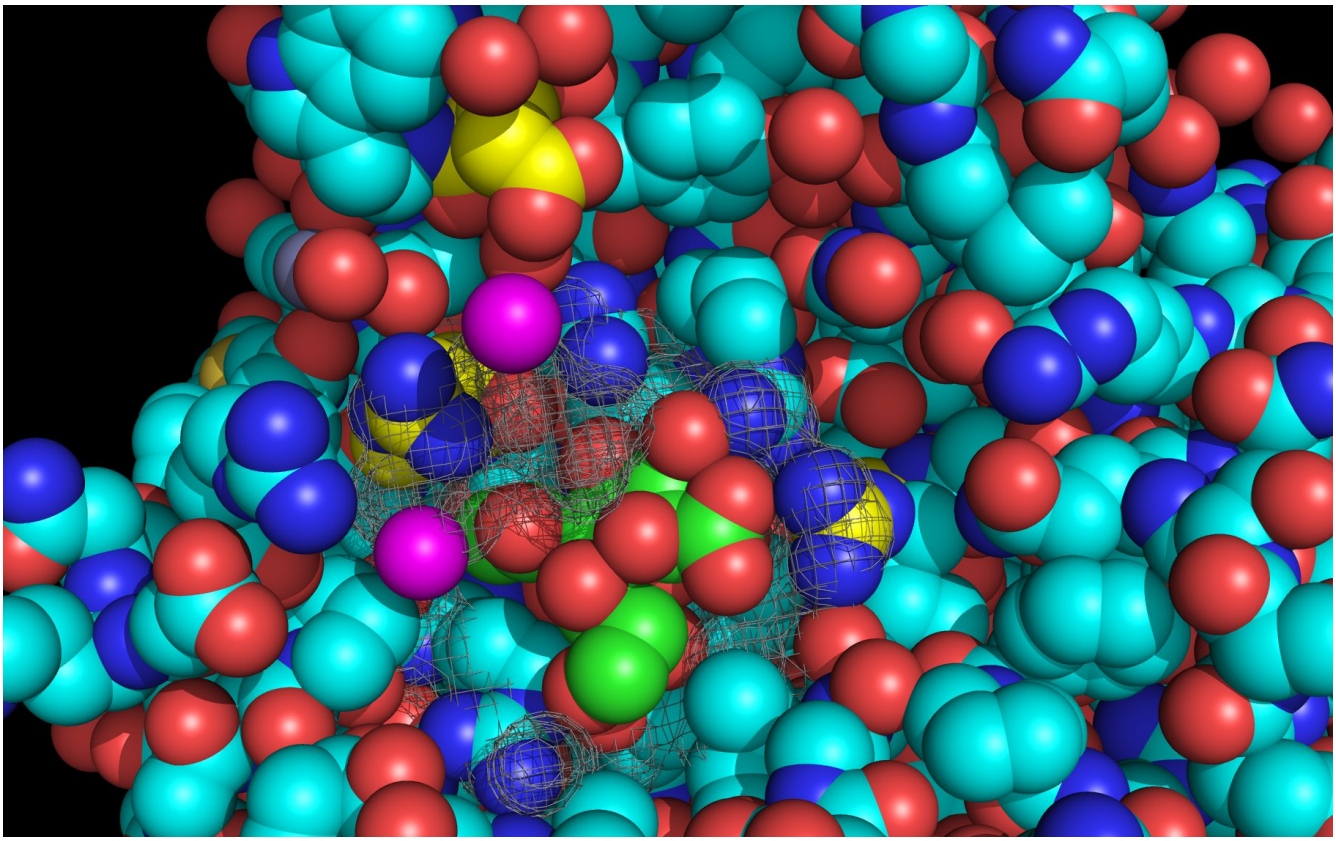
Weininger, A.; Weininger S.;
"Finding a sialic acid binding site in a highly divergent N10 neuraminidase"
Weininger Works Technical Notes (2013) Aug 5;3:1-19



Finding a binding site without sequence identity

The bat influenza virus N10 neuraminidase has 20 to 27 percent sequence identity with other influenza virus sialidases. wwavePDB analysis suggests that the bat influenza virus N10 neuraminidase is a viral enzyme that has maintained its sialic acid binding site without maintaining sequence homology with other influenza virus sialidases. The following pictures and description document the finding of key catalytic residues and the building of a putative sialic acid binding site using wwavePDB and the Weininger Works' program twwistPDB.

Shown here is sialic acid in the wwavePDB-identified putative sialic acid binding site of the bat viral influenza N10 neuraminidase. Publications[6,7] of the structures (4VFK[6] and 4GEZ[7]) of this bat viral influenza N10 neuraminidase have not identified this site and indicate that the N10 neuraminidase does not process MUNANA. wwavePDB analysis delivers a putative sialic acid binding site with functional features consistent with catalysis. This study suggests that the N10 neuraminidase may function under conditions different from the consensus neuraminidases. The wwavePDB-identified putative sialic acid binding site may not be able to bind and process MUNANA as the large size of MUNANA would prevent E153 to E156 loop movement.

wwavePDB produces "WWaveMarkers™, sets of atoms that are used to reorient structures relative to one another. See the discussion of WWaveMarkers™ and the atoms used for neuraminidase

reorientation. WWaveMarkers™ were used to orient the N1, N6, N8, and N9 neuraminidases and associated bound compounds (sialic acid, oseltamivir, zanamivir, and peramivir) relative to one another. wwavePDB also identifies atoms in the promximity of bound compounds; the combined surfaces of these atoms are called "WWaveCores™".

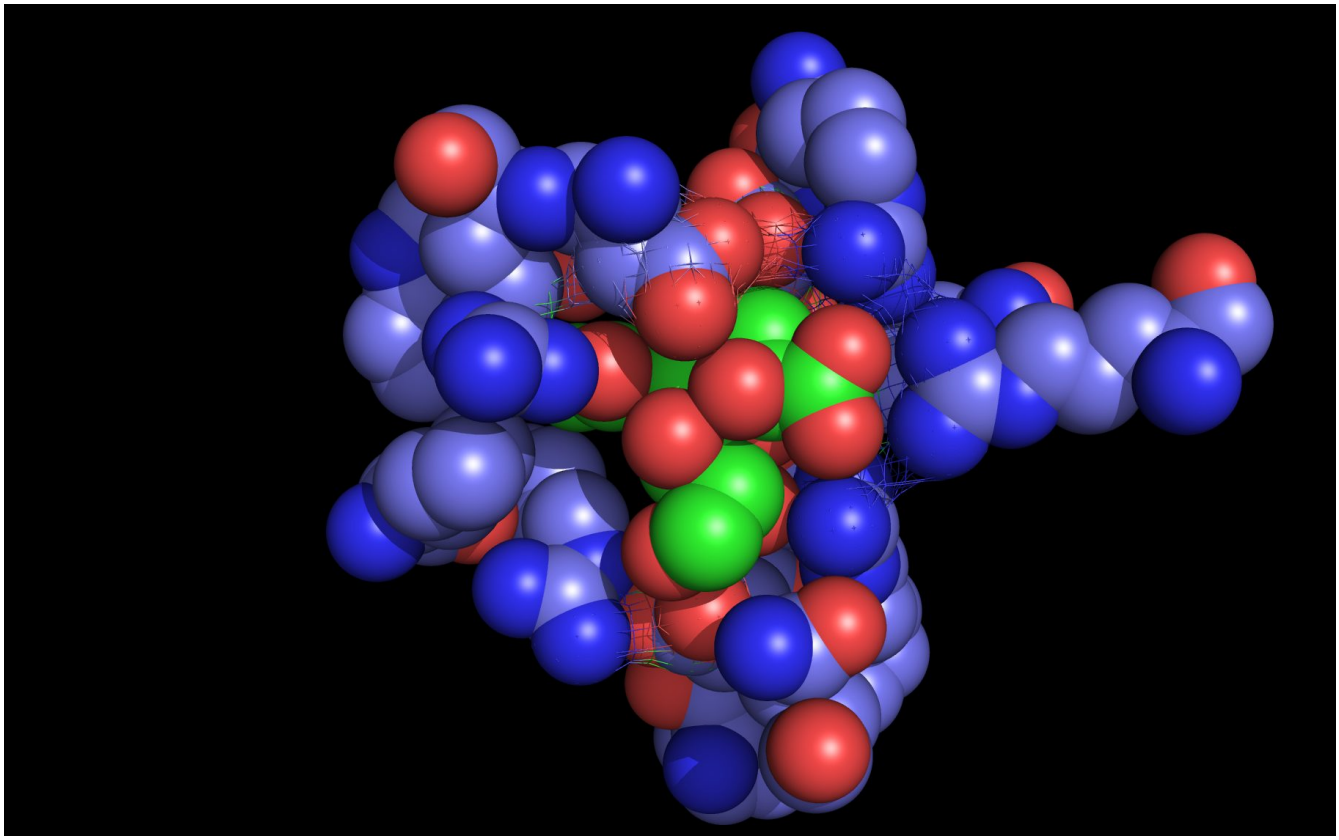
WWaveCores™ were produced by wwavePDB from analysis of the N1, N6, N8, and N9 neuraminidases and their bound compounds. Sialic acid was oriented relative to the WWaveCores™. The N10 structure was reoriented onto the N6 WWaveCores™ using WWaveMarkers™. 1W1X[1] and 4VFK[6] were used as reference structures for identifying the components of the N10 neuraminidase putative sialic acid binding site. Selected side chain positions in 4VFK[6] were altered in order to place wwavePDB-identified 4VFK[6] atoms into WWaveCores™. The sialic acid binding site was formed by reorienting wwavePDB-identified N10 neuraminidase side chain atoms to fill the consensus neuraminidase WWaveCores™. The twwistPDB-reoriented positions of the residues of 4GEZ[7] are substantially the same as 4VFK[6] but the 4GEZ[7] is not shown in the example images because its structure is so close to 4VFK[6] as to add no value in the description.

Selected bound water oxygens are colored magenta.

wwavePDB-reoriented sialic acid spheres are color-coded according to element (C N O).

wwavePDB-reoriented mesh WWaveCores™ are colored grey.

wwavePDB-selected and reoriented 4VFK[6] N10 neuraminidase residue spheres are color-coded according to element (C N O) with the exception that the E153, R178, and R406 residue atom spheres coordinating the sialic acid are colored (C N O).



Using wwavePDB to define the substrate binding site

The neuraminidase structures are first deconstructed by wwavePDB. wwavePDB outputs the features of a sialic acid binding site as sets of atoms. These sets of atoms are used to make WWaveCores™ and WWaveMarkers™.

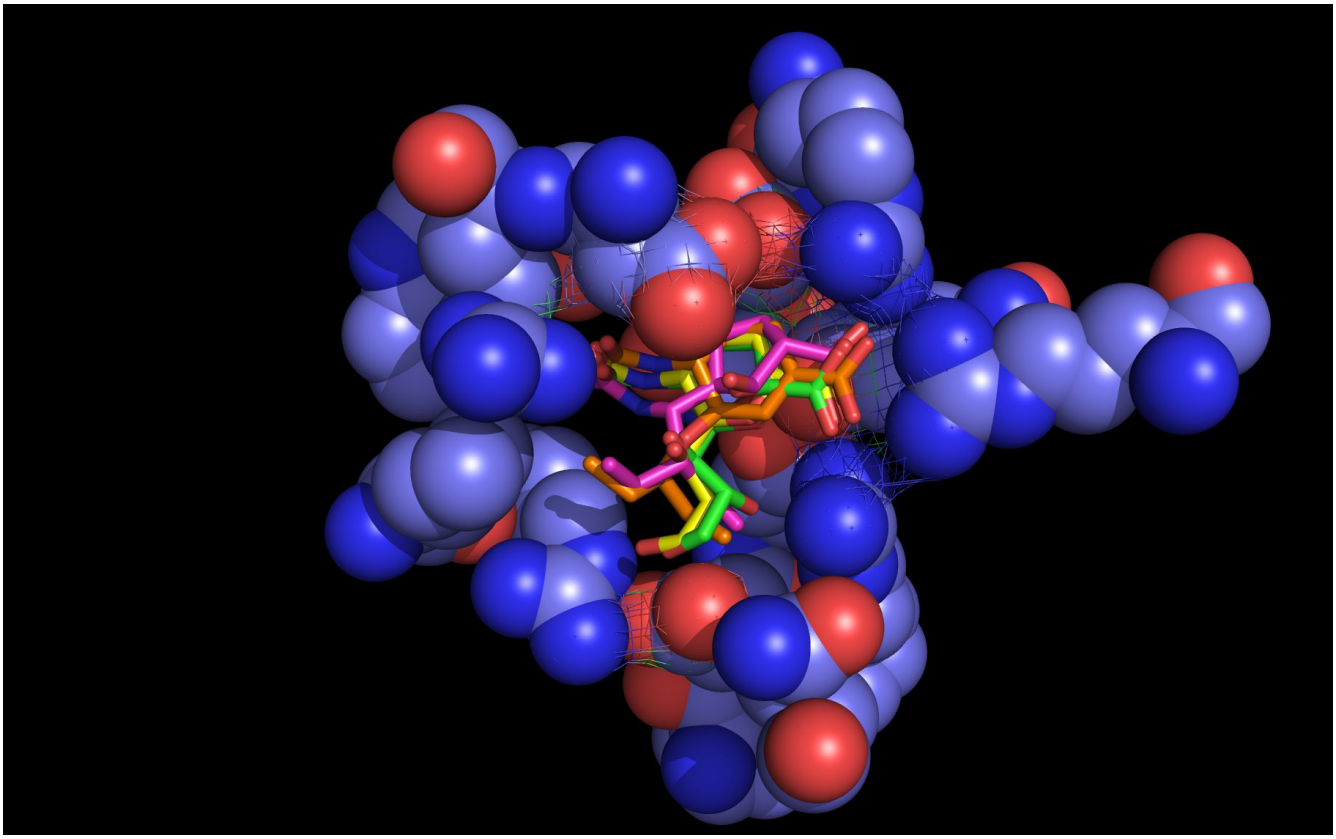
Shown here are:

> wwavePDB-selected residues from an N6 neuraminidase that have been co-crystallized with sialic acid
>
> Mesh N6 WWaveCores™ that enclose a subset of the wwavePDB-identified atoms from the N6 neuraminidase that are in contact with the sialic acid

wwavePDB-reoriented sialic acid spheres are color-coded according to element (C N O).

wwavePDB-reoriented mesh WWaveCores™ are color-coded according to element (C N O).

1W1X[1] selected and reoriented N6 neuraminidase binding site residue spheres are color-coded according to element (C N O).



Using WWave to define inhibitor binding

Shown here are:

WWave-selected residues from an N6 neuraminidase that have been co-crystallized with sialic acid

Mesh N6 WWaveCores™ that enclose a subset of the WWave-identified atoms from the N6 neuraminidase that are in contact with the sialic acid

Inhibitor compounds (oseltamivir, zanamivir, and peramivir) that bind certain N1, N6, N8 and N9 neuraminidases reoriented relative to sialic acid using their neuraminidase WWaveMarkers™ using the Weininger Works' program twwistPDB

WWave-reoriented sialic acid sticks are color-coded according to element (C N O).

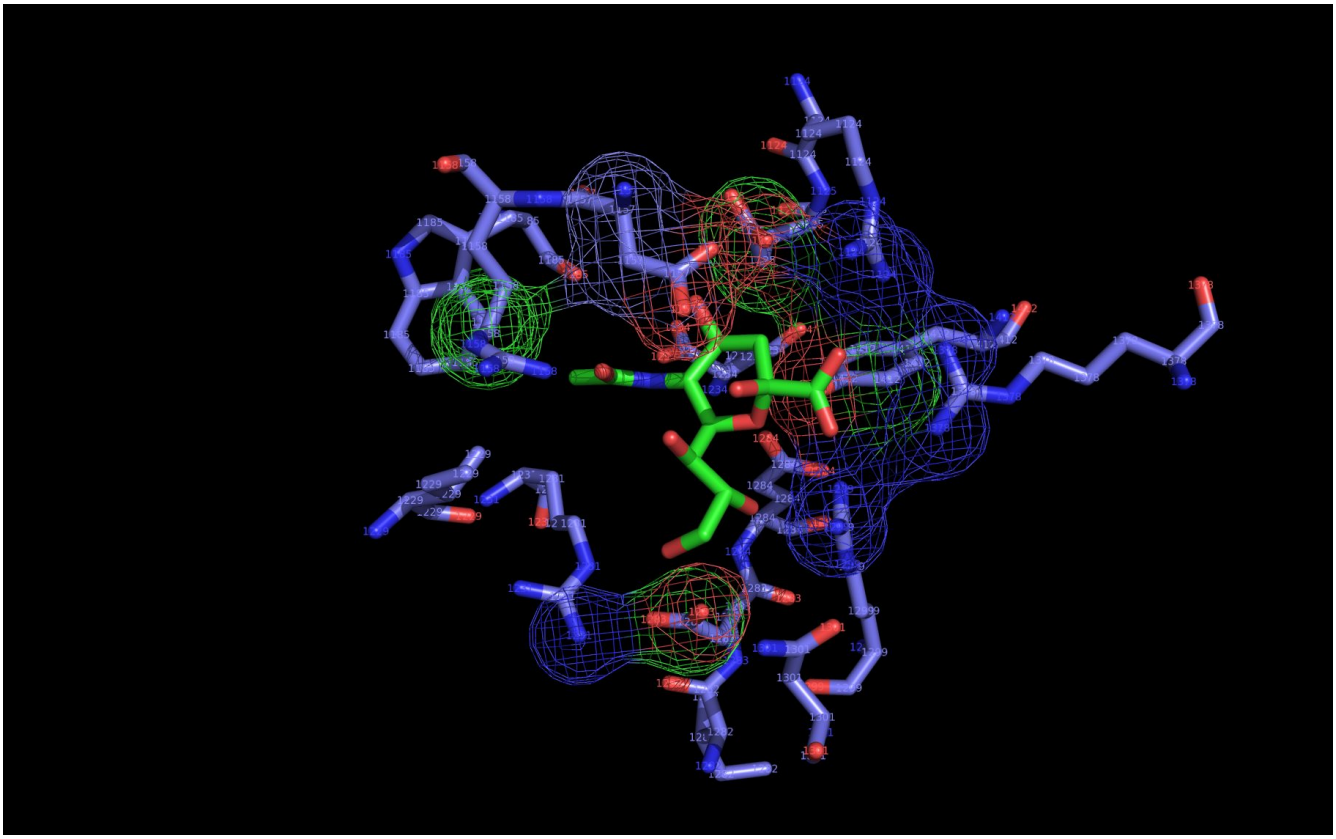
WWave-reoriented oseltamivir sticks are color-coded according to element (C N O).

WWave-reoriented zanamivir sticks are color-coded according to element (C N O).

WWave-reoriented peramivir sticks are color-coded according to element (C N O).

WWave-reoriented mesh WWaveCores™ are color-coded according to element (C N O).

1W1X[1] selected and reoriented N6 neuraminidase binding site residue spheres are color-coded according to element (C N O).



Using WWave to deconstruct the binding site

The WWaveCores™ represent target positions for WWave-identified N10 neuraminidase residue atoms. If target positions within the WWaveCores™ are able to be occupied by N10 neuraminidase residue atoms, then a putative binding site for sialic acid may be constructed. The WWave-selected N6 neuraminidase residue atoms contributing to the WWaveCores™ shown are labelled with residue numbers.

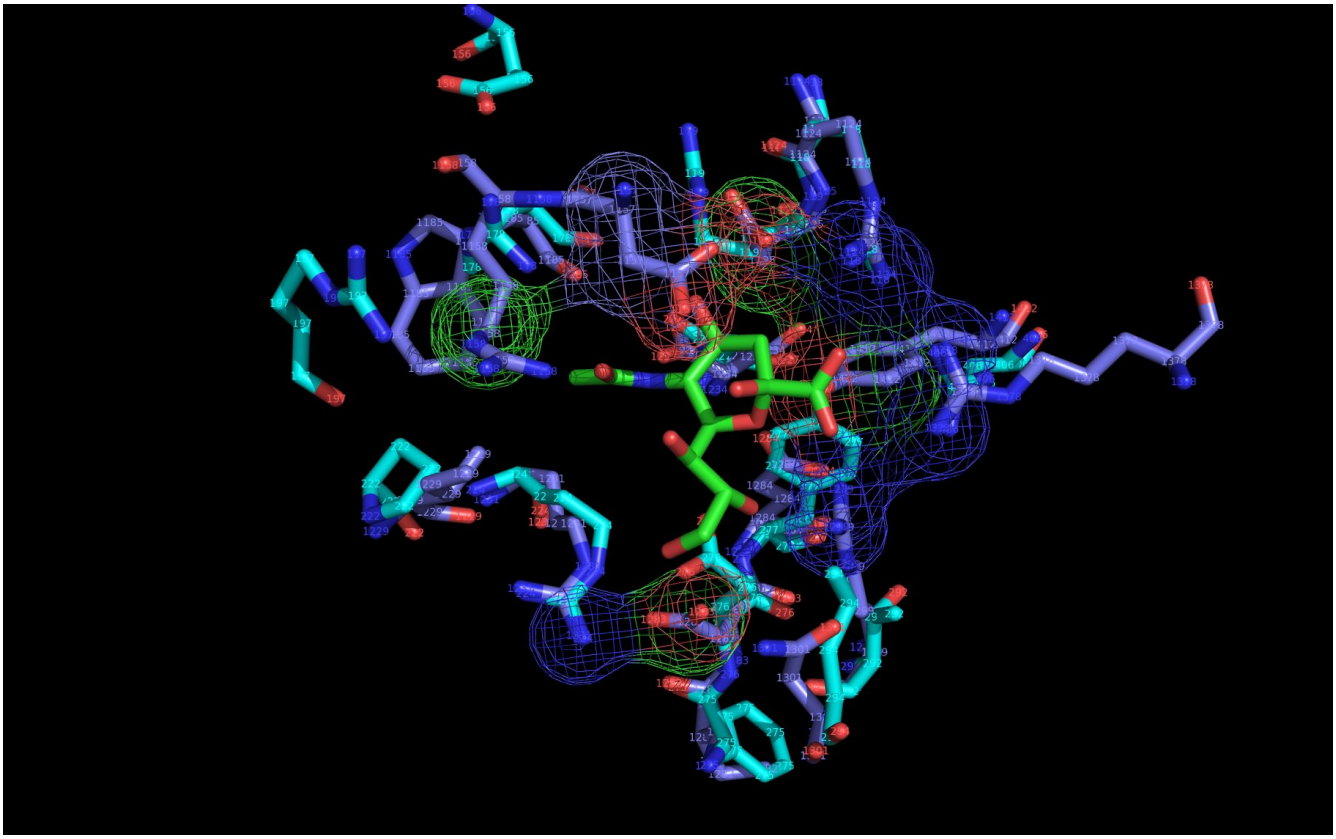
Shown here are:

> WWave-selected, numbered residues from an N6 neuraminidase that have been co-crystallized with sialic acid
>
> Mesh N6 WWaveCores™ that enclose a subset of the WWave-identified atoms from the N6 neuraminidase that are in contact with the sialic acid

WWave-reoriented sialic acid sticks are color-coded according to element (C N O).

WWave-reoriented mesh WWaveCores™ are color-coded according to element (C N O).

1W1X[1]-selected and reoriented N6 neuraminidase binding site residue sticks are color-coded according to element (C N O) and numbered.



Filling the WWaveCores™ to construct the binding site

The WWaveCores™ volume represents the target positions for WWave-identified N10 neuraminidase residue atoms. N10 residues have been repositioned to occupy the WWaveCores™. The N6 and N10 neuraminidase atoms are labelled with the residue number. The sialic acid is positioned relative to the N6 WWaveCores™.

The N10 neuraminidase residues that contribute atoms to the WWaveCores™ that define the sialic acid binding site are positioned differently in the N10 neuraminidase relative to the consensus neuraminidases. Despite this difference in position, the N10 neuraminidase residues can form a binding site with all of the features of a classic sialidase binding site. The N10 neuraminidase has all of the necessary residues in place to bind and produce the transition state of the enzyme for nucleophilic attack of the sialic acid. The constructed N10 neuraminidase sialic acid binding site, however, can not bind the inhibitors oseltamivir, zanamivir, or peramivir.

Shown here are:

WWave-selected, numbered residues from an N6 neuraminidase that have been co-crystallized with sialic acid

Mesh N6 WWaveCores™ that enclose a subset of the WWave-identified atoms from the N6 neuraminidase that are in contact with the sialic acid

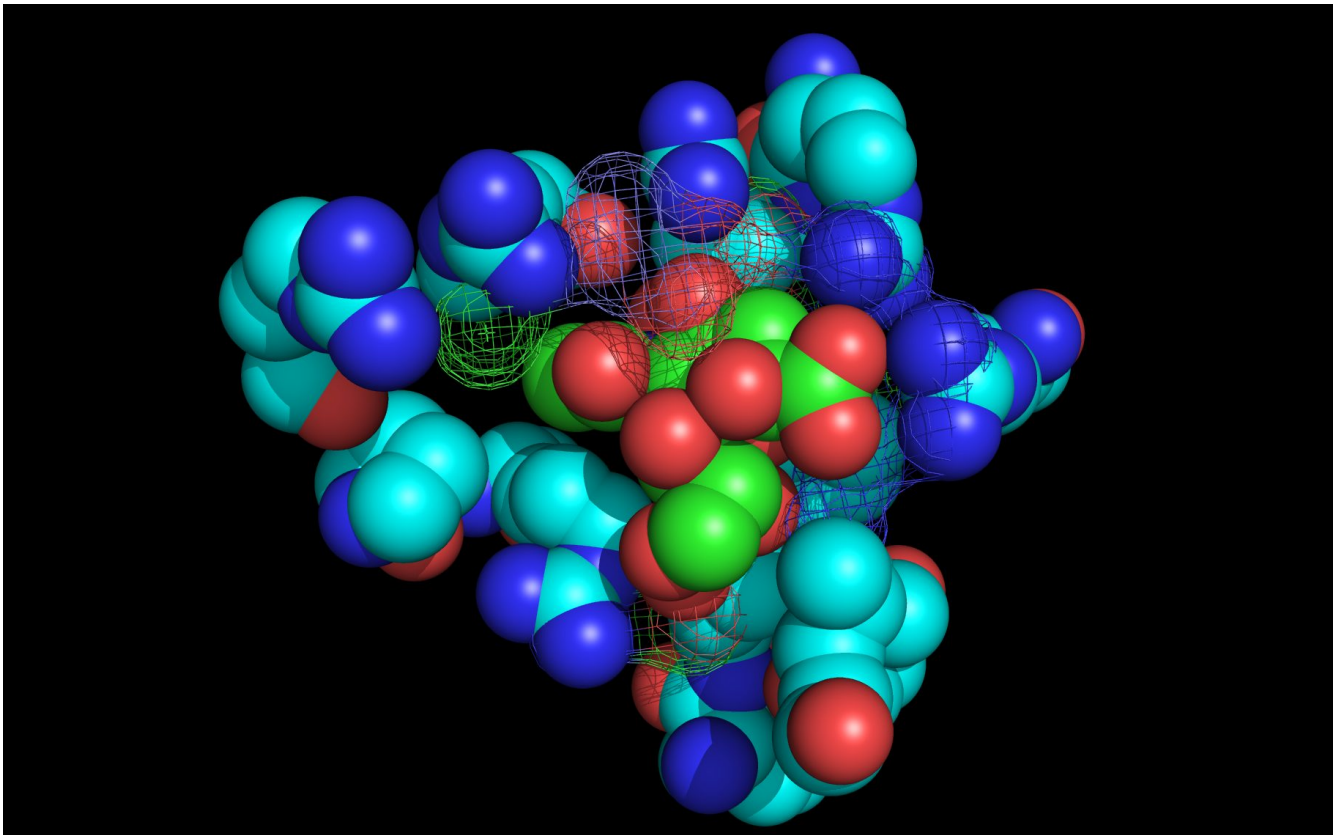
WWave-selected and reoriented 4VFK[6] residues whose side-chains have been reoriented so that key atoms identified by WWave are placed in the WWaveCores™ volume

WWave-reoriented sialic acid sticks are color-coded according to element (C N O).

WWave-reoriented mesh WWaveCores™ are color-coded according to element (C N O).

1W1X[1]-selected and reoriented N6 neuraminidase binding site residue sticks are color-coded according to element (C N O) and numbered.

WWave-selected and reoriented 4VFK[6] N10 neuraminidase residue sticks are color-coded according to element (C N O).



Using WWave to understand structural differences

One of the important differences between the N6 and N10 neuraminidases is that the N6 neuraminidase sialic acid coordinating residues D151 and R152 have been replaced in the N10 neuraminidase by residues that are no longer in the same loop. The WWaveCores™ volume occupied by the consensus D151 and R152 (unfilled red mesh and blue mesh WWaveCores™ to the right of the unfilled green volume) in the N6 neuraminidase are not occupied in the N10 neuraminidase absent the movement of the E153 - E156 loop. There are two N10 neuraminidase arginines shown in the upper left (R178) and far left (R197). R178 of the N10 neuraminidase can move into position to interact with the sialic acid in place of R152 in the N6 neuraminidase. E153 from the N10 neuraminidase can move into position from the E153 - E156 loop and replace the consensus D151 (1157 in the N6 neuraminidase). In N10 neuraminidase, the consensus tryptophan, W178 (W1185 in the N6 neuraminidase) is changed to R178. R178 of the N10 neuraminidase is not impeded by any tryptophan atom (unfilled green mesh WWaveCores™).

Shown here are:

WWave-selected residues from the N10 neuraminidase with twwistPDB-positioned sialic acid

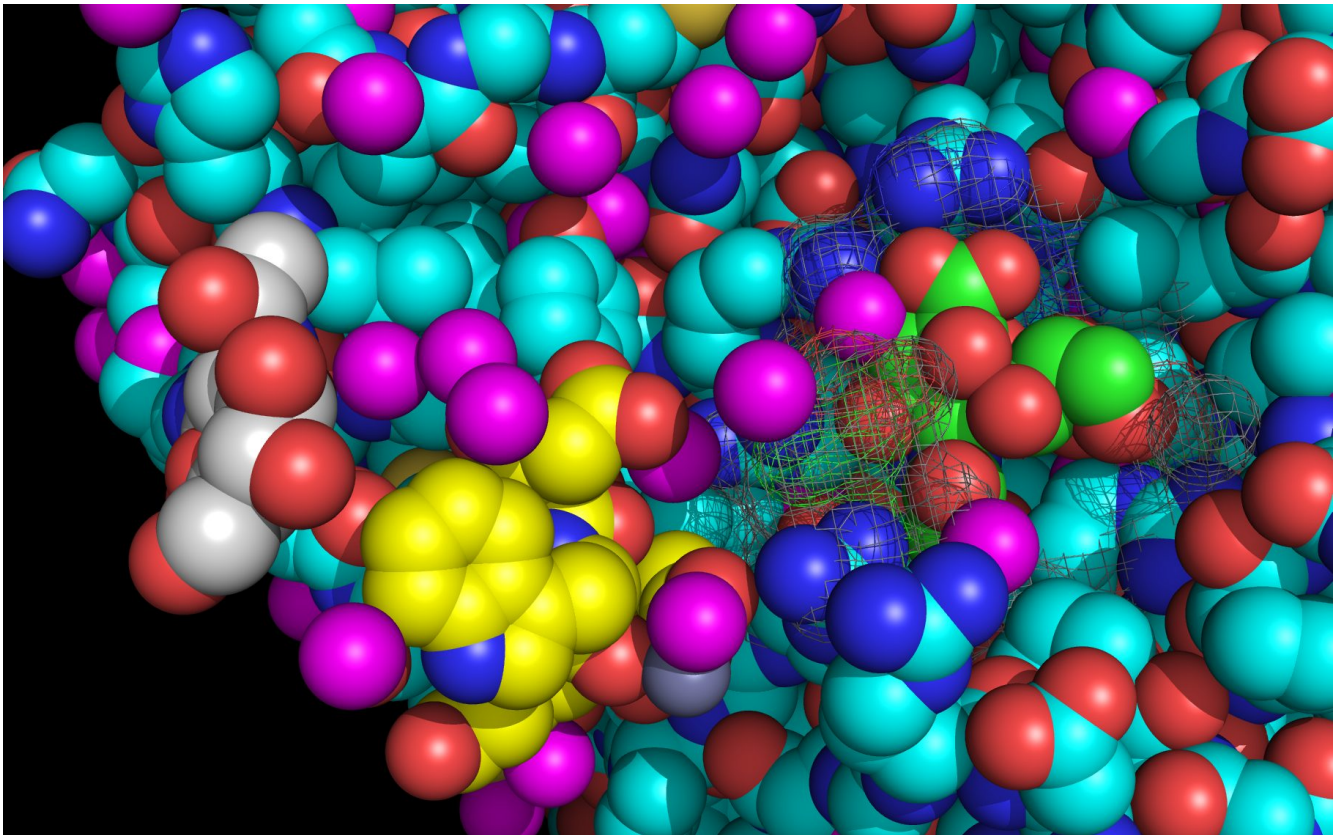
Mesh N6 WWaveCores™ that enclose a subset of the WWave-identified atoms from the N6 neuraminidase that are in contact with the sialic acid

WWave-selected and reoriented 4VFK[6] residues whose side-chains have been reoriented so that key atoms identified by WWave are placed in the WWaveCores™ volume

WWave-reoriented sialic acid spheres are color-coded according to element (C N O).

WWave-reoriented mesh WWaveCores™ are color-coded according to element (C N O).

WWave-reoriented 4VFK[6] N10 neuraminidase residue spheres are color-coded according to element (C N O).



Using WWave to understand functional differences

Researchers have published[6,7] that this N10 neuraminidase does not process MUNANA. The structure of the WWave-identified sialic acid binding site suggests an explanation.

An examination of the WWave-identified putative binding site shows that the N10 neuraminidase has all of the necessary residues in place to bind and stabilize the transition state of the enzyme. Shown above on the left are the carbon atoms (colored yellow) of the N10 neuraminidase residues E153 and R178. N10 neuraminidase residues E153 and R178 replace N1, N6, and N8 neuraminidase consensus residues D151 and R152 in the loop absent from the N10 neuraminidase.

In N1, N6, N8, and N9 neuraminidases, E152 is in a loop that can move the D151 and R152 consensus residues into contact with sialic acid. In the N6 neuraminidase, the consensus residues D151 and R152 are numbered D1157 and R1158.

In the N10 neuraminidase, residue E153 is in a loop containing residues E153 - E156. In the crystal structure, the E155 - E156 loop in the N10 neuraminidase, that replaces the consensus D151 - E152 loop in the N1, N6, N8, and N9 neuraminidases, is constrained by stabilizing molecules. There is a zinc atom coordinated by the loop residues. There is also a bound N-acetyl-d-glucosamine (NAG) shown as white spheres. There are also a number of bound waters. When the waters are displaced, the E153 from N10 neuraminidase can coordinate the sialic acid in place of consensus D151. Unrestrained loop movement that closes the configuration, stabilizes the transition state, and assists in ejecting cleaved products is critical to enzyme function. If any component of the substrate prevents this movement, it will prevent enzyme function.

Shown here are:

WWave-selected residues from the N10 neuraminidase with twwistPDB-positioned sialic acid

Mesh N6 WWaveCores™ that enclose a subset of the WWave-identified atoms from the N6 neuraminidase that are in contact with the sialic acid

WWave-selected and reoriented 4VFK[6] residues whose side-chains have been reoriented so that key atoms identified by WWave are placed in the WWaveCores™ volume

Residues E153, W154, E155, and E156 are color coded (C N O)

Bound water oxygens are colored magenta.

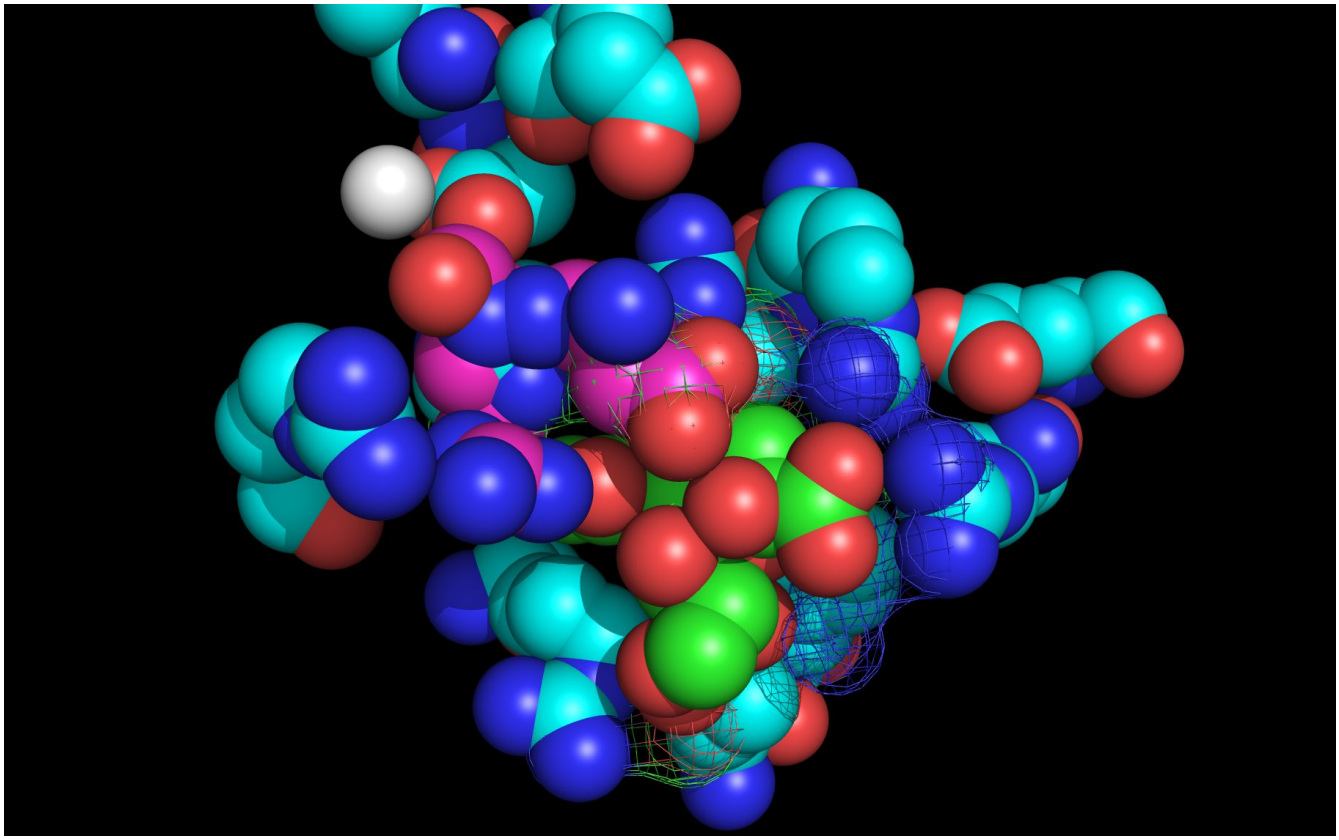
The zinc atom is shown as a grey sphere.

NAG molecule spheres are color-coded according to element (C N O).

WWave-reoriented sialic acid spheres are color-coded according to element (C N O).

WWave-reoriented mesh WWaveCores™ are color-coded according to element (C N O).

WWave-selected and reoriented 4VFK[6] N10 neuraminidase residue spheres are color-coded according to element (C N O), with the exception that E153, W154, E155 and E156 are colored (C N O).



Using WWave to understand sequence divergence

The consensus neuraminidase residues D151 and R152, missing in the N10 neuraminidase but superimposed on the WWave identified N10 binding site, show the close and critical contact of these residues with sialic acid.

Shown here are:

Sialic acid in the constructed WWaveCores™-defined N10 neuraminidase sialic acid binding site.

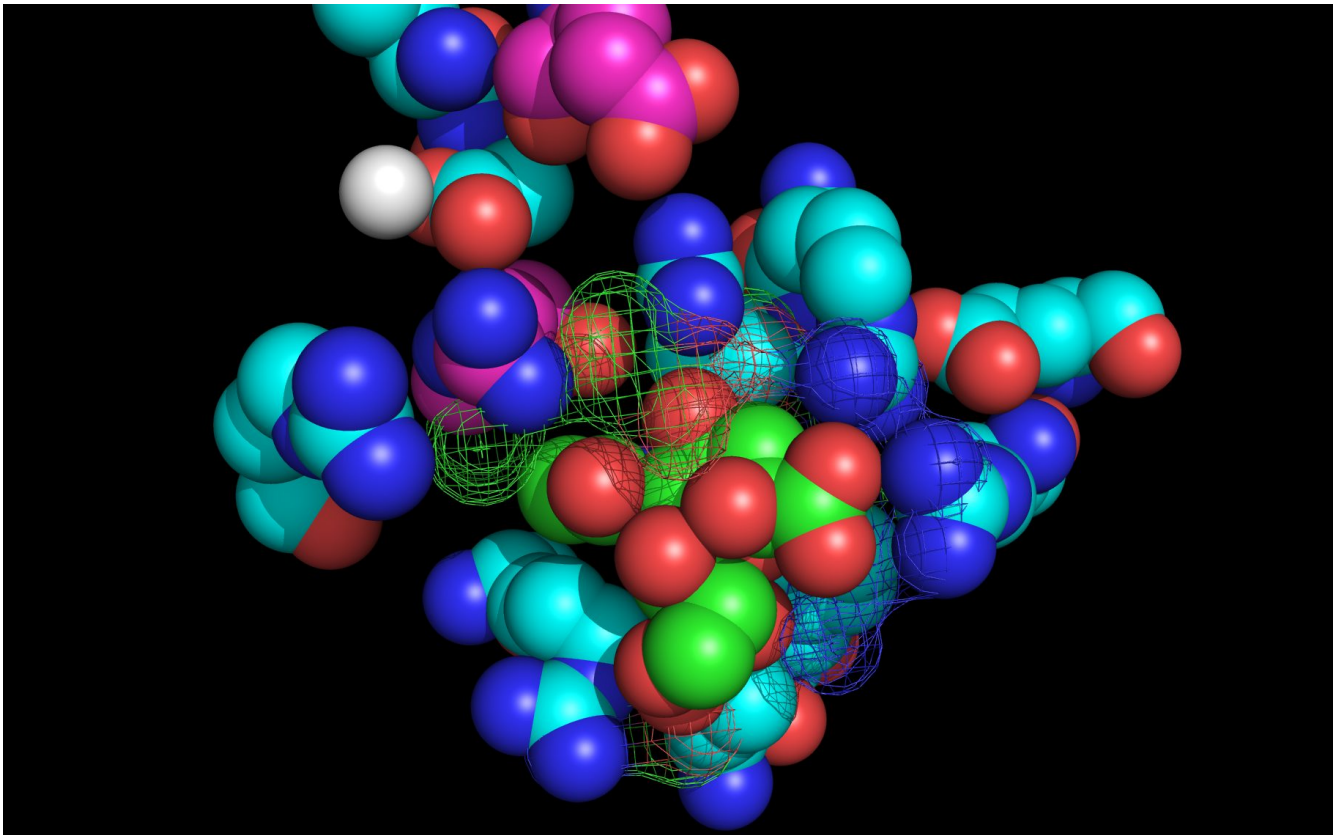
Consensus residues D151 and R152 (numbered D1157 and R1158 in the N6 neuraminidase), color coded (C N O) and superimposed on the WWave-identified N10 binding site.

WWave-reoriented sialic acid spheres are color-coded according to element (C N O).

WWave-reoriented mesh WWaveCores™ are color-coded according to element (C N O).

WWave-selected and reoriented 4VFK[6] N10 neuraminidase residue spheres are color-coded according to element (C N O).

1W1X[1]-selected and reoriented N6 neuraminidase residues D1157 and R1158 spheres are color-coded according to element (C N O) and numbered.



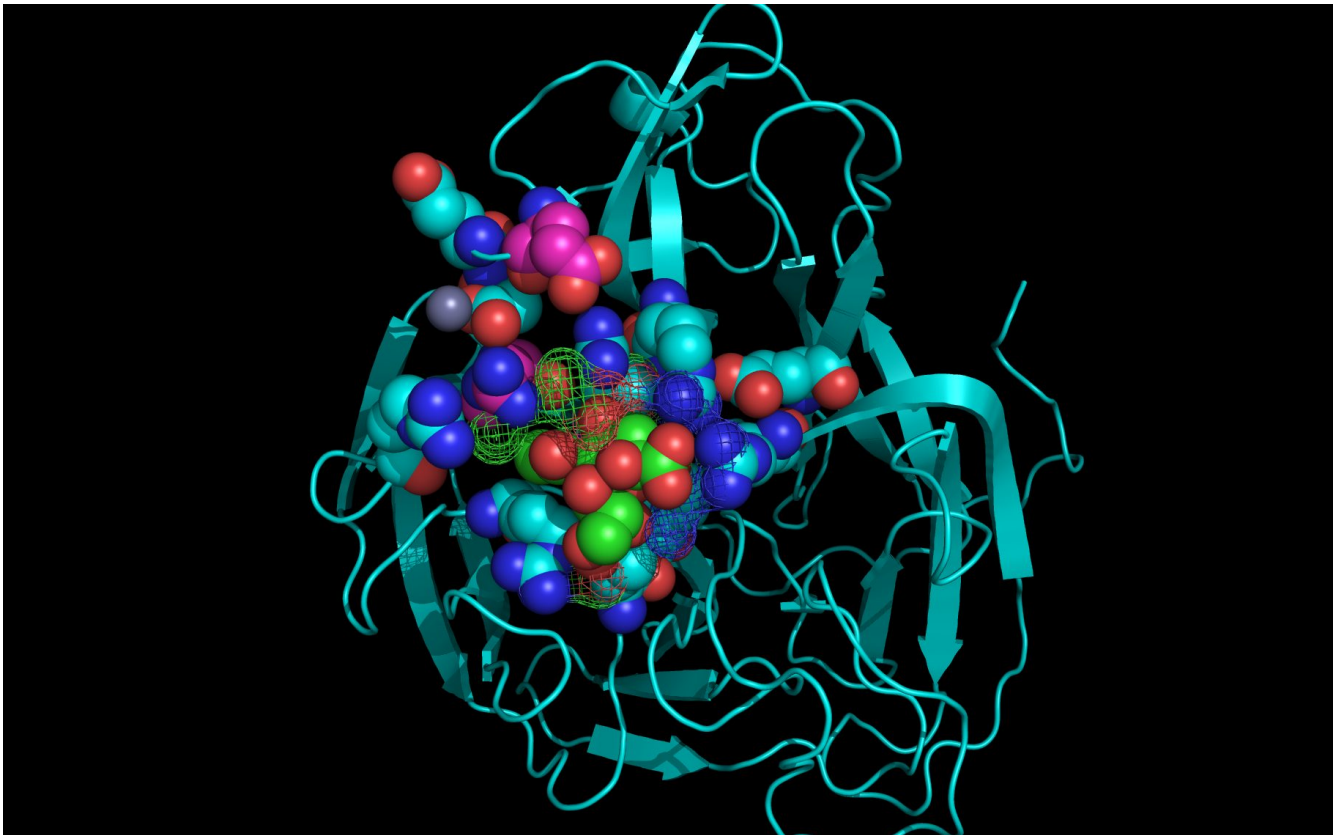
Using WWave to understand sequence divergence

Shown are N10 residues E153 and R178 (color coded C N O) which can move into the same positions in the N6 WWaveCores™ as consensus residues D151 and R152.

WWave-reoriented sialic acid spheres are color-coded according to element (C N O).

WWave-reoriented mesh WWaveCores™ are color-coded according to element (C N O).

WWave-selected and reoriented 4VFK[6] N10 neuraminidase residue spheres are color-coded according to element (C N O) with the exception that the E153 and R178 carbons are color coded magenta.



Using WWave to isolate elements of function

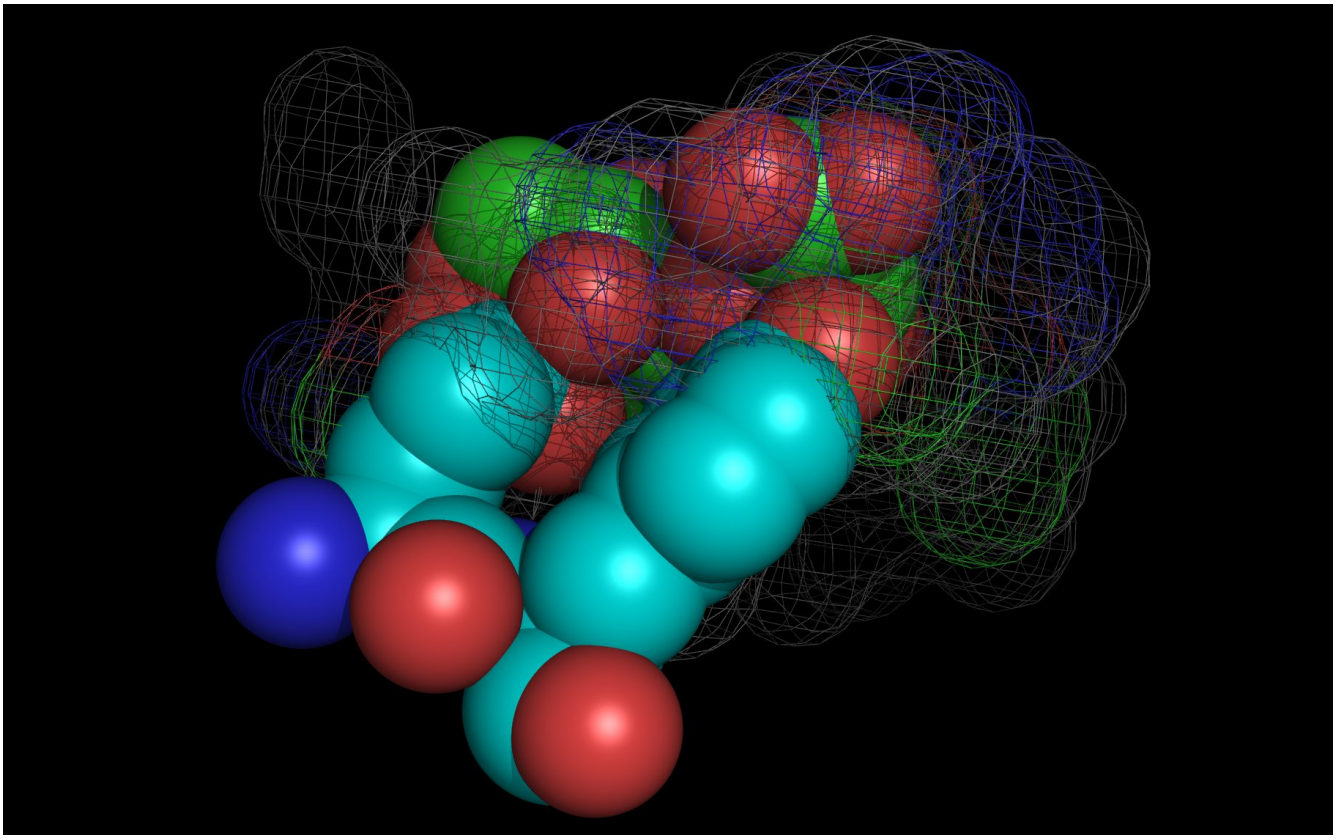
A cartoon of the N10 neuraminidase structure shows the N10 neuraminidase residue E153 (in the loop containing E153 - E156) which can move into the same position in the N6 WWaveCores™ as consensus residue D151. N10 neuraminidase residue R178 can move into the same position in the N6 neuraminidase WWaveCores™ as consensus residue R152.

WWave-reoriented sialic acid spheres are color-coded according to element (C N O).

WWave-reoriented mesh WWaveCores™ are color-coded according to element (C N O).

WWave-selected and reoriented 4VFK[6] N10 neuraminidase residue spheres are color-coded according to element (C N O) with the exception that the E153 and R178 carbons are color coded magenta.

WWave-reoriented N10 neuraminidase cartoon is colored cyan.



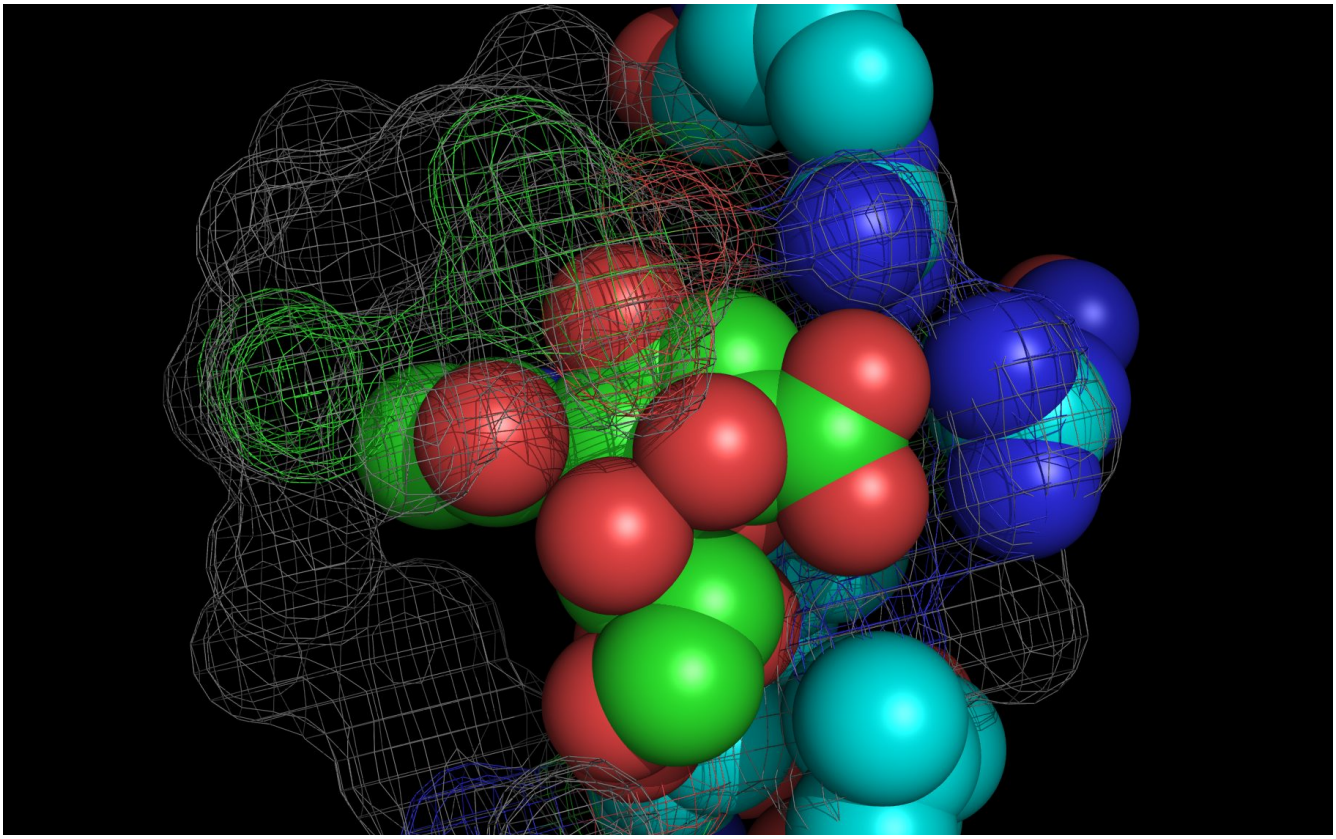
Filling WWaveCores™ with the binding site atoms

The N10 residue E276 and Y277 side chains have been reoriented to place certain atoms in the WWaveCores™ similarly to the consensus neuraminidases. The OH-contributing consensus residue Y406 is replaced by the N10 neuraminidase residue Y277. Reoriented residue Y277 can make contact with the sialic acid using its OH; this key transition state contact is maintained. In particular, the OH of residue Y277 is positioned to set up nucleophilic attack of the sialic acid with the adjacent residue E276.

WWave-reoriented sialic acid spheres are color-coded according to element (C N O).

WWave-reoriented mesh WWaveCores™ are color-coded according to element (C N O).

WWave-selected and reoriented 4VFK[6] N10 neuraminidase residue spheres are color-coded according to element (C N O).



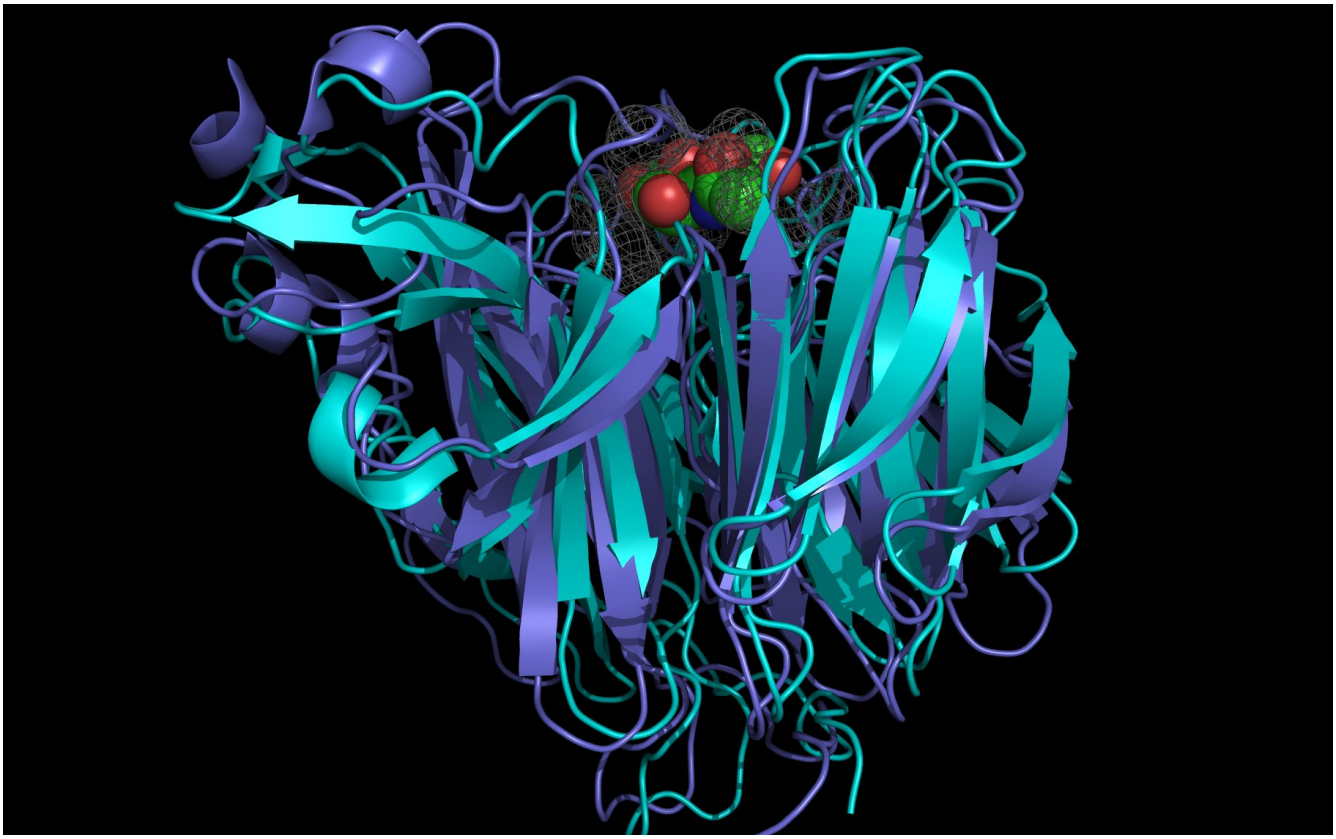
Using WWave to understand catalysis and inhibition

The N10 residue side chains have been reoriented to place certain atoms in the WWaveCores™ similarly to the N6 neuraminidase. The problematic consensus R292 (in the N6 neuraminidase this residue is labelled R1299) whose mutation to lysine confers resistance to oseltamivir, zanamivir, and peramivir has been mutated further by the bat virus N10 neuraminidase in position 292 to threonine (R292T) and coupled to a change at position 294 (N294L). Although the N10 neuraminidase has removed the arginine stabilization of sialic acid by one of the three arginine residues forming contact with the O1A and O1B oxygen atoms of the sialic acid; this change may not adversely affect enzyme function. The R292T mutation coupled to the N294L mutation ensures that oseltamivir, zanamivir, and peramivir will not bind to the N10 neuraminidase. The consensus residue R371 (residue 1378 in the N6 neuraminidase) is replaced by residue R406 in the N10 neuraminidase. Residue R406 is in a position to coordinate the O1A and O1B oxygens on the sialic acid, a critical enzymatic function.

WWave-reoriented sialic acid spheres are color-coded according to element (C N O).

WWave-reoriented mesh WWaveCores™ are color-coded according to element (C N O).

WWave-selected and reoriented 4VFK[6] residues T292 and L294 (bottom right), R406 (middle right), and R118 (top right) residue spheres from the N10 neuraminidase are color-coded according to element (C N O).



Using WWave to find corresponding features

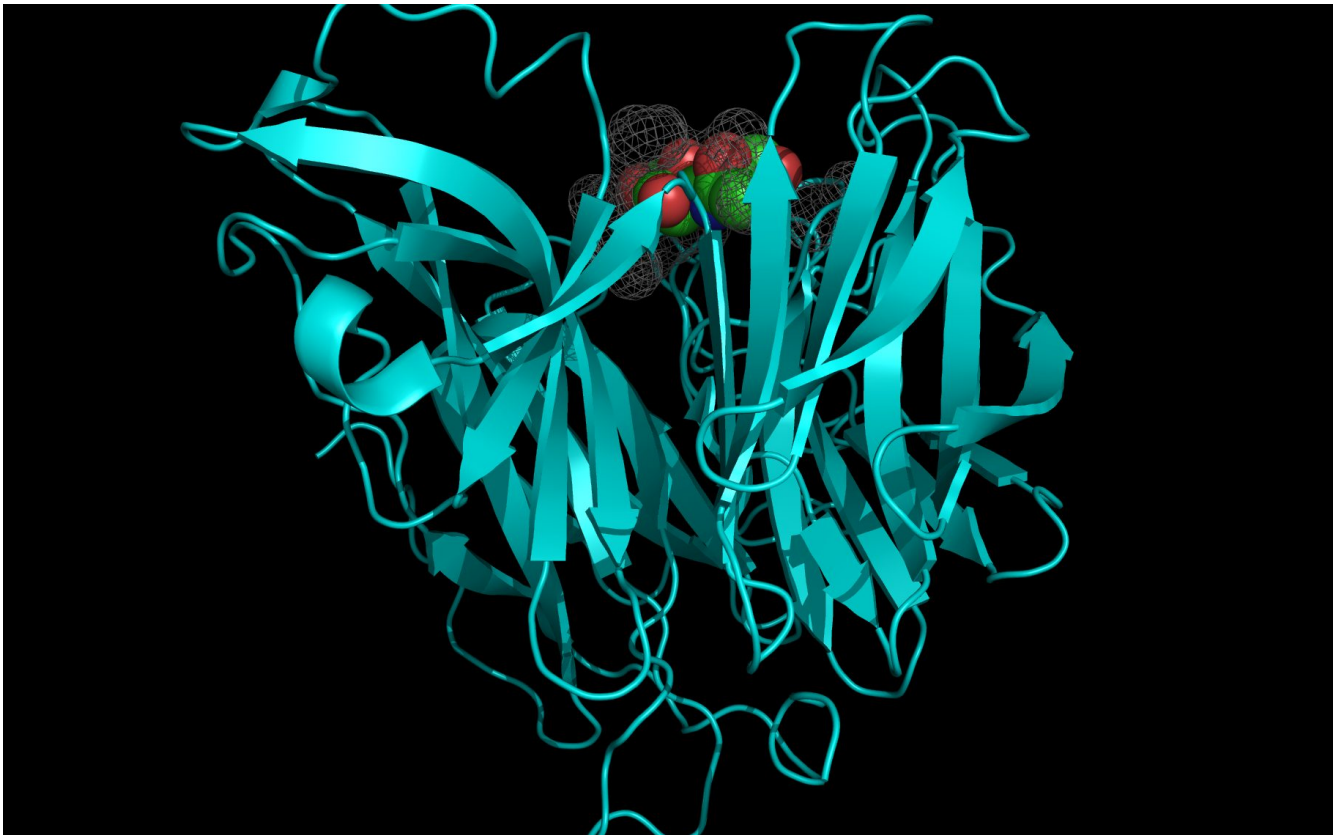
Shown here are WWave-reoriented N6 and N10 neuraminidase cartoons and sialic acid in WWaveCores™ (grey mesh). The WWave-orientation of the N10 and N6 neuraminidase structures shows the close structural correspondences between the structures.

WWave-reoriented sialic acid spheres are color-coded according to element (C N O).

WWave-reoriented mesh WWaveCores™ are colored grey.

WWave-reoriented 4VFK[6] N10 neuraminidase cartoon is color-coded cyan.

WWave-reoriented 1W1X[1] N6 neuraminidase cartoon is color-coded purple.



Using WWave to understand binding through structure

WWave can find binding sites in sequence divergent targets, a critical pharmaceutical design capability.

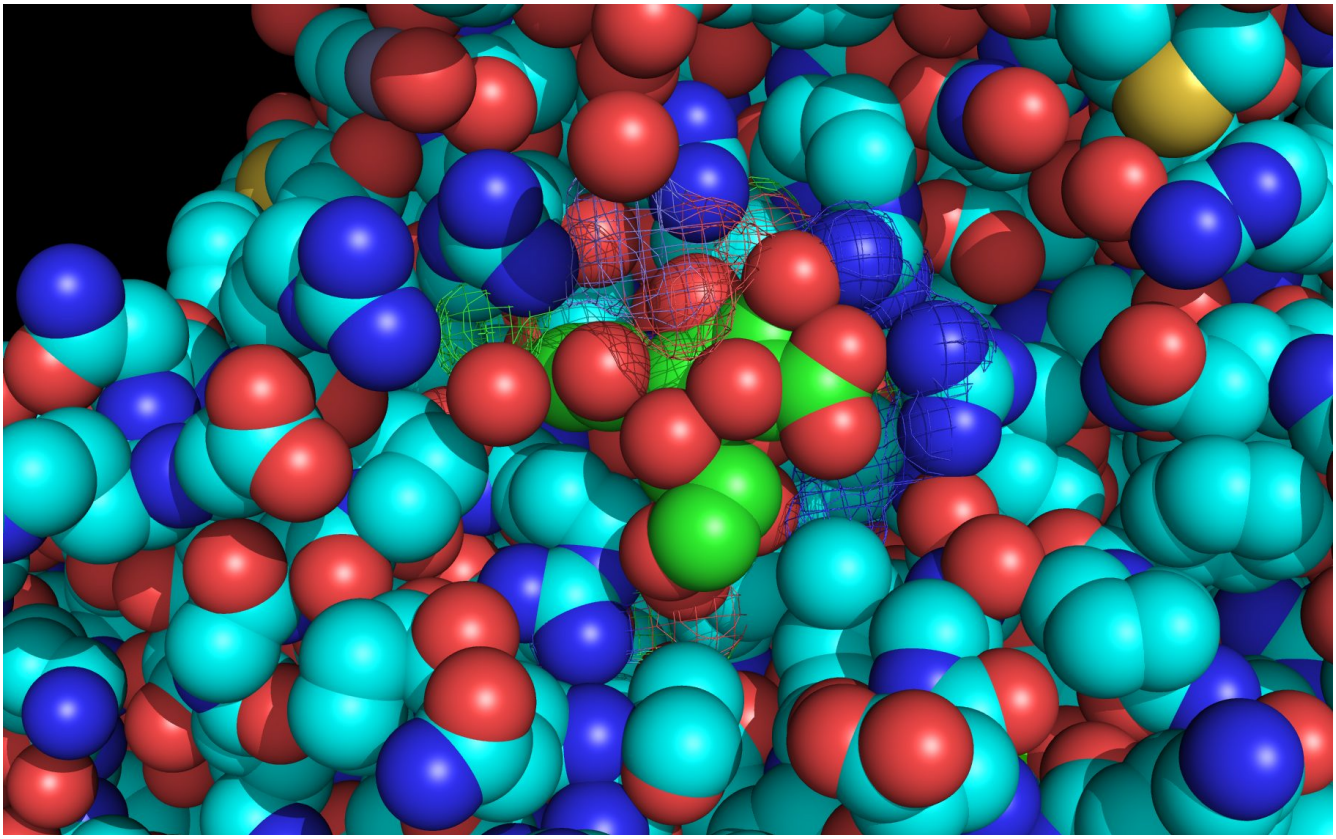
Summary of the study:

> WWave analysis of N10 neuraminidase identifies a putative binding site with all of the features of an active site.
>
> WWave analysis of N10 neuraminidase suggests possible reasons for the difference in activity of the N10 neuraminidase under conditions that permit N6 neuraminidase activity.

WWave-reoriented sialic acid spheres are color-coded according to element (C N O).

WWave-reoriented mesh WWaveCores™ are colored grey.

WWave-reoriented 4VFK[6] N10 neuraminidase cartoon is color-coded according to element (C N O).



WWave – a critical pharmaceutical design tool

Shown here is the WWave identified and constructed putative sialic acid binding site of the bat influenza N10 neuraminidase.

WWave-reoriented sialic acid spheres are color-coded according to element (C N O).

WWave-reoriented mesh WWaveCores™ are colored grey.

WWave-reoriented 4VFK[6] N10 neuraminidase spheres are color-coded according to element (C N O S).

WWave – where drug discovery surfaces™