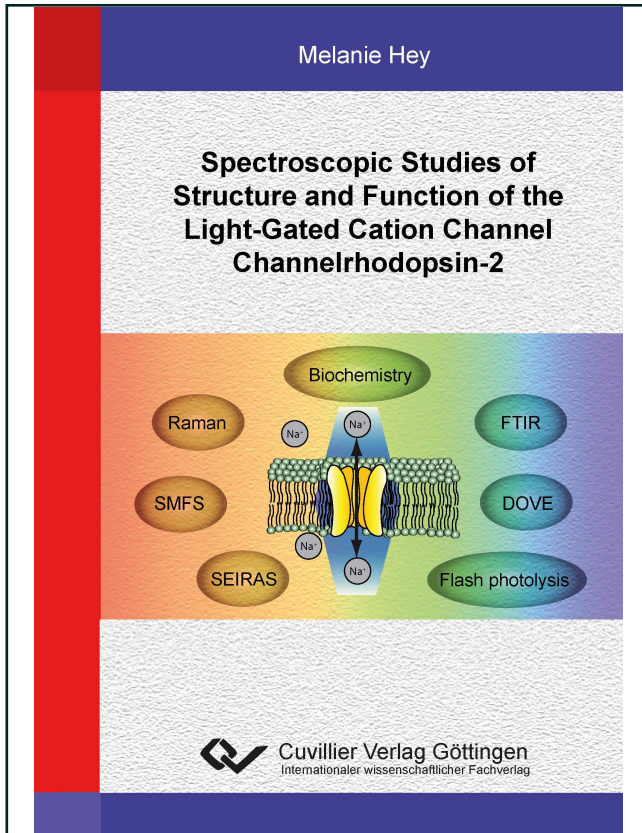




Melanie Hey (Autor)

# Spectroscopic Studies of Structure and Function of the Light-Gated Cation Channel Channelrhodopsin-2



<https://cuvillier.de/de/shop/publications/6598>

Copyright:

Cuvillier Verlag, Inhaberin Annette Jentsch-Cuvillier, Nonnenstieg 8, 37075 Göttingen,  
Germany

Telefon: +49 (0)551 54724-0, E-Mail: [info@cuvillier.de](mailto:info@cuvillier.de), Website: <https://cuvillier.de>

# Contents

<b>1</b>	<b>Introduction</b>	<b>1</b>
<b>2</b>	<b>Channelrhodopsin-2</b>	<b>3</b>
2.1	Structure of Channelrhodopsin-2 . . . . .	4
2.2	The N-terminal domain of Channelrhodopsin-2 . . . . .	5
2.2.1	The N-terminal domain of Channelrhodopsin-2 <i>in vitro</i> . . . . .	6
2.2.2	Functional mechanism of nChannelrhodopsin-2 . . . . .	8
2.2.3	Optogenetics . . . . .	10
2.3	The C-terminal domain of Channelrhodopsin-2 . . . . .	12
2.4	Channelrhodopsin-2 samples . . . . .	13
<b>3</b>	<b>Biochemical Methods</b>	<b>15</b>
3.1	Heterologous expression of ChR2 . . . . .	16
3.2	Membrane isolation . . . . .	18
3.3	Protein purification . . . . .	18
3.4	Sodium dodecylsulfate polyacrylamide gel electrophoresis . . . . .	20
3.5	Reconstitution into lipid vesicles . . . . .	20
<b>4</b>	<b>Spectroscopic Methods</b>	<b>23</b>
4.1	UV/Vis absorption spectroscopy . . . . .	24
4.1.1	Experimental setup for flash photolysis . . . . .	25
4.1.2	Absorption spectrum of nChR2-wt . . . . .	27
4.1.3	Photocycle of nChR2-wt . . . . .	28

4.1.4	Conducting UV/Vis experiments . . . . .	32
4.2	Vibrational spectroscopy . . . . .	33
4.2.1	Theoretical background of vibrational spectroscopy . . . . .	34
4.3	Fourier transform infrared spectroscopy . . . . .	36
4.3.1	FTIR spectroscopy on proteins . . . . .	38
4.3.2	Light-induced FTIR difference spectroscopy on ChR2 . . . . .	40
4.3.3	Conducting FTIR experiments in transmission . . . . .	43
4.4	Surface-enhanced FTIR (difference) absorption spectroscopy . . . . .	44
4.4.1	SEIRAS: Experimental setup . . . . .	45
4.4.2	SEIRAS results on Sensory rhodopsin II . . . . .	46
4.4.3	Conducting SEIRAS experiments . . . . .	47
4.5	Resonance Raman spectroscopy . . . . .	50
4.5.1	Resonance Raman: Experimental setup . . . . .	52
4.5.2	Resonance Raman spectroscopy on retinal proteins . . . . .	52
4.5.3	Example of pre-resonance Raman spectroscopy on nChR2 . . . . .	53
4.5.4	Conducting pre-resonance Raman experiments . . . . .	53
4.6	Optimization of DOVE spectroscopy . . . . .	55
4.6.1	Theoretical background of DOVE spectroscopy . . . . .	59
4.6.2	DOVE spectroscopy: Experimental setup . . . . .	61
4.6.3	DOVE spectroscopy on peptides and proteins . . . . .	62
4.6.4	Conducting DOVE spectroscopic experiments . . . . .	65
4.7	Single molecule force spectroscopy . . . . .	69
4.7.1	SMFS: Experimental Setup . . . . .	72
4.7.2	SMFS on microbial rhodopsins . . . . .	73
4.7.3	Performance of SMFS measurements . . . . .	73
4.8	Data processing . . . . .	77
<b>5</b>	<b>Biochemical Results</b>	<b>79</b>
5.1	Improvement of purity of nChR2 samples . . . . .	79
5.2	Expression and purification of nChR2 variants . . . . .	82
5.3	Discussion of biochemical results . . . . .	84
<b>6</b>	<b>Spectroscopic Results on Channelrhodopsin-2</b>	<b>89</b>
6.1	The retinal binding pocket of ChR2 . . . . .	89
6.2	The DC gate in ChR2 . . . . .	93
6.3	Proton transfer in ChR2 . . . . .	103
6.3.1	Results of time-resolved pH indicator experiments . . . . .	103

---

6.3.2	Results on ChR2-E123D . . . . .	112
6.4	Discussion of spectroscopic results on ChR2 . . . . .	116
6.4.1	Kinetic studies of ChR2 . . . . .	117
6.4.2	Channeling mechanism in ChR2 . . . . .	119
6.4.3	Proton transfer in ChR2 . . . . .	122
<b>7</b>	<b>Application and Modification of Advanced Techniques</b>	<b>135</b>
7.1	Results for surface-enhanced IR spectroscopy on ChR2 . . . . .	135
7.2	Results for doubly-vibrationally enhanced spectroscopic experiments	141
7.2.1	Signal enhancement by gold nanoparticles . . . . .	141
7.2.2	Signal enhancement by nanostructured gold surface . . . . .	143
7.3	Results for SMFS on ChR2 . . . . .	148
7.3.1	SMFS results on NpSRII . . . . .	148
7.3.2	SMFS results on ChR2 . . . . .	149
7.4	Discussion . . . . .	154
7.4.1	Discussion of SEIRAS results . . . . .	155
7.4.2	Discussion of DOVE results . . . . .	157
7.4.3	Discussion of SMFS results . . . . .	161
<b>8</b>	<b>Summary and Outlook</b>	<b>169</b>
<b>9</b>	<b>Zusammenfassung</b>	<b>173</b>
<b>A</b>	<b>Supplementary Information</b>	<b>I</b>
A.1	Sequence alignment of ChR2, C1C2 and BR . . . . .	I
A.2	Results on cChR2 . . . . .	I
A.2.1	The C-terminal domain of ChR2 . . . . .	I
A.2.2	Methods applied to cChR2 . . . . .	III
A.2.3	Results on cChR2 . . . . .	VIII
A.3	Gel filtration runs on Superdex200 . . . . .	XI
A.4	HPLC results of retinal extraction . . . . .	XII
A.5	Dependence of BXB signal on buffer concentration . . . . .	XIII
A.6	Absorption of thiol-binding to gold nanoparticles . . . . .	XIV
A.7	Electrochemical cleaning of the gold surface for DOVE . . . . .	XIV
A.8	Additional force curves on ChR2 in PG . . . . .	XVI
A.9	Material . . . . .	XVII
A.9.1	Optical filters used in FTIR . . . . .	XVII

---

A.9.2	Material und buffer lists . . . . .	XVIII
<b>B</b>	<b>Liste der Publikationen und Lebenslauf</b>	<b>XXV</b>
<b>C</b>	<b>Danksagung</b>	<b>XXIX</b>
<b>D</b>	<b>Erklärung über die selbständige Anfertigung der Arbeit</b>	<b>XXXI</b>