Introduction

The consensus opinion that cell surface proteins could be anchored in the plasma membrane via covalent linkage to phospholipid emerged in 1985 through an amalgamation of data from several laboratories. Since the mid-1970s data from the groups of Ikezawa and Low showed that the treatment of cells or membranes with bacterial phosphatidylinositol-specific phospholipase C (PI-PLC) results in the selective release of certain membrane proteins such as alkaline phosphatase, acetylcholinesterase (AChE) and 5'-nucleotidase (reviewed by Low, 1987). In an extensive study Low showed that PI-PLC released alkaline phosphatase could no longer re-bind to membranes and concluded that it was originally anchored through a tight, and probably covalent, linkage to phosphatidylinositol (PI) phospholipid (Low and Zilversmit, 1980). However in the absence of direct chemical evidence this proposal was largely ignored. The first chemical evidence for covalent linkage of phospholipid components to protein came from work on the rat Thy-1 antigen by Williams and colleagues (Campbell et al, 1981) who found stoichiometric amounts of ethanolamine and fatty acid attached to C-terminal proteolysis fragments. Subsequently Holder showed that ethanolamine is in amide linkage to the C-terminal amino acid carboxyl group of Trypanosoma brucei variant surface glycoprotein (VSG) polypeptide and associated with a novel carbohydrate moiety (Holder and Cross, 1981; Holder, 1983). However the VSG as conventionally isolated was water soluble (sVSG) with no evidence of a hydrophobic membrane insertion site to account for its original stable association with the trypanosome plasma membrane. This enigma was was explained by the elegant study of Cardoso de Almeida and Turner (1983) who isolated the amphiphilic membrane binding form of VSG (mfVSG) for the first time. They showed that trypanosomes contain a potent
enzyme which rapidly converts mFVSG to sVSG unless specifically inhibited during VSG isolation. Together with Holder's data it was concluded from this work that the membrane anchor of VSG was lipid in nature. Subsequent chemical analysis supported this view (Ferguson & Cross 1984, Ferguson et al, 1985a) and a partial structure defining the term glycosyl-phosphatidylinositol (GPI) was elucidated (Ferguson et al, 1985b). At the same time compositional analyses of Torpedo AChE (Puterman et al, 1985), Thy-1 (Tse et al, 1985) and erythrocyte AChE (Roberts & Rosenberry, 1895) demonstrated the covalent association of GPI components with these proteins. Since then over forty examples of GPI anchored eukaryotic proteins have been described (reviewed recently by Ferguson & Williams, 1988; Low 1989; Cross, 1990).

The elucidation of GPI anchor structure
As an example of the analytical strategies employed to determine GPI structure the example of the GPI anchor of T. brucei VSG will be used. The GPI anchor of the group 1 VSG MITat1.4 was the first to be structurally solved. Based on the known partial structure shown at the top of Fig 1 a strategy was developed to solve the structure using proton nuclear magnetic resonance spectroscopy (1H NMR), mass spectrometry and chemical and enzymatic modifications as principle tools (Ferguson et al, 1988a). Of these techniques, NMR, provides the most information but requires reasonable quantities (>50nmoles) of material in a suitable form, i.e. freely water soluble and fairly small (<10kD). Clearly mFVSG itself fails both of these criteria. It was necessary therefore to remove the diacyl-glycerol (DAG) portion, already known to be sn-1,2-dimyristyl glycerol (Ferguson et al, 1985a; Schmitz et al, 1986), to produce sVSG and subsequently remove all the protein except for the C-terminal amino acid by Pronase digestion. The resulting fragment known as the soluble form C-terminal glycopeptide (sCt-gp, see Fig 1) is ideal for NMR analysis.

High resolution Fourier transform NMR using high-field (500MHz) instruments has become an invaluable tool in the determination of complex carbohydrate structure. Many of the modern techniques were introduced to solve asparagine-linked glycoprotein oligosaccharides. Where previously solved structures are available a one-dimensional NMR spectrum "fingerprint" can be compared with reference spectra to give rapid