Splicing removes non-coding sequences (introns) from pre-mRNA to generate mRNA template for protein production. The splicing proceeds through two separate catalytic reactions, both catalyzed by the spliceosome at a single catalytic center. Thus, after completing the first reaction, the generated lariat intermediate needs to reposition within the catalytic center to make space for the second reaction, in which the 3'SS needs to be placed at the active site. Obviously, any mistakes in selecting the precise borders of introns (5' and 3' splice sites, SS) lead to incorrect production of mRNA, causing severe problems for the cell. We are interested in the mechanisms by which the spliceosome selects the 3'SS and properly positions it at the catalytic center for catalysis. In particular, we would like to understand what happens when a poor or mutated 3'SS enters the spliceosome and how can we modulate these events.
Because the structure and function of the catalytic center are highly conserved in all eukaryotes, we chose to study these issues in yeast, keeping in mind, that the obtained results describe also the function of human spliceosomes. We rely on simple, yet precise methods of yeast genetic analyses, which allows us to monitor dynamic splicing events in a living cell.

We noticed that in the presence of high levels of 3'SS mutant introns, yeast cells display significant growth defects. We suggest that this is because the spliceosomes processing such mutant 3'SS introns are trapped in the conformation compatible with the first of the two reactions but are unable to move to the second. This would reduce the pool of spliceosomes available for splicing of endogenous introns, causing growth defects.
We will test this hypothesis and look for ways to correct these defects. One way would be to reduce the number of 3'SS introns, a different way would be to help the spliceosomes to reject or discard these ‘poisonous’ introns. We speculate that spliceosomes trapped by mutant 3'SS lariat intermediates can be rescued by a quality control system, in which specific within the catalytic center compete with binding of the intron, discarding mutant introns. To study this discard mechanism, we will alter the putative RNA motifs implicated in the discard of mutant 3'SS introns.
Our results suggest that the 3'SS motif, PyAG, is recognized and positioned at the catalytic center twice: first, in a ‘waiting room’ before the second step, during the first catalytic reaction, and then again during the second reaction, when it is positioned at the active site for catalysis. We will identify and characterize these binding sites for 3'SS at the catalytic center to better understand how the spliceosome controls the fidelity of splice site selection.

Together, our experiments will test the existence of previously unknown RNA:RNA interactions and their possible function in the discard of lariat intermediates as well as those recognizing and positioning the 3'SS at the catalytic center. If confirmed, these findings would represent a major discovery with significant implications for the control of splicing fidelity. The proposed model of the quality control of splicing at the catalytic phase represents an entirely new view of the process, that has never before been investigated. Growth defects caused by trapped spliceosomes may represent a general type of defects in eukaryotic cells under certain conditions. Thus, our work may suggest a ‘treatment’ for this problem and may be used in the future development of new therapeutic strategies for genetic diseases based on analogous mechanisms, paving the way to a new class of general therapeutic solutions for various splicing-based diseases.