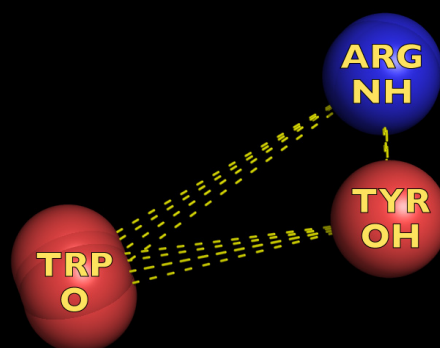


Weininger, A.; Weininger S.
“Using WWaveMarkers™ and building WWaveCores™”
Weininger Works Technical Notes (2013) Jul 22;2:1-19



Conserved Geometry in the Neuraminidase Binding Pocket

Neuraminidase active site mapping using wwavePDB and twistPDB

In this example, one set of WWaveMarkers™ was derived from wwavePDB analysis of six closely related neuraminidase structures. 3B7E² was used as the reference structure onto which the other neuraminidase structures were mapped. A seventh structure, 4FVK⁶, and an eighth structure, 4GEZ⁷, were of a highly divergent N10 neuraminidase from a bat virus. The first set of WWaveMarkers™ could not be used with the N10 neuraminidase structures due to mutations of N10 neuraminidase residues whose atoms were in the first set of WWaveMarkers™. A second set of WWaveMarkers™ was derived from the wwavePDB analysis of 4FVK⁶ and 4GEZ⁷ relative to the reference structure 3B7E². The structures analyzed in this study were:

- 1W1X¹ neuraminidase subtype N6 in complex with sialic acid
- 2HTU³ neuraminidase subtype N8 in complex with peramivir (BioCryst)
- 2HU4³ neuraminidase subtype N1 in complex with oseltamivir (Roche)
- 3B7E² neuraminidase subtype N1 (1918 a/brevig) in complex with zanamivir (GSK)
- 2QWA⁴ neuraminidase subtype N9 with R292K mutation
- 1A14⁵ neuraminidase subtype N9 with engineered VH and VL antibody domains bound
- 4FVK⁶ neuraminidase subtype N10 with divergent sequence
- 4GEZ⁷ neuraminidase subtype N10 with divergent sequence

The first set of neuraminidase WWaveMarkers™ (atom serial # - residue - residue #) were:

PDB	NH	O	OH
3B7E ²	#269 ARG 118	#744 TRP 178	#2468 TYR 406
1W1X ¹	#3308 ARG 1124	#3779 TRP 1185	#5497 TYR 1412
2HTU ³	#279 ARG 118	#753 TRP 180	#2489 TYR 411
2HU4 ³	#270 ARG 118	#741 TRP 178	#2458 TYR 406
2QWA ⁴	#269 ARG 118	#744 TRP 178	#2531 TYR 406
1A14 ⁵	#296 ARG 118	#774 TRP 178	#2533 TYR 406

The distances (D, in Angstroms) between the first set of neuraminidase WWaveMarkers™ are:

PDB	D(O ↔ NH)	D(O ↔ OH)	D(NH ↔ O)
3B7E ²	744 ↔ 269: 9.706	744 ↔ 2468: 8.834	269 ↔ 2468: 4.321
1W1X ¹	3779 ↔ 3308: 9.961	3779 ↔ 5497: 8.654	3308 ↔ 5497: 4.267
2HTU ³	753 ↔ 279: 9.987	753 ↔ 2489: 8.617	279 ↔ 2489: 4.552
2HU4 ³	741 ↔ 270: 9.732	741 ↔ 2458: 8.735	270 ↔ 2458: 4.329
2QWA ⁴	744 ↔ 269: 9.919	744 ↔ 2531: 8.464	269 ↔ 2531: 4.261
1A14 ⁵	774 ↔ 269: 10.558	774 ↔ 2533: 8.587	269 ↔ 2533: 4.773

The first set of neuraminidase WWaveMarkers™ are shown in the image above color coded according to element (**N** **O**).

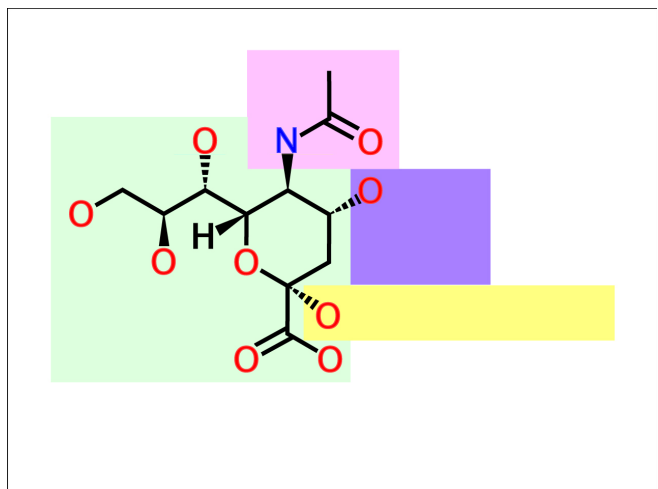
The second set of neuraminidase WWaveMarkers™ (atom serial # - residue - residue #) were:

PDB	O	O	O
3B7E ²	#262 ARG 118	#1089 ARG 224	#1487 GLU 276
4FVK ⁶	#3169 ARG 118	#3974 ARG 224	#4385 GLU 276
4GEZ ⁷	#8859 ARG 111	#9664 ARG 215	#10075 GLU 267

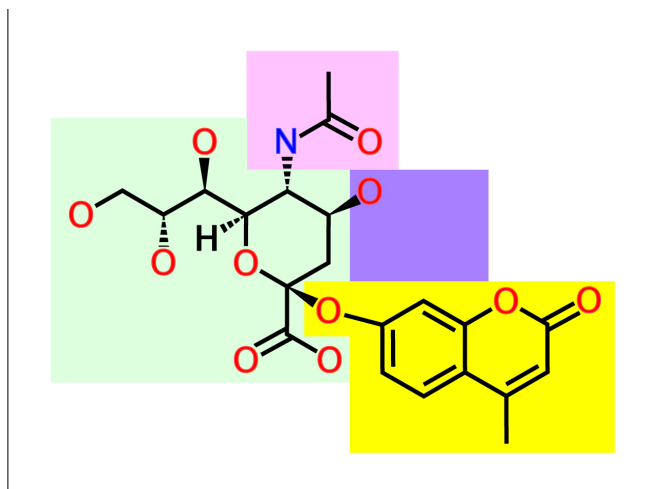
The distances (D, in Angstroms) between the second set of neuraminidase WWaveMarkers™ are:

PDB	D(O ↔ NH)	D(O ↔ OH)	D(NH ↔ O)
3B7E ²	262 ↔ 1089: 14.730	262 ↔ 1487: 15.001	1089 ↔ 1487: 8.332
4FVK ⁶	3169 ↔ 3974: 14.197	3169 ↔ 4385: 15.190	3974 ↔ 4385: 8.793
4GEZ ⁷	8859 ↔ 9664: 14.178	8859 ↔ 10075: 15.353	9664 ↔ 10075: 8.924

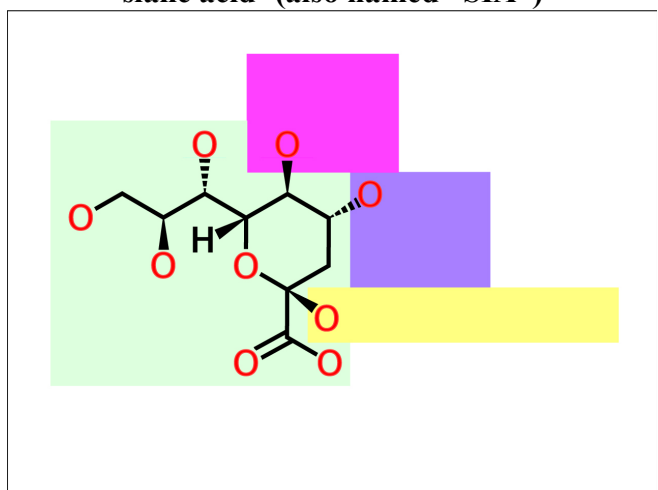
Neuraminidase binding compounds



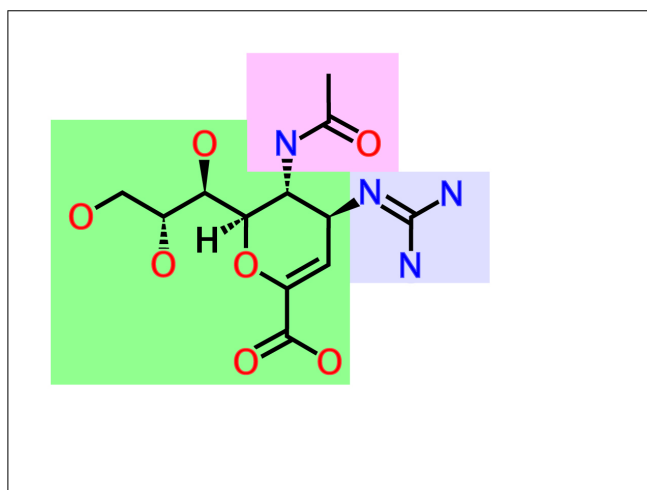
sialic acid¹ (also named “SIA”)



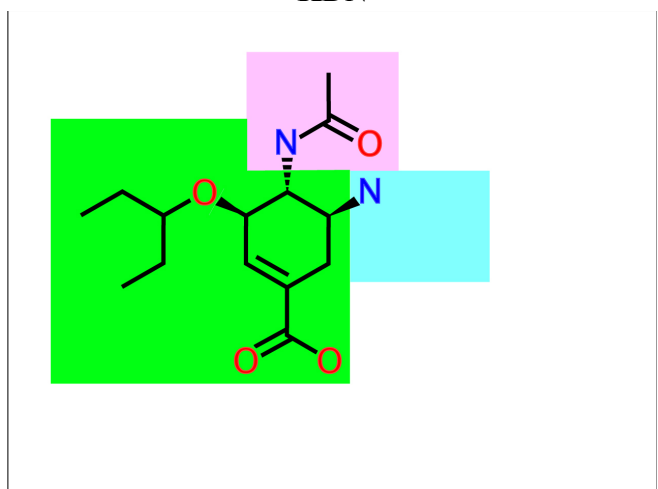
MUNANA



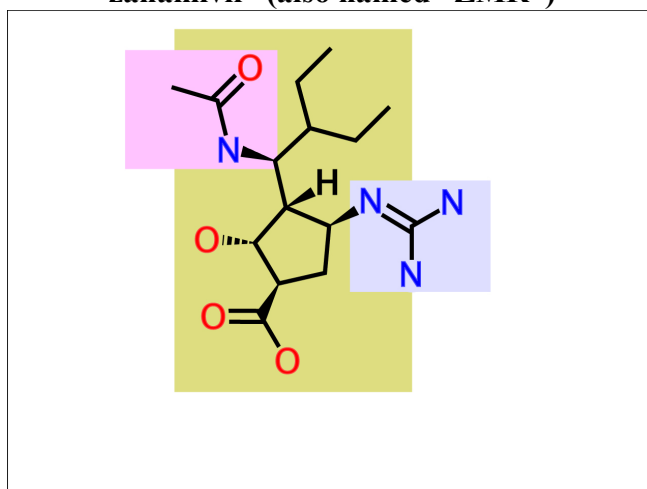
KDN



zanamivir² (also named “ZMR”)



oseltamivir³ (also named “G39”)



peramivir³ (also named “BCZ”)

(The following SMILES highlight colors match above binding compound structure highlight colors.)

sialic acid (also named "SIA")

IUPAC Name: (2R,4S,5R,6R)-5-acetamido-2,4-dihydroxy-6-[(1R,2R)-1,2,3-trihydroxypropyl]oxane-2-carboxylic acid

SMILES: CC(=O)N[C@H]1[C@H]([C@H](O)[C@H](O)CO)O[C@](C(=O)O)(O)C[C@@H]1O

MUNANA (also named "MUS")

IUPAC Name: (2S,4S,5R,6R)-5-acetamido-4-hydroxy-2-(4-methyl-2-oxochromen-7-yl)oxy-6-[(1R,2R)-1,2,3-trihydroxypropyl]oxane-2-carboxylic acid

SMILES: CC(=O)N[C@H]1[C@H]([C@H](O)[C@H](O)CO)O[C@H](C(=O)O)(Oc2cc3oc(=O)cc(C)c3cc2)C[C@@H]1O

KDN

IUPAC Name: (2S,4S,5R,6R)-2,4,5-trihydroxy-6-[(1R,2R)-1,2,3-trihydroxypropyl]oxane-2-carboxylic acid

SMILES: O[C@H]1[C@H]([C@H](O)[C@H](O)CO)O[C@](O)(C(=O)O)C[C@@H]1O

zanamivir (also named "ZMR")

IUPAC Name: (2R,3R,4S)-3-acetamido-4-(diaminomethylideneamino)-2-[(1R,2R)-1,2,3-trihydroxypropyl]-3,4-dihydro-2H-pyran-6-carboxylic acid

SMILES: CC(=O)N[C@H]1[C@H]([C@H](O)[C@H](O)CO)OC(C(=O)O)=C[C@@H]1N=C(N)N

oseltamivir (also named "G39")

IUPAC Name: (3R,4R,5S)-4-acetamido-5-amino-3-pentan-3-yloxycyclohexene-1-carboxylic acid

SMILES: CC(=O)N[C@H]1[C@H](OC(CC)CC)C=C(C(=O)O)C[C@@H]1N

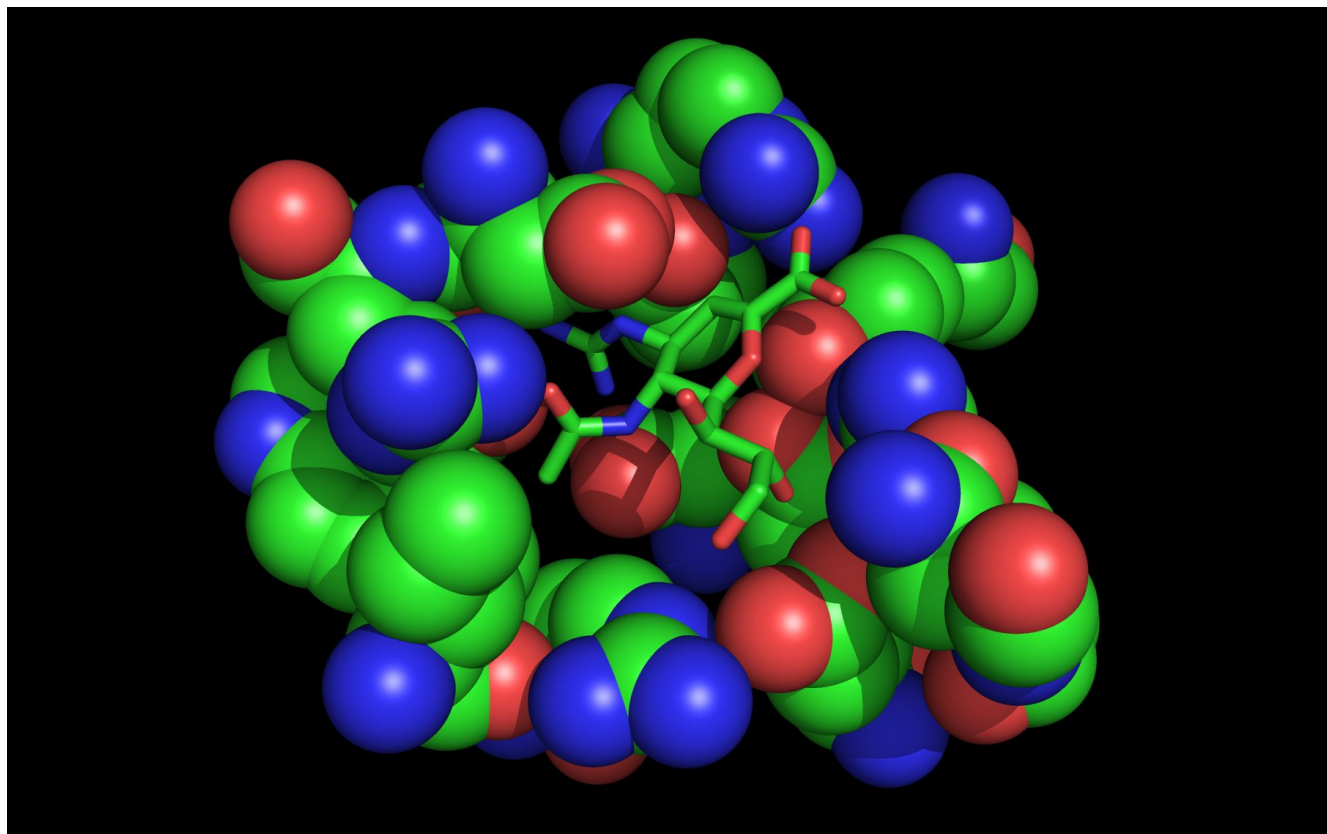
peramivir (also named "BCZ")

IUPAC Name: (1S,2S,3S,4R)-3-[(1S)-1-acetamido-2-ethylbutyl]-4-(diaminomethylideneamino)-2-hydroxycyclopentane-1-carboxylic acid

SMILES: CC(=O)NN[C@@H](C(CC)CC)[C@@H]1[C@H](O)[C@@H](C(=O)O)C[C@H]1N=C(N)N

References

- ¹ 1W1X (neuraminidase complexed with sialic acid).
Rudino-Pinera, E.; Tunnah, P.; Crennell, S.J.; Webster, R.G.; Laver, W.G.; Garman, E.F.;
“The Crystal Structure Of Type A Influenza Virus Neuraminidase Of The N6 Subtype
Reveals The Existence Of Two Separate Neu5ac Binding Sites”
To Be Published/REVDAT 2H24-FEB-09 1W1X
- ² Protein Data Bank ID: 3B7E (neuraminidase complexed with zanamivir).
Xu, X.; Zhu, X.; Dwek, R.A.; Stevens, J.; Wilson, I.A.;
“Structural characterization of the 1918 influenza virus H1N1 neuraminidase.”
J. Virology (2008) 82: 10493-10501
- ³ 2HTU (neuraminidase complexed with peramivir).
2HU4 (neuraminidase complexed with oseltamivir).
Russell, R.J.; Haire, L.F.; Stevens, D.J.; Collins, P.J.; Lin, Y.P.;
Blackburn, G.M.; Hay, A.J.; Gamblin, S.J.; Skehel, J.J.;
“The structure of H5N1 avian influenza neuraminidase suggests new opportunities
for drug design.”
Nature (2006) 443: 45-49
- ⁴ 2QWA (R292K mutant neuraminidase).
Varghese, J.N.; Smith, P.W.; Sollis, S.L.; Blick, T.J.; Sahasrabudhe, A.;
McKimm-Breschkin, J.L.; Colman, P.M.;
“Drug design against a shifting target: a structural basis for resistance
to inhibitors in a variant of influenza virus neuraminidase.”
Structure (1998) 6: 735-746
- ⁵ 1A14 (neuraminidase complexed with VH and CL antibody domains).
Malby, R.L.; McCoy, A.J.; Kortt, A.A.; Hudson, P.J.; Colman, P.M.;
“Three-dimensional structures of single-chain Fv-neuraminidase complexes.”
J.Mol.Biol. (1998) 279: 901-910
- ⁶ 4FVK (divergent neuraminidase isolated from bats).
Li, Q.; Sun, X.M.; Li, Z.X.; Liu, Y.; Vavricka, C.J.; Qi, J.X.; Gao G.F.;
“Structural and functional characterization of neuraminidase-like molecule n10
derived from bat influenza a virus.”
Proc.Natl.Acad.Sci.USA (2012) 109: 18897-18902
- ⁷ 4GEZ (divergent neuraminidase isolated from bats).
Zhu, X.; Yang, H.; Guo, Z.; Yu, W.; Carney, P.J.; Li, Y.; Chen, L.M.;
Paulson, J.C.; Donis, R.O.; Tong, S.; Stevens, J.; Wilson, I.A.;
“Crystal structures of two subtype N10 neuraminidase-like proteins from
bat influenza A viruses reveal a diverged putative active site.”
Proc.Natl.Acad.Sci.USA (2012) 109: 18903-18908

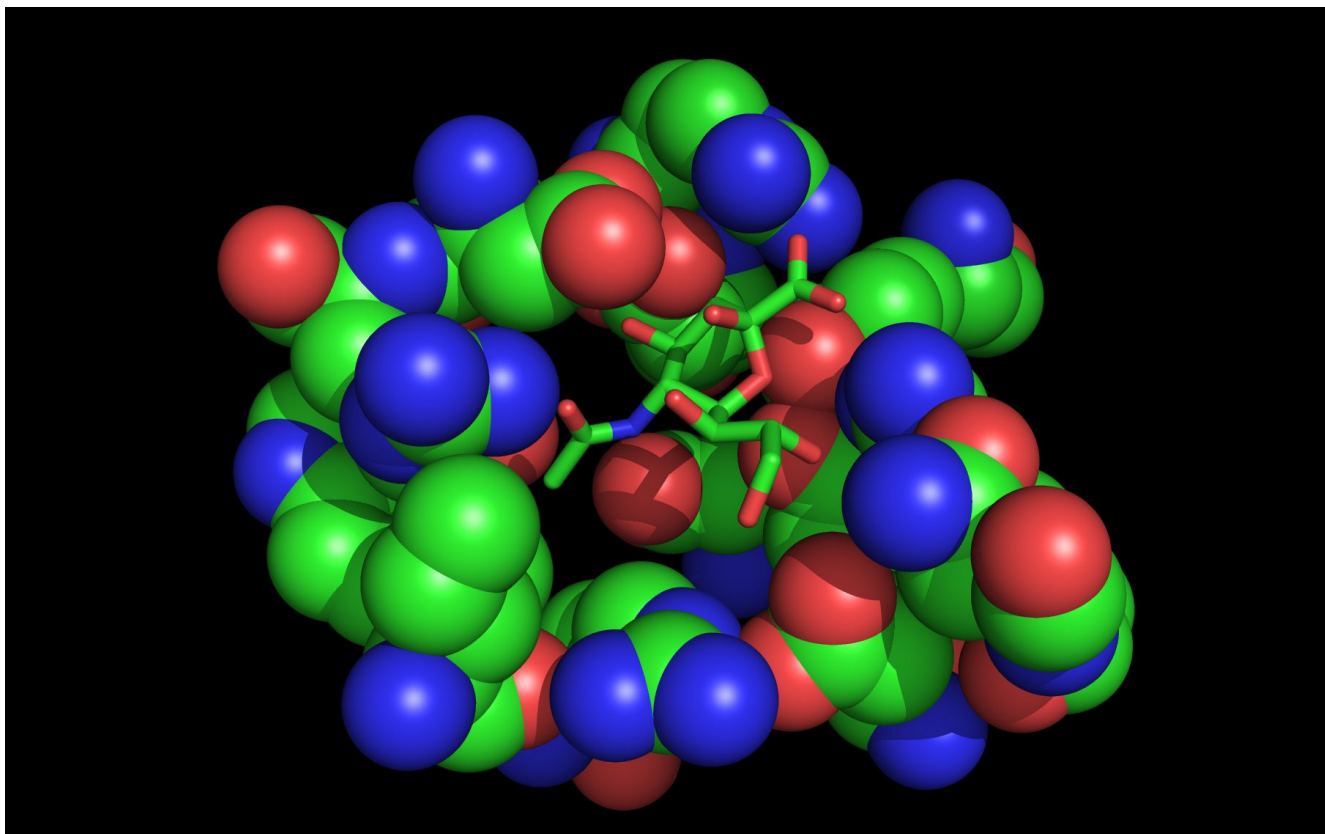


wwavePDB-identified neuraminidase residues binding zanamivir

Shown here are the wwavePDB-identified neuraminidase residues (spheres) that contact bound zanamivir atoms (sticks) from 3B7E².

Zanamivir is used as the reference structure and maintains its coordinate system in this example.

The atom spheres and molecule sticks are color coded according to element (**C** **N** **O**).

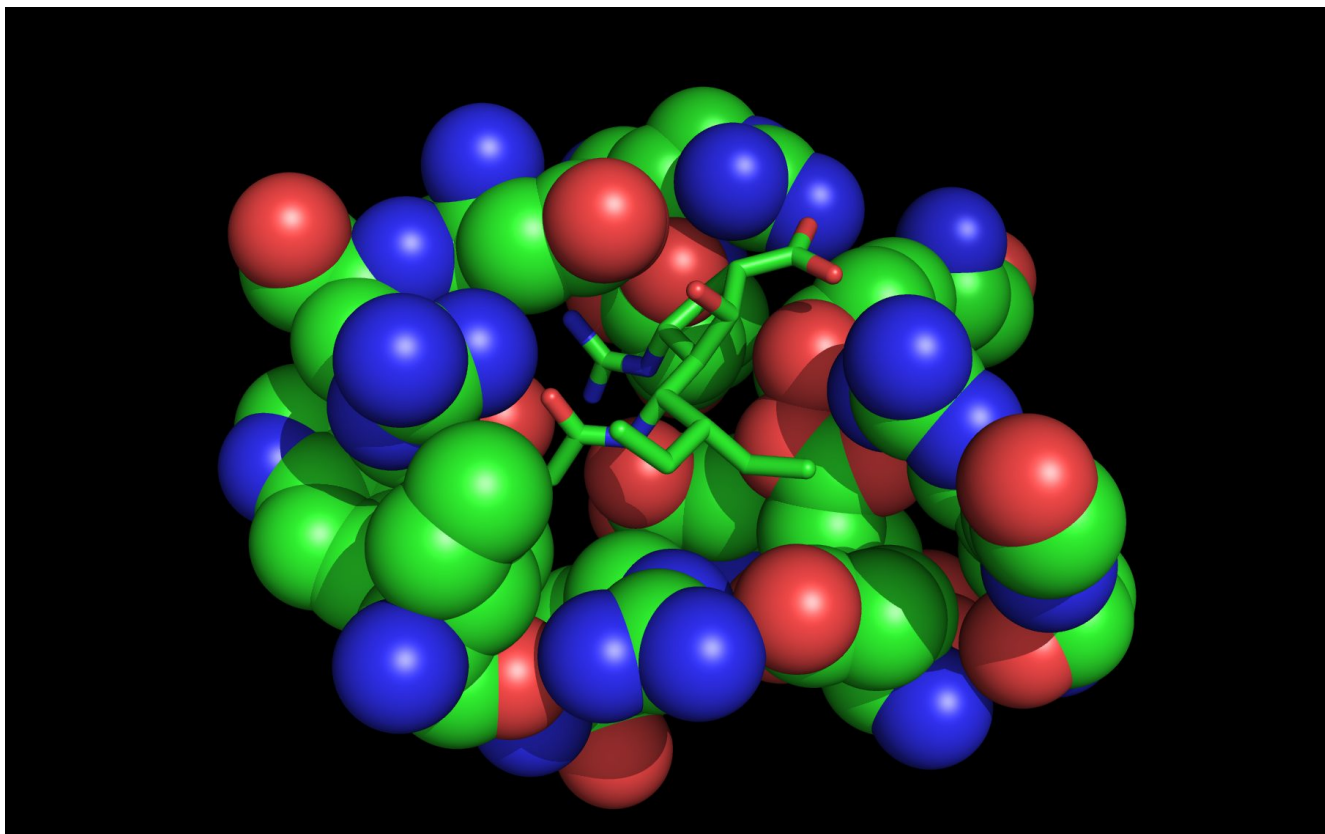


wwavePDB-identified neuraminidase residues binding sialic acid

Shown here are the wwavePDB-identified neuraminidase residues (spheres) that contact bound sialic acid atoms (sticks) from 1W1X¹.

1W1X¹ was mapped onto 3B7E² by twistPDB.

The atom spheres and molecule sticks are color coded according to element (C N O).

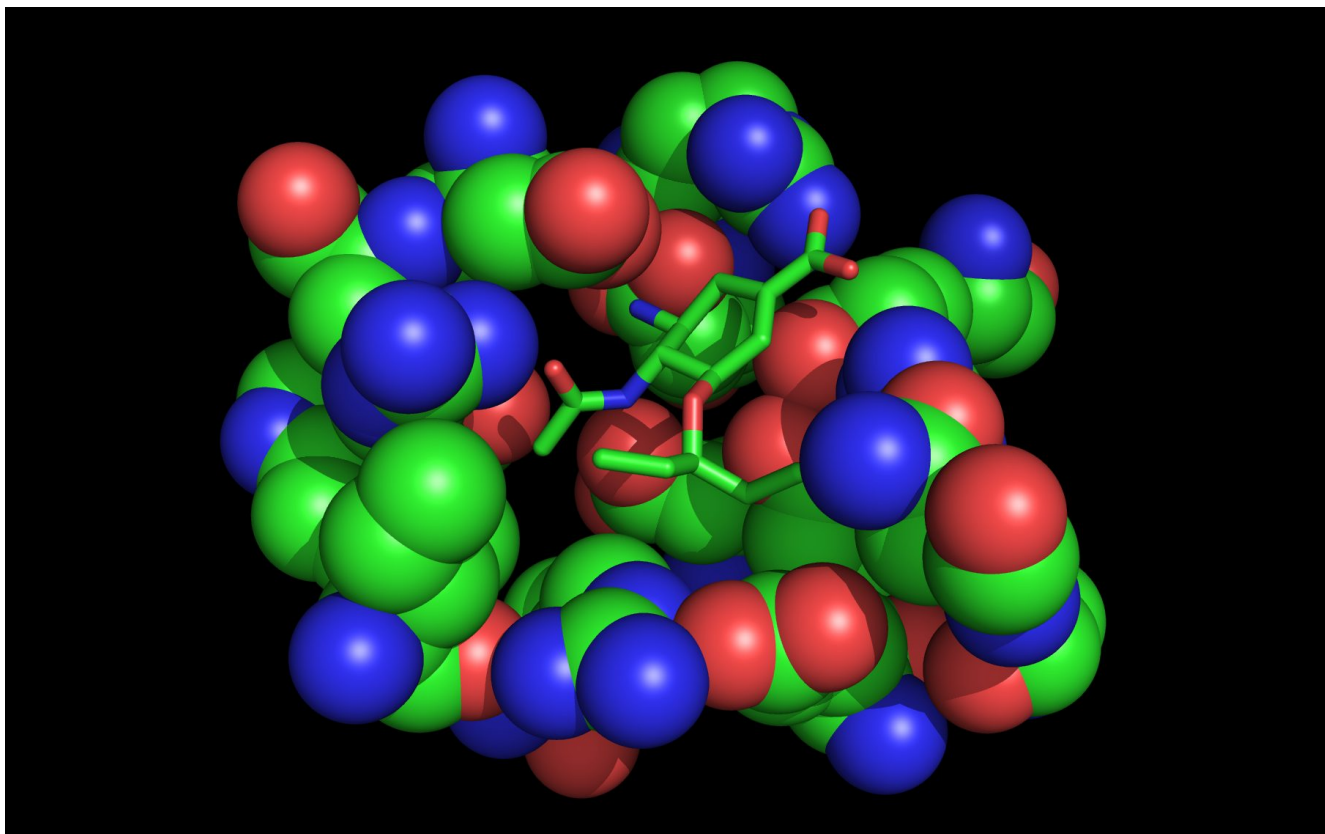


wwavePDB-identified neuraminidase residues binding peramivir

Shown here are the wwavePDB-identified neuraminidase residues (spheres) that contact bound peramivir atoms (sticks) from 2HTU³.

2HTU³ was mapped onto 3B7E² by twwistPDB.

The atom spheres and molecule sticks are color coded according to element (C N O).

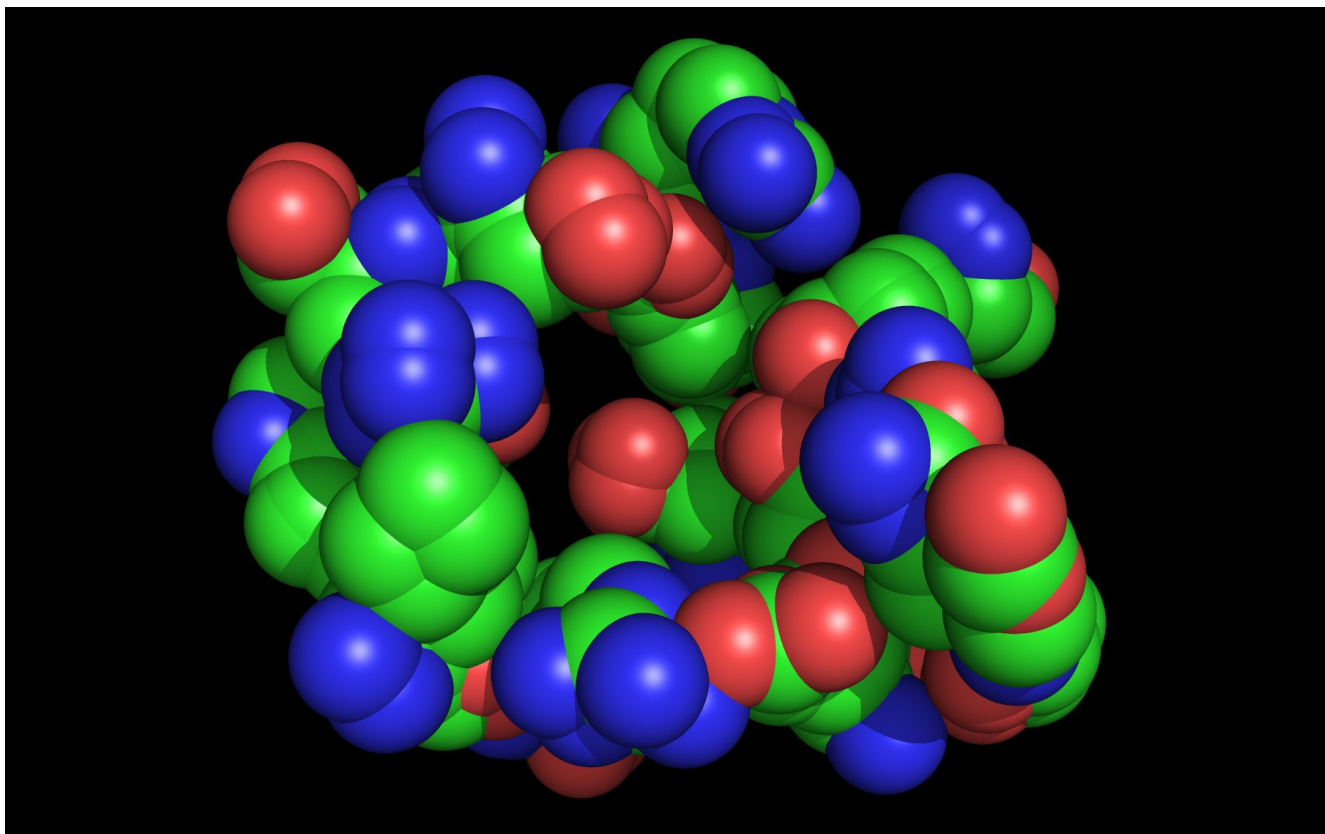


wwavePDB-identified neuraminidase residues binding oseltamivir

Shown here are the wwavePDB-identified neuraminidase residues (spheres) that contact bound oseltamivir atoms (sticks) from 2HU4³.

2HU4³ was mapped onto 3B7E² by twwistPDB.

The atom spheres and molecule sticks are color coded according to element (C N O).

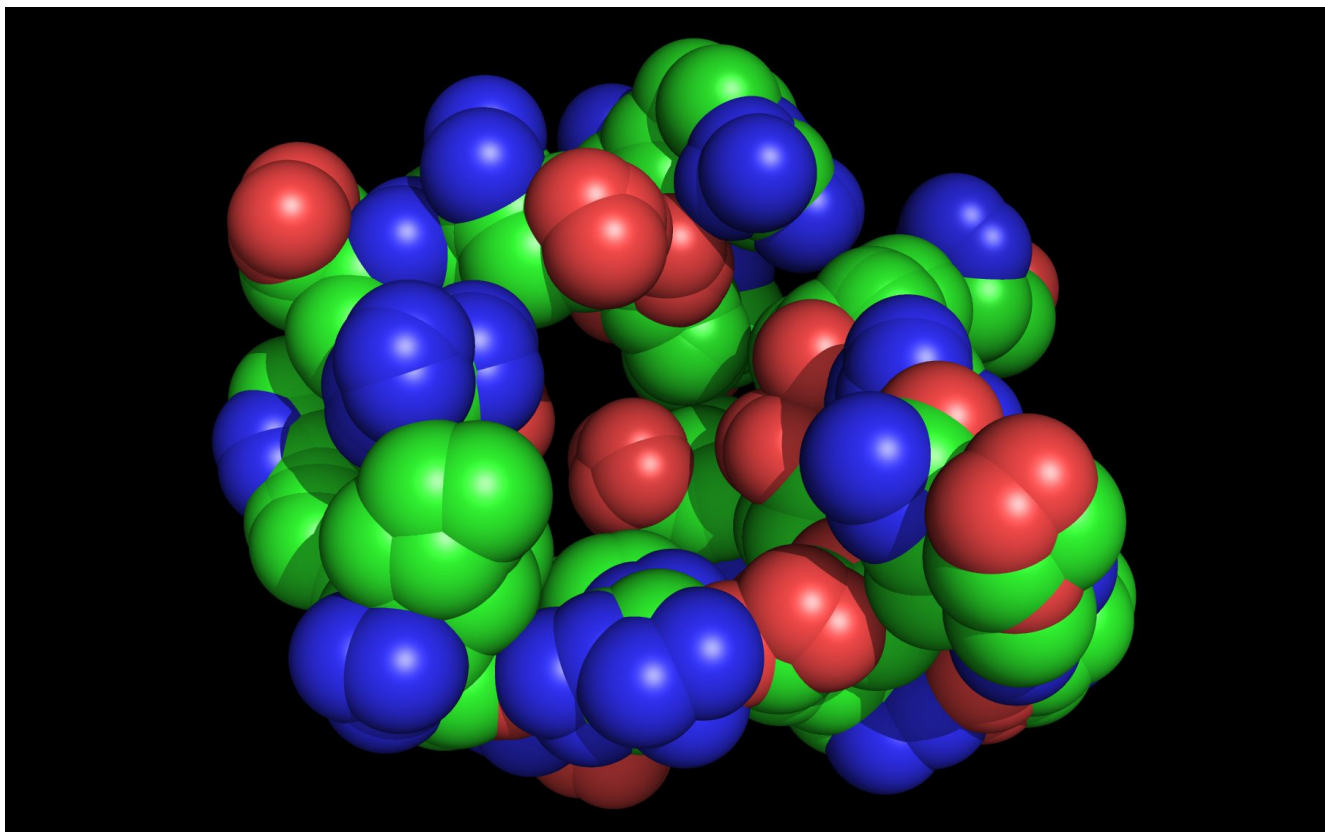


Neuraminidase WWaveCores™ from two structures

Shown here are neuraminidase WWaveCores™ from two structures.

3B7E²-selected and reoriented N1 neuraminidase residue spheres are color-coded according to element (C N O).

2HU4³-selected and reoriented N1 neuraminidase residue spheres are color-coded according to element (C N O).



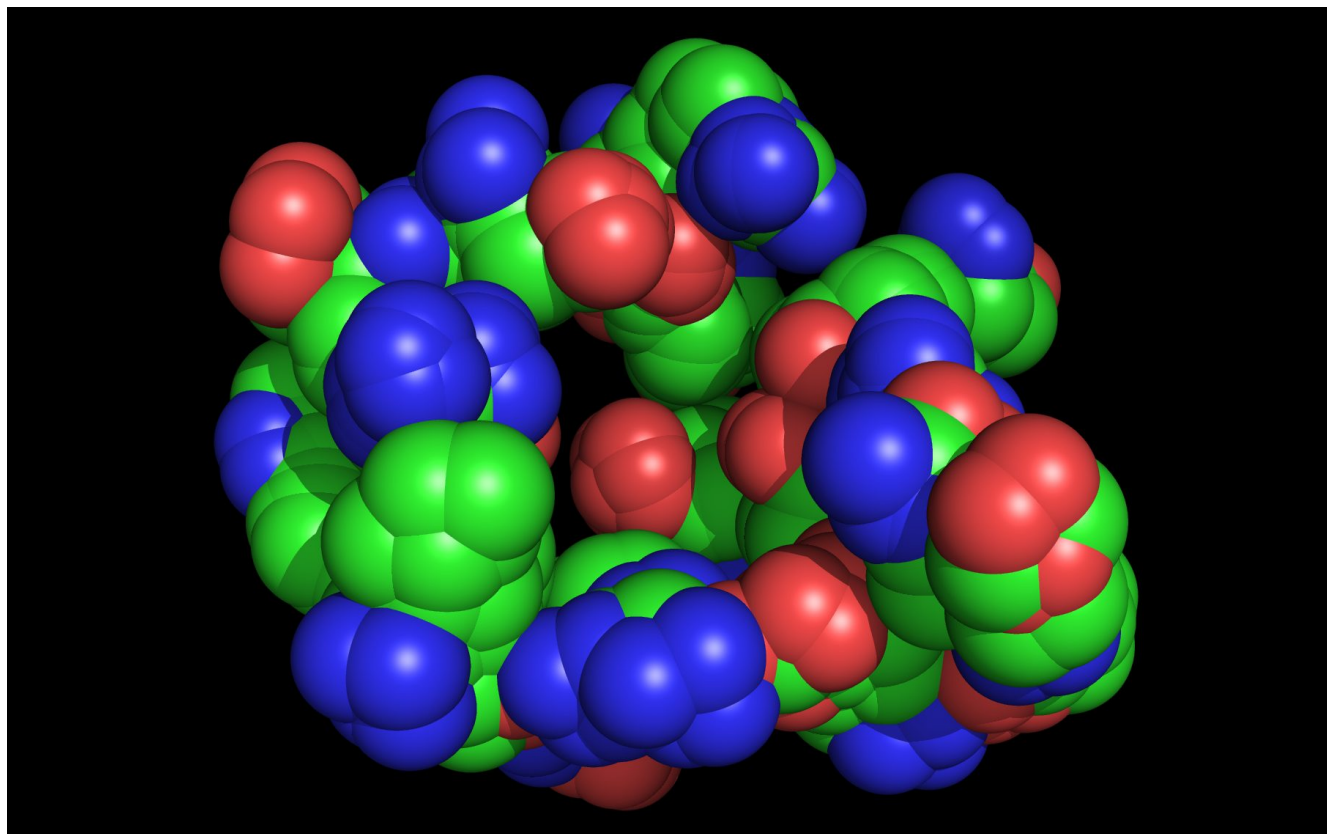
Neuraminidase WWaveCores™ from three structures

Shown here are neuraminidase WWaveCores™ from three structures.

3B7E²-selected and reoriented N1 neuraminidase residue spheres are color-coded according to element (C N O).

2HU4³-selected and reoriented N1 neuraminidase residue spheres are color-coded according to element (C N O).

2HTU³-selected and reoriented N8 neuraminidase residue spheres are color-coded according to element (C N O).



Neuraminidase WWaveCores™ from four structures

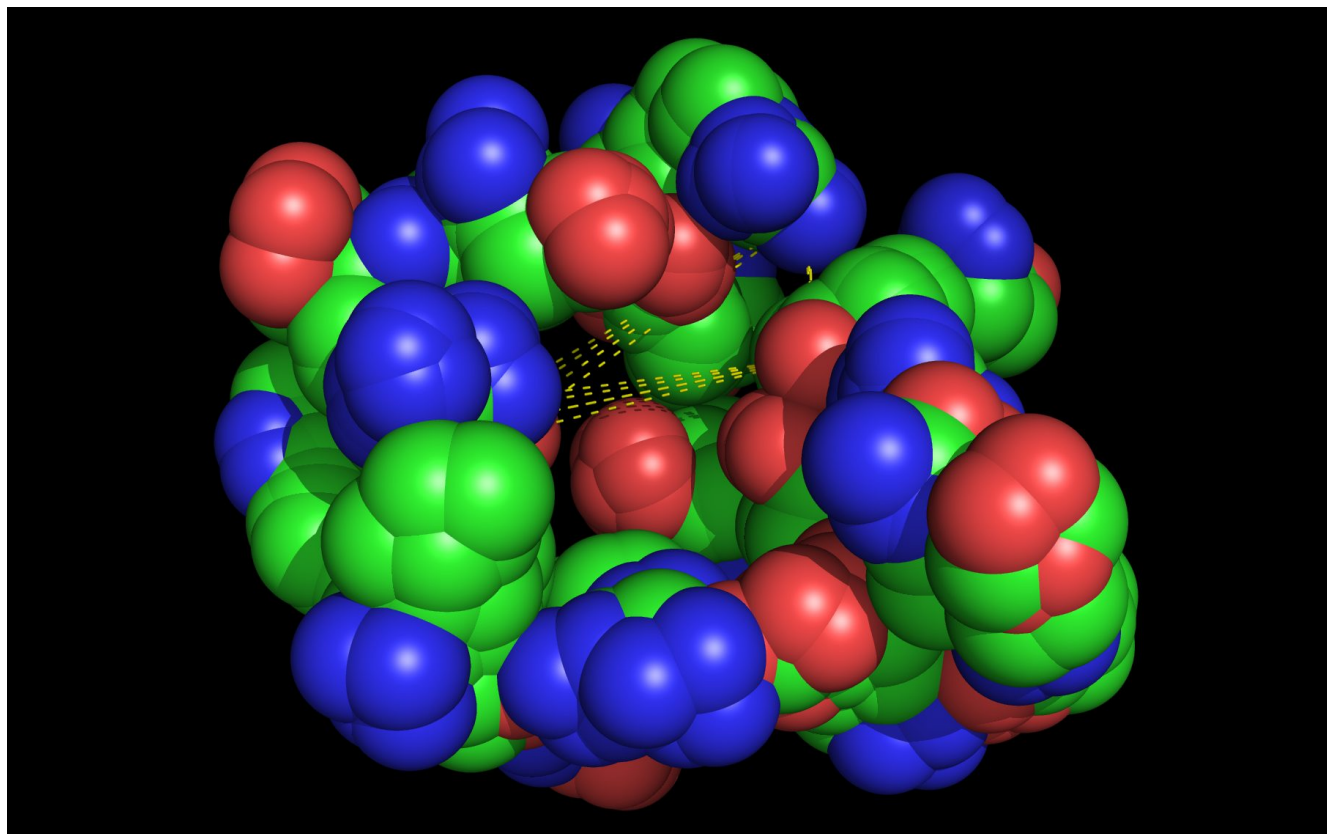
Shown here are neuraminidase WWaveCores™ from four structures.

3B7E²-selected and reoriented N1 neuraminidase residue spheres are color-coded according to element (C N O).

2HU4³-selected and reoriented N1 neuraminidase residue spheres are color-coded according to element (C N O).

2HTU³-selected and reoriented N8 neuraminidase residue spheres are color-coded according to element (C N O).

1W1X¹-selected and reoriented N6 neuraminidase residue spheres are color-coded according to element (C N O).



Neuraminidase WWaveCores™ with WWaveMarkers™

Shown here are neuraminidase WWaveCores™.

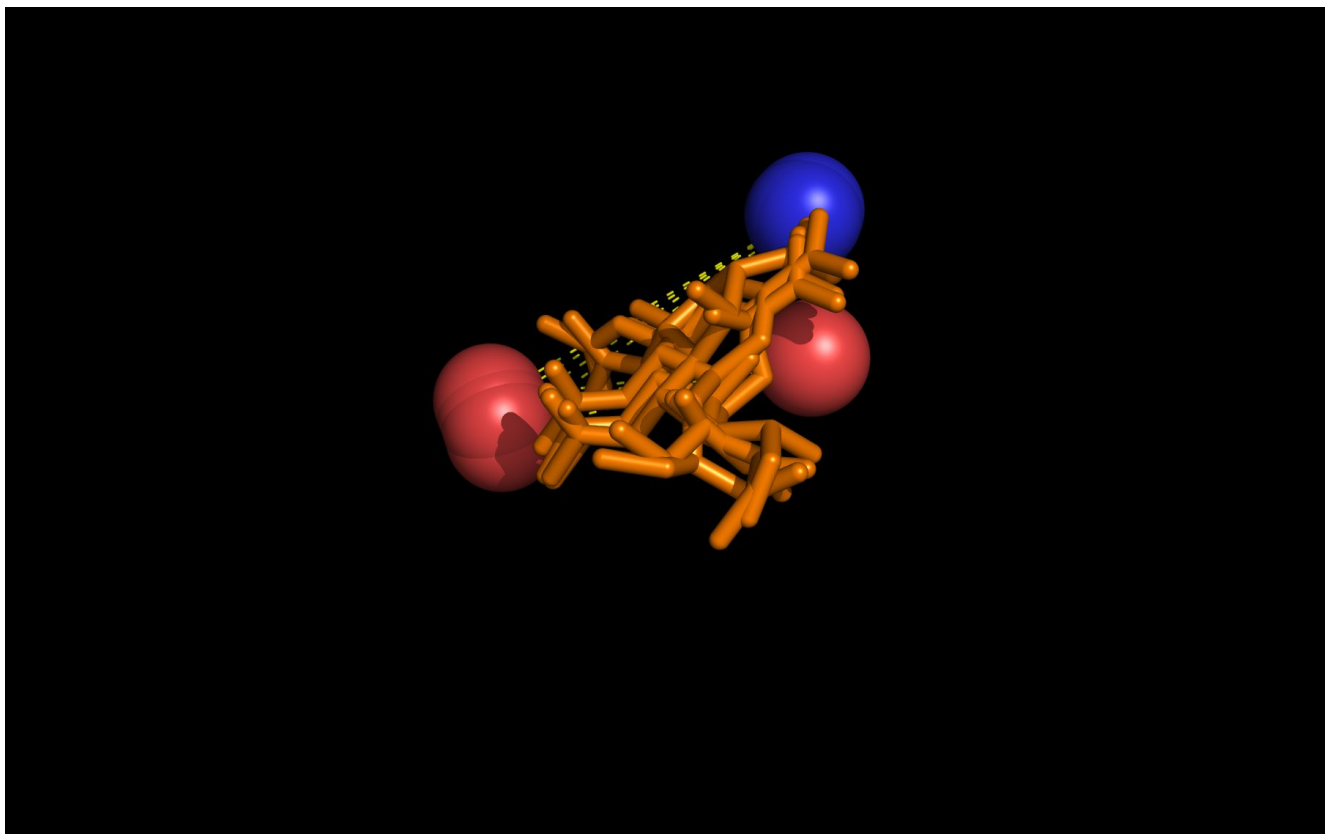
Yellow lines are drawn between the WWaveMarkers™.

3B7E²-selected and reoriented N1 neuraminidase residue spheres are color-coded according to element (C N O).

2HU4³-selected and reoriented N1 neuraminidase residue spheres are color-coded according to element (C N O).

2HTU³-selected and reoriented N8 neuraminidase residue spheres are color-coded according to element (C N O).

1W1X¹-selected and reoriented N6 neuraminidase residue spheres are color-coded according to element (C N O).



Neuraminidase-bound molecules and WWaveMarkers™

Shown here are the twtwistPDB-reoriented neuraminidase bound molecules and the neuraminidase WWaveMarkers™.

Yellow lines are drawn between the WWaveMarkers™.

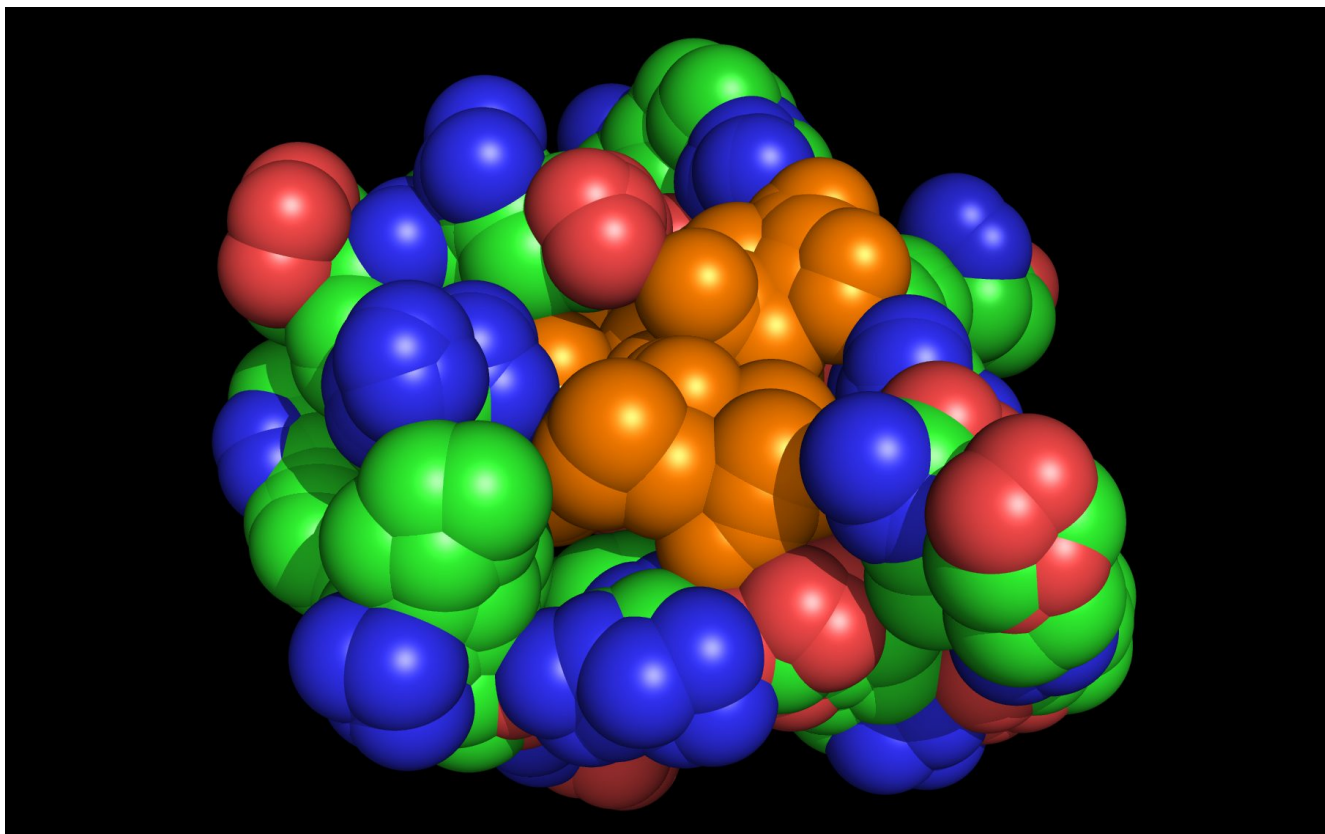
The WWaveMarkers™ atom spheres are color coded according to element (N O).

3B7E²-reoriented zanamivir sticks are colored orange.

2HU4³-reoriented oseltamivir sticks are colored orange.

2HTU³-reoriented peramivir sticks are colored orange.

1W1X¹-reoriented sialic acid sticks are colored orange.



WWaveCores™: neuraminidase and bound molecules

Shown here are neuraminidase WWaveCores™ enclosing neuraminidase-bound molecule WWaveCores™.

3B7E²--selected and reoriented N1 neuraminidase residue spheres are color-coded according to element (C N O).

2HU4³-selected and reoriented N1 neuraminidase residue spheres are color-coded according to element (C N O).

2HTU³-selected and reoriented N8 neuraminidase residue spheres are color-coded according to element (C N O).

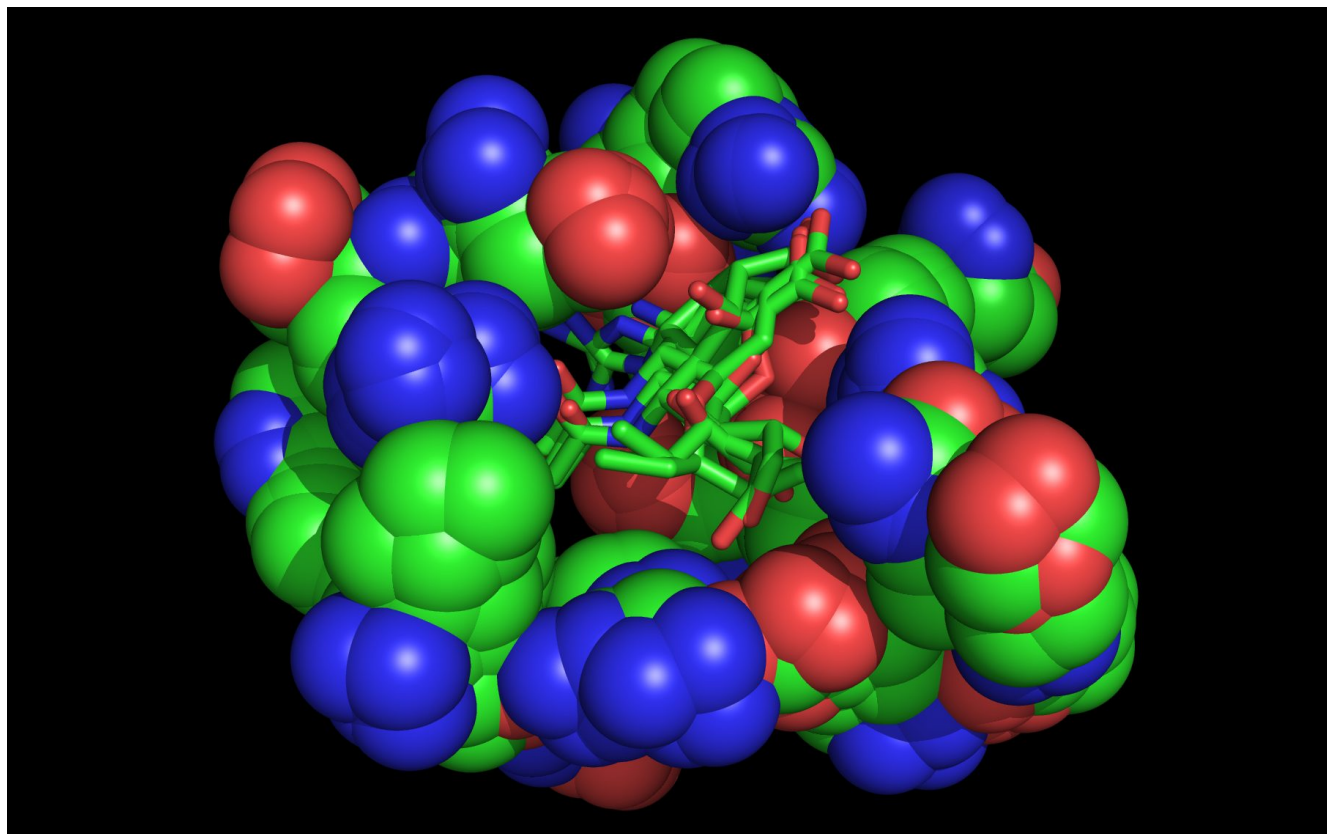
1W1X¹-selected and reoriented N6 neuraminidase residue spheres are color-coded according to element (C N O).

3B7E²-reoriented zanamivir spheres are colored orange.

2HU4³-reoriented oseltamivir spheres are colored orange.

2HTU³-reoriented peramivir spheres are colored orange.

1W1X¹-reoriented sialic acid spheres are colored orange.



Neuraminidase WWaveCores™ and bound molecules

Shown here are neuraminidase WWaveCores™ enclosing neuraminidase-bound molecules.

3B7E²--selected and reoriented N1 neuraminidase residue spheres are color-coded according to element (C N O).

2HU4³--selected and reoriented N1 neuraminidase residue spheres are color-coded according to element (C N O).

2HTU³--selected and reoriented N8 neuraminidase residue spheres are color-coded according to element (C N O).

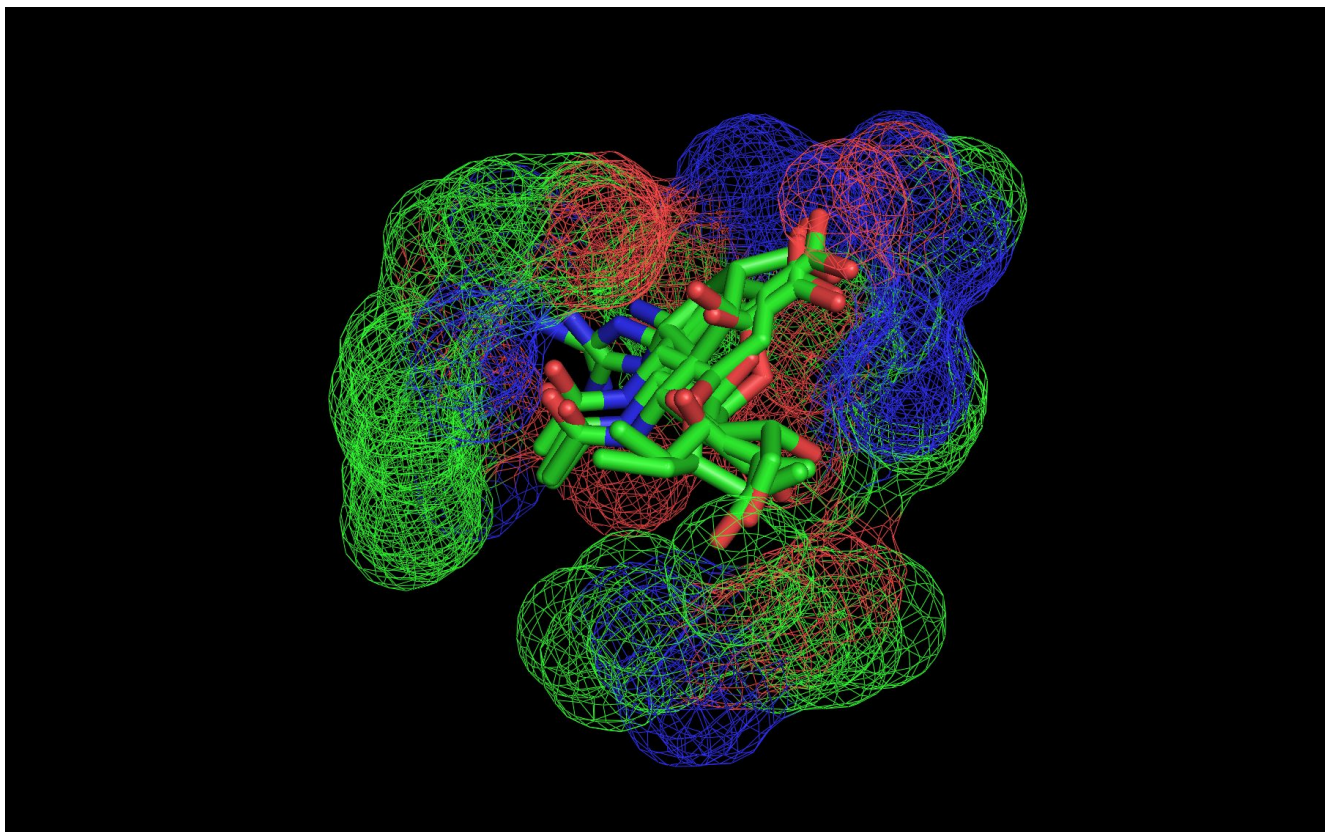
1W1X¹--selected and reoriented N6 neuraminidase residue spheres are color-coded according to element (C N O).

3B7E²--reoriented zanamivir sticks are color-coded according to element (C N O).

2HU4³--reoriented oseltamivir sticks are color-coded according to element (C N O).

2HTU³--reoriented peramivir sticks are color-coded according to element (C N O).

1W1X¹--reoriented sialic acid sticks are color-coded according to element (C N O).



Mesh contact WWaveCores™ and bound molecules

Shown here are mesh neuraminidase contact atom WWaveCores™ enclosing neuraminidase-bound molecules.

The mesh surrounding 3B7E²-selected and reoriented N1 neuraminidase residues is color-coded according to element (C N O).

The mesh surrounding 2HU4³-selected and reoriented N1 neuraminidase residues is color-coded according to element (C N O).

The mesh surrounding 2HTU³-selected and reoriented N8 neuraminidase residues is color-coded according to element (C N O).

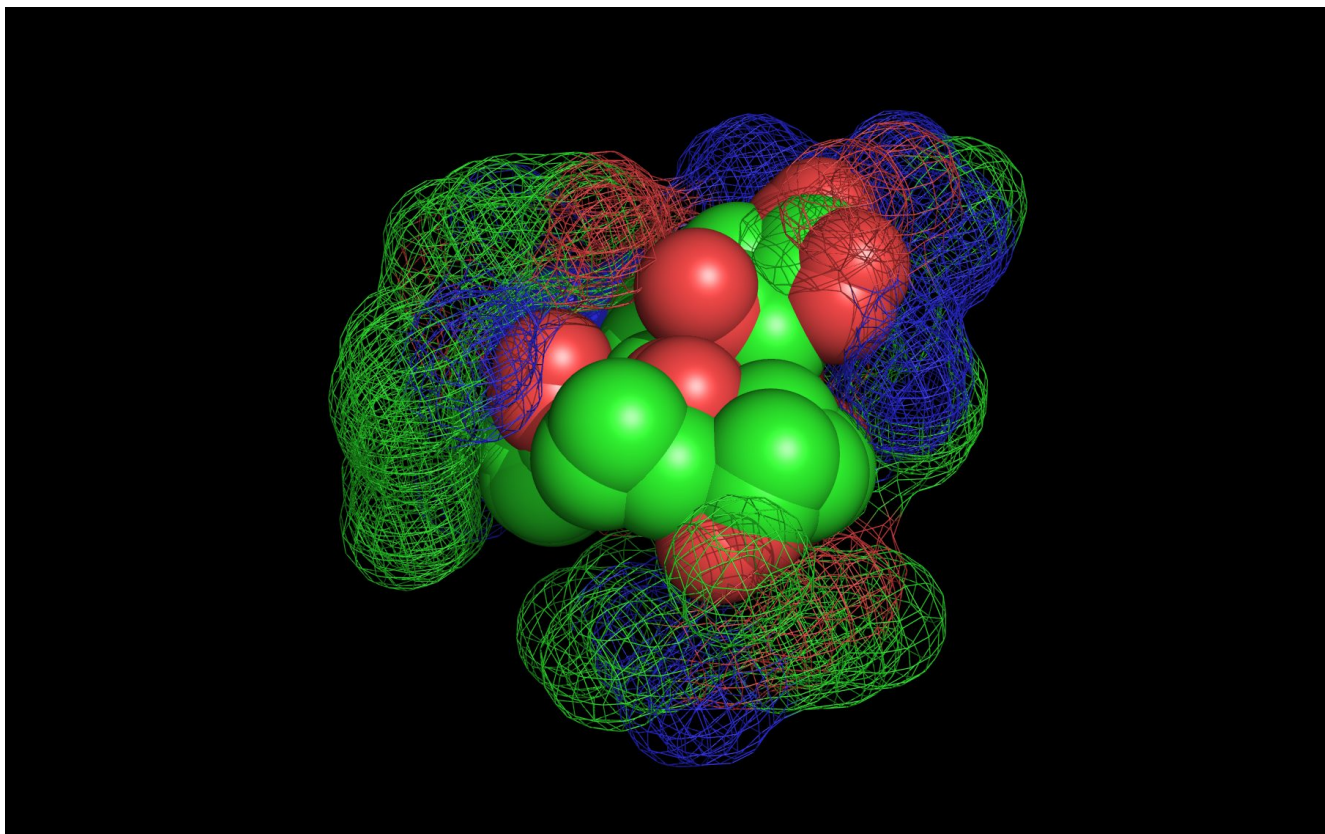
The mesh surrounding 1W1X¹-selected and reoriented N6 neuraminidase residues is color-coded according to element (C N O).

3B7E²-reoriented zanamivir sticks are color-coded according to element (C N O).

2HU4³-reoriented oseltamivir sticks are color-coded according to element (C N O).

2HTU³-reoriented peramivir sticks are color-coded according to element (C N O).

1W1X¹-reoriented sialic acid sticks are color-coded according to element (C N O).



Mesh contact WWaveCores™ and bound molecules

Shown here are mesh neuraminidase contact atom WWaveCores™ enclosing neuraminidase-bound molecules.

The mesh surrounding 3B7E²-selected and reoriented N1 neuraminidase residues is color-coded according to element (C N O).

The mesh surrounding 2HU4³-selected and reoriented N1 neuraminidase residues is color-coded according to element (C N O).

The mesh surrounding 2HTU³-selected and reoriented N8 neuraminidase residues is color-coded according to element (C N O).

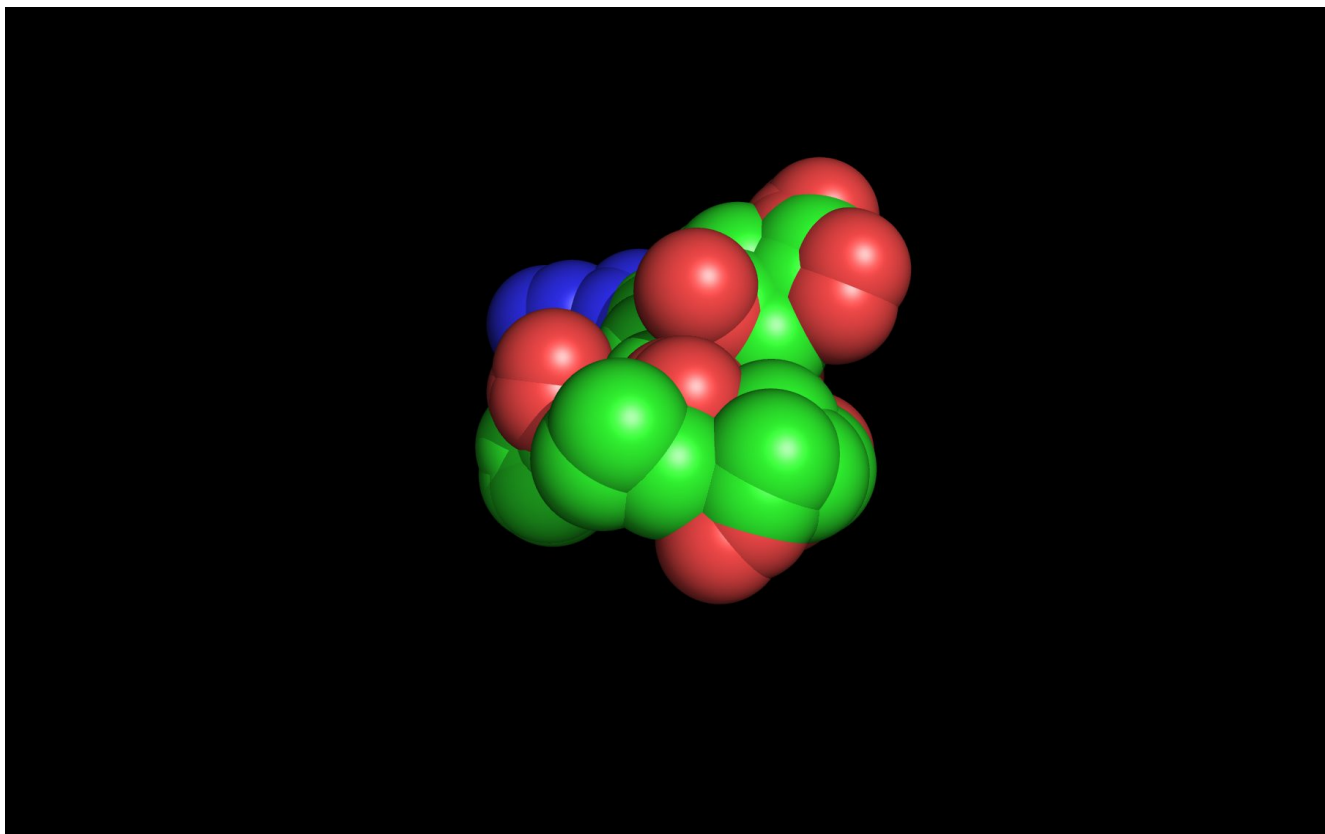
The mesh surrounding 1W1X¹-selected and reoriented N6 neuraminidase residues is color-coded according to element (C N O).

3B7E²-reoriented zanamivir spheres are color-coded according to element (C N O).

2HU4³-reoriented oseltamivir spheres are color-coded according to element (C N O).

2HTU³-reoriented peramivir spheres are color-coded according to element (C N O).

1W1X¹-reoriented sialic acid spheres are color-coded according to element (C N O).



Neuraminidase bound molecule WWaveCores™

Shown here are the neuraminidase bound molecule WWaveCores™.

3B7E²-reoriented zanamivir spheres are color-coded according to element (C N O).

2HU4³-reoriented oseltamivir spheres are color-coded according to element (C N O).

2HTU³-reoriented peramivir spheres are color-coded according to element (C N O).

1W1X¹-reoriented sialic acid spheres are color-coded according to element (C N O).