



Stefanie Ahl (Autor)  
**Optical Biosensors in a New Design**

New Platforms for Optical Biosensing

Stefanie Elisabeth Ahl



Cuvillier Verlag Göttingen

<https://cuvillier.de/de/shop/publications/1722>

Copyright:

Cuvillier Verlag, Inhaberin Annette Jentzsch-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. Introduction .....</b>   | <b>1</b>  |
| 1.1 Biosensors .....   | 1         |
| 1.2 Aim of the study .....   | 2         |
| 1.3 References .....   | 4         |
| <b>2. Methods for surface characterization .....</b>   | <b>5</b>  |
| 2.1 Theoretical Background – Surface Plasmon Resonance (SPR) Spectroscopy.....   | 5         |
| 2.1.1 Excitation of Propagating Surface Plasmons (p-SPR).....  | 5         |
| 2.1.1.1. Optical properties of materials .....   | 5         |
| 2.1.1.2. Prism coupling .....  | 7         |
| 2.1.1.3. SPR signal .....  | 8         |
| 2.1.1.4. Influence of the excitation wavelength to the SPR signal .....  | 8         |
| 2.1.1.5 Changes in the dielectric due to adsorbates leading to changes in the plasmon resonance minimum.....             | 9         |
| 2.1.2 Excitation of Localized Surface Plasmons (l-SPR) .....   | 10        |
| 2.2 Basics of Cyclic Voltammetry (CV) .....  | 11        |
| 2.3 Introduction to Electrochemical Impedance Spectroscopy (EIS) theory .....  | 15        |
| 2.4 Scanning Electron Microscopy (SEM) .....   | 19        |
| 2.5 Autocorrelation.....   | 20        |
| 2.6 References .....   | 21        |
| <b>3. Experimental Section .....</b>   | <b>22</b> |
| 3.1 Instrumental - SPR setup.....  | 22        |
| 3.2 Modifications of the SPR setup: Halogen lamp plus monochromator.....   | 25        |
| 3.3 Instrumental – CV/EIS- setup .....   | 26        |
| 3.4 Further instruments .....  | 27        |
| 3.4.1 Plasma cleaner .....   | 27        |
| 3.4.2 Surface profiler .....   | 27        |
| 3.4.3 UV/VIS/NIR Spectrometer.....   | 28        |
| 3.5 Preparation of Evaporated Gold (EG) films.....   | 29        |
| 3.6 Preparation of Template Stripped Gold (TSG) films .....  | 29        |
| 3.7 Preparation of silane monolayers .....   | 29        |
| 3.8 Materials .....  | 31        |
| 3.9 References .....   | 33        |
| <b>4. Nanoporous gold (NPG) membrane .....</b>   | <b>34</b> |
| 4.1 Advantage of Porous Gold - new plasmonic material and the aim of the study .....                                     | 34        |
| 4.2 Fabrication of Random Nanoporous Gold Substrates.....  | 37        |
| 4.2.1 Cleaning of the glass slides.....  | 37        |
| 4.2.2 Silanization of the glass slides .....   | 37        |
| 4.2.3 Wet-chemical acid etching of the decorative gold leafs .....   | 37        |
| 4.2.3.1 Execution of dealloying .....  | 38        |
| 4.2.4 Electrochemical dealloying.....  | 40        |
| 4.3 Scanning Electron Microscopy as a tool to visualize the NPG morphology .....   | 41        |
| 4.4 Two Dimensional Autocorrelation to determine the typical structure size of the NPG for different etching times ..... | 43        |

|   |    |
|---|----|
| 4.5 Cyclic voltammetry and electrical impedance spectroscopy as methods to determine the surface area of NPG substrates.....            | 47 |
| 4.6 Simultaneous Excitation of Propagating and Localized Surface Plasmon Resonance in Nanoporous Gold Membranes (p-SPR and l-SPR) ..... | 54 |
| 4.6.1 Multilayer architecture built on NPG and flat gold substrates.....  | 61 |
| 4.6.2 Environmental refractive index changes to NPG (glyceroltest) .....  | 68 |
| 4.6.3 Layer by layer (LbL) deposition of charged dendrimers .....   | 72 |
| 4.6.4 Layer by layer (LbL) deposition of avidin and antiavidin .....  | 77 |
| 4.7 Application of NPG  |    |
| The Protein-Tethered Lipid Bilayer established on the Nanoporous Gold Substrate .....   | 85 |
| 4.7.1 Characterization of the layer formation by SPR and EIS .....  | 88 |
| 4.7.2 Activation of the Cytochrome C Oxidase.....   | 91 |
| 4.8 Conclusion.....   | 93 |
| 4.9 References .....  | 96 |

## **5. Gold/Silica Composite Inverse Opals ..... 101**

|  |     |
|--|-----|
| 5.1 Advantage of gold/silica composite inverse opals - new plasmonic material and the aim of the study ..... | 101 |
| 5.2 Fabrication of gold/silica composite inverse opals .....   | 102 |
| 5.3 Surface plasmon resonance features of gold/silica composite inverse opals .....                          | 106 |
| 5.4 Conclusion and Outlook.....  | 107 |
| 5.5 References .....   | 109 |

## **6. Epitope mapping to identify the centrin sequence interacting to transducin..... 111**

|  |     |
|--|-----|
| 6.1 Processes of optical signaling .....                       | 111 |
| 6.1.1. The vertebrate visual signal transduction cascade ..... | 112 |
| 6.1.2. Light and dark adaptation .....                         | 114 |
| 6.1.3. Barrier hypothesis.....                                 | 116 |
| 6.2 Characteristics of Transducin.....                         | 119 |
| 6.3 Centrins in vertebrate cells .....                         | 121 |
| 6.4 Motivation .....   | 125 |
| 6.5 Development of the sensor architecture .....               | 127 |
| 6.5.1. Commercial CM5 sensor chip (Biacore) .....              | 127 |
| 6.5.2. Peptide P19 matrix.....                                 | 131 |
| 6.5.3. Combined mPEG thiol matrix .....                        | 134 |
| 6.6 Regeneration of the sensor surface.....                    | 136 |
| 6.7 Further experimental optimization .....                    | 137 |
| 6.8 SPR results of centrin-transducin interactions .....       | 137 |
| 6.8.1. Centrin 1 (Cen1p) constructs .....                      | 138 |
| 6.8.2. Centrin 3 (Cen3p) isoform.....                          | 141 |
| 6.9 Conclusion and Outlook .....                               | 142 |
| 6.10 References .....  | 145 |

## **7. Summary ..... 151**

## **8. Appendix ..... 153**

|   |     |
|---|-----|
| 8.1 Summary of advantages and disadvantages of NPG at a glance..... | 153 |
| 8.2 Supporting material for chapter 6.....                          | 154 |

|   |     |
|---|-----|
| 8.3 Table of standard amino acid abbreviations..... | 155 |
| 8.4 List of Abbreviations.....                      | 156 |
| 8.5 List of Figures .....                           | 159 |
| 8.6 List of Tables.....                             | 162 |

**Acknowledgements.....****164**

**Curriculum vitae .....****166**