Antibody Markup Language (AbML)
Format Description V1.3

June 14, 2024

V1.0 — 10th February 2022
V1.1 — 30th June 2022
V1.2 — 2nd October 2023
V1.3 — 14th June 2024

1 General

- Whitespace (including line breaks) is ignored except within comments
- The system is case insensitive except for the comments
- The term 'domain', as used in this document, is a general term for a region of the protein and can refer to flexible linkers and hinge regions as well as formal protein domains.

2 Antibody Domain Types

VL	Variable Light
CL	Constant Light
VH	Variable Heavy
VHH	Camelid single VH domain
CH1	Constant Heavy 1
H	Hinge
CH2	Constant Heavy 2
CH3	Constant Heavy 3
CH4	Constant Heavy 4
CHS	Part of CH3 or CH4, but used where it makes disulphides
J	A J-chain (IgM or IgA)

3 TCR Domain Types

VA	Variable alpha
CA	Constant alpha
VB	Variable beta
CB	Constant beta
VG	Variable gamma
CG	Constant gamma
VD	Variable delta
CD	Constant delta

4 Additional Domain Types

L	Linker
X	Extra domain
C	Chemical conjugation

5 Domain Peptide Connectivity

Working from N-terminus to C-terminus, connectivity is indicated with a `-`. Chains are separated by a `|`.

e.g.

```
VL-CL|VH-CH1-H-CH2-CH3
```

6 Domain Identifiers and Interactions

After any **Domain Type**, a numeric **Domain Identifier** may be indicated in parentheses. These will normally be used sequentially.

e.g. for a normal antibody:

```
VL(1)-CL(2) | VH(3)-CH1(4)-H(5)-CH2(6)-CH3(7) |
VL(8)-CL(9) | VH(10)-CH1(11)-H(12)-CH2(13)-CH3(14)
```

Interactions between domains are indicated by a ':' followed by a comma-separated list of interacting **Domain Identifiers**.

e.g. for a normal antibody

```
VL(1:3)-CL(2:4) | VH(3:1)-CH1(4:2)-H(5:12)-CH2(6:13)-CH3(7:14) |
VL(8:10)-CL(9:11) | VH(10:8)-CH1(11:9)-H(12:5)-CH2(13:6)-CH3(14:7)
```

If a domain interacts with multiple other domains, then these are specified in a comma-separated list.
e.g. in an IgM

```
J(81:8,80)
```

7 Disulfides

The number of **Disulfides** occurring between interacting domains can be indicated in curly brackets. Note that **Disulfides** must follow a domain **Interaction** indicator.

e.g. for a normal antibody

```
VL(1:3)-CL(2:4){1} | VH(3:1)-CH1(4:2){1}-H(5:12){2}-CH2(6:13)-CH3(7:14) |
VL(8:10)-CL(9:11){1} | VH(10:8)-CH1(11:9){1}-H(12:5){2}-CH2(13:6)-CH3(14:7)
```

If a domain interacts with multiple other domains and makes hydrogen bonds with them, then these are specified in a comma-separated list.
e.g. in an IgM

```
J(81:8,80){1,1}
```

8 Specificity

For multi-specific antibodies, the **Specificity** is indicated with a `.x` after the **Domain Type**. e.g. `VL.a`, `VL.b`. A domain having multiple specificities is indicated with `.x...` e.g. `VL.ab` for two specificities, etc.

9 Linkers

Linkers (indicated by an `L`) simply occur within the sequence of domains. A **Linker** may be followed by (**Domain Identifier** / **Interaction**) information optionally followed by **Disulphide** information and/or a **Comment**.

The comment keyword `LENGTH:` is reserved for indicating the length of a **Linker**.

e.g.

- `L(5), L(5:10), L(5:10){1}`
- `L[LENGTH:20]`
- `L(5:10){1}[LENGTH:15]`

10 Extra Domains (X)

An **Extra Domain** (i.e. a non-immunoglobulin domains) is indicated with the **Domain Type** `X`. An **Extra Domain** may be followed by (**Domain Identifier** : **Interaction**) information, optionally followed by **Disulphide** information and/or a comment. Typically a `[TYPE:xxx]` comment will be included to indicate the type of the extra domains. (See **Comments**, below)

11 Chemical Moieties (C)

Chemical Moieties are chemical cross linkers indicated with the Domain Type `C` and used to join two or more protein domains (note that these are *not* conjugation linkers for ADCs).

Chemical Moieties may be followed by (**Domain Identifier** : **Interaction**) information, optionally followed by a comment. Typically a `[TYPE:xxx]` comment will be included to indicate the type of the chemical moiety. (See **Comments**, below)

12 Modifications

Specific and general domain modifications can be indicated with the following symbols which must appear immediately after a **Domain Type**:

^	specific ADC site
>	a 'knob' for domain pairing
@	a 'hole' for domain pairing
+	a positive charge for domain pairing
-	a negative charge for domain pairing (note this is an underscore, since the - is reserved for connections between domains)
!	used after a CH2 domain to indicate that it is not glycosylated
*	a general modification (which may then be explained by a comment)
\verb[ADC]‘	a non-specific ADC conjugation (when appended to the end of expression as a separate chain)

e.g.

```
CH3>(7:14)
CH3@(14:7)
```

Where a modification occurs to a variable domain where the **Specificity** is also indicated, the modification is described before the **Specificity**.

e.g.

```
VL*.a
```

Thus a bispecific antibody using a knob-into-hole for heavy chain pairing and charges for light chain pairing might be:

```
VL.a(1:3)-CL+(2:4){1} |
VH.a(3:1)-CH1_(4:2){1}-H(5:12){2}-CH2(6:13)-CH3>(7:14) |
VL.b(8:10)-CL_(9:11){1} |
VH.b(10:8)-CH1+(11:9){1}-H(12:5){2}-CH2(13:6)-CH3@(14:7)
```

A modification in CH2 to enhance FcRn binding would be:

```
CH2*[MOD:ENHANCEFCRN]
```

A non-specific ADC modification would be:

```
VL.a(1:3)-CL(2:4){1} |
VH.a(3:1)-CH1(4:2){1}-H(5:12){2}-CH2(6:13)-CH3(7:14) |
VL.b(8:10)-CL(9:11){1} |
VH.b(10:8)-CH(11:9){1}-H(12:5){2}-CH2(13:6)-CH3(14:7) |
[ADC]
```

13 Split Domains

[*Added in V1.3*]

Some recent antibody-based drugs have introduced 'split domains' — i.e. cases in which some other domain has been inserted within an antibody domain (e.g. within a CDR).

This is now described in AbML by adding `'1`, `'2`, etc., to describe the parts of the antibody domain with the insert being an `X` domain, typically with linkers either side. For example:

```
VL'1(1)-L(2)-X(3)-L(4)-VL'2(1)-CL(4)
```

Note that the two parts of the `VL` domain have the same domain number (1 in this case).

14 Comments

Comments (each preceded by a keyword) may be added in square brackets and appear last in the set of qualifiers after a **Domain Type**. e.g. `VL.a(1:3)[ANTI:CD3]`

Multiple comments may appear as a comma-separated list, or in separate sets of square brackets. e.g. `VL*.a(1:3)[ANTI:CD3,MOD:PI]` or `VL*.a(1:3)[ANTI:CD3][MOD:PI]`

The following keywords are currently allowed for comments:

ANTI:	Gives the specificity (free text)
MOD:	Used to indicate the type of a modification - only a restricted list is allowed
TYPE:	Used with **Extra Domains** and **Chemical Moieties** to indicate what they are - only a restricted list is allowed
LENGTH:	The length of a domain (typically of a **Linker**)
NOTE:	Any other comment (free text, must appear last in a list of comments)

14.1 TYPE - allowed keywords

The following keywords are reserved for **Extra Domain** types:

TYPE:ZIPPER	a leucine zipper
TYPE:FUSION	a fusion protein
TYPE:OTHER	a type of extra domain not explained by any reserved keywords (explained in a NOTE comment)

The following keywords are reserved for **Chemical Moiety** types:

TYPE:OPDM	a thiol-thiol chemical crosslinker (orthophenylenedimaleimide)
TYPE:SPDP	an amine-amine chemical crosslinker (succinimidyl 3-(2-pyridyldithio)propionate)
TYPE:SMCC	a thiol-amine chemical crosslinker (succinimidyl 4-(N-maleidomethyl)cyclohexane-1-carboxylate)
TYPE:OTHER	a type of extra domain not explained by any reserved keywords (explained in a NOTE comment)

14.2 MOD - allowed keywords

MOD:ENHANCEFCRN	a modification to enhance FcRn binding
MOD:ENHANCEADCC	a modification to increase antibody dependent cell-mediated cytotoxicity
MOD:STRANDEXCHANGE	a modification for strand exchange engineered domains
MOD:DISULPHIDE	a modification for additional disulphide bonds
MOD:DISULFIDE	a modification for additional disulphide bonds
MOD:REMDISULPHIDE	a modification for removal of disulphide bonds
MOD:REMDISULFIDE	a modification for removal of disulphide bonds
MOD:PI	a modification to alter the isoelectric point
MOD:CONJUGATION	a modification for a specific conjugation site
MOD:HEXAMER	a hexamer formation of IgG
MOD:NOFCGR	a modification to reduce FcRn binding
MOD:NOPROTEINA	a modification to reduce ProteinA binding
MOD:NOOX	a modification to reduce oxidation
MOD:NOADCC	a modification to reduce antibody dependent cell-mediated cytotoxicity
MOD:NOCDC	a modification to reduce complement dependent cytotoxicity
MOD:NOADCP	a modification to reduce antibody dependent cellular phagocytosis
MOD:NOADCCCDC	a modification to reduce ADCC and CDC
MOD:NOGLYCOS	a modification to remove glycosylation site (other than the CH2 one which has its own symbol)
MOD:NOADE	a modification to remove prevent antibody dependent enhancement of viral uptake
MOD:NOAGG	a modification to reduce aggregation
MOD:NOPROT	a modification to reduce proteolysis
MOD:REMCYS	a modification to remove free cysteine or a disulphide
MOD:STABILIZATION	a modification for stabilization
MOD:AFFINITY	a modification to increase or decrease affinity
MOD:OTHER	a modification not explained by any reserved keywords (explained in a NOTE comment)

15 Sequence Data

While AbML is designed for indicating domain connectivity and interactions, sequence data can also be associated with domains using the `ASEQ()` and `DSEQ()` keywords (for amino acid and DNA sequences respectively). These are used after the end of the standard AbML. The domain number is given in parentheses followed by and = sign and the sequence ending with a semicolon. For example, to specify the amino acid sequence of domain 1, you would use:

```
ASEQ(1)=EVQLQQSGAELMKPGASVKISCKASGYTFSDYWIEWVKQRPGHGLEWIGEILPGSGSTNY
        HERFKGKATFTADTSSSTAYMQLNSLTSEDSGVYYCLHGNYDFDGWGQGTTLTV;
```