

MSnbase development

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Abstract

This vignette describes the classes implemented in *MSnbase* package. It is intended as a starting point for developers or users who would like to learn more or further develop/extend **pSet**.

Keywords: Mass Spectrometry (MS), proteomics, infrastructure.

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Foreword

MSnbase is in an early development (see section 4 for details about packages and version used in this vignette). Although main data structures have been thought out and are meant to be compatible with other existing mature infrastructure in the Bioconductor project, changes may occur in the future. Current functionality will evolve and new one will be added. Although at an early stage, this package is released in the hope that it may foster new developments in proteomics data analysis within R by providing a common infrastructure. Several package developers working with mass spectrometry and proteomics data met at the Bioconductor Developer Meeting Europe¹ held in Heidelberg in November 2010, and agreed to combine efforts. This library is one attempt to do so.

You are welcome to contact me for questions, bugs, typos or suggestions about *MSnbase*. If you wish to reach a broader audience for general questions about proteomics analysis using R, you may want to use the Bioconductor mailing list².

1 Introduction

This document is not a replacement for the individual manual pages, that document the slots of the *MSnbase* classes. It is a centralised high-level description of the package design.

MSnbase aims at being compatible with the *Biobase* infrastructure Gentleman et al. (2004). Many meta data structures that are used in *eSet* and associated classes are also used here. As such, knowledge of the *Biobase development and the new eSet* vignette³ would be beneficial.

The initial goal is to use the *MSnbase* infrastructure for labelled quantitation using reporter ions (iTRAQ (Ross et al., 2004) and TMT (Thompson et al., 2003)). Spectral counting should be trivial to apply with current features, as long as identification data is at hand. Currently, no effort is invested to streamline label-free quantitative proteomics, although some effort has been done to keep the infrastructure flexible enough to accommodate more designs.

2 *MSnbase* classes

All classes have a `__classVersion__` slot, of class `Versioned` from the *Biobase* package. This slot documents the class version for any instance to be used for debugging and object update purposes. Any change in a class implementation should trigger a version change.

¹<http://bioconductor.org/help/course-materials/2010/HeidelbergNovember2010/>

²<https://stat.ethz.ch/mailman/listinfo/bioconductor>

³The vignette can directly be accessed with `vignette("BiobaseDevelopment", package="Biobase")` once *Biobase* is loaded.

2.1 pSet: a virtual class for raw mass spectrometry data and meta data

This virtual class is the main container for mass spectrometry data, i.e spectra, and meta data. It is based on the `eSet` implementation for genomic data. The main difference with `eSet` is that the `assayData` slot is an environment containing any number of `Spectrum` instances (see section 2.6).

One new slot is introduced, namely `processingData`, that contains one `MSnProcess` instance (see section 2.4). and the `experimentData` slot is now expected to contain MIAPE data (see section 2.5). The `annotation` slot has not been implemented, as no prior feature annotation is known in shotgun proteomics.

```
> getClass("pSet")
```

```
Virtual Class "pSet" [package "MSnbase"]
```

```
Slots:
```

Name:	assayData	phenoData	featureData
Class:	environment	NAnnotatedDataFrame	AnnotatedDataFrame

Name:	experimentData	protocolData	processingData
Class:	MIAxE	AnnotatedDataFrame	MSnProcess

Name:	.__classVersion__
Class:	Versions

```
Extends: "Versioned"
```

```
Known Subclasses: "MSnExp"
```

Future work Currently, few setters have been implemented.

2.2 MSnExp: a class for MS experiments

`MSnExp` extends `pSet` to store MS experiments. It does not add any new slots to `pSet`. Accessors and setters are all inherited from `pSet` and new ones should be implemented for `pSet`. Methods that manipulate actual data in experiments are implemented for `MSnExp` objects.

```
> getClass("MSnExp")
```

```
Class "MSnExp" [package "MSnbase"]
```

```

Slots:

Name:      assayData      phenoData      featureData
Class:      environment NAnnotatedDataFrame AnnotatedDataFrame

Name:      experimentData      protocolData      processingData
Class:      MIAxE      AnnotatedDataFrame      MSnProcess

Name:      __classVersion__
Class:      Versions

Extends:
Class "pSet", directly
Class "Versioned", by class "pSet", distance 2

```

2.3 MSnSet: a class for quantitative proteomics data

This class stores quantitation data and meta data after running `quantify` on an `MSnExp` object. The quantitative data is in form of a $n \times m$ matrix, where n is the number of features/spectra originally in the `MSnExp` used as parameter in `quantify` and m is the number of reporter ions (see section 2.7).

This prompted to keep a similar implementation as the `ExpressionSet` class, while adding the proteomics-specific annotation slot introduced in the `pSet` class, namely `processingData` for objects of class `MSnProcess` (see section 2.4).

The `MSnSet` class extends the virtual `eSet` class to provide compatibility for `ExpressionSet`-like behaviour. The experiment meta-data in `experimentData` is also of class `MIAPE` (see section 2.5). The `annotation` slot, inherited from `eSet` is not used.

```
> getClass("MSnSet")
```

```
Class "MSnSet" [package "MSnbase"]
```

```

Slots:

Name:      experimentData      processingData      qual
Class:      MIAPE      MSnProcess      data.frame

Name:      assayData      phenoData      featureData
Class:      AssayData AnnotatedDataFrame AnnotatedDataFrame

Name:      annotation      protocolData      __classVersion__
Class:      character AnnotatedDataFrame      Versions

Extends:

```

```

Class "eSet", directly
Class "VersionedBiobase", by class "eSet", distance 2
Class "Versioned", by class "eSet", distance 3

```

2.4 MSnProcess: a class for logging processing meta data

This class aims at recording specific manipulations applied to `MSnExp` or `MSnSet` instances. The `processing` slot is a `character` vector that describes major processing. Most other slots are of class `logical` that indicate whether the data has been centroided, smoothed, ... although many of the functionality is not implemented yet. Any new processing that is implemented should be documented and logged here.

It also documents the raw data file from which the data originates (`files` slot) and the *MSnbase* version that was in use when the `MSnProcess` instance, and hence the `MSnExp`/`MSnSet` objects, were originally created.

```
> getClass("MSnProcess")
```

```
Class "MSnProcess" [package "MSnbase"]
```

```
Slots:
```

Name:	files	processing	merged	cleaned
Class:	character	character	logical	logical
Name:	removedPeaks	smoothed	trimmed	centroided
Class:	character	logical	numeric	logical
Name:	normalised	MSnbaseVersion	__classVersion__	
Class:	logical	character	Versions	

```
Extends: "Versioned"
```

2.5 MIAPE: Minimum Information About a Proteomics Experiment

The Minimum Information About a Proteomics Experiment ([Taylor et al., 2007, 2008](#)) MIAPE class describes the experiment, including contact details, information about the mass spectrometer and control and analysis software.

Raw data is currently imported from `mzXML` files ([Pedrioli et al., 2004](#)) < using the `xcms::rampRawData` and `xcms::rampRawDataMSn` functions from the *xcms* package ([Smith et al., 2006](#)). These functions do not give access to the meta data. New importer functions are under development (see for instance *mzR*⁴) that will hopefully give programmatic access to meta data stored in the

⁴<https://github.com/sneumann/mzR/blob/master/DESCRIPTION>

data file to populate the MIAPE object.

```
> getClass("MIAPE")
```

Class "MIAPE" [package "MSnbase"]

Slots:

Name:	title	url
Class:	character	character
Name:	abstract	pubMedIds
Class:	character	character
Name:	samples	preprocessing
Class:	list	list
Name:	other	dateStamp
Class:	list	character
Name:	name	lab
Class:	character	character
Name:	contact	instrumentModel
Class:	character	character
Name:	instrumentManufacturer	instrumentCustomisations
Class:	character	character
Name:	softwareName	softwareVersion
Class:	character	character
Name:	switchingCriteria	isolationWidth
Class:	character	numeric
Name:	parameterFile	ionSource
Class:	character	character
Name:	ionSourceDetails	analyser
Class:	character	character
Name:	analyserDetails	collisionGas
Class:	character	character
Name:	collisionPressure	collisionEnergy

```

Class:          numeric          character

Name:          detectorType      detectorSensitivity
Class:          character         character

Name:          __classVersion__
Class:          Versions

Extends:
Class "MIAxE", directly
Class "Versioned", by class "MIAxE", distance 2

```

2.6 Spectrum *et al.*: classes for MS spectra

Spectrum is a virtual class that defines common attributes to all types of spectra. MS1 and MS2 specific attributes are defined in the Spectrum1 and Spectrum2 classes, that directly extend Spectrum.

The choices of attributes has been dictated by the `xcms:::rampRawData` and `xcms:::rampRawDataMSn` functions and what data from the mzXML file they gave access to. It is expected that some hopefully minor changes might come up here when migrating to other data import packages, that allow random access to mzXML data and support mzML (Martens et al., 2010).

```
> getClass("Spectrum")
```

```
Virtual Class "Spectrum" [package "MSnbase"]
```

```
Slots:
```

```

Name:          msLevel          peaksCount          rt          acquisitionNum
Class:          integer          integer          numeric          integer

Name:          scanIndex          mz          intensity          fromFile
Class:          integer          numeric          numeric          integer

```

```

Name:  __classVersion__
Class:  Versions

```

```
Extends: "Versioned"
```

```
Known Subclasses: "Spectrum2", "Spectrum1"
```

```
> getClass("Spectrum1")
```

```
Class "Spectrum1" [package "MSnbase"]
```

```
Slots:
```

Name:	polarity	msLevel	peaksCount	rt
Class:	integer	integer	integer	numeric

Name:	acquisitionNum	scanIndex	mz	intensity
Class:	integer	integer	numeric	numeric

Name:	fromFile	__classVersion__
Class:	integer	Versions

```
Extends:
```

```
Class "Spectrum", directly
```

```
Class "Versioned", by class "Spectrum", distance 2
```

```
> getClass("Spectrum2")
```

```
Class "Spectrum2" [package "MSnbase"]
```

```
Slots:
```

Name:	merged	ms1scan	precursorMz
Class:	numeric	integer	numeric

Name:	precursorIntensity	precursorCharge	collisionEnergy
Class:	numeric	integer	numeric

Name:	msLevel	peaksCount	rt
Class:	integer	integer	numeric

Name:	acquisitionNum	scanIndex	mz
Class:	integer	integer	numeric

Name:	intensity	fromFile	__classVersion__
Class:	numeric	integer	Versions

```
Extends:
```

```
Class "Spectrum", directly
```

```
Class "Versioned", by class "Spectrum", distance 2
```

2.7 ReporterIons: a class for isobaric tags

The iTRAQ and TMT (or any other peak of interest) are implemented `ReporterIons` instances, that essentially defines an expected MZ position for the peak and a width around this value as well a names for the reporters.

```
> getClass("ReporterIons")
```

```
Class "ReporterIons" [package "MSnbase"]
```

```
Slots:
```

Name:	name	reporterNames	description	mz
Class:	character	character	character	numeric

Name:	col	width	__classVersion__
Class:	character	numeric	Versions

```
Extends: "Versioned"
```

2.8 NAnnotatedDataFrame: multiplexed AnnotatedDataFrames

The simple expansion of the `AnnotatedDataFrame` classes adds the `multiplex` and `multiLabel` slots to document the number and names of multiplexed samples.

```
> getClass("NAnnotatedDataFrame")
```

```
Class "NAnnotatedDataFrame" [package "MSnbase"]
```

```
Slots:
```

Name:	multiplex	multiLabels	varMetadata	data
Class:	numeric	character	data.frame	data.frame

Name:	dimLabels	__classVersion__
Class:	character	Versions

```
Extends:
```

```
Class "AnnotatedDataFrame", directly
```

```
Class "Versioned", by class "AnnotatedDataFrame", distance 2
```

3 Miscellaneous

Unit tests *MSnbase* implements unit tests with the *testthat* package.

Processing methods Methods that process raw data, i.e. spectra should be implemented for `Spectrum` objects first and then `eapply`'ed (or similar) to the `assayData` slot of an `MSnExp` instance in the specific method.

Speed and memory requirements Raw mass spectrometry file are generally several hundreds of MB large and most of this is used for binary raw spectrum data. As such, data containers can easily grow very large and thus require large amounts of RAM. This requirement may be tackled in the future by avoiding to load the raw data into memory and using random access to the content of `mzXML`/`mzML` data files on demand. When focusing on reporter ion quantitation, a direct solution for this is to trim the spectra using the `trimMz` method to select the area of interest and thus substantially reduce the size of the `Spectrum` objects. This is illustrated on page ?? of the `MSnbase-demo` vignette.

The independent handling of spectra is ideally suited for parallel processing. This will be added soon.

4 Session information

- R version 2.13.1 (2011-07-08), i386-pc-mingw32
- Locale: LC_COLLATE=C, LC_CTYPE=English_United States.1252, LC_MONETARY=English_United States.1252, LC_NUMERIC=C, LC_TIME=English_United States.1252
- Base packages: base, datasets, grDevices, graphics, grid, methods, stats, tools, utils
- Other packages: Biobase 2.12.2, MSnbase 1.0.7, Rcpp 0.9.6, cacheSweave 0.6, codetools 0.2-8, filehash 2.2, formatR 0.2-3, getopt 1.16, ggplot2 0.8.9, highlight 0.2-5, optparse 0.9.1, parser 0.0-13, pgfSweave 1.2.1, plyr 1.6, proto 0.3-9.2, reshape 0.8.4, stashR 0.3-4, tikzDevice 0.6.1
- Loaded via a namespace (and not attached): IRanges 1.10.6, digest 0.5.0, xcms 1.26.1

References

Robert C. Gentleman, Vincent J. Carey, Douglas M. Bates, Ben Bolstad, Marcel Dettling, Sandrine Dudoit, Byron Ellis, Laurent Gautier, Yongchao Ge, Jeff Gentry, Kurt Hornik, Torsten Hothorn, Wolfgang Huber, Stefano Iacus, Rafael Irizarry, Friedrich Leisch, Cheng Li, Martin Maechler, Anthony J. Rossini, Gunther Sawitzki, Colin Smith, Gordon Smyth, Luke Tierney, Jean Y. H. Yang, and Jianhua Zhang. Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol*, 5(10):-80,

2004. doi: 10.1186/gb-2004-5-10-r80. URL <http://dx.doi.org/10.1186/gb-2004-5-10-r80>.
- Lennart Martens, Matthew Chambers, Marc Sturm, Darren Kesner, Fredrik Levander, Jim Shofstahl, Wilfred H Tang, Andreas Römpp, Steffen Neumann, Angel D Pizarro, Luisa Montecchi-Palazzi, Natalie Tasman, Mike Coleman, Florian Reisinger, Puneet Souda, Henning Hermjakob, Pierre-Alain Binz, and Eric W Deutsch. mzml - a community standard for mass spectrometry data. *Molecular & Cellular Proteomics : MCP*, 2010. doi: 10.1074/mcp.R110.000133.
- Patrick G A Pedrioli, Jimmy K Eng, Robert Hubley, Mathijs Vogelzang, Eric W Deutsch, Brian Raught, Brian Pratt, Erik Nilsson, Ruth H Angeletti, Rolf Apweiler, Kei Cheung, Catherine E Costello, Henning Hermjakob, Sequin Huang, Randall K Julian, Eugene Kapp, Mark E McComb, Stephen G Oliver, Gilbert Omenn, Norman W Paton, Richard Simpson, Richard Smith, Chris F Taylor, Weimin Zhu, and Ruedi Aebersold. A common open representation of mass spectrometry data and its application to proteomics research. *Nat. Biotechnol.*, 22(11):1459–66, 2004. doi: 10.1038/nbt1031.
- Philip L. Ross, Yulin N. Huang, Jason N. Marchese, Brian Williamson, Kenneth Parker, Stephen Hattar, Nikita Khainovski, Sasi Pillai, Subhakar Dey, Scott Daniels, Subhasish Purkayastha, Peter Juhasz, Stephen Martin, Michael Bartlett-Jones, Feng He, Allan Jacobson, and Darryl J. Pappin. Multiplexed protein quantitation in *saccharomyces cerevisiae* using amine-reactive isobaric tagging reagents. *Mol Cell Proteomics*, 3(12):1154–1169, Dec 2004. doi: 10.1074/mcp.M400129-MCP200. URL <http://dx.doi.org/10.1074/mcp.M400129-MCP200>.
- C.A. Smith, E.J. Want, G. O’Maille, R. Abagyan, and G. Siuzdak. Xcms: Processing mass spectrometry data for metabolite profiling using nonlinear peak alignment, matching and identification. *Analytical Chemistry*, 78:779–787, 2006.
- Chris F. Taylor, Norman W. Paton, Kathryn S. Lilley, Pierre-Alain Binz, Randall K. Julian, Andrew R. Jones, Weimin Zhu, Rolf Apweiler, Ruedi Aebersold, Eric W. Deutsch, Michael J. Dunn, Albert J. R. Heck, Alexander Leitner, Marcus Macht, Matthias Mann, Lennart Martens, Thomas A. Neubert, Scott D. Patterson, Peipei Ping, Sean L. Seymour, Puneet Souda, Akira Tsugita, Joel Vandekerckhove, Thomas M. Vondriska, Julian P. Whitelegge, Marc R. Wilkins, Ioannis Xenarios, John R. Yates, and Henning Hermjakob. The minimum information about a proteomics experiment (mipe). *Nat Biotechnol*, 25(8):887–893, Aug 2007. doi: 10.1038/nbt1329. URL <http://dx.doi.org/10.1038/nbt1329>.
- Chris F Taylor, Pierre-Alain Binz, Ruedi Aebersold, Michel Affolter, Robert Barkovich, Eric W Deutsch, David M Horn, Andreas Höijmer, Martin Kussmann, Kathryn Lilley, Marcus Macht, Matthias Mann, Dieter Mäijler,

- Thomas A Neubert, Janice Nickson, Scott D Patterson, Roberto Raso, Kathryn Resing, Sean L Seymour, Akira Tsugita, Ioannis Xenarios, Rong Zeng, and Randall K Julian. Guidelines for reporting the use of mass spectrometry in proteomics. *Nat. Biotechnol.*, 26(8):860–1, 2008. doi: 10.1038/nbt0808-860.
- Andrew Thompson, Jürgen Schäfer, Karsten Kuhn, Stefan Kienle, Josef Schwarz, Günter Schmidt, Thomas Neumann, R Johnstone, A Karim A Mohammed, and Christian Hamon. Tandem mass tags: a novel quantification strategy for comparative analysis of complex protein mixtures by MS/MS. *Anal. Chem.*, 75(8):1895–904, 2003.