

This presentation is about the design and modeling of macrocyclic RNA targeting agents for Myotonic Dystrophy Type 1 (also known as DM1).

DM1 is a trinucleotide disease that causes the weakening of muscles. DM1 is characterized by DNA with expanded CTG and CAG repeats that form these hairpin structures. These structures cause the transcription of RNA with CAG and CUG repeats which can then undergo RAN translation to form toxic proteins. These toxic RNA also cause the sequestration of MBNL1, an alternative splicing regulator. That protein's removal leads to the misplicing of RNA.

Our goal was to prevent the sequestration of MBNL1 by interfering with the toxic RNA by replacing the U-U mismatch interactions in the RNA with a ligand's instead.

Specifically, macrocyclic ligands with either melamine or diaminopurine as the U-U mismatch targeting agent. Down here on the right are all the ways melamine (in blue) can bind with the U bases (in black). These dotted lines are hydrogen bonds. So in (a) and (b) we see melamine forming 6 hydrogen bonds with the U base in a triplet pattern where it fits inside the RNA. (c) is a slightly different binding situation where the melamine fits slightly below instead of between the U bases in a wobble base pair and creates 4 hydrogen bonds. (d) is a bonding scenario where the U base actually flips out and the melamine takes its place. We'll be modeling the flip out interactions in the future, but for now we'll be focusing on the other three.

This is one of the more promising melamine macrocycles from our modeling. Here you can see the macrocycle fit inside the CUG RNA repeat molecule. The C and G bases are colored grey while the U bases are colored green. These red atoms represent oxygen, so here on this side corresponds to here on this side with the two esters. The nitrogen, on the other hand, is blue, so the right side corresponds to the right side. Our macromolecule maintained a total of 8 out of the theoretical 12 hydrogen bonds between the melamine units and the U bases. Here you can see it in more detail. This top melamine maintained 3 hydrogen bonds while the bottom one maintained 5. This bottom one especially looks very similar to how we hope melamine will bind to U bases, which gives us hope for future directions.

We designed our macromolecules with either two melamine or two diaminopurine units connected with two linkers of different lengths. We varied the number of methylene units at a, b, c, and d, on the left, to find which linker lengths led to the most hydrogen bonding with the U base and were the most stable. We will then take those most promising molecules and evaluate the ease with which they can be synthesized. Then hopefully we can actually synthesize and then test those macrocycles to see if our models are valid.

Our results in this presentation have focused on melamine in triplet interactions with the U bases, but we plan on comparing the success of this model with the flip out model, and then with diaminopurine macrocycle variants, since diaminopurine is also known to

bind to U bases in similar ways as seen on the right.

In conclusion, we designed macrocyclic ligands to bind to the U-U mismatches in toxic RNA using melamine or diaminopurine as a targeting agent. We started modeling the melamine macrocycles and the promising hydrogen bonding will inform future modeling variants. We hope to combine these models with experimental results to shed light on RNA-ligand interactions. Hopefully the combined results can be used to help future study of U-U mismatch recognition units and possibly trinucleotide repeat diseases in general.

And with that, I conclude this presentation and would like to acknowledge the School of Chemical Sciences, especially the Department of Chemistry and the Department of Chemical Engineering, Steven C. Zimmerman, and the Zimmerman Group, especially the Drug Discovery Team. And then I would like to thank our sponsors, the NIH, MDA, and 3M. Thank you for listening.