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# COMMENTARY

# Turning an Opinion Inside-Out: Rees and Eisenberg's Commentary (Proteins 2000;38:121–122) on "Are Membrane Proteins 'Inside-Out' Proteins?" (Proteins 1999;36:135–143)

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In any scientific discussion it is paramount to recognize what is agreed upon and what are the areas of contention. We are happy to conclude that Rees and Eisenberg in their recent commentary[1] agree with us[2] that membrane proteins are not "inside-out" proteins. They also concede[1] that their original article, describing the use of the hydrophobic moments to model and understand membrane protein structure,[3] is conceptually incorrect, because it is based on the "inside-out" nature of membrane proteins as its foundation. The disagreement is based upon whether Rees and Eisenberg's 1989 *Science* article entitled "Hydrophobic organisation of membrane proteins"[4] is also a proponent of the "inside-out" nature of membrane proteins. Rees and Eisenberg, now claim[1] that their intentions in their 1989 *Science* article were to discredit the "inside-out" nature of membrane proteins. We could not disagree more, in fact Rees and Eisenberg's 1989 *Science* article is one of the strongest and most influential proponents of the "inside-out" nature of membrane proteins as we readily demonstrate below.

The original concept for the "inside-out" model for membrane proteins was introduced by Engelman and Zaccai,[5] after a neutron diffraction study of bacteriorhodopsin. In referring to bacteriorhodopsin it is stated that[5]:

" . . . the protein is inside-out compared with normal distribution of polar and non-polar amino-acids found in soluble proteins."

Although this model may have been useful to describe some of the unusual properties of this archaean protein, it is clearly of limited applicability to the structures solved since. For the currently published high-resolution structures, hydrophobic organization is a poor indicator of the orientation of transmembrane helices.[6,2]

Rees and Eisenberg's 1989 *Science* article[4] describes the study of the hydrophobic organization of *Rhodobacter sphaeroides* PRC and is highly supportive of the "inside-out" nature of membrane proteins. Indeed, one only needs to look at the first two sentences of the abstract in order to come to this conclusion:

"Membrane-exposed residues *are more hydrophobic* than buried interior residues in the transmembrane regions of the photosynthetic reaction center from *Rhodobacter sphaeroides*. This hydrophobic organisation is *opposite* to that of water-soluble proteins."

If the residues in the exterior *are more hydrophobic* than the residues in the core, and this situation is reversed from water soluble proteins (which are "outside-out"), how can PRC not be "inside-out" according to Rees and Eisenberg?[4] To reiterate, since water soluble proteins are "outside-out" and the hydrophobic organization of the PRC is *opposite* to that, then PRC cannot be "outside-out" as well. The above quotation from Rees and Eisenberg's PRC study unambiguously specifies a direction for the TM helix amphipathicity, whereby the more polar residues are oriented towards the core of the protein.

Polarity is a relative concept, in which one should compare the polarity of a solute with the polarity of its solvent. Water is less polar than brine, TM helices are less polar than water, and decane is less polar than TM helices. Rees and Eisenberg's analysis indicated that, compared to the lipid solvent, PRC has a polar core.[4] The magnitude of the core hydrophobicity may be comparable to the cores of aqueous domains, but given that our interests were concerned with the residues within the plane of the lipid bilayer, the PRC article does indicate that the core was more polar than the exterior of the protein and the lipid environment. In our analysis a different conclusion was reached: The polarities of the core and exterior of mem-

*Abbreviations:* PDB, protein data bank; TMD, transmembrane domain; PRC, photosynthetic reaction center.

The commentary entitled "Turning An Opinion Inside-Out: Rees and Eisenberg's Commentary on 'Are Membrane Proteins Inside Out?' " by Stevens and Arkin, published in this issue, is in reality a rebuttal to a commentary by Rees and Eisenberg (Turning a Reference Inside-Out: Commentary on an Article by Stevens and Arkin Entitled: "Are Membrane Proteins 'Inside-Out' Proteins?"; Proteins 2000;38:121–122) to an article by Arkin and Stevens (Are Membrane Proteins "Inside-Out" Proteins?; Proteins 1999;36:135–143). The rebuttal should have been published in the same issue as the commentary, but owing to an error on the part of the editor, this did not happen. Apologies to all those involved.

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brane proteins are indistinguishable. The reasons for the difference between the two studies are twofold:

(I) Our analysis was based on a much larger data set than that used by Rees and Eisenberg which consisted of only one (non-homologous) protein. The hydrophobic moments calculated by Rees and Eisenberg for proteins other than PRC are of no consequence. This is due to the fact that the correlation between the direction of these moments and the helix accessibility vectors cannot be made, because the protein structure is unknown.
(II) The algorithms used in our analysis did not take protein-specific, often charged co-factor–binding residues into account.

In no way do we suggest that the polar core of PRC is comparable in magnitude to the surface of aqueous proteins. We think it obvious that a discussion of a polar core should be taken in relation to the polarity of the environment, for it is widely known that, irrespective of amphipathicity, TM helices are generally hydrophobic in nature. It is this fact that enables their detection by hydropathy algorithms.[7]

To conclude, we contend that not only did Rees and Eisenberg's PRC article not discredit the "inside-out" model as they now claim, "our very article[4] that discredited this model 10 years ago",[1] but in fact strengthened it. It is for this reason that we[2] and so many others have cited it when referring to the paradigm describing membrane proteins as "inside-out." As an example we quote but a few key references in which the "inside-out" nature of membrane proteins is referred to and the reference provided is Rees and Eisenberg's 1989 *Science* article:[4] " . . . bilayer exposed residues of membrane proteins are more hydrophobic that the interior residues . . . ";[8] " . . . since transmembrane helices within membranes are widely noted to contain examples of polar and charged residues, they can also pack through hydrogen bonding and electrostatic interactions.";[9] "The lateral position of helices is determined by the strength of the inter-helix binding estimated from the polar interaction field . . . ".[10]

## REFERENCES


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