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The Life and Work of Dr Robert Bittman (1942-2014)

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Short Title: A tribute to Dr Robert Bittman

Abstract--With the passing of Dr. Robert Bittman on the 1st Oct 2014 from cancer, the lipid field lost its most distinguished lipid chemist researcher. Herein, we provide a brief account of Dr. Robert Bittman's research achievements over a 50 year career. Dr. Robert Bittman's lasting legacies to the fields of lipid membrane biophysics, cholesterol trafficking and cellular functions of phospholipids and sphingolipids are truly inestimable.

Key words: Cholesterol, sphingolipids, phospholipids, ceramide, membrane biophysics, anti-cancer

Dr Robert Bittman's genius was in chemical design and eye to spot a complex biological problem that required solving. Many scientists benefited enormously from Bob's vocation. Some of the major breakthroughs in our current understanding of the role of lipids in constituting complex biophysical membranes, sterol transfer and participation in cellular communication networks were made achievable by unique chemical reagents devised by Bob. Bob published 322 peer-reviewed papers, contributed 64 book chapters and filed 19 US patents. This prolific output reflects the energy and enthusiasm that Bob brought to integrating chemistry with topical biological research problems. For this effort, Bob was funded continuously by the NIH grant mechanism from 1973 to the beginning of 2014, including a distinguished MERIT award from the National Heart Lung and Blood Institute from 1986.

Bob enrolled at the City University of New York at the age of sixteen, where he majored in chemistry in 1962, and then completed a PhD in chemistry at University of California, Berkeley, under the mentorship of Andrew Streitwieser in 1965. For postdoctoral training, Bob joined the laboratory of Nobel Laureate Manfred Eigen at the Max Planck Institute for Physical Chemistry in Gottingen, Germany in 1965. His first paper was published in 'The Proceedings of the National Academy of Sciences'; a citation classic that Bob was immensely proud of. This study involved deciphering the allosteric mechanism involved in binding of nicotinamide adenine dinucleotide to D-glyceraldehyde-3-phosphate dehydrogenase, employing analysis of temperature-jump relaxation kinetics (Kirschner et al. 1966). In 1966, Bob joined the faculty of CUNY and became a Distinguished Professor of Chemistry and Biochemistry of Queens College and the Graduate Centre of CUNY in 1988. Initially Bob focused on interaction of proteins, such as filipin with sterol-containing lipid vesicles and cellular membranes using spectral and electron microscopy and stop flow kinetic and equilibrium analysis (Bittman et al. 1974). Bob published on the distribution of cholesterol

between the outer and inner halves of the lipid bilayer of mycoplasma cell membranes (Bittman & Rottem, 1976) and the lack of effect of ester or ether linkages in phosphatidylcholine on the ability of cholesterol to reduce bilayer permeability (Bittman et al. 1984). There was also determination of the rates of rapidly exchanging cholesterol and phospholipid pools in sphingomyelin- and phosphatidylcholine-containing cells (Clejan et al. 1984).

From the mid-1990's Bob's interest in the role of lipids as mediators of cell function flourished. He was instrumental in developing synthetic routes for platelet activating factor analogues and assessing their biological activity in cancer cells (Bittman et al. 1987; Salari et al. 1992). Bob also developed a highly productive collaboration of Dr. Gilbert Arthur on the synthesis and biological assessment of ether lipids. These ether lipids function as anti-tumour agents. For instance, ET18-OCH3 induced inhibition of cell proliferation, Raf association with the membrane, and mitogen activated protein kinase activation (Zhou et al. 1996). In the early 1990's, Bob also collaborated with Dr. Richard Kolesnick in a foray into sphingolipid signalling. This resulted in the demonstration that ceramide inhibits diacylglycerol kinase in cells (Younes et al. 1992), thereby establishing a reciprocal relationship between diacylglycerol and ceramide. This was a very novel concept at that time, given its significance as a major factor in defining life or death of cells. The collaboration also resulted in the synthesis of additional sphingolipids that enabled study of the structural/functional relationship of a newly-identified ceramide-activated protein kinase that was involved in mediating the effect of tumour necrosis factor alpha on differentiation of monocytic cancer cells (Joseph et al. 1993).

Ceramide 1-phosphate had been characterised before, but Bob developed chemical synthetic routes from ceramide to produce this lipid (Byun et al. 1994). With Drs. Antonio Gomez-Muñoz and David Brindley there was further characterisation of the biological activity of

ceramide 1-phosphate, specifically in fibroblasts where it stimulates proliferation (Gomez-Muñoz et al. 1995). This interest in ceramide biology grew exponentially over time, and Bob published many papers on novel synthetic routes for caged ceramide 1-phosphate (Lankalapall et al. 2009), phytoceramide and phytosphingosine (He et al. 2000), *trans* double bond sphingolipid analogues: Δ^4 and $\Delta^{4,6}$ ceramides (Chun et al. 2001) and fluorescent lactosylceramide stereoisomers (Liu and Bittman, 2006). There were also studies involving the use of α-C-galactosylceramide analogues to induce TH1-biased responses in human natural killer T-cells (Lu et al. 2006) and on characterisation of the recognition of CD1d-*C*-galactosylceramide by natural killer T-cell receptor (Patel et al. 2011).

Bob further demonstrated that sphingomyelin modulates transbilayer distribution of galactosylceramide in phospholipid membranes (Mattjus et al. 2002). Using fluorescence quenching assays, there was also an assessment of the effect of ceramide on lipid raft organisation and sterol content (Megha et al. 2007). At this time, Bob also synthesised BODIPY-cholesterol to visualise sterol trafficking in cells (Hölttä-Vuori et al. 2008). Indeed, BODIPY-cholesterol has recently been used to demonstrate that N-myc downstream-regulated gene 1 (NDRG1) regulates low density lipoprotein (LDL) uptake by LDL receptor (Pietiäinen et al. 2013).

Bob expansively turned his hand to synthesis of analogues of lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P), bioactive lipids that bind to families of G-protein coupled receptors that function to programme cell responses, such as proliferation and migration. In this regard, Bob established collaboration with Dr. Gabor Tigyi. This resulted in synthesis of inhibitors of LPA receptors (Bittman et al. 1996), ligands (alkyl ether analogues or unsaturated acyl forms of LPA) of peroxisome proliferator-activated receptor γ that promote neointimal formation (Zhang et al. 2004) and carba analogues of cyclic phosphatidic acid,

which were used as selective inhibitors of autotaxin to block cancer cell invasion and metastasis (Baker et al. 2006).

Bob's interest in S1P expanded in 2003, to collaboration with many cutting edge research groups working in the area. Seminal findings during this period included studies on the uptake and metabolism of S1P in lung endothelial cells (Zhao et al. 2007), inhibition of ceramide synthase by the sphingosine analogue FTY720 (Berdyshev et al. 2009), identification of erythrocytes as a major reservoir of S1P (Bode et al. 2010), and development of a novel assay for S1P lyase activity using labelled BODIPY-sphingosine 1-phosphate (Bandhuvula et al. 2009). A highlight in this area was the synthesis of sphingadienes and characterisation of their biological activity in cancer. Collaboration with Dr. J. Saba revealed that sphingadienes induce colon cancer cell death by blocking AKT translocation to plasma membrane, promoting apoptosis and autophagy (Fyrst et al. 2009). Sphingadienes also down-regulated the Wnt signalling program, which when dysregulated is a key driver of colon cancer tumourigenesis (Kumar et al. 2012). Most recently, these workers found that up-regulation of S1P lyase by dietary sphingadienes reduces colon carcinogenesis and is associated with down-regulation of S1P and Signal Transducer Activator of Transcription 3 (STAT3), instigating relief from STAT3 activated miRNAs that suppress anti-oncogenes (Degagné et al. 2014).

In the midst of all this activity, Bob developed a productive collaboration with Drs. Nigel and Susan Pyne, using the FTY720 scaffold (FTY720 is characterised as a functional antagonist of the $S1P_1$ receptor marketed as the first oral treatment of relapsing multiple sclerosis under the trade name, Gilenya^{TM}) to develop inhibitors of sphingosine kinase 1 and 2 (SK1 and SK2). This included identification of (*R*)-FTY720 methyl ether, a selective SK2 inhibitor that prevents formation of actin-enriched lamellipodia typical of a migratory phenotype in breast cancer cells (Lim et al. 2011) and induces death of T cell acute lymphoblastic leukaemia cells

(Evangelisti et al. 2014). The collaboration also resulted in extensive analysis of structural activity relationships of SK1 selective inhibitors, including modelling into SK1 to establish optimal binding modalities (Baek et al. 2013). Such advances may significantly impact future development of SK inhibitors for therapeutic treatment of cancer. As Bob's research accomplishments are many and his collaborations widespread, we apologise to those contributors who are not mentioned here.

Bob's research achievements were recognised by several plenary lectures (in Japan and Finland), and by awards including the Avanti Award of the American Society of Biochemistry and Molecular Biology in 2003 and a Fellowship from the AAAS in 2004. He served on many Editorial Boards and NIH panels such as Organic Reactions (Secretary or Co-Secretary, 1968-2014), Journal of Lipid Research (1985-2006), Biophysics Section of New York Academy of Sciences (Vice-Chairman, 1975-76; Chairman, 1977-78), N.I.H. Biophysical Chemistry Study Section, 1983, 1996, N.I.H. Panel on Minority Biomedical Research Support, 1991, N.I.H. Panel on the National Cooperative Drug Discovery Group, 1991 and N.I.H. Panel - Program Project Reviews, 2000. Bob was an excellent and patient teacher and mentor. He trained over 20 post-doctoral and 23 graduate students. Bob coordinated a Membrane Lipid Biochemistry course at the City University of New York that featured leading investigators in the lipid field. Both authors of this tribute had the pleasure to be involved in this teaching and one could recognise the high degree of respect he was regarded by his students. Bob was a terrific tennis player and had an abiding interest in the performing arts. He is survived by his wife, Marlene, and two children. All of us will greatly miss Bob, who was, among other things, a distinguished scholar, an excellent teacher and mentor, and a very good friend and colleague.

The City University of New York has established a memorial fund to honour Bob's legacy to be used for the support and development of Chemistry and Biochemistry students at Queens

College. Description of the Robert Bittman Memorial Fund can be found by visiting https://qccommunity.qc.cuny.edupages/funds/memory-of-dr-robert-bittman.

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