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We are not alone: The iMOP initiative and its roles in a Biology and Disease driven Human Proteome Project.

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Abstract

The Human Proteome is nearly fully mapped and will provide a knowledge base to accelerate our understanding of how proteins and protein networks can affect human health and disease. However, providing solutions to human health challenges will likely fail if insights are exclusively based on studies of human samples and human proteomes. In recent years, it has become evident that human health depends on an integrated understanding of the many species that make human life possible. These include the classic model organisms that enable studies of biological mechanisms as well as the food species, pathogens and commensal microorganisms which are essential to human life. The Human Proteome Organization (HUPO) initiative on multi-organism proteomes (iMOP) works to support proteome research undertaken on non-human species which remain widely under-studied compared to the progress in human proteome research. This perspective argues the need for further research on multiple species that impact human life. We also present an update on recent progress in model organisms, microbiota, and food species, and outline how iMOP activities could lead to a more inclusive approach for the human proteome project (HPP) to better support proteome research aimed at improving human health and furthering knowledge on human biology.

1. Introduction: goals and progress of iMOP

The human proteome project (HPP) was successful in achieving significant advances in coverage and detection of human proteins including the innumerable proteoforms generated from the restricted genetically encoded sequences by means of posttranslational modifications. This steady increase in knowledge is an essential aspect in our understanding of how biological mechanisms supports health and disease in the human organism. Achieving the first draft of a human proteome was made possible by organising the HPP into work domains which focused on the specific organs and cell types of the human body ¹. This approach not only increased the protein coverage of the complete human proteome, but also provided steady progress to explain fundamental knowledge of organ functions in health and disease ².

In recent years, it has become clear that only studying the human organism only presents a fraction of our understanding of human health and physiology ³. Since humans are in constant interaction with their environment, both inorganic and living, we can no longer neglect organisms and species for which human life, health and disease depends. A fact highlighted by the coevolution of humans with their surrounding living environments ^{4,5}. While such analyses should include obvious organisms such as pathogens, it also needs to encompass species that are responsible for our food such as crops and farm animals ⁶, as well as the countless symbiotic microorganisms associated with human organs ⁷. We now have evidence that symbiotic microbes are involved in operations as diverse as nutrient uptake, biosynthesis, immune response, cognition, tissue development, and cancer ⁸⁻¹¹.

From both practical and ethical points of view, many studies aimed at exploring human disease are unable to be performed on human subjects. This can range from the inability to collect samples from vital organs, due to experiments that require genetic modifications, issues related to sampling over entire life spans or even long term sampling. Sampling over extended periods is essential to describe mechanisms involved in aging or how specific nutrients affect onset and progression of specific diseases like cancer, autoimmunity, and food allergies. For such studies, model organisms have been and are still essential to achieve insight into the mechanisms of human health and disease ¹².

The iMOP initiative was launched in 2011 ¹³, with the aim to enforce focus on non-human proteome research, and to bring awareness of the need for integrated multi-organism research

within the clinically oriented human proteomics community ¹⁴. The need to establish iMOP was based on a recognition that progress in understanding human biology and health is hampered by a relatively slow progress in proteome research on the many non-human species on which all human life and health depends ¹⁵. These include most importantly food species (farm animals and plants), pathogens, commensal microorganisms, but also novel model species that have unique adaptations resulting in a constrained metabolism (e.g. hibernation, starvation, drought) or life under extreme conditions. Such variability enables us to extend basic biological principles to situations that would be impossible to replicate in human systems, ultimately informing us about the dynamics of biological systems ^{16,17}. Studies of clinical samples alone are unlikely to provide the medical and biological insight we seek for dramatic and fundamental improvements to human life. Today, much non-human proteomic research is presented throughout different HUPO initiatives, but within the iMOP initiative, there is the potential to drive progress in essential non-human proteomes to what has been accomplished with the HPP initiative ¹.

A major goal for the iMOP initiative is to harmonize technologies across the species-oriented communities. Since proteome methods will often require modification for each species studied, we aim to alleviate the efforts a new species must undergo to provide data that is compatible with human centric proteomics. Many of these approaches were developed through HUPO and include data representation standards ¹⁸, analysis of mass spectrometry data (CompMS) and public data repositories and exchanges ¹⁹ all of which would enable the direct integration of human and non-human proteomics data which is essential to enable an understanding of their interaction ^{13, 14, 20}.

Over the past 5 years, the iMOP initiative has organized workshops and parallel sessions within the framework of the annual HUPO congress ²¹, and contributed to shaping a biology and disease driven HPP pillar. We have connected research groups across multiple species-oriented proteome communities, including those with focus on classic and new model organisms; on microbial proteomes; and brought together research communities that have individually worked with specific agricultural species and within environmental biology ²²⁻²⁹. This cross-species connectivity has been a successful action since these research domains share many of the same daily challenges of developing technologies within relatively small and scattered research domains.

To progress with the goals of iMOP we need a closer involvement from the larger HUPO community to address the challenges shared across the multiple non-human proteome communities. The most important challenges are i) a severe lack of funding for fundamental research on non-human biology research domains; ii) lack of funds hampers progress of research and technology development (e.g. mass spectrometry and bioinformatics) for many species; iii) small research groups in non-human fields results in a limited number of scientists joining HUPO and engaging with the iMOP community. These problems have been further compounded since research areas within the iMOP frame are spread across several overlapping initiatives such as those focussed on microbes, food and nutrition ³⁰ or disease, which collectively support many non-human systems in their focus.

Five years after the establishment of iMOP, it is timely to discuss the need and the progress of the original iMOP objectives and examine whether the iMOP initiative would be better integrated into other HUPO subdivisions such as the Biology/Disease component of HPP (B/D-HPP) ³¹. This status paper presents views from those working in non-human proteomics and their efforts to unite biological disciplines across this field. We highlight some of the roadblocks that have contributed to a relatively slow progress of proteome research within fields of basic biology, and highlight the many ways these fields and proteome research are essential to progress research that supports human life and health. With these views, we also hope to invite the broader HUPO community into the discussion of how multi-species proteomes can improve the human proteome research field, to deliver solutions to societal challenging and to human life and health on a global scale, which indeed are the long-term goals of HPP.

2. Proteomes from biology – what is the value for human proteomics?

The value of model organisms for advancing biomedical research has a long history with such systems having many advantages ¹². Some obvious advantages are that they can be cultivated under defined conditions, can be genetically modified for directed functional studies and many have short life cycles. These and many other factors make them valuable tools to study biological situations in more detail than it would be possible with humans. This holds true for many biological and biochemical experiments and in general also for proteomic studies ³². Here we outline examples highlighting how research on model organisms can support the objectives of the B/D-HPP initiative.

An area where a direct interaction between humans and non-human proteomics often occurs are the fields of infection diseases and inflammation. While both the genetic and proteomic background of the host are important, it is equally important to consider this information for the microorganism. During infections, the proteomic repertoire of the infectant can vary from proteases that aid the manipulation of a hosts defense ³³ to mechanisms that enable it to subvert the hosts biology for its own purpose ³⁴. Thus, a detailed knowledge of the functional potential of a microorganism and virus is essential to understand human disease and the associated defense mechanisms to enable the development of therapeutic options.

In recent years, there has been a steady increase in knowledge around how human life and wellbeing are dependent on microorganisms ³. Complex microbial communities are found throughout the human body from our skin to our intestinal tracts ³⁵. The knowledge about the fine regulation of these populations, the regulation of their interaction with the human host by exchange of molecular information and, in case of diseases, the role both of host and microbial factors leading to the disturbance of the balance are key for precision medicine in the future. For example, the development of inflammatory bowel disease is not only attributable to human genetic factors but is also triggered by the composition and metabolic activity of the gut microbiome ³⁶. Understanding the complex mechanisms of the development of such diseases cannot be achieved by conducting research on humans, organs or cell cultures alone as it also requires tremendous efforts to also analyze the microbiota. Thus, other aspects that affect microbiomes, such as nutrition and lifestyle (e.g. smoking), also need to be considered.

An emerging field with a high relevancy for human health and disease is aging. Numerous diseases are related to aging, such as the neurodegenerative diseases Alzheimer's and Parkinson's. On the other hand, knowing the biological mechanisms of healthy aging (e.g. the influence of nutrition), has great potential to increase public health which would have tremendous social and economic impacts. However, longitudinal studies on single human individuals is not straightforward to perform due to their longer life cycles. Here, model organisms such as *Caenorhabditis elegans*³⁷ are a valuable tool as they possess very short life cycles and can thus be used for directed biochemical studies during aging as well as examining the influence of environmental and nutritional factors.

Overall, the study of proteomes (and metabolomes) of model organisms is a highly suitable means to decipher basic biological principles that are the result of direct impacts and interactions of other organisms with humans. Such approaches will be vital to better understand human health and disease. Such obvious outcomes clearly justify an initiative like iMOP within the context of the human proteome organization. Moreover, these arguments could be distributed among researchers working in non-human communities that currently do not attend HUPO conferences. For such interactions to occur, it is essential to develop common languages, starting from a common nomenclature for proteins to easily exchangeable database structures to better facilitate scientific communications.

3. Antimicrobial Resistance (AMR): an emerging research need

Antibiotics revolutionized human medicine but many drugs have a limited lifespan as over time microbes will develop a resistance. Currently this is happening at an unforeseen rate, with the World Health Organization claiming that a lack of efficient antibiotics is a major threat to human health ³⁸. Antimicrobial resistance (AMR) is currently one of the most significant challenges to human health. The rapid expansion of multi-resistant bacteria or "superbugs" is a direct consequence of accelerated use of antibiotics. The emergence of *Escherichia coli* resistant to third generation cephalosporin has increased by more than 500 % in 10 years ³⁹. With a significant proportion of antibiotics being used by the food animal industry ⁴⁰, solving the antibiotics crisis will depend on solving the current crisis in farm animal health (discussed in more details in the next section). Funders and scientists must prioritize farm animal health research at the same level as human health research, to protect future antibiotic resources. Today this is very far from reality. In the previous decade, it was estimated that in the U.S. around 5 % of its national health expenditure is spent on biomedical research (around \$101 billion per year). In contrast, during the same period the U.S. Department of Agriculture provided the equivalent of 0.001 % of U.S. livestock and poultry sales in external research funds ⁴¹. The desperate underfunding of veterinary research in the U.S. is highlighted by the fact that in 2007 less than 0.04 % of U.S. Department of Agriculture resources was spent on agriculturally important animals ⁴². Not much is likely to change in the short term since there has been a stagnation in agricultural research funding by the U.S. government over the past few decades and a shift to the private sector ⁴³. Understandably industry-based research will likely focus on applied outcomes, however with stagnating funds, research that leads to fundamental understandings of processes such as host response to pathogens is likely to be undertaken by fewer research groups.

Meeting the need for new antibiotics and reducing the use of current antibiotics requires new lines of research, including fundamental research into zoonosis, studies of pathogen biology and comparative infection studies of humans and animals. A major challenge for innovations in antibiotic therapy is that gut health depends on complex interactions of both human/animal genetics and environmental factors like food and gut bacteria. Specific animal models to allow studies of animal health and host-pathogen cross-talk at the molecular levels are needed for directing the design of novel therapeutics, but such animal models are rare, costly, and challenging to study. Recent work with both humans and pigs, have demonstrated that gene-variants can protect against *E. coli* infections by determining the structure of glycan receptors

to control bacterial colonization of the gut, but little is known about the specific interactions between glycans and bacteria ²⁷. Clearly, proteome research of these non-human species is needed, in part to study specific host-pathogen crosstalk at the molecular level, but also for making available panels of accurate health measures for monitoring the health state of relevant animal models and for delivering proof of concept when new drugs, or alternatives like pre- and pro-biotics are being tested for their efficiency in protecting both animals and humans against pathogens.

4. Farm animal proteomes, and the many ways it links to human health.

As discussed above, industrial farm animal production presents challenges to the health of both farm animals and humans. Thus, building fundamental knowledge of farm animal biology is essential to provide new insights into human health. Here we reflect on some of the direct links between human and animal health, and highlight how farm animal proteome resources are essential for progress towards sustainable farm animal production methods that will benefit of human health.

i. Sustainable food production

Meat, milk and eggs represent a major protein source of the human diet, both in high meat consuming western societies and throughout the developing world where milk and eggs are fundamental to a healthy diet ⁴⁴. Modern farm animal biology is determined by genetic selection. Industrialized breeding strategies has been very successful in increasing the productivity of farm animals, but is also responsible for the collateral decline of animal health, animal welfare and food safety ⁴⁵. For example, the feed conversion rate of the Danish Landrace pigs, and likewise, the average milk yield of Holstein cows, has been approximately doubled since 1950, by genetic selection of traits that regulate metabolism and growth ^{46, 47}. However, since biochemical pathways of metabolism and immunity are closely connected, the past few decades of genomic selection strategies have narrowly targeted productivity traits. These include factors like weight gain and milk yield, which are easily monitored and correlated to gene variants available in the breeding stock. However, such a selection process will also affect the host response pathways that allow the animal to fight pathogens ⁴⁸. The unintended side-effects of intensive breeding today are clear, with unacceptably high animal mortality rates and a reliance on antibiotics ^{49, 50}. Current farm animal proteome research has mainly been aimed at marker discovery used to monitor early onset of diseases ⁵¹, but more research is needed to enable advanced techniques such as selected reaction monitoring (SRM) to deliver breakthroughs at the scale available for human studies ⁵². SRM based monitoring of animal health could support a more sustainable genetic selection of future livestock, to achieve animals with a robust balance between productivity and health traits. An application for SRM based monitoring of bovine host response lies in the potential for routine daily monitoring of an animal's immune response to pathogens by linking health markers to daily milking as has been done for genetic markers ⁵³. Such an approach could support a re-balancing of cow genetics, to achieve a more reasonable trade-off between productivity and health in future milk breeds.

ii. Zoonosis

Over 60 % of all human pathogens originate from animals and while some instances are highly publicized such as SARS and H5N1, the area is largely neglected ⁵⁴. As discussed above, comparative studies between humans and animals are essential to deliver the best possible measures to control infectious diseases.

iii. The decline of antibiotic efficiency

The speed of AMR is exacerbated by the massive amounts of antibiotics needed to maintain industrial production of farm animals ⁵⁵. As discussed above, industrialized animal farming, based on intensive genetic selection, which together with very large populations and high animal density, has set the stage for accelerated outbreak of infectious diseases, which require frequent use of antibiotics. The current lack of monitoring of both pathogens and an individual animal's health state leads to administration of antibiotics often with very little gain ⁴¹. For this reason, better monitoring of pathogens, virulence factors and animal health states need to be developed for species-specific proteome based monitoring methods.

iv. The role of farm animals as model organisms

The value of farm animals as model organisms for human disease is underappreciated despite well-established examples such as pig models, which better reflect human metabolism and neurology than rodent models ⁵⁶. However, new gene-editing methods like CRISPR could advance farm animal research by making available new and precisely designed animal models allowing fundamental research and support high precision breeding for health traits in farm animals ⁵⁷. For farm animal and crop sciences, the new era of high precision gene editing is of particular interest, because in contrast to existing genetics technologies, a CRISPR based approach promises precise optimization of gene variants without the unintended editing of collateral gene positions. This was recently demonstrated by a high precision knock in of a bovine macrophage protein variant, which improved the animal's resistance to respiratory infections ⁵⁸. However, new breeds and gene variant animals must be evaluated by thorough phenotypic studies to evaluate effects to their health, and for their environmental impact, and for this, the application of proteome markers for health (e.g. inflammation markers) are essential, as discussed above.

5. Can advances in crop research and proteomics enable us to feed the world?

Securing crop production under challenging environments to meet future demand depends on innovations in all layers of plant research, breeding and agronomy. Estimates indicate that crop production must double by 2050 to meet global demand ⁵⁹. Our capacity to significantly increase production will severely be hampered by our need to use marginal lands ⁶⁰ and by the compounding effects of changing climate ⁶¹. Advances in molecular biology in the past two decades have begun to see crop breeding embracing genotyping approaches rather than traditional phenotyping methods of the past to assist in the identification of agriculturally important quantitative trait loci (QTL). With the development of next generation sequencing (NGS), we are now able to leverage genetic information from the entire genome to map complex traits down to the resolution of a single nucleotide ⁶². Given this backdrop, does proteomics have a role to play in developing elite cultivars and defining new and important agricultural traits?

Proteins are distinct from both genomic and transcriptomic information that are currently being exploited by NGS for molecular breeding as they are the functional contents of cells. Thus the proteome of an organism often has a poor correlation between expression of genes and transcripts. Also as the genome of an organism is expressed and translated to proteins, the complexity rapidly increases with multiple functional roles of distinct proteoforms being encoded by single genes. This points to a vital role for proteome driven marker-assisted breeding to enable the harnessing of this complexity for enhanced plant performance. The current generation of mass spectrometers have the sensitivity and resolution to dig deep into proteome, overcoming many of the limitations that existed in the previous decade. Recently, several large-scale proteomic studies have been conducted on the important food crop *Triticum aestivum* (wheat). In this section, we will briefly explore the extent to which wheat proteomics can assist in the future development of one of the world's most important food crops.

Wheat is grown on more land than any other global crop with over 700 million tonnes produced annually, providing ~20% of daily calories and protein for over 60% of the world's population. Analysis of the bread wheat genome is complicated by the fact that it is hexaploid having originated from three diploid donors. This has made sequencing its genome extremely difficult and only recently has a reference assembly been made available using the Chinese spring cultivar in combination with data from 18 other cultivars ⁶³. This lack of a highly

refined and cohesive genetic resource has greatly limited proteomic studies seeking to map genetic markers and QTLs. However, now with a sequenced genome, proteomics will be an essential tool to enable the rapid interpretation of the hexaploid genome as between 80% to 90% is repetitive sequences and these must be characterised as functional or non-functional genes ⁶³. Most previous proteomic surveys conducted in wheat have examined responses to stress targeting the discovery of new regulators and mechanisms ^{64, 65}. More recently, an effort was made to better relate emerging genomic resources with proteomics through the development of a proteo-genomic map of wheat ⁶⁶. This resource enables the interpretations of the complex ORFeome as a result of the hexaploidy genome, as one can quickly assess which isoforms of a particular protein are expressed. This is particularly important as many enzymes and metabolic pathways were first characterized in model plants or plants with less complicated genomes than those in the Poaceae. This information can be used to develop SRM transitions to discriminate between isoforms as a result of duplication events. For example, the first step of fatty acid synthesis in plants is carried out by acetyl-CoA carboxylase and in Arabidopsis this is carried out by a four subunit heteromeric >650kDa membrane associated complex in the plastid and two isoforms of a >500kDa homodimeric complex outside the plastid. In contrast, in wheat only the >500kDa homodimeric complex is present and the complex hybridization history of wheat has resulted in 15 isoforms of this enzyme with widely different expression patterns.

Thus, while there have been clear delays in enabling proteomics to be used in many of the most important global crop species, the recent availability of an assembled genome, modern mass spectrometers and their utilization in generating resources such as the wheat proteo-genomic map are indicative of pipelines initially developed for model organisms. Consequently, it is likely that proteomics will start to play a larger role in molecular breeding programs in crops such as wheat and play an important role in securing global food supplies.

6. Conclusion

- We are not alone: Human health from the OneHealth perspective. We have above discussed the importance of the non-human proteomes and how these directly link to HPP and human health.
- We suggest a need for a community driven building a MOPP (Multi Organism Proteome Project), to meet some of the needs suggested above.
- The MOPP should gain from the experience harvested in the HPP.
- How can the current HPP support by making MOPP progress at the same priority and progress as the currently much flagged HPP.
- How can iMOP help to re-balance research progress in the relevant MOP fields (future plans, goals, and milestones for your initiative, and the development of a list of priority).
- Most importantly: How can we reach the non-proteome but species oriented communities, to have their support?
- The HUPO has the power to make also non-human proteome development at priority. In fact, only the (relatively) well-funded human field has the power to drive this field

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