Computational Design of Macrocycles to Modulate TNF-α

Paola Moyano-Gómez[1,2], Pankaj Kumar Singh[1,2], Pekka Roivas[1,3], Shelly Pathania[1,2], Elmeri Jokinen[4], J. Santeri Puranen[4], Ulla Pentikäinen[1,3], Olli T. Pentikäinen[1,2,4]

[1]*Institute of Biomedicine, Integrative Physiology and Pharmacology, University of Turku, 20014, Turku, Finland*

[2]*InFLAMES Research Flagship, University of Turku, 20014, Turku, Finland*

[3]*Turku Bioscience Centre, University of Turku, FI-20520, Turku, Finland*

[4]*Aurlide Ltd, Lemminkäisenkatu 14A, 20520, Turku, Finland*

Macrocyclic peptides combine high affinity and selectivity with the ability to modulate challenging protein–protein interactions [1]. Tumor necrosis factor alpha (TNF-α), a central immune regulator implicated in autoimmune, cancer, and neurological diseases, remains difficult to target with conventional small molecules and is primarily addressed using monoclonal antibodies [2]. Computationally designed macrocyclic peptides generated via ring-closing metathesis represent a promising alternative for targeting this clinically important cytokine [3].

Here, 58 peptide building blocks, including standard, N-methylated, and D-amino acids, were computationally assembled into flexible macrocycles. Tri- and tetrapeptide combinations yielded approximately 230 million 3D conformers. These were docked into TNF-α crystal structures using PLANTS [4] and refined through three molecular dynamics simulations with AMBER24.

This computational discovery platform identified TNF-α–binding macrocycles. All 39 experimentally tested compounds demonstrated binding, with 11 exhibiting dissociation constants (K_D) below 30 μM. Microscale thermophoresis (MST) binding data correlated with differential scanning fluorimetry (DSF) thermal shifts, where larger ΔT_m values indicated stronger and more stable interactions [5]. Extended incubation times in MST proved critical for accurately capturing binding behavior, particularly for larger macrocycles.

Overall, this study presents a computation-guided strategy for generating amino acid–based macrocycles that bind TNF-α using a limited set of building blocks and a single cyclization approach. This platform offers a scalable route to accelerate ligand discovery for challenging cytokine targets and can be expanded through broader chemistries and machine learning.

Bibliography :

[1] E.M. Driggers; S.P. Hale; J. Lee; N.K. Terrett. Nat. Rev. Drug Discov. 7 (2008) 608–624.
[2] B.B. Aggarwal. Nat. Rev. Immunol. 3 (2003) 745–756.
[3] C.J. White; A.K. Yudin. Nat. Chem. 3 (2011) 509–524.
[4] O. Korb; T. Stützle; T.E. Exner. J. Chem. Inf. Model. 49 (2009) 84–96.
[5] F.H. Niesen; H. Berglund; M. Vedadi. Nat. Protoc. 2 (2007) 2212–2221.