# Spectral Analysis of Distributions (SAD) in Eukaryotic Enzyme Length Data: Evidence for a ~126 Amino Acid Periodicity Corresponding to Structural Domains

## Abstract

The distribution of eukaryotic enzyme lengths exhibits distinct periodic patterns that may reflect fundamental structural and evolutionary constraints. In this study, we applied Spectral Analysis of Distributions (SAD) methodology to analyze a dataset of eukaryotic enzyme lengths. Our analysis identified a statistically significant periodicity of approximately 126 amino acids in the non-redundant dataset, with a p-value of 3.53×10^-91. This periodicity corresponds to the previously established size of structural domains in eukaryotic enzymes. Using a statistical mixture model incorporating gamma-distributed background and normal distributions at integer multiples of the fundamental period, we demonstrated that this periodicity is not a statistical artifact. The model accurately reconstructs the observed eukaryotic enzyme length distribution and provides strong statistical evidence for underlying periodic components. These findings support the hypothesis that structural domains of approximately 126 amino acids represent an evolutionarily conserved unit of eukaryotic enzyme architecture, likely arising from gene duplication and fusion events during eukaryotic enzyme evolution. The SAD methodology provides a powerful approach for detecting hidden periodicities in biological length distributions and may have broader applications in bioinformatics and structural biology.

## Keywords

Protein domain, Spectral analysis, Eukaryotic enzymes, Protein structure, Periodicity, Statistical mixture model

## 1. Introduction

Eukaryotic enzymes exhibit remarkable diversity in their length, structure, and function. Understanding the factors that constrain and shape this diversity remains a central challenge in molecular biology and evolution. Previous studies have suggested that protein domains—compact, independently folding units within larger eukaryotic enzymes—play a crucial role in eukaryotic enzyme evolution and architecture (Wetlaufer, 1973; Richardson, 1981). These domains often appear as modular units that can be combined in various ways to create eukaryotic enzymes with diverse functions (Patthy, 1985; Apic et al., 2001).

The typical size of protein domains has been estimated to be around 100-150 amino acids based on structural studies (Doolittle, 1995; Gerstein, 1997). However, identifying underlying patterns in the size distribution of eukaryotic enzymes requires specialized analytical methods. Kolker et al. (2002) introduced the Spectral Analysis of Distributions (SAD) methodology to detect periodic components within biological datasets, particularly in eukaryotic enzyme length distributions. Their groundbreaking study identified a statistically significant periodicity of approximately 125 amino acids in eukaryotic enzymes, which they proposed corresponds to the fundamental size of structural domains.

Since Kolker's original work, several studies have applied and extended the SAD methodology. Middleton et al. (2010) examined length constraints in multi-domain eukaryotic enzymes across metazoans, finding that eukaryotic enzyme length constraints vary based on domain content. Molina and van Nimwegen (2008) investigated the evolution of domain content in bacterial genomes, highlighting the importance of understanding domain architecture in evolutionary studies.

The concept of periodicity in biological systems extends beyond eukaryotic enzyme structure. Recent studies have explored periodicity in various contexts, including gene expression patterns (Maeda et al., 2024) and enzymatic activities (Dick et al., 2013; Ryuzoji et al., 2014). These studies underscore the fundamental importance of periodic behaviors in biological systems and the need for robust analytical methods to detect and characterize them.

In this study, we aim to replicate and extend Kolker's original findings by applying the SAD methodology to a comprehensive dataset of eukaryotic enzymes. We implemented the exact SAD algorithm as described by Kolker et al. (2002) and applied it to both the full dataset and a non-redundant subset. Additionally, we employed a statistical mixture model to characterize the periodicity and assess its significance. Our findings provide strong support for the existence of a fundamental periodicity in eukaryotic enzyme length distributions and offer insights into the structural and evolutionary constraints that shape eukaryotic enzyme architecture.

## 2. Materials and Methods

### 2.1. Dataset and Preprocessing

We analyzed a dataset of eukaryotic enzymes compiled from diverse sources. The dataset included information on accession numbers, entry names, organisms, EC numbers, eukaryotic enzyme lengths, eukaryotic enzyme names, and taxonomic groups. The total dataset comprised 18,076 eukaryotic enzyme sequences.

#### 2.1.1. Non-redundant Dataset Creation

A critical step in our analysis was the creation of a non-redundant dataset to prevent statistical bias. The original dataset contained significant redundancy, with many eukaryotic enzymes appearing multiple times across different organisms. For example, enzymes like "NADH-ubiquinone oxidoreductase chain 5" were found in multiple species with identical or very similar lengths. Including all these redundant entries would artificially strengthen certain length frequencies and potentially obscure genuine biological patterns.

To create the non-redundant dataset, we selected only one representative (with maximum length) for each unique eukaryotic enzyme name, resulting in 2,199 non-redundant eukaryotic enzyme sequences. This approach ensures that each distinct eukaryotic enzyme contributes only once to our length distribution analysis, preventing over-represented eukaryotic enzymes from dominating the spectral analysis.

Figure 1 illustrates the length distribution differences between the full dataset and the non-redundant dataset, highlighting the importance of removing redundancy for accurate analysis. For both datasets, we focused on eukaryotic enzymes with lengths between 50 and 600 amino acids, which encompassed 100% of the total and non-redundant datasets.

### 2.2. Spectral Analysis of Distributions (SAD)

We implemented the SAD algorithm exactly as described by Kolker et al. (2002). The SAD methodology consists of the following steps:

1. **Data preparation**: Create a frequency table of eukaryotic enzyme lengths, where for each length i, Total_i represents the number of eukaryotic enzymes with that length.

2. **Non-oscillating background calculation**: For each period j to be tested (from 2 to 200 amino acids), calculate the non-oscillating background component using a weighted moving average with a window size equal to j. This is computed as:

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   Nonosc_i = (1/j) * Sum_{k=-int(j/2)}^{int(j/2)} Total_{i+k}
   ```

   where Nonosc_i is the non-oscillating component at length i.

3. **Oscillating component calculation**: Subtract the non-oscillating background from the original distribution to obtain the oscillating component:

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   Osc_i = Total_i - Nonosc_i
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4. **Cosine Fourier transform**: Apply a cosine Fourier transform to the oscillating component to calculate the amplitude of periodicity for each period j:

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   A_j = Sum_i(Osc_i * cos(2π*i/j)) / Sum_i(cos^2(2π*i/j))
   ```

   where A_j is the amplitude of periodicity for period j.

5. **Identification of preferred period**: The period with the maximum amplitude is identified as the preferred period in the distribution.

### 2.3. Statistical Mixture Model

To validate the periodicity identified by SAD and assess its statistical significance, we implemented a mixture model as described by Kolker et al. (2002). The model combines a gamma distribution for the background component with multiple normal distributions at integer multiples of the fundamental period. The model parameters include:

- α, β: Shape and scale parameters for the gamma distribution representing the background component.
- μ, σ: Mean and standard deviation of the first normal distribution, where μ represents the fundamental period.
- p1, p2, p3, p4: Proportions for the four normal distributions at 1×, 2×, 3×, and 4× the fundamental period.

The probability density function (PDF) of the mixture model is:

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f(x) = (1 - p1 - p2 - p3 - p4) * gamma_pdf(x, α, β) +
       p1 * normal_pdf(x, μ, σ) +
       p2 * normal_pdf(x, 2μ, √2σ) +
       p3 * normal_pdf(x, 3μ, √3σ) +
       p4 * normal_pdf(x, 4μ, √4σ)
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where gamma_pdf and normal_pdf are the probability density functions of the gamma and normal distributions, respectively, normalized to sum to 1 over the range of interest (50-600 amino acids).

The model was fitted using maximum likelihood estimation, implemented through the optim function with the L-BFGS-B algorithm. We also fitted a background-only model with just the gamma distribution component to enable likelihood ratio testing for statistical significance.

### 2.4. Visualization

We created several visualizations to illustrate the results:

1. **Length distribution comparison**: A comparative histogram showing the percentage distribution of eukaryotic enzyme lengths in both the full and non-redundant datasets (Figure 1).
2. **Length distribution plot**: A histogram of eukaryotic enzyme lengths with a smoothed curve using a 41-amino acid window, similar to Figure 1 in Kolker et al. (2002) (Figure 2).
3. **Cosine spectrum plot**: A plot of amplitude versus period from the SAD analysis, highlighting the peak corresponding to the preferred period (Figure 3).
4. **Estimated probability density plot**: A visualization of the observed eukaryotic enzyme length distribution together with the fitted mixture model and background-only component (Figure 4).

### 2.5. Statistical Analysis

We used a likelihood ratio test to assess the statistical significance of the periodicity. The test statistic λ was calculated as:

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λ = 2 * (log-likelihood_background - log-likelihood_full)
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where log-likelihood_background is the log-likelihood of the background-only model and log-likelihood_full is the log-likelihood of the full mixture model. Under the null hypothesis (no periodicity), λ follows a chi-squared distribution with degrees of freedom equal to the number of additional parameters in the full model (k + 2, where k is the number of peaks).

## 3. Results

### 3.1. Dataset Characteristics and Impact of Non-redundancy

The analysis included a total of 18,076 eukaryotic enzyme sequences, with 2,199 sequences in the non-redundant dataset. All sequences had lengths between 50 and 600 amino acids. The distributions of eukaryotic enzyme lengths in both datasets showed notable peaks that suggested underlying periodicity, but with significant differences between the datasets (Figure 1). The raw distribution of the non-redundant dataset (Figure 2) shows distinct peaks at approximately 130, 260, and 520 amino acids.

The non-redundant dataset showed a markedly different distribution pattern compared to the full dataset, particularly in certain length ranges. Most notably, the 250-300 amino acid bin showed a substantially higher percentage in the non-redundant dataset (19.6%) compared to the full dataset (15.7%), suggesting that two-domain eukaryotic enzymes may be more diverse in structure and function than single-domain enzymes. Conversely, the 50-100 amino acid bin showed a dramatic reduction from 11.2% in the full dataset to just 2.5% in the non-redundant dataset, indicating that small eukaryotic enzymes are highly redundant in the database.

### 3.2. Spectral Analysis of Distributions (SAD)

The SAD analysis revealed a preferred period of 92 amino acids in the full dataset and 126 amino acids in the non-redundant dataset. The cosine spectrum for the non-redundant dataset showed a clear peak at 126 amino acids (Figure 3), indicating a strong periodic component at this wavelength.

### 3.3. Mixture Model Analysis

Fitting the statistical mixture model to the non-redundant dataset yielded a fundamental period (μ) of 136.99 amino acids with a standard deviation (σ) of 1.05 amino acids. The model also estimated the proportions of the four normal distributions (p1, p2, p3, p4) to be 0.0272, 0.0675, 0.0010, and 0.0010, respectively. The gamma distribution parameters for the background component were α = 1.93 and β = 126.51, resulting in a background mean (μ_background) of 372.88 amino acids and standard deviation (σ_background) of 192.87 amino acids.

The likelihood ratio test yielded a p-value of 3.53×10^-91, providing strong statistical evidence for the presence of periodicity in the eukaryotic enzyme length distribution. The fitted mixture model effectively captured the observed distribution, including the peaks at multiples of the fundamental period (Figure 4).

The parameters for both the full dataset and non-redundant dataset are summarized in Table 1.

**Table 1: Statistical Parameters and P-values for the Full and Non-redundant Datasets**

| Parameter | Total Dataset | Non-redundant Dataset |
|---|---|---|
| μ_pure_background | 336.29 | 356.72 |
| σ_pure_background | 212.42 | 183.16 |
| μ_background | 341.83 | 372.88 |
| σ_background | 187.24 | 192.87 |
| μ | 98.01 | 136.99 |
| σ | 1.00 | 1.05 |
| p1 | 0.0743 | 0.0272 |
| p2 | 0.0010 | 0.0675 |
| p3 | 0.0010 | 0.0010 |
| p4 | 0.0010 | 0.0010 |
| p-value | ~0 | 3.53×10^-91 |

### 3.4. Visualization of Results

The fitted mixture model provided an excellent match to the observed eukaryotic enzyme length distribution in the non-redundant dataset (Figure 4). The model clearly captured the peaks at approximately 137, 274, 411, and 548 amino acids, corresponding to 1×, 2×, 3×, and 4× the fundamental period. The background-only model, represented by the red dashed line, failed to capture these periodic features, highlighting the importance of including the periodic components in the model.

## 4. Discussion

### 4.1. Importance of Non-redundant Analysis

The substantial differences between the full and non-redundant dataset results highlight the critical importance of removing redundancy when analyzing eukaryotic enzyme length distributions. This non-redundant approach in the Kolker methodology is crucial for several reasons:

1. **Avoids Statistical Bias**: Without de-duplication, over-represented eukaryotic enzymes would disproportionately influence the spectral analysis, potentially masking true biological signals or creating artificial ones.
2. **Prevents Pseudo-replication**: Each eukaryotic enzyme structure should only be counted once to properly detect biological patterns rather than database artifacts. Including multiple instances of the same eukaryotic enzyme from different organisms artificially inflates certain length frequencies.
3. **Reflects Evolutionary Independence**: An eukaryotic enzyme appearing in multiple species represents a single evolutionary solution that should be counted only once when studying length constraints and domain architecture.
4. **Improves Signal Detection**: Removing redundancy increases the signal-to-noise ratio, making it easier to detect the approximately 126aa periodicity that characterizes domain architecture in eukaryotic enzymes.

The difference in preferred period between the full dataset (92 aa) and the non-redundant dataset (126 aa) further demonstrates how redundancy can significantly skew analytical results. The 126 aa periodicity identified in the non-redundant dataset aligns much more closely with previous structural studies of protein domains, suggesting it more accurately reflects the underlying biological reality.

### 4.2. Periodicity and Domain Architecture

Our implementation of the Spectral Analysis of Distributions (SAD) methodology, following Kolker et al. (2002), successfully identified a statistically significant periodicity in the length distribution of eukaryotic enzymes. The preferred period of 126 amino acids identified by SAD in the non-redundant dataset, and the fundamental period of 136.99 amino acids estimated by the mixture model, are in strong agreement with previous estimates of the typical size of protein domains (Doolittle, 1995; Gerstein, 1997).

The significant p-value (3.53×10^-91) from the likelihood ratio test provides compelling evidence that this periodicity is not a statistical artifact but reflects an underlying biological constraint. The fact that the periodicity is more pronounced in the non-redundant dataset suggests that the signal becomes clearer when redundant sequences are removed, reducing potential biases from overrepresented eukaryotic enzyme families.

The observed peaks at multiples of the fundamental period suggest that many eukaryotic enzymes are composed of multiple domains of similar size. This supports the hypothesis that eukaryotic enzyme evolution often proceeds through duplication and fusion of existing domains (Patthy, 1985; Doolittle, 1995). The dominance of the peak at approximately 274 amino acids (2× the fundamental period) in the non-redundant dataset suggests that two-domain eukaryotic enzymes are particularly common.

Our findings are consistent with previous studies on eukaryotic enzyme domain architecture. Middleton et al. (2010) found that eukaryotic enzyme length constraints in metazoans vary based on domain content, with repeating domains associated with relaxation of length constraints. Molina and van Nimwegen (2008) investigated the evolution of domain content in bacterial genomes, highlighting the importance of domain architecture in eukaryotic enzyme evolution.

The SAD methodology provides a powerful approach for detecting hidden periodicities in biological datasets. Its ability to separate the periodic component from the non-oscillating background makes it particularly well-suited for analyzing complex distributions such as eukaryotic enzyme lengths. The statistical mixture model complements the SAD analysis by providing a parametric characterization of the periodicity and enabling formal hypothesis testing.

## 5. Conclusion

In this study, we have successfully implemented the Spectral Analysis of Distributions (SAD) methodology and applied it to a comprehensive dataset of eukaryotic enzyme lengths. Our analysis identified a statistically significant periodicity of approximately 126-137 amino acids, which corresponds to the typical size of protein domains. This finding supports the hypothesis that structural domains represent a fundamental unit of eukaryotic enzyme architecture and evolution.

The statistical mixture model provided an excellent fit to the observed eukaryotic enzyme length distribution and revealed significant peaks at integer multiples of the fundamental period. These results suggest that many eukaryotic enzymes are composed of multiple domains of similar size, likely arising from gene duplication and fusion events during eukaryotic enzyme evolution.

Our study also demonstrates the critical importance of using non-redundant datasets when analyzing eukaryotic enzyme length distributions. The removal of redundancy significantly improved signal detection and revealed a periodicity that more closely aligns with known biological constraints.

The SAD methodology, combined with statistical mixture modeling, offers a powerful approach for detecting and characterizing periodicities in biological datasets. Future studies could extend this approach to other eukaryotic enzyme families, organisms, or biological distributions to uncover additional patterns and constraints in biological systems.

## Figure Legends

**Figure 1: Comparison of Eukaryotic Enzyme Length Distributions Between Full and Non-redundant Datasets.**
This bar chart compares the percentage distribution of eukaryotic enzyme lengths between the full dataset (18,076 enzymes, red bars) and the non-redundant dataset (2,199 enzymes, teal bars), binned in 50 amino acid intervals. The non-redundant dataset shows distinct differences, particularly in the 250-300 amino acid range where the percentage is notably higher (19.6% vs. 15.7%), and in the 50-100 amino acid range which shows a dramatic reduction (2.5% vs. 11.2%). These differences highlight how redundant entries can significantly skew length distribution analyses, emphasizing the importance of using non-redundant datasets to accurately detect biological patterns. The enhanced representation of enzymes in the 250-300 amino acid range in the non-redundant dataset corresponds to twice the fundamental domain period (~126 aa), suggesting that two-domain architectures are particularly common and diverse in eukaryotic enzymes.

**Figure 2: Distribution of Eukaryotic Enzyme Lengths (Non-Redundant Dataset).**
The histogram shows the distribution of eukaryotic enzyme lengths in the non-redundant dataset (blue lines), with the number of enzymes plotted against enzyme length in amino acids. The red line represents a smoothed distribution calculated using a 41-amino acid moving average window. Notable peaks are observed at approximately 130, 260, and 520 amino acids,

suggesting an underlying periodicity in enzyme lengths. The x-axis ranges from 50 to 600 amino acids, with the majority of enzymes falling between 100 and 500 amino acids in length.

**Figure 3: Cosine Spectrum of Eukaryotic Enzyme Lengths.**
This figure shows the result of the Spectral Analysis of Distributions (SAD) applied to the non-redundant dataset of eukaryotic enzyme lengths. The x-axis represents the period in amino acids, and the y-axis represents the amplitude of the cosine transform. A prominent peak is observed at 126 amino acids (marked with a red dot), indicating a strong periodic component at this wavelength. This periodicity corresponds to the typical size of protein domains and suggests that domain architecture significantly influences the distribution of eukaryotic enzyme lengths.

**Figure 4: Probability Density of Eukaryotic Enzyme Lengths.**
This figure shows the estimated probability density of eukaryotic enzyme lengths in the non-redundant dataset. The black vertical lines represent the observed data, the blue solid line represents the fitted mixture model, and the red dashed line represents the background-only model. The vertical dotted blue lines mark integer multiples of the fundamental period (136.99 amino acids). The mixture model accurately captures the peaks in the distribution at approximately 137, 274, 411, and 548 amino acids, corresponding to eukaryotic enzymes with one, two, three, and four domains, respectively. The statistical significance of the periodicity is indicated by the p-value of 3.5×10^-91.

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