Chem 572
Discussion Session #2 Due January 22, 2016
“Crystal Structure of a DNA Catalyst”

Name:________________________

Please complete all questions. Do your work on additional sheets of paper and staple to the back of this sheet. Do not put your answers on this page. You may work with your classmates on this and all discussion and homework assignments. I will grade the same three questions for all students and post the answers to the questions after they are due.


Define these terms: (Feel free to use other resources and to work together)

1.) Ligation- The joining of two molecules via a phosphodiester linkage such as between two RNA or DNA molecules

2.) Phosphorothioate- A phosphate functional group in which one of the oxygen molecules has been exchanged for a sulfur molecule

3.) Pseudoknot- A secondary structure in which pairing occurs between a loop and another region of the nucleic acid polymer

4.) Define at least 5 more terms that you had to look up while reading the paper. Feel free to write a complete list if you wish

General Questions:

1. The authors state that “fundamental mechanistic understanding of [DNA catalysts] is lacking in the absence of three-dimensional models at atomic resolution.” Why is it important to have high resolution structures of DNA catalysts or other catalytic polymers when trying to understand how they function?

Three dimensional structures are important in mechanistic studies since they allow researchers to more accurately assign potential roles to specific residues. The lack of high quality structures means that only inferences can be drawn from any experimental results about the roles of residues. The three dimensional structure is key to assigning specific roles to residues since the location and orientation of the residues can give clues on what their potential roles are. Loss of function could be attributed to either altering an important catalytically residue or loss of a structural residue.

2. Figure 3d and Extended Data Figure 2 shows the fraction of RNA ligated over time with different A-1 modifications. Why did certain modifications result in no observable
ligation, 2’deoxy and 2’O-CH3, while other modifications, 2’-NH2 and 2’-Fluoro, showed slowed ligation?

The ligation reaction is dependent on the 3’ hydroxyl of A-1 acting as a nucleophile and attacking the alpha phosphate of G1 so reactions without a proper nucleophile will show no ligation. The 2’ deoxy modification has a hydrogen atom which cannot hydrogen bond to align the 3’OH nucleophile (note H-bond of A-1 2’OH in Figure 3b, bottom); it also might give the wrong sugar pucker. Similarly the 2’ O-CH3 modification cannot form this hydrogen bond. The 2’-NH2 can form some hydrogen bonding and so rate is less affected. The 2’-F modification is highly electronegative with lone pairs and so can correct the sugar pucker to N and help with alignment.

3. Exchanging a non-bridging oxygen atom for a sulfur atom creates a phosphorothioate group and gives the phosphate a special property. What is this special property and what does the S_P and R_P notation mean?

The introduction of the sulfur creates a chiral center and leads to the formation of a distereomer. S_P means S chirality around the phosphate while R_P means R chirality around the phosphate.

4. In order to probe the possibility of metal ions in the catalytic mechanism, phosphorothioate analogs are often used. In this study, a phosphorothioate analog is inserted between dC12 and dA13 and the reaction is run in the presence of three different metal ions, Mg^{2+}, Mn^{2+} and Cd^{2+}. If there was a metal ion that was interacting with the non-bridging oxygen atoms between dC12 and dA13, how would the introduction of a phosphorothioate analog alter the rate of ligation in the presence of Mg^{2+} and Cd^{2+}?

With Mg^{2+} the rate of the reaction would decrease compared to the normal DNA catalyst since Mg^{2+} is more oxyphilic than thiophilic and so would have weaker interactions with the phosphorothioate analog than the oxy-DNA catalyst. With Cd^{2+} the rate of reaction would be rescued since Cd^{2+} is more thiophilic than it is oxyphilic and so would interact strongly with the phosphorothioate analog.

5. In figure 1d a number of interactions are drawn among the various nucleotides of the DNA catalyst and RNA substrates. The interaction between dG8 and dG26 involves the Watson-Crick face of dG8 to the Hoogsteen dG26. Draw the interaction between these two residues.
6. The authors mention that compensatory mutagenesis of the DNA catalyst at the ligation junction was able to activate previously inert RNA substrates. What is meant by compensatory mutagenesis and why did this activate the inert RNA substrates?

Compensatory mutagenesis is the mutation of specific residues, in this case specific nucleotides, used to restore interactions that were lost by a previous mutation. Originally dT29 forms a base pair with the G1 residue which allowed G1 to be in the proper location and orientation for catalysis to occur. RNA substrates which had C1 or U1 most likely could not form a proper base pair and so would not be in the proper location or orientation. The introduction of a dG29 and dA29, respectively, introduced Watson-Crick interactions that allowed base pairing with either C1 or U1 respectively thereby orientating the pyrimidine into the proper placement.

7. In a few sentences describe the overarching conclusions that can be drawn from this study, including any broad-scale applications.

Similar to RNA, DNA is able to form complex tertiary folds that can support catalysis and supports a larger conformational diversity compared to RNA. In addition the study also provides a rationalization into how substrates are recognized and for the regiospecific bond formation. This work raises additional questions about DNA’s potential role in the prebiotic evolution of life and the role of single-strand DNA in today’s organisms. Also shows fundamentally that any polymer, if single stranded and evolvable, can do catalysis. Also suggests the 2’OH might be as or more important for restricting sugar pucker, and reducing the conformational entropy of RNA folding, than for H-bonding.