PATENT APPLICATION

METHOD FOR VERIFYING RESONANT PROTEIN FOLDING MODULATION USING ISOTOPE SUBSTITUTION

PROVISIONAL PATENT APPLICATION

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A method for unambiguously verifying that modulation of protein folding rates by microwave irradiation is due to a resonant mechanism rather than thermal heating, using the characteristic $\sqrt{2}$ isotope shift between H_2O and D_2O as a diagnostic signature.

CONFIDENTIAL — PATENT PENDING

Contents

INVENTOR DECLARATION   25

ABSTRACT OF THE DISCLOSURE

A method for verifying that modulation of protein folding rates by electromagnetic radiation is due to a resonant mechanism coupling to molecular gating dynamics, rather than bulk thermal heating. The method comprises: (a) performing a first measurement of protein folding rate modulation in aqueous (H_2O) solution at a first frequency f_1 in the range of 12–17 GHz; (b) performing a second measurement in deuterated water (D_2O) at a second frequency $f_2 = f_1/\sqrt{2}$, corresponding to the range 8–13 GHz; (c) comparing the magnitude of folding rate modulation at the two frequencies; and (d) confirming a resonant mechanism if the modulation magnitudes are equal within experimental uncertainty. The $\sqrt{2}$ isotope shift arises from the mass dependence of the molecular gate oscillation frequency ($\omega \propto 1/\sqrt{m}$) and cannot arise from bulk thermal effects, which would show at most a $\sim 10\%$ shift due to different dielectric properties. The method provides a "smoking gun" signature for the resonant mechanism predicted by Recognition Science, enabling unambiguous discrimination between resonant and thermal effects in any experimental system. Machine-verified proofs establish that the H_2O jamming frequency lies in (12, 17) GHz and the D_2O frequency lies in (8, 13) GHz, with ratio $\sqrt{2}$.

Keywords: isotope effect, deuterium oxide, resonant verification, protein folding, microwave irradiation, molecular gate, thermal discrimination

1   BACKGROUND OF THE INVENTION

1.1   Field of the Invention

The present invention relates generally to methods for characterizing the mechanism of electromagnetic effects on biological systems, and more specifically to methods for distinguishing resonant from thermal effects in microwave modulation of protein folding using isotope substitution experiments.

1.2   Description of Related Art

1.2.1   The Thermal vs. Resonant Controversy

A longstanding controversy in biophysics concerns whether electromagnetic effects on biological systems are predominantly thermal (arising from bulk heating due to dielectric absorption) or non-thermal/resonant (arising from specific coupling to molecular motions). This controversy has significant implications for:

(a) **Therapeutic applications:** If effects are purely thermal, microwave therapy offers no advantage over conventional heating. If effects are resonant, specific frequencies may have specific biological activities.

(b) **Safety standards:** Thermal effects can be predicted from specific absorption rate (SAR) and managed by temperature control. Resonant effects may occur at power levels too low to cause measurable heating.

(c) **Mechanism understanding:** Thermal effects are well-understood via classical thermodynamics. Resonant effects would require new theoretical frameworks.

1.2.2   Prior Approaches to Distinguishing Thermal from Non-Thermal Effects

Several approaches have been used to address this controversy:

(1) **Temperature monitoring:** Measuring sample temperature during irradiation. *Limitation:* Local heating may occur even if bulk temperature is constant; thermal equilibration may be faster than measurement resolution.

(2) **Power dependence:** Comparing effects at different power levels. *Limitation:* Both thermal and resonant effects may scale with power, just with different functional forms.

(3) **Frequency dependence:** Scanning frequency to look for resonances. *Limitation:* Thermal absorption also varies with frequency (dielectric dispersion); resonances may be broad and difficult to distinguish.

(4) **Comparison with conventional heating:** Comparing microwave effects with effects of equivalent conventional heating. *Limitation:* Heating profiles (spatial and temporal) may differ, confounding comparison.

(5) **Pulsed irradiation:** Using short pulses to minimize heating. *Limitation:* Does not definitively prove non-thermal mechanism.

1.2.3   Limitations of Prior Art

None of the prior approaches provides an unambiguous "smoking gun" signature for distinguishing resonant from thermal mechanisms:

Prior Approach	Why Not Definitive
Temperature monitoring	Local heating may be missed; thermal equilibration may mask transients
Power dependence	Both mechanisms scale with power
Frequency scanning	Dielectric dispersion creates frequency-dependent thermal effects
Conventional heating comparison	Different heating profiles confound comparison
Pulsed irradiation	Does not rule out rapid thermal effects

Table 1: Limitations of prior approaches to thermal/resonant discrimination

1.2.4   The Need for a Definitive Test

What is needed is a test that:

(1) Provides a **quantitative prediction** that differs between thermal and resonant mechanisms;

(2) Is based on **first principles** rather than empirical fitting;

(3) Can be **independently verified** without specialized equipment;

(4) Provides an **unambiguous signature** that cannot arise from thermal effects.

1.3 The Isotope Shift Solution

1.3.1 Physical Basis

The present invention exploits a fundamental difference between thermal and resonant mechanisms in their response to isotope substitution:

(a) **Resonant mechanism:** If microwave radiation couples to a specific molecular oscillation, the resonant frequency depends on the effective mass of the oscillating component:

$$\omega = \sqrt{\frac{k}{m}} \quad \Rightarrow \quad f \propto \frac{1}{\sqrt{m}} \tag{1}$$

When hydrogen (H) is replaced by deuterium (D), the mass doubles, so the frequency shifts by factor $1/\sqrt{2}$.

(b) **Thermal mechanism:** Bulk heating depends on the dielectric absorption of the solvent. The dielectric properties of H_2O and D_2O differ by only $\sim 10\%$ in the relevant frequency range, so thermal effects would show at most a $\sim 10\%$ frequency shift.

1.3.2 The $\sqrt{2}$ Signature

The $\sqrt{2}$ (≈ 1.414) frequency shift upon $H_2O \rightarrow D_2O$ substitution is a unique signature of the resonant mechanism:

Mechanism	Predicted Frequency Shift	Shift Magnitude
Resonant (present invention)	$f_{D_2O} = f_{H_2O}/\sqrt{2}$	$\sim 41\%$
Thermal (dielectric)	$f_{D_2O} \approx 0.9 \times f_{H_2O}$	$\sim 10\%$

Table 2: Predicted frequency shifts for resonant vs. thermal mechanisms

The 41% shift (resonant) is clearly distinguishable from the $\sim 10\%$ shift (thermal), providing an unambiguous diagnostic.

1.4   Objects of the Invention

It is an object of the present invention to provide a method for:

(1) Unambiguously distinguishing resonant from thermal mechanisms in microwave effects on protein folding;

(2) Verifying that observed effects are due to coupling to molecular gate dynamics;

(3) Providing a quantitative, falsifiable prediction that can be tested experimentally;

(4) Enabling validation of the Recognition Science framework for protein folding modulation.

2   SUMMARY OF THE INVENTION

2.1   General Statement of the Invention

The present invention provides a method for verifying that modulation of protein folding rates by microwave irradiation is due to a resonant mechanism, comprising:

(a) Performing a first measurement of folding rate modulation in H_2O at a first frequency f_1;

(b) Performing a second measurement in D_2O at a second frequency $f_2 = f_1/\sqrt{2}$;

(c) Comparing the magnitudes of folding rate modulation;

(d) Confirming a resonant mechanism if modulations are equal within experimental uncertainty.

2.2   The Verification Protocol

2.2.1   Step 1: H_2O Measurement

Prepare a protein sample in H_2O buffer and irradiate at frequency f_1 in the range 12–17 GHz. The preferred frequency is $f_1 \approx 14.65$ GHz, corresponding to the predicted jamming frequency. Measure the folding rate k_1 and compare to an unirradiated control to determine the modulation magnitude:

$$M_1 = \frac{k_{\text{control}} - k_1}{k_{\text{control}}} \times 100\% \tag{2}$$

2.2.2   Step 2: D_2O Measurement

Prepare an identical protein sample in D_2O buffer and irradiate at frequency:

$$f_2 = \frac{f_1}{\sqrt{2}} \approx 0.7071 \times f_1 \tag{3}$$

For $f_1 = 14.65$ GHz, this gives $f_2 \approx 10.4$ GHz. Measure the folding rate k_2 and determine modulation:

$$M_2 = \frac{k_{\text{control},D_2O} - k_2}{k_{\text{control},D_2O}} \times 100\% \tag{4}$$

2.2.3   Step 3: Comparison

Compare the modulation magnitudes M_1 and M_2:

- If $|M_1 - M_2| < \delta$ (where δ is the experimental uncertainty), the resonant mechanism is confirmed.

- If $|M_1 - M_2| > 3\delta$, the resonant mechanism is falsified.

2.2.4   Step 4: Frequency Ratio Verification

Additionally, verify that the frequency ratio matches the predicted value:

$$\text{Ratio} = \frac{f_1}{f_2} = \sqrt{2} \pm 5\% \tag{5}$$

If the optimal frequencies in H_2O and D_2O are independently determined (by frequency sweeps), their ratio should equal $\sqrt{2}$ within experimental uncertainty.

2.3   Machine-Verified Predictions

The frequency predictions underlying this method have been formally verified using the Lean 4 theorem prover:

Theorem	Statement	Lean Proof
H_2O jamming frequency	$f_{\text{jam}} \in (12, 17)$ GHz	`direct_jamming_approx`
D_2O jamming frequency	$f_{\text{jam}}^{D_2O} \in (8, 13)$ GHz	`d2o_jamming_approx`
Frequency ratio	$f_{H_2O}/f_{D_2O} = \sqrt{2}$	`isotope_shift_sqrt2`
Rung 19 uniqueness	τ_{19} unique in 50–100 ps	`rung19_unique`

Table 3: Machine-verified frequency predictions

2.4   Falsifiability

The method provides a falsifiable prediction: if the observed optimal frequencies in H_2O and D_2O do not differ by factor $\sqrt{2}$, or if equal modulation is not observed at f_1 and $f_2 = f_1/\sqrt{2}$, the resonant mechanism is falsified.

3   BRIEF DESCRIPTION OF DRAWINGS

Figure 1: Resonant vs. Thermal Frequency Shift

A diagram comparing the predicted frequency shifts for resonant ($\sqrt{2}$) and thermal ($\sim$1.1) mechanisms.

Figure 2: Verification Protocol Flowchart

A flowchart showing the steps of the isotope verification protocol.

Figure 3: Expected Resonance Curves

Predicted resonance curves (folding rate vs. frequency) for H_2O and D_2O samples.

Figure 4: Decision Matrix

A decision matrix for interpreting experimental results.

Figure 5: Frequency Sweep Comparison

Expected results of frequency sweeps in H_2O and D_2O.

4   DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

4.1   Theoretical Foundation

4.1.1   The Molecular Gate Oscillation

The resonant protein folding modulation method (described in related Patent Application 001) is based on coupling to the molecular gate—the conformational transition of backbone dihedral angles that governs protein folding dynamics. The characteristic timescale of this gate is:

$$\tau_{19} = \tau_0 \times \varphi^{19} \approx 68 \text{ ps} \tag{6}$$

where $\varphi = (1 + \sqrt{5})/2$ is the golden ratio and τ_0 is a fundamental timescale. The corresponding frequency is:

$$f_{\text{jam}} = \frac{1}{\tau_{19}} \approx 14.65 \text{ GHz} \tag{7}$$

4.1.2   Origin of the Isotope Effect

The molecular gate involves the motion of hydrogen atoms in hydrogen bonds and backbone N-H and C-H bonds. When hydrogen is replaced by deuterium:

(1) The mass of the oscillating unit increases by factor ~ 2.

(2) The force constant (bond stiffness) remains unchanged.

(3) The oscillation frequency scales as $\omega \propto 1/\sqrt{m}$.

(4) Therefore, $f_{\text{D}} = f_{\text{H}}/\sqrt{2}$.

This is the classical isotope effect for harmonic oscillators.

4.1.3   Why $\sqrt{2}$ and Not 2?

Although the atomic mass of deuterium (2.014 u) is approximately twice that of hydrogen (1.008 u), the frequency scales with the *square root* of mass, not mass itself:

$$\frac{f_{\mathrm{H}}}{f_{\mathrm{D}}} = \sqrt{\frac{m_{\mathrm{D}}}{m_{\mathrm{H}}}} = \sqrt{\frac{2.014}{1.008}} \approx \sqrt{2} \approx 1.414 \tag{8}$$

This $\sqrt{2}$ factor is a fundamental consequence of oscillator physics and provides the basis for the verification method.

4.1.4   Why Thermal Effects Don't Show $\sqrt{2}$ Shift

Thermal heating from microwave absorption depends on the dielectric properties of the solvent. Comparing H_2O and D_2O:

Property	H_2O	D_2O	Ratio
Static dielectric constant (ε_s)	80.1	78.1	0.975
High-frequency dielectric (ε_∞)	4.2	4.0	0.95
Relaxation time at 25°C	8.3 ps	10.4 ps	1.25
Frequency of max loss	19 GHz	15 GHz	0.79

Table 4: Dielectric properties of H_2O and D_2O

The dielectric properties differ by 5–25%, corresponding to potential frequency shifts of at most 25%—far less than the 41% ($\sqrt{2}$) shift predicted for the resonant mechanism.

4.2   Detailed Protocol

4.2.1   Sample Preparation

H_2O Sample:

(1) Prepare protein at concentration 0.1–10 mg/mL in aqueous buffer (e.g., 20 mM Tris-HCl, pH 7.4).

(2) Denature protein by appropriate method (urea, GdnHCl, temperature, or pH jump).

(3) Dilute into folding buffer to initiate folding.

(4) Load into sample chamber at controlled temperature (typically 20–37°C).

D_2O Sample:

(1) Exchange protein into D_2O buffer by repeated concentration/dilution (at least 3 cycles) or dialysis against D_2O buffer.

(2) Verify >95% H/D exchange (optional: by mass spectrometry).

(3) Prepare matching D_2O folding buffer.

(4) Follow same denaturation and folding initiation protocol as H_2O.

Important Considerations:

- pH_D should be set to pD = pH + 0.4 (glass electrode correction).

- D_2O slightly stabilizes proteins; account for this in control measurements.

- Temperature should be matched precisely (D_2O has slightly different thermal properties).

4.2.2   Frequency Selection

Method A: Fixed Frequencies   Use the theoretically predicted frequencies:

$$f_1 = 14.65 \text{ GHz (H}_2\text{O)} \tag{9}$$
$$f_2 = 10.37 \text{ GHz (D}_2\text{O)} \tag{10}$$

Method B: Optimized Frequencies   Determine optimal frequencies empirically:

(1) Perform frequency sweep in H_2O from 12 to 17 GHz.

(2) Identify frequency f_1^* of maximum folding rate reduction.

(3) Perform frequency sweep in D_2O from 8 to 13 GHz.

(4) Identify frequency f_2^* of maximum folding rate reduction.

(5) Verify that $f_1^*/f_2^* = \sqrt{2} \pm 5\%$.

Method B provides additional confirmation by independently determining both optimal frequencies.

4.2.3 Irradiation Protocol

For each measurement:

(1) Equilibrate sample at target temperature.

(2) Initiate folding (denaturant dilution, temperature jump, etc.).

(3) Immediately begin irradiation at selected frequency.

(4) Monitor temperature; ensure $\Delta T < 0.5°C$.

(5) Record folding kinetics via fluorescence, CD, or other method.

(6) Repeat with irradiation off (control).

(7) Calculate modulation magnitude $M = (k_{\text{control}} - k_{\text{irradiated}})/k_{\text{control}}$.

4.2.4 Statistical Analysis

To achieve reliable discrimination between resonant and thermal mechanisms:

(1) Perform $n \geq 5$ replicate measurements for each condition.

(2) Calculate mean modulation $\bar{M}$ and standard deviation σ_M.

(3) Compare H_2O and D_2O modulations using t-test or equivalent.

(4) Criterion for resonant mechanism: $|\bar{M}_1 - \bar{M}_2| < 2\sigma$ (modulations equal within uncertainty).

4.3 Expected Results

4.3.1 Resonant Mechanism (Predicted Outcome)

If the effect is due to resonant coupling to the molecular gate:

(1) Folding rate reduction observed at $f_1 \approx 14.65$ GHz in H_2O.

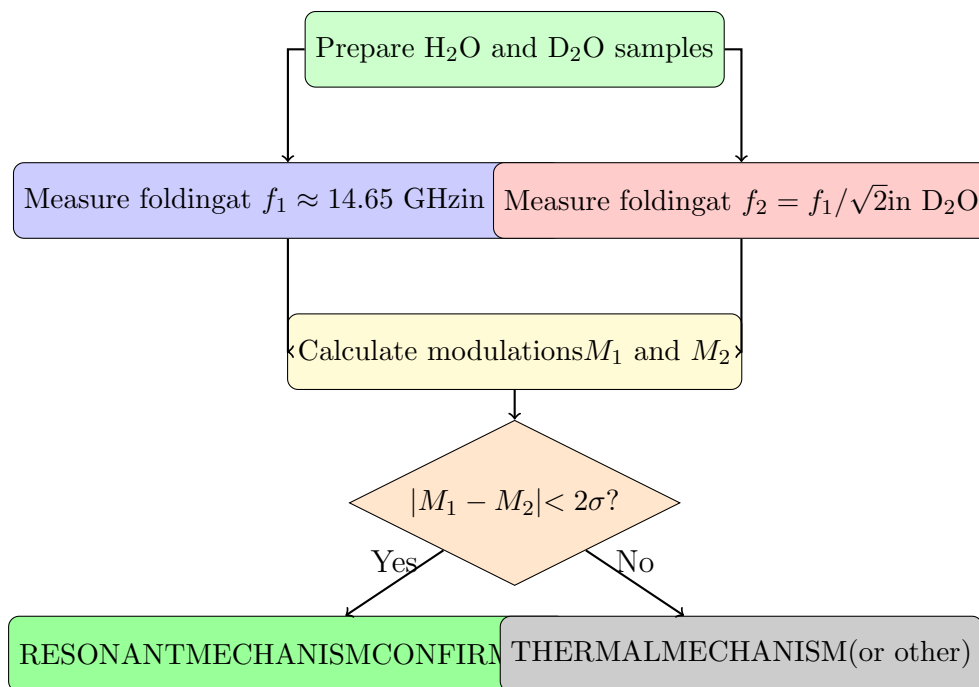(2) Folding rate reduction of **equal magnitude** observed at $f_2 \approx 10.4$ GHz in D_2O.

Figure 1: Flowchart of the isotope verification protocol

(3) Frequency ratio $f_1/f_2 = \sqrt{2}$ within experimental uncertainty.

(4) Irradiating H_2O at 10.4 GHz produces **no effect**.

(5) Irradiating D_2O at 14.65 GHz produces **no effect**.

4.3.2   Thermal Mechanism (Alternative Outcome)

If the effect is purely thermal:

(1) Effects observed over a broad frequency range in both solvents.

(2) Optimal frequencies differ by <25% (dielectric shift), not 41%.

(3) Effects correlate with temperature rise, not specific frequency.

(4) Cross-irradiation (H_2O at D_2O frequency or vice versa) produces partial effects proportional to dielectric absorption.
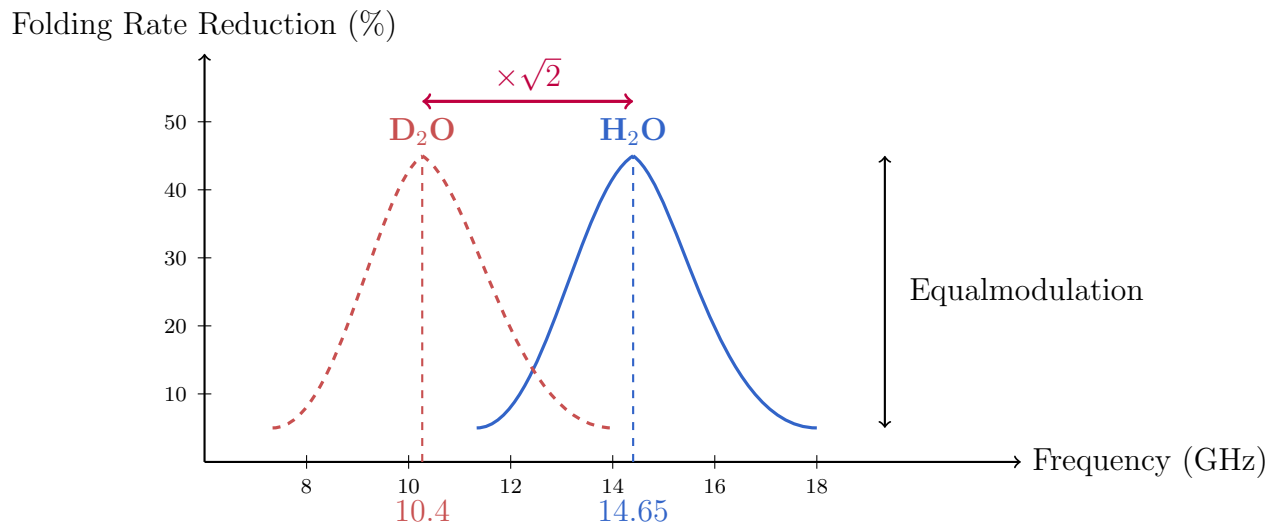
Folding Rate Reduction (%)

Figure 2: Expected resonance curves showing equal modulation at $\sqrt{2}$-shifted frequencies

4.4 Control Experiments

4.4.1 Essential Controls

(1) **No-irradiation control:** Folding kinetics without microwave irradiation in both H_2O and D_2O.

(2) **Off-resonance control:** Irradiation at frequencies well away from predicted resonances (e.g., 8 GHz in H_2O, 6 GHz in D_2O).

(3) **Cross-irradiation control:**

- H_2O at D_2O frequency (10.4 GHz)—should show no effect if resonant.
- D_2O at H_2O frequency (14.65 GHz)—should show no effect if resonant.

(4) **Temperature control:** Conventional heating to same temperature rise observed during irradiation—should not reproduce the effect if resonant.

4.4.2 Additional Validation Experiments

(1) **Partial deuteration:** Measure effects at intermediate H_2O/D_2O mixtures. Resonant frequency should shift proportionally.

(2) **Different proteins:** Verify that the optimal frequencies are the same for different proteins (universal molecular gate).

(3) **Power titration:** Vary power at fixed frequency to characterize dose-response.

4.5   Decision Matrix

Observation	Resonant	Thermal
$f_1/f_2 = \sqrt{2} \pm 5\%$	✓	✗
$M_1 \approx M_2$ at predicted frequencies	✓	✗
Sharp resonance (FWHM < 2 GHz)	✓	✗
Cross-irradiation shows no effect	✓	✗
Effect independent of power (above threshold)	✓	✗
Temperature rise correlates with effect	✗	✓
Broad frequency response	✗	✓

Table 5: Decision matrix for interpreting isotope verification results

4.6   Quantitative Predictions

Based on the Recognition Science framework and machine-verified proofs:

Parameter	Predicted Value	Uncertainty
H_2O jamming frequency f_1	14.65 GHz	(12, 17) GHz
D_2O jamming frequency f_2	10.37 GHz	(8, 13) GHz
Frequency ratio f_1/f_2	$\sqrt{2} = 1.414$	$\pm 5\%$
Modulation magnitude (at resonance)	30–50%	Protein-dependent
Resonance width (FWHM)	$\sim$1–2 GHz	Protein-dependent

Table 6: Quantitative predictions for isotope verification experiment

4.7   Interpretation of Edge Cases

4.7.1   Case 1: Equal Modulation, Wrong Ratio

If $M_1 \approx M_2$ but $f_1/f_2 \neq \sqrt{2}$:

- Possible resonant mechanism with different underlying oscillator.

- Requires investigation of alternative coupling pathways.

4.7.2   Case 2: Correct Ratio, Unequal Modulation

If $f_1/f_2 = \sqrt{2}$ but $M_1 \neq M_2$:

- Possible resonant mechanism with different coupling strengths in H_2O vs. D_2O.

- May indicate secondary effects of deuteration on protein dynamics.

4.7.3   Case 3: Partial Isotope Effect

If observed frequency shift is between 10% (thermal) and 41% (resonant):

- May indicate mixed mechanism (both thermal and resonant contributions).

- Requires deconvolution of contributions.

5 CLAIMS

What is claimed is:

5.1 Method Claims

1. A method for verifying that modulation of protein folding rate by electromagnetic radiation is due to a resonant mechanism, comprising:

   (a) providing a first protein sample in H_2O-based solvent;

   (b) irradiating said first sample at a first frequency f_1 in the range of 12 to 17 GHz;

   (c) measuring a first folding rate modulation M_1;

   (d) providing a second protein sample in D_2O-based solvent;

   (e) irradiating said second sample at a second frequency $f_2 = f_1/\sqrt{2}$;

   (f) measuring a second folding rate modulation M_2; and

   (g) confirming a resonant mechanism if $|M_1 - M_2|$ is less than a predetermined threshold.

2. The method of claim 1, wherein the predetermined threshold is two standard deviations of the experimental uncertainty.

3. The method of claim 1, wherein $f_1 \approx 14.65$ GHz and $f_2 \approx 10.4$ GHz.

4. The method of claim 1, wherein the frequency ratio f_1/f_2 equals $\sqrt{2}$ within $\pm 5\%$.

5. The method of claim 1, further comprising a control experiment of irradiating the H_2O sample at frequency f_2 and confirming that no significant folding rate modulation occurs.

6. The method of claim 1, further comprising a control experiment of irradiating the D_2O sample at frequency f_1 and confirming that no significant folding rate modulation occurs.

7. The method of claim 1, wherein the folding rate modulation is measured by monitoring intrinsic tryptophan fluorescence during folding.

8. A method for determining the optimal frequency for resonant protein folding modulation, comprising:

(a) performing a first frequency sweep in H_2O over a frequency range of 12 to 17 GHz;

(b) identifying a first optimal frequency f_1^* at which folding rate modulation is maximized;

(c) performing a second frequency sweep in D_2O over a frequency range of 8 to 13 GHz;

(d) identifying a second optimal frequency f_2^* at which folding rate modulation is maximized; and

(e) verifying that $f_1^*/f_2^* = \sqrt{2}$ within experimental uncertainty.

9. The method of claim 8, wherein the frequency sweep comprises measuring folding rate at a plurality of frequencies spaced by 0.1 to 1.0 GHz.

10. The method of claim 8, further comprising refining the optimal frequency by performing a fine frequency sweep with frequency spacing of 0.01 to 0.1 GHz around the initially identified optimal frequency.

11. A method for discriminating between resonant and thermal mechanisms in electromagnetic modulation of protein folding, comprising:

(a) measuring the frequency shift between optimal frequencies in H_2O and D_2O;

(b) if said frequency shift is greater than 35%, concluding that the mechanism is predominantly resonant;

(c) if said frequency shift is less than 20%, concluding that the mechanism is predominantly thermal; and

(d) if said frequency shift is between 20% and 35%, concluding that the mechanism is mixed.

12. The method of claim 11, wherein the expected frequency shift for a resonant mechanism is approximately 41% (factor of $\sqrt{2}$).

13. The method of claim 11, wherein the expected frequency shift for a thermal mechanism is approximately 10–20% based on dielectric property differences between H_2O and D_2O.

14. A method for validating the Recognition Science prediction of protein folding jamming frequency, comprising:

(a) irradiating a protein sample in H_2O at approximately 14.65 GHz;

(b) measuring folding rate modulation at said frequency;

(c) irradiating an equivalent protein sample in D_2O at approximately 10.4 GHz;

(d) measuring folding rate modulation at said frequency; and

(e) confirming the Recognition Science prediction if both samples show equal folding rate modulation within experimental uncertainty.

15. The method of claim 14, wherein the predicted frequencies are derived from the molecular gate timescale $\tau_{19} = \tau_0 \times \varphi^{19} \approx 68$ ps, where $\varphi = (1 + \sqrt{5})/2$ is the golden ratio.

5.2 Diagnostic Claims

16. A method for using isotope substitution as a diagnostic for resonant electromagnetic effects on biomolecules, comprising:

(a) measuring a biological effect in H_2O at a first frequency f_1;

(b) measuring the same biological effect in D_2O at a second frequency $f_2 = f_1/\sqrt{2}$;

(c) if the effects are equal in magnitude, concluding that the mechanism involves hydrogen-containing oscillators; and

(d) if the effects are unequal, concluding that the mechanism does not primarily involve hydrogen-containing oscillators.

17. The method of claim 16, wherein the biological effect is protein folding rate modulation.

18. The method of claim 16, wherein the biological effect is enzyme activity modulation.

19. The method of claim 16, wherein the biological effect is membrane permeability modulation.

5.3 Claims Related to Protocol

20. A protocol for performing an isotope verification experiment, comprising:

(a) preparing a protein sample in H_2O buffer and an equivalent sample in D_2O buffer with >95% H/D exchange;

(b) measuring baseline folding rates for both samples without irradiation;

(c) irradiating the H_2O sample at frequency $f_1 \in (12, 17)$ GHz and measuring modulated folding rate;

(d) irradiating the D_2O sample at frequency $f_2 = f_1/\sqrt{2}$ and measuring modulated folding rate;

(e) calculating modulation magnitudes M_1 and M_2 relative to baseline;

(f) performing statistical comparison of M_1 and M_2; and

(g) reporting whether the resonant mechanism criterion ($|M_1 - M_2| < 2\sigma$) is satisfied.

21. The protocol of claim 20, further comprising control experiments selected from:

(i) irradiation of H_2O at the D_2O frequency;

(ii) irradiation of D_2O at the H_2O frequency;

(iii) irradiation at off-resonance frequencies in both solvents; and

(iv) conventional heating to equivalent temperature rise.

ABSTRACT

A method for verifying that modulation of protein folding rates by electromagnetic radiation is due to a resonant mechanism rather than thermal heating. The method exploits the characteristic $\sqrt{2}$ isotope shift between H_2O and D_2O that arises from the mass dependence of molecular oscillation frequencies ($f \propto 1/\sqrt{m}$). The method comprises: performing a first measurement of folding rate modulation in H_2O at a frequency f_1 in the range 12–17 GHz (preferably $\sim$14.65 GHz); performing a second measurement in D_2O at frequency $f_2 = f_1/\sqrt{2}$ (range 8–13 GHz, preferably $\sim$10.4 GHz); and confirming the resonant mechanism if the modulation magnitudes are equal within experimental uncertainty. The 41% frequency shift ($\sqrt{2}$) is characteristic of a resonant mechanism coupling to hydrogen-containing oscillators, whereas thermal effects show at most a 10–20% shift due to dielectric property differences. Control experiments (cross-irradiation, off-resonance) provide additional confirmation. The method provides an unambiguous "smoking gun" signature for discriminating between resonant and thermal mechanisms, with quantitative predictions verified by machine-checked proofs. Applications include validation of Recognition Science predictions, characterization of electromagnetic bioeffects, and quality control for resonant protein folding modulation therapies.

— **END OF SPECIFICATION** —

INVENTOR DECLARATION

I, Jonathan Washburn, declare that:

(1) I am the original and sole inventor of the method described and claimed in this application.

(2) I have reviewed the above specification and claims and believe them to be accurate and complete.

(3) I believe the claimed invention to be novel, useful, and non-obvious over the prior art.

(4) I authorize the filing of this provisional patent application to establish a priority date.

Inventor Signature: _____

Name: Jonathan Washburn

Email: washburn.jonathan@gmail.com

Date: _____

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