One-bead-one-compound combinatorial libraries for the discovery of ligands towards SIRPα and checkpoint CD47 proteins

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https://doi.org/10.17952/37EPS.2024.P1137

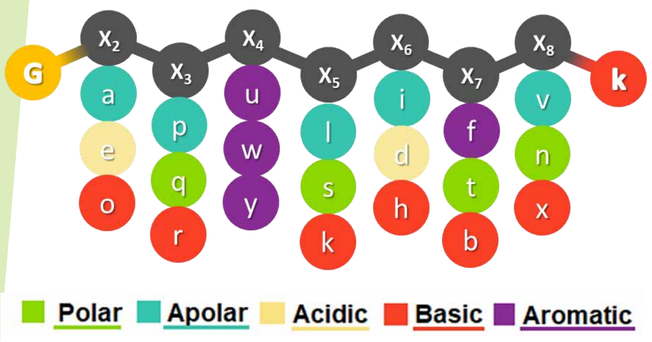
Abstract

Library screening is a powerful tool to find new peptide-based drug candidates, allowing rapid identification of ligands from a large number of diverse compounds. Among these methods, one-bead-one-compound (OBOC) peptide libraries, encoding for lineal, branched or cyclic peptides, including natural and unnatural amino acids, hold great potential.

CD47 is a glycoprotein expressed on the surface of cells. Its interaction with signal-regulating protein alpha (SIRPα) of dendritic cells induces an antiphagocytic signal, known as “don’t-eat-me” signal. This mechanism is not only exploited by certain tumours to escape immune surveillance, but some pathogens, such as the *Plasmodium malariae*, induce CD47 overexpression. Since the activation of CD47 expression is not related to the pathogen-host specific interaction, CD47 appears as a therapeutic target to treat a wide range of infection agents. In these terms, peptides might be used to inhibit this interaction and enhance phagocytosis.

Our main objective is to discover new protease resistant peptides with affinity for the CD47/SIRPα axis, so as to inhibit their interaction. We decided to use and design linear and cyclic OBOC library comprised non-proteinogenic amino acids. After synthesis of these libraries, they are evaluated using affinity selection-mass spectrometry (AS-MS) to identify binders for the proteins of interest.

One-bead-one-compound (OBOC) library



Percentage of sequences identified by nLC-MS/MS

His-Tagged SIRPα protein expression

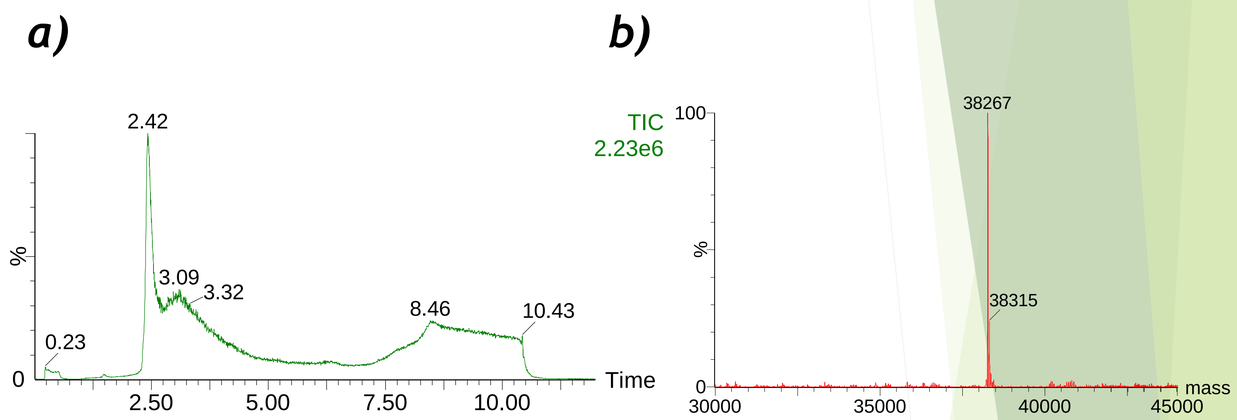
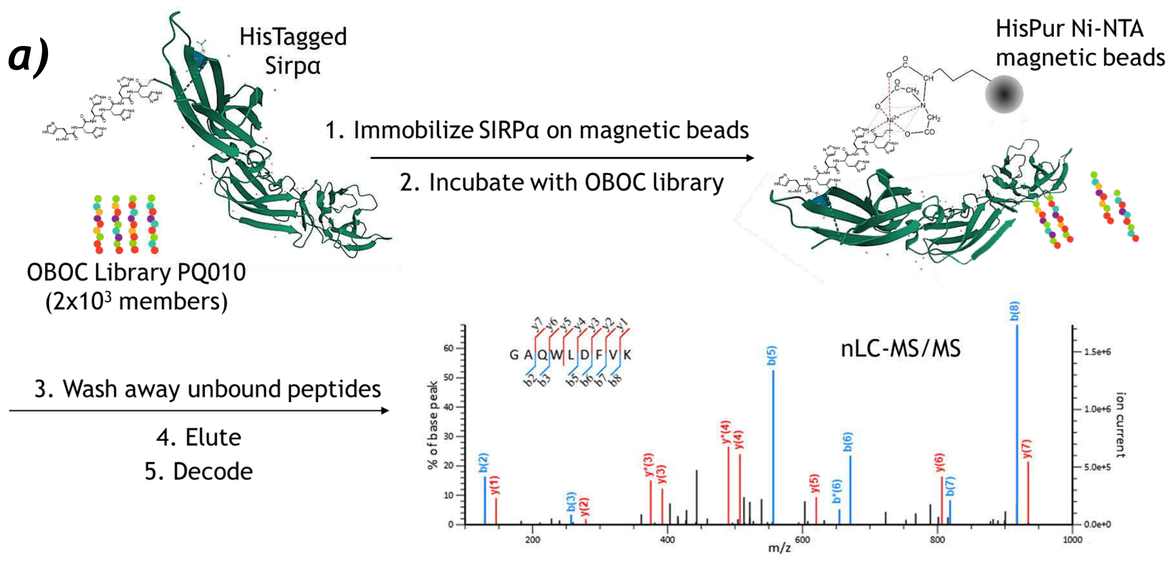


***Figure 1*. OBOC Library synthesized**. Three different D-amino acids have been introduced in seven positions of the sequence. 2187 possible peptides. After analysis via nLC-MS/MS, most of the peptide were detected.

***Figure 2*. Characterization of His-Tagged SIRPα. a)** Chromatogram of SIRPα, showing a retention time of 2.42 minutes **b)** Mass spectra after deconvolution, the molecular weight obtained matches the theoretical one (3264.67 Da). The protein was successfully obtained.

Affinity Selection-Mass Espectrometry (AS-MS) assay



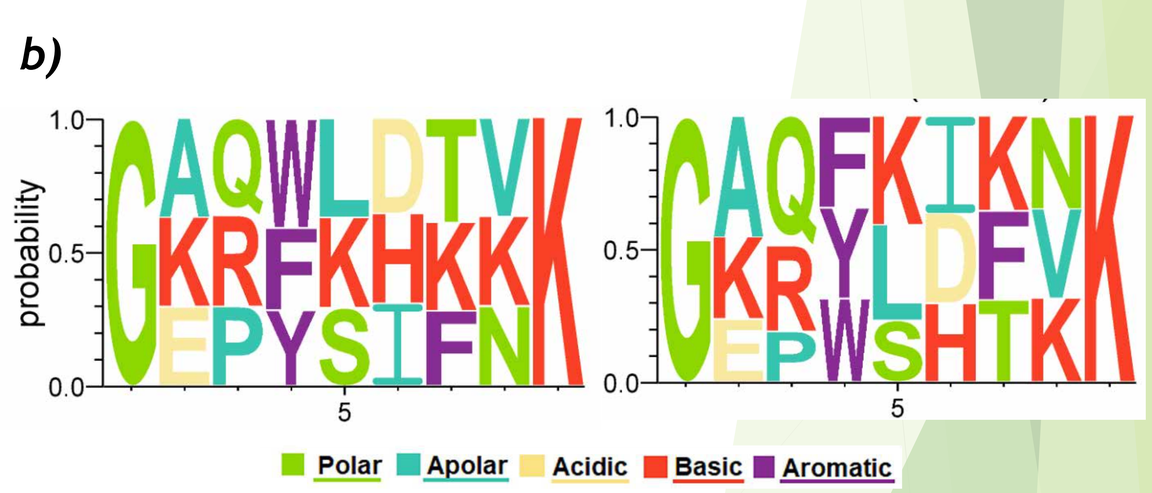


***Figure 3*. a) Workflow of AS-MS assay**. The OBOC library is incubated with immobilized His-SIRPα. After incubation and wash, hits are decode by MS analysis**. b) Positional frequency plot** showing theoretical possibilities of finding the selected amino acids in each position**.** Assay performed at different OBOC library concentrations (1 and 10 nM respectively). None of the positions are biased towards an specific type of amino acid.

Conclusions

·A OBOC protease-resistant peptide library has been designed, its synthesis has been performed by SPPS and its characterization was done by nLC/MS-MS, identifying 92% of the population.

·AS-MS assay was performed using SIRPα against OBOC library, but results show the need of using larger libraries to identify posible hits.

Future Perspectives

·Methods to optimize analysis of the elution from the AS-MS assays are being developed and other approaches are being taken into consideration as an alternative for the analysis of this library.

·Larger OBOC libraries have been synthesized and are ready to be tested.

References

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[2*] Antibodies 2020, 9(3), 44.*
[3] *Nat. Commun. 2020, 11, 3183.*