What is differential accessibility?

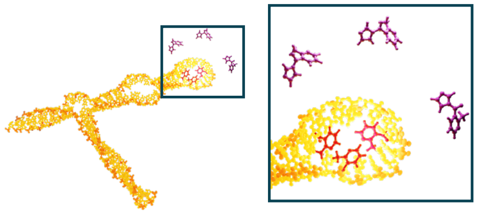
RNA secondary structure greatly impacts the efficacy of an RNA therapeutic. Highly structured RNA often has greater stability and a longer shelf life, but less structured RNA can have greater translation and protein production. Determining an RNA's secondary structure with structure probing assays is important for developing a successful therapeutic.

Besides the secondary structure itself, identifying where molecules, such as small molecules, RNA binding proteins (RBPs), and antisense oligonucleotides (ASOs), are able to bind to RNA is important for RNA therapeutic development. Fortunately, researchers can perform differential accessibility analysis with the data from structure-probing assays to uncover where these molecules bind to RNA.

Gathering data to use differential accessibility

Differential accessibility analyzes and compares two secondary structure-probing datasets of the same RNA under different conditions. To use differential accessibility, researchers first need to create these datasets using structure-probing assays. These assays measure the flexibility of the RNA at a single nucleotide level, which is quantified as a reactivity score. Higher scores mean that the nucleotide base is likely unpaired and accessible, while lower scores mean that the base is likely paired.

Assays like Eclipsebio's eSHAPE can make these measurements when the RNA is under different conditions. For example, one dataset can have measurements of the RNA in vitro, or free from cellular environmental factors. Another dataset can have measurements in cellulo where the RNA is in a cell and exposed to the proteins and other materials in the cellular environment. Other examples of conditions that could be analyzed are the presence or absence of an ASO or small molecule.

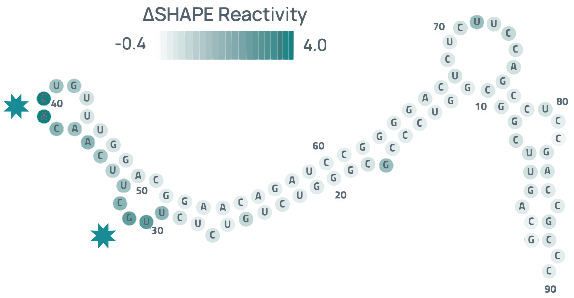


Unpaired nucleotide bases, such as those in an open loop in an RNA secondary structure, are exposed and able to be bound by small molecule therapies.

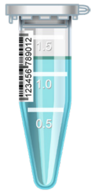
Using differential accessibility

Once a researcher has two datasets under different conditions (in this example, in vitro and in cellulo), they can analyze differential accessibility to compare the two conditions and look for molecule binding. These insights are generated by comparing the mutation rates of the in vitro and in cellulo bases to determine differential reactivity.

Bases where the in cellulo sample is less reactive than in vitro suggest that the base is paired or already has a molecule bound to it in the cellular environment's RNA structure. Bases where the in cellulo sample is more reactive than in vitro suggest that cellular factors rearrange the RNA structure to make it more accessible than the naked in vitro RNA. The bases in this region may be free to bind with RBPs or small molecules, making them potential targets for siRNA, ASO, or other small molecule therapies.



In this differential accessibility analysis, the dark blue, starred bases have decreased reactivity in cells, consistent with binding by a cellular factor. Both sites are known to interact with the RBP IRP1.



In vitro

In cellulo

eSHAPE and differential accessibility at Eclipsebio

At Eclipsebio, we can directly measure RNA secondary structure with eSHAPE. This allows us to determine structure in any condition, including in vitro and in cellulo. We can use these data to perform differential accessibility analysis, revealing binding sites for delivering effective RNA therapeutics.

Interesting in learning more about the structure and behavior of your RNA? Contact us today.

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