

**THE MEASUREMENT AND PHYSICS OF VIBRATIONAL
STARK EFFECTS**

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I certify that I have read this thesis and that it is in my opinion fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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THE MEASUREMENT AND PHYSICS OF VIBRATIONAL STARK EFFECTS**Steven S. Andrews, Ph.D.****Stanford University, 2001****Advisor: Steven G. Boxer****Abstract**

Weak electric fields cause minor changes to molecular infrared absorption spectra, called the vibrational Stark effect. It has been used here to study electric fields in proteins, and the physics of molecular vibrations.

Samples were immobilized in frozen solvents, with isotropic orientations, and analyzed with an FTIR and applied electric fields up to 1 MV/cm. An optical liquid nitrogen immersion cryostat was designed, making it convenient to use unsealed samples with direct electrical connections. Cryogen bubbling and schlieren near the sample is prevented with a jacket of liquid nitrogen at relatively low pressure around the sample chamber. Data collection methods include ones using a DC electric field, an AC field that is synchronized to the interferometer, and a step-scan method with lock-in amplification, of which the DC method has the lowest noise.

Stark effects were measured for the stretching vibration of CO bound to the myoglobin heme iron, yielding a tuning rate of $2.4/f \text{ cm}^{-1}/(\text{MV}/\text{cm})$, which is 4 times larger than for unbound CO (f is the local field correction factor). Spectra are reported as a function of pH, for various mutants, and for similar systems, resulting in a span of 60 cm^{-1} for the CO frequencies but similar Stark tuning rates, indicating that the frequency serves as a probe of the local electrostatic field. Using it this way, the matrix field in myoglobin parallel to the CO bond changes by about 8 MV/cm upon histidine protonation.

The physical origins of vibrational Stark effects were investigated with spectra for the C-N stretch mode of several small nitriles. Tuning rates range from $0.2/f$ to $0.7/f$ $\text{cm}^{-1}/(\text{MV}/\text{cm})$, with aromatic compounds towards the high end and symmetric dinitriles towards the low end. Most quadratic Stark effects are small and negative, while transition polarizabilities are positive and significantly affect Stark lineshapes. Symmetric dinitrile tuning rates decrease with increasing conjugation of the connecting bridge, due to improved mechanical coupling. Perturbation models show that Stark tuning rates arise from a combination of bond anharmonicity and the effect of an electric field on bond strengths. Transition polarizability arises from altered partial charges on atoms in a field.

Approved for publication:

By: _____

For the Department of Chemistry

Preface

When I started my graduate studies, the lobby of the Keck building was empty. Then, one day during my fourth year, I walked in the front door to discover a large bronze cube, covered with grooves, dents, bumps, and an embedded sphere; the cube was smashed on one of its corners into a cracking bronze base. It appeared as though an alien spaceship had crashed in the lobby during the night. However, there weren't any little green men running around and the glass ceiling was still intact, so I surmised that it must be artwork. This was confirmed by nearby plaques that read "Arnaldo Pomodoro, Italy b. 1926. Cube, 1964-67, Bronze. Given in memory of Pamela Djerassi, Class of 1971, by her parents, 1977.78" and, of course, "Please do not touch."

As I walked upstairs towards the lab, I thought about Arnaldo Pomodoro spending four years creating this bronze cube, and about the fact that I had spent four years trying to measure a vibrational Stark effect. In many ways, the end results aren't that different and even the processes of creating them have many similarities. Neither his cube nor this thesis will save lives, produce energy, create stuff, pollute, or do anything else that technologically developed societies are supposed to do. Instead, both results are trying, in their small ways, to inspire thought and to provide enlightenment. Good science is a work of art, where the results, whether concepts, equations, or images, bring out the underlying structure of nature and are aesthetically pleasing in themselves. While I don't claim that any of the science here is profound, I hope that you, the reader, will find it to be interesting and perhaps even aesthetic.

As with the cube, I would like to dedicate this work to the memory of someone; in this case, it is to my father, who has profoundly influenced my life in work, in play, and in my outlook on life. Unlike the cube though, there is no need for "Please do not touch." Instead, this thesis was written to be read (although I know that few people will actually read it), so please thumb through it, crease the binding, and mark it up. As long as there are computers and printers, more copies can always be produced.

Many people have contributed to this research. First and foremost, Steve Boxer has been an excellent advisor; he gave me the freedom to pursue my own interests, he provided insightful and accurate criticism, he continually assembles a talented and

dynamic research group, and he provided my funding. During my first year or two, Arun Chattopadhyay, Josh Goldsmith, Jay Groves, and Josh Salafsky introduced me to Stark effect and biophysics research. After several years of failed experiments and dead-end projects, things finally started working and someone was needed to help collect and interpret data. I was fortunate that Eunice Park was looking for a project at that time. She made rapid progress with the myoglobin Stark effect project and she provided me with an incentive to make the vibrational Stark hardware and fitting program useful for others. Since almost the day I started work in this lab, I have enjoyed frequent scientific discussions with everyone in the group. While these discussions often led to distractions to the research in this thesis, I have been grateful for the opportunity to learn about, among other things, molecular biology, electron transfer theory and experiment, and lipid bilayer diffusion, formation, and detection. Throughout this research, Pat Pietz has been invaluable with purchasing and administrative matters. I have also been fortunate to have access to the professional shops on campus, where Joe Rolfe, Manny Gutierrez, and Karlheinz Merkle helped me design and build the hardware for vibrational Stark spectroscopy. Several people deserve recognition for their contributions to the lab infrastructure. Melissa Thomas and Joe Johnson kept the safety people happy, and Bob Hu and Li Kung kept our computers running smoothly.

Several groups of people were not directly related to this research, but it would not have happened without them. My parents instilled in me a curiosity of the natural world and have provided a lot of guidance for many years. Many professors at Dartmouth, at the Woods Hole Oceanographic Institution, and at Stanford introduced me to much more of the natural world and gave me the tools to pursue those interests. While studying here, many friends have made these last several years especially enjoyable, including those from the Stanford outdoors community, those from the co-ops, fellow chemists, and others. With them, I have gotten to explore nearly all of the California rivers, as well as a good fraction of the rest of the Sierras.

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