# Crystal Structure of a Pre-Catalytic Group II Intron from *Pylaiella littoralis* (LSU.I2)

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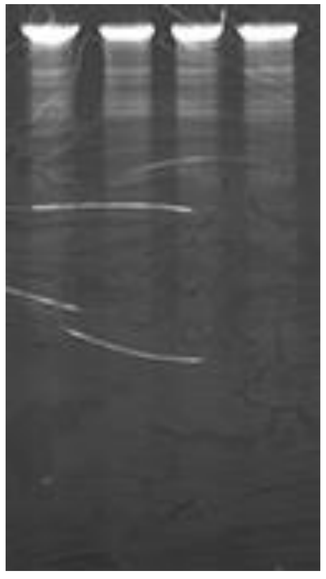
## Abstract

Group II introns are ribozymes that can self-splice; they do not need the assistance of the spliceosome. Group II introns differ from group I introns in that they form a lariat, or loop, which is caused by an adenosine's 2' hydroxyl group attacking and reacting with the 5' phosphate group of the first guanosine of the intron. The Toor lab focuses on finding the structures of these introns through the aide of x-ray crystallography. Our experiment focused on a group II intron from the mitochondria of a brown algae called *Pylaiella littoralis* which was mutated in four different ways so the RNA would not splice. This was done so that the pre-catalytic structure could be found. The catalytic triad which binds to magnesium within the RNA (AGC) was mutated with labels corresponding to which nucleotide was changed. For example, the label 123 would mean all three nucleotides in the triad were changed, specifically to GAU. The DNA was put into *E. coli* via a plasmid.

## Methods

In order to harvest the DNA sequence, a maxiprep was performed using four *E. coli* cultures which already contained the plasmid DNA. The restriction enzyme BamHI was added. The DNA template, buffer, nucleoside triphosphates, RNA polymerase, and pyrophosphatase and triton X-100 were combined to synthesize the RNA *in vitro*. It was left in a water bath for three hours at 37˚C. Once this was complete, calcium chloride and DNase were added to break down the DNA. Likewise, Proteinase K was added for an hour at 37˚C, leaving only the RNA in its full structure. It was left at 4˚C for 20 minutes, then spun down at maximum speed at 4˚C for ten minutes. The supernatant was removed with a syringe and afterwards filtered with a syringe filter. the RNA was then purified using a centrifugal filter with a 100kD cutoff to concentrate the RNA. This was done six times in order to exchange the buffer.



Once the RNA was filtered, it was run on a 4% polyacrylamide gel to see how pure the RNA was. When it was determined that the RNA sample was pure, it was set on a tray to crystallize. Spermine was added and 48 different chemical conditions were tested for each RNA sample in the hopes that at least one would promote crystallization. The tray was set at room temperature for one week before it was looked at under a microscope.

## Observation of crystals from P.li CT 12 (GAC)

The best crystals by far came from the *Pylaiella littoralis* (P.li.) 12 group. Although the crystals were small, there were relatively many. However, only 11 of the 48 drops actually contained any reasonable crystals. Condensation on the top of the tray was a determining factor in deciding whether or not there were crystals because it was difficult to see through and the lid could not be opened until it was time to put the crystals in the x-ray beam line (of which I will not be present to witness). P.li CT 12 was the third group to be observed.

## Observation of crystals from P.li CT 13 (GGU)

The crystals from this P.li. sample were small and the best crystals were barely visible under the microscope. However, this was the second best sample, with eight drops containing potential crystals. Unfortunately, condensation fogged the top of the tray for most of the wells, so it was difficult to see the water droplets underneath. For most of the uncondensed wells, there were only a few with any sort of crystal structures. For others, there appeared a brown, unidentified object. Many of the drops were given a pass if they contained anything which even remotely resembled a crystal. This was the first sample to be inspected.

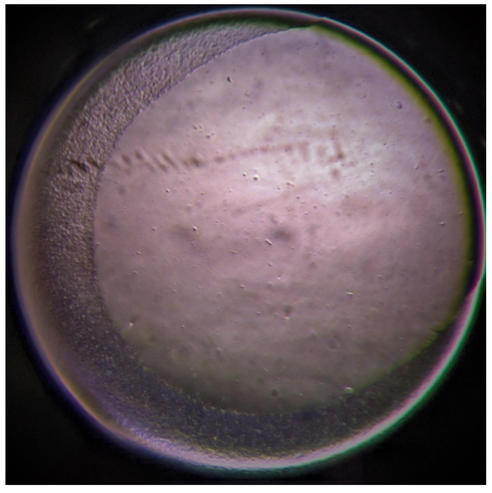
## Observation of crystals from P.li CT 23 (AAU)

The third best results came from P.li 23. There was more condensation than for the previous two samples, but it did seemed to contain a fair amount. There was still condensation at the top which made it difficult to see underneath, but there was some potentially useful material inside which seemed clear, if not highly microscopic in size. This tray was the second to be inspected.

## Observation of crystals from P.li CT 123 (GAU)

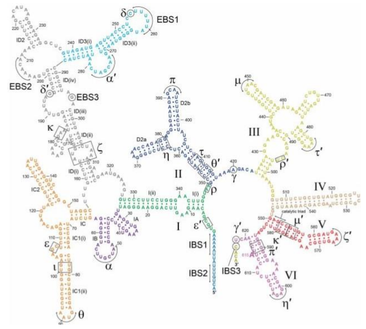
The least amount of crystals found in any group was in P.li 123. Only two drops contained what could be remotely classified as crystals. Very few of the drops were visible under the dense condensation and the ones that were visible could not be accurately said to have many crystals, if any. This sample was the last to be inspected

## Sample image



This droplet, as viewed under a microscope, contains what could potentially be microscopic crystals of group II introns. The crystals may be the small clear "pellets" scattered within the water droplet.

## Secondary structure of the group II intron of *Pylaiella littoralis*




This diagram shows the secondary structure of the group II intron. On Domain V, there is a catalytic triad. This triad forms the active site of the ribozyme. In order to attain the pre-catalytic structure of the RNA, this triad had to be changed using the sequence GAU. The separate RNAs tested were based on which nucleotides were changed. 12 means the first two nucleotides were changed. 13 means the first and third nucleotides were changed to this sequence and so on.

Domains are expressed as Roman numerals and tertiary interactions are expressed as Greek characters.

## Discussion and Conclusions

The crystallization of this RNA intron was a long process which yielded few satisfactory results. Although the *P.li.LSU.I2* intron did seem to crystallize in some cases (about 14% of the time in total), not all the drops were accounted for because of heavy condensation on the top of the tray and some unidentified objects, possibly contaminants, were found in the tray. Almost all of the crystals found were small enough that they could be easily glossed over if one did not look clearly enough. It became necessary to check over some droplets a second time to ensure there was a crystal forming inside. The drops were therefore judged very leniently to maximize potential yield.

As for the experiment itself, it was an interesting experience to see firsthand the mechanisms behind the information in any Biology or Chemistry textbook play out in real-world applications. Many fundamentals of biochemistry became more clear as they were presented, such as charged ions, polarization, hydrophobia and hydrophilia, and the functions of a molecular structure.

## References

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