PATENT C (Draft): Calibration and Verification of Frequency-Selective
Microwave Modulation of Protein Folding (Non-Thermal Discrimination)

Method Specification and Starter Claim Set

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Status: Technical draft for counsel; **not legal advice**.
Related internal documents: `docs/JAMMING_PATENT_OUTLINES.md`; `docs/JAMMING_PROTOCOL.md`;
`docs/RS_JAMMING_FREQUENCY_PAPER.pdf`; `docs/RS_PROTEIN_FOLDING_BASELINE_PAPER.pdf`.
Note on examples: any "Example" describing expected outcomes is **prophetic** unless explicitly
stated as experimentally observed.

Abstract (Patent)

Disclosed are methods for calibrating, identifying, and verifying frequency-selective modulation of
protein folding in aqueous samples under microwave irradiation, while distinguishing such modulation
from dielectric heating. In embodiments, a calibration workflow comprises: measuring frequency-
dependent coupling and/or absorbed power in an applicator and sample cell; establishing a heating
baseline of a solvent and/or buffer; performing a temperature-controlled frequency sweep while
measuring one or more folding metrics; computing a residual response after controlling for heating;
identifying a resonant frequency window; and verifying the window using off-resonance controls
and/or an isotope shift experiment in D_2O. In embodiments, the workflow produces a resonant
frequency estimate, bandwidth, effect size, and a confidence score, and may transition to a lock
mode for subsequent experiments.

1 Field of the invention

The present disclosure relates to calibration and verification methods for microwave irradiation
experiments on biomolecular samples, and more particularly to methods that identify and validate
frequency-selective folding modulation while controlling for bulk thermal effects and coupling artifacts.

2 Background

Microwave irradiation of aqueous samples frequently produces broad, frequency-dependent heating
due to dielectric loss in water and buffer components. In the Ku band and nearby regimes, heating
and temperature gradients can dominate observed changes in biomolecular kinetics and stability. As

a result, identifying a genuine frequency-selective (resonance-like) effect requires an experimental and analytical workflow that explicitly separates narrowband effects from the baseline heating curve.

The art lacks widely adopted, reproducible calibration procedures that (i) generate a frequency-response curve for a folding metric under controlled temperature, (ii) incorporate matched-heating and off-resonance controls, and (iii) optionally verify a solvent-mass dependence (e.g., D_2O shift) that can strengthen interpretation and reproducibility.

3 Summary of the invention

In one aspect, a method is provided to identify a frequency-selective effect of microwave irradiation on a protein folding metric. The method comprises: establishing a heating baseline; performing a temperature-controlled frequency sweep while measuring a folding metric; computing a frequency-response curve; identifying a resonant frequency window as a localized deviation from the heating baseline and/or matched-heating controls; and verifying the window using one or more off-resonance controls and/or isotope shift validation.

In embodiments, the method further provides an automated analysis routine that produces a resonant frequency estimate, bandwidth, effect size, and confidence score, and that selects a frequency for subsequent "lock mode" operation.

4 Brief description of drawings

Drawings are not included in this draft. Typical filing figures include:

- Fig. 1: flowchart of calibration/verification workflow.

- Fig. 2: example coupling calibration versus frequency (e.g., reflection coefficient).

- Fig. 3: heating baseline curve (temperature rise or absorbed power) versus frequency.

- Fig. 4: folding metric versus frequency (raw) and residual (after baseline removal).

- Fig. 5: fit of residual response to a peak model (e.g., Lorentzian) with extracted center and width.

- Fig. 6: comparison plots: on-window vs off-window frequencies under matched heating.

- Fig. 7: H_2O versus D_2O frequency shift comparison.

5 Detailed description

5.1 Definitions

Unless otherwise stated:

- **Folding metric**: any measurable observable correlated with folded fraction or folding kinetics, including CD ellipticity (e.g., 222 nm), fluorescence, FRET, NMR, IR, light scattering, or other spectroscopy.

- **Heating baseline**: a model or measurement describing the expected change in the folding metric attributable to bulk temperature change and/or absorbed power as a function of frequency.

- **Matched-heating control**: a control condition in which power/duty cycle is adjusted so that the bulk temperature trajectory (or absorbed power estimate) matches a reference condition.

- **Off-resonance control**: one or more frequencies outside a candidate resonant window used to estimate a null response under matched heating.

- **Residual response**: a difference between (i) observed folding metric response and (ii) expected response from the heating baseline or matched-heating controls.

- **Resonant window**: a bounded frequency interval where the residual response is significantly different from off-resonance controls.

- **Confidence score**: a quantitative indicator of how likely the observed residual response is to represent a localized, repeatable effect rather than noise/heating artifacts (e.g., peak-to-baseline ratio, repeatability, fit quality, or statistical significance).

5.2 Calibration workflow (core embodiment)

In one embodiment, the calibration and verification workflow comprises:

1. **Instrument/coupling calibration.** Measure frequency-dependent coupling of the applicator and sample cell. In embodiments, measure incident/reflected power and/or reflection coefficient as a function of frequency to characterize delivered field strength.

2. **Blank heating baseline.** Using buffer without protein (or a reference sample), apply irradiation across a frequency sweep and record bulk temperature trajectories. This produces a baseline heating curve as a function of frequency and power.

3. **Temperature-controlled sweep with protein.** Prepare a protein sample at a fixed temperature setpoint (e.g., $25\,°C$). Sweep frequency across a target band while maintaining bulk temperature within a tolerance (e.g., $\pm 0.2\,°C$). Measure the folding metric at each frequency (or continuously).

4. **Matched-heating sweep (control).** Repeat the sweep while adjusting power and/or duty cycle so that the bulk temperature trajectory (or absorbed power estimate) matches across frequencies. This enables subtraction of purely thermal effects.

5. **Compute residual response.** Compute a residual response versus frequency by subtracting the heating baseline or matched-heating control response from the raw folding metric response.

6. **Identify candidate resonant window.** Identify a localized peak/dip in the residual response. In embodiments, fit a peak model (e.g., Gaussian or Lorentzian) to estimate center frequency, width, and effect size.

7. **Verification by off-resonance controls.** Measure the folding metric at one or more off-resonance frequencies under matched heating to confirm that the effect is localized and not broadly present.

8. **Optional verification by isotope shift.** Repeat the above steps in D_2O buffer and compare the identified resonant window to that in H_2O, determining whether a reproducible shift exists.

9. **Lock mode.** If verified, operate at the identified frequency and duty cycle for subsequent experiments (e.g., kinetic measurements, power-dependence curves).

5.3   Residual computation embodiments

Residual from matched heating.   In one embodiment, the residual response at frequency f is:

$$R(f) = M_{\mathrm{on}}(f) - M_{\mathrm{matched}}(f),$$

where M is a folding metric and M_{matched} is measured under a matched-heating control.

Residual from heating baseline model.   In one embodiment, a baseline model predicts a thermal contribution $B(f)$ to the folding metric (based on measured temperature rise, absorbed power, or a calibration curve), and:

$$R(f) = M_{\mathrm{on}}(f) - B(f).$$

5.4   Peak identification embodiments

Peak model fit.   In one embodiment, a peak model (e.g., Lorentzian) is fit:

$$R(f) \approx \frac{A\gamma^2}{(f - f_0)^2 + \gamma^2} + c,$$

extracting center frequency f_0, half-width γ, amplitude A, and baseline c.

Non-parametric identification.   In one embodiment, the resonant window is identified by comparing residual values to an off-resonance distribution using a threshold criterion (e.g., exceeds the off-resonance mean by k standard deviations) and requiring repeatability across runs.

5.5   Confidence scoring embodiments

In one embodiment, a confidence score combines:

- effect size (peak-to-off-resonance contrast),
- fit quality (e.g., R^2 of peak model),
- repeatability across replicate sweeps,
- thermal stability (temperature drift within tolerance),
- control separation (off-resonance controls show reduced residual).

6   Examples (prophetic unless otherwise stated)

Example 1: Ku-band sweep with matched heating

Prepare a fast-folding protein in buffer at $25\,^{\circ}\mathrm{C}$ and measure a fluorescence folding metric. Perform a sweep from $14.0\,\mathrm{GHz}$ to $15.2\,\mathrm{GHz}$ in $0.05\,\mathrm{GHz}$ steps under closed-loop temperature control. Repeat the sweep using matched-heating controls. Compute residual response and identify whether a localized peak/dip exists.

Example 2: Off-resonance verification

After identifying a candidate window near 14.65 GHz, measure the folding metric at two off-resonance frequencies (e.g., near 11.5 GHz and 18.6 GHz) under matched heating. Confirm that residual effects are reduced outside the window.

Example 3: D_2O shift

Repeat Example 1 in D_2O buffer. Identify whether the candidate window shifts in center frequency relative to H_2O.

7 Claims (starter set; for counsel refinement)

What follows is a technical starter claim set to guide drafting. Counsel should rewrite for jurisdiction, support, and scope.

1. A method of identifying a frequency-selective effect of microwave irradiation on protein folding, comprising:

   (a) providing an aqueous sample comprising a protein;

   (b) irradiating the aqueous sample with narrowband microwave radiation at a plurality of frequencies while maintaining a bulk temperature of the aqueous sample within a temperature tolerance;

   (c) measuring a folding metric at the plurality of frequencies to generate a frequency-response curve; and

   (d) identifying a resonant frequency window based on the frequency-response curve.

2. The method of claim 1, further comprising establishing a heating baseline by irradiating a reference sample and recording a temperature response as a function of frequency.

3. The method of claim 2, further comprising computing a residual response by subtracting an expected thermal contribution from the frequency-response curve.

4. The method of claim 1, further comprising performing a matched-heating control in which microwave power and/or duty cycle is adjusted to match a bulk temperature trajectory across at least two different frequencies.

5. The method of claim 1, further comprising verifying the resonant frequency window by measuring the folding metric at one or more off-resonance control frequencies under matched heating.

6. The method of claim 5, wherein the off-resonance control frequencies comprise at least one frequency below and at least one frequency above the resonant frequency window.

7. The method of claim 1, wherein identifying the resonant frequency window comprises fitting a peak model to a residual response curve to estimate a center frequency and a bandwidth.

8. The method of claim 1, further comprising generating a confidence score based on effect size and repeatability across replicate sweeps.

9. The method of claim 1, further comprising selecting an operating frequency within the resonant frequency window and operating the microwave radiation at the operating frequency in a lock mode for subsequent folding experiments.

10. The method of claim 1, wherein the plurality of frequencies comprise frequencies in a Ku-band range.

11. The method of claim 1, wherein the plurality of frequencies comprise a sweep across $14.0\,\mathrm{GHz}$ to $15.2\,\mathrm{GHz}$.

12. The method of claim 1, wherein the temperature tolerance is $\pm 0.2\,^\circ\mathrm{C}$ or tighter.

13. The method of claim 1, further comprising repeating the method in a D_2O-containing aqueous sample and determining an isotope-dependent shift of the resonant frequency window.

14. A non-transitory computer-readable medium comprising instructions that, when executed by one or more processors, cause the one or more processors to:

    (a) ingest frequency-response measurements of a folding metric and corresponding temperature data;

    (b) compute a residual response by controlling for heating;

    (c) fit a peak model or apply a threshold criterion to identify a resonant frequency window; and

    (d) output a center frequency, a bandwidth, and a confidence score.