-(Agl-βTrp-Agl-βAla)$_2$-NH_2	2	>200	-0.15
Ac-(Agl-βTrp-Agl-βAla)$_3$-NH_2	3	167±5	0.43

Sequence	s (µM)	LogP	
Ac-(Agl-αPhe-Agl-αAla)$_3$-NH_2	117 ± 5	0.30	x 8
Ac-(Agl-βPhe-Agl-βAla)$_3$-NH_2	860 ± 5	-0.42	

Figure 10 : Series of hydrophobic sides chains Agl-βAA oligomers (left) and solubility and LogP values (right).

Most of the oligomers exhibit excellent water solubility and possess LogP values ≤ 0. Only the full Ile series possesses LogP > 0. Oligomers containing β-Ala and β-Phe are 8 times more soluble in phosphate buffer compared to Agl-αAA oligomers.

Conclusion and Perspectives

A new family of foldamers based on the Agl motif has been developed. When the oligomers contain α-amino acids, a ribbon-like structure is formed. The presence of Trp and Arg side chains on each face of the ribbon grants the oligomers the ability to penetrate cells. This property has been utilized to successfully vectorize the KLA proapoptotic peptide in MDA-MB-231 cells. Replacing α-amino acids with β-amino acids results in a novel structure, a 12-Helix that is stable in water. Surprisingly, this family of oligomers exhibits high solubility in water despite the exclusive presence of hydrophobic side chains. We further investigate these unique physicochemical properties to attempt to link specific structures to their solubility.

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