REPORT

Report on participation of ICMR International Fellow (ICMR-IF) in Training/ Research abroad.

1. Name and designation of ICMR - IF : SARIPELLA SRIKRISHNA, PROFESSOR

2. Address : Department of Biochemistry, Institute of Science, BANARAS HINDU UNIVERSITY, VARANASI, Uttar Pradesh, INDIA - 221005

3. Frontline area of research in which training/research was carried out : Cryo Electorn Microscopy & Imaging of Cancer cells by Cryo-Electron Tomography Technique

4. Name & address of Professor and host institute : **Dr. Wah Chiu, Professor of Bioengineering**, Microbiology and Immunology, Photon Science, Stanford University Director, Division of CryoEM and Bioimaging, SSRL, SLAC National Accelerator Laboratory, Bio-X, School of Medicine, **STANFORD UNIVERSITY, CA, USA**.

5. Duration of fellowship : 6 Months (11th January to 10th July 2019)

6. Highlights of work conducted : Append

i) Technique/expertise acquired :

1. Received excellent training on advanced CryoEM Microscopy facility at SLAC (Stanford Linear Accelerator Center), Stanford University, CA, USA.

2. Attended two S^2C^2 workshops (Cryo-EM Training for beginners during January 22nd through 25th and Cryo-EM Imaging Process workshop during March 11th through 13th, 2019), sponsored by NIH, USA, on single particle structure salvation and imaging work flows.

3. Learned about sample preparation for CryoEM, grid preparation by rapid plung freeze method using Vitrobot technology for both single particle and whole cells (cancer cells)

4. Learnt about Relion work flow for single particle structure determination, 2D and 3D classifications and 3D reconstruction of protein molecules.

5. Tomography of isolated mitochondria from glioblastoma cancer cells and whole cell imaging of different organelles like mitochondria and Ribosomes in intact glioblastoma cells.

6. Identified novel aggregates of about 50 nm size inside the mitochondria of glioblastoma cancer cells. These aggregates are abundantly seen in cancer cells treated with translational inhibitor.

7. Reconstruction of tomograms was done and annotations of the tomograms are under process.

8. Sub tomogram averaging is done in some cases where large vesicles like structures are filled with ribosome like particles. Sub-tomogram averaging has more or less confirmed them to be ribosomes. Further validation is under process.

9. Negative staining done on isolated mitochondria and with some isolated septin proteins.

10. Culture of cancer cells on holy carbon 2/2 gold grids was done for whole cell tomograms.

ii) Research results, including any papers, prepared/submitted for publication : **One manuscript is under preparation**

Cryo Electron- tomography (Cryo-ET) is a recent and powerful technological advance by which the architecture of cells, viruses, Complex molecular machines, Proteins, Enzymes, DNA, RNA and other macro molecular complex structures under Cryogenic (-185^{0}C) temperatures at near-atomic resolutions in its native state; an advance previously impossible with traditional techniques such as X-ray crystallography. In this case, samples are preserved in its original state on holy carbon 2/2 gold grids using cryo-vitrification process, entire procedure right from sample preparation, processing and imaging is done at cryogenic temperatures, and sub cellular structures are determined by subtomogram averaging method.

CryoEM/ET is employed to study the post translational route for import of proteins into mitochondria through TIM -TOM super complex. There is a mounting evidence of data accumulating in support of localized synthesis of proteins on the surface of mitochondrial outer membrane by cytosolic ribosomes, but the co-localization of cytosolic ribosomes with TOM complex was not resolved till date. Our current results based on CryoET and biochemical assays reveal that cytosolic ribosomes interact specifically with the TOM complex and binding of nascent chain is critical for cytosolic ribosome recruitment and stabilization on mitochondrial membrane and promoting protein synthesis and transportation into mitochondria.

In this context we have tested the involvement of cytosolic ribosome specific translational events in mitochondria of glioblatoma cancer cells by using CryoET, Correlative Light and Electron Microscopy and other biochemical assays. In this study, the untreated (without translation inhibitor treatment) glioblastoma cells were considered as control group and drug treated cells were considered

as treatment group. The translation inhibitor treated cells interestingly showed very prominent and consistent phenotype of stress body like aggregates inside the mitochondria, close to the cristae and this feature was strikingly enhanced to several folds higher in presence of eukaryotic translation inhibitor (see figures 1 and 2).

Cryo-Electron Tomograms of intact cancer cells revealed the presence of large granular aggregates closely associated with cristae regions of Mitochondria. In control group of cancer cells these aggregates appeared at basal level with low percentage (about 20 to 25% showing aggregates and remaining 70 to 75 percent mitochondria are not showing such aggregates: n=28). On the other hand, interestingly the treatment group showed aggregates in 97% Mitochondria (n=72). The same phenomenon is observed when isolated mitochondria from glioblastoma cancer cells were subjected to translation inhibitor treatment. Both the *in situ* and *ex-vivo* studies showed same results. These Cryo tomography studies and biochemical data are strongly suggesting that the aggregation phenomenon is tightly coupled to the translation event. This typical aggregated feature of glioblastoma cancer cell mitochondria could be a potential drug/ therapeutic target or diagnostic target.

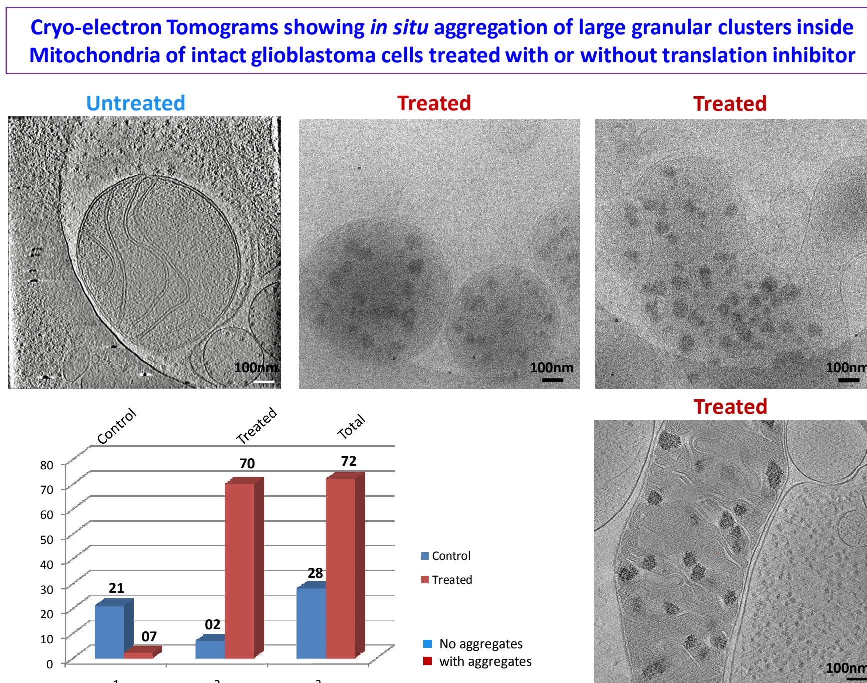

Figure. 1. Cryo Electron tomograms of glioblastoma cells showing control mitochondria without aggregates in untreated cells and very prominent large aggregates/ stress bodies inside the mitochondria of drug treated cancel cells. Histogram shows the statistical significance.

Tilt series sub-tomogram averaging of particles of giant vesicle in drug treated cells

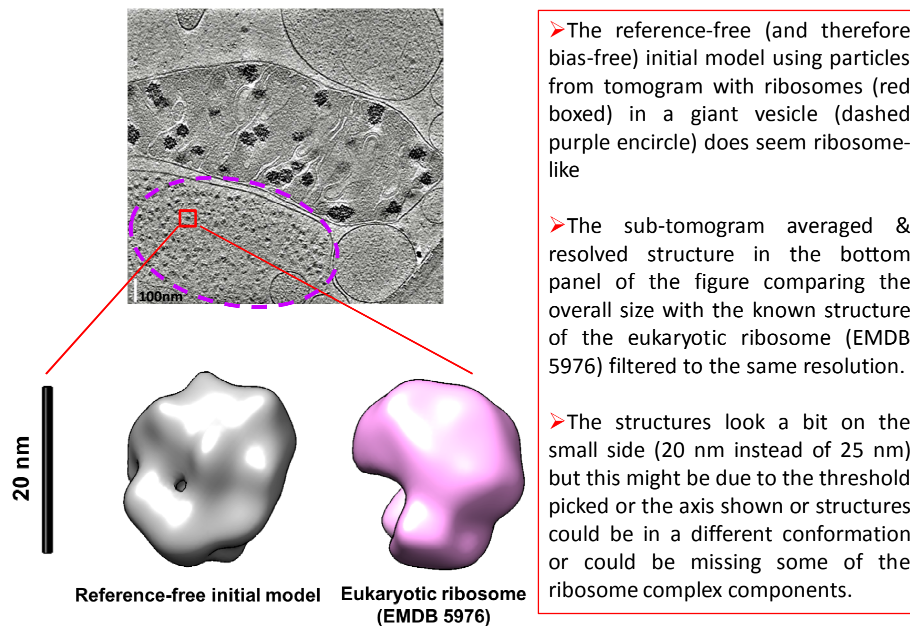

Figure. 2. Cryo Electron tomogram of glioblastoma cells showing sic mitochondria with electron dense aggregates and a large sac like vesicle filled with ribosome like particles. Lower panel showing sub-tomogram averaging of these particles by reference free initial model and confirmed them as ribosomes.

The intact whole cell Mitochondria data collected by Cryo Elecron Tomography is very authentic and it is very tough to acquire such fantastic tomograms from intact cancer cells. It will be interesting to see if this feature is common in rest of the cancer types and also other disease conditions. Further work is in progress at Stanford at the moment. Further, molecular and biochemical characterization and the mechanism behind the formation of these aggregates is in progress. Results will be compiled and published soon. Over all, the work done by me along with our collaborative team yielded an excellent piece of information which will be useful to target and treat cancer.

iii) Proposed utilization of the experience in India:

As ICMR International Fellow, I have received an excellent hands on training in handling 200 keV Talos Arctica and 300 keV Krios CryoEM (Cryo Electron Microscopy) systems at world's most advanced

CryoEM facility in SLAC (Stanford Linear Accelerator Laboratory), Stanford University, CA, USA. I worked extensively on Cryogenic Transmission Electron Microscopy Technology during my 6 months stay over here. During this period, I also attended two international CryoEM workshops supported by NIH, and had an opportunity to interact with eminent scientists in the field of CryoEM and crystallography. I learnt sample preparation, preparation of grids by plunge freeze vitrobot technology and CryoEM image processing (both single particle analysis and Tomography of organelles and whole cells) workflow etc. I have actively participated in the weekly group meetings, attended popular talks time to time and also presented my research work in group meetings. I have established a collaborative research team between our CryoEM group and Stanford Medicine department and did interesting piece of work. The Stanford medicine department and SLAC CryoEM team lead by my mentor Prof. Wah Chiu is very much interested to have a long term collaborative association with my ongoing cancer research work here at Banaras Hindu University, India.

The experience gained by me on CryoEM and CryoElectron Tomography techniques at Stanford University and the ongoing research collaboration with them would greatly help me to establish this advanced facility in India. At the moment this cutting edge tomography technology is not available in India though CryoEM facility for single particle analysis is recently established at NCBS and IISc, Bangalore. This powerful technology with unprecedented resolution of $<2A^0$ will be greatly help to resolve structures and addressing many challenging biomedical problems associated with variety of diseases endemic to India. We can effectively screen and solve structures of various viral/ bacterial and other diseases causing pathogenic strains specific to various diseases like Cancer, Encephalitis, TB, Malaria, Hepatitis, Chickungunya, Dengue, other predominant life style diseases and genetic disorders in India. Further, the outcome would lead us to devise diagnostic, prognostic and therapeutic interventions in future. Over all, the expertise gained during this tour not only gave me a deep level of experience in a wide range of techniques but will also help me in developing the necessary skills in biomedical research in India.

In this direction, I have already proposed to establish a CryoEM/ET facility here at Banaras Hindu University. The proposal is under serious consideration and soon it will be established to foster the need of researchers across India.

Signature of ICMR-IF