IR and Raman spectroscopy

Peter Hildebrandt

Content

1. Basics of vibrational spectroscopy

2. Special approaches

3. Time-resolved methods

IR and Raman spectroscopy - vibrational spectroscopy:

$\Rightarrow$ *probing well-defined vibrations of atoms within a molecule*


Siebert &
Hildebrandt, 2007)
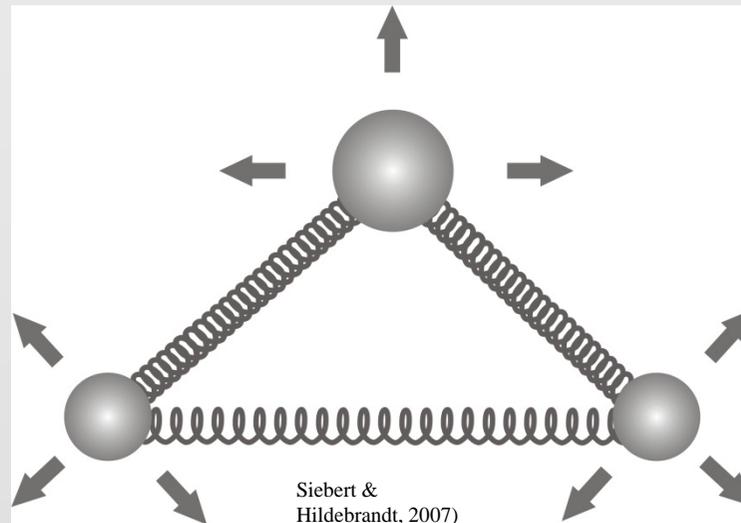
Motions of the atoms in a molecule are not random!

$\Rightarrow$ well-defined number of vibrational degrees of freedom

$\Rightarrow$ 3N-6 and 3N-5 for non-linear and linear molecules, respectively

What controls the molecular vibrations and how are they characterized?

Definition of the molecular vibrations: ⇒ *Eigenwert problem* ⇒ ***normal modes***

for a non-linear N-atomic molecule: ⇒ 3N-6 normal modes

example: benzene
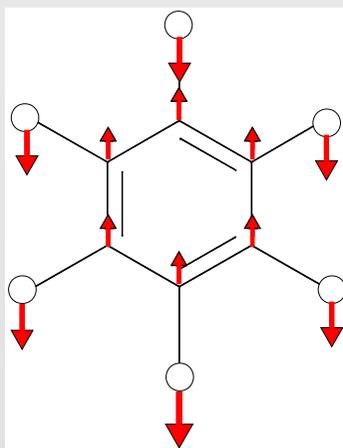
in each **normal mode**:
all atoms vibrate with the same frequency but different amplitudes

$\nu_1(A_{1g})$: 3062 cm^{-1} $\nu_{14}(E_{1u})$: 1037 cm^{-1}

Thus:
➢   normal modes are characterised by frequencies (given in cm^{-1})
➢   and the extent by which individual atoms (or coordinates) are involved.

Determination of normal modes – a problem of classical physics

Approach:
- ❖ Point masses connected with springs
- ❖ Harmonic motion

Siebert &
Hildebrandt, 2007)
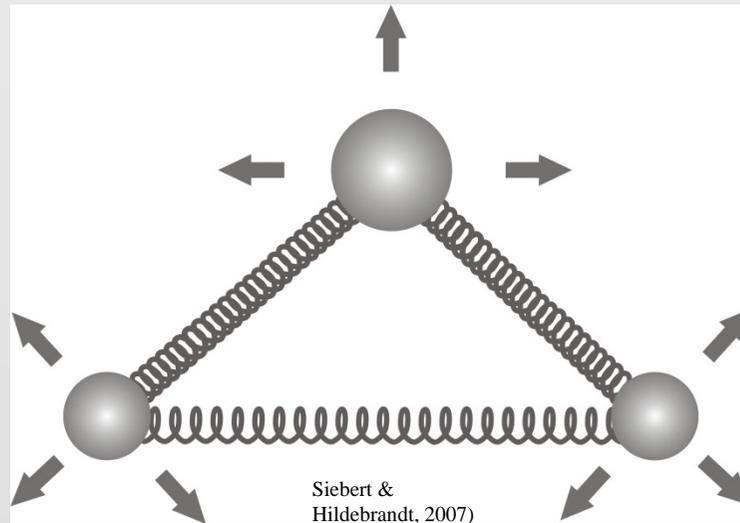
Crucial parameters determining the normal modes:

- Geometry of the molecule (spatial arrangement of the spheres)

- strength of the springs (force constants)

☞ very sensitive fingerprint of the molecular structure

A. Describing the movement of the atoms in terms of mass-weighted Cartesian displacement coordinates

e.g. $\Delta x_i = x_i - a_i$ (i: all atoms)

Δx_i

Δy_i

Δz_i

Siebert &
Hildebrandt, 2007)

Mass-weighted Cartesian displacement coordinates

$$q_1 = \sqrt{m_1}\Delta x_1 \qquad q_2 = \sqrt{m_1}\Delta y_1 \qquad q_3 = \sqrt{m_1}\Delta z_1 \qquad q_4 = \sqrt{m_2}\Delta x_2 \qquad \text{etc.}$$
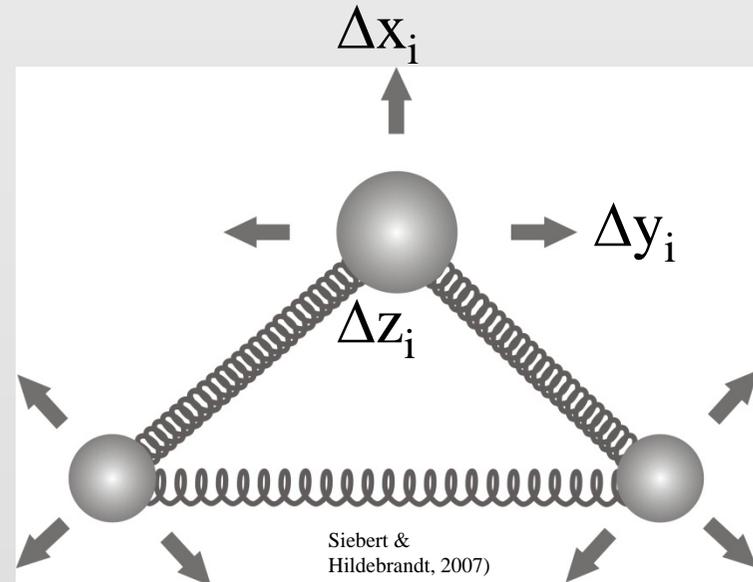
B. Expressing the kinetic and potential energy

kinetic energy T

$$T = \frac{1}{2} \sum_{i=1}^{3N} \dot{q}_i^2$$

potential energy V for small displacements (harmonic approximation)

$$V \cong \frac{1}{2} \sum_{i,j=1}^{3N} f_{ij} q_i q_j$$

Total energy:

$$E = T + V$$

Newton´s equation of motion

$$0 = \ddot{q}_j + \sum_{i,j=1}^{3N} f_{ij} q_i$$

C. Solving the eigenwert problem

Set of 3N linear second-order differential equations

$$q_i = A_i \cos\left(\lambda^{1/2} t + \varepsilon\right) \qquad 0 = \sum_{i=1}^{3N} f_{ij} A_i - A_j \lambda$$

Secular determinant:

$$0 = \begin{vmatrix} f_{11}-\lambda & f_{12} & f_{13} & ..... & f_{1,3N} \\ f_{21} & f_{22}-\lambda & f_{23} & ..... & f_{2,3N} \\ f_{31} & f_{23} & f_{33}-\lambda & ..... & f_{3,3N} \\ ... & & & & \\ f_{3N,1} & f_{3N,2} & f_{3N,3} & ..... & f_{3N,3N}-\lambda \end{vmatrix}$$

3N solutions, 3N frequencies

Removal of 6 solutions für $f_{ij}=0$ (translation, rotation)

3N-6 non-zero solutions for frequencies

D. Coordinate transformation:

Cartesian coordinates to *normal coordinates*

$$Q_k = \sum_{i=1}^{3N} l_{ki} x_i$$

One normal mode accounts for one normal mode – unique relationship!

Simplifies the mathematical and theoretical treatment of molecular vibrations but is not illustrative!

Intuitive coordinates – *internal coordinates*:

$$S_t = \sum_{i=1}^{3N} B_{ti} \Delta x_i$$

❖ Stretching

❖ Bending

❖ Out-of-plane deformation

❖ Torsion

E. Solving the eigenwert problem

Constructing the G- und F-Matrix and inserting into Newton´s equation of motion

$$G_{t,t'} = \sum_{\alpha=1}^{N} \frac{1}{m_\alpha} s_{t,\alpha} s_{t`,\alpha} \qquad\Longrightarrow\qquad \left| G - F^{-1}\lambda \right| = 0$$

Example: three-atomic molecule

$$G = \begin{vmatrix} G_{11} & G_{12} & G_{13} \\ G_{21} & G_{22} & G_{23} \\ G_{31} & G_{32} & G_{33} \end{vmatrix}$$
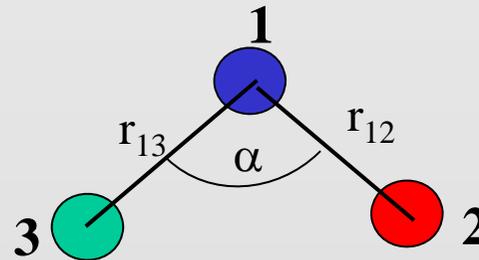
G-Matrix known, if the structure is known

$$F = \begin{vmatrix} F_{11} & F_{12} & F_{13} \\ F_{21} & F_{22} & F_{23} \\ F_{31} & F_{32} & F_{33} \end{vmatrix}$$

**Solving the FG-matrix
leads to (3N-6) λ solutions**

F-Matrix *a priori* unknown

Main problem:

How to determine the force constant matrix?

→ Quantum chemical calculations

Objectives of the theoretical treatment of the vibrational problem:

→ Calculating vibrational spectra rather than analysing vibrational spectra

→ Comparing the experimental vibrational spectra with spectra calculated for different structures

Quantum chemical methods

→ Optimizing the geometry for a molecule

→ Calculating the force field

→ Solving the normal mode problem
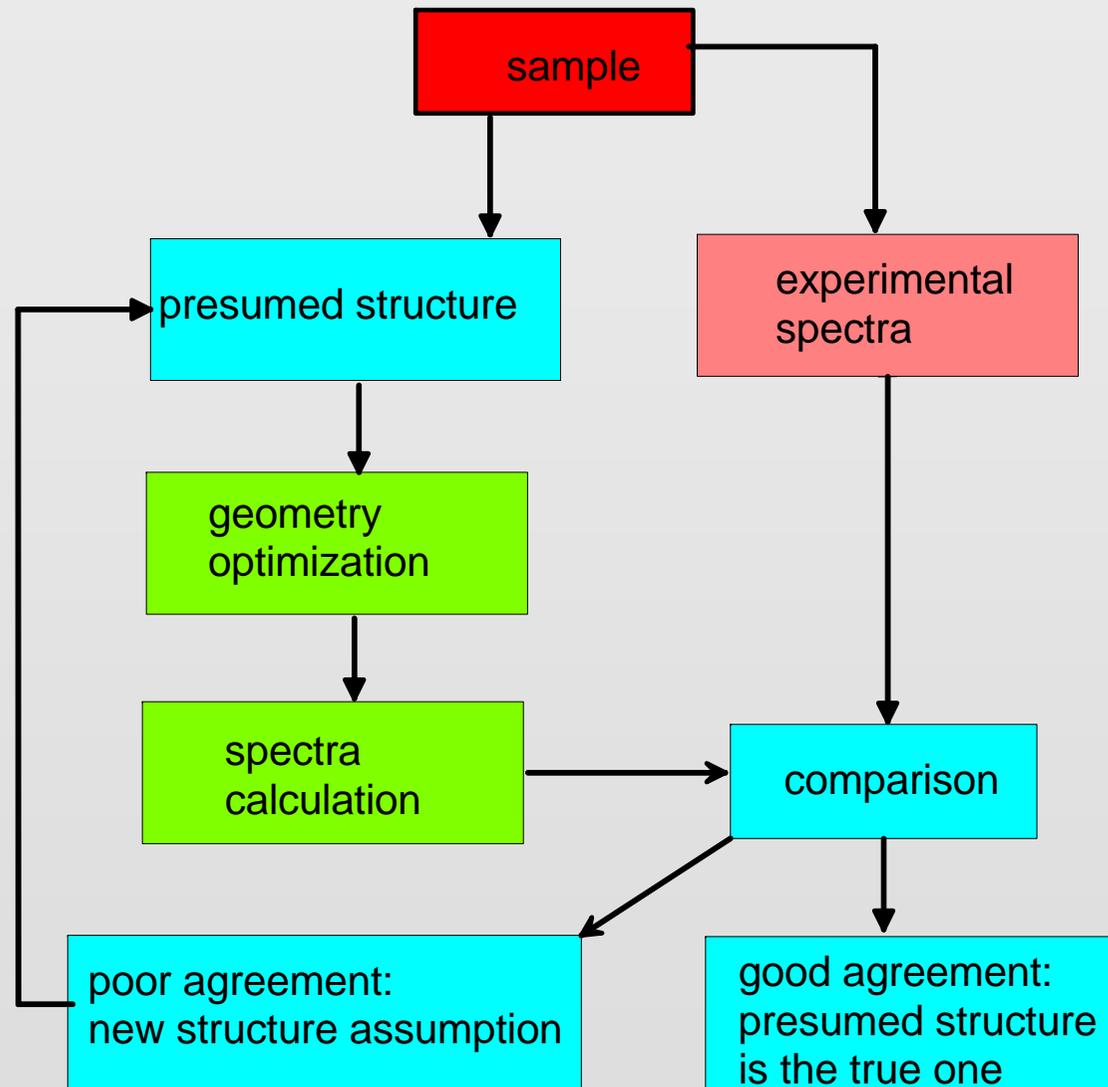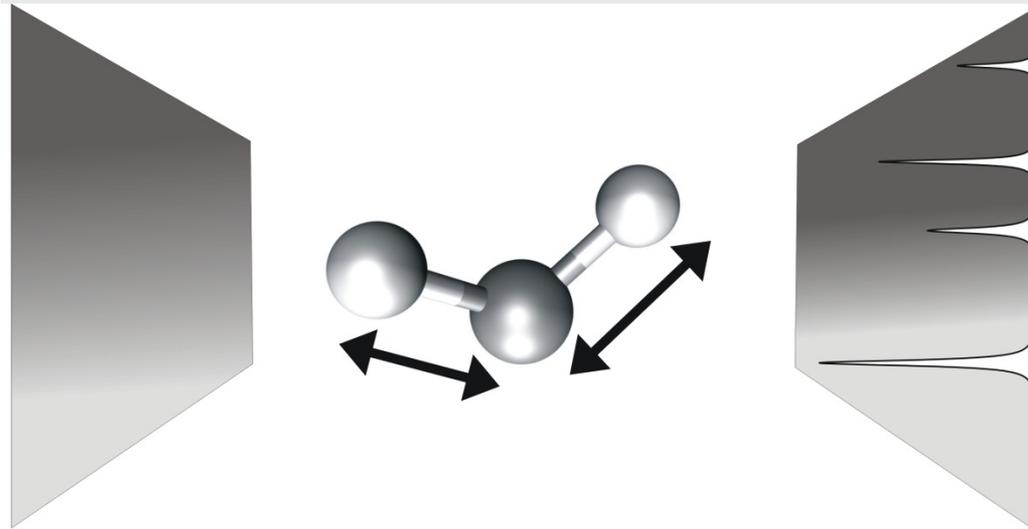
sample

presumed structure

experimental spectra

geometry optimization

spectra calculation

comparison

poor agreement: new structure assumption

good agreement: presumed structure is the true one

Infrared spectroscopy
direct absorption of photons

Raman spectroscopy
inelastic scattering of photons

ν_0

ν_{Ra}

ν_0

Infrared spectroscopy
direct absorption of photons

Raman spectroscopy
inelastic scattering of photons

white
light

white light
$- \nu_n$

ν_0 $\nu_0 - \nu_n$

$h \nu_n$
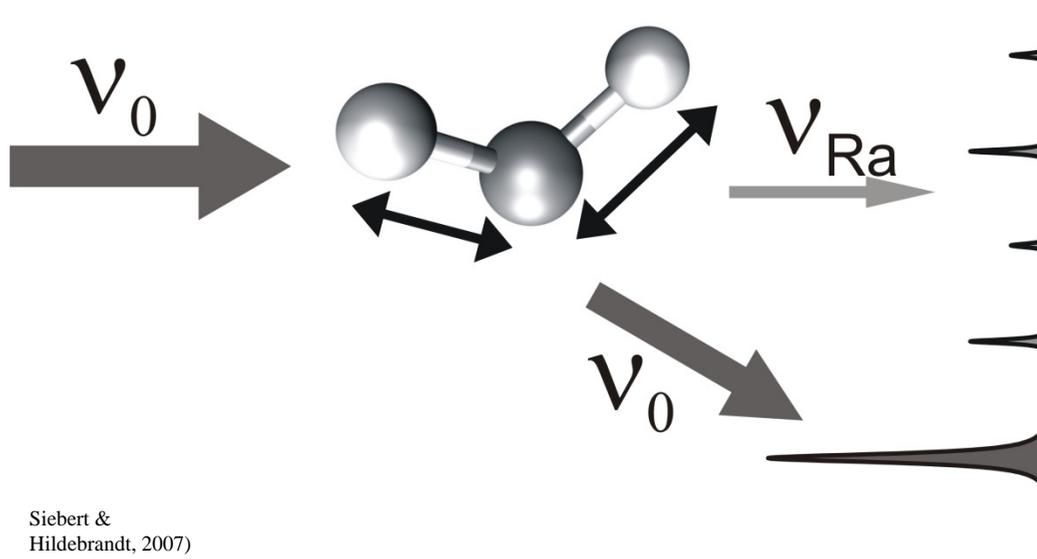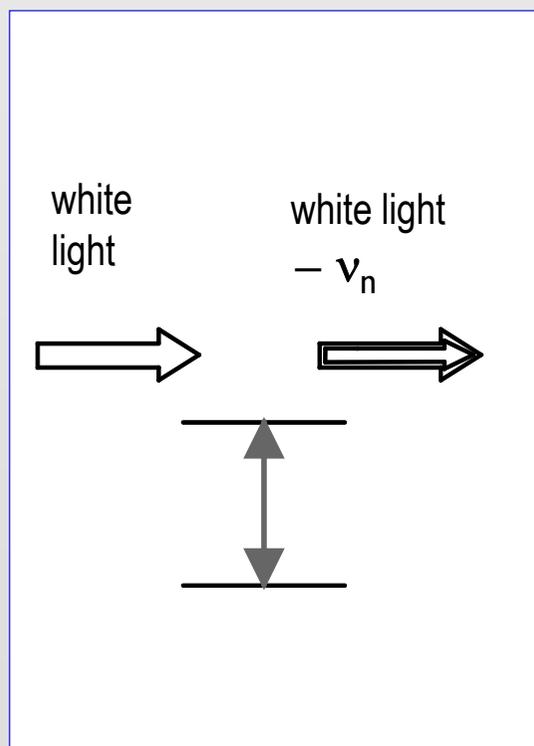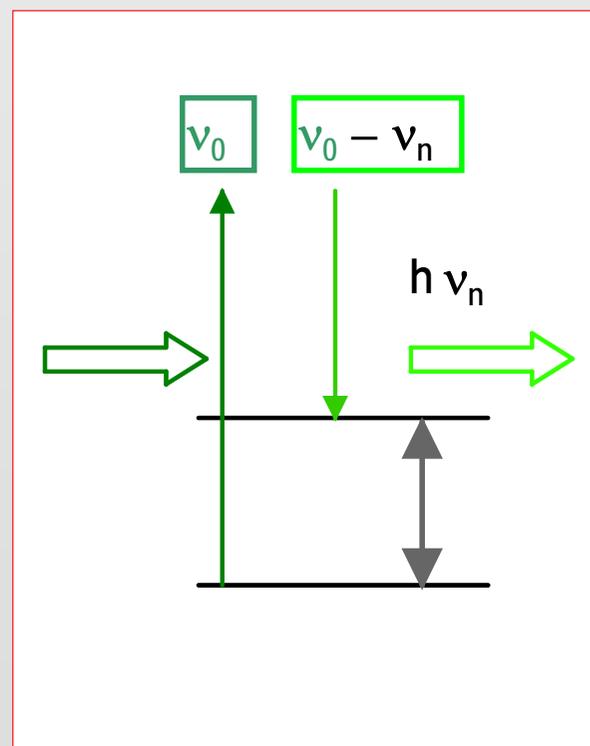
ν_n : normal mode frequency

$\rightarrow$ detected intensity

$\Rightarrow$ *interferogram: I=f(x)*

source

fixed mirror

beam
splitter

movable mirror

sample

detector

OPTICAL
DELAY
0
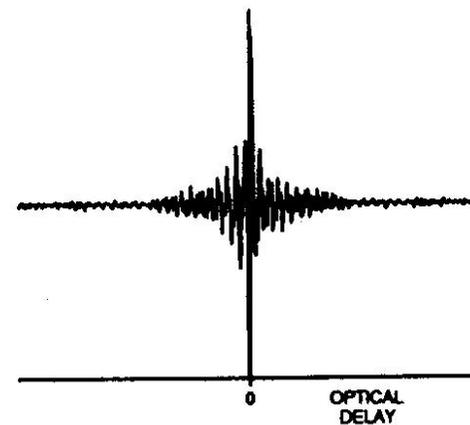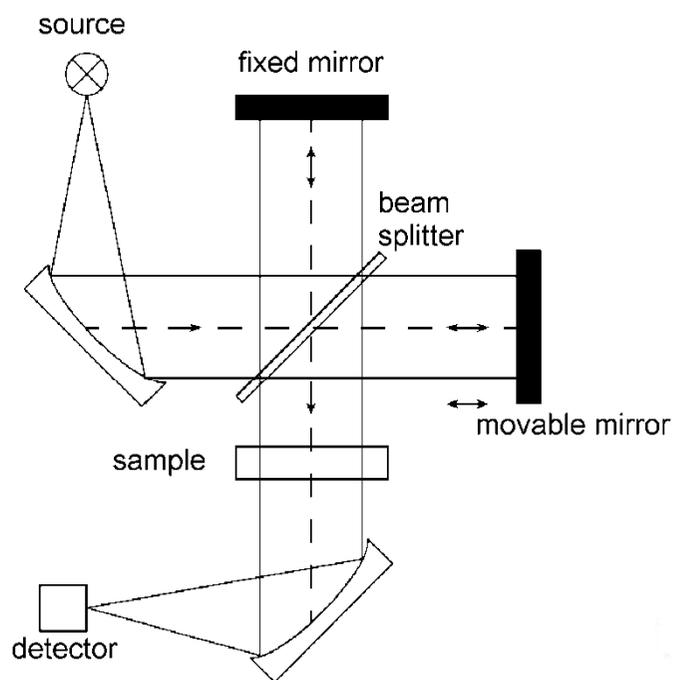
$\rightarrow$ Fourier transformation

$\Rightarrow$ *spectrum: I = f(ν)*
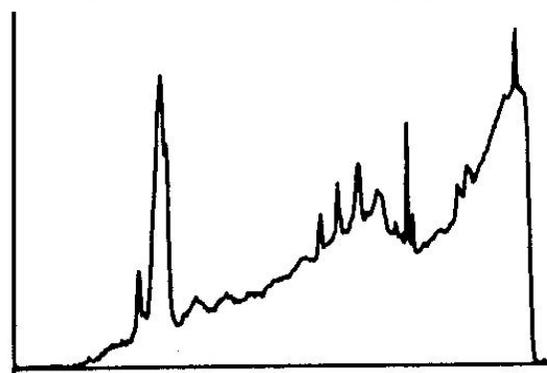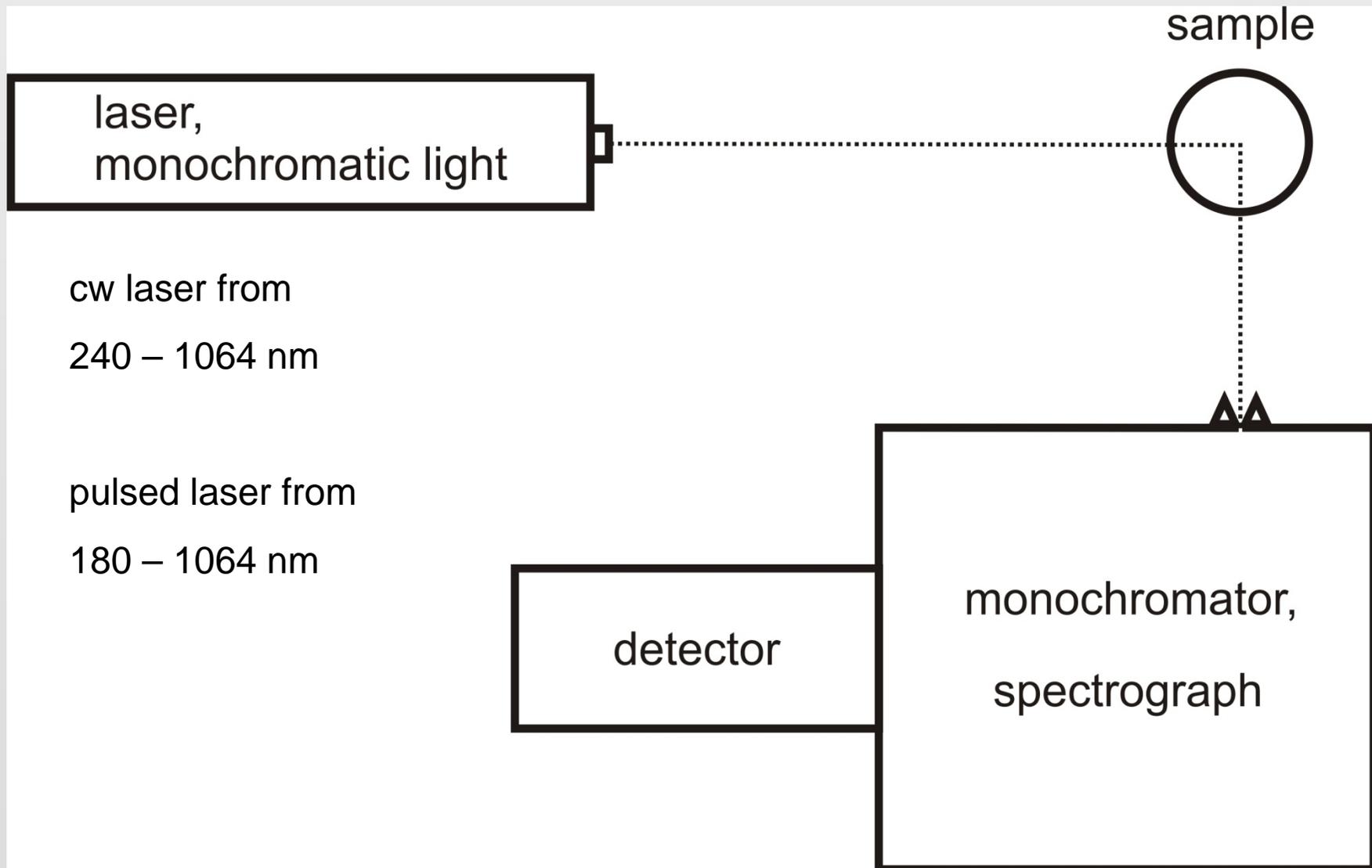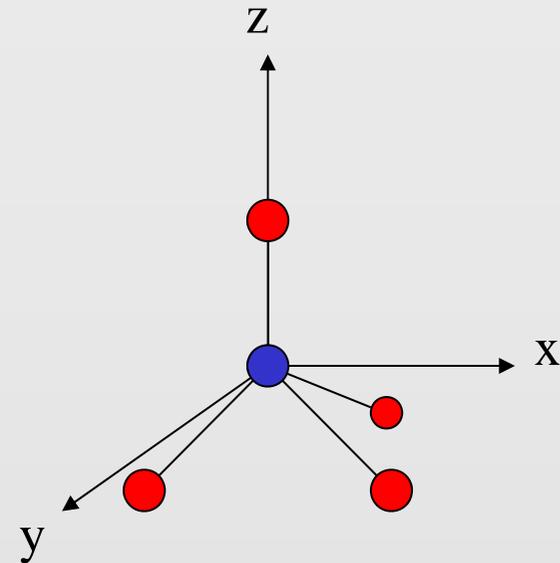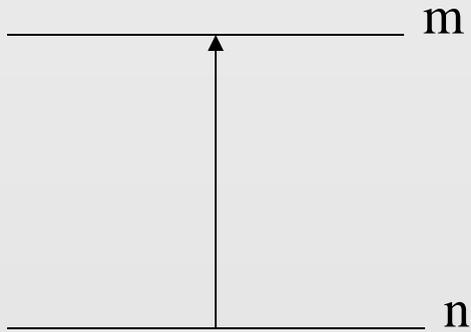
$$I_{mn}(Q_k) \propto [\mu_{mn}]_{xyz}^2$$

$$[\mu_{mn}]_x = \left\langle \psi_m^* \left| \hat{\mu}_x \right| \psi_n \right\rangle$$

$$\mu_x = \mu_x^0 + \sum_{i=1}^{3N-6} \left(\frac{\partial \mu_x}{\partial Q_k} \right)_0 Q_k$$

$$[\mu_{mn}]_x = \mu_x^0 \left\langle \psi_m^* \psi_n \right\rangle + \sum_{k=1}^{3N-6} \left(\frac{\partial \mu_x}{\partial Q_k} \right)_0 \left\langle \psi_m^* \left| Q_k \right| \psi_n \right\rangle$$

= 0,
orthogonality

$\neq 0$, if dipole
moment varies
with Q_k

$\neq 0$, if m
= n $\pm$ 1
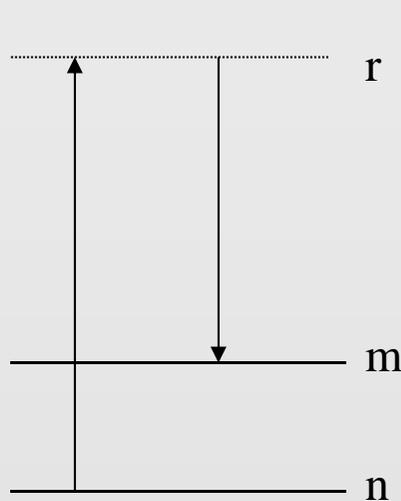
Oszillating EM radiation $\longrightarrow$ $\vec{E} = \vec{E}_0 \cos(2\pi v_0 t)$

induces dipole in the molecule $\longrightarrow$ $\vec{\mu}^{ind}_{xyz} = \alpha \vec{E}$

Raman intensity $\qquad I_{mn}(Q_k) \propto \left[\mu^{ind}_{mn}\right]^2_{xyz} = \left[\alpha_{mn}\right]^2_{xyz} \left|\vec{E}\right|^2$

Calculating the scattering tensor by second–order perturbation theory

$$\left[\alpha_{xyz}\right]_{mn} = \frac{1}{h} \sum_r \left(\frac{\langle m|M_\rho|r\rangle \langle r|M_\sigma|n\rangle}{v_r - v_n - v_0 + i\Gamma_r} + \frac{\langle m|M_\sigma|r\rangle \langle r|M_\rho|n\rangle}{v_r - v_n + v_0 + i\Gamma_r} \right)$$

Intrinsic problem of Raman and IR spectroscopy:

$\rightarrow$ *low sensitivity and selectivity*

Therefore:

- Resonance Raman spectroscopy

- surface enhanced resonance Raman spectroscopy

- IR difference spectroscopy

- surface enhanced infrared absorption difference spectroscopy

A historical example: Bacteriorhodopsin

IR difference spectra: *structural changes induced by a reaction*

Siebert et al. 1985
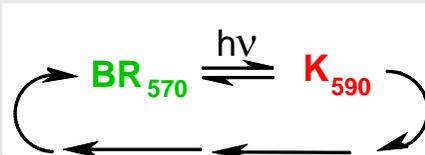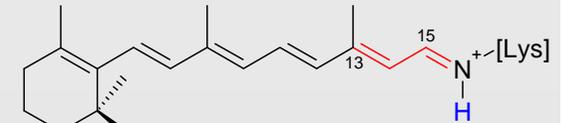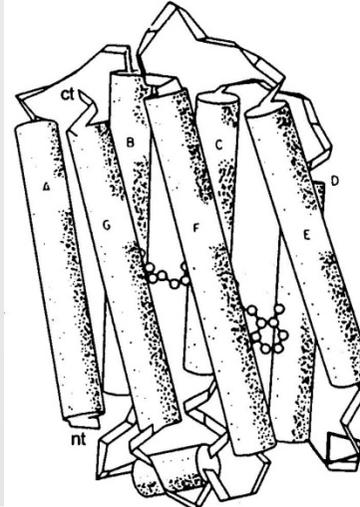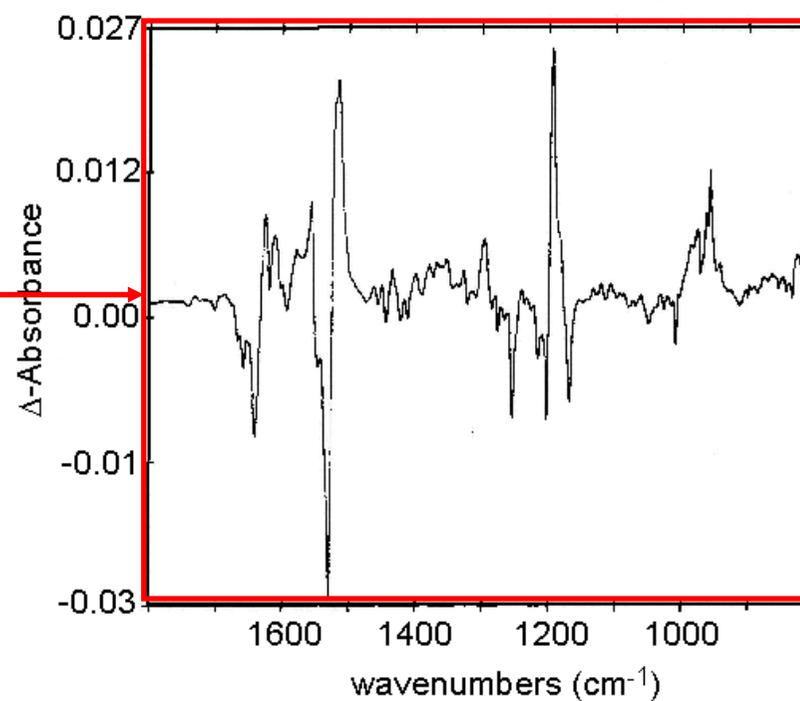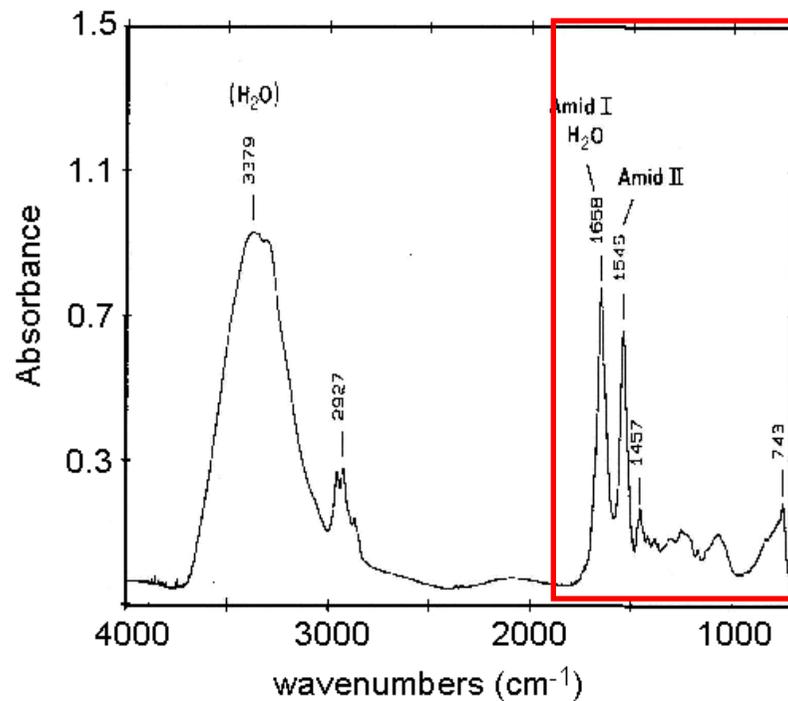
2.2. Resonance Raman spectroscopy

Raman   vs.   **resonance Raman**

electronically excited state

ν_0 $\nu_0 - \nu_n$ ν_0 $\nu_0 - \nu_n$

$h \nu_n$ $h \nu_n$

electronic ground state
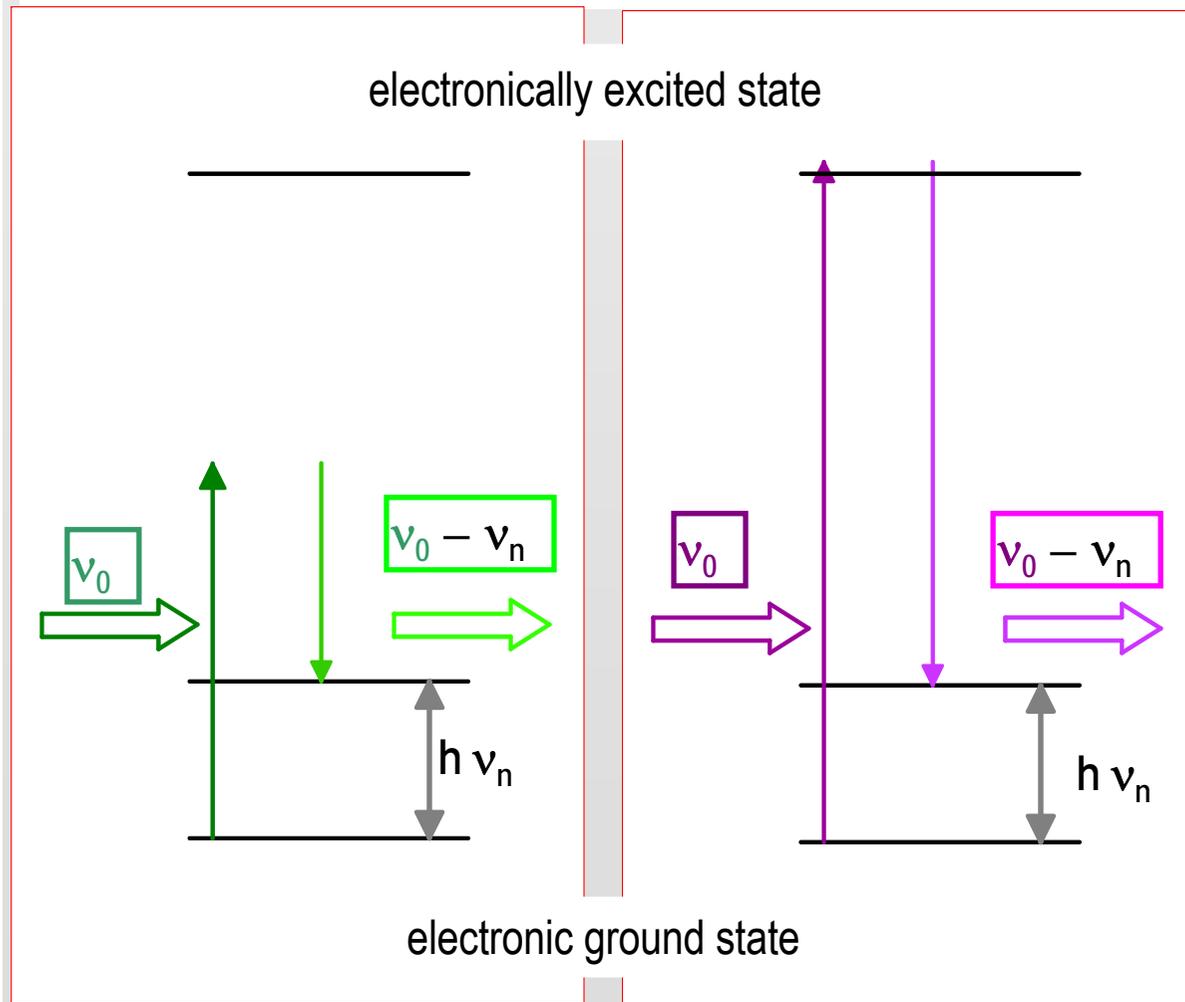
Resonance Raman (RR):

$\Rightarrow$ enhancement of the vibrational bands of a chromophore upon excitation in resonance with an electronic transition

Resonance Raman intensities
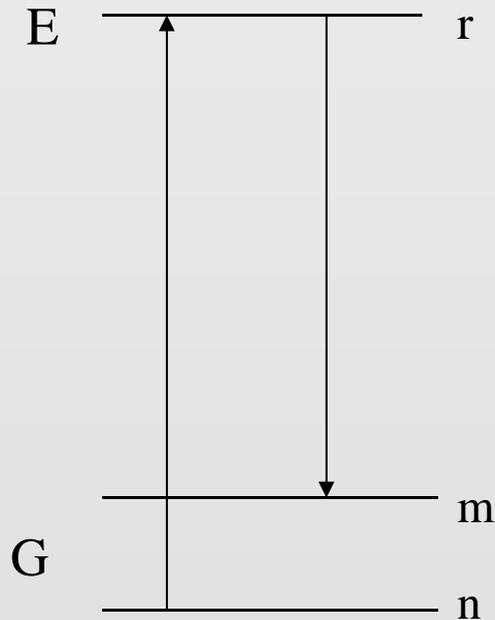
$$I_{mn}(Q_k) \propto \left[\alpha_{mn}\right]^2_{xyz} \left|\vec{E}\right|^2 \qquad \text{Raman intensity}$$

$$\left[\alpha_{xyz}\right]_{mn} = \frac{1}{h}\sum_r \left(\frac{\langle m|M_\rho|r\rangle\langle r|M_\sigma|n\rangle}{\nu_r - \nu_n - \nu_0 + i\Gamma_r} + \frac{\langle m|M_\sigma|r\rangle\langle r|M_\rho|n\rangle}{\nu_r - \nu_n + \nu_0 + i\Gamma_r} \right)$$

for $\nu_0 \to \nu_{EG}$

approximation for
strong transitions $\longrightarrow$

$$\left[\alpha_{xyz}\right]_{mn} = \frac{1}{h}\sum_r \left(\frac{M_{EG,\rho}M_{EG,\sigma}\langle m|r\rangle\langle r|n\rangle}{\nu_{EG} - \nu_0 + i\Gamma_r} \right)$$

$$\left[\alpha_{xyz}\right]_{mn} = \frac{1}{h}\sum_{r}\left(\frac{M_{EG,\rho}M_{EG,\sigma}\langle m|r\rangle\langle r|n\rangle}{\nu_{EG} - \nu_0 + i\Gamma_r}\right)$$

Non-zero Franck-Condon factor products only for modes including coordinates with an excited state displacement

Siebert &
Hildebrandt, 2007)

internal coordinate
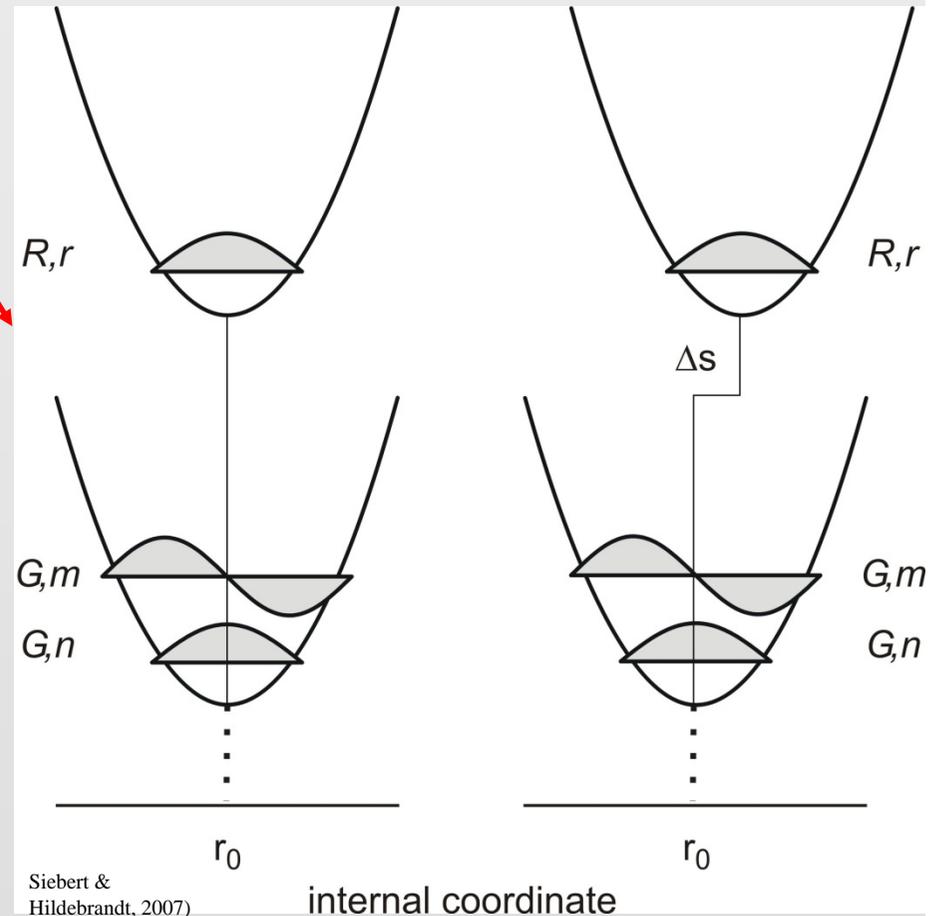
$$\left[\alpha_{xyz}\right]_{mn} \propto \frac{M_{EG,\rho}M_{EG,\sigma}\Delta s\,\nu_k}{\left(\nu_{EG} - \nu_0 + i\Gamma_r\right)\left(\nu_{EG} - \nu_0 + \nu_k + i\Gamma_r\right)}$$

bacteriorhodopsin
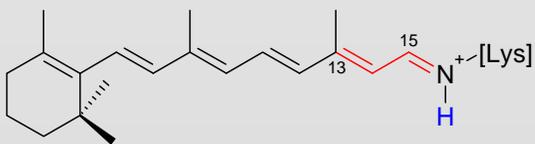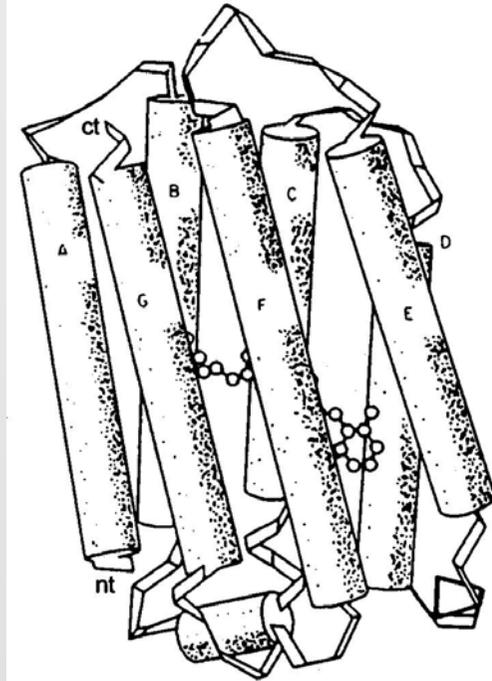
Resonance Raman
selectively probes the
vibrational bands of a
chromophore in a
macromolecular matrix
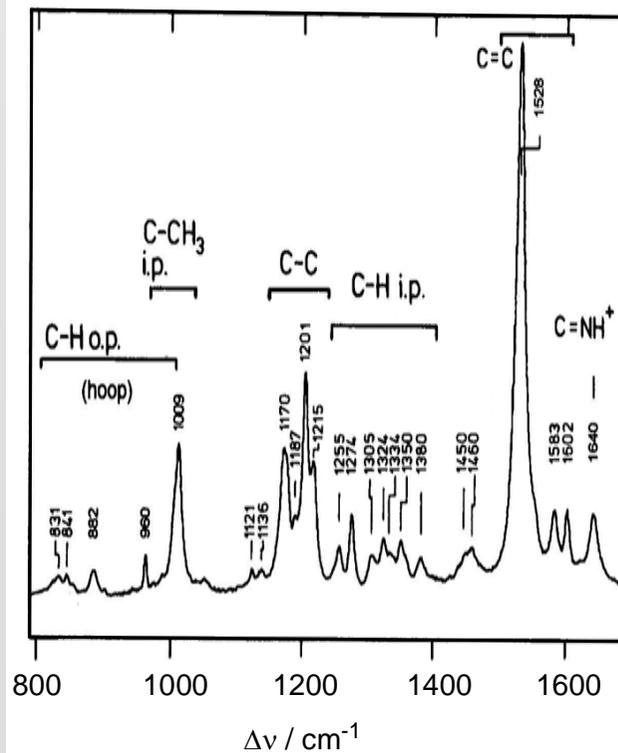
λ / nm

resonant excitation

non-resonant excitation
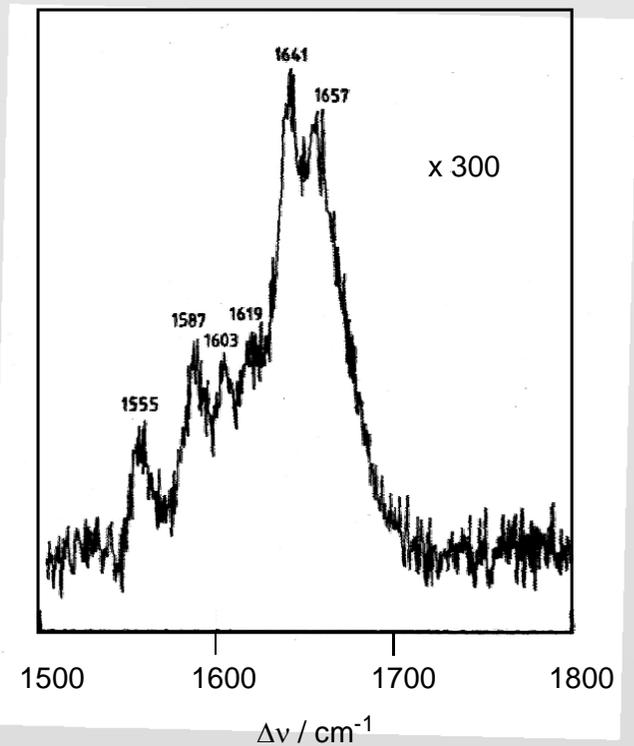
C=C

1528

C-CH$_3$
i.p.

C-C

C-H i.p.

C=NH$^+$

C-H o.p.

(hoop)

1009

1170
1187
1215

1201

1255
1274

1305
1324
1334
1350
1380

1450
1460

1583
1602
1640

831
841

882

960

1121
1136

x 300

1641

1657

1587
1603

1619

1555

$\Delta\nu$ / cm^{-1}

$\Delta\nu$ / cm^{-1}

15

13

N^{+} [Lys]

H

Althaus et al. 1995

Observation:

molecules adsorbed on rough (nm-scale) Ag or Au surface experience an enhancement of the Raman scattering – surface enhanced Raman (SER) effect.

SER-active systems:

- Electrochemically roughened electrodes
- Colloidal metal particles
- Evaporated (sputtered) or (electro-)chemically deposited metal films
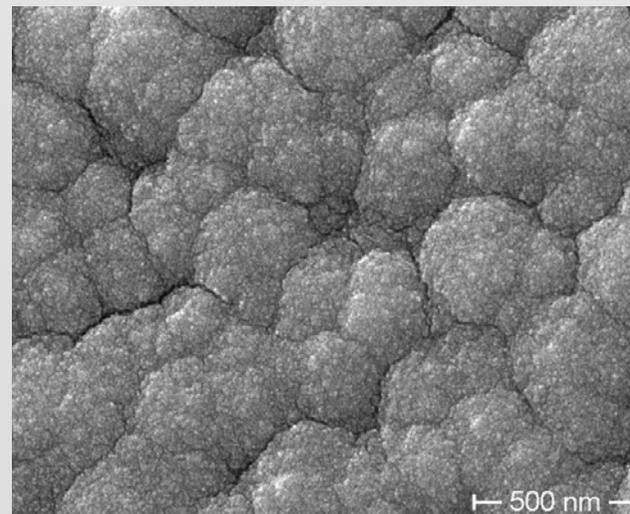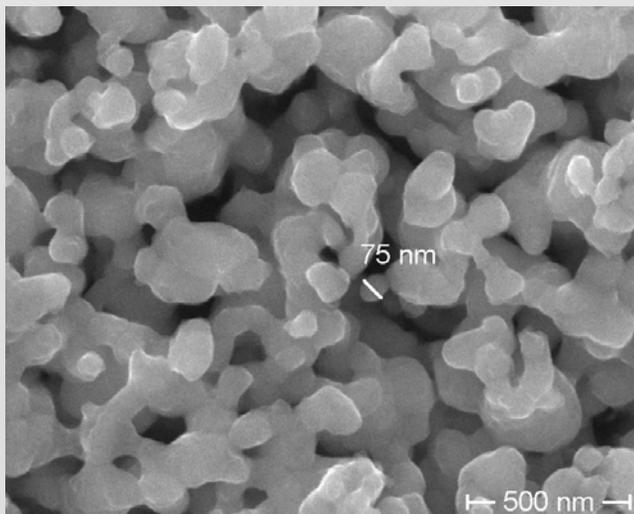
Theorie - SER:

➢ Delocalised electrons in metals can undergo collective oscillations (plasmons) that can be excited by electromagnetic radiation

➢ Eigenfrequencies of plasmons are determined by boundary conditions
   - Morphology
   - Dielectric properties

Upon resonant excitation, the oscillating electric field of the radiation field $\vec{E}_0(v_0)$
Induces an electric field in the metal $\vec{E}_{ind}(v_0)$

$$\vec{E}_{tot}(v_0) = \vec{E}_0(v_0) + \vec{E}_{ind}(v_0)$$ Total electric field

$$F_E(v_0) = \frac{\left|\vec{E}_0(v_0) + \vec{E}_{ind}(v_0)\right|}{\left|\vec{E}_0(v_0)\right|} = \left|1 + 2g_0\right|$$ Enhancement factor for the field at the incident frequency

The magnitude of the enhancement depends on the frequency-dependent dielectric properties of the metal

$$g_0 = \frac{\tilde{\varepsilon}_r(\nu_0) - 1}{\tilde{\varepsilon}_r(\nu_0) + 2}$$

$\tilde{\varepsilon}_r(\nu_0)$ complex dielectric constant

$$\tilde{\varepsilon}_r(\nu_0) = \frac{\varepsilon_{re}(\nu_0) + i\varepsilon_{im}(\nu_0)}{n_{solv}^2}$$

If real part ➜ -2 and imaginary part ➜ 0, largest enhancement

In a similar way, one may derive a field enhancement for the Raman scattered light $\vec{E}_{Ra}(\nu_0 \pm \nu_k)$ which depends on $\vec{E}_{tot}(\nu_0)$

Since the intensity is proportional to the square of the electric field strength, the SER enhancement factor is given by:

$$F_{SER}(\nu_0 \pm \nu_k) = \left[(1 + 2g_0)(1 + 2g_{Ra}) \right]^2$$

Total enhancement ca. 1o^5 - 10^6

SERR and SEIRA:

➢ all photophysical processes at metal surfaces can be enhanced via the frequency-dependent electric field enhancement

➢ *combination of RR and SER*: surface enhanced resonance Raman – **SERR**: excitation in resonance with both

➔ an electronic transition of the adsorbate and

➔ the surface plasmon eigenfrequency of the metal

Single-molecule sensitivity!

➢ IR - Absorption: surface enhanced infrared absorption – SEIRA

enhancement of the incident electric field in the infrared! (Au, Ag)

$$\vec{E}_{tot}(v_0) = \vec{E}_0(v_0) + \vec{E}_{ind}(v_0)$$ Total electric field

Total enhancement thus ca. less than the square root of the SER effect:100 – 1000
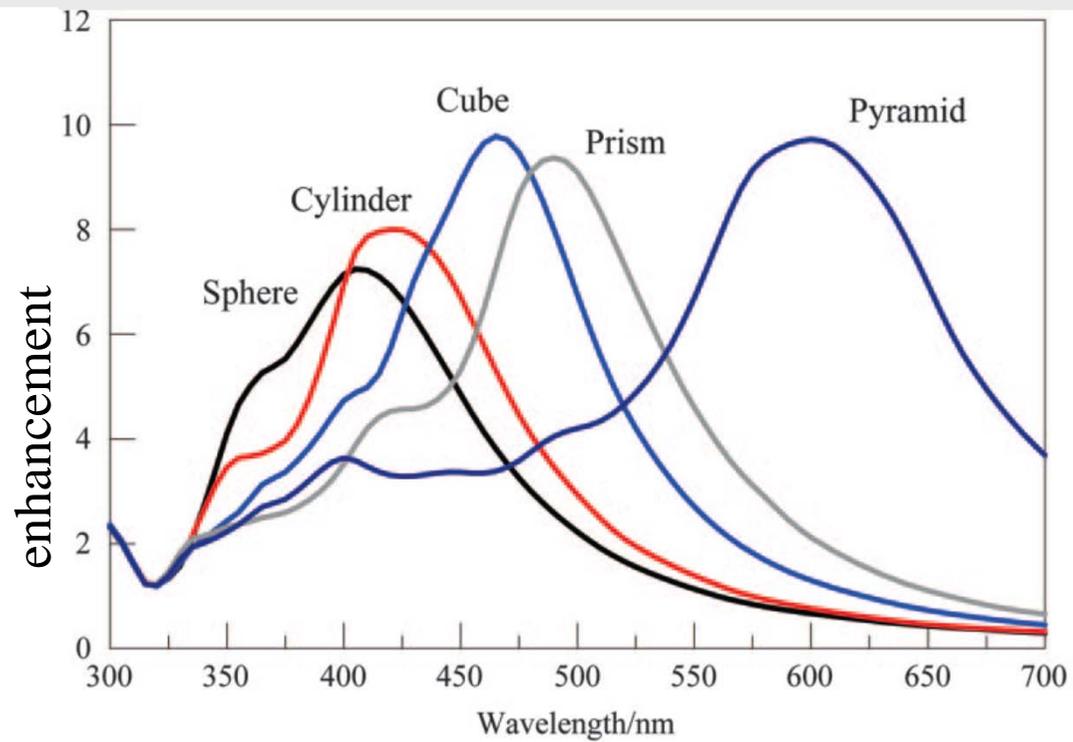
Calculations of the enhancement factor for various geometric shapes (Ag)

Siebert & Hildebrandt, 2007)
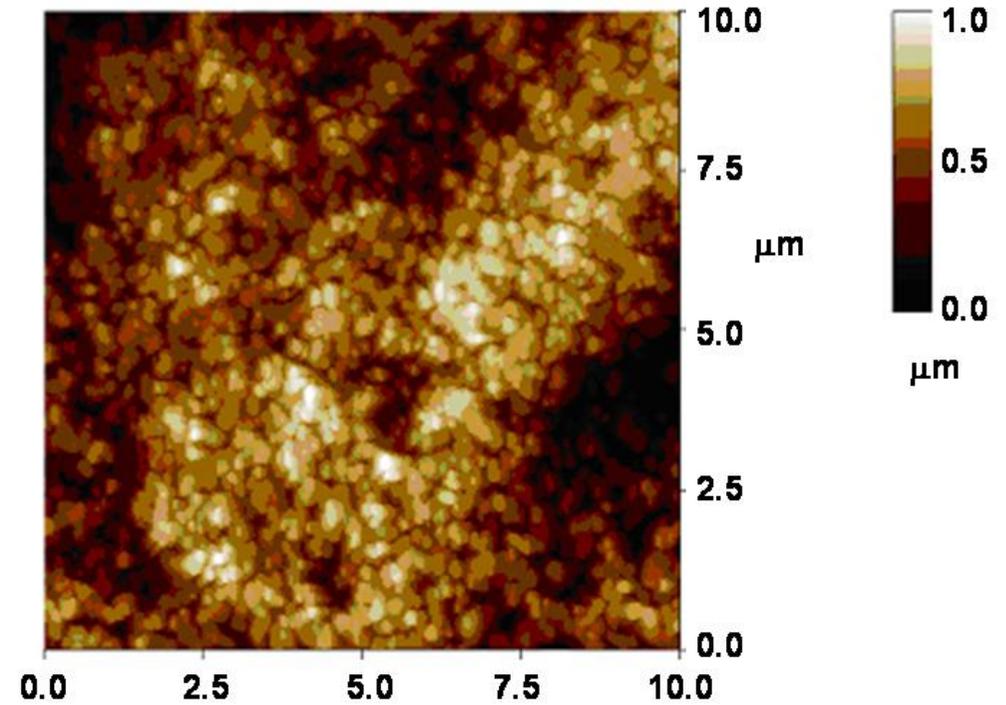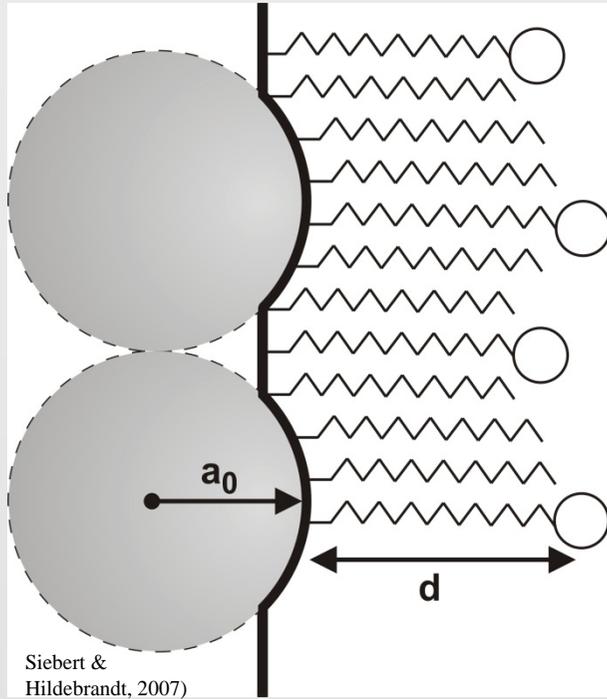
Distance-dependence of the SER and SEIRA effect

$$F_{SER}(d) = F_{SER}(0) \cdot \left(\frac{a}{a+d} \right)^{12}$$

$$F_{SEIRA}(d) = F_{SEIRA}(0) \cdot \left(\frac{a}{a+d} \right)^{6}$$

Siebert & Hildebrandt, 2007)
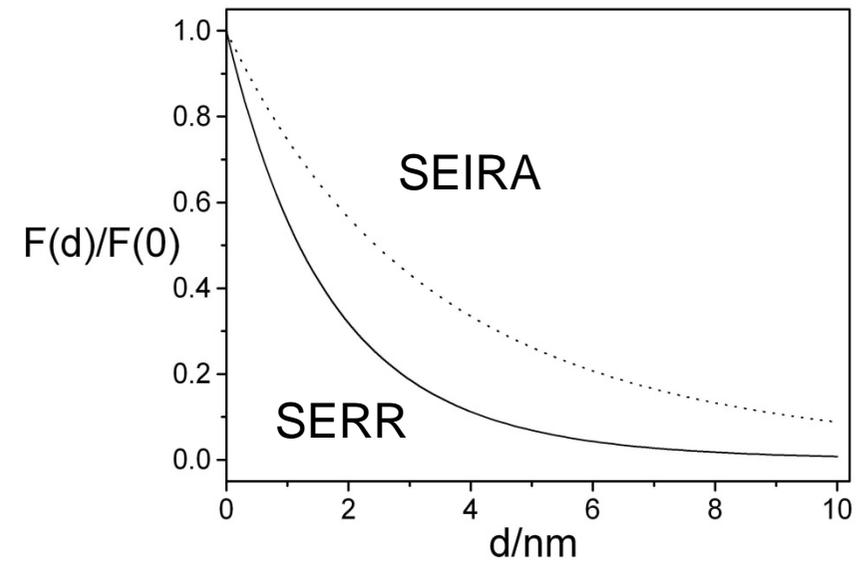
… and how to overcome them

Plasmon resonance of polydisperse nanostructures
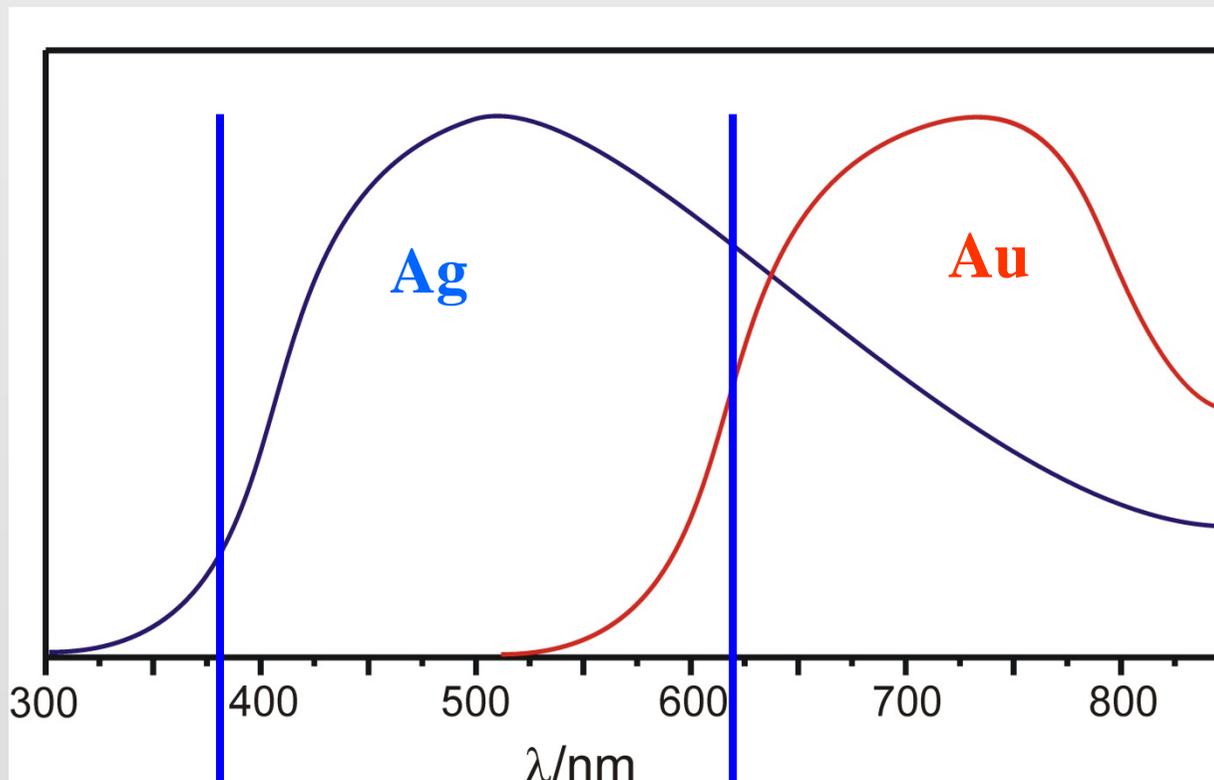
Preferred spectral range for combining RR and SER

But: Au displays a much broader electrochemical potential range and is chemically more stable

Layered hybrid devices

Electrochemical roughening of Ag electrode
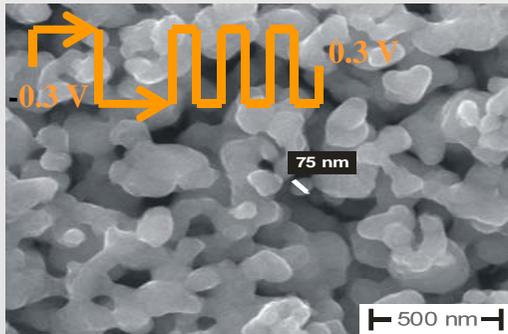
Coating by a dielectric layer (SAM, SiO_2)
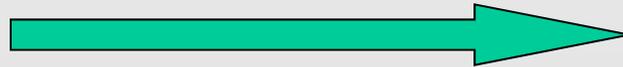
Deposition of a metal film or semiconductor film (Au, Pt, TiO_2)

C-O
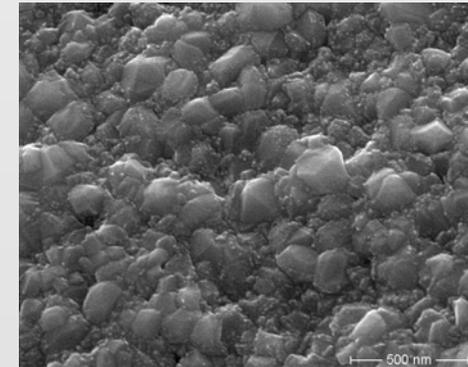
Functionalisation of the outer metal layer for protein binding
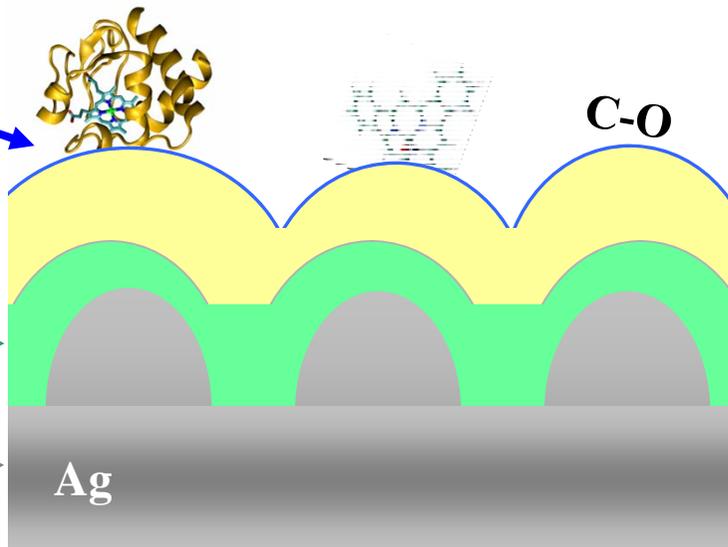
Metal film: ca. 20 nm

dielectric layer: 2 – 30 nm

Nanostructured Ag
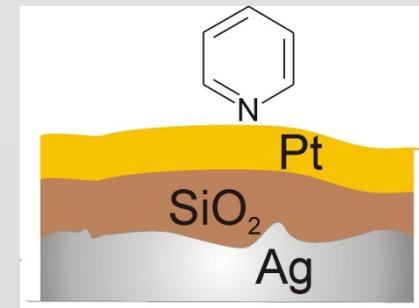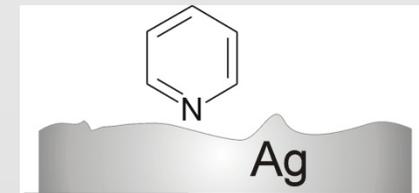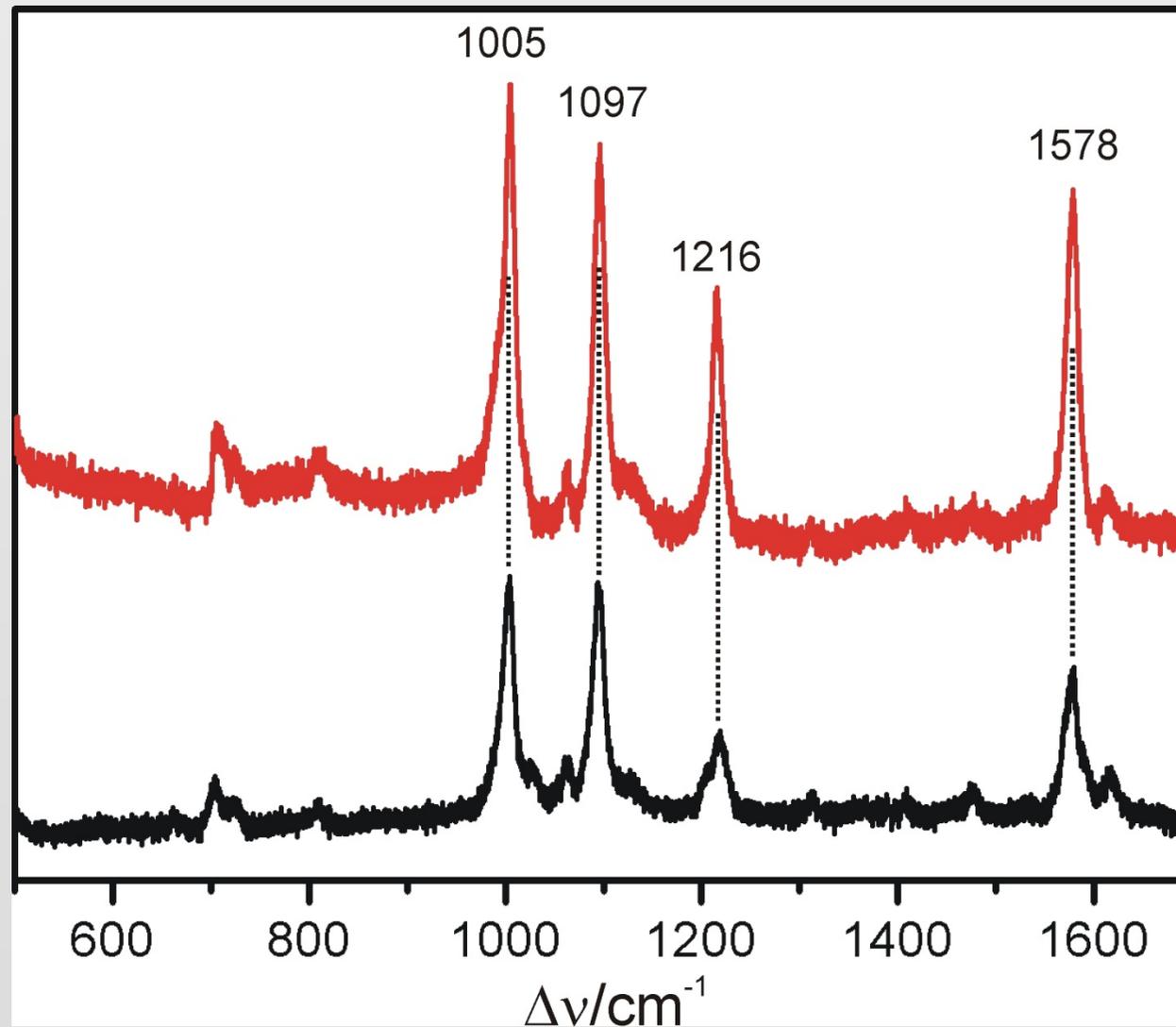
Ag

Layered hybrid devices
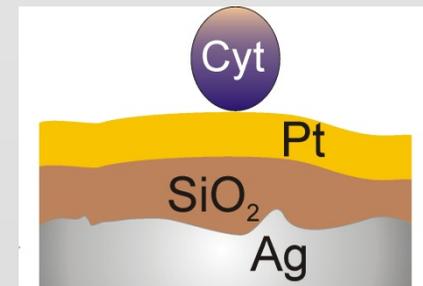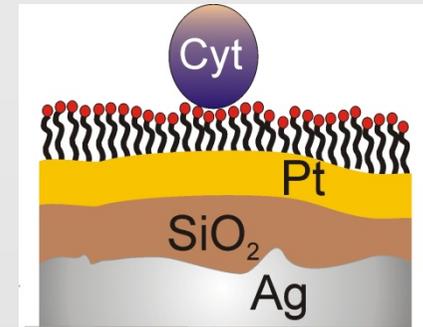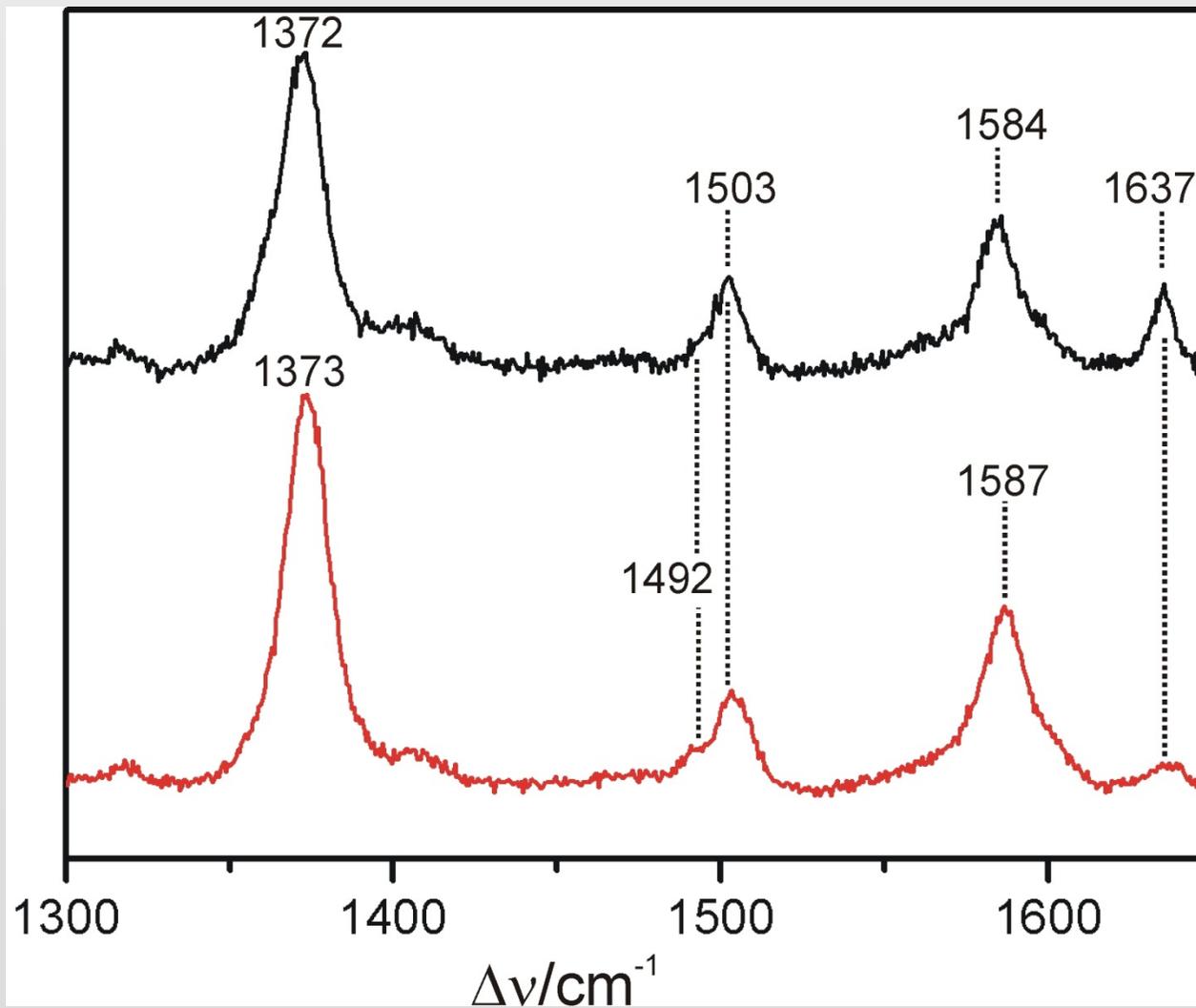
Layered hybrid devices

Layered devices

Distance-dependence of the enhancement

experimental
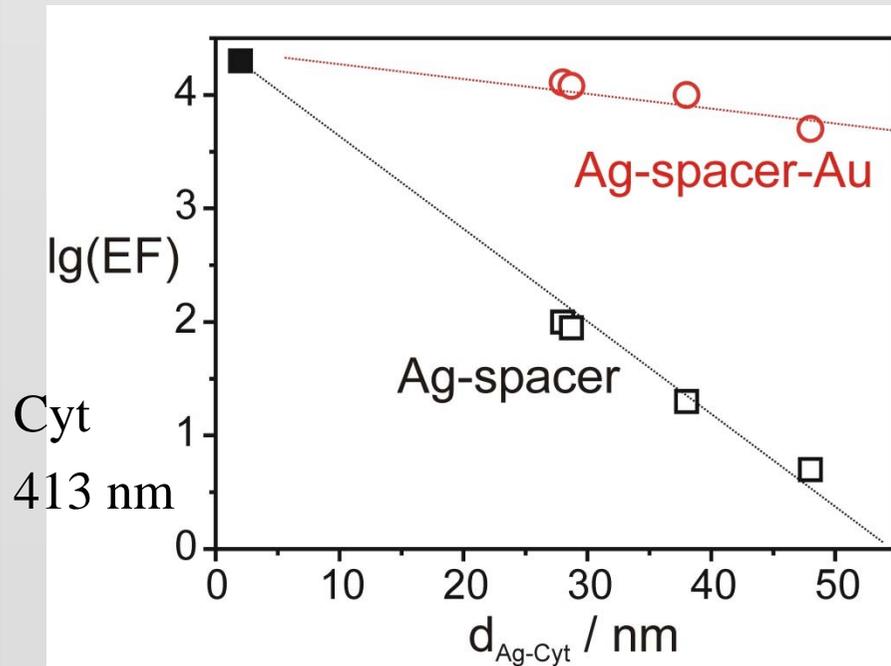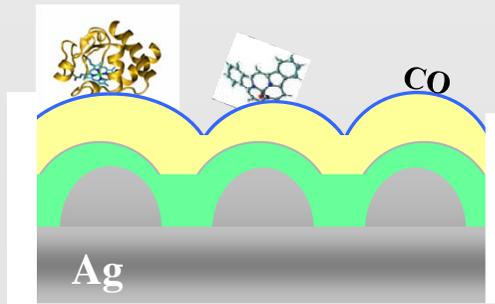
theoretical

$$g_0 = |\frac{\mathbf{E}(\mathbf{r}, \omega)}{\mathbf{E}_0}|^2 = |\nabla\psi(\mathbf{r}, \omega)|^2$$
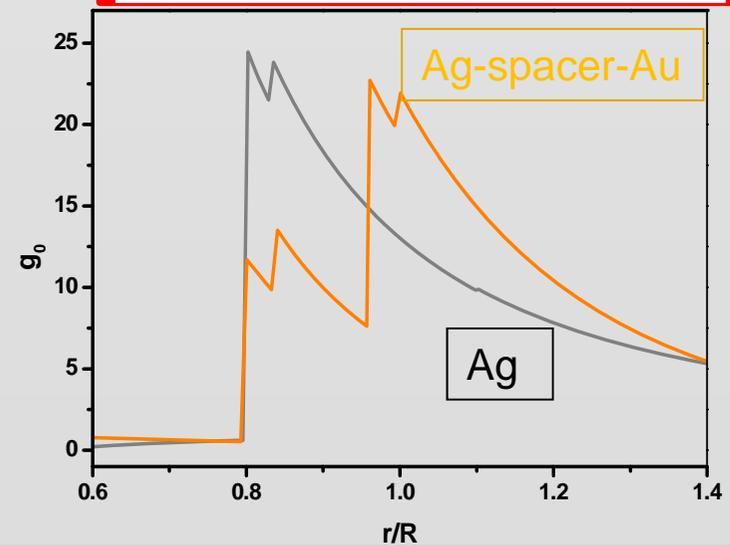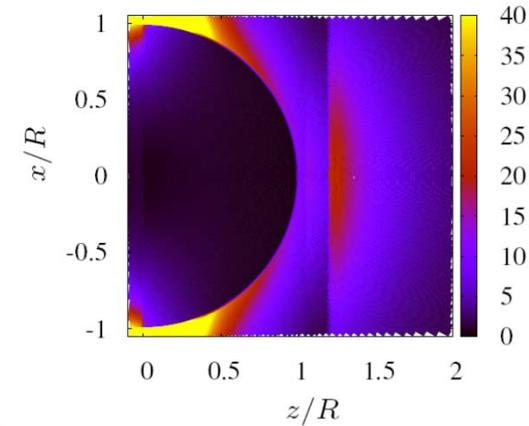
Layered devices

Potential application for in-situ studies in heterogeneous catalysis
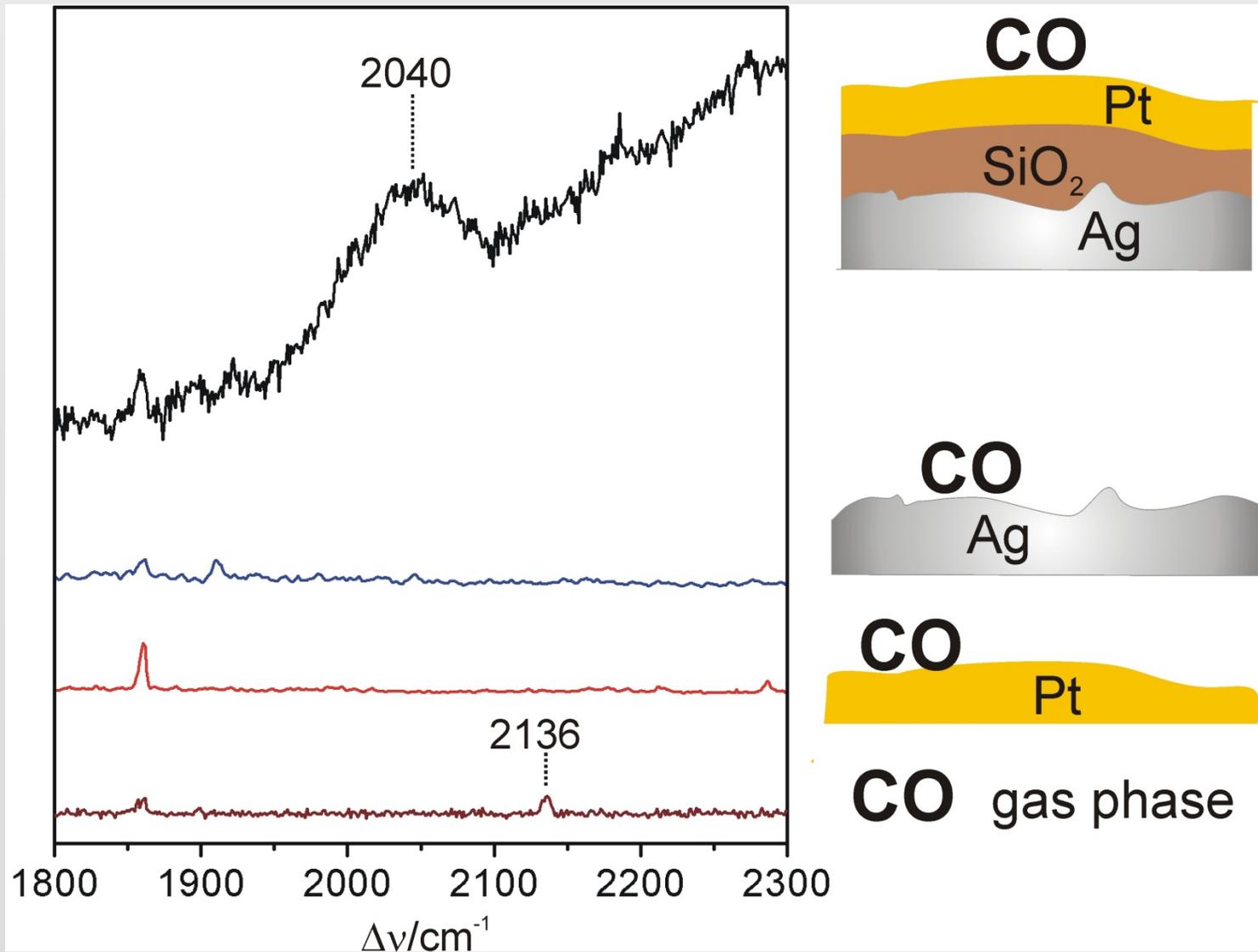
probing electron transfer processes

SERR
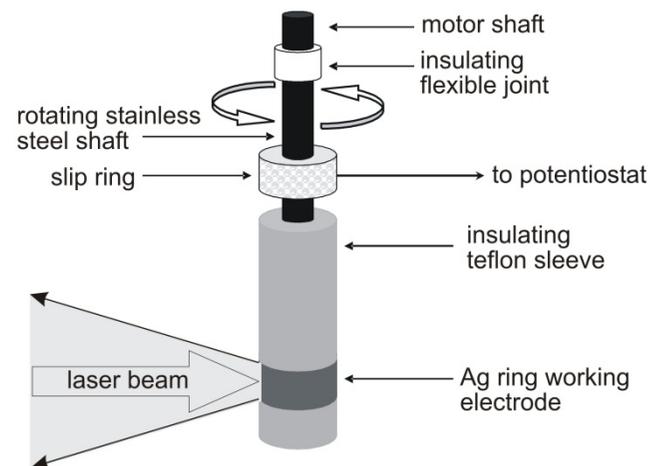
SEIRA
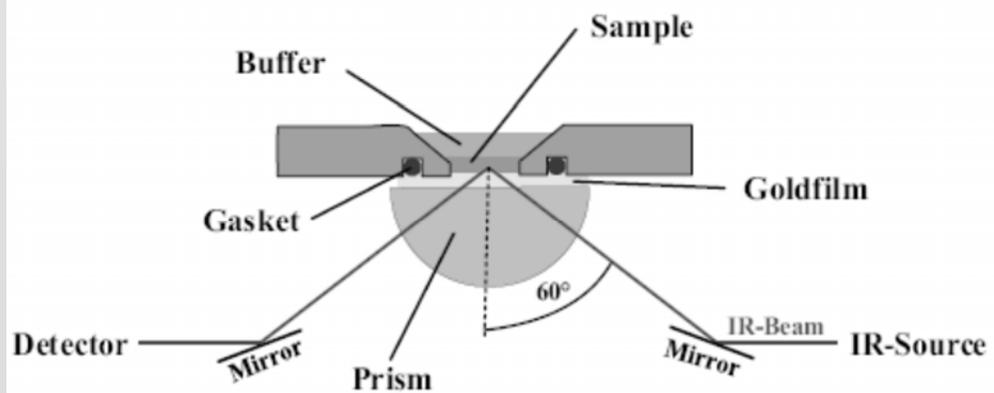
Siebert & Hildebrandt, 2007)
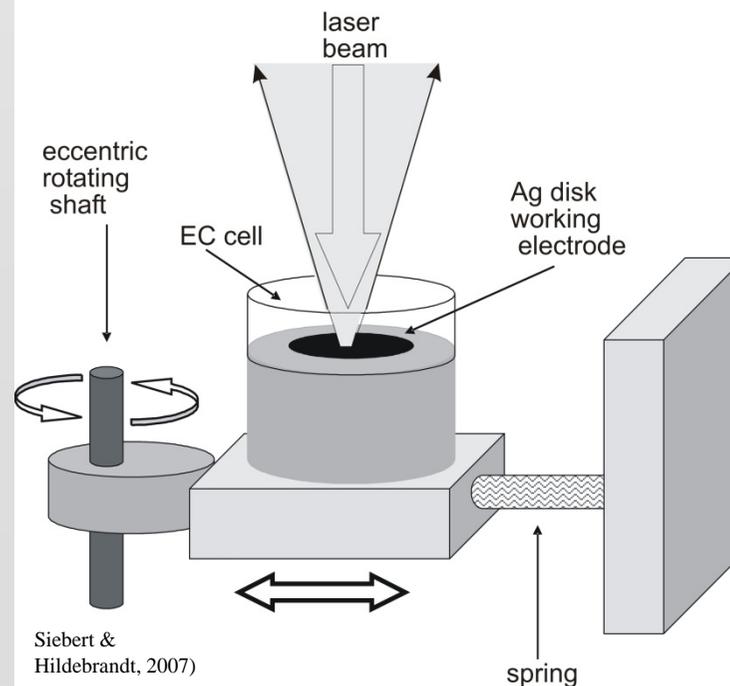
Siebert & Hildebrandt, 2007)

SERR: applicable to proteins bound to biocompatibly coated metal surfaces

Immobilization of cytochrome *c* on "membrane models"

Electrostatic (CO_2^-, PO_3^{2-}, NH_3^+)
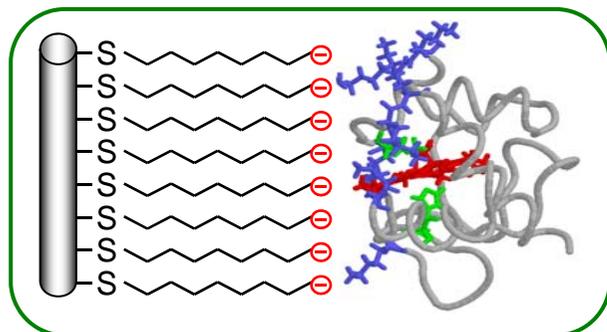
Hydrophobic (e.g. CH_3)
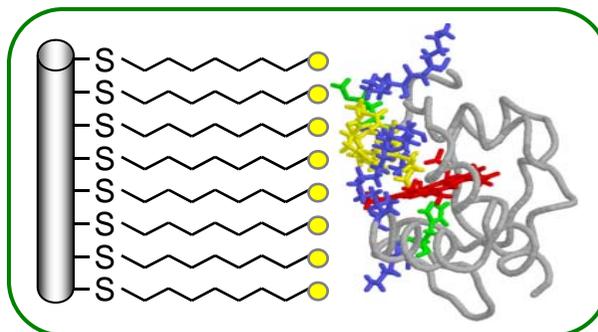
Polar (e.g. OH)
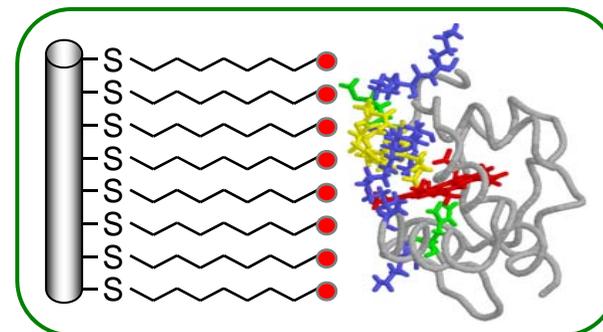
Mixed SAMs (e.g. OH/CH_3)
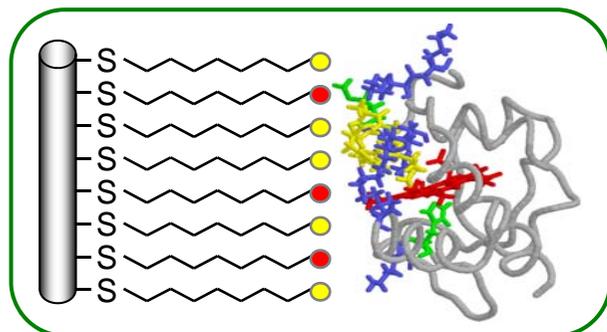
Covalent (cross linking)
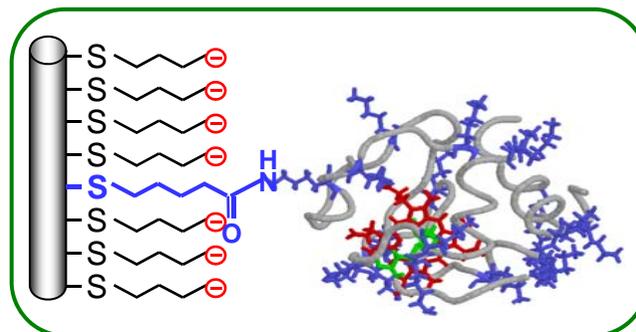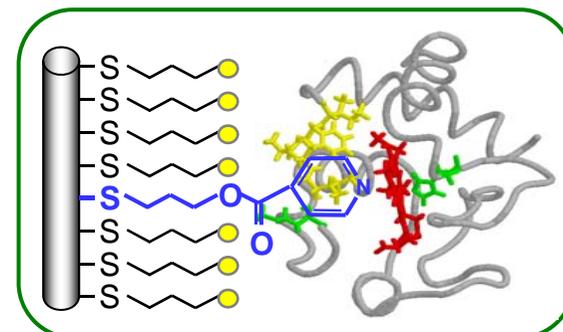
Coordinative (e.g. Py)

Example: redox processes of cytochrome c
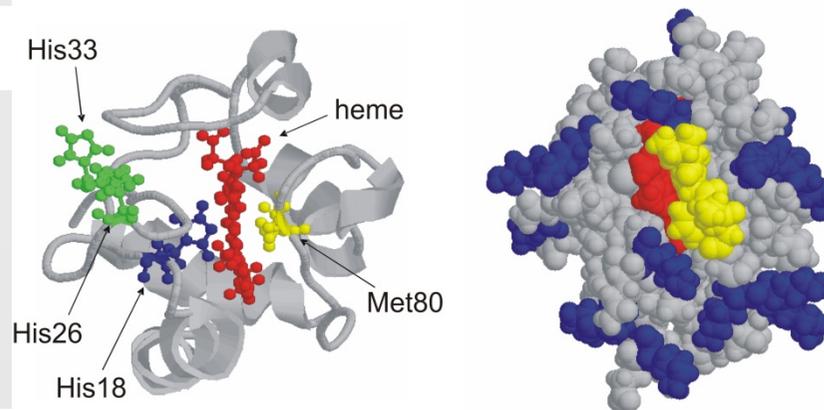
Potential-dependent SERR measurements to probe the redox equilibrium of the immobilised protein
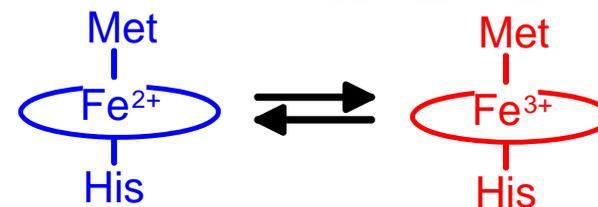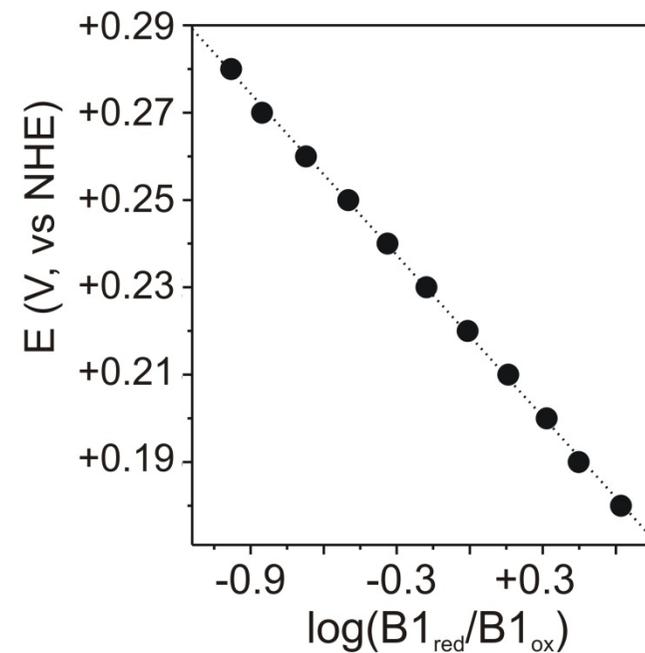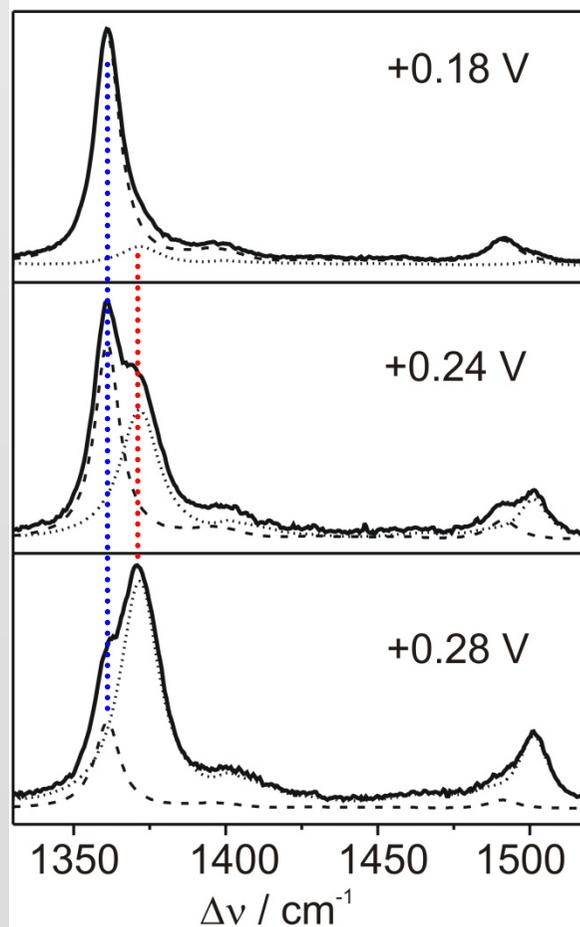
3. Time-resolved methods

3.1.   Principles of time-resolved IR and RR experiments

3.2.   Time-resolved pump-probe Raman spectroscopy with cw excitation

3.3.   Time-resolved IR experiments

3.4.   Rapid mixing techniques and time-resolved spectroscopy

3.5.   Potential-jump time-resolved SERR and SEIRA spectroscopy

3.6.   Time-resolved techniques - summary

3. Time-resolved methods

Principle approaches:

	Time scale	Method	comments
Resonance Raman	> 100 ns	Cw excitation	Low photon flux
	> 10 ps	Pulsed excitation	High photon flux
	> 100 fs	Stimulated Raman	Very demanding set-up
IR	> 1 ms	Rapid scan	
	> 10 ns	Step scan	
	> 100 fs	transient absorption	Very demanding set-up

Triggering the processes to be studied by

→ light (photo-processes or photoinduced release of reactands)

→ temperature, pressure, or potential jump

→ rapid mixing with the reaction partner

Pump-probe experiments with cw excitation

Time resolution $\quad \Delta t = \dfrac{\Delta s}{v} \quad$ for $\quad \Delta s_{min} \approx d_{laser} \qquad \Delta t_{min} \approx 100 ns$

Pump-probe experiments with pulsed excitation

Time resolution $\Delta t = \dfrac{\Delta s}{c}$ for $\Delta s_{min} \approx 1 mm$ $\Delta t_{min} \approx 3 ps$

Sensory rhodopsin II from Natronobacterium pharaonis

Sensory rhodopsin II from Natronobacterium pharaonis

Challenge:

Time-resolved approach must cover a dynamic range of more than six decades

→ Gated-cw pump probe experiments

Siebert &
Hildebrandt, 2007)

M$_{400}$ formation

M$_{400}$ decay

M$_{400}$ kinetics

Same results as for transient absorption spectroscopy

- Rapid scan

  measuring consecutive interferograms (ca. 10 ms)

- Step scan

  Measuring signal decays after each mirror step (< 100 ns)

Siebert &
Hildebrandt, 2007)

Example: photocycle of bacteriorhodopsin

Gerwert et al.)

Example: photocycle of bacteriorhodopsin

Gerwert et al.)

$$A + B \xrightarrow{\text{mixing}} C \xrightarrow{t_1} D \xrightarrow{t_2} etc$$

Rapid mixing of components A and B with $t_{mix} \approx 0.1 - 5\ ms$ .....
.
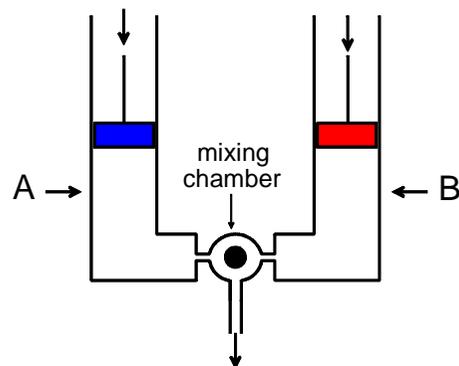
A →   mixing
      chamber   ← B

.... *and monitoring the reaction using the ....*

....*stopped-flow method*
time resolution: $\Delta t = t_{mix} + \delta$

mixing chamber

A → ← B

observation

stop
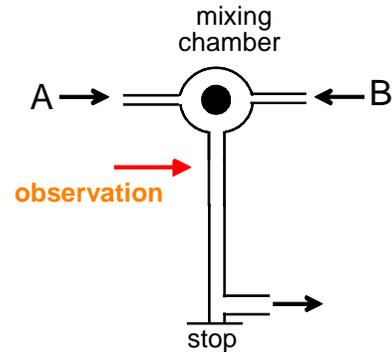
rapid scan RR and FT IR ($\delta = 10$ *ms*)

... *continuous-flow method*
time resolution: $\Delta t = t_{mix} + \delta$ *with*

mixing chamber

A → ← B

Δs

observation
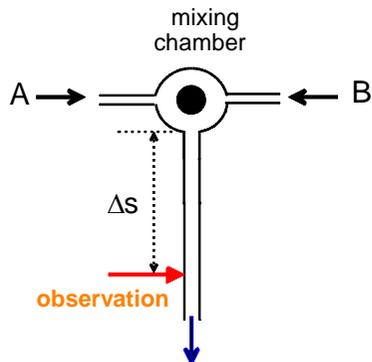
$\rightarrow RR$ *spectroscopy* ($\delta_{min} < t_{mix}$)

.... *freeze-quench method*
time resolution: $\Delta t = t_{mix} + t_{cool}$ *with* $t_{cool} \approx 2$ *ms*

mixing chamber

A → ← B

Δs

isopentane
-120°C →
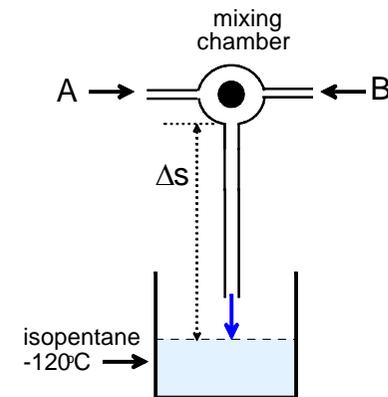
$\rightarrow RR$ *spectroscopy*

Example: re-folding of cytochrome c

→ rapid mixing of **unfolded cytochrome *c* in GuHCl** with **a GuHCl-free solution**

→ continuous-flow method with RR detection
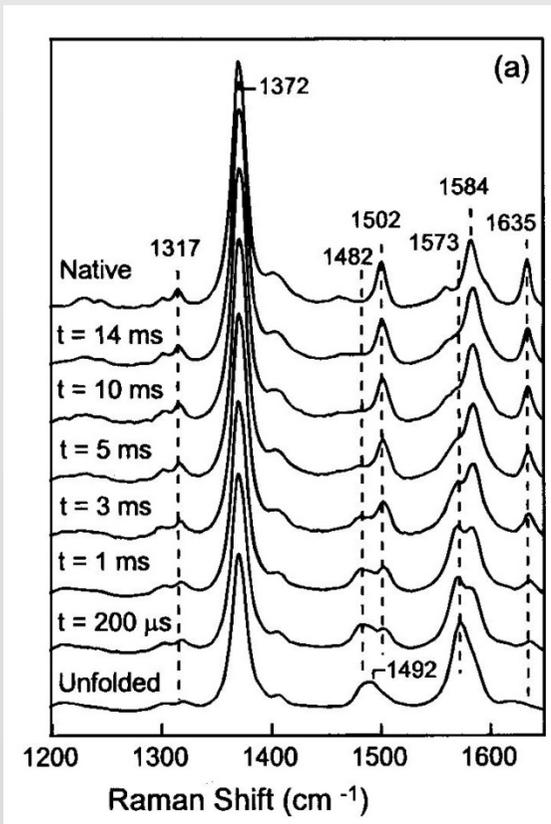
Rousseau et al. 1998
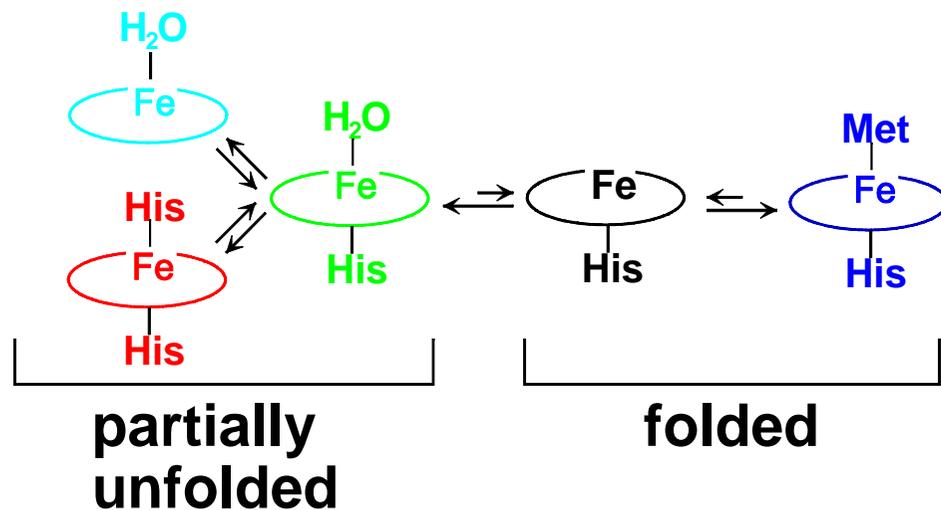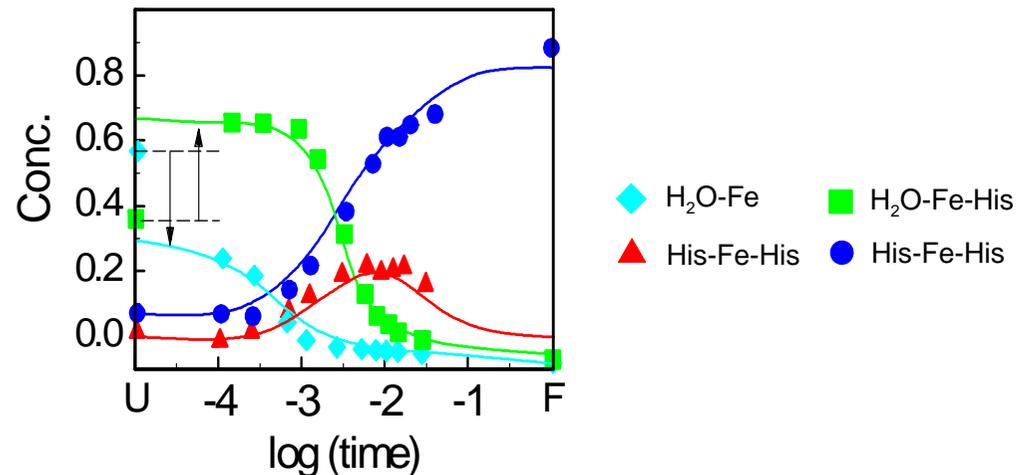
for probing the dynamics of interfacial processes

- Rapid potential jump to perturb the equilibrium of protein immobilised on an electrode

- probing the relaxation process by TR SERR or step-scan or rapid scan SEIRA

- time resolution limited by the reorganisation of the electrical double layer
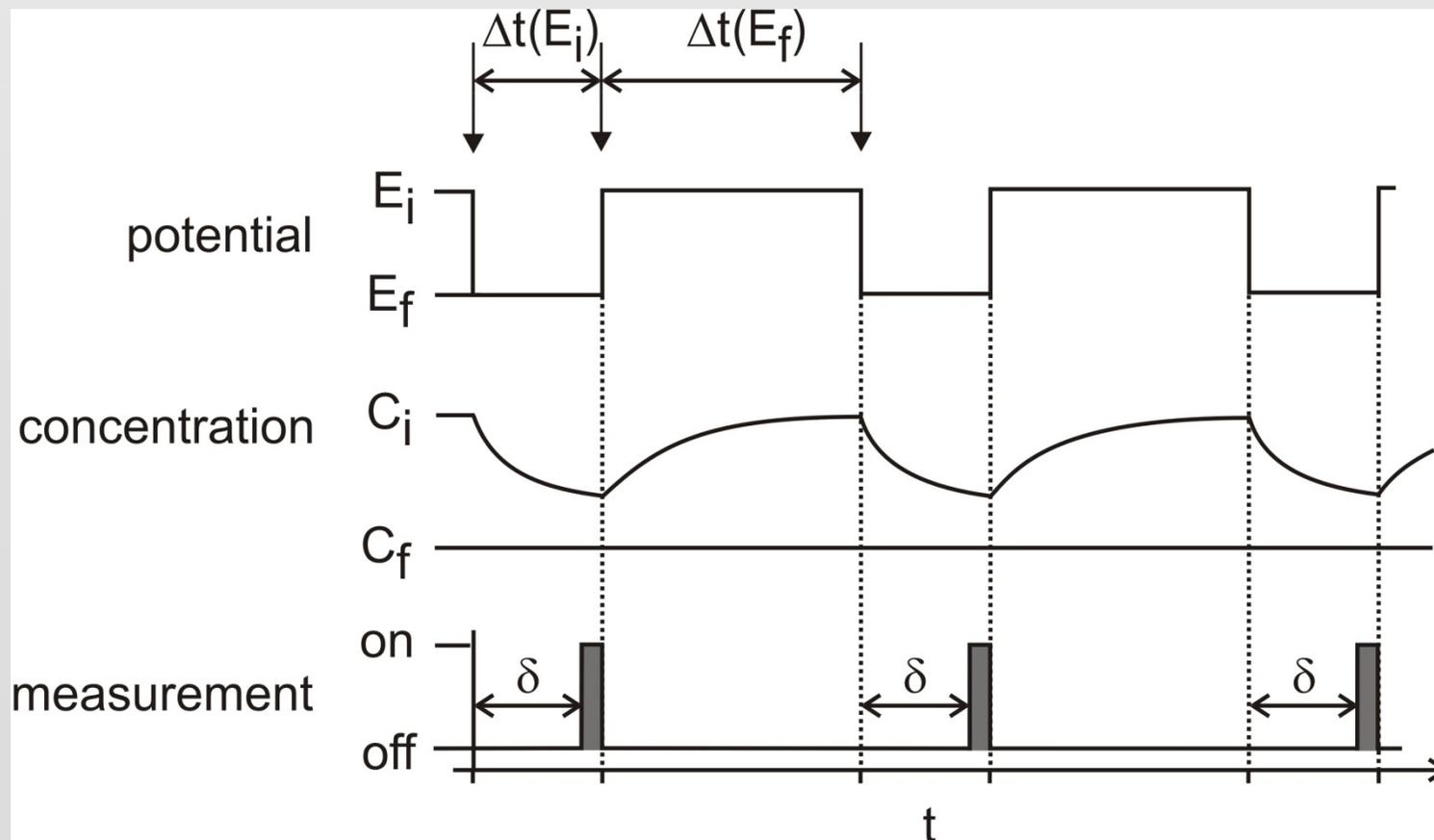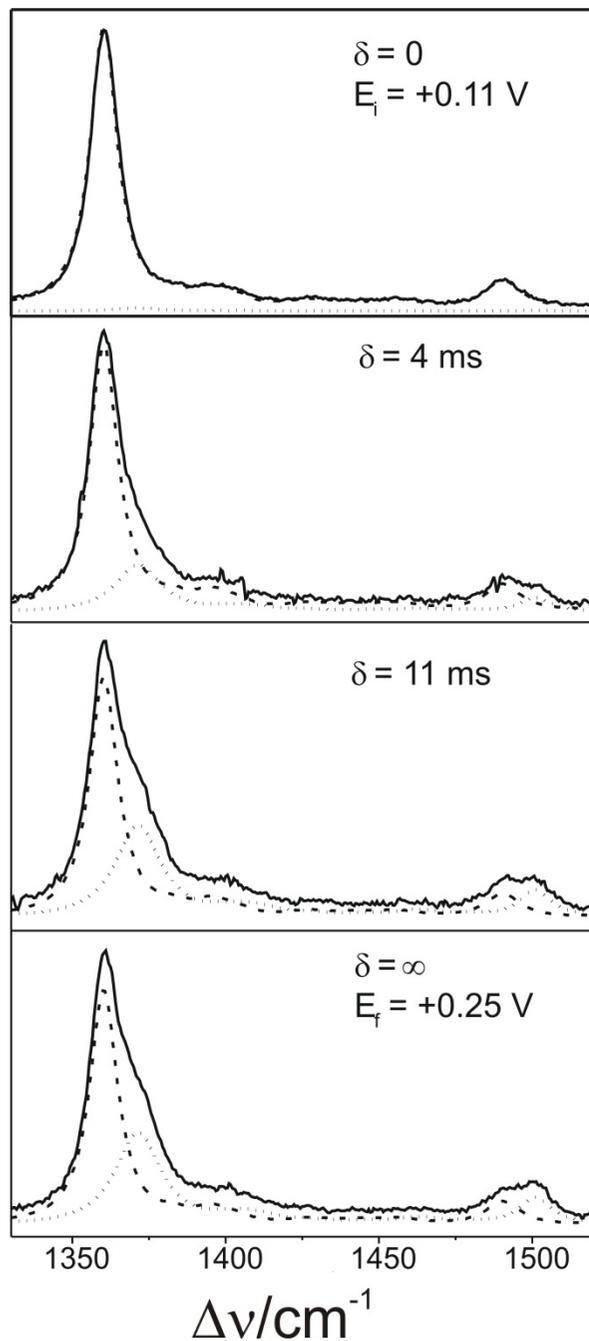
Example: interfacial redox process of cytochrome c

$\rightarrow$ one-step relaxation process

$B1_{red} \rightleftharpoons B1_{ox}$

Met — Fe^{2+} — His $\rightleftharpoons$ Met — Fe^{3+} — His

Example: interfacial redox process of cytochrome c

$\rightarrow$ one-step relaxation process

$B1_{red}$ $B1_{ox}$

Met   Met

Fe^{2+} $\rightleftharpoons$ Fe^{3+}

His   His

$\delta = 0$
$E_i = +0.11$ V

$\delta = 4$ ms

$\delta = 11$ ms

$\delta = \infty$
$E_f = +0.25$ V

$\Delta\nu/cm^{-1}$

$\ln[c_{Red}(\delta)/c_{Red}(0)]$

δ(seconds)

C16

C2
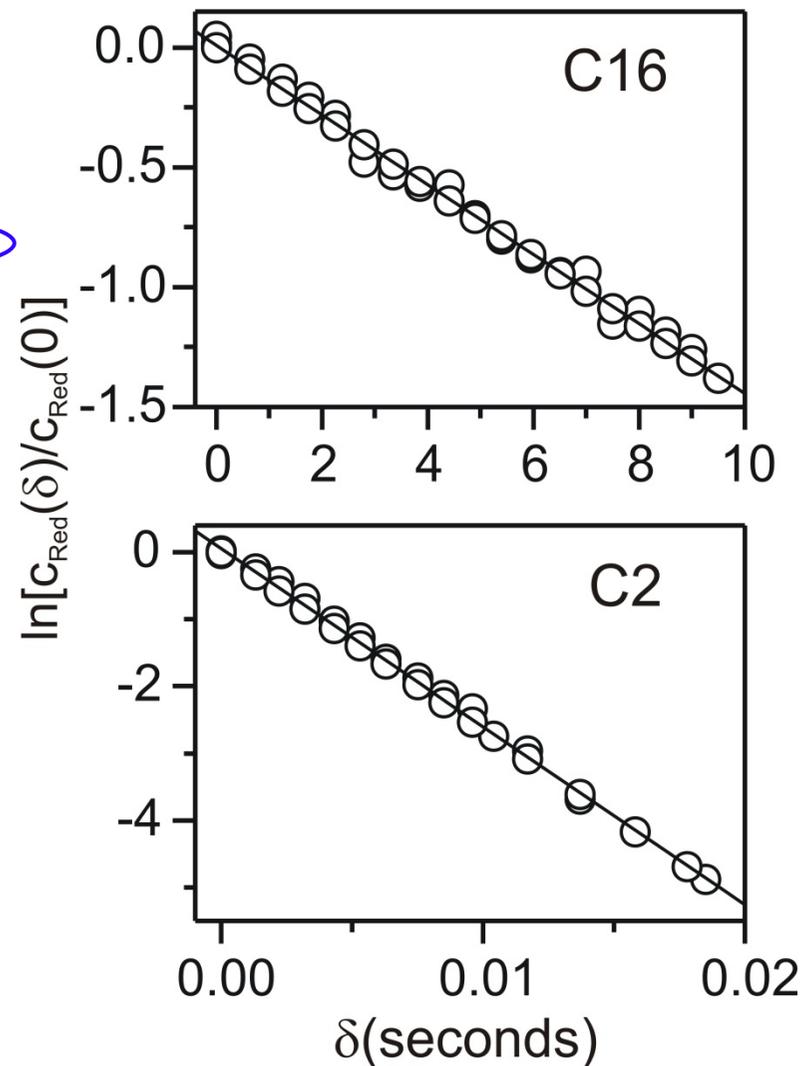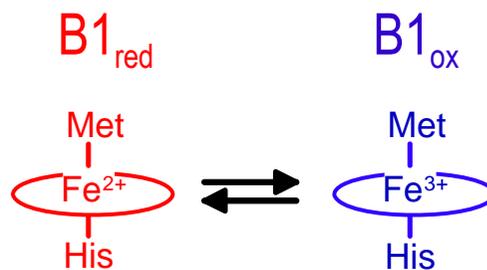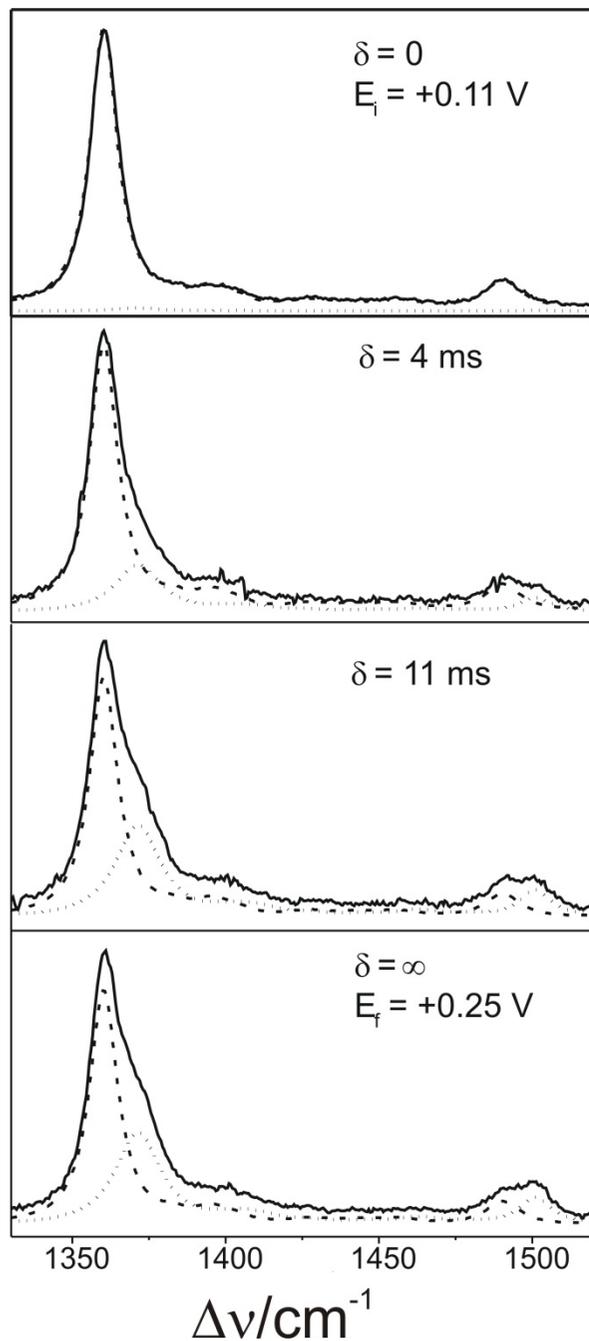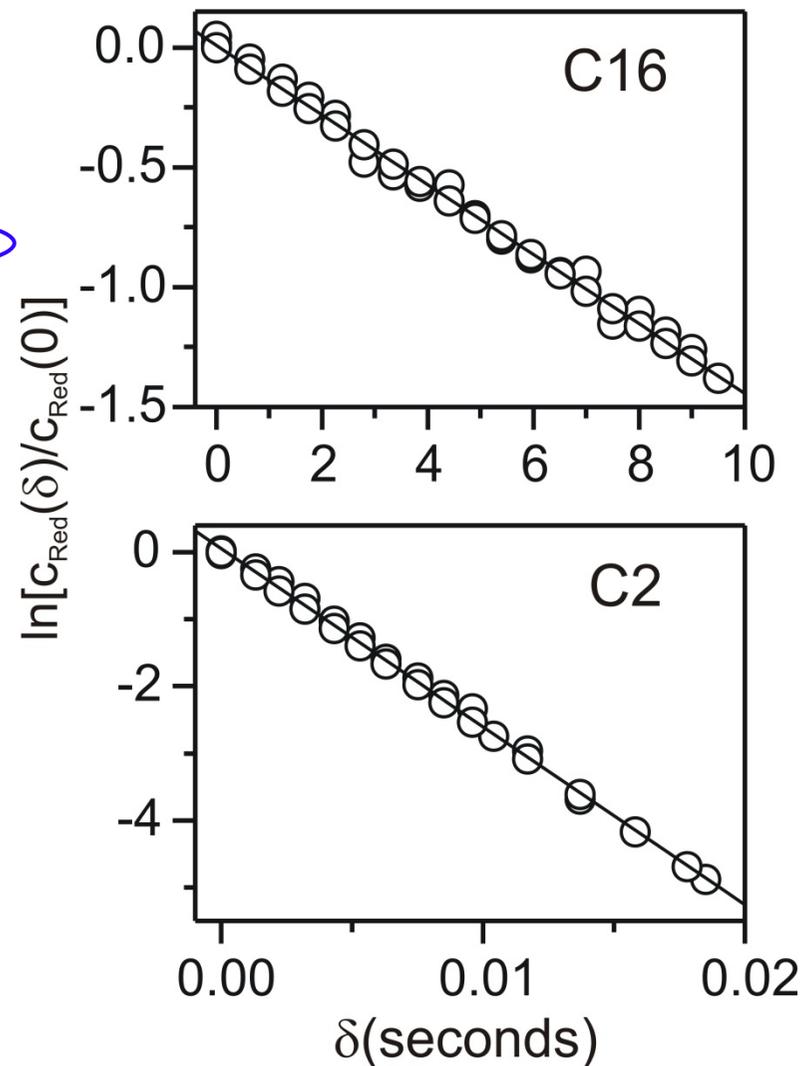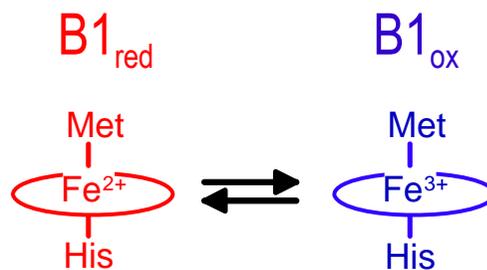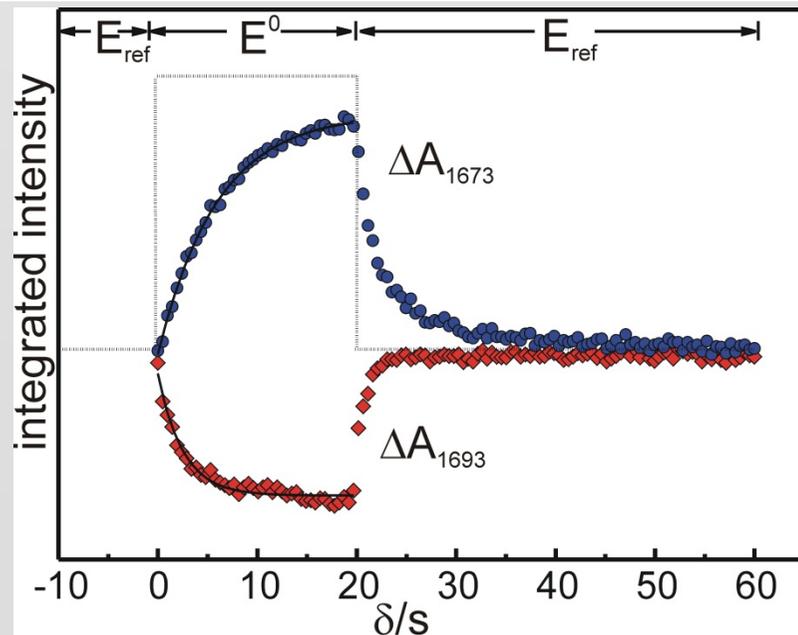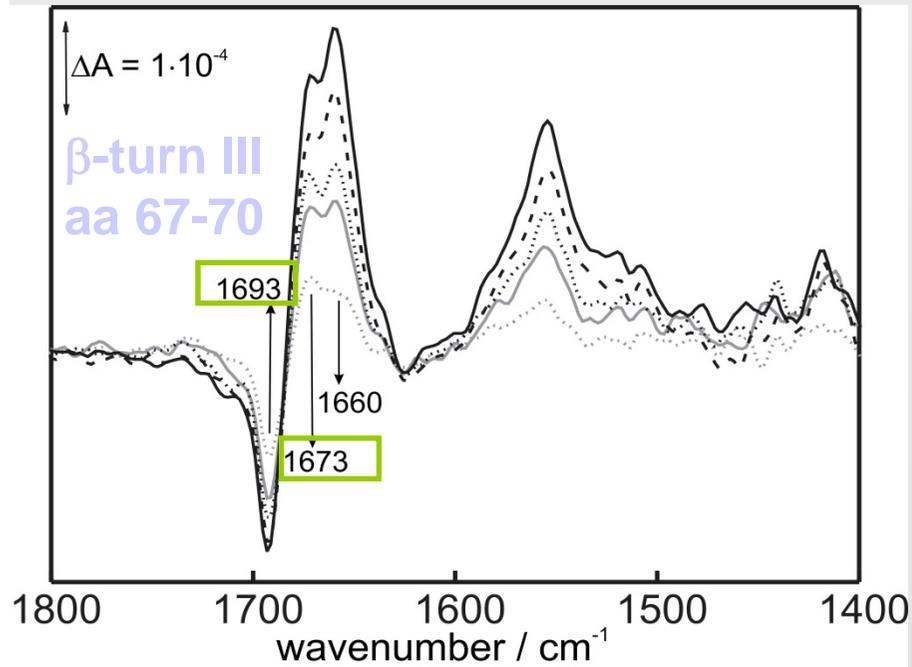
Protein structural changes monitored
by SEIRA spectroscopy
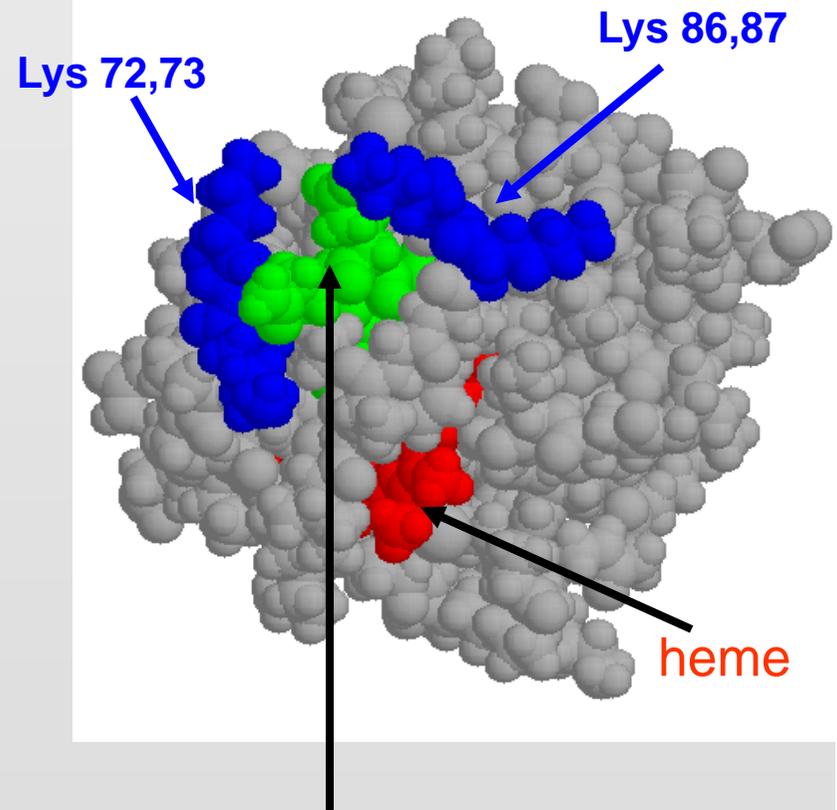
Amide I band changes of the β-turn III segment 67-70 occur simultaneously with electron transfer

RR	Photoinduced processes	cw (> 100 ns) pulsed (> 10 ps)	Photoreceptors Ligand binding bimolecular reactions with caged compounds
IR	Pump-probe	rapid scan (> 10 ms) step scan (> 100 ns)	
RR	Bimolecular reactions Rapid mixing	cw (> 100 μs)	Protein folding Enzymatic reactions Ligand binding
IR		rapid scan (> 10 ms)	
SERR	Potential-dependent processes at electrodes	cw (> 10 μs)	Re-orientation Conformational transitions electron transfer
SEIRA	Potential-jump	rapid scan (> 10 ms) step scan (> 10 μs)	

TUTORIALS IN BIOPHYSICS

Friedrich Siebert and Peter Hildebrandt   WILEY-VCH

Vibrational Spectroscopy in Life Science

The authors describe basic theoretical concepts of vibrational spectroscopy, address instrumental aspects and experimental procedures, and discuss experimental and theoretical methods for interpreting vibrational spectra. It is shown how vibrational spectroscopy provides information on general aspects of proteins, such as structure, dynamics, and protein folding. In addition, the authors use selected examples to demonstrate the application of Raman and IR spectroscopy to specific biological systems, such as metalloproteins, and photoreceptors. Throughout, references to extensive mathematical and physical aspects, involved biochemical features, and aspects of molecular biology are set in boxes for easier reading.

Ideal for undergraduate as well as graduate students of biology, biochemistry, chemistry, and physics looking for a compact introduction to this field.

From the Contents:
- Introduction
- Introduction to infrared absorption and Raman spectroscopy
- Instrumentation
- Experimental Techniques
- Proteins
- Heme proteins
- Non-heme metalloproteins

Wiley Tutorials in Biophysics is a series edited by A. Herrmann (Institute of Biology/Biophysics, Humboldt-University Berlin) and K.-P. Hofmann (Charité, Humboldt-University Berlin). Biophysics is the branch of physics focused on the study of biological systems. This series address the key issues within this rapidly growing field of research.

Friedrich Siebert is Professor for Biophysics at the University of Freiburg. He studied physics in Freiburg and Hamburg, receiving his PhD in solid-state physics. Since his diploma thesis he is working with different methods of vibrational spectroscopy. In 1972 he changed to biophysics, establishing the method of static and time-resolved infrared difference spectroscopy. Current research interests are photo-biological systems, membrane proteins and receptors, surface-enhanced techniques, time-resolved IR techniques.

Peter Hildebrandt received his PhD in chemistry from the Universität Göttingen in 1985. After a post-doc stay in Princeton, he worked in research institutes in Göttingen, Mülheim, and Lisboa. Since 2003 he is Professor for Physical Chemistry and Biophysical Chemistry at the Technische Universität Berlin. His research is dedicated to vibrational spectroscopy of biological systems, focussing on Raman spectroscopic techniques applied to redox proteins and photoreceptors.

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Most of the figures shown in this presentation have been taken from this book