

Structural Aspects of Therapeutic Enzymes to Treat Metabolic Disorders

Tse Siang Kang^{1,2} and Raymond C. Stevens^{1*}

¹Department of Molecular Biology, The Scripps Research Institute, La Jolla, California; ²Department of Pharmacy, National University of Singapore, Singapore

Communicated by Johannes Zschocke

Received 4 June 2009; accepted revised manuscript 7 August 2009.

Published online 29 September 2009 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/humu.21111

ABSTRACT: Protein therapeutics represents a niche subset of pharmacological agents that is rapidly gaining importance in medicine. In addition to the exceptional specificity that is characteristic of protein therapeutics, several classes of proteins have also been effectively utilized for treatment of conditions that would otherwise lack effective pharmacotherapeutic options. A particularly striking class of protein therapeutics is exogenous enzymes administered for replacement therapy in patients afflicted with metabolic disorders. To date, at least 11 enzymes have either been approved for use, or are in clinical trials for the treatment of selected inherited metabolic disorders. With the recent advancement in structural biology, a significantly larger amount of structural information for several of these enzymes is now available. This article is an overview of the correlation between structural perturbations of these enzymes with the clinical presentation of the respective metabolic conditions, as well as a discussion of the relevant structural modification strategies engaged in improving these enzymes for replacement therapies. *Hum Mutat* 30:1591–1610, 2009. © 2009 Wiley-Liss, Inc.

KEY WORDS: enzyme replacement therapy; protein therapeutics; metabolic disease; protein misfolding; lysosomal storage disease

Introduction

Proteins are unique macromolecules involved in a variety of key physiological processes ranging from structural and contractile roles in the formation of supporting and muscle tissues, to biochemical processes including receptor channel formation and signal transduction, to transport functions by hemoglobin or albumin, as well as a plethora of other highly specialized functions such as immunomodulation and catalysis of reactions. For this reason, it is often the defect or deficiency of these functionally distinct molecules that form the basis of severe pathological conditions. Conversely, it is also the exceptional specificity and potency of proteins that placed them in a pharmacologically

favorable position over conventional small-molecule chemical therapeutic agents [Leader et al., 2008].

Proteins as a whole, present as a trove of potential therapeutics, with the structure–function relationship playing a critical role in the biological activity and potency of the protein to the target receptor. To date, approximately 130 proteins and peptides have been approved for clinical use in the United States and in European countries, with many more in developmental stages [Leader et al., 2008; Rader, 2007]. In early 2008, Leader et al. [2008] reviewed the list of protein therapeutics approved as therapeutic agents, and these agents were classified according to their pharmacological and functional applications. Among the list of proteins approved by the U.S. Food and Drug Administration (FDA), over 30 products are based on the unique subset of enzymes therapeutics [Rader, 2007], which in addition to possessing the trait of impeccable affinity to their respective target receptors, are also responsible for the catalysis of complex biochemical reactions.

Based on their therapeutic applications, we further classified enzymes that are currently approved for use as therapeutics or have entered clinical trials into two major groups. The focus of this article is placed on the first group of therapeutic enzymes (Table 1), which serves the niche purpose of enzyme replacement therapy (ERT) in patients afflicted by hereditary metabolic diseases, in which genetic defects result in the structural defect or absence of endogenous enzymes critical for metabolic pathways. These metabolic defects in turn, result in the accumulation of substrates and/or its intermediates, leading to pathological states. The second group of therapeutic enzymes alleviates symptoms of pathological conditions by augmenting existing endogenous pathways, or by exerting novel catalytic functions (Table 2). Group II therapeutic enzymes have been successfully utilized in a variety of pathological conditions ranging from oncology, to neuromuscular dysfunctions, to hemostasis, and even cosmetic surgery.

The concept of ERT was first suggested more than 40 years ago by De Duve and Waiteaux for the treatment of lysosomal storage disorders [Rohrbach and Clarke, 2007]. Prior to the advent of ERT, patients with metabolic conditions faced strict dietary restriction and symptomatic management of the conditions [Holtzman, 1970]. However, for metabolic diseases such as lysosomal storage diseases that could not be alleviated through these measures, patients are often faced with drastic treatment modalities and these conditions are frequently fatal [Beutler, 2006a; de Fost et al., 2003]. The first ERT product, Alglucerase (Ceredase), was first approved by the FDA in 1991 for the treatment of Gaucher disease (MIM# 230800) (glucocerebrosidase; MIM# 606463, GBA; EC 3.2.1.45) [Barton et al., 1991], and

Additional Supporting Information may be found in the online version of this article.

*Correspondence to: Raymond C. Stevens, The Scripps Research Institute, Molecular Biology, 10550 North Torrey Pines Road, La Jolla, CA 92037.

E-mail: stevens@scripps.edu

Table 1. Group I Therapeutic Enzymes

Enzyme	Trade name	Therapeutic application, mode of action	Structure (PDB code)	Reference
Glucocerebrosidase	Ceredase [®] , Cerezyme [®] , Velaglugerase, prGCD	Gaucher disease: degradation of glucosylceramide	X-ray (1OGS)	Barton et al. [1991]; Grabowski et al. [1995]
α -galactosidase	Replagal, Fabrazyme [®]	Fabry disease: degradation of globotriaosylceramide	X-ray (1R47)	Eng et al. [2001]; Schiffmann et al. [2001]
Acid α -glucosidase	Myozyme [®] , Lumizyme TM	Pompe disease: degradation of glycogen at 1.4- and 1.6-glycosidic linkages	Homology model	Amalfitano et al. [2001]
α -L-iduronidase	Aldurazyme [®]	Hurler and Hurler-Scheie forms of mucopolysaccharidosis I: hydrolysis of unsulfated α -L-iduronosidic linkages in glucosaminoglycans, heparin sulfate, and dermatan sulfate	Homology model (1Y24)	Kakkis et al. [2001]
Iduronate-2-sulfatase	Elaprase [®]	Hunter Disease (MPS II): cleavage of sulfate group from the 2-position of L-iduronic acid present on glycosaminoglycan, dermatan sulfate, and heparan sulfate	Homology model	Muenzer et al. [2007]
N-acetyl-galactosamine-4-sulfatase	Naglazyme [®]	Mucopolysaccharidosis VI: cleaving of terminal sulfate from sulfated glycoaminoglycans, glycoproteins, and glycolipids	X-ray (1FSU)	Hopwood et al. [2006]
Arylsulfatase A	Metazyme TM	Metachromatic leukodystrophy: cerebroside 3-sulfate	X-ray (1AUK)	Lukatela et al. [1998]
Acid sphingomyelinase	rhASM	Niemann-Pick disease: hydrolysis of sphingomyelin to phosphocholine and ceramide	Partial homology model	Miranda et al. [2000]
Tissue-nonspecific alkaline phosphatase fusion protein	ENB 0040	Hypophosphatasia: hydrolysis of phosphomonoester including phosphoethanolamine, inorganic pyrophosphate, and pyridoxal 5-phosphate	Homology model	Millan et al. [2008]
Porphobilinogen deaminase	Porphozym	Acute intermittent porphyria: third catalytic step of heme biosynthesis pathway in which four porphobilinogen molecules are condensed into tetrapyrrole	Homology model	Louie et al. [1992]
Phenylalanine ammonia lyase	rAvPAL-PEG	Phenylketonuria: degradation of phenylalanine	X-ray (3CZO)	Wang et al. [2008]

Table 2. Group II Therapeutic Enzymes*

Enzyme	Trade name	Function	Origin
Thrombosis and hemostasis			
Alteplase	Activase [®]	Conversion of plasminogen to plasmin	Recombinant human tissue plasminogen activator
Reteplase	Retivase [®]	Conversion of plasminogen to plasmin	Recombinant variant of human tissue plasminogen activator (nonglycosylated kringle 2 and protease domain)
Tenecteplase	TNKase [®]	Conversion of plasminogen to plasmin, with increased specificity compared to Activase and Retivase	Recombinant variant of human tissue plasminogen activator (T103D, D117Q, tetraalanine 296–299 substitutions)
Urokinase	Abbokinase [®]	Conversion of plasminogen to plasmin	Human neonatal kidney cells
Streptokinase	Streptase [®]	Conversion of plasminogen to plasmin	Group C β -hemolytic streptococci bacteria
Anistreplase	Eminase [®]	Conversion of plasminogen to plasmin, with prolonged duration of action compared to streptokinase	Anisoylated derivative of plasminogen-streptokinase activator complex
Drotrecogin- α	Xigris [®]	Antithrombotic effect by inhibiting Factors Va and VIIIa; antiinflammatory effects	Recombinant human activated protein C
Factor VIIa oncology	Novoseven [®]	Activation of extrinsic coagulation pathway	Recombinant human coagulation Factor VIIa
L-Asparaginase	ELSPAR [®]	Exogenous asparaginase activity, used in for treatment of acute lymphocytic leukemia	<i>Escherichia coli</i> L-asparaginase type EC-2 enzyme
PEGylated L-asparaginase	Oncaspar [®]		
Carboxypeptidase	Voraxaze TM (orphan drug status)	Cleaves folates and related compounds containing folic acid moieties, used in chemotherapy	Recombinant <i>Variovorax paradoxus</i> carboxypeptidase G2 enzyme
Rasburicase	Elitek [®]	Enzymatic oxidation of uric acid into allantoin	Recombinant <i>Aspergillus flavus</i> urate oxidase
Others			
Adenosine deaminase	Adagen [®]	Metabolism of adenosine in SCID patients	PEGylated purified bovine adenosine deaminase
Botulism toxin type A	Botox [®]	Cleaves SNAP25 at neuromuscular junction, disrupting SNARE complex and preventing acetylcholine release	<i>Clostridium botulinum</i> toxin
Botulinum neurotoxin type A complex	Dysport [®]		
Botulism toxin type B	Myobloc [®]	Cleaves synaptobrevin at neuromuscular junction, disrupting SNARE complex and preventing acetylcholine release	<i>Clostridium botulinum</i> toxin
Hyaluronidase	Hylenex TM Amphadase [®] Hydase TM Vitrase [®]	Hydrolysis of hyaluronic acid	Recombinant human hyaluronidase Bovine hyaluronidase Bovine hyaluronidase Ovine hyaluronidase

*From Leader et al. [2008] and Rader [2007].

took the form of human glucocerebrosidase laboriously purified from human placenta. This pioneering application of ERT was met with stunning success, and ERT was subsequently developed for several other forms of metabolic disorders including Pompe disease (MIM# 232300) (acid α -glucosidase; MIM# 606800; GAA; EC 3.2.1.20), Hurler, Scheie, and Hurler-Scheie forms of mucopolysaccharidosis Type I (MIM#s 607014, 607015, and 607016) (α -L-iduronidase; MIM# 252800; IDUA; EC 3.2.1.76), Mucopolysaccharidosis Type II (MIM# 309900) (iduronate-2-sulfatase, IDS; EC 3.1.6.13), mucopolysaccharidosis Type VI (MIM# 253200) (N-acetylgalactosamine-4-sulfatase; MIM# 611542, ARSB; 3.1.6.1), and Fabry disease (MIM# 301500) (α -galactosidase; MIM# 300644, GLA; EC 3.2.1.22) [Leader et al., 2008]. In addition, enzyme candidates for ERT in metachromatic leukodystrophy (MIM# 250100) (arylsulfatase A; MIM# 607574, ARSA; EC 3.1.6.8) [Lukatela et al., 1998]; Niemann-Pick disease (MIM#s 257200, 607616) (acid sphingomyelinase; MIM# 607608, SMPD1; 3.1.4.41) [Miranda et al., 2000]; hypophosphatasia (MIM# 146300) (tissue-nonspecific alkaline phosphatase; MIM# 171760, TNSAP; EC 3.1.3.1) [Millan et al., 2008]; acute intermittent porphyria (MIM# 176000) (porphobilinogen deaminase; MIM# 609806, HMBS; EC 4.3.1.8) [Sardh et al., 2007]; and phenylketonuria (PKU; MIM# 261600) (enzyme substitution with phenylalanine ammonia lyase, EC 4.3.1.5) [Sarkissian et al., 2008] have also been developed and entered Phase II clinical trials.

The current strategy of ERT involves exogenous administration of native enzymes deficient in patients afflicted with the metabolic disease states. Despite the fact that the target patient population for each of the therapeutic enzymes is comparatively limited compared to conventional pharmaceutical products, and is typically one of the most costly products [Wraith, 2006], this specialized subset of biological therapeutics is rapidly gaining grounds. Due to the massive medical costs involved in ERT (estimated >\$200,000 USD per patient per year) [Beutler, 2006b], there is a continuing effort in improving the catalytic and therapeutic efficacy of these enzymes as well as optimizing the usage of these enzymes. Several protein engineering efforts have shown that by strategically engineering and refining the enzymes' architecture, it is theoretically possible to redesign the enzymes' catalytic and biochemical characteristics [Penning and Jez, 2001; Toscano et al., 2007]. The wealth of structural information advanced in the recent years for several of these therapeutic enzymes have positioned them as potential candidates for rational protein engineering and refinement of enzyme architecture to enhance catalytic activity and site-specific delivery. In addition, several other enzyme candidates for the treatment of specific metabolic diseases are in development. These include β -glucuronidase for mucopolysaccharidosis VII [LeBowitz et al., 2004; Orii et al., 2005], galactosylceramidase for globoid cell leukodystrophy (GLD) or Krabbe disease [Lee et al., 2005], N-acetylgalactosamine-6-sulfate sulfatase for Morquio syndrome [Tomatsu et al., 2008], sulfamidase for mucopolysaccharidosis IIIa (Sanfilippo A syndrome) [Gliddon and Hopwood, 2004], and α -mannosidase for α -mannosidosis [Roces et al., 2004], which are all in preclinical studies. However, these preliminary agents fall beyond the scope of the current review and are not discussed in this work. Herein, we review some of the available structural information for therapeutic enzymes that are currently approved or in/beyond phase II clinical trials for the treatment of metabolic conditions.

Glucocerebrosidase in Gaucher Disease

Gaucher disease is the most prevalent form of lysosomal storage disorder [Brady et al., 1965], affecting approximately 1:40,000 to

1:60,000 in the general population [Grabowski, 1993]. This autosomal recessive condition [Brady et al., 1965] results in defects in the peripheral membrane enzyme glucocerebrosidase (GlcCerase, acid β -glucosidase, d-gluco-N-acyl-sphingosine glucosylceramide [Sawkar et al., 2005]), preventing the degradation of glucosylceramide [Brady et al., 1965]. The resulting intracellular accumulation of glucosylceramide in macrophages leads in turn to hepatomegaly, splenomegaly, anemia, skeletal complications, and neurological complications [Zhao and Grabowski, 2002]. One of the major advances in the management of Gaucher disease came with the effective management of disease state through ERT using purified (Ceredase[®]), and subsequently recombinant glucocerebrosidase (Cerezyme[®]). Glucocerebrosidase is a 497-residue monomeric enzyme with the mature unglycosylated polypeptide having a molecular weight of ~56 kDa [Berg-Fussman et al., 1993], and final glycosylated molecular weight between 59 and 67 kDa [Brumshtein et al., 2006; Dvir et al., 2003; Grace et al., 1994]. For glucocerebrosidase to be functional, the enzyme needs to undergo a reconformation into the active form by interaction with negatively-charged head groups of lipids such as phosphatidylserine [Basu et al., 1985; Qi and Grabowski, 1998] and saposin C [Qi and Grabowski, 1998; Wilkening et al., 1998]. Cerezyme[®] contains a single amino acid substitution of R495 H relative to the native enzyme due to a cloning artifact, which was concluded to bear no impact on the structural or biochemical properties of the enzyme [Dvir et al., 2003; Friedman et al., 1999; Weinreb, 2008].

The three-dimensional (3D) structure of Cerezyme[®] partially deglycosylated with N-glycosidase F was elucidated in 2003, with its crystal structure refined to 2.0 Å [Dvir et al., 2003]. The structure of the fully-glycosylated Cerezyme[®] was subsequently solved, and found to be virtually identical to the previous structure [Brumshtein et al., 2006]. The overall architecture of the enzyme is comprised of three noncontiguous domains. Domain I is made up of residues 40 to 66 and 422 to 453 of the translated protein sequence, and comprises a three-stranded antiparallel β -sheet as well as an amino terminal strand. The immunoglobulin-like Domain II is formed by two β -sheets made up of residues 69 to 114 and 470 to 536, while the $(\beta/\alpha)_8$ TIM barrel Domain III is the key catalytic domain made up of residues 115 to 420 and 455 to 469 [Dvir et al., 2003]. Residue E379 was identified as the nucleophile to the catalytic site, while residue E274 acted as the acid/base for the completion of catalytic reaction [Fabrega et al., 2000; Miao et al., 1994]. A discontinuous series of seven aromatic residues (F167, W218, Y283, F285, Y352, W420, and F436) was also noted to line the entrance of the active site pocket, possibly involved in substrate recognition [Dvir et al., 2003]. The entrance to the active site was noted to be in the proximity of two loops (S384–E388 as loop 1, and V433–D438 as loop 2) that form a lid to the active site entrance [Premkumar et al., 2005].

The glucocerebrosidase's structure consists of seven cysteine residues, of which C43, C55, C57, and C62 are involved in disulfide bridge formation within Domain I [Dvir et al., 2003]. Mutagenesis studies confirmed that these disulfide interactions are critical in maintaining catalytically viable forms of the enzyme. Although C287 and C381 are not involved in disulfide bridge formation, serine-substituted analogs have compromised or abolished catalytic activities. The remaining C167 is the only cysteine residue that is not shown to be directly implicated in the activity of the enzyme [Zhao and Grabowski, 2002].

A total of five potential glycosylation sites [Asn-Xaa-(Ser/Thr)] have been identified on glucocerebrosidase, namely N58, N98, N185, N309, and N501. Site-directed mutagenesis demonstrated

that except for N501, the remaining four sites are N-glycosylated [Berg-Fussman et al., 1993]. Activities of p.N98Q, p.N185Q, and p.N309Q were comparable to wild type, but were drastically reduced for p.N58Q, p.N501Q, and p.N501D. The poor catalytic activities of N501 mutants were not entirely unexpected considering the proximity of the asparagine residue to R502, a known mutation site [Berg-Fussman et al., 1993; Hong et al., 1990]. These results suggest that the nature of the amino acid at glucocerebrosidase's first glycosylation site, as well as the occupancy of specific glycosylation sites are critical for the catalytic activity of the enzyme. Glycosylation at N58 has also been suggested to act as a nidus for the correct conformation of the native protein [Berg-Fussman et al., 1993; Liou et al., 2006]. Further, trimming of the oligosaccharide residues to the mannosyl core at the various glycosylation sites facilitated mannose receptor-mediated uptake of the enzyme into macrophages [Doebber et al., 1982]. For this reason, the initial placental enzyme and the subsequent recombinant enzyme were trimmed to the core mannose to improve uptake into the reticuloendothelial system [Furbish et al., 1981]. Subsequent efforts to express glucocerebrosidase containing terminal mannose structures of various sizes at the four glycosylation sites in several alternative expression systems did not show any distinct biochemical pharmacological advantages over Cerezyme[®] [Van Patten et al., 2007]. Interestingly, while the deglycosylation of N185 resulted in the decreased thermostability of the glucocerebrosidase, deglycosylation of glucocerebrosidase with a p.N58D mutation was noted to be hyperresponsive to activation by negatively-charged phospholipid (NCP) [Berg-Fussman et al., 1993]. The most common mutation p.N409S variant [Beutler and Gelbart, 1993] which is responsible for mild, nonneuronopathic Gaucher disease, was also noted to have a greater activation effect by NCP [Zhao and Grabowski, 2002].

To date, over 200 different mutations implicated to Gaucher disease have been documented [Beutler et al., 2005; Hruska et al., 2008; Steet et al., 2006]. In contrast to mild mutations, which are generally associated with Type I nonneuronopathic Gaucher disease, patients with severe or null mutations typically present with Type II or Type II neuronopathic Gaucher disease. In Dvir et al. [2003], the authors illustrated the distribution of the different mutations reported in GeneDis Human Genetic Disease Database (www.tau.ac.il/lifesci/bioinfo/genedis/gaucher/gaucher.html) across all three domains in the structure of glucocerebrosidase. It was suggested that the severe phenotypic effects of selected mutations correlated to their proximity to critical structural features, including the seven aromatic residues lining the entrance of the active-site pocket and the active site, immunoglobulin-like Domain II, and the interface of Domain II and III.

The review article by Beutler et al. [2005] provided an update on the list of mutations to 236 single or combined mutations. Of the 50 mutations sites that resulted in severe Gaucher disease, 11 reside in Domain I, six were observed in the hydrophobic core of the immunoglobulin-like Domain II, while the remaining occurred within the catalytic Domain III (Fig. 1A). Of the 11 severe mutation sites from Domain I, five are located on loop 2 of the lid to the active site, falling within a 5-Å radius of the aromatic lining. Nine out of the 33 mutation sites within Domain III reside within 5 Å of either the active site E379 nucleophile, acid/base E274, or the seven aromatic residues (F167, W218, Y283, F285, Y352, W420, and F436) lining the entrance of the active site (Fig. 1B). Interestingly, while a high density of mutation points occur on the entrance of the active site pocket, only a single severe

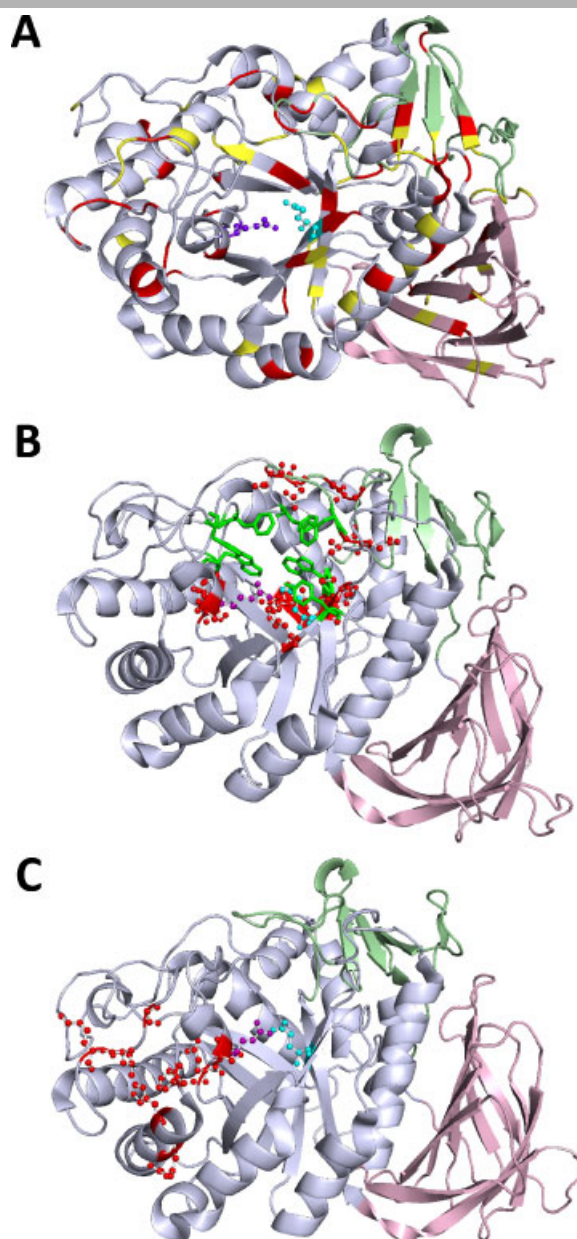


Figure 1. **A:** Overall mutation for glucocerebrosidase (PDB code 10GS). The structure of glucocerebrosidase is divided into Domain I (pale green), Domain II (pink), and Domain III (blue-white). The residues shown in ball-and-stick representation are active-site residues E274 (purple) and E379 (cyan), respectively. Mutations resulting in severe disease are indicated in red, while those resulting in mild disease are indicated in yellow. Null mutations or frameshift mutations are not indicated in this diagram. **B:** Severe mutation sites around active-site pocket of glucocerebrosidase; 9 out of the 33 severe mutation sites (red ball-and-sticks) located within catalytic Domain III are situated within 5 Å proximity of the seven aromatic residue (green sticks) lining the entrance of active site. **C:** Severe mutation sites around $\alpha 3$ and $\alpha 4$ helices of glucocerebrosidase Domain III.

mutation point (p.Y343C/M) was found on the posterior surface of TIM barrel motif of Domain III.

Another cluster of severe mutation sites was found in the region of $\alpha 3$ and $\alpha 4$ helices of the TIM barrel catalytic Domain III. These sites include p.G234W, p.L236F, p.K237E, p.E272 M, p.H294Q, and p.R296Q, all falling within a 5-Å radius of one another

(Fig. 1C). While it is not surprising to note that mutations at or around the active site result in impairment of the enzyme's functionality, the observed cluster of mutations in Figure 1C is indicative of suggested functional roles of $\alpha 3$ and $\alpha 4$ helices of Domain III.

While certain mutations that result in drastic changes to the charge or size of the side chains of the residues concerned will obviously be detrimental to the structure and function of the enzyme, the effects of certain conservative substitutions (e.g., p.L483R, p.V433L, and p.H350R, resulting in severe disease) were certainly unexpected. More importantly, it is interesting to note that although a total of 33 mutations are found on the 319-residue Domain III, nearly one-half of these mutations occurred in these two foci. In contrast, the 37 mutation sites resulting in mild Gaucher disease are distributed throughout the three domains, with no specific focus point. These observations suggest that the locations, in addition to the type of mutation, are critical in determining the phenotypic outcome of Gaucher disease.

α -Galactosidase in Fabry Disease

Fabry disease is the second most prevalent lysosomal storage disorder, and is the second metabolic condition to be approved for treatment with ERT [Garman and Garboczi, 2004]. The reported incidence of Fabry disease in newborn males ranges from 1:40,000 to nearly 1:4,000 [Spada et al., 2006]. This lysosomal storage disease is caused by an X-linked recessive inheritance, leading to the mutation of α -galactosidase and the subsequent accumulation of globotriaosylceramide substrate within lysosomes [Scriver, 2001]. Patients with the classical form of Fabry disease are typically devoid of the functional α -galactosidase enzyme, and present with severe symptoms including chronic pain of extremities, corneal clouding, hypohidrosis, liver and kidney impairment, and vascular and cardiac deterioration. A milder form of the condition presents in patients who retain residual galactosidase activity, and usually exhibit milder clinical manifestations [Scriver, 2001]. To date, nearly 500 different types of mutation have been reported on the GLA gene (www.hgmd.cf.ac.uk/ac/index.php).

α -Galactosidase is a homodimeric protein, with each monomer having 398 amino acid residues. The mature polypeptide sequence is posttranslationally N-glycosylated for recognition of the enzyme to mannose 6-phosphate receptors on endosomes that are responsible for the transportation of the 46 kDa mature enzyme into lysosomes [Sugawara et al., 2008a]. It had also been suggested that similar to glucocerebrosidase, the activity of α -galactosidase is also positively influenced by effector proteins. However, instead of saposin C, which was noted to be essential for the former case, α -galactosidase is activated by saposin B, a closely-related analog derived from the same precursor [Kase et al., 1996, 2000; Kretz et al., 1990; Sun et al., 2008].

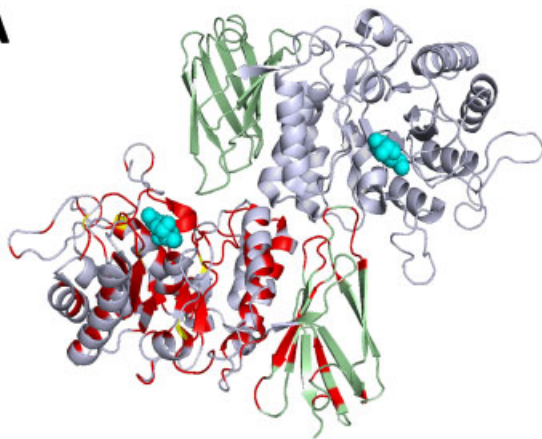
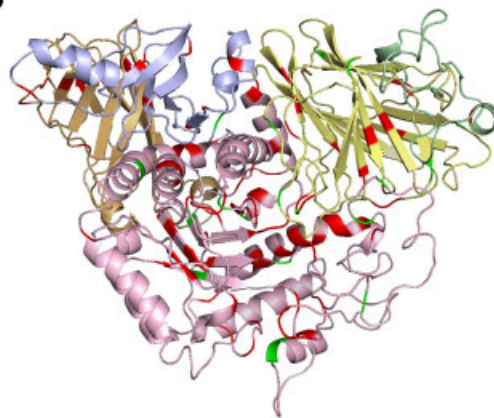
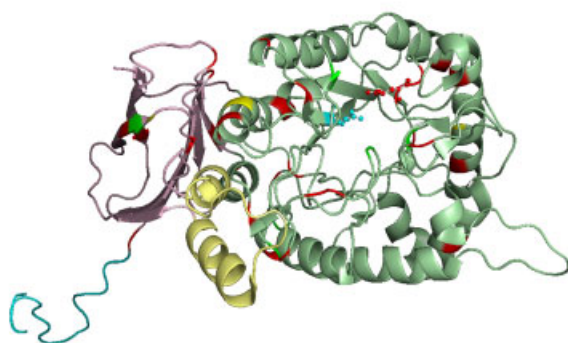
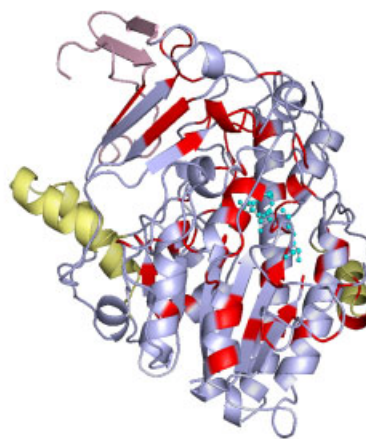
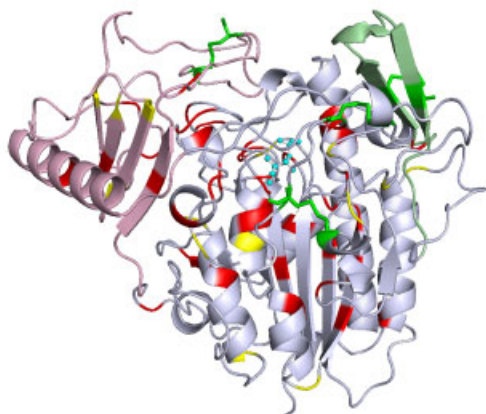
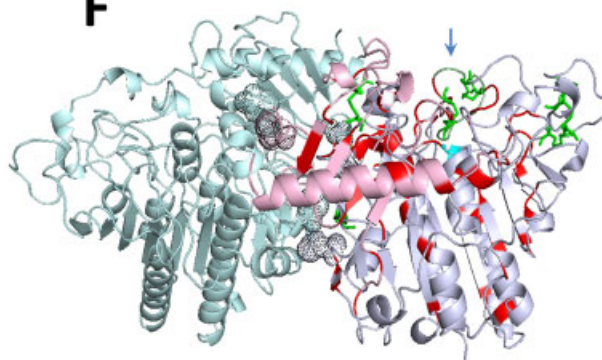
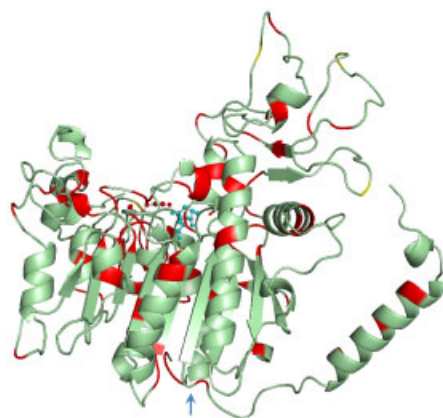
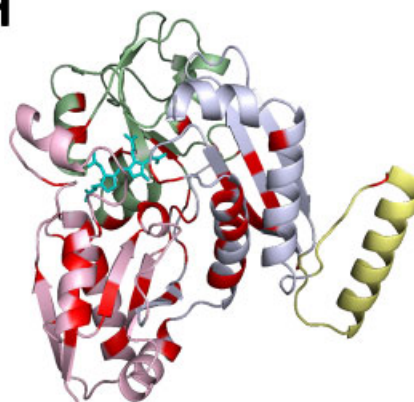
Two recombinant glycosylated α -galactosidases are currently approved for the treatment of Fabry disease [Eng et al., 2001; Schiffmann et al., 2001]. Amino acid sequences for both of these products are identical, but exhibited differences in glycosylation due to the expression in different cell lines. In 2004, Garman and Garboczi [2004] elucidated the 3D structure of Replagal[®] (agalsidase alfa), the α -galactosidase expressed in genetically engineered human cell lines. The crystal structure was determined to a resolution of 3.25 Å, revealing the structure of the homodimer. Each monomer is comprised of two domains, with residues 32 to 330 forming the catalytic (β/α)₈ TIM barrel Domain I and residues 331 to 429 forming the β sandwich Domain II. The first 31 residues belong to the signal sequence and

are not considered in part of the structure. The active site of each monomer is made up by residues W47, D92, D93, Y134, K168, D170, E203, L206, Y207, R227, D231, D266, and M267, as well as C142, which forms a disulfide bridge with C172. Except for E203 and L206, all remaining 13 active site residues were conserved across 40 homologous sequences of galactosidases [Garman and Garboczi, 2004]. D170 acts as the nucleophile to the catalytic reaction, while deprotonation of a water molecule by D231 completes the catalytic reaction by attacking the reaction intermediate.

A total of 12 cysteine residues and three *cis* proline residues are present in the sequence of the mature α -galactosidase, including C142, which is also involved in the formation of the active site. Ten cysteines are involved in forming disulfide bridges present in the structure of each monomer (C52–C94, C56–C63, C142–C172, C202–C223, and C378–C382). Incidentally, mutation for any of the cysteine residues involved in disulfide bridge formation results in Fabry disease [Ashley et al., 2001; Ashton-Prolla et al., 2000; Blanch et al., 1996; Blaydon et al., 2001; Davies et al., 1996; Eng et al., 1993, 1994, 1997; Galanos et al., 2002; Germain and Poenaru, 1999; Okumiya et al., 1995; Ploos van Amstel et al., 1994; Rodriguez-Mari et al., 2003; Schafer et al., 2005; Shabbeer et al., 2002; Topaloglu et al., 1999; Yasuda et al., 2003]. C90 and C174 are present as free cysteines [Garman and Garboczi, 2004], and are neither involved in the formation in the dimer, nor near the hydrophobic interface of the two monomers. Not surprisingly, these are the only two cysteine residues not implicated in Fabry disease.

A total of four potential glycosylation sites (N139, N192, N215, and N408) are found on each monomer of α -galactosidase. Unlike N408, which is unoccupied, N139, N192, and N215 are conserved glycosylation sites and are typically glycosylated in the mature protein [Scriver, 2001]. Elimination of any two of the glycosylation sites has been shown to result in unstable enzymes that result in the degradation of the protein before transport to lysosomes can be completed [Ioannou et al., 1998; Scriver, 2001]. In contrast, glycosylation site 3 (N215) has been demonstrated to be the critical glycosylation site required for the solubility, stability, and enzymatic activity of α -galactosidase [Ioannou et al., 1998].

Of the more than 430 mutations that have been reported for α -galactosidase, over 294 mutations represented missense mutations (www.hgmd.cf.ac.uk/ac/index.php). Schaefer et al. [2005] recently examined the correlation between the genotypic and phenotypic profiles of 545 patients in 157 families monitored under the Fabry Outcome Survey. Several earlier publications have suggested that residues affected by mutation are not restricted to the region of the active site but distributed throughout the two domains of the enzyme structure (Fig. 2A) [Garman, 2007]. By using the crystal structure of human α -galactosidase and the current mutation database, 47 severe mutation sites were found within a 10-Å radius of the substrate galactose, while 36 and 21 severe mutation sites were found within a similar radius of active site residues D170 and D231, respectively. An extensive number of buried mutation sites was also observed in the human α -galactosidase structure [Garman, 2007; Garman and Garboczi, 2002, 2004], with foci around the β -strands of the TIM barrel core as well as the domain interface (Fig. 2A). Mutations resulting in the classical phenotypic form of Fabry disease were noted to be disruptive to the hydrophobic core of the enzyme [Garman and Garboczi, 2002]. These observations, commensurate with earlier observations that tryptophan and cysteine residues have the highest mutation rates within the α -galactosidase structure [Garman, 2007; Garman and Garboczi, 2002], indicate that structural disruption is likely to be one of the main reasons for loss of enzymatic activity in the mutant analogues.

A**B****C****D****E****F****G****H**

Acid α -Glucosidase in Pompe Disease

Pompe disease is an autosomal recessive condition arising from the deficiency of the lysosomal enzyme acid α -glucosidase (GAA), with an estimated incidence of Pompe disease ranging from 1:40,000 to 1:300,000 [Scriver, 2001]. GAA catalyses the hydrolysis of glycogen and other natural α -glucans by hydrolyzing α -1,4- and α -1,6-glucosidic bonds. Consequently, the deficiency of GAA results in the intracellular accumulation of glycogen in various tissues, with the largest impact on cardiac, respiratory, and skeletal muscles [Hers, 1963; Scriver, 2001]. Until the recent approval of a recombinant form of GAA, Myozyme[®] (alglucosidase alfa), no effective form of therapy was previously available for the alleviation of Pompe disease [Geel et al., 2007].

Based on amino acid sequence similarity, GAA has been classified under Family 31 of glycosyl hydrolases [Ernst et al., 2006; Frandsen and Svensson, 1998; Henrissat and Bairoch, 1993]. Although a recombinant form of GAA has been approved for use in ERT of Pompe disease, the structure of GAA has not been reported to date. By constructing homology models based on partial crystallographic data from another Family 31 glycosyl hydrolase, Ma1A (residues 152–604) from *Sulfolobus solfataricus*, Tajima et al. [2007] proposed the 3D structure of the catalytic domain (residues 344–795) of GAA. The constructed model of the catalytic domain of GAA showed a $(\beta/\alpha)_8$ TIM barrel motif, a common motif seen in lysosomal hydrolase active sites [Durand et al., 1997]. Of the seven N-linked glycosylation sites present in the 952-residue precursor sequence, three were present in the catalytic domain constructed, and were found to be exposed on the surface of the molecule. The sites at N882 and N925 were ultimately cleaved off in the proteolytic maturation steps. It was also demonstrated that except N233, which is critical for the enzyme's stability, localization, and maturation, elimination of glycosylation at the remaining six glycosylation sites was noncritical [Hermans et al., 1993]. Interestingly, N652 was located in a region stabilized by the disulfide bridge C647–C658 [Tajima et al., 2007].

By comparison with the substrate binding residues of Ma1A, residues D404, D518, R600, D616, and H674 were determined to be critical for substrate binding and catalysis in GAA [Tajima

et al., 2007]. These deductions concur with earlier literature concluding that D518 is the nucleophile for the catalysis reaction [Hermans et al., 1991], as well as previous work by Durand et al. [1997], which predicted that key catalytic residues of lysosomal hydrolases reside on the C-terminal ends of β -strands of the TIM barrel motif.

In order to gain perspective of the structural features of the remaining domains of GAA not examined by Tajima et al. [2007], we constructed another homology model using the automated mode of homology modeling on the Swiss-Model Comparative Protein Modeller Server [Schwede et al., 2003]. Template selection was performed by a “first approach mode,” in which human intestinal maltase-glucoamylase (NtMGAM; PDB code 2QLY) [Sim et al., 2008], another member of Family 31 glycosyl hydrolases, was selected by the server. As compared to the 30% sequence identity between GAA and Ma1A used in the previous homology model by Tajima et al. [2007], the NtMGAM is the first eukaryotic structure of Family 31 glycosyl hydrolases, and has a 42.8% identity to GAA. Sequence alignment and homology modeling were automatically generated based on residues 84–952 of the GAA sequence (Supp. Fig. S1). The model of GAA overlaps well with the structures of NtMGAM, as well as Ma1A α -glucosidase used as template in the previously reported homology model, with root mean square deviation (RMSD) of 0.056 Å and 1.36 Å, respectively (Supp. Fig. S2). Similar to the structure of the template NtMGAM, the model of GAA could be divided into a Trefoil Type P domain, an N-terminal β -sandwich domain, a $(\beta/\alpha)_8$ TIM barrel motif catalytic domain, a proximal C-terminal domain, and a distal C-terminal domain (Supp. Fig. S3).

A total of 111 missense point mutation sites were identified on the sequence of GAA [Kroos et al., 2008] (www.pompecenter.nl). Of these, 92 sites results in severe or unknown clinical presentation, and 19 resulted in mild or intermediate Pompe disease. Aside from 33 sites of severe/unknown severity that generally lie within the hydrophobic cores of the four noncatalytic domains, the remaining 59 mutation sites with severe/unknown effects are localized within the 451-residue catalytic domain (Fig. 2B). Not surprisingly, most of the mutation sites either were either buried within the hydrophobic core of the domain, or along

Figure 2. Overall mutation of enzymes. Unless otherwise stated, mutation sites resulting in severe, moderate, and mild forms of the metabolic conditions are highlighted in red, green, and yellow, respectively. Mutations resulting from splicing, deletion, truncation, and frameshifts are not included in the diagrams. **A:** Dimeric GLA (PDB Code 1R47), with Domain I in blue-white, Domain II in pale-green, and galactose in cyan. For clarity, mutation sites are only marked on 1 of the 2 monomers. **B:** Structural domains to the homology model of GAA is divided into the Trefoil Type-P domain (light green), C-terminal domain (light yellow), catalytic domain (light pink), proximal N-terminal domain (light blue), and distal N-terminal domain (light orange). **C:** Structure of the homology model of IDUA (PDB Code 1Y24) may be divided into the catalytic domain (pale green), β -sandwich domain (pale pink), C-terminal chain (cyan), as well as a small α -helix domain (pale yellow). Active site residues E299 and E182 are presented in cyan and red ball-and-stick, respectively. **D:** Known missense mutation sites for IDS are highlighted in red, while purported active site residues D45, D46, K135, and D334 are shown in cyan ball-and-sticks. **E:** Structure of ARSB (PDB Code 1FSU) comprises of a larger N-terminal domain (blue white) and a smaller C-terminal domain (light pink). A putative recognition element is colored in light green. Catalytic C91 residue is presented in cyan ball-and-sticks while disulfide bridges are represented in green sticks. **F:** The structure of ARSA (PDB Code 1AUK) in neutral pH exists in dimeric form. The monomer on the right is color-coded into its structural domains, with the core β -sandwich domain in pale blue and the C-terminal beta sheet domain in pale pink. The loop region corresponding to the putative recognition element of 4-S is highlighted in pale green and marked with an arrow. For clarity, mutation sites (red) and disulfide bridges (green sticks) are only marked in a single monomer. FGly located in the catalytic pocket is marked in cyan. Interactions between the two monomers occur through three direct hydrogen bond contacts (dots) as well as 25 water-mediated hydrogen contacts. Side chain of residues interacting with other monomer via hydrogen bonds are represented in dots [Lukatela et al., 1998]. **G:** Mutation sites on TNSALP resulting in severe hypophosphatasia are highlighted in red, while those resulting in >10% residual enzymatic activity are highlighted in yellow. Active site residues are presented in ball-and-stick. C-terminal segment of TNSALP (indicated with blue arrow) involved in GPI formation is not presented in model. **H:** Homology model of HMBS. Domain I is colored in pale pink, Domain II in pale green, Domain III in pale blue. Dipyrromethane cofactor bound to active site in Domain III is shown in cyan sticks. Extra α -helix not seen in the *E. coli* template (PDB code 1PDA) is marked in yellow. All point mutation sites on HMBS are marked in red.

the β -strands of the TIM barrel core. Of particular note is the of α_4 helix region of the TIM barrel domain, which has eight mutation sites within the stretch of 24 residues. In contrast, mutation sites that resulted in mild/intermediate clinical phenotypes were typically located on loop regions exposed toward the surface. Further, 7 out of 19 mild mutation sites are located on loops of the 216-residue C-terminal β -sheet domain, while the remaining 12 reside within the predominant catalytic domain. A particular mutation site of interest is p.M439K, which although residing on the β -strands that form the active-site pocket, only results in a mild clinical phenotype. Interestingly, a short N-terminal region of the GAA model corresponding to the Trefoil Type P domain seen in NtMGAM contains a mutation site (C103G) that is disruptive enough to result in severe Pompe disease. The exact function of this Trefoil Type P domain in the corresponding section of NtMGAM is unknown [Sim et al., 2008]. However, considering the fact that the first 83 residues of GAA are not included in the alignment and modeling, this putative Trefoil Type P domain in the GAA model could well be a portion of a larger and functionally relevant domain.

Noting the similarity between the overall architecture of GAA's homology model with both eukaryotic and prokaryotic members of the Family 31 glycosyl hydrolases, it would be interesting to explore the localization of the various analogs in an animal model. Further, unlike the previously-discussed lysosomal enzymes that are targeted for delivery into lysosomes via mannose-6-phosphate receptors, the structural and molecular mechanism by which GAA is targeted to muscle cells remains to be elucidated [Scriver, 2001]. Therefore, this information reiterates the necessity of obtaining the 3D structure of the GAA enzyme.

α -L-iduronidase in Mucopolysaccharidosis Type I

Mucopolysaccharidosis Type I (MPS I) is an autosomal recessive lysosomal storage disorder that results in the deficiency of α -L-iduronidase (IDUA), a glycosyl hydrolase necessary for the cleavage of terminal α -iduronic acid residues from glycosaminoglycan, heparin sulfate, and dermatan sulfate. The estimated incidence of MPS I in British Columbia and Northern Ireland is approximately 1:76,000 and 1:300,000 newborns [Scriver, 2001]. Depending on the severity of the mutation, the consequent accumulation of glycoaminoglycans results in a spectrum of multisystemic clinical symptoms, ranging from mild clinical phenotype (Scheie's syndrome), to intermediate phenotype (Hurler/Scheie's syndrome), and the most severe clinical presentation, termed Hurler's syndrome [Rempel et al., 2005]. A recombinant form of IDUA, Aldurazyme[®] (aronidase), has recently been approved for use in the treatment of MPS I [Kakkis et al., 2001].

IDUA belongs to Family 39 of glycosyl hydrolases [Nieman et al., 2003], and is synthesized in a 653-residue precursor form of ~76 kDa, which is subsequently posttranslationally glycosylated and proteolytically cleaved to the mature monomeric form [Myerowitz and Neufeld, 1981]. Recombinant IDUA expressed by CHO cells indicated that all six glycosylation sites present on the IDUA are utilized, with phosphorylated mannose oligosaccharide on glycosylation sites 3 and 6 [Zhao et al., 1997].

Although the 3D structure of IDUA has not been reported thus far [Ruth et al., 2000], the crystal structure of β -D-xylosidase from *Thermoanaerobacterium saccharolyticum* was reported in 2004 [Yang et al., 2004]. A single subunit of the β -D-xylosidase dimer molecule (PDB code 1PX8) was subsequently used as a template for the homology modeling of monomeric IDUA (PDB code

1Y24) [Rempel et al., 2005]. However, due to the significant difference in sequence length (IDUA is 153 residues longer), not all sections of IDUA can be reliably modeled and interpreted from the existing β -D-xylosidase structure. The most noticeable limitation would be the segments between residues 1–35, and 523–653, which could not be reliably modeled into the crystal structure [Rempel et al., 2005]. Nonetheless, the homology model provided an insight to the structural features of Family 39 glycosyl hydrolases, and is the closest current prediction of IDUA's structure.

As with the other lysosomal enzymes described earlier, the catalytic domain of IDUA's model also presents as a $(\beta/\alpha)_8$ TIM barrel motif with the active-site residues similarly predicted to reside at the C-terminal ends of the β -strands [Durand et al., 1997]. This predominant domain is closely associated with a smaller C terminal β -sandwich domain (Fig. 2C). The β -sandwich domain is comprised of two twisted β -sheets made up of a six-stranded antiparallel β -sheet, and a mixed three-stranded β -sheet. Except for the N-terminal β -strand, which forms the first strand of the mixed three-stranded β -sheet, nearly the entire β -sandwich is made up of residues C-terminus to the catalytic domain. A small α -helical domain made up of two antiparallel helices observed in the original β -D-xylosidase structure was also seen in the IDUA model, inserted between β -strands 16 and 17 of the β -sandwich [Yang et al., 2004]. The C-terminal β -sandwich domain is trailed by a short linear chain. However, due to the fact that the native sequence of IDUA has an additional 523–653 segment that was not incorporated into the model, the exact conformation and function of the final C-terminal chain remains ambiguous [Rempel et al., 2005]. Spatial alignment with the corresponding active site of the β -D-xylosidase structure, corroborated with earlier biochemical studies by Nieman et al. [2003], indicate that the active site nucleophile and acid/base residue are E299 and E182, respectively.

Unlike Gaucher disease and Fabry disease, which have more extensive databases of mutations of the enzymes concerned, the number of point mutants documented for IDUA is comparatively lower. Excluding the mutations that resulted in premature termination and truncation of the protein, approximately 46 point-mutation sites have been reported (www.hgmd.cf.ac.uk/ac/index.php) [Rempel et al., 2005; Sugawara et al., 2008b; Terlato and Cox, 2003]. Of these, 32 sites resulted in the severe Hurler's syndrome, seven resulted in the intermediate form of Hurler-Scheie's syndrome, and the remaining seven resulted in the attenuated form of Scheie's syndrome. However, of this list, at least six severe mutation sites, one intermediate mutation site, and one mild mutation site could not be visualized in the available homology model.

Earlier analysis by Rempel et al. [2005] and Sugawara et al. [2008b] correlated the structural implications of certain mutations with the phenotypic-genotypic presentation of the patients. While certain mutations such as p.E182 K, which substitutes the critical active site nucleophile, or p.R89Q, which displaces the activator for the nucleophile, clearly result in defective enzyme and severe clinical outcome, structural consequence to most of the remaining mutations were less conclusive. Nonetheless, it was concluded that most of the severe mutations generally lie within the core of the protein, while the attenuated forms were mostly related to mutations occurring at the molecule's surface exposed regions [Rempel et al., 2005; Sugawara et al., 2008b]. Interestingly, unlike the previously-discussed lysosomal storage diseases, none of the currently known mutation sites of IDUA reside on the critical β -strands that form the catalytic TIM barrel domain of the enzyme's structural model. While the current model provides an

indication of the structural features of IDUA, the presence of several severe mutation sites on the ~130 residues left out from the current homology model is suggestive of critical functionalities of these regions. Further, the homology model was constructed based on the sequence of the precursor 653-residue polypeptide, and not the extensive proteolytically matured form of the enzyme. These observations indicate the need for constant update of mutation database, as well as reiterate the urgent need to confirm the genotypic–phenotypic correlations with precise structural data of the enzyme.

Iduronate-2-Sulfatase in Mucopolysaccharidosis Type II

Iduronate-2-sulfatase (IDS) is a member of the sulfatase family that is responsible for the cleavage of sulfate group from the 2-position of L-iduronic acid present on glycosaminoglycan such as dermatan sulfate and heparan sulfate [Scriver, 2001]. The X-linked recessive condition of mucopolysaccharidosis Type II (Hunter's syndrome) results in the deficiency of IDS, leading to symptoms such as mental retardation, skeletal deformities, as well as multiorgan and tissue involvement that can potentially lead to fatality [Sukegawa-Hayasaka et al., 2006]. The incidence of Hunter's syndrome ranges from 1:132,000 in the United Kingdom, to as high as 1:34,000 in Israel [Scriver, 2001]. As with several other forms of lysosomal storage disorders, Hunter's syndrome may present in a spectrum of phenotypic severity, largely dependent on the mutations involved. The only treatment modality available for Hunter's syndrome is a recombinant form of IDS, Elaprase® (idursulfase), which has been recently approved for use as an ERT [Muenzer et al., 2007].

The full-length gene of IDS encodes for a 550-amino acid polypeptide that is posttranslationally glycosylated and phosphorylated to a ~90-kDa precursor form [Froissart et al., 1995]. A total of eight potential glycosylation sites, at N31, N115, N144, N246, N280, N325, N513, and N537, have been identified on the precursor molecule. While N-glycosylation have been observed at all eight sites and shown to be crucial for the maturation and enzymatic activity of IDS [Millat et al., 1997b], Millat et al. [1997a] reported the sequential elimination of the glycosylation sites on the precursor molecule and concluded that none of these sites were essential for lysosomal targeting. The glycosylated precursor form is then proteolytically processed into a final 45 kDa mature form by proteolytic removal of eight residues after the 25-residue signal sequence, and cleavage at residue 456 to remove a 95-residue C-terminal polypeptide segment [Wilson et al., 1990].

Although the 3D structure of IDS has not been elucidated, several groups have previously reported on the homology modeling of IDS using structural templates of arylsulfatase A, arylsulfatase B, and N-acetylgalactosamine-6S sulfatase [Kim et al., 2003; Sukegawa-Hayasaka et al., 2006]. The model reported by Sukegawa-Hayasaka et al. [2006] consisted of a larger domain with a 10-stranded β -sheet sandwiched between α -helices, as well as a smaller domain made up of a four-stranded antiparallel β -sheet with an orthogonal α -helix. With reference to other lysosomal enzymes, the putative catalytic residues of IDS included D45, D46, C84, K135, and D334; of these, C84 was suggested to be posttranslationally modified to formylglycine during maturation of the enzyme [Schmidt et al., 1995; Sukegawa-Hayasaka et al., 2006]. Through the use of these homology models, the structural consequences of several mutations were correlated to the function of the enzyme [Kim et al., 2003; Sukegawa-Hayasaka et al., 2006]. In Kim et al. [2003], it was suggested that the majority of 20 point mutations examined resided in the periphery of the enzyme

model. In contrast, Sukegawa-Hayasaka et al. [2006] reported that most of the eight mutations examined were associated with either the hydrophobic core of the structure, or in proximity to the catalytic residues. However, the overall distribution of mutations was not described in either of the models.

To date, over 340 mutations have been reported to result in Hunter's syndrome (www.hgmd.cf.ac.uk/ac/index.php) [Froissart et al., 2007]. Of these, 141 missense mutations occurring at 96 point residue sites have been reported. In view of the lack of actual structural data of IDS, but increasing overall structural information on the sulfatase family as a whole, we performed homology modeling for IDS on the Swiss-Model Comparative Protein Modeller Server [Schwede et al., 2003] based on the “first approach mode.” The putative sulfatase from *Bacteroides thetaiotaomicron* (PDB code 3P5Q) was identified by the server as the template with highest level of homology. The RMSD between arylsulfatase B (PDB code 1FSU) used in other homology modeling and the sulfatase from the sulfatase from *Bacteroides thetaiotaomicron* used in the current homology modeling is 2.70 Å. Except for three helices on the flanks of the structure, as well as an additional β -hairpin cumulating to a 6-stranded antiparallel β -sheet with an existing C-terminal β -sheet, the structure of the modeled IDS mirrors closely to the template molecule (Supp. Fig. S4).

Not surprisingly, the majority of the mutations were within the hydrophobic cores of the helix– β -sheet interfaces, or at/in close proximity to the purported active site residues D45, D46, C84, K135, and D334 (Fig. 2D) [Sukegawa-Hayasaka et al., 2006]. Except for D46, which has not been reported as a mutation site, the remaining four purported active site residues are known mutation sites. Further, 22 additional severe mutation sites were noted within a 5-Å radius around these active-site residues. This observation is not unlike the trend of mutations seen in other lysosomal storage diseases described earlier. Interestingly, except for p.N181I and p.L182P that reside on the end of 1 of the 3 additional helices, none of the mutation sites were noted on the additional β -hairpin at the C-terminus or the remaining helices seen on the flanks of the model. It should, however, be noted that proteolytic maturation of the enzyme to the final 45-kDa form requires the removal of the C-terminal 95-residue chain of the precursor molecule. This segment coincides with the six-stranded antiparallel β -sheet at the C-terminus that harbors several mutation sites implicated in Hunter's syndrome.

In conclusion, the homology models offer an insight to the structural features that may be present on the enzyme's architecture. However, the requirement for posttranslational proteolysis and modification also indicate that conformation is critical for the activity of the enzyme. Therefore, until concrete structural data of IDS becomes available, phenotype–genotype correlations should be made with prudence.

N-Acetylgalactosamine-4-Sulfatase in Mucopolysaccharidosis Type VI

Mucopolysaccharidosis Type VI (MPS VI), also known as Maroteaux-Lamy syndrome, is a rare autosomal recessive condition with an incidence of approximately 1:360,000 births, resulting from the deficiency of N-acetylgalactosamine-4-sulfatase (ARSB; previously known as arylsulfatase B). Similar to the 10 other known mammalian sulfatases, ARSB is responsible for the degradation of sulfated glycoaminoglycans, glycoproteins, and glycolipids [Scriver, 2001]. Consequently, deficiency of ARSB leads to intralysosomal accumulation and urinary excretion of dermatan sulfate, chondroitin sulfate, and other glycosaminoglycans.

Similar to the other forms of lysosomal storage disease discussed earlier, MPS VI exhibits a spectrum of clinical presentations, depending on the extent of ARSB deficiency or impairment [Saito et al., 2008]. However, unlike several other types of lysosomal storage diseases, MPS VI does not involve the central nervous system. A recombinant form of N-acetylgalactosamine-4-sulfatase, Naglazyme® (galsulfase), has recently been approved as an ERT for the treatment of MPS VI [Hopwood et al., 2006].

The structure of ARSB was elucidated by Bond et al. [1997], and the ARSB monomer is comprised of an N-terminal domain with 10-stranded β -sheet flanked by a series of helices on both sides, forming an α/β sandwich domain [Bond et al., 1997; Ghosh, 2005]. The comparatively smaller C-terminal domain consists of four-stranded antiparallel β -sheet with an α -helix lying perpendicularly across the solvent exposed surface [Bond et al., 1997]. The precursor ARSB molecule comprises of 533 residues, and maturation of the ARSB molecule requires the glycosylation of the precursor molecule and removal of the first 38 residues forming the signal peptide, followed by subsequent cleavage of the C-terminal end of residues 423 and 465 to generate the three final chains (43 kDa α -chain; 8 kDa β -chain; and 7 kDa γ -chain) of the mature enzyme [Kobayashi et al., 1992].

The active site of ARSB is located in a narrow cleft on the surface of the molecule, formed between the 10-stranded β -sheet core and the flanking helices immediately C-terminus to the β -sheet. Other than C91, which is posttranslationally modified to form the C $^{\alpha}$ -formylglycine (FGly) critical for the catalytic machinery of the enzyme [Bond et al., 1997; Brooks et al., 1995; Recksiek et al., 1998], the active site of ARSB is lined with a series of conserved residues [Bond et al., 1997]. These conserved residues include D53, D54, P93, S94, R95, K145, H147, H242, D300, N301, and K318, which are similarly identified in corresponding regions of other members of the sulfatase family [Waldow et al., 1999]. Residues R95, K145, H147, H242, and K318 are involved in the binding and stabilization of the sulfate ester substrate that binds to the active site. The side chains of D53, D54, D300, and N301 are involved in calcium ion coordination in the metal binding site located at the base of the substrate binding pocket [Bond et al., 1997].

Aside from C91, which is involved in the formation of the active site FGly, the remaining eight cysteine residues are involved in the formation of disulfide bridges that are responsible for linking the three nonidentical chains of the enzyme. C121–C155 and C181–C192 are intrachain disulfide bridges within the α -chain, while C117–C521 and C405–C447 are responsible for bridging α -chain to β -chain and α -chain to γ -chain, respectively [Bond et al., 1997; Kobayashi et al., 1992]. Except for C192, which has been noted to be a mutation site that result in mild MPS VI, all three other cysteine residues involved in intrachain disulfide bridges have not been implicated in the condition. Interestingly, while mutations of C117, C447, or C521 result in severe MPS VI [Karageorgos et al., 2007], mutation of C405 results in an attenuated form of the disease [Jin et al., 1992]. In addition, five N-linked glycosylation sites are present on the mature polypeptide sequence; except for N279, N426, and N366, it is unclear whether N188 and N458 are glycosylated [Bond et al., 1997]. To the best of our knowledge, no mutational studies to examine these glycosylation sites have been reported.

A total of 61 point mutation sites [Karageorgos et al., 2007; Saito et al., 2008] have been highlighted on the structure presented in Figure 2E, with the 46 mutation sites resulting in severe MPS VI and 15 sites resulting in mild MPS VI (www.hgmd.cf.ac.uk/ac/index.php). Mutations such as p.D54N, p.R95Q, p.G144R,

p.W146R/S/L, p.D300E, and p.G302R are situated within the proximity of the active site or metal ion binding sites, and expectedly result in severe forms of MPS VI. Saito et al. [2008] had also examined structural changes associated with several of these mutations and reported that most of the sites that result in severe MPS VI have a low solvent accessible surface area, or are associated with drastic changes near structurally important areas. Although the specific functions of each of the structural domains in the enzyme have not been clearly elucidated, the mutation sites that result in MPS VI are generally distributed throughout the entire structure of the molecule, mostly buried within the hydrophobic cores of each domain.

Arylsulfatase A in Metachromatic Leukodystrophy

Metachromatic leukodystrophy is a rare autosomal recessive metabolic disorder, arising from the deficiency of arylsulfatase A (ARSA). The overall incidence of metachromatic leukodystrophy is generally low, affecting approximately 1:150,000 in France and Germany. However, incidence may be as high as 1:2,500 and 1:6,000 in isolated gene pools such as the Eskimos or Navajo Indians [Scriver, 2001]. The consequent intralysosomal accumulation of the cerebroside 3-sulfate substrate results in the progressive demyelination of the central and peripheral nervous system, leading to severe neurological impairment [Scriver, 2001]. Although a recombinant form of ARSA is currently under clinical trials (Metazym; renamed HGT-1111 in 2008) (<http://clinicaltrials.gov>) as an ERT, no treatment modality is currently available for this fatal metabolic condition.

ARSA is a member of the sulfatase family, and possesses several similarities to other members of this family of lysosomal enzymes. As with most other lysosomal enzymes, posttranslational processing of the 507-residue preprotein includes cleavage of the signal sequence, as well as high-mannose oligosaccharide glycosylation. Glycosylation was observed at N158 and N184, but the third N350 glycosylation site remains unoccupied [Chruszcz et al., 2003]. Despite the fact that ARSA only shares ~50% sequence similarity to other sulfatases, the crystal structure of ARSA showed that the secondary structure, active site topology, and overall conformation are highly similar to those observed in the structure of ARSB, the human sulfatase implicated in MPS VI [Lukatela et al., 1998; von Bulow et al., 2001]. The structure of ARSA also consists of a large core α/β sandwich domain of a 10-stranded β -sheet flanked by several helices, as well as a smaller four-stranded C-terminal β -sheet with a closely associated orthogonal helix. As mentioned earlier, the active site of sulfatases are lined by a series of conserved residues that are important for the formation of the metal-ion coordinating site and the critical FGly moiety. In this case, the magnesium ion is coordinated by D29, D30, D281, and N282. H125 assists in the cleavage of the enzyme-substrate intermediate, while K123, K302, H229, and S150 contribute to the binding of the sulfate substrate [Waldow et al., 1999]. A total of 15 cysteine residues are present in the sequence of ARSA. Of these, 12 are involved in disulfide bond formation, C38 and C294 exist as free thiols, and C69 is posttranslationally modified to FGly of the active site [Lukatela et al., 1998].

Unlike ARSB which exists as monomer, ARSA exists as dimer in neutral pH and further oligomerizes into an octamer in acidic pH. ARSA's orthogonal α -helix is important for the interdigitation of hydrophobic side chains at the dimer interface. Three direct monomer–monomer contacts (H328–P42, S43–S432, and Y439–T408) and 25 water-mediated hydrogen bond contacts were also involved in the formation of the dimer interface. This dimer

interface is mediated by the short β 13 strands (residues 325–327) of the core β -sheet, and four loops including 32–44, 286–300, 406–414, and 430–439. Subsequent oligomerization of the dimers occur via interactions between the long α -helix I (residues 450–469) that lies orthogonal to each monomer, as well as protonation/deprotonation of E424 [Lukatela et al., 1998].

The majority of the 76 missense mutation sites documented to cause metachromatic leukodystrophy were noted to cluster around the active site pocket, or in the loop regions on the surface of the molecule where the active site cleft is situated (Fig. 2F) (www.hgmd.cf.ac.uk/ac/index.php) [Simonaro et al., 2002]. These include p.D29N, p.G32S, p.L68P, p.G122S, p.H227Y, p.P231T, and p.D281Y that will potentially upset the coordination of the metal ion or substrate binding, as well as other buried mutation sites that disrupt the hydrophobic core of the molecule. In contrast, except for p.G119R, p.A212V, p.F219V, and p.T274 M, none of the remaining 72 mutation sites reside in the regions close to the posterior surface of the active site pocket. Interestingly, eight mutation sites (including 2 out of the 4 cysteine residues forming the two disulfide bridges in this segment) were noted in the short segment between residue 152–172, corresponding to the proximity of the putative recognition element in ARSB's structure. This indicates that the conformation of the small putative recognition segment is possibly critical for the activity of ARSA. In contrast, the corresponding segment was noted to be extruded out of the ARSB's surface [Bond et al., 1997], but no known mutation sites have been noted on this segment.

The cohesion of the monomers via the short β -strand and four loops at the dimer interface through the cumulated effect of hydrogen bonds was reported to be very strong [Lukatela et al., 1998]. Therefore, it was not surprising to note a total of 11 mutation points in the dimer interface. In particular, the loop region between residues 286–300 contained 7 of the 11 noted mutations. The ability of these dimers to form octamers in acidic media was also reported to be critical in yielding an active form of the ARSA in the harsh lysosomal environment [Marcao et al., 2003]. For example, the C300F mutant causes the generation of a free thiol at C414, resulting in an unnatural dimerization through disulfide bridge formation with a second monomer, and the consequent severe impairment of octamerization of ARSA [Marcao et al., 2003]. This theory was further supported by the analysis of the p.P426L and p.P425 T mutants, which, although they did not result in major structural alteration, resulted in a failure in oligomerization and the consequent unstable enzymes in lysosomes [Marcao et al., 2003; von Bulow et al., 2002].

Acid Sphingomyelinase in Niemann-Pick Disease

The sphingomyelin phosphodiesterase 1 (SMPD1) gene encodes for two proteins of the same sequence but with distinct posttranslational modifications [Schissel et al., 1996]. The zinc-stimulated secretory form of sphingomyelinase catalyzes the extracellular hydrolysis of sphingomyelin to phosphorylcholine and ceramide, while the second form of acid sphingomyelinase (ASM) hydrolyzes the same substrate intralysosomally [Brady et al., 1966]. A deficiency of ASM causes massive accumulation of sphingomyelin in lysosomes, resulting in a lipid storage disorder termed Niemann-Pick disease that exhibits a broad range of phenotypic presentations. Patients presenting with the severe infantile, neurological form (Type A) of the Niemann-Pick disease deteriorates rapidly and often do not survive beyond the first 3 years of life. Type B Niemann-Pick disease patients present with minimal neurodegeneration and milder overall clinical presenta-

tions [Seto et al., 2004]. The occurrence of Type A Niemann-Pick disease is observed throughout the world, but has been observed to be more frequent in Jewish populations, with an incidence of 1:40,000 newborns, but is likely to be even higher considering the propensity for spontaneous abortions [Scriver, 2001].

The 629-residue protein encoded by the SMPD1 gene consists of a signal peptide [Schuchman et al., 1991], a saponin B domain (residues 87–165) [Ponting, 1994], a phosphodiesterase domain (residues 201–472) [Seto et al., 2004], and a carboxy-terminal region (residues 490–620) that does not possess a specific functional motif [Seto et al., 2004]. The amino acid sequence of ASM possesses six N-glycosylation sites (N88, N177, N337, N397, N505, and N522), in which N505 is not occupied, while the substitution of either of the last two sites inhibited the formation of the active enzyme [Ferlinz et al., 1997]. Similar to other acidic enzymes that are selectively delivered to lysosomes, the mannose-6-phosphate moiety on the glycoprotein recognizes the mannose-6-phosphate receptor on lysosomes for specific delivery into these intracellular organelles [Ferlinz et al., 1997]. Ten putative phosphorylation sites and six disulfide bonds (out of 17 cysteine residues) were also reported [Lee et al., 2007]. Interestingly, while it is not surprising that the disulfide linkages are critical for maintaining the stability and overall conformation of the enzyme, it was unexpected that activation of ASM requires the deletion of the free cysteine residue C629 to acquire full catalytic activity [Qiu et al., 2003]. Finally, a series of proteolytic cleavages at various sites of the precursor molecule is also needed in order to generate the mature ~52-kDa ASM [Hurwitz et al., 1994].

Although the implication of ASM in Niemann-Pick disease had been identified for more than half a century [Brady et al., 1966], the structure of ASM has not been elucidated. In an attempt to better understand the structural characteristic of ASM, Seto et al. homology modeled the phosphodiesterase domain (residues 201–472) of ASM based on purple acid phosphatase (PAP) [Seto et al., 2004]. Due to the low level of homology of ASM to the structure of known human protein, the structural template for homology modeling was identified through multiple sequence alignment search engines and fold recognition methodologies. PAP was ultimately chosen as the template for the phosphodiesterase domain of ASM. However, with only ~15% homology between the two proteins, the remaining sections of ASM could not be modeled. Although over 80 missense mutations have been identified (www.hgmd.cf.ac.uk/ac/index.php), we are not aware of any reports attempting to correlate the structural features of ASM with genotypic presentations of Niemann-Pick disease.

Although there is no currently approved ERT for the treatment of Niemann-Pick disease, the recombinant form of human ASM, is currently undergoing clinical trials (<http://clinicaltrials.gov>). In attempt to target specific tissues affected by the condition, Garnacho et al. [2008] has also recently reported an unconventional method of delivery ASM to the lungs of murine Niemann-Pick models using intercellular adhesion molecule-1-targeted polymer nanocarriers.

Tissue-Nonspecific Alkaline Phosphatase in Hypophosphatasia

Hypophosphatasia is a hereditary metabolic disorder caused by the deficiency of tissue-nonspecific alkaline phosphatase (TNSALP), consequently resulting in defective bone mineralization that can present in various levels of clinical severity ranging from as mild as loss of dentition, to the most severe perinatal form that is nearly always fatal [Fraser, 1957]. As with most other

metabolic disorders, hypophosphatasia has been observed in various populations across the world. However, hypophosphatasia is particularly prevalent in the inbred Mennonite family in Manitoba Canada, with an incidence of 1:2,500 newborns, as compared to the 1:100,000 incidence for the whole of Canada [Scriver, 2001].

TNSALP anchors to the plasma membrane of osteoblasts' matrix vesicles through a posttranslationally added glucosylphosphatidylinositol moiety (GPI), forming a specialized sheltered environment in which inorganic phosphates are hydrolyzed for the formation of hydroxyapatite bone crystals [Anderson, 1995; Jemerson and Low, 1987]. Of the four known alkaline phosphatase isoforms present in man, the placental (PLAP), germ cell (GCAP), and intestinal alkaline phosphatases exhibit tissue specificity and are 90 to 98% homologous to one another. In contrast, the TNSALP, as its name suggests, is found in several tissue types and bears only about 50% homology to the three tissue-specific isoforms [Harris, 1990]. Although the crystal structure of TNSALP has not been elucidated, the crystal structure of PLAP was reported by Le Du et al. [2001] at 1.8 Å, and serves as a representative of this class of closely-related alkaline phosphatases. The 524-residue amino acid sequence of the TNSALP was homology modeled with PLAP as the structural template on the Swiss-Model Comparative Protein Modeller Server [Schwede et al., 2003] on "first approach mode."

Except for a short C-terminal stretch that is not present in the homology model, the model of TNSALP is highly similar to the monomeric subunit of the homodimeric PLAP template with a root mean square deviation (RMSD) of 0.0058 Å (Supp. Fig. S5). The homology model and sequences of TNSALP and PLAP indicate the conservation of potential glycosylation sites, the four metal ion-binding sites, key catalytic residues (corresponding to D110, S111, and R186 in PLAP), as well as the overall conformation of the dimeric interface are conserved in the homology model. Briefly, the core segment of the molecule consists of a mixed 10-stranded β -sheet, flanked by several short α -helices on one surface, and the active site domain on the other. A metal-binding domain (residues 211–289) is seen on the surface of the monomer opposite to the dimer interface. An interfacial "crown domain" is formed by residues 371–431, and has been identified to be crucial for the catalytic activity of TNSALP [Numa et al., 2008]. The N-terminal helix that was described in PLAP to be remote from the rest of the monomer and to interact with the second monomer was also observed in TNSALP, orientated in an identical conformation [Le Du et al., 2001].

More than 150 mutations have been implicated with hypophosphatasia. Of these, 110 mutations represent missense/nonsense mutations occurring at 89 residues (Fig. 2G) (www.hgmd.cf.ac.uk/ac/index.php) [Mornet, 2000]. Mutations at 84 sites resulted in severe hypophosphatasia, while the remaining five sites resulted in TNSALP with more than 10% residual activity. Based on the clustering of mutations around various regions of the TNSALP model, Mornet et al. [2001] proposed five regions of the enzyme that are likely to result in hypophosphatasia. The first region is the active site pocket in which residues critical for catalysis or $\text{Zn}^{2+}/\text{Mg}^{2+}$ coordination reside. These mutations include p.A111T, p.G120R, p.H171R/Y, and p.G334D [Di Mauro et al., 2002]. It would also be expected that mutations to residues in a second region forming the active site valley (p.T134N, p.R136H, p.E191K/G, and p.R450H/C) would result in a similar impact on the enzyme's activity [Mornet et al., 2001]. The third region involves the calcium ion binding site, in which mutations to either the metal coordinating residues (E235, E291, and D306), or residues

in close proximity (R223, R272, P292, and M295) resulted in hypophosphatasia. The final two mutation clusters include the "Crown Domain" region, as well as the dimeric interface. The "Crown Domain," specifically the collagen binding loop (residues 422–452), contains the mutations p.V423A, p.G426C, p.Y436H, p.S445P, p.R450C, and p.R450H that severely impairs the activity of the enzyme [Mornet et al., 2001]. Mutations at the dimeric interface such as p.R391C, p.A399S, p.D406G, and p.N417S, as well as p.A33V and p.A40V on the remote N-terminal helix involved in monomer interaction, were also shown to result in hypophosphatasia [Di Mauro et al., 2002; Mornet et al., 2001].

As described earlier, TNSALP is anchored to the plasma membrane of specialized vesicles in the osteoblast via a GPI moiety located on the C-terminal end of the enzyme. This specific localization of the enzyme hindered effective production and purification of the recombinant enzyme for characterization. In a recent study by Di Mauro et al. [2002], GPI was eliminated to result in a secreted form of the enzyme while leaving the kinetic parameters of the mutant enzymes largely unaffected. This strategy verified that deletion of the GPI anchor is not detrimental to the activity of the enzyme, as well as dramatically improved the production of soluble recombinant enzyme. Further work by Nishioka et al. [2006] demonstrated that replacement of the GPI anchor moiety with a stretch of acidic oligopeptide (L-aspartic acid or L-glutamic acid) resulted in the selective delivery of TNSALP to bone tissues. This strategy further led to the development of a human TNSALP variant with a C-terminal extension of a human immunoglobulin G (IgG) Fc region for rapid purification, as well as a deca-aspartate sequence for targeting to bone tissues. The variant was shown to be effective in preventing skeletal manifestations of hypophosphatasia in TNSALP-null murine models [Millan et al., 2008]. The engineered TNSALP variant (ENB-0040) has recently entered clinical trials (<http://clinicaltrials.gov>), with the potential of becoming one of the first ERTs for a metabolic condition unrelated to lysosomal storage disorder.

Porphobilinogen Deaminase in Acute Intermittent Porphyria

Acute intermittent porphyria (AIP) arises from the deficiency of the enzyme porphobilinogen deaminase (hydroxymethylbilane synthase [HMBS]) involved in the third step of the heme biosynthesis pathway, condensing four porphobilinogen molecules into a linear tetrapyrrole [Scriver, 2001]. The incidence of AIP in most parts of the world is approximately 1:200,000 [Scriver, 2001]. Unlike other metabolic conditions discussed earlier, AIP is the result of autosomal dominant inheritance, and leads to the accumulation of the heme precursors 5-aminolaevulinic acid and porphobilinogen [Wood et al., 1995]. AIP is clinically characterized by acute episodes of gastrointestinal and neuropathic symptoms, while being clinically unremarkable between episodes.

The structure of *E. coli* HMBS was first elucidated by Louie et al. [1002], and was shown to consist of three domains. Domain I and Domain II possess similar overall conformations, each with a mixed β -sheet core with α -helices packed on each face of the strands. In contrast, Domain III consists of an antiparallel β -sheet with one surface covered with three α -helices. The connecting strands and limited number of interdomain non-covalent interactions suggest that the domains might be flexible in nature. The dipyrromethane cofactor, which is assembled from substrate porphobilinogen, binds to C261 of the substrate binding pocket in Domain III through the cleft formed between Domains I

and II of the enzyme [Brownlie et al., 1994]. In addition, the dipyrromethane cofactor interacts with HMBS by extensive hydrogen-bonding and ion-pair interaction with active-site residues. Residues K98, D99, S147, R149, T145, R150, and R173 interact with the side chain of the cofactor C1 ring; while residues R26, S28, D99, R167, G168, L170, and R173 interact with the cofactor C2 ring [Wood et al., 1995].

E. coli HMBS bears 43% sequence homology with human HMBS, and was expected to possess conserved structural features [Brownlie et al., 1994]. A homology model of the 313-residue human HMBS was generated on the Swiss-Model Comparative Protein Modeller Server [Schwede et al., 2003] based on “first approach mode,” and the generated model of human HMBS was found to be remarkably similar to the template *E. coli* HMBS (PDB code 1PDA), with a RMSD of 0.19 Å. All structural features in the template structure were essentially conserved in the modeled human HMBS, with an additional helix (residue 299–325) inserted on the solvent-exposed flank of Domain III (Fig. 2H).

More than 120 mutations have been noted on the human HMBS gene (www.hgmd.cf.ac.uk/ac/index.php). Of these, 65 sites affected by missense point mutations were identified on the created homology model. Nearly all residues identified as critical for interacting with the dipyrromethane cofactor were implicated in causing AIP. D99 was previously suggested to be critically involved in the catalysis, C261Y results in the elimination of the binding site for the cofactor, and R167Q/W results in the accumulation of enzyme-substrate intermediate but halts the progress of the reaction [Wood et al., 1995]. Other mutations interrupting the binding of the dipyrromethane cofactor include p.R26C, p.S28N, p.K98R, p.D99G/N/H, p.R149Q/L, and p.R173W/Q.

The three domains of *E. coli* HMBS were reported to be maintained by a series of interdomain hydrogen bonds and salt bridges [Louie et al., 1992]. These interactions were similarly observed in the homology model of human HMBS, and include hydrogen bonding between E216 and E250 and R116 and T241, as well as ionic interaction between R116 and E250. AIP was reported for patients with mutations at 3 (p.R116Q/W, p.G216D, and p.E250A/K/Q/V) out of the 4 residues involved in the formation of this interdomain hinge. A large number of the remaining mutations resulted from the disruption of the hydrophobic core regions due to steric clashes or drastic volume changes resulting from side chain substitutions (e.g., p.L177R, p.T269I, p.A122G, and p.A31 T) [Louie et al., 1992].

A recombinant form of porphobilinogen deaminase (PorphozymTM) was previously developed by Zymenex (Hillerød, Denmark; www.zymenex.com) for potential use as an enzyme replacement therapeutic for AIP. Although PorphozymTM exhibited the expected enzymatic activity on porphobilinogen, was shown to have a favorable safety profile, and is well-tolerated by patients, the development of the product was hampered by the acute nature of the target condition compounded by issues of patient variability and lack of targeting of the enzyme to the liver. The development of PorphozymTM was subsequently discontinued, and is not likely to resurface until specific targeting of the enzyme can be achieved.

Phenylalanine Hydroxylase and Phenylalanine Ammonia Lyase in Phenylketonuria

Phenylketonuria (PKU) is an autosomal recessive condition, in which the accumulation of phenylalanine in the system results in

aberration of various physiological processes, the most severe of which is the irreversible impairment of physical and intellectual development. PKU has an incidence of approximately 1:15,000 newborns, and is one of the most commonly reported forms of metabolic disorder [Scriver, 2001]. While several mutations to genes relating to the enzymes involved in the biosynthesis and regeneration of the tetrahydrobiopterin (BH₄) cofactor were found to be responsible for PKU state in some patients, the majority of hyperphenylalaninemia patients exhibit classical PKU, which is the direct result of impairment or deficiency of a functional phenylalanine hydroxylase (PAH). Consequently, because of the extensive population screened, PKU is also one of the most well-studied monogenic metabolic conditions.

The human liver PAH is a 452-residue protein that exists in a pH-dependent equilibrium of nonheme homodimers and homotetramers [Martinez et al., 1995]. Although the structure of the full-length PAH has not been solved, high-resolution crystal structures for several truncated variants of PAH, as well as a composite model of the full-length PAH, are available [Erlandsen et al., 1997; Fusetti et al., 1998; Kobe et al., 1999]. Each PAH monomer has an approximate molecular weight of 50 kDa and comprises three structural domains: residues 1–142 form the N-terminal regulatory domain, residues 143–410 form the catalytic domain, and the C-terminal tetramerization domain is made up of residues 411–452 [Kobe et al., 1999]. The α/β -sandwich motif regulatory domain consists of four-stranded antiparallel sheets flanked by two helices and the catalytic domain. The catalytic domain consists of a “basket-like” arrangement of 14 helices and eight β -sheets, with 28 out of the 34 residues lining the active site being hydrophobic residues. A key component to the active site is a catalytic iron that is coordinated by H285, H290, and E330, as well as three water molecules. The C-terminal tetramerization domain is made up of a two-stranded antiparallel sheet followed by a long α -helix [Erlandsen and Stevens, 1999].

To date, nearly 600 documented mutations resulting in PKU have been reported (www.hgmd.cf.ac.uk/ac/index.php; www.pahdb.mcgill.ca). Of these mutations, 365 missense mutations occur at 216 sites on the protein sequence (Fig. 3). Based on the structural information of the truncated variants of PAH, the most prevalent single-point mutations resulting in PKU were attributed to mutations that disrupt the active site, critical structural residues, residues involved in the interdomain or dimer/tetramer interfaces, as well as those involving the N-terminal autoregulatory sequence. As these information have been extensively discussed in several earlier reviews, they are not detailed herein [Erlandsen et al., 2003; Erlandsen and Stevens, 1999; Pey et al., 2007].

Considering the fact that the offending substrate in PKU is phenylalanine present in the patient's plasma, PKU appears to be readily amendable to ERT that has been extremely successful in several other metabolic disorders. Various groups had attempted the delivery of PAH by strategies such as PEGylation, as well as fusion with a transduction domain [Eavri and Lorberboum-Galski, 2007; Gamez et al., 2004]. However, the complex regulatory requirements of PAH, overall instability of the molecule, and the obligate requirement for a cofactor makes it a challenging candidate for development into an ERT [Sarkissian et al., 1999]. An alternative approach to the alleviation of classical PKU is enzyme substitution therapy with phenylalanine ammonia lyase (PAL; EC 4.3.1.24) derived from bacterial or yeast origins.

PALs convert L-Phe into the harmless metabolite trans-cinnamic acid, which is converted into hippurate and readily excreted in the urine [Hoskins et al., 1984]. Unlike PAH, PALs are



Figure 3. Structure of human phenylalanine hydroxylase model. A composite model was derived based on the structures of catalytic/regulatory domains of PAH (PDB 1PHZ) and catalytic/tetramerization domains (PDB 2PAH) [Erlandsen et al., 1997; Fusetti et al., 1998; Kobe et al., 1999]. The catalytic domain is presented in green, while the regulatory and tetramerization domains are colored in light pink and light blue, respectively. The catalytic iron of PAH's active site is represented by the blue sphere. For clarity, only mutation sites on a single monomer are presented. Mutation sites resulting in unknown or severe clinical phenotypes are marked in red. Mutation sites resulting in mild clinical phenotypes are marked in yellow.

independent of obligatory cofactors for its catalytic activity and are devoid of any specific regulatory controls. Deamination of L-Phe relies on a 4-methylidene-imidazole-5-one (MIO) prosthetic group that is produced by the posttranslational condensation of a sequential Ala-Ser-Gly triad within the enzyme [Sarkissian et al., 1999; Wang et al., 2005]. Administration of *Rhodospirium toruloides* PAL (RtPAL), derived from the yeast *Rhodospirium toruloides*, to murine PKU models have already demonstrated reduction in plasma Phe levels, reinforcing the feasibility of utilizing PAL as a potential enzyme therapeutic for the treatment of PKU [Hoskins et al., 1984; Sarkissian et al., 1999]. Recently, *Anabaena variabilis* PAL (AvPAL), derived from cyanobacterium *Anabaena variabilis*, was shown to have similar biochemical characteristics with superior thermal stability and protease resistance as compared to RtPAL [Moffitt et al., 2007; Wang et al., 2008]. By optimizing AvPAL with PEGylation, we previously

demonstrated that PEG-AvPAL exhibits reduced immunogenicity and improved pharmacokinetic characteristics that are suitable for development into a potential enzyme therapeutic [Sarkissian et al., 2008]. This molecule has recently entered into phase II clinical trials for the treatment of PKU (<http://clinicaltrials.gov>), and may potentially be the first enzyme substitution therapeutic developed for the treatment of hereditary metabolic conditions.

Conclusions

In this review, we have discussed the structural information of the enzymes used for ERT in 11 hereditary conditions. Aside from the catalytic domain and active site pocket of the enzymes, the functions of most of the structural domains of these enzymes are not well characterized. Further, mutation sites that render the enzymes inactive are generally widely distributed throughout the

various domains of the enzyme. Due to likely compensatory variations of residues involved in the intramolecular interactions, not all of the effects of point mutants could be adequately rationalized. Nonetheless, point mutations that abolish biological activity of these enzymes may be generally classified into the following types: (1) Disruption of the hydrophobic core. Several of the lysosomal enzymes share common structural frameworks. For example, lysosomal hydrolases such as glucocerebrosidase, α -galactosidase, and acid α -glucosidase possess a conserved $(\beta/\alpha)_8$ TIM barrel catalytic domain [Durand et al., 1997], while the sulfatases such as iduronate-2-sulfatase, N-acetylgalactosamine-4-sulfatase, and arylsulfatase A possess an α/β sandwich catalytic domain [Ghosh, 2005]. In nearly all cases, disruptions to the hydrophobic cores of the catalytic domains or interfaces of the domains were noted to result in severe phenotypes [Dvir et al., 2003; Garman, 2007; Louie et al., 1992; Lukatela et al., 1998; Saito et al., 2008; Sugawara et al., 2008b; Sukegawa-Hayasaka et al., 2006; Tajima et al., 2007]. (2) Disruption of the multimer interface. Dimer formation was also noted to be crucial for the biological activity of TNSALP [Mornet et al., 2001], and both dimer and octamer formation were important for a stable and functional arylsulfatase A [Marcao et al., 2003; von Bulow et al., 2002]. (3) Disruption to functionally relevant regions. Disruptions to active-site residues of the enzymes invariably resulted in abolishment of catalytic activity of the enzymes. In addition, disruptions to conserved residues in proximity to the active site pocket, such as the case of the sulfatases, or metal binding sites of sulfatases and TNSALP were also detrimental to the enzymes. (4) Substitutions to/from conformationally constraining residues such as proline or cysteine frequently resulted in the disruption in overall conformation of the enzyme. (5) N-glycosylation is a critical form of posttranslational modification that is involved in various stages of biosynthetic maturation of lysosomal enzymes. While not all N-glycosylation sites are necessarily absolutely conserved across the various types of lysosomal enzymes, substitutions of these carbohydrate moieties are frequently detrimental to the enzymes' catalytic activities, lysosomal delivery, as well as folding/maturation pathways. (6) Premature degradation of partially misfolded enzymes. Several of these enzymes require proteolytic maturation steps to attain the fully-functional mature protein. In certain cases, mutations to the precursor sequence results in misfolding and rapid degradation of the misfolded protein. For example, p.P86L, p.S333L, p.S349I, p.R468Q, and p.R468L mutations to iduronate-2-sulfatase resulted in severe phenotypes with only 73 to 76 kDa precursors detected [Sukegawa-Hayasaka et al., 2006]. Through the use of monoclonal antibodies to epitopes expressed at various stages of arylsulfatase A biosynthesis, Poeppel et al. [2005] also examined the correlation of premature degradation with misfolding of proteins, and suggested that metabolic conditions arising from premature degradation of partially misfolded enzymes could represent a form of "conformational disease" [Carrell and Lomas, 1997].

With the advancement of structural biology in the recent decades, critical information on the structure-function relationships of these enzymes is becoming increasingly available. In this article, we have reviewed how perturbations to specific structural features of these enzymes resulted in the clinical states of the metabolic conditions concerned. However, except for selected literature reporting the usage of these enzymes in the clinical settings [Al Sawaf et al., 2008; Charrow, 2009; Hughes et al., 2008; Pastores, 2008; van der Ploeg and Reuser, 2008; Wraith, 2008], information correlating the structural features of the enzymes to

the delivery of the enzyme in the in vivo system, as well as the consequent safety and efficacy of the final therapeutic enzyme highlights direction for significant improvements.

Future Directions

ERT for lysosomal storage diseases marked a major milestone in the treatment of metabolic conditions. However, advancement for this class of therapeutic enzymes has been hampered by a series of hurdles. First, the requirement for posttranslational proteolytic processing of several lysosomal enzymes suggests that a conformational change is required for optimal substrate binding/access and catalysis. Although structural data is now available for several of these therapeutic enzymes, the unknown functions of the various structural domains have made it particularly challenging for enzyme redesign. Further, the 3D structures for over one-half of the enzymes currently in clinical use remain to be elucidated (Table 1). Second, analysis of the structural and biochemical correlation of the mutants indicate a broad distribution of mutations throughout the various domains of the structures of the native enzymes, making structural modification an uphill task.

The next hurdle to development of ERT for lysosomal storage diseases concerns the transport of these enzymes into lysosomes. The need for organelle-specific delivery of the enzymes adds a further barrier to the utilization of these enzymes. In specific subsets of lysosomal diseases such as Pompe disease, the target skeletal muscles forms a large portion of body mass, and are separated from the circulatory system by the blood-muscle layer [McVie-Wylie et al., 2008], and the inability to target the exogenously administered enzyme can result in losses of over 80% of the administered dose [Geel et al., 2007]. The success of ERT is largely dependent on delivery of a sufficient amount of enzymes to the target tissue, and the inability of these therapeutic enzymes to cross the blood-brain-barrier seriously limits the applications of these exogenous enzymes in treating the most severe forms of several lysosomal storage disorders that are associated with neurological symptoms.

Despite that fact that the first ERT has been utilized for more than a decade, no form of redesign of native enzymes or usage of analogs from alternative sources has been reported. Although the use of GAA derived from *Aspergillus niger* has been reported for the treatment of MPS VI, this preliminary attempt called for high doses of recombinant enzymes over long durations [Geel et al., 2007]. This approach was further compromised by the risk of immunogenicity and the lack of guarantee for lysosomal delivery. Considering the fact that only a small amount of enzyme or residual enzymatic activity is needed for preventing disease progression in several of these conditions [Doebber et al., 1982], a more pragmatic approach would be to optimize enzyme targeting, as well as minimize premature degradation of partially-active mutant enzymes. In another work, Silverman et al. [2001] rationally mutated 142 residues of the 182 examined residues in the 250-residue $(\beta/\alpha)_8$ TIM barrel catalytic domain of a bacterial triosephosphate enzyme to be comprised of solely of a seven-letter amino-acid alphabet. Interestingly, such comparatively drastic substitution did not result in significant loss of biological activity of the enzyme. Such an example of advancement in protein engineering promises a future of structural insights and potential reengineering of lysosomal enzymes.

A possible approach to improving these therapeutic enzymes would perhaps be the manipulation of specificity of enzyme delivery. Glycosylation of the mature enzymes has been noted in

all of the above-discussed lysosomal enzymes [Berg-Fussman et al., 1993; Kobayashi et al., 1992; Millat et al., 1997b; Scriver, 2001; Zhao et al., 1997] for selective delivery of the enzymes into lysosomes. The successful glycoengineering of acid α -glucosidase with higher level of mannose-6-phosphate [McVie-Wylie et al., 2008], and exposing of the mannose core of glucocerebrosidase [Doebber et al., 1982] for targeting to the lysosomal mannose-6-phosphate receptor indicate that a similar strategy may be applied to other exogenous enzymes utilized for treatment of other lysosomal storage diseases. Glycosylation at specific sites, the nature of carbohydrate moieties and phosphorylation states, and putative positive influences of certain mutations such as N19D on the activation of glucocerebrosidase [Berg-Fussman et al., 1993] suggest that manipulation of glycosylation state might be a possible approach in optimizing enzyme function and specificity. This is especially valid since many lysosomal enzymes share common synthesis and processing pathways, involve extensive posttranslational modification such as glycosylation, acquisition of phosphomannosyl residues for lysosomal targeting via mannose-6-phosphate receptor, and proteolytic cleavage [Kornfeld, 1986]. Furthermore, glycosylation has been known to improve the overall stability of proteins [Sola and Griebenow, 2009], and have been utilized to improve the circulation half-life and activity of erythropoietin analogues [Sinclair and Elliott, 2005]. Several other alternative approaches independent of the mannose-6-phosphate receptor pathway have also been attempted in improving the “targeting” and delivery of therapeutic enzymes to specific delivery sites. For example, the use of a stretch of acidic oligopeptide dramatically enhanced the selective delivery of TNSALP to bone tissues [Nishioka et al., 2006]. Fusion of IGF-II fragments to the β -glucuronidase improves the delivery of enzyme to storage in glomerular podocytes and osteoblasts [LeBowitz et al., 2004], while attachment of the HIV Tat protein transduction domain to the same enzyme improved delivery to renal tubules, retina, and bone [Orii et al., 2005].

Although intravenous administration of appropriate exogenous enzymes can halt or alleviate systemic symptoms to several of the lysosomal storage diseases, the most severe forms of lysosomal storage diseases are frequently associated with central nervous system involvement that is not responsive to the current treatment modality. Although intrathecal or intraventricular administrations of certain therapeutic enzymes such as α -L-iduronidase or glucocerebrosidase are already under investigation [Dickson et al., 2007; Lonser et al., 2007; Pastores, 2008], these techniques may not be sufficient in providing adequate doses of the

administered enzyme to the large areas of the brain due to a dilution effect. Lonser et al. [2007] recently reported an image-guided convective-enhanced direct delivery of therapeutic proteins to afflicted tissues of the brain. Despite the fact that this method holds the potential for direct delivery of enzymes to the affected tissues in the brain, this method is invasive in nature and more studies are required to prove the feasibility for use as a long-term therapeutic option. An alternative approach that has been recently reported involves the fusion of IDUA with a chimeric monoclonal antibody to the human insulin receptor to transport a fusion protein across the blood-brain-barrier via a “Trojan horse” approach [Boado et al., 2008].

An important upcoming therapeutic approach is the use of low-molecular-weight chemical entities. These include synthetic cofactors such as sapropterin dihydrochloride (Kuvan[®]) for phenylketonuria patients who are responsive to the obligatory cofactor tetrahydrobiopterin [Levy et al., 2007], as well as chemical chaperones that interact with folding intermediates and facilitate folding into the correct conformation [Kolter and Wendeler, 2003]. Such an approach would be particularly useful when ERT is ineffective in providing neurological benefits for severe forms of lysosomal storage disorders. To date, at least six different small chemical pharmacological chaperones/low-molecular-weight chemicals have been identified to facilitate the refolding of mutant proteins or for substrate reduction therapy by inhibiting glycosphingolipid biosynthesis (Table 3). Unlike the therapeutic enzymes, the distinct advantage of these chemical entities lies in the potential of crossing the blood-brain-barrier and interacting with mutant enzymes trapped in misfolded conformations, thereby preventing premature degradation by the endoplasmic reticulum, and potentially enhancing the folding, stability, and any remnant activity of the mutant enzyme [Brady, 2006]. This characteristic is of particular importance since the more severe neuronopathic forms of several lysosomal conditions are typically unresponsive to ERT.

The understanding of the structure–function relationship of known mutations will be instrumental in the determination of the correct therapeutic approach. For example, mutations that destabilize the protein may be alleviated by providing chaperone therapy, while mutations that led to ablation of enzymatic activity will clearly be more responsive to ERT. By correlating the degree of structural aberration of the mutation to α -galactosidase, Shin et al. [2008] recently mapped the predictability of response of mutated GAA to pharmacological chaperones, reinforcing the feasibility of this strategy. While it is uncertain which of these

Table 3. Pharmacological Chaperones and Low-Molecular-Weight Chemicals

Chemical	Trade name/code	Therapeutic application, mode of action	Reference
Migalastat hydrochloride	Amigal TM	Fabry disease: pharmacological chaperone	Benjamin et al. [2009]
Isofagomine tartrate	Plicera TM	Gaucher disease: pharmacological chaperone	Lieberman et al. [2007]
1-deoxynojirimycin hydrochloride	HGT-3510 (Amicus Therapeutics)	Pompe disease: pharmacological chaperone	Okumiyu et al. [2007]
Miglustat	Zavesca [®]	Gaucher disease: substrate reduction therapy; also demonstrated positive effect in other lysosomal storage disorders such as Tay-Sachs disease	Bembi et al. [2006]; Lachmann and Platt [2001]; Pastores [2006]
Pyrimethamine	PYR	GM2 gangliosidosis: pharmacological chaperone	Maegawa et al. [2007]
Sapropterin dihydrochloride	Kuvan [®]	Phenylketonuria: synthetic cofactor	Levy et al. [2007]

non-ERT approaches will ultimately prove to provide the ideal therapeutic modality for alleviating lysosomal storage diseases, it is clear that structural information for the enzymes concerned would be pivotal for the rationale design of superior therapeutics.

Acknowledgments

We are grateful to Dr. Jeremiah Stanson Joseph for his invaluable advice on homology modeling, and Angela Walker for manuscript preparation. T.S. Kang is supported by the National University of Singapore Overseas-Postdoctoral Fellowship.

References

- Al Sawaf S, Mayatepek E, Hoffmann B. 2008. Neurological findings in Hunter disease: pathology and possible therapeutic effects reviewed. *J Inher Metab Dis* 31:473–480.
- Amalfitano A, Bengur AR, Morse RP, Majure JM, Case LE, Veerling DL, Mackey J, Kishnani P, Smith W, McVie-Wylie A, and others. 2001. Recombinant human acid alpha-glucosidase enzyme therapy for infantile glycogen storage disease type II: results of a phase I/II clinical trial. *Genet Med* 3:132–138.
- Anderson HC. 1995. Molecular biology of matrix vesicles. *Clin Orthop Relat Res* 266–280.
- Ashley GA, Shabbeer J, Yasuda M, Eng CM, Desnick RJ. 2001. Fabry disease: twenty novel alpha-galactosidase A mutations causing the classical phenotype. *J Hum Genet* 46:192–196.
- Ashton-Prolla P, Tong B, Shabbeer J, Astrin KH, Eng CM, Desnick RJ. 2000. Fabry disease: twenty-two novel mutations in the alpha-galactosidase A gene and genotype/phenotype correlations in severely and mildly affected hemizygotes and heterozygotes. *J Invest Med* 48:227–235.
- Barton NW, Brady RO, Dambrosia JM, Di Bisceglie AM, Doppelt SH, Hill SC, Mankin HJ, Murray GJ, Parker RI, Argoff CE, and others. 1991. Replacement therapy for inherited enzyme deficiency—macrophage-targeted glucocerebrosidase for Gaucher's disease. *N Engl J Med* 324:1464–1470.
- Basu A, Prenc E, Garrett K, Glew RH, Ellingson JS. 1985. Comparison of N-acyl phosphatidylethanolamines with different N-acyl groups as activators of glucocerebrosidase in various forms of Gaucher's disease. *Arch Biochem Biophys* 243:28–34.
- Bembi B, Marchetti F, Guerci VI, Ciana G, Addobbati R, Grasso D, Barone R, Cariati R, Fernandez-Guillen L, Butters T, Pittis MG. 2006. Substrate reduction therapy in the infantile form of Tay-Sachs disease. *Neurol* 66:278–280.
- Benjamin ER, Flanagan JJ, Schilling A, Chang HH, Agarwal L, Katz E, Wu X, Pine C, Wustman B, Desnick RJ, Lockhart DJ, Valenzano KJ. 2009. The pharmacological chaperone 1-deoxygalactonojirimycin increases alpha-galactosidase A levels in Fabry patient cell lines. *J Inher Metab Dis* 32:424–440.
- Berg-Fussman A, Grace ME, Ioannou Y, Grabowski GA. 1993. Human acid beta-glucosidase. N-glycosylation site occupancy and the effect of glycosylation on enzymatic activity. *J Biol Chem* 268:14861–14866.
- Beutler E, Gelbart T. 1993. Gaucher disease mutations in non-Jewish patients. *Br J Haematol* 85:401–405.
- Beutler E, Gelbart T, Scott CR. 2005. Hematologically important mutations: Gaucher disease. *Blood Cells Mol Dis* 35:355–364.
- Beutler E. 2006a. Gaucher disease: multiple lessons from a single gene disorder. *Acta Paediatr Suppl* 95:103–109.
- Beutler E. 2006b. Lysosomal storage diseases: natural history and ethical and economic aspects. *Mol Genet Metab* 88:208–215.
- Blanch LC, Meaney C, Morris CP. 1996. A sensitive mutation screening strategy for Fabry disease: detection of nine mutations in the alpha-galactosidase A gene. *Hum Mutat* 8:38–43.
- Blaydon D, Hill J, Winchester B. 2001. Fabry disease: 20 novel GLA mutations in 35 families. *Hum Mutat* 18:459.
- Boado RJ, Zhang Y, Zhang Y, Xia CF, Wang Y, Pardridge WM. 2008. Genetic engineering of a lysosomal enzyme fusion protein for targeted delivery across the human blood-brain barrier. *Biotechnol Bioeng* 99:475–484.
- Bond CS, Clements PR, Ashby SJ, Collyer CA, Harrop SJ, Hopwood JJ, Guss JM. 1997. Structure of a human lysosomal sulfatase. *Structure* 5:277–289.
- Brady RO, Kanfer JN, Shapiro D. 1965. Metabolism of glucocerebrosides. II. Evidence of an enzymatic deficiency in Gaucher's disease. *Biochem Biophys Res Commun* 18:221–225.
- Brady RO, Kanfer JN, Mock MB, Fredrickson DS. 1966. The metabolism of sphingomyelin. II. Evidence of an enzymatic deficiency in Niemann-Pick disease. *Proc Natl Acad Sci USA* 55:366–369.
- Brady RO. 2006. Enzyme replacement for lysosomal diseases. *Annu Rev Med* 57:283–296.
- Brooks DA, Robertson DA, Bindloss C, Litjens T, Anson DS, Peters C, Morris CP, Hopwood JJ. 1995. Two site-directed mutations abrogate enzyme activity but have different effects on the conformation and cellular content of the N-acetylgalactosamine 4-sulphatase protein. *Biochem J* 307(Pt 2):457–463.
- Brownlie PD, Lambert R, Louie GV, Jordan PM, Blundell TL, Warren MJ, Cooper JB, Wood SP. 1994. The three-dimensional structures of mutants of porphobilinogen deaminase: toward an understanding of the structural basis of acute intermittent porphyria. *Protein Sci* 3:1644–1650.
- Brumshtein B, Wormald MR, Silman I, Futerman AH, Sussman JL. 2006. Structural comparison of differently glycosylated forms of acid-beta-glucosidase, the defective enzyme in Gaucher disease. *Acta Crystallogr D Biol Crystallogr* 62 (Pt 12):1458–1465.
- Carrell RW, Lomas DA. 1997. Conformational disease. *Lancet* 350:134–138.
- Charrow J. 2009. Enzyme replacement therapy for Gaucher disease. *Expert Opin Biol Ther* 9:121–131.
- Chruszcz M, Laidler P, Monkiewicz M, Ortlund E, Lebiada L, Lewinski K. 2003. Crystal structure of a covalent intermediate of endogenous human arylsulfatase A. *J Inorg Biochem* 96:386–392.
- Davies JB, Eng CM, Hill JA, Malcolm S, MacDermot K, Winchester B, Desnick RJ. 1996. Fabry disease: fourteen alpha-galactosidase A mutations in unrelated families from the United Kingdom and other European countries. *Eur J Hum Genet* 4:219–224.
- de Fost M, Aerts JM, Hollak CE. 2003. Gaucher disease: from fundamental research to effective therapeutic interventions. *Neth J Med* 61:3–8.
- Di Mauro S, Manes T, Hesse L, Kozlenkov A, Pizauro JM, Hoylaerts MF, Millan JL. 2002. Kinetic characterization of hypophosphatasia mutations with physiological substrates. *J Bone Miner Res* 17:1383–1391.
- Dickson P, McEntee M, Vogler C, Le S, Levy B, Peinovich M, Hanson S, Passage M, Kakkis E. 2007. Intrathecal enzyme replacement therapy: successful treatment of brain disease via the cerebrospinal fluid. *Mol Genet Metab* 91:61–68.
- Doebber TW, Wu MS, Bugianesi RL, Ponpipom MM, Furbish FS, Barranger JA, Brady RO, Shen TY. 1982. Enhanced macrophage uptake of synthetically glycosylated human placental beta-glucocerebrosidase. *J Biol Chem* 257:2193–2199.
- Durand P, Lehn P, Callebaut I, Fabrega S, Henrissat B, Mornon JP. 1997. Active-site motifs of lysosomal acid hydrolases: invariant features of clan GH-A glycosyl hydrolases deduced from hydrophobic cluster analysis. *Glycobiology* 7:277–284.
- Dvir H, Harel M, McCarthy AA, Tokar L, Silman I, Futerman AH, Sussman JL. 2003. X-ray structure of human acid-beta-glucosidase, the defective enzyme in Gaucher disease. *EMBO Rep* 4:704–709.
- Eavri R, Lorberbaum-Galski H. 2007. A novel approach for enzyme replacement therapy. The use of phenylalanine hydroxylase-based fusion proteins for the treatment of phenylketonuria. *J Biol Chem* 282:23402–23409.
- Eng CM, Resnick-Silverman LA, Niehaus DJ, Astrin KH, Desnick RJ. 1993. Nature and frequency of mutations in the alpha-galactosidase A gene that cause Fabry disease. *Am J Hum Genet* 53:1186–1197.
- Eng CM, Niehaus DJ, Enriquez AL, Burgert TS, Ludman MD, Desnick RJ. 1994. Fabry disease: twenty-three mutations including sense and antisense CpG alterations and identification of a deletion hot-spot in the alpha-galactosidase A gene. *Hum Mol Genet* 3:1795–1799.
- Eng CM, Ashley GA, Burgert TS, Enriquez AL, D'Souza M, Desnick RJ. 1997. Fabry disease: thirty-five mutations in the alpha-galactosidase A gene in patients with classic and variant phenotypes. *Mol Med* 3:174–182.
- Eng CM, Guffon N, Wilcox WR, Germain DP, Lee P, Waldek S, Caplan L, Linthorst GE, Desnick RJ. 2001. Safety and efficacy of recombinant human alpha-galactosidase A—replacement therapy in Fabry's disease. *N Engl J Med* 345:9–16.
- Erlandsen H, Fusetti F, Martinez A, Hough E, Flatmark T, Stevens RC. 1997. Crystal structure of the catalytic domain of human phenylalanine hydroxylase reveals the structural basis for phenylketonuria. *Nat Struct Biol* 4:995–1000.
- Erlandsen H, Stevens RC. 1999. The structural basis of phenylketonuria. *Mol Genet Metab* 68:103–125.
- Erlandsen H, Patch MG, Gamez A, Straub M, Stevens RC. 2003. Structural studies on phenylalanine hydroxylase and implications toward understanding and treating phenylketonuria. *Pediatrics* 112(6 Pt 2):1557–1565.
- Ernst HA, Lo Leggio L, Willemoes M, Leonard G, Blum P, Larsen S. 2006. Structure of the *Sulfolobus solfataricus* alpha-glucosidase: implications for domain conservation and substrate recognition in GH31. *J Mol Biol* 358: 1106–1124.
- Fabrega S, Durand P, Codogno P, Bauvy C, Delomenie C, Henrissat B, Martin BM, McKinney C, Ginns EI, Mornon JP, Lehn P. 2000. Human glucocerebrosidase: heterologous expression of active site mutants in murine null cells. *Glycobiology* 10:1217–1224.
- Ferlinz K, Hurwitz R, Mocall H, Lansmann S, Schuchman EH, Sandhoff K. 1997. Functional characterization of the N-glycosylation sites of human acid sphingomyelinase by site-directed mutagenesis. *Eur J Biochem* 243:511–517.

- Frandsen TP, Svensson B. 1998. Plant alpha-glucosidases of the glycoside hydrolase family 31. Molecular properties, substrate specificity, reaction mechanism, and comparison with family members of different origin. *Plant Mol Biol* 37:1–13.
- Fraser D. 1957. Hypophosphatasia. *Am J Med* 22:730–746.
- Friedman B, Vaddi K, Kreston G, Mahon E, Cataldo JR, McPherson JM. 1999. A comparison of the pharmacological properties of carbohydrate remodeled recombinant and placental-derived beta-glucocerebrosidase: implications for clinical efficacy in treatment of Gaucher disease. *Blood* 93:2807–2816.
- Froissart R, Millat G, Mathieu M, Bozon D, Maire I. 1995. Processing of iduronate 2-sulphatase in human fibroblasts. *Biochem J* 309(Pt 2):425–430.
- Froissart R, Da Silva IM, Maire I. 2007. Mucopolysaccharidosis type II: an update on mutation spectrum. *Acta Paediatr Suppl* 96:71–77.
- Furbish FS, Steer CJ, Krett NL, Barranger JA. 1981. Uptake and distribution of placental glucocerebrosidase in rat hepatic cells and effects of sequential deglycosylation. *Biochim Biophys Acta* 673:425–434.
- Fusetti F, Erlandsen H, Flatmark T, Stevens RC. 1998. Structure of tetrameric human phenylalanine hydroxylase and its implications for phenylketonuria. *J Biol Chem* 273:16962–16967.
- Galanos J, Nicholls K, Grigg L, Kiers L, Crawford A, Becker G. 2002. Clinical features of Fabry's disease in Australian patients. *Intern Med J* 32:575–584.
- Gamez A, Wang L, Straub M, Patch MG, Stevens RC. 2004. Toward PKU enzyme replacement therapy: PEGylation with activity retention for three forms of recombinant phenylalanine hydroxylase. *Mol Ther* 9:124–129.
- Garman SC, Garboczi DN. 2002. Structural basis of Fabry disease. *Mol Genet Metab* 77:3–11.
- Garman SC, Garboczi DN. 2004. The molecular defect leading to Fabry disease: structure of human alpha-galactosidase. *J Mol Biol* 337:319–335.
- Garman SC. 2007. Structure-function relationships in alpha-galactosidase A. *Acta Paediatr Suppl* 96:6–16.
- Garnacho C, Dhami R, Simone E, Dziubla T, Leferovich J, Schuchman EH, Muzykantov V, Muro S. 2008. Delivery of acid sphingomyelinase in normal and Niemann-Pick disease mice using intercellular adhesion molecule-1-targeted polymer nanocarriers. *J Pharmacol Exp Ther* 325:400–408.
- Geel TM, McLaughlin PM, de Leij LF, Ruiters MH, Niezen-Koning KE. 2007. Pompe disease: current state of treatment modalities and animal models. *Mol Genet Metab* 92:299–307.
- Germain DP, Poenaru L. 1999. Fabry disease: identification of novel alpha-galactosidase A mutations and molecular carrier detection by use of fluorescent chemical cleavage of mismatches. *Biochem Biophys Res Commun* 257:708–713.
- Ghosh D. 2005. Three-dimensional structures of sulfatases. *Methods Enzymol* 400:273–293.
- Glidden BL, Hopwood JJ. 2004. Enzyme-replacement therapy from birth delays the development of behavior and learning problems in mucopolysaccharidosis type IIIA mice. *Pediatr Res* 56:65–72.
- Grabowski GA. 1993. Gaucher disease. Enzymology, genetics, and treatment. *Adv Hum Genet* 21:377–441.
- Grace ME, Newman KM, Scheinker V, Berg-Fussman A, Grabowski GA. 1994. Analysis of human acid beta-glucosidase by site-directed mutagenesis and heterologous expression. *J Biol Chem* 269:2283–2291.
- Harris H. 1990. The human alkaline phosphatases: what we know and what we don't know. *Clin Chim Acta* 186:133–150.
- Henrissat B, Bairoch A. 1993. New families in the classification of glycosyl hydrolases based on amino acid sequence similarities. *Biochem J* 293(Pt 3):781–788.
- Hermans MM, Kroos MA, van Beemelen J, Oostra BA, Reuser AJ. 1991. Human lysosomal alpha-glucosidase. Characterization of the catalytic site. *J Biol Chem* 266:13507–13512.
- Hermans MM, Wisselaar HA, Kroos MA, Oostra BA, Reuser AJ. 1993. Human lysosomal alpha-glucosidase: functional characterization of the glycosylation sites. *Biochem J* 289(Pt 3):681–686.
- Hers HG. 1963. alpha-Glucosidase deficiency in generalized glycogen storage disease (Pompe's disease). *Biochem J* 86:11–16.
- Holtzman NA. 1970. Dietary treatment of inborn errors of metabolism. *Annu Rev Med* 21:335–356.
- Hong CM, Ohashi T, Yu XJ, Weiler S, Barranger JA. 1990. Sequence of two alleles responsible for Gaucher disease. *DNA Cell Biol* 9:233–241.
- Hopwood JJ, Bate G, Kirkpatrick P. 2006. Galsulfase. *Nat Rev Drug Discov* 5:101–102.
- Hoskins JA, Holliday SB, Greenway AM. 1984. The metabolism of cinnamic acid by healthy and phenylketonuric adults: a kinetic study. *Biomed Mass Spectrom* 11:296–300.
- Hruska KS, LaMarca ME, Scott CR, Sidransky E. 2008. Gaucher disease: mutation and polymorphism spectrum in the glucocerebrosidase gene (GBA). *Hum Mutat* 29:567–583.
- Hughes DA, Elliott PM, Shah J, Zuckerman J, Coghlan G, Brookes J, Mehta AB. 2008. Effects of enzyme replacement therapy on the cardiomyopathy of Anderson-Fabry disease: a randomised, double-blind, placebo-controlled clinical trial of agalsidase alfa. *Heart* 94:153–158.
- Hurwitz R, Ferlinz K, Vielhaber G, Moczał H, Sandhoff K. 1994. Processing of human acid sphingomyelinase in normal and I-cell fibroblasts. *J Biol Chem* 269:5440–5445.
- Ioannou YA, Zeidner KM, Grace ME, Desnick RJ. 1998. Human alpha-galactosidase A: glycosylation site 3 is essential for enzyme solubility. *Biochem J* 332 (Pt 3):789–797.
- Jemmerson R, Low MG. 1987. Phosphatidylinositol anchor of HeLa cell alkaline phosphatase. *Biochemistry* 26:5703–5709.
- Jin WD, Jackson CE, Desnick RJ, Schuchman EH. 1992. Mucopolysaccharidosis type VI: identification of three mutations in the arylsulfatase B gene of patients with the severe and mild phenotypes provides molecular evidence for genetic heterogeneity. *Am J Hum Genet* 50:795–800.
- Kakkis ED, Muenzer J, Tiller GE, Weber L, Belmont J, Passage M, Izykowski B, Phillips J, Doroshov R, Walot I, Hoft R, Neufeld EF. 2001. Enzyme-replacement therapy in mucopolysaccharidosis I. *N Engl J Med* 344:182–188.
- Karageorgos L, Brooks DA, Pollard A, Melville EL, Hein LK, Clements PR, Ketteridge D, Swidler SJ, Beck M, Giugliani R, Harmatz P, Wraith JE, Guffon N, Leão Teles E, Sá Miranda MC, Hopwood JJ. 2007. Mutational analysis of 105 mucopolysaccharidosis type VI patients. *Hum Mutat* 28:897–8903.
- Kase R, Bierfreund U, Klein A, Kolter T, Itoh K, Suzuki M, Hashimoto Y, Sandhoff K, Sakuraba H. 1996. Only sphingolipid activator protein B (SAP-B or saposin B) stimulates the degradation of globotriaosylceramide by recombinant human lysosomal alpha-galactosidase in a detergent-free liposomal system. *FEBS Lett* 393:74–76.
- Kase R, Bierfreund U, Klein A, Kolter T, Utsumi K, Itoha K, Sandhoff K, Sakuraba H. 2000. Characterization of two alpha-galactosidase mutants (Q279E and R301Q) found in an atypical variant of Fabry disease. *Biochim Biophys Acta* 1501:227–235.
- Kim CH, Hwang HZ, Song SM, Paik KH, Kwon EK, Moon KB, Yoon JH, Han CK, Jin DK. 2003. Mutational spectrum of the iduronate 2 sulfatase gene in 25 unrelated Korean Hunter syndrome patients: identification of 13 novel mutations. *Hum Mutat* 21:449–450.
- Kobayashi T, Honke K, Jin T, Gasa S, Miyazaki T, Makita A. 1992. Components and proteolytic processing sites of arylsulfatase B from human placenta. *Biochim Biophys Acta* 1159:243–247.
- Kobe B, Jennings IG, House CM, Michell BJ, Goodwill KE, Santarsiero BD, Stevens RC, Cotton RG, Kemp BE. 1999. Structural basis of autoregulation of phenylalanine hydroxylase. *Nat Struct Biol* 6:442–448.
- Kolter T, Wendeler M. 2003. Chemical chaperones—a new concept in drug research. *Chembiochem* 4:260–264.
- Kornfeld S. 1986. Trafficking of lysosomal enzymes in normal and disease states. *J Clin Invest* 77:1–6.
- Kretz KA, Carson GS, Morimoto S, Kishimoto Y, Fluharty AL, O'Brien JS. 1990. Characterization of a mutation in a family with saposin B deficiency: a glycosylation site defect. *Proc Natl Acad Sci USA* 87:2541–2544.
- Kroos M, Pomponio RJ, van Vliet L, Palmer RE, Phipps M, Van der Helm R, Halley D, Reuser A. 2008. Update of the Pompe disease mutation database with 107 sequence variants and a format for severity rating. *Hum Mutat* 29:E13–E26.
- Lachmann RH, Platt FM. 2001. Substrate reduction therapy for glycosphingolipid storage disorders. *Expert Opin Invest Drugs* 10:455–466.
- Le Du MH, Stigbrand T, Taussig MJ, Menez A, Stura EA. 2001. Crystal structure of alkaline phosphatase from human placenta at 1.8 Å resolution. Implication for a substrate specificity. *J Biol Chem* 276:9158–9165.
- Leader B, Baca QJ, Golan DE. 2008. Protein therapeutics: a summary and pharmacological classification. *Nat Rev Drug Discov* 7:21–39.
- LeBowitz JH, Grubb JH, Maga JA, Schmiel DH, Vogler C, Sly WS. 2004. Glycosylation-independent targeting enhances enzyme delivery to lysosomes and decreases storage in mucopolysaccharidosis type VII mice. *Proc Natl Acad Sci USA* 101:3083–3088.
- Lee WC, Courtenay A, Troendle FJ, Stallings-Mann ML, Dickey CA, DeLucia MW, Dickson DW, Eckman CB. 2005. Enzyme replacement therapy results in substantial improvements in early clinical phenotype in a mouse model of globoid cell leukodystrophy. *FASEB J* 19:1549–1551.
- Lee CY, Tamura T, Rabah N, Lee DY, Ruel I, Hafiane A, Iatan I, Nyholt D, Laporte F, Lazure C, Wada I, Krimbou L, Genest J. 2007. Carboxyl-terminal disulfide bond of acid sphingomyelinase is critical for its secretion and enzymatic function. *Biochemistry* 46:14969–14978.
- Levy HL, Milanowski A, Chakrapani A, Cleary M, Lee P, Trefz FK, Whitley CB, Feillet F, Feigenbaum AS, Bechuk JD, Christ-Schmidt H, Dorenbaum A; Sapropterin Research Group. 2007. Efficacy of sapropterin dihydrochloride (tetrahydrobiopterin, 6R-BH4) for reduction of phenylalanine concentration in patients with phenylketonuria: a phase III randomised placebo-controlled study. *Lancet* 370:504–510.
- Lieberman RL, Wustman BA, Huertas P, Powe Jr AC, Pine CW, Khanna R, Schlossmacher MG, Ringe D, Petsko GA. 2007. Structure of acid beta-glucosidase with pharmacological chaperone provides insight into Gaucher disease. *Nat Chem Biol* 3:101–107.

- Liou B, Kazimierzczuk A, Zhang M, Scott CR, Hegde RS, Grabowski GA. 2006. Analyses of variant acid beta-glucosidases: effects of Gaucher disease mutations. *J Biol Chem* 281:4242–4253.
- Lonser RR, Schiffman R, Robison RA, Butman JA, Quezado Z, Walker ML, Morrison PF, Walbridge S, Murray GJ, Park DM, Brady RO, Oldfield EH. 2007. Image-guided, direct convective delivery of glucocerebrosidase for neuroopathic Gaucher disease. *Neurology* 68:254–261.
- Louie GV, Brownlie PD, Lambert R, Cooper JB, Blundell TL, Wood SP, Warren MJ, Woodcock SC, Jordan PM. 1992. Structure of porphobilinogen deaminase reveals a flexible multidomain polymerase with a single catalytic site. *Nature* 359:33–39.
- Lukatela G, Krauss N, Theis K, Selmer T, Gieselmann V, von Figura K, Saenger W. 1998. Crystal structure of human arylsulfatase A: the aldehyde function and the metal ion at the active site suggest a novel mechanism for sulfate ester hydrolysis. *Biochemistry* 37:3654–3664.
- Maegawa GH, Tropak M, Buttner J, Stockley T, Kok F, Clarke JT, Mahuran DJ. 2007. Pyrimethamine as a potential pharmacological chaperone for late-onset forms of GM2 gangliosidosis. *J Biol Chem* 282:9150–9161.
- Marcao A, Azevedo JE, Gieselmann V, Sa Miranda MC. 2003. Oligomerization capacity of two arylsulfatase A mutants: C300F and P425T. *Biochem Biophys Res Commun* 306:293–297.
- Martinez A, Knappskog PM, Olafsdottir S, Doskeland AP, Eiken HG, Svebak RM, Bozzini M, Apold J, Flatmark T. 1995. Expression of recombinant human phenylalanine hydroxylase as fusion protein in *Escherichia coli* circumvents proteolytic degradation by host cell proteases. Isolation and characterization of the wild-type enzyme. *Biochem J* 306(Pt 2):589–597.
- McVie-Wylie AJ, Lee KL, Qiu H, Jin X, Do H, Gotschall R, Thurberg BL, Rogers C, Raben N, O'Callaghan M, Canfield W, Andrews L, McPherson JM, Mattaliano RJ. 2008. Biochemical and pharmacological characterization of different recombinant acid alpha-glucosidase preparations evaluated for the treatment of Pompe disease. *Mol Genet Metab* 94:448–455.
- Miao S, McCarter JD, Grace ME, Grabowski GA, Aebersold R, Withers SG. 1994. Identification of Glu340 as the active-site nucleophile in human glucocerebrosidase by use of electrospray tandem mass spectrometry. *J Biol Chem* 269:10975–10978.
- Millan JL, Narisawa S, Lemire I, Loisel TP, Boileau G, Leonard P, Gramatikova S, Terkeltaub R, Camacho NP, McKee MD, Crine P, Whyte MP. 2008. Enzyme replacement therapy for murine hypophosphatasia. *J Bone Miner Res* 23:777–787.
- Millat G, Froissart R, Maire I, Bozon D. 1997a. Characterization of iduronate sulphatase mutants affecting N-glycosylation sites and the cysteine-84 residue. *Biochem J* 326(Pt 1):243–247.
- Millat G, Froissart R, Maire I, Bozon D. 1997b. IDS transfer from overexpressing cells to IDS-deficient cells. *Exp Cell Res* 230:362–367.
- Miranda SR, He X, Simonaro CM, Gatt S, Dagan A, Desnick RJ, Schuchman EH. 2000. Infusion of recombinant human acid sphingomyelinase into Niemann-Pick disease mice leads to visceral, but not neurological, correction of the pathophysiology. *FASEB J* 14:1988–1995.
- Moffitt MC, Louie GV, Bowman ME, Pence J, Noel JP, Moore BS. 2007. Discovery of two cyanobacterial phenylalanine ammonia lyases: kinetic and structural characterization. *Biochemistry* 46:1004–1012.
- Mornet E. 2000. Hypophosphatasia: the mutations in the tissue-nonspecific alkaline phosphatase gene. *Hum Mutat* 15:309–315.
- Mornet E, Stura E, Lia-Baldini AS, Stigbrand T, Menez A, Le Du MH. 2001. Structural evidence for a functional role of human tissue nonspecific alkaline phosphatase in bone mineralization. *J Biol Chem* 276:31171–31178.
- Muenzer J, Gucaavas-Calikoglu M, McCandless SE, Schuetz TJ, Kimura A. 2007. A phase I/II clinical trial of enzyme replacement therapy in mucopolysaccharidosis II (Hunter syndrome). *Mol Genet Metab* 90:329–337.
- Myerowitz R, Neufeld EF. 1981. Maturation of alpha-L-iduronidase in cultured human fibroblasts. *J Biol Chem* 256:3044–3048.
- Nieman CE, Wong AW, He S, Clarke L, Hopwood JJ, Withers SG. 2003. Family 39 alpha-L-iduronidases and beta-D-xylosidases react through similar glycosyl-enzyme intermediates: identification of the human iduronidase nucleophile. *Biochemistry* 42:8054–8065.
- Nishioka T, Tomatsu S, Gutierrez MA, Miyamoto K, Trandafirescu GG, Lopez PL, Grubb JH, Kanai R, Kobayashi H, Yamaguchi S, Gottesman GS, Cahill R, Noguchi A, Sly WS. 2006. Enhancement of drug delivery to bone: characterization of human tissue-nonspecific alkaline phosphatase tagged with an acidic oligopeptide. *Mol Genet Metab* 88:244–255.
- Numa N, Ishida Y, Nasu M, Sohda M, Misumi Y, Noda T, Oda K. 2008. Molecular basis of perinatal hypophosphatasia with tissue-nonspecific alkaline phosphatase bearing a conservative replacement of valine by alanine at position 406. Structural importance of the crown domain. *FEBS J* 275:2727–2737.
- Okumiyu T, Ishii S, Kase R, Kamei S, Sakuraba H, Suzuki Y. 1995. Alpha-galactosidase gene mutations in Fabry disease: heterogeneous expressions of mutant enzyme proteins. *Hum Genet* 95:557–561.
- Okumiyu T, Kroos MA, Vliet LV, Takeuchi H, Van der Ploeg AT, Reuser AJ. 2007. Chemical chaperones improve transport and enhance stability of mutant alpha-glucosidases in glycogen storage disease type II. *Mol Genet Metab* 90: 49–57.
- Orii KO, Grubb JH, Vogler C, Levy B, Tan Y, Markova K, Davidson BL, Mao Q, Orii T, Kondo N, Sly WS. 2005. Defining the pathway for Tat-mediated delivery of beta-glucuronidase in cultured cells and MPS VII mice. *Mol Ther* 12:345–352.
- Pastores GM. 2006. Miglustat: substrate reduction therapy for lysosomal storage disorders associated with primary central nervous system involvement. *Recent Pat CNS Drug Discov* 1:77–82.
- Pastores GM. 2008. Laronidase (Aldurazyme): enzyme replacement therapy for mucopolysaccharidosis type I. *Expert Opin Biol Ther* 8:1003–1009.
- Penning TM, Jez JM. 2001. Enzyme redesign. *Chem Rev* 101:3027–3046.
- Pey AL, Stricher F, Serrano L, Martinez A. 2007. Predicted effects of missense mutations on native-state stability account for phenotypic outcome in phenylketonuria, a paradigm of misfolding diseases. *Am J Hum Genet* 81:100–1024.
- Ploos van Amstel JK, Jansen RP, de Jong JG, Hamel BC, Wevers RA. 1994. Six novel mutations in the alpha-galactosidase A gene in families with Fabry disease. *Hum Mol Genet* 3:503–505.
- Poeppel P, Habetha M, Marcao A, Bussow H, Berna L, Gieselmann V. 2005. Missense mutations as a cause of metachromatic leukodystrophy. Degradation of arylsulfatase A in the endoplasmic reticulum. *FEBS J* 272:1179–1188.
- Ponting CP. 1994. Acid sphingomyelinase possesses a domain homologous to its activator proteins: saposins B and D. *Protein Sci* 3:359–361.
- Premkumar L, Sawkar AR, Boldin-Adamsky S, Tokar L, Silman I, Kelly JW, Futerman AH, Sussman JL. 2005. X-ray structure of human acid-beta-glucosidase covalently bound to conduritol-B-epoxide. Implications for Gaucher disease. *J Biol Chem* 280:23815–23819.
- Qi X, Grabowski GA. 1998. Acid beta-glucosidase: intrinsic fluorescence and conformational changes induced by phospholipids and saposin C. *Biochemistry* 37:11544–11554.
- Qiu H, Edmunds T, Baker-Malcolm J, Karey KP, Estes S, Schwarz C, Hughes H, Van Patten SM. 2003. Activation of human acid sphingomyelinase through modification or deletion of C-terminal cysteine. *J Biol Chem* 278:32744–32752.
- Rader RA. 2007. Biopharmaceutical products in the U.S. and European markets, 6th edition. Rockville, MD: BioPlan Associates.
- Recksiek M, Selmer T, Dierks T, Schmidt B, von Figura K. 1998. Sulfatases, trapping of the sulfated enzyme intermediate by substituting the active site formylglycine. *J Biol Chem* 273:6096–6103.
- Rempel BP, Clarke LA, Withers SG. 2005. A homology model for human alpha-L-iduronidase: insights into human disease. *Mol Genet Metab* 85:28–37.
- Roces DP, Lullmann-Rauch R, Peng J, Balducci C, Andersson C, Tollersrud O, Fogh J, Orlacchio A, Beccari T, Saftig P, von Figura K. 2004. Efficacy of enzyme replacement therapy in alpha-mannosidosis mice: a preclinical animal study. *Hum Mol Genet* 13:1979–1988.
- Rodriguez-Mari A, Coll MJ, Chabas A. 2003. Molecular analysis in Fabry disease in Spain: fifteen novel GLA mutations and identification of a homozygous female. *Hum Mutat* 22:258.
- Rohrbach M, Clarke JT. 2007. Treatment of lysosomal storage disorders: progress with enzyme replacement therapy. *Drugs* 67:2697–2716.
- Ruth L, Eisenberg D, Neufeld EF. 2000. alpha-L-iduronidase forms semi-crystalline spherulites with amyloid-like properties. *Acta Crystallogr D Biol Crystallogr* 56(Pt 4):524–528.
- Saito S, Ohno K, Sugawara K, Sakuraba H. 2008. Structural and clinical implications of amino acid substitutions in N-acetylgalactosamine-4-sulfatase: insight into mucopolysaccharidosis type VI. *Mol Genet Metab* 93:419–425.
- Sardh E, Reijkjaer L, Andersson DE, Harper P. 2007. Safety, pharmacokinetics and pharmacodynamics of recombinant human porphobilinogen deaminase in healthy subjects and asymptomatic carriers of the acute intermittent porphyria gene who have increased porphyrin precursor excretion. *Clin Pharmacokinet* 46:335–349.
- Sarkissian CN, Shao Z, Blain F, Peevers R, Su H, Heft R, Chang TM, Scriver CR. 1999. A different approach to treatment of phenylketonuria: phenylalanine degradation with recombinant phenylalanine ammonia lyase. *Proc Natl Acad Sci USA* 96:2339–2344.
- Sarkissian CN, Gamez A, Wang L, Charbonneau M, Fitzpatrick P, Lemontt JF, Zhao B, Vellard M, Bell SM, Henschell C, Lambert A, Tsuruda L, Stevens RC, Scriver CR. 2008. Preclinical evaluation of multiple species of PEGylated recombinant phenylalanine ammonia lyase for the treatment of phenylketonuria. *Proc Natl Acad Sci USA* 105:20894–20899.
- Sawkar AR, Adamski-Werner SL, Cheng WC, Wong CH, Beutler E, Zimmer KP, Kelly JW. 2005. Gaucher disease-associated glucocerebrosidases show mutation-dependent chemical chaperoning profiles. *Chem Biol* 12:1235–1244.
- Schaefer E, Mehta A, Gal A. 2005. Genotype and phenotype in Fabry disease: analysis of the Fabry Outcome Survey. *Acta Paediatr Suppl* 94:87–92; discussion 79.
- Schaefer E, Baron K, Widmer U, Deegan P, Neumann HP, Sunder-Plassmann G, Johansson JO, Whybra C, Ries M, Pastores GM, Mehta A, Beck M, Gal A. 2005.

- Thirty-four novel mutations of the GLA gene in 121 patients with Fabry disease. *Hum Mutat* 25:412.
- Schiffmann R, Kopp JB, Austin 3rd HA, Sabnis S, Moore DF, Weibel T, Balow JE, Brady RO. 2001. Enzyme replacement therapy in Fabry disease: a randomized controlled trial. *JAMA* 285:2743–2749.
- Schissel SL, Schuchman EH, Williams KJ, Tabas I. 1996. Zn²⁺-stimulated sphingomyelinase is secreted by many cell types and is a product of the acid sphingomyelinase gene. *J Biol Chem* 271:18431–18436.
- Schmidt B, Selmer T, Ingendoh A, von Figura K. 1995. A novel amino acid modification in sulfatases that is defective in multiple sulfatase deficiency. *Cell* 82: 271–278.
- Schuchman EH, Suchi M, Takahashi T, Sandhoff K, Desnick RJ. 1991. Human acid sphingomyelinase. Isolation, nucleotide sequence and expression of the full-length and alternatively spliced cDNAs. *J Biol Chem* 266:8531–8539.
- Schwede T, Kopp J, Guex N, Peitsch MC. 2003. SWISS-MODEL: an automated protein homology-modeling server. *Nucleic Acids Res* 31:3381–3385.
- Scriver CR. 2001. The metabolic and molecular basis of inherited disease. New York: McGraw-Hill/Medical Publishing Division.
- Seto M, Whitlow M, McCarrick MA, Srinivasan S, Zhu Y, Pagila R, Mintzer R, Light D, Johns A, Meurer-Ogden JA. 2004. A model of the acid sphingomyelinase phosphoesterase domain based on its remote structural homolog purple acid phosphatase. *Protein Sci* 13:3172–3186.
- Shabbeer J, Yasuda M, Luca E, Desnick RJ. 2002. Fabry disease: 45 novel mutations in the alpha-galactosidase A gene causing the classical phenotype. *Mol Genet Metab* 76:23–30.
- Shin SH, Kluepfel-Stahl S, Cooney AM, Kaneski CR, Quirk JM, Schiffmann R, Brady RO, Murray GJ. 2008. Prediction of response of mutated alpha-galactosidase A to a pharmacological chaperone. *Pharmacogenet Genomics* 18:773–780.
- Silverman JA, Balakrishnan R, Harbury PB. 2001. Reverse engineering the (beta/alpha)₈ barrel fold. *Proc Natl Acad Sci USA* 98:3092–3097.
- Sim L, Quezada-Calvillo R, Sterchi EE, Nichols BL, Rose DR. 2008. Human intestinal maltase-glucoamylase: crystal structure of the N-terminal catalytic subunit and basis of inhibition and substrate specificity. *J Mol Biol* 375:782–792.
- Simonaro CM, Desnick RJ, McGovern MM, Wasserstein MP, Schuchman EH. 2002. The demographics and distribution of type B Niemann-Pick disease: novel mutations lead to new genotype/phenotype correlations. *Am J Hum Genet* 71:1413–1419.
- Sinclair AM, Elliott S. 2005. Glycoengineering: the effect of glycosylation on the properties of therapeutic proteins. *J Pharm Sci* 94:1626–1635.
- Sola RJ, Griebenow K. 2009. Effects of glycosylation on the stability of protein pharmaceuticals. *J Pharm Sci* 98:1223–1245.
- Spada M, Pagliardini S, Yasuda M, Tükel T, Thiagarajan G, Sakuraba H, Ponzzone A, Desnick RJ. 2006. High incidence of later-onset Fabry disease revealed by newborn screening. *Am J Hum Genet* 79:31–40.
- Steet RA, Chung S, Wustman B, Powe A, Do H, Kornfeld SA. 2006. The iminosugar isofagomine increases the activity of N370S mutant acid beta-glucosidase in Gaucher fibroblasts by several mechanisms. *Proc Natl Acad Sci USA* 103:13813–13818.
- Sugawara K, Ohno K, Saito S, Sakuraba H. 2008a. Structural characterization of mutant alpha-galactosidases causing Fabry disease. *J Hum Genet* 53:812–824.
- Sugawara K, Saito S, Ohno K, Okuyama T, Sakuraba H. 2008b. Structural study on mutant alpha-L-iduronidases: insight into mucopolysaccharidosis type I. *J Hum Genet* 53:467–474.
- Sukegawa-Hayasaka K, Kato Z, Nakamura H, Tomatsu S, Fukao T, Kuwata K, Orii T, Kondo N. 2006. Effect of Hunter disease (mucopolysaccharidosis type II) mutations on molecular phenotypes of iduronate-2-sulfatase: enzymatic activity, protein processing and structural analysis. *J Inherit Metab Dis* 29:755–761.
- Sun Y, Witte DP, Ran H, Zamzow M, Barnes S, Cheng H, Han X, Williams MT, Skelton MR, Vorhees CV, Grabowski GA. 2008. Neurological deficits and glycosphingolipid accumulation in saposin B deficient mice. *Hum Mol Genet* 17:2345–2356.
- Tajima Y, Matsuzawa F, Aikawa S, Okumiyama T, Yoshimizu M, Tsukimura T, Ikekita M, Tsujino S, Tsuji A, Edmunds T, Sakuraba H. 2007. Structural and biochemical studies on Pompe disease and a “pseudodeficiency of acid alpha-glucosidase.” *J Hum Genet* 52:898–906.
- Terlato NJ, Cox GF. 2003. Can mucopolysaccharidosis type I disease severity be predicted based on a patient’s genotype? A comprehensive review of the literature. *Genet Med* 5:286–294.
- Tomatsu S, Montano AM, Ohashi A, Gutierrez MA, Oikawa H, Oguma T, Dung VC, Nishioka T, Orii T, Sly WS. 2008. Enzyme replacement therapy in a murine model of Morquio A syndrome. *Hum Mol Genet* 17:815–824.
- Topaloglu AK, Ashley GA, Tong B, Shabbeer J, Astrin KH, Eng CM, Desnick RJ. 1999. Twenty novel mutations in the alpha-galactosidase A gene causing Fabry disease. *Mol Med* 5:806–811.
- Toscano MD, Woycechowsky KJ, Hilvert D. 2007. Minimalist active-site redesign: teaching old enzymes new tricks. *Angew Chem Int Ed Engl* 46:3212–3236.
- van der Ploeg AT, Reuser AJ. 2008. Pompe’s disease. *Lancet* 372:1342–1353.
- Van Patten SM, Hughes H, Huff MR, Piepenhagen PA, Waire J, Qiu H, Ganesa C, Reczek D, Ward PV, Kutzko JP, Edmunds T. 2007. Effect of mannose chain length on targeting of glucocerebrosidase for enzyme replacement therapy of Gaucher disease. *Glycobiology* 17:467–478.
- von Bulow R, Schmidt B, Dierks T, von Figura K, Usón I. 2001. Crystal structure of an enzyme-substrate complex provides insight into the interaction between human arylsulfatase A and its substrates during catalysis. *J Mol Biol* 305:269–277.
- von Bulow R, Schmidt B, Dierks T, Schwabauer N, Schilling K, Weber E, Usón I, von Figura K. 2002. Defective oligomerization of arylsulfatase A as a cause of its instability in lysosomes and metachromatic leukodystrophy. *J Biol Chem* 277:9455–9461.
- Waldow A, Schmidt B, Dierks T, von Bulow R, von Figura K. 1999. Amino acid residues forming the active site of arylsulfatase A. Role in catalytic activity and substrate binding. *J Biol Chem* 274:12284–12288.
- Wang L, Gamez A, Sarkissian CN, Straub M, Patch MG, Han GW, Striepeke S, Fitzpatrick P, Scriver CR, Stevens RC. 2005. Structure-based chemical modification strategy for enzyme replacement treatment of phenylketonuria. *Mol Genet Metab* 86:134–140.
- Wang L, Gamez A, Archer H, Abola EE, Sarkissian CN, Fitzpatrick P, Wendt D, Zhang Y, Vellard M, Bliesath J, Bell SM, Lemontt JF, Scriver CR, Stevens RC. 2008. Structural and biochemical characterization of the therapeutic *Anabaena variabilis* phenylalanine ammonia lyase. *J Mol Biol* 380:623–635.
- Weinreb NJ. 2008. Imiglucerase and its use for the treatment of Gaucher’s disease. *Expert Opin Pharmacother* 9:1987–2000.
- Wilkening G, Linke T, Sandhoff K. 1998. Lysosomal degradation on vesicular membrane surfaces. Enhanced glucosylceramide degradation by lysosomal anionic lipids and activators. *J Biol Chem* 273:30271–30278.
- Wilson PJ, Morris CP, Anson DS, Occhiodoro T, Bielicki J, Clements PR, Hopwood JJ. 1990. Hunter syndrome: isolation of an iduronate-2-sulfatase cDNA clone and analysis of patient DNA. *Proc Natl Acad Sci USA* 87: 8531–8535.
- Wood S, Lambert R, Jordan PM. 1995. Molecular basis of acute intermittent porphyria. *Mol Med Today* 1:232–239.
- Wraith JE. 2006. Limitations of enzyme replacement therapy: current and future. *J Inherit Metab Dis* 29:442–447.
- Wraith JE. 2008. Enzyme replacement therapy with idursulfase in patients with mucopolysaccharidosis type II. *Acta Paediatr Suppl* 97:76–78.
- Yang JK, Yoon HJ, Ahn HJ, Lee BI, Pedelacq JD, Liong EC, Berendzen J, Laivenieks M, Vieille C, Zeikus GJ, Voadlo DJ, Withers SG, Suh SW. 2004. Crystal structure of beta-D-xylosidase from *Thermoanaerobacterium saccharolyticum*, a family 39 glycoside hydrolase. *J Mol Biol* 335:155–165.
- Yasuda M, Shabbeer J, Benson SD, Maire I, Burnett RM, Desnick RJ. 2003. Fabry disease: characterization of alpha-galactosidase A double mutations and the D313Y plasma enzyme pseudodeficiency allele. *Hum Mutat* 22:486–492.
- Zhao H, Grabowski GA. 2002. Gaucher disease: perspectives on a prototype lysosomal disease. *Cell Mol Life Sci* 59:694–707.
- Zhao KW, Faull KF, Kakkis ED, Neufeld EF. 1997. Carbohydrate structures of recombinant human alpha-L-iduronidase secreted by Chinese hamster ovary cells. *J Biol Chem* 272:22758–22765.