Application Note

Dr Vassilis Koronakis, Department of Pathology, University of Cambridge

Salmonella Uptake Studies

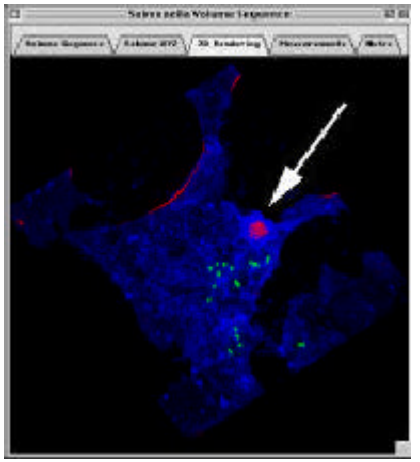
Salmonella species are pathogens of man and animals which commonly cause severe gastroenteritis. The pathogen triggers its own uptake into non-phagocytic mammalian cells. Work in the laboratory of Dr Vassilis Koronakis (Department of Pathology, University of Cambridge) is directed towards understanding the mechanisms by which the pathogen triggers its own uptake into non-phagocytic mammalian cells.

Dr Richard Hayward and Matthew Garner are investigating the contribution of cholesterol to the *Salmonella* entry mechanism. They have been able to establish that uptake of *Salmonella* into cholesterol depleted HeLa cells was dramatically reduced, indicating the importance of cholesterol in the uptake process. During further experiments, HeLa cells were infected with *Salmonella* expressing GFP and cholesterol and F-actin were visualized using filipin and Texas-red conguated phalloidin respectively. It was found that the cholesterol relocated and accumulated at the sites of bacterial entry in every infected cell.

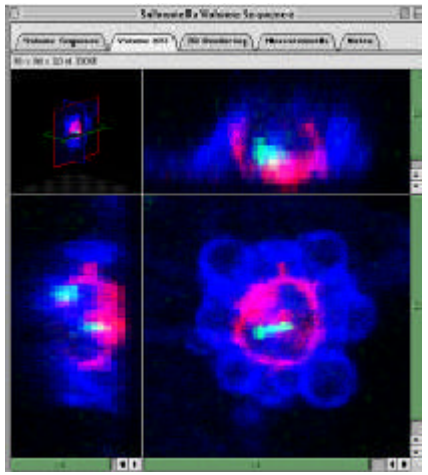
Investigation of the bacterial entry sites in infected fibroblasts revealed remarkable cholesterol-rich entry associated surface protrusions, which extended beyond the level of the cell surface. Drs Hayward and Garner acquired a confocal z series of images through these novel structures and used ***Volocity*** to create a three dimensional reconstruction of the novel structures.

Using ***Volocity*** they were clearly able to see that the cholesterol-rich exterior of the structure envelops a central “cup” of F-actin that in turn surrounds the invading bacteria. The images here show the complete structure of the infected fibroblast with the entry site which is evident as a filipin labeled protrusion (blue) surrounding an F-actin cup like structure (red). However, analysis of the z series using the XYZ view in ***Volocity*** allowed Drs Hayward and Garner to step through the structure plane by plane, revealing the the GFP labeled *Salmonella* bacterium at the center of the protrusion.

Further analysis by these researchers will address the role of cholesterol in the entry process in more detail, which will allow further insights into the complex bacterial internalization mechanism.



One view in ***Volocity*** of three color volume showing novel cholesterol/F-actin structure (marked with arrow)



XYZ view in ***Volocity*** showing GFP labeled bacterium at the center of the novel structure