From Department of Medical Biochemistry and Biophysics
Karolinska Institutet, Stockholm, Sweden

Plurifaceted Proteomics in Studying Cellular Dynamics and Action Mechanisms of Anticancer Drugs

Pierre Sabatier

Karolinska Institutet

Stockholm 2021

Published by Karolinska Institutet.
Printed by Universitetsservice US-AB, 2021

ISBN 978-91-8016-247-0

Plurifaceted Proteomics in Studying Cellular Dynamics and Action Mechanisms of Anticancer Drugs
THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Pierre Sabatier

The thesis will be defended in public at Eva & George Klein lecture hall, floor 3 Biomedicum, Solnavägen 9, Solna, May 28th, 2021 at 13.00 PM

Principal Supervisor:
Professor Roman Zubarev
Karolinska Institutet
Department of Medical Biochemistry and Biophysics
Division of Physiological Chemistry I

Co-supervisor:
Professor Elias Arnér
Karolinska Institutet
Department of Medical Biochemistry and Biophysics
Division of Biochemistry

Opponent:
Professor Bernhard Küster
Technical University of Munich
Department of Molecular Life Sciences
Chair of Proteomics and Bioanalytics

Examination Board:
Professor Jonas Bergquist
Uppsala University
Department of Chemistry-BMC
Division of Analytical Chemistry

Professor Adil Mardinoglu
KTH
Department of Protein Science
Division of Systems Biology

Associate Professor Leopold Luna Ilag
Stockholm University
Department of Materials and Environmental Chemistry

To my family.

ABSTRACT

Mass spectrometry (MS)-based proteomics has developed tremendously in recent years and was the leading technology for many novel methods to study protein chemistry. Contrary to classical approaches based on Western blot, MS-based approaches are mostly unbiased. In addition to protein expression levels, today several protein chemical properties can be examined by MS-based proteomics making it unique in comparison to transcriptomics approaches. These properties include post-translational modifications (PTMs), protein localization, synthesis/degradation, and lastly protein thermal stability, adding novel dimensions or facets to characterize the proteome. However, these facets are difficult to combine as they are mainly orthogonal and are therefore often analyzed separately. This thesis presents a simplified and higher throughput version of current protein stability analyses and showcases the advantages of combined/merged analysis of proteomics facets including our new method, as well as expression and redox proteomics to study anticancer treatments and cellular dynamics.

In **paper I**, we studied the dynamics of cancer cells in vitro with and without anticancer treatment over the course of 48 h by monitoring protein expression every 6 h. We discovered that naturally occurring proteome variations are on par with anticancer treatment killing 50% of the cells after 48 h. Then, we acquired a deep proteomics dataset of untreated HCT116 and A375 cell lines. Surprisingly, we observed downregulation of proteins involved in cell division and upregulation of proteins involved in metabolism as early as 12 h after treatment, suggesting that growth inhibition happens earlier than usually assumed and even at low cell confluence.

In paper II we developed the proteome integral solubility alteration (PISA) assay that increases the throughput of pre-existing drug target deconvolution methods based on protein stability/solubility measurements and reduces the analysis time and cost as well as sample requirements. We provided theoretical calculations showing that the integral of the curve correlates well with melting temperature estimations in Thermal Proteome Profiling (TPP) and tested our assumptions with publicly available TPP datasets. Then we performed a proof of principle experiment using the well-studied methotrexate (MTX) and 5-fluorouracile (5-FU) as test drugs highlighting the targets as outliers with our method. Furthermore, we demonstrated that PISA assay can also be used for concentration series analysis as in 2D-TPP. Finally, we showcased the higher throughput of PISA compared to TPP by simultaneously analyzing nine drugs in one multiplexed analysis.

In paper III we used a combination of chemical proteomics approaches to study the target and mechanism of action (MOA) of Auranofin (AF) (Ridaura®). Functional Identification of Target by Expression Proteomics (FITExP), TPP, and redox proteomics combined highlighted that thioredoxin reductase 1 (TXNRD1) is indeed a top target hit of AF, and that the main MOA of the drug is through disruption of the redox balance in the cell. Finally, we showed that protein thermal shifts can be associated with altered cysteine oxidation levels in

proteins, suggesting that TPP is suitable to study disulfide bond formation/reduction and map some cysteines to the active sites of sulfiredoxin 1 (SRXN1) and peroxiredoxin 5 (PRDX5) as examples. Overall, our study demonstrates that using only one of the proteomics methods is not sufficient to accurately pinpoint drug target and MOA, but a combination of multiple complementary methods should be used instead.

Following the success of our strategy in **paper III**, we decided to utilize the same strategy in **paper IV** using PISA developed in **paper II** instead of TPP, to study two novel inhibitors of TXNRD1, namely TRi-1 and TRi-2. For these studies, we used the mouse cancer cell lines B16-F10 and Lewis lung carcinoma (LLC) to provide a comprehensive analysis of the therapeutic effects of the inhibitors. Since we showed that Txnrd1 (the mouse version of the human TXNRD1 protein) is a main target of AF, we decided to use it as our reference point to evaluate the specificity of the two new compounds. AF had a broader effect on the proteome than TRi-1 and TRi-2 and had more target hits in PISA analyses. This suggested that AF has lower specificity than TRi-1 and TRi-2. TRi-1 was the most specific Txnrd1 inhibitor with a better target ranking in FITExP and the least target hits in PISA followed by TRi-2 and AF. Thus, we showed that TRi-1 and TRi-2 are indeed more specific inhibitors of Txnrd1. In addition to these findings, we also highlighted that only AF triggers a high nuclear factor erythroid 2-related factor 2 (Nrf2) antioxidant response, suggesting that this response is not necessarily Txnrd1-dependent. Finally, we detected selenocysteine-specific elongation factor (Eefsec), mini-chromosome maintenance complex-binding protein (Mcmbp), glycogen synthase kinase-3 (Gsk3) a and b, as target hits of AF, which would explain at least partially three different effects of AF treatment. Collectively, our data represents a resource for redox biologists interested in Txnrd1 inhibition. Our approach provides a framework for target deconvolution using proteomics approaches.

Finally, in **paper V** we studied the dynamics of the proteome in transition between various cell types. We reprogrammed human foreskin fibroblasts (hFF) into induced pluripotent stem cells (iPSCs), which we differentiated through embryoid bodies (EBs) formation. We examined protein expression and stability after each cell type transition using PISA-Express, a new version of the PISA assay developed in **paper II**. We merged the readout from protein expression and thermal stability in one analysis using Sankey diagrams to detect changes in protein properties during proteome transitions resulting in the ProteoTracker web interface (http://www.proteotracker.genexplain.com/). Using this innovative analysis, we discovered that ribosomes are less stable in pluripotent stem cells (PSCs) compared to differentiated cells and that this difference stems from the deficiency of one ribosome maturation factor, Shwachman-Bodian-Diamond syndrome protein (SBDS). Knock-down (KD) of SBDS slowed down translation and increased expression of the master pluripotency markers homeobox protein NANOG (NANOG), and octamer-binding transcription factor 4 (OCT4). Collectively, we developed a new method for simultaneous analysis of protein thermal stability and expression, a new analysis and visualization tool, and provided evidence that control of translation through ribosome biogenesis is a natural mechanism used by PSCs to maintain the pluripotency state.

LIST OF SCIENTIFIC PAPERS

I. **Pierre Sabatier**, Amir A. Saei, Shiyu Wang and Roman A. Zubarev. Dynamic Proteomics Reveals High Plasticity of Cellular Proteome: Growth-Related and Drug-Induced Changes in Cancer Cells are Comparable. Proteomics. https://doi.org/10.1002/pmic.201800118.

II. Massimiliano Gaetani*, **Pierre Sabatier***, Amir A. Saei*, Christian M. Beusch, Zhe Yang, Susanna L. Lundström and Roman A. Zubarev. Proteome Integral Solubility Alteration: A High-Throughput Proteomics Assay for Target Deconvolution. Journal of Proteome Research. https://doi.org/10.1021/acs.jproteome.9b00500.

III. Amir A. Saei, Hjalmar Gullberg*, **Pierre Sabatier***, Christian M. Beusch*, Katarina Johansson, Bo Lundgren, Per I. Arvidsson, Elias S. J. Arnér and Roman A. Zubarev. Comprehensive chemical proteomics for target deconvolution of the redox active drug auranofin. Redox Biology. https://doi.org/10.1016/j.redox.2020.101491.

IV. **Pierre Sabatier**, Christian M. Beusch, Roman A. Zubarev* and Elias S. J. Arnér*. Comprehensive chemical proteomics reveal that the new TRi-1 and TRi-2 compounds are more specific thioredoxin reductase 1 inhibitors than Auranofin [manuscript].

V. **Pierre Sabatier**, Christian M. Beusch, Amir A. Saei, Mike Aoun, Noah Moruzzi, Niels Leijten, Magnus Nordenskjöld, Patrick Micke, Diana Maltseva, Alexander G. Tonevitsky, Vincent Millischer, J. Carlos Villaescusa, Sandeep Kadekar, Per-Olof Berggren, Oscar Simonson, Karl-Henrik Grinnemo, Rikard Holmdahl, Sergey Rodin* and Roman A. Zubarev*. Plurifaceted proteomics method identifies key regulators of translation during stem cells maintenance and differentiation [manuscript].

*: These authors contributed equally.

SCIENTIFIC PAPERS NOT INCLUDED IN THE THESIS

I. Amir A. Saei, **Pierre Sabatier**, Ülkü G. Tokat, Alexey Chernobrovkin, Mohammad Pirmoradian and Roman A. Zubarev. Comparative proteomics of dying and surviving cancer cells improves the identification of drug targets and sheds light on cell life/death decisions. Molecular and cellular proteomics. https://doi.org/10.1074/mcp.RA118.000610.

II. Amir A. Saei, Christian M. Beusch*, Alexey Chernobrovkin*, **Pierre Sabatier***, Bo Zhang*, Ülkü G. Tokat, Eleni Stergiou, Massimiliano Gaetani, Ákos Végvári and Roman A. Zubarev. ProTargetMiner as a proteome signature library of anticancer molecules for functional discovery. Nature Communications. https://doi.org/10.1038/s41467-019-13582-8.

III. Amir A. Saei*‡, Christian M. Beusch*, **Pierre Sabatier**, Juan A. Wells, Hassan Gharibi, Zhaowei Meng, Alexey Chernobrovkin, Sergey Rodin, Katja Näreoja, Ann-Gerd Thorsell, Tobias Karlberg, Qing Cheng, Susanna L. Lundström, Massimiliano Gaetani, Ákos Végvári, Elias S. J. Arnér, Herwig Schüler and Roman A. Zubarev‡. System-wide identification and prioritization of enzyme substrates by thermal analysis. Nature Communications. https://doi.org/10.1038/s41467-021-21540-6.

IV. Gonzalo F. Lahore‡, Bruno Raposo, Marie Lagerquist, Claes Ohlsson, **Pierre Sabatier**, Bingze Xu, Mike Aoun, Jaime James, Xiaojie Cai, Roman A. Zubarev, Kutty S. Nandakumar and Rikard Holmdahl‡. Vitamin D3 receptor polymorphisms regulate T cells and T cell-dependent inflammatory diseases. PNAS. https://doi.org/10.1073/pnas.2001966117.

*: These authors contributed equally.

CONTENTS

1 INTRODUCTION....5
1.1 Deconvolution strategies in drug development and stem cell research....5
1.2 Chemical proteomics in studying targets and mechanisms of action of small molecule inhibitors....6
1.3 Analytical dimensions and methods in mass spectrometry-based proteomics and chemical proteomics....6
1.3.1 Chemical probe-based methods....6
1.3.2 Shotgun analysis in chemical proteomics....8
1.3.3 Advanced approaches based upon protein expression analyses....9
1.3.4 Study of PTMs....11
1.3.5 Methods based on limited proteolysis....13
1.3.6 Protein thermal stability measurements....14
1.4 Proteomics atlases....16
2 RESEARCH AIMS....19
2.1 Main objectives....19
2.2 Specific aims of the thesis....19
3 MATERIALS AND METHODS....21
3.1 Cell culture....21
3.2 Label-free proteomics....21
3.3 TMT labeling....21
3.4 High-pH reversed-phase peptide fractionation....22
3.5 Mass spectrometry analysis....23
3.6 Protein quantification....23
3.7 Data analysis....24
4 RESULTS and discussion....25
4.1 Dynamic proteomics reveals high plasticity of cellular proteome: growth-related and drug-induced changes in cancer cells are comparable....25
4.1.1 Proteome changes in the control group are equivalent to drug treatment at IC50 after 48 h....25
4.1.2 Proteome changes are not due to chemical treatment and are not cell line-specific....26
4.1.3 Proteins involved in cell division and metabolism are the most regulated over time in *in vitro* cell culture....26
4.2 Proteome Integral Solubility Alteration: A High-Throughput Proteomics Assay for Target Deconvolution....28
4.3 Comprehensive chemical proteomics for target deconvolution of the redox active drug auranofin....31
4.3.1 Combination of chemical proteomics methods predicts auranofin targets and mechanism of action....31

4.3.2 Peptide cysteine oxidation levels link to thermal stability alterations. 32
4.4 Comprehensive chemical proteomics reveals that the new TRi-1 and TRi-2 compounds are more specific thioredoxin reductase 1 inhibitors than Auranofin. 34
4.4.1 A combination of chemical proteomics approaches shows that auranofin has a wider effect on the cellular proteome than TRi-1 and TRi-2 and all treatments trigger oxidative stress. 35
4.4.2 TRi-1 and TRi-2 are more specific Txnrd1 inhibitors than auranofin. 36
4.4.3 Auranofin target landscape recapitulates some of the observed effects of the drug. 37
4.5 Plurifaceted proteomics method identifies key regulators of translation during stem cells maintenance and differentiation 39
4.5.1 Protein stability and expression measurements distinguish cell types and various degrees of pluripotency. 40
4.5.2 ProteoTracker interface visualizes protein trajectories in stability and expression between cell types. 41
4.5.3 PSCs have lower amounts of mature ribosomes than differentiated cells. 42
4.5.4 Proteins involved in late steps of ribosome maturation are downregulated in iPSCs compared to differentiated cells. 43
4.5.5 SBDS knock-down slows down differentiation of iPSCs. 44
5 CONCLUSIONS and future perspectives 47
6 ACKNOWLEDGEMENTS 51
7 REFERENCES 55

LIST OF ABBREVIATIONS

2D-TPP	2-Dimensional Thermal Proteome Profiling
5-FU	5-fluorouracile
ABPP	Activity-Based Protein Profiling
ACN	acetonitrile
AF	auranofin
CAMP	camptothecin
CCCP	Compound-Centric Chemical Proteomics
CETSA	Cellular Thermal Shift Assay
DMSO	dimethylsulfoxide
EB	embryoid body
ESC	embryonic stem cell
FA	formic acid
FITExP	Functional Identification of Target by Expression Proteomics
GO	Gene Ontology
hFF	human foreskin fibroblast
iPSC	induced pluripotent stem cell
KD	Knock-down
LC-MS/MS	liquid chromatography coupled to tandem mass spectrometry
LLC	Lewis lung carcinoma
LysC	lysyl endopeptidase LysC
MOA	mechanism of action
MS	mass spectrometry
MS/MS	tandem mass spectrometry
MS-CETSA	mass spectrometry-based Cellular Thermal Shift Assay
MTX	methotrexate
NADPH	nicotinamide adenine dinucleotide phosphate
NANOG	homeobox protein NANOG
NRF2	human nuclear factor erythroid 2-related factor 2

Nrf2	mouse nuclear factor erythroid 2-related factor 2
OCT4	octamer-binding transcription factor 4
PCTL	paclitaxel
PISA	Proteome Integral Solubility Alteration
PPI	protein-protein interaction
PRDX5	human peroxiredoxin 5
PTM	post-translational modification
SBDS	Shwachman-Bodian-Diamond syndrome
SILAC	Stable Isotope Labeling by Amino acids in Cell culture
SRXN1	human sulfiredoxin 1
Tm	melting temperature
TMT	Tandem Mass Tag
TMT10	Tandem Mass Tag 10-plex
TPP	Thermal Proteome Profiling
TPP-CCR	Thermal Proteome Profiling-Compound Concentration Range
TPP-TR	Thermal Proteome Profiling-Temperature Range
TXNRD1	human thioredoxin reductase 1
Txnrd1	mouse thioredoxin reductase 1

1 INTRODUCTION

1.1 DECONVOLUTION STRATEGIES IN DRUG DEVELOPMENT AND STEM CELL RESEARCH

Only 7% of the compounds entering phase I clinical trials end up being approved for therapy (Dowden & Munro, 2019). Seventy-nine percent of these drugs failed for efficacy and safety reasons during the 2016-2018 period and these numbers were similar in previous years (Arrowsmith & Miller, 2013; Harrison, 2016), highlighting the gap between preclinical studies in cell lines and animal models and human clinical trials. Part of the problem lies in overinterpretation of the results, bias, and inaccurate characterization of the compounds' target landscape, and mechanism of action (MOA) (Colquhoun, 2014; Halsey et al., 2015). Target deconvolution is present in every phase of drug development in both target-based and phenotypic drug discovery (Noberini & Bonaldi, 2019) (Figure 1), but even for drugs used in the clinic, off-targets are still discovered today due to the development of new methods (Klaeger et al., 2016; M. M. F. Savitski et al., 2014). However, not all discovered off-targets have a negative impact since they can also lead to repurposing of the drug for treating other diseases or help in compound optimization. Consequently, the pharmaceutical industry would benefit from the development of better preclinical methods for accurate characterization of the target landscape and MOA of lead compounds.

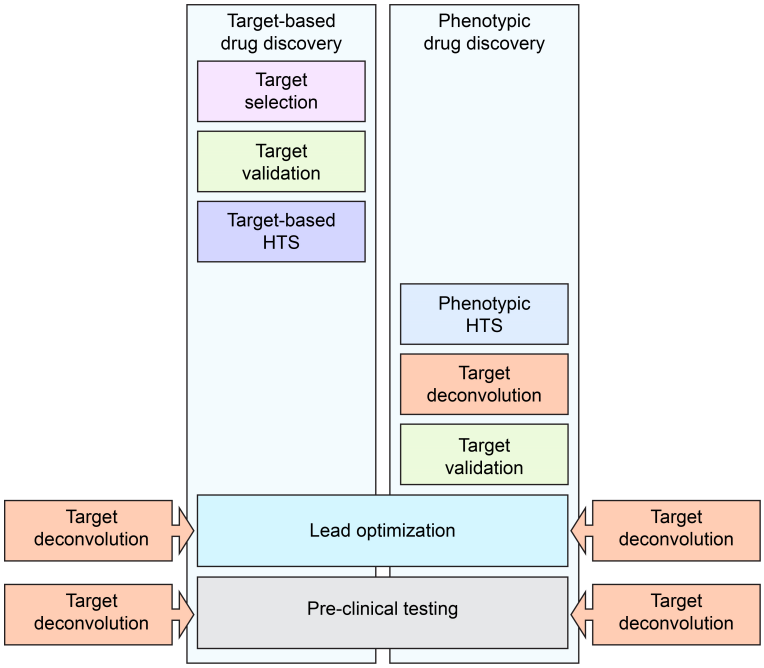

Figure 1. Target deconvolution in drug discovery. Scheme of the different stages of drug discovery. Target deconvolution is involved throughout drug discovery in phenotypic drug discovery while it is involved in the late stages for target-based drug discovery. HTS: High-throughput screen. Redrawn by permission from Springer Nature Customer Service Centre GmbH: Springer Nature, Nature Structural & Molecular Biology, Epigenetic drug target deconvolution by mass spectrometry–based technologies, (Noberini & Bonaldi, 2019).

Conversely, discovering new mechanistic targets that could be affected by chemicals or other molecules to achieve the desired effect in diseases or cell biology is also valuable. For example, in the stem cell field and regenerative medicine, the ability to maintain and expand specific types of stem cells and/or direct their differentiation into specific cell types is of utmost importance to accelerate the development and approval of stem cells-based therapies (Poulos, 2018). Cocktails of small molecule inhibitors and growth factors are used to differentiate stem cells in various cell types and tissues such as airway epithelial cells (Wong

et al., 2012), megakaryocytes (Nakamura et al., 2014), intestinal tissue (Spence et al., 2011), cardiomyocytes (Lian et al., 2012), nephron organoids (Morizane et al., 2015), neurons (Lu et al., 2016; Nolbrant et al., 2017), pancreatic β cells (Pagliuca et al., 2014) and retina cells (Osakada et al., 2009). This is also relevant in precision medicine and targeted therapies where treatments are adapted based on the patients' genetics and where the knowledge of key mechanistic proteins can help choosing optimal treatments (Collins & Varmus, 2015; Lamberti et al., 2018; Srivastava et al., 2018). Understanding the underlying mechanisms of diseases and cell type transitions is crucial for developing more effective drug and cell-based therapies. Consequently, there is a need for more efficient methods to deconvolute drug targets and cellular mechanisms for the development of novel therapies.

1.2 CHEMICAL PROTEOMICS IN STUDYING TARGETS AND MECHANISMS OF ACTION OF SMALL MOLECULE INHIBITORS

Standard approaches of chemical proteomics employ affinity pull-downs or chromatography methods using drugs or probes that bind to specific enzymes, usually coupled to SDS-PAGE or mass spectrometry analysis (Jeffery & Bogyo, 2003). However, mass spectrometry-based proteomics methods have become increasingly powerful in recent years by enabling the possibility to monitor thousands of proteins simultaneously (Hornburg et al., 2014; Pirmoradian et al., 2013; Wei et al., 2016; Zhou et al., 2013). In addition, mass spectrometry offers unique possibilities to study in an unbiased manner various aspects of protein chemistry, such as post-translational modifications (PTM), small molecules-protein interactions and protein-protein interactions (PPI) as well as protein structure and thermal stability (J. X. Huang et al., 2019; Lomenick et al., 2009; Molina et al., 2013; Saei et al., 2021; M. M. F. Savitski et al., 2014; Schopper et al., 2017). Thus, the chemical proteomics field expanded to the broader use of mass spectrometry-based approaches in general. Now, chemical proteomics is defined as a multidisciplinary field that aims to study how small-molecule compounds bind to proteins and modulate their function (Tao & Zhang, 2019), primarily focused on drug target deconvolution and target discovery.

1.3 ANALYTICAL DIMENSIONS AND METHODS IN MASS SPECTROMETRY-BASED PROTEOMICS AND CHEMICAL PROTEOMICS

1.3.1 Chemical probe-based methods

Initially, chemical proteomics encompassed the use of specifically designed probes to study enzyme-substrate or drug-proteins interactions. Activity-based and affinity-based were the main types of probes. The functions of many proteins remain unknown, and the discovery of new enzymes is crucial for both fundamental biology and drug discovery. The development of Activity-Based Protein Profiling (ABPP) (Yongsheng Liu et al., 1999) was a major step forward to address the issue. In ABPP, specific probes are designed to target a particular feature of a family of enzymes (Figure 2). New protein functions can be assigned using this approach. The probes consist of a reactive group that binds covalently to a specific domain of

an enzyme class (in most cases to the catalytic domain). It is connected by a linker to a tag providing an identifier for proteins that are bound to the probe, which is also used for pulling down the proteins followed by identification using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Probes for numerous enzyme types have been designed over the years and have been very successful in assigning new enzymatic functions to proteins (Barglow & Cravatt, 2007; Zweerink et al., 2017). Such probes have also facilitated drug discovery via the establishment of a high-throughput platform (Bachovchin et al., 2009). For instance, using this platform, Bachovchin et al. discovered new inhibitors of GSTO1 and RRBP9 (Bachovchin et al., 2009).

While ABPP is mainly focused on enzymatic activity, the second type of probes used in chemical proteomics is affinity purification-based chemical probes that focus on target deconvolution. This approach is called Compound-Centric Chemical Proteomics (CCCP) (Figure 2). In this approach, the reactive group is replaced by a drug that binds to a specific or to multiple protein targets (Chan et al., 2004; Shi et al., 2012). Usually, the compound is immobilized on beads (or on a resin). The beads are incubated with a cell lysate or a protein mixture, washed extensively and proteins bound to the drug are subjected to proteolytic digestion to release peptides that are analysed and identified by LC-MS/MS. One of the disadvantages of this method is that proteins can beads without interaction with the drug and remain even after extensive washing. Additionally, other proteins that are interacting with the real target of the drug can be pulled down along thus creating false positive identifications (J. Wang et al., 2016). One more disadvantage of the method is that the design of the probe can alter the activity of the drug and binding affinity to its target. Therefore, the activity of the probe needs to be confirmed by comparison with the unmodified drug before starting experiments. Some compounds do not even allow for the addition of a linker. Finally, in affinity purification, the target proteins are not covalently bound, making the washing steps and accurate pull down a challenging task.

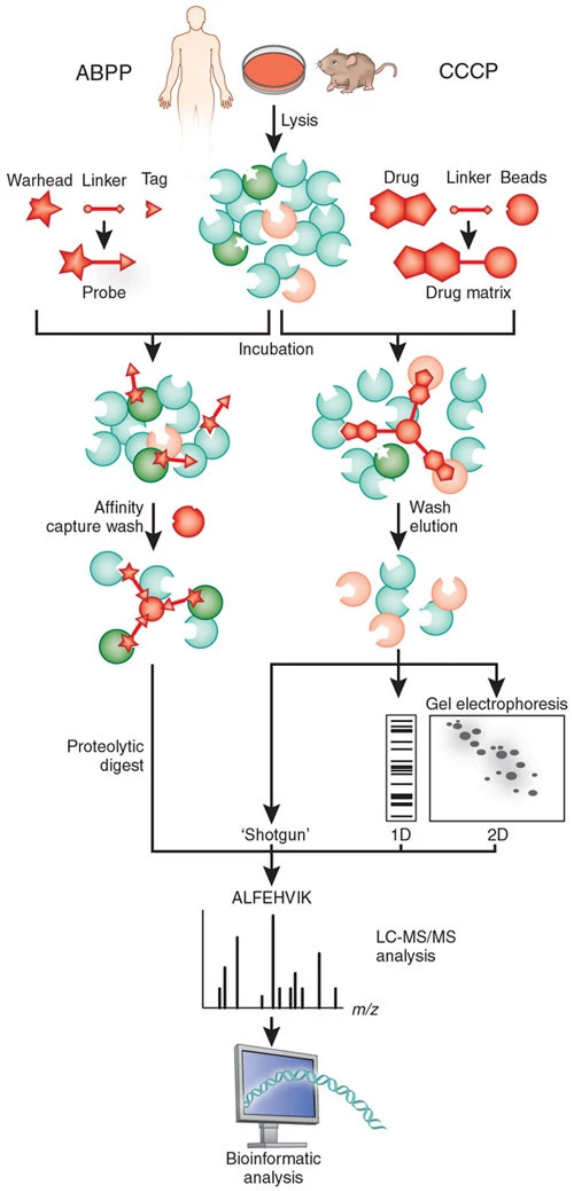

Figure 2. Probe-based chemical proteomics methods. Schematic view of typical workflows in Activity-Based Protein Profiling (ABPP) and Compound-Centric Chemical Proteomics (CCCP). Reprinted by permission from Springer Nature Customer Service Centre GmbH: Springer Nature, Nature Chemical Biology, Target profiling of small molecules by chemical proteomics, (Rix & Superti-Furga, 2009).

An example of an affinity purification strategy is the use of kinobeads (Bantscheff et al., 2007). They consist of more than a hundred kinase inhibitors and ATP-mimetics immobilized on beads. These drugs/probes bind to the ATP binding pocket of kinases and purine-binding proteins and can capture a substantial fraction of these proteins present in a cell lysate. Contrary to other affinity-based approaches, kinobeads do not aim to determine the targets of the drugs bound to the beads. Instead, a tested "free" kinase inhibitor is also added to the cell lysate and will compete with the kinobeads to bind to its targets. Then, the targets of the tested drug are deconvoluted by measuring the concentration of proteins bound to the kinobeads. It is also possible to determine affinity curves using a concentration range of the tested drug. The advantage of this approach is that there is no need to chemically modify the compound of interest. Kinobeads have been successfully used to characterize kinase inhibitors and particularly in a study involving 243 kinase inhibitors used in clinics (Klaeger et al., 2017). Kinobeads have been also employed to confirm a common off-target of kinase inhibitors, ferrochelatase (FECH) (Klaeger et al., 2016).

The main drawback of ABPP and CCCP lies in the design of the probes or the drug immobilization, which can be labour-intensive. The design of the probes is also important to avoid false positive or false negative results. The advantages of these approaches are that they are unbiased and can be coupled with stable isotope or isobaric labelling to increase their accuracy and throughput (Bantscheff et al., 2007; Ong et al., 2009).

1.3.2 Shotgun analysis in chemical proteomics

Another classical workflow in chemical proteomics is a shotgun analysis of cell lines or animal and human tissues after treatment with a drug, to monitor the effect on the cellular proteome (Fatima et al., 2009; Voruganti et al., 2013). The widely accepted hypothesis behind such experiments is that the effect of the chemical is reflected in the variation of protein concentration within the MOA of the drug and feedback loops (Chakrabarty et al., 2012; Wan et al., 2007). The most common method is based on the bottom-up approach where proteins from cells or tissue lysates are digested, and the resulting peptide mixture is analysed by mass spectrometry (McDonald & Yates, 2003; Swanson & Washburn, 2005; Wu & MacCoss, 2002) (Figure 3). Both label-free and labelling methods exist for protein quantification, with the latter being more commonly used. The main reason is that label-free techniques are still limited by the analysis throughput and analysis depth due to the dynamic range of protein concentrations in cells.

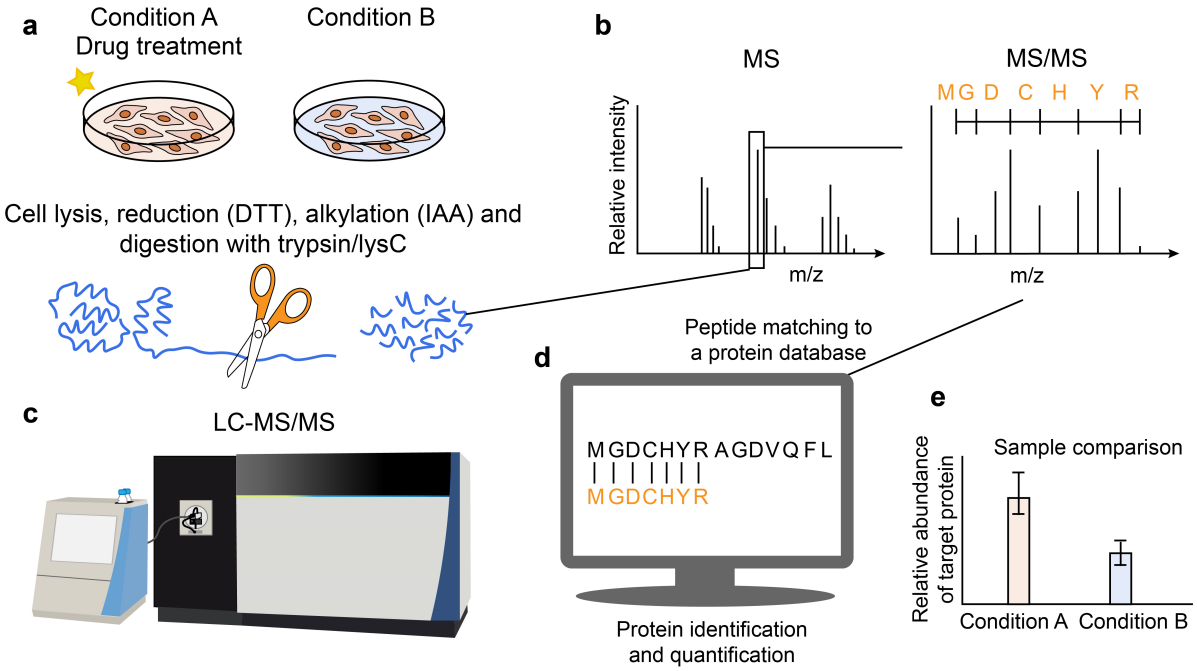

Figure 3. Shotgun (bottom-up) chemical proteomics workflow. (a) Cells are treated with a drug, then lysed to extract the proteins. Proteins are then reduced with dithiothreitol (DTT), alkylated with iodoacetamide (IAA), and digested into peptides with trypsin and/or lysyl endopeptidase (lysC). (b) The peptide mixture is analysed by LC-MS/MS for each sample independently. (c) Precursors are selected in a full scan (MS) and fragmented for MS/MS. (d) The MS/MS spectra are matched to a peptide database to identify the protein of origin. Quantification of a protein is performed using the intensity of all its identified peptides corresponding to their signal in MS. (e) Differences in relative abundance between conditions A (drug-treated) and B highlights potential targets of the treatment.

Several labelling technologies have been developed over the years and currently, SILAC (Ong et al., 2002), iTRAQ (Ross et al., 2004), and TMT (Thompson et al., 2003) are the most widely used labelling approaches allowing for multiplexing of up to 16 samples (J. Li et al., 2020; Thompson et al., 2019), greatly increasing the speed of the analysis. Moreover, since samples are prepared simultaneously, they can be fractionated off-line prior to LC-MS/MS. One of the limitations of reversed-phase liquid chromatography coupled to MS/MS is that mass spectrometers are not fast enough to process the enormous number of peptides that are eluted simultaneously and only the most abundant peptides are usually identified. This can hamper the detection of low abundant proteins such as those involved in signal transduction. One way to solve this issue is to pre-fractionate the samples with an orthogonal dimension to low-pH reversed-phase chromatography. Consequently, samples are now routinely fractionated using isoelectric focusing (Branca et al., 2014; Pirmoradian et al., 2014) or the semi-orthogonal high-pH reversed-phase liquid chromatography (H. Wang et al., 2015; F. Yang et al., 2012) reaching high proteome coverage in a reasonable analysis time which would be difficult to achieve using contemporary label-free technologies.

1.3.3 Advanced approaches based upon protein expression analyses

FITExP (Chernobrovkin et al., 2015; Gaetani & Zubarev, 2019; R. F. S. S. Lee et al., 2017a; Saei et al., 2019) employs shotgun proteomics for the identification of drug MOA, adding a

specificity component to the conventional protein concentration measurements. In FITExP, several cell lines are treated with multiple drugs targeting various proteins (Figure 4). Then, the proteome signature of each compound is captured by LC-MS/MS analysis and is contrasted against the signatures of all the other compounds in each cell line to highlight specific features and eliminate cell type-specific response and default cells detoxification response to foreign chemicals. The authors successfully showed that MTX known target, dihydrofolate reductase (DHFR), was identified as the top target candidate. The authors also identified TYMS, the target of 5-FU, and demonstrated that it affects ribosome biogenesis as a new mechanistic insight. Later, FITExP was used to study metallodrugs (R. F. S. S. Lee et al., 2017b) and evaluate the cytotoxicity and pro-inflammatory effects of nanoparticles on monocytes (Tarasova et al., 2017). We recently modified FITExP by including matrix-detached cells in the analysis and made it easier to visualize using OPLS-DA models (Saei et al., 2018) (Figure 4). Finally, we built a database of anticancer drug response for 56 compounds in A549 adenocarcinoma cells and three datasets with increased analytical depth for 9 of the drugs in MCF-7, RKO, and A549 cells (Saei et al., 2019). The data can be explored through the ProTargetMiner web interface using OPLS models (http://protargetminer.genexplain.com).

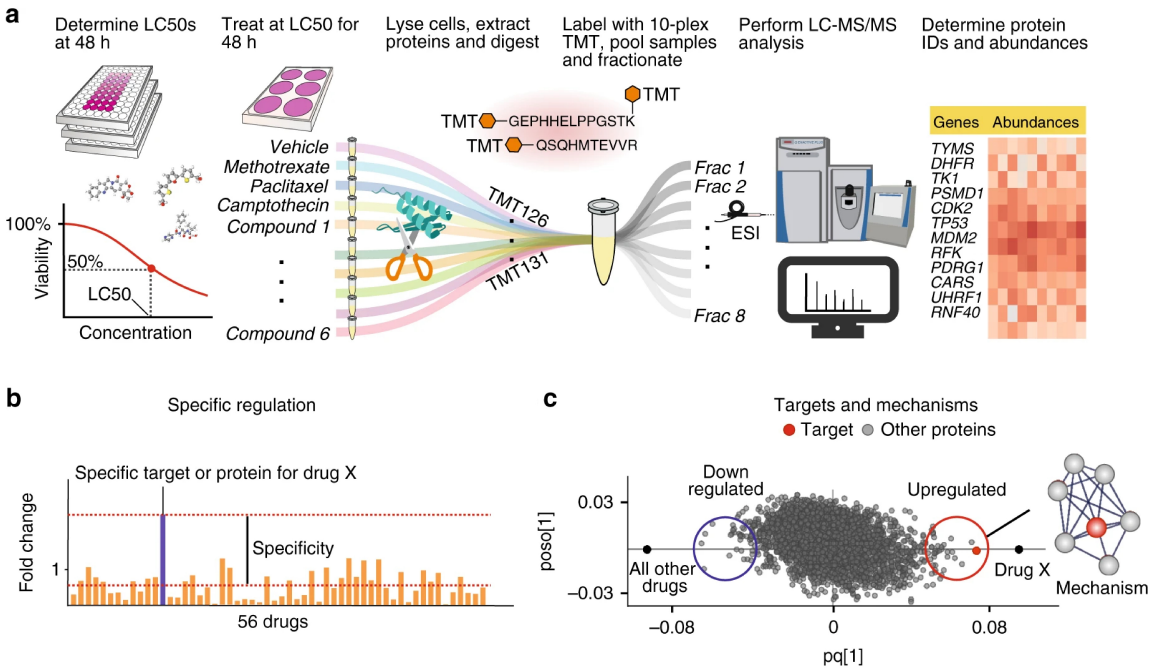

Figure 4. ProTargetMiner workflow using FITExP analysis. (a) Preparation of a dataset for FITExP analysis. The first step is to determine the IC50 of the compounds in the desired cell lines. Then cells are treated with IC50 including reference drugs, in this case, methotrexate (MTX), camptothecin (CAMP), and paclitaxel (PCTL) in addition to the compounds of interest and the vehicle. Then cells are lysed, proteins are digested, and the resulting peptides are labelled with TMT10, off-line fractionated, and analysed by LC-MS/MS. These steps can be done label-free and without off-line fractionation. (b) Specific regulation of the target or target candidate for the drug of interest in comparison to other drugs. (c) OPLS-DA models highlight the mechanism of action and target of the drug of interest by contrasting it against all other drugs or against the reference drugs. Figure adapted from (Saei et al., 2019).

Stable Isotope Labelling by Amino acids in Cell culture (SILAC) is a type of metabolic labelling that can be utilized to tag proteins with stable isotopes in cells and *in vivo* for multiplexing and enhanced accuracy of protein abundances measurements (Krüger et al., 2008; Ong et al., 2002; Sury et al., 2010). However, SILAC is not only used for these features but also for the measurement of protein synthesis and degradation rates (Doherty et al., 2009). Indeed, pulse and pulse-chase SILAC strategies were rapidly implemented where cells or animals are fed with lysine and arginine labelled with stable isotopes of carbon and nitrogen. In pulse SILAC, the first labels are replaced by a second label at a specific timing, to quantify the portion of newly synthesized and degraded proteins over a certain period allowing to also calculate synthesis and degradation rates (Ong, 2012). SILAC can be combined with TMT for increasing multiplexing capacities, but only at the expense of high computational power and complicated spectra interpretation (Welle et al., 2016). Recently, Savitski et al. developed multiplexed proteome dynamics profiling (mPDP) (M. M. Savitski et al., 2018) for studying protein turnover in cells and, particularly, in the context of drug-mediated protein degradation. This is relevant for studying drug MOA and disease mechanisms (Fierro-Monti et al., 2013; Somasekharan et al., 2012).

Lastly, subcellular fractionation methods coupled to LC-MS/MS can dissect the cellular proteome down to sub organelles and protein complexes using differential centrifugations and label-free or isotope labelling (Dunkley et al., 2004; Geladaki et al., 2019; Itzhak et al., 2017). Since these methods were developed to be compatible with mass spectrometry, they enable unbiased detection of proteins present in specific cell locations. This is particularly valuable since cellular localization is fundamental to maintain the normal function of a protein and altered localization has been associated with diseases including cancer (Hung & Link, 2011; Rogaia et al., 1997; Xin et al., 2014). Several studies have systematically classified proteins based on their expression and cellular localization and provided comprehensive visualization of the data (Christoforou et al., 2016; Itzhak et al., 2016; Joshi et al., 2019; Orre et al., 2019). These studies provide tools and unbiased spatial information on the organization of the cellular proteome in various cell types and stimulated conditions that are otherwise only available via time-consuming Western blotting or imaging techniques such as fluorescence microscopy.

1.3.4 Study of PTMs

One of the biggest challenges in the proteomics field is to accurately detect and quantify changes in post-translational modifications (PTMs) (M. S. Kim et al., 2016). The possibility of simultaneous detection of thousands of proteins makes LC-MS/MS the most appealing technology for large-scale studies of PTMs. Nevertheless, most of the developed methods focus solely on one type of PTM and are based on enrichment of the native modification or enrichment of a tag specifically binding to the modification (Thygesen et al., 2018). Computational tools for the detection of any PTMs in complex proteomics samples have also been implemented (Devabhaktuni et al., 2019; M. M. Savitski et al., 2006). Yet, these workflows are mainly efficient for stable modifications only. In addition, the PTM occupancy is rather low in most cases, hindering their identification. As a result, mostly unmodified peptides are identified.

Redox proteomics faces similar issues. Several mass spectrometry-based technologies aimed at studying various proteins' redox-related modifications have also been implemented for irreversible modifications (J. Yang et al., 2016). Nonetheless, studying other types of modifications is challenging due to their rapid dynamics. Indeed, these PTMs can often be enzymatically reverted by the antioxidant systems. Moreover, some of these PTMs occur *in vitro* during sample preparation such as oxidation of methionine that is routinely added to proteomics search engines in shotgun experiments due to its high occurrence rate (Nielsen et al., 2006). These characteristics stress the need to "freeze" the modification on the protein. This approach can be similar to the strategy used in phosphoproteomics where kinases and phosphatases are heat-inactivated to avoid the removal of the phosphorylation (Humphrey et al., 2018).

The importance of disulfide bond formation for protein structure promoted the development of labelling-based strategies to study system-wide cysteine oxidation. Among such labels, iodoTMT (Qu et al., 2014), iTORC (Albertolle et al., 2019), CysPat (H. Huang et al., 2016), and SICyLIA (Van Der Reest et al., 2018), were successful in addressing the issue since the chemical tags can be added directly to the lysis buffer. Indeed, these tags irreversibly bind to reduced cysteines preventing further modification during sample preparation. The first step is to label reduced cysteines in the cells with the first tag (Figure 5). Then, oxidized cysteines are reduced with a reducing reagent such as DTT and labelled with a second tag. The proportion of oxidized to reduced cysteines in different conditions is accurately measured by the reporter ion intensity (Figure 5). One of the drawbacks of these methods is that they erase or disregard the information about the identity of the modifications that are present on the cysteines. Reversible cysteine modifications include disulfide formation (Sevier & Kaiser, 2002), nitrosylation (Fernando et al., 2019), sulfenylation (Devarie-Baez et al., 2016), glutathionylation (Grek et al., 2013), persulfidation (Kasamatsu et al., 2016), polysulfidation (Kasamatsu et al., 2016) and palmitoylation (Jin et al., 2021). Cysteine labelling methods summarize all these modifications into a single readout. In addition, hardly reversible or irreversible cysteine modifications such as sulfinylation and sulfonylation (Beedle et al., 2016; Chauvin & Pratt, 2017; Van Bergen et al., 2014), will be completely left out of the analysis due to the impossibility to reduce them and to subsequently label the reduced thiol (Day et al., 2021). Consequently, the interpretation of the results of such analysis should always be considered with caution as they could be subjected to over or misinterpretation. Finally, cysteine-containing peptides need to be detected. This is not always the case in LC-MS/MS-based proteomics, since only a fraction of the peptides from a protein can be measured. One unique advantage of CysPat is that the tag contains a phosphate group and thus the enrichment strategy is the same as in phosphoproteomics, allowing one to study in parallel the crosstalk between redox signalling and phosphorylation cascades.

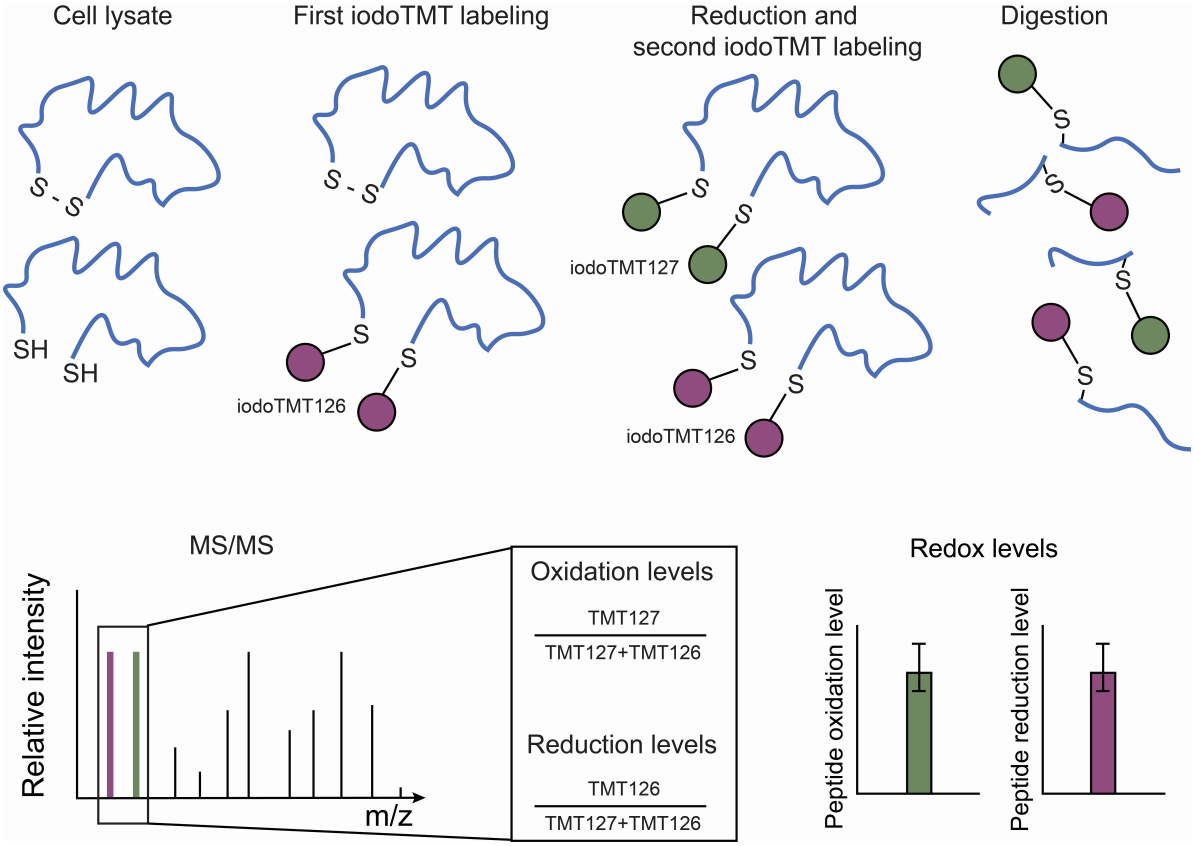

Figure 5. Labelling of cysteine-containing proteins by iodoTMT. Scheme of the labelling strategy used in redox proteomics to measure cysteine oxidation levels in peptides. Cells are lysed and a first TMT is added to bind to the reduced thiol on cysteines. Then oxidized cysteines are reduced with a reducing agent such as DTT and labelled with a second TMT. Proteins are digested and peptides are analysed by LC-MS/MS. The MS/MS intensity from both TMTs allows calculation of the oxidation and reduction levels for each peptide.

1.3.5 Methods based on limited proteolysis

One of the unique advantages of mass spectrometry-based proteomics over other existing technologies is that it enables the proteome-wide study of protein conformational changes reflected on protein stability. Several methods have been developed to address the issue, for instance, Drug Affinity Responsive Target Stability (DARTS) (Lomenick et al., 2009). This technique is based on the same principle as pulse proteolysis classically used with Western blot (Park & Marqusee, 2005). DARTS relies on the fact that the binding of a drug will cover enzyme-cutting sites resulting in a difference in levels of the covered peptides in shotgun proteomics compared to a non-treated condition. Since only peptides that are in contact with the chemical are affected, it is also possible to pinpoint the location of the binding site. DARTS has been routinely used for detecting binding events of small molecules on proteins or protein-protein interactions in multiple diseases and organisms (Cai et al., 2018; Gautam et al., 2018; Geng et al., 2019; Lazarev et al., 2018; Q. Li et al., 2018; Nampoothiri & Rajanikant, 2019; Rodriguez-Furlan et al., 2017; Tanie et al., 2018; Z. Yang et al., 2017).

Another method that relies on a similar principle is Limited Proteolysis coupled to MS analysis (LiP-MS) (Feng et al., 2014; Schopper et al., 2017). Usually, trypsin or lysyl endopeptidase (LysC) is used to produce peptides needed for bottom-up analysis. Here the first digestion with a broad protease such as proteinase K produces unspecific peptides

enabling the detection of structural changes. LiP-MS was employed in yeast to study conformational alterations occurring in around 300 proteins upon changes of growth conditions (Feng et al., 2014). Interestingly, the authors of the study could detect both subtle and more permanent conformational changes. Subsequently, the authors studied changes in protein conformation associated with heat shock and showed that a specific subset of proteins is denatured when cells reach temperatures that begin to reduce their fitness (Leuenberger et al., 2017). Since then, LiP-MS was used to highlight protein-metabolite and protein-protein interactions (Piazza et al., 2018), to detect the binding site of a drug (Piazza et al., 2020) or nanoparticle (Duan et al., 2019) on a protein and, in general, to detect chemical modifications that do not necessarily alter protein levels (Cappelletti et al., 2021; de Souza & Picotti, 2020; Pepelnjak et al., 2020). However, one of the drawbacks of both DARTS and LiP-MS is that only a fraction of the proteome can be analysed due to the limitations of enzymatic digestion. In addition, specific peptides mapped at the site of the conformational change are sometimes not identified. These caveats greatly cripple the use of these methods in a high throughput context and for analysis of low abundant proteins.

1.3.6 Protein thermal stability measurements

The Cellular Thermal Shift Assay (CETSA) has been developed to monitor protein thermal stability using Western blot (Jafari et al., 2014; Molina et al., 2013). CETSA is based on a previously described technique to detect the binding of a chemical to a purified protein by measuring changes in thermal stability (Pace & McGrath, 1980; Pantoliano et al., 2001). Briefly, cell lysates, whole cells, or animals (Molina et al., 2013) are treated with the compound of interest and vehicle control. Then, samples are aliquoted and heated with a temperature range, usually from 37 to 67°C, which will cause proteins to gradually unfold and aggregate. The aggregates are cleared by centrifugation and the soluble fraction is then analysed by Western blot to record the melting profile of the proteins of interest. A thermal shift between the samples treated with the drug and the samples treated with the vehicle highlights protein-drug interaction. Importantly, animal experiments (and cell experiments to some extent) also include pharmacokinetics aspects that are crucial for drug development. Nonetheless, this approach relies on *a priori* knowledge of drug targets. The existence of suitable antibodies against the target of interest is also a requirement in CETSA. Recently, the method was used to screen for thymidylate synthase inhibitors in a high throughput setup and successfully identified new inhibitors (Almqvist et al., 2016).

TPP or MS-CETSA was adapted from CETSA as an LC-MS/MS-based method, turning CETSA into a proteome-wide and unbiased approach (M. M. F. Savitski et al., 2014) (Figure 6). Using TMT10 labelling, the authors were able to record protein melting profile of >7000 proteins and to accurately identify multiple targets of kinases inhibitors. Moreover, one of the advantages of TPP is that it can measure downstream effects of protein target inhibition in the cell experiments and differentiate them from direct interaction with the drug in the lysate experiment. 2-dimensional TPP (2D-TPP) was also introduced to simultaneously monitor temperature as well as drug concentration-dependent protein unfolding (Becher et al., 2016) (Figure 6). TPP discovered FECH as the off-target of several kinase inhibitors, explaining light-sensitivity experienced by treated patients (M. M. F. Savitski et al., 2014). The method

was also used to study the binding of metabolites, such as ATP and GTP and was adapted to increase the recovery of membrane-bound proteins using a low concentration of detergent (Reinhard et al., 2015). Later, TPP was adapted for deconvolution of targets of antibiotics in bacteria (Mateus et al., 2018), was used to study ROS effect on protein structure (Sun et al., 2019), and correlated thermal stability changes to phosphorylation (J. X. Huang et al., 2019). In a more biological context, TPP was employed to monitor protein stability changes associated with different phase-transitions of the cell cycle (Becher et al., 2018; Dai et al., 2018). Finally, TPP was used to study the effect of genetic mutations on protein thermal stability (Mateus et al., 2020; Peck Justice et al., 2020) and was used to monitor drug-protein interaction in treated animals (Perrin et al., 2020).

Overall TPP opens new possibilities in mass spectrometry-based chemical proteomics because of its versatility and adds a new dimension in studying protein characteristics. However, there is a lot of room for improvement of the method as it lacks throughput and, therefore, is costly to use. Another drawback is that TPP involves arbitrary curve fitting in most cases (Franken et al., 2015; Jarzab et al., 2020; Kalxdorf et al., 2021; Leijten et al., 2021; Mateus et al., 2018; Peck Justice et al., 2020; Reinhard et al., 2015) (Figure 6). The problem of this model is that the sigmoidal curve fitting equation is designed for proteins that have an expected and predefined melting behaviour characterized by a decrease in solubility with an increase of temperature, which is not the case for all proteins. The same issue also arises for highly stable proteins that do not unfold at the maximum temperature. This inevitably leads to an absence of curves and estimated melting temperature (Tm) for a lot of proteins. New methods for the analysis of TPP output are under development to overcome this limitation. In 2D-TPP for example, (Becher et al., 2018) established a scoring system relying on a bootstrap algorithm and FDR calculations to set up cut-offs for significance. However, this approach still relies on complex calculations and the field would benefit from a simplified method that could be widely used.

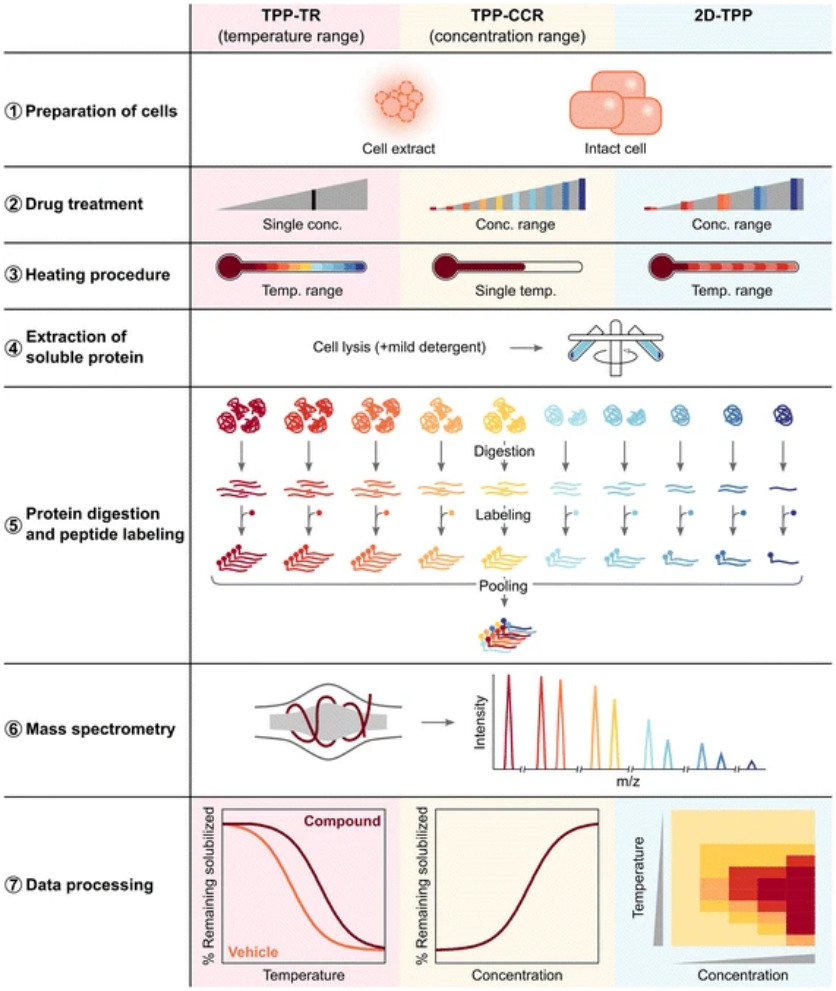

Figure 6. Scheme of the various versions of TPP. 1) Intact cells or cell extracts are prepared. 2) Then drug treatment is performed either in single (TPP-TR) or multiple concentrations (TPP-CCR and 2D-TPP). 3) Samples are heated with a single temperature (TPP-CCR) or a temperature range (TPP-CCR and 2D-TPP). 4) Cell lysis is performed and followed by centrifugation to eliminate protein aggregates. 5) Soluble proteins are digested, then peptides are labelled with isobaric tags, and samples are multiplexed. 6) Samples are analysed by LC-MS/MS. 7) Data processing involves sigmoidal curve fitting for TPP-TR and TPP-CCR while 2D-TPP uses 2-dimensional heatmaps of protein FC and a different scoring system (Becher et al., 2018). Reprinted from (Mateus et al., 2016).

1.4 PROTEOMICS ATLASES

With technical development in proteomics, most conventional approaches have improved in throughput and analytical depth, which opened the possibility of creating atlases or resources of increasing complexity. These resources were focused on various systems and proteomics dimensions. In some resources, protein expression was measured in various cell types or tissues and authors have measured protein expressions in up to 375 different cancer cell lines (H. J. Johansson et al., 2019; Nusinow et al., 2020; D. Wang et al., 2019). We also produced the ProTargetMiner resource (Saei et al., 2019). ProTargetMiner is the first connectivity map-like resource in proteomics and allows to explore protein expression variation upon anticancer treatment of 56 drugs with some measured in several cell lines. Expression measurements were also used to determine protein subcellular localization with multiple comprehensive resources (Christoforou et al., 2016; Itzhak et al., 2017; Joshi et al., 2019; Orre et al., 2019). In another study focused on drug target deconvolution, kinobeads were utilized to evaluate the target landscape of 234 kinase inhibitors approved for therapeutic use in clinics or involved in clinical trials (Klaeger et al., 2017). Now that other dimensions have been defined in proteomics, other atlases such as the Meltome atlas have been produced (Jarzab et al., 2020) where the authors measured protein thermal stability using TPP across several cell lines and 13 different species, from archaea to human. The functional landscape of E. Coli was also studied by analysing 121 genetic alterations in E. Coli using TPP (Mateus

et al., 2020). Finally, a combination of multiple disciplines including genome engineering led to a tour de force analysis of 1311 CRISPR-edited cell lines to study protein localization (Cho et al., 2021). These extensive resources are valuable for the proteomics community as well as for biological sciences in general as they offer the possibility to other researchers to analyse pre-existing multidimensional datasets without the cost of producing them. However, while increased throughput and analytical depth allowed for such studies, they are only possible in a handful of research laboratories as they demand a consequent analysis time, material and they increase the data analysis burden. Therefore, new developments in higher throughput methods and simplified analysis and visualization tools are still highly needed.

2 RESEARCH AIMS

2.1 MAIN OBJECTIVES

The main objectives of the present work were to develop new mass-spectrometry-based proteomics methods and visualization strategies and combine them with pre-existing methods to study the cellular dynamics and the mechanisms of anti-cancer compounds. These methods were developed to uncover the unknown mechanisms of action and targets of promising anti-cancer treatments as well as to discover biomarkers playing a role in cell type transitions that can be targeted to modulate cell fates.

2.2 SPECIFIC AIMS OF THE THESIS

1- To study cellular dynamics in *in vitro* cell culture using proteomics methods (**papers I and V**).
2- To further develop proteomics approaches for drug target deconvolution and for target discovery (**papers II and V**).
3- To combine proteomics approaches to uncover molecular clues on drug treatment and cellular events (**papers III, IV, and V**).
4- To merge proteomics analysis and create a new data visualization tool (**paper V**).

3 MATERIALS AND METHODS

3.1 CELL CULTURE

We mainly used human and mouse cancer cell cultures which were maintained in a medium consisting either of DMEM or RPMI supplemented with 2 mM L-glutamine when not included in the formulation, 100 units/ml of penicillin/streptomycin mix, and 10% FBS, this was the case for **papers I, II, and III**. In **paper IV** the medium was supplemented with an additional 250 nM sodium selenite to avoid potential variation in selenium supplementation from different FBS batches (Leist et al., 1996). Finally, in **paper V** only two cancer cell lines were used throughout the article, and hFF, iPSCs, human embryonic stem cells (ESC), neurons, and EBs were used there. For pluripotent stem cells and differentiation protocols, a medium was specially formulated as described in the method sections of the articles. Various coating materials were also used including laminin 521, laminin 511, poly-L-ornithine, gelatin, and Matrigel as well as various small molecule inhibitors.

3.2 LABEL-FREE PROTEOMICS

Label-free proteomics is the classical quantification approach used in shotgun proteomics. In short, peptides are extracted from the cells with detergent (SDC, SDS) and/or chaotropic agents (Urea) then proteins are reduced using DTT and alkylated using iodoacetamide (IAA), digested overnight by LysC and for 6 h by sequencing grade trypsin. Then samples are desalted using C18 resins and injected into a liquid chromatography system coupled to a mass spectrometer. In this case, each individual sample is analyzed separately. The absence of intermediate steps in the protocol makes it faster than labeling-based methods for sample preparation. Label-free proteomics was used in **papers I, III, and V.**

3.3 TMT LABELING

Contrary to label-free proteomics, in label-based shotgun approaches, samples are labeled with a chemical tag. This allows us to identify the samples and multiplex them to save LC-MS/MS analysis time and reduced the missing values between samples in the same set. In **papers I, II, III, IV, and V** we used a combination of various TMTs, TMT10 in all articles, TMT11 in **paper V,** and iodoTMT in **papers III and IV**. Classical TMTs react to primary amines while iodoTMTs comprise a cysteine reactive group that binds to cysteines (Figure 7). Instead of measuring peptide abundance, sequential iodoTMT labeling allows the measurement of cysteine oxidation levels on peptides. The protocol is different for both versions. For TMT samples after cell lysis, equal amounts of proteins are reduced with DTT, alkylated with IAA, and samples are cleaned using methanol chloroform protein precipitation to exchange buffer, and then they are resuspended into 8M urea and EPPS. Since TMTs label free amine groups, buffers such as ammonium bicarbonate or Tris cannot be used for labeling. Then, samples are digested overnight with LysC and then 6 h with trypsin. Subsequently, TMTs were added corresponding to four times the protein amount in the

samples and ACN is added to the samples to a concentration of 20% to facilitate labeling. The reaction is quenched using 0.5% hydroxylamine and samples are combined. For PISA-Express a pooled sample corresponding to one-tenth of each sample was created as a linker, labeled with TMT11, and used for normalizing protein abundances between TMT11 sets.

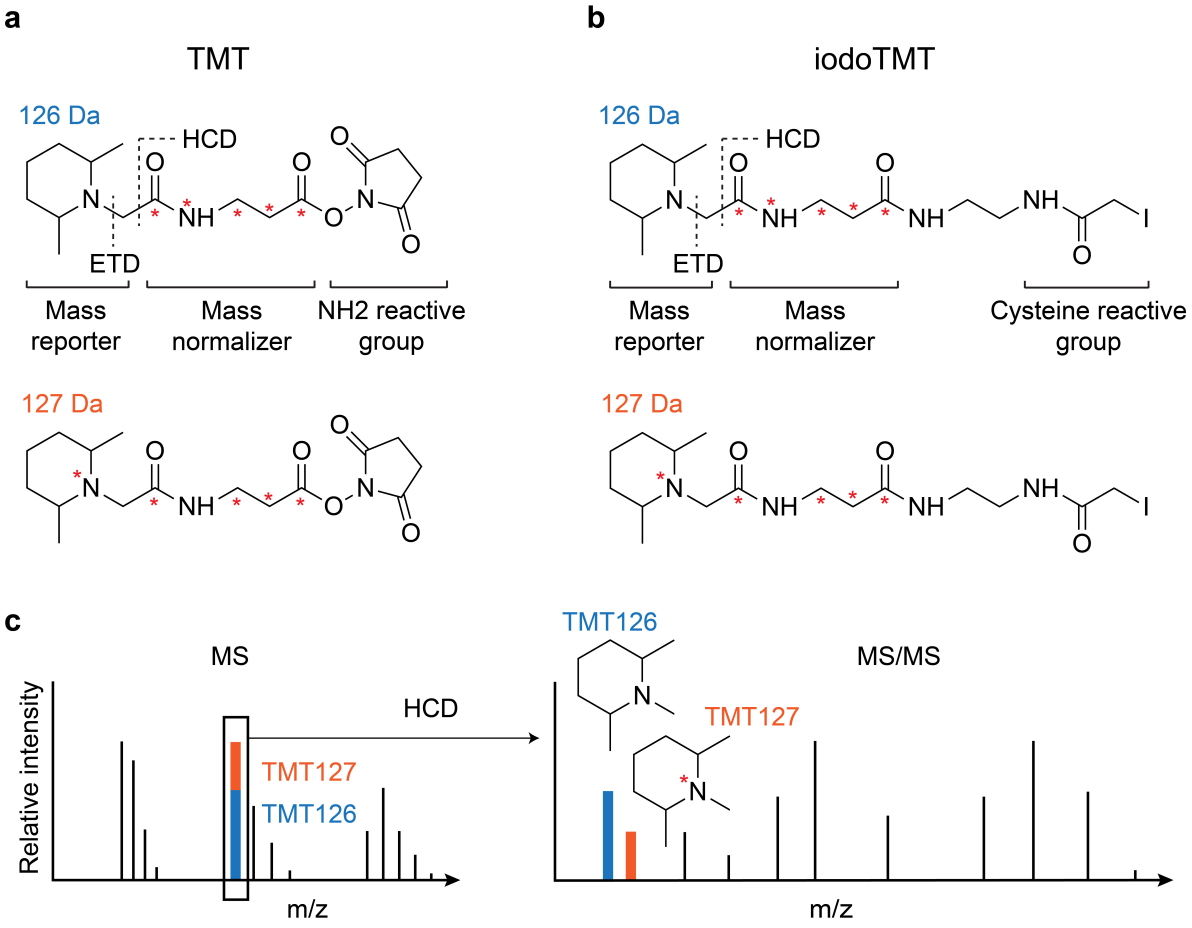

Figure 7. Structure of the Tandem Mass Tag. The isobaric mass tag consists of a mass reporter, a mass normalizer, and a reactive group. The difference between TMT (a) and iodoTMT (b) lies in the reactive group. TMT reacts to primary amines (NH2) while iodoTMT reacts to cysteines exclusively and thus measurements are limited to cysteine-containing peptides only. A different number of carbon-13 and nitrogen-15 (red star) are positioned on the mass reporter to identify different tags thanks to the mass difference. The weight of the tag is balanced by the mass normalizer in the opposite way so that the overall mass is the same for each tag. Because of this, only one mass is detected in a full MS spectrum for a mixture of the same peptides labeled with different TMTs (c). The mass reporters are detached during ETD or HCD fragmentation for MS/MS and each of them produces a unique mass signal. The intensity of the reporter ion signal corresponds to the abundance of the peptide allowing deconvolution of the multiplexed samples and relative quantification.

3.4 HIGH-PH REVERSED-PHASE PEPTIDE FRACTIONATION

Sample multiplexing allowed for off-line pre-fractionation of the samples. One of the main issues in data-dependent acquisition is that the dynamic range of protein abundance leads to the overrepresentation of abundant proteins in spectrums. Thus, to circumvent this problem samples can be fractionated with an orthogonal method to reversed-phase low-pH chromatography to split the LC gradient into several containing a portion of the peptide

population only. One of the most common methods is high-pH reversed-phase chromatography that is semi-orthogonal to its low pH counterpart. This is what was used in all the articles presented in this thesis, either using Pierce™ High pH Reversed-Phase Peptide Fractionation Kit (Thermo Fisher Scientific) resulting in 8 fractions (**papers III and V**) or 100 to 250 µg of peptides were injected into an UltimateTM 3000 RSLCnano System (Dionex) equipped with an XBridge Peptide BEH 25 cm column of 2.1 mm internal diameter, packed with 3.5 µm C18 beads with 300 Å pores (Waters). Peptides were eluted with a buffer A (20 mM NH_4OH in H_2O) and B (100% ACN) from 1% to 63% B and collected into 96 fractions which were then concatenated into 12-24 (**papers I, II, IV, and V**).

3.5 MASS SPECTROMETRY ANALYSIS

Various instruments and LC systems were used in the articles presented in this thesis. In terms of the mass spectrometers, we used Orbitrap Q Exactive, Orbitrap Q Exactive plus, Orbitrap Q Exactive HF, Orbitrap Elite, Orbitrap Fusion, and Orbitrap Fusion Lumos (all from Thermo Fisher Scientific). In terms of LC systems, we used the EASY-nLC-1000 system (Thermo Fisher Scientific) and the nanoflow Dionex Ultimate 3000 UPLC system (Thermo Fisher Scientific). Both systems were connected to an EASY-spray column (50 cm, 75 µm inner diameter, 2 µm PepMap C18 beads of 100 Å size). Peptides were resuspended into 0.1% formic acid (FA) and 2% acetonitrile (ACN) in H_2O buffer and around 1 µg of peptide per sample or fraction was injected into the LC system. The gradient consisted of a B phase (98% ACN, 2% H_2O, 0.1% FA) ranging from 2% to 26-36%. The acquisition was performed on data-dependent mode and the various parameters are detailed in the method section of each article. The major difference was that the resolution minimum for MS/MS had to be higher for TMT-labeled samples (minimum 35 000) and the isolation window lower 0.7 to 1.6 Da while it was 3 Da for label-free analysis.

3.6 PROTEIN QUANTIFICATION

All LC-MS/MS data were analyzed with several versions of MaxQuant software using the Andromeda search engine. For TMT/iodoTMT-based experiments, MS/MS quantification was selected while LFQ was selected for label-free. Carbamidomethylation of cysteine was selected as a fixed modification for samples that were not labeled with iodoTMT. For iodoTMT, no fixed modification was selected, iodoTMT was selected as MS/MS quantification instead. Oxidation of methionine and deamidation of asparagine and glutamine were selected as variable modifications for all types of samples. Trypsin/P was selected as the enzyme with up to two missed cleavages. The FDR was set to 1% for peptides and proteins and for the rest of the parameters we used the default settings of the software. The “Match between runs” option was selected with a time window of 0.7 min and an alignment time window of 20 min for each analysis including multiple samples or TMT sets. Finally, data were searched against either human or mouse protein databases from Uniprot. The reference is indicated in the method section of the articles.

3.7 DATA ANALYSIS

Quantification data from MaxQuant were analyzed with Excel and R. SIMCA was used for OPLS-DA modeling. Raw protein abundances (or intensity of TMT reporter ion) for each sample were normalized by the total abundance of all proteins in the sample except for TPP experiments where protein abundances were normalized by the average abundance of the proteins in the 37ºC and 41ºC temperature points in the corresponding TMT10 set. In PISA-Express, each protein abundance was also normalized by the corresponding TMT11 linker protein abundance prior to sample comparison, to remove batch effects.

4 RESULTS AND DISCUSSION

4.1 DYNAMIC PROTEOMICS REVEALS HIGH PLASTICITY OF CELLULAR PROTEOME: GROWTH-RELATED AND DRUG-INDUCED CHANGES IN CANCER CELLS ARE COMPARABLE.

In **paper I**, we studied cellular proteome dynamics in *in vitro* cancer cell cultures treated by anti-cancer drugs, vehicle and untreated. The rationale behind these experiments is that *in vitro* cell culture is widely used throughout biological sciences and it is considered a stable and controlled system. Time series analysis usually contains time-matched controls, but it is not always the case as the effects of cell culture variations on the proteome are often overlooked since they are not necessarily visible phenotypically. Here we aimed to investigate the magnitude of these proteome changes and compare them to changes associated with anticancer drug treatment at IC50 concentration.

4.1.1 Proteome changes in the control group are equivalent to drug treatment at IC50 after 48 h.

To study the magnitude of proteome changes over time, we treated HCT116 cancer cells with MTX, paclitaxel (PCTL) at IC50, and the equivalent concentration of vehicle, dimethylsulfoxide (DMSO), for 48 h and collected samples at the start of the experiment and every 6 h. We measured protein abundances using label-free proteomics for 2116 different proteins quantified in all samples. To monitor proteome variation, we plotted the abundance changes using PCA (Figure 8a). We observe clear separation from the control sample group after 12 h and even some noticeable separation after 6 h of treatment for MTX and PCTL. The x-axis corresponding to PC1 seemed to reflect the variation over time and MTX had the higher variation after 48 h of treatment. Strikingly, the control group starts to also show separation after 12 h. The changes in the DMSO-treated control group at 48 h are on par with those in PCTL treatment. This was confirmed by examining the number of proteins showing significant changes at the various time points, where DMSO control numbers were on par with PCTL treatment (Figure 8b). This was particularly unexpected since the treatments that we used were at IC50 and thus highly lethal for the cells, while the concentration of DMSO was 4.5 ppm likely to be negligible. These changes in the control group are gradual with a maximum at 48 h and are likely to arise from phenomena such as cell division, degree of confluence, acidification of the medium.

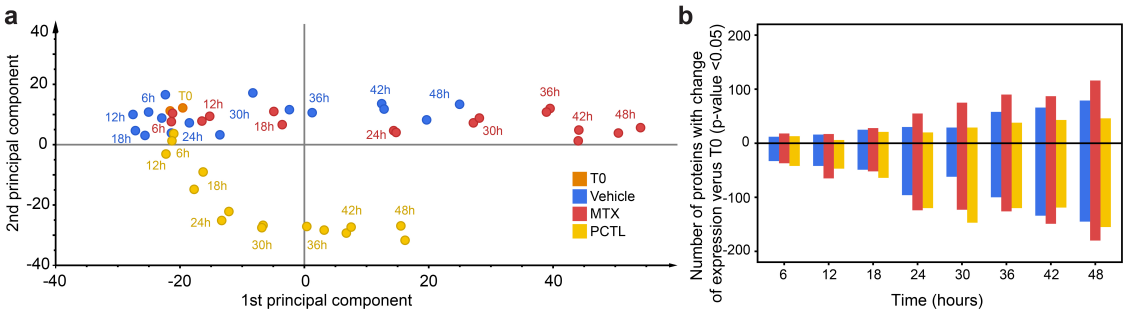

Figure 8: Proteome variations upon chemical treatment. (a) PCA of the 2116 proteins that were quantified in all samples for MTX, PCTL, and vehicle treatments (DMSO). (b) Number of proteins with p-value <0.05 calculated using an unpaired *t*-test against T0. Figure adapted from (Sabatier et al., 2018).

4.1.2 Proteome changes are not due to chemical treatment and are not cell line-specific.

Since in the pilot experiment the control group was still treated with DMSO we decided to study changes associated only with cell growth. To investigate naturally occurring variation in *in vitro* cell culture, we did not treat the cell lines that were tested this time. Additionally, to demonstrate that these changes are not cell-line specific only, we monitored the proteome of A375 and HCT116 for 48 h, collecting samples every 12 h starting 24 h after plating. To obtain a deep picture of the proteome evolution we chose to label samples with TMT10 labeling and multiplex them. This allowed us to fractionate the samples using high-pH reversed-phase peptide fractionation. We obtained quantitative data for 8898 proteins identified with at least two peptides. To evaluate proteome changes we only considered reproducibly quantified proteins with a coefficient of variation < 15% between replicates resulting in 6053 proteins. The resulting expression changes comparing each time point to the start of the experiment (T0) show that the proteomes of both cell lines start to change as early as 12 h and continue to gradually drift until 48 h (Figure 9). Importantly, the magnitude of the changes was similar in both cell lines. Thus, we concluded that the general proteome changes happen regardless of chemical treatment and are not cell line dependent.

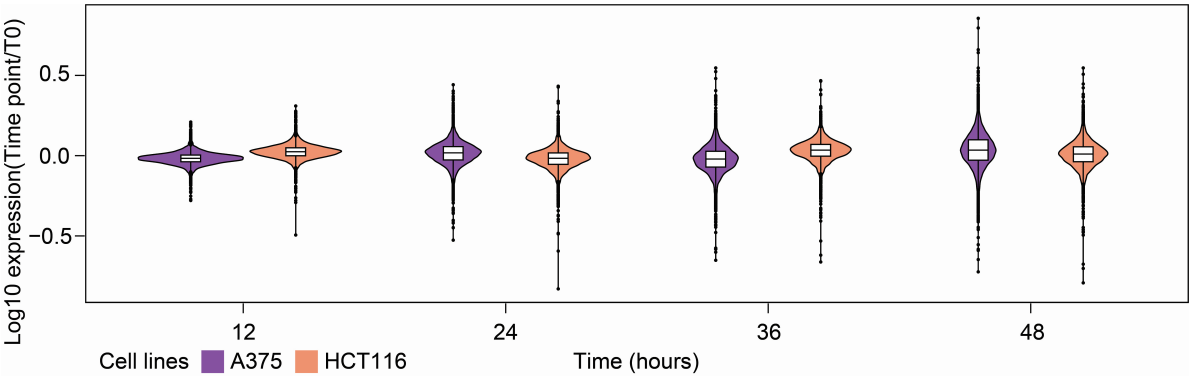

Figure 9. Gradual expression changes in A375 and HCT116 cells during *in vitro* culture. Violin plots of log10 scaled expression fold change (FC) against the starting point T0. Figure adapted from (Sabatier et al., 2018).

4.1.3 Proteins involved in cell division and metabolism are the most regulated over time in *in vitro* cell culture.

Finally, to investigate in detail whether specific pathways were activated or silenced over the course of the experiment, we used hierarchical clustering on the log scaled fold change (FC) at each time point against T0 followed by pathways enrichment using GO analysis of the clusters highlighted on the heatmap (Figure 10). The heatmap highlights pathways that are gradually upregulated and downregulated over time in both cell lines as well as cell line-specific regulated pathways. Among the common regulated pathways, those that were downregulated include mainly proteins that are linked to cell division, DNA replication, and ribosome biogenesis while upregulated pathways encompassed proteins involved in the oxidation-reduction process, cellular respiration, and organic acid metabolism. Therefore, the general trend was downregulation of pathways linked to cell division and upregulation of

pathways linked to energy production and metabolism. Strikingly, proteins involved in cell division were downregulated already starting from 12 h. This data, in light of the low confluence of cells suggest that cell division is affected even during what is commonly considered the exponential growth phase.

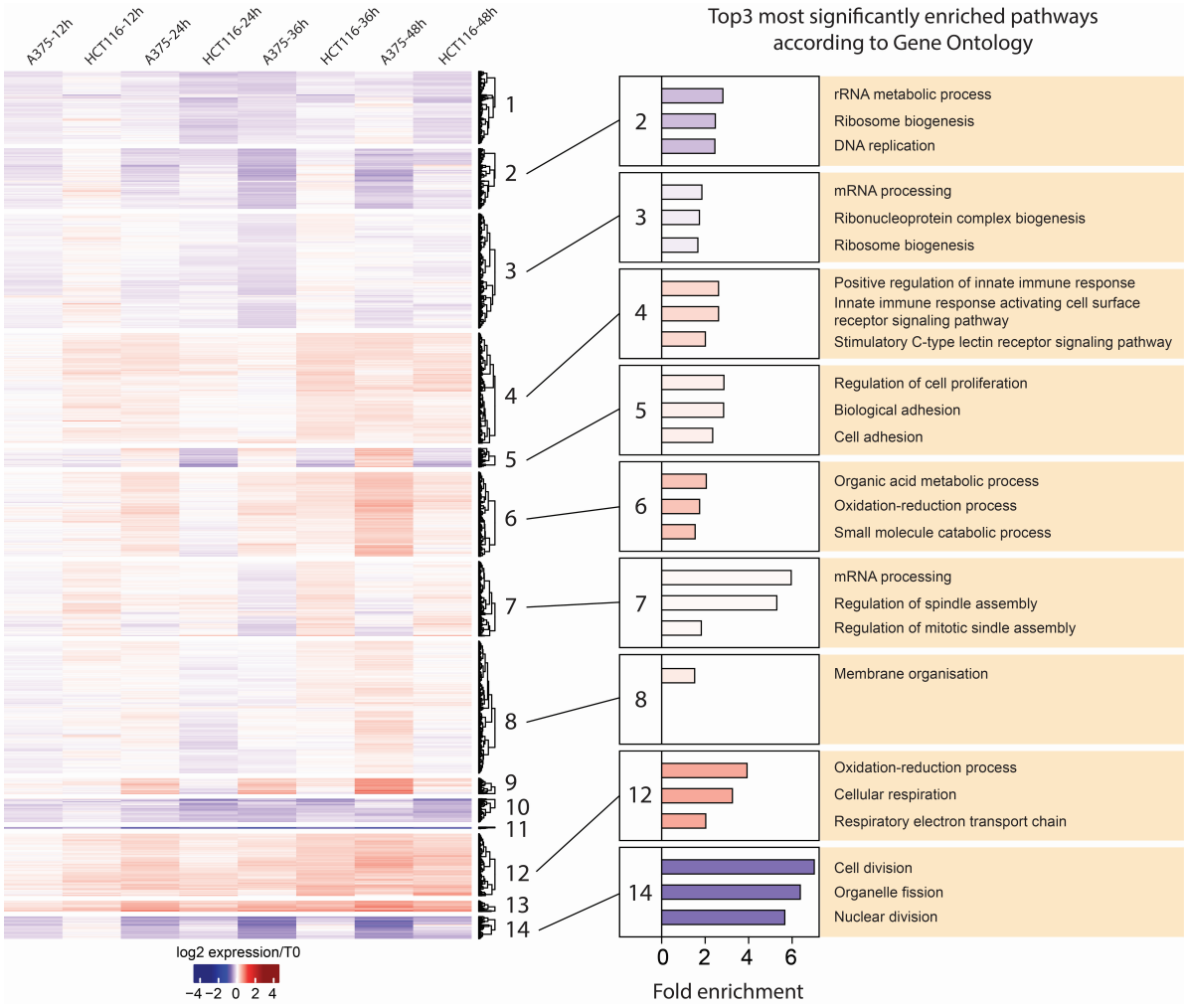

Figure 10. Proteome variation in A375 and HCT116. Heat map of mean protein log2 scaled FC at each time point compared to T0 (left) and fold enrichment of GO processes, with bars colored according to the average log2 scaled FC compared to T0 at 48 h for both cell lines combined (right). Figure reused from (Sabatier et al., 2018).

Conclusions

We studied the evolution of the cellular proteome over time in *in vitro* cell cultures treated with anticancer drugs MTX and PCTL, with DMSO and untreated. Our observations show that naturally occurring variations of the proteome are on par with variations induced by drug treatment at IC50. We also demonstrate that these changes are not cell type-dependent and emphasize the use of time-matched control in cell experiments. Importantly, the expression of proteins involved in the cell cycle and cell division was altered even at a low confluence. Finally, we provide a magnitude for this change as well as a deep dataset that can be explored for studying proteome changing happening over time in *in vitro* cell culture.

4.2 PROTEOME INTEGRAL SOLUBILITY ALTERATION: A HIGH-THROUGHPUT PROTEOMICS ASSAY FOR TARGET DECONVOLUTION.

Approaches based on measurement of protein solubility following a gradient or stepwise increase of the protein solubility-altering factor often rely on curve-fitting as a readout. TPP is one of the most prominent examples of such methods (Franken et al., 2015; M. M. F. Savitski et al., 2014). In 1D TPP (TPP-TR or TPP-CCR), cells or cell lysates can either be subjected to a fixed drug concentration and a temperature range or vice versa. While in 2D-TPP both a varying drug concentration and temperature gradient are used (Becher et al., 2016). In many cases, variations in protein thermal stability are primarily estimated based on the melting temperature (Tm) difference of a protein between two conditions. The Tm corresponds to the temperature where 50% of the pool of a protein is soluble and 50% is insoluble compared to the lowest temperature of the range. Tms are used to visualize the entire dataset on volcano plots for example, while curves can only be individually inspected. One of the issues with this approach is that only proteins with sigmoidal melting behavior can have a Tm estimate and thus many proteins that do not follow this pattern are disregarded in the analysis. Another problem is that in the 1D version, for one replicate, up to 10 temperature or concentration points are measured both for the control and treated condition while the Tm analysis generates only one data point. Thus, in **paper II**, we aimed to develop an alternative to curve-fitting and integral-based methods in proteomics and particularly protein solubility-based methods by combining the samples over a focused temperature range prior to analysis (Figures 11a, b, and c). The resulting method, Proteome Integral Solubility Alteration (PISA) assay considerably increases the throughput and reduces the amount of material and instrumental cost needed for such studies, and provides an easy readout.

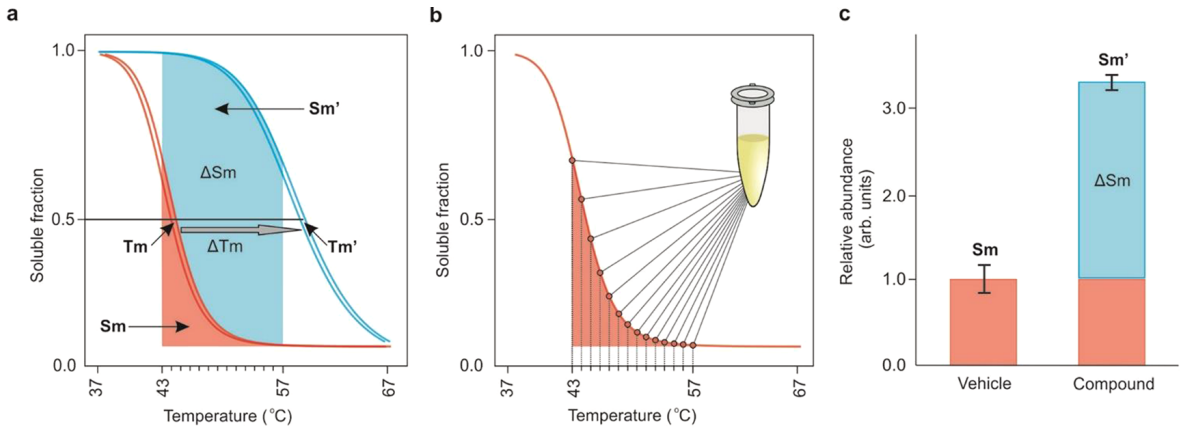

Figure 11. Schematic of PISA compared to TPP. (a) A focused temperature range is selected in the sigmoidal part of protein precipitation curves. Sm, Sm' and ΔSm being the area under the curve of the vehicle, compound and the difference between these two areas, respectively. (b) Samples corresponding to multiple temperature points are pooled. (c) ΔSm highlights the difference in the area under the curve between the vehicle and control. Adapted with permission from (Gaetani et al., 2019). Copyright (2019) American Chemical Society.

To verify that our approach could work, we first used theoretical calculations and calculations based on the available TPP datasets. We demonstrate that calculating the difference between the integral of the curves and the Tm differences highly correlate with R^2 of 0.999, 0.999 for theoretical measurements of temperature range and concentration range experiments, respectively. Then we also show that Tm differences and integral differences also correlate using data from published datasets with R^2 of 0.897 and 0.975 for TPP in cell and cell lysate, respectively (M. M. F. Savitski et al., 2014) (Figure 12a-f). This confirmed that analysis of the integral is as valuable as the analysis of the Tm.

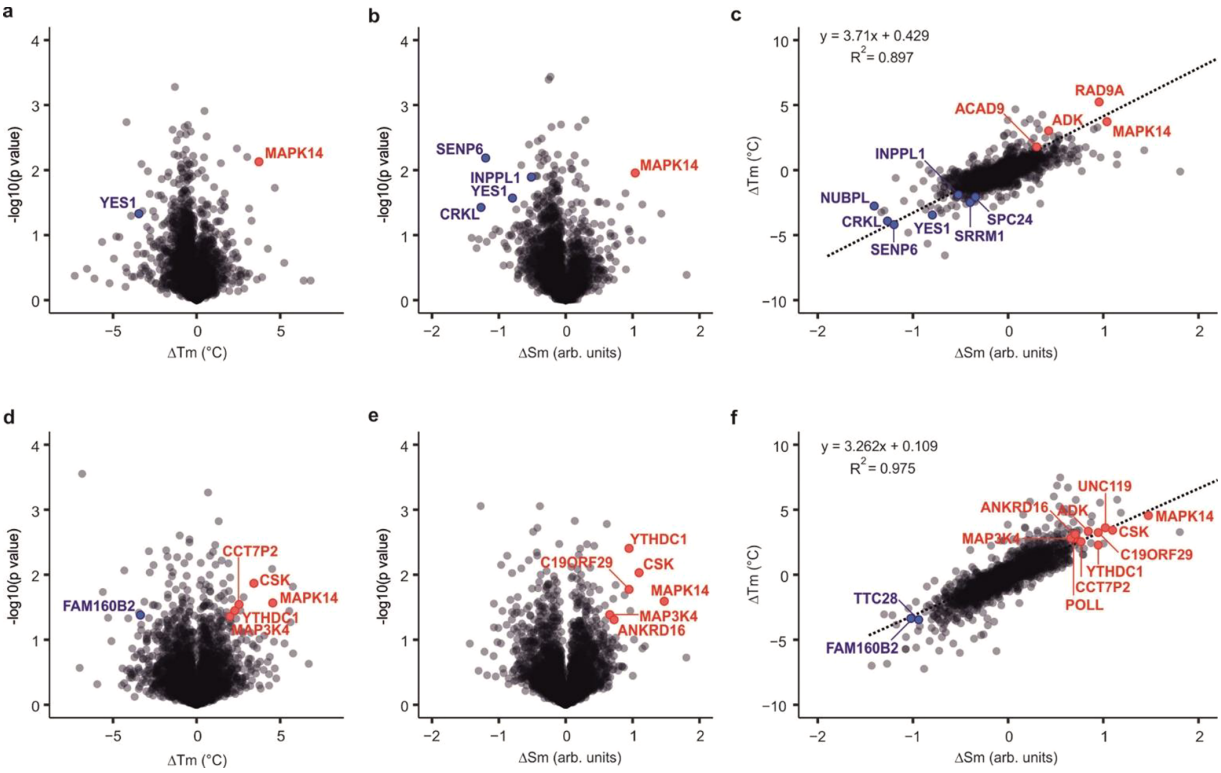

Figure 12. Theoretical calculations using pre-existing TPP datasets. (a), (b), (c) Volcano plots of ΔTm, ΔSm and 2-dimensional plot of ΔSm against ΔTm of intact cells treated with 5 µM dasatinib. (d), (e), and (f) same plots but in cell lysate. Data were extracted from a study by (M. M. F. Savitski et al., 2014). Reprinted with permission from (Gaetani et al., 2019). Copyright (2019) American Chemical Society.

Then we used well-studied drugs MTX and 5-FU as model drugs for our proof-of-concept experiment. We show using PISA with a focused temperature range, that DHRF and TYMS, respective targets of MTX and 5-FU, are identified as outliers in cell and lysate experiments (Figure 13). To showcase the higher throughput of the method, we analyzed 9 drugs in one TMT10 set per replicate and highlighted some of their targets. Finally, we show that concentration range analysis can be added to the measurements using only 3 samples (control, temperature range, concentration range) hosting an entire experiment in triplicate in one TMT10 set.

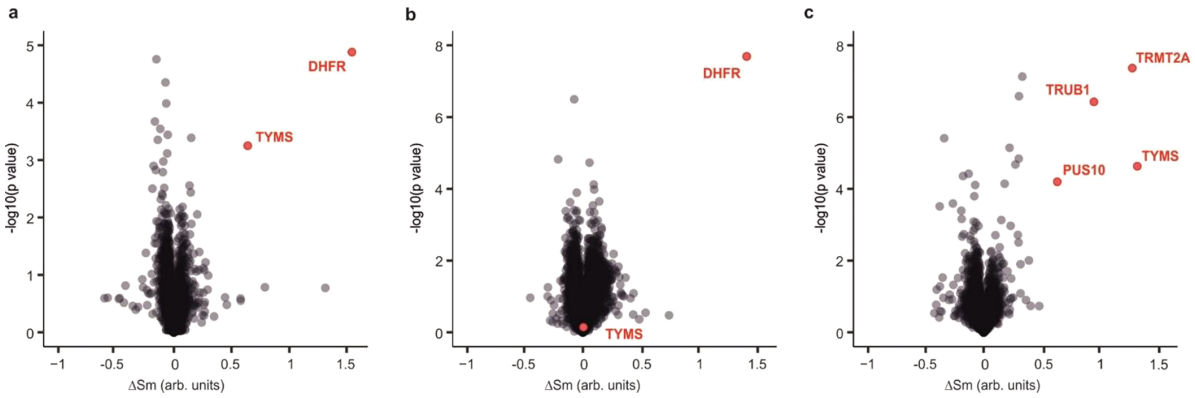

Figure 13. 1D PISA highlights targets of methotrexate and 5-fluorouracile. 1D PISA analysis of A549 treated with MTX in intact cell (a) and cell lysate (b). (c) 1D PISA analysis of A498 cells treated with 5-FU. Reprinted with permission from (Gaetani et al., 2019). Copyright (2019) American Chemical Society.

Conclusions

We developed a higher throughput assay for condensing data from dose-response curves and demonstrated its potential using TPP analysis as an example. The assay also uses less material than TPP, greatly reducing the cost of such analysis. The temperature range can be easily adapted to better fit a particular cell system as in TPP. This type of strategy can be used in various contexts such as in limited proteolysis, in various protein precipitation strategies including chaotropic agents, pH, temperature, pressure, salt concentration. In summary, these advantages make PISA analysis highly suitable for large-scale studies that are becoming a routine in the proteomics field. Here we also show that PISA can be complementary to FITExP in a first attempt to combine our in-house methods.

4.3 COMPREHENSIVE CHEMICAL PROTEOMICS FOR TARGET DECONVOLUTION OF THE REDOX ACTIVE DRUG AURANOFIN.

In **paper III**, we combined our in-house FITExP analysis with TPP and redox proteomics to study in detail the targets and MOA of AF. AF has been used primarily in the treatment of rheumatoid arthritis (Borg et al., 1988; Graham et al., 1991; N. H. Kim et al., 2010). However, it has regained interest in recent years as a potential anti-cancer drug and is now/was in clinical trials for several types of cancer (https://clinicaltrials.gov/). Multiple targets and MOAs have been described (De Luca et al., 2013; N. Liu et al., 2014; Tian et al., 2019) for the drug and particularly it is known to bind to and inhibit TXNRD1 (Marzano et al., 2007; Stafford et al., 2018; Zhang et al., 2019). Here we took on the task of elucidating the targets and MOA of AF in detail using chemical proteomics approaches.

4.3.1 Combination of chemical proteomics methods predicts auranofin targets and mechanism of action.

First, we performed FITExP analysis using MTX and PCTL as referenced drugs in HCT116, A375, and RKO cell lines, TPP in HCT116 cells, and cell lysate and redox proteomics in HCT116 (Figures 14a, b, and c). Proteins were ranked according to their regulations in each of the experiments and the individual rankings were combined into a final ranking. The top three proteins in the combined ranks were NFKB2, CHORDC1, and TXNRD1, respectively (Figure 14d). The proteins showed alteration in either or all analyses of expression, oxidation level, and thermal stability (Figures 14e, f, and g). The presence of TXNRD1 in the top 3 of the combined ranking of all analyses performed confirmed the utility of the combined approach for drug target deconvolution since it is known as a target of AF. Indeed, TXNRD1 ranked at best at 15^{th} position in FITExP analysis demonstrating that individual methods failed to accurately deconvolute it as a very top target hit. Additionally, NFKB2 is responsible for at least a part of AF anti-inflammatory effect and is known to be regulated by the TXRND1 system (Espinosa & Arnér, 2019; Flohé et al., 1997; Jeong et al., 2009), which is likely to explain its presence as the number 1 target hit. CHORDC1 presence as the number 2 target hit was mainly due to its high ranking in redox proteomics suggesting this might come from a downstream effect of TXNRD1 inhibition. Finally, the combined ranking for all methods was subjected to GO enrichment analysis. The results show that “oxidoreductase” is the top pathway, demonstrating that disruption of the redox balance is also part of AF general MOA (Figure 14h).

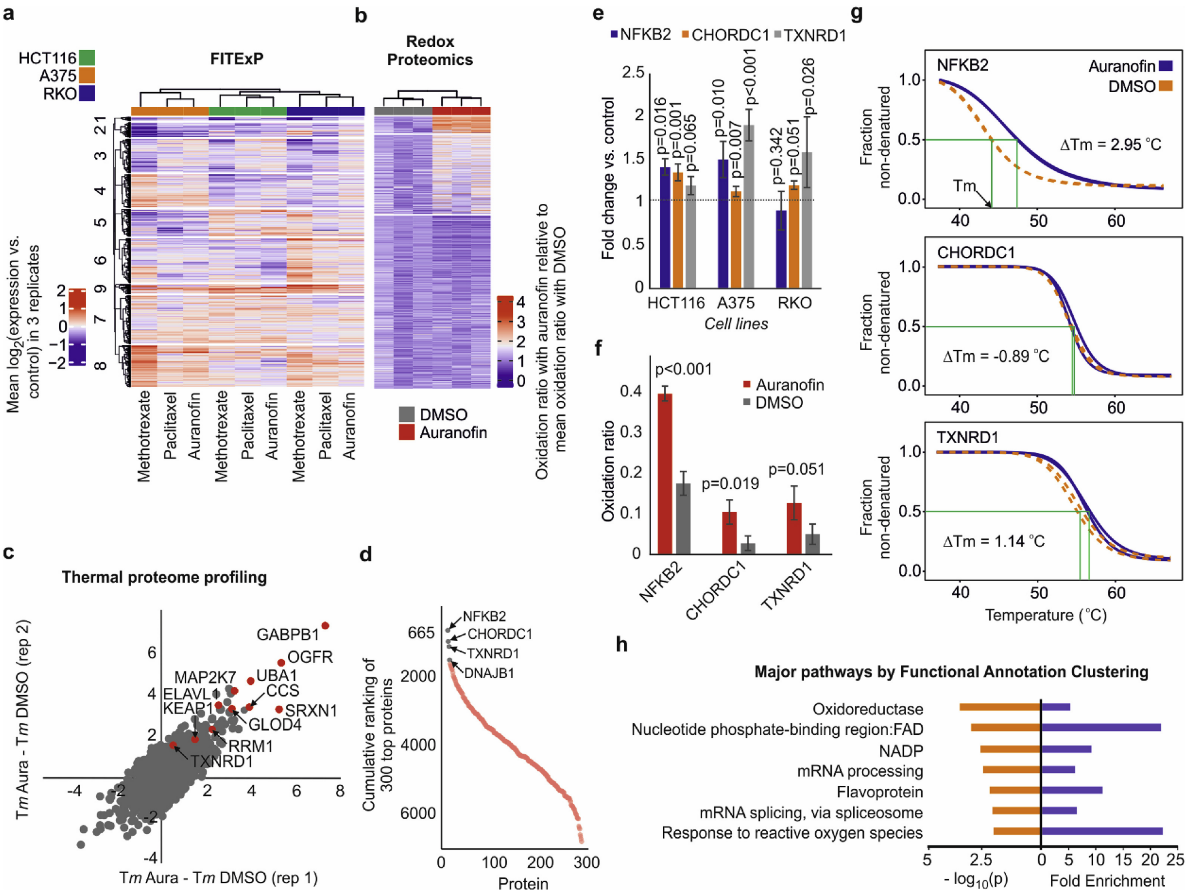

Figure 14. A combination of FITExP, TPP, and redox proteomics highlights auranofin target and mechanism of action. Hierarchical clustering of log2 scaled FC in FITExP (a) and cysteine oxidation ratios in redox proteomics (b). (c) TPP of auranofin in HCT116 cells. (d) Combined target ranking. (e), (f), (g) expression level, cysteine oxidation, and melting curves of the top 3 hits in ranking. (h) GO enrichment analysis using DAVID of the top 15 proteins in the combined ranking. Figure reused from (Saei et al., 2020).

4.3.2 Peptide cysteine oxidation levels link to thermal stability alterations.

Since TXNRD1 exerts many of its functions by reducing disulfide bonds in target proteins, we thought that modulation of such strong interaction would affect the overall stability of protein structure. Thus, we asked whether an increase or decrease in cysteine oxidation levels could be linked to changes in protein thermal stability. We plotted cysteine-containing peptides oxidation levels and highlighted peptides from proteins having a significant Tm shift in TPP analysis in cells. In total, 11 proteins that had altered thermal stability also had peptides with an increase or decrease in cysteine oxidation levels (Figure 15a).

Next, we asked whether these peptides could be attributed to the active sites of the enzyme, which would demonstrate that inhibition of TXNRD1 modulates their function. Particularly peptides covering the active sites of SRXN1 and PRDX5 had increased cysteine oxidation levels and both proteins showed altered thermal stability in TPP with Tm of +4.5 ºC and -1.7 ºC compared to DMSO treated control, respectively (Figures 15b and c). This showed that TPP could be used to detect post-translational modifications in accordance with previous observations (Becher et al., 2018; J. X. Huang et al., 2019; Sun et al., 2019).

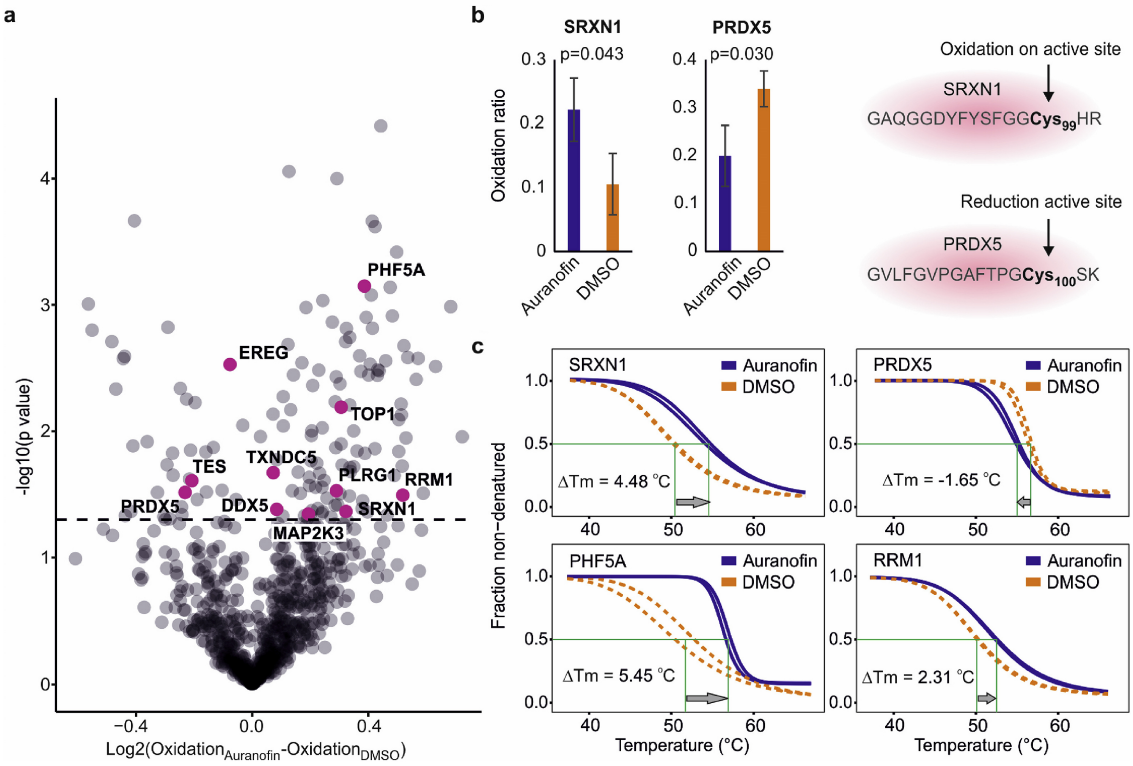

Figure 15: Cysteine oxidation levels and protein thermal stability. (a) Volcano plot of cysteines oxidation levels compared to DMSO control, peptides belonging to proteins that also show thermal shifts in TPP are colored in purple. (b) Peptides from SRXN1 and PRDX5 showing a significant change in oxidation level ($p < 0.05$) and sequence of the peptides highlighting the cysteine on the active site of the enzymes. (e) Melting curves of SRXN1, PRDX5, PHF5A, and RRM1. Figure adapted from (Saei et al., 2020).

Conclusions

A combination of chemical proteomics methods can uncover the target and MOA of a redox-regulating drug. Importantly, when analyzing the results of each method individually, the main target of AF treatment, TXNRD1 is not a top target hit and they failed to identify it as a putative target. However, when all methods were combined, TXNRD1 appeared as a top 3 candidate demonstrating the power of combined analysis. Lastly, we show that thermal shift can be linked to alteration of cysteine oxidation in proteins increasing the versatility of protein thermal stability measurements to detect PTMs.

4.4 COMPREHENSIVE CHEMICAL PROTEOMICS REVEALS THAT THE NEW TRI-1 AND TRI-2 COMPOUNDS ARE MORE SPECIFIC THIOREDOXIN REDUCTASE 1 INHIBITORS THAN AURANOFIN.

Following the success of our strategy in **paper III**, we decided to further investigate redox-modulating anti-cancer compounds. Two new Txnrd1 inhibitors, namely TRi-1 and TRi-2, have been recently identified and shown to have anticancer efficacy in mouse cancer models (Stafford et al., 2018) but their target landscape and MOA have not been investigated yet using unbiased approaches. Since we already studied Auranofin in human cancer cells and highlighted that its main mechanism of killing cells is likely to involve alteration of redox balance via Txnrd1 inhibition, we used AF as a reference point for the two other drugs. Considering the translational importance of our results for the understanding of effects in mouse cancer models, we used a similar combination of approaches with the treatment of two mouse cancer cell lines with several proteomics approaches, including FITExP, redox proteomics, and PISA developed in **paper II** instead of TPP. Additionally, we capitalized on our recent study of TXNRD1 substrates which showed that adding nicotinamide adenine dinucleotide phosphate (NADPH) as a co-factor with recombinant TXNRD1 enables the detection of disulfide bonds reduction by TXNRD1 in target proteins by TPP analysis (Saei et al., 2021). Thus, we added NADPH to one of the PISA in cell lysate experiment, representing another layer of information that could help reduce the number of potential false positive protein hits, by cross-validating the results with the other two PISA assays in cells and cell lysate. Finally, we previously showed that using OPLS-DA modeling to contrast one treatment against other treatments allows deconvoluting the drug MOA (Saei et al., 2018, 2019). Therefore, we used this approach in FITExP adding MTX, CAMP, and PCTL to contrast with our drugs of interest. The overall scheme of the experimental strategy is described in Figure 16.

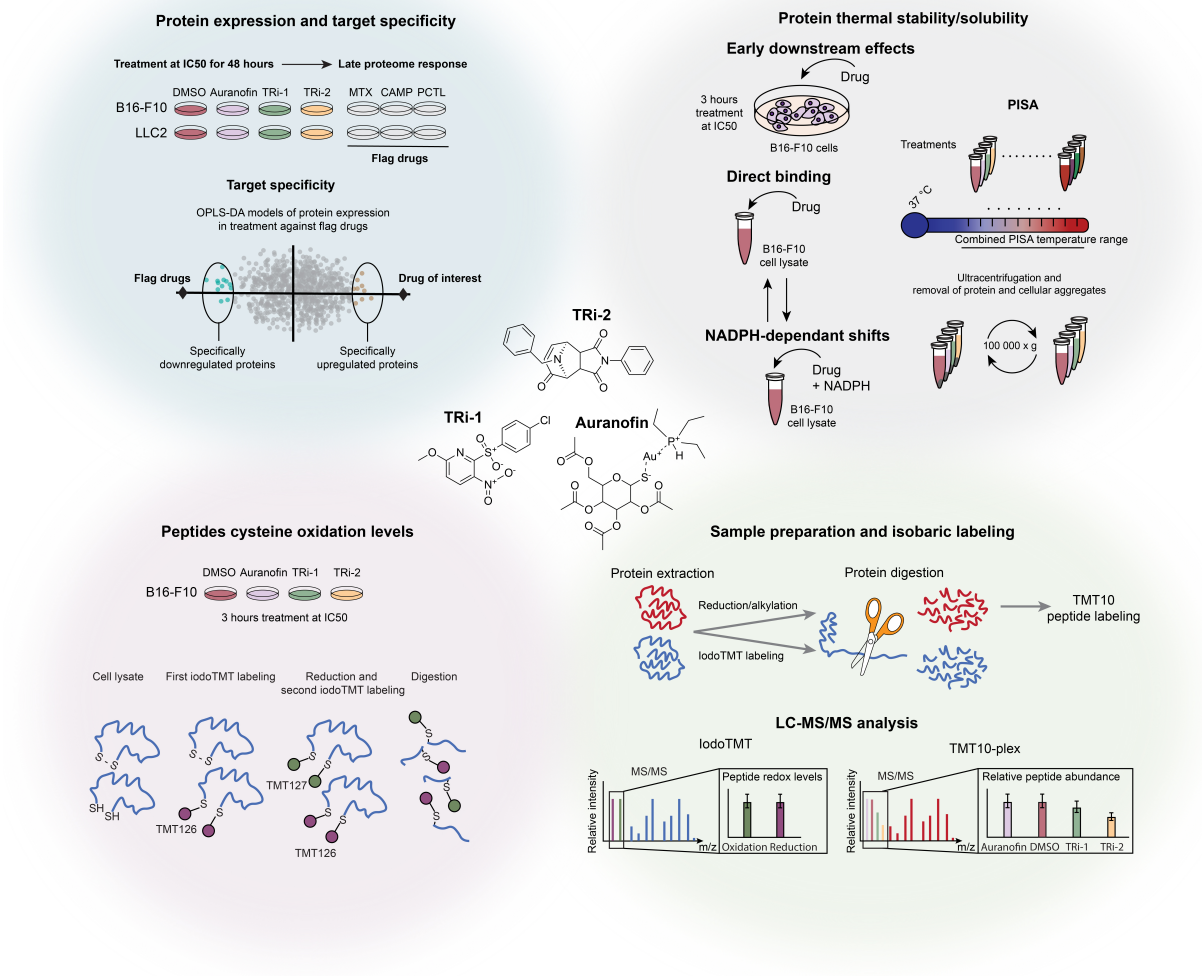

Figure 16. Combined chemical proteomics workflow. Protein expression and target specificity are measured by FITExP, PISA is used to evaluate early downstream effects, direct binding, and NADPH-dependent thermal shifts induced by the treatments. Redox proteomics measures cysteine oxidation levels in each treatment using iodoTMT labeling.

4.4.1 A combination of chemical proteomics approaches shows that auranofin has a wider effect on the cellular proteome than TRi-1 and TRi-2 and all treatments trigger oxidative stress.

First, we investigated the effect of each compound on the cellular proteomes of B16-F10 and LLC2 in FITExP and B16-F10 cell line only in PISA in cells and redox proteomics. We used B16 and LLC mouse cell lines as they can be used as tumor models in *in vivo* mouse experiments (D'Arcy et al., 2011; Kelkka et al., 2013; Winkelmann et al., 2006). Also, both the melanoma B16 and the lung adenocarcinoma LLC cells were earlier suggested to be dependent upon Txnrd1 for viability or tumor progression in mice (Hwang et al., 2015; Yoo et al., 2006; Zou et al., 2014). AF induced much higher variation in protein expression after 48h of treatment compared to TRi-1 and TRi-2 (Figure 17a). GO analysis of the proteins that were significantly upregulated in both B16-F10 and LLC2 cell lines in each treatment shows that all treatment increases of proteins involved in oxidative stress (Figure 17b). Although AF had the proteins showing the widest thermal stability shifts, TRi-1 had a higher number of

proteins shifting in cells while TRi-2 had the least effect on protein thermal stability (Figure 17c). Finally, all treatments had significantly higher cysteine oxidation levels than the DMSO control. TRi-2 showed the highest mean cysteine oxidation levels in peptides then Auranofin and finally TRi-1 (Figure 17d). Thus, redox proteomics showed that all treatments trigger oxidative stress and expression proteomics showed that they increase expression levels of proteins involved in oxidation-reduction processes probably as an adaptation of the cell to the treatments.

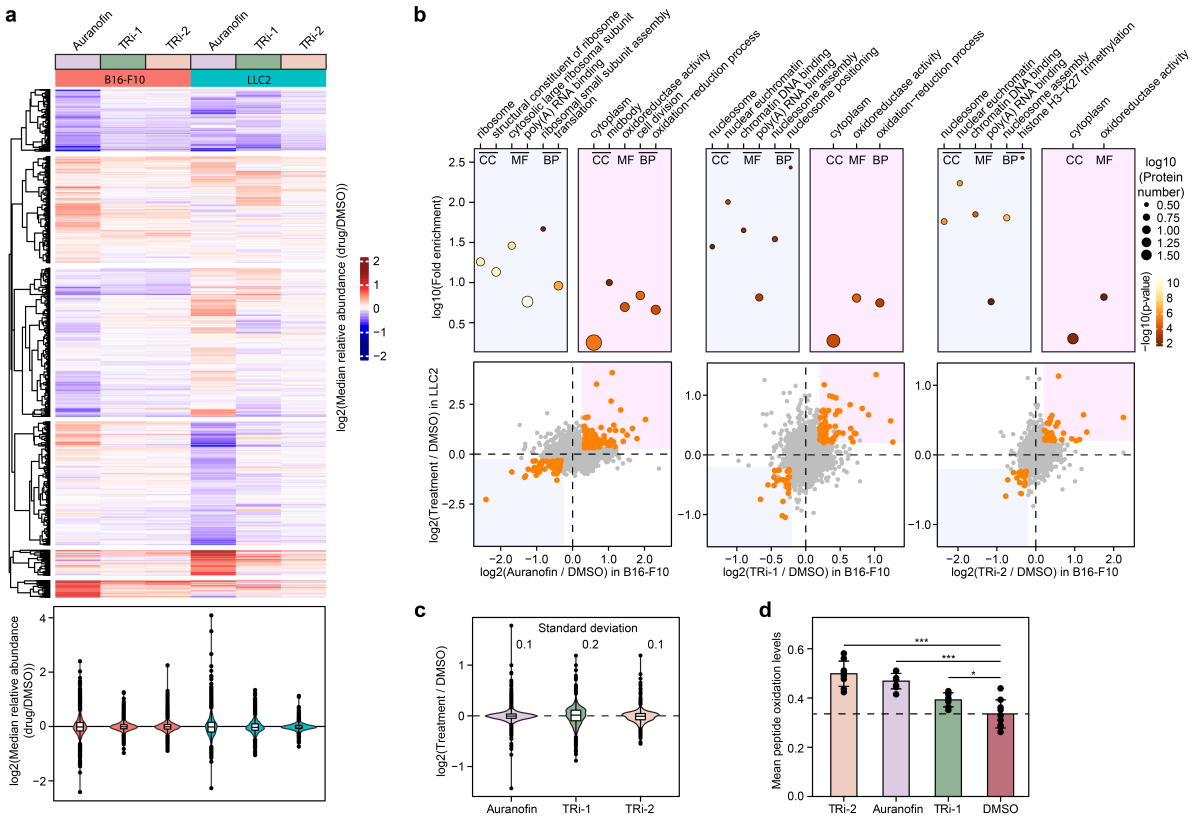

Figure 17. Protein expression, stability, and cysteine oxidation alterations induced by auranofin, TRi-1, and TRi-2. (a) Hierarchical clustering and violin plots of expression FC of each drug against DMSO control in B16-F10 and LLC2 cell lines. (b) Two-dimensional plots of protein expression FC of each treatment in B16-F10 against LLC2 (bottom) and GO pathways enrichment of the top up and downregulated proteins in both cell lines. (c) Violin plots of Sm FC from PISA analysis in B16-F10 cells. (d) Mean cysteine oxidation in treated samples, measured by redox proteomics in B16-F10 cell line. p-values were calculated using an unpaired *t*-test, error bars represent the standard deviation of the mean.

4.4.2 TRi-1 and TRi-2 are more specific Txnrd1 inhibitors than auranofin.

We then used OPLS-DA models to highlight the specificity of the drugs toward Txnrd1, by contrasting each treatment against reference drugs MTX, camptothecin (CAMP), and PCTL in B16-F10 and LLC2, as in ProTargetMiner (Saei et al., 2018, 2019). The OPLS models highlight the specific regulation of various proteins involved in oxidation-reduction mechanisms (Figure 18). Txnrd1 ranked 28th, 76th, and 2810th in TRi-1, TRi-2, and AF treatments, respectively. Additionally, combined PISA analysis highlights 15, 37, and 38 target hits for TRi-1, TRi-2, and AF, respectively (Figure 19 a). Thus, TRi-1 and TRi-2 are

more specific inhibitors of Txnrd1 than AF. Interestingly AF triggered high Nrf2 response as shown previously (Dunigan et al., 2018; Fuse et al., 2018; K. Johansson et al., 2017; N. H. Kim et al., 2010; Kipp et al., 2017), but not TRi-1 and TRi-2 suggesting that Txnrd1 inhibition alone may not necessarily be enough to activate Nrf2. This is broadly in line with previous suggestions (Cebula et al., 2015), but suggests that Txnrd1 inhibition may combined with an additional, yet unknown, factor in order for Nrf2 activation to be triggered.

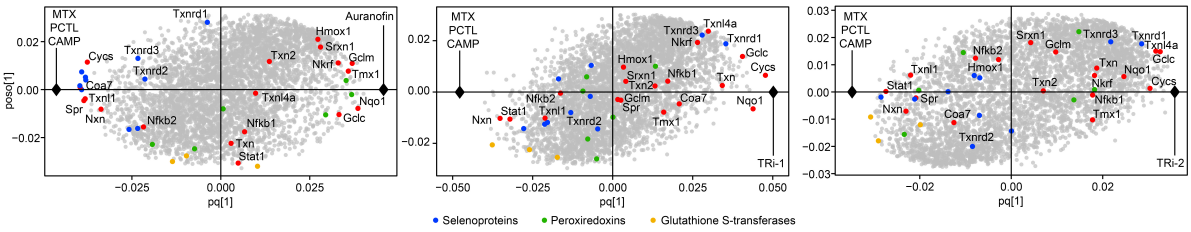

Figure 18. FITExP analysis using OPLS-DA models of auranofin, TRi-1, and TRi-2. OPLS-DA models contrasting AF, TRi-1, and TRi-2 cell lines against MTX, CAMP, and PCTL treatments in B16-F10 and LLC2, various redox-related enzymes are highlighted including Txnrd1.

4.4.3 Auranofin target landscape recapitulates some of the observed effects of the drug.

The target landscape of AF included Eefsec, Mcmbp, Gsk3a, and Gsk3b (Figures 19a-d). Incidentally, AF also led to specific and strong downregulation of selenoproteins as previously shown (Talbot et al., 2008), which has not been shown for TRi-1 and TRi-2. Selenoprotein P (SELENOP) depletion is known to inhibit the synthesis of selenoproteins and likely through decreased availability of selenium for Eefsec-dependent selenoprotein translation (Burk et al., 2008; Chang et al., 1990; Christensen et al., 1994; Mostert et al., 2003; Olsson et al., 1993). Thus, we hypothesized that AF inhibits Eefsec leading to a decrease in selenium incorporation in selenoproteins and their downregulation as a late response. Then, AF had Mcmbp as target hit stabilized in all three PISA experiments while members of the MCM complex were mostly destabilized. Mcmbp is known to disassemble the MCM complex likely by destabilizing each member (Nishiyama et al., 2011) and proteins that are in complexes are usually more stable than as free subunits (Tan et al., 2018). Mcmbp KD leads to the accumulation of DNA double-strand breaks (Quimbaya et al., 2014; Sedlackova et al., 2020) as does AF treatment (Boullosa et al., 2021). Thus, we hypothesized that the increased DNA double-strand breaks upon AF treatment stems from its effect on Mcmbp. Finally, AF altered the stability of Gsk3a and Gsk3b. Inhibition of these proteins leads to anti-inflammatory effects (Schrecengost et al., 2018) and AF was used in the treatment of rheumatoid arthritis (Borg et al., 1988; Graham et al., 1991; N. H. Kim et al., 2010). Thus, anti-inflammatory properties of AF might originate in part from inhibition of Gsk3a and Gsk3b.

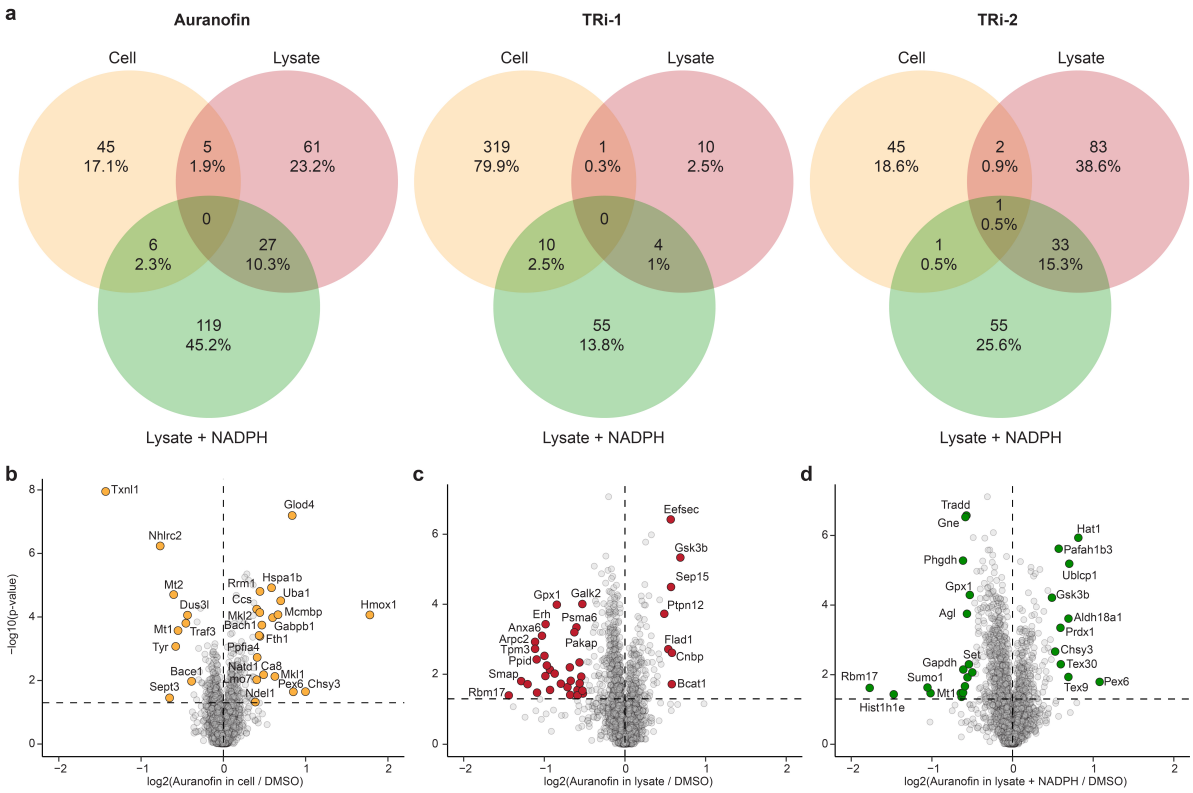

Figure 19. Combined PISA analysis. (a) Venn diagrams showing the number of shared target hits in each PISA in B16-F10 cells, cell lysate, and lysate + NADPH for AF, TRi-1, and TRi-2. (b), (c), (d) Volcano plots of Sm FC of Auranofin in PISA in cells, cell lysate, and lysate + NADPH, respectively.

Conclusions

Here we present a follow-up study of **paper III** adding our recently developed PISA and OPLS-DA modeling to our strategy for target deconvolution. We used AF and the recently discovered Txnrd1 inhibitors TRi-1 and TRi-2 and identified effects that are specific to Txnrd1 inhibition and drug-specific effects. According to our combined analysis, TRi-1 and TRi-2 are more specific inhibitors of Txnrd1. Additionally, our analysis highlight targets of AF treatment that explain part of its MOA in cells. Taken together our results and experimental design further demonstrate, in accordance with **paper III**, that a combination of chemical proteomics approaches is needed for obtaining comprehensive data about each treatment target and MOA as each analysis yielded additional layers of information. Additionally, using drugs that have Txnrd1 in common helped us deconvolute some of the mechanisms. Thus, a combination of chemical proteomics methods and drugs having the same targets proved to be valuable both for solving biological questions and to deconvolute specific drug mechanisms.

4.5 PLURIFACETED PROTEOMICS METHOD IDENTIFIES KEY REGULATORS OF TRANSLATION DURING STEM CELLS MAINTENANCE AND DIFFERENTIATION

We showcased the methods that we developed in **paper III** and **paper IV** by investigating anti-cancer drugs targeting a protein which proved to be challenging due to its specific properties. In **paper V** we aimed to combine protein stability and expression dimensions in a more comprehensive analysis instead of studying them separately. First, we further develop PISA and employed it to investigate biological questions that are not involving drug treatment. The rationale behind that was to show that a similar combination of methods used in drug target deconvolution could also help deconvolute molecular mechanisms that happen during transitions of cells from one cell type to another. We reprogrammed hFF into iPSCs and differentiated them randomly through EB formation. We monitored protein expression and thermal stability after each transition and added human ESCs as control as well as well studied colon cancer cell line RKO that is dividing as fast as PSCs to verify that the differences that we observe are not coming from division rate bias (Figure 20). Thus, we obtained protein stability and expression data for 5 cell types in one TMT10 set. The general idea was that we could obtain an additional layer of information from the thermal stability measurement that is not accessible through protein expression only and could help us discover new specific features of PSCs.

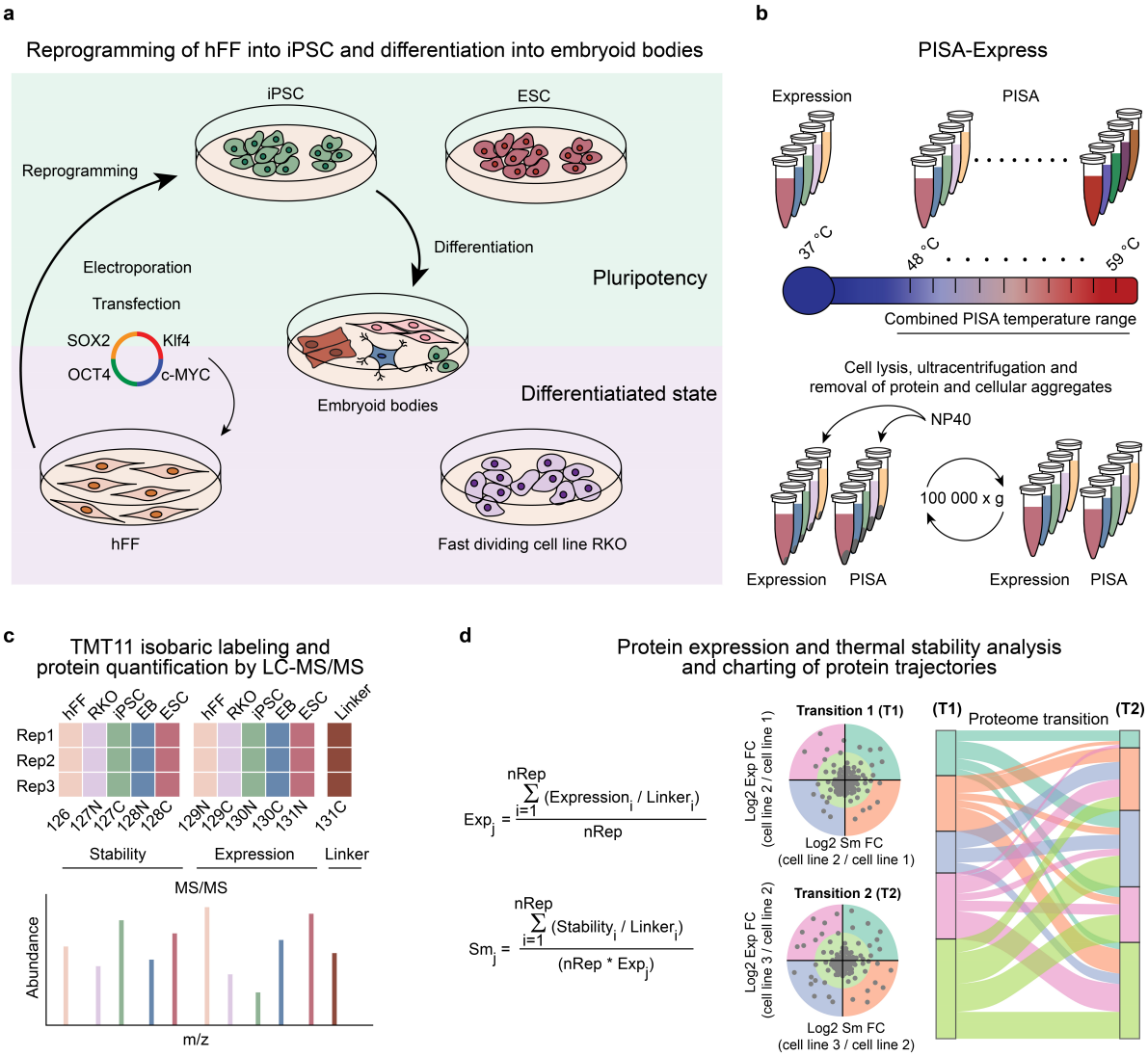

Figure 20. PISA-Express workflow. (a) hFF were reprogrammed into iPSCs using the four Yamanaka factors (Takahashi & Yamanaka, 2006), then differentiated into EB. (b) Cell aliquots were heated, combined, lysed with NP40, and freezing-thawing and insoluble protein fraction was removed by ultracentrifugation. (c) Samples were multiplexed, and one-tenth of each sample was pooled to create a linker used for normalizing protein abundances between sets during data analysis. (d) Sm and Exp were calculated and plotted against each other, the resulting protein position of the plots determines their movement on the Sankey diagram.

4.5.1 Protein stability and expression measurements distinguish cell types and various degrees of pluripotency.

First, we asked whether thermal stability could distinguish cell types as efficiently as protein expression and showed that the separation by principal component analysis (PCA) is similar. Moreover, iPSCs and ESCs were almost indistinguishable from each other on the PCA confirming our model. Strikingly, iPSCs and ESCs were separated on one side of the PCA, with hFF and RKO on the other side and EBs in an intermediate position in both stability and expression demonstrating that both parameters can separate various degrees of stemness. Indeed, EBs consist of a group of differentiated, progenitor stem and pluripotent cells so they still retain in part features of their parental iPSCs (Figures 21a and b). Additionally, the log scaled stability (Sm) and expression (Exp) FC of each cell line against iPSCs did not correlate, demonstrating that the two measurements are independent (Figure 21c).

4.5.2 ProteoTracker interface visualizes protein trajectories in stability and expression between cell types.

This prompted us to design a visualization approach to simultaneously study protein thermals stability and expression changes associated with the transition from one cell type to another. We used a mock transition following a pluripotency scale starting with iPSCs as our initial point then to EBs and finally hFF as terminally differentiated cells. What we wanted to see is whether we could distinguish general features of differentiated cells compared to pluripotent cells. However, observing this many proteins simultaneously (7778) on two dimensions (stability and expression) and another pseudo-time dimension, proved to be difficult. So, we opted to split the 2-dimensional plots into 5 sectors according to their log2 scaled protein thermal stability FC and expression FC for EBs versus iPSCs and hFF versus EBs (Figure 21d). The 5 sectors corresponded to proteins that are stabilized and upregulated (A), stabilized and downregulated (B), destabilized and downregulated (C), and destabilized and upregulated (D) (Figure 22). To assign proteins in their respective quadrant, a combined Fisher score was calculated if proteins passed the test (combined p-value <0.05) they were placed in the sector corresponding to their stability and expression FC sign, otherwise they were assigned to sector E thus comprising proteins that are not significantly changing. Finally, we used Sankey diagrams to monitor the transition of each protein from one sector to another following the transitions from one cell type to another, resulting in the ProteoTracker web interface (http://www.proteotracker.genexplain.com/) (Figure 22).

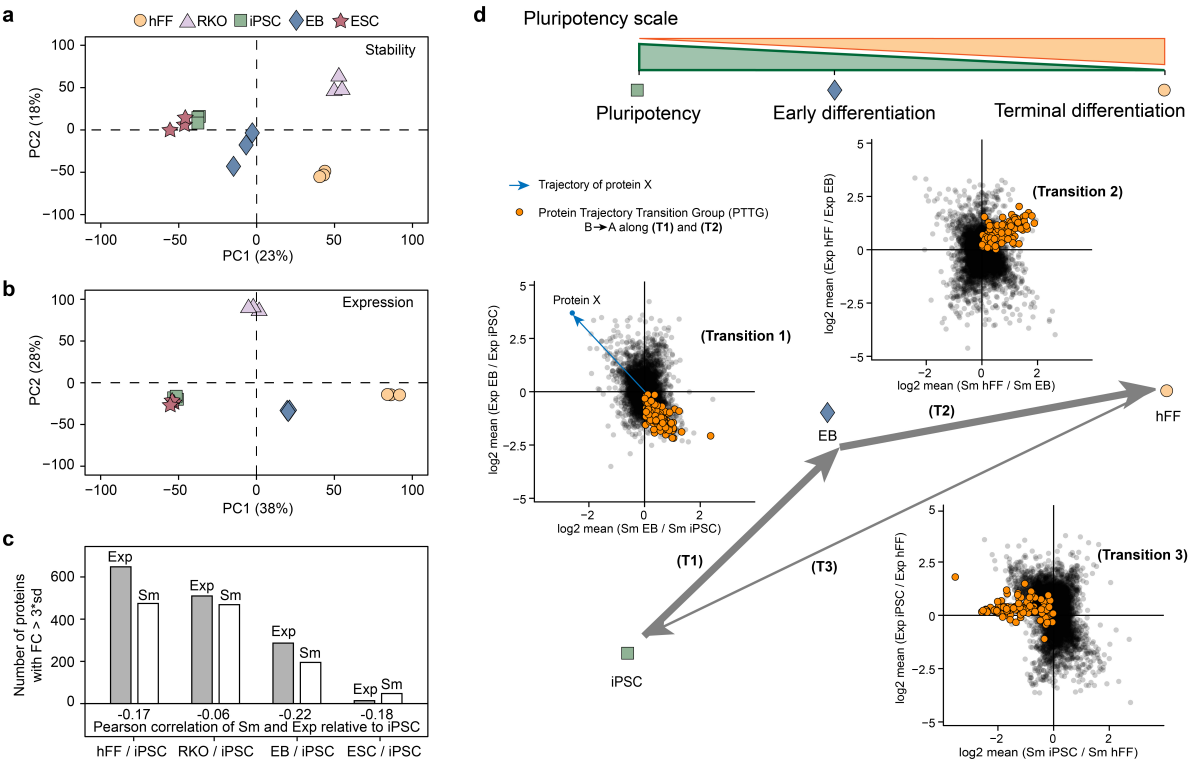

Figure 21. Protein thermal stability and expression changes between cell types. (a) and (b) PCA of normalized stability and expression measurements in hFF, RKO, iPSC, EB, and ESC. (c) Number of proteins with FC > 3*standard deviation and Pearson correlation of Sm and Exp FC. (d) Protein trajectories in cell type transitions were defined as positions in a 2D plot of log2 FCs for Sm and Exp compared to the original type. Transition (T1) was from pluripotency (iPSC) to early differentiation (EB), and (T2) from EB to terminal differentiation (hFF).

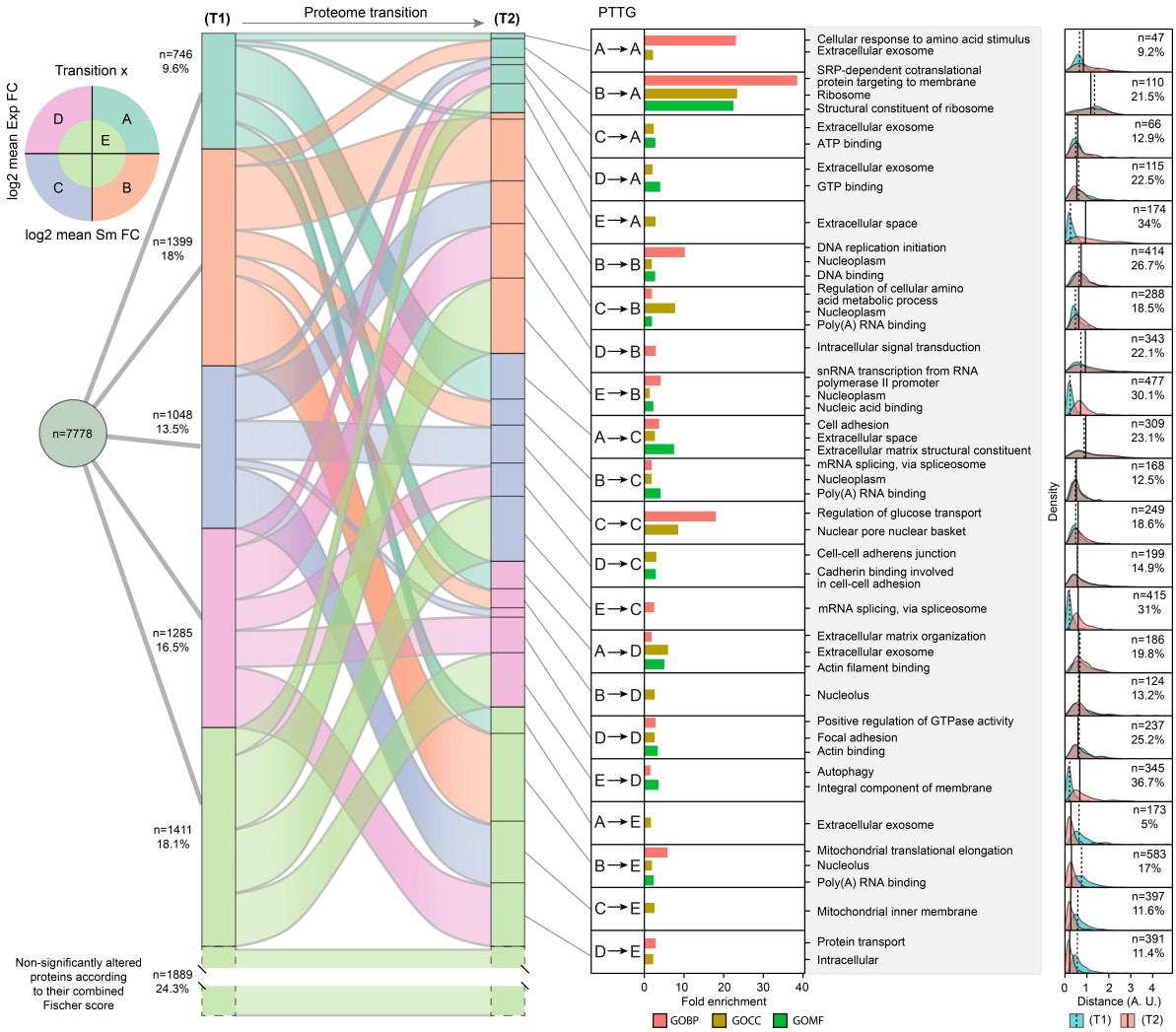

Figure 22. Sankey diagram maps protein trajectories during proteome transitions. Sankey diagram based on assigning to a significant sector (A - D) a protein trajectory in (T1) and (T2) (Figure 21d) when the combined p-value for changes in stability and expression was <0.05, and to an insignificant sector E otherwise. Each protein trajectory transition group (PTTG) of proteins undergoing a transition from a sector X in (T1) to a sector Y in (T2) (25 PTTGs in total) was submitted to a Gene Ontology (GO) enrichment analysis with all quantified proteins as background. For each sector in PTTG, the density distribution of the distances on the 2D plot in (T1) and (T2) is plotted and the percentage of proteins transiting from any sector in T1 to sector Y is calculated, and their number is given.

4.5.3 PSCs have lower amounts of mature ribosomes than differentiated cells.

We performed pathways enrichment on the various proteome trajectories that we termed proteome trajectory transition group (PTTG) to design the transition of a protein group from sector to sector. Strikingly PTTG from B to A contained most ribosomal proteins (Figures 22e and 23a). We observed no differences in ribosomal protein stability or expression between iPSCs and ESCs (Figure 23 b). However, ribosomal proteins were stabilized in all differentiated cells compared to iPSCs and significantly changed in expression only in EBs (Figures 23 c, d, and e). Since almost all ribosomal proteins showed the same trend, we hypothesized that it stems from a difference in the number of assembled ribosomes which would explain the increased stability in differentiated cells. We studied ribosome density

profiles between RKO and iPSC which have a close dividing rate since it can influence the profile. RKO showed much more assembled ribosomes. Additionally, iPSCs had the lowest translation rate compared to hFF and RKO.

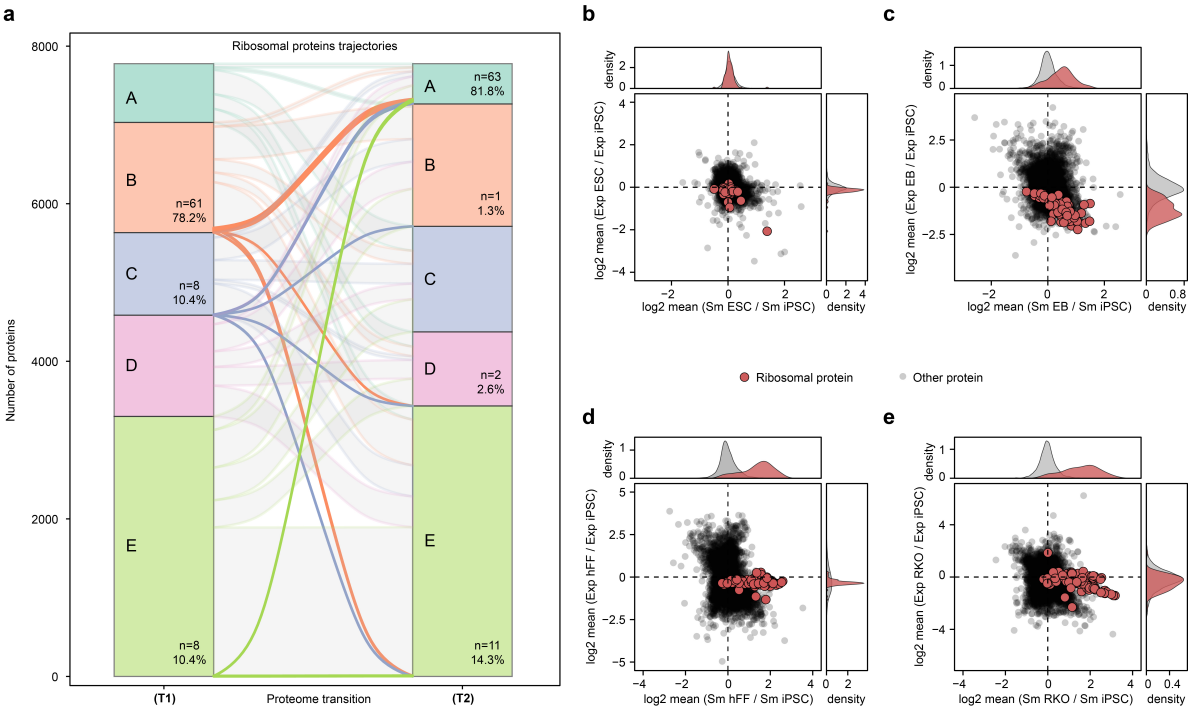

Figure 23. Ribosomal proteins are stabilized in differentiated cells. (a) Sankey diagram highlighting the movements of ribosomal proteins between the EB/iPSC to hFF/EB transition. (b), (c), (d), and (e) Two-dimensional plots of Sm and Exp FC of ESC, EB, hFF, and RKO against iPSC.

4.5.4 Proteins involved in late steps of ribosome maturation are downregulated in iPSCs compared to differentiated cells.

Since we found a difference in the quantity of assembled ribosomes leading to a higher translation rate in differentiated cells, we asked whether this could come from a defect within the ribosome assembly line. We studied ribosomal maturation factors expression in several cell lines against iPSCs including hFF, EBs, neurons differentiated from our iPSCs, RKO and HT29. Strikingly, we only see 11 factors that were downregulated on average in iPSCs compared to all the cell lines and the most downregulated is SBDS which ejects EIF6 with the help of the GTPase EFL1 from the 60S subunit as a late step of ribosome assembly, leading to association with 40s and translationally competent ribosomes (Finch et al., 2011; Menne et al., 2007; Weis et al., 2015) (Figure 24a). Incidentally, both EFL1 and EIF6 were among the 11 downregulated factors (Figure 24a). However, SBDS is the only ribosome maturation factor that was downregulated in every cell line in comparison to iPSCs (Figure 24b). Paradoxically, iPSCs had higher levels of ribosomal proteins' expression than other cell lines. Then, constitutive repression of SBDS expression could be a mechanism used by PSCs to maintain a lower pool of translationally competent ribosomes while maintaining assembled subunits as we see in the ribosome density profile (slightly higher level of 40S and a higher level of 60S).

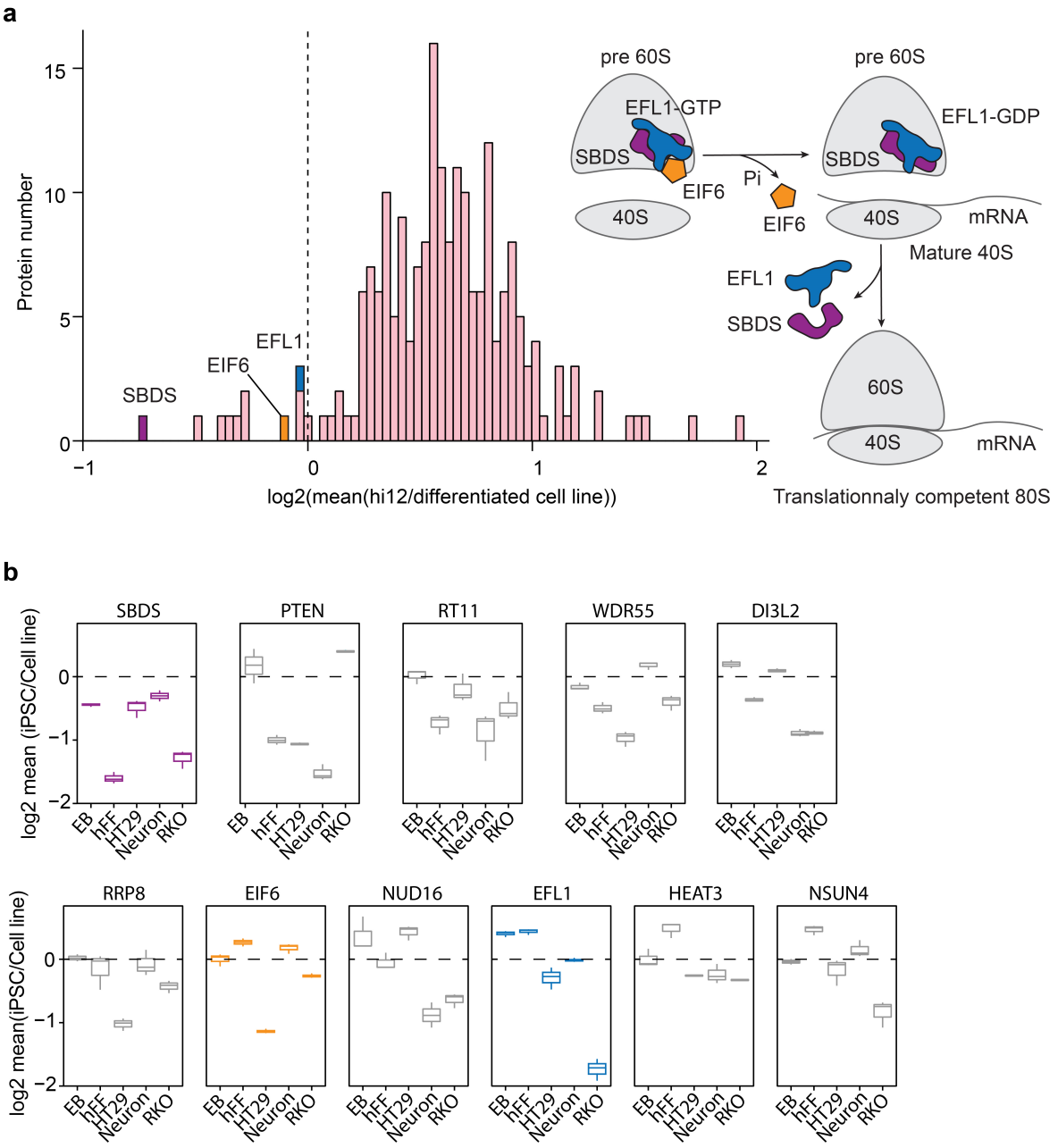

Figure 24. Expression of ribosome maturation factors. (a) Mean expression FC of ribosome maturation factors in iPSC against EB, hFF, HT29, Neurons, and RKO and scheme of the late step of ribosome maturation. (b) Expression FC of the 11 most downregulated ribosome maturation factors in iPSC against each other cell lines. Error bars represent the standard deviation of the mean, n=3.

4.5.5 SBDS knock-down slows down differentiation of iPSCs.

Finally, we investigated the effect of SBDS inhibition on cellular differentiation, our objective was to show that repressing SBDS expression slows down differentiation of iPSCs since they would need higher levels of SBDS and functional ribosomes to differentiate properly. Thus, we differentiated iPSCs into embryoid bodies for three days and then plated them to trigger random differentiation for three more days before treatments with siRNA for three days. We then measured protein expression and mRNA levels. SBDS expression level had an opposite behavior to pluripotency markers OCT4 and NANOG as well as compared to the average expression of ribosome maturation factors and ribosomal proteins highlighting

that SBDS level anticorrelates with pluripotency markers and other proteins from the same pathway. SBDS KD leads to downregulation of proteins involved in developmental pathways suggesting that it slowed down differentiation. To further confirm this observation and study the effect of SBDS KD in another system, we plated our iPSCs onto Matrigel instead of laminin 521. Matrigel is known to allow more spontaneous differentiation than culture on laminin (Rodin et al., 2014), and our cells were not adapted to the system so they spontaneously differentiated even more. We measured mRNA expression of SBDS, OCT4, NANOG, and of several lineage-specific markers GATA4, SOX17, PAX6, and SOX7. SBDS KD leads to a significant increase in mRNA levels of OCT4 and a decrease of all the markers apart from GATA4 which did not change (Figure 25). Collectively, our data showed that SBDS KD can be used for slowing down differentiation and potentially help maintain PSCs *in vitro* culture.

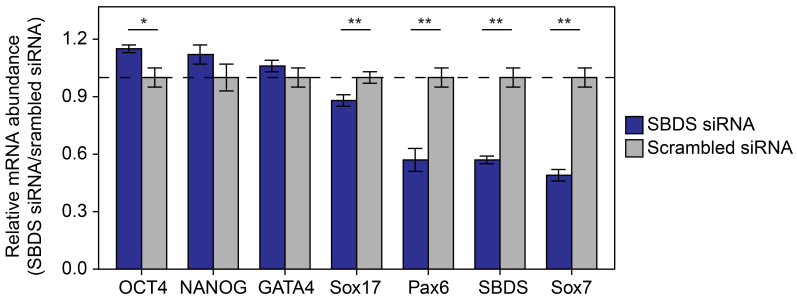

Figure 25. SBDS siRNA knock-down in iPSCs plated on Matrigel. Ratios of mRNA levels of pluripotency markers and several lineage-specific markers as well as SBDS measured by qRT-PCR against the scrambled siRNA control. Error bars represent the standard deviation of the mean, n=3.

Conclusions

Here we further develop PISA analysis by adding an expression-based correction to the stability measurement. This correction is important for samples that potentially have a difference in protein expression, otherwise, the readout of the PISA assay would be an amalgam of stability and expression changes. The other advantage is that the expression measurement can be used as a standalone measurement and this idea was the basis of the PISA-Express methodology presented here. In addition to PISA-Express, we also developed an online visualization and analysis tool, ProteoTracker, that can be used to explore our and own, uploaded data for analysis. Any proteomics dataset containing two-dimensional measurements along a third dimension like time for instance can be analyzed through our interface offering new possibilities for the proteomics community. Here we also demonstrate for the first time that a combination of protein stability and expression measurement in a purely biological context can lead to biomarker discovery by highlighting SBDS as a key controller of translation in PSCs. SBDS KD could be used as a tool to culture iPSCs *in vitro*. Moreover, since several types of tissue-resident stem cells also exhibit low translation rates it could come in handy to adapt them to *in vitro* culture, this warrants further investigation.

5 CONCLUSIONS AND FUTURE PERSPECTIVES

Proteomics is a fast-evolving field that has been more and more used and trusted in biology and medical sciences in recent years. When the work presented in this thesis started, the emphasis in the proteomics field was mainly on analytical depth. How many proteins can we identify with a theoretically illimited amount of sample material (Bekker-Jensen et al., 2017)? Initially, the classical experiment was label-free (Hornburg et al., 2014; Nagaraj et al., 2012; Pirmoradian et al., 2013), and this was mainly replaced by multiplexing strategies to quantify more proteins (McAlister et al., 2014; Zhou et al., 2013). Indeed, sample multiplexing did not necessarily reduce the instrumental time because off-line fractionation was employed to reach high numbers of quantified proteins. This is reflected in the low number of proteins identified in the first label-free analysis of **paper I** with 2116 proteins common in all samples, while 8898 proteins common in all samples were quantified in the second multiplexed analysis with similar analysis time. The field was already investigating features that are only available in proteomics such as studies of protein PTMs or protein synthesis/degradation. However, the focus shifted, even more, when protein numbers attained in one analysis were sufficient for the field to stand its ground against transcriptomics analysis (Bekker-Jensen et al., 2017). Analytical depth is now a problem more akin to single-cell proteomics, where proteomics once again tried to catch up in the race against transcriptomics. Reduced instrumental time and higher throughput of mass spectrometry analysis are underway and have seen tremendous improvements (Bian et al., 2020; Messner et al., 2021; Meyer et al., 2020). For the rest of the proteomics field, a large portion of the research attention is now on:

- reduced sample amount.
- development of complementary unbiased approaches to protein expression measurements.
- analysis and visualization of datasets of ever-increasing complexity.

These technical hurdles are particularly relevant in biology where accurate and alternative parameters measurements of protein properties are crucial to properly interpret molecular mechanisms or explain observed phenotypes. In addition, the most challenging and interesting biological materials are rarely available in large amounts. Finally, studies involving a larger and larger number of samples such as databases and atlases are becoming a routine in proteomics (Cho et al., 2021; Jarzab et al., 2020; H. J. Johansson et al., 2019; Mateus et al., 2020; Nusinow et al., 2020; D. Wang et al., 2019) and we have also added our contribution (Saei et al., 2019). In this thesis we introduce methods and experimental strategies that contribute to overcoming some of these challenges.

First, PISA developed in **paper II** paves the way for higher throughput studies of protein thermal stability and protein solubility measurements in general. PISA also requires less sample material than TPP. Using PISA in **paper IV** instead of TPP allowed us to analyze the three drugs and vehicle control in duplicates in one set and obtain data for four replicates of the three experiments in six TMT sets corresponding to 60 TMT sets in a classical 1D-TPP analysis involving 10 temperature points per samples. This is also relevant in **paper V**, where we could monitor simultaneously protein expression and thermals stability in five cell types in one TMT10 set per replicate. Even though we used less material and reduced the

instrumental time that we would need in a classical TPP analysis, we did not use small sample amounts per se, and this should be addressed in future studies.

Next, we combined the methodologies that we developed and existing methods to elucidate drug MOA and to study cellular dynamics (**papers I, III, IV, and V**). In **paper I**, we showed that the naturally occurring cell variations in *in vitro* cell culture over time are on par with those of cells treated with anticancer compounds at IC50. These results stress the use of time-matched controls and highlight the high plasticity and dynamics of the cellular proteome even in a stable environment that is *in vitro* cell culture. In **papers III** and **IV** we studied the target landscapes and MOA of several redox-regulating anti-cancer compounds using a combination of chemical proteomics approaches namely FITExP, TPP/PISA, and redox proteomics. We demonstrate that one method is not enough for accurately deconvoluting MOA of AF but considering the complementarity and overlap between the three methods we could pinpoint the target TXNRD1. We also demonstrate that disulfide bond formation or reduction can lead to alteration of proteome thermal stability by directly comparing TPP and redox proteomics readouts which were later confirmed using the newly developed SIESTA (Saei et al., 2021). Lastly, in **paper IV** we highlight the usefulness of analyzing the effect of multiple drugs having a target in common, on the proteome. This helped us to establish the specificity of each of the compounds toward their common target and deconvolute effects that are due to the inhibition of the common target from other effects. This study serves as an example of combining attributes of chemical proteomics methods and of the compounds themselves and could be used as a model for evaluating compounds that are structural or functional analogs in drug development.

Going one step further, after demonstrating that protein stability and expression measurements (or FITExP analysis) are orthogonal (**papers II, III, and V**) we decided to merge the two dimensions or facets in one analysis resulting in PISA-Express. One of the disadvantages of the current visualization and analysis tools available in multifaceted proteomics is that they tend to compress information (McInnes et al., 2018; Van Der Maaten & Hinton, 2008). Visualizing the behavior of all proteins in two parameters without reduction of the information is easy but extracting the evolution of these parameters along a third parameter like pseudo-time or even a three ways comparison is not trivial. Here, we were interested in monitoring both stability and expression simultaneously for all proteins and particularly the changes of protein properties in both parameters for comparing several successive conditions (cell types in our case). ProteoTracker allows visualizing if a protein or group of proteins have significantly altered thermal stability and expression in one cell type compared to another and tracks their behavior during successive cell type transitions. These characteristics are rather unique in proteomics. In summary, ProteoTracker represents our attempt to overcome some challenges in data visualization and analysis and should be improved in the future to include additional analyses. We also used a pseudo-cell transition where we studied cell lines that were static apart from differentiating EBs, even though they were reprogrammed and then differentiated from the same parental line. Monitoring differentiation of more defined cell lineages at various steps of differentiation should showcase the utility of the interface more than in the current study and should also be investigated in the future.

Finally, the work presented here displays analyses that are unique to the proteomics world (**papers II, III, IV, and V**). Protein expression levels can be estimated through measurements of mRNA levels. However, protein and mRNA levels do not always correlate perfectly due to post-transcriptional events (Yansheng Liu et al., 2016). This is particularly true when the cell undergoes strong phenotypic alterations such as in differentiation, division, stress response such as in response to chemical treatment (Cheng et al., 2016; Fournier et al., 2010; Jayapal et al., 2008; Kristensen et al., 2013; M. V. Lee et al., 2011; Maier et al., 2011; Vogel et al., 2011), as in **papers I, II, III, IV, and V**. Furthermore, only proteomics allows one to study post-translational events such as PTM, PPI, protein degradation and so on. Measurement of protein thermal stability is a powerful tool for observing multiple protein properties simultaneously. Indeed, TPP can measure changes in protein physico-chemical properties and so far, it was shown that TPP can detect ligand binding, PPI, and PTM. All these parameters can be theoretically monitored at once, the difficulty is then to find out the cause of protein stability alterations when using TPP in a biological system. In **paper V** we deconvolute and explain the difference in stability of ribosomes between PSCs and differentiated cells. We also link thermal stability alterations of histones and proteins involved in glycolysis and oxidative phosphorylation to differences in cell metabolism and chromatin compaction. Control of translation through modulation of ribosome biogenesis is an example of post-transcriptional control and altered stability of ribosomes could not be highlighted other than with proteomics analysis. Therefore, efforts on developing methods that are unique to the field and provide complementary information to already existing measurements are valuable to biologists (**papers II and V**). Particularly, in **paper V** we further set the stage for using protein thermal stability as an alternate measurement to protein expression to elucidate molecular mechanisms in biological studies. This was already initiated by the work of Becher et al. and Dai et al. by studying the different phases of the cell cycle using TPP/MS-CETSA (Becher et al., 2018; Dai et al., 2018). However, now that the utility of TPP/PISA in cell biology has been established using relatively accessible cellular systems, more challenging systems should be investigated in future work. More defined and rare cell types are described by cell biologists where the differences in protein expression are minimal or almost absent from one cell type to another and could often be missed by conventional analysis. Adding protein thermal stability measurements to expression measurements can greatly increase the value and discovery potential in such studies and should become more and more popular in the future.

6 ACKNOWLEDGEMENTS

First, I would like to express my deepest gratitude to my supervisor Prof. **Roman Zubarev**. You are the only person who replied positively to my request when I was looking for an internship for my master thesis… and here we are 6 years later. Thank you for offering me an opportunity as an intern student first, then as a research assistant, and finally as a Ph.D. student. I owe most of my scientific development to you and I have learned tremendously in your lab. Specifically, your influence was key in improving my critical thinking, which is a prerequisite in science. Oddly enough, I often heard other students complaining about how stressful and tough a Ph.D. could be, mine was enjoyable, and to me, it is a testimony to your qualities as a teacher, scientific mentor, and researcher.

To my co-supervisor Prof. **Elias Arnér**, thank you so much for your help and advice and for fixing things on extremely short notices sometimes. Congratulations on bringing this Knut and Wallenberg Redox consortium together and organizing the meetings and scientific discussions, it was always interesting. You were a great help on many aspects, and I hope that we will continue to collaborate in the future. I would also like to thank some members of your lab, **Belén Espinosa**, **Qing Cheng**, **Dorian Cheff**, **Markus Dagnell**, for discussions and collaborations. Thank you also, **Markus**, for accepting to be the chairperson on the day of my defence.

A special thanks go to Associate Prof. **Sergey Rodin**, my other scientific mentor, and friend. It was inspiring to witness the development of your scientific career. I could never thank you enough for all the help, guidance as well as good company. Thank you for motivating me and pushing me when it was needed and especially sending me on missions to unknown territories to scavenge for lab equipment or material. It helped me in so many aspects of my scientific work and outside. I am truly blessed to have been able to work with you and I believe that the best is to come.

To all the members of the Hematopoietic stem cells Knut and Wallenberg funded consortium, **Svetlana Soboleva**, **Mark van der Garde**, **Anna Rydström**, **Hooi Min Tan Grahn**, **Sara Warsi**, **Els Mansell**, **Valgardur Sigurdsson**, **Agatheeswaran Subramaniam**, Prof. **Stefan Karlsson**, Prof. **Jonas Larsson** and Prof. **Björn Nilsson**. Thank you all for the discussions, scientific or not, and the nice time in Lund. Particularly, I would like to thank Prof. **Stefan Karlsson** for being such a nice and welcoming host on two separate occasions in addition to organizing the yearly meetings, I was always looking forward to it. Thank you, **Anna**, **Hooi Min**, **Mark**, **Svetlana,** and **Els** for the fun discussions during these meetings and the ensuing dinners.

To newly appointed Prof. **Kenichi Miharada**, first of all, I would like to congratulate you on becoming a professor. We always had great scientific discussions and collaborations that sparked my interest in developing new approaches. One of the central works of this thesis would not have been shaped in the same way if not for the challenges that you brought to the

table during our collaborations. Thank you very much for this, I wish you the best of luck in your new position and I hope that we will stay in touch.

There are several persons that I would like to thank for allowing me to use their lab equipment and/or helped me in some ways. Prof. **Jesper Haeggström**, **Mikaela Mårback**, **Aleksandra Pajak**, Assistant Prof. **Joanna Rorbach**, group leader **Christoph Freyer** and Prof. **Rikard Holmdahl**. I also owe late Prof. **Arne Holmgren**. Thank you very much for disregarding the fact that a random person was working in your lab at times and being so patient and helpful with me. Meeting such positive persons as you helped me tremendously throughout my Ph.D.

To the people that I met here and there at Karolinska and sometimes at pubs, in no particular order, I would like to thank **Shane Wright**, **Luca D. Retattino**, **Gabriele Girelli**, **Rubin Joshi**, **Eva Daskalaki**, **Christopher Grigsby**, **Jean Aymard Nzigou Mandouckou**, **Joep Titulaer**, **Roberta Filograna**, **Antonio Checa**, **Hector Gallart-Ayala**, **Hassan Foroughi**, Associate Prof. **Robert Schnell**. **Shane**, you bring the fun and enthusiasm everywhere you go, hopefully, we will have more opportunities to gather later this year, I am looking forward to it.

Cheers to my friends in Stockholm, **Sigitas Kruopys**, **Cristina Loghin**, **Iryna Petrusha**, **Joanna Glass**, **Laurent Courty,** and **Pablo Lopez**, for all the chill and fun. You helped me to disconnect and that was needed at times. Thank you for your friendship, which I hope will be lifelong.

Je remercie **Florian Barré** pour les années de folie que l'on a passé en GenPro et pour le surplus de motivation que tu m'as apporté pendant le master. Je me souviendrai toute ma vie de nos après-midi bombardements, suivis par les soirées agrémentées de cocktails maison… il doit encore y avoir quelques belles collections de nos CV dans les facs européennes à l'instar des albums Panini! Notre collocation à Copenhague restera l'un de mes meilleurs souvenir. Merci de m'avoir fait découvrir Maastricht, dernièrement. Merci également à ta moitié **Fanny Boyaval** ainsi qu'à **Antoine Caillau**d et à **Thomas Sénard** pour tous les bons moments passé dès mon arrivée à Stockholm, particulièrement les aventures sur mon rafiot, PA. Enfin, j'adresse tous mes remerciements à la fine équipe, Dr. **Valentin Cognet** et el señor **Gaetan Pikon**, ne changez rien les gars, vous êtes les meilleurs!

I would like to express my deepest gratitude to my university teachers in Lille. Prof. **Michel Salzet**, Prof. **Isabelle Fournier**, Associate Prof. **Maxence Wisztorski**, Associate Prof. **Julien Frank** and Assistant Prof. **Christophe Tastet**. Before joining the GenPro program, I was fed up with my studies and just wanted to be done with it and find a job. Thank you for inspiring me to pursue a Ph.D. and research in general. I would never have followed this path otherwise. I would also like to thank Prof. **Anne Chotteau** for convincing me to enroll in the program, during an interview that ended up shaping my early carrier path. I am also grateful to Prof. **Michael Lisby**, who supervised me during the internship of my first year of masters

at the University of Copenhagen, and who greatly contributed to developing my research interest.

I am indebted to **Victoria Balabanova**, **Chad Tunell**, and **Alessandra Nanni** for all the valuable help with administrative and informatics works, it really made my life easier.

Cheers to the whole gang of 9D (mostly the immunologists) past and present, **Michael Bonner**, **Ulrika Norin**, **Jaime James**, **Gonzalo Fernandez Lahore**, **Ana Do Carmo Oliveira Cuelho**, **Alexander Krämer**, **Vilma Urbonaviciute**, **Laura Romero Castillo**, **Erik Lönnblom**, **Edit Szodorai**, **Yibo He**, **Amit Saxena**, **Daniëlle Vaartjes**, for all the fun, parties, scientific discussions, collaborations… you name it, during these past years. Life in Biomedicum would have been boring without you all.

I extend my thanks to **Noah Moruzzi** and **Francesca Lazzeri-Barcelo** for interesting collaborations as well as scientific discussions. I wish that all the collaborative work in science was as efficient as ours! Best of luck to you two in your respective careers. I would also like to thank Assistant Prof. **Michael Landreh** and **Margit Kaldmäe** for a nice collaboration and **Michael** for being one of the jury members during my half-time.

My genuine thanks to all current and former members of the Zubarev's lab, **Xueshu Xie**, **Natalyia Tarasova**, **Xuepei Zhang**, **Jijing Wang**, **Jimmy Rodríguez**, **Mohammad Pirmoradian**, **Piliang Hao**, **Hassan Gharibi**, **Jimmy Ytterberg**, **Dorothea Rutishauser**, **Susanna Lundström**, **Rahul Pandey**, **Piliang Hao**, **Hongyu Xie**, **Luciano Di Stefano**, **Alexander Manoylov**, **Andrea Fossati**, **Vincent Gémard**, **Niels Leijten**, **Shiyu Wang**, **Neil Rumachik**, **Marcus Lundin**, **Mohsen Gasemi**, **Ülkü Guler Tolkat**, **Sasha Zdanovich**, **Marjan Abbasi**, **Qinyu Jia**, **Robert Kehrber**, **Giorgia Palano**, for making this lab a nice work environment over the years.

Particularly I owe a lot to some of the previous lab members that have helped me tremendously when I arrived and over the years. **Alexey Chernobrovkin**, you took the time to prepare R lessons for us and I wish to let you know that at least it was not for naught. I continued to work with it, and I hope that you appreciate the fact that all the graphs and analyses presented in this thesis would not have been possible without your teachings. **Juan Astorga-Wells**, talking science with you always made me want to build stuff and I owe a lot of my daring to make new custom-made lab tools to you. **Bo Zhang**, you are a great friend, and I will always remember this new year's road trip to Copenhagen. Hopefully, your car will not… damn these automatic transmissions! Thank you, for allowing me to blow up some steam with foosball matches fought tooth and nail. Ultimately, we still had to bow down to the dream team Alexey and Juan in our foosball tournament final. Shout out to you all!

To our two core facility managers **Akos Vegvari**, **Massimiliano Gaetani**, I would like to express my gratitude for all the help, scientific collaborations, discussions, and fun over the years. To **Marie Ståhlberg** and **Carina Palmberg**, thank you for everything, you are the soul of the lab. **Carina**, you are the sweetest person that I know, and it still amazes me that

after 6 years I have never seen you angry at someone considering all the troubles that we regularly bring to the lab… how is this even possible?! **Marie**, I always had fun talking with you and you have helped me so much with everything. This thesis would have been so much harder if both of you were not around. I learned a ton from you, and you were usually the first persons I would go to when I needed help. You even took the time to help me train my broken Swedish, I truly appreciate it, tack så jättemycket!

To our two wonderful ex-students **Alexandra Alexandridou** and **Eleni Stergiou**, thank you for all the fun and positive vibe. I wish you the best for the future. I would like to thank **Amir Ata Saei** for all the help, hangouts, parties, and more. You played a big part in the making of this thesis. **Mike Aoun** for being a great gym buddy and bringing fun and excitement to everyday life at Biomedicum, keep it up! Thank you, **Michael Bonner**, for bringing your cool character, for always being up for random discussions around a coffee about everything and nothing, and for the exploration of Stockholm's food scene along with **Christian Beusch,** and **Ulrika Norin**. Finally, a special thanks to **Christian Beusch** for being a great friend and always being there and ready to help. I loved the sarcasm, hangouts, discussions, and suspicious culinary compositions. The latest part of this work could not have been done without your help. I wish you all the best even if I am convinced that you have a brilliant career ahead of you!

I would like to thank **Roman Zubarev**, **Elias Arnér**, **Sergey Rodin**, **Christian Beusch,** and **Michael Bonner** for proofreading this thesis.

À la mif, **Alexandre Brocard**, **Johann Lemarie**, **Bastien Dauphin**, **Guillaume Gosset**, mon ami d'enfance **Jérémy Felix** et mon ami de longue date **Alexandre Noblecourt**. Malgré la distance, on trouve quand même le temps de se voir et de s'organiser des vacances d'anthologie. J'ai hate de voir ce que cette année nous reserve!

My deepest thanks to my dearest, **Katerina Motso**, for this amazing year despite the Corona pandemic. You appeared in my life recently, but it immediately felt like you were always here. You were wonderful mental support during this sprint of thesis writing and you made this whole experience so much more fun and relaxing than it should have been. Thank you for everything.

Enfin, je remercie ma famille, qui n'est absolument pas étonnée de mon intérêt pour les sciences et particulièrement mes **parents**. Vous m'avais inculqué les valeurs de la vie et du travail. Vous m'avez toujours encouragé malgré les hauts et les bas de mon parcours scolaire et m'avez poussé à travailler pour accomplir les objectifs que je me suis fixé. Je ne vous serais jamais assez reconnaissant pour tout ce que vous avez fait pour moi. Ensuite, à mes deux inspirations et meilleurs amis, mes deux frères, **Jean-Michel** et **Guillaume**. On en aura suffisamment plaisanté, vous pouvez oficiellement m'appeler Bobby maintenant! Les années passent mais votre soutien inconditionnel persiste. Je vous remercie d'avoir toujours été présents pour moi, je n'y serais jamais arrivé sans vous tous. Je vous aime du fond du coeur et je vous dédie cette these.

7 REFERENCES

Albertolle, M. E., Glass, S. M., Trefts, E., & Guengerich, F. P. (2019). Isotopic tagging of oxidized and reduced cysteines (iTORC) for detecting and quantifying sulfenic acids, disulfides, and free thiols in cells. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.AC118.007225

Almqvist, H., Axelsson, H., Jafari, R., Dan, C., Mateus, A., Haraldsson, M., Larsson, A., Molina, D. M., Artursson, P., Lundbäck, T., & Nordlund, P. (2016). CETSA screening identifies known and novel thymidylate synthase inhibitors and slow intracellular activation of 5-fluorouracil. *Nature Communications*. https://doi.org/10.1038/ncomms11040

Arrowsmith, J., & Miller, P. (2013). Trial Watch: Phase II and Phase III attrition rates 2011-2012. In *Nature Reviews Drug Discovery*. https://doi.org/10.1038/nrd4090

Bachovchin, D. A., Brown, S. J., Rosen, H., & Cravatt, B. F. (2009). Identification of selective inhibitors of uncharacterized enzymes by high-throughput screening with fluorescent activity-based probes. *Nature Biotechnology*. https://doi.org/10.1038/nbt.1531

Bantscheff, M., Eberhard, D., Abraham, Y., Bastuck, S., Boesche, M., Hobson, S., Mathieson, T., Perrin, J., Raida, M., Rau, C., Reader, V., Sweetman, G., Bauer, A., Bouwmeester, T., Hopf, C., Kruse, U., Neubauer, G., Ramsden, N., Rick, J., … Drewes, G. (2007). Quantitative chemical proteomics reveals mechanisms of action of clinical ABL kinase inhibitors. *Nature Biotechnology*. https://doi.org/10.1038/nbt1328

Barglow, K. T., & Cravatt, B. F. (2007). Activity-based protein profiling for the functional annotation of enzymes. *Nature Methods*. https://doi.org/10.1038/nmeth1092

Becher, I., Andrés-Pons, A., Romanov, N., Stein, F., Schramm, M., Baudin, F., Helm, D., Kurzawa, N., Mateus, A., Mackmull, M.-T. T., Typas, A., Müller, C. W., Bork, P., Beck, M., & Savitski, M. M. (2018). Pervasive Protein Thermal Stability Variation during the Cell Cycle. *Cell*, *173*(6), 1495-1507.e18. https://doi.org/10.1016/j.cell.2018.03.053

Becher, I., Werner, T., Doce, C., Zaal, E. A., Tögel, I., Khan, C. A., Rueger, A., Muelbaier, M., Salzer, E., Berkers, C. R., Fitzpatrick, P. F., Bantscheff, M., & Savitski, M. M. (2016). Thermal profiling reveals phenylalanine hydroxylase as an off-target of panobinostat. *Nature Chemical Biology*. https://doi.org/10.1038/nchembio.2185

Beedle, A. E. M., Lynham, S., & Garcia-Manyes, S. (2016). Protein S-sulfenylation is a fleeting molecular switch that regulates non-enzymatic oxidative folding. *Nature Communications*, *7*(1), 1–10. https://doi.org/10.1038/ncomms12490

Bekker-Jensen, D. B., Kelstrup, C. D., Batth, T. S., Larsen, S. C., Haldrup, C., Bramsen, J. B., Sørensen, K. D., Høyer, S., Ørntoft, T. F., Andersen, C. L., Nielsen, M. L., & Olsen, J. V. (2017). An Optimized Shotgun Strategy for the Rapid Generation of Comprehensive Human Proteomes. *Cell Systems*. https://doi.org/10.1016/j.cels.2017.05.009

Bian, Y., Zheng, R., Bayer, F. P., Wong, C., Chang, Y. C., Meng, C., Zolg, D. P., Reinecke, M., Zecha, J., Wiechmann, S., Heinzlmeir, S., Scherr, J., Hemmer, B., Baynham, M., Gingras, A. C., Boychenko, O., & Kuster, B. (2020). Robust, reproducible and quantitative analysis of thousands of proteomes by micro-flow LC–MS/MS. *Nature*

Communications. https://doi.org/10.1038/s41467-019-13973-x

Borg, G., Allander, E., Lund, B., Berg, E., Brodin, U., Pettersson, H., & Trang, L. (1988). Auranofin improves outcome in early rheumatoid arthritis. Results from a 2-year, double blind, placebo controlled study. *Journal of Rheumatology*.

Boullosa, L. F., Van Loenhout, J., Flieswasser, T., De Waele, J., Hermans, C., Lambrechts, H., Cuypers, B., Laukens, K., Bartholomeus, E., Siozopoulou, V., De Vos, W. H., Peeters, M., Smits, E. L. J., & Deben, C. (2021). Auranofin reveals therapeutic anticancer potential by triggering distinct molecular cell death mechanisms and innate immunity in mutant p53 non-small cell lung cancer. *Redox Biology*, 101949. https://doi.org/10.1016/j.redox.2021.101949

Branca, R. M. M., Orre, L. M., Johansson, H. J., Granholm, V., Huss, M., Pérez-Bercoff, A., Forshed, J., Käll, L., & Lehtiö, J. (2014). HiRIEF LC-MS enables deep proteome coverage and unbiased proteogenomics. *Nature Methods*. https://doi.org/10.1038/nmeth.2732

Burk, R. F., Hill, K. E., Nakayama, A., Mostert, V., Levander, X. A., Motley, A. K., Johnson, D. A., Johnson, J. A., Freeman, M. L., & Austin, L. M. (2008). Selenium deficiency activates mouse liver Nrf2-ARE but vitamin E deficiency does not. *Free Radical Biology and Medicine*. https://doi.org/10.1016/j.freeradbiomed.2008.01.016

Cai, Y., Zheng, Y., Gu, J., Wang, S., Wang, N., Yang, B., Zhang, F., Wang, D., Fu, W., & Wang, Z. (2018). Betulinic acid chemosensitizes breast cancer by triggering ER stress-mediated apoptosis by directly targeting GRP78 article. *Cell Death and Disease*. https://doi.org/10.1038/s41419-018-0669-8

Cappelletti, V., Hauser, T., Piazza, I., Pepelnjak, M., Malinovska, L., Fuhrer, T., Li, Y., Dörig, C., Boersema, P., Gillet, L., Grossbach, J., Dugourd, A., Saez-Rodriguez, J., Beyer, A., Zamboni, N., Caflisch, A., de Souza, N., & Picotti, P. (2021). Dynamic 3D proteomes reveal protein functional alterations at high resolution in situ. *Cell*. https://doi.org/10.1016/j.cell.2020.12.021

Cebula, M., Schmidt, E. E., & Arnér, E. S. J. (2015). TrxR1 as a potent regulator of the Nrf2-Keap1 response system. In *Antioxidants and Redox Signaling*. https://doi.org/10.1089/ars.2015.6378

Chakrabarty, A., Sánchez, V., Kuba, M. G., Rinehart, C., & Arteaga, C. L. (2012). Feedback upregulation of HER3 (ErbB3) expression and activity attenuates antitumor effect of PI3K inhibitors. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.1018001108

Chan, E. W. S., Chattopadhaya, S., Panicker, R. C., Huang, X., & Yao, S. Q. (2004). Developing photoactive affinity probes for proteomic profiling: Hydroxamate-based probes for metalloproteases. *Journal of the American Chemical Society*. https://doi.org/10.1021/ja047044i

Chang, M., Burgess, J. R., Scholz, R. W., & Reddy, C. C. (1990). The induction of specific rat liver glutathione S-transferase subunits under inadequate selenium nutrition causes an increase in prostaglandin F(2α) formation. *Journal of Biological Chemistry*. https://doi.org/10.1016/s0021-9258(19)39376-7

Chauvin, J.-P. R., & Pratt, D. A. (2017). On the Reactions of Thiols, Sulfenic Acids, and Sulfinic Acids with Hydrogen Peroxide. *Angewandte Chemie International Edition*,

56(22), 6255–6259. https://doi.org/10.1002/anie.201610402

Cheng, Z., Teo, G., Krueger, S., Rock, T. M., Koh, H. W., Choi, H., & Vogel, C. (2016). Differential dynamics of the mammalian mRNA and protein expression response to misfolding stress . *Molecular Systems Biology*. https://doi.org/10.15252/msb.20156423

Chernobrovkin, A., Marin-Vicente, C., Visa, N., & Zubarev, R. A. (2015). Functional Identification of Target by Expression Proteomics (FITExP) reveals protein targets and highlights mechanisms of action of small molecule drugs. *Scientific Reports*, *5*(1), 11176. https://doi.org/10.1038/srep11176

Cho, N. H., Cheveralls, K. C., Brunner, A.-D., Kim, K., Michaelis, A. C., Raghavan, P., Kobayashi, H., Savy, L., Li, J. Y., Canaj, H., Kim, J. Y., Stewart, E. M., Gnann, C., McCarthy, F., Cabrera, J. P., Brunetti, R. M., Chhun, B. B., Dingle, G., Hein, M. Y., … Francisco, S. (2021). OpenCell: proteome-scale endogenous tagging enables the cartography of human cellular organization. *BioRxiv*.

Christensen, M. J., Nelson, B. L., & Wray, C. D. (1994). Regulation of glutathione S-transferase gene expression and activity by dietary selenium. *Biochemical and Biophysical Research Communications*. https://doi.org/10.1006/bbrc.1994.1923

Christoforou, A., Mulvey, C. M., Breckels, L. M., Geladaki, A., Hurrell, T., Hayward, P. C., Naake, T., Gatto, L., Viner, R., Arias, A. M., & Lilley, K. S. (2016). A draft map of the mouse pluripotent stem cell spatial proteome. *Nature Communications*. https://doi.org/10.1038/ncomms9992

Collins, F. S., & Varmus, H. (2015). A New Initiative on Precision Medicine. *New England Journal of Medicine*. https://doi.org/10.1056/nejmp1500523

Colquhoun, D. (2014). An investigation of the false discovery rate and the misinterpretation of p-values. *Royal Society Open Science*. https://doi.org/10.1098/rsos.140216

D'Arcy, P., Brnjic, S., Olofsson, M. H., Fryknäs, M., Lindsten, K., De Cesare, M., Perego, P., Sadeghi, B., Hassan, M., Larsson, R., & Linder, S. (2011). Inhibition of proteasome deubiquitinating activity as a new cancer therapy. *Nature Medicine*. https://doi.org/10.1038/nm.2536

Dai, L., Zhao, T., Bisteau, X., Sun, W., Prabhu, N., Lim, Y. T., Sobota, R. M., Kaldis, P., & Nordlund, P. (2018). Modulation of Protein-Interaction States through the Cell Cycle. *Cell*, *173*(6), 1481-1494.e13. https://doi.org/10.1016/j.cell.2018.03.065

Day, N. J., Gaffrey, M. J., & Qian, W. J. (2021). Stoichiometric thiol redox proteomics for quantifying cellular responses to perturbations. In *Antioxidants* (Vol. 10, Issue 3, p. 499). MDPI AG. https://doi.org/10.3390/antiox10030499

De Luca, A., Hartinger, C. G., Dyson, P. J., Lo Bello, M., & Casini, A. (2013). A new target for gold(I) compounds: Glutathione-S-transferase inhibition by auranofin. *Journal of Inorganic Biochemistry*. https://doi.org/10.1016/j.jinorgbio.2012.08.006

de Souza, N., & Picotti, P. (2020). Mass spectrometry analysis of the structural proteome. In *Current Opinion in Structural Biology*. https://doi.org/10.1016/j.sbi.2019.10.006

Devabhaktuni, A., Lin, S., Zhang, L., Swaminathan, K., Gonzalez, C. G., Olsson, N., Pearlman, S. M., Rawson, K., & Elias, J. E. (2019). TagGraph reveals vast protein modification landscapes from large tandem mass spectrometry datasets. *Nature Biotechnology*. https://doi.org/10.1038/s41587-019-0067-5

Devarie-Baez, N. O., Silva Lopez, E. I., & Furdui, C. M. (2016). Biological chemistry and functionality of protein sulfenic acids and related thiol modifications. *Free Radical Research*, *50*(2), 172–194. https://doi.org/10.3109/10715762.2015.1090571

Doherty, M. K., Hammond, D. E., Clague, M. J., Gaskell, S. J., & Beynon, R. J. (2009). Turnover of the human proteome: Determination of protein intracellular stability by dynamic SILAC. *Journal of Proteome Research*. https://doi.org/10.1021/pr800641v

Dowden, H., & Munro, J. (2019). Trends in clinical success rates and therapeutic focus. In *Nature reviews. Drug discovery*. https://doi.org/10.1038/d41573-019-00074-z

Duan, Y., Liu, Y., Coreas, R., & Zhong, W. (2019). Mapping molecular structure of protein locating on nanoparticles with limited proteolysis. *Analytical Chemistry*. https://doi.org/10.1021/acs.analchem.9b00482

Dunigan, K., Li, Q., Li, R., Locy, M. L., Wall, S., & Tipple, T. E. (2018). The thioredoxin reductase inhibitor auranofin induces heme oxygenase-1 in lung epithelial cells via Nrf2-dependent mechanisms. *American Journal of Physiology - Lung Cellular and Molecular Physiology*, *315*(4), L545–L552. https://doi.org/10.1152/ajplung.00214.2018

Dunkley, T. P. J., Watson, R., Griffin, J. L., Dupree, P., & Lilley, K. S. (2004). Localization of organelle proteins by isotope tagging (LOPIT). *Molecular and Cellular Proteomics*. https://doi.org/10.1074/mcp.T400009-MCP200

Espinosa, B., & Arnér, E. S. J. (2019). Thioredoxin-related protein of 14 kDa as a modulator of redox signalling pathways. In *British Journal of Pharmacology* (Vol. 176, Issue 4, pp. 544–553). John Wiley and Sons Inc. https://doi.org/10.1111/bph.14479

Fatima, N., Chelius, D., Luke, B. T., Yi, M., Zhang, T., Stauffer, S., Stephens, R., Lynch, P., Miller, K., Guszczynski, T., Boring, D., Greenwald, P., & Ali, I. U. (2009). Label-free global serum proteomic profiling reveals novel celecoxib-modulated proteins in familial adenomatous polyposis patients. *Cancer Genomics and Proteomics*.

Feng, Y., De Franceschi, G., Kahraman, A., Soste, M., Melnik, A., Boersema, P. J., De Laureto, P. P., Nikolaev, Y., Oliveira, A. P., & Picotti, P. (2014). Global analysis of protein structural changes in complex proteomes. *Nature Biotechnology*. https://doi.org/10.1038/nbt.2999

Fernando, V., Zheng, X., Walia, Y., Sharma, V., Letson, J., & Furuta, S. (2019). S-Nitrosylation: An Emerging Paradigm of Redox Signaling. *Antioxidants*, *8*(9), 404. https://doi.org/10.3390/antiox8090404

Fierro-Monti, I., Racle, J., Hernandez, C., Waridel, P., Hatzimanikatis, V., & Quadroni, M. (2013). A novel pulse-chase SILAC strategy measures changes in protein decay and synthesis rates induced by perturbation of proteostasis with an Hsp90 inhibitor. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0080423

Finch, A. J., Hilcenko, C., Basse, N., Drynan, L. F., Goyenechea, B., Menne, T. F., Fernández, Á. G., Simpson, P., D'Santos, C. S., Arends, M. J., Donadieu, J., Bellanné-Chantelot, C., Costanzo, M., Boone, C., McKenzie, A. N., Freund, S. M. V. V., Warren, A. J., Gonzalez Fernandez, A., Simpson, P., … Warren, A. J. (2011). Uncoupling of GTP hydrolysis from eIF6 release on the ribosome causes shwachman-diamond syndrome. *Genes & Development*, *25*(9), 917–929. https://doi.org/10.1101/gad.623011

Flohé, L., Brigelius-Flohé, R., Saliou, C., Traber, M. G., & Packer, L. (1997). Redox regulation of NF-kappa B activation. *Free Radical Biology and Medicine*, *22*(6), 1115–

1126. https://doi.org/10.1016/S0891-5849(96)00501-1

Fournier, M. L., Paulson, A., Pavelka, N., Mosley, A. L., Gaudenz, K., Bradford, W. D., Glynn, E., Li, H., Sardiu, M. E., Fleharty, B., Seidel, C., Florens, L., & Washburn, M. P. (2010). Delayed correlation of mRNA and protein expression in rapamycin-treated cells and a role for Ggc1 in cellular sensitivity to rapamycin. *Molecular and Cellular Proteomics*. https://doi.org/10.1074/mcp.M900415-MCP200

Franken, H., Mathieson, T., Childs, D., Sweetman, G. M. A. A., Werner, T., Tögel, I., Doce, C., Gade, S., Bantscheff, M., Drewes, G., Reinhard, F. B. M. M., Huber, W., & Savitski, M. M. (2015). Thermal proteome profiling for unbiased identification of direct and indirect drug targets using multiplexed quantitative mass spectrometry. *Nature Protocols*, *10*(10), 1567–1593. https://doi.org/10.1038/nprot.2015.101

Fuse, Y., Endo, Y., Araoi, S., Daitoku, H., Suzuki, H., Kato, M., & Kobayashi, M. (2018). The possible repositioning of an oral anti-arthritic drug, auranofin, for Nrf2-activating therapy: The demonstration of Nrf2-dependent anti-oxidative action using a zebrafish model. *Free Radical Biology and Medicine*, *115*, 405–411. https://doi.org/10.1016/j.freeradbiomed.2017.12.022

Gaetani, M., Sabatier, P., Saei, A. A., Beusch, C. M., Yang, Z., Lundström, S. L., & Zubarev, R. A. (2019). Proteome Integral Solubility Alteration: A High-Throughput Proteomics Assay for Target Deconvolution. *Journal of Proteome Research*, *18*(11), 4027–4037. https://doi.org/10.1021/acs.jproteome.9b00500

Gaetani, M., & Zubarev, R. A. (2019). Functional Identification of Target by Expression Proteomics (FITExP) . In *Mass Spectrometry-Based Chemical Proteomics*. https://doi.org/10.1002/9781118970195.ch10

Gautam, J., Banskota, S., Lee, H., Lee, Y. J., Jeon, Y. H., Kim, J. A., & Jeong, B. S. (2018). Down-regulation of cathepsin S and matrix metalloproteinase-9 via Src, a non-receptor tyrosine kinase, suppresses triple-negative breast cancer growth and metastasis. *Experimental and Molecular Medicine*. https://doi.org/10.1038/s12276-018-0135-9

Geladaki, A., Kočevar Britovšek, N., Breckels, L. M., Smith, T. S., Vennard, O. L., Mulvey, C. M., Crook, O. M., Gatto, L., & Lilley, K. S. (2019). Combining LOPIT with differential ultracentrifugation for high-resolution spatial proteomics. *Nature Communications*. https://doi.org/10.1038/s41467-018-08191-w

Geng, J., Liu, W., Gao, J., Jiang, C., Fan, T., Sun, Y., Qin, Z. H., Xu, Q., Guo, W., & Gao, J. (2019). Andrographolide alleviates Parkinsonism in MPTP-PD mice via targeting mitochondrial fission mediated by dynamin-related protein 1. *British Journal of Pharmacology*. https://doi.org/10.1111/bph.14823

Graham, G. G., Champion, G. D., & Ziegler, J. B. (1991). The antirheumatic gold complexes: Considerations of chemical form in studies on their mechanism of action. In *Inflammopharmacology*. https://doi.org/10.1007/BF02735391

Grek, C. L., Zhang, J., Manevich, Y., Townsend, D. M., & Tew, K. D. (2013). Causes and consequences of cysteine s-glutathionylation. In *Journal of Biological Chemistry* (Vol. 288, Issue 37, pp. 26497–26504). Elsevier. https://doi.org/10.1074/jbc.R113.461368

Halsey, L. G., Curran-Everett, D., Vowler, S. L., & Drummond, G. B. (2015). The fickle P value generates irreproducible results. In *Nature Methods*. https://doi.org/10.1038/nmeth.3288

Harrison, R. K. (2016). Phase II and phase III failures: 2013-2015. *Nature Reviews Drug Discovery*. https://doi.org/10.1038/nrd.2016.184

Hornburg, D., Drepper, C., Butter, F., Meissner, F., Sendtner, M., & Mann, M. (2014). Deep proteomic evaluation of primary and cell line motoneuron disease models delineates major differences in neuronal characteristics. *Molecular and Cellular Proteomics*, *13*(12), 3410–3420. https://doi.org/10.1074/mcp.M113.037291

Huang, H., Petersen, M. H., Ibañez-Vea, M., Lassen, P. S., Larsen, M. R., & Palmisano, G. (2016). Simultaneous enrichment of cysteinecontaining peptides and phosphopeptides using a cysteine-specific phosphonate adaptable tag (CysPAT) in combination with titanium dioxide (TiO2) chromatography. *Molecular and Cellular Proteomics*. https://doi.org/10.1074/mcp.M115.054551

Huang, J. X., Lee, G., Cavanaugh, K. E., Chang, J. W., Gardel, M. L., & Moellering, R. E. (2019). High throughput discovery of functional protein modifications by Hotspot Thermal Profiling. *Nature Methods*, *16*(9), 894–901. https://doi.org/10.1038/s41592-019-0499-3

Humphrey, S. J., Karayel, O., James, D. E., & Mann, M. (2018). High-throughput and high-sensitivity phosphoproteomics with the EasyPhos platform. *Nature Protocols*. https://doi.org/10.1038/s41596-018-0014-9

Hung, M. C., & Link, W. (2011). Protein localization in disease and therapy. *Journal of Cell Science*. https://doi.org/10.1242/jcs.089110

Hwang, G. H., Ryu, J. M., Jeon, Y. J., Choi, J., Han, H. J., Lee, Y. M., Lee, S., Bae, J. S., Jung, J. W., Chang, W., Kim, L. K., Jee, J. G., & Lee, M. Y. (2015). The role of thioredoxin reductase and glutathione reductase in plumbagin-induced, reactive oxygen species-mediated apoptosis in cancer cell lines. *European Journal of Pharmacology*, *765*, 384–393. https://doi.org/10.1016/j.ejphar.2015.08.058

Itzhak, D. N., Davies, C., Tyanova, S., Mishra, A., Williamson, J., Antrobus, R., Cox, J., Weekes, M. P., & Borner, G. H. H. (2017). A Mass Spectrometry-Based Approach for Mapping Protein Subcellular Localization Reveals the Spatial Proteome of Mouse Primary Neurons. *Cell Reports*. https://doi.org/10.1016/j.celrep.2017.08.063

Itzhak, D. N., Tyanova, S., Cox, J., & Borner, G. H. H. (2016). Global, quantitative and dynamic mapping of protein subcellular localization. *ELife*. https://doi.org/10.7554/eLife.16950

Jafari, R., Almqvist, H., Axelsson, H., Ignatushchenko, M., Lundbäck, T., Nordlund, P., & Molina, D. M. (2014). The cellular thermal shift assay for evaluating drug target interactions in cells. *Nature Protocols*. https://doi.org/10.1038/nprot.2014.138

Jarzab, A., Kurzawa, N., Hopf, T., Moerch, M., Zecha, J., Leijten, N., Bian, Y., Musiol, E., Maschberger, M., Stoehr, G., Becher, I., Daly, C., Samaras, P., Mergner, J., Spanier, B., Angelov, A., Werner, T., Bantscheff, M., Wilhelm, M., … Kuster, B. (2020). Meltome atlas—thermal proteome stability across the tree of life. *Nature Methods*, *17*(5), 495–503. https://doi.org/10.1038/s41592-020-0801-4

Jayapal, K. P., Philp, R. J., Kok, Y. J., Yap, M. G. S., Sherman, D. H., Griffin, T. J., & Hu, W. S. (2008). Uncovering genes with divergent mRNA-protein dynamics in Streptomyces coelicolor. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0002097

Jeffery, D. A., & Bogyo, M. (2003). Chemical proteomics and its application to drug

discovery. In *Current Opinion in Biotechnology*. https://doi.org/10.1016/S0958-1669(02)00010-1

Jeong, W., Jung, Y., Kim, H., Park, S. J., & Rhee, S. G. (2009). Thioredoxin-related protein 14, a new member of the thioredoxin family with disulfide reductase activity: Implication in the redox regulation of TNF-α signaling. In *Free Radical Biology and Medicine* (Vol. 47, Issue 9, pp. 1294–1303). Free Radic Biol Med. https://doi.org/10.1016/j.freeradbiomed.2009.07.021

Jin, J., Zhi, X., Wang, X., & Meng, D. (2021). Protein palmitoylation and its pathophysiological relevance. *Journal of Cellular Physiology*, *236*(5), 3220–3233. https://doi.org/10.1002/jcp.30122

Johansson, H. J., Socciarelli, F., Vacanti, N. M., Haugen, M. H., Zhu, Y., Siavelis, I., Fernandez-Woodbridge, A., Aure, M. R., Sennblad, B., Vesterlund, M., Branca, R. M., Orre, L. M., Huss, M., Fredlund, E., Beraki, E., Garred, Ø., Boekel, J., Sauer, T., Zhao, W., … Lehtiö, J. (2019). Breast cancer quantitative proteome and proteogenomic landscape. *Nature Communications*. https://doi.org/10.1038/s41467-019-09018-y

Johansson, K., Cebula, M., Rengby, O., Dreij, K., Carlström, K. E., Sigmundsson, K., Piehl, F., & Arnér, E. S. J. (2017). Cross Talk in HEK293 Cells between Nrf2, HIF, and NF-κB Activities upon Challenges with Redox Therapeutics Characterized with Single-Cell Resolution. In *Antioxidants and Redox Signaling* (Vol. 26, Issue 6, pp. 229–246). Mary Ann Liebert Inc. https://doi.org/10.1089/ars.2015.6419

Joshi, R. N., Stadler, C., Lehmann, R., Lehtiö, J., Tegnér, J., Schmidt, A., & Vesterlund, M. (2019). TcellSubC: An Atlas of the Subcellular Proteome of Human T Cells. *Frontiers in Immunology*. https://doi.org/10.3389/fimmu.2019.02708

Kalxdorf, M., Günthner, I., Becher, I., Kurzawa, N., Knecht, S., Savitski, M. M., Eberl, H. C., & Bantscheff, M. (2021). Cell surface thermal proteome profiling tracks perturbations and drug targets on the plasma membrane. *Nature Methods*. https://doi.org/10.1038/s41592-020-01022-1

Kasamatsu, S., Nishimura, A., Morita, M., Matsunaga, T., Abdul Hamid, H., & Akaike, T. (2016). Redox Signaling Regulated by Cysteine Persulfide and Protein Polysulfidation. *Molecules*, *21*(12), 1721. https://doi.org/10.3390/molecules21121721

Kelkka, T., Pizzolla, A., Laurila, J. P., Friman, T., Gustafsson, R., Källberg, E., Olsson, O., Leanderson, T., Rubin, K., Salmi, M., Jalkanen, S., & Holmdahl, R. (2013). Mice lacking NCF1 exhibit reduced growth of implanted melanoma and carcinoma tumors. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0084148

Kim, M. S., Zhong, J., & Pandey, A. (2016). Common errors in mass spectrometry-based analysis of post-translational modifications. In *Proteomics*. https://doi.org/10.1002/pmic.201500355

Kim, N. H., Oh, M. K., Park, H. J., & Kim, I. S. (2010). Auranofin, a gold(I)-containing antirheumatic compound, activates Keap1/Nrf2 signaling via Rac1/iNOS signal and mitogen-activated protein kinase activation. *Journal of Pharmacological Sciences*. https://doi.org/10.1254/jphs.09330FP

Kipp, A. P., Deubel, S., Arnér, E. S. J., & Johansson, K. (2017). Time- and cell-resolved dynamics of redox-sensitive Nrf2, HIF and NF-κB activities in 3D spheroids enriched for cancer stem cells. *Redox Biology*, *12*, 403–409.

https://doi.org/10.1016/j.redox.2017.03.013

Klaeger, S., Gohlke, B., Perrin, J., Gupta, V., Heinzlmeir, S., Helm, D., Qiao, H., Bergamini, G., Handa, H., Savitski, M. M., Bantscheff, M., Médard, G., Preissner, R., & Kuster, B. (2016). Chemical Proteomics Reveals Ferrochelatase as a Common Off-target of Kinase Inhibitors. *ACS Chemical Biology*. https://doi.org/10.1021/acschembio.5b01063

Klaeger, S., Heinzlmeir, S., Wilhelm, M., Polzer, H., Vick, B., Koenig, P. A., Reinecke, M., Ruprecht, B., Petzoldt, S., Meng, C., Zecha, J., Reiter, K., Qiao, H., Helm, D., Koch, H., Schoof, M., Canevari, G., Casale, E., Re Depaolini, S., … Kuster, B. (2017). The target landscape of clinical kinase drugs. *Science*. https://doi.org/10.1126/science.aan4368

Kristensen, A. R., Gsponer, J., & Foster, L. J. (2013). Protein synthesis rate is the predominant regulator of protein expression during differentiation. *Molecular Systems Biology*. https://doi.org/10.1038/msb.2013.47

Krüger, M., Moser, M., Ussar, S., Thievessen, I., Luber, C. A., Forner, F., Schmidt, S., Zanivan, S., Fässler, R., & Mann, M. (2008). SILAC Mouse for Quantitative Proteomics Uncovers Kindlin-3 as an Essential Factor for Red Blood Cell Function. *Cell*. https://doi.org/10.1016/j.cell.2008.05.033

Lamberti, M. J., Wilkinson, M., Peña, Y., Getz, K., & Beltre, C. (2018). Preparing for precision medicine. *Contract Pharma*. https://doi.org/10.1056/nejmp1114866

Lazarev, V. F., Sverchinsky, D. V., Mikhaylova, E. R., Semenyuk, P. I., Komarova, E. Y., Niskanen, S. A., Nikotina, A. D., Burakov, A. V., Kartsev, V. G., Guzhova, I. V., & Margulis, B. A. (2018). Sensitizing tumor cells to conventional drugs: HSP70 chaperone inhibitors, their selection and application in cancer models. *Cell Death and Disease*. https://doi.org/10.1038/s41419-017-0160-y

Lee, M. V., Topper, S. E., Hubler, S. L., Hose, J., Wenger, C. D., Coon, J. J., & Gasch, A. P. (2011). A dynamic model of proteome changes reveals new roles for transcript alteration in yeast. *Molecular Systems Biology*. https://doi.org/10.1038/msb.2011.48

Lee, R. F. S. S., Chernobrovkin, A., Rutishauser, D., Allardyce, C. S., Hacker, D., Johnsson, K., Zubarev, R. A., & Dyson, P. J. (2017a). Expression proteomics study to determine metallodrug targets and optimal drug combinations. *Scientific Reports*, *7*, 1590. https://doi.org/10.1038/s41598-017-01643-1

Lee, R. F. S. S., Chernobrovkin, A., Rutishauser, D., Allardyce, C. S., Hacker, D., Johnsson, K., Zubarev, R. A., & Dyson, P. J. (2017b). Expression proteomics study to determine metallodrug targets and optimal drug combinations. *Scientific Reports*, *7*, 1590. https://doi.org/10.1038/s41598-017-01643-1

Leijten, N. M., Bakker, P., Spaink, H. P., den Hertog, J., & Lemeer, S. (2021). Thermal Proteome Profiling in Zebrafish Reveals Effects of Napabucasin on Retinoic Acid Metabolism. *Molecular and Cellular Proteomics*. https://doi.org/10.1074/MCP.RA120.002273

Leist, M., Raab, B., Maurer, S., Rösick, U., & Brigelius-Flohé, R. (1996). Conventional cell culture media do not adequately supply cells with antioxidants and thus facilitate peroxide-induced genotoxicity. *Free Radical Biology and Medicine*, *21*(3), 297–306. https://doi.org/10.1016/0891-5849(96)00045-7

Leuenberger, P., Ganscha, S., Kahraman, A., Cappelletti, V., Boersema, P. J., Von Mering, C., Claassen, M., & Picotti, P. (2017). Cell-wide analysis of protein thermal unfolding

reveals determinants of thermostability. *Science*. https://doi.org/10.1126/science.aai7825

Li, J., Van Vranken, J. G., Pontano Vaites, L., Schweppe, D. K., Huttlin, E. L., Etienne, C., Nandhikonda, P., Viner, R., Robitaille, A. M., Thompson, A. H., Kuhn, K., Pike, I., Bomgarden, R. D., Rogers, J. C., Gygi, S. P., & Paulo, J. A. (2020). TMTpro reagents: a set of isobaric labeling mass tags enables simultaneous proteome-wide measurements across 16 samples. *Nature Methods*. https://doi.org/10.1038/s41592-020-0781-4

Li, Q., Cao, L., Tian, Y., Zhang, P., Ding, C., Lu, W., Jia, C., Shao, C., Liu, W., Wang, D., Ye, H., & Hao, H. (2018). Butyrate suppresses the proliferation of colorectal cancer cells via targeting pyruvate kinase M2 and metabolic reprogramming. *Molecular and Cellular Proteomics*. https://doi.org/10.1074/mcp.RA118.000752

Lian, X., Hsiao, C., Wilson, G., Zhu, K., Hazeltine, L. B., Azarin, S. M., Raval, K. K., Zhang, J., Kamp, T. J., & Palecek, S. P. (2012). Robust cardiomyocyte differentiation from human pluripotent stem cells via temporal modulation of canonical Wnt signaling. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.1200250109

Liu, N., Li, X., Huang, H., Zhao, C. C., Liao, S., Yang, C., Liu, S., Song, W., Lu, X., Lan, X., Chen, X., Yi, S., Xu, L., Jiang, L., Zhao, C. C., Dong, X., Zhou, P., Li, S., Wang, S., … Liu, J. (2014). Clinically used antirheumatic agent auranofin is a proteasomal deubiquitinase inhibitor and inhibits tumor growth. *Oncotarget*. https://doi.org/10.18632/oncotarget.2113

Liu, Yansheng, Beyer, A., & Aebersold, R. (2016). On the Dependency of Cellular Protein Levels on mRNA Abundance. In *Cell*. https://doi.org/10.1016/j.cell.2016.03.014

Liu, Yongsheng, Patricelli, M. P., & Cravatt, B. F. (1999). Activity-based protein profiling: The serine hydrolases. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.96.26.14694

Lomenick, B., Hao, R., Jonai, N., Chin, R. M., Aghajan, M., Warburton, S., Wang, J., Wu, R. P., Gomez, F., Loo, J. A., Wohlschlegel, J. A., Vondriska, T. M., Pelletier, J., Herschman, H. R., Clardy, J., Clarke, C. F., & Huang, J. (2009). Target identification using drug affinity responsive target stability (DARTS). *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.0910040106

Lu, J., Zhong, X., Liu, H., Hao, L., Huang, C. T. L., Sherafat, M. A., Jones, J., Ayala, M., Li, L., & Zhang, S. C. (2016). Generation of serotonin neurons from human pluripotent stem cells. *Nature Biotechnology*. https://doi.org/10.1038/nbt.3435

Maier, T., Schmidt, A., Güell, M., Kühner, S., Gavin, A. C., Aebersold, R., & Serrano, L. (2011). Quantification of mRNA and protein and integration with protein turnover in a bacterium. *Molecular Systems Biology*. https://doi.org/10.1038/msb.2011.38

Marzano, C., Gandin, V., Folda, A., Scutari, G., Bindoli, A., & Rigobello, M. P. (2007). Inhibition of thioredoxin reductase by auranofin induces apoptosis in cisplatin-resistant human ovarian cancer cells. *Free Radical Biology and Medicine*. https://doi.org/10.1016/j.freeradbiomed.2006.12.021

Mateus, A., Bobonis, J., Kurzawa, N., Stein, F., Helm, D., Hevler, J., Typas, A., & Savitski, M. M. (2018). Thermal proteome profiling in bacteria: probing protein state in vivo . *Molecular Systems Biology*. https://doi.org/10.15252/msb.20188242

Mateus, A., Hevler, J., Bobonis, J., Kurzawa, N., Shah, M., Mitosch, K., Goemans, C. V., Helm, D., Stein, F., Typas, A., & Savitski, M. M. (2020). The functional proteome landscape of Escherichia coli. *Nature*. https://doi.org/10.1038/s41586-020-3002-5

Mateus, A., Määttä, T. A., & Savitski, M. M. (2016). Thermal proteome profiling: unbiased assessment of protein state through heat-induced stability changes. *Proteome Science*, *15*(1), 13. https://doi.org/10.1186/s12953-017-0122-4

McAlister, G. C., Nusinow, D. P., Jedrychowski, M. P., Wühr, M., Huttlin, E. L., Erickson, B. K., Rad, R., Haas, W., & Gygi, S. P. (2014). MultiNotch MS3 enables accurate, sensitive, and multiplexed detection of differential expression across cancer cell line proteomes. *Analytical Chemistry*. https://doi.org/10.1021/ac502040v

McDonald, W. H., & Yates, J. R. (2003). Shotgun proteomics: Integrating technologies to answer biological questions. *Current Opinion in Molecular Therapeutics*.

McInnes, L., Healy, J., & Melville, J. (2018). UMAP: Uniform Manifold Approximation and Projection for Dimension Reduction. *ArXiv*. http://arxiv.org/abs/1802.03426

Menne, T. F., Goyenechea, B., Sánchez-Puig, N., Wong, C. C., Tonkin, L. M., Ancliff, P. J., Brost, R. L., Costanzo, M., Boone, C., & Warren, A. J. (2007). The Shwachman-Bodian-Diamond syndrome protein mediates translational activation of ribosomes in yeast. *Nature Genetics*, *39*(4), 486–495. https://doi.org/10.1038/ng1994

Messner, C. B., Demichev, V., Bloomfield, N., Yu, J. S. L., White, M., Kreidl, M., Egger, A. S., Freiwald, A., Ivosev, G., Wasim, F., Zelezniak, A., Jürgens, L., Suttorp, N., Sander, L. E., Kurth, F., Lilley, K. S., Mülleder, M., Tate, S., & Ralser, M. (2021). Ultra-fast proteomics with Scanning SWATH. *Nature Biotechnology*. https://doi.org/10.1038/s41587-021-00860-4

Meyer, J. G., Niemi, N. M., Pagliarini, D. J., & Coon, J. J. (2020). Quantitative shotgun proteome analysis by direct infusion. *Nature Methods*. https://doi.org/10.1038/s41592-020-00999-z

Molina, D. M., Jafari, R., Ignatushchenko, M., Seki, T., Larsson, E. A., Dan, C., Sreekumar, L., Cao, Y., & Nordlund, P. (2013). Monitoring drug target engagement in cells and tissues using the cellular thermal shift assay. *Science*. https://doi.org/10.1126/science.1233606

Morizane, R., Lam, A. Q., Freedman, B. S., Kishi, S., Valerius, M. T., & Bonventre, J. V. (2015). Nephron organoids derived from human pluripotent stem cells model kidney development and injury. *Nature Biotechnology*. https://doi.org/10.1038/nbt.3392

Mostert, V., Hill, K. E., Ferris, C. D., & Burk, R. F. (2003). Selective induction of liver parenchymal cell heme oxygenase-1 in selenium-deficient rats. *Biological Chemistry*. https://doi.org/10.1515/BC.2003.076

Nagaraj, N., Kulak, N. A., Cox, J., Neuhauser, N., Mayr, K., Hoerning, O., Vorm, O., & Mann, M. (2012). System-wide perturbation analysis with nearly complete coverage of the yeast proteome by single-shot ultra HPLC runs on a bench top orbitrap. *Molecular and Cellular Proteomics*. https://doi.org/10.1074/mcp.M111.013722

Nakamura, S., Takayama, N., Hirata, S., Seo, H., Endo, H., Ochi, K., Fujita, K. I., Koike, T., Harimoto, K. I., Dohda, T., Watanabe, A., Okita, K., Takahashi, N., Sawaguchi, A., Yamanaka, S., Nakauchi, H., Nishimura, S., & Eto, K. (2014). Expandable megakaryocyte cell lines enable clinically applicable generation of platelets from human

induced pluripotent stem cells. *Cell Stem Cell*. https://doi.org/10.1016/j.stem.2014.01.011

Nampoothiri, S. S., & Rajanikant, G. K. (2019). miR-9 Upregulation Integrates Post-ischemic Neuronal Survival and Regeneration In Vitro. *Cellular and Molecular Neurobiology*. https://doi.org/10.1007/s10571-018-0642-1

Nielsen, M. L., Savitski, M. M., & Zubarev, R. A. (2006). Extent of modifications in human proteome samples and their effect on dynamic range of analysis in shotgun proteomics. *Molecular and Cellular Proteomics*. https://doi.org/10.1074/mcp.M600248-MCP200

Nishiyama, A., Frappier, L., & Méchali, M. (2011). MCM-BP regulates unloading of the MCM2-7 helicase in late S phase. *Genes and Development*. https://doi.org/10.1101/gad.614411

Noberini, R., & Bonaldi, T. (2019). Epigenetic drug target deconvolution by mass spectrometry–based technologies. *Nature Structural and Molecular Biology*, *26*(10), 854–857. https://doi.org/10.1038/s41594-019-0279-x

Nolbrant, S., Heuer, A., Parmar, M., & Kirkeby, A. (2017). Generation of high-purity human ventral midbrain dopaminergic progenitors for in vitro maturation and intracerebral transplantation. *Nature Protocols*. https://doi.org/10.1038/nprot.2017.078

Nusinow, D. P., Szpyt, J., Ghandi, M., Rose, C. M., McDonald, E. R., Kalocsay, M., Jané-Valbuena, J., Gelfand, E., Schweppe, D. K., Jedrychowski, M., Golji, J., Porter, D. A., Rejtar, T., Wang, Y. K., Kryukov, G. V., Stegmeier, F., Erickson, B. K., Garraway, L. A., Sellers, W. R., & Gygi, S. P. (2020). Quantitative Proteomics of the Cancer Cell Line Encyclopedia. *Cell*. https://doi.org/10.1016/j.cell.2019.12.023

Olsson, U., Lundgren, B., Segura-Aguilar, J., Messing-Eriksson, A., Andersson, K., Becedas, L., & De Pierre, J. W. (1993). Effects of selenium deficiency on xenobiotic-metabolizing and other enzymes in rat liver. *International Journal for Vitamin and Nutrition Research*.

Ong, S. E. (2012). The expanding field of SILAC. In *Analytical and Bioanalytical Chemistry*. https://doi.org/10.1007/s00216-012-5998-3

Ong, S. E., Blagoev, B., Kratchmarova, I., Kristensen, D. B., Steen, H., Pandey, A., & Mann, M. (2002). Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. *Molecular & Cellular Proteomics : MCP*. https://doi.org/10.1074/mcp.M200025-MCP200

Ong, S. E., Schenone, M., Margolin, A. A., Li, X., Do, K., Doud, M. K., Mani, D. R., Kuai, L., Wang, X., Wood, J. L., Tolliday, N. J., Koehler, A. N., Marcaurelle, L. A., Golub, T. R., Gould, R. J., Schreiber, S. L., & Carr, S. A. (2009). Identifying the proteins to which small-molecule probes and drugs bind in cells. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.0900191106

Orre, L. M., Vesterlund, M., Frings, O., Fredlund, E., Lehtiö, J., Minus Orre, L., Pan, Y., Arslan, T., Zhu, Y., & Woodbridge, A. F. (2019). SubCellBarCode: Proteome-wide Mapping of Protein Localization and Relocalization Molecular Cell Resource SubCellBarCode: Proteome-wide Mapping of Protein Localization and Relocalization. *Molecular Cell*.

Osakada, F., Ikeda, H., Sasai, Y., & Takahashi, M. (2009). Stepwise differentiation of pluripotent stem cells into retinal cells. *Nature Protocols*.

https://doi.org/10.1038/nprot.2009.51

Pace, C. N., & McGrath, T. (1980). Substrate stabilization of lysozyme to thermal and guanidine hydrochloride denaturation. *Journal of Biological Chemistry*.

Pagliuca, F. W., Millman, J. R., Gürtler, M., Segel, M., Van Dervort, A., Ryu, J. H., Peterson, Q. P., Greiner, D., & Melton, D. A. (2014). Generation of functional human pancreatic β cells in vitro. *Cell*. https://doi.org/10.1016/j.cell.2014.09.040

Pantoliano, M. W., Petrella, E. C., Kwasnoski, J. D., Lobanov, V. S., Myslik, J., Graf, E., Carver, T., Asel, E., Springer, B. A., Lane, P., & Salemme, F. R. (2001). High-density miniaturized thermal shift assays as a general strategy for drug discovery. *Journal of Biomolecular Screening*. https://doi.org/10.1089/108705701753364922

Park, C., & Marqusee, S. (2005). Pulse proteolysis: A simple method for quantitative determination of protein stability and ligand binding. *Nature Methods*. https://doi.org/10.1038/nmeth740

Peck Justice, S. A., Barron, M. P., Qi, G. D., Sagara Wijeratne, H. R., Victorino, J. F., Simpson, E. R., Vilseck, J. Z., Wijeratne, A. B., & Mosley, A. L. (2020). Mutant thermal proteome profiling for characterization of missense protein variants and their associated phenotypes within the proteome. *Journal of Biological Chemistry*. https://doi.org/10.1074/jbc.RA120.014576

Pepelnjak, M., de Souza, N., & Picotti, P. (2020). Detecting Protein–Small Molecule Interactions Using Limited Proteolysis–Mass Spectrometry (LiP-MS). In *Trends in Biochemical Sciences*. https://doi.org/10.1016/j.tibs.2020.05.006

Perrin, J., Werner, T., Kurzawa, N., Rutkowska, A., Childs, D. D., Kalxdorf, M., Poeckel, D., Stonehouse, E., Strohmer, K., Heller, B., Thomson, D. W., Krause, J., Becher, I., Eberl, H. C., Vappiani, J., Sevin, D. C., Rau, C. E., Franken, H., Huber, W., … Bergamini, G. (2020). Identifying drug targets in tissues and whole blood with thermal-shift profiling. In *Nature Biotechnology*. https://doi.org/10.1038/s41587-019-0388-4

Piazza, I., Beaton, N., Bruderer, R., Knobloch, T., Barbisan, C., Chandat, L., Sudau, A., Siepe, I., Rinner, O., de Souza, N., Picotti, P., & Reiter, L. (2020). A machine learning-based chemoproteomic approach to identify drug targets and binding sites in complex proteomes. *Nature Communications*. https://doi.org/10.1038/s41467-020-18071-x

Piazza, I., Kochanowski, K., Cappelletti, V., Fuhrer, T., Noor, E., Sauer, U., & Picotti, P. (2018). A Map of Protein-Metabolite Interactions Reveals Principles of Chemical Communication. *Cell*. https://doi.org/10.1016/j.cell.2017.12.006

Pirmoradian, M., Budamgunta, H., Chingin, K., Zhang, B., Astorga-Wells, J., & Zubarev, R. A. (2013). Rapid and deep human proteome analysis by single-dimension shotgun proteomics. *Molecular and Cellular Proteomics*, *12*(11), 3330–3338. https://doi.org/10.1074/mcp.O113.028787

Pirmoradian, M., Zhang, B., Chingin, K., Astorga-Wells, J., & Zubarev, R. A. (2014). Membrane-assisted isoelectric focusing device as a micropreparative fractionator for two-dimensional shotgun proteomics. *Analytical Chemistry*. https://doi.org/10.1021/ac404180e

Poulos, J. (2018). The limited application of stem cells in medicine: A review. In *Stem Cell Research and Therapy*. https://doi.org/10.1186/s13287-017-0735-7

Qu, Z., Meng, F., Bomgarden, R. D., Viner, R. I., Li, J., Rogers, J. C., Cheng, J., Greenlief, C. M., Cui, J., Lubahn, D. B., Sun, G. Y., & Gu, Z. (2014). Proteomic quantification and site-mapping of S -nitrosylated proteins using isobaric iodoTMT reagents. *Journal of Proteome Research*. https://doi.org/10.1021/pr401179v

Quimbaya, M., Raspé, E., Denecker, G., De Craene, B., Roelandt, R., Declercq, W., Sagaert, X., De Veylder, L., & Berx, G. (2014). Deregulation of the Replisome Factor MCMBP Prompts Oncogenesis in Colorectal Carcinomas through Chromosomal Instability. *Neoplasia (United States)*. https://doi.org/10.1016/j.neo.2014.07.011

Reinhard, F. B. M., Eberhard, D., Werner, T., Franken, H., Childs, D., Doce, C., Savitski, M. M. F., Huber, W., Bantscheff, M., Savitski, M. M. F., & Drewes, G. (2015). Thermal proteome profiling monitors ligand interactions with cellular membrane proteins. *Nature Methods*. https://doi.org/10.1038/nmeth.3652

Rix, U., & Superti-Furga, G. (2009). Target profiling of small molecules by chemical proteomics. *Nature Chemical Biology*, *5*(9), 616–624. https://doi.org/10.1038/nchembio.216

Rodin, S., Antonsson, L., Hovatta, O., & Tryggvason, K. (2014). Monolayer culturing and cloning of human pluripotent stem cells on laminin-521–based matrices under xeno-free and chemically defined conditions. *Nature Protocols*, *9*(10), 2354–2368. https://doi.org/10.1038/nprot.2014.159

Rodriguez-Furlan, C., Zhang, C., Raikhel, N., & Hicks, G. R. (2017). Drug Affinity Responsive Target Stability (DARTS) to Resolve Protein–Small Molecule Interaction in Arabidopsis. *Current Protocols in Plant Biology*. https://doi.org/10.1002/cppb.20062

Rogaia, D., Grignani, F., Carbone, R., Riganelli, D., LoCoco, F., Nakamura, T., Croce, C. M., Di Fiore, P. P., & Pelicci, P. G. (1997). The localization of the HRX/ALL1 protein to specific nuclear subdomains is altered by fusion with its eps15 translocation partner. *Cancer Research*.

Ross, P. L., Huang, Y. N., Marchese, J. N., Williamson, B., Parker, K., Hattan, S., Khainovski, N., Pillai, S., Dey, S., Daniels, S., Purkayastha, S., Juhasz, P., Martin, S., Bartlet-Jones, M., He, F., Jacobson, A., & Pappin, D. J. (2004). Multiplexed protein quantitation in Saccharomyces cerevisiae using amine-reactive isobaric tagging reagents. *Molecular and Cellular Proteomics*. https://doi.org/10.1074/mcp.M400129-MCP200

Sabatier, P., Saei, A. A., Wang, S., & Zubarev, R. A. (2018). Dynamic Proteomics Reveals High Plasticity of Cellular Proteome: Growth-Related and Drug-Induced Changes in Cancer Cells are Comparable. *Proteomics*. https://doi.org/10.1002/pmic.201800118

Saei, A. A., Beusch, C. M., Chernobrovkin, A., Sabatier, P., Zhang, B., Tokat, Ü. G., Stergiou, E., Gaetani, M., Végvári, Á., & Zubarev, R. A. (2019). ProTargetMiner as a proteome signature library of anticancer molecules for functional discovery. *Nature Communications*. https://doi.org/10.1038/s41467-019-13582-8

Saei, A. A., Beusch, C. M., Sabatier, P., Wells, J. A., Gharibi, H., Meng, Z., Chernobrovkin, A., Rodin, S., Näreoja, K., Thorsell, A.-G., Karlberg, T., Cheng, Q., Lundström, S. L., Gaetani, M., Végvári, Á., Arnér, E. S. J., Schüler, H., & Zubarev, R. A. (2021). System-wide identification and prioritization of enzyme substrates by thermal analysis. *Nature Communications*.

Saei, A. A., Gullberg, H., Sabatier, P., Beusch, C. M., Johansson, K., Lundgren, B., Arvidsson, P. I., Arnér, E. S. J., & Zubarev, R. A. (2020). Comprehensive chemical proteomics for target deconvolution of the redox active drug auranofin. *Redox Biology*. https://doi.org/10.1016/j.redox.2020.101491

Saei, A. A., Sabatier, P., Güler Tokat, Ü., Chernobrovkin, A., Pirmoradian, M., & Zubarev, R. A. (2018). Comparative proteomics of dying and surviving cancer cells improves the identification of drug targets and sheds light on cell life/death decisions. *Molecular &Amp; Cellular Proteomics*. http://www.mcponline.org/content/early/2018/03/23/mcp.RA118.000610.abstract

Savitski, M. M. F., Reinhard, F. B. M. M., Franken, H., Werner, T., Savitski, M. M. F., Eberhard, D., Molina, D. M., Jafari, R., Dovega, R. B., Klaeger, S. S., Kuster, B., Nordlund, P., Bantscheff, M., & Drewes, G. (2014). Tracking cancer drugs in living cells by thermal profiling of the proteome. *Science*, *346*(6205), 1255784. https://doi.org/10.1126/science.1255784

Savitski, M. M., Nielsen, M. L., & Zubarev, R. A. (2006). ModifiComb, a new proteomic tool for mapping substoichiometric post-translational modifications, finding novel types of modifications, and fingerprinting complex protein mixtures. *Molecular and Cellular Proteomics*. https://doi.org/10.1074/mcp.T500034-MCP200

Savitski, M. M., Zinn, N., Faelth-Savitski, M., Poeckel, D., Gade, S., Becher, I., Muelbaier, M., Wagner, A. J., Strohmer, K., Werner, T., Melchert, S., Petretich, M., Rutkowska, A., Vappiani, J., Franken, H., Steidel, M., Sweetman, G. M., Gilan, O., Lam, E. Y. N., … Bantscheff, M. (2018). Multiplexed Proteome Dynamics Profiling Reveals Mechanisms Controlling Protein Homeostasis. *Cell*. https://doi.org/10.1016/j.cell.2018.02.030

Schopper, S., Kahraman, A., Leuenberger, P., Feng, Y., Piazza, I., Müller, O., Boersema, P. J., & Picotti, P. (2017). Measuring protein structural changes on a proteome-wide scale using limited proteolysis-coupled mass spectrometry. *Nature Protocols*. https://doi.org/10.1038/nprot.2017.100

Schrecengost, R. S., Green, C. L., Zhuang, Y., Keller, S. N., Smith, R. A., Maines, L. W., & Smith, C. D. (2018). In vitro and in vivo antitumor and anti-inflammatory capabilities of the novel GSK3 and CDK9 inhibitor ABC1183s. *Journal of Pharmacology and Experimental Therapeutics*. https://doi.org/10.1124/jpet.117.245738

Sedlackova, H., Rask, M. B., Gupta, R., Choudhary, C., Somyajit, K., & Lukas, J. (2020). Equilibrium between nascent and parental MCM proteins protects replicating genomes. *Nature*. https://doi.org/10.1038/s41586-020-2842-3

Sevier, C. S., & Kaiser, C. A. (2002). Formation and transfer of disulphide bonds in living cells. In *Nature Reviews Molecular Cell Biology* (Vol. 3, Issue 11, pp. 836–847). Nature Publishing Group. https://doi.org/10.1038/nrm954

Shi, H., Zhang, C. J., Chen, G. Y. J., & Yao, S. Q. (2012). Cell-based proteome profiling of potential Dasatinib targets by use of affinity-based probes. *Journal of the American Chemical Society*. https://doi.org/10.1021/ja208518u

Somasekharan, S. P., Stoynov, N., Rotblat, B., Leprivier, G., Galpin, J. D., Ahern, C. A., Foster, L. J., & Sorensen, P. H. B. (2012). Identification and quantification of newly synthesized proteins translationally regulated by YB-1 using a novel Click-SILAC approach. *Journal of Proteomics*. https://doi.org/10.1016/j.jprot.2012.08.019

Spence, J. R., Mayhew, C. N., Rankin, S. A., Kuhar, M. F., Vallance, J. E., Tolle, K., Hoskins, E. E., Kalinichenko, V. V., Wells, S. I., Zorn, A. M., Shroyer, N. F., & Wells, J. M. (2011). Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. *Nature*. https://doi.org/10.1038/nature09691

Srivastava, P. K., Eyll, J. van, Godard, P., Mazzuferi, M., Delahaye-Duriez, A., Steenwinckel, J. Van, Gressens, P., Danis, B., Vandenplas, C., Foerch, P., Leclercq, K., Mairet-Coello, G., Cardenas, A., Vanclef, F., Laaniste, L., Niespodziany, I., Keaney, J., Gasser, J., Gillet, G., … Johnson, M. R. (2018). A systems-level framework for drug discovery identifies Csf1R as an anti-epileptic drug target. *Nature Communications*. https://doi.org/10.1038/s41467-018-06008-4

Stafford, W. C., Peng, X., Olofsson, M. H., Zhang, X., Luci, D. K., Lu, L., Cheng, Q., Trésaugues, L., Dexheimer, T. S., Coussens, N. P., Augsten, M., Ahlzén, H. S. M., Orwar, O., Östman, A., Stone-Elander, S., Maloney, D. J., Jadhav, A., Simeonov, A., Linder, S., & Arnér, E. S. J. (2018). Irreversible inhibition of cytosolic thioredoxin reductase 1 as a mechanistic basis for anticancer therapy. *Science Translational Medicine*. https://doi.org/10.1126/scitranslmed.aaf7444

Sun, W., Dai, L., Yu, H., Puspita, B., Zhao, T., Li, F., Tan, J. L., Lim, Y. T., Chen, M. W., Sobota, R. M., Tenen, D. G., Prabhu, N., & Nordlund, P. (2019). Monitoring structural modulation of redox-sensitive proteins in cells with MS-CETSA. *Redox Biology*. https://doi.org/10.1016/j.redox.2019.101168

Sury, M. D., Chen, J. X., & Selbach, M. (2010). The SILAC fly allows for accurate protein quantification in vivo. *Molecular and Cellular Proteomics*. https://doi.org/10.1074/mcp.M110.000323

Swanson, S. K., & Washburn, M. P. (2005). The continuing evolution of shotgun proteomics. In *Drug Discovery Today*. https://doi.org/10.1016/S1359-6446(05)03450-1

Takahashi, K., & Yamanaka, S. (2006). Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. *Cell*. https://doi.org/10.1016/j.cell.2006.07.024

Talbot, S., Nelson, R., & Self, W. T. (2008). Arsenic trioxide and auranofin inhibit selenoprotein synthesis: Implications for chemotherapy for acute promyelocytic leukaemia. *British Journal of Pharmacology*. https://doi.org/10.1038/bjp.2008.161

Tan, C. S. H., Go, K. D., Bisteau, X., Dai, L., Yong, C. H., Prabhu, N., Ozturk, M. B., Lim, Y. T., Sreekumar, L., Lengqvist, J., Tergaonkar, V., Kaldis, P., Sobota, R. M., & Nordlund, P. (2018). Thermal proximity coaggregation for system-wide profiling of protein complex dynamics in cells. *Science*, *359*(6380), 1170–1177. https://doi.org/10.1126/science.aan0346

Tanie, Y., Tanabe, N., Kuboyama, T., & Tohda, C. (2018). Extracellular neuroleukin enhances neuroleukin secretion from astrocytes and promotes axonal growth in vitro and in vivo. *Frontiers in Pharmacology*. https://doi.org/10.3389/fphar.2018.01228

Tao, W. A., & Zhang, Y. (2019). Mass spectrometry-based chemical proteomics. In *Mass Spectrometry-Based Chemical Proteomics*. https://doi.org/10.1002/9781118970195

Tarasova, N. K., Gallud, A., Ytterberg, A. J., Chernobrovkin, A., Aranzaes, J. R., Astruc, D., Antipov, A., Fedutik, Y., Fadeel, B., & Zubarev, R. A. (2017). Cytotoxic and Proinflammatory Effects of Metal-Based Nanoparticles on THP-1 Monocytes

Characterized by Combined Proteomics Approaches. *Journal of Proteome Research*, *16*(2), 689–697. https://doi.org/10.1021/acs.jproteome.6b00747

Thompson, A., Schäfer, J., Kuhn, K., Kienle, S., Schwarz, J., Schmidt, G., Neumann, T., & Hamon, C. (2003). Tandem mass tags: A novel quantification strategy for comparative analysis of complex protein mixtures by MS/MS. *Analytical Chemistry*. https://doi.org/10.1021/ac0262560

Thompson, A., Wölmer, N., Koncarevic, S., Selzer, S., Böhm, G., Legner, H., Schmid, P., Kienle, S., Penning, P., Höhle, C., Berfelde, A., Martinez-Pinna, R., Farztdinov, V., Jung, S., Kuhn, K., & Pike, I. (2019). TMTpro: Design, Synthesis, and Initial Evaluation of a Proline-Based Isobaric 16-Plex Tandem Mass Tag Reagent Set. *Analytical Chemistry*. https://doi.org/10.1021/acs.analchem.9b04474

Thygesen, C., Boll, I., Finsen, B., Modzel, M., & Larsen, M. R. (2018). Characterizing disease-associated changes in post-translational modifications by mass spectrometry. In *Expert Review of Proteomics*. https://doi.org/10.1080/14789450.2018.1433036

Tian, S., Siu, F. M., Lok, C. N., Fung, Y. M. E., & Che, C. M. (2019). Anticancer auranofin engages 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) as a target. *Metallomics*. https://doi.org/10.1039/c9mt00185a

Van Bergen, L. A. H., Roos, G., & De Proft, F. (2014). From thiol to sulfonic acid: Modeling the oxidation pathway of protein thiols by hydrogen peroxide. *Journal of Physical Chemistry A*, *118*(31), 6078–6084. https://doi.org/10.1021/jp5018339

Van Der Maaten, L., & Hinton, G. (2008). Visualizing Data using t-SNE. In *Journal of Machine Learning Research* (Vol. 9, Issue 86). http://jmlr.org/papers/v9/vandermaaten08a.html

Van Der Reest, J., Lilla, S., Zheng, L., Zanivan, S., & Gottlieb, E. (2018). Proteome-wide analysis of cysteine oxidation reveals metabolic sensitivity to redox stress. *Nature Communications*. https://doi.org/10.1038/s41467-018-04003-3

Vogel, C., Silva, G. M., & Marcotte, E. M. (2011). Protein expression regulation under oxidative stress. *Molecular and Cellular Proteomics*. https://doi.org/10.1074/mcp.M111.009217

Voruganti, S., Lacroix, J. C., Rogers, C. N., Rogers, J., Matts, R. L., & Hartson, S. D. (2013). The anticancer drug AUY922 generates a proteomics fingerprint that is highly conserved among structurally diverse Hsp90 inhibitors. *Journal of Proteome Research*. https://doi.org/10.1021/pr400321x

Wan, X., Harkavy, B., Shen, N., Grohar, P., & Helman, L. J. (2007). Rapamycin induces feedback activation of Akt signaling through an IGF-1R-dependent mechanism. *Oncogene*. https://doi.org/10.1038/sj.onc.1209990

Wang, D., Eraslan, B., Wieland, T., Hallström, B., Hopf, T., Zolg, D. P., Zecha, J., Asplund, A., Li, L., Meng, C., Frejno, M., Schmidt, T., Schnatbaum, K., Wilhelm, M., Ponten, F., Uhlen, M., Gagneur, J., Hahne, H., & Kuster, B. (2019). A deep proteome and transcriptome abundance atlas of 29 healthy human tissues. *Molecular Systems Biology*. https://doi.org/10.15252/msb.20188503

Wang, H., Sun, S., Zhang, Y., Chen, S., Liu, P., & Liu, B. (2015). An off-line high pH reversed-phase fractionation and nano-liquid chromatography-mass spectrometry method for global proteomic profiling of cell lines. *Journal of Chromatography B:*

Analytical Technologies in the Biomedical and Life Sciences. https://doi.org/10.1016/j.jchromb.2014.10.031

Wang, J., Gao, L., Lee, Y. M., Kalesh, K. A., Ong, Y. S., Lim, J., Jee, J. E., Sun, H., Lee, S. S., Hua, Z. C., & Lin, Q. (2016). Target identification of natural and traditional medicines with quantitative chemical proteomics approaches. In *Pharmacology and Therapeutics*. https://doi.org/10.1016/j.pharmthera.2016.01.010

Wei, W., Luo, W., Wu, F., Peng, X., Zhang, Y. Y., Zhang, M., Zhao, Y., Su, N., Qi, Y., Chen, L., Zhang, Y. Y., Wen, B., He, F., & Xu, P. (2016). Deep Coverage Proteomics Identifies More Low-Abundance Missing Proteins in Human Testis Tissue with Q-Exactive HF Mass Spectrometer. *Journal of Proteome Research*, *15*(11), 3988–3997. https://doi.org/10.1021/acs.jproteome.6b00390

Weis, F., Giudice, E., Churcher, M., Jin, L., Hilcenko, C., Wong, C. C., Traynor, D., Kay, R. R., & Warren, A. J. (2015). Mechanism of eIF6 release from the nascent 60S ribosomal subunit. *Nature Structural & Molecular Biology*, *22*(11), 914–919. https://doi.org/10.1038/nsmb.3112

Welle, K. A., Zhang, T., Hryhorenko, J. R., Shen, S., Qu, J., & Ghaemmaghami, S. (2016). Time-resolved analysis of proteome dynamics by tandem mass tags and stable isotope labeling in cell culture (TMT-SILAC) Hyperplexing. *Molecular and Cellular Proteomics*. https://doi.org/10.1074/mcp.M116.063230

Winkelmann, C. T., Figueroa, S. D., Rold, T. L., Volkert, W. A., & Hoffman, T. J. (2006). Microimaging characterization of a B16-F10 melanoma metastasis mouse model. *Molecular Imaging*. https://doi.org/10.2310/7290.2006.00011

Wong, A. P., Bear, C. E., Chin, S., Pasceri, P., Thompson, T. O., Huan, L. J., Ratjen, F., Ellis, J., & Rossant, J. (2012). Directed differentiation of human pluripotent stem cells into mature airway epithelia expressing functional CFTR protein. *Nature Biotechnology*. https://doi.org/10.1038/nbt.2328

Wu, C. C., & MacCoss, M. J. (2002). Shotgun proteomics: Tools for the analysis of complex biological systems. *Current Opinion in Molecular Therapeutics*.

Xin, Z., Yamaguchi, A., & Sakamoto, K. (2014). Aberrant expression and altered cellular localization of desmosomal and hemidesmosomal proteins are associated with aggressive clinicopathological features of oral squamous cell carcinoma. *Virchows Archiv*. https://doi.org/10.1007/s00428-014-1594-6

Yang, F., Shen, Y., Camp, D. G., & Smith, R. D. (2012). High-pH reversed-phase chromatography with fraction concatenation for 2D proteomic analysis. In *Expert Review of Proteomics*. https://doi.org/10.1586/epr.12.15

Yang, J., Carroll, K. S., & Liebler, D. C. (2016). The expanding landscape of the thiol redox proteome. In *Molecular and Cellular Proteomics*. https://doi.org/10.1074/mcp.O115.056051

Yang, Z., Kuboyama, T., & Tohda, C. (2017). A systematic strategy for discovering a therapeutic drug for Alzheimer's disease and its target molecule. *Frontiers in Pharmacology*. https://doi.org/10.3389/fphar.2017.00340

Yoo, M. H., Xu, X. M., Carlson, B. A., Gladyshev, V. N., & Hatfield, D. L. (2006). Thioredoxin reductase 1 deficiency reverses tumor phenotype and tumorigenicity of lung carcinoma cells. *Journal of Biological Chemistry*.

https://doi.org/10.1074/jbc.C600012200

Zhang, X., Selvaraju, K., Saei, A. A., D'Arcy, P., Zubarev, R. A., Arnér, E. S., & Linder, S. (2019). Repurposing of auranofin: Thioredoxin reductase remains a primary target of the drug. *Biochimie*. https://doi.org/10.1016/j.biochi.2019.03.015

Zhou, F., Lu, Y., Ficarro, S. B., Adelmant, G., Jiang, W., Luckey, C. J., & Marto, J. A. (2013). Genome-scale proteome quantification by DEEP SEQ mass spectrometry. *Nature Communications*, *4*. https://doi.org/10.1038/ncomms3171

Zou, T., Lum, C. T., Lok, C. N., To, W. P., Low, K. H., & Che, C. M. (2014). A binuclear gold(I) complex with mixed bridging diphosphine and bis(N-heterocyclic carbene) ligands shows favorable thiol reactivity and inhibits tumor growth and angiogenesis in vivo. *Angewandte Chemie - International Edition*. https://doi.org/10.1002/anie.201400142

Zweerink, S., Kallnik, V., Ninck, S., Nickel, S., Verheyen, J., Blum, M., Wagner, A., Feldmann, I., Sickmann, A., Albers, S. V., Bräsen, C., Kaschani, F., Siebers, B., & Kaiser, M. (2017). Activity-based protein profiling as a robust method for enzyme identification and screening in extremophilic Archaea. *Nature Communications*. https://doi.org/10.1038/ncomms15352