Needle in the haystack: miRNA structure/spectra relationships

Towards a molecular description of the conformational ensemble of miRNA

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In Short

- microRNAs are essential molecules in cell regulation and promising candidates as novel anti-tumor therapeutics
- The delivery of microRNAs to the organism requires protection from degradation and structural changes
- Through andvanced large scale atomistic simulations we will provide an overview on the structural variability and stability of these molecules
- By comparing with experimental Circular Dichroism measurements, we will build a general structural/spectral relationship, to instruct future research

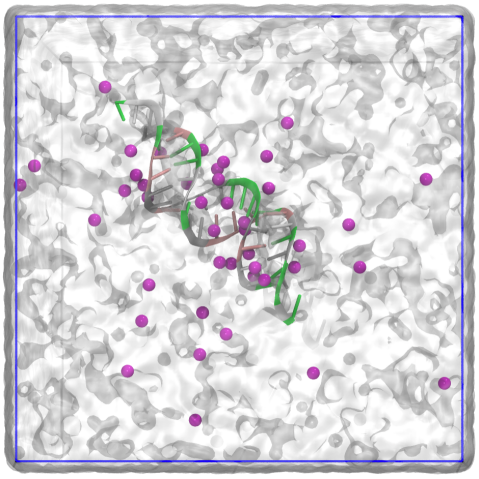

Figure 1: *Idealized duplex of hsa-miR-145 based on structure prediction*

Errors in cell signaling, which cause an uncontrolled cell proliferation, can develop from misexpressions or dysfunctions of microRNA (miRNA). MicroRNAs are small endogenous RNAs, approximately 22 nucleotides in length, which regulate eukaryotic gene expression on the post-transcriptional level. They work as a guidance for mRNA, in order to prevent it from expressing the wrong proteins. The related mechanism is called silencing, which can occur through two cellular mechanisms: cleavage of target mRNA or translation inhibition.[1]

Only a small number of human miRNAs have been functionally characterized and many of these regulate cancer-related processes such as cell growth and differentiation, and therefore, potentially function as oncogenes. Indeed, due to a dysfunction of miRNA, processes or molecules are not regulated any more. The missing regulation can, for example, affect oncogenes or tumor suppressor genes mediating cell cycle regulation, apoptosis, senescence, metabolism, angiogenesis and metastasis.[1]

Gene therapy is a kind of targeted therapy, that uses genetic material to modify the signaling pathways and thereby the gene expression within cells. The introduction of miRNAs into human cells could provide an efficacious therapeutic approach to inhibit tumour progression.[2] The delivery of miRNAs has been tested with functionalized gold nanoparticles, combined with either the stem-loop or hairpin structure of a miRNA. More recently chitosan-miRNA (CS-miRNA) complexes have been proposed as delivery systems, and their structure?function relationship has been investigated.[3]

MicroRNA 145, in particular, is known to be dysregulated in endometriosis. Delivered to the cells, it has been found to inhibit breast cancer and endometriotic cell invasiveness, marking this reagent as a potential candidate for novel antimetastatic therapeutic applications. To this purpose, the cell-relevant part of this miRNA is considered for delivery.[3] One strand (hsa-miR-145-5p) is the one having medical effectiveness. To increase stability, it is delivered together with its stabilizing partner (hsa-miR-145-3p), forming a duplex. Determining the stability and structure of both the individual components and their duplex is therefore of paramount importance to advance the therapeutic applications of this miRNA.

The structure of RNA plays a crucial role for its functionality in biological systems as well as for the functionality of the cell-relevant components. The 3D conformation of RNA molecules is generally mediated by a complex interplay of sequence depending interaction between the nucleotides,[4] in which the presence of every hydrogen-bond plays a crucial role. These interactions are mediated by the intrinsic conformational propensities of the backbone. The canonical helices provide a major contribution towards the thermodynamic

stability. However, more than twelve consecutive pairs are rarely found for RNA, contradicting their necessary flexibility. The characteristic features of the backbone are the preferential occupancy of specific torsion angle regions, while other ranges remain less populated, and furthermore a high correlation between these torsional angles.

Among solution-based methods, electronic Circular Dichroism (CD) spectroscopy is widely used for identifying and quantifying the secondary structures of solvated biomolecules, including nucleic acids. The electronic structure of RNA is complex based on electron dislocalization due to hyperconjugate effects. With respect to CD spectroscopy the most dominant contributions arise from the nucleic bases, which have strong adsorption bands between 190 and 300 nm. We provide here our own experimental characterization of changes in the CD spectra based on temperature ramping experiments for hsa-miR-145-5p, hsa-miR- 145-3p and their duplex. Interpretation is however not straightforward due to a lack of atomistic information on the conformational origin of the specific signals.

Molecular dynamics (MD) simulations in principle provide a powerful tool to access RNA dynamics at virtually unlimited space and time resolution, and can be performed in an environment very similar to that used in CD. However, their result is often not satisfactory mostly due to the short accessible timescales and the inaccuracy of the employed force fields. The former issue can be tackled using enhanced sampling methods.

Due to its importance for the functionality of cancer treatment, hsa-miR-145, and its components will be analyzed in greater depth combining our Circular Dichroism spectroscopy experiments with enhanced sampling MD. The calculations of CD spectra from simulated ensembles, will be used to gain novel understanding on the origin of the spectral changes with increasing temperature for miRNA.

Particularly, the general question we aim at answering during the proposed project is: What is the undelaying conformational variability of miRNAs structures and how does it impact the structural/CD spectra relationship?

We plan to tackle this question by:
1) Experimentally characterizing the thermal stability of the three miRNA species (hsa-miR-145-5p, hsa-miR-145-3p and their duplex) via CD spectroscopy;
2) Simulating the denaturation of the three species;
3) Sampling, by advanced enhanced sampling simulations, the conformational variability of miRNA in solution;
4) Calculating the joint CD observable based on the atomistic information on the conformers, building a structural/spectral relationship.

We believe that the obtained results, although obtained for a selected miRNA duplex and its single strands, will be of general interest within the larger cancer research and drug delivery communities, adding atomistic understanding to experimental results. This will provide essential information for the progress of miRNA-based therapeutics from laboratory research to further stages in pharmaceutical design and production.

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More Information

[1] A. Esquela-Kerscher, F.J. Slack, S. Oncomirs, *Nat. Rev. Cancer* **6**, 259?269 (2006). doi: 10.1038/nrc1840

[2] Y. Chen, D.-Y. Gao, S. Huang, *Adv. Drug Deliv. Rev* **81**, 128?141 (2015). doi: 10.1016/j.addr.2014.05.009.

[3] B. Santos-Carballal, et. al., *Sci. Rep.* **5**, 13567 (2015). doi:10.1038/srep13567.

[4] V. Mlýnský, G. Bussi, *Curr. Opin. Struct. Biol.* **49**, 63?71 (2018). doi: 10.1016/j.sbi.2018.01.004