# Ultrastructural & Analytical Methods in Life Sciences

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## Freeze-Fracture Replica Immunolabeling revealed insights into the dynamics of urothelial plaques during blood-urine barrier formation *in vitro*

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The high-resolution imaging technique of freeze-fracture electron microscopy combined with immunogold labelling, i.e. freeze-fracture replica immunolabeling technique (FRIL) targets membrane proteins (or less commonly, the lipids) on an outer surface and deep within the cells or tissues. In the FRIL technique, conventional freeze-fracture replicas are first prepared; the biological material is then dissociated using sodium dodecylsulphate. The remaining layer of cellular components is so thin that it is transparent to the electron beam of electron microscope. The proteins or lipids are then localized by immunogold labelling, which reveals their spatial distribution superimposed upon a standard planar freeze-fracture view of the membrane interior. Moreover, because samples are freeze fractured prior to immunogold labelling step, both membrane leaflets of the plasma membrane (PM) and those of intracellular membranes are accessible for labelling [1].

As an illustration how FRIL has recently contributed to advances in cellular and molecular medicine, we will present the spatial organization of plasma membrane (PM) proteins uroplakins into urothelial plaques in urothelial cells (UCs) *in vitro*. Mouse and porcine UC cultures were prepared as described previously [3, 4]. After 2 months in culture, we performed FRIL experiments. The formation of urothelial plaques was additionally evaluated by molecular and ultrastructural analysis and by measuring TER.

By revisiting the localization of uroplakins in cultured UCs with the FRIL technique, the first unequivocal evidence for uroplakin-positive urothelial plaques in primary mouse and secondary porcine UC cultures has been achieved "Figure 1.". Apart from highly specific immunolabeling of molecular epitops, FRIL provides also new insights into dynamics of molecular aggregates, such as urothelial plaques, which contribute to the blood-urine barrier, the tightest and most impermeable barrier in the body.

To sum up, our findings illustrate that the information provided by FRIL is unique; without its wider application, substantial gaps in our knowledge of how membranes, organelles, cells, tissues and organs function in health and disease will remain.

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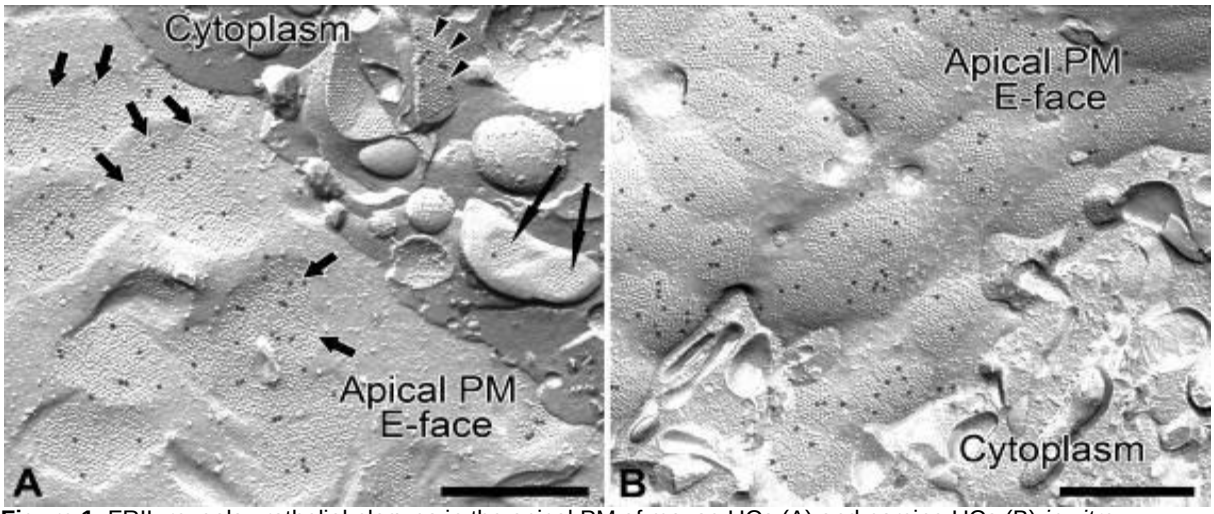


**Figure 1.** FRIL reveals urothelial plaques in the apical PM of mouse UCs (A) and porcine UCs (B) *in vitro*. Immunogold labelling for uroplakins is seen in urothelial plaques on the E faces of the apical PM, and also on E faces of discoidal-fusiform vesicles (DFV) membranes (arrowheads and long arrows in A). The number and ordering of uroplakin particles in urothelial plaques varies between DFVs. The DFVs marked with arrowheads, bear more uroplakin particles than those marked with long arrows. This could be interpreted as the sequential assembly of uroplakin particles into DFV membranes. Variations in the number of uroplakin particles in the urothelial plaques are also seen in the apical PM (A, B), suggesting that the gradual aggregation of small urothelial plaques into larger ones is not only limited to DFVs but still takes place in the apical PM (thick arrows). The edges of urothelial plaques appear rounded or straight. Bars, 500 nm.