Streamlining Receptor Allosterism Data Analysis

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Overview

The data analysis involved in screening drug candidates for receptor allosterism is a challenge for drug companies, typically involving several software systems and many manual steps. The purpose of this poster is to show how positive allosteric modulation (PAM) data can be analysed using one tool, Analyze™, a cloud based system designed for a wide variety of drug discovery data analysis applications.

Introduction

An allosteric modulator is a molecule that influences a target receptor by binding to an allosteric site that is topographically distinct from the orthosteric or primary binding site. Drugs targeting allosteric sites can work with natural signaling mechanisms rather than causing activation independently which can lead to desensitization. In addition, allosteric sites tend to be less conserved between receptor subtypes and therefor offer the potential for drugs with greater selectivity and less side-effects.

A general model for receptor allosterism is given below. It was adapted from Zhang, 2015 with the constitutive efficacy term removed (i.e., χ=0).

$$\%E_{max} = 100\frac{E}{E_{max}} = 100\frac{E_{app}{}^{n}}{E_{app}{}^{n} + K_{app}{}^{n}}$$

$$K_{app} = K_A K_B + AK_B + BK_A + \alpha AB$$

$$E_{app} = \tau_A AK_B + \tau_B BK_A + \alpha\beta\tau_A AB$$

where:

E	:	measured system response
E_{max}	:	maximal system response
A	:	agonist concentration
B	:	modulator concentration
System-specific parameters		
K_A	:	agonist affinity
τ_A	:	agonist efficacy
n	:	exponent
Modulator-specific parameters		
K_B	:	modulator affinity
τ_B	:	modulator efficacy
α	:	affinity modifier
β	:	efficacy modifier

Methods

Simulation

An experiment testing 100 virtual compounds was simulated using the plate layout below (Figure 1). Briefly, 2 compounds were tested in each plate at 16 concentrations (1.0e5 to 3.2e-4 nM, half-log dilutions). At each concentration, 11 agonist concentrations were used (100 to 0.001 nM, half-log dilutions), for a total of 176 data points per compound. DMSO controls contained neither agonist nor modulator. Agonist controls contained a saturating concentration of agonist and no modulator.

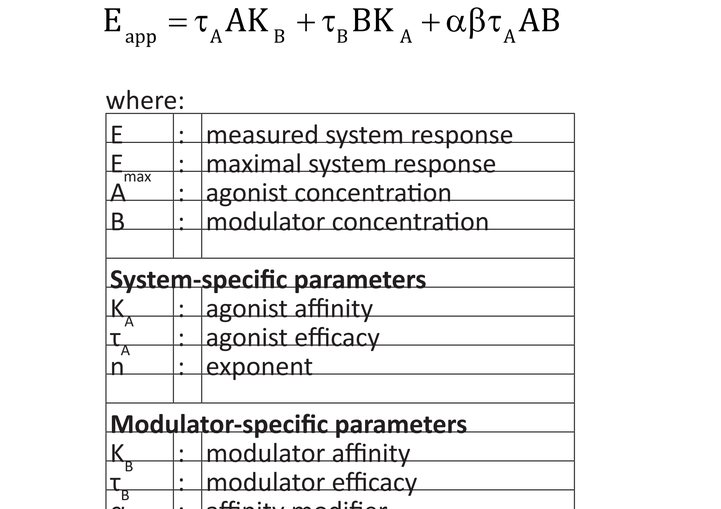

Figure 1: Plate layout

Test compound response was normalized using control medians and scaled by a predetermined maximum relative response of the agonist control ($\%A_{Max}$), as follows:

$$\%E_{max} = \%A_{max}\frac{E - \tilde{D}}{\tilde{A} - \tilde{D}}$$

where:

E	:	measured system response
$\%E_{max}$	:	scaled and normalized system response
$\%A_{max}$	:	agonist control maximum relative response
$\tilde{A}$	:	agonist control median
$\tilde{D}$	:	DMSO control median

For physical experiments, the value of $\%A_{max}$ should be determined separately or estimated. A theoretical value of 95% was used here ($100\ \tau_A/(\tau_A+1)$), corresponding to a nearly full agonist.

Response data for the virtual compounds was simulated using fixed system-specific parameters and, for each compound, randomly generated modulator-specific parameters (except β, which was fixed at 1) designed to be consistent with PAM. K_A, τ_A, and n were set at 200, 19, and 1, respectively. Randomly generated values for K_B, α, and τ_B were log-normally distributed and centered at 100 nM, 50, and 0.05, respectively. Values were further constrained so that K_B/α was between 0.1 and 1000, and α was greater than 5.

Response and background noise were introduced so that the signal %CV ranged from 2.5% in DMSO wells to 5% at maximum response. This noise level resulted in an average Z'-Factor of 0.77 for the 50 plates.

Data Analysis

The 50 data files produced by the simulation (one per plate) were read into Analyze™ to create a single assay run where non-linear curve fitting was performed. For curve fitting, 6 of the 7 parameters were unconstrained, while β was fixed at 1, as suggested when testing for PAM using a full agonist (Zhang, 2015). There were no other constraints on the fitter and no manual intervention to aid the system. Figure 2 shows the interactive curve fitter with data for one of the virtual compounds.

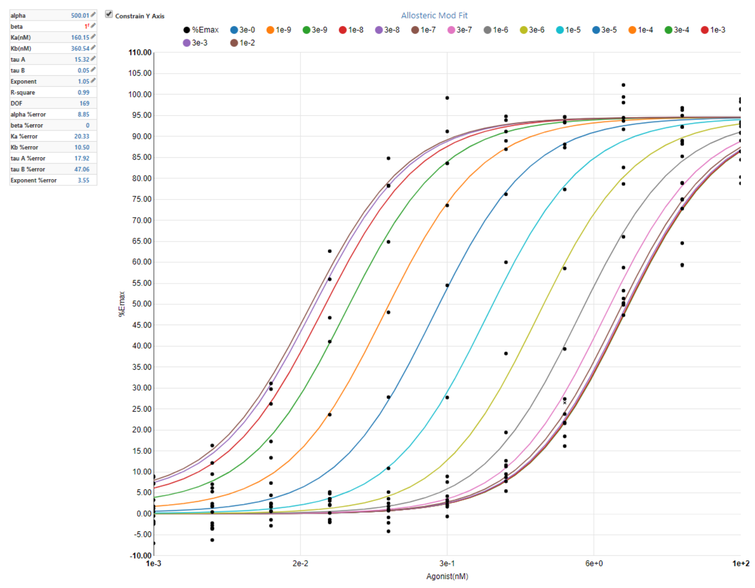

Figure 2: Analyze™ interactive curve fitting

Results

Figure 3 shows a random sample of the virtual compound fits (16 of 100). Figure 4 shows the correlation between the three modulator-specific fitted parameters (measured) and the values used for simulation (actual). There was good correlation for K_B and α, while the prediction of τ_B was poor (r^2=0.67). Additionally, there was a clearly a trend showing less agreement at lower τ_B values.

In an attempt to better predict τ_B, fitting was repeated with two additional parameters fixed, K_A and τ_A. There was only a marginal improvement in r^2 (0.73) and the same trend appeared. Interestingly, there was also no obvious improvement in the other predictions. When all 7 parameters were unconstrained, only K_B remained well predicted, as r^2 for α dropped dramatically (0.39). This was expected as β cannot be reliably determined when $\beta\tau_A$ > ~20 (Zhang, 2015).

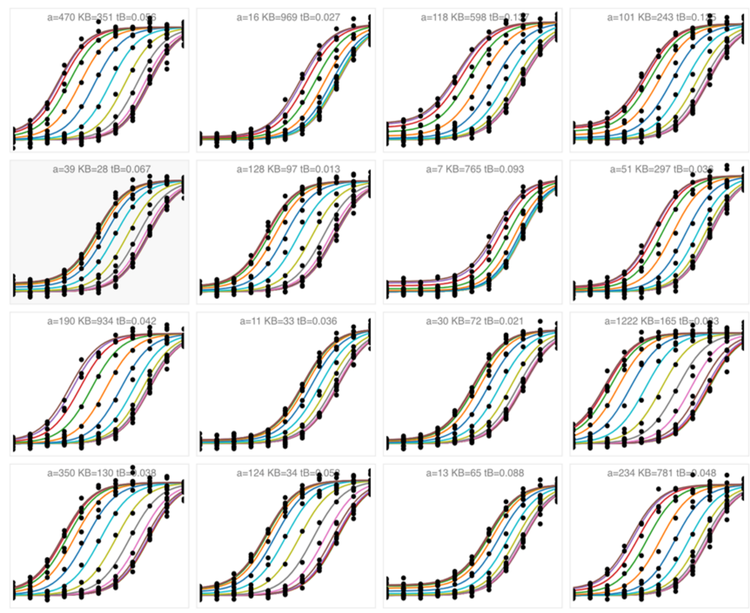

Figure 3: Representative fits from simulation (16 of 100)

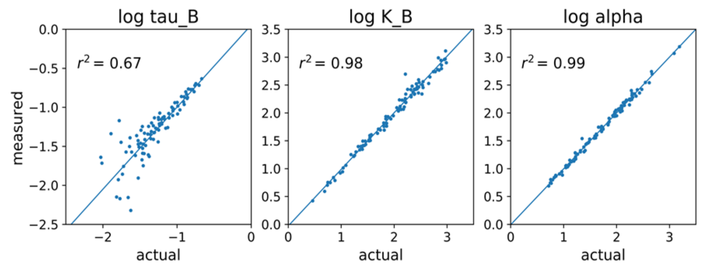

Figure 4: Agreement between simulated (actual) and fitted (measured) parameters

Conclusions

- Using a strong agonist and fixing at β =1 allowed good prediction of K_B and α.
- Low values of τ_B (<0.1) were not well predicted, even when 3 of 7 parameters were fixed. This was likely due to the noise introduced into the simulation, even though it was relatively small (2.5% CV in DMSO controls). In practice, a very low signal-to-noise ratio would be needed for better estimates of τ_B.
- Not fixing any parameters only allowed good prediction of K_B.

References

Zhang R, Kavana M. Quantitative analysis of receptor allosterism and its implication for drug discovery (2015) *Expert Opinion on Drug Discovery*. 10:763–780.

Tools

Modeling and simulation were done using the Python programming language. Data normalization and curve fitting were done using Analyze™ (Scigilian Software Inc.)

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