Cells receive diverse stimuli from their surroundings and process them into distinct physiological responses through signal transduction pathways. Mitogen-activated protein kinase pathways are responsible for many cellular functions. The overarching goal of this thesis was to study the effects of mutations on MAPK signaling function using complementary systems biology and genetic engineering methods.

First, I asked how mutations could rescue a decrease in signaling caused by overexpression. I identified several variants of the yeast MAPK, Fus3, that rescued mating function in an overexpression background. In addition to finding Fus3 variants that rescued pathway function by improving kinase activity, I found variants containing premature stop codons (PSC), which are naturally readthrough, that rescued pathway function. The location of the mutated residue in the protein structure and the identity of the residue play a role in determining the impact of the PSC on pathway function. These results suggest that one way in which selection can compensate for protein overexpression is by introducing PSCs at permitted positions.

Next, I quantified the effects of mutations on yeast mating and HOG signaling. I used the information theory measure of mutual information to measure signaling and introduced change in mutual information as the measure of the effects of mutations on signaling. I showed that information transmission in HOG signaling is more robust to spontaneous mutations than mating signaling. I controlled for some of the possible reasons for this difference, redundancy and mutational target size, and found that although HOG signaling mutational robustness is compromised without the redundancy, it is still more robust than the mating signaling suggesting that other mechanisms play a role in maintaining this mutational robustness. Finally, I did a direct comparison of the effects of the mutations in a shared component on information
transmission in the pathways and found that large effect mutations in this protein have large effects on amount of mutual information in both signaling pathways and that some of these effects are opposite.

Overall, in this thesis work, I used various mutagenesis methods to test the effects of mutations on signaling in living cells. In the first part, I used an engineered random mutant library of a protein kinase to assess the functional significance of mutations on a yeast MAPK pathway. In the second part, along with genetic engineering, I used experimental evolution to quantify the mutational robustness of two yeast MAPK pathways. We found both non-intuitive and intuitive results and ways to quantify both. Applying the tools of systems biology and genetic engineering is a comprehensive and complementary approach to answering evolutionary questions.