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Structure, Biological Functions and Applications of the AB₅ Toxins

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Abstract

AB₅ toxins are important virulence factors for several major bacterial pathogens, including *Bordetella pertussis*, *Vibrio cholerae*, *Shigella dysenteriae* and at least two distinct pathotypes of *Escherichia coli*. The AB₅ toxins are so termed because they comprise a catalytic A-subunit, which is responsible for disruption of essential host functions, and a pentameric B-subunit that binds to specific glycan receptors on the target cell surface. The molecular mechanisms by which these AB₅ toxins cause disease have been largely unraveled, including recent insights into a novel AB₅ toxin family, subtilase cytotoxin (SubAB). Furthermore, AB₅ toxins have become a valuable tool for studying fundamental cellular functions, and are now being investigated for potential applications in the clinical treatment of human diseases.

Getting from A to B of AB₅ toxins

AB₅ toxins are an important weapon in the armory of virulence factors deployed by major bacterial pathogens, which collectively kill over a million people each year. *Bordetella pertussis*, which produces pertussis toxin (Ptx), is the aetiological agent of whooping cough, an acute respiratory infection that continues to cause high global morbidity and mortality in spite of the availability of effective vaccines. Cholera toxin (Ctx) produced by *Vibrio cholerae* and the closely related heat labile enterotoxins (LT) produced by enterotoxigenic *Escherichia coli* (ETEC) are principally responsible for the copious watery diarrhoea that is the hallmark of gastrointestinal infection with the respective organism. Cholera is endemic in Asia, but causes epidemics in nearly all parts of the globe ¹. Indeed, it continues to represent a major threat to global human health, with the 2008 outbreak in Zimbabwe resulting in the deaths of over 4000 people. ETEC infection is highly prevalent in developing countries, where it is a major killer of young children. Visitors to regions where ETEC disease is endemic are also at high risk, hence the term “travellers’ diarrhoea”. ETEC diarrhoea is also a major problem for the livestock industry ². Shiga toxin (Stx) is produced by *Shigella dysenteriae* and Shiga

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toxigenic *E. coli* (STEC), which also cause serious gastrointestinal disease in humans, ranging from diarrhoea to haemorrhagic colitis and life-threatening systemic sequelae such as the haemolytic uraemic syndrome (HUS). These manifestations are largely attributable to Stx-mediated damage to microvascular endothelial cells in the gut, kidneys and brain^{3,4}. Outbreaks of STEC disease including HUS are common in developed countries (40 in the United States in 1999)⁵; HUS has a 5-10% mortality rate for children and 35% for adults⁵ and STEC infections are estimated to cause 500 deaths each year in the United States². The high prevalence and severity of all these bacterial infections emphasizes the importance of gaining a full understanding of the mode of action of the AB₅ toxins that are central to disease pathogenesis.

Over the past two decades, approximately 30 crystal structures of AB₅ toxins have been determined, including the complete holotoxins from the various pathogenic bacteria as well as the *apo* and *holo* forms of the individual A-subunit and B-subunit components⁶. These major breakthroughs provided significant structural insights into the biological function and catalytic activity of the holotoxins. Based on sequence homology and the specific A-subunit catalytic activity, the AB₅ toxins have been classified into four families (Figure 1). The B-subunit forms a ring-shaped pentamer that is responsible for the binding to the host cell surface, whereas the catalytic A-subunit disrupts the host's cellular machinery. Despite sharing a similar structural architecture, the various AB₅ family members can differ in their host cell surface receptor specificity, catalytic activity and intracellular trafficking. Here we review the various structural characteristics shared by the AB₅ toxins and their implications in terms of toxicity, cellular targeting, and their use as cell biology reagents and potential therapeutics.

The A-subunit: catalytic domain

The A-subunit of the bacterial AB₅ toxins is a single polypeptide composed of two domains (A1 and A2) that are also linked together *via* a disulfide bond. The A1 domain comprises the catalytic domain responsible for the toxicity to the host cell. The A2 domain consists of an α -helix that penetrates into the central pore of the pentameric B-subunit, thereby non-covalently anchoring the A- and B-subunits together to create the holotoxin (Figure 1). This unusual arrangement is a common structural feature of all AB₅ toxins, as first revealed by the crystal structure of LT from *E. coli* (for review see⁶). To date, the A-subunit crystal structures of seven members of the various AB₅ toxin families have been determined either in their *apo*, *holo* or mutant forms (Figure 1)⁷.

The AB₅ toxins are subdivided into families according to A-subunit sequence homology and catalytic activity. The cholera toxin family (which includes Ctx and LT), and the Ptx family, trigger the ADP-ribosylation of the G_s α and G_i α proteins in the cytosol, disrupting the respective G-protein signal transduction pathways. This results in an increase in intracellular cAMP levels and dysregulation of ion transport mechanisms⁶. The LT toxins have been serologically distinguished into two groups, termed type I and type II, the latter being further divided into LT-IIa and LT-IIb⁸. The A-subunits of Ctx, LT-I and LT-II share approximately 55-80 % amino acid sequence identity⁶, whereas the sequence identity between the catalytic subunit of Ptx (referred to as the S1 subunit) and the A-subunits of Ctx or LT is much lower (15-20%).

The Stx family members have RNA N-glycosidase activity, and inhibit eukaryotic protein synthesis by cleaving a specific adenine base from 28S rRNA, thereby causing cell death. STEC produce two major Stx classes (Stx1 and Stx2). Stx1 is essentially identical to classical Stx produced by *S. dysenteriae* (a single amino acid difference), and is about 60% identical at the amino acid level to Stx2, which also exists as several subtypes⁹. Accordingly the three-dimensional structures of Stx and Stx2 revealed few structural differences¹⁰. Interestingly

however, people infected with Stx2-producing STEC strains are more likely to develop HUS than if they were infected by Stx1-producing STEC; this is most likely due to increased cytotoxicity of Stx2 for microvascular endothelial cells ^{3, 11}.

The recent discovery of an additional STEC AB₅ toxin, whose A-subunit shares homology with subtilase-like serine proteases and differs significantly from other AB₅ toxins, led to the establishment of a fourth AB₅ family ^{12, 13} (Figure 1). This subtilase cytotoxin (SubAB) was discovered in an O113:H21 STEC strain, which was responsible for an outbreak of HUS in South Australia in 1998, and has since been detected in numerous other STEC serotypes ^{3, 14, 15}. SubAB is extremely cytotoxic *in vitro* for a range of cell types, and it is more toxic than Stx for Vero cells (kidney epithelial cells). It is also lethal for mice when injected intraperitoneally, producing pathological features that strongly resemble HUS in humans ^{12, 16}. Subtilase family proteases are characterised by a conserved catalytic triad (comprising distinct Asp, His, and Ser motifs); substitution of the critical catalytic Ser in SubA with Ala abolished toxicity, indicating that the SubA subunit acts as a protease ^{12, 16}. Proteomic and functional analysis subsequently revealed that BiP (also termed GRP78) is the specific molecular target for SubA ¹³. Unlike most subtilases, structural studies revealed that SubA possessed an unusually deep active site cleft (Figure 2), providing a basis for its exquisite substrate specificity for BiP ¹³, a highly conserved endoplasmic reticulum (ER) chaperone essential for survival of eukaryotic cells. BiP also maintains the permeability barrier of the ER membrane and plays a crucial role in the unfolded protein response (UPR) as the ER stress-signalling master regulator ¹⁷. Thus, cleavage of BiP has fatal consequences for the cell. Collectively, these recent findings for SubA reveal a novel mechanism in which bacterial toxins can induce cell death, *via* the specific proteolytic abrogation of chaperone function.

In order to reach their molecular targets in the cytosol, the Ptx, Stx, Ctx and LT toxins recognize their cognate cell surface receptor *via* the pentameric B-subunit; this recognition event triggers endocytosis, followed by retrograde transport via the Golgi to the ER (Figure 3) ¹⁸. In the ER, protein disulfide isomerase (PDI) unfolds the A-subunit (A1) ¹⁹, which is then retro-translocated into the cytosol *via* the Sec61 channel ²⁰, or via the derlin-1–Hrd1 complex ^{21–23}. It then undergoes refolding and finally causes cellular toxicity. In Ctx and LT, the very end of the A2 domain contains an ER-targeting motif (KDEL or RDEL). However, this motif is clearly not essential, as it is not present in Stx. Furthermore, CtxB and LT-B undergo retrograde transport efficiently in the absence of their respective A subunits ²⁴. SubAB also lacks a KDEL motif, and furthermore, the localization of its molecular target in the ER lumen implies that SubAB does not require retro-translocation into the cytosol to induce toxicity. Within the target cell, SubAB follows a retrograde pathway similar to that previously observed for Stx and Ctx. However, unlike Stx, Ctx and LT, which engage both clathrin-dependent and lipid raft pathways, trafficking of SubAB in Vero cells was shown to be entirely clathrin-dependent ²⁵. The route through the Golgi is also distinct, with SubAB exploiting a novel p115–golgin-84-independent, COG–Rab6–COPI-dependent mechanism, and unlike Stx, retrograde transport is not dependent on the endosomal sorting nexins, SNX1 and SNX2 ²⁶.

The B₅ receptor-binding domain: Same fold, different specificity

The B-subunits of the AB₅ toxins recognise glycan receptors displayed on the cell surface. Receptor specificity is critical for the pathogenic process, as it determines host susceptibility, tissue tropism, and the nature and spectrum of the resultant pathology. In the past two decades, an increasing number of X-ray structures of AB₅ toxin B-subunits, determined in their *apo* form as well as in complex with their respective A-subunits and various ligands or inhibitors, have been solved. This has provided crucial structural insights into the receptor specificity and mode of binding, which has informed inhibitor design ^{6, 27, 28}. Despite low sequence identity, the B-subunits of the Cholera (Ctx, LTI and LTII), Shiga, and SubAB toxin families share the

common oligosaccharide/oligonucleotide binding fold (OB) consisting of a five-stranded β -barrel flanked by an α -helix that assembles to form a homo-pentameric ring-shaped scaffold (Figure 4). By contrast, the B subunit of Ptx is a hetero-pentamer comprising four different monomers (S2, S3, two copies of S4, and S5). Despite sharing a common fold, the cell surface receptor specificity of the AB₅ toxins varies from one family to the other and also within the same family. In the cholera toxin family, the Ctx and LT-I B pentamers specifically recognise the oligosaccharide moiety of the ganglioside GM1²⁹, whereas the LT-IIa and LT-IIb B subunits show affinity for gangliosides GD1b and GD1a, respectively³⁰. Stx/Stx1 and most variants of Stx2 bind the oligosaccharide component of the glycosphingolipid Gb₃, which is expressed on the surface of the microvascular endothelium as well as kidney tubular epithelium, whereas a variant toxin, Stx2e, binds preferentially to Gb₄³. Glycan array (<http://www.functionalglycomics.org/static/consortium/consortium.shtml>) and subsequent functional studies have revealed that the recently discovered SubAB has unusual binding specificity for glycans terminating in α -3-linked *N*-glycolylneuraminic acid (Neu5Gc)³¹. This sialic acid is not synthesised by humans (Box 1), but it is incorporated into the cell surface *via* dietary intake, with the most common source of Neu5Gc being dairy products and red meat. Ironically, these foods are the most common sources of STEC contamination, and so dietary choices can increase both the susceptibility of an individual to the toxin as well as the risk of infection with the pathogen that produces it³¹.

Box 1. Why have humans lost the ability to synthesise Neu5Gc?

Neu5Gc and Neu5Ac are sialic acids (Sia) that differ by a single hydroxyl group. They are attached to the termini of cell surface glycans of all vertebrates and are essential for development⁶⁷. Moreover, they are major targets of many pathogens⁶⁸. In humans the major Sia is Neu5Ac due to disruption of the gene (*CMAH*) encoding CMP-Neu5Ac hydroxylase, which converts Neu5Ac to Neu5Gc. This mutation occurred approximately 2 million years ago, after separation of the hominid lineage from the great apes⁶⁹. By contrast, Neu5Gc is the most abundant Sia in other mammals, including the great apes⁷⁰. Loss of Neu5Gc-containing glycans in humans was probably due to infection by pathogens, in particular malaria (*Plasmodium falciparum*), one of the most devastating human pathogens⁷¹. A recent study analyzing different strains of *P. reichenowi*, which infects chimpanzees and gorillas, suggested that it gave rise to all strains of *P. falciparum*⁷². In *P. reichenowi*, the well-characterized red-blood cell binding protein EBA-175, which is required for infection, prefers Neu5Gc whereas *P. falciparum* EBA-175 prefers Neu5Ac⁷¹. This switch in Sia preference was due to humans losing the ability to synthesise Neu5Gc, perhaps as a means to provide relief from infection.

Not only do the AB₅ toxin B subunits show a broad variety in terms of receptor specificity, but the location and the number of binding sites for these glycans can also differ markedly (Figure 5). Interestingly, SubAB, LT and Ctx contain five receptor-binding sites (one per monomer), whereas Stx contains up to fifteen binding pockets (three per monomer) presenting various affinities³². The receptor-binding site of the cholera toxin family members Ctx and LT is conserved and buried in a deep pocket formed by the L45, L3 α and N-terminal loops located at the bottom of the B-subunit³³. The receptor GM1 makes extensive interactions with the B-subunit resulting in high affinity for the ganglioside³⁴. CtxB binds exclusively to GM1 whereas the LT B-subunit can also recognise *N*-acetylglucosamine-terminating glycolipids and glycoproteins³⁵ and certain blood group ABO antigens³⁶. Furthermore, the crystal structures of the B-subunit of a hybrid between CtxB and LT-IB in complex with a blood group A antigen analogue reveals a novel binding specificity and a binding site distinct from the GM1 site³⁷. More recently, the X-ray structure of the LT-I B-subunit showed that LT-I could bind the blood group A antigen ligand in the same shallow binding groove halfway down the subunit³⁸. The

study provided important structural insight for the blood group antigen recognition by the enterotoxin and supported the idea that specificity of LT or even Ctx could play a role in the blood group dependence observed in ETEC infections and cholera.

In the SubAB toxin, the non-human Neu5Gc also binds in a shallow pocket located halfway down the side of the B-subunit and formed by the $\beta 1$ and $\beta 4$ strands. The structure of SubB resembles the S2, S3 and S5 subunits of Ptx, with the S2 and S3 subunits also forming a similar binding pocket that binds sialylated glycoproteins (Figure 5)³¹. Interestingly, the architecture of the active site of SubB provided a basis for understanding its unique specificity for Neu5Gc, and mutation of these key active-site residues abrogated binding and cytotoxicity³¹. Collectively, these findings for SubAB provide a novel paradigm wherein a bacterial toxin's receptor can be generated by metabolic incorporation of an exogenous factor derived from food.

AB₅ toxins as innovative cellular tools

The highly specific actions of the AB₅ toxin catalytic subunits have been exploited both to investigate the roles of the targeted processes in cellular physiology, and to specifically manipulate the associated signaling pathways. Most notably, the specific ADP-ribosylation of G_s and G_i by Ctx/LT and Ptx, respectively, has had a major impact on our understanding of the roles of G-protein-coupled receptor signaling in diverse physiological processes^{39, 40}. Experiments have typically involved treating cells with exogenous holotoxin *in vitro*, but in recent years, the role of G_{i/o} signaling *in vivo* has been examined using transgenic mice expressing the Ptx catalytic S1 subunit⁴¹. The B-subunit pentamers of the AB₅ toxins, particularly StxB and CtxB, have also proved to be highly valuable tools for detailed characterization of receptor-mediated endocytic pathways in eukaryotic cells (for review see²⁴).

The immunomodulatory and mucosal adjuvant properties of Ctx/LT are also well-established (reviewed in^{42, 43}). The Ctx/LT holotoxins elicit strong mucosal responses to co-administered antigens, through a combination of increasing the permeability of the mucosal epithelium, upregulating co-stimulatory molecule expression and enhancing presentation on antigen presenting cells, promoting isotype differentiation in B cells, and exerting an array of effects on T cell proliferation and cytokine production⁴². Both A- and B-subunits contribute to these properties, and the precise ganglioside binding specificity of the respective B-subunits has been associated with differences in T helper type 1 (Th1) versus T helper type 2 (Th2) responses elicited by the two holotoxins⁴⁴. The isolated Ctx/LT B subunits have much reduced adjuvant activity, unless directly conjugated to the heterologous antigen⁴². CtxB has also been used to promote mucosal tolerance to autoantigens or allergens⁴². For example, patients with Behcet's disease experienced significantly reduced numbers of uveitis relapses following oral administration of CtxB conjugated to an autoantigenic peptide derived from the human 60 kDa heat shock protein⁴⁵. More recent experiments demonstrated that administration of CtxB to the airways of allergic and naïve mice can suppress airway allergen-triggered eosinophilia, Th2 cytokine synthesis and bronchial hyperreactivity. These effects were both preventative and curative, and were mediated by stimulation of antigen-specific IgA production by B cells⁴⁶.

StxB also has potent immunomodulatory properties and has been used to deliver exogenous antigens to the major histocompatibility complex (MHC) Class I pathway of antigen presenting cells (which express the StxB receptor Gb₃), thereby eliciting a strong cell mediated immune response (reviewed by⁴⁷). This property is mediated by the capacity of StxB to direct retrograde trafficking of conjugated antigens from endosomes to the ER compartment, enabling loading of the processed antigen onto nascent Class I MHC molecules prior to display on the cell

surface. Thus, StxB has great potential as a non-living, non-toxic vaccine vector, which has already been demonstrated to impart anti-tumour protective immunity in mice ⁴⁷. Its capacity to elicit cell-mediated immunity also points to potential for immunoprophylaxis and therapy of infectious diseases.

The exquisite specificity of the recently discovered SubAB toxin for BiP, and its capacity abolish BiP function *in vitro* and *in vivo*, also make it a powerful cell biological tool, particularly for investigating the roles of BiP and ER stress in a range of cellular processes. As BiP is essential for the survival of eukaryotic cells, *Bip*^{-/-} cell lines and animal models are not available. Moreover, RNA knock-down approaches do not completely suppress BiP expression in transfected cells. However, treatment with SubAB results in rapid degradation of nearly all cellular BiP in a wide variety of cell lines, effectively mimicking a knock-out phenotype. The toxin has already been used to examine the role of BiP and/or ER stress in ER-associated degradation in HeLa cells ⁴⁸, and regulation of gap junction function in mesangial cells ⁴⁹. SubAB has also been employed to demonstrate the critical role of BiP in assembly and egress of cytomegalovirus from infected cells ⁵⁰ and in production and processing of dengue virus proteins ⁵¹. Both viruses upregulate BiP during infection, and SubAB treatment significantly reduces infectious virus release. Like Ctx/LT, SubAB might also have immunomodulatory activities, affecting T-cell activation and inflammatory responses of a variety of cell types ^{52, 53}. The toxin also preferentially inhibits secretion of immunoglobulins by activated murine B lymphocytes, leaving cytokine secretion relatively unscathed. SubAB preferentially cleaved newly synthesized BiP in these cells, and the C-terminal BiP fragment remained tightly bound to nascent immunoglobulin light chains, trapping them in the ER compartment ⁵⁴. SubAB treatment also caused transient Akt phosphorylation and nuclear factor-kappaB (NF-κB) activation in rat renal tubular epithelial cells, effects which were mediated via the activating transcription factor 6 (ATF6) branch of the UPR ⁵⁵. Interestingly however, at sub-cytotoxic concentrations, SubAB has anti-inflammatory properties and can inhibit lipopolysaccharide (LPS)-mediated NF-κB activation in a murine macrophage cell line, as well as protect mice from LPS-induced endotoxic lethality and experimental arthritis ⁵⁶.

AB₅ toxins as novel therapeutics

In addition to their potential use as immunomodulatory agents, AB₅ toxins could be used as therapeutic agents against a range of diseases. In particular, their specific catalytic functions, their cell binding specificities, and their intracellular trafficking properties can be exploited in the treatment of cancer. Many tumours exhibit aberrant glycosylation patterns, enabling them to be targeted by AB₅ toxin B-subunits, which exhibit high glycan binding specificity. StxB, for example, binds with high specificity to Gb₃ (also referred to as CD77), surface expression of which is enhanced in a broad range of cancer cells, including colon, pancreatic, ovarian, breast and testicular cancers, as well as in myelomas, lymphomas, astrocytomas and meningiomas ^{47, 57-59}. Thus, StxB has the capacity to target tumours *in vivo* preferentially over normal tissues, and then mediate uptake of holotoxin and intracellular delivery of the toxic A-subunit, which then inhibits cellular protein synthesis and kills the cancer cell. Indeed several studies have demonstrated inhibition by Stx holotoxin of tumour growth *in vivo* ^{47, 60, 61}. Nevertheless, Gb₃ is also expressed in a number of normal cells/tissues, particularly the kidneys, microvasculature endothelial cells, and CD77-positive dendritic cells and B lymphocytes, necessitating careful dose optimization. Notwithstanding the therapeutic potential of the Stx holotoxin, StxB has the capacity to deliver other toxic payloads or drugs, as well as molecular imaging reagents ^{24, 62, 63}.

SubAB also has potential as an anti-cancer therapeutic. Many tumours up-regulate its unique substrate BiP in response to the ER stress induced by their own rapid growth. Such up-regulation is a crucial anti-apoptotic mechanism for the tumour and it is also linked to metastatic

potential and resistance to chemotherapy⁶⁴. Thus, if SubA can be specifically targeted to these tumours, it has the potential to inactivate a cellular process that is crucial for survival of the cancer cell. Fortuitously, the glycan specificity of SubB might enable such targeting. SubB binds preferentially to glycans terminating in α 2-3-linked Neu5Gc, a sugar that humans cannot synthesise. Humans can assimilate Neu5Gc from dietary sources, but in cancer patients, it is preferentially incorporated into the tumours⁶⁵. As in the case of Stx, careful dosing would be required if SubAB holotoxin were to be deployed, as there is the potential for toxicity to normal tissues expressing traces of Neu5Gc taken up from the diet, or through weaker, but nevertheless potentially significant, interactions with more ubiquitously distributed α -3-linked Neu5Ac glycans. Alternatively, SubA alone could be delivered via a more tumour-specific targeting molecule, such as epidermal growth factor (EGF), as its receptor (EGFR) is expressed at high levels in many tumours, including melanomas and gliomas, as well as breast, prostate, and ovarian cancers. Indeed, an EGF–SubA fusion protein was recently constructed, and it was internalized efficiently by tumour cells in an EGFR-dependent fashion. Moreover, treatment with EGF–SubA killed EGFR-positive rat glioma and human breast and prostate cancer cells at picomolar concentrations⁶⁶. Cell lines expressing moderate to high levels of EGFR, which is associated with invasiveness and metastatic potential, were the most susceptible. EGF–SubA acted synergistically with drugs, such as thapsigargin, that induce ER stress, enabling effective deployment at concentrations well below the cytotoxicity threshold for each component. EGF–SubA is also efficacious *in vivo*, significantly inhibiting tumour growth in mouse xenograft models of human breast and prostate cancer⁶⁶.

Concluding remarks

AB₅ toxins are key virulence factors of several major bacterial pathogens that cause worldwide morbidity and mortality. Early studies on AB₅ toxins classified the toxins into three families; however, the recent discovery of the SubAB toxin resulted in the establishment of a fourth member of this family. Unique among other AB₅ toxins, the A-subunit of this toxin is a subtilase-like serine protease that specifically cleaves BiP, and unexpectedly, SubB binds a glycan that humans cannot synthesise, but which is incorporated from our diet. This novel SubAB toxin has raised several exciting questions including: i) Are there other AB₅ toxins that possess different catalytic A-subunits? ii) How did SubA evolve its highly specific activity? iii) How does the OB fold of the B-pentamer evolve different binding sites and glycan specificities?

Through investigating how these toxins cause disease they have become essential reagents in a biochemist's toolbox for investigating the process of cell trafficking and immune cell function. The ability of AB₅ toxins to bind specific glycans has enabled experiments to directly assess the physiological role of the various glycans (eg GM1 that is recognized by Ctx). The ability to evolve the glycan specificity of the B-pentamer could also have potential benefit in the use of AB₅ toxins in a clinical setting. The continued investigation into the AB₅ family should lead to greater understanding of cellular trafficking and the specific physiological role of defined glycoconjugates, which will ultimately lead to increased use as potential therapies for a range of human diseases.

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Glossary

BiP	(Binding immunoglobulin protein): also called Grp78 (Glucose regulated protein); BiP is an essential ER resident chaperone that plays a major role in the assistance of newly synthesised proteins to acquire correct folding and quaternary structure.
ETEC and STEC	Enterotoxigenic and Shiga Toxigenic <i>E. coli</i> strains are common <i>E. coli</i> infections. ETEC produces the heat-labile (LT) and is the most common cause of travellers' diarrhea. The Shiga-Toxin secreting <i>E. coli</i> STEC also causes gastrointestinal disease that can lead to the life threatening syndrome HUS (renal failure)
HUS	(haemolytic uraemic syndrome): a disease characterised by haemolytic anemia and acute renal failure. This syndrome occurs predominantly in children and is preceded by diarrhoea caused by the shiga-toxin producing <i>E. coli</i> . The toxin preferentially targets the kidneys by inhibiting protein synthesis leading to cell necrosis or apoptosis. The most common route of infection in children is via undercooked meat containing bacteria
Neu5Gc	N-glycolylneuraminic acid is a terminating sialic acid glycan not synthesised by humans as they do not possess the gene necessary for its synthesis. Neu5Gc is abundant in dairy products and red meats, which ironically are the foods the most commonly contaminated by STEC
SubAB	abbreviated name for the recently established 4 th member of AB ₅ toxin family. It was named 'subtilase cytotoxin' (SubAB) because the A-subunit of the toxin shares sequence homology with a subtilase-like serine protease of <i>Bacillus anthracis</i> and exhibits protease activity towards the ER chaperone BiP, a protein playing a key role for cell survival
UPR	(Unfolded Protein Response): a cellular stress response and is critical for the maintenance of cellular function in the ER. Under stress conditions, proteins in the ER can misfold or aggregate. To prevent protein aggregation and/or misfolding, the ER activates the UPR system, thus enabling an increase in the capacity for protein folding

Figure 1. Crystal structures of members of the four recognised AB₅ toxin families

The B-subunit is represented as molecular surface. The A-subunits of SubAB, Ctx and LT, Stx and Ptx are shown in cartoon representation and coloured according to the respective catalytic activity (Light blue for subtilase activity, light green for ADP-ribosylase activity and purple for RNA N-glycosidase activity). The common structural element (Helix A2) is coloured in red, and the level of sequence identity of the A-subunit inside a family is also indicated.

Figure 2. Molecular surface representations of a broad specificity protease and the SubAB A-subunit

The position of the catalytic triad (His-Asp-Ser) in both X-ray structures is coloured in red. The Subtilisin Carlsberg (*Pdb code*: 1C3L) is a typical broad specificity protease with a shallow active site. The loops composed of the amino acid residues 81-88 and 234-239 forming a deeper binding cleft in SubAB are coloured in yellow and pale green, respectively.

Figure 3. [SC1]Schematic representation of the glycan surface recognition of the various AB₅ toxins and their respective trafficking pathways into the cell

The toxins' A-subunits are shown as a pentagon and each subunit is coloured according to the specific catalytic activity (blue for protease activity, green and magenta for ADP-ribosyltransferase and N-glycosidase activities, respectively). The toxins bind to the cell-surface *via* their respective glycan receptors, where they are internalized to the early endosomal (EE) compartment. The names of these glycan receptors are indicated and inserted into schematic rings located on the surface of the cell and coloured according to their nature (grey and pale green for glycoproteins and glycolipids, respectively). The toxins are then trafficked to the Golgi then onto the endoplasmic reticulum (ER) where the A-subunit of the Stx, Ctx,

LT and Ptx are separated from the B-pentamer. They are then exported out of the ER, where they are able to attack their respective molecular targets (the G-protein (brown triangle), the 28S ribosomal RNA, and the ribosome (teal[SC2])). In the case of SubAB, it is unknown whether SubA is separated from SubB; however the purified SubA and SubAB can cleave BiP (grey) *in vitro* ³¹.

Figure 4. The pentameric B-subunit of the AB5 toxin

a) Overall architecture of the pentameric B-subunit of SubAB shown in two different orientations. Each monomer is coloured differently. b) Close up view of a monomer forming the common oligosaccharide/oligonucleotide (OB) fold. The monomer is coloured according to the secondary structural elements (helices, gold; β -strands, purple).

Figure 5. Molecular surface representations of the B-subunits of Ctx, Stx1, SubAB, Ptx and LT-I with bound glycans

The Gb₃ analogue (PK-MCO) is shown bound to Stx1. The antagonist BMSC-0011 (*in blue*) is shown bound to LT-I and Ctx. The second binding site of LT-I is also shown with the blood group A antigen analogue (*in green*) bound in a similar position as in SubAB, whereas Neu5Gc and Neu5Ac α -3Gal are shown bound to SubAB and Ptx, respectively. A close-up view of the interactions for SubAB–Neu5Gc and Ptx–Neu5Ac α 2-3Gal is also represented. For clarity, only the amino acid residues involved in direct hydrogen bonds (dashed red lines) with the two ligands are shown. The position of the extra hydroxyl in Neu5Gc and the equivalent position in Neu5Ac α 2-3Gal are highlighted in cyan.