K15 **Molecular Infection: The Invasion Complex between Internalin of Listeria monocytogenes and human E-cadherin.** Wolf-Dieter Schubert,[a] Thomas Wollert,[a] Claus Urbanke[b] and Dirk W. Heinz[a], [a]*Structural Biology, German Research Centre for Biotechnology, Mascheroder Weg 1, 38124 Braunschweig, Germany, and* [b]*Institute of Biophysical Chemistry, Hannover Medical School, Carl-Neuberg-Str. 1, D-30623 Hannover, Germany. E-mail: wds@gbf.de*

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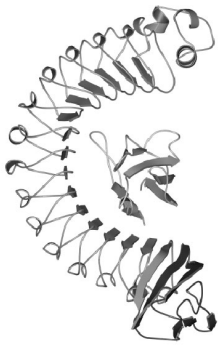
The bacterium *Listeria monocytogenes* is a food-borne human pathogen. It infects humans by inducing its own uptake into epithelial cells of the intestine - although these are normally non-phagocytic. Recognition, adhesion and invasion of intestinal epithelial cells is mediated by a single listerial surface protein, Internalin (InlA), through specific interaction with the host cell receptor E-cadherin.

We have solved the crystal structure of the functional domain of InlA both uncomplexed and in complex with the extracellular, N-terminal domain of human E-cadherin (hEC1).

In the complex between InlA and hEC1, the superhelically twisted leucine rich repeat (LRR) domain of InlA surrounds and specifically recognizes hEC1. Site-directed mutagenesis, analytical ultracentrifugation and Biacore experiments indicate that binding affinity is remarkably weak yet highly specific: Pro16 of hEC1, a major determinant for human susceptibility to *L. Monocytogenes* infection is essential for intermolecular recognition. Ca^{2+} was found to stabilize the complex. Structurally, this is corroborated by a Ca^{2+}-binding site bridging the two proteins. This indicates that complex formation in the intestine is favoured by high Ca^{2+}-concentrations whereas low, intracellular concentrations induce dissociation, freeing the bacterium and allowing it to move through the eukaryotic cell.



Our studies thus provide detailed insights into the molecular deception *L. monocytogenes* employs to exploit the host E-cadherin signal cascade through the expression of a single surface protein.

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K16 **From Crystal Structure to Molecular Recognition Principles: Mining in Crystal Data as a Prerequisite for Drug Design.** Gerhard Klebe, *Inst. of Pharmaceutical Chemistry, Univ. of Marburg, Marbacher Weg 6, D35032 Marburg, Germany, Fax +49 6421 282 8994. E-mail: klebe@mailer.uni-marburg.de*

The requirements a ligand has to meet in order to bind to a protein are defined by the shape and interaction properties of the residues exposed to the binding site of a target protein. Since protein-ligand binding is a process of mutual molecular recognition, rational drug design is greatly concerned with understanding the principles of molecular recognition. Systematic evaluation of geometries of protein-ligand complexes by statistical means provides a powerful tool to retrieve and correlate information about recognition patterns with respect to protein binding. To efficiently access such data, we have developed Relibase [1,2] as a database system particularly tailored to handle protein-ligand related problems, e.g. the induced adaptation of proteins upon ligand binding, the role of water in the binding process, the mapping of hot-spots of ligand binding or analyzing the versatile molecular recognition properties of functional groups.

The function of proteins is almost invariably linked with the specific recognition of substrates and ligands in given binding pockets, thus proteins of related function should share comparable recognition properties exposed to these pockets. We have developed the new module Cavbase for Relibase that stores protein cavities in terms of simple surface-exposed physicochemical properties [3]. These descriptors allow for fast retrieval of proteins with functional relationships independent of a particular sequence or fold homology. The approach also allows to detect unexpected cross-reactivity of ligands among unrelated proteins. The classification of binding pockets across protein family members allows to elucidate selectivity determinants.

In order to investigate the issue of selectivity and specificity in protein-ligand interactions from an experimental point of view, we study the step-wise reconstruction of binding pockets in related proteins being members of a particular protein family. As examples, the binding site of human factor Xa has been introduced in the structurally related trypsins or aldose reductase is gradually transferred into aldehyde reductase by site-directed mutagenesis. In case of the serine proteases, three sequential regions were selected as representing the major structural differences between the ligand-binding sites. Crystals of the variants were obtained in the presence of several different inhibitors [4,5]. The affinity data of these ligands together with the determined binding modes provide a first insight to factorize the different contributions to binding and helps to understand selectivity determining features.

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