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Using MTBindingSim as a tool for experimental planning and interpretation

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Abstract

MTBindingSim is a program that enables users to simulate experiments in which proteins bind to microtubules or other polymers under various binding models. The purpose of MTBindingSim is to help researchers and students gain an intuitive understanding of binding behavior and design experiments to distinguish between different binding mechanisms. MTBindingSim is open-source, freely available software and can be found at bindingtutor.org/mtbindingsim.

This chapter first describes the capabilities of MTBindingSim, including the experimental designs and protein binding models that it simulates, and then discusses two examples in which MTBindingSim is utilized in an experimental context. In the first, MTBindingSim is used to investigate potential explanations for unusual behavior observed in the binding of the neuronal protein Tau to microtubules, demonstrating that some potential explanations are incompatible with the experimental data. In the second example, MTBindingSim is used to design experiments to examine the question of whether the plus-end tracking protein EB1 binds preferentially to the microtubule seam.

Introduction

Microtubules (MTs) are a protein polymer that are a fundamental part of the cell cytoskeleton and are involved in many critical cellular processes, such as cell division, maintenance of cell polarity, and cargo transport (Amos & Schlieper, 2005; Desai & Mitchison, 1997; Howard & Hyman, 2003; Lansbergen & Akhmanova, 2006). MTs interact with a vast, highly-coordinated array of Microtubule-Associated Proteins (MAPs) to carry out these functions (Lansbergen & Akhmanova, 2006). Researchers often investigate the interactions between MAPs and MTs by investigating their interactions *in vitro*. In particular, many MAP-MT interactions have been probed using binding assays (e.g., Gupta et al., 2009, 2010; Zhu et al., 2009), which are powerful experimental tools often used in studies involving MTs. However, the results of binding studies can be difficult to interpret in cases where the MAP-MT interaction is not a simple 1:1 binding interaction with a single, well-defined dissociation constant (K_D). To assist researchers in interpreting MAP-MT binding experiments, Philip *et al.* have written a program to model MAP-MT binding interactions, MTBindingSim (Philip, Pence, & Goodson, 2011). MTBindingSim is also a useful tool to help researchers train their intuition about MAP-MT binding and to aid in the design of new experiments that would distinguish between different binding mechanisms. While the text below focuses on using MTBindingSim to study interactions between MAPs

and MTs, some of the models used in MTBindingSim can be used to simulate binding to any polymer, and still others are suitable for modeling general protein-ligand interactions.

MTBindingSim

MTBindingSim simulates experimental data for various MAP-MT binding mechanisms (described below) according to user-specified values for the total MAP and MT concentrations and the binding dissociation constant(s). MTBindingSim was written in MATLAB and can be run either in MATLAB or as an independent program that does not require MATLAB on Windows or Mac OS. MTBindingSim is open-source software and the MATLAB code and independent programs are freely available at bindingtutor.org/mtbindingsim. A user manual with detailed instructions on the operation of MTBindingSim and the mathematical calculations used in simulating the binding curves is also available. Some text of this section has been adapted from the user manual.

Experimental Designs in MTBindingSim

MTBindingSim can plot simulated curves from three different experimental designs. The most common experimental method for investigating MAP-MT binding is a cosedimentation assay, where MT polymer and MAPs are mixed in known quantities, incubated to allow the proteins to bind to equilibrium, and then centrifuged, which causes the MTs and any bound MAPs to separate out of solution (the supernatant) and form a pellet. The supernatant and pellet fractions are then assayed to determine the protein concentration in each fraction by SDS-PAGE and densitometry analysis. The cosedimentation method relies on the assumption (verified by appropriate controls) that the MTs will form a pellet regardless of any MAPs that may be bound and that the MAPs will not spin down in the absence of binding to MTs. While the simulated curves in MTBindingSim were designed with cosedimentation assays in mind, the data will be the same for any method of investigating MAP-MT binding, such as fluorescence anisotropy, SPR, or tryptophan fluorescence. MTBindingSim follows the standard convention of writing the concentration of MTs, [MT], to mean the concentration of polymerized tubulin dimers (the “MT polymer”). MAP-MT binding experiments are usually performed in the presence of MT stabilizers such as taxol, so the concentration of unpolymerized tubulin dimers will be minimal and can be neglected. Experiments performed with a high concentration of unpolymerized tubulin dimers will present additional complications in interpreting binding curves and cannot be modeled with MTBindingSim.

Vary [MT]:

In this assay the concentration of A (a generic MAP) is held constant and the concentration of MT is varied. This is the “standard” binding experiment. The fraction of A bound to MT is graphed on the y-axis. The user can choose either the total MT concentration or the free MT concentration to be graphed on the x-axis. Note that when the x-axis is set to [MT] free, this experiment shows the familiar Langmuir isotherm. The quick method of determining K_D from the concentration of MT where 50% of A is bound *only* works when the x-axis plots [MT] free, not when it plots [MT] total.

Vary [A]:

In this assay the concentration of MT is held constant and the concentration of A is varied. There are three possible ways of graphing data from this kind of simulation. The first is a Scatchard plot where the x-axis is [A] bound and the y-axis is [A] bound/[A] free. In this kind of plot, binding data will be linear for a simple 1:1 interaction, and a curved line indicates cooperativity or other types of non-simple binding. The other two plots have [A] bound on the y-axis and the x-axis set to either [A] free or [A] total.

Competition:

In this assay there are two MT binding proteins, A and B. The concentration of A and MT is held constant and the concentration of B is varied. The y-axis is the fraction of A bound and the x-axis is the total concentration of B. In the present version of MTBindingSim, this kind of experiment can only be simulated when both A and B bind to MTs with simple 1:1 binding.

Binding Models in MTBindingSim

The binding models simulated in MTBindingSim were chosen to reflect commonly seen or hypothesized mechanisms of MAP-MT binding. While MTBindingSim was written specifically with MAP-MT binding in mind, most of its models have broader applicability. Only the “seam and lattice binding” model is MT-specific as it relies on the unique structure of the MT. The “MAPs dimerize” and “pseudocooperativity” models are valid for any protein-polymer or ligand-polymer interaction. The other models are valid for any protein-protein or ligand-protein interaction. The calculations performed by MTBindingSim are based on the definitions of the dissociation constants and mass balances for each binding model. Detailed derivations of the equations used in MTBindingSim can be found in the user manual, available at bindingtutor.org/mtbindingsim.

Binding ratio:

Some MAPs appear to interact with MTs at ratios differing from 1 MAP: 1 tubulin dimer. MTBindingSim allows for such situations by including the binding ratio, n , as a user-specified input. The binding ratio is defined as $n \times A$: 1 tubulin dimer in all models in MTBindingSim. MTBindingSim includes n in its calculations by setting the concentration of initial available binding sites to the concentration of free polymerized tubulin dimers ($[MT]$ free) and only taking the binding ratio into account when calculating how many binding sites have been occupied by a binding event.

First order binding (Figure 1a):

In this model the MAP A binds to MTs in a 1 MAP:1 tubulin dimer fashion with a dissociation constant K_{AMT} . In this model all AMT interactions are identical.

Seam and lattice binding (Figure 1b):

In this model A can bind to either the MT seam (α - β lateral tubulin contacts, forming 1/13 of the number of tubulin dimers) or the lattice (α - α and β - β lateral tubulin contacts, forming 12/13 of the tubulin dimers) with dissociation constants K_{AS} and K_{AL} , respectively.

MAPs dimerize (Figure 1c):

In this model A can exist either as a monomer or as a dimer, both of which can bind the MTs with dissociation constants K_{AMT} and K_{AAMT} respectively. The dissociation constant for the A-A dimerization is K_{AA} .

Pseudocooperativity:

The standard model of cooperative binding cannot be applied to polymers, so we have substituted this with a “pseudocooperativity” model. In this model, each A that binds to a MT site with a dissociation constant of K_{AMT} alters a second MT site so that it becomes MT*, which has a dissociation constant for A of K_{AMT}^* .

MAPs bind MT-bound MAPs (Figure 1d):

In this model the binding of A to a MT with a dissociation constant of K_{AMT} creates a new binding site for another A to bind to the previous MT-bound A with a dissociation constant of K_{AA} . This allows a second A to bind without taking up another MT site.

Two MAPs bind MT-bound MAPs (Figure 1e):

This model is an extension of the “MAPs bind MT-bound MAPs” model, where the binding of A to a MT with a dissociation constant of K_{AMT} then allows two more As to bind to the MT-bound A with a dissociation constant of K_{AA} . An examination of this model and the “MAPs bind MT-bound MAPs” model shows that further A-A binding on the MT surface will follow a similar pattern, ultimately resulting in a non-convergent infinite series, which cannot be modeled by the equations used in MTBindingSim (this would be a polymerization).

Two binding sites:

In this model there are two independent binding sites on the MT for an A with dissociation constants K_{AMT1} and K_{AMT2} .

Using MTBindingSim Example 1: Tau-MT Binding

Tau is a MAP that binds all along the length of MTs and stabilizes the MT structure. Despite multiple investigations into the binding of Tau to microtubules, it is unclear how many tubulin dimers one Tau protein binds to or what is the Tau-MT dissociation constant (K_D), because different publications report different answers. For example, measured K_D values range from <100nM up to >1 μ M (Ackmann, Wiech, & Mandelkow, 2000; Goode, 1994; Gustke, Trinczek, Biernat, Mandelkow, & Mandelkow, 1994; Kar, Fan, Smith, Goedert, & Amos, 2003; Makrides, Massie, Feinstein, & Lew, 2004).

Ackmann *et al.* (Ackmann et al., 2000) and more recently Duan and Goodson (Duan & Goodson, 2012) have shown that the amount of Tau that binds to 0.031 μ M MTs continues to rise as the Tau concentration is increased (data from Duan and Goodson is shown in Figure 2a). If a binding ratio of 1 Tau: 1 tubulin dimer is assumed, the data appear to show that Tau is supersaturating the MTs (the concentrations of cosedimented Tau end up being greater than the concentrations of total MTs).

Ackmann et al., (2000) suggested that these observations could be explained by the idea that Tau binds to MT-bound Tau. Further experimental support for this idea was provided by cross-linking and AFM experiments by Makrides *et al.* (Makrides et al., 2003). In this proposed model, Tau binds to the MT and a second Tau protein is then able to bind the first, already MT-bound, Tau protein. This would result in Tau oligomers forming on the MT surface (approximately 2–3 Tau proteins binding each other on the MT surface). Both Tau-MT and Tau-Tau binding would contribute to the amount of Tau that is measured in the pellet fraction of a cosedimentation assay, which would normally be interpreted as Tau bound to MTs.

Could this type of model explain the data? Intuitively, it is appealing. MTBindingSim can be used to investigate this question in a more quantitative way. To start, it is a good idea to investigate how proteins interact according to a simple binding model. To do this, the standard “first order binding” model is employed, in which Tau binding only to MTs is simulated. For the curve shown in Figure 2b, the K_D was set to 0.1 μ M based on reasonable estimates from other Tau-MT binding experiments (data not shown). As shown in Figure 2b, the amount of Tau that binds to the MTs saturates at 1 μ M.

Next, the behavior of models in which Tau binds to MT-bound Tau can be examined. Figure 2c shows the amount of Tau that binds to 1 μ M MTs using the “MAPs bind MT-bound MAPs” model. Three different scenarios are shown: the MAP-MT binding affinity is weaker than the MAP-MAP binding, the MAP-MT and the MAP-MAP binding affinities are equal, and the MAP-MT binding affinity is stronger than the MAP-MAP affinity. Figure 2c shows that in any scenario in which the one MAP is allowed to bind each MT-bound MAP, all curves saturate at 2 μ M bound MAP, unlike what happens with the data that are shown in Figure 2a.

To test the hypothesis that the data in Figure 2a can be explained by adding an additional layer of Tau-Tau interaction, the “Two MAPs bind MT-bound MAPs” model was used with the same binding affinities as those set in Figure 2c (Figure 2d). The results of Figure 2d are similar to those shown in Figure 2c in that the curves saturate; the amount of MAP bound simply saturates at 3 μ M instead of 2 μ M. More importantly, Figure 2c and Figure 2d together demonstrate that for any small number of Tau-Tau interactions at the MT surface there will be a clear upper bound limit in the amount of Tau bound. This behavior is in contrast to the continual rise in the amount of Tau bound seen in the experimental data of Figure 2a.

These observations suggest that a model based on Tau-MT interactions and finite Tau-Tau oligomerization is not able to explain the data observed in Figure 2a. There must be another interaction occurring in solution between the Tau and MT proteins that has not yet been

accounted for by the model. Duan and Goodson have recently used fluorescence microscopy to provide evidence that this “additional process” is formation of Tau-only filaments that are induced by the presence of MTs. The presence of these filaments confounds the MT cosedimentation assays by removing free Tau from solution (causing an apparent reduction of Tau-MT affinity) and by sedimenting independently of MTs (increasing the apparent affinity) (Duan and Goodson, 2012).

This work shows the value of MTBindingSim as a tool for interpreting binding data. Using MTBindingSim demonstrated that the observations of supersaturation in the Tau-MT experiments could not be due to Tau oligomerization at the MT surface as had previously been suggested. This conclusion opened up consideration to other possible explanations for the observed Tau-MT behavior and ultimately led to the microscopy studies that revealed the cause of the puzzling binding data. Without the aid of MTBindingSim, it would have been more difficult to confidently reject the hypothesis of Tau oligomerization on the MT surface and then move on to other possibilities.

Using MTBindingSim Example 2: Does Human EB1 Bind to the MT Seam or Lattice?

One class of MAPs of particular interest is the microtubule plus-end tracking proteins (+TIPs). One +TIP that has been of particular interest is EB1, which is considered the “core” of the +TIP network (Akhmanova & Steinmetz, 2008). There are conflicting reports in the published literature about the nature of EB1-MT binding interactions and whether EB1 has a preference for the MT seam. The MT seam is a region where the lateral contacts between tubulin dimers are α - β , in contrast to the lattice, where the lateral contacts between tubulin dimers are α - α and β - β . The seam and lattice are thus chemically distinct. Sandblad *et al.* published binding data that they interpreted as evidence that EB1 binds strongly to the MT seam and weakly, if at all, to the lattice (Sandblad *et al.*, 2006). However, more recent electron microscopy and fluorescence microscopy experiments by Maurer *et al.* have contradicted Sandblad *et al.*'s conclusions, providing strong evidence that EB1 binds to the entire MT and specifically avoids the seam (Maurer, Fourniol, Bohner, Moores, & Surrey, 2012).

How can these reports be reconciled? Using MTBindingSim simulations, it can be shown that there are other possible interpretations of the Sandblad binding data (Figure 3A) (Ottenweller *et al.*, 2012). To take a biochemical approach to resolving the question of whether EB1 binds to the lattice or seam and provide additional evidence on the specific question of how human EB1 binds to MTs, Ottenweller *et al.* (2012) used MTBindingSim to identify a set of experimental conditions that should distinguish between a model where EB1 binds strongly to the MT seam with weak to no lattice binding, and one where EB1 binds to the entire MT with a moderate affinity. The resulting theoretical binding curves are shown in Figures 3b and 3c (Ottenweller *et al.*, 2012). These data lead to the prediction that if EB1 binds strongly to the seam, a moderate concentration of EB1 will saturate the available binding sites, and as the concentration of EB1 increases, the fraction of EB1 that is bound to MTs at a given MT concentration will fall. In contrast, if EB1 binds to the entire lattice with

moderate or weak affinity, the overall concentration of free MT sites will change little as the concentration of EB1 moves up or down, leading a situation where the fraction of EB1 bound to MTs stays relatively constant. Note that these graphs are plotted against total [MT] because calculating the free [MT] cannot be done without making assumptions about the EB1-MT binding ratio. Using these simulated binding curves as a guide, Ottenweller *et al.* have recently provided strong biochemical evidence that human EB1 does not bind to the MT seam (Ottenweller et al., 2012).

Conclusion

MTBindingSim is software designed to assist researchers in designing and interpreting binding experiments as well as in training their intuition about how MAP-MT binding interactions will behave. This program has been very helpful when investigating puzzling MAP-MT binding behavior and in designing experiments to distinguish between different models of MAP-MT binding. For further information on how to use MTBindingSim, see the user manual at bindingtutor.org/mtbindingsim. MTBindingSim is under active development and the developers welcome suggestions from other researchers for new features or models for MTBindingSim or for changes to the user interface (see website for contact details). A more general version of MTBindingSim, BindingTutor, for use as an educational tool, is also currently under development and will be available at bindingtutor.org/bindingtutor when it is complete.

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Figure 1:

Cartoons illustrating binding models from MTBindingSim. (a) First order binding. (b) Seam (1/13 of the MT polymer) and lattice (12/13 of the MT polymer) binding. (c) MAPs dimerize, where a MAP can exist either as a monomer or as a dimer, each with different affinities for the MT. (d) MAPs bind MT-bound MAPs, where the binding of a MAP to the MT creates a new MAP binding site. (e) 2 MAPs bind MT-bound MAPs, where each MAP bound to the MT surface can bind two more MAPs.

Figure 2:

Tau-MT binding assay and simulations from MTBindingSim. (a) Data from a Tau-MT cosedimentation binding assay (reproduced from Duan and Goodson (Duan & Goodson, 2012)). (b) MTBindingSim simulation of a first order binding relationship with a K_D of 0.1 μM. (c) MTBindingSim simulation using the MAPs bind MT-bound MAPs model, with the MAP-MAP K_D less than (green line), equal to (red line) and greater than (blue line) the MAP-MT K_D . In interpreting these curves, it is important to remember that a smaller K_D value corresponds to a higher affinity. (d) MTBindingSim simulation using the 2 MAPs bind MT-bound MAPs model with the MAP-MAP K_D less than (orange line), equal to (teal line) and greater than (purple line) the MAP-MT K_D .

(a)

(b)

(c)

Figure 3:

Does EB1 bind preferentially to the MT seam? (a) Data extracted from Sandblad *et al.* (Sandblad et al., 2006) (green triangles), with overlaid curves corresponding to different binding models as generated by MTBindingSim. The blue curve shows a simulated curve for strong seam binding ($K_D = 0.001 \mu\text{M}$) with 60% active EB1 protein, while the purple curve illustrates the data expected for weak binding ($K_D = 10 \mu\text{M}$) to the entire MT. These curves show that both binding models are similarly consistent with the data. (b) Curves from MTBindingSim with strong seam binding ($K_D = 1 \mu\text{M}$) and weak lattice binding ($K_D = 1 \text{ M}$) at 1, 2, and 4 μM EB1. (c) Curves from MTBindingSim with first order binding to the entire MT lattice ($K_D = 13 \mu\text{M}$) at 1, 2, and 4 μM EB1. Figures are adapted from Ottenweller *et al.* (Ottenweller et al., 2012).