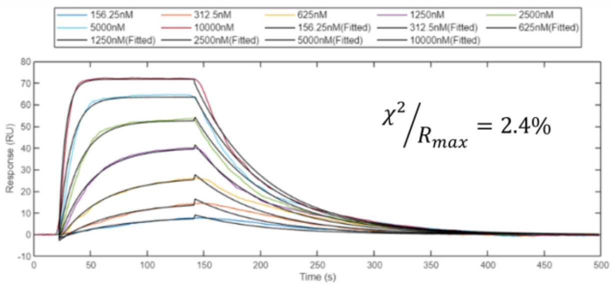
SPR Data Analysis Metrics: Definition and Evaluation Criteria for Chi-square (χ^2) (Understanding the '10% of R_{max}' Guideline)

1. Overview

The **χ^2(Chi-square)** value is often the first metric encountered when assessing the quality of kinetic fitting in Surface Plasmon Resonance (SPR) analysis. While many researchers understand that the χ^2 value should be minimized, there is frequently confusion regarding the exact threshold for acceptance or how this value varies in relation to signal magnitude.

This document reviews the mathematical definition of χ^2 as calculated by SPR software and explains the rationale behind the **"10% (or 5%) of R_{max} guideline"** commonly adopted in academia and industry, providing a framework for its correct interpretation.



Result

#	Name	Concentration	KD (M)	kon (1/(M*s))	koff (1/s)	Rmax (RU)	KA (1/M)	BI (RU)	Chi2 (RU^2)
1	Furosemide	156.25nM	1.03e-06	1.58e+04	1.62e-02	76.59	9.72e+05	-1.75	1.85
2	Furosemide	312.5nM	1.03e-06	1.58e+04	1.62e-02	76.59	9.72e+05	-2.92	1.85
3	Furosemide	625nM	1.03e-06	1.58e+04	1.62e-02	76.59	9.72e+05	-2.35	1.85
4	Furosemide	1250nM	1.03e-06	1.58e+04	1.62e-02	76.59	9.72e+05	-1.99	1.85
5	Furosemide	2500nM	1.03e-06	1.58e+04	1.62e-02	76.59	9.72e+05	-1.70	1.85
6	Furosemide	5000nM	1.03e-06	1.58e+04	1.62e-02	76.59	9.72e+05	-0.13	1.85
7	Furosemide	10000nM	1.03e-06	1.58e+04	1.62e-02	76.59	9.72e+05	2.22	1.85

[Figure 1. Kinetic analysis result]

2. Definition and Significance of χ^2 (Chi-square)

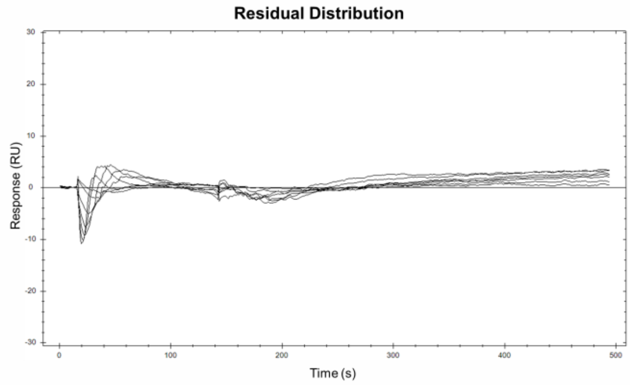
In SPR analysis software (e.g., iMSPR, Biacore Evaluation, ProteOn Manager), the χ^2 value functions more as a physical metric indicating the **"magnitude of the error between theoretical and measured values,"** rather than as a traditional statistical test statistic.

2.1 Mathematical Definition

χ^2 is defined as the average of the squared **residuals**—which represent the differences between the experimentally **measured values** and the mathematically calculated **fitted values**.

$$\chi^2 = \frac{\sum (r_f - r_x)^2}{N - p}$$

(where r_f is the fitted value, r_x is the measured value, N is the number of data points, and p is the number of fitted parameters)



[Figure 2. Residual distribution]

2.2 Significance of the Unit [RU^2]

It is crucial to note that the unit of χ^2 is [RU^2], which represents the **square** of the response unit.

Theoretically, the ideal χ^2 value corresponds to the square of the instrument's system noise. For example, if the instrument's baseline noise is 0.3 RU, the χ^2 value for an ideal fit should be approximately 0.09 RU^2.

3. Correlation Between Signal Magnitude (R_{max}) and χ^2

Many users encounter a phenomenon where "the χ^2 value increases drastically as the signal magnitude increases, even when the fit appears visually good." This is not an indication of poor data quality, but rather a natural occurrence stemming from the mathematical properties of χ^2.

3.1 Why Does χ^2 Surge as the Signal Increases?

In actual experiments, experimental artifacts inevitably increase in proportion to the signal (R_{max}). (e.g., buffer effects, minute concentration errors, mass transport effects, etc.)

- **Increase in Residuals:** If R_{max} increases 10-fold, the magnitude of the residuals (errors) can be assumed to increase approximately 10-fold.
- **Quadratic Effect of χ^2:** Since χ^2 represents the **square** of the residuals, a 10-fold increase in residuals results in a **100-fold** increase in the χ^2 value.

Therefore, in experiments with high immobilization levels, it becomes impossible to assess data quality based solely on the absolute value of χ^2.

4. Meaning of the "10% of R_{max}" Criterion

Due to this signal dependence, the SPR industry has adopted an empirical guideline that utilizes the ratio relative to the maximum response (R_{max}, rather than relying on absolute numerical values.

4.1. Is this an Academic or Theoretical Standard?

No, it is not. Rather than being a statistically rigorously proven standard, it is more accurately described as an **"Empirical Guideline"** or accepted practice that has evolved based on data accumulated over a long period among SPR researchers. A general absolute cut-off is difficult because χ^2 varies according to the signal level [1-2].

4.2. Criteria Commonly Used in Practice (5% vs. 10%)

Different criteria regarding the ratio relative to R_{max} may be applied depending on the researcher or the experimental objective.

- Stringent Criterion (< 5%)
  - The Biacore T200 Official Software Handbook suggests that, **"as a rough guideline, the χ^2 value should be less than 5% of R_{max}. "** [3]
  - This represents a conservative standard that requires highly precise experimental conditions.
- Practical Criterion (< 10%)
  - Bio-Rad's ProteOn analysis guide and numerous academic papers (e.g., Talanta, Malaria Journal) adopt the criterion that **"a fit is considered acceptable when the χ^2 value is less than or equal to 10% of R_{max}. "** [4-6]
  - This is a more general-purpose standard that accommodates realistic errors often encountered during high R_{max} experiments or screening processes.

5. Conclusion: Guide for Correct Interpretation

While χ^2 is an important metric indicating the reliability of the data, it is not an absolute pass/fail criterion. Therefore, it is reasonable to interpret the value as follows:

- **Relative Evaluation**: One should check the ratio relative to R_{max} rather than relying solely on the absolute χ^2 number.
- **Acceptance Range**:
  - Generally, data is considered to have an acceptable fit for analysis when the χ^2 value is less than or equal to **10% of R_{max}**.
  - If higher precision is required, a target of less than **5%** may be set.
- **Important Considerations**:
  - The ratio criteria proposed in this document (10% or 5%) are strong **empirical indicators**. However, flexible interpretation considering the experimental context is necessary rather than excessive reliance on a single metric.
  - To ensure the reliability of kinetic analysis, it is recommended to consider **Visual Inspection** alongside the χ^2 value. Furthermore, various statistical metrics—such as **SE (Standard Error), RMSE (Root Mean Square Error, $\approx \sqrt{\chi^2}$), and U-value (Uniqueness value)**—could be considered to analyze the **Goodness of Fit** from multiple perspectives [2, 5].

6. References

1. **SPR Pages**. Data fitting – theory / validation. (Available at: http://www.sprpages.nl)
2. Önell, A. Et al., **"Kinetic determinations of molecular interactions using Biacore—minimum data requirements for efficient experimental design"**, Journal of molecular recognition, Vol. 18:307-317 (2005).
3. **Biacore T200 Software Handbook** (Edition AA), Cytiva (formerly GE Healthcare), p.159.
4. **Bio-Rad Bulletin 6300**, "Guide to SPR Data Analysis on the ProteOn XPR36 System".
5. Battaglia, F. et al., **"Detection of canine and equine procalcitonin for sepsis diagnosis in veterinary medicine by Surface Plasmon Resonance"**, Talanta, Vol. 230, 122297 (2021).
6. Dutta, S. et al., **"Strain-transcending neutralization of malaria parasite by antibodies against Plasmodium falciparum enolase"**, Malaria Journal, Vol. 17, Article number: 304 (2018).