Signed details of the excellence in research work for which the Sun Pharma Research Fellowship is claimed, including references & illustrations (Max. 2.5 MB). The candidate should duly sign on the details

Characterizing reverse transsulfuration* (RTS) *pathway enzyme in Mtb

Metabolism of cysteine and methionine amino acids is critical for the survival of the most successful human pathogen, *Mycobacterium tuberculosis* (*Mtb)*. Cysteine functions as a biosynthetic precursor for essential cofactors such as Fe-S clusters and a primary antioxidant buffer, mycothiol. Fe-S cluster proteins and mycothiol coordinate *Mtb*'s ability to respire, resist oxidative stress, counteract anti-TB drugs, and establish infection. Therefore, it is essential to understand the pathways that *Mtb* utilizes to generate cysteine and its subsequent utilization to produce Fe-S clusters and mycothiol. We discovered that *Mtb* generates cysteine via the reverse transsulfuration pathway (RTS). We have biochemically, biophysically, and genetically characterized the first rate-limiting enzyme of the RTS pathway, cystathionine beta-synthase (Cbs). An important aspect of this work was done in collaboration with Prof. Somnath Dutta, IISc. Dr. Dutta's group characterized Cbs by biophysical techniques, TEM and single-particle cryo-electron microscopy (cryo-EM) based atomic model determination of tetrameric assembly of native and S-adenosyl methionine (SAM)-bound *Mtb* Cbs, which provided unprecedented insight into the mechanism of allosteric activation of *Mtb* Cbs. We also discovered that SAM depletion results in the degradation of Cbs, which triggers a non-canonical pathway for methionine biosynthesis in *Mtb*. Inhibition of RTS and the non-canonical methionine biosynthesis pathway killed *Mtb* by inducing oxidative stress. Lastly, Cbs is essential for *Mtb* to cause infection during HIV-TB coinfection.

Role of Fe-S cluster biogenesis systems in Mtb

In another skilled effort, we identified Fe-S cluster biogenesis machineries (Suf and IscS) in *Mtb* and showed its requirement for mycobacterial respiration, metabolism, and survival in mice. The iron-sulfur cluster containing proteins are important for essential processes such as energy production by respiration, and also enable the bacteria to survive harsh conditions of the lungs and cause infection. Our studies discovered the mechanisms that *Mtb* uses to build these iron-sulfur clusters. Iron-sulphur clusters are mainly produced by the SUF operon in *Mtb*. However, there is another single gene called IscS that can also produce the clusters. We asked why would the bacterium need both? To solve this mystery, we generated a mutant version of *Mtb* that lacked the IscS gene. We found that under normal and oxygen limiting conditions, iron-sulphur clusters are produced mainly by the action of the IscS gene. However, when the bacterium faces a lot of oxidative and nitrosative stress, the iron atoms of the clusters become oxidized and released, damaging the clusters. Therefore, there is an increased demand for producing more clusters, which switches on the SUF operon. The absence of the IscS gene led to severe disease in the infected mice, rather than a persistent, chronic infection typically seen with wild-type *Mtb*. We found that in the absence of the IscS gene, the SUF operon is highly activated albeit in an unregulated fashion during infection in response to host-generated nitric oxide (NO), leading to hypervirulence. Depleting both IscS and SUF system dramatically reduced persistence of *Mtb* in mice. Therefore, the IscS gene keeps the activation of the SUF operon in check, causing persistence in TB.

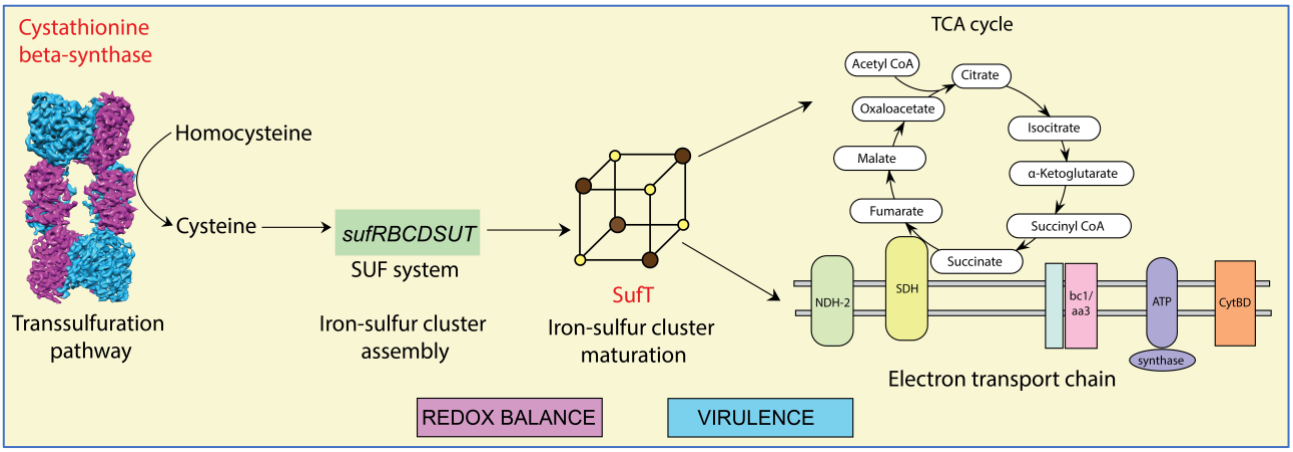

Figure 1: Cryo-EM structure of Mtb CBS and its involvement in sulfur metabolism along with Suf and IscS Fe-S cluster biogenesis systems.

Capturing redoxosome of Mtb

Free radicals in the form of reactive oxygen species (ROS) are produced during cell metabolism. Cells need a way to protect themselves from the harmful effects of overaccumulation of ROS. All cells, human to bacterial, have protective mechanisms. Interfering with the protective mechanisms in bacterial cells is a way to make bacterial cells susceptible to antibiotics. Using a combination of an intracellular redox biosensor with transposon mutagenesis, we have identified 368 genes/proteins that keeps ROS below toxic levels inside *Mycobacterium tuberculosis* (the organism causing TB) and, therefore, can potentially play a role in resisting anti-TB drugs. One of them, Rv0158, maintains low ROS levels by integrating carbon metabolism with redox balance in TB bacteria, making bacteria resistant to anti-TB drugs. Rv0158 could be targeted to improve the efficacy of current anti-TB drugs and to reverse drug resistance.

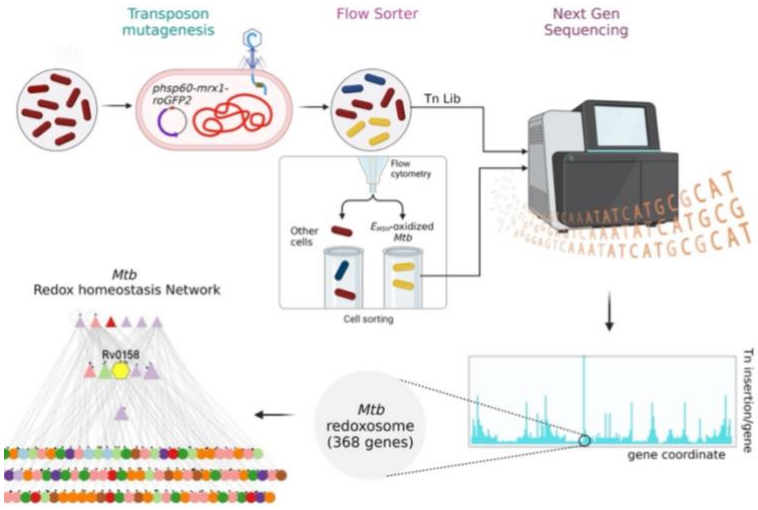

Figure 2: Integrating transposon mutagenesis, redox biosensor, and deep sequencing to capture redoxosome of Mtb.

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Novel strategies to investigate Human Immunodeficiency Virus (HIV) Latency.

HIV, the causative agent of AIDS, is responsible for 0.6 million deaths and 1.7 million new infections in 2019 (https://www.unaids.org/en/resources/fact-sheet). Despite advances in antiretroviral therapy (ART), the persistence of latent but replication-competent HIV is a major barrier to cure. The viral reservoirs are mainly present within different subsets of memory $CD4^+$ T cells and known to be localized in several lymphoid organs. Upon ART treatment, the plasma viral load is suppressed to below the detection limit of current clinical assays, but HIV persists in a latent state, which leads to viral rebound upon therapy interruption. Therefore, there is an urgent need to understand the mechanistic basis of HIV latency and then to use this knowledge for developing newer therapeutic strategies against persistent HIV infection.

Since HIV-1 has an unusual ability to lie dormant for decades despite highly active ART, we first aimed to see inside the HIV-1 infected cells during infection. This is likely to help optimization of therapy outcome. To do this, we developed a non-invasive biosensor that can measure what is going on inside the HIV-1 infected cells in real-time. The biosensor measures the levels of major cellular antioxidant, glutathione that acts as a shield to protect from harmful chemicals called as oxidants such as hydrogen peroxide. We discovered that as long as cells maintain robust levels of glutathione they successfully control HIV in the latent state. We further discovered that a modest increase in oxidants triggers HIV-1 reactivation. One of the strategies for HIV-1 eradication involves stimulation of HIV-1 reactivation in latent cells followed by immune clearance (shock-and-kill). Our work raises the possibility of adopting shock-and-kill strategy to induce virus reactivation by an oxidative stress inducing compound followed by killing with ART or by the immune system.

Artificial antioxidant Nanozyme to suppress HIV-1 reactivation

Our study with the non-invasive biosensor indicates that an increase in the levels of oxidant-hydrogen peroxide reactivates virus from latency and helps in subsequent replication and perpetuation of infection. On this basis, we thought that efficiently detoxify hydrogen peroxide might results in a long-term suppression of virus or locking of virus in the latent state- a strategy

termed as block-and-lock. We prepared a ultrathin nanosheet of vanadium pentoxide that mimics the activity of an enzyme (glutathione peroxide) involved in detoxifying hydrogen peroxide. These nanoparticles are termed as antioxidant nanozymes. When we treated cells derived from HIV patients harbouring virus with the antioxidant nanozyme plus ART combination, latency was induced faster and subsequent reactivation was suppressed when ART was stopped. Our data indicate that combining currently used ART with vanadium pentoxide nanozyme could be more effective in preventing HIV reactivation in humans.

Additionally, long-term use of ART causes side effects which involves generation of oxidative stress. Therefore, combining an antioxidant nanozyme with the current ART could help in reducing the side effects caused by the drugs. However, while the nanozymes were found to be harmless in cells tested in our laboratory, further extensive studies are needed to understand if they can have other side effects once they are introduced inside the human body.

Tackling HIV using Hydrogen sulfide gas

We found that a clinically used antioxidant N-acetyl cysteine (NAC) suppresses HIV reactivation from a latently infected cells. Later it was reported that NAC partly acts by releasing hydrogen sulfide (H_2S) gas, which also a potent antioxidant and in low amounts help improve the function of mitochondria and reduces inflammation. We discovered that reactivation of HIV-1 from latency is associated with depletion in the endogenous levels of H_2S. We further found that as long as cells maintains higher levels of H_2S, virus remains locked in the latent state. Interfering with a main cellular enzyme responsible for H_2S production results in breakdown of latency and reactivation of HIV. We observed a direct effect of H_2S on suppressing HIV reactivation and replication along with all its other beneficial effects, such as maintenance of mitochondrial health and dissipation of oxidative stress.

Lastly, exposing latent cells derived from the HIV infected individuals with a combination of a pharmacological donor of H_2S (GYY4137) and ART locked HIV in a latent state and prevented virus rebound when ART was stopped. Our study open the door to supplementing clinically used ART with chemical donors of H_2S to lock HIV in a state of deep latency, potentially improving the lives of millions infected with the virus. Since H_2S donors are already undergoing clinical trials for other diseases, they can quickly be repurposed for HIV treatment.

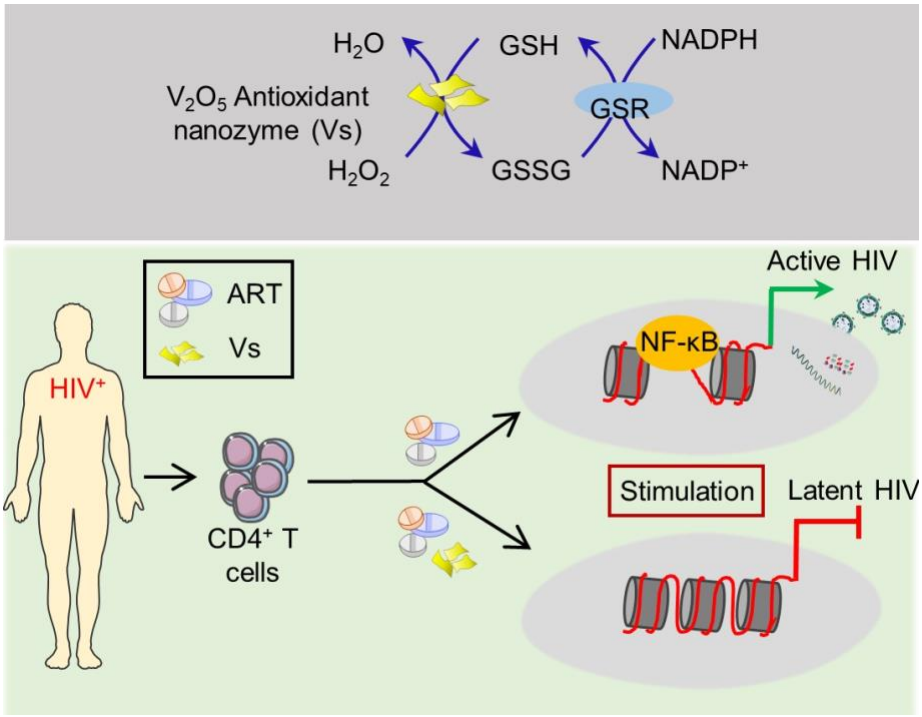

Figure 4: V_2O_5 antioxidant nanosheet (Vs) blocks HIV reactivation

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Patent

Composition and methods for managing viral infections/diseases (IP59400, Indian Patent filed-2023).

(Amit Singh- Signature)