Betatrophin/Lipasin/C19orf80: In Silico Approach for Protein-Based Biomaterial Marker in Metabolic Syndrome and Colorectal Cancer Through Computational-Based Study

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Abstract. This computational material-based study aimed to provide a comprehensive data with the molecular interaction between betatrophin and its target as the confirmation of the primary role of betatrophin on serological lipid profile alteration and metabolic syndrome progression. We analyzed the binding behavior of betatrophin and LPL using docking model. The underlying molecular interaction between betatrophin and lipoprotein lipase (LPL) binding site results in the inhibitory activity of betatrophin on LPL. Our in Silico data showed that betatrophin could regulate that essential enzyme and suggested indirectly to restrict TG level during the circadian cycle in the human circulation. To sum up, betatrophin or RIFL shows as a promising future early biomarker for obesity and cancer linked metabolic syndrome. Hence, this preliminary computational material analysis data provided a hallmark for the future development of betatrophin as the clinical biomaterial marker in metabolic syndrome and gastrointestinal cancer.

Keywords: Betatrophin, In Silico, biomaterial, marker, metabolic syndrome, colorectal cancer

1. Introduction
Metabolic syndrome is the combination of several metabolic perturbations including dyslipidemia, glucose intolerance, abdominal obesity, elevated blood pressure (BP) that can leads to type 2 diabetes mellitus (T2DM), stroke, vascular disease, and other definite types of cancers [1-3]. Lifestyles nowadays tend to consume simple carbohydrates, and high-fat diet in almost everyday; the low-physical activity, alcohol consumption, smoking, and insufficient sleep contribute to high rates of this evidence [4-5]. Recent studies tend to find an early potential biomarker to prevent thee cases, and the result showed that the serological biomarker is elected as the most used clinical approach for diagnosis. That is why
conducting studies about serological biomarker of metabolic syndrome is promising for cancer treatments [6-8].

Betatrophin is a liver and adipose tissue derived hormone that is also referred as lipasin, angiopoietin-like protein 8 (ANGPTL8), TD26, chromosome 19 open reading frame 80 (C19orf80), and re-feeding induced fat and liver (RIFL) has been known to be correlated in triglyceride (TG) and glucose metabolism [9-11]. The previous study showed that betatrophin implicated on lipid and glucose metabolism of mice either proliferation of pancreatic beta-cells [12-13]. Betatrophin protein is distributed into most tissues through circulation; it will be highly expressed by feeding and suppressed by fasting [14]. After refeeding, fatty acids from triglyceride (TG)-rich lipoproteins will fulfill adipose tissue, then inside of adipose tissues fatty acids cleaved from TG-lipoprotein by lipoprotein lipase enzyme (LPL) [15]. Fasting effectively reduces LPL activity and TG-fatty acids transferred into heart and skeletal muscle for oxidation process [10,15]. Betatrophin and the other two protein angiopoietin-like such as ANGPTL3 and ANGPTL4 act together to inhibit LPL activity by releasing its N-terminal domain and increasing plasma TG levels [16]. The previous study revealed that patients with T2DM witnessed a higher level of serum betatrophin [17] and in obese MetS patients, compared to subjects with healthy individual and normal glucose tolerance [18].

Compared to those wet laboratory results, till now computational-based study or in silico approach to the revealed molecular interaction of Betatrophin remain unclear. The ‘real’ synthesis and screening of compound in wet laboratory methods are mostly pricey, complicated, and need much accuracy, so otherwise alternative methods to replace in vitro studies are necessary. In silico methods are widely used to represent virtual word data analysis, hypothesis, models, and design. The need of concrete research or bioassay would be completed by examining the basic concept through computational screens and models [19-21].

2. Methods
The protein sequences of Betatrophin (ANGPTL8) and Lipoprotein lipase (LPL) were obtained from the database on www.uniprot.org, and the ID is Q6UXH0 and P06858. Both of sequences were modeled to a 3D structure using the homology modeling method on Swiss Model web-server, to comparing the query sequences with the templates located on the database and resulted in homologous model formation [22-26]. The structure was validated by RAMPAGE server (mordred.bioc.cam.ac.uk/~rapper/rampage.php) [27], then obtaining the 3D structure of each of these proteins to the possible molecular binding simulation between Betatrophin and LPL. Binding site prediction of each protein using the COFACTOR server (zhanglab.ccmb.med.umich.edu/COFACTOR) [28-30]. Simulation of molecular docking was performed on Cluspro's web-server (cluspro.bu.edu) and visualizing the docking results in the PyMol software to confirm Betatrophin's binding position with the LPL [31-35]. The docking results were fixed using two web-servers, FireDock (bioinfo3d.cs.tau.ac.il/FireDock/index.html) [42-43] and HADDOCK (haddock.science.uu.nl/services/HADDOCK2.2/) [45-46].

3. Results and Discussion

3.1. Results
UniProt is a specific database, and it contains the information about proteins that have been published in previous studies. This information is related to the names and function of gene along with the protein, also enzyme-specific information such as catalytic activity-residues, cofactors including binding sites, significant domains and its role, subcellular location, substrate ions, protein interactions, and pattern of expression [36]. Our study used this database for sample preparation then we obtained sequences sample (Figure 1) of Betatrophin (ANGPTL8) protein with ID Q6UXH0 and Lipoprotein lipase (LPL) with ID P06858, each of these sequences has a length of 198-mer and 475-mer. The sequences enter the 3D structure modeling stage for further analysis.
The homology modeling method was aimed to construct the 3D protein structure; this method works based on alignment process between query sample with a template, so we obtained homologous protein structure; the similarity of a protein model with the template is very similar if the score is 20% or more [37]. After we attained a sample of Betatrophin and LPL sequences, we used homology modeling on Swiss-Model web-server that resulted in modeling score for a Betatrophin protein; the value was 31.48% which had similarity with template while the value of LPL was about 30.14%, so both had high resemblance value. The validation of the model results was done using RAMPAGE web-server, and the results showed that both proteins had a favored region score of more than 90% so it can be concluded that the model was valid [27]. The secondary protein structures showed that Betatrophin is composed of α-helix and coil, while LPL is formed of α-helix, β-sheet, and coil (Figure 2).

**Figure 1.** Fasta format of protein sequences from UniProt. (A) Betatrophin (B) Lipoprotein lipase

**Figure 2.** Molecular visualization of protein modeling results and structural validation. (A) Betatrophin (B) LPL, both shown in the cartoons and secondary structures (α-helix, β-sheet, and coil) are red, yellow, and green.
After the modeling and validation process, we predicted the binding sites on both target proteins. The used parameters were Cscore<sup>LB</sup>, a score of confidence which should be about 0-1 to meet the requirement of the domain to be predicted as positive binding sites, then BS-score which is the similarity of binding sites between structures or template sequences with query, this score should be >1 to predict the high similarity with the template [29-30]. The results of this study indicated that the highest number of Cscore<sup>LB</sup> and BS-scores on the Betatrophin protein was in the amount of 0.04 and 0.97 with the sequence positions (S82, A88, Q89, R92, A93, L95, L96, E97, Q99, M100, E102, D103, A111 (G76, F77, G133, H151, S152, L153, R163, R164, T165, N166, A128, E110, P180, E187, R190, D192, D195, D205, I206, P208, F209, G214, F215, G216, M217, H263, N404, V407, I409, E440), both the protein targets are shown in PyMol software with rigid surface structures and performed color selection (Table 1 and Figure 3).

Table 1. The value of Cscore<sup>LB</sup> and BS-score for Betatrophin and LPL proteins.

|        | Betatrophin |        | LPL     |
|--------|-------------|--------|---------|
| Cscore<sup>LB</sup> | BS-score    | Cscore<sup>LB</sup> | BS-score |
| 0.04   | 0.97        | 0.72   | 1.94    |
| 0.03   | 0.66        | 0.44   | 1.90    |
| 0.03   | 0.59        | 0.44   | 1.88    |
| 0.02   | 0.70        | 0.39   | 1.66    |
| 0.02   | 0.64        | 0.39   | 1.65    |
| 0.02   | 0.60        | -      | -       |

In the next step, we did molecular docking analysis to determine the possibility of betatrophin binding protein with LPL receptor. Docking is a method to interact between molecules with one another to determine the lowest energy produced when a stable complex is formed due to docking events [38]. Cluspro is a docking web-server that works using the rigid docking body method by ensuring interaction between the proteins with each other superimpose and each position is capable of producing the lowest different energy binding scores, while this type of docking can be used to confirm or simulate a protein binding to a specific receptor [40]. Our results showed that some Betatrophin ligand binding positions for different LPL domains were displayed in PyMol software in the form of rigid surfaces structure with color selection (Figure 4); the lowest energy obtained was -728 KJ/mol (Table 2). That energy might be required to form a stable complex between Betatrophin and LPL because the molecular docking method serves to determine the shape of molecular conformation oriented to the ligand binding position in the protein-specific domain [31].

Figure 3. Structure visualization of predicted binding sites scores for a protein target. Predicted binding sites region showed with red color on these protein surfaces (A) Betatrophin and (B) LPL.
Table 2. The lowest energy for complexes molecule Betatrophin and LPL.

| Macromolecule | Binding Positions | Lowest Energy (kJ/mol) |
|---------------|-------------------|------------------------|
| Betatrophin-LPL | 1                 | -693.3                 |
| Betatrophin-LPL | 2                 | -703.5                 |
| Betatrophin-LPL | 3                 | -654.2                 |
| Betatrophin-LPL | 4                 | -728.8                 |
| Betatrophin-LPL | 5                 | -6356                  |

The Cluspro web-server groups the 1000 best docking models and each has different free energy. The resulted model is also grouped based on the total RMSD value [44]. Our study used 5 Cluspro docking models selected based on the possibility of a Betatrophin binding position in the predicted binding site at LPL and the lowest binding energy. The binding position of number 4 was chosen because it corresponds to a predetermined parameter, then the fixed docking process will be performed on the molecular compound of the binding position. Some parameters were used in the fixed process on FireDock web-server that works based on the flexible method of docking and scoring process through fast rigid-body docking algorithm. FireDock performs scores based on the chemical binding interaction function that occurs in the molecular complex, but basically this web-server works with a large scale in the flexible fixing process and scoring on the docking online. The parameters used in FireDock are Van der Waals (VdW) which comprises attractive-repulsive, atomic contact energy (ACE), and hydrogen bond (HB) [42-43]. High Ambiguity Driven DOCKing proteins (HADDOCK) is a flexible docking server used for modeling complex biomolecular interactions. This server can perform the latch-protein docking process and docking proteins. The fixing interface process on the HADDOCK server aims to determine the electrostatic interaction as well as i-RMSD, occurring in the molecular complexes formed [45-46].

Figure 4. Visualization of a binding position for ligand and receptor. The green colors and cyans in the surfaces structure are LPL and Betatrophin.
The result of fixing the docking process on FireDock server obtained about 5 ranks of the best bond energy position resulted in rank 1 and obtained Van der Waals attractive energy (VdW) in the amount of -29.72 kcal/mol, the repulsive of 23.05 kcal/mol, atomic contact of 1.28 kcal/mol, hydrogen bond of -4.11 kcal/mol. Rank 2 for the attractive VdW of -14.86 kcal/mol, the repulsive of 5.49 kcal/mol, atomic contact of -3.68 kcal/mol, and hydrogen bond of -1.20 kcal/mol. Rank 3 has an attractive energy VdW of -22.01 kcal/mol, the repulsive VdW 13.72 kcal/mol, atomic contact of -3.56 kcal/mol, and hydrogen bond of -2.04 kcal/mol. Rank 4 has an attractive energy VdW of -32.41 kcal/mol, the repulsive VdW of 13.28 kcal, 4.96 kcal/mol atomic contact, and hydrogen bond of -4.03 kcal/mol. Rank 5 has an attractive energy VdW of -12.69 kcal/mol, the repulsive of 3.68 kcal/mol, atomic contact -1. of 26 kcal/mol, and -0.31 kcal/mol (Figure 5). After fixing docking process on FireDock, we obtained the complex model of a betatrophin-LPL molecule with the best chemical interaction that was at rank 1, the model was downloaded and then put into the server HADDOCK to know the electrostatic interaction that occurred. The result of fixing interface showed that the energy of electrostatic interaction in a betatrophin-LPL molecular complex was -89.8 kcal/mol with the iRMSD value of 0.3 Å (Figure 6).

Figure 5. The rank predicted of chemical interaction in Betatrophin and LPL binding site for position number four. The circled area is predicted the region of their chemical interactions.
3.2. Discussion

Previous research used UniProt database to pass LPL sequence samples for LPL protein structure modeling using homologous modeling with MODELLER software using homology modeling method; the modeling result showed that about 83% of domains conserved proteins were identified, and the model was validated using plot Ramachandran. Hence, about 94% of the amino acid residues were in the preferred area. Once it was successfully performed on the LPL domain performed using the CASTP server, the predicted results that found some amino acid residues that contribute to the active side were GLY6, ILE8, ASN10, GLY14, LEU18, SER20, GLN22, CYS28, THR32, HIS151, PRO180, GLY250, PRO309, VAL321, PHE321 [39]. The samples in our study were obtained from UniProt with ID Betatrophin (ANGPTL8) and Lipoprotein lipase (LPL) with ID Q6UXH0 and P06858 with the length of 198-mer and 475-mer. The 3D modeling on Swiss-Model Berlin was obtained by Betatrophin and LPL model with the similarity score of 31.48% and 30.14% with the same template because the scores were above 20% [37], and then this model quality was checked using RAMPAGE web-server to obtain the value of favored area greater than 90%, the second Berlin model was valid. Betatrophin 3D structure modeling (ANGPTL8) using PHYRE, MUSTER, QUARK, and Rosetta was then validated using RAMPAGE server. Modeling and validation results showed that the structure had a similarity score of more than 20% in the 3mtt ID PDB template and about more than 90% was identified in the favored region [40].

The validity test was continued for the binding site prediction test using the COFACTOR web-server, the results showed that there were approximately 20 positions of amino acid residues predicted binding sites at Betatrophin and 30 in LPL, both of which have predictive values according to the used parameters [29-30]. However, there was an amino acid predicted binding site residues in LPL domains with the same predicted position listed in previous studies [39], like HIS151 and PRO180. The molecular process of docking was done on the Cluspro web-server, showing that the lowest docking energy between Betatrophin and LPL was about -728.8 KJ/mol. The molecular visualization results in the PyMol software showed that there was a position of amino acid residues at the binding sites that allowed both proteins interact, like GLU179, PRO180, ILE206, and PRO208 positions in the LPL with ARG92, LEU133, GLU134, and VAL135 on Betatrophin, so the position of amino acid residues used both proteins to interact directly to form the Betatrophin-LPL complex in stable condition (Figure 7). Some previous studies used Cluspro servers to ensure binding energy and biological activity in the target proteins when simulated undergoing direct binding. Docking using the Cluspro server was used to
determine the possibility of bundle gp350/20 on Epstein-Barr with a CR3 receptor, gp350/20 binds to domain 1 and domain 2 with the lowest energy -899.9 kJ/mol allowing for binding conformation between gp350 and CR3 [41]. Molecular docking simulation could prove the binding of osteocalcin and GPRC6A receptor activation in β cells, the results of the docking scores showed that the protein-ligand complex formed produced the lowest binding energy of -1227.4 kJ/mol. GPRC6A receptor activation occurs [44]. Based on the previous researches, we can concluded that the docking protein method could be used to simulate a protein binding with other proteins directly to produce certain biological activity, for example to the activation or inhibition of a protein depending on the purpose of the study.

The Cluspro web-server docking results showed the possibility of Betatrophin-LPL direct bindings, but the results were still rigid or rigid. The process of fixing docking on FireDock with the transformation files are necessary to make the sample looks more flexible, the results showed the best rank (rank 1) that was Betatrophin-LPL complex has energy interaction Van der Waals consisting of an attractive of -29.72 kcal/mol and a repulsive of 23.05 kcal/mol; the Van der Waals interaction produced at rank 1 was more stable and allowed the formation of Betatrophin-LPL molecular complexes because they had a far-reaching energy difference of -6.67 kcal/mol than rank 2 ie -9.37 kcal/mol, -8.29 kcal/mol rank 3, -19.13 kcal/mol rank 4, and rank 5 of -9.01 kcal/mol. So at rank 1, the interaction of Van der
Waals was very possible to form the Betatrophin-LPL molecular complex because it had binding energy which is almost close to 1 kcal/mol. The Van der Waals interaction energy that created only about 1 kcal/mol, was just slightly higher than the average thermal energy of each molecule with a temperature of 25 °C; the Van der Waals interaction was weaker than hydrogen bonds that have an energy of about 1-2 kcal/mol in solution. Van der Waals interactions are composed of attractive and repulsive, both of which are sufficient to mediate enzyme binding to their substrate or antibody bonds with each specific antigen [47]. Molecular docking using the FireDock web-server demonstrated the position of Phe-3 and Trp-43 amino acids on highly-efficient CD4 mutations to inhibit gp120 binding with binding energies of -113.8 and -101.7 kJ/mol; the binding energy in docking very closely related to Gibbs-free energies that are increasingly negative in value can indicate the inhibitory efficiency of gp120, and the results of the FireDock simulation suggest that Van der Waals interactions take a crucial role in the binding of CD4-gp120 and maintain the stability of the interactions of both proteins [48].

Hydrogen bonds play an important role in macromolecular stabilization; these bonds are strong although they are still below ionic. The type of hydrogen bonding to help stabilize the protein three-dimensional structure is nonlinear hydrogen bonds and multiple hydrogen bonds, both of which play an important role in forming large biological molecules architecture. The strength of hydrogen bonds in nucleic acids and proteins are about 1 to 2 kcal/mol, but when there is an interaction between proteins with each other, the hydrogen bond strength can reach less than 5 kcal/mol [48]. The stability of the protein when the side of a particular side undergoes a mutation, with reference to the conclusions of previous studies regarding hydrogen bonds of about 151 hydrogen bonds formed in 15 proteins, the arrangement of hydrogen bonds contributes to energy of about 1.1 to 0.8 kcal/mol for protein stability for bonding inter-complex is getting stronger [49]. The hydrogen bonds contribute very well to the stability of proteins and hydrogen bonds by the side chain with the peptide group contributing equally to the stability of the protein [50]. The energy of the hydrogen bond interaction in the LPL-Betatrophin molecule complex in the best rank was in rank 1 with the most negative energy compared to the other rank of -4.11 kcal/mol. So it can be ascertained that the hydrogen bond interaction at rank 1 can guarantee Betatrophin-LPL complex stability as it is more negative and keeps the molecule's complexes binding.

The rank 1 model was then calculated for the electrostatic energy formed on the HADDOCK web server; the results showed that about -89.8 kcal/mol with an iRMSD value of 0.3 iRMSD is the interaction distance of the atoms contributing to the molecular complex that is formed [45-46]. Electrostatic energy contributes to the interaction between molecules with each other as an example of the interaction of the formation of stable antibody-antigen complexes [51]. The energy is generated when the atoms have a distance of approximately 10 Å [52]. So the rank 1 model has a low atomic interaction spacing compared to the reference to allow the electrostatic energy to be formed to be very negative as a result of the Betatrophin-LPL complex that can form stable.

4. Conclusion
In summary, Betatrophin (ID Q6UXH0) and LPL (ID P06858) can be remodeled into 3D structure protein and should be validated before simulation binding and docking step. Both protein structures we used had a favored region score about more than 90% which was valid based on RAMPAGE web-server. Simulation binding step by COFACTOR server showed that betatrophin and LPL have some of possible binding sites that are similar to template sequences which showed as colored rigid surface structure based on PyMol software. The energy binding scores of both proteins were measured by Cluspro web-server and revealed 5 results visualisation of possible binding position with -728 KJ/mol (4th position) as the lowest energy required to form a stable complex. Then, from the 4th position visualization complex, the molecular binding interaction measured by FireDock which resulted in 5 rank prediction sites, and the last was the energy of electrostatic by HADDOCK which resulted in -89.8 kcal/mol with the iRMSD value of 0.3 Å. It can be concluded that based on in the silico method, the binding of Betatrophin ligand and LPL receptor is assumed can reduce triglyceride level.
References

[1] Aleksandrova, K. et al. Metabolic Syndrome and Risks of Colon and Rectal Cancer: The European Prospective Investigation into Cancer and Nutrition Study. Cancer Prevention Research 4, 1873–1883 (2011).

[2] Harlid, S., Myte, R. & Van Guelpen, B. The Metabolic Syndrome, Inflammation, and Colorectal Cancer Risk: An Evaluation of Large Panels of Plasma Protein Markers Using Repeated, Prediagnostic Samples. Mediators of Inflammation 2017, 1–9 (2017).

[3] Liu, D. et al. Increased circulating full-length betatrophin levels in drug-naïve metabolic syndrome. Oncotarget 8, (2017).

[4] Bhanushali, C. J. et al. Association between Lifestyle Factors and Metabolic Syndrome among African Americans in the United States. Journal of Nutrition and Metabolism 2013, 1–6 (2013).

[5] McCULLOUGH, A. J. Epidemiology of the metabolic syndrome in the USA: The metabolic syndrome. Journal of Digestive Diseases 12, 333–340 (2011).

[6] Falahi, E., Khalkhali Rad, A. H. & Roosta, S. What is the best biomarker for metabolic syndrome diagnosis? Diabetes & Metabolic Syndrome: Clinical Research & Reviews 9, 366–372 (2015).

[7] Santoro, N. & Weiss, R. Metabolic syndrome in youth: current insights and novel serum biomarkers. Biomarkers in Medicine 6, 719–727 (2012).

[8] Srikanthan, K., Feyh, A., Visweshwar, H., Shapiro, J. I. & Sodhi, K. Systematic Review of Metabolic Syndrome Biomarkers: A Panel for Early Detection, Management, and Risk Stratification in the West Virginian Population. International Journal of Medical Sciences 13, 25–38 (2016).

[9] Tseng, Y.-H., Yeh, Y.-H., Chen, W.-J. & Lin, K.-H. Emerging Regulation and Function of Betatrophin. International Journal of Molecular Sciences 15, 23640–23657 (2014).

[10] Wang, Y. et al. Mice lacking ANGPTL8 (Betatrophin) manifest disrupted triglyceride metabolism without impaired glucose homeostasis. Proceedings of the National Academy of Sciences 110, 16109–16114 (2013).

[11] Yamada, H. et al. Circulating betatrophin is elevated in patients with type 1 and type 2 diabetes. Endocrine Journal 62, 417–421 (2015).

[12] Li, Y. & Teng, C. Angiopoietin-like proteins 3, 4 and 8: regulating lipid metabolism and providing new hope for metabolic syndrome. Journal of Drug Targeting 22, 679–687 (2014).

[13] Wang, H. et al. The Effects of Serum ANGPTL8/betatrophin on the Risk of Developing the Metabolic Syndrome – A Prospective Study. Scientific Reports 6, (2016).

[14] Fu, Z. et al. Elevated circulating lipasin/betatrophin in human type 2 diabetes and obesity. Scientific Reports 4, (2015).

[15] Beigneux, A. P. et al. Glycosylphosphatidylinositol-Anchor High-Density Lipoprotein-Binding Protein 1 Plays a Critical Role in the Lipolytic Processing of Chylomicrons. Cell Metabolism 5, 279–291 (2007).

[16] Zhang, R. A dual role of lipasin (betatrophin) in lipid metabolism and glucose homeostasis: consensus and controversy. 10 (2014).

[17] Xie, X. et al. Associations of betatrophin levels with irisin in Chinese women with normal glucose tolerance. Diabetology & Metabolic Syndrome 7, (2015).

[18] Crujeiras, A. B. et al. Interplay of atherogenic factors, protein intake and betatrophin levels in obese–metabolic syndrome patients treated with hypocaloric diets. International Journal of Obesity 40, 403–410 (2016).

[19] Estrada, E. & Peña, A. In silico studies for the rational discovery of anticonvulsant compounds. Bioorganic & Medicinal Chemistry 8, 2755–2770 (2000).

[20] Harm, M. & Green, R. Chemoinformatics - a new name for an old problem? 5

[21] Kaur, M. et al. In Silico discovery of transcription factors as potential diagnostic biomarkers of ovarian cancer. BMC Systems Biology 5, 144 (2011).
[22] Waterhouse, A. et al. SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Research* **46**, W296–W303 (2018).

[23] Bienert, S. et al. The SWISS-MODEL Repository—new features and functionality. *Nucleic Acids Research* **45**, D313–D319 (2017).

[24] Guex, N., Peitsch, M. C. & Schwede, T. Automated comparative protein structure modeling with SWISS-MODEL and Swiss-PdbViewer: A historical perspective. *ELECTROPHORESIS* **30**, S162–S173 (2009).

[25] Benkert, P., Biasini, M. & Schwede, T. Toward the estimation of the absolute quality of individual protein structure models. *Bioinformatics* **27**, 343–350 (2011).

[26] Bertoni, M., Kiefer, F., Biasini, M., Bordoli, L. & Schwede, T. Modeling protein quaternary structure of homo- and hetero-oligomers beyond binary interactions by homology. *Scientific Reports* **7**, (2017).

[27] Lovell, S. C. et al. Structure validation by Ca geometry: $\phi, \psi$ and Cβ deviation. *Proteins: Structure, Function, and Bioinformatics* **50**, 437–450 (2003).

[28] Zhang, C., Freddolino, P. L. & Zhang, Y. COFACTOR: improved protein function prediction by combining structure, sequence and protein–protein interaction information. *Nucleic Acids Research* **45**, W291–W299 (2017).

[29] Roy, A., Yang, J. & Zhang, Y. COFACTOR: an accurate comparative algorithm for structure-based protein function annotation. *Nucleic Acids Research* **40**, W471–W477 (2012).

[30] Roy, A. & Zhang, Y. Recognizing Protein-Ligand Binding Sites by Global Structural Alignment and Local Geometry Refinement. *Structure* **20**, 987–997 (2012).

[31] Kozakov, D. et al. The ClusPro web server for protein–protein docking. *Nature Protocols* **12**, 255–278 (2017).

[32] Kozakov, D. et al. How good is automated protein docking?: Automated Protein Docking. *Proteins: Structure, Function, and Bioinformatics* **81**, 2159–2166 (2013).

[33] Kozakov, D., Brenke, R., Comeau, S. & Vajda, S. PIPER: An FFT-based Protein Docking Program with Pairwise Potentials. *arXiv:q-bio/0605018* (2006).

[34] Comeau, S. R., Gatchell, D. W., Vajda, S. & Camacho, C. J. ClusPro: an automated docking and discrimination method for the prediction of protein complexes. *Bioinformatics* **20**, 45–50 (2004).

[35] Comeau, S. R., Gatchell, D. W., Vajda, S. & Camacho, C. J. ClusPro: a fully automated algorithm for protein–protein docking. *Nucleic Acids Research* **32**, W96–W99 (2004).

[36] Leinonen, R. et al. UniProt archive. *Bioinformatics* **20**, 3236–3237 (2004).

[37] Venclovas, Č. & Margelevičius, M. Comparative modeling in CASP6 using consensus approach to template selection, sequence-structure alignment, and structure assessment. *Proteins: Structure, Function, and Bioinformatics* **61**, 99–105 (2005).

[38] Zsoldos, Z., Reid, D., Simon, A., Sajjad, S. B. & Johnson, A. P. eHiTS: A new fast, exhaustive flexible ligand docking system. *Journal of Molecular Graphics and Modelling* **26**, 198–212 (2007).

[39] Torabizadeh, M., Munawar, T. M., Prasad, M., Rayalu, D. J. & Lakshmidri, K. Computational modeling and drug designing of lipoprotein lipase (LPL). 9

[40] Siddiqua, A. et al. Structural characterization of ANGPTL8 (betatrophin) with its interacting partner lipoprotein lipase. *Computational Biology and Chemistry* **61**, 210–220 (2016).

[41] Sitompul, L. S., Widodo, N., Djati, M. S. & Utomo, D. H. Epitope mapping of gp350/220 conserved domain of Epstein barr virus to develop nasopharyngeal carcinoma (npc) vaccine. *Bioinformation* **8**, 479–482 (2012).

[42] Andrusier, N., Nussinov, R. & Wolfson, H. J. FireDock: Fast interaction refinement in molecular docking. *Proteins: Structure, Function, and Bioinformatics* **69**, 139–159 (2007).

[43] Mashiach, E., Schneidman-Duhovny, D., Andrusier, N., Nussinov, R. & Wolfson, H. J. FireDock: a web server for fast interaction refinement in molecular docking. *Nucleic Acids Research* **36**, W229–W232 (2008).
[44] Pi, M. *et al.* Evidence for Osteocalcin Binding and Activation of GPRC6A in β-Cells. *Endocrinology* **157**, 1866–1880 (2016).

[45] van Zundert, G. C. P. *et al.* The HADDOCK2.2 Web Server: User-Friendly Integrative Modeling of Biomolecular Complexes. *Journal of Molecular Biology* **428**, 720–725 (2016).

[46] Wassenaar, T. A. *et al.* WeNMR: Structural Biology on the Grid. *Journal of Grid Computing* **10**, 743–767 (2012).

[47] Lodish, H. *et al.* Molecular Cell Biology. 4th edition. in *Molecular Cell Biology, 4th edition* (W. H Freeman, 2000).

[48] Teoh, T., Salmah, I. & Tang, J. Molecular Dynamics and Docking of Biphenyl: A Potential Attachment Inhibitor for HIV-1 gp120 Glycoprotein. *Tropical Journal of Pharmaceutical Research* **13**, 339 (2014).

[49] Nick Pace, C., Scholtz, J. M. & Grimsley, G. R. Forces stabilizing proteins. *FEBS Letters* **588**, 2177–2184 (2014).

[50] Pace, C. N. *et al.* Contribution of hydrogen bonds to protein stability: Hydrogen Bonds and Protein Stability. *Protein Science* **23**, 652–661 (2014).

[51] Norel, R., Sheinerman, F., Petrey, D. & Honig, B. Electrostatic contributions to protein-protein interactions: Fast energetic filters for docking and their physical basis. *Protein Science* **10**, 2147–2161 (2008).

[52] Maleki, M., Vasudev, G. & Rueda, L. The role of electrostatic energy in prediction of obligate protein-protein interactions. *Proteome Science* **11**, S11 (2013).

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