

# Contents

<b>Zusammenfassung</b>	<b>xi</b>
<b>Summary</b>	<b>xiii</b>
<b>1 Introduction and Motivation</b>	<b>1</b>
<b>2 Model membranes</b>	<b>5</b>
2.1 Introduction to lipids . . . . .	5
2.2 Lipid systems studied in this work . . . . .	8
2.3 Overview of model membrane systems . . . . .	10
2.4 Preparation of supported lipid bilayers . . . . .	14
2.5 Preparation of pore-spanning membranes . . . . .	17
2.6 Preparation of giant unilamellar vesicles . . . . .	18
2.6.1 Electroformation . . . . .	19
2.6.2 PVA-assisted swelling . . . . .	24
<b>3 Methods for studying lipid diffusion in membranes</b>	<b>27</b>
3.1 Lipid diffusion . . . . .	27
3.2 Commonly used methods for studying lipid diffusion in membranes	31
3.2.1 Fluorescence recovery after photobleaching (FRAP) . . . . .	31
3.2.2 Fluorescence correlation spectroscopy (FCS) . . . . .	32
3.2.3 Single particle tracking (SPT) . . . . .	33
3.3 Interferometric scattering microscopy (iSCAT) . . . . .	38
3.3.1 Principle of iSCAT . . . . .	38
3.3.2 Point spread function . . . . .	40
3.3.3 Particle contrast . . . . .	42
3.3.4 Axial sensitivity . . . . .	47
3.3.5 Signal-to-noise ratio . . . . .	47
3.3.6 Gold nanoparticles as a scattering label in iSCAT . . . . .	48
3.4 Labeling of lipids with gold nanoparticles . . . . .	49

3.5	Experimental arrangement . . . . .	52
3.5.1	ISCAT and fluorescence imaging . . . . .	52
3.5.2	Optical magnification . . . . .	54
3.5.3	Micropipette aspiration . . . . .	55
<b>4</b>	<b>Data analysis of single particle trajectories</b>	<b>57</b>
4.1	Image background correction . . . . .	57
4.2	Particle tracking and localization . . . . .	60
4.2.1	Tracking algorithm . . . . .	60
4.2.2	Localization precision of the particle position . . . . .	61
4.2.3	Trajectory reconstruction . . . . .	63
4.3	Statistical analysis of diffusion trajectories . . . . .	72
4.3.1	Estimation of the diffusion coefficient . . . . .	72
4.3.2	Detecting changes in the diffusion behavior . . . . .	87
<b>5</b>	<b>Lipid diffusion in supported lipid bilayers</b>	<b>91</b>
5.1	Membrane quality . . . . .	91
5.2	Detection of multimobility diffusion in DOPC SLBs . . . . .	94
5.3	Detection of transient, nanoscale confinements . . . . .	97
5.4	Dependence of lipid diffusion behavior on lipid concentration . . . . .	102
5.5	Influence of the particle size on the diffusion behavior . . . . .	108
5.6	Influence of camera exposure time and frame rate on the estimated diffusion coefficient . . . . .	113
5.7	High speed particle tracking . . . . .	115
5.8	Atomic force microscopy for measuring membrane topography . . . . .	118
<b>6</b>	<b>Lipid diffusion in pore-spanning membranes</b>	<b>127</b>
6.1	Gold nanoparticle tracking on PSMs . . . . .	128
6.2	Lipid diffusion in free-standing vs. supported membrane regions . . . . .	131
6.3	Nanoscale confinements on supported membrane regions . . . . .	136
6.4	Influence by the pore rim . . . . .	138
6.5	Detecting changes in diffusivity by a changing point analysis . . . . .	140
<b>7</b>	<b>Lipid diffusion in giant unilamellar vesicles</b>	<b>147</b>
7.1	Membrane quality . . . . .	147
7.2	Effects of sucrose on particle–lipid interaction . . . . .	148
7.3	Lipid diffusion in GUVs prepared in buffer solution . . . . .	150

7.4	Influence of camera exposure time and frame rate on the estimated diffusion coefficient . . . . .	154
<b>8</b>	<b>Studying lipid diffusion around the phase transition temperature</b>	<b>157</b>
8.1	Temperature-dependent measurements on SLBs . . . . .	159
8.1.1	Heating device for temperature-dependent iSCAT-SPT measurements . . . . .	159
8.1.2	Behavior of lipid-bound GNPs around the phase transition temperature . . . . .	161
8.1.3	Dependence of the phase transition temperature on lipid density . . . . .	168
8.1.4	Formation of lipid vesicles, patches and tubes . . . . .	169
8.1.5	Diffusion in membrane patches and lipid tubes . . . . .	176
8.2	Temperature-controlled diffusion studies on GUVs . . . . .	180
<b>9</b>	<b>Conclusion and Outlook</b>	<b>183</b>
9.1	Comparison of lipid diffusion among the three model systems . . . . .	183
9.2	Concluding remarks . . . . .	185
9.3	Future prospects . . . . .	187
	<b>Appendix - Detection and tracking of biological nanoprobe on GUVs</b>	<b>191</b>
A1	Tat peptide decorated polymer particles . . . . .	191
A2	HCMV Dense bodies . . . . .	194
A3	Proteo-SUVs containing synaptic fusion proteins . . . . .	198
	<b>Bibliography</b>	<b>209</b>