Stepping into Catalysis

Kinetic and Mechanistic Investigations of Photo- and Electrocatalytic Hydrogen Production with Natural and Synthetic Molecular Catalysts

DANIEL STREICH
In light of its rapidly growing energy demand, human society has an urgent need to become much more strongly reliant on renewable and sustainable energy carriers. Molecular hydrogen made from water with solar energy could provide an ideal case. The development of inexpensive, robust and rare element free catalysts is crucial for this technology to succeed. Enzymes in nature can give us ideas about what such catalysts could look like, but for the directed adjustment of any natural or synthetic catalyst to the requirements of large scale catalysis, its capabilities and limitations need to be understood on the level of individual reaction steps. This thesis deals with kinetic and mechanistic investigations of photo- and electrocatalytic hydrogen production with natural and synthetic molecular catalysts. Photochemical hydrogen production can be achieved with both *E. coli* Hyd-2 [NiFe] hydrogenase and a synthetic dinuclear [FeFe] hydrogenase active site model by ruthenium polypyridyl photosensitization. The overall quantum yields are on the order of several percent. Transient UV-Vis absorption experiments reveal that these yields are strongly controlled by the competition of charge recombination reactions with catalysis. With the hydrogenase major electron losses occur at the stage of enzyme reduction by the reduced photosensitizer. In contrast, catalyst reduction is very efficient in case of the synthetic dinuclear active site model. Here, losses presumably occur at the stage of reduced catalyst intermediates. Moreover, the synthetic catalyst is prone to structural changes induced by competing ligands such as secondary amines or DMF, which lead to catalytically active, potentially mononuclear, species. Investigations of electrocatalytic hydrogen production with a mononuclear catalyst by cyclic voltammetry provide detailed kinetic and mechanistic information on the catalyst itself. By extension of existing theory, it is possible to distinguish between alternative catalytic pathways and to extract rate constants for individual steps of catalysis. The equilibrium constant for catalyst protonation can be determined, and limits can be set on both the protonation and deprotonation rate constant. Hydrogen bond formation likely involves two catalyst molecules, and even the second order rate constant characterizing hydrogen bond formation and/or release can be determined.

**Keywords:** Artificial photosynthesis, photocatalysis, electrocatalysis, hydrogen production, proton reduction, hydrogenase, iron complex, active site model, catalytic mechanism, transient absorption, spectroscopy, electrochemistry, cyclic voltammetry

Daniel Streich, Uppsala University, Department of Chemistry - Ångström, Physical Chemistry, Box 523, SE-751 20 Uppsala, Sweden.
Science without religion is lame. Religion without science is blind.

– Albert Einstein
List of papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I Light Driven Hydrogen Production in Ruthenium Polypyridyl Photosensitized *E. coli* Hyd-2 [NiFe] Hydrogenase Studied by Steady State and Time Resolved UV-Vis Absorption Spectroscopy
D. Streich, B.J. Murphy, F. Armstrong, L. Hammarström
*Manuscript in preparation*

II High-Turnover Photochemical Hydrogen Production Catalyzed by a Model Complex of the [FeFe]-Hydrogenase Active Site
D. Streich, Y. Astuti, M. Orlandi, L. Schwartz, R. Lomoth, L. Hammarström, S. Ott

III Comparing the Reactivity of Benzenedithiolate- versus Alkyldithiolate-Bridged Fe$_2$(CO)$_6$ Complexes with Competing Ligands
D. Streich, M. Karnahl, Y. Astuti, C.W. Cady, L. Hammarström, R. Lomoth, S. Ott

IV Benchmarking Molecular Catalysts for Multi-Electron Redox Processes – Kinetic and Mechanistic Information from Voltammetric Data
D. Streich, R. Lomoth
*Submitted manuscript*

Reprints were made with permission from the publishers.
**Contribution report**

**Paper I**
Main responsibility for all experiments and data analyses except for the enzyme preparation and the mass spectrometry. Major contributions to the interpretation of the results and the writing of the manuscript.

**Paper II**
Major contribution to all experiments and data analyses as well as the interpretation of the results and the writing of the manuscript.

**Paper III**
Contribution to all spectroscopic characterizations except for the EPR spectroscopy, to the interpretation of the results and to the writing of the manuscript.

**Paper IV**
Main responsibility for all experiments as well as the major part of the simulations and data analyses. Major contributions to the interpretation of the results and the writing of the manuscript.

I have contributed to the discussions of all these papers.
Contents

1 Introduction............................................................................................................. 13
  1.1 General background ......................................................................................... 13
  1.2 General objectives and approach .................................................................... 14

2 Fundamentals ........................................................................................................ 16
  2.1 Properties and production of molecular hydrogen ........................................ 16
  2.2 Sustainable hydrogen production with molecular catalysts ....................... 18
    2.2.1 General aspects of water splitting .............................................................. 18
    2.2.2 Mechanistic aspects of proton reduction catalysis .................................... 20
    2.2.3 Electronic states, oxidation states and thermodynamics ............................ 21
    2.2.4 Reaction kinetics and kinetics of electron transfer ................................. 25
    2.2.5 Molecularity and reaction order ............................................................... 27

3 Materials and methods ......................................................................................... 31
  3.1 Studied systems and their components ......................................................... 31
    3.1.1 Ruthenium polypyridyl photosensitizers .................................................. 31
    3.1.2 Hydrogenase enzymes ............................................................................ 32
    3.1.3 Synthetic molecular catalysts ................................................................. 35
  3.2 Steady state and transient absorption spectroscopy ....................................... 37
    3.2.1 UV-Vis spectroscopy ................................................................................ 38
    3.2.2 IR spectroscopy ...................................................................................... 40
    3.2.3 EPR spectroscopy ................................................................................... 41
  3.3 Electrochemistry ............................................................................................... 43
    3.3.1 Cyclic voltammetry .................................................................................. 43
    3.3.2 Bulk electrolysis and spectroelectrochemistry ......................................... 47

4 Specific objectives, results and discussion ............................................................ 48
  4.1 Photocatalytic hydrogen production with natural E. coli Hyd-2 [NiFe] hydrogenase (Paper I) .......................................................... 48
    4.1.1 Possibilities and challenges .................................................................... 48
    4.1.2 Photocatalytic activity ............................................................................ 50
    4.1.3 Electron transfer processes inside the enzyme ....................................... 52
    4.1.4 Covalently photosensitized systems ....................................................... 53
  4.2 Photocatalytic hydrogen production with a synthetic dinuclear [FeFe] hydrogenase active site model (Papers II and III) ...................... 54
    4.2.1 Photocatalytic activity ............................................................................ 54
4.2.2 Mechanistic information and comparison to Hyd-2 ..... 56
4.2.3 Catalyst and photosensitizer stability ....................... 59
4.2.4 Bridge dependent reactivity .................................. 61
4.2.5 Implications for catalytic hydrogen production ........... 62

4.3 Electrocatalytic hydrogen production with a synthetic
mononuclear [FeFe] hydrogenase active site model (Paper IV) . 64
4.3.1 Electrochemical methods as complements to
spectroscopy ................................................................ 64
4.3.2 Mechanistic and kinetic information from cyclic
voltammetry .................................................................. 65
4.3.3 Implications for the electrocatalytic hydrogen
production with a mononuclear [FeFe] hydrogenase
active site model ............................................................ 67

5 Summary and outlook ......................................................... 70

Mekanistiska och kinetiska studier av foto- och elektrokatalytisk
vätgasproduktion med naturliga och syntetiska molekylära katalysatorer ... 72
Mechanistische und kinetische Untersuchungen der photo- und
elektrokatalytischen Wasserstoffherstellung mit natürlichen und
synthetischen molekularen Katalysatoren ........................................... 75

Acknowledgements ................................................................ 79

References ............................................................................. 81
### Abbreviations

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td>transfer coefficient (unit free)</td>
</tr>
<tr>
<td>γ</td>
<td>activity coefficient (unit free)</td>
</tr>
<tr>
<td>ε</td>
<td>extinction coefficient (in M$^{-1}$ cm$^{-1}$)</td>
</tr>
<tr>
<td>ε$_0$</td>
<td>vacuum permittivity (unit free)</td>
</tr>
<tr>
<td>ε$_s$</td>
<td>low frequency (static) dielectric constants (unit free)</td>
</tr>
<tr>
<td>ε$_{op}$</td>
<td>high frequency (optical) dielectric constants (unit free)</td>
</tr>
<tr>
<td>η</td>
<td>overpotential (in V)</td>
</tr>
<tr>
<td>η$_{inj}$</td>
<td>catalyst reduction branching ratio (unit free)</td>
</tr>
<tr>
<td>η$_{inj}$</td>
<td>(cumulative) catalyst reduction yield (unit free)</td>
</tr>
<tr>
<td>κ$_{el}$</td>
<td>electronic transmission coefficient (unit free)</td>
</tr>
<tr>
<td>λ</td>
<td>reorganization energy (in J mol$^{-1}$) or wavelength (in m)</td>
</tr>
<tr>
<td>λ$_i$</td>
<td>inner sphere reorganization energy (in J mol$^{-1}$)</td>
</tr>
<tr>
<td>λ$_o$</td>
<td>outer sphere reorganization energy (in J mol$^{-1}$)</td>
</tr>
<tr>
<td>μ$_B$</td>
<td>Bohr magneton (in J T$^{-1}$)</td>
</tr>
<tr>
<td>ν</td>
<td>scanrate (in V s$^{-1}$) or frequency (in s$^{-1}$)</td>
</tr>
<tr>
<td>φ</td>
<td>quantum yield (unit free)</td>
</tr>
<tr>
<td>˜ν</td>
<td>wavenumber (in cm$^{-1}$)</td>
</tr>
<tr>
<td>τ</td>
<td>lifetime (in s)</td>
</tr>
<tr>
<td>A</td>
<td>surface area (in m$^2$)</td>
</tr>
<tr>
<td>A$^-$</td>
<td>conjugate base to acid HA</td>
</tr>
<tr>
<td>a$_A$</td>
<td>radius of electron acceptor (in m)</td>
</tr>
<tr>
<td>a$_D$</td>
<td>radius of electron donor (in m)</td>
</tr>
<tr>
<td>A$_{ox}$</td>
<td>oxidized state of electron acceptor</td>
</tr>
<tr>
<td>A$_{red}$</td>
<td>reduced state of electron acceptor</td>
</tr>
<tr>
<td>Abs</td>
<td>absorbance (unit free)</td>
</tr>
<tr>
<td>bdt</td>
<td>1,2-benzenedithiolate</td>
</tr>
<tr>
<td>B$_0$</td>
<td>magnetic field (in T)</td>
</tr>
<tr>
<td>Bn</td>
<td>benzyl</td>
</tr>
<tr>
<td>Cat$_{ox}$</td>
<td>oxidized state of catalyst</td>
</tr>
<tr>
<td>Cat$_{red}$</td>
<td>reduced state of catalyst</td>
</tr>
<tr>
<td>CV</td>
<td>cyclic voltammetry or cyclic voltammogram</td>
</tr>
<tr>
<td>Cy</td>
<td>cyclohexyl</td>
</tr>
<tr>
<td>d</td>
<td>path length (in m)</td>
</tr>
<tr>
<td>D</td>
<td>diffusion coefficient (in m$^2$ s$^{-1}$)</td>
</tr>
<tr>
<td>D$_{exc}$</td>
<td>excited state of electron donor</td>
</tr>
<tr>
<td>D$_{ox}$</td>
<td>oxidized state of electron donor</td>
</tr>
<tr>
<td>D$_{red}$</td>
<td>reduced state of electron donor</td>
</tr>
<tr>
<td>DMF</td>
<td>N-N$'$-dimethylformamide</td>
</tr>
<tr>
<td>e</td>
<td>elementary charge (in C)</td>
</tr>
</tbody>
</table>
- electron
- standard (reduction) potential (in V)
- formal (reduction) potential (in V)
- vertex potential (in V)
- anodic peak potential (in V)
- cathodic peak potential (in V)
- electron paramagnetic resonance
- ethyl
- force constant (N m$^{-2}$)
- Faraday constant (in C mol$^{-1}$)
- the ferrocinium/ferrocene couple
- standard Gibbs free energy (in J mol$^{-1}$)
- reaction free energy change (in J mol$^{-1}$)
- activation energy (in J mol$^{-1}$)
- electronic coupling (in cm$^{-1}$)
- proton (or hydron)
- acid with conjugate base A$^{-}$
- E. coli Hyd-2, [NiFe] hydrogenase
- electric current (in A)
- cathodic peak current (in A)
- anodic peak current (in A)
- cathodic peak current (in A)
- irradiation intensity (in W m$^{-2}$)
- infrared spectral range
- intersystem crossing
- diffusive flux (in M s$^{-1}$)
- first order rate constant (in s$^{-1}$)
- pseudo first order rate constant (in s$^{-1}$)
- second order rate constant (in M$^{-1}$ s$^{-1}$)
- Boltzmann constant (in J K$^{-1}$)
- (pseudo) first order catalytic rate constant (in s$^{-1}$)
- first order catalytic rate constant (in s$^{-1}$)
- second order catalytic rate constant (in M$^{-1}$ s$^{-1}$)
- electron transfer rate constant (in s$^{-1}$)
- electron transfer rate constant (in M$^{-1}$ s$^{-1}$)
- standard rate constant (in cm s$^{-1}$)
- ligand
- ligand to metal charge transfer
- decadic logarithm
- natural logarithm
- methyl
- metal to ligand charge transfer
- number of electrons
- optical density (unit free)
- oxidized state
- phenyl
PS  ground state photosensitizer
*PS  excited state photosensitizer
PS_{ox}  oxidized state photosensitizer
PS_{red}  reduced state photosensitizer
r_{AD}  electron donor–acceptor distance (in m)
Red  reduced state
[Ru(bpy)_3]^{1+}  Tris(2,2’-bipyridyl)ruthenium(I)
[Ru(bpy)_3]^{2+}  Tris(2,2’-bipyridyl)ruthenium(II)
[Ru(bpy)_3]^{3+}  Tris(2,2’-bipyridyl)ruthenium(III)
[Ru(dm-bpy)_2(MI-phen)]^{2+}  Bis(4,4’-dimethyl-2,2’-bipyridyl)(5-maleiimido-1,10-phenanthroline)ruthenium(II)
SHE  standard hydrogen electrode
t  time (in s)
T  absolute temperature (in K)
TOF  turnover frequency (in product per catalyst per second)
TON  turnover number (in product per catalyst)
UV-Vis  ultra-violet and visible spectral ranges
W  energy (in eV or J mol^{-1})
Figure 1. Without words.
1. Introduction

1.1 General background

Modern society is characterized by its rapidly growing energy demand and an energy supply chain that is mainly based on the utilization of fossil and nuclear fuels [1]. This bears severe environmental and socio-economic risks, since the availability of these fuels is limited both in terms of absolute amounts and geographical accessibility and also because their use is immediately (particulate matter, NO\textsubscript{x}, SO\textsubscript{x}, etc.) and obliquely (greenhouse gas emissions, nuclear waste, etc.) damaging to the environment [2, 3].

Renewable, easily and widely accessible, non-pollutive energy sources and carriers have therefore become very desirable and solar energy plays a key role in this context\textsuperscript{1}. In one hour the sun delivers about 445 EJ \textsuperscript{2} of energy to planet earth [4], which is equivalent to $\approx 120 \%$ of the world’s total energy consumption in 2010 [5]. This energy can be harvested either directly in the form of sunlight or indirectly as biomass, wind, water or wave energy. The annual conversion of solar energy to biomass by green plants and other photosynthetic organisms amounts to approximately 3000 EJ of which roughly 1 \% is used as a fuel [4]. The utilization of biomass and waste contribute on average about 12 \% to the world’s total energy consumption [5]. However, the biomass fraction can be higher than 80 \% in less industrialized countries, where, for example, burning wood or other readily available biomass is an important energy source. Under such conditions competition for feed-stocks and arable soil as well as irreversible environmental damage (e.g. desertification) due to excessive use can pose serious issues for this otherwise attractive renewable energy carrier [6, 7].

Since all other forms of solar energy do not come as easily storable and transportable energy carriers, they are subject to significant temporal variations, which makes energy conversion and storage an inevitable and crucial issue for their utilization. Virtually all commercially available solar energy conversion technologies not based on heat (solar thermal energy) or biomass (pellet fuel, biodiesel, etc.) produce electricity (photo-voltaics, hydro-electric, wind and wave power, etc.), even though the latter contributed only 17.7 \%\textsuperscript{3} to the global energy consumption in 2010 [5].

\textsuperscript{1} geothermal (996 EJ/a [4]) and tidal (94 EJ/a [4]) energies will not be further discussed because of their limited geographical and technological accessibility

\textsuperscript{2} 1 EJ = 10\textsuperscript{18} J $\approx 2.8 \cdot 10\textsuperscript{11}$ kWh

\textsuperscript{3} in several countries (Haiti, Nigeria, Ethiopia, etc.) this fraction was only $\approx 1$ \% [8]
Electricity can be stored in batteries. But their energy densities and cyclabilities frequently render them too costly for many intermediate and large scale stationary applications. Also, both battery costs and recharging times still discourage potential buyers of electric vehicles.

If solar energy is stored in chemical bonds as liquid or gaseous fuels, there is the potential for very high energy density and cyclability, and recharging times are not an issue. Molecular hydrogen is of particular interest in this context because water is the only resource required to produce this energy rich compound and water is at the same time the only product generated upon its utilization as a fuel. Solar hydrogen, therefore, has the potential to become the key to a globally accessible, renewable and non-pollutive energy supply.

The major challenge in the production of solar fuels in general and solar hydrogen in particular is the development of catalysts that make the underlying processes energy efficient. The ideal catalyst would be composed of low cost materials and possess high robustness, specificity and activity. In nature, enzymes based on exclusively earth-abundant elements catalyze the water splitting half reactions important for hydrogen production with high specificity and activity. Therefore, it is tempting to conduct studies that provide a deeper insight into the mode of operation of these enzymes and of synthetic mimics of their bio-inorganic catalytically active sites. The idea is to identify concepts relevant to the robustness, specificity and activity of these systems and make use of them for the development of improved natural or artificial catalytic systems and components.

1.2 General objectives and approach

The focus of this thesis is on catalysts for the hydrogen production half reaction. The development and verification of methods that allow the study and comparison of natural and artificial catalysts at the level of individual elementary reactions occupies a central position and constitutes the major part of this thesis. In this context the following questions arise:

- Is continuous irradiation photochemical hydrogen production possible with ruthenium polypyridyl photosensitized hydrogenases and synthetic active site model complexes?
- What limits the respective quantum yields and rates of hydrogen production in such photochemical schemes?
- Which role does the stability of the catalyst and the auxiliary components play in a particular catalytic system?
- How many metal centers are necessary for catalysis?
• What are the limits on efficiency and rate of hydrogen production that are actually imposed by the catalyst?

• What kind of insight can be gained about catalytic mechanisms?

These questions are addressed in this work by investigations of a [NiFe] hydrogenase from *E. coli* and on both dinuclear and mononuclear synthetic model complexes of the [FeFe] hydrogenase active site. Time resolved and steady state UV-Vis absorption spectroscopy, IR spectroscopy, EPR spectroscopy and electrochemical methods are employed and interesting details about, for example, hydrogen production quantum yields, catalyst and auxiliary component stability as well as the molecularity of hydrogen bond formation are obtained and discussed.
2. Fundamentals

2.1 Properties and production of molecular hydrogen

Frequently, strong emphasis is put on hydrogen’s extraordinary heat of combustion, which is superior to all other chemical fuels. The energetic gain associated with the bonding of an electron to a nucleus – if reduced to mere electro-statics – depends on the effective nuclear charge per electron, which is maximal in hydrogen atoms [9]. The fact that hydrogen atoms at the same time feature the highest electron to nucleon ratio results in a gravimetric energy density that is three times higher for molecular hydrogen than for common liquid hydrocarbon fuels like gasoline or diesel (Table 2.1).

Table 2.1. Mass and energy densities of molecular hydrogen and liquid hydrocarbon fuels$^a$.

<table>
<thead>
<tr>
<th></th>
<th>density (kg/m$^3$)</th>
<th>energy density (kWh/m$^3$)</th>
<th>kWh/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>hydrogen (gas, 298 K)</td>
<td>0.09</td>
<td>3.55</td>
<td>39.4</td>
</tr>
<tr>
<td>hydrogen (liquid, 21 K)</td>
<td>70.9</td>
<td>2790</td>
<td>39.4</td>
</tr>
<tr>
<td>hydrocarbon fuels (liquid, 298 K)</td>
<td>$\approx$ 800</td>
<td>$\approx$ 10500</td>
<td>13.1</td>
</tr>
</tbody>
</table>

$^a$hydrogen data from [4], hydrocarbon energy density from [9], approximate hydrocarbon density based on specifications of EN 228 and EN 590, all energy densities are based on upper heating values.

However, a glance at the volumetric energy densities in Table 2.1 reveals that whenever spatial limitations play a role, as is the case in most of the classical fuel-dependent applications e.g. in the transportation sector, hydrogen can only be attractive in a compressed at 350 – 700 bar, liquefied at $< 21$ K, 1 atm, cryo-compressed at $\geq 20$ K, $\leq 350$ bar or chemically bound as metal or mixed hydride form. Compression, pre-cooling and liquefaction processes are associated with energetic costs on the order of several kWh per kg H$_2$, which considerably reduces the effective hydrogen energy density for the corresponding forms of hydrogen [10]. Metal and mixed hydrides have the potential for hydrogen mass fractions $\geq 10\%$ but these materials are still very much under development. Their stability, cyclability as well as the controlled loading with and release of hydrogen are typical issues that yet need to be resolved [11].

Considering further that the three major processes by which molecular hydrogen is produced on industrial scale today – steam reforming, partial oxidation and the Kværner process – all require substantial thermal energy inputs,
utilize fossil fuel resources and yield CO, CO\textsubscript{2} or elementary amorphous carbon as side products, it is clear that hydrogen’s attractiveness in the context of renewable energy technologies must have other reasons.

The real strength of molecular hydrogen is that, unlike any other chemical fuel, its entire production and consumption cycle can be based on one of the most abundant and non-hazardous resources on our planet: water. Its use as energy carrier is thus potentially free of any harmful side-products such as particulate matter, greenhouse gases, NO\textsubscript{x}, SO\textsubscript{x} or radio-active waste. If care is taken not to compete for the supply of drinking water, the use of hydrogen as energy carrier would likely greatly reduce the environmental burden and the socio-economic risks characterizing today’s energy supply chain.

There are four major categories of processes by which molecular hydrogen can be renewably and sustainably produced from water [1, 12, 13]:

- Thermochemical cycles (based on renewable thermal energy)
- Photobiological processes (modified natural photosynthesis)
- Photochemical processes (artificial photosynthesis)
- Electrolysis (based on renewable electricity)

Common to all of these processes is that catalysts are required to make them energy efficient under conditions accessible with renewable energy sources. Inorganic salts able to withstand temperatures exceeding 1000 °C are typical for thermochemical cycles. Photobiological processes are based on microorganisms equipped with specific enzymes as catalysts. Homogeneous molecular catalysts as well as heterogeneous, mesoporous and nanoparticle catalysts in bulk are encountered in both photochemical processes and electrolysis, even though the latter is traditionally associated with the use of metal and amalgam electrodes catalyzing the water splitting half reactions.

Molecular catalysts play a special role because, with the exception of thermochemical cycles, they can be used in all of the above processes. They are structurally well defined and in this way enable targeted modification. This is a prerequisite for the study of structure-function relationships and thus knowledge driven (directed) catalyst design and development. The work presented in this thesis is therefore focused on natural and synthetic catalysts that fall into this category (Sections 3.1.2 and 3.1.3) with special emphasis on their applicability in photo- and electrocatalytic hydrogen production.
2.2 Sustainable hydrogen production with molecular catalysts

2.2.1 General aspects of water splitting

The most important aspects of catalytic hydrogen production from water are summarized in Figure 2.1. The overall water splitting reaction

\[ 2\text{H}_2\text{O} \rightarrow \text{O}_2 + 2\text{H}_2 \]  \hspace{1cm} (2.1)

is comprised of two half reactions,

\[ 2\text{H}_2\text{O} \rightarrow \text{O}_2 + 4\text{H}^+ + 4e^- \]  \hspace{1cm} (oxidation) \hspace{1cm} (2.2)

\[ 4\text{H}^+ + 4e^- \rightarrow 2\text{H}_2 \]  \hspace{1cm} (reduction) \hspace{1cm} (2.3)

each of which is characterized by a standard reduction potential, \( E^\circ \), which is a measure of the propensity of the oxidized reactant(s) to take up electrons (Section 3.3). As with all potentials, these standard reduction potentials need to be compared to some kind of reference. The reduction of protons to hydrogen at a Pt electrode, the so called standard hydrogen electrode (SHE), is

\[ [14, 15, 16] \]

\[ ^2 \text{defined in the direction Ox} + e^- \rightarrow \text{Red}, \text{Ox} \text{ and Red being the oxidized and reduced reactant(s); standard conditions: } 25 \, ^\circ \text{C}, 1 \, \text{M}, 1 \, \text{atm}, \text{pH} = 0 \]
probably the most prominent example of such a reference. Reduction potentials in this work either refer to SHE or to the ferrocinium/ferrocene couple, \( \text{Fc}^{+/0} \), which has a standard reduction potential of +400 mV vs. SHE [14]. If the standard reduction potentials for two half reactions with respect to the same reference are known, they can be used to judge which direction the overall reaction will proceed. Electrons will only move spontaneously from reduced reactants at low potential (e.g. \( \text{H}_2 \)) towards oxidized reactants at a more positive reduction potential (e.g. \( \text{O}_2 \)). Thus, the oxidation of hydrogen with oxygen to form water is a spontaneous process setting free an energy equivalent to

\[
W_{\text{out}} = \Delta G = -n \cdot F \cdot \Delta E = -237.2 \text{ kJ/mol} \quad (2.4)
\]

per mole of hydrogen as electrons move towards lower potential energy \( W \) (note the reversed sign convention for \( W \) and \( E^0 \) in Figure 2.1). Therefore, hydrogen production from water requires a quantity

\[
|W_{\text{in}}| \geq |W_{\text{out}}| \quad (2.5)
\]

of energetic input. This energy can be provided in different ways. In electrolysis low energy holes can accept electrons from the oxidation catalyst, \( \text{Cat}_{\text{ox}} \), while high energy electrons can be donated to the reduction catalyst, \( \text{Cat}_{\text{red}} \). These electrons can be generated at two electrodes by means of electrical energy using a potentiostat. In this case the "Engine" levels in Figure 2.1 correspond to the anode\(^4\) and cathode\(^5\) materials, respectively. In photobiological and photochemical processes \( W_{\text{in}} \) stems from light that is absorbed by one or several so called photosensitizers (Section 3.1.1). Here, the "Engine" levels in Figure 2.1 would correspond to low energy holes and high energy electrons located on photooxidized and photoreduced molecules, respectively, or on a molecule in an excited state\(^6\) (Section 2.2.3).

In practice, \(|W_{\text{in}}|\) will be significantly larger than \(|W_{\text{out}}|\). Two factors contribute to the so called overpotentials, \( \eta_{\text{ox}} \) and \( \eta_{\text{red}} \), which result in energy losses for both half reactions. On the one hand, each of the catalysts works at a certain operating potential, which only ideally is identical to the standard reduction potential of the corresponding half reaction. On the other hand, driving electron transfer in the desired direction at an appreciable rate requires an additional amount of energy. Since there is hardly anything that can be done about the latter, the catalyst operating potentials are crucial for the minimization of energetic losses. This underscores the importance of catalyst characterization and development.

The simplicity of Figure 2.1 and of the chemical formula for water splitting (Equation 2.1) may give the impression that designing a catalytic system will

---

\(^3\) \( G \): Gibbs free energy, \( F \): Faraday constant, \( n \): number of electrons transferred per reaction

\(^4\) electrode at which oxidation takes place

\(^5\) electrode at which reduction takes place

\(^6\) excitons in case of a semiconductor photosensitizer
be comparatively simple. This, however, is not the case. Kinetic and thermodynamic factors that affect catalysis as well as multiple mechanistic pathways that may be undertaken by the catalytic system must also be considered. These concepts will be elaborated upon in the next sections.

2.2.2 Mechanistic aspects of proton reduction catalysis

Figure 2.2. Catalytic matrix for the proton reduction half reaction of water splitting. The coordinate system in the top left corner relates the reactions on the ligand, L, and the metal center, Cat, of a hypothetical molecular catalyst, LCat, to the directions in space. Reductions of LCat by an external electron donor are indicated by electrons, e\(^{-}\). Protonations by an external acid (e.g. water) are indicated by protons, H\(^{+}\). Note, this scheme should, in fact, include at least six ligand reduced and six ligand protonated species, but for clarity only two of each are shown.

Since this thesis focuses on the proton reduction half reaction of water splitting, a closer look at important mechanistic, thermodynamic and kinetic aspects of this reaction is necessary to appreciate its complexity and identify and understand concepts relevant to the characterization of multi-electron redox catalytic systems.

Two electrons and two protons must be brought together in order to generate a molecule of hydrogen. Figure 2.2 illustrates possible intermediates and pathways through which this can be achieved with the help of a hypothetical molecular catalyst, LCat. The mechanistic manifold is a three-dimensional matrix with direct metal reduction, metal protonation, ligand reduction and ligand protonation reactions. For an intramolecular catalytic mechanism that is first order in catalyst concentration there are two possible pathways to H\(_2\) production. Firstly, via an at least doubly reduced and singly protonated cat-
alyst hydride species which reacts with a proton in solution. Secondly, via an at least doubly reduced and doubly protonated catalyst species in which two H-atoms or a hydride and a proton can react with each other.

Protons and electrons originate from external sources but it is important to realize that they can be transferred between the different catalyst intermediates, as well. This implies that overall catalysis can proceed via a broad spectrum of intermolecular mechanisms that are second order in catalyst concentration. Here, two at least singly reduced and protonated catalyst intermediates would react with each other.

Note that one particular catalyst, LCat, has the possibility of reacting via several intra- and intermolecular channels and can even change the preferred mechanism depending on the properties of the other components of the catalytic system, i.e. the experimental conditions. The fitness of a catalyst is often measured by the overall yield and the rate of hydrogen production given by the quantities of turnover number (TON) and turnover frequency (TOF), respectively. TON and TOF analysis can assess catalytic experimental conditions as a whole, but frequently cannot give direct insight into catalyst performance. Therefore, both the judgement about whether catalysis is really limited by the catalyst and the assessment of catalyst performance necessarily require more detailed mechanistic insight on the level of individual reaction steps.

2.2.3 Electronic states, oxidation states and thermodynamics

A catalytic system for hydrogen production not only comprises a catalyst but also an acid, HA, an electron donor, D, and, possibly, a photosensitizer, PS. The latter is particularly important for light driven catalysis and transient absorption spectroscopy, because the catalysts studied in this thesis are not in essence photoactive. Figure 2.3 shows that these additional components lead to a further increase in the mechanistic complexity of a catalytic system. Photons get absorbed and protons and electrons transferred between the different components during catalysis and all these processes are associated with changes in the electronic and oxidation states of the respective reactants. A sound knowledge about these states and their distinct thermodynamic and spectroscopic properties is very important for the design of a catalytic system that can work in the desired reaction. This knowledge can also help to limit the scope of possible mechanistic pathways by a clever choice of the experimental conditions.

Figure 2.4 shall serve as a basis for the qualitative discussion of different electronic and oxidation states the components of a catalytic system might be in. The impact of the electronic or oxidation state on thermodynamic properties important for (light driven) hydrogen production will be highlighted.

---

8 note that a particular catalytic system will typically be designed in a way to allow only one of the electron transfer reactions proposed in Figure 2.3 to occur.
Extended catalytic matrix including proton transfer and various possible electron transfer reactions from external donors. Protons are fed into the catalytic reactions from an acid, HA, producing conjugate base, A⁻ (top left). Electrons can be provided in three different ways. Firstly, via direct chemical reduction by a reduced state electron donor, D_{red}, producing oxidized state electron donor, D_{ox} (top right). Secondly, via transfer from a reduced state photosensitizer, PS_{red}, obtained from reductive quenching of excited state photosensitizer, *PS, by D_{red} (bottom right). Thirdly, via direct transfer from *PS, producing oxidized state photosensitizer, PS_{ox}, which is rereduced to ground state photosensitizer, PS, by D_{red} (bottom left). Note that both the second and the third variant require the absorption of photons to generate *PS from PS. Note further that D_{red} can be replaced by an electrode poised at a sufficiently negative potential.

Moreover, mutual relationships between different thermodynamic properties will be addressed.

Regarding a photosensitizer, PS, all states depicted in Figure 2.4 are potentially relevant for photocatalysis. If one electron of a hypothetical two electron ground state PS molecule is raised to a higher energy molecular orbital by absorption of a photon of matching energy, a spin conserved singlet excited state is formed. The separation of spins into two orbitals leads to a stabilization of both singly occupied orbitals by an amount equivalent to the spin pairing energy. This stabilization is arbitrarily assumed here to affect both electrons to the same extent. Intersystem crossing can convert a singlet to a triplet excited state, in which a decrease in spin correlation usually leads to a further overall stabilization. Featuring both an electron at higher energy and a hole at lower energy interestingly makes the singlet and triplet excited states simultaneously...
Figure 2.4. Schematic diagram of a molecule in different electronic and oxidation states. Electrons are represented by up (↑) and down (↓) spins occupying one of three molecular orbitals at different electronic energies, $W_{el}$. Dashed arrows indicate possible electronic transitions giving rise to the absorption of electromagnetic radiation (h·ν).

more reducing and more oxidizing than the ground state. Thus, a ground state PS incapable of reducing a catalyst or oxidizing an electron donor may well be able to do so in an excited state. This is the basis for light triggered electron transfer as required for both continuous irradiation photochemical hydrogen production and transient absorption spectroscopy.

Removal or addition of electrons gives rise to oxidized and reduced states, respectively, and the change in effective nuclear charge per electron can lead to substantial changes in electronic energies for all electrons in the molecule. The lower energy orbitals become stabilized in the oxidized state compared to the ground and excited states, whereas the additional electron in the reduced state leads to a destabilization of these orbitals\(^9\). This renders the oxidized state substantially more oxidizing and the reduced state considerably more reducing than the ground and the excited states. If a PS is not able to transfer an electron to a catalyst from its ground or one of its excited states, it can possibly do so from its reduced state, obtained by reductive quenching of one of its excited states with an electron donor.

With respect to a catalyst that is not intrinsically photoactive, mainly the reduced and oxidized states in Figure 2.4 will be relevant for catalysis. Two aspects are important in this context. Firstly, the transfer of a second electron to a catalyst is typically much more difficult than the transfer of the first electron. An exception to this is the case where the first reduction of a catalyst

\(^9\) electronic effects on the vacant highest energy orbital in Figure 2.4 are assumed to be negligible
results in major structural rearrangements inside the catalyst, so that the second reduction takes place at milder potentials. In such a case the reduction potentials are said to be inverted. Secondly, a catalyst’s affinity for protons, i.e. its basicity or acidity, will change as its electron configuration is altered. In terms of the Brønsted definition of acidity, the equilibrium

$$\text{AH} + \text{H}_2\text{O} \rightleftharpoons \text{A}^- + \text{H}_3\text{O}^+ \quad (2.6)$$

between an acid AH and its conjugate base A$^-$ is characterized by an equilibrium constant

$$K_a = \frac{[\text{A}^-][\text{H}_3\text{O}^+]}{[\text{AH}]} \quad (2.7)$$

which is typically reported in terms of

$$pK_a = -\log(K_a) \quad (2.8)$$

and thus higher acidity of the acid is indicated by a lower p$K_a$ value. Analogously, high basicity of the conjugate base is connected to low p$K_b$ values with

$$pK_b = 14 - pK_a \quad (2.9)$$
in aqueous solution. Increasing a catalyst’s electron density e.g. by reduction typically raises its basicity substantially. Inversely, protonation of a catalyst can be a viable strategy to facilitate subsequent catalyst reduction steps. Consequently, the reduction potential and the p$K$ of a catalyst in a particular oxidation state are mutually dependent.

![Figure 2.5. Thermodynamic cycle consisting of two protonation (horizontal) and reduction (vertical) equilibria characterized by p$K_{a,1}$ and p$K_{a,2}$ and the reduction potentials $E_1^0$ and $E_2^0$.](image)

This mutual dependence can be quantified by means of a closed thermodynamic cycle as depicted in Figure 2.5. Since the free energy $G$ is a state function, the free energy changes associated with the two protonation and reduction equilibria must sum up to zero. Using the definitions

$$\Delta G^0 = -n \cdot F \cdot E^0 \quad (2.10)$$

and

$$\Delta G^0 = -R \cdot T \cdot \ln(K_a) = 2.3 \cdot R \cdot T \cdot pK_a \quad (2.11)$$
implies
\[-F \cdot E_0^0 + 2.3 \cdot R \cdot T \cdot pK_{a,1} = -F \cdot E_2^0 + 2.3 \cdot R \cdot T \cdot pK_{a,2}\] (2.12)
for \(n_1 = n_2 = 1\), which gives
\[
\Delta E^0 = 59 \text{mV} \cdot \Delta pK_a
\] (2.13)
at 25 °C, where \(\Delta E^0 = E_2^0 - E_1^0\) and \(\Delta pK_a = pK_{a,2} - pK_{a,1}\). An important conclusion from this is that for every \(pK_a\) unit difference between \(\text{LCat}\) and \(\text{LCat(e}^-)\), \(\text{LCat(H}^+)\) will be more easily reduced by 59 mV compared to \(\text{LCat}\).

2.2.4 Reaction kinetics and kinetics of electron transfer

\[\text{Figure 2.6. Potential energy surface diagrams of a general electron transfer reaction. Energies are expressed in terms of free energy, G, or electronic energy, } W_{\text{el}}. \text{ Electrons are represented as up (1) and down (1) spins. }\]

\[\text{a) The reaction proceeds along a one-dimensional reaction coordinate from the reactants, } A_{\text{red}} \text{ and } D_{\text{ox}}, \text{ to the products, } A_{\text{ox}} \text{ and } D_{\text{red}}, \text{ via a higher energy transition state. The change in electronic energy upon electron transfer matches the driving force, } \Delta G^0. \text{ The height of the activation barrier, } \Delta G^\# \text{, depends on the energy of the transition state and determines the rate of (adiabatic) electron transfer.}\]

\[\text{b) Adiabatic versus non-adiabatic electron transfer. Possible reactant (dark gray) and product (light gray) state configurations are defined by parabolic energy surfaces as functions of a one-dimensional nuclear coordinate. The electronic coupling term } H_{\text{AD}} \text{ determines the extent of splitting of the energy surfaces at the crossing point of the reactant and product state energy surfaces.}\]

\[^{10}[15, 18, 19, 22]\]
A thermodynamic driving force (positive $\Delta E^0$ or negative $\Delta G^0$) is necessary for any reaction to occur spontaneously. Having a driving force does not necessarily ensure that a reaction will proceed at an appreciable rate. Reaction rates depend on the activation barrier, either in the form of an overpotential, $\eta$, or an activation energy, $\Delta G^\dagger$, that energetically separates the reactants from the products. To illustrate this point a general electron transfer reaction can be considered. The potential energy surfaces for such a reaction are shown in Figure 2.6. In order to proceed from the reduced donor, $D_{\text{red}}$, and oxidized acceptor, $A_{\text{ox}}$, to the oxidized donor, $D_{\text{ox}}$, and reduced acceptor, $A_{\text{red}}$, the reactants need to come together to form an encounter complex wherein an electron can be transferred. Structural rearrangements in the reactants and the surrounding solvent need to take place to achieve the transition state configuration, such that crossover to the products proceeds with conservation of energy. The relative position of the reactant and product state energy minima defines the driving force, $\Delta G^0$. The energy difference, $\Delta G^\dagger$, between the reactant state minimum and the transition state determines the rate, $k_{eT}$, at which the electron transfer reaction will proceed according to the Arrhenius type equation

$$k_{eT} = \kappa_{el} \cdot \nu_{TS} \cdot e^{-\frac{\Delta G^\dagger}{k_B T}}$$

(2.14)

as originally described by Marcus and Sutin [23]. Here $\kappa_{el}$ is the electronic transmission coefficient, $\nu_{TS}$ is the frequency of passage through the transition state, $k_B$ is the Boltzmann constant and $T$ is the absolute temperature. The product of $\nu_{TS}$ and the exponential term corresponds to the frequency of visiting the transition state configuration at a given temperature, while $\kappa_{el}$ is a measure of the probability of crossing the barrier in the transition state.

If $\kappa_{el} \approx 1$, electron transfer occurs quantitatively when the nuclear coordinate of the transition state is achieved. In Figure 2.6a this becomes manifest in a continuous energy surface connecting reactants and products. More generally, the reactant and product energy surfaces need to be represented by intersecting parabolas as functions of a one-dimensional nuclear coordinate (Figure 2.6b). The extent of splitting into two continuous energy surfaces is determined by the electronic coupling, $H_{AD}$, between donor and acceptor in the transition state, which depends on the overlap of the vibrational wavefunctions in the reactant and product state. For strong coupling ($\kappa_{el} \approx 1$) the electron transfer step is said to be adiabatic. At the other extreme lies nonadiabatic electron transfer, for which $\kappa_{el} << 1$. Here the nuclear coordinate of the transition state will typically have to be visited many times before a crossing from the reactant to the product energy surface occurs. For this case the modified Arrhenius expression

$$k_{eT} = \frac{2 \cdot \pi}{\hbar} \cdot (H_{AD})^2 \cdot \frac{1}{\sqrt{4 \cdot \pi \cdot \lambda \cdot k_B \cdot T}} \cdot e^{\frac{(\Delta G^0 + \lambda)^2}{4 \cdot \lambda \cdot k_B \cdot T}}$$

(2.15)
relates the electron transfer rate $k_{\text{el}}$ to the electronic coupling term $H_{\text{AD}}$, the reorganization energy $\lambda$ and the activation energy

$$\Delta G^\ddagger = \frac{(\Delta G^0 + \lambda)^2}{4 \cdot \lambda}$$

(2.16)

$\Delta G^\ddagger$ and $H_{\text{AD}}$ can be determined experimentally from the change in $k_{\text{el}}$ with respect to temperature. If $\lambda$ is not known, it can be estimated using

$$\lambda = \lambda_i + \lambda_o = \frac{1}{2} \sum_k f_k \cdot (r_{0R} - r_{0P})^2 + \left(\frac{(n \cdot e)^2}{4 \cdot \pi \cdot \varepsilon_0} \left( \frac{1}{2 \cdot a_D} + \frac{1}{2 \cdot a_A} - \frac{1}{r_{AD}} \right) \left( \frac{1}{\varepsilon_{op}} - \frac{1}{\varepsilon_s} \right) \right)$$

(2.17)

In this expression the inner sphere reorganization energy component, $\lambda_i$, which is the energy required to distort bondlengths and angles in the reagents, is expressed in terms of reduced force constants, $f_k$, and reactant and product state equilibrium bond lengths, $r_{0R}$ and $r_{0P}$. The outer sphere or solvent reorganization energy, $\lambda_o$, is characterized by the transferred charge, $n \cdot e$, the vacuum dielectric constant, $\varepsilon_0$, the solvent’s high- and low-frequency dielectric constants, $\varepsilon_{op}$ and $\varepsilon_s$, the donor and acceptor radii, $a_D$ and $a_A$, and the donor-acceptor distance, $r_{AD}$. This analysis treats the solvent as dielectric continuum and assumes a spherical molecule geometry.

2.2.5 Molecularity and reaction order\textsuperscript{11}

How the rate of a reaction depends on the reactant concentrations is determined by the reaction molecularity and the reaction order. If a reaction involves $k$ molecules of a certain type of reactant, it is said to be $k$-molecular with respect to that reactant. If the reaction is an elementary reaction\textsuperscript{12}, the corresponding rate law will also be $k$th order in this reactant. Since the collision of reactants becomes increasingly unlikely with higher orders of molecularity, most reactions do not exceed second order dependence in any of the reactants. The following elementary reactions and rate laws are therefore of particular interest:

$$\text{A} \rightarrow \text{products}; \quad \frac{d [\text{A}]}{dt} = -k_1 \cdot [\text{A}]$$

(2.18)

$$\text{A} + \text{B} \rightarrow \text{products}; \quad \frac{d [\text{A}]}{dt} = -k_2 \cdot [\text{A}] \cdot [\text{B}] \approx -k'_1 \cdot [\text{A}]$$

(2.19)

$$2 \cdot \text{A} \rightarrow \text{products}; \quad \frac{d [\text{A}]}{dt} = -2 \cdot k_2 \cdot [\text{A}]^2$$

(2.20)

\textsuperscript{11}[16, 18, 19, 24]

\textsuperscript{12}a reaction that cannot be split up further into smaller steps
The reaction and rate law in Equation 2.18 correspond to a real first order decay of reactant \( A \), for which \( k_1 \) is a first order rate constant in units of \( s^{-1} \). Starting at an initial concentration \([A]_0\) the concentration \([A]\) at time \( t \) is given by

\[
[A] = [A]_0 \cdot e^{-k_1 \cdot t} \tag{2.21}
\]

In Equation 2.19 the overall second order reaction can be approximated by a pseudo first order reaction when \([B] \gg [A]\) such that \([B] \approx [B]_0\) is essentially constant. The pseudo first order rate constant is then defined as \( k_1' = k_2 \cdot [B]_0 \) and a simple replacement of \( k_1 \) with \( k_1' \) in Equation 2.21 affords the corresponding time dependence of \([A]\). Rate constants can be extracted from exponential fits to plots of \([A] \) vs. \( t \) or from linear fits to plots of \( \ln([A]) \) vs. \( t \) (Figure 2.7a and b).

If \( A \) is consumed by multiple (pseudo) first order reactions simultaneously, the observed decay will still be monoexponential with

\[
k_1 = \sum_i k_1^{(i)} \tag{2.22}
\]

or

\[
k_1' = \sum_i k_1^{(i)'} \tag{2.23}
\]

respectively, and the component rate constants are only accessible if further information about the formation of specific products is available.

A homo-molecular second order decay is described by Equation 2.20. Here, the concentration of \([A]\) at time \( t \) is described by

\[
\frac{1}{[A]} = \frac{1}{[A]_0} + 2 \cdot k_2 \cdot t \tag{2.24}
\]

where \([A]_0\) is the initial concentration of \( A \) and \( k_2 \) is a second order rate constant in units of \( M^{-1} \cdot s^{-1} \). Multiple second order homo-molecular reactions of reactant \( A \) lead to the same kind of analytical function describing its decay, with the observed second order rate being the sum of the individual rates. Second order rate constants can be obtained by fitting plots of \([A] \) vs. \( t \) with equations of type

\[
[A] = \frac{[A]_0}{1 + 2 \cdot k_2 \cdot t} \tag{2.25}
\]

derived from Equation 2.24, or by linear fitting of a plot of \( 1/[A] \) vs. \( t \) (Figure 2.7a and c).

As shown in Figure 2.7, plots of \( \ln[A] \) and \( 1/[A] \) vs. \( t \) can also be used to qualitatively distinguish between first and second order behavior. The former yield straight lines for first order reactions but bent curves in case of second order reactions (Figure 2.7 b). Conversely, second order reactions give rise to straight lines whereas first order reactions lead to upward bent curves in the
Figure 2.7. Different plots of (pseudo) first order (black curves) and second order (gray curves) order decays of reactant A as defined in Equations (2.18–2.20). The initial concentrations $[A]_0$ are identical in both cases and the numerical values of the (pseudo) first and second order rate constants correspond to $1/10$ of the full time axis.

latter type of plot (Figure 2.7 c). Alternatively, initial rates obtained at different initial reactant concentrations can be used to determine the order of a reaction. This, however, requires that the concentrations of the reactants of interest can be varied easily and independently, which is often not the case. Further complications to these simple kinds of graphical and initial rate analyses can arise for example by equilibria that need to be taken into account, by higher order reactions involving different reactants or by combinations of different order reactions involving the same reactant. If analytical equations cannot be obtained and reaction conditions cannot be modified to achieve simple kinetics, simulation by numerical integration of the corresponding systems of differential equations can be another approach for obtaining rate constants.

The importance of reaction order and molecularity in the context of catalysis lies in the fact that catalysis typically comprises a number of sequential (and even parallel) reactions of different order, many of which are undesired (charge recombination, degradation, etc.). The different concentration dependencies and physical limits applying to first and higher order rate constants often allow for manipulations of a catalytic system via the reactant concentrations. Moreover, the determination of rate constants can elucidate which molecular properties could be tuned in order to favour desired over undesired reactions.

An illustrative example is given in Figure 2.8. The doubly reduced and doubly protonated catalyst species $\text{LCat}(\text{H}^+)_2(e^-)_2$ can undergo either irreversible inactivation by reaction with another molecule of the same species or productive catalytic turnover. As apparent from Figure 2.7, second order reactions outcompete the first order reactions at high reactant concentrations. At low reactant concentrations, however, first order catalytic turnover will prevail. Also, first order rate constants can be as high as $10^{14}$ s$^{-1}$, whereas sec-

---

$^{13}$imposed by the upper limit of typical bond vibration frequencies
Scond order rate constants for bimolecular reactions are limited to values $\leq 10^{10}$ M$^{-1}$ s$^{-1}$. In addition to working at lower catalyst concentrations, modifications on the catalyst resulting in increased $k_1$ would thus be another way of favouring catalytic turnover instead of degradation. Modifications that facilitate catalyst reduction or protonation would, counterintuitively, have no effect or even disfavor catalytic turnover, since this would likely lead to increased LCat(H$^+$)$_2$(e$^-$)$_2$ concentrations.

In summary, catalysis of the seemingly simple proton reduction half reaction is mechanistically demanding and the design of an efficient system requires careful balancing of thermodynamic and kinetic properties of all its components. Of particular importance are the different redox and protonation states of the catalyst. The identification as well as the thermodynamic and kinetic characterization of at least the rate limiting step(s) is of paramount importance for catalyst assessment. Without this understanding, a comparison between catalysts reacting along different pathways bears the risk of serious misinterpretations and oversight of promising compounds.

---

Figure 2.8. Second order degradation reaction competing with first order catalytic turnover.
3. Materials and methods

3.1 Studied systems and their components

3.1.1 Ruthenium polypyridyl photosensitizers

Many catalysts cannot be driven directly with light. Photosensitizers that are able to transfer electrons upon absorption of light are typically required to achieve light driven catalysis in these cases. The use of photosensitizers also provides the possibility to trigger catalytic processes of interest with very short flashes of light, enabling the temporal investigation of these processes with nano to femtosecond resolution.

The range of photosensitizers extends from heterogeneous materials [25] to organic [26] and metal-organic [27, 28] molecules. For analytical purposes molecular photosensitizers are most convenient because they have well defined structural, photophysical and photochemical properties that can often be tuned and modified with relative ease to suit the demands of a particular application [29, 30]. Due to their robustness, versatility and extraordinarily well documented properties [31, 32], ruthenium polypyridyl complexes are of particular interest. Figure 3.1 shows the ruthenium polypyridyl complexes used as photosensitizers for the studies presented in this thesis. 

\[
\text{[Ru(bpy)}_3\text{]}^{2+} \quad \text{(Figure 3.1a)}
\]

is used diffusing freely in solution for experiments described in Sections 4.1 (Paper I) and 4.2 (Paper II). Maleimide functionalized [Ru(dm-bpy)]_2(MI-phen)]^{2+} (Figure 3.1b) is used for covalent cysteine-specific photosensitization in Section 4.1 (Paper I).

The most important photophysical and electrochemical properties of this kind of photosensitizer are summarized in Figure 3.2. Broad metal to ligand charge transfer (MLCT) absorption bands centered at around 450 nm enable excitation to a singlet \( ^1 \text{MLCT} \) state by visible light. Ultrafast intersystem crossing (ISC) generates a triplet \( ^3 \text{MLCT} \) excited state, which at room temperature has a lifetime of about 0.5-1 \( \mu \)s and leads to phosphorescent emission with quantum yields of roughly 2-10\% and emission maxima at ca. 600 nm. As discussed in the context of electronic and oxidation states in Section 2.2.3, Figure 2.4, both reduction and oxidation of the \( ^3 \text{MLCT} \) excited state are facilitated by an amount roughly corresponding to the excited state energy \( E_{0-0} \approx 2.1 \) eV. Exact absorption maxima, excited state lifetimes, emission quantum yields and emission maxima depend on the solvent and the ligand substitutions but vary only slightly between the two complexes in Figure 3.1. These photosensitizers are robust with respect to excitation, reduction and oxidation.
and the excited, reduced and oxidized states have characteristic UV-Vis absorption properties which allow for spectroscopic distinction and monitoring of interconversion processes between them and the ground state as discussed in more detail in Section 3.2.1.

3.1.2 Hydrogenase enzymes

In the early 1930s Stephenson and Stickland postulated [33, 34] and further elaborated on [35] the existence of hydrogenase enzymes, based on evidence
for microbial hydrogen oxidation activity in river muds. Today three families of these enzymes, most frequently termed [NiFe], [FeFe] and [Fe] hydrogenases according to the number and type of metal atoms in their catalytically active site, are known to occur in a broad variety of microorganisms [36, 37]. All hydrogenases have the ability to activate molecular hydrogen, but they vary not only in their active site compositions but also in their prevailing functions and properties. [NiFe] hydrogenases are capable of catalyzing both hydrogen oxidation and proton reduction at maximum turnover frequencies from 1 to 1000 s\(^{-1}\) and from 5 to 5000 s\(^{-1}\), respectively [38]. Members of the [FeFe] hydrogenase family catalyze proton reduction at maximum turnover frequencies from 500 to 5000 s\(^{-1}\) and hydrogen oxidation at turnover frequencies from 6000 to 40000 s\(^{-1}\) [38]. In this way both [NiFe] and [FeFe] hydrogenases are able to provide the two functionalities that are important for their utilization by microorganisms. On the one hand, they allow the use of molecular hydrogen as energy and electron source, while on the other hand they enable redox balancing by dumping electrons and preventing accumulation of excessive amounts of reductive equivalents. The third hydrogenase family is somewhat exceptional in that it catalyzes the transfer of hydrides to and from an organic molecule called methylenetetrahydromethanopterin rather than the hydrogen/proton interconversion reaction [39].

Considering the extraordinary catalytic capabilities of these enzymes, it is not surprising that there is an extraordinary scientific interest in the regulation, maturation, stability, structure and function of many hydrogenases from all three families. In Section 4.1 (Paper I) catalytic investigations of the Hyd-2 [NiFe] hydrogenase from *Escherichia coli* (*E. coli*) will be presented. While an experimental structure of this particular hydrogenase has not been determined, a close analog from *Desulfovibrio gigas* (*D. gigas*) is shown in Figure 3.3. This structure highlights the most important structural properties and components of this class of enzymes. A small (39.7 kDa) and a large (62.5 kDa) subunit make up the overall protein fold. The [NiFe] active site is part of the large subunit and buried in the hydrophobic core of the enzyme at the interface between the two subunits. Proton and hydrogen diffusion to and from the active site is enabled by channels to the solvent [41, 42, 43]. Two [4Fe4S] clusters and one [3Fe4S] cluster are nicely aligned inside the small subunit making up an electron relay for the transfer of electrons to and from the active site [44]. According to their distance to the latter, these three clusters are typically called proximal, medial and distal [FeS] clusters or [4Fe4S]\(_p\), [3Fe4S]\(_m\) and [4Fe4S]\(_d\), respectively.

The identity, structure and thermodynamic properties of many oxidation and protonation states of the [NiFe] hydrogenase active site are known in much detail [45]. Several studies show how important the [FeS] clusters are in the context of catalytic bias and oxygen tolerance [46, 47, 48, 49]. In contrast, very little is known about dynamic properties of [NiFe] hydrogenases like the kinetics of transitions between catalytic intermediates or the electron transfer
Figure 3.3. X-ray crystal structure of *D. gigas* [NiFe] hydrogenase (PDB code 1FRV [40]). *C*α-traces are shown as black (small subunit) and yellow (large subunit) ribbons. [FeS] cluster and active site atoms are represented as yellow (S), orange (Fe), red (O), blue (N), green (Ni) and gray (C) spheres. Approximate courses of putative [41, 42, 43] substrate and product diffusion channels are indicated by black (e⁻), red (H⁺) and blue (H₂) arrows.

through the chain of [FeS] clusters. A possible reason for this is likely the difficulty in obtaining sufficient quantities of enzyme for advanced kinetic studies. Many hydrogenases occur in organisms that are difficult or impossible to manipulate and grow under laboratory conditions in reasonably short times, and often heterologous expression in suitable host organisms is required. Proper folding as well as [FeS] cluster and active site assembly can easily be impaired by over-expression, especially in heterologous hosts, in which important cofactors might be missing. Also, some hydrogenases are membrane bound or associated, posing problems for extraction and purification, which are already demanding in any case, owing to the oxygen sensitivity of the majority of hydrogenases. Thus, the preparation and purification of functional enzyme is very challenging and requires a know-how that bridges many disciplines of science.

Only in recent years has the scope of analytical techniques for the study of hydrogenases begun to extend beyond steady-state spectroscopy, with a number of attempts to drive the catalytic reactions by light [50, 51, 52, 53, 54, 55]. These studies show that light driven catalysis can be achieved but detailed kinetics of elementary steps in intramolecular electron transfer remain elusive. Since these processes are expected to be very fast [44], their study requires methods that allow not only for synchronized, fast triggering but also for the
tracking of individual electrons at high time resolution. As described in more
detail in Section 3.2, transient UV-Vis absorption spectroscopy combined with
ruthenium polypyridyl photosensitization is very promising in this context and
the results of a study using this method on Hyd-2 [NiFe] hydrogenase will be
discussed in Section 4.1 (Paper I).

3.1.3 Synthetic molecular catalysts

First reports on the synthesis of iron and nickel based carbonyl complexes by
Reihlen and Zimmermann [56] date back to 1928, three years before the exis-
tence of hydrogenase enzymes was postulated (Section 3.1.2). With the first
crystallographic structures of [NiFe], [FeFe] and [Fe] hydrogenases in 1995
[40], 1998 [57] and 2008 [58], respectively, the field of thiol-bridged [NiFe],
[FeFe] and [Fe] organo-metallic synthetic chemistry has experienced a renais-
sance. Inspired by the alluring proton/hydrogen interconversion capabilities of
the enzymes (Section 3.1.2), hundreds of active site models have been made
ever since, which have been covered in a number of excellent recent reviews
[21, 59, 60, 61].

Structural models aiming for maximum geometrical resemblance to the ac-
tive sites have proven valuable in particular for identifying the bridge head
hetero-atom in [FeFe] hydrogenases to be a nitrogen [62, 63]. Functional
models, conceived not only for gaining a better understanding of the cata-
sytic processes taking place inside the enzymes but also for the develop-
ment of high-performance biomimetic catalysts, have advanced our knowl-
dge about the roles of bridging and terminal hydrides [64, 65] as well as
proton shuttling functionalities [66] in proton/hydrogen interconversion. Re-
cently, a number of structurally similar iron and nickel based complexes were
reported to efficiently catalyze hydrogen oxidation [67] and/or proton reduc-
tion [68, 69, 70, 71].

Biomimetic catalysts are very promising for becoming viable alternatives to
noble metal catalysts like platinum and are capable already today of competing
with, or even outperforming most other types of molecular catalysts, such as
cobalt based organo-metallics [72, 73, 74, 75].

Figure 3.4. Synthetic di- and mononuclear iron carbonyl complexes.
The identification of the mechanistic, thermodynamic and kinetic limitations of natural hydrogenases as well as synthetic proton reduction catalysts is crucial if promising candidates are to be developed further. This is not a trivial undertaking and the major part of the work presented in this thesis deals with different aspects of the mechanistic, thermodynamic and kinetic assessment of catalysts that fall into one of three categories of iron based active site models (Figure 3.4). A dinuclear hexacarbonyl complex (Figure 3.4a) is discussed in Section 4.2 in terms of its applicability for light driven hydrogen production (Paper II). In the same section hexacoordinate mononuclear complexes (Figure 3.4b) are addressed as potential catalytically active intermediates of dinuclear complexes reacting with intermediate field ligands (Paper III). Finally, a detailed mechanistic and kinetic characterization of hydrogen production with a pentacoordinate mononuclear complex (Figure 3.4c) by cyclic voltammetry is presented in Section 4.3 (Paper IV).
3.2 Steady state and transient absorption spectroscopy

As discussed in Section 2.2.3, reduction/oxidation and protonation/deprotonation reactions lead to changes in the electronic configuration of the components of a catalytic system, which can often result in measurable differences in the absorption of electromagnetic radiation. Most relevant to the investigation of the systems in this thesis are absorption changes in the UV-Vis, near IR, IR and microwave range. The corresponding concepts and techniques will be briefly discussed in the following sections. Before that, the concepts of steady state and transient absorption spectroscopy will be explained.

In steady state absorption spectroscopy a continuous probe light irradiates the sample with spectral intensities $I_0(\lambda)$ at different wavelengths $\lambda$ (Figure 3.5a). The extent of light absorption by a given analyte A is typically reported as absorbance, Abs, or optical density, OD, defined as

$$\text{Abs}(\lambda) = \text{OD}(\lambda) = -\log\frac{I(\lambda)}{I_0(\lambda)}$$

The Lambert-Beer law

$$\text{Abs}(\lambda) = \varepsilon_A(\lambda) \cdot c_A \cdot d$$

relates absorbance to the analyte’s extinction coefficient, $\varepsilon_A$, concentration, $c_A$ and the pathlength, d, for the light passing through the sample. Extinction

\[1\{18, 19, 22, 76, 77\]
spectra visualize the absorption properties of a given analyte in terms of $\varepsilon$ vs. $\lambda$, i.e. independent on concentration and pathlength. Figure 3.5b provides an example of an extinction spectrum for a hypothetical stochiometric mixture of an oxidized electron acceptor, $A_{\text{ox}}$, and a reduced electron donor, $D_{\text{red}}$. Corresponding extinction spectra for the reduced electron acceptor, $A_{\text{red}}$, oxidized electron donor, $D_{\text{ox}}$, and the excited state of the reduced electron donor, $D_{\text{exc}}$, are given in Figure 3.5c. For simplicity all species are assumed to absorb only at one wavelength ($\lambda_A$ or $\lambda_D$). The absolute value of the extinction coefficients are identical for $A_{\text{ox}}$, $D_{\text{red}}$ and $D_{\text{ox}}$, and virtually zero for $D_{\text{exc}}$. Assuming an electron transfer reaction

$$D_{\text{exc}} + A_{\text{ox}} \rightarrow D_{\text{exc}} + A_{\text{red}} \quad (3.3)$$

will occur upon excitation of $D_{\text{ox}}$ to $D_{\text{exc}}$ with a short pulse of light, transient absorption spectroscopy can be used to monitor and characterize the kinetics of the reaction (Figure 3.5d). Here, the absorption or extinction difference of the sample at a given time after excitation with respect to the absorption or extinction before excitation is monitored and expressed in terms of $\Delta\text{Abs}(\lambda)$, $\Delta\text{OD}(\lambda)$ or $\Delta\varepsilon(\lambda)$. Transient spectra at different times after the excitation pulse are obtained by plotting one of these quantities vs. wavelength $\lambda$ (Figure 3.5e). For the example above, a negative signal would be detected at $t_0$ and $\lambda_D$, because excitation has rapidly converted $D_{\text{ox}}$ to non-absorptive $D_{\text{exc}}$. Somewhat later, e.g. at $t_{1/2}$, this so-called bleach has diminished and a positive signal has appeared at $\lambda_A$ due to the formation of $A_{\text{red}}$, which absorbs more strongly than $A_{\text{ox}}$. At $t_{\text{end}}$ all $D_{\text{exc}}$ has reacted with $A_{\text{ox}}$ and since $D_{\text{ox}}$ and $D_{\text{red}}$ are indistinguishable, now only the larger positive $A_{\text{red}}$ signal remains. When the two absorption bands at $\lambda_A$ and $\lambda_D$ are monitored with respect to time, the kinetic traces in Figure 3.5f are obtained, and the lifetimes or electron transfer rates can be determined from suitable fits to these traces.

### 3.2.1 UV-Vis spectroscopy

Typical electronic transitions (Figure 2.4) have energies which can be addressed by spectroscopy in the UV-Vis and near IR ranges. The most relevant UV-Vis absorption signals in the context of this thesis are based on the ruthenium polypyridyl photosensitizers and the hydrogenase [FeS] clusters. These photosensitizers have distinct electronic absorption spectra in their oxidized, reduced and excited states with $\Delta\varepsilon$ as high as $\pm 2 \cdot 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ compared to the ground state. In Figure 3.6a the corresponding difference extinction spectra of these states with respect to the ground state are shown for $[\text{Ru(bpy)}_3]^{2+}$. The $*[\text{Ru(bpy)}_3]^{2+}$ excited state has enhanced absorption at about 360 nm and reduced MLCT absorption around 450 nm, giving rise to corresponding positive and negative features in the difference spectrum. The broad negative feature peaking at about 610 nm is not due to diminished absorption of the excited
state but rather to excited state emission. The \([\text{Ru(bpy)}_3]^{1+}\) reduced state also shows enhanced absorption at around 360 nm and reduced absorption in the MLCT region. However, a characteristic absorption enhancement peaking at about 510 nm renders it easily distinguishable from any of the other states. In contrast to the reduced and excited states, the \([\text{Ru(bpy)}_3]^{3+}\) oxidized state is characterized by diminished absorption over almost the complete range of wavelengths.

In their oxidized states \([\text{FeS}]\) clusters have rather broad and unstructured ligand to metal charge transfer (LMCT) absorption bands, extending far into the visible range of the spectrum. These LMCT bands disappear almost completely upon reduction giving rise to broad negative features in the corresponding difference spectra with \(\Delta \varepsilon\) on the order of \(2 \sim 4 \cdot 10^3 \text{ M}^{-1} \text{ cm}^{-1}\) per Fe (Figure 3.6b). The different types of \([\text{FeS}]\) clusters occurring in [NiFe] hydrogenases should differ somewhat in their absorption properties so that the negative band in the difference spectra of [4Fe4S] clusters peaks at about 420 nm whereas the somewhat broader negative feature of [3Fe4S] clusters has its peak at roughly 490 nm.

Steady state UV-Vis absorption spectrophotometers nowadays have detection limits on the order of \(\text{OD} \approx 10^{-4}\), allowing for extraordinarily sensitive detection of analytes. With detection limits on the order of at least \(\Delta \text{OD} \approx 10^{-3}\) typical instruments for transient UV-Vis absorption spectroscopy are not quite as sensitive but are still capable of detecting concentrations as low as 0.1 \(\mu\text{M}\) and 0.5 \(\mu\text{M}\) of photosensitizer and [FeS] cluster Fe equivalents, respectively, at a pathlength of 1 cm.

Figure 3.6. Transient (difference) UV-Vis absorption spectra. a) Difference extinction spectra of \(*[\text{Ru(bpy)}_3]^{2+}\) excited, \([\text{Ru(bpy)}_3]^{1+}\) reduced and \([\text{Ru(bpy)}_3]^{3+}\) oxidized states with respect to \([\text{Ru(bpy)}_3]^{2+}\) ground state (based on [78]). b) Difference absorption spectra of reduced peptide bound [4Fe4S] and [3Fe4S] clusters with respect to corresponding oxidized clusters normalized to the respective minima (based on [79] and [80], respectively).
IR spectroscopy probes both vibrational and vibrational-rotational (vib-rot) transitions. Many of the transition energies for molecular vibrations correspond to wavenumbers on the order of several hundreds to a few thousand reciprocal centimeters (cm\(^{-1}\)) giving rise to signal-rich finger-prints in this wavenumber range. In systems containing a large number of atoms it is frequently the case that individual signals are difficult to isolate, assign and characterize. Vibrations involving multiply-bonded hetero-atoms often give much more distinct signals at higher wavenumbers, which can be more easily assigned and studied. Particularly interesting in the context of this thesis are the stretching vibrations of the cyanide (CN\(^-\)) and carbonyl (CO) ligands coordinated to the iron centers in both the hydrogenase [NiFe] active site and the synthetic di- and mononuclear iron complexes.

As schematically depicted in Figure 3.7a for a typical hexacoordinate mononuclear iron dithiolate complex, the CO stretching vibrations give rise to intense absorption bands in the range from 1900 to 2100 cm\(^{-1}\). Both cyanide and carbonyl coordinate to the metal via the carbon atom. In this way the metal-carbon bond can be stabilized by back donation of electron density from the metal d-orbitals into the empty anti-bonding \(\pi^*_\text{CN}\) or \(\pi^*_\text{CO}\) orbitals, respectively (Figure 3.7b). This \(\pi\)-backbonding at the same time destabilizes the carbon-nitrogen or carbon-oxygen bond, making the corresponding vibrational frequencies sensitive to the electron density around the metal center. Removing
the electron withdrawing Cl substituents on the dithiolate bridge as depicted in Figure 3.7a increases the electron density around the iron for the Cl-free complex and thus increases the amount of \( \pi \)-backbonding, shifting the CO stretching vibration bands to lower wavenumbers. In addition to substituents and ligands, protonation/deprotonation as well as reduction/oxidation events have an impact on the electron density around the metal centers. Whenever these processes lead to detectable shifts in the IR absorption bands, IR spectroscopy can be employed to study them.

### 3.2.3 EPR spectroscopy

As apparent from Figure 2.4, excitation, oxidation or reduction of a molecule often generates paramagnetic species with unpaired electrons. These unpaired electrons can align in different ways with respect to an external magnetic field. The different alignments, which are degenerate in the absence of a magnetic field, give rise to non-degenerate energy levels between which transitions can be induced by the absorption of electromagnetic radiation. As indicated in Figure 3.8a, the transition energy, which is equivalent to the energy splitting, \( \Delta E \), is proportional to the strength, \( B_0 \), of the external field, the Bohr magneton\(^2\), \( \mu_e \), and the g-factor, \( g \). The latter is very close to the g-factor of a free electron\(^3\) but sensitive to the environment of a particular bound electron, so that experimental g-factors can provide information about the orbital an unpaired electron resides in.

![EPR spectroscopy](image)

**Figure 3.8.** EPR spectroscopy. a) Splitting of energy levels in an external magnetic field of strength \( B_0 \) (Zeeman effect). b) Example of an isotropic EPR signal.

In contrast to the absorption spectroscopies described above, the probe in EPR spectroscopy is typically monochromatic microwave radiation and spectra are obtained by scanning the external magnetic field. Depending on the

\[
\Delta E = g \mu_e B_0
\]

\[
E = \pm \frac{1}{2} g \mu_e B_0
\]

\[
E = - \frac{1}{2} g \mu_e B_0
\]

![Graph](image)

\[
9.27400968(20) \cdot 10^{-24} \text{J T}^{-1} = 5.7883818066(38) \cdot 10^{-5} \text{eV T}^{-1}
\]

\[
2.002319
\]
microwave frequency employed, the terms X-band (8-10 GHz), Q-band (≈ 35 GHz), etc. are frequently used to describe and distinguish between particular EPR experiments. Microwave radiation, polarized perpendicular to the magnetic field, is required to detect signals from half-integer spin systems, whereas parallel polarization gives access to integer spin systems. Spectra are usually reported in terms of the first derivative of the absorption vs. magnetic field strength or g-factor (Figure 3.8b). The interaction of unpaired electrons with magnetic nuclei is termed hyperfine coupling and leads to signals consisting of multiple lines, reflecting the transition energies associated with the different possible relative positions of the electronic and nuclear spins. Depending on molecular symmetry, an unpaired electron may be characterized by up to three different g-factors (one for each of the three coordinate axes) which can lead to asymmetrically distorted EPR signals. Simple signals from systems described by three identical g-factors are called isotropic, whereas signals containing two and three different g-factors are termed axial and rhombic, respectively. Analysis of these various parameters can provide a wealth of information about paramagnetic species.
3.3 Electrochemistry

If an electrode is employed as electron donor in lieu of a chemical reductant, a catalytic system can be restricted to even fewer components than discussed above. Under these conditions only a catalyst and an acid are required. Electrochemical techniques like cyclic voltammetry (CV) thus allow to focus even more on the properties and performance of a particular catalyst, e.g. for catalytic hydrogen production.

3.3.1 Cyclic voltammetry

A typical setup for cyclic voltammetry and other kinds of electrochemical measurements is schematically depicted in Figure 3.9a. A counter, working

![Diagram of cyclic voltammetry setup](image)

**Figure 3.9.** Electrochemistry instrumentation and theoretical background of cyclic voltammetry. a) Instrumentation consisting of a sample compartment containing oxidized and reduced analyte, Ox and Red, in supporting electrolyt solution and three electrodes controlled by a potentiostat. b) Potential-time profile for linear-sweep cyclic voltammetry. The potential is varied at a constant scanrate, \( \nu \), from an initial value beyond the formal potential, \( E^{0'} \), of the Ox/Red couple to the vertex potential, \( E_{\lambda} \), and back. c) Typical cyclic voltammograms expected for reversible reduction/oxidation of the Ox/Red couple (solid gray line) and a catalytic reaction of a catalyst \( L_{\text{Cat}} \) in presence of acid (dashed gray line). The indicated time points \( t_1 - t_7 \) relate the different points of the reversible wave to the potential-time profile in b). d) Concentration profiles of species Ox with respect to the distance \( x \) from the working electrode at different times \( t_1 - t_4 \) as referring to the potential-time profile in b).

\[^4[15, 81, 82]\]
and reference electrode are immersed in an analyte solution and attached to a potentiostat controlling their potentials with respect to the reference electrode. For stable referencing, the current through the reference electrode is kept very low at all times. The counter electrode is set to whatever potential is required to sustain the current and potential at the working electrode. To prevent contamination of the analyte solution by products and components of the counter and reference electrodes, the sample compartment can be separated from these electrodes by saltbridges that prevent crossover of chemicals while allowing current to pass.

At the working electrode interconversion between oxidized (Ox) and reduced (Red) analyte can be achieved by setting the working electrode to suitable values. As mentioned earlier, an electron transfer reaction of type

$$\text{Ox} + n\cdot e^- \rightleftharpoons \text{Red}$$

(3.4)

is thermodynamically characterized by a standard potential $E^0$. In practice the formal potential

$$E^{0'} = E^0 + \frac{R \cdot T}{n \cdot F} \ln \frac{\gamma_{\text{Ox}}}{\gamma_{\text{Red}}}$$

(3.5)

is more convenient, because it implicitly includes the activity coefficients $\gamma_{\text{Ox}}$ and $\gamma_{\text{Red}}$ of species Ox and Red applying to the given experimental conditions. The formal potential straightforwardly connects the equilibrium potential $E_{eq}$ to the bulk concentrations of oxidized and reduced analyte, $[\text{Ox}]_{\text{bulk}}$ and $[\text{Red}]_{\text{bulk}}$, via the Nernst equation

$$E_{eq} = E^{0'} + \frac{R \cdot T}{n \cdot F} \ln \frac{[\text{Ox}]_{\text{bulk}}}{[\text{Red}]_{\text{bulk}}}$$

(3.6)

Kinetically, a heterogeneous one-electron transfer reaction is characterized by the exchange current

$$i_s = F \cdot A \cdot k_s \cdot [\text{Ox}]_{\text{bulk}} \cdot \exp \left[ -\frac{\alpha \cdot F \cdot (E_{eq} - E^{0'})}{R \cdot T} \right]$$

$$= F \cdot A \cdot k_s \cdot [\text{Red}]_{\text{bulk}} \cdot \exp \left[ \frac{(1 - \alpha) \cdot F \cdot (E_{eq} - E^{0'})}{R \cdot T} \right]$$

(3.7)

in which the standard rate constant, $k_s$, is a measure of the facility or sluggishness of the electron transfer step and the transfer coefficient $0 \leq \alpha \leq 1$ defines the symmetry of the activation barrier. The observed overall current, $i$, at an applied potential, $E$, and analyte concentrations, $[\text{Ox}]_{x=0}$ and $[\text{Red}]_{x=0}$, is given by the Butler-Volmer equation

$$i = F \cdot A \cdot k_s \cdot [\text{Ox}]_{x=0} \cdot \exp \left[ -\frac{\alpha \cdot F \cdot (E - E^{0'})}{R \cdot T} \right]$$

$$- F \cdot A \cdot k_s \cdot [\text{Red}]_{x=0} \cdot \exp \left[ \frac{(1 - \alpha) \cdot F \cdot (E - E^{0'})}{R \cdot T} \right]$$

(3.8)
where the first and second term correspond to the cathodic and anodic components of the current, respectively. Reformulation of Equation 3.8 using Equations 3.7 and 3.6 yields the current-overpotential equation

\[
i = i_s \cdot \frac{[\text{Ox}]_{x=0}}{[\text{Ox}]_{\text{bulk}}} \cdot \exp \left[ -\frac{\alpha \cdot F}{R \cdot T} \cdot \eta \right] - i_s \cdot \frac{[\text{Red}]_{x=0}}{[\text{Red}]_{\text{bulk}}} \cdot \exp \left[ \frac{(1 - \alpha) \cdot F}{R \cdot T} \cdot \eta \right]
\]

(3.9)

where \( \eta = E - E_{\text{eq}} \) corresponds to the applied overpotential. For sufficiently large overpotentials there will be observable currents dominated by either the cathodic or the anodic term. In cyclic voltammetry the variations of these currents with the potential applied to the working electrode are measured.

In a typical experiment on a solution of Ox, the applied potential, \( E \), is scanned from a starting value above, to a vertex potential, \( E_{\lambda} \), below the formal potential, \( E^{0'} \), and back, at a constant scanrate, \( \nu = \frac{dE}{dt} \), as shown in Figure 3.9b. Assuming that the Ox/Red couple is fully reversible, a reversible wave like the grey solid curve in Figure 3.9c is obtained. Going from the potential at \( t_1 \) to the potential at \( t_2 \) the cathodic current increases in amplitude as expected from Equation 3.9, because the cathodic term becomes non-zero and the anodic term is negligible as long as \([\text{Red}]_{x=0} \approx 0\). When the potential is scanned beyond the formal potential, \( E^{0'} \), from \( t_2 \) to \( t_3 \) the current reaches an extreme and finally goes back in amplitude from \( t_3 \) to \( t_4 \). This phenomenon is due to an effect neglected in Equation 3.9: limitation of the current by diffusion. This limitation sets in when the cathodic reaction becomes so fast that \([\text{Ox}]_{x=0} \) drops to values \( \ll [\text{Ox}]_{\text{bulk}} \). Under these conditions the observed current is determined by the flux, \( J \), of fresh Ox from the bulk towards the working electrode as determined by Fick’s First Law

\[
J = -D_{\text{Ox}} \cdot \left[ \frac{\partial [\text{Ox}]}{\partial x} \right]_{x=0}
\]

(3.10)

where \( D_{\text{Ox}} \) is the diffusion coefficient and \( [\partial [\text{Ox}] / \partial x]_{x=0} \) the concentration gradient of species Ox at the electrode surface. As shown in Figure 3.9d, the amplitude of this gradient increases up to the point where \([\text{Ox}]_{x=0} = 0\) is reached and then goes down as the concentration gradient gradually extends further into the bulk \((t_3 \rightarrow t_4)\). When the potential scan is inverted at time \( t_4 \) and vertex potential, \( E_{\lambda} \), the surface concentrations of Ox and Red are \([\text{Ox}]_{x=0} = 0\) and \([\text{Red}]_{x=0} \approx [\text{Red}]_{\text{bulk}}\), which leads to a current response from \( t_4 \) to \( t_7 \) due to the reoxidation of Red that is roughly symmetric to the curve observed during the forward scan.

For a fully reversible wave the cathodic peak has an amplitude

\[
i_{0c} = -n \cdot 0.4463 \cdot F \cdot A \cdot [\text{Ox}]_{\text{bulk}} \cdot \sqrt{D_{\text{Ox}}} \cdot \sqrt{\frac{F \cdot \nu}{R \cdot T}}
\]

(3.11)
is located at

\[ E_{pc} = E^{0'} - 1.11 \cdot \frac{R \cdot T}{n \cdot F} \]  \hspace{1cm} (3.12)

and separated by

\[ \Delta E = E_{pc} - E_{pa} \geq 2.20 \cdot \frac{R \cdot T}{n \cdot F} = \frac{56.5}{n} \text{ mV at 25 } ^\circ \text{C} \]  \hspace{1cm} (3.13)

from the anodic peak potential, \( E_{pa} \). Even though equality in Equation 3.13 strictly only applies if the forward scan extends infinitely beyond \( E_{pc} \), deviations become negligible as soon as the vertex potential is at least 90 mV beyond \( E_{pc} \). The anodic peak current, \( i_{0a} \), can be determined using the extrapolated forward scan as baseline and should be approximately equal to \( i_{0c} \). In fact, both the peak separation, \( \Delta E \), and the ratio of peak currents, \( i_{0c}/i_{0a} \), are convenient criteria for testing the reversibility of an electrode reaction. In addition, the former can be used to determine the number of transferred electrons, \( n \). Thus, cyclic voltammetry for example allows the determination of \( E^{0'} \) and \( n \) for a new catalyst in a very simple and straightforward way, and provides information about the reversibility of oxidation and reduction reactions.

However, the real beauty of cyclic voltammetry lies in the fact that homogeneous reactions such as degradation, dimerization, protonation/deprotonation or catalytic turnover that are coupled to heterogeneous electron transfer reactions lead to deviations from ideal reversible behavior of the i-E curves. From these deviations qualitative and quantitative mechanistic and kinetic information can be inferred. An interesting example is the catalytic reaction introduced in Figure 2.8, in which catalyst LCat is the analyte and will undergo repeated catalytic cycles provided that the electrode potential is sufficiently reducing and protons from a suitably strong acid are available. If the solid gray curve in Figure 3.9c is assumed to be a reversible wave for LCat in absence of acid, enabling catalysis by the addition of acid would qualitatively lead to the dashed grey curve. Due to the repeated cycling of LCat the cathodic current would become substantially enhanced and the reverse oxidation peak would disappear. In such a case, the observed catalytic peak current can generally be described by equations of the form

\[ i_p = x_e \cdot F \cdot A \cdot [LCat]_{bulk} \cdot \sqrt{D_{Lcat}} \cdot \sqrt{k_{cat}} \]  \hspace{1cm} (3.14)

where \( x_e \) is a stochiometric factor related to the number of electrons transferred per catalyst and catalytic cycle, and \( k_{cat} \) is a real or pseudo first order rate constant characterizing the rate determining step(s) under the respective experimental conditions. The enhancement factors, \( i_p/i_{0c} \), are independent of electrode surface area, \( A \), and diffusion coefficient, \( D_{LCat} \), and plots of \( i_p/i_{0c} \) vs. the square root of the acid concentration, \( \sqrt{C_{HA}^{0}} \), can be used for the identification of rate determining steps and the mechanistic and kinetic characterization of proton reduction catalysts, as discussed in more detail in Section 4.3 (Paper IV).
3.3.2 Bulk electrolysis and spectroelectrochemistry

Two more electrochemical techniques need to be introduced at this point: bulk electrolysis and spectroelectrochemistry. In contrast to cyclic voltammetry where minute amounts of analytes and substrates are converted for analytical purposes, these techniques aim for large, often quantitative, scale conversions in order to obtain detectable amounts of products. This is typically achieved by the use of large surface area working electrodes (e.g. Pt mesh or glassy carbon rod electrodes) set to constant potentials at which the desired reaction(s) take place at considerable rates. In the context of proton reduction catalysis bulk electrolysis typically describes an experiment in which the amount of hydrogen produced electrocatalytically by a certain catalyst is to be quantified by means of the charge passed through the working electrode or by direct detection e.g. with gas chromatography or with a hydrogen detector.

In spectroelectrochemistry the quantitative conversion of a compound to a different oxidation state is combined with the detection of the concomitant changes in absorption of electromagnetic radiation. Any of the spectroscopic techniques described above can in principle be applied. The absorption spectra for a compound in its various oxidation states can be obtained and used for example to construct reference difference spectra required for the interpretation of transient absorption spectra (Section 3.2, Figures 3.5 and 3.6).

For further reading on electrochemical techniques the reader is referred to corresponding literature and textbooks about electrochemistry [15, 82] and cyclic voltammetry [81].
4. Specific objectives, results and discussion

4.1 Photocatalytic hydrogen production with natural E. coli Hyd-2 [NiFe] hydrogenase (Paper I)

4.1.1 Possibilities and challenges

With the information available today about the structural and thermodynamic (i.e. static) properties of the many different states found in [NiFe] hydrogenases (Section 3.1.2 and [45]), it is about time that progress is made towards the kinetic (i.e. dynamic) characterization of the interconversion processes taking place between different states. Ultimately, only kinetic measurements can provide unambiguous information on whether and how states are linked to each other by elementary reactions, how fast these are, if competition with other reactions plays a role and which steps actually limit the overall chain of events (Sections 2.2.4 and 2.2.5).

Considering the fact that EPR has been most extensively used in the past to study not only a broad range of different active site states but also various types of [FeS] clusters in hydrogenases and other proteins [83], time resolved EPR might appear to be the most obvious choice for kinetic measurements. However, there are several drawbacks connected to this technique. In principle, the time-resolution limit in EPR measurements is dictated by the inverse of the microwave frequency employed as probe. In X-band EPR this would be \( \approx (10 \text{ GHz})^{-1} = 10^{-10} \text{ s} \). In practice about 10 ns [84] are feasible, but most state-of-the-art instruments will not be able to go to higher time resolutions than \( \mu \text{s} \) [85, 86]. Even though the overall catalytic rates observed in [NiFe] hydrogenases suggest gross limitations on \( \mu \text{s} \) to ms time scales, many interesting processes, in particular intramolecular electron transfer reactions, are likely to be significantly out of reach for time resolved EPR. Furthermore, the low temperatures usually required for decent signal-to-noise ratios make EPR experiments under more realistic, close to ambient conditions difficult if not impossible. Finally, many states cannot be observed because they are EPR silent.

Time resolved IR spectroscopy has similar issues with the availability of high time resolution instruments and inactive species. In particular, the investigation of [FeS] clusters is impossible by IR spectroscopy due to the lack of IR active groups with distinct spectral features. Also, the fact that enzymes typically come in aqueous solution poses problems to this technique, since water strongly absorbs IR light and therefore substantially impairs the signal to noise ratio making surface enhancement techniques mandatory [87, 88, 89].
Yet another alternative to purely spectroscopic studies for kinetic information are dynamic electrochemical techniques. These techniques are typically applied to hydrogenases adsorbed [90] or covalently attached [91] to an electrode. The steady state currents observed at different static or scanned potentials provide direct information about the catalytic activity of the enzyme and enable the determination of important enzyme properties like switching potentials, substrate affinities and the sensitivity towards inhibitors [92, 93]. Challenges that may be encountered with these techniques are the instability of the protein films and difficulties in controlling and determining the amount of surface attached enzyme that is actually catalytically active [94, 95, 96]. Major drawbacks in the context of characterizing elementary electron transfer steps inside the enzyme are two-fold: triggered transfer of individual electrons is not feasible with these methods and the observed currents can only address overall rates of electron transfer.

In contrast to all the techniques discussed above, UV-Vis absorption spectroscopy might not immediately come to mind as the most viable candidate for detailed kinetic and mechanistic investigations of enzymes. Distinct absorption bands in the UV are often obscured by large protein absorption backgrounds and metal co-factor LMCT or MLCT bands that often occur in the visible region of the spectrum tend to be broad and unstructured so that detailed quantifications may not be possible. Nevertheless, very small spectral changes can rather easily be picked up in UV-Vis absorption measurements. Moreover, instruments with ns time resolutions are widely available, and even sub-picosecond time resolution can be called standard today. Further, literature reports on the UV-Vis absorption properties of [3Fe4S] clusters [80, 97], [4Fe4S] clusters [79, 97] and [NiFe] active site models [98, 99] suggest that the spectroscopic distinction between these entities and between at least some of their respective different oxidation states should be possible. Therefore, time resolved UV-Vis spectroscopy is a promising technique to resolve kinetics of dynamic protein processes. In fact, this technique is particularly interesting for the study of electron transfer processes through the [FeS] clusters and from the [FeS] clusters to the active site, since these are likely to be inaccessible by EPR, IR and electrochemical techniques (vide supra).

In practice, the experimentalist attempting to apply time resolved UV-Vis spectroscopy to the study of hydrogenase enzymes faces a number of challenges in addition to the difficulty of obtaining sufficient quantities of enzyme as already discussed in Section 3.1.2. Hydrogenases are not in essence photosenzymes, so an additional photosensitizer and an electron donor are required to enable triggering of the catalytic processes by light. Electron donor, photosensitizer and catalyst need to be compatible in terms of solubility, stability, thermodynamic and kinetic properties as well as orthogonal with respect

---

1 spectral resolutions are on the order of a few nanometers (≈ few kJ mol⁻¹), sensitivities are on the order of 10⁻⁴ absorbance units
to their spectroscopic features. Hydrogenases are typically oxygen-sensitive, which makes work in an anaerobic environment mandatory. Additionally, protein aggregation in the sample solution can cause scattering of light, which can lead to challenges in obtaining a clear baseline in spectroscopic measurements. Also, reference spectra on different oxidation and protonation states of [FeS] clusters and the [NiFe] active site are at present very scarce, making the unambiguous assignment of observed signals challenging.

That interesting results can be obtained despite these difficulties will be shown in the following sections, in which the investigation of hydrogen production catalysis with ruthenium polypyridyl photosensitized *E. coli* Hyd-2 [NiFe] hydrogenase (Figure 4.1) by steady state and time resolved UV-Vis absorption spectroscopy is discussed.

### 4.1.2 Photocatalytic activity

Only in recent years have attempts been made to induce the photocatalytic processes in hydrogenase enzymes by light. Golbeck et al. in several studies report about using the photosystem I enzyme complex from *Synechococcus* as photosensitizer for light driven hydrogen production with *C. acetobutylicum*
[FeFe] hydrogenase [51, 53]. Linking these components by means of a molecular wire, “solar hydrogen-producing bionanodevices” can be obtained that supercede natural photosynthesis in terms of productive electron transfer rates [52]. Unfortunately, the investigated systems so far provide no spectroscopic handles for monitoring electron transfer processes.

King and co-workers utilize different CdTe and CdS quantum dots for hydrogenase photosensitization [100]. Hydrogen production quantum yields of up to 20% can be obtained in a self-assembled system of CdS and C. acetobutylicum [FeFe] hydrogenase [54]. The major focus of these studies is on enabling high-efficiency light driven hydrogen production and the investigation of properties related to the quantum dot materials. Only for a system comprising CdTe and T. roseopersicina [NiFe] hydrogenase has spectroscopic information about processes inside the hydrogenase been obtained [55]. The spectroscopic characterization of individual electron transfer steps is, however, difficult to achieve in such a system, since the heterogeneous nature of the photosensitizer is associated with poor control over the photosensitizer/hydrogenase interface and the number of electrons transferred to the enzyme per excitation flash. The use of [Ru(bpy)$_3$]$_2^{2+}$ as an alternative photosensitizer that donates one electron at a time and can be functionalized for site-specific covalent attachment does not appear to be an option for this particular system considering the very low quantum yield of 0.02%.

In contrast, [Ru(bpy)$_3$]$_2^{2+}$ photosensitization is feasible with E. coli Hyd-2 [NiFe] hydrogenase, and results in quantum yields of ca. 6% (Paper I). Transient UV-Vis absorption spectroscopy shows that the excited photosensitizer is first reductively quenched by ascorbate with an efficiency, $\eta_{\text{quench}} = 77\%$, and subsequently reduces hydrogenase with a (cumulative) yield, $\eta_{\text{inj}} \approx 10\%$. These results suggest an overall quantum yield for Hyd-2 reduction, $\eta_{\text{tot}} = \eta_{\text{quench}} \cdot \eta_{\text{inj}}$, of 7.7%, which is slightly higher than the quantum yield of continuous irradiation photochemical hydrogen production.

Charge recombination between reduced photosensitizer and ascorbyl radical is characterized by a second order rate constant $k_{\text{rec}} = 4 \pm 2 \cdot 10^9 \text{ M}^{-1}\text{s}^{-1}$. This is about two orders of magnitude higher than the second order rate constant $k_{\text{el}}$ for Hyd-2 reduction, which is estimated to be $\approx 4 \cdot 10^7 \text{ M}^{-1}\text{s}^{-1}$. These results appear reasonable considering the fact that productive collisions between an electron donor and Hyd-2 are restricted to a rather small fraction of the total surface area of Hyd-2. An interesting implication is that, in analogy, the rate constants for recombination between reduced Hyd-2 species and ascorbyl radical are likely to be on the order of $k_{\text{el}}$ rather than similar to $k_{\text{rec}}$. Therefore, these recombination reactions should negligibly contribute to the competition with catalytic turnover, provided that the concentrations of reduced Hyd-2 species do not significantly exceed the concentration of [Ru(bpy)$_3$]$^{1+}$. Given the corresponding initial concentrations this applies to the laser flash experiments. That this also holds for the continuous irradiation experiments is questionable without further knowledge about
the (pseudo) steady state concentrations of $[\text{Ru(bpy)}_3]^{1+}$ and possible reduced Hyd-2 species. This issue will be discussed in more detail in Section 4.2 in the context of a general summary about continuous irradiation experiments and a comparison between Hyd-2 and a synthetic catalyst.

4.1.3 Electron transfer processes inside the enzyme

![Figure 4.2](image-url)

Figure 4.2. Chemical and light induced reduction of wild-type Hyd-2. a) Scatter corrected difference spectra for the chemical reduction of Hyd-2 with hydrogen monitored by steady state UV-Vis absorption spectroscopy. In the inset the normalized absorption changes after 70 min of hydrogen exposure are compared to normalized difference spectra for individual [3Fe4S] and [4Fe4S] clusters inside protein scaffolds based on data from [80] and [79], respectively (Figure 2 in Paper I). b) Difference spectra with respect to $[\text{Ru(bpy)}_3]^{1+}$ photogenerated by 470 nm 10 ns flash excitation of a sample containing $[\text{Ru(bpy)}_3]^{2+}$ as photosensitizer, ascorbic acid/ascorbate as electron donor and Hyd-2 (Figure 4 in Paper I).

As shown in Figure 4.2a, a marked gradual decrease in UV-Vis absorption with a maximum amplitude at 420 nm is observed when Hyd-2 is exposed to hydrogen. By comparison to literature data on peptide incorporated [4Fe4S] and [3Fe4S] clusters [79, 80] these signals can be assigned to the reduction of [4Fe4S] clusters in Hyd-2. This comparison also suggests that the medial [3Fe4S] cluster of Hyd-2 is already reduced before exposure of the enzyme to hydrogen.

In Section 3.2.1 it was mentioned that the absorption changes associated with [4Fe4S] cluster reduction are expected to be on the order of $10^4$ M$^{-1}$ cm$^{-1}$ [79]. The laser flash experiments suggest that 0.5 µM electrons get transferred to Hyd-2 upon excitation of the photosensitizer. The corresponding amounts of reduced [FeS] clusters should be detectable in these experiments. The fact that no reduced [FeS] cluster signals are observed is therefore interpreted such that their accumulation to detectable quantities is prevented by electron transfer through the [FeS] clusters to the [NiFe] active site being
much faster than Hyd-2 reduction by freely diffusing reduced photosensitizer. An interesting and unexpected observation seems to corroborate this hypothesis. The spectra in Figure 4.2b show rather sharp transient signals that are centered at 470 and 530 nm, respectively, and appear on \( \mu \text{s} \) timescales, similar to Hyd-2 reduction. These signals cannot originate from the reduction of the [FeS] clusters, the photosensitizer, the electron donor or any amino acid in Hyd-2. In contrast, bands of similar shape and spectral position are associated with the reduction of Ni in spectroelectrochemical studies on certain Ni and NiFe complexes [98, 99]. Therefore, the observed signals are tentatively assigned to reduced active site Ni species, even though more detailed optical absorption reference data on the different oxidation and protonation states of [NiFe] hydrogenase active sites or suitable models thereof will be required for verification. If this assignment is correct, the observation of these signals sets an upper limit of ms or less for electron transfer reactions inside Hyd-2. This agrees with the pseudo first order rate constant, \( k'_{\text{inj}} = k_{\text{el}} \cdot [\text{Hyd-2}]_0 \), which is \( \approx 500 \, \text{s}^{-1} \) for \( [\text{Hyd-2}]_0 = 12 \, \mu \text{M} \) and \( k_{\text{el}} \approx 4 \cdot 10^7 \, \text{M}^{-1} \, \text{s}^{-1} \).

4.1.4 Covalently photosensitized systems

If the lack of transient optical signals due to reduced [FeS] cluster in the experiments above is really due to rapid inter-[FeS] cluster electron transfer, access to these reactions can only be gained by overcoming the diffusion limitation of the Hyd-2 reduction step. To achieve this, the Hyd-2 reductant needs to be attached to the enzyme. Ideally this attachment would be in a well defined place in close proximity to the [FeS] clusters.

Mass spectrometric analysis shows that a maleimide functionalized ruthenium photosensitizer can be specifically attached \textit{via} a thioether linkage (Figure 4.3a) to artificially introduced cysteines located close to the medial and distal [FeS] cluster, respectively, in two Hyd-2 variants (Figure 4.3b-c). The photosensitizer has photophysical and photochemical properties similar to [Ru(bpy)$_3$]$^{2+}$ and its attachment does not impair the light-independent hydrogen production activity of the enzyme variants. Unfortunately, the light driven hydrogen production activity of the covalently photosensitized variants is very poor and steady state and time resolved UV-Vis absorption experiments are indicative of decomposition and possibly also non-productive quenching of the photosensitizer. Further investigations will show how these issues need to be addressed but the fact that both [FeS] clusters and active site species are detectable by UV-Vis absorption techniques suggests that the overall approach of using these techniques for the detailed kinetic mapping of catalytic electron transfer processes inside Hyd-2 [NiFe] hydrogenase is promising.
Figure 4.3. Photosensitizer and Hyd-2 variants for covalent photosensitization. a) The maleimide functionalization allows for specific attachment of [Ru(dm-bpy)$_2$(MIPhen)]$^{2+}$ to cysteine thiol groups via a thioether linkage. b)-c) Hydrogenase variants with artificially introduced cysteines. The enzyme structure and color coding is the same as in Figure 3.3 with the exception of the atoms in the NiFe active site, which are all red here. Amino acid residues whose atoms are represented as blue spheres correspond to cysteines in Hyd-2 that are potentially located close to the solvent exposed surface. Amino acid residues whose atoms are represented as yellow spheres indicate the locations at which cysteines were artificially introduced. b) BJM-2 variant with an artificial cysteine close to the medial [3Fe4S] cluster. c) BJM-3 variant with an artificial cysteine close to the distal [4Fe4S] cluster.

4.2 Photocatalytic hydrogen production with a synthetic dinuclear [FeFe] hydrogenase active site model (Papers II and III)

4.2.1 Photocatalytic activity

As mentioned in Section 3.1.3, quite a share of dithiolate bridged dinuclear iron carbonyl complexes have been synthesized as models of the [FeFe] hydrogenase active site since the determination of the enzyme’s structure. The few of them that had been tested for their light driven hydrogen production performance at the time Paper II was published, disillusioned those who had been hoping for rates on the order of thousands per second as suggested by the activity of the enzyme template. Apparent turnover numbers with respect
to the bulk catalyst concentration of barely 5 at rates < 2 H₂ per catalyst per hour were the very best that could be achieved before catalysis ceased [101, 102]. [Fe₂(μ-Cl₂bdt)(CO)₆] (Cl₂bdt = 3,6-dichloro-benzene-1,2-dithiolate) promised to be an interesting candidate for breaking this record, because Felton et al. [103] had found that the closely related [Fe₂(μ-bdt)(CO)₆] (bdt = benzene-1,2-dithiolate) featured fully reversible reductive electrochemistry and Schwartz et al. [104] had shown that the introduction of electron withdrawing Cl-substituents shifted the reduction potential to a much more attractive value of -1.2 V vs. Fe⁺/⁰.

**Figure 4.4.** Ru(bpy)₃ photosensitized [Fe₂(μ-Cl₂bdt)(CO)₆] with ascorbic acid as proton source and sacrificial electron donor for continuous irradiation photochemical hydrogen production and transient absorption spectroscopy. Oxidation states and formal charges are omitted for clarity.

Indeed, photochemical hydrogen production experiments employing [Fe₂-(μ-Cl₂bdt)(CO)₆] as catalyst show that [FeFe] hydrogenase active site models can do better (Paper II). Photocatalysis at maximum rates of 3.7 H₂ per catalyst per minute corresponding to a quantum yield of approximately 3% can be achieved in presence of [Ru(bpy)₃]²⁺ as photosensitizer with ascorbic acid/ascorbate acting simultaneously as proton source and sacrificial electron donor, yielding in total up to 200 molecules of H₂ per catalyst (Figure 4.4). The turnover frequencies and numbers are strongly dependent on the photon absorption rate as suggested by experiments in which the photosensitizer concentration was varied. Likewise, they depend on pH in such a way that increasing pH values lead to higher turnover frequencies and also to accelerated ceasing of catalytic activity.
4.2.2 Mechanistic information and comparison to Hyd-2

Figure 4.5. Decay of photogenerated $[\text{Ru(bpy)}_3]^{1+}$ in absence (black dots) and presence (red dots) of 14 μM $[\text{Fe}_2(\mu-\text{Cl}_2\text{bdt})(\text{CO})_6]$. The samples contain 14 μM $[\text{Ru(bpy)}_3]\text{Cl}_2$ and 0.1 M ascorbic acid in deoxygenated 1:1 DMF:water and were excited with a 10 ns laser flash at 470 nm (Paper II, Figure 2). The data in a) and b) were obtained at pH 3.5, whereas the data in c) and d) were acquired at pH 5.5. Magenta and yellow lines correspond to linear fits, from which $k_{el}$ and $k_{rec}$ were determined in the same way as described for Hyd-2 in the supporting information of Paper I. For the inset plots in a) and c) $\Delta\varepsilon = 6700 \text{ M}^{-1} \text{cm}^{-1}$ was used for $[\text{Ru(bpy)}_3]^{1+} - [\text{Ru(bpy)}_3]^{2+}$.

Transient UV-Vis absorption experiments help to understand some of the phenomena observed during the photochemical hydrogen production experiments. The kinetics of the *$[\text{Ru(bpy)}_3]^{2+}$ excited state decay and the concomitant $[\text{Ru(bpy)}_3]^{1+}$ formation reveal that reductive quenching of *$[\text{Ru(bpy)}_3]^{2+}$ by ascorbic acid/ascorbate occurs with an efficiency close to unity at pH 5.5 but with only about 50% efficiency at pH 3.5. Ascorbic acid has a $pK_a = 4.1$ in water, which implies that the fraction of ascorbate is significantly higher at pH 5.5, where reductive quenching is more efficient. This suggests that ascorbate is the actual electron donating species rather than ascorbic acid and that the pH dependent photoactivity observed in the continuous irradiation ex-
periments is at least partly attributable to the pH sensitivity of the reductive quenching reaction.

Most importantly, the halflife for the decay of $[\text{Ru(bpy)}_3]^{1+}$ is substantially reduced in presence of $[\text{Fe}_2(\mu-\text{Cl}_2\text{bdt})(\text{CO})_6]$, which is a clear indication that electrons are being transferred to the catalyst. More detailed analyses, yet to be published, of these $[\text{Ru(bpy)}_3]^{1+}$ decay kinetics are summarized in Figure 4.5 and provide information about the rate constants for catalyst reduction and charge recombination between $[\text{Ru(bpy)}_3]^{1+}$ and ascorbyl radical. These analyses were performed in analogy to the analyses of the corresponding Hyd-2 data as discussed in Section 4.1.3 and detailed in the supporting information of Paper I. A second order rate constant $k_{\text{rec}} \approx 1-2 \cdot 10^9 \text{ M}^{-1} \text{ s}^{-1}$ is obtained for the charge recombination reaction from the inset plots in Figure 4.5a and c. $k_{\text{rec}}$ seems to be moderately pH dependent and the fact that it is lower than in the Hyd-2 system suggests that the charge recombination reaction also shows some solvent dependent behavior. From the slopes of the plots in Figure 4.5b and d, a pH independent pseudo first order rate constant $k_1' \approx 1 \cdot 10^4 \text{ s}^{-1}$ can be obtained for the electron transfer reaction from $[\text{Ru(bpy)}_3]^{1+}$ to the catalyst. The determination of $k_1'$ is somewhat impeded by the interference of a positive transient absorption signal growing in on a timescale of a few ms. Interestingly, this signal seems to grow in more rapidly at lower pH, and is therefore tentatively assigned to the protonation of a reduced catalyst species.

The (cumulative) catalyst reduction yield, $\eta_{\text{inj}}$, suggests that, by the time the interfering signal starts to occur, 70-80% of the photogenerated $[\text{Ru(bpy)}_3]^{1+}$ have already transferred an electron to the catalyst at pH 3.5 and 5.5. The overall quantum yield for electron transfer to the catalyst, $\eta_{\text{tot}} = \eta_{\text{quench}} \cdot \eta_{\text{inj}}$, thus amounts to 35 and 80% at pH 3.5 and 5.5, respectively.

![Figure 4.6. Simplified one electron catalytic scheme. The identities of the reactants and products are specified in the text. Concentrations are indicated by brackets.](image-url)
Interestingly, the quantum yields in continuous irradiation hydrogen production are lower for $[\text{Fe}_2(\mu-\text{Cl}_2\text{bdt})(\text{CO})_6]$ than for Hyd-2, even though based on the corresponding $\eta_{\text{tot}}$ one would expect the opposite ($\eta_{\text{tot}}(\text{Hyd-2}) \approx 10\%$). Figure 4.6 may help to understand this seemingly counterintuitive observation and put the results of this and the previous sections into a more general context. For simplicity reasons, catalysis is reduced to a one electron process. $[\text{Ru}(\text{bpy})_3]^{1+}$ (Ru$^{1+}$) and ascorbyl radical (Ascorbyl) are generated pairwise from $[\text{Ru}(\text{bpy})_3]^{2+}$ (Ru$^{2+}$) and ascorbate (Ascorbate), at a rate determined by the product of the rate of light absorption, $k_{\text{abs}}$, and the quenching efficiency, $\eta_{\text{quench}}$. Photogenerated Ru$^{1+}$ can feed an electron into the catalytic cycle at a rate determined by its own concentration, [Ru$^{1+}$], the concentration of oxidized catalyst, [Cat$_{\text{ox}}$], and the second order rate constant, $k_{\text{el}}$. Alternatively, it can recombine with Ascorbyl at a rate characterized by the reactant concentrations and the second order rate constant, $k_{\text{rec}}$. Assuming that all possible reduced catalyst species, Cat$_{\text{red}}$, behave in the same way, their recombination with Ascorbyl can be combined into one reaction characterized by the second order rate constant, $k_{\text{rec2}}$. Both recombination options compete with catalysis and can contribute to a decrease in the overall quantum yield. The overall extent and individual contributions depend on the particular reactant concentrations and rate constants. The rate, $v_{\text{cat}}$, of hydrogen formation and Cat$_{\text{ox}}$ regeneration depends on the nature of the rate limiting catalytic step and thus on the concentration of either Cat$_{\text{ox}}$ or Cat$_{\text{red}}$.

Interesting conclusions can be drawn from this scheme. Firstly, if catalysis is limited by catalyst reduction, recombination reactions between Cat$_{\text{red}}$ and Ascorbyl will be negligible, because Cat$_{\text{red}}$ will not accumulate to significant concentrations. Secondly, the observed hydrogen production rate is a function of the concentration of some rate limiting catalyst intermediate. The steady state concentration of such an intermediate is determined by the balance between its formation and its productive (catalysis) and unproductive (e.g. recombination) depletion reactions. This balance is, however, not uniquely defined and various possibilities exist for why the steady state concentration of the intermediate might have a certain value. Consequently, the observed hydrogen production rate or turnover frequency (TOF) is potentially ambiguous. Thirdly, since Ru$^{1+}$ and Ascorbyl are generated pairwise, the latter can accumulate as soon as electrons from Ru$^{1+}$ are incorporated into hydrogen. The extent and rate of this accumulation are determined on the one hand by the rate, $v_{\text{decomp}}$, of Ascorbyl decomposition, which depends, on the concentration of Ascorbyl, and on the other hand by the rate of all other reactions contributing to the formation or depletion of Ascorbyl.

With this in mind, there are at least two possible explanations for the unexpected relative hydrogen production quantum yields for Hyd-2 and $[\text{Fe}_2(\mu-\text{Cl}_2\text{bdt})(\text{CO})_6]$. Firstly, the pseudo steady state concentration of Ascorbyl is either higher with $[\text{Fe}_2(\mu-\text{Cl}_2\text{bdt})(\text{CO})_6]$ than with Hyd-2. Secondly, the
[Fe₂(μ-Cl₂bdt)(CO)₆] catalytic intermediates are more susceptible to recombination with Ascorbyl. The rate constants determined for catalysis with [Fe₂-(μ-Cl₂bdt)(CO)₆] and Hyd-2, respectively, actually indicate that any or both of these explanations might apply. First, k_{rec} is actually somewhat lower for [Fe₂(μ-Cl₂bdt)(CO)₆] than for Hyd-2, which would facilitate Ascorbyl accumulation. Second, k_{el} is on the order of k_{rec} for [Fe₂(μ-Cl₂bdt)(CO)₆]. In analogy to the corresponding discussion about the likely similarity of k_{el} and k_{rec2} in Hyd-2, this would imply that, compared to the catalytic intermediates of Hyd-2, the [Fe₂(μ-Cl₂bdt)(CO)₆] intermediates have an almost two orders of magnitude larger k_{rec2}. The gain in catalyst reduction rate is thus likely balanced by an evenhanded increase in recombination rate. In light of this, it is not surprising that the overall quantum yield in photochemical hydrogen production is more or less the same regardless of whether [Fe₂(μ-Cl₂bdt)(CO)₆] or Hyd-2 is used as catalyst, despite transfer of the first electron to the former being much faster.

An important lesson from all this is that the quantities and rates of hydrogen production observed in continuous irradiation experiments are the result of an intricate balance between a complex set of reactions. For the prediction and optimization of a complete photocatalytic system, this set of reactions needs to be understood at a very detailed level, including as many rate constants and concentrations as possible. Unfortunately, the assignment and characterization of catalytic intermediates has so far been impeded for the systems studied here by the fact that the catalyst concentrations cannot be further increased under catalytic conditions. Regarding Hyd-2, it is unlikely that substantially higher concentrations are feasible without protein aggregation becoming an issue. In contrast, with respect to synthetic catalysts such as [Fe₂(μ-Cl₂bdt)-(CO)₆] efforts are currently being made to establish an all-organic photocatalytic framework, in which catalyst concentrations on the order of mM are possible.

4.2.3 Catalyst and photosensitizer stability

The ideal electron donor for photochemical hydrogen production should very rapidly and irreversibly decompose as soon as it has transferred an electron to the excited photosensitizer. In contrast, maximum stability is desired for the other components of the catalytic system, such as the photosensitizer and the catalyst. In this context, steady state UV-Vis and IR spectroscopy experiments can provide interesting information. In contrast to the Hyd-2 photocatalytic system, photosensitizer stability does not seem to be an issue in the [Fe₂(μ-Cl₂bdt)(CO)₆] system. As apparent from Figure 4.7a, the absorption spectrum of [Ru(bpy)₃]²⁺ barely changes under irradiation conditions emulating the continuous irradiation hydrogen production experiments.
Figure 4.7. Photosensitizer and catalyst stability under continuous irradiation. The legends refer to irradiation times with light emulating the conditions of photochemical hydrogen production with $[\text{Fe}_2(\mu-\text{Cl}_2\text{bdt})(\text{CO})_6]$. a) Stability of $[\text{Ru(bpy)}_3]^{2+}$ in 1:1 DMF:water in presence of 0.1 M ascorbic acid. Difference spectra with respect to the spectrum before irradiation are given in the inset. b) Stability of $[\text{Fe}_2(\mu-\text{Cl}_2\text{bdt})(\text{CO})_6]$ in neat DMF.

Striking, however, are changes in the IR spectra of $[\text{Fe}_2(\mu-\text{Cl}_2\text{bdt})(\text{CO})_6]$ irradiated in neat DMF solution (Figure 4.7b). These show unmistakably that $[\text{Fe}_2(\mu-\text{Cl}_2\text{bdt})(\text{CO})_6]$ gradually converts to iron carbonyl products. The conversion seems to occur in different stages which eventually lead to catalytically inactive products at irradiation times > 100 min. Interestingly, the largest changes in the original IR spectra occur very early, on the timescale of only a few minutes. This suggests that by the time the maximum continuous irradiation photochemical hydrogen production activities are achieved (Paper II, Figure 1), a major fraction of $[\text{Fe}_2(\mu-\text{Cl}_2\text{bdt})(\text{CO})_6]$ has already been converted to one or several early products. Therefore, a number of questions arise:

- Does $[\text{Fe}_2(\mu-\text{Cl}_2\text{bdt})(\text{CO})_6]$ just go to pieces or does it react with something else?
- What does $[\text{Fe}_2(\mu-\text{Cl}_2\text{bdt})(\text{CO})_6]$ react with?
- What are the products?
- Are any of the products catalytically active?

More detailed investigations, as presented in Paper III, help to address these questions. UV-Vis and IR spectroscopy experiments suggest that $[\text{Fe}_2(\mu-\text{Cl}_2\text{bdt})(\text{CO})_6]$ does not just fall to pieces. Secondary amine species are likely reactants inducing $[\text{Fe}_2(\mu-\text{Cl}_2\text{bdt})(\text{CO})_6]$ structural changes by ligand-substitution reactions. DMF is known to be subject to thermal decomposition yielding exactly these kinds of species [105, 106] and very similar IR absorption changes are observed both in neat DMF solution and in acetonitrile solutions.
to which diethylamine (HNEt$_2$) is added (Figure 4.8). In contrast, [Fe$_2$(µ-Cl$_2$-bdt)(CO)$_6$] is stable in neat acetonitrile.

![IR spectra](image)

**Figure 4.8.** IR spectra of 5 mM [Fe$_2$(µ-Cl$_2$-bdt)(CO)$_6$] (Figures 3a and 4, Paper III). a) In deoxygenated acetonitrile (CH$_3$CN) solution at 0, 20, 60, 120 min after addition of 0.1 M HNEt$_2$. b) In deoxygenated DMF solution at 0, 30, 60, 90, 150, 180 min after sample preparation.

### 4.2.4 Bridge dependent reactivity

When it comes to the nature of the suggested ligand-substitution reactions and the corresponding products, it is important to realize that there are interesting differences in the reactivity of dinuclear dithiolate bridged iron hexacarbonyl complexes that depend on the one hand on the dithiolate bridge and on the other hand on the competing ligand. The corresponding reaction pathways and intermediates are summarized in Figure 4.9.

Alkyl-dithiolate bridged complexes (b)1 are unreactive with respect to weak donor ligands like HNEt$_2$ or DMF and only form dinuclear ligand-substituted species (b)3(L) with stronger donor ligands like cyanide (CN$^-$) or trimethyl phosphine (PMe$_3$). In contrast, aryl-dithiolate bridged (b)1 specimen appear to be able to react with both strong and weak donor ligands, yielding both di- and mononuclear products of type (b)3(L) and (b)6(L). The fragmentation into mononuclear products is likely controlled by the electronic properties of the aryl-dithiolate bridge rather than by disproportionation or the size of the chelate ring comprising the bridge and the irons. The latter is corroborated by the observation that alkyl-dithiolate bridged (b)1 complexes do not react to mononuclear (b)6(L) even if they have the same chelate ring size as an aryl-dithiolate bridged complex that does react to mononuclear (b)6(L). The hypothesis that disproportionation is of minor importance for driving fragmentation is supported by the fact that a magnetically uncoupled Fe$_1$ species, (Cl$_2$bd$t)7$(L), is observed by EPR spectroscopy when [Fe$_2$(µ-Cl$_2$-bdt)(CO)$_6$] is
exposed to DMF or HNEt$_2$. This species is likely an intermediate along pathway B between (b)$_2$(L) and (b)$_4$(L), suggesting that fragmentation precedes disproportionation.

In summary, the data suggest that structural changes in [Fe$_2$(µ-Cl$_2$bdt)(CO)$_6$] are induced by weak donor competing ligands, which lead to the formation of a potentially mononuclear Fe$^+$ (Cl$_2$bdt)7(L) species carrying both the dithiolate bridge and at least two CO ligands. Due to its lability, unfortunately, no further information can be obtained about the mass or structure of this species.

4.2.5 Implications for catalytic hydrogen production

As already mentioned, a considerable fraction of [Fe$_2$(µ-Cl$_2$bdt)(CO)$_6$] has presumably already undergone substantial structural changes by the time maximum hydrogen production activity is observed in the continuous irradiation experiments. Due to the low solubility of the catalyst in the catalytic solvent mixture, IR spectra cannot be obtained under photocatalytic conditions. However, a comparison of the corresponding IR spectra in Figure 4.7 b) with those in Figure 4.8 suggests that a considerable amount of (Cl$_2$bdt)7(L) has likely accumulated after about 10 min of irradiation. Moreover, continuous irradiation hydrogen production experiments on acetonitrile solutions of [Fe$_2$(µ-Cl$_2$bdt)(CO)$_6$] show that photocatalytic hydrogen production takes place, regardless of whether these solutions were incubated with 0.1 M HNEt$_2$ or not. A tempting interpretation of these observations is, that either both (Cl$_2$bdt)7(L) and [Fe$_2$(µ-Cl$_2$bdt)(CO)$_6$] are catalytically active, or - even more tantalizingly
that \((\text{Cl}_2\text{bdt})_{7}(L)\) is the actual catalyst and \([\text{Fe}_2(\mu-\text{Cl}_2\text{bdt})(\text{CO})_6]\) only functions as a precatalyst. If \((\text{Cl}_2\text{bdt})_{7}(L)\) really was a mononuclear species, this would imply that only one iron center is necessary for catalytic hydrogen production. That this is indeed the case will be discussed in the next chapter based on the kinetic and mechanistic study of electrocatalysis with a mononuclear iron catalyst, as presented in Paper IV.
4.3 Electrocatalytic hydrogen production with a synthetic mononuclear [FeFe] hydrogenase active site model (Paper IV)

4.3.1 Electrochemical methods as complements to spectrosocopy

Continuous irradiation photochemical hydrogen production experiments are important to test whether a catalyst actually functions in a given photocatalytic scheme or not. The amounts and rates of hydrogen production observed in such experiments are, however, strongly dependent on the rate of photon absorption and determined by the intricate interplay of the catalyst with all the other components of the catalytic system rather than the intrinsic catalytic performance of the catalyst alone. In particular the sensitive balance between the rates of catalysis, charge recombination reactions and electron donor decomposition seriously complicate the extraction of information about the exact reasons for why a given photocatalytic system performs better or worse than another. Importantly, the competition between catalysis and charge recombination for photogenerated reductant will in practice most frequently make it impossible to overcome limitations by photon flux and to drive catalysis anywhere near a catalyst’s limit. Consequently, alternative approaches are necessary whenever these intrinsic catalyst limits are of interest, as should be the case in the context of directed catalyst improvement.

In order to focus on the catalyst, it is beneficial to remove as many auxiliary components as possible from the catalytic system. Intuitively, the most obvious strategy is to narrow the range of components down to catalyst and substrate (acid) and provide electrons via an electrode. Bulk electrolysis experiments, in which catalysis is driven by reduction of the catalyst at a certain constant potential (Section 3.3.2), provide information about whether the catalyst is active or not. The dependence of the observed catalytic rates on parameters like the applied potential, the sample cell geometry (i.e. the ratio of electrode surface area to sample volume) as well as convective and diffusive mass transport, however, impede the extraction of information about properties of the catalyst that could be compared and used for directed improvement, also in this approach [15, 82].

In contrast, cyclic voltammetry can provide this kind of information. Based on the variation of current-potential responses with catalyst and substrate concentration on the one hand, and the scanrate on the other hand, both mechanistic and kinetic details can be inferred from experimental data [81]. By extension of existing theory, it is possible to distinguish between alternative catalytic pathways and to extract rate constants for individual steps of catalysis. The development of these extensions and their verification by simulations constitute one of the main results of this thesis and will be discussed in the following sections, based on Paper IV.
4.3.2 Mechanistic and kinetic information from cyclic voltammetry

Figure 4.10. a) Schematic $i_p/i_0$ vs. $\sqrt{C_{HA}}$ plot (Figure 4, Paper IV). Three zones are highlighted in different shades of gray. The expressions inside the plot refer to the definitions of $i_p/i_0$ in the respective zones. Critical concentrations for the transitions between zones are given below the plot. b) Schematic representation of the two most simple alternatives for two step catalysis (Scheme 1 b), Paper IV. For hydrogen production, $X = Y = H^+$ from acid HA.

As pointed out in Section 3.3.1, plots of enhancement factors $i_p/i_0$ vs. $\sqrt{C_{HA}}$ are a valuable tool for the analysis of cyclic voltammetry data on electrocatalytic hydrogen production. For simplicity, these plots will from now on be referred to as $i_p/i_0$ plots, where $i_0$ is used synonymously with $i_{0c}$ (Section 3.3.1). The schematic curve in Figure 4.10a shows that these plots can be divided into three zones. This behaviour relates to both existing theory [81] for catalysis along pathway A (Figure 4.10b) as well as extensions thereof for catalysis along pathway B.

In zone I, $C_{HA}$ is so low that catalyst protonation is too slow to give any significant current enhancement. The observed peak currents are therefore $\approx i_0$, yielding apparent enhancement factors of $\approx 1$. In zone II, protonation is sufficiently fast for catalysis to enhance the observed current noticeably. Since protonation limits the rate of catalysis in this zone, the apparent catalytic rate constant, $k_{cat}$ (Equation 3.14), is defined as the product of $C_{HA}$ and a second order protonation rate constant, $k_{prot}$, yielding

$$\frac{i_p}{i_0} = \frac{x_e}{n \cdot 0.446} \cdot \sqrt{\frac{R \cdot T}{F \cdot V}} \cdot \sqrt{C_{HA} \cdot k_{prot}}$$

by combination of Equations 3.11 and 3.14. The curve ideally is a straight line in this zone and $k_{prot}$ can be extracted from its slope, substituting $x_e/n = 2$ in
case of catalysis via pathway A and $x_e/n = 1$ in case of catalysis via pathway B. Mechanistically most valuable is zone III. Here, $C^0_{HA}$ is so high that protonation no longer limits catalysis and the enhancement factors reach a limiting value, independent of $C^0_{HA}$. Depending on whether the catalytic step(s) following protonation are first (pathway A) or second (pathway B) order in catalyst, these limiting enhancement factors can be defined as

$$\left( \frac{i_p}{i_0} \right)_{\text{lim},A} = \frac{2}{0.446} \cdot \sqrt{\frac{R \cdot T}{F \cdot \nu}} \cdot \sqrt{k^{(1)}_{\text{cat}}}$$

and

$$\left( \frac{i_p}{i_0} \right)_{\text{lim},B} = \frac{1}{0.446} \cdot \sqrt{\frac{R \cdot T}{F \cdot \nu}} \cdot \sqrt{\frac{4}{3} \cdot k^{(2)}_{\text{cat}} \cdot C^0_{\text{cat}}}$$

where $k^{(1)}_{\text{cat}}$ and $k^{(2)}_{\text{cat}}$ are first and second order rate constants and $C^0_{\text{cat}}$ is the catalyst concentration. Since only $(i_p/i_0)_{\text{lim},B}$ depends on $C^0_{\text{cat}}$, a distinction between pathways A and B is possible by means of experiments, in which $C^0_{\text{cat}}$ is varied. When the mechanism is known, $k^{(1)}_{\text{cat}}$ or $k^{(2)}_{\text{cat}}$ can be extracted from experimental data using Equation 4.2 or 4.3.

A further extension of the existing theory has to do with an important issue, which is often neglected. Equations 4.1 – 4.3 are strictly only applicable to conditions under which zones II and III are well defined and separated ($k_{\text{cat}}/k_{\text{prot}} \rightarrow \infty$). If this is not the case, zone III may extend far into zone II leading to artificially decreased slopes and values for $k_{\text{prot}}$ that may be underestimated by several orders of magnitude. Under such circumstances, it will be advisable to include the transition region into the data analysis. Instead of extracting $k_{\text{prot}}$ and $k_{\text{cat}}$ from the slope in zone II and the limit in zone III, separately, the complete curve can be fitted with

$$\left( \frac{i_p}{i_0} \right)_{\text{lim},A} = \frac{2}{0.446} \cdot \sqrt{\frac{R \cdot T}{F \cdot \nu}} \cdot \left[ \frac{(C^0_{HA} \cdot k_{\text{prot}})^r \cdot (k^{(1)}_{\text{cat}})^r}{(C^0_{HA} \cdot k_{\text{prot}})^r + (k^{(1)}_{\text{cat}})^r} \right]^{0.5/r}$$

or

$$\left( \frac{i_p}{i_0} \right)_{\text{lim},B} = \frac{1}{0.446} \cdot \sqrt{\frac{R \cdot T}{F \cdot \nu}} \cdot \left[ \frac{(C^0_{HA} \cdot k_{\text{prot}})^r \cdot \left( \frac{4}{3} \cdot C^0_{\text{cat}} \cdot k^{(2)}_{\text{cat}} \right)^r}{(C^0_{HA} \cdot k_{\text{prot}})^r + \left( \frac{4}{3} \cdot C^0_{\text{cat}} \cdot k^{(2)}_{\text{cat}} \right)^r} \right]^{0.5/r}$$

for pathway A and B catalysis, respectively. The exponent, $r$, is a measure of the curvature in the transition region and can be used as a quality criterion for the rate constants obtained from the fit. Only if $r$ falls into a narrow interval that depends on the catalytic pathway, can both $k_{\text{prot}}$ and $k_{\text{cat}}$ be determined from the same curve. If $r$ is too high, zone II is not well defined and $k_{\text{prot}}$ will be unreliable. The limits of $r$ for which $k_{\text{prot}}$ can be determined within an error margin of $\leq 50\%$ are:
• $r \leq 0.6$ for pathway A, if $k_1 \leq k_2$
• $r \leq 0.95$ for pathway A, if $k_2 \leq k_1$
• $r \leq 0.8$ for pathway B

Conversely, the analysis of a substantial number of simulations that are not included in Paper IV suggests that $k_{\text{cat}}$ will be determined within an error margin of $\leq 20\%$ for $r \geq 0.55$.

Interestingly, the critical concentrations for the zone transitions in Figure 4.10a imply that the zone separation can to some extent be manipulated by the choice of the experimental conditions. A decrease in scanrate, for example, leads to a broadening of zone II at the expense of zones I and III. If catalysis proceeds via pathway B, zone II can also be broadened by increasing $C_{\text{cat}}^0$. Conversely, high scanrates and low $C_{\text{cat}}^0$ can facilitate the observation of a well defined zone III.

Consequently, cyclic voltammetry is a powerful technique that allows to distinguish between catalytic mechanisms, extract rate constants for several individual steps and even evaluate their quality. All this, without prior knowledge about the catalytic mechanism. However, without thorough assessment, substantially wrong results can be obtained, if for example equations are applied to the wrong mechanism or if the impact of zone III on zone II is neglected.

4.3.3 Implications for the electrocatalytic hydrogen production with a mononuclear [FeFe] hydrogenase active site model

As an example of how the theory discussed above can be applied to real data, the pentacoordinate mononuclear complex [(bdt)Fe(PPh$_2$N$_{X}$PPh$_2$)(CO)] (Figure 4.11a) is investigated by cyclic voltammetry at two different $C_{\text{cat}}^0$, a range of different $C_{\text{HA}}^0$ and various scanrates in Paper IV. This complex is known from a previous study by Beyler et al. [107] to have a pK$_a$ of about 7.3 and to feature electrocatalytic hydrogen production activity also with weak acids in acetonitrile. The latter makes [(bdt)Fe(PPh$_2$N$_{X}$PPh$_2$)(CO)] particularly suitable for analysis, because the catalyst can only become protonated by a weak acid after it has been reduced first. This ensures that the large matrix of possible reaction pathways depicted in Figure 2.2 will in good approximation be simplified to the pathways in Figure 4.10b.

The experimental results for electrocatalytic hydrogen production from chloroacetic acid (pK$_a = 15.3$ in acetonitrile [108]), using [(bdt)Fe(PPh$_2$N$_{X}$PPh$_2$)(CO)] as catalyst, are displayed in the form of scanrate normalized $i_P/i_0$ plots in Figure 4.11b and c. Equations 4.1 – 4.5 suggest that, scanrate normalization should lead to superimposable curves in each plot, respectively. Preliminarily neglecting that this is not the case, the curves at 50 V s$^{-1}$ clearly show that limiting enhancement factors can be reached and that these depend on $C_{\text{cat}}^0$. 67
Figure 4.11. Catalyst characterization by cyclic voltammetry. a) Chemical structure of the characterized catalyst, [(bdt)Fe(PPh2NPh2)(CO)] (Ph = phenyl). b)-c) Experimental scanrate normalized $i_p/i_0$ plots for $C_0^{\text{cat}} = 50 \mu\text{M}$ (b) and $C_0^{\text{cat}} = 200 \mu\text{M}$ (c) at $\nu = 0.1$ (□), 1 (■), 10 (○) and 50 (●) V s$^{-1}$ (Figure 7, Paper IV).

In fact, they are proportional to $\sqrt{C_0^{\text{cat}}}$, which is interpreted such that catalysis proceeds via pathway B. Fits using Equation 4.5 consistently yield a $k^{(2)}_{\text{cat}}$ of $1 \cdot 10^8 \text{M}^{-1} \text{s}^{-1}$, a $k_{\text{prot}}$ on the order of $1 \cdot 10^5 \text{M}^{-1} \text{s}^{-1}$ and an $r > 1$. This suggests that the value determined for $k^{(2)}_{\text{cat}}$ is reliable, whereas $k_{\text{prot}}$ is likely substantially underestimated.

The fact that the curves in each plot are not superimposable can be explained by the protonation reaction having a very low equilibrium constant, $K_1$. The concentration of conjugate base, $A^-$ will determine the extent of competition between forward and backward proton transfer under such conditions. Because the absolute number of catalytic turnovers is larger at small scanrates, $A^-$ reaches higher concentrations and more strongly decreases the apparent protonation rate by accelerating the deprotonation reaction. Consequently, a gradual decrease in the scanrate normalized $i_p/i_0$ curves is observed for decreasing scanrates.

Simulations using $k^{(2)}_{\text{cat}} = 1 \cdot 10^8 \text{M}^{-1} \text{s}^{-1}$ consistently yield $K_1 = 10^{-2}$, provided that $k_1 \geq 5 \cdot 10^6 \text{M}^{-1} \text{s}^{-1}$. Since backward proton transfer is based on diffusion controlled collisions between protonated catalyst and $A^-$, $k_{-1}^t$ has to be $\leq 10^{10} \text{M}^{-1} \text{s}^{-1}$ in acetonitrile. The equilibrium constant thus allows
to confine $k_1$ to the range $5 \cdot 10^6 \leq k_1 \leq 10^8 \text{ M}^{-1} \text{ s}^{-1}$ and $k_{-1}$ to the interval $5 \cdot 10^8 \leq k_{-1} \leq 10^{10} \text{ M}^{-1} \text{ s}^{-1}$. In addition, the pK$_a$ value for the reduced catalyst can be estimated to be 13.3.

As mentioned above, Beyler et al. [107] report a pK$_{a,1}$ of 7.3 for unreduced [(bdt)Fe(P$_{Ph_2}$N$^X$P$_{Ph_2}$)(CO)]. Moreover, they observe a catalytic wave at a peak potential, $E_p$, of -1.32 V vs. Fc$^{+/0}$. If this wave is interpreted as originating from catalysis by protonated [(bdt)Fe(P$_{Ph_2}$N$^X$P$_{Ph_2}$)(CO)], $E_p$ can be assumed to be identical or very close to the formal reduction potential of protonated [(bdt)Fe(P$_{Ph_2}$N$^X$P$_{Ph_2}$)(CO)]. A thermodynamic cycle analysis according to Figure 2.5 with pK$_{a,1} = 7.3$, $E_1^0 = -1.66$ V vs. Fc$^{+/0}$, pK$_{a,2} = 13.3$ and $E_2^0 = -1.32$ V vs. Fc$^{+/0}$ agrees well with Equation 2.13 corroborating the results obtained from the analysis above.

Studies with other acids are currently in preparation in order to acquire further experimental evidence for the correctness of the interpretation of the observed scanrate dependence. Possibly, such studies can also provide information about how $k_1$ and $k_{-1}$ are affected by a change in acid pK$_a$. Depending on whether or not forward and backward proton transfer are affected to the same extent by the acid pK$_a$, conclusions could be drawn about whether this kind of iron complex behaves like an Eigen acid [109]. This would be interesting from a fundamental science perspective. In the context of catalyst development, it will be important to verify that this kind of analysis can be applied to a broad range of different catalysts. If this is the case, the rate constants accessible by this approach would be objective parameters of catalyst performance that could be compared and utilized for catalyst improvement.
5. Summary and outlook

Ruthenium polypyridyl photosensitization enables light driven hydrogen production with both *E. coli* Hyd-2 [NiFe] hydrogenase and a synthetic dinuclear [FeFe] hydrogenase active site model in aqueous solution close to neutral pH. The quantum yields for photocatalytic hydrogen production under continuous irradiation are on the order of several percent. The turnover frequencies (TOFs) and turnover numbers (TONs) depend on the rate of photon absorption suggesting that these photocatalytic systems likely operate far away from the limits imposed by the catalysts themselves. Electron transfer to the catalyst and competition of productive catalytic reactions with charge recombination or decomposition reactions probably have a strong impact on the overall photocatalytic performance. The quantum yields, TOFs and TONs provide no information about the individual contributions of these processes and are therefore potentially ambiguous. The disambiguation of the information contained in these parameters is crucial for directed development and improvement of catalysts and requires more detailed kinetic and mechanistic investigations.

Transient UV-Vis absorption spectroscopy reveals that photocatalytic turnover in the hydrogenase is likely limited by the rate of electron injection, possibly in combination with catalytic steps taking place inside the active site, when a ruthenium polypyridyl photosensitizer is supplied as a freely diffusing species. Electron transfer through the [FeS] clusters appears to be much faster than Hyd-2 reduction, preventing the build-up of any observable quantities of transiently reduced [FeS] cluster species. Since clear signals for [FeS] clusters reduction can be obtained when Hyd-2 is chemically reduced by H₂, the study of electron transfer processes between the [FeS] clusters with transient UV-Vis absorption spectroscopy should be feasible but probably requires facilitation of electron injection by covalent photosensitization of the enzyme at engineered sites close to the [FeS] clusters. Corresponding enzyme variants and functionalized ruthenium polypyridyl photosensitizers have been prepared, but quenching and aggregation issues need to be resolved before further progress towards the study of electron transfer process inside the enzyme can be made.

Similar experiments with the synthetic dinuclear iron based catalyst demonstrate efficient transfer of a first electron to the catalyst. In this system, the decreased overall quantum yield is likely due to charge recombination between the oxidized electron donor and one or several reduced catalytic intermediates. More details on the catalytic mechanism can, however, not be obtained because the experimentally accessible catalyst concentrations preclude the formation of analyzable quantities of these catalytic intermediates. This
issue is currently addressed by the development of an all-organic photocatalytic framework that allows for experiments in which the accessible catalyst concentration range is substantially extended.

Studies on the reactivity and stability of this kind of catalyst indicate that the presence of weak donor ligands such as secondary amines or DMF can induce the formation of mononuclear iron species. This reactivity requires an aromatic dithiolate bridge and is not observed in corresponding alkyl-dithiolate bridged compounds. Potentially mononuclear intermediates feature light driven hydrogen production activity, suggesting that one iron center is sufficient for proton reduction catalysis.

Investigations of a pentacoordinate mononuclear iron catalyst by cyclic voltammetry in acetonitrile solution provide better control over catalyst stability than the photocatalytic studies and eliminate issues with charge recombination reactions involving oxidized electron donor species. As verified by simulations, it is possible to extend the existing theory about homogeneous multi-electron catalysis coupled to heterogeneous electron transfer at an electrode in such a way that different catalytic mechanisms can be distinguished and rate constants for individual catalytic steps can be extracted from cyclic voltammetry data. Moreover, the curvatures of $i_p/i_0$ plots provide information about the quality of these extracted rate constants. The experimental data for the studied catalyst suggest that, at low to intermediate chloroacetic acid concentrations, catalysis is limited by a protonation equilibrium and that the deprotonation reaction needs to be accounted for in the analysis. The scanrate dependence of the $i_p/i_0$ plots allows to determine the protonation equilibrium constant and to set limits on the protonation and deprotonation rate constants. At higher acid concentrations catalysis no longer depends on acid concentration and instead becomes limited by a catalytic step that is second order in catalyst concentration, implying that H–H bond formation likely involves two catalyst molecules. Importantly, even the second order rate constant for this catalytic step can be reliably determined as suggested by the strong curvatures and clearly defined plateaus of the $i_p/i_0$ plots. Experiments with various other acids and catalysts are currently under progress and in planning to verify these results and test the versatility of the approach.

Overall, these results can hopefully inspire and guide further studies on similar systems and facilitate the assessment and development of better catalysts so that hydrogen will become a major energy carrier already in the near future.
Mekanistiska och kinetiska studier av foto- och elektrokatalytisk vätskagasproduktion med naturliga och syntetiska molekylära katalysatorer

“Vatten, delat i sina beståndsdelar genom elektricitet. Ja, mina vänner, jag tror att vattnet en dag blir använt som bränsle. En dag kommer fartygens och lokomotivens ångpannor att drivas med vätte och syre i stället för kol. Så länge vår jord är bebodd, ska dess invånare aldrig lida brist på vare sig ljus eller värme. Vatten är framtidens kol.”

Så skrev den franska författaren Jules Verne år 1874 i sin fängslande roman *Den hemlighetsfulla ön* [110]. Nästan 150 år senare verkar vi fortfarande inte vara mycket närmare denna vision än då. Det är nedslående att behöva erkänna detta, fram för allt med tanke på de allt mer uppenbara miljöpåverknignar som människans användande av kol och andra fossila bränslen innebär. Men vad är det egentligen som gör det så svårt för oss att kunna använda oss av vatten på det sätt som Jules Verne föreställde sig? Svaret på denna fråga har att göra med att klyvningen av vatten i sina beståndsdelar, vätgas och syrgas, inte sker av sig självt om bara man tillför vatten (elektrisk) energi. Hjälpmedel behövs, som underlätta de underliggande kemiska processerna. Dessa hjälpmedel kallas för katalysatorer, och i samband med vätskagasproduktion kan man föreställa sig en katalysator som någon slags tratt. På samma sätt som en tratt kan användas för att blanda till exempel vatten och äpplejuice i en flaska, används en vätskakatalysator för att fånga in och slå ihop vägarsens beståndsdelar, två protoner och två elektroner, för att bilda vätska (Figur 5.1).

En bra tratt eller katalysator skall vara billig, stabil, ofarlig1 och kunna blanda både bra och snabbt2. Platina är en väldigt bra vätskakatalysator och används därför mest inom industrin idag. Men med hänsyn till hur dyr och sällsynt platina är, är det uppenbart att det behövs alternativ om man vill producera vätska på stor skala. I naturen finns det enzym som är utomordentligt bra vätskakatalysatorer, så kallade hydrogenaser. De använder sig av järn eller nickelför att bilda vätska och dessa ämnen finns det riktigt av här på jorden. Om man vill utveckla gångbara alternativ till platinakatalysatorer är det därför mycket intressant att ta reda på hur bra och på vilket sätt de naturliga katalysatorerna, samt konstgjorda modeller av dessa, fungerar.

---

1 det verkar inte vara någon särskilt bra idé att blanda vatten och äpplejuice i en blytratt  
2 vi vill nog inte behöva vänta i två dagar på vår vatten-äpplejuice blandning om vi är törstiga
Figur 5.1. Vätgaskatalysatorer slår ihop elektroter (e\(^-\)) och protoner (H\(^+\)) för att bilda vätgas (H\(_2\)). a) En serie av schematiska “tratt”-vätgaskatalysatorer som skiljer sig åt med både hänsyn till utseende och hur snabbt de bildar vätgas. b) Molekylstruktur på en hydrogenas. Protoner och elektroner flyttar in i hydrogenasens kärna där de släs ihop till vätgas i det så kallade aktiva sätet. c) Molekylstruktur på en model av hydrogenasens aktiva sätet.

För att kunna göra det, behöver man använda sig av olika mätmetoder, som dels berättar om hur bra en viss katalysator är jämfört med en annan och dessutom ger information om varför den ena är bättre än den andra. Figur 5.1a visar ett enkelt exempel för att illustrera några viktiga aspekter i samband med sådana mätningar och jämförelser. Om man endast mäter bredden på nedre delen av trattarna I och I*, så drar man troligtvis slutsatsen att denna bredd inte har någon särskilt stor effekt på hur snabbt vätgas (H\(_2\)) blandas ihop från protoner (H\(^+\)) och elektroner (e\(^-\)), och en jämförelse med tratt II verkar bestyrka denna hypotes. Tittar man inte på tratt II*, går man miste om att effekten av bredden på trattens nedre del hänger tätt ihop med vinkeln på trattens övre del. I trattarna I och I* är det i huvudsak den övre öppningen som avgör prestandan och det hjälper därför inte mycket till att vidga den nedre delen. I motsats till detta, fungerar infångandet av elektroner och protoner mycket bättre med trattarna II och II*, som har en större övre öppning. Här är det trattens nedre
öppning som avgör prestandan. För att kunna dra korrekta slutsatser, är det alltså oerhört viktigt att veta vilken egenskap som är avgörande för hur bra eller dålig en viss katalysator är. Dessutom är det viktigt att endast jämföra katalysatorer som begränsas av samma egenskap(er). I samband med kemiska reaktioner och katalys benämns sådana begränsningar vanligtvis (hastighets) begränsande steg, och identifieringen samt kategoriseringen av dess utgör kärnan av denna avhandling.

Avhandlingen omfattar studier av vägtagprodukton katalyserad av ett hydrogenas (Figur 5.1b) eller av ett antal olika konstgjorda modeller (Figur 5.1c) och drivkraften bakom dessa processer har kommit från antingen ljus eller av elektricitet. Ett resultat av dessa undersökningar är att både hydrogenaset och en av modellerna kan drivas med ljus. Med hydrogenaset är det infångandet av elektroner som begränsar vägtagproduktonen. Detta gör det svårt att få tydliga resultat med de tillgängliga mätmetoderna. För att få tag i fler detaljer om vad som händer inne i hydrogenaset behövs det därför ytterligare förbättringar av metoder och experimentella betingelser, vilket är en sak man ska syssla med i framtiden. Problemen med den syntetiska modellen är lite annorlunda. Den är ganska duktig på att fånga in elektroner, men tyvärr verkar den tappar bort en stor del av dem under katalysens gång. Det är alltså som om tratten hade ett eller flera hål som gör att det läcker åt fel riktning. Ett intressant framtida projekt är att identifiera och "täppa igen" dessa hål. En annan utmaning med den syntetiska modellen är att den inte är särskilt stabil och därmed slutar fungera efter en viss tid. Mer detaljerade undersökningar avslöjade att olika varianter av dessa modeller bryts ned på olika sätt och det tyder på att vissa av nedbrytningsprodukterna också är aktiva katalysatorer. En av dessa nedbrytningsprodukter visar sig vara en väldigt aktiv katalysator när den drivas med hjälp av elektricitet och frödjupade studier av den ger viktig information om mekanismen bakom den katalytiska processen. Till exempel hur snabbt protoner fångas upp och att två stycken katalysatorer behöver samarbeta för att bilda vägtag.

Sammantaget kan resultaten från denna avhandling förhoppningsvis bidra till att det inte dröjer ytterligare 150 år, innan Jule Verne’s vision går i uppfyllelse, så att vi människor kan fortsätta använda den energi vi behöver, men utan att samtidigt förstöra miljön på det sättet som vi gör idag.
Mechanistische und kinetische Untersuchungen der photo- und elektrokatalytischen Wasserstoffherstellung mit natürlichen und synthetischen molekularen Katalysatoren

“Das Wasser ist die Kohle der Zukunft. Die Energie von morgen ist Wasser, das durch elektrischen Strom zerlegt worden ist. Die so zerlegten Elemente des Wassers, Wasserstoff und Sauerstoff, werden auf unabsehbare Zeit hinaus die Energieversorgung der Erde sichern.”


Ein guter Trichter oder Katalysator zeichnet sich dadurch aus, dass er billig, stabil und ungefährlich als auch gut und schnell mischen kann. Platin ist ein sehr guter Wasserstoffkatalysator und wird daher heutzutage auch am

3 einen Katalysator aus Blei zum Mischen von Wasser und Apfelsaft zu benutzen ist wohl keine so gute Idee
4 wir wollen ja nicht zwei Tage lang auf unsere Apfelschorle warten, wenn wir durstig sind

ich illustrieren lassen. Misst man lediglich die Breite der unteren Öffnung der Trichter I und I*, zieht man vermutlich den Schluss, dass diese Breite keinen besonders großen Einfluss darauf hat, wie schnell Wasserstoff (H₂) aus Protonen (H⁺) und Elektronen (e⁻) gebildet wird, und ein Vergleich mit Trichter II scheint diese Hypothese zu untermauern. Sieht man sich nicht auch Trichter II* an, dann entgeht einem, dass der Einfluss der Breite der unteren Trichteröffnung mit dem Öffnungswinkel der oberen Trichteröffnung zusammenhängt. In den Trichtern I und I* ist es im Wesentlichen die obere Öffnung, die die Geschwindigkeit der Wasserstoffbildung bestimmt, weshalb es nicht viel hilft, die untere Öffnung breiter zu machen. Im Gegensatz dazu ist das Einfangen von Elektronen und Protonen sehr viel leichter mit den Trichtern II und II*, die eine größere obere Öffnung haben. Hier bestimmt die Breite der unteren Öffnung, wie schnell Wasserstoff gebildet wird. Deshalb ist es unglaublich wichtig zu wissen, welche Eigenschaft dafür verantwortlich ist, wie gut oder schlecht ein bestimmter Katalysator ist. Außerdem sollte man, um falsche Schlussfolgerungen zu vermeiden, nur Katalysatoren miteinander vergleichen, die durch die selbe(n) Eigenschaft(en) in ihre Wasserstoffbildungskapazität eingeschränkt werden. Im Zusammenhang mit chemischen Reaktionen und Katalyse nennt man solche Einschränkungen üblicherweise geschwindigkeitsbestimmende Schritte, und die Identifikation und Charakterisierung solcher Schritte bildet den Kern dieser Doktorarbeit.

katalytischen Prozesses, beispielsweise, wie schnell Protonen vom Katalysator eingefangen werden oder, dass zwei Katalysatoren zusammenarbeiten müssen, um Wasserstoff zu bilden.

Insgesamt können die Ergebnisse dieser Doktorarbeit hoffentlich dazu beitragen, dass es keine weiteren 150 Jahre dauert ehe Jule Verne’s Vision in Erfüllung geht, sodass wir Menschen weiterhin die Energimengen verbrauchen können, die wir benötigen, allerdings ohne dabei unsere Umwelt weiterhin derart zu zerstören, wie es heute der Fall ist.
I would like to thank...

...my main supervisor, Leif Hammarström, for taking the risk of converting a biologist into a physical chemist. I very much hope that you don’t regret it and that I haven’t disappointed you.

...my deputy supervisor, Reiner Lomoth, for his guidance during my thesis work. I so much appreciate all the scientific discussions we have had and very much hope that there will be more of them to come.

...my former deputy supervisor, Jan Davidsson, for his help with regard to my teaching duties and for the awesome KemFys summer parties.

...Susanne Karlsson, for taking care of me when I first arrived at the Ångström Laboratories and for being a role model to me.

...Michele Orlandi, for acting as my scientific tutor during the first part of my PhD studies and for being a really dear friend.

...Prof. Fraser Armstrong and Bonnie Murphy, for their good collaboration on the hydrogenase project.

...the opponent for my thesis defence, Prof. Clifford Kubiak, for taking on the responsibility of scrutinizing my thesis.

...Starla Glover and Todd Markle for proofreading my thesis.

...Erik Göransson and Jonas Petersson for proofreading the Swedish summary to my thesis.

...the C F Liljewalchs Stiftelse for a generous travel stipend that allowed me to attend a conference in Canada.

...Sven Johansson, for always lending a hand.

...Susanne Söderberg, Åsa Furberg, Maria Leijon, Inger Carlberg, Laila Fältman and Jessica Stålberg for all their help with so many administrative issues.

...all my colleagues and friends at work, for making the Ångström Laboratories such a special place.

...all my friends in different places of the world, for being with me despite the long distance between us.
...Terry Pratchett and Ephraim Kishon for keeping me laughing.

...my wife’s family and my own family, for their immeasurable support during all this time. Danke, für alles, was ihr für mich getan habt. Ohne euch wäre das hier nicht möglich!

...my wife, Kathi, and my daughter, Maya Sofia, for being the best wife and daughter I could ever imagine. Ich habe euch unglaublich fest lieb und bin einfach nur dankbar für jeden neuen Tag, den ich mit euch verbringen darf.
References


[31] A. Juris, V. Balzani, F. Barigelletti, S. Campagna, P. Belser, and A. von...


2005.


J. Verne, Den hemlighetsfulla ön. 1874.

J. Verne, Die geheimnisvolle Insel. 1874.
A doctoral dissertation from the Faculty of Science and Technology, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology.