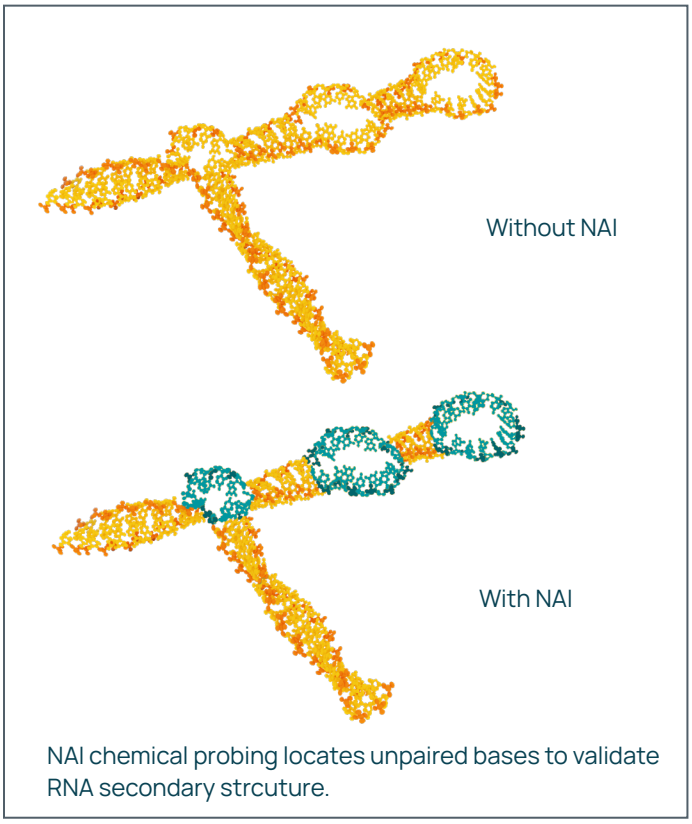
What are reactivity scores?

RNA secondary and tertiary structure play vital roles in biological functions like translation, stability, and gene expression. Different structures can impact how and where proteins bind to RNA as well as how stable the RNA is during delivery through lipid nanoparticles. If drug developers can gain insights on the structure of their RNA, they can design more precise and stable therapeutics.

At Eclipsebio, we use our eSHAPE assay to reveal insights about an RNA's secondary structure. For eSHAPE, samples are split, and half undergo chemical probing with NAI (2-methyl-nicotonic acid imidazole), which leads to the formation of chemical adducts in positions of unpaired bases. The other half of the sample is then probed with control DMSO (dimethyl sulfoxide). Both the NAI and DMSO samples are then reverse transcribed, and anywhere that NAI reacted with the RNA will appear as a mutation after sequencing.

After the NAI and DMSO samples are sequenced, the mutation rates are analyzed to calculate the reactivity score for each base.

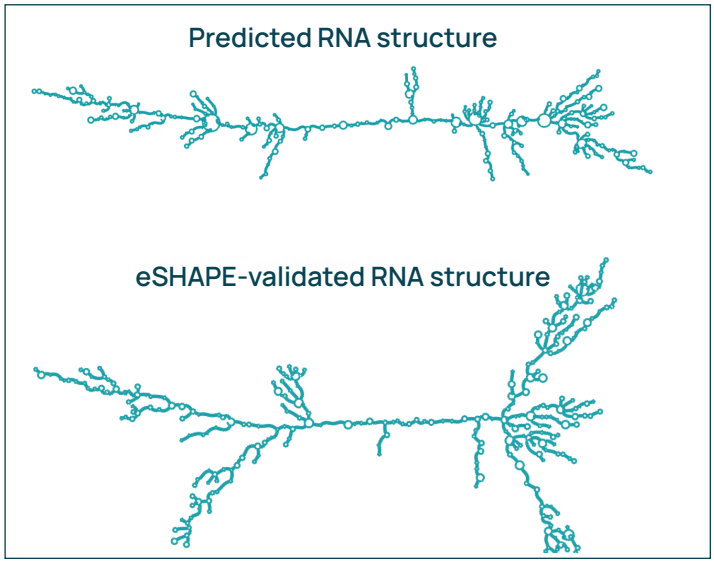


NAI chemical probing locates unpaired bases to validate RNA secondary strcuture.

Reactivity scores

Reactivity scores represent the flexibility of the RNA. With eSHAPE, the reactivity score for each base is calculated by subtracting the DMSO control RNA mutation rate by the NAI mutation rate. A lower reactivity score means the base is paired and likely already a part of an RNA structure. A higher reactivity score means the base is unpaired, such as within a loop. On endogenous RNAs, these unpaired regions can form druggable pockets for small molecules.

In addition, reactivity scores guide RNA folding software to more accurate predictions because eSHAPE directly measures RNA structure.



eSHAPE at Eclipsebio

By directly measuring RNA secondary structure with eSHAPE, developers can gain greater insights on how to make their therapeutics more effective. Revealing structure in the RNA's natural environment allows developers to see how cellular effects impact their RNA. eSHAPE also shows developers how the RNA changes in different conditions, such as when the RNA is in manufacturing compared to when it is delivered in an LNP. This is especially important for determining the stability of a therapeutic.

eSHAPE can also compare the reactivity scores of RNA under different conditions, showing developers where accessible protein binding sites are located with a gene. These accessible sites can be targeted for therapeutics.

Interested in seeing the accurate structure of your RNA? Contact Eclipsebio to get started.