Two are not enough: Extra base pairs in nucleic acids and their applications

Prof. Dr. S. Kath-Schorr

University of Cologne, Department of Chemistry, Cologne, Germany

To study folding, structure, localization and transport of specific cellular RNAs, new approaches in functionalizing and tracking RNAs in vitro and in their native cellular environment are required. For almost all biophysical studies, the site-specific introduction of reporter groups into the RNA of interest is essential. The synthesis of site-specifically modified long RNA molecules, which cannot entirely be prepared via solid phase synthesis due to its limitations in length, has remained a challenge. We employ a chemo-enzymatic approach by *in vitro* transcription based on an expanded genetic alphabet to incorporate various functional groups into large RNAs at specific positions. For this, unnatural hydrophobic base pairs are introduced into DNA to direct the site-specific introduction of unnatural ribonucleoside triphosphates during RNA transcription. By this approach alkene modifications, in particular norbornene and cyclopropene moieties, are enzymatically incorporated at specific positions into RNA by *in vitro* transcription allowing posttranscriptional labeling via additive-free inverse electron-demand Diels-Alder (iEDDA) cycloaddition reactions. We demonstrate that site-specific labeling by *in vitro* transcription is feasible for large, naturally occurring RNA molecules with complex foldings. We extended our approach to incorporate nitroxide spin labels site-specifically into RNAs to study their folding by electron paramagnetic resonance spectroscopy. Recent results on site-specific fluorophore and spin labeling of long non-coding RNAs and the site-specific modification of mRNAs will be presented.