The Reinhard Lab

Epidermal Growth Factor Receptors are important cancer biomarkers, and advancing our understanding of their mechanisms is critical. **Dr Björn Reinhard** elucidates the exploratory work of his laboratory on epidermal growth factor receptors and the applications for their exciting technological developments

Could you give an idea of who comprises the Reinhard Lab, what led to its formation and your current research goals?

The Reinhard Lab, based within the Boston University Photonics Center, develops novel nanotechnologies that can provide quantitative information about biological processes. Presently we are trying to get a quantitative understanding of how the spatial distribution of EGFR influences its signalling activity.

Why have you opted to use plasmon coupling between gold nanoparticle labelled EGFRs in living cells in real time to investigate the dynamics of EGFR oligomerisation? What makes this method ideal for your study?

Gold and silver nanoparticles have long been used as labels in high resolution electron microscopy. However, these studies involved a complex sample preparation that often requires sample dehydration, and they are limited in temporal resolution. With Plasmon Coupling Microscopy (PCM) technology, we now have the opportunity to monitor sub-diffraction limit distances in living cells under physiological conditions in an optical microscope without limitation in observation time. Noble metal nanoparticles have much higher optical signals than conventional dyes and, even more importantly, they have an almost unlimited lifetime. While these advantages also apply, for instance, to quantum dots, noble metal nanoparticles have the additional advantage that the individual particles strongly interact with each other once they approach each other to length scales of their diameter. These distance-dependent electromagnetic interactions lead to collective spectral responses which are detected in PCM and which enable us to measure distances and distance changes on nanometre and tens of nanometre length scales.

Why is it important to work on length scales between 1 – 100 nm when monitoring distances between EGFRs?

The textbook model for EGFR activation assumes the association of two EGFR molecules upon ligand addition. A series of recent studies has, however, indicated that EGFR also shows a larger scale association into tetramers, or maybe even larger clusters. In order to be able to investigate these larger scale associations and their organisation into even higher order organised structures, it is necessary to monitor distances on the tens of nanometre length scale.

Will you be using or developing any other innovative approaches in your work?

Implementing PCM has required innovations not only in the development of the actual microscopy, but also in various fields of material science and nanotechnology. One aspect that made a systematic investigation of EGFR-clustering possible with PCM was the development of nanoparticle labels that are stable under physiological conditions and that bind with high affinity to their cellular target. The cell surface is a complex hybrid material that contains a coating of proteins and sugars which complicates efficient labelling with nanoparticle labels. Our labelling strategies have universal applicability and, in the future, will be instrumental in investigating the spatial distribution of a broad range of cell surface receptors.

I also want to stress that our research into plasmon coupling has provided a better understanding of the underlying electromagnetic mechanisms that govern the distance-dependent electromagnetic interactions in clusters of coupled gold or silver nanoparticles. This knowledge has enabled the development of new electromagnetic materials with applications in biosensing and light harvesting.

To what extent do you encourage collaboration in your work? How has your research benefited from work partnerships and strategies to attract young researchers?

Having a multidisciplinary research team with expertise in biophysics, biochemistry, and nanotechnology is absolutely essential for this project. The composition of our team reflects this need. Furthermore, our laboratory benefits from the intellectual environment at the Photonics Center and at Boston University, where collaboration is really the norm. The Boston University Cancer Center and our colleagues at the Boston University Medical School provide very valuable input to our research, as well as to the training of our students.

I think that the interface between biology and nanotechnology offers tremendous prospects for overcoming longstanding problems in the life sciences. To realise this great potential, a well-educated work force is absolutely key. To attract students to this research area we have in the last years organised a nanocamp where we invite students (~25 students every summer) from different high schools in the Boston area to participate in research related to this project. We hope that by inspiring young students to perform research in the area of nanobiotechnology, some of them consider a career in this emerging key technology.

Sending the right signals

Cell signalling and its role in the development of carcinogenic cells is a highly complex field. **The Reinhard Laboratory** of Boston University is dedicated to exploring these relationships and providing technological solutions

DR BJÖRN REINHARD

STANDING JUST A few blocks from the waterfront of the Charles River Basin in downtown Boston, the unassuming building that houses the Boston University Chemistry Department belies none of the work that is conducted behind its doors.

Contained inside the Photonics Center is the Nano-Bio Interface Laboratory, otherwise known as the Reinhard Lab. This laboratory is tasked with the design, implementation and characterisation of novel nanotechnologies that can provide quantitative information about key biological processes. Its membership is comprised of a diverse range of researchers and graduate students with background in physical, material and biological sciences who are all seeking innovative solutions to address exigent biological problems that cannot be overcome with conventional technologies.

AN ADVANCED APPROACH

Under the charge of Professor Björn Reinhard, the Reinhard Lab is a leader in the production of new tools for imaging and manipulating inorganic and biological materials, and currently has nine graduates and three postdoctoral fellows collaborating on the development of photonic and plasmonic technologies for cancer biomarker sensing and imaging. The team is particularly focused on using electromagnetic interactions between noble metal nanoparticles to map the spatial distribution of cancer-related cell surface receptor densities. Through this study, they are endeavouring to ascertain the role that spatial distribution or topology of the epidermal growth factor receptor (EGFR) plays in controlling the signalling activity of the cancer cell. This research transcends the capabilities of current technologies, allowing the team to probe interactions between cell surface receptors on the nanometre to micron length scale in their work.

STATE-OF-THE-ART FACILITIES

The Reinhard Lab shares a 10 storey building with the other members of the Boston University Photonics Center and subsequently has access to a wealth of technologies, including electron microscopy, nanofabrication, material characterisation, and imaging facilities.

With such state-of-the-art resources available, the Reinhard Lab has been able to develop nanoparticle immunolabels that provide the means for a highly efficient localisation of particular antigens within tissues and for labelling cell surface receptors.

Chief amongst their technological achievements is the creation of Plasmon Coupling Microscopy (PCM) a potentially revolutionary imaging tool that offers enhanced usability: "PCM monitors interparticle separation on deeply subdiffraction limit length scales by analysing the colorimetric information of labels," explains Reinhard. "A great strength of PCM is its simplicity. It can be implemented with the imaging technologies that are commonly available in most biological laboratories."

The conscious development of PCM and its inherent simplicity means that only a conventional darkfield microscope, a camera, and an optical splitter (which allows the detection of spectral shifts through colorimetric imaging) are required to integrate it with existing technologies. Reinhard believes that the relative ease of PCM makes it to researchers, particularly those in the field of life sciences, where imaging is becoming more and more important. Furthermore, PCM technology has very promising potential in the study of EGFR and, subsequently, cancer research.

LEADING EGFR STUDIES

EGFR is a transmembrane protein that serves as a surface receptor for growth factors. Ligand binding promotes activation of the receptor, triggering cell division that, when abnormal, can cause abnormal cell growth and cause cancer.

The Reinhard Lab's current efforts are dedicated to producing a quantitative understanding of how the spatial distribution of EGFR influences its signalling activity, with the goal of better cancer characterisation in the future.

It is a well-established fact that individual EGFR proteins require interaction with another EGFR protein or another member of the epidermal growth factor receptor family to initiate signalling. Yet, as Reinhard explains, some essential questions still remain over this signalling activity, which he intends to investigate: "What is still not understood in sufficient detail is what role the plasma membrane plays in this signalling process by generating 'hot spots' that are locally enriched in EGFR. Our major aim is to apply PCM to map the spatial distribution of the receptors on the surface of living cells and, thus, to characterise the EGFR organisation on length scales ranging from a few nanometres to microns".

Reinhard and his collaborators are also conducting a simultaneous study into the dynamics of the EGFR organisation on these length scales in order to increase knowledge of the mechanisms that instigate the formation of EGFR hot spots in the plasma membrane and to characterise their lifetime.

RESULTS TO DATE

The Reinhard Lab's unique approach has already yielded some exciting results in this field. The high spatial resolution of their imaging approach has enabled them to map the spatial distribution of EGFR on a myriad of locations on the cell surface. This has helped the group to better understand the mechanism underlying EGFR signalling, as Reinhard elaborates: "In the selected cancer model system, we found interesting differences in the spatial distribution of EGFR density. Our studies show that the EGFR receptors are not randomly distributed but that they are significantly clustered, and it seems that the actin network of the cytoskeleton contributes to the patterning of the local density of the receptor".

From this, the lab were able to identify that EGFR density is higher on filopodia – the slender cytoplasmic, actin bundle projections that extend beyond the leading edge of lamellipodium in migrating cells, and which form focal adhesions with the substratum, linking it to the cell surface – than on the main cell body. While the exact role of filopodia is still a matter of debate, Reinhard's research is consistent with a role of these projections as sensor antennas that detect growth factors in remote areas from the cell body. A pronounced role of filopodia in guiding the directed motion of cancer cells due to chemotaxis has significant implication for future research in combating cancer. Cells with a high motility that allows them attack healthy tissues are a hallmark of aggressive cancers.

THE IMPACT

While the immediate aim of these investigations is to offer an unprecedented insight into the role that the spatial EGFR organisation on these length scales play in signalling and cell development, it has very real implications for clinical cancer studies and treatment, given that EGFR expression levels are used as prognostic indicators for various forms of cancer.

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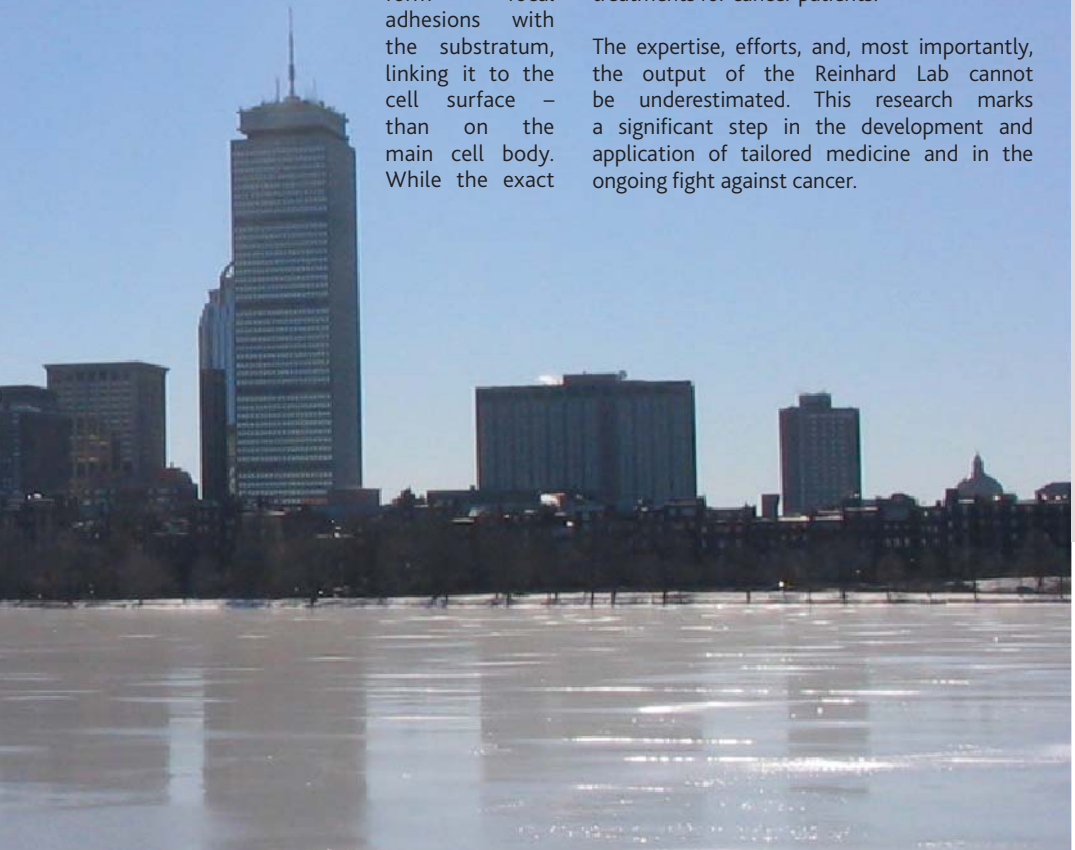
Through the Reinhard Lab's ongoing studies into the sheer surface expression, the spatial organisation of the receptors on the surface and in determining the signalling activity of the receptor, the research group are concurrently developing new technologies that could, eventually, contribute to better, clearer, characterisation of cancer cells, and, thus, help to provide more effective and tailored treatments for cancer patients.

The expertise, efforts, and, most importantly, the output of the Reinhard Lab cannot be underestimated. This research marks a significant step in the development and application of tailored medicine and in the ongoing fight against cancer.



INTELLIGENCE

THE REINHARD LAB

OBJECTIVES

The Reinhard Lab aims to develop a plasmon coupling based imaging modality to characterise the spatial distribution of cell surface receptors on living cells under physiological condition. They will then apply this technology to map the spatial distribution of the Epidermal Growth Factor Receptor (EGFR) on the cellular surfaces. Finally, they seek to elucidate the role of the spatial EGFR organisation and its dynamics on the EGFR signalling activity.

KEY COLLABORATORS

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