Chemical proteomics to understand the mechanism and side effects of antibiotics.

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Many of the antimicrobials that are in widespread use today were discovered through studies that screened naturally occurring molecules for their antimicrobial activity, followed by further chemical development of the identified molecules to give them the desired properties to make them good drugs. Surprisingly, in some cases exactly how the drugs work is not well understood. Some of these drugs also have side effects that limit their use and often result in patients failing to take the drugs, or completing the course of antibiotics, resulting in both significant costs of wasted drugs and increased development of resistance in the microbes. The reason for these side effects is also largely unknown. In some cases, how the microbes become resistant to drugs is also unknown [1].

Chemical proteomics [2] is a method that allows us to study the proteins in both microbe and human cells that the drugs interact with, and by knowing these we can start to identify how they work, how bacteria evade their effects, and what causes the side-effects in humans. Chemical proteomics works by immobilizing the drug onto a solid support and fishing in cell extracts for proteins that bind to the drug. We can then use this information to identify the proteins responsible for the effects we see. By using different drugs from the same class, we can also start to understand how the modifications in their structure changes their effects.

The project will initially study two classes of antimicrobials, the tetracyclines and aminoglycosides, which are widely used in the clinic for the treatment of a range of infections. Gentamycin is a widely used aminoglycoside antibiotic active against a range of Gram-negative pathogens, including those causing serious infections such as meningitis, pneumonia and septicaemia. Tetracyclines such as doxycycline are widely used to treat bacterial and protozoal infections, and recent development of the tetracyclines has provided one of the few drugs available for treating multidrug resistance microbes, tigecycline. However, surprisingly the detailed mechanisms of action of these drugs remains unclear [3], and the potential for synergy is poorly explored. Identifying the targets is vital for the further development of new antibiotics.

The aminoglycosides exhibit significant side effects, such as nephrotoxicity and inner ear damage, but the cause of these are unknown. Tetracyclines have been found to affect a number of other cellular pathways that suggests further protein targets that have not yet been identified, and adverse reactions, while mild, can be significant. Side effects result in problems with compliance of patients with drug regiments, often with a failure to use the drugs or to complete the full course, wasting valuable resource and resulting in further development of drug resistance. Identifying mammalian proteins that these drugs bind to will lead to a better understanding of the causes of side effects and enable these to be designed out

Both the tetracyclines and aminoglycosides are amenable to immobilization on solid beads using chemical methods, and these will be used to capture interacting proteins from cell lysates of bacterial pathogens and their antimicrobial resistant strains (*Escherichia coli* and *Pseudomonas aeruginosa)*, and human endothelial and liver cell lines Interacting proteins will be identified by quantitative proteomics methods using the modern, highly sensitive chromatography and mass spectrometry methods.

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