Virtual screening for ligands with predefined dynamic allosteric response

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Allosteric regulation is the coupling between distant sites in biomolecules: An action at one site can affect the function at another site. Targeting allosteric regulation in biomolecules is a promising strategy in drug discovery, due to advantages over conventional orthosteric ligands. [1] However, the identification of novel allosteric pockets is complicated by the variety of allosteric mechanisms, differing by the extent of conformational change upon ligand binding. Particularly, dynamic allostery, which can occur in the absence of conformational change, [2] is difficult to detect from static crystal structures alone. Here, we present an efficient approach to probe dynamic allostery in biomolecules by constructing fuzzy ligands as surrogates for "true" ligands, with which an allosteric response is studied by deducing altered stability characteristics from rigidity analysis.

We probed the performance of the fuzzy ligand approach on the AsteX diverse set [3] containing 85 protein-ligand complexes, including 20 allosterically regulated proteins. For the *apo* states generated by removing the original ligand in Maestro, pockets were calculated using PocketAnalyzer^{PCA}. [4] Fuzzy ligands were generated by mapping the possible interactions of the binding pocket to build a rod-like structure that connects the interacting atoms placed in the pocket. Altered long-range stability characteristics upon binding of a ligand were computed by the Constraint Network Analysis (CNA) approach, which aims at characterizing biomolecular flexibility and rigidity for linking structure and function. [5] The fuzzy ligands were validated I) in terms of their influence on network rigidity compared to the "true" ligand and II) to what extent pharmacophore models based on them allow for a successful retrieval of binders in retrospective virtual screenings on DUD-E datasets. [6]

Altered per-residue stability characteristics from rigidity analysis of our fuzzy ligands are in agreement with those from "true" ligands. In 62% of all cases, the calculated per-residue energies correlate with $R^2 \geq 0.50$. The virtual screening results based on fuzzy ligands perform equally well or outperform, in 29% of the cases, the true ligands' results. In all, analyzing unexplored pockets with fuzzy ligands could thus be a promising step towards identifying novel allosteric drug targets and drugs.

[1] R. Nussinov, C.-J. Tsai, *Cell*, **2013**, *153*, 293-305
[2] A. Cooper, D.T. Dryden, *Eur. Biophys. J.*, **1984**, *11*, 103-109
[3] M.J. Hartshorn, M.L. Verdonk, G. Chessari, S.C. Brewerton, W.T.M. Mooij, P.N. Mortenson, C.W. Murray, *J. Med. Chem.*, **2007**, *50*, 726-741
[4] I.R. Craig, C. Pfleger, H. Gohlke, J.W. Essex, K. Spiegel, *J. Chem. Inf. Model.*, **2011**, *51*, 2666-2679
[5] C. Pfleger, P.C. Rathi, D.L. Klein, S. Radestock, H. Gohlke, *J. Chem. Inf. Model.*, **2013**, *53*, 1007-1015
[6] M.M. Mysinger, M. Carchia, J.J. Irwin, B.K. Shoichet, *J. Med. Chem.*, **2012**, *55*, 6582-6594