Structure and Function of RNA

BII Annual Conference 2022

Roland G. Huber
Louis DeFalco Jr
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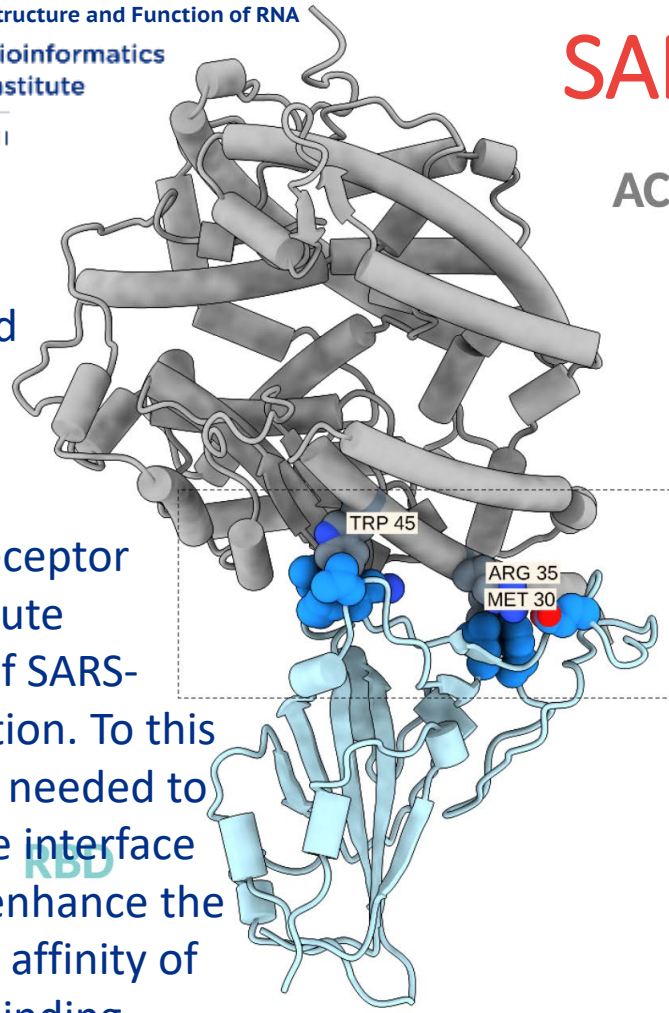
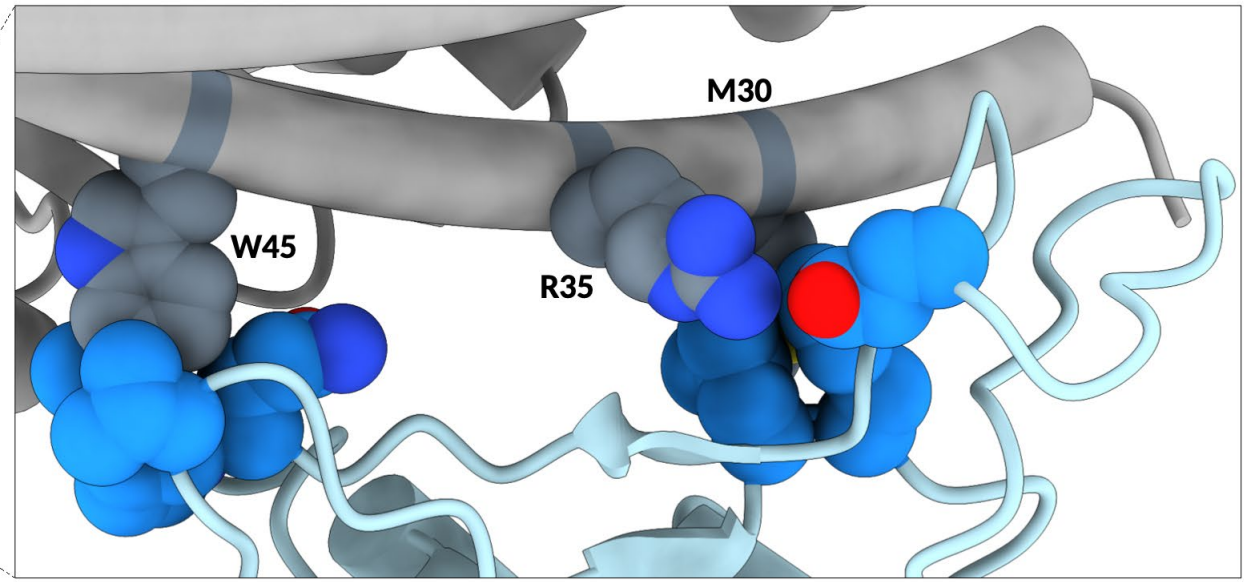
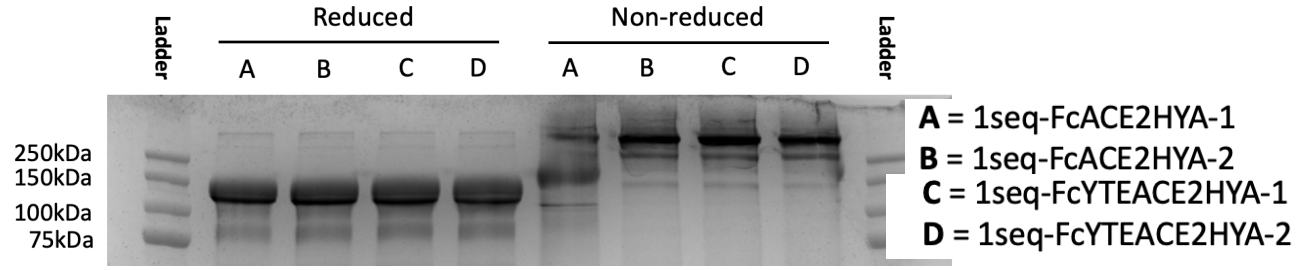
SARS-CoV-2 Decoy

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

Singapore Patent Application 10202112143T **(01 Nov 2021)**

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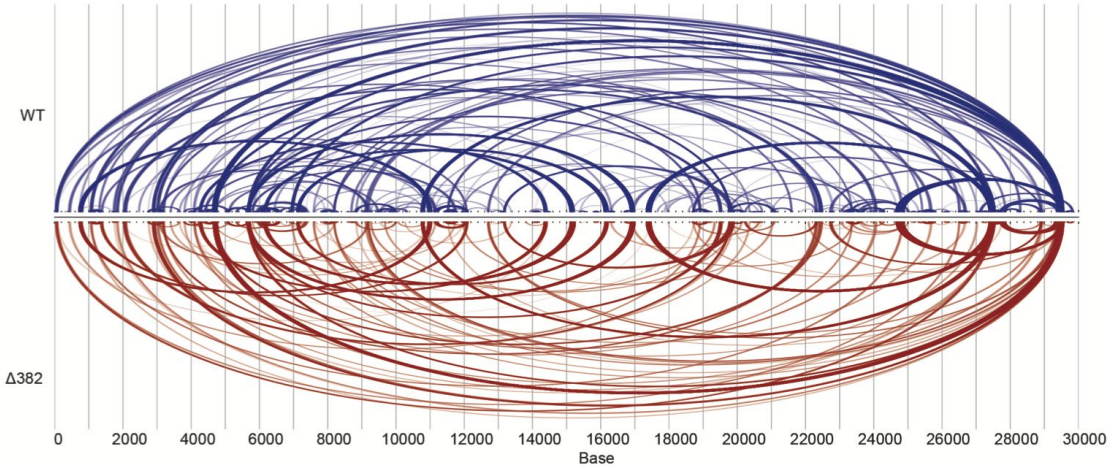
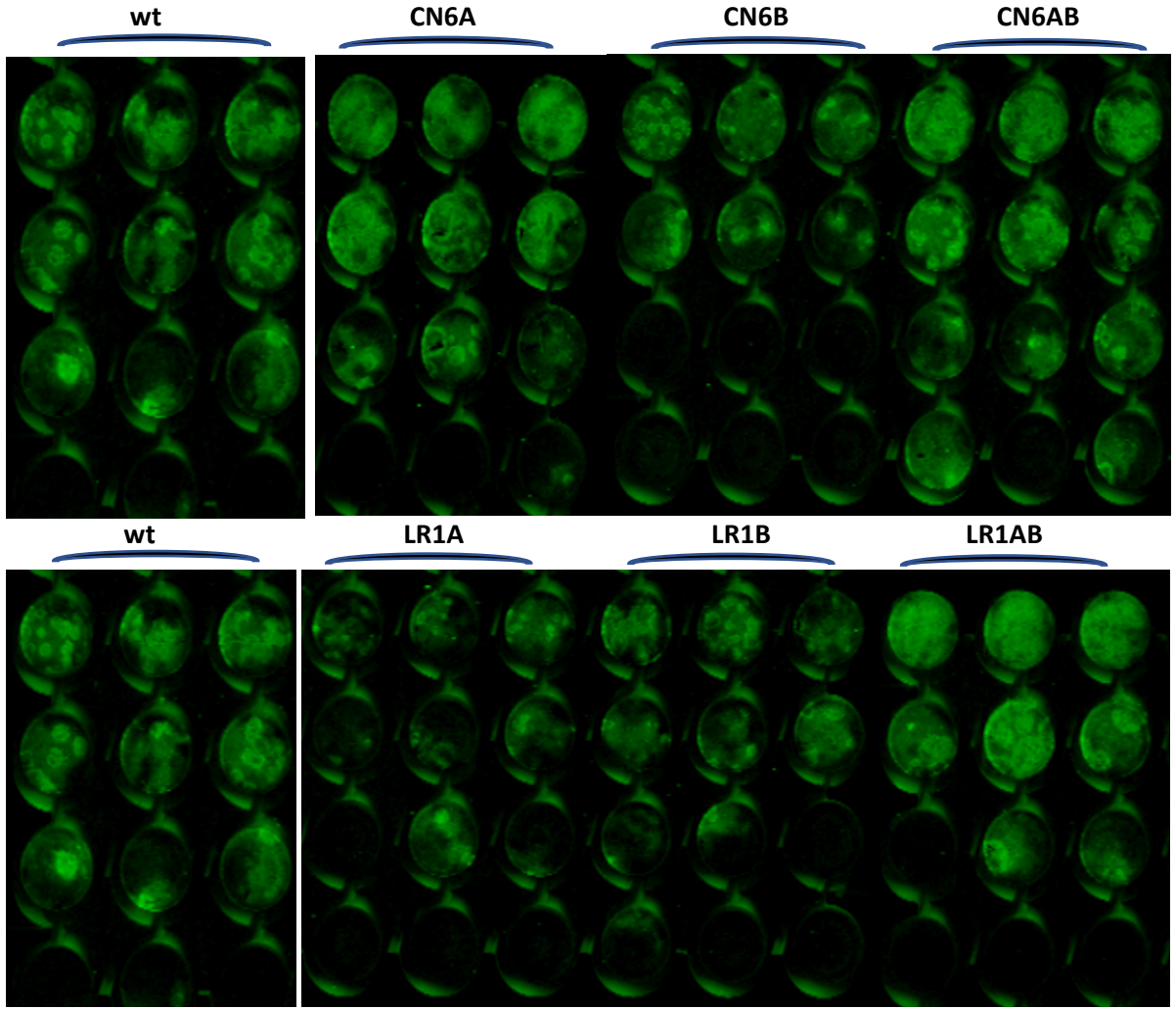
SARS-CoV-2 Genome Structure

We identified crucial structural elements in the genome of SARS-CoV-2 through chemical cross-linking, SHAPE probing and usage of Nanopore sequencing to analyze the individual subgenomic transcripts of the virus.
This allowed us to design mutations within these elements that disrupt these structures and cause significant attenuation of viral fitness. Mutations were designed on both sides of the strand and tested as A/B, and A+B to confirm the effect is related to the predicted structures. Our study offers the potential to design attenuated strains and targeted therapies toward these elements.





Yang SL et al. Nature Communications 12 (1) 1-15. (**2021)**

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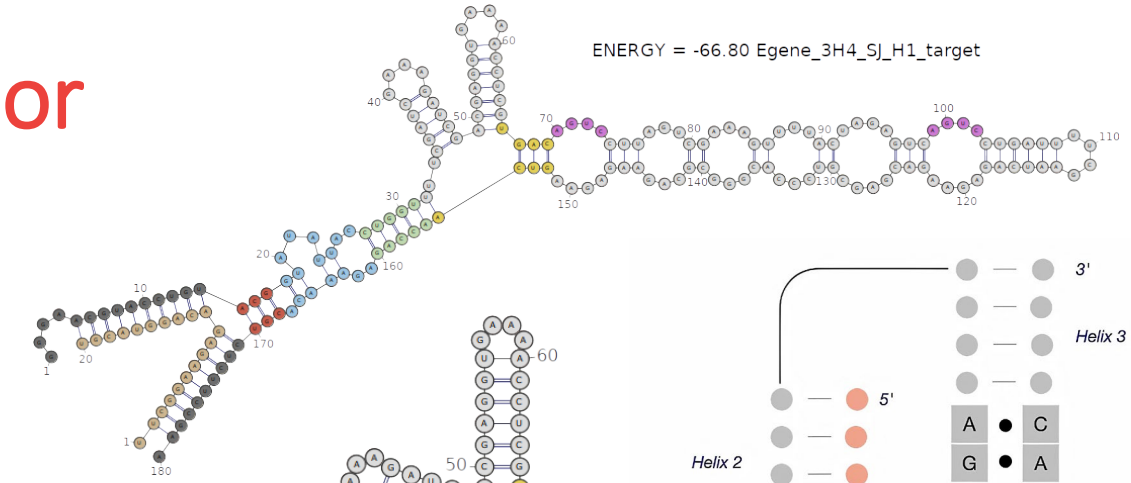
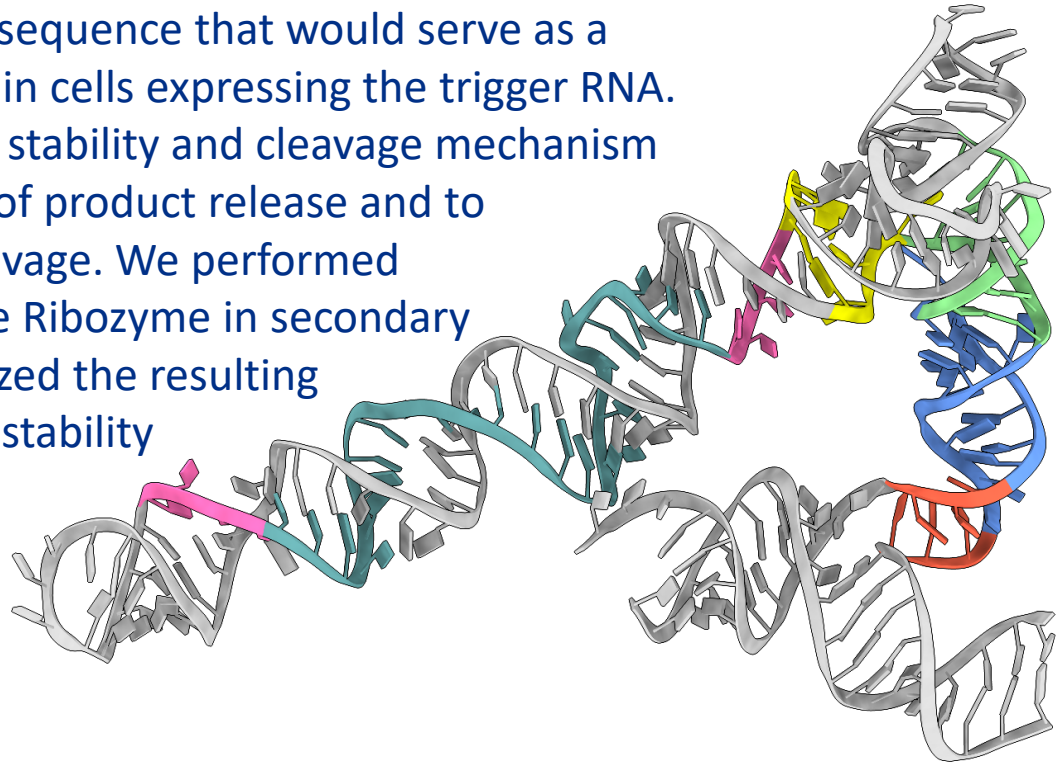
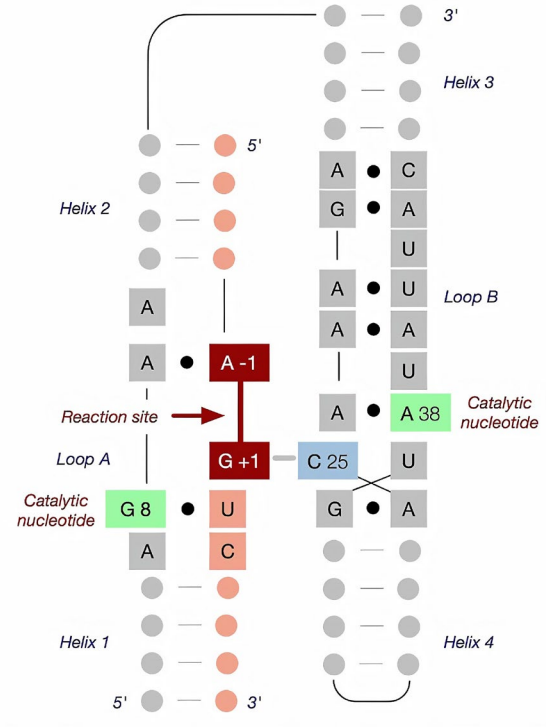
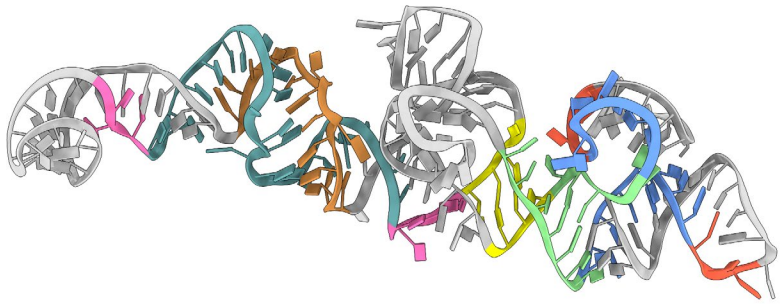
Ribozyme Biosensor

The lab of Sherry Aw designs RNA constructs for diagnostic and therapeutic purposes. We contribute to the design of an autocatalytic ribozyme triggered by specific small RNAs. This strategy offers the opportunity to create biosensors via the release of fluorescent probe RNAs in the presence of disease-specific small RNAs. Alternatively, the constructs can release any specific small sequence that would serve as a targeted therapeutic only in cells expressing the trigger RNA. Our team investigates the stability and cleavage mechanism to improve the efficiency of product release and to minimize background cleavage. We performed extensive modelling of the Ribozyme in secondary structure space and analyzed the resulting Ensembles with regard to stability and energetics. We also proceeded to model the cleavage dynamics using MD simulations.









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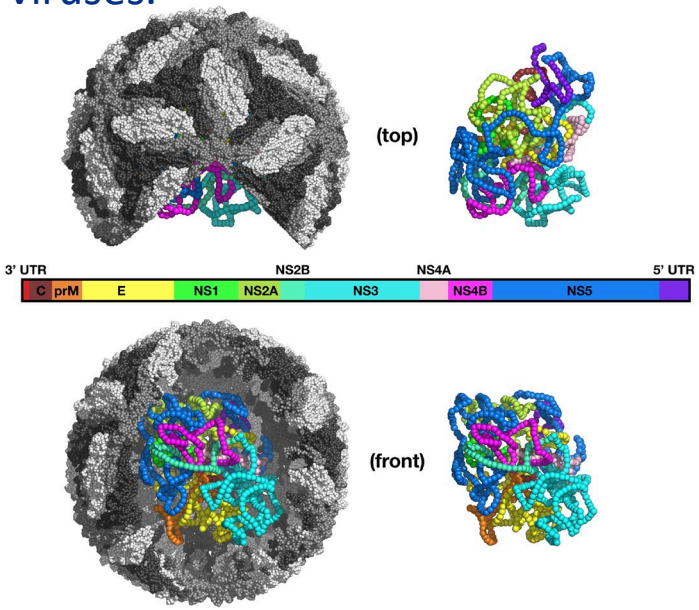
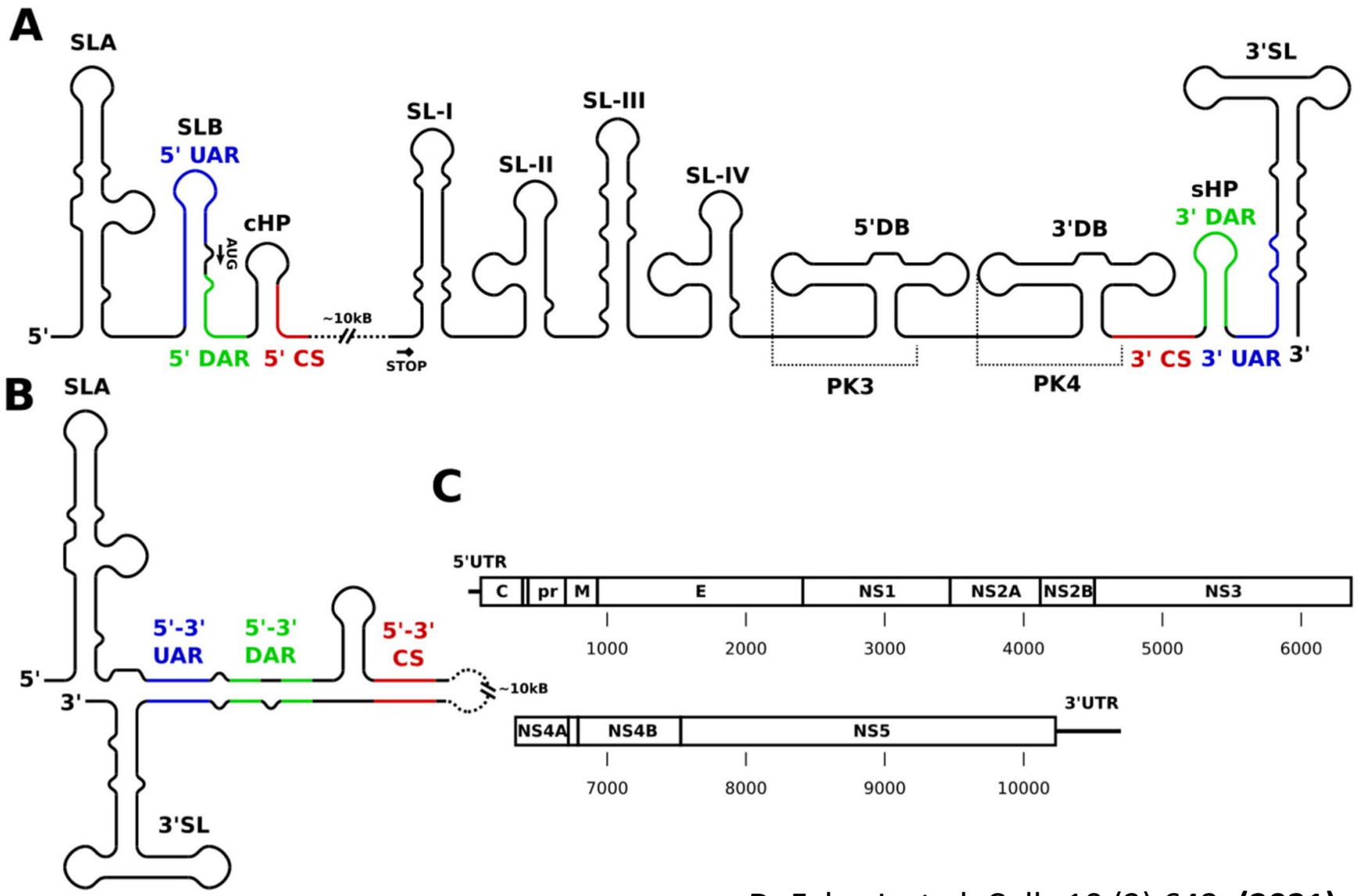
Flavivirus Genome Structures

We collated a review of the cause and effect of flavivirus genome circularization. Flaviviruses are dependent on 5’ and 3’ circularization motifs for their replication. Our review highlighted recent work by our own and other labs that shed light on the functional role circularization plays for these viruses.





DeFalco L et al. Cells 10 (3) 642. **(2021)**

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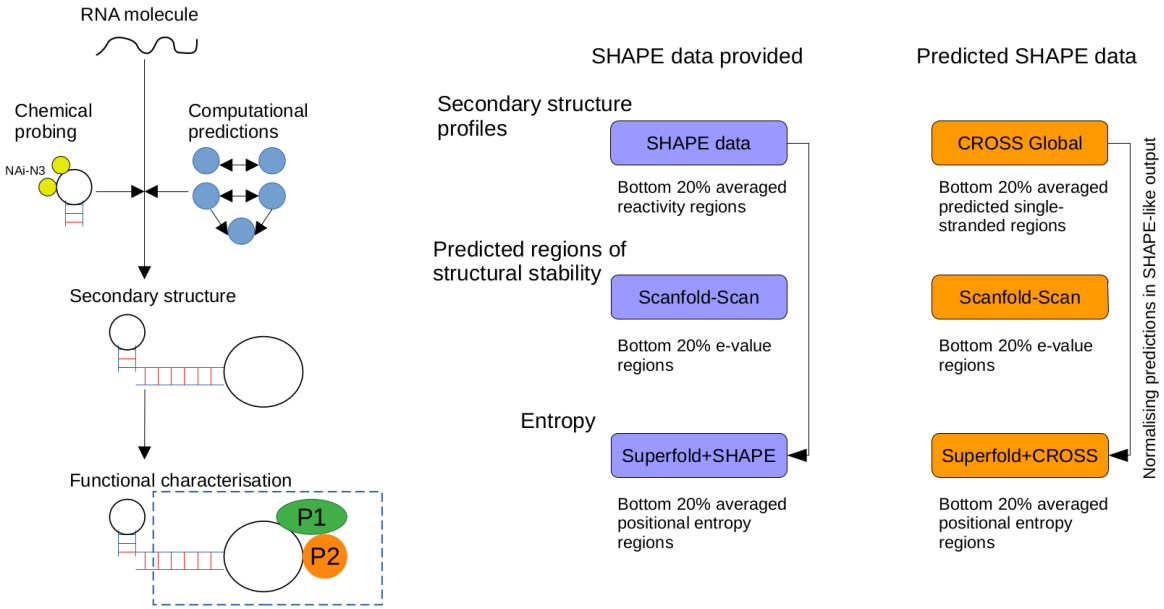
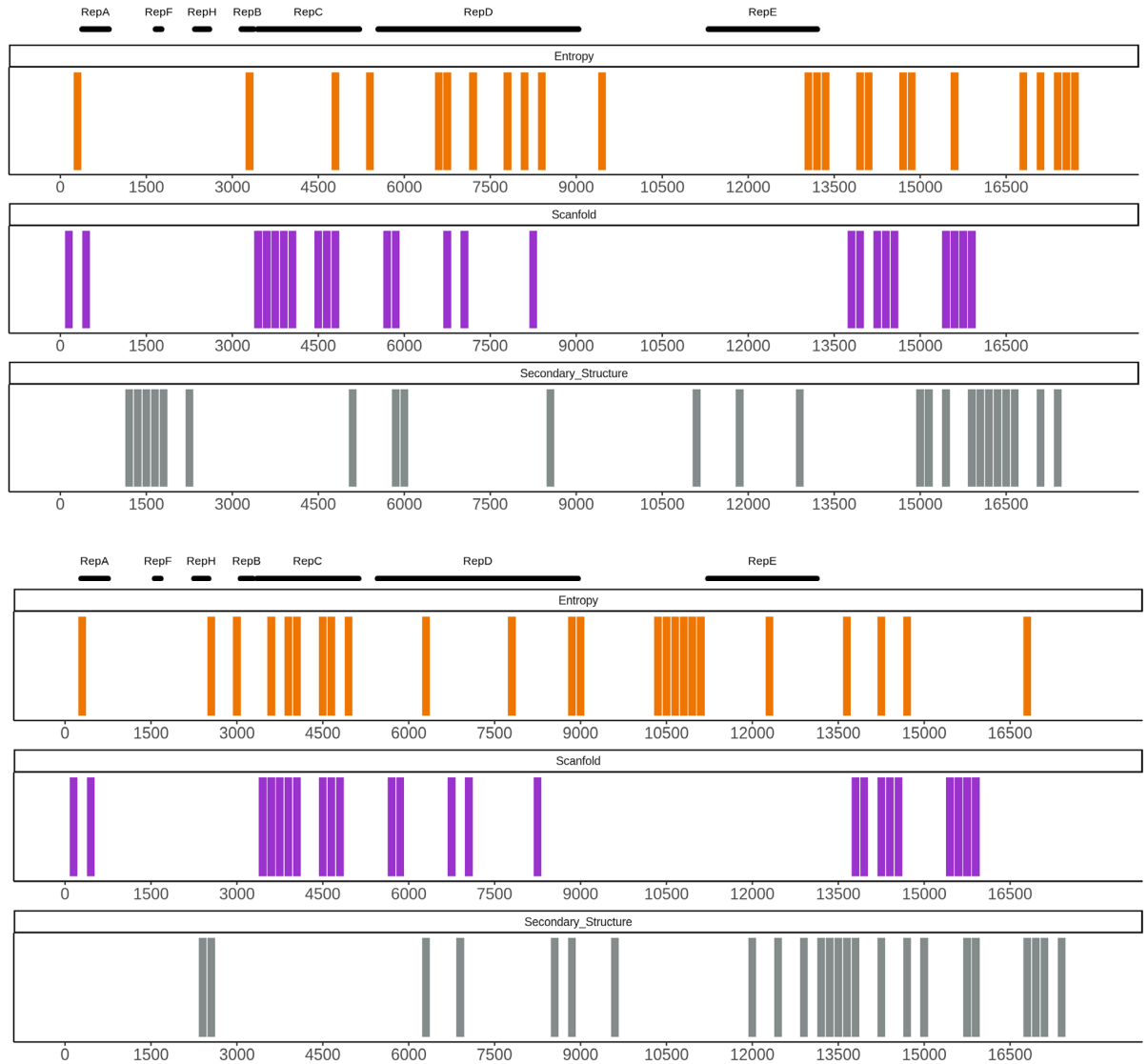
RNAvigator





We constructed a computational pipeline to streamline the analysis of structure probing data and secondary structure modelling in the analysis of RNA structure. In the absence of experimental reactivity data, we include CROSS data that uses machine learning to impute reactivity from sequence. We show that our pipeline is able to identify known functional RNA segments in viral genomes, mRNAs and lncRNAs.

Delli Ponti R et al. Under Revisions **(2022)**

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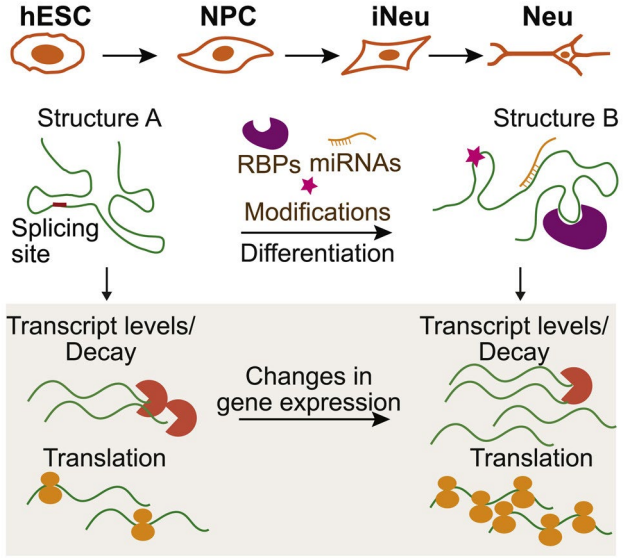
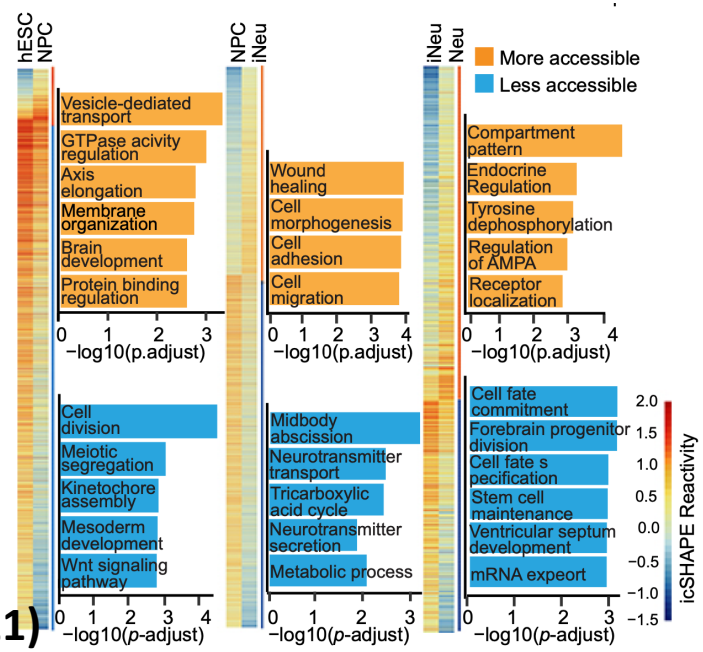
Neurogenesis

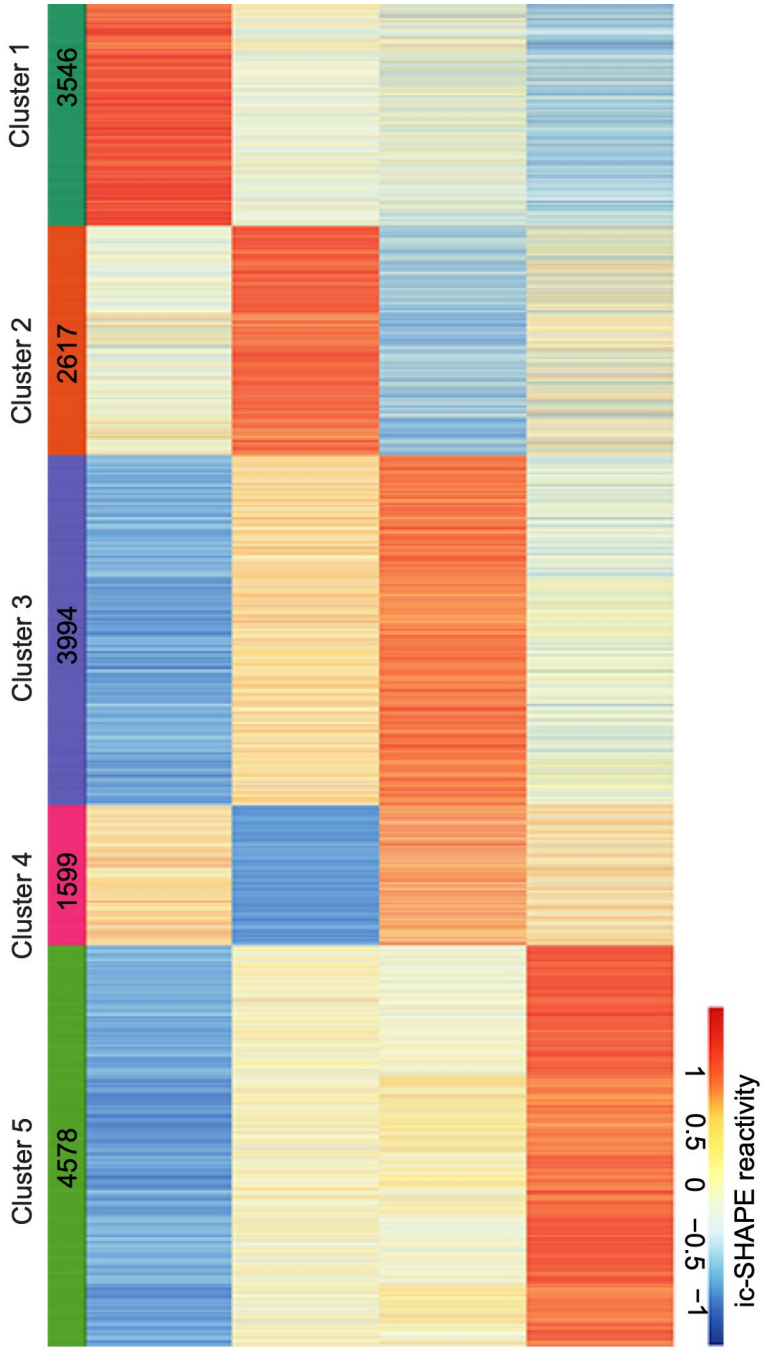
We traced the structure of transcripts through the process of neuronal cell differentiation. We found that a significant number of transcripts change their structure, particularly within their UTRs throughout differentiation. This is an interesting mechanism of gene regulation as such structures can regulate decay of transcripts and translation initiation.

We find that transcripts are generally more homogeneous in structure within stem cells while the diversity increases throughout differentiation. We identified specific clusters associated with changes in structure that are uniquely more single-stranded at particular cell stages which is likely indicative of active translation.

Modification of sites associated with RNA-binding proteins to alter their structure showed that these structure changes can affect protein binding.







Wang J et al. Molecular Cell 81 (23) 4942-4953. (**2021**)

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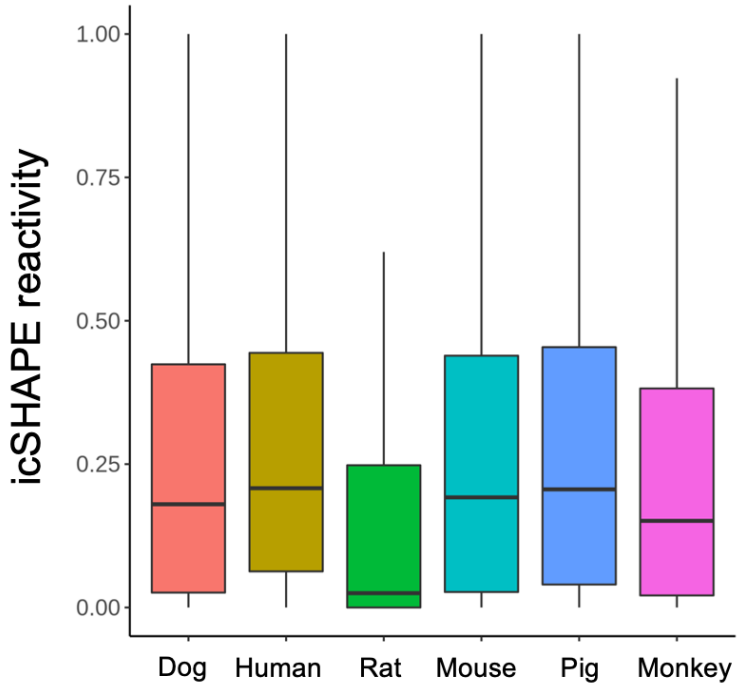
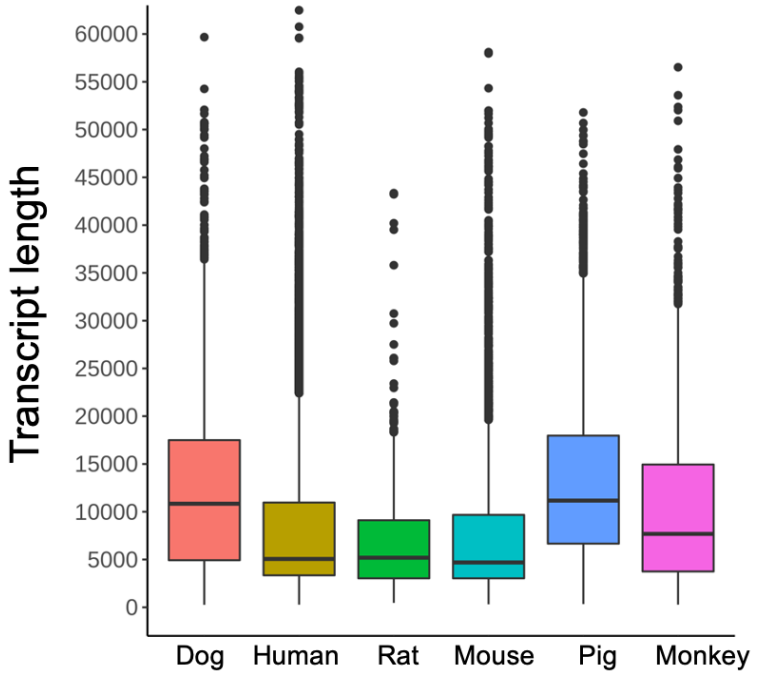
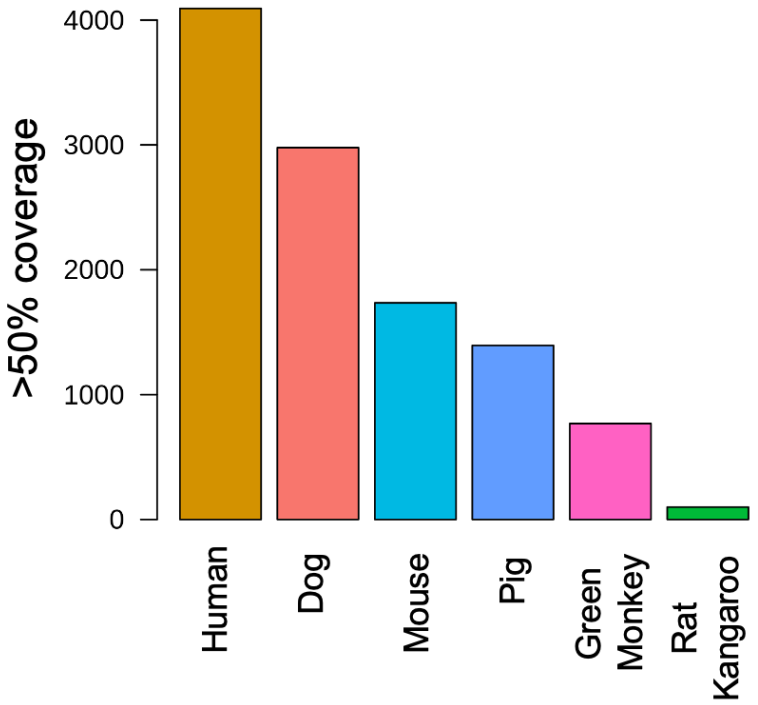
Evolution of RNA structure

Conceived as a follow-up to the neurogenesis project, we are currently investigating the evolutionary conservation of mRNA and ncRNA structure in mammals. We were able to identify a high number of orthologous genes and our collaborators performed full-transcriptome structure probing. We are currently comparing structures of all covered orthologous transcripts between species to identify conserved structural elements despite sequence changes.

Species	Total Genes	>50%	>80%	Orthogroups of 9 species	Orthogroups (9 species) >50%	Orthogroups (9 species) >80%
Human	23'325	12'795 (55%)	5'333 (23%)	6'508 (28%)	4'093 (17%)	2'149 (9%)
Green Monkey	1'972	1'082 (55%)	481 (24%)	1'401 (71%)	768 (39%)	359 (18%)
Mouse	7'891	4'840 (61%)	2'285 (29%)	2'583 (33%)	1'735 (22%)	990 (13%)
Dog	6'265	4'475 (71%)	2'563 (41%)	3'978 (63%)	2'979 (48%)	1'824 (29%)
Pig	3'841	2'103 (55%)	1'011 (26%)	2'473 (64%)	1'394 (36%)	694 (18%)
Rat Kangaroo	832	149 (18%)	21 (2%)	596 (71%)	99 (12%)	12 (1%)







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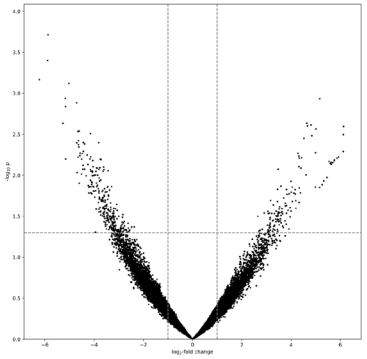
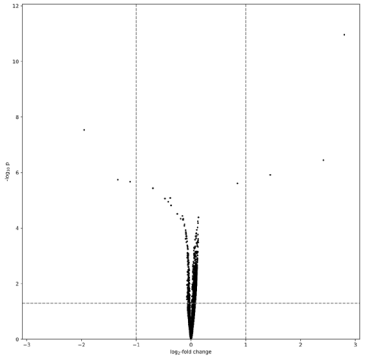
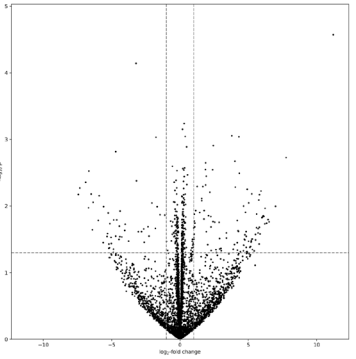
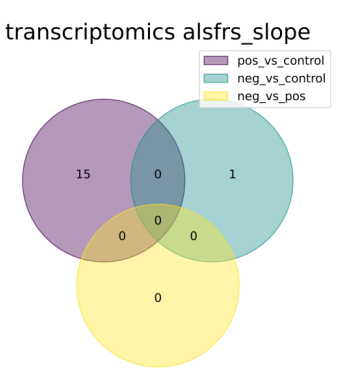
ALS Multi-Omics

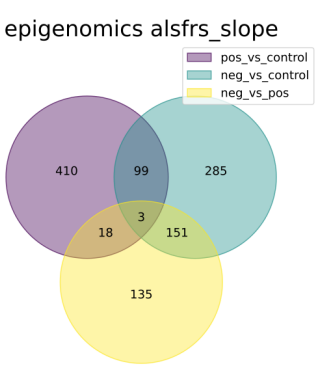


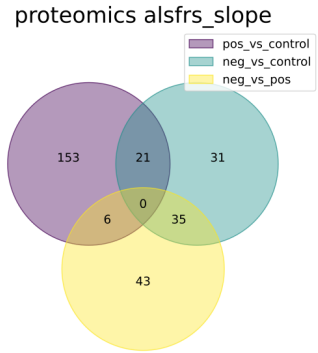


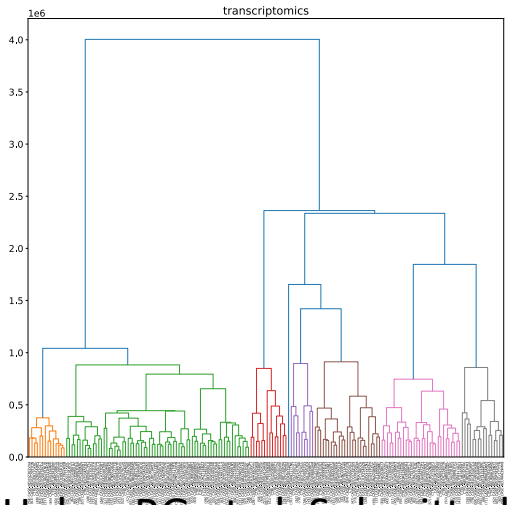


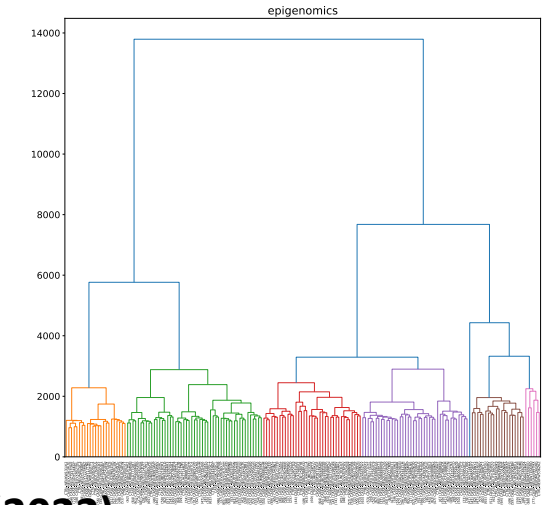
This project aims to identify diagnostic and prognostic biomarkers for Amyotrophic Lateral Sclerosis. ALS is highly heterogeneous in presentation and currently relies on a purely clinical diagnosis. Moreover, specific patient sub-populations have divergent outcomes with regard to speed of progression and involvement of cognitive-behavioural aspects.
Using public multi-omics data, our study used both a top-down analysis relying on clinical criteria for stratification of contrasts and a bottom-up approach based on clustering patients based on genetic information and subsequently identifying interesting clinical sub-populations with distinct outcomes.

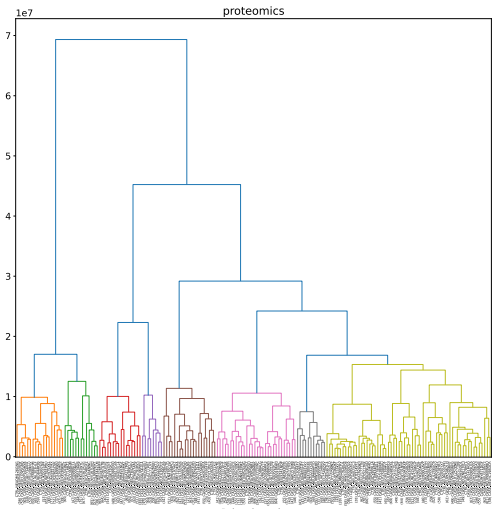


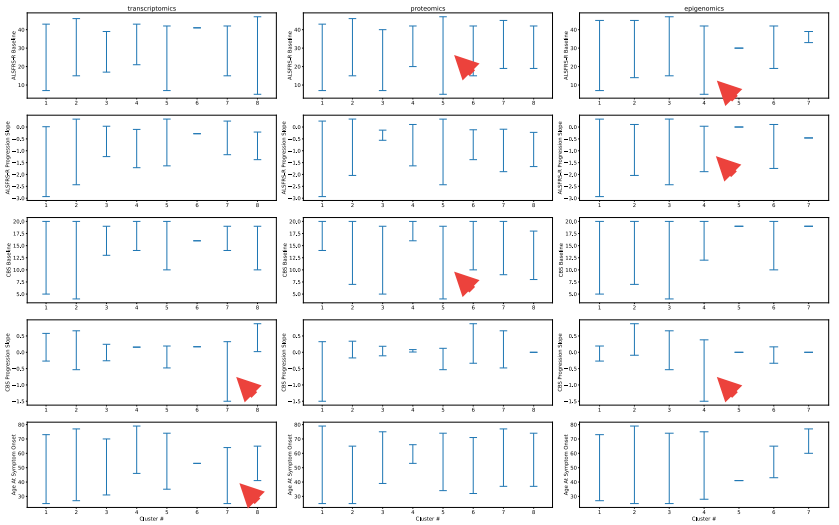












Huber RG et al. Submitted **(2022)**

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HSC Multi-Omics

PBS/LPS/*Bacteroides*

4w recovery

0

CLP

Secondary infection

Evaluate immune response of HSCs and derived myeloid cells:

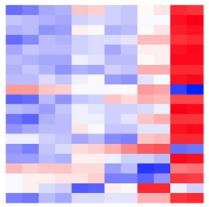
- Survival
- Inflammatory cytokine panel

Young or Old mice

16h & 1 month (acute – chronic)

Single Cell Analysis of HSCs/myeloid cells

ATAC-seq	Chromatin Structure
RNA-seq	Quantitative Transcriptome
Mass cytometry	Epigenetic Marks



Differential gene expression
Differential accessibility
Differential epigenetic markers

CHIP-seq (Cut&Tag) ongoing

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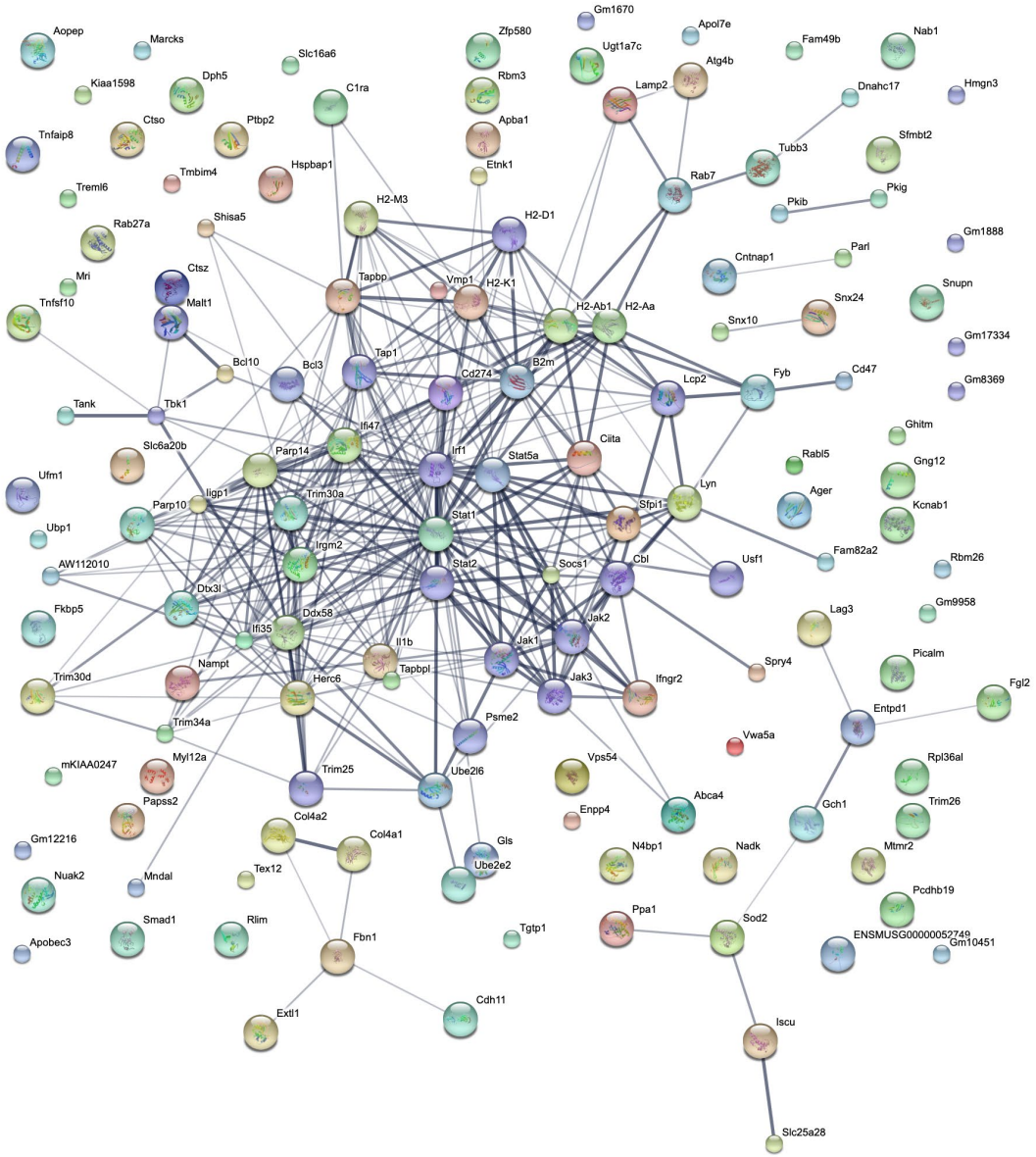
HSC Multi-Omics



Biological Process (GO)			
pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
GO:0006955	immune response	28	2.15e-11
GO:0006952	defense response	30	2.8e-11
GO:0045087	innate immune response	21	5.42e-11
GO:0002376	immune system process	34	9.55e-09
GO:0051707	response to other organism	22	5.59e-08
			(more ...)

Molecular Function (GO)			
pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
GO:0046977	TAP binding	4	0.000231
GO:0005515	protein binding	53	0.00276
GO:0042605	peptide antigen binding	4	0.00288
GO:0046979	TAP2 binding	2	0.0417

Cellular Component (GO)			
pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
GO:0030666	endocytic vesicle membrane	8	4.89e-06
GO:0030670	phagocytic vesicle membrane	7	1.36e-05
GO:0042611	MHC protein complex	5	1.55e-05
GO:0045335	phagocytic vesicle	7	0.00011
GO:0030139	endocytic vesicle	8	0.000726
			(more ...)

KEGG Pathways			
pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
04612	Antigen processing and presentation	10	5.16e-09
05168	Herpes simplex infection	14	5.16e-09
05164	Influenza A	13	6.52e-09
05152	Tuberculosis	12	7.85e-08
05162	Measles	11	8.13e-08
			(more ...)

PFAM Protein Domains			
pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
PF01017	STAT protein, all-alpha domain	3	0.000964
PF02864	STAT protein, DNA binding domain	3	0.000964
PF02865	STAT protein, protein interaction domain	3	0.000964
PF05049	Interferon-inducible GTPase (IIGP)	4	0.00109
PF00017	SH2 domain	5	0.00184
			(more ...)

INTERPRO Protein Domains and Features			
pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
IPR000980	SH2 domain	6	0.000733
IPR003006	Immunoglobulin/major histocompatibility complex, conserved site	6	0.000733
IPR001217	Transcription factor STAT	3	0.000794
IPR012345	STAT transcription factor, DNA-binding, subdomain	3	0.000794
IPR013799	STAT transcription factor, protein interaction	3	0.000794
			(more ...)

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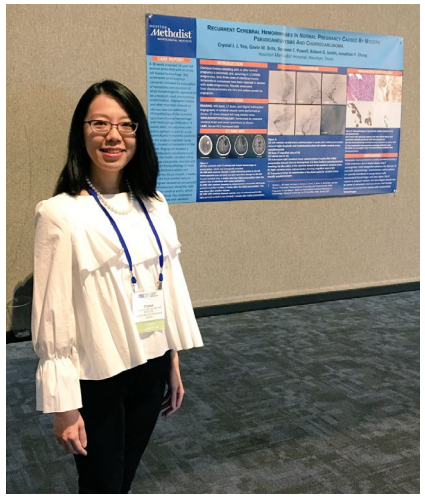

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