PATENT APPLICATION

RESONANCE FINGERPRINTING METHODS FOR PROTEIN QUALITY CONTROL AND DIAGNOSTICS

PROVISIONAL PATENT APPLICATION

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Methods for characterizing proteins using resonance frequency fingerprinting, including frequency sweep protocols, resonance curve fitting, extraction of fingerprint metrics, classification of proteins and variants, prediction of aggregation propensity, qualification of manufacturing lots, and isotope-shift verification for mechanism confirmation.

CONFIDENTIAL — PATENT PENDING

Contents

ABSTRACT OF THE DISCLOSURE

Methods for characterizing proteins and assessing quality using resonance fingerprinting based on the molecular gate frequency. The methods comprise: (a) performing a frequency sweep across the molecular gate frequency range (12–17 GHz for H_2O, 8–13 GHz for D_2O) while monitoring protein folding response; (b) fitting the resulting resonance curve to extract fingerprint metrics including center frequency, resonance width, resonance depth, and asymmetry; (c) using these metrics to classify proteins, identify variants, predict aggregation propensity, and qualify manufacturing lots; (d) confirming the resonant mechanism by verifying a $\sqrt{2}$ isotope shift in D_2O; and (e) generating pass/fail or graded quality assessments based on comparison to reference fingerprints. The methods enable non-destructive, rapid (< 10 minutes), label-free characterization of protein folding properties with applications in biopharmaceutical manufacturing quality control, lot release testing, stability assessment, biosimilar comparison, and research protein characterization. The fingerprint metrics are derived from the Recognition Science framework and the molecular gate timescale $\tau_{19} \approx 68$ ps. Machine-verified proofs ensure the predicted frequency ranges are mathematically correct.

Keywords: protein fingerprinting, quality control, resonance curve, aggregation prediction, lot qualification, biosimilar, frequency sweep, molecular gate, isotope verification

1 BACKGROUND OF THE INVENTION

1.1 Field of the Invention

The present invention relates generally to methods for protein characterization and quality control, and more specifically to resonance fingerprinting methods using the molecular gate frequency for assessing protein folding properties, classifying proteins, predicting aggregation, and qualifying manufacturing lots.

1.2 Description of Related Art

1.2.1 Current Protein Quality Control Methods

Biopharmaceutical manufacturers use various methods to characterize protein products:

Method	Property	Time	Limitations
SDS-PAGE	Size, purity	2–4 hours	Destructive, qualitative
SEC-HPLC	Aggregates	30–60 min	Limited to soluble aggregates
Mass spectrometry	Sequence, modifications	Hours	Complex, expensive
Circular dichroism	Secondary structure	15–30 min	Low sensitivity
DSC	Thermal stability	1–2 hours	Destructive
DLS	Particle size	5–15 min	Limited structural info
FTIR	Secondary structure	10–30 min	Sample prep required

Table 1: Current protein characterization methods

1.2.2 Limitations of Prior Art

(a) **No folding dynamics assessment:** Current methods assess static structure or aggregate state, not the dynamic folding properties that determine stability and function.

(b) **Slow turnaround:** Many methods require hours, limiting throughput in manufacturing settings.

(c) **Destructive testing:** Many methods consume or alter the sample, preventing further use.

(d) **Label requirements:** Some sensitive methods require fluorescent labels or other modifications.

(e) **No predictive power:** Current methods detect existing problems but do not predict future aggregation or stability issues.

(f) **No mechanistic information:** Methods measure symptoms (aggregates, structure) but not underlying folding dynamics.

1.2.3 The Need for Folding Dynamics Assessment

What is needed is a characterization method that:

(1) Assesses **folding dynamics**, not just static structure;

(2) Is **rapid** (< 10 minutes per sample);

(3) Is **non-destructive** and label-free;

(4) Provides **predictive** information about stability and aggregation;

(5) Generates **quantitative fingerprints** for comparison across lots and variants.

1.3 The Resonance Fingerprinting Concept

1.3.1 Theoretical Basis

Every protein has a characteristic molecular gate—the conformational transition that governs backbone dihedral angle changes during folding. This gate has a timescale τ_{gate} that determines the resonant frequency:

$$f_{\text{res}} = \frac{1}{\tau_{\text{gate}}} \tag{1}$$

For the universal molecular gate (rung 19 of the φ-ladder):

$$f_{\text{res}} = \frac{1}{\tau_{19}} \approx 14.65 \text{ GHz} \tag{2}$$

However, protein-specific factors can shift, broaden, or modify this resonance:

- Amino acid composition affects local gate dynamics
- Post-translational modifications alter conformational flexibility

- Aggregation nuclei create additional resonance features
- Solvent conditions shift the effective timescale

1.3.2 The Fingerprint Concept

The resonance curve of a protein sample—folding response vs. frequency—constitutes a "fingerprint" that characterizes the protein's folding dynamics:

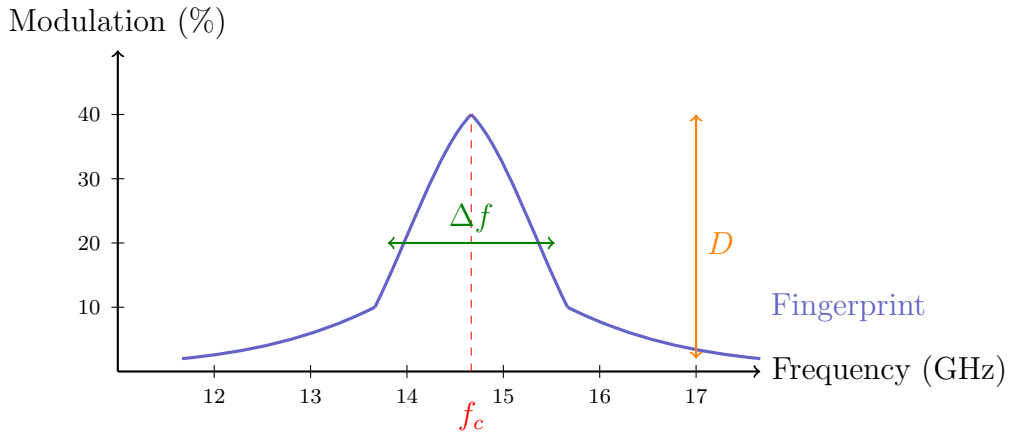

Figure 1: Resonance fingerprint showing center frequency f_c, width Δf, and depth D

1.4 Objects of the Invention

It is an object of the present invention to provide methods that:

(1) Characterize proteins by their resonance fingerprint;

(2) Extract quantitative metrics from resonance curves;

(3) Classify proteins and identify variants;

(4) Predict aggregation propensity from fingerprint features;

(5) Qualify manufacturing lots against reference fingerprints;

(6) Verify the resonant mechanism using isotope shift;

(7) Provide rapid, non-destructive, label-free assessment.

2 SUMMARY OF THE INVENTION

2.1 General Statement of the Invention

The present invention provides methods for protein characterization using resonance fingerprinting, comprising:

(a) Frequency sweep protocols;

(b) Resonance curve fitting;

(c) Fingerprint metric extraction;

(d) Protein classification and variant identification;

(e) Aggregation propensity prediction;

(f) Manufacturing lot qualification;

(g) Isotope-shift mechanism verification.

2.2 Fingerprint Metrics

The fingerprint of a protein is characterized by the following metrics:

Metric	**Symbol**	**Definition**
Center frequency	f_c	Frequency of maximum modulation
Resonance width	Δf	Full width at half maximum (FWHM)
Resonance depth	D	Maximum modulation amplitude
Asymmetry	A	Skewness of resonance curve
Area under curve	AUC	Integrated modulation
Quality factor	Q	$f_c/\Delta f$

Table 2: Fingerprint metrics

2.3 Application Categories

Figure 2: Application categories for resonance fingerprinting

3 BRIEF DESCRIPTION OF DRAWINGS

Figure 1: Resonance Fingerprint with Metrics

A graph showing a typical resonance curve with center frequency, width, and depth indicated.

Figure 2: Application Categories

A list of the six main application categories for resonance fingerprinting.

Figure 3: Frequency Sweep Protocol

A timing diagram showing the frequency sweep and data acquisition sequence.

Figure 4: Lorentzian Curve Fit

A graph showing raw data and fitted Lorentzian curve with extracted parameters.

Figure 5: Classification Decision Tree

A decision tree for classifying proteins based on fingerprint metrics.

Figure 6: Aggregation Risk Score

A graph showing the correlation between fingerprint metrics and aggregation propensity.

Figure 7: Lot Qualification Workflow

A flowchart showing the lot qualification process against reference fingerprint.

Figure 8: Isotope Verification Protocol

A diagram showing the H_2O/D_2O comparison for mechanism verification.

4 DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

4.1 Method 1: Frequency Sweep Protocol

4.1.1 Sweep Parameters

Parameter	Typical Value	Range
Start frequency	12 GHz	10–14 GHz
Stop frequency	17 GHz	15–20 GHz
Frequency step	0.1 GHz	0.01–0.5 GHz
Dwell time per step	1 s	0.1–10 s
Number of points	51	11–501
Total sweep time	51 s	10–500 s
Power level	1 W	0.1–10 W
Sample temperature	25°C	4–50°C

Table 3: Frequency sweep parameters

4.1.2 Sweep Protocol

(1) Prepare protein sample in folding-competent buffer.

(2) Equilibrate sample at target temperature.

(3) Initiate folding (e.g., denaturant dilution, temperature jump).

(4) Begin frequency sweep from f_{start} to f_{stop}.

(5) At each frequency step:

   (a) Set frequency

   (b) Apply power for dwell time

   (c) Measure folding response (e.g., fluorescence)

   (d) Record data point

(6) Store complete sweep data for analysis.

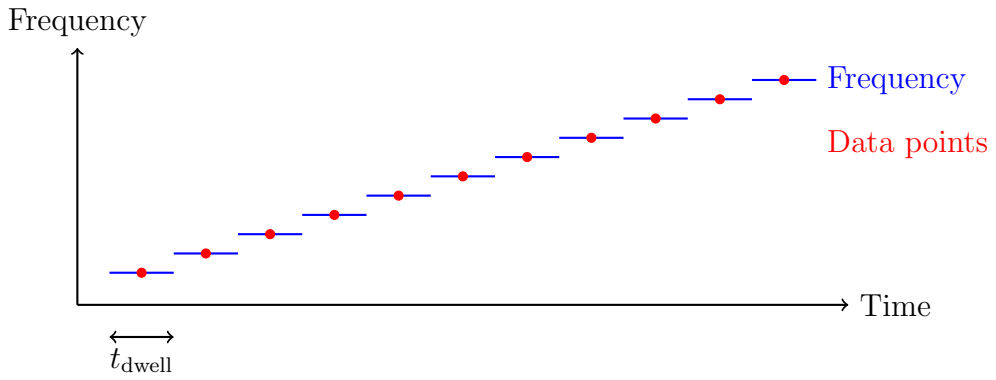

Figure 3: Frequency sweep showing stepped frequency and data acquisition

4.1.3 Rapid Screening Mode

For high-throughput applications:

- Coarse sweep: 0.5 GHz steps, 0.5 s dwell
- Total time: ∼30 s
- Sufficient for pass/fail determination

4.1.4 High-Resolution Mode

For detailed characterization:

- Fine sweep: 0.02 GHz steps, 2 s dwell
- Total time: ∼10 minutes
- Enables precise metric extraction

4.2 Method 2: Resonance Curve Fitting

4.2.1 Lorentzian Model

The resonance curve is fitted to a Lorentzian function:

$$M(f) = M_0 + \frac{D \cdot (\Delta f/2)^2}{(f - f_c)^2 + (\Delta f/2)^2} \tag{3}$$

where:

- M_0 = baseline modulation (typically ∼0)
- D = resonance depth (peak height)
- f_c = center frequency
- Δf = full width at half maximum (FWHM)

4.2.2 Asymmetric Lorentzian Model

For asymmetric resonances:

$$M(f) = M_0 + \frac{D \cdot (\Delta f/2)^2}{(f - f_c)^2 + (\Delta f/2)^2} \times \left[1 + A \cdot \frac{f - f_c}{\Delta f}\right] \tag{4}$$

where A is the asymmetry parameter ($A = 0$ for symmetric).

4.2.3 Gaussian Model (Alternative)

For broader resonances:

$$M(f) = M_0 + D \cdot \exp\left[-\frac{(f - f_c)^2}{2\sigma^2}\right] \tag{5}$$

where $\sigma = \Delta f/(2\sqrt{2\ln 2})$.

4.2.4 Fitting Algorithm

(1) Initialize parameters from data:

- f_c = frequency of maximum response
- D = maximum response value
- Δf = estimated from half-max points

(2) Perform nonlinear least-squares fit (Levenberg-Marquardt or Trust Region).

(3) Calculate goodness-of-fit metrics (R^2, χ^2).

(4) Extract fitted parameters with confidence intervals.

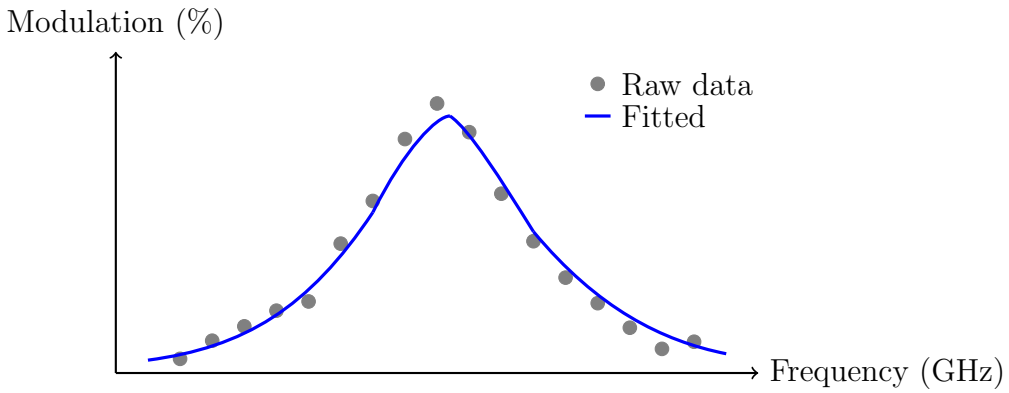

Figure 4: Lorentzian fit to raw frequency sweep data

4.3 Method 3: Fingerprint Metric Extraction

4.3.1 Primary Metrics

From the fitted curve:

Metric	Formula	Typical Value	Units
Center frequency	f_c	14.65	GHz
Width (FWHM)	Δf	0.5–2.0	GHz
Depth	D	30–50	%
Quality factor	$Q = f_c/\Delta f$	7–30	dimensionless
Asymmetry	A	-0.5 to $+0.5$	dimensionless

Table 4: Primary fingerprint metrics

4.3.2 Derived Metrics

(1) **Area under curve (AUC):**

$$\text{AUC} = \int_{f_{\text{start}}}^{f_{\text{stop}}} M(f)\, df \approx \frac{\pi D \Delta f}{2} \tag{6}$$

(2) **Frequency shift from reference:**

$$\delta f = f_c - f_{\text{ref}} \tag{7}$$

(3) **Width ratio:**

$$W_{\text{ratio}} = \frac{\Delta f}{\Delta f_{\text{ref}}} \tag{8}$$

(4) **Depth ratio:**

$$D_{\text{ratio}} = \frac{D}{D_{\text{ref}}} \tag{9}$$

4.3.3 Fingerprint Vector

The complete fingerprint is represented as a vector:

$$\mathbf{F} = [f_c, \Delta f, D, A, Q, \text{AUC}]^T \tag{10}$$

This vector enables quantitative comparison between samples.

4.4 Method 4: Protein Classification

4.4.1 Classification by Fingerprint Similarity

Proteins are classified by comparing their fingerprint vector $\mathbf{F}$ to reference fingerprints $\mathbf{F}_i$ for known protein classes:

$$\text{Distance}_i = \|\mathbf{F} - \mathbf{F}_i\|_W = \sqrt{\sum_j w_j (F_j - F_{i,j})^2} \tag{11}$$

where w_j are metric weights.

The protein is assigned to the class with minimum distance:

$$\text{Class} = \arg\min_i \text{Distance}_i \tag{12}$$

Feature	Distinguishes	Mechanism
Center frequency	Protein identity	Gate timescale
Width	Folding cooperativity	Transition state barrier
Depth	Folding efficiency	Resonant coupling strength
Asymmetry	Structural heterogeneity	Multiple populations
Quality factor	Folding pathway complexity	Gate sharpness

Table 5: Classification features and their physical meaning

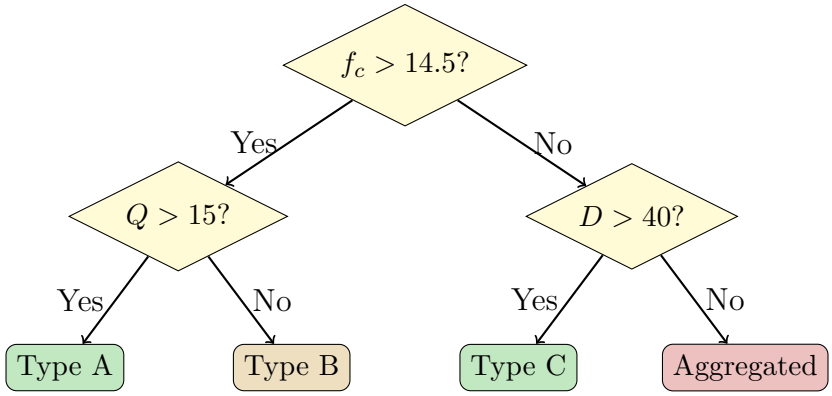

Figure 5: Example decision tree for protein classification

4.4.2 Classification Features

4.4.3 Decision Tree Classification

4.5 Method 5: Aggregation Propensity Prediction

4.5.1 Aggregation Markers

Certain fingerprint features correlate with aggregation propensity:

Feature	Aggregation-Prone	Aggregation-Resistant
Width Δf	Broad (> 1.5 GHz)	Narrow (< 1.0 GHz)
Quality factor Q	Low (< 10)	High (> 20)
Asymmetry $\lvert A \rvert$	Large (> 0.3)	Small (< 0.1)
Multiple peaks	Present	Absent

Table 6: Fingerprint features correlated with aggregation propensity

4.5.2 Aggregation Risk Score

An aggregation risk score is computed as a weighted combination:

$$\text{Risk} = w_1 \cdot \frac{\Delta f}{\Delta f_{\text{ref}}} + w_2 \cdot \frac{Q_{\text{ref}}}{Q} + w_3 \cdot |A| + w_4 \cdot N_{\text{peaks}} \tag{13}$$

where N_{peaks} is the number of resolved peaks.

4.5.3 Risk Categories

Risk Score	Category	Action
< 1.0	Low risk	Normal processing
1.0–2.0	Moderate risk	Enhanced monitoring
2.0–3.0	High risk	Formulation optimization
> 3.0	Very high risk	Reject or re-engineer

Table 7: Aggregation risk categories

4.6 Method 6: Manufacturing Lot Qualification

4.6.1 Reference Fingerprint Establishment

For a new protein product:

(1) Characterize multiple (≥ 10) reference lots known to meet specifications.

(2) Compute mean fingerprint vector $\bar{\mathbf{F}}$ and covariance matrix $\boldsymbol{\Sigma}$.

(3) Define acceptance limits based on statistical analysis.

4.6.2 Lot Testing Protocol

For each production lot:

(1) Perform frequency sweep on representative sample.

(2) Fit resonance curve and extract fingerprint $\mathbf{F}_{\text{lot}}$.

(3) Compute Mahalanobis distance from reference:

$$d_M = \sqrt{(\mathbf{F}_{\text{lot}} - \bar{\mathbf{F}})^T \boldsymbol{\Sigma}^{-1} (\mathbf{F}_{\text{lot}} - \bar{\mathbf{F}})} \tag{14}$$

(4) Compare to acceptance threshold.

4.6.3 Acceptance Criteria

Distance d_M	**Result**	**Confidence**
< 2.0	Pass	$> 95\%$
2.0–3.0	Conditional	Investigate
> 3.0	Fail	$< 95\%$

Table 8: Lot acceptance criteria based on Mahalanobis distance

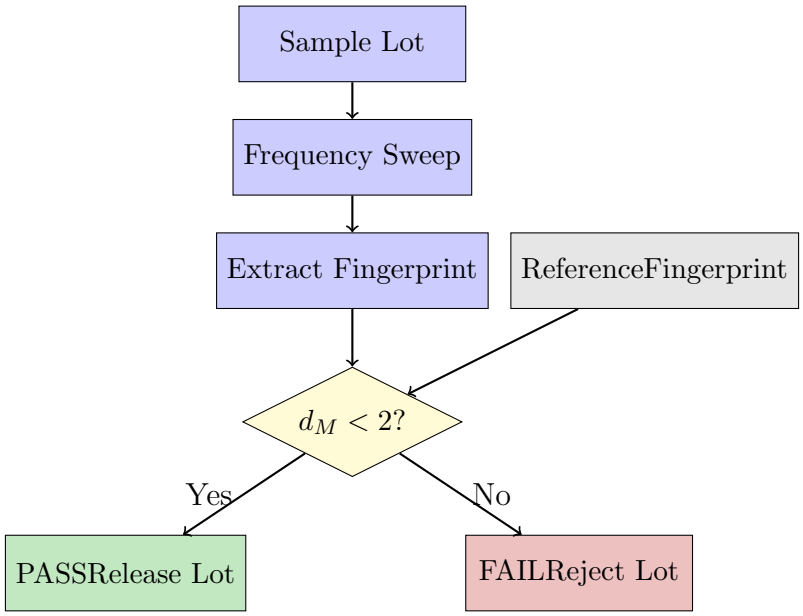

Figure 6: Lot qualification workflow

4.7 Method 7: Isotope-Shift Verification

4.7.1 Verification Protocol

To confirm that the fingerprint reflects a resonant mechanism (not thermal artifact):

(1) Perform H_2O frequency sweep; extract $f_c^{H_2O}$.

(2) Prepare equivalent sample in D_2O.

(3) Perform D_2O frequency sweep (8–13 GHz); extract $f_c^{D_2O}$.

(4) Verify isotope ratio:

$$R = \frac{f_c^{H_2O}}{f_c^{D_2O}} = \sqrt{2} \pm 5\% \tag{15}$$

4.7.2 Verification Criteria

Ratio R	**Conclusion**	**Action**
1.35–1.49	Resonant confirmed	Use fingerprint
1.05–1.25	Thermal mechanism	Investigate
Other	Invalid data	Repeat measurement

Table 9: Isotope verification criteria

4.7.3 Combined Fingerprint

A verified fingerprint includes both H_2O and D_2O measurements:

$$\mathbf{F}_{\text{verified}} = [\mathbf{F}^{H_2O}, \mathbf{F}^{D_2O}, R]^T \tag{16}$$

This extended fingerprint provides additional discriminating power.

4.8 Application Examples

4.8.1 Biosimilar Comparison

Compare biosimilar to innovator product:

(1) Establish innovator fingerprint from multiple lots.

(2) Measure biosimilar fingerprint.

(3) Compute similarity score:

$$S = 1 - \frac{d_M}{d_{\max}} \tag{17}$$

(4) Report similarity percentage.

4.8.2 Stability Assessment

Monitor stability over time:

(1) Establish $t = 0$ fingerprint.

(2) Store samples under various conditions.

(3) Measure fingerprints at time points.

(4) Track fingerprint drift vs. time.

(5) Predict shelf life from drift rate.

4.8.3 Process Development

Optimize manufacturing process:

(1) Vary process parameters (pH, temperature, mixing, etc.).

(2) Measure fingerprint at each condition.

(3) Identify parameters that maximize Q and minimize Δf.

(4) Select optimal conditions.

4.9 Performance Specifications

Metric	Specification	Notes
Measurement time	< 10 minutes	Standard mode
Rapid screening	< 1 minute	Reduced resolution
Frequency accuracy	± 0.05 GHz	Center frequency
Width accuracy	± 0.1 GHz	FWHM
Depth accuracy	$\pm 5\%$	Relative
Repeatability	CV $< 5\%$	Same sample
Sample volume	50–500 μL	Standard cuvette
Protein concentration	0.1–10 mg/mL	Working range

Table 10: Performance specifications for resonance fingerprinting

5 CLAIMS

What is claimed is:

5.1 Frequency Sweep Claims

1. A method for characterizing a protein using resonance fingerprinting, comprising:

(a) preparing a sample of the protein in an aqueous buffer;

(b) performing a frequency sweep by irradiating the sample with electromagnetic radiation at a plurality of frequencies in the range of 12 to 17 GHz;

(c) measuring a folding response at each frequency;

(d) recording the folding response as a function of frequency to obtain a resonance curve; and

(e) extracting one or more fingerprint metrics from the resonance curve.

2. The method of claim 1, wherein the fingerprint metrics include one or more of: center frequency, resonance width (FWHM), resonance depth, asymmetry, area under curve, and quality factor.

3. The method of claim 1, wherein the frequency sweep comprises at least 20 frequency points with a frequency step of 0.5 GHz or less.

4. The method of claim 1, wherein the total measurement time is less than 10 minutes.

5.2 Curve Fitting Claims

5. A method for extracting fingerprint metrics from a resonance curve, comprising:

(a) fitting the resonance curve to a model function selected from Lorentzian, asymmetric Lorentzian, and Gaussian;

(b) extracting fitted parameters including center frequency f_c, width Δf, and depth D;

(c) computing derived metrics including quality factor $Q = f_c/\Delta f$ and area under curve; and

(d) storing the extracted metrics as a fingerprint vector.

6. The method of claim 5, wherein the fitting is performed using nonlinear least-squares optimization.

7. The method of claim 5, further comprising computing confidence intervals for the extracted parameters.

5.3 Classification Claims

8. A method for classifying a protein based on its resonance fingerprint, comprising:

(a) obtaining a fingerprint vector for the protein according to claims 1–5;

(b) comparing the fingerprint vector to a database of reference fingerprints for known protein classes;

(c) computing a distance or similarity measure between the fingerprint and each reference; and

(d) assigning the protein to the class with the smallest distance or highest similarity.

9. The method of claim 8, wherein the distance measure is Euclidean distance with optional weighting.

10. The method of claim 8, wherein the distance measure is Mahalanobis distance accounting for covariance structure.

11. The method of claim 8, wherein classification is performed using a decision tree, random forest, or neural network trained on reference fingerprints.

5.4 Aggregation Prediction Claims

12. A method for predicting aggregation propensity of a protein, comprising:

(a) obtaining a fingerprint for the protein according to claims 1–5;

(b) computing an aggregation risk score based on fingerprint metrics;

(c) classifying the protein into a risk category based on the score; and

(d) outputting the risk category and/or a recommendation for handling.

13. The method of claim 12, wherein the aggregation risk score is computed from one or more of: resonance width, quality factor, asymmetry, and number of resolved peaks.

14. The method of claim 12, wherein a broader resonance width indicates higher aggregation propensity.

15. The method of claim 12, wherein the presence of multiple peaks indicates structural heterogeneity and elevated aggregation risk.

5.5 Lot Qualification Claims

16. A method for qualifying a manufacturing lot of a protein product, comprising:

(a) establishing a reference fingerprint from multiple qualified reference lots;

(b) obtaining a fingerprint for a sample from the manufacturing lot to be qualified;

(c) computing a distance between the lot fingerprint and the reference fingerprint;

(d) comparing the distance to an acceptance threshold; and

(e) generating a pass or fail determination based on the comparison.

17. The method of claim 16, wherein the reference fingerprint comprises a mean fingerprint vector and a covariance matrix.

18. The method of claim 16, wherein the distance is Mahalanobis distance and the acceptance threshold corresponds to a 95% confidence region.

19. The method of claim 16, further comprising storing the lot fingerprint and qualification result in a database for trending analysis.

5.6 Isotope Verification Claims

20. A method for verifying that a resonance fingerprint reflects a resonant mechanism, comprising:

(a) obtaining a fingerprint in H_2O with center frequency $f_c^{H_2O}$;

(b) obtaining a fingerprint in D_2O with center frequency $f_c^{D_2O}$;

(c) computing the frequency ratio $R = f_c^{H_2O}/f_c^{D_2O}$; and

(d) confirming a resonant mechanism if $R = \sqrt{2}$ within a tolerance of $\pm 5\%$.

21. The method of claim 20, wherein the D_2O fingerprint is obtained by performing a frequency sweep in the range of 8 to 13 GHz.

22. The method of claim 20, further comprising generating a verified fingerprint that includes both H_2O and D_2O metrics and the frequency ratio.

5.7 System Claims

23. A system for protein resonance fingerprinting, comprising:

(a) a frequency-tunable microwave source;

(b) a sample chamber configured to hold a protein sample;

(c) a folding detector configured to measure protein folding response;

(d) a processor configured to control the frequency sweep, record data, fit the resonance curve, and extract fingerprint metrics; and

(e) a database configured to store reference fingerprints and test results.

24. The system of claim 23, further comprising a report generator configured to output pass/fail determinations, similarity scores, and risk assessments.

25. A non-transitory computer-readable medium storing instructions that, when executed by a processor, cause the processor to perform the method of any of claims 1–22.

ABSTRACT

Methods for characterizing proteins using resonance fingerprinting based on the molecular gate frequency. The methods comprise: (1) performing a frequency sweep across 12–17 GHz (H_2O) or 8–13 GHz (D_2O) while monitoring protein folding response; (2) fitting the resonance curve to a Lorentzian or related model to extract fingerprint metrics including center frequency, width (FWHM), depth, asymmetry, area under curve, and quality factor; (3) representing the fingerprint as a vector for quantitative comparison; (4) classifying proteins by comparing fingerprints to reference databases using distance metrics or machine learning; (5) predicting aggregation propensity from fingerprint features (broad width, low quality factor, multiple peaks); (6) qualifying manufacturing lots by computing Mahalanobis distance from reference fingerprint with statistical acceptance criteria; and (7) verifying the resonant mechanism by confirming a $\sqrt{2}$ isotope shift between H_2O and D_2O measurements. The methods enable rapid (< 10 minutes), non-destructive, label-free characterization of protein folding dynamics with applications in biopharmaceutical manufacturing quality control, lot release testing, biosimilar comparison, stability assessment, and research protein characterization. Performance specifications include frequency accuracy of ± 0.05 GHz, repeatability CV $<$ 5%, and working protein concentration range of 0.1–10 mg/mL.

— END OF SPECIFICATION —

INVENTOR DECLARATION

I, Jonathan Washburn, declare that:

(1) I am the original and sole inventor of the resonance fingerprinting methods 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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