Protein Science best papers for 2021

The winners of the 2021 *Protein Science* Best Paper awards are **Chelsea Vickers**, from the School of Biological Sciences, Victoria University of Wellington, New Zealand, and **Ryan M Woloschuk** and **Paul MM Reed**, both from the Department of Chemistry, University of Toronto, Canada.

Chelsea Vickers is a postdoctoral fellow with Wayne Patrick at Victoria University in Wellington, and she received her PhD from the University of Waikato, in New Zealand. Her paper in *Protein Science* concerns what she and her colleagues refer to as the "enzymatic dark matter" within the tree of life (Vickers CJ, Fraga D, Patrick WM. Quantifying the taxonomic bias in enzymology. *Protein Sci.* 2021 Apr;30(4):914-921. doi: 10.1002/pro.4041). Using bioinformatic tools, Vickers and her coauthors analyze the extent to which enzymes in different branches of life have been characterized. They use the BRaunschweig ENzyme DAtabase (BRENDA), a compendium of enzymatic data (specifically, Michaelis-Menten constants), to map the limits of biochemical exploration along the different branches of the tree of life, and they find that many branches are completely unexplored (the "enzymatic dark matter", Figure 1). They find that the enzymes that have been characterized biochemically are from a limited number of species and phyla, reflecting the historical development of biochemistry and the use of model organisms, as well as a focus on human biology.

The most important conclusion of this paper is that there is an enormous untapped opportunity to discover new enzymes in life, as the results of deep-sequencing efforts keep pouring in. As one of the reviewers of the paper puts it, "the kinetic data in the BRENDA database present enzymologists with a survey of which enzymes occur on earth, and what kinetic parameters are typical. However, as the authors argue, if the database is heavily biased, then any take-home messages we try to infer from this database will not be representative of what is possible for enzymatic activity in the biosphere". Another reviewer complimented the authors by saying that this "is a very interesting and thought provoking paper. I enjoyed reading it. It's an important point, we work on a few model enzymes and the (non-metabolomic) genomic databases are the tip of the iceberg, so what are we studying?".

Her mentor, Wayne Patrick has this to say about Chelsea Vickers: "It has been an absolute privilege hosting Chelsea in my research group. She has pushed me to think differently about my own research and she has been a superb role model for my postgraduate students. I am excited to see what future contributions she makes to protein science!" Patrick goes on to provide some context for the work by noting that "It was a fun and fascinating project during the Covid lockdowns here in New Zealand. The other author, Dean Fraga, is a Professor at a liberal arts college in Ohio (Wooster) who had a highly unusual sabbatical here — locked down in a suburban AirBnB for three months! We were *supposed* to be doing crystallography, but the three of us ended up drinking beer over Zoom and pondering enzymes across the tree of life. I'm excited you found it as interesting to read as we found it to conceive and write".

Ryan Woloschuk and **Paul Reed** are both graduate students, working under the mentorship of Prof. Andrew Woolley at the University of Toronto (see photographs). Their paper in *Protein Science* concerns the design of a photoswitchable binding protein (Woloschuk RM, Reed PMM, Jaikaran ASI, Demmans KZ, Youn J, Kanelis V, Uppalapati M., Woolley, GA. Structure-based design of a photoswitchable affibody scaffold. *Protein Sci* 2021 doi: 10.1002/pro.4196). The design is based on the Z domain, a small three-helix bundle that is a versatile scaffold for the design of variants that bind with high affinity and specificity to desired targets. To make the Z domain photoswitchable, Woloschuk, Reed, and their colleagues adapted a strategy first exploited by Stewart Loh and his group, which is to create a fusion protein in which the two proteins that are joined together exhibit positive or negative cooperativity in folding. If one of the proteins is easily converted between folded and unfolded states by the action of an external agent, then the folding, and therefore the function, of the second protein becomes responsive to that agent. Woloschuk, Reed, and colleagues describe the fusion of a Z domain to photoactive yellow protein, which is well folded in the dark, but adopts a molten globule state when exposed to blue light. In their designed fusion protein, the Z domain can fold into a stable structure only when the photoactive yellow protein is destabilized. As a result, exposure to blue light activates the binding capacity of the Z domain to an IgG molecule (Figure 2).

The paper by Wolloschuk, Reed, *et al*., provides a thorough biophysical and functional characterization of the designed photoswitchable binding protein. As one of the reviewers of the paper notes, "The function of the switches is thoroughly characterized through a range of approaches from yeast two-hybrid to protein NMR. In a field were many papers lack a clear design framework and the constructed switches are minimally characterized, this work stands out." Another reviewer states that "… the NMR structural experiments and yeast assays are compelling, and they support the proposed switching mechanism in convincing fashion."

Wolloschuk and Reed's mentor, Andrew Woolley, was enthusiastic about the collaborative effort that led to this paper, noting that "It was such a delight to work with Ryan and Max, discussing the conceptual, as well as the practical parts of this effort. Proposing and criticizing ideas together makes the process of science fun".

The following two papers were chosen as runners up:

1. Siegert A (Anna), Rankovic M, Favretto F, Ukmar-Godec T, Strohaker T, Becker S, Zweckstetter M.

Interplay between tau and α-synuclein liquid-liquid phase separation.

Protein Sci. 2021 Jul;30(7):1326-1336. doi: 10.1002/pro.4025. Epub 2021 Jan 28.

2. Gonzalez-Ordenes F (Felipe), Bravo-Moraga F (Felipe), Gonzalez E, Hernandez-Cabello L, Alzate-Morales J, Guixe V, Castro-Fernandez V.

Crystal structure and molecular dynamics simulations of a promiscuous ancestor reveal residues and an epistatic interaction involved in substrate binding and catalysis in the ATP-dependent vitamin kinase family members.

Protein Sci. 2021 Apr;30(4):842-854. doi: 10.1002/pro.4040. Epub 2021 Feb 17.

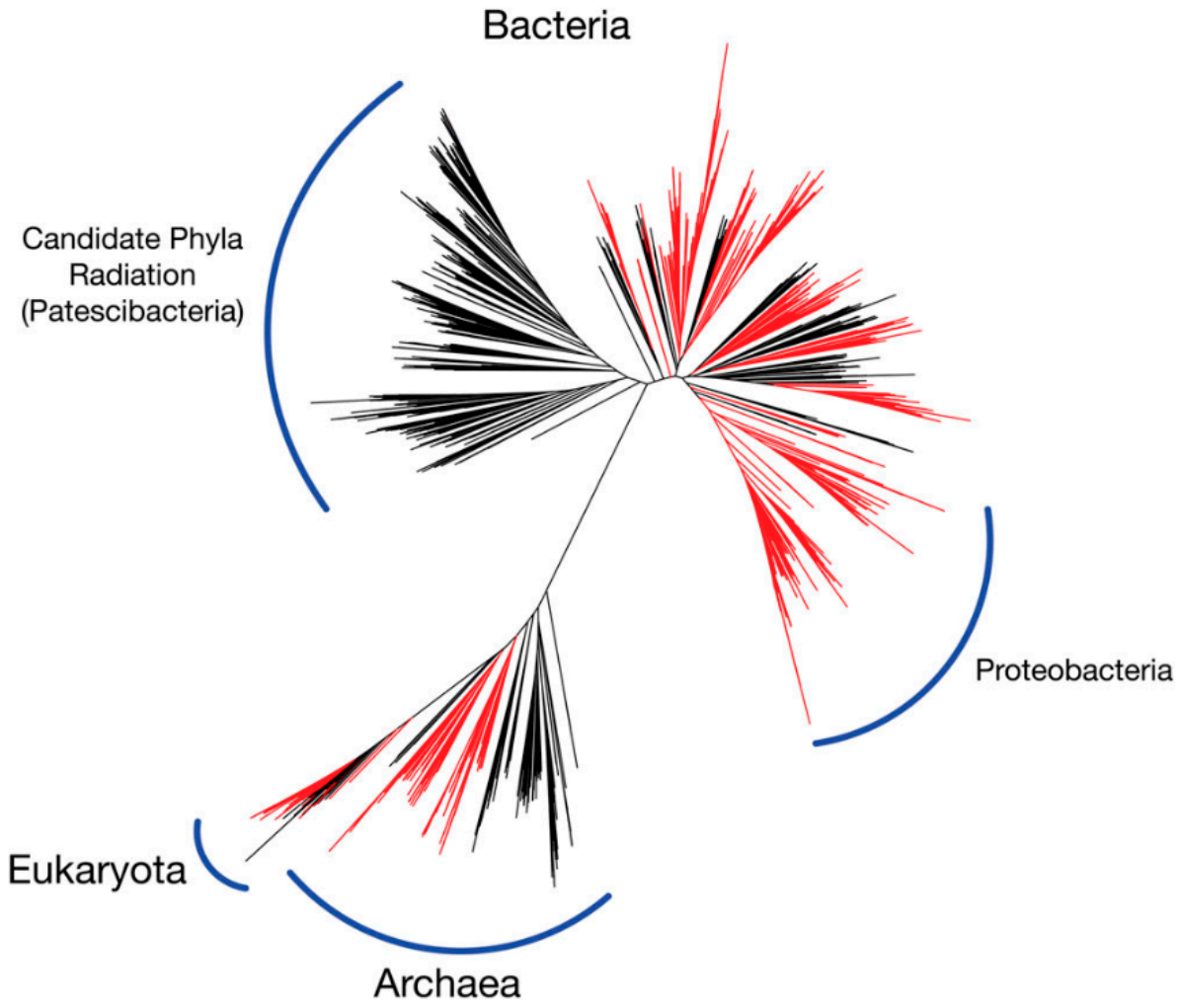

Figure 1. A phylogentic tree of life, with branches colored red when there is at least one entry in the BRENDA database for enzymes in organisms in that branch. From Figure 1 of Vickers CJ, Fraga D, Patrick WM. *Protein Sci.* 2021. doi: 10.1002/pro.4041.

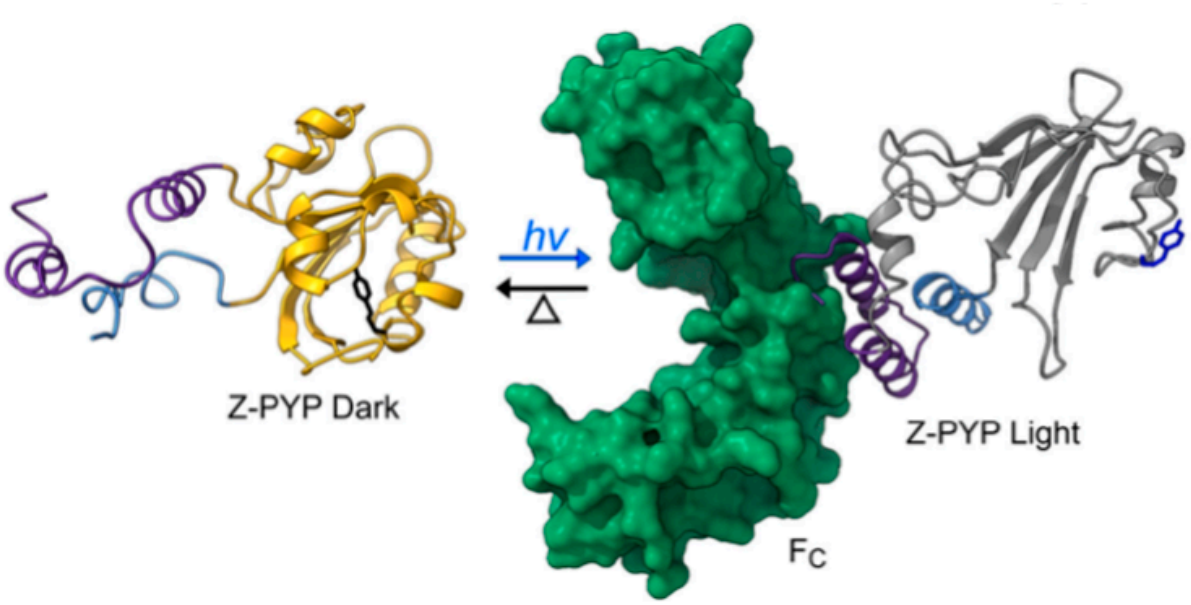

Figure 2 Mechanism of Z-PYP, a chimera in which photoactive yellow protein (PYP) is inserted into a loop of the Z-domain (blue and purple). In the dark, PYP is folded (yellow) and prevents folding of the Z-domain. Exposure to blue light results in the destabilization of PYP (gray), enabling folding of the Z-domain, which can then bind to the Fc portion of an IgG molecule (green). Adapted from Figure 1C of Woloschuk, Reed, *et al.* (*Protein Sci* 2021 doi: 10.1002/pro.4196).