Bachelor Degree Thesis

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Computational-aided Drug Discovery in Protein-Protein Interactions: Defining a simple protocol and testing it in Notch signaling

INTRODUCTION TO COMPUTATIONAL-AIDED DRUG DISCOVERY:

Computational-aided drug discovery (CADD) is the implementation of computer technologies in the drug development cycle (Figure 1). Its role is mainly to improve and accelerate the hit discovery process, while it reduces the high costs associated with experimental methods. CADD also plays an important role in lead optimization and drug rational design. A widely used method in computational hit discovery is the structure-based approach, which is the implemented strategy in protein-protein interactions drug discovery.

Structure-based drug discovery relies on the knowledge of proteins structures and explicit modelling of both chemicals and biological entities. The standard computational approach is **molecular dockings**. This methods sample ligand conformations from virtual compound libraries (chemicals or fragments) to dock them within the binding site of the biological structure and using scoring functions (simplified energetic functions) to predicted binding affinities. Among many strategies is the **pharmacophore-based screening**, in which pharmacophore hypothesis defines the virtual 3D arrangement of ligand's key physicochemical proprieties for the interaction with its biological target. These features are used as a scaffold or map to screen compounds libraries.

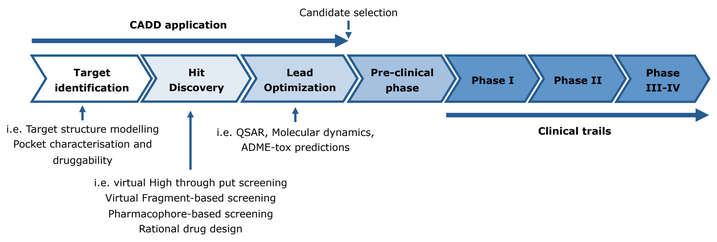


FIGURE 1: The drug discovery and development cascade and CADD role (1).

TARGETING PROTEIN-PROTEIN INTERACTIONS: PROMISING BUT CHALLENGING

Protein-protein interactions (PPIs) have a crucial role in cell signalling transduction and the execution of cellular functions, offering new therapeutic opportunities to treat pathologies such as transplant rejection (i.e. IL2/IL-2Rα) or cancer (i.e. Bcl-2/BAK).

PPIs have singular features and different chemical spaces from conventional targets (i.e. enzymes), for this reason targeting PPIs with small molecules can be challenging (Figure 2). The main druggability challenges for PPIs are:

- **Large interaction surface** (1500-3000 Å²)
- **Surface flatness**: lack of grooves and cavities.
- **Flexibility**: surfaces are plastic and conformational changes.
- **Electrostatic** and **solvent exposition**.

The presence of small regions in the interaction surface called **hotspots** (600 Å²) that confer most of the binding energy offers a key druggability advantage.

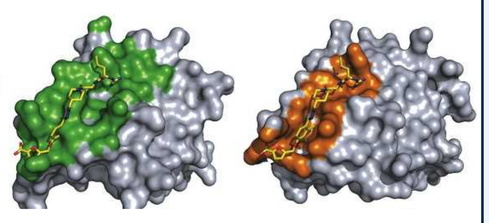


FIGURE 2: IL-2Rα bound to the small molecule SP4206. The **green** surface on the left represents the IL-2 interaction with the receptor, while on the right the **orange** represents the small molecule contact surface (2).

OBJECTIVE:

- Define a simple computational approach using free and user-friendly software compatible with the computer power of a regular laptop in order to target PPIs based on the current knowledge on computational development of PPIs' inhibitors.
- Proof of concept for the first functional steps in the protocol using Jagged1-ligand and Notch-receptor interaction.

METHODOTHODLOGY AND SYSTEM SELECTION

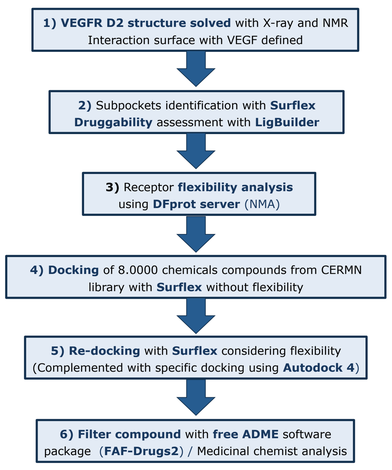
Methodology followed to define the protocol:

1. Definition of the system to use as a proof of concept.
2. Intensive bibliographic search for computational drug discovery examples in PPIs.
3. Identification of key steps required for the cascades and search for free-software or webservers to apply a strategy like the one presented in figure 3.
4. Test the different programs and webservers in this proof of concept system to test their function in protein-protein interactions and choose the more suitable for the propose of the project.

Selection of Jagged1-Notch interaction as a case of study:

Notch signalling is a cell-cell communication system involved in cell proliferation, cell death, differentiation, among other functions. **Jagged1 is the most characterised Notch-ligand in terms of structure and functions**. The solved structures have a maximum resolution of 2.38 Å (PDB ID: 4CC0) and the interaction surface has been described. In addition, Jagged1-Notch interaction is a **relevant therapeutic target** to develop novel cancer treatments due to its role in tumour progression and cancer cells microenvironment control.

FIGURE 3: Computational hit discovery strategy to inhibit VEGFR-D2 domain interaction with VEGF (3).



RESULTS AND DISCUSSION:

PPIs target characterization

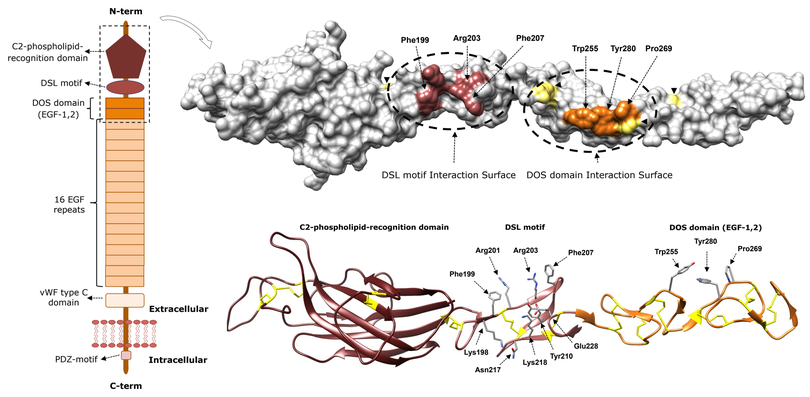


FIGURE 4: Jagged1 structure and interaction surface in the N-terminal domain definition (PDB ID: *4CC0.A)* (4).

- Target PPIs should have **previous structural information and the interaction regions characterized** to not add complexity and lose reliability.
- Hotspots and their druggability can be computationally characterised using webservers like DoGsitescorer or Fpocket, docking chemical probes or docking both proteins.
- The interaction surface of Jagged1 has been experimentally (residues mutagenesis) and computationally (proteins docking) defined in the N-terminal region (figure 4). The DSL motif has been postulated to be a relevant hotspot, where mutations in its residues led to the loss of function.

Virtual Fragment-based screening and druggability

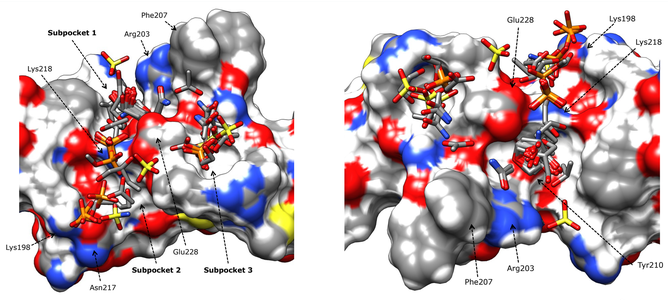


FIGURE 6: Fragments docked with CrystalDock along the DSL motif (RMSD < 2Å).

The fragment-based screening **provides a more versatile screening for PPIs** as it is based on docking small chemical fragments, **covering wider chemical spaces** than using small molecules. The application of CrystalDock offers an **alternative approach for fragment-based screening to conventional free tools** like DOCK or AutoDock.

CrystalDock calculations show docked fragments along the DSL motif groove and in the associate subpocket 3, which have significant polar features (figure 6). These results might confirm motif's druggability, but it could be affected by the relevant polar interactions of Glu228 in subpockets 1 and 2.

PPIs target characterization:
Structural and target interface information

Protein surface flexibility analysis
(Normal Mode Analysis (NMA))

Virtual Fragment-based screening and druggability
CrystalDock

Ligand-Protein pharmacophore hypothesis
ZINCPharmer

Re-docking hit-compounds
AutoDock Vina in UCSF Chimera (Flexible)

Energy minimization (UCSF chimera)
ADME-tox (FAF-drug3)

Protein surface flexibility analysis

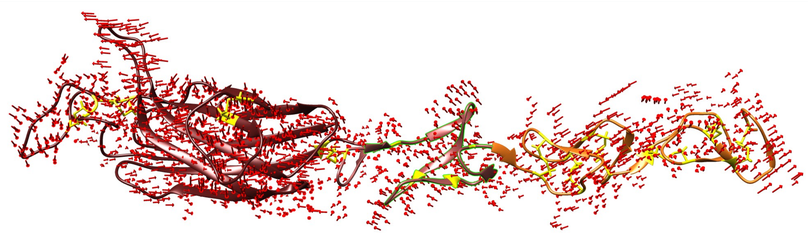


FIGURE 5: Normal mode analysis applying elastic network modes with UCSF chimera. Mode 6: 0.95 Hz. Arrows show the positive displacement.

PPIs surface are dynamic and **flexibility assessment is crucial to study target pocket motion and its impact on druggability**.

Normal Modes Analysis determines the different modes of vibration of protein structures, where each mode correspond to atoms vibrating in a specific frequency, describing with several modes protein flexibility. The low-frequency modes provide information about proteins global motion.

These calculations can be performed using the UCSF Chimera platform or webservers like FlexServ, DFprot or ElNémo. **The NMA calculations show considerable motion along Jagged1 structure**. The DSL motif remains apparently stable, but residues flanking the groove like Lys198 and Glu228 are rearranged when the pocket is deformed for the global movement. This could have implications in druggability.

Ligand-protein pharmacophore hypothesis

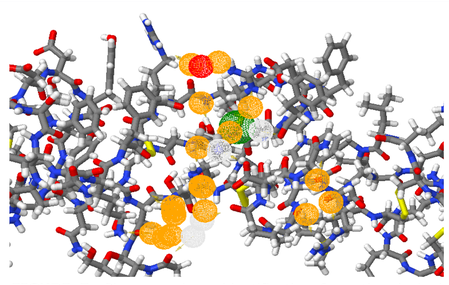
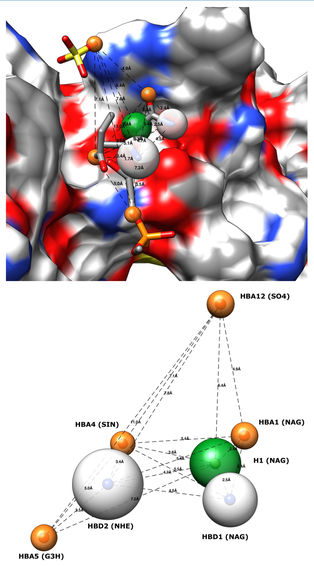


FIGURE 7: Pharmacophore identification from the docked fragments interacting with the surface pocket using the ZINCPharmer online interface.



(Up-Left) Global pharmacophore definition for the DSL motif. (Up/Bottom-right) An example of possible pharmacophore hypothesis for future research screening with ZINC database.

Hydrophobic (H: **green**), Hydrogen bond donor (HBD: **grey**), Hydrogen bond acceptor (HBA: **orange**).

CONCLUSIONS AND PERSPECTIVES:

- **Drug discovery in PPIs is currently an evolving field** with a promising future for their notorious implications in human diseases and druggable potential. Although several computational screenings strategies have been reported to trigger PPIs, there is still not a defined procedure better than the others. It is probably a consequence of the PPIs singularity, challenging features and their still not completely clear chemical space.
- **A simplified computational strategy to target PPIs using free and user-friendly software is proposed**. The most important steps (pocket characterisation, druggability, flexibility, fragment-based docking and pharmacophore generation) have been **successfully tested in the case of study (Jagged1)**. The tested steps set up the protocol's base and provide confidence of its working possibilities. However, **future validations and the full implementation of the protocol has to be preformed**.
- **The last steps proposed in the protocol are a crucial validation phase**. The re-docking step using AutoDock Vina followed by the energy minimization of ligand-protein interaction will prove the proper binding interaction of selected hits from the pharmacophore screening and discard possible steric clashed. Finally, an ADME-tox filtering step with FAF-drug3 webserver is suggested to discard potential toxic compounds and select final hits with more optimal pharmacokinetic features.
- Although real concerns on Jagged1 druggability exist, the strategy here reported applied in Jagged 1 could represent the first step for a future drug discovery research campaign to identify novel compounds triggering Notch/Jagged1 signaling in cancer.

REFRERENCES:

1. Sliwoski, G. et.al. Computational Methods in Drug Discovery. *Pharmacol. Rev.*; 2014, **66**:334-395.
2. Wells, J. a, & McClendon, C. L. Reaching for high-hanging fruit in drug discovery at protein-protein interfaces. *Nature*; 2007, ***450***:1001–1009. (Image from figure 3; **Poster License number**: 3634761449161, **Thesis License number**: 3638380500812)
3. Gautier, B. et.al. Targeting the proangiogenic VEGF-VEGFR protein-protein interface with drug-like compounds by in silico and in vitro screening. *Chemistry & Biology*; 2011, **18**:1631–9.
4. Cordle, J. et.al.. A conserved face of the Jagged/Serrate DSL domain is involved in Notch trans-activation and cis-inhibition. *Nature Structural & Molecular Biology*; 2008, **15**:849–57.

ACKNOWLEDGMENT

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