RESEARCH INNOVATION VALUE

Michal Kamenicky; Maria Bräuer; Katrin Volk; Kamil Ödner; Christian Klein; Norbert Müller

Targeting protein-protein interactions: A hot topic in drug discovery

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Abstract

The functioning of living organisms is to a large extent dependent on the interplay between the biomolecules they are composed of. Protein-protein interactions (PPIs) represent a basic mechanism that regulates this interplay. Consequently, in the past few years the search for active compounds that have a therapeutic influence on protein-protein interactions has been intensified. In most cases these compounds are inhibitors of these interactions. The present work focuses on our attempt to combine methods from molecular and structural biology with computational approaches to identify small organic compounds that are potential protein-protein interaction inhibitors.

Keywords:

Protein-protein interaction, acyl carrier protein, acyl carrier protein synthase, NMR, recombinant proteins, synthetic peptides, pharmacophore models, virtual screening

Introduction

The biological target used is the complex of ACP (acyl carrier protein) with ACPS (acyl carrier protein synthase) from Staphylococcus aureus. ACP is a crucial molecule in fatty acid biosynthesis, as the growing fatty acid is esterified to acyl-carrier protein. Holo ACP, like CoA, contains a phosphopantetheine group that forms thioesters with acyl groups. The phosphopantetheine phosphoryl group is esterified to a Ser OH group of ACP; the transfer of phosphopantetheine from CoA to apo-ACP to form the active holo-ACP is catalyzed by ACP synthase (Voet / Voet, 2011). Thus, interrupting or disturbing the ACP-ACPS protein-protein interaction should lead to inhibition of fatty acid biosynthesis, since this interaction is crucial in correctly positioning the Ser OH group to be esterified with phosphopantetheine (Parris et al. 2000).

Our study system is ACP-ACPS from Staphylococcus aureus. Both proteins show a high homology, not only to other cocci, at a sequence level. Moreover, available X-ray and NMR structures suggest that this

homology is highly conserved also at a structural level. Therefore, we can expect that compounds which inhibit the bacterial ACP-ACPS interaction should have antibiotic activity.

To analyze the ACP-ACPS interaction more thoroughly, both proteins were produced recombinantly in E. coli. Peptides containing parts of the proteins (ACP or ACPS) were synthetized and interaction studies of these peptides with the whole proteins were performed using biolayer-interferometry. ACPS-derived peptides binding to ACP were identified.

For binding ACPS peptides, NMR structures could be obtained. In Fig. 1 one of these structures is shown:

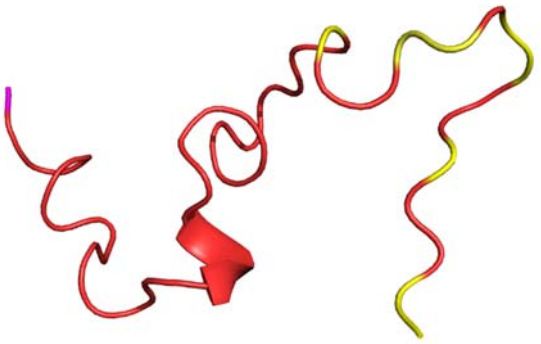

Figure 1: CS23D solution structure of a synthetic peptide of ACPS that binds to ACP. The backbone colored in **yellow** represents residues which interact with ACP.

Moreover, from STD NMR experiments (Mayer / Meyer, 1999) the residues of the ACPS peptide interacting with ACP could be determined. They are marked in yellow on the backbone of the peptide in Fig. 1.

When matched to the available X-ray structure of the ACP-ACPS complex from Staphylococcus aureus (PDB code: 4DXE), all the interacting residues of the ACPS-derived peptide (as colored in Fig. 1) are located at the interface between the two proteins. They are marked in magenta in Fig. 2:

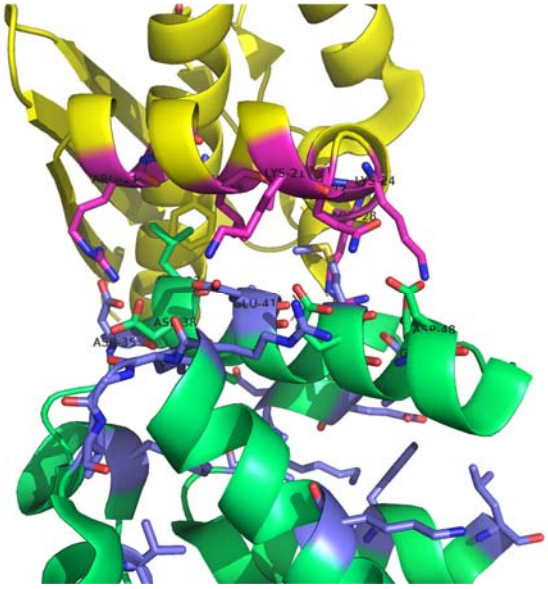

Figure 2: ACP-ACPS complex based on the X-ray structure from PDB (4DXE). **Yellow**: ACPS, **green**: ACP, **magenta**: interacting residues of the ACPS-derived peptide as determined from the STD NMR experiment; **blue**: residues for which NMR peaks disappear or shift during the titration experiment.

To get a deeper insight to the binding between ACP and ACPS, NMR titration experiments with ACPS were performed. A typical spectrum is shown in Fig.3.

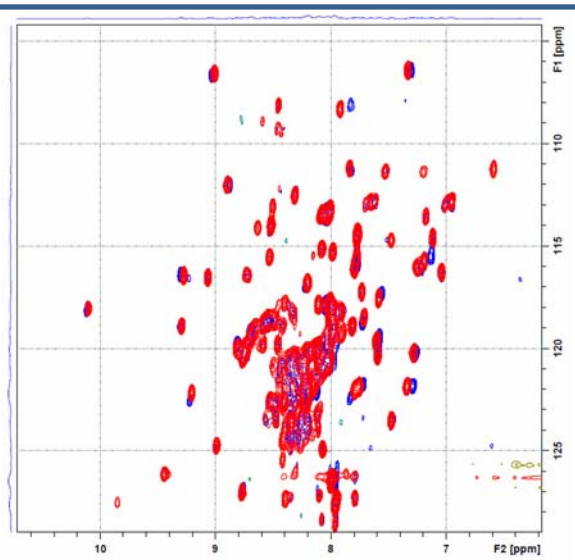

Figure 3: ^{1}H-^{15}N HSQC of His-ACP titrated with ACPS protein. The red spectrum denotes ACP without ACPS; blue peaks ACP after titration with ACPS.

From these spectra positional changes/movements of residues in the protein (ACP) upon binding of ACPS can be deduced. They are marked in blue on ACP in Fig. 2.

One of the final goals of the experiments described above is to use the available structural information for deriving pharmacophore models, which in turn can then be employed for virtual screening. Pharmacophore models describe molecules in more abstract terms than atoms, namely by so called pharmacophoric features. The latter are properties of atoms or groups rather than the atoms or groups themselves. Commonly used pharmacophoric features are hydrogen bond donors and acceptors, charged moieties or hydrophobic/aromatic moieties. Pharmacophore models are spatial arrangements of such pharmacophoric features and hence can be used as queries for searching large virtual molecular libraries for retrieving compounds that have similar spatial pharmacophoric arrangements. In other words, pharmacophore models derived from known protein-ligand or protein-protein complexes have the potential to retrieve hits from a virtual screening experiment (Horvat, 2011).

So far we have derived pharmacophore models starting from the available ACP-ACPS complex of Staphylococcus aureus (4DXE), including information from the NMR experiments: while the STD experiment suggests that all residues of ACPS from the protein-protein interface are involved in the interaction, the titration experiment indicates that – at least for ACP – some of them might be more important, namely those for which the peaks shift or disappear. These residues can be prioritized when building the pharmacophore models. In Fig. 4 one typical model is shown:

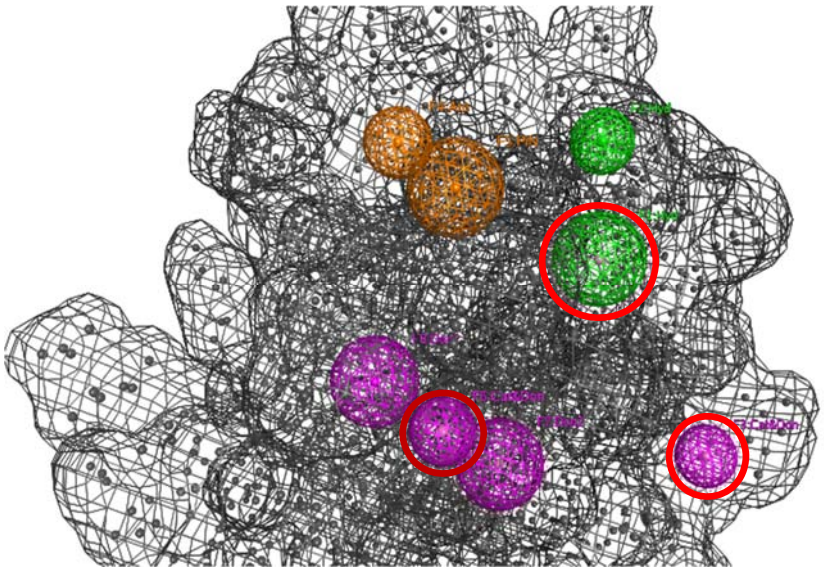

Figure 4: Pharmacophore model derived from 4DXE. **Green**: hydrophobic features, **magenta**: cations & donors, **orange**: aromatic, **grey**: excluded volumes. The features marked with red circles are required as essential considering NMR information

This model was built considering ACPS as the ligand and ACP as the receptor, i.e., the features shown were derived considering the residues colored in magenta in Fig. 2. However, we derived also models were ACP was considered the ligand and ACPS the receptor.

Models derived as briefly delineated above were then used to perform virtual screening experiments. The database screened was ZINC (Irwin / Shoichet, 2005), more precisely the “Drugs Now” subset (version 2014-11-24) which is composed of 10,639,555 drug-like molecules as defined by the Lipinski-rules (Lipinski et al., 2001). Several interesting hits were found which are going to be evaluated subsequently.

References

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