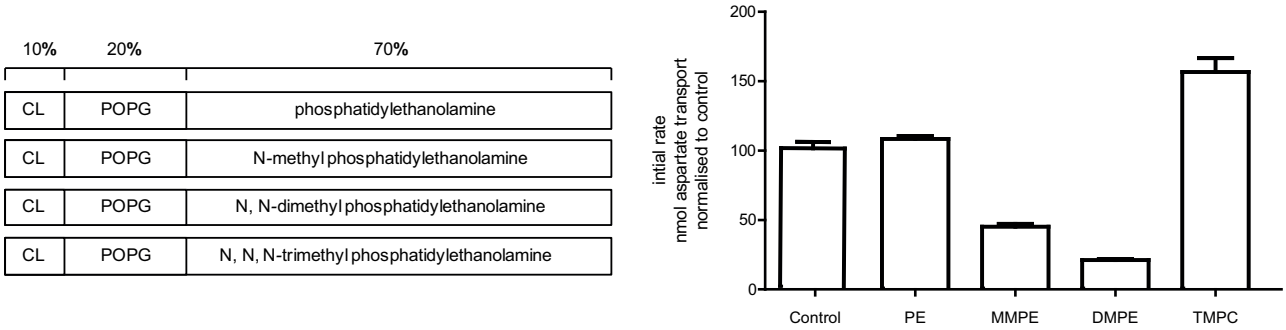
Conformational dynamics of an archaeal aspartate transporter are determined by the lipid bilayer

B.C. McIlwain, R.J. Vandenberg and R.M. Ryan, Transporter Biology Group, Discipline of Pharmacology and Bosch Institute, University of Sydney, NSW 2006, Australia.

Glutamate transporters, or Excitatory Amino Acid Transporters (EAATs), are concentrative solute carriers that use steep gradients of Na^+ to sequester glutamate and aspartate intracellularly to terminate the action of these neurotransmitters (Danbolt, 2001). Detailed structural information for the EAATs remains elusive, as X-ray crystallography on mammalian membrane-bound proteins remains difficult. For this reason, a homologous protein has been identified which provides structural insights into the transport mechanisms and kinetics of the EAATs.

The Na^+/aspartate symporter from *Pyrococcus horikoshii*, GltPh, has been crystallized in a variety of states representing static conformations of the transport cycle (Boudker *et al.*, 2007). The aim of this study is to determine whether the activity of GltPh is influenced by the lipid bilayer. Purified GltPh was reconstituted into liposomes comprised of 10% cardiolipin, 20% phosphatidylglycerol and 70% of phosphatidylethanolamine or its mono-, di- or tri- methylated derivatives (see Figure). Radiolabelled ^{3}H-L-aspartate uptake studies were conducted and the activity in these different lipid compositions compared to a well-documented control condition (Ryan *et al.*, 2009).



The Figure demonstrates the initial rate of transport in each of the experimental conditions, normalized to the control condition, and highlights the significant differences in transport supported where only subtle differences in the primary lipid species exist (mono-, di- and tri-methyl substitutions). When the primary lipid is phosphatidylethanolamine, the rate of uptake is comparable to control (108 ± 4%), but decreases with both mono- and di-methyl substitution (45 ± 4 and 21 ± 1 % of control, respectively). The rate of uptake is recovered and exceeds the control condition when the primary lipid is tri-methylated phosphatidylethanolamine (156 ± 24 % of control). The differences in transport rate can not be explained by changes in aspartate affinity for the transporter, with the mono-, di- and tri-methylated phosphatidylethanolamine lipids displaying aspartate EC50s of 38 ± 4, 67 ± 12 and 48 ± 7 nM, respectively, which is similar to control (46 ± 13 nM). Similarly, the affinity for Na^+ remains unchanged with 3.5 ± 1 mM for both mono- and di-methyl phosphatidylethanolamine, which is similar to published results for control (3.9 ± 0.3 mM).

The ability for GltPh transport rate to be affected without altering affinities for co-transported Na^+ or substrate indicate that conformational changes in the transport cycle have been affected, with the lipid bilayer determining the favourability of conformational transitions.

Boudker O, Ryan RM, Yernool D, Shimamoto K, Gouaux E (2007) Coupling substrate and ion binding to extracellular gate of sodium-dependent aspartate transporter. *Nature* **445:** 387-393.

Danbolt, NC (2001) Glutamate uptake. *Progress in Neurobiology* **65:** 1-105.

Ryan RM, Compton EL, Mindell JA (2009) Functional characterization of a Na^+-dependent aspartate transporter from Pyrococcus horikoshii. *Journal of Biological Chemistry* **284:** 17540-17548.