

which has progressed to phase I/II (Seymour et al., 2002). Both mannose and ASGR expression is, however, found on both cancerous and normal cells (Hashida et al., 2001). E-selectin, a receptor exclusively expressed in endothelial cells, has been targeted using Sialyl Lewis-X-coated PLGA microparticles containing fluorescent dyes (Eniola et al., 2002). Saccharide-based targeting employed in non-viral gene delivery is discussed in Section 1.5.3.

#### **1.3.2.4 Peptide-Targeted Delivery**

Peptide-targeted delivery has a basis in nature as many peptides are used as attachment ligands by bacteria and viruses. The use of peptides as ligands for receptor-targeting has been investigated by several groups (reviewed in Shadidi & Sioud, 2003). The approach chosen for this study uses peptides identified from the binding region of uPA. Peptide ligands have a number of advantages. These include their lower antigenic potential, making them less likely to cause an immune reaction. They are also easier to synthesise and characterise. Their smaller size means multiple peptides could be attached to a single nanoparticle conferring multivalent attachment.

The use of phage display libraries to determine binding peptides for tumour targets is a powerful method that has shown much success (Nilsson et al., 2000). This method is akin to combinatorial chemistry producing large numbers of potential molecules and selecting for them by their activity, in this case binding to the cell of interest. Essentially the technique involves the recombinant insertion of a peptide motif into the coat of the phage, application to cells and harvesting of attached phage, and the process repeated to purify the most effective phage (Nilsson et al., 2000).

Arap et al. (2002) targeted prostate cancer with a short peptide sequence (SMSIARL) derived from a prostate homing phage. This phage peptide was found to be in 15 times greater amounts in mouse prostate than a control peptide, signifying that targeting had been successful (Arap et al., 2002). In a fusion between this peptide and a proapoptotic peptide they found tissue destruction in the prostate and delayed development of cancer in prostate cancer prone transgenic mice (Arap et al., 2002). Phage display experiments are usually carried out using cell culture and the results are therefore less physiologically relevant. *In vivo* selection of phage has also been developed with great success (Reviewed in Trepel et al., 2002). However, the *in vivo* panning is made in animal models and therefore may have less specificity for human

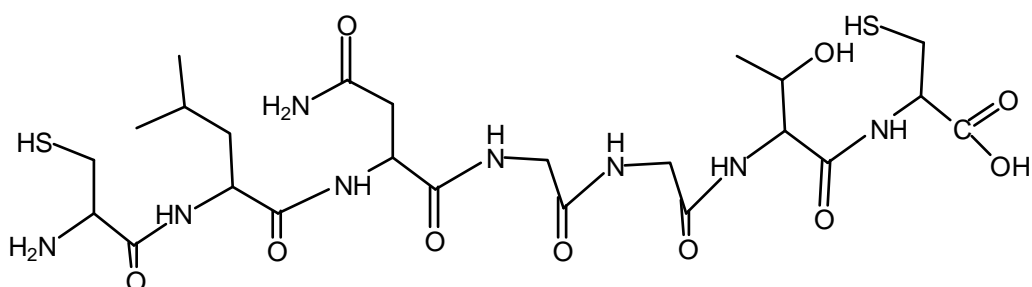
targets. This limitation of murine models may have been overcome using *ex vivo* panning on human umbilical veins. The latter model has identified a heptapeptide that bound 5 times more efficiently to HUVEC (human umbilical vascular endothelial cells) than to B16F10 (murine melanoma) and other cell lines (Maruta et al., 2003).

The most widely investigated receptor family for targeting with peptide ligands is the integrin family. These receptors are expressed on the neo-vasculature of various tumours and are involved in adhesion and cell signalling (Vander et al., 1994). Their peptide ligands are defined as arginine-glycine-aspartic acid (RGD) peptides, and comprise a range of linear and cyclic peptides containing the RGD motif (Arap et al., 1998). Arap et al. (1998) found that mice treated with RGD-targeted doxorubicin had greater survival than mice treated with doxorubicin alone in a murine tumour model. Many other targets are being investigated with peptide ligands targeting the tumour vasculature, cancer cell surface and surface immunoglobulins (reviewed in Shadidi & Sioud, 2003).

Such peptide ligands can be easily prepared in bulk quantities using peptide synthesis. Peptide synthesis is a procedure that involves the activation of carboxylic acids and their reaction with amines to produce a peptide bond. However, as aa contain both a carboxylic acid group and an amino group the amine is first protected to prevent unwanted reactions. This protected aa can then be activated and reacted with an unprotected aa to give the dipeptide. The protecting group is then removed resulting in the dipeptide product desired. The synthesis of peptides and proteins was simplified by Merrifield who won the Nobel Prize in chemistry in 1984 for developing an automated solid phase peptide synthesis (Solomons, 1996). In this advance the C-terminal aa is attached to a resin and the subsequent aa added in deprotection, washing, addition with activator, washing, deprotection etc. until all aa have been added and the peptide is cleaved from the resin (as described in Section 2.3.10).

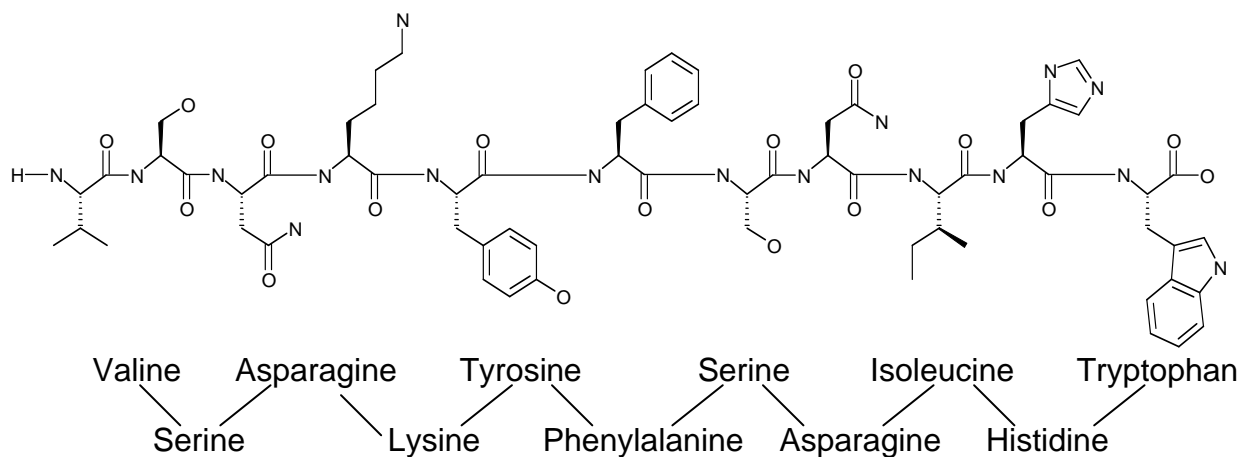
The peptides studied in this thesis, CLNGGTC (u7) and VSNKYFSNIHW (u11) (Fig. 1.5), were identified and selected from the binding region of uPA (Appella et al., 1987). Their smaller size compared with uPA means that one would expect less disruption in the formation of polyplexes. The use of such peptides also removes the catalytic activity of uPA, preventing degradation of the extracellular matrix during use. u7 has been seen to increase the uptake of adenovirus in human airway epithelia (Drapkin et al., 2000).

A)



Cysteine-----Leucine---Asparagine---Glycine---Glycine---Threonine---Cysteine

B)

**Figure 1.5 – Chemical structure of peptide ligands for uPAR**

Panel A) u7, single letter amino acid sequence = CLNGGTC B) u11, single letter amino acid sequence = VSNKYFSNIHW

The peptide increased adenoviral uptake when applied in solution and when conjugated to PEG attached to the viral capsid (Drapkin et al., 2000). Peptide-based targeting employed in non-viral gene delivery is discussed in more detail in Section 1.5.3.

Having discussed the need for improved therapy and the methods employed to target therapies, the uPA receptor and its use as a novel target are now examined.

#### **1.4 Rationale for the Choice of uPAR as a Target**

A cancer cell receptor of increasing interest for targeting is uPAR. Though currently less well understood than many of the other targets being investigated, this receptor may show advantages for targeting either for novel therapeutics or delivery systems. In this section the role, presence and targeting potential of uPAR are described.

##### **1.4.1 Physiological Role and Functions of uPAR**

uPAR, also designated CD87, it is a glycosylphosphatidyl inositol (GPI)-linked receptor of approximately 55 kDa. This GPI linkage means that the protein is attached to the cell membrane at the C-terminal aa only (Fig. 1.6). It is composed of three similar disulphide bonded domains (Fig. 1.7; (Blasi & Carmeliet, 2002)). The crystal structure of the soluble (cleaved at the GPI link) receptor was recently solved with a non-natural peptide ligand antagonist bound (AE147) (Llinas et al., 2005). There is a central cavity 19 Å deep formed between the three domains where the antagonist peptide binds (Llinas et al., 2005). It has been proposed that uPA also binds in this cavity (Llinas et al., 2005). The primary function of uPAR is to bind uPA, which catalyses plasminogen activation to plasmin (Ramage et al., 2003). Plasmin is a serine protease that hydrolyses peptide bonds in fibrin clots and thus prevents thrombosis (Fig. 1.8; (Stryer, 1995)).

By binding uPA at the surface of the cell, uPAR focalises the activity of uPA. It is suggested that this enables cell migration through the digestion of extracellular molecules (Blasi & Carmeliet, 2002). The binding of uPA to uPAR is a high affinity interaction with a  $K_d$  in the low nM range 0.1 - 17 nM (Picone et al., 1989). This makes it particularly attractive in the concept of receptor targeting. The affinity of uPA for uPAR expressed in a (uPAR) transfected cell line (LB6, murine fibroblast) was reported as 1-10 nM (Roldan et al., 1990).