Invited Speaker

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Prof. Dr. Ralf Jungmann[1]
[1]Max Planck Institute of Biochemistry, Martinsried, Germany, [2]LMU Munich, Munich, Germany
1176 Revealing the molecular architecture of the cell using Ultrastructure Expansion Microscopy (U-ExM)
Prof. Paul Guichard
1179 Dynamics of molecules at the nanoscale: from RESOLFT to STARSS
Ilaria Testa

Oral Presentation

630 Characterisation of red fluorescent protein FLIM properties and comparison with novel StayGold live cell imaging
Dr. Philipp Tripal[1], MSc Hanan Said[2], Dr. Benjamin Schmid[1], Dr. Ralph Palmisano[1]
[1]Optical Imaging Competence Centre, Friedrich-Alexander Universität, Erlangen, Germany, [2]University of Applied Science, Coburg, Germany
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Prof Michelle Peckham[1], Dr Alistair Curd[1], Dr Ruth Hughes[1], Dr Heather Martin[1], Dr Anna Tang[1], Dr Francine Parker[1], Dr Christian Tiede[1], Dr Darren Tomlinson[1]
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479 Nanoscale T-cell membrane protein imaging across complex topography using accelerated large depth-of-field localisation microscopy
Dr Aleks Ponjavic[1,2], Dr Alex Carr[3], Dr Edward Sanders[3], Dr Mafalda Santos[4], Dr Edward Jenkins[4], Dr James McColl[3], Prof David Klenerman[3], Prof Simon Davis[4], Prof Steven Lee[3]
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Patrick Byers[1,2], M.Sc. Thomas Kellerer[1], Prof. Dr. Thomas Huser[2], Prof. Dr. Thomas Hellerer[1]
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Charlotte Clews[1], Dr Scott Dillon[2], Dr Fabio Nudelman[3], Professor Colin Farquharson[1], Dr Louise Stephen[1]
[1]The Roslin Institute, The University of Edinburgh, Edinburgh, United Kingdom, [2]Yusuf Hamied Department of Chemistry, Cambridge, United Kingdom, [3]Department of Chemistry, The University of Edinburgh, Edinburgh, United Kingdom
784 Microglial contactology–mapping the connection network of microglia from the micrometer to the nanometer range
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[1]Neuroimmunology Laboratory, HUN-REN Institute of Experimental Medicine, Budapest, Hungary, [2]János Szentágothai, Semmelweis University Doctoral School of Neuroscience, Budapest, Hungary
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Valeriia Grudtsyna[1]
[1]Niels Bohr Institute, Copenhagen, Denmark
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[1]Department of Materials Science and Engineering/ McMaster University, Hamilton, Canada, [2]Chemical Engineering and Materials Science, University of Minnesota–Twin Cities, Minneapolis, USA, [3]Canadian Centre for Electron Microscopy, McMaster University, Hamilton, Canada
970 Ultrastructural and chemical analysis of human Locus coeruleus using correlative microscopy and mass spectrometry
Zahraa BERRO[1], Dr Maria Angels Subirana[2], Dr Clementine Warres[3], Dr Tom Wirtz[1], Dr Tatjana Taubitz[1,4], Dr Jean-Nicolas Audinot[1], Dr Fabio A. Zucca[5], Dr Michel Mittelbronn[6,7], Dr Luigi Zecca[5], Dr Antje Biesemeier[1]
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1023 FRET and TIRF microscopy for single molecule characterisation of synergistic antimicrobial peptides in artificial bilayers
Miss Hannah Baird[1], Dr William David Jamieson[1], Dr Oliver Castell[1]
[1]School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, United Kingdom
1025 A novel pipeline to elucidate the adaptation response to the G2/M DNA damage checkpoint
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[1]Department of Experimental Oncology - European Institute of Oncology IRCCS , Milano, Italy
1062 Novel nanobody-based tools for studying the synaptic vesicle life cycle
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1158 Time ecology method

Marion Bitsch[1]

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Late Poster Presentation

1234 Interactions between Titanium Dioxide Particles and Wood Cell Wall Ultrastructurea

Petr Svora[1,2], Sylwia Krystyna Svorová Pawelkowicz[3], Petra Ecorchard[4], Jan Duchoň[1], Alena Schieberová[5]

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1273 Understanding and modifying cell wall permeability to facilitate cellular nanoparticle uptake

Tommaso Angeletti[1], Grmay Lilay H[1], Søren Husted[1]

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1327 Label-free evaluation of apoptotic cell death in pancreatic cancer cells using stimulated Raman scattering microscopy

Dr. Sanghee Nah[1], Suji Baek[2], Seung-Hae Kwon[1], Kang Pa Lee[2], Byung Seok Moon[3]

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1328 Evaluation of pH-sensitive drug delivery systems for lung cancer therapy via multiplex fluorescence microscopy

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1333 Localization of SHP-1 in Natural Killer Cells Across Education

Sophia Hald[1], PhD Thuy Thanh Luu[1,2], Petter Höglund[1]

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From DNA Nanotechnology to biomedical insight: Towards single-molecule spatial omics

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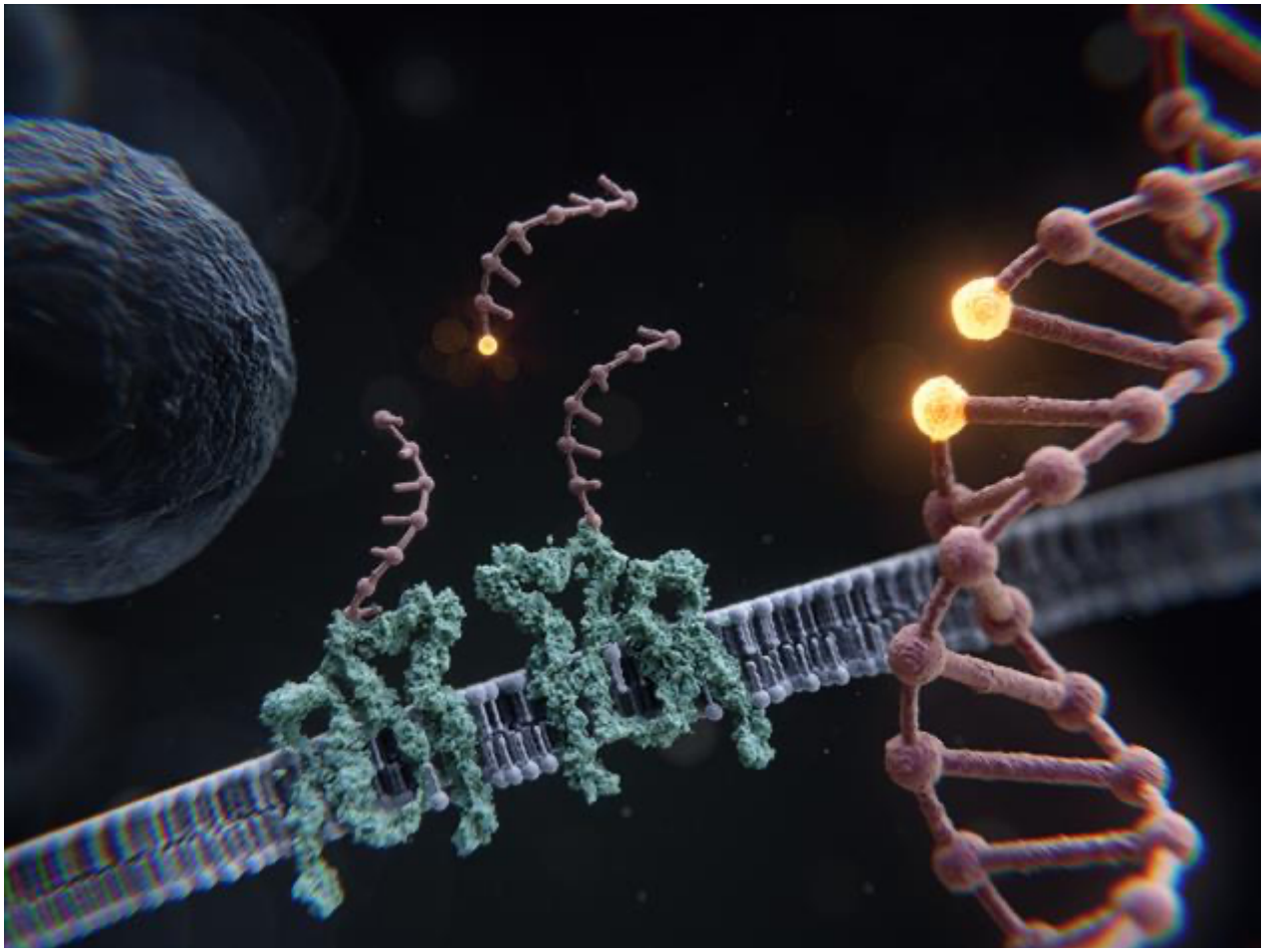
LS-03 (1), Lecture Theater 4, august 26, 2024, 15:00 - 16:00

Super-resolution fluorescence microscopy is a powerful tool for biophysical and biological research. The transient binding of short fluorescently labeled oligonucleotides (DNA-PAINT) can be leveraged for easy-to-implement multiplexed super-resolution imaging that achieves molecular-scale resolution across large fields of view. This seminar will introduce recent technical advancements in DNA-PAINT including approaches that achieve sub-10-nm spatial resolution and spectrally unlimited multiplexing in whole cells followed by recent developments in novel protein labeling probes such as Slow Off-rate Modified Aptamers (SOMAmers), that have the potential to facilitate DNA-barcoded labeling of much of the proteome within intact cellular environments. Applications of these new approaches will be discussed in cell surface receptor imaging and neuroscience. Visualization and quantification of cell surface receptors at thus far elusive spatial resolutions and levels of multiplexing yield fundamental insights into the molecular architecture of surface receptor interactions thus enabling the future development of more refined “pattern”-based therapeutics. A key approach in implementing these methods has been to leverage standard off-the-shelf fluorescence microscopy hardware as a tool for spatial omics, thus democratizing the ability to visualize most biomolecules and probe their network-wide interactions in single cells, tissues, and beyond with single-molecule-based "Localizomics".



Keywords:

Super-resolution microscopy, DNA Microscopy

Reference:

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Nature (2023).

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Up to 100-fold speed-up and multiplexing in optimized DNA-PAINT
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Modified aptamers enable quantitative sub-10-nm cellular DNA-PAINT imaging
Nature Methods (2018).

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Revealing the molecular architecture of the cell using Ultrastructure Expansion Microscopy (U-ExM)

Prof. Paul Guichard

LS-03 (2), Lecture Theater 4, august 27, 2024, 10:30 - 12:30

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Dynamics of molecules at the nanoscale: from RESOLFT to STARSS

Ilaria Testa

LS-03 (2), Lecture Theater 4, august 27, 2024, 10:30 - 12:30

Molecular interactions and complex formation are the base of cellular processes and therefore human activities. However, it is very hard with state-of-the-art techniques to precisely, efficiently and specifically follow their formation directly in physiological relevant system such as living cells. The goal of this project is to develop a novel apparatus for measuring the formation of large molecular complexes with unprecedented level of precision and in living cells, providing new capabilities in the functional aspects of imaging.
Measuring rotational diffusion properties of biological macromolecules is an extremely useful tool in cell biology because of its high sensibility to molecular weight changes, giving direct insight into several biological processes such as molecular binding, enzymatic activity, and protein complex formation. Here, we present a novel methodology based on the time-resolved fluorescence anisotropy experiment (TR-FA), and named Super Time-resolved Fluorescence Anisotropy with Switchable States (STARSS), which aims to measure complex formation in living cells by providing rotational accuracy far beyond the current fluorescence lifetime temporal limit. STARSS translates powerful and fundamental ideas from the field of super-resolution microscopy, which deals with increasing the precision of the determination of the spatial coordinates of fluorescent probes, into rotational accuracy. We used STARSS to measure the maturation state of viral-like particles as well as chromatin packing and the oligomerization of protein in cells.

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Characterisation of red fluorescent protein FLIM properties and comparison with novel StayGold live cell imaging

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LS-03 (1), Lecture Theater 4, august 26, 2024, 15:00 - 16:00

Red fluorescent proteins (RFPs) have become indispensable tools in molecular and cell biology, facilitating a wide range of applications including live cell imaging, protein tracking, and gene expression analysis. However, the increasing availability of RFP variants poses a challenge in selecting the most suitable probe for specific experimental requirements. In this study, we conducted a comprehensive comparative analysis of eight commonly used RFPs: mCherry, mCherry2, mCarmine, mKate2, TagRFP, mScarlet, mScarlet-I, and mScarlet-H, with a focus on their fluorescence lifetime properties.

We employed time-correlated single photon counting (TCSPC) and fluorescence lifetime imaging microscopy (FLIM) techniques to characterize the fluorescence lifetimes of these RFPs. Our results revealed significant variations in fluorescence lifetimes among the tested RFPs. mCherry exhibited the shortest fluorescence lifetime, followed by mCherry2 and mCarmine. In contrast, mKate2, TagRFP, mScarlet, mScarlet-I, and mScarlet-H displayed longer fluorescence lifetimes, with mScarlet-H demonstrating the longest lifetime among the tested variants.

Furthermore, we investigated the potential of fluorescence lifetime imaging (FLIM) as a tool to distinguish between these RFPs in live cell imaging experiments. By analyzing the fluorescence decay curves and calculating the average fluorescence lifetimes for each RFP, we observed distinct lifetime signatures that enable discrimination between the RFP variants. Additionally, we evaluated the photostability and brightness of these RFPs under similar experimental conditions, providing comprehensive insights into their performance characteristics.

Our findings highlight the importance of considering fluorescence lifetime properties when selecting RFPs for specific imaging applications. The ability to distinguish between RFP variants based on their fluorescence lifetimes expands the toolkit for multicolor imaging and facilitates the development of advanced imaging techniques such as fluorescence lifetime imaging microscopy (FLIM) and Förster resonance energy transfer (FRET) assays. Moreover, the availability of RFPs with diverse fluorescence lifetime characteristics enhances the versatility and applicability of fluorescence-based imaging approaches in biological research.

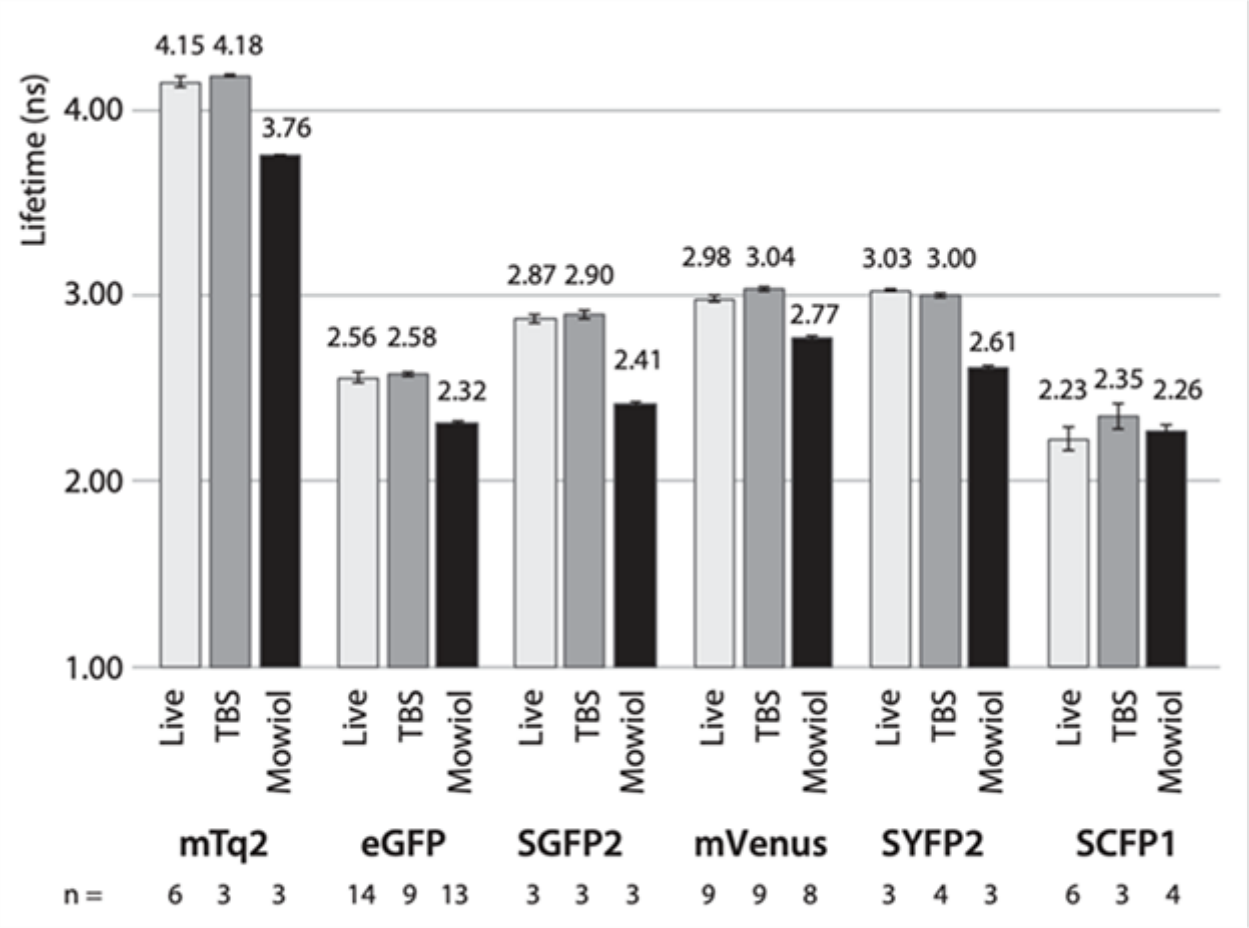
In conclusion, this comparative analysis provides valuable insights into the fluorescence lifetime properties of commonly used RFPs, paving the way for informed selection of RFP probes tailored to the requirements of specific imaging experiments.

Subsequently, we extended our investigation to compare the performance of tagRFP against a novel StayGold fluorescent protein (FP), despite its established suitability for super resolution spinning disc laser scanning microscopy. Our comparative analysis revealed the superiority of StayGold over tagRFP in several key aspects.

Firstly, StayGold exhibited enhanced photostability compared to tagRFP, enabling prolonged imaging sessions with minimal photobleaching effects. This attribute is particularly advantageous for long-term live cell imaging studies where maintaining fluorescence signal intensity over extended periods is critical.

Secondly, StayGold demonstrated superior brightness and signal-to-noise ratio (SNR) when visualized under super resolution spinning disc laser scanning microscopy. The improved brightness of StayGold allowed for enhanced image clarity and resolution, enabling the detection of finer cellular structures and dynamics with greater precision.

Furthermore, StayGold exhibited favorable fluorescence lifetime properties, with a distinct fluorescence decay profile that distinguishes it from tagRFP. This unique characteristic not only facilitates multiplexing experiments but also provides additional flexibility in fluorescence imaging applications.
Moreover, we explored the functional capabilities of tagRFP and StayGold by tagging them to different actin markers. TagRFP was attached to Lifeact as an actin marker, while StayGold was either tagged to F-tractin or utrophin as an actin skeleton marker. Our results demonstrated the suitability of StayGold for visualizing actin structures with high specificity and resolution, surpassing the performance of tagRFP in this context.
In conclusion, while red fluorescent proteins (RFPs) are traditionally favored for imaging deeper tissue layers due to their longer wavelength, our study suggests that StayGold may emerge as the superior alternative, particularly for long-term live cell or organoid imaging using super resolution spinning disc laser scanning microscopy (LSM).
Despite the conventional advantage of RFPs in imaging deeper tissues, StayGold offers distinct advantages that make it a compelling choice for specific applications. Its enhanced photostability, superior brightness, and favorable fluorescence lifetime properties, as demonstrated in our comparative analysis, position StayGold as an ideal candidate for long-term imaging studies where sustained signal intensity and minimal photobleaching are critical factors.
Moreover, StayGold's ability to achieve high-resolution imaging of cellular structures, such as the actin cytoskeleton, coupled with its suitability for super resolution spinning disc LSM, further enhances its utility in dynamic imaging scenarios. The precise and specific labeling provided by StayGold, particularly when tagged to actin markers like F-tractin or utrophin, underscores its potential for visualizing intricate cellular processes with exceptional clarity and resolution.
While RFPs remain valuable tools for certain imaging applications, our findings suggest that StayGold may offer unique advantages in the context of long-term live cell or organoid imaging, especially when coupled with advanced microscopy techniques such as super resolution spinning disc LSM. Continued exploration and optimization of StayGold FP hold promise for further advancements in fluorescence microscopy and biomedical imaging, providing researchers with enhanced capabilities for studying complex biological systems with unprecedented detail and precision.



Determined fluorescence lifetime of the different fluorophore variants in HeLa cells. The FLT was measured with TCSPC FLIM. The FLT of live cells (Live), fixed cells embedded in TBS (TBS) and fixed cells embedded in Mowiol (Mowiol) was determined.

Keywords:

SR-SpinningDisc-LSM
FLIM
Red-and-Green-FPs

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Using super-resolution imaging to understand protein organization within Z-discs of striated muscle.

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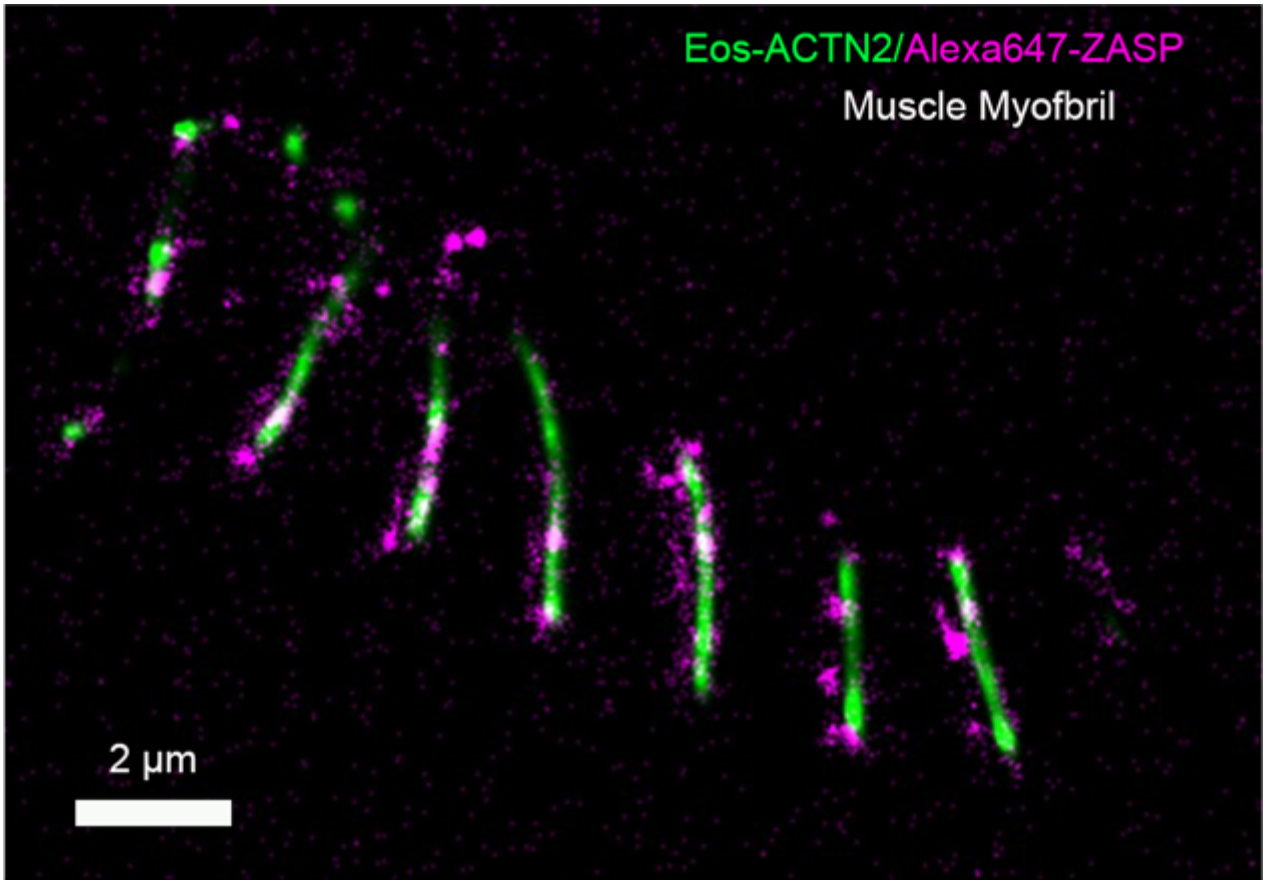
LS-03 (1), Lecture Theater 4, august 26, 2024, 15:00 - 16:00

The Z-disc is a complex structure found at the ends of muscle sarcomeres in striated muscle. It is approximately 100nm in width and comprises over 30 different proteins, which play key structural and signaling roles. However, we have little understanding into how these proteins are organized. Electron microscopy approaches have so far been unable to resolve the positions of most of these proteins. Light microscopy approaches are hampered by low resolution, failure of antibodies to penetrate the dense Z-disc structure (Parker et al., 2023), and the large size and flexibility of antibodies is not well suited to super-resolution fluorescence microscopy approaches. Affimers, are small (~10kDa, 3-4 nm in size) non-antibody binding proteins, developed at Leeds, that overcome the challenges of imaging the organization of proteins within the Z-disc (Cordell et al., 2022; Tiede et al., 2017). Their small size means that they penetrate the Z-disc better than antibodies and that they only introduce a small linkage error, as fluorescent dye molecules conjugated to Affimers are close to the epitope of interest.
Here, we have used Affimers to begin to determine protein organization within the Z-disc, using dSTORM. We isolated Affimers against at >10 different Z-disc protein epitopes, targeting titin, α-actinin-2 (ACTN2), ZASP (LIM domain-binding protein 3), myotilin, capping protein, and others. To achieve this, we expressed and purified domains using E.coli from each of these proteins, using a 'BAP' tag (biotinylation site) to ensure that the proteins are biotinylated as they are expressed, and a HIS tag for purification. Purified proteins were used in a phage display library to isolate binders, which were then subcloned, an unique C-terminal cysteine added for subsequent conjugation of the purified Affimers to fluorophores via a maleimide linkage, and the Affimers expressed and purified from E.coli (Tiede et al., 2017). Purified, dye labelled (e.g. Alexa 647 for dSTORM and iPALM) Affimers were used to label myofibrils isolated from mouse or pig hearts and imaged using an Abbelight dSTORM microscope. Alternatively, Affimers were fused to mEos3, expressed and purified and used in labelling. The resulting image datasets (xyz localisation) were analysed using PERPL to gain a better understanding of their organisation with Z-discs (Curd et al., 2015). We have now successfully tested these Affimers in STED, dSTORM and in iPALM, and the results reveal that Affimers penetrate the Z-disc much better than antibodies, and the overall organisation of specific Z-disc proteins with high (~15nm or better), X,Y and Z resolution.



Keywords:

dSTORM, Affimers, super-resolution imaging,

Reference:

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Nanoscale T-cell membrane protein imaging across complex topography using accelerated large depth-of-field localisation microscopy

Dr Aleks Ponjavic[1,2], Dr Alex Carr[3], Dr Edward Sanders[3], Dr Mafalda Santos[4], Dr Edward Jenkins[4], Dr James McColl[3], Prof David Klenerman[3], Prof Simon Davis[4], Prof Steven Lee[3]
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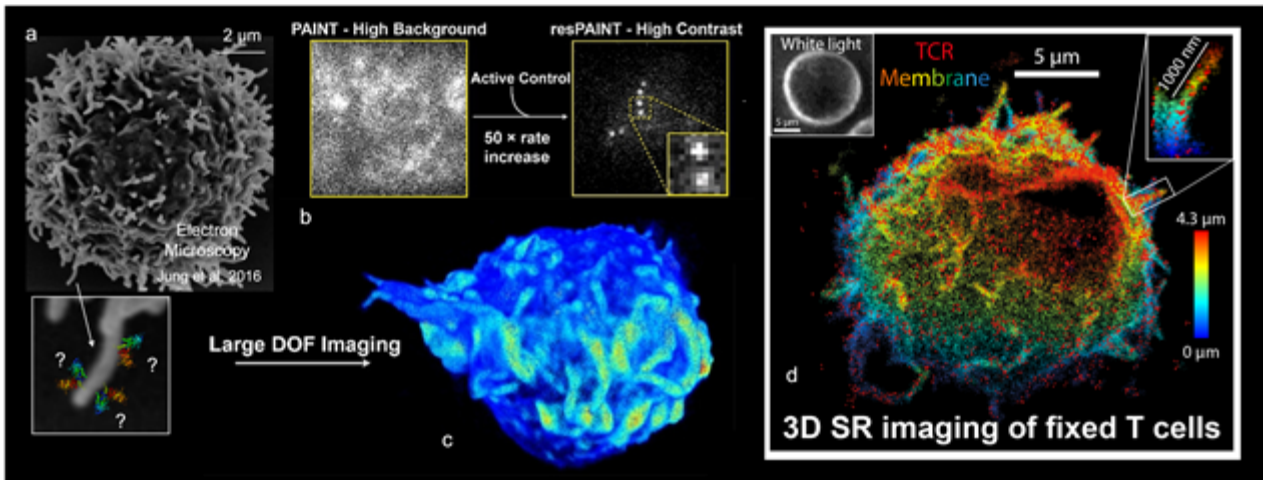
LS-03 (2), Lecture Theater 4, august 27, 2024, 10:30 - 12:30

Background incl. aims
The nanoscale topography of cells plays a key role in a variety of biological processes, particularly for T cells, where finger-like structures scan the environment to sense pathogens. Conventional imaging often considers individual protein distributions, which can result in perceived clustering due to the membrane curvature. Without considering topography, it becomes impossible to decipher protein distributions and therefore its role in biological processes. Imaging cell topography (Fig. 1a) with localisation microscopy in 3D is thus important, but challenging, due to the relatively large scales involved and the requirement to achieve sufficient localisation densities. Point accumulation for imaging in nanoscale topography (PAINT) can be used overcome these challenges, but is typically hampered by high background, particularly for large depth-of-field 3D imaging.

Methods
Here we use actively controlled probes to greatly increase the effective concentration in PAINT, without influencing the background. By building up a concentrated and reversible reservoir of photoactivatable or spontaneously blinking probes the localisation rate of PAINT can be greatly accelerated. We combine this method with double-helix point spread function engineering that enables large depth-of-field localisation microscopy, which we apply to image the cell membrane of fixed T cells. Finally, the membrane imaging is combined with conventional membrane protein localisation microscopy to characterise nanoscale T-cell protein distributions across the complex topography of the cell membrane.

Results
We demonstrate that our approach (resPAINT) can increase the localisation rate of PAINT up to 50-fold without increasing background (Fig. 1b), which is essential for achieving suitable localisation densities to study membrane topography. We then show that our approach is suitable for imaging topography as we apply it to volumetric super-resolution imaging of entire T-cell membranes (Fig. 1c). Finally, we achieve correlative membrane and protein imaging (Fig 1d) to resolve the distribution of key T-cell membrane proteins: the T-cell receptor, the phosphatase CD45 and the microvilli marker CD62. By considering the local distribution of neighbouring proteins and membrane topography, we demonstrate how eigenvector analysis can be used to decipher preferential organisation and clustering of membrane proteins.

Conclusion
We have developed a technique for performing correlative membrane topography and membrane protein imaging, which we show is key for understanding how proteins distribute at the nanoscale. This represents a new approach to understanding how proteins organise and redistribute to achieve important biological functions, such as the recognition of pathogens by the adaptive immune response.



Keywords:

Super-resolution, Single-molecule, Membrane proteins, Biophysics

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Two-photon line-scanning structured illumination microscopy (LIL-SIM) for super-resolution imaging in deep tissue

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LS-03 (2), Lecture Theater 4, august 27, 2024, 10:30 - 12:30

Background incl. aims
In the field of cellular imaging, the demand for sub-cellular resolution and three-dimensional (3D) visualization of biological specimens has been a driving force behind the development of various microscopy techniques. This study explores a novel approach of combining structured illumination microscopy (SIM)1,2 and two-photon line scanning (2P-LS) microscopy to provide super-resolution imaging in thick biological tissue. We apply our method, named LIL-SIM, to cardiovascular artery tissue and tree trunk to demonstrate the penetration capability of our imaging system.

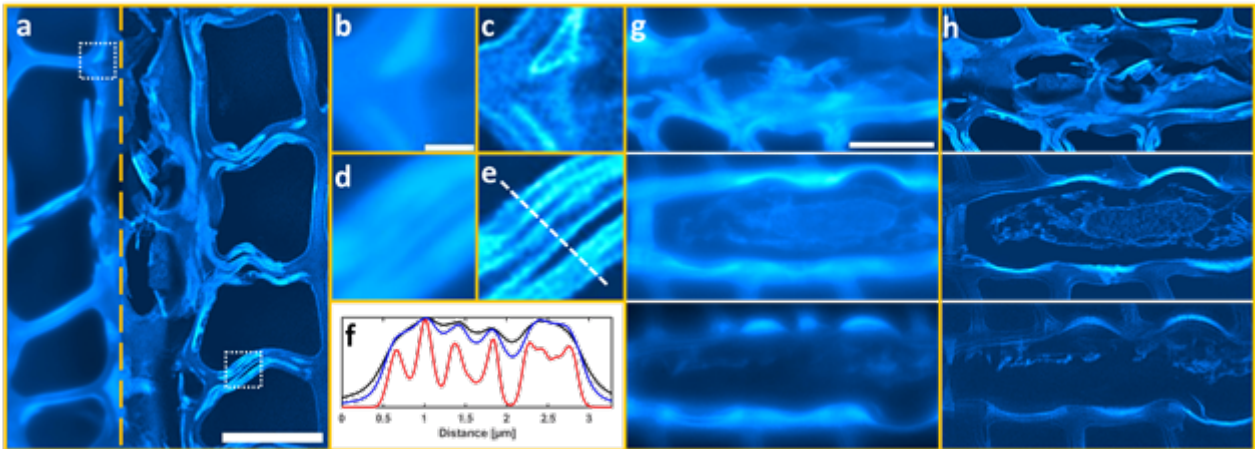
Methods
Structured Illumination Microscopy (SIM) is a well-established super-resolution method, breaking the diffraction limit by introducing a spatially modulated light pattern to the sample. However, SIM's performance traditionally decreases in highly scattering media typical of biological tissues. Conversely, Two-photon (2P) microscopy provides exceptional depth penetration due to its employment of near-infrared light, reducing scattering, and targeting excitation to the focal volume, thus limiting photodamage. Despite the advantages gained by combining 2P microscopy and SIM, the necessity for camera-based detection lowers the modulation contrast of the pattern in thick biological specimens and reduces the signal to background ratio (SBR) of the acquired SIM patterns. This leads to artefacts in the reconstructed images due to insufficient modulation contrast of the generated patterns. Interestingly, the SBR can be increased by confocalization of the emitted fluorescence emission, restricting out of focus contributions of scattered photons. This leads to increased SBR and high modulation depth of the generated SIM patterns in thick biological tissue, enabling volumetric super-resolution imaging.

Results
We validate the super-resolution capability of our microscope by imaging tissue samples composed of pine wood and tree trunk, with z-depths of around 40 µm. For all experiments, we generated SIM patterns with five phase shifts to acquire homogeneous illumination of the sample and three rotation angles for an isotropic resolution enhancement. This amounts to a total of 15 acquired images for one SIM frame. Averaging of the 15 acquired frames leads to a homogeneously illuminated intensity distribution, which we refer to as the 2P widefield image in the following. As seen in Fig. 1a), LIL-SIM increases the resolution compared to diffraction limited 2P widefield imaging while simultaneously obtaining better optical sectioning by line confocalization. The 2P widefield image is blurred by the contribution of excited fluorophores close to the focal plane due to scattering. By the application of LIL-SIM and following computation of the acquired images with fairSIM3 , the resolution is effectively doubled. LIL-SIM insets 1c) and e) show clear resolution improvement of nanostructures over the acquired 2P widefield structures 1b) and d). The line plot comparison in Fig. 1f) shows a FWHM distance of 384 nm in the 2P widefield image and 150 nm for 2P-LS-SIM.

Conclusion
Our results demonstrate that the fusion of SIM and 2P-LS microscopy leads to an imaging modality capable of resolving structures down to 150 nm in thick biological specimens. We provide data

analyzing imaging parameters, e.g. penetration depth, modulation depth of the illumination grating and phase stability of the illumination patterns and present reconstructed super-resolution images of thick tissue samples, e.g. cardiovascular artery and pine trunk.

Fig. 1 a) 2P widefield and LIL-SIM comparison. b-e) insets and f) line graph demonstrate resolution improvement. g-h) 2P widefield and LIL-SIM with increasing z-depth (10, 20 and 30 µm). Scale bars a) 10 µm, b) 2 µm, g) 5 µm.



Keywords:

Structured Illumination microscopy, Two-photon microscopy

Reference:

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A new approach for direct visualization of unlabeled lipid nanoparticles for intracellular pathway analysis

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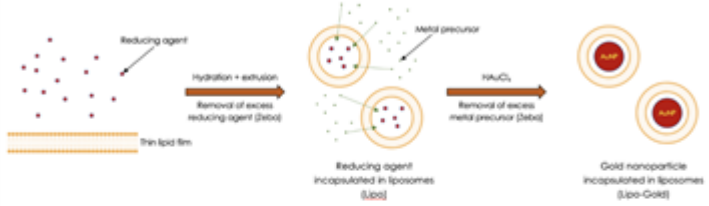
LS-03 (2), Lecture Theater 4, august 27, 2024, 10:30 - 12:30

Background incl. aims
Lipid Nanoparticles (LNPs) are the most clinically advanced delivery vector for both small molecules (liposomes) and genetic material payloads, such as siRNA or mRNA (ionizable cationic LNPs).[1] Their recent development has unlocked a wide range of therapeutic approaches, overcoming the limitations of naked genetic payloads and of previous delivery strategies.[2] This is testified by the fact that the majority of clinically approved nano-systems are of lipidic nature.[3] However, much is still unclear about the release of genetic material in the cytoplasm upon LNPs cell internalization and trafficking in the endo-lysosomal compartment. This process, known as endosomal escape, is a crucial step of the delivery process but to date is inefficient and often considered the bottleneck for the clinical efficacy of LNPs.[4] Most of the methods for detection and quantification of endosomal escape are either indirect assays, usually based on detection of endosome damage indicators, or rely on fluorescent labelling of LNPs. The latter, however, has proven to significantly alter the surface properties of nanoparticles and thus their biodistribution and cell interaction properties.[5] While the direct imaging of LNPs in biological samples is impossible given the nature and elemental composition of LNPs, which are practically identical to the composition of cells. To overcome these limitations, a new hybrid Liposomal-Gold nano-system (namely Lipo-Gold) has been here developed for the direct analysis of cellular interaction and intracellular trafficking of LNPs.
Methods
The Lipo-Gold have been synthetized by a modified thin lipid film hydration method: specifically, the lipid film composed by saturated phospholipid DPPC, cholesterol and PEGylated lipid DSPE-PEG (2000 Da), has been hydrated with an aqueous solution of biocompatible reducing agent, namely ascorbic acid (vitamin C). The vesicles encapsulating the reducing agent have been extruded to the desired dimension and a size-exclusion chromatography column has been employed for the quick removal of free ascorbic acid. Upon addition of metal precursor, HAuCl4, AuNPs spontaneously and quickly formed in the LNPs aqueous compartment. The formation of hybrid Lipid-Gold nanoparticles has been optimized and characterized by quantification of encapsulated ascorbic acid, UV/Vis spectroscopy, dynamic light scattering (DLS), nanoparticles tracking analysis (NTA) and electron microscopy techniques. Then, MTS cytotoxicity assay, flow cytometry, confocal and hyperspectral darkfield microscopy, as well as TEM, have been used to evaluate the cellular biocompatibility and cell interaction properties of the nanoparticles and to verify whether the hybrid Lipo-Gold system could be used as a model for the study of LNPs.
Results
The optimization of the methods resulted in formation of very monodispersed ~130nm LNPs encapsulating 15-35nm AuNPs. The resulting nano-system has proven to be highly biocompatible through MTS cytotoxicity assay after 24 or 48 hours even at very high concentrations. Unlabeled hybrid Lipid-Gold nanoparticles have been employed for the analysis of cellular internalization by TEM and hyperspectral darkfield microscopy, while fluorescent labeling with DiD dye (Ex/Em: 647/663nm) have been used to compare the properties of the hybrid system in comparison with empty LNPs. Both flow cytometry and confocal microscopy clearly showed that the cellular

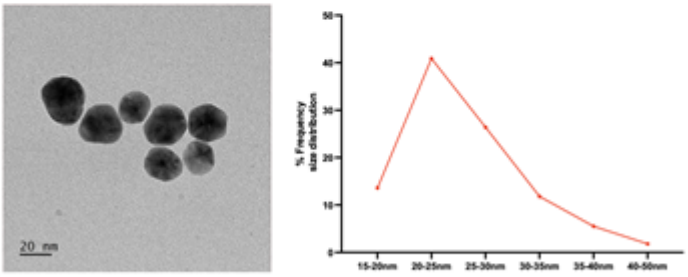
internalization and intracellular localization is not altered upon AuNPs synthesis as compared to empty LNPs, both quantitatively and qualitatively. This proves that the hybrid Lipid-Gold nano-system is a potential good model for the direct analysis of LNPs interaction with cells, taking advantage of AuNPs optical properties.

Conclusion

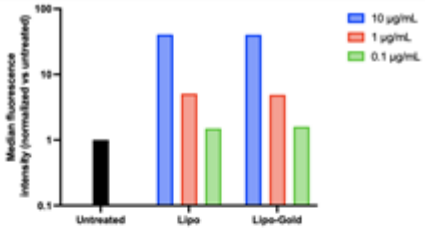
This work resulted in the development of a hybrid Lipo-Gold nano-system that showed promising results towards its employment as a reporter system for the visualization of LNPs cellular internalization and intracellular trafficking. This could enable further and easier optimization of Lipid Nanoparticle platform, enhancing the efficacy of the endosomal escape and thus the overall clinical relevance. Furthermore, Dynamic TEM measurements are ongoing on this system, with the aim of getting real-time visualization of the intracellular path of nanoparticles in response to an external optical/thermal trigger. The method will be developed in standard biological samples prepared for electron microscopy analysis (i.e. dehydrated cells included with epoxy resin), thus with a low biological relevance. However, the method would be a proof of concept that could then be employed in state-of-the-art, and more biologically relevant, techniques such as Cryo-TEM for the time-resolved, high-resolution analysis of intracellular trafficking of LNPs in biological samples.



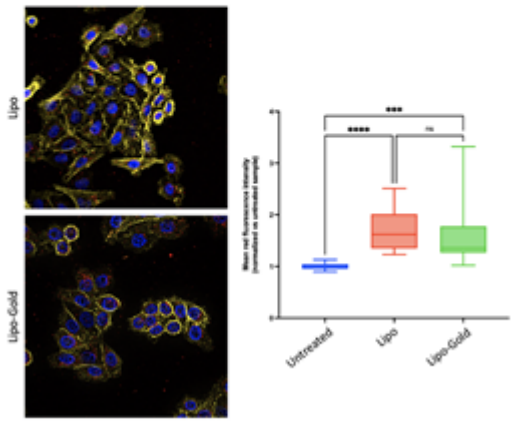
1. Schematic representation of the hybrid nano-system synthesis method



2. TEM image and size distribution of encapsulated AuNPs (n=100)



3. Flow cytometry analysis of HeLa cells incubated with DiD-labeled nanoparticles at increasing concentrations for 3h



4. Confocal microscopy analysis of HeLa cells incubated with nanoparticles 0.1 µg/mL for 1h

Keywords:

Lipid-Nanoparticles, Cellular internalization, Endosomal escape

Reference:

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The study of spatial relationship between Restrictor complex and RNA-Pol II through Expansion Microscopy

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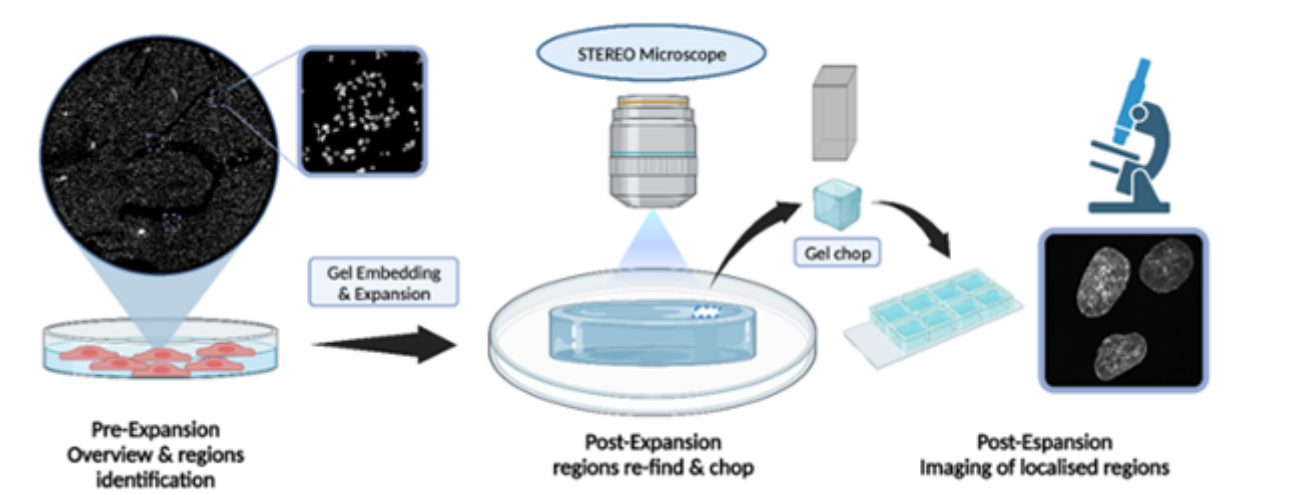
LS-03 (2), Lecture Theater 4, august 27, 2024, 10:30 - 12:30

Background incl. aims
In recent years, there have been continuous research efforts to optimize the experimental Expansion Microscopy (ExM) protocol for studying spatial correlation among different proteins. Our focus has been on developing an effective method to relocate the same cells acquired in pre-expansion onto expanded gels, enabling precise calculation of expansion and deformation factors. We then applied ExM and colocalization analysis to uncover the spatial relationship between the Restrictor complex and RNA-Pol II. The Restrictor complex, comprising ZC3H4, a putative RNA-binding protein, and WDR82, an adapter protein that interacts with RNA-Pol II Ser5-p, terminates extragenic transcription arising from active enhancers and promoters, thereby mitigating the pervasive capacity of transcription of the genome, which may lead to genomic instability (1). However, the exact molecular mechanism of action of the Restrictor complex remains poorly understood.

Methods
HCT-116 WT and HA-WDR82 HCT-116 cells were seeded on 35 mm MatTek dishes before being processed for indirect immunofluorescence. ZC3H4, WDR82, and RNA-Pol II Ser5-p or RNA-Pol II Ser2-p were fluorescently labeled. A scratch with no symmetry for rotation and reflection on adhered cells, together with a pre-expansion overview of the sample, were used to identify pre-expansion imaging areas. Samples were then processed for ExM according to (2). After expansion, the same areas were identified under a stereo microscope based on their relative position with respect to the scratch (Fig.1). Both pre- and post-expansion images were acquired using a Yokogawa spinning disk confocal system (CSU-W1, Nikon Europe B.V.). Colocalization was assessed using Pearson's coefficient calculation and object-based colocalization analysis.

Results
Expansion factor, deformation, fluorescence intensity decrease upon expansion, and both 2D and 3D colocalization were evaluated. Across three experiments, an expansion factor up to 6 and an average deformation factor of 9% were obtained. Overall, the colocalization results obtained with ExM revealed a stronger spatial correlation between the Restrictor complex and RNA-Pol II Ser5-p compared to RNA-Pol II Ser2-p, consistent with molecular, genomic, STocastical Optical Reconstruction microscopy (3) and Structured Illumination Microscopy observations previously obtained in our laboratory.

Conclusion
Our ExM imaging workflow efficiently facilitated the acquisition of identical areas both before and after expansion, thus providing essential control over the expansion protocol. Considering that the expansion factor directly influences the ultimate resolution of any ExM experiment, it is crucial to consistently account for the precise expansion factor of each sample to ensure fair comparisons among different samples. The colocalization analysis of ExM images further supported the concept that Restrictor-mediated transcription termination primarily affects initiating and early-elongating RNA-Pol II complexes.



Keywords:

ExM, colocalization, extragenic transcription

Reference:

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PHOSPHO1-mNeonGreen reporter cells are a robust model to study matrix vesicle biogenesis during osteoblast-driven mineralisation

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Poster Group 1

Background and aims: Bio-mineralisation is an essential process in which bone cells initiate the laying down of mineral within the extracellular matrix (ECM) to form an organic-inorganic composite material that is both tough and stiff to support the physiological demands of bone. The delicate balance of inorganic phosphate and pyrophosphate concentrations is a major determinant of the rate of ECM mineralisation. This process is under the control of tissue non-specific alkaline phosphatase (TNAP) and PHOSPHO1, key phosphatases found in a specific set of extracellular vesicles termed “matrix vesicles” which are postulated to drive mineralisation. The biogenesis of matrix vesicles remains unclear, in part due to lack of imaging options available for such small vesicles, and the delicate nature of calcium phosphate mineral. This study aims to develop a robust imaging system that will allow us to study the mechanisms underpinning matrix vesicle biogenesis, and the process of mineral deposition within the ECM.

Methods: Mineralisation was studied in the osteoblast cell line MC3T3. The calcium-binding compound, Alizarin red was used to confirm mineral deposition, and gene and protein expression assessed via qPCR and protein across the time course. Key matrix vesicle phosphatase PHOSPHO1 was tagged with mNeonGreen and live imaging performed with pseudo-super resolution (LSM880) and super resolution (ELYRA) microscopy in live and fixed cells. Reporter cell lines were generated using Fluorescence-activated Cell Sorting (FACS) with a Bigfoot Spectral Cell Sorter, and validated for matrix mineralisation capability and fusion protein expression. Organelle markers and immunofluorescence allowed the localisation of PHOSPHO1-mNeonGreen to be followed throughout the cell.

Results: PHOSPHO1-mNeonGreen expressing cells are a robust model for imaging the intracellular stages of matrix vesicle production and biogenesis by osteoblasts. Using fluorescent PHOSPHO1 as a marker, we have followed the trafficking pathway of MV precursors in the endoplasmic reticulum to the trans-Golgi network; a process involving RAB8 vesicles and driven by microtubules. This suggests a RAB GTPase-dependant method of intracellular vesicle packaging and subsequent MV secretion by the osteoblast. Furthermore, confocal imaging shows PHOSPHO1 positive matrix vesicles appear to be released at actin-rich sites at the osteoblast cell membrane, possibly linked to focal adhesion sites. Time-lapse confocal imaging of cells surrounding mineralised nodules produced in vitro shows small PHOSPHO1-mNeonGreen objects present and interacting at the mineral-cell membrane boundary, suggesting evidence of matrix vesicle activity. Preliminary evidence with a TNAP-mKate2+PHOSPHO1-mNeonGreen double reporter line shows co-localisation of both matrix vesicle markers on intracellular objects, with object size and localisation as supporting evidence for potentially an MVB driven method of MV release.

Conclusions: PHOSPHO1-mNeonGreen expressing osteoblasts are a valuable and robust model for studying the process of bone mineralisation in vitro and allow our first insights into the intracellular steps of matrix vesicle biogenesis.

Keywords:

Super-resolution microscopy, cell matrix mineralisation

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Microglial contactology–mapping the connection network of microglia from the micrometer to the nanometer range

Phd Anett Dóra Máté-Schwarcz[2], Dr. Csaba Cserép[1], Dr. Eszter Szabadits[1], Dr. Ádám Dénes[1]
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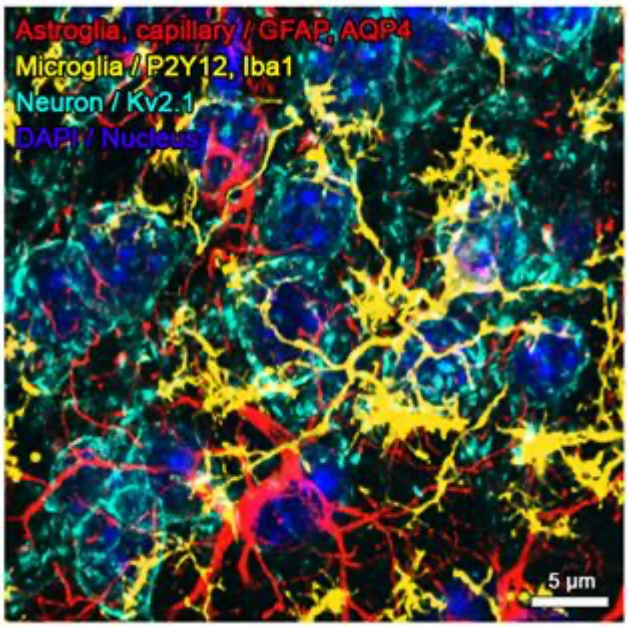
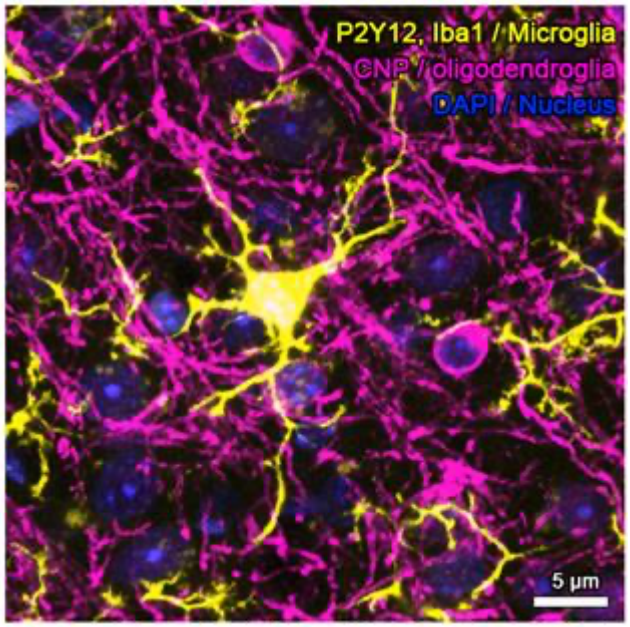
Poster Group 1

The dynamic development of microscopic techniques offers new opportunities for the comprehensive study of biological processes. However, the application of methods covering a wide resolution range requires the right questions to be asked and the definition of a precise target, without which it is difficult to obtain relevant results. In the following, I would like to demonstrate the application of microscopic modalities with different resolution capabilities in the field of neuroscience by highlighting examples from my own work.
Our research focuses on microglial cells which are the main immune cells of the brain. These cells are essential for the normal functioning of the brain and play an important role in maintaining brain homeostasis, proper development and function of neurons, the formation of their synaptic connections and the regulation of inflammatory processes. They also play a role in the control of cerebral blood flow. Carrying out these complex tasks requires continuous communication between the microglia and the other cells of the brain. However, our current understanding of the cell-cell interactions of microglia with neurons, other glial cells and blood vessel cells is rather incomplete.
We uses different microscopic methods, ranging from micrometre magnification to nanometre resolution, to reveal the connectivity of microglial cells. Starting from the lower resolution, we first investigated the location and heterogeneity of glial cells (microglia, astrocytes, oligodendrocytes) and neurons in the cerebral cortex using slide scanner microscope. With this method, we can obtain information on the distribution and quantity of the cells under investigation in a small but fast way for many samples. We then used a higher-resolution confocal laser scanning microscope to take a closer look at how many different cells a microglial cell interacts with at a given time point and how many different cells it interacts with. Then, to achieve nanometre resolution, we used scanning electron microscopy serial section tomography to verify that the putative contact sites visualised by diffraction-limited confocal microscopy are real direct contacts, where the plasma membranes of microglia and other cells are brought within nanometre proximity of each other. Furthermore, these images can be used to reveal the intracellular ultrastructure characteristic of the different contacts.
In order to study the dynamics and function of these connections, we use two-photon microscopy in vivo, in a departure from the mainly anatomical microscopy techniques used so far.Our studies will allow us to determine the lifetime, stability and signalling pathway involved in the connection of each contact.
By combining these different microscopic techniques, we can gain a complete view of both the anatomical structure and the functional role of microglial cells in their interactions with other cells. Our studies will be carried out in mouse, human adult and elderly samples, which will help us to understand the subcellular processes underlying ageing and related neurodegenerative neurodegenerative diseases such as Alzheimer's disease.
The project KDP-12-10/PALY-2022 was funded by the Ministry of Culture and Innovation with support from the National Research Development and Innovation Fund under the KDP-2021 grant scheme. The project was supported by the Richter Gedeon Talentum Foundation (1103 Budapest, Gyömrői u. 19-21.).





Keywords:

microglia, SEM, CLSM, somatic junction

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Physical determinants of YAP mechanotransduction in multicellular assemblies

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Poster Group 1

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Background incl. aims
Cells within tissues constantly receive biochemical and mechanical cues. Biochemical cues are evenly distributed across a monolayer due to the uniform spread of growth factors in the culture environment. Mechanical cues are highly localized, arising from cellular processes such as cell divisions, extrusions, and interactions with neighboring cells, involving pushing and pulling. These cues are sensed and interpreted by individual cells through a process called mechanotransduction. Depending on the input, cells make decisions that impact their micro-environment, creating a feedback loop regulating tissue homeostasis.1

The Hippo signaling pathway plays a crucial role in modulating cellular responses. Various upstream affectors have been shown to regulate it. One important constituent of the Hippo pathway is "Yes-associated protein 1" (YAP). When the Hippo pathway is on, YAP is phosphorylated and is permitted to enter the nucleus. When the Hippo pathway is off, YAP can shuttle into the nucleus and regulate gene transcription by interaction with DNA binding factors. 2

Nuclear compression, either independently or by disrupting the connection between the nucleoskeleton and the cytoskeleton, has been demonstrated to effectively regulate the localization of YAP. 3 YAP enters the cell nucleus through nuclear pores. The expansion of nuclear pores is linked to the shape of the nucleus. When the nucleus is elongated and stretched, the nuclear pores tend to widen. 4

The response of YAP to mechanical cues has been studied in the context of single cells or in multicellular contexts with high and low local density conditions. However, we found YAP expression and local cell density to be highly heterogeneous across the MDCK monolayer at the two seeding densities that we chose. Thus, we decided to characterize YAP activation across the MDCK monolayer in terms of local cell density.

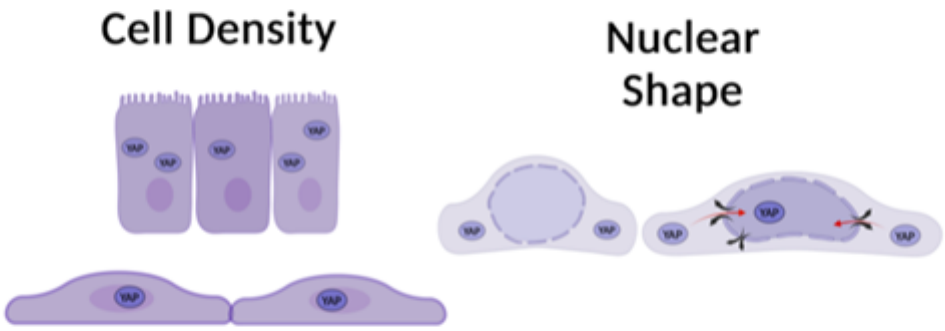
Methods
Madin-Darby canine kidney (MDCK) cells are cultured at a high and low global cell density. When a propper monolayer is formed, cells are fixed and immunostained with YAP antibodies and Hoechst. The samples are imaged using a spinning-disc confocal microscope. Z-stacks are taken to reconstruct the cells in 3D. The nuclear stacks are segmented using Cellpose for extraction of nuclear shape properties. The focal plane is segmented in 2D to calculate the local cell density and YAP nuclear/cytoplasmic (n/c) ratio.

By manipulating e-cadherin expression using an e-cadherin KO MDCK cell line as well as altering actomyosin cytoskeleton integrity with Latrunculin A and ROCK-mediated myosin contractility with Y-27632, we aim to untangle their individual contributions from local cell density on YAP activation.

Results
YAP activation is determined by local cell density. A clear relationship can be observed between the YAP nuclear to cytoplasmic (n/c) ratio and local cell density. On average, YAP activation steadily decreases with an increasing number of neighboring cells. In contrast to the single-cell scenario, where less spread cells have larger nuclear volumes, cells with tall and high nuclei are found at lower local cell densities. These trends are preserved when performing perturbation experiments, highlighting the importance of local cell density and nuclear shape in the context of YAP activation and potentially also other processes.

Conclusion
By manipulating e-cadherin expression using an e-cadherin KO MDCK cell line as well as altering actomyosin cytoskeleton integrity with Latrunculin A and ROCK-mediated myosin contractility with Y-27632, we aim to untangle their individual contributions from local cell density on YAP activation.

Theme: Mechanotransduction in multicellular assemblies
Subtheme: Power of microscopy and 2D/3D image analysis



Keywords:

Mechanotransduction, YAP, 2D, 3D segmentation

Reference:

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Examining dislocation and stacking faults within Hybrid MBE-Grown (110)-oriented RuO2 films

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Poster Group 1

Background

Thanks to its high electrical conductivity and exceptional thermal and chemical stability, rutile ruthenium dioxide (RuO2) has attracted significant attention across a range of applications in energy storage and microelectronics [1]. Research indicates that RuO2 behaves as a Dirac nodal line semi-metal with flat band surface states, and it theoretically demonstrates a novel crystal Hall effect [2], thus reasserting itself as a quantum material [3]. Particularly intriguing is the rutile phase of ruthenium dioxide (RuO2), which epitaxially oriented along the (110) direction on rutile titanium dioxide (TiO2) substrates, displays remarkable anisotropic behavior, possibly originating from a 2.2% tensile strain along the [1$\bar{1}$0] direction and a 4.9% compressive strain along the [001] direction. This anisotropy, stemming from the interaction between crystallographic orientations and epitaxial strain, provides fertile ground for both fundamental research and technological exploration [4,5].
Transmission electron microscopy (TEM) researchers to probe the atomic-scale structure and defects at interfaces with unprecedented precision. In this context, the interface between RuO2 and TiO2 in (110)-oriented films has garnered considerable attention.
In this work, we characterize (110)-oriented RuO2 films on TiO2 substrates using TEM techniques. Specifically, we focus on the detection and analysis of dislocations and stacking faults within the RuO2 film, as well as the variations in defect density along different in-plane directions.

Methods

An 11 nm Hybrid MBE-grown rutile film of RuO2 oriented along (110) on rutile TiO2 (110) substrate was used in this research. Transmission electron microscopy (TEM) samples were prepared using a Focused Ion Beam Scanning Electron Microscope (FIB-SEM) equipped with a Ga ion gun (Thermo FEI Helios 5 FIB). A tungsten (W) and carbon (C) film were deposited on the area of interest before FIB milling to protect the surface from ion damage. A double-corrected TFS Spectra Ultra HRTEM/STEM (operated at 300 keV) is used to acquire high annular angle dark field (HAADF) micrographs and Energy-dispersive X-ray spectroscopy (EDS).

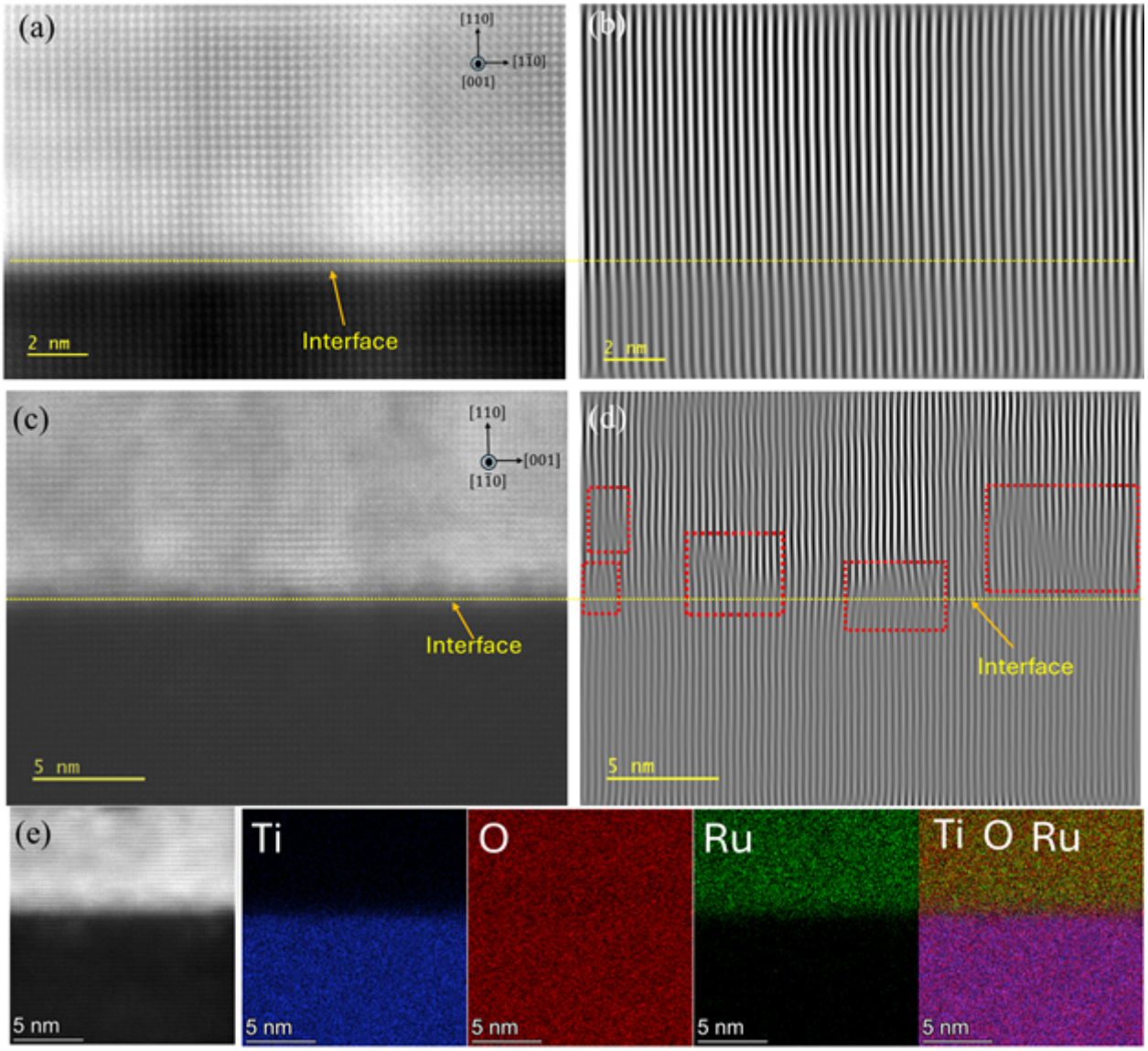
Results

Figure 1 displays High-Angle Annular Dark Field (HAADF) micrographs across the RuO2/TiO2(110) interface along [001] and [1$\bar{1}$0] zone axes. The accompanying EDS elemental maps in Fig. 1 revealed the presence of a Ru-oxide film on the surface of TiO2. Inverse FFT techniques were used to unveil defects during atomic-scale investigations. Interestingly, it can be observed from Fig. 1(b) and (c) that there are no defects at the RuO2/TiO2(110) interface along the [001] zone axis. However, various defects become apparent at the interface when changing the zone axis from [001] to [1$\bar{1}$0].
Severe lattice distortions were observed at the interface between RuO2/TiO2 (110) along [1$\bar{1}$0] zone axis. In the corresponding enlarged lattice, dislocation loop, stacking faults, and dense dislocation arrays were observed. All were caused by strain induced lattice distortion in [1$\bar{1}$0] direction. An exploration as the origin on these defects and their confinement to the tensile strain zone axis is underway.

Conclusion

Aberration-corrected scanning transmission electron microscopy (STEM) operating at 300 keV was employed to analyze the atomic-scale interface of RuO2 and TiO2 (110) along both the [001] and [1$\bar{1}$0] zone axes. Utilizing the inverse FFT method, various dislocations and defects were identified. Specifically, dislocation arrays and dislocation loops were observed within the RuO2 film along the [1$\bar{1}$0] zone axis. Furthermore, stacking faults were observed near the RuO2/TiO2 interface along the [1$\bar{1}$0] zone axis, potentially attributed to strain effects.

Acknowledgements
The authors would like to thank US AFOSR for financial support of this work, and the Canadian center for Electron Microscopy (CCEM) for their technical support.



Keywords:

STEM, RuO2, TiO2, Dislocations

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Ultrastructural and chemical analysis of human Locus coeruleus using correlative microscopy and mass spectrometry

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Poster Group 1

Background and aims: Among the characteristic hallmarks observed in the human brain during aging is the neuronal accumulation of neuromelanin (NM) pigment and iron in substantia nigra (SN) and locus coeruleus (LC), the two brain areas mainly targeted in Alzheimers and Parkinsons disease (PD). The neuromelanin pigment accumulates inside the catecholaminergic neurons of SN and LC and there particularly within specific organelles (with an average size of 1.5 µm), called neuromelanin organelles. Within these organelles, neuromelanin clusters with with lipid and protein-based partitions of less than 100 nm each
Several spectroscopic studies investigated the role of neuromelanin pigments and metal dyshomeostasis in the SN during aging [1] and a found a correlation of both with disease progression. However, conducting chemical analysis on LC proves to be challenging due to the small size of the tissue. It would necessitate a substantial number of specimens which are difficult to collect. Moreover, only few studies attempted high lateral resolution subcellular (re)distribution analyses of iron storage in brain tissue, e.g. for SN [2] in order to find out where exactly the metal is stored on a subcellular basis.
Therefore, the aim of this work is to develop a workflow to study the ultrastructural metal distributions in NM organelles of LC on individual human tissue sections using novel high resolution analytical approaches based on secondary ion mass spectrometry (SIMS) performed on Focused Ion Beam instruments (FIB-SIMS)..o Chemical analysis were corroborated by CLEM (Abbrev.) to correlate the finding with the cellular and molecular changes that take place in relation to PD.
Methods: A total of 11 human LC tissues were collected from elderly (n=9) and PD (n=2) specimen (60-80 years old) and were either formalin fixed paraffin embedded (FFPE), Epon-embedded or just as fresh frozen. Tissue sections were investigated by light, fluorescence and electron microscopy for high resolution imaging and immunohistochemistry, respectively. The standard chemical analysis was performed by analytical electron microscopy and mass spectrometric imaging (MSI), using the CAMECA NanoSIMS 50 with the ability for isotopic identification and highest sensitivity and mass resolution and a lateral resolution down to 50-100 nm. Sub organellar distribution of metals in NM organelles was addressed on selected samples using FIB-SIMS with lateral resolution < 20 nm for SIMS and < 1nm for secondary electron (SE) imaging developed at LIST. A combined “TEM like” ultrastructural investigation together with SIMS is possible on the so-called npSCOPE, an in house novel cryoFIB-SIMS platform with SE, SIMS and scanning transmission ion microscopy (STIM) detectors [2]. Taking the advantage of the cryo-chamber of the npSCOPE, it can also be used to

perform respective analyses on frozen-hydrated brain samples and thereby minimizing preparation artefacts.
Results: A semi-quantitative SIMS approach was implemented here where a single tissue slice was imaged first using CLEM to identify the region of interest in LC tissues of both elderly control and PD subjects. Quantitative EDX maps and qualitative SIMS maps were acquired for regions of interest. Counts/ pixel ratios were calculated to get a semi-quantitative data set that shows different signal abundances for SIMS. Respective data on healthy elderly specimen showed that the melanic moiety of the NM organelle is composed by a mixture of eumelanin/ pheomelanin units with a sulfur/nitrogen ratio of (XXX), as identified by the sulphur signal which derives from the benzothiazines of the pheomelanin part of the NM. NM organells showed increased signal for iron, calcium, and aluminium, in comparison to the lipid moiety of the NM organelles or the cytoplasmic surrounding areas.
Interestingly, copper and zinc signal were at or below the detection limit, which is totally contrast to the isolated NM analysed using the Electron Paramagnetic resonance spectroscopy (EPR) that showed an actual accumulation of these two elements [1].
The higher spatial resolution of LIST's FIB-SIMS instrument in comparison to the nano-SIMS could more precisely identify the localization of these metals and non-metals in different sub-organellar compartments of the NM organelle.
In addition, PO2 signal which is an indicator of phosphorus rich areas like myelin sheath, nucleus and lipids was also week in the lipid moiety and lipid bodies of the NM organelle, which might strongly agree with the fact that the lipid portion of NM is mainly composed of dolichols, and this is to be proved by lipidomic profiling. Another method of molecular profiling is done by IHC, in order to identify the differential expression of the markers related to inflammation, PD and iron storage proteins, and in order to identify the regions that showed, by our elemental analysis, to be rich in iron but are not a NM organelle, who could be glial cells or ferritin rich areas.

Conclusion: We analysed the sub-cellular localization of certain marker elements for subcellular structures (PO for myelin), NM organelle compartments (based on Cl, S, CN, O signal distribution) in addition to the metal loading into these compartments in elderly controls and PD patients using routine and novel high-resolution chemical and structural imaging techniques in the NM in addition to its molecular proteomic and lipidomic profiling. Continued work will be done to identify more the significant differences in LC tissues of PD patients compared to healthy controls at the molecular and elemental levels, with investigations on fresh frozen samples for a better close to native analysis of PD pathophysiology also including molecular MSI,also done at high resolution, like MALDI and TOF-SIMS.

Keywords:

Correlative electron microscopy, SIMS, Parkinson

Reference:

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1023

FRET and TIRF microscopy for single molecule characterisation of synergistic antimicrobial peptides in artificial bilayers

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Poster Group 1

Background incl. aims
Droplet Interface Bilayers (DIBs) function as artificial membrane models for electrical and optical measurements [1]. This project uses total internal reflection fluorescence (TIRF) microscopy and Förster Resonance Energy Transfer (FRET) in DIBs to investigate the dynamics of peptides and proteins interacting with membranes. Membrane pores have vital roles in neuronal and cardiac signalling, as well as in the mechanism of certain antibiotics.
The lytic antimicrobial peptides Magainin 2 (Mag2) and PGLa, part of the immune system of the African clawed frog (Xenopus laevis), have a synergistic antimicrobial effect when applied to Gram-negative bacteria in a 1:1 ratio. The aim of this project is to uncover the mechanism behind this synergy at the membrane level.
Methods
When an agarose surface and a droplet are submerged in a lipid/oil environment, a monolayer spontaneously self-assembles at the aqueous-oil interface. DIBs form when the lipid monolayers make contact. The protein or peptide of interest, contained within the droplet, can then form pores in this bilayer. Ions flux through these pores, and this pico-ampere current can be measured by electrophysiology. Simultaneously, TIRF microscopy is used to observe ion flux by illuminating the fluorescent calcium binding dye, Fluo-8, with totally internally reflected laser light.
Alternatively, the single-molecule dynamics of fluorescently tagged peptides on the membrane surface can be investigated. By tagging PGLa with a donor fluorophore and Mag2 with an acceptor fluorophore, single molecule FRET with TIRF microscopy can act as a so called "molecular ruler"[3]. When the donor fluorophore is being excited and is less than 10nm from the acceptor fluorophore, a non-radiative energy transfer can occur from the donor to the acceptor, causing the acceptor to fluoresce. This offers distance-dependent insights into the interaction and dynamics of Mag2-PGLa. The formation of heterodimers and higher order structures is of particular interest in these experiments.
Results
Spatiotemporal data from electrophysiology and TIRF experiments has revealed that the Mag2-PGLa pore is a highly dynamic pore that visits multiple conductance states. Single molecule FRET data of Mag2 and PGLa has been obtained for the first time, confirming the existence of Mag2-PGLa heterodimers. Data suggests that very few Mag2-PGLa heterodimers exist at any time. These experiments have allowed better understanding of the interaction of the peptides with one another and with the membrane, thus shining a light on the mechanism behind the Mag2-PGLa synergy.
Conclusion
DIBs, TIRF microscopy, and single-molecule FRET have been employed to investigate the dynamics of Mag2 and PGLa interacting with one another and the membrane. Experimental results reveal the highly dynamic nature of the Mag2-PGLa pores and confirm the existence of Mag2-PGLa heterodimers. These findings provide valuable insight into the molecular mechanism driving the Mag2-PGLa synergy. Future work will focus on achieving pseudo-simultaneous pore-imaging, electrophysiology, and FRET of Mag2 and PGLa.

Keywords:

TIRF FRET AMPs DIBs

Reference:

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1025

A novel pipeline to elucidate the adaptation response to the G2/M DNA damage checkpoint

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Poster Group 1

Background

The DNA damage checkpoint (DDC) is a surveillance mechanism evolved to preserve genome integrity in response to DNA lesions. Checkpoints halt cell cycle progression to provide cells with the opportunity to repair the damage before dividing. When the DNA damage is successfully repaired, the checkpoint is satisfied and cells can resume the division in a process called checkpoint recovery. Conversely, when the DNA damage is irreparable, cells eventually die. Possibly, a few cells reenter the cell cycle with damaged DNA. This process, known as adaptation to the DDC, poses a threat to genomic stability, as daughter cells can accumulate genomic aberrations. Indeed, evidence exists that adaptation contributes to genomic instability and may drive the selection of therapy-resistant cells in oncology patients. Despite the dangerous nature and the clinical relevance of the process, adaptation is largely overlooked and has not been investigated in depth, likely because of the difficulty in experimentally tackling the question. Most commonly, adaptation is explored either by assessing the phosphorylation status of DNA damage checkpoint proteins, detectable solely by Western blot analysis on bulk population. Since adaptation is an asynchronous process (cells undergo adaptation with their own kinetics) and partial (only a fraction of the population adapts), we set to investigate adaptation in single cells by live-cell imaging measuring cell cycle parameters, with the ultimate goal of enhancing the reproducibility and reliability of adaptation studies.

Methods

We investigated adaptation to the G2/M DNA damage checkpoint in Saccharomyces cerevisiae, a highly amenable microorganism, whose genome is easily manipulated. Since in yeast checkpoint activation blocks cells in metaphase by preventing sister chromatid separation and segregation, via inhibition of cohesin cleavage and spindle elongation, respectively, these two processes can be considered the cell cycle events defining adaptation. To follow cohesin and spindle elongation, the Scc1 subunit of the cohesin complex was tagged with GFP (SCC1-yEGFP) while alpha-tubulin was tagged with the mCherry protein (mCherry-TUB1). In our experimental setup, cells are synchronized in G1 and released in presence of unrepairable DNA damage in a microfluidic chamber for live imaging where cells are immobilized and continuously supplemented with fresh media. Images are taken every 7.5 minutes for 20 hours. To analyze the resulting movies, we developed a Python script to automatically segment and measure the mitotic spindle for each cell at each timeframe, as well as to measure the SCC1 signal intensity. Anaphase onset (i.e., adaptation) is defined as the first timepoint where either reduction of the SCC1 signal or spindle elongation was observed. We applied our script to all imaged cells, gaining information about the timing of adaptation for each cell, and the overall percentage of adapting cells in the population.

Results

As a proof of concept, we analyzed wild-type (WT) cells (i.e. adaptation-proficient), as well as cells carrying mutations known to result in an adaptation-defective phenotype, including the well-characterized cdc5-ad mutant. In WT cells, we were able to discriminate adapting cells from arrested ones in the heterogeneous population, while in cdc5-ad mutant cells, we detected only metaphase arrested cells, as expected for an adaptation defective mutant. Interestingly, certain mutants previously classified as adaptation-defective in the literature were discovered to be capable of

adaptation but unable to progress through subsequent stages of the cell cycle. These data, alongside highlighting the reliability of our approach in discriminating checkpoint-arrested cells from those that escaped the checkpoint surveillance in the whole population, strongly support the idea that assessing cell cycle progression is key to understanding the molecular bases of adaptation.

Conclusion
Given the limits associated with both the experimental procedures in use and more generally with population-based experiments, we propose to investigate adaptation by live-cell imaging monitoring cell cycle parameters. Taken together, by coupling the power of live-cell imaging, which allows to investigate dynamic processes at a single-cell level, with the power of cell cycle parameters, which provide a clear picture of cellular behaviors, could provide direct insights into the molecular processes underlying adaptation and survival in cells that divide with damaged DNA, thus contributing to making the study of adaptation reproducible and reliable.

Keywords:

live imaging, DNA damage

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Novel nanobody-based tools for studying the synaptic vesicle life cycle

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Poster Group 1

Synaptic vesicles are important organelles in neurotransmission and their precise composition is essential for information transfer from neuron to neuron. While many aspects about their functions at the synapse are known, open questions remain regarding how these stoichiometrically highly-precise organelles are formed and maintained to mature to functional SVs that are able to release neurotransmitters at the synapse. To accurately study SV biogenesis, it is important to maintain the highly-precise stoichiometry of SV components in place and untouched. This renders experiments involving overexpression of a particular SV protein of difficult interpretation and instead requires endogenous expression.
One powerful approach to access endogenous localization, distribution and movement of proteins are intrabodies: small probes based on high-affinity nanobodies that can be expressed inside the cell. Here, I present the characterization of an intrabody against the SV Ca2+-sensor Synaptotagmin-1 (Syt1), that was named iNbSyt1. As shown in our recently published paper (https://doi.org/10.1002/smtd.202300218), this intrabody enables not only the direct live imaging of SVs (mScarlet-/mNeonGreen-iNbSyt1), but also allows to detect single action potentials thanks to a highly sensitive synaptically localized Ca2+-sensor, jGCaMP8s-iNbSyt1. Meanwhile, expression of iNbSyt1 does not affect vesicle mobility, synaptic localization, or fusion capacity, which makes this highly modifiable tool optimal to study synaptic processes in living neurons without genetic perturbation of the SV protein target. Likewise, we show that the underlying nanobody also is an ideal probe for several super-resolution imaging techniques, including STED microscopy, Structured Illumination Microscopy (SIM), DNA-PAINT and Expansion Microscopy.
Overall, the NbSyt1 is a versatile small imaging probe that offers adaptation to divers experimental requirements and will find a broad use in the microscopy field.

Keywords:

neuroscience, nanobody, synaptic vesicle, live-imaging

Reference:

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Time ecology method

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Poster Group 1

"[One] cannot solve a problem with the same level of thinking that created it" said Professor Albert Einstein. A new modeling [1] developed as 3 layers 4:3 dimensions frames may contribute to understanding how macromolecular complexes ultrastructure can influence cell physiology, and the reverse. As those 3 frames, 4D:OLI:LOI, connect to the concept of time and the dynamic perception of the flows in time ecology [2].
Are labeled [3] microorganisms oscillatory responses healthy or would those entrain inflammatory loop cascades? Is it fundamental, to gain an understanding of live cells, those forming living systems, to account for time and sound?
Background incl. aims.
The LOI (Location-Ownership-Internalization) dynamic conceptual positioning, enveloped in OLI (in existence since 1977), a static envelop, and the 4 structuring environmental dimensions, the chemotaxis motives of the environmentally active actor, as the: market, resources, efficiency (in existence since 1974) and strategic asset: associated with the notion of the time internalization, are various theoretical tools to conceptualize about time, timing, and transitions inside living systems. Internalization is a synonym for assimilation, for instance to quantify the assimilation of radioactive labeled or stable-isotope-labeled substrate (in single cell) to study the cell ecophysiology [3]. Combining LOI:OLI:4D frames together form a wider system view, as if several foreign languages would be simultaneously used to characterize one unique situation. This approach could be more specifically used to distinguish between macroscopic and microscopic entropy, to develop inferential statistics (see Results).
One purpose would be to conceptualize the formation of sound matter, based on a theoretical understanding of past literatures [4] to close a gap between cognitive psychology and the perception of sound as music.
Methods.
Imaging of microorganisms can be performed using the expansion microscopy to visualize intra- and extra-cellular components as well as the cellular ultrastructural context (the environment). Based on the tridimensional thinking described and the understanding of HOH molecules configurations dynamics [1] an understanding of time ownership could be formed from a multidimensional spectral sensing of 3 dimensions: hearing (sound goes faster than neural transmission speed. [2]), color vision and olfaction (most odorants do not contain nitrogen). The research could also focus on linear thinking, or one single ribbon, with the focus on the role of neuropeptides (one CKK, -4 or -8S), and NO; neuropeptides as signaling molecules, e.g. bombesin like peptides, can modify nerve impulse. CCK may have a universally conserved role in cognition depending on how significant lipids are in individuals' diet. I could defend the relevance of odd-chain fatty acids for the stability of thinking (gluconeogenesis) about complex stressful situations.
Time to be owned must be internalized. An envelope is thus necessary as a rigid frame to enable the dynamics to become dense enough to be observed.
The logic for dynamics to emerge is: first a location (minimal viable cavitation) will be required for resources to be gathered, which may become internalized or future resources would first be owned, before to become further internalized.
Results.

Accounting for time in research would imply to reveal time soft matter, beyond time measurement, dynamics. Or unpacking signals from noise using gentle ultraweak-photon emission intensities as non-invasive [3] spectroscopic tool for diagnosis of internal states.
Going back to the Viennese physicist Ludvig Boltzmann's aim to discover one objective interpretation of the law of increase of entropy in terms of microscopic mechanical properties of the system, the LOI [1] model of time internalization could be applied to understanding thermodynamics: hypotheses could be formulated regarding variables causing structural or transactional flows failure. The flow is driven by the hopping from electrons: a quantum-mechanical tunneling. Hopping is a long-range transfer across redox chains (oxidized radicals) using multi-step tunnelling. The process is required in several natural enzymes, it involves vibrational mode from hydrogen tunneling, producing volatile organic compounds.
Conclusions.
"Thermodynamic quantities should be assigned, not to single systems, but to ensembles of systems having a given probability to occupy this or that point in […] space." [5]. The LOI [1] dynamics based on the dipole moment of HOH mirror important biological models. Those would be forming, or contributing to form, the 'awareness' or 'consciousness' factor of human cognition, or a 'Zeitgeist'. The "chance factors" forms the major influence from the surrounding. It is important to account for the constant novelty provided by one surrounding. These can be observed through taking a standard view (proposed here). A system view is a simultaneously joint exploration of dynamics and the intersections: the fluids inside their enveloping structures. A system view can be systematically developed around 3 dimensions, as the dynamic framework: LOI, which is also embedded inside a static 3 dimensional frame: OLI.

Keywords:

Time ecology. Time internalization. Ecophysiology.

Reference:

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Interactions between Titanium Dioxide Particles and Wood Cell Wall Ultrastructurea

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Poster Group 1

TiO_2 is known for its photoactivity and chemically inert behavior. It is widely used in many applications as a pigment or as a UV light absorber. We were interested in what happen when the particle of TiO_2 is in contact with the wood matter and irradiated by UV light. For the experiments, two wood species were chosen: beech (Fagus sylvatica) and pine (Pinus sylvestris). Molecular and physical modifications in coated and uncoated wood exposed to UV radiation were investigated with Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR) and transmission electron microscopy (TEM). UV-VIS spectroscopy was used to describe the absorption of UV light by the TiO_2 planar particles chosen for the experiment. It was demonstrated that TiO_2 coating protects wood against photodegradation to a limited extent. TEM micrographs showed fissures in the wood matter around clusters of TiO_2 particles in beech wood.

Keywords:

TiO_2, TEM, UV-VIS, FTIR-ATR

Reference:

Ref. Svorová Pawelkowicz, S. et al.(2022) Nanomaterials.12,2678.

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Understanding and modifying cell wall permeability to facilitate cellular nanoparticle uptake

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Poster Group 1

Background incl. aims
The green transition of agriculture requires an increased crop productivity accompanied by a low environmental impact. However, poor nutrient use efficiency (NUE) and the environmental footprint of conventional fertilizers hamper this process.
The advances within nano-biotechnology have paved the way for the development of next-generation nanofertilizers. Foliar application of nanofertilizers allows farmers to bypass the soil matrix, where a large fraction of nutrients becomes unavailable to the plant due to chemical fixation, microbial immobilization, leaching to water bodies or volatilized to the atmosphere. Moreover, these nanofertilizers can be tailored with a range of smart properties, which enable them to deliver nutrients more efficiently to the plant.
In order to increase the efficiency of foliar nanofertilization, fundamental understanding of how these nanoparticles (NPs) interact with plant tissue is essential. After foliar application, NPs can cross the leaf surface through stomata to reach the apoplast. Here, NPs must remain intact to deliver their nutrient cargo inside mesophyll cells or travel through the phloem towards developing plant tissues. Further cellular internalization of NPs requires passage through the cell wall (CW) and plasma membrane. The CW has a size exclusion limit of 10-20 nm and, thus, it represents a major barrier for NP cellular uptake.
Zwitterions have been shown to loosen the tight cell wall networks of plant tissue in a biocompatible manner. Zwitterions destabilize hydrogen bonds in the cellulose matrix of CW's and thereby enhancing its permeability.
We hypothesize that CW porosity is the major barrier for NP uptake at the single cell level. Therefore, our aim is to utilize zwitterions to enhance CW porosity and facilitate internalization of polyacrylic acid coated manganese oxide (PAA-MnO) NPs.
In this innovative approach, the impact of zwitterions on NPs uptake is investigated in single-cell cultures with a combination of Confocal Laser Scanning Microscopy (CLSM), Transmission Electron Microscopy (TEM) and Laser Ablation Inductively Coupled Plasma Mass Spectometry (LA-ICP-MS).

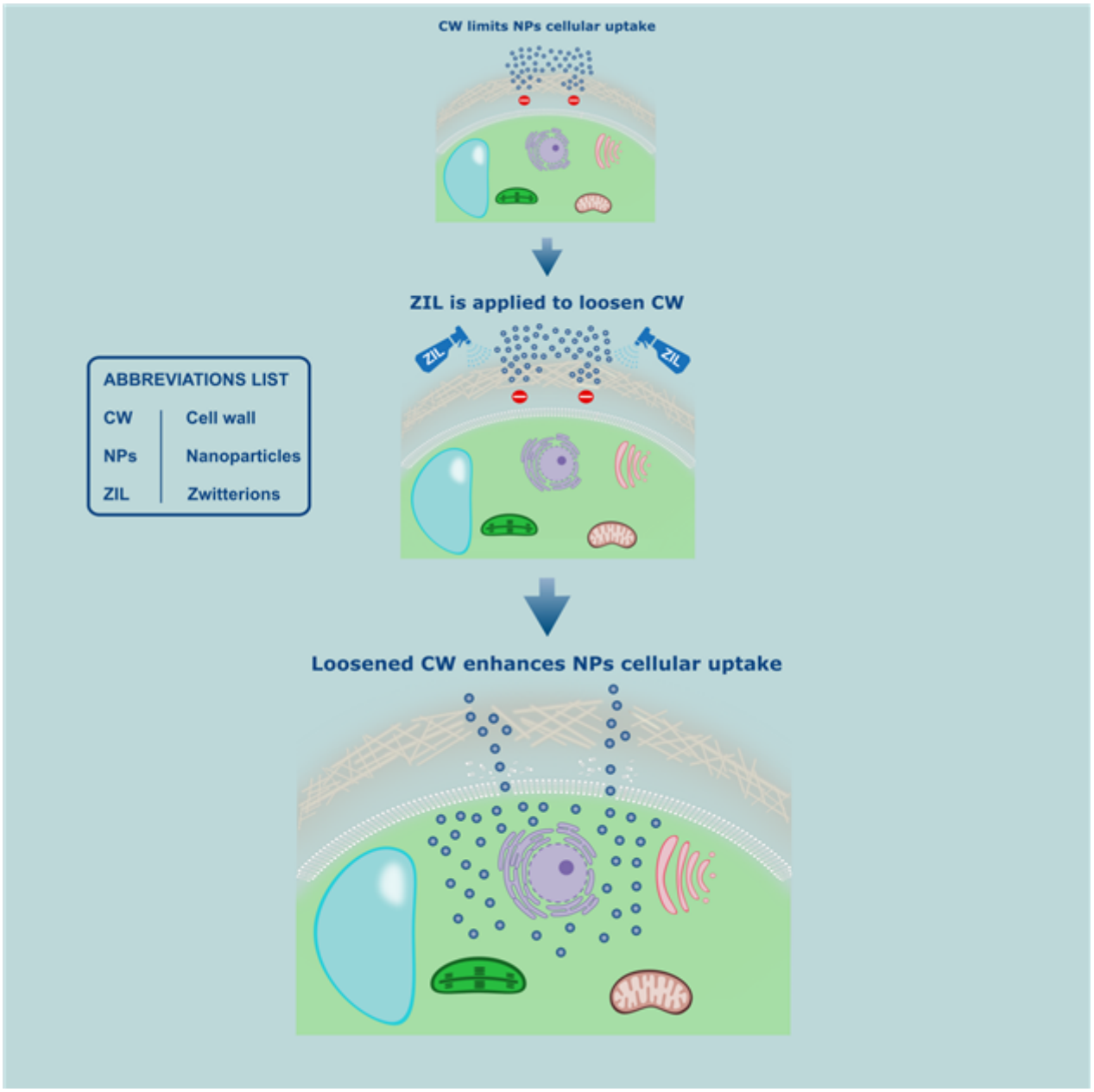
Methods
For the single-cell experiments, Tobacco BY-2 cells (Nicotiana Tabacum cv. Bright Yellow 2) were used.
PAA-MnO NPs were synthesized according to an established protocol and labeled with Dil dye. PAA-MnO NPs exhibited a hydrodynamic dyameter of ~ 20 nm and a zeta potential of -20 mv.
BY-2 cells were either incubated with only PAA-MnO NPs or pre-incubated (30 minutes) with PAA-MnO NPs and then treated with zwitterions. Cells were imaged with CLSM after several time points of interest. The same experimental setup is repeated to image the interaction between BY-2 cells and Ce spiked PAA-MnO NPs, using TEM and LA-ICP-MS.

Results
BY-2 cells treated only with PAA-MnO NPs for 3 hours exhibited a pronounced fluorescent signal around the cell edges. Additionally, a faint fluorescent signal was also visible within the cells, with a halo-like pattern surrounding the cell nucleus. The intensity of the signal inside the cell increased when cells were imaged after 4 hours of incubation with PAA-MnO NPs. Interestingly, BY-2 cells

incubated with a combination of PAA-MnO NPs and zwitterions for 3 hours were characterized by a strong fluorescent signal inside the cell, localized in the cytosol, around the cell nucleus and tracing actin filaments. A mild fluorescent signal was also observed around the cell perimeters. The intensity of the fluorescent signal inside the cell increased when the incubation time was prolonged to 4 hours.

Conclusions

Our findings suggest that the CW is a significant barrier to NPs internalization as it limits the uptake of 20 nm spherical NPs, while zwitterions facilitate NPs uptake in BY-2 cells. The mechanism of plasma membrane penetration is still unclear and warrants for further investigation. Furthermore, CLSM, TEM and LA-ICP-MS are powerful techniques to study the processes of NP internalization at the single cell level.



Keywords:

Nanofertilizers, single-cells, zwitterions, cell-wall, bioimaging.

Reference:

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Label-free evaluation of apoptotic cell death in pancreatic cancer cells using stimulated Raman scattering microscopy

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Poster Group 1

Anti-cancer drug treatment has been a standard approach for managing aggressive human pancreatic cancer. Effective, label-free monitoring of cell death is crucial for assessing drug efficacy and elucidating the mechanism of apoptosis. In this study, we utilized stimulated Raman scattering (SRS) microscopy to evaluate individual pancreatic cancer cells treated with a newly synthesized hydroxyl group-introduced chalcone derivative and the commercially available gemcitabine. SRS microscopy, by detecting inelastic vibration scattering of biomolecules, provided high-resolution imaging data revealing morphological and compositional alterations during apoptotic cell death. SRS imaging identified distinctive apoptotic features, including cellular shrinkage and membrane blebbing, without the need for staining. Notably, treatment with hydroxyl group-introduced chalcone derivative induced more pronounced structural and biomolecular changes, such as increased lipid droplets and decreased protein levels, compared to gemcitabine during apoptotic cell death.

Keywords:

Pancreatic cancer, Apoptosis, SRS microscopy

Reference:

S. Baek et al., A novel chalcone derivative exerts anticancer effects by promoting apoptotic cell death of human pancreatic cancer cells, Bioorganic & Medicinal Chemistry (2023) 93, 117458.

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Evaluation of pH-sensitive drug delivery systems for lung cancer therapy via multiplex fluorescence microscopy

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Poster Group 1

Non-small cell lung cancer (NSCLC) is characterized by the presence of tumor-associated macrophages that can polarize into a tumor-promoting phenotype, along with elevated levels of folate receptor beta (FRβ). Additionally, calpain-2 (CAPN2) is widely expressed in NSCLC and contributes to tumor progression. To enhance anticancer treatment efficacy and minimize side effects, developing innovative drug delivery systems with precise targeting and controlled release is essential. Here, we employed multiplex fluorescence microscopy to demonstrate that pH-sensitive, FRβ-targeted liposome-based nanoparticle drug delivery systems facilitate precise targeting and acid-responsive release in NSCLC. Our findings reveal that folate-mediated targeting of FRβ in tumor-promoting macrophages and NSCLC cells significantly suppresses tumor growth, while the stimulus-responsive release mechanism reduces drug-related toxicity. Furthermore, multiplex fluorescence imaging reveals that the combined use of docetaxel (an anticancer agent) and doxycycline (an anti-CAPN2 agent) with FRβ-targeted pH-sensitive liposomes exhibits a synergistic effect in suppressing tumor progression.

Keywords:

Multiplex fluorescence microscopy, lung cancer

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Localization of SHP-1 in Natural Killer Cells Across Education

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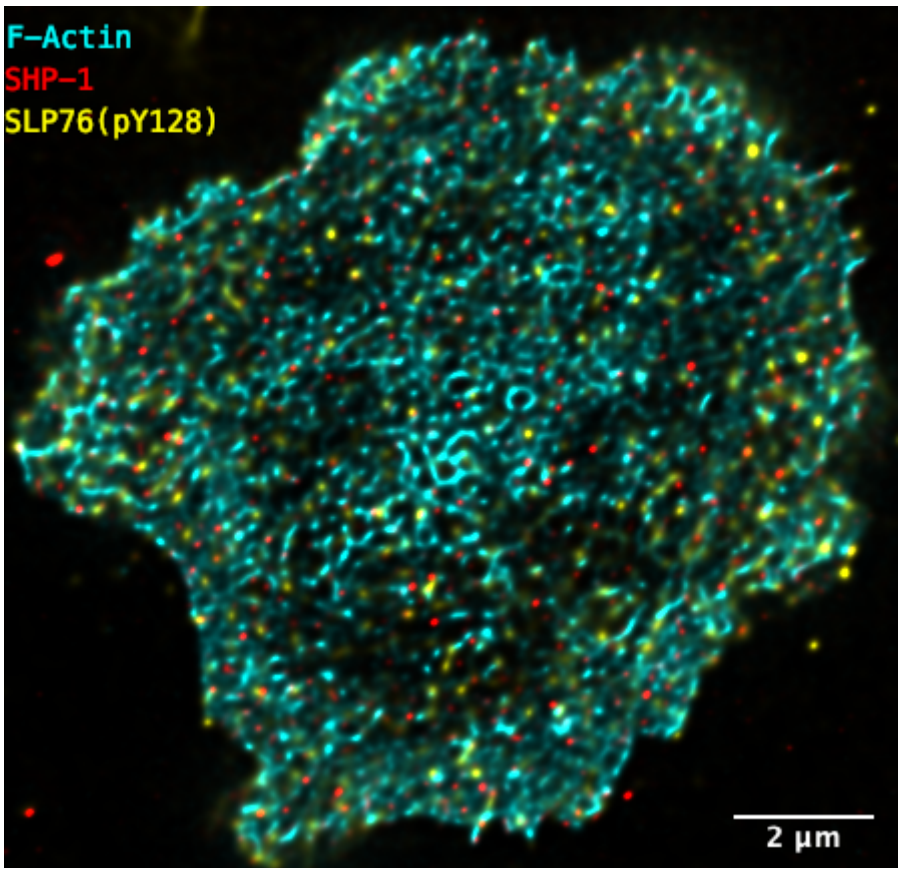
Poster Group 1

Natural killer cells learn to differentiate self from non-self cells through a process called education, wherein inhibitory receptors on natural killer cells interact with self-MHC class I molecules on healthy cells, leading to the acquisition of functional competence. Inhibitory receptors transmit signals via the recruitment of Src homology region 2 domain-containing phosphatase-1 (SHP-1), which downregulates activating signals, suggesting a role of SHP-1 in NK cell education.
Our recent super-resolution imaging studies have demonstrated a link between SHP-1 expression, localization, and morphology in activating synapses. Educated natural killer cells exhibited reduced SHP-1 expression and localized less to activating synapses compared to uneducated natural killer cells, indicating that SHP-1 may regulate the NK cell education process.
Interestingly, we also observed that the educating receptor Ly49A associates more extensively with SHP-1 in resting natural killer cells of uneducated cells compared to educated cells, suggesting a role for Ly49A in regulating SHP-1 involvement with activation synapses.
To further elucidate the role of SHP-1 in natural killer cell education we aim to develop novel techniques of investigating the natural killer cell synapses in real time. We will utilize high-resolution live cell imaging and super resolution techniques on primary cells and in vitro systems. In these studies, we will investigate SHP-1 correlations, localization, and dynamics, using continuously developed and refined analysis scripts to optimize and automate the data analysis process.
These investigations aim to provide a deeper understanding of SHP-1 regulation in natural killer cells and may lead to new therapeutic strategies for natural killer cell-related diseases.



Keywords:

Localization, STED, Confocal, Immunology

Reference:

Schmied L, Luu TT, Søndergaard JN, et al. SHP-1 localization to the activating immune synapse promotes NK cell tolerance in MHC class I deficiency. Sci Signal. 2023;16(780):eabq0752. doi:10.1126/scisignal.abq0752