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13. ABSTRACT (Maximum 200 words)

We are using pulsed and continuous-wave dielectric spectroscopy for sensing DNA and proteins with high frequency techniques. This is rarely used for proteins and other macromolecules due to their low permittivity. Water, however, is a strong dielectric and the shells of bound water surrounding a macromolecule in solution may be used as a reporter for changes in its conformation or activity. Such measurements have been pursued with resonant cavities or planar waveguides, but optical correlation has been lacking. An alternative is to employ a planar resonant slot antenna, using a network analyzer to measure the solution's properties in reflection. The slot antenna's window permits passage of a light beam, enabling simultaneous dielectric and optical spectroscopic measurements. Here we report two systems, both resonant in the 4-20 GHz regime. In the first, unfolding/folding thermodynamics of a protein in solution were measured using a slot antenna affixed to a UV/VIS cuvette. In the second system, a slot antenna was incorporated into the cell holder of a fluorescence polarization instrument and used to measure binding of synthetic hormones to various receptors. Results from dielectric measurements are in good agreement with results from optical spectroscopies.

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Biomolecular sensing, dielectric spectroscopy, terahertz techniques, protein folding

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(1) List of papers submitted or published under ARO sponsorship during this reporting period. List the papers, including journal references, in the following categories:

(a) Manuscripts submitted, but not published

Kimberly M. Taylor and D.W. van der Weide, "Protein Conformational Changes Detected in the Microwave Regime Using a Coaxial Fed Planar Slot Antenna," submitted to *Biophysical Journal.*

Papers published in peer-reviewed journals

K. Taylor and D.W. van der Weide, "Ultra-Sensitive Microwave Detection of Protein Conformational Changes," regular-length talk to be presented at IEEE Microwave Theory and Techniques International Microwave Symposium, Fort Worth TX, 2004 and to be subsequently published in *IEEE Trans. Microwave Theory Tech.*

M. K. Choi, K. Taylor, A. Bettermann, and D.W. van der Weide, "Broadband 10–300 GHz stimulus-response sensing for chemical and biological entities," Physics in Medicine and Biology, vol. 47, pp. 3777–3787, 2002.

M. K. Choi, K. Taylor, A. Bettermann, and D.W. van der Weide, "Spectroscopy with Electronic Terahertz Techniques for Chemical and Biological Sensing," Chapter 2, Volume II. Emerging Scientific Applications and Novel Device Concepts, Terahertz Sensing Technology, Editors: D. L. Woolard, M. S. Shur and W. R. Loerop, World Scientific, 2003.

D. W. van der Weide, "Electronic Sources and Detectors for Wideband Sensing in the Terahertz Regime," in Sensing with terahertz radiation, vol. 85, Springer series in optical sciences, D. M. Mittleman, Ed.: Springer, 2003.

D.W. van der Weide, "Applications and outlook for electronic terahertz technology," Invited paper OSA Optics and Photonics News, April 2003.

M. K. Choi, K. Taylor, A. Bettermann, and D.W. van der Weide, "Spectroscopy with Electronic Terahertz Techniques for Chemical and Biological Sensing," Chapter 2, Volume II. Emerging Scientific Applications and Novel Device Concepts, Terahertz Sensing Technology, Editors: D. L. Woolard, M. S. Shur and W. R. Loerop, World Scientific, 2003.

(b) Papers published in non-peer-reviewed journals or in conference proceedings

Papers presented at meetings, but not published in conference proceedings

D. van der Weide, K. Taylor, C. Paulson, D. Lagally, A. Karbasi, S. Ramachandran, B. Butler, and P. Gustafson, "SPM spectroscopy of biological and low-dimensional systems," presented at New Phenomena in Mesoscopic Systems 6 Surfaces and Interfaces in Mesoscopic Devices 4, Maui, HI, 2003

D.W. van der Weide, "Terahertz sensing for explosives detection," *FOI-Sweden (Swedish Defense Agency)*, Linköping, Sweden, 29 August 2003

D.W. van der Weide, "Terahertz sensing technology," *FFI-Norway (Norwegian Defense Agency)*, Oslo, Norway, 1 September 2003

D.W. van der Weide, "Spectroscopy with electronic terahertz techniques for chemical and biological sensing," *Joint European Research Centre*, Ispra, Italy, 12 June 2003

D.W. van der Weide, "Detection of explosives, chemical and biological hazards with terahertz systems," *Gordon Conference on Illicit Substance Detection: Explosives*, Il Ciocco, Barga, Italy, 8-13 June 2003.

D.W. van der Weide, "Detection of chemical and biological hazards with terahertz systems," *The terahertz gap: the generation of far-infrared radiation and its applications*, Royal Society Scientific Discussion Meeting, London, England, 4-5 June, 2003.

K. Taylor and D. W. van der Weide, "Combined Microwave And Optical Spectroscopy of Solution Protein Conformation and Ligand Binding", Biophysical Society Annual Meeting, 2003.

D.W. van der Weide, "Detection of explosives with terahertz systems," *Expert workshop on Explosive Detection Techniques for use in Mine Clearance and Security Related Requirements*, Lake Bled, Slovenia, 2-4 June, 2003.

Invited speaker at 27th Annual Great Lakes Biomedical Conference, "Applications of nanotechnology," April 4, 2003.

K. Taylor and D. W. van der Weide, "Microwave sensing of protein conformation and binding," presented at American Physical Society March Meeting, Austin TX, 2003.

- D.W. van der Weide, "All electronic terahertz spectroscopy," 2003 OSA Ultrafast Electronic and Optoelectronic Topical Meeting, January 15-17, 2003, in Washington DC.

(2) "Scientific personnel" supported by this project and honors/awards/degrees received

Name	Function	Honors	Awards	Degrees
Van der Weide, Daniel	PI	None this term	None this term	N/A
Choi, Min	Grad student	None this term	None this term	Ph.D. (one year to go)
Gustafson, Patrick	Grad student	None this term	None this term	M.S.E.E. (near completion)
Taylor, Kimberly	Grad student	None this term	None this term	Ph.D. (9 months to go)
Bettermann, Alan	Researcher	None this term	None this term	N/A

(3) "Report of inventions" (by title only)

Microwave dielectric spectroscopy method and apparatus (van der Weide and Taylor)

(4) "Scientific progress and accomplishments" (Description should include significant theoretical or experimental advances)

(see continuation sheet)

(5) "Technology transfer" (any specific interactions or developments which would constitute technology transfer of the research results). Examples include patents, initiation of a start-up company based on research results, interactions with industry/Army R&D Laboratories or transfer of information which might impact the development of products.

Technology is being developed further by a cooperative R&D arrangement with a startup company.

Ultra-Sensitive Microwave Detection of Protein Conformational Changes

Kimberly M. Taylor and Daniel van der Weide

The focus of our research during the grant period has been the use of dielectric spectroscopy—particularly the resonant properties of antennas and other structures—in the radiofrequency to gigahertz range to detect changes in protein conformation. Our results in the past have been successful. Between 2000 and 2001, we designed, built and tested planar coaxial-fed slot antennas resonant in the 1-20 GHz range. Our initial slot antennas were designed to be used simultaneously with UV/VIS spectroscopy. These antennas were used to monitor the unfolding and refolding of a small globular protein (bovine pancreatic ribonuclease) in solution. Dielectric "spectra" varied with temperature; "spectra" at particular temperature could be fit to a series of Lorentzian peaks, and the position of particular peak could be plotted versus temperature. We obtained similar thermodynamic parameters (midpoint temperature T_m and enthalpy ΔH_m) from both dielectric and UV/VIS measurements. These experiments have been published in conference proceedings[1; 2]. A second antenna, designed to be used simultaneously with fluorescence anisotropy measurements, was used to measure kinetics and thermodynamics of the binding of β-estradiol and a fluorescent derivative (fluormone) to the ligand-binding domain of human estrogen receptor β. Perhaps because this antenna was resonant in a higher frequency range (10-20 GHz instead of 0.5-6.5 GHz), individual peaks positions varied little with temperatures, but peak amplitude could be used to assay changes in the protein conformation. Similar kinetic (k_{on} for fluormone binding) and thermodynamic (binding constant K_D for binding of both fluormone and unlabelled estradiol) parameters were obtained from the two techniques.

More recent studies have focused on exploration of the limits of detection using the slot antenna system. In particular, we are interested in possible interactions between the microwave radiation and the protein. A 1999 paper has suggested that microwave radiation may enhance both the folding and denaturation rates for globular proteins[3]. This enhancement, if present, would be reflected as changes in the thermodynamic parameters obtained during equilibrium unfolding/refolding experiments. Using our bovine pancreatic

ribonuclease (RNase A) model, we performed equilibrium unfolding/refolding experiments over a variety of pH, concentration and network analyzer power conditions.

Variation of pH was intended to establish that the dielectric phenomena observed was not simply a function of the buffer or other experimental conditions. For acidic pH, the midpoint temperature T_m and unfolding enthalpy ΔH_m decrease with decreasing pH; the slope of the dependence of enthalpy on T_m yields the heat capacity ΔC_p. We intended to observe three things with these experiments: 1) the expected variation of T_m and ΔH_m with pH using both UV/VIS and dielectric spectroscopies; 2) estimation of ΔC_p from thermodynamic parameters at the different pH conditions. The results of these experiments are summarized in Table 1. Note that only the midpoint temperature is displayed. Unfolding/refolding enthalpies were much noisier, particularly for the VNA data.

Table 1: Midpoint temperature for unfolding of RNase A at different pH

pH	Midpoint Temperature T_m (°C)		
	UV/VIS alone	UV/VIS with VNA	VNA
2.5	39.79 ± 0.50	40.18 ± 0.50	40.86 ± 2.16
3.0	46.89 ± 0.50	47.65± 0.50	46.50 ± 1.68
3.5	54.84 ± 0.50	55.06 ± 0.50	52.73 ± 1.32
4.0	62.25 ± 0.50	64.01 ± 0.50	56.41 ± 0.86
4.5	63.23 ± 0.50	65.17 ± 0.50	56.81 ± 3.80
5.0	63.42 ± 0.50	64.21 ± 0.50	61.95 ± 2.58

Similar midpoint temperatures were obtained from UV/VIS and VNA data. Generally, thermodynamic parameters from the VNA are similar to, but lower than, the UV/VIS data. The heat capacity obtained from VNA measurements (1.239 ± 0.726 kcal mol^{-1} K^{-1}), though rather high in error, compares well to the value obtained from differential scanning calorimetry (1.454 ± 0.122 kcal mol^{-1} K^{-1})[4].

Variation of protein concentration had two purposes: 1) to test the limits of sensitivity of the technique; 2) to assay whether the protein became destabilized by the microwave power at low concentrations. Midpoint

temperatures obtained at concentrations from 19 pM to 680 μM are shown in Table 2. Note that the UV/VIS signal became too small to detect below 8.8 μM.

Table 2: Variation of midpoint temperature with protein concentration (all data at pH 3.5)

RNase A Concentration	Midpoint temperature (°C)		
	UV/VIS Alone	UV/VIS with VNA	VNA
680 μM	54.59 ± 0.50	56.56 ± 0.50	50.91 ± 2.16
78 μM	53.33 ± 0.50	54.10 ± 0.50	51.49 ± 2.78
8.8 μM	52.84 ± 1.00	53.15 ± 1.00	56.09 ± 1.00
1.0 μM	---	---	49.36 ± 3.32
110 nM	---	---	54.09 ± 1.83
13 nM	---	---	53.44 ± 4.27
1.5 nM	---	---	46.66 ± 3.68
170 pM	---	---	54.78 ± 2.01
19 pM	---	---	49.04 ± 5.01
Average	53.59 ± 1.00	54.60 ± 1.76	51.76 ± 3.08

Midpoint temperature detected using the VNA, although considerably higher in error than the data from UV/VIS spectroscopy, shows no concentration-dependent decrease. The average T_m obtained from VNA measurements over all concentrations compares well with the T_m obtained from earlier measurements at the same pH.

Note that thermodynamic parameters could be obtained at concentration as low as 19 pM (equivalent to approximately 0.3 ng/mL). UV/VIS and circular dichroism spectroscopy measurements on this protein can only be obtained to approximately 120 μg/mL (equal to 9 mM for this protein). The only common technique that can be used to obtain thermodynamic data at similar concentrations is fluorescence spectroscopy; published reports have used concentrations as low as 10 ng/mL (0.8 nM)[5]. Our technique is usable over a wider concentration range than any almost other known biophysical technique.

A series of refolding/unfolding experiments at constant RNase A concentration and buffer conditions, but varying power network analyzer power levels, were also obtained. These experiments indicated that

thermodynamic parameters do not vary with power level, although the data gets increasing noisy at low power. This result confirms that the protein is not stabilized or destabilized by the presence of microwave power.

Results from these three series of experiments will be presented at the June 2004 International Microwave Society Meeting in Ft. Worth, TX. In addition, we applied for a patent for our antenna system in 2001[6]; it is currently in the process of final approval.

Future experiments will refine the antenna design and explore additional aspects of protein conformational change. New antennas have been designed and etched and are currently being tested; we have improved the quality factor Q and greatly reduced the S/N ratio of data. We have also designed and are currently testing a VNA-only system for titration experiments; this system can be used to measured chemical denaturation or ligand binding events.

1. Taylor, K. M. & van der Weide, D. W. (2001). *Instrumentation for Air Pollution and Global Atmospheric Monitoring, Boston, MA.*
2. Taylor, K. M. & van der Weide, D. W. (2001). *9th International Conference on Terahertz Electronics, Charlottesville, VA.*
3. Bohr, H. & Bohr, J. (2000). Microwave-enhanced folding and denaturation of globular proteins. *Phys. Rev. E* 61, 4310-4314.
4. Pace, C. N., Grimsley, G. R., Thomas, S. T. & Makhatadze, G. I. (1999). Heat capacity change for ribonuclease A folding. *Protein Sci.* 8, 1500-1504.
5. Abel, R. L., Haigis, M. R., Park, C. & Raines, R. T. (2002). Fluorescence assay for the binding of ribonuclease A to the ribonuclease inhibitor protein. *Anal. Biochem.* 306, 100-107.
6. van der Weide, D. W. & Taylor, K. M. (2001). Microwave dielectric spectroscopy method and apparatus, United States.

Figure 1: Representative return loss spectra for 78 μM RNase A at pH 3.5. From bottom to top, the temperatures are 19.78, 39.55, 59.30 and 78.96 °C.

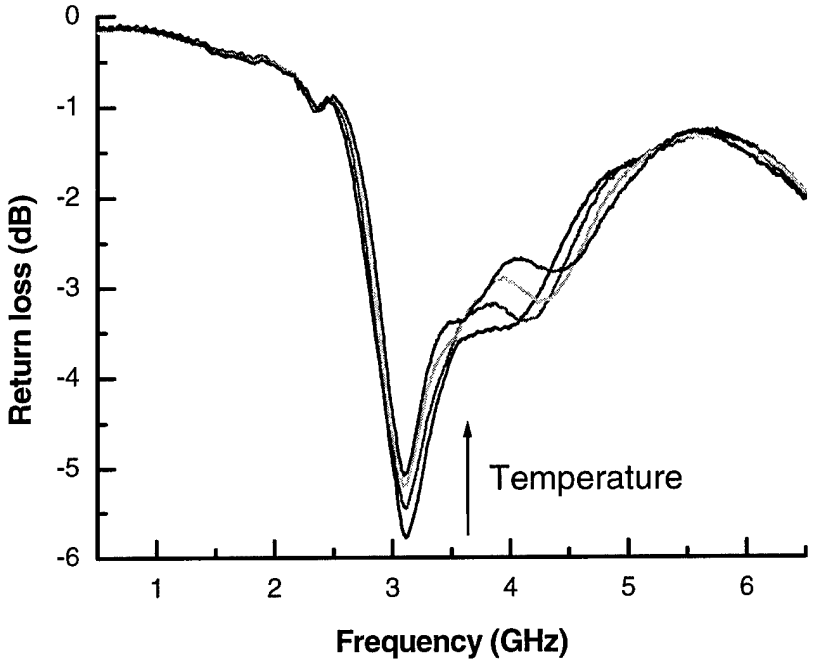

Figure 2: Variation in peak position vs. temperature for a selected peak. Protein concentration and pH are the same as in figure 1. Error bars indicate the uncertainty in peak position from the fit of the return loss spectrum to Lorentzians.

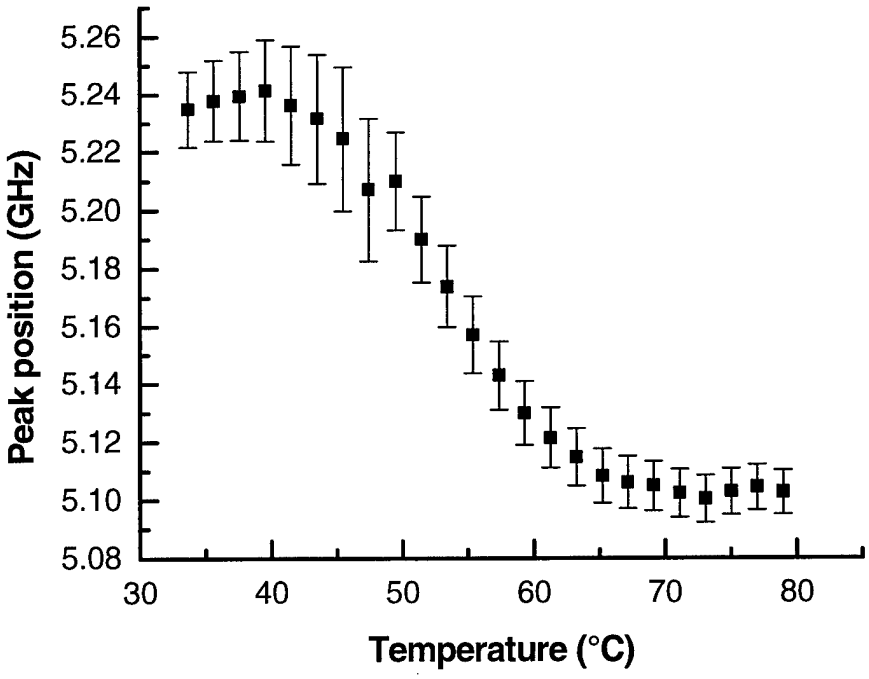
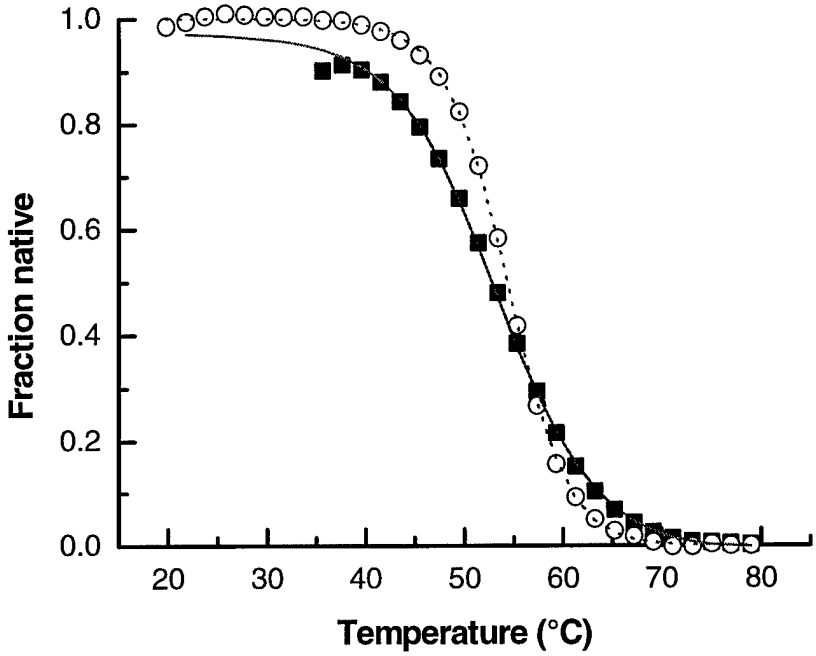
Figure 3: Analysis of peak positions from Figure 2 (solid symbols) and UV/VIS absorbance data (open symbols). Thermodynamic parameters from the peak position data were Tm equals 51.38 ± 1.37°C and ΔHm equal to 47.67 ± 5.83 kcal/mol. For the UV/VIS data, Tm was 53.44 ± 0.60 °C and ΔHm was 67.35 ± 3.00 kcal/mol.