protein, with information of how often each residue are included in binding sites of high scoring docking models. We applied multiple methods of machine learning to discriminate binders and non-binders using those profiles as input. In this poster we present an evaluation of our method by applying it to proteins of Protein Docking Benchmark ver. 5.0, bacterial chemotaxis, and host-virus protein interactions. We further applied our method to analyze proposed host-virus PPI interaction surfaces by post-docking analysis to compare surfaces of PPIs between the host proteins.

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***In Silico* Screening for Chemical Scaffolds as Suitable Natural Inhibitors of Kinesin EG5 Divulges Morelloflavone, a Biflavonoid, as Potential Anti-cancer Compound**
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Natural products remain a source of chemical scaffold pool for drug design and development. Kinesin Eg5 has emerged as a clinical target for anticancer agents. In our search for potent natural inhibitors of kinesin Eg5, we employed *in silico* tools to screen selected diverse chemical scaffolds (compounds) that were obtained from medicinal plants. Surprisingly, the molecular interaction analysis for the selected compounds adjudged morelloflavone (a biflavonoid) as a potential ATP-noncompetitive inhibitor of kinesin Eg5 protein which occupied the putative L5/α2/α3 allosteric pocket on the protein. Compared to STLC with binding energy value -10.0 Kcal/mol, morelloflavone displayed binding energy value of -10.2 Kcal/mol and a 90 percent binding site similarity. It is also worth noting that morelloflavone was embedded within the cavity formed by amino acid residues Ile-136, Glu-116, Glu-118, Trp-127, Gly-117, Ala-133, Glu-215, Leu-214, Tyr-211 and hence, displayed a reliable tendency to block the enzymatic catalysis of kinesin Eg5 allosterically. The compound established hydrogen bonds with Glu-118 and Tyr-211 having minimum length of 2.97Å and hydrophobic interactions occurred with alkyl side chain of residues Gly-117, Glu-116, Ala-218, Ile-136, Arg-119 and Asp-130 while π-stacking interaction is observed between the aromatic ring of morelloflavone and Arg-119. These interactions anchored morelloflavone into the binding site. The results obtained in this work indicate the strong affinity and inhibitory potential of this compound on kinesin Eg5, hence lending credence to the yet untapped anticancer capacities of morelloflavone. We therefore suggest *in vitro* and *ex vivo* evaluation of this compound as anticancer agent targeting kinesin Eg5 protein.

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Development of Postprocessing Method of Protein-Ligand Docking using Interaction Fingerprint
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Protein-ligand docking is an important method in Structure-based Drug Discovery [1]. Although many programs have been developed for docking [2], the accuracy is still insufficient due to the difficulty in the scoring function [3]. Interaction fingerprint is one of the solutions, which generate fingerprints of ligands using the interactions between the ligand and the protein. Interaction fingerprints use the information of known compounds so that compounds that have similar interaction to the known active ligands are expected to find through the virtual screening. However, existing interaction fingerprints such as SIFt [4] and SPLIF [5] only assess the existence or the distance of the interactions and do not consider the strength correctly. In this study, we made a new scoring function of protein-ligand docking called SIEVE-Score (Similarity of Interaction Energy VEctor-Score), which can consider the strength of each interaction explicitly. SIEVE-Score is calculated based on the similarity of the interaction energy vector, which is the list of interaction energy between the ligand and each residue of the protein. We also evaluate the accuracy of virtual screening using SIEVE-Score after the docking by Glide [6].

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Optical Spectroscopy: CD, UV-VIS, Vibrational, Fluorescence I

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New Thiol-Reactive Eu-Complex for Distance Measurements by LRET
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Crystallography and NMR spectroscopy are ideally suited to resolve the 3D structures of biomolecules but the material and time demand for each structure is high. Fluorescence resonance energy transfer (FRET) provides less structural information but is better suited to study conformational changes and structure-function relationships by screening a large number of mutants or experimental conditions. Moreover, FRET allows for real time monitoring of conformational changes induced by specific ligands. Usually, FRET yields only a crude estimate of the donor-acceptor distance, due to the fact that the relative orientation of donor and acceptor are rarely known. Luminescence resonance energy transfer (LRET) is much better suited for distance measurements because the orientation factor (and thus the Forster distance) are known, and because energy transfer is measured by a change of lifetime, rather than of signal intensity. In LRET-experiments the ideal donors are highly stable Eu/Tb-complexes, with a single lifetime that is not influenced by attaching the complex to a biomolecule. Several terpyridine-based Eu-complexes described in literature have promising properties concerning uniform lifetimes after protein labeling but all described complexes have rather long linkers which prevent accurate distance measurements. In this study, a new terpyridine-based Eu-complex with maleimide very close to the metal ion center was synthesized and found to have ideal properties for distance measurement by LRET: After linking to the single cysteine of BSA, the complex showed a quantum yield of 30%, a single lifetime of 1.2 ms, comparable to the best known Eu-complexes, and the lifetime was unaffected by phosphate or EDTA. In conclusion, this new Eu complex appears ideally suited for reliable measurement of intra- or intermolecular distances.

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A Novel FRET Technique to Characterize the Oligomerization State of Protein-Protein Interactions
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Protein-protein interactions are the fundamental driving force of numerous cellular processes and cell signaling pathways. Characterizing whether proteins interact as dimers, trimers, or higher oligomers is essential to understanding these interactions. Several microscopy and advanced imaging techniques relying on Förster resonance energy transfer (FRET) between identical fluorophores (homo-FRET) have been developed to estimate protein stoichiometry. The increased FRET in oligomers is detected by measuring depolarization or emission time. Homo-FRET methods have a strong advantage in requiring only a single fluorophore, greatly simplifying sample preparation in comparison to conventional hetero-FRET methods. However, most homo-FRET methods require sophisticated imaging equipment, and both theoretical models and applications have been restricted to the study of membrane-bound proteins. Using a simple bulk homo-FRET and laser photobleaching approach, we demonstrate the feasibility of characterizing the oligomerization state of an interacting protein in-vitro. To simulate oligomers in a proof of concept, we constructed an extensive repertoire of fusion proteins with 1-6 consecutive green fluorescent protein (GFP) domains. We show how the resulting homo-FRET (measurable via steady-state anisotropy or fluorescence polarization) is proportional to the oligomerization state of proximal GFP domains. For the first time, this is demonstrated with soluble proteins. In both membrane and soluble proteins, oligomerization increases FRET and therefore anisotropy. However for soluble proteins oligomerization also slows fluorophore rotation, leading to a size-dependent decrease in anisotropy. Through gradual photobleaching of