Structure and Function of RNA

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- Investigate roles of RNA in regulation, in virology, as enzymes, as vaccines
- Identify conserved and functional structures in RNA
- Identify functional RNA-RNA interactions
- Predict spatial RNA structures as novel drug targets
- Improve methods of RNA structure measurement by NMR
- Industry collaborations in the RNA therapeutics and vaccine areas (UVAXX Pte Ltd, AAVACC Pte Ltd, Skyhawk Therapeutics Inc.)
- Local Collaborations: NUS, NTU, NEA, A*STAR GIS, SigN, IBN, IMCB
- International Collaborations: Cambridge, Harvard, UCSF Kumamoto, Lisbon, Tallin, Lund, Innsbruck

Selected publications

Huber, R. G.; Lim, X. N.; Ng, W. C.; Sim, A. Y. L.; Poh, H. X.; Shen, Y.; Lim, S. Y.; Sundstrom, K. B.; Sun, X.; Aw, J. G.; et al. **Structure Mapping of Dengue and Zika Viruses Reveals Functional Long-Range Interactions.** Nat. Commun. 2019, 10 (1), 1408.

Kam, Y.-W.; Lee, C. Y.-P.; Teo, T.-H.; Howland, S. W.; Amrun, S. N.; Lum, F.-M.; See, P.; Kng, N. Q.-R.; Huber, R. G.; Xu, M.-H.; et al. **Cross-Reactive Dengue Human Monoclonal Antibody Prevents Severe Pathologies and Death from Zika Virus Infections.** JCI Insight 2017, 2 (8).

Huber, R. G.; Margreiter, M. A.; Fuchs, J. E.; Von Grafenstein, S.; Tautermann, C. S.; Liedl, K. R.; Fox, T. **Heteroaromatic Pi-Stacking Energy Landscapes.** J. Chem. Inf. Model. 2014, 54 (5), 1371–1379.

Petrlova, J.; Hansen, F. C.; van der Plas, M. J. A.; Huber, R. G.; Morgelin, M.; Malmsten, M.; Bond, P. J.; Schmidtchen, A. **Aggregation of Thrombin-Derived C-Terminal Fragments as a Previously Undisclosed Host Defense Mechanism.** Proc. Natl. Acad. Sci. U. S. A. 2017, 114 (21), E4213–E4222.

Marzinek, J. K.; Huber, R. G.; Bond, P. J. **Multiscale Modelling and Simulation of Viruses.** Curr. Opin. Struct. Biol. 2020, 61, 146–152.

Boon, P. L. S.; Saw, W. G.; Lim, X. N.; Raghuvamsi, P. V.; Huber, R. G.; Marzinek, J. K.; Holdbrook, D. A.; Anand, G. S.; Grüber, G.; Bond, P. J. **Partial Intrinsic Disorder Governs the Dengue Capsid Protein Conformational Ensemble.** ACS Chem. Biol. 2018, 13 (6), 1621–1630.

Chambers, J. E.; Kubánková, M.; Huber, R. G.; López-Duarte, I.; Avezov, E.; Bond, P. J.; Marciniak, S. J.; Kuimova, M. K. **An Optical Technique for Mapping Microviscosity Dynamics in Cellular Organelles.** ACS Nano 2018, 12 (5).

Chang, H.-H.; Huber, R. G.; Bond, P. J.; Grad, Y. H.; Camerini, D.; Maurer-Stroh, S.; Lipsitch, M. Systematic **Analysis of Protein Identity between Zika Virus and Other Arthropod-Borne Viruses.** Bull. World Health Organ. 2017, 95 (7).

Maurer-Stroh, S.; Mak, T.; Ng, Y.; Phuah, S.; Huber, R. G.; Marzinek, J. K.; Holdbrook, D. A.; Lee, R. T.; Cui, L.; Lin, R. T. **South-East Asian Zika Virus Strain Linked to Cluster of Cases in Singapore, August 2016.** Eurosurveillance 2016, 21 (38).

Röck, R.; Mayrhofer, J. E.; Torres-Quesada, O.; Enzler, F.; Raffeiner, A.; Raffeiner, P.; Feichtner, A.; Huber, R. G.; Koide, S.; Taylor, S. S.; et al. **BRAF Inhibitors Promote Intermediate BRAF(V600E) Conformations and Binary Interactions with Activated RAS.** Sci. Adv. 2019.

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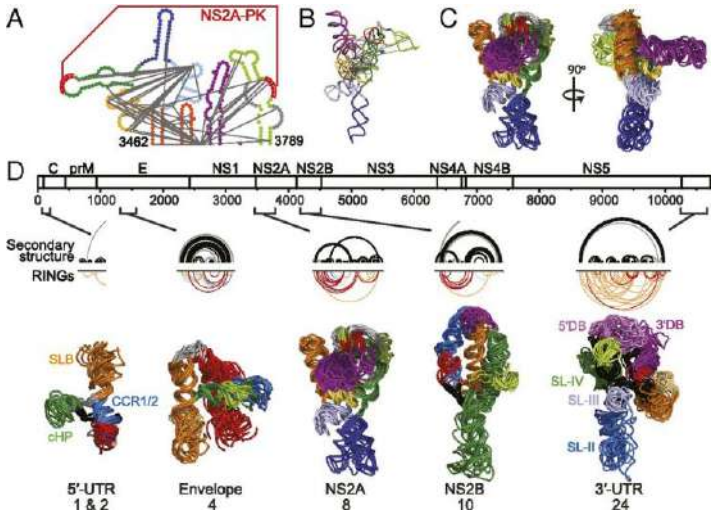
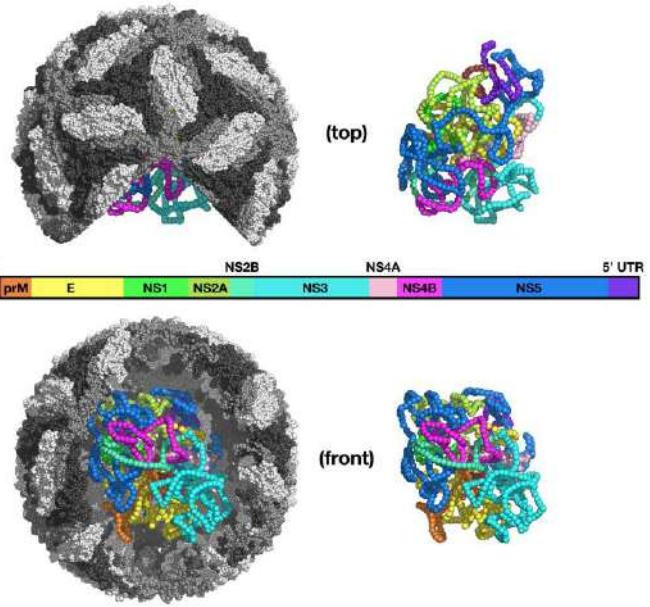
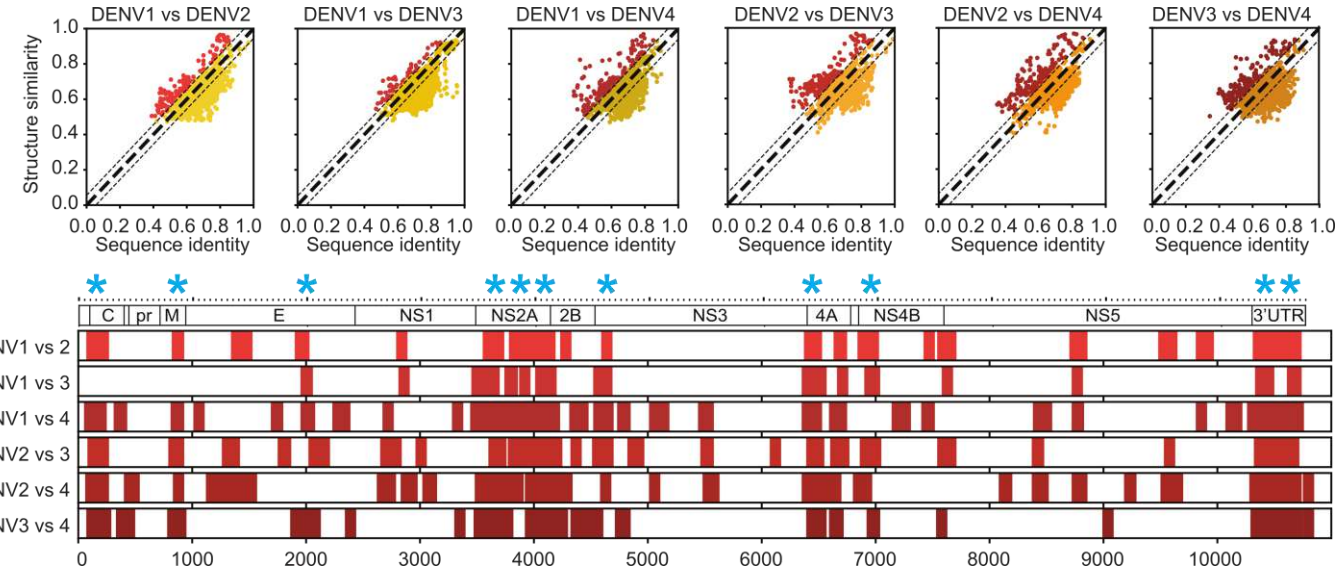
Flaviviruses

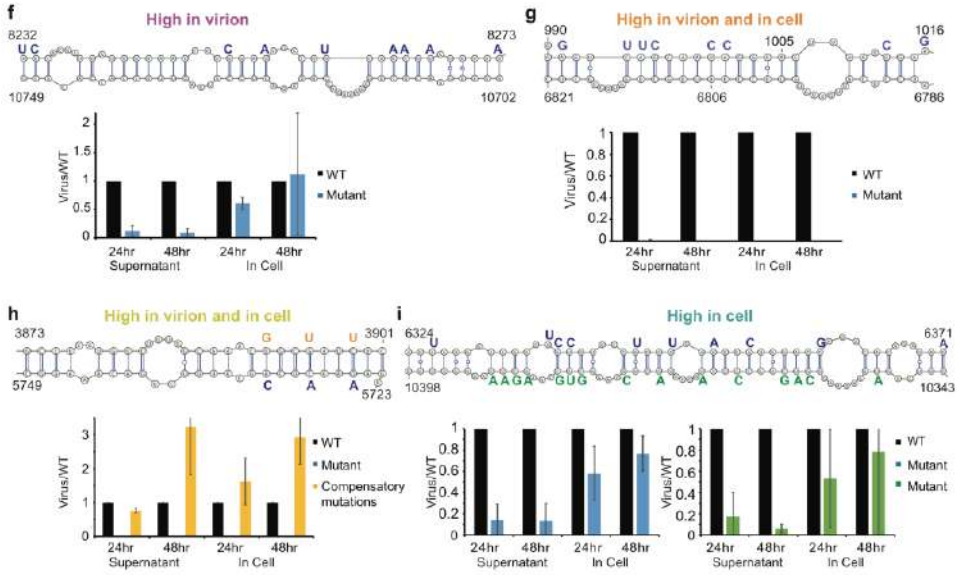
We identified crucial conserved structural elements in the RNA genome of Dengue and Zika viruses that may be amenable to targeting by RNA or conventional therapeutics. Bioinformatic analysis of genomic structure enabled by chemical probing, crosslinking and sequencing experiments alongside structural models of the genome elucidated potential targets within the genome and these sites were verified to be functional structures by synonymous mutagenesis experiments and (where possible) attempted rescue through compensatory mutations that reconstitute the targeted structures.





We are currently working to target identified structures with antisense-oligonucleotides and small molecules to demonstrate their viability as targets for drug discovery.





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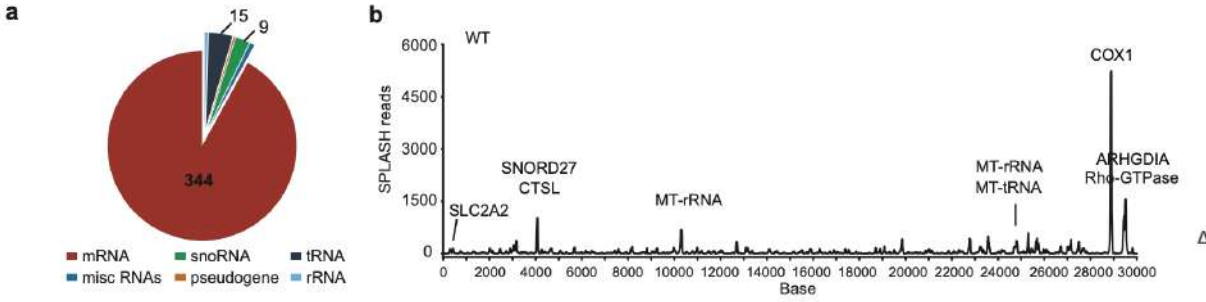
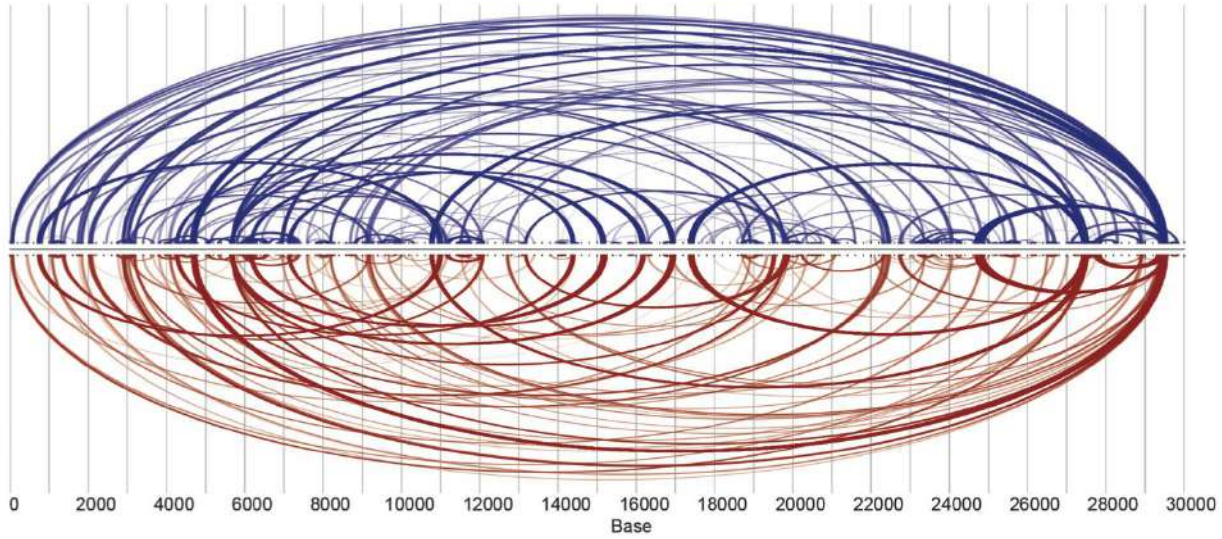
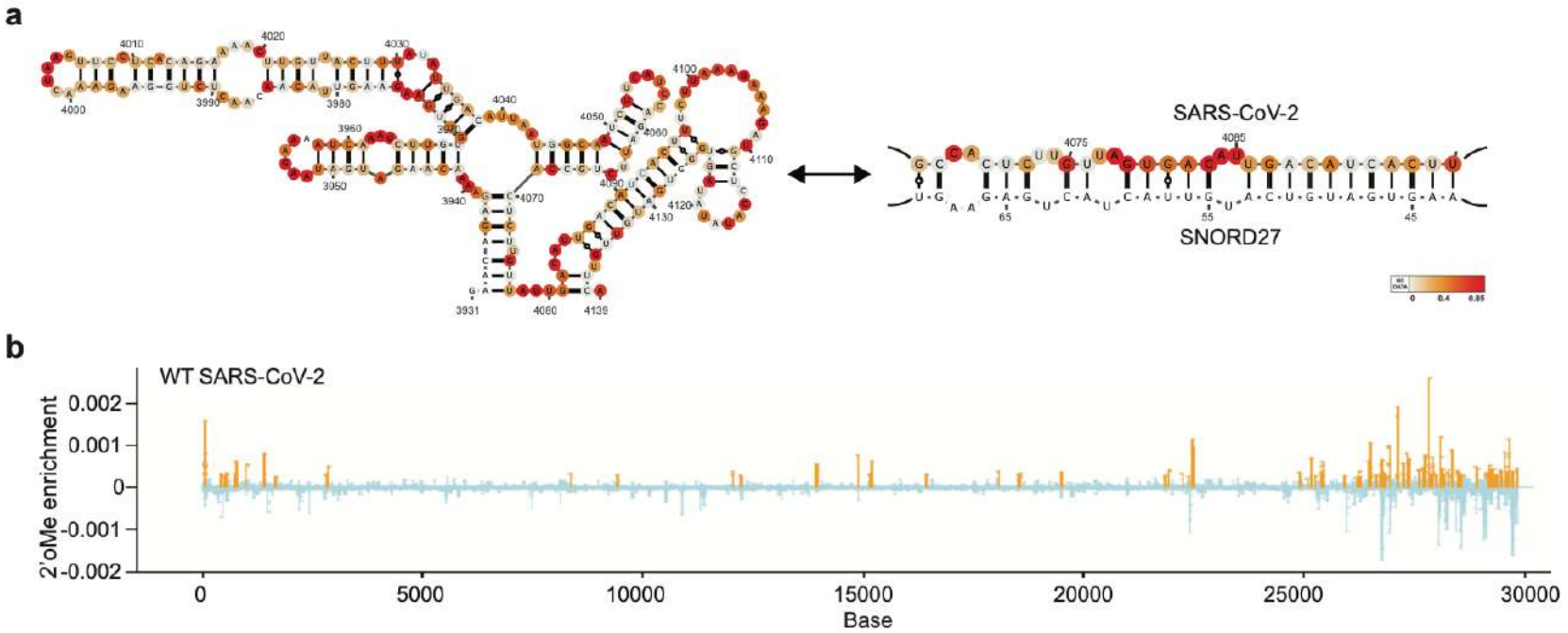
SARS-CoV-2 I





We have studied the SARS-CoV-2 genome structure and host-virus interactions as well as the interface between the spike protein RBD and the host receptor ACE2. Our study of genome host interactions show a complex network of snoRNA and mtRNA interaction partners. Particularly we find that snoRNA27 binding is associated with extensive methylation of the viral genome which we postulate is thus protected from the extensive degradation of the transcriptome observed in the host. Additionally, our use of nanopore sequencing technology allowed us to identify individual structures associated with the distinct subgenomic transcripts of SARS-CoV-2.



With Yue Wan, GIS

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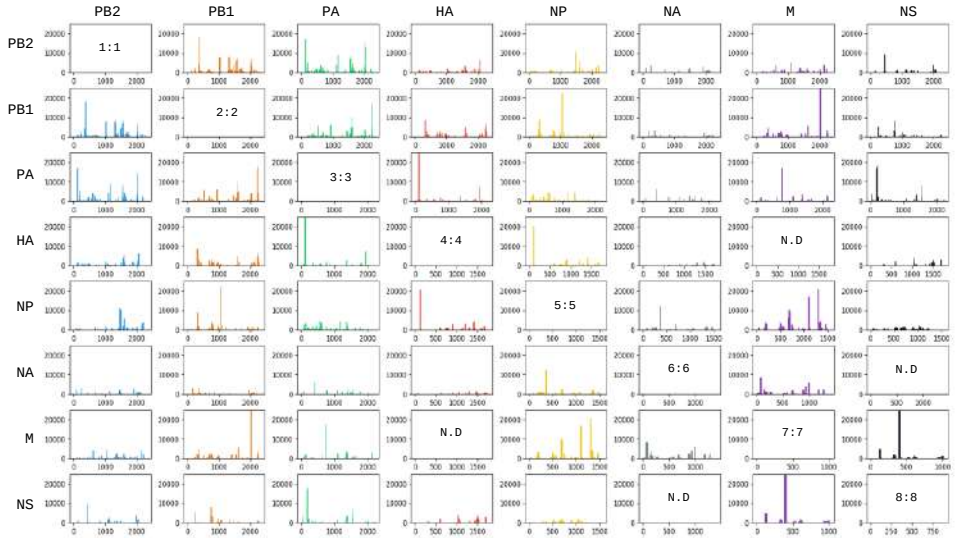
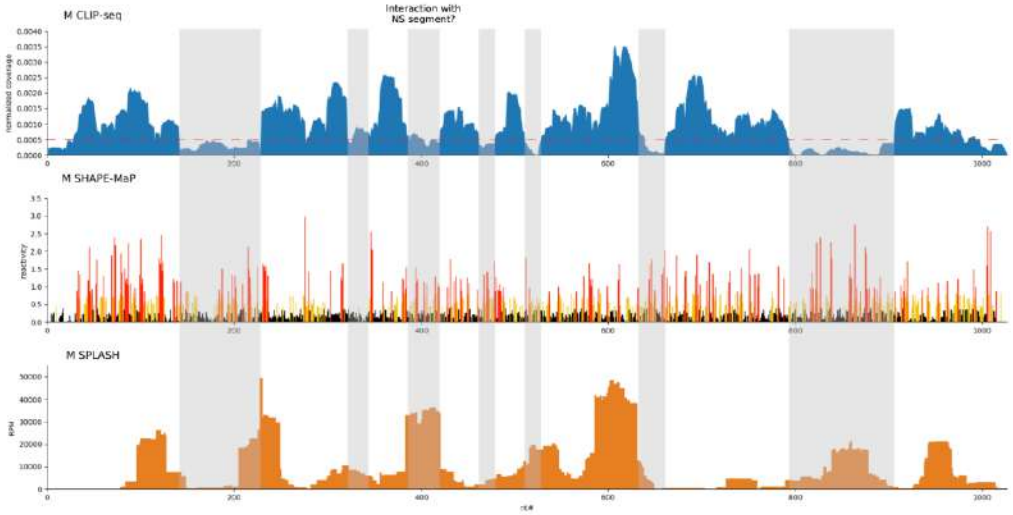
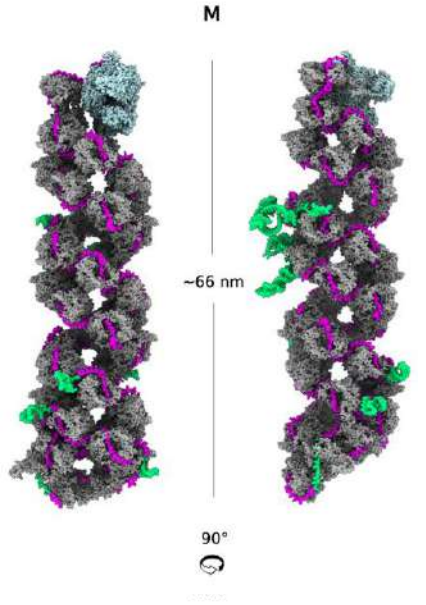
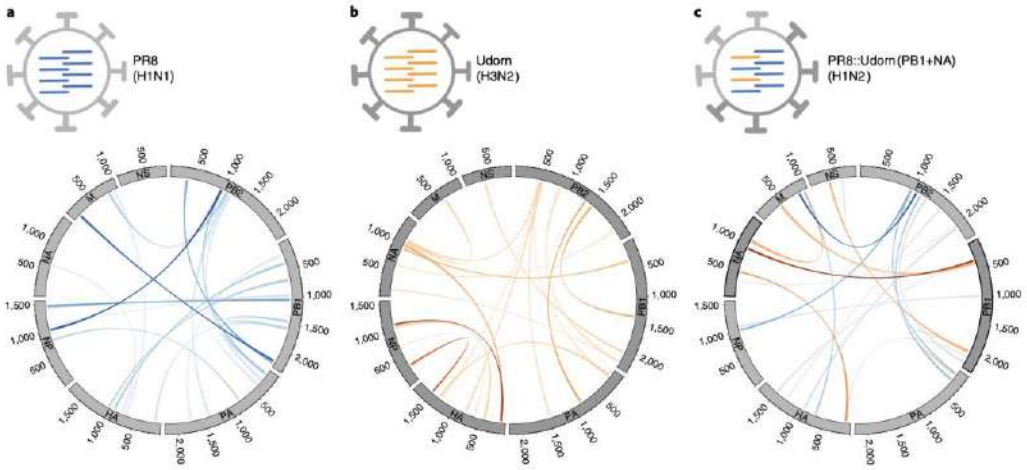
Influenza Reassortment



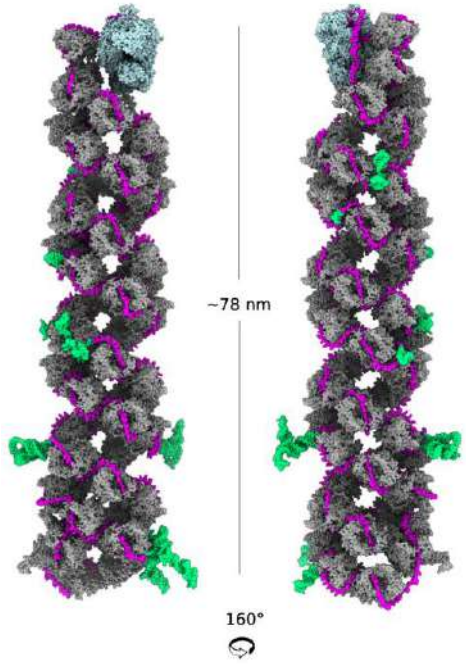






Influenza viruses feature a (-)-sense single-stranded RNA genome segmented into 8 distinct parts, with each part of RNA bound to a helical nucleoprotein assembly. A key step in the lifecycle of Influenza viruses is the packaging of the correct 8 segments into a nascent virion. Recent evidence has shown that specific RNA-RNA interactions are involved in the association of these segments and provide distinct interaction sites to recognize each other.

We have constructed detailed structural models comprising of viral RNA, the viral nucleoproteins and the viral polymerases. EM studies of these assemblies have allowed us to integrate near-atomic resolution models of the protein components with RNA structures modelled based on cross-linking and chemical probing data of the Influenza genome.



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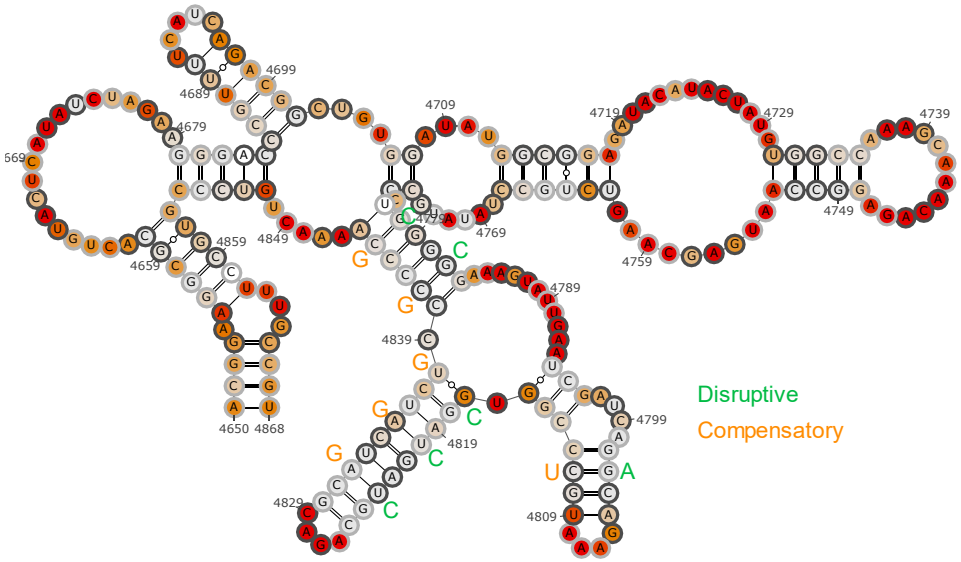
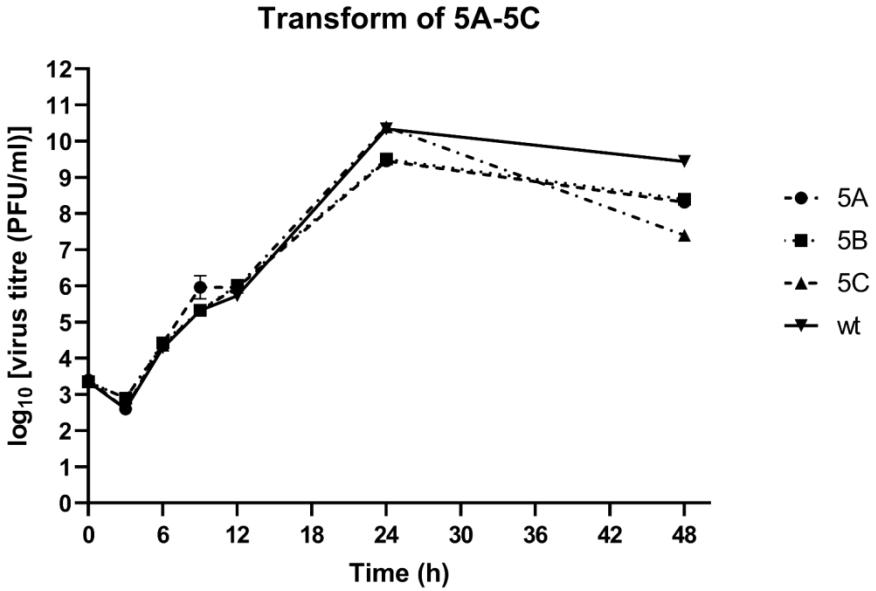
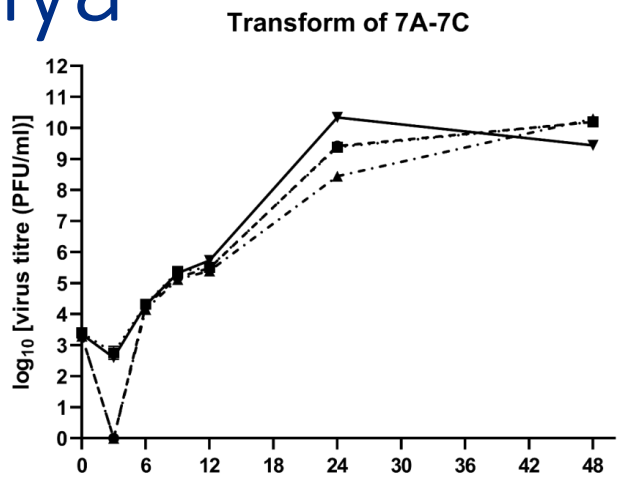
Other Viruses: Chikungunya

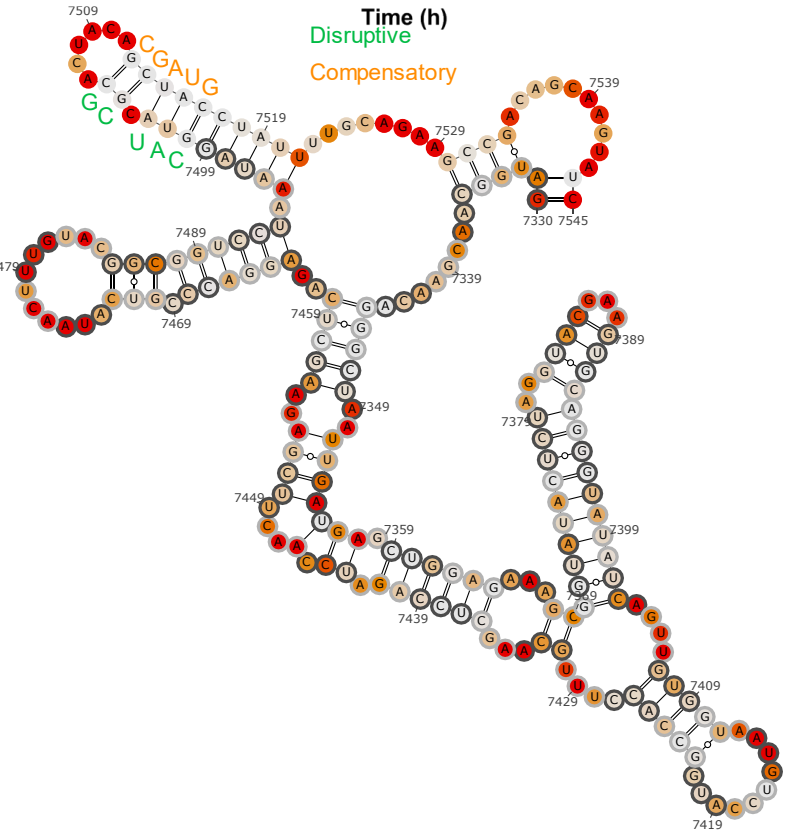




We found functional structures in the genome of Chikungunya virus. This is particularly remarkable as in general, alpha-virus genomes are quite resilient to changes in nucleotide sequence and even full codon optimization can result in viable virions. The genome of Chikungunya virus produces a subgenomic segment in cells that is the dominant species. To this purpose, a subgenomic promotor is present at around position 7500. Modifications of this promotor have interesting effects on the phenotype that suggest that the recognition is both sequence- and structure-dependent. It is as of now unclear which viral proteins are involved in the expression of the subgenomic strand.

With Wan Yue, GIS & Dahai Luo, NTU





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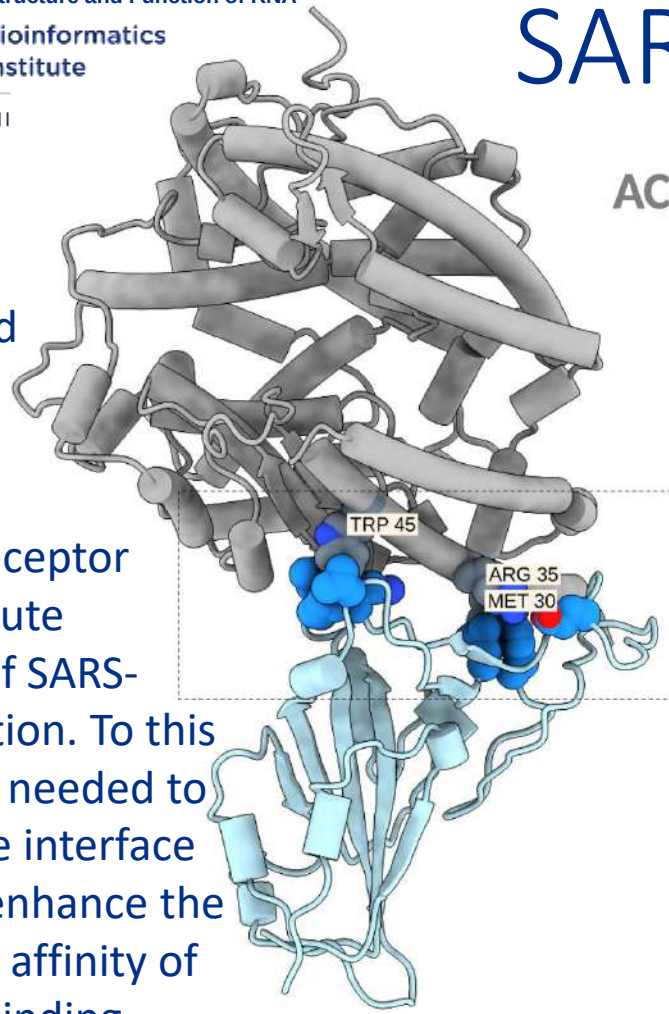
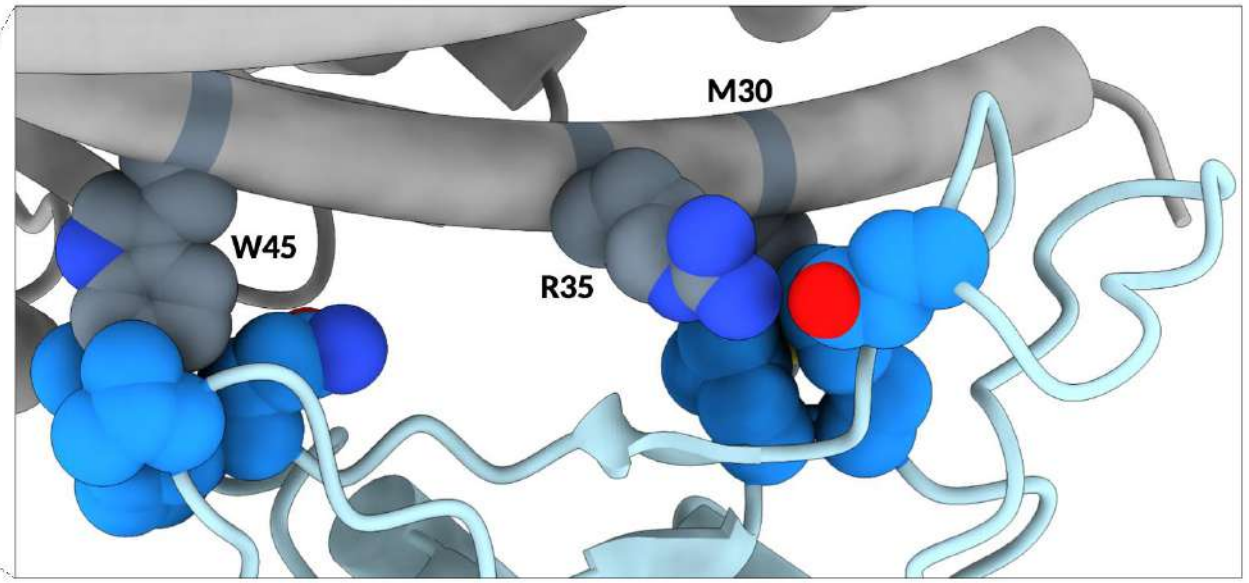
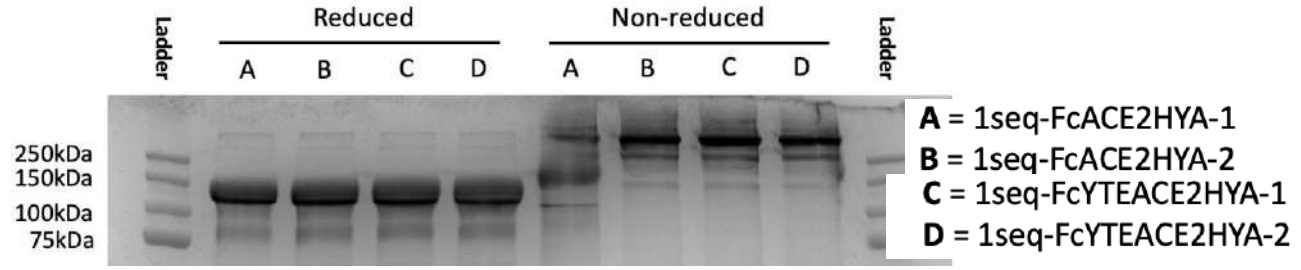
SARS-CoV-2 II

We intended to design a decoy of the human cell-entry receptor ACE2 for acute treatment of SARS-CoV-2 infection. To this purpose we needed to optimize the interface of ACE2 to enhance the kinetics and affinity of Spike-RBD binding.







We identified several effective mutations that were subsequently synthesized and evaluated, which proved the effectiveness of our proposed changes. ACE2-YHA showed improved affinity and faster binding kinetics over WT. The construct has shown to be effective in neutralizing SARS-CoV-2 in cell culture and is currently undergoing production optimization.

With Cheng-I Wang, SigN.& Yuan Sheng Yang, BTI

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Other projects

- Time-dependent and cell type-dependent structures in Enteroviruses
- Multi-omics: Imprinting of immune memory in hematopoietic stem cells
- Multi-omics: Pathways in spinal muscular atrophy
- Phage genome assembly
- Viral marine pathogens in Aquaculture
- Pseudo-contact shifts in NMR for RNA structure and dynamics measurements
- Ribozyme-based biosensors
- Feature detection and structure prediction using ML/AI
- RNA-protein interactions

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- PhD in Computational Biology (2014)
- Research Visits at Roche (Basel, CH), Yale (CT, USA)
- 72 publications, cited ~1300 times, h-index 22
- Group leader at BII since 04/2019
- Competitive grants: Austrian Academy of Sciences, BMRC YIG, A*STAR CDA, AMED Japan, A*STAR UIBR
- Industry collaborations in the RNA therapeutics and vaccine areas (UVAXX Pte Ltd, AAVACC Pte Ltd, Skyhawk Therapeutics Inc.)
- Local Collaborations: NUS, NTU, NEA, A*STAR GIS, EDDC, SigN, BTI, IMCB
- International Collaborations: Cambridge, Harvard, UCSF, Kumamoto, Lisbon, Tallin, Lund, Innsbruck