IDENTIFICATION AND CHEMO-CATALOGING OF MULTI-TARGET DRUGS

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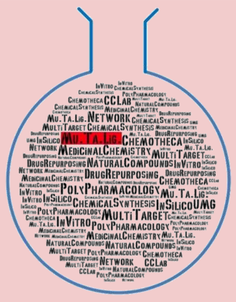
Project outline

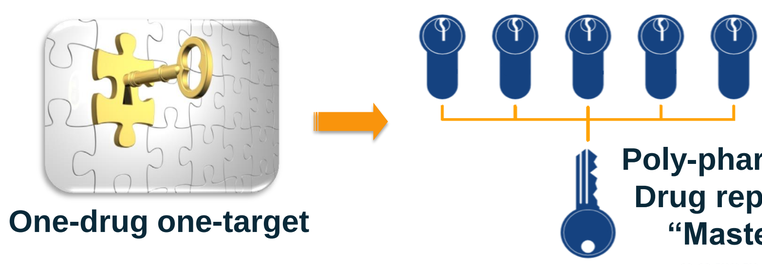


A promising approach for treating complicated diseases is related to the identification and to the chemo-cataloging of multiple therapeutic targets modulator molecules. Multi-target drugs (MTD) have triggered the interest of both academia and pharmaceutical companies.[1] By means of *in silico* techniques, MTD will be selected from: (a) known drugs, (b) substances endowing biological activity but unknown pharmacodynamics, and (c) natural products. Finally, the molecules will be stored into a web accessible database whose development is an integral part of the research project: the **Mu.Ta.Lig Chemotheca**, developed within the COST ACTION CA15135[2] framework. A pharmacophore based virtual screening will be performed taking into account all target models available into the *Protein Data Bank (PDB)*. Finally, for each of the selected compounds, tentative Phase I metabolites will be generated, using *in silico* techniques. Theoretical metabolites will be subjected to the same pharmacodynamic investigation of the precursors and, therefore, cataloged.

Progress report – First year

The research activity of this year has focused on the collection and characterization of chemical libraries to be virtually screened against 3D models of the therapeutic targets. Two key points will characterize this project:

1. The **Multi-Target Drug Discovery (MTDD)** paradigm, the identification of multiple therapeutic targets modulators. This could be particularly relevant in multifactorial diseases, such as cancer, that requires new generation therapeutic tools. This strategy could provide both a better efficacy and a reduction of side effects;
2. The therapeutic **Repositioning** of known drugs, particularly interesting in industry.



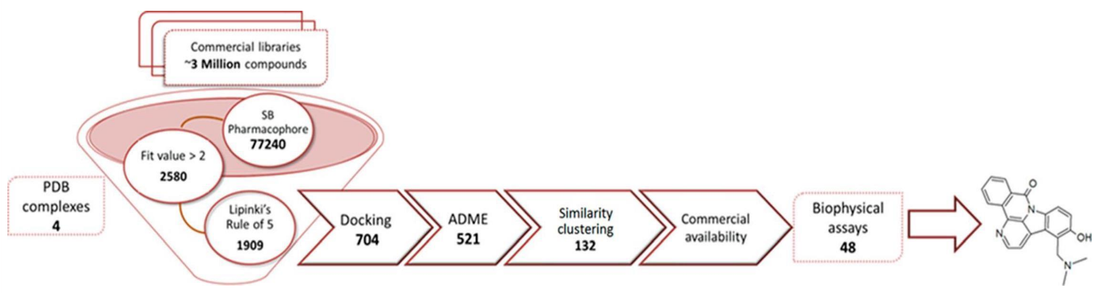
These are also the objectives of Net4Science (N4S) an academic spin-off of the Computational Pharmaceutical Chemistry Laboratory (CCLab).

www.net4science.com

Multi-target drug Discovery

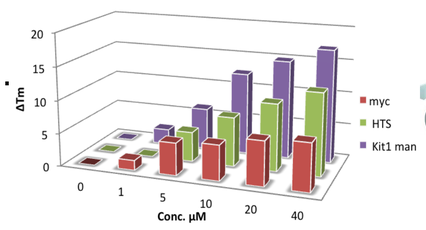
Targeting G-quadruplex-forming DNA (G4) is a very attractive goal in cancer treatment.[3] This structure is located at the telomere level, ribosomal DNA (rDNA) and oncogen promoters.[4]

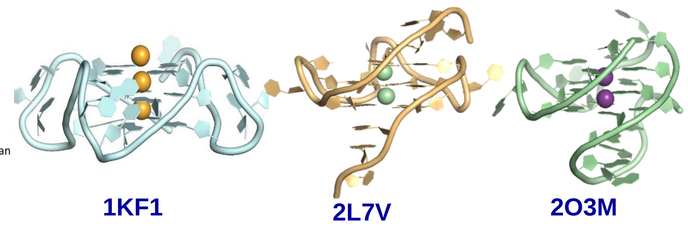
The identification of multi-target ligands for multiple sequences of G4 DNA, is interesting. Starting from a known compound,[5] other models of pharmacophores were generated and were included in a "*focused library*" of 12 promising compounds of naphthyridine.



- Docking simulations using the G4 sequences *of h-telo, c-myc* and *c-kit1* (PDB codes: 1KF1, 2L7V, 2O3M, respectively);
- Fluorescence Resonance Energy Transfer (FRET) and Circular dichroism (CD) experiments were carried out.

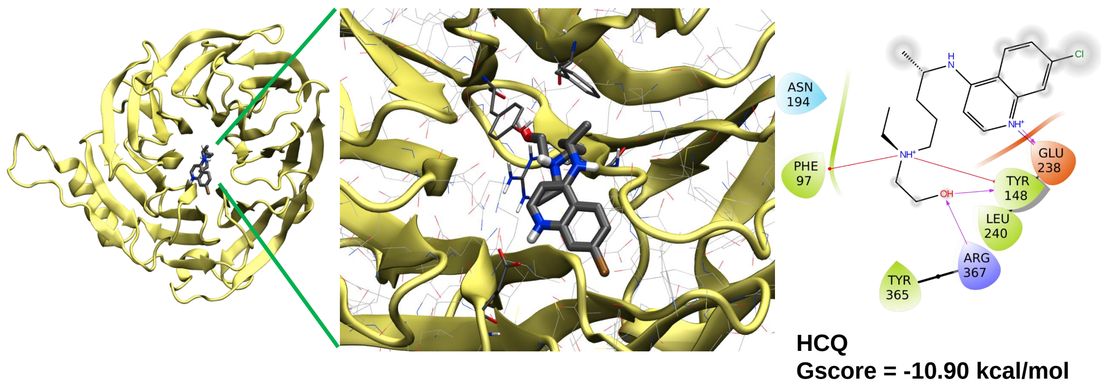
Moreover, further investigations are in progress also on other G4 sequences to confirm the multi-target properties of these compounds.





Drug repurposing

Polycomb Repressive Complex 2 (PRC2), is a regulator of epigenetic states required for development and homeostasis. In particular, it trimethylates histone H3 at lysine 27 (H3K27me3), which leads to gene silencing and it is dysregulated in many cancers.[6] PRC2 activity is further enhanced by the binding of EED and EZH2 subunits. Therefore, we targeted the EED allosteric domain in order to selectivity inhibit PRC2. The 5K0M PDB structure was selected as EED model, and we performed an high throughput *in silico* screening of a database containing FDA approved drugs.

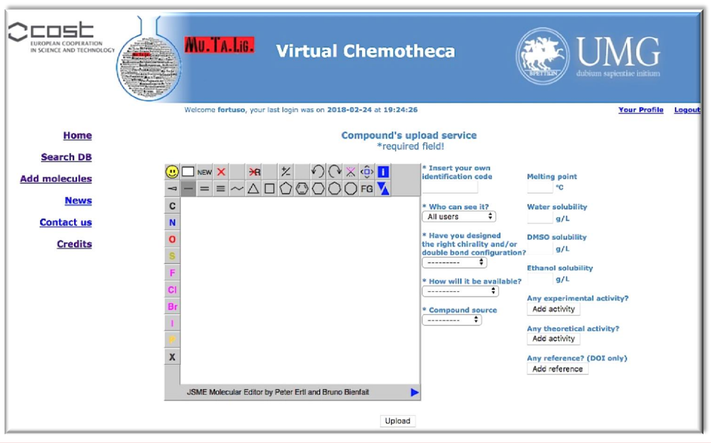


HCQ
Gscore = -10.90 kcal/mol

The binding affinity for each of compounds against our target was predicted using Glide Extra Precision (XP) protocol. The most promising predicted compound was **Hydroxychloroquine (HCQ).**

Mu.Ta.Lig. Virtual Chemotheca

At present, the only available multi-target oriented database is the Chemotheca, available at chemotheca.unicz.it



Programming languages

For contributing to the development of Chemotheca, I have written a Python-based software for the qualitative analysis of the PDB crystallographic models. The software, daily used at CCLab, allows a more accurate selection of the therapeutic target models to be used in research projects.

```
rafcat@ws09 /home/CCLab/rafcat/provapython : comp_dpi -h

Compute DPI of PDB file(s) according to:
Acta Crystallographica D Biological Crystallographica, 2002, 58, 792-797

USAGE : comp_dpi <file.pdb> [file2.pdb] [...]


rafcat@ws09 /home/CCLab/rafcat/provapython : comp_dpi p
pdb/            pdb_analyzer.py* pp.py*          pro.py*
rafcat@ws09 /home/CCLab/rafcat/provapython : comp_dpi pdb/*.pdb
PDB     DPI
4hvb    0.488 [BEST]
5wby    0.817
4jsv    0.964
4jt5    1.008
```

References

1 Ramsay RR, Popovic-Nikolic MR, Nikolic K, Uliassi E, Bolognesi ML, *Clinical and Translational Medicine*, **2018;** 7:3

2 http://www.mutalig.eu

3 Xu, H.; Di Antonio, M.; McKinney, S. *et al.*, *Nat. Commun.*, **2017**, *8*, 14432.

4 Huppert, J.L. *Biochimie,* **2008**, *90*, 1140-1148.

5 Rocca, R.; Costa, G.; Artese, A. *et al.*, *ChemMedChem,* **2016**, *11,* 1-14.

6 He, Y. *et al*. *Nat Chem Biol.* **2017**, *13(4):389-395*.

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