b. In order of importance, list of ten best papers of the candidate, highlighting the important discoveries/contributions described in them briefly (not to exceed 3000 words)

1. Anand K, Tripathi A, Shukla K, Malhotra N, Jamithireddy AK, Jha RK, Chaudhury SN, Rajmani RS, Ramesh A, Nagaraja V, Gopal B, Nagaraju G, Seshayee ASN, **Singh A**. *Mycobacterium tuberculosis* SufR Responds to Nitric oxide via its 4Fe-4S cluster and Regulates Fe-S cluster Biogenesis for Persistence in Mice. ***Redox Biol***. 2021. 102062.

Transcription factor SufR (*Rv1460*) senses NO via its 4Fe–4S cluster and promotes persistence of *Mtb* by mobilizing the Fe–S cluster biogenesis system; *suf* operon (*Rv1460-Rv1466*). Analysis of anaerobically purified SufR by UV visible spectroscopy, circular dichroism, and iron sulfide estimation confirms the presence of a 4Fe–4S cluster. Atmospheric O_2 and H_2O_2 gradually degrade the 4Fe–4S cluster of SufR. Furthermore, electron paramagnetic resonance (EPR) analysis demonstrates that NO directly targets SufR 4Fe–4S cluster by forming a protein bound dinitrosyl-iron-dithiol complex. DNase I footprinting, gel shift, and *in vitro* transcription assays confirm that SufR directly regulates the expression of the *suf* operon in response to NO. Consistent with this, RNA sequencing of *MtbΔsufR* demonstrates deregulation of the *suf* operon under NO stress. Strikingly, NO inflicted irreversible damage upon Fe–S clusters to exhaust respiratory and redox buffering capacity of *MtbΔsufR*. Lastly, *MtbΔsufR* failed to recover from a NO-induced non-growing state and displayed persistence defect inside immune activated macrophages and murine lungs in a NO-dependent manner. Data suggest that SufR is a sensor of NO that supports persistence by reprogramming Fe–S cluster metabolism and bioenergetics.

2. Singh S, Ghosh S, Pal VK, Munshi MH, Shekhar P, Murthy DTN, Mugesh G and **Singh A.** Antioxidant nanozyme counteracts HIV-1 by modulating intracellular redox potential. ***EMBO Mol Med***. 2021. e13314.

Vanadium pentoxide (V_2O_5) nanosheets functionally mimic natural glutathione peroxidase activity to mitigate ROS associated with HIV-1 infection without adversely affecting cellular physiology. Using genetic reporters of glutathione redox potential and hydrogen peroxide, we showed that V_2O_5 nanosheets catalyze ROS neutralization in HIV-1 infected cells and uniformly block viral reactivation and replication. Mechanistically, V_2O_5 nanosheets suppressed HIV-1 by affecting the expression of pathways coordinating redox balance, virus transactivation (e.g., NF-κB), inflammation, and apoptosis. Importantly, a combination of V_2O_5 nanosheets with a pharmacological inhibitor of NF-κB (BAY11-7082) abrogated reactivation of HIV-1. Lastly, V_2O_5 nanosheets inhibit viral reactivation upon prostratin stimulation of latently infected $CD4^+$ T cells from HIV-infected patients receiving suppressive antiretroviral therapy. Our data successfully revealed the usefulness of V_2O_5 nanosheets against HIV and suggested nanozymes as future platforms to develop interventions against infectious diseases.

3. Tyagi P, Pal V, Agrawal R, Srinivasan S, Singh, S and **Singh A.** *Mycobacterium tuberculosis* reactivates HIV-1 via exosomes mediated resetting of cellular redox potential and bioenergetics. ***mBio.*** 2020. 11; e03293

Exosomes secreted by macrophages infected with *M. tuberculosis*, including drug resistant clinical strains, reactivated HIV-1 by inducing oxidative stress. Mechanistically, *M. tuberculosis* specific exosomes realigned mitochondrial and nonmitochondrial oxygen consumption rates (OCR) and modulated the expression of host genes mediating oxidative stress response, inflammation, and HIV-1 transactivation. Proteomics analyses revealed the enrichment of several host factors (e.g., HIF-1α, galectins, and Hsp90) known to promote HIV-1 reactivation in *M. tuberculosis* specific exosomes. Treatment with a known antioxidant N-acetyl cysteine (NAC) or with inhibitors of host factors galectins and Hsp90 attenuated HIV-1 reactivation by *M. tuberculosis* specific exosomes. Our findings uncover new paradigms for understanding the redox and bioenergetics bases of HIV *M. tuberculosis* coinfection, which will enable the design of effective therapeutic strategies.

4. Mishra R, Kohli S, Malhotra N, Bandhyopadhyay P, Mehta M, Munshi M, Adiga V, Ahuja VK, Shandil RK, Rajmani RS, Seshasayee ASN and **Singh A**. Targeting redox heterogeneity to counteract drug tolerance in replicating *Mycobacterium tuberculosis*. ***Sci Transl Med.*** 2019. 11 (518)

Phagosomal acidification alters the redox physiology of *Mtb* to generate a population of replicating bacteria that display drug tolerance during infection. RNA sequencing of this redox altered population revealed the involvement of iron

sulfur (Fe-S) cluster biogenesis, hydrogen sulfide (H_2S) gas, and drug efflux pumps in antibiotic tolerance. The fraction of the pH and redox dependent tolerant population increased when *Mtb* infected macrophages with actively replicating HIV-1, suggesting that redox heterogeneity could contribute to high rates of TB therapy failure during HIV-TB coinfection. Pharmacological inhibition of phagosomal acidification by the antimalarial drug chloroquine (CQ) eradicated drug tolerant *Mtb*, ameliorated lung pathology, and reduced post-chemotherapeutic relapse in *in vivo* models. The pharmacological profile of CQ (C_{max} and AUC_{last}) exhibited no major drug-drug interaction when coadministered with first line anti TB drugs in mice. Our data establish a link between phagosomal pH, redox metabolism, and drug tolerance in replicating *Mtb* and suggest repositioning of CQ to shorten TB therapy and achieve a relapse free cure.

5. Chawla M, Mishra S, Anand K, Parikh P, Mehta M, Vij M, Verma T, Singh P, Jakkala K, Verma HN, AjitKumar P, Ganguli M, Narain Seshasayee AS and **Singh A**. Redox dependent condensation of the mycobacterial nucleoid by WhiB4. ***Redox Biol***. 2018. 13(19): 116-133

Intracellular redox sensor, WhiB4, dynamically links genome condensation and oxidative stress response in *Mtb*. Disruption of WhiB4 affects the expression of genes involved in maintaining redox homeostasis, central metabolism, and respiration under oxidative stress. Notably, disulfide linked oligomerization of WhiB4 in response to oxidative stress activates the protein's ability to condense DNA. Further, overexpression of WhiB4 led to hypercondensation of nucleoids, redox imbalance and increased susceptibility to oxidative stress, whereas WhiB4 disruption reversed this effect. In accordance with the findings in vitro, ChIP-Seq data demonstrated non-specific binding of WhiB4 to GC rich regions of the *Mtb* genome. Lastly, data indicate that WhiB4 deletion affected the expression of ~ 30% of genes preferentially bound by the protein, suggesting both direct and indirect effects on gene expression. We propose that WhiB4 structurally couples *Mtb*'s response to oxidative stress with genome organization and transcription.

6. Mehta M, Agarwal N and **Singh A**. *Mycobacterium tuberculosis* WhiB3 maintains redox homeostasis and survival in response to reactive oxygen and nitrogen species. ***Free Radic Biol Med***. 2018. 27(131): 50-58.

***Mtb*Δ*whiB3* is acutely sensitive to oxidants and to nitrosative agents. Using a genetic biosensor of cytoplasmic redox state (Mrx1-roGFP2) of *Mtb*, we show that WhiB3 facilitates recovery from ROS (cumene hydroperoxide and hydrogen peroxide) and RNS (acidified nitrite and peroxynitrite). Also, *Mtb*Δ*whiB3* displayed reduced survival inside RAW 264.7 macrophages. Consistent with the role of WhiB3 in modulating host pathogen interaction, we discovered that WhiB3 coordinates the formation of early human granulomas during interaction of *Mtb* with human peripheral blood mononuclear cells (PBMCs). Altogether, our study provides empirical proof that WhiB3 is required to mitigate redox stress induced by ROS and RNS, which may be important to activate host/bacterial pathways required for the granuloma development and maintenance.**

7. Mishra S, Shukla P, Bhaskar A, Anand K, Baloni P, Jha RK, Mohan A, Rajmani RS, Nagaraja V, Chandra N and **Singh A**. Efficacy of β-lactam/β-lactamase inhibitor combination is linked to WhiB4 mediated changes in redox physiology of *Mycobacterium tuberculosis*. ***eLife***. 2017. 26(6): e25624

***Mycobacterium tuberculosis* (*Mtb*) expresses a broad spectrum b-lactamase (BlaC) that mediates resistance to one of the highly effective antibacterials, b-lactams. Nonetheless, b-lactams showed mycobactericidal activity in combination with b-lactamase inhibitor, clavulanate (Clav). However, the mechanistic aspects of how *Mtb* responds to b-lactams such as Amoxicillin in combination with Clav (referred as Augmentin [AG]) are not clear. Here, we identified cytoplasmic redox potential and intracellular redox sensor, WhiB4, as key determinants of mycobacterial resistance against AG. Using computer based, biochemical, redox biosensor, and genetic strategies, we uncovered a functional linkage between specific determinants of b-lactam resistance (e.g. b-lactamase) and redox potential in *Mtb*. We also describe the role of WhiB4 in coordinating the activity of b-lactamase in a redox dependent manner to tolerate AG. Disruption of WhiB4 enhances AG tolerance, whereas overexpression potentiates AG activity against drug resistant *Mtb*. Our findings suggest that AG can be exploited to diminish drug resistance in *Mtb* through redox based interventions.**

8. Mehta M, Rajmani RS and **Singh A**. *Mycobacterium tuberculosis* WhiB3 Responds to Vacuolar pH induced Changes in Mycothiol Redox Potential to Modulate Phagosomal Maturation and Virulence. ***J Biol Chem***. 2016. 291(6): 2888-903

The ability of *Mycobacterium tuberculosis* to resist intraphagosomal stresses, such as oxygen radicals and low pH, is critical for its persistence. Here, we show that a cytoplasmic redox sensor, WhiB3, and the major M. tuberculosis thiol, mycothiol (MSH), are required to resist acidic stress during infection. WhiB3 regulates the expression of genes involved

in lipid anabolism, secretion, and redox metabolism, in response to acidic pH. Furthermore, inactivation of the MSH pathway subverted the expression of whiB3 along with other pH specific genes in *M. tuberculosis*. Using a genetic biosensor of mycothiol redox potential (E_{MSH}), we demonstrated that a modest decrease in phagosomal pH is sufficient to generate redox heterogeneity in E_{MSH} of the *M. tuberculosis* population in a WhiB3 dependent manner. Data indicate that *M. tuberculosis* needs low pH as a signal to alter cytoplasmic E_{MSH}, which activates WhiB3 mediated gene expression and acid resistance. Importantly, WhiB3 regulates intraphagosomal pH by down regulating the expression of innate immune genes and blocking phagosomal maturation. We show that this block in phagosomal maturation is in part due to WhiB3 dependent production of polyketide lipids. Consistent with these observations, *Mtb*Δ*whiB3* displayed intramacrophage survival defect, which can be rescued by pharmacological inhibition of phagosomal acidification. Last, *Mtb*Δ*whiB3* displayed marked attenuation in the lungs of guinea pigs. Altogether, our study revealed an intimate link between vacuolar acidification, redox physiology, and virulence in *M. tuberculosis* and discovered WhiB3 as crucial mediator of phagosomal maturation arrest and acid resistance in *M. tuberculosis*.

9. Bhaskar A, Munshi M, Khan SZ, Fatima S, Arya R, Jameel S and **Singh A**. Measuring glutathione redox potential of HIV-1-infected macrophages. ***J Biol Chem***. 2015. 290(2): 1020-38

Redox signaling plays a crucial role in the pathogenesis of human immunodeficiency virus type-1 (HIV-1). Here, we exploited a roGFP based specific bioprobe of glutathione redox potential (E_{GSH}; Grx1-roGFP2) and measured subcellular changes in E_{GSH} during various phases of HIV-1 infection using U1 monocytic cells (latently infected U937 cells with HIV-1). We show that although U937 and U1 cells demonstrate significantly reduced cytosolic and mitochondrial E_{GSH} (approximately −310 mV), active viral replication induces substantial oxidative stress (E_{GSH} more than −240 mV). Furthermore, exposure to a physiologically relevant oxidant, hydrogen peroxide (H_2O_2), induces significant deviations in subcellular E_{GSH} between U937 and U1, which distinctly modulates susceptibility to apoptosis. Using Grx1-roGFP2, we demonstrate that a marginal increase of about −25 mV in E_{GSH} is sufficient to switch HIV-1 from latency to reactivation, raising the possibility of purging HIV-1 by redox modulators without triggering detrimental changes in cellular physiology. Importantly, we show that bioactive lipids synthesized by clinical drug resistant isolates of *Mycobacterium tuberculosis* reactivate HIV-1 through modulation of intracellular E_{GSH}. Finally, the expression analysis of U1 and patient peripheral blood mononuclear cells demonstrated a major recalibration of cellular redox homeostatic pathways during persistence and active replication of HIV.

10. Bhaskar A, Chawla M, Mehta M, Parikh P, Chandra P, Bhave D, Kumar D, Carroll KS and **Singh A**. Reengineering redox sensitive GFP to measure mycothiol redox potential of *Mycobacterium tuberculosis* during infection. ***PLoS Pathog***. 2014. 10(1): e1003902

***Mycobacterium tuberculosis* (*Mtb*) survives under oxidatively hostile environments encountered inside host phagocytes. To protect itself from oxidative stress, *Mtb* produces millimolar concentrations of mycothiol (MSH), which functions as a major cytoplasmic redox buffer. Here, we introduce a novel system for real time imaging of mycothiol redox potential (E_{MSH}) within *Mtb* cells during infection. We demonstrate that coupling of *Mtb* MSH dependent oxidoreductase (mycoredoxin-1; Mrx1) to redox sensitive GFP (roGFP2; Mrx1-roGFP2) allowed measurement of dynamic changes in intramycobacterial E_{MSH} with unprecedented sensitivity and specificity. Using Mrx1-roGFP2, we report the first quantitative measurements of E_{MSH} in diverse mycobacterial species, genetic mutants, and drug resistant patient isolates. These cellular studies reveal, for the first time, that the environment inside macrophages and subvacuolar compartments induces heterogeneity in E_{MSH} of the *Mtb* population. Further application of this new biosensor demonstrates that treatment of *Mtb* infected macrophage with anti-tuberculosis (TB) drugs induces oxidative shift in E_{MSH}, suggesting that the intramacrophage milieu and antibiotics cooperatively disrupt the MSH homeostasis to exert efficient *Mtb* killing. Lastly, we analyze the membrane integrity of *Mtb* cells with varied E_{MSH} during infection and show that subpopulation with higher E_{MSH} are susceptible to clinically relevant antibiotics, whereas lower E_{MSH} promotes antibiotic tolerance. Together, these data suggest the importance of MSH redox signaling in modulating mycobacterial survival following treatment with anti-TB drugs. We anticipate that Mrx1-roGFP2 will be a major contributor to our understanding of redox biology of *Mtb* and will lead to novel strategies to target redox metabolism for controlling *Mtb* persistence.**