Functional characterisation of P-type ATPases using solid-supported membrane based electrophysiology

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P-type ATPases are a large, ubiquitous and varied family of membrane proteins that are involved in many transport processes in virtually all living organisms (Bublitz *et al*., 2011). These membrane proteins couple the energy provided by ATP hydrolysis to the active transport of various ions across biological membranes. A specific feature of P-type ATPases is the formation of a phosphorylated intermediate state during their enzymatic cycle.

Solid supported membranes (SSM) have been employed for the functional characterisation of P-type ATPases, *e.g.* sarcoplasmic reticulum (SR) Ca^{2+}-ATPase and Na^+,K^+-ATPase (Tadini-Buoninsegni *et al*., 2008). The SSM, consisting of a hybrid alkanethiol/phospholipid bilayer supported by a gold electrode, is a convenient model system for a biological membrane. An advantageous feature of the SSM is its high mechanical stability which allows fast solution exchange at the membrane surface (Schulz *et al*., 2008). Proteoliposomes or native membranes (vesicles or fragments) incorporating the ATPase are adsorbed on the SSM surface and are subjected to a rapid substrate concentration jump. The substrate concentration jump activates the ATPase and the charge displacement concomitant with the transport activity of the enzyme is recorded as a current transient *via* capacitive coupling (Tadini-Buoninsegni & Fendler, 2015).

Charge transfer in P-type ATPases was investigated by SSM-based electrophysiology in order to gain insights into the ion transport mechanism. In the case of SR Ca^{2+}-ATPase, the SSM technique provided useful information for a detailed characterisation of the enzyme's transport cycle, especially as concerns Ca^{2+} binding and Ca^{2+}/H^+ exchange (Tadini-Buoninsegni *et al*., 2006). More recently, SSM measurements were performed on bacterial and human Cu^+-ATPases (Tadini-Buoninsegni *et al*., 2010; Mattle *et al*., 2015) to demonstrate electrogenic Cu^+ ion displacement across the ATPase protein. While the SSM-based technique is well-suited for the investigation of fundamental questions concerning the transport mechanism of membrane transporters, it is also a promising platform technology for drug screening and development.

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