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PROJECT REPORT:
THE BRITISH SCHOLARSHIP TRUST

Project specifications	
Title	**Radical enzymes**
Field	**Computational chemistry**
Supervisor	**Christof Jäger**
Institution	**University of Nottingham**
Duration	**2 months**
Start date	**1st October 2018**
End date	**1st December 2018**
Budget	**1800 £**

In 2018, I was awarded with the British Scholarship Trust (BST) grant within which I have spent two months in the Faculty of Engineering at the University of Nottingham (UoN) as a visiting researcher where I closely collaborated with Dr. Christof Jäger (CJ). Soon after my arrival I was successfully enrolled in the Sustainable Process and Technologies (SPT) group and introduced into the novel computer-based methodologies used to study the radical stabilities in the enzyme framework. My research at the UoN was divided into three major parts and performed as follows:

(i) I mostly worked on a computational enzyme design project, more specifically on one of CJs research focus areas about understanding radical control and stability in radical *S*-adenosylmethionine (SAM) enzymes. In this project I performed extensive molecular dynamics (MD) simulations and quantum mechanical/molecular mechanical (QM/MM) hybrid calculations on a glycyl radical enzyme (GRE) pyruvate formate lyase (PFL) and its activating enzyme (PFL-AE);

(ii) I participated in a side-project by performing QM calculations of ammonium-orthoester cryptands with emphasis on their reactivity and intrinsic flexibility;

(iii) I was helping students in the group with several projects, i.e. a mechanistic study of hydrogenation of CO_2 by substituting iridium for zinc in the carbonic anhydrase using multiscale QM/MM approach.

In summary, as a result of this study visit I presented my current PhD research progress on the SPT group seminar which was held on a weekly basis. The valuable comments from colleagues and professors in the group have helped me to focus my present research and to obtain new ideas for extending the research and knowledge in the field of GREs.

Furthermore, the main project **(*i*)** yielded a preliminary draft of the manuscript with intriguing and useful findings about the influence of peptide substrate binding on the dynamics of PFL-AE and on the stability of radical species formed in its active site. The key findings of this research were that the stability of PFL-AE is highly impacted by the peptide substrate binding near SAM in the active site of an enzyme. In particular the binding of the substrate near the active site stabilizes the enzyme itself, which also confirms earlier experimental studies performed on PFL-AE. We also found that, when glycine residue from the peptide substrate is mutated to (*S*)-alanine, the complex between AE and the peptide becomes less stable, while if we mutate this central glycine to enantiomeric form of (*R*)-alanine, AE is more likely to bind this peptide and catalyze the alanyl radical formation. We have also established hypothesis on the dynamical aspects of the inactivation mechanisms of the metal cluster placed in PFL-AE interior which are initiated by the absence of the peptide substrate. The further idea is to connect with the experimental group of Prof. J. Broderick at the Montana State University in the US who has already showed interest in collaboration on this topic and has impressive knowledge on the system behavior.

The results obtained within the second **(*ii*)** part of the research were included in the recently accepted manuscript in a highly-ranked general-chemistry journal, namely *J. Am. Chem. Soc.*, followed by excellent reviewer response highlighting the work as highly important. This publication was of great importance for me, because I was also listed as one of the co-authors on that paper.

This BST award and the study visit at the UoN yielded interesting findings in the field of molecular modelling, while also being a great opportunity for me to establish connections for my future scientific path. I

was strongly encouraged, by the colleagues at the UoN, to continue my scientific career in academia; obtaining the BST and participating as a member of the research group in Nottingham was of great importance for my current postgraduate studies because I obtained better insights into the mechanisms of radical enzymes and the dynamical requisites for such processes. The study visit was also a great opportunity to directly interact with successful scientists in the field of theoretical and computational chemistry in the UK, especially Dr. CJ and Dr. Anna Croft, who both have expertise in similar topics closely related to my scientific interests. I also enjoyed being integrated into the SPT group with its closer focus on enzyme and metabolic engineering towards industrial applications. Both working at the UoN and the BST award enabled me to work on an interesting project for a reasonable period of time, which resulted in exciting observations and was of great value for both the Croatian and the UK groups. After obtaining my PhD I want to continue already established successful collaboration with Dr. CJ by applying for a post-doctoral position as a fellow researcher at the UoN. I already took a step towards that by applying for the Royal Society grant, namely Newton International Fellowships 2019 together with the co-applicant Dr. CJ at the UoN. This fellowship provides the opportunity for the best early stage post-doctoral researchers from all over the world to work at UK research institutions for a period of two years.

In brief, this BST grant was an amazing experience and a great chance for me to spend some time abroad and get to know people from the field. I would recommend applying for the BST award to anyone who wishes to extend their scientific network and knowledge on particular topics, and maybe to build new exciting friendships for life.

Marko Hanževački

Zagreb, 5/20/2019