This Week's Citation Classic®

Pinto da Silva P & Branton D. Membrane splitting in freeze-etching: covalently bound ferritin as a membrane marker. *J. Cell Biol.* 45:598-605. 1970.
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This paper showed that freeze-fracture splits biological membranes. The outer and inner surfaces of erythrocyte membranes were labeled with covalently bound ferritin, freeze-fractured, and "etched" to sublime away surrounding ice. Surface markers (ferritin) were absent from fracture faces but were present on the surfaces of unfractured membranes exposed by "etching" of ice. [The *SCI*® indicates that this paper has been cited in over 495 publications.]

Split Membranes Integrate Concepts

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I thought of and did this work when I was a graduate student in D. Branton's laboratory at Berkeley. I had arrived in the fall of 1966 from Portugal, then under a benignly provincial, Fascist rule, and the post-Free-Speech Movement atmosphere at Berkeley, with its myriad little tables distributing every shade of exotic-left propaganda, was a wonderland. Berkeley soon became another kind of wonderland; our campus was the center of attention due to student opposition to the war in Vietnam. During this time I got my share of tear gas, sometimes piped into our laboratories through the ventilation system.

Our proof that biological membranes were split along an interior plane had two consequences: (1) it showed that biomembranes were composed of a bilayered continuum that was locally interrupted by particles (which we hypothesized to be proteins) intercalated across the apolar matrix of the bilayer, and (2) it allowed the interpretation of images of freeze-fractured membranes. Our view was the synthesis of antithetic concepts: (1) membranes as lipid bilayers with adsorbed proteins (as held by J.F. Danielli and J.D. Robertson), and (2) membranes as planar aggregates of lipid/protein subunits (as proposed by F. Sjostrand, D. Green, S.J. Singer, and A.A. Benson). Our proposal (which I presented at the International Botanical Congress, Seattle, August 1969) proved correct and became the basis for the fluid-mosaic model of membranes (S.J. Singer and G. Nicolson).

Over the next few years, I used freeze-etching as a molecular cytochemical approach to establish the topology and chemical nature of intramembrane particles (IMPs). I showed that, in erythrocyte membranes, the IMPs correspond to transmembrane proteins that reach to the outer surface where they expose antigens,[1] negative charges, and lectin receptors. I showed, also, that the IMPs (and, therefore, transmembrane proteins) could move translationally along the plane of the membrane.[2] In 1974 Garth Nicolson and I proposed that both glycophorin and Band 3 (the anion transport protein) were components of the IMPs.[3]

I believe I introduced two words into the vocabulary of membranologists: intercalation and topology. In Portugal I studied basic concepts of geometric topology. I felt (and still feel) that the study of topology of a system precedes that of its structure. Thus, to me, questions such as the existence or not of bilayers, and whether IMPs corresponded to molecules intercalated across or buried within the apolar matrix of the membrane, dealt with topology, as also did those related to the distribution of apolar/polar domains in intercalated proteins. The solutions that I proposed in 1971 and 1972[2] held, and they explained the vertical stability as well as the potential rotational and translational freedom of transmembrane proteins.

Since 1979 my laboratory has developed other combinations of freeze-fracture and cytochemistry:[4,5] fracture-label (to label split membrane "halves"), fracture-permeation (to study intermolecular spacing in cytomatrices), and label-fracture (to relate immunogold surface labels to freeze-fracture images). With our recent discovery of fracture-flip,[6] images of membrane fracture faces are replaced by extended, high-resolution views of actual membrane surfaces.

I see biological membranes as instances of form modulated by structure. Having faced for 20 years the enigmatic, beautiful, and distant micrographs of fractured membrane faces—the outcome of a process—the recent discovery that a mere "flip"[6] can so easily reveal the actual surfaces of biomembranes makes me think that, one day, the superreal that touches and hides just behind the "skin" of our visual reality will, with sleight of hand, reveal that masked, yet almost familiar, face that is welded to every moment of our lives.

1. **Pinto da Silva P, Douglas S D & Branton D.** Localization of A antigen sites on human erythrocyte ghosts. *Nature* 232:194-6, 1971. (Cited 175 times.)
2. **Pinto da Silva P.** Translational mobility of the membrane intercalated particles of human erythrocyte ghosts: pH-dependent, reversible aggregation. *J. Cell Biol.* 53:777-87, 1972. (Cited 330 times.)
3. **Pinto da Silva P & Nicolson G.** Freeze-etch localization of concanavalin A receptors to the membrane intercalated particles in human erythrocyte membranes. *Biochim. Biophys. Acta* 363:311-9, 1974. (Cited 265 times.)
4. **Pinto da Silva P.** Topology, dynamics and molecular cytochemistry of integral membrane proteins: a freeze-fracture view. (Harris J R & Horne R W, eds.) *Electron microscopy of proteins. Volume 6. Membranous structures.* London: Academic Press, 1987. p. 2-38.
5. ———. Molecular cytochemistry of freeze-fractured cells: freeze-etching, fracture-label, fracture-permeation, and label-fracture. *Advan. Cell Biol.* 1:157-90, 1987.
6. **Andersson-Forsman C & Pinto da Silva P.** Fracture-flip: new high resolution images of cell surfaces after carbon stabilization of freeze-fractured membranes. *J. Cell Sci.* 90:531-41, 1988.

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