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Interrogating (anti)microbial mechanisms using vibrational spectroscopy – from electrostatic effects in catalysis to virus-membrane interactions

Vibrational spectroscopy is a highly sensitive method that delivers detailed chemical information by probing molecular vibrations. As such, it has become an invaluable tool in the field of (bio)physical chemistry for the investigation of structural changes from subtle hydrogen bonding reorganizations to large-scale rearrangements in secondary structures of biomolecules [1]. Recent advances enabled to go even beyond such *structural* investigations and interrogate the consequences of *electrostatic* interactions or perturbations. This is achieved by both the experimental quantification of electrostatic forces via the vibrational Stark effect (VSE) [2] or the in-situ control of electrostatic conditions using surface-sensitive spectroelectrochemical approaches [3]. This unique "electrostatic resolution" of vibrational spectroscopy is a particularly powerful feature, as many phenomena in the condensed phase are thought to have a significant electrostatic origin [4] – among them biomedically relevant processes. Utilizing the versatility of vibrational spectroscopic methods, we provide a physicochemical perspective on one of today's largest biomedical challenges, the understanding of molecular mechanisms and catalytic effects in (anti)microbial infections and resistances [5, 6]. Many aspects of this research, in particular those relating to electrostatic effects in catalysis, are generalizable and hold large potential to be transferred into the design of nanoreactors with catalytic properties.

1. Exploring electrostatic catalysis in antibiotic resistance using vibrational Stark effect spectroscopy

Various physical models have been developed to describe the unprecedented catalytic activities of enzymes, which can accelerate chemical reactions by as much as 10^{17}-fold [7]. Among these models, the concept of electrostatic catalysis (EC, or electric field-driven catalysis) has emerged as one of the most important catalytic mechanisms [4]. In EC, active site electric fields interact preferentially with the charge distribution in the transition state to lower activation barriers (Fig. 1A), providing a powerful, physicochemical framework to tune catalytic efficiency, or reactivity in general.

Interestingly, the model reaction at a carbonyl shown in Fig. 1B (top) is reminiscent of the most important mechanism of antibiotic resistance: the catalytic hydrolysis of β-lactam antibiotics, which is associated with about one million death worldwide [5]. We aimed to test if EC underlies antibiotic resistance. To this end, we utilized the VSE, a physical phenomenon that enables to quantify electric field strengths at the location of so-called VSE reporter groups via shifts of their vibrational wavenumber [2]. Here, the reactive β-lactam C=O group acts as the VSE reporter. This is demonstrated by an IR absorption peak shift of -60 cm^{-1} (Fig. 2B, bottom) along the solvatochromic trend by exposing the β-lactam C=O to a range of electrostatic solvent environments, from the apolar hexane to polar water [8]. Surprisingly, when the β-lactam C=O of the drug penicillin G is bound to the active site of the β-lactamase enzyme TEM-1, which confers antibiotic resistance, an even larger peak shift of -140 cm^{-1} is observed [9, 10]. Utilizing VSE-based calibrations (via VSE spectroscopy in defined electric fields, and molecular dynamics simulations with high level description of electrostatics using the AMOEBA force field) [8], we converted these peak shifts to the electric field scale (see top axis in Fig. 2B, bottom) revealing that the enzymatic active site exerts an immense electric field of -1.7 V/Å onto the β-lactam C=O; one of the strongest active site electric fields determined to date. This field reduces the activation barrier by 46 kJ/mol, accelerating the reaction electrostatically by 10^7-fold [11]. Importantly, according to convention, the negative sign implies a stabilizing C=O-dipole–field interaction, which provides direct experimental evidence that transition state stabilization within the framework of EC underlies the catalytic mechanism of antibiotic resistance.

Since the β-lactam C=O reports on the electrostatics at a specific location in the active site (with Å-resolution), significant contributions from other regions could go undetected. Assessing such effects requires the use of orthogonal VSE probes, which absorb in different spectral regions and can be introduced into the drug or the protein matrix. Towards this strategy, we perform in-depth characterizations of several VSE reporters, such as the C≡N with its stretching vibration at ~2100 cm^{-1} (vs. C=O stretches at ~1700 cm^{-1}; see Fig. 1C). Despite the widespread

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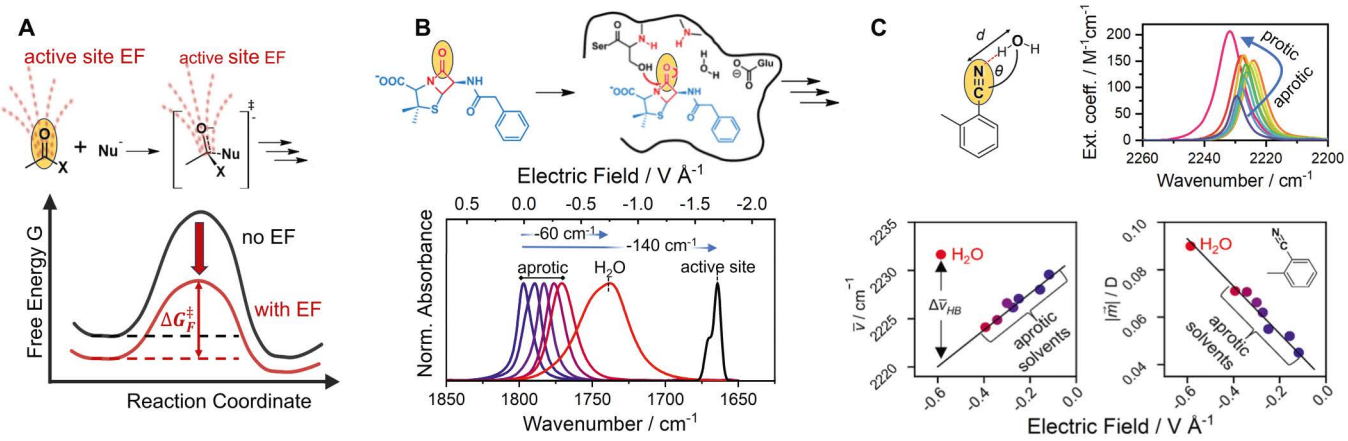

Fig. 1: A) Electrostatic catalysis exemplified by a reaction at a carbonyl group. **(Top)** Electric fields (red dashed lines) interact with the charge distribution of the reactive molecule, where in particular the C=O experiences a charge reorganization towards the transition state. **(Bottom)** Active site electric fields – when properly oriented – can preferentially stabilize the reaction's transition state and lower the activation barrier. **B – Top)** The catalytic reaction underlying antibiotic resistance against β-lactam drugs (here penicillin G is shown) occurs at the reactive β-lactam C=O group (highlighted in yellow). **(Bottom)** This C=O group can be used as a VSE probe to measure electric fields via the C=O stretch; the solvatochromic trend from hexane to water as solvent causes a shift by -60 cm^{-1}, which can be used to translate the wavenumber axis to an electric field axis (bottom vs top axes). Inside the enzyme active site, the C=O stretch wavenumber is shifted by -140 cm^{-1}, consistent with an immense electric field of -1.7 V/Å. **C – top)** Nitriles (C≡N) show a more complicated behavior because they are sensitive to the presence and geometry of hydrogen bonds (HB). This leads to a redshift in aprotic, but a blueshift in protic environments when the polarity is increased; at the same time the peak intensity increases monotonically. **(Bottom)** Via a dual analysis of C≡N wavenumber ($\bar{\nu}$) and transition dipole moment ($|\vec{m}|$, proportional to the square-root of intensity), it is possible to quantify local electric fields (right plot) and HB geometry via the HB blueshift ($\Delta\bar{\nu}_{HB}$; left plot). Data in B and C reused from refs. [8, 9, 15, 17] with permission. Copyright 2021 – 2024 American Chemical Society / the authors.

use of C≡N groups as VSE reporters [2, 12–15], their analysis has been complicated due to an anomalous hydrogen bond blueshift. Combining experimental and computational IR spectroscopy, we established the dual use of IR peak position and intensity (more precisely, the transition dipole moment) of the C≡N stretching to quantify simultaneously the electric field and the hydrogen bond geometry at the C≡N group [16, 17]. Together with CF_n, CD and other groups [18–20], a versatile set of orthogonal VSE probes is available, enabling the experimental quantification of electrostatic forces in solvents, at electrode interfaces, or in biological settings.

2. Tracking molecular events during viral infection at model membranes using surface-enhanced infrared absorption (SEIRA) spectroscopy

Viral infection processes offer multiple vantage points for antiviral treatment, but effective drugs are only available for a limited number of viral diseases [6]. This motivates the development of new spectroscopic methods that can provide molecular-level insights into viral action and inhibition to explore new drug targets. A common feature among many such targets is their localization at biointerfaces – either at the viral lipid membrane itself or in the interactions with the host cell membrane.

Previously, we have developed combinations of surface-enhanced infrared absorption (SEIRA) spectroscopy with bilayer lipid membrane (BLM) systems, which can mimic such biointerfaces (Fig. 2A and C) [3]. In SEIRA spectroscopy, a nanostructured Au film enhances IR signals by up to 10^3-fold via a plasmonic mechanism with a surface-sensitivity of roughly 10 nm [3]. Constructing the BLM within this enhanced region provides the unique advantage of selectively monitoring processes at or within the membrane [3] (note that the Au film can also act as an electrode to control interfacial electrostatics, transmembrane potentials, or redox processes [21–23]). Now, we investigated potential antiviral drug targets in the Influenza A virus (IAV) as a proof-of-concept for future applications. One general antiviral drug target is presented by so-called viroporins, i.e. viral ion channels which exhibit key functions during viral infection [24]. Constructing a model system of the IAV membrane on the SEIRA Au film (Fig. 2A), we tracked the activity of its viroporin, the protonation-dependent M2 proton channel [25]. The spectra revealed highly specific features consistent with large-scale reorientation of α-helices upon channel opening (Fig. 2B). Comparison with density-functional-theory-based computational spectra – accounting for the transition dipole moment vectors of the helical amide modes and the polarization direction in SEIRA – enabled us to quantitatively describe the change in the opening angle of the channel [21, 25]. Strikingly, these spectral features disappeared in the presence of the inhibitor Rimantadine, providing a direct spectroscopic marker to track the function and inhibition of viroporins from pathogenic viruses in future studies.

Other popular antiviral strategies aim to target viral binding to receptors at or viral fusion with the host membrane. To study these events for IAV, we inverted the experimental setup and constructed a host-cell membrane mimic on the SEIRA Au surface, incorporating glycosylated lipids as viral receptors (Fig. 2C) [26]. Because IAV particles are ~100 nm in diameter, the ~10 nm surface sensitivity of SEIRA allowed us to specifically probe the relevant interfacial interactions between the virus and the host membrane mimic. Furthermore, constructing the host membrane model using deuterated lipids, we utilized the vibrational isotope effect to distinguish between spectral markers of viral and host model membrane (Fig. 2D). Upon triggering viral fusion via a pH change – an essential event preceding the release of viral genetic material – we dissected relevant structural changes of viral proteins and both membranes. Traditionally, such events are monitored by fluorescence-based assays, which require ex-

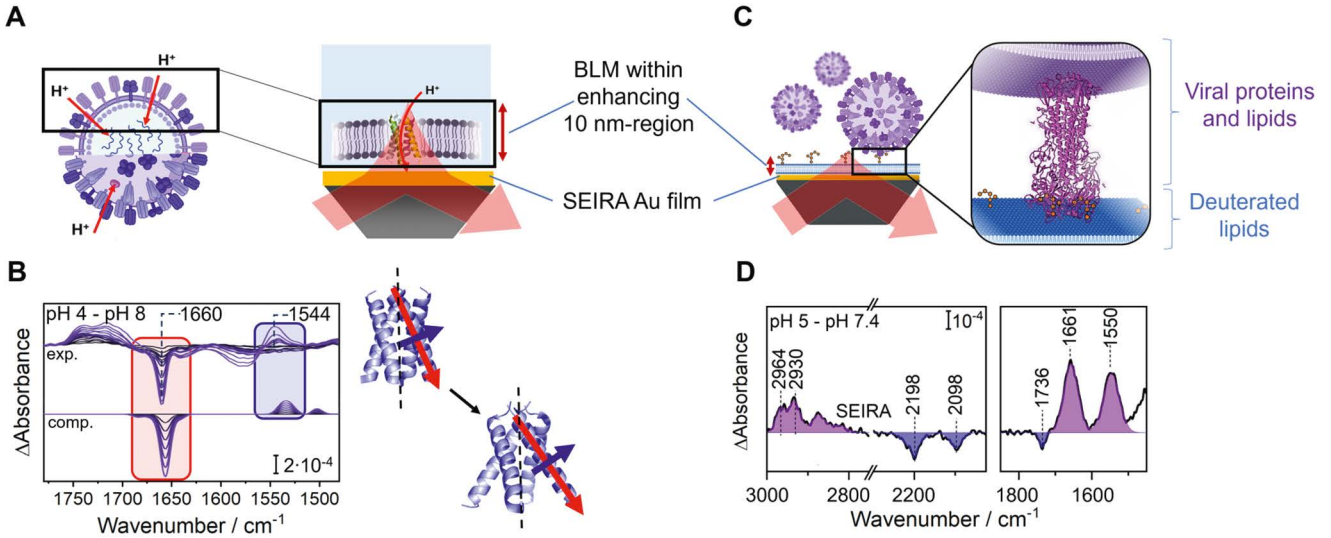

Fig. 2: A). In SEIRA spectroscopy, a model membrane can be constructed on a Au film that provides a surface-enhancement within the first 10 nm – here, a minimal model of the Influenza A viral membrane containing the proton transporting M2 channel. B) SEIRA spectra of the opening of the M2 channel show a specific spectral feature of amide backbone bands with inverted intensity (amide I in red; amide II in blue; top experimental spectrum). This spectral feature originates from the reorientation of the channel's α-helices due to their transition dipole moments (red and blue arrows) changing their orientation with respect to the vertical axis (dashed lines) – this is consistent with computational spectra obtained via density functional theory (bottom computational spectra). C) Inverting the system, the interaction of whole viruses with a host membrane model can be monitored within the enhancing 10 nm-region. D) Using deuterated lipids for membrane model enables to separate spectral information on the viral protein (purple, 1700 – 1500 cm^{-1}), viral lipid (purple, 3000 – 2800 cm^{-1}), and model membrane (blue, 2250 – 2000 cm^{-1}). Virus models are from BioRender; PDB IDs 6BLK, 6B0C, 6Y5G; data in B and D reused from refs. [25, 26].

ogenous dye labeling [27]. In contrast, our SEIRA-based strategy is fully label-free, exploiting the inherent vibrational fingerprint of viral constituents, while embedded in functional virus particles.

3. An outlook towards the nanoscale – IR detection of single viruses and nanosolvation phenomena

As of now, both strategies described above rely on the detection of an ensemble of molecules. For instance, while SEIRA enables to detect virus-membrane interactions in a label-free fashion, spectra of many virus particles are recorded simultaneously [26] – this contrasts with fluorescence-based assays, which require the introduction of dyes but provide the sensitivity to monitor single particles or molecules [27]. The use of atomic force microscopy (AFM)-based IR nanoscopic methods can overcome this limitation (Fig. 3). In scattering-type scanning near-field optical microscopy (sSNOM) and its nanoFTIR spectroscopy mode, the AFM tip acts as a local scatterer, enabling detection of IR spectral information from an area as small as 20 nm × 20 nm – thus achieving (super-)resolution well below the diffraction limit [28, 29]. As the size of the smallest viruses is around 20 nm, these IR nanoscopic methods will enable extending the presented SEIRA-based approach towards single-virus particle experiments. Moreover, IR nanoscopy can enhance our understanding of the concept of electrostatic catalysis as a general application in chemistry, as it is not exclusive to enzymes as described above. For instance, it provides the potential to specifically enhance chemical reactions in (nano)solvation environments as exemplified by investigations at interfaces or in microdroplets [30] providing the basis for the development of nanoreactors with tunable reactivity. The nanoscale resolution of IR nanoscopy provides the capability to probe zeptoliter-sized volumes and, by this, generate unique insights into such nanosolvation phenomena.

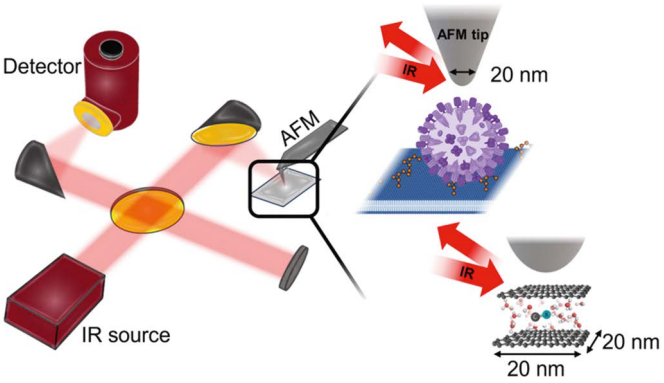

Fig. 3: IR nanoscopy uses an atomic force microscopy (AFM) tip to record IR images and IR spectra with a spatial resolution of 20 nm (left: a schematic set-up of an asymmetric Michelson interferometer combined with an AFM). The spatial resolution is compatible with the detection of single viruses (top right) or the investigation of phenomena in zeptoliter-sized volumes (bottom right).

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QUOTES

"It actually does not take much to be considered a difficult women. That's why there are so many of us."

Jane Goodall (primatologist & anthropologist)

Prof. Dr. Jacek Kozuch

Jacek Kozuch studied chemistry from 2004 to 2009 with a scholarship of the Studienstiftung des deutschen Volkes and obtained his doctoral degree in physical chemistry in 2013 under the supervision of Prof. Dr. Peter Hildebrandt, both at the Technische Universität Berlin. After an early independent postdoctoral research period in Berlin, he joined the group of Prof. Dr. Steven G. Boxer at Stanford University, USA, as a DFG research scholar from 2016 to 2019. In 2020, he established a junior research group at the Physics Department of the Freie Universität Berlin with the support of the Sonderforschungsbereich 1078, developing vibrational (nano-)spectroscopic methods for the investigation of biomedically relevant processes, for which he was awarded with the Nernst-Haber-Bodenstein prize 2025 of the Deutsche Bunsen-Gesellschaft. Since 2025, he is professor for physical chemistry at the Technische Universität Braunschweig. His research focusses on the development and use of experimental (and computational) vibrational spectroscopic methods to elucidate fundamental physicochemical concepts in catalysis in the condensed phase and in biomolecular processes during (anti)microbial action and resistance, and protein misfolding; with a particular focus on electrostatic effects that fine-tune activity.

QUOTES

On perspectives and "aha" moments.

"I find it fascinating that you can look at the same problem from different perspectives and approach it using different methods."

"Of course, the most rewarding part is the "Aha" moment, the excitement of discovery and enjoyment of understanding something new, the feeling of being on top of a hill, and having a clear view. But most of the time, doing mathematics for me is like being on a long hike with no trail and no end in sight!"

Mariyam Mirzakhani, Mathematician and Fields medal winner

"The more we know, the more we realize there is to know."

Jennifer Doudna (biochemist, Nobel Laureate in Chemistry 2020)