Molecular Dynamics study of the UQ construct of West Nile Virus NS2B/NS3 protease and it's interactions with inhibitors.

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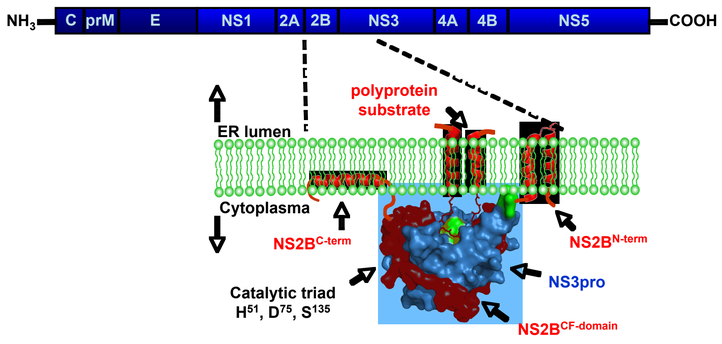
1. Flavivirus Introduction

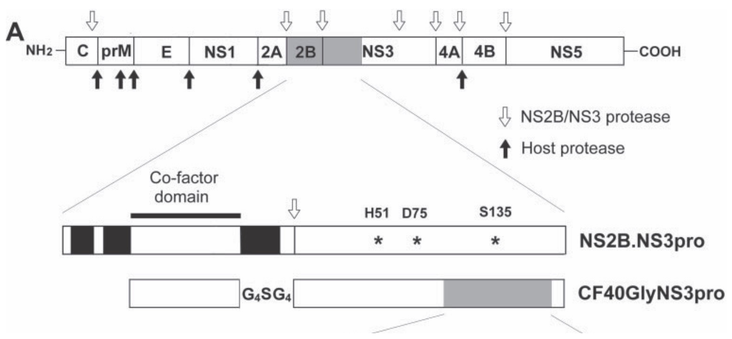
- Genus within the *Flaviviridae*
- Over 70 species
- Positive stranded RNA viruses
- Primarily transmitted to mammals by mosquitoes and ticks
- Generally cause a mild febrile illness
- Severe forms often fatal, involving either encephalitic or haemorrhagic pathologies.
- All contain a NS2B/NS3 two-component protease crucial for viral replication

2. A membrane-bound protease



- The protease is tethered to the lumenal side of the ER membrane in infected cells
- The transmembrane tethers are the α-helical portions of the NS2B protein.
- NS2B forms a loop that cradles NS3 close to the membrane.
- Viral protein substrates are also membrane bound

3. The UQ construct

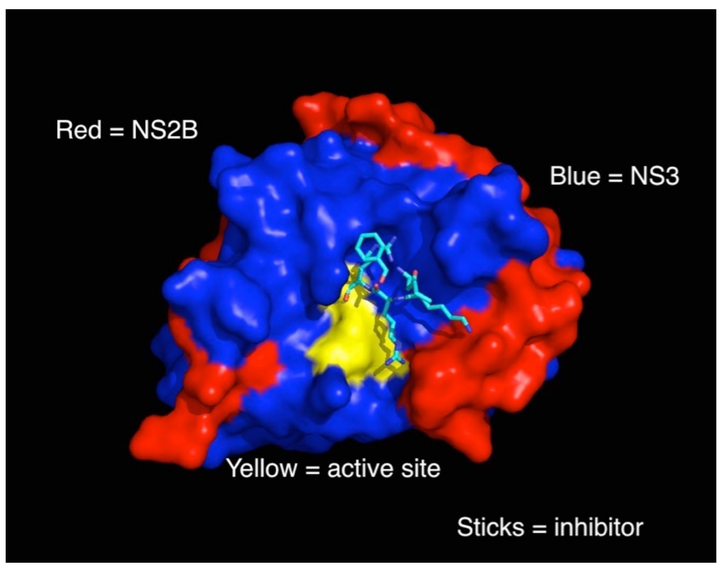


- Originally developed for Dengue, a construct with a flexible GGGGSGGGG linker enabled production of soluble protein for *in vitro* studies,[1] crystal structures[2]
- Constructs with G_nSG_n linkers have been successfully extended to multiple other viruses including WNV, ZIKV, MVEV, KUN, TBEV, JEV, YFV

4. Crystal Structures

- These constructs enabled structures of several viral proteases to be solved
- Dengue (2fom, 2vbc, 3l6p, 3lkw, 3u1i, 3u1j, 4m9f, 4m9i, 4m9k, 4m9m, 4m9t,
- WNV (2fp7, 2ggv, 2ijo, 3e90,[2] 2yol, 5idk, 2ggw)
- ZIKV (5lc0, 5t1v, 5gj4, 5gxj)
- These structures are of great importance for computer-based drug design and homology modelling of other flaviviral proteases.

5. Features *and failures* of the crystal structures



Structure 3e90[2]

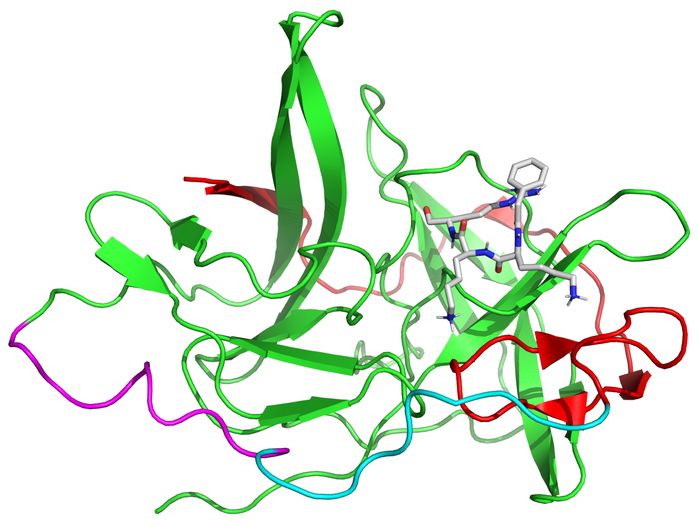
- The WNV NS2B protein encircles NS3 and interacts with inhibitors
- NS2B highly flexible in absence of inhibitors and moves away from active site
- The flexible linker usually entirely missing in the electron density along with N-terminus of NS3 and some of the NS2B C-terminus
- Critical NS2B residues involved in inhibitor and substrate binding often also missing.

6. Uncertiancies

- Location of linker uncertain
- Interactions of inhibitors with linker uncertain
- Does the "missing" linker impede access of inhibitors to the active site
- *Need a useful model of the entire active species*
- Can we use these synthetic constructs with confidence?
- Or are alternate bimolecular constructs without linkers more biorelevant?[3]

7. The New "UQ" WNV model

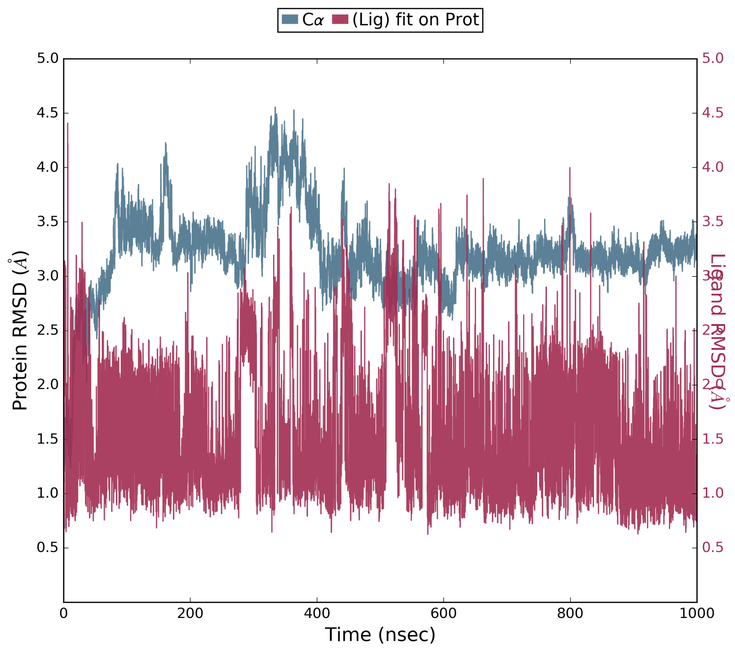
- The missing residues from the 3e90 structure were added using Schrödinger Suite 2016-3/Prime and whole protein minimised.
- More potent (Ki=9nM) inhibitor PhAcKKR-aldehyde added and docked with GOLD

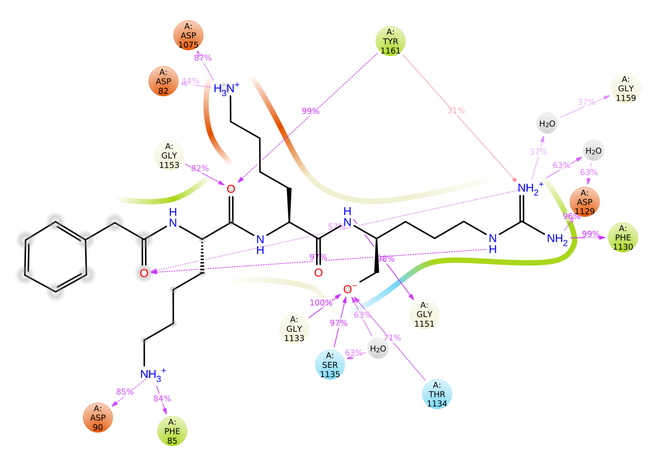


Red=NS2B; Green=NS3; Magenta=missing NS3 N-terminus; Cyan=Linker, Grey Sticks=aldehyde Inhibitor

8. Docking and Molecular Dynamics

- The whole flexible region extends from the last β-strand of NS2B, through the linker to the N-terminus of NS3
- Ran 1μs Molecular dynamics using Schrödinger Desmond
- The flexible linker was indeed flexible, but did make multiple transient contacts to NS3, particularly between 300-400 ns
- The inhibitor was very stable throughout the simulation





- A number of important protein-ligand contacts were maintained throughout the simulation

9. Conclusions

- The flexible G_nSG_n linker does not impede access to the active site
- The linker doesn't directly interact with the inhibitor.
- Linked constructs remain valid biochemical tools

References

1. Leung, D.; Schroder, K.; White, H.; Fang, N. X.; Stoermer, M. J.; Abbenante, G.; Martin, J. L.; Young, P. R.; Fairlie, D. P., Activity of recombinant dengue 2 virus NS3 protease in the presence of a truncated NS2B co-factor, small peptide substrates, and inhibitors. *J. Biol. Chem.* **2001**, *276 (49)*, 45762-71.

2. Robin, G.; Chappell, K.; Stoermer, M.J.; Hu, S.H.; Young, P.R.; Fairlie, D.P.; Martin, J.L. Structure of West Nile virus NS3 protease: ligand stabilisation of the catalytic conformation, *J. Mol. Biol.* **2009**, *385*, 1568-1577.

3. Kuiper, B. D.; Slater, K.; Spellmon, N.; Holcomb, J.; Medapureddy, P.; Muzzarelli, K. M.; Yang, Z.; Ovadia, R.; Amblard, F.; Kovari, I. A.; Schinazi, R. F.; Kovari, L. C., Increased activity of unlinked Zika virus NS2B/NS3 protease compared to linked Zika virus protease. *Biochem Biophys Res Commun* **2017**, *492 (4)*, 668-673.

4. Shannon, A. E.; Chappell, K. J.; Stoermer, M. J.; Chow, S. Y.; Kok, W. M.; Fairlie, D. P.; Young, P. R., Simultaneous uncoupled expression and purification of the Dengue virus NS3 protease and NS2B co-factor domain. *Protein expression and purification* **2016**, *119*, 124-9.

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