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RESEARCH NOTE

UPDATE DOCLASP - Docking ligands to target proteins using spatial and electrostatic congruence extracted from a known holoenzyme and applying simple geometrical transformations [version 2; peer review: 2 approved with reservations]

Sandeep Chakraborty ^{1,2}¹Plant Sciences Department, University of California, Davis, CA, 95616, USA²Department of Biological Sciences, Tata Institute of Fundamental Research, Mumbai, 400 005, India**V2** First published: 31 Oct 2014, 3:262
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<https://doi.org/10.12688/f1000research.5145.2>Latest published: 16 Jun 2016, 3:262
<https://doi.org/10.12688/f1000research.5145.3>**Abstract**

The ability to accurately and effectively predict the interaction between proteins and small drug-like compounds has long intrigued researchers for pedagogic, humanitarian and economic reasons. Protein docking methods (AutoDock, GOLD, DOCK, FlexX and Glide to name a few) rank a large number of possible conformations of protein-ligand complexes using fast algorithms. Previously, it has been shown that structural congruence leading to the same enzymatic function necessitates the congruence of electrostatic properties (CLASP). The current work presents a methodology for docking a ligand into a target protein, provided that there is at least one known holoenzyme with ligand bound - DOCLASP (Docking using CLASP). The contact points of the ligand in the holoenzyme defines a motif, which is used to query the target enzyme using CLASP. If there are no significant matches, the ligand cannot be docked in the protein. Otherwise, the holoenzyme and the target protein are superimposed based on congruent atoms. The same linear and rotational transformations are also applied to the ligand, thus creating a unified coordinate framework having the holoenzyme, the ligand and the target enzyme. This provides the docked ligand in the target enzyme. Previously, CLASP was used to predict and validate (in vivo) the inhibition of phosphoinositide-specific phospholipase C (PI-PLC) from *Bacillus cereus* by two dipeptidyl peptidase-IV (DPP4) inhibitors - vildagliptin and K-579. In the current work, vildagliptin was docked to the PI-PLC structure complexed with myo-inositol using

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1. Rahul Banerjee, Saha Institute of Nuclear Physics, Kolkata, India**2. Amarda Shehu**, George Mason University, Fairfax, USA

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DOCLASP. The docked ligand is free from steric clashes and interacts with the same side chain residues that bind myo-inositol, providing corroboration of the validity of the proposed methodology.

Keywords

protein, docking ligand, congruence

Corresponding author: Sandeep Chakraborty (sanchak@gmail.com)

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UPDATE Amendments from Version 1

The updated version notes the usage of DOCLASP in correlating the ability of VP24 protein from Ebola and Marburg viruses to bind human karyopherin to their immune suppression mechanism and pathogenicity.

See referee reports

Introduction

The ability to computationally predict protein-ligand interactions with accuracy is an invaluable asset, since it allows for large scale screening at minimal costs^{1,2}. Consequently, computational methods that predict the favorable conformation of a protein-ligand complex have been the focus of intense research over the last few decades³. Protein docking methods are a subset of these methods, characterized by their ability to score a large number of possible conformations using fast algorithms. Among these, five programs - AutoDock⁴, GOLD⁵, DOCK⁶, FlexX⁷ and Glide⁸ - are the most cited, although it should be noted that ‘the number of citations of a given paper is no measure of quality of the corresponding protein-ligand docking software program’⁹. Typically, a protein-ligand docking program has two distinct phases - conformational sampling (or searching) and scoring¹⁰. Despite the significant progress in the field, there are several challenges arising from protein or ligand flexibility, entropic considerations or the presence of water molecules that need to be addressed¹¹.

Previously, the conservation of spatial and electrostatic properties in cognate pairs of residues in the catalytic site of proteins with the same functionality has been used to develop a computational method (CLASP) for detecting binding and catalytic sites¹²⁻¹⁵. In the current work, this methodology has been extended by proposing a method for docking ligands into target proteins - DOCLASP (Docking using CLASP). DOCLASP takes as input a set of proteins with known structures which bind a particular ligand, and a target protein into which the ligand is to be docked. Each of these holo structures is used to define a motif consisting of the first four residues making non-hydrophobic interactions. These motifs are used to query the target protein, using an enhanced version of the search engine used by CLASP that uses precompiled databases¹⁶, and significant congruent matches are identified. These significant matches in the target protein are now superimposed to the binding residues (the motif) in the corresponding holoenzyme(s), thus creating a unified coordinate framework formed by the holoenzyme, the ligand and the target enzyme. This gives us the docked ligand to the target protein, which is outputted as a Pymol formatted file. In essence, the holoenzyme is replaced with the target enzyme if the contact points have a good spatial and electrostatic match in the target enzyme by aligning the congruent atoms. Thus, DOCLASP leverages the implicit search and scoring functions in CLASP to rank possible conformations.

The native activity of phosphoinositide-specific phospholipase C (PI-PLC) was previously shown to be inhibited by two dipeptidyl peptidase-IV (DPP4) inhibitors - vildagliptin (LAF-237) at micromolar concentrations, and K-579 at nanomolar concentrations using *in vitro* experiments based on CLASP analysis¹⁵. Since ‘comparing

docking programs can be difficult’⁹, the DOCLASP methodology is validated by docking vildagliptin to the PI-PLC structure in complex with myo-inositol¹⁷. The docked ligand is free from steric clashes and interacts with the exact side chain residues that bind myo-inositol, providing corroboration of the validity of the proposed methodology. Thus, the current work presents a fast methodology for docking ligands into protein structures based on spatial and electrostatic congruence of known binding sites to putative binding targets.

Materials and methods

DOCLASP uses the basic hypothesis of CLASP - the non-triviality of the spatial and electrostatic congruence in cognate pairs seen across different structures of the same catalytic function, which is extended to the related concept of ligand binding¹². It takes as input a set of M proteins with known structures (Equation 1) which bind a particular ligand (Lig), and a target protein into which Lig is to be docked (P_{target}). Each of these M holo structures is used to define a motif consisting of N ($=4$) residues (Equation 2), taking the first four closest non-hydrophobic interactions into account (Algorithm 1).

$$\Phi_{proteins}^{Lig} = \{P_1, P_2 \dots P_M\} \quad (1)$$

$$\Phi_{motifs}^{P_i} = \{R_1, R_2 \dots R_4\} \quad (2)$$

Each position of the motif has a set of amino acids specified to allow for stereochemically equivalent matches at that particular position (Equation 3), such that while matching amino acid type of r_i should belong to $GROUP_i$.

$$\Phi_{groups} = \{GROUP_1, GROUP_2 \dots GROUP_4\} \quad (3)$$

Previously, the K sets of N residues were obtained in P_{target} using an exhaustive search procedure similar to the one used in SPASM¹⁸. An enhanced algorithm now precompiles all possible motifs of a set ($N=4$ in this case) of predefined amino acid residues from a protein structure that occur within a specified distance¹⁶, and selects the appropriate ones based on each motif (Equation 4). Any match below a user defined threshold score (S_{thresh}) is discarded.

$$\begin{aligned} \Phi_{matches}^{P_i} &= \{M_1^{P_i}, M_2^{P_i} \dots M_K^{P_i}\}, \\ \forall (j = 1 \dots K) [M_j^{P_i} &= \{r_1, r_2 \dots r_N\}, \forall (p = 1 \dots N) [AminoAcidType(r_p) \in GROUP_p]], \quad (4) \\ [CScore_{M_1^{P_i}} < CScore_{M_2^{P_i}}, CScore_{M_2^{P_i}} < CScore_{M_3^{P_i}} \dots], \\ CScore_{M_K^{P_i}} &< S_{thresh} \end{aligned}$$

In Case $\Phi_{matches}^{P_i}$ is null, the ligand Lig can not be docked to the target protein P_{target} . $M_1^{P_i}$, the first element, has the minimum $CScore$ and represents the putative binding site in P_{target} based on the holoenzyme P_i . The set of putative binding sites $\Phi_{bindsite}$ is thus defined (Equation 5).

$$\Phi_{bindsite} = \{M_1^{P_1}, M_1^{P_2} \dots M_1^{P_M}\} \quad (5)$$

Each element of $\Phi_{bindsite}$ ($M_1^{P_i}$) is now superimposed to the corresponding holoenzyme, based on the motif binding Lig in P_i . In order to superimpose these motifs, linear and rotational transformations

Algorithm 1. GetMotif(): Choose n closest atoms, excluding hydrophobic interactions

```

Input: Protein :
Input: Ligand :
Input: n: number of closest atoms to choose
Output:  $\phi_{motif} = \{atom_1 \cdots atom_n\}$ 
begin
  /* Output Motif */
   $\phi_{motif} = \emptyset$  ;
  /* Accepted atom pairs - exclude hydrophobic interactions*/
   $\phi_{AcceptedAtomPair} = [O-N, N-O, O-H, H-O, O-O, N-N, N-H, H-N, S-H, H-S]$  ;
   $ATOMS_{Lig} = \text{atoms of all residues of } Ligand$  ;
  /* Initial radius in Å */
  Radius = 2.5 ;
  foreach  $atom_i$  in  $ATOMS_{Lig}$  do
     $\phi_{atoms} = \text{ProteinAtomsWithinRadiusOfLigandAtom}(Protein, atom_i, Radius)$ ;
    foreach  $atom_j$  in  $\phi_{atoms}$  do
      if  $atom_i - atom_j$  is in  $\phi_{AcceptedAtomPair}$  then
        InsertInMotifSet( $atom_j, \phi_{motif}$ );
        if ( $\phi_{motif} == n$ ) then
          last ;
        end
      end
    end
    /* increment radius by 0.1 Å */
    Radius = Radius + 0.1;
  end
  return  $\phi_{motif}$  ;
end

```

are applied on all atoms such that the first three atoms lie on the same plane ($Z=0$), the first atoms are the origin of the coordinate axis and the second atoms lie on the Y axis. This creates a unified coordinate framework having the holoenzyme, the ligand and the target enzyme, thus providing the docked ligand in the target enzyme. Essentially, the holoenzyme is replaced with the target enzyme if the contact points have a good spatial and electrostatic match in the target enzyme by aligning the congruent atoms. This docked ligand is now outputted as a Pymol formatted file.

The DOCLASP package is written in Perl on Ubuntu. Hardware requirements are modest - all results here are from a simple workstation (2GB ram) and runtimes were a few minutes at the most. Adaptive Poisson-Boltzmann Solver (APBS) and PDB2PQR packages were used to calculate the potential difference between the reactive atoms of the corresponding proteins^{19,20}. The APBS

parameters and electrostatic potential units were set as described previously in¹². All protein structures were rendered by PyMol (<http://www.pymol.org/>).

Results and discussion

Previous CLASP analysis of the spatial and electrostatic properties of active site residues in PI-PLC from *B. cereus* indicated that it is a prolyl peptidase, which was also validated by *in vitro* experiments¹³. Subsequently, it was shown that PI-PLC is inhibited by two dipeptidyl peptidase-IV (DPP4) inhibitors - vildagliptin (LAF-237) at micromolar concentrations, and K-579 at nanomolar concentrations. Since there are no DPP4 structures solved which ligand K-579, a DPP4 protein structure in complex with vildagliptin (PDBid:3W2TA)²¹ provided the five closest atoms in the protein (E205, E206, S630, Y662 and Y547) (see Methods) that make non-hydrophobic interactions with the ligand (Table 1).

Table 1. Residues in dipeptidyl peptidase-IV (DPP4) holoenzyme (PDBid:3W2TA) that have non-hydrophobic interactions with the bound DPP4 inhibitor vildagliptin. Interactions are sorted based on the distance. R/A/LA/D: Residue number/Atom of the residue/Atom of ligand/distance between the interacting atoms (in Å). For example, 'S630/OG/N2/2.4' means that the atom OG from Ser630 is at 2.4 Å from the N2 atom of vildagliptin in PDBid:3W2TA.

R/A/LA/D	R/A/LA/D	R/A/LA/D	R/A/LA/D	R/A/LA/D
S630/OG/N2/2.4	E205/OE1/N12/2.8	Y662/OH/O20/3	E206/OE2/N12/3	Y547/OH/N2/3.1

A subset of these atoms might be sufficient to ligand vildagliptin in the target protein. Thus, $\binom{5}{4} = 5$ motifs, each with four atoms, were created using the five closest atoms in the protein. The binding of ligands is known to induce electrostatic and spatial perturbations in the binding site. The spatial and electrostatic perturbations induced by the vildagliptin binding is shown by comparing the apo (PDBid:2OQIA) and the holoenzyme (PDBid:3W2TA) in [Table 2](#). Hence, the electrostatic and spatial profile of the motif were obtained from the apo DPP4 enzyme (PDBid:2OQIA), and then used for querying the PI-PLC apo structure (PDBid:1PTDA).

These motifs were used to query the PI-PLC structure using an enhanced algorithm (PREMONITION) that precompiles all motifs

in a database¹⁶. [Table 3](#) shows the best matches obtained in the PI-PLC structure for the five partial motifs. All these matches have significant electrostatic congruence. The root mean square deviation (RMSD) have low values - however, this is a deceptive metric since these deviations are averaged out. The maximum pairwise distance is another metric to discriminate the spatial congruence, and should be used in combination with the RMSD value. All of these above mentioned matches have significant maximum pairwise distance deviation. Also, three matches do not comprise of active site residues (motifs 2, 3 and 4). However, it can be seen that the first and fifth matches comprises of active site residues (involved in the binding of myo-inositol in PDBid:1PTGA), and have three residues (Asp67, Asp198 and Trp178) in common.

Table 2. Changes in the conformation of the binding site due to vildagliptin (a DPP4 inhibitor) binding. We compare the pairwise distance and electrostatic potential difference (EPD) changes in the apo (PDBid:2OQIA) and holo (PDBid:3W2TA) enzymes. Note that the pairwise distance between these atoms change in the ligand free PDB (2OQIA) as compared to the protein with bound inhibitor (2BUBA). For example, the distance between E205OE2 and E206OE2 (pair ab) changes from 3.9 Å to 5.6 Å. Also, there is a definite change in the EPD between E205OE2 and E205OE2 (pair ab). D = Pairwise distance in Å. PD = Pairwise potential difference. The electrostatic potential are in dimensionless units of kT/e where k is Boltzmann's constant, T is the temperature in K and e is the charge of an electron.

PDB	Active site atoms (a,b,c,d)		ab	ac	ad	bc	bd	cd
2OQIA	GLU205OE2, GLU206OE2, SER630OG, TYR662OH,	D	3.9	7.9	4.0	10.1	3.5	7.3
apo		PD	75.7	-193.4	-94.5	-269.1	-170.2	98.9
3W2TA	GLU205OE2, GLU206OE2, SER630OG, TYR662OH,	D	5.6	9.1	6.1	9.4	5.1	5.6
holo		PD	-74.4	-307.9	-159.8	-233.5	-85.5	148.1

Table 3. Querying PI-PLC using partial motifs derived from the atoms in vildagliptin that make contact to the DPP4 enzyme (PDBid:3W2TA). The comparison is done using apo enzymes (PDBid:1PTDA for PI-PLC and PDBid:2OQIA for DPP4), since the binding of a ligand induces spatial and electrostatic changes in the active site. The fifth motif has the least rmsd deviation, and comprises of active site residues (involved in the binding of myo-inositol in PDBid:1PTGA). Out of the best matches in the other four motifs, three do not comprise of active site residues (motifs 2, 3 and 4). Motif 1 has a reasonably significant match, and has three residues (Asp67, Asp198 and Trp178) in common with the best match for Motif 5. These three residues from PI-PLC (Asp67, Asp198 and Trp178) and corresponding three residues from DPP4 (Glu205, Glu206 and Tyr662) were used to superimpose DPP4 and PI-PLC. N = Motif number. D = Pairwise distance in Å. PD = Pairwise potential difference. Rmsd = Root mean square deviation. Max = maximum pairwise distance deviation. APBS writes out the electrostatic potential in dimensionless units of kT/e where k is Boltzmann's constant, T is the temperature in K and e is the charge of an electron.

PDB	N	Active site atoms(a,b,c,d)		ab	ac	ad	bc	bd	cd	Rmsd/Max (Å)
2OQIA	1	S630OG, E205OE1, Y662CZ, E206OE1	D	9.5	7.8	11.2	7.2	6.6	5.8	0.9/5.1
			PD	237	-104	228	-341	-9	332	
1PTDA		S113OG, D67OD1, W178CZ2, D198OD1	D	4.3	6.7	10.1	6.7	7.7	4.9	
			PD	195	-79	277	-275	81	357	
2OQIA	2	S630OG, E205OE1, Y662CZ, Y547CZ	D	9.5	7.8	5.4	7.2	11.3	9.3	0.8/3.3
			PD	237	-104	-241	-341	-478	-137	
1PTDA		S244OG, D274OD1, Y275CZ, Y246CZ	D	11.3	7.2	8.1	7.0	11.6	5.9	
			PD	312	-104	-171	-417	-484	-67	
2OQIA	3	S630OG, E205OE1, E206OE1, Y547CZ	D	9.5	11.2	5.4	6.6	11.3	10.4	1.3/7.0
			PD	237	228	-241	-9	-478	-469	
1PTDA		S129OG, D126OD1, E125OE1, Y164CZ	D	7.3	12.5	6.7	5.5	12.9	17.4	
			PD	172	262	-232	90	-405	-495	
2OQIA	4	S630OG, Y662CZ, E206OE1, Y547CZ	D	7.8	11.2	5.4	5.8	9.3	10.4	0.8/3.3
			PD	-104	228	-241	332	-137	-469	
1PTDA		S244OG, Y275CZ, D274OD1, Y246CZ	D	7.2	11.3	8.1	7.0	5.9	11.6	
			PD	-104	312	-171	417	-67	-484	
2OQIA	5	E205OE1, Y662CZ, E206OE1, Y547CZ	D	7.2	6.6	11.3	5.8	9.3	10.4	0.7/4.0
			PD	-341	-9	-478	332	-137	-469	
1PTDA		D67OD1, W178CZ2, D198OD1, Y200CZ	D	6.7	7.7	11.6	4.9	9.6	6.3	
			PD	-275	81	-318	357	-42	-400	

Thus, these three residues from PI-PLC (Asp67, Asp198 and Trp178) and corresponding three residues from DPP4 (Glu205, Glu206 and Tyr662) were used to superimpose DPP4 and PI-PLC. Table 4 shows the congruence of these residues. The corresponding holo structures - PDBid:3W2TA for DPP4, and PDBid:1PTGA for PIPLC - were used for the superimposition. This superimposition applies geometric transformations such that Asp67OD1 and Glu205OE2 were at the center of the coordinate axis (coordinates = [0,0,0]), Asp198OD1 and Glu206OE2 lies on the X-Y axis (i.e. Y coordinate is 0) and Tyr662CZ and Trp178CZ2 were on the X-Y plane (i.e. Z coordinate is 0). Figure 1 shows the superimposed proteins. It is observed that (Asp67, Asp198 and Trp178) overlaps well with (Glu205, Glu206 and Tyr662).

These transformations were also applied to the vildagliptin molecule, and this resulted in a docked structure for this molecule into the PI-PLC protein. Figure 2 shows the vildagliptin docked into the PI-PLC structure which is complexed with myo-inositol

(PDBid:1PTGA). The distances of the atoms in vildagliptin and myo-inositol that interact (excluding hydrophobic interactions) to the first ten residues in the PI-PLC structure are shown in Table 5. It is interesting to note that the residues shown in Table 5 are all part of side chain residues in close contact with the myo-inositol ring (shown in Figure 7¹³). Further validation was obtained by observing that both Arg69/NH1 and His32/NE2 interact with atom O4 in vildagliptin, and Arg69/NH2 and His32/NE2 interact with O2 in myo-inositol in the PI-PLC structure. The Pymol script for visualizing the docking (SupplementaryPymol.p1m) and a movie (SupplementaryMovie.avi) are also provided as Supplementary information.

It is important to comment on the previous hypothesis of a nucleophilic serine being responsible for the inhibition of PI-PLC using vildagliptin. The electrostatic and spatial profile of the motif 4 from DPP4 is compared to the electrostatic and spatial profile of matching active site residues in PI-PLC in Table 6, including serine in the

Table 4. Spatial and electrostatic congruence of a three residue partial motif.
The match is significant and comprises active site residues (involved in the binding of myo-inositol in PDBid:1PTGA). Pair 'ab' is considered to be electrostatically congruent since the PD values are close to zero, and can be considered almost equipotential. This is expected for atoms of the same type from the same residue (GLU205OE1/GLU206OE1 and ASP67OD1/ASP198OD1). These three residues from PI-PLC (Asp67, Asp198 and Trp178) and corresponding three residues from DPP4 (Glu205, Glu206 and Tyr662) were used to superimpose DPP4 and PI-PLC. D = Pairwise distance in Å, PD = Pairwise potential difference. APBS writes out the electrostatic potential in dimensionless units of kT/e where k is Boltzmann's constant, T is the temperature in K and e is the charge of an electron.

PDB	Atoms(a,b,c)		ab	ac	bc
2OQIA	GLU205OE1, GLU206OE1, TYR662CZ,	D	6.6	7.2	5.8
		PD	-9.3	-341.5	-332.2
1PTDA	ASP67OD1, ASP198OD1, TRP178CZ2,	D	7.7	6.7	4.9
		PD	81.8	-275.7	-357.6

Figure 1. Superimposing PI-PLC (PDBid:3W2TA in green) and DPP4 (PDBid:2OQOA in cyan). Three residues from PI-PLC (Asp67, Asp198 and Trp178 in yellow) were superimposed to the corresponding three residues from DPP4 (Glu205, Glu206 and Tyr662 in red). Asp67OD1 and Glu205OE2 is at the center of the coordinate axis (coordinates = [0,0,0]) (in black), Asp198OD1 and Glu206OE2 lies on the X-Y axis (i.e. Y coordinate is 0) and Tyr662CZ and Trp178CZ2 are on the X-Y plane (i.e. Z coordinate is 0). Asp67, Asp198 and Trp178 in the PI-PLC protein overlaps well with Glu205, Glu206 and Tyr662 from DPP4, but the Ser234-Ser630 pair is not spatially congruent.

Figure 2. Docking vildagliptin to the PI-PLC structure in complex with myo-inositol (PDBid:1PTGA). It can be seen that vildagliptin fits into the binding site of PI-PLC. It also makes non-hydrophobic contacts to the residues in the protein similar to those made by myo-inositol (Table 5).

Table 5. Atoms of myo-inositol and vildagliptin that make contact to the residues of the PI-PLC structure (PDBid:1PTGA). These interactions exclude hydrophobic interactions, and the first closest ten atoms are chosen. Out of ten, seven residues obtained by docking vildagliptin using DOCLASP are seen to be equivalent to those that are known to bind myo-inositol to the PI-PLC structure¹³, while two more have the same amino acid type (marked by asterisks). Only one pair has a different amino acid type (Tyr200 for myo-inositol and Glu117 for vildagliptin). Further validation is obtained by observing that both Arg69/NH1 and His32/NE2 both interact with atom O4 in vildagliptin, and Arg69/NH2 and His32/NE2 interact with O2 in myo-inositol in the PI-PLC structure.

myo-inositol	vildagliptin	Same?
ASP198/OD1/O3/2.6	ASP198/OD2/N12/3.1	Y
ARG163/NH2/O5/2.7	ARG163/NE/O20/1.1	Y
HIS32/NE2/O2/2.9	HIS32/NE2/O4/4.5	Y
ARG69/NH2/O2/3.3	ARG69/NH1/O4/1.0	Y
LYS115/NZ/O5/3.4	LYS115/NZ/O4/4.9	Y
TRP178/NE1/O4/4.0	TRP178/NE1/N15/2.2	Y
ASP33/OD1/O2/4.1	ASP33/OD1/O4/2.8	Y
SER234/OG/O3/4.8	SER113/OG/O20/5.3	*
ASP180/OD1/O6/4.5	ASP67/OD2/O4/3.8	*
TYR200/OH/O6/4.4	GLU117/OE1/O4/4.1	N

comparison. It can be seen that Ser630 in DPP4 has a significant spatial difference as compared to Ser234 in PI-PLC (pair 'bc' has a difference of 5 Å), and also a reasonable electrostatic difference (pair 'ac' has a difference of 144 PD units). The relatively large distance over which Ser234 in PI-PLC interacts with myo-inositol (4.8 Å) indicates that Ser234 is not directly involved in the binding of the ligand. However, it is responsible for creating the electrostatic milieu that is required for other interacting residues to attain their appropriate potential. Even for DPP4, many inhibitors do not interact with the nucleophilic Ser630 (manuscript in preparation) - although the vildagliptin molecule does²¹. Thus, the previous conjecture of a nucleophilic serine being *directly* responsible for the binding of DPP4 inhibitors to PI-PLC, as implied by the catalytic triad congruence, is incorrect¹³. However, this serine is indirectly responsible for driving the neighboring residues to an appropriate state. Spatial constraints are an additional discriminator.

DOCLASP was also used recently²² to dock human karyopherin to the VP24²³ protein of the Reston Ebola strain using the VP24 from Zaire Ebola²⁴ as a template, and demonstrate that a single mutation might be one of the critical factors responsible for the non-pathogenic nature of Reston Ebola in humans^{25,26}.

Table 6. Potential and spatial congruence of the residues binding vildagliptin in DPP4 structure (PDBid:2OQIA) to the putative binding site in PI-PLC structure (PDBid:1PTDA). Both the structures are apo enzymes, since the binding of a ligand induces spatial and electrostatic changes in the active site. Ser630 in DPP4 has a significant spatial difference as compared to Ser234 in PI-PLC (pair 'bc' has a difference of 5 Å), and also a reasonable electrostatic difference (pair 'ac' has a difference of 144 PD units). D = Pairwise distance in Å. PD = Pairwise potential difference. APBS writes out the electrostatic potential in dimensionless units of kT/e where k is Boltzmann's constant, T is the temperature in K and e is the charge of an electron.

PDB	Active site atoms(a,b,c,d)		ab	ac	ad	bc	bd	cd
2OQIA	GLU205OE1, GLU206OE2, SER630OG, TYR662CZ,	D	5.2	9.5	7.2	10.1	4.2	7.8
		PD	31.7	-237.4	-341.5	-269.1	-373.2	-104.1
1PTDA	ASP67OD1, ASP198OD1, SER234OG, TRP178CZ2,	D	7.7	8.2	6.7	5.7	4.9	9.2
		PD	81.8	-93.7	-275.7	-175.6	-357.6	-182.0

The number of docking methods available is such that even a detailed review could only provide a partial list of currently available docking methods⁹. The current work presents a template based, static method that leverages the spatial and electrostatic properties of the binding site. The definitive advantage of a static method can only be highlighted by emphasizing the known limitations of conformational sampling of the protein structure^{27,28}. The problem is indeed exacerbated by the plasticity of the drug itself^{29–31}. While DOCLASP is completely ineffectual in the absence of such a database, unlike *de novo* methods, it benefits from the burgeoning database of protein-ligand structures³². The conservation of electrostatic properties, extracted using APBS/PDB2PQR, is the strongest argument in favor of DOCLASP.

There are several limitations in the method. Firstly, it can be applied to those compounds which are bound to proteins whose structures have been solved. Additionally, it requires the structure of the apoenzyme, as this is used to extract the query motif considering the structural and electrostatic changes induced by ligand binding. However, with an ever increasing number of protein structures being solved, this is not a severe limitation since most proteins with ligands also have their apo structures solved. Furthermore, the lack

of congruent matches leads DOCLASP to return a null result. This can be overcome by relaxing constraints, for example by checking for spatial congruence only. Finally, it is required to develop an energy function which will be able to discriminate poorly docked structures that have either significant steric clashes or are docked on the surface of the protein.

To summarize, this work presents an implicit method for docking ligands to proteins, in which the search and scoring are implicit in the CLASP algorithm. One significant limitation of this method is the requirement of template protein structures in complex with the given compound. As future work, I intend to incorporate the flexibility of the ligand and protein to add further discrimination.

Competing interests

No competing interests were disclosed.

Grant information

The author(s) declared that no grants were involved in supporting this work.

Supplementary materials

Pymol script for visualizing the docking and movie.

The file “SupplementaryPymol.p1m” contains the Pymol file for viewing vildagliptin docked to PIPLC and the file “SupplementaryMOVie.avi” contains a movie showing a 360 rotation of the ligand docked to PIPLC.

[Click here to access the data.](#)

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Amarda Shehu

Department of Computer Science, George Mason University, Fairfax, VA, USA

This article serves as a proof of concept. It tests the idea, previously published, that if spatial and electrostatic similarities between cognate pairs of residues are predictive of catalytic function, then aligning two proteins to match 4 cognate pairs of residues ought to identify the binding site in a novel/target protein. The alignment process additionally provides the pose of the ligand in the target protein, if the matching is conducted with a holoenzyme.

As a proof of concept, the article is scientifically sound.

Major observations:

1. It would be desirable to provide more testing sets. That is, expand the evaluation to more than binding of vildagliptin to PI-PLC. It is trivial to do so. The authors can use known protein-ligand complex cases, treat them as unknown effectively, and test whether they are able to reproduce the binding pose for the ligand.
2. Expanding the test set will also improve the evaluation of the proposed DOCLASP protocol. The selected case study is presented in detail, but how would one determine the effectiveness of the method at a large scale? What would be summary results on application of DOCLASP on a set of test cases?
3. The author states that a limitation of the method, as a template-based one, is the availability of protein-ligand pairs with known structures. Given the growth in structural data, this is bound to be less of a limitation now as opposed to ten years ago. However, what needs to be discussed is the sensitivity of the method. Is the author reliant on structures with good resolution? What would this be? If an X-ray structure has 2.5Å or lower resolution as opposed to 1.5, should the results be trusted less?
4. Following on above, if two different poses are obtained by matching with two different holoenzymes, and one of the holoenzymes has resolution 2.5 but the other has resolution 1.5, should this be considered in what pose is recommended for the ligand in the binding

protein? What is the reliance of predictions on quality of input?

5. In case of pose discrepancies from diversity of quality of holoenzymes, would energetic minimization help converge to the right pose? This would be an interesting experiment to conduct. In particular, the experiment can suggest that a specific threshold in quality is needed for the minimization to converge to the right pose.

Minor:

1. Consider using a different symbol to denote the number of holoenzymes, M , from the symbol used to denote motif, M . In particular, this can cause confusions regarding equation (5). $P1...PM$ relates to the set of holoenzymes, whereas $M1^{PM}$ relates to best-scoring motif extracted from the last holo-enzyme, PM .
2. Given that there are M holoenzymes, it is unclear whether the method produces M possible poses for the ligand, or if these poses are filtered or joined in some way to provide a super pose.
3. Consider using passive form rather than active "I" form when summarizing future work.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 06 Jun 2016

Sandeep Chakraborty, University of California, Davis, CA, USA

Dear Dr Shehu,

I would like to thank you for taking the time to review this paper, and providing constructive criticism on the overall manuscript. I have revised the manuscript keeping these in mind. Below I outline a point-by-point response to your comments.

This article serves as a proof of concept. It tests the idea, previously published, that if spatial and electrostatic similarities between cognate pairs of residues are predictive of catalytic function, then aligning two proteins to match 4 cognate pairs of residues ought to identify the binding site in a novel/target protein. The alignment process additionally provides the pose of the ligand in the target protein, if the matching is conducted with a holoenzyme. As a proof of concept, the article is scientifically sound.

I greatly appreciate the positive comment.

Major observations: 1. It would be desirable to provide more testing sets. That is, expand the evaluation to more than binding of vildagliptin to PI-PLC. It is trivial to do so. The authors can use known protein-ligand complex cases, treat them as unknown

effectively, and test whether they are able to reproduce the binding pose for the ligand.

Since, DOCLASP uses templates from holoenzymes, re-liganding them back would be trivially correct all the time. However, I have added two testcases. The first docks phenylthiourea to polyphenol oxidases (PPO) from walnut (JrPPO1). In a recent paper on the walnut genome sequence (<http://onlinelibrary.wiley.com/doi/10.1111/tpj.13207/>), DOCLASP was used to dock phenylthiourea to the modelled structure of JrPPO1. Incidentally, the structure of this protein was solved while the walnut genome manuscript was in review (PDBid:5CE9). In the current work, docking phenylthiourea to this structure resulted in almost the same pose. Secondly, a comprehensive analysis of the binding of suramin to different non-homologous proteins from the PDB database has been presented, and DOCLASP was used to dock suramin to a phospholipase A2-like protein. This highlights the non-specific binding of some ligands, and also the complexity of modelling a flexible ligand like suramin.

2. Expanding the test set will also improve the evaluation of the proposed DOCLASP protocol. The selected case study is presented in detail, but how would one determine the effectiveness of the method at a large scale? What would be summary results on application of DOCLASP on a set of test cases?

A large scale analysis is desirable, but difficult for an individual independent researcher. Fortunately, the f1000 allows theoretically infinite revisions. This will be work in progress.

3. The author states that a limitation of the method, as a template-based one, is the availability of protein-ligand pairs with known structures. Given the growth in structural data, this is bound to be less of a limitation now as opposed to ten years ago. However, what needs to be discussed is the sensitivity of the method. Is the author reliant on structures with good resolution? What would this be? If an X-ray structure has 2.5Å or lower resolution as opposed to 1.5, should the results be trusted less? 4. Following on above, if two different poses are obtained by matching with two different holoenzymes, and one of the holoenzymes has resolution 2.5 but the other has resolution 1.5, should this be considered in what pose is recommended for the ligand in the binding protein? What is the reliance of predictions on quality of input?

Empirically, I would assume that the sensitivity of this method would improve with lower resolution, but I do not know how to establish that scientifically. DOCLASP also depends on APBS for computing electrostatic potential, and that is known to improve with lower resolution.

5. In case of pose discrepancies from diversity of quality of holoenzymes, would energetic minimization help converge to the right pose? This would be an interesting experiment to conduct. In particular, the experiment can suggest that a specific threshold in quality is needed for the minimization to converge to the right pose.

Energetic minimization would certainly help in resolving steric clashes. Since there are

several well established methods that can be applied to the output PDB from DOCLASP, I have not discussed these in the current paper.

Minor: 1. Consider using a different symbol to denote the number of holoenzymes, M, from the symbol used to denote motif, M. In particular, this can cause confusions regarding equation (5). P1...PM relates to22 the set of holoenzymes, whereas M1PM relates to best-scoring motif extracted from the last holo-enzyme, PM.

Done (number of holoenzymes is denoted by Z).

2. Given that there are M holoenzymes, it is unclear whether the method produces M possible poses for the ligand, or if these poses are filtered or joined in some way to provide a super pose.

If there are multiple holoenzymes, DOCLASP will generate a pose for each (assuming there is a significant match of the motif from the holoenzyme). Currently, there is no method to create a single 'super pose' from these.

3. Consider using passive form rather than active "I" form when summarizing future work.

Done.

I appreciate your consideration of the revised manuscript, and look forward to hearing back from you.

best wishes,
Sandeep

Competing Interests: No competing interests were disclosed.

Reviewer Response 20 Jun 2016

Amarda Shehu, George Mason University, Fairfax, USA

I will be keen to see the progress of this work, particularly with regards to an integrative setting that considers various structural models and further interface with energetic minimization protocols. I do sympathize with the comment on an independent researcher aiming to share research progress in spite of large-scale evaluation settings. F1000 is the right venue for these types of dissemination.

Competing Interests: No competing interests were disclosed.

Reviewer Report 20 January 2015

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Rahul Banerjee

Crystallography and Molecular Biology Division, Saha Institute of Nuclear Physics, Kolkata, India

The author has presented a novel docking algorithm based on the principle that spatial and electrostatic properties in cognate pairs of residues are conserved in the catalytic site of proteins endowed with the same function. Thus, in case the crystal structure of a protein-ligand complex is available, it can be used to dock the same ligand into the active site of a homologous protein (with identical catalytic activity). The author has tested the algorithm by docking vildagliptin into the active site of PI-PLC.

1. Examination of Figure 2 and Table 5 show quite a few short contacts between the docked ligand and amino acid chains. Under such circumstances the author could consider the inclusion of an energy minimization protocol to relieve the short contacts.
2. The author considers the method validated by the above mentioned docking exercise. However, the author could consider a case where the actual crystal structure of the solution exists and the rmsd between the experimental solution and the solution obtained by DOCLASP could be provided to give some estimate of the accuracy of the docked poses.

Given the fact that the DOCLASP solution abounds in short contacts, further improvements could be made in the pose.

3. On page 3, two lines below Equation 4, a more explicit account of CScore could be given in the manuscript.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 06 Jun 2016

Sandeep Chakraborty, University of California, Davis, CA, USA

Dear Dr Banerjee,

I would like to thank you for taking the time to review this paper, and for your positive comments. I also apologize for the time taken to respond to your comments, a delay that took place due to the time taken to find another reviewer. Please find my detailed responses to your comments below.

The author has presented a novel docking algorithm based on the principle that spatial and electrostatic properties in cognate pairs of residues are conserved in the

catalytic site of proteins endowed with the same function. Thus, in case the crystal structure of a protein-ligand complex is available, it can be used to dock the same ligand into the active site of a homologous protein (with identical catalytic activity). The author has tested the algorithm by docking vildagliptin into the active site of PI-PLC. 1. Examination of Figure 2 and Table 5 show quite a few short contacts between the docked ligand and amino acid chains. Under such circumstances the author could consider the inclusion of an energy minimization protocol to relieve the short contacts. 2. The author considers the method validated by the above mentioned docking exercise. However, the author could consider a case where the actual crystal structure of the solution exists and the RMSD between the experimental solution and the solution obtained by DOCLASP could be provided to give some estimate of the accuracy of the docked poses. Given the fact that the DOCLASP solution abounds in short contacts, further improvements could be made in the pose.

Since, DOCLASP uses templates from holoenzymes, re-liganding them back would be trivially correct all the time. Energy minimizations would certainly help in resolving the steric constraints. Such methods are standard, and can be applied to the output PDB of DOCLASP, but have not been done in the current version. I will consider including this in the future. Instead, I have included two additional test cases, DOCLASP was previously applied to the binding of phenylthiourea to polyphenol oxidases (PPO) from walnut (JrPPO1) (<http://onlinelibrary.wiley.com/doi/10.1111/tpj.13207/>). Incidentally, the structure of this protein was solved while the walnut genome manuscript was in review (PDBid:5CE9). Here, phenylthiourea was docked to this solved structure, resulting in almost the same pose as the one in which DOCLASP docked phenylthiourea to the SWISSMODEL-modelled structure of JrPPO1. Secondly, a comprehensive analysis of the binding of suramin to different non-homologous proteins from the PDB database has been presented, and DOCLASP was used to dock suramin to a phospholipase A2-like protein. This highlights the non-specific binding of some ligands, and also the complexity of modelling a flexible ligand like suramin.

3. On page 3, two lines below Equation 4, a more explicit account of CScore could be given in the manuscript.

I have modified the methods section to give a more explicit explanation of CScore.

I appreciate your consideration of the revised manuscript, and look forward to hearing back from you.

best wishes,
Sandeep

Competing Interests: No competing interests were disclosed.

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