Featuring cutting edge research projects and enabling technologies of the Netherlands Proteomics Centre
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Frontpage: Stem Cells
Bioartist Charlotte Jarvis meets her ‘second self’ through donated body cells that were transformed into stem cells and eventually ‘active brain’, ‘beating heart’ and ‘flowing blood’ vessels (see Project Ergo Sum, p27).

The Netherlands Proteomics Centre (NPC) is a strategic collaboration of research groups from seven universities, four academic medical centres and several research institutes and biotech companies. With a scientific programme addressing key areas of proteomics in several projects, and specialised ‘research hotels’, the NPC performs high-quality research and knowledge transfer in an international context. The NPC is part of the Netherlands Genomics Initiative.

In NPC Highlights researchers present progress and results from NPC projects of the scientific programme and the research hotels. NPC Highlights is published by the Netherlands Proteomics Centre.

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Welcome  The Netherlands Proteomics Centre (NPC) operates in its last year under the umbrella of the Netherlands Genomics Initiative (NGI). With gratitude and proud I’m looking back on what the NPC community has achieved over the last decade, driven by great enthusiasm of the involved researchers, continuous technological break-troughs and a significant amount of funding from NGI.

At the same time I’m looking forward with great expectations. Proteomics technologies have become a vast part of today’s biological and biomedical research and we have built up a world-wide competitive infrastructure in the Netherlands. This infrastructure and expertise in proteomics will remain available for the Dutch life sciences field and we will sustain our effort to bring together and promote proteomics research in the Netherlands.

The core of the NPC will be continued via the NWO large-scale research facility Proteins At Work. The five-year programme will start in early 2014 and has a 13.5 million euro budget. The aim is to make high-level technology, equipment and expertise for studying proteins in cells and tissues available to biological and biomedical researchers in the Netherlands. You can read more about this programme, which builds upon the NPC and the European large-scale proteomics facility PRIME-XS, on page 24.

In this issue we will also report on some of our highlights, addressing scientific research, valorisation, education, bioart and science communication.

Naturally we are looking forward to the NPC Progress Meeting! On 10 & 11 February 2014 we will once again hold our annual meeting in the Media Plaza, Utrecht. A great number of internationally renowned speakers will give lectures, in addition to other activities. Please visit the NPC website for details of the programme as well as for abstract submission and registration, and mark your calendar to make sure you will be there!

Albert Heck, scientific director NPC
DNA is everywhere

The D&A Store, active throughout the Netherlands from October 2012 till September 2013 and in which the Netherlands Proteomics Centre participated, was a great experience! Divided over 13 locations around the country, including a D&A Store booth at the Vrijmarkt in Utrecht, the D&A store distributed over 4300 personal DNA profiles on T-Shirts and 4900 goodie bags to over 10,000 visitors of the D&A Store.

In addition, the D&A store welcomed over 15,300 visitors on DNAstore.nl and got 18,300 page views on the D&A Store Facebook page. With 41 press reports on the radio, television and internet, the news value of over €130,600 was enormous. With a spectacular ending during the "Nacht van de Kunst en Kennis" in Leiden, the D&A store looks back on a successful campaign, in which they demonstrated to the Netherlands: DNA is everywhere, you are DNA.

The NPC congratulates NPC researchers Duangnapa Kovanic, Adja Zoularu-Djayoon, Pauline Krijgsheld, Christian Frese, Rick van Nuland and Marit Terweij on successfully defending their thesis.

Duangnapa Kovanic, Utrecht University, 13 May 2013
A proteomics investigation of anchored PKA-RI signaling
Adja Zoumaro-Djayoon, Utrecht University, 15 May 2013
A phosphoproteomics view at human pluripotent stem cells
Pauline Krijgsheld, Utrecht University, 4 September 2013
Sporulation inhibited secretion in Aspergillus Niger
Christian Frese, Utrecht University, 6 September 2013
Development and application of novel electron transfer dissociation-based technologies for proteomics
Rick van Nuland, UMC Utrecht, 24 October, 2013
Menin/MLL complexes in chromatin regulation
Marit Terweij, Netherlands Cancer Institute, 5 November 2013
Investigating epigenome dynamics the rite way

The Human Proteome Organisation has awarded its Discovery Award in Proteomic Sciences to Albert Heck, scientific director of the Netherlands Proteomics Centre. The award recognises a scientist for an eminent single discovery in the field of proteomics. Heck received the award on 18 September 2013 at the HUPO World Congress in Yokohama, Japan, because he has implemented innovative mass spectrometric methods with an unique emphasis on protein post-translational modifications and interactions.

Are you a PhD student, a postdoc or a Master student and are you interested to pursue your career in one of the many Life Sciences companies? Do you want to know more about the business field and your career opportunities? Then participate in the unique programme of the international BioBusiness summer school.

The five-day intensive programme of the BioBusiness Summer School introduces you to the business world of Life Sciences. Not only will you learn about all important business topics, you will also have ample networking opportunities and meet many representatives of leading Life Sciences companies. In 2014 the BioBusiness Summer School will be held from June 16th until June 20th.

More information: www.biobusinesssummerschool.nl
On 17 August 2013 Christine Mummery, professor of Developmental Biology at LUMC and project leader at the Netherlands Proteomics Centre, held a lecture on stem cell research at Lowlands University. Hundreds of festival visitors crammed into the Echo Tent for her lecture on groundbreaking results based on this type of research. Mummery showed how she tries to grow slices of human hearts. Not for transplantation, but for testing medicines and for research on diseases of the heart.

Cancer Drug Discovery Initiative

The Antoni van Leeuwenhoek/Netherlands Cancer Institute, Leiden Institute of Chemistry and Pivot Park Screening Centre have signed a letter of intent, committing them to sharing their knowledge and infrastructure under the auspices of the Cancer Drug Discovery Initiative (CDDI). The aim of the CDDI over the next three years is to discover two so-called ‘lead series’ which have the potential to deliver a candidate drug. In addition to the development of new drugs, the CDDI will also be investing in educating and training a new generation of researchers who are specialised in all the latest developments and technologies and who understand the ‘drug discovery process’. Initiators of the project are NPC project leaders Sjaak Neefjes (NKI), Huib Ovaa (NKI), Hermen Overkleeft (LU) and Stan van Boeckel.

Access to facilities of Proteins At Work

In 2012 the programme Proteins At Work, coordinated by Prof. Dr. Albert Heck, has received financing from NWO to set up a large-scale research facility. The project in which various Dutch research institutes collaborate, received 13.5 million euro. As from January 2014 Proteins At Works provides open access to researchers in the life sciences from academia (universities and hospitals) and industry (pharma and nutrition, biotechnology SME’s). Researchers can request access to the facilities via the website of Proteins At Work (www.proteinsatwork.nl). You can also find information on how to apply and submit a project proposal on this website.

Proteins At Work builds upon the Netherlands Proteomics Centre and the European large-scale proteomics facility PRIME-XS.
Trying to grasp the essence of Maarten Altelaar’s research isn’t easy. With his background in analytical chemistry and mass spectrometry and his papers on new proteomics techniques it is tempting to label him a technology and methodology developer, and his role as manager of the NPC Analytical Hotel in Utrecht supports this. But then there is his recently awarded Netherlands Organisation for Scientific Research (NWO) Vidi proposal entitled ‘Network medicine; quantifying proteome wide crosstalk’, which focuses on understanding the interplay (or ‘crosstalk’) between proteins, pathways and post-translational modifications in cancer, particularly melanoma. This is not about theoretical model systems, but rather measurements on actual melanoma cell lines and patient samples. One of the aims is to reveal how these cells develop drug resistance — a real-life clinical problem.

“My research operates on the intersection of biology and pure methodology-technology development,” Altelaar explains. “I think in methods, but I’m always driven by a biological question. Methodology development as such is not my thing.” Biological questions that provoke his curiosity can cover a variety of topics, but he mostly works on neurobiology and more recently cancer. “Through our ongoing collaborations with the Netherlands Cancer Institute, I came into contact with Daniël Peper and his work on cellular senescence and melanoma. It turned out that this offers a very good model for my aim to develop proteomics tools that can measure protein crosstalk. That is why collaborating with different researchers is so important.”

Cellular defense
Melanoma appeals to Altelaar because there are multiple signalling pathways involved in disease onset and progression. The fact that drug resistance is common in melanoma patients points to a large array of ‘rewiring’ options. Apparently, melanoma cells have numerous ways of circumventing the inhibitory effect of drugs by rerouting along different pathways. Interestingly, at the same time cells have several natural defense mechanisms against oncogenic stress. One of these mechanisms, in the case of the common melanoma mutation BRAF V600E, is cellular senescence, which leads to arrest of cell growth, halting tumor formation, and is observed in humans as nevi (birthmarks).

All this cellular maneuvering suggests intensive communication on the level of the proteome. Protein communication often runs via post-translational modifications (PTMs), such as phosphorylation, methylation or ubiquitination. So far, studies of proteome dynamics have primarily focused on changes in protein expression upon a perturbation, but Altelaar aims to broaden the spectrum to include PTMs. “Proteins continuously
“My mind is always busy trying to find ways to realise my ideas. That is why academic research appeals to me”

undergo modifications, but also continuously induce modifications in others. The incredible dynamics of PTMs seriously complicate reliable measurements. Another difficulty is that PTMs are used to activate and de-activate proteins, while the modification itself doesn’t tell you what the function is. You have to measure over time to deduce the effect of a PTM. Currently, we are only able to measure one type of PTM, for example only phosphorylation. That obscures insight into PTM crosstalk. We know one PTM can provoke or inhibit another PTM and therefore one of my main objectives with the Vidi grant is to develop proteomics technologies that will allow us to measure all types of PTMs in coherence. We need these approaches to unravel PTM crosstalk.”

Blocking the highway
It’s tough developing new technologies to study a complex system with partially unknown qualitative and quantitative composition — not to mention one with many interactions between unknowns. Altelaar provides an example. “We take a cancer cell line as our starting point and we induce a perturbation, for example by adding a kinase inhibitor. Then we execute a quantitative proteomics measurement to determine changes in protein expression upon this perturbation and we use phosphoproteomics to determine the direct effect of the inhibitor. Certain PTMs will not occur anymore but others may emerge. With the current technologies, we can map the global proteome to a very large extent, however for PTMs this still remains to be answered. Repeating such measurements at several moments provides stepwise insight into the effect of that particular inhibitor.”

According to Altelaar, a kinase inhibitor is an obvious choice for the source of the perturbation, because genomics-based studies have put the spotlight on kinases as the new targets for cancer drugs. But there may be smarter ways to block cancer cells.

“Kinase pathways are the cellular highways. Blocking a highway creates a huge and immediate effect, but the cell will do everything to find alternative routes, resulting in drug resistance. I think we should concentrate more on ‘downstream’ options, smaller interactions that might seem less important, but which may be much more influential because the cell has no alternatives available. It could very well be that inhibiting a minor player results in a stronger or even permanent clinical effect.”

That ambition brings us back to the essence of his Vidi proposal: mapping proteome crosstalk. “Only when all interactions between proteins and PTMs have been revealed can we use the proteome map to find the smallest alleyways.”

Create your freedom
The main aim behind the Vidi grant is to enable early career researchers to set up their own line of research. When asked why he decided to participate in this competition he seems to be caught off-guard, as if he cannot imagine a reason not to take this opportunity. ”My mind is always busy trying to find ways to realise my ideas. That is why academic research appeals to me; it is the freedom you have to let your own questions be your guide.” Hence declining a job offer from pharma giant Novartis after completing his PhD. “I wanted to at least try to find that freedom for my ideas. When I talked to [scientific director of the NPC] Albert Heck about a postdoc position I quickly knew that I would find the space to work out new ideas.” And his drive to explore new topics will not stop now that he has the means to focus on his crosstalk project. ”You need a broad view. My PhD had nothing to do with proteomics, but I read about it and talked to people in the field. Ideas come from new areas, new contacts. Combining biological questions with technological possibilities remains a source of inspiration. Right now, I am already thinking about the next proposal, the next idea, the next opportunity.”

Career

Maarten Altelaar
2002 MSc Analytical Chemistry, VU, Amsterdam
2007 PhD, AMOLF institute, Amsterdam
2008 NPC Hotel Manager, Analytical Hotel Utrecht
2011 Assistant professor Biomolecular Mass Spectrometry and Proteomics, Utrecht University
2011 Board member of the Dutch society for Mass Spectrometry (NWMS),
2011 PRIME-XS editor for the access site Utrecht

Awards
2013 NWO Vidi grant
2013 ERC interview-support grant Utrecht University (250 k€).
2013 TI Pharma SME Partnership 2012; in collaboration with, PamGene, Pepscan, and the Dutch Cancer Institute (NKI) (495 k€).

Recent key publications
of carbamylated peptides present in the inflamed joints of human RA patients as well as mice suffering from experimental arthritis. To date we have performed full proteome analysis, but we are now also developing reagents that would allow the targeted identification of all carbamylated (and citrullinated) proteins prior to MS analyses in order to study these modified proteins more efficiently.

The combined efforts of experts in serology, immunology, arthritis and mass spectrometry-based proteomics have been successful in identifying the first sets of carbamylated proteins in inflamed joints, providing insight into how antibodies against post-translationally modified proteins contribute to disease processes like arthritis.

Autoantibodies Despite many years of intensive research, the cause of RA remains unknown. However, several biomarkers, especially autoantibodies, have been identified that provide insight into the aetiology of RA. The identification of antibody responses against post-translationally modified (citrullinated) proteins (ACPA) in particular changed the RA landscape [1].
Comparing ACPA-negative with ACPA-positive patients revealed that the latter especially presented severe joint destruction [2]. ACPA are also predictive for future development of RA in patients with complaints about joint pain (arthralgia) [3]. Unfortunately, ACPA are only present in around 60% of the patients. Clearly there is a need for additional biomarkers to identify people at risk of developing RA [4]. Taking into account the specific reactivity against citrullinated proteins in RA, we argued that other post-translational modifications might also be targeted. We selected carbamylation, since this process leads to the conversion of lysine into homocitrulline, a structure chemically very similar to citrulline (see Figure 1).

Post-translational modification (PTM) of proteins in the body is widespread and has an impact on protein function. However, in some cases these modified proteins may initiate an unwanted immune response leading to autoimmune reactions. In 2009, a collaborative NPC project including immunologists, chemists and mass spectrometrist was started in order to obtain comprehensive knowledge about the role of PTM in the initiation and perpetuation of an autoimmune response. “Joint efforts led to the discovery of anti-CarP antibodies, which has been found to be an interesting biomarker for rheumatoid arthritis,” explains Leendert Trouw, assistant professor of Immunology at LUMC. Anti-CarP antibodies bind to carbamylated proteins, which result from PTM of lysine to homocitrulline. This post-translational carbamylation is closely related to post-translational citrullination, which implies the modification of arginine to citrulline. The latter modification has been extensively studied over the past decade and has provided much insight into the autoimmune response in rheumatoid arthritis (RA). Antibodies against citrullinated protein antigens (ACPAs) have been shown to be a useful biomarker for RA. “Unfortunately, ACPAs are only present in around 60% of the patients,” says Trouw. “Therefore, the discovery of anti-CarP antibodies as an additional biomarker has been very promising and might improve our pathophysiological insight.”

Proteomics methods were employed to characterize autoantibody response in RA. “To understand the pathologic mechanism, it is necessary to detect and to identify the antigens to which the autoantibodies can bind in the inflamed joints of RA patients,” explains Peter van Veenen, head of the mass spectrometry group of the Department Immunohematology and Blood Transfusion at LUMC. “By using full proteome analysis we succeeded in showing the presence of both carbamylated and citrullinated protein antigens in joint tissue. Currently, we are developing a targeted mass spectrometric approach to further distinguish these antigens and to specifically identify citrulline and homocitrulline.” Anti-CarP antibodies are already detectable in the years prior to disease. Furthermore, the presence of anti-CarP antibodies is associated with subsequent severe joint destruction. These findings provided promising clinical relevance. We will now further focus our attention on celiac disease and type 1 diabetes,” the researchers conclude.

What this research is about:

**Joint research reveals new biomarker**

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**Figure 1 | Citrulline and homocitrulline.**

Citrullination is the term used for the post-translational modification of the amino acid arginine in a protein into the amino acid citrulline. This reaction is performed by enzymes called peptidylarginine deiminases (PADs). Carbamylation (homocitrullination) involves the non-enzymatic reaction of urea-derived cyanate with free NH$_2$-groups on lysine (Lys) residues to yield homocitrulline. This process can be enhanced in vivo by myeloperoxidase (MPO), the enzyme responsible for the inflammation-driven carbamylation of proteins.
observed that anti-CarP antibodies are already detectable up to 10 years prior to disease onset [Shi, J. et al., article submitted]. In addition, we observed that the presence of anti-CarP antibodies in patients who visit the doctor with joint pain (arthralgia) but not yet arthritis is associated with the future development of RA [6]. Furthermore, we observed the presence of anti-CarP antibodies in children suffering from arthritis, so-called Juvenile Idiopathic Arthritis, JIA [7].

The presence of anti-CarP antibodies in serum samples obtained at the diagnosis of the disease, in ACPA-negative RA patients, was associated with subsequent severe joint destruction as compared to the patients who are negative for both antibodies (Shi et al. PNAS 2011).

Enzymatic treatment is performed with trypsin. During the 20 min iodoacetamide treatment artificial carbamylation might occur. Although we have not been able to detect artificial carbamylation in our procedures, we now use $^{13}$C-urea to exclude any doubt. After data-dependent mass spectrometric analysis of the whole proteome, carbamylated peptides are identified by the database search software. After manual inspection of the carbamylated peptide hits, the candidate peptides are synthesized and the tandem mass spectra of the ‘natural’ peptide and its synthetic counterpart are compared and should be a perfect match.

**Targeted approach** In a second approach, we are currently developing a targeted approach to specifically label (homo)citrulline-containing peptides based on glyoxal based-tagging [10], prior to mass spectrometric measurement. Design of a ‘smart’ label yielding a uniquely recognizable pattern in the mass spectra helps in finding the carbamylated peptides (see Figure 3). Note that the light and heavy labelled peptides coelute. Ultimately, the reagent used in the second approach will be coupled to a solid support to specifically enrich the carbamylated peptides from the complex peptide pool.

We have compared the ‘soft’ tissue of the inflamed joint to the more resistant matrix/cartilage/bone components of the inflamed joint and observed most carbamylated proteins to be localized to the matrix components, suggesting that especially long-lived proteins can accumulate post-translational modifications like homocitrulline. Carbamylated proteins were also identified in samples of human RA patients, and next to a set of modified matrix molecules several plasma proteins were also found to be carbamylated. These hits are currently being used for optimization of the anti-CarP ELISA and therefore, unfortunately, we cannot as yet provide a list of the identified molecules.

**Mass spectrometric characterisation** We have used mass spectrometry to identify the presence of carbamylated antigens in joint-tissue. Mass spectrometric characterisation initially involved the solubilisation of selected clinical material in an adapted FASP2 procedure [9] for full proteome studies, from which carbamylated peptides are selected. In this procedure SDS solubilized joint material, e.g. synovium, is washed on a 30 kDa filter to remove low molecular weight material, which might interfere with downstream mass spectrometric analysis. Following buffer exchange to 8 M urea, iodoacetamide treatment is performed for 20 minutes at room temperature to block free SH-groups, after which the buffer is again exchanged to ammonium bicarbonate.

![Figure 2](image.png) **Figure 2** | Antibodies against citrullinated (ACP) and carbamylated proteins (anti-CarP). In the ACPA negative RA population around 16% are positive for anti-CarP antibodies and these patients display more severe joint destruction as compared to the patients who are negative for both antibodies (Shi et al. PNAS 2011).

![Figure 3](image.png) **Figure 3** | Design of a ‘smart’ label. Specific labelling of a carbamylated peptide with a glyoxal derivative. Mixing of this glyoxal derivative (top left) and its heavy labeled counterpart (4 deuterium atoms $\Delta$=4.025 Da) bottom left), here in a 2 to 3 ratio, allows the generation of specific peak patterns that can be easily picked up from complex mixtures.
Further sub-classification  The identification of antibodies directed against carbamylated proteins in RA now allows a further sub-classification of the heterogeneous RA patient population. Anti-CarP antibodies are present before disease onset and are of predictive value for identifying people at risk of developing RA later in life. The presence of anti-CarP antibodies is associated with more severe joint destruction, but how this is mediated is still unknown. With the identification of carbamylated proteins in the inflamed joints of mice and man, we are beginning to understand how these antibodies could contribute to the pathogenesis of RA.

In addition, the newly identified in vivo carbamylated proteins can serve as novel targets in assays aimed at identifying clinically relevant anti-CarP antibody responses. In addition, research on this post-translation modification represents an interesting test case for the identification of low abundant modifications in a complex biological matrix, both for ‘direct’ mass spectrometric analysis and (targeted) sample pretreatment methodology.

Multidisciplinary research  This project is a clear example of a ‘from bench to bedside’ type of project; a close collaboration of various disciplines (immunology, mass spectrometry, peptide synthesis and chemical synthesis), with clear and applicable results. This approach offers new chances for a better understanding of the disease and the development of diagnostic and prognostic tools for the (early) detection of rheumatoid arthritis.

References
3 Bos, W.H. *et al.* (2010) Arthritis development in patients with arthralgia is strongly associated with anti-citrullinated peptide synthesis and chemical synthesis), with clear and applicable results. This approach offers new chances for a better understanding of the disease and the development of diagnostic and prognostic tools for the (early) detection of rheumatoid arthritis.

Research team
LUMC
From left: Jing Shi, Peter van Veelen, George Janssen and Leendert Trouw.
Not on the photo: Rene Toes.

**summary**

We have identified antibodies against carbamylated antigens. These anti-CarP antibodies are present in 45% of the RA patients and associate with severe joint destruction. The presence of anti-CarP in patients suffering from joint pain is associated with future development of RA. How anti-CarP antibodies contribute to RA is currently unknown, but here we have studied the presence and identity of carbamylated proteins in the inflamed joints of RA patients and mice suffering from arthritis.

Using optimized mass spectrometric analyses, we have been able to identify carbamylated proteins in the inflamed joints of mice and man. Most carbamylation is taking place on long-lived proteins such as matrix molecules but does also occur on plasma proteins. The identified proteins will now be used to develop more specific assays.

Overall we can now conclude that in RA patients we observe the presence of anti-CarP antibodies and the presence of carbamylated proteins in the inflamed joint, possibly indicating that anti-CarP antibodies contribute to the observed joint destruction by binding to their antigens in the joint.
A proteomic approach to identify the key regulators of chemotaxis

A central problem in cell biology lies in understanding how small-scale biochemical interactions generate large-scale organization and cellular structure. Eukaryotic cells move and navigate in gradients of diffusive molecules. This process, called chemotaxis, is a complex cellular process involving a multitude of signalling pathways and molecules. Research on chemotaxis has progressed substantially, mainly through studies targeting specific genes or pathways. Here we describe the use of a more comprehensive proteomic approach to study and identify critical components and missing links in chemotaxis.

Chemotaxis is one of the fascinating processes in modern cell biology. Chemotaxis, or directional movement towards extracellular gradient of chemicals, is fundamentally important for processes as diverse as finding nutrients, tracking bacterial infections, and organizing the embryonic structure [1]. Defects in chemotaxis are critically linked to the progression of many diseases including asthma, atherosclerosis, cancer and other chronic inflammatory diseases. Therefore, it is important to gain further insight into the mechanisms involved in chemotaxis.

Model system Dictyostelium cells resemble small amoebae. Since they move and feed in an amoeboid manner, they represent an ideal model system to study chemotaxis. The organism is genetically tractable with the ease of making gene disruptions and inducible expression, and at the same time it can be grown to large quantities for biochemical analyses.

Most importantly, the key pathways regulating chemotaxis are conserved between Dictyostelium and human. During the vegetative phase of their life cycle Dictyostelium cells are attracted by folate secreted by the bacteria on which they feed. Upon starvation, Dictyostelium cells undergo dramatic changes in gene expression pattern, starting to secrete cAMP and simultaneously sensing and chemotaxing towards the cAMP secreted by neighbouring cells. After about 6 hours an aggregate centre of approximately 100,000 cells is formed around the initiation point (see Figure 1). The cell differentiation finally ends with the formation of a fruiting body composed of a stalk of dead cells with a spore head on top. Dictyostelium cells can detect very low extracellular gradients of chemoattractant [2]. In fact, at the lower limit of chemotaxis only 200 of the 40,000 cAMP receptors present on the surface of one cell are occupied; these are mainly concentrated at the front half of the cell where about 5-fold more occupied receptors are present compared to the back half of the cell. The central question in our research is to understand how the cell responds to such minute chemical signals and which signalling pathways are involved.
During the last couple of years much progress has been made in elucidating the mechanism of chemotaxis [3]. It is now clear that chemotaxis is mediated by a complex network of interconnecting signalling pathways, amplification loops, and feedback control mechanisms. An in vivo study using Dictyostelium mutants revealed a basal signalling module consisting of heterotrimeric and monomeric G-proteins [4], which act as molecular switches cycling between a GDP-bound inactive and GTP-bound active form.

The first step in initiating chemotaxis is the binding of the chemoattractant to cell-surface G-protein coupled receptors (GPCRs). GPCRs are coupled to heterotrimeric G-protein, which are composed of Gα, Gβ and Gγ subunits. Upon ligand binding, GPCRs undergo a conformational change, which enables activation of the heterotrimeric G-protein by GDP to GTP exchange, resulting in the dissociation into Gα-GTP and a Gβγ dimer, both of which can provide activation of the monomeric G-protein Ras at the leading edge. Ras activation is sufficient for the local activation of the cytoskeleton, with F-actin predominantly located at the front of the cell.

**Minimal chemotaxis pathway** During the last couple of years much progress has been made in elucidating the mechanism of chemotaxis [3]. It is now clear that chemotaxis is mediated by a complex network of interconnecting signalling pathways, amplification loops, and feedback control mechanisms. An in vivo study using Dictyostelium mutants revealed a basal signalling module consisting of heterotrimeric and monomeric G-proteins [4], which act as molecular switches cycling between a GDP-bound inactive and GTP-bound active form.

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and myosin filaments at the side and the rear of the cell, resulting in cell polarization and chemotaxis (see Figure 2). Although this work has contributed to the understanding of chemotaxis, it has also raised many new interesting questions. How is the heterotrimeric G-protein cycle regulated? What is the mechanism by which heterotrimeric G-proteins induce Ras activation? What are the other components of the core pathway? How is G-protein signalling coupled to activation of cytoskeletal elements and subsequently cell movement?

Proteomic approach To answer these questions and gain further insight into the mechanism of chemotaxis, we have used a proteomic-based strategy (see Figure 2). In the first step of this approach, recombinant heterotrimeric and monomeric G-proteins were expressed and purified from bacteria as glutathione S-transferase (GST)-fusion proteins. To identify both regulators and downstream effectors, the G-proteins were first loaded either with non-hydrolysable GTP (GppNHp) or with GDP and then used as bait in a pull-out study from Dictyostelium cell lysate. The resulting interactome was analysed by SDS-PAGE and tryptic in-gel digestion followed by LC-MS/MS. The identified proteins included most of the binding partners that were previously published, indicating that the experimental set-up was working. However, in addition to those, we found new putative regulators of G-protein signalling and new putative effectors involved in the regulation of development and chemotaxis. Subsequently we confirmed the interactions of a subset of proteins and characterized their function by biochemical and genetic analysis using the Dictyostelium model.

Crucial role for Ric8 In the screen performed with Gαi proteins as bait, we identified a Dictyostelium homologue of human Ric8 as potential binding partner [5]. Although the exact function and mechanism of mammalian Ric8 is unclear, recent studies have shown that it is implicated in the Gαi mediated regulation of olfactory signalling, neural function and embryogenesis [6]. Biochemical studies showed that Dictyostelium Ric8 binds directly and specifically to free Gαi-GDP and that Ric8 stimulates the exchange of Gαi-GDP back to the active GTP-bound form. In this way Ric8 enhances receptor-mediated activation of heterotrimeric G-proteins, resulting in amplification of the perceived signal.

Figure 2 Basal chemotaxis pathway. Binding of a signalling molecule to the cell-surface receptor results in the activation and dissociation of the heterotrimeric G-protein complex, which provides prolonged activation of Ras and F-actin at the leading edge of the cell, thereby inducing chemotaxis. To understand the model completely, it is essential to identify regulators and downstream effectors of Gαi and Ras proteins.

In vivo studies revealed that Ric8 is critical for G-protein activation and breaking of Ras signalling symmetry (hence inducing more Ras activation at the front of the cell than at the back) during development and chemotaxis in Dictyostelium (see Figure 1 and Figure 3). These findings emphasize the importance of mammalian Ric-8 in the regulation of G-protein signalling and suggest a similar function and mechanism of Ric8-mediated excitation. Furthermore, it will be interesting to determine whether the same role and mechanism hold true for other critical mammalian G-protein mediated processes.

New Rap effectors Rac and Rap are small G-proteins belonging to the Rho and Ras families of monomeric G-proteins, respectively. They are involved in development and chemotaxis in Dictyostelium, but their exact role is not fully understood. RapA is essential and it is implicated in several processes throughout the life cycle. In early development and in chemotaxis competent cells, RapA induces pseudopod formation by activating PI3K, and it regulates substrate attachment and myosin disassembly via the serine/threonine kinase Phg2.

RapA is also important in late development. However, until recently nothing was known about downstream effectors of RapA playing a role in the progression of multicellular development. In vivo experiments, on the other hand, had shown that cells expressing constitutively active RapA also exhibit a high level of Rac activation. Rac proteins and Rho G-proteins in general were initially implicated in actin-regulated processes; more recently, however, Rho has been shown to act in numerous other pathways as well, including cytokinesis and development.

Based on a cellular pull-out with active RapA as bait, we identified the Rac guanine nucleotide exchange factor, GxcC, among the interacting proteins [7]. GxcC binds directly and specifically to active RapA; subsequently it binds and activates a subset of Rac proteins. With these experiments we were able to demonstrate that GxcC provides a direct link between the two important G-protein pathways Rap and Rac in Dictyostelium. Most importantly, deletion studies revealed that cells lacking GxcC have severe developmental defects, suggesting that this pathway is the first downstream node in the regulation of multicellular development of Dictyostelium cells.
Promising approach  Chemotaxis is a very complicated process involving a complex network of signalling pathways and molecules. The research work presented here shows that the use of Dictyostelium as model system, in combination with affinity-purification and mass spectrometry based proteomic, provides an excellent strategy to gain new insights into the molecular mechanisms underlying regulation of chemotaxis and cellular development.

Figure 3 | Ric8 regulates activation of Gα-proteins. Ligand binding leads to activation and disassociation of heterotrimeric GDP-bound Gαβγ into Gα-GTP and Gβγ. Due to intrinsic GTPase activity Gα-GTP is converted back to Gα-GDP. Ric8 binds to Gα-GDP before it can reassociate with Gβγ to form the inactive heterotrimer, facilitating its conversion back to the active form, thereby extending Gα activation. Ric8-mediated activation of Gα is critical for development, chemotaxis, activation of Ras and symmetry breaking in Dictyostelium. A comparable mechanism could hold for similar mammalian G-protein mediated processes.

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This protein research project is a collaboration between the Department of Cell Biochemistry and NPC Analytical Hotel Groningen.

summary  Genetic and biochemical techniques have been used over the years as tools to study the detailed mechanism of chemotaxis. Although these studies have resulted in the identification of several key signalling pathways, many important molecular nodes remain unknown to present. In this report we describe a simple but effective proteomic approach to identify these missing links.

By using different recombinant purified G-proteins as bait in pull-down screens from Dictyostelium lysate followed by protein identification by mass-spectroscopy, we have identified the protein Ric8 as a non-receptor activator of Gα-proteins.

Ric8 amplifies Gα signalling, which is essential for chemotaxis and development. Additionally, using the small G-protein Rap as bait, we were able to discover the protein GxcC, which plays a fundamental role in cellular development by linking the Rap and Rac mediated pathways. A proteomics approach is essential for the identification of new key regulators and downstream effectors of G-protein signalling and will provide more complete insight into the complex signal transduction mechanisms regulating chemotaxis and cellular development.
Affinity purification combined with mass spectrometry (AP-MS) has become a popular technique to study protein-protein interactions. However, discrimination between specific and non-specific interacting proteins can be difficult since the latter are often highly abundant. A database has recently been set up from these negative (AP-MS) controls originating from twelve different laboratories. This database can now help researchers find the genuine protein-protein interactions in an objective way.

Affinity pulldown using epitope-tagged proteins is a well-established biochemical technique to identify protein-protein interactions [1]. Mass-spectrometry, on the other hand, allows minute quantities of proteins to be identified and is beginning to replace the western blot for protein detection. Combining affinity purification (AP) with mass-spectrometry (MS) has therefore become increasingly popular. However, one common pitfall for AP-MS is the large number of co-purified nonspecific interactors. To discern bona-fide interactors from these background contaminants, ‘negative control’ purifications are usually conducted. These ‘mock’ purifications are performed with the same epitope-tag, support resin and cell line, but without expression of the ‘bait’ proteins. Generally, the number of negative controls in one experiment is insufficient to comprehensively capture and characterize all these contaminants. The second problem arises from the lack of a convenient and universal scheme to score and rank each interaction. Until a short time ago, there was no unified measure to objectively select genuine protein interactors from a list of proteins identified from AP-MS experiments and thereby avoid ‘cherry-picking’. Moreover, a common scoring system would standardize the comparison of AP-MS data generated at different times or from different laboratories. The CRAPome was created precisely in order to tackle these two issues [2].

CRAPome is an international collaborative effort involving twelve proteomics labs. CRAPome is an acronym for ‘Contaminant Repository for Affinity Purification’. It is a database (www.crapome.org, see Figure 1) that collects and annotates negative controls generated by affinity purification (AP) coupled with mass spectrometry (MS) experiments. CRAPome also provides a service that allows users to upload their own AP-MS data so as to score identified protein interactors against negative controls, which can either be derived from user-generated or database-derived experiments.

Improving AP-MS experiments Firstly, the CRAPome accommodates a comprehensive collection of background contami-
“Protein-protein interactions are essential for virtually every process in a living cell. Therefore information about these interactions is crucial to our understanding of cellular processes and diseases,” according to Teck Yew Low from the NPC. There are many methods to detect these interactions, but a widely-adopted approach is ‘affinity purification- mass spectrometry’ (AP-MS). Although it is a very good method, it is not very easy to establish which of the identified proteins are real interacting partners because there are often a lot of non-specific binding proteins. The first step of this method, affinity purification, involves fishing with a protein of interest (the bait) in a lysed cell mixture. Putative binding partners (prey) will stick to the bait and non-specific binding proteins can be washed away. In the second step the prey proteins are disengaged from the bait and analyzed by mass spectrometry to determine their identity.

"Not all non-specific proteins can be washed away in the affinity purification step because some of these proteins occur in high abundance in a cell and tend to stick to everything, no matter how much you wash," says Low. "Negative control experiments are not sufficient to filter out all contaminants. Therefore deciding what the threshold is between specific and non-specific remains subjective." To help researchers deal with this problem, two scientists, Anne-Claude Gingras from Toronto, Canada and Alexey Nesvizhskii from Michigan, USA decided to set up a database with AP-contaminants. This database called "Contaminant Repository for Affinity Purification" (CRAPome) now contains data from 360 experiments from 12 different laboratories.

The Netherlands Proteomics Centre is one of the laboratories that provided data to CRAPome and served as a beta-test site for the optimization and proper use of the database and its software tools. Low: "The CRAPome database now allows researchers to upload their own data and controls and compare them with the controls from the database. Software scoring tools from the database help to decide more objectively between genuine interacting and non-specific binding proteins.” Low hopes that in future more researchers will upload their data to the database in order to improve the CRAPome.

Creation of CRAPome CRAPome collects studies from twelve laboratories in the form of 360 raw MS files. These raw files are processed with a uniform analysis pipeline prior to association of metadata covering fourteen controlled vocabularies (CVs). Mapping of the protein identifiers to NCBI Gene IDs is performed for each experiment, and spectral count information is parsed to the relational database. This architecture also allows for further expansion in the future by incorporating nants from ‘negative controls’ associated with a variety of purification schemes and sample sources that come from different laboratories. This prior knowledge is thoroughly characterized and is carefully annotated based on fourteen categories such as organisms, cell/tissues types, affinity-support, epitope tags etc., so that users can make a variety of choices to evaluate their own protein interaction data. Secondly, it also incorporates a scoring scheme called Significance Analysis of INTeractome (SAINT) score [3] as well as a more generic Fold Change (FC) score, which allows users to score their current data against their own negative controls and/or the prior knowledge.

Useful database for analysis of protein-protein interactions

CRAPome helps improve AP-MS experiments

What this research is about:

Figure 1 | Website. The repositories are currently available for Homo sapiens and Saccharomyces cerevisiae. The computational tools are freely accessible at http://www.crapome.org/.
more negative control data.

Abundant proteins By correlating quantitative proteomics data from HEK293 cell line with the frequency of occurrence of each identified protein within the CRAPome datasets, we found that high abundance proteins tend to occur in higher frequency, meaning that high abundance proteins have a higher propensity to be background contaminants. Not surprisingly, this small group consists of keratin, cytoskeletal proteins such as actin and tubulins, translation elongation factors and histones, and they appear in more than 50% of the CRAPome datasets regardless of experimental conditions. We also discovered that CRAPome data primarily cluster according to experimental conditions. This means that a large proportion of these contaminants differ from one set of experiments to another depending on, among other things, the cell/tissue types, subcellular fractions, the epitope-tag and the affinity resins used.

In characterising these background contaminants, one therefore needs to consider both the overall frequency of detection and the experimental conditions. Thus, based on important determinants of background behaviour, all experiments in the CRAPome can be annotated using fourteen CVs. These fourteen attributes allow users to select the most appropriate negative controls to evaluate their experiments. Apart from the frequency of occurrence in the CRAPome and experimental conditions, another critical parameter to consider is the abundance level of the candidates. For example, if a protein is detected at a high frequency (many CRAPome datasets) and low abundance (low spectral counts) in the CRAPome, but is detected with a high spectral count in bait purifications performed by a user, it is more likely to be a true interactor than if it is always detected with high abundance in the CRAPome.

Workflows Researchers can access the CRAPome web resource in three workflows:

1. Query selected proteins
   Users can submit queries consisting of protein or gene identifiers and retrieve summaries of the occurrence of queried entries.

2. Create contaminant lists
   The users can select the list of desired controls and download the resulting tables of contaminants.

3. Analyze user data
   The users can upload and analyze their own data using selected CRAPome controls and/or their own controls. The users then upload their data in the specified format (or use previously uploaded data). Upon selection of baits and controls, analysis is performed with SAINT and/or a simpler Fold Change calculation. These scoring tools create lists of interacting partners, ranked by confidence. Previously

Figure 2 | The CRAPome project.
1. First, raw mass spectrometry files for negative-control runs as well as detailed experimental protocols and mapping information were submitted by individual contributors to the CRAPome administrator. 2. Raw mass spectrometry files are first converted to mzXML and analyzed by X!Tandem and the Trans-Proteomic Pipeline (TPP); counts are extracted for protein quantification, and the CRAPome administrator performs a quality-control check. 3. Released high-quality runs (data) are associated with experimental descriptions and protocols (metadata) by the CRAPome administrator in consultation with the data provider. 4. The CRAPome database is queried by external users via the web interface.
(Adapted from Mellacheruvu, D. et al., see [2])

Figure 3 | Overview of the CRAPome workflow.
1. Proteins are queried against the CRAPome by inputting one of several identifiers, which are mapped to corresponding gene symbols. Different views enable exploration of the contaminant profile of each queried protein, either as a summary table (2) or in graphical formats (3).
(Adapted from Mellacheruvu, D. et al., see [2])
reported interactions documented in the interaction database aggregator iRefIndex (version 9.0) are also mapped onto user data.

Scoring schemes The CRAPome implements two complementary scoring strategies based on quantitative comparisons of prey abundance levels in bait purifications against the distribution of prey abundances across a set of negative controls. While SAINT performs advanced statistical modelling of the input bait-prey spectral count data and reports a posterior probability of true interaction, a simpler FC calculation is based on computing the ratio of average normalized spectral counts in bait purifications versus negative controls. Besides the standard FC score (FC-A), a secondary, more stringent score (FC-B) is also computed. Both FC and SAINT calculations are run in parallel using the facile CRAPome interface, and comparison of their relative performances for each of the tested baits can be assessed by a Receiver Operating Characteristic (ROC) analysis provided via the CRAPome interface.

Role of NPC researchers NPC researchers – Vincent A. Halim and Teck Yew Low – contributed a substantial part of the CRAPome data [4, 5, 6]. Together with Shabaz Mohammed and Professor Albert J.R. Heck, as well as the CRAPome project coordinators located in Michigan and Toronto, they implemented the metadata, protocols and experimental annotation. NPC also served as beta-test site for the optimization and proper use of the CRAPome. Among the contributory datasets from NPC, one originated from work that led to the discovery of the role of Lgr5 in enhancing Wnt signalling, a pathway that is intimately linked to cancers and the adult stem cells.

Room for improvement While negative controls for AP-MS experiments are often viewed as supportive data that are disposable, we think that it is actually a gold mine for the interactomics community. Significantly, each epitope-tag negative control is generic, hence useful for filtering the background from any bait protein subjected to the same purification schemes. By systematically organizing and characterizing these generic controls performed under different conditions by different labs, we can actually obtain useful information to enable us to make well-informed plans and post-acquisition analysis of our AP-MS experiments. Thus, the CRAPome facilitates interactomics research by providing prior knowledge regarding contaminants, besides standardizing the scoring and evaluation process for each candidate.

Of course there is still room for improvement. In the current version, spectral counting is used as the sole quantification tool. Extension of the system to other types of quantification such as MS1 intensity-based quantitation would further discriminate between background contaminants and true interactors. Moreover, to make the CRAPome a truly universal resource, we anticipate that more researchers will contribute their negative control data to the CRAPome. This would certainly improve the comprehensiveness of the database in addition to expanding the current species beyond H. sapiens or S. cerevisiae.

References

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Research team NPC researchers – Vincent Halim, Teck Yew Low, Shabaz Mohammed and Albert Heck – contributed a substantial part to the CRAPome project. They implemented metadata, protocols and experimental annotation. NPC also served as beta-test site for the optimization and proper use of the CRAPome.

summary
Affinity purification coupled with mass spectrometry (AP-MS) is a widely used approach for the identification of protein-protein interactions. However, for any given protein of interest, determining which of the identified polypeptides represent bona fide interactors versus those that are background contaminants (for example, proteins that interact with the solid-phase support, affinity reagent or epitope tag) is a challenging task. The standard approach is to identify nonspecific interactions using one or more negative-control purifications, but many small-scale AP-MS studies do not capture a complete, accurate background protein set when available controls are limited. Fortunately, negative controls are largely bait independent. Hence, aggregating negative controls from multiple AP-MS studies can increase coverage and improve the characterization of background associated with a given experimental protocol. A database with negative AP-controls called the contaminant repository for affinity purification (the CRAPome) was therefore set up. The benefits and use of the database for scoring protein-protein interactions are described.
Omics2Image wins Venture Challenge Spring 2013

25,000 euro for IonPix camera that makes molecular photo’s

The Dutch Life Sciences SME Event on June 14 proved to be a successful day for life sciences venture Omics2Image. The start-up company, founded by NPC theme leader Ron Heeren, was named the winner of the Venture Challenge Spring 2013 and took home 25,000 euro to further develop their venture. Omics2Image created the IonPix camera that makes ‘molecular photo’s’ and detects 262,000 mass spectra simultaneously.

The finalists of the Venture Challenge were each granted one minute to pitch their business plan during the Dutch Life Sciences SME Event in Maarssen. The start-up companies ranged from automated image analysis, pain relieve for arthritis patients, to a sustainable way to turn coffee waste into food compounds. The jury, composed of investors and experienced biotech entrepreneurs, judged their business plans on product, IP position, market needs, team, presentation and ability to answer questions from the jury.

Omics2Image did best, was the jury’s verdict. “There is a great need for mass spectrometry that is faster and more accurate. They can complete in 6 hours what normally takes 95 days, that’s a huge difference,” chairman Carine van den Brink said afterwards. “Omics2Image has a great combination of background in tech development and business knowledge. They already have a product and have the power to market it.”

The camera that Omics2Image developed can detect more molecules than ever before, in an instance. “We take molecular photo’s of tissue, for example from breast cancer”, Ron Heeren explains. “To investigate which type of tissue it is, researchers normally use a labeling technique, which only detects one protein a time. Our IonPix Camera makes a picture and detects all molecules at once; not only the proteins, also the lipids, fatty acids, the DNA molecules, and many more.” The IonPix camera is an add-on for existing mass spectrometers or can be integrated in a company’s own device to enhance the system and make it more sensitive and remarkably faster. “We’ve already sold one.”

The team gained many new insights, even though the company was already founded, says Odile Basedow, who is also part of the Omics2Image-team, along with Chris Rétif and Hans Roeland Poolman. “Our marketing strategy was significantly improved during the Venture Challenge. We particularly learned a great deal on finance. The experts that contributed to the challenge gave us a clear overview of what is needed to run a successful company.” The contribution of fellow contestants is also very relevant, Basedow says. “Everyone is focused on their own ventures; questions from other teams keep you alert and make sure you tell a comprehensible story.”

The Omics2Image team received a cheque of 25,000 euro to further develop the future. From left to right: Hans Roeland Poolman, Ron Heeren, Odile Basedow and Chris Rétif.

About the Venture Challenge:
The Venture Challenge offers advice and coaching on essential elements for setting up a life sciences business. In two 3-day workshops, participating teams are continuously challenged by the facilitators and each other to strengthen their business plan and improve their pitch. The team that presents the best business plan is awarded 25,000 euro to further develop their venture.
BioBusiness Summer School

Each summer since 2008, Hyphen Projects has organized the BioBusiness Summer School in Amsterdam for life sciences students to learn more about potential career opportunities and avenues open to them. The intensive 5-day programme schedules lectures from industry experts, recruiters and subject specialists. This year the Netherlands Proteomics Centre helped some of their researchers to attend, and we spoke to two of them - Gemma Moiset (University of Groningen) and Roel Wilting (Netherlands Cancer Institute).

The first talk was by Prof. dr. Eric Claassen, who gave an ‘Introduction to Biobusiness’. He describes himself as "10% Academic - 90% Entrepreneur," and Gemma said she “really liked his enthusiastic talk” and the way he gave “a very good overview of the biotech industry emphasising the importance of the relationships with the academic world”. Roel took away a similar message, telling us, “Summer School made very clear that in the process from scientific discovery to a product on the market, academia and industry need each other … [as] initial scientific discovery must be followed by very long and costly developmental and testing phases before most products become available to the public.”

Of the other lectures over the week, Gemma felt that the most interesting part for her was the one-on-one talks with experts from a range of Biotech areas. She talked to Peter Bartens, Senior Policy Advisor at Nefarma, and Henk Vietor, Managing Director at Drug Discovery Factory, who shared their career experiences, answered questions and gave her some sound advice. Roel enjoyed hearing from Dr. Edwin Moses, CEO of Ablynx, who told an informative timeline of the financing of the company, from start-up, to IPO and beyond.

Understanding small companies
For Roel, the school offered him “a great insight into what kind of life science businesses exist, what kind of products they develop, what their business models are, and how the world of life science companies works.” As a researcher, he found it very helpful to get an understanding of “how it works on the other side.” He says that he was surprised to learn that “it is the small start-up companies that come up with the most innovative and revolutionary ideas and products, which are bought by the big pharmaceutical companies when the products seem to be really working.”

Overall, the programme was very helpful to Gemma in making decisions about her future career and helped shaped her opinion of working in industry. Beforehand her opinion “was that companies only care about the money, and this deprives scientists of the freedom to perform good research.” After hearing the talks however she says, “now I understand that while ‘money is king’ companies must heed the needs of the society.” What’s more, she tells us, “I now feel more confident and have a better overview on how things work in the Biotech industry. I would definitely recommend it to all PhDs and postdocs that are wondering how it is on ‘the other side’.” Asked if there are any changes that Roel would recommend for the BioBusiness Summer School to make for next year, he said, “It was very well organised with plenty of really interesting speakers and I learned a lot during most sessions, so basically nothing much should be changed, except maybe turning the airconditioning down a bit.” Thus, we congratulate Hyphen on another successful year, but also throw down the gauntlet for climate control.

Ample networking opportunities and interesting meetings with representatives of leading Life Sciences companies.
Working in Industry

Academia or industry? That’s the big question facing young researchers as they look to take the next rung on their career ladder. At the Netherlands Proteomics Centre, there’s a pretty even split — last year 43% took a job in the industrial sector while 57% continued in to pursue academic work. Though there are similarities between them, in many ways they are worlds apart, with differing project durations, working group size and focuses. So how does one decide?

We spoke to two researchers to find out more. Ioana Barbu works at DSM Food Specialties as an Associate Scientist Analysis, and we asked her why she made the move to industry. "While there is very interesting research to be done in academia, often you are working alone. I missed working in a team and this was one of the most important reasons for me to move to industry."

The type of work differs too, of course — Ioana now works on a number of projects simultaneously, some of which are short-term, and some of which are ongoing. Overall, she says: "This variation of projects and the fact that they do not last for years is a big change. I prefer it, it gives me more satisfaction."

Advantages to each

Rebecca Burnley (formerly Rose) is in a slightly more unusual position, as she remains a Postdoctoral Research Fellow although she is now employed within the discovery research department of the pharmaceutical company UCB, so she has a foot in both the academic and industrial worlds. "I work alongside scientists who are focussed on driving the projects in our pipeline, but my focus is on distinct areas and specific projects with a strong research flavour." On how it differs from the academic environment of her previous position at Utrecht University, Rebecca says: "In industry, you are a smaller part of a larger team, but often working more directly towards a very specific and exciting real-world application," and the benefit of this is that the end goal is "a very meaningful and important therapeutic product."

Of course, many positions in industry are more steadfastly separated from academic research than Rebecca’s, so we asked her what she might miss if she were working purely in an industrial setting. "I am personally interested in quite fundamental science, as well as its applications, and there can be less opportunity to perform ‘pure’ research studies [in industry] … I would most miss the freedom to follow my own research path.” However, she counterpoints this with the argument that academia lacks the resources of industrial work, and that without the targets that come with commercial territory, one can be "less likely to make a direct or timely impact in a meaningful application or product."

We asked both Ioana and Rebecca what they would say to others deciding which trajectory to take their career in. Both agreed that it is a difficult decision to make, with Ioana advising that researchers “should try to talk with as many people as possible that have a job similar to the one they desire.” Rebecca concurred that it is a very individual process, and "particularly important to find a role that suits you,” especially since "some roles are very flexible and varied and have a large element of creative thinking, research and investigation [whereas others] are more routine and methodical, requiring organisation and very precise and reliable lab skills.” In conclusion then, it is a tough choice, but one that can be very rewarding.
Over the past ten years, many researchers filed a patent, based on knowledge that was developed during their NPC research project. What happens to these patents when filed and when does it lead to an actual patent? An investigation revealed that from all patents resulting from the first phase of NPC (2003-2008), 26% is granted, 30% is abandoned and 44% is still pending. For the second phase of NPC (2009-now) 12% of the patents is abandoned and 88% is still pending for approval. Patenting of an invention is a long and costly process. When looking at the patents that are granted, it took between 2 and 7 years from the initial filing date (priority date) to the date that the patent was awarded. The granted patents are all owned by different companies, which is a good sign: the granted patents have found their way towards commercialization.

- Granted: 26%
- Abandoned: 30%
- Pending: 44%

**Status of NPC Patent applications (2009-2013)**
- Abandoned: 12%
- Granted: 88%

Patenting of an invention is a long and costly process. When looking at the patents that are granted, it took between 2 and 7 years from the initial filing date (priority date) to the date that the patent was awarded. The granted patents are all owned by different companies, which is a good sign: the granted patents have found their way towards commercialization.

Funding options

Are you looking for a valorisation grant, financial support for your start-up company or grants for Public-Private Partnerships? We have made an overview for you with available grants and deadlines. Please visit our website for more information. www.netherlandsproteomicscentre.nl/funding

**LSH Plaza: Frontrunner in Public-Private Partnerships**

The Netherlands is a frontrunner in Public-Private Partnership (PPP), working on innovative solutions for health challenges. The PPP is not a goal in and of itself. Rather, the PPP is founded on the belief that today’s solutions require multiple organizations to work together. PPPs combine top research groups from universities, research institutes and academic medical centers with global companies, medium-sized enterprises and high-tech start-ups to improve therapies — often with support of health foundations — and to boost life sciences research and development. The sector has the ambition to contribute to affordable, high-quality healthcare. The figure below provides links to a number of PPPs.

PFPs: **String of Pearls, Lifelines** (in Dutch)
- Patient information
- Biobanks
- e-Health
- Clinical Decision Support

PFPs: **CTMM, Cytrion**
- Molecular Imaging
- Lab-on-a-chip Diagnostics
- Minimal invasive technology

PFPs: **TI Pharma, Virgo, NeuroBisik**
- Medicines
- Vaccins

PFPs: **CTMM**
- Proteomics
- Metabolomics
- System Biology
- Bioinformatics

PFPs: **BMM, NIRM, DCTI**
- Biomaterial for implants
- Stem Cell Therapies

Niaba BioBusiness Masterclass

Niaba is organising the BioBusiness Masterclass for the fifth time in a row in 2014. The masterclass is a unique course aiming for ambitious entrepreneurs in the field of biotechnology, life sciences and chemical. Are you aiming to start your own company or to grow to a management position within your current work? Then this is the course for you! For more information and registration, please go to: http://www.masterclassbiobusiness.nl/

**Intellectual property: facts and figures**
Proteins At Work

Access to state-of-the-art proteomics technology, equipment and expertise

In the past decade, several countries have recognised the importance of proteomics in understanding the biology of humans, animals, plants, pathogens and model organisms and as such have made great funding contributions to proteomics-related research and infrastructures. As a result, the field has matured immensely and today offers incredibly powerful tools to study the biology of cells, tissues and whole organisms, and to reveal the causes of disease processes at the most fundamental level.

Through the Netherlands Proteomics Centre (funded by the Netherlands Genomics Initiative (NGI)) the Netherlands was able to achieve a leading role in developing these enabling proteomics technologies, promoting them to be adopted within the Dutch life science community. Following the successful pioneering stage of the NPC, Albert Heck and colleagues from leading life science institutes realised that in the next decade access to high-quality proteomics facilities and expertise would become indispensable in enabling further major scientific breakthroughs.

In response to the 2011 call for a National Roadmap for Large-scale Research Facilities, which aims to implement the European ESFRI Roadmap in the Dutch scientific landscape, they jointly applied for inclusion in the Roadmap and for financial support for Proteins At Work. With this, they wanted to catalyse a much larger proteomics-related research programme, with access to large-scale state-of-the-art proteomics facilities, thus allowing the community to chart simultaneously and quantitatively all proteins in human cells and tissues, in order to answer complex biological questions.

In March 2012 the Proteins At Work programme was awarded 13.5 million euro by the Netherlands Organisation for Scientific Research (NWO). In total, five programmes in the Netherlands received financing from NWO within the National Road Map for Large-scale Research Facilities, for which 80 million euro was made available.

About Proteins At Work
Proteins At Work will make high-level technology, equipment and expertise for studying proteins in cells and tissues available to the biological and biomedical researchers in the Netherlands. The programme is a collaboration between Utrecht University, the UMC Utrecht, the Hubrecht Institute, Erasmus Medical Centre Rotterdam and the Netherlands Cancer Institute (NKI), and builds upon the NPC and the European large-scale proteomics facility, PRIME-XS. Proteins At Work will run from early 2014 to 2019.

Research programme
Proteins At Work has a research programme for proteomics technology development and highly demanding application projects. At the core site in Utrecht, researchers will develop novel proteomics technology which is required to answer the biomedical questions of the future. In addition, dedicated researchers will be employed on selected proteomics application projects, in close collaboration with biomedical researchers. These projects, which will be selected on scientific quality and their large demand for proteomics experiments, will be jointly coordinated between Proteins At Work and partner institutes throughout the Netherlands.

Access to facilities
Proteins At Work will provide open access to state-of-the-art proteomics technology, equipment and expertise to researchers in the Life Sciences from academia and industry. It will consist of a core facility at Utrecht University, with smaller out-stations at the NKI, the Erasmus Medical Centre Rotterdam and the University Medical Centre Utrecht. Researchers can get more information and request access to the facilities at the Proteins At Work website (www.proteinsatwork.nl).
Profiles of the four facilities

**Utrecht University Facility**
The facility in Utrecht, an expert centre for state-of-the-art proteomics analysis, will develop and implement innovative mass spectrometric methods for efficient and detailed characterisation of biomolecules in relation to their biological function. Scientific focus ranges from single protein identifications to the analysis of complex, whole-cell lysate samples and determination of posttranslational modifications.

**Netherlands Cancer Institute Facility**
The facility at the Dutch Cancer Institute will house two high-resolution LC-MS setups, optimised for biomolecular analysis. Main focus of the facility is state-of-the-art proteomics research on a wide range of cancer related biological processes. Scientific focus ranges from single protein identifications, protein-protein interaction networks and the analysis of complex, whole-cell lysate samples, as well as the determination of post-translational modifications.

**Erasmus Medical Centre Rotterdam Facility**
The facility will be embedded within the Erasmus MC Proteomics Center (part of the Department of Biochemistry). It will offer proteomic and mass spectrometric services for the Dutch academic research community. All projects will be on a collaborative basis. Researchers can consult the experienced staff on study design and data interpretation. The facility also develops proteomics methodologies based on mass spectrometry and protein separation techniques for qualitative and quantitative analysis of (sub)proteomes. In addition, it will provide mass spectrometry and proteomics services for the local scientific community as well as for external researchers.

**University Medical Centre Utrecht Facility**
The facility in UMC Utrecht will provide state-of-the-art proteomics methods to aid fundamental, translational and clinical research into proteins, protein modifications and biomarkers relevant for health and disease.
EuPA Open Proteomics is the new Open Access journal of the European Proteomics Association. Expanding and complementing the society affiliate Journal of Proteomics, it covers the complete spectrum of proteome sciences from basic, fundamental to applied, translational proteomics, from microorganisms, over plants, and (model) animal species to human.

Particularly welcomed are manuscripts reporting on:

- **Novel Methodological and Technical Developments, including Bioinformatics and Data Processing**
- **Proteomic Reference Data Sets**
- **Mass Spectrometry Imaging**
- **Pathway Analysis**
- **Peptidomics**
- **Lipidomics**
- **Glycomics**

EuPA Open Proteomics is aimed at international proteomics scientists, both academic and industrial. It will also publish official EuPA reports and notes.

**No publication charges in 2013**

*The author publication open access charge will be waived for all papers submitted in 2013.*

For more information, please visit:

www.elsevier.com/locate/euprot
Ergo Sum Exhibition

Work at the intersection of art and science

The Netherlands Proteomics Centre’s artist-in-residence Charlotte Jarvis has spent the last year working on her new project, Ergo Sum. It is now finished and on display as part of the Netherlands Genomics Initiative’s Designers & Artists 4 Genomics Award (DA4GA) at the old Raamsteeg building in Leiden that once housed Naturalis.

To recap, the project saw Charlotte creating a ‘second self’ through donated tissues that were transformed into stem cells and eventually ‘active brain’, ‘beating heart’ and ‘flowing blood’ vessels. She spent months working with prof.dr. Christine Mummery and her lab to bring her partial doppelganger to life, and many more devising an aesthetically pleasing incubator to display the cell samples.

Projects like this are always subject to change, so we asked Charlotte how the final show differed from what she had first conceived. “It was much more dramatic than I had originally thought. The incubator and film installation were exhibited in a completely black room with a soundtrack of my breathing and heartbeat, which was something I decided upon relatively late in the project - I think it felt like quite a theatrical experience walking into the space. You could also hear the heartbeat from some distance away, so there was some sense of anticipation or perhaps even apprehension as you approached the installation - that was a bonus.”

Realising ideas

Of course, there is often a gap somewhere between the scope of an artist’s ideas and the bounds of what is possible in the lab. Looking back, Charlotte tells us that the hardest part of the project was building the incubator to house her living samples in. The problem was that “scientific incubators look like big fridges, but I wanted one with a glass front, doors and mirrored surfaces.” Not to be so easily defeated, she set about having one built especially. The custom incubator had to meet many of the specifications of a lab model — “you need to feed CO₂ into the chamber, heat it and ventilate it. All the levels had to be just right to keep the samples alive.” In the end, it all worked out, but “there were a few points along the way where it looked like it might not be possible or that it would arrive too late for the opening. It kept me on my toes!”

Charlotte’s work was unveiled at the DA4GA show, which opened on 14th September. She enjoyed the opening, but says she was “nervous about showing the final piece to the scientists I have been working with. They have put so much time and energy into the project and I really wanted them to like the installation and to appreciate what I was trying to do with it. Luckily they did!” The team of researchers that worked with Charlotte were crucial to the artwork’s success, and they are maintaining the project now that she is back in England (her home country). “We have two sets of samples that rotate between the gallery and the lab. The samples are fed in the sterile conditions of the lab where they get some TLC before roughing it in the gallery incubator. It’s rather a large job for the scientists to commit to - for which I am extremely grateful!”

Now that Charlotte’s involvement in Ergo Sum is largely complete, she has begun work on her next project, called Music of the Spheres, with Dr. Nick Goldman from the European Bio-informatics Institute. She summarises it as “utilising new bioinformatics technology developed by Nick to encode a new musical recording by Mira Calix into DNA,” and then “suspending the DNA in soap solution which will be used to blow bubbles.” Charlotte says that she hopes to bring that piece to fruition in 2014.
In this NPC HighLights we provide a short list of papers that appeared recently in some of the top journals and to which NPC participants contributed. With the guarantee of being by far not comprehensive, this overview shows some elegant ground-breaking research.

Selection of bone metastasis the primary tumor stroma


Cancer Biology and Genetics Program, Memorial Sloan-Kettering Cancer Center, New York, NY 10065, USA.

How organ-specific metastatic traits arise in primary tumors remains unknown. Here, we show a role of the breast tumor stroma in selecting cancer cells that are primed for metastasis in bone. Cancer-associated fibroblasts (CAFs) in triple-negative (TN) breast tumors skew heterogeneous cancer cell populations toward a predominance of clones that thrive on the CAF-derived factors CXCL12 and IGF1. Limiting concentrations of these factors select for cancer cells with high Src activity, a known clinical predictor of bone relapse and an enhancer of PI3K-Akt pathway activation by CXCL12 and IGF1. Carcinoma clones selected in this manner are primed for metastasis in the CXCL12-rich microenvironment of the bone marrow. The evidence suggests that stromal signals resembling those of a distant organ select for cancer cells that are primed for metastasis in that organ, thus illuminating the evolution of metastatic traits in a primary tumor and its distant metastases.

Relative quantification of proteasome activity by activity-based protein profiling and LC-MS/MS.


Activity-based protein profiling (ABPP) is a functional proteomics technique for directly monitoring the expression of active enzymes in cell extracts and living cells. The technique relies on irreversible inhibitors equipped with reactive groups (warheads) that covalently attach to the active site of enzymes and fluorescent or affinity tags for imaging and purification purposes, respectively. Here, a high-throughput and robust protocol for high-resolution quantitative activity-based proteasome profiling is described. We use both panreactive and subunit-specific fluorescent activity-based probes (ABPs) to quantify the proteasome activity in living cells, in the presence or absence of the potent proteasome inhibitor bortezomib. Active proteasome subunits from cell lysates are affinity-purified via a biotinylated ABP. Purification from live cells involves a two-step ABP approach using a reagent with a cell-permeable azide-warhead and postlysis installation of biotin. By means of liquid chromatography-mass spectrometry (LC-MS)-based proteomics, we can accurately identify the enriched proteins and the active site peptides of the enzymes, and relatively quantify all the proteasome activities in one experiment. The fluorescence ABPP protocols takes 2-3 d, and approximately 8-10 d are needed to complete the entire protocol.

The CRAPome: a contaminant repository for affinity purification-mass spectrometry data


Department of Pathology, University of Michigan, Ann Arbor, Michigan, USA.

Affinity purification coupled with mass spectrometry (AP-MS) is a widely used approach for the identification of protein-protein interactions. However, for any given protein of interest, determining which of the identified polypeptides represent bona fide interactors versus those that are background contaminants (for example, proteins that interact with the solid-phase support, affinity reagent or epitope tag) is a challenging task. The standard approach is to identify nonspecific interactions using one or more negative-control purifications, but many small-scale AP-MS studies do not capture a complete, accurate background protein set when available controls are limited. Fortunately, negative controls are largely bait independent. Hence, aggregating negative controls from multiple AP-MS studies can increase coverage and improve the characterization of background associated with a given experimental protocol. Here we present the contaminant repository for affinity purification (the CRAPome) and describe its use for scoring protein-protein interactions. The repository (currently available for Homo sapiens and Saccharomyces cerevisiae) and computational tools are freely accessible at http://www.crapome.org/.

http://www.crapome.org/.
Signatures of mutational processes in human cancer

Alexandrov, L.B., et al  
Cancer Genome Project, Wellcome Trust Sanger Institute, 
Wellcome Trust Genome Campus, Hinxton, Cambridgeshire 
CB10 1SA, UK.

All cancers are caused by somatic mutations; however, understanding of the biological processes generating these mutations is limited. The catalogue of somatic mutations from a cancer genome bears the signatures of the mutational processes that have been operative. Here we analysed 4,938,362 mutations from 7,042 cancers and extracted more than 20 distinct mutational signatures. Some are present in many cancer types, notably a signature attributed to the APOBEC family of cytidine deaminases, whereas others are confined to a single cancer class. Certain signatures are associated with age of the patient at cancer diagnosis, known mutagenic exposures or defects in DNA maintenance, but many are of cryptic origin. In addition to these genome-wide mutational signatures, hypermutation localized to small genomic regions, 'kataegis', is found in many cancer types. The results reveal the diversity of mutational processes underlying the development of cancer, with potential implications for understanding of cancer aetiology, prevention and therapy.

Carbamylation and antibodies against carbamylated proteins in autoimmunity and other pathologies

Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands.

Carbamylation is a non-enzymatic post-translational modification in which cyanate binds to molecules containing primary amine or thiol groups and forms carbamyl groups. Cyanate is in equilibrium with urea in body fluid and increased carbamylation was first reported in patients with increased urea levels such as patients suffering renal diseases. Next, increased carbamylation related to inflammation has also been described in other conditions such as cardiovascular disease. Recently, a new consequence of carbamylation has been observed: induction of an autoantibody response. We identified anti-carbamylated protein (anti-CarP) antibodies in rheumatoid arthritis (RA) patients and in patients having 'pre-RA' symptoms; arthralgia. The presence of anti-CarP antibodies in arthralgia patients is associated with an increased risk of developing RA. The presence of anti-CarP antibodies in RA patients is associated with more severe joint damage in RA patients who do not have anti-citrullinated protein antibodies. It is currently unknown to what extent carbamylation and/or the formation of anti-CarP antibodies contributes to the disease processes of chronic diseases such as renal diseases, cardiovascular diseases and RA. This review summarizes the current knowledge on carbamylation and the formation of anti-CarP antibodies and discusses their possibly important implications.

Histone Chaperone NAP1 Mediates Sister Chromatid Resolution by Counteracting Protein Phosphatase 2A

Department of Biochemistry and Centre for Biomedical Genetics, Erasmus University Medical Center, Rotterdam, The Netherlands.

Chromosome duplication and transmission into daughter cells requires the precisely orchestrated binding and release of cohesin. We found that the Drosophila histone chaperone NAP1 is required for cohesin release and sister chromatid resolution during mitosis. Genome-wide surveys revealed that NAP1 and cohesin co-localize at multiple genomic loci. Proteomic and biochemical analysis established that NAP1 associates with the full cohesin complex, but it also forms a separate complex with the cohesin subunit stromalin (5A). NAP1 binding to cohesin is cell-cycle regulated and increases during G2/M phase. This causes the dissociation of protein phosphatase 2A (PP2A) from cohesin, increased phosphorylation of SA and cohesin removal in early mitosis. PP2A depletion led to a loss of centromeric cohesion. The distinct mitotic phenotypes caused by the loss of either PP2A or NAP1, were both rescued by their concomitant depletion. We conclude that the balanced antagonism between NAP1 and PP2A controls cohesin dissociation during mitosis.

Other highlighted publications

Isogenic human pluripotent stem cell pairs reveal the role of a KCNH2 mutation in long-QT syndrome.  
EMBO J (in press)

CCAT2, a novel long non-coding RNA in breast cancer: expression study and clinical correlations.  
PMID: 24077681.

Quantitative Proteomic Analysis of Microdissected Breast Cancer Tissues: Comparison of Label-Free and SILAC-based Quantification with Shotgun, Directed, and Targeted MS Approaches.  
KRAS and BRAF mutation status in circulating colorectal tumor cells and their correlation with primary and metastatic tumor tissue.

Profiling of diet-induced neuropeptide changes in rat brain by quantitative mass spectrometry.

Suits, F., Fehniger, T.E., Végvári, A., Marko-Varga, G., Horvatovich, P.
Correlation queries for mass spectrometry imaging.

Bronsema, K.J., Bischoff, R., van de Merbel, N.C.
High-Sensitivity LC-MS/MS Quantification of Peptides and Proteins in Complex Biological Samples: The Impact of Enzymatic Digestion and Internal Standard Selection on Method Performance.

Quantitative dissection and stoichiometry determination of the human SET1/MLL histone methyltransferase complexes.

Peng, M., Scholten, A., Heck, A.J., van Breukelen, B.
Identification of Enriched PTM Crosstalk Motifs from Large-Scale Experimental Data Sets.

Meinema, A.C., Poolman, B., Veenhoff, L.M.
Quantitative analysis of membrane protein transport across the nuclear pore complex.

Multivalent Engagement of TFIID to Nucleosomes.

Barasa, B., Slijper, M.
Challenges for red blood cell biomarker discovery through proteomics.

van Nuland, R., van Schaik, F.M., Simonis, M., van Heesch, S., Cuppen, E., Boelens, R., Timmers, H.M., van Ingen, H.
Nucleosomal DNA binding drives the recognition of H3K36-methylated nucleosomes by the PSIP1-PWWP domain.

Characterization of Electron Transfer Dissociation in the Orbitrap Velos HCD Cell.

Innovations in studying in vivo cell behavior and pharmacology in complex tissues - microvascular endothelial cells in the spotlight.
Cell Tissue Research (2013) Sept 27

Recombination-Induced Tag Exchange (RITE) Cassette Series to Monitor Protein Dynamics in Saccharomyces cerevisiae.

Boichenko, A.P., Govorukhina, N., van der Zee, A.G., Bischoff, R.
Multidimensional separation of tryptic peptides from human serum proteins using reversed-phase, strong cation exchange, weak anion exchange and fused-core fluorinated stationary phases.

Generation of induced pluripotent stem cells from human foetal fibroblasts using the sleeping beauty transposon gene delivery system.

Chitinases CtcB and Cfcl modify the cell wall in sporulating aerial mycelium of Aspergillus niger.

Wösten, H.A., van Veluw, G.J., de Bekker, C., Krijgsheld, P.
Heterogeneity in the mycelium: implications for the use of fungi as cell factories.
We cordially invite you to attend the NPC Progress Meeting 2014. As in previous years outstanding international and national scientists will present their view on the newest developments in proteomics with lectures, parallel sessions, NPC project posters and an exhibition. The meeting is sponsored by: AB Sciex, Advion, Agilent, Bruker, Cell Signaling, MS Vision, PRIME-XS, Promega, Shimadzu Benelux, Thermo Scientific, U-Protein Express.

We look forward to seeing you at the meeting!

**Registration & abstract deadline: 17 January 2014**

www.netherlandsproteomicscentre.nl

progressmeeting@npc.genomics.nl

Confirmed speakers include

- Maarten Altelaar
- Cathy Costello
- Edwin Cuppen
- Petra van Damme
- Jeroen Demmers
- Alain van Dorsselaer
- Ron Heeren
- Charlotte Jarvis
- Simone Lemeer
- Kathryn Lilley
- Geert Mommen
- Zoltan Takats
- Michiel Vermeulen
- Roman Zubarev
Two years of art at the NPC

On 16 December my death will mark the end of two years as Artist in Residence at the Netherlands Proteomics Centre. On that date my heart will stop beating, my blood vessels will collapse and my brain, already half dead, will completely cease to function.

I think I’ll celebrate with a stiff gin and tonic.

Ergo Sum is the second project I have made in collaboration with the NPC. We have created a ‘second self’ made from a collage of synthesised body parts that are currently living in an incubator in an exhibition in Leiden. On 16 Dec ‘she’ gets switched off, and what is left – a metal and glass incubator – will get shipped back to me empty. I think I might keep it in my flat – with the doors open like a waiting reliquary.

Two years of working with the NPC has afforded me some extraordinary opportunities. Alongside my doppelganger living in Leiden, we have made ‘forbidden fruit’, sprayed with DNA encoding for the Universal Declaration of Human Rights (for the project Blighted by Kenning). I accidentally left one of them on the Tube. It was never returned. I hope it tempted someone.

Aside from the projects and physical artworks themselves, the most valuable thing I have gained from this residency is a greater understanding of how extraordinary science is made. What has struck me most is the dedication required; the exhaustive attention to detail and unwavering focus. I used to fancy that if art school had not seemed quite so appealing I could have been a scientist. I now know that was a pretty arrogant assumption to make. I simply would not have the patience or stamina.

What I can do however is celebrate it. There is a great amount of critique in modern art – that is something I applaud and believe to be essential, but alongside saying what you don’t agree with I think there is also a space for stating what you DO believe in. I sometimes feel that it can be too easy to criticise without ever putting yourself in a position where your own opinions can be dissected. Recently, through working with the NPC, I have become interested in putting myself in the position of saying “this is what I want the future to be”.

Charlotte Jarvis
Artist and film maker
NPC artist in residence
2011-2013