

Tellurium Notebooks - An Environment for Dynamical Model Development, Reproducibility, and Reuse

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Abstract

The considerable difficulty encountered in reproducing the results of published dynamical models limits validation, exploration and reuse of this increasingly large biomedical research resource. To address this problem, we have developed Tellurium Notebook, a software system that facilitates building reproducible dynamical models and reusing models by 1) supporting the COMBINE archive format during model development for capturing model information in an exchangeable format and 2) enabling users to easily simulate and edit public COMBINE-compliant models from public repositories to facilitate studying model dynamics, variants and test cases. Tellurium Notebook, a Python-based Jupyter-like environment, is designed to seamlessly inter-operate with these community standards by automating conversion between COMBINE standards formulations and corresponding in-line, human-readable representations. Thus, Tellurium brings to systems biology the strategy used by other literate notebook systems such as Mathematica. These capabilities allow users to edit every aspect of the standards-compliant models and simulations, run the simulations in-line, and re-export to standard formats. We provide several use cases illustrating the advantages of our approach and how it allows development and reuse of models without requiring technical knowledge of standards. Adoption of Tellurium should accelerate model development, reproducibility and reuse.

Author summary

There is considerable value to systems and synthetic biology in creating reproducible models. An essential element of reproducibility is the use of community standards, an often challenging undertaking for modelers. This article describes Tellurium Notebook, a tool for developing dynamical models that provides an intuitive approach to building and reusing models built with community standards. Tellurium automates embedding human-readable representations of COMBINE archives in literate coding notebooks, bringing to systems biology this strategy central to other literate notebook systems such as Mathematica. We show that the ability to easily edit this human-readable representation enables users to test models under a variety of conditions, thereby providing a way to create, reuse, and modify standard-encoded models and simulations, regardless of the user's level of technical knowledge of said standards.

Introduction

Multiscale dynamical modeling requires the ability to build large, comprehensive, and complex models of biological systems. Examples include the *Mycoplasma genitalium* whole-cell model [1] and the central metabolism of *E. coli* [2]. These models are often composed of many submodels. Typically, submodels are developed and validated by other research teams. Indeed, without the ability to reuse existing models, constructing larger models becomes impractical.

Being able to reuse tools and techniques developed by others is a hallmark of science. Poor reproducibility of biomedical experimental studies has been recognized as a major impediment to scientific progress [3, 4]. Much of the focus on poor reproducibility has been on wet lab experiments. However, barriers to reproducibility is also a significant problem in computational studies [5–8]. In recognition of this problem, **reproducibility** has become a central focus of scientific software [9, 10]. The general experience of researchers in the field of modeling suggests that a similar problem in poor reproducibility also exists for biomodels. Difficulty in model reproducibility can result from a published model not being deposited in a public repository or from differences in the deposited model and the actual model used for published simulations. In addition, it is difficult for researchers to utilize and modify public models because the standards are not human-readable. This state of affairs imperils continued progress with developing and exploiting biological models.

We propose that reproducible computational studies must satisfy two requirements. First, they must be *transparent*; that is, researchers must be able to inspect and understand the details of the model and the computational experiments. With transparency, researchers can check assumptions and explore variations in computational studies. Second, computational studies must be *exchangeable*; that is, it must be possible for a study done in one computational environment to be done in another computational environment and produce comparable results. For a study to be exchangeable means that other researchers can make use of and build on the published results in their computational environment.

In order to be transparent and exchangeable, a computational model and any simulation experiments must be encoded in a standard format that separates the reusable part of a model and its simulations (i.e. parameters, processes, and kinetics) from the implementation used to simulate it (i.e. the numerical methods and algorithms used to generate results). Models can be described using the Synthetic Biology Markup Language (SBML) [11] or CellML [12] standards. These standards support models based on ordinary differential equations (ODEs), stochastic master equations, and constraint-based modeling [13]. Simulations can be described using the Simulation Experiment Description Markup Language (SED-ML) [14], which encodes the types of simulations, either time-course simulations or steady state computations, that should be run on a model. SED-ML allows specifying the exact numerical algorithms needed to run a simulation using the KiSAO ontology [15], which includes widely used ODE (e.g. LSODA [16, 17], CVODE [18]) and stochastic solvers (e.g. Gillespie direct method [19], Gibson algorithm [20]).

In order to facilitate exchanging models and simulations between software tools, SED-ML simulations and SBML / CellML models can be packaged together using COMBINE archives [21]. However, few authoring tools exist for SED-ML and COMBINE archives [22, 23]. Furthermore, existing resources require technical knowledge of standards, restricting use of these standards by the modeling community at large. Therefore, an authoring tool is needed that allows a wider range of users to create and edit COMBINE archives containing both models and simulations. We propose that the authoring tool should satisfy five requirements:

1. It should represent the models or simulation specifications in a human-readable form. 52
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2. It should allow the user to easily edit this human-readable representation. 54
3. It should allow the user to provide narrative, annotations, or comments in order to improve transparency. 55
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4. It should translate the specifications into an implementation that can be used to run simulations. 57
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5. It must be capable of repackaging the model and/or simulation in a standard form that is usable by other tools. 59
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To address these requirements, we have developed the Tellurium Notebook environment, which extends the literate notebook concept used by tools like Jupyter [24] and Mathematica [25] to support community standards in systems biology. Whereas Jupyter notebooks contain code and narrative cells, Tellurium adds a third cell type for representing models and simulations encoded as standards. Our tool allows modeling studies to be constructed in a notebook environment and exported using community standards. This workflow provides both transparency, through a literate notebook, and exchangeability, through seamless, fluid support for standards. 61
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Tellurium supports embedding human-readable representations of SBML [26] and SED-ML [27] directly in cells. These cells can be exported as COMBINE archives which are readable by other tools. We refer to this human-readable representation as *inline OMEX* (after Open Modeling and EXchange, the encoding standard used by COMBINE archives). Inline OMEX cells operate in much the same way as code cells, i.e. they have syntax highlighting and are executable. Executing an inline OMEX cell runs all SED-ML simulations in the cell, producing any plots or reports declared in the SED-ML. A major advantage of this approach is that it offers a means of authoring transparent, exchangeable modeling studies without requiring technical knowledge of standards. 69
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Results 79

We demonstrate the benefits for reproducibility provided by Tellurium Notebook with case studies. In the first case study, we explore the impact of variations in the value of a parameter in a model of yeast. Such explorations are frequently done to determine if a model is applicable to conditions beyond those in the original model, an important consideration for testing model validity. The second case study evaluates if a model implementation produces results that are comparable to those in the original study via a series of tests which cover important dynamical properties of the model. 80
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Case Study 1: Assessing Previously Unexplored Model Parameters 87 88

In order to meet our requirements for reproducibility, it is not sufficient to simply recreate a simulation. Rigorous reproducibility requires the ability to reuse, expand, and test existing models under a variety of circumstances. This first case study shows how Tellurium facilitates conducting new experiments with an existing model, including the packaging of the model and the experiments as a COMBINE archive. The study is based on a model of autonomous metabolic oscillations in yeast and associated numerical studies [28]. The model has a cooperativity parameter m for which no 89
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specific justification is provided. We explore a range of values of m to understand how
this parameter influences the dynamics in the model.

When grown in continuous culture, yeast exhibit oscillations that can be maintained
for months and can cause the temporal separation of many cellular functions in a
synchronized way [29]. This type of dynamics belongs to a broader class of coupled
oscillators that are thought to be highly important in the organization of circadian
rhythms and play a role in the regulation of diurnal physiological activity in various
species [30, 31]. Wolf, et al. [28] developed a model that uses metabolic coupling
mediated in part by the H_2S pathway, one of the known contributors to respiratory
oscillations in yeast [29], to explain experimentally observed synchronized oscillations in
yeast cultures.

The model contains 21 reactions and is available via the BioModels repository as
BIOMD0000000090 [32]. The reaction v11a is a key part of the H_2S pathway. As shown
in Fig 1, standard-encoded SBML for this reaction is difficult to decipher, much less
comprehend. As with other authoring tools, Tellurium provides a concise,
human-readable abstraction of this encoding. However, unlike other tools, this
abstraction covers the entire SBML model and even covers entire COMBINE archives,
as we show shortly. We focus on the human readable representation of the reaction
v11a, including the reaction kinetics using the Hill coefficient m .

Clearly, readability is essential for model transparency. However, readability is
essential for model reuse as well. To demonstrate this, we convert this SBML-only
model into a COMBINE archive containing both SBML portions describing the model
and SED-ML portions describing the simulation. We then show how Tellurium's
human-readable format permits easy modification of the published model and
simulations contained in the COMBINE archive.

In order to create a SED-ML specification for this model, we need to define four
steps in the workflow, which correspond to distinct elements in SED-ML: (1) model
definition, (2) simulation, (3) task specification, and (4) output generation. For (1),
models can be defined in Tellurium's human-readable format by referencing SBML or
CellML files in the same COMBINE archive, with the option of including parameter
replacements. For (2), SED-ML simulations can be either timecourse simulations or
steady state computation, and can reference a specific algorithm (e.g. LSODA), or a
generic implementation using the KiSAO ontology [15]. Tellurium uses predefined
keywords such as `lsoda` (an ODE solver implementation [34]) to refer to popular
implementations. In SED-ML, simulations are specified independently from models.
This allows model and simulation elements to be reused in different combinations. For
(3), SED-ML uses task elements to describe these combinations. Finally, the output
elements of (4) can be plots or reports and allow users to access the output of tasks.
Tellurium's human-readable format allows defining a SED-ML model by instantiating
the same SBML model with different parameter values (m in this example) using the
syntax:

```
mymodel = model "wolf2001" with param1=value2, param2=value2 ...
```

with the param/value pairs being replaced by the corresponding parameter ids and
values respectively. We use this syntax to instantiate five copies of the model and
explore the values $m = 1, 2, 4, 8, 16$. Since we do not know *a priori* if the value of m
affects the timescale of the dynamics, we also create two simulations using different
durations. Finally, we create a task for each model/simulation combination and plot the
results on their respective timescales. Fig 2 shows that the value of m drastically affects
the dynamical behavior of the system, abolishing the periodicity of the oscillations at
 $m = 8$ and ceasing them entirely at $m = 16$. Smaller values of m also affect the phase

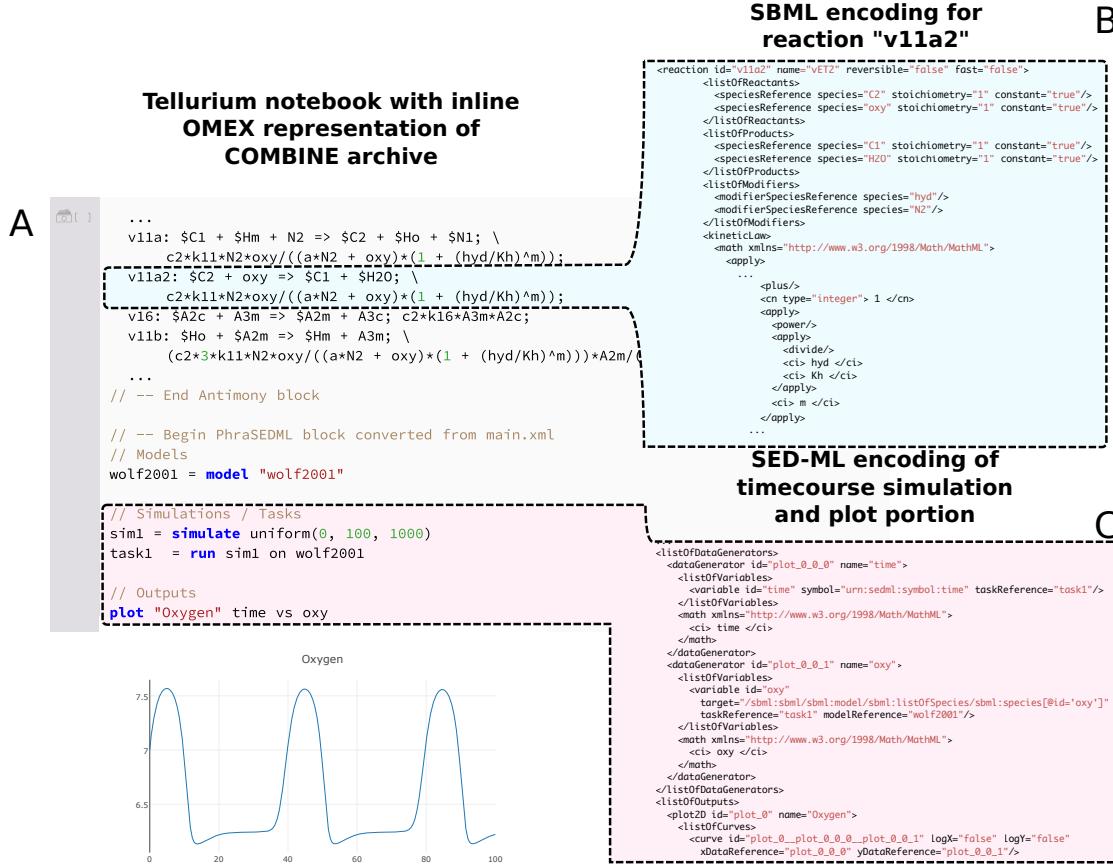


Fig 1. A comparison of Tellurium's human-readable representation of a COMBINE archive shown in a Tellurium notebook (A) and excerpts from the equivalent SBML (B) and SED-ML (C) encodings. Tellurium's in-line OMEX format contains human-readable representations of both SBML and SED-ML (A). (B) contains the SBML encoding for a single reaction. The single-line human-readable form of this reaction is highlighted in part (A) for comparison. Using the SBML encoding, it is difficult to modify the reaction stoichiometry or kinetic law, whereas this task is easy in Tellurium. This example makes use of a model of respiratory oscillations in yeast [28] and is available as a COMBINE archive [33]. Note, when using Tellurium, only the human-readable representation is displayed and can be modified by the user to automatically generate modifications in the hidden underlying standard representations.

and amplitude of the oscillations.

This case study shows that Tellurium provides an efficient means of converting SBML models into exchangeable COMBINE archives containing simulation components. Furthermore, COMBINE archives can contain important dynamical information about the model, such as the influence of the parameter m that we explored in this study. In order to demonstrate exchangeability of this study, we have exported it to the SED-ML Web Tools [22] (Fig S1) and iBioSim [35] (Fig S2).

Case Study 2: Reproducibility Through In-depth Variational Studies

Reproducibility requires that a model implementation produces results consistent with the original study, especially if a different authoring tool is used. In order to provide criteria for judging whether a model reproduction is consistent with the original, a set of testing criteria are required, similar to the concept of unit testing in software. However, researchers seldom perform extensive checks on the dynamics of models before using them. This is due in part to the lack of tool support for easily modifying and producing variants of models and simulations encoded in exchangeable formats. Tellurium’s authoring features enable modelers to encode dynamical unit tests in COMBINE archives, thereby providing a way to verify that a model has been correctly reproduced.

For this case study, we reproduce a highly-detailed model of syncytial nuclear divisions in the *Drosophila* embryo [36] through testing the model’s dynamics under different conditions. In many insect species, the embryo enters a period of rapid mitotic division without cytokinesis [37] immediately following fertilization. In *Drosophila*, 13 of these divisions occur within 3 hours of fertilization [36]. These divisions are regulated by metaphase promoting factor (MPF), a complex between cyclin (specifically the cyclin CycB in this model) and cyclin-dependent kinases (Cdk). CycB subunits tend to be the limiting factor in complex formation, and are thought to regulate mitotic division. CycB availability is controlled by the anaphase promoting complex (APC), which targets CycB for degradation. However, in *Drosophila*, the levels of CycB appear to remain high during the first 8 mitotic divisions [38]. This observation can be reconciled with known mechanisms by assuming that CycB degradation only occurs in the vicinity of the mitotic spindle [36, 39, 40], despite the absence of a nuclear envelope during the mitotic divisions. To account for this hypothetical local degradation of CycB, the model artificially separates the cytoplasm into two “compartments,” with a cytoplasmic compartment representing the cell and a nuclear compartment representing the volume in the vicinity of the mitotic spindle.

As a starting point, we use the COMBINE archive encoding of this model by Scharm and Tourè [41]. This archive contains SBML derived from biomodel BIOMD0000000144¹, which is intended to reproduce Fig 1 of [36]. However, the archive does not contain more extensive tests of the model’s dynamics, such as whether the model can be used to reproduce several other simulations described in the paper. The initial variant encoded by the COMBINE archive and shown in Fig 3B and C is based on a model with a constant level of the phosphatase String, whereas in reality String levels change over the course of the mitotic cycles. String regulates MPF via a positive feedback loop, and has been shown to peak at the seventh or eighth cycle of the mitotic divisions [36]. To account for this, Calzone et al. [36] posited that String mRNA is degraded by a hypothetical factor “X,” causing the synthesis rate of String to drop over time. Therefore, we have modified the SED-ML of the original COMBINE archive [41] as follows to include the synthesis and degradation of String. We are able to reproduce Fig 3 of [36] by making these modifications to the original COMBINE archive:

¹<https://www.ebi.ac.uk/biomodels-main/BIOMD0000000144>

A

```

...  

// -- End Antimony block  

// -- Begin PhraSEDML block converted from main.xml  

// Create five models with different Hill  

// coefficients to explore the effect of m  

model_m_1 = model "wolf2001" with m=1  

model_m_2 = model "wolf2001" with m=2  

model_m_4 = model "wolf2001" with m=4  

model_m_8 = model "wolf2001" with m=8  

model_m_16 = model "wolf2001" with m=16  

// The value of m may affect the timescale of the  

// dynamics, so simulate on two different timescales  

sim_short = simulate uniform(0, 100, 5000)  

sim_long = simulate uniform(0, 200, 5000)  

// Run each simulation / Hill coef. combo  

m1_short = run sim_short on model_m_1  

m2_short = run sim_short on model_m_2  

m4_short = run sim_short on model_m_4  

m8_short = run sim_short on model_m_8  

m16_short = run sim_short on model_m_16  

m1_long = run sim_long on model_m_1  

m2_long = run sim_long on model_m_2  

m4_long = run sim_long on model_m_4  

m8_long = run sim_long on model_m_8  

m16_long = run sim_long on model_m_16  

// Plot the results  

plot "Oxygen (short duration)" m1_short.time vs m1_short.oxy, m2_short.oxy,  

m4_short.oxy, m8_short.oxy, m16_short.oxy  

plot "Oxygen (long duration)" m1_long.time vs m1_long.oxy, m2_long.oxy, m4_long.oxy,  

m8_long.oxy, m16_long.oxy  

// -- End PhraSEDML block

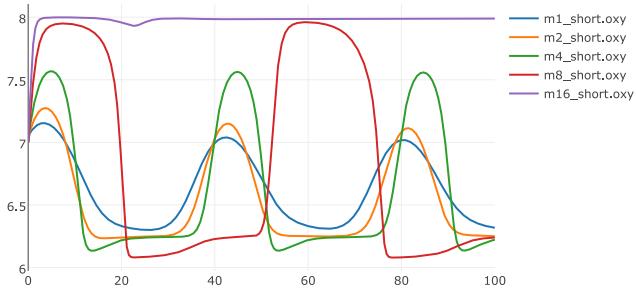
```

Instantiate model with different
Hill coefficients

Create two simulations with
different time scales

B

Oxygen (short duration)



C

Oxygen (long duration)

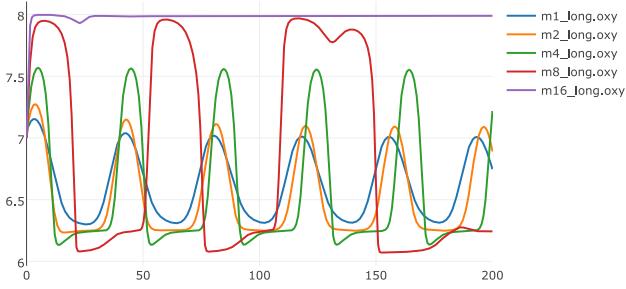


Fig 2. An example of using Tellurium to edit the respiratory oscillations model introduced in Fig 1. To investigate the effect of the Hill coefficient m , we used Tellurium's human-readable representation of SED-ML to create five instantiations of the model using values of 1, 2, 4, 8, and 16 for m (A). We then simulated each of these instantiations on two different timescales and plotted the respective results for short (B) and long (C) simulations. Tellurium's COMBINE archive support allows this model and simulation to be exported to other tools, as shown in Fig S1

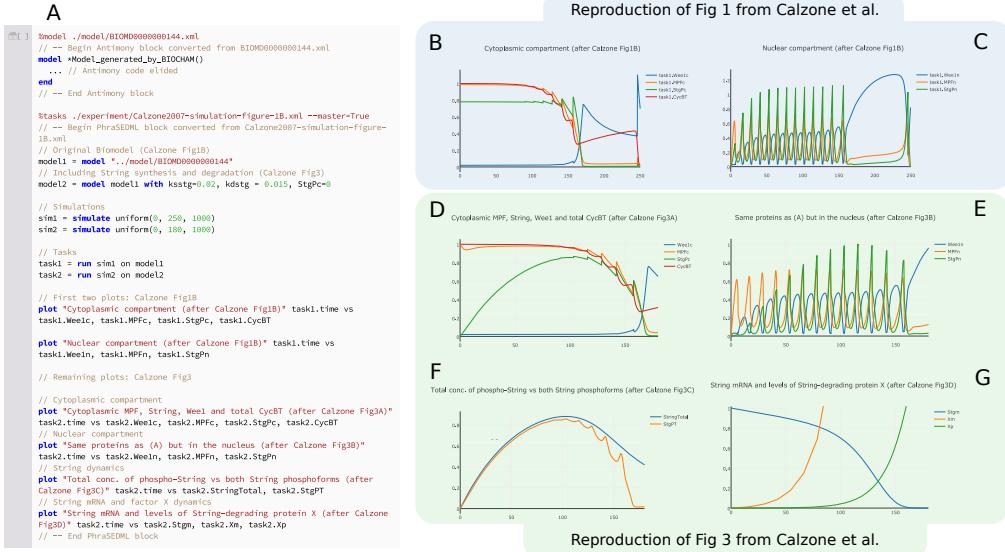


Fig 3. Using Tellurium to reproduce model variants in [36] and repackage as a COMBINE archive. To demonstrate the use of COMBINE archives for encoding model variants, we began with a COMBINE archive describing a single variant of this model without String synthesis or degradation [41], which reproduces Fig 1B of [36] (plots B and C here). We then used Tellurium to add a variant describing String degradation, which reproduces Fig 3 of [36] (plots D through G here). Plots B and D show the cytoplasmic compartment of the model. Plots C and E show the nuclear compartment (defined as the spatial region around the mitotic spindle). Plot F shows the levels of total String and its phosphorylated state. Plot G shows the level of String mRNA and protein factor X, which degrades String mRNA. Note the y-axis scale on plot G was manually adjusted to show the mRNA dynamics. The subplots in this figure intentionally have different durations, after Calzone et al [36]. The model in [36] was authored using BIOCHAM [42]. Our model reproductions that reproduce these plots are available as a COMBINE archive [43].

- Enable synthesis and degradation of String by setting the parameters `ksstg=0.02` 197
`kdstg=0.015` respectively. 198
- Set the initial concentration of total String to zero by setting `StgPc=0`. 199
- Compute the total amount of unphosphorylated String by adding the rule 200
`StgT := (1 - N*E_1)*Stgc + N*E_1*Stgn.` 201
- Compute the total amount of String in the cell by adding the rule 202
`StringTotal := StgPT + StgT.` 203

Tellurium makes it easy to encode both the original variant, without String synthesis and degradation, and the variant including these terms in a COMBINE archive [43]. Fig 3 shows the results of executing this COMBINE archive in Tellurium. We have thus expanded the dynamical test cases for this model, as it now reproduces two simulations from two different variants described by the original authors (Fig 1 and 3 of [36]), enabling better coverage of the model's dynamics. 204
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In order to gain insight into the regulatory mechanism controlling the mitotic divisions, and understand the transitions that control the exact number of these divisions, Calzone et al. performed a one-parameter bifurcation analysis [36]. Bifurcation analyses probes the number and position of steady states and other types of attractors as a function of a parameter. The oscillations shown in Fig 3 are the result of discrete division events, and the behavior shown does not represent a limit cycle. However, the model can be shown to exhibit limit cycle behavior by 1) removing all discrete events and 2) fixing the number of divisions by introducing the variable C as a cycle counter. The number of nuclear compartments is then given by $N = 1.95^C$ (1.95 is a scaling factor described in [36]). For a given cycle number C , MPF exhibits limit cycle oscillations, although the amplitude and period of these oscillations changes with the cycle number. At low cycle number, Calzone et al. observed that these oscillations were dominated by the negative feedback effect of cyclin degradation, whereas for large cycle number ($C \geq 12$), positive feedback via control of phosphorylated MPF by the kinase Wee and phosphatase String contributes to the oscillations. 210
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SED-ML does not support bifurcation analysis, precluding us from reproducing that part of the study in an exchangeable format. However, it is still possible to test the change in regulatory shift from negative to positive feedback. Instead of a bifurcation diagram, we compare the limit cycle behavior of the original model to a model variant with reduced Wee and String activation and deactivation rates. This slows the timescale of the positive feedback component of the model. Fig 4 compares the behavior of the original model at early and late cycle numbers with the variant containing attenuated positive feedback. Whereas the normal model exhibits stable limit cycle oscillations at both $C = 1$ and $C = 12$, the oscillations in the attenuated model are transient at late cycles ($C = 12$) but not at early cycles ($C = 1$). This observation suggests that String and Wee dynamics are indeed crucially important for late cycle oscillations, but not for early cycle oscillations, confirming the shift in regulatory mechanism. These simulation thus form a third set of unit tests for the model, encoded as a COMBINE archive [44]. 225
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In summary, using Tellurium's editing capabilities, we have created an extensive set of unit tests for dynamical behavior this model, which we exported as a COMBINE archive and imported into another tool as shown in Fig S3. Creating these tests required a means of quickly editing and expanding upon both the SBML and SED-ML embedded in the COMBINE archive. Tellurium's notebook approach allows us to satisfy these requirements, and provides an integrated workflow for testing the dynamical behavior of the model. 238
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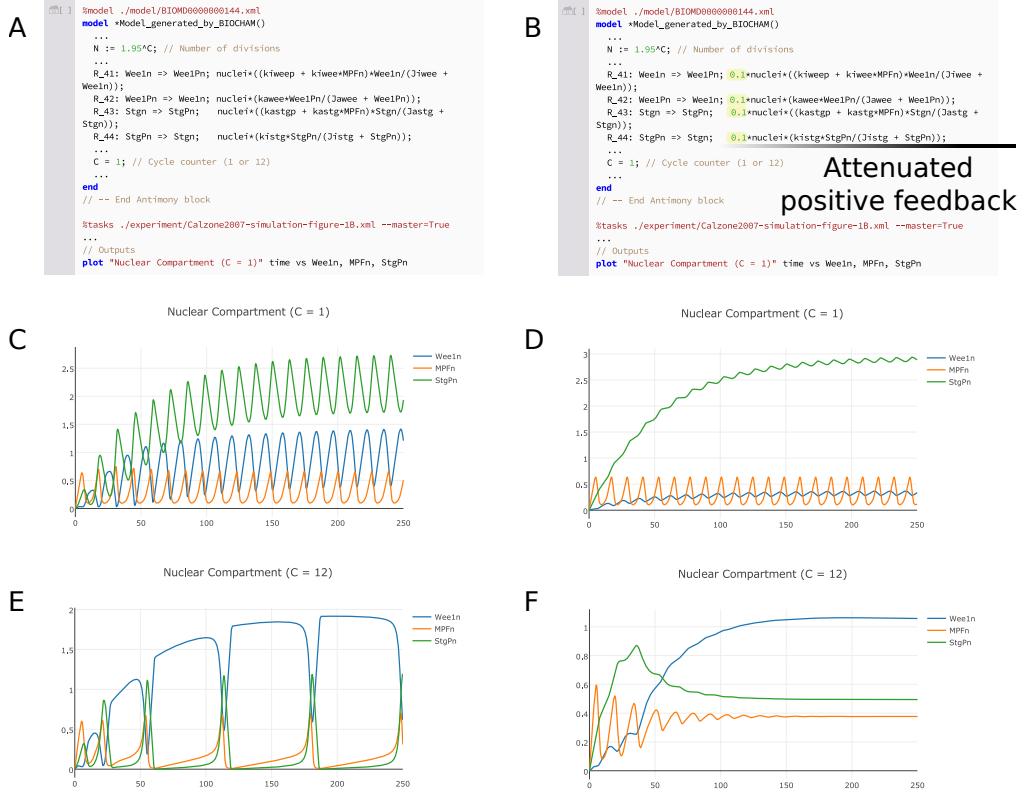


Fig 4. Testing the shift in regulatory mechanism of mitotic oscillations. To verify the observation [36] that the number of mitotic divisions in the *Drosophila* embryo is governed by a shift from negative to positive feedback, we first removed all discrete events and introduced the variable C such that $N = 1.95^C$. We then compared the limit cycles produced by this eventless model (left) with those produced by a variant with attenuated positive feedback from the regulators Wee and String (right). Attenuation was achieved by decreasing the rates of the phosphorylation and dephosphorylation of Wee and String. The original model exhibits stable limit cycle oscillations for both early cycles (C), which are putatively dominated by negative feedback, and late cycles (E), which are putatively dominated by positive feedback. The attenuated model only exhibits stable oscillations at early cycles (D), suggesting that positive feedback does indeed play a role in late cycle oscillations (F). Our model reuse and modification study is available as a COMBINE archive that reproduces the figure shown and facilitates further modification and reuse [44].

Interoperability Concerns & Test Cases	245
In order to achieve the exchangeability requirement of reproducibility, broad standards compliance is necessary. A small number of test cases, such as the first two case studies, is not sufficient to ensure interoperability with other software. During Tellurium's development, we gathered a number of COMBINE archive exemplars from the literature, other software tools, and our own archives. We have provided these archives as a resource to other developers by making them publicly available online. The test archives are structured to separate examples with advanced SED-ML features from those with basic SED-ML usage, enabling tool developers to implement incremental support for the standard. Table S1 lists all test archives and how to obtain them.	246 247 248 249 250 251 252 253 254
Advanced SED-ML Support	255
In order to address the requirement of broad standards compliance, we tested Tellurium against a set of tests provided by the SED-ML Web Tools [22]. These tests utilize advanced features of the SED-ML standard, and are designed to demonstrate the standard's coverage of different types of analysis. Table S2 lists all files used in this test set, and Fig S6 shows the results of exporting these files to Tellurium and back again.	256 257 258 259 260
SBML Test Suite COMBINE Archives	261
The SBML Test Suite [45] is a collection of dynamical models along with expected trajectories designed to test software tools for compliance with the SBML standard. Each test case contains a SBML model, simulation parameters encoded in SED-ML, expected trajectories encoded as a common-separated values (CSV) file, and graphical plots for reference. We converted each of these 1196 test cases into COMBINE archives containing the SBML models, SED-ML simulations, and CSV expected results and used these COMBINE archives as a benchmark for Tellurium's support for standards. The results of this benchmark are shown in Table S3.	262 263 264 265 266 267 268 269
Discussion	270
In order for the conclusions of a research study to be valid, the models used in the study must be reliable. Using SED-ML to reproduce the dynamics of a model and compare these dynamics with expected values adds crucial value to the integrity and validity of studies that reuse or expand on the model. As an exchangeable format, SED-ML is confined to the intersection of the most common features available in dynamical modeling tools, which leaves out certain useful types of analysis (e.g. bifurcation analysis). However, we argue that the use case of SED-ML is not to serve as a replacement for current analysis methods. Instead, SED-ML is a tool to test the dynamical behavior of models before using them. For example, while we were not able to reproduce the bifurcation analysis of the mitotic division study [36] in an exchangeable format, we were able to verify the observations regarding the shift in regulatory mechanism, and in doing so gained new insight from this alternative approach. A researcher may also wish to verify that the model reproduces certain expected behaviors. For example, if the model is expected to exhibit switch-like behavior, does this behavior occur at the correct input threshold? For models with feedback, such as integral feedback control [46], does the output exhibit robustness in the presence of perturbations? These types of validation require expert knowledge of the system. While there are tools and resources to help with this, the most important point for conveying this information to other researchers is to encode it as transparently and lucidly as possible, which is achieved using the literate notebook approach described here.	271 272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 288 289 290

Tellurium’s approach of blending standards with literate coding enables researchers to create rich, detailed workflows incorporating community standards. Tellurium allows the models and simulations from these notebooks to be shared with other tools via COMBINE archives. This allows other users to import these models and simulations and reproduce them using independently developed software tools. This is consistent with our original definition of reproducibility, as it enables robust cross-validation of results between tools, as opposed to simply repeating a previous simulation. It also helps ensure that the tools themselves are accurate and free of idiosyncrasies that could affect the analysis results. Model repositories such as BioModels [47,48], JWS Online [49], and the CellML model repository [50] have enabled widespread support for the SBML and CellML standards. We believe that better tool support for SED-ML and COMBINE archives will help create a trend toward better adoption of these formats by repositories.

Comparison with Existing Software

Many dynamical modeling tools support exchanging models via the SBML format, including COPASI [51,52], SBW [53], iBioSim [35], PathwayDesigner [54], CellDesigner [55,56], VCell [57–59], CompuCell3D [60], PySCeS [61], BioNetGen [62], and PySB [63]. These tools have diverse feature sets and intended use cases, such as tissue modeling (CompuCell3D), rule-based modeling of molecular complexes (BioNetGen, PySB, VCell), and general modeling and simulation (all others). The tools also have different forms of user interaction, such as graphical user interfaces (COPASI, iBioSim, VCell) and graph-based network editors (CellDesigner, PathwayDesigner). Python-based tools such as PySCeS [61] and PySB [63] can be used with a Jupyter notebook, but do not feature integration of standards with the notebook itself. In general, Tellurium is useful when the user wishes to interactively edit and test standard-encoded models and simulations or produce presentations and PDFs of modeling studies.

Tellurium’s Python foundation makes it easy to combine with other Python-based software such as PySCeS, COBRApy [64], and PySB. There are also many specialized Python packages for specific tasks such as moment closure approximation for stochastic models [65], parameter estimation [66], Bayesian inference [67], and estimating rate laws and their parameter values [68].

In biomedical research, certain tools have been created specifically to facilitate reproducible research. One such tool is Galaxy [69]. Galaxy is a web-based tool which allows users to create workflows describing experiments, e.g. metagenomic studies [70]. A similar tool with a focus on web services and which supports SBML-based workflows is Taverna [71]. Galaxy and Taverna allow users to annotate each step of the workflow, which provides a way for others to follow and understand the chain of reasoning used in the workflow’s construction. This satisfies the requirement of transparency, as it allows users to view the sequence of steps used to produce a result. Although this approach is very different from a literate notebook in terms of the way the user interacts with the system, it shares the goal of allowing the user to see the sequence of steps used to produce a result and interrogate the specific procedure used in each of the steps. Galaxy and Taverna also allow users to share workflows via the web. However, neither tool attempts to directly address the problem of exchangeability with other software tools.

VisTrails [72] is another workflow system based on visual design. VisTrails focuses primarily on generating rich, three-dimensional diagrams and visualizations based on input data and a specific sequence of steps. VisTrails also saves all changes made to a workflow and allows users to view previous versions, a concept termed “retrospective provenance” [73]. However, this approach also lacks exchangeability. Furthermore, while graphical tools may be more accessible because they abstract away the underlying algorithms, it can be difficult to isolate and correct software errors when a step fails due

to bad input or an internal error.	342
Many other research software systems make use of notebooks, and some incorporate special extensions. StochSS [74], the GenePattern Notebook [75], the SAGE math system [76], and the commercial Mathematica software [25] all utilize notebooks which are specially tailored or feature special extensions for each respective application.	343 344 345 346
However, none of these approaches attempt to solve the problem we address: workflow integration with exchangeable standards. Our usage of the literate notebook approach is intended to satisfy two specific requirements, which are distinct from other use cases: 1) to make these standards easy for humans to read, understand, and modify, without requiring expert knowledge of the technical specifications of the standards, and 2) provide an integrated workflow which facilitates exchangeability with other software.	347 348 349 350 351 352
The notebook approach used by Tellurium also has disadvantages. For example, it is difficult to use notebooks with a version control system in a meaningful way. Furthermore, large or complex analyses can be difficult to orchestrate using notebooks, as interacting with a large notebook with many cells can be cumbersome. Nevertheless, we believe that Tellurium's approach is highly useful in many crucial use cases, including testing models, experimenting with model variants, and as a final step in producing an analysis for other researchers in a transparent, visual presentation.	353 354 355 356 357 358 359
Conclusion	360
In order to build larger, more complete, and more accurate dynamical models of cells and tissues, it will be necessary to reuse models of subsystems. This is currently very difficult due to the time-consuming and laborious process of manually reconstructing models from the literature, or manually verifying third-party SBML models. Tellurium provides support for encapsulating both a model and its dynamics in a community-developed standard format, the COMBINE archive. This archive can contain the model as well as a number of simulations which test various dynamical properties of the model. Tellurium allows users to create COMBINE archives easily from SBML models, or import and modify preexisting COMBINE archives.	361 362 363 364 365 366 367 368 369
Tellurium integrates SBML, SED-ML, and COMBINE archives within a notebook environment, making it exceptionally easy for users to work with these standards, and obviating the need for users to understand the technical specifications of the standards. The availability of authoring tools such as Tellurium will make it possible for model repositories to begin implementing support for SED-ML and COMBINE archives. Indeed, the JWS Online repository [49] already has support for exporting COMBINE archives of models and simulations, which can be read by Tellurium. We hope that other databases will follow suit so that it will be possible to automatically extract dynamical information from these repositories.	370 371 372 373 374 375 376 377 378
Tellurium's human-readable representation of COMBINE archives is highly important for facilitating model modification as we describe here. This feature enables researchers to experiment with models using alternate parameterizations in order to test the dynamical behavior of the models under varying conditions. We hope that this will lead to more robust models which lead to biological insight by providing predictions under a wide range of circumstances, as with the case studies presented here.	379 380 381 382 383 384
Future Work	385
There is a clear need to support exchangeability of simulation experiments in order to allow researchers to build larger, better tested, and more comprehensive models. Tellurium's built-in support for exchangeability comes from the SBML and SED-ML	386 387 388

standards. This allows Tellurium to support the widest possible range of software tools, 389
but also prevents exchanging studies not covered by SED-ML’s vocabulary of 390
predefined simulation types. Due to delays associated with standardizing and 391
implementing features, SED-ML tends to lag several years behind other systems which 392
do not rely on standardization. Thus, SED-ML has the advantage of stable support 393
from a wide range of tools, but has the disadvantage of lacking the flexibility to encode 394
custom studies based on recent advancements in model simulation. 395

In order to provide a more flexible platform for encoding simulation studies, new 396
solutions are need. One such solution would be to extend SED-ML with generic 397
scripting capabilities. Another solution would be to build an alternative platform for 398
exchanging simulation experiments. For example, the SESSL [77] software tool also 399
provides a means for encoding and exchanging simulations. Whereas SED-ML uses a 400
standardized XML schema to describe simulations, SESSL uses a 401
domain-specific-language implemented using the Scala programming language. This 402
allows users to mix in Scala code to access features not yet available via SESSL’s public 403
interface. However, this approach is not language-agnostic and is tied to Scala and its 404
low-level execution engine. The SED-ML standard, in contrast, does not constrain the 405
low-level operation of its implementations. 406

In this paper, we have argued for modelers to construct “unit tests” for dynamical 407
models by including model variants as in the study by Calzone et al. [36]. We have 408
shown that these variants are easy to construct and encode in COMBINE archives using 409
Tellurium, but we have not addressed how to validate these tests in an automated way. 410
Due to simulation algorithm differences between tools and the presence of multiple 411
steady states in some models, performing a direct numeric comparison between steady 412
state values or timecourse traces may be too fragile to be useful. 413

The BIOCHAM software tool [42] employs an interesting solution by using temporal 414
logic constructs to make assertions about properties of model timecourse dynamics. 415
Using this approach, it would be possible, for instance, to make semi-quantitative 416
assertions such as “species X exhibits oscillations with a period of $100 \pm 50\text{mHz}$ ”. 417
These logical constructs could be used in lieu of a direct numerical comparison to 418
validate the dynamics of a model. A practical solution to the problem of validating 419
model timecourse dynamics would likely make use of semi-quantitative assertions such 420
as “Is the number of oscillations of X at least 10,” “Does Y exhibit a peak value of at 421
least 100 nM,” or “Does the response time of the system fall within a certain range?” 422

However, we believe that several important questions remain before such a validation 423
method will be useful in practical contexts, such as what is the minimal set of formal 424
logic expressions sufficient to capture any useful assertions, and what are the best 425
practices for encoding assertions? For example, should the assertions strive to use 426
relative relationships between model quantities, such that reparameterizing the model 427
does not affect the assertions, or should they be valid only for a single given 428
parameterization? In the former case, how should model variations be generated to test 429
assertions? We believe that implementing automated testing of dynamical models 430
requires addressing these questions in a well thought-out way. Until then, we believe 431
that manually comparing results encoded as COMBINE archives as in the studies 432
presented here will provide immediate benefits to reproducibility. For moderate-size 433
models such as the Calzone study, we have shown that this approach is a practical 434
solution. 435

Availability

Tellurium Notebook is available as a standalone app (tellurium.analogmachine.org) 436
or as a collection of Python packages hosted on the Python Package Index 437
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(pypi.python.org) for 64-bit versions of Mac OS X, Windows, and Linux. The
Tellurium Python packages support Python 2.7, 3.4, 3.5, and 3.6. The notebook app
comes bundled with Python 3.6 and all requisite packages. The source code of
Tellurium (github.com/sys-bio/tellurium) is licensed under the Apache license,
version 2.0. Tellurium incorporates or makes use of other software, such as *nteract*,
Plotly (<http://plot.ly>), *Python*, *libSBML*, *libSEDML*, and others, which are licensed
under their respective terms. See tellurium.analogmachine.org for links to
installation instructions, documentation (tellurium.readthedocs.io), and the source
code (github.com/sys-bio/tellurium). 439
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Chris Myers for their help and guidance in diagnosing and fixing interoperability
problems. 448
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Supporting information

All COMBINE archives used in this paper can be obtained at
<https://github.com/0u812/tellurium-combine-archive-test-cases>. 462
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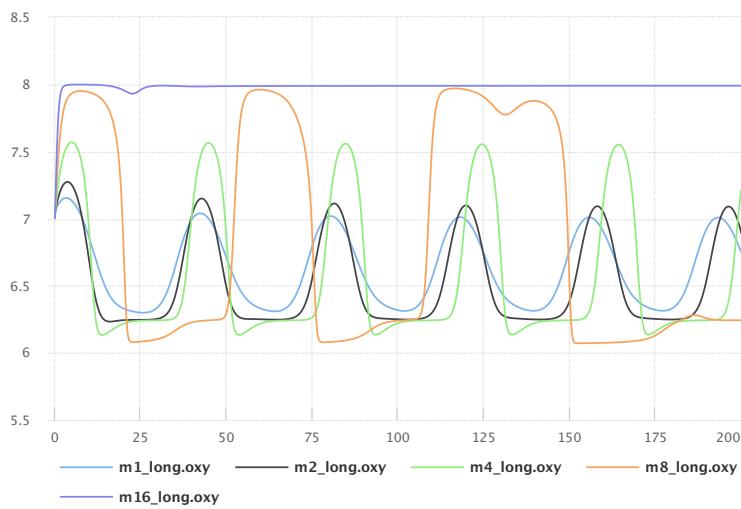
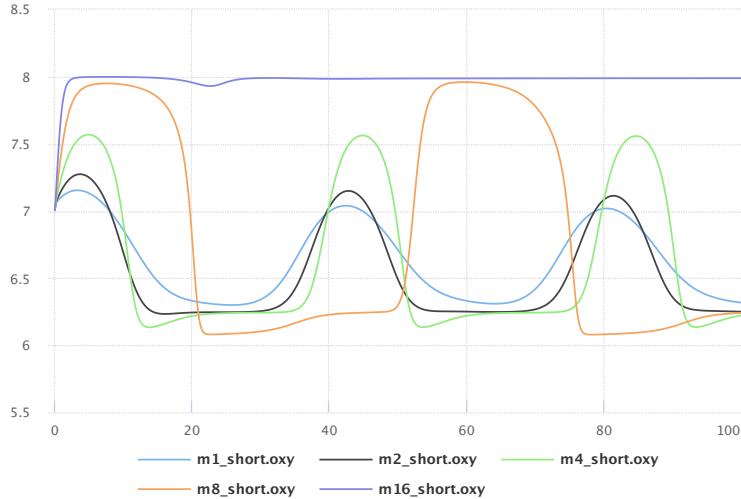


Fig S1. Demonstrating exchangeability of COMBINE archives containing SBML and SED-ML. The respiratory oscillation case study in Fig 2 was exported to a COMBINE archive from Tellurium, imported into the SED-ML Web Tools [22], and used to generate plots to verify that the simulation results were identical to Fig 2. The COMBINE archive used to create this example can be found online [78].

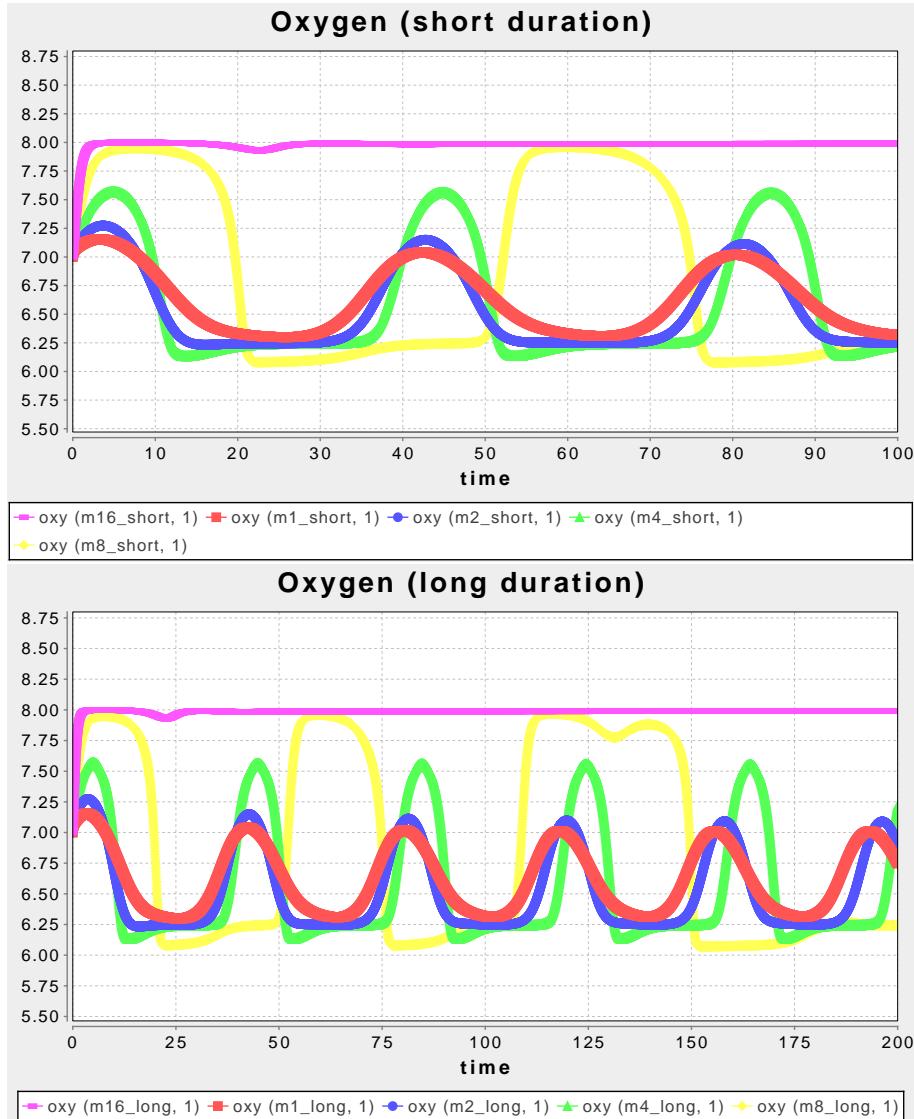


Fig S2. A second test of exchangeability. To demonstrate exchangeability between multiple tools, the same Hill coefficient case study as shown in Fig S1 was exported to iBioSim [35] and used to produce identical plots. This shows that COMBINE archives are sufficiently flexible to be exchanged between different tools, despite the limited number of tools which currently support the format.

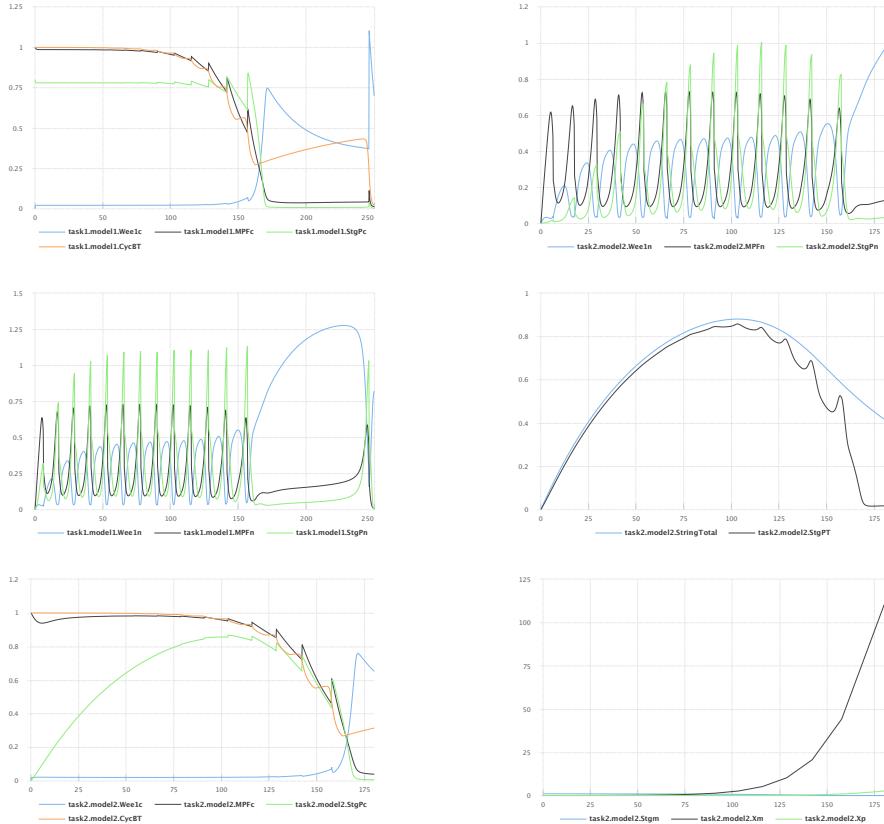


Fig S3. Demonstrating exchangeability of the second case study. To show that the extended set of simulations from Fig 3 can be exchanged with other tools via a COMBINE archive, we exported the study in Fig 3 to the SED-ML Web Tools and verified that the plots were identical to Fig 3.

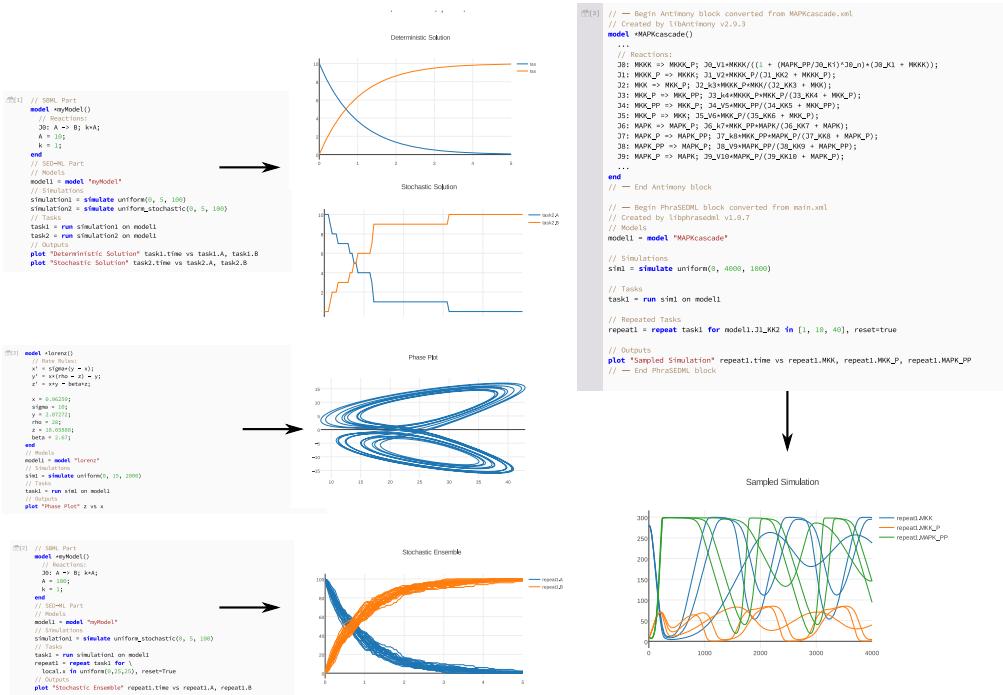


Fig S4. Examples of Tellurium’s inline OMEX format for specifying COMBINE archives. These examples start with very simple cases and build on these cases with progressively more advanced features. The first example contains a simple two-species model and simulated using a deterministic and stochastic solver. The second example shows a phase plot. The third example shows multiple stochastic traces. The fourth example shows a one-dimensional parameter scan. All of these examples are available via a Tellurium notebook, which can be accessed by clicking on “File” → “Open Example Notebook” → “COMBINE Archive Basics” from within the Tellurium notebook viewer.

```

model *BIOMD00000000012()
...
// Assignment Rules:
beta :=tau_mRNA /tau_prot;
alpha0 :=a0_eff*tau_prot/(ln(2)*KM);
a0_tr :=ps_0 *60;
alpha :=(Tr *eff*tau_prot/(ln(2)*KM));
a_tr :=(ps_a -ps_0 )*60;
t_ave :=tau_mRNA /ln(2);
kd_mRNA :=ln (2)/tau_mRNA;
kd_prot :=ln (2)/tau_prot;
k_til :=eff /t_ave;
// Reactions:
Reaction1 :X => ;kd_mRNA *X;
Reaction2 :Y => ;kd_mRNA *Y;
Reaction3 :Z => ;kd_mRNA *Z;
Reaction4 :=>PX ;k_til *X;
Reaction5 :=>PY ;k_til *Y;
Reaction6 :=>PZ ;k_til *Z;
Reaction7 :PX => ;kd_prot *PX;
Reaction8 :PY => ;kd_prot *PY;
Reaction9 :PZ => ;kd_prot *PZ;
Reaction10 : ==>X ;a0_tr +a_tr *KM^n/(KM^n +PZ ^n);
Reaction11 : ==>Y ;a0_tr +a_tr *KM^n/(KM^n +PX ^n);
Reaction12 : ==>Z ;a0_tr +a_tr *KM^n/(KM^n +PY ^n);
...
end
// Models
model1 =model "BIOMD00000000012"
model2 =model model1 with ps_0 = 1.3e-05,ps_a = 0.013
// Simulations
simulation1 =simulate uniform (0, 1000, 1000)
simulation1.algorithm =kisao .88
simulation2 =simulate uniform_stochastic (0, 1000, 1000)
simulation2.algorithm =kisao .27
// Tasks
task1 =run simulation1 on model1
task2 =run simulation1 on model2
// Outputs
plot "protein numbers per time point" task1.time vs task1.PX,task1.PY,task1.PZ
plot "protein numbers per time point - damped oscillations" task2.time vs task2.PX,
task2.PY,task2.PZ
plot "Normalized Plot" task1.PX/max(task1.PX) vs task1.PY/max(task1.PY),task1.PY /
max(task1.PY) vs task1.PZ/max(task1.PZ),task1.PZ/max(task1.PZ) vs task1.PX /
max(task1.PX)
// Names
model1 is "Repressilator-regular oscillations"
model2 is "Damped oscillations"
task1 is "Oscillation using a deterministic simulator"
task2 is "Damped oscillations using a deterministic simulator"

```

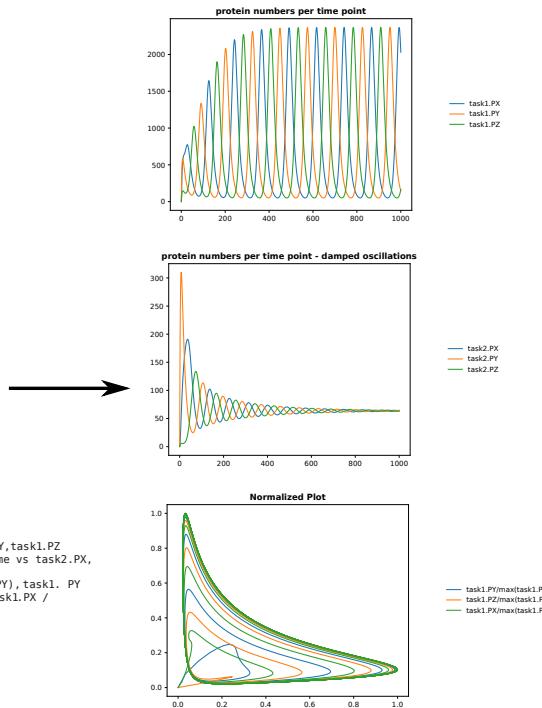


Fig S5. A normative example of a COMBINE archive introduced in the original paper describing the COMBINE archive format [21]. This example contains the repressilator model [79], a damped oscillation variant showing the modification of model parameters using SED-ML, and a phase plot of the undamped system.

Table S1. Combine Archive Test Cases.

demos: A set of models and SED-ML simulations of increasing complexity, in order to facilitate incremental support of the standard (4 archives, shown in Fig S4).
real-models: A selection of BioModels converted to COMBINE archives containing the associated SED-ML simulations (2 archives).
published: COMBINE archives published in the literature, including normative examples from the original paper introducing COMBINE archives [21] shown in Fig S5 (2 archives).
swt: Examples from the SED-ML Web Tools [22] demonstrating advanced features of SED-ML (5 archives).
sbml-test-suite: COMBINE archive encodings of the entire SBML test suite (1196 archives).

We collected all COMBINE archives used during development of Tellurium in an online repository on GitHub [80]. These archives serve to test Tellurium’s standards compliance, but they may also allow other tool developers to better support COMBINE archives. We have therefore organized the test cases into different categories, from toy examples using progressively more advanced features of SED-ML, to BioModels, and finally advanced SED-ML usage. The test suite draws archives from a wide range of sources: publications [21, 81], other tools (e.g. the SED-ML Web Tools [22]), the SBML Test Suite encoded as COMBINE archives, and archives developed by our group. The COMBINE test suite contains archives ranging from basic examples to advanced usage of the SED-ML standard. To verify exchangeability, we have manually tested importing these archives into our software and also into the SED-ML Web Tools. The SBML test cases were too numerous to test in this way, so a subset of archives were tested with the SED-ML Web Tools whereas the full set of archives was tested with Tellurium using a Tellurium notebook [82].

Table S2. Advanced SED–ML Tests (Provided by SED–ML Web Tools [22]).

<code>repeated_stochastic_traces.omex</code> :	Repeated runs of a stochastic simulation, overlaying the results of each run.
<code>repeated_stochastic_traces.omex</code> :	Scanning the steady state of a model as a function of a parameter.
<code>pulse_experiment.omex</code> :	Representing a generalized, time-varying forcing function in SED–ML.
<code>pulse_experiment.omex</code> :	Generalized, time-varying forcing function in SED–ML.
<code>timecourse_scan.omex</code> :	Time-course plot of different parameterizations of a model.
<code>nested_scan.omex</code> :	Nested steady state scan, iterating over two parameters.

The SBML test suite was converted into COMBINE archives using the provided notebook <https://github.com/0u812/tellurium-combine-archive-test-cases/blob/master/sbml-test-suite/convert-to-combine-arch.ipynb>. These SBML test cases are automatically converted into COMBINE archives containing the expected results, which are then converted by Tellurium into inline OMEX and simulated. A “failed” test refers to a case where the numeric simulation results diverge from the expected values. An “unsupported” test refers to a test that uses features not available in our simulator (libroadrunner) or the inline OMEX strings.

Table S3. Tellurium SBML Test Suite Results.

Test Range	Number Passing	Number Failing	Unsupported
1-100	91	3	6
101-200	90	1	9
201-300	90	1	9
301-400	99	1	0
401-500	97	3	0
501-600	59	3	38
601-700	77	1	22
701-800	86	6	8
801-900	41	2	10
901-1000	41	2	57
1001-1196	0	0	95
Totals	816	25	254

The SBML test suite was converted into COMBINE archives using the provided notebook <https://github.com/0u812/tellurium-combine-archive-test-cases/blob/master/sbml-test-suite/convert-to-combine-arch.ipynb>. These SBML test cases are automatically converted into COMBINE archives containing the expected results, which are then converted by Tellurium into inline OMEX and simulated. A “failed” test refers to a case where the numeric simulation results diverge from the expected values. An “unsupported” test refers to a test that uses features not available in our simulator (libroadrunner) or the inline OMEX strings.

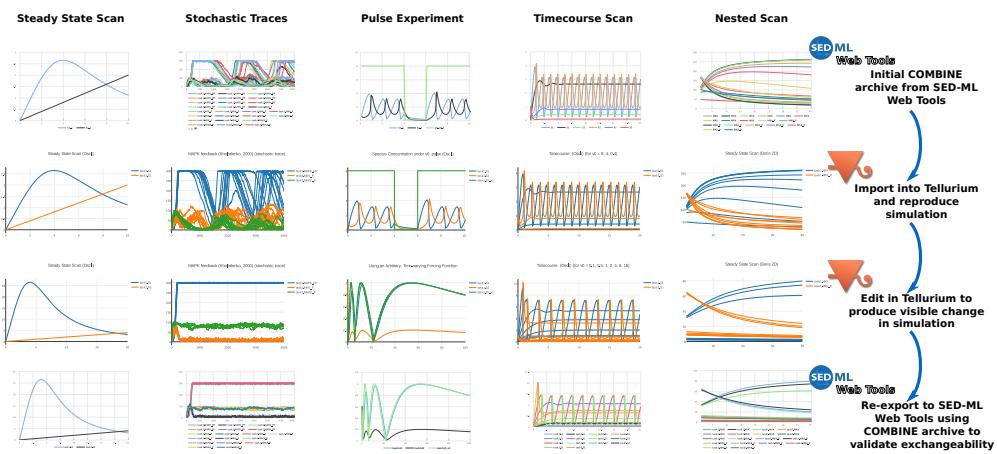


Fig S6. Round-tripping the SED-ML Web Tools examples [22]. In order to demonstrate broad support for standards, we conducted a series of tests utilizing advanced usage of SED-ML. The first row shows the original example rendered in the SED-ML Web Tools. The second row shows the same example imported into Tellurium. The third row shows the simulation after editing the model in Tellurium. Finally, the fourth row shows the result of re-exporting the example to the SED-ML Web Tools using a COMBINE archive.

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