REPORT

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

1. Name and designation of ICMR-IF: **Dr. Saroj Kumar**
   Additional Professor

2. Address: Department of Biophysics
   All India Institute of Medical Sciences
   Ansari Nagar, New Delhi-110029

3. Frontline area of research in which: training/research was carried out: Neurodegenerative diseases, Biophysics

4. Name & address of Professor: and host institute: **Prof. Fredrik Nikolajeff**
   Professor and Head of Subject
   Biomedical Engineering
   Department of Health, Education & Technology
   Luleå University of Technology, Luleå, Sweden

5. Duration of Fellowship: 3 months (28-03-2023 to 26-06-2023)

6. Highlights of the work conducted:

i. Technique/Expertise acquired:

The fellowship offered me an exceptional opportunity to gain comprehensive exposure to the academic and research environment within Prof. Fredrik Nikolajeff's esteemed research laboratory and his dedicated research group. This experience provided valuable insights into the operational aspects of various research methodologies, while also serving as a platform for scientific meetings, discussions, laboratory visits, and participation in scientific conference. First and foremost, during my time in Prof. Nikolajeff's lab, I had the privilege of observing and learning about FTIR spectroscopy using state-of-the-art instrumentation (Vertex 70 FTIR Spectrometer). Moreover, I was able to share my expertise on FTIR spectroscopy with the lab members, which will significantly contribute to my ongoing research endeavors as I possess a dedicated FTIR setup at my parent institute. This exposure enabled me to conduct FTIR and LC-MS/MS analyses on total lipids extracted from salivary exosomes. The outcomes of this work will prove instrumental in my ICMR and DHR extramural research projects (ICMR Ad-hoc project: 2020-1194, DHR GIA: GIA/2020/000595). Additionally, Prof. Nikolajeff's collaborative research group, in association with the Umeå Centre for Electron Microscopy in Sweden, provided access to advanced instrumentation facilities such as the High-Resolution Cryo-TEM (Titan Krios 300 kV). Being exposed to this cutting-edge facility allowed me to perform cryo-electron microscopy experiments on salivary exosomes, facilitating an invaluable opportunity to observe and acquire knowledge about this state-of-the-art methodology. Furthermore, this

fellowship provided me with the chance to participate in the prestigious annual meeting of the International Society for Extracellular Vesicles held in Seattle, USA, where I had the privilege of presenting and sharing my research work in collaboration with Prof. Nikolajeff. Moreover, I established a collaboration with Dr. Shailesh Chouhan, a researcher from the Department of Systems and Space Engineering at Luleå University of Technology in Sweden. Together, we are working on developing an Artificial Intelligence (AI) tool using machine learning techniques to predict and screen early cases of Alzheimer's and Parkinson's disease based on our research findings (ICMR Ad-hoc project: 2020-1194, DHR GIA: GIA/2020/000595). This collaboration further enriched my fellowship experience and broadened the scope of my research contributions. Overall, the fellowship provided me with a platform to employ an integrated approach, leveraging the expertise and advanced techniques available in these esteemed facilities. The technical proficiency gained throughout this fellowship will be invaluable as I return to India, where I aim to expand and explore exosomal research, ultimately enhancing diagnostic and therapeutic approaches for neurodegenerative diseases.

ii. Research results, including any papers, prepared/submitted for publication:

Exosome Isolation: We used our developed methodology for exosome isolation from biological samples (ICMR Patent Application Number: 202211022537, April 16, 2022) to isolated exosomes from saliva samples of healthy controls and Parkinson's disease patients (ICMR Ad-hoc project: 2020-1194, DHR GIA: GIA/2020/000595).

Cryo-Electron Microscopy: Cryo-EM was used for direct visualization of salivary exosomes. To prepare samples for cryo-EM study lacey carbon EM grids were used. 3 µl of the aqueous solution of the sample was used and plunge-frozen using Vitrobot. The samples were studied in a cryo-electron microscope Titan Krios (300 kV) at Umeå Centre for Electron Microscopy, Sweden. The morphological properties of salivary exosomes in PD patients were observed under cryo-EM. I was able to observe exosomes with lipid bilayers and internal vesicle structures with size ranges of 100-200nm (Fig. 1).

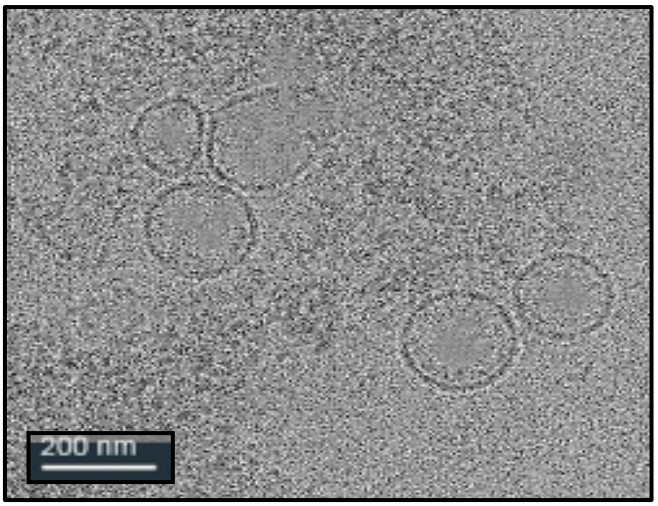

Figure 1: Representative cryo electron micrograph of salivary exosomes.

Lipidomics of Exosomes using FTIR spectroscopy: The chloroform-methanol method was used to isolate pure lipids from saliva samples of control and PD patient. Primarily, FTIR spectroscopy was performed on salivary exosomes to observe the changes in lipids' composition. We primarily focused on spectral region of 1100-3900cm-1 which represent structural changes in lipids (Fig. 2). In this region, I observed differences in FTIR spectra of total lipid isolated from salivary exosomes in control and PD patients, specifically at wave numbers 2956, 1377, 1300, 1090 cm^{-1}. These changes represent increased aldehydes and carboxylic acid in the lipid contents of exosomes in PD patients, therefore, disease-associated oxidative stress in PD is clearly indicated.

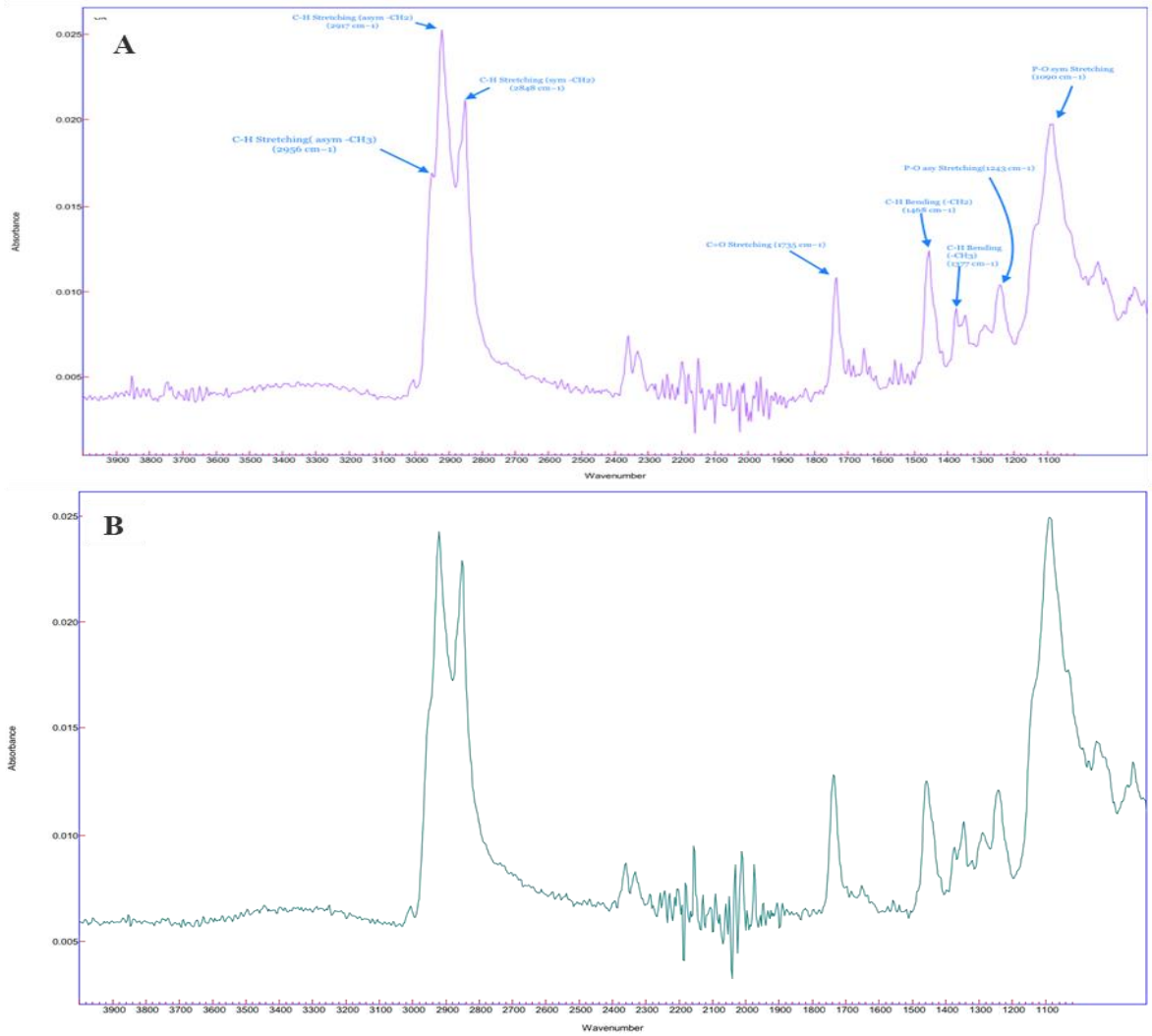

Figure 2: FTIR spectroscopy analysis of lipids isolated from salivary exosomes from control (A) and PD patient (B).

Lipidomics of Exosomes using LC-MS/MS: Additionally, mass spectrometry was performed on lipids isolated from salivary exosomes. The total lipid was isolated using chloroform-methanol method. A total of 16 lipids were identified after LC-MS/MS experiment; and 9 phosphoglycerols, 3 phosphatidylethanolamines, 2 phosphatidylinositols, 1 ceramide and 1 stearoylethanolamide were identified. The descriptive information is mentioned in Table 1 and Figure 3. Further analysis of this data is undergoing.

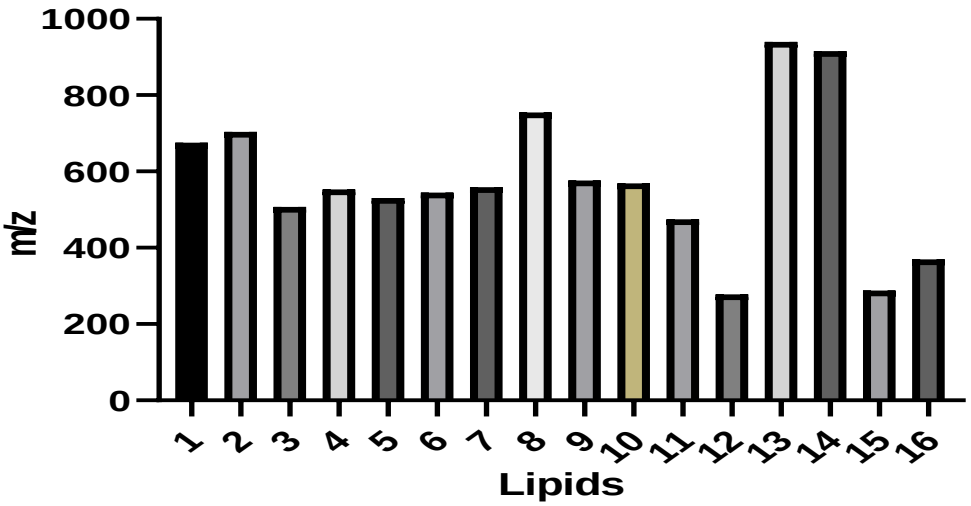

Figure 3: Lipid analysis of total lipids isolated from salivary exosomes (for details of the lipids at x-axis, please refer to table 1)

Table 1: Lipid analysis of total lipids isolated from salivary exosomes.

S. No.	Lipid	Formula	Annot. DeltaMass [ppm]	Calc. MW	m/z	RT [min]	MS2
1	Phosphoglycerol	C33 H65 O10 P	-4.33	652.429	675.418	18.619	DDA for preferred ion
2	Phosphoglycerol	C35 H69 O10 P	-3.21	680.461	703.45	19.265	DDA for preferred ion
3	Phosphoglycerol	C22 H45 O9 P	-2.27	484.279	507.268	16.581	DDA for preferred ion
4	Phosphoglycerol	C26 H43 O9 P	-1.48	530.264	553.253	16.717	DDA for preferred ion
5	Phosphoglycerol	C24 H49 O9 P	-1.95	512.31	530.344	16.58	DDA for other ion
6	Phosphoglycerol	C25 H51 O9 P	-1.46	526.326	544.36	16.581	DDA for other ion
7	Phosphoglycerol	C26 H53 O9 P	-2.47	540.341	558.375	16.58	DDA for other ion
8	Phosphoglycerol	C40 H81 O9 P	-3.63	736.559	754.593	16.663	DDA for other ion
9	Phosphoglycerol	C24 H49 O9 P	0.3	512.312	576.328	16.717	DDA for other ion
10	Phosphatidylethanolamine	C30 H50 N O7 P	0.35	567.333	568.34	14.202	DDA for preferred ion
11	Phosphatidylethanolamine	C23 H40 N O7 P	0.56	473.255	474.262	13.99	DDA for preferred ion
12	Phosphatidylethanolamine	C29 H48 N O7 P	5.24	553.32	277.667	16.943	DDA for other ion
13	Phosphatidylinositol	C49 H89 O13 P	-4.68	916.6	939.589	18.077	DDA for other ion
14	Phosphatidylinositol	C49 H87 O13 P	-3.41	914.585	915.593	18.075	DDA for preferred ion
15	Ceramide	C16 H33 N O3	-4.34	287.245	288.252	14.586	DDA for preferred ion
16	Stearoyl ethanolamide	C20 H41 N O2	1.51	327.314	369.349	15.965	DDA for preferred ion

Conference: Vaibhav Sharma, **Saroj Kumar**, Sanskriti Rai, Fredrik Nikolajeff. "Dissecting the Multiomics Atlas of Extracellular vesicles in Parkinson's Disease". International Society for Extracellular Vesicles – Annual Meeting 2023, Seattle, USA. https://doi.org/10.1002/jev2.12329

iii. Proposed utilization of the experience in India:

The fellowship served as a platform for fostering collaborations with Swedish colleagues, allowing me to gain valuable insights into the exosome's rich information content and its mechanistic relevance in the development of innovative early-stage diagnostics and treatment strategies for neurodegenerative diseases. The exposure to FTIR spectroscopy and LC-MS/MS during the fellowship will be highly beneficial for my ongoing research endeavors, particularly considering my possession of a dedicated FTIR setup in the parent institute. These techniques will contribute significantly to lipidomic analysis of exosomes, facilitating the development of early-stage screening methods for neurodegenerative illnesses. Furthermore, the fellowship presented an opportunity to observe and learn cutting-edge cryo-electron microscopy (cryo-EM) techniques for studying exosomes. This knowledge will be instrumental in conducting such experiments at my home institution, which boasts a dedicated electron microscopy facility. Additionally, the fellowship enabled a collaboration that focuses on developing an AI tool utilizing machine learning techniques to predict and screen early cases of Alzheimer's and Parkinson's disease based on our research findings. The outcomes of this collaboration will have a profound impact on the field of neurodegenerative disease research. Moreover, the results and expertise gained from the fellowship will play a pivotal role in my ICMR and DHR extramural research projects (ICMR Ad-hoc project: 2020-1194, DHR GIA: GIA/2020/000595). These collaborative efforts and acquired techniques will not only benefit my parent institute but also facilitate the implementation of advanced research methodologies with state-of-the-art equipment within the institute, empowering future generations of researchers.

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Signature of ICMR-IF

(Dr. Saroj Kumar)

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