## 2. Overview/Abstract

Viral infections such as the ongoing COVID-19 pandemic take millions of human lives each year. Our immune system combats viral infections, but it can fail, or be co-opted to promote disease. Chemical therapies, immunotherapy and vaccines are helpful, but they can drive resistance and fail as viruses evolve, and they may be outright useless against newly emerging viruses. Moreover, some may have harmful side effects. Thus, developing radically new, effectively programmable approaches for studying and treating viral diseases could maximize beneficial impact while minimizing cost, time and human effort. To complement the natural human immune system and help it prevail, we will construct programmable, synthetic sense-defense genetic circuitry entirely different from human immunity, based on recently discovered, RNA-guided RNA-cleaving bacterial Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) immunity. Many engineered CRISPR systems exist, but with uncontrolled locations, expression and activity, risking cellular disruptions and burden and/or unwanted off-target effects. In contrast, the new human CRISPR (hCRISPR) systems we propose will have (i) minimal or no activity without viral transcriptome perturbation; (ii) automatic activation only when needed in individual cells; (iii) harmless genomic insertion; (iv) external control by chemicals for added safety. We envision hCRISPR as the basis of future programmable systems that counteract not just viral RNA, but unwanted RNA deviations in cancer, heritable genetic diseases and aging.

Our *long-term goal* is to control transcriptional aberrations by engineered genetic systems. *Here*, our goal is to develop engineered human and green monkey cell lines with integrated, programmable synthetic gene circuits that can sense and counteract infection by SARS-CoV-2 and possibly other RNA viruses.

To achieve this overall goal, we will pursue two **Specific Goals**:

**Goal 1:** To design and optimize automatic RNA virus sensor and responder gene circuits;

**Goal 2:** To test and optimize RNA virus sensors and responders against real viruses *in vitro*.

This work is significant and innovative because the synthetic gene circuits and cell lines we develop will be novel, broadly applicable tools for sensing and counteracting the presence of viral RNA in human cells. The implications can be far-reaching, including automatically sensing and responding to RNA deviations in cancer, viral infections and genetic diseases.

This work will be conducted by a highly qualified interdisciplinary team with complementary expertise: a PI with quantitative biology, physics and bioengineering background, and a human virologist Co-Investigator.