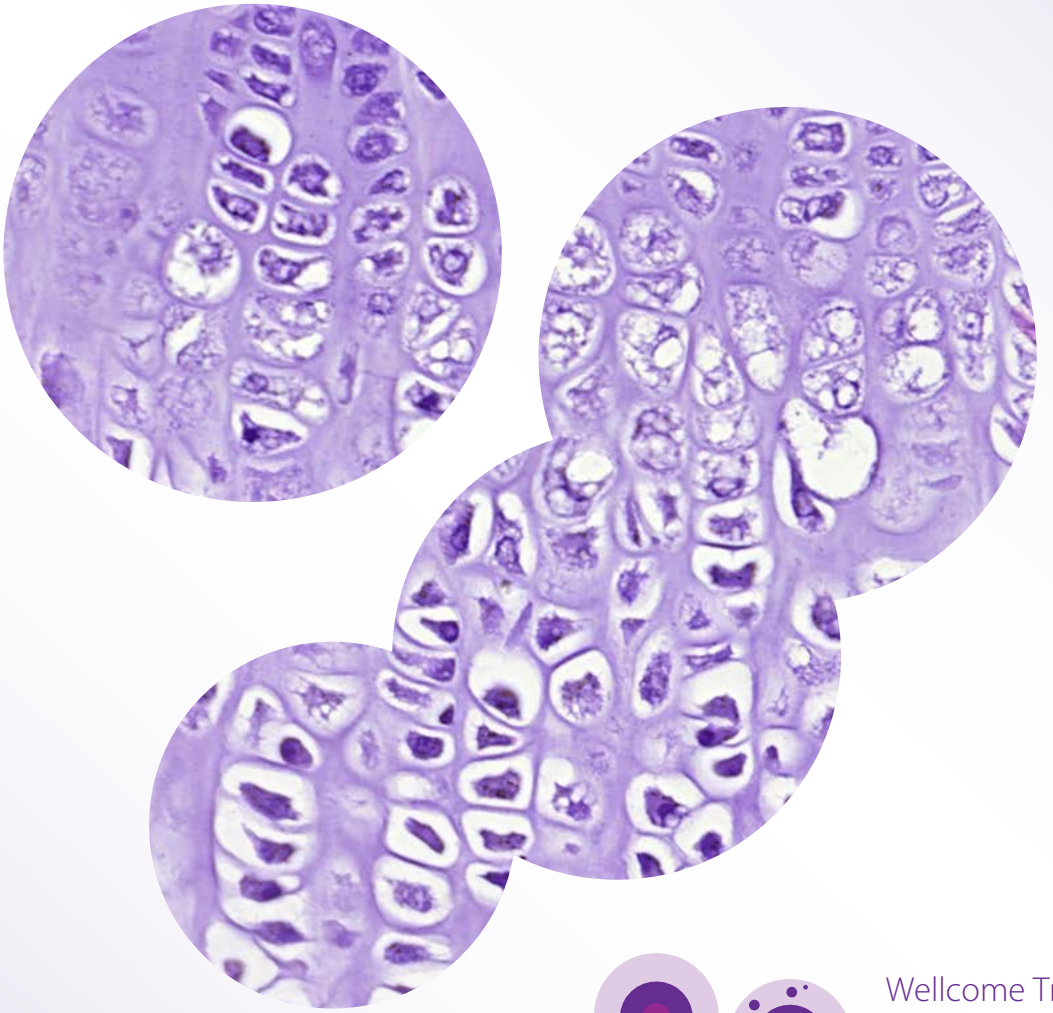
MANCHESTER 1824

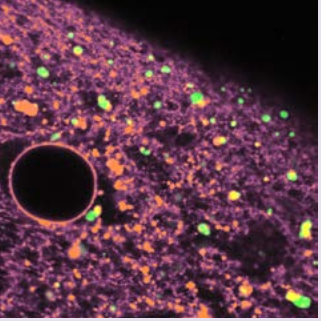
The University of Manchester

2014



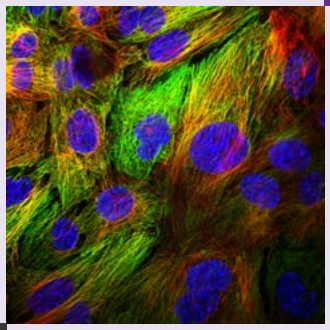
Wellcome Trust Centre for

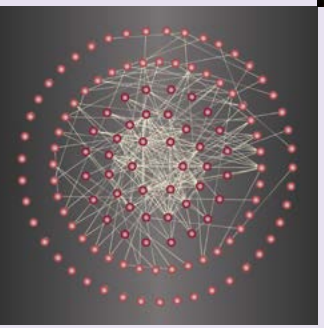
Cell-Matrix Research



Overview of Cell-Matrix Research

The extracellular matrix is the material outside of cells that creates the three-dimensional framework supporting tissues. The 'matrix' is found around and between all cells, providing the microenvironment in which cells live. Matrix functions at different dimensions, providing the macro-scale structural and protective framework necessary for tissues/ organ function and at the molecular-scale, intimate contacts, signals and interactions that govern cell behaviour.



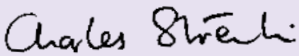


Matrix is dynamic, constantly being assembled, maintained and remodelled by cells, and it presents the physical and biological cues that are essential for different cell functions.

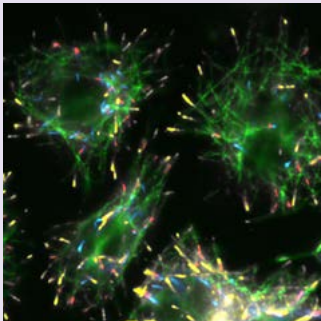
Cell-matrix interactions are crucial for nearly every aspect of body function. Due to this close connection between cells and the matrix, failures in the extracellular matrix are linked to many acute and chronic diseases, including: cancers, fibrosis of the heart, liver and kidney, lung diseases, osteoarthritis, tendinopathies, as well as inherited musculoskeletal conditions.

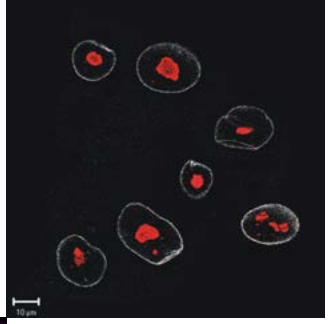
The research in the Cell-Matrix Centre is focussed into three major research areas. These will provide paradigm-shifting changes in the understanding of cell-matrix biology over the next decade. They are aimed at delivering novel and unique opportunities for medical advances in tissue regeneration, for the protection of tissues from inflammation and fibrosis, and for the repair and recovery of key nanomechanical functions.

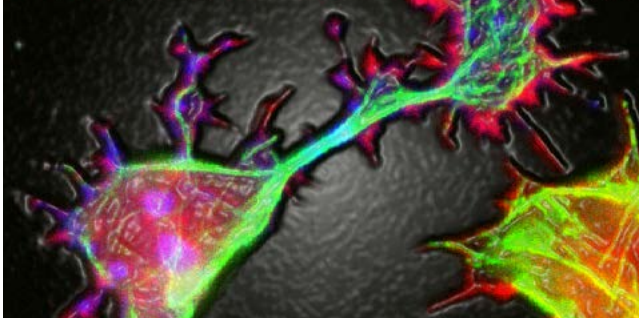
Around 130 biomedical researchers work at the Centre, including principal investigators, postdoctoral scientists, PhD students, technical and support staff. In this brochure, we highlight the outstanding discoveries of our scientists during 2013; the key advances that our core facilities support in our research; the international conferences we've organised; the visitors we have welcomed to the Centre; and the new ways in which we are interacting with the public.

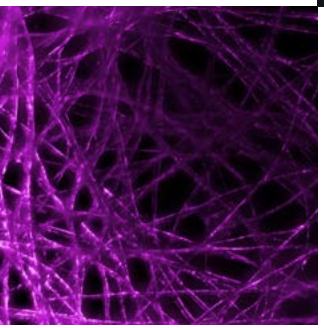


Charles Streuli
Director









Mission Statement

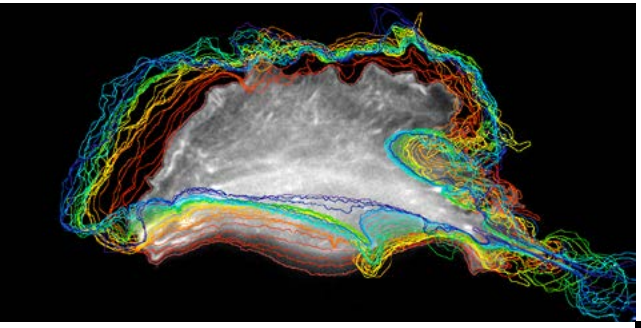
Our scientific mission is to understand how cells make their extracellular matrix microenvironment, and how they integrate matrix-derived biological and physical cues to build and repair tissues. By doing so we will discover how changes in cell-matrix interactions cause and exacerbate major human pathologies, and we will exploit these findings for disease prevention, diagnosis and therapy.

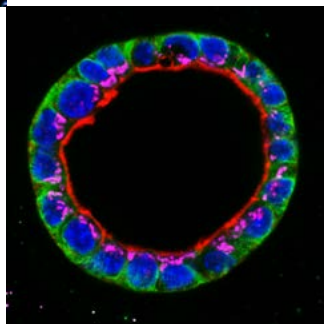
Our work focuses on three specific areas in cell-matrix biology.

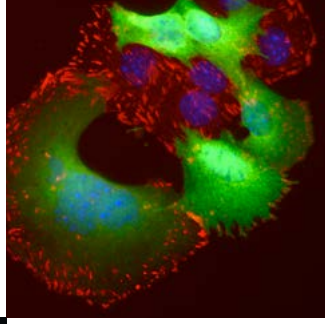
Cellular Matrix Microenvironment - We are using state-of-the-art approaches to discover how 3D matrix organisation and cell-matrix interactions regulate cell programing and behaviour in normal tissue homeostasis and how changes in matrix compromise cell function in disease.

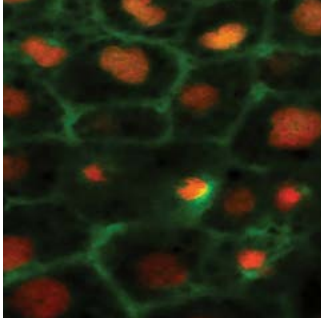
Matrix Immunobiology - We are discovering new areas by which the extracellular matrix controls innate and adaptive immune responses and influences host-parasite interactions, and how the matrix forms an essential part of the immunomodulatory function of mesenchymal stem cells.

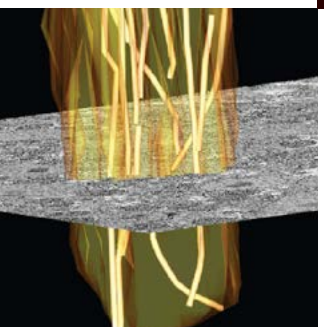
Mechanobiology of Matrix - We are designing new approaches to reveal how tissues are formed from the nano through to the macro scale, to understand how matrix determines tissue physical properties, how this regulates cell behaviour and how altered tissue stiffness can drive pathology.







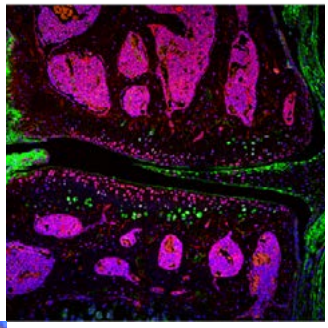




Research themes within the Centre

Cellular Matrix Microenvironment

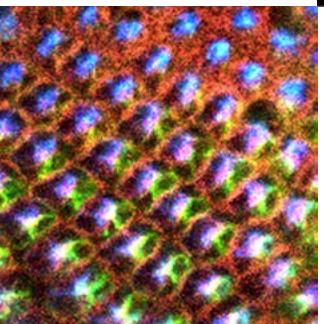
Cells in all our tissues assemble and are embedded within an extracellular matrix- microenvironment. By remodelling this matrix throughout life, cells fine-tune the extrinsic signals that are essential to maintain healthy cell and tissue function. However, sudden or progressive degenerative changes in matrix organisation and cell-matrix interactions cause or exacerbate many major diseases as well as ageing.



Our research vision is to determine the molecular mechanisms by which cells assemble and remodel their three-dimensional matrix, and how the matrix generates signals to control cellular behaviour.

To achieve these aims, we are discovering: cellular mechanisms of matrix assembly; cell-matrix interactions and signals regulating stem cell fate; matrix control of growth factor bioavailability; mechanisms of cell migration in tissues; integrin signals that control epithelial polarity; the control of cell fate by integrin trafficking; and mechanisms linking mutations in matrix-associated genes to a broad range of skeletal diseases.

In the longer term, our research will facilitate strategies to replace defective tissues through targeted stem cell therapy and will lead to the design of new treatments for diseases arising from inherited skeletal disorders, defective wound healing and malignant cancers.



How epithelial cells know which way up they are

Milk is made in the mammary gland in epithelial ball-like structures called alveoli, from which it is squeezed into the ductal plumbing network of the tissue in order for babies to feed.

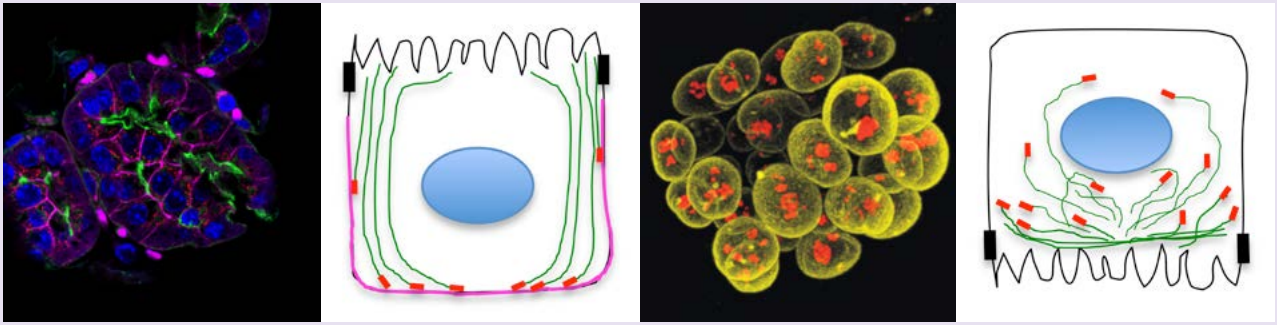
This process requires correct topological secretion of milk proteins, fats and sugars into the central, luminal space of alveoli. Epithelial cells are polarised with a top (apical) surface that faces the lumen and a bottom (basal) surface that is adjacent to the rest of the tissue. In order for milk to be secreted properly, the polarity of the epithelial cells needs to be in the correct orientation. However, until now it was not known how epithelial cells know which way up they are.

Discovery: Here we discovered that a transmembrane receptor protein called β1-integrin determines the polarised orientation of epithelia. Integrins binds cells to the main constituent of their surroundings, the extracellular matrix. We used genetic methods to delete the gene for β1-integrin in mammary gland, and found that the cells were the wrong way round, so the tissue became disorganised. Additionally, we observed that β1-integrin was needed to align intracellular architectural microtubules into an apical-basal orientation. This process then influences the transport machinery of the cell to internalise apical membrane components that are incorrectly located on the basal membrane. By default, the top surface then forms on the opposite side of the cell.

Importance: This study provides new molecular clues to explain how epithelial cells know which way up they are. Moreover, when epithelial cells loose their polarity, they resemble the kind of tissue structure that forms in the early stages of cancer. This suggests that there may be a link between altered cell organisation and the formation of breast tumours. We hope that our work to understand cell polarity could ultimately lead to better diagnosis for cancer patients.

Charles Streuli

Akhtar, N. and Streuli, C.H. (2013). An integrin-ILK-microtubule network orients cell polarity and lumen formation in glandular epithelium. **Nat Cell Biol.** 27-17 ,15.



Cell movement explained by molecular recycling

Many cells in our body have the capacity to migrate through the dense network of fibres that surrounds them. This process is essential for repairing wounds, combatting infection, and maintaining tissue function.

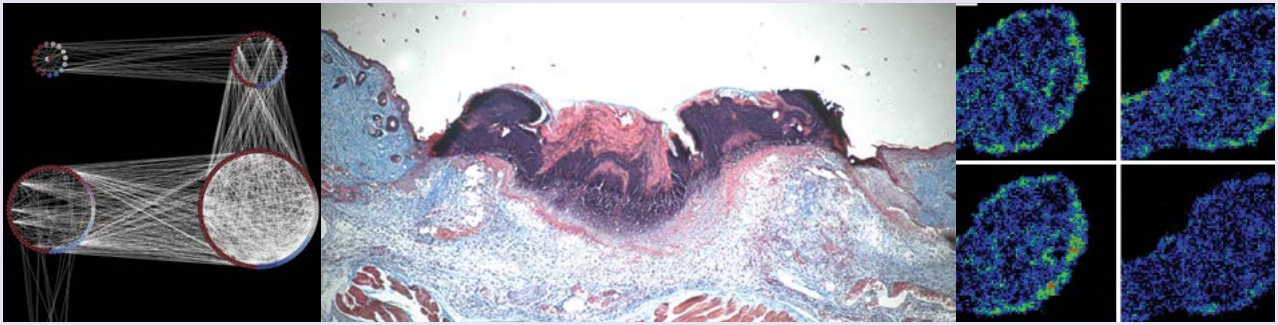
As cell movement is so important, it is perhaps not surprising that it is disrupted in many diseases including cancer, vascular disorders and chronic inflammatory disease. Cells have a group of molecules on their surface, called integrins. These molecules are able to grab hold of the fibres surrounding the cell, and allow the cell to drag itself through tissue. However, there are several types of integrin on the cell surface and they all have different properties; some hold very tightly to fibres whereas others bind quite weakly. In order for cells to crawl efficiently, the cell needs to control precisely which integrins are able to bind the fibres.

Discovery: We have uncovered one way in which cells dynamically control integrins, as cells explore their environment. Rather like any environmentally conscious individual, cells don't like waste. Instead of using the integrins once, and then disposing of them, cells adopt a recycling scheme - this helps minimise waste and conserve energy. Integrins are internalised from the cell membrane, moved to an intracellular store and, when the time is right, recycled back to the cell surface, where they can bind the surrounding fibres. We have shown that another molecule on the cell surface, called syndecan-4, is able to detect and interpret subtle changes in the cell's surroundings. Syndecan-4, decodes the vast array of signals outside the cell and functions as a molecular switch to dictate whether the strong or weak binding integrins are recycled. By regulating where and when the different integrins are delivered to the cell surface, syndecan-4 precisely regulates cell movement and exploration.

Importance: This study provides fundamental insight into how cells migrate within the body. As syndecan-4 plays a key role in regulating wound healing, we hope that ultimately this work will inform the development of novel therapeutic strategies to improve wound healing. Intriguingly, many of the molecules identified in this study also have key roles in regulating cancer invasion, so it will now be important to test whether this mechanism is involved in tumour progression and metastasis.

Martin Humphries

Morgan, M.R., Hamidi, H., Bass, M.D., Warwood, S., Ballestrem, C. and Humphries, M.J. (2013). Syndecan-4 phosphorylation is a control point for integrin recycling. **Dev Cell.** 24, 472-85.



Cancer cell invasion: the third way

Metastasis is the spread of cancer cells from the site of a primary tumour to distant organs, and is the leading cause of death in cancer patients. In order to spread into surrounding tissues, cancer cells migrate through the extracellular matrix meshwork of proteins that surrounds them.

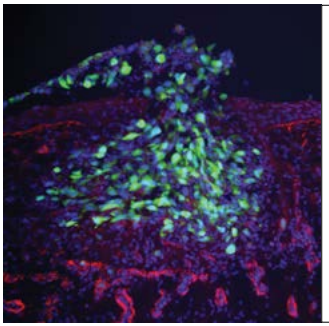
Migration requires that the cells change their shape and reorganise their internal cytoskeleton. This is controlled by both physical signals from the matrix via integrin receptors, and by chemical cues via growth-factor receptors. However, how the combined signals from integrins and growth factor receptors control the cytoskeleton during cell migration is not fully understood.

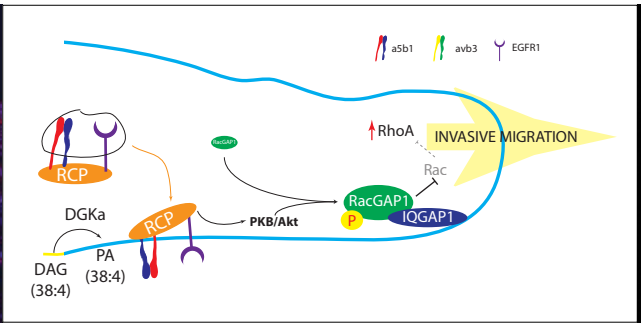
Discovery: We have shown that during cell movement, intracellular transport of integrins by Rab-coupling protein (RCP) determines the localised action of growth-factor receptors and extension of long processes that invade the matrix. Signals at the front of the cell attract two regulatory proteins called RacGAP1 to IQGAP1. One of these proteins, RacGAP1 controls RhoGTPase signaling, inactivating Rac and activating RhoA, to reorganise the actin cytoskeleton into spikes that drive the cell forward into the ECM.

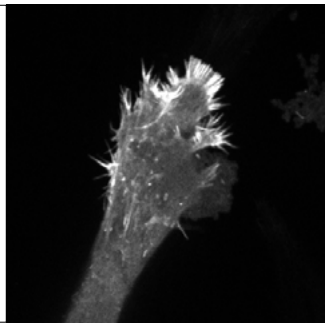
Importance: Until now, invasion of individual cancer cells has been broadly categorised as 'mesenchymal' or 'amoeboid'. The mesenchymal mode of migration occurs by elongation of the cell and activation of Rac, which creates waves of actin cytoskeleton at the front edge of the cell. In amoeboid migration, rounded cells move by activating RhoA, which pushes the membrane into 'blebs'. We have now uncovered a third mode of invasion, which we call 'pseudopodial' migration. This is characterised by protrusion of long extensions in the direction of migration, and tipped by the formation of new bursts of cytoskeletal spikes. Understanding the exact ways that cancer cells move within the ECM will help to develop new drugs that prevent cell migration and reduce the mortality caused by metastasis.

Pat Caswell

Jacquemet, G., Green, D.M., Kriegsheim, A., Humphries, M.J., Norman, J.C. and Caswell, P.T. (2013). RCP-driven $\alpha 5\beta 1$ recycling suppresses Rac activity through the RacGAP1-IQGAP1 complex to permit local activation of RhoA which drives invasive migration. **J Cell Biol.** 202, 917-35.







Healthy cells work hard to avoid preprogrammed suicide

Almost all cells are preprogramed to commit suicide if they become damaged. This process, called apoptosis, is tightly regulated to ensure that the death programme is activated in cells that need to die.

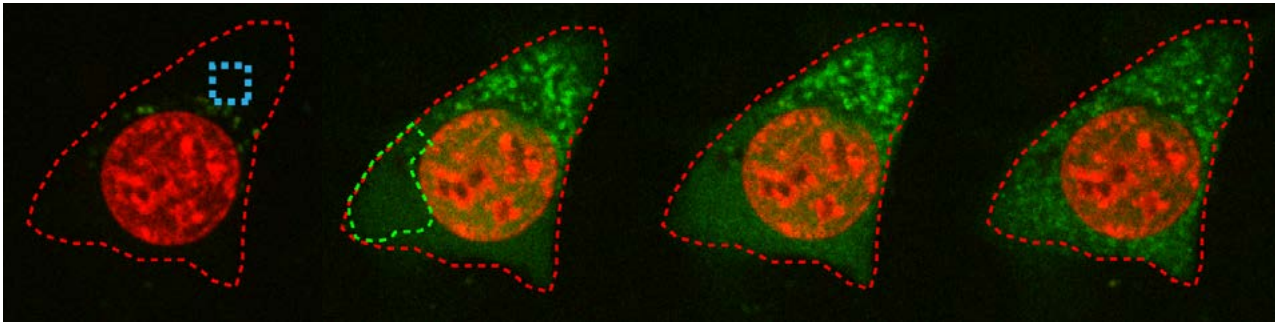
Apoptosis is controlled by mitochondria, whose primary function is to generate ATP. Mitochondria also contains cytochrome *c*, which can kill cells by apoptosis if it is released into the cytosol. When cells become damaged, a group of proteins termed the Bcl-2 family form holes in the mitochondrial outer membrane, thus releasing cytochrome *c* to kill the cell. Current models of how Bcl2- proteins are regulated suggest that they exist in a latent, inactive state in healthy cells, but become activated after cellular damage or after loosing contact with matrix. One Bcl2-protein, called Bax is found in the cytosol in healthy cells. It was previously thought that when a cell activates its apoptosis program, Bax moves from the cytosol to mitochondria, where it releases cytochrome *c*. However our new work challenges this view.

Discovery: Here we have discovered that, rather than actively turning on the process of apoptosis, cells have to continually suppress it to stay alive. By looking at cells in real time, we found that Bax constantly associates with mitochondria. However, in healthy cells it is removed back to the cytosol before it has time to form pores for releasing cytochrome *c*. We found that the signaling pathways that normally keep cells alive, including integrin-regulated adhesion pathways, perpetually remove Bax from the mitochondrial membrane. When survival pathways are prevented, Bax accumulates on mitochondria simply because it is no longer being removed.

Importance: The prevailing view of apoptosis is one in which key regulators are actively turned on when a cell is damaged. However, our research indicates that the reality is the opposite. Indeed the default decision of a cell is to activate apoptotic proteins. Healthy cells make an effort to avoid this, for example by attaching to extracellular matrix. Furthermore, the signaling pathways that control the removal of Bax from mitochondria are often activated in cancer.

Andrew Gilmore

Schellenberg, B., Keeble, J.A., Walker, S., Owens, T.W., Foster, F., Tanianis-Hughes, J., Brennan, K., Streuli, C.H. and Gilmore, A.P. (2013). Bax exists in a dynamic equilibrium between the cytosol and mitochondria to control apoptotic priming.
Mol Cell. 71-959 ,49.



Increasing the effectiveness of an anti-melanoma drug

Melanoma is responsible for over 90% of skin cancer deaths. Today the most prominent genetic changes in melanoma have been characterised. This helped to identify a group of proteins called the MAP-kinase signalling module as the main driver of melanoma development.

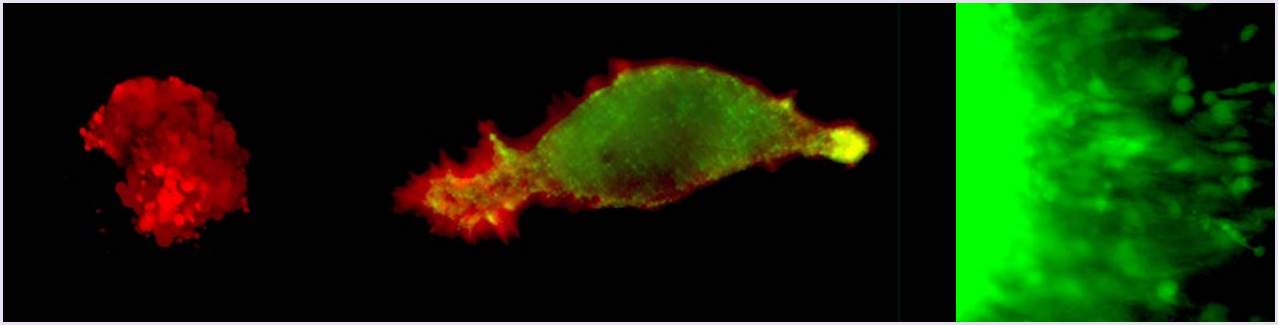
Currently inhibitors for the protein MEK, which is one component of this module, are being evaluated in the clinic. However, MAP-kinase signalling is important for all cells and not only for melanoma cells. Therefore, higher doses of the drug are required for a good response, which leads to strong side effects. This means that any mechanism that can be targeted to increase the effectiveness of MEK inhibitors has the potential to improve the treatment of melanoma.

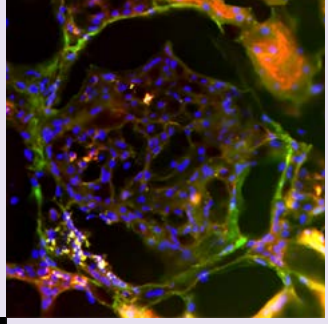
Discovery: We discovered that targeting a protein called SMURF2 primes melanoma cells to the cancer-cell killing effects of the MEK inhibitor 'Selumetinib'. Removing SMURF2 from melanoma cells allows Selumentinib to kill melanoma cells within a shorter time, i.e. 24 hours instead of vs 3-4 days, and at ~100 fold lower concentrations. Moreover, when we removed SMURF2 from cells it did not affect normal cells, but it greatly enhanced Selumetinib's anti-tumour activity in mice with melanoma.

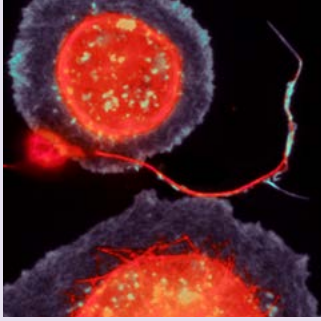
Importance: Melanoma is the fifth most common cancer in the UK, but it is notorious for being resistant to most treatments. The recent discovery of the role of the MAP-kinase signalling module in melanoma has led to the development of a whole new group of drugs, such as the MEK-inhibitors that are now tested in the treatment of melanoma. However, identifying other drugs to use in combination with MEK inhibitors will provide a much more powerful and ultimately more successful approach to treating melanoma. Such combinations will also reduce the toxicity of the MEK-inhibitors currently used, and this means that cancer treatments are less harmful to patients. Our findings provide a possible first step in the development of such combination therapies.

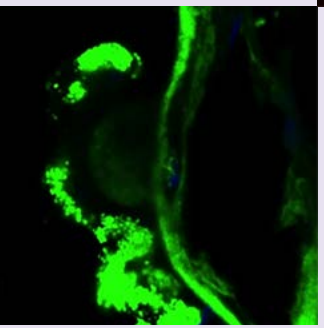
Claudia Wellbrock

Smith, M., Ferguson, J., Arozarena, I., Hayward, R., Marais, R. Chapman, A., Hurlstone, A. and Wellbrock, C. (2013) Effect of SMURF2 Targeting on Susceptibility to MEK Inhibitors in Melanoma. **J Natl Cancer Inst.** 105, 33-46.









Research themes within the Centre

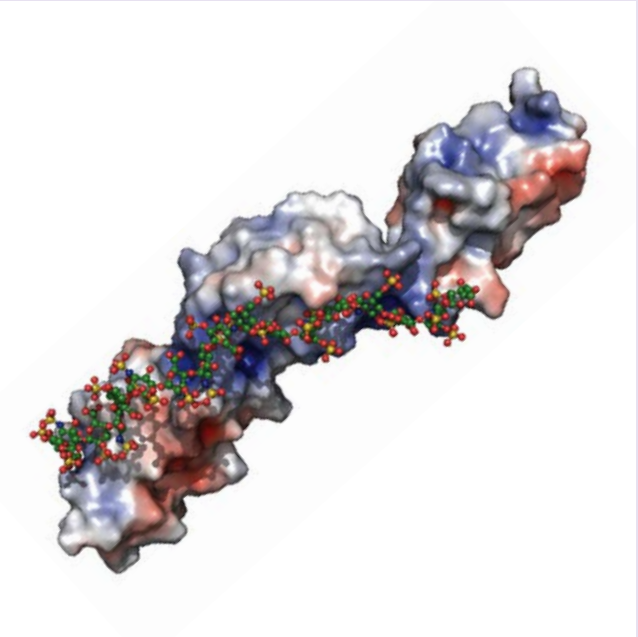
Matrix Immunobiology

The extracellular matrix has long been known to have an important role in leukocyte adhesion and migration. However, the matrix also plays a key role in controlling other aspects of immune function that become deregulated with age, infection and disease.

Our research vision is to determine how extracellular matrices act to control innate and adaptive immune responses and to regulate the immunomodulatory functions of multipotent stromal cells.

To achieve these aims, we are discovering: how matrix governs immune responses through control of TGFβ, chemokines, and complement; mechanisms by which the epithelial mucosal matrix controls host-parasite interactions and regulates the intestinal biome; how matrix interactions guide the immunomodulatory functions of stem cells; and how the changes in matrix structure that cause altered immune function drive pathology in the macula of the eye and in cartilage.

In the longer term, our research will lead to new ways to exploit mesenchymal stem cells in applications in regenerative medicine, and to devise new treatments for chronic inflammatory diseases and fibrosis, for colitis, for age-related macular degeneration, and for the control of parasitic nematode infections.



Tissue specific regulation of the immune system by eye and kidney matrix

Our innate immune system provides a first line of defense against many different types of microorganisms. This includes the "complement cascade", which has an important role in the destruction of pathogens as well as removal of the body's own dead cells.

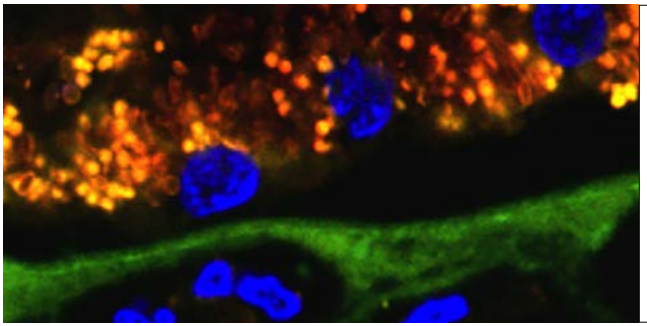
However unwanted activation of complement can damage healthy tissues, and needs to be carefully controlled. A protein termed complement factor H (CFH) can distinguish a host cell from an invading bacteria. CFH does this by recognizing particular molecular patterns on host cell surfaces and in the matrix. This allows it to bind to our own tissues forming a protective coating that stops inappropriate complement activation. CFH contains two pattern-recognition regions that can recognize a diverse family of sugar molecules (called GAGs) found on all host tissues and in the matrix. These two regions are termed CCP7 and CCP19-20.

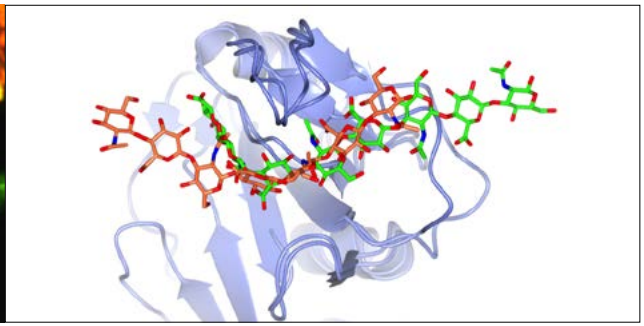
Discovery: Previously we showed that a common genetic variant within the CCP7 region impairs the binding of CFH to a matrix component within the eye, called Bruch's membrane. This may explain why this variant is associated with a higher risk of getting Age-related Macular Degeneration (AMD), which is the major cause of blindness in the developed world. Conversely, variants in CCP19-20 are associated with atypical hemolytic uremic syndrome (aHUS), a rare kidney disease that affects children. The variants that predispose to aHUS do not increase the risk of AMD or vice versa, and we have now found out why. We discovered that the CCP7 region plays the principle role in host tissue recognition in the human eye, whilst the CCP19-20 region makes the major contribution to the binding of CFH in the human kidney. Furthermore these two regions of CFH have different GAG-binding specificities and it is also likely that human eye and kidney tissues contain GAGs of different composition that act like molecular postcodes. In this way, the particular structure of the ECM within the eye and kidney determines precisely how and where CFH molecules will bind, tuning its function in a tissue specific manner.

Importance: Our work paves the way for development of tissue-specific therapeutic strategies for diseases where complement is regulated incorrectly.

Tony Day

Clark, S.J., Ridge, L.A., Herbert, A.P., Hakobyan, S., Mulloy, B., Lennon, R., Wurzner, R., Morgan, B.P., Urhin, D., Bishop, P.N. and Day, A.J. (2013). Tissue-specific host recognition by complement factor H is mediated by differential activities of its glycosaminoglycan-binding regions. **J Immunol**. 190, 2049-2057.





On a mission to tackle parasitic worm infections

Infection with intestinal parasitic worms is a major global health problem, with billions of people infected world-wide. Often these worms (known as helminths) develop a long-lasting chronic infection, due to the inability to mount the correct type of immune response that would normally expel the parasite.

This infection results in severe morbidity and health problems, which have been heavily linked to the poverty of affected regions. Because infections with these intestinal parasites are usually chronic, it is likely that helminths are able to influence the immune system to prevent their expulsion. Therefore, understanding the cellular and molecular pathways that regulate the immune response during helminth infection is crucial in identifying novel therapeutic targets for these poorly managed infections.

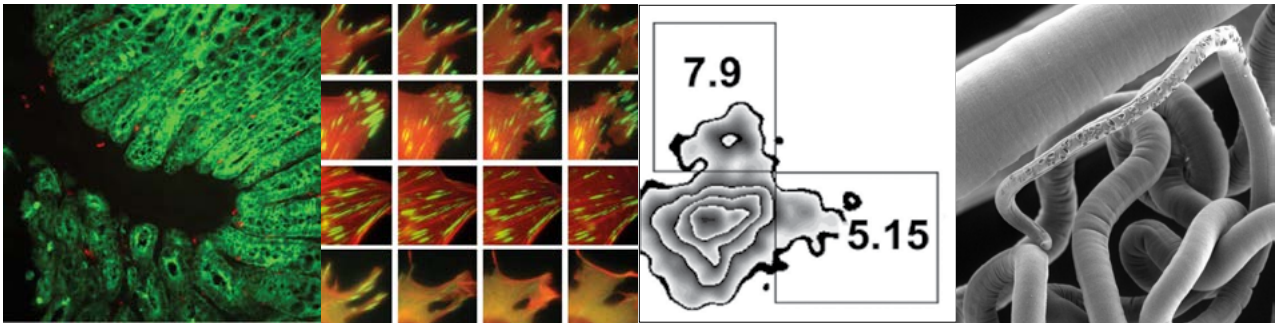
Discovery: Using a well-established mouse model of chronic helminth infection, we discovered that a protein called transforming growth factor beta (TGFβ) signals to T-cells early during the development of chronic infection, and that blocking this signal protects mice from infection.

We have uncovered a mechanism and a cell type that controls this TGFβ signalling system during the development of a chronic infection. When a protein called integrin αvβ8 is absent from dendritic cells of the immune system, TGFβ cannot be activated to signal to T-cells and mice are then able to mount a protective (type 2) immune response resulting in worm expulsion.

Importance: Helminth infections are normally treated with anti-helminthic drugs, which kill the parasite. However, rapid re-infection with worms often occurs and drug resistance is common. Our research provides new insights into how these chronic infections develop, and it has identified new molecular targets for the long-term prevention of chronic helminth infection.

Mark Travis

Worthington, J.J., Klementowicz, J.E., Rahman, S., Czajkowska, B.I., Smedley, C., Waldmann, H., Sparwasser, T., Grencis, R.K. and Travis, M.A. (2013). Loss of the TGFβ-Activating Integrin αvβ8 on Dendritic Cells Protects Mice from Chronic Intestinal Parasitic Infection via Control of Type 2 Immunity. **PLoS Pathog.** 9, e1003675.



Intestinal mucus provides a battleground to protect against parasite infections

Gastrointestinal parasitic worm infections cause significant morbidity, affecting up to a third of the world's population and their domestic pets and livestock. Mucus is a gel-like extracellular matrix that blankets the surface of the intestine.

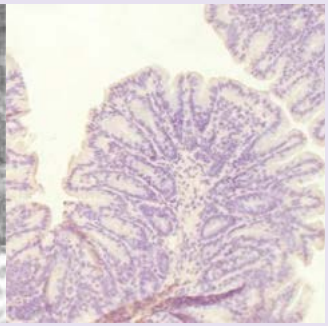
Mucous forms a protective barrier based on a network of mucin glycoproteins, and is an important part of our innate immune system. The whipworm Trichuris is closely associated with the intestinal mucus barrier. We have previously established that a specific type of mucin (Muc5ac) affects the viability of the worms, and plays a significant role in their expulsion from the gut in a mouse model. Mice unable to produce Muc5ac are unable to expel the worms.

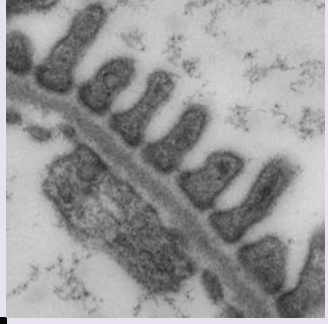
Discovery: By comparing mice that get long-term chronic infections with other mice that are able to expel the worms from the intestine, we have uncovered a new role for products secreted by the worms. We have discovered that protease enzymes secreted by whipworms can disrupt the mucin network that gives mucus its barrier properties. However, we found that these proteases are unable to degrade Muc5ac, the mucin that is only present in the mucus barrier in mice able to expel the worms. Moreover, we showed that mice able to expel worms also produced anti-proteases to combat the worm products.

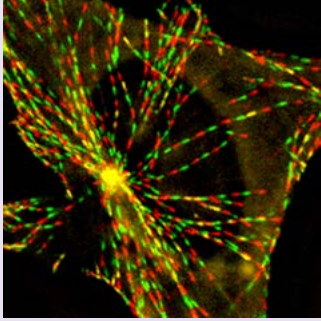
Importance: Our work suggests that these protease enzymes may be released by the worm as part of a regime to improve its niche within the intestines and to survive in the host. However, the host is able to counteract by producing molecules that protect the mucus barrier from degradation, and which are detrimental to the viability of the worm. We hope that by exploiting chinks within the host-parasite relationship, we will be able to develop novel strategies to tackle this global health problem.

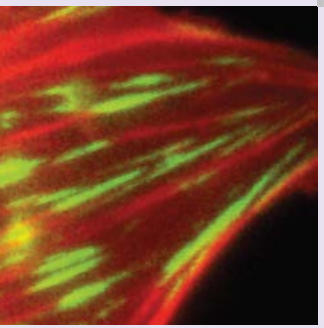
Dave Thornton

Hasnain, S., Grencis, R.K. and Thornton, D.J. (2012). Serine protease(s) secreted by thenematode Trichuris muris degrade the mucus barrier. **PLoS Neg Trop Dis.** 6, e1856.





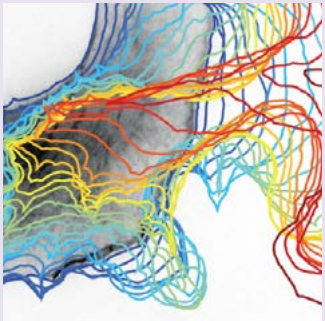




Research themes within the Centre

Mechanobiology of Matrix

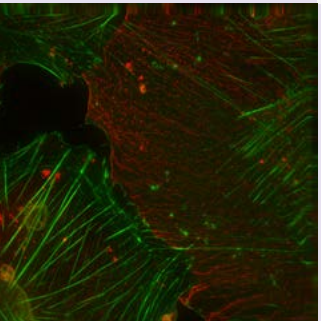
Mechanical forces contribute to all levels of vertebrate development, morphology, and tissue function. Cells exert tension on the surrounding extracellular matrix to guide tissue assembly, they migrate through mechanically varied and biophysically complex matrices, and cell fate and phenotype are governed by matrix stiffness. Furthermore, altered biomechanical properties of the matrix in disease and ageing can have profound consequences on the health and function of cells and tissues.



Our research vision is to obtain an integrated mechanistic understanding of how cells make and maintain a highly organised and biomechanically complex matrix, and how forces arising within the cellular niche are sensed and transduced into intracellular pathways controlling cell fate and tissue assembly.

To achieve these aims, we are discovering: the structural basis of matrix elasticity; how cells generate and maintain tension and how matrix organisation determines stress relaxation; the mechanisms underpinning the sensing of elasticity; the biomechanical pathways that control mitochondrial function; mechanical regulation of mitotic spindle orientation; and the links between biomechanics and circadian transcriptional rhythms within musculoskeletal and epithelial tissues.

In the longer term, our research will establish principles to understand the basis of diseases caused and exacerbated by abnormal matrix biomechanics and altered cellular mechanotransduction, including fibrosis, kidney failure, and musculoskeletal diseases such as tendinopathies and osteoarthritis. Moreover, they will provide new opportunities for risk assessment, early detection of disease and new candidate treatments for a broad range of “mechanopathies”.



Uncovering how collagen fibrils are manufactured and transported

Collagen is the main structural protein in connective tissues in animals and exists as ordered bundles of fibrils in tendons, ligaments, bone and skin.

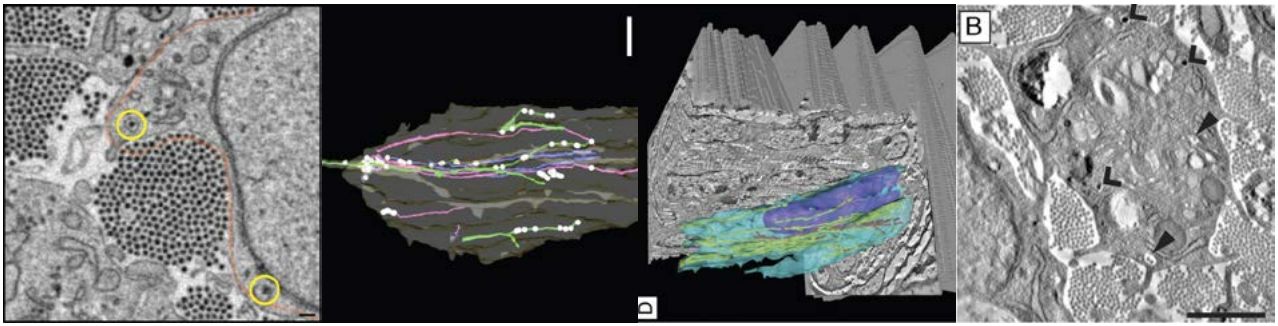
Collagen fibrils can be several millimeters in length, making them the longest, largest and most size-variable polymers known. Until now, the precise cellular location of their manufacture and the transport of these large proteins was not understood.

Discovery: Here we used serial block face scanning electron microscopy to generate high resolution 3D images of embryonic tendon tissue. We found that collagen fibrils are assembled in specialised cell membrane structures, called fibripositors, and are transported at the surface of tendon cells during development. The transport of the fibrils was dependent on cellular force produced by the molecular motor non-muscle myosin II.

Importance: We show that fibripositors are a non-muscle myosin II (NMII)-dependent mechanical interface between the actinomyosin machinery and the extracellular matrix. Understanding the location of fibril assembly is important to allow treatment of diseases where there is too much fibril assembly (fibrosis) or deregulated fibril assembly (scarring). This unique mechanism of collagen fibril transport could form the basis of future studies into fundamental processes of development, including tissue morphogenesis and in the study of conditions including wound healing and fibrosis.

Karl Kadler

Kalson, N.S., Starborg, T., Lu, Y., Mironov, A., Humphries, S.M., Holmes, D.F. and Kadler, K.E. (2013) Non-muscle myosin II powered transport of newly-formed collagen fibrils at the plasma membrane. **PNAS**. 110, E4743–E4752.



Chondrocytes have circadian rhythmicity that dampens with age

Cartilage is the tissue that lines the articulating surfaces of joints, allowing smooth gliding of the joint surfaces and providing shock absorbing properties. In osteoarthritis, the cartilage becomes damaged and joint movement is compromised and frequently painful.

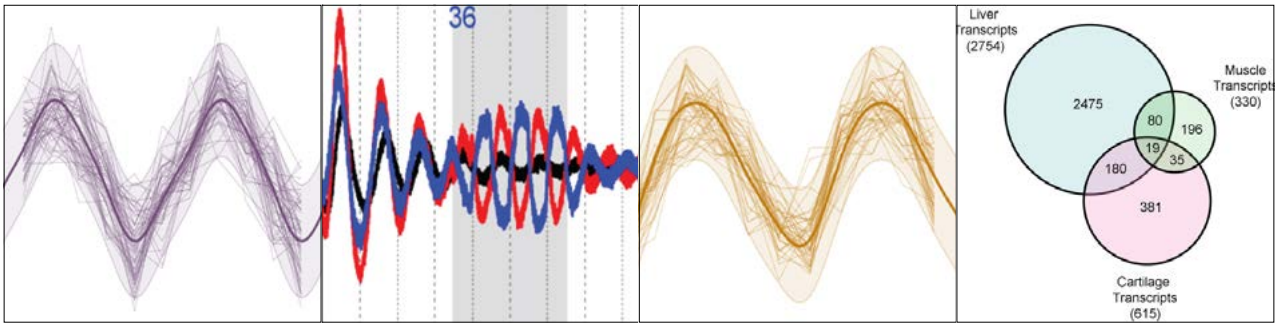
Many studies have shown that osteoarthritis results from complex combinations of genetic and environmental factors. Increasing age is one of the major susceptibility factors, although how age causes increased susceptibility remains unknown.

Discovery: We have now discovered that chondrocytes, which are the cells responsible for the synthesis and maintenance of cartilage, exhibit cell autonomous circadian clocks. The chondrocyte clock drives the transcription of around 4% of all the genes expressed in cartilage, with a 24 hour period. Most of the genes controlled in this manner are chondrocyte-specific and involved in cartilage homeostasis, and many encode matrix proteins and proteinases. We found that the chondrocyte circadian clock is regulated by hormones and by changes in body temperature. Moreover in cartilage, the amplitude of rhythmic gene expression is dampened in aged tissue, and the expression of core circadian clock genes becomes disrupted during osteoarthritis.

Importance: The results reveal for the first time that circadian rhythm controls the expression of key chondrocyte genes involved in cartilage homeostasis, some of which have already been associated with osteoarthritis. The changes in oscillations in ageing and osteoarthritis suggests that cartilage may be less able to maintain itself as we age, perhaps explaining our increased susceptibility to osteoarthritis. Future work aims to identify a causal link between changes in chondrocyte rhythm and osteoarthritis, with a view to using drugs or timed heat therapy to restore the clock in ageing cartilage as possible future therapies for osteoarthritis.

Qing-Jun Meng

Gossan, N., Zeef, L., Hensman, J., Hughes, A., Bateman, J.F., Rowley, L., Little, C.B., Piggins, H.D., Rattray, M., Boot-Handford, R.P. and Meng, Q.J. (2013).The circadian clock in chondrocytes regulates genes controlling key aspects of cartilage homeostasis. **Arthritis Rheum.** 65, 2334-45.



Vinculin provides stability when tension increases

Cells sense their extracellular environment using integrin receptors. Integrins are linked to the cell's intracellular cytoskeleton via proteins arranged in large multi-protein complexes called focal adhesions.

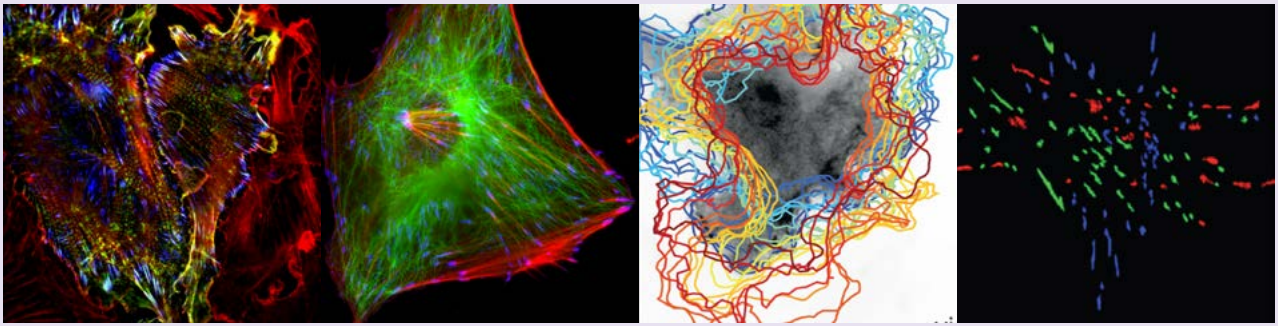
The physical properties of the extracellular matrix control fundamental cellular processes, such as cell division and migration. However the molecular basis of how integrins transduce mechanical stimuli from the matrix, and how this leads to a coordination of focal adhesions, is unclear.

Discovery: We have now discovered that vinculin, one of the key proteins in focal adhesions, is a tension-regulated switch and master regulator of mechanically controlled focal adhesion dynamics. We found that as intracellular tension increases, vinculin is recruited to focal adhesions, where it becomes activated. This activation process leads to increased stability of cell-matrix interactions and activation of core integrin signaling components. Using mutant forms of vinculin, we found that its ability to bind to actin has a key role in the cells' response to mechanical stimuli. The data suggest a tension-relaxation cycle in which physical forces stabilize adhesions through coordinated recruitment and activation of other focal adhesion components to activated vinculin. Once tension is released, vinculin reverts to an inactive state, leading into the release of focal adhesion proteins. Any perturbation of this cycle of activation and inactivation of vinculin would lead to aberrant cell motility and cell polarization.

Importance: The appropriate cellular responses to mechanical forces originating within the matrix are important for maintaining tissue integrity and organ function. Even at the earliest stages of development, differences in mechanical environments determine how cells behave and what cell types they will become in the adult. Many tissues, including the lung, kidney and blood vessels are constantly exposed to varying physical stresses and strains, and failure of cells to respond to mechanical stimuli leads to serious health problems. Our work is important because it reveals that vinculin is a key regulator of force-induced cellular responses.

Christoph Ballestrem

Carisey, A., Tsang,R., Greiner, A.M., Nijenhuis, N., Heath, N., Nazgiewicz, A., Kemkemer, R., Derby, B., Spatz, J. and Ballestrem, C. (2013). Vinculin Regulates the Recruitment and Release of Core Focal Adhesion Proteins in a Force-Dependent Manner. **Curr Biol.** 81-271 ,23.



The stress of inherited skeletal diseases

In order to function correctly, newly synthesised proteins must be folded correctly. The folding process often requires a particular type of protein called a chaperone. When a protein contains a mutation that alters the normal amino acid sequence, protein misfolding can occur.

We have previously shown that some mutations in the genes encoding extracellular matrix proteins trigger a stress response within the endoplasmic reticulum (ER) called the unfolded protein response (UPR). This leads to skeletal dysplasia. The UPR involves increased expression of chaperones and folding-associated proteins, however not all the components of this response pathway are known.

Discovery: We have now discovered that mutations in matrilin-3 and collagen-10, which cause two different types of chondrodysplasia, result in the expression of two new ER stress response proteins, Armet and Creld2. Both these proteins become secreted as part of the UPR, and one of them, Creld2, helps to resolve malformed disulphide bonds.

Importance: Our work reveals that the repertoire of ER stress response proteins induced by the expression of mutant extracellular matrix proteins is finely tuned to the nature of the mutation. So even though mutations in different matrix genes can result in the same skeletal dysplasia, they induce subtly different ER stress responses. This means that future therapies to treat skeletal dysplasias, particularly in children, will need to be tailored carefully to the specific nature of the inherited disease.

Ray Boot-Handford

Hartley, C.L., Edwards, S., Mullan, L., Bell, P.A., Fresquet, M., Boot-Handford, R.P. and Briggs, M.D. (2013) Armet/Manf and Creld2 are components of a specialised ER stress response provoked by inappropriate formation of disulphide bonds: implications for genetic skeletal diseases. **Hum Mol Genet.** 22, 5262-75.

How nature builds elasticity into the matrix

Elastin is the main protein in mammals that endows tissues such as skin, lungs and blood vessels with their elastic properties.

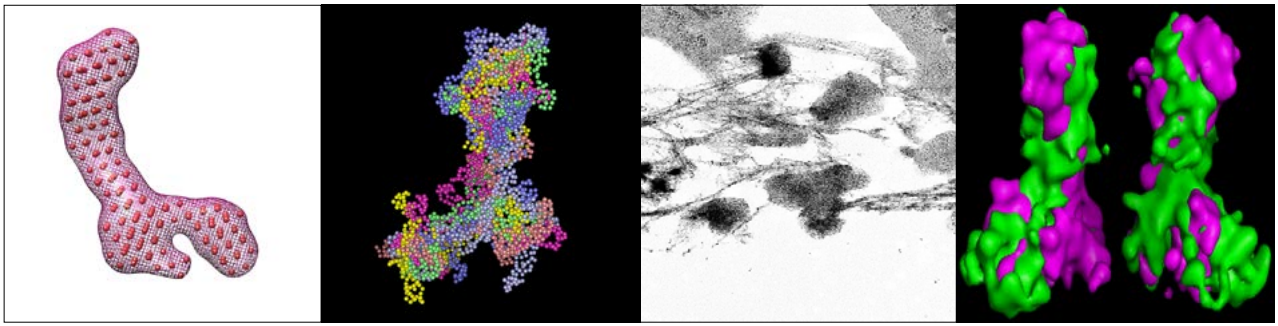
Elastin is produced during embryonic development, and under normal conditions, it should last us a lifetime. Large blood vessels such as the aorta are able to use the elastic properties of elastin to expand and contract with every pulse, and to repeat this for billions of heartbeats. The soluble building block of elastin is the molecule tropoelastin.

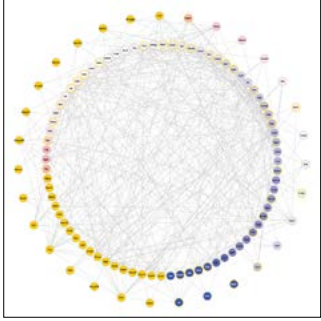
Discovery: We identified for the first time, a region in tropoelastin called the "bridge" region that links the two ends of the molecule, the coiled N-terminus and cell-binding C-terminus. We found that making changes to the bridge region reduces the capability of tropoelastin to assemble into elastin and to bind to cells. Disrupting the bridge region also increases the flexibility of the tropoelastin molecule.

Importance: The use of elastin-based biomaterials for tissue engineering is becoming increasingly popular in regenerative medicine applications. Our work on how the properties of the different regions of tropoelastin contribute to its function is opening up new ways to develop improved synthetic 'elastin-like' polymers. It is also revealing how changes in elastic tissue structure and function contribute to human diseases, and will suggest new approaches to alleviate the problems this causes in ageing and in patients with connective tissue disorders.

Clair Baldock

Yeo, G.C., Baldock, C., Tuukkanen, A., Roessle, M., Dyksterhuis, L.B., Wise, S.G., Matthews, J., Mithieux, S.M., Weiss, A.S. (2012). The tropoelastin bridge region positions the cell-interactive C-terminus and contributes to elastic fiber assembly. **Proc Natl Acad Sci U S A.**, 109, 2878-2883.

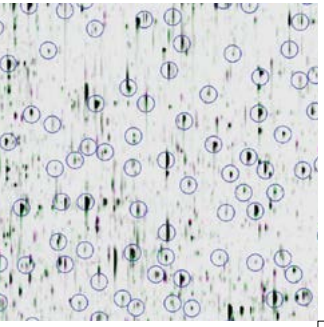


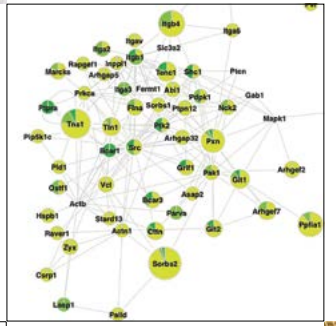


Core Facilities

Biological Mass Spectrometry

The Biological Mass Spectrometry facility is one of the largest of its type in Europe, with 6 staff including a dedicated informatition and a complete suite of mass spectrometers and sample preparation technologies that supports work within the Centre. The mass spectrometry equipment available includes; Orbitrap Elite; Thermo Velos Pro; Applied Biosystems 4000 Q-Trap; Bruker Ultraflex II MALDI TOF-TOF; Agilent 7890GC with 5975C mass spectrometric detector; Agilent 6520 Q-TOF.



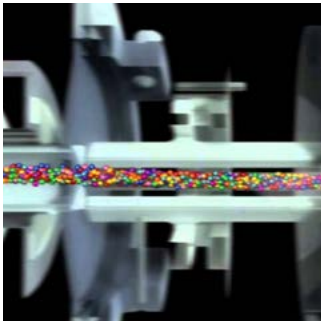


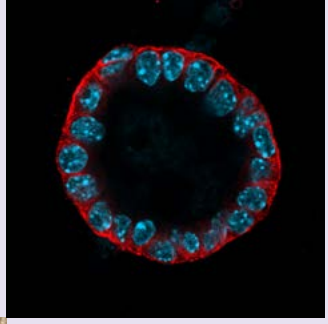
Sample preparation technologies include; 1 and 2D gel electrophoresis, preparative PAGE (Expedeon GelFree 8100), isoelectric focusing (Agilent Offgel); standard and micro scale HPLC purification systems).

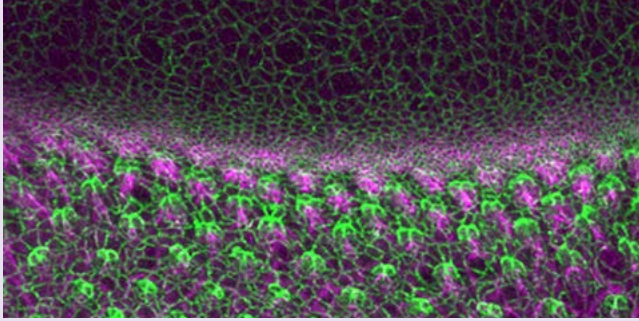
The facility supports all aspects of the research process from project specific advice on experimental design and sample preparation, to performing the analyses and aid in data interpretation. It is also involved in developing new methods in areas such as high resolution separations, characterising modifications, and absolute protein quantification. The facility benefits from regular investment and will be expanding its capabilities for targeted protein identification and quantification in 2014.

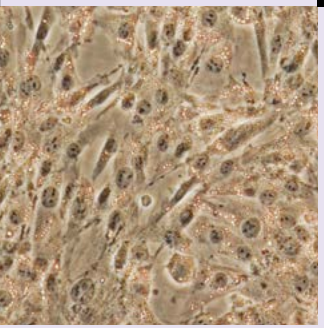
New discoveries:

- Novel targets of p38 signalling in the adhesion-control of apoptosis in breast epithelia
- Dynamics of adhesion complex assembly and disassembly, and phospho-signalling pathways triggered on adhesion
- Identification of the glomerular matrisome of the kidney
- Quantification of mucin processing in the clinical environment



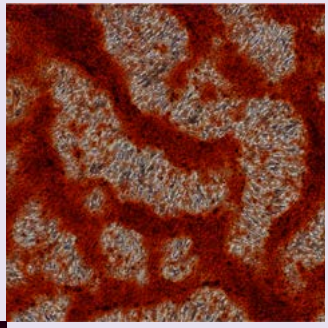


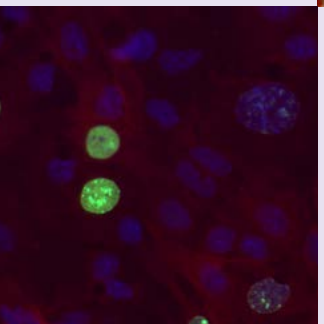




Bioimaging and Force Microscopy

The Bioimaging Facility provides access to a wide range of microscopes from simple manual fluorescent microscopes through to in vivo multiphoton confocals. Most of our systems are set up for live cell imaging and are capable of performing photokinetic studies such as FRAP and photo-activation.





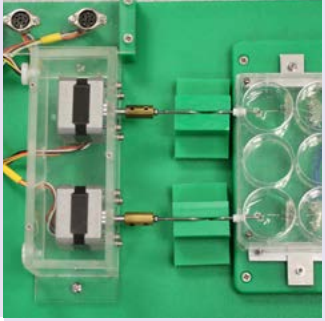
The Bioimaging Facility provides access to a wide range of microscopes from simple manual fluorescent microscopes through to in vivo multiphoton confocals. Most of our systems are set up for live cell imaging and are capable of performing photokinetic studies such as FRAP and photo-activation.

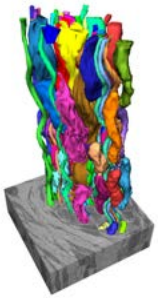
We currently have 5 point scanning confocals, 2 spinning disc confocals, 3 deconvolution microscopes, TIRF, laser autofocus based live cell imaging, laser microdissection, a digital slide scanning microscope and a high content screening platform. We also have a range of atomic force microscopes including a Bruker Multimode 8 and Bioscope, and JPK CellHesion.

The facility provides help with the design, imaging and analysis of experiments, as well as novel data analysis algorithms for image segmentation; object tracking; and fluorescence correlation spectroscopy.

New methodologies developed:

- Use of wavelets to identify cells in bright field illumination microscopy for cell tracking
- Matlab based colour segmentation to automatically quantify the extent of staining in labelled histology slides and cell density in tissue culture
- Fluorescence correlation spectroscopy to quantify peptide uptake in cells, protein aggregation phenomena, and to identify receptor dimerization in cell signaling
- High content screening to develop a novel anti-angiogenic therapeutics
- Novel setups for label free live cell imaging, e.g. interference reflection microscopy
- The use of light sheet fluorescence microscopy to image multicellular microorganisms in 3-D
- Development of automated fluorescence in situ hybridization analysis to analyse the correlation between chromatin architecture and gene function



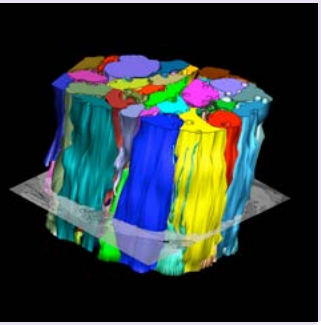


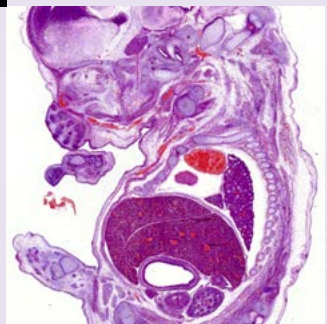
3D Electron Microscopy

The Electron Microscope Facility houses three transmission electron microscopes and an environmental scanning electron microscope. We also have equipment for preparing samples for cryo-electron microscopy, which can be visualised on our FEI Polara TEM.

A recent acquisition is the Gatan 3view machine, based within an FEI Quanta FEG 250 SEM, which allows us to easily generate serial section reconstructions through significant volumes of tissues and constructs.

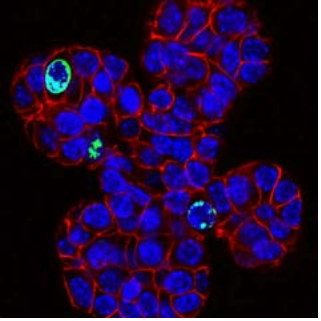
The facility has expertise in single particle reconstruction, offering insight into protein folding without the need for crystallisation; electron tomography provides high-resolution reconstruction of a tissue samples.

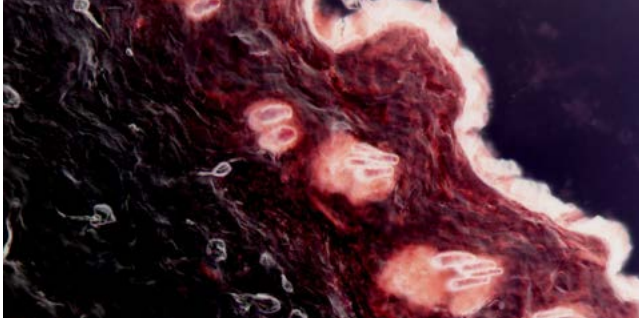




Ongoing projects/new discoveries:

- Single particle TEM, combined with solution X-ray scattering, to study the structure of regulators of TGFbeta and BMP signalling
- The 3view machine in combination with electron tomography to examine individual collagen VI microfibrils surrounding the cartilage cells
- 3view serial sections to identify and track individual collagen fibrils through an entire tendon
- 3D electron microscopy and TEM to examine the structure and organisation of spindle pole bodies and centriole positioning during cell division
- Visualisation of the Glomerular basement membrane in 3 dimensions
- Examination of flagella formation during cell division in Trypanosome Brucei





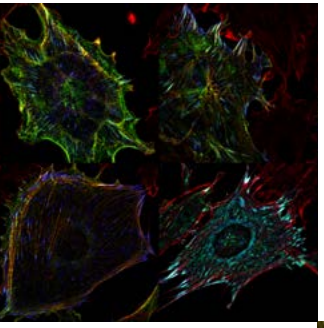
Biomolecular Analysis

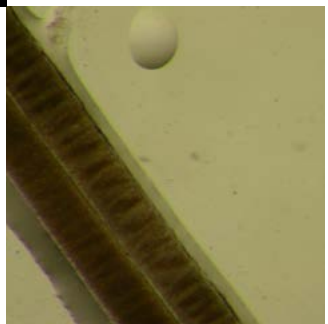
The Biomolecular Analysis Facility is a state-of-the-art resource for the biophysical characterisation of molecular hydrodynamics, multi-domain protein solution structures and molecular interactions. We are one of the largest single facilities of its type in the UK with three dedicated staff members. The facility has helped resolve the dynamics and solution structures of several complex molecules within the matrix such as PTX-3, PLA2R and BMP-1 using combinatorial hydrodynamics techniques.

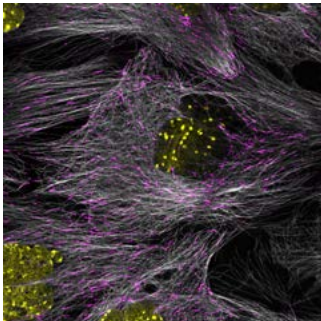
The facility has also developed nano-scale biological surfaces for the investigation of protein interactions on biological membranes to aid investigations of hierarchical binding events and complex assembly.

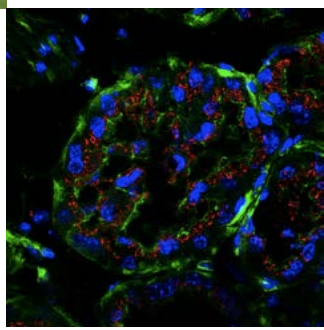
New discoveries:

- Low-resolution solution structures of tropoelastin, collagen VI and BMP-1 using analytical ultracentrifugation, bead modelling and light scattering in conjunction with EM and SAXS
- Discovery of the zinc binding site in matrillin-3 and its effect in stabilizing the molecule which helps us understand the disease mechanism in MED
- Analysis of protein-ligand interactions by Surface Plasmon Resonance. This includes: fibrillin-1 with heparin; the binding of TSG-6 to chemokines, which inhibits their interaction with ECM; and binding of TSG-6 to the heavy chains of inter-alpha-inhibitor that plays a crucial in the formation of the cumulus extracellular matrix during ovulation
- Solution structure using hydrodynamic techniques of the PLA2R receptor found on the cell surface of podocytes and the generation of peptide inhibitors of the auto-antibody against PLA2R in the disease membranous nephropathy
- Hydrodynamics of mucin molecules, and the solution structure of the globular N-terminus of the mucin respiratory molecule, Muc5B

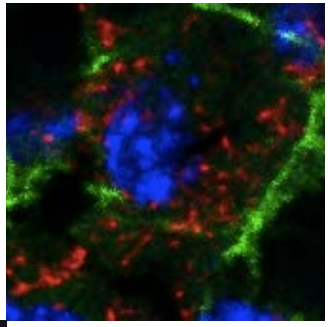








The Centre's 2013 conference was jointly organised with the Manchester Collaborative Centre for Inflammation Research. *Get Connected 3: Immuno Matrix* was focused on the role of the extracellular matrix in inflammation, the immune system and inflammatory disease. The *Get Connected* conferences aim to provide a forum for young scientists from across Europe to present their research stories to an international audience.



"Immuno Matrix: The Dynamic Interplay between the Immune System and the Extracellular Matrix"

Get Connected 3

Organisers:

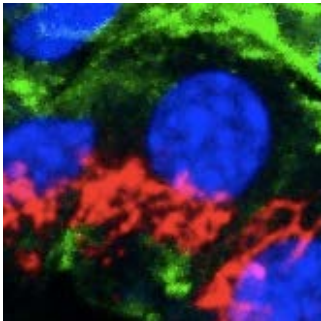
Alex Langford-Smith (Day lab) and Amy Saunders (MCCIR)

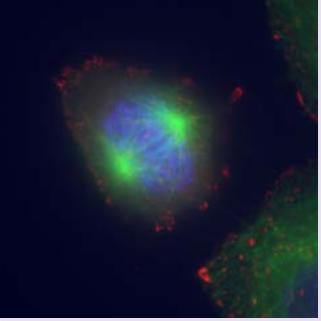
Keynote speakers

Anna Blom, Lund University
Tony Day, University of Manchester
Richard Grencis, University of Manchester
Thomas Krieg, Cambridge University
Isabelle Maridonneau-Parini, University of Toulouse
Liliana Schaefer, Goethe University
Dylan Edwards, University of East Anglia

Session topics

ECM in Immune Homeostasis and Wound Healing
Role of the ECM in Leukocyte Migration
Gut Matrix and Inflammation
ECM in Inflammatory Disease
MMPs in Health and Disease
Cell Matrix Signalling
ECM-Innate immune system cross talk





Mechanotransduction conference

In 2013, the Cell-Matrix Centre also held the first joint symposium with the Mechanobiology Institute in Singapore. The conference, *Interfaces and Transitions in Cell and Tissue Dynamics*, brought together PIs from Manchester University with PIs, students and postdocs from MBI, with the aim of establishing research links and forging new collaborations.

"Interfaces and Transitions in Cell and Tissue Dynamics"

Organisers:

Christoph Ballestrem (Cell-Matrix Centre)
and Ronen Zaidel-Bar (MBI)

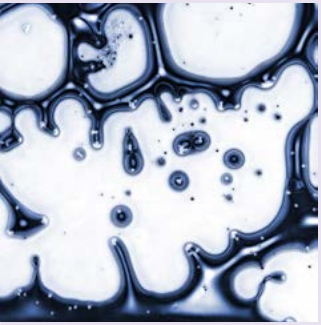
Keynote Speakers:

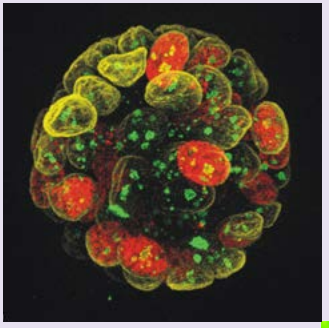
Cell-Matrix Centre
Christoph Ballestrem
Ray Boot-Handford
Andrew Gilmore
Karl Kadler
Rachel Lennon
Charles Streuli

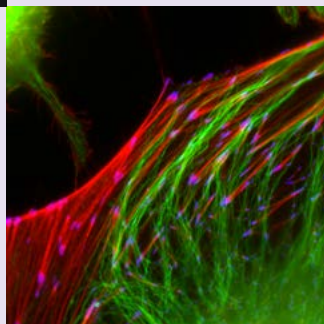
Mechanobiology Institute:
Mohan Balasubramanian
Alexander Bershadsky
Low Boon Chuan
Nils Gauthier
Pakorn Tony Kanchanawong
Wu Min
Fumio Motegi
Michael Sheetz
GV Shivashankar
Yusuke Toyama
Virgile Viasnoff
Ronen Zaidel-Bar

Guest Speakers:

Andrew Chisholm (UCSD)
Dorit Hanein (Burnham Institute)
Andreas Prokop (Manchester)
Herbert Schiller (Max Planck, Munich)







Visitors to the Centre in 2013

Andrew Copp, UCL Institute of Child Health, *Neural tube defects – genetics, development and prevention*

John Couchman, Copenhagen, *Regulating tumour cell behaviour through cell surface heparan sulphate*

Patrick Derksen, University Medical Center Utrecht, *Context dependent regulation of breast cancer metastasis by p120-catenin*

Filippo Giancotti, Memorial Sloan-Kettering Cancer Center, *Mechanisms underlying the reactivation of disseminated breast cancer cells*

Alex Gould, NIMR, *Food for thought: nutrients and neural stem cells*

Anthony Graham, King's College London, *Tales from the pharynx; how my ontogeny ate my phylogeny*

Denis Headon, Edinburgh, *Periodic patterning during skin development*

Corinne Houart, King's College London, *Temporal control of signalling centres in regulation of forebrain size and complexity*

David Ish-Horowicz, London Research Institute, *Genetic and kinetic studies of transcript metabolism and transport in vivo*

David Jackson, Oxford, *New insights into hyaluronan receptors in lymph and blood*

Laura Machesky, CRUK Beatson Institute, *Role of the actin bundling protein fascin in invasion and metastasis*

Alfonso Martinez Arias, Cambridge, *What are mouse ES cells missing in culture when trying to make embryos?*

Jeffrey Miner, Washington University, *The Glomerular Basement Membrane: Focus on the Filter*

Nick Monk, Sheffield, *Modelling cell fate decisions in fluctuating environments*

Thomas Mueller, Julius-von-Sachs Institute, *Can promiscuous ligand-receptor interactions encode for ligand-specific signals?*

Nipam Patel, Berkeley, California, *Developmental Insights from the Study of Emerging Model Systems*

Matthew Pickering, Imperial College London, *How does complement damage the kidney?*

Miguel del Pozo, CNIC Madrid, *Regulation of caveolar domain plasticity and trafficking*

Jordan Raff, Oxford, *Centrosome and asymmetric stem cell divisions: size does matter*

Sara Rankin, Imperial College London, *Mobilizing stem cells from the bone marrow - a pharmacological approach*

Gerhard Sengle, Cologne, *Integration of BMP growth factor signaling by fibrillin microfibrils*

Helen Skaer, Cambridge, *Taking shape; morphogenesis of fly renal tubules*

Vic Small, Institute of Molecular Biotechnology, *Puching cells and pathogens with actin*

Daniel St Johnston, Cambridge, *Epithelial polarity, spindle orientation and cancer*

Didier Stainier, MPI for Heart & Lung Research, *Curing diabetes one fish at a time; the long road to translational research*

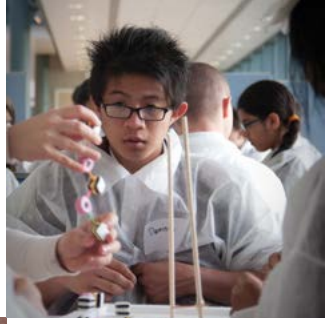
Karl Tryggvason, Karolinska Institutet, *Laminins - Extracellular modulators of stem cells and cell lineages*

John Wallingford, University of Texas at Austin, *Planar Cell Polarity: From cell biology to human disease*

Fiona Watt, Cambridge, *Stem cell-niche interactions in mammalian epidermis*

Sara Wickstrom, MPI for Biology of Ageing, *Integrin signaling in tissue morphogenesis*

Tom Wight, Benaroya Research Institute, *Targeting the Extracellular Matrix and the Control of Inflammation*



Public Engagement

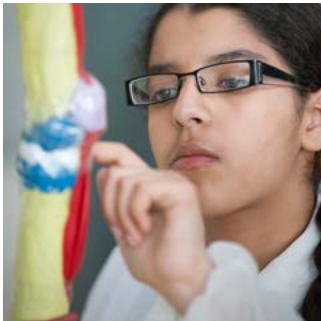
Public Programmes at the Cell-Matrix Centre

2013 was an exciting year for the Centre's Public Programme. Continuing with our ever popular schools programme, we hosted many students from across Greater Manchester from Key Stage 3 to adult learners in various study days, work experience and research-focussed placements.

Additionally, we have started a new Matrix Book Club. Carefully selecting texts that touch on a certain aspect of the Centre's research programme, we offer the text to local book clubs, and then head out a month later to discuss the whys and wherefores of the text in the context of contemporary cell- matrix research. This programme is ongoing into 2014 and so far has meant we have had the opportunity to share our research with previously unmet audiences.

In an exciting new venture with a local screen print artist, the Centre research images and stories will be captured in glorious screen print and exhibited in Manchester's Northern Quarter in Spring 2014. By drawing the public in with striking images, we then aim to tell the research story which inspired the image and run follow-up workshops and discussion groups around the art work.

The Centre was part of the Natural History Museum's 'Science Uncovered' event in September 2013, in London. Over 10 000 visitors explored all things science for the evening. Researchers from across the Centre's main research themes shared their passion, fascination and findings with an insatiable public through 3D models, images, movies and dialogue.



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Strategic Award