Towards Identifying Novel Allosteric Drug Targets using a "Dummy" Ligand Approach

Susanne M.A. Hermans, Christopher Pfleger and Holger Gohlke*

Department of Mathematics and Natural Sciences, Institute for Pharmaceutical and Medicinal Chemistry, Heinrich-Heine-University, Düsseldorf, Germany

**Email: gohlke@uni-duesseldorf.de*

Allosteric regulation is the coupling between separated sites in biomacromolecules such that an action at one site changes the function at a distant site. The identification of novel allosteric pockets is complicated by the large variation in allosteric regulation, ranging from rigid body motions to disorder/order transitions, with dynamically dominated allostery in between.[1] Here, we present a new and efficient approach to probe information transfer through proteins in the context of dynamically dominated allostery that exploits "dummy" ligands as surrogates for allosteric modulators.

In a preliminary study to test the general feasibility, the approach was applied to conformations extracted from a MD trajectory of the *holo* and *apo* structures of LFA1. The grid-based PocketAnalyzer program[2] is used to detect putative binding sites. "Dummy" ligands were generated for each detected pocket along the ensemble. Finally, the Constraint Network Analysis (CNA) software, which links biomacromolecular structure, (thermo-)stability, and function, is used to probe the allosteric response by monitoring altered stability characteristics of the protein due to the presence of the "dummy" ligand.[3–5] The results were compared to those of the *holo* structure with the bound allosteric ligand to validate the "dummy" ligand approach.

Remarkably, the usage of "dummy" ligands almost perfectly reproduced the results obtained from the known allosteric effector. Although it turned out that the intrinsic rigidity of the "dummy" ligands over-stabilizes the LFA1 structure, these results are already encouraging. Even for the LFA1 apo structures, where the allosteric pocket is partially closed, the results are in agreement with known allosteric effectors. Overall, the results obtained from the validation of the "dummy" ligand approach are encouraging. This suggests that our "dummy" ligand approach for the characterization of unexplored allosteric pockets is a promising step towards identifying novel drug targets.

[1] H.N. Motlagh, J.O. Wrabl, J. Li, V.J. Hilser, *Nature*, **2014**, *508*, 331–339.
[2] I.R. Craig, C. Pfleger, H. Gohlke, J.W. Essex, K. Spiegel, *J. Chem. Inf. Model.*, **2011**, *51*, 2666-2679
[3] C. Pfleger, P.C. Rathi, D.L. Klein, S. Radestock, H. Gohlke, *J. Chem. Inf. Model.*, **2013**, *53*, 1007-1015
[4] D.M. Krüger, P.C. Rathi, C. Pfleger, H. Gohlke, *Nucleic Acids Res.*, **2013**, *41*, 340-348
[5] C. Pfleger, *Doctoral Thesis, Heinrich-Heine-University Düsseldorf*, **2014**