Ultrasensitive nonlinear vibrational spectroscopy of complex molecular systems

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CHAPTER 1

INTRODUCTION

1.1 HISTORY OF SPECTROSCOPY

1.1.1 OPTICAL SPECTROSCOPY

Optical spectroscopy is one of the most important and influential instruments in the toolbox of a scientist. It provides a unique possibility to link a macroscopic observation, dispersed light, to microscopic properties such as the atomic composition, the presence of specific chemical groups and even the molecular structure.

Newton is often referred to as the first spectroscopist[1,2] since he coined the term spectrum. Additionally, he demonstrated that sunlight is composed of multiple colors by dispersing a ray into its rainbow spectrum and recombining it again using a set of prisms.[3] In the beginning of the 19th century Wollaston improved Newtons approach and was the first to discover that, upon close inspection, the continuous spectrum of the sun is interrupted by sharp dark bands.[4] Although the precise origin of these (Fraunhofer) lines remained a mystery for another hundred years, their presence hinted at a way of distinguishing light sources with a similar appearance, like stars, by characterizing their spectrum. In 1822 Herschel observed that different substances burn at distinct colors and noted that even extremely "minute" quantities of certain elements can be detected by the hue of the flame.[5] Bunsen and Kirchhoff combined the previous two observations by demonstrating that the low pressure gas of a substance absorbs and emits light of exactly the same color.[6] After cataloging the absorption lines of most of the alkali and earth alkali metals, they went on and used their new realization to prove the existences of cesium and rubidium[7] by identifying, until then, unassigned emission lines in water from the village Dürkheim and in the mineral lepidolite.

Kirchhoff's rules established the possibility to describe the atomic composition of stars, light years away, by matching their absorption lines with the cataloged emission lines of all known elements. Equipped with this knowledge, Jules Jansen and Norman Lockyer proved the existence of helium in 1868 by observing the sun spectrum and identifying a so far unclassified sharp yellow line.[8] This was the first

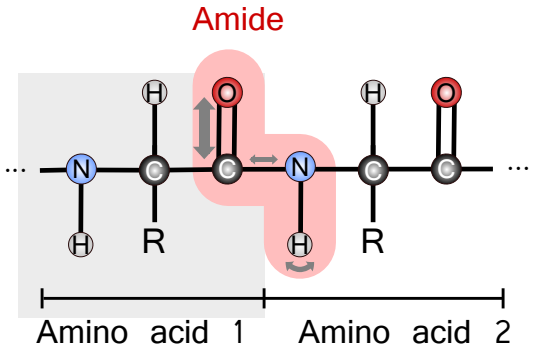

FIGURE 1.1. Two linked amino acids. The amide I mode involves the amide group (highlighted in red) and arises mainly from the C=O-stretching vibration with contributions from the C–N-stretching and N–H-bending vibration.[9]

time that an element was discovered on a celestial body other than the earth. Since then, the discovery of most elements has either been led or confirmed by means of optical spectroscopy.

1.1.2 INFRARED SPECTROSCOPY

The electromagnetic spectrum has more to offer than just the visible. Herschel showed already in 1800 that there is invisible radiation beyond the red part of the solar spectrum (the infrared), carrying enough energy to heat thermometers above room temperature.[10] Nevertheless, it took almost a century until the exploration of this new invisible part of the spectrum began. In 1882, Abney and Festing invented a new photographic compound which was sensitive in the near infrared (<1.2 μm). They studied different organic compounds and discovered extinction lines similar to the ones found in the visible. These lines were highly correlated with the presence of certain chemical groups which led to initial speculations that infrared spectroscopy is less sensitive to the presence of specific elements but instead rather interacts with the collective motion of atoms.[11] This theory was confirmed by Coblentz who can be seen as the father of infrared spectroscopy. Not only was he the first to measure more than a hundred organic compounds in the whole mid-infrared range (1-15 μm) and to coin the term mid-infrared; but he was also the first to provide experimental evidence for the connection between fundamental vibrations and molecular structure.[12] Only years later Rose published a paper where he concisely stated that the structural groups of hydrocarbons have a specific absorption frequency and a fixed cross section independent of the compound they are part of. This laid the foundation for the qualitative and quantitative analysis of arbitrary samples using infrared spectroscopy.[13,14] In the 1940s the American petroleum and rubber industry became intensely interested in a fast and efficient method to identify,

monitor and distinguish compounds with very similar atomic composition but a different chemical structure such as crude oil and its refined derivatives. They chose infrared spectroscopy which by then showed very promising indications of being chemically specific and capable of identifying chemical bonds and groups, and even capable of providing some structural information such as the distinction of various structural isomers. The industrial application fueled the development of new, easy to use spectrometers, which led to the dispersion of infrared spectroscopy into other industries and academic research.

1.1.3 PROTEIN SPECTROSCOPY

Arguably one of the most powerful applications of infrared spectroscopy is the investigation of the three-dimensional structure of proteins. Proteins and peptides are the molecular machines in our bodies and they orchestrate almost every biological process from DNA replication[15] to mood regulation.[16,17] The key aspect to their functionality is their three-dimensional form.[18] Small variations in their structure can already have severe impact on their efficiency or, even worse, result in diseases, such as sickle-cell anemia[19] or progeria syndrome[20a]. Therefore, the precise knowledge of the (correctly folded) protein structure is of key interest, not only for medical purposes, but for a fundamental understanding of biology.

One way by which infrared spectroscopy can provide information about the protein structure is via the spectrum of the amide I vibration. The amide I vibration is one of the characteristic modes of the peptide bonds inside proteins. It mainly corresponds to the stretching of the backbone carbonyl group (C=O, ∼80%) with additional contributions from the C–N-stretching and N–H in-plane bending vibration (see Figure 1.1).[9] This mode has a rather large infrared extinction coefficient, absorbing at ∼1650 cm^{-1}, and is extremely sensitive to changes in the backbone environment, such as hydrogen bonding. In principle, a protein which consists of n amino acid residues contains $n-1$ amide I oscillators. The spectrum of a well-structured protein usually looks very different from the spectrum of an isolated amide I vibration. The reason for this difference is the coupling between the individual amide groups inside a protein, which leads to collective oscillations of the involved amide I vibrations. The frequencies of these collective oscillations depend sensitively on the three-dimensional organization of the residues inside the protein, and therefore on the secondary structure.[21]

Figure 1.2 shows how the amide I spectra can be used to distinguish between the three most common secondary-structure motifs: random coil, α-helices and β-sheets. Usually, unstructured domains (random coils) show a broad featureless band at 1639–1654 cm^{-1}, α-helices a relatively narrow band at 1642–1660 cm^{-1} and β-sheets mostly show two distinct resonances, with one intense peak around 1630 cm^{-1} and a weak one at ∼1680 cm^{-1}.[21] These spectral signatures allow not only a qualitative but also a quantitative analysis of the protein architecture.[22,23]

[a] premature aging

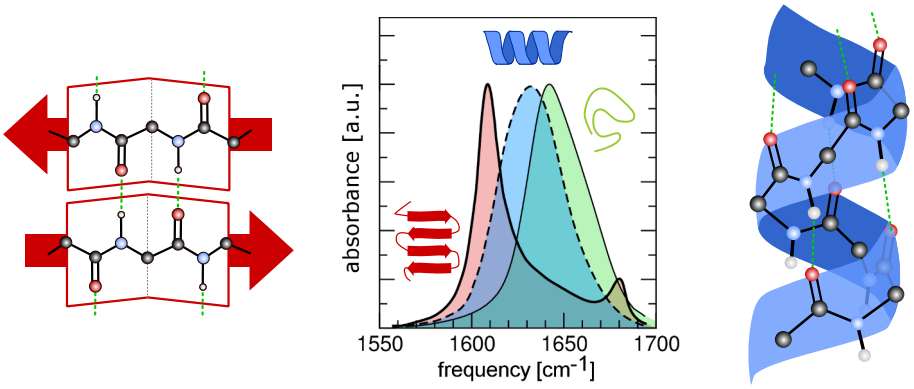

FIGURE 1.2. Infrared spectra of the amide I region. Spectrum (center) of the same peptide (poly-l-lysine) in three distinct secondary structure conformations (spectra adapted from[24]). The different spectral shapes of β-sheets (red, left) and α-helices (blue, right) originate from distinct hydrogen bonding patterns and phase relations of the involved amide groups.

1.2 NOVEL DEVELOPMENTS IN PROTEIN INFRARED SPECTROSCOPY

1.2.1 TWO-DIMENSIONAL INFRARED SPECTROSCOPY

One limitation of infrared spectroscopy is that information-rich spectral windows, like the amide I region, are often crowded with absorption bands originating from various chemical groups and/or different secondary structure motifs. This overlap of many resonances can result in hard to interpret, featureless and broad lineshapes. A second limitation is that although most chemical groups have an infrared signature, it is very difficult to obtain information about the spatial organization of the groups from the linear spectrum. In other words, the linear spectrum does not allow one to unambiguously determine how different vibrational modes are coupled to each other.

Both shortcomings can be lifted by moving from steady state linear infrared spectroscopy to ultrafast two-dimensional infrared (2DIR) spectroscopy. In 2DIR spectroscopy ultra-short laser pulses are used to excite molecular vibrations and to follow their relaxation dynamics. The excitation acts like a short-term tag which labels an oscillator and allows to track it on a femtosecond timescale. Similar to nuclear magnetic resonance (NMR) spectroscopy, the spectra in 2DIR spectroscopy are spread along two dimensions where one axis marks the excitation and the other the detection frequency. This frequency dispersion by itself can greatly simplify the assignment of the observed vibrational bands. Furthermore, interacting chemical groups give rise to distinct spectral features, i.e. cross-peaks, which carry information about the underlying coupling mechanism and the relative orientation of the involved

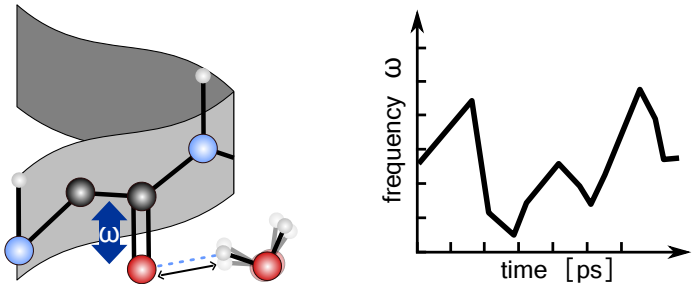

FIGURE 1.3. Rapid motions of solvent molecules in close vicinity of the peptide backbone cause the amide I vibrational frequency ω to fluctuate. 2DIR spectroscopy can be used to access the timescale of these fluctuations, and thereby the solvent exposure, by monitoring the time-dependent lineshapes (Section 2.5.4).

vibrational modes.

Many studies have demonstrated that 2DIR spectroscopy is capable of extracting direct structural information about the investigated system. In one of the earliest 2DIR experiments, Woutersen and Hamm used polarization-resolved 2DIR to study the spatial structure of the model peptide trialanine.[25] By studying the polarization dependence of the cross-peaks between the alanines and assuming a planar geometry for the amide units, they could determine the absolute angles between the three residues. In a different set of studies the group of Rubtsov looked at the picosecond dynamics of cross-peaks in various molecules and demonstrated that the time-dependence of the cross-peak intensity can be used to measure the atomic distance between chemical groups with angstrom precision.[26]

In addition to providing structural information, 2DIR is extremely sensitive to protein-solvent interactions. The rapid motions of solvent molecules close to the protein cause fluctuations in the amide I vibrational frequency (Fig. 1.3) resulting in inhomogeneous broadening of the spectrum. Since the time resolution of 2DIR spectroscopy is higher than the timescale of most solvent motions, also the solvent-induced spectral diffusion can be followed in real time. The group of Hochstrasser used a combination of isotope-labelling and 2DIR to study the time scale of the frequency variation of most of the amide I vibrations in β-amyloid fibrils. Fast variations were only observed for a small subset of residues, which they assigned to the presence of mobile water molecules inside the otherwise dry protein[27](Fig. 1.4). These results indicate that 2DIR is capable of detecting individual, structurally significant water molecules in the vicinity of the protein backbone.

Intrinsically disordered peptides In recent years, it became increasingly obvious that many proteins are not perfectly well structured. On the contrary, it is currently assumed that 35% of the proteins in the human proteome contain extended intrinsically disordered domains.[28] These unstructured regions allow the proteins to display a large conformational diversity, and thereby scaffold and bind with numerous interaction sites. Furthermore, the high flexibility often leads to a

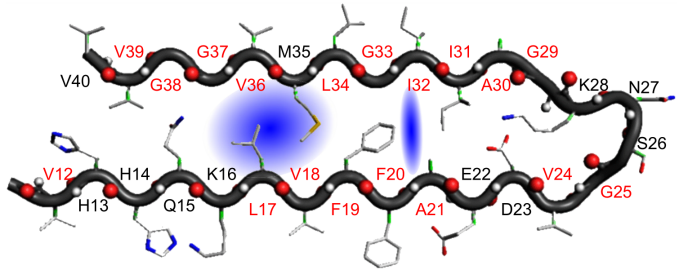

FIGURE 1.4. Schematic representation of one monomer unit of Aβ40 fibril. The measurement of the frequency fluctuations of individually labeled residues (red) with 2DIR suggested the presence of individual water atoms (blue), contained inside the otherwise dry protein.[27]

low binding affinity resulting in fast interprotein interactions, making proteins containing intrinsically disordered domains a key element in many cellular signal-processing pathways.[29] Malfunction of intrinsically disordered proteins (IDP) has been linked to a variety of human maladies including diabetes, cardiovascular and neurodegenerative diseases and most cancer varieties.[30,31] Oftentimes the reason for the malfunction originates in misfolding of the proteins into a nonfunctional, insoluble aggregate. A detailed understanding of the aggregation process and, in general, the physiochemical properties of the unstructured domains, is essential for the development of possible cures. Unfortunately, fast progress has been limited due the extreme flexibility of these proteins.

Most IDPs occupy a large and fairly flat energetic landscape which allows them to rapidly interconvert between different conformations and which prevent high quality crystal formation. This makes them an extremely difficult subject for conventional experimental methods to study protein structure, such as x-ray crystallography and NMR spectroscopy.[32] Therefore, ultrafast experimental methods can be very useful to extract direct structural information.

Elastin-like polypeptides (ELPs) were proposed as a minimal model system for the investigation of IDPs since they possess many physical, chemical and biological properties common to IDPs.[33] ELPs are intrinsically disordered biopolymeres which can undergo a temperature-induced and aggregation-like phase transition. In Chapter 4 we will employ the structural specificity and picosecond time-resolution of two-dimensional infrared spectroscopy to investigate the inverse-temperature transition of ELPs and to identify residual structural elements.

1.2.2 EFFORTS TO ENHANCE IR SENSITIVITY

Although infrared absorption spectroscopy provides a powerful tool to study biomolecules in solution, it is severely limited when it comes to the investigation of nanometer-sized samples, like self-assembled monolayers, supported lipid bilayers (and possibly anchored membrane proteins), or in general ultrathin films. The origin for this limitation is twofold: most chemical groups have a relatively small

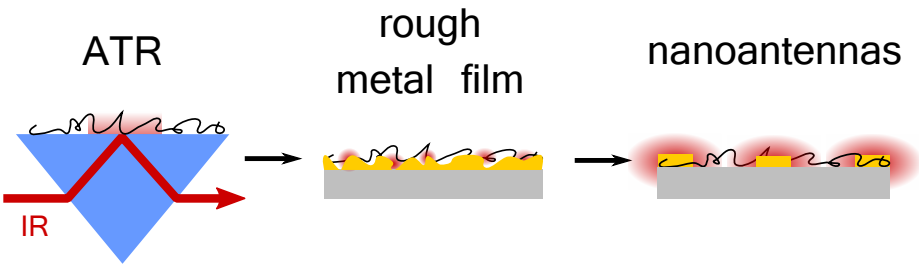

FIGURE 1.5. Efforts to enhance the infrared absorption of (ultra-)thin films (see text for discussion).

absorption cross-section in the infrared and the high solvent-to-film ratio causes significant background absorption.

A common approach to mitigate these problems is to employ attenuated total reflection infrared spectroscopy (ATR-IR). In ATR-IR the infrared light is passed through an optically dense crystal and internally reflected from a surface onto which the sample is deposited. The reflection is accompanied by an evanescent field which extends into the sample volume. The resulting short optical path lengths of around 0.5–3 μm allows one to study samples with a strong solvent absorption[34] . In addition, the optical path length is highly reproducible (because it does not rely on the use of a sample spacer), so that the solvent background can be subtracted very accurately. Although this method can greatly boost the sensitivity for adsorbed thin films, it is generally insufficient to achieve monolayer detection. In 1974, Fleischman et al. showed that monolayer sensitivity can be obtained in Raman scattering experiments by attaching the studied molecules to a roughened silver film, introducing the new method of surface enhanced Raman spectroscopy (SERS).[35] Half a decade later, it was demonstrated that similar results can be achieved in infrared spectroscopy by adsorbing molecules to a metal film deposited on top of an infrared transparent substrate,[36] although with significantly less signal enhancement. The signal enhancement in surface enhanced infrared absorption spectroscopy (SEIRA) originates from nanometer sized features and the strong curvature of the roughened metal film which leads to strong local electric fields.

In recent years, nanofabrication methods became commonly available which allowed the replacement of the metal films with specifically engineered metal nanostructures. These resonant nanoantennas act like little lenses, strongly focusing the infrared light into subwavelength volumes, thereby strongly enhancing the vibrational signals from molecules adsorbed to the nanoantennas. The current generation of SEIRA can thus achieve zeptomolar sensitivity[37,38] and has been incorporated in 'on-chip' designs paving the way for a new class of biological sensors.[39]

Despite these advances, most studies so far have been limited to exploring surface enhancement in linear infrared spectroscopy. Obviously, it would be highly beneficial to also apply these surface-enhancement techniques for increasing the sensitivity of nonlinear infrared spectroscopy. This would, for example, make it possible to study the structure of membrane proteins that are embedded in surface-attached

membranes. A large part of this thesis is dedicated to the development of the technique of Nanoantenna Enhanced Nonlinear Infrared Spectroscopy (NENIS).

1.3 OUTLINE OF THE THESIS

Chapter 2 presents the theory on which the experiments described in this thesis are based. The first part of the chapter deals with the theory of surface plasmons. In the second part of the chapter non-linear light conversion is discussed, and the fundamentals of linear and nonlinear infrared spectroscopy are introduced. The last part of the chapter introduces the experimental observables of 2DIR spectroscopy and discusses the information they provide. In Chapter 3 the experimental setup that has been used to collect data for this thesis is described. In Chapter 4 the inverse temperature transition of elastin-like peptides is studied with time resolved 2DIR. Chapter 5 introduces a novel method to perform nonlinear vibrational spectroscopy on nanoscale volumes with the assistance of infrared nanoantennas. The capabilities of this method are demonstrated by recording the 2DIR spectrum of a 5 nm thick film of polymethylmethacrylate. In Chapter 6 an analytical model for nanoantenna enhanced vibrational spectroscopy is introduced and the implications of local-field effects are discussed. In Chapter 7 we use 2DIR spectroscopy to study the molecular motion of the organic cation in methylammonium lead iodide perovskites, and in Chapter 8 we investigate how this motion is affected by the substitution of the halide anions in the material.