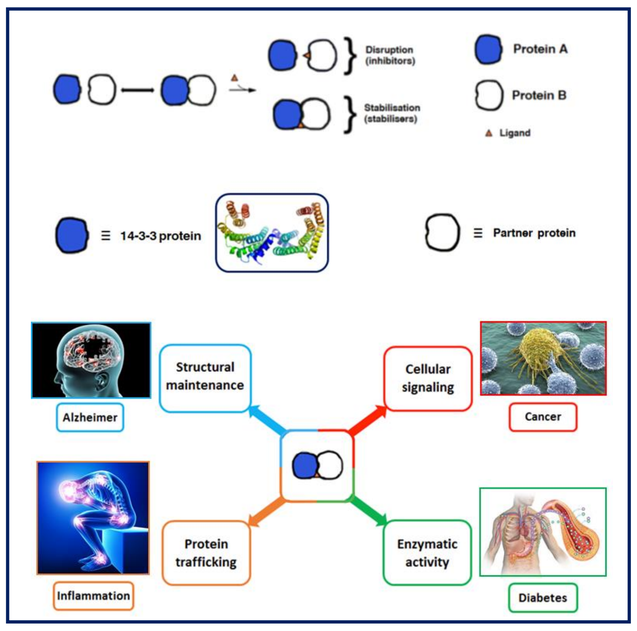
Fragment-based approach applied to the discovery of protein-protein interactions stabilisers

Dario Valenti,[a] Stanimira Hristeva,[a] Christian Ottmann,[b] Anna Karawajczyk [a]

[a] Medicinal Chemistry, Taros Chemicals GmbH & Co. KG, Emil-Figge-Straße 76a, 44227 Dortmund, Germany
[b] Department of Biomedical Engineering, Technische Universiteit Eindhoven, Den Dolech 2, 5612 AZ Eindhoven, The Netherlands
E-mail: dvalenti@taros.de

STABILISERS OF PROTEIN-PROTEIN INTERACTIONS



Protein-protein interactions (**PPIs**) are involved in a wide range of human biological processes and related possible pathologies. Therefore, PPIs' modulation by small molecules is a crucial topic in drug discovery.
Modulation of protein-protein interactions means to push the equilibrium between the free proteins and the complex toward one of these two states (Figure 1). There are two strategies to accomplish that: inhibition and stabilisation.
In contrast to the inhibition of PPIs, the stabilisation approach has not yet been exploited in a systematic way. Thus, the **TASPPI** consortium is aiming to **identify chemical stabilizers** for providing new crucial therapeutic strategies in the **Horizon 2020** key areas of "**Ageing Population**" (neurodegeneration and cancer) and "**Increased Burden of Chronic Diseases**" (metabolic disorders and inflammation).
The main targets of the consortium are 14-3-3 protein and its complexes with different protein partners.

Figure 1.
14-3-3 protein's crystal structure from Ottmann et al., Bioorg. Med. Chem. **2013**, 21, 4058

Given its ubiquitous and adapting nature, 14-3-3 protein is able to bind several different partners being involved in the already mentioned high impact pathologies.
Despite the protein-protein complexes are indicated to be very challenging targets, we decided to apply a fragment-based approach to develop new stabilisers.
The Taros' internal fragments collection was nominated for being the starting point of the design and development process, as it is composed of novel and three-dimensional structures fitting properly with the principles of the chosen approach. This set will serve the consortium members for primary screenings and it will be then subjected to further structural elaborations resulting in a final lead-like molecule.
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.

TASPPI – an ETN consortium consisting of 6 academic and 5 industrial partners.

THE FRAGMENT-BASED APPROACH

The aim of fragment-based strategy is to use low molecular weight entities as starting point for building "piece-by-piece" a ligand for a chosen target (Figure 2). For fulfilling this task a strict cooperation between a wide range of techniques and different drug discovery experiences is required.
An example of the fragment-based approach workflow is illustrated in Figure 3. It begins with **selection of the target** (red) and **fragment libraries** to test (yellow). Afterwards the chosen libraries will be exposed to a **primary screening** (green). In this stage, the availability of different techniques – see lower box in Figure 3 – gives the possibility to identify a pool of fragment hits able to bind the target.

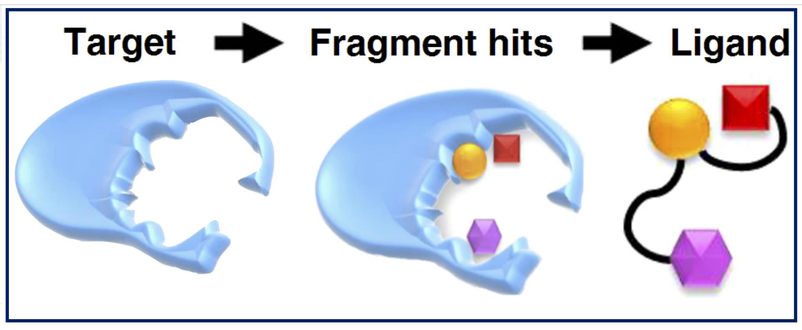


Figure 2

Figure 2. adapted from Joseph-McCarthy et al., J. Chem. Inf. Model. **2014**, 54, 693.
Figure 3. Abell et al., Biochemistry **2012**, 51, 4990.
Figure 4. adapted from Joseph-McCarthy et al., J. Chem. Inf. Model. **2014**, 54, 693.

The identified structures would exhibit a good Ligand Efficiency (values > 0.3) but a weak affinity towards the target (magnitude order of mM). During the hit identification the most reliable technique will be chosen for providing an overview on both the hit's treatable chemical space and binding mode. The data would make possible to proceed with the **hits' structural elaboration** (blue). Synthetic chemistry is applied in the enrichment of the hits according to one or a combination of the main **developing strategies: fragment growing, linking and merging** (Figure 4). The desired outcome of this building-up step is the improved hit molecule (binding affinity magnitude order of nM) that will facilitate the hit-to-lead transition.

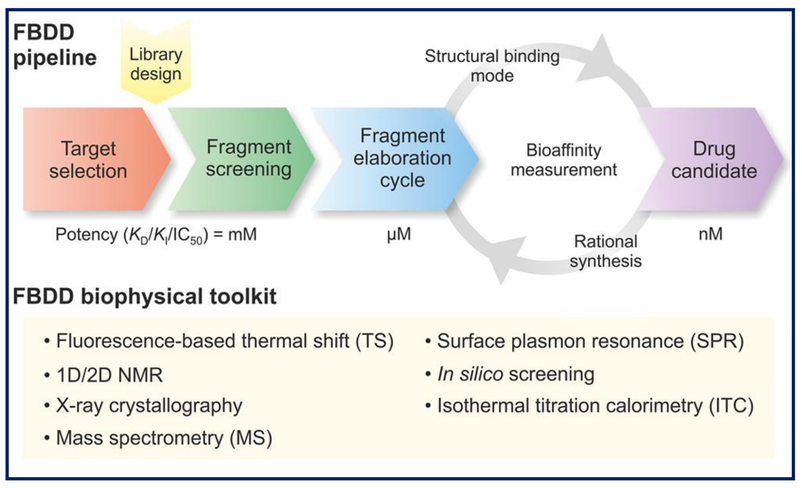


Figure 3

The biological activity of newly synthesised compounds will be evaluated through **biochemical/biophysical and cellular assays** (grey cycle). At last, it will be possible to conduct a structure-activity relationship study on the library in order to identify a **candidate with a good balance between pharmacokinetics and pharmacodynamics** (purple).

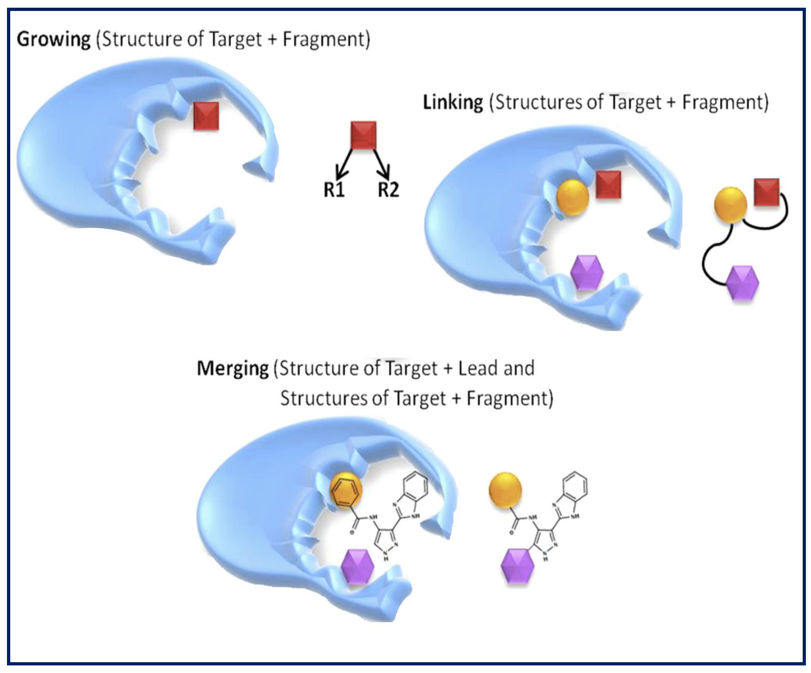


Figure 4

TAROS' FRAGMENTS COLLECTION

Taros' fragments collection contains a wide range of **novel chemical entities** designed applying orthogonal enumeration and synthesised according to an already established and reliable chemistry. The structures were designed with the aim to obtain a high diversification level that allows further structural elaborations.
All the members of this collection present an unprecedented **structural complexity** and high **three-dimensionality.**
These properties are related to the preponderance of systems rich in sp^3-hybridized carbon atoms (Fsp^3-rich) incorporated into the structures in order to achieve different saturations of their rings and/or of their substituents.

There are described a few examples of novel designed and synthesised spyro, bridged and fused polycycles presenting **different saturation** and **conjugation** in Figure 5**.**
The presence of saturated systems (60% of fragments with Fsp^3 > 0.4) leads to the **improvement** of our structures' **3D properties** in a fashionable way that might provide successful results for a challenging target as PPIs.
Moreover, ideas that highly experienced medicinal chemists poured in the selection of suitable structures to the collection are illustrated by some of the physico-chemical properties showed by charts A and B. Those charts highlight the **good balance** in the structures **between molecular weight, clogP, number of rotatable bonds and Hydrogen-bond acceptors or donor groups** according to the fragment-based approach principles ("Rule of 3").
The possibility for further elaborations of these structures is guaranteed by the several diversification points included into the selected structures. Moreover, the different substitution patterns would provide deeper inside into the attractivity of the chemical space and the hot spots considered the specific target.
Thus, these characteristics provide versatile starting points and ample opportunities for further chemical exploration and growth during the hit-to-lead phase.

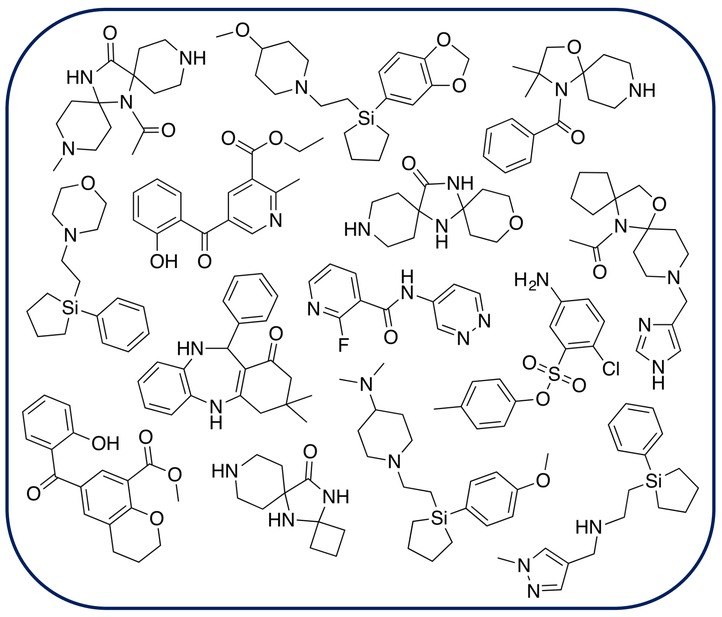


Figure 5. Examples of chemical entities present in the Taros' in-house collection of fragments.

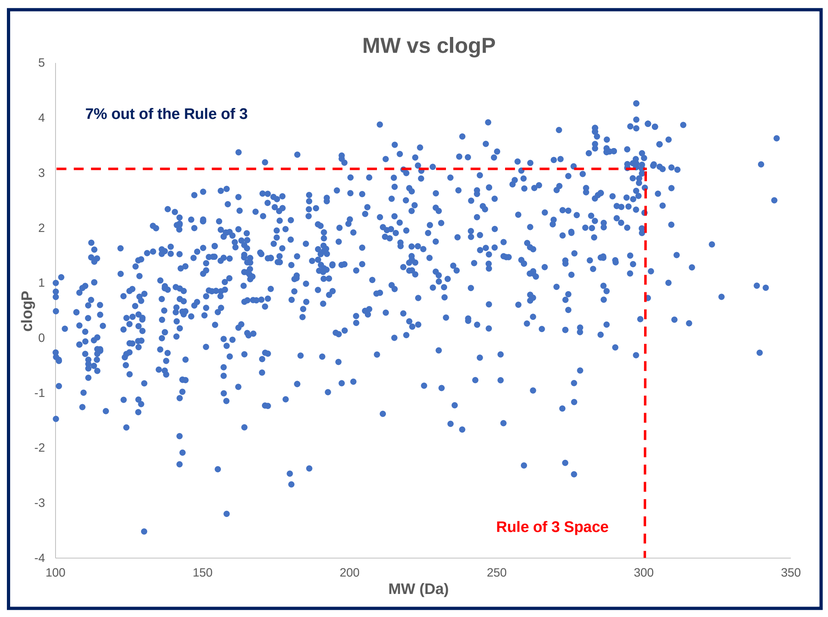


Chart A. Molecular weight and clogP of the Taros' fragments collection members.

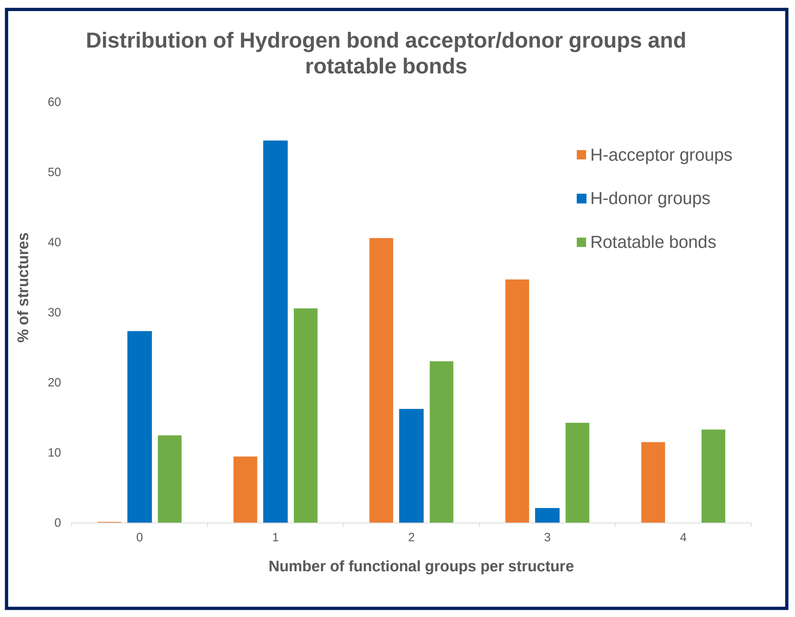


Chart B. Distribution of H-bond acceptor/donor groups and rotatable bonds into the Taros' fragments collection.

This work is supported by the Initial Training Network, funded by the H2020 Marie Curie Actions of the European Commission under Grant Agreement 675179