Harvey Thomas McMahon

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Present Position: Tenured Group Leader
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EDUCATION:
University:
1983-1987 Trinity College, Dublin. B.A.Mod in Biochemistry, First Class Honours.
1987-1990 Dundee University, Scotland. Ph.D in Neurochemistry. Thesis title 'Glutamate neurotransmitter: new insights into the release mechanism'.

HONOURS and AWARDS:
Associate of the London College of Music- A.L.C.M. piano.
EMBO member 2005.
The Sackler International Prize in Biophysics 2006.
The Royal Society 2008

COMMUNITY:
School governor 2005-2006 and involvement in a range of initiatives, from providing holidays for mentally handicapped children, to providing support for international students in Cambridge.

CAREER
1990-1991: Postdoctoral research position following on from a PhD with Prof. David Nicholls, Dundee University, Dundee, Scotland.

During my PhD with Prof. Nicholls I had worked on the pharmacology and mechanics of glutamate transmitter release. It was not clear at this point that glutamate was primarily released from synaptic vesicles. I demonstrated biphasic kinetics of glutamate exocytosis in a calcium-dependent manner after sustained depolarisation and proposed that the two phases were due to docked and reserve vesicle pools. I also observed a more efficient release of transmitter with calcium channel opening than with non-specific calcium entry and thus provided evidence for a direct coupling mechanism between calcium entry and vesicle release. Both of these observations have been reinforced in many subsequent papers in the literature. I introduced the use of (inwardly-rectifying) potassium channel blockers as a means to mimic action potentials and achieve a more physiological stimulus of transmitter release in isolated nerve terminals. This is now also a standard protocol for this

preparation. I showed the mechanism of action of a-latrotoxin in inducing glutamate release, the different inhibitory effects of tetanus and botulinum toxins on glutamate exocytosis, and the effects of anoxia and ischaemia on the reversal of glutamate transporters. All of this work culminated in the writing of many papers and an invited review for BBA in 1991 on *The Bioenergetics of Transmitter Release*. This review covered many of the bioenergetic considerations that were a theme throughout the work of my Ph.D. I was awarded a medal for this work and delivered an ESN honorary lecture in Dublin in 1992. I also demonstrated the techniques developed during these years at a number of international neuroscience workshops.

1991-1995: Howard Hughes Research Fellow with Prof. Thomas Südhof, UTSW, Dallas, USA.

Here I worked on the idea that synaptic mechanisms are not unique but simply specialisations of more ubiquitous processes. In line with this I cloned *Cellu*brevin (a homologue of *synapto*brevin) and showed its involvement in vesicle trafficking outside the synapse. I went on to work on the SDS resistance of the SNARE complex and the differential sensitivities of SNARE proteins to tetanus and botulinum toxins when present in complexes (in collaboration with Heiner Niemann), and thus explained the distinct *in vivo* effects of these toxins. The SDS resistance of the SNARE complex is now used as a standard protocol to detect stable SNARE complex formation by those working on synaptic vesicle fusion mechanisms. I then followed up the SDS resistance of the SNARE complex by finding an additional protein doublet that co-migrated with synaptobrevin on SDS-PAGE and was thus missed as part of the SNARE complex. I sequenced and cloned the proteins and named these complexin A and B. I went on to work on the function of complexins in the regulation of SNARE assembly and vesicle release. Many papers have since been published on these proteins and they have been shown to function in calcium-dependent membrane fusion. Finally, I started to make a number of knock-out mouse models and completed one for synaptophysin.

1995-present (tenured in 2000):

Staff scientist and group leader at the Medical Research Council Laboratory of Molecular Biology, Cambridge, England.

At the LMB I changed my main focus of research from exocytosis to start working on *molecular mechanisms of endocytosis*. We initially started by combining structural and functional approaches to clathrin-mediated endocytosis to determine how this process works at a molecular level. With time we became interested in the ways in which proteins could effect *membrane bending,* and this naturally led us into defining novel mechanisms of vesicle budding and to the study of how many different pathways of vesicle trafficking can be coordinated. Thus we now study *protein networks* from a cell biologist's point of view. We have also recently been deciphering how membrane fusion can possibly occur without proteins that can

effect membrane curvature stress at an appropriate focus point. These interests are highlighted in the accompanying resumé of research.

Web Pages

For details of current research interests see: http://www.endocytosis.org/. I maintain these pages as an educational and social forum and I am also a regular contributor to the online encyclopaedia at http://www.wikipedia.org/.

Reviewing and committee memberships

I frequently review manuscripts for Nature, Science, Cell and the Journal of Cell Biology (and many others). I advise Nature on difficult manuscripts. I review grants for the Wellcome Trust, NIH, NSF, BBSRC, and MRC. I sit on the finance and seminars committees for the MRC.

Science and the community: I initiated and continue to run a competition with school students to develop a deeper interest in science. This is aimed at 16-18 year olds who study both science and art in their final years at secondary school. We are aiming for a mutual education process where their art will help us to simplify concepts and our teaching will enlighten the students about current exciting areas of science and inform their art to make it accurate and informative. Local sixth formers are invited to submit a piece of art work depicting an aspect of neuroscience and the winning entrant (judged by professional artists) is invited for a summer placement in my lab as an artist in residence. The initiative is called '*Imagining the Brain*' and details can be found at http://www.endocytosis.org/ImaginingTheBrain/ along with some information on winners and images from competition participants.

To give back to the local Sixth Form schools this initiative has involved members of my lab delivering a series of neuroscience lectures and basic science talks. We also take work experience students for placements during the summer and grasp any opportunity to demonstrate the exciting and challenging nature of a scientific career to young people. I participate in lectures to postgraduate students both within our own institute and in other MRC institutes. I also frequently teach on EMBO and FEBS practical courses, some of which also involve practical demonstrations.

Harvey T. McMahon – Publications

Summaries of the contents of all these papers can be found on http://www.endocytosis.org/publica/papers.html

1. Nicholls, D.G., Barrie, A.P., McMahon, H.T., Tibbs, G. and Wilkinson, R. Mechanisms of Glutamate Exocytosis from Isolated Nerve Terminals. In, Nato ASI Series H: Cell Biology: "Receptors, Membrane Transport and Signal Transduction" **29**, 147-161 (1988).

2. McMahon, H.T., Kauppinen, R. and Nicholls, D.G. The energetics of Ca-dependent release from synaptosomes and the effect of experimental anoxia, hypoglycaemia and ischaemia. Biochem. Soc. Trans. **16**, 880-881 (1988).

3. Kauppinen, R.A., McMahon H.T., and Nicholls, D.G. Calcium-dependent and calcium-independent glutamate release, energy status and cytosolic free calcium concentration in isolated nerve terminals following *in vitro* hypoglycaemia and anoxia. Neuroscience **27**, 175-182 (1988).

4. Tibbs, G., Barrie, A.P., Van Mieghem F., McMahon, H.T. and Nicholls, D.G. Repetitive action potentials in isolated nerve terminals in the presence of 4-aminopyridine: effects on cytosolic free Ca^{2+} and glutamate release. J. Neurochem. **53**, 1693-1699 (1989).

5. McMahon, H.T., Barrie, A.P., Lowe, M. and Nicholls, D.G. Glutamate release from guinea pig synaptosomes: Extra-cellular glutamate may exert a positive feedback by reuptake-induced depolarisation rather than receptor activation. J. Neurochem. **53**, 71-79 (1989).

6. Kauppinen, R.A., McMahon, H.T., and Nicholls, D.G. The effects of experimental anoxia on glycolysis, energy status, cytosolic free calcium, and glutamate release from isolated nerve terminals, in *Cerebral Ischaemia and Calcium* (Hartmann A. and Kuschinsky W. eds.) Springer, Berlin, 1990.

7. McMahon,H.T. and Nicholls, D.G. Glutamine and aspartate loading of guinea pig cerebral cortical synaptosomes: a re-evaluation of effects on Ca^{2+}-dependent excitatory amino acid release. J. Neurochem. **54**, 373-380 (1990).

8. McMahon, H.T., Rosenthal, L., Meldolesi, J and Nicholls, D.G. α-Latrotoxin releases both vesicular and cytoplasmic glutamate from isolated nerve terminals. J. Neurochem. **55**, 2039-2047 (1990).

9. McMahon, H.T. and Nicholls, D.G. The relationship between cytoplasmic free Ca^{2+} and the release of glutamate from synaptosomes. Biochem. Soc. Trans. **18**, 375-377 (1990).

10. Verhage, M., McMahon, H.T. Boomsna, Wiegant and Nicholls, D.G. Release of amino acids, cholecystokinin and noradrenaline from isolated hippocampal terminals show differing preferences for localised versus delocalised Ca^{2+} entry. Neuron **6**, 517-524 (1991).

*11. McMahon, H.T. and Nicholls, D.G. Transmitter glutamate release from isolated nerve terminals: evidence for biphasic release and triggering by localized Ca^{2+}. J. Neurochem. **56**, 86-94 (1991).

12. McMahon, H.T. and Nicholls, D.G. The Bioenergetics of Neurotransmitter Release (invited review). BBA **1059**, 243-264 (1991).

*13. McMahon, H.T., Foran, P., Dolly, J.O., Verhage, M., Wiegant, V.M. and Nicholls, D.G. Tetanus and Botulinum toxins type A and B inhibit glutamate, GABA, aspartate and met-enkephalin release from synaptosomes: Clues to the locus of action. J. Biol.Chem. **267**, 21338-21343 (1992).

14. McMahon, H.T. and Nicholls, D.G. Barium-evoked glutamate release from guinea-pig cerebrocorical synaptosomes. J. Neurochem. **61**, 110-115 (1993).

15. Robinson, P.J., Sontag, J-M., Liu, J-P., Fyske, E.M., Slaughter, C., McMahon, H.T. and Südhof, T.C. Dynamin GTPase regulated by protein kinase C phosphorylation in nerve terminals. Nature **365**, 163-166 (1993).

16. Link, E., McMahon, H., Fischer von Mollard, G., Yamasaki, S., Niemann, H., Südhof, T.C. and Jahn, R. Cleavage of cellubrevin by tetanus toxin does not affect fusion of early endosomes. J. Biol.Chem. **268**, 18423-18426 (1993).

*17. McMahon, H.T., Ushkaryov, Y.A., Edelmann, L., Link, E., Binz, T., Niemann, H., Jahn, J. and Südhof, T.C. Cellubrevin is a ubiquitous tetanus-toxin substrate homologous to a putative synaptic vesicle fusion protein. Nature **364**, 346-349 (1993).

18. Ullrich, B., Li, C., Zhang, J.Z., McMahon, H.T., Anderson, R.G.W., Geppert, M. and Südhof, T.C. Functional properties of multiple synaptotagmins in brain. Neuron **13**, 1020 (1994).

*19. Hayashi, T., McMahon, H.T., Yamasake, S., Binz, T., Hata, Y., Südhof, T. C. and Niemann, H. Synaptic vesicle membrane fusion complex: Action of Clostridial neurotoxins on assembly. EMBO J. **13**, 5051-5061 (1994).

20. McMahon, H.T. and Südhof, T.C. Synaptic core complex of synaptobrevin, syntaxin and SNAP25 forms a high affinity αSNAP binding site. J. Biol. Chem. **270**, 2213-2217 (1995).

*21. McMahon, H.T., Missler, M., Li,.C. and Südhof, T.C. Complexins: Cytosolic proteins that regulate SNAP receptor function. Cell **83**, 111-119 (1995).

22. McMahon, H.T., Bolshakov, V.Y., Janz, R., Hammer, R.E., Siegelbaum, S.A. and Südhof, T.C. Synaptophysin, a major synaptic vesicle protein, is not essential for neurotransmitter release. Proc. Natl. Acad. Sci. USA **93**, 4760-4764 (1996).

23. McMahon, H.T., Wigge, P. and Smith, C. Clathrin interacts specifically with amphiphysin and is displaced by dynamin. FEBS Lett. **413**, 319-322 (1997).

24. Wigge, P., Vallis, Y. and McMahon, H.T. Inhibition of receptor-mediated endocytosis by the amphiphysin SH3 domain. Curr. Biol. **7**, 554-560 (1997).

25. Wigge, P., Köhler, K.,Vallis, Y., Doyle, C.A., Owen, D., Hunt, S.P. and McMahon, H.T. Amphiphysin heterodimers: potential role in clathrin-mediated endocytosis. Mol. Biol. Cell **8**, 2003-2015 (1997).

*26. Marks, B. and McMahon, H.T. Calcium triggers calcineurin-dependent synaptic vesicle recycling in mammalian nerve terminals. Curr. Biol. **8**, 740-749 (1998).

*27. Owen, D.J., Wigge, P., Vallis, Y., Moore, J.D.A., Evans, P.R. and McMahon, H.T. Crystal structure of the amphiphysin-2 SH3 domain and its role in the prevention of dynamin ring formation. EMBO J. **17**, 5273-5285 (1998).

28. Wigge, P. and McMahon, H.T. The amphiphysin family of proteins and their role in endocytosis at the synapse. Trends Neurosci. **21**, 339-344 (1998).

29. Wigge, P. and McMahon, H.T. Amphiphysins. In: Guidebook to the Cytoskeletal and Motor Proteins, (T. Kreis and R. Vale, eds.), pp. 502-504, Oxford University Press 1998.

*30. Owen, D.J., Vallis, Y., Noble, M.E.M., Hunter, J.B., Dafforn, T. R., Evans, P. R. and McMahon, H.T. A structural explanation for the binding of multiple ligands by the α-adaptin appendage domain. Cell **97**, 805-815 (1999).

31. Stowell, M.H.B., Marks, B., Wigge, P. and McMahon, H.T. Nucleotide-dependent conformational changes in dynamin: evidence for mechanochemical molecular spring. Nature Cell Biol. **1**, 27-32 (1999).

32. Vallis, Y., Wigge, P., Marks, B., Evans, P.R. and McMahon, H.T. Importance of the pleckstrin homology domain of dynamin in clathrin-mediated endocytosis. Curr. Biol. **9**, 257-260 (1999).

33. Marsh, M. and McMahon, H.T. The structural era of endocytosis. Science **285**, 215-220 (1999).

34. McMahon, H.T. Endocytosis: An assembly protein for clathrin cages. Curr. Biol. **9**, R332-R335 (1999).

35. Owen, D.J., Vallis, Y., Pearse, B.M.F., McMahon, H.T. and Evans, P.R. The structure and function of the β2-adaptin appendage domain. EMBO J. **19**, 4216-4227 (2000).

36. Wigge, P. and McMahon, H.T. Synaptic vesicle recycling: multiple pathways and functions in the nervous system. In: Endocytosis, (M. Marsh, ed.), pp. 204-217, Oxford University Press, 2000.

*37. Ford, M.G.J., Pearse, B.M.F., Higgins, M.K., Vallis, Y., Owen, D.J., Gibson, A., Hopkins, C.R., Evans, P.R. and McMahon, H.T. Simultaneous binding of Ptdlns(4,5)P_2 and clathrin by AP180 in the nucleation of clathrin lattices on membranes. Science **291**, 1051-1055 (2001).

*38. Marks, B., Stowell, M.H.B., Vallis, Y., Mills, I.G., Gibson, A., Hopkins, C.R. and McMahon, H.T. GTPase activity of dynamin and resulting conformation change are essential for endocytosis. Nature **410**, 231-235 (2001).

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essential for retromer-mediated endosome-to-TGN transport. J. Cell. Sci. **118**, 4527-4539 (2005).

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analysis of FCHo2 F-BAR domain: a dimerising and membrane recruitment module that effects membrane curvature. Structure **15**, 839-852 (2007).

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68. Lene E. Olesen, Eva M. Schmid, Marijn G. J. Ford, Yvonne Vallis, M. Madan Babu, Peter Li, Ian G. Mills, Philip R. Evans, (Harvey T. McMahon and Gerrit J.K. Praefcke) (2008) Solitary and repetitive binding motifs for the AP2 complex alpha-appendage in amphiphysin and other accessory proteins. J.Biol.Chem. **283,** 5099-5109. First published on line in 2007, JBC Papers in Press, November 6, 2007, DOI 10.1074/jbc.M708621200.

69. Gary J. Doherty and Harvey T. McMahon (2008) Mediation, Modulation, and Consequences of Membrane-Cytoskeleton Interactions. Annual Review in Biophysics **37,** 65-95. First published online as a Review in Advance on December 3, 2007 (doi:10.1146/annurev.biophys.37.032807.125912).

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71. Sascha Martens and Harvey T. McMahon (2008) Mechanisms of membrane fusion: disparate players and common principles. Nature Reviews Molecular Cell Biology, **9,** 543-556 (July issue). Advance online publication, 21 May 2008 | doi:10.1038/nrm2417, PMID: 18496517

72. Felix Campelo, Harvey T. McMahon and Misha M. Kozlov (2008) The hydrophobic insertion mechanism of membrane curvature generation by proteins. Biophys J. 2008 May 30. [Epub ahead of print] doi:10.1529/biophysj.108.133173, PMID: 18515373

73. Richard Lundmark, Gary J. Doherty, Yvonne Vallis, Brian J. Peter and Harvey T. McMahon (2008) Arf family GTP loading is activated by, and generates, positive membrane curvature. Biochemical Journal 414, 189-194.

Highlights of 20 papers above marked with an asterix

Defining a role for membrane curvature stress in membrane fusion[64]. Synaptotagmin-1 is the major calcium sensor for synaptic vesicle exocytosis and the mechanism by which it controls vesicle fusion in response to calcium influx at the synapse has been intensely studied for many years. We discovered that synaptotagmin can drive extreme positive membrane curvature upon binding to membranes[64]. We showed that it performs this by inserting hydrophobic residues of its C2 domains into the hydrophobic phase of the synaptic membrane, and showed that the calcium-dependent induction of membrane curvature by synaptotagmin-1 is essential for the promotion of SNARE-dependent membrane fusion. We proposed that the extreme membrane curvature induced by synaptotagmin-1 results in the induction of curvature stress, thereby reducing the otherwise high energy barrier to membrane fusion. Synaptotagmin-related molecules with multiple linked C2 domains are ubiquitous and we have shown that some of these can also modulate membrane curvature. Thus our results suggest that the promotion of membrane fusion by multiple C2 domain-containing proteins by the induction extreme positive curvature may be a widespread phenomenon in biology.

First structural and mechanistic insights into membrane remodelling by EHD ATPases[67]. Dynamin superfamily NTPase members actively remodel membranes but molecular details on these proteins have hitherto been lacking. We have solved the structure of the full-length multidomain family member EHD2 NTPase and based on its structure we performed *in vitro* and *in vivo* assays showing how an EHD oligomer is formed and how perpendicular curvature stresses on a membrane surface can result in remodelling of membranes by the formation of oligomeric EHD rings[67]. In our previous studies in this field we worked on mammalian dynamin-1, which is the major neuronal protein involved in the mechanics of endocytic synaptic vesicle retrieval following exocytosis. We demonstrated a change in helix pitch for a dynamin oligomer on GTP hydrolysis[31]. This led to the proposal of a ‘poppase’ mechanism for the action of dynamin in vesicle membrane scission. In a follow up paper in Nature we demonstrated that dynamin is a mechanochemcial GTPase[38] and provided strong evidence for the mechanical role of dynamin in membrane scission.

Discovery of Serine-Threonine acetylation – mechanism of action of the bacterial toxin YopJ[62].
As part of our interest in cellular signalling processes, we examined the mechanism of action of the toxin YopJ produced by the bacillus *Yersinia pestis* responsible for plague. The activity of YopJ allows the pathogen to overcome cellular defences by blocking multiple signalling pathways of mammalian cells – including the mitogen activated protein kinase (MAP kinase) and NFkappaB pathways. Our results[62] identified that YopJ elaborates an enzymatic activity that leads to the O-acetylation of

key serine and threonine residues on kinases of both pathways that results in the inhibition of their activities. We are currently exploring the possibility of this post translational modification having a wider role in mammalian cell biology.

Finding general principles underlying biological pathways through the study of protein networks. Over the years we have been taking a novel approach to clathrin-mediated endocytosis by studying the organization and functioning of this process, to understand how the protein machinery is structured in space and time. To this end we have crystallized key organising domains and examined how they interact with other proteins[30,35,37,41,50,61]. When viewing the pathway as a whole functioning unit we found that the pathway is organized around hub proteins which control progression in vesicle formation, the flexibility and fidelity of cargo incorporation, and the approximate time course of events during vesicle formation. We believe through such approaches that we have an initial glimpse of the underlying design principles of this pathway[66]. We have proposed that the principles we derived should be applied to other biological systems and should help in experimental design and interpretation. We are now interested in protein-based decision making in networks, the maturation of protein interactions, and how and when pathways become irreversibly committed.

First structure and functional characterization of a BAR domain[49]. The BAR module is a dimerization and membrane curvature sensing domain[49]. From its 'structure signature' we identified its presence in many protein families where, in combination with other protein domains, it can help target and concentrate proteins to membrane sub-regions defined by curvature and lipid specificity. The initial paper was published as a Science article[49] and Nature subsequently asked for a full review[59]. This review was accompanied by a short letter to Nature on another BAR family member[55] and a year later we published the structure of the second member of the family[60]. At this point we refined the nomenclature showing that the presence of an N-terminal amphipathic helix dramatically altered the properties of this 'N-BAR' unit[60]. Our previous work has already highlighted the function of these proteins in clathrin-mediated endocytosis[e.g.27] and in muscle T-tubule development[39]. Over the past year we have published the structure of a distinct protein module with similarity to N-BAR domains. This module was called F-BAR and its curvature specificity was found to be different from that of N-BARs[65]. Again this module is found in many protein families. From database mining we can now define the 'BAR Superfamily', which contains at least 3 subfamilies, and we have ascribed curvature sensitive dimerization on membrane binding as function to many of these proteins (invited review for Cell in progress).

Structure and functional characterization of an ENTH domain in clathrin-coated pit formation[41]. An outstanding question in the membrane trafficking field is how transport vesicles of high curvature can be formed in the cell. We approached this problem by examining clathrin-coated vesicle formation and reasoned that an

accessory protein that interacted directly with the membrane was a likely candidate to have a function in curvature induction. By screening these proteins we found that epsin had a potent effect on membrane curvature *in vitro* (in the presence of phosphatidylinositol (4,5) bisphosphate). We solved the structure of the epsin1 ENTH domain in combination with its ligand, phosphatidylinositol (4,5) bisphosphate (published as a Nature article[41]). We found that ligand binding induced the folding of an amphipathic helix that inserted into the membrane. This provided the explanation for curvature induction since the helix acted like a wedge in the membrane. This membrane insertion and bending occurs in concert with clathrin polymerization which stabilizes the curvature in the forming clathrin-coated pit. We showed that this domain is also present in other proteins that define families capable of sculpting membranes[45]. This was a seminal paper on membrane bending and many other investigators have since shown the presence of amphipathic helices in other proteins families and confirmed their role in curvature generation.

***First structure of an ANTH domain and demonstration of its membrane binding capability*[37].** Clathrin-coated vesicles form from phosphatidylinositol (4,5) bisphosphate-enriched membranes yet the canonical inositol lipid binding domains (PH, PX and FYVE domains) are not found in the accessory proteins associated with clathrin attachment to membranes. We screened for other inositol lipid specificity domains in the accessory proteins and as a result we were able to define (structurally and functionally) another phosphatidylinositol (4,5) bisphosphate-binding domain previously recognized by pfam as an ANTH domain[37]. We showed that this domain in AP180 functions to target this clathrin polymerizing protein to sites of endocytosis in synapses, where it recruits/polymerizes clathrin into defined structures which we called 'caps'. Previously it was believed that AP2 adaptors were the principal clathrin recruitment proteins. However, the ubiquitous form of this protein, CALM (clathrin-associated myeloid leukaemia protein), is the major clathrin recruitment analogue in non-neuronal tissues. This paper includes a description of the original 'lipid monolayer assay' which is a novel methodology for visualization of clathrin polymerization[37].

***First structure of an accessory protein binding domain of adaptor complexes (appendage domain)*[30].** This work marked the beginning of our studies into how molecular pathways might be organized (protein network organization) and developed our understanding of the modular organization of clathrin-coated vesicle assembly[50,61]. We went on to solve the structures of several of these domains, understanding how they interact with ligands, and the structural basis behind their high-affinity yet readily-reversible interactions. Our studies defined the structural basis of ligand binding and have wide implications as these domains are found in all adaptor complexes, in some of the COP vesicle components and in GGA proteins.

***First demonstration of the calcium stimulation of endocytosis in synapses*[26].** It was previously believed that endocytosis was coupled to exocytosis, but this study showed

that endocytosis at the synapse is directly triggered by calcium (at least in part by the activation of the protein phosphatase, calcineurin).

***Cloning and characterization of complexins*[21]**. The SNARE complex is widely believed to be ubiquitous membrane fusion molecules. We identified complexins as being tightly associated with the SNARE proteins in synaptic vesicle fusion, and suggested that they would regulate complex assembly[21]. In knock-out models we also showed that complexins affected the calcium dependence of vesicle fusion[40].

***SDS-resistance as an assay for the assembly of SNARE complexes*[19]**. In this collaborative paper with Heiner Niemann we showed for the first time that assembled SNARE complexes are very stable even to the presence of SDS. This allowed us to show differential sensitivity of assembled versus unassembled SNARE proteins to botulinum toxins. This SDS resistance assay has been used in hundreds of subsequent papers, and has laid the basis for being able to distinguish two functional states of these important proteins.

***Cloning and characterization of cellubrevin*[17]**. This paper introduced the idea that synaptic mechanisms were not unique but simply specializations of more ubiquitous processes. Thus synaptic proteins were predicted to have ubiquitous counterparts that will have analogous functions.

***Proposal for the stimulation of vesicular release of glutamate at synapses by localized calcium influx and the direct effects of neurotoxins on the release mechanism*[11,13]**. The coupling of calcium channels and vesicle release sites was proposed here on the basis of the differential sensitivity of small synaptic vesicles to depolarization-induced calcium influx versus ionophore-carried calcium influx[11]. We also showed that large dense-core vesicles and small synaptic vesicles have distinct calcium sensitivities. Botulinum neurotoxins and tetanus toxin were also shown to directly affect neurotransmitter exocytosis at synapses.

Work in progress:

***Novel membrane trafficking pathways*[56,60].** We have shown how the membrane is deformed during clathrin-coated vesicle formation with the aid of ENTH, BAR-containing and dynamin family proteins. We have also shown the widespread distribution of BAR superfamily members and how they can bind to many different membrane curvatures. We are now applying this information to probe for molecularly undefined vesicle budding pathways whose existence has long been appreciated. For example, in the synapse where clathrin-coated vesicle retrieval may be too slow to keep pace with exocytosis we can show that a fast mode of retrieval is ablated by manipulation of another BAR family member (unpublished)[60]. We previously showed that only a slow component of vesicle retrieval is clathrin-mediated[56]. We are also investigating other BAR family members that underlie cell motility and the accompanying membrane trafficking in non-neuronal cells (unpublished).