A. SPECIFIC AIMS

Both gram negative and gram-positive bacteria are developing resistance to key antibiotics at an alarming pace. During the 10 year span from 1990 to 1999, the prevalence of ciprofloxacin resistant isolates grew as much as 5-fold in enterobacteria that cause gastroenteritis, meningitis, and wound infections(cite). There are multiple mechanisms by which resistance can arise, including single point mutations that cause changes in the drug target so that it no longer binds, changes in cell wall or membrane permeability to the drug, and many others(cite). While these adaptations permit the cell to survive antibiotic exposure, they often have a side effect: they result in sub-optimal cellular functioning and cell survival in *non-antibiotic environments* when compared to the wild-type. When the antibiotic is removed, this implies that the resistant strains would be less fit to survive than the original strain, and would remain more susceptible to elimination by the host immune system. However, contrary to this theory, it is observed that resistant strains frequently regrow within the host as vigorously as the original drug-susceptible strain. In our preliminary data, we've generated streptomycin-resistant strains of *E. coli* that demonstrate this phenomenon. We exposed the wild-type strain to streptomycin challenges at a concentration that allowed only a small fraction of the population to survive, and after several cycles of exposure, we obtained a strain that had a 4X increase in minimal inhibitory concentrations. Compared to the wild-type, the resistant strain had a population doubling of about 1.4X slower than the wild-type. By then performing a process of selecting the fastest growing resistant cells and exposing them to streptomycin, repeating this through 20 cycles, we obtained an *E. coli* strain that was both streptomycin resistant and had a doubling rate equal to the parent wild-type strain. WHY THE READER SHOULD CARE. WHAT IS THE THEORY. WHO ARE THE APPLICANTS. HOW ARE THEY GOING TO DO IT.

This process of compensating for the deleterious effects of the adaptation antibiotic resistance is due to *compensatory mutations*. These are secondary mutations elsewhere in the genome that offset the effect of the primary mutation. If we could eliminate or inhibit the ability of cells to develop these compensatory mutations, it would leave the resistant strains weakened, and more vulnerable to elimination. However, at the present time, very little is known about the nature, locations, and targets for these mutations, preventing forward progress on strategies that may block the compensatory mutations from occurring. In this exploratory R21 proposal, we are proposing a proof-of-principle study using the latest genome sequencing in combination with proteomic technologies to identify compensatory mutations occurring in gram-negative *E. coli* strains exposed to streptomycin. Our goal is to map out the compensatory mutations that have occurred, and to begin analyzing the putative mechanisms of their action via pathway analysis tools like KEGG. Our group has 10 years of experience with proteomic methods, 5 years of experience working with antibiotic resistant strains, and 3 years of experience performing comparative genome resequencing of antibiotic resistant bacterial strains. We will apply this experience in the following specific aims:

1. To identify mutations that correspond with growth rate compensation in streptomycin resistant *E. coli*, we will use the Hi-Seq and related genome re-sequencing methods to map the locations and types of mutations that occur in growth-rate compensated strains derived as described in our preliminary results.
2. To determine the regulatory effects on expressed proteins due to the compensatory mutations in strep resistant *E. coli*, we will apply modern shotgun proteomic methods to identify proteins that are upregulated, down-regulated, and those whose sequence is directly changed by mutation.
3. To combine the sequencing and proteomic data to determine pathways through which the compensatory mutations elicit their positive growth effects, we will use the KEGG tool to map out the putative effects of each mutation identified in aim 1 in combination with its putative effects measured in aim 2.

In this exploratory proposal, we expect to obtain a unique map of mutations that can then be used in a subsequent R01-scale, hypothesis-driven project that thoroughly explores and validates the effects of the significant compensatory mutations identified in this study via a combination of targeted gene knock outs and recovery experiments. Ultimately, these studies will tease apart the complete pathways by which compensatory

mutations operate to optimize bacterial growth, knowledge that we can use to better target bacterial populations for reduction or elimination.