

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.

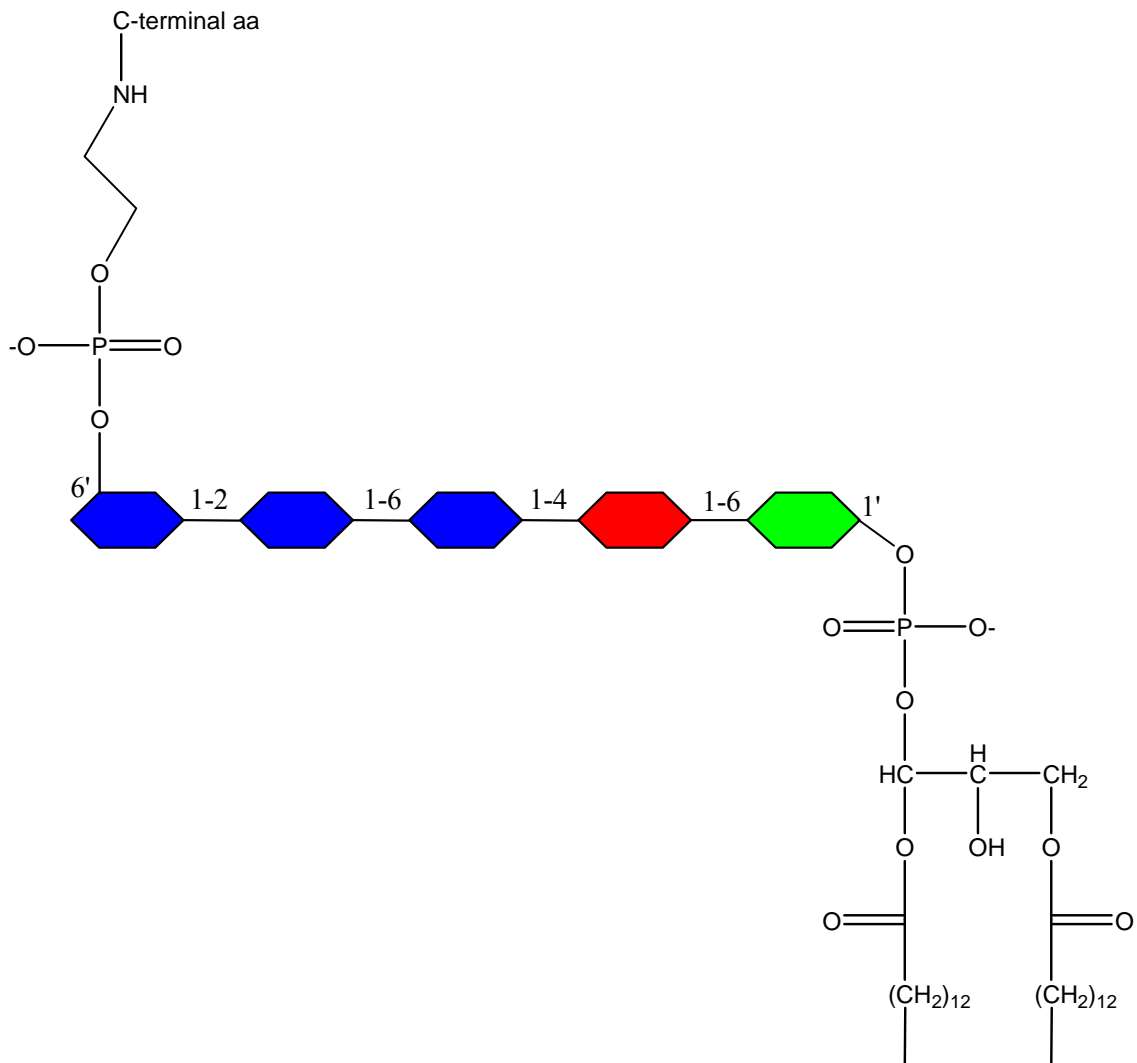
#### **1.4 Rationale for the Choice of uPAR as a Target**

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**

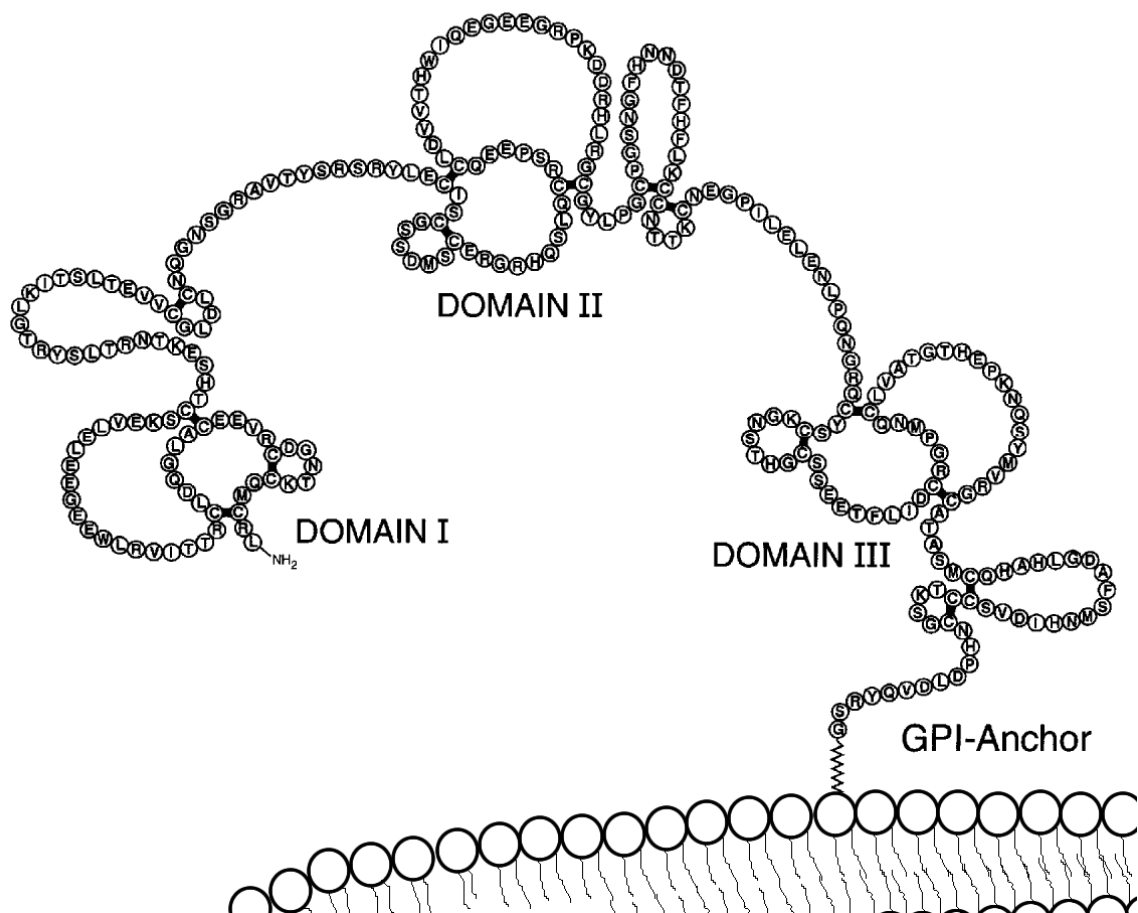
uPAR, also designated CD87, it is a glycosylphosphatidyl inositol (GPI)-linked receptor of approximately 55 kDa. This GPI linkage means that the protein is attached to the cell membrane at the C-terminal aa only (Fig. 1.6). It is composed of three similar disulphide bonded domains (Fig. 1.7; (Blasi & 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).



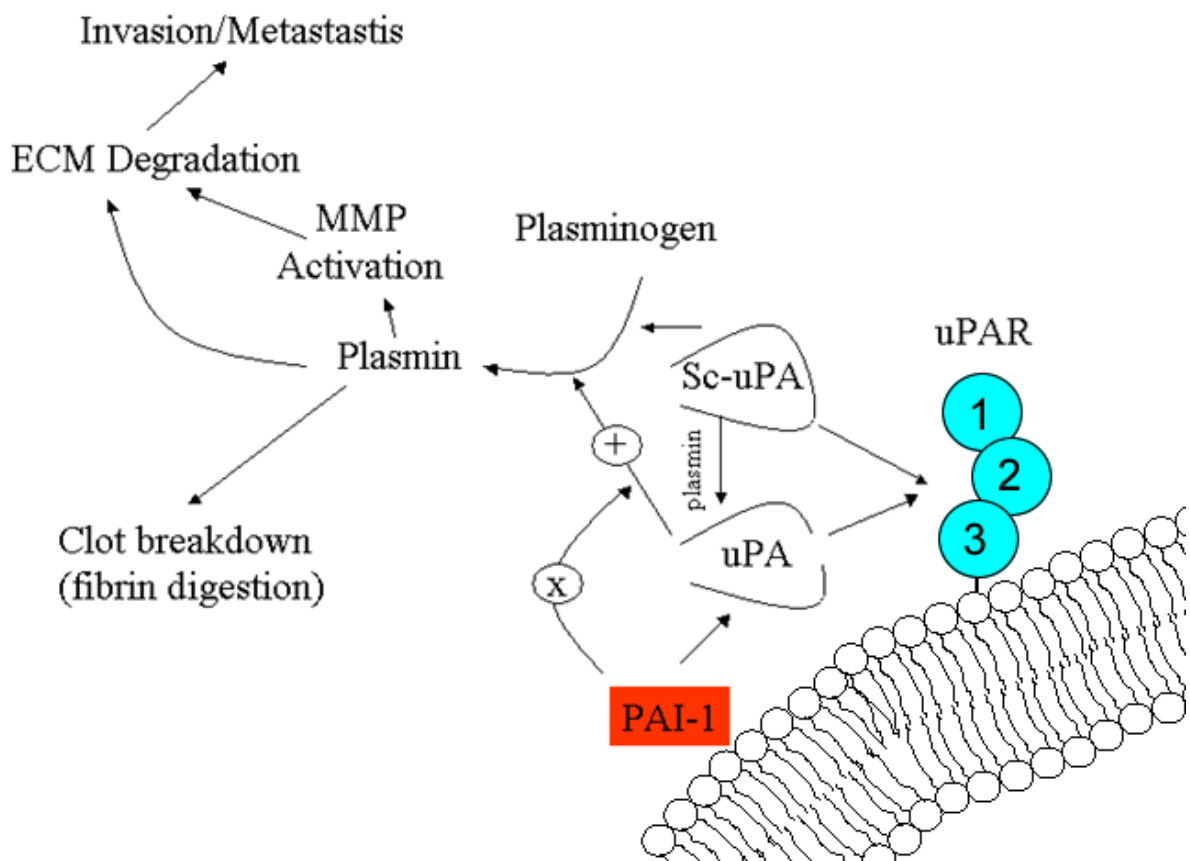
**Figure 1.6 - GPI anchor structure**

Diagrammatic representation of the general structure of a GPI linkage. Blue hexagons represent mannose, red hexagon represents glucosamine and green hexagon represents inositol. The saccharide linkage is shown between the sugars. The oligosaccharide unit is variable between GPI linked proteins, the glycerol phosphate and phosphoethanolamine units are conserved (adapted from Doering et al., 1990).



**Figure 1.7 - Amino acid sequence and predicted structure of uPAR**

uPA binds to domain I and may form a composite binding with domain II. Black bars between cysteine residues indicate disulphide connections (adapted from Ploug et al., 2002).



**Figure 1.8 - Physiological role of the uPA system**

Key; Sc-uPA = Single chain (pro) urokinase plasminogen activator, uPA = urokinase plasminogen activator, uPAR = urokinase plasminogen activator receptor (having domains 1,2 & 3), PAI-1 = plasminogen activator inhibitor 1, ECM = Extra cellular matrix, MMP = Matrix metalloproteinases (Compiled from: Blasi & Carmeliet, 2002, Ploug et al., 2002, Vander et al., 1994)

uPAR is endogenously expressed on the surface of many normal cell types including: blood neutrophils, eosinophils, monocytes, bone marrow, CD34 negative myelomonocytic precursors, mast cells, fibroblasts, fetal thyroid cells, and endothelial cells (Lanza et al., 1998, Nykjaer et al., 1994b, Plesner et al., 1997, Sillaber et al., 1997). This expression is a disadvantage for targeting as these cells would also take up the targeted therapeutic. However, receptor expression on these cells is lower than in cancerous cells.

uPA is secreted as pro-uPA or sc-uPA which are the single chain form with a molecular weight of 55 kDa (Ghosh et al., 2000b). This secretion is performed by many cell types including neutrophils, endothelial and epithelial cells (Abraham et al., 2003, Ghosh et al., 2000a). The single chain protein is cleaved by plasmin at the Lys<sup>158</sup>-Ile<sup>159</sup> site to give an activated two chain form linked by cystine bonds (Mazar et al., 1999). In addition to activation by plasmin, activation of sc-uPA by kallikrein, mast cell tryptase, T cell-associated serine proteinase and cathepsin B are also reported (Ghosh et al., 2000b, Kobayashi et al., 1991).

uPA binds to domain I in the amino terminal portion of uPAR (Fig. 1.7). It was later suggested that domains I and III form a composite binding site for uPA (Blasi & Carmeliet, 2002), and, as suggested by Llinas et al. (2005), all three domains may make a cavity which binds uPA. Soluble uPAR is formed through the cleavage of the GPI anchor (Blasi & Carmeliet, 2002). Cleavage of uPAR can be made by several different enzymes including trypsin, chymotrypsin, human neutrophil elastase, phospholipase C (Ploug et al., 2002) and plasmin itself producing the fragments domain 1, domain 2/domain 3 (Montuori et al., 2002). As uPAR is a GPI linked receptor it does not have an intracellular signalling domain, which is the case for many other receptors (Wise et al., 2002).

Plasminogen activator inhibitor (PAI-1) is the primary physiological inhibitor of uPA and tissue-type plasminogen activator. It is a multi-functional serine protease inhibitor (serpin) protein that is also involved in many other physiological and pathophysiological processes (Wind et al., 2002). PAI-1 binds to uPA (but not sc-uPA) stopping its catalytic activity both in circulation and when attached to the receptor (Goretzki & Mueller, 1997). It can both inhibit invasion (through inactivation of proteolytic activity) and promote it (through disruption of cell adhesion) (Bajou et al., 2004, Degryse et al., 2001, Deng et al., 1996).

In addition to the many specific components of the uPA system shown in Fig. 1.8, internalisation of uPAR is complex. It proceeds after the recruitment of other cell membrane components (Blasi, 1997, Blasi & Carmeliet, 2002, Nykjaer et al., 1998, Nykjaer et al., 1997, Nykjaer et al., 1994a). There appears to be many mechanisms by which uPAR internalisation can occur. An understanding of the endocytic fate is important to the proposed use of uPAR as a target and it is discussed in detail in Section 1.4.3.

#### ***1.4.2 Evidence for Over-Expression of uPAR in Cancer***

It has been shown that uPAR is over-expressed in a variety of cancers including: monocytic, myelogenous and megakaryocytic leukemias (Lanza et al., 1998, Plesner et al., 1994), bladder transitional carcinomas (Carriero et al., 1997), thyroid (Hudson & McReynolds, 1997), stomach (Plebani et al., 1997), liver (De Petro et al., 1998), pleura (Shetty & Idell, 1998), lung (Morita et al., 1998), pancreatic (Taniguchi et al., 1998), ovarian (Sier et al., 1998) cancers and glioblastomas (Mori et al., 2000). The upregulation of uPAR has also been shown in prostate cancer cell lines by microarray analysis (Han et al., 2002). This group found an increase (2.2x to 10.3x) in several cell lines in comparison with normal pancreas cells (Han et al., 2002). Lanza et al. (1998) found that those patients with acute myeloid leukaemia (M5 French-British-American subtype) having the worst survival rates showed a significant increase in uPAR levels ( $>12 \times 10^3$  antibody binding capacity (ABC)/cell compared to  $<12 \times 10^3$  ABC/cell) in their tumour (Lanza et al., 1998). It was also shown that high uPAR expression was associated with chromosome abnormalities and disease relapse (Lanza et al., 1998). Similarly, Suzuki et al. (1998) reported that expression of uPAR in colorectal carcinomas correlated with increasing disease severity, whereas there was no detectable expression in matched normal cells.

The number of uPAR receptors expressed per cell has been determined using radioligand binding assays for several cell lines. U937 (human histocytic lymphoma monocyte) cells have ~31000 receptors/cell and MCF-7 (human breast cancer epithelial) cells have ~4600 receptors/cell (Rajagopal & Kreitman, 2000). All prostate cancer cells (Gleason score 4-9) tested by Garilov et al. (2001) were positive for uPAR protein expression. The Gleason score, or grade, is an indicator of how differentiated the tumour cells are. Well differentiated cells (i.e. normal cells) have a score of 1 and poorly differentiated cells have higher scores. Overall it is apparent that in many cancers