

We are encouraged that Cooper, Occhipinti & Boron (Cooper *et al.*, 2015) agree that CO<sub>2</sub> passage through membrane proteins (“gas channels”) should not be considered a universal phenomenon. We draw attention to our recent findings (Hulikova & Swietach, 2014) that membranes in tissue-growths of different cellular origin are not rate-limiting for CO<sub>2</sub> transport. This indicates that gas channels are not obligatory for CO<sub>2</sub> exchange across membranes.

Cooper *et al* have proposed a sliding scale of gas channel-dependence using examples from several cell types, ranked by cholesterol content (a factor believed to decrease CO<sub>2</sub> permeability), but without considering differences in the size of unstirred layers (USLs). Since USLs remain ill-defined (Missner & Pohl, 2009), it is not possible to determine whether slow CO<sub>2</sub> transport in cholesterol-rich cells is due to slow permeation across the membrane or adjoining USLs. Resistance across USLs cannot be changed by permeation through gas channels, therefore cholesterol content alone is not a robust predictor of gas channel-dependence. Without information on USLs, the presumption that “low  $P_{M,CO_2}$  represents  $P_{Back,CO_2}$ ” cannot be made confidently. Moreover, the notion that cholesterol decreases gas diffusivity has been challenged by studies showing no effect of cholesterol on the transversal diffusivity of hydrophobic solutes (Zocher *et al.*, 2013).

In contrast to the controversial effect of cholesterol on CO<sub>2</sub> transport, it is beyond doubt that resistance across the lipid matrix will be higher in membranes containing a near-confluent matrix of integral proteins. These integral proteins are also likely to produce a highly tortuous nano-environment on either side of the membrane due to their protrusions. Insertion of aquaporins may provide a route for CO<sub>2</sub> passage (i.e. a *bona fide* channel); alternatively, insertion may loosen the density of impermeable protein protrusions that otherwise obstruct access to the membrane. Under the latter mechanism, aquaporins (and other gas channel-candidates) would increase CO<sub>2</sub> transport, without functioning as gas channels. To distinguish these modes of action, a more complete understanding of the juxta-membraneous environment is warranted.

In conclusion, an assessment of the role of gas channels in CO<sub>2</sub> transport awaits accurate determination of the resistances to CO<sub>2</sub> flow imposed by the lipid matrix, unstirred layers (USLs) and membrane proteins. In the meantime, it is important to agree on naming conventions and we make two suggestions. Firstly, measurements of CO<sub>2</sub> movement in and out of cells need not represent *membrane* CO<sub>2</sub> permeability ( $P_{M,CO_2}$ ), if the rate-limiting barrier is outside the membrane. The absence of CO<sub>2</sub> transport across the apical pole of epithelial cells may not solely be due to low  $P_{M,CO_2}$ . Secondly, since USLs are not part of membranes, the concept of an ‘*apparent membrane permeability*’ is misleading, and a more general term (e.g., apparent CO<sub>2</sub> permeability) is proposed.

## Recommendations for experts who can comment on the crosstalk

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