

SBML Level 3 Package: Flux Balance Constraints (‘fbc’)

Brett G. Olivier PhD

b.g.olivier@vu.nl

Systems Bioinformatics
VU University Amsterdam
Amsterdam, NH, The Netherlands

Frank T. Bergmann PhD

fbergmann@caltech.edu

Computing and Mathematical Sciences
California Institute of Technology
Pasadena, CA, US

Matthias König PhD

konigmatt@googlemail.com

Institute for Theoretical Biology
Humboldt Universität zu Berlin
Berlin, DE

Version 3, Release 1, Proposal 1 π

April 12, 2021

The latest release, past releases, and other materials related to this specification are available at
[http://sbml.org/Documents/Specifications/SBML_Level_3/Packages/Flux_Balance_Constraints_\(flux\)](http://sbml.org/Documents/Specifications/SBML_Level_3/Packages/Flux_Balance_Constraints_(flux))

This release of the specification is available at
<http://identifiers.org/combine.specifications/sbml.level-3.version-1.fbc.version-3.release-1>



Contents

| | | |
|----------|--|-----------|
| 1 | Introduction and motivation | 3 |
| 1.1 | Proposal corresponding to this package specification | 3 |
| 1.2 | Tracking number | 4 |
| 1.3 | Package dependencies | 4 |
| 1.4 | Document conventions | 4 |
| 2 | Background | 5 |
| 2.1 | Problems with current SBML approaches | 5 |
| 2.2 | Past work on this problem or similar topics | 5 |
| 3 | Proposed syntax and semantics | 6 |
| 3.1 | Namespace URI and other declarations necessary for using this package | 6 |
| 3.2 | Primitive data types | 6 |
| 3.2.1 | Type FbcType | 6 |
| 3.3 | The extended Model class | 7 |
| 3.3.1 | The FBC listOfObjectives | 8 |
| 3.3.2 | The FBC listOfGeneProducts | 9 |
| 3.3.3 | A note on units | 9 |
| 3.4 | The extended Species class | 9 |
| 3.5 | The FBC GeneProduct class | 10 |
| 3.6 | The FBC Objective class | 11 |
| 3.7 | The FBC FluxObjective class | 12 |
| 3.8 | The extended Reaction class | 13 |
| 3.9 | The FBC GeneProductAssociation class | 14 |
| 3.10 | The FBC Association class | 16 |
| 3.11 | The FBC GeneProductRef class | 16 |
| 3.12 | The FBC And class | 17 |
| 3.13 | The FBC Or class | 17 |
| 4 | Proposal for FBC version 3: HARMONY 2020 | 18 |
| 4.1 | The extended Model class (updates specification Section 3.3) | 18 |
| 4.1.1 | Type FbcVariableType | 18 |
| 4.1.2 | The FBC listOfUserConstraints | 18 |
| 4.2 | The extended Species class (updates specification Section 3.4) | 20 |
| 4.3 | The FBC FluxObjective class | 21 |
| 4.4 | The FBC UserConstraint class | 22 |
| 4.5 | The FBC UserConstraintComponent class | 23 |
| 4.6 | The FBC ListOfKeyValuePairs class | 24 |
| 4.7 | The FBC KeyValuePair class | 25 |
| 5 | Illustrative examples of the FBC syntax | 27 |
| 5.1 | Example one: the basic FBC syntax | 27 |
| 5.1.1 | Kinetic model description | 27 |
| 5.1.2 | Flux Bounds | 28 |
| 5.1.3 | Objective function | 28 |
| 5.1.4 | Complete worked example | 29 |
| 6 | Best practices | 32 |
| 6.1 | Examples contrasting the current SBML L2 encoding with L3 and FBC | 32 |
| 6.2 | An example of a strict FBC model (XML) | 35 |
| A | Validation of SBML documents | 37 |
| A.1 | Validation and consistency rules | 37 |
| B | The Systems Biology Ontology and the sboTerm attribute | 45 |
| | Acknowledgments | 46 |
| | References | 47 |

1 Introduction and motivation

Constraint-based modeling is a widely accepted methodology used to analyze and study biological networks on both a small and whole organism (genome) scale. Due to their large size these models are generally underdetermined and constraint-based optimization methods (such as linear or mixed integer convex optimization) are used to analyze them. Optimization is assumed to occur within a defined set of constraints (e.g. stoichiometric, metabolic) and bounds (e.g. thermodynamic, experimental and environmental) on the values that the solution fluxes can obtain.

Perhaps the most well known (and widely used) analysis method is Flux Balance Analysis (FBA) which is performed on Genome Scale Metabolic Reconstructions (GSR's; [Oberhardt et al. 2009](#)). Using FBA a target flux is optimized (e.g. maximizing a flux to biomass or minimizing ATP production) while other fluxes can be bounded to simulate a selected growth environment or specific metabolic state.

As constraint-based models are generally underdetermined and few or none of the kinetic rate equations, flux capacity constraints and related parameters are known it is crucial that a model definition includes the ability to define optimization parameters such as objective functions, flux bounds and constraints. Currently this is not possible in the Systems Biology Markup Language (SBML) Level 2 or Level 3 core specification ([Hucka et al., 2011, 2003](#)).

The question of how to encode constraint-based (also referred to as steady state or FBA) models in SBML is not new. However, advances in the methods used to construct genome scale constraint-based models and the wider adoption of constraint-based modeling in biotechnological/medical applications have led to a rapid increase in both the number of models being constructed and the tools used to analyze them.

Faced with such growth, both in number and diversity, the need for a standardized data format for the definition, exchange and annotation of constraint-based models has become critical. As the core model components (e.g. species, reactions, stoichiometry) can already be efficiently described in SBML (with its associated active community, software and tool support) the Flux Balance Constraints package aims to extend SBML Level 3 core by adding the elements necessary to encode current and future constraint-based models.

1.1 Proposal corresponding to this package specification

This specification for Flux Balance Constraints in SBML Level 3 Version 1 is based on the proposal, by this documents authors, located at the following URL:

[http://sbml.org/Community/Wiki/SBML_Level_3_Proposals/Flux_Balance_Constraints_Proposal_\(2012\)](http://sbml.org/Community/Wiki/SBML_Level_3_Proposals/Flux_Balance_Constraints_Proposal_(2012))

The tracking number in the SBML issue tracking system ([SBML Team, 2010](#)) for Flux Balance Constraints package activities is 3154219. The version of the proposal used as the starting point for this specification is the version of March 2012. Previous versions of the current proposal are:

Version 3 (March 2012)

[http://sbml.org/Community/Wiki/SBML_Level_3_Proposals/Flux_Balance_Constraints_Proposal_\(2012\)](http://sbml.org/Community/Wiki/SBML_Level_3_Proposals/Flux_Balance_Constraints_Proposal_(2012))

Version 2 (March 2011)

http://sbml.org/Community/Wiki/SBML_Level_3_Proposals/Flux_Constraints_Proposal

Version 1 (February 2010)

<http://precedings.nature.com/documents/4236/version/1>

Details of related, earlier, independent proposals are provided in [Section 2](#).

1.2 Tracking number

As initially listed in the SBML issue tracking system under:

http://sourceforge.net/tracker/?func=detail&aid=3154219&group_id=71971&atid=894711.

1.3 Package dependencies

The Flux Balance Constraints package adds additional attributes and classes to SBML Level 3 Version 1 Core and has no dependency on any other SBML Level 3 package.

1.4 Document conventions

Following the precedent set by the SBML Level 3 Core specification document, we use UML 1.0 (Unified Modeling Language; Eriksson and Penker 1998; Oestereich 1999) class diagram notation to define the constructs provided by this package. We also use color in the diagrams to carry additional information for the benefit of those viewing the document on media that can display color. The following are the colors we use and what they represent:

- **Black:** Items colored black in the UML diagrams are components taken unchanged from their definition in the SBML Level 3 Core specification document.
- **Green:** Items colored green are components that exist in SBML Level 3 Core, but are extended by this package. Class boxes are also drawn with dashed lines to further distinguish them.
- **Blue:** Items colored blue are new components introduced in this package specification. They have no equivalent in the SBML Level 3 Core specification.

We also use the following typographical conventions to distinguish the names of objects and data types from other entities; these conventions are identical to the conventions used in the SBML Level 3 Core specification document:

AbstractClass: Abstract classes are classes that are never instantiated directly, but rather serve as parents of other object classes. Their names begin with a capital letter and they are printed in a slanted, bold, sans-serif typeface. In electronic document formats, the class names defined within this document are also hyperlinked to their definitions; clicking on these items will, given appropriate software, switch the view to the section in this document containing the definition of that class. (However, for classes that are unchanged from their definitions in SBML Level 3 Core, the class names are not hyperlinked because they are not defined within this document.)

Class: Names of ordinary (concrete) classes begin with a capital letter and are printed in an upright, bold, sans-serif typeface. In electronic document formats, the class names are also hyperlinked to their definitions in this specification document. (However, as in the previous case, class names are not hyperlinked if they are for classes that are unchanged from their definitions in the SBML Level 3 Core specification.)

Something, otherThing: Attributes of classes, data type names, literal XML, and generally all tokens *other* than SBML UML class names, are printed in an upright typewriter typeface. Primitive types defined by SBML begin with a capital letter; SBML also makes use of primitive types defined by XML Schema 1.0 (Biron and Malhotra, 2000; Fallside, 2000; Thompson et al., 2000), but unfortunately, XML Schema does not follow any capitalization convention and primitive types drawn from the XML Schema language may or may not start with a capital letter.

For other matters involving the use of UML and XML, we follow the conventions used in the SBML Level 3 Core specification document.

2 Background

2.1 Problems with current SBML approaches

While there is currently no official way of encoding constraint-based models in SBML L2 there have been pragmatic approaches used by a variety of groups and applications. Arguably the most comprehensive and widely used format is that used by the COBRA toolbox (Becker et al., 2007) where the metabolic reaction network is well defined using SBML's **Reaction** and **Species** classes. However, other FBA specific model components such as flux bounds and the reactions that take part in the objective function are less well defined. For example, in this case **LocalParameter** elements are used which (implicitly) rely on all tools knowing and using the same naming convention for the parameter **id**'s. Furthermore, reaction annotations are generally stored as tool specific HTML key-value pairs in a **Notes** element which has routinely led to different research groups and software using in-house and/or tool specific ways to describe the same information. An example of such an annotation is the widely used 'gene protein association'. While a step in the right direction, this encoding is not suitable for direct translation into SBML Level 3.

It is perhaps worth noting that, while SBML Level 2 does have a construct known as **Constraint**, its function is traditionally limited to measuring and reporting a model **variable's** behavior in time. In contrast, the Flux Balance Constraints package considers a model at steady state and therefore time invariant. Instead it makes use of **Parameter** elements to define the allowable range that a steady-state flux may attain. Therefore 'flux bounds' and **Constraint** elements should be considered complementary to one another. Furthermore, certain attributes that were widely used by the constraint-based modeling community such as the **Species** attribute **charge** were removed in later versions of SBML. This has had the effect that a significant number of constraint-based modelling and metabolic flux analysis software still make use of SBML Level 2 Version 1.

2.2 Past work on this problem or similar topics

The problem of describing and annotating FBA models in SBML has been raised at various times in the past few years. In this regard there are two known putative proposals one by Karthik Raman and the other by the Church Laboratory. As far as we are aware these proposals never developed beyond their initial presentation at SBML forums/hackathons. In 2009 the discussion was reopened at the SBML Forum held in Stanford, an initiative which has subsequently developed into the current active package proposal and this document. In reverse chronological order these are:

Brett Olivier (2009) SBML Level 3 FBA package discussion

http://sbml.org/images/4/4a/Olivier_sbml_forum_2009_09_04.pdf

Karthik Raman (2005) Flux annotations in SBML

<http://sbml.org/images/d/d9/Raman-flux-annotations.pdf>

Church laboratory (pre 2005) Metabolic flux model annotations

http://sbml.org/Community/Wiki/Old_known_SBML_annotations_list

3 Proposed syntax and semantics

In this section, we define the syntax and semantics of the Flux Balance Constraints package for SBML Level 3 Version 1. We expound on the various data types and constructs defined in this package, then in Section 5 on page 27, we provide complete examples of using the constructs in an example SBML model.

3.1 Namespace URI and other declarations necessary for using this package

Every SBML Level 3 package is identified uniquely by an XML namespace URI. For an SBML document to be able to use a given SBML Level 3 package, it must declare the use of that package by referencing its URI. The following is the namespace URI for this version of the Flux Balance Constraints package for SBML Level 3 Version 1:

```
"http://www.sbml.org/sbml/level3/version1/fbc/version2"
```

In addition, SBML documents using a given package must indicate whether understanding the package is required for complete mathematical interpretation of a model, or whether the package is optional. This is done using the attribute **required** on the `<sbml>` element in the SBML document. For the Flux Balance Constraints package, the value of this attribute must be set to **"false"**.

The following fragment illustrates the beginning of a typical SBML model using SBML Level 3 Version 1 and this version of the Flux Balance Constraints package:

```
<?xml version="1.0" encoding="UTF-8"?>
<sbml xmlns="http://www.sbml.org/sbml/level3/version1/core" level="3" version="1"
  xmlns:fbc="http://www.sbml.org/sbml/level3/version1/fbc/version2" fbc:required="false">
```

3.2 Primitive data types

Section 3.1 of the SBML Level 3 Version 1 Core specification defines a number of primitive data types and also uses a number of XML Schema 1.0 data types (Biron and Malhotra, 2000). More specifically we make use of **integer**, **double**, **string**, **SId** and **SIdRef**. In addition we make use of a new primitive: the enumeration **FbcType**, see Figure 1 for the interrelation between these entities.

The **SId** type is used as the data type for the identifiers of **GeneProduct** (Section 3.5), **GeneProductRef** (Section 3.11), **GeneProductAssociation** (Section 3.9), **FluxObjective** (Section 3.7) and **Objective** (Section 3.6) classes. In all cases where the primitive data type **SId** is used in the Flux Balance Constraints package it is used unchanged from its description in SBML Level 3 Version 1 Core. When used as the type of a **fbc:id** attribute, that ID is added to the core **SId** namespace, and must continue to follow those rules for uniqueness: no **fbc:id** may duplicate any other **fbc:id**, nor the **id** of any **Model**, **FunctionDefinition**, **Compartment**, **Species**, **Reaction**, **SpeciesReference**, **ModifierSpeciesReference**, **Event**, or **Parameter**, nor the **package:id** of any other SBML Level 3 package element that is also defined as being in the **SId** namespace.

In the Flux Balance Constraints package the **ListOfObjectives** has an attribute of type **SIdRef** that is used to refer to an 'active' **Objective** as does the extended **Reaction** class which defines two attributes referring to flux capacity constraints. Finally, the **GeneProductRef** class declares an attribute of type **SIdRef** which references a **GeneProduct** which itself contains an attribute that can refer to a **Species**.

3.2.1 Type FbcType

The Flux Balance Constraints package defines a new enumerated type **FbcType** which represents the optimization sense of the objective function. It can have one of the following two values **"maximize"** or **"minimize"**.



Figure 1: A UML representation of the Flux Balance Constraints package. Derived from **SBBase**, the FBC classes inherit support for constructs such as SBML **Notes** and **Annotation**'s. The [association] element name is the name of the class, de-capitalized. In this case, the possible values are "and", "or", or "geneProductRef". See [Section 1.4](#) for conventions related to this figure. The individual classes are further discussed in the text.

3.3 The extended **Model** class

The **SBML Model** class is extended by adding a mandatory boolean attribute **strict** as well as optional lists: **listOfObjectives** and **listOfGeneProducts**. A **Model** may contain at most one of each of these lists.



Figure 2: A UML representation of the extended **SBML Model** class used in the Flux Balance Constraints package. See [Section 1.4](#) for conventions related to this figure.

The attribute **strict**

The mandatory attribute **strict**, of type **boolean**, is used to apply an additional set of restrictions to the model. The **strict** attribute ensures that the Flux Balance Constraints package can be used to encode legacy FBA models expressible as Linear Programs (LP's) with software that is unable to analyze arbitrary mathematical expressions. In addition it ensures that a 'strict' model is fully described and mathematically consistent, for example, by ensuring that all fluxes have a valid upper or lower bound.

This is accomplished by defining a set of restrictions which come into effect if **strict** is set to "true":

- Each **Reaction** in a **Model** must define attributes **lowerFluxBound** and **upperFluxBound** with each pointing to a valid **Parameter** object defined in the current **Model**.
- Each **Parameter** object referred to by the **Reaction** attributes **lowerFluxBound** and **upperFluxBound** must have their **constant** attribute set to "true" and its **value** attribute set to a **double** value which may not be "NaN".
- **SpeciesReference** elements of **Reactions** must have their **stoichiometry** attribute set to a **double** value that is neither "NaN" nor "-INF" nor "INF". In addition their **constant** attribute must be set to "true".
- **InitialAssignment** elements may neither target the **Parameter** elements referenced by the **Reaction** attributes **lowerFluxBound** and **upperFluxBound** nor any **SpeciesReference**.
- All defined **FluxObjective** elements must have their **coefficient** attribute set to a **double** value that is neither "NaN" nor "-INF" nor "INF".
- A **Reaction** **lowerFluxBound** attribute may not point to a **Parameter** with a value of "INF".
- A **Reaction** **upperFluxBound** attribute may not point to a **Parameter** with a value of "-INF".
- For all reactions, the value of a **lowerFluxBound** must be less than or equal to the value of the **upperFluxBound**.

While it is not compulsory for a 'strict' FBC model to define an **Objective**, doing so does allow it to be formulated as an LP and optimized, however, this decision is left to the modeler. Note that all other properties of the elements referred to in this list are as specified in the relevant SBML Level 3 Version 1 Core and FBC specifications.

Alternatively, if the value of the **strict** attribute is "false" then none of these restrictions apply and the model creator can choose to define FBC models that are not necessarily encodable as a LP. For example, if **strict** is "false" the **InitialAssignment** construct may be used to set any valid numerical entity, including **Parameter** values and stoichiometric coefficients, with any **double**. In addition, **Parameter** elements are no longer required to be flagged as 'constant' thus allowing for an FBC model's use in alternative, hybrid modeling strategies.

3.3.1 The FBC listOfObjectives

As shown in [Figure 1](#) the **ListOfObjectives** is derived from **SBase** and inherits the attributes **metaid** and **sboTerm**, as well as the subcomponents for **Annotation** and **Notes**. Unlike most other **SBML ListOf__** classes, **ListOfObjectives** introduces an additional required attribute **activeObjective**. The **ListOfObjectives** must contain at least one **Objective** (defined in [Section 3.6](#)).

The activeObjective attribute

This attribute is of type **SIdRef** and can only refer to the **id** of an existing **Objective**. This required attribute exists so that when multiple **Objective**'s are included in a single model, the model will always be well described i.e., there is a single, primary objective function which defines a single optimum and its associated solution space.

3.3.2 The FBC listOfGeneProducts

As shown in Figure 1 the **ListOfGeneProducts** is derived from **SBase** and inherits the attributes **metaid** and **sboTerm**, as well as the subcomponents for **Annotation** and **Notes**. The **ListOfGeneProducts** must contain at least one **GeneProduct** (defined in Section 3.5).

3.3.3 A note on units

The main unit definitions that should be considered when using the Flux Balance Constraints package are the global model definitions of "extent" and "time" as all FBC flux related classes (i.e., **FluxObjective** implicitly attain the same unit as the **Reaction** that they reference). More details on units can be found in their respective class definitions.

3.4 The extended Species class

The Flux Balance Constraints package extends the SBML Level 3 Version 1 Core **Species** class with the addition of two attributes **charge** and **chemicalFormula**.

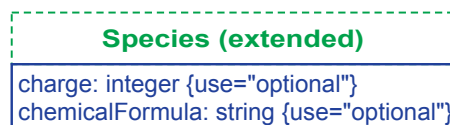


Figure 3: A UML representation of the extended **SBML Species** class used in the Flux Balance Constraints package. See Section 1.4 for conventions related to this figure.

The charge attribute

The optional attribute **charge** which contains a signed **integer** referring to the **Species** object's charge and is defined as it was in the **SBML** Level 2 Version 1 specification: "The optional field charge takes an integer indicating the charge on the species (in terms of electrons, not the SI unit coulombs)."

The chemicalFormula attribute

The optional attribute **chemicalFormula** containing a **string** that represents the **Species** objects elemental composition.

```

<species metaid="meta_M_atp_c" id="M_atp_c" name="ATP" compartment="Cytosol"
  boundaryCondition="false" initialConcentration="0" hasOnlySubstanceUnits="false"
  fbc:charge="-4" fbc:chemicalFormula="C10H12N5O13P3"/>
  
```

While there are many ways of referring to an elemental composition the purpose of the **chemicalFormula** attribute is to allow reaction balancing and validation which is particularly important in constraint-based models.

The format of **chemicalFormula** must consist only of atomic names (as in the Periodic Table) or user defined compounds either of which take the form of a single capital letter followed by zero or more lowercase letters. Where there is more than a single atom present, this is indicated with an integer. With regards to order (and enhance inter-operability) it is recommended to use the Hill system order (Hill, 1900, 2012).

Using this notation the number of carbon atoms in a molecule is indicated first, followed by the number of hydro-

H2O4S C2H5Br BrH
C10H12N5O13P3 CH3I

Table 1: Examples of chemical formulas written using the Hill System. As described in [Section 4.2](#)

gen atoms and then the number of all other chemical elements in alphabetical order. When the formula contains no carbon; all elements, including hydrogen, are listed alphabetically.

3.5 The FBC **GeneProduct** class

GeneProduct is a new FBC class derived from **SBML SBase** that inherits **metaid** and **sboTerm**, as well as the sub-components for **Annotation** and **Notes**. The purpose of this class is to define a single gene product. It implements two required attributes **id** and **label** as well as two optional attributes **name** and **associatedSpecies**.

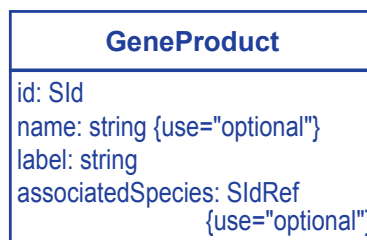


Figure 4: A UML representation of the Flux Balance Constraints package **GeneProduct** class. For a complete description see [Figure 1](#) as well as [Section 1.4](#) for conventions related to this figure.

The **id** and **name** attributes

A **GeneProduct** has a required attribute **id** of type **SId** and an optional attribute **name** of type **string**. The unique **id** attribute is required to enable a **GeneProduct** to be referenced from a **GeneProductRef** used in a **GeneProductAssociation**.

The **label** attribute

The primary purpose of a **GeneProduct** is to uniquely reference a gene or implied gene product. As there is, currently, no restriction on the format of these references they cannot be assumed to conform to an **SBML SId** syntax. Therefore the Flux Balance Constraints package defines the required attribute **label**, of type **string**, for this purpose.

While ideally some form of restriction could, in principle, be placed on the value of **label**, at this point in time it is only possible to suggest that this attribute's value should conform to the definition of an **SId**. For example, consider an existing GPR annotation, as encountered in legacy **SBML** Level 2 encoded models:

```
<p>GENE_ASSOCIATION: (Rv0649)</p>
```

this can now be formally (and unambiguously) encoded as:

```

<fbc:geneProduct metaid="meta_gene_1" fbc:id="gene1" fbc:label="Rv0649"
fbc:associatedSpecies="s_Rv0649">
<annotation>
<rdf:RDF xmlns:rdf="http://www.w3.org/1999/02/22-rdf-syntax-ns#"
xmlns:dc="http://purl.org/dc/elements/1.1/" xmlns:dcterms="http://purl.org/dc/terms/"
xmlns:vCard="http://www.w3.org/2001/vcard-rdf/3.0#"
xmlns:bqbiol="http://biomodels.net/biology-qualifiers/"
xmlns:bqmodel="http://biomodels.net/model-qualifiers/">
<rdf:Description rdf:about="#meta_gene_1">
  
```

```

<bqbiol:is>
  <rdf:Bag>
    <rdf:li rdf:resource="http://identifiers.org/kegg.genes/mtu:Rv0649"/>
  </rdf:Bag>
</bqbiol:is>
</rdf:Description>
</rdf:RDF>
</annotation>
</fbc:geneProduct>

<species id="s_Rv0649" compartment="Cytosol" hasOnlySubstanceUnits="false"
  boundaryCondition="true" constant="true"/>

```

Furthermore, it is a highly recommended ‘best practice’ that a **GeneProduct** be annotated using the inherited MIRIAM compliant **SBML Annotation** mechanism. Doing so will help reduce the dependence and ambiguity of using an overloaded, semantically meaningful **label** attribute and enhance interoperability. For an example of this approach see [Section 6.1](#).

The associatedSpecies attribute

A **GeneProduct** may, optionally, refer to a **Species** so as to provide compatibility with the Manchester style encoding of gene-protein associations. In this case the attribute **associatedSpecies** is of type **SIdRef** and, if defined, must point to an existing **Species** in the model.

3.6 The FBC Objective class

The FBC **Objective** class is derived from **SBML SBBase** and inherits **metaid** and **sboTerm**, as well as the subcomponents for **Annotation** and **Notes**. An integral component in a complete description of a steady-state model is the so-called ‘objective function’ which generally consist of a linear combination of model variables (fluxes) and a sense (direction). In the FBC package this concept is succinctly captured in the **Objective** class.

The id and name attributes

An **Objective** has a required attribute **id** of type **SId** and an optional attribute **name** of type **string**.

The type attribute

The required **type** attribute contains an **FbcType** type which represents the sense of the optimality constraint and can take one of two values:

$maximize \mapsto \text{“maximize”}$
 $minimize \mapsto \text{“minimize”}$

The listOfFluxObjectives element

The element **listOfFluxObjectives** which contains a **ListOfFluxObjectives** is derived from and functions like a typical **SBML ListOf__** class with the restriction that it must contain one or more elements of type **FluxObjective** (see [Section 3.7](#)). This implies that if an **Objective** is defined there should be at least one **FluxObjective** contained in a **ListOfFluxObjectives**.



Figure 5: A UML representation of the Flux Balance Constraints package **Objective** class. For a complete description see [Figure 1](#) as well as [Section 1.4](#) for conventions related to this figure.

Encoding the **Objective**

The Flux Balance Constraints package allows for the definition of multiple model objectives with one being designated as active (see [Section 3.6](#)) as illustrated in this example:

```
<fb:listOfObjectives fb:activeObjective="obj1">
  <fb:objective fb:id="obj1" fb:type="maximize">
    <fb:listOfFluxObjectives>
      <fb:fluxObjective fb:reaction="R101" fb:coefficient="1"/>
    </fb:listOfFluxObjectives>
  </fb:objective>
  <fb:objective fb:id="obj2" fb:type="minimize">
    <fb:listOfFluxObjectives>
      <fb:fluxObjective fb:reaction="R102" fb:coefficient="-2.5"/>
      <fb:fluxObjective fb:reaction="R103" fb:coefficient="1"/>
    </fb:listOfFluxObjectives>
  </fb:objective>
</fb:listOfObjectives>
```

Note how both **Objective** instances differ in **type** and each contains different set of **FluxObjectives** (see [Section 3.7](#)). For an example of how the **Objective** relates to the description of the underlying mathematical model please see [Section 5.1.3](#).

3.7 The FBC **FluxObjective** class

The FBC **FluxObjective** class is derived from **SBML SBBase** and inherits **metaid** and **sboTerm**, as well as the sub-components for **Annotation** and **Notes**.

The **FluxObjective** class is a relatively simple container for a model variable weighted by a signed linear coefficient.

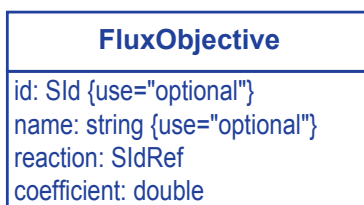


Figure 6: A UML representation of the Flux Balance Constraints package **FluxObjective** class. For a complete description see [Figure 1](#) as well as [Section 1.4](#) for conventions related to this figure.

The **id** and **name** attributes

A **FluxObjective** has two optional attributes: **id** an attribute of type **SId** and **name** an attribute of type **string**.

The reaction and coefficient attributes

The required **reaction** is of type **SIdRef** and is restricted to refer only to a **Reaction** while the **coefficient** attribute holds a **double** referring to the coefficient that this **FluxObjective** takes in the enclosing **Objective**. For example the objective **Maximize: 1 R1 + 2 R2** would be encoded as

```
<fbc:listOfObjectives fbc:activeObjective="obj1">
  <fbc:objective fbc:id="obj1" fbc:type="maximize">
    <fbc:listOfFluxObjectives>
      <fbc:fluxObjective fbc:reaction="R1" fbc:coefficient="1"/>
      <fbc:fluxObjective fbc:reaction="R2" fbc:coefficient="2"/>
    </fbc:listOfFluxObjectives>
  </fbc:objective>
</fbc:listOfObjectives>
```

Units

As described above the **FluxObjective** defined here as $n \cdot J$ where the **coefficient** (n) is dimensionless and the **value** (J) takes the units of the **reaction** flux i.e., “extent per time”. Therefore, the **FluxObjective** ($n \cdot J$) has the unit “extent per time” where the units of reaction “extent” and “time” are defined globally.

3.8 The extended Reaction class

The Flux Balance Constraints package extends the SBML Level 3 Version 1 Core **Reaction** class with the addition of a new optional element **GeneProductAssociation** as well as two optional attributes **lowerFluxBound** and **upperFluxBound**.

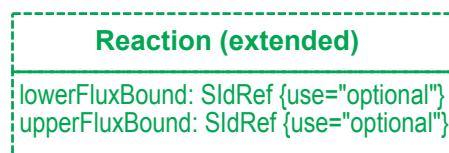


Figure 7: A UML representation of the extended **SBML Reaction** class used in the Flux Balance Constraints package. For a complete description see [Figure 1](#) as well as [Section 1.4](#) for conventions related to this figure.

The attributes **lowerFluxBound** and **upperFluxBound**

The optional attributes **lowerFluxBound** and **upperFluxBound** of type **SIdRef** are used to specify the lower and upper flux bounds for a **Reaction** flux.

The attributes must refer to an existing **Parameter** in the model and in the case that equal bounds are required for a reaction, both attributes should point to the same **Parameter**. The Flux Balance Constraints package specifies Systems Biology Ontology (SBO) terms that can be used to define the character of such a **Parameter**, please see the appendix describing SBO for more details.

Using a **Parameter** in this way makes it possible for other SBML elements to interact with these parameters depending on the value of the **fbc:strict** attribute of the **Model** (see also [Section 3.3](#) on page 7).

For example, if the value of the **strict** attribute is “**false**”, even in the case of a constant **Parameter** (i.e. SBML parameters that have their **constant** attribute set to “**true**”) the value of a flux bound’s value could be calculated using an **InitialAssignment**. Should the parameter not be constant, then its value can be additionally updated by all SBML Level 3 Version 1 Core constructs (for example **EventAssignment**, **AssignmentRule** and **AlgebraicRule**). However none of the aforementioned applies when the **strict** attribute is set to “**true**”.

Encoding the flux bounds

To generate a list of (in)equalities for each reaction, out of the references in **upperFluxBound** and **lowerFluxBounds**, one first resolves the reference to the underlying **Parameter**. If they both point to the same element, the equality will be of the form of Equation 1. Alternatively two inequalities are derived, as shown in Equations 2 and 3:

$$\text{reaction} = \text{value} \quad (1)$$

$$\text{reaction} \geq \text{lowerFluxBound value} \quad (2)$$

$$\text{reaction} \leq \text{upperFluxBound value} \quad (3)$$

In SBML Level 3 Version 1 with FBC Version 2 this is encoded as:

```
<listOfParameters>
  <parameter constant="true" id="R1b" value="1.2"/>
  <parameter constant="true" id="R2b" value="-1.2"/>
  <parameter constant="true" id="negInf" value="-INF"/>
  <parameter constant="true" id="posInf" value="INF"/>
  <parameter constant="true" id="R5b" value="1"/>
</listOfParameters>

<listOfReactions>
  <reaction id="R1" fbc:lowerFluxBound="negInf" fbc:upperFluxBound="R1b" ... />
  <reaction id="R2" fbc:lowerFluxBound="R2b" fbc:upperFluxBound="posInf" ... />
  <reaction id="R3" fbc:lowerFluxBound="negInf" fbc:upperFluxBound="posInf" ... />
  <reaction id="R4" fbc:lowerFluxBound="negInf" fbc:upperFluxBound="posInf" ... />
  <reaction id="R5" fbc:lowerFluxBound="R5b" fbc:upperFluxBound="R5b" ... />
</listOfReactions>
```

3.9 The FBC **GeneProductAssociation** class

The Flux Balance Constraints package defines a **GeneProductAssociation** class that derives from **SBase** and inherits the attributes **metaid** and **sboTerm** as well as the subcomponents for **Annotation** and **Notes**. As shown in Figure 1 the **GeneProductAssociation** class extends **Reaction** with one or more genes (or gene products). Where more than one gene (or gene product) is present in an association they are written as logical expressions and thereby related to one another using logical ‘and’ and ‘or’ operators.

The id and name attributes

A **GeneProductAssociation** has two optional attributes: **id** an attribute of type **SId** and **name** an attribute of type **string**.

The association element

Each **GeneProductAssociation** contains a single **Association**, however, as described in Section 3.10 an **Association** is an abstract class that implies that an **association** will always contain an instance of one of its sub-classes: **And**, **Or** or **GeneProductRef**.

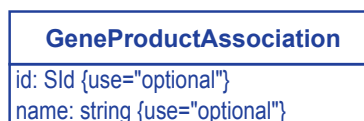


Figure 8: A UML representation of the Flux Balance Constraints package **GeneProductAssociation** class. For a complete description see Figure 1 as well as Section 1.4 for conventions related to this figure.

Encoding the *GeneProductAssociation*

As described in [Section 3.9](#), the **GeneProductAssociation** is simply a container that contains one of three types of **Association** either holding a single **GeneProductRef** or two or more **Association** elements in an **And** or **Or** relationship. For example, the following typical gene–protein association expression from the BiGG database *E. coli* reconstruction (iJR904; [Reed et al. 2003](#); [Schellenberger et al. 2010](#))

((B3670 and B3671) or (B0077 and B0078) or (B3768 and B3769 and B3767))

can now be encoded as:

```
<fbc:listOfGeneProducts>
  <fbc:geneProduct fbc:id="g_b3670" label="b3670" />
  <fbc:geneProduct fbc:id="g_b3671" label="b3671" />
  <fbc:geneProduct fbc:id="g_b0077" label="b0077" />
  <fbc:geneProduct fbc:id="g_b0078" label="b0078" />
  <fbc:geneProduct fbc:id="g_b3768" label="b3768" />
  <fbc:geneProduct fbc:id="g_b3769" label="b3769" />
  <fbc:geneProduct fbc:id="g_b3767" label="b3767" />
</fbc:listOfGeneProducts>

<reaction id = "R_ACHBS" ... >
  <fbc:geneProductAssociation fbc:id="ga_29">
    <fbc:or>
      <fbc:and>
        <fbc:geneProductRef fbc:geneProduct="g_b3670"/>
        <fbc:geneProductRef fbc:geneProduct="g_b3671"/>
      </fbc:and>
      <fbc:and>
        <fbc:geneProductRef fbc:geneProduct="g_b0077"/>
        <fbc:geneProductRef fbc:geneProduct="g_b0078"/>
      </fbc:and>
      <fbc:and>
        <fbc:geneProductRef fbc:geneProduct="g_b3768"/>
        <fbc:geneProductRef fbc:geneProduct="g_b3769"/>
        <fbc:geneProductRef fbc:geneProduct="g_b3767"/>
      </fbc:and>
    </fbc:or>
  </fbc:geneProductAssociation>
</reaction>
```

3.10 The FBC **Association** class

The Flux Balance Constraints package defines an abstract **Association** class that is derived from **SBase** and inherits the attributes **metaid** and **sboTerm**, as well as the subcomponents for **Annotation** and **Notes**. It represents either a single gene, or a collection of genes in a logical expression and is only ever instantiated as one of its subclasses: **GeneProductRef** (Section 3.11), **And** (Section 3.12) and **Or** (Section 3.13).

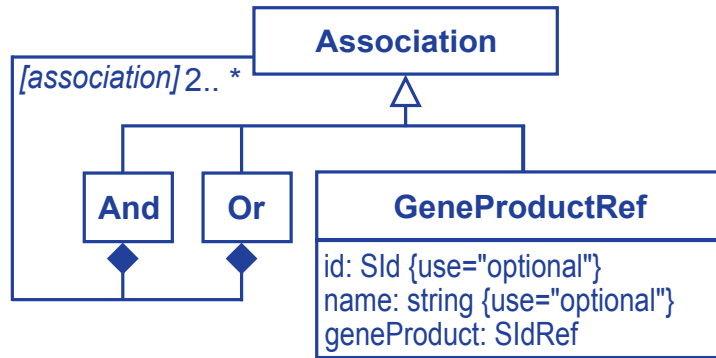


Figure 9: A UML representation of the Flux Balance Constraints package **Association** and derived classes. The *[association]* element name is the name of the class, de-capitalized. In this case, the possible values are "and", "or", or "geneProductRef". For a complete description see Figure 1 as well as Section 1.4 for conventions related to this figure.

3.11 The FBC **GeneProductRef** class

The Flux Balance Constraints package defines a **GeneProductRef** class that references a **GeneProduct** declared in **ListOfGeneProducts**, a child of **Model** (see Section 3.5). It is derived from an **Association** and thereby inherits the **SBase** attributes **metaid** and **sboTerm**, as well as the subcomponents for **Annotation** and **Notes** as described in Figure 9.

The **id** and **name** attributes

A **GeneProductRef** has two optional attributes: **id** an attribute of type **SId** and **name** an attribute of type **string**.

The **geneProduct** attribute

The required **geneProduct** attribute of type **SIdRef** references a **GeneProduct** element declared in the **ListOfGeneProducts**.

3.12 The FBC **And** class

The Flux Balance Constraints package defines an **And** class that is derived from an **Association** and thereby inherits the **SBase** attributes **metaid** and **sboTerm**, as well as the subcomponents for **Annotation** and **Notes** as described in Figure 9. This class represents a set of two or more associations that are related in an order independent ‘*and*’ relationship.

The **elements** *element*

Each **And** must contain two or more instances (not necessarily of the same type) of any **Association** subclass (**And**, **Or**, **GeneProductRef**).

```
<reaction id = "R_ACACCT" ... >
  <fbc:geneProductAssociation fbc:id="ga_18">
    <fbc:and>
      <fbc:geneProductRef fbc:geneProduct="g_b3670"/>
      <fbc:geneProductRef fbc:geneProduct="g_b3671"/>
    </fbc:and>
  </fbc:geneProductAssociation>
</reaction>
```

3.13 The FBC **Or** class

The Flux Balance Constraints package defines an **Or** class that represents a gene (or gene product) and is derived from an **Association** and thereby inherits the **SBase** attributes **metaid** and **sboTerm**, as well as the subcomponents for **Annotation** and **Notes** as described in Figure 9. This class represents a set of two or more **Association** elements related in an order independent ‘*or*’ relationship.

The **elements** *element*

Each **Or** must contain two or more instances (not necessarily of the same type) of any **Association** subclass (**And**, **Or**, **GeneProductRef**).

```
<reaction id = "R_ABTA" ... >
  <fbc:geneProductAssociation fbc:id="ga_16">
    <fbc:or>
      <fbc:geneProductRef fbc:geneProduct="g_b2662"/>
      <fbc:geneProductRef fbc:geneProduct="g_b1302"/>
    </fbc:or>
  </fbc:geneProductAssociation>
</reaction>
```

4 Proposal for FBC version 3: HARMONY 2020

The proposed Flux Balance Constraints package version 3 extends the definition of the [FluxObjective](#), extends the definition of the `chemicalFormula`, redefines `charge` as a double, defines a [UserConstraint](#) and adds a generic [KeyValuePair](#) annotation.

The extensions and modifications contained in this document are proposed as version 3 of the Flux Balance Constraints package. This proposal is been formulated through open discussions on the FBC PWG mailing list and intense discussion during HARMONY 2018 and HARMONY 2020.

At HARMONY 2020 it was decided that the name 'UserConstraint' should be changed to something else.

Please note that where specification text (or UML) has been modified and diverges from the Flux Balance Constraints package version 2 specification, it has been colored red.

4.1 The extended Model class (updates specification [Section 3.3](#))

The `SBML Model` class is extended by a `listOfUserConstraints` of which it may contain at most one, see [Figure 13](#).

4.1.1 Type `FbcVariableType`

The Flux Balance Constraints package defines a new enumerated type `FbcVariableType` which represents the index of a variable that occurs in either the [FluxObjective](#) or [UserConstraintComponent](#). It contains the following two values, "linear" or "quadratic".

4.1.2 The FBC `listOfUserConstraints`

As shown in [Figure 13](#) the [ListOfUserConstraints](#) is derived from `SBase` and inherits the attributes `metaid` and `sboTerm`, as well as the subcomponents for `Annotation` and `Notes`. The [ListOfUserConstraints](#) must contain at least one [UserConstraint](#) (defined in [Section 4.4](#)).

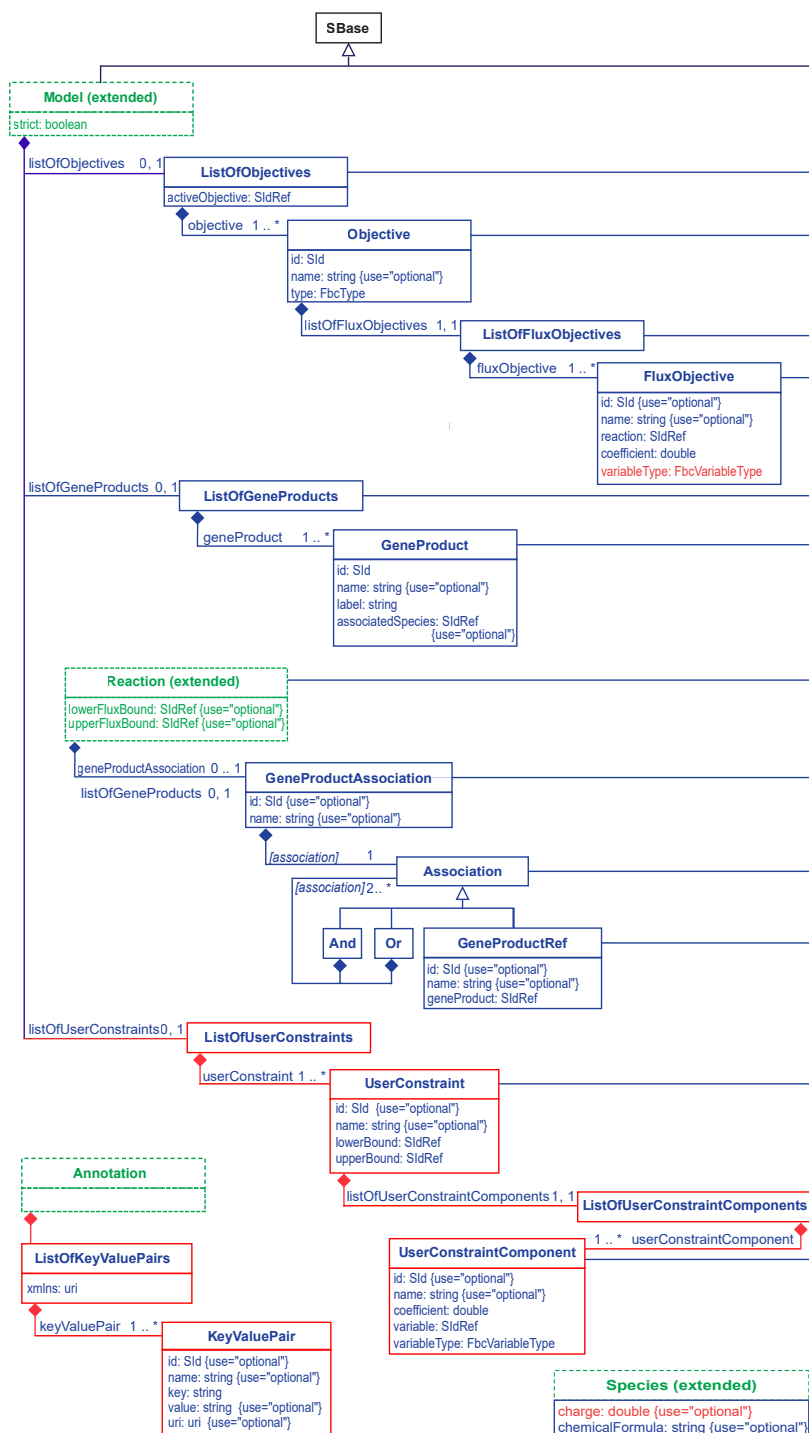


Figure 10: A UML representation of the Flux Balance Constraints package version three. Derived from **SBase**, most FBC classes inherit support for constructs such as SBML **Notes** and **Annotation**'s. The [association] element name is the name of the class, de-capitalized. In this case, the possible values are "and", "or", or "geneProductRef". See [Section 1.4](#) for conventions related to this figure. The individual classes are further discussed in the text.

4.2 The extended Species class (updates specification [Section 3.4](#))

The Flux Balance Constraints package extends the SBML Level 3 Version 1 Core **Species** class with the addition of two attributes **charge** and **chemicalFormula**.

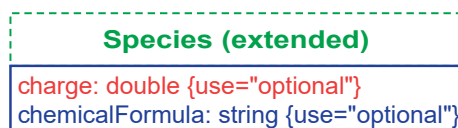


Figure 11: A UML representation of the extended **SBML Species** class used in the Flux Balance Constraints package. See [Section 1.4](#) for conventions related to this figure.

The charge attribute

The optional attribute **charge** contains a signed double referring to the **Species** object's charge (in terms of electrons, not the SI unit coulombs). Note, that unlike FBC versions one and two a **Species** may, for the purposes of charge, be interpreted as a pseudoisomer or aggregate molecule and may assume a non-integer value. Non-integer charges should be used with caution as their use may have unintended side-effects, for example, with respect to the accuracy of reaction balancing.

The chemicalFormula attribute

The optional attribute **chemicalFormula** containing a **string** that represents the **Species** objects elemental composition.

```

<species metaid="meta_M_atp_c" id="M_atp_c" name="ATP" compartment="Cytosol"
boundaryCondition="false" initialConcentration="0" hasOnlySubstanceUnits="false"
fbc:charge="-4" fbc:chemicalFormula="C10H12N5O13P3"/>

<species metaid="meta_M1" id="M1" compartment="C" boundaryCondition="false"
initialConcentration="0" hasOnlySubstanceUnits="false" fbc:charge="0"
fbc:chemicalFormula="RCONH2"/>

<species metaid="meta_M2" id="M2" compartment="c" boundaryCondition="false"
initialConcentration="0" hasOnlySubstanceUnits="false" fbc:charge="0"
fbc:chemicalFormula="C2H4O2(CH2)n"/>
  
```

While there are many ways of referring to an elemental composition, the purpose of the **chemicalFormula** attribute is to enable reaction balancing and validation, something of particular importance in constraint-based models.

The format of the **chemicalFormula** should, whenever possible, consist only of atomic names (as in the Periodic Table). Similarly, for enhanced inter-operability, the element order should be arranged according to the Hill system (see [Table 2](#)) ([Hill, 1900, 2012](#)). Using this notation the number of carbon atoms in a molecule is indicated first,

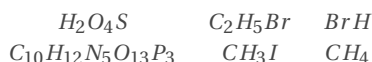


Table 2: Examples of chemical formulas written using the Hill System. As described in [Section 4.2](#)

followed by the number of hydrogen atoms and then the number of all other chemical elements in alphabetical order. When the formula contains no carbon; all elements, including hydrogen, are listed alphabetically. Where there is more than a single atom present, this is indicated with an integer that follows the element symbol.

However, in certain situations it does become necessary to use a generic symbol in a user defined compound. For example, such symbols can include R and X and have the general form of a single capital letter followed by zero or more lowercase letters. In addition, the undefined parenthesised group index $(\dots)_n$ may also be used. Note that



Table 3: Examples of chemical formulas written using allowed non-Hill symbols, as described in [Section 4.2](#).

in this case only the subscript n is allowed, integer values $(\dots)_2$ and expressions such as $(\dots)_{n-1}$ are considered invalid.

However, the use of R, X and $(\dots)_n$ is not advised, as any **Reaction** in which such a **Species** occurs cannot necessarily be balanced. Therefore, any **chemicalFormula** that contains any of the aforementioned, non-Hill compatible symbols will raise a ‘best practices’ warning on model validation.

4.3 The FBC **FluxObjective** class

The FBC **FluxObjective** class is derived from **SBML SBBase** and inherits **metaid** and **sboTerm**, as well as the subcomponents for **Annotation** and **Notes**. The **FluxObjective** class is a relatively simple container for a model variable that

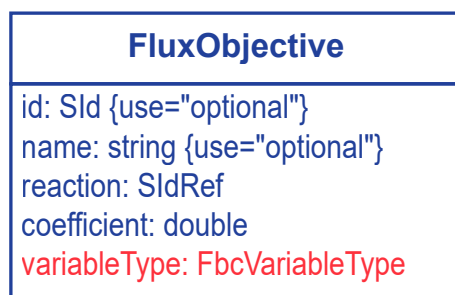


Figure 12: A UML representation of the Flux Balance Constraints package **FluxObjective** class. For a complete description see [Figure 1](#) as well as [Section 1.4](#) for conventions related to this figure.

can be expressed as a ‘linear’ or ‘quadratic’, weighted by a signed linear coefficient.

The **id** and **name** attributes

A **FluxObjective** has two optional attributes: **id** an attribute of type **SId** and **name** an attribute of type **string**.

The **reaction** and **coefficient** attributes

The required **reaction** is of type **SIdRef** and is restricted to refer only to a **Reaction** while the **coefficient** attribute holds a **double** referring to the coefficient that this **FluxObjective** takes in the enclosing **Objective**.

The **variableType** attribute

The required **variableType** attribute contains a **FbcVariableType** that represents the index to which a variable is raised in a **FluxObjective**. For example, where J_x represents a steady-state flux the **FbcVariableType** defines either a “linear”, J_x^1 or “quadratic”, J_x^2 term.

Flux objectives: example code

An objective with purely linear terms in LP format: Maximize: 1 R1 + 2 R2

```

<fbc:listOfObjectives fbc:activeObjective="obj1">
  <fbc:objective fbc:id="obj1" fbc:type="maximize">
    <fbc:listOfFluxObjectives>
      <fbc:fluxObjective fbc:reaction="R1" fbc:coefficient="1" fbc:variableType="linear"/>
      <fbc:fluxObjective fbc:reaction="R2" fbc:coefficient="2" fbc:variableType="linear"/>
    </fbc:listOfFluxObjectives>
  </fbc:objective>
</fbc:listOfObjectives>

```

Similarly, an objective with a quadratic term in LP format: Minimize: $1 R_1 + [4 R_2^2]/2$

```

<fbc:listOfObjectives fbc:activeObjective="obj2">
  <fbc:objective fbc:id="obj2" fbc:type="minimize">
    <fbc:listOfFluxObjectives>
      <fbc:fluxObjective fbc:reaction="R1" fbc:coefficient="1" fbc:variableType="linear"/>
      <fbc:fluxObjective fbc:reaction="R2" fbc:coefficient="2" fbc:variableType="quadratic"/>
    </fbc:listOfFluxObjectives>
  </fbc:objective>
</fbc:listOfObjectives>

```

Units

As described above the linear **FluxObjective** defined here as $n \cdot J$ where the **coefficient** (n) is dimensionless and the **value** (J) takes the units of the **reaction** flux i.e., “extent per time”. Therefore, the linear **FluxObjective**, ($n \cdot J$) has the unit $\frac{\text{extent}}{\text{time}}$ where the units of reaction “extent” and “time” are defined globally. Analogously, in the case of a quadratic **FluxObjective**, $n \cdot J^2$ this would be $\frac{\text{extent}^2}{\text{time}^2}$.

4.4 The FBC **UserConstraint** class

The FBC **UserConstraint** class is derived from **SBML SBase** and inherits **metaid** and **sboTerm**, as well as the sub-components for **Annotation** and **Notes**. Its purpose is to define non-stoichiometric constraints, that is constraints that are not necessarily defined by the stoichiometrically coupled reaction network. In order to achieve we defined a new type of linear constraint, the **UserConstraint**.

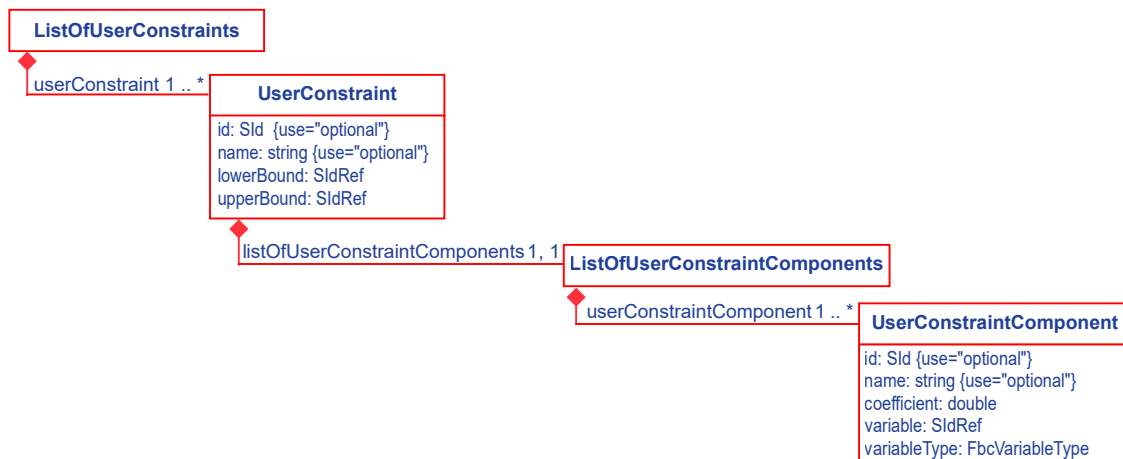


Figure 13: A UML representation of the **SBML Model** class extended in the Flux Balance Constraints package by the **ListOfUserConstraints**. See Section 1.4 for conventions related to this figure.

Analogous to the attributes described in Section 3.8 the **lowerBound** and **upperBound** form the boundaries of the

UserConstraint.

$$\text{UserConstraint} = \text{value} \quad (4)$$

$$\text{UserConstraint} \geq \text{lowerBound value} \quad (5)$$

$$\text{UserConstraint} \leq \text{upperBound value} \quad (6)$$

Defining either an equality if both `lowerBound` and `upperBound` refer to the same parameter (Equation 4) or set of inequalities (Equations 4 and 5).

The `UserConstraint` contains a `ListOfUserConstraintComponents` representing a linear combination of `UserConstraintComponents`. Similar to a `FluxObjective` each `UserConstraintComponent` contains a coefficient–variable pair where the `coefficient` refers to a `Parameter`. In addition to a `Reaction` a `UserConstraintComponent` allows the `variable` to refer to non-constant `Parameter` thus allowing the definition of non-reaction, artificial, variables.

The id and name attributes

A `UserConstraint` has an optional `id` of type `SId` and an optional attribute `name` of type `string`.

The lowerBound attribute

The required `lowerBound` attribute contains an `SIdRef` that references a `Parameter` which contains the lower boundary value of the `UserConstraint`.

The upperBound attribute

The required `upperBound` attribute contains an `SIdRef` that references a `Parameter` which contains the upper boundary value of the `UserConstraint`.

The listOfUserConstraintComponents element

The element `listOfUserConstraintComponents` which contains a `ListOfUserConstraintComponents` is derived from and functions like a typical `SBML ListOf__` class with the restriction that it must contain one or more elements of type `UserConstraintComponent` (see Section 4.5). This implies that if a `UserConstraint` is defined there should be at least one `UserConstraintComponent` contained in a `ListOfUserConstraintComponents`.

4.5 The FBC `UserConstraintComponent` class

The FBC `UserConstraintComponent` class is derived from `SBML SBBase` and inherits `metaid` and `sboTerm`, as well as the subcomponents for `Annotation` and `Notes`. The `UserConstraintComponent` class is a relatively simple container for a variable and a variable type specifier which is weighted by a signed coefficient.

The id and name attributes

An `UserConstraintComponent` has an optional `id` of type `SId` and an optional attribute `name` of type `string`.

The coefficient attribute

The required `coefficient` attribute contains an `SIdRef` that is restricted to reference only a constant `Parameter` which holds the coefficient value. In `strict` mode a `Parameter` whose `SId` is referenced by a `coefficient`, as in the case of a `FluxObjective` `coefficient`, has to be constant and not take the value `NaN` or $\pm\text{inf}$.

The variable attribute

The required `variable` attribute contains an `SIdRef` that is restricted to reference the `SId` of either a `Reaction` or a non-constant `Parameter`. Conversely, if such non-constant `Parameter`'s `SId` is referenced by a `UserConstraintComponent`'s `variable` attribute it may not be referenced by any `coefficient`, `lowerFluxBound` or `upperFluxBound` attribute.

The variableType attribute

The required `variableType` attribute contains a `FbcVariableType` that indicates whether a variable should be considered as 'linear' or 'quadratic'.

User constraints: example code

The following example illustrates the encoding of the following two **UserConstraints**:

$$RGLX - RXLG = 5 \quad (7)$$

$$2 \cdot Avar - RGDP \geq 2 \quad (8)$$

```
<listOfParameters>
  <parameter id="uc1" value="5" constant="True"/>
  <parameter id="uc2lb" value="2" constant="True"/>
  <parameter id="uc2ub" value="INF" constant="True"/>
  <parameter id="ucco1a" value="1" constant="True"/>
  <parameter id="ucco1b" value="-1" constant="True"/>
  <parameter id="ucco2a" value="2" constant="True"/>
  <parameter id="ucco2b" value="-1" constant="True"/>
  <parameter id="Avar" value="NaN" constant="False"/>
</listOfParameters>

<fbc:listOfUserConstraints>
  <fbc:userConstraint fbc:id="uc1" fbc:lowerBound="uc1" fbc:upperBound="uc1">
    <fbc:listOfUserConstraintComponents>
      <fbc:userConstraintComponent fbc:coefficient="ucco1a" fbc:variable="RGLX"
        variableType="linear"/>
      <fbc:userConstraintComponent fbc:coefficient="ucco1b" fbc:variable="RXLG"
        variableType="linear"/>
    </fbc:listOfUserConstraintComponents>
  </fbc:userConstraint>
  <fbc:userConstraint fbc:id="uc2" fbc:lowerBound="uc2lb" fbc:upperBound="uc2ub">
    <fbc:listOfUserConstraintComponents>
      <fbc:userConstraintComponent fbc:coefficient="ucco2a" fbc:variable="Avar"
        variableType="linear"/>
      <fbc:userConstraintComponent fbc:coefficient="ucco2b" fbc:variable="RGLX"
        variableType="linear"/>
    </fbc:listOfUserConstraintComponents>
  </fbc:userConstraint>
</fbc:listOfUserConstraints>
```

4.6 The FBC ListOfKeyValuePairs class

The **ListOfKeyValuePairs**, see Figure 14 for details, forms the basis of a controlled annotation defined by the Flux Balance Constraints package. This element defines a 'structured note' or 'descriptive list' of keys and associated values.

```
<annotation>
  <listOfKeyValuePairs xmlns="http://sbml.org/fbc/keyvaluepair">
    <keyValuePair key="keyX" uri="https://tinyurl.com/ybyr7b62" value="47"/>
    <keyValuePair key="ZZkey" uri="urn:tinyurl.com:example:kvp" value="level_5"/>
    <keyValuePair key="x-factor" uri="https://tinyurl.com/ybyr7b62"
      value="intangible_metaphysical_property"/>
  </listOfKeyValuePairs>
</annotation>
```

As such it is analogous to the official **SBML** RDF annotation used to support MIRIAM annotations, as defined in the **SBML** specification documents. When an annotation that declares the `xmlns http://sbml.org/fbc/keyvaluepair` then it must have the format specified here. Tools may choose to support reading and interpreting the content as

described, but may optionally ignore the annotation and merely round trip it with any other third party annotations. As is the case with the RDF/MIRIAM annotations, support for **ListOfKeyValuePair**s will be included in the **SBML** support libraries. The **ListOfKeyValuePair**s function like a typical **SBML ListOf** class with the restriction

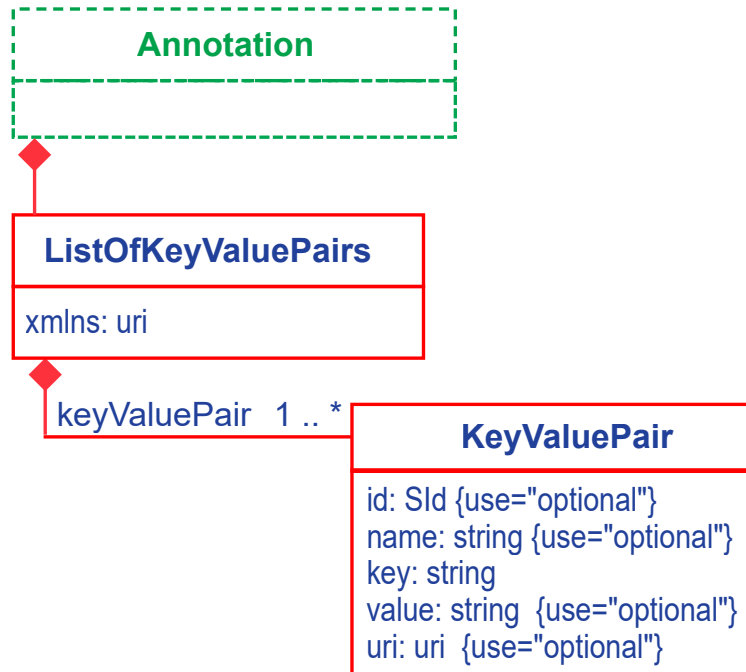


Figure 14: A UML representation of the **SBML SBBase** class extended in the Flux Balance Constraints package by the **ListOfKeyValuePair**. See Section 1.4 for conventions related to this figure.

that it must contain one or more elements of type **KeyValuePair** (see Section 4.7). In addition it defines a single mandatory attribute, **xmlns**, which identifies the annotation as belonging to the Flux Balance Constraints package.

The **xmlns** attribute

The **xmlns** is a mandatory component of the **ListOfKeyValuePair**, is of the type **uri** and must have the value <http://sbml.org/fbc/keyvaluepair>.

4.7 The FBC **KeyValuePair** class

The FBC **KeyValuePair** class' sole purpose is to define a key-value pair with an optional extended key definition.

The **KeyValuePair** defines a single mandatory attribute the **key** as well as four optional attributes: **value**, **uri**, **id** and **name**.

The **id** and **name** attributes

A **KeyValuePair** has two optional attributes: **id** an attribute of type **SId** and **name**, an attribute of type **string**.

The **key** attribute

The **key** is the mandatory component of the **KeyValuePair** pair and is of type **string**. It has the special property that every **key** in an enclosing **ListOfKeyValuePair** must be unique.

| Type | Example | Description |
|------------|---|---|
| urn | urn:tinyurl.com:example:kvp | a tool specific namespace declaration |
| url | https://tinyurl.com/ybyr7b62 | a url identifying a set of key definitions |

Table 4: Examples of how the **uri** attribute can be used to identify **key** definitions by **urn** or **url**.

The **value** attribute

The optional **value** attribute is of **string** and contains the value associated with a particular **key**. If not present, the **KeyValuePair** is defined as having no value.

The **uri** attribute

The optional attribute **uri** is of type **uri**. This attribute identifies a resource that defines the associated **key** component of the **KeyValuePair**, see Table 4 for examples. As a best practice, it is highly recommended that all tools implementing a **KeyValuePair** also implement support for the **uri** attribute.

5 Illustrative examples of the FBC syntax

This section contains a worked example showing the encoding of a model suitable for Flux Balance Analysis using the Flux Balance Constraints package.

5.1 Example one: the basic FBC syntax

5.1.1 Kinetic model description

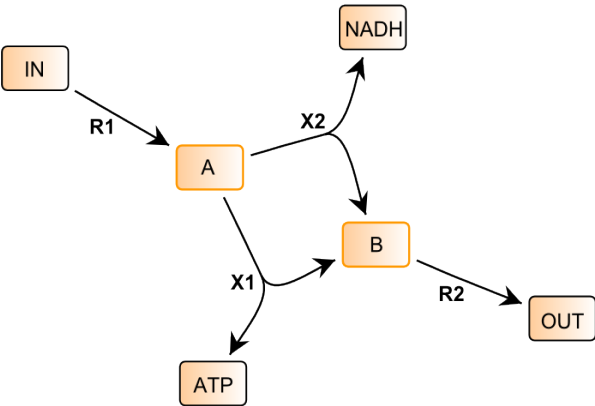


Figure 15: FBC syntax example: a simple four reaction pathway. The reactions are R1, R2, X1, X2 with fixed species IN, OUT, ATP, NADH and variable species A, B.

As shown in Figure 15 this example is a simple four reaction pathway that transforms metabolite IN to OUT. The model was created and analyzed using the SBW Flux Balance FBC implementation and CBMPy (Bergmann, 2012; Olivier, 2012). In SBML each reaction is represented as a chemical process transforming reactants to products, e.g. reaction R1 is encoded in XML as (see also the complete example provided at the end of this section):

```
<reaction id="R1" reversible="false" fast="false">
  <listOfReactants>
    <speciesReference species="IN" stoichiometry="1" constant="true"/>
  </listOfReactants>
  <listOfProducts>
    <speciesReference species="A" stoichiometry="1" constant="true"/>
  </listOfProducts>
</reaction>
```

Using the reagent identity and stoichiometry it is possible to compactly describe this network in terms of its reaction stoichiometry as shown in Table 5 where each reaction is represented as a column. Each row in the stoichiometric matrix represents the differential equation describing the change of a variable Species. In SBML such variable Species have the attribute boundaryCondition set to “false”. This is in contrast to “fixed species” (where the attribute boundaryCondition is set to “true”) which do not appear in the stoichiometric matrix. Please see the SBML Level 3 Version 1 Core specification for more details.

| | R1 | R2 | X1 | X2 |
|---|----|----|----|----|
| A | 1 | 0 | -1 | -1 |
| B | 0 | -1 | 1 | 1 |

Table 5: Example one: stoichiometric matrix, N

While the stoichiometry contains the structural properties of the reaction network the full description of a biological model can, for example, be described as a set of ordinary differential equations (ODE's). Of course other formalisms do exist, but here we will concentrate exclusively on kinetic models where the change in concentration of each variable component in the system ($\frac{ds}{dt}$) is a non-linear function of the rates of the reactions which either create or consume it (the product of the stoichiometric matrix, \mathbf{N} and the vector of reaction rates, \mathbf{v}).

$$\frac{ds}{dt} = \mathbf{N}\mathbf{v} \quad (9)$$

The formulation of the kinetic model, as shown in Equation 9 is typical of the kind that can already be described using SBML Level 3 Version 1 Core where the vector \mathbf{v} would contain rate equations as a function of parameters and variable species. In a steady-state, constraint-based model these rates are considered unknowns and the system of equations can be rewritten as a set of linear constraints (see Equation 10):

$$\mathbf{N}\mathbf{J} = 0 \quad (10)$$

Note that the rate vector \mathbf{v} is now represented as the steady-state flux vector \mathbf{J} . However, in order to perform a typical steady-state analysis such as flux balance analysis (FBA) we need to include more information into the model description. SBML Level 3 Version 1 Core does not have an unambiguous way of encoding either a capacity constraint or an objective function and for this we need to use the additional constructs provided by the Flux Balance Constraints package. In the following sections the same model data is shown encoded as XML and as a linear program (LP) in the commonly used IBM™ CPLEX format.

5.1.2 Flux Bounds

A capacity constraint or flux bound describes the limits that the flux through a certain reaction may attain at steady state. In this example the maximum limit (upper bound) on the flux through reaction *R1* is set to be one with the minimum value (lower bound) set to zero (with an arbitrary unit of flux). In LP format this may be written as:

```
Bounds
R1 <= 1.0
R1 >= 0.0
```

the same information encoded as XML:

```
<listOfParameters>
  <parameter id="R1l" constant="true" value="0" sboTerm="SBO:0000625" />
  <parameter id="R1u" constant="true" value="1" sboTerm="SBO:0000625" />
</listOfParameters>

...

<reaction id="R1" reversible="false" fast="false"
  fbc:upperFluxBound="R1l" fbc:upperFluxBound="R1u">
  <listOfReactants>
    <speciesReference species="IN" stoichiometry="1" constant="true"/>
  </listOfReactants>
  <listOfProducts>
    <speciesReference species="A" stoichiometry="1" constant="true"/>
  </listOfProducts>
</reaction>
```

5.1.3 Objective function

This describes a target which can be maximized or minimized: in this example the flux through reaction *R2* will be *maximized*.

```

Maximize
objective1_objf:  + 1.0 R2

```

the same information encoded as XML:

```

<fbc:listOfObjectives fbc:activeObjective="objective1">
  <fbc:objective fbc:id="objective1" fbc:type="maximize">
    <fbc:listOfFluxObjectives>
      <fbc:fluxObjective fbc:reaction="R2" fbc:coefficient="1"/>
    </fbc:listOfFluxObjectives>
  </fbc:objective>
</fbc:listOfObjectives>

```

5.1.4 Complete worked example

To conclude we show how the complete model described in [Figure 15](#) encoded as both an LP and as XML. Formulated as an LP the problem can be written as:

```

\\ Example one LP format

Maximize
objective1_objf:  + 1.0 R2

Subject To
A: + R1 - X1 - X2 = 0.0
B: - R2 + X1 + X2 = 0.0

Bounds
0.0 <= R1 <= 1.0
0.0 <= R2 <= +inf
0.0 <= X1 <= +inf
0.0 <= X2 <= +inf

END

```

Solving this we find that maximization of flux through $R2$ gives an optimal solution $R2 = 1$, shown in Equation 11, with one possible solution for J.

$$\begin{pmatrix} 1 & 0 & -1 & -1 \\ 0 & -1 & 1 & 1 \end{pmatrix} \begin{pmatrix} 1.0 \\ 1.0 \\ 0.0 \\ 1.0 \end{pmatrix} = 0 \quad (11)$$

Finally we provide the complete model, described above, encoded using the Flux Balance Constraints package:

```

<?xml version="1.0" encoding="UTF-8"?>
<sbml xmlns="http://www.sbml.org/sbml/level3/version1/core"
  xmlns:fbc="http://www.sbml.org/sbml/level3/version1/fbc/version2"
  level="3" version="1" fbc:required="false" sboTerm="SB0:0000624">
  <model id="fbcSpecExample1" timeUnits="time" fbc:strict="true">
    <listOfUnitDefinitions>
      <unitDefinition id="volume">
        <listOfUnits>
          <unit kind="litre" exponent="1" scale="0" multiplier="1"/>
        </listOfUnits>
      </unitDefinition>
      <unitDefinition id="substance">
        <listOfUnits>
          <unit kind="mole" exponent="1" scale="0" multiplier="1"/>
        </listOfUnits>
      </unitDefinition>
    </listOfUnitDefinitions>
  </model>

```

```

<unitDefinition id="time">
  <listOfUnits>
    <unit kind="second" exponent="1" scale="0" multiplier="1"/>
  </listOfUnits>
</unitDefinition>
</listOfUnitDefinitions>
<listOfCompartments>
  <compartment id="compartment" spatialDimensions="3" size="1" units="volume" constant="true"/>
</listOfCompartments>
<listOfSpecies>
  <species id="IN" compartment="compartment" initialConcentration="0" substanceUnits="substance"
    hasOnlySubstanceUnits="false" constant="false" boundaryCondition="true"/>
  <species id="OUT" compartment="compartment" initialConcentration="0" substanceUnits="substance"
    hasOnlySubstanceUnits="false" constant="false" boundaryCondition="true"/>
  <species id="A" compartment="compartment" initialConcentration="0" substanceUnits="substance"
    hasOnlySubstanceUnits="false" constant="false" boundaryCondition="false"/>
  <species id="B" compartment="compartment" initialConcentration="0" substanceUnits="substance"
    hasOnlySubstanceUnits="false" constant="false" boundaryCondition="false"/>
  <species id="ATP" compartment="compartment" initialConcentration="0" substanceUnits="substance"
    hasOnlySubstanceUnits="false" constant="false" boundaryCondition="true"/>
  <species id="NADH" compartment="compartment" initialConcentration="0" substanceUnits="substance"
    hasOnlySubstanceUnits="false" constant="false" boundaryCondition="true"/>
</listOfSpecies>
<listOfParameters>
  <parameter id="R1u" constant="true" value="1" sboTerm="SBO:0000625" />
  <parameter constant="true" id="irrLow" value="0" sboTerm="SBO:0000625" />
  <parameter constant="true" id="posInf" value="INF" sboTerm="SBO:0000626" />
</listOfParameters>
<listOfReactions>
  <reaction id="R1" reversible="false" fast="false"
    fbc:lowerFluxBound="irrLow" fbc:upperFluxBound="R1u" >
    <listOfReactants>
      <speciesReference species="IN" stoichiometry="1" constant="true"/>
    </listOfReactants>
    <listOfProducts>
      <speciesReference species="A" stoichiometry="1" constant="true"/>
    </listOfProducts>
  </reaction>
  <reaction id="R2" reversible="false" fast="false"
    fbc:lowerFluxBound="irrLow" fbc:upperFluxBound="posInf" >
    <listOfReactants>
      <speciesReference species="B" stoichiometry="1" constant="true"/>
    </listOfReactants>
    <listOfProducts>
      <speciesReference species="OUT" stoichiometry="1" constant="true"/>
    </listOfProducts>
  </reaction>
  <reaction id="X1" reversible="false" fast="false"
    fbc:lowerFluxBound="irrLow" fbc:upperFluxBound="posInf" >
    <listOfReactants>
      <speciesReference species="A" stoichiometry="1" constant="true"/>
    </listOfReactants>
    <listOfProducts>
      <speciesReference species="ATP" stoichiometry="1" constant="true"/>
      <speciesReference species="B" stoichiometry="1" constant="true"/>
    </listOfProducts>
  </reaction>
  <reaction id="X2" reversible="false" fast="false"
    fbc:lowerFluxBound="irrLow" fbc:upperFluxBound="posInf" >
    <listOfReactants>
      <speciesReference species="A" stoichiometry="1" constant="true"/>
    </listOfReactants>
    <listOfProducts>
      <speciesReference species="B" stoichiometry="1" constant="true"/>
      <speciesReference species="NADH" stoichiometry="1" constant="true"/>
    </listOfProducts>
  </reaction>

```

```
</reaction>
</listOfReactions>
<fbc:listOfObjectives fbc:activeObjective="objective1">
  <fbc:objective fbc:id="objective1" fbc:type="maximize">
    <fbc:listOfFluxObjectives>
      <fbc:fluxObjective fbc:reaction="R2" fbc:coefficient="1"/>
    </fbc:listOfFluxObjectives>
  </fbc:objective>
</fbc:listOfObjectives>
</model>
</sbml>
```

1
2
3
4
5
6
7
8
9
10
11
12

6 Best practices

In this section, we illustrate a number of practices for using and interpreting various constructs in the Flux Balance Constraints package. These recommendations are non-normative, ignoring them will not render a model invalid, rather they are suggested as ways of enhancing model inter-operability. For a description of the additional SBO terms relevant to FBC models please see the SBO appendix.

6.1 Examples contrasting the current SBML L2 encoding with L3 and FBC

These examples contrast some elements of an existing model, iJR904 from the BiGG Database encoded in the COBRA SBML Level 2 Version 1 format (Becker et al., 2007; Reed et al., 2003; Schellenberger et al., 2010) that have been translated into SBML Level 3 Version 1 FBC Version 2.

Objective function definition

SBML Level 2 objective function

```
<reaction id="R_BiomassEcoli" name="BiomassEcoli" reversible="false">
  <kineticLaw>
    <math xmlns="http://www.w3.org/1998/Math/MathML">
      <ci>FLUX_VALUE</ci>
    </math>
    <listOfParameters>
      <parameter id="LOWER_BOUND" value="0" units="mmol_per_gDW_per_hr"/>
      <parameter id="UPPER_BOUND" value="999999" units="mmol_per_gDW_per_hr"/>
      <parameter id="OBJECTIVE_COEFFICIENT" value="1" />
      <parameter id="FLUX_VALUE" value="0" units="mmol_per_gDW_per_hr"/>
    </listOfParameters>
  </kineticLaw>
</reaction>
```

The SBML Level 3 objective function

```
<fbc:listOfObjectives fbc:activeObjective="obj1">
  <fbc:objective fbc:id="obj1" fbc:type="maximize">
    <fbc:listOfFluxObjectives>
      <fbc:fluxObjective fbc:reaction="R_BiomassEcoli" fbc:coefficient="1"/>
    </fbc:listOfFluxObjectives>
  </fbc:objective>
</fbc:listOfObjectives>
```

Species definition

SBML Level 2 Species annotation version 1

Examine the SBML Level 2 Version 1 **Species** definition. Note how the **name** attribute is overloaded with the chemical formula in a tool specific way.

```
<species id="M_atp_c" name="ATP_C10H12N5O13P3"
  compartment="Cytosol" charge="-4" />
```

SBML Level 2 Species annotation version 2

A variation of the previous syntax that appeared in later models.


```

<species id="M_atp_c" name="ATP" compartment="c">
  <notes>
    <body xmlns="http://www.w3.org/1999/xhtml">
      <p>FORMULA: C10H12N5O13P3</p>
      <p>CHARGE: -4</p>
    </body>
  </notes>
</species>

```

The SBML Level 3 FBC Species attributes

With the adoption of **SBML** FBC these **Species** properties can now be unified into a common format.

```

<species metaid="meta_M_atp_c" id="M_atp_c" name="ATP" compartment="Cytosol"
  boundaryCondition="false" initialConcentration="0" hasOnlySubstanceUnits="false"
  fbc:charge="-4" fbc:chemicalFormula="C10H12N5O13P3"/>

```

Reaction definition and flux bounds

SBML Level 2 Reaction

```

<reaction id="R_GTHS" name="glutathione_synthetase" reversible="false">
  <notes>
    <html:p>Abbreviation: R_GTHS</html:p>
    <html:p>EC Number: 6.3.2.3</html:p>
    <html:p>SUBSYSTEM: Cofactor and Prosthetic Group Biosynthesis</html:p>
    <html:p>Equation: [c] : atp + glucys + gly --> adp + gthrd + h + pi</html:p>
    <html:p>Confidence Level: 0</html:p>
    <html:p>LOCUS:b2947#ABBREVIATION:gshB#ECNUMBERS:6.3.2.3#</html:p>
    <html:p>NAME:glutathione synthase#ABBREVIATION:GshB#</html:p>
    <html:p>GENE ASSOCIATION: (b2947)</html:p>
  </notes>
  <listOfReactants>
    <speciesReference species="M_atp_c" stoichiometry="1"/>
    <speciesReference species="M_glucys_c" stoichiometry="1"/>
    <speciesReference species="M_gly_c" stoichiometry="1"/>
  </listOfReactants>
  <listOfProducts>
    <speciesReference species="M_adp_c" stoichiometry="1"/>
    <speciesReference species="M_gthrd_c" stoichiometry="1"/>
    <speciesReference species="M_h_c" stoichiometry="1"/>
    <speciesReference species="M_pi_c" stoichiometry="1"/>
  </listOfProducts>
  <kineticLaw>
    <math xmlns="http://www.w3.org/1998/Math/MathML">
      <ci>FLUX_VALUE</ci>
    </math>
    <listOfParameters>
      <parameter id="LOWER_BOUND" value="0" units="mmol_per_gDW_per_hr"/>
      <parameter id="UPPER_BOUND" value="999999" units="mmol_per_gDW_per_hr"/>
      <parameter id="OBJECTIVE_COEFFICIENT" value="0" />
      <parameter id="FLUX_VALUE" value="0" units="mmol_per_gDW_per_hr"/>
    </listOfParameters>
  </kineticLaw>
</reaction>

```

The SBML Level 3 FBC Reaction

As an example of a good annotation practice the EC number stored in the **Notes** element has been converted into MIRIAM compliant RDF. The Flux Balance Constraints package also facilitates the structured definition and use of gene protein associations and flux capacity constraints.

```

<reaction metaid="meta_R_GTHS" id="R_GTHS" name="glutathione_synthetase" reversible="false"
  fbc:lowerFluxBound="R_GTHS_l" fbc:upperFluxBound="R_GTHS_u">
  <annotation>
    <rdf:RDF xmlns:rdf="http://www.w3.org/1999/02/22-rdf-syntax-ns#"
      xmlns:dc="http://purl.org/dc/elements/1.1/" xmlns:dcterms="http://purl.org/dc/terms/"
      xmlns:bqbiol="http://biomodels.net/biology-qualifiers/"
    >
      <rdf:Description rdf:about="#meta_R_GTHS">
        <bqbiol:is>
          <rdf:Bag>
            <rdf:li rdf:resource="http://identifiers.org/ec-code/6.3.2.3"/>
          </rdf:Bag>
        </bqbiol:is>
      </rdf:Description>
    </rdf:RDF>
  </annotation>
  <fbc:geneProductAssociation fbc:id="gpr_GTHS">
    <fbc:geneProductRef fbc:geneProduct="g_b2947"/>
  </fbc:geneProductAssociation>
  <listOfReactants>
    <speciesReference constant="true" species="M_atp_c" stoichiometry="1"/>
    <speciesReference constant="true" species="M_glucys_c" stoichiometry="1"/>
    <speciesReference constant="true" species="M_gly_c" stoichiometry="1"/>
  </listOfReactants>
  <listOfProducts>
    <speciesReference constant="true" species="M_adp_c" stoichiometry="1"/>
    <speciesReference constant="true" species="M_gthrd_c" stoichiometry="1"/>
    <speciesReference constant="true" species="M_h_c" stoichiometry="1"/>
    <speciesReference constant="true" species="M_pi_c" stoichiometry="1"/>
  </listOfProducts>
</reaction>
<fbc:listOfGeneProducts>
  <fbc:geneProduct metaid="meta_g_b2947" fbc:id="g_b2947" fbc:label="b2947">
    <annotation>
      <rdf:RDF xmlns:rdf="http://www.w3.org/1999/02/22-rdf-syntax-ns#"
        xmlns:dc="http://purl.org/dc/elements/1.1/" xmlns:dcterms="http://purl.org/dc/terms/"
        xmlns:bqbiol="http://biomodels.net/biology-qualifiers/"
      >
        <rdf:Description rdf:about="#meta_g_b2947">
          <bqbiol:isEncodedBy>
            <rdf:Bag>
              <rdf:li rdf:resource="http://identifiers.org/ncbigene/947445"/>
            </rdf:Bag>
          </bqbiol:isEncodedBy>
        </rdf:Description>
      </rdf:RDF>
    </annotation>
  </fbc:geneProduct>
</fbc:listOfGeneProducts>
<listOfParameters>
  <parameter id="R_GTHS_l" constant="true" sboTerm="SBO:0000625" value="0" sboTerm="SBO:0000625" />
  <parameter metaid="meta_R_GTHS_u" id="R_GTHS_u" constant="true" value="inf" sboTerm="SBO:0000625">
    <annotation>
      <rdf:RDF xmlns:rdf="http://www.w3.org/1999/02/22-rdf-syntax-ns#"
        xmlns:dc="http://purl.org/dc/elements/1.1/" xmlns:dcterms="http://purl.org/dc/terms/"
        xmlns:bqbiol="http://biomodels.net/biology-qualifiers/"
      >
        <rdf:Description rdf:about="#meta_R_GTHS_u">
          <bqbiol:isDescribedBy>
            <rdf:Bag>
              <rdf:li rdf:resource="http://identifiers.org/pmc/PMC193654"/>
            </rdf:Bag>
          </bqbiol:isDescribedBy>
        </rdf:Description>
      </rdf:RDF>
    </annotation>
  </parameter>
</listOfParameters>

```

6.2 An example of a strict FBC model (XML)

This section highlights the best practices for a complete FBC Version 2 model. To improve readability, detailed annotations as described in Section [Section 6.1](#) and unit definitions have been omitted.

```
<?xml version="1.0" encoding="UTF-8"?> <sbml
xmlns="http://www.sbml.org/sbml/level3/version1/core"
xmlns:fbc="http://www.sbml.org/sbml/level3/version1/fbc/version2"
level="3" version="1" fbc:required="false" sboTerm="SBO:0000624">
<model id="fbcSpecExample1" timeUnits="time" fbc:strict="true">
  <listOfCompartments>
    <compartment id="compartment" spatialDimensions="3" size="1" units="volume" constant="true"/>
  </listOfCompartments>
  <listOfSpecies>
    <species id="IN" compartment="compartment" initialConcentration="0" substanceUnits="substance"
      hasOnlySubstanceUnits="false" boundaryCondition="true" constant="false"/>
    <species id="OUT" compartment="compartment" initialConcentration="0" substanceUnits="substance"
      hasOnlySubstanceUnits="false" boundaryCondition="true" constant="false"/>
    <species id="A" compartment="compartment" initialConcentration="0" substanceUnits="substance"
      hasOnlySubstanceUnits="false" boundaryCondition="false" constant="false"/>
    <species id="B" compartment="compartment" initialConcentration="0" substanceUnits="substance"
      hasOnlySubstanceUnits="false" boundaryCondition="false" constant="false"/>
    <species id="ATP" compartment="compartment" initialConcentration="0" substanceUnits="substance"
      hasOnlySubstanceUnits="false" boundaryCondition="true" constant="false"/>
    <species id="NADH" compartment="compartment" initialConcentration="0" substanceUnits="substance"
      hasOnlySubstanceUnits="false" boundaryCondition="true" constant="false"/>
  </listOfSpecies>
  <listOfParameters>
    <parameter id="lb" name="arbitrary_lower_bound" constant="true" value="-INF"
      sboTerm="SBO:0000626" />
    <parameter id="ub" name="arbitrary_upper_bound" constant="true" value="INF"
      sboTerm="SBO:0000626" />
    <parameter id="R1l" name="lower_bound" constant="true" value="0"
      sboTerm="SBO:0000625" />
    <parameter id="R1u" name="uptake_upper_bound" constant="true" value="1"
      sboTerm="SBO:0000625" />
    <parameter id="X2l" name="export_only_lower_bound" constant="true" value="0"
      sboTerm="SBO:0000625" />
  </listOfParameters>
  <fbc:listOfGeneProducts>
    <fbc:geneProduct fbc:id="g1" fbc:name="dog_gene" fbc:label="PetGeneDB1"/>
    <fbc:geneProduct fbc:id="g2" fbc:name="cat_gene" fbc:label="PetGeneDB2"/>
    <fbc:geneProduct fbc:id="g3" fbc:name="mouse_gene" fbc:label="PetGeneDB3"/>
    <fbc:geneProduct fbc:id="g4" fbc:name="bird_gene" fbc:label="PetGeneDB4"/>
  </fbc:listOfGeneProducts>
  <listOfReactions>
    <reaction id="R1" reversible="false" fast="false"
      fbc:lowerFluxBound="R1l" fbc:upperFluxBound="R1u" >
      <fbc:geneProductAssociation>
        <fbc:geneProductRef fbc:geneProduct="g1"/>
      </fbc:geneProductAssociation>
      <listOfReactants>
        <speciesReference species="IN" stoichiometry="1" constant="true"/>
      </listOfReactants>
      <listOfProducts>
        <speciesReference species="A" stoichiometry="1" constant="true"/>
      </listOfProducts>
    </reaction>
    <reaction id="R2" reversible="true" fast="false"
      fbc:lowerFluxBound="lb" fbc:upperFluxBound="ub">
      <fbc:geneProductAssociation fbc:id="andGPR">
        <fbc:and>
          <fbc:geneProductRef fbc:geneProduct="g1"/>
          <fbc:geneProductRef fbc:geneProduct="g4"/>
        </fbc:and>
      </fbc:geneProductAssociation>
```

```

<listOfReactants>
  <speciesReference species="B" stoichiometry="1" constant="true"/>
</listOfReactants>
<listOfProducts>
  <speciesReference species="OUT" stoichiometry="1" constant="true"/>
</listOfProducts>
</reaction>
<reaction id="X1" reversible="true" fast="false"
  fbc:lowerFluxBound="lb" fbc:upperFluxBound="ub" >
  <fbc:geneProductAssociation fbc:id="orGPR">
    <fbc:or>
      <fbc:geneProductRef fbc:geneProduct="g2"/>
      <fbc:geneProductRef fbc:geneProduct="g3"/>
    </fbc:or>
  </fbc:geneProductAssociation>
  <listOfReactants>
    <speciesReference species="A" stoichiometry="1" constant="true"/>
  </listOfReactants>
  <listOfProducts>
    <speciesReference species="ATP" stoichiometry="1" constant="true"/>
    <speciesReference species="B" stoichiometry="1" constant="true"/>
  </listOfProducts>
</reaction>
<reaction id="X2" reversible="true" fast="false"
  fbc:lowerFluxBound="X2l" fbc:upperFluxBound="ub" >
  <fbc:geneProductAssociation fbc:id="allGPR">
    <fbc:or>
      <fbc:and>
        <fbc:geneProductRef fbc:geneProduct="g1"/>
        <fbc:geneProductRef fbc:geneProduct="g4"/>
      </fbc:and>
      <fbc:and>
        <fbc:geneProductRef fbc:geneProduct="g1"/>
        <fbc:geneProductRef fbc:geneProduct="g3"/>
      </fbc:and>
    </fbc:or>
  </fbc:geneProductAssociation>
  <listOfReactants>
    <speciesReference species="A" stoichiometry="1" constant="true"/>
  </listOfReactants>
  <listOfProducts>
    <speciesReference species="B" stoichiometry="1" constant="true"/>
    <speciesReference species="NADH" stoichiometry="1" constant="true"/>
  </listOfProducts>
</reaction>
</listOfReactions>
<fbc:listOfObjectives fbc:activeObjective="objective1">
  <fbc:objective fbc:id="objective1" fbc:type="maximize">
    <fbc:listOfFluxObjectives>
      <fbc:fluxObjective fbc:reaction="R2" fbc:coefficient="1"/>
    </fbc:listOfFluxObjectives>
  </fbc:objective>
</fbc:listOfObjectives>
</model>
</sbml>

```

A Validation of SBML documents

A.1 Validation and consistency rules

This section summarizes all the conditions that must (or in some cases, at least *should*) be true of an SBML Level 3 Version 1 model that uses the Flux Balance Constraints package. We use the same conventions as are used in the SBML Level 3 Version 1 Core specification document. In particular, there are different degrees of rule strictness. Formally, the differences are expressed in the statement of a rule: either a rule states that a condition *must* be true, or a rule states that it *should* be true. Rules of the former kind are strict SBML validation rules—a model encoded in SBML must conform to all of them in order to be considered valid. Rules of the latter kind are consistency rules. To help highlight these differences, we use the following three symbols next to the rule numbers:

- ☑ A checked box indicates a *requirement* for SBML conformance. If a model does not follow this rule, it does not conform to the Flux Balance Constraints specification. (Mnemonic intention behind the choice of symbol: “This must be checked.”)
- ▲ A triangle indicates a *recommendation* for model consistency. If a model does not follow this rule, it is not considered strictly invalid as far as the Flux Balance Constraints specification is concerned; however, it indicates that the model contains a physical or conceptual inconsistency. (Mnemonic intention behind the choice of symbol: “This is a cause for warning.”)
- ★ A star indicates a strong recommendation for good modeling practice. This rule is not strictly a matter of SBML encoding, but the recommendation comes from logical reasoning. As in the previous case, if a model does not follow this rule, it is not strictly considered an invalid SBML encoding. (Mnemonic intention behind the choice of symbol: “You’re a star if you heed this.”)

The validation rules listed in the following subsections are all stated or implied in the rest of this specification document. They are enumerated here for convenience. Unless explicitly stated, all validation rules concern objects and attributes specifically defined in the Flux Balance Constraints package.

🔍 For convenience and brevity, we use the shorthand “**fbc:x**” to stand for an attribute or element name **x** in the namespace for the Flux Balance Constraints package, using the namespace prefix **fbc**. In reality, the prefix string may be different from the literal “**fbc**” used here (and indeed, it can be any valid XML namespace prefix that the modeler or software chooses). We use “**fbc:x**” because it is shorter than to write a full explanation everywhere we refer to an attribute or element in the Flux Balance Constraints package namespace.

General rules about this package

- fbc-10101** ☑ To conform to the Flux Balance Constraints package specification for SBML Level 3 Version 1, an SBML document must declare the use of the following XML Namespace: “<http://www.sbml.org/sbml/level3/version1/fbc/version2>”. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.1 on page 6.)
- fbc-10102** ☑ Wherever they appear in an SBML document, elements and attributes from the Flux Balance Constraints package must be declared either implicitly or explicitly to be in the XML namespace “<http://www.sbml.org/sbml/level3/version1/fbc/version2>”. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 1, Section 3.1 on page 6.)

General rules about identifiers

- fbc-10301** ☑ (Extends validation rule #10301 in the SBML Level 3 Version 1 Core specification.) Within a **Model** the values of the attributes **id** and **fbc:id** on every instance of the following classes of objects must be unique across the set of all **id** and **fbc:id** attribute values of all such objects in a model: the **Model** itself, plus all contained **FunctionDefinition**, **Compartment**, **Species**,

Reaction, **SpeciesReference**, **ModifierSpeciesReference**, **Event**, and **Parameter** objects, plus the **GeneProduct**, **Objective**, **FluxObjective** and **GeneProductAssociation** objects defined by the Flux Balance Constraints package. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.2 on page 6.)

- fbc-10302** ✓ The value of a **fbc:id** attribute must always conform to the syntax of the SBML data type **SId**. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.2 on page 6.)

Rules for the extended SBML class

- fbc-20101** ✓ In all SBML documents using the Flux Balance Constraints package, the **SBML** object must include a value for the attribute **fbc:required** attribute. (References: SBML Level 3 Version 1 Core, Section 4.1.2.)
- fbc-20102** ✓ The value of attribute **fbc:required** on the **SBML** object must be of the data type **boolean**. (References: SBML Level 3 Version 1 Core, Section 4.1.2.)
- fbc-20103** ✓ The value of attribute **fbc:required** on the **SBML** object must be set to “false”. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.1 on page 6.)

Rules for extended Model object

- fbc-20201** ✓ There may be at most one instance of each of the following kinds of objects within a **Model** object using Flux Balance Constraints: **ListOfGeneProducts** and **ListOfObjectives**. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.3 on page 7.)
- fbc-20202** ✓ The various **ListOf__** subobjects with an **Model** object are optional, but if present, these container object must not be empty. Specifically, if any of the following classes of objects are present on the **Model**, it must not be empty: **ListOfGeneProducts** and **ListOfObjectives**. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.3 on page 7.)
- fbc-20203** ✓ (This validation rule does not apply in Flux Balance Constraints Version 2.)
- fbc-20204** ✓ Apart from the general notes and annotation subobjects permitted on all SBML objects, a **ListOfObjectives** container object may only contain **Objective** objects. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.3 on page 7.)
- fbc-20205** ✓ (This validation rule does not apply in Flux Balance Constraints Version 2.)
- fbc-20206** ✓ A **ListOfObjectives** object may have the optional attributes **metaid** and **sboTerm** defined by SBML Level 3 Core. Additionally the **ListOfObjectives** must contain the attribute **fbc:activeObjective**. No other attributes from the SBML Level 3 Core namespace or the Flux Balance Constraints namespace are permitted on a **ListOfObjectives** object. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.3 on page 7.)
- fbc-20207** ✓ The value of attribute **fbc:activeObjective** on the **ListOfObjectives** object must be of the data type **SIdRef**. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.3.1 on page 9.)
- fbc-20208** ✓ The value of attribute **fbc:activeObjective** on the **ListOfObjectives** object must be the identifier of an existing **Objective**. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.3.1 on page 9.)

- fbc-20209** ✓ A **Model** object must have the required attribute **fbc:strict**. No other attributes from the SBML Level 3 Flux Balance Constraints namespaces are permitted on a **Model** object. (Reference: SBML Level 3 Specification for Flux Balance Constraints Version 1, Section 3.3 on page 7.)
- fbc-20210** ✓ The attribute **fbc:strict** on a **Model** must have a value of data type **boolean**. (Reference: SBML Level 3 Specification for Flux Balance Constraints Version 1, Section 3.3 on page 7.)
- fbc-20211** ✓ Apart from the general notes and annotation subobjects permitted on all SBML objects, a **ListOfGeneProducts** container object may only contain **GeneProduct** objects. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.3 on page 7.)
- fbc-20212** ✓ A **ListOfGeneProducts** object may have the optional attributes **metaid** and **sboTerm** defined by SBML Level 3 Core. No other attributes from the SBML Level 3 Core namespace or the Flux Balance Constraints namespace are permitted on a **ListOfGeneProducts** object. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.3 on page 7.)

Rules for extended Species object

- fbc-20301** ✓ A **SBML Species** object may have the optional attributes **fbc:chemicalFormula** and **fbc:charge**. No other attributes from the Flux Balance Constraints namespaces are permitted on a **Species**. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 1, Section 3.4 on page 9)
- fbc-20302** ✓ The value of attribute **fbc:charge** on the **SBML Species** object must be of the data type **integer**. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.4 on page 9).
- fbc-20303** ✓ The value of attribute **fbc:chemicalFormula** on the **SBML Species** object must be set to a **string** consisting only of atomic names or user defined compounds and their occurrence. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.4 on page 9.)

Rules for Objective object

- fbc-20501** ✓ An **Objective** object may have the optional SBML Level 3 Core attributes **metaid** and **sboTerm**. No other attributes from the SBML Level 3 Core namespace are permitted on an **Objective**. (References: SBML Level 3 Version 1 Core, Section 3.2.)
- fbc-20502** ✓ An **Objective** object may have the optional SBML Level 3 Core subobjects for notes and annotations. No other elements from the SBML Level 3 Core namespace are permitted on an **Objective**. (References: SBML Level 3 Version 1 Core, Section 3.2.)
- fbc-20503** ✓ An **Objective** object must have the required attributes **fbc:id** and **fbc:type** and may have the optional attribute **fbc:name**. No other attributes from the SBML Level 3 Flux Balance Constraints namespace are permitted on an **Objective** object. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.6 on page 11.)
- fbc-20504** ✓ The attribute **fbc:name** on an **Objective** must be of the data type **string**. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.6 on page 11.)
- fbc-20505** ✓ The attribute **fbc:type** on an **Objective** must be of the data type **FbcType** and thus its value must be one of “**minimize**” or “**maximize**”. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.6 on page 11.)

- fbc-20506** ✓ An **Objective** object must have one and only one instance of the **ListOfFluxObjectives** object. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.6 on page 11.)
- fbc-20507** ✓ The **ListOfFluxObjectives** subobject within an **Objective** object must not be empty. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.6 on page 11.)
- fbc-20508** ✓ Apart from the general notes and annotation subobjects permitted on all SBML objects, a **ListOfFluxObjectives** container object may only contain **FluxObjective** objects. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.6 on page 11.)
- fbc-20509** ✓ A **ListOfFluxObjectives** object may have the optional **metaid** and **sboTerm** defined by SBML Level 3 Core. No other attributes from the SBML Level 3 Core namespace or the Flux Balance Constraints namespace are permitted on a **ListOfFluxObjectives** object. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.6 on page 11.)

Rules for FluxObjective object

- fbc-20601** ✓ A **FluxObjective** object may have the optional SBML Level 3 Core attributes **metaid** and **sboTerm**. No other attributes from the SBML Level 3 Core namespace are permitted on a **FluxObjective**. (References: SBML Level 3 Version 1 Core, Section 3.2.)
- fbc-20602** ✓ A **FluxObjective** object may have the optional SBML Level 3 Core subobjects for notes and annotations. No other elements from the SBML Level 3 Core namespace are permitted on a **FluxObjective**. (References: SBML Level 3 Version 1 Core, Section 3.2.)
- fbc-20603** ✓ A **FluxObjective** object must have the required attributes **fbc:reaction** and **fbc:coefficient**, and may have the optional attributes **fbc:id** and **fbc:name**. No other attributes from the SBML Level 3 Flux Balance Constraints namespace are permitted on a **FluxObjective** object. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.7 on page 12.)
- fbc-20604** ✓ The attribute **fbc:name** on a **FluxObjective** must be of the data type **string**. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.7 on page 12.)
- fbc-20605** ✓ The value of the attribute **fbc:reaction** of a **FluxObjective** object must conform to the syntax of the SBML data type **SIdRef**. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.7 on page 12.)
- fbc-20606** ✓ The value of the attribute **fbc:reaction** of a **FluxObjective** object must be the identifier of an existing **Reaction** object defined in the enclosing **Model** object. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 1, Section 3.7 on page 12.)
- fbc-20607** ✓ The value of the attribute **fbc:coefficient** of a **FluxObjective** object must conform to the syntax of the SBML data type **double**. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.7 on page 12.)
- fbc-20608** ✓ When the value of the **Models** **fbc:strict** attribute is “**true**”, the value of the attribute **fbc:-coefficient** of a **FluxObjective** object must not be set to “**NaN**”, “**-INF**” or “**INF**”. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.3 on page 7.)

Rules for extended Reaction object

- fbc-20701** ✓ There may be at most one instance of a **GeneProductAssociation** within a **Reaction** object using Flux Balance Constraints. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.8 on page 13.)
- fbc-20702** ✓ A **SBML Reaction** object may have the optional attributes **fbc:lowerFluxBound** and **fbc:upperFluxBound**. No other attributes from the Flux Balance Constraints namespaces are permitted on a **Reaction**. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.8 on page 13)
- fbc-20703** ✓ The attribute **fbc:lowerFluxBound** of a **Reaction** must be of the data type **SIdRef**. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.8 on page 13.)
- fbc-20704** ✓ The attribute **fbc:upperFluxBound** of a **Reaction** must be of the data type **SIdRef**. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.8 on page 13.)
- fbc-20705** ✓ The attribute **fbc:lowerFluxBound** of a **Reaction** must point to an existing **Parameter** in the model. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.8 on page 13.)
- fbc-20706** ✓ The attribute **fbc:upperFluxBound** of a **Reaction** must point to an existing **Parameter** in the model. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.8 on page 13.)
- fbc-20707** ✓ When the value of the **Models fbc:strict** attribute is “**true**”, a **Reaction** must define the attributes **fbc:lowerFluxBound** and **fbc:upperFluxBound**. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.3 on page 7.)
- fbc-20708** ✓ When the value of the **Models fbc:strict** attribute is “**true**”, the **Parameter** objects referred to by the attributes **fbc:lowerFluxBound** and **fbc:upperFluxBound** must have their **constant** attribute set to “**true**”. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.3 on page 7.)
- fbc-20709** ✓ When the value of the **Models fbc:strict** attribute is “**true**”, the **Parameter** objects referred to by the attributes **fbc:lowerFluxBound** and **fbc:upperFluxBound** must have a defined value for their **value** attribute, which may not be “**NaN**”. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.3 on page 7.)
- fbc-20710** ✓ When the value of the **Models fbc:strict** attribute is “**true**”, the **Parameter** objects referred to by the attributes **fbc:lowerFluxBound** and **fbc:upperFluxBound** may not be targeted by an **InitialAssignment**. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.3 on page 7.)
- fbc-20711** ✓ When the value of the **Models fbc:strict** attribute is “**true**”, the value of the **Parameter** object referred to by the attribute **fbc:lowerFluxBound** may not have the value “**INF**”. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.3 on page 7.)
- fbc-20712** ✓ When the value of the **Models fbc:strict** attribute is “**true**”, the value of the **Parameter** object referred to by the attribute **fbc:upperFluxBound** may not have the value “**-INF**”. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.3 on page 7.)

- fbc-20713** ✓ When the value of the **Models** `fbc:strict` attribute is “true”, the value of the **Parameter** object referred to by the attribute `fbc:lowerFluxBound` must be less than or equal to the value of the **Parameter** object referred to by the attribute `fbc:upperFluxBound`. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.3 on page 7.)
- fbc-20714** ✓ When the value of the **Models** `fbc:strict` attribute is “true”, the `constant` attribute of **SpeciesReference** elements of a **Reaction** must be set to “true”. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.3 on page 7.)
- fbc-20715** ✓ When the value of the **Models** `fbc:strict` attribute is “true”, the value of a **SpeciesReference**’s `stoichiometry` attribute must not be set to “NaN”, “-INF” or “INF”. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.3 on page 7.)
- fbc-20716** ✓ When the value of the **Models** `fbc:strict` attribute is “true”, the **SpeciesReference** elements of a **Reaction** may not be targeted by an **InitialAssignment**. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.3 on page 7.)

Rules for GeneProductAssociation object

- fbc-20801** ✓ A **GeneProductAssociation** object may have the optional SBML Level 3 Core attributes `metaid` and `sboTerm`. No other attributes from the SBML Level 3 Core namespace are permitted on a **GeneProductAssociation**. (References: SBML Level 3 Version 1 Core, Section 3.2.)
- fbc-20802** ✓ A **GeneProductAssociation** object may have the optional SBML Level 3 Core subobjects for notes and annotations. No other elements from the SBML Level 3 Core namespace are permitted on a **GeneProductAssociation**. (References: SBML Level 3 Version 1 Core, Section 3.2.)
- fbc-20803** ✓ A **GeneProductAssociation** object may have the optional attributes `fbc:id` `fbc:name`. No other attributes from the SBML Level 3 Flux Balance Constraints namespace are permitted on a **GeneProductAssociation** object. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.9 on page 14.)
- fbc-20804** ✓ The attribute `fbc:id` on a **GeneProductAssociation** must be of the data type `SId`. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.9 on page 14.)
- fbc-20805** ✓ A **GeneProductAssociation** object must have one and only one of the concrete **Association** objects: **GeneProductRef**, **And** or **Or**. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.9 on page 14.)
- fbc-20806** ✓ The attribute `fbc:name` on a **GeneProductAssociation** must be of the data type `string`. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.9 on page 14.)

Rules for GeneProductRef object

- fbc-20901** ✓ A **GeneProductRef** object may have the optional SBML Level 3 Core attributes `metaid` and `sboTerm`. No other attributes from the SBML Level 3 Core namespace are permitted on a **GeneProductRef**. (References: SBML Level 3 Version 1 Core, Section 3.2.)
- fbc-20902** ✓ A **GeneProductRef** object may have the optional SBML Level 3 Core subobjects for notes and annotations. No other elements from the SBML Level 3 Core namespace are permitted on a **GeneProductRef**. (References: SBML Level 3 Version 1 Core, Section 3.2.)
- fbc-20903** ✓ A **GeneProductRef** object must have the required attribute `fbc:geneProduct` and may have the optional attribute `fbc:id`. No other attributes from the SBML Level 3 Flux Balance Con-

straints namespace are permitted on a **GeneProductRef** object. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.11 on page 16.)

fbc-20904 ✓ The attribute **fbc:geneProduct** on a **GeneProductRef** must be of the data type **SIdRef**. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.11 on page 16.)

fbc-20908 ✓ The attribute **fbc:geneProduct** on a **GeneProductRef** if set, must refer to **id** of a **GeneProduct** in the **Model**. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.11 on page 16.)

Rules for And object

fbc-21001 ✓ An **And** object may have the optional SBML Level 3 Core attributes **metaid** and **sboTerm**. No other attributes from the SBML Level 3 Core namespace are permitted on an **And**. (References: SBML Level 3 Version 1 Core, Section 3.2.)

fbc-21002 ✓ An **And** object may have the optional SBML Level 3 Core subobjects for notes and annotations. No other elements from the SBML Level 3 Core namespace are permitted on an **And**. (References: SBML Level 3 Version 1 Core, Section 3.2.)

fbc-21003 ✓ An **And** object must have two or more concrete **Association** objects: **GeneProductRef**, **And**, or **Or**. No other elements from the SBML Level 3 Flux Balance Constraints namespace are permitted on an **And** object. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.12 on page 17.)

Rules for Or object

fbc-21101 ✓ An **Or** object may have the optional SBML Level 3 Core attributes **metaid** and **sboTerm**. No other attributes from the SBML Level 3 Core namespace are permitted on an **Or**. (References: SBML Level 3 Version 1 Core, Section 3.2.)

fbc-21102 ✓ An **Or** object may have the optional SBML Level 3 Core subobjects for notes and annotations. No other elements from the SBML Level 3 Core namespace are permitted on an **Or**. (References: SBML Level 3 Version 1 Core, Section 3.2.)

fbc-21103 ✓ An **Or** object must have two or more concrete **Association** objects: **GeneProductRef**, **And**, or **Or**. No other elements from the SBML Level 3 Flux Balance Constraints namespace are permitted on an **Or** object. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.13 on page 17.)

Rules for GeneProduct object

fbc-21201 ✓ A **GeneProduct** object may have the optional SBML Level 3 Core attributes **metaid** and **sboTerm**. No other attributes from the SBML Level 3 Core namespace are permitted on a **GeneProduct**. (References: SBML Level 3 Version 1 Core, Section 3.2.)

fbc-21202 ✓ A **GeneProduct** object may have the optional SBML Level 3 Core subobjects for notes and annotations. No other elements from the SBML Level 3 Core namespace are permitted on a **GeneProduct**. (References: SBML Level 3 Version 1 Core, Section 3.2.)

fbc-21203 ✓ A **GeneProduct** object must have the required attributes **fbc:id** and **fbc:label** may have the optional attributes **fbc:name** and **fbc:associatedSpecies**. No other attributes from the SBML Level 3 Flux Balance Constraints namespace are permitted on a **GeneProduct** object. (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.5 on page 10.)

| | | |
|--------------------|---|----------------|
| fbc-21204 ✓ | The attribute fbc:label on a GeneProduct must be of the data type string . (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.5 on page 10.) | 1 2 3 |
| fbc-21205 ✓ | The attribute fbc:label on a GeneProduct must be unique among the set of all GeneProduct elements defined in the Model . (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.5 on page 10.) | 4 5 6 |
| fbc-21206 ✓ | The attribute fbc:name on a GeneProduct must be of the data type string . (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.5 on page 10.) | 7 8 9 |
| fbc-21207 ✓ | The attribute fbc:associatedSpecies on a GeneProduct must be the identifier of an existing Species defined in the enclosing Model . (References: SBML Level 3 Package Specification for Flux Balance Constraints, Version 2, Section 3.5 on page 10.) | 10 11 12 |

B The Systems Biology Ontology and the **sboTerm** attribute

The following text on the usage of SBO has been extracted from the SBML Level 3 Version 1 Core specification and is provided here for your convenience. Please consult the official documentation for more details. Table 6 shows the additional SBO terms specified by the Flux Balance Constraints package.

| SBML Element | SBO Name | SBO term |
|--------------|------------------------|-------------|
| Document | flux balance framework | SBO:0000624 |
| Parameter | flux bound | SBO:0000625 |
| Parameter | default flux bound | SBO:0000626 |

Table 6: SBML components and the additional types of SBO terms specified in the Flux Balance Constraints package that may be assigned to them. Note that the important aspect here is the set of specific SBO identifiers, not the SBO term names, because the names may change as SBO continues to evolve. See the text and SBO online resources for detailed descriptions on the meaning and usage of these terms.

The values of **id** attributes on SBML components allow the components to be cross-referenced within a model. The values of **name** attributes on SBML components provide the opportunity to assign them meaningful labels suitable for display to humans. The specific identifiers and labels used in a model necessarily must be unrestricted by SBML, so that software and users are free to pick whatever they need. However, this freedom makes it more difficult for software tools to determine, without additional human intervention, the semantics of models more precisely than the semantics provided by the SBML object classes defined in other sections of this document.

An approach to solving this problem is to associate model components with terms from carefully curated controlled vocabularies (CVs). This is the purpose of the optional **sboTerm** attribute provided on the SBML class **SBase**. The **sboTerm** attribute always refers to terms belonging to the Systems Biology Ontology¹. In this section, we discuss the **sboTerm** attribute, SBO, the motivations and theory behind their introduction, and guidelines for their use.

SBO is not part of SBML; it is being developed separately, to allow the modeling community to evolve the ontology independently of SBML. However, the terms in the ontology are being designed keeping SBML components in mind, and are classified into subsets that can be directly related with SBML components such as reaction rate expressions, parameters, and a few others, see below. The use of **sboTerm** attributes is optional, and the presence of **sboTerm** on an element does not change the way the model is *interpreted*. Annotating SBML elements with SBO terms adds additional semantic information that may be used to *convert* the model into another model, or another format. Although SBO support provides an important source of information to understand the meaning of a model, software does not need to support **sboTerm** to be considered SBML-compliant.

Although the use of SBO can be beneficial, it is critical to keep in mind that the presence of an **sboTerm** value on an object *must not change the fundamental mathematical meaning* of the model. An SBML model must be defined such that it stands on its own and does not depend on additional information added by SBO terms for a correct mathematical interpretation. SBO term definitions will not imply any alternative mathematical semantics for any SBML object labeled with that term. Two important reasons motivate this principle. First, it would be too limiting to require all software tools to be able to understand the SBO vocabularies in addition to understanding SBML. Supporting SBO is not only additional work for the software developer; for some kinds of applications, it may not make sense. If SBO terms on a model are optional, it follows that the SBML model *must* remain unambiguous and fully interpretable without them, because an application reading the model may ignore the terms. Second, we believe allowing the use of **sboTerm** to alter the mathematical meaning of a model would allow too much leeway to shoehorn inconsistent concepts into SBML objects, ultimately reducing the interoperability of the models.

¹<http://biomodels.net/SBO/>

Acknowledgments

We would like to thank everyone who contributed, in various ways, to the development of both the original proposal and this specification.

For financial/travel/technical support and highly productions discussions we thank especially (in alphabetical order): Michael Hucka (CalTech, USA), Ursula Kummer (Heidelberg University, Germany), Herbert Sauro (University of Washington, USA) and Bas Teusink (VU University Amsterdam, The Netherlands).

A special word of thanks to Sarah M. Keating for her critical reading of this document and development of the libSBML MATLAB bindings and SBML Toolbox.

We also would like to thank (in alphabetical order) the people involved in the development of the Flux Balance Constraints package. Andreas Dräger, Ali Ebrahim, Ronan Fleming, Ben Heavner, Daniel Hyduke, Sarah Keating, Matthias Koenig, Nicolas Le Novère, Chris Myers, Thomas Pfau, Nicolas Rodriguez, Kieran Smallbone, Lucian Smith, Neil Swainston, Alex Thomas, all past and present members of the FBC Package Working Group and contributors to the discussion on the FBC mailing list, the libSBML and JSBML development teams and all others who contributed to discussions on various occasions. If we have not added you to this list and you feel you should be here please contact us.

References

- Becker, S. A., Feist, A. M., Mo, M. L., Hannum, G., Palsson, B. O., and Herrgard, M. J. (2007). Quantitative prediction of cellular metabolism with constraint-based models: the COBRA Toolbox. *Nat. Protocols*, 2(3):727–738. 1750-2799 10.1038/nprot.2007.99 10.1038/nprot.2007.99.
- Bergmann, F. T. (2012). Sbw flux balance. Available via the World Wide Web at <https://github.com/fbergmann/FluxBalance>.
- Biron, P. V. and Malhotra, A. (2000). XML Schema part 2: Datatypes (W3C candidate recommendation 24 October 2000). Available via the World Wide Web at <http://www.w3.org/TR/xmlschema-2/>.
- Eriksson, H.-E. and Penker, M. (1998). *UML Toolkit*. John Wiley & Sons, New York.
- Fallside, D. C. (2000). XML Schema part 0: Primer (W3C candidate recommendation 24 October 2000). Available via the World Wide Web at <http://www.w3.org/TR/xmlschema-0/>.
- Hill, E. A. (1900). On a system of indexing chemical literature; adopted by the classification division of the U.S. patent office. *Journal of the American Chemical Society*, 22(8):478–494.
- Hill, E. A. (2012). Wikipedia: The Hill System. Available via the World Wide Web at http://en.wikipedia.org/wiki/Hill_system.
- Hucka, M., Bergmann, F. T., Hoops, S., Keating, S. M., Sahle, S., and Wilkinson, D. J. (2011). Available via the World Wide Web at <http://sbml.org/Documents/Specifications>.
- Hucka, M., Finney, A., Sauro, H., Bolouri, H., Doyle, J., Kitano, H., Arkin, A., Bornstein, B., Bray, D., Cornish-Bowden, A., Cuellar, A., Dronov, S., Gilles, E., Ginkel, M., Gor, V., Goryanin, I., Hedley, W., Hodgman, T., Hofmeyr, J., Hunter, P., Juty, N., Kasberger, J., Kremling, A., Kummer, U., Novère, N. L., Loew, L., Lucio, D., Mendes, P., Minch, E., Mjolsness, E., Nakayama, Y., Nelson, M., Nielsen, P., Sakurada, T., Schaff, J., Shapiro, B., Shimizu, T., Spence, H., Stelling, J., Takahashi, K., Tomita, M., Wagner, J., and Wang, J. (2003). The Systems Biology Markup Language (SBML): a medium for representation and exchange of biochemical network models. *Bioinformatics*, 19(4):524–31.
- Oberhardt, M. A., Palsson, B. O., and Papin, J. A. (2009). Applications of genome-scale metabolic reconstructions. *Molecular Systems Biology*, 5:320.
- Oestereich, B. (1999). *Developing Software with UML: Object-Oriented Analysis and Design in Practice*. Addison-Wesley.
- Olivier, B. G. (2012). PySCeS CBMPy: PySCeS Constraint Based Modelling in Python. Available via the World Wide Web at <http://cbmpy.sourceforge.net>.
- Reed, J. L., Vo, T. D., Schilling, C. H., and Palsson, B. O. (2003). An expanded genome-scale model of Escherichia coli K-12 (iJR904 GSM/GPR). *Genome Biol*, 4(9):R54.
- SBML Team (2010). The SBML Issue Tracker. Available via the World Wide Web at <http://sbml.org/issue-tracker>.
- Schellenberger, J., Park, J., Conrad, T., and Palsson, B. (2010). BiGG: a Biochemical Genetic and Genomic knowledgebase of large scale metabolic reconstructions. *BMC Bioinformatics*, 11(1):213.
- Thompson, H. S., Beech, D., Maloney, M., and Mendelsohn, N. (2000). XML Schema part 1: Structures (W3C candidate recommendation 24 October 2000). Available online via the World Wide Web at the address <http://www.w3.org/TR/xmlschema-1/>.