# Sialic acid acquisition in bacteria – one substrate, many transporters.

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Abbreviations: ABC, ATP-binding cassette; TRAP, tripartite ATP-independent periplasmic; MFS, major facilitator superfamily; SSS, sodium solute symporter; Neu5Ac, *N*-acetyl neuraminic acid; Neu5Gc, *N*-glycoly neuraminic acid; SBP, substrate binding protein.

**Abstract**

The sialic acids are a family of 9-carbon sugar acids found predominantly on the cell-surface glycans of humans and other animals within the Deuterostomes and are also used in the biology of a wide range of bacteria that often live in association these animals. For many bacteria sialic acids are simply a convenient source of food, while for some pathogens they are also used in immune evasion strategies. Many bacteria that use sialic acids derive them from the environment and so are dependent on sialic acid uptake. In this mini-review I will describe the discovery and characterisation of bacterial sialic acids transporters, revealing that they have evolved multiple times across multiple diverse families of transporters, including the ATP-binding cassette (ABC), tripartite ATP-independent periplasmic (TRAP), major facilitator superfamily (MFS) and sodium solute symporter (SSS) transporter families. In addition there is evidence for protein-mediated transport of sialic acids across the outer membrane of Gram negative bacteria, which can be coupled to periplasmic processing of different sialic acids to the most common form, β-D-*N*-acetylneuraminic acid (Neu5Ac) that is most frequently taken up into the cell.

**Introduction**

The sugar acid, *N*-acetylneuraminic acid (Neu5Ac), is the most common sialic acid found in nature and plays key roles in the biology of many organisms[1–5]. In humans and other higher eukaryotes Neu5Ac is often the terminal sugar on glycans and is consequently involved in many cell-cell interactions in the body. Likewise bacteria that have evolved to make a living in humans, and other animals within the Deuterostomes, have also learned to exploit sialic acid as both a nutrient and also to help in immune evasion [1,3,4].

The involvement of sialic acid in the biology of bacteria has been dominated by work in human pathogens[5]. Some of these such as *Campylobacter jejuni* and *Neisseria meningitidis* use sialic acid to alter their cell surface and have dedicated biosynthetic pathways to enable them to produce sialic acid independent of the host[4]. However, many other pathogens and commensals lack this biosynthetic capacity, and are therefore dependent on host-derived sialic acid. Some synthesise and secrete sialidase enzymes to help liberate sialic acid from the host cell surface, while others scavenge what free sialic acid they can find[4].

Bacteria have multiple types of transporters to move small molecules across their inner cytoplasmic membrane. Many of these use an energy source to actively concentrate the molecule inside the cell. The two most widely used families of transporters in bacteria, both of which are mentioned in this review, are the ATP-binding cassette (ABC) transporters that use direct ATP binding and hydrolysis to drive uptake (so called primary transporters) and the major facilitator superfamily (MFS) transporters that use pregenerated ion gradients, usually H+ or $Na^+$, to energise the concentrative movement of substrates across the membrane. The main focus of this short article is to review all published information on experimentally characterised sialic acid transporters in bacteria, within a historical timeline, and then to reflect on the diversity of transporters that have evolved for this purpose across multiple bacterial genera (Fig. 1). Gram negative bacteria that utilise sialic acid also need to move this across their outer membrane too and recent work on movement across the outer membrane will also be included.

## The first transporters – major facilitator superfamily.

The first bacterial sialic acid transporter to be experimentally characterised was in 1985, through the work of Eric Vimr and colleagues [6]. This was in a lab strain of the model organism and gut commensal *Escherichia coli* K-12, which is able to grow on Neu5Ac as the sole source of carbon. Mutants of *E. coli* were isolated that were unable to transport [$H^3$]-Neu5Ac into the cell, which mapped to a locus they called *nanT*, for *N*-acetylneuraminic transporter, that encodes an MFS transporter[7] (Fig. 1). The protein has been purified and reconstituted into proteoliposomes where it behaves as a classical $H^+$-coupled secondary transporter[8], consistent with earlier *in vivo* studies in a related strain[9]. The protein itself is unusual for an MFS family member as it contains 14 predicted transmembrane helices (TMH), instead of the usual 12 TMH see in MFS transporters[7]. The structure is not known, but it will likely resemble other MFS transporters and of published structures it is most similar to the *Staphylococcus epidermidis* glucose transporter[10] and *Escherichia coli* D-xylose transporter XylE[11]. The additional 2 TMH sit at TMH 7-6, between the two 6 TMH repeats that constitute the MFS fold, but their role in the transport process and/or its regulation is currently unknown. Homologues of NanT are found throughout a wide range of human pathogens within the

Enterobacteriaceae including the genera *Salmonella, Yersinia*, *Citrobacter* and *Chronobacter*[12], but none of these have been experimentally characterised. Homologues are also seen in the Bacteroidetes which have been studied including the NanT protein in the oral pathogen *Tannerella forsythia* by the Stafford group[13,14], who have also demonstrated a role for sialic acid uptake for biofilm formation and also survival when exposed to epithelial cells in this organism[14]. A role for NanT in sialic acid uptake has also been demonstrated in the closely related gut bacterium *Bacteroides fragilis*[15].

## Second time lucky – the TRAP transporters.

Further identification of sialic acid transporters in bacteria followed from a number of groups interested in how the Gram negative human pathogen *Haemophilus influenzae* colonises the host. Sialic acid was already known to be important for this bacterium to persist in the host, via an immune evasion mechanism whereby the bacterium uses host-derived sialic acid to coat its own surface lipo-oligosaccharide (LOS), resulting in evasion from innate immune response[16,17]. Identification of the sialic acid catabolic genes in this bacterium revealed the genes for a tripartite ATP-independent periplasmic (TRAP) transporter of unknown function [18–20]. Deletion of genes for either the binding protein component, *siaP*, or the membrane components, *siaQM*, resulted in loss of sialic acid uptake and subsequent sialylation of the LOS and also decreased resistance to growth in the presence of human serum [20,21]. Later a direct role for the TRAP transporter for virulence in a chinchilla model of otitis media was demonstrated, confirming the importance of this single system to the function of *H. influenzae* as a pathogen[22]. This sialic acid transporter has now become the most well studied TRAP transporter in biology. The substrate binding protein (SBP) for this system, SiaP, (Fig. 1) binds a single molecule of sialic acid with high affinity in the low µM to nM range, similar to other TRAP SBPs[21,23–25]. The structure of *H. influenzae* SiaP is also known, being first TRAP SBP to be solved[23] and homologues from a non-typeable strain of *H. influenzae*, *Vibrio cholerae*, *Pasteurella multocida* and *Fusobacterium nucleatum* have now been published[24,25]. The role of different binding site mutants has been investigated, identifying key roles for conserved arginines in the binding site for the coordination of carboxylate group of Neu5Ac[24,26]. The membrane domains of this system, SiaQM, which are fused into a 17 transmembrane helix-containing protein have also been purified and the whole transporter reconstituted and demonstrate to function as a unidirectional high-affinity $Na^+$-dependent secondary transporter[8].

The orthologous SiaPQM system from the human pathogen *Vibrio cholerae* has also been well studied as it was first discovered to be encoded within a pathogenicity island, VPI-2, in a number of toxigenic strains[27,28]. Further work from Fidelma Boyd's group have demonstrated experimentally that sialic acid catabolism is linked to successful host colonisation[29]. Like *H. influenzae* the SiaPQM system is the sole Neu5Ac transporter in this bacterium[30,31], but the system differs from that in *H. influenzae* as the SiaQM subunits are encoded by separate genes rather than fused into a single *siaQM* gene and this true tripartite system was used to demonstrate that all three components of the transporter are essential for function[32]. Additionally sialic acid uptake by an orthologous SiaPQM system has also been shown to be important in two other related Pasteurellaceae, namely *Pasteurella multocida* [33–35] and *Vibrio vulnificus*[36].

## Three's a crowd – ABC transporters for sialic acid

In the same year as the discovery of SiaPQM in *H. influenzae,* there followed the characterisation of a sialic acid transporter from its cousin *Haemophilus ducreyi*[37]. Surprisingly this was not a TRAP transporter, but was instead an ABC transporter (Fig. 1)[37]. It was essential for sialic acid uptake and subsequent modification of the LPS via sialylation. The transporter is encoded by a 4 gene operon, named *satABCD* (for *s*ialic *a*cid *t*ransport), although its functional organisation is unusual for ABC transporters in that the third gene, *satC*, encodes a fused permease and nucleotide binding domain[37] (Fig. 1). Sat-type ABC transporters were predicted in a range of other related bacteria like *Haemophilus somnus* and *Actinobacillus pleuropneumoniae*, but also in selected Gram positives. One of these is the soil bacterium *Corynebacterium glutamicum*, where the function of its *satABCD* system has been experimentally verified and shown to be essential to enable this bacterium to use sialic acid as a sole source of carbon[38]. The binding protein in these systems, SatA, is a member of the cluster C group of oligopeptide-binding proteins[39,40] and there are no currently published structures to understand how the protein coordinates its ligand. More recently a role for a Sat-like ABC transporter in the *nan* cluster of the Gram positive commensal bacterium *Bifidobacterium breve* UCC2003 has been discovered and named *nanBCDF* (which are in fact direct orthologues of *satABCD*) and mutant strains with a disruption in the *nanB* gene, encoding the binding protein subunit, lose the ability to grow on sialic acids as a sole carbon source [41].

A second distinct group of ABC systems specific for sialic acid have been discovered in another subset of Gram positive bacteria, first in *Streptococcus pneumoniae*[42,43], which has been named *satABC*, but are structurally different and lack their own dedicated nucleotide binding protein (Fig. 1), a common feature of carbohydrate ABC transporters in the CUT1 family in which this belongs. Instead they use another ATPase, MsmK, that energises SatABC and also other carbohydrate ABC transporters in this pathogen[44]. The binding protein, SatA (SP1681), is a member of cluster D group of carbohydrate binding proteins[39], and so differs from the *H. ducreyi* SatA. A similar system has been shown to be essential for sialic acid uptake in the Group B Streptococcus (GBS)[45].

## Going forth – SSS transporters

While investigating the sialic acid catabolic genes in *Salmonella enterica* serovar Typhimurium, two gene clusters containing likely genes involved in utilisation of sialic acid were found. One contains a *nanT* gene like in *E. coli*, while the second, that is liked to other sialic acid processing (*nanM*) and outer membrane transport (*nanC*) genes was an uncharacterised member of the sodium solute symporter family (SSS)[46]. Using an *E. coli* transport deletion strain for sialic acid (TD Sialic acid) that is unable to grow on Neu5Ac as the sole carbon source, the heterologously expressed gene, *STM1128*, was able to restore growth, demonstrating a Neu5Ac transport function[47]. This system was not named at the time, but I suggest that the name SiaT is used to represent sialic acid transporters in the SSS family (Fig. 1). This system is also seen in *Vibrio fisheri*, which is known to grow on Neu5Ac[46] and in *Lactobacillus sakei* where a role in uptake has been demonstrated[48]. Also, in other Gram positives there are examples in both *Staphylococcus aureus*[49] and *Clostridium perfringens*[50], which are the only obvious sialic acid transporters from the known families in these organisms. More recently the function of the SSS sialic transporter present in *Clostridium difficile* has been demonstrated to be essential for sialic acid uptake and also for post-antibiotic mediated colonisation of the mouse intestine by this important human pathogen[51]. In this important publication from the Sonnenburg group the transporter has been named *nanT*, which is confusing as it is not homologous to the *nanT* transporters of the MFS system and the name *siaT* should be used

instead. This is also the case with the *S. aureus* protein which was called NanT but is in fact a SiaT[49] While this study show the SSS transporter to be a critical determinant of host colonisation, it is of note that in *Streptococcus pneumoniae*, which has multiple potential sialic acid transporters, the SSS system (SP1328) appears to not be essential for sialic acid transport, while the SatABC system appears to be the main system[42].

## Transport across the outer membrane

Movement of sialic acid across the outer membrane (OM) of Gram negative bacteria is also important and there are now some examples of specific transporters in the outer membrane for sialic acid (as opposed to the cell just being dependent on diffusion through porins). The first system characterised was in *E. coli* and is the NanC porin[52,53], which appears to be involved in sialic acid uptake and is part of the sialic acid inducible *nanCMS* operon in *E. coli*. More recently a more complex system has been identified by the Stafford group in *Tannerella forsythia* and *Bacteroides fragilis*, which features a bi-partite outer membrane protein complex of NanO and NanU[13] . NanO is a TonB-dependent OM porin so by using this energised system can actively catalyse uptake into the periplasm[54]. The NanU protein is a very high affinity sialic acid binding protein that sits on the surface of the OM, presumably in a complex with NanO to initially bind sialic acid before transport by NanO[54]. Once across the outer membrane, Gram negative bacteria often also use a periplasmic sialic acid mutarotase that allows the cell to rapidly convert the α-anomer of Neu5Ac that is released by the action of host or other microbial sialidases, to the β-anomer that is recognise by the transporter in the inner membrane[55].

## Substrate range in known sialic acid transporters

Despite sialic acid being a term that represents a wide range of related nonulosonic acids, almost all studies on transport have focussed on the most common sialic acid, *N*-acetylneuraminic acid (Neu5Ac) (Fig. 1). A recent study in *E. coli* demonstrated that the related sialic acid *N*-glycolyl neuraminic acid (Neu5Gc) could be used as a carbon source [6,56], which is dependent on NanT and which uses the same catabolic pathway as the cell uses for Neu5Ac. This has also been recently demonstrated for NanT in *Tannerella forsythia* also[13]. The SiaPQM system also recognises Neu5Gc [23] and there is experimental evidence that *H. influenzae* can actually catabolise Neu5Gc[57], which in humans is only present from the diet. However, there are many other modified forms of sialic acids that have not been studied that may require their own transporter. For acetylated sialic acids it appears that Gram negative bacteria secrete to the periplasm an *O*-acetylesterase, NanS, (Fig. 1) that removes the acetyl group(s) before transport[58] and some bacteria like Bacteroidetes might secrete this enzymes to aid in sialidase action, which is inhibited by *O*-acetylation[59]. However, for other modifications like phosphorylation, sulfation and methylation there is no experimental characterisation of how (and even if) they are utilised by bacteria[3,60]. There is clearly a vast amount of new biology to be discovered in terms of how different bacteria utilise the diversity of sialic acids present in nature, particularly in the complex environments of animal guts where host-derived sialic acids are abundant and many bacteria compete for a limited supply of this carbon and nitrogen containing food.

## Conclusions

In summary, the past decade has given us a hugely improved understanding of the diversity and importance of sialic acid transporters in bacteria, taking our knowledge from the *E. coli nanT* transporter to now having data on over a dozen different experimentally characterised systems from four different transport families (Fig. 2) and from a diverse range of organisms. It is intriguing that biology has chosen from such a wide range of transporter types for sialic acid uptake. While both ABC and TRAP transporters are high affinity, they differ in how they are energised between ATP hydrolysis (primary transporter) and membrane potential (secondary transporters), respectively, consequently having different energy costs to the cell per molecule of Neu5Ac transported. Likewise the MFS, TRAP and SSS systems are all secondary transporters, but vary in the coupling ions that they use. Also within the ABC transporters it is clear that specificity for sialic acid has evolved twice, demonstrating the importance of this molecule in biology. It would not be surprising if other new sialic acid transporters of new types were discovered with our exponentially increasing knowledge of bacterial genomes. Finally, to end with a plea. There are still no structures for the complete transporters for any of the sialic acid-specific systems described in this review (Fig. 1), a major challenge for structural biology for the next decade. It is clear that for many pathogens sialic acid transport is critical for host colonisation and a clearer mechanistic understanding of transporter function offers new routes to drug design and potential new treatments for numerous dangerous pathogens.

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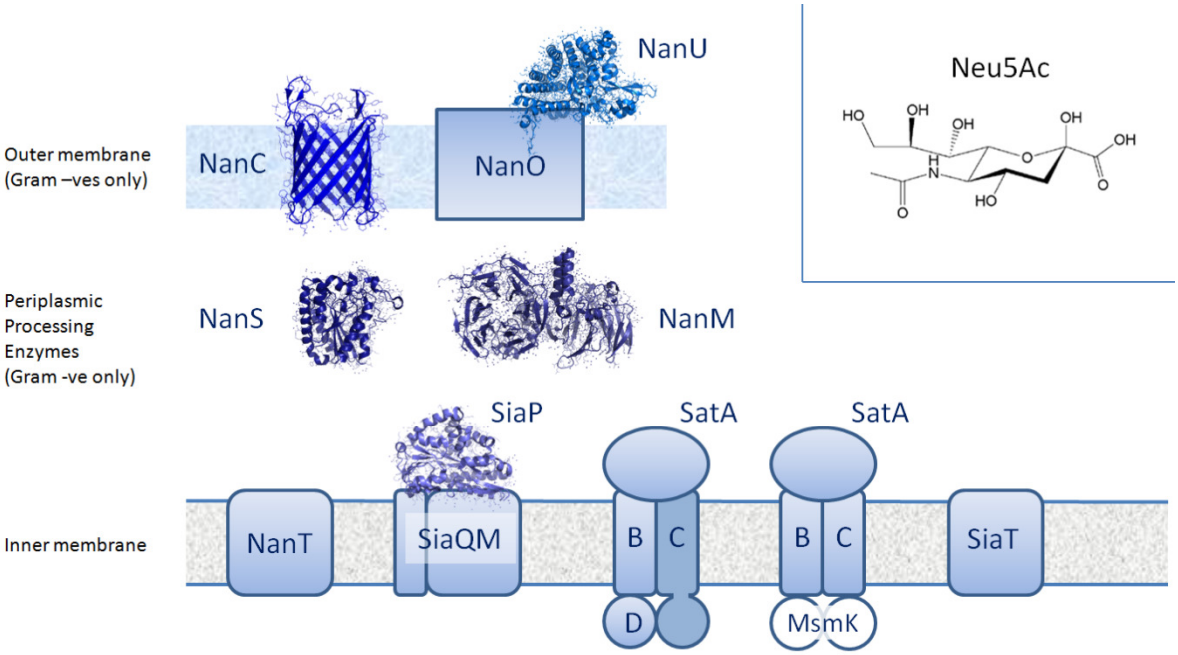


| Transporter family | MFS | TRAP | ABC | | SSS |
|---|---|---|---|---|---|
| Binding protein type | | Cluster E | Cluster C | Cluster D | |
| Archetypal system | *Ec* NanT | *Hi* SiaPQM | *Hd* SatABCD | *Sp* SatABC | *St* SiaT |

*Figure 1.* Schematic representation of the five experimentally defined types of bacterial sialic acid transporters in the inner membrane, two in the outer membrane and two proteins involved in periplasmic processing of sialic acid. Known structures are shown where available at approximately the same scale. Inset: the chemical structure of the common sialic acid Neu5Ac.

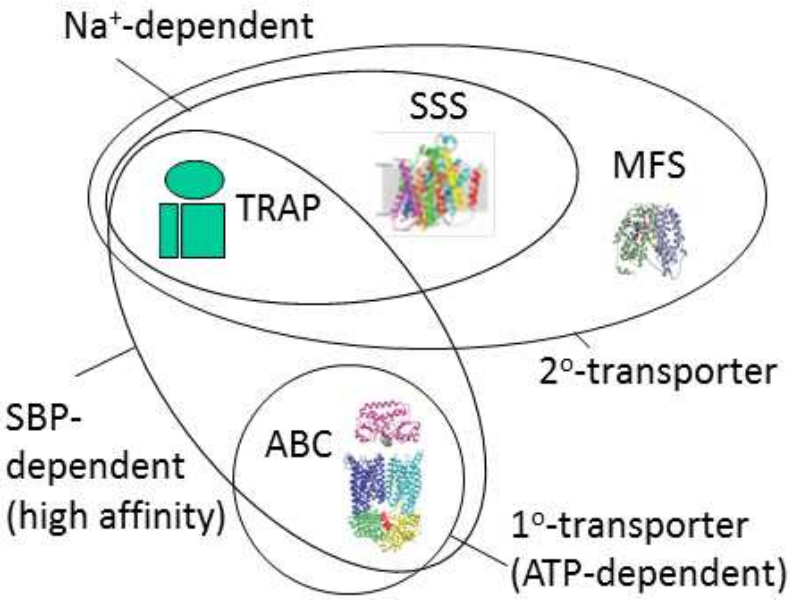


*Figure 2.* Summary of the functional differences between the four known families of bacterial transporter for sialic acid. Representative structures of family members are shown for illustrative purposes only.

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