

Research

Our group is currently focused on two aspects at IMCB:

- (A) Platform technologies for high-throughput modulation of the human transcriptome;
- (B) RNA therapeutics: from target discovery to lead candidates.

(A) Platform technologies for high-throughput modulation of the human transcriptome

Our interests lie in the modulation of specific RNA processes and events. To induce the desired modulations, we use *shAON* (steric hindrance antisense oligonucleotide), which is an entirely chemically-modified ssRNA that binds to a target RNA specifically via Watson-Crick bonds, to effect steric hindrance. Depending on where and when it binds the target RNA, a *shAON* can be directed to competitively block RNA binding proteins (RNA-BPs) (Figure A) or to alter the local RNA structure (Figure B).

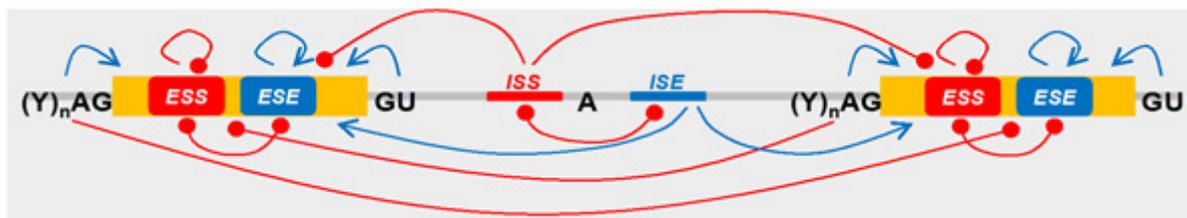


Figure A. Schematic diagram depicting putative RNA-BP binding sites on a RNA Multiple opposing and/or synergistic RNA-BPs bind to their respective putative motifs in the regulation of exon recognition and splicing. The eventual inclusion or exclusion of an exon in a mature mRNA depends on the interplay between the promoting (blue arrows) and inhibiting (red hammerheads) RNA-BPs. Through blockade of specific RNA-BP motifs or by altering the local structure of the RNA, the splicing of an exon can be modulated. Legend: ESE – exonic splicing enhancer, ESS – exonic splicing silencer, ISE – intronic splicing enhancer, ISS – intronic splicing silencer. The conserved exon splice site bases are indicated.

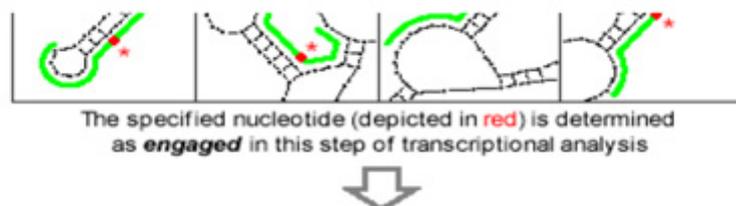


Figure B. Schematic diagram depicting altered local RNA structures by an shAON The local RNA structure can be altered by an shAON binding to the same target site (indicated in green) at different steps of transcription.

A rational approach in the design of highly efficacious and efficient *shAONs* is a requisite to scale the modulation to the human transcriptome. We develop and validate mathematical,

computational and statistical models to simulate the dynamics of biophysical, biochemical and biological properties of a RNA molecule and as well as its interactions with RNA binding proteins during its lifecycle. We apply insights from the models analyses to predict sites and opportune windows at which an *shAON* can effectively and efficiently induce the following types of modulation:

- Exon inclusion and exclusion;
- Alternate 3' and 5' splice sites usage;
- Alternate initiation and termination codon usage;
- Mutually exclusive exon selection;
- Intron retention and restoration;
- Circular exons from back-splicing;
- Transcript stability (half-life);
- Transcript intracellular transport.

Our platform technologies encompass the models and methods for the rational design of *shAONs* specific to each of the above modulations, and the extensive *shAON* libraries.

(B) RNA therapeutics: from target discovery to lead candidates

Our *shAON* libraries and rational *shAON* design methods allow us to undertake an integrative approach that begins at target discovery and validation, to drug design and development. As both the target-screening and the therapeutic *shAONs* share the same molecular mechanism, the vertical workflow enables a rapid and smooth transition to the drug discovery stage, which is merely a (rational) re-design of the hit *shAON* to meet therapeutic requirements. This further reduces the investment and lowers the project risk. Another feature of our technology is precision – by screening for and drugging selective transcripts instead of all the gene products.

Following are some novel therapeutic modalities that we are working on:

i. NMD (nonsense-mediated decay) modality

We are constructing a comprehensive NMD-inducing *shAON* library for phenotype-based transcript screening. Being biologically-inert, NMD-inducing *shAON* mediates target suppression in a non-catalytic manner unlike siRNA and gapmer, which activate the Dicer/Dorsal complex and RNase-H respectively. Moreover, both siRNA and gapmer have limited capacity for chemical modifications without abolishing their biological activities. For these reasons, *shAON* has superior target binding specificity and *in vivo* stability, and exhibits a clear and distinct dose-response curve (Figure C).

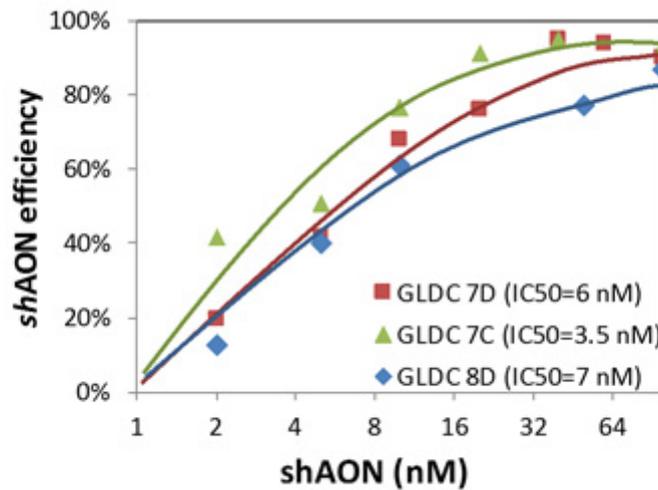


Figure C. Dose response curve of three lead shAONs inducing NMD of GLDC transcript

ii. Bi-therapeutics modality

Deep RNA sequencing has uncovered genome-wide alternative and aberrant splicing events and differential expression of spliced-isoforms in disease pathology. Interestingly, isoforms from a gene can manifest antagonistic functions (Figure D). The therapeutic strategy is then to reverse or undo the pathological splice event and switches the expression of the “diseased isoform” to the “normal isoform”. The shAON is considered bi-therapeutic because it eliminates the former while restoring the latter isoform, simultaneously.

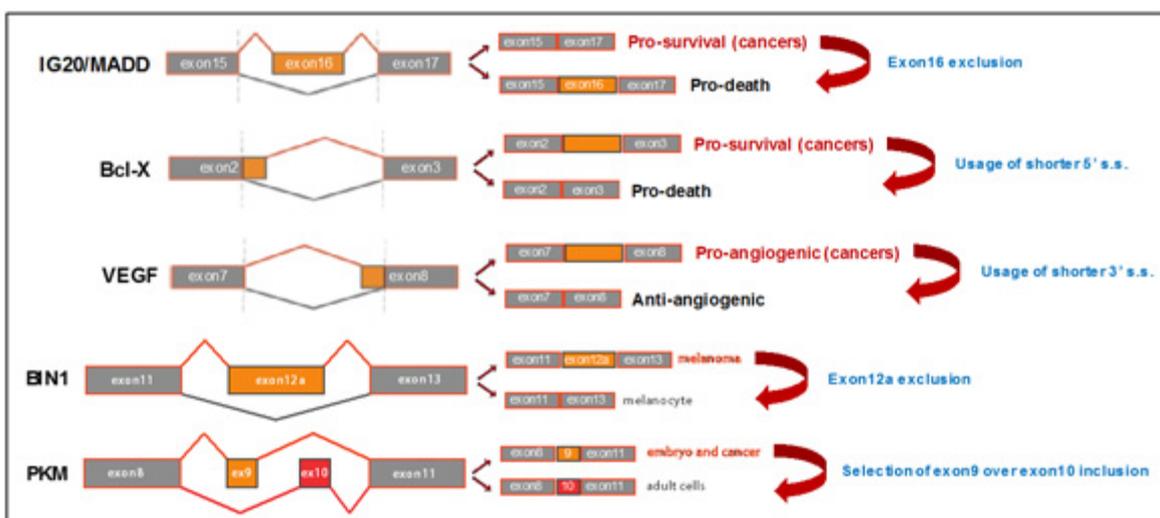


Figure D. Antagonistic functions between isoforms from a gene in cancers

We build various types of splice-switching shAON libraries:

- Skipping of every coding and non-coding exons in the human genome;
- Inducing and reversing each annotated splice event in the human genome;
- Custom libraries that tailored to novel splice events and isoforms uncovered from RNA sequencing.

The libraries are fed into our 2-Way Splice-Switching Screening Platform (2WS³) for performing phenotype-based splice-event/isoform screening (Figure E). A two-way screen will discover splice-events or isoforms as targets with mechanistic insights and with higher certainty (by lowering false-positives).

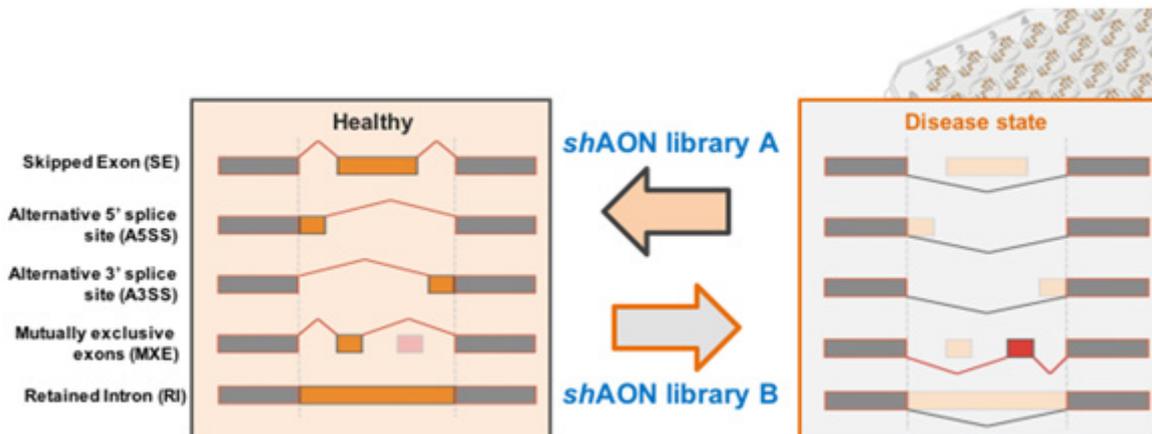


Figure E. 2-Way Splice-Switching Screening Platform for performing phenotype-based splice-event/isoform screening

Note: The other focus is in the development of theoretical and computational frameworks for the rational perturbation of complex biological networks to achieve specific systems properties or dynamics. Potential applications include generation of biological hypotheses, identification of drug targets with therapeutic index or resistance hedging, and rewiring of biological pathways for cell-based manufacturing of biologics or chemical compounds. Please contact me if you are interested to know more or to collaborate.