Activity-based protein profiling - celebrating the groundbreaking contributions of Benjamin Cravatt

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Editorial

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Activity-Based Protein Profiling – Celebrating the Groundbreaking Contributions of Benjamin Cravatt

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We are excited to introduce the special issue of the Israel Journal of Chemistry dedicated to the awarding of the Wolf Prize in Chemistry 2022 to Benjamin Cravatt for his "contributions to understanding the chemistry of cellular communication and inventing chemical methodologies to study the role of carbohydrates, lipids, and proteins in such biological processes". To celebrate Ben's countless achievements, we have invited several of his previous trainees, collaborators and colleagues to contribute to this special issue. With a focus on "Activity-based protein profiling", this collection is designed to highlight the widespread impact of Ben's innovative chemoproteomic tools within chemistry, biology and pharmaceutical science.

Activity-based protein profiling (ABPP) is a chemoproteomic method for the global analysis of protein function and protein-ligand interactions within native biological systems that was pioneered by the groups of Ben Cravatt and Matt Bogyo around the turn of the millennium.[1–2] This special issue, which aims to highlight the many different developments in this research field, begins with a perspective by Ben himself, providing an insightful personal account of past achievements, current applications and future directions in the field of ABPP (B. F. Cravatt, https://doi.org/10.1002/ijch.202300029). The article by Howard Hang, Ben's colleague at Scripps Research, provides an additional perspective on Ben's achievements and the sustained and continuous impact of Ben's research within the chemical biology community (H. C. Hang, https://doi.org/10.1002/ijch.202200066).

The special issue continues with two broad review articles that give an overview of different facets of the field of activity-based protein profiling. In this context, Vinogradova and coworkers give a global overview of the tools, applications and translational potential of ABPP technologies with a focus on studying different states of proteins (K. A. Scott, T. L. Zhang, S. Y. Xi, B. Ngo, E. V. Vinogradova, https://doi.org/10.1002/ijch.202200101). Besides being able to study the activity of proteins globally, it is also highly desirable to elucidate effects based on different locations within the cell. Therefore, Moellering and coworkers focus on the discussion of the application of these technologies to illuminate the spatial regulation of protein activities within cells (C. S. Swenson, K. Smitha Pillai, A. J. Carlos, R. E. Moellering, https://doi.org/10.1002/ijch.202200104).

In the further sections, the special issue focusses on the many different applications that are enabled by ABPP. Early developments in the ABPP field were largely focused on the development and application of fluorophosphonate (FP)-based and related probes for characterizing the serine hydrolase enzyme family. This research area continues to be a very active and fruitful application field for ABPP. The review article by Adibekian and coworkers highlights recent developments in serine hydrolase activity probes with a specific focus on the reactive groups used and their applications in understanding serine hydrolase function (B. Racioppo, N. Qiu, A. Adibekian, https://doi.org/10.1002/ijch.202300016). Furthermore, the review by Birner-Gruenberger and coworkers gives detailed insights into the application of these probes to study lipid hydrolases (S. E. Honeder, T. Tomin, M. Schinagl, R. Pfleger, J. Hoehlschen, B. Darnhofer, M. Schittmayer, R. Birner-Gruenberger, https://doi.org/10.1002/ijch.202200078). In the review article by van der Stelt and coworkers, the use of ABPP to further our understanding of the endocannabinoid system and to develop chemical compounds that modulate this system is highlighted (N. Zhu, A. P. A. Janssen, M. van der Stelt, https://doi.org/10.1002/ijch.202200115). Demonstrating the diverse application for ABPP and serine hydrolase profiling, Pezacki and coworkers provide an overview on the use of FP probes for understanding microRNA function during viral infection (P. Evers, J. P. Pezacki, https://doi.org/10.1002/ijch.202200088). The reviews on serine hydrolase profiling

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with ABPP are nicely complemented by two research articles. The first by Verhelst and coworkers describes the synthesis of an FP-alkyne ABPP probe and its further elaboration for dual color serine hydrolase labeling (J. Yang, D. Korovesis, S. Ji, J. P. Kahler, R. Vanhoutte, S. H. L. Verhelst, https://doi.org/10.1002/ijch.202200094). The second by Bachovchin and coworkers describes the elucidation of the substrate scope of the serine proteases DPP8/9 directly in the lysate of human cells (A. Bhattacharjee, D. A. Bachovchin, https://doi.org/10.1002/ijch.202200117).

The demonstration of the utility of ABPP tools within the serine hydrolase family spurred the development of activity-based probes for a diverse array of protein families. The review by Breinbauer and coworkers describes the generation of ABPP tools to investigate oxidases and reductases with a special focus on the specific reactive groups that can be used to study these target families as exemplified by functional groups that are converted into electrophiles by oxidation (L. Krammer, R. Breinbauer, https://doi.org/10.1002/ijch.202200086). Furthermore, the review by Huang and coworkers focuses on tools to study glycan-binding and -processing proteins as well as glycosylated proteins (Z. Vilen, A. E. Reeves, M. L. Huang, https://doi.org/10.1002/ijch.202200097).

Traditional activity-based probes rely on chemical probes that bind to specific proteins within a defined protein family. More recent work within the field also utilizes reactivity-based profiling, where proteins are grouped not by functional class, but by the presence of highly nucleophilic amino acids – a concept also pioneered by Ben's group with the isoTOP-ABPP platform.[3–4] Reactivity-based profiling has been widely utilized to study cysteine-mediated protein activities and Wang and coworkers provide a detailed review of the use of quantitative chemoproteomics in this context (W. Xiao, Y. Chen, C. Wang, https://doi.org/10.1002/ijch.202200100). These reactivity-based profiling tools have lately been expanded to many other amino acids beyond cysteine (for a review see *e.g.*[5]). As an example, Hsu and coworkers in their review provide an overview of profiling methods for reactive tyrosines within the proteome (J. W. Brulet, A. M. Ciancone, K. Yuan, K.-L. Hsu, https://doi.org/10.1002/ijch.202300001).

One of the many important applications of ABPP is the ability to screen for inhibitors within a native proteome, thereby concurrently evaluating inhibitor potency and selectivity. These studies also routinely identify a multitude of new ligandable binding sites in proteins that are amenable to covalent ligands.[4] Several reviews in this special issue summarize and provide future outlooks on the role of ABPP in ligand discovery. Parker and coworkers provide insight into fragment-based ligand and target discovery using many of the tools of ABPP as well as affinity- and reactivity-based profiling (I. Forrest, C. G. Parker, https://doi.org/10.1002/ijch.202200098). Additionally, Zhang and coworkers provide specific insights into case studies of covalent ligand discovery including traditional target-focused methods and global profiling against a complex proteome (C. Ngo, A. Ekanayake, C. Zhang, https://doi.org/10.1002/ijch.202200105). Marto and coworkers explicitly focus on the power of competitive ABPP and give some exciting case studies of covalent ligand discovery that were facilitated by ABPP and chemoproteomics (H. Zhu, M. Sharafi, W. Pin Teh, A. S. Bratt, S. J. Buhrlage, J. A. Marto, https://doi.org/10.1002/ijch.202200113). While many of the traditional ABPP probes modify protein active sites and, thereby, inhibit protein function, in a related method, known as ligand-directed chemistry, covalent protein labeling is directed by ligands to generate traceless protein modification without inhibiting the protein's function. This concept is discussed in the review by Hamachi and coworkers with a specific focus on the chemistries used and the biological applications (S. Sakamoto, I. Hamachi, https://doi.org/10.1002/ijch.202200077). To round up this ligand discovery section, a research paper by Bogyo and coworkers describes the synthesis of a library of cyclic peptides bearing fluorosulfate-based covalent warheads and the exploration of its targets in complex human proteomes (F. F. Faucher, D. Abegg, P. Ipock, A. Adibekian, S. Lovell, M. Bogyo, https://doi.org/10.1002/ijch.202300020).

ABPP has also been broadly utilized to identify protein activities that are dysregulated in numerous disease states. One important application in this context is the use of ABPP to investigate protein activities in prokaryotic biology and during bacterial infections. The review article by Hatzios and coworkers focuses on the use of ABPP to study post-translational modifications and enzyme activities in the host during infection (R. Ramanathan, S. K. Hatzios, https://doi.org/10.1002/ijch.202200095). The review article by Chang and coworkers describes ABPP methods that allow studying protein functions in prokaryotic cells with a focus on bacterial physiology, pathogenesis and metabolism (K. P. Malarney, P. V. Chang, https://doi.org/10.1002/ijch.202200076). To round up this section, the review by Wright and coworkers discusses the application of ABPP to characterize bacteria in host-associated and environmental microbiomes (A. T. Wright, L. A. Hudson, W. L. Garcia, https://doi.org/10.1002/ijch.202200099).

Together, this collection of contributions serves to highlight the diverse applications of ABPP in discovering new biology and developing new pharmacological tools. Besides his academic excellence, the impact and translational nature of Ben's research is additionally highlighted by the many pharmaceutical and biotech companies that he has advised and founded. The broad reach of Ben's science is undeniable, but his impact on science reaches well beyond his own research achievements. Ben has been a tremendous mentor to younger scientists throughout his career. His immense impact on training and inspiring younger scientists is underscored by the myriads of alumni from his lab now holding leading roles in

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both academia and industry. Ben's boundless energy and contagious enthusiasm for science has always inspired us and will continue to inspire us into the future. We are excited to honor the highly deserving recognition of Ben and his achievements through the Wolf Prize in Chemistry 2022 and hope that this issue celebrating his award provides a snapshot of everything that he has accomplished as well as a glimpse of the promising future that is yet to come.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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