Journal of proteome research

Reviews

pubs.acs.org/jpr

Terms of Use

The Role and Importance of Glycosylation of Acute Phase Proteins with Focus on Alpha-1 Antitrypsin in Acute and Chronic Inflammatory Conditions

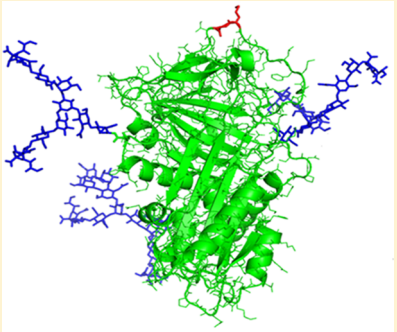
Cormac McCarthy,*[,†] Radka Saldova,[‡] Mark R Wormald,[§] Pauline M. Rudd,[‡] Noel G. McElvaney,[†] and Emer P. Reeves[†]

[†]Respiratory Research Division, Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin 9, Ireland

[‡]NIBRT GlycoScience Group, The National Institute for Bioprocessing Research and Training, University College Dublin, Dublin 4, Ireland

[§]Department of Biochemistry, Oxford Glycobiology Institute, University of Oxford, Oxford OX1 3QU, U.K.

ABSTRACT: Acute phase proteins (APPs) are a group of circulating plasma proteins which undergo changes quantitatively or qualitatively at the time of inflammation. Many of these APPs are glycosylated, and it has been shown that alterations in glycosylation may occur in inflammatory and malignant conditions. Changes in glycosylation have been studied as potential biomarkers in cancer and also in chronic inflammatory conditions and have been shown to correlate with disease severity in certain conditions. Serine protease inhibitors (serpins), many of which are also APPs, are proteins involved in the control of proteases in numerous pathways. Alpha-1 Antitrypsin (AAT) is the most abundant serpin within the circulation and is an APP which has been shown to increase in response to inflammation. The primary role of AAT is maintaining the protease/antiprotease balance in the lung, but it also possesses important anti-inflammatory and immune-modulating properties. Several glycoforms of AAT exist, and they possess differing properties in regard to plasma half-life and stability. Glycosylation may also be important in determining the immune modulatory properties of AAT. The review will focus on the role and importance of glycosylation in acute phase proteins with particular attention to AAT and its use as a biomarker of disease. The review describes the processes involved in glycosylation, how glycosylation changes in differing disease states, and the alterations that occur to glycans of APPs with disease and inflammation. Finally, the review explores the importance of changes in glycosylation of AAT at times of inflammation and in malignant conditions and how this may impact upon the functions of AAT.



Glycosylated Alpha-1 Antitrypsin

KEYWORDS: *alpha-1-antitrypsin, glycosylation, inflammation*

INTRODUCTION

Serine protease inhibitors (Serpins) are the largest superfamily of protease inhibitors, membership of which is based upon the presence of a single common core domain consisting of three β-sheets and eight to nine α-helices.[1,2] There are 36 confirmed serpins in humans, among these are alpha-1 antitrypsin (AAT), alpha-1 chymotrypsin (ACT), C1-inhibitor and antithrombin (AT) which play important roles in control of proteases involved in inflammatory, complement, and coagulation pathways, respectively.[3,4] Serpins are relatively large molecules that employ unique extensive conformational changes to the core domain to inhibit proteases.[2] For example, the majority of proteins fold naturally into their most stable state; however, AAT is produced in a metastable form with lower conformational stability which is essential for its biological function. In the reactive center loop there are 20 amino acids that are cleaved by the target protease,[5,6] resulting in an inhibitory noncovalent Michaelis stable complex between protease and serpin. Indeed, AAT is the archetypal member of the serpin superfamily and is the most abundant endogenous serine protease inhibitor in the blood. AAT is also an acute phase protein,[7] the levels of which become elevated within hours of developing inflammation or postinfection[8] and is known to be elevated in numerous conditions ranging from acute coronary syndrome to postsurgery.[9,10] In addition to antiprotease activity, AAT demonstrates unique anti-inflammatory properties affecting several cell types and modulating inflammation caused by both host and microbial factors.[11,12]

The AAT molecule is a polypeptide chain composed of 394 amino acids and is post-translationally modified by glycosylation in the endoplasmic reticulum (ER). *N*-glycosidically linked oligosaccharides are added at three asparagine residues at positions 70, 107, and 271 on the peptide[13,14] in the ER, and the precursor oligosaccharide is modified in the ER and Golgi. AAT is synthesized and secreted primarily in the liver by hepatocytes[15,16] but is also synthesized and secreted from macrophages,[17] monocytes,[18] neutrophils,[12] and intestinal[19]

Received: February 14, 2014
Published: June 3, 2014

and bronchial epithelial cells.[20] The primary function of AAT is as an antiprotease and is important in the protease/antiprotease balance, protecting connective tissue in the lung from degradation by serine proteases including neutrophil elastase.[21] This role of AAT is most apparent in the genetic condition of AAT deficiency (AATD) in which patients are at a high risk of developing emphysematous lung disease at a young age.[22] The most common variants associated with both lung and liver disease are the Z (Glu342Lys) and S (Glu264Val) mutations, caused by the substitution of glutamic acid for lysine or valine at positions 342 and 264 of the polypeptide, respectively.[23−26] The Z allele causes the most severe plasma deficiency, and the mutant Z variant (Z-AAT) occurs in >95% individuals with AAT deficiency.[27] AATD is the only proven genetic risk factor for the development of chronic obstructive pulmonary disease and individuals who are heterozygous for AATD and also smoke, are at increased risk of developing lung disease.[28] Treatment for AATD currently consists of infusion of purified human plasma AAT (60 mg per kilogram of body weight per week) and is now used in parts of Europe and North America in treatment of AATD individuals.[29] The clinical efficacy of intravenous AAT augmentation therapy for AATD individuals has been evaluated in a number of studies, with increasing evidence of a benefit from treatment, primarily in decreasing the loss of lung density[30−33] and in slowing the loss of FEV1;[34,35] however, the cost-effectiveness of this therapy remains debatable.[36]

The role of AAT as an antiprotease and its role in lung and liver disease have been extensively studied with a large number of reviews published in this area.[22,37−39] Many recent reviews have focused on the anti-inflammatory and immune modulatory properties of AAT and its possible novel therapeutic implications.[11,40,41] Although the presence of a large number of different glycoforms of AAT have been known to exist and were initially studied in the mid-1970s,[42] there have been few studies addressing the role of glycosylation in both AAT and other serpins until recently. This re-emerging interest in glycosylation appears to have followed evidence generated in the middle of the past decade that highlighted the possible importance of glycosylated AAT used in augmentation therapy for AATD, demonstrating that glycosylation of AAT improved both the stability and functional efficacy of treatment.[43,44]

In this review we will first give an overview of glycosylation, explaining the processes involved, and describe the importance of glycosylation in health and in disease. Focus will then turn to the importance of glycosylation changes in APPs and serpins with an emphasis on AAT. The review will then detail the changes and importance of glycosylation in AAT and how it is linked to function and altered in various disease states.

Overview of Glycosylation

Glycosylation is a process in which carbohydrate residues are attached to proteins, and it is the most complex post-translational modification that peptides undergo.[45] Protein synthesis is under genetic control, however, as glycosylation is a posttranslational process, involving some 600 proteins, significant variation and heterogous glycoforms of peptides exist.[46] Protein glycosylation throughout all species is an extremely complex process and includes *N*-glycosylation, *O*-glycosylation, *C*-mannosylation, phosphoglycation, and glypiation (GPI anchors). At least 13 different monosaccharides, 8 amino acids, and 16 enzymes are involved in forming approximately 41 varying types of glycosidic bonds.[47] There is a range of important functions for specific sugars involved in the glycosylation process within the ER; they protect certain residues from cleavage by proteases,[48] and they are important in certain peptide folding pathways acting as recognition structures.[46] For the purpose of this review we will primarily discuss the process of *N*-glycosylation in humans as this is the type of glycosylation that AAT and other serpins undergo. *N*-glycosylation is the addition of *N*-linked oligosaccharides, known as glycans, to a protein requiring the transfer of a common precursor to the nitrogen side chain of an asparagine residue.

Process of *N*-glycosylation

The most commonly found saccharide−peptide bond is the β-glycosylamine linkage of *N*-acetylglucosamine (GlcNAc) to asparagine (Asn);[49] first described in 1961,[50] this bond serves as a site for oligosaccharide attachment and is found in plasma proteins, cell surface receptors, enzymes, and immunoglobulins.[47] Initiation of *N*-glycosylation occurs in the ER, as a peptide leaves the ribosome and the NH_2 terminus signal sequence translocates the nascent glycoprotein into the ER lumen. A peptide that possesses the amino acid triplet (sequon) of AsnXSer or AsnXThr (if X is not Pro) can undergo *N*-glycosylation. Glycosylation occurs when this sequon interacts with oligosaccharyltransferase, a heteroligomeric ER membrane complex,[51] and a dolichol diphosphate oligosaccharide precursor.[46] In this process a 14-sugar oligosaccharide, $Glc_3Man_9GlcNA_2$, is transferred from the precursor to the nitrogen of Asn, an event which occurs before protein folding is complete. The initial modification step is the removal of three glucose residues attached to the terminal mannose of the $Glc_3Man_9GlcNA_2$ precursor by α-glucosidase I and α-glucosidase II.[52] The removal of one to four α-1,2-linked mannose residues by α-mannosidase I occurs at the time of or following protein folding and deglucosylation.[46] The extent to which a peptide is glycosylated depends on multiple factors: peptide specific factors including protein structure, proximity of sequon to N or C terminus,[53] proximity of certain amino acids to the sequon,[54,55] and cellular factors such as oligosaccharyltransferase levels, dolichol diphosphate levels[56] and enzyme levels.[57]

Following initial processing in the ER, protein glycosylation continues in the Golgi apparatus where the sequential addition of monosaccharide residues occurs. This processing is both controlled by protein structure[58] and initiated by *N*-acetylglucosaminyl (GlcNAc) transferase I, which substitutes *N*-acetyl-glucosamine (GlcNAc) onto the α-1,3-arm of the glycan $Man_5GlcNAc_2$.[59] The further processing of the glycan groups into branching complex sugars depends on the levels of the enzyme, cell type, and 3-D structure of the protein. If further branching is possible, this is mediated by GlcNAc transferase II forming bi-antennary structures and GlcNAc transferases IV and V responsible for tri-antennary and tera-antennary complexes.[46,59] Branching is blocked by the insertion of a GlcNAc residue at a bisecting position between two arms by GlcNAc transferase III.[60] Termination of chain prolongation usually occurs following the addition of a sialic acid residue to terminal galactose. The heterogeneity of glycosylation and subsequent glycan groups processed in the Golgi is cell specific and has been demonstrated to differ in certain cell types such as cancer cells[61,62] and under different conditions such as in response to inflammation.[63]

dx.doi.org/10.1021/pr500146y | J. Proteome Res. 2014, 13, 3131−3143

Purposes of Glycosylation and Role of Glycan Groups

Glycosylation of proteins increases their stability by protecting proteins from proteolysis and degradation,[64] and in some cases specific oligosaccharide groups confer improved stability upon proteins.[65] Subsequently the plasma half-life of proteins is often prolonged for glycosylated proteins when compared to the corresponding under-glycosylated or nonglycosylated peptides.[66] Glycans are also fundamentally important to protein folding and polymerization as glycosylation is a cotranslational process occurring as the protein folds, with certain oligosaccharide variations also conferring differences in relaxation and mobility.[67] Glycan groups also modulate protein steric interactions[64,68] and specific oligosaccharides can impart specific functions to proteins.[69] It has been shown that different branching of glycan groups can communicate variation in affinities for receptors and play a role in controlling plasma half-life.[70,71] The importance of glycans in the immune system has been extensively described, with major roles in cell to cell adhesion and recognition signals for antibodies.[72,73] Glycan groups are also recognized when differentiating human cell-surface proteins from yeast or bacteria, hence, playing a pivotal role in the innate immune system.[46]

Glycosylation in health, variations among individuals and influence of environmental factors

To understand the glycosylation changes in disease, we should first describe glycosylation in the healthy state. Glycosylation can be affected by numerous factors such as sugar nucleotide concentrations, types of glycoenzymes, and their expression levels in the specific cells; it is a complex process including 600 proteins involved together with transcription factors.[74] Applying recently developed high-throughput technology hydrophilic interaction liquid chromatography with fluorescence detection (HILIC-fluorescence),[75] an initial study based on a cohort of 1008 individuals revealed that the median difference between minimal and maximal levels of individual plasma *N*-glycan levels is over 6-fold, reflecting a structural diversity that is higher for *N*-glycans than for any other macromolecule.[76] This large study has also shown that heritability varies widely across glycans, and several environmental factors, including smoking, were identified that were associated with changes in some glycan structures.[76]

Both age and gender have strong modifying effects on *N*-glycosylation. Nongalactosylated glycans and glycoforms containing bisecting GlcNAc are increased and core (α-1,6) fucosylated glycans are decreased with increasing age.[76,77] Among these observed age-related alterations the most evident were present in women, particularly associated with the transition through menopause.[78] The glycan features that differ between men and women include antennary, (outer arm, α-1,3) fucosylation, degree of branching, level of nongalactosylated, tetragalactosylated glycans and bi-antennary nongalactosylated.[78] These gender-specific differences may be explained by hormonal variances. It is known that the level of estrogen correlates negatively with the sialyl Lewis x (sLe^x) antigen, galactosylation and sialylation of immunoglobulin G (IgG) increases during pregnancy and core fucosylated bi-antennary glycans are associated with usage of oral contraceptives.[79] The first successful attempt to connect genome-wide association studies (GWAS) to the study of genetic regulation of *N*-glycans from human plasma glycoproteins was done on a population study of 2000 people, with glycan structures characterized by HILIC-fluorescence.[78] The results indicated that a large part of the observed variability is under genetic control, since all measured environmental and lifestyle factors explained less than 5% of the variance in most of the glycans.[78] Lauc et al. demonstrated significant association of bi-antennary *N*-linked glycan A2 with single nucleotide polymorphisms in the fucosyltransferase 8 (FUT8) gene and estrogen receptor 2 beta (ESR2) gene.[80] HNF1a was described as a master regulator of fucosylation by tight regulation of fucosyltransferases expression.[81] Genetic variants in the FUT8 and FUT6 influence mainly glycan structures containing core and outer arm fucosylation.[81] This finding is supported by their known biological function.[82]

Smoking and Glycosylation

Tri-antennary, tetra-antennary, and outer arm fucosylated (including sLe^x antigen) human plasma protein glycans are increased, while core fucosylated, bi-antennary, monogalactosylated and nongalactosylated glycans are decreased in smokers compared to nonsmokers.[76,78,83,84] These changes reflect increased *N*-glycan branching and outer arm fucosylation in smokers and may be associated with smoking-related inflammation as these changes are associated with inflammatory processes.[74,85] On the other hand, decrease in monogalactosylated and nongalactosylated glycans could be the consequence of decreased IgG concentration, since higher consumption of cigarettes was reported to result in decreased IgG levels.[86] Core fucosylation was also decreased in smokers, confirming the observation of decreased FUT8 activity in mice exposed to cigarette smoke.[78]

Glycosylation in Disease

Glycosylation is altered in many diseases such as acute and chronic inflammatory diseases (sepsis, pancreatitis, congenital disorders of glycosylation, rheumatoid arthritis, diabetes), infection (HIV/AIDS), or malignancy.[74] There are several recent publications that focus on *N*-glycans and *O*-glycans as disease markers.[87] Alterations in the degree of branching, levels of sialylation and fucosylation in *N*-glycans and changes in *O*-glycans in mucins have been associated with diseases.[87] Glycans are crucial for the immune response, and some of the most important interactions between virus, bacteria, and the immune system are governed by protein−glycan interactions. Additionally, as glycans are involved in important recognition events, an altered glycome can lead to autoimmune diseases.[88]

Inflammation is a complex biological response to harmful stimuli, such as infection, damaged cells, physical trauma, or malignancy. Every inflammatory process is accompanied by numerous changes at the site of inflammation and many systemic physiological and biochemical changes.[89] A large number of cytokines from the inflamed site travel systemically and stimulate hepatocytes, the acute phase response is triggered, and the synthesis and glycosylation of acute phase proteins circulating in serum is altered.[89,90] The concentration of acute phase proteins increases (positive acute phase proteins) or decreases (negative acute phase proteins) by at least 25% during inflammation.[89] Two of the most reported alterations in the serum *N*-glycome during inflammation are increases in branching (tri- and tetra-antennary glycans) and in levels of sLe^x antigen,[74] which is involved in attachment of leukocytes to E-selectins.[74]

The inflammatory response can be divided into two stages: acute inflammation, which is triggered as an immediate response to damage or injury, and chronic inflammation. In a study by Gornik et al., the serum *N*-glycome from sepsis and

dx.doi.org/10.1021/pr500146y | J. Proteome Res. 2014, 13, 3131−3143

acute pancreatitis was monitored during the first 8 days of the disease.[91] Sepsis is the clinical syndrome resulting from infection, while acute pancreatitis is a systemic inflammatory response without bacterial infection. Results revealed that the level of high mannose structures decreased with the progression of both sepsis and pancreatitis.[91] These types of *N*-glycan structures can be found on the C3 component of complement, one of the positive acute-phase proteins. Branching (tri-antennary and tetra-antennary structures) and ratio of outer arm fucosylated glycans to core fucosylated glycans were increased in both diseases,[91] and implicated in immune modulation.[92] In pancreatitis, there were also increased bi-antennary glycans with bisecting GlcNAc and trisialylated tri-antennary glycan containing sLe^x.[91]

Chronic inflammatory diseases include allergic conditions as well as autoimmune diseases such as rheumatoid arthritis, chronic inflammatory bowel disease (ulcerative colitis, Crohn's disease), and neurodegenerative diseases such as schizophrenia.[74] In rheumatoid arthritis, there is a decrease in galactosylation on IgG, altered glycosylation on alpha-1-acid glycoprotein (AGP), increased branching in *N*-glycans in transferrin, and increase in expression levels of haptoglobin (HPT) as well as in its fucosylation, sialylation, and branching.[74] In schizophrenia, an increase in tri-antennary trisialylated glycans with sLe^x has been reported in male schizophrenia patients, but the level decreased in female patients compared to controls.[93] The alteration of the serum *N*-glycome in cirrhotic patients includes an increase in structures with bisecting GlcNAc, core fucosylation, and neutral glycans.[94] Chronic liver fibrosis patients with hepatitis C virus infection and hepatocellular carcinoma patients with hepatitis B virus infection showed a high level of core fucosylation[95,96] and it was proposed that the increase of core fucosylation associated with chronic liver damage could be a high risk factor for developing cancer.[97] Increase of core fucosylated nongalactosylated bi-antennary glycans in IgG *N*-glycans and highly sialylated forms of HPT were described in Crohn's patients.[98] Chronic pancreatitis patients showed increased branching and levels of sLe^x on acute phase proteins and increase in bisecting GlcNAc and tri-antennary glycans on transferrin.[99]

Glycosylation of Acute Phase Proteins

The major acute phase proteins (APPs) are glycosylated, and include AGP, HPT, ACT, AAT, fetuin (FET), transferrin (TFR), and fibrinogen. The most significant APP glycosylation changes that have been described include alterations of the number of antennae and of the extent/type of fucosylation, changes in sialylation especially the expression of the carbohydrate antigen sLe^x.[100] A summary of the glycosylation of acute phase proteins and the alterations of glycosylation in diseases is shown in Table 1. Bi-antennary glycans on AGP increase in acute inflammation, and there is an inconsistency in reports whether they increase or decrease as the disease become chronic.[100] Branching increases on HPT in ovarian cancer compared to controls and in prostate cancer compared to benign prostatic hyperplasia.[100] Tetra-antennary structures increase on AGP and HPT in both chronic pancreatitis and advanced pancreatic cancer patients.[99] SLe^x type fucosylation increases on different APPs including AGP, HPT, ACT and AAT in acute inflammation, chronic inflammation (rheumatoid arthritis, inflammatory bowel disease, diabetes mellitus) and in advanced cancer (ovarian, breast, prostate, lung and pancreatic).[100] AGP fucosylation was also proposed as a marker of progression and prognosis in different types of malignancies.[101] It has recently been reported that complement C3 (positive APP) shows an increase in α2,6-sialic acid and in fucosylation in plasma from colorectal cancers in comparison to adenoma and healthy patients.[102] AGP and HPT showed an increased core fucosylation in lung cancer patients compared to healthy controls.[103] Pancreatic cancer patients have also shown an increase of HPT core fucosylation compared to controls and chronic pancreatitis, suggesting that this modification could be cancer associated.[100]

N-Glycosylation of Alpha-1 Antitrypsin

N-glycosidically linked oligosaccharides of AAT are found at three asparagine residues at 70, 107, and 271 on the peptide; these carbohydrate groups represent approximately 12−16% of the weight of AAT[104] (Figure 1). There are multiple genetic variations of AAT which differ according to their electrophoretic properties and in their concentration found in plasma. A number of these are associated with pronounced deficiency of AAT in the circulation; the most important is the Z mutation.[105] The most commonly found form of AAT in humans is the M phenotype which has normal levels of the protein and is at no increased risk of lung or liver disease.[22] Multiple isoforms of AAT are known to exist, and these differ according to the glycan groups found at these three sites (Figure 2).[106] These are readily seen on isoelectric focusing which is used to phenotype individuals suspected of AATD.[107] There are nine known glycoforms of M AAT, M0−M8, which differ according to their glycan groups. The more negatively charged glycoforms have a greater number of branched glycan groups, and hence an increased number of sialic acid residues. In all the glycoforms there are di-antennary glycan groups at positions 46 and 247, and the branching glycans at position 83 differentiate the separate glycoforms (Figure 3). M6 contains a di-antennary glycan at position 83, while M4 contains a tri-antennary glycan at this position and M2 a tetra-antennary glycan. M7 and M8 contain glycans identical to those of M4 and M6, respectively, but theses differ in size due to a 5-amino acid deletion.[13] There is no reported glycoanalysis of M0, M1, M3, or M5 in the literature; these glycoforms are less abundant and not as readily identifiable using proteomic techniques. Similar to other glycoproteins the *N*-glycosylation process primarily occurs in the ER, and final branching of the glycans occurs in the Golgi apparatus. Relevant to Z-AAT, the Golgi apparatus is essential in processing AAT as it has been demonstrated that the Golgi can glycosylate misfolded proteins that are otherwise not secreted from the cell.[108] It has also been shown that the glycosylation of AAT differs between serum and hepatocytes,[42] and this may be important in Z AAT in which there is a failure to fully secrete the protein from hepatocytes.[24,109] During the branching of glycans into tri-antennary or tetra-antennary groups the addition of a fucose group can form a sLe^x epitope.[13,14] The sLe^x epitope consists of a sialic acid residue α-2,3-linked to galactose with fucose α-1,3-linked to GlcNAc. This can be used to identify these isoforms and may have a role as a biomarker[110] which will be discussed in a later section.

The General Role of *N*-Glycosylation in Alpha-1 Antitrypsin

In many glycoproteins the role of oligosaccharides is multifaceted, and AAT is no different. Glycans in general are important for protein folding, stability, flexibility, and signaling. In many proteins the importance of glycans for adequate protein folding[111] has been demonstrated; however, this has

dx.doi.org/10.1021/pr500146y | J. Proteome Res. 2014, 13, 3131−3143

Table 1. Acute Phase Glycoproteins Altered in Disease and Their *N*-Glycosylation

acute phase glycoprotein	glycosylation	glycosylation changes	disease
alpha-1 acid glycoprotein (AGP)	**5 *N*-glycosylation sites**	increase in bi-antennary glycans	acute inflammation[100]
	N33- di- or tri-antennary, core and outer arm fucosylated, sialylated[156]	increase in total fucosylation	cancer progression and prognosis[101]
	N56 di-, tri-, or tetra-antennary, sialylated[156]	increase in sLe^x (outer arm fucosylation	acute inflammation,[63,157,158] chronic inflammation (e.g., diabetes)[157−160]
	N72 di-, tri-, or tetra-antennary, core and outer arm fucosylated, sialylated[156]		ovarian cancer,[161] pancreatic cancer, and pancreatitis,[99] lung cancer,[162] breast cancer,[147] diabetes and acute inflammation[158]
	N93 tri- or tetra-antennary, core and outer arm fucosylated, sialylated; tetra-antennary glycans can have more than one fucose[156]		
	N103 di-, tri-, or tetra-antennary, core and outer arm fucosylated, sialylated; tetra-antennary glycans can have more than one fucose[156]	increase in core-fucosylation	lung cancer,[103] pancreatic cancer[99]
		increase in tetra-antennary glycans	chronic pancreatitis and pancreatic cancer,[99] diabetes and acute inflammation[158]
haptoglobin (HPT)	**4 *N*-glycosylation sites**	increase in sialylation	Crohn's disease, rheumatoid arthritis, stomach, ovarian, and breast cancer,[98] lung cancer[164]
	N184: bi-antennary, mono- and di-sialylated glycans[163]	increase in branching	ovarian cancer, prostate cancer[100,163]
	N207, N211: bi- and tri-antennary, mono-, di-, and tri-sialylated and many fucosylated containing sLe^x epitope[163]	increase in tetra-antennary glycans	chronic pancreatitis and pancreatic cancer patients[99]
	N241: bi- and tri-antennary, mono-, di-, and tri-sialylated and many fucosylated containing sLe^x epitope[163]	increase in sLe^x (outer arm fucosylation)	pancreatitis[99] and pancreatic cancer,[165,166] ovarian cancer,[161,167,168] prostate cancer,[163] lung cancer,[162] prostate cancer,[163] breast cancer,[147] acute inflammation,[159] chronic inflammation[159,169]
		increase in core fucosylation	lung cancer,[103] pancreatic cancer[99]
		increase in fucosylation (total)	Crohn's disease, rheumatoid arthritis, stomach, ovarian, and breast cancer,[98,167,168] lung cancer,[164] pancreatic cancer[165]
		increase in galactosylation and GlcNAc content	ovarian cancer[167]
alpha-1 antitrypsin (AAT)	**3 *N*-glycosylation sites**	increase in sLe^x (outer arm fucosylation)	ovarian cancer, breast cancer,[170] pancreatitis[99]
	N70-bi-antennary di-sialylated, little tri-antennary trisialylated, few core or outer arm fucosylated[13]		alpha-1 antitrypsin deficiency[123]
	N107-bi-, tri-, and tetra-antennary, di,- tri-, and tetra-sialylated, some core and outer arm fucosylated[13]		
	N271-bi-antennary di-sialylated, some core fucosylated[13] (Figures 1 and 3)	increase in core fucosylation	alpha-1 antitrypsin deficiency[123]
anti-chymotrypsin (ACT)	**6 *N*-glycosylation sites**	increase in sLe^x (outer arm fucosylation)	acute inflammation and chronic inflammation,[159] ovarian cancer,[161] breast cancer[147]
	N33, N93, N106, N127, N186, N271		
	disialyl bi-antennary, tri-sialyl tri-antennary and di-sialyl tri-antennary, tri- and tetra-antennary glycans have been identified[171]		
complement C3	**3 *N*-glycosylation sites**	increase in α-2,6-sialic acid and in fucosylation	colorectal cancers in comparison to adenoma and healthy patients[102]
	N85, N939, (N1617-not glycosylated[172]		
	high mannosylated glycans (M5−M9)[173]		
transferrin	**2 *N*-glycosylation sites**	increase in sLe^x (outer arm fucosylation)	pancreatitis[99]
	N432, N630		
	bi-antennary, tri-antennary, high mannosylated, mono-, di-, and tri-sialylated, some core and some outer arm fucosylated[99]	increased branching	pancreatitis[99]

dx.doi.org/10.1021/pr500146y | J. Proteome Res. 2014, 13, 3131−3143

Table 1. continued

	glycosylation	glycosylation changes	disease
acute phase glycoprotein			
fetuin	**2*N*-glycosylation sites**	increase in sLe^x (outer arm fucosylation)	pancreatitis[99]
	N156: bi- and tri-antennary, fucosylated[174]		
	N176: bi- and tri-antennary[174]	increased branching	pancreatitis[99]
	Bi-, tri-, tetra-antennary, mono-, di-, and tri-sialylated, some core and some outer arm fucosylated[99]		

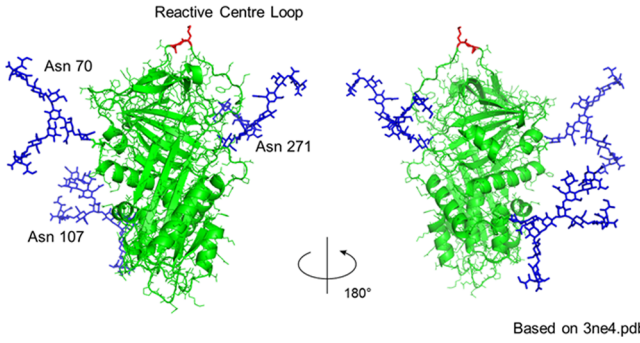


Figure 1. Molecular model of glycosylated alpha-1 antitrypsin. Green - peptide; blue - glycans; red - reactive center loop (peptide linkage) (residues M382-S383). Methods: Molecular modeling was performed on a Silicon Graphics Fuel workstation using InsightII and Discover software (Accelrys Inc., San Diego, U.S.A.). Figures were produced using the program Pymol.[150] Protein structures used for modeling were obtained from the PDB database.[151] The structure of glycosylated alpha-1 antitrypsin was based on the crystal structure of human alpha-1 antitrypsin.[152] *N*-linked glycan structures, chosen on the basis of the sequencing results (Figure 3, glycoform M4), were generated using the database of glycosidic linkage conformations[153] and in vacuo energy minimization to relieve unfavorable steric interactions. The Asn-GlcNAc linkage conformations were based on the observed range of crystallographic values,[154] the torsion angles around the Asn Cα−Cβ and Cβ−Cγ bonds then being adjusted to eliminate unfavorable steric interactions between the glycans and the protein surface.

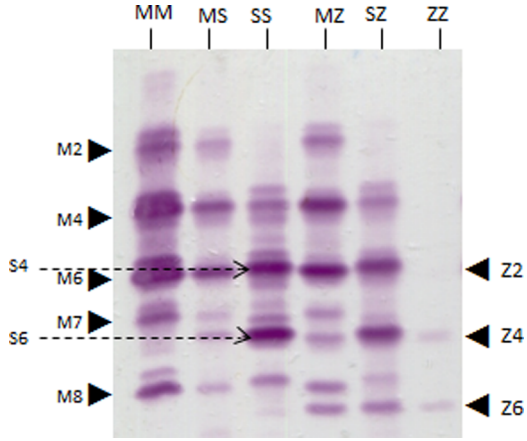


Figure 2. Alpha-1 antitrypsin phenotypes Multiple isoforms of AAT are known to exist, and these differ according to the *N*-glycan groups. These are readily seen on isoelectric focusing gels which are used to phenotype individuals suspected of AATD. Isoelectric focusing patterns for MM, SS, or heterozygous MZ and MS serum compared to serum of a ZZ-AATD individual are shown. The isoform numbers for the M (left of gel), S (dotted line), and Z variants (right of gel) are indicated. A Sebia isoelectric focusing kit was employed for AAT phenotyping over a linear pH gradient of 4.2−4.9 with the Hydrasys System as previously described.[155]

not been specifically demonstrated for AAT[112] and may be related to the position of the glycan groups and also to the nature of serpins and their metastable state. It has been shown that mutant forms of AAT which carry different glycans compared to wild-type AAT may be proteolytically degraded more rapidly and preferentially in the ER.[113,114] In most proteins glycosylation increases the stability and reduces the flexibility of a protein. However, serpin proteins are present in

dx.doi.org/10.1021/pr500146y | *J. Proteome Res.* 2014, 13, 3131−3143

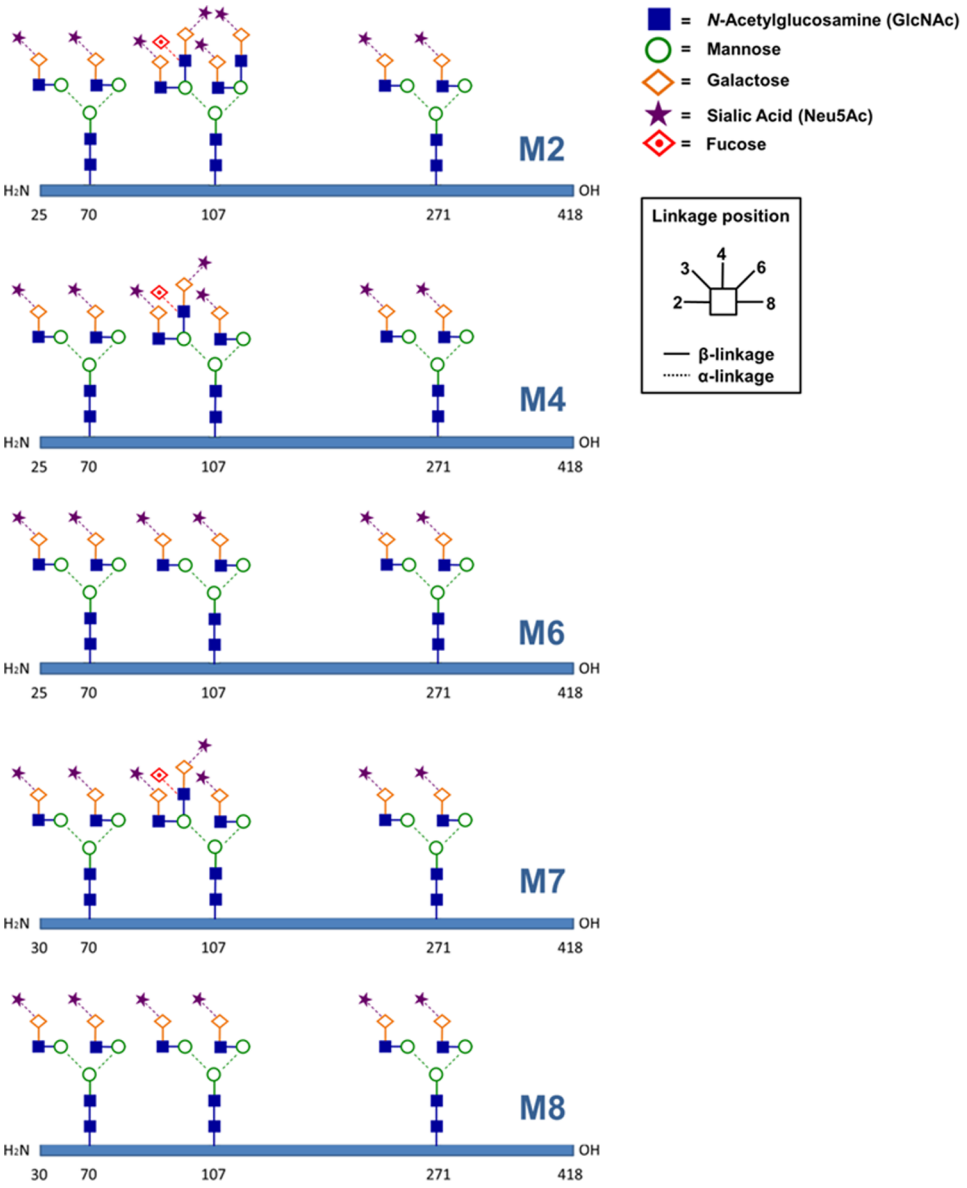


Figure 3. *N*-Linked glycan structures associated with native isoforms of AAT. The AAT molecule is composed of 394 amino acids and is post-translationally modified by *N*-glycosidically linked oligosaccharides at three asparagine residues at positions 70, 107, and 271. *N*-glycans of AAT are composed of sialic acids, galactose, *N*-acetyl-glucosamine, and mannose and exist in di-, tri, or tetra-antennary structures on native AAT protein.

the circulation in a metastable state; this is essential for their interaction with proteases as they undergo conformational changes when they bind these proteases.[115,116] Loss of glycosylation has not been shown to alter the metastable state of AAT; thus, the glycan groups do not increase the stability of AAT, and this may be due to the fact that the glycosylation sites are distanced from the reactive center loop. However, it has been shown that glycosylation does increase the flexibility of the protein in its dynamic state.[117]

Glycosylation is critical for protecting proteins from proteolysis, and in AAT this extends the half-life of the protein and also prevents aggregation.[118,119] The importance of glycosylation for both extending the half-life and preventing polymerization in recombinant AAT used for replacement therapy has been studied extensively. It is known that recombinant AAT (RC-AAT) is glycosylated differently from human purified AAT (HP-AAT),[43,44,120] and in vitro studies demonstrated that HP-AAT was more resistant to polymerization than RC-AAT.[121] Whether glycosylation of Z-AAT is important in the context of polymers forming in the liver of patients with AATD remains to be seen. In line with this thought it has been shown that glycosylation is needed for secretion of AAT from monocytes[122] and that the glycosylation of AAT differs between serum and hepatocytes[42] so glycan groups may play a vital role in the accumulation of AAT in the liver of AATD patients. Indeed, our own work in the area has shown that the *N*-glycans found on Z-AAT protein from AATD individuals differ from those seen on M-AAT. Results revealed a significantly increased percentage of both core and outer arm fucosylated glycans on the Z-AAT of healthy AATD individuals.[123]

Inflammation and Alpha-1 Antitrypsin

Although the classical function of AAT is to act as a protease inhibitor, there is significant evidence now that AAT plays a major role in the immune system and is involved in systemic inflammation, possessing anti-inflammatory activity against

dx.doi.org/10.1021/pr500146y | J. Proteome Res. 2014, 13, 3131−3143

neutrophils, macrophages, monocytes, mast cells, and lymphocytes.[11] The role of AAT in inflammation is multifaceted, and it has been shown to affect leukotriene B_4,[124] TNF-α,[125] IL-8,[12] IFN-γ and IL-1-β.[126,127] AAT is involved in modulating both neutrophil chemotaxis in response to IL-8 and immune complex signaling[12] and apoptosis[128] and has potent anti-apoptotic properties in other cells.[129] AAT modulates neutrophil chemotaxis by binding IL-8 and subsequently inhibiting signaling through the CXCR1 receptor pathway[12] In this later study our results demonstrated that non-glycosylated RC-AAT did not possess the same anti-inflammatory effect on this pathway as glycosylated HP-AAT. The importance of glycosylation in the immune modulatory role of AAT can be seen in several studies; one in which the use of aerosolized transgenic AAT which was differentially glycosylated to native AAT demonstrated a limited effect on neutrophil elastase (NE) activity and inflammation,[130] while two studies in which glycosylated native AAT was delivered through aerosol demonstrated a decrease in the levels of IL-8 and TNF-α and reduced NE activity.[126,131] However, a study in which recombinant AAT, which was nonglycosylated, delivered by aerosolization, showed this nonglycosylated form of AAT was efficient at inhibiting NE.[132] Thus, it appears that the glycans of AAT are more important for the immune modulatory functions rather than for anti-elastase properties.

AAT can modulate the activity of both cytokines and chemokines, but in turn, AAT is itself regulated by inflammatory cytokines. Previous studies have demonstrated that the production of AAT in hepatocytes, human bronchial epithelial cells, monocytes, and macrophages can be up-regulated by IL-6, IFN-β2, lipo-polysaccharide (LPS), IL-1β, NE, and TNF-α.[133−137] Not only is the increased production of AAT controlled by these cytokines but they also control the extent to which the protein is glycosylated. Monocytes alter the glycosylation of both AAT and anti-chymotrypsin through the release of IL-1α/β, TNF-α, TGF-β, and IL-6.[138] The role of IL-6 to induce production of AAT has been shown in a multitude of inflammatory conditions and has also been shown to significantly alter the glycosylation of AAT. This latter point has been demonstrated in severe burn injuries,[139] hepatoma cell lines,[140] and psoriatic arthritis[141] among several other inflammatory conditions. Additional cytokines shown to either increase or reduce the number of branched oligosaccharides of AAT include IFN-γ, IL-1, leukemia inhibitory factor (LIF), TNF-α, and TGF-β.[142] This phenomenon highlights the importance of AAT as an acute phase protein that is not only significantly elevated during inflammation but also changes in glycoforms play a role in response of AAT during acute inflammation. In line with this theory, a study comparing AAT from hepatoma-derived cells to that from lung-derived epithelial cells in response to oncostatin M, an analogue of IL-6, demonstrated upregulation of AAT production in both cell types; however, the two differed in glycosylation pattern. This latter result may indicate differences in functional properties of AAT derived from distinct systems/organs.[143] This is particularly important in the role that AAT may have as a useful biomarker in both inflammatory conditions and in malignant disease. Of particular interest is the change in core fucosylation and the addition of sLe^x epitope.[99]

α1-Antitrypsin as a Biomarker of Inflammation and Malignancy

AAT levels are commonly elevated in response to myocardial infarction, surgery, bacterial infection, viral infection, and pancreatitis among many other conditions.[9,99] It is not just increased production of AAT modulated by cytokines that occurs during episodes of inflammation but there is also a significant change in the percentage of branched glycans. This was initially examined in hepatocellular carcinoma and hepatoma, since AAT is synthesized by hepatocytes. AAT produced by these cells and by other adenocarcinoma cell types was noted to have a higher molecular weight, indicating a greater number of carbohydrate groups[144] with increased proportion of fucosylation and branching (tri-antennary glycans). In hepatocellular carcinoma there is an increased number of sLe^x epitopes, and the core fucosylation on AAT is more specific than the standard α-fetoprotein levels which is the current standard biomarker for this condition.[110] Changes in outer arm fucosylation also appear to occur in inflammation and predicts the development of hepatocellular carcinoma in cirrhotic liver disease,[145] especially in patients infected with Hepatitis B virus.[96]

Alterations in glycosylation of AAT have also been shown in other adenocarcinoma cell types including lung, prostate, and gastrointestinal tract,[146] and increased levels of sLe^x epitope indicate an increased risk of breast cancer progression.[147] Additional studies have suggested that certain genotypes of AAT predispose patients to lung cancer development and this may be due to an imbalance in the levels of AAT and NE.[148] Apart from deficient states and abnormal phenotypes, the differences in glycosylation of AAT in all individuals may be a potential biomarker for lung carcinoma.[149] Similar to other malignancies the presence of increased number of glycans containing sLe^x epitope appears to be a potential biomarker and can be noted even as early as stage I lung cancer.[83]

■ CONCLUSION

This review article has explained the processes of glycosylation of proteins and focused on acute phase proteins. Primarily this review describes the role and importance that glycosylation plays in the function of AAT and how changes in glycosylation patterns of AAT may have potential as a biomarkers in both inflammatory disorders and malignancy. The importance of glycosylation in AAT extends beyond its natural functional capacities and changes in disease states. The use of glycan signatures of AAT and other APPs may be useful as biomarkers in a range of inflammatory conditions, both in acute and chronic disease as well as in malignant conditions. The role of glycosylation in AAT primarily appears to be in affecting how the protein acts as a modulator of the immune system, and hence, it is an interesting area for future research. This research may be involved in developing biomarkers, new therapeutic uses of AAT, and understanding the broader role of serpins and all APPs.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: cmccarthy@rcsi.ie. Tel: +35318093796.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank the U.S. Alpha One Foundation, the Medical Research Charities Group/Health Research Board Ireland (MRCG/2013/1) and the Program for Research in Third Level Institutes (PRTLI) administered by the Higher Education Authority. R.S. acknowledges funding from the European Union Seventh Framework Programme (FP7/2007-2013) under Grant Agreement 260600 ("GlycoHIT").

■ GLOSSARY OF ABBREVIATIONS

AAT: alpha-1 antitrypsin
AATD: alpha-1 antitrypsin deficiency
ACT: alpha-1 chymotrypsin
AGP: alpha-1 acid glycoprotein
APPs: acute phase proteins
Asn: asparagine
AT: antithrombin
ER: endoplasmic reticulum
FET: fetuin
FEV1: forced expiratory volume in 1 s
FUT: fucosyltransferase
Glc: glucose
GlcNAc: *N*-acetylglucosamine
GWAS: genome-wide association studies
HILIC: hydrophilic interaction liquid chromatography
HPT: haptoglobin
IFN: interferon
IgG: immunoglobulin G
IL: interleukin
LPS: lipopolysaccharide
Man: mannose
MS: mass spectrometry
NA: neuraminic acid
NE: neutrophil elastase
Pro: proline
Ser: serine
Serpin: serine protease inhibitor
sLex: sialyl Lewis x
TFR: transferrin
TGF-β: transforming growth factor beta
Thr: threonine

■ REFERENCES

(1) Law, R. H.; Zhang, Q.; McGowan, S.; Buckle, A. M.; Silverman, G. A.; Wong, W.; et al. An overview of the serpin superfamily. *GenomeBiology* **2006**, *7* (5), 216.
(2) Gettins, P. G. Serpin structure, mechanism, and function. *Chem. Rev.* **2002**, *102* (12), 4751−4804.
(3) Huntington, J. A. Serpin structure, function and dysfunction. *J. Thromb. Haemostasis* **2011**, *9* (Suppl1), 26−34.
(4) Gooptu, B.; Lomas, D. A. Conformational pathology of the serpins: themes, variations, and therapeutic strategies. *Ann. Rev. Biochem.* **2009**, *78*, 147−176.
(5) Elliott, P. R.; Lomas, D. A.; Carrell, R. W.; Abrahams, J. P. Inhibitory conformation of the reactive loop of alpha 1-antitrypsin. *Nat Struct Biol.* **1996**, *3* (8), 676−681.
(6) Elliott, P. R.; Pei, X. Y.; Dafforn, T. R.; Lomas, D. A. Topography of a 2.0 A structure of alpha-1-antitrypsin reveals targets for rational drug design to prevent conformational disease. *Protein Sci.* **2000**, *9* (7), 1274−1281.
(7) Huber, R.; Carrell, R. W. Implications of the three-dimensional structure of alpha-1-antitrypsin for structure and function of serpins. *Biochemistry* **1989**, *28* (23), 8951−8966.
(8) Perlmutter, D. H. Alpha-1-antitrypsin deficiency: diagnosis and treatment. *Clin. Liver Dis.* **2004**, *8* (4), 839−859 viii-ix.
(9) Voulgari, F.; Cummins, P.; Gardecki, T. I.; Beeching, N. J.; Stone, P. C.; Stuart, J. Serum levels of acute phase and cardiac proteins after myocardial infarction, surgery, and infection. *Br. Heart J.* **1982**, *48* (4), 352−356.
(10) Correale, M.; Totaro, A.; Abruzzese, S.; Di Biase, M.; Brunetti, N. D. Acute phase proteins in acute coronary syndrome: An up-to-date. *Cardiovasc. Hematol. Agents Med. Chem.* **2012**, *10* (4), 352−361.
(11) Bergin, D. A.; Hurley, K.; McElvaney, N. G.; Reeves, E. P. Alpha-1 antitrypsin: A potent anti-inflammatory and potential novel therapeutic agent. *Arch. Immunol. Ther. Exp. (Warsz)* **2012**, *60* (2), 81−97.
(12) Bergin, D. A.; Reeves, E. P.; Meleady, P.; Henry, M.; McElvaney, O. J.; Carroll, T. P.; et al. Alpha-1 Antitrypsin regulates human neutrophil chemotaxis induced by soluble immune complexes and IL-8. *J. Clin Invest.* **2010**, *120* (12), 4236−4250.
(13) Kolarich, D.; Weber, A.; Turecek, P. L.; Schwarz, H. P.; Altmann, F. Comprehensive glyco-proteomic analysis of human alpha1-antitrypsin and its charge isoforms. *Proteomics* **2006**, *6* (11), 3369−3380.
(14) Mills, P. B.; Mills, K.; Johnson, A. W.; Clayton, P. T.; Winchester, B. G. Analysis by matrix assisted laser desorption/ionisation-time of flight mass spectrometry of the post-translational modifications of alpha 1-antitrypsin isoforms separated by two-dimensional polyacrylamide gel electrophoresis. *Proteomics* **2001**, *1* (6), 778−786.
(15) Eriksson, S.; Alm, R.; Astedt, B. Organ cultures of human fetal hepatocytes in the study of extra-and intracellular alpha1-antitrypsin. *Biochim. Biophys. Acta* **1978**, *542* (3), 496−505.
(16) Koj, A.; Regoeczi, E.; Toews, C. J.; Leveille, R.; Gauldie, J. Synthesis of antithrombin III and alpha-1-antitrypsin by the perfused rat liver. *Biochim. Biophys. Acta* **1978**, *539* (4), 496−504.
(17) Mornex, J. F.; Chytil-Weir, A.; Martinet, Y.; Courtney, M.; LeCocq, J. P. Crystal RG. Expression of the alpha-1-antitrypsin gene in mononuclear phagocytes of normal and alpha-1-antitrypsin-deficient individuals. *J. Clin. Invest.* **1986**, *77* (6), 1952−1961.
(18) Carroll, T. P.; Greene, C. M.; O'Connor, C. A.; Nolan, A. M.; O'Neill, S. J.; McElvaney, N. G. Evidence for unfolded protein response activation in monocytes from individuals with alpha-1 antitrypsin deficiency. *J. Immunol.* **2010**, *184* (8), 4538−4546.
(19) Perlmutter, D. H.; Daniels, J. D.; Auerbach, H. S.; De Schryver-Kecskemeti, K.; Winter, H. S.; Alpers, D. H. The alpha 1-antitrypsin gene is expressed in a human intestinal epithelial cell line. *J. Biol. Chem.* **1989**, *264* (16), 9485−90.
(20) Cichy, J.; Potempa, J.; Travis, J. Biosynthesis of alpha1-proteinase inhibitor by human lung-derived epithelial cells. *J. Biol. Chem.* **1997**, *272* (13), 8250−8255.
(21) Greene, C. M.; McElvaney, N. G. Proteases and antiproteases in chronic neutrophilic lung disease - relevance to drug discovery. *Br. J. Pharmacol.* **2009**, *158* (4), 1048−1058.
(22) Kelly, E.; Greene, C. M.; Carroll, T. P.; McElvaney, N. G.; O'Neill, S. J. Alpha-1 antitrypsin deficiency. *Respir. Med.* **2010**, *104* (6), 763−772.
(23) Curiel, D. T.; Chytil, A.; Courtney, M. Crystal RG. Serum alpha 1-antitrypsin deficiency associated with the common S-type (Glu264——Val) mutation results from intracellular degradation of alpha 1-antitrypsin prior to secretion. *J. Biol. Chem.* **1989**, *264* (18), 10477−10486.
(24) Lomas, D. A.; Evans, D. L.; Finch, J. T.; Carrell, R. W. The mechanism of Z alpha 1-antitrypsin accumulation in the liver. *Nature* **1992**, *357* (6379), 605−607.
(25) Ferrarotti, I.; Thun, G. A.; Zorzetto, M.; Ottaviani, S.; Imboden, M.; Schindler, C.; et al. Serum levels and genotype distribution of alpha1-antitrypsin in the general population. *Thorax* **2012**, *67* (8), 669−674.
(26) Carroll, T. P.; O'Connor, C. A.; Floyd, O.; McPartlin, J.; Kelleher, D. P.; O'Brien, G.; et al. The prevalence of alpha-1 antitrypsin deficiency in Ireland. *Respir. Res.* **2011**, *12*, 91.

(27) Brantly, M.; Nukiwa, T.; Crystal, R. G. Molecular basis of alpha-1-antitrypsin deficiency. *Am. J. Med.* **1988**, *84* (6A), 13−31.
(28) Molloy, K.; Hersh, C. P.; Morris, V. B.; Carroll, T. P.; O'Connor, C. A.; Lasky-Su, J. A. Clarification of the Risk of COPD in Alpha-1 Antitrypsin Deficiency PiMZ Heterozygotes. *Am. J. Respir. Crit. Care Med.* **2014**, *189* (4), 419−427.
(29) Abboud, R. T.; Ford, G. T.; Chapman, K. R. Alpha1-antitrypsin deficiency: a position statement of the Canadian Thoracic Society. *Canadian Respir. J.* **2001**, *8* (2), 81−88.
(30) Chapman, K. R.; aBurdon, J. G.; Piitulainen, E.; Sanhaus, R. A.; Seersholm, N.; Stocks, J. M.; Huang, L.; Edelman, J. M.; McElvaney, N. G., IV Alpha1 Antitrypsin (A1AT) Preserves Lung Density In Homozygous Alpha1 Antitrypsin Deficiency (A1ATD); A Randomized, Placebo-Controlled Trial American journal of respiratory and critical care medicine. *C20. Late Breaking Abstracts in Clinical Trials.* **2013**, A6069.
(31) Dirksen, A.; Piitulainen, E.; Parr, D. G.; Deng, C.; Wencker, M.; Shaker, S. B.; et al. Exploring the role of CT densitometry: a randomised study of augmentation therapy in alpha1-antitrypsin deficiency. *Eur. Respir. J.* **2009**, *33* (6), 1345−1353.
(32) Dirksen, A.; Dijkman, J. H.; Madsen, F.; Stoel, B.; Hutchison, D. C.; Ulrik, C. S.; et al. A randomized clinical trial of alpha(1)-antitrypsin augmentation therapy. *Am. J. Respir. Crit. Care Med.* **1999**, *160* (5 Pt 1), 1468−1472.
(33) Chapman, K. R.; Stockley, R. A.; Dawkins, C.; Wilkes, M. M.; Navickis, R. J. Augmentation therapy for alpha1 antitrypsin deficiency: A meta-analysis. *COPD* **2009**, *6* (3), 177−184.
(34) Wencker, M.; Fuhrmann, B.; Banik, N.; Konietzko, N. Longitudinal follow-up of patients with alpha(1)-protease inhibitor deficiency before and during therapy with IV alpha(1)-protease inhibitor. *Chest* **2001**, *119* (3), 737−744.
(35) Seersholm, N.; Wencker, M.; Banik, N.; Viskum, K.; Dirksen, A.; Kok-Jensen, A.; et al. Does alpha1-antitrypsin augmentation therapy slow the annual decline in FEV1 in patients with severe hereditary alpha1-antitrypsin deficiency? Wissenschaftliche Arbeitsgemeinschaft zur Therapie von Lungenerkrankungen (WATL) alpha1-AT study group. *Eur. Respir. J.* **1997**, *10* (10), 2260−2263.
(36) McCarthy, C.; Dimitrov, B. D. Augmentation therapy for alpha-1 antitrypsin deficiency–not enough evidence to support its use yet! *COPD* **2010**, *7* (3), 234.
(37) Stoller, J. K.; Aboussouan, L. S. A review of alpha1-antitrypsin deficiency. *Am. J. Respir. Crit. Care Med.* **2012**, *185* (3), 246−259.
(38) Greene, C. M.; Miller, S. D.; Carroll, T.; McLean, C.; O'Mahony, M.; Lawless, M. W.; et al. Alpha-1 antitrypsin deficiency: a conformational disease associated with lung and liver manifestations. *J. Inherited Metab. Dis.* **2008**, *31* (1), 21−34.
(39) Tuder, R. M.; Janciauskiene, S. M.; Petrache, I. Lung disease associated with alpha1-antitrypsin deficiency. *Proc. Am. Thorac. Soc.* **2010**, *7* (6), 381−386.
(40) Ekeowa, U. I.; Marciniak, S. J.; Lomas, D. A. Alpha(1)-antitrypsin deficiency and inflammation. *Expert Rev. Clin. Immunol.* **2011**, *7* (2), 243−252.
(41) Mulgrew, A. T.; Taggart, C. C.; McElvaney, N. G. Alpha-1-antitrypsin deficiency: current concepts. *Lung* **2007**, *185* (4), 191−201.
(42) Jeppsson, J. O.; Larsson, C.; Eriksson, S. Characterization of alpha1-antitrypsin in the inclusion bodies from the liver in alpha 1-antitrypsin deficiency. *New Engl. J. Med.* **1975**, *293* (12), 576−579.
(43) Cowden, D. I.; Fisher, G. E.; Weeks, R. L. A pilot study comparing the purity, functionality and isoform composition of alpha-1-proteinase inhibitor (human) products. *Curr. Med. Res. Opin.* **2005**, *21* (6), 877−883.
(44) Kolarich, D.; Turecek, P. L.; Weber, A.; Mitterer, A.; Graninger, M.; Matthiessen, P.; et al. Biochemical, molecular characterization, and glycoproteomic analyses of alpha(1)-proteinase inhibitor products used for replacement therapy. *Transfusion* **2006**, *46* (11), 1959−1977.
(45) Spiro, R. G. Glycoproteins. *Adv. Protein Chem.* **1973**, *27*, 349−467.
(46) Rudd, P. M.; Dwek, R. A. Glycosylation: heterogeneity and the 3D structure of proteins. *Crit. Rev. Biochem. Mol. Biol.* **1997**, *32* (1), 1−100.
(47) Spiro, R. G. Protein glycosylation: nature, distribution, enzymatic formation, and disease implications of glycopeptide bonds. *Glycobiology* **2002**, *12* (4), 43R−56R.
(48) Klausner, R. D.; Sitia, R. Protein degradation in the endoplasmic reticulum. *Cell.* **1990**, *62* (4), 611−614.
(49) Montreuil, J. Primary structure of glycoprotein glycans: Basis for the molecular biology of glycoproteins. *Adv. carbohydr. chem. biochem.* **1980**, *37*, 157−223.
(50) Johansen, P. G.; Marshall, R. D.; Neuberger, A. Carbohydrates in protein. 3. The preparation and some of the properties of a glycopeptide from hen's-egg albumin. *Biochem. J.* **1961**, *78*, 518−527.
(51) Silberstein, S.; Gilmore, R. Biochemistry, molecular biology, and genetics of the oligosaccharyltransferase. *FASEB J.* **1996**, *10* (8), 849−858.
(52) Hubbard, S. C.; Ivatt, R. J. Synthesis and processing of asparagine-linked oligosaccharides. *Annu. Rev. Biochem.* **1981**, *50*, 555−583.
(53) Livi, G. P.; Lillquist, J. S.; Miles, L. M.; Ferrara, A.; Sathe, G. M.; Simon, P. L.; et al. Secretion of N-glycosylated interleukin-1 beta in Saccharomyces cerevisiae using a leader peptide from Candida albicans. Effect of N-linked glycosylation on biological activity. *J. Biol. Chem.* **1991**, *266* (23), 15348−15355.
(54) Kasturi, L.; Eshleman, J. R.; Wunner, W. H.; Shakin-Eshleman, S. H. The hydroxy amino acid in an Asn-X-Ser/Thr sequon can influence N-linked core glycosylation efficiency and the level of expression of a cell surface glycoprotein. *J. Biol. Chem.* **1995**, *270* (24), 14756−14761.
(55) Bause, E. Structural requirements of N-glycosylation of proteins. Studies with proline peptides as conformational probes. *Biochem. J.* **1983**, *209* (2), 331−336.
(56) Carson, D. D.; Earles, B. J.; Lennarz, W. J. Enhancement of protein glycosylation in tissue slices by dolichylphosphate. *J. Biol. Chem.* **1981**, *256* (22), 11552−11557.
(57) Van Schaftingen, E.; Jaeken, J. Phosphomannomutase deficiency is a cause of carbohydrate-deficient glycoprotein syndrome type I. *FEBS Lett.* **1995**, *377* (3), 318−320.
(58) Parekh, R. B.; Tse, A. G.; Dwek, R. A.; Williams, A. F.; Rademacher, T. W. Tissue-specific N-glycosylation, site-specific oligosaccharide patterns and lentil lectin recognition of rat Thy-1. *EMBO J.* **1987**, *6* (5), 1233−1244.
(59) Schachter, H. Biosynthetic controls that determine the branching and microheterogeneity of protein-bound oligosaccharides. *Biochem. Cell Biol.* **1986**, *64* (3), 163−181.
(60) Harpaz, N.; Schachter, H. Control of glycoprotein synthesis. Processing of asparagine-linked oligosaccharides by one or more rat liver Golgi alpha-D-mannosidases dependent on the prior action of UDP-N-acetylglucosamine: alpha-D-mannoside beta 2-N-acetylglucosaminyltransferase I. *J. Biol. Chem.* **1980**, *255* (10), 4894−4902.
(61) Itzkowitz, S. H.; Bloom, E. J.; Kokal, W. A.; Modin, G.; Hakomori, S.; Kim, Y. S. Sialosyl-Tn. A novel mucin antigen associated with prognosis in colorectal cancer patients. *Cancer* **1990**, *66* (9), 1960−1966.
(62) Hiraizumi, S; Takasaki, S; Ohuchi, N; Harada, Y; Nose, M; Mori, S Altered glycosylation of membrane glycoproteins associated with human mammary carcinoma. *Jpn. J. Cancer Res.* **1992**, *83* (10), 1063−1072.
(63) De Graaf, T. W.; Van der Stelt, M. E.; Anbergen, M. G.; van Dijk, W. Inflammation-induced expression of sialyl Lewis X-containing glycan structures on alpha 1-acid glycoprotein (orosomucoid) in human sera. *J. Exp. Med.* **1993**, *177* (3), 657−666.
(64) Rudd, P. M.; Joao, H. C.; Coghill, E.; Fiten, P.; Saunders, M. R.; Opdenakker, G.; et al. Glycoforms modify the dynamic stability and functional activity of an enzyme. *Biochemistry* **1994**, *33* (1), 17−22.
(65) Leatherbarrow, R. J.; Dwek, R. A. Binding of complement subcomponent C1q to mouse IgG1, IgG2a and IgG2b: A novel C1q binding assay. *Mol. Immunol.* **1984**, *21* (4), 321−327.

(66) Flintegaard, T. V.; Thygesen, P.; Rahbek-Nielsen, H.; Levery, S. B.; Kristensen, C.; Clausen, H.; et al. N-glycosylation increases the circulatory half-life of human growth hormone. *Endocrinology.* **2010**, *151* (11), 5326−36.
(67) Wormald, M. R.; Rudd, P. M.; Harvey, D. J.; Chang, S. C.; Scragg, I. G.; Dwek, R. A. Variations in oligosaccharide-protein interactions in immunoglobulin G determine the site-specific glycosylation profiles and modulate the dynamic motion of the Fc oligosaccharides. *Biochemistry.* **1997**, *36* (6), 1370−1380.
(68) Mori, K.; Dwek, R. A.; Downing, A. K.; Opdenakker, G.; Rudd, P. M. The activation of type 1 and type 2 plasminogen by type I and type II tissue plasminogen activator. *J. Biol. Chem.* **1995**, *270* (7), 3261−3267.
(69) Kornfeld, S. Structure and function of the mannose 6-phosphate/insulinlike growth factor II receptors. *Annu. Rev. Biochem* **1992**, *61*, 307−30.
(70) Pepys, M. B.; Rademacher, T. W.; Amatayakul-Chantler, S.; Williams, P.; Noble, G. E.; Hutchinson, W. L.; et al. Human serum amyloid P component is an invariant constituent of amyloid deposits and has a uniquely homogeneous glycostructure. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91* (12), 5602−5606.
(71) Lee, Y. C.; Townsend, R. R.; Hardy, M. R.; Lonngren, J.; Arnarp, J.; Haraldsson, M.; et al. Binding of synthetic oligosaccharides to the hepatic Gal/GalNAc lectin. Dependence on fine structural features. *J. Biol. Chem.* **1983**, *258* (1), 199−202.
(72) Rudd, P. M.; Elliott, T.; Cresswell, P.; Wilson, I. A.; Dwek, R. A. Glycosylation and the immune system. *Science* **2001**, *291* (5512), 2370−2376.
(73) Rudd, P. M.; Wormald, M. R.; Stanfield, R. L.; Huang, M.; Mattsson, N.; Speir, J. A.; et al. Roles for glycosylation of cell surface receptors involved in cellular immune recognition. *J. Mol. biol.* **1999**, *293* (2), 351−366.
(74) Marino, K, Saldova, R, Adamczyk, B, Rudd, P. M. Changes in serum N-glycosylation profiles: Functional significance and potential for diagnostics. In *Carbohydrate Chemistry: Chemical and Biological Approaches*; Rauter, A. P., Ed.; RSC Publishing: Londoon, 2012.
(75) Royle, L.; Campbell, M. P.; Radcliffe, C. M.; White, D. M.; Harvey, D. J.; Abrahams, J. L.; et al. HPLC-based analysis of serum N-glycans on a 96-well plate platform with dedicated database software. *Anal. Biochem.* **2008**, *376* (1), 1−12.
(76) Knezevic, A.; Polasek, O.; Gornik, O.; Rudan, I.; Campbell, H.; Hayward, C.; et al. Variability, heritability and environmental determinants of human plasma N-glycome. *J. Proteome Res.* **2009**, *8* (2), 694−701.
(77) Yamada, E.; Tsukamoto, Y.; Sasaki, R.; Yagyu, K.; Takahashi, N. Structural changes of immunoglobulin G oligosaccharides with age in healthy human serum. *Glycoconjugate J.* **1997**, *14* (3), 401−405.
(78) Knezevic, A.; Gornik, O.; Polasek, O.; Pucic, M.; Redzic, I.; Novokmet, M.; et al. Effects of aging, body mass index, plasma lipid profiles, and smoking on human plasma N-glycans. *Glycobiology* **2010**, *20* (8), 959−969.
(79) Saldova, R.; Huffman, J. E.; Adamczyk, B.; Muzinic, A.; Kattla, J. J.; Pucic, M.; et al. Association of medication with the human plasma N-glycome. *J. Proteome Res.* **2012**, *11* (3), 1821−1831.
(80) Lauc, G.; Huffman, J.; Hayward, C.; Knezevic, A.; Polasek, O.; Gornik, O.; et al. Genome-wide association study identifies FUT8 and ESR2 as co-regulators of a bi-antennary N-linked glycan A2 (GlcNAc2Man3GlcNAc2) in human plasma proteins. *Nat. Precedings* **2009**, DOI: 10101/npre.2009.2864.1.
(81) Lauc, G.; Essafi, A.; Huffman, J. E.; Hayward, C.; Knezevic, A.; Kattla, J. J.; et al. Genomics meets glycomics: The first GWAS study of human N-Glycome identifies HNF1alpha as a master regulator of plasma protein fucosylation. *PLoS Genet.* **2010**, *6* (12), e1001256.
(82) Sharp, L. K.; Mallya, M.; Kinghorn, K. J.; Wang, Z.; Crowther, D. C.; Huntington, J. A.; et al. Sugar and alcohol molecules provide a therapeutic strategy for the serpinopathies that cause dementia and cirrhosis. *FEBS J.* **2006**, *273* (11), 2540−2552.
(83) Arnold, J. N.; Saldova, R.; Galligan, M. C.; Murphy, T. B.; Mimura-Kimura, Y.; Telford, J. E.; et al. Novel glycan biomarkers for the detection of lung cancer. *J. Proteome Res.* **2011**, *10* (4), 1755−1764.
(84) Vasseur, J. A.; Goetz, J. A.; Alley, W. R., Jr.; Novotny, M. V. Smoking and Lung Cancer-induced Changes in N-Glycosylation of Blood Serum Proteins. *Glycobiology* **2012**, DOI: 10.1093/glycob/cws108.
(85) Gornik, O.; Lauc, G. Glycosylation of serum proteins in inflammatory diseases. *Dis. Markers* **2008**, *25* (4−5), 267−278.
(86) McMillan, S. A.; Douglas, J. P.; Archbold, G. P.; McCrum, E. E.; Evans, A. E. Effect of low to moderate levels of smoking and alcohol consumption on serum immunoglobulin concentrations. *J. Clin. Pathol.* **1997**, *50* (10), 819−822.
(87) An, H. J.; Kronewitter, S. R.; de Leoz, M. L.; Lebrilla, C. B. Glycomics and disease markers. *Curr. Opin Chem. Biol.* **2009**, *13* (5−6), 601−607.
(88) Arnold, J. N.; Wormald, M. R.; Sim, R. B.; Rudd, P. M.; Dwek, R. A. The impact of glycosylation on the biological function and structure of human immunoglobulins. *Annu. Rev. Immunol.* **2007**, *25*, 21−50.
(89) Gabay, C.; Kushner, I. Acute-phase proteins and other systemic responses to inflammation. *New Engl. J. Med.* **1999**, *340* (6), 448−454.
(90) Medzhitov, R. Origin and physiological roles of inflammation. *Nature* **2008**, *454* (7203), 428−435.
(91) Gornik, O.; Royle, L.; Harvey, D. J.; Radcliffe, C. M.; Saldova, R.; Dwek, R. A.; et al. Changes of serum glycans during sepsis and acute pancreatitis. *Glycobiology* **2007**, *17* (12), 1321−1332.
(92) Bone, R. C. Immunologic dissonance: a continuing evolution in our understanding of the systemic inflammatory response syndrome (SIRS) and the multiple organ dysfunction syndrome (MODS). *Ann. Int. Med.* **1996**, *125* (8), 680−687.
(93) Stanta, J. L.; Saldova, R.; Struwe, W. B.; Byrne, J. C.; Leweke, F. M.; Rothermund, M.; et al. Identification of N-glycosylation changes in the CSF and serum in patients with schizophrenia. *J. Proteome Res.* **2010**, *9* (9), 4476−4489.
(94) Klein, A. Human total serum N-glycome. *Adv. Clin Chem.* **2008**, *46*, 51−85.
(95) Callewaert, N.; Van Vlierberghe, H.; Van Hecke, A.; Laroy, W.; Delanghe, J.; Contreras, R. Noninvasive diagnosis of liver cirrhosis using DNA sequencer-based total serum protein glycomics. *Nature Med.* **2004**, *10* (4), 429−434.
(96) Liu, X. E.; Desmyter, L.; Gao, C. F.; Laroy, W.; Dewaele, S.; Vanhooren, V.; et al. N-glycomic changes in hepatocellular carcinoma patients with liver cirrhosis induced by hepatitis B virus. *Hepatology* **2007**, *46* (5), 1426−1435.
(97) Chen, C.; Schmilovitz-Weiss, H.; Liu, X. E.; Pappo, O.; Halpern, M.; Sulkes, J.; et al. Serum protein N-glycans profiling for the discovery of potential biomarkers for nonalcoholic steatohepatitis. *J. Proteome Res.* **2009**, *8* (2), 463−470.
(98) Goodarzi, M. T.; Turner, G. A. Reproducible and sensitive determination of charged oligosaccharides from haptoglobin by PNGase F digestion and HPAEC/PAD analysis: glycan composition varies with disease. *Glycoconjugate J.* **1998**, *15* (5), 469−75.
(99) Sarrats, A.; Saldova, R.; Pla, E.; Fort, E.; Harvey, D. J.; Struwe, W. B.; et al. Glycosylation of liver acute-phase proteins in pancreatic cancer and chronic pancreatitis. *Proteomics Clin. Appl.* **2010**, *4* (4), 432−448.
(100) Peracaula, R.; Sarrats, A.; Rudd, P. M. Liver proteins as sensor of human malignancies and inflammation. *Proteomics Clin. Appl.* **2010**, *4* (4), 426−431.
(101) Hashimoto, S.; Asao, T.; Takahashi, J.; Yagihashi, Y.; Nishimura, T.; Saniabadi, A. R.; et al. alpha1-acid glycoprotein fucosylation as a marker of carcinoma progression and prognosis. *Cancer* **2004**, *101* (12), 2825−2836.
(102) Qiu, Y.; Patwa, T. H.; Xu, L.; Shedden, K.; Misek, D. E.; Tuck, M.; et al. Plasma glycoprotein profiling for colorectal cancer biomarker identification by lectin glycoarray and lectin blot. *J. Proteome Res..* **2008**, *7* (4), 1693−1703.
(103) Ueda, K.; Katagiri, T.; Shimada, T.; Irie, S.; Sato, T. A.; Nakamura, Y.; et al. Comparative profiling of serum glycoproteome by

sequential purification of glycoproteins and 2-nitrobenzensulfenyl (NBS) stable isotope labeling: a new approach for the novel biomarker discovery for cancer. *J. Proteome Res.* **2007**, *6* (9), 3475−3483.
(104) Mega, T.; Lujan, E.; Yoshida, A. Studies on the oligosaccharide chains of human alpha 1-protease inhibitor. II. Structure of oligosaccharides. *J. Biol. Chem.* **1980**, *255* (9), 4057−61.
(105) Hodges, L. C.; Laine, R.; Chan, S. K. Structure of the oligosaccharide chains in human alpha 1-protease inhibitor. *J. Biol. Chem.* **1979**, *254* (17), 8208−12.
(106) Packer, N. H.; MR, W. I.; Golaz, O.; Lawson, M. A.; Gooley, A. A.; Hochstrasser, D. F.; et al. Characterization of human plasma glycoproteins separated by two-dimensional gel electrophoresis. *Biotechnology (NY)* **1996**, *14* (1), 66−70.
(107) Mills, K.; Mills, P. B.; Clayton, P. T.; Johnson, A. W.; Whitehouse, D. B.; Winchester, B. G. Identification of alpha(1)-antitrypsin variants in plasma with the use of proteomic technology. *Clin. Chem.* **2001**, *47* (11), 2012−2022.
(108) Torossi, T.; Fan, J. Y.; Sauter-Etter, K.; Roth, J.; Ziak, M. Endomannosidase processes oligosaccharides of alpha1-antitrypsin and its naturally occurring genetic variants in the Golgi apparatus. *Cell. Mol. Life Sci.* **2006**, *63* (16), 1923−1932.
(109) Foreman, R. C. Alpha 1-antitrypsin deficiency–A defect in secretion. *Biosci. Rep.* **1987**, *7* (4), 307−311.
(110) Comunale, M. A.; Rodemich-Betesh, L.; Hafner, J.; Wang, M.; Norton, P.; Di Bisceglie, A. M.; et al. Linkage specific fucosylation of alpha-1-antitrypsin in liver cirrhosis and cancer patients: Implications for a biomarker of hepatocellular carcinoma. *PloS One* **2010**, *5* (8), e12419.
(111) Trombetta, E. S. The contribution of N-glycans and their processing in the endoplasmic reticulum to glycoprotein biosynthesis. *Glycobiology* **2003**, *13* (9), 77R−91R.
(112) Parodi, A. J. Role of N-oligosaccharide endoplasmic reticulum processing reactions in glycoprotein folding and degradation. *Biochemical J.* **2000**, *348* (Pt 1), 1−13.
(113) Hosokawa, N.; Tremblay, L. O.; You, Z.; Herscovics, A.; Wada, I.; Nagata, K. Enhancement of endoplasmic reticulum (ER) degradation of misfolded Null Hong Kong alpha1-antitrypsin by human ER mannosidase I. *J. Biol. Chem.* **2003**, *278* (28), 26287−94.
(114) Wu, Y.; Swulius, M. T.; Moremen, K. W.; Sifers, R. N. Elucidation of the molecular logic by which misfolded alpha 1-antitrypsin is preferentially selected for degradation. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100* (14), 8229−34.
(115) Whisstock, J. C.; Skinner, R.; Carrell, R. W.; Lesk, A. M. Conformational changes in serpins: I. The native and cleaved conformations of alpha(1)-antitrypsin. *J. Mol. Biol.* **2000**, *296* (2), 685−699.
(116) Huntington, J. A.; Whisstock, J. C. Molecular contortionism - on the physical limits of serpin 'loop-sheet' polymers. *Biological Chem.* **2010**, *391* (8), 973−982.
(117) Sarkar, A.; Wintrode, P. L. Effects of glycosylation on the stability and flexibility of a metastable protein: the human serpin alpha(1)-antitrypsin. *Int. J. Mass Spectrom.* **2011**, *302* (1−3), 69−75.
(118) Shental-Bechor, D.; Levy, Y. Folding of glycoproteins: toward understanding the biophysics of the glycosylation code. *Curr. Opin. Struct. Biol.* **2009**, *19* (5), 524−33.
(119) Varki, A. Biological roles of oligosaccharides: All of the theories are correct. *Glycobiology* **1993**, *3* (2), 97−130.
(120) Casolaro, M. A.; Fells, G.; Wewers, M.; Pierce, J. E.; Ogushi, F.; Hubbard, R.; et al. Augmentation of lung antineutrophil elastase capacity with recombinant human alpha-1-antitrypsin. *J. Appl. Physiol* **1987**, *63* (5), 2015−23.
(121) Kwon, K. S.; Yu, M. H. Effect of glycosylation on the stability of alpha1-antitrypsin toward urea denaturation and thermal deactivation. *Biochim. Biophys. Acta* **1997**, *1335* (3), 265−72.
(122) Gross, V.; vom Berg, D.; Kreuzkamp, J.; Ganter, U.; Bauer, J.; Wurtemberger, G.; et al. Biosynthesis and secretion of M- and Z-type alpha 1-proteinase inhibitor by human monocytes. Effect of inhibitors of glycosylation and of oligosaccharide processing on secretion and function. *Biol. Chem. Hoppe-Seyler* **1990**, *371* (3), 231−238.
(123) McCarthy, C.; Saldova, R.; O'Brien, M. E.; Bergin, D. A.; Carroll, T. P.; Keenan, J. Increased Outer Arm and Core Fucose Residues on the N-Glycans of Mutated Alpha-1 Antitrypsin Protein from Alpha-1 Antitrypsin Deficient Individuals. *J. Proteome Res.* **2014**, *13* (2), 596−605.
(124) O'Dwyer, C. A. M.; Reeves, N. G.; Alpha-1, E. P. antitrypsin inhibits leukotriene B4 neutrophil signalling through a mechanism that involves direct complexation of the two molecules. *Am. J. Respir. Crit. Care Med.* **2013**, *187*, A2741.
(125) Bergin, D. A.; Reeves, E. P.; Hurley, K.; Wolfe, R.; Jameel, R.; Fitzgerald, S.; et al. The Circulating Proteinase Inhibitor alpha-1 Antitrypsin Regulates Neutrophil Degranulation and Autoimmunity. *Sci. Transl. Med.* **2014**, *6* (217), 217ra1.
(126) Griese, M.; Latzin, P.; Kappler, M.; Weckerle, K.; Heinzlmaier, T.; Bernhardt, T.; et al. Alpha 1-antitrypsin inhalation reduces airway inflammation in cystic fibrosis patients. *Eur. Respir. J.* **2007**, *29* (2), 240−250.
(127) Kalis, M.; Kumar, R.; Janciauskiene, S.; Salehi, A.; Cilio, C. M. Alpha 1-antitrypsin enhances insulin secretion and prevents cytokine-mediated apoptosis in pancreatic beta-cells. *Islets* **2010**, *2* (3), 185−189.
(128) Al-Omari, M.; Korenbaum, E.; Ballmaier, M.; Lehmann, U.; Jonigk, D.; Manstein, D. J.; et al. Acute-phase protein alpha1-antitrypsin inhibits neutrophil calpain I and induces random migration. *Mol. Med.* **2011**, *17* (9−10), 865−874.
(129) Zhang, B.; Lu, Y.; Campbell-Thompson, M.; Spencer, T.; Wasserfall, C.; Atkinson, M.; et al. Alpha1-antitrypsin protects beta-cells from apoptosis. *Diabetes* **2007**, *56* (5), 1316−1323.
(130) Martin, S. L.; Downey, D.; Bilton, D.; Keogan, M. T.; Edgar, J.; Elborn, J. S. Safety and efficacy of recombinant alpha(1)-antitrypsin therapy in cystic fibrosis. *Pediatr. Pulmonol.* **2006**, *41* (2), 177−183.
(131) McElvaney, N. G.; Hubbard, R. C.; Birrer, P.; Chernick, M. S.; Caplan, D. B.; Frank, M. M.; et al. Aerosol alpha 1-antitrypsin treatment for cystic fibrosis. *Lancet* **1991**, *337* (8738), 392−394.
(132) Hubbard, R. C.; McElvaney, N. G.; Sellers, S. E.; Healy, J. T.; Czerski, D. B. Crystal RG. Recombinant DNA-produced alpha 1-antitrypsin administered by aerosol augments lower respiratory tract antineutrophil elastase defenses in individuals with alpha 1-antitrypsin deficiency. *J. Clin. Invest.* **1989**, *84* (4), 1349−1354.
(133) Perlmutter, D. H.; May, L. T.; Sehgal, P. B. Interferon beta 2/interleukin 6 modulates synthesis of alpha 1-antitrypsin in human mononuclear phagocytes and in human hepatoma cells. *J. Clin. Invest.* **1989**, *84* (1), 138−144.
(134) Perlmutter, D. H.; Schlesinger, M. J.; Pierce, J. A.; Campbell, E. J.; Rothbaum, R. J.; Schwartz, A. L. Induction of the stress response in alpha 1-antitrypsin deficiency. *Trans. Assoc. Am. Physicians* **1988**, *101*, 33−41.
(135) Perlmutter, D. H.; Travis, J.; Punsal, P. I. Elastase regulates the synthesis of its inhibitor, alpha 1-proteinase inhibitor, and exaggerates the defect in homozygous PiZZ alpha 1 PI deficiency. *J. Clin. Invest.* **1988**, *81* (6), 1774−1780.
(136) Knoell, D. L.; Ralston, D. R.; Coulter, K. R.; Wewers, M. D. Alpha 1-antitrypsin and protease complexation is induced by lipopolysaccharide, interleukin-1beta, and tumor necrosis factor-alpha in monocytes. *Am. J. Resp. Crit. Care Med.* **1998**, *157* (1), 246−255.
(137) Boutten, A.; Venembre, P.; Seta, N.; Hamelin, J.; Aubier, M.; Durand, G.; et al. Oncostatin M is a potent stimulator of alpha1-antitrypsin secretion in lung epithelial cells: modulation by transforming growth factor-beta and interferon-gamma. *Am. J. Respir. Cell Mol. Biol.* **1998**, *18* (4), 511−520.
(138) Mackiewicz, A.; Sobieska, M.; Kapcinska, M.; Mackiewicz, S. H.; Wiktorowicz, K. E.; Pawlowski, T. Different capabilities of monocytes from patients with systemic lupus erythematosus and rheumatoid arthritis to induce glycosylation alterations of acute phase proteins in vitro. *Ann. Rheum. Dis.* **1992**, *51* (1), 67−72.
(139) Pos, O.; van der Stelt, M. E.; Wolbink, G. J.; Nijsten, M. W.; van der Tempel, G. L.; van Dijk, W. Changes in the serum concentration and the glycosylation of human alpha 1-acid glycoprotein and alpha 1-protease inhibitor in severely burned

persons: relation to interleukin-6 levels. *Clin. Exp. Immunol.* **1990**, *82* (3), 579−582.
(140) Mackiewicz, A.; Rose-John, S.; Schooltink, H.; Laciak, M.; Gorny, A.; Heinrich, P. C. Soluble human interleukin-6-receptor modulates interleukin-6-dependent N-glycosylation of alpha 1-protease inhibitor secreted by HepG2 cells. *FEBS Lett.* **1992**, *306* (2−3), 257−261.
(141) Saso, L.; Valentini, G.; Giardino, A. M.; Spadaro, A.; Riccieri, V.; Zoppini, A.; et al. Changes of glycosylation of serum proteins in psoriatic arthritis, studied by enzyme-linked lectin assay (ELLA), using concanavalin A. *Biochem. Mol. Biol. Int.* **1998**, *46* (5), 867−875.
(142) Mackiewicz, A.; Laciak, M.; Gorny, A.; Baumann, H. Leukemia inhibitory factor, interferon gamma and dexamethasone regulate N-glycosylation of alpha 1-protease inhibitor in human hepatoma cells. *Eur. J. Cell Biol.* **1993**, *60* (2), 331−336.
(143) Kulig, P.; Cichy, J. Acute phase mediator oncostatin M regulates affinity of alpha1-protease inhibitor for concanavalin A in hepatoma-derived but not lung-derived epithelial cells. *Cytokine* **2005**, *30* (5), 269−274.
(144) Kataoka, H.; Seguchi, K.; Inoue, T.; Koono, M. Properties of alpha 1-antitrypsin secreted by human adenocarcinoma cell lines. *FEBS Lett.* **1993**, *328* (3), 291−295.
(145) Arnold, J. N.; Saldova, R.; Hamid, U. M.; Rudd, P. M. Evaluation of the serum N-linked glycome for the diagnosis of cancer and chronic inflammation. *Proteomics* **2008**, *8* (16), 3284−3293.
(146) Rostenberg, I.; Guizar-Vazquez, J.; Penaloza, R. Altered carbohydrate content of alpha1-antitrypsin in patients with cancer. *J. Natl. Cancer Inst.* **1978**, *61* (4), 961−965.
(147) Abd Hamid, U. M.; Royle, L.; Saldova, R.; Radcliffe, C. M.; Harvey, D. J.; Storr, S. J.; et al. A strategy to reveal potential glycan markers from serum glycoproteins associated with breast cancer progression. *Glycobiology* **2008**, *18* (12), 1105−1118.
(148) Sun, Z.; Yang, P. Role of imbalance between neutrophil elastase and alpha 1-antitrypsin in cancer development and progression. *Lancet Oncol.* **2004**, *5* (3), 182−190.
(149) Yang, P.; Bamlet, W. R.; Sun, Z.; Ebbert, J. O.; Aubry, M. C.; Krowka, M. J.; et al. Alpha1-antitrypsin and neutrophil elastase imbalance and lung cancer risk. *Chest* **2005**, *128* (1), 445−452.
(150) Schrodinger, L. *The PyMOL Molecular Graphics System*. Version 1.3r1 ed.; Schrodinger, LLC; 2010.
(151) Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; et al. The Protein Data Bank. *Nucleic Acids Res.* **2000**, *28* (1), 235−242.
(152) Patschull, A. O.; Segu, L.; Nyon, M. P.; Lomas, D. A.; Nobeli, I.; Barrett, T. E.; et al. Therapeutic target-site variability in alpha1-antitrypsin characterized at high resolution. *Acta Crystallogr. Sect. F* **2011**, *67* (Pt 12), 1492−7.
(153) Wormald, M. R.; Petrescu, A. J.; Pao, Y. L.; Glithero, A.; Elliott, T.; Dwek, R. A. Conformational studies of oligosaccharides and glycopeptides: complementarity of NMR, X-ray crystallography, and molecular modelling. *Chem. Rev.* **2002**, *102* (2), 371−86.
(154) Petrescu, A. J.; Milac, A. L.; Petrescu, S. M.; Dwek, R. A.; Wormald, M. R. Statistical analysis of the protein environment of N-glycosylation sites: implications for occupancy, structure, and folding. *Glycobiology* **2004**, *14* (2), 103−14.
(155) Zerimech, F.; Hennache, G.; Bellon, F.; Barouh, G.; Lafitte, J. J.; Porchet, N.; et al. Evaluation of a new Sebia isolectrofocusing kit for α_1-antitrypsin phenotyping with the Hydrasys System. *Clin Chem. Lab Med.* **2008**, *46* (2), 260−3.
(156) Fournier, T.; Medjoubi, N. N.; Porquet, D. Alpha-1-acid glycoprotein. *Biochim. Biophys. Acta* **2000**, *1482* (1−2), 157−71.
(157) Higai, K.; Aoki, Y.; Azuma, Y.; Matsumoto, K. Glycosylation of site-specific glycans of alpha1-acid glycoprotein and alterations in acute and chronic inflammation. *Biochim. Biophys. Acta* **2005**, *1725* (1), 128−35.
(158) Higai, K.; Azuma, Y.; Aoki, Y.; Matsumoto, K. Altered glycosylation of alpha1-acid glycoprotein in patients with inflammation and diabetes mellitus. *Clin. Chim. Acta* **2003**, *329* (1−2), 117−125.
(159) Brinkman-van der Linden, E. C.; de Haan, P. F.; Havenaar, E. C.; van Dijk, W. Inflammation-induced expression of sialyl LewisX is not restricted to alpha1-acid glycoprotein but also occurs to a lesser extent on alpha1-antichymotrypsin and haptoglobin. *Glycoconjugate J.* **1998**, *15* (2), 177−182.
(160) Al Murri, A. M.; Bartlett, J. M.; Canney, P. A.; Doughty, J. C.; Wilson, C.; McMillan, D. C. Evaluation of an inflammation-based prognostic score (GPS) in patients with metastatic breast cancer. *Br J. Cancer* **2006**, *94* (2), 227−230.
(161) Saldova, R.; Royle, L.; Radcliffe, C. M.; Abd Hamid, U. M.; Evans, R.; Arnold, J. N.; et al. Ovarian cancer is associated with changes in glycosylation in both acute-phase proteins and IgG. *Glycobiology* **2007**, *17* (12), 1344−1356.
(162) Kossowska, B.; Ferens-Sieczkowska, M.; Gancarz, R.; Passowicz-Muszynska, E.; Jankowska, R. Fucosylation of serum glycoproteins in lung cancer patients. *Clin. Chem. Lab Med.* **2005**, *43* (4), 361−369.
(163) Fujimura, T.; Shinohara, Y.; Tissot, B.; Pang, P. C.; Kurogochi, M.; Saito, S.; et al. Glycosylation status of haptoglobin in sera of patients with prostate cancer vs. benign prostate disease or normal subjects. *Int. J. Cancer* **2008**, *122* (1), 39−49.
(164) Hoagland, L., 4th; Campa, M. J.; Gottlin, E. B.; Herndon, J. E., 2nd; Patz, E. F., Jr. Haptoglobin and posttranslational glycan-modified derivatives as serum biomarkers for the diagnosis of nonsmall cell lung cancer. *Cancer* **2007**, *110* (10), 2260−8.
(165) Okuyama, N.; Ide, Y.; Nakano, M.; Nakagawa, T.; Yamanaka, K.; Moriwaki, K.; et al. Fucosylated haptoglobin is a novel marker for pancreatic cancer: A detailed analysis of the oligosaccharide structure and a possible mechanism for fucosylation. *Int. J. Cancer* **2006**, *118* (11), 2803−2808.
(166) Nakano, M.; Nakagawa, T.; Ito, T.; Kitada, T.; Hijioka, T.; Kasahara, A.; et al. Site-specific analysis of N-glycans on haptoglobin in sera of patients with pancreatic cancer: A novel approach for the development of tumor markers. *Int. J. Cancer* **2008**, *122* (10), 2301−2309.
(167) Thompson, S.; Dargan, E.; Turner, G. A. Increased fucosylation and other carbohydrate changes in haptoglobin in ovarian cancer. *Cancer Lett.* **1992**, *66* (1), 43−48.
(168) Thompson, S.; Turner, G. A. Elevated levels of abnormally-fucosylated haptoglobins in cancer sera. *Br J. Cancer.* **1987**, *56* (5), 605−610.
(169) Turner, G. A. Haptoglobin. A potential reporter molecule for glycosylation changes in disease. *Adv. Exp. Med. Biol.* **1995**, *376*, 231−238.
(170) Goodarzi, M. T.; Turner, G. A. Decreased branching, increased fucosylation and changed sialylation of alpha-1-proteinase inhibitor in breast and ovarian cancer. *Clin. Chim. Acta* **1995**, *236* (2), 161−171.
(171) Laine, A.; Hachulla, E.; Strecker, G.; Michalski, J. C.; Wieruszeski, J. M. Structure determination of the glycans of human-serum alpha 1-antichymotrypsin using ^{1}H-NMR spectroscopy and deglycosylation by *N*-glycanase. *Eur. J. Biochem./FEBS* **1991**, *197* (1), 209−215.
(172) Solis, D.; Feizi, T.; Yuen, C. T.; Lawson, A. M.; Harrison, R. A.; Loveless, R. W. Differential recognition by conglutinin and mannan-binding protein of *N*-glycans presented on neoglycolipids and glycoproteins with special reference to complement glycoprotein C3 and ribonuclease B. *J. Biol. Chem.* **1994**, *269* (15), 11555−62.
(173) Crispin, M. D.; Ritchie, G. E.; Critchley, A. J.; Morgan, B. P.; Wilson, I. A.; Dwek, R. A.; et al. Monoglucosylated glycans in the secreted human complement component C3: Implications for protein biosynthesis and structure. *FEBS Lett.* **2004**, *566* (1−3), 270−274.
(174) Halim, A.; Nilsson, J.; Ruetschi, U.; Hesse, C.; Larson, G. Human urinary glycoproteomics; attachment site specific analysis of N- and O-linked glycosylations by CID and ECD. *Mol. Cell. Proteomics* **2012**, *11* (4), M111 013649.