Electronic Supplementary Material (ESI) for Faraday Discussions

Cell cycle dependent changes in membrane stored curvature elastic energy: evidence from lipidomic studies

Charlotte V. Hague, Anthony D. Postle, George S. Attard, Marcus K. Dymond

Supplementary Content

The method for generating and testing proxy determination from Equations 3 and 4 is briefly described. Asynchronous quantitative lipidomic data was test for conformance to a ratio control system of nonbilayer to bilayer lipids. This is achieved using Equation 3, in the body text, reproduced above, under the 'postulated control system' heading. Initially, lipidomic data from HL60 cells grown in an oleic acid enriched medium were used to generate a set of parameters that would classify the lipidomic data into two sets, either bilayer, or non-bilayer. Each set of parameters enabled a weighting factor (w) for stored elastic stress contributions to be generated. Each molecular species of lipid has its own w value which multiplied by its concentration, gives a measure of its contribution to stored elastic stress (either as a type 0 or type II lipid). Allocating lipids as type II or type 0 is based on whether their w values are greater or lesser than the w value of a lipid pivot species (L_p), such that if w_m equals $w\ (L_p)$ it is type 0.

The variables c_{h1}, c_{h2} describe the contribution of lipid chain to stored elastic stress and h_g describes the headgroup contribution, Equation 4 body. Previously we conditioned our parameter sets w.r.t. the number of unsaturations in the chain such that $c_{h(0)}<c_{h(1)}<c_{h(2)}<c_{h(3)}<c_{h(4)}<c_{h(5)}<c_{h(6)}$, where the bracketed subscript denotes the number of unsaturations. Head groups were biased in the order PS(h_g)<PC(h_g)<PI(h_g)<PE(h_g)<PA(h_g)<DAG(h_g). In this coarse grain model only unsaturations in the hydrocarbon chain increase a lipid's propensity to negative curvatures and increase stored elastic stress. We use the following notation to describe our lipids, which effectively have no chain length contribution to negative curvature preference, PE 0:1, where the first two letters describe the headgroup class and the two numbers describe the number unsaturations in each chain. Once a set of parameters had been generated for HL60 cells grown in oleate, these parameters were tested on HL60 cells grown in normal media and finally in HeLa cells. The coefficient of variance for each cell line was used as a measure of ratio control; parameter sets that gave low coefficients of variance across all cell lines were reported.

From *in vitro* studies it is clear that the pivot species that should emerge as the best is POPE, PE 0:1 in our modelling notation, since it forms a lamellar phase at 37°C, with a transition to inverse hexagonal at 75°C. In contrast DOPE forms an inverse hexagonal phase at 37°C and is therefore a type II lipid. In this simple model the hydrocarbon chain contribution to stored elastic stress increased with the number of unsaturations. In previous studies we found the signature of conservation of stored elastic stress to be in evidence since the coefficient of variance of about 10% was the lower limit of ratio control across all the cell types and samples. These emerged from parameter sets where the pivot species was a PE with a single unsaturation in 1 chain, i.e. POPE. Full details have been published previously (Dymond et al.).