Talking translational genomics

Marie Curie Fellowship recipient **Mahtab Nourbakhsh** has been conducting important research that brings together different strands of investigation into the regulation of cytokine genes, translational genomics and chemical biology. She outlines the timeliness of the study

Can you outline the primary objectives of the 'Antagonists of Protein-Protein Interactions' (APPI) project?

The multidisciplinary project creates a link between disease gene identification through translational genomics, the development of cell-based assays, and chemical biology research. The project has four principal missions: first, we utilise association studies on susceptibility genes of cytokine and cytokine receptors in complex inflammatory diseases or solid tumours. Currently, our main focus lies on End Stage Renal Disease (ESRD), gastritis and gastric cancer. Second, we develop and evaluate *in vivo* and *in vitro* analysis systems for protein-protein and protein-DNA interaction patterns to elucidate the potential function of the identified polymorphisms. Third, we establish cell-based assays relevant to inflammation and Angiogenesis with utility for chemical biology applications. This includes the design of reporter plasmids and their stable integration in relevant cell lines. Following evaluation, we adopt these primary assays to 384-well microtiter plate format high throughput screening (HTS). Fourth, we perform HTS to discover and evaluate biologically active compounds.

Can you shed light on the timeliness of the project? In what sense is the need to understand and develop treatments for inflammatory and infectious diseases greater than ever before?

The need to understand and develop treatments for inflammatory and infectious diseases is immense. Within the next 10 years, many antibiotics currently used for treating bacterial infections will no longer be effective because of microbial resistance. Very few treatments for viral infections exist to date. Recent events have made clear the need for an understanding of the molecular mechanisms of disease. A wide variety of chronic inflammatory diseases, including rheumatoid arthritis and inflammatory bowel diseases, are linked to dysfunctional host responses to pathogens – an abnormal expression or production of cytokines or an irregular reaction to cytokines. This is also manifest in multiple sclerosis, a progressive neurodegenerative disease.

What are the underlying principles and aims of translational genomics? What does it entail, and how have advances made through the Human Genome Project contributed to the development of this field of study?

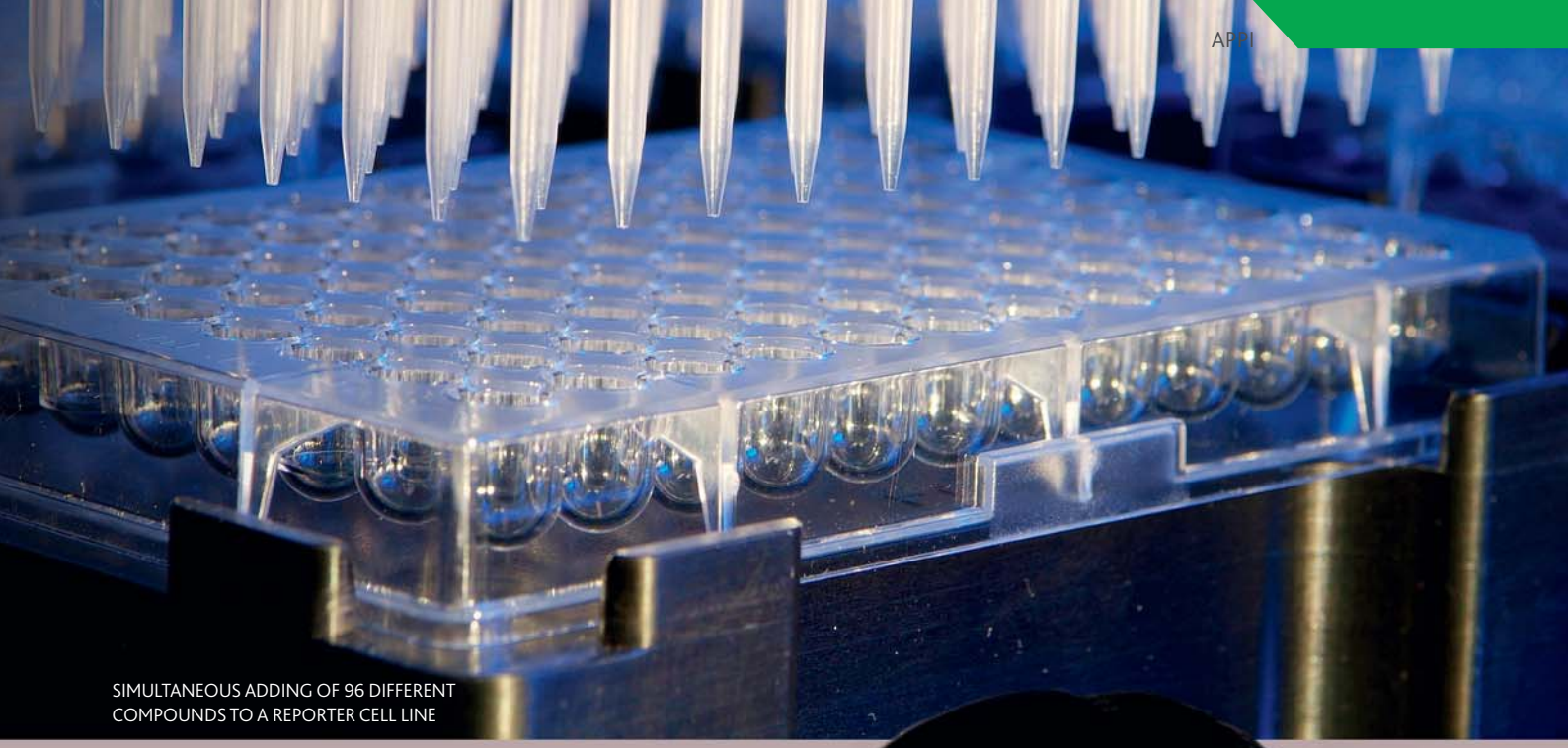
The ability to better diagnose, treat, and ultimately cure disease in the 21st Century will depend on two things: understanding the genetic cause of disease, and the ability to translate this information into new diagnostic tests and therapeutics. Thanks to the mapping of the human genome, clinical practice is shifting from treatment based on symptoms to treatment based on each person's unique genetic makeup – in other words, personalised medicine. Translational research is the process of translating basic scientific discoveries into clinical applications such as new diagnostics and treatments; it serves as a bridge between lab bench discoveries and the patient bedside. Information collected at the patient bedside can circle back to the laboratory to fuel additional discoveries.

As an emerging, post-genomic discipline, what perspective does chemical biology adopt in tackling key problems in the life sciences?

After a disease is assigned to a genetic defect, we need a powerful tool to search for new molecules and drugs to combat the outcome of the genetic defect. Chemical biology is a powerful tool to search for natural compounds and chemically modify them to optimise their effect. Chemical biology is the basis for designing new drugs.

To what extent would you dispute the suggestion that traditional genetic and biochemical approaches are no longer sufficient to process or take advantage of the vast amount of information emerging from translational genomics studies?

The need for traditional methods and technologies in molecular biology and biochemistry, as well as their permanent advancement and improvement, are greater than ever before. A recent editorial in *Nature Genetics* suggested that there should be more significant investment in functional characterisation of identified genetic dispositions, and this requires methods in biochemistry, molecular biology and immunology. So it is generally accepted that identification of genetic polymorphisms which frequently coincide with a certain phenotype or disease is not enough. As simple as it sounds, genetic polymorphisms can be determined, but can't be simply eliminated. The goal is to learn of the direct consequences of a genetic disposition in order to find a new, more powerful and more specific strategy to combat related diseases. The traditional



SIMULTANEOUS ADDING OF 96 DIFFERENT COMPOUNDS TO A REPORTER CELL LINE

methods and their steady development are indispensable in our learning of the consequences of genetic dispositions and the design of new strategies to target a disease.

What do you consider to be the benefits of employing a multidisciplinary approach in your research?

There are many benefits in terms of the outcome of a project, of course. This is based on an additive effect between the disciplines and the principle of collaboration. It's a fact that in all disciplines there is a self-driven tendency for improvement and development of existing methods. In my opinion, the most important benefit of a multidisciplinary project is that the methods, tools and skills, when used together, offer new directions. This is of immense benefit and opens new spheres to everyone in the future and not only for the project participants or the outcome of a multidisciplinary project.

What role has the European Research Council (ERC) played in the development of the project?

This project would not be possible without the generous support of the ERC, which makes an enormous effort to encourage European research institutes to develop better strategies to establish themselves as more effective players in an increasingly competitive global market.

ROBOTIC SYSTEM FOR HIGH THROUGHPUT SCREENING OF COMPOUNDS

Sights on cytokines

The development of cell-based models for protein-protein interactions is a crucial step which could take us closer to new treatments for a number of chronic inflammatory and infectious diseases, including rheumatoid arthritis and multiple sclerosis. The **APPI** project has provided a firm base on which further research can now build

MANY ANTIBIOTICS IN current use are predicted to lose efficacy over time, as microbial resistance increases in the bacterial infections they are intended to treat. Drug-resistant strains of some pathogens have already appeared, and very few treatments for viral infections exist to date. Moreover, several deadly viral agents are on the rise, threatening increasingly large numbers of people worldwide. A number of chronic inflammatory and infectious diseases, including rheumatoid arthritis, inflammatory bowel diseases and multiple sclerosis, have been linked to dysregulated cytokine expression or sustained responses to cytokines. These diseases are extremely debilitating and are becoming increasingly common in Western society. Generally speaking, the investigation and combat of inflammatory and infectious diseases ties in with basic molecular biology, signal transduction and cytokine gene regulation, as the vast majority of cytokine regulatory networks found in cells, are based on protein-protein interactions. Professor Mahtab Nourbakhsh's research seeks to develop indicative cell-based models for protein-protein interactions that cause abnormalities in cytokine expression and cytokine response.

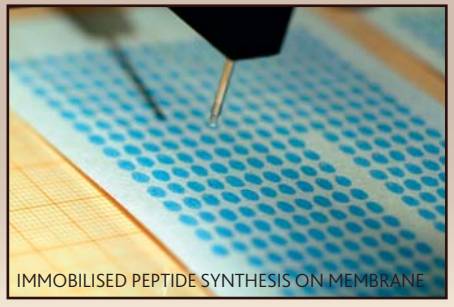
COLLABORATIVE COMMUNITY

Nourbakhsh's project, 'Antagonists of Protein-Protein Interactions' (APPI), has been conducted in collaboration with the Helmholtz Centre for Infection Research (HZI), Braunschweig, Germany, and the Translational Genomics Research Institute (TGen), Arizona, where Nourbakhsh is an adjunct faculty member. The multidisciplinary project has three main prongs:

- Translational Genomics: identification of polymorphisms in cytokine genes associated with End-stage Renal Disease (ESRD) or gastric cancer
- Molecular Biology: functional characterisation of gene polymorphisms by gene expression and protein-protein interaction
- Chemical Biology: development of cell-based assays and search for natural or synthetic compounds

TGen provides the tools necessary both to identify the genes that play a role in hereditable diseases, and to understand the genetic changes contributing to disease progression and resistance to therapy. Through partnering with academic, clinical, and corporate entities, TGen's mission is to deliver these discoveries to the patient bedside as improved healthcare interventions. Its labs are staffed by teams of researchers focused on making genomic discoveries in common diseases and disorders in the areas of oncology, neurogenomics and metabolic disease.

Alongside this, HZI focuses on the programme 'Infection and Immunity', the goal of which is to solve the challenges in infection research, and to make a contribution to public health with new strategies for the prevention and therapy ofinfectious diseases. The discovery of new chemical substances that have the potential to be applied in the fight against infectious pathogens is a primary goal of the Department of Chemical Biology. In pursuit of this goal, researchers at HZI follow the empirical process of combinatorial chemistry and parallel or serial testing (screening), and are also involved in the development of suitable technologies and bio assays for high-throughput screening (HTS).



IMMOBILISED PEPTIDE SYNTHESIS ON MEMBRANE

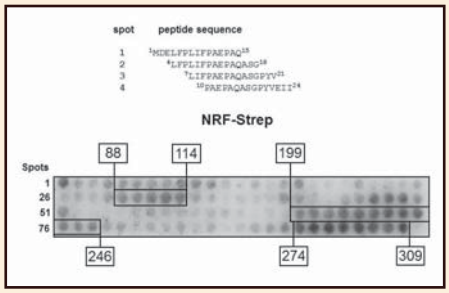
SAMPLES AND TOOLS

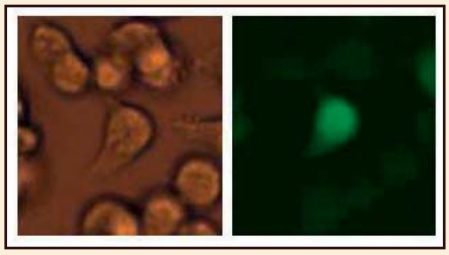
One challenge for Nourbakhsh has been to find the suitable DNA-sample collections within the budget of the APPI project. The Genetics of Kidneys in Diabetes (GoKinD) study supported the project with a DNA collection and clinical information from more than 3,000 adults with long-term Type 1 diabetes, with or without kidney disease, along with their parents. Their contribution was invaluable to Nourbakhsh: "I'm most grateful to the GoKinD organisation who have conducted the collection with no costs," she states with enthusiasm. Another DNA collection with clinical information from 395 adults, including 116 gastric cancer, 142 high-risk gastritis and 94 healthy patients, was provided by The Clinic of Gastroenterology, Hepatology and Infectious Diseases of Otto von Guericke-University Magdeburg, Germany. After collecting the required sample collections, a further challenge was the management of the enormous amount of generated data and their statistical analysis. For this, Nourbakhsh used commercially available data analysis software, SNP & Variation Suite (SVS) from Golden Helix, which runs on conventional desktop computers and is a powerful, integrated collection of high-performance analytic tools for managing, analysing, and visualising large-scale, complex genomic data.

MULTIPLE OUTCOMES

Diabetes is the most common cause for patients requiring renal replacement therapy, accounting for approximately 30 and 45 per cent of cases in Europe and the U.S. respectively. In the GoKinD study, distinct single-nucleotide polymorphisms (SNPs) were identified in CXCL8 and CXCL4V1 chemokine genes, which are associated with measures of kidney function in Type-1-Diabetes (T1D) patients. These polymorphisms lead to an increased CXCL8 and a reduction in CXCL4V1 expression in kidney cells. Following functional characterisation of these polymorphisms, Nourbakhsh designed relevant cell-based assays and screened different compound libraries for small interfering molecules. Two secondary metabolites from myxobacteria were found which inhibit the expression of CXCL8. Currently, Nourbakhsh and her team are preparing patent claims for the medical use of both metabolites.

They have also developed a database that collects accumulating information on altered structural and functional organisation of protein-protein interactions in inflammatory diseases. The team has tried to discover the important protein motifs that are involved in pro-inflammatory protein-protein interactions. Their main focus recently has been the interacting motifs in NF-κB p65 protein, and NF-κB repressing factor (NRF). The NF-κB p65 protein affects important genes which induce inflammation in response to multiple signals, including inflammatory cytokines and bacterial and viral products. The function of NF-κB p65 is regulated by a direct binding and interaction with NRF. The researchers use peptide-mapping experiments to discover interaction motifs. Therefore, they chemically synthesise short fragments of NRF protein, peptides, and immobilised them on a membrane. In the next step, they incubated the membrane with labelled NF-κB p65 protein to detect the interacting regions of NRF. Using an inverse approach, they discovered three protein motifs in NF-κB p65 protein. The researchers transferred the candidate peptides into living cells and measure their effects by inflammation or bacterial or viral infections. Highly effective peptides hint at promising targets for new inhibitors of inflammation.





In Europe and the U.S., 90 per cent of gastric cancers are advanced when diagnosed and 70-90 per cent are attributed to *H. pylori* infection. In her gastric cancer study, Nourbakhsh has used similar approaches to analyse the effect of the associated gene polymorphisms on the level of protein expression and the downstream signalling pathways in gastric cells. The data is very promising and Nourbakhsh hopes that the project will soon lead to the design of new therapeutic strategies.

LOOKING TO THE PUBLIC

As Nourbakhsh is keen to illustrate, the project has also played an important role in fostering and developing the skills of young researchers: "The project offers, but also benefits from, training for PhD students and postdocs in many ways," she states with enthusiasm. APPI's work has involved the development and distribution of educational materials for students, teachers and the general public, about the goals, benefits and chances in translational genomics, and about the prerequisites of this research work. Translational Genomics Research depends decidedly on the willingness of not only patients, but also healthy individuals, to donate DNA samples, as the significance of a genetic variation for a particular disease is measured by its paucity in a healthy cohort population. It is widely held that the more knowledge the general public has about the necessity of – and chances in – this research field, the more likely they are to donate DNA.

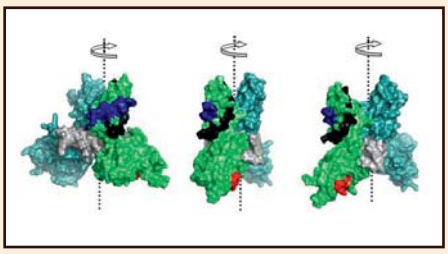
A FUTURE OF POSSIBILITY

The scope of Nourbakhsh's project is much broader than APPI, which has now reached the end of its funding. It has demonstrated its timeliness, relevance and significance not only to the future of research in the field, but to wider implications for future health. It could be the first step towards developing an up-to-date platform for the research activities that Nourbakhsh has been conducting. Whilst this is not planned to take place at HZI, Nourbakhsh is excited about finding a partner for the next phase of the project: "The next goal is to find another European institute which is indeed dedicated to integrate such a platform upon the end phase of the next reintegration grant," she explains. The future looks promising, and we shall watch with anticipation to see how the project develops.

FIGURE 1. PEPTIDE MAPPING OF NF-KAPPAB P65 (LEFT)

FIGURE 2. FLUORESCENCE LABELED PEPTIDES INTRODUCED INTO CELLS (BELOW LEFT)

FIGURE 3. THREE-DIMENSIONAL STRUCTURE MAP OF NF-KAPPAB MOTIFS INTERACTING WITH NRF (BELOW)



INTELLIGENCE

APPI

ANTAGONISTS OF PROTEIN-PROTEIN INTERACTIONS

OBJECTIVES

This project aims to identify and characterise genetic polymorphisms that cause abnormalities in protein interactions, and contribute to cancer and inflammatory diseases. The results serve as a basis for the development of cell-based assays with utility for high-throughput screening and chemical biology as powerful tools for the discovery and evaluation of biologically active compounds.

PARTNERS

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Translational Genomics Research Institute (TGen), Arizona, USA

FUNDING

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PROFESSOR MAHTAB NOURBAKHSH completed her PhD at the University of Braunschweig, Germany, before gaining her Habilitation for Molecular Pharmacology in 2006. She is recipient of the Marie Curie Fellowship for senior scientists, and Research Project Leader at the Collaborative Research Centre 566 at Hannover Medical School.