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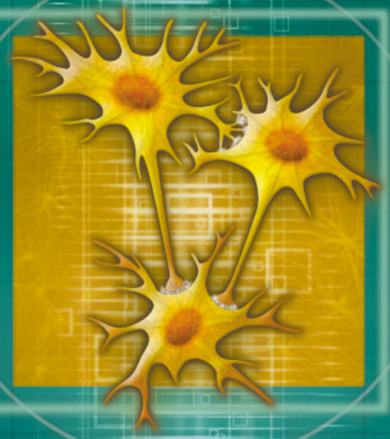

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Biacore's SPR technology in neuroscience

Biacore's SPR technology is now firmly established in neurological research. This booklet reviews some recent papers both from clinical laboratories in which Biacore® is used to measure neurological disease markers in complex biological matrices and basic research facilities aimed at delineating the basic molecular mechanisms behind neuropathies. Examples of published work citing Biacore applications in neuroscience can be found on the regularly updated reference section on Biacore's website at http://www.biacore.com.

Alzheimer's disease and amyloid plaque fomation

C. W. Cairo, A. Strzelec, R. M. Murphy and L. L. Kiessling
Biochemistry 41: 8620-9 (2002).

B. Bohrmann, L. Tjernberg, P. Kuner, S. Poli, B. Levet-Trafit, J. Naslund, G. Richards, W. Huber, H. Dobeli and C. Nordstedt
J Biol Chem 274: 15990-5 (1999).

R. L. Naylor, A. G. Robertson, S. J. Allen, R. B. Sessions, A. R. Clarke, G. G. Mason, J. J. Burston, S. J. Tyler, G. K. Wilcock and D. Dawbarn
Biochem Biophys Res Commun 291: 501-7 (2002).

H. Vorum, C. Jacobsen and B. Honore
FEBS Lett 465: 129-34 (2000).

The rationale for a Biacore study on Alzheimer's disease by Christopher Cairo and colleagues at the University of Wisconsin in Madison (Cairo *et al*, 2002) was to identify small peptides that tightly bind and inhibit the toxic neurodegenerative effects of β-amyloid (Aβ) in the diseased brain. Although Aβ aggregates are present in neurotoxic lesions, it is not known if they are causative. Short peptides directed to the self-recognition motifs in Aβ were synthesized and Biacore® 2000 was used to screen the affinities of the interactions.

Presenting a uniform binding target

Solution-based methods for screening Aβ inhibitors consume large amounts of the target and they are limited by the tendency of Aβ to aggregate in solution and thus present multiple target forms. This problem is avoided in Biacore assays because the target is immobilized on a planar sensor chip surface at a density and in an orientation that can be carefully controlled by the user. A 26-amino acid fragment of Aβ comprising amino acids 10 to 35 with a C-terminal cysteine residue was immobilized at low density on Pioneer Chip B1 primed by maleimide chemistry and thus presented a uniform surface. Further, inter-assay variability was low as a single chip surface could be regenerated several times and re-used for all test interactions. A range of concentrations of each peptide, based on variations of a 5 amino acid sequence corresponding to the central hydrophobic domain of Aβ known to be responsible for self association was injected over Aβ on the chip surface for 500 seconds and the response at equilibrium (R_{eq}) was plotted against concentration to calculate affinity constants.

Consolidation of Biacore data in cell-based assays

Of several peptides based on multiple length C-terminal extensions of a KLVFF pentapeptide in the central hydrophobic domain of Aβ (which generally bound more efficiently than the original pentapeptide to Aβ), a pattern emerged in which the affinity for Aβ was sensitive to site-specific positioning of positively charged lysine residues. In the Biacore assay, peptides in which 3 of these residues were separated from the basic sequence by 3 negatively charged residues had a 14-fold lower affinity for Aβ, while the affinity of a peptide of similar size bearing 3 directly adjacent lysine residues was almost unchanged.

The peptides were then tested for cytotoxicity in microtiter assays by incubating Aβ with neuroblastoma cells in the presence of peptides. Compounds with an affinity dissociation constant (K_D) less than 50 μM protected against toxicity by greater than 80% in viability assays while the ability to protect gradually diminished with reducing affinity (Table 1). It is hoped that these peptides will be useful as probes in investigations of the mechanisms underlying amyloid plaque formation and in the design of drugs.

Peptide sequence#	K_D (μM)	Viability (%)+
KLVFFRRRRRR	40	> 90
KLVFFKKKKKK	40	80-90
KLVWWKKKKKK	40	80-90
KLVFFKKKK	37	70-80
KLVFWKKKKKK	65	70-80
KLVFFKK	80	70-80
KLVFFKKKEEE	90	60-70*
KKKKLVFF	180	60-70*
KLVFFEKEKEK	300	60-70*
KKKKKK	400	60-70*
KLVFFEEEKKK	1300	60-70*

Table 1: Peptides based on C-terminal extensions to a central KLVFF fragment derived from the central hydrophobic domain of Aβ were tested for their affinity to Aβ immobilized on Pioneer Chip B1. Affinity (K_D) is related to the ability of the same peptide to inhibit Aβ-mediated cell cytotoxicity in a cell-based assay. #K=lysine, L=leucine, V=valine, F=phenylalanine, R=arginine; W=tryptophan, E=glutamic acid. +Cellular viability of human neuroblastomas cells was assessed in a microtiter assay using an MTT assay in which cells were exposed to Aβ in the presence or absence of peptides. *This is a similar level of viability seen after treatment with negative controls.

Biacore's SPR technology is proposed as a reliable means to measure the affinity of small peptides for Aβ, a protein that that is notoriously difficult to handle in solution based assays. Such data may help predict the suitability of candidate peptides as inhibitors of Aβ toxicity in Alzheimer's disease.

Albumin: a natural inhibitor of Aβ polymerization

Although Aβ protein is expressed by almost all nucleated cells, it is not known why the protein is deposited to form lesions exclusively in tissues of the central nervous system (CNS). The most common plasma proteins are also present in cerebrospinal fluid (CSF), but at much lower concentrations. One study reported the use of Biacore's SPR technology in a screening strategy to find out if any proteins common to both plasma and CSF could bind Aβ protein and thus potentially inhibit deposition (Bohrmann *et al*, 1999). Two forms of Aβ protein are known; one with 40 amino acids and a longer variant that contains two extra C-terminal residues (Aβ 1-42). The latter polymerizes more readily into plaque-forming fibrils. Two parallel immobilization strategies were followed to monitor the binding of plasma/CSF proteins to both forms of Aβ protein. Plasma and CSF proteins were tested for their capacity to inhibit Aβ polymerization in a microtiter assay in which Aβ protein or Aβ 1-42 were immobilized as templates to which further Aβ-protein was added in the presence of the test protein. This revealed that the substances that most effectively inhibited polymerization were albumin, α 1-antitrypsin, IgA and IgG, proteins found only at low concentrations in CSF. The authors suggest that albumin, at its normal concentration in plasma, may bind and thus prevent the deposition of polymerized Aβ protein in amyloid plaques. In inflammation, levels of albumin in CSF may be sufficiently reduced to abrogate this protective function, enabling the formation of β - amyloid.

Inhibitors of apoptosis

The findings of Dr Dave Dawbarn's group at the University of Bristol may help accelerate the search for mimetics of cell survival factors (Naylor *et al*, 2002). In Alzheimer's disease, there is reported to be a selective loss of the catalytic form of TrkB, a tyrosine kinase receptor for certain neurotrophins. TrkB is expressed on numerous structures of the central and peripheral nervous systems. Reported functions of the ligated receptor include axonal outgrowth and neurotransmitter release, while one ligand of TrkB, brain-derived neurotrophic factor (BDNF) is a known neuronal survival factor. Naylor *et al* used Biacore® 3000 to identify the extracellular docking site for neurotrophins on TrkB. Specifically, the role of the membrane proximal Ig-like domain of TrkB was investigated. Recombinant Ig-like domain was immobilized on Sensor Chip CM5 by amine coupling. In order to calculate the affinities of BDNF and neurotrophin-4 (NT-4) for TrkB, concentration series of recombinant analytes were run over the immobilized ligands, giving K_Ds of 0.79 nM and 0.26 nM, respectively while the related neurotrophin NT-3 bound with much lower affinity (K_D of 18 nM). This short report therefore suggests that the juxta-membrane Ig-like domain of TrkB is responsible for the high-affinity binding of BDNF and NT-4 for TrkB and that it may thus find an application in screening for mimetics of BDNF in the treatment of neurodegenerative diseases.

In addition to Aβ protein, serum amyloid P component (SAP) is also found in amyloid deposits and, like Aβ protein, tends to self-associate in the presence of calcium. Calumenin is one of a family of endoplasmic reticulum proteins that coordinate calcium in maintaining the structural integrity of helix-loop-helix motifs in certain proteins. One study elegantly demonstrates how Biacore can be used to define the interaction and characterize the conditions

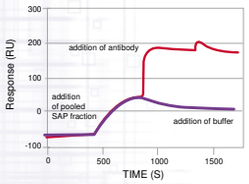

Figure 1: Verification of SAP-binding to immobilized calumenin by the additional binding of anti-SAP anitbody

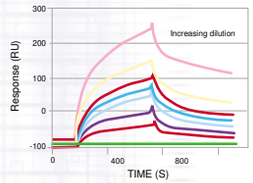

Figure 2: Binding of SAP to immobilized calumenin

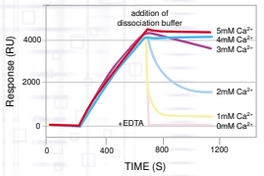

Figure 3: After formation of the calumenin:SAP complex, Ca^{2+} is necessary to maintain the complex as shown by the effect on the dissociation rate by the exclusion of Ca^{2+} from the dissociation buffer.

necessary for this process to occur (Vorum *et al*, 2000). An unidentified binding partner for calumenin was first detected when a crude placental extract was run over recombinant calumenin immobilized on an affinity column in the presence of calcium. After elution, one 30-kDa band was isolated by PAGE. The protein was internally sequenced by tryptic digestion and identified as SAP. Calumenin was then immobilized on Sensor Chip CM5 and SAP was run as the analyte. The results confirmed that SAP is a ligand for calumenin and that the formation and stability of the complex requires calcium. It is therefore possible that given the tendency of both SAP and calumenin to form insoluble complexes, calumenin may participate in amyloidosis, the pathological process by which amyloid is deposited in the CNS in patients with Alzheimer's disease (Figures 1-3).

Spinal muscular atrophy

P. J. Young, N. Man, C. L. Lorson, T. T. Le, E. J. Androphy, A. H. Burghes and G. E. Morris
Hum Mol Genet 9: 2869-77 (2000).

P. J. Young, P. M. Day, J. Zhou, E. J. Androphy, G. E. Morris and C. L. Lorson
J Biol Chem 277: 2852-9 (2002).

Spinal muscular atrophy (SMA) is predominantly a childhood disease of motor neurons in the spinal cord, affecting the voluntary muscles and causing the impediment of functions such as locomotion and head and neck control. The disease is strongly linked to mutations in the SMN1 (survival motor neuron 1) gene. The work of Philip Young and colleagues at Arizona State University (Young *et al*, 2002) suggests that the clinical symptoms of the disease may be due to increased motor neuron death through the inability of mutated SMN protein to dimerize, and thereby sequester and inhibit the pro-apoptotic function of the tumor suppressor protein, p53.

Biacore: a new and rapid alternative to immunoprecipitation

An elegant strategy was followed in which Biacore® X was used initially to confirm conventional immunoprecipitation data using an antibody to p53 showing that SMN associated with p53. This finding was substantiated using Biacore in sequential binding experiments that demonstrated how the technology might be used as an alternative to immunoprecipitation and may represent a more convenient tool for detecting molecular interactions in whole cell extracts (Figure 4). Briefly, anti-p53 antibody was coupled to Sensor Chip CM5. Next, whole cell extracts from SMN-transfected cells (treated with a protease inhibitor that protects the labile p53) were passed over the chip, followed by exposure to anti-SMN antibody. Cell extracts that did not contain detectable levels of p53 were used in parallel and demonstrated the specificity of the interaction.

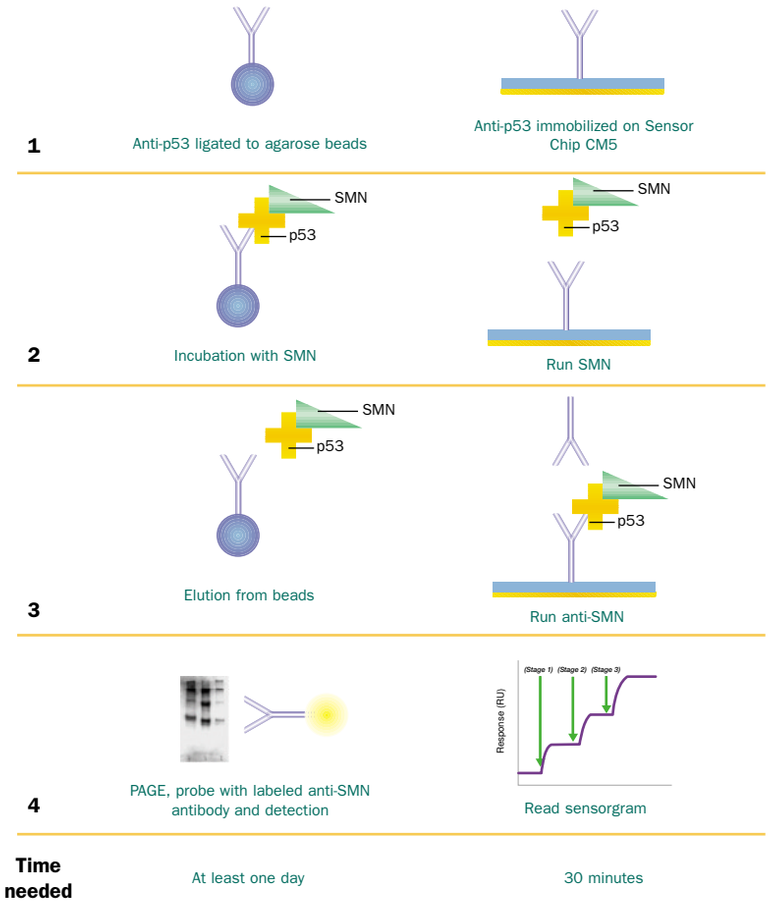

Figure 4: Comparison between immunoprecipitation/blotting and Biacore in the detection of molecular complexes. Both assays reveal that SMN protein derived from whole cell extracts is associated with p53 in the cell. Results achieved by immunoprecipitation are available within 1 day whereas the Biacore assay takes minutes.

Biacore was used in sequential binding experiments that demonstrated how the technology might be applied as an alternative to immunoprecipitation.
"It is straightforward and significantly faster than the standard technique.": Dr Christian Lorson, Arizona State University.

Molecular consequences of disease-causing mutations

SMA patient-derived mutant proteins were also tested for p53 binding. Two SMN proteins carrying C-terminal mutations found in patients with severe type I SMA and a peptide from a patient with less severe type II SMA were immobilized on Sensor Chip CM5 and exposed to p53. The extent of reduction in SMN peptide binding strongly correlated with the clinical subset. This defect is probably attributable to the inability of the SMN mutant proteins to efficiently self-associate. Young *et al* then applied the sequential Biacore approach to show that it is this very inability to self-associate that may prevent SMN from sequestering and inhibiting p53 function. In these experiments, p53 failed to bind to immobilized monomeric SMN and was only induced to bind when additional dimeric SMN was immobilized to the same chip (Table 2 and Figure 5).

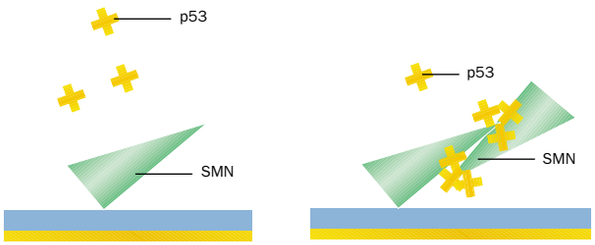

Figure 5: Non-self associated SMN cannot sequester p53. A Biacore assay was used to show that p53 bound only weakly to monomeric SMN (A), a configuration that is likely to dominate in SMA patients of various degrees of severity (see Table 2). When dimeric SMN was immobilized on Sensor Chip CM5 (B), p53 binding was greatly increased.

SMN protein form	Binding to wildtype SMN (% of wildtype)
Wildtype	100%
Truncated SMN[1]	33%
Two type I mutations[2]	20% and 31%
Type II mutation[3]	57%

Table 2: Mutated SMN protein fails to self-associate efficiently. Wildtype SMN was immobilized on Sensor Chip CM5 and exposed to wildtype, truncated or point-mutated SMN protein. [1]Truncated SMN is the primary product of the *SMN2* allele, which is present in SMA patients. [2]Type I mutations were isolated from severe SMA. [3]Type II mutations were isolated from mild SMA

These findings are supported by results from indirect confocal immunofluorescence microscopy experiments showing that SMN and p53 co-localize in Cajal bodies (sites of small nuclear ribonucleoprotein particles involved in RNA splicing) of normal fibroblasts, but not in those derived from SMA patients. In the patient fibroblasts SMN levels are greatly reduced, and in this cellular context, activated p53 can be observed within the nucleolus, suggesting that SMN may play a role in targeting p53 to Cajal bodies.

Selecting antibodies as molecular probes

There is a strong correlation between the severity of SMA and levels of SMN protein. The function of SMN protein is unclear although, as stated above, it is thought to be involved in mRNA splicing. To determine a possible mechanism to explain why mis-sense mutations in SMN should compromise protein function and hence cause disease, Biacore was used to discover how native peptide fragments of SMN, defined by whole, discrete exons, interact with each other (Young *et al*, 2000). To identify and select the tools necessary to map these protein interactions, a panel of 22 monoclonal antibodies was raised to full-length SMN protein and tested using Biacore's SPR technology for immunoreactivity against the peptides encoded by each exon in the cloned gene. The antibodies were immobilized on Sensor Chip CM5 via rabbit anti-mouse IgG and screened with all fragments. Antibodies were thus identified that specifically recognized certain peptides within the protein and that could then be used to characterize intra-chain interactions that may determine conformational settings of functional importance in full-length protein. By immobilizing each peptide fragment and screening with exon-defined peptides, the authors defined the fragments of SMN that possessed the potential to interact with each other. Figure 6 shows how an experiment was constructed that gave a wealth of information about the molecular events determining the function of this protein. A monoclonal antibody that recognized the peptide product of exon "A" alone was immobilized on Sensor Chip CM5. Subsequent injections of different peptides showed that the capture of A enabled exon peptides "B1" and "B2" to bind sequentially in the same experiment. These data show not only that A appears to form a scaffold to allow the assembly of B1 and B2, but that the functional protein product is a result of the creation of a novel epitope formed by the combination of B1 and B2.

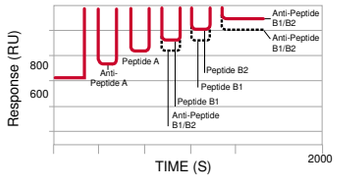

Figure 6: Sensorgram showing how, by using a combinatiion of carefully specified monoclonal antibodies and recombinant peptide fragments, Biacore's SPR technology can provide a powerful means of helping to determine the order of assembly of protein structures.

Regulation of the inflammatory response in neuropathies

B. Catimel, A. M. Scott, F. T. Lee, N. Hanai, G. Ritter, S. Welt, L. J. Old, A. W. Burgess and E. C. Nice
Glycobiology 8: 927-38 (1998).

Gangliosides are membrane-spanning glycolipids which are expressed in most nucleated cells and are enriched in neurons. Their expression is associated with certain neuropathies including human neuroectodermal cancers and Alzheimer's disease. Biacore has been used to develop a simple assay for the detection of anti-ganglioside antibodies (Catimel *et al*, 1998). One requirement of the technique is that the gangliosides are presented in a consistent and appropriate orientation for antibody characterization i.e. that the sugar moiety presented on cells is available to the analyte *in vitro* and which is thus an accessible target for therapeutic monoclonal antibodies. The gangliosides were immobilized via the lipid tail, as using the more common aldehyde coupling technique via the carbohydrate domain may mask the epitope. The authors present a simple and rapid protocol, in which a stable surface is created that can be readily reconditioned and reused. Gangliosides were injected over the unmodified carboxymethyldextran surface of Sensor Chip CM5, while a control ganglioside was covalently immobilized by photoactivation for comparison. Although overall immobilization levels were low compared with those achieved by covalent linkage, the molar level of binding of the antibodies (specific binding) was much higher. Complete dissociation of antibody from gangliosides was achieved by treatment with 10 mM NaOH and a similar binding level of antibody was repeatedly re-established on subsequent immobilizations. The immobilized ganglioside itself was easily removable by treatment with detergent or propanol.

The development of humanized antibodies for neuroblastoma therapy

K. Nakamura, Y. Tanaka, K. Shitara and N. Hanai
Cancer Immunol Immunother 50: 275-84 (2001).

Phase I clinical trials, in which humanized anti-ganglioside monoclonal antibodies were tested in children with neuroblastomas, have shown that a host immune response is still induced to the murine component of the antibody. For this reason, it is desirable to engineer fully humanized antibodies that retain the murine specificity-determining regions, but from which the flanking V-region sequences have been replaced with human counterparts (Nakamura *et al*, 2001). This produces a theoretically less immunogenic protein in which the murine component is reduced from 30% to 10%. Nakamura *et al* based the characterization of the interaction between these humanized antibodies and immobilized gangliosides GD2 and GD3 on the protocol of Catimel *et al* (above). Their Biacore experiments revealed that humanization altered the association and dissociation rates of the antibodies in ways that are otherwise difficult if not impossible to predict. Biacore's SPR technology thus provides information about the molecular mechanisms underlying functional changes in humanized antibodies, a critical part of the development of novel antibodies for human immunotherapy.

Neurotransmitter release

S. Quetglas, C. Iborra, N. Sasakawa, L. De Haro, K. Kumakura, K. Sato, C. Leveque and M. Seagar
EMBO J 21: 3970-9 (2002).

Biacore's SPR technology has been used to provide the molecular mechanisms to support a proposal of how neuronal synaptic vesicles fuse with the plasma membrane to release neurotransmitters. Vesicle-associated membrane protein (VAMP-2 or synaptobrevin) is anchored in the vesicle membrane and can associate with two other proteins in the plasma membrane. Interaction with these two plasma membrane-linked proteins called SNAP-25 and syntaxin-1 leads to formation of a complex comprising a parallel array of α-helices that twists into a super helix. The two membranes are thus brought together to promote fusion of the lipid interfaces. Although the mechanisms that drive synaptic vesicle fusion with the neuronal plasma membrane are not known, the process is Ca^{2+}-dependent. Calmodulin, a cytosolic calcium sensor protein, is implicated in several membrane fusion processes in other mammalian models and in yeast. Specifically, Stephanie Quetglas and colleagues (INSERM Unit 464) in collaboration with Raymond Miquelis (UMIM) at the IFR Jean Roche, Université de la Méditerranée in Marseille (Quetglas *et al*, 2002) have shown that calmodulin binds to a C-terminal VAMP-2 sequence located close to its anchor in the vesicle membrane. They used cell-based assays and Biacore to test some of the discrete molecular steps within this process. In testing several protein:protein interactions as well as protein:lipid interactions using Biacore® X and Biacore® 3000, they have also given some indication of the broad scope of the technology.

The role of Ca^{2+} in the calmodulin/VAMP interaction

The requirement for Ca^{2+} to support calmodulin binding to VAMP was tested by passing calmodulin over a VAMP/GST fusion protein captured on a Biacore sensor chip in the presence of increasing concentrations of Ca^{2+}. Significant binding occurred only in the presence of Ca^{2+} at concentrations above 0.3 µM. Mutations, designed according to consensus sequences in other calmodulin-binding proteins, were tested for their capacity to inhibit calmodulin binding. Compared with the wildtype peptide, those bearing mutations aimed at eliminating basic charges or in altering the hydrophobic quality of the consensus were severely impaired in their affinity for immobilized calmodulin (10-fold and 300-fold inhibition, respectively). In a further Biacore assay, liposomes were immobilized on Pioneer Chip L1 and shown to bind VAMP-derived peptides. Again the mutants, particularly those with reduced hydrophobic quality, bound lipids only weakly. Finally, in a competition assay in which VAMP was linked to agarose beads, increasing concentrations of calmodulin were able to displace labelled liposomes bound to VAMP in the presence of Ca^{2+}. Taken together these results suggest that lipids and calmodulin share a binding motif on VAMP.

Vesicle fusion and exocytosis

The mutants were then tested for function in two cell-based assays. Firstly, cultured bovine chromaffin cells (neuroendocrine cells that are useful as an *in vitro* model for the induction of vesicle fusion and exocytosis) were pulsed with 100 mM KCl and release of the neurotransmitter catecholamine was measured using carbon fiber amperometry. Catecholamine release was reduced by 80% in the presence of wildtype calmodulin-binding peptides, suggesting that the VAMP peptides sequestered endogenous calmodulin and prevented exocytosis. In contrast, mutant VAMP peptides (non-calmodulin-binding) affected exocytosis only very weakly.

Secondly, PC12 cells were treated with tetanus toxin, which cleaves VAMP at a site immediately N-terminal to the calmodulin binding site, inhibiting vesicle fusion and hence exocytosis. When cells were transfected with mutant VAMP that cannot be cleaved by the toxin but which still binds calmodulin, membrane fusion and exocytosis were restored. However, the introduction of additional mutations (ie. those which inhibited calmodulin and lipid binding in Biacore assays) abolished rescue of Ca^{2+}-dependent exocytosis.

This study elegantly demonstrates how concise affinity data measurements on Biacore can be given functional significance in cell-based assays. Mutant peptides were designed according to database information, binding behaviour was tested using Biacore's SPR technology and functionality was confirmed in cell-based assays. The authors have used Biacore to provide quantitative affinity data in support of a highly credible hypothesis for the regulation of neurotransmitter release.

Receptor:ligand interactions and signal transduction in neuroscience

R. A. McCarthy, J. L. Barth, M. R. Chintalapudi, C. Knaak and W. S. Argraves
J Biol Chem 277: 25660-7 (2002).

Y. Miyagi, T. Yamashita, M. Fukaya, T. Sonoda, T. Okuno, K. Yamada, M. Watanabe, Y. Nagashima, I. Aoki, K. Okuda, M. Mishina and S. Kawamoto
J Neurosci 22: 803-14 (2002).

The pleiotropic signaling molecule, sonic hedgehog *(Shh)* is involved in several aspects of neurodevelopment including maturation of ventral neural cells and patterning of the ventral neural tube. It functions by binding to a receptor called *Ptc*, which is normally embedded in the neural cell membrane in close association with a signal transducing co-receptor called *Smo*, an interaction that maintains *Smo* in a dormant state. On binding to *Shh*, however, the membrane-bound complex dissociates and leaves *Smo* in a monomeric and active state.

Kinetic analysis of the interaction between *Shh* and megalin

Megalin, a member of the LDL receptor family is critical in neurodevelopment with megalin-deficient mouse embryos suffering numerous defects causing reduced viability of neural epithelia. In fact, the phenotype is similar to mice deficient in *Shh* or *Smo* including apoptosis of neural epithelial cells. Scott Argraves and colleagues at the Medical University of South Carolina in Charleston (McCarthy *et al*, 2002) showed by confocal microscopic analysis that megalin was co-expressed during neural development with *Shh* or known receptors for *Shh* and thus suggested that a functional relationship may exist between *Shh* and megalin. Binding between *Shh* and megalin was confirmed by ELISA and by a ligand or RAP (a specific megalin inhibitor) competition assay.

The Argraves group performed a kinetic analysis of the interaction between the biologically active N-terminus of *Shh* and megalin using Biacore® 3000. Megalin was immobilized on Sensor Chip CM5 by amine coupling. A concentration range of *Shh*:GST fusion proteins were passed over the ligand with or without 1 mM $CaCl_2$ and the effect of pH on the dissociation rate of the complex was tested by washing in buffers of varying acidity. In the presence of Ca^{2+}, the interaction between *Shh*:GST and megalin had an affinity dissociation constant (K_D) of 21 nM. Acidic pH made little difference to the dissociation rate suggesting that the complex would remain stable within the acidic environment of endosomes. It is therefore possible that the *Shh*:megalin complex functions in endosome-mediated transcytosis to enable distal signalling. This might possibly be a mechanism for cell differentiation in development e.g. in the maturation of cells in the ventral region of the neural tube that differentiate to ventral nerves, a process dependent on both *Shh*-mediated signal transduction and megalin expression. In cell based assays *Shh*:GST was only taken up into cells in the absence of RAP suggesting that endocytosis occurs after interaction at the cell surface

Purkinje cell function

The glutamate receptor δ2 (GluRδ2) is exclusively expressed on the dendritic synapses of Purkinje cells, structurally unique cells that are among the most organizationally complex neurons in the mammalian nervous system. Although they receive an enormous amount of data from neurons of the spinal cord and elsewhere, they are nevertheless able to convert this input into interpretable information and are, in fact, the sole channel of output from the cerebellar cortex. That GluRδ2 is limited to these cells possibly implies a function in this extraordinary feat of information processing. Susumu Kawamoto and his research group at Yokohama City University School of Medicine (Miyagi *et al*, 2002) showed that an intracellular protein called delphilin co-localizes with GluRδ2 at parallel fiber (input)-Purkinje cell (output) synapses. Analysis using Biacore® 2000 showed that recombinant delphilin PDZ domains bind to the C-terminus of GluRδ2 with moderate affinity. They also showed that delphilin bound profilin, an actin-binding protein, with very slow dissociation (k_d of 4.09 x 10^{-4} s^{-1}). It is possible, therefore, that delphilin acts as a molecular bridge linking GluRδ2 to the actin cytoskeleton via profilin with rapid turnover of the GluRδ2:delphilin interaction contributing to the capacity of the Purkinje cell to process a very large amount of input data.

Physiological functions of nerve growth factor

G. M. Ross, I. L. Shamovsky, S. B. Woo, J. I. Post, P. N. Vrkljan, G. Lawrance, M. Solc, S. M. Dostaler, K. E. Neet and R. J. Riopelle

J Neurochem 78: 515-23 (2001).

The "chemical space" or physico-chemical composition of the microenvironment of the binding site between ligand and receptor is critical for optimal interaction. Ionic strength and pH, for example, affect non-covalent bonds and so by varying the buffer composition, judgments can be made on the nature of the interaction. Biacore's SPR technology was used to find out if local variations in pH changed the capacity of Zn^{2+} and Cu^{2+} to modulate NGF binding to its receptor (Ross *et al*, 2001). To do this, Zn^{2+} and Cu^{2+} were included in running buffers with pH varying from 5.5 to 7.4, mimicking local conditions in cerebral acidosis, a condition frequently arising after stroke or traumatic insult. This pH range also covers the pKa value of histidine, the position and electrostatic status of which are known to influence cation binding. The authors prepared and immobilized recombinant TrkA on Sensor Chip CM5. The K_D for the interaction between nerve growth factor (NGF) and TrkA was calculated over a pH range covering the pKa value of histidine. This revealed that Zn^{2+}, but not Cu^{2+}, lost its ability to bind NGF and inhibit its interaction with TrkA under acidic conditions. The physiological consequences of this may depend on the cell type and context; TrkA-expressing cells in an acidic environment and needing NGF for survival may well benefit from the inactivation of Zn^{2+}, whereas the effects might be detrimental if NGF, in contrast, initiates signals leading to cell death.