Developing novel tools to guide the discovery of new cell-permeable, on-target anti-infective compounds.

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As the current covid-19 pandemic continues to show, infectious diseases can pose serious threats to public health systems around the world. On the bright side, the response to the current crisis demonstrated that public and private sectors can work together to deliver solutions, such as vaccines, at unimaginably fast speeds. Unfortunately, the current crisis also highlighted that making novel, small-molecule-based anti-infectives is extremely hard. These must be developed not only to address the next virus-inflicted pandemic, but also to address current unmet medical needs in infectious diseases. For example, the World Health Organization has continuously warned of an eminent post-antibiotic era, in which bacterial infections will no longer be treatable using currently available antibiotics. Likewise, diseases previously limited to certain parts of the globe, such as leishmaniosis in the tropics, are expanding their geographic range due to climate and environmental changes caused by man-made actions. Unfortunately, the availability of microbial genomes and the development of sophisticated genetic tools to identify essential genes have not yet delivered the expected renaissance in anti-infective discovery. This lack of success, especially for Gram-negative bacteria and intra-cellular pathogens, is likely to result from the incredible challenges posed to compound permeability by the microbial cell envelope and the amazing plasticity of pathogenic microbes, which makes inactivating single enzymes an often-ineffective therapeutic strategy. At UNICAMP, my group has been using the vast collection of publicly-available structures in the PDB to design and develop novel methods to identify cell-permeable compounds having on-target activity in cells. Currently-available methods to study compound permeability and retention in microbes are often not amenable to high-throughput formats and most cannot be used with living cells in culture or do require compounds to be modified. Here I will share our advances in developing a new fluorescence-based strategy to investigate compound permeability and retention in live, whole microbial cells. This assay is based on resonance energy transfer (RET) between a luciferase-fused target protein and a bioluminescence RET (BRET) probe. Competitively displacing the BRET probe from the luciferase-fused target informs on the binding affinity and residence time of unmodified compounds to their molecular targets in live microbial cells in culture. As opposed to current methods, this assay is within easy reach of the research community working on new anti-infectives, as it does not require expensive equipment, intensive sample manipulation nor that test compounds be modified. We have used this assay to identify, from within compound libraries, novel ligands of essential enzymes in pathogenic bacteria (Escherichia coli and Mycobacterium abscessus) and in Leishmania, an intracellular eukaryotic parasite. We expect that similar assays can be developed to expedite the discovery of new anti-infectives for a broad range of microbial pathogens.