RNA Kissing Loop Kinetic Analysis

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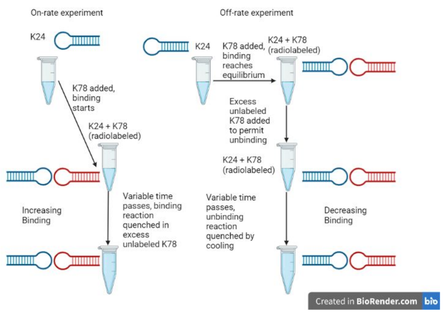
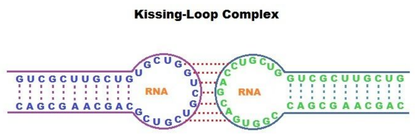
Abstract

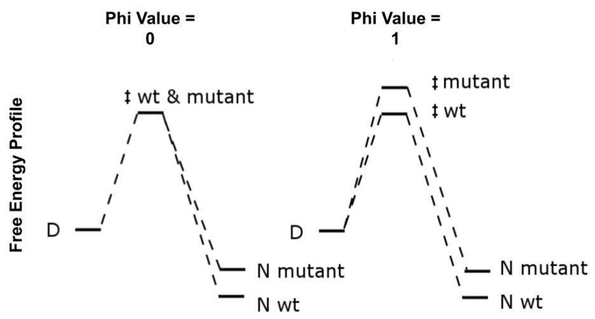
Kissing loops are important interactions between RNA strands in many structured RNAs including retroviral genomes and engineered riboswitches. Despite research on the thermodynamics of these interactions, their kinetics are still poorly understood. For this reason we are performing Phi analysis on an engineered stable kissing loop to determine information about its transition state. By understanding the kinetics of kissing loop formation in isolation, we hope to be able to compare this data to its kinetics when inserted into tetrahymena ribozyme. If it behaves similarly in isolation and in a structured RNA context, it will show modularity and perhaps be useful in RNA engineering applications.

Background

-Kissing loops form between the loops of RNA hairpins. If internal to one molecule, known as pseudoknot
-Small hairpins known to be hyperstable were ordered (structure in Results section)
-Point mutants ordered to determine stability and perform Phi analysis
-Data for on-rate and off-rates gathered via time course reactions







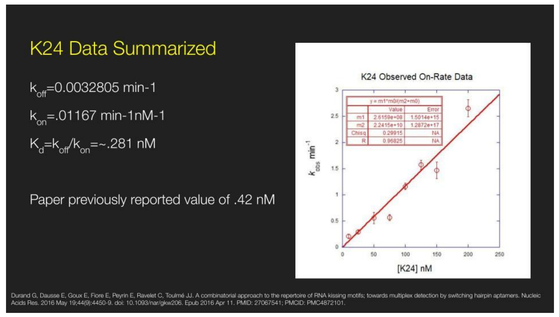
Results

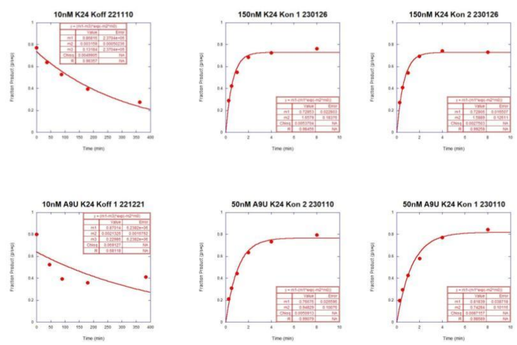
-The WT K24 kissing loop has had on-rate and off-rate calculations performed, and the dissociation constant calculated of .281 nM is similar to that previously reported, .42 nM

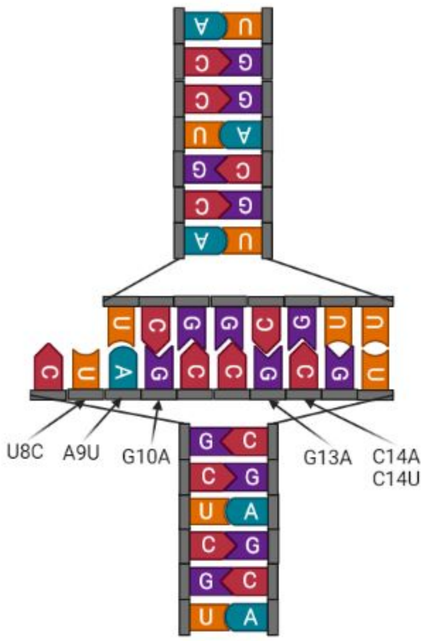
-Representative data for on-rates and off-rates of WT and mutant are pictured along with on-rate and off-rate graphs generated in Kaleidagraph software

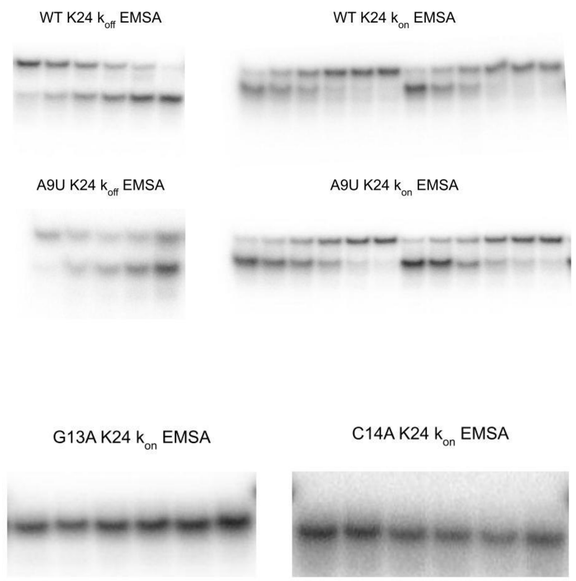
-Some mutants are so destabilizing that they do not form kissing loops. Both of these mutants are in the 13th and 14th positions. Wobble pairing restores kissing loop formation at position 14

-Kissing loops of more than 3 base pairs have previously been reported to perform thermodynamically similar to extended duplexes; if nearest neighbor rules for RNA duplexes are used, these positions would carry the largest malus, providing a logical explanation for why these mutants are not observable



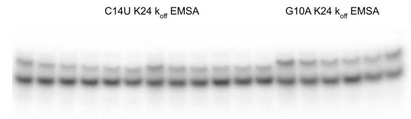






Conclusions

-Base pairs which destabilize the complex the most are the same that would be predicted to have the largest thermodynamic malus by nearest neighbor rules, providing a logical basis for future mutant designs
-K24 kinetics have been measured and a matching dissociation constant to that previously reported was calculated
-Recent k_{off} data shows C14U runs faster than other mutants; this suggests possible structural differences based on formation of base pairs



Future Directions

-Small amounts of on-rate data and off-rate data still required for mutants
-Next, the kissing loop will be put into the context of tetrahymena ribozyme; measurements will be made again, and if they mirror those in isolation kissing loop analyzed can be used as an ‘RNA module’ with potential applications to riboswitch design.

Acknowledgments

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