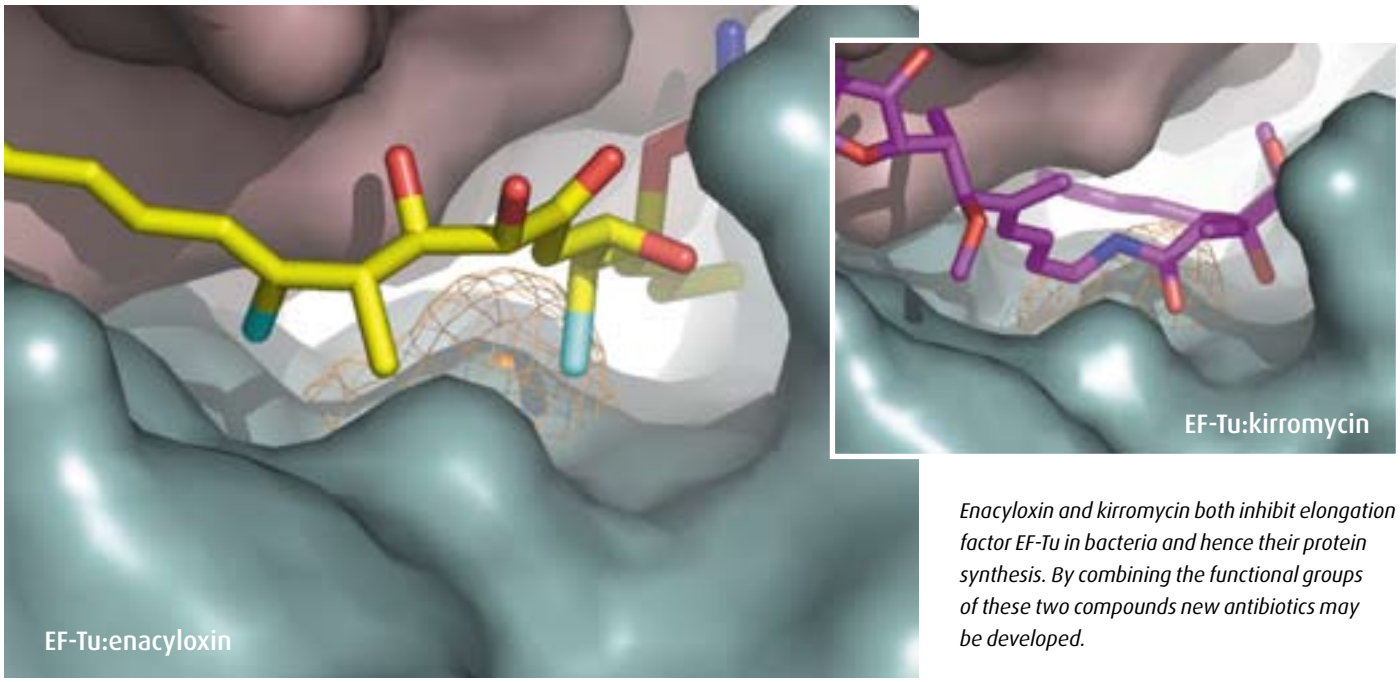
Drug design at the atomic level



Enacyloxin and kirromycin both inhibit elongation factor EF-Tu in bacteria and hence their protein synthesis. By combining the functional groups of these two compounds new antibiotics may be developed.

The atomic structures of promising drug targets complexed with experimental medicines can be revealed by X-ray crystallography. At iNANO such crystal structures are exploited in the development of new antibiotics and a promising prodrug strategy to treat hormone-insensitive prostate cancer.

By Anne-Marie Lund Jensen, Morten Grøftehauge and Poul Nissen

In biology and medicine structure at the atomic level equals function. Drug molecules must fit into their receptors as a hand in a glove to exercise their therapeutic effect, and medicines should only interact with specific targets to avoid undesired side effects. One way to achieve these goals is to determine the three-dimensional atomic structures of drug receptors and analyse their interaction with a drug.

Most drug targets in the human body are proteins, and the Protein Data Bank currently holds about 32.000 atomic structures. They have mainly been determined by X-ray crystallography, which provides a direct three-dimensional image of the molecular structure. X-ray crystallography has become an integrated tool of molecular biology, and new frontiers such as membrane proteins and multicomponent complexes are now being challenged. These are the most common drug targets, and solving their structures may open a bounty-land of opportunities to go from basic research to nanotechnological application, such as rational drug design at the atomic level.

An important question is, however, whether a given protein is at all a useful target for medical drugs? Nature often gives a good indication. An example is the bacterial translation elongation factor EF-Tu, which is indispensable for protein synthesis. Some bacteria such as Streptomyces and Frauteria gain an edge in their environment by releasing EF-Tu inhibitors to fight other bacteria. The interesting point is the attacked bacteria develop resistance only with difficulty because the price of introducing changes in EF-Tu is high, generally making the mutants unfit compared to the wild type bacteria. Thus, EF-Tu is a "proven target" for antibiotics tested in the powerful selection of nature. Drugs that inactivate EF-Tu may therefore circumvent a major health problem of increasing importance - the limited number of available antibiotics to fight infectious multiresistant bacteria.

Another "proven target" is the mammalian calcium pump, which is the subject of our attempts to devise a prodrug strategy against prostate cancer. When the calcium pump does not work properly, the affected cell commits suicide by apoptosis.

EF-Tu as target for new antibiotics

EF-Tu is a universally conserved protein and a critical component of the protein synthesis machinery. It brings tRNA with amino acids, the building blocks of proteins, to the protein factory of the cell, the ribosome. The natural bacterial inhibitors, kirromycin and enacyloxin, impose a specif-

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ic conformation of the EF-Tu protein, which then becomes trapped on the ribosome and blocks protein synthesis, killing the bacteria. However, these and all other known EF-Tu-targeting antibiotics are very difficult to synthesise and not attractive as clinical drugs.

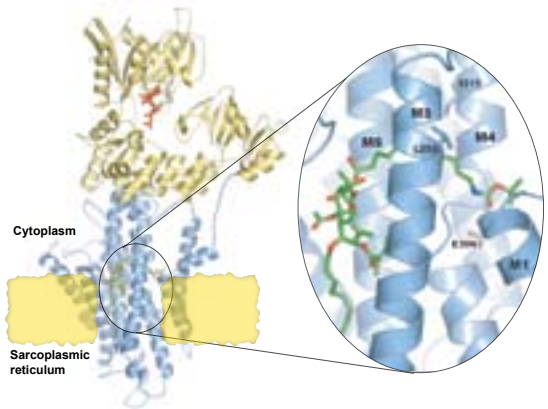
We have been able to determine crystal structures of EF-Tu in complex with kirromycin and enacyloxin, and we observe that kirromycin and enacyloxin bind at overlapping binding sites. In collaboration with the chemists of iNANO we now exploit the design and synthesis of new compounds, combining the functional groups of kirromycin and enacyloxin. In this way we seek to identify a lead compound for the development of new antibiotics.

A prodrug strategy for treating prostrate cancer

The compound thapsigargin is produced by the plant Thapsia garganica, and it is a potent inhibitor of the calcium pump inducing apoptosis in any affected cell due to loss of control of Ca2+-mediated signalling. The plant is therefore toxic and avoided by grassing animals.

Together with local collaborator Jesper Vuust Møller as well as Søren Brøgger Christensen at the Pharmaceutical University of Denmark and international collaborators, we now investigate the prospects of using thapsigargin in a prodrug strategy against prostate cancer. The hormone-insensitive forms of prostate cancer are slowly proliferating and resistant to traditional chemotherapeutics that generally target fast-dividing cells. A new cure is therefore needed. The prodrug strategy is based on the fusion of a thapsigargin derivative with a peptide extension. The peptide makes the compound inactive in the body, but when the peptide is cut off, the drug becomes cytotoxic. Here is the trick: Only an enzyme specific to prostate cells is able to make the cut, and the thapsigargin derivative is therefore only released to kill prostate cells - in the prostate gland or in prostate cancer tissue anywhere in the body.

We have determined structures of the calcium pump complexed with thapsigargin derivatives with the aim of improving drug properties of the active component of the prodrug. The structures identify hotspots that can be further exploited by modification, and we aim at developing new cancer drugs in the future.



The calcium pump, Ca2+-ATPase, is a transmembrane protein. When the pump is inactivated by derivatives of thapsigargin, the cell commits suicide. The binding site of the drug is shown to the right.