A. SPECIFIC AIMS

Bacteria can adapt rapidly to deleterious effects of antibiotics, posing the specter of a major public health crisis. A key component of the adaptive process is the occurrence of compensatory mutations in the genome that offset the fitness costs associated with drug resistance, which can stabilize drug resistant genes in a bacterial population. Resistance stabilization potentially contributes to the emergence of virtually untreatable pathogens such as multi-drug resistant *Mycobacterium tuberculosis* and *E. coli,* or vancomycin-resistant enterococci. Most of the primary mutations responsible for antimicrobial drug resistance have been identified, but many compensatory mutations remain unidentified[1],**WHO CARES???** because practical methods for studying the rapidly occurring *genome-wide* changes are limited. Locating these adaptive mutations requires an approach that examines global changes in the organism and can trace them to the genome.

Our long-term goal is to integrate proteomics, genomics, and computing in a systems biology fashion to provide tools for examining and modeling microbial evolution. **WHO CARES???** *Herein we propose a proof-of-principle test of a novel combination of proteomic methods,* applying them t o the problem of determining the multiple phenotypic and genomic changes(?????) that occur during adaptive evolution to offset the fitness costs of antibiotic resistance. Our rationale is that mass spectrometry (MS) now has the capability to examine intact protein masses with sub-Dalton accuracy, allowing us to examine proteins for many possible mass changes due to the effects adaptive evolution. Our goal is to take advantage of this and other recent proteomic technologies to identify variations in the proteome that can be mapped to an origin in the genome. Our team has expertise in proteomics, genomics, microbiology and computational biology, making us well-suited to a cross-disciplinary project like this.

The Specific Aim of this proposal is to determine the multiple genome changes that SEQUENCING!!! occur during adaptive evolution compensating for the fitness costs associated with an antibiotic resistance mutation in *E. coli*. Our test system will include streptomycin sensitive wild-type *E. coli* MG1655; a streptomycin-resistant/low-fitness mutant of the wild type parent, and an experimentally evolved fitness-compensated derivative of the resistant mutant. This set of proof-of principle experiments will examine the expressed proteome focusing on both *compositional* differences in proteins (mass or chemical property changes) due to underlying genetic changes, and for events that differentially switch protein expression on or off, such as phase variation[2-4]. We plan to apply a novel combination of the following approaches:

a. To provide a map of proteins that have been switched on or off, and to possibly reveal compositional changes resulting from adaptive evolution, comparative 2-dimensional gel electrophoresis will be applied. Protein spots that are present/absent across our derivatively-evolved strains, or shifted in either dimension (mass/pI) will be digested, analyzed by mass spectrometry, and mapped to their genomic origin using our software. The identified gene loci encoding the differentially changed proteins will be sequenced to determine if the observed protein changes originate from mutations in or near the encoding gene.

b. To provide a high-accuracy examination of protein changes resulting from genomic mutations we will apply comparative multi-dimensional liquid chromatography separations coupled with electrospray-ionization mass spectrometry (ESI-MS). Fractions corresponding to regions of chromatograms displaying differences across our evolved derivative strains will be examined by ESI-MS to survey constituent intact proteins for mass differences. A portion of the same fractions will be digested into peptides for MS to identify the protein's genomic locus. The identified genomic loci will be sequenced to determine if the observed protein changes originate from mutations in their encoding gene. While this sub-aim presents a much greater technical challenge than a), it is anticipated to provide greatly enhanced detail regarding the specific nature of protein changes occurring in adaptive evolution. **YOU ARE SO SMART! (BUT, I'M NOT GOING TO FUND YOU!)**

We anticipate that this work will demonstrate the utility of a novel systems biology approach for examining the interrelationships among the networks of components in a cell, and to determine the nature of coordinated changes to those components during adaptive evolution. We also expect the work to reveal how adaptive

evolution contributes to the stabilization of drug resistance genes in a bacterial population, providing an important resource for researchers focused on managing antibiotic drug resistance.

REJECTION!!!!!!!! GAME OVER