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Cover Image: migrating cancer cells, courtesy of Prof. Laura Machesky. B16 melanoma cells depleted of the actin cytoskeletal protein NckAP1 invading into Matrigel collectively left to right and stained with phalloidin (actin filaments, magenta), DAPI (yellow, nucleus) and tubulin (cyan). Photo credit: Jamie Whitelaw- postdoctoral fellow funded by MEGA-FLIM.
In the October edition,

Professor Michelle Peckham
Newsletter Editor

Items for the newsletter should be e-mailed to m.peckham@leeds.ac.uk
The Committee

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Pietro Cicuta

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Mark Leake (new!)

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New Members
Margarita Staykova
Marco Mazza

And many thanks to our retiring members: Susan Cox (our honorary secretary), Marisa Martin-Fernandez and Rosalind Allen for all their hard work on the committee.
The Chair’s commentary

Dear Group,

as we enter an ever more unknown phase of the post-referendum era, with uncertainty in eligibility for ERC and other European funding, and evidence of some colleagues and junior staff leaving with others deciding not to come, let me focus on more positive news.

The UKRI “umbrella” has taken an important concrete step, ringfencing £15 million for research in the Physics of Life, see https://epsrc.ukri.org/funding/calls/physicsoflifecollab/. This is funded the first of two calls. The first call had grants selected by a panel this March, with some of the grants still recruiting now. It is envisaged that a second call will be announced in 2021 for 2022 grant start dates. Our IOP group, with others, notably the EPSRC Physics of Life Network, had been advocating for such a cross-council call in the area of basic science, for some time. There is a lot to learn and improve on all sides (the deadlines were so tight that postal peer review in the first call was not possible) but probably the most significant aspect of which we know so far is the sheer number of applications submitted (86, of which 8 funded). The total applied for was >£155M, £14.6 was funded. We all know that when success rates are less than ~25%, proposals that absolutely should be funded are being turned away. So - the good news here is that there is a thriving community, and clearly a need for progressing at this interface, felt both from the physical and biological ends, and finally a mechanism for funding this. It can be improved and hopefully grown to a sensible, regular and physiological level.

The Group continues to grow and promote activities. Of the IOP groups linked to research areas, we are the best gender balanced (a quantitative analysis will follow soon) and have a healthy age distribution, amongst the highest fraction of young members. We have regular conferences but also many one-off meetings and workshops. Please get in touch with any of the committee members if you would like to propose an event at IOP within this remit. We usually need to know by end of August in order to budget for the following calendar year.

We are also thinking of new initiatives.

Launching now a "short video" competition, which we hope will create a nice collection to represent activity in biological physics in the UK – details so far:

- Max 3 minutes
- Deadline 27th March
- Must be self-produced by early career (PhDs, postdocs, Fellows)
- Permission of PI must be obtained
- Competition is open to members of the Biological Physics group
- Video should be pitched to level of a physics undergraduate
- Prizes (TBC)

best wishes,

Pietro Cicuta (Chair)
The EPSRC funded 8 projects in the first round of funding from the ‘Physics of Life’ strategic fund from April 1st 2019. The aim of these grants is to foster cross-disciplinary research between physics and biology.

The funded projects were:

**Stochastic fluctuations during mammary development and breast cancer morphogenesis**  
G Salbreux, C Dunsby, AA Behrens (The Francis Crick Institute)

**The physics of antimicrobial resistance**  
J Hobbs, S Foster, N Zenkin, RJ Allen, W Vollmer, P Cicuta (University of Sheffield)

**Health assessment across biological length scales for personal pollution exposure and its mitigation (INHALE)**  
CC Pain, F Chung, P Kumar, J Lischner, AE Porter, Y Guo, D Arvind, I Adcock (Imperial College London)

**Biological metamaterials for enhanced noise control technology**  
MW Holderied, R Craster, B Drinkwater, D Robert (University of Bristol)

**Transcription and nuclear phase transitions**  
D Hebenstreit, V Kantsler, L Dyson, RC Ball (University of Warwick)

**MEGA-FLIM: quantum technologies for megapixel time-resolved imaging and control across biological scales**  
L Machesky, DFA Faccio, R Insall, N Gadegaard, A Harvey, J Taylor (University of Glasgow)

**Biological physics of protein clustering in epigenetic memory and transcriptional control**  
C Dean, M Howard (John Innes Centre) and MC Leake (University of York)

**Molecular Mechanics of Enzymes**  
F Vollmer, JA Littlechild, N Gow, CP Winlove (University of Exeter)

Three of the funded groups have summarized their research below, for this issue of the newsletter. Hopefully more to come soon!

**MEGA-FLIM: quantum technologies for megapixel time-resolved imaging and control across biological scales**  
Laura M Machesky 1,2, Jamie Whitelaw 1,2, Daniele Faccio 3, Robert H. Insall 1,2, Jonny Taylor 3, Andy Harvey 3, Nikolaj Gadegaard 4, 1. CRUK Beatson Institute, 2. Institute of Cancer Sciences, 3. School of Physics and Astronomy, 4. School of Engineering, University of Glasgow

Visualising biological processes at molecular resolution large cell collectives such as embryos, organoids and tumours is required to understand how these complex systems behave. It is increasingly clear that measuring protein activities and interactions during signalling, cell-environment interactions and regulation of gene expression, is crucial to understanding the biology of healthy cells and mechanisms of disease. Förster resonance energy transfer (FRET), combined with fluorescence lifetime imaging (FLIM) allows individual protein-protein interactions to be specifically interrogated in real time using fluorescent reporter probes. Additionally, optogenetics provides control of biological processes. However, biological insight is currently limited by our inability to image morphology and chemical processes in large 3D multicellular collectives simultaneously. These limitations can only be breached by increasing pixel counts, widening the imaging field and developing improved time resolution.

Our cross disciplinary group seek to build upon the physics of cutting-edge quantum imaging techniques to develop a new sensitive FRET/FLIM imaging system, capable of megapixel widefield imaging. We are working together with QuantIC in Glasgow [https://quantic.ac.uk/](https://quantic.ac.uk/) and Horiba Glasgow [http://www.horiba.com/uk/manufacturing/glasgow/](http://www.horiba.com/uk/manufacturing/glasgow/) to develop relevant technology that we hope will make an impact internationally. Together with bespoke probes, this will enable live imaging and optogenetic control of multicellular structures in three dimensions and at unprecedented spatial and temporal resolution. Crucially, our system will use computational data fusion from next-generation single-photon cameras (very high picosecond temporal, but poor spatial resolution) and high pixel-density CMOS and EMCCD cameras (no or very poor temporal resolution). This unique combination will allow unprecedented wide-field vision and thus permit FLIM measurements and optogenetic manipulation of large biological systems. New methods of computational imaging will provide real time detection of sparse 3D structures and smart synchronised one-photon and two-photon light-sheet imaging of moving and densely packed cell collectives.

These systems will be useful to address multiple biological questions, but our first focus will be on how collectively moving cells use self-generated chemical and force gradients to steer. Previous studies in these areas have mainly used tissue cultured cells on two dimensional rigid substrates. We will use organoids, tumouroids and mouse embryos expressing novel FRET and optogenetic probes at endogenous levels with CRISPR and lentiviral technology, allowing precise measurement and perturbation of both physical force and chemical signals. This technology is in its infancy, but some tools are available. For example, a FRET reporter mouse for ERK (extracellular signal-related kinase) revealed kinetics of signalling
during wound healing in live mouse skin (Aoki et al., Dev. Cell, 2017). This is just the start, as we will use and develop probes for other kinases, small GTPases and mechanosensitive molecules (e.g. nuclear-cytoskeletal linkers, vinculin, Yap/Taz, p38MAPK). Unbiased approaches will help to identify new key pathways (e.g. RNA-sequencing, proteomics). The ultimate test of knowledge-based models is reconstructing systems using engineered circuits and interventions. Key questions include: How do extracellular gradients of signalling molecules, together with physical forces, guide migration in embryos and tumours? Can we engineer signalling and mechanosensing circuits that control shape and dynamics of cell collectives in predictable ways? Can we thus use physics to advance synthetic biology? Our unique team of optical physicists, bioengineers and biologists is poised to overcome current barriers, unifying physics, engineering and biology for landmark discoveries.

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Stochastic fluctuations during mammary development and breast cancer morphogenesis
Guillaume Salbreux and Axel Behrens - The Francis Crick Institute
Chris Dunsby - Imperial College London

We all know from everyday experience that parts of our body have different shapes. Some of this variance is due to differences in genes, but some is also due to natural variability. This variability, where the same genetic program within cells can lead to slightly different results, arises from stochastic physical processes in cells. At present the variability exhibited by tissues is not well understood. In this project we will quantify and analyse the stochasticity underlying the adoption of three-dimensional shapes by multicellular structures. Part of the variability that we intend to explore arises from how cells exert forces and interact mechanically with each other, and part of it arises from the dynamics of stem cells. Cells use their cytoskeleton, an internal architecture capable of exerting forces, to move relative to each other. In addition, stem cells ensure that tissues function properly by dividing and giving rise to different cell types. For example, stem cells replace damaged cells during the repair of injured organs. Also in cancer there are stem cells, so call cancer stem cells, and these cancer stem cells are believed to be required for cancer to spread to other sites in the body (metastasis) and are also linked to the re-emergence of cancer (relapse) after therapy.

It is not currently possible to investigate the position and the behaviour of all cells in a living animal organ to address the origin of tissue shape variability. In the last few years it has become possible however to culture small organ-like structures and cancers in 3 dimensions, in so-called organoids. The cellular functions and interplay in organoids are similar to what is observed in a living animal, thus organoids represent a unique system to study the collective behaviour of cells. Here we will look at healthy and cancerous mammary gland organoids, as breast cancer is a very common disease and affects 1 in 7 woman.

To explore tissue shape variability in mammary gland organoids, we will develop a system to image organoids over a prolonged period of time and use it to investigate where the stem cells are, how they divide, what type of progeny cells they generate, and how stem and progeny cells exert forces inside the organoid. This imaging will be performed using a custom-built light sheet fluorescence microscope. We will use methods from physical sciences and numerical simulations to understand how the uncertainty in cellular behaviour results in variability of tissue shapes. We will develop simulations of tissue growth in 3D taking into account changes in cell types, cell division, and forces generated at the cellular level, and use results inferred from experiments to decompose the origin of tissue variability. The EPSRC grant is a fantastic opportunity for us to develop this multi-disciplinary approach to identify fundamental principles governing organ and cancer development.
Advanced theory and experiment from the Physics of Life to understand how protein clustering can 
regulate the output of genes and modify DNA as stored memory
Mark C. Leake 1

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The functional importance of protein clustering in cells is now a major focus in molecular biology. Clustering 
can be generated by different mechanisms including ordered oligomerization or by liquid-liquid phase 
separation, processes with an intrinsic physical origin. Recent interest has centred around key roles for 
clustering in the regulation and memory of transcriptional. Different types of clusters regulate these processes, 
but the biophysical mechanisms underlying their formation are not understood. Here I discuss our recent and 
planned research to explore these physical origins of clustering using a model system in Arabidopsis plant root 
tips.

The Physics of Life question of how epigenetic memory and transcription are controlled

Our research into this fascinating biological-physical system involves a highly interdisciplinary UK research 
consortium of Caroline Dean (plant biologist, John Innes Centre), Martin Howard (theory biophysicist, John 
Innes Centre) and Mark Leake (experimental biophysicist, University of York), combining expertise in 
developmental timing in plants and epigenetic regulation, ground-breaking mechanistic studies of chromatin 
regulation, mathematical modelling in biology of problems in cell and developmental biology including 
epigenetic dynamics, and experimental single-molecule biophysics tools enabling transformative insights into 
functional interactions of proteins with DNA. Our work was recently enabled from an EPSRC fund in the UK 
dedicated to furthering our understanding of the "Physics of Life" (1) in a grant entitled ‘Biological physics of 
protein clustering in epigenetic memory and transcriptional control’.

Our key research challenge is to investigate the biophysical origin, functional importance and interactions of 
different sets of protein clusters regulating a model plant epigenetic switching system. By focusing on one 
gene we can investigate how different biophysical clustering mechanisms are functionally integrated. In 
 essence, we plan to exploit a well-characterized single gene system - regulation of the Arabidopsis gene 
FLOWERING LOCUS C (FLC), which plays a key role in timing the developmental transition to reproduction 
in Arabidopsis (2). Due to sustained research on FLC over decades, we now have unparalleled understanding 
of the components that act together to quantitatively regulate its expression, and it has become a ‘model’ locus 
in the international epigenetics/transcription fields. FLC expression is repressed by prolonged cold, repression 
that is “remembered” even after cessation of cold. This memory of environmental cold exposure is an 
epigenetic process, mediated through the Polycomb repressive pathway, a conserved pathway from plants to 
flies to humans (3).

Repression of Polycomb-silenced genes is believed to be mediated not by repressive networks of transcription 
factors, but by covalent modifications to the histone proteins which tightly associate with the DNA. Specifically, 
tri-methylation of lysine 27 of histone H3 (H3K27me3) is the key mark which carries the silencing information. 
However, storage of epigenetic information in the chromatin via histone modifications faces a severe 
perturbation at DNA replication, where the parental histones are shared randomly (in pairs) between the two 
daughter DNA strands, with the deficit made up by unmarked histones. This process leads to a dilution of 
histone modification levels by a factor of two on average, but with significant small number fluctuations arising 
from the relatively low number of histones (~50) wrapping individual genes. To overcome this dilution, the 
enzymatic complex (Polycomb Repressive Complex 2, PRC2) that methylates H3K27me3 utilises a read-write 
mechanism, whereby it can recognise H3K27me3, allosterically self-activate and add more H3K27me3 to 
 nearby nucleosomes. Previous statistical mechanics modelling of these read-write feedbacks has 
demonstrated that such a module can robustly store epigenetic memory, but only in a digital, all-or-nothing 
form. For example, silencing mediated by high H3K27me3 levels is rebuilt after DNA replication by strong read-
write feedbacks, recapitulating the digitally silenced. Past work in the Dean and Howard groups, measuring 
FLC protein levels in single cells, has shown that Polycomb-mediated memory of cold exposure is stored 
locally in the chromatin around the FLC gene in a digital, mode, with increasing cold increasing the fraction of 
digitally silenced loci, as theoretically predicted.

Recent work has, however, called into question whether histone modifications are, by themselves, sufficient 
to ensure accurate memory maintenance through DNA replication. Mutants in components of the 
everolutoarily-conserved Polycomb silencing system (LHP1 and CLF) have restricted histone modifications at 
FLC to a very narrow region, comprising only around 6 histones. This restriction should cause rapid loss of 
memory, with complete loss of all marks at DNA replication with a probability of 1/8 (probability of independent 
loss of all three histone pairs). However, we found that the memory, although not fully stable, was much more 
persistent than predicted, an observation also found in the fly system. This result points towards the existence 
of other memory elements that function in addition to histone modifications. A key hypothesis to test in our new 
work of this is that these additional memory elements take the form of sticky, oligomerising proteins that are 
retained and inherited by the daughter DNA strands after DNA replication. Such a hypothesis is consistent 
with ChIP (Chromatin Immunoprecipitation) data that indicate several Polycomb accessory proteins, including 
VIN3 and VRN5 (likely functional equivalents of the mammalian MTF2 and PHF1 PRC2 accessory proteins.
that act at many Polycomb loci), progressively bind to a specific location of the FLC gene, the so-called
nucleation region, in cold temperatures. Preliminary structural data have also pointed towards the existence
of specific domains within VIN3 and VRN5 that facilitate oligomerisation, with oligomerisation of these proteins
also observed in vitro. Furthermore, confocal images of VIN3-GFP also indicate the presence of discrete
protein clusters.

Prior to cold exposure, FLC is also regulated by a separate repressive ("Autonomous") pathway involving RNA-
processing proteins. This pathway mediates an antisense-mediated chromatin silencing mechanism, involving
co-transcriptional regulators and functionally important antisense transcripts (termed "COOLAIL"). However,
how these factors act together to achieve quantitative co-transcriptional regulation of FLC is still not
understood. Genetic screens/analysis have uncovered factors important in setting FLC expression levels.
These include the RNA-binding protein FCA, as well as other RNA processing factors, such as FPA and FY.
Recently, we discovered that FCA forms dynamic, phase-separated compartments which are essential for
proper FLC regulation (4). Phase separated FCA droplets were observed to fuse, with FRAP studies indicating
that FCA exchanged rapidly with the surrounding nucleoplasm. Phase separation was consistent with the FCA
protein containing structurally disordered regions. Furthermore, phase separation required a coiled coil protein
FL2, which co-localized with FCA in the droplets. Mutations in FL2 dramatically reduced the number and
size of the FCA bodies. Crucially, the FL2 mutant was found to be defective in the same RNA processing
steps as FCA, indicating that FCA bodies are functionally important for FCA-mediated RNA processing.
Despite these observations, the mechanistic role of the droplets remains unknown. A second key goal of new
research is therefore to elucidate the physical basis and functional importance of the phase separated
compartments for FLC repression.

Specific future research aims
In the last two years the epigenetics and transcriptional regulation fields have converged on the recognition of
the functional importance of protein clusters. However, only by understanding the physics of differing protein
clusters, both protein oligomerization and liquid-liquid phase separation, will we progress our mechanistic
understanding of gene regulation. FLC provides a highly appropriate, regulated, single gene target with which
to study different mechanisms of protein clustering and how they are integrated to influence quantitative
transcription and epigenetic switching of one locus. Our tightly focused proposal is therefore extremely timely
and could lead a step change in our understanding of these vital biological processes. Our interdisciplinary
planned research breaks down into 2 primary objectives:

1. How is epigenetic memory stably maintained beyond histone read-write feedbacks? We hypothesise that
self-reinforcing, protein oligomerization plays a key role in the memory of repression. We therefore propose
an interdisciplinary three-track approach: develop statistical mechanics models of the biological physics of
protein oligomers localised to the gene; use genetic and structural analyses of the proteins involved to isolate
and perturb key oligomerising features; and use single-molecule, super-resolution imaging to examine whether
such oligomers are persistent at the locus and in what numbers. The likely low numbers of proteins (tens)
involved in the oligomer will make this project challenging, but one that could revolutionise our understanding
of the biophysics of epigenetic memory.

2. How do phase-separated droplets regulate gene repression? We hypothesise that the droplets may prolong
protein interactions through increased dwell times and reduced diffusion, thus facilitating RNA processing.
To investigate this question, we will perform genetic and structural analysis of component proteins, probe droplet
dynamics by precise biophysical measurements, and use these results to develop statistical mechanics models
of phase separation, and of their internal reaction-diffusion dynamics. This work will provide direct evidence
for phase-separating compartments in modulating the transcription of a key gene.

How are we proposing to address these challenging Physics of Life questions?:

1) How is epigenetic memory stably maintained beyond histone read-write feedbacks?

1.1 Theoretical modeling of oligomerization. We will develop a new statistical mechanics model of protein
oligomerization, working together with conventional H3K27me3 read-write feedback, to generate more stable
epigenetic memory. To simplify matters, we will initially consider only a single protein species, acting as a
composite for the nucleating proteins VIN3 and VRN5. We will assume slow nucleation of the oligomerized
cluster due to rare simultaneous binding events, followed by strong auto-recruitment to maintain the nucleated
state. These dynamics will be layered on top of the more standard read/write H3K27me3 dynamics
agonized by transcription. The model will be fit to existing time-course ChIP data on histone modification
and protein levels in the nucleation region, during and after the cold, in both wild-type and mutants (LHP1 and
CLF) with a narrowed region of H3K27me3. The model will be implemented using a spatial Gillespie
framework, fully incorporating low-copy number fluctuation effects. As determined by FRAP data, the model
may need to accommodate relatively fast turnover of individual elements in a situation where the entire
oligomeric structure is collectively stable over timescales of days. We will model the partitioning of the
oligomerized proteins onto daughter stands during the replication. The logic is conceptually similar to histone
modification inheritance, i.e. we assume that the complexes are distributed randomly to each strand, after
which the feedbacks fill in the missing proteins. Occasionally, however, these feedbacks will fail due to
inheritance of an insufficient number of proteins and the metastably silenced state will be lost. The average
total number of inherited protein will then be predicted such that the lifetime of the metastable state matches
our earlier experiments (~10 days), to be compared with experiments. We will also compare model output with cases where oligomerisation has been impaired but not eliminated, as well as generalise to more complex cases where we will model VIN3/VRN5 separately.

1.2 Testing the functional consequences of perturbed oligomerisation

Our work to date shows that two domains (VEL and FNIII) within the homologues VRN5 and VIN3 are important for oligomerization. We envisage that head-to-tail oligomerization of the C-terminal VEL domain results in locally high concentration of protein. This would promote a conformational switch on the chromatin involving cross-linking of fibrils through domain swapping of the FNIII domain. Our hypothesis is that a stochastic conformationally-induced oligomerization event of VIN3/VRN5 assembles a VEL-PRC2 complex and generate a switch to a nucleated silenced state. Self-templating mechanisms promoting a similar conformational change on incoming proteins would then constitute the feedbacks providing the metastable memory function. We will therefore generate mutations designed to impede or block completely the dimerization/cross-linking and stably transform these into plants to assay their consequence on memory. We will also undertake a comprehensive analysis of post-translational modifications of VIN3, VRN5 oligomerization domains (including phosphorylation, arginine methylation, SUMO, ubiquitin, O-GlcNAc), mutating their sites and again assaying functional consequences in stably transformed plants. In addition to testing function through assaying FLC expression and through protein/H3K27me3 ChIP at FLC we will generate lines carrying both wild-type and mutated VIN3-mScarlet and VRN5-Venus, as well as FLC tagged with lacO, to which we will target LacI-mTurquoise. The lacO repeats enable us to identify the FLC locus in the nucleus: the lacO repeats have been introduced downstream of the transcription unit thus avoiding barrier issues to transcription.

1.3 Testing the oligomerisation hypothesis using single-molecule experiments.

We will develop a new optical microscopy capability using physics approaches to enable rapid stoichiometric quantification and 3D tracking of individual dynamic protein clusters deep into native root tissue. State-of-the-art super-resolution imaging of tissues has been enabled in particular through recent progress of lattice light sheet microscopy, however this technology is limited to relatively thin samples and to slow cellular dynamics with effective frames rates for monitoring whole cells of at best ~1 Hz. Here we instead develop highly inclined angle illumination by modifying an existing single-molecule microscope (Fig. 1), using Bessel beam excitation to minimise optical aberration due to diffraction and adaptive optics to correct for refractive inhomogeneity through multiple cell layers in native root tips. These methodologies will be coupled to use of astigmatism imaging to determine precise 3D coordinates of tracked fluorescent particles. Using narrowfield excitation of ~10μm width as previously developed by us (4-7), but with the new capability of precision-corrected imaging at depths of tens of microns, will enable millisecond sampling with single-molecule sensitivity of fluorescently labelled protein constructs in individual native root tip cells, without the requirement for an expensive commercial super-resolution microscopy system. We will optimise this methodology first using isolated root cells, prior to using native root tips prepared with standard preparation protocols already optimised at JIC for confocal microscopy imaging. Using the fluorescently tagged proteins/FLC locus, both wild-type and mutated, we will use dual- and triple-colour super-resolution single-molecule tracking to monitor the simultaneous localization of the FLC gene locus from the 3’ lacO array, as well as VIN3 and VRN3 relative to FLC and to each other. Tracking the FLC gene location is essential as VIN3 and VRN5 will likely have many other genomic targets and it will be essential
to track clusters specifically at \textit{FLC} that may be linked to epigenetic memory. This methodology will enable us to characterize respective mobility and dwell time distributions of \textit{VIN3/VRN5} and the various mutated forms. This will inform which features are particularly important to hold the protein-based memory through DNA replication, the key moment when histone-based memory is perturbed. To determine the composition of the clusters we will perform precise molecular counting of each protein component using step-wise photobleaching, developed by us previously, monitoring time-resolved changes to cluster number, location and composition throughout all stages of the cell cycle. We will also determine how temperature influences changes in cluster composition and dynamics in wild-type and mutants with defective oligomerization and altered post-translational modifications, using vernalisation temperatures of 5°C up to 30°C. We will also perform Förster Resonance Energy Transfer (FRET) measurements utilising an alternating laser excitation (ALEX) system we have already developed on individual clusters to determine relative conformational and distance changes between \textit{VIN3/VRN5}. These results and quantification will be fed back into the statistical physics model. We will also develop AI-based machine learning algorithms to enable real-time pattern recognition of cell images for keeping track of multiple regions of the root tip over extended imaging periods of up to several days, as well as facilitating automated sample drift correction.

2) \textbf{How do phase-separated droplets regulate gene repression?}

2.1 Molecular biology of FCA phase separated droplets. Our previous work on the autonomous pathway suggested that \textit{COOLAIR} transcription in the warm is limited to the G1/S phase of the cell cycle, just before DNA replication. This transcription generates an R-loop structure at \textit{FLC}, where an RNA molecule invades double-stranded DNA to form an RNA-DNA hybrid. We envisage that this structure generates conflict with the DNA replication machinery (the “replisome”), stalling replication and providing a window of opportunity for chromatin modifiers to associate to the replisome for subsequent spreading of silencing marks; this provides a mechanism to ensure faithful inheritance of chromatin modifications every cell cycle. The genetically identified factors FCA, FY and other RNA processing factors function to resolve R-loops releasing the replisome. The spreading of chromatin modifiers with progressing replisomes establishes the chromatin domain covering the locus, which influences both transcription initiation and elongation. Our recent work shows that FLL2, a coiled coil protein, is required for FCA, FY and other RNA processing factors to form a phase separated compartment at the R-loop/replisome stall (4). Phase separation is likely to depend on weak, multivalent interactions involving the different protein constituents, post-translational modifications and RNA molecules. We will mutate predicted salt bridge interactions in FLL2, the key RNA-binding amino acid residues in the FCA RRM domain and both arginine methylated and phosphorylated sites in FCA. Using leaf infiltration transfection experiments, combined with selected stable transgenic plant analyses, we will determine the importance of these residues, and the RNA binding, on the droplet formation, size and lifetime. We will also generate fluorescently tagged versions of FCA and FLL2 for molecular probing experiments, using mScarlett/Venus, and compare to the photoswitchable mEos2. Lastly, we will use an MS2-ligand fluorescence tagging system to image the nascent \textit{COOLAIR} transcript, exploring the use of Janelia photoactivated Fluros and Halotag ligands, and ideally also the chromatin-embedded R-loop, in live cells. This work will enable us to understand the spatial relationships and dynamics of the different components of the phase separated compartments.

2.2 Probing the biophysical properties of FCA phase separated droplets. We will use dual- and triple-colour single-molecule fluorescence tracking on live root tips to determine the roles of RNA-binding protein FCA, FLL2 and \textit{COOLAIR} RNA as scaffolds for clustering, using fluorescent protein cell strains. We will use the photoswitchable mEos2 dye variants to map out the mobility of proteins within each liquid droplet and determine the local spatial dependence of liquid viscosity to test if there are shell structures interfacing with the droplet surface to aid droplet integrity and stability. We will quantify turnover of separate molecular components using fluorescence recovery after photobleaching to determine the kinetics of molecular recruitment into droplets, parameters which we will feed into theoretical models. We will also use the \textit{lacO-LacI-mTurquoise FLC} locus to track \textit{FLC} localisation relative to the droplets. We will perform these assays against a background of several mutants in which specific kinase and methylation sites have been deleted in order to determine their influence of liquid droplet formation. We will also perform FRET measurements on individual liquid droplets to determine relative orientations and displacement between pairs of all available fluorescently-labelled molecular components, in order to determine how changes in the structural architecture of clusters are influenced by the cell cycle phase, and potentially by DNA replication stalls.

2.3 Theoretical modelling of formation and kinetic properties of FCA phase separated droplets. In this objective we will use the kinetic droplet properties, to develop a simple reaction-diffusion model of the key repressive droplet components, including FCA and FLL2. In particular, we will investigate the hypothesis that the function of the droplet is to raise the concentration of key repressive elements to effect efficient silencing of the locus. Key ingredients will be the mobilities and concentrations of the relevant proteins in the droplet, as well as of the \textit{FLC} locus itself, as well as any observed droplet sub-structure. We will particularly focus on whether there is a critical size threshold for the droplet where \textit{FLC} is confined for long enough periods of time in an environment with enhanced repressor concentrations to deliver effective silencing. One possible model outcome is that even in the droplet environment, repression is still too weak due to low concentrations at the locus. In that case, we will investigate the possibility of further sub-droplet oligomerisation at the locus, forging
a synergy between the two modes of clustering. If time permits, we will also embark on a more fundamental theoretical study of the dynamics of droplet formation and growth (rather than taking these as assumed kinetic properties, as above), nucleated by a transcription-replication conflict. These studies could take as a useful starting point, the dynamics of centriole-nucleated centrosome formation, linking out-of-equilibrium reaction-diffusion dynamics with equilibrium phase separation.

**Discussion**

By the end of this new project we aim to have developed deep mechanistic understanding of the biophysical origin and functional importance of two types of protein clusters. New insights from the project will be immediately important for focused applications to flowering time in plants, as well as to the biological physics field. However, our results will also be much more widely relevant, as protein clustering is prevalent in numerous aspects of sub-cellular organisation. In the long-term, fundamental concepts emerging from this project will, we believe, have benefits to human health. In the shorter term, in the Leake group, the experimental biophysics tools that will be developed using bioimaging hardware and AI-based analysis will enable high-throughput molecular-level quantification in native tissue samples. This ability will impact multiple biosensing applications in plants and animals of relevance to many UK-based research teams in academia, as well as in commercial biomedical and pharma sectors. This type of research is impossible to do without a highly integrated approach spanning experimental and theoretical methods, with key expertise required in both the life and physical sciences. It therefore needs a big, interdisciplinary consortium, big support from reviewers and funding panels, and big pockets from the funders. Ultimately though the end result can be a genuine transformation into our understanding of the science behind one of the most basic of biological processes, and so armed with this knowledge great impacts may come into future areas of health and biomedicine.

**Acknowledgments**

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**References**


Meeting Report:

Co-Organisers: Peter McClintock & Aneta Stefanovska

Oscillatory processes in biology are special. They differ markedly from those analysed over the centuries by physicists and mathematicians in that their amplitudes, frequencies and phases vary in time. The underlying reason is of course that living systems are open, subject to their environments, and having continuing inputs and outputs of matter and energy. In the language of mathematics, they are non-autonomous. Analysing such oscillations is challenging because the traditional approaches (e.g. Fourier based) either fail completely or require particular care in application.

To confront this challenge, 56 researchers from 17 countries came together for an intensive 3-day interdisciplinary meeting in Chicheley Hall, Buckinghamshire. They included physicists, mathematicians, engineers, computer scientists, information theorists, biologists, physiologists, and clinicians. Their aim was to discuss the physics of systems far from equilibrium, to share their understanding of biological oscillators in a diversity of different systems, to pool their experience of the latest methods introduced to analyse the signals that they generate, and to consider applications, especially in medicine.

Contributions encompassed the huge range of frequencies and length scales over which biological oscillations manifest themselves, and considered both the theoretical and experimental aspects of the phenomena. The relevant length scales run from the sub-cellular (e.g. calcium and glycolytic oscillations), through cellular (e.g. endothelial) and organ (e.g. heart and brain) levels up to whole organism and beyond, to populations. Frequencies include the range from circadian, through metabolic (0.01 Hz), cardiac (1 Hz) up to the fastest EEG frequencies (arguably around 500 Hz). All of these oscillations share common features with attendant difficulties in their analysis.

Alex Webb (Cambridge) gave a keynote presentation on the dynamical plasticity of circadian rhythms in mutant plants, and how they contribute to carbon homeostasis through the regulation of starch turnover. Several talks followed, also on oscillations at the cellular level, covering glycolysis in yeast and HeLa cervical cancer cells, as well as modelling metabolic dynamics.

The theory of non-autonomous oscillators was addressed by several speakers. Robert MacKay (Warwick) gave a keynote talk on normal hyperbolicity in networks of oscillators, including non-autonomous ones, leading to a framework for treating synchronisation. Peter Kloeden (Tuebingen, Germany) gave a keynote on the mathematics of attractors (forward and pullback) in non-autonomous systems, in the context of discrete-time dynamical systems, i.e. non-autonomous difference equations.

Constantino Tsallis (Centro Brasileiro de Pesquisas Física, Brazil, and Santa Fe Institute, USA), in another keynote talk, discussed how Boltzmann-Gibbs statistical mechanics now needs to be extended to encompass
complex systems (financial, linguistic, geophysical and astrophysical, as well as living). He proposed the introduction of nonadditive entropies as one way forward.

The most considered topic was the cardiovascular system which, arguably, provides one of the best and most striking demonstrations of non-autonomous dynamics in physiology. It can be traced back to Hales’ 1773 report of heart rate in a horse being modulated by respiratory oscillations. Much later, in the 1990s, Aneta Stefanovska and co-workers applied wavelet analysis to blood flow dynamics, thereby revealing the existence of many (at least 6) cardiovascular oscillations. Each of them was characterised by a particular frequency, and was subject to modulation by the others. The oscillations seemed to manifest themselves, to a greater or lesser extent, in seemingly quite different measured quantities (e.g. arterial blood pressure, and respiratory oscillations, as well as the microvascular blood flow). Each oscillation is attributable to a particular physiological process, e.g. myogenic, neurogenic and endothelial activities – in addition to the more obvious cardiac and respiratory rhythms, thus naturally linking the macroscopic and microscopic (cellular) levels. These oscillations were extensively discussed. So also were the inter-oscillator interactions, which can now be described in terms of coupling functions. Brain (EEG) oscillations interact with each other and sometimes with cardiovascular rhythms. These interactions, too, can be characterised by coupling functions, and provided additional topics for debate.

Biological oscillations are important, not only for their own intrinsic interest, but also for numerous potential applications. Those discussed included diabetes, assessment of endothelial reactivity, heart failure, hypertension, melanoma, dementia, malaria, and anaesthesia – all of which can exploit the altered oscillatory amplitudes and/or frequencies and/or changes in couplings. The fact that most of the physiological oscillations can be accessed noninvasively promises huge advantages for future developments in wearable technology.

A short excursion was squeezed into the programme, so that participants could visit (nearby) Bletchley Park and the National Museum of Computing, inter alia to appreciate Alan Turing’s World War II decryption environment and view the related exhibits, as well as to see the Enigma, Bombe and Colossus machines in action.

The final wrapping-up session, chaired by Aneta Stefanovska and Robert MacKay, tried to take stock of the stage reached and consider how best to move forward. It started from the fact of living systems being open and operating far from equilibrium. It was agreed that, although we do not yet have a theory appropriate for such systems, we do now have some ingredients. Living systems are often treated as Hamiltonian, though inappropriately. In reality, they do certainly need to be analysed within the framework of non-autonomous systems. The latter is not yet quite ready for practical use, but it is getting there. We also need to take account of the fact that the biological oscillators of living systems always operate within finite time windows (e.g. between birth and death) and this can mean that the traditional asymptotic approach will fail. In particular, current theories are mostly based on the asymptotic approach and need revision. The most important outcome of the meeting was probably a shared awareness of the problems faced, their universality and importance, and the progress currently being made in addressing them.
Upcoming Meetings

Invited Speakers

- Martin Ackermann, ETH Zurich, Switzerland
- Rosalind Allen, University of Edinburgh, UK
- Omar Bayraktar, Wellcome Sanger Institute, UK
- Diana Fusco, University of Cambridge, UK
- Robert Klose, University of Oxford, UK
- Luke Mackinder, University of York, UK
- Erik Miska, University of Cambridge, UK
- Namiko Mitara, University of Copenhagen, Denmark
- Wolfram Moebius, University of Exeter, UK
- Andrea Weisse, Imperial College London, UK

Key dates

Abstract submission deadline: past

Early registration deadline:
28 October 2019

Registration deadline:
29 November 2019
Meeting for John Trinick and Gerald Offer: University of Leeds, January 10th 2020
Two highly regarded researchers in the ‘muscle’ field, died this year – Professor John Trinick and Professor Gerald Offer. John Trinick did his degree in physics, and then a PhD in biochemistry, and his research focussed on the structure of muscle proteins. Gerald did his degree in Natural Sciences at Cambridge, and then a PhD in biochemistry. We are planning a meeting to honour their life and work, and look forward to future developments.

*Coiled coils, myosin, titin and striated muscle: A reflection on the contributions of John Trinick and Gerald Offer.*

January 10th 2020
University of Leeds
John Trinick and Gerald Offer were leading researchers in the field of coiled coils, myosin, titin and skeletal muscle. Both researchers passed away this year, and the main reason for holding the meeting is to celebrate their contributions to the field, with a look back into how the field has developed over the past 50 years, and to look forward to new exciting developments that build on their contributions. Our invited speakers reflect this diversity of aims, and include many people who have worked with John and Gerald over their careers, reflecting their rich diversity of research. John made many contributions to the development of electron microscopy, using it to visualise titin and myosin. Gerald was an expert in myosin and coiled coils.

ORGANISERS: Michelle Peckham, Steve Muench and Peter Knight (University of Leeds, UK), and Prof. Howard White (Eastern Virginia Medical School, USA)

Provisional list of Speakers
Howard White  East Virginia Medical School
KW Ranatunga  University of Bristol
Dek Woolfson  University of Bristol
Pauling Bennett  King’s College London
Charlotte Scarff  University of Leeds
Michelle Peckham  University of Leeds
Roger Craig  University of Massachusetts
Ken Taylor  Florida State University

Details to follow
Registration will open on October 1st 2019. There will be a small registration fee of £30 to cover the costs of catering. Registrations will open here: https://www.rms.org.uk/discover-engage/event-calendar/meeting-for-john-trinick-and-gerald-offer.html