



Dear Sir/Madam

It is my great pleasure to write this recommendation letter for Mr. Sundaram Acharya who works as a PhD student in my lab at CSIR-IGIB. I have interacted with him closely as his PhD supervisor for more than three years and observed in him keen scientific acumen and approach to problem solving. He has so far worked on a project that required independent design and implementation of research questions in a field with little prior experience in my lab and has made promising progress.

Sundaram is working on a project aimed at improving the efficiency and specificity of an orthogonal Cas9 protein. His work on protein engineering has led to the discovery of an improved Cas9 variant which is soon going towards clinical trials in India. This work is in preparation for communication. During the process, he has closely worked towards biochemical characterization of proteins and performed a wide range of studies pertinent to its future clinical applications. Due to the nature of the project, he has contributed to setting up and standardizing several new techniques which has added to our expertise as a lab. He has also collaborated and networked with other groups in the process. He has published in top scientific journals and has a patent to his credit. Below is the summary of the work carried out by him:

Title : Rational design of an enhanced genome editing protein for therapeutic gene correction and diagnostics

Summary:

RNA-guided, programmable CRISPR-Cas9 nucleases is a facile genome engineering platform heralding a new dawn in the field of therapeutic gene correction and disease detection. However, the off-targeting effect of the most widely used system of Cas9 from *Streptococcus pyogenes* (SpCas9) and the low editing efficacy of its engineered derivatives thwart its application in the clinical settings. Our group has developed a new genome editing technology by using an orthologous Cas9 system from *Francisella novicida* (FnCas9) which has negligible affinity towards off-target DNA substrate thereby giving specificity in DNA interrogation which we published in PNAS, 2019 where Sundaram is a joint first author. This property has been utilized for developing the CRISPR diagnostic platforms namely FnCas9 Editor Linked Uniform Detection Assay (FELUDA) and Rapid Variant Assay (RAY) which were also co-authored by him. Low cellular targeting efficiency of FnCas9 in the human genome is hindering its therapeutic applications. We have taken a structure-guided rational protein engineering approach to develop an improved protein variant which is better suited for targeting human genome in terms of activity and target range while keeping the intrinsic

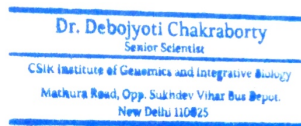
specificity of its DNA interrogation unaltered, thereby providing the essential robustness in the system.

Taken together, we developed a robust genome editing and diagnostics platform by engineered FnCas9 variants which is led by Sundaram.

In my opinion he belongs to the top 1% among his peers and he holds a very bright career ahead. The recognition of the prestigious Sun Pharma Science Foundation Science Scholar Award will surely motivate him to do better work in years to come. I am enthusiastically nominating him for this award and wish him all the best.

Thanks!

Best,



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