CPB Cambridge Centre for Physical Biology

Annual Meeting

13th January 2025

Old Divinity School at St John's College

Programme

Afternoon

13:30 – 14:00 – Registration
14:00 – 14:05 – Welcome
14:05 – 14:45 – **Keynote Lecture** Prof. Timothy Saunders, University of Warwick, UK
"Deciphering how complex organ form emerges in development"
14:45 – 15:20 – *Coffee Break + Posters*

CPB Prizes Session

15:20 – 15:40 – Akaash Kumar, Research Associate, MRC Laboratory of Molecular Biology
"Shedding light on the molecular interactomes of fast, complex biological processes using multispectral imaging with uncompromised spatiotemporal resolution"
15:40 – 16:00 – Maria Julia Maristany, Research Associate, Department of Chemistry
"Biomolecular Condensates Through a Computational Microscope"
16:00 – 16:20 – Leonardo Mancini, Herchel Smith postdoctoral fellow, Department of Physics
"Surface versus volume synthesis governs growth-dependent efficacy of a β-lactam antibiotic"
16:20 – 17:00 – *Coffee Break + Posters*

Invited Speakers

17:00 – 17:25 – Evgeny Zatulovskiy, Department of Biochemistry, University of Cambridge
"Cell size regulation: molecular mechanisms and functional implications"
17:25 – 17:50 – Teuta Pilizota, Department of Physics, University of Cambridge
"Escherichia coli's Responses to Osmotic Pressure Challenges"

17:50 – 18:00 – **Closing Remarks**
18:00 – 19.15 – Poster Session + Prize Announcement
19-15 – 20:00 – Reception

Abstracts

Keynote Lecture

14:05 – 14:45
Prof. Timothy Saunders, University of Warwick, UK

"Deciphering how complex organ form emerges in development"

Abstract: Our internal organs have specific three-dimensional morphologies essential for their efficient function. Yet, we know remarkably little about the physical processes underlying their morphogenesis. Here, I present quantitative analysis of early muscle formation in zebrafish. This system is highly accessibly to live imaging and is enabling us to decipher the biophysical mechanisms helping to build the first skeletal muscle structures.

CPB Prizes Session

15:20 – 15:40
Akaash Kumar, Research Associate, MRC Laboratory of Molecular Biology

"Shedding light on the molecular interactomes of fast, complex biological processes using multispectral imaging with uncompromised spatiotemporal resolution"

My research is highly interdisciplinary. For instance, the development of the multispectral imaging hardware required principles of physics (optics) and engineering where I optically simulated the light paths to maximise the imaging performance (using Zemax OpticStudio), mechanically designed the hardware (using SolidWorks) and built/aligned the system. The processing algorithm required coding (Python) employing mathematical principles related to image formation and deconvolution operations. Finally, I successfully integrated the hardware and algorithm to study complex intracellular processes (endosomal sorting) requiring advanced molecular biology techniques, including the development of novel polycistronic plasmids and computationally-designed receptor binders, as well as image analysis pipelines.

15:40 – 16:00
Maria Julia Maristany, Research Associate, Department of Chemistry

"Biomolecular Condensates Through a Computational Microscope"

My work intersects biology, physics, and computational chemistry. I employ physics-based computational models to understand complex biological systems, like molecular condensates, exploring how molecular interactions drive organization and phase behaviour, and providing a multiscale perspective to explain condensates' biological functionality rooted in first principle physics. My findings reveal how different physical properties of single biological molecules, like valency and flexibility, regulate their condensation and biological function. Collaborations with groups in Cambridge, UT Southwestern, and MBL ensure predictions are backed by in vitro and in vivo experiments. My computational techniques then enhance experimental resolution and link molecular to mesoscale phenomena.

16:00 – 16:20
Leonardo Mancini, Herchel Smith postdoctoral fellow, Department of Physics

"Surface versus volume synthesis governs growth-dependent efficacy of a β-lactam antibiotic"

Our research bridges biology and physics by showing how bacterial survival under antibiotic treatment is influenced by physical principles like surface-to-volume ratio. The work demonstrates that the conceptual and methodological approach offered by physics can be both suitable and efficacious for addressing biological questions. Indeed, as showcased here, certain mechanisms of biology might actually only be accessible when the cell is considered at the system level, and not just as the sum of its molecular parts. Our findings, therefore, show that interdisciplinary approaches can be key to understand the vast complexity of biology.

Invited Speakers

17:00 – 17:25
Evgeny Zatulovskiy, Department of Biochemistry, University of Cambridge

"Cell size regulation: molecular mechanisms and functional implications"

Cell size is the most fundamental and evident, yet poorly understood, physical property of all cells. While different cell types in the body vary in size by multiple orders of magnitude, cells of a given type are typically very uniform in size, suggesting that cell size is tightly controlled and crucial for cell function. Although the cell size homeostasis is a century-ago observation, surprisingly little is known about how cell size affects specific aspects of cell physiology and what molecular mechanisms regulate animal cell size. We have identified a long-sought molecular mechanism controlling size in proliferating human cells. Specifically, we found that cell growth dilutes the retinoblastoma protein (RB), a key inhibitor of the cell division cycle. Once RB is diluted to a critical threshold concentration, cells commit to division, thereby ensuring that division occurs only after a cell has grown to its target size. Using gene editing and quantitative live-cell microscopy, we further demonstrated that smaller-born cells compensate for their reduced size by delaying division, allowing them to grow further and dilute RB to the appropriate threshold. This finding highlights how cell size can influence important cellular processes by modulating the concentrations of their key regulatory proteins. Our follow-up studies demonstrated that changes in cell size alter the concentrations of dozens of proteins regulating crucial cellular processes, from cytoskeleton organisation and metabolism to DNA repair and cellular ageing. These findings provide a rationale for why cells must tightly regulate their size to maintain proper function.

17:25 – 17:50
Teuta Pilizota, Department of Physics, University of Cambridge

"Escherichia coli's Responses to Osmotic Pressure Challenges"

Ordinarily, the total solute concentration within the bacterial cell is higher than that of the environment, resulting in a positive osmotic pressure on the cell wall (termed turgor pressure). Bacterium Escherichia coli responds to both increases and decreases in external concentrations. An increase in external osmolarity results in water efflux from the cell interior, causing cellular volume to shrink and osmotic pressure to drop to zero. E. coli responds by actively accumulating specific solutes (osmolytes), which causes reentry of water, cell volume increase, and recovery of osmotic pressure. However, recovered cells continue to grow slower. A downward shift in external osmolarity causes fast water influx into the cell. As a result, the osmotic pressure increases and the cell expands in a nonlinear fashion, increasing the inner membrane tension and activating the nonspecific export of solutes through mechanosensitive channels. As the solutes leave the cell, so does the water inside enabling the cell to recover original volume and pressure.
I will present our experimental and theoretical results that fully explain the response to downward shift in external osmolarity, and some unexpected discoveries we made along the way. I will also give a brief summary of our, more than a decade-long, attempt at explaining the reduced growth rates after upward shifts in external osmolarity, which is still not successful.

Posters

1- **Eva Kreysing**, Department of Physiology, Development and Neuroscience, University of Cambridge

"The electrical maturation of neurons is regulated by tissue mechanics via the mechanosensitive ion channel Piezo1"

During the development of the nervous system, neurons grow axons and dendrites to connect with other cells. As neurons become integrated into the neural network, they mature and develop electrical activity. While mechanical interactions between neurons and their environment are critical for axon growth and pathfinding, the role of mechanical cues in the electrical maturation of neurons, and thus the formation of circuits in the developing brain, remain unexplored. Here, we cultured rat hippocampal neurons on substrates with different mechanical properties and found that electrical activity developed earlier on soft hydrogels compared to stiff hydrogels. This stiffness-dependent neuronal maturation was mediated by the mechanosensitive ion channel Piezo1. Using RNA sequencing, pathway analysis and Western blots, we identified a downstream signalling cascade responsible for the differential expression of neurotransmitter receptors. Finally, we found that stiffening of the developing Xenopus brain leads to impaired synapse formation in vivo. Our findings highlight the critical role of mechanical signals in neuronal maturation and suggest that local brain tissue stiffness is a critical parameter for circuit formation in the developing brain.

2- **Paula Garcia Galindo**, Department of Chemical Engineering and Biotechnology, University of Cambridge

"RNA plasticity emerges as an evolutionary response to fluctuating environments"

Phenotypic plasticity refers to the ability of a single genotype to produce multiple distinct phenotypes. Using the computationally tractable genotype-phenotype (GP) map of RNA secondary structures, we model RNA phenotypic plasticity using the Boltzmann distribution of secondary structures for each genotype. Through evolutionary simulations that involve periodic environmental switching on the GP map, we reveal that RNA phenotypes can adapt to these fluctuations towards an optimal plasticity. The optimal phenotypes exhibit near-equal Boltzmann probabilities of distinct structures, each representing the fittest structure for each alternating environment. Our findings demonstrate that phenotypic plasticity, a widespread biological phenomenon, is a fundamental evolutionary response to changing environments.

3- **Lakshmi Balasubramaniam**, Gurdon Institute, University of Cambridge

"Cellular scale cytoskeleton guides migration of extra-embryonic tissue during early avian development"

Early avian embryonic development occurs through large scale polonaise movements in the epiblast and outward migration of the extra-embryonic tissue. While much is known about how polonaise movements affect embryonic development, the role of outward growth of extra-embryonic tissue during embryonic development is poorly understood. In this work, we show that the cells around the embryo edge adhere onto the vitelline membrane thereby recruiting their cytoskeletal machinery during this outward motion. We show that outward pushing forces through lamellipodial / filopodial formation restricted by microtubule alignment mediate the outward motion of extra-embryonic tissue. In addition, acto-myosin cable formation at the edge of the migrating extra-embryonic tissue modulates the frequency and size of protrusions being formed keeping embryonic growth under check. This checkpoint mechanism ensures size maintenance of the embryo during development. Improper size growth of the embryo through removal of the extra-embryonic tissue led to deformities in body axis elongation highlighting the importance of the extra-embryonic tissue as a mechanical support that guides epiblast development. In the second part, we also show that the extra-embryonic tissue has a role in remodelling apical ECM which in turn acts as a glue between cells and the different tissue layers forming as embryonic development progresses. Through a combination of live imaging, electron microscopy and drug-based inhibitions we describe the function of extra-embryonic tissue as a mechanical guide that maintains epiblast growth thereby ensuring proper embryonic development.

4- **Joel Hochstetter**, Gurdon Institute, University of Cambridge

"Time-lapse intravital imaging unveils proliferative heterogeneity and correlated cell fate decisions in mouse interfollicular epidermis"

In the maintenance of skin interfollicular epidermis, the identity and fate behaviour of the stem cell population remains in debate. In some studies, it is argued that a single equipotent progenitor population captures the main features of the dynamics, whereas others support the idea of multiple discrete proliferative compartments. To challenge the competing one and two progenitor cell models, we perform long-term time lapse imaging of mouse ear-skin epidermis tracing clones for up to 6 months. We compare both ,"zero-dimensional" models from the literature, with a novel spatial Voronoi model that incorporates mechanics, allowing a more realistic picture of homeostatic clonal dynamics in squamous tissues. We find that one progenitor models are insufficient to capture fine-grained features of static and temporal clonal dynamics, even when realistic division time distributions and sister correlations are considered. However, we find that a model with a stem cell and transit amplifying progenitor (TA) population can capture correlations in both static clonal distributions and transitions between states in our data. In this picture, clones comprise pairs of stem and TA cells, with highly coordinated decision timing. Our data also illuminates significant biological variability in clonal dynamics between male and female mice, which can be explained within the framework of our two-progenitor model.
Based on these results, we are starting to unravel the molecular mechanisms underpinning cell identity and the feedback mechanisms regulating cell fate decisions of the multiple progenitor populations in the ear skin epidermis.

5- **Tal Agranov**, Department of Applied Mathematics and Theoretical Physics, University of Cambridge

"Uncovering remarkable tuning to criticality in the assembly of actomyosin cortex in C. elegans"

How does a biological system manage to produce long time scales that vastly outlast intrinsic biochemical rates, yet are not infinite? This challenge features in various biological tasks involving memory and sensing. In this work, we uncover how this manifests in the cellular assembly of a C. elegans embryo. High-resolution imaging reveals that the cell's actin cortex formation is preceded by a stage where thousands of highly branched actin structures transiently grow and disassemble. Some structures grow orders of magnitude past their intrinsic degradation time, yet without proliferating. We uncover how an overlooked bifurcation in the underlying biochemical dynamics can account for the huge lifetime disparity. We show how a simple mechanism based on resource competition can explain how this process spontaneously self-tunes to the vicinity of this dynamical bifurcation.

6- **Argyris Zardilis**, Sainsbury Laboratory, University of Cambridge

"Learning a physical growth model from combined transcriptional, morphology timeseries"

Flowers are the most geometrically complex organs in plants. Like any developmental process, their morphogenesis is a multi-scale process so the data that we collect are also multi-scale, typically expression profiles of molecular species either through microscopy or sequencing and morphology timeseries. How do we go from this multi-scale dynamic data to physical mechanistic models? Here, we first learn an integration of these two spaces using unsupervised techniques and then learn a plausible elasto-viscoplastic model for this process.

7- **Ivan Lobaskin**, Department of Applied Mathematics and Theoretical Physics and Gurdon Institute, University of Cambridge

"Morphometrics of the developing human lung"

The human lung is a structurally highly complex organ. The question of how this structure emerges during development has long fascinated biologists and mathematicians. Due to technical limitations, the study of human samples has so far been restricted to adult and embryonic stages. Crucially, this leaves a gap in data for the pseudoglandular stage (PCW 7,Äî17), during which most of the bronchial airway tree develops. Capitalising on recent advances in tissue clearing and image processing methods, we are now able to provide a comprehensive dataset of human lung samples throughout the pseudoglandular stage. Further, by applying computational data analysis methods, we produce a quantitative reference of the growth dynamics. We find that there are two main branching modes, which lay down the main framework of the tree and fill in the available volume, respectively. Also, surprisingly, we find that the statistical properties of the lengths of growing tips are better explained by an asymmetric growth process, rather than simple, symmetric bifurcation.

8- **Marta Urbanska**, Department of Physiology, Development and Neuroscience, University of Cambridge

"The impact of cell mechanics on cell fate and morphogenesis in a 3D model of mammalian gastrulation"

During early embryonic development, pluripotent cells differentiate, change shapes, and rearrange spatially to form the blueprint of the adult body. Traditionally, the orchestration of cell fate commitment in the developing embryo has been attributed to biochemical signalling pathways. More recently, the biophysical properties of cells and their environment have emerged as important players in this process. Gastruloids, derived from three-dimensional aggregates of mouse embryonic stem cells, are emerging as a robust system that models key features of early post-implantation development. During gastruloid growth, the originally symmetric and genetically unpatterned clusters of cells become spatially organised (anterior-posterior polarization), elongated structures containing cells representing all three embryonic germ layers. The mechanism behind this spontaneous organisation is not fully understood. Strikingly, we showed that the expression of constitutively active ezrin, a protein that attaches actin filaments to the plasma membrane and governs cell surface mechanics, prevents gastruloid elongation. Even though the perturbed gastruloids remain spherical, cell fate commitment towards the mesendodermal lineage is observed. We are actively exploring which biochemical and biophysical factors within the ezrin-CA expressing gastruloids are altered and contribute to the observed morphogenetic defect. This work will shed light on the interplay between cell surface mechanics, cell fate, and morphogenetic movements in developing gastruloids, and contribute to answering the long-standing question in developmental biology of how physical determinants integrate with biochemical signalling to drive embryogenesis.

9- **Iskra Yanakieva**, Department of Physiology, Development and Neuroscience, University of Cambridge

"Cell shape noise strength regulates shape dynamics during cell spreading in epithelial-to-mesenchymal transition"

Cellular shape is intimately linked to cell function and state, and transitions between cell states are tightly coupled to shape changes. While the biochemical signalling and gene expression changes that drive state transitions have been extensively studied, the biomechanical mechanisms that drive the accompanying cell shape changes remain less well understood.
We investigate cell shape dynamics in 3D during epithelial-to-mesenchymal transition (EMT), a key process in development and homeostasis, in which epithelial cells adopt spread morphologies and become more motile. To characterise cell shape dynamics, we employ live-cell imaging and morphometric analysis. Using stochastic inference, we extract the morphogenetic landscape underlying EMT. We show that within this landscape, EMT-associated cell spreading can be represented as a transition between shape attractors. Strikingly, we observe a peak in cell shape noise strength at the time of cell spreading, and show that higher shape noise accelerates the transition between shape attractors. Our findings suggest that efficient cell spreading in EMT requires a change in the balance of actin protrusivity, contractility, and membrane tension. To understand the mechanisms that determine shape attractors and cell shape noise, we investigate actin reorganisation and cell mechanical properties throughout EMT.
Overall, by combining quantitative imaging, morphometric analysis, and stochastic modelling with biomechanical measurements and molecular perturbations, our project creates a comprehensive understanding of the physical and biomolecular basis of EMT-associated cell shape change. Moreover, the description of cell shape changes using our stochastic inference approach provides a novel conceptual framework for investigations of cell morphology in general.

10- **Ana Hernandez**, Department of Physiology, Development and Neuroscience, University of Cambridge

"Mechanics, Matrix and Fate. A tale of the Tail"

Vertebrate embryos grow and pattern their body axis in an anterior-to-posterior direction, guided by a pool of multipotent cells in the posterior progenitor zone (PZ). This highly dynamic region is regulated by WNT and FGF signaling pathways, but the heterogeneity and motility of PZ cells led to the hypothesis that mechanical properties, particularly a stiffness gradient within the PZ, also contribute to fate specification. To test this, I examined PZ stiffness patterns and evaluated how perturbations impact differentiation potential.
Using atomic force microscopy (AFM), I demonstrated that the PZ exhibits distinct mechanical properties correlating with cell identity, with an anterior-to-posterior stiffness gradient. This finding was validated using our lab's custom Tissue Force Microscope (TiFM), which directly probes mechanical properties in situ. Investigating the extracellular matrix (ECM) composition of the PZ revealed an absence of fibers but the presence of fibronectin, laminin, and fibrillin puncta. Inhibiting matrix metalloproteinases (MMPs) to prevent ECM degradation unexpectedly reduced stiffness in the neural-mesodermal competence (NMC) region, disrupting the stiffness gradient. This mechanical alteration impaired axis elongation and biased PZ differentiation towards neural fates, as shown by gene expression analysis.
These findings suggest that the mechanical environment of the PZ plays a crucial role in body axis patterning. Disruption of PZ stiffness leads to elongation defects and altered cell fate specification.

11- **Oliver Meacock**, School of Biosciences, The University of Sheffield

"Topological defects drive self-sorting of mixed-activity bacterial monolayers"

Many microbes live in dense, multi-species communities in which their functional capabilities are largely determined by adjacent partners. For example, toxin secretion may severely reduce the growth of nearby strains, while production of metabolites that can be utilised by other species may enhance neighbours' growth. Spatial structure is therefore a key determinant of the properties of microbial ecosystems. Here, we use a combination of theoretical analyses, individual-based models and experiments with the pathogen *P. aeruginosa* to investigate the impact of microbial motility on spatial structure. In contrast to expectations, we find that undirected motility can drive segregation of strains once they grow to a high enough density to generate collective behaviours. We show that topological defects, positions where cells of differing orientations meet, are at the heart of this phenomenon: high-motility strains push into comet-like +1/2 defects, while lower-motility strains become enriched around -1/2 defects. Though phenomenologically similar to previous observations of density disparities around +1/2 and -1/2 defects in mono-population systems, we show that the anisotropic friction mechanism thought to drive these density effects does not explain our observations. Instead, we attribute segregation in our system to its mixed polar/nematic symmetry. We find that +1/2 defects are highly polarised regions that migrate at a speed determined by the high-activity cells, with lower-activity cells therefore being left behind and lost from the defect tail. These findings show that the physics of active matter has critical implications for the social lives of microbes.

12- **Amir Porat**, Sainsbury Laboratory, University of Cambridge

"Integrating Growth, Mechanics, and Genetic Control in Plant Morphogenesis"

Plant morphogenesis arises from an intricate feedback loop between growth, mechanics, and genetic regulation. A key question in plant development is understanding how morphogens such as auxin, WUS, CLV3, and cytokinin influence growth. In this project, I analyse the morphoelastic effects of these morphogens using a multiscale, physics-based modelling approach.
I focus on two organs with distinct symmetries: the apical hook, a slender, rod-like structure with pronounced macroscopic curvature, and the shoot apical meristem (SAM), a dome-shaped stem cell niche from which organs form periodically. By leveraging the self-similar growth kinematics of these organs, I address an "inverse morphoelastic problem"—determining the elastic profiles and growth laws that may drive the observed kinematics, and how these are modulated by spatiotemporal morphogen patterns.
In the SAM, I explore explicit feedback loops coupling morphogens to cell wall properties, such as elastic strain and stiffness, to explain size regulation, mutant phenotypes, and responses to biochemical treatments. For the apical hook, I use macroscopic morphoelastic rod models to show that the shape dynamics can be fully captured by a one-way wave equation describing the curvature of the centreline. Using this framework, I further investigate how residual stresses influence the growth-driven movement.

13- **Ruizhe Li**, Department of Engineering, University of Cambridge

"Host cell cycle and ribosomal resources drive phage infection outcomes"

The efficacy of bacteriophages is intrinsically linked to the physiological state of their bacterial hosts. However, identifying which physiological factors dominate and how they regulate the infection progression has been challenging. This problem stems from the inherent heterogeneity between individual cells and their dynamic physiological states. Traditionally, the field has relied on bulk interactions and measurements to infer key parameters like "average" lysis time and burst size, which obscure the dynamics of infection progression and its dependency on infected bacterial physiology.
To tackle this, we developed a microfluidic imaging assay for tracking phage infection steps in individual bacterial cells. This method enabled us to investigate how bacterial physiology shapes infection dynamics. Our findings uncovered that the burst size of phage infection is predominantly determined by the host's translation capacity. Particularly, we identified the bacterial cell cycle as a critical factor influencing infection efficiency - something that cannot be captured through bulk assays. By perturbing the cell cycle with division inhibitors, we demonstrated how shifts in physiological states can influence phage infection dynamics.
Understanding the physiological factors governing phage infection progression opens many strategies for steering the infection kinetics by perturbing cell physiology. This insight helps to potentiate phages for their applications in biocontrol, including tackling antimicrobial resistant pathogens.

14- **Sebastian W. Krauss**, Department of Chemical Engineering and Biotechnology, University of Cambridge

"Exploring DNA Linkers for Biomimetic Cell Adhesion of Red Blood Cells"

Sebastian W. Krauss, Roger Rubio-Sánchez, Bortolo M. Mognetti, Lorenzo Di Michele, and Pietro Cicuta

Ligand-receptor interactions are fundamental to cellular membrane dynamics, influencing a range of processes like cell-cell signaling, wound healing, and viral infections. The affinity and specificity of these molecular interactions govern how adjacent membranes recognize, bind, and respond to one another, ultimately determining the strength and stability of membrane contacts. To better understand these mechanisms, we developed a biomimetic approach that grants precise control over the strength of interactions between complementary receptors in opposing membranes. Our strategy employs short membrane-anchored amphiphilic DNA nanostructures featuring single-stranded 'sticky-ends', which through complementary sequences, are designed to bind labelled objects, providing a programmable platform for membrane-membrane interactions [1]. We implemented our method to functionalize the surface of red blood cells (RBCs) with complementary DNA, resulting in the formation of cellular aggregates, with tunable morphologies, ranging from doublets to star-like geometries, depending on mixing ratios and cell density. Additionally, we used DNA-functionalized particles to selectively bind RBCs. By tuning the length of the DNA sticky-ends, we precisely controlled interaction strength, enabling RBCs to progressively envelop beads. Furthermore, we employed optical tweezers to observe the rapid formation of strong bonds in situ [manuscripts in preparation]. This model system offers a versatile platform to understand the forces and dynamics of RBC aggregation and their interactions with pathogens, such as Plasmodium species responsible for malaria. Moreover, the ability to fine-tune interaction strengths opens new possibilities for developing synthetic tissues with programmable adhesion properties.

15- **Alice Yuen**, Department of Genetics, University of Cambridge

"Investigating morphogenetic flow patterns during symmetry breaking in early development using zebrafish blastoderm explants"

Gastrulation requires the coordination of a diverse set of cell behaviours that together generate tissue-level flows to drive morphogenesis. Whether the observed tissue flows can be generated in a manner that is robust to embryo geometry is unknown. We use zebrafish blastoderm explants as our model system because it is genetically tractable, easy to culture, and spontaneously breaks symmetry to elongate and become axially patterned. Since the embryonic yolk is absent, these explants are transparent, allowing high-resolution, long-term imaging using lightsheet microscopy. To extract information about tissue-level flows, we have developed tools to conduct three-dimensional particle velocimetry analysis. In parallel, we have applied theoretical modelling to predict 3D flow dynamics in our model system. We find that, prior to explant elongation, there are polonaise movements-like symmetric flows, and that the onset of elongation coincides with the interruption of such flows. Our computational modelling indicates that converging and internalising flows are active forces that synergise to break these symmetric flows and sustain elongation. Consistently, we observe cell movements associated with both convergence and internalisation during explant elongation. Currently, we are experimentally testing whether convergence and internalisation act independently or synergistically as predicted. Together, our work allows us to uncover different types of behaviours, such as those that are retained upon disruption of pre-gastrula morphology (convergence and internalisation), and those that appear to emerge as a property of altering tissue geometry (polonaise movements).

16- **Jose Ignacio Perez Lopez**, Yusuf Hamied Department of Chemistry, University of Cambridge

"HISTONE VARIANTS MODULATE CHROMATIN PHASE TRANSITIONS BY ALTERING NUCLEOSOME STABILITY AND CHROMATIN STRUCTURE"

Eukaryotic cells store genetic information within the nucleus as chromatin, a condensed protein-DNA polymer. The basic unit of chromatin, the nucleosome, consists of DNA wrapped around histone proteins. The higher-level organization of chromatin complexes is critical for regulating gene expression, yet the physicochemical mechanisms driving this organization remain poorly understood. One such mechanism is the replacement of native histones for histone variants. In this study, we upgraded our multiscale coarse-grained chromatin model and performed molecular dynamics simulations of chromatin arrays containing different histone variants. Our simulations reveal that histone variants, like CENP-A and H2A.Z, critically regulate the biophysical properties of chromatin— including nucleosome stability, internucleosomal interactions, and chromatin structure—in agreement with published experimental data. Additionally, we show that by transforming chromatin structure, histone variants can change significantly the phase behavior of chromatin condensates. We present a ready-to-use workflow to incorporate any histone variants into our multiscale chromatin model and probe their effect on chromatin phase transitions. By simulating chromatin at different levels of resolution, our workflow can be used to decode how epigenetics shape chromatin's physicochemical properties to regulate its emergent properties.

17- **Marie de la Burgade**, Department of Physiology, Development and Neuroscience, University of Cambridge

"Investigating the trunk neural crest leader cell's asymmetric division in vivo"

Cells dividing in tissues must push against other cells around them. In vitro studies have shown that physical confinement causes mechanical stress during mitosis, leading to asymmetric cell divisions and mitotic errors. However, the effects of mechanical forces on mitosis have not yet been studied in vivo. During this PhD, I explored the impact of mechanical stress and signalling on asymmetric divisions and cell fate decisions in vivo, using translucent zebrafish embryos to visualise migrating cells directly. I focused on trunk neural crest leader (TNC) leader cells, which migrate collectively through tight interstitial spaces, forming chains guided by a larger leader cell. These cells divide asymmetrically, giving rise to a larger distal daughter which becomes the new leader, and a smaller, slower proximal daughter with follower behaviour. Later, the distal daughter gives rise to a sympathetic neuron, while the proximal daughter becomes glia. In this project, I investigated how cell shape and environmental constraints affect asymmetric cell divisions, revealed local differences in contractility during mitosis, and uncovered signalling mechanisms impacting the division and subsequent fate decisions in the zebrafish TNC.

18- **Matt French**, Department of Genetics, University of Cambridge

"A toolkit for mapping cell identities in relation to neighbours reveals Notch-dependent heterogeneity within the caudal epiblast"

Patterning of cell fates is central to embryonic development, tissue homeostasis, and disease. Methods to quantify patterning can give insights into the logic by which cell-cell interactions orchestrate changes in cell fate. However, it is challenging to quantify patterning when graded changes in identity occur over complex 3D trajectories, or where different cell states are intermingled. Furthermore, comparing patterns across multiple individual embryos, tissues, or organoids is difficult because these often vary in shape and size.
Here we present a toolkit of computational approaches to tackle these problems. These strategies are based on measuring properties of each individual cell in relation to the properties of its local neighbours in order to quantify patterning, and on using embryonic landmarks in order to compare these patterns between embryos. We use this toolkit to characterise patterning of cell identities within the caudal lateral epiblast of E8.5 embryos, revealing local patterning in emergence of early mesoderm cells that is sensitive to inhibition of Notch activity.

19- **Apolline Delahaye**, Department of Genetics, University of Cambridge

"Engineering gastrulation cell behaviours in vitro by modulating ECM components"

During gastrulation, early embryos undergo the specification and reorganization of their germ layers. This transformation necessitates the mesoderm's transition from an epithelial to a mesenchymal state (EMT). Central to this process is the remodelling of the extracellular matrix (ECM) by mesodermal cells, which in turn, reciprocally regulates cell fate. However, studying the developmental impacts of the ECM presents challenges due to its complex protein composition and overlapping functions, impeding traditional knockout methodologies. Alternatively, in vitro models such as gastruloids could be utilized for studying ECM during gastrulation; however, current models fail to fully replicate the EMT at both morphological and cellular behaviour levels. To address this, we employ mouse embryonic stem cell gastruloids cultured on ECM proteins involved in embryonic development. Utilizing a reporter cell line, we track pluripotency exit and mesoderm activation, along with high-throughput imaging and computational analysis of live imaging data. This enables quantification of gene expression changes, morphological alterations, and cell migration dynamics. Notably, our observations reveal cell migration on fibronectin, collagen I and IV, only after experimentally inducing mesoderm, while on laminin gastruloid flatten and generate protrusions rich in myosin cables. Furthermore, the ECM attachment preferences remarkably correlate with their temporal appearance in the embryo. These suggest that we can induce typical mesoderm cell behaviours (convergent extension, or cell migration) in gastruloids by interchanging ECM proteins. Finally, we show that using this system we can potentially modulate morphogenesis via interfering with cell behaviours, and more specifically lamellipodia formation.

20- **Anna Tarodi**, Department of Physics, University of Cambridge

"The Matrix – Motility Lifestyle Switch in Bacillus subtilis"

Bacteria exhibit collective phenomena, such as differentiation into distinct phenotypes and division of labour. These properties contribute to their success in their biofilm state, in which they exist as multicellular communities attached to surfaces. Biofilms are found in resistant bacterial infections and in numerous microbiomes in nature.
Therefore, investigating the steps leading to the collective state is key for improving understanding of health and disease linked to bacteria. My research involves one of these steps, the matrix-motility lifestyle switch that governs the entry into to the matrix-producing cell phenotype present in the multicellular state.
In Bacillus subtilis, the switching between motile and sessile cells arises even in the absence of external fluctuations that uncovered the involvement of a bistable switch in the genetic circuitry [1][2].
My research focuses on elucidating the internal and external factors regulating the motility-matrix transition in bacterial biofilm formation by connecting single-cell level cell fate decisions with emergent patterns of gene-expression on biofilm level. To this end I employ microfluidic devices, trapping individual cells to follow single-cell gene expression of motility- and matrix-related proteins for obtaining growth and switching dynamics. Recently, I have managed to reproduce the qualitative dynamics of the switching as described in the paper by Norman et al., under constant nutrient conditions.

[1] Norman, T., Lord, N., Paulsson, J. et al. Memory and modularity in cell-fate decision making. Nature 503, 481–486 (2013). https://doi.org/10.1038/nature12804
[2] Nathan D. Lord et al. ,Stochastic antagonism between two proteins governs a bacterial cell fate switch. Science366,116-120(2019).DOI:10.1126/science.aaw4506