

Contents

Summary	iii
Zusammenfassung	v
List of Figures	x
List of Tables	xii
List of Abbreviations	xiii
1 Introduction	1
1.1 Proteomics background.....	1
1.2 Mass spectrometry.....	1
1.2.1 Principle, key inventions and types.....	2
1.2.2 Linear ion trap Orbitrap hybrid mass spectrometer.....	2
1.2.3 Quadrupole Orbitrap hybrid mass spectrometer.....	3
1.3 Liquid chromatography mass spectrometry based proteomics.....	4
1.4 Mass spectrometry based quantitative proteomics.....	8
1.4.1 Relative label-free quantification.....	10
1.4.2 Relative quantification based on stable isotope labeling.....	11
1.4.2.1 Enzymatic labeling.....	11
1.4.2.2 Chemical labeling.....	12
1.4.2.2.1 Chemical labeling using ICAT.....	12
1.4.2.2.2 Chemical labeling using iTRAQ and TMT.....	13
1.4.2.3 Metabolic labeling.....	14
1.4.2.3.1 Metabolic labeling using SILAC.....	15
1.4.3 Absolute quantification using stable isotope label.....	16
1.4.3.1 Absolute quantification using AQUA.....	17
1.4.3.2 Absolute quantification using PSAQ.....	17
1.4.3.3 Absolute quantification using QconCATs.....	17
1.5 Quantitative techniques orthogonal to mass spectrometry.....	18
1.5.1 Quantification by two-dimensional gel electrophoresis.....	18
1.5.2 Quantification by Western blotting.....	18
2 Motivation and aims of the thesis	20
2.1 Motivation for the thesis work.....	20
2.2 Aims of the thesis.....	21
3 Results and Discussion	22
3.1 MS Western: protein quantification concept and workflow.....	22
3.2 Validation and properties of MS Western.....	26
3.3 Benchmarking MS Western quantification.....	33

3.4 Conclusion.....	43
4 Histone protein dynamics during zebrafish embryogenesis.....	44
4.1 Biological background.....	44
4.2 Absolute quantification of Histone proteins from zebrafish embryo.....	45
4.3 Conclusion.....	52
5 Impact of diet on the local and global proteome in <i>Drosophila</i> Eye.....	53
5.1 Project rationale.....	53
5.2 Biological background.....	53
5.3 Proteomics background in <i>Drosophila</i>	57
5.4 Lipidomics background in <i>Drosophila</i>	57
5.5 Quantification of <i>Drosophila</i> eye lipidome and proteome.....	57
5.5.1 Study design.....	58
5.5.2 Electron Microscopy of <i>Drosophila</i> eye.....	59
5.5.3 Quantification of Lipids from <i>Drosophila</i> eye.....	61
5.5.4 Absolute quantification of proteins by MS Western.....	65
5.5.5 Label-free quantification of <i>Drosophila</i> eye proteome.....	80
5.5.6 Conclusion.....	89
6 Conclusion and perspective.....	90
7 Material and Methods.....	91
7.1 Chemicals and reagents.....	91
7.2 Protein standard and amino acids.....	91
7.3 GeLC-MS/MS.....	91
7.4 Data processing for protein identification and quantification.....	92
7.5 Expression and labeling of chimeric protein.....	92
7.6 Absolute quantification of recombinant histone H4 protein.....	93
7.7 Quantification of spiked protein mix.....	93
7.8 Knockdown experiments in HeLa cells.....	94
7.9 Analysis of the dilution series of HeLa cells protein extracts.....	94
7.10 Absolute quantification of histones in zebrafish embryos by MS Western.....	95
7.11 Absolute quantification of histones in zebrafish embryos by WB on Li-COR Odyssey system.....	96
7.12 <i>Drosophila</i> diet experiment.....	96
7.12.1 <i>Drosophila</i> culture.....	96
7.12.2 <i>Drosophila</i> diets.....	96
7.12.3 Electron microscopy of <i>Drosophila</i> eye.....	97
7.12.4 Lipid extraction and shotgun lipidomics analysis of <i>Drosophila</i> eye...97	
7.12.5 Protein extraction and GeLC-MS/MS analysis of <i>Drosophila</i> eye	

proteome.....	98
Appendix.....	99
Bibliography.....	110
Acknowledgements.....	124
Anlage 1.....	125
Anlage 2.....	126