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## Publications

Pierson J, Ziese U, Sani M, Peters PJ. Exploring vitreous cryo-section-induced compression at the macromolecular level using electron cryo-tomography; 80S yeast ribosomes appear unaffected. *J Struct Biol.* 2010

Sani M, Houben EN, Geurtsen J, Pierson J, de Punder K, van Zon M, Wever B, Piersma SR, Jiménez CR, Daffé M, Appelmelk BJ, Bitter W, van der Wel N, Peters PJ. Direct visualization by cryo-EM of the mycobacterial capsular layer: a labile structure containing ESX-1-secreted proteins. *PLoS Pathog.* 2010;6:e1000794

Barker N, Huch M, Kujala P, van de Wetering M, Snippert HJ, van Es JH, Sato T, Stange DE, Begthel H, van den Born M, Danenberg E, van den Brink S, Korving J, Abo A, Peters PJ, Wright N, Poulson R, Clevers H. *Lgr5(+ve) stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro.* *Cell Stem Cell.* 2010;6:25-36

Weerdenburg EM, Peters PJ, van der Wel NN. How do mycobacteria activate CD8+ T cells? *Trends Microbiol.* 2010;18:1-10

## NANOBIOLOGY

The clue of the treatment of various diseases is inside biological nanomachines. Investigating these machines becomes more attractive and serious when there is a possibility to realize these experiments without isolation. Obtaining high resolution images at 3nm from cryo-electron microscopy tomograms from native cell will give immense information. Besides our work on mycobacteria we have therefore made a main focus in our research line on visualization of nanomachines in their native cellular environment and strive to integrate this in cancer and mycobacteria research.

The cellular nanocosm is made up of numerous types of macromolecular complexes or biological nanomachines. These form functional modules that are organized into complex subcellular networks. Information on the ultra-structure of these nanomachines has mainly been obtained by analyzing isolated structures, using imaging techniques such as X-ray crystallography, NMR, or single particle electron microscopy (EM). Yet there is a strong need to image biological complexes in a native state and within a cellular environment, in order to gain a better understanding of their functions. Emerging methods in EM are now making this goal reachable. Cryo-electron tomography bypasses the need for conventional fixatives, dehydration and stains, so that a close-to-native environment is retained. As this technique is approaching macromolecular resolution, it is possible to create maps of individual macromolecular complexes. Atomic structures made by X-ray and NMR can be 'docked' or fitted into the lower resolution particle density maps to create a macromolecular atlas of the cell under normal and pathological conditions. The majority of cells, however, are too thick to be imaged in an intact state and therefore methods such as 'high pressure freezing' and 'cryo-sectioning of unperturbed vitreous fully hydrated samples' have been introduced for electron tomography. This year we continued on improving methods for visualizing nanomachines in a close-to-physiological, cellular context. EM is in a renaissance and with a Dutch team of colleagues we obtained grants to create a national centre of large infrastructure where two Titan Krios cryo-TEMs will be located. For more information see [www.necen.nl](http://www.necen.nl).

Cryo-electron tomography of vitreous cryo-sections is the most suitable method for exploring the 3D organization of biological samples that are too large to be imaged in an intact state. Producing good quality vitreous cryo-sections, however, is challenging. We focused on the major obstacles to success: contamination in and around the microtome, and attachment of the ribbon of sections to an electron microscopic grid support film. The conventional method for attaching sections to the grid has involved mechanical force generated by a crude stamping or pressing device, but this disrupts the integrity of vitreous cryo-sections. Furthermore, attachment is poor, and parts of the ribbon of sections are often far from the support film. This results in specimen instability during image acquisition and subsequent difficulty with aligning projection images. We have implemented a protective glove box surrounding the cryo-ultramicrotome that reduces the humidity around and within the microtome during sectioning. We also introduced a novel way to attach vitreous cryo-sections to an EM grid support film using electrostatic charging with a major effort of the Dutch company 'Simco' as a gesture of charity. The ribbon of vitreous cryo-sections remains in place during transfer and storage and is devoid of stamping related artifacts. We illustrated these improvements by exploring the structure of putative cellular 80S ribosomes within 50nm, vitreous cryo-sections of *Saccharomyces cerevisiae*.

We then explored vitreous cryo-section-induced compression at the macromolecular level using electron cryo-tomography and focused on the 80S ribosomes. Vitreous cryo-section-induced compression influences the interpretation and the reliability of electron microscopy images and tomographic reconstructions. We showed this year that electron cryo-tomographic reconstructions of vitreous cryo-sections show that 80S ribosomes, both intracellular and in an isolated state, appear able to resist section-induced compression.

## Publications (continued)

Pierson J, Fernández JJ, Bos E, Amini S, Gnaegi H, Vos M, Bel B, Adolfsen F, Carrascosa JL, Peters PJ. Improving the technique of vitreous cryo-sectioning for cryo-electron tomography: electrostatic charging for section attachment and implementation of an anti-contamination glove box. *J Struct Biol.* 2010;169:219-25

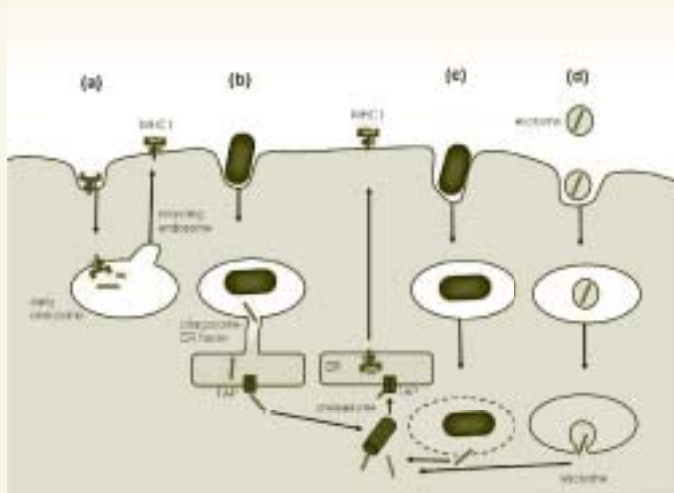


Figure 5: Proposed cross-presentation pathways from literature.

**Mycobacteria** Inflammation is a localized protective reaction to injury or infection, but it also has a pathogenic role in many diseases, including cancer. Whereas acute inflammation is critical for host defense, chronic inflammation can contribute to tumorigenesis and metastatic progression. The latter, including macrophages and lymphocytes, are important elements of the tumour microenvironment.

*Mycobacterium tuberculosis* is a devastating pathogen that kills >1.5 million people each year, but controlled use of its cell wall activates macrophages in ways that can be harnessed for therapy. For example, *M. bovis* Bacille Calmette-Guérin (BCG) is one of the most widely used antitumor adjuvant therapies in humans. Injection of BCG into the bladder mediates regression of transitional cell carcinomas by stimulating a vigorous local immune response, bathing tumors in cytokines and activated immune cells. That is why we focused earlier this year on writing a review called “How do mycobacteria activate T cells?”

CD8(+) T cells are activated upon presentation of antigens from the cytosol.

Therefore, it was unclear how pathogenic mycobacteria could prime this type of lymphocyte, given that these microbes were thought to remain in phagosomes and, hence, be shielded from the host cytosol. Recently, we showed that pathogenic mycobacteria can enter the cytosol through translocation from phagolysosomes (Cell 2008), providing a direct mechanism for CD8(+) T cell priming. However, this mechanism does not apply to other mycobacteria such as BCG, which do not enter the cytosol. We discussed the different hypotheses to explain the induction of CD8(+) T cell responses in mycobacterial infections.

A lot of effort went into the direct visualization by cryo-EM of the mycobacterial capsular layer. The cell envelope of mycobacteria, a group of Gram positive bacteria, is composed of a plasma membrane and a Gram-negative-like outer membrane containing mycolic acids. In addition, the surface of the mycobacteria is coated with an ill-characterized layer of extractable, non-covalently linked glycans, lipids and proteins, collectively known as the capsule, whose occurrence is a matter of debate. By using plunge freezing cryo-electron microscopy technique, we were able to show that pathogenic mycobacteria produce a thick capsule, only present when the cells were grown under unperturbed conditions and easily removed by mild detergents. This detergent-labile capsule layer contains arabinomannan, alpha-glucan and oligomannosyl-capped glycolipids. Further immunogenic and proteomic analyses revealed that *Mycobacterium marinum* capsule contains high amounts of proteins that are secreted via the ESX-1 pathway. Finally, cell infection experiments demonstrated the importance of the capsule for binding to cells and dampening of pro-inflammatory cytokine response. Together, this study showed a direct visualization of the mycobacterial capsular layer as a labile structure that contains ESX-1-secreted proteins.

We continued our successful collaboration with the laboratory of Hans Clevers (Hubrecht Institute, Utrecht).

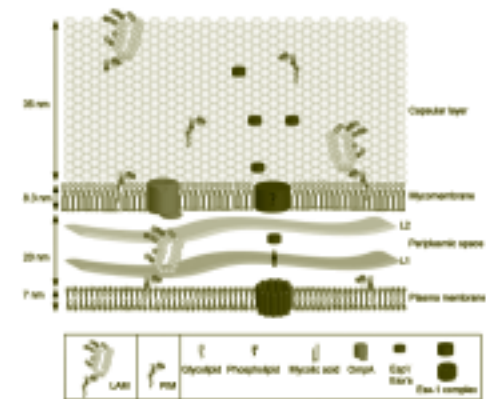


Figure 6: The spatial organization of the mycobacterial cell envelope exhibiting the capsule. This scheme represents the relative size and organization of the different layers of the envelope including the plasma membrane, mycomembrane, periplasmic space and capsular layer. The positions of some of the constituents analyzed are depicted.