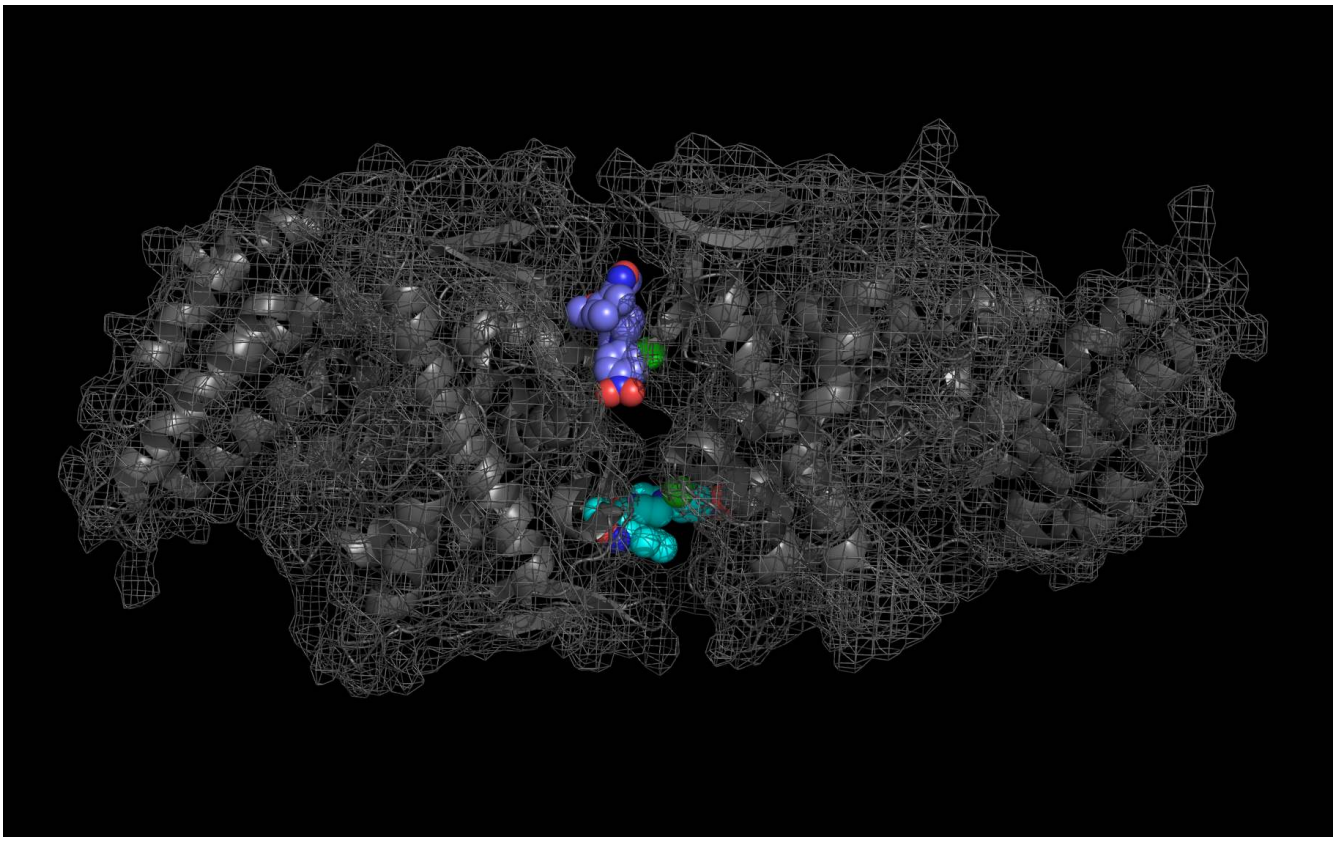
Weininger, A.; Weininger S.;
"Finding an alternate binding site in a nucleoprotein trimer-trimer interface"
Weininger Works Technical Notes (2013) Sept 10;4:1-22



wwavePDB alternative (upper) and crystal (lower) LGH sites

Shown above are two monomers of LGH: one LGH is shown bound to two nucleoprotein monomers (grey mesh and cartoon) in the reported crystallographic position (bottom LGH with cyan carbon spheres) and one LGH in shown bound in the wwavePDB-identified putative alternative binding position (top LGH with purple carbon spheres).

The following pictures and description document the finding and building of an alternative binding site for a class of influenza A nucleoprotein binding compounds that have been examined as drug candidates[1,2,3]. Kao et al.[1] were the first to discover that a small molecule compound, nucleozin, triggered the aggregation of nucleoprotein, inhibited its nuclear accumulation with nanomolar effectiveness, protected mice against lethal challenges of H5N1, and produced a mutation in the nucleoprotein, Y289H, conferring resistance to nucleozin. Su et al.[2] identified a number of small molecule compounds that also protected against lethal challenges but also induced resistance mutations. Su et al.[2] found that nucleozin related compound, 3061, induced nucleoprotein aggregation and produced the nucleoprotein mutation Y52H. Gerritz at al.[3] subsequently also reported nucleoprotein aggregation induced by additional nucleozin-related compounds and associated induced mutations. Nucleoproteins were co-crystallized with several compounds (BMS-885986[5], BMS-883559[6], BMS-885838[7], BMS-831780[8], and LGH[4]) and the x-ray structures were reported. The crystallized

compounds were reported to bridge between monomers with the long axis of the compound positioned orthogonally to the plane of the nucleoprotein monomer-monomer interface. The long axis of superimposed nucleozin, compound 3061, and the crystallized compounds in the wwavePDB-identified alternative binding site is oriented in the the plane between the nucleoprotein monomers. In the process of using wwavePDB to look for on-target and off-target binding sites for this class of compounds, alternative putative binding sites for this class of compounds were identified. LGH in the crystal structure orientation and LGH in the alternative wwavePDB-identified binding site are shown above, relative to the plane of the dimer-dimer interface.

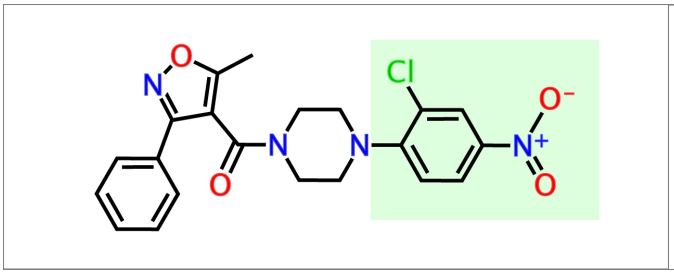
An alternative on-target nucleoprotein binding site identified by wwavePDB has features consistent with binding this class of compounds and the reported mutant data[1,2,3]. The wwavePDB-identified putative on-target nucleoprotein binding site has favorable stereochemistry. The wwavePDB-identified binding site is positioned along the interface between nucleoprotein trimers. The wwavePDB-identified binding site resides in a position that is roughly 90 degrees rotated and slightly shifted from the configuration reported in the crystal structures[4,5,6,7,8]. wwavePDB used the crystal structure[9] of a nucleoprotein trimer (with no compound bound and with a R416A mutation) to build WWaveCores™ for a trimer-trimer interface. The WWaveCores™ defining the binding sites for the compounds (nucleozin[1], compound 3061[2], BMS-885986[5], BMS-883559[6], BMS-885838[7], BMS-831780[8], and LGH[4]) were built by maintaining the trimer structure with the exception that three residue side chains were moved. The wwavePDB-identified putative alternative binding sites supplies detail that is consistent with, and may be important for, the interpretation of the reported mutant data[1,2,3]. Alternative wwavePDB-identified binding sites, whether for nucleozin or non-nucleozin related compounds, may represent new, well-defined, putative targets for nucleoprotein drug design. This example shows the utility of wwavePDB in finding and building alternative on-target binding sites for drug candidates that can be tested.

3RO5[4] nucleoprotein dimer cartoon and surface mesh is colored grey.

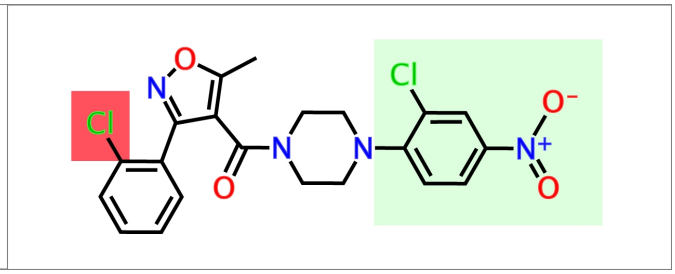
LGH molecule spheres oriented as in 3RO5[4] are color-coded according to element (C N O Cl).

LGH molecule spheres reoriented by twwistPDB into WWaveCores™ are color-coded according to element (C N O Cl).

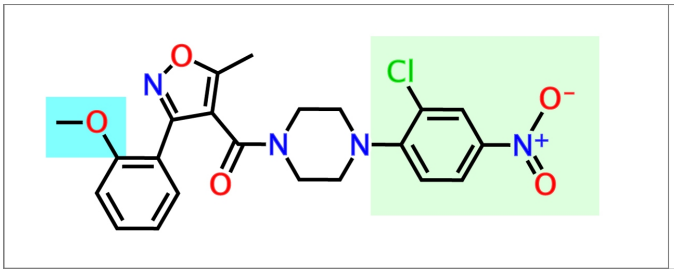
Nucleoprotein binding compounds



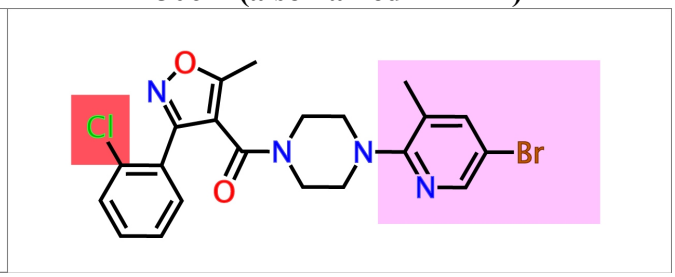
nucleozin[1]



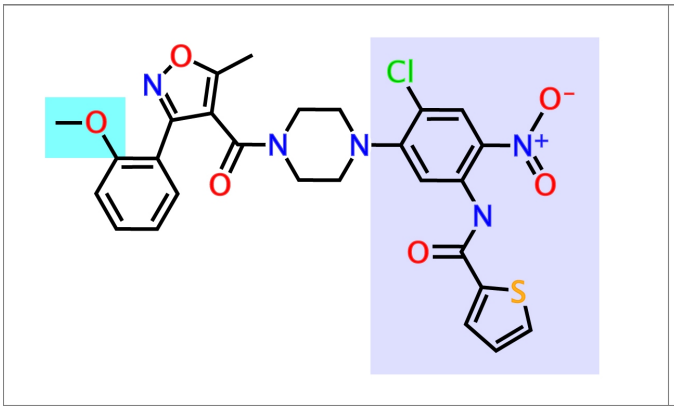
3061[2] (also named "FA-2")



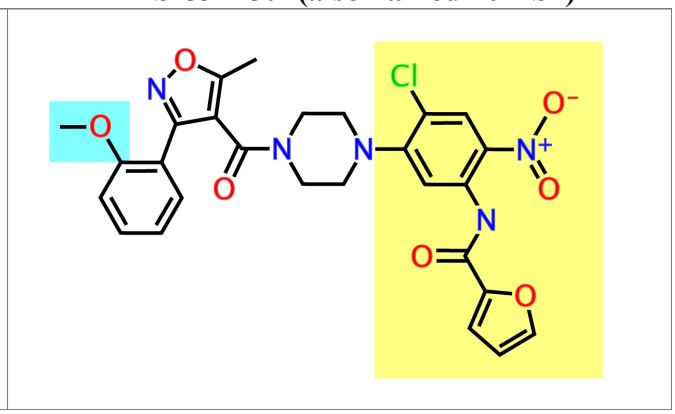
LGH[4]



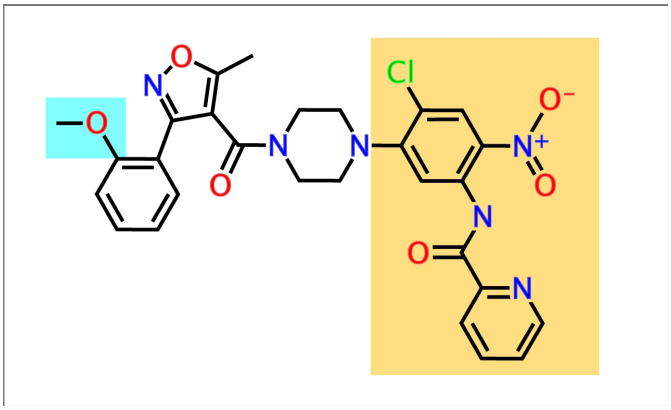
BMS-831780[8] (also named "0MS")



BMS-883559[6] (also named "0MH")



BMS-885986[5] (also named "0MF")



BMS-883559[6] (also named "0MH")

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

nucleozin
CAS Number: 341001-38-5
IUPAC Name: [4-(2-chloro-4-nitrophenyl)piperazin-1-yl]-(5-methyl-3-phenyl-1,2-oxazol-4-yl)methanone
SMILES: c1ccccc1c2noc(C)c2C(=O)N3CCN(CC3)c4ccc(cc4Cl)[N+](=O)[O-]

3061 (also named "FA-2")
IUPAC Name: 3-[3-[5-[5-(4,5-dihydroxy-6-methyloxan-2-yl)oxy-4-hydroxy-6-methyloxan-2-yl]oxy-4-hydroxy-6-methyloxan-2-yl]oxy-14-hydroxy-10,13-dimethyl-1,2,3,4,5,6,7,8,9,11,12,15,16,17-tetradecahydrocyclopenta[a]phenanthren-17-yl]-2H-furan-5-one
SMILES: Clc1ccccc1c2noc(C)c2C(=O)N3CCN(CC3)c4ccc(cc4Cl)[N+](=O)[O-]

LGH
IUPAC Name: [4-(2-chloro-4-nitrophenyl)piperazin-1-yl]-[3-(2-methoxyphenyl)-5-methyl-1,2-oxazol-4-yl]methanone
SMILES: COc1ccccc1c2noc(C)c2C(=O)N3CCN(CC3)c4ccc(cc4Cl)[N+](=O)[O-]

BMS-831780 (also named "0MS")
IUPAC Name: [4-(5-bromo-3-methylpyridin-2-yl)piperazin-1-yl]-[3-(2-chlorophenyl)-5-methyl-1,2-oxazol-4-yl]methanone
SMILES: Clc1ccccc1c2noc(C)c2C(=O)N3CCN(CC3)c4c(C)cc(Br)cn4

BMS-883559 (also named "0MH")
IUPAC Name: N-[4-chloro-5-[4-[3-(2-methoxyphenyl)-5-methyl-1,2-oxazole-4-carbonyl]piperazin-1-yl]-2-nitrophenyl]thiophene-2-carboxamide
SMILES: COc1ccccc1c2noc(C)c2C(=O)N3CCN(CC3)c4cc(NC(=O)c5sccc5)c(cc4Cl)[N+](=O)[O-]

BMS-885986 (also named "0MF")
IUPAC Name: N-[4-chloranyl-5-[4-[[3-(2-methoxyphenyl)-5-methyl-1,2-oxazol-4-yl]carbonyl]piperazin-1-yl]-2-nitro-phenyl]furan-2-carboxamide
SMILES: COc1ccccc1c2noc(C)c2C(=O)N3CCN(CC3)c4cc(NC(=O)c5occc5)c(cc4Cl)[N+](=O)[O-]

BMS-885838 (also named "0MR")
IUPAC Name: N-[4-chloro-5-[4-[3-(2-methoxyphenyl)-5-methyl-1,2-oxazole-4-carbonyl]piperazin-1-yl]-2-nitrophenyl]pyridine-2-carboxamide
SMILES: COc1ccccc1c2noc(C)c2C(=O)N3CCN(CC3)c4cc(NC(=O)c5ncccc5)c(cc4Cl)[N+](=O)[O-]

References

[1] Kao, R.Y.; Yang, D.; Lau, L.S.; Tsui, W.H.; Hu, L.; Dai, J.; Chan, M.P.; Chan, C.M.; Wang, P.; Zheng, B.J.; Sun, J.; Huang, J.D.; Madar, J.; Chen, G.; Chen, H.; Guan, Y.; Yuen, K.Y.;\l "Identification of influenza A nucleoprotein as an antiviral target."\l *Nat.Biotechnol.* (2011) Jun;28;(6) 600-605

[2] Su, C.Y.; Cheng, T.J.; Lin, M.I.; Wang, S.Y.; Huang, W.I.; Lin-Chu, S.Y.; Chen, Y.H.; Wu, C.Y.; Lai, M.M.; Cheng, W.C.; Wu, Y.T.; Tsai, M.D.; Cheng, Y.S.; Wong, C.H.; "High-throughput identification of compounds targeting influenza RNA-dependent RNA polymerase activity." *Proc.Natl.Acad.Sci.USA* (2010) Nov 9;107(45): 19151-19156

[3] Gerritz, S.W.; Cianci, C.; Kim, S.; Pearce, B.C.; Deminie, C.; Discotto, L.; McAuliffe, B.; Minassian, B.F.; Shi,S.; Zhu, S.; Zhai, W.; Pendri, A.; Li, G.; Poss, M.A.; Edavettal, S.; McDonnell, P.A.; Lewis, H.A.; Maskos, K.; Mörtl, M.; Kiefersauer, R.; Steinbacher, S.; Baldwin, E.T.; Metzler, W.; Bryson, J.; Healy, M.D.; Philip, T.; Zoeckler, M.; Schartman, R.; Sinz, M.; Leyva-Grado, V.H.; Hoffmann, H.H.; Langley, D.R.; Meanwell, N.A.; Krystal, M. "Inhibition of influenza virus replication via small molecules that induce the formation of higher-order nucleoprotein oligomers." *Proc.Natl.Acad.Sci.USA* (2011) Sep 13;108: 15366-15371

[4] Protein Data Bank ID: 3RO5.
Crystal structure: influenza a H1N1 virus nucleoprotein with ligand (LGH)
NP Source: Influenza A/WSN/1933: H1N1
Authors: Pearce, B.C.; Edavettal,S.; McDonnell,P.A.; Lewis, H.A.; Steinbacher, S.; Baldwin,E.T.; Langley, D.R.; Maskos, K.; Mörtl, M.; Kiefersauer, R.
Reference: Gerritz, S.W.; Cianci, C.; Kim, S.; Pearce, B.C.; Deminie, C.; Discotto, L.; McAuliffe, B.; Minassian, B.F.; Shi,S.; Zhu, S.; Zhai, W.; Pendri, A.; Li, G.; Poss, M.A.; Edavettal, S.; McDonnell, P.A.; Lewis, H.A.; Maskos, K.; Mörtl, M.; Kiefersauer, R.; Steinbacher, S.; Baldwin, E.T.; Metzler, W.; Bryson, J.; Healy, M.D.; Philip, T.; Zoeckler, M.; Schartman, R.; Sinz, M.; Leyva-Grado, V.H.; Hoffmann, H.H.; Langley, D.R.; Meanwell, N.A.; Krystal, M.
"Inhibition of influenza virus replication via small molecules that induce the formation of higher-order nucleoprotein oligomers."
Proc.Natl.Acad.Sci.USA (2011) Sep 13;108: 15366-15371

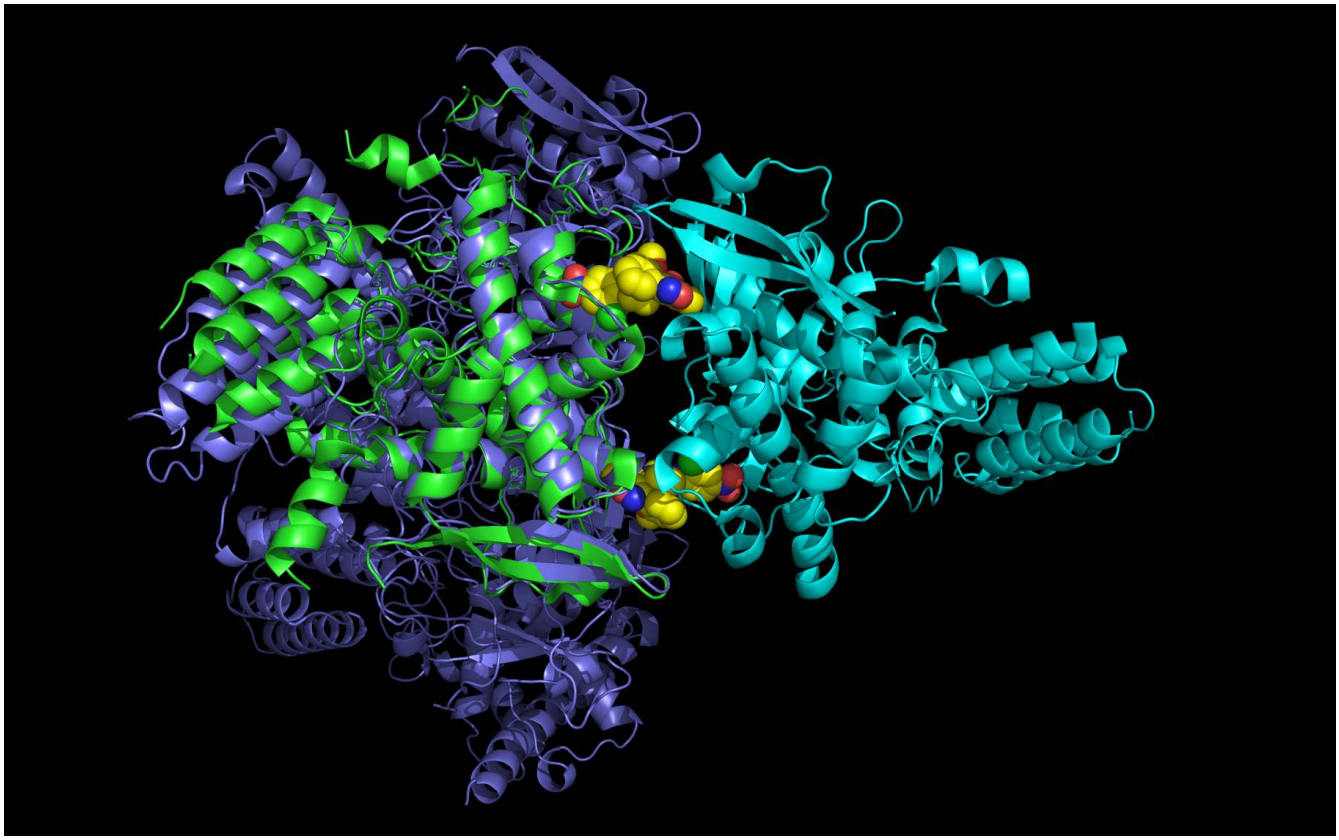
[5] Protein Data Bank ID: 4DYA.
Crystal structure: WSN/A influenza nucleoprotein with BMS-885986 ligand bound
NP Source: Influenza A/WSN/1933: H1N1
Authors: Lewis, H.A.; Baldwin, E.T.; Steinbacher, S.; Maskos, K.; Mörtl, M.; Kiefersauer, R.; Edavettal,S.; McDonnell, P.A.; Pearce, B.C.; Langley, D.R.;
"Crystal structure of WSN/A influenza nucleoprotein with BMS-885986 ligand bound"
Reference: To Be Published.

[6] Protein Data Bank ID: 4DYB.
Crystal structure: WSN/A influenza nucleoprotein with BMS-883559 ligand bound
NP Source: Influenza A/WSN/1933: H1N1
Authors: Lewis, H.A.; Baldwin, E.T.; Steinbacher, S.; Maskos, K.; Mörtl, M.; Kiefersauer, R.; Edavettal,S.; McDonnell, P.A.; Pearce, B.C.; Langley, D.R.;
"Crystal structure of WSN/A influenza nucleoprotein with BMS-883559 ligand bound"
Reference: To Be Published.

[7] Protein Data Bank ID: 4DYN.\J
Crystal structure: WSN/A influenza nucleoprotein with BMS-885838 ligand bound
NP Source: Influenza A/WSN/1933: H1N1
Authors: Lewis, H.A.; Baldwin, E.T.; Steinbacher, S.; Maskos, K.; Mörtl, M.; Kiefersauer, R.; Edavettal,S.; McDonnell, P.A.; Pearce, B.C.; Langley, D.R.;
"Crystal structure of WSN/A influenza nucleoprotein with BMS-885838 ligand bound"
Reference: To Be Published.

[8] Protein Data Bank ID: 4DYP.
Crystal structure: WSN/A influenza nucleoprotein with BMS-831780 ligand bound
NP Source: Influenza A/WSN/1933: H1N1
Authors: Lewis, H.A.; Baldwin, E.T.; Steinbacher, S.; Maskos, K.; Mörtl, M.; Kiefersauer, R.; Edavettal,S.; McDonnell, P.A.; Pearce, B.C.; Langley, D.R.;
"Crystal structure of WSN/A influenza nucleoprotein with BMS-831780 ligand bound"
Reference: To Be Published.

[9] Protein Data Bank ID: 3ZDP.
Crystal structure: R416a Monomeric nucleoprotein of influenza A virus
NP Source: Influenza A/WSN/1933: H1N1
Authors: Chenavas, S.; Ruigrok; R.W.H.; Crépin,T.
Reference: Chenavas, S.; Estrozi, L.F.; Slama-Schwok, A.; Delmas, B.; Di Primo, C.; Baudin, F.; Li, X.; Crépin, T.; Ruigrok, R.W.H.;
"Monomeric nucleoprotein of influenza A virus."
Plos Pathog. (2013) 9(3): 3275-3284



Crystal structure NP dimer and LGHs on NP trimer

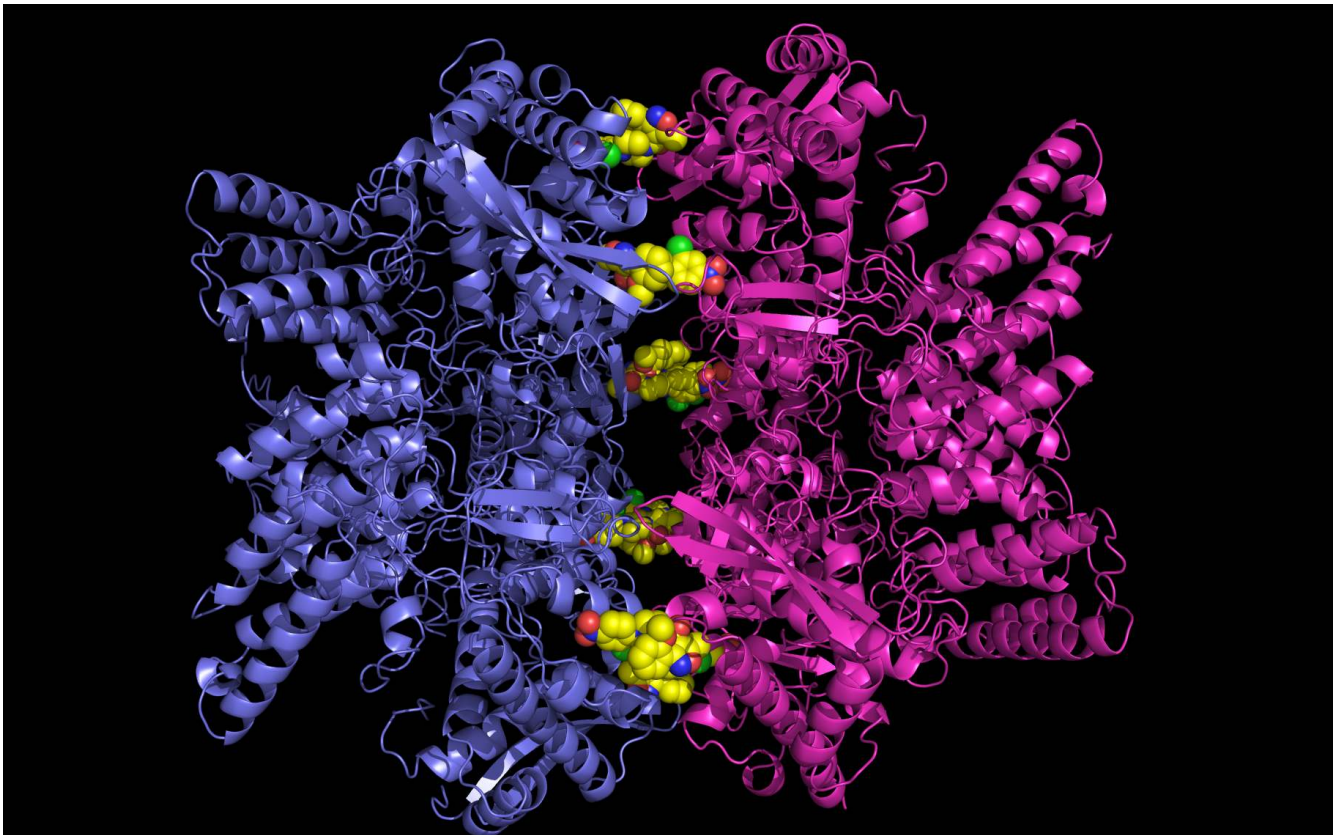
Shown here are the nucleoprotein dimer (green and cyan cartoon) and two LGH molecules (yellow spheres) from the reoriented onto the 3ZDP[9] nucleoprotein trimer (purple cartoon) structure using the Weininger Works' program twwistPDB. WWaveMarkers™ that were close to the LGH compound binding site were used by the Weininger Works' program twwistPDB for the reorientation.

3RO5[4] nucleoprotein chain A cartoon is colored green.

3RO5[4] nucleoprotein chain B cartoon is colored cyan.

3ZDP[9] nucleoprotein trimer cartoon is colored purple.

LGH molecule spheres are color-coded according to element (C N O S Cl).



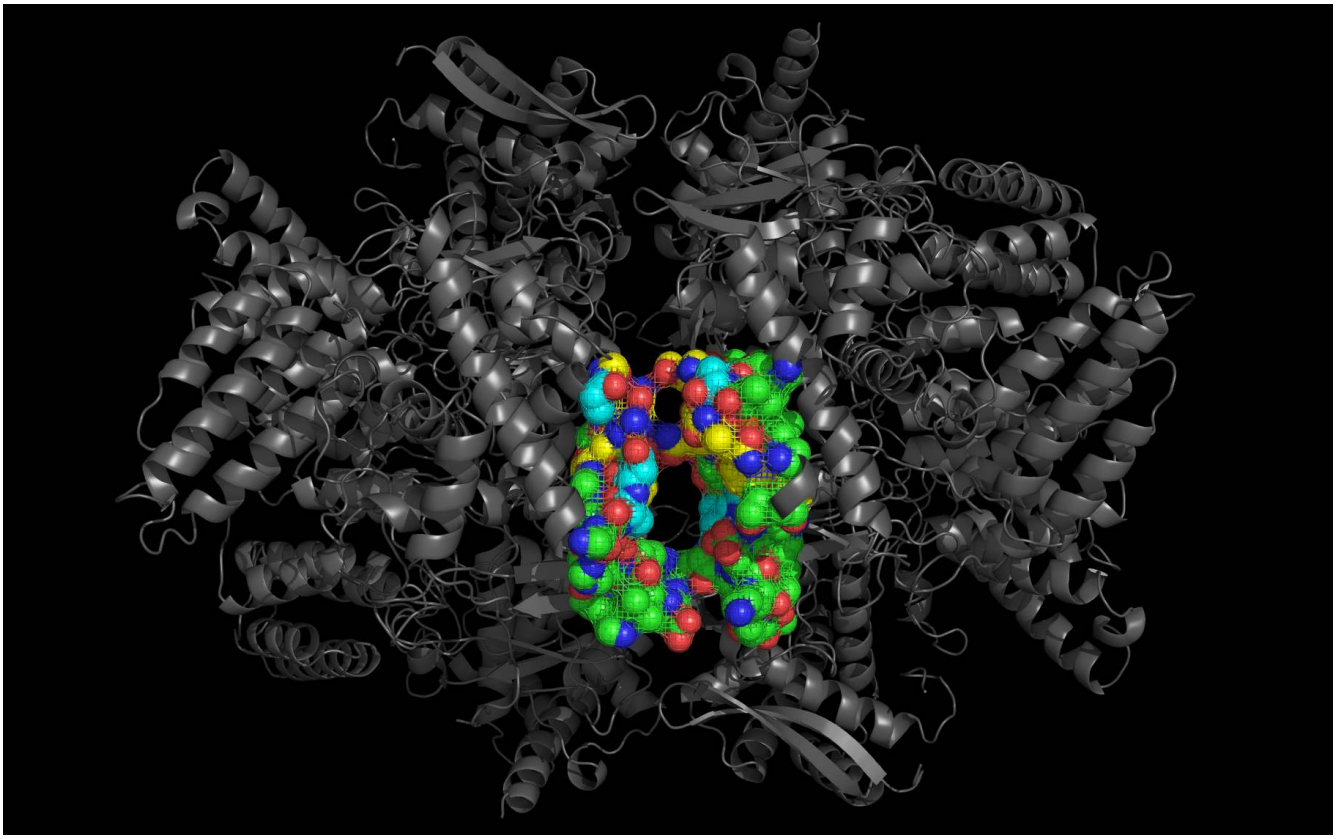
Two NP trimers with crystal structure-oriented LGHs

Shown here are two 3ZDP[9] nucleoprotein trimers reoriented and spaced using 3RO5[4]. The LGH spheres are in the same position relative to the opposing monomers as in the 3RO5[4] structure (i.e., not yet in the alternative binding position). The spacing and reorientation of the nucleoprotein trimers was achieved by using the Weininger Works' program twwistPDB to reorient three 3RO5[4] dimer structures onto two 3ZDP[9] trimer structures. This results in a spacing between trimers in the resulting hexamer that is the same as the spacing between dimer monomers in 3RO5[4]. The LGH spheres are in the same position relative to the opposing monomers as in the 3RO5[4] structure.

One 3ZDP[9] nucleoprotein trimer cartoon is colored purple.

The other 3ZDP[9] nucleoprotein trimer cartoon is colored magenta.

LGH molecule spheres are color-coded according to element (C N O S Cl).



Trimer-trimer contact WWaveCores™ on NP hexamer

Shown here is the 3ZDP[9] structure-based, 3RO5[4] structure-reoriented, and wwavePDB-generated nucleoprotein hexamer (grey cartoon). Also shown are the trimer-trimer interface partial WWaveCores™ for the area where mutants have been identified to alter compound binding. The green, yellow, and cyan spheres are carbons of nucleoprotein residues. The residues with yellow carbons and cyan carbons are residues that have been observed to mutate.[1,2,3] Yellow carbons mark "sensitive" residues whose mutations result in a greater than three-fold change in IC_{50} compared with WT and Cyan carbons mark "tolerant" residues whose mutations result in a less than three-fold change in IC_{50} compared with WT.[1,2,3] The residues with green carbons are not characterized for mutation sensitivity or tolerance.

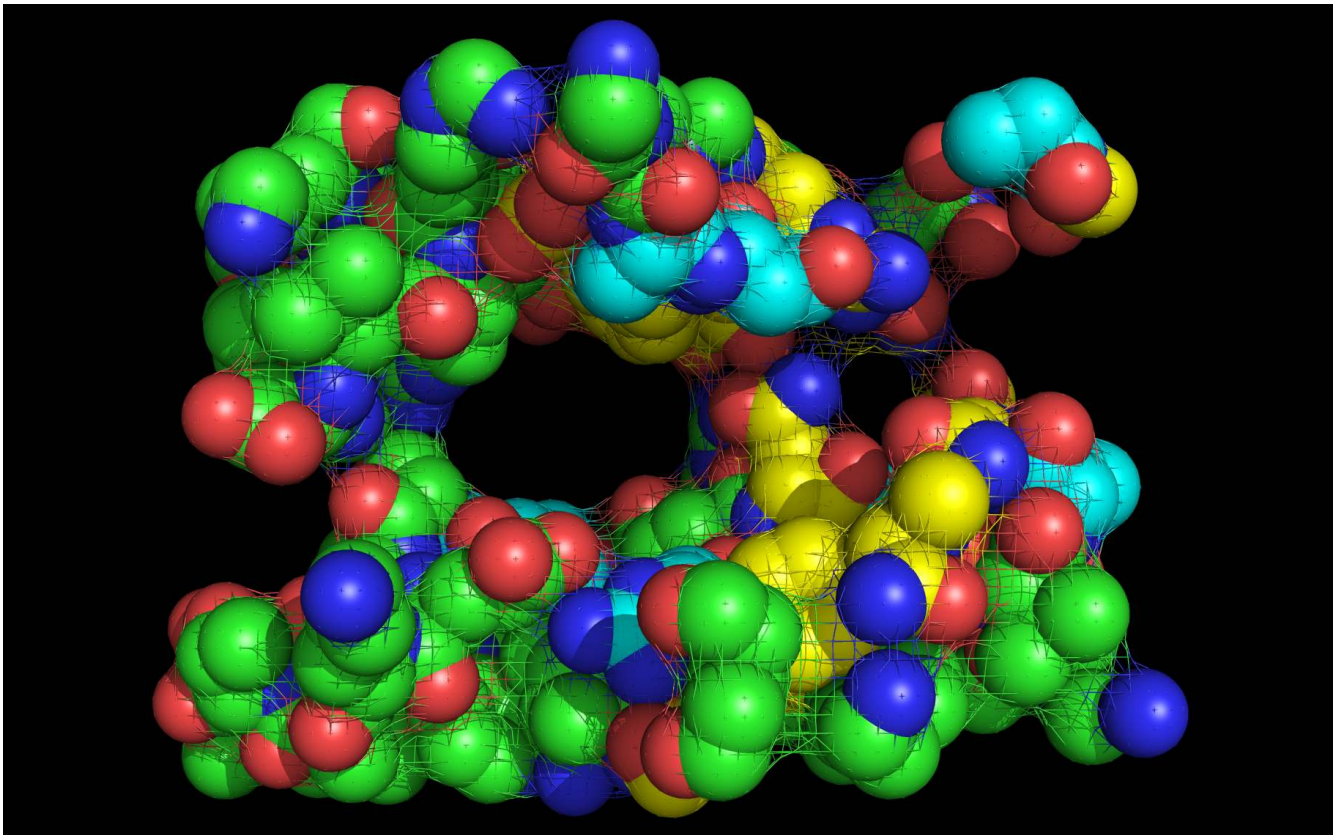
Nucleoprotein hexamer cartoon is colored grey.

Nucleoprotein molecule spheres and mesh WWaveCores™ are color-coded according to element and whether the residue is "sensitive", "tolerant", or "not characterized".

Sensitive residues are color coded according to element (C N O S).

Tolerant residues are color coded according to element (C N O S).

Residues not characterized with respect to mutation are color coded according to element (C N O S).



Trimer-trimer contact WWaveCores™ showing mutants

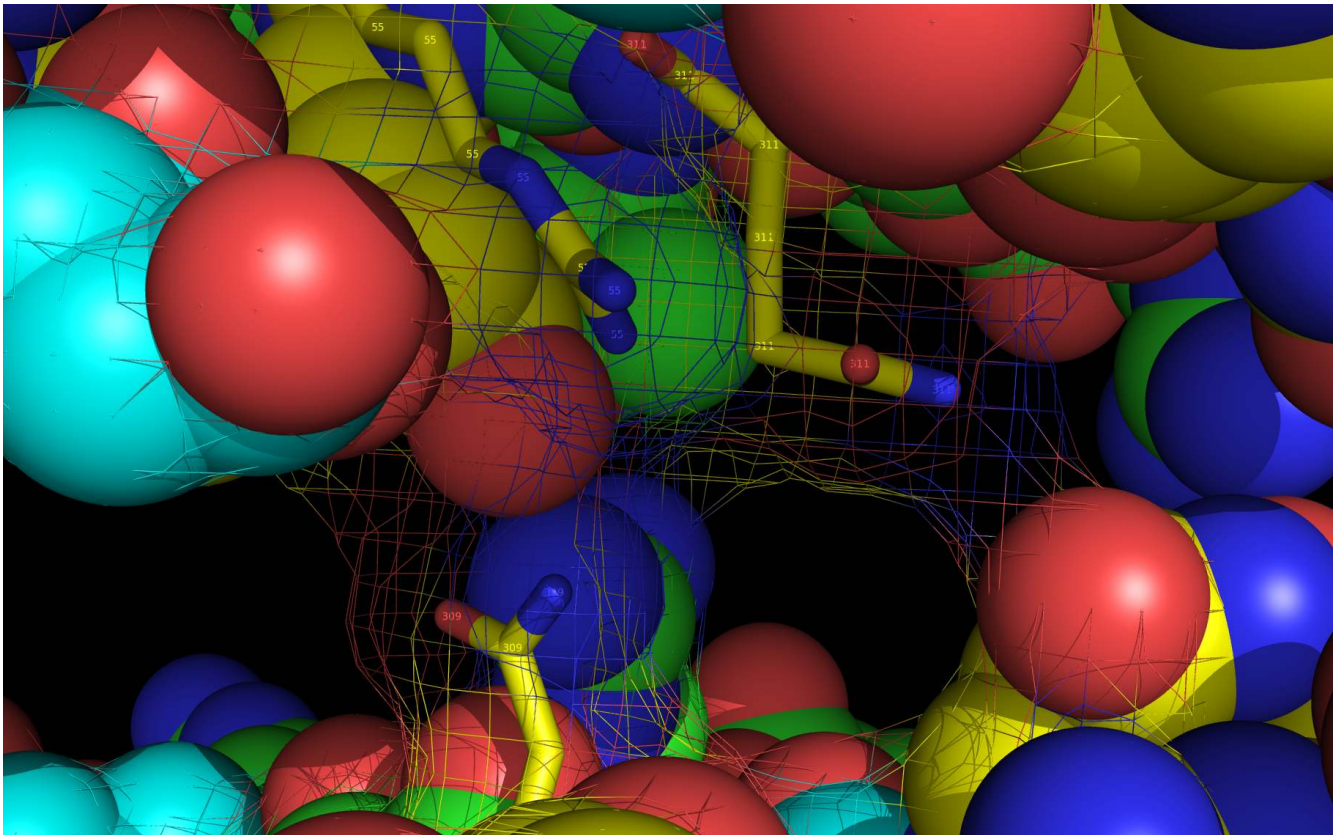
Shown here are partial WWaveCores™ for the area where "sensitive" and "tolerant" mutants have been identified to alter compound binding.[1,2,3] "Sensitive" residues are shown with yellow carbons: Y52, E53, R55, A284, S287, G288, Y289, D302, L306, N309, Q311, Y313 and S376. "Tolerant" residues are shown with cyan carbons: S50, D51, S283, A286, R305, S310, and L466.

Nucleoprotein molecule spheres and mesh WWaveCores™ are color-coded according to element and whether the residue is "sensitive", "tolerant", or "not characterized".

Sensitive residues are color coded according to element (C N O S).

Tolerant residues are color coded according to element (C N O S).

Residues not characterized with respect to mutation are color coded according to element (C N O S).



NP WWaveCores™ optimized by side chain repositioning

Three nucleoprotein residue side chains were moved to modify the WWaveCores™ to optimize the fit of the wwavePDB study compounds (nucleozin[1], compound 3061[2], BMS-885986[5], BMS-883559[6], BMS-885838[7], BMS-831780[8], and LGH[4]) in the WWaveCores™. Residue side chains R55, N309, and Q311 were moved to optimize the WWaveCores™ that define the alternative binding site for the compounds. Other than these three side chains, none of the other protein atoms in the nucleoprotein hexamer were moved. Residue N309 was moved onto the nucleoprotein surface into a position where it could better fit WWaveCores™ for the compounds. R55 was moved slightly to better coordinate the binding of either an oxygen or bromine compound atom. Q311 was moved slightly to better coordinate with the R55 on the same chain and Q311 on the opposite chain.

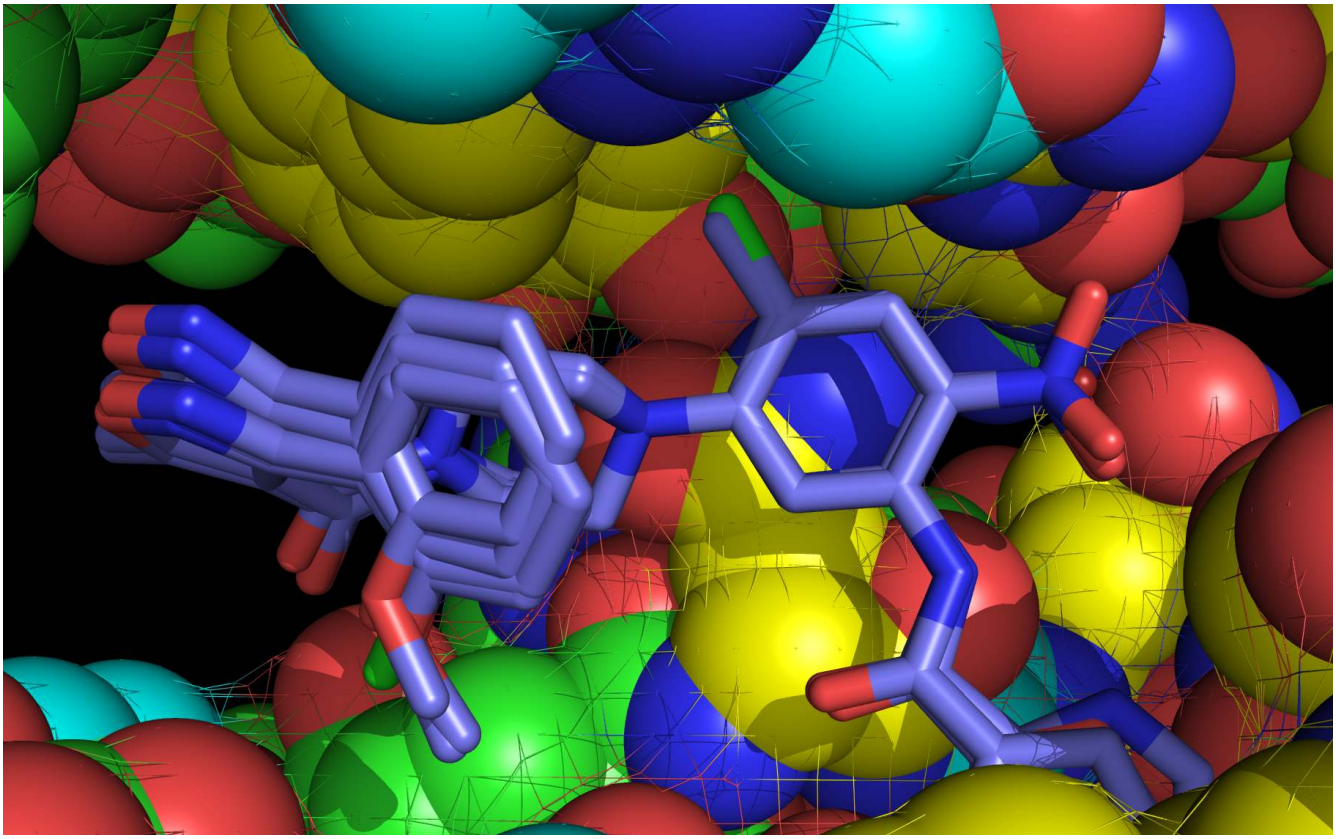
Nucleoprotein residue sticks whose side chain positions were altered to fit WWaveCores™ are "sensitive" and are color-coded according to element (C N O S).

Nucleoprotein molecule spheres and mesh WWaveCores™ are color-coded according to element and whether the residue is "sensitive", "tolerant", or "not characterized".

Sensitive residues are color coded according to element (C N O S).

Tolerant residues are color coded according to element (C N O S).

Residues not characterized with respect to mutation are color coded according to element (C N O S).



NP WWaveCores™, mutant, and compound positions

Shown are the reoriented and superimposed nucleozin[1], compound 3061[2], BMS-885986[5], BMS-883559[6], BMS-885838[7], BMS-831780[8], and LGH[4] compounds in the wwavePDB-identified and built alternative binding site.

Nucleozin[1], compound 3061[2], BMS-885986[5], BMS-883559[6], BMS-885838[7], BMS-831780[8], and LGH[4] were reoriented relative to WWaveCores™ between the 3ZDP[9] trimers. None of the BMS-885986[5], BMS-883559[6], BMS-885838[7], BMS-831780[8], and LGH[4] atom configurations were altered from the crystal structures other than spatial reorientation. In this alternative binding site defined by the WWaveCores™, the compounds are reoriented in the plane between the two trimers. This alternative binding site is orthogonal to the crystal structure binding configurations[4,5,6,7,8]. Compounds positioned in the WWaveCores™ defining the alternate binding site have extensive contacts with sensitive residues.

The repositioned side chain of the N309 nucleoprotein residue contributes favorable contacts on the "floor" of the binding site. The N309 repositioned residue (whose side chain nitrogen can be seen through the compound rings) is well-suited to optimally fill the space below the compound without interfering with compound binding. The N309 residue is sensitive and is known to mutate to lysine. An N309K mutation would interfere with compound binding in this alternative binding site partly because a lysine is bulky and at this position cannot move completely out of the way of the compound.

Residues Y52, R55, and Q311 line the long axis of the molecule at the top of the picture and have been determined to be sensitive residues[1,3]. Y52 contributes important contacts for ring structures at one end

of the molecule (far left). R55 coordinates bound compounds at the other end of the compound (far right). R55 coordinates an oxygen or bromine atom at the other end of the compound (far right). Q311 moves slightly from its crystal structure position and can coordinate an R55 from its own chain and another Q311 from the adjacent chain. Observed reduction in binding upon mutation of Q311 to arginine, aspartic acid, or asparagine, may be due to elimination of the residue bridging provided by Q311 in the alternative binding site. The Q311R, Q311D, and Q311N mutations would also disrupt the coordination of the binding of the compound oxygen or bromine in the alternative binding site.

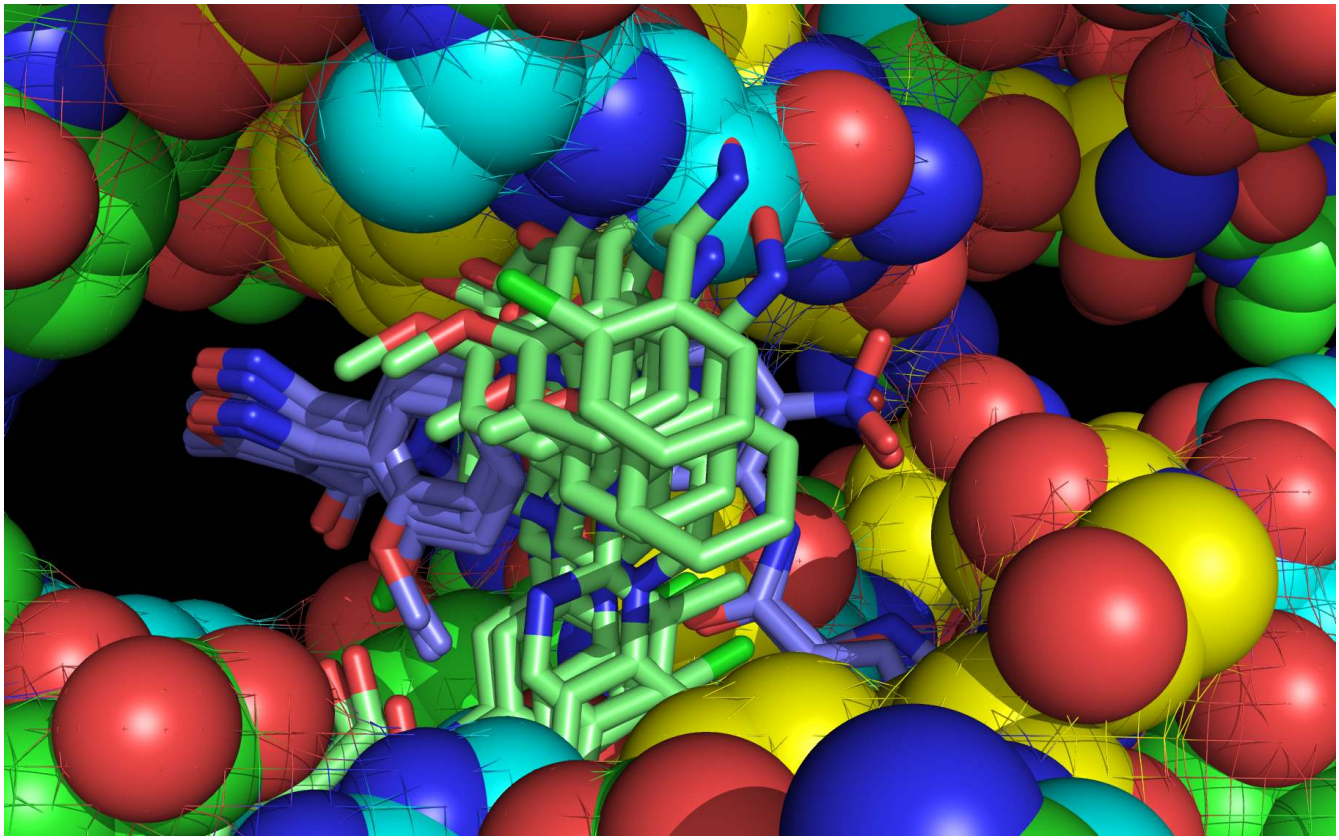
wwavePDB-reoriented compounds (nucleozin[1], compound 3061[2], BMS-885986[5], BMS-883559[6], BMS-885838[7], BMS-831780[8], and LGH[4]) sticks are color-coded according to element (C N O S Cl Br).

Nucleoprotein molecule spheres and mesh WWaveCores™ are color-coded according to element and whether the residue is “sensitive”, “tolerant”, or “not characterized”.

Sensitive residues are color coded according to element (C N O S).

Tolerant residues are color coded according to element (C N O S).

Residues not characterized with respect to mutation are color coded according to element (C N O S).



wwavePDB alternative and crystal compound positions

Shown is a set of compounds (nucleozin[1], compound 3061[2], BMS-885986[5], BMS-883559[6], BMS-885838[7], BMS-831780[8], and LGH[4]) reoriented in the WWaveCores™-defined alternative binding site (purple carbons). Also shown is a second set of compounds (BMS-885986[5], BMS-883559[6], BMS-885838[7], BMS-831780[8], and LGH[4]) oriented in the binding site as reported in the crystal structures[4,5,6,7,8] (light green carbons). The WWaveCores™-defined alternative binding site is positioned orthogonally to the crystal structure[4,5,6,7,8] binding sites.

Crystal structure-oriented compound (BMS-885986[5], BMS-883559[6], BMS-885838[7], BMS-831780[8], and LGH[4]) sticks are color-coded according to element (C N O S Cl Br).

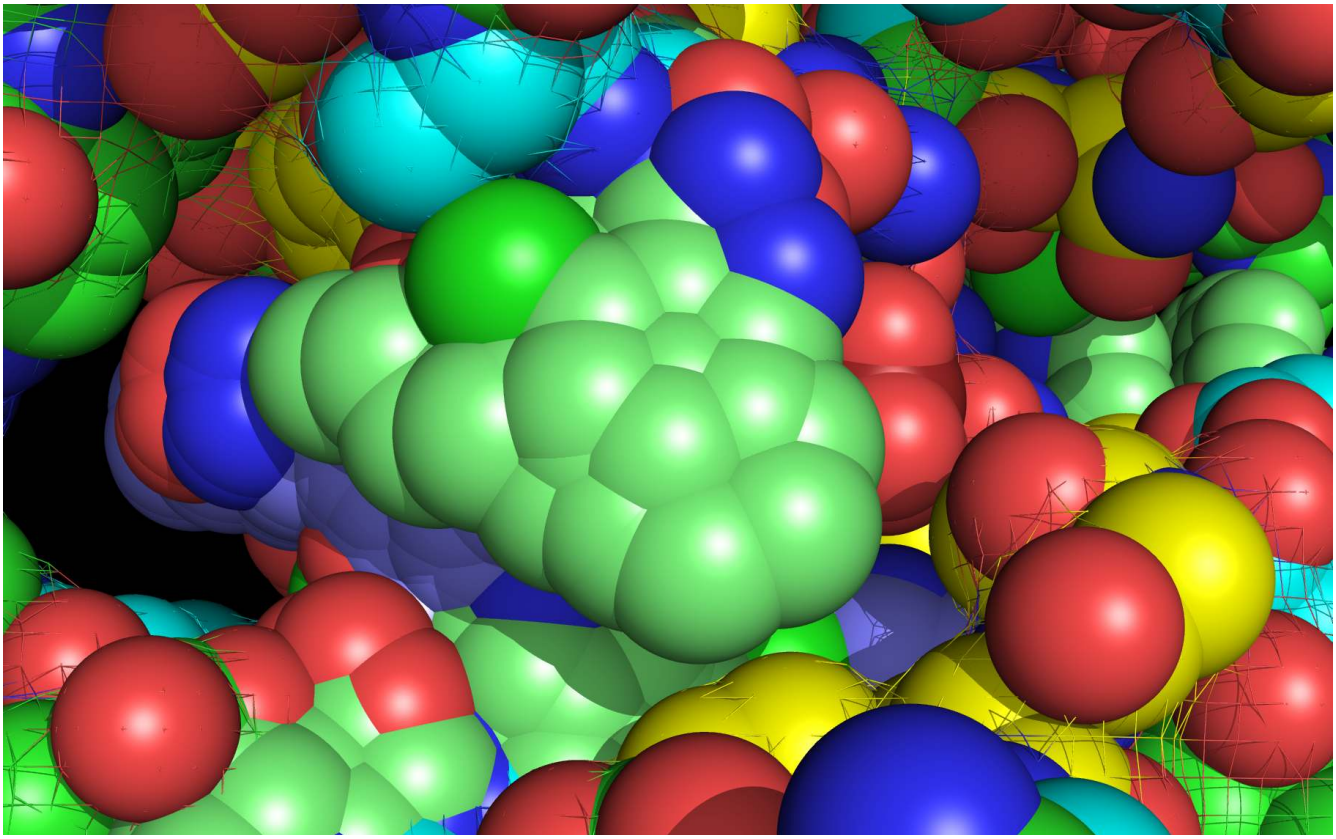
wwavePDB-reoriented compounds (nucleozin[1], compound 3061[2], BMS-885986[5], BMS-883559[6], BMS-885838[7], BMS-831780[8], and LGH[4]) sticks are color-coded according to element (C N O S Cl Br).

Nucleoprotein molecule spheres and mesh WWaveCores™ are color-coded according to element and whether the residue is "sensitive", "tolerant", or "not characterized".

Sensitive residues are color coded according to element (C N O S).

Tolerant residues are color coded according to element (C N O S).

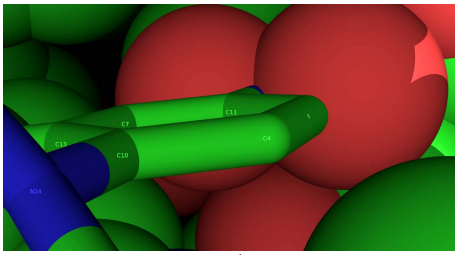
Residues not characterized with respect to mutation are color coded according to element (C N O S).



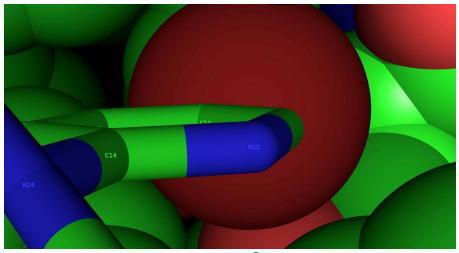
WWaveCores™ for alternative and crystal positions

Shown above are WWaveCores™ for a set of compounds (nucleozin[1], compound 3061[2], BMS-885986[5], BMS-883559[6], BMS-885838[7], BMS-831780[8], and LGH[4]) reoriented in the WWaveCores™-defined alternative binding site. Also shown are WWaveCores™ for a second set of compounds (BMS-885986[5], BMS-883559[6], BMS-885838[7], BMS-831780[8], and LGH[4]) oriented in the binding site as reported in the crystal structures[4,5,6,7,8]. The WWaveCores™-defined alternative binding site is positioned orthogonally to the crystal structure[4,5,6,7,8] binding sites. The volume of all compounds positioned in the alternative binding site identified by wwavePDB and their respective positions in the crystal structure[4,5,6,7,8] overlap.

In contrast to the wwavePDB-identified alternative binding site, the NH1 and NH2 atoms of LGH (in the 3RO5[4] crystal structure, below left) and the bromine atom of BMS-831780 (in the 4DYP[8] crystal structure, below right) are positioned in a pocket lined with a number of hydrophobic and negative atoms.



LGH in the 3RO5[4] crystal structure



831780 in the 4DYP[8] crystal structure

Crystal structure-oriented compound (BMS-885986[5], BMS-883559[6], BMS-885838[7], BMS-831780[8], and LGH[4]) spheres are color-coded according to element (C N O S Cl Br).

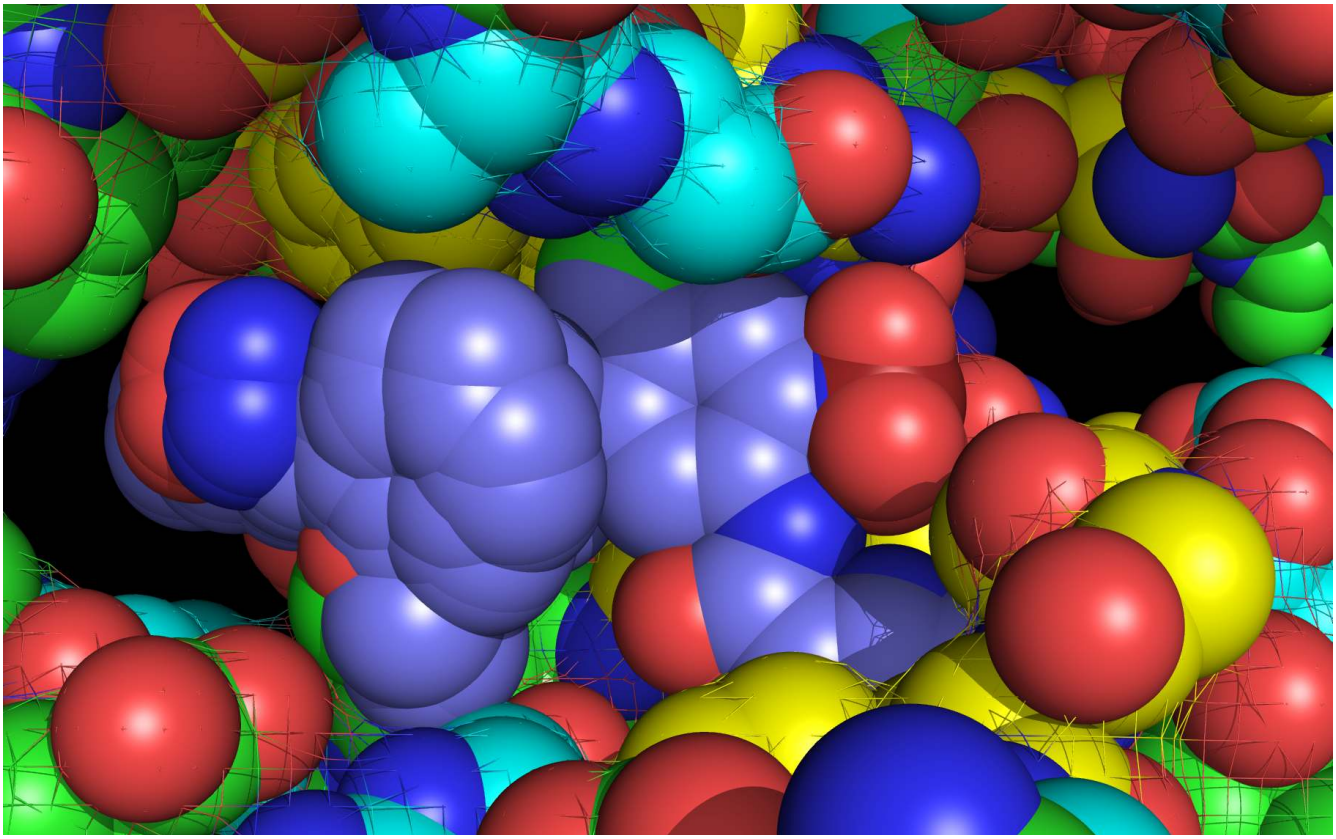
wwavePDB-reoriented compounds (nucleozin[1], compound 3061[2], BMS-885986[5], BMS-883559[6], BMS-885838[7], BMS-831780[8], and LGH[4]) spheres are color-coded according to element (C N O S Cl Br).

Nucleoprotein molecule spheres and mesh WWaveCores™ are color-coded according to element and whether the residue is “sensitive”, “tolerant”, or “not characterized”.

Sensitive residues are color coded according to element (C N O S).

Tolerant residues are color coded according to element (C N O S).

Residues not characterized with respect to mutation are color coded according to element (C N O S).



WWaveCores™ for compounds in alternative positions

Shown are WWaveCores™ for a set of compounds (nucleozin[1], compound 3061[2], BMS-885986[5], BMS-883559[6], BMS-885838[7], BMS-831780[8], and LGH[4]) \ reoriented in the WWaveCores™-defined alternative binding site. This alternative binding site is bracketed by two tyrosine residues (Y52 lining the upper left WWaveCores™ and Y289 lining the lower right WWaveCores™, both with yellow carbon spheres) on opposite sides of the dimer interface. This alternative binding site is also bracketed by N309 (top right yellow spheres) and S288 (bottom right yellow spheres). The slightly repositioned R55 residue NH1 and NH2 (top middle right blue spheres) coordinates compound terminal oxygen or bromine (top middle right red spheres or red-brown spheres). Y52, Y289, N309, S288 and R55 are all sensitive residues. The residues lining the WWaveCores™ provide well-matched hydrophobic binding sites for the hydrophobic compound rings. The volumes of all of the compounds fit within the nucleoprotein WWaveCores™ with excellent stereochemistry.

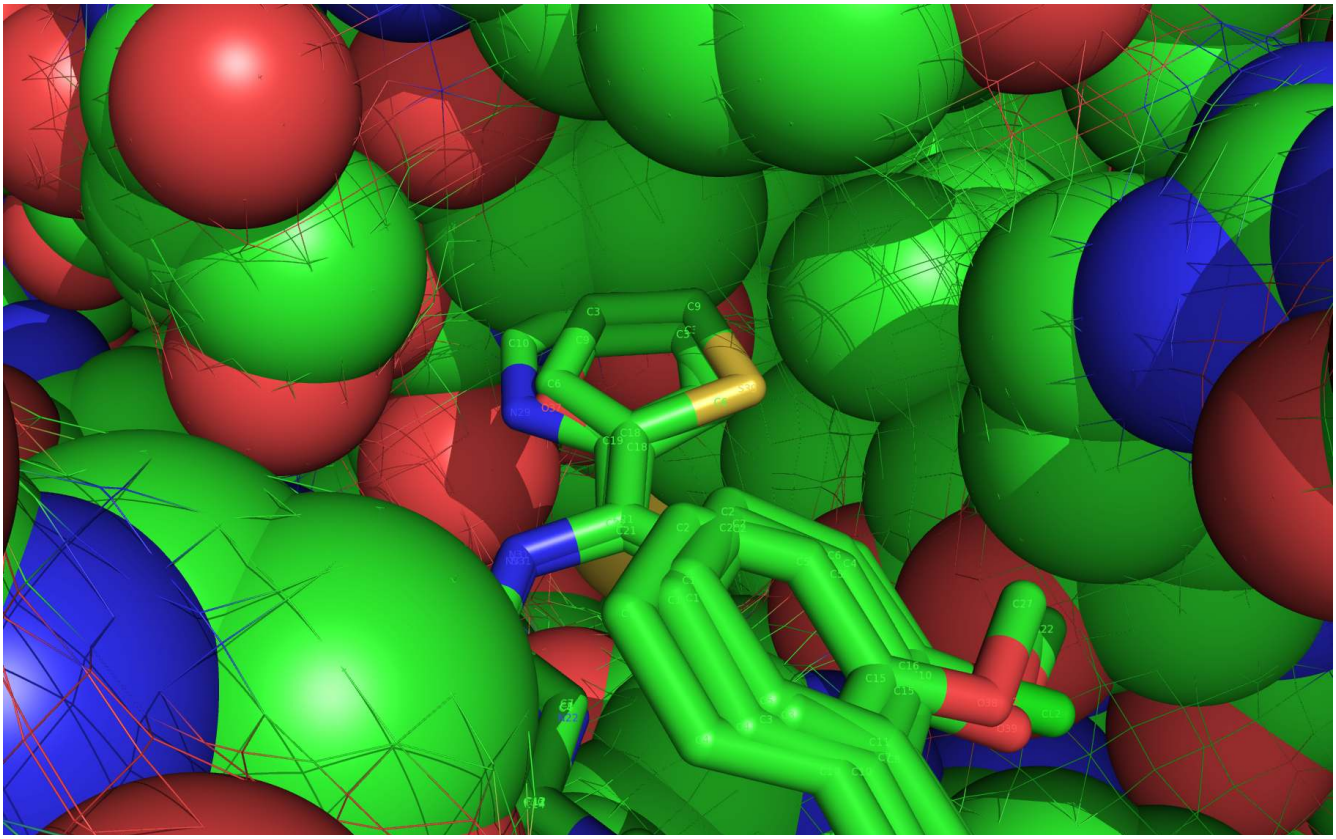
wwavePDB-reoriented compounds (nucleozin[1], compound 3061[2], BMS-885986[5], BMS-883559[6], BMS-885838[7], BMS-831780[8], and LGH[4]) spheres are color-coded according to element (C N O S Cl Br).

Nucleoprotein molecule spheres and mesh WWaveCores™ are color-coded according to element and whether the residue is “sensitive”, “tolerant”, or “not characterized”.

Sensitive residues are color coded according to element (C N O S).

Tolerant residues are color coded according to element (C N O S).

Residues not characterized with respect to mutation are color coded according to element (C N O S).

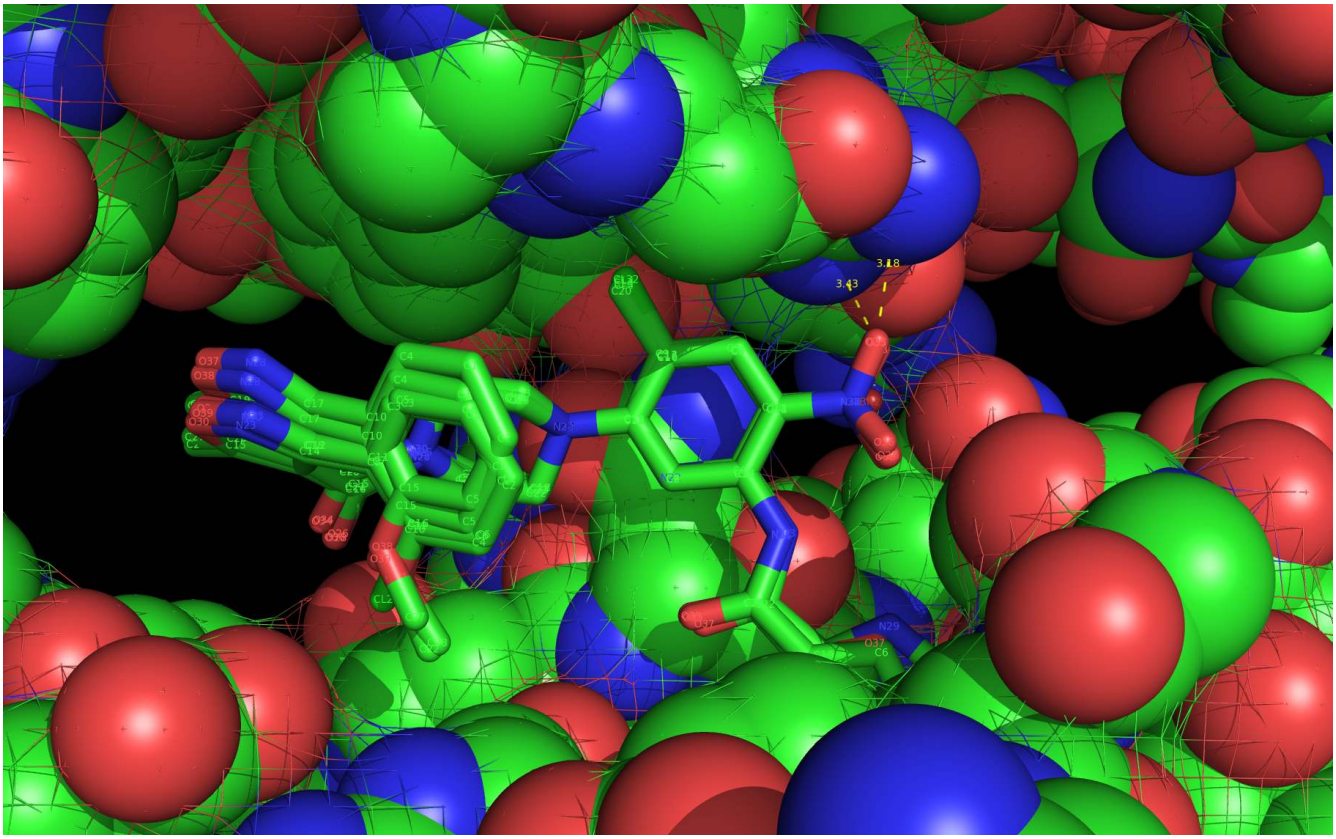


NP WWaveCores™ and compounds in alternative NP site

Shown is a set of compounds (nucleozin[1], compound 3061[2], BMS-885986[5], BMS-883559[6], BMS-885838[7], BMS-831780[8], and LGH[4]) reoriented in the WWaveCores™-defined alternative binding site. This alternative binding site provides a large hydrophobic site that can accommodate large hydrophobic rings, including rings with large sulfur atoms (colored gold). The binding site defined by the WWaveCores™ is lined with hydrophobic residues that have been found[2,3] to be "sensitive": A284, S287, Y289, D302 and L306. The TYR289 (green and red spheres immediately above and above right of the compounds) has the latitude to reposition itself over the lower middle compound rings.

wwavePDB-reoriented compounds (nucleozin[1], compound 3061[2], BMS-885986[5], BMS-883559[6], BMS-885838[7], BMS-831780[8], and LGH[4]) sticks are color-coded according to element (C N O S Cl).

Nucleoprotein molecule spheres and partial WWaveCores™ mesh are color-coded according to element (C N O S).



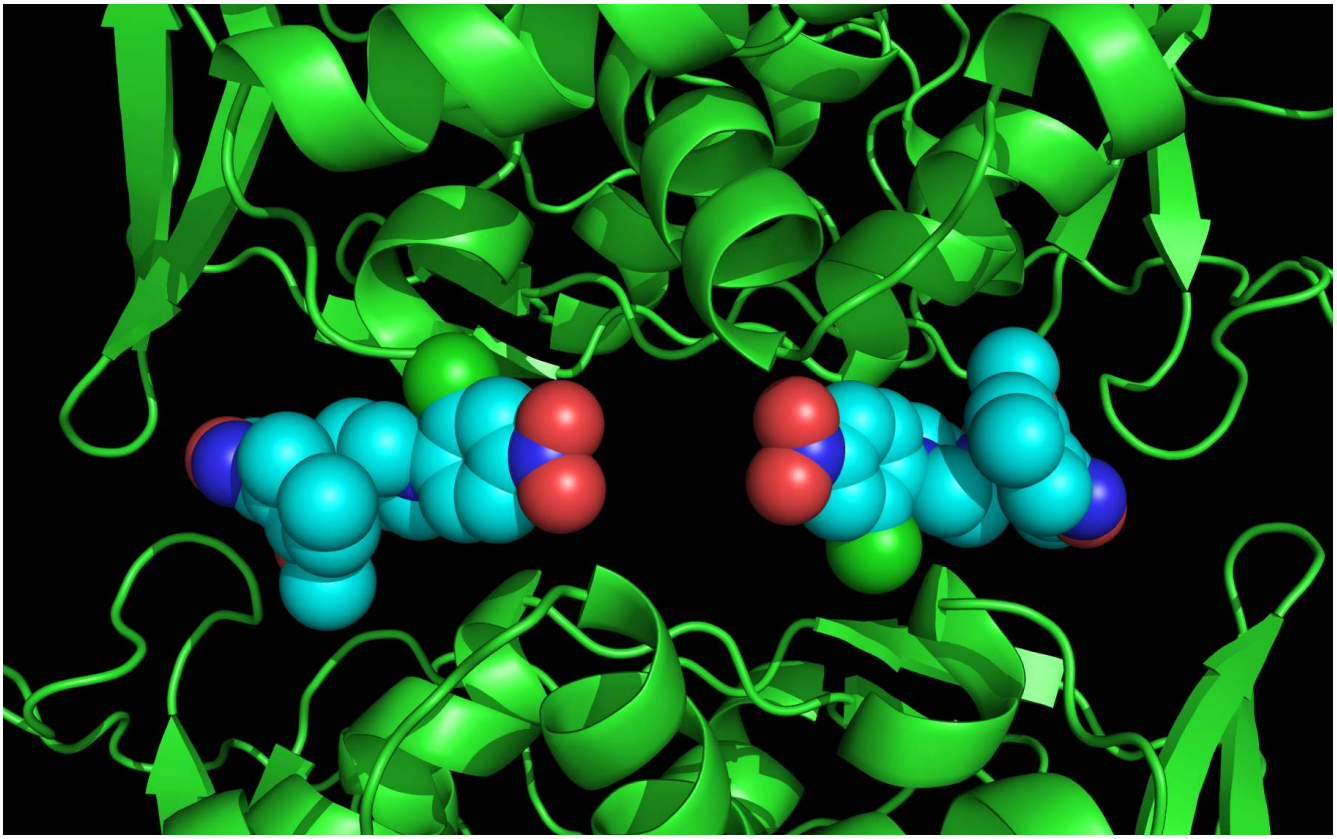
NP WWaveCores™ and compounds in alternative NP site

Shown is a set of compounds (nucleozin[1], compound 3061[2], BMS-885986[5], BMS-883559[6], BMS-885838[7], BMS-831780[8], and LGH[4]) reoriented in the WWaveCores™-defined alternative binding site.

R55 coordinates bound compounds in the wwavePDB-identified alternative binding site. Lines and distances between R55 side chains NH1 and NH2 and a compound oxygen are shown with dashed yellow lines.

wwavePDB-reoriented compounds (nucleozin[1], compound 3061[2], BMS-885986[5], BMS-883559[6], BMS-885838[7], BMS-831780[8], and LGH[4]) sticks are color-coded according to element (C N O S Cl).

Nucleoprotein molecule spheres and partial WWaveCores™ mesh are color-coded according to element (C N O S).

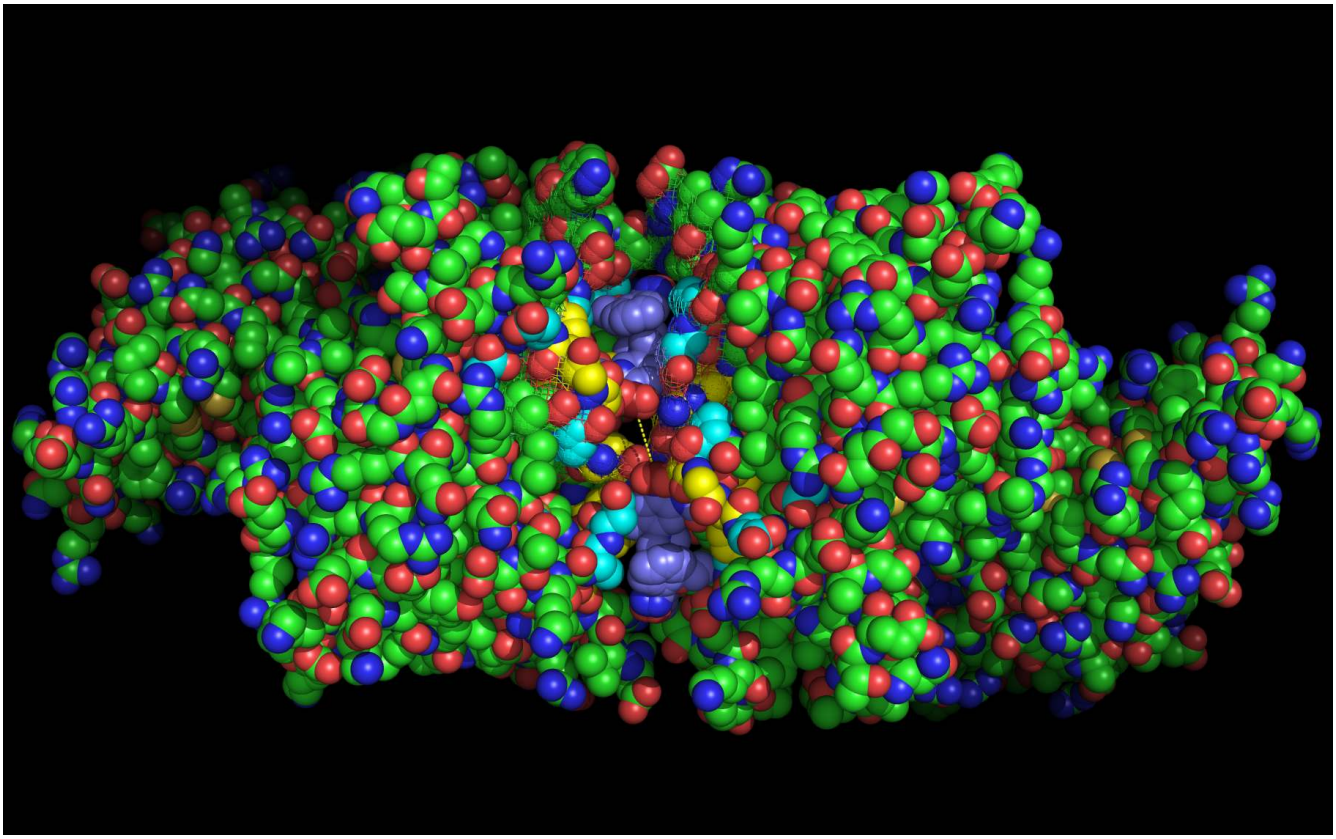


wwavePDB alternative NP sites with head to head LGHs

Shown are LGH molecules bound in the orientation dictated by the WWaveCores™. In the alternative nucleoprotein dimer binding sites defined by the WWaveCores™, the twin oxygens in the bound LGH molecules face each other.

Nucleoprotein dimer cartoon is colored green.

wwavePDB-reoriented compounds LGH^4 spheres in the alternative binding configuration are color-coded according to element (C N O Cl).



wwavePDB alternative NP sites with head to head LGHs

Shown are the wwavePDB-repositioned nucleozin[1], compound 3061[2], BMS-885986[5], BMS-883559[6], BMS-885838[7], BMS-831780[8], and LGH[4] superimposed compounds in the alternative nucleoprotein binding site identified by wwavePDB. The BMS-831780 bromines are separated by approximately 8 angstroms as indicated by dashed yellow lines.

wwavePDB-reoriented compounds (nucleozin[1], compound 3061[2], BMS-885986[5], BMS-883559[6], BMS-885838[7], BMS-831780[8], and LGH[4]) spheres are color-coded according to element (C N O S Cl Br).

Nucleoprotein molecule spheres and mesh WWaveCores™ are color-coded according to element and whether the residue is "sensitive", "tolerant", or "not characterized".

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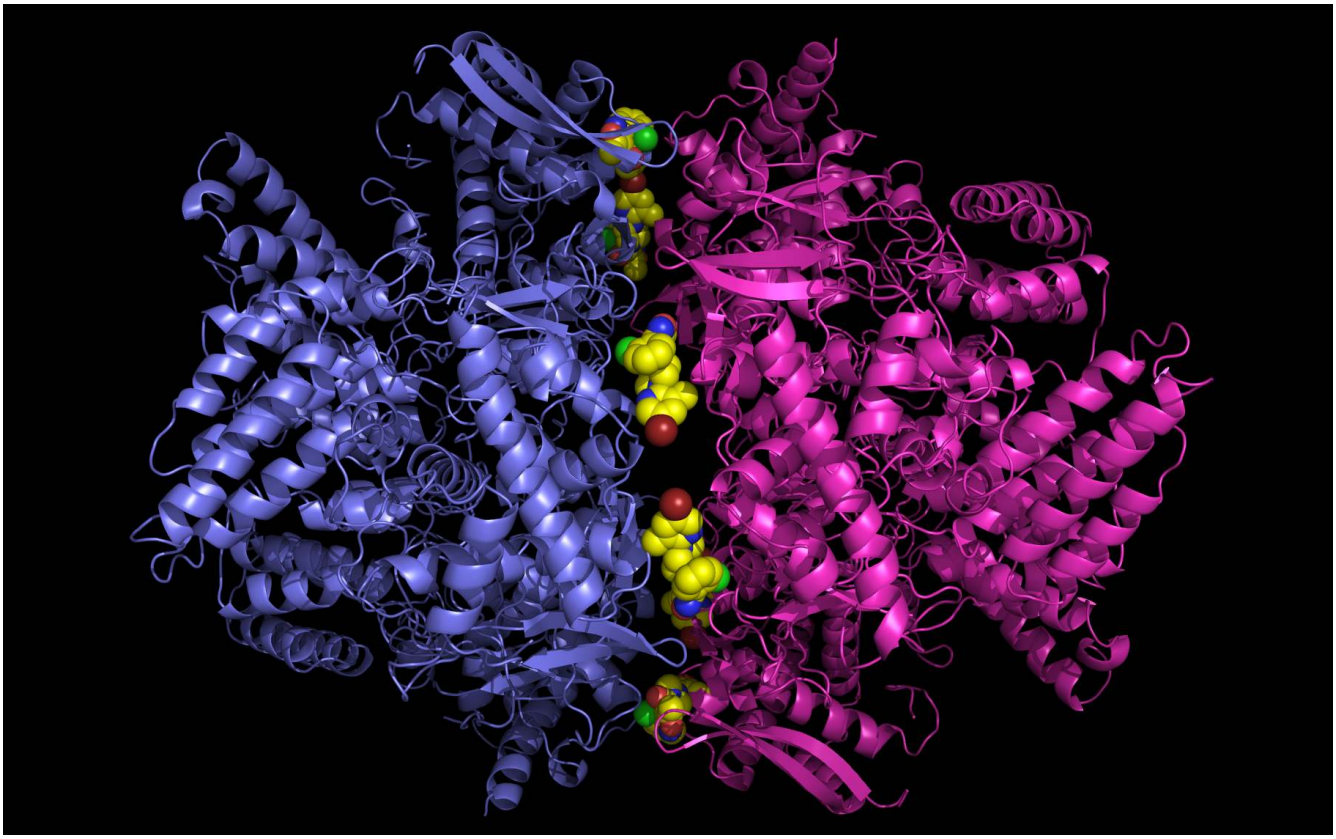
Tolerant residues are color coded according to element (C N O S).

Residues not characterized with respect to mutation are color coded according to element (C N O S).



Compounds in alternative NP sites between trimers

Shown are six BMS-831780 molecules in the putative alternative binding site positions in the constructed nucleoprotein hexamer. The repositioned BMS-831780 compounds are orthogonal to the reported compound crystal structure positions[4,5,6,7,8] and have excellent stereochemistry relative to the nucleoprotein hexamer. The bromines are separated by approximately 8 angstroms. There are a number of ways of testing the usefulness of this alternative nucleoprotein binding site for drug design. A linker between opposing BMS-831780 compounds would confirm this alternative binding site. A linkage between the two opposing BMS-831780 compounds might be acheived through a condensation reaction with the terminal bromines.

One 3ZDP[9] nucleoprotein trimer cartoon is colored purple.

The other 3ZDP[9] nucleoprotein trimer cartoon is colored magenta.

BMS-831780 molecule spheres are color-coded according to element (C N O S Cl Br).

BMS-831780 is: [4-(5-bromanyl-3-methyl-pyridin-2-yl)piperazin-1-yl]-[3-(2-chlorophenyl)-5-methyl-1,2-oxazol-4-yl]methanone.

BMS-831780 in SMILES: Cc1onc(c2ccccc2Cl)c1C(=O)N3CCN(CC3)c4ncc(Br)cc4C