Seventh HFSP Annual Awardees Meeting
Twin Waters, Queensland, Australia
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By Martin Reddington and Geoff Richards

Twin Waters

The Human Frontier Science Program ventured into the southern hemisphere for the first time for the Seventh Awardees Annual Meeting reflecting the recent expansion of its membership. Delegates had little trouble navigating via Brisbane to the Twin Waters Resort on the Sunshine Coast of Australia for the meeting held from the 18-21 July 2007, with support from the Australian National Health and Medical Research Council. While retaining the now traditional prestigious opening and closing plenary lectures, there were several innovations in the format which included the presentation of 25 talks and 98 posters by HFSP awardees, 5 chalkboard sessions and an imaging discussion workshop. Contrary to the practice of recent meetings there was a deliberate attempt at 'mixing and matching' attendees. By abandoning the traditional thematic sessions for the oral presentations the audience was exposed to a rich mixture of scientific problems and approaches very much in the interdisciplinary spirit of the program. To compensate for this mixing, a series of chalkboard sessions on selected topics on the first day ensured that members from similar disciplines met early in the meeting so as to find those whose interests matched their own. These thematic sessions fostered informal discussions throughout the meeting.

This report is a sample of a wide ranging series of presentations which emphasized the importance of interdisciplinary research for the understanding of complexity in living organisms. The opening plenary lecture was a fascinating talk on honeybee navigation by **Mandyam Srinivasan** from the Queensland Brain Institute, a compelling combination of biology and engineering, which captured the imagination of both specialists and those for whom the problems were entirely novel. The closing lecture was from **Roger Kornberg** from Stanford who won the 2006 Nobel Prize in chemistry "for his studies of the molecular basis of eukaryotic transcription". Roger Kornberg thus continued the tradition of past HFSP awardees who have since been awarded the Nobel Prize generously giving of their time to inspire current young awardees at the annual meeting.

More than a honey machine

A major theme in the session on the first morning was animal navigation. In his plenary lecture, subtitled "More than a honey machine", **Mandyam Srinivasan** presented an overview of the remarkable vision and navigation systems of bees, which allow them to find their way "despite the fact that they carry a brain that weighs less than a milligram and has far fewer neurons than our own brains." A theme throughout his talk was the way in which the visual system allows bees to

manoeuvre and navigate in a 3D world. The compound eyes of insects are too close together to enable them to create a stereoscopic image and they have therefore evolved other strategies, using image motion cues to infer object distance and to perceive the world in 3D.

Srinivasan described how bees are able to manoeuvre, for instance when flying through small gaps, by comparing the "optic flow" (i.e. the rate at which the image moves) in the two eyes. By manipulating the amount of optic flow when bees flew through a patterned tunnel by moving the walls at different rates, their closeness to the wall could be changed. The animals appear to be determining their course by maintaining the same optic flow in the two eyes. This mechanism is quite robust and is not affected by the fineness or contrast of the patterns, an important characteristic in the wild since a bee may be confronted with different patterns, for instance on the bark of two tree trunks that she needs to navigate between. Similarly, optic flow is also used to determine the flight speed: the bees regulate their flight speed by keeping the global velocity of the image constant.

The dependence of optic flow on the bee's distance from a surface and the flight speed comes into play beautifully in allowing it to orchestrate the complicated process of landing. Srinivasan and colleagues showed that there is a clear linear relationship between the horizontal velocity and height from the ground as a bee comes in to land. Thus "when the animal is flying high it is flying fast and when it is flying low it is flying slow". As it approaches the ground it therefore slows down, reaching almost zero speed on touchdown. "This is a simple and elegant way to land since you don't have to know how fast you are travelling at any instant of time, you don't have to know how far away the ground is - all you have to do is to keep the image velocity of the ground constant so you automatically make a smooth touchdown" explained Srinivasan: "This is a beautiful biological autopilot, not the kind of thing an engineer would put in an aircraft ".

Srinivasan then turned his attention to another question: How does a bee know how far she has flown? This question is of fundamental significance for the foraging behaviour of the bees. It has been known since Antiquity that, on finding a food source, a bee will return to the hive and other bees will then fly to the same source. It was only in the last century, however, that Karl von Frisch established how the information is passed on from the scout to the recruit bees. Von Frisch was awarded the Nobel Prize for Physiology or Medicine in 1973 for decoding the "language" of the bee. On returning to the hive after locating a food source, the forager (scout) bee starts to dance. In the words of von Frisch from his Nobel Lecture: "I was astonished to see that all foragers from nearby performed round dances, while long-distance foragers did tail-wagging dances". The round dance is performed if the food source is close to the hive, but beyond about 50m it is replaced by the "waggle dance", which transmits information about both the direction (the direction of the dance) and distance (the duration of the tail wagging phase) of the food source from the hive.

The dancing behaviour of the bees provides an excellent opportunity to study the bee's odometer. As Srinivasan put it "the dance provides a window into the bee's mind, so to speak - you can tap into the bee's perception of how far it thinks it has flown". By moving food sources and changing the visual texture of the path, it became clear that bees estimate how far they have flown by integrating the amount of image motion, another demonstration of the importance of optic flow. Further, using the waggle dance as an indicator of the function of the odometer in their natural environment, it became clear that the bees also use visual cues to navigate in the wild. Thus there is now

strong evidence that, despite some initially sceptical voices, the bees are indeed responding directly to the symbolic information encoded in the dance as originally proposed by Von Frisch.

In the final part of the talk, Srinivasan showed his true interdisciplinary colours (he is an electrical engineer by training) by describing how the principles discussed above can be used to build flying robots. "We do the robotics for two reasons" he explained. "First to see if we can implement in vehicles some of what we learned from the insects in terms of navigational principles and see if they behave in a reasonable way so as to have more faith that we understand what is really going on. Also, by using some of these biologically inspired approaches, we might even be able to do something that turns out to be useful". Indeed, emulating the principle of optic flow in robots did result in machines that were able to use optic flow to regulate the height at which they hovered (in the case of a helicopter) or flew (as in an aircraft), raising exciting prospects for the development of new classes of flying vehicles.

Seeing the Earth's magnetic field

In the second scientific presentation of the meeting, the topic shifted from the bees to the birds with a fascinating and exemplary interdisciplinary talk on the mechanism underlying the avian magnetic compass by Program Grant holders, zoologist **Roswitha Wiltschko** and physicist **Thorsten Ritz**. It has been known for the last 40 years since the pioneering work of Wolfgang Wiltschko that migrating European robins orientate using the Earth's magnetic field. Although many hypotheses have been formulated to account for this phenomenon, two main ideas have received attention by both experimentalists and theoreticians: the magnetite hypothesis, according to which particles of iron oxide play a role and, more recently, the radical pair hypothesis, involving molecules sensitive to weak magnetic fields. Roswitha Wiltschko and Thorsten Ritz presented studies using behavioural measurements on the orientation of robins in a magnetic field to probe the characteristics of the magnetic compass experimentally, a process they have dubbed "behavioural resonance spectroscopy". In their experimental approach, migratory robins are captured and placed in a laboratory apparatus that allows their directional preference to be measured. The magnetic sensitivity of the birds' orientation preference can then be investigated by changing the magnetic field around the apparatus with the help of a coil system.

In a paper published in the first issue of the new HFSP Journal[1], Wiltschko and Ritz clearly showed that anaesthetising magnetite-containing cells in the upper beak of the robins had no effect on their orientation preference, indicating that magnetite does not play a significant role in the compass of this species. Their further studies therefore explored experimentally the alternative, radical pair hypothesis. This mechanism depends first on a light-dependent electron transfer from a donor to an acceptor with formation of a radical pair in a singlet state, i.e. where the spins of the two electrons are aligned in opposite directions. As shown by Ritz and the third member of the grant team, **Christiane Timmel** in 2003, in the presence of a weak magnetic field the spins of the two electrons can become aligned in the same direction (the "triplet state"); the consequence of this is that the radicals live longer thus affecting the chemistry of the

[1] HFSP Journal **1**(1), 41-48 (2007) (http://hfspj.aip.org)

molecules carrying the radical pairs. In 2000, in a theoretical "summer project" during Ritz' PhD studies, Ritz and his doctoral mentor, Klaus Schulten postulated that such an event occurring in a visual pigment could conceivably affect signal transduction in the visual pathways, thus allowing the animals to "see" the magnetic field.

By changing the intensity and frequency of oscillating magnetic fields and observing the orientation preferences of the robins, Wiltschko and Ritz were able to delineate some of the biophysical characteristics of the magnetic compass. Further, comparing the diagnostic behavioural results to spin resonance spectroscopy studies of molecules in solution, they could identify the basic properties of candidate molecular carriers of such radical pair transitions. The results of these studies point strongly to the existence of a radical pair mechanism underlying the avian magnetic compass and show that the properties of the putative magnetic receptors are consistent with those of the cryptochromes. These blue-green pigments do indeed occur in the avian retina and have suitable quantitative biophysical properties to make them strong candidates.

So far very few species have been tested for orientation mechanisms involving radical pairs and it remains to be seen how widespread this phenomenon really is. That it is not universal was shown in an accompanying poster using blind mole rats that did not respond to oscillating magnetic fields and are presumably using a different mechanism to orientate, possibly magnetite. However, the domestic chick does appear to use a radical pair mechanism. Interestingly, the cryptochromes are widely spread, even in plants, and Wiltschko and Ritz showed tantalizing preliminary experiments in *Arabidopsis*, opening exciting possibilities to study the magnetoreceptor molecules with powerful combinations of physics and molecular genetics.

This kind of collaboration between scientists from different disciplines is a hallmark of the HFSP grant programs, but was also evident in the projects of younger scientists where chemistry is making a major contribution.

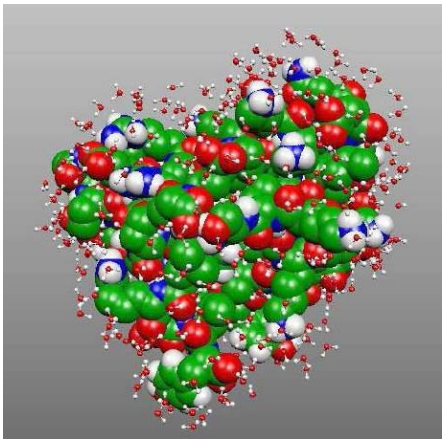
Deciphering biological systems using chemical probes

Mathieu Pucheault trained initially in organic chemistry and catalysis at the ENS in Paris before obtaining one of the very first HFSP Cross-Disciplinary Fellowships to visit the laboratory of Craig Crews at Yale to explore his interests in biology. Crews runs an interdisciplinary laboratory looking at the application of chemical biology to investigate intracellular signaling pathways and, as Pucheault pointed out, this proved to be the ideal place to make his own transition to biological systems. He has developed a system using small molecules to induce specific protein degradation. In his presentation he explained the principles of the PROTAC molecules (Proteolysis Targeting Chimeras) that induce selective ubiquitination of a protein that leads to its proteolysis. The chemist must design an artificial linker to bring an E3 ligase and the target protein into close proximity, thus promoting its polyubiquitination which in turn will recruit it to the 26S proteasome where degradation occurs. As a proof of principle, Pucheault showed his experiments with a synthetic linker between the Androgen Receptor (AR) and MDM2 (an E3 ligase) which leads to specific degradation of AR in vivo. Pucheault has now returned to France to the University of Rennes where he is applying his new expertise to the study of protein-protein interactions.

Another young scientist coming from organic chemistry and now dissecting a biological problem is **Gonen Ashkenasy**, currently a Career Development Awardee with a young laboratory in the Chemistry Department of the Ben Gurion University in Israel. After a PhD at the Weizmann Institute, he spent several years training in chemical biology with Reza Ghadiri at the Scripps Institute, including three years as an HFSP Long term Fellow, before returning home to Israel. Ashkenasy's interests are in molecular networks and his approach is to start from a synthetic system so as to avoid the many unknowns that are generated when tampering with a natural system. He presented the design of peptide-based self-organized non-linear networks whose readout is via the production of peptides produced by template-directed coiled-coil auto and cross-catalytic ligation. The network can be manipulated by chemical triggers (substrate and catalyst levels, salts and pH) and its components can be induced to behave as logic gates. His approach is intended to give an understanding of the consequence of manipulations which may be as fine as changes in the energy of a single chemical bond resulting from changes in pH or salt concentration. In parallel in his laboratory, as part of his CDA project, Ashkenasy is designing and constructing novel synthetic proteins by the chemical synthesis of short component peptides followed by their ligation.

The Dance of Water

In his poetically titled talk "The THz dance of water with proteins", grant holder **Martin Gruebele** from the Department of Chemistry and Center for Biophysics and Computational Biology at the University of Illinois described how the use of far red IR radiation of THz frequencies can provide information about the contribution of surface water to the structure and energetics of proteins. Proteins are associated with water, which can be trapped in folds and crevices in the folded protein structure or in an ordered state around the protein surface due to a hindrance of the rotation of water molecules in the presence of the large protein. Techniques such as X-ray diffraction and NMR can yield information about the structure and/or dynamics of trapped water and of water close to the surface, but are not useful for looking at more distant, global effects of the protein on water dynamics.



Gruebele described an interdisciplinary approach to this problem using advanced spectroscopic techniques and theoretical molecular dynamics simulations carried out in collaboration with grant holders **Martina Havenith** and **David Leitner**. The grant team found that effects of proteins on water involving many hundreds of water molecules could be detected up to about 0.2 nm from protein surfaces. Their investigations provide direct evidence that, given the high concentration of proteins and other macromolecules in living cells, virtually all water molecules in the cell are constrained. Water dynamics were also altered by denaturing the proteins or by changing the amino acid composition of the protein surface or the protein backbone. THz spectroscopy can thus be used to probe both the local structure of water after point mutations of the protein and the global structure of the solvent around the proteins. Finally, Gruebele showed preliminary results indicating that THz sources

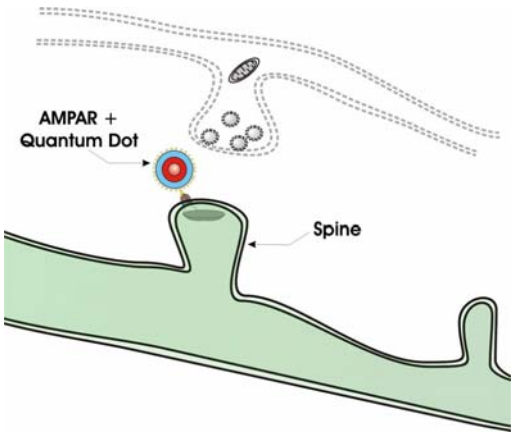
could even be used in near field imaging to map out the structure of water at subcellular levels in whole cells.

In the ensuing discussion, Gruebele was asked about the functional significance of these observations. He speculated that the structure of water around proteins could be of enormous significance by affecting the way in which small molecules approach macromolecules through the shell of hydration, maybe even being guided to their sites of action.

Fusion forces

In an approach to the way physical barriers are overcome in biological systems, physicist **Eric Perez** from Paris described experiments aimed at understanding the energetics of membrane fusion. The fusion of membranes plays an important role in several biological processes including the vesicular release of neurotransmitters from synapses. The proteins involved in this process have been characterized and a large family of proteins called the SNARE proteins have been shown to play a critical role in the process of membrane fusion. Membrane fusion does not happen passively, however. An energetic barrier has to be surmounted to bring closer the two opposing membranes and to curve them. Perez described experiments in which SNARE proteins (syntaxin and SNAP25 on one surface and vamp2 on the other) were bound to lipids on solid surfaces to study the relationship between interaction energy and distance between the surfaces as they are brought together. Binding was specific to the SNARE proteins and the binding energy was found to correspond closely with the energy needed to fuse outer but not inner leaflets (hemifusion) of pure lipid bilayers. The experiments described by Perez provide a sound basis for investigating the physics of membrane fusion in more detail but studying other regulatory factors. Such investigations are essential to understand in quantitative terms these vital cellular processes.

Insights into the cell



From many abstracts submitted for the meeting, it was apparent that there were exciting developments in the field of imaging drawing on a wide range of expertise; chemists for preparing new labels, biologists for delivery systems for the labels and in vivo observations and physicists and mathematicians for the analysis of the observations. These different elements were to be found both in the formal talks and posters, but were also a major topic in the chalkboard session “Issues and techniques in macromolecular structure and function” (where labels for studying protein complexes were discussed extensively) and in the workshop on molecular imaging technologies entitled ”What would you like to see ? ”.

In his presentation of their Young Investigator project on synaptic remodeling in neuronal communication, **Paul De Koninck** from Quebec spoke of the interest of obtaining smaller quantum dots, as improved labels for tracking single neurotransmitter receptors. One of the aims of the project is to follow the accumulation of post-synaptic receptors at synapses, where they are needed, via lateral diffusion and trapping. The development of the quantum dots is using the skills of the chemist in their team, **Benoit Dubertret** in Paris with the aim of enabling them to produce labeled receptors that are not physically constrained from entering the synaptic cleft. At the other end of

the process, De Koninck emphasized how **Paul Wiseman**, a physicist, was indispensable for analyzing the data they obtained at the microscope (see below) and, amongst other things, distinguishing between the fluorescent blinking of the quantum dots and their real passage in and out of the focal plane during transport. This multidisciplinary approach is allowing them to investigate the post-synaptic mechanisms responsible for trapping receptors at the synapse.

CDA holder **Adi Mizrahi**, from Jerusalem, spoke of their studies on neurogenesis in the adult mouse olfactory bulb. They are using lentivirus labeling via stereotaxic injections and high resolution in vivo two-photon microscopy to study the dynamic behavior of dendritic trees as well as the distribution of synapses over time. Although during early development these are highly ordered, they are continuously changing location along the dendritic shafts when studied over several days and indeed remain structurally dynamic months after incorporation into the network. The overall plasticity of the adult-born neuronal population as revealed by these studies was indeed striking.

Long term fellow **Iain Cormack,** now with Coherent Scotland in Glasgow, whose thesis was concerned with laser development, presented his work on innovative multi-photon microscopy in the Institute of Photonic Science (ICFO) in Barcelona. This is a further example of a recent trend where innovation in microscopy is being driven by academic centers of imaging excellence rather than by traditional manufacturers. The reason is that such centers work side by side with biologists and as a result know their current needs and future dreams. If they have assembled the necessary interdisciplinary skills they can then immediately undertake development 'at the bench'. This is more efficient than the traditional industrial approach of designing a 'better' microscope and then convincing biologists to buy it and indeed many manufacturers have made co-operation agreements with such centers. Cormack's work was centered on the use of an ultrashort pulse laser source which helps to reduce both the cost and the size of the laser source in a multi-photon microscope. As beam control and analysis of the beam's interaction with the sample is critical, the project also involved the development of characterization techniques for accurate phase and amplitude measurements. Using the microscope they have been able to use focused pulses to modulate the biological machinery of cortical growth cones and which will open up more generally applicable biomedical applications.

Looking into the future

The imaging workshop was concerned with the "cutting edge" of imagery – both theoretical considerations of the tools available and illustrations of what can be achieved with current approaches

Everett Lipman's (UCSB, California, in collaboration with **Benjamin Schuler**, Zurich, and **Olgica Bakajin**, LLNL) talk on the use of FRET in following protein folding started with a 'physicist's view of a protein' which sees the model protein as a disordered chain tagged at both ends. Their set up allows them to observe the fluorescence of individual molecules in dilute solution by confocal microscopy and to investigate the effects of buffer conditions that can be finely manipulated in a microfluidic mixer. By determining the percentage of individual molecules in which the tags are brought into proximity by folding thus allowing energy transfer between the tags and a positive FRET signal, they can map the distribution of individual structures as a function of the experimental conditions. By using the mixing capacities of the microfluidic mixer, it is possible to release proteins from the denatured state. The kinetics of folding can then be followed by observing individual molecules at a known distance (and thus, via the flow rate, a known time) from the dilution event that initiates refolding.

In his talk Paul De Koninck had spoken of the need for physicists to analyze the data and this was taken up by team member **Paul Wiseman** who described ways of sifting information using image correlation spectroscopy (ICS) techniques. These methods are extensions of the original fluorescence correlation spectroscopy (FCS) which relied on observing fluorescently labeled molecules within a small laser beam focal volume and collecting intensity fluctuations as the molecules emitted fluorescence as they entered the beam focus. With time, it became apparent that the temporal FCS approach was missing some key spatial information that could be obtained on a laser scanning confocal microscope and using ICS. However, within the limits of typical imaging rates, the time scale of movements that could be analyzed was limited to rather slower moving or tethered molecules such as membrane proteins. Over the past few years, starting with FCS and now moving into a variety of ICS approaches, physicists have taken up this problem so as to calculate from the available data the underlying behavior of the molecules under study, including those with faster rates of transport including directed transport via vector mapping by space-time correlation. As well, it has involved a departure from 'real space' methods which instead use analysis in reciprocal space. This involves Fourier transformation of the images followed by calculation of k-space-time correlation functions. This allows the separation of photophysical factors such as QD blinking or probe photobleaching from the transport fluctuations of the molecules being followed so as to allow analysis of the movements themselves rather than 'artifacts' that come from the complex emission photophysics. While Wiseman's presentation was very clear and the basic principles easy to follow, the majority of the biologists in the audience understood the need to know a friendly physicist in order to undertake cutting edge image analysis projects.

Daniel Choquet presented data on a new approach to in vivo single molecule imaging. A recurrent problem is that when labels are made smaller, so as to reduce physical effects on the marked molecule, the time for observation is also reduced, often to a few seconds. The new approach is based on the use of gold nanoparticles rather than fluorescent molecules. They are stable and have long been used as labels in electron microscopy. Traditionally they have been observed by their properties to scatter light, but as they get smaller their absorption of light is a more interesting characteristic. To do this, the group of Brahim Lounis developed a two beam approach for photo-thermal imaging, the first laser is tuned to the gold particle's absorption spectra and heats it, which causes a change in the refractive index in a 'shell' around the particle. The second, probe beam detects these changes in index and hence the particle. In practice the probe beam is scattered by the particle and this interferes with the incoming beam and measurements are derived from the interference between the beam and the light diffracted by the particle. However this approach requires scanning and conventionally is too slow for in vivo detection of marked molecules which appear as tracks in the integrated image. In a close collaboration, the groups of Lounis and Choquet developed a solution to track receptors in live cells. Laurent Cognet implemented a GPS style triangulation approach to track the marked molecule rather than scanning the whole field. Although this approach will enable the use of 2-3 nm stable gold particles, the problem now lies in attaching these to the molecule of interest as the current antibody link is some 12 nm in size. While there are chemical approaches that look promising, there is still a basic problem in that mitochondria give a thermal signal similar to that of the gold particles and this causes an unfortunate background for studies in living cells. The solution may lie in substituting the gold particles by other metals with a distinct thermal spectrum.

Recent Developments

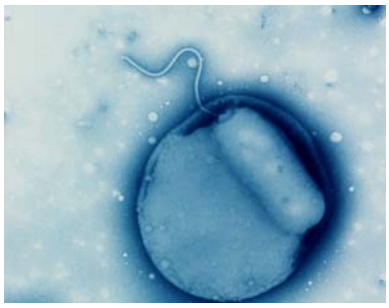
Although advanced imagery was central to many of the presentations, its importance was paramount in studies of development which is clearly, at the minimum, a 4D process. This was apparent in the presentations of several long term fellows who were looking at fundamental processes in development.

Julien Vermot working with Scott Fraser at Caltech has been looking at the mechanisms that break symmetry in the zebrafish so as to give left-right asymmetry in the distribution of organs. Several groups, working with mice or zebrafish have been interested in the role of cilia beating as a means of creating asymmetry either by redistributing a signaling molecule or by creating a flow that can be differentially sensed by mechanosensors on the opposite sides of the body. With his colleagues Vermot has used high speed imaging and 4D reconstructions to follow the beating of cilia in the Kupffer vesicle of the zebrafish, a vesicle that disappears after the 3 hour critical period for breaking symmetry. This approach required, in addition to adaptations of the microscope, image processing tools developed in the lab to analyze the data. Modeling expertise was sought for the general problem of fluid flow in a confined volume so as to enable them to model the forces necessary to disrupt flow symmetry. By laser ablation they were able to release cytoplasm from nearby cells which they then used to trace particle movement in the region of the cilia in 4D. Finally they were able to verify the effect by following asymmetric calcium release, thus confirming the establishment of left-right asymmetry. In the discussion there was a question as to whether cilia beating was itself the primary determinant of asymmetry or whether it served to reinforce an even earlier decision.

In her postdoctoral work in Utrecht, **Karen Lyle** used cell tracking software to analyze data on cell velocity and cell migration obtained using time lapse phase contrast microscopy to follow epithelial cell movements. Her project looked at growth factor-induced cell scattering as a consequence of changes in cell-cell and cell-extracellular matrix adhesion following the modulation of adhesion receptors. In the specific case of Epac/Rap1 regulation, the dominant factor appeared to involve cell-extracellular matrix adhesion complexes rather than cell-cell complexes. Rap1 appears to decrease cell migration rates by increasing focal adhesion lifetime, that is modulating the stability of the multi-protein complexes that connect the actin cytoskeleton to the extracellular matrix. This Rap1 effect did not seem to be dependent on the regulation of integrin affinities.

Working in Marcos Gonzalez-Gaitan's laboratory, **Max Fürthauer** has been looking at the problems of communication between cells which must occur at the time of both symmetrical and asymmetrical cell division in *Drosophila*. SARA (Smad Anchor for Receptor Activation) an endosomal protein has previously been shown in the lab to ensure equal partitioning of the TGFbeta signal in symmetrically dividing wing cells. Using advanced microscopy and appropriately labeled proteins, Fürthauer has concentrated on the distribution of SARA in the cell lineage of the mechanosensory bristles, a well characterized system of asymmetrical cell division. In these, the subset of SARA-positive early endosomes segregates specifically into those cells that will activate Notch signaling and is thus a clear case of asymmetric organelle segregation. These studies were undertaken in living sensory organ precursor cells with labels which enabled them to follow different components of the Notch signaling pathway as well as those of other endosome components which showed that this is indeed a SARA specific phenomenon. In addition, SARA overexpression causes cell fate transformations indicative of ectopic Notch signalling. This suggests that SARA-containing endosomes are important for biased Notch pathway activation following asymmetric cell division.

The Cinderella of bacteria



Liz Sockett from the University of Nottingham gave the meeting participants a fascinating and penetrating insight into the life of a tiny but voracious bacterial predator, Bdellovibrio bacteriovorus. These bacteria were discovered only in 1962 and remain relatively little studied, as Sockett put it: "the Cinderella of bacteria". They attach to their prey, penetrate the outer membrane and enter the periplasm where they digest the intracellular components of the prey to grow and divide, eventually lysing the host. Sockett described experiments geared to understanding the interaction of Bdellovibrio with its prey cells, in particular the surface proteins involved. By using a combination of proteomics with transcript profiling, several proteins could be identified that were enriched in the predatory attach stage of the life cycle. Sockett described in particular the protein Bd3000, a member of the so-called RTX family of proteins that are translocated out of the Bdellovibrio, to interact with the outer membrane of the bacterial prey. Gene inactivation considerably slowed down the process of prey killing but did not eliminate it indicating that Bd3000 and related proteins are not essential for prey entry although they do contribute to it. Further, generation of slowly swimming Bdellovibrio by removing flagellar proteins also reduced the effectiveness of predation. However, flagellar motion is not itself essential for infection of the prey since a mutant that is unable to swim was still able to infect prey if the two were located in close contact in culture. Thus flagellar motility is not required to achieve prey penetration but is crucial for efficient prey location. Infection is instead mediated by the "pili", tiny filamentous structures that can be ratcheted in and out of the "nose" of the Bdellovibrio. Apart from their intrinsically interesting parasitic life cycle and surprisingly large genome, the specificity of Bdellovibrio for pathogenic bacteria and the lack of any effect on eukaryotic cells makes them particularly interesting therapeutic candidates as potential "living antibiotics".

The ins and outs of higher cognitive function

In a funding program dedicated to complex biological systems, studies of the mechanisms underlying higher level cognitive functions have a natural place. Several talks concerned ways in which the brains of higher mammals, including man, respond to and process sensory stimuli.

How can the brain hear shapes?

Long-Term Fellow **Amir Amedi** from Harvard University presented a fascinating account of his research with Alvaro Pascual-Leone, William Stern and Peter Meijer into cross-modal sensory processing. It is well known that the form and location of objects, the "what" and the "where" of the visual system, are processed using different pathways in the brain. Part of the lateral occipital cortex, known as LOtv (in the "what" pathway) is activated during object recognition using both visual and tactile stimuli, indicating common processing pathways independent of the sensory input. Visual and tactile modalities are rich in shape information, so the question thus arises whether other sensory modalities can also be commandeered to allow object recognition in the absence of visual input. Using a sophisticated set of hardware and software to make a human-machine interface that transforms visual images into patterns of sound, called "The vOICe", Amedi showed how both sighted and blind individuals could be trained to recognize shapes by the soundscapes generated. Functional imaging indicated that several brain regions were

activated, including LOtv. Furthermore, in contrast to other areas, LOtv was not activated by arbitrary association between soundscapes and object identity (i.e. without extracting shape information). Taken together this suggests that LOtv is driven by shape information regardless of the sensory modality used. These studies not only provide fascinating insights into the way the brain processes shape information but also give hope that "vision" may be restored by using such sensory substitution devices in blind individuals.

The smile of the Mona Lisa

"Is she smiling at you" asked **Alessandro Treves**, showing a slide of the enigmatic expression of the Mona Lisa, "That depends on your experience of her". Treves, a computational neuroscientist from the International School of Advanced Studies in Trieste, Italy continued with the theme of object recognition in his presentation of a multi-pronged study of the way facial expressions are processed by the brain, involving non-invasive functional imaging in humans, computational modeling and single cell recordings in non-human primates. Using morphed images of faces, Treves and his HFSP-funded grant team showed that the way we interpret an ambiguous face is affected by our prior exposure to an emotional or a neutral facial expression of the same face. Thus, adaptation to a fearful expression makes subjects relatively insensitive to it. This effect is especially pronounced after long prior exposure to the priming stimulus but also occurs with short, almost subliminal stimuli, perhaps suggesting that some pathways involved in processing faces and facial expressions might bypass the cortex going directly to the amygdala, an area known to be involved in processing emotional stimuli.

Treves then considered whether general neural network principles could be elucidated that would give insights into the possible mechanisms involved in the response to facial expressions. Using a so-called "attractor" model – an attractor is a stable network state towards which a system will converge, in this case a state representing an emotional or neutral expression - he constructed a simple neural network model including parameters representing neuronal fatigue and a mask disrupting further processing. This model was able to mimic the after-effect of a priming stimulus such that the network output was biased to the opposite attractor. Where could such a network be located in the brain? In collaboration with team member **Ray Dolan** and colleagues in London, a combination of functional magnetic imaging and magnetoencephalography showed late after-effects in the medial temporal lobe, downstream from the areas mediating the initial processing of faces. In a final approach, **Bharathi Jagadeesh** and colleagues showed that, when monkeys were presented with morphs and single unit recordings were made, the responses were graded in a way that was consistent with convergence to an attractor state.

Taken together these different approaches suggest that the adaptation to a priming stimulus might be explained by fatigue-prone attractor networks, once their dynamics are released from merely reflecting afferent input. The data from monkeys suggests that the mechanisms we use for interpreting the emotional content of facial expressions involves common cortical mechanisms that we share with other mammals. Or, in other words, as Treves ended his talk "It might not take a human to appreciate the Mona Lisa".

Here's looking at you

A further exploration of how the brain reacts to sensory input was described by Long-Term Fellow **Roy Mukamel** from the University of California, Los Angeles. In 1996, Giacomo Rizzolatti and colleagues at the University of Parma in Italy discovered neurons in monkey brains, which they dubbed "mirror neurons", that responded not only when the monkey carried out an action but also when it observed another monkey or a human perform the same action. Since then, functional imaging studies in humans have shown patterns of activation that correlate with the regions activated in monkeys. However, functional imaging techniques are limited in their low temporal and spatial resolution. Mukamel and his colleagues in the laboratory of Marco Iacoboni and Itzhak Fried have taken advantage of a unique opportunity to investigate mirror neurons using single unit recordings in volunteer epileptic human subjects who are undergoing electrophysiological monitoring to locate their epileptic foci before surgery. These ongoing studies are extending those previously performed in monkeys. In the human brain, different classes of cells could be identified, for instance some that responded with excitation during the execution of an action but were inhibited during observation. Unlike in the monkey, some cells responded only with inhibition raising the interesting possibility that some cells might be involved in suppressing unwanted mimicry.

How the worm turns

The complexity of the brain presents immense challenges to understanding the cellular networks underlying behavior. Scientists have therefore looked for simpler model organisms to understand behavior at the cellular level. One of these is the nematode worm *Caenorhabditis elegans.* This worm lives in soil and its directional movement depends upon gradients in the concentration of oxygen in its natural habitat. Its nervous system has been completely mapped and consists of only about 7000 synaptic connections between clearly identified neurons. Long-Term Fellow **Manuel Zimmer** from the laboratory of Cori Bargmann at Rockefeller University in New York presented elegant studies on the oxygen-sensitive behavior of *C. elegans.* Changing the oxygen concentration stepwise leads to a decrease in the rate of mobility of these worms both when oxygen increases or decreases. Using genetic mutants, previous work has shown that the signaling molecule, soluble guanylate cyclase plays a role in this behavior. *C. elegans* has 7 different forms of this enzyme. Unlike their counterparts in mammalian systems, they have a specific oxygen binding site. In mutants lacking specific forms of the enzyme, Zimmer and colleagues were able to identify specific enzyme isotypes responsible for the response to increasing or decreasing oxygen levels that were differentially expressed in putative sensory neurons. Further, using a new microfluidics chamber that allows individual worms to be immobilized, developed with Nikos Chronis who is now at the University of Michigan, Zimmer was able to demonstrate that these sensory neurons were indeed activated by changing oxygen levels. These clear studies provided a powerful demonstration of the use of simple organisms as models for studying neuronal networks responsible for specific behaviors.

The nuts and bolts of transcription

Inviting Nobelists, who have been associated with the HFSP, to talk at the annual meeting has become a tradition and the closing plenary lecture was given by **Roger Kornberg**, who was awarded the 2006 Nobel Prize in Chemistry. As Torsten Wiesel said in his introduction to the presentation, we are always grateful that recent Laureates find time in their busy schedule to come and address the HFSP community.

Underlying beliefs of the HFSP are that both interdisciplinary approaches and advances in methods are essential to our progress in understanding fundamental biological processes. This was clearly illustrated by Kornberg in the first part of his presentation which described advances in the dissection of polymerase II gene transcription over a period of three decades. After an initial training in chemistry, during his postdoctoral studies Kornberg turned his attention to the problem of gene regulation using essentially a structural biology approach. However when describing the challenges that he and his collaborators had met in unraveling the story, it became clear that progress came via a combination of biochemistry, chemistry, genetics, molecular biology and structural biology at a time when in most universities these disciplines were to be found in distinct departments. While the biochemical identification of the components of the polymerase II complex was achieved relatively early, because of their size, as well as the impressive number of regulatory proteins interacting with the minimal complex, the problem was orders of magnitude above what was possible using the then routine structural approaches. By the time his group had identified 'mediator' as an essential transcription factor interacting with both the minimal complex and the transcription activating factors (TAFs), in the early 90s, they were faced with a total of some 60 polypeptides assembled at the promoter of each actively transcribed eukaryotic gene. In dissecting interactions between the components of the complex, the genetics of the yeast system was particularly powerful and fortunately, because of evolutionary conservation, applicable to studies of the complexes in other organisms such as *Drosophila* and man. Obtaining the structures required technical innovations as even the initial studies of the polymerase core, which might be considered the basic platform for the assembly of the complex, were beyond the existing techniques. Kornberg described the development of 2D crystallography using lipid mono-layers as a support which over a decade ultimately led to the first 3D crystals for X-ray analysis. However the then current X-ray technology had itself to be improved significantly to handle crystals of these larger complexes. Indeed his group had to use new heavy atom compounds for the analysis as the existing compounds weren't useful.

After this rapid summary of how the foothills were climbed, Kornberg gave a detailed view from the present summit. He gave a guided tour of the transcription cycle within the polymerase complex showing the position of the DNA, the nascent RNA chain and its exit from the complex. From the initial studies, specificity appeared a problem as calculating the energies of hydrogen bonds alone would not account for the accuracy of transcription. In consequence, Kornberg spent rather more time explaining the role of the relatively recent discovery of the mobile 'trigger loop' within the complex which is essential for specificity. The 'trigger loop' fits tightly in the presence of the correct ribonucleotide so as to allow its incorporation into the growing chain. There again, genetics enabled the dissection of the multiple interactions of the loop both by explaining the basis of the consequences of known mutations and modeling the consequences of systematic mutations in the loop and its surroundings. As well as participating in nucleotide selection, the trigger loop also interacts with the bridge helix which appears to be acting as a molecular ratchet allowing physical progress along the template strand as transcription proceeds.

These studies are complicated because the functional structures of individual components are influenced by their interactions with neighboring peptides. As an example, Kornberg discussed the interactions between the growing DNA-RNA hybrid and the TFIIB finger which occupy similar locations on the polymerase surface. Their associations with polymerase are not incompatible until the RNA chain reaches 5 nucleotides in length. Indeed up to that point the B finger promotes stability of the initiation complex, however thereafter there is competition. If the B finger 'wins' then initiation aborts, if B is ejected then the polymerase 'escapes' from the promoter and can go on to complete transcription. At the other end of the B factor one can look at its interactions with the TATA box binding protein (TBP) and their combined interactions with DNA. The structure in this region accounts for the finding from the study of very many eukaryotic genes that the transcription start site is some 25 to 30 base pairs downstream of the TATA box.

By way of summary Kornberg showed how the current picture of a poised transcription initiation complex had been assembled, emphasizing the different techniques that had contributed to understanding the structures of individual components and their interactions, which in turn modify their structure and functional capabilities. As a challenge for the future, he finished with the problem of understanding how 'mediator' interacts both with this complex and with the regulatory transcription activating factors – studies that will require further methodological advances.

Genome Organization – men and marsupials

A strong tradition in Australian research is in genome organization and genetic mapping. This was illustrated first by a presentation by **Nick Martin** who described the use of twin studies in the mapping and characterization of quantitative trait loci (QTL) involved in cognition. He explained that part of their current research program in Brisbane derives from an international collaboration seeded by an HFSP grant in the late 90s looking at polymorphisms underlying variation in human cognitive abilities. Later studies eventually led to a localisation of these QTLs in discrete chromosome regions, but until recently it was difficult to go further in the analyses. Advances in genome wide scanning using 100 or 500K snp chips are now proving indispensable for the more detailed analysis of these regions and it is expected that such approaches eventually will pin-point the genes involved.

While Nick Martin was concerned with finding genes that are useful for brain function, the after-dinner speaker **Jenny Graves** was targeting 'useless' genomic regions and in her opening remarks suggested that males might like to leave her talk as one of her principal conclusions would be that the Y chromosome was of such little use that it would soon be eliminated. Despite this frontal challenge the whole group remained to follow her fascinating talk on chromosome structure and gene content in species endemic to Australia. Although she described them as weird, she went on to explain that they provide a unique opportunity in understanding the evolution of genomes as many of the more conventional species studied are either too far apart or somewhat too close in terms of evolution. As Graves said at one point, for some evolutionary studies 'Kangaroos are just right'. Using chromosome painting techniques her laboratory has rapidly elucidated the karyotypes and evolutionary history of chromosome segments in spectacular Australian species such as Tasmanian Devils, duckbilled platypus and many other endemic species. In fact, so far ranging have been their studies, her lab obtained a reputation for 'road-kill genomics' as their

techniques can be applied to virtually any source of material. A major theme of these studies is the chromosomal basis of sex determination and they have shown with both endemic species and others worldwide that the situation is far more complex than the familiar ‘simple’ XY system of mammals or ZW of birds and reptiles. Her recurring conclusion at different points in her presentation was that the Y chromosome was not as enduring and as important as some like to believe and that in humans it might be completely eliminated in the next 9 million years. Jenny Graves finished with the declaration that ‘Weird is good’ because their studies on animals that were previously considered to be too unusual to merit serious study had in fact revealed much about the underlying biological mechanisms of sex in evolution. There was a certain relief among the “Y-carriers” at the end of her talk. As Torsten Wiesel remarked in his thanks, given the time-scale involved, this loss should not be a serious worry for members of the audience.

The annual HFSP meetings bring together both awardees and others who have been associated with the program for several years as members of the Board of Trustees, Council of Scientists and Review Committees. It is a time to reflect collectively on the health of the program and to measure progress in the initiatives introduced in the scientific programs since 2000. Efforts have been made during this period to enhance the interdisciplinary nature of the Program by changing the criteria for the review of research grants and encouraging fellows to acquire new expertise during their postdoctoral training. As in previous meetings, it was a pleasure to see the enthusiastic participation of so many young scientists in a meeting where the scientific invitations are limited to awardees (and who cannot send post-docs to represent them). Many were of course fellowship holders and amongst these there were the signs of the emergence of an understanding of the need for broader training, a trend stimulated perhaps by the establishment of Cross Disciplinary Fellowships. As to the grant teams, who present their reports some 3-4 years after the review cycle that led to their funding, there is necessarily a delay in seeing the full consequences of initiatives. In informal discussions there was a clear and unsolicited consensus that interdisciplinarity was very much present this time and that this aspect of the Program has truly come of age.