Grant Application to BBSRC and MRC

Summary:
Mycobacteria tuberculosis is one of the most bad-named bacteria for the deadly disease it caused in human. Emergence of the multi-drug resistant strains of the pathogen is calling for novel therapies urgently. One possible route is to employ *Mycobacteriophage*, a natural consumer of the pathogen, to kill the bacteria but previous efforts have been hampered by a lack of knowledge in bacterial immune system and in *Mycobacteriophage* diversity[1]. We seek to address these problems by analysing the bacterial immune system in *M. bovis,* a less pathogenic but related species, with the aid of the newly sequenced phage genomes.

Background and Importance:
Tuberculosis is a contagious pulmonary disease affecting 10 million people worldwide, of which 2 million died from it. Whereas treatment with antibiotics has been successful, the emergence of multi-antibiotic resistant strains is increasingly serious. Previous proposal of *Mycobacteriophage*-based therapy that employ virus to kill the bacteria specifically has remained impractical in lack of a suitable strain that can infect both *M. Smegmatis* and *M. tuberculosis* simultaneously[1]. Recent research on the wide-spread CRISPR/Cas bacterial/archeal has furthered our understanding of bacterial immunity towards phage infection[2], which may guide a better attempt in finding a such strain.

Novelty:
Recently, an intact type III CRISPR/Cas system has been identified[3] in the genome of *M. tuberculosis* and *M. bovis,* the less pathogenic yet biologically closely related strain, but not in *M. Smegmatis*, highlighting the difference between their immune systems. On the other hand, number of *Mycobacteriophage* genomes has exploded from 100 in 2010 to 1360 in 2017[4], as a result of SEA-PHAGES programme, identifying promising potential phage targets for native CRISPR/Cas system in *M. bovis* (unpublished data). These two advances warrant a further investigation to elucidate the immune system of *M. bovis.*

Aims and Objectives:
Although genetically identified, the role of CRISPR/Cas system in *Mycobacteria* remains unclear. We aim to characterise the system and test whether it indeed confers immunity towards targeted phage DNA. The conclusion may then be mapped to *M. tuberculosis* given the strong similarity between their CRISPR/Cas systems. Additionally, we aim to construct a recombinant strain of *M. Smegmatis* with a mentioned CRISPR/Cas system functionally integrated to mimic the immune system of *M. bovis,* in order to enable rapid and safe experiments in the future.

Methods:
We aim to test the functionality of the mentioned CRISPR/Cas system in *M. bovis* with both phage challenging assay and plasmid challenging assay, in native strains and in those with knockouts of CRISPR/Cas system. A functional CRISPR/Cas should reject phage/plasmid in a sequence specific fashion. The used target sequence will be produced from a bioinformatic analysis to identify conserved proto-spacers. If successful, we then seek to integrate this CRISPR/Cas system into *M. Smegmatis,* and test for its functionality more extensively. Gene editing is conducted with integration-proficient vectors[5]. Experiments with *M. bovis* will be done in category 2 facility.

1. Hatfull, G. F. Mycobacteriophages: Windows into Tuberculosis. *PLoS Pathog.* (2014). doi:10.1371/journal.ppat.1003953
2.Tamulaitis, G. Type III CRISPR-Cas Immunity : Major Differences Brushed Aside. **25,** 49–61 (2017).
3.He, L., Fan, X. & Xie, J. Comparative genomic structures of Mycobacterium CRISPR-Cas. *J. Cell. Biochem.* (2012). doi:10.1002/jcb.24121
4.Mycobacteriophage genomes at PhagesDB. Available at: http://phagesdb.org/hosts/genera/1/.
5.Hatfull, G. F. *The Secret Lives of Mycobacteriophages*. *Advances in Virus Research* **82,** (Elsevier Inc., 2012).