

Contents

Preface XIII

List of Contributors XV

Part One General Information 1

- 1 Half-Life Modulating Strategies—An Introduction** 3
Roland E. Kontermann
- 1.1 Therapeutic Proteins 3
- 1.2 Renal Clearance and FcRn-Mediated Recycling 3
- 1.3 Strategies to Modulate Plasma Half-Life 7
- 1.3.1 Strategies to Increase the Hydrodynamic Radius 9
- 1.3.2 Strategies Implementing FcRn-Mediated Recycling 13
- 1.4 Half-Life Extension Strategies Applied to a Bispecific Single-Chain Diabody—A Case Study 15
- 1.5 Conclusion 18
References 19
- 2 Pharmacokinetics and Half-Life of Protein Therapeutics** 23
Bernd Meibohm
- 2.1 Introduction 23
- 2.2 Basic Principles of Pharmacokinetics 24
- 2.2.1 Primary Pharmacokinetic Parameters 24
- 2.2.2 Secondary Pharmacokinetic Parameters 25
- 2.3 Pharmacokinetics of Protein Therapeutics 27
- 2.3.1 Absorption of Protein Therapeutics 28
- 2.3.2 Distribution of Protein Therapeutics 29
- 2.3.3 Elimination of Protein Therapeutics 31
- 2.3.3.1 Proteolysis 31
- 2.3.3.2 Renal Protein Metabolism 32
- 2.3.3.3 Hepatic Protein Metabolism 33
- 2.3.3.4 Receptor-Mediated Protein Metabolism and Target-Mediated Drug Disposition 34

- 2.3.3.5 The Role of the Neonatal Fc Receptor (FcRn) in the Disposition of Protein Therapeutics 35
- 2.4 Summary and Conclusions 35
- References 37

Part Two Half-Life Extension through Chemical and Post-translational Modifications 39

- 3 Half-Life Extension through PEGylation 41**
Simona Jevševar and Menči Kunstelj
- 3.1 Introduction 41
- 3.2 Preparation and Physico-Chemical Characterization of PEGylated Proteins 44
 - 3.2.1 Selection of PEG Reagents and PEGylation Chemistry 44
 - 3.2.1.1 Random PEGylation 45
 - 3.2.1.2 Site-Specific PEGylation 46
 - 3.2.2 Purification of PEGylated Proteins 48
 - 3.2.3 Physico-Chemical Characterization of PEG Reagents and PEG-Protein Conjugates 49
 - 3.2.3.1 Characterization of PEG Reagents 49
 - 3.2.3.2 Characterization of PEG-Protein Conjugates 50
- 3.3 Pharmacokinetic (PK) Behavior of PEGylated Proteins 50
 - 3.3.1 Administration Route of PEGylated Proteins 51
 - 3.3.2 Elimination Properties of PEGylated Proteins 51
 - 3.3.3 Biodistribution Properties of PEGylated Proteins 54
 - 3.3.4 Increased Circulation Lifespan of PEGylated Proteins 54
- 3.4 Safety of PEGylated Proteins 55
- 3.5 Conclusions 56
- References 56
- 4 Half-Life Extension of Therapeutic Proteins via Genetic Fusion to Recombinant PEG Mimetics 63**
Uli Binder and Arne Skerra
- 4.1 Introduction 63
- 4.2 Mechanisms to Retard Kidney Filtration Using Conjugates of Drugs with Polymers 65
- 4.3 Naturally Occurring Repetitive Amino Acid Sequences 67
- 4.4 Gelatin-Like Protein Polymers 68
- 4.5 Elastin-Like Polypeptides 69
- 4.6 Polyanionic Polymers 70
- 4.7 Genetic Polymers™ 70
- 4.8 XTEN Polypeptides 71
- 4.9 Glycine-Rich Homo-Amino-Acid Polymers 72
- 4.10 PASylation® Technology 73

4.11	Conclusions and Outlook	75
	References	78
5	Half-Life Extension through O-Glycosylation	81
	<i>Fuad Fares</i>	
5.1	Introduction	81
5.2	The Role of O-Linked Oligosaccharide Chains in Glycoprotein Function	83
5.3	Designing Long-Acting Agonists of Glycoprotein Hormones	84
5.3.1	Construction of Chimeric Genes and Expression Vectors	86
5.3.2	Expression of Chimeric Genes	86
5.3.3	Bioactivity of Designed Long-Acting Glycoproteins	87
5.3.3.1	Follicle-Stimulating Hormone (FSH)	87
5.3.3.2	Thyrotropin (TSH)	88
5.3.3.3	Erythropoietin	89
5.3.3.4	Growth Hormone (GH)	90
5.4	Conclusions and Summary	92
	References	92
6	Polysialic Acid and Polysialylation to Modulate Antibody Pharmacokinetics	95
	<i>Antony Constantinou, Chen Chen, and Mahendra P. Deonarain</i>	
6.1	Introduction	95
6.2	Polysialic Acid in Nature	97
6.3	PSA Biosynthesis and Biodegradation	101
6.4	Pharmacological Effects of PSA	103
6.5	PSA Conjugation: Polysialylation for Therapeutic Applications	103
6.6	Summary	108
	References	109
7	Half-Life Extension through HESylation®	117
	<i>Thomas Hey, Helmut Knoller, and Peter Vorstheim</i>	
7.1	Introduction	117
7.2	Hydroxyethyl Starch (HES)	118
7.2.1	Production and Characteristics	118
7.2.2	HES Parameters	119
7.2.2.1	Mean Molecular Weight	119
7.2.2.2	Molar Substitution (MS)	120
7.2.2.3	Other Parameters	120
7.3	Clinical Use of HES	120
7.4	HES Metabolism and Toxicology	121
7.4.1	Metabolic Pathways	121
7.5	HESylation® – Conjugation of Hydroxyethyl Starch to Drug Substances	123
7.5.1	The Origin of HES Protein Coupling	123

7.5.2	Going from Multivalent to Site-Specific Functionalization of HES by Selective Oxidation of the Reducing End	124
7.5.3	HES Derivatives Based on Non-Oxidized HES	125
7.6	HES–Protein Conjugates—Two Case Studies	127
7.6.1	Erythropoietin Polymer Conjugates	127
7.6.1.1	Erythropoietin Products on the Market	127
7.6.1.2	Chemistry of Polymer Modified Erythropoietin	127
7.6.1.3	<i>In Vitro</i> Activity of Polymer-Modified Erythropoietin Variants	128
7.6.1.4	<i>In Vivo</i> Activity of Polymer-Modified Erythropoietin Variants	129
7.6.2	Polymer-Modified Interferon α Variants	130
7.6.2.1	PEGylated Interferon α Products on the Market	130
7.6.2.2	HESylation of rhIFN α -2b	131
7.6.2.3	<i>In Vitro</i> Activity of HESylated rhIFN α -2b	134
7.6.2.4	Pharmacokinetics of HES-IFN α Compared with PEGasys	135
7.6.2.5	Viscosity: HES Compared with PEG	135
7.7	Summary and Conclusion	136
	References	137

Part Three Half-Life Modulation Involving Recycling by the Neonatal Fc Receptor 141

8	The Biology of the Neonatal Fc Receptor (FcRn)	143
	<i>Jonghan Kim</i>	
8.1	Homeostasis of Albumin and Immunoglobulin	143
8.2	Neonatal Fc Receptor Biochemistry	145
8.3	FcRn Function: Recycling	147
8.4	FcRn Function: Transport	149
8.5	FcRn Function: Mucosal Immune	150
8.6	Therapeutic Implications of FcRn	151
8.7	Conclusions	152
	References	152
9	Half-Life Extension by Fusion to the Fc Region	157
	<i>Jalal A. Jazayeri and Graeme J. Carroll</i>	
9.1	Introduction	157
9.2	Immunoglobulin G	158
9.2.1	The Fc Region	163
9.2.2	The FcReceptor	163
9.2.3	Fc-Mediated Antibody Functions and Their Optimization	166
9.3	Strategies to Increase Cytokine Serum Stability and Half-Life	167
9.3.1	Fc-Fusion Dimeric	168
9.3.1.1	Protein Domains Fused to Fc	168
9.3.2	Fc-Fusion Monomeric	169
9.3.3	Fc-Peptide Fusion Protein (Peptibody)	170

9.3.4	Other Antibody-Engineered Constructs	170
9.3.4.1	Fab Fusions	170
9.3.4.2	Antibody without the Fc Region (Diabodies)	171
9.4	Methods to Construct Fc-Fusion Dimeric Proteins	172
9.4.1	Polymerase Chain Reaction (PCR) Approach	172
9.4.2	Fc-Plasmid Vectors	172
9.4.3	Design Considerations	173
9.4.3.1	Choice of Linkers	173
9.4.3.2	Codon Optimization	174
9.5	Choice of Host for Expression	174
9.5.1	Expression in Bacteria	174
9.5.2	Expression in Mammalian Cells	175
9.5.3	Expression in Insect Cells	175
9.5.4	Expression in Yeast	176
9.6	Purification of Fc Fusion Proteins	176
9.7	Demonstration of Biological Activity in Fc Constructs	
	<i>In Vitro</i> and <i>In Vivo</i>	177
9.8	Pharmacokinetics	177
9.9	Applications of Fc Fusion Proteins	178
9.9.1	Therapeutic Proteins	178
9.9.2	Protein/Cytokine Traps	178
9.9.3	Gene Therapy	178
9.9.4	Drug Delivery	179
9.9.5	Research Tool	179
9.9.6	Tumor Targeting	180
9.10	Immunogenicity	180
9.11	Conclusion	181
	References	182
10	Monomeric Fc Fusion Technology: An Approach to Create Long-Lasting Clotting Factors	189
	<i>Jennifer A. Dumont, Xiaomei Jin, Robert T. Peters, Alvin Luk, Glenn F. Pierce, and Alan J. Bitonti</i>	
10.1	Introduction	189
10.2	Neonatal Fc Receptor and Interaction with Immunoglobulin G	189
10.3	Traditional Fc Fusion Proteins	191
10.4	Monomeric Fc Fusion Proteins Show Improved Biologic Properties	192
10.4.1	EPOFc as a Prototype Construct	193
10.4.2	Clotting Factor Fc Fusions for the Treatment of Hemophilia	194
10.4.2.1	Recombinant Factor IX-Fc Fusion Protein (rFIXFc)	195
10.4.2.2	Recombinant Factor VIII-Fc Fusion Protein (rFVIII-Fc)	200
10.5	Summary	202
	Acknowledgments	203
	References	203

11	The Diverse Roles of FcRn: Implications for Antibody Engineering	207
	<i>E. Sally Ward and Raimund J. Ober</i>	
11.1	Introduction	207
11.2	FcRn: Early Characterization and Diverse Expression Patterns	207
11.3	The Molecular Details of FcRn–IgG Interactions	208
11.4	FcRn Is Expressed Ubiquitously throughout the Body Where It Serves Multiple Functions	209
11.5	The Cell Biology of FcRn and Its Intracellular Transport of IgG	210
11.6	The Molecular Determinants of FcRn Trafficking	212
11.7	Engineering IgG–FcRn Interactions	213
11.8	Inhibitors of FcRn Function	215
11.9	Engineering Mice with Altered FcRn Function	216
11.10	Concluding Remarks	216
	Acknowledgments	216
	References	216
12	Half-Life Extension by Fusion to Recombinant Albumin	223
	<i>Hubert J. Metzner, Thomas Weimer, and Stefan Schulte</i>	
12.1	Introduction	223
12.2	Recombinant Albumin Fusion Proteins	225
12.2.1	Mode of Action	227
12.2.2	Practical Applications	227
12.2.3	Advantages	228
12.2.4	Challenges	228
12.2.5	Therapeutic Potential	229
12.2.5.1	Fusion to Small Proteins and Peptides	229
12.2.5.2	Fusion to Cytokines	231
12.2.5.3	Fusion to Complex Proteins	232
12.3	Albumin Fusion to Complex Proteins	233
12.3.1	Recombinant Fusion Protein Linking Coagulation Factor VIIa with Albumin (rVIIa-FP)	233
12.3.2	Recombinant Fusion Protein Linking Coagulation Factor IX with Albumin (rIX-FP)	234
12.3.3	Butyrylcholinesterase (BChE)	234
12.4	Recombinant Albumin Fusion Technology	234
12.4.1	Recombinant Fusion Protein Linking Coagulation Factor VIIa with Albumin (rVIIa-FP)	234
12.4.2	Recombinant Fusion Protein Linking Coagulation Factor IX with Albumin (rIX-FP)	235
12.4.3	Albutropin	237
12.5	Technological Advantages and Challenges	237
12.6	Pharmacokinetics of Recombinant Albumin Fusion Proteins	238
12.6.1	Recombinant Fusion Protein Linking Coagulation Factor VIIa with Albumin (rVIIa-FP)	238
12.6.2	Recombinant Fusion Protein Linking Coagulation Factor IX with Albumin (rIX-FP)	238

12.6.3	Albutropin	238
12.7	Preclinical Efficacy	239
12.7.1	Recombinant Fusion Protein Linking Coagulation Factor VIIa with Albumin (rVIIa-FP)	239
12.7.2	Recombinant Fusion Protein Linking Coagulation Factor VIIa with Albumin (rVIIa-FP)	240
12.7.3	Albutropin	240
12.8	Clinical Efficacy	240
12.8.1	Albuferon	240
12.9	Future Perspectives	241
12.10	Conclusion	241
	Acknowledgments	242
	References	242
13	AlbudAb™ Technology Platform – Versatile Albumin Binding Domains for the Development of Therapeutics with Tunable Half-Lives	249
	<i>Christopher Herring and Oliver Schon</i>	
13.1	Introduction	249
13.2	The Domain Antibody	251
13.3	Key Considerations for AlbudAb™-Based Molecules	252
13.4	Challenges of Albumin as a Target	253
13.5	Interactions of Albumin with AlbudAbs™	255
13.6	Bio-Analytical Characterization of AlbudAb™ Leads	256
13.6.1	Versatility	256
13.6.2	Affinity to Serum Albumin and Potency	257
13.6.3	Solution State	258
13.6.4	Thermal Stability and Aggregation Resistance	258
13.7	Production of AlbudAb™ Fusions	259
13.8	Purification of AlbudAbs™	260
13.9	Biodistribution of AlbudAbs™	260
13.9.1	Pharmacokinetics and Efficacy of AlbudAb™ Fusions	262
13.10	Summary and Conclusion	265
	References	266
14	Half-Life Extension by Binding to Albumin through an Albumin Binding Domain	269
	<i>Fredrik Y. Frejd</i>	
14.1	Introduction	269
14.2	Albumin Binding Domains and Engineered Derivatives	270
14.3	Albumin Binding Domains and Half-Life Extension <i>In Vivo</i>	272
14.4	Albumin Binding Domains and Immunogenicity	277
14.5	Bispecific Albumin Binding Domains for Novel Target Binding and Long Half-Life	278
14.6	Conclusion	279
	Acknowledgments	280
	References	280

15	Half-Life Extension by Binding to Albumin through Small Molecules	285
	<i>Sabrina Trüssel, Joerg Scheuermann, and Dario Neri</i>	
15.1	Albumin and Albumin Binders	285
15.2	Albumin-Binding Insulin Derivatives	289
15.3	“Albu” Tagging Technology	291
15.4	Concluding Remarks	294
	References	294

Part Four Half-Life Extension with Pharmaceutical Formulations 297

16	Half-Life Extension with Pharmaceutical Formulations: Liposomes	299
	<i>Astrid Hartung and Gerd Bendas</i>	
16.1	Rationale	299
16.2	Prospects of Liposomes as Drug Carriers and Their Pharmacokinetic Properties	300
16.3	Entrapment of Therapeutically Relevant Proteins in PEGylated Liposomes	302
16.4	Noncovalent Complex Formation of Proteins and PEGylated Liposomes	307
16.5	Conclusions	310
	References	311
17	Half-Life Extension with Pharmaceutical Formulations: Nanoparticles by the Miniemulsion Process	315
	<i>Katharina Landfester, Anna Musyanovych, and Volker Mailänder</i>	
17.1	Introduction	315
17.2	Polymeric Nanoparticles	317
17.3	Particles Obtained by Radical Polymerization and Their Functionalization	317
17.4	Other Polyreactions in Miniemulsion	321
17.5	Formation of Nanocapsules and Their Functionalization	323
17.6	Encapsulation of Markers and Detection of Nanoparticles in Biological Systems	327
17.6.1	Fluorescent Component as Marker	328
17.6.2	Magnetite as Marker	331
17.7	Release of Materials	333
17.8	Conclusion	334
	Acknowledgment	335
	References	336

Index 341