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Development of Ultra-Sensitive Dielectric Spectrometers and Chemical Techniques for the Detection, Characterization, and Cataloging of Peptide and Protein Structural Motifs.

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Project Summary

The project will develop novel instrumentation and techniques to detect and resolve the dielectric responses, derived from the permanent dipole moments, of the intramolecular secondary structure and structural motifs of peptides and proteins. It will develop a set of custom dielectric spectrometers to encompass the frequency range of 10 μHz to 1 GHz. Commercially-available lock-in amplifiers and dynamic-signal analyzers will be connected to custom preamplifiers and high-resolution temperature-controlled capacitive sample cells managed by custom software for instrument automation and real-time data analysis to create the most sensitive, precise, and accurate dielectric spectrometers available to date. Advanced searches of the Protein Databank will ensure that well-characterized model peptide and protein systems are selected for the study.

The early goals will decrease undesired effects, such as electrode polarization in an aqueous environment, fringe, stray, and parasitic effects, and the Maxwell-Boltzmann distribution due to thermal agitation. High temperature studies, 20 to 300°C, will be performed under the denaturation of the protein. These studies should partially melt the protein's core, thus reducing its internal viscosity and allow dielectric measurements of its intramolecular structural motifs. Low temperature studies, -88 to 20°C, should reduce the noise due to thermal agitation and provide insight into the interactions of embedded water molecules within the protein. Further studies of proteins dissolved in low-dielectric gels in aqueous solutions will be used to create highly viscous semi-solids to rotationally and translationally immobilize the protein macromolecules.

The intellectual merit encompasses two primary goals. First, it will improve the limits of modern instrumentation, and the chemical and environmental techniques, required to observe and resolve the intramolecular dielectric responses within peptides and proteins. Second, the expected dielectric spectra, generated from the first goal, will increase the empirical knowledge of the intramolecular charge distributions and polarizabilities of the secondary structural motifs. For instance, high temperature studies will reveal enthalpies and activation energies of the intermotif dampening forces and of the molten core. Also, low temperature studies will reveal relaxation and resonance processes of internal hydration layers within peptides and proteins.

The broader impact of the project will range from a greater understanding of the intramolecular dielectrics, dynamics, and energetics of peptides and proteins to enzymatic manipulation of proteins using externally applied electric fields. For instance, the before mentioned intramolecular empirical data can dispel myths of dielectric continuum models and support charge heterogeneity of the molten core in proteins. Additionally, the empirical data will provide valuable activation energy, motif flexibility, intermotif dampening, and relaxation data for further theoretical models leading to intermotif and interchain interactions, and thus protein folding energetics and dynamics. Accurate theoretical studies would promote the fields of biophysics, biochemistry, and proteomics. Ultimately, it is conceivable that an external electric field can impose complex waveforms on proteins to manipulate their enzymatic activity, therefore affecting micro- and molecular cell biology, immunology, endocrinology, pharmacology, bioengineering, and nanotechnology.

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Project Description

a. Research Activities

The proposed research will design, develop, build, and calibrate four dielectric spectrometers and develop environmental and chemical techniques for the purpose of detecting and characterizing dielectric responses of intramolecular sub-domains, which form the components of the net dipole moment, and ultimately the intra- and interchain dielectric responses of intramolecular secondary structure and structural motifs of peptides and proteins.

The instrumentation will comprise a collection of commercially-available and custom preamplifiers, lock-in amplifiers, and dynamic-signal analyzers coupled to custom cylindrically-symmetric coaxial capacitor-based sample cells and interfaced to personal computers (PCs) for overall control of instrumentation, sample environmental control, and data acquisition and real-time analysis. The proposed project will design and construct four instruments; Dielectric Spectrometers 2 through 5 (DS2-DS5). They will calibrate and reproduce dielectric spectra against one another and include the frequency range of 10 μHz to 1 GHz. A summary is as follow:

	Lock-In Amplifier	Dynamic-Signal Analyzer
Low Frequency (f < 100 kHz)	**DS2**	**DS3**
High Frequency (f > 100 kHz)	**DS4**	**DS5**

It is only in recent years that all of the necessary tools have become available, with mature and stable technology, for the complete success of this project. This includes the electronics, instruments, temperature equipment, computers, software development capabilities, and the Protein Databank (PDB) [1] as outlined in the following description. In short, the project includes studies of high resolution temperatures, with a range of –88 to 300°C and stability of 0.01°C, pH variations for side R-group perturbations, and rotational immobilization using semi-solid low-dielectric gels. The Dielectric Spectrometer 1 (DS1) generated intriguing results with gelatin, myoglobin, and hemoglobin, which is further discussed under “b.2. Current Research and Instrumentation”.

a.1. Precise and Accurate Measurement and Confirmation of Dielectric Signals

Theoretically, the measurement of any physical quantity requires the mapping from a source set of an empirical domain onto image elements [2] which provide an accurate representation of the physical quantity. Practically, a dielectric spectrometer will electronically measure changes of an applied electric field, which correspondingly represents electric polarizations of the sample. The capacitive sample cell, and its corresponding electronic support, is the basis of the proposed spectrometers. The electronic support will consist of extremely sensitive, precise, and accurate commercially-available electronics that have never before been used together for studying the intramolecular dielectric responses of peptides and proteins, herein regarded simply as proteins.

Technically, the proposed electronics will act as the bridge between the dielectric sample cell, where the actual physical measurement is conducted, and the final reproducible data set. This proposal encompasses four dielectric spectrometers, the DS2 to DS5. Each proposed spectrometer has different frequency ranges, full-spectral run times, and sensitivities. Although the spectrometers have different capabilities, they will be used to find or resolve “areas of interest” within each of the samples of proteins. Since high-resolution temperature and frequency studies are proposed, it will be most efficient to first find “areas of interest” using low-resolution techniques using fast acquisition

methods. Then, once potential "areas" are identified, use the slow high-resolution methods, which will be more sensitive and accurate, to further resolve and confirm the low resolution techniques.

Two dynamic-signal analyzers for the DS3 and DS5 will be used for the low resolution studies. Both spectrometers will have two modes of operation. The first mode will pulse the capacitive cells with an excitation voltage and perform a fast-Fourier transform (FFT) to convert the time to the frequency domain of the detected response, thereby creating a fast admittance spectrum. This mode was developed in 1976 by Wada *et. al.* [3], in which dielectric spectra were obtained in milliseconds and has the potential of observing fast transients. In this mode, the proposed DS3 and DS5 will encompass the frequency range of 10 μHz to 1 GHz. The second mode will increment through each frequency, called sine-swept mode, to obtain a single measurement and accumulate the data to generate the final spectrum. Although the second sine-swept mode is much slower than the first FFT mode, it allows for greater sensitivity, superior signal stability, and specifies a frequency range of 10 μHz to 240 MHz. Both modes will allow multiple and simultaneously parallel sample cells to create a normalized comparison of the sample versus the reference for the final dielectric spectrum.

The DS3 is based on the Stanford Research SR785 Dynamic Signal Analyzer. It has its own arbitrary and sinusoidal function generator and the standard model will perform both modes of operation. Its specified frequency range is DC to 102 kHz with a 145 dB dynamic reserve. This spectrometer will be operational first because it requires the least software development.

The DS5 is an analyzer based on the Tektronix TDS5104 1-GHz Digital Phosphor Oscilloscope, the Fluke PM6681R Rb-based Frequency Counter, and the Agilent 33250A and Tektronix AFG3252 Arbitrary Function Generators. This collection of devices will be encapsulated into a single dynamic-signal analyzer with the abovementioned capabilities and both modes of operation. This will be accomplished by developing a custom software application in National Instruments' LabView. LabView comes standard with advanced real-time computational, transform, and statistical functions to convert these separate devices into a single user-friendly spectrometer.

For instance, the software will have the option of choosing either the first FFT or the second sine-swept mode. For the first mode, LabView will be programmed using its FFT functionality to convert the time domain digital waveforms from the oscilloscope to the frequency domain and display the real-time spectrum. The second sine-swept mode will use the 11-digit resolution of the Rb-based frequency counter and the time domain digital sine-waves of the oscilloscope to accurately lock-in on the excitation frequency, therefore rejecting noise, increasing the sensitivity, and generating an accurate spectrum. It will have a frequency range of 10 μHz to 240 MHz and a 1 μHz resolution. Additionally, the DS5 will allow for higher detection tolerances, leading to nonlinear effects in the dielectric studies on proteins. Preliminary tests with only the oscilloscope and LabView have already demonstrated the above capabilities for the DS5.

Once the "areas of interest", those of temperature and frequency, are identified with the above fast analyzers for a particular protein sample, then high resolution studies will be performed with the DS2 and DS4 to accurately resolve and confirm the temperature and frequency responses. The DS2 and DS4 spectrometers are based on lock-in amplifier technologies which have greater dynamic reserve and sensitivity than either the DS3 or DS5. Modern lock-in amplifiers are digitally-based extremely narrow notch-filters that focus on only a single frequency at a time, therefore these spectrometers will only work in sine-swept mode but have a high common mode rejection ratio.

The DS2 is based on the Stanford Research SR850 DSP Dual-Phase Lock-In Amplifier. It will cover the low frequency range of 1 mHz to 102 kHz, 0.1 mHz resolution, and has a built-in sinusoidal function generator for excitation of the sample cell. The DS4 will cover the high frequency range of 25 kHz to 200 MHz, and is based on the Stanford Research SR844 DSP Dual-

Phase Lock-In Amplifier. The latter amplifier will also require the Tektronix AFG3252 Arbitrary Function Generator for excitation of the sample cells.

Each of the DS2 and DS4 spectrometers will require three lock-in amplifiers. The first of the three amplifiers will acquire the admittance of a short circuit, the second will calibrate an admittance on a load impedance or reference cell, and the third will measure the admittance of the sample cell. This technique is similar to that employed by Gross *et. al.* [4], except the three amplifiers will run in parallel and simultaneously. Essentially, the first amplifier will negate any effects due to electronics or cables, and the second and third to the dielectric differences between the sample and the reference cell. The complex dielectric spectrum of the sample is its admittance normalized by the complex division by the reference admittance. This calibration technique will also be designed into the DS3 and DS5 spectrometers.

Cadence OrCad circuit analysis software will be used to simulate noisy signals from virtual sample cells. The specifications of modern operational and instrumental amplifiers from Analog Devices and Texas Instruments will be entered into the software to simulate various designs and multiple device packages for potential preamplifier designs. The best configuration will be chosen for the particular dielectric spectrometer which will lead to fabrication of the printed-circuit board (PCB). PCB preamplifiers will be created to ensure that additional stray or parasitic effects are not introduced into the experiments. Each of the four proposed dielectric spectrometers will require their own custom preamplifiers.

In general, low-noise differential current-to-voltage converters or preamplifiers will be created for high frequency studies, above 1 Hz, and low-noise differential charge-to-voltage converters or preamplifiers will be created for low frequency studies, below 1 Hz. A charge-to-voltage preamplifier is essentially an integrating amplifier which contains capacitors for the amplifier's feedback. These will increase the accuracy by counting the charges emitted by the sample cell at the low frequencies. The current-to-voltage preamplifiers will ensure that the current impedances are properly matched to the voltage inputs of the lock-in amplifiers or dynamic-signal analyzers.

The custom PCB preamplifiers will also contain ground shunts for both the source and the detector sides of the sample cells. It was found with the current DS1 that superposition of the previous frequency, in sine-swept mode, introduces noise into the final measurement of the lock-in amplifier. This is especially true at less than 0.1 Hz. It is proposed to shunt all electrical energy of the cabling and sample cell conductive plates to ground for a few seconds before running the next frequency of the spectral run. This will ensure that any charge and current in the lines and electrodes are only for the single frequency under study, therefore, avoiding any superposition effects.

And finally, all sample cells, cabling, and preamplifiers will require proper shielding and grounding to ensure that ambient noise is not introduced into the experiments. Guard rings will be designed into the sample cells to negate fringe effects and ensure that the measured electric fields are only those of the samples, as further detailed under "a.3. Sample Cells".

In summary, four dielectric spectrometers, the DS2 through DS5, are proposed that are based on lock-in amplifier or dynamic-signal analyzer technologies. Custom design and fabrication of the preamplifiers and environmentally-controlled sample cells will be coupled to the amplifiers and analyzers with all of this instrumentation encapsulated and controlled by custom computer software. The software will also acquire all data and utilize real-time statistical and graphical analysis and perform the necessary computations to acquire and store the final dielectric spectra.

a.2. Computer Control and Data Acquisition

Each dielectric spectrometer will interface to a personal computer (PC). Each PC will interface to the amplifiers or analyzers using a General Purpose Interface Board (GPIB), Ethernet, or Universal Serial Bus (USB), depending on the capabilities of the equipment. Switching mechanisms within the preamplifiers will be controlled by digital interface boards. To reduce cost, each computer will use the Microsoft Windows XP Operating System, with National Instruments LabView Full Development System, version 8. All electronic and instrument control will be with custom LabView applications. Real-time data acquisition, as well as, statistical, least-squares regression and fitting, and graphical analysis, and dielectric spectral display and storage will also occur within the LabView applications. This capability is vital for determining a settled stable admittance, especially at frequencies below 1 Hz, as realized with the DS1.

The current DS1 is based on the above LabView control. This application will be ported and modified to accommodate the proposed DS2 through DS4. This will save seven months of programming on the three spectrometers. The proposed DS5 will require a new application because of its innovative design of encapsulating the various electronic devices into a broad-frequency dynamic-signal analyzer and a lock-in amplifier, depending on the mode of the experiment.

In summary, a total of four personal computers running LabView will be interfaced to the respective electronics and instruments of each of the four proposed dielectric spectrometers. Since spectra require careful monitoring and control, the LabView applications will completely automate the admittance runs, control the temperatures, and process the dielectric spectra of the samples.

a.3. Sample Cells

The sample cell is where the physical quantity begins its mapped representation to the collected data. Since the electric polarization of matter is based on the separation of charge, leading to dipoles and multi-poles, it will be affected by an applied electric field. The applied field is the experimental probe used to study the electric polarization within any sample or material. Changes in the electric field represent changes in the electric polarization and charge distribution within the material, hence the physical nature of impedance, admittance, and dielectric spectroscopy.

The sample cell will consist of an insulating body and two conducting electrodes, separated by a controlled distance. At any particular instant, one electrode will be positively or negatively charged to a certain magnitude. The second electrode will have the opposite charge and equal magnitude, thus an electric field is created between the space of the two electrodes. If an insulating material, a dielectric, is placed between these charged plates, then the applied electric field will cause polarization or charge separation of the material, thus increasing the measured displacement field.

Dielectric spectroscopy has been developing since 1897 [5] and five polarization phenomena have been observed: **(1)** Maxwell-Wagner for heterogeneous phases, **(2)** electrode polarization, **(3)** orientational due to permanent dipole moments in polar molecules, and inductional due to **(4)** atomic and **(5)** electronic polarizabilities. The latter two occur at extremely rapid rates and are far beyond the frequency range for dielectrics of structural motifs; therefore they will not be discussed further.

The expected complex dielectric response from the intra- and interchain structural motifs of proteins will be from the permanent dipole moments inherent to those motifs and the electronegative bonding, therein. Therefore, the basic development for the sample cells will be to increase this effect while decreasing all other effects, namely the Maxwell-Wagner and more importantly, electrode polarization effects. This will ensure that such effects do not mask the motif dipole moments of proteins.

Maxwell-Wagner [6 7] effects, or interfacial polarizations, are based on the charge buildup at the boundary of two heterogeneous phases with different impedances. For example, DNA exhibits a

huge permittivity, contrary to the absence of permanent dipole moments. The observed permittivity has been attributed to interfacial polarization, and has also been observed in nonpolar latex and colloidal suspensions. This effect exhibits anomalous dispersion with very slow relaxation times that mimic the behavior of Debye relaxation for polar molecules [8]. Although this effect may mask the expected structural motif dielectric response, the effect may prove valuable in studying the dynamics of intermotif and interchain permittivity and internal and external hydration layers of proteins.

Electrode polarization is due to the pseudo-ordering of ions and molecules around the electrodes to form an electrical double layer. Since the electrodes carry charge to deliver the applied electric field, mobile ions and polar molecules will orient themselves to the electrode charge source, thus forming an ordered electrical shielding layer at the electrode surface. The electronic shielding imposed by electrode polarization is frequency dependent and can impose a significant measurable permittivity, thus potentially overwhelming and masking the measured permittivity of the bulk sample, within a specific frequency range.

The proposed work will investigate a number of techniques to reduce or negate the effect of electrode polarization. Four well-established techniques are proposed herein: **(1)** four-terminal electrodes as used by Klaassen [2] and Takashima [6], **(2)** adjusting the ratio of electrode separation versus the electrode area as used by Grosse [9] and Schwan [10], thereby increasing the bulk response, **(3)** surface etching and roughening of the electrode as used by Grant [8], and **(4)** coating the electrode with Pt-black as used by Schwan [11], Takashima [12], Tirado [13], and Grosse [4 9], in which Tirado [13] reports a decrease of EP by 2 to 3 orders of magnitude.

The applied electric field will experience boundary conditions at the edges of the electrodes. These fringe effects will be present but not measured by incorporating grounded guard rings into the sample cells' detector-side electrode. Essentially, this electrode will be smaller than the power-side electrode and will comprise an extra insulating ring and an outer conducting guard ring. The power-side electrode will create the fringe fields and the guard ring will shunt them to ground. Therefore, the inner detector-side electrode is only absorbing the uniform electric field through the bulk sample.

Although thermoelectric coolers and heating coils are inexpensive methods for temperature control, they will emit DC or AC noise that will disrupt the dielectric responses of the samples. Therefore, precision liquid coolers and heaters will be employed to control the temperature of the sample cells. The liquids are nonpolar solvents with miniscule electrical conductivity; therefore a single cooler or heater should service a few immersed sample cells. The primary liquid for these applications will be derivatives of silicone oil, which is an electrically insulating nonpolar liquid with a dielectric constant of less than 3 [14]. It is proposed to submerge the sample cells in immersion baths of this silicone oil. This will provide optimum thermal contact with the samples without contaminating their dielectric signals.

Julabo USA, Inc. is the foremost manufacturer of such liquid coolers and heaters. The models proposed for this development include the temperature range of -88 to 300°C, with a stability of 0.01°C. The controllers will facilitate extremely stable high-resolution temperature studies for the proposed cold near-frozen studies and the below protein denaturation studies where the tertiary structure is melted but the secondary structure remains intact, as further discussed in "a.4. Peptide and Protein Samples" and "b.1.ii. Structure of and Permanent Dipole Moments in Peptides and Proteins".

In summary, the sample cells will be electrically-conductive cylindrically-symmetric capacitive plates separated by a discreet distance and held firmly by an electrically-insulated body. The protein samples will be between the two capacitive plates. Grounded guard rings will be employed to negate fringe effects and four techniques will be explored to reduce electrode polarization. And finally, nonpolar liquid immersion baths will be employed for accurate high-resolution temperature studies on the protein samples.

a.4. Peptide and Protein Samples and Studies

The Protein Databank (PDB) [1] is a free and searchable internet site at Rutgers University, NJ, containing over 34k protein submissions. The protein structures submitted to the PDB are near atomic-resolution 3D maps characterized by either X-ray crystallography or nuclear magnetic resonance (NMR). The PDB is an established library of 3D protein structures. The PDB will be extensively searched to find model proteins for this research. The proteins chosen for the study will be previously characterized by complimentary methods and commercially-available, i.e. Sigma-Aldrich. The initial studies will comprise proteins with a high content of pure α-helices, preferably with only a few sub-domains which contain no ligands or porphyins. Such model systems will facilitate the observation and assignment of the intramolecular structural motifs.

Initial protein samples will be studied in dry powdered and in pure aqueous forms. Powdered protein samples have the advantage of having constrained rotational movement, which will have the effect of decreasing the dielectric response from the molecular net dipole moment. Dry samples will also produce negligible electrode polarization effects because mobile charge or ion carriers will not exist, as previously found studying hemoglobin with the current DS1.

Salt and pH variations on aqueous solutions will confirm previously reported studies of the molecular net dipoles of proteins and further characterize electrode polarization effects. The permanent dipole moments of the protein's structural motifs will be perturbed by the localized acidic or basic R-groups of the amino acids whose backbone atoms create those motifs. These perturbations will be studied by varying the pH of the solution. At least the three extremes can be evaluated; acidic, neutral, and basic solutions. Such pH variations of the solution will charge or uncharge the side R-groups accordingly, depending on the exposure of the R-group to the solution, its pK_a value, and the pH of the solution.

The most important studies of this project may be the high-resolution high-temperature experiments below the denaturation point of the protein where the tertiary structure is partially or fully melted and elastic, flexible, or fully open, but while the secondary structural motifs remain fully intact. Since the α-helical and β-sheet motifs have extensive hydrogen-bond lattices and such lattices have a high enthalpy, the hydrophilic/hydrophobic interactions compressing the tertiary molten core structure should release at a lower temperature than the structural motifs. This is the "area of interest" for these studies. It is expected that with stable resolved warming of the protein that the tertiary structure and molten core will soften, thereby allowing the intact secondary structural motifs some torsional degrees-of-freedom in which to respond to the applied electric field. These studies will be performed using the Julabo liquid heaters as described in "a.3. Sample Cells". Since these studies are temperature dependent, activation energies of the tertiary melt should lend information on the degree of crystallization of the molten core, through an Arrhenius approach, while proving the strengths, relaxation rates, and heterogeneity of the permanent dipole moments of the intramolecular structural motifs of proteins.

It is expected that the molecular net dipole will mask the dielectric responses of the intramolecular motifs. It is conceivable that the torque and rotational inertia of the whole protein will require immobilization to decrease the net dipole which will, in turn, enhance the dielectric responses of the intramolecular structural motifs. Therefore, it is proposed to rotationally immobilize the proteins with three techniques: **(1)** semi-solid chemical matrices, **(2)** thermal effects, and **(3)** molecular alignment using magnetic fields in conjunction with the latter two techniques. Essentially, these techniques will increase the viscosity of the medium, thereupon "freezing" the macromolecules rotational inertia.

The first technique is to embed the protein in a highly-viscous semi-solid chemical matrix to impede the rotational motion of the protein. Gelatins and agaroses, collectively known as gels, form

semi-solid aqueous environments that are commonly used in biochemical research, i.e. electrophoresis and chromatography. The latter techniques use gels to slow the translational mobility for protein separation and purification. It is proposed to use these gels in higher concentrations to also inhibit the rotational inertia of the proteins. These gels are also known to have low dielectric signatures as proven by electrophoresis. Gels are the most obvious choice for the proposed protein study because they imitate *in vivo* environments. Other highly viscous substances may also be investigated, such as ionic or quaternary ammonium liquids. Of course, prior to protein studies, various commercially-available high-purity gels will be characterized with the proposed dielectric spectrometers.

The second technique will use low temperature studies, in the range of –88 to 0°C, in conjunction with trehalose, a sugar commonly known as "nature's antifreeze". Studies below the freezing point of water will reduce its thermal agitation on the proteins, thereby decreasing the normally broad Maxwell-Boltzmann distribution and thus environmental noise contributing the dielectric response of the protein. This combination of low temperatures and trehalose should create a pseudo frozen, highly viscous solution that is not crystallized, to reduce the agitation due to the water while simultaneously constraining the protein under study.

If required, we will also study the effects of magnetic alignment of the protein's net dipole on their dielectric responses. Scheraga *et.al.* [15] suggest that dipoles of the structural motifs, most notably α-helices, have a general alignment to the net dipole of the folded protein. Therefore, by aligning the net dipole of the protein, this should increase the dielectric signal from a greater population of motif aligned dipole moments. The magnetic alignments may also be used in conjunction with the gels to impede the rotation once magnetic alignment is applied.

a.5. Task Analysis and Time-Line

Prior to funding, and after submission of this proposal, the test protocol of proteins will be accumulated to act as real model systems to achieve the proposed goals. The Protein Databank (PDB) [1] will be extensively searched for protein candidates with high percentages of structural motifs. All candidates will be commercially-available, i.e. Sigma Aldrich. The initial candidates will have a high α-helical, zero β-sheet, and low hairpin turn content because of their strong inherent dipole moments. The next candidates will have a high β-sheet, parallel and anti-parallel, zero α-helical, and low turn content because of their weak dipole moments. And finally, candidates of mixed α-helical and β-sheet content will be scheduled to gauge the gradient of responses between the α to β extremes. All protein candidates will have zero ligand and porphyrin content to reduce any potential interference of the motif's dielectric responses. Acidic and basic side R-groups, along with their relative positions to the structural motifs, will also be considered in the test models and poised for further pH studies. The proposed protein protocol will be complete prior to funding. See section "b.1.ii. Structure of and Permanent Dipole Moments in Peptides and Proteins" for more information.

After funding, it is estimated that the first 12 to 18 months will be devoted to the design and development of the sample cells, custom circuitry, and software integration with the commercially-available amplifiers and analyzers. The sample cells will require integration of the liquid temperature controls, metallurgy, nonpolar and Pt-black coatings, as well as, proper guarding and shielding of the sample cells and cabling. The preamplifiers, wiring, sample cells, and software for the DS2 through DS4 will require about 3 to 6 months, with porting of the existing DS1 software to these three DS. The DS5, however, will require an additional 8 to 12 months because the software will be created from first principles. Therefore, the DS2 through DS4 will be built immediately after funding with the initial studies on metallurgy and nonpolar and Pt-black coatings, owing to the reduction of electrode polarization, following shortly after their development.

After the first DS is built, immediate testing may commence on the protein candidates. Since a spectrum may require days to run, parallel development will continue during the initial protein tests. All in all, between the proposed high-resolution temperature studies, the semi-solid chemical matrices, and the protocol of the protein candidate, actual dielectric measurements of the proteins, in their various environments, will require approximately 24 to 36 months. Specific personnel roles are detailed under the section, "d. Project and Management Plans."

b. Description of Research Instrumentation and Need

b.1. Rationale of Technical Feasibility and Developmental Foundations

b.1.i. Theory and Measurement of Dielectric Relaxation

The classic work "Polar Molecules" in 1929 by Peter Debye [16] formalized the complex dielectric spectra, and anomalous dispersion and absorption, of polar molecules. C. J. F. Böttcher later modernized the polarization of molecules in static and alternating electric fields in his two-volume publication, "Theory of Electric Polarization" [17] [18]. Essentially, the increase in magnitude and change of phase of the displacement field D, is due to the polarization of matter as compared to the *in vacuo* electric field, E, as proven by Maxwell in 1865 [19], or

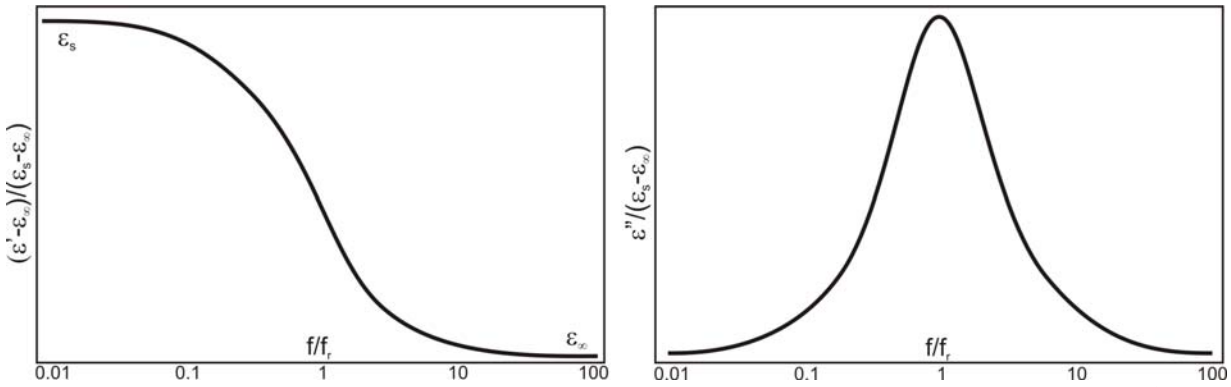
$$\hat{D} = \varepsilon_0 \left[\varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_\infty}{1 + i\omega\tau} \right] E_0 e^{i\omega t} \qquad \text{(Eq. 1)}$$

where τ is the relaxation time, ω is the incident angular frequency or $\omega=2\pi f$, ε_s is the static permittivity, ε_∞ is the permittivity at optical frequencies, and ε_0 is the permittivity of free space. Therefore, the observed frequency dependent increase of the displacement field D, as compared to E, is due to the introduction of an insulating material, as seen by the second term.

Comparing Equation 1 with the *in vacuo* electric field equation, and solving for the real and imaginary components of the complex permittivity for relaxation processes of any dielectric material yields

$$\varepsilon' = \varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_\infty}{1 + (\omega\tau)^2} \quad \text{and} \quad \varepsilon'' = \frac{(\varepsilon_s - \varepsilon_\infty)(\omega\tau)}{1 + (\omega\tau)^2} \qquad \text{(Eq. 2)}$$

where ε', the real component of the complex permittivity, yields the frequency-dependent dielectric constant and ε'', the imaginary component, yields the energetic absorption or dielectric loss from the applied electric field. The latter complex dielectric relaxation yields the normalized real and imaginary components, respectively, as shown below



(Fig. 1)

where the right plot represents the anomalous dispersion, a decreasing permittivity with increasing frequency, and the left plot represents the absorption or energetic loss of the applied electric field. The product, $\omega\tau$, is unity at the center of the relaxation transition.

Microscopically, dielectric data simultaneously provide the strength of the dipole moment, through the change in the magnitude of the real permittivity, and the relaxation rate of the molecule's rotational motion, which is derived from the frequency of the transition [20]. Debye [16] determined the relaxation rate of the dipole to be related to

$$\tau_{\text{int rinsic}} = \frac{4\pi a^3 \eta}{kT}$$

(Eq. 3)

or the relaxation rate τ is related to the volume of the molecule a^3, the viscosity of the solvent η, and the temperature T of the solution. Hence, a frequency scan will reveal the relaxation time, leading to an understanding of the effective molecular size of the responding dipole and the viscosity of the surrounding environment.

The electric permittivity, or frequency-dependent dielectric constant, normalizes the sample cell geometry by the relation

$$\varepsilon^*(\omega) = \frac{C^*}{C_0^*} = \frac{Y^*}{Y_0^*} = \frac{Z_0^*}{Z^*} = \varepsilon' - i\varepsilon''$$

(Eq. 4)

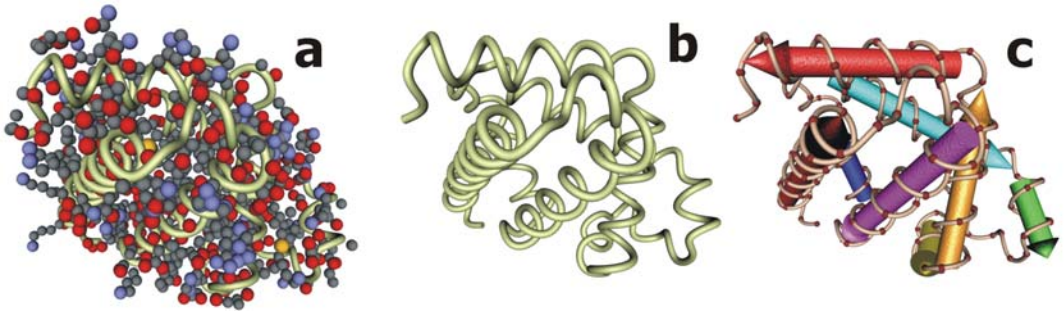
or the complex dielectric permittivity ε^* is the complex capacitance C^* of the sample divided by that of the same *in vacuo* reference cell $C_0{}^*$, or similarly for the admittance Y^*, or its inverted impedance Z^*. Admittance Y^* is the measured complex current I^* over the applied voltage V and impedance Z^* is the inverse of admittance [21]. This is the foundation of the classic and well-proven techniques of admittance, impedance, modulus, and dielectric spectroscopy [22].

b.1.ii. Structure of and Permanent Dipole Moments in Peptides and Proteins

Early X-ray crystallographic data revealed four levels of substructure within peptides and proteins, herein known as simply proteins. The four structures are primary, secondary, tertiary, and quaternary structures [23] [24] [25]. The primary structure is the linear amino acid sequence of each chain within the protein. This extended structure is highly energetic and initial folding of the primary into

sub-domains, called the secondary structure, occurs to form ubiquitous motifs. The most common energy reducing structural motifs are α-helices and β-sheets, for these motifs form extensive hydrogen bond lattices. For instance, if the protein is destined to have only α-helices, then the secondary structure would roughly be linear but compressed with sub-domains of spiral-staircase coils. These coils are the α-helices. The tertiary structure would form by additional folding of the non-helical domains so that the α-helices fold in on themselves in a compact 3D globular structure. Additional bonding, van der Waals, and hydrophilic/hydrophobic interactions with the aqueous environment further compresses the protein in which the interior becomes a molten core, or liquid-crystal polymeric center. Quaternary structure occurs when multiple peptide chains bond together to form a larger protein structure. Many proteins do not have quaternary structure because they are composed of only a single peptide chain.

Figure 2 was included to aid in visualizing the secondary and tertiary structures of a protein, in this case myoglobin and was directly derived from the Protein Databank (PDB) [1], entry 1A6G. Myoglobin is an O_2 transport enzyme composed of a single peptide chain with 151 amino acids. It forms eight α-helical segments which comprise 76.16% of the total protein and surround an organic Iron-based porphyrin ring. Figure 2a is a representation of myoglobin's true atomic positions, excluding hydrogen and porphyrin atoms. The primary backbone of the protein has been highlighted by a yellow tube in which the remainder of the atoms are its amino acid side R-groups. Figure 2b is only the backbone of myoglobin. Its eight α-helical motifs are clearly depicted as spiral staircases. This is the secondary structure of myoglobin. The segments between the α-helices are either random coils or hair-pin turns and these fold to form the final 3D globular sphere. This is the tertiary structure of myoglobin.



(Fig. 2)

The driving forces for the formation of secondary structure are steric hindrance and electrostatic interactions of inherent dipole moments. Concentrating on the latter, the typical amide or peptide bond carries a dipole moment of 3.71 Debyes ($D = 10^{-18}$ esu cm = 3.33564×10^{-30} C m) [26] [23]. The moment of water is 1.85 D [27] [28] [29] and that of Hydrogen cyanide is 2.93 D. Therefore in comparison, the amide bond imposes a large dipole moment and thus the primary structure possesses the greatest potential energy. The reduction of the potential energy due to the amide bond dipoles contributes to the folding of the secondary structures.

Experimental and theoretical studies [20] [30] [31] [32] report that the dipole moment of an α-helix is 4 to 5 D per residue or 14.4 to 18 D per helical turn. This may seem ironic since an increase in the dipole strength per residue is apparent compared to the abovementioned 3.71 D for a peptide bond. However, these studies propose that polarization effects occur, which increase the dipole strength, while minimizing the overall potential energy due to hydrogen bond lattice formation in the folding of the α-helix. Every n^{th} residue forms a hydrogen bond with every $n+4^{th}$ residue in the helix, with the net dipole of an α-helix in roughly the direction of the primary axis. Figure 2c shows the eight dipoles that are formed due to the eight α-helical motifs for myoglobin. The dipoles range in

strength from 28 to 130 D in which these large differences must be resolvable by dielectric spectroscopy. The molecular configurations of β-sheets tend to produce permanent dipole moments of near 0 D [23 24 25 32].

Dielectric studies by Oncley *et. al.* [33] on native freely-suspended proteins in solution reveal relaxation times ranging from 16 ps to 2.5 μs, which translate to frequencies on the order of tens to hundreds of kHz. Application of the Debye relation, Equation 3, reveals that the high viscosity of the molten-core of globular proteins will greatly lower the observed frequency of intramolecular structural motifs, for a higher viscosity leads to an increased relaxation time and thus a lower observed transitional frequency. Therefore, it is expected to observe the transitional frequencies in the μHz to kHz range for the intra- and interchain structural motifs.

Conceivably, if the liquid-crystal molten core of the protein is gently melted or relaxed by thermal or chemical techniques, as detailed in section "a.4. Peptide and Protein Samples and Studies", then the proposed spectrometers should detect and resolve the individual torques and subsequent absorptions of these freer-floating motifs, especially for varying α-helices. For instance, if high-resolution temperature studies are performed below the denaturation temperature of the protein, specifically, where the tertiary structure is semi- or fully melted but the secondary structure remains intact, then the secondary structural motifs should absorb the applied electric field at a particular frequency. This temperature range should be possible since the enthalpies of hydrogen bond lattices, that of structural motifs, is higher then those of van der Waals or hydrophilic/hydrophobic interactions. The temperature of the fractionally melted tertiary structure should lend itself to the activation energies of the molten core, through an Arrhenius approach, and the frequency of the absorption to its relaxation rate and intrachain dampening forces. Hence the basis of this proposal and its spectrometer development.

Intramolecular electrostatics perform vital roles in the structure and function of the protein [34]. Such electrostatics contribute to, "enzyme catalysis, electron transfer, proton transport, ion channels, ligand binding, macromolecular assembly, and signal transduction." [35] Warshel *et. al.* [35] concluded on the failures of dielectric continuum models, such as the Kirkwood-Tanford treatment, that use unrealistically large dielectric constants to compensate for charged R-groups. It is apparent that continuum models are a first approximation designed to reduce computational expense, especially since simple heteroatomic organic molecules generally have π-conjugation, resonance structures, and heterogeneous electrostatic distributions and thus intramolecular permanent dipole moments. It is only reasonable that proteins mimic the same chemistry. There are recent trends of theoretical studies, both *ab initio* [32] and semi-empirical [15] using the PDB, migrating towards models of heterogeneous charge distributions and backbone structural motif dipoles in peptides and proteins.

The empirical data derived from this work would include the frequency-dependent permanent dipole moments of the structural motifs. These values will be directly applicable to theoreticians. Also, the high-resolution below denaturation temperature studies, through an Arrhenius approach, would reveal the activation energies of the melted tertiary but intact secondary structure. Further studies can disseminate the enthalpy and entropy of such mechanisms. Together, these will reveal the dampening forces and intermotif interactions that can be directly used by theoretical and computational researchers to further our concepts and understanding of peptides and proteins.

In summary, since X-ray crystallography, NMR, and electron microscopy can not directly characterize the electrostatic properties of proteins, dielectric spectroscopy (DS) should be developed into a modern complimentary technique. DS can provide direct empirically-based intramolecular electrostatic and dipolar data on proteins to allow for further development of more accurate theoretical models. The need for this complimentary discipline is crucial to the future of proteomics and all surrounding implications and effects, such as protein folding and energetics, enzymatic activity, and drug interaction.

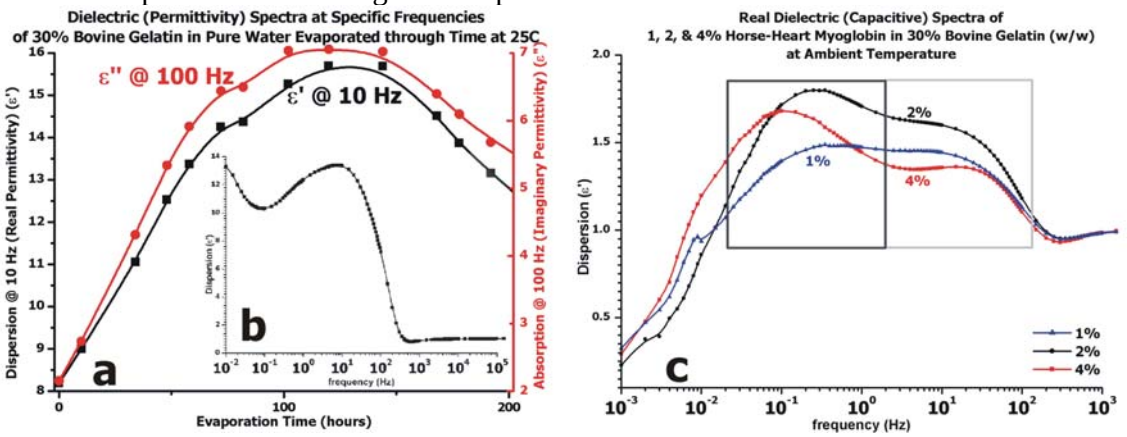
b.2. Current Research and Instrumentation

The Principle Investigator (PI) has actively pursued the current research for three years. Prior theoretical and experimental results provide a strong basis for this endeavor and the PI has invested his own personal funds and time into the development of the Dielectric Spectrometer 1 (DS1). Additionally, six corporations, Ametek Signal Recovery (formerly Perkin-Elmer), Tektronix, National Instruments, 3M, Millipore, and Mettler-Toledo, have contributed equipment and technical assistance to the development of the DS1.

The DS1 is based on a highly sensitive lock-in amplifier, the Ametek 7265 DSP Dual-Phase Lock-In Amplifier. Its high common mode rejection inherently filters noise and extraneous signals. The 7265 has a frequency range of 1 mHz to 250 kHz, a resolution of 1 mHz, and a current sensitivity of 2 fA. This is more sensitive than any commercially-available impedance analyzer or dielectric spectrometer, i.e. those manufactured by Agilent, Novocontrol, and Solartron.

The DS1 employs the current-to-voltage (I-V) mode of impedance spectroscopy, in which a frequency dependent voltage is applied across a parallel-plate capacitive sample cell using a sine-wave function generator. The lock-in amplifier then detects the current. The generator and amplifier are connected to a computer using a General Purpose Interface Board (GPIB), with custom software written in National Instruments' LabView Full v7.1. The software encapsulates the entire instrument into a fully-automated user-friendly computer application. The application automatically obtains admittance spectra using the sine-swept mode. The application sets the frequency, measures and waits for a settled and stable amperage, and then averages and stores the real-time current measurements. The application will iterate the latter process for each resolved frequency within the specified range and generate a real-time complex impedance or admittance spectrum. Dielectric spectra are obtained by complex division of the sample over the reference admittance spectra.

Gelatin is a protein with miniscule α-helical and β-sheet content and thus a negligible overall dipole moment. It was used to encapsulate myoglobin in a highly-viscous environment to impede its rotational motion. Studies began with calibrating the gelatin in pure water. The studies culminated in a final study of initially 30% bovine gelatin in pure water (w/w) and the sample was allowed to evaporate undisturbed over an eight day period. Figure 3b, the inset, shows the real dielectric spectrum for the initially prepared sample. It is a smooth function in which the peak at 10 Hz is due to the electrode polarization of water with no apparent response from gelatin itself. Figure 3a shows the real permittivity at 10 Hz, black squares, and the imaginary loss at 100 Hz, red circles, over the eight days of evaporation. Both real and imaginary responses show similar trends and confirm other concentration dependent studies of gelatin in pure water.

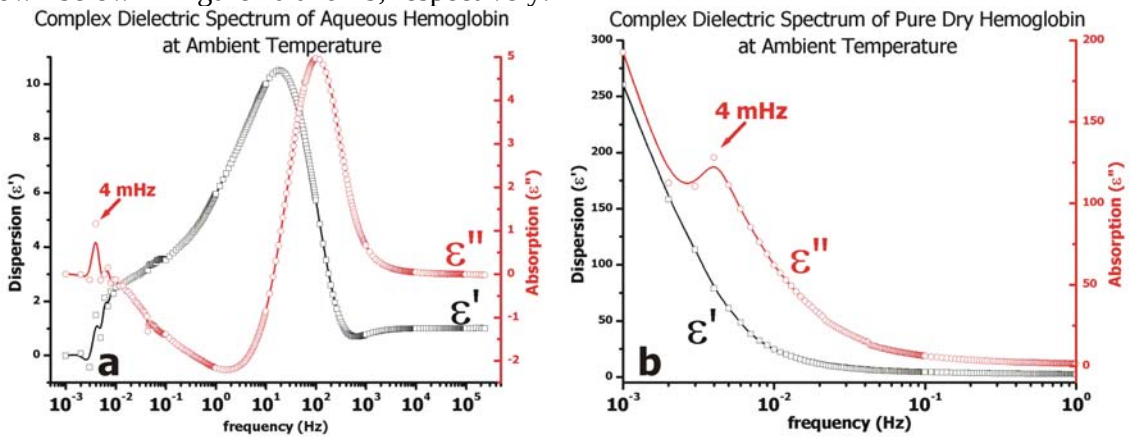


(Fig. 3)

Horse-heart myoglobin was then studied in 30% bovine gelatin. Figure 3c shows two regions of interest. The right silver box around 10 Hz is water interacting with the electrode, as it did in the gelatin study of Figure 3b, and the left gray box around 0.1 Hz is due to myoglobin. The 0.1 Hz response is completely absent from the gelatin response of Figure 3b. There is some deviation of the absolute permittivity, especially of the 2% myoglobin, due to inconsistent evaporation. The relative responses, however, of the 0.1 Hz myoglobin peaks to the 10 Hz water peak is apparent. Essentially, the vertical ratios of the 1% myoglobin to water is 1:1 (blue), that of the 2% is 2:1 (black), and that of the 4% is 4:1 (red). Therefore, the myoglobin in gelatin studies clearly show a concentration dependence of the myoglobin peaks at 0.1 Hz without interference from its gelatin environment.

These myoglobin results indicate the viability of using gels as rotational immobilizers for lower concentrations of proteins under study, as proposed under "a.4. Peptide and Protein Samples and Studies". Although the problem of electrode polarization needs to be addressed in this project, the study also emphasizes the importance of studying the effects of water, and its interactions, with the protein under study, whether it is internal or external hydration layers of the protein. It is this potential of studying the dielectric responses of hydration layers of the protein that the low temperature studies were proposed by immobilizing the protein in a non-crystallized trehalose solution down to –88°C, as also detailed in the latter mentioned section, a.4.

Similarly, DS1 produced complex dielectric spectra of aqueous and dry bovine hemoglobin, as shown below in Figure 4a and 4b, respectively.



(Fig. 4)

where the left data represents 10% (w/w) aqueous bovine hemoglobin for the full frequency range of 1 mHz to 250 kHz. The large broad dispersion around 10 to 100 Hz is due to electrode polarization with water, as again seen with the above gelatin and myoglobin data. However, the data at less than 20 mHz has never been observed before and may represent a dielectric response from hemoglobin. To confirm the aqueous results, Figure 4b shows the dielectric spectrum for pure dry hemoglobin. An obvious and strong primary absorption peak at 4 mHz arises, with additional harmonic or overtone peaks around 8, 12, and 16 mHz as observed in aqueous solution. Additionally, the real dispersion of the aqueous sample increases with frequency, indicative of normal dispersion, and may represent resonant, as opposed to relaxation, processes.

In March 2005, an Italian research group, Bruni *et. al.* [36] [37] publicly reported similar dielectric responses with hydrated lysozyme, emphasizing the absorption in the mHz range. These are the first studies investigating the behavior of biologically-based proton glasses using dielectric spectroscopy. Their research reports of a proton glass transition that is observable "due to proton displacements along hydrogen-bonded water molecules absorbed on the protein surface, with ionizable groups

[amino acid R-groups] as sources of migrating protons." [37] A primary feature of the transition is in their temperature-dependent Arrhenius approach, that of rate versus the inverse of temperature, they found a non-Arrhenius temperature dependence of the dielectric relaxation rate. Such a result is indicative of a distribution of relaxation rates as first given by the Kohlrausch-Williams-Watts (KWW) nonexponential decay equation. The KWW behavior was observed by this researcher, Lukacs *et. al.* [38] [39], with the temperature dependent studies of a liquid-crystalline random copolyester, an LCP. It is no surprise that proteins should mimic the behavior of LCPs. However, it is expected that the temperature dependence of proteins is more complicated than LCPs because of their lack of polymeric order as derived from extrusion, and additional complications due to side R-groups, hydrophilic/hydrophobic interactions, and globular nature.

At least the cited Bruni results validate the above myoglobin and hemoglobin studies, in that interfacial water leads to important protein dynamics and enzymatic activity. The proposed high resolution, 1/100°C, temperature studies will target the internal and external hydration layers, via the proposed low temperature studies, and the glassy or liquid-crystalline phase transition of the molten core, via the proposed high temperature studies, of the PDB cross-referenced model proteins. The proposed combination of 3D structural searches and information from the PDB, custom dielectric spectrometers, and chemical and temperature studies on peptides and proteins have never been attempted or reported. The time for such studies has arrived.

b.3. Justification of Proposed Versus Commercially-Available Instrumentation

Our research of published dielectric instrumentation concerning biologically-oriented research reveals that researchers employ commercially-available network or impedance analyzers. The research groups are Samouillan *et. al.* [40], Bruni *et. al.* [41], Facer *et. al.* [42], Grosse *et. al.* [4] [9], Weingartner *et. al.* [43], Bordi *et. al.* [44], Kubisz *et. al.* [45], Smith *et. al.* [46], and Risuleo *et. al.* [47]. In this arena, three manufacturers are referenced; Agilent Technologies (formerly Hewlett-Packard (HP)), NovoControl Technologies, and Solartron Analytical.

Agilent provides high frequency ([20,110G] Hz) analyzers with poor sensitivity (>20 μA) for the industrial purposes of electronic component testing. The analyzers are not designed for low frequency studies or low-voltage excitation studies and thus do not require high sensitivity. NovoControl provides ultra-low to medium frequency ([3μ,20M] Hz) analyzers with excellent sensitivity (>10fA) with an exceedingly high cost and extensive sample cell interfacing will be required with their instruments. Solartron provides ultra-low to medium frequency ([10μ,32M] Hz) analyzers with excellent sensitivity (>10fA) but their products carry an unreliable reputation.

Conversations and correspondence with Paul Moses at the Center for Dielectric Studies at Pennsylvania State University confirms the latter synopsis of commercially-available instrumentation. These interactions have confirmed the direction of using the proposed Stanford Research lock-in amplifiers and dynamic-signal analyzers with custom preamplifiers. The proposed instrumentation has not been used in the studies of peptides and proteins, however, established dielectric laboratories, such as that at Pennsylvania State University, have used lock-in technologies for dielectric studies on materials, i.e. ceramics, polymers, etc., for decades. Our own cursory studies on the DS1 using the Ametek 7265 DSP Lock-In Amplifier (i.e. see the latter section) has proven the potential and feasibility of the proposed instrumentation for observing and characterizing the intramolecular structural motifs of peptides and proteins.

c. Impact of Infrastructure Projects

infinite quanta, inc. is a nonprofit 501(c)(3) organization formed for the purpose of performing pure scientific research. Officially, its purpose is, "to perform pure scientific research and develop innovative and applied scientific instrumentation for the general purpose of acquiring constructive scientific knowledge and the distribution of such pure knowledge and applied innovation through various forms of information transmission, i.e., scientific journals, video, internet, etc." The Articles of Incorporation, the I.R.S. tax-exempt application and ruling, as well as, the corporate bylaws are located at http://iquanta.iquanta.info:97/organization/corporate.

The organization is composed of cross-discipline individuals with formal training and experience ranging from molecular biology and physics, to engineering, computer programming, and instrumentation. Our organization will grow to encompass personnel and collaborations, both commercial and academic, of an innovative, intellectual, creative, and scientific spirit regardless of age, gender, ethnicity, creed, sexual orientation, or religion.

d. Project and Management Plans

The "Task Analysis and Time-Line" section of this proposal is under section a.5.

Stephen Lukacs is the Principle Investigator for the proposed project. He will be responsible for carrying out the research objectives, as well as, the overall coordination of the research effort. He will be in charge of the budget, the purchasing of equipment and hardware, and any fiscal audits. He will also develop the computer applications. He will chair weekly or biweekly group meetings. These will be the primary venue for measuring the project's status and progress, educating group members for mutual understanding of the project in general, and brain-storming to resolve problems and issues.

Travis Carter and Bob Macias will work together to design, simulate, fabricate, test, and calibrate any electronic components and custom circuits required for the project. This will include testing and standardizing any off-the-shelf electronic components, custom circuits, instrumentation, and temperature control equipment. They will fabricate specially designed low-noise circuits and integrate those circuits with commercially-available amplifiers and instrumentation to create the complete DS2 through DS5 spectrometers.

Jonathan Rose and Walter Petersen will choose the proper materials, design, machine, and construct the custom sample cells. Integration of the sample cells with the circuits, instrumentation, and fluid and temperature control systems lies at the heart of the spectrometers, therefore, all members of the team will need to collaborate during the design of the sample cells. The initial group meetings will focus on this critical aspect of the project.

Once the four proposed spectrometers are constructed, Carter and Macias will ensure that the electronics and instruments remain properly calibrated and maintained to specifications and expectations. Lukacs and Rose will maintain the computer applications, prepare samples, and examine the spectra of the peptide and protein samples. Lukacs will process, analyze, and interpret the data and develop new samples, or sample characteristics, for further studies.

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Biographical Sketches

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EDUCATION August 1985 December 1991	Bachelor of Science degrees in Microbiology/Molecular Biology, Chemistry, Physics, and a Mathematics minor, from the University of Central Florida (UCF), Orlando, FL.
August 1993 October 1999	Ph.D. in Physical Chemistry from Rutgers University, Piscataway, NJ. Advisor: Frederick H. Long. Thesis Title: Development and Implementation of Optical Instrumentation for the Investigation of a Liquid Crystalline Random Copolyester.
EXPERIENCE May 1986 September 1986	University of Central Florida, Orlando, FL. Dr. David Kuhn. I studied master gene control via enzyme markers in developing fruit fly larvae. This work involved DNA recombination, and protein and DNA purification techniques.
August 1990 August 1991	University of Central Florida, Orlando, FL. Dr. Binayak Dutta-Roy. This position specialized in cold temperature physics, electronics, data acquisition and control, lasers, optics, and electro-optic detectors to study protein dynamics and hyperfine spectroscopy.
September 1991 December 1991	University of Central Florida, Orlando, FL. Dr. Lee Chow. This work determined the speed of light using innovative picosecond timing techniques and classical time of flight measurements.
September 1991 September 1992	Free Electron Laser (FEL) Group at the Center for Electro-Optics and Lasers (CREOL), Orlando, FL. This position specialized in high voltage electronics, CAD, ultra-high vacuum techniques, and high energy particles and lasers.
August 1992 February 1993	Rudolph Research, Inc., Flanders, NJ. This position involved the research and production of custom optical systems for ellipsometers, polarimeters, and refractometers.
February 1993 August 1993	Load Star Systems, Newton, NJ. This position involved creating a new account receivables software system through their proprietary operating system and assembler-based database language.
August 1993 October 1999	Ph.D. Candidate in Rutgers University, Piscataway, NJ. I specialized in the design and construction of laser, optical, spectrometers, and near-field microscope equipment for the study of polymer films and interfaces. In addition to the latter research, I also taught primarily college freshmen and seniors in general, analytical, instrumental, and physical chemistry.

June 1991 Present	Infinite Quantum, Inc., Yulee, FL. President and Head Developer. The foundation of this work began in 1985 as a computer consultant and programmer in Orlando, FL. This work involves the development of logistical systems for fleet maintenance. This system is based on the two-tier client/server database model using 32-bit versions of Fierbird SQL Server and Borland Delphi. This system is featured at http://www.iquanta.com.
May 2002 Present	Infinite Quanta, Inc., Yulee, FL. Founder and President. A non-profit scientific research organization that specializes in the development of scientific instrumentation and chemical techniques for the biophysical and biochemical fields.
ABILITIES Computer Development and Software	National Instruments LabView and Mathworks MatLab. Borland Delphi v1 & above, Pascal v7, and C and C++. ISIS Draw v2.2, Mathematica v2 & above, Maple v9 & above, Origin v5 & above, Igor. Nemetschek Vectorworks CAD Designer v12.
AWARDS	2nd Place in the 1985 Florida State Science Fair. 4th Place in the 36th International Science and Engineering Fair. 1998 William Rieman Award for teaching excellence at Rutgers University, with a honorable mention in 1997.
PUBLICATIONS	U.S. Copyright. Fleet Dynamics Client/Server 32. 1994 – 2006. U.S. Patent #4,772,274. Feminine Hygiene Apparatus. 1988. Lukacs, Stephen J., *Journal of Physical Chemistry B*, Temperature-Dependent Photophysical Properties of a Liquid-Crystalline Random Copolyester, 2001, 105(17), 3372-3377. Lukacs, Stephen, *Rutgers University Press*, Development and Implementation of Optical Instrumentation for the Investigation of a Liquid Crystalline Random Copolyester, 1999. Lukacs, S.L. Cohen, S.M., Long, F.H., *Journal of Physical Chemistry B*, Optical properties of a liquid-crystalline random copolyester, 1999, 103(32), 6648-6652. Burton, J.C., Sun, L., Pophristic, M., Lukacs, S.J., Long, F.H., *Journal of Applied Physics*, Spatial characterization of doped SiC wafers by Raman spectroscopy, 1998, 84(11), 6268-6273. Chow, L., Lukacs, S., Hopkins, K., *European Journal of Physics*, "Speed of light" measurement using BaF_2 scintillation detectors, 1994, 15, 49-52.

Travis J. Carter
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EDUCATION

Florida Atlantic University, Graduate Studies in Electrical Engineering – Introduction to EMC, Clayton Paul (3 quarter hours).

Florida Tech, Masters in Space Systems, 1993 – Curriculm provides a broad technical overview of space systems. Courses included Space Power Systems, Satellite Communications, Remote Sensing and Guidance Navigation Control.

Auburn University, Bachelor in Electrical Engineering, 1989 - Senior electives included Introduction to EMC, Henry Ott and Antenna Theory.

Other Training: IEEE Symposium 1993, 1994, 1995, 1997, 1998; 1999, Space Radiation Symposium 2000, "EMC: Designing Digital Equipment for Compliance", Compliance Engineering; "Conference on Lightning and Surge Protection", U. of Florida; "Lightning Protection Seminar", Dr. Peter Hasse; "Grounding and Bonding of Building Electrical Systems", NTT; "Introduction to EMC", R&B Enterprises .

EXPERIENCE

President / Licensed Professional Engineer.
Engineered Solutions of Amelia, Inc.; Amelia Island, FL.
September 1997 to Present
Providing electrical contracting and professional engineering services in the design, installation and testing of custom integrated electrical systems for residential, commercial, and industrial applications. System designs include Application Specific Industrial Control Boxes, Wired/Wireless Video, Voice and Data Networks, Distributed Audio and Medical Waste Disposal System and Lightning /Surge Protection.

December 2003 to September 2004
Engineering Manager
Unison Industries; Jacksonville, FL
Engineering manager of Technical Support Group including engineers and technicians responsible for development and certification testing of all product lines. Test support includes EMI, vibration and thermal testing in addition to operational performance testing. Support also includes failure/root cause analysis and full documentation. Role as a senior technical support staff member has remained as a function as well as the EMC/Certification expert for the company. Responsibilities also include capital equipment acquisition, long term planning and test facility management across all plant locations.

December 1994 to December 2003

Senior Staff EMC/Electrical Engineer
Unison Industries; Jacksonville, FL
EMC/Certification expert for the company. Providing EMC design and test support for all electronic products which consist of ignition systems for aerospace and industrial turbine engines. The EMC requirements include EMI , ESD, power transients and lightning protection designs for AC and DC circuits. Responsibilities also include electrical design, troubleshooting, and certification (UL/CE/FCC/CSA/ETL) of high voltage capacitive discharge products for the ignition systems. Performed as the lead engineer developing a new electrical satellite propulsion system for NASA.

April 1989 to November 1994

EMI/TEMPEST Test Engineer.
Harris Electronic Systems Sector; Palm Bay, FL
Responsible for supervising and performing EMI/EMP/ESD/TEMPEST tests on telemetry data links, communication systems, and data processing equipment. Duties included developing proposals; preparing test procedures/reports; managing cost accounts; performing EMI/EMP/ESD/TEMPEST tests; and assisting in failure analysis and redesign of non-compliant equipment. Company expert in the testing of RF transmitters and receivers for performance characteristics such as spurious responses, channel isolation, and receiver sensitivity which required extensive knowledge of RF design and test equipment. Functioned as EMI Technical Representative for test laboratory from November 1992 in addition to performing the following tasks in other Harris divisions.

September 1993 to November 1994

Electrical Design Engineer.
Harris Government Aerospace Systems Division; Palm Bay, FL.
Member of the Army's Multiple Launch Rocket System (MLRS) Improved Fire Control Panel (FCP) design team. The FCP is a customized X486 computer which includes a keyboard, display, hard drive and four circuit card assemblies (CCA). Responsibilities included electrical design of a digital and analog electrical design of a CCA and the interconnect backplane internal wiring harness. The CCA provided the following interfaces: VMEbus; SCSI bus; keyboard; serial; various discretes.

October 1991 to November 1992

EMI Design Engineer.
Harris Government Aerospace Systems Division; Palm Bay, FL.
Team member of the Audio/Video Distribution System and Automated Test Set for the NASA Space Station. Responsibilitie for EMI design of the fiber optic based audio distribution units (3), performing development testing and automated test set design.

LICENSES / MEMBERSHIPS

Licensed Professional Engineer for the State of Florida, NARTE Certified EMC Engineer, Florida State Certified Electrical Systems Contractor.

PUBLICATIONS

Switch Mode Power Supplies: An EMI Engineer's Point of View, IEEE Southcon Electronics Conference, March 1994.

Bob Macias
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(904) 321-3418 xmacias@comcast.net

EDUCATION 1960 - 1961	DeVry Technical Institute. Graduate of two year engineering program.
EXPERIENCE 1953 - 1955	Detroit News. United Press Photographer, Editorial Department.
1955 - 1959	U. S. Army Corp of Engineers. Combat photographer.
1961 - 1962	Zenith. Development of test equipment for sub-miniature audio amplifiers.
1963 - 1965	Motorola. Field Test Engineer responsible for diagnostic equipment for the hearing impaired.
1965 - 1968	Started a business venture using a novel molding process which allowed fabrication of a hearing aid within a customer's ear in less than two hours using common dental materials.
1968 - 1970	Bendix. Testing and developing of procedures for microwave weather systems used in commercial airplanes.
1970 - 1995	Sensormatic Electronics. Developed test strategies in the production area for printed circuit boards, test methods for silicone wafers, wrote computer programs for microchip testing, and developed equipment for testing magnetic and optical devices.
2000 - 2006	Consultant on electronics used in the study of molecular properties of peptides and proteins.

Walter A. Petersen
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EDUCATION 1956	Bachelor of Science degree in Metallurgical Engineering from the Polytechnic University of New York
1963	Master of Science degree in Metallurgical Engineering from the Polytechnic University of New York
EXPERIENCE 1956 -1960	Grumman Aircraft Engineering, Bethpage, NY. Failure analysis and materials specification for airframe components.
1960 – 1984	International Nickel Company, Sterling Forest, NY. Research Metallurgist specializing in welding research; Patent Agent for Corporate Patents & Licensing Group; Principal Metallurgist Processing Research; Senior Project Manager Product Development & Marketing. Devised welding consumables, filed and prosecuted patent applications; invented weldable, low melting point stainless and nickel base alloys and coinage alloys; marketed oxide dispersion strengthened nickel base alloys for gas turbines.
1984-1987	General Electric Aircraft Engines, Cincinnati, Ohio. Manager Advanced Quality and Metallic Materials Marketing. Marketing and preparation of military proposals.
1987 – 1996	Polymet, Cincinnati, Ohio. Director R&D and Quality Assurance. Created process for weld repairing single crystal gas turbine blades. Designed continuous wire grinding machine. Prepared ISO quality assurance system.
1996 - 2006	Self employed Consultant. Welding wire processing, quality assurance, machine design, chemical industry security, proposal preparation.
PUBLICATIONS & PATENTS	12 U.S. patents, 18 publications.

Jonathan M. Rose
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Sussex, NJ 07461
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EDUCATION 1984 – 1991	Bachelor of Science degree in Computer Science, Mathematics and Physics, Rutgers University, New Brunswick, NJ.
EXPERIENCE 2000 – 2006	Farious Net Solutions, Sussex, NJ. Owner/President. Provide custom hardware and software solutions for large-scale server installations, high speed network and data storage technologies.
1992 – 2001	Rutgers University, New Brunswick, NJ. Co-Adjunct Professor. Taught classes in computer architecture design and development of microprocessors and Internet/networking technologies.
1999 – 2001	Diamond Machine. Prototype Designer. Computer aided design of parts for computerized milling machines.
PATENT	U. S. Patent #6,904,364, Navcell Pier-to-Pier GPS

Budget Justification

The budget includes all projected equipment, materials, supplies, personnel, consultants, and administration required for the 36 month development for the proposed Dielectric Spectrometers 2 through 5, DS2 to DS5, and environmental and chemical techniques, as discussed in the "Project Description" and also "b.3. Justification of Proposed Versus Commercially-Available Instrumentation". All costs have been aggressively minimized while maintaining the specifications of the equipment and level of expertise in order to achieve the goals of the proposed project.

The bulk of the equipment expenses are for the electronics from Stanford Research, the freezers and heaters from Julabo, the signal sources and a frequency counter from Agilent, Tektronix, and Fluke, and the test and measurement equipment from Tektronix. The electronics from Stanford Research comprise six lock-in amplifiers and one dynamic-signal analyzer. Although the total cost for this equipment is $59k, it is comparable or far less expensive than any single analyzer or spectrometer from Agilent, NovoControl, or Solartron. In fact, the quoted $59k from Stanford Research will purchase the necessary electronics for three of the four proposed spectrometers, the DS2 to DS4, instead of a single unit from the three before mentioned manufacturers. For instance, a single base-model Agilent spectrum analyzer starts at $48k, a NovoControl dielectric spectrometer is $105k, and a Solartron impedance analyzer is $46k. The necessary electronics from Stanford Research will form a foundation that is far more stable and reliable, with greater precision, accuracy, and resolution, than any of the before mentioned commercially-available analyzers or spectrometers. This has been confirmed by conversations with Paul Moses at the Center for Dielectric Studies at Pennsylvania State University. Therefore, with equitable treatment of all accessories and modules, the three proposed spectrometers, DS2 to DS4, that will be based on the Stanford Research amplifiers and analyzer will be built for the price of just one commercially-available analyzer or spectrometer.

The liquid freezers and heaters from Julabo comprise another $44k. Thermoelectric coolers and heating coils were seriously considered for this project because of their low cost. However, these will introduce electrical noise and bias into the dielectric measurements and will not maintain the temperature stability required for the proposed goals. The Julabo temperature equipment provides a stability of 0.01°C or 0.02°C, depending on the model, and the very low dielectric nature of silicone oil will ensure that no electrical noise or bias is introduced into the sample cells due to temperature control. No other known manufacturer or method can provide the thermal stability or electrical insulation required to achieve the thermal goals of this project.

Signal sources and a frequency counter from Agilent, Tektronix, and Fluke, totaling $46k, are also required to excite the sample cells and precisely measure the frequency for the four proposed dielectric spectrometers, DS2 to DS5. The signal sources, or arbitrary function generators, will generate sinusoidal voltages in the range of 1 μHz to 240 MHz, with broader frequency ranges provided by square pulses via Fourier series. The frequency counter has a highly stable Rubidium time-base and will provide 11 digits of resolution in the range of 1 μHz to 1.3 GHz.

Test and measurement equipment will also be required for the project. The resistivity, capacitance, and inductance (RCL) meter from Fluke, totaling $6.5k, will accurately measure the capacitance of new sample cells and the conductance of new electrodes. The Tektronix digital oscilloscope and probes, totaling $55k, are required for characterizing the signals from the function generators, sample cells, samples themselves, operational and instrumental amplifiers, custom preamplifiers, printed circuit boards, lock-in amplifiers, dynamic-signal analyzers, and other components, electronics, and equipment. The oscilloscope is an essential fundamental test and measurement tool for seeing those signals and noises from the various components and equipment.

Each of the four proposed spectrometers will require it own computer. Therefore, a total of four Dell computers and two APC universal power supplies are required for the four spectrometers,

totaling $21k, in which a 4-port KVM switch will be used requiring only two monitors for the four computers, as opposed to the quoted eight monitors. This will save $4.4k. Computer interfacing hardware, data acquisition, and real-time statistical, transform, and graphical software from National Instruments and Mathworks will total $11k. These packages are required to run and automate the sample environments and experimental batches, as well as, analyze and graphically report on the data and status of the runs. Both National Instruments LabView and Mathworks MatLab are industry standards for research-level instrument control, data acquisition, and data analysis.

Additional required software will be for circuit analysis and electronic simulations from Cadence, for sample cell design from Nemetschek, and data analysis and linear regression from OriginLab, all totaling $12.5k. And finally, some wet-chemistry equipment is required for peptide and protein sample preparation from Eppendorf, Mettler-Toledo, and Millipore, totaling $16.7k. This equipment includes volumetric pipettes for liquid handling and measurement, a centrifuge for protein washing, a pH and conductivity meter for sample preparation and calibration, and filters for the already owned water purifiers.

It is difficult to provide approximations or near estimates for sample cell fabrication, preamplifier PCB fabrication, and protein and chemical supplies, however a value around $65k has been factored in for these necessities. The total equipment, hardware, and material expenses are $338k for the full three years of development. Official manufacturer quotes are included in the "Supplementary Documents" of this proposal.

Industrial contributions to our nonprofit pure research endeavor have reduced this budget by over $62k in hardware and equipment. These contributions made the DS1 spectrometer a reality and the results are outlined under "b.2. Current Research and Instrumentation". I, Stephen Lukacs, have worked without charge for the last three years, and will donate the space for, this project.

The salaries and consultant fees have been trimmed to their absolute minimum. Only two employees will work for the full three years of research and development, Lukacs and Macias. They will perform the majority of the routine experiments. Lukacs will receive an annual salary of $60k for a full 40 hours per week, which is a less-than-market salary. Macias will receive an annual salary of $10k for 10 hours per week. Consultants will be employed for the development thrust during the first 18 months. Carter, Rose, and Petersen will develop the electronics, fabricate the sample cells, and provide metallurgical input, respectively. The consultant fees will total $62.5. Therefore, the total salary and consultant fees for the entire three years of development is $272.5k.

And finally, administrative, travel, and other foreseen expenses have been factored into the budget. Since Lukacs owns the 600 ft^2 space slated for this project, rent will not be required or charged to the project. The temperature controllers will probably use quite a bit of electricity, especially since the four spectrometers will be automatically running experiments 24 hours a day, 7 days a week, utilities have been factored into the budget. Also, some small legal and accounting fees for the nonprofit requirement of infinite quanta are included. These will total $18k for the entire three years of development. Publication subscriptions have been included for a total of $9k and project-related travel at $15k.

Although the total budget is for $653k, it is far less expensive when compared to previous budgets. It is hoped that NSF realizes that the potential data, science, and understanding derived from the proposed project will far outweighs its cost.

Facilities, Equipment, and Other Resources

The Principal Investigator, Stephen Lukacs, owns a new 600 ft^2 space which will be used, without charge, for the proposed project. It is laboratory space with full electrical and water utilities. This will provide enough room to house the equipment and hardware, perform the necessary development, and run the routine experiments for the proposed project. The space will include an office.

Major technical and scientific corporations have donated over $62k in equipment and hardware for the advancement of this project. Mettler-Toledo donated the AT261 analytical balance for sample preparation. Millipore Corporation donated the Elix5 and Milli-Q water purification systems to provide the purest water possible for aqueous solutions of peptide and protein samples. This contribution has made a marked difference in reducing conductivity of the samples and thus decreasing the undesired electrode polarization effect during the dielectric studies of peptides and proteins.

Tektronix donated the TDS5104 4-Channel 1-GHz Digital Phosphor Oscilloscope. This oscilloscope will be embedded in the proposed Dielectric Spectrometer 5, DS5, and will act as the primary analog-to-digital waveform converter for dynamic-signal analysis and sine-swept mode experiments. National Instruments has donated GPIB interface hardware and cables, as well as, LabView v7.1 software. This made the Dielectric Spectrometer 1, DS1, possible and provided the dielectric results on gelatin, myoglobin, and hemoglobin, as outlined in "b.2. Current Research and Instrumentation". This interface equipment and software will be used with the proposed Fluke RCL meter for initial electrode, sample cell, and reference calibrations.

The Principal Investigator has donated three computers to the project for the purpose of administration, financial tracking, proposal and publication preparation, web site development, data analysis and presentation, and theoretical calculations. The data analysis will include least-squares regression and peak-fitting of dielectric data. The theoretical calculations can include potential dielectric simulations of peptides and proteins with the AMBER or GROMACS software packages.

The proposed project will not require any clinical or animal facilities. The proposed project is not concerned with organisms of any kind, simply peptides and proteins.