

การติดตามการเปลี่ยนแปลงทางโครงสร้างจากการรีดอกซ์ในไซโทโครมซีโดยสเทชันนารี  
และโทมรีโซล์วเซอร์เฟสเอ็นฮานซ์อินฟราเรดแอบซอร์บชันสเปกโทรสโกปี



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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต

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ปีการศึกษา 2551

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

REDOX-LINKED STRUCTURAL CHANGES IN CYTOCHROME C PROBED  
BY STATIONARY AND TIME-RESOLVED SURFACE ENHANCED  
INFRARED ABSORPTION SPECTROSCOPY

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A Dissertation Submitted in Partial Fulfillment of the Requirements  
for the Degree of Doctor of Philosophy Program in Chemistry

Department of Chemistry

Faculty of Science

Chulalongkorn University

Academic year 2008


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510688


Thesis Title                    REDOX-LINKED      STRUCTURAL      CHANGES      IN  
CYTOCHROME C PROBED BY STATIONARY AND  
TIME-RESOLVED SURFACE ENHANCED INFRARED  
ABSORPTION SPECTROSCOPY  
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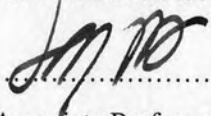
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of the Requirements for the Doctoral Degree

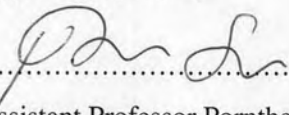
  
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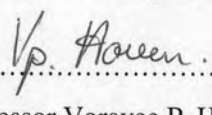
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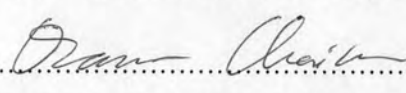
  
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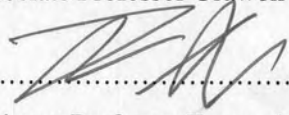
  
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ัญญัติ วิจัยเรื่องสกุล : การติดตามการเปลี่ยนแปลงทางโครงสร้างจากการรีดอกซ์ในไซโทโครมซี โดยสเปกตรัมอินฟราเรดและไทม์รีโซลว์เซอร์เฟสเอนฮานซ์อินฟราเรดแอบซอร์บชันสเปกโทรสโกปี. (REDOX-LINKED STRUCTURAL CHANGES IN CYTOCHROME C PROBED BY STATIONARY AND TIME-RESOLVED SURFACE ENHANCED INFRARED ABSORPTION SPECTROSCOPY) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : รศ. ดร. สนอง เอกสิทธิ์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม : ดร. อิงโก เชปเกอร์, 136 หน้า.

เซอร์เฟสเอนฮานซ์อินฟราเรดแอบซอร์บชัน (เซียร์รา) สเปกโทรเคมีไฟฟ้าเพื่อศึกษาโปรตีนที่ถูกตรึงอยู่มีข้อได้เปรียบทางด้านความไวของการตอบสนองที่ผิวหน้าและสามารถตรวจสอบโครงสร้างหลักของโปรตีนได้ สำหรับการตรึงแบบไฟฟ้าสถิตนั้น ไซโทโครมซีถูกตรึงอยู่บนขั้วไฟฟ้าที่ทำด้วยทองโดยการยึดจับกับสารประกอบคาร์บอกซิลอัลเคนไทออลที่เรียงตัวกันเป็นชั้นเดียว (SAMs) ในงานวิจัยนี้วิธีเซียร์ราได้ถูกนำมาใช้เพื่อศึกษาการเปลี่ยนแปลงทางโครงสร้างของ SAMs, การเปลี่ยนแปลงทางโครงสร้างและทิศทางการจัดตัวของไซโทโครมซีจากการรีดอกซ์ และเพื่อศึกษาสถานะที่ผิดปกติของไซโทโครมซี นอกจากนั้นการประยุกต์ใช้ไทม์รีโซลว์เซอร์เฟสเอนฮานซ์อินฟราเรดสเปกโทรสโกปีเพื่อติดตามพลศาสตร์ของไซโทโครมซีที่เกิดควบคู่กับกระบวนการรีดอกซ์ได้ตีพิมพ์เป็นครั้งแรก

การเปลี่ยนแปลงทางสเปกตรัมของ SAMs ภายใต้การควบคุมศักย์ไฟฟ้าแสดงให้เห็นการเปลี่ยนแปลงแบบผันกลับภายในช่วงศักย์ไฟฟ้าที่สนใจ (-0.1 ถึง +0.2 โวลต์) ซึ่งไม่รบกวนการตรวจสอบไซโทโครมซีที่ยึดจับกับ SAMs อย่างไรก็ตามการเปลี่ยนแปลงทางสเปกตรัมแบบไม่ผันกลับสามารถตรวจพบที่ศักย์ไฟฟ้าในช่วงดังกล่าว โดยขึ้นกับความยาวของ SAMs โครงสร้างบีตาเทริน 3 (1673 ซม<sup>-1</sup>) และ บีตาเทริน 2/แอลฟาอีลิกซ์ (1660 ซม<sup>-1</sup>) ของเฟร์ริกไซโทโครมซีแสดงการเปลี่ยนแปลงทิศทางการจัดตัวที่พื้นผิวภายใต้อิทธิพลความเข้มของสนามไฟฟ้าซึ่งพบว่ามีความสัมพันธ์กับการเปลี่ยนแปลงทางโครงสร้างจากสภาวะธรรมชาติที่ 1 สู่สภาวะผิดปกติที่ 2 สเปกตรัมที่ขึ้นกับศักย์ไฟฟ้าของสภาวะผิดปกติที่ 2 แตกต่างจากสเปกตรัมของสภาวะที่ 1 อย่างชัดเจน โดยแสดงแถบสเปกตรัมเชิงซ้อนที่กว้าง อันเป็นผลจากการเปลี่ยนแปลงโครงสร้างตติยภูมิและการปรับเปลี่ยนอันตรกิริยาของพันธะไฮโดรเจนซึ่งเกี่ยวข้องกับการเคลื่อนที่ของโครงสร้างบีตาเทริน 2/แอลฟาอีลิกซ์ (1659 ซม<sup>-1</sup>) และ โครงสร้างที่ไม่เป็นระเบียบ (1645 ซม<sup>-1</sup>) ในการสร้างโครงสร้างที่ผิดปกติที่ 2 ค่าศักย์ไฟฟ้ารีดอกซ์ของสภาวะที่ 1 ที่ได้จากเทคนิคเซียร์ราและไซคลิกโวลแทมเมตรี (ซีวี) ไม่แตกต่างอย่างมีนัยสำคัญจากค่าศักย์ไฟฟ้ารีดอกซ์ของไซโทโครมซีในสารละลาย การวัดด้วยวิธีซีวีแสดงให้เห็นกระบวนการถ่ายโอนอิเล็กตรอนแบบผันกลับได้และอัตราการถ่ายโอนอิเล็กตรอนขึ้นกับฟังก์ชันของ SAMs ไม่เป็นแบบเลขชี้กำลัง การเปลี่ยนแปลงทางโครงสร้างของโปรตีนที่เกิดจากการถ่ายโอนอิเล็กตรอนถูกติดตามด้วยเทคนิคเรปิดสแกนและสเปกตรัมเซียร์ราสเปกโทรสโกปีร่วมกับเทคนิคการปรับเปลี่ยนศักย์ไฟฟ้าแบบข้ามกระโดด พบว่าการเปลี่ยนแปลงของพีกที่ 1693 และ 1673 ซม<sup>-1</sup> มีค่าเท่ากับอัตราการถ่ายโอนอิเล็กตรอน ส่วนอัตราการเปลี่ยนแปลงของพีกที่ 1660 ซม<sup>-1</sup> จะมีค่าน้อยกว่าสองเท่า จากการศึกษาพบว่าไทม์รีโซลว์เซอร์เฟสเอนฮานซ์อินฟราเรดสเปกโทรสโกปีได้ให้ข้อมูลเพิ่มเติมเกี่ยวกับพลศาสตร์และกลไกของกระบวนการรีดอกซ์ของโปรตีนที่บริเวณรอยต่อระหว่างเฟสสองเฟส ดังนั้นจึงเป็นส่วนสมบูรณ์ของผลที่ได้จากการวิเคราะห์ที่ผิวหน้าด้วยเทคนิคอื่นๆ

ภาควิชา.....เคมี.....

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ลายมือชื่อนิสิต.....

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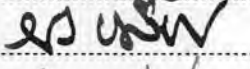
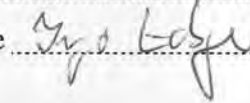
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## 4573814123 : MAJOR CHEMISTRY  
 KEYWORDS : SEIRA / TIME-RESOLVED SEIRA / PROTEIN DYNAMICS /  
 CYTOCHROME C

NATTAWADEE WISITRUANGSAKUL : REDOX-LINKED STRUCTURAL  
 CHANGES IN CYTOCHROME C PROBED BY STATIONARY AND  
 TIME-RESOLVED SURFACE ENHANCED INFRARED ABSORPTION  
 SPECTROSCOPY. ADVISOR : ASSOC. PROF. SANONG EKGASIT, PH.D.,  
 CO-ADVISOR : INGO ZEBGER, PH.D., 136 pp.

The application of surface enhanced infrared absorption (SEIRA) spectro-electrochemistry for studying an immobilized protein yields advantages with respect to its surface sensitivity and probing the whole protein backbone. For an electrostatic immobilization, cytochrome c (Cyt-c) was adsorbed on a Au electrode via a binding to self-assembled monolayers (SAMs) of variable-length  $\omega$ -carboxyl alkanethiols. In this work, stationary SEIRA measurements were performed to elucidate the structural changes of the SAMs, the redox-linked structural and orientational changes of the immobilized Cyt-c, and to study the non-native B2 state of Cyt-c. For the first time, the application of time-resolved SEIRA spectroscopy was reported to probe the protein dynamics coupled with the redox processes of Cyt-c.

Potential dependent spectral changes of the SAMs revealed only reversible variations within the potential range of interest (-0.1 to +0.2 V), which do not interfere with the investigation of Cyt-c bound to these SAMs. However, depending on the SAM-lengths, irreversible spectral changes could be found also at certain potentials outside the mentioned range. The  $\beta$ -turn III ( $1673\text{ cm}^{-1}$ ) and  $\beta$ -turn II/ $\alpha$ -helix ( $1660\text{ cm}^{-1}$ ) segments of ferric Cyt-c revealed orientational changes with respect to the surface under the influence of the electric field strengths, which were found to be correlated with the conformational transition from the native B1 to the non-native B2 state. The potential dependent spectra of the B2 conformation obviously differ from that of the B1 state. It reveals broad complex bands caused by an alteration of the tertiary structure and an arrangement of the hydrogen-bonding interaction involving the movement of peptide backbone of  $\beta$ -turn II/ $\alpha$ -helix (ca.  $1659\text{ cm}^{-1}$ ) and unordered (ca.  $1645\text{ cm}^{-1}$ ) structures for the formation of non-native B2 conformation. The redox potentials of the B1 state determined by stationary SEIRA spectroscopy and cyclic voltammetry (CV) are not significantly different from that in solution. CV measurements demonstrate a reversible electron transfer (ET) process and a non-exponential distance-dependence of the ET rate. On the basis of characteristic of redox state sensitive amide I bands, the protein structural changes triggered by the ET are monitored by rapid scan and step scan SEIRA spectroscopy in combination with the potential jump technique. Whereas the temporal evolution of the conjugate bands at  $1693$  and  $1673\text{ cm}^{-1}$  displays the same rate constants as ET rate, the time-dependent changes of the  $1660\text{-cm}^{-1}$  band are slower by about a factor of 2. The study demonstrates that time-resolved SEIRA spectroscopy provides additional information about the dynamics and mechanism of interfacial processes of redox proteins, thereby complementing the results obtained from other surface-sensitive techniques.

Department : ..... Chemistry ..... Student's Signature   
 Field of Study : ..... Chemistry ..... Advisor's Signature   
 Academic Year : ..... 2008 ..... Co-Advisor's Signature 

## ACKNOWLEDGEMENTS

The financial support via the Royal Golden Jubilee (RGJ) Ph.D. program, grant contract number: PHD/0107/2545, from the Thailand Research Fund (TRF)/Thailand between 2002 to 2008 and the DAAD/RGJ scholarship/Germany between 2004 to 2005 are gratefully acknowledged.

The experiments in this thesis were carried out at Max-Volmer Laboratorium in the Technical University of Berlin (TU-Berlin)/Germany between 2004 and 2008. The financial supports from the Volkswagen-Foundation (I/80816) and the DFG (Cluster of Excellence “Unifying Concepts in Catalysis”)/Germany are gratefully acknowledged.

Deepest gratitude to the host at TU-Berlin, Prof. Dr. Peter Hildebrandt, for giving me the great opportunity to work at one of the famous vibrational spectroscopic groups for four years, as well as his excellent advices and comments. I have greatly enjoyed the innovative and challenging atmosphere in his laboratory.

My Ph.D. research would never be successful without my thesis co-advisor, Dr. Ingo Zebger. I would especially like to thank for his invaluable guidance, always available for discussions any time, his patience and strong effort in training me the research work, and providing me such a warm hospitality during my stay in Berlin. His untiring way of encouragement and support will be forever thankful.

Special thanks go to Dr. Jiu-Ju Feng for the critical suggestion in electrochemistry, especially her kindly helpfulness for the EIS measurements and data evaluation. The nice time we had shared at TU-Berlin is sincerely appreciated.

Thanks the colleagues at Hildebrandt group for interesting discussions and creating the great atmosphere in the office and the laboratory. Thanks also the present and former SRU members for the overlasting friendship.

All in all, my Ph.D. would never be successful without my thesis advisor, Assoc. Prof. Dr. Sanong Ekgasit, who always gives me the excellent experienced suggestion in the most important moments of making the right decisions and the great opportunity for scientific life.

Thanks Siroj Payarkawong for the spiritual supports. Finally, I am affectionately grateful to my wonderful parent and the endearing brothers and sister for their wholehearted support, understanding, and unconditional love.

# CONTENTS

	Pages
ABSTRACT IN THAI .....	iv
ABSTRACT IN ENGLISH.....	v
ACKNOWLEDGEMENTS.....	vi
CONTENTS.....	vii
LIST OF FIGURES.....	xi
LIST OF TABLES.....	xix
LIST OF ABBREVIATIONS.....	xx
LIST OF SYMBOLS.....	xxii
CHAPTER I INTRODUCTION.....	1
CHAPTER II THEORETICAL BACKGROUND.....	4
2.1 Infrared Absorption Spectroscopy.....	4
2.1.1 Fourier Transform Infrared Spectroscopy.....	6
2.1.2 Time-Resolved Fourier Transform Infrared Spectroscopy: Step Scan and Rapid Scan Mode.....	8
2.1.2.1 Time-Resolved Step Scan FT-IR Spectroscopy.....	8
2.1.2.2 Time-Resolved Rapid Scan FT-IR Spectroscopy.....	10
2.1.3 Attenuated Total Reflection Fourier Transform Infrared Spectroscopy.....	10
2.1.4 Surface Enhanced Infrared Absorption Spectroscopy.....	12
2.2 Proteins.....	15
2.2.1 Protein Structure.....	15
2.2.2 Infrared (Difference) Spectroscopy of Peptides and Proteins...	17
2.2.3 Band Assignments of the Protein Secondary Structure in the IR Active Region.....	19
2.3 Investigated Protein: Cytochrome c.....	20

2.3.1 Structure of the Monoheme Cytochrome c.....	20
2.3.2 Structural Changes in Cytochrome c.....	21
2.4 Electrochemical Interfaces.....	24
2.4.1 Electrode Reactions.....	24
2.4.2 Electrode/Solution Interface.....	24
2.4.3 Modification of Metal Electrode by Self-Assembled Monolayers.....	26
2.4.3.1 Formation of Self-Assembled Monolayers of Organosulfur Compounds on Metal Surfaces.....	26
2.4.3.2 Properties of Self-Assembled Monolayers of $\omega$ -carboxylalkanethiols (C1 to C15).....	28
2.4.4 Interfacial Potential Distribution.....	29
2.5 Electron Transfer.....	31
2.5.1 General Considerations.....	32
2.5.2 Homogeneous Electron Transfer.....	32
2.5.2.1 Classical Marcus Theory.....	32
2.5.2.2 Reorganization Energy.....	33
2.5.2.3 Quantum Mechanical Treatment.....	34
2.5.3 Heterogeneous Electron Transfer.....	36
 CHAPTER III EXPERIMENTAL METHODS.....	 40
3.1 Materials and Reagents.....	40
3.1.1 Chemicals.....	40
3.1.2 Buffer Solution.....	40
3.2 Electrode Preparation.....	41
3.2.1 Preparation of the SEIRA Active Surface.....	41
3.2.2 Electrochemical Cleaning of the SEIRA Active Surface.....	41
3.2.3 Optimization of the Gold Surface Preparation.....	42
3.2.4 Self-Assemble Monolayers.....	43



	Pages
3.3 SEIRA Spectro-Electrochemical Measurements.....	44
3.3.1 SEIRA Spectro-Electrochemical Set-Up.....	44
3.3.2 Immobilisation of Cytochrome c.....	44
3.3.3 Stationary SEIRA Investigations.....	45
3.3.3.1 Self-Assembled Monolayers.....	45
3.3.3.2 Native B1 State of Cytochrome c.....	46
3.3.3.3 non-Native B2 State of Cytochrome c.....	46
3.3.4 Time-Resolved SEIRA Measurements.....	47
3.4 Cyclic Voltammetry Measurements.....	48
3.5 Data Evaluation.....	49
 CHAPTER IV RESULTS AND DISCUSSION.....	 50
 4.1 Stationary SEIRA: Self-Assembled Monolayers of $\omega$ -Carboxylalkanethiols.....	 50
4.1.1 Spectroscopic Characterization of the Structural Changes of Monolayers under the Electric Field.....	 50
4.1.2 The Potential Range of Reversible Changes in SEIRA Difference Spectra of $\omega$ -carboxylalkanethiol Self-Assembled Monolayers.....	 53
4.1.3 Stability and Reorientation of Short Monolayers (C1-SAM)...	64
4.1.4 Spectral Contributions of Monolayers.....	66
4.2 Stationary SEIRA Investigations: Cytochrome c.....	69
4.2.1 Characterization of Cytochrome c.....	69
4.2.2 Redox-Linked Structural Changes of Immobilised Cytochrome c.....	 71
4.2.3 Redox Potential of Native B1 Cytochrome c.....	77
4.3 Electric Field Induced Conformational Transition of the Immobilised Cytochrome c.....	 80
4.4 Non-Native B2 Conformation of Cytochrome c.....	88

	Pages
4.4.1 Conformational and Redox Equilibria between the Native and non-Native State of Cytochrome c.....	88
4.4.2 Characterization of the non-Native B2 Conformation of Cytochrome c.....	91
4.5 Time-Resolved SEIRA Spectro-Electrochemistry.....	96
4.6 Cyclic Voltammetry.....	103
4.7 Electric Field Effect on the Interfacial Redox Process.....	107
 CHAPTER V CONCLUSION.....	 109
 5.1 Structural Changes of $\omega$ -carboxyl alkanethiols SAMs.....	 109
5.2 Structural and Orientational Changes of Immobilized Cyt-c on Gold Electrode.....	110
5.3 non-Native B2 Conformation of Cytochrome c.....	110
5.4 Time-Resolved SEIRA Spectro-Electrochemistry.....	111
 REFERENCES.....	 112
APPENDIX.....	123
CURRICULUM VITAE.....	136

## LIST OF FIGURES

		Pages
2.1	Layout of a Michelson interferometer.....	7
2.2	The retardation-dependence in time, which is characterized via the interferogram in step-scan (a) and rapid-scan (b) modes.....	9
2.3	A three-dimensional (3D) plot of the intensity versus the optical path difference and time. The interferograms are recorded as a function of time at each sampling positions.....	9
2.4	Schematic illustration of the beam leading to the total internal reflection phenomenon and IRE configurations for experimental ATR setups: (a) single-reflection variable angle hemicylinder crystal and (b) multiple-reflection planar crystal.....	11
2.5	The decay pattern of evanescent wave under ATR condition.....	12
2.6	A) The structure of an $\alpha$ -amino acid, and B) peptide bond linking two amino acids.....	15
2.7	The secondary protein structures. (A) Model of the $\alpha$ -helix structure. (B) A three-stranded $\beta$ -sheet which contains one anti-parallel and one parallel strand. Hydrogen bonds are indicated with red lines (antiparallel strands) and blue lines (parallel strands) connecting the oxygen receptor and hydrogen. (C) A turn structure...	17
2.8	Structure of the heme in Cyt-c covalently attached to the protein.....	20
2.9	Structure of iron complexes in Cyt-c considering the axial ligands and the related energy levels for $d_6$ iron. The reduced forms of Cyt-c: low-spin (left) and high-spin (right) configurations of iron complexes.....	22
2.10	The globular (a) and three-dimensional (b) structures of cytochrome c with the front surface view according to the Brookhaven Databank file "1hrc.pdb".....	22
2.11	Models of the electrical double layer at the electrode/solution interface: (a) Helmholtz, (b) Gouy-Chapman, (c) Stern, and (d) Grahame models.....	25

2.12	Thiols with variable alkyl chain length ( $n$ ) and tail group ( $X$ ) form ordered monolayers by self-assembly on gold electrode. $n \geq 1$ and $X =$ e.g. $\text{CO}_2\text{H}$ , $\text{CN}$ , $\text{OH}$ , $\text{CH}_3$ , pyridine, etc.....	27
2.13	Schematic representation of an interfacial potential distribution at electrode/SAM/protein/electrolyte interface.....	29
2.14	Profile of the Gibbs energy surfaces for reactant (R) and product (P).	32
3.1	The cyclic voltammogram of an electrochemically cleaned Au-surface. The Au-film was formed at a temperature controlled of $65^\circ\text{C}$ and a deposition time of 60 s.....	42
3.2	(Left) The time-dependent SEIRA spectra of a C3-SAM and (Right) the plot of the peak intensities of $\text{C}=\text{O}$ stretching vibration of the $1711\text{-cm}^{-1}$ band as function of absorption time for different Au-film preparation; (a) $55^\circ\text{C}$ 60 s, (b) $55^\circ\text{C}$ 120 s, (c) $75^\circ\text{C}$ 60 s, (d) $65^\circ\text{C}$ 90 s and (e) $65^\circ\text{C}$ 60 s.....	43
3.3	The SEIRA spectro-electro-chemical cell consists of (a) a Si ATR-crystal, (b) a thin Au-film working electrode, (c) a teflon coated o-ring, (d) a Au plate for the contact to the working electrode, (e) a Ag/AgCl reference electrode, (f) a Pt counter electrode, (g) a gas inlet, (h) a gas outlet and (i) a sample inlet.....	45
4.1	The potential-dependent SEIRA difference spectra of C2-, C3-, C6-, C11- and C16-SAMs. The spectra were taken at an electrode potential of $+0.15\text{ V}$ (the reference spectrum was measured at $-0.1\text{ V}$ ). The experiments were carried out at the ionic strength of 22 mM (pH 7.0). The time/temperature dependent effects in an ATR FT-IR spectrum of air were measured with a Si-prism.....	52
4.2	Example of series of the potential-dependent SEIRA difference spectra of C6-SAM measured at $-0.20, -0.15, -0.10, -0.05, 0.00, 0.05, 0.10, 0.15,$ and $0.20\text{ V}$ . The reference spectrum was collected at $-0.10\text{ V}$ . The experiments were carried out at the ionic strength of 22 mM (pH 7.0). Each spectrum was measured after waiting at the certain respective potential for 60 s (3 min/spectrum).....	54
4.3	The series of reversible spectral changes of C6-SAM at the reference	

	potential of -0.10 V after the potential jumps to (a) -0.20 V, (b) -0.15 V, (c) -0.05 V, (d) 0.00 V, (e) 0.05 V, (f) 0.10 V, (g) 0.15 V, and (h) 0.20 V, respectively; recalculated relative to the reference spectrum before the respective potential jump.....	55
4.4	The reversible spectral changes of monolayers at the reference potential of -0.10 V for C2-, C3-, C6-, C11- and C16-SAMs; recalculated relative to the reference spectrum before the respective potential jump.....	56
4.5	The series of irreversible spectral changes of C2-SAM at the reference potential of -0.10 V after potential jumps to (a) -0.20 V, (b) -0.15 V, (c) -0.05 V, (d) 0.00 V, (e) +0.05 V, (f) +0.10 V, (g) +0.15 V and (h) +0.20 V, respectively; recalculated relative to the reference spectrum before the potential jump.....	59
4.6	Series of the potential-dependent SEIRA difference spectra of 2-SAM measured at -0.20, -0.15, -0.05, 0.00, 0.05, 0.10, 0.15 and 0.20 V, respectively. The reference spectrum was collected at -0.10 V. Each spectrum was measured after waiting at a certain potential for 60 s (3 min/spectrum).....	60
4.7	The potential-dependent SEIRA difference spectra of C11-SAM measured at +0.4, +0.2, -0.2, -0.4, -0.6 and -0.7 V, respectively. The reference spectrum was collected at +0.1 V. The experiments were carried out at the ionic strength of 22 mM (pH 7.0). Each spectrum was measured after waiting at a certain potential for 60 s (3 min/spectrum).....	61
4.8	The series of irreversible spectral changes of C11-SAM at the reference potential of +0.10 V after potential jumps to (a) +0.4 V, (b) +0.2, (c) -0.2, (d) -0.4, (e) -0.6 and (f) -0.7 V, respectively; recalculated relative to the reference spectrum before the potential jump. The experiments were carried out at an ionic strength of 22 mM (pH 7.0). Each spectrum was measured after waiting at a certain potential for 60 s (3 min/spectrum).....	62
4.9	The irreversible changes of the potential-dependent SEIRA	

	difference spectra of C3-SAM after jumps to very negative potentials measured at +0.20, -0.30 and -0.40 V, respectively. The reference spectrum was taken at -0.10 V. The irreversible changes in the monolayer spectra measured at -0.10 V are obvious after recalculating it relative to the reference spectrum before the potential jump. The spectra series at varied electrode potentials is displayed here (from the top to the bottom).....	63
4.10	The potential-dependent SEIRA difference spectra of C2-SAM at +0.15 and +0.20 V, respectively. The reference spectrum was collected at -0.10 V.....	64
4.11	A schematic of the monolayer of mercaptoacetic acid adsorbed (a) with carboxylate and thiolate group and (b) only with the thiol group; e.g. on a Ag surface.....	65
4.12	The potential-dependent SEIRA difference spectra (Cyt-c fully oxidized at +0.10 V minus fully reduced at -0.10 V); the overlay of the C2-SAM (top) and C16-SAM (bottom) difference spectra with and without the presence of Cyt-c.....	67
4.13	The plot of peak intensities as function of the electrode potential of the corresponding pure monolayer spectra (square, open) and additionally adsorbed Cyt-c (square, solids).....	68
4.14	The absorbance spectra (top) and their second (2 <sup>nd</sup> )-derivatives (bottom) of Cyt-c in a bulk-solution, and immobilized on C11-SAM and bare Au-electrode. All spectra were measured at the open circuit potential (OCP), which is always >0 V.....	70
4.15	SEIRA difference spectra of Cyt-c immobilised on C16-SAM coated Au electrode at different ionic strengths (22 and 66 mM). The Spectra of the oxidized (positive bands) and reduced state (negative bands) were measured at +0.1 and -0.1 V, respectively.....	72
4.16	SEIRA difference spectra of Cyt-c immobilised on a C11-SAM coated Au electrode at different ionic strengths (22 and 66 mM). The spectra of the oxidized (positive bands) and reduced state (negative bands) were measured at +0.1 and -0.1 V, respectively.....	73

- 4.17 SEIRA difference spectrum of Cyt-c adsorbed on a C11-SAM coated Au electrode compared with the redox-induced IR difference spectrum of Cyt-c in solution. The spectra of the oxidized (positive bands) and reduced state (negative bands) were measured at +0.1 and -0.1 V, respectively. The experiments were carried out at an ionic strength of 22 mM (pH 7.0)..... 74
- 4.18 SEIRA difference spectra of Cyt-c adsorbed on C11-SAM coated Au electrode, obtained as a function of potential. The reference spectrum of the reduced state was measured at -0.1 V. The experiments were carried out at an ionic strength of 66 mM (pH 7.0)..... 78
- 4.19 Potential-dependence of the peak height of the SEIRA difference bands at 1673 (triangles) and 1660  $\text{cm}^{-1}$  (circles) as obtained from the potential-dependent SEIRA difference spectra. The solid line represents the fit of the Nernst equation to the experimental data..... 79
- 4.20 The SEIRA difference spectra of Cyt-c adsorbed on a Au-electrode and on SAM with various chain lengths. The spectra of the oxidized (positive bands) and reduced state (negative bands) were measured at +0.1 and -0.1 V, respectively. The experiments were carried out at an ionic strength of 22 mM (pH 7.0)..... 81
- 4.21 The normalized SEIRA difference spectra of Cyt-c adsorbed on a Au-electrode (gray, dash), and on C2- (light gray, solid), C3- (gray, solid), C6- (black, dot), C11- (black, dash) and C16-SAM (black, solid). The spectra of the oxidized (positive bands) and reduced state (negative bands) were measured at +0.1 and -0.1 V, respectively. The experiments were carried out at an ionic strength of 22 mM (pH 7.0)..... 82
- 4.22 The normalized SEIRA difference spectra of Cyt-c adsorbed on C2-, C3-, C6-, C11- and C16-SAM at selected electrode potentials; +0.02 (black, dash), +0.06 (gray, solid) and +0.10 V (black, solid). The reference spectrum was recorded at -0.10 V. The experiments were carried out at an ionic strength of 22 mM (pH 7.0)..... 83
- 4.23 Plot of the relative band intensities of 1672 (circle, open) and

- 1660(circle, close)  $\text{cm}^{-1}$  characteristic for the oxidized state relative to the band at 1693  $\text{cm}^{-1}$  in the reduced state as a function of electrode potential for Cyt-c adsorbed on  $\omega$ -carboxylalkanethiol with different chain lengths. The reference spectrum was recorded at -0.10 V..... 84
- 4.24 The normalized SEIRA difference spectra of Cyt-c adsorbed on a Au-electrode covered with phosphate anions after Cyt-c adsorption at OCP (solid) and at -0.20 V (dash), with respected to the 1693- $\text{cm}^{-1}$  band. The spectra of the oxidized (positive bands) and reduced state (negative bands) were measured at +0.1 and -0.1 V, respectively. The experiments were carried out at an ionic strength of 22 mM (pH 7.0)..... 85
- 4.25 A series of the potential-dependent SEIRA difference spectra of Cyt-c adsorbed to the bare Au-electrode measured at the potential of -0.475, -0.45, -0.4, -0.3, -0.2, -0.1, 0.0, 0.1 and 0.15 V, respectively. The reference spectrum was recorded at -0.5 V. Spectra were measured in the equilibrium state..... 89
- 4.26 The potential-dependent peak intensities of the 1660- $\text{cm}^{-1}$  band of Cyt-c immobilized on the bare Au-electrode covered with phosphate anions. The redox couples of Cyt-c are fitted to the summation of three Nernst functions represented by the continuous line. The reference spectrum was recorded at -0.5 V. The spectra were measured in the equilibrium state..... 90
- 4.27 The potential-dependent SEIRA difference spectra of Cyt-c adsorbed to: C6-SAM measured at a potential of +0.02 V (-0.10 V as reference) with (a) a “perfect” and (b) non-perfect coverage of the SAM, and adsorbed to the bare Au-electrode measured at a potential of (c) 0.0 V (-0.1 V as reference) and (d) -0.30 V at equilibrium state (-0.5 V as reference)..... 93
- 4.28 The potential-dependent SEIRA difference spectra (ox. minus red.) of Cyt-c in bulk solution (a), and adsorbed to the “bare” Au-electrode covered with phosphate anions measured at -0.30 V (b) and



- +0.10 V (c) using the spectrum at -0.50 V as reference. The measurements of the bound state were done under equilibrium conditions..... 94
- 4.29 The 2<sup>nd</sup>-derivative spectra of potential-dependent SEIRA difference spectra (ox. minus red.) of Cyt-c in bulk solution (a), and adsorbed to the “bare” Au-electrode covered with phosphate anions measured at -0.30 V (b) and +0.10 V (c) using the spectrum at -0.50 V as reference. The measurements of the bound state were done under equilibrium conditions..... 95
- 4.30 Rapid-scan SEIRA difference spectra of Cyt-c on C16-SAM coated Au electrodes using the spectrum measured at -0.1 V as a reference. The individual traces represent the difference spectra obtained at 1 (gray, dotted), 3 (gray, solid), 5 (black, dotted), 10 (black, dashed), and 20 (black, solid) s after the potential jump from -0.1 V to the redox potential (+0.04 V). The experiments were carried out at an ionic strength of 66 mM (pH 7.0)..... 97
- 4.31 Step-scan SEIRA difference spectra of Cyt-c on C11-SAMcoated Au electrodes using the spectrum measured at -0.1 V as a reference. The individual traces represent the difference spectra obtained at 5 (gray, dotted), 10 (gray, solid), 20 (black, dotted), 40 (black, dashed), and 70 (black, solid) ms after the potential jump from -0.1 V to the redox potential (+0.04 V). The experiments were carried out at an ionic strength of 66 mM (pH 7.0)..... 98
- 4.32 Kinetic traces of the time-evolution of the SEIRA bands at 1693 cm<sup>-1</sup> (gray squares; reduced), 1673 cm<sup>-1</sup> (white circles, oxidised), and 1660 cm<sup>-1</sup> (dark gray triangles, oxidised) obtained from the rapid scan and step scan SEIRA spectroscopic measurements of Cyt-c on C16-SAM (top) and C11-SAM (bottom), respectively (see Figure 4.30 and 4.31)..... 99
- 4.33 Model for the reaction mechanism of the interfacial redox process of Cyt-c immobilised on SAM-coated electrodes. The reaction pathway studied in this work is highlighted by the bold arrows.  $k_{rot}^{ox}$

- and  $k_{rot}^{red}$  refer to the relaxation constants for rotational diffusion, monitored by TR SERR spectroscopy, whereas  $k_{prot}^{ox}$  and  $k_{prot}^{red}$ , derived from SEIRA measurements, denote the rate constants of the protein structural changes to adapt the equilibrium structure of the reduced and oxidised Cyt-c, respectively.  $k_{ET}^{ox}$  and  $k_{ET}^{red}$  are the standard heterogeneous electron transfer rate constants at zero driving force (see eq (4.1))..... 102
- 4.34 CVs of immobilised Cyt-c on C6-SAM (top) at scan rates of 1, 2, 3, 4, 5 and 6  $Vs^{-1}$ , respectively; on C11-SAM (middle) at scan rates of 0.05, 0.10, 0.2, 0.4, 0.8 and 1.0  $Vs^{-1}$ , respectively; and on C16-SAM (bottom) at scan rates of 1, 2, 4, 7.5 and 10  $mVs^{-1}$ , respectively. The experiments were carried out at an ionic strength of 66 mM (pH 7.0)..... 105
- 4.35 Relationship between peak current and scan rate derived from the CVs of immobilised Cyt-c on C6-SAM (top), on C11-SAM (middle) and on C16-SAM (bottom). Anodic and cathodic currents are given by close and open symbols, respectively. The experiments were carried out at an ionic strength of 66 mM (pH 7.0)..... 106

**LIST OF TABLES**

	Pages
2.1 Chain lengths and surface $pK_a$ values of $\omega$ -carboxylalkanethiol monolayers as function of methylene groups.....	28
4.1 Relaxation constants for the potential jump from -0.1 V to the redox potential for Cyt-c immobilised on coated electrodes as determined by rapid scan and step scan SEIRA spectroscopy.....	100

## LIST OF ABBREVIATIONS

2 <sup>nd</sup>	: second
3D	: three-dimensional
5cHS	: five-coordinate high spin
6cHS	: six-coordinate high spin
6cLS	: six-coordinate low spin
ATR	: attenuated total reflection
C1	: mercaptoacetic acid
C2	: 3-mercaptopropionic acid
C5	: 6-Mercaptohexanoic acid
C10	: 11-mercaptoundecanoic acid
C15	: 16-mercaptohexadecanoic acid
CV	: cyclic voltammogram
Cyt-c	: cytochrome c
EF	: electric field
ET	: electron transfer
FCWD	: Franck Condon weighted density
FT-IR	: Fourier transform infrared
His	: histidine
IR	: infrared
IRE	: internal reflection element
Lys	: lysine
M	: molarity
Met	: methionine
MSEF	: mean square electric field
Phe	: phenylalanine

PZC	: potential of zero charge
RC	: redox center
RR	: resonance Raman
SAM	: self-assembled monolayer
SEIRA	: surface enhanced infrared absorption
SERR	: surface enhanced resonance Raman spectroscopy
SER	: surface enhanced Raman
Si	: silicone
SNR	: signal to noise ratio
Try	: tryrosine
UV-VIS	: ultra-violet visible
V	: voltage

## LIST OF SYMBOLS

$\Delta A$	: absorbance change
$\alpha$	: alpha
$\beta$	: beta
$\omega$	: omega
$\nu$	: scan rate
$\tau$	: relaxation time
$\Gamma$	: surface coverage
$I$	: ionic strength
$\lambda$	: wavelength
$\lambda$	: reorganization energy
$\lambda_{in}$	: inner reorganization energy
$\lambda_{out}$	: outer reorganization energy
$\theta_c$	: critical angle
$\text{\AA}$	: angstrom
$d_p$	: penetration depth
$A_{real}$	: real surface area
$A_{geom}$	: geometric area
Ag	: silver
Au	: gold
$E$	: electrode potential
$E^0$	: redox potential
$E_{ref}$	: reference potential
$E_f$	: final potential

$E_p$	:	potential of peak separation
$E_{PZC}$	:	potential of zero charge
$I_p$	:	average peak current
$k_{ET}$	:	electron transfer rate constant
$k_{prot}$	:	protein relaxation constant
$k_{rot}$	:	protein rotation constant
$n$	:	number of electron transfer
$Q_{exp}$	:	experimental charges
$Q_{the}$	:	theoretical charges
$R_f$	:	roughness factor