Structural insights into the *Danio rerio* aquaglyceroporin 3b

Samuel Hjorth-Jensen[1], Peng Huang[1], François Chauvigné[2], Joan Cerdà[2] and Karin Lindkvist-Petersson[1,3]

[1]Department of Experimental Medical Science, Lund University, Lund.

[2]Institut de Recerca i Tecnologia Agroalimentàries (IRTA) – Institut de Ciències del Mar, Consejo Superior de Investigaciones Científicas (CSIC), Barcelona, Spain.

[3]LINXS - Institute of Advanced Neutron and X-ray Science, Lund, Sweden.

Aquaglyceroporins are integral membrane proteins that transport water and small solutes such as glycerol across cells membranes, playing key roles in cellular homeostasis and signalling. Enhancing membrane permeability to cryoprotectants is critical for successful cryopreservation of large cells and tissues, and ectopic expression of aquaglyceroporins has been shown to improve cryoprotectant uptake and post-thaw survival. The zebrafish aquaglyceroporin 3b (DrAqp3b) exhibits high permeability to ethylene glycol but is strongly regulated by pH, remaining active at neutral pH and inactive under acidic conditions.

Here, we present high-resolution cryo-EM structures of DrAqp3b in both open and closed states. At pH 8.0, the channel adopts an open conformation with ethylene glycol bound within the pore. At pH 5.5, an extracellular loop collapses into the channel, occluding the pore and preventing solute transport. This pH-dependent gating mechanism is conserved among orthologs, including human and rat aquaporin 3 (AQP3), and likely involves protonation of a key aspartate residue (Asp163).

In addition to ethylene glycol, DrAqp3b transports and is autoregulated by hydrogen peroxide (H_2O_2), a key reactive oxygen species. Functional assays in Xenopus oocytes demonstrated up to a 3-fold increase in H_2O_2 permeability at low-to-moderate concentrations, with reduced transport at higher levels (>500 µM). This H_2O_2-induced closure was first demonstrated in human AQP3 and suggests a feedback mechanism to limit oxidative stress. Given its high expression in zebrafish skin, DrAqp3b may help fine-tune H_2O_2 signalling in response to injury.

These findings provide mechanistic insight into aquaglyceroporin regulation, highlighting their potential both for novel cryopreservation strategies and for modulating H_2O_2 signalling in wound healing.