



Supervisor' review of dissertation thesis by Sameer Dixit

Sameer was pretty lucky right from the start, as he became a PhD student in frame of a very prestigious and competitive RNPnet project funded by the EU (first such funding in our lab), bringing together 15 European labs with interest in RNA-binding proteins, from one perspective or the other. This went with an unusual salary (and I won't say more) and some other goodies, such as covered traveling all over Europe to the RNPnet meetings, special trainings, interactions with interesting and/or wise people etc. The funding was, however, only for 2.5 years, the rest each lab had to cover from other sources.

With Hassan, who helped me a lot to write the proposal and also participated in some of the RNPnet meetings, we proposed to use this opportunity to establish at that time a cutting-edge methods termed CLIP, iCLIP and iCLAP (more on these in Sameer's talk). It would shift the field of RNA editing if people were able to show exactly which RNAs, pre-edited, never-edited, minimally-edited and/or pan-edited) are bound by which proteins. We also knew that those are really challenging technologies that have been, until then, shown to work in trypanosomes just once. In frame of the RNPnet consortium, however, Sameer got the opportunity to learn them in a dedicated RNA lab at Max Planck Institute in Dresden, and we could consult possible roadblocks with people who co-discovered these methods, such as Jernej Ule from London.

Sameer knew quite a lot already when he joined us, and he was also a fast learner in our and the collaborating labs. He single handedly established the iCLIP protocol, in our lab and started applying it to our favorite proteins involved in RNA editing in *T. brucei*. Moreover, due to my appointments and also the fact that I knew close to nothing about these methods, he had to rely on himself and our colleagues abroad. What turned out even more challenging than performing the protocol was a bioinformatics analysis of the tons of sequences he generated. Sameer went to a lab in Frankfurt am Main, which specializes in iCLIP read analysis and came back with confidence that he will manage to decode what the data says. It took him longer than we initially thought but eventually he succeeded. These efforts resulted in a story of two related RNA binding proteins MRB8170 and 4160 that we and collaborators from the above-mentioned lab submitted to NAR. We got extensive reviews and a chance to resubmit but then the resubmission was kicked out. We paid a high price for not excluding reviewers (do exclude them). In any case, it was eventually nicely published in mBio.

Sameer then focused on MRP1, a component of the MRP1/2 complex that our lab is interested in for at least 15 years, of course with major brakes, and the



function of which remains despite significant efforts pretty obscure. The obtained iCLIP results are nice and provide a straightforward story, which Sameer again submitted to NAR, we again got encouraging reviews, yet requiring so much additional work that, due to time limits, we decided to submit to RNA, and are waiting for what they'll say.

Sameer and Zhenqiu also wanted to find out if RNA is bound by a very interesting protein called Nudix hydrolase, and if so, which one. Unfortunately, despite major efforts, which showed that indeed some RNA molecules are bound by this protein, the libraries repeatedly failed and we had to scrap the project. Similar fate happened to an effort in which Paula and Sameer wanted to establish whether in *T. brucei* cytosolic aconitase is an RNA binding protein, as it apparently is in *Saccharomyces cerevisiae* and probably also in humans. Due to recurrent problems with protein tagging and obtained antibodies, they had to give up.

Fortunately, other projects look much more promising. The really cool one is the identification of totally unexpected (at least for us, Juan Alfonzo – as always – seems to be a visionary in this respect) RNA targets of ADAT2, which Sameer identified with Mary Anne Rubio from Ohio State University during her stay in our lab. He was not only a dedicated and attentive teacher of the method to Mary Anne, but also to Rachel Simpson, who came from the State University of New York in Buffalo to master the technique with him. I am very optimistic about a follow-up on this. One experiment Sameer committed to do before moving on for a post-doc is to look for which RNAs will cross-link with ADAT2 in the bloodstream stage. Interestingly, Mary Anne's iCLIP library data were sitting for a long time on a backburner, because people who analyzed them for her and Juan in Ohio did not find anything particularly interesting. It was only when Sameer had another look at the data, when he found the highly specific binding to the 3' region of a hexose transporter and two variant surface glycoproteins. Curiously, these proteins share a putative variability in their amino acid sequences.

Finally, Sameer did experiments, in which he showed that the protein REG9.1, functionally connected with the key transition of long slender bloodstream stages into the so-called stumpy forms, is also an RNA binding protein and it will be the task of Pragya Tripathi, a brand new PhD student who is now being supervised by Sameer, to move this project to a safe harbor.

When we were looking for a PhD student in frame of the RNPnet, we really wanted to find someone highly motivated, who would take full advantage



of such a unique opportunity. After the years spent together, I am happy to say that in Sameer we found such a guy. He is driven by interest, cares a lot about what he is doing, wants to have a career in science, has only a limited respect for dogmas and has the courage to go into uncharted territory. Importantly, he is also a nice person to have around, always optimistic and with a smile.

As all of us, he also has limits, and a personal task for him in the future is to work on them. I will mention those I have noticed, in no particular order. One is to be a collaborator, ready to help others or partake on tasks that have to be done for the general benefit of the lab. While Sameer is very friendly, outgoing and lately the one arranging parties after the departure of Tomáš Skalický (now in hands of one lady in this room), he was also, due mostly to his project, a stand-alone person, so working more as a member of a team will be a bit of a challenge for him. Putting things on paper is another one. Until recently, he thought that writing is after all not that important, that what matters most is the raw data, challenging the mainstream, discussing the results etc. Only now, and PhD thesis and the reviewers are particularly good for that, started he to realize that writing matters a lot, that one has to be meticulous not only with executing the experiment and presenting the data, but also in the formulations, clarity of writing, style, and such unimportant things like grammar and typos. But that's just to say that there is always something to improve, especially when one wants to strive in the challenging, but first of all very enjoyable environment science provides.

In all those years, Sameer became my friend, so my review can't be totally impartial. Yet after all, that's to different extent, the case with every PhD student. Anyway, I guess he will keep fond memories of South Bohemia and some of its inhabitants in his future – and I believe successful – scientific and private life.

Finally, I would like to state that the presented dissertation thesis fulfils, in my opinion, all postulations and I recommend it to be accepted as a partial fulfillment of the requirements for the degree of Doctor of Philosophy at the Faculty of Biology of the University of South Bohemia.

Thanks Sameer for your friendship and great work – so far, while I still expect more...

March 4, 2018 in České Budějovice



Julius Lukes