STATEMENT OF NEED (SON)

1.0 TITLE

Purchase of NMR and Q-Band Electron Paramagnetic Resonance Equipment Upgrades to Expand Research Capabilities and Replace End-of-Life Instrumentation

2.0 BACKGROUND

The National Institutes of Health (NIH), National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Laboratory of Chemical Physics (LCP), conducts advanced research in biomolecular structure, conformational dynamics, and molecular mechanisms using nuclear magnetic resonance (NMR) spectroscopy and electron paramagnetic resonance (EPR) spectroscopy. The advanced NMR and EPR instrumentation in the LCP support mission-critical biomedical research.

The federally conducted research programs focus on:

- Structural and dynamic characterization of proteins and nucleic acid
- Mechanisms of phosphorylation and regulation in intrinsically disordered proteins
- Transient conformational states associated with ligand and substrate binding
- Sparsely-populated excited states of proteins associated with macromolecular recognition, allostery, assembly and fibril formation
- Long-range distance measurements in biomolecules using pulsed Q-band EPR techniques

This research supports NIH's mission to advance fundamental knowledge of biological systems and to improve understanding of disease mechanisms relevant to diabetes, metabolic disorders and related conditions, and neurodegenerative conditions associated with protein fibril formation and aggregation leading to a variety of amyloidosis. High-field 600-MHz NMR spectrometers equipped with cryogenic probe technology and Q-band pulsed EPR spectrometers capable of Double Electron-Electron Resonance (DEER) measurements are essential infrastructure for this research. These complementary methods provide distance measurements and structural and dynamic information that is not obtainable by other biophysical techniques. NMR provides atomic resolution information that allows almost every atom in a protein to be probed. While one of the main sources of structural information provided by NMR relates to interproton distances separated by less than 5-10 Å, pulsed Q-band EPR spectroscopy, particularly Double

Electron–Electron Resonance (DEER) enables distance measurements between pairs of paramagnetic centers ranging from 20-160 Å. When combined with rapid mixing/freeze-quenching methods, DEER measurements can provide critical insight into kinetics and mechanisms of conformational transitions.

4.0 SPECIAL ORDER REQUIREMENTS

4.1 PRODUCT FEATURES / SALIENT CHARACTERISTICS

The following product features and characteristics are required for this acquisition.

4.1.1 BASE REQUIREMENT (Base Quantity)

4.1.1.1 Cryoplatform Replacements

- Quantity two (2) current-generation cryoplatform units equivalent to or newer than Bruker Biospin CCU05.
- Full compatibility with existing Bruker Neo 600-MHz spectrometers (one located in Blg 5,B2-20, Bruker S/N 5074790, NIH Decal 02315430; the other located in Bldg 50/B204, Bruker S/N 421134, NIH Decal 02212774) and associated cryoprobes currently installed in the NMR facility.
- Equivalent or improved cooling performance relative to the current end-of-life Bruker Biospin CCU04 systems.
- Designed for integration without requiring modification to existing magnets, consoles, cryoprobes, or laboratory infrastructure.
- Manufacturer-supported systems with available service parts and long-term support.

Compatibility with existing spectrometers is mandatory. The Government seeks replacement cryogenic platforms only; acquisition of alternative systems requiring modification or replacement of existing spectrometers would not be cost-effective and would create unacceptable operational disruption.

4.1.1.2 100-W Solid-State Amplifier (SSA) and Q-Band Resonator (Base Requirement)

A 100-W solid-state amplifier and associated Q-band loop resonator are required to replace a failing 300-W traveling wave tube amplifier on the existing Bruker Excelsys E580 34 GHz EPR spectrometer currently installed in the laboratory.

Operational Need

The existing Bruker Excelsys E580 Q-band EPR spectrometer is used to carry out Double Electron-Electron Resonance (DEER) measurements for probing molecular structure and dynamics through long-range distance measurements (20–160 Å) between pairs of spin labels. When combined with rapid mixing/freeze-quench methodologies, this system is particularly powerful for elucidating the kinetics and mechanisms of conformational changes associated with ligand and substrate binding in proteins.

The current E580 spectrometer is equipped with a 300-W traveling wave tube (TWT) amplifier and complementary resonator. The TWT amplifier is progressively failing due to sustained heavy use and is approaching end-of-life status. Traveling wave tube amplifiers are no longer being manufactured and therefore cannot be repaired or replaced with equivalent units.

Required specifications

The replacement system shall meet the following minimum requirements:

- Output power: 100 W solid-state amplifier.
- Fully compatible with the existing Bruker E580 Q-band (34 GHz) spectrometer operating at approximately 34 GHz.
- Capable of delivering performance equivalent to or better than the existing TWT-based system for DEER pulse sequences.
- Designed for continuous operation consistent with high-throughput DEER measurements.
- Loop-gap resonator operating at 34 GHz with a B1 conversion factor greater than 12 G/√W when critically coupled and greater than 5 G/√W when overcoupled; a resonator bandwidth greater than 400 MHz (overcoupled); an operating temperature range from 2.5 K to room temperature; an integrated temperature sensor; optical access capability; and a probe leak rate less than 10^{-8} Torr·L·s^{-1}.

- **Compatibility and Integration**
- The SSA and resonator must integrate with the existing E580 Q-band spectrometer without modification of the console or microwave bridge.
- The system must be supported by manufacturer service and parts availability

4.1.1.3 600-MHz Small-Volume (3 mm) Cryoprobe with 1H/13C/15N/19F/31P capability

The Vendor shall provide a 600-MHz cryoprobehead meeting the following minimum performance specifications is required:

Compatibility

Must be:

- fully compatible with existing Bruker Neo 600-MHz spectrometers (and specifically the Bruker 600 MHz spectrometer located in Bldg 6/B127, Bruker S/N 421138, NIH Decal 02172949).
- Compatible with existing cryogenic infrastructure.
- Capable of integration without modification to existing console hardware.

The Government currently operates Bruker Neo 600-MHz spectrometers. To ensure interoperability and avoid unnecessary duplication of capital equipment, the probe must be compatible with the installed systems. The probe extends the capabilities by enabling observation of 31P and 19F nuclei at high sensitivity. These capabilities are essential for the study of structural effects of protein phosphorylation, and for in-cell NMR experiments of 19F-tagged molecules.

Performance specifications:

Signal-to-Noise Ratios

- ^{1}H sensitivity greater than 1000:1 (0.1% Ethylbenzene, 200 Hz noise)

- Sucrose sensitivity greater than 200:1 (2 mM sucrose in H_2O/D_2O, 1 s acquisition)
- ^{13}C sensitivity greater than 60:1 (ASTM standard)

These minimum sensitivity thresholds are required to conduct experiments on low-concentration biological samples, including intrinsically disordered proteins and phosphorylated species. Lower sensitivity would result in excessive instrument time, increased operating costs, and potentially infeasible experiments.

Pulse Width Specifications

- 1H pulse width < 10 µs
- 19F pulse width < 10 µs
- 31P pulse width <15 µs
- 2H pulse width < 150 µs
- 13C pulse width < 12 µs
- 15N pulse width < 25 µs

These pulse performance metrics are required to ensure compatibility with established experimental protocols and to maintain spectral quality consistent with ongoing NIH-funded projects.

Lineshape and Resolution (1% Chloroform Sample)

- 1H non-spinning lineshape < 1 / 8 / 20 Hz
  (50% / 0.55% / 0.11%)

High spectral resolution is required for structural and dynamic analyses of proteins where small chemical shift differences are biologically meaningful.

z-Gradient Specifications

- Gradient strength > 50 G/cm (maximum current 10 A)

- Gradient recovery time < 100 µs (85% signal amplitude)

These gradient specifications are required to support multidimensional experiments and diffusion measurements central to current research programs.

Sample Volume

- 3-mm configuration to accommodate limited sample volumes while maximizing sensitivity.

Many biological samples are available only in limited quantities. A 3-mm cryoprobe reduces sample requirements while maintaining high sensitivity, improving experimental efficiency and reducing reagent costs.

4.1.2 OPTIONAL REQUIREMENTS

The Government may, at its discretion, exercise the following options.

4.1.2.1 OPTIONAL EQUIPMENT – Q-Band Pulsed EPR Spectrometer with Cryogen-Free Cryomagnet and DEER Capability

The Government requires one compact Q-band pulsed EPR spectrometer capable of advanced Double Electron-Electron Resonance (DEER) measurements and integration with rapid freeze-quench methodologies.

Capacity

The currently installed Q-band EPR spectrometer is operating continuously (24 hours per day, 7 days per week) in support of ongoing NIH-funded research programs. Due to this sustained full utilization, the existing instrument cannot accommodate:

- New method development
- Extended acquisition experiments
- Tm-edited and T1-edited DEER measurements

- Measurements required to determine true intensities at zero evolution time
- Experiments aimed at resolving and delineating multimeric species

Many of these experiments require extensive signal averaging and long measurement times to obtain statistically reliable peak intensities in distance probability distributions derived from Q-band DEER data.

Required Performance Characteristics

The instrument shall meet or exceed the following minimum characteristics:

Frequency and Experimental Capability

- Q-band pulsed EPR operation (~34 GHz)
- Optimized for DEER (PELDOR) and DQC measurements
- Capability to perform advanced pulse sequences, including shaped pulses (WURST, HS, OCT)
- High-fidelity pulse generation via arbitrary waveform generator (AWG) with ≥12-bit amplitude resolution and sampling rate >2 GS/s
- Digital demodulation detection architecture to eliminate baseline artifacts and DC offsets

High-fidelity pulse shaping and digital detection are required for Tm-edited and T1-edited DEER experiments and for accurate determination of zero-time intensities in multimeric systems.

Sensitivity and Distance Measurement Performance

- High-sensitivity Q-band loop-gap probe capability
- Demonstrated high modulation depth and sensitivity for DEER measurements
- Capability for DQC measurements providing enhanced sensitivity at low concentrations

High sensitivity is required to accurately determine peak intensities in distance probability distributions, particularly when analyzing heterogeneous or multimeric protein systems.

Temperature Range

- Variable temperature operation from approximately 2 K to 325 K

Low-temperature operation near 2 K is required to maximize spin polarization, enhance phase memory times, and enable advanced pulsed experiments not feasible at higher temperatures.

Cryogen-Free Operation

- Integrated cryogen-free superconducting magnet and cryostat system

Cryogen-free operation reduces operational burden, eliminates dependence on liquid helium supply, and supports continuous 24/7 research activity.

Rapid Freeze-Quench Compatibility

The system must support experiments utilizing rapid mixing and freeze-quenching methodologies with dead times less than 0.5 milliseconds.

Rapid freeze-quench capability is required to investigate kinetics and mechanisms of ligand- and substrate-induced conformational changes in proteins. Such studies:

- Require large numbers of samples (one per time point)
- Require extensive acquisition time per time point

- Demand high stability and reproducibility

The existing Q-band instrument lacks sufficient availability to support these large-scale kinetic studies.

Footprint and Laboratory Constraints

- Compact footprint approximately 3 ft × 4 ft (0.9 m × 1.2 m)

Laboratory space within the NMR/EPR facility is severely limited. A compact system is required to permit installation without renovation, relocation of existing instrumentation, or interruption of ongoing research.

Integration and Support Requirements

- System must be fully integrated, turnkey, and supported by manufacturer service and parts availability.
- Software must support automated acquisition, hardware-implemented phase cycling, and high-throughput workflows

4.1.2.2 OPTIONAL EQUIPMENT – Additional Cryoplatforms

- Four (4) additional CCU05 (or newer equivalent) cryoplatforms to replace additional CCU04 cryoplatforms approaching end-of-life status. The 4 NMR spectrometers associated with these cryoplatforms are as follows: 600 MHz spectrometer in Bldg 6/B127, Bruker S/N 421138, NIH Decal 02172949; 700 MHz spectrometer in Bldg 6/B127, Bruker S/N 5077343, NIH Decal 02315427; 500 MHz spectrometer in Bldg 6/B127, bruker S/N 5058431, NIH Decal 02275426; 500 spectrometer in Bldg 50/B204, Bruker S/N 428585, NIH Decal # 02172945.
- Shall be fully compatible with installed NMR systems and associated cryoprobes.

- Shall support continuous high-duty-cycle laboratory operation.

4.2 DELIVERY / INSTALLATION

The Vendor shall deliver and install the equipment at NIH main campus in Bethesda, MD.

The Vendor shall remove all packing materials and debris left from delivery.

Delivery and installation requirements shall be coordinated with the LCP.

4.3 TRAINING

No training is required. End users are fully experienced in the operation of NMR and pulsed EPR instrumentation.