A Software for a New Methodology of Kinetics Analyses of Protein-Ligand Interaction

Masaki Yamamura¹
masaki@genome.ist.i.kyoto-u.ac.jp

Takuma Shiraki²
tshiraki@protein.osaka-u.ac.jp

Takashi S. Kodama³
seeder@gene.med.osaka-u.ac.jp

Tatsuo Nakagawa⁴
tatsuo@unisoku.co.jp

Natsuhiro Ichinose¹
ichinose@genome.ist.i.kyoto-u.ac.jp

Osamu Gotoh¹
o.gotoh@i.kyoto-u.ac.jp

¹ Department of Intelligence Science and Technology, Graduate School of Informatics, Kyoto University, Yoshida-Honmachi, Sakyo-ku, Kyoto 606-8501, Japan,
² Protein Science Institute, Osaka University, 3-2 Yamadaoka, Suita, Osaka 565-0871, Japan
³ Department of Medical Genetics, Osaka University Medical School, 2-2 Yamadaoka, Suita, Osaka, 565-0871, Japan
⁴ UNISOKU CO., LTD. 2-4-3 Kasugano, Hirakata, OSAKA, 573-0131, Japan

Keywords: SPECTRAC, kinetic analysis, optimization problem

1 Introduction

SPECTRAC is a new scheme for analyzing the reaction kinetics of a protein and its binding ligand. Using the stopped flow rapid scan absorption spectrometer of UNISOKU RSP-1000, we can obtain the absorbances at multiple wavelengths in 100ms, and observe the spectral change during a chemical reaction as a series of multi-wavelength data. Here we report an algorithm engaged for the computational analysis of this scheme. The task of the kinetic profile analysis can be treated as a simple optimization problem to minimize the least square error. The problem can be divided into smaller pieces of linear least square optimization problems, and this idea helps us to solve the whole problem quickly. We applied the method to the experimental data for the binding reaction between a nuclear receptor PPAR-γ and its ligand PGJ2. For the reaction, a kinetic model shown below is supposed [1,2]. Assuming this reaction scheme, we analyzed an experimental data set that consists of 43 wavelengths across 50 time points by our novel software. The calculation was successfully converged within a couple of minutes on a personal computer. The result of the analysis showed that our new methodology would be applicable to reaction kinetics with complex intermediates, whose optical characteristics cannot be acquired by ordinary biochemical experiments.

A + B \rightarrow C \xrightarrow{k_1} D

2 Methods

The kinetics models used in the software generate sequential values for the concentrations of the protein, substrates, products, and their intermediate complexes. The differential equations which describe the kinetic models cannot always be solved analytically, and hence we solved them numerically with the Runge-Kutta algorithm. The chosen model gives simulated data of components, and the problems can be formalized as an optimization problem:

\[ \text{Minimize} \quad \sum_{i, \lambda} (\text{obs}_i^{\lambda} - \sum_m \text{eps}(m)_i^{\lambda} \cdot \text{con}(m)_i^{\lambda})^2 \]

subject to \( k_n > 0, \text{eps}_i^{\lambda} > 0 \),
where \( \text{obs} \) indicates an observed absorbance at the time point \( t \) and the wavelength \( \lambda \), \( \text{eps} \) indicates the molar extinction coefficient \( \varepsilon \) of the compound \( m \), \( \text{con} \) indicates a molar concentration of \( m \) generated from a kinetic model, and \( k_n \) indicates the kinetic constant.

As mentioned above, in our algorithm the whole optimization problem can be divided into partial linear optimization problems and the solutions for each of them are given with Householder triangularization. From the point of view of this solution, the objective function can be seen as the function whose variables are only kinetic constants, \( k_n (n=1,2,\ldots) \). To optimize the whole problem with the kinetic constants rapidly, the dichotomizing search method is chosen for this analysis.

## 4 Results

An example of our analysis applied to the binding reaction between PPAR-\( \gamma \) and PGJ2 is shown in fig. 1 and 2. The fitting calculation was well converged over the wavelength range from 250 to 390nm at all time points. The estimated spectrum for each of PPAR-\( \gamma \) and PGJ2 is consistent with the observed spectrum measured for each reactant independently (data not shown). The result revealed the significance of our new methodology to analyze stopped flow data with multiple wavelengths almost simultaneously.

![Fig. 1. The estimated time course of fractional concentration of each component.](image1)

The time course of the fractional concentration of each component was calculated with the reaction model described in Section 1.

![Fig. 2. The estimated spectra of the components.](image2)

The final value of the objective function was \( 5.67622 \times 10^{-7} \). The values of kinetic parameters of \( k_1 \) and \( k_2 \) were estimated 4.70772 and \( 5.54998 \times 10^{-3} \).

## 4 Discussions

Although the kinetic model we used in the present study is a very simple one, our method can be extended to incorporate more complicated kinetic models. Using a series of test data, we are examining the performance of our method in detail with respect to the time cost and the robustness against noises.

## References
