What are siRNA off-targets?

Small interfering RNAs (siRNAs) offer huge potential in RNA therapeutics because they can silence specific genes. However, siRNAs sometimes silence the incorrect gene through off-target binding, causing unforeseeable effects.

How siRNAs silence genes

siRNAs silence genes through RNA interference (RNAi). For naturally produced siRNAs, 20-24 nucleotide-long segments of double-stranded RNA (dsRNA) are produced by cutting long dsRNA using a Dicer ribonuclease. The siRNA then binds with an RNA-induced silencing complex (RISC), and the siRNA's sense or "passenger" strand degrades, leaving behind the antisense or "guide" strand. The guide ideally binds along the full length of the sequence to a specific mRNA target, leading RISC to cleave the mRNA.

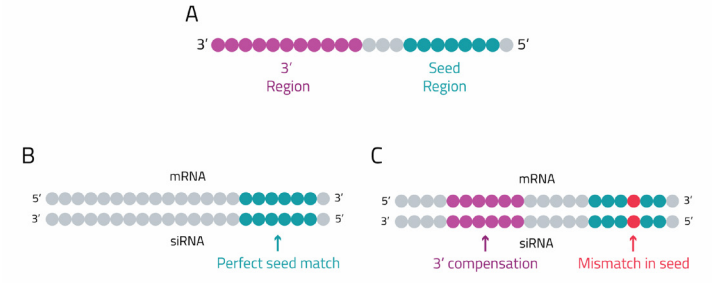
siRNA therapeutics use the same cellular machinery, except the synthetic siRNA is typically delivered as a duplex that is loaded into RISC. After loading, one strand acts as a guide and triggers degradation of the target.

How off-targets form

Sometimes, the siRNA guide strand can bind to a non-targeted region of mRNA that has some, but not all, of the same sequence as the guide strand. This is called partial binding.

Partial binding causes siRNA to bind like an miRNA. With miRNA-like binding, the siRNA often binds using a seed sequence which is located from nucleotide bases 2 – 7 or 8 of the guide strand. If there are mismatched bases between the siRNA and mRNA in the seed region, the 3' region of the siRNA may compensate akin to non-canonical miRNA binding. This binding can lead to RNA degradation or act as a steric block to prevent protein translation, reducing gene expression in genes where expression wasn't intended to be reduced. In a therapeutic, this effect could have harmful consequences.

Off-targets and degradation also occur if the passenger strand is loaded into the RISC instead of the guide strand.



Methods to address off-targets

Fortunately, there are methods to reduce siRNA off-targets. Chemically modifying the siRNA can reduce the likelihood of causing off-targets effects, such as 2'-O-methylation to reduce immunogenicity and phosphorothioate linkage to increase resistance to nuclease degradation. 5'-(E)-vinyl phosphonate can improve potency, and ribose modifications can increase binding affinity; both can increase siRNA stability.

When the double-stranded siRNA is loaded into the RISC, whichever siRNA strand has a less thermodynamically stable 5' end is identified as a guide strand. Thus, by modifying the 5' end of the guide strand to be less stable, developers can optimize the RISC to load the guide strand.

Finally, pooling siRNA, or using siRNAs that target multiple regions in the same mRNA, can reduce off-targets by limiting the chance of a high concentration of a specific seed region that can incorrectly bind to mRNA.

Finding off-targets at Eclipsebio

To evaluate how effective any of these methods are for reducing off-targets, developers need to know where and how siRNAs are binding to mRNA. At Eclipsebio, we offer ways to gather this information. Our miR-eCLIP+ assay directly identifies siRNA off-targets by immunoprecipitating the RISC, ligating the siRNAs to their target site, and sequencing the results, generating transcriptome-wide siRNA binding data. When we add our eRibo Pro assay, we can profile ribosomes to determine whether siRNA off-target effects result in changes at the protein level.

In addition, AI models can help predict off-target binding. Our eVERSE platform offers AI-ready data to help you train your models to improve their siRNA off-target predictions.

Ready to identify siRNA off-targets? Contact Eclipsebio to get started.