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GENERATION OF A DATASET FOR STUDYING LIGAND EFFECT ON HOMODIMER INTERFACE

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TABLE OF CONTENTS

1. ABSTRACT

Protein dimer interfaces (homodimer – same polypeptide and heterodimer – different polypeptide) display geometric and chemical properties that give the non-covalent assembly its stability and specificity. Therefore, it is important to understand the molecular principles of dimer interaction. Several studies on homodimer interaction are available. However, a study on the effect of ligands (i.e. non-peptide compounds) on subunit interactions is not available. Hence, we generated a dataset of 62 identical homodimer pairs (one structure determined with an interface ligand and the other without an interface ligand) and analyzed the effect of interface ligands on dimer interface. The analysis suggests that homodimer interfaces having ligands are less hydrophobic with small interface area compared to those without ligands. We also found that ligands occupying ≤ 7% interface area have negligible effect on dimer interaction.

2. INTRODUCTION

Homodimers are the simplest examples of non-covalent self-assembly. The homodimer interface, defined as protein surface involved in subunit contacts, has been widely examined to unravel the principles of protein-protein complexations (1). Analysis of homodimer structures has provided reasonable insight to the molecular principles of homodimer interaction (1-5). These studies deal with protein component of the interfaces and disregard ligand components (i.e. non-peptide compounds, containing biological and non-biological components). Therefore, the effects of interface ligands on homodimer interfaces are not known.

Nooren and Thornton, studied transient state protein-protein interactions using complexes without interface ligands (6). Likewise, Bahadur and colleagues discarded homodimers with ligands occupying more than

5% (a randomly chosen cut-off value) of interface area for homodimer analysis (7). Therefore, it is of interest to standardize the "ligand interface area cut-off value" (LIACV) for discarding homodimers with ligands during homodimer analysis. We created a dataset consisting of 62 identical homodimer pairs (IDP), where one dimer structure was determined with interface ligands (target dimer) and the other without interface ligands (reference dimer). In this article, we describe a rationale to select homodimer structures with interface ligands for studying the effect of ligands on homodimer interaction.

3. MATERIALS AND METHODS

3.1. Dataset creation

We selected 1324 high resolution (≤2.5Å) homodimers (each monomer is >100 residues) using the information in biological unit record from Protein databank (PDB) (8). The dataset was subject to rigorous analysis for the creation of an IDP dataset using a methodology (Figure 1) described below.

Step 1: Identification of homodimers with and without ligands.

Interface area (2) was calculated twice for 1324 homodimer structures using NACCESS (an accessible surface area calculation program) with and without ligands (toggle –H option in NACCESS) (9). The interface area calculated with ligands is designated as B_L ($Å^2$) and the interface area calculated without ligands is designated as B ($Å^2$). Hence, we defined L, ligand occupation to interface area, using the following equation:

$$L\ (\%) = (B - B_L)/B \quad (1)$$

Based on the value of L, homodimers were clustered into two categories (< 1 and ≥ 1). Homodimers with L < 1 are considered as those without interface ligands (or their effect on interface is negligible). Homodimers with L ≥ 1 are considered as those with interface ligands. In this dataset (Figure 2), we identified 698 homodimers with L < 1 (designated thereafter as HDL^-) and 626 homodimers with L ≥ 1 (designated thereafter as HDL^+). Thus, two subsets of HDL^- and HDL^+ were created.

Step 2: Identification of IDPs

The 1324 homodimer sequences were clustered using HSSP (homology derived secondary structures of proteins) at ≥70 cut-off value (10). This procedure created 585 clusters (each cluster containing highly homologous sequences), where 321 clusters contained at least two sequences each. The entries in each of these 321 clusters were compared with HDL+ and HDL- group members using PDB ID. Subsequently, the clusters were further grouped into three categories, namely (1) category I (154 clusters consisting of at least one member from each HDL+ and HDL-), (2) category II (65 clusters consisting of all members from HDL-) and (3) category III (102 clusters consisting of all members from HDL+). We used category I members (154 clusters) and created 483 identical pairs in all combinations, such that each pair consists of one member of HDL^+ and HDL^- from the same cluster. We further divided these pairs into 9 groups based on the value of L ranging from 1 to 10 with a step size of 1 as shown in Figure 1. In this grouping, dimers with L > 10 in HDL^+ group do not have an identical dimer pair in HDL^- group and are therefore discarded. Then the redundant pairs in each group were removed with an HSSP cutoff value of ≤ 5**.** We thus created 69 IDPs consisting of one member from HDL^+ and the other from HDL^- (Figure 1). The HDL^+ member in each IDP is thereafter, called the target dimer and HDL^- member in each IDP is thereafter, called the reference dimer. Subsequently, we calculated the difference of interface areas (ΔB) between reference and target dimer of each IDP using the equation:

$$\Delta B = (B_r - B_t)/B_r \quad (2)$$

where B_r is the interface area for reference dimer and B_t is that of the target dimer. It is found that most IDP pairs have ΔB values in the range of -6 to +16%. However, 7 pairs have ΔB values in the range of -18% to -200% and these target dimers have significantly larger interface area than reference dimers (due to conformational flexibility). Hence, these 7 pairs were eliminated, resulting in 62 IDP for further analysis (Table 1).

3.2. Determination of LIACV

It is known that protein interfaces are affected by factors (e.g. surface ligands and the conformational flexibility of interacting surfaces forming the interface) other than interface ligands. The effect of these factors on interface is considered as background noise in this study. We used category II members consisting of 65 HDL- clusters to define IDPBN (IDP for background noise) structures (Figure 1). The redundant pairs were removed at a HSSP cutoff value of ≤ 5. Thus, we created a non-redundant dataset consisting of 25 IDPBN (Table 2). The selected pairs consist of one dimer with the smallest interface area and the other with the largest interface area from each cluster. The interface properties of IDP members were compared with the IDPBN to determine LIACV.

4. RESULTS

4.1. Effect of interface ligands on interface area

The effect of interface ligands on interface area is studied by measuring L and ΔB in 62 IDPs (Figure 3). Figure 3 shows that ΔB range from -6% to 16%. The interface area of reference dimers is predominantly larger than target dimers and their differences (%ΔB) increase with ligand occupation (%L) to interface area with a weak correlation co-efficient (R = 0.36). The mean value of ΔB (Figure 4a) and standard deviation (SD) about the mean value of ΔB (Figure 4b) are shown for each of the 9 IDP groups clustered based on the value of L. Figure 4a shows that mean ΔB increases with L and it is significantly large for IDP groups 7 - 9. The mean for groups 1 - 6 is less than the mean for IDPBN. SD about mean ΔB is consistently small for IDP groups 1-7 and SD is high for groups 8 and 9 (Figure 4b). These SD values are generally larger than the SD in IDPBN.

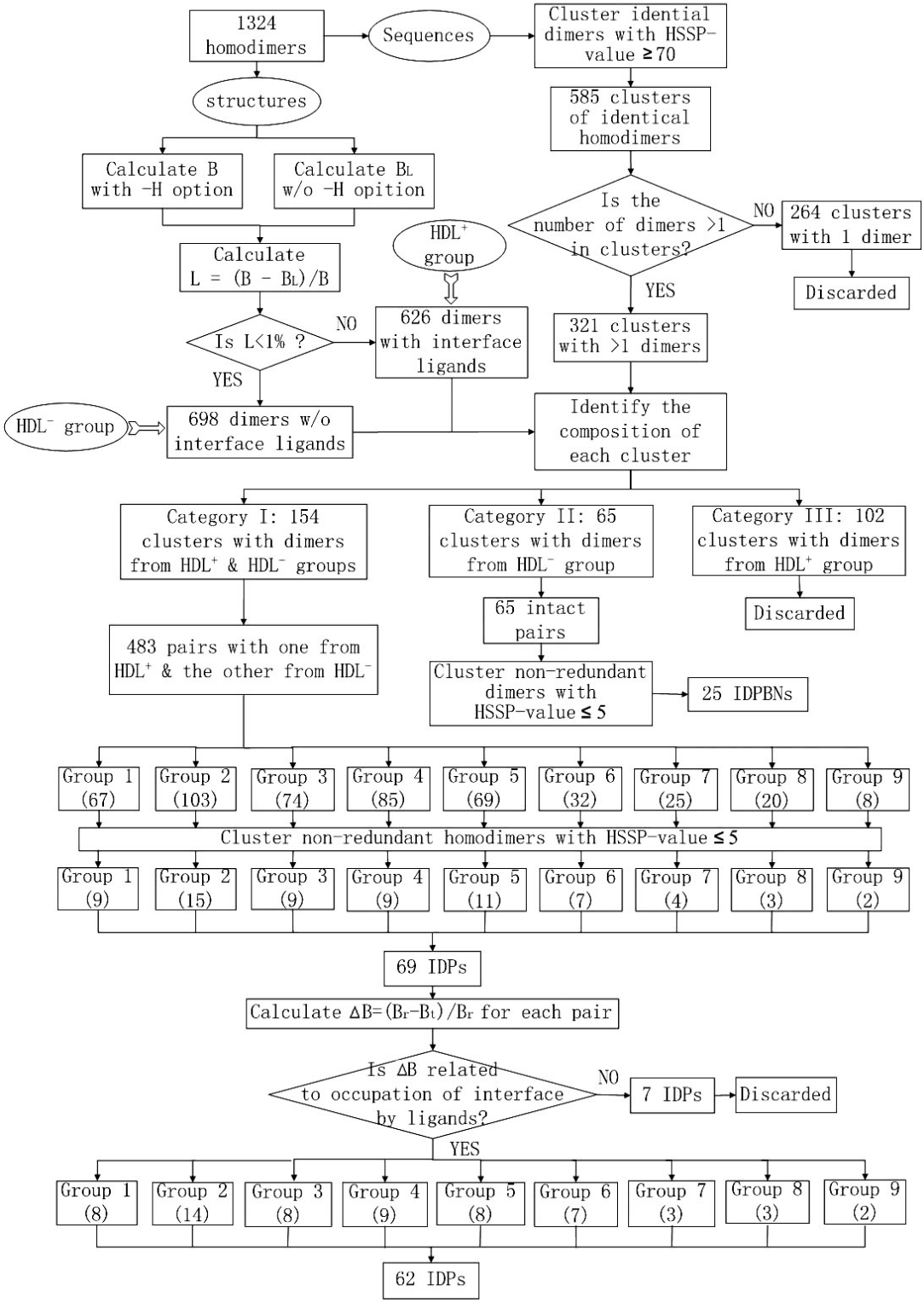

Figure 1. The flowchart describing the creation of 62 IDPs (identical homodimer pairs) is shown. 698 homodimers without interface ligands are designated as HDL^- group and 626 homodimers with interface ligands are designated as HDL^+ group. IDPBN refers to identical homodimer pairs as background noise. B_r refers to the interface area of the reference dimer (from HDL^- group) and B_t refers to that of the target dimer (from HDL^+ group). ΔB is the difference of interface areas between reference and target dimers of each IDP.

Table 1. Identical homodimer pairs (IDP) of reference and target structures

Protein name	Reference		Target		
IDP group 1 (8 pairs)	PDB ID	B(Å²)	PDB ID	B(Å²)	Interface ligand names
Autocrine Motility Factor	1IRICD	12884	1IRIAB	12952	erythose-4-phosphate
Transthyretin	1F41	1782	1E4H	1749	glycerol[1]
Recombination Endonuclease VII	1EN7	6604	1E7D	6845	zinc ion
Neopullulanase	1J0I	5552	1J0K	5256	isopanose
Triosephosphate Isomerase	1R2RAB	3414	1R2RCD	3411	2-amino-2-hydroxymethyl-propane-1,3-diol
Fructose 1,6-Bisphosphatase/Inositol Monopho	1LBV	2387	1LBX	2401	d-myo-inositol-1-phosphate
Glucosamine-Phosphate N-Acetyltransferase	1I12BD	5734	1I1DBC	5807	n-acetyl-d-glucosamine-6-phosphate
Siroheme Synthase	1PJT	12307	1PJQ	12337	phosphoserine
IDP group 2 (14 pairs)					
Recombination Endonuclease VII	1EN7	6604	1E7L	6702	zinc ion & sulfate ion[1]
Triosephosphate Isomerase	1TCD	2967	1CI1	3036	Hexane
ADP-Ribose Pyrophosphatase	1G0S	7678	1KHZ	7147	α-β methylene adp-ribose
Transthyretin	1F41	1782	1BM7	1748	2-[[3-(trifluoromethyl)phenyl]amino] benzoic acid
L-3-Hydroxyacyl-Coa Dehydrogenase	1F14	3223	1F12	3266	3-hydroxybutanoyl-coenzyme a
Neopullulanase	1J0I	5552	1J0J	5235	Maltotetraose
Quinolinate Acid Phosphoribosyl Transferase	1QPOAB	4843	1QPRAB	5146	phthalic acid
D-Amino Acid Aminotransferase	2DAB	4617	1G2W	4553	acetate ion
Hiv-1 Integrase	1BIS	2993	1EXQ	3090	sulfate ion[1]
Cyanovirin-N	1L5E	6543	1L5B	6306	2-[n-cyclohexylamino]ethane sulfonic acid[1] & sodium ion[1]
Beta-Lactamase Oxa-10	1E3UBD	2553	1E4DAC	2636	lysine nz-carboxylic acid & sulfate ion[1]
Siroheme Synthase	1PJT	12307	1PJS	12414	nicotinamide-adenine-dinucleotide & phosphate ion[1]
Orotidine Monophosphate Decarboxylase	1LP6	1595	1LOL	1570	xanthosine-5'-monophosphate
Branched-Chain Amino Acid Aminotransferase	1EKV	4679	1KT8	4720	L-isoleucine
IDP group 3 (8 pairs)					
Glutathione S-Transferase	1EOG	2369	1PX6	2417	gluthathione & 2-(n-morpholino)-ethanesulfonic acid
Fructose 1,6-Bisphosphatase/Inositol Monopho	1LBV	2387	1LBY	2302	fructose-6-phosphate
Quinolinate Acid Phosphoribosyl Transferase	1QPOAB	4843	1QPREF	5138	phthalic Acid
Glucosamine-Phosphate N-Acetyltransferase	1I12BD	5734	1I1DAD	5843	n-acetyl-d-glucosamine-6-phosphate
Alcohol Dehydrogenase E Chain	1HEU	3306	1QV6	3397	nicotinamide-adenine-dinucleotide
Phosphotriesterase	1EZ2	2741	1I0D	2599	ethylene glycol[1]
Probable Fosfomycin Resistance Protein	1NNR	5177	1LQO	5211	thallium ion[1]
Nitric Oxide Synthase	1ED6	5595	1DMJ	5610	5,6-Cyclic-Tetrahydropteridine
IDP group 4 (9 pairs)					
ADP-Ribose Pyrophosphatase	1G0S	7678	1G9Q	7600	ADP-ribose
Beta-Lactamase Oxa-10	1E3UBD	2553	1E4DBD	2650	sulfate ion[1]
Fructose 1,6-Bisphosphatase/Inositol Monopho	1LBV	2387	1LBZ	2258	fructose-1,6 bisphosphate
Beta-Ketoacyl Synthas	1DD8AB	5784	1EK4AB	5788	lauric acid
Ribonuclease, Seminal	1R5D	3784	1R5C	3827	2'-deoxycitidylyl(3',5')-2'-deoxyadenosine
Cyanovirin-N	1L5E	6543	1M5J	6341	a-d-mannose
Orotidine Monophosphate Decarboxylase	1LP6	5595	1LOSAB	5575	6-azauridine 5'-monophosphate
Alcohol Dehydrogenase E Chain	1HEU	3306	1HSO	3295	nicotinamide-adenine-dinucleotide
Nitric Oxide Synthase	1ED6	3161	1D1X	3061	(6r,1'r,2's)-5,6,7,8 tetrahydrobiopterin
IDP group 5 (8 pairs)					
Beta-Lactamase Oxa-10	1E3UBD	2553	1E3UAC	2597	1,2-ethanediol[1]
Fructose 1,6-Bisphosphatase/Inositol Monopho	1LBV	2387	1G0I	2290	1,2,3,4,5,6-hexahydroxy-cyclohexane
L-3-Hydroxyacyl-Coa Dehydrogenase	1F14	3223	1F0Y	3235	acetoacetyl-coenzyme a
Cyanovirin-N	1L5E	6543	1M5M	6315	α -d-mannose & 2-[n-cyclohexylamino]ethane sulfonic acid
Transthyretin	1F41	1782	1IIK	1710	β-mercaptoethanol[1]
Glutathione S-Transferase A1	1K3O	2860	1K3L	2956	s-hexylglutathione
Phosphotriesterase	1EZ2	2741	1I0B	2595	ethylene glycol & sodium ion
Aspartate 1-Decarboxylase	1PPY	1790	1PQF	1739	s-oxy cysteine
IDP group 6 (7 pairs)					
Thymidylate Synthase	1F28CD	4104	1F28AB	4113	2'-deoxyuridine 5'-monophosphate
Transthyretin	1F41	1782	1III	1713	β-mercaptoethanol[1]
Undecaprenyl Pyrophosphate Synthetase	1V7U	3531	1UEH	3529	magnesium ion
Glutathione S-Transferase A1	1K3O	2860	1ML6	2910	mgsta2-2 Toward benzo[A]Pyrene-(7R,8S)-diol-(9S,10R)-epoxide
Glucosamine-Phosphate N-Acetyltransferase	1I12AC	5569	1I21AB	5240	Selenomethionine[1]
Protein Maf	1EX2	1078	1EXC	1048	deoxyuridine-5'-triphosphate
Dtdp-4-Dehydrorhamnose 3,5-Epimerase	1DZR	2773	1DZT	2797	3'-o-acetylthymidine-5'-diphosphate & glycerol[1]
IDP group 7 (3 pairs)					
Transthyretin	1F41	1782	1IJN	1712	β-mercaptoethanol[1]
Glutathione S-Transferase A1	1K3O	2860	1K3Y	2845	s-hexylglutathione
Glucosamine-Phosphate N-Acetyltransferase	1I12BD	5734	1I21AB	5240	Selenomethionine[1]
IDP group 8 (3 pairs)					
Capsid Protein P40: Assemblin Protease	1IED	3059	1WPO	2729	Selenomethionine[1]
Aspartate 1-Decarboxylase	1PPY	1790	1PT1	1905	sulfate anion
Glucosamine-Phosphate N-Acetyltransferase	1I12BD	5734	1I21XY	5335	Selenomethionine[1]
IDP group 9 (2 pairs)					
Cytosine Deaminase	1P6O	2944	1OX7	2480	Selenomethionine[1]
Aspartate 1-Decarboxylase	1PPY	1790	1PQH	1810	malonic acid & s-oxy cysteine

B = interface area; [1] indicates non-biological ligand

4.2. Effect of interface ligands on interface hydrophobicity

It is our interest to study the effect of interface ligands on interface hydrophobicity. This is done by calculating the properties of interface chemical groups. These groups are categorized as non-polar (carbon atoms) and polar (nitrogen, oxygen and sulphur atoms) groups. Table 3 shows the non-polar fractions of interface area in target and reference dimers for each IDP group. On average, the non-polar fraction is greater in reference structures than target structures. This suggests that hydrophobic interface in target structures are disturbed by interface ligands. The differences between them are 0.7 in groups 1-3, 0.1 in groups 4-6 and above 1.7 in groups 7-9. These differences are generally greater than the differences (0.3) in IDPBN structures. Thus, the effect of interface ligands on non-polar fractions of interface dominates over the other factors. These observations indicate that the dimer interface with ligands is less hydrophobic than that without interface ligands and the distinction increases with ligands occupation.

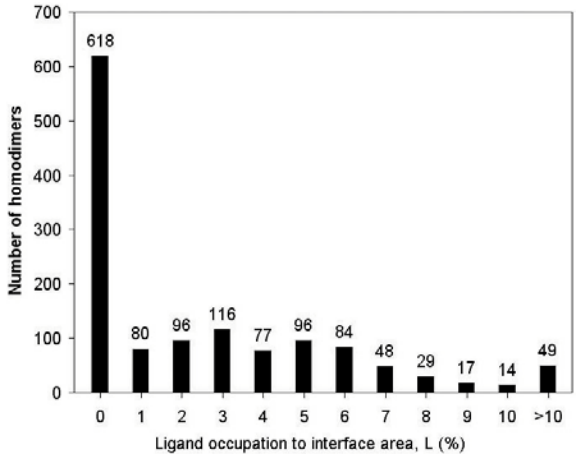

Figure 2. Distribution of 1324 homodimers based on %L, a measure of ligand occupation to interface area.

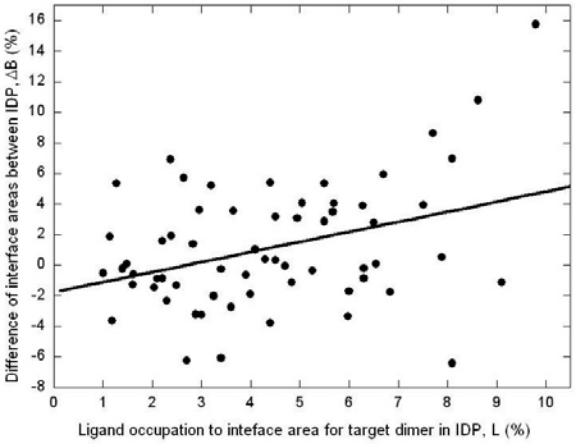

Figure 3. The relationship between ΔB and %L is shown for 62 IDP. The correlation co-efficient r is 0.36.

4.3. Effect of interface ligands on interface residue composition

The effect of interface ligands was studied on interface residue composition. The similarity between the residue composition of target/reference interfaces in each IDP group was quantified by calculating their Euclidean distance Δf (11):

$$(\Delta f)^2 = 1/19 \sum_{i=1}^{20} \left(f_i - f_i^{'} \right)^2 \quad (3)$$

where f_i and $f_i^{'}$ are the percent contribution of residue type i to the interface area of target and reference dimers, respectively. Figure 5 shows that Δf is < 0.2 for groups 1-4, 0.45 for groups 5-6 and > 0.9 for groups 7-9. This suggests that Δf increases with L. It also shows that the Δf for groups 4-9 are greater than background noise in IDPBN.

5. DISCUSSION

Protein homodimers are structural assemblies formed by the association of identical monomers to create active centers for biological reactions. Therefore, it was of great interest to understand the principles of homodimer association. These associations are generally governed by weak interactions. Several efforts have been made to study homodimer interaction using structural data and many structural features are found important (1-7). The Protein databank also contains many homodimers containing small molecule ligands at the subunit interface (Figure 2). These ligands contribute to subunit association by both positive and negative effect. However, the effect on ligand on interface properties has been neglected when %L is <5% in previous studies (9,11). The 5% cut-off was randomly selected in these studies. Therefore, it is our interest to standardize the cut-off value of %L to neglect ligand effect on interface. This feature is called the "ligand interface area cut-off value" (LIACV). We created a dataset of 62 identical homodimer pairs, designated as IDP (Table 1). Each pair consists of a homodimer with interface ligands and a homodimer without interface ligands. We also created a dataset of homodimer pairs, designated as IDPBN, to determine background noise (Table 2). Each pair in this dataset consist of identical homodimers with varying interface conformational flexibility, such that one homodimer has large interface area and other has small interface area. It should also be noted that homodimer pairs in IDPBN do not contain interface ligands, yet exhibit flexible interface conformation. These datasets were created from an initial set of 1324 homodimers using the methodology described in the methods section (Figure 1). These two datasets were independently studied using parameters, such as (1) interface area, (2) interface hydrophobicity, and (3) interface residue composition. These interface properties were also studied for increasing values of %L, a measure of ligand occupation at the homodimer interface. The mean change in interface area between homodimers with (target dimers) and without (reference dimers) interface ligands (ΔB) is less than the background noise (2.3) when %L is less than 7% (Figure 4a). The SD about the mean difference is also small when %L is less than 7% (Figure 4b). It should be noted that the SD about the mean is also large with a larger mean ΔB when %L is ≥ 7%. It is also found that most IDP groups showed a change in interface non-polar fraction larger than the background noise of 0.2 (Table 3). However, IDP groups with %L ≥ 7% showed significant difference compared to the background noise. The Euclidean distance (Δf) interface residue composition between target and reference structures is also found to be larger than background noise when %L is ≥ 4% (Figure 5). The difference of hydrogen bond between target and reference structures is not related to the increase in %L (Figure 6). Thus, we show that the difference between target and reference homodimer interface properties is less than the background noise when %L < 7%. Therefore, the effect of interface ligand on interface properties such as (1) interface area, (2) interface hydrophobicity, and (3) interface residue composition is negligible when %L is < 7%.

Table 2. 25 IDPBN set

PDB entry	Protein	B(Å^2)
1BIZ / 1BIS	Hiv-1 Integrase	2841 / 2992
1CZ3 / 1D1G	Dihydrofolate Reductase	3011 / 3127
1D8IBC / 1D8HAB	Mrna Triphosphatase Cet1	4089 / 4307
1DAP / 3DAP	Diaminopimelic Acid Dehydrogenase	5267 / 5319
1DD8AB / 1DD8CD	β-Ketoacyl [Acyl Carrier Protein] Synthase I	5784 / 5839
1E3F / 1F41	Transthyretin	1709 / 1782
1EBH / 2ONE	Enolase	3575 / 3703
1I0S / 1I0R	Conserved Hypothetical Protein	4373 / 4376
1IED / 1IEG	Capsid Protein P40: Assemblin Protease	3059 / 3067
1IRICD / 1JIQCD	Autocrine Motility Factor	12884 / 12949
1LBW / 1LBV	Fructose 1,6-Bisphosphatase/Inositol Monophosphatase	2323 / 2387
1MI3AB / 1K8CAB	Xylose Reductase	2407 / 2419
1MVP / 2RSP	Rous Sarcoma Virus Protease	2915 / 2982
1OBU / 1OBQ	Crustacyanin C1 Subunit	2209 / 2241
1P5R / 1P5H	Formyl-Coenzyme A Transferase	12214 / 12396
1Q1E / 1Q1BAB	Maltose/Maltodextrin Transport ATP-Binding Protein Malk	2161 / 2290
1QRK / 1GGY	Coagulation Factor Xiii	4246 / 4423
1R2SAB / 1R2T	Triosephosphate Isomerase	3404 / 3435
1P18 / 1P19AB	Hypoxanthine Phosphoribosyltransferase	3300 / 3327
1SES / 1SET	Seryl-tRNA Synthetase	4466 / 4536
1SLCAB / 1SLBAB	Galectin-1 (S-Lectin)	1059 / 1111
1SMN / 1QL0	Nuclease	1735 / 1782
1UQ5 / 1UQ4	Ricin	1828 / 1892
1URA / 1ALK	Alkaline Phosphatase	7565 / 7665
1V26 / 1V25	Long-Chain-Fatty-Acid-Coa Synthetase	5726 / 5728
2NAD / 2NAC	Nad-Dependent Formate Dehydrogenase	7590 / 7599

The interface areas for IDPBN pairs are separated by a forward slash.

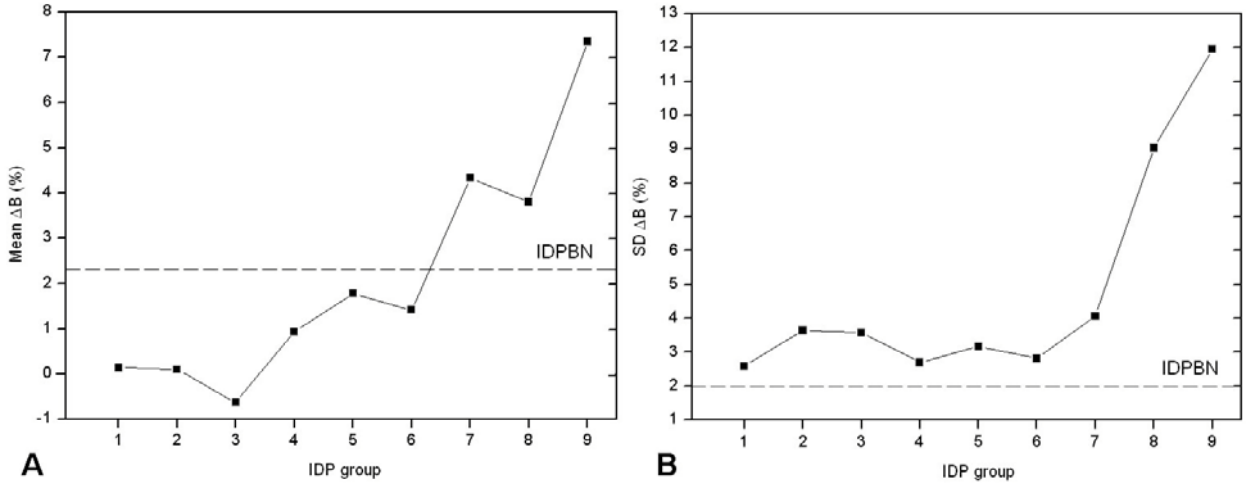

Figure 4. A: The mean % ΔB (difference between target and reference structures) for each IDP group is shown. The dash line through 2.3 is a measure of background noise. B: The standard deviation (SD) about the mean ΔB of each IDP group is given. The SD value for IDPBN set is 1.9 (dash line).

Table 3. Non-polar fractions of interface area in IDP groups

Group Name	Reference (%)	Target (%)	Difference (%)[1]
IDP group 1	64.6	64.0	0.6
IDP group 2	66.3	65.5	0.8
IDP group 3	65.2	64.6	0.6
IDP group 4	64.9	65.1	-0.2
IDP group 5	64.9	64.8	0.1
IDP group 6	62.0	61.7	0.3
IDP group 7	64.4	62.7	1.7
IDP group 8	65.1	61.5	3.6
IDP group 9	57.5	54.9	2.6

[1] Difference between reference and target structures

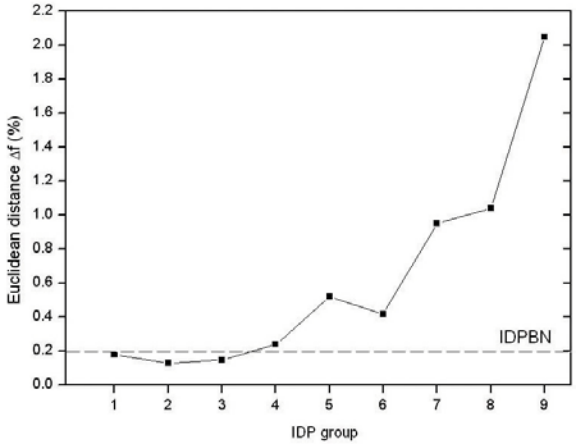

Figure 5. The Euclidean distance Δf between target and reference dimers for each IDP group is shown. The dash line through 0.2 is a measure of background noise.

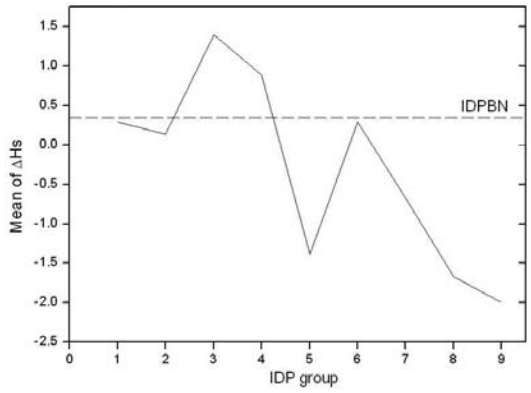

Figure 6. The difference in interface hydrogen bonds (ΔH) between target and reference structure is shown for each IDP group. The hydrogen bonding between subunits was calculated using HBPLUS (12). Data suggest no significant correlation between ΔH and %L.

6. CONCLUSIONS

The challenge in understanding the principles of protein dimer associations are multifaceted. Dimer associations are either homo/hetero dimer in nature. This association is critical in the formation of active reaction centers. The active centers are important for substrate binding. Therefore, it is essential to study disturbances caused by substrate binding to dimer interfaces. Here, we created a dataset of 62 identical homodimer pairs consisting of one dimer with a bound interface ligand and the other without any interface ligand. Analysis of this dataset suggests that bound ligands significantly affect homodimer interfaces when the ligands occupy ≥ 7% of interface area. In previous studies, the effect of ligand on interface was neglected at a random cut-off of 5%. This study shows that ligand effect on homodimer interface can be neglected if ligands occupy <7% of interface area. It should be noted that this observation is based on a dataset of 62 identical homodimer pairs.

7. ACKNOWLEGEEMNTS

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Abbreviations: HSSP, homology derived structures of proteins; IDP, identical homodimer pair; IDPBN, identical homodimer pair as background noise; PDB, protein data bank

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