FRAGMENT-BASED APPROACH APPLIED TO THE DISCOVERY OF PROTEIN-PROTEIN INTERACTIONS STABILISERS

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PPIs STABILISATION OPPURTUNITIES BY FRAGMENT-BASED APPROACH: THE TAROS LIBRARY

Protein-protein interactions (PPIs) are constituents of numerous biological pathways and offer therapeutic intervention points into different pathologies such as cancer[1], inflammation[2], neurodegenerative[3] and metabolic diseases[4].
Particularly, stabilisation of complexes showed to play a key role in biological pathways offering an additional access point to their modulation.
Since this approach has not yet been explored in a systematic way, the TASPPI (TArgeted small-molecule Stabilisation of Protein-Protein Interactions) consortium[5] aims to identify chemical PPI stabilisers in order to develop new crucial therapeutic strategies in the treatment of the disease areas mentioned above.

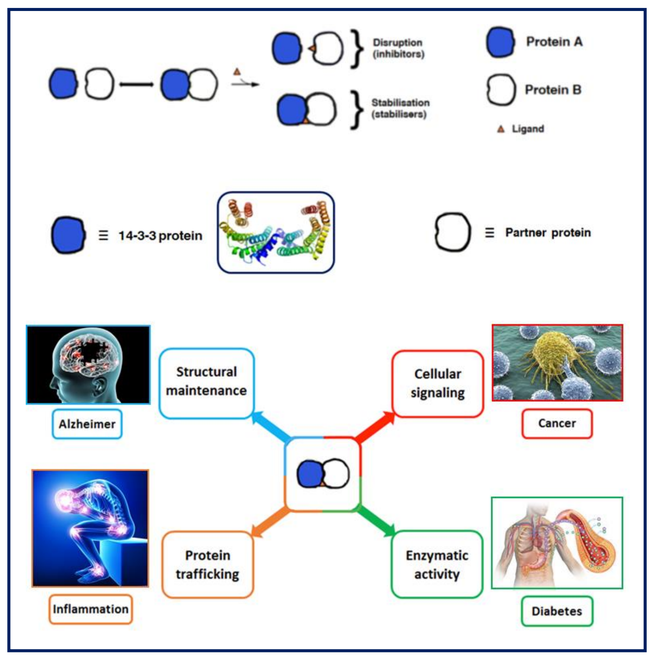

Figure 1. 14-3-3 complexes stabilisation and therapeutic implications.
14-3-3 protein's crystal structure from Ottmann, C. et al. Bioorg. Med. Chem. 21 (**2013**).

The main targets of the consortium are 14-3-3 protein and its complexes with different protein partners.
These complexes have already been proven to be widely involved in the already mentioned high impact patologies (Figure 1).
Given the intricacy of the interactions occurring between the complex members' surfaces, fragment-based approach has been considered the most suitable strategy to face this challenge / address the stabilisation.
The Taros' internal fragments collection was nominated for being the starting point of the design and development process.

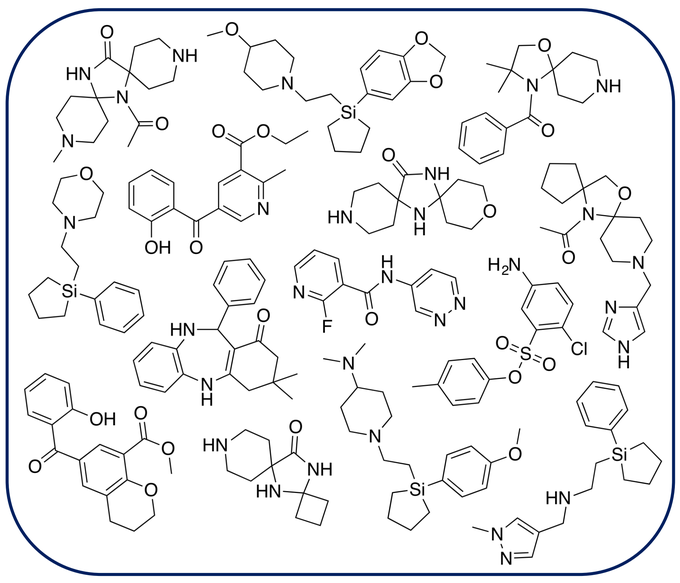

Figure 2. Examples of chemical entities present in the Taros' in-house collection of fragments.

This set is composed by novel structures presenting a good three-dimensionality balance and fitting properly with the principles of the chosen approach[6].
The collection has served the consortium members for primary screenings that identified multiple hits for different 14-3-3 complexes enabling the progress toward hit-to-lead elaboration.
The physico-chemical properties of the selected set of fragments are presented together with some examples of novel fragments coming from the Taros Chemical's proprietorial collection (Figures 2 and 3).
To date, the Taros fragment collection contains 1.370 molecules and offer ample possibilities to be further enriched.

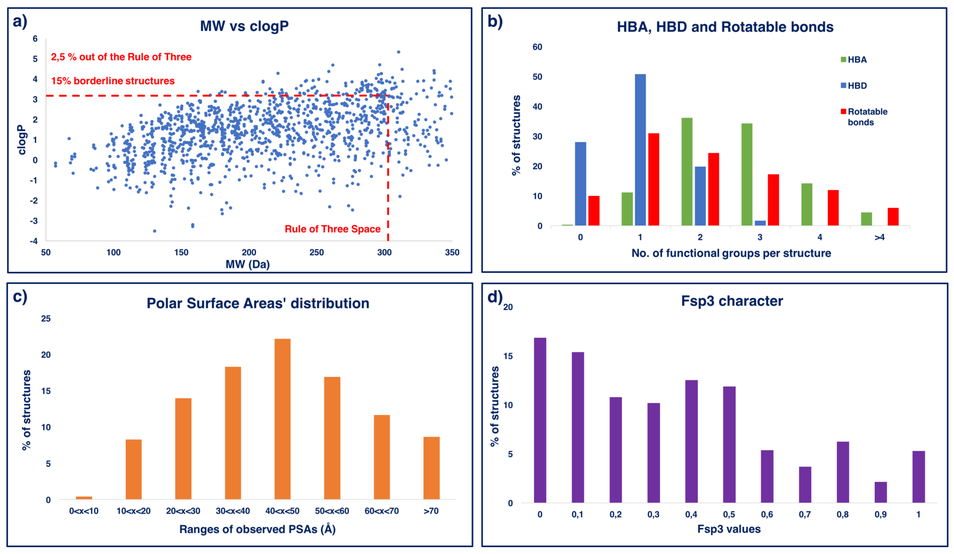

Figure 3. Overview of the Taros fragment collection physico-chemical properties. **a)** Distribution of MW and clogP, **b)** Hydrogen bond-acceptors **(HBA),** Hydrogen bond-donors **(HBD)** and **Rotatable bonds**, **c)** Polar Surface Areas **(PSA)** and **d)** three-dimensionality **(Fsp3 character)** of the fragment entities.

FRAGMENT LIBRARY EXPANSION: RATIONAL DESIGN OF NEW ENTITIES

In order to further enrich the collection, new libraries were designed and synthesized using both single reactions and parallel set-up for further diversification of the central cores.
The new fragments' design was inspired by two main sources: natural compounds and known scaffolds in drug discovery.
In the example of Nicotine-like structures (Figure 4), these two sources were merged during the design phase and produced an interesting new Biocore[7] – the 1,3,5-trisubstitued triazole – which showcases a new horizon in fragment design.
The three exit points created on the last core are directed towards three different spatial vectors (Figure 5).
The systematic probing for favorable interactions by introduction of a small substituent (or no substituent) at one exit vector whilst diversifying the other two exit points may allow for the rapid identification of the target space by using only one binding core. This concept could be termed "SAR by BioCores".

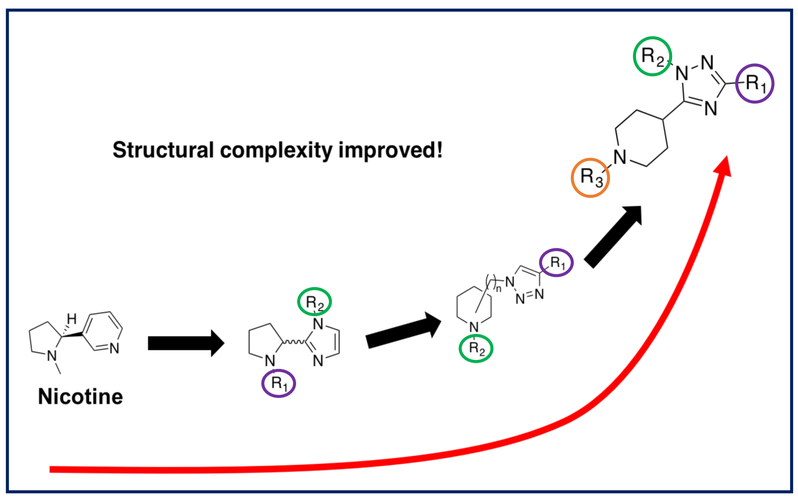

Figure 4. Design of novel Nicotine-like fragments maintaining its pharmacophoric features and adding exit points for further diversification.

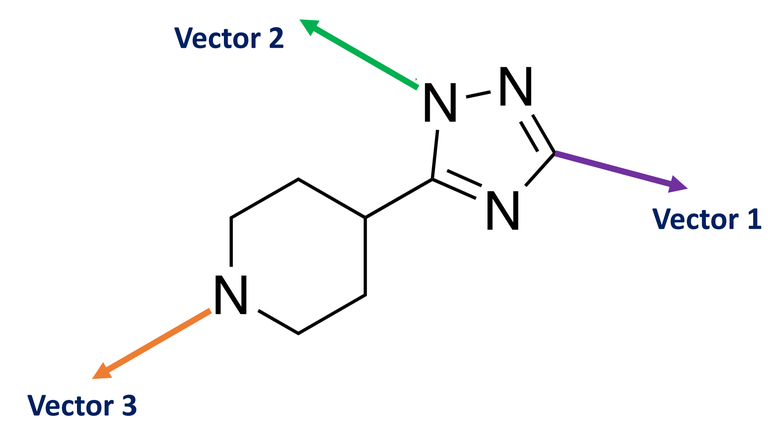

Figure 5. An example of the last stage of Nicotine-like fragments design introducing the "SAR by BioCores" concept.

PRIMARY SCREENING RESULTS FOR NOVEL PPIs STABILISERS BY FRAGMENT-BASED APPPROACH

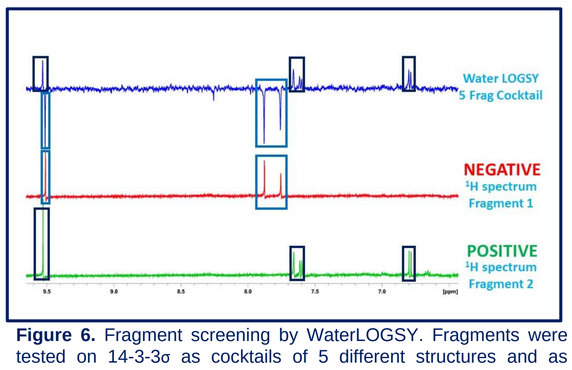

Figure 6. Fragment screening by WaterLOGSY. Fragments were tested on 14-3-3σ as cocktails of 5 different structures and as singletons.

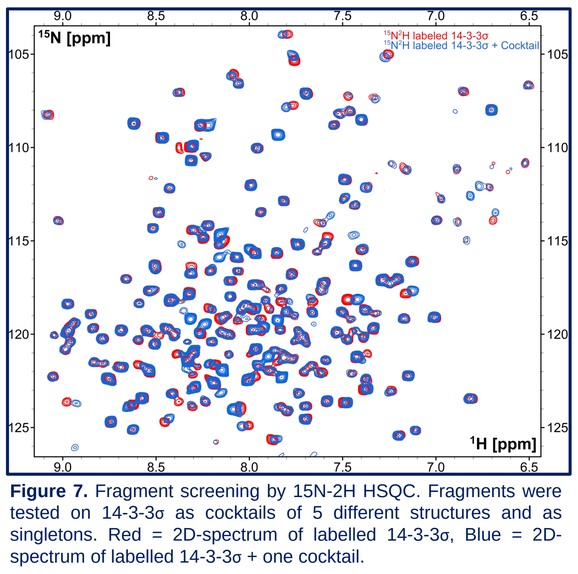

Figure 7. Fragment screening by 15N-2H HSQC. Fragments were tested on 14-3-3σ as cocktails of 5 different structures and as singletons. Red = 2D-spectrum of labelled 14-3-3σ, Blue = 2D-spectrum of labelled 14-3-3σ + one cocktail.

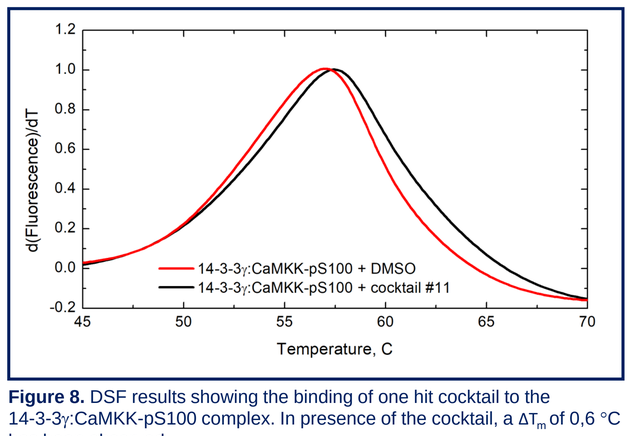

Figure 8. DSF results showing the binding of one hit cocktail to the 14-3-3γ:CaMKK-pS100 complex. In presence of the cocktail, a ΔT_m of 0,6 °C has been observed.
Red = 14-3-3γ:CaMKK-pS100 complex,
Black = 14-3-3γ:CaMKK-pS100 complex + hit cocktail 11.

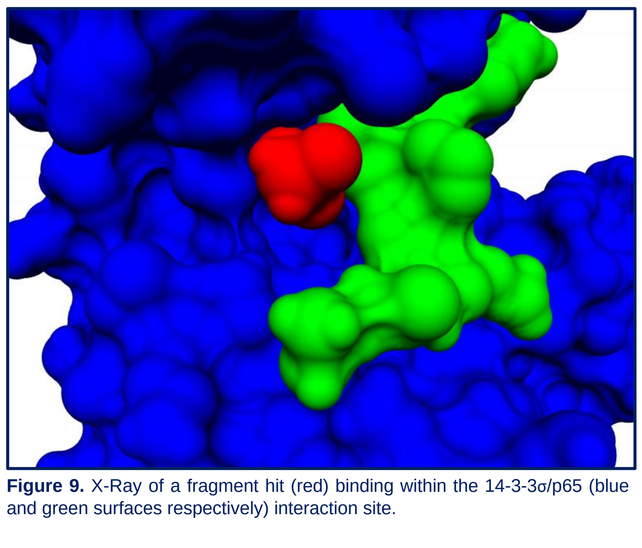

Figure 9. X-Ray of a fragment hit (red) binding within the 14-3-3σ/p65 (blue and green surfaces respectively) interaction site.

References:
1) Waterman, M. J. F. et al. Nat. Genet. 19 (**1998**); 2) Aguilera, C. et al. J Cell Sci 119 (**2006**); 3) Hashiguchi, M. et al. J. Biol. Chem. 275 (**2000**); 4) Datta, S. R. et al. Mol. Cell 6 (**2000**); 5) TASPPI http://wwww.tasppi.eu/; 6) Congreve, M. et al. Drug Discov. Today 8 (**2003**); 7) Kombarov, R. et al. Mol. Divers. 14 (**2010**).

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