Atomic structure of the translation regulatory protein NS1 of bluetongue virus

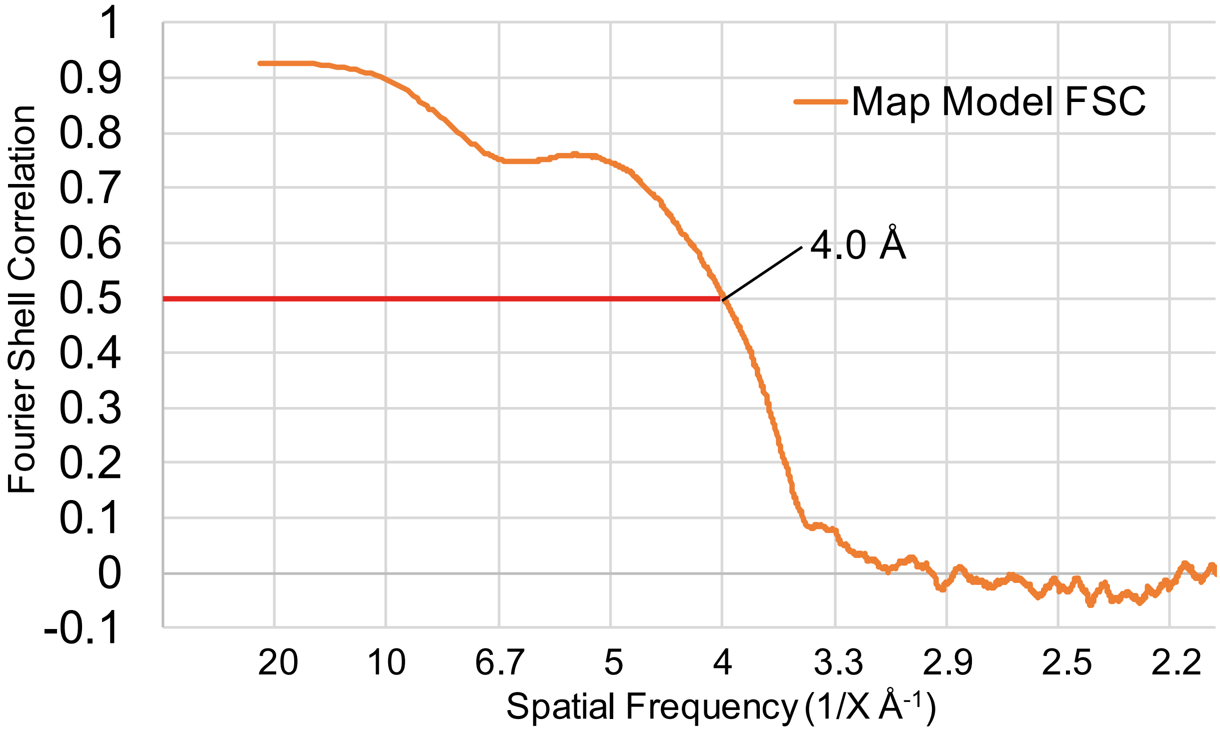
Adeline Kerviel[1,5], Peng Ge[2,5], Mason Lai[2,3,5], Jonathan Jih[2], Mark Boyce[1], Xing Zhang[2,4], Z. Hong Zhou[2,3,*], Polly Roy[1,*]

[1]Department of Pathogen Molecular Biology, London School of Hygiene and Tropical Medicine, United Kingdom
[2]California NanoSystems Institute, University of California, Los Angeles (UCLA), Los Angeles CA 90095, USA
[3]Department of Microbiology, Immunology & Molecular Genetics, UCLA, CA 90095, USA

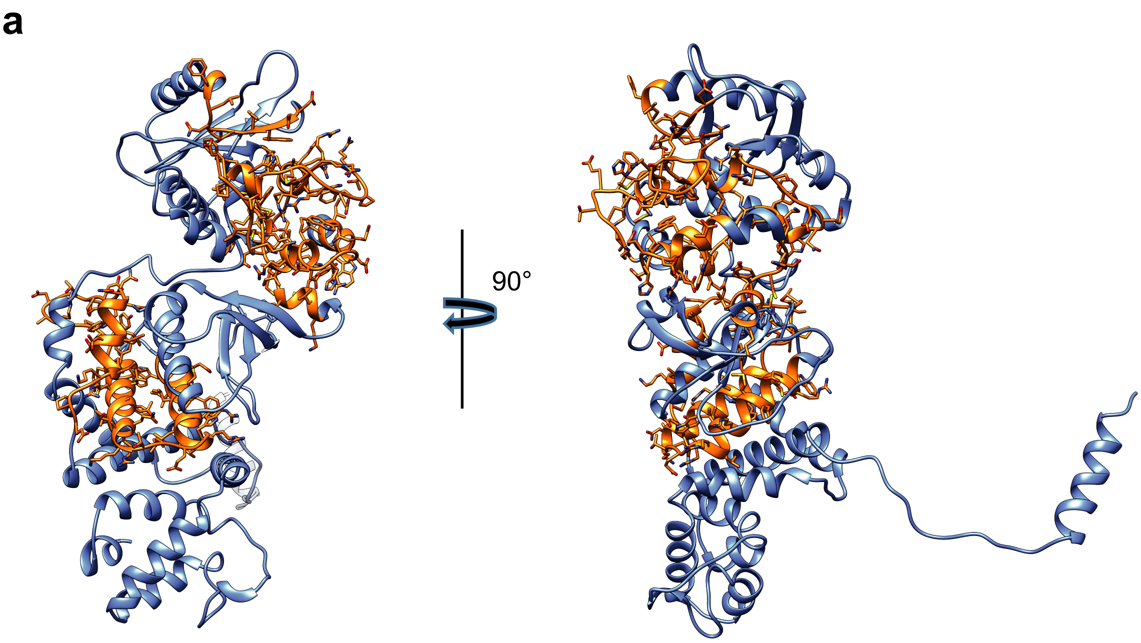
[4]Current Address: Center of Cryo Electron Microscopy, Department of Biophysics, Zhejiang University School of Medicine, Hangzhou, China

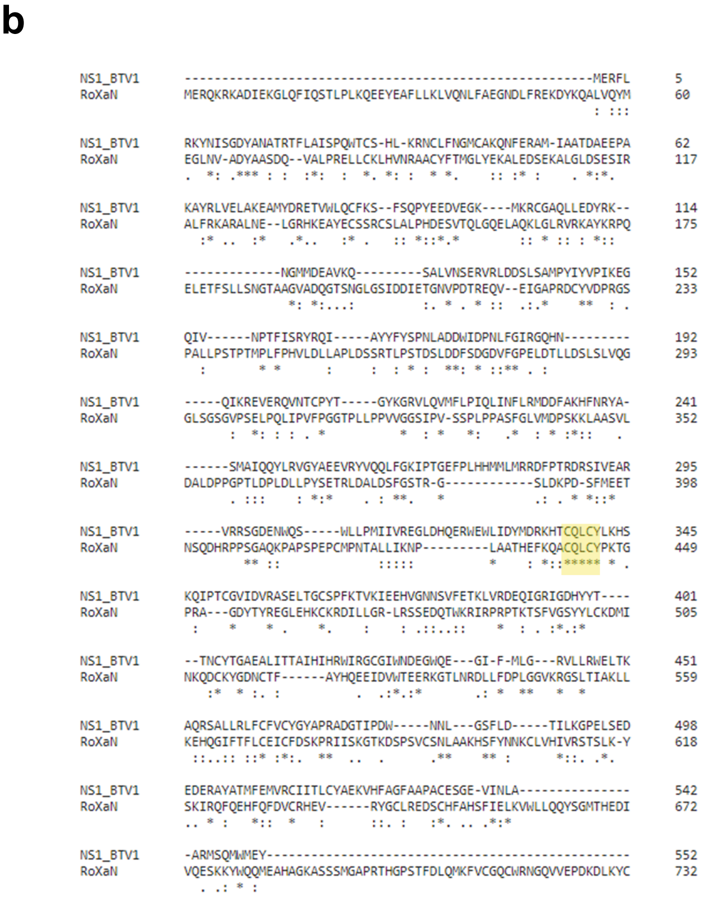
[5]These authors contributed equally: Adeline Kerviel, Peng Ge, Mason Lai.

Supplementary Information



Supplementary Figure 1: Fourier shell correlation (FSC) between the cryoEM density map and our atomic model showing a resolution of 4.0 Å.





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NS1_BTV1        -------------------------------------------------------MERFL      5
RoXaN           MERQKRKADIEKGLQFIQSTLPLKQEEYEAFLLKLVQNLFAEGNDLFREKDYKQALVQYM      60
                                                                       : :::

NS1_BTV1        RKYNISGDYANATRTFLAISPQWTCS-HL-KRNCLFNGMCAKQNFERAM-IAATDAEEPA      62
RoXaN           EGLNV-ADYAASDQ--VALPRELLCKLHVNRAACYFTMGLYEKALEDSEKALGLDSESIR      117
                .  *: .*** : :  :*:  :  *. *: :  * *.    :: :* :    . *:*.

NS1_BTV1        KAYRLVELAKEAMYDRETVWLQCFKS--FSQPYEEDVEGK----MKRCGAQLLEDYRK--      114
RoXaN           ALFRKARALNE--LGRHKEAYECSSRCSLALPHDESVTQLGQELAQKLGLRVRKAYKRPQ      175
                  :* ..  :*   .*..   :* .   :: *::*.*        :: * :: : *::

NS1_BTV1        -------------NGMMDEAVKQ---------SALVNSERVRLDDSLSAMPYIYVPIKEG      152
RoXaN           ELETFSLLSNGTAAGVADQGTSNGLGSIDDIETGNVPDTREQV--EIGAPRDCYVDPRGS      233
                              *: *:...:         :. * . * ::  .:.*    **  : .

NS1_BTV1        QIV------NPTFISRYRQI-----AYYFYSPNLADDWIDPNLFGIRGQHN---------      192
RoXaN           PALLPSTPTMPLFPHVLDLLAPLDSSRTLPSTDSLDDFSDGDVFGPELDTLLDSLSLVQG      293
                  :       * *      :     :  : * :  **: * ::** . :

NS1_BTV1        -----QIKREVERQVNTCPYT-----GYKGRVLQVMFLPIQLINFLRMDDFAKHFNRYA-      241
RoXaN           GLSGSGVPSELPQLIPVFPGGTPLLPPVVGGSIPV-SSPLPPASFGLVMDPSKKLAASVL      352
                      :  *: : : . *          *  : *   *:   .*  : * :*::   .

NS1_BTV1        ------SMAIQQYLRVGYAEEVRYVQQLFGKIPTGEFPLHHMMLMRRDFPTRDRSIVEAR      295
RoXaN           DALDPPGPTLDPLDLLPYSETRLDALDSFGSTR-G------------SLDKPD-SFMEET      398
                      . :::    : *:*    . : **.   *            .: . * *::*

NS1_BTV1        -----VRRSGDENWQS-----WLLPMIIVREGLDHQERWEWLIDYMDRKHTCQLCYLKHS      345
RoXaN           NSQDHRPPSGAQKPAPSPEPCMPNTALLIKNP---------LAATHEFKQACQLCYPKTG      449
                        ** ::             :::::          *    : *::***** * .

NS1_BTV1        KQIPTCGVIDVRASELTGCSPFKTVKIEEHVGNNSVFETKLVRDEQIGRIGDHYYT----      401
RoXaN           PRA---GDYTYREGLEHKCKRDILLGR-LRSSEDQTWKRIRPRPTKTSFVGSYYLCKDMI      505
                 :    *    * .    *.    :    : .::..::    *  : . :*.:*

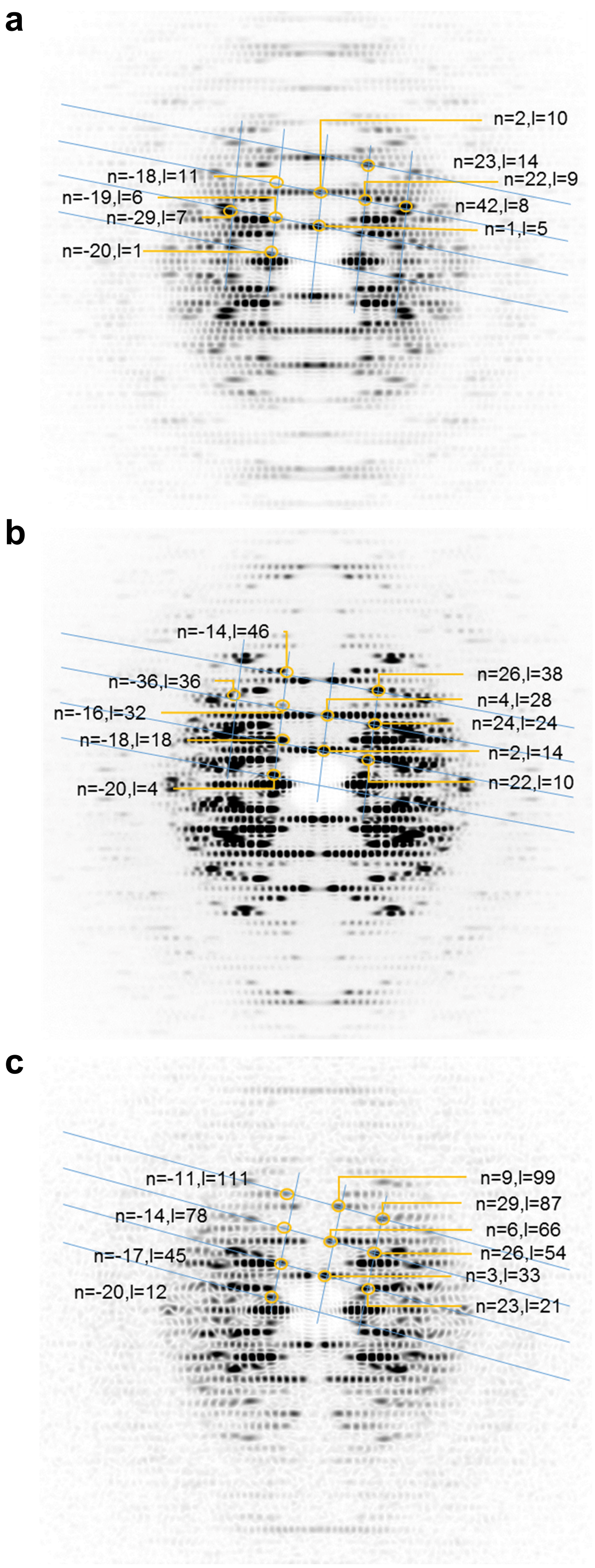
NS1_BTV1        --TNCYTGAEALITTAIHIHRWIRGCGIWNDEGWQE---GI-F-MLG---RVLLRWELTK      451
RoXaN           NKQDCKYGDNCTF------AYHQEEIDVWTEERKGTLNRDLLFDPLGGVKRGSLTIAKLL      559
                   :*  * :. :          .  .:*.:*       .: *  **   *  *

NS1_BTV1        AQRSALLRLFCFVCYGYAPRADGTIPDW-----NNL---GSFLD-----TILKGPELSED      498
RoXaN           KEHQGIFTFLCEICFDSKPRIISKGTKDSPSVCSNLAAKHSFYNNKCLVHIVRSTSLK-Y      618
                 ::..:: ::* :*:.  **  ..  .      .**    ** :      *::. .*.

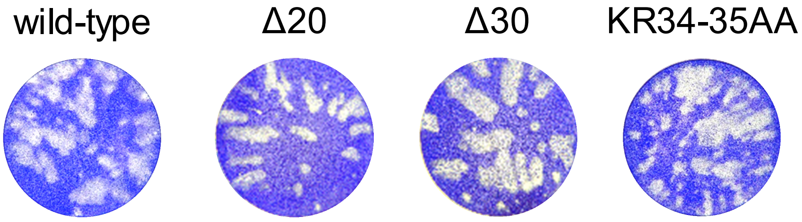
NS1_BTV1        EDERAYATMFEMVRCIITLCYAEKVHFAGFAAPACESGE-VINLA---------------      542
RoXaN           SKIRQFQEHFQFDVCRHEV------RYGCLREDSCHFAHSFIELKVWLLQQYSGMTHEDI      672
                .. * :   *::  *   :      ::. :   :*. .. .*:*

NS1_BTV1        -ARMSQMWMEY-------------------------------------------------      552
RoXaN           VQESKKYWQQMEAHAGKASSSMGAPRTHGPSTFDLQMKFVCGQCWRNGQVVEPDKDLKYC      732
                  . .: * :
```

Supplementary Figure 2: Possible conservation between BTV NS1 and rotavirus X protein associated with NSP3 (RoXaN). (a) Two orthogonal views of the NS1 atomic model. Areas exhibiting sequence similarity to RoXaN are highlighted in orange with sidechains shown. Note the concentration of disjointed regions with sequence similarity along surfaces of the head and body domains. (b) Multiple sequence alignment of NS1 and RoXan using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/). The whole sequence of BTV1 NS1 and the first 732 residues of RoXaN are shown (respective accession numbers: AGW27484 and Q9UGR2). Motif 2 of NS1 and its similarity with RoXaN is highlighted in yellow.



Supplementary Figure 3: Selection rule for all three helical forms of class 2 diameter helices (tier II) (a, b, c).



Supplementary Figure 4: Recovery of Δ20, Δ30 and KR34-35AA mutant viruses by reverse genetics as compared to the wild-type virus. Experiment was repeated twice independently with similar results.

	Bluetongue Virus Non-structural protein 1 (EMDB-0383) (PDB 6N9Y)
Data collection and processing	
Magnification	130,000x
Voltage (kV)	300
Electron exposure (e–/Å^2)	30
Defocus range (μm)	1.2-4
Pixel size (Å)	1.104
Symmetry imposed	helical
Initial particle images (no.)	87,190
Final particle images (no.)	7,793
Map resolution (Å)	4.0
FSC threshold	0.5
Map resolution range (Å)	200-3.7
Refinement	
Initial model used (PDB code)	*De novo*
Model resolution (Å)	4.0
FSC threshold	0.5
Model resolution range (Å)	80-4.0
Map sharpening *B* factor (Å^2)	160
Model composition	
Non-hydrogen atoms	4527
Protein residues	552
Ligands	0
B factors (Å^2)	
Protein	30
Ligand	
R.m.s. deviations	
Bond lengths (Å)	0.008
Bond angles (°)	0.979
Validation	
MolProbity score	1.77
Clashscore	4.80
Poor rotamers (%)	0.42
Ramachandran plot	
Favored (%)	90.91
Allowed (%)	9.09
Disallowed (%)	0

Supplementary Data Table 1: Cryo-EM data collection, refinement and validation statistics.