# A computational protocol to Fragment-Based Drug Design at PDB scale

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## Introduction:

Computational methods are needed to mine efficiently all the available 3D structures of ligands complexed to proteins, both treated as a whole and as smaller fragments to increase the likelihood of fragment hopping from one target to another.

MED-SuMo [2,3], a target based drug design tool, offers a procedure to effectively characterize the protein binding site. This tool is based on the identification of local shape and chemical similarities in the target binding site with other proteins (with their co-crystallized ligands).

In this work, we've used MED-SuMo on a fragment database derived from the PDB. We report here (1) the protocol to generate a library of fragments likely to bind to a given target (2) an application to the drugability of an ATP binding protein, an Histidine Protein Kinase. One of the most promising applications of this protocol is its potential to identify hits and leads against targets of low or borderline drugability. The lack of deep active site pocket can be overridden by comparing the whole surface of a protein to a database of fragmented pdb's ligand.

## Fragment-based Drug Design Protocol:

### 1)The fragment database

- Local Shape and Surface Chemical Functions around the fragments (6.0 Å) are stored as 3D graphs in a MED-SuMo database
- The Whole PDB had been processed
- 82k ligands are fragmented into 405k fragments. MSQ fragmentation from 1DI9 is shown in *Fig.1.*

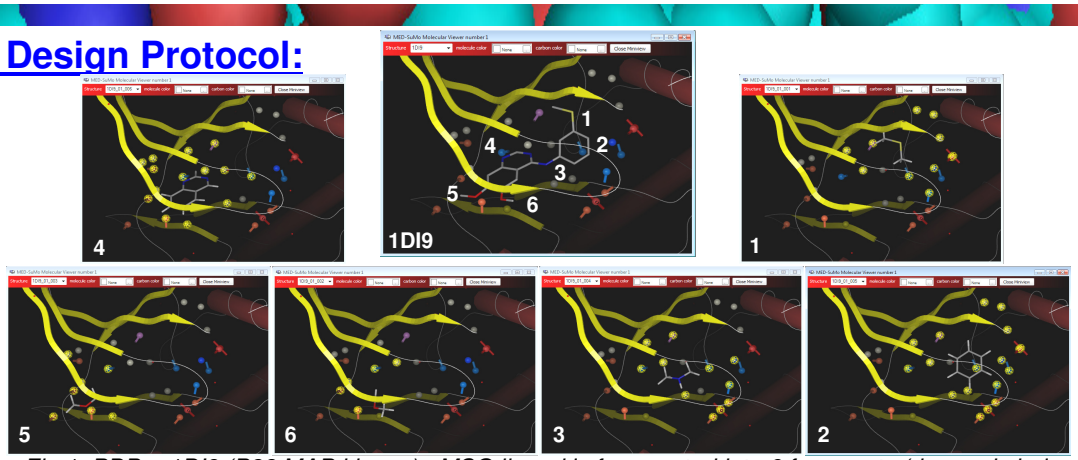


*Fig.1. PDB = 1DI9 (P38 MAP kinase) ; MSQ ligand is fragmented into 6 fragments (ring and chain assemblies), numbered from 1 to 6 in the figure. Surface Chemical Functions are shown around the whole MSQ and are highligted around each fragment, describing the local environement.*

### 2)Fragment hopping from the pdb to your protein of interest

- The Structural superposition is based on a 3D graph of protein Surface Chemical Features (MED-SuMo software). An example is shown in *Fig.2.*
- The Hit list is clusturized according to the fragment environnement signature, i.e. subpocket. Drug design can be focused to a specific subpocket. (*Fig. 4*)

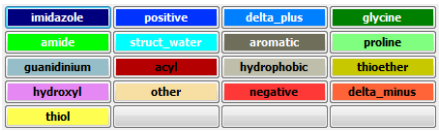


*Fig. 3.: Color coding of MED-SuMo Surface Chemical Features.*

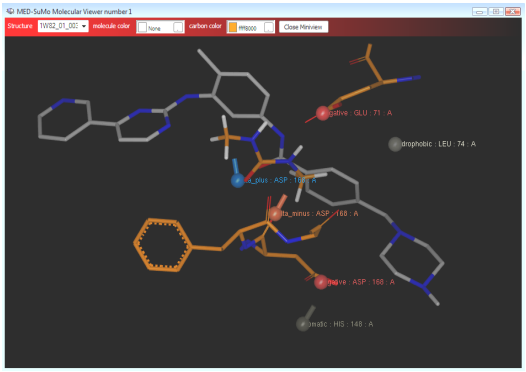

*Fig. 2.: fragment hopping from a P38 MAP kinase to Abl kinase. Query = 1IEP (Abl kinase) Hit = 1W82 urea fragment. The ligand C=O is conserved and the H-bond to N-H D of DFG-out motif is conserved.*

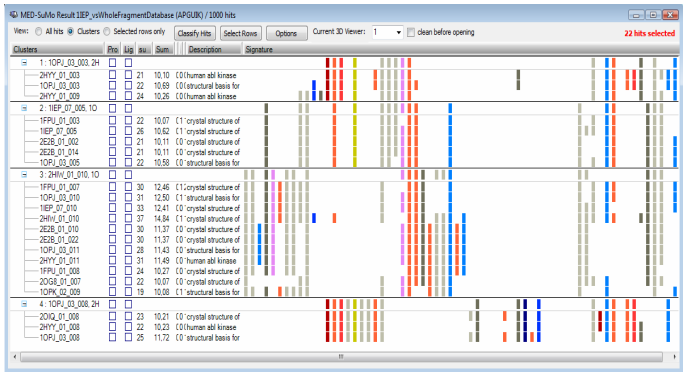

*Fig. 4.: clusterized Hit list obtained through a dendrogram available in the MED-SuMo GUI.*

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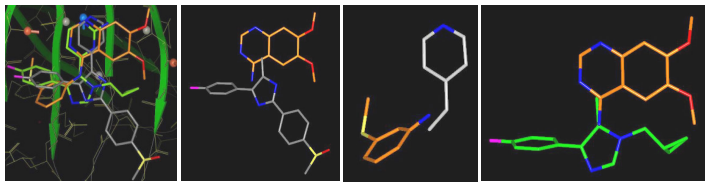
# Applications:

## 1) Intra-family drug design

- Application to 1BMK, a P38 MAP kinase pdb file
- The protocol reproduces the results published by Pierce et al.[5]: a known active ligand is hybridized (1BL6) and new scaffolds are proposed (*Fig. 5* )
- This new protocol leads to hundreds of compatible fragments from the kinome (*Fig. 6*)

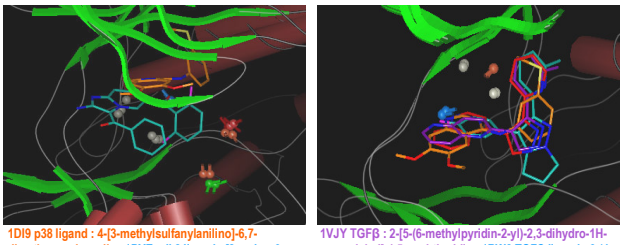
Same site (p38)



1DI9 p38 ligand : 4-[3-methylsulfanylanilino]-6,7-dimethoxyquinazoline 1A9U p38 ligand : 4-[5-(4-fluoro-phenyl)-2-(4-methanesulfinyl- phenyl)-3H-imidazol-4-yl]-pyridine 1BMK p38 ligand : 4-(fluorophenyl)-1-cyclopropylmethyl-5-(2- amino-4-pyrimidinyl)imidazole

*Fig. 5: overlay of 3 co-crystallized ligands from p38 MAP kinase. From left to right: overlay of ligands from 1DI9 (orange), 1A9U (grey), 1BMK (green) ; 2 cases of hybridization of 1DI9 and 1A9U ligands ; hybridization of 1DI9 and 1BMK ligands. This work agrees with the work published by Pierce et al. [5].*

Similar site



1DI9 p38 ligand : 4-[3-methylsulfanylanilino]-6,7-dimethoxyquinazoline 1PYE cdk2 ligand : [2-amino-6-(2,6-difluoro-benzoyl)-imidazo[1,2- a]pyridin-3-yl]-phenyl-methanone

1VJY TGFβ : 2-[5-(6-methylpyridin-2-yl)-2,3-dihydro-1H-pyrazol-4-yl]-1,5-naphthyridine 1RW8 TGFβ ligand : 3-(4-fluorophenyl)-2-(6-methylpyridin-2-yl)- 5,6-dihydro-4H-pyrrolo[1,2-b]pyrazole1PY5 TGFβ : 4-(3-pyridin-2-yl-1H-pyrazol-4-yl)quinoline 1DI9 p38 ligand : 4-[3-methylsulfanylanilino]-6,7-dimethoxyquinazoline

*Fig. 6: LEFT overlay of a cdk2 ligand and a p38 MAP kinase ligand. RIGHT overlay of three tgf-β ligand and a p38 MAP kinase ligand. These are two examples of the hybridization of ligands from similar binding site*

## 2) Inter-family drug design

- Application to 2CH4, a CheA bacterial protein histidine kinase file. Assessment of structural similarity with other purine binding proteins like hsp90, grp94, gyrase b is required for fragment based drug design of CheA inhibitors (potential antibiotics). An example from gyrase is shown in *Fig.7.* Sequence identity between CheA and gyrase b is below 13% (PSI-Blast iteration 5).

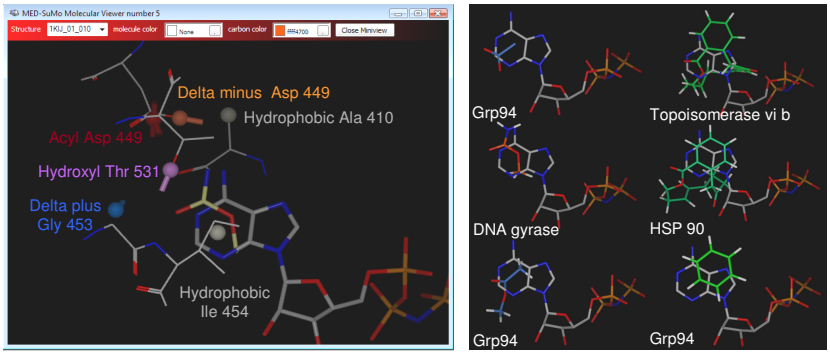



*Fig. 7: left view, 2CH4 (CheA Histidine kinase) is overlaid to 1KIJ (DNA gyrase) with MED-SuMo. The Small fragment 1KIJ (yellow) is from NOV cocristalized in 1KIJ (DNA gyrase) and the larger fragment is ANP, the cocristalized ligand of 2CH4. Common Surface chemical features between 2CH4 and 1KIJ are shown as colored ball&sticks. Numbering of AA according to 2CH4. Right view, tiled representation of a few compatible fragments from 2FYP (grp94), 2HKJ (topoisomerase vi-b), 1KIJ (DNA gyrase), 2IWU (hsp90), 2GFD (grp94) and 2GFD (grp94). All of them are in the 2CH4 reference frame and only ANP is shown.*

# Conclusions:

Intra-family (kinase to kinase) and Inter-family (from one ATP binding to another) fragment hopping is conducted at PDB scale. Validation on P38 MAP kinase is obtained by comparison to the pioneering work of Pierce et al. [5]. This new protocol applies to ATP binding proteins, e.g. Protein Histidine Kinase. Other applications of MED-SuMo include drugability of exposed binding sites and protein-protein interactions.

# References: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# Appendix: MED-SuMo-GUI to lauch query and browse/classify results: