Laboratory of Biotechnology at the National Institute of Chemistry

From Molecular Immunity to Synthetic Biology:

Applying Basic Science to Solve Health Problems

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The Department of Biotechnology underwent a significant transformation six years ago, when several researchers from the Laboratory for Molecular Modelling and NMR Spectroscopy joined the Laboratory of Industrial Mycology. The laboratory was later renamed the Department of Biotechnology, as the focus of its research shifted along with the acquisition of new instruments for advanced biochemical techniques and an increase in the number of PhD students. Researchers devote considerable effort to education, as several members have teaching commitments at the Faculty of Chemistry and Chemical Technology at the University of Ljubljana and all of the members participate in student training, particularly at the PhD level, but also at the undergraduate level (see below). The department is continuously engaged in EU projects starting with the 5th Framework Programme in the area of antimicrobial and immunomodulatory peptides and prion proteins, and previously also in engineering metabolic pathways. The departmental programme, "Molecular biotechnology: from dynamics of biological systems to applications", was selected by the Slovenian Research Agency among the top Slovenian research programmes for the year 2005.

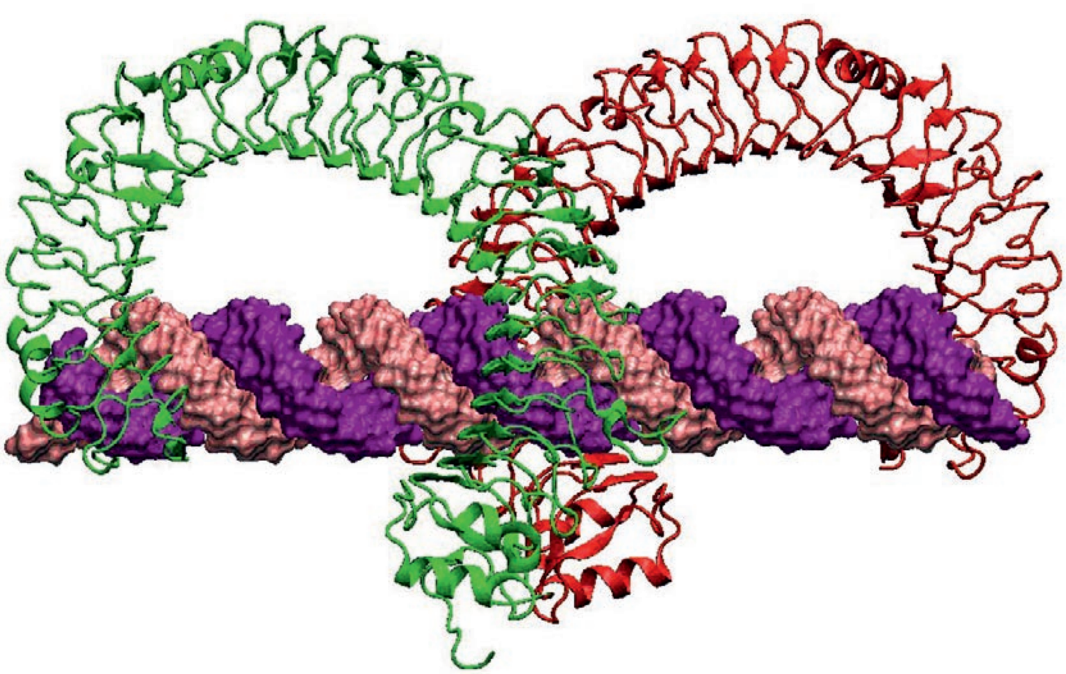

Figure 4: Recognition of viral double-stranded RNA by Toll-like receptor 3 dimer, proposed and experimentally confirmed in the Department of Biotechnology at NIC. The existence of two binding sites on the ectodomain of TLR3 provides differentiation between long RNA duplexes, characteristic of viruses, and endogenous short RNA molecules.

Research topics

Research at the Department of Biotechnology can be broadly defined as biochemical processes, such as biomolecular recognition and cellular signal transduction, which have potential applications for health (biotechnological, medicinal, and pharmaceutical). The main research topics are the molecular mechanisms of innate immunity, mechanisms and design of antimicrobial agents and conformational diseases involving amyloids. Members of the department successfully combine methods of atomic resolution, such as high-resolution NMR and bioinformatics, with techniques of biochemistry, molecular and cell biology, microbiology and biophysics. We are particularly interested in the mechanisms of molecular recognition and signal transduction at the molecular level, especially involving molecular patterns characteristic of pathogenic microorganisms (PAMPs) recognised by pattern recognition receptors (PRRs). To this end, we use instrumental methods with high resolution and sensitivity, such as nuclear magnetic resonance (NMR) and fluorescent spectroscopy using fluorimeters, luminometers, a circular dichroism spectrometer, a fluorescent cell sorter and a laser confocal microscope. Research at the department also includes work on the metabolism of microorganisms, where again we find parallels in the human organism in pathological processes such as cancer.

Molecular mechanism of response to infections

The largest group of researchers is involved in research on immunological recognition of bacterial infections and some other inflammatory processes. Among the cellular receptors that sense infection, we are currently studying TLR3 and TLR4 with its co-receptor MD-2. The TLR4/MD-2 complex senses the presence of extremely small amounts of bacterial endotoxin, which causes a strong response of the organism invaded by the bacteria and can lead to sepsis. Sepsis is the major cause of death in intensive care units throughout the world, with high rates of mortality, particularly in elderly and immunocompromised patients. There is a strong need for effective therapy or prevention of sepsis. It is only in recent years, with the discovery of Toll-like receptors, that the mechanisms of initiation of sepsis are becoming understood. We approach this problem in two parallel ways: the first approach is the study of cellular receptors that recognise bacterial endotoxin, which could lead to the possible inhibition of this process, and the second approach is to design compounds, particularly peptides, that bind to endotoxin and prevent activation of receptors, and would require rational design, based on high-resolution structures determined by NMR.

Several years ago, we pioneered identification of the segments of MD-2 that

Mechanisms of the natural immune system: molecular modelling and biochemistry hand-in-hand unravel how the cellular receptor discriminates between viral and the body's own dsRNA

Our immune system is able to recognise infection by microorganisms by detecting components of pathogens that are different from our own molecules, which is the basis of the innate immune system. Toll-like receptors are a family of cellular proteins that recognise specific molecular patterns of pathogens. Double-stranded RNA occurs in the process of viral replication inside infected cells. On the other hand, our cells contain short segments of double-stranded RNA, which should not, and does not, activate the immune system. How does the body distinguish between long (viral, harmful) and short (its own, normal) RNA? It has been known that TLR3 receptor binds dsRNA, and previous work has determined the crystal structure of the binding domain and binding site for RNA. We reasoned, however, that this mechanism was not satisfactory since it could not distinguish between short and long RNA duplexes. We used molecular modelling and predicted the existence of a second binding site on the extracellular domain of TLR3, which requires dsRNA longer than 40 base pairs for binding and activation, because it must span the distance between binding sites on two TLR3 molecules. Experiments using cell lines expressing mutated TLR3 confirmed this hypothesis, which is very important for the potential design of drugs directed towards better vaccines, antiviral defence, anti-inflammatory or anticancer uses.

Figure 1: Members of the Department of Biotechnology at the National Institute of Chemistry.

are responsible for the recognition of endotoxin. More recently, we have prepared and experimentally tested the molecular model of MD-2, which has been in most aspects corroborated with recent determination of its crystal structure. We have recently characterised natural polymorphisms of MD-2, which interestingly occur only in populations of European descent. One of the polymorphisms significantly impairs cellular response to endotoxin. This discovery is most relevant for the responsiveness of cells that do not produce MD-2, such as epithelial cells in the respiratory tract, which are constantly exposed to high concentrations of endotoxin. We have also identified several inhibitors of MD-2, synthetic and of natural origin, as promising therapeutic targets to prevent inflammation caused by endotoxin.

Antimicrobial and endotoxin-neutralising peptides

The second area of research on preventing the harmful effects of bacterial endotoxin is based on our expertise in high-resolution NMR spectroscopy, and the use of the National NMR centre, with 800, 600 and 300 MHz instruments, located at our institute. We have determined several structures of peptides in complex with endotoxin and in a membrane-mimetic environment that have allowed us to design improved generations of antimicrobial and endotoxin-neutralising peptides that selectively target bacterial membranes. With our collaborators in the ANEPID EU research project, we were able to improve the efficiency of lipopeptides in the animal model by several orders of magnitude, with the best compounds being more effective than the "gold standard" polymyxin B, but importantly lacking its toxicity. As part of our research on antibacterial agents, in recent years we have prepared a set of lipopolyamines which combine antimicrobial and endotoxin-neutralising activity and, in a report with particular resonance outside of specialised circles, we have characterised and determined the target site of the catechin from green tea – we have discovered that it inhibits bacterial gyrase.

Molecular mechanism of prion diseases

Conformational diseases are caused by changes in particular protein conformation, which transforms into a structure that forms aggregates, such as amyloids, that are connected with pathology. Best known among those are prion diseases, which are unique in that replication and transmission to other cells and organisms does not require nucleic acids. We are interested in the molecular mechanism of transformation of prion protein (PrP) with potential application for prevention of pathological transformation of PrP, and for faster and more sensitive diagnosis of infectivity, and are participating within the TSEUR EU research project. Through binding of a compound of natural origin, curcumin, we discovered the possibility of inhibiting the conversion of prion protein by means of binding to the structural intermediate between the native and fibrillised form of PrP. Based on analysis of the spatial structure of PrP and its biochemical properties, we prepared mutants of PrP, some of which were resistant to conversion, while others transform faster, which can be important for health, and also for preparation of a quicker and more sensitive test for detection of infectivity of biological material.

Synthetic biology as the link between research and education

In 2006 and 2007, our researchers mentored student research teams for the preparation of a research project in the field of synthetic biology at the iGEM (international Genetically Engineered Machines) competition. In 2006, we designed and successfully implemented a genetic circuit, which restricts the cellular response to stimulation with bacterial components that can lead to sepsis. This was achieved by inserting a negative feedback loop,

Figure 3: Finalists of the iGEM competition 2006 on the stage at the Kresge auditorium at MIT in November 2006. Imperial College team in blue shirts (second place), Slovenian team in green shirts (first place) and Princeton University on the right (third place). The winning project of the Department of Biotechnology at NIC prepared a genetic device to decrease excessive response to bacterial infection aimed at preventing sepsis.

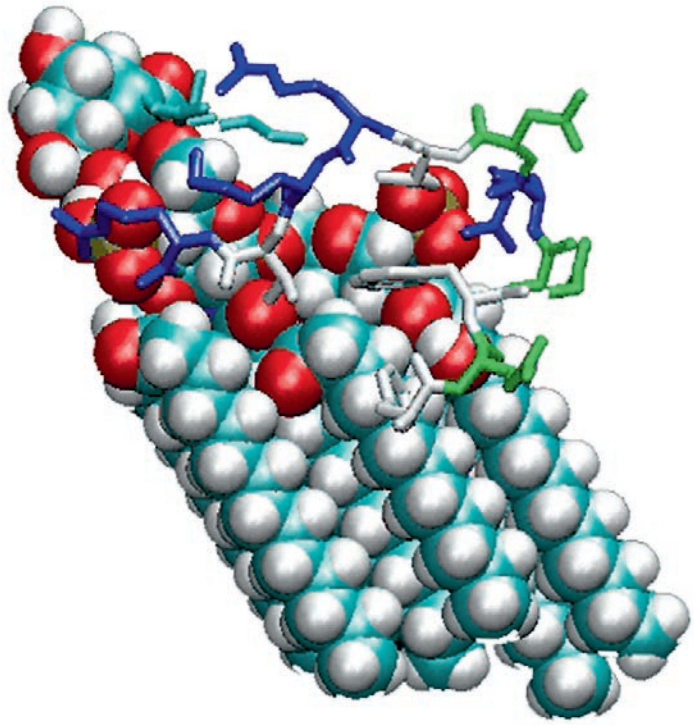

Figure 2: Structure of the antimicrobial peptide LF11 in complex with endotoxin determined by high-resolution NMR spectroscopy. Peptide structure is important for the rational design of peptides with improved biological activity.

activated by inflammation and leading to the inhibition of excessive stimulation. The objective of the project in 2007 was to prepare the defence of human cells against HIV-I virus that would be insensitive to viral mutations. This objective was achieved in such a way that the viral detection was based on the viral function independently of particular amino acid sequences which can mutate, leading to drug resistance or evasion of the immune system (see frame for details). At the 2006 competition, 33 teams participated; this increased to 56 teams in 2007, coming from all over the world to compete at the jamboree at MIT. Among participants were the most prominent universities such as Harvard, Stanford, Berkeley, Princeton, MIT (from the USA); Cambridge, ETH, Paris, Freiburg (from Europe), and Asian teams from Bangalore, Tokyo, Daejong and four top Chinese universities. In 2006, our team achieved an outstanding success by winning the first place (Grand Prize) with a project on a synthetic biology approach towards treatment of sepsis, and in 2007 a new team of students repeated the success by qualifying among the six finalists and winning first place among the projects in the field of Health and Medicine. The success attracted significant media attention in Slovenia and abroad, and contributed to awareness about the importance and capabilities of Slovenian bioscience and university education. The mentors received the Prometheus of Science Award for scientific communication of the Slovenian Scientific Foundation and the Socrates Award for achievements in high-school education.

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