‘cryo-EM IN INDIA’ WEBINAR Q&A

Q. Thank you for setting Sci-ROI up. This is an excellent platform to connect with Indian and International scientists alike. I would like to contribute and be involved in future such sessions. Whom do I contact? (asked by Vamseedhar Rayaprolu)

A. Please send an email to research.ops.india@gmail.com with your ideas.

Q. To all panelist one most important thing that you would suggest to anyone planning to start a lab for cryoEM in India. (asked by Manoj Saxena)

A. Patience

A. Have a plan for funding the facility.

Q. In the current COVID and the post-COVID world, what kind of funding opportunities can we expect? Most of the governments are pouring money into Corona research. This is hampering other basic science research. Is that a trend that you see in India right now and expect to see in the future too? (asked by Vamseedhar Rayaprolu)

A. Yes, they are pouring money into COVID. A wise thing to do is to combine your basic research with infectious disease.

A. It appears that things are coming back to the way they were before COVID19 in terms of funding non-COVID research.

Q. Can the local engineers handle all the brands or is only Thermo fisher? (asked by Swetha Vijayakrishnan)

A. At the moment, ThermoFisher (ICON analytical is the agent) is better equipped with engineers. JEOL has only one 200 kV for LS (yet) and they are depended on engineers from Singapore.

A. Local engineers are typically alway tied to one of the comapnies. So, it’s hard to imagine how they could install machines from both companies.

A. I know most of the ICON analytical engineers from 2004. They are very good and helpful.

Q. Since I did my PhD in cryo-EM (ribosome) from Singapore, I have close ties with Singapore. What is the possibility that I screen my membrane protein grids in in-house 200kV (India), and sent it out to Krios in Singapore? How should I fund a student going to Singapore once a while? How frequent can he/she travel? -Tofayel (asked by Tofayel Ahmed)

A. Why would you want to go to Singapore when we will have 300 kV microscopes here in the India. There is also ESRF -Grenoble (DBT is part of this) and it is free including shipping of dewar. If you are comfortable with Singapore then you can write grants to funding agency to travel (but they might not fund as they will say there are facilities here in India).

A. Thank you Dr. Vinoth, I just wasn't sure how busy the only Krios in India is. ESRF-Gronoble is a new information for me. Thank you!

Q. Do you have any suggestions how we can speed up the bureaucratic process for equipment funding? (asked by Swetha Vijayakrishnan)

A. We have to work with the funding agencies. Typically, most of the funding agencies are very receptive and helpful.

A. Host Institutes also have a big role to play. The IITD facility was funded by a private trust through the Institute. Funding agencies like DST and DBT are very helpful.

Q. Is there a national center where you can apply for microscope time, something like a national lab beam line in USA. If so, how much is the back log for microscope time.. Thanks Manoj (asked by Manoj Saxena)

A. Both IISc and NCBS are National Facilities, applications are through web portal. The back log is 2-3 months (given the COVID constraints, it is bit longer).

Q. Are there plans to buy the new cost effective microscope Tundra Cryo-TEM from Thermofisher? (asked by Nandish Khanra)

A. Probably not immediately. Because, the stage (as I understand) has limited range and can do only single particle and not microED/tomography. Also, this is on the expensive side.

A. Not sure if this is really cost effective for the returns. At best, you can get to a 3.5A structure with a perfectly optimized sample and system. This is most often not the case. Maybe the panelists can add to this

Q. In the USA, we have access to XSEDE (and others) and we apply for computation time that is given to us for free. Is there a similar national computational resource available for data analysis? If not, is that something that the community would be interested in investing? (asked by Vamseedhar Rayaprolu)

A. Computational resources are usually institute based. IITD has a HPC facility which is available to us. Similar facilities are available at other institutes.

Q. What are the challanges for screening cryoET grids and subsequent data collection in Indian scenario ? (asked by Mintu Chandra)

A. Currently, it is challenging as not many places have screening microscopes and freezing of specimens. But in the next years this should get better as we will have more microscopes.

Q. Hi Vinoth, How much is the waiting time to collect a data? (asked by Deivanayagabarathy vinayagam)

A. If grids are not screened then it delays. I would put the wait between 2-3 months but with more EM (Delhi, Hyderabad) arriving, it might come down.

Q. In addition to a good instrumentation set up, undertaking cryo-EM or cryo-ET requires a robust GPU set up to quickly make sense of the data a K3 detector spews out. In quite a lot of places in the west, institutes do heavily invest in massive communal computational facilities to do that. My question is that in cities which have a substantial amount of PIs doing cryo-EM in India, are there any plans to set up these kind of communal computational infrastructure in these institutes..? (asked by Abhinav Koyamangalth Vadakkepat)

A. The BLiSc has a reasonable computational facility and it has worked well so far.

Q. Are researchers in India allowed and are able to get funding from sources outside India? How are these funding sources affected by government rules?(asked by Vamseedhar Rayaprolu)

A. Yes, EMBO, HFSP, Wellcome etc., can be applied. As far as I know, these are not affected by rules.

Q. Is there a website which compiles all resources for cryo-EM and opportunity in India?

A. This is an excellent point. We should do something like this.

A. thanks Arun.. A central hub would go long way in helping those in India and those wishing to go back to in India.

Q. What is the feasibility of working on Single particle analysis of small proteins (~100 kDa) of both Soluble and membrane proteins in India at the moment and what would be the future aspect in this direction? (asked by Vivekanand Malviya)

A. It is challenging but doable. For example, we need a lot of sample optimization for GPCR samples.

Q. Vinoth, are you allowed to say what toys you are looking to get in the grant you mentioned? (asked by Shwetha Vijayakrishnan)

A. Yes, HPF, CryoLM, FIB, Ultra microtome, volumescope to name a few.

Q. What kind of (a) computational resources and (b) data storage infrastructures available for researchers at NCBS, IISc and other national labs in India? In short, how is everyone dealing with cryo-EM data processing and data storage in India? Thanks (asked by Saikat Chowdhury)

A. In the grant we did ask for both storage and processing. The DBT mandate is to keep data for 10 years, so storage is the major issue. Processing (with cryosparc and Relion) becoming faster reasonable GPU workstations or nodes work well. But outside of NCBS, they have not dealt with big data and it is still at educational stage.

Q. Could an NIH style of cryo-em center be setup in India? (NIH has setup 3 centers in the US at Oregon, NY, and Stanford). The center would provide "free" service for researchers. That model has worked well here. That way, I think, the headache of obtaining the scopes, building shock proof buildings etc could be centralized. (asked by Narasimha Kumar)

A. The facilities in NCBS is like that, which is currently free but if funding is not extended then we will have to charge the users.

Q. How much of method optimization is possible given the sparsity of the microscopes? (asked by Vamseedhar Rayaprolu)

A. Several institutions have microscopes that can be used for smaple screening, for example, by negative staining.

Q. Vinoth, is it possible to get trained on cryo-EM during PhD and apply for the post of application scientist just after PhD i.e without a post-doc?

A. Yes

A. Yes

A. Sure!

Q. How about getting funding for maintenance and consumables for cryo EM.. things like grids are not easily available..why not have a central facility in India which buy these stuffs in large amount and help users who doesn't have them ?

A. There is a delay in getting grids etc. but you get everything. But, a centralized resource would be great.

Q. How does one recuperate costing for service contracts especially for equipment like screening microscopes that are not a national facility? Is it factored into the initial grant?(asked by Shwetha Vijayakrishnan)

A. The first 3-5 years of service contract can be built in the purchase price. After that, usually the institute pitches in.

Q. To all panelist how much flexibility is there in the number of conditions (age/ reservations) that are laid out in the applications for new positions- given the scarcity of the experts in such areas like cryoEM. (asked by Manoj Saxena)

A. I am not sure in other institutes, NCBS has no age restrictions.

Q. Hello everyone, would like to know how do you see the cryo-EM and other related regimes in the next 5-10 years in India? Regards, Anahita (asked by Anahita Vispi Bharda)

A. Very promising, we should have critical mass build up in various places.

A. Agreed, the outlook is very promising.

Q. Arun, you mentioned critical mass for use of equipment could be a problem. If you do find partner institutions that are not very nearby, how do you decide where the equipment will be housed? (asked by Shwetha Vijayakrishnan)

A. It all depends on the team - mutual discussion and seeing which partner can provide the space, and take the responsibility for the overhead.

Q. That problem of data is not unique to India, but would be good to know how much data you are handling easily. some numbers would be great @ Vinoth (asked by Manoj Saxena)

A. For external users, we just copy the data and give. for internal users, we have dedicated space (disks) and choice of CPU/GPU to process (dedicated queues for these). Since we have only K2 and FIII, the data collection is not as fast as K3 or beam-shift (strategy rather than stage). Rough estimate (depending on data collection set up - no. of frames stored) is 1-1.5 TB/day, which in many cases is sufficient to get very good structure.

Q. Are the institutes hosting the microscopes (say NCBS, IISc, IITD or CCMB) interested in investing/ hiring facility managers or scientists to run the microscopes and train their post docs or PhDs in data collection and processing? If so, is it possible for these facility managers to run independent projects of their own? (asked by Abhijith Radhakrishnan)

A. This is something that has to happen. In fact, some institutions have already advertised to hire facility manager.

Q. Hi, I do cryo-electron tomography and I think I would need all the 'toys' (as Dr. Vinoth put it) to image in vivo and be efficient about it. For people like me who are on the fence about moving to India right away but strongly thinking about it in the near future, are there ways to collaborate with scientists currently in India to apply for funding from DBT and get the ball rolling. (asked by Shrawan Kumar Mageswaran)

A. Yes, once you chose where to go (North/South/East/West), there are now number of institutes that will be very receptive for writing grants together. In my case, this is what happened, was part of grant before I joined.

Q. I am a post-doctoral research associate with two years of experience and planning to go back to India in another one year. I have gained experiences in Hydrogen deuterium exchange mass spectrometry, Native mass spectrometry, Fast photochemical oxidation of proteins and other biophysical techniques. We use these techniques in combination with Xray crystallography or CryoEM to add confidence to the findings.
Can you comment on my options in India, preferably academic positions in Delhi?
Thank you, (asked by Ravi Kant)

A. Hi Ravi, Institutes in and around Delhi like NII, RCB, ICGEB, IITD, JNU etc do have mass spec, X-ray crystallograpgy and cryoEM facilities. All setups may not be available at one Institute, but it is quite possible to use the central research facilities at neighbouring institutes.

A. Thank you Dr. Banerjee, which are the departments in IITD, where i can apply for a assistant professor positions? Can i send you an email after the session for more details?

A. Sure please do. IITD has a rolling advertisement mode, you may apply at any time. There are three bio-related departments - Kusuma School, DBEB and CBME. We can discuss more via email.

Q. How is CryoEM community or structural biology thinking about the Alpha Fold which is showing promising future and especially Google has ability can spread it easily in India because it is attractive as it doesnot need any expensive hardware?
Another follow up question is, is there any plans for developing new machine learning algorthims in India? (asked by Venkata Dandey)

A. Yes, there are plans to develop (or being developed) AI. AlphaFold is great but still it can't do complexes or predict dynamics (yet). So, CryoEM is valuable in this sense.

A. I feel like Alphafold still has a long way to go. There are many things Alphafold cannot solve right now. It also depends on MSA and isn't necessarily denovo. I cannot predict ligand interactions as far as I know and that I think is a key factor for correct structure and, more

importantly, function determination. Alphafold is definitely a great step forward but I think it still needs a lot of work.

Q. Is screening microscope available in Mumbai-Pune region? if not is there any plan to buy one?(asked by Nikhil Bharambe)

A. Two proposal were submitted from Pune/Mumbai in the recent SERB call.

Q. Any specific opportunities in Hyderabad? Which microscope is CCMB being upgraded to?(asked by Vamseedhar Rayaprolu)

A. TIFR-H and Uni-H are opportunities. CCMB is getting a Talos-Arctica.

Q. Other than 4 microscopes mentioned in India, is any more microscopes are coming in next 2-3 years, other CSIR or IIT or DBT labs?(asked by Nagesh Peddada)

A. Hopefully. There are plans to have more.

Q. Q for Dr. vinothkumar. Is NCBS or any vendors in India planning to make or fabricate cryo holey grids in India? (asked by Saikat Chowdhury)

A. We are trying to.

Q. Do the users pay for the use of the national facility and any consumable?(asked by Manoj Saxena)

A. At the moment, the facilty in BLiSc is free but in the future we may need to charge. We do charge for consumables, mainly to make people realise the costs involved.

Q. How is the recent private universities are panning out to be in field of cryo-EM or structural biology? Recently, there has been quite a buzz about private universities getting involved in biological research. (asked by Dilip Kumar)

A. There are places which are investing significantly. For example, Ashoka University.

A. IITD and Ashoka campuses are very close by, so that is an advantage. It will be good to have such private-public university cooperation in other places in India

Q. Vinoth, can you give more info about the freezing system that is built in your lab? Is there a new thing compared to existing systems? (asked by Venkata Dandey)

A. just basic plunger at the moment. Your spottion is something we should make here (at lower cost). The chameleon (SPT laptech) is very expensive - ~ 3 crores.

A. building inexpensive plunger inhouse is great!! Thanks for taking the initiative. More and more cheap microscopes are getting built, there is a high need of cheaper plunging machines

which can help prescreen the grids before checking in the scope. ofcourse, I as well hate to buy a 3 crore machine. Even we cannot buy it at NIH, at least there is no strong reason to spend. I totally agree that cryoem need a machine with price tag of atleast < 5 lacs.
A.

Q. On International Collaborations, I would indicate that in January this year we approached several Institutes as Instuct-ERIC (Instruct is the European Res. Inf. on Structural Biology) to explore collaboratios. Since then "the world" has somehow slowed down, but we will catch up in the future! (Jose-Maria Carazo, Instruct-ES)

A. Hi Jose-Maria, thank you for this information. Is this only possible at the institute level? or are there grants that individuals could apply for and use that at a Indian host institution?

Q. '@Arun How is the cryoEM scene in around UP region. Are there any new facilities coming up with Krios 3 and with screeing facilities. (asked by Manoj Saxena)

A. We are trying to have on at IITK together with other institutions in the region. Stay tuned.

Q. For SPA, what is a typical timeframe in India to go from purified protein to a final structure? (asked by Vamseedhar Rayaprolu)

A. The fastest we have done from gene to structure is 9 days, including the wait time to confirm the sequence.

A. That's impressive given the bottle necks. But, not to be cynical, is this influenced by the fact that you have access to a Krios almost immediately? What may be the typical timeframe for other places?

Q. Is there any possibility of reinstalling scopes from international institutions that are upgrading their own facilities? These might not be Krios 300 KV, but perhaps JEOL 300KV or similar might be available for transfer. (asked by Swati Manjari)

A. Extremely difficult. The paper work to clear and the hesitancy by vendors to transport is a bottleneck.

Q. Are you allowed to do public engagement with schools and universities to get students into structural biology especially EM as an exciting field? (asked by Shwetha Vijayakrishnan)

A. Absolutely. I have gone around to talk about these things, even in Hindi.

A. Yes, we do very often. This told me that structural/cell biology is not taught well.

A. Absolutely. I have been teaching at colleges and universities, and the response has been great!

Q. Are there any reliable method (couriers or shippers) of shipping vitrified grids within India to the national cryo-EM centers and also abroad to ESRF?(asked by Saikat Chowdhury)

A. Expensive but available.

A. ESRF pays for it. DHL and Fedex do it. I just sent one to eSRF (not my samples) and reached in 3 days in good condition.

A. Good to know. Is FedEx or DHL doing the same domestically? What is the situation for domestic shipment of vitrified grids?

A. Domestically, it is difficult as they are not used to. Bluedart (DHL) has done from Pune to BLR but students travel by bus overnight.

A. DHL for Grenoble. Usually, students visit National Facilities with dewars or samples.

Q. How many more 300kv microscopes are coming and whereabouts in India?(asked by Shwetha Vijayakrishnan)

A. The 2nd one in Delhi around the corner (Manidipa can confirm). Stay tuned for more.

A. A Titan Krios has been ordered for IITD. Should be installed by mid 2021.

Q. We still have to develop the collaboration further. We thought we could go faster, but it has been impossible. At this stage we need to have few ad clear interloquotors that could themselves reach the Indian community. (asked by Jose-Maria Carazo)

A. @Jose-Maria Happy to participate and help in anyway I can. Please connect.

Q. Here in the US, there is an unofficial location map for Krioses (https://tinyurl.com/y2sg4h2j). Is it possible to create something similar for India? (asked by Vamseedhar Rayaprolu)

A. Its a great idea, we should do that once we have enough Krioses…

A. Or just for any microscopes (Screening and high-res). It would be a good resource for prospective faculty and students alike.

Q. Is there a forum to contact the panelists in the future? (asked by Vamseedhar Rayaprolu)

A. I think it should be ok to contact directly.

Q. As a starting PI in India, is it harder to get postdocs than phd students in India? Is that more possible through external grants? Do you negotiate with the institute that recruits you for personnel?(asked by Shwetha Vijayakrishnan)

A. Getting postdocs is difficult is becuase of clear career path for them. Also, the place (city) is also important to attract them. Institutes do not generally give much in terms of personnel.

A. Some institutes have a postdoc program..IITD has one. Also, there are several national postdoc programs sponsored by DST, DBT etc

Q. Continuing the point raised by Vinoth about the Tomograph, at NIEHS, our group postdoc(Johnathan) developed super fast tomography data collection and he uses Serial EM. He and me are always open to help setup that system in any part of the world. I works very well especially on a 300 Kv Krios. Please let me know if any of your group are interested. We also have a fully automated grid screening protocols developed for SerialEM (asked by Venkata Dandey)

A. Will keep in touch to get the macros for Serial EM to serious EM

Q. We all said and heard we need more trained people and will be easier for securing more fundings for more equipments but where are most of the most institutes stands in supporting with adequate facilities for structural biology be it Cryo EM or X-ray Crystallography?(asked by Rahul Jaiswal)

A. Not very strong. We need to raise money to run these facilities either through funding agency or through user fees.