Contributions to Science

1. **Discovery of the release of free docosahexaenoic acid (DHA) and arachidonic acid during seizures or ischemia by a phospholipase A2 (PLA2) driven event key for cell function.** In Dr. Nicolas Bazan's first lab in 1968-1970 (Clarke Institute of Psychiatry, University of Toronto, Canada) using rapid brain sampling and a simple preparative isolation technique that he developed in combination with GLC, Bazan found DHA and AA to be released during ischemia at rates comparable to maximal hormonal lipolytic stimulation. He observed negligible amounts of unesterified (free) DHA and arachidonic acid (AA) in the control brains, in contrast with several publications showing relatively large pool sizes of these fatty acids in the free form. Since he observed that the low abundance brain triglycerides were unchanged, he proposed that the fatty acids have to be released from membrane PLs by means of PLA2. Because of the high rate of release, he thought that synaptic activation could be involved, and sure enough he found that seizures elicit a similar release of the fatty acids. This has often been referred as the "Bazan effect" (Citation Classic, "Neural Stimulation or Onset of Cerebral Ischemia Activates Phospholipase A2": Current Contents, Life Sciences, 1991). At that time Hans Olaf Bang and Jorn Dyerberg found beneficial health effects of diet rich in omega-3 fatty acids. They visited Invit villages in northwest Greenland and observed that the people in those places that ate whales, seals and fish rarely have heart attacks and do have other health benefits. So Bazan began pondering upon the significance of brain DHA function and release under various conditions, as described below. Many unexpected outcomes have evolved including the finding that the phospholipid-mediator, platelet-activating factor (PAF), modulates hippocampal excitatory synaptic transmission, presynaptic glutamate release and is a retrograde messenger of long-term potentiation enhancing memory formation. PAF synthesis involves DHA and AA release. So he began connecting the initial findings with synaptic signaling and function.

2. **Identification of molecular mechanisms in the supply and retention of DHA in the CNS.** The essential omega-3 fatty acid family member, DHA, is avidly retained in the CNS where it attains the highest concentrations in the human body. While studying its release, several years ago, Bazan began using the retina as a nature-made brain slice, because its differentiated neurons (photoreceptor cell) are enriched in DHA and its neuronal circuitry makes it an integral part of the CNS. In the early 1970s, his laboratory uncovered an unusually high content of DHA in retina diacylglycerol and phosphatidic acid, prompting the suggestion that the "vertebrate retina… [has] developed a metabolic differentiation… to sustain photoreceptors…" particularly during outer segment renewal (1973). Morover, he identified phosphatidic acid synthesis, enriched in DHA, as a route for phospholipid biosynthesis rather than the acylation/re-acylation cycle. Also during that time, he and his graduate students (particularly M. Aveldańo) discovered "supraenoic molecular species" (containing more than one DHA per molecule) of phospholipids that display high rates of synthesis in photoreceptors. Then he stumbled on new mechanisms regarding how DHA is acquired to reach such unique endowment in the CNS. Thus, he identified the liver-to-retina (and brain) "long loop" for DHA supply (1985-89) and a retinal pigment epithelium/photoreceptors intercellular "short loop" for DHA retention in photoreceptors (1990-91). This recycling by means of the short loop is similar to one of retinoids, and he postulated it to be critical for photoreceptor survival; hence, its breakage leads to retinal degenerations. Bazan found that Usher's Syndrome patients have DHA shortage in the blood, implicating the long loop in retinal degenerations (1986). This observation, among others from his lab, led him to further explore the role of DHA in photoreceptor degeneration. Very recently, Bazan's lab and collaborators, which include Dr. Dennis Rice, led the discovery of a specific transmembrane protein (Adiponectin Receptor 1) for uptake/retention in RPE cells and photoreceptors necessary for cell functional integrity (2015). This AdipoR1-protein is not a G-protein, and he demonstrated that the ligand is not the cognate ligand, adiponectin. He showed that AdipoR1 represents a key molecular switch in the conversion of DHA to a photoreceptor-specific molecular species of phosphatidylcholine that are decreased in age related macular degenerations. In fact, when Bazan and collaborators genetically ablated the protein, retinal degeneration ensued (2015).

3. **Contributed to the discovery of the first docosanoid, Neuroprotectin D1 (NPD1).** Bazan then demonstrated that DHA (22 C and 6 double bonds) is the precursor for pro-homeostatic cell survival mediators: the docosanoids (22 C, in contrast to the 20C-eicosanoids from AA). He coined the term docosanoids in 1984. He and his colleagues, which include Dr. Charles Serhan, discovered the synthesis and bioactivity of, NPD1 (10R,17S-dihydroxy-docosa-4Z,7Z,11E,13E,15Z,19Z-hexaenoic acid), in 2003-2004. NPD1 arrests apoptosis in RPE cells at the pre-mitochondrial level, and is neuroprotective in brain ischemia-reperfusion and in cellular models of Alzheimer's disease. Thus, he and his colleagues coined the name **neuroprotectin D1** for this first-identified docosanoid. Several labs including his showed that NPD1 is a stress/injury response mediator made on demand that counteracts disruptions of cellular homeostasis, and is an active participant in a well-concerted process that effectively modulates neuroinflammation. Esterified-DHA from PLs is cleaved by PLA2, releasing DHA, followed by NPD1 synthesis. Bazan also showed that 15-LOX-1 catalyzes DHA enzymatic oxygenation and conversion into NPD1 (2010), and that neurotrophins stimulate this process (e.g., BDNF, NGF, PEDF) (2007). He and his colleagues found enhanced cytosolic PLA$_2$ expression and decreased free-DHA in short post-mortem sampled CA1 hippocampal region of early stages of Alzheimer's disease (AD) with a concomitant 25-fold decrease in NPD1 (2005). Then he showed that NPD1 promotes down-regulation of pro-inflammatory genes and of pro-apoptotic Bcl-2 proteins, and promotes neuronal and glial cell survival from Aβ toxicity. Among the molecular targets that he found for this bioactive lipid is the triggering of dephosphorylation of Bcl-xL in a PP2A-dependent fashion during oxidative stress that as result induces cell survival (2012).

4. **Discovery of DHA protective bioactivity in ischemic stroke and epileptogenesis: identification of novel neuronal-specific molecular mechanisms.** Following the early observation of ischemia-mediated released DHA, Bazan and his colleagues demonstrated that DHA induces cell survival, modulates neuroinflammation, and activates long-term restoration of synaptic circuits in models of epileptogenesis (2011-2015). Thus the release has beneficial effects. DHA improves recovery after experimental ischemic stroke and through its conversion into NPD1 to halt homeostasis disruptions and cell death signals (2011). Increased NPD1 synthesis, as a consequence of DHA administration in the stroke MCAo model, prompts selective neuronal cREL translocation followed by BIRC3 expression, resulting in remarkable neurological recovery (2015). Thus, cREL was translocated into the nucleus to a greater extent in DHA-treated animals, suggesting that NPD1 produced by the conversion of systemically-administered DHA acts through cREL-mediated BIRC3 transcriptional activation to elicit its neuroprotective bioactivity. In his cellular model, Bazan also found that when the cREL protein increases, it leads to survival and a decrease in p65/RelA, in response to NPD1 (2015). These findings are helping further unravel the endogenous signaling that sustains cellular integrity, thus providing a greater understanding of these mechanisms that could lead to novel precise therapeutic approaches for neuroprotection.

5. **Development of novel synthetic compounds.** By effective collaborations with medicinal chemists, Bazan contributed to the development of: a) a non-toxic paracetamol analog (a new 'Tylenol'), b) novel PAF receptor antagonists and c) novel docosanoids with Prof. Nicos A. Petasis from the University of Southern Callifornia, CA. The phospholipid mediator PAF accumulates in the brain in ischemic stroke and in turn activates the transmembrane PAF receptor (PAF-R). Excessive accumulation of PAF activates the receptor, triggering a myriad of pro-neuroinflammatory events that include: enhanced excitotoxicity by stimulating glutamate release, inhibition of ionotropic GABA receptor, apoptosis, induction of matrix metalloproteinases 1 and 9, activation of COX-2 transcription, and complement activation. Thus PAF-R antagonists have the potential of curtailing critical events of neuroinflammatory signaling activated by PAF. Bazan developed low molecular weight PAF-R antagonists; the series is called LAU compounds (Louisiana Alcala Universities, reflecting the collaboration of his lab with Prof Alvarez–Builla from the University of Alcala, Spain). One of the members of this series, LAU-0901 downregulates neuroinflammation and reduces the volume of the penumbra after MCAo. Thus LAU PAF-R antagonists are highly neuroprotective in experimental ischemic stroke. Bazan also developed a series of acetaminophen (APAP)

analogs, 2-(1,1-dioxido-3-oxo-1,2-benzisothiazol-2(3H)-yl)-N-(4-hydroxyphenyl) alkanecarboxamides, bearing a heterocyclic moiety linked to the p-acylaminophenol fragment. Unexpectedly, these compounds maintained the *in vivo* analgesic profile, while the characteristic hepatotoxicity of APAP was reduced. Analgesic efficacy was comparable to that of APAP. Overall, these compounds display a favorable safety profile as an orally delivered analgesic.