iNEXT workshop on Integrated methodologies and approaches for structural biology

Name of Speaker: **Peter J. Peters & the M4I nanoscopy & CryoSol-World teams**

University / Research Institute / Department: **The Maastricht Multimodal Molecular Imaging institute**

Title of Lecture: **Cryo-EM beauty and benefit for drug discovery and vaccine design: our achievements with T7SS in Mycobacterium tuberculosis**

Abstract:

We found that after prolonged infection in macrophages and dendritic cells, M. tuberculosis translocates from phago-lysosomes to the cytosol and kills the host cell a few days later, while the BCG vaccine strain fails to translocate (Cell; 129:1287). This process is dependent on a novel type VII secretion system (T7SS) (Abdallah AM et al., Nat. Rev. Microbiol. 5:883, Abdallah AM et al., J. Immunol. 2187:4744 and Houben et al., Cell Microbiol. 14:1287).

Since my move to Maastricht University, where I was invited to establish a new Institute (www.maastrichtuniversity.nl/m4i), we use cryo-EM single-particle analysis (SPA) and cryo-EM tomography to investigate recombinant purified proteins of individual gene products from the T7SS. In addition, we are purifying the entire intact T7SS structure using biochemical methods for 3D reconstruction.

We also generate thin lamellae of infected cells using cryo-FIB/SEM technology and electron tomography. The workflow starts with a high precision of localization light microscope, with live cell imaging of cell cultures on an EM carrier, which can be used throughout the complete workflow in order to prevent loss of orientation and sample contamination or destruction. Once a specific event or location of a GFP-tagged protein of the T7SS has been identified by light microscopy and preserved by cryo-fixation using jet freezing, the vitrified sample is re-examined for GFP expression. After transferring the sample to the cryo-FIB/SEM, the identified region is thinned down to approximately 150 nm without artifacts, using the focused ion beam (FIB). Samples are then transferred to the cryo-TEM for high-resolution cryo-tomography. The cryo-SPA data of the T7SS will then be docked onto the images from vitreous lamellae to construct a macromolecular map of the T7SS within the host cell.

The broader objective is to gain insight into the structure and function of the mechanism for T7SS-mediated translocation. This knowledge should lay the groundwork for the development of novel antibiotics and better vaccines against tuberculosis, still the most deadly infectious disease.

We are also developing the next generation vitrification machine for proteins and cells which we call the VitroJet. I will provide a status update of our developments, and present structures solved from samples prepared with this device.

Research Profile:

Peter Peters is a distinguished university professor and co-directs the Maastricht Multimodal Molecular Imaging Institute studying native unfixed cells with 3D cryo-electron tomography to visualize molecular machines in the context of organelles. His team discovered that mycobacteria causing tuberculosis move from the phagosome into the cytosol (top 10 cited in tuberculosis in the last 10 years). He initiated the Netherlands Centre for Electron Nanoscopy (NeCEN). His group aims to resolve the type VII secretion system of Mycobacterium tuberculosis within the phagolysosome in order to design better drugs and vaccines. He was instrumental in improving cryo-immunogold EM and vitreous cryo-sectioning methods. He initiated a new start-up CryoSol-World https://www.cryosol-world.com that will produce the next generation vitrification devices called Vitrojet. Vitrobots, developed in Maastricht have been distributed to more the 500 cryo-EM labs worldwide. His research has been reported in 120 articles with more than 28.000 citations. www.maastrichtuniversity.nl/m4i

Three selected publications:

1. *M. tuberculosis* and *M. leprae* translocate from the phagolysosome to the cytosol in myeloid cells. van der Wel N, Hava D, Houben D, Fluitsma D, van Zon M, Pierson J, Brenner M, **Peters PJ**. *Cell*. 2007;129(7):1287–1298. DOI: 10.1016/j.cell.2007.05.059
2. Visualization of a short-range Wnt gradient in the intestinal stem-cell niche. Farin HF, Jordens I, Mosa MH, Basak O, Korving J, Tauriello DVF, de Punder K, Angers S, **Peters PJ**, Maurice MM, Clevers H. *Nature*. 2016;530(7590):340-+. DOI: 10.1038/nature16937
3. Reg4^{+} deep crypt secretory cells function as epithelial niche for Lgr5^{+} stem cells in colon. Sasaki N, Sachs N, Wiebrands K, Ellenbroek SIJ, Fumagalli A, Lyubimova A, Begthel H, van den Born M, van Es JH, Karthaus WR, Li VSW, Lopez-Iglesias C, **Peters PJ**, van Rheenen J, van Oudenaarden A, Clevers H. *P Natl Acad Sci USA*. 2016;113(37):E5399–E5407. DOI: 10.1073/pnas.1607327113

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