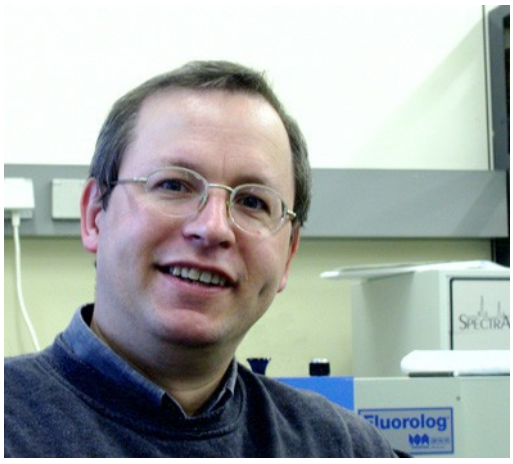
A special focus on intercellular mediators

Transmembrane proteins constitute around one third of all cellular proteins. Around half of all drugs that are currently on the market target the function of a specific class of transmembrane proteins, i.e. the G-protein coupled receptors. However, little is yet known about how transmembrane proteins are integrated into the membranes and how they are folded. Dr. Jörg H. Kleinschmidt hopes to shed light into the mechanisms of membrane protein folding by using bacterial membrane proteins of E. coli or Fusobacterium nucleatum, amongst others. His team also investigates the role of molecular chaperones, which are a class of proteins that prevent the misfolding of proteins to an inactive state.



Dr. Jörg Kleinschmidt is a researcher at the University of Constance
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The steric conformations and the functions of membrane proteins mark still a large number of relatively unknown spots on the map of scientists, especially in biochemical terms. A major reason for this is that it is difficult to isolate membrane proteins in sufficient quantities for structural and functional studies, since they occur naturally only in very small quantities. Due to their lipid environment, membrane proteins have a hydrophobic surface and are hard to solubilise without a major loss in biological activity. "The heterologous overexpression, i.e. the expression and isolation of foreign membrane proteins from host organisms such as bacteria, yeast or insect cells, is often toxic or leads to the misfolding of the proteins," said Dr. Jörg Kleinschmidt. Kleinschmidt's group of researchers is investigating the folding mechanism of a membrane protein class that has a barrel-like structure. These proteins are known as β-barrel membrane proteins. Moreover, the biophysicist and his team are also focusing on the interactions between membrane proteins and specialist proteins, including molecular chaperones that assist in the folding of proteins.

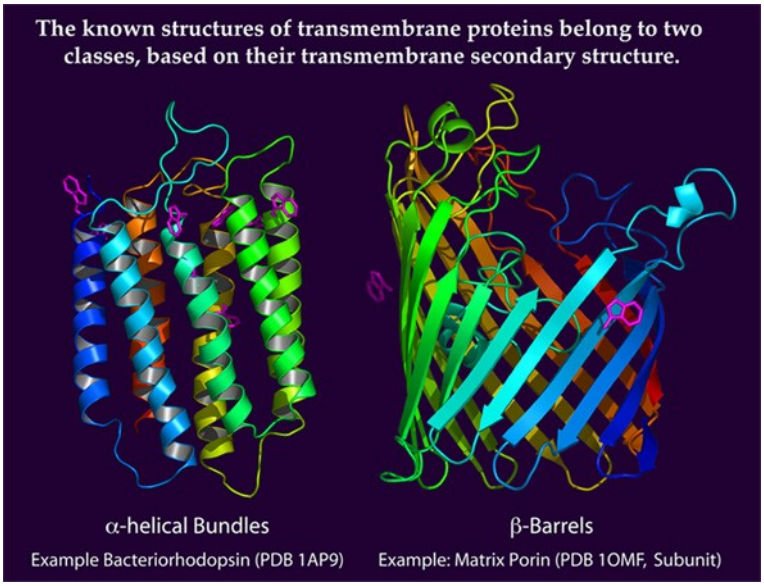
"It is of great advantage for our investigations that β-barrel membrane proteins can be unfolded in aqueous solutions containing high concentrations of chaotropic substances like guanidinium chloride. The interactions with such chemical denaturants result in a nearly complete loss of the secondary structure of β-barrel membrane proteins," explains Dr. Jörg Kleinschmidt going on to add "when denaturing agents are diluted in the presence of detergent micelles, which are ensembles of detergent molecules, or in the presence of lipid bilayers, which are the lipid structures of vesicles or liposomes, many of these proteins refold without necessarily requiring chaperones. Therefore, the folding mechanisms of β-barrel membrane proteins can be investigated more easily than folding mechanisms of the more hydrophobic α-helical membrane proteins.

The researchers are extremely interested in finding out why the proteins fold rather spontaneously into model membranes or detergent micelles, while in cells, they require the presence of certain proteins to do so. The analysis of the folding mechanism of a protein is no easy task since a protein can theoretically assume a large number of different conformations, whereas only one of these conformations leads to the proper function of this protein. "Many calculations and huge computer capacities are required to explore the functional conformation of a protein. To date, there is no general algorithm available that can accurately predict the 3-dimensional structure of a protein based on its primary amino acid sequence," says Dr. Jörg Kleinschmidt highlighting the need to investigate the principles of protein folding. The exploration of these principles helps the development of improved algorithms that enable more accurate predictions of the 3-dimensional structures of proteins. Kleinschmidt also explains that the process is easier in the case of transmembrane regions of membrane proteins, since the lipid bilayer into which the membrane proteins are embedded, leads to restrictions in potential protein conformations.

Chaperones determine the speed of folding

Currently, the researchers' analyses focus on bacterial membrane proteins, including OmpA from *E. coli* and FomA from *Fusobacterium nucleatum* as well as eukaryotic membrane proteins, including voltage-dependent anion-selective channels in mitochondria or the human tachykinin receptor NK_2R, a 7 α-helix bundle transmembrane protein that mediates the contraction of smooth muscle tissue.

Dr. Jörg Kleinschmidt and his team have discovered that the folding mechanism of β-barrel membrane proteins is characterised by a synchronous formation of the secondary and tertiary structure of the β-barrel, and that the rate of folding depends on several factors, like, e.g., on the hydrophobic thickness of the lipid bilayer of the membrane. "We have also characterised soluble complexes of the Skp (seventeen kilo-Dalton protein) chaperone from *E. coli* using unfolded outer membrane proteins of different bacteria and have described the influence of chaperones on membrane protein folding," added the researcher from Constance. However, depending on the protein, and in the case of membrane proteins also depending on the membrane, the rates of protein folding can differ considerably. Soluble proteins can fold within a few milliseconds, whereas β-barrel membrane proteins take seconds or minutes to fold. Proteins contained in lipid bilayers, which serve as model membranes, fold within a few minutes or several hours. Folding rates depend on the concentration of membrane proteins and membranes.



Two structurally different classes of transmembrane proteins are being focussed on in Kleinschmidt's investigations: α-helical and β-barrel-shaped proteins.
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The composition of the membrane, for example the lipid species or the hydrophobic thickness of the lipid bilayer can be decisive when the concentrations are identical. Another essential aspect also relates to the presence of chaperones. "The folding rate of β-barrel membrane proteins increases considerably in the presence of chaperones, i.e. proteins that prevent the misfolding of proteins," said Kleinschmidt. The folding speed also depends on the size of the protein and on its physical properties.

Besides the periplasmic chaperone Skp from *E. coli*, the researchers were also able to biochemically elucidate the effects of two other chaperones on membrane folding. They are also characterising the effects of the components of a membrane protein complex in bacteria, which is necessary for membrane protein folding. "In order to develop refolding processes of α-helical transmembrane proteins, we have developed a heterologous expression system for the human tachykinin receptor NK_2R, which is important for the contraction of smooth muscles," explains Dr. Jörg Kleinschmidt.

Structure formation in three steps

Researchers have been able to decipher several basic mechanistic principles related to structure formation and folding of transmembrane proteins as well as to characterise important factors that are involved in these processes. They found that the integration of membrane proteins and their folding can take place co-translationally as well as post-translationally. "The co-translational folding requires the presence of a membrane-spanning secretion complex (termed Sec-complex), which consists of three subunits," said Kleinschmidt. This complex enables the integration and folding of membrane proteins with α-helical transmembrane structure. For this class of membrane proteins, the experimental observations have led to a three-stage model in which the α-helices of the transmembrane domain initially insert into the membrane independently from each other, before they laterally associate within the plane of the lipid bilayer of the membrane. In the third stage, the proteins form peripheral structure elements or bind prosthetic groups such as retinal in rhodopsin or heme groups in cytochromes.

The outer membrane proteins of bacteria or of cell organelles like mitochondria or chloroplasts, which have a β-barrel transmembrane structure, fold according to other principles. "In bacteria, the outer membrane proteins are transported into the periplasm through the SecYEG transmembrane protein complex, where they bind to soluble chaperones before they assemble into the outer membrane," said Dr. Jörg Kleinschmidt. In cells, the integration of proteins into the outer membrane requires another protein complex. However, the researchers' model experiment showed that various β-barrel membrane proteins can spontaneously insert into certain membranes without requiring extra proteins. "The properties of the membrane lipids are very important in this process," said Kleinschmidt.

With new analysis methods to the goal

In order to analyse the folding of membrane proteins as well as protein-protein interactions, the team led by Dr. Kleinschmidt uses molecular biology, biochemical and biophysical methods. The coupling of the preparation of biochemically labelled point mutants of the relevant proteins and spectroscopic methods like fluorescence or electron spin resonance spectroscopy has proven especially suitable for the researchers' investigations. "For example, we have developed time-resolved fluorescence quenching methods to examine insertion of membrane proteins into membranes containing spectroscopically labelled lipids," explained the researcher from the University of Constance. The conformational changes in membrane proteins can also be investigated in a time-resolved manner using double-labelled proteins. In order to determine whether β-barrel membrane proteins also function after reactivation by insertion and folding into membranes, the researchers perform single-channel conductance recordings on folded transmembrane proteins that function as ion channels. The team also uses other spectroscopic methods, for example circular dichroism spectroscopy to determine the secondary structure of membrane proteins.

Looking for industrial partners for pharmacologically interesting receptors

At present, Kleinschmidt and his team are working with a Danish company that uses aquaporin membrane proteins to produce filters for making highly pure water. "We have provided this company with another membrane porin for tests and developments," said Kleinschmidt, whose research group would be very interested to cooperate with companies, for example to investigate the folding and function of membrane-bound receptors. The researchers from Constance have recently developed a method to isolate milligram amounts of the tachykinin receptor NK_2R. "This receptor is of major pharmacological interest since it is assumed that tachykinins trigger for example the hyperreactivity of bronchia in asthma, irritable bowel syndrome or cystitis," said Dr. Jörg Kleinschmidt. In order to characterise in detail the folding and function of NK_2R, Kleinschmidt hopes to find an industrial partner to finance a doctoral thesis.

Membrane proteins as biosensors

The researchers are also focussed on designing modified or even completely new membrane proteins with new properties. For example, the properties of β-barrel transmembrane proteins can be modified by exchanging amino acid residues or by various other modifications. β-barrel membrane proteins can also be used as biosensors. "Some of these proteins are selective transmembrane channels that enable the transport of soluble substances, for example nutrients such

as sugar, across a membrane," said Dr. Jörg Kleinschmidt.

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