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The conserved domain database in 2023

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ABSTRACT

NLM's conserved domain database (CDD) is a collection of protein domain and protein family models constructed as multiple sequence alignments. Its main purpose is to provide annotation for protein and translated nucleotide sequences with the location of domain footprints and associated functional sites, and to define protein domain architecture as a basis for assigning gene product names and putative/predicted function. CDD has been available publicly for over 20 years and has grown substantially during that time. Maintaining an archive of pre-computed annotation continues to be a challenge and has slowed down the cadence of CDD releases. CDD curation staff builds hierarchical classifications of large protein domain families, adds models for novel domain families via surveillance of the protein 'dark matter' that currently lacks annotation, and now spends considerable effort on providing names and attribution for conserved domain architectures. CDD can be accessed at https://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml.

INTRODUCTION AND OVERVIEW

CDD aims to collect a comprehensive set of protein and domain family models, and it does allow for considerable redundancy in the model set, to ensure good coverage of the protein space. Models that provide significantly overlapping annotation are clustered into protein domain superfamilies, and when domain annotation fails to exceed critical model-specific score thresholds, CDD by default reports superfamily annotation rather than individual model hits. For each model, we compute a consensus sequence, which is used for display purposes only, and reflects the length of the position-specific score matrix (PSSM). While consensus sequences are visible and made available, CDD is not a sequence collection, but is rather meant to enrich the annotation of existing sequence collections. The current CDD version, v3.20, contains 59 693 protein- and protein domain-models obtained from Pfam (1) version 34, SMART (2), the COGs collection (3), TIGRFAMS (4), the NCBI Protein Clusters collection (5), NCBIfam (6) and CDD's in-house data curation effort (7) (about 1600 new or updated models). For CDD v3.20, the fixed assumed size of the domain model database has again been increased to match the current size of the model collection, resulting in marginally higher E-values reported by RPS-BLAST (8). The upcoming CDD release v3.21 will contain a revised COGs collection, Pfam version 35 and around 500 new or updated models from CDD's in-house curation. Table 1 details the composition of CDD release v3.20 and the contributions from each source database.

The sizes of these source databases vary considerably, as they were assembled for distinct purposes, and may only consider subsets of the protein space, such as limited by taxonomy. Neither of them is a complete accounting of protein and protein domain families, and neither is the aggregate of many resources, as (1) available protein sequence collections are expanding rapidly and becoming more diverse and (2) limited curation resources impact further growth.

Table 2 shows the 20 largest classifications for common and functionally diverse domain families that have recently been updated or added to CDD. Hierarchical classifications are revisited as curation resources permit. Quite commonly, the analysis of novel 'dark matter' families suggests membership in a previously established superfamily classification, for example, or the availability of newly determined 3D structures suggests changes to the multiple sequence alignments that may have an impact on classification details. Over 4700 models curated by the CDD group have been newly published or updated since CDD release v3.16. As of now, a total of 42 937 site annotations are available on 15 943 out of 18 882 CDD staff-curated domain models. Sequence patterns have been recorded for 4971 of these site annotations, so that pattern matching can be used to

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Table 1. Composition of the CDD model collection

Data source	Version	Number of models
Pfam	34	19 178
CDD in-house curation	3.20	18 882
Protein Clusters	(25 October 2021)[a]	10 140
COGs	1	4871
TIGRFAM	15	4488
NCBIfams	(25 October 2021)	1125
SMART	6*	1009
CDD superfamily clusters	3.20	4541

[a]Recent changes to the protein clusters and SMART collections were the removal of models considered redundant.

Table 2. The largest domain family hierarchies created or updated since CDD release v3.17

Root	Models	Name
cd14964	592	seven-transmembrane G protein-coupled receptor superfamily
cd00590	586	RNA recognition motif (RRM) superfamily
cd13968	585	Catalytic domain of the Protein Kinase superfamily
cd00162	417	RING finger (Really Interesting New Gene) domain and U-box domain superfamily
cd00174	328	Src Homology 3 domain superfamily
cd00900	327	Pleckstrin homology-like domain
cd00196	311	Beta-grasp ubiquitin-like fold
cd00096	291	Immunoglobulin domain
cd01165	242	BTB/POZ domain superfamily
cd00648	239	Type 2 periplasmic binding fold superfamily
cd15489	221	PHD finger superfamily
cd00083	202	basic Helix Loop Helix (bHLH) domain superfamily
cd01391	191	Type 1 periplasmic binding fold superfamily
cd06174	185	Major Facilitator Superfamily
cd14494	171	Cys-based protein tyrosine phosphatase and dual-specificity phosphatase superfamily
cd17912	170	N-terminal helicase domain of the DEAD-box helicase superfamily
cd08368	159	LIM domain superfamily
cd00014	158	calponin homology (CH) domain superfamily
cd00194	157	UBA domain-like superfamily
cd00105	153	K homology (KH) RNA-binding domain, type I

The table lists the root node of each hierarchy, the number of models in the hierarchy (including the root node and intermediate nodes if present), and the name of the protein domain (super) family.

decide whether annotation can be mapped on to individual sequences.

We identify as ‘dark matter’ those protein sequences in a collection of non-redundant representatives from a taxonomically diverse set, which do not yet have conserved domain annotation via CDD. These are clustered into sequence-similar groups, and multiple sequence alignment models are created for clusters that contain either (1) sequences obtained from experimentally determined 3D structure, or (2) sequences associated with publications, unless these publications describe very large sequence sets such as complete genomes. These alignment models together with the sources that may provide functional information are triaged by curation staff and selected for inclusion in CDD if generic functional information can be provided, as a minimum.

SARS-CoV-2

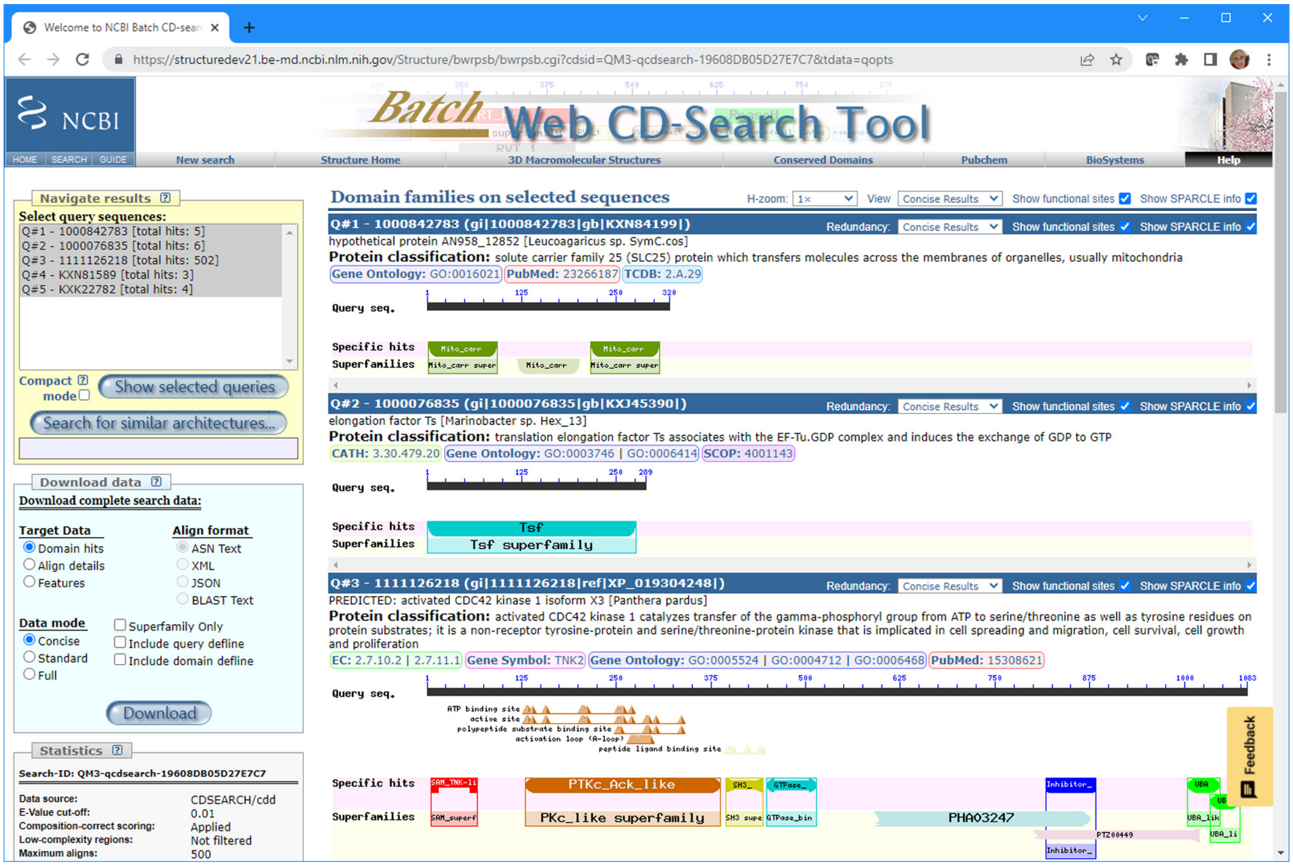
In early 2020, CDD version v3.18 was released and re-released a short time later, as new curated models were added to provide complete annotation of the SARS-CoV-2 proteins. A list of those proteins mapped to domain models and experimentally determined 3D structures is available via https://www.ncbi.nlm.nih.gov/Structure/SARS-CoV-2.html. As a result of this work, a detailed classification of the conserved catalytic core domain of the RNA-dependent RNA polymerase (RdRp) from the positive-sense single-stranded RNA [(+)ssRNA] viruses and closely related viruses has been provided under the root accession cd23167.

Model-specific word score thresholds

Beginning with the release of CDD v3.19, we have been optimizing RPS-BLAST (8) search databases for performance. The BLAST heuristics implemented in RPS-BLAST uses initial matches of query three-letter words via a large lookup table that records matches on individual database PSSMs (position-specific score matrices) crossing a word-score threshold. Initially, a uniform word-score threshold was used across the entire search database. The selection of the word-score threshold implies a trade-off between search speed and sensitivity. The threshold affects the size of the lookup table, which is directly linked to search speed. A large fraction of the time spent by the BLAST heuristic goes into exploring initial word hits and resulting local alignments that will eventually be abandoned and not reported. By raising the word-score threshold, many of these futile cycles can be avoided. The majority of the models included in the CDD collection do not require a very low word-score threshold in order to be identified as matches by members of the respective protein (domain) family. We have implemented a simple procedure to determine suitable model-specific thresholds, which uses a list of bona-fide family members, either as provided by the model data source (such as Pfam or COGs), or as detected by RPS-BLAST with significant score when using a low default word-score threshold. We then titrate the word-score threshold up to a point where RPS-BLAST will still detect matches for >99% of those family members. Overall, this change had very little impact on concise domain annotation (which favors the best-scoring hits) and derived domain architectures. We were able to reduce RPS-BLAST runtime for typical searches by a factor of 3, approximately. This has helped to keep up with the rapid growth of the sequence databases while still providing pre-computed results for a large set of representative sequences.

SPARCLE superfamily architectures

We define protein domain architectures as the sequential (N- to C-terminal) list of one or more domain footprints annotated on a protein sequence. SPARCLE, which stands for ‘Subfamily Protein Architecture Labeling Engine’, groups proteins by domain architecture, and now considers both specific and superfamily domain architectures. Superfamily architectures collect proteins that have



https://structuredev21.be-md.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi?cdsid=QM3-qcdsearch-19608DB05D27E7C7&tdata=qopts

Navigate results

Select query sequences:

Q#1 - 1000842783 [total hits: 5]
Q#2 - 1000076835 [total hits: 6]
Q#3 - 1111126218 [total hits: 502]
Q#4 - KXN81589 [total hits: 3]
Q#5 - KXK22782 [total hits: 4]

Compact mode □ Show selected queries

Search for similar architectures...

Download data

Download complete search data:

Target Data: ● Domain hits ○ Align details ○ Features

Align format: ○ ASN Text ○ XML ○ JSON ○ BLAST Text

Data mode: ● Concise ○ Standard ○ Full

□ Superfamily Only
□ Include query defline
□ Include domain defline

Download

Statistics

Search-ID: QM3-qcdsearch-19608DB05D27E7C7

Data source:	CDSEARCH/cdd
E-Value cut-off:	0.01
Composition-correct scoring:	Applied
Low-complexity regions:	Not filtered
Maximum aligns:	500

Domain families on selected sequences

H-zoom: 1× View: Concise Results Show functional sites ☑ Show SPARCLE info ☑

Q#1 - 1000842783 (gi|1000842783|gb|KXN84199|) Redundancy: Concise Results Show functional sites ✓ Show SPARCLE info ✓

hypothetical protein AN958_12852 [Leucoagaricus sp. SymC.cos]

Protein classification: solute carrier family 25 (SLC25) protein which transfers molecules across the membranes of organelles, usually mitochondria

Gene Ontology: GO:0016021 | PubMed: 23266187 | TCDB: 2.A.29

Q#2 - 1000076835 (gi|1000076835|gb|KXJ45390|) Redundancy: Concise Results Show functional sites ✓ Show SPARCLE info ✓

elongation factor Ts [Marinobacter sp. Hex_13]

Protein classification: translation elongation factor Ts associates with the EF-Tu.GDP complex and induces the exchange of GDP to GTP

CATH: 3.30.479.20 | Gene Ontology: GO:0003746 | GO:0006414 | SCOP: 4001143

Q#3 - 1111126218 (gi|1111126218|ref|XP_019304248|) Redundancy: Concise Results Show functional sites ✓ Show SPARCLE info ✓

PREDICTED: activated CDC42 kinase 1 isoform X3 [Panthera pardus]

Protein classification: activated CDC42 kinase 1 catalyzes transfer of the gamma-phosphoryl group from ATP to serine/threonine as well as tyrosine residues on protein substrates; it is a non-receptor tyrosine-protein and serine/threonine-protein kinase that is implicated in cell spreading and migration, cell survival, cell growth and proliferation

EC: 2.7.10.2 | 2.7.11.1 | Gene Symbol: TNK2 | Gene Ontology: GO:0005524 | GO:0004712 | GO:0006468 | PubMed: 15308621

Figure 1. BATCH CD-Search results formatted for a few query sequences demonstrate the availability of domain architecture information (under the heading 'Protein classification'), as well as transferable attributes assigned to each architecture, on top of domain footprint annotation and functional sites associated with some of the domain models. The protein classification information and site annotations can be toggled off for a sparse display focusing on domain footprints only.

Table 3. URLs and other resources associated with the CDD project

URL	Description
https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi	CD-Search interface utilizing the RPS-BLAST algorithm and the model database, and to the CDART database of pre-computed domain annotation
https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi	BATCH CD-Search interface utilizing the RPS-BLAST algorithm and the model database, and to the CDART database of pre-computed domain annotation. Up to 1000 protein queries may be submitted per request, and the size of queries is restricted to no more than 40 000 residues.
https://www.ncbi.nlm.nih.gov/cdd	Entrez search interface to CDD
https://ftp.ncbi.nih.gov/pub/mmdb/cdd	CDD FTP site, see README file for content
https://ftp.ncbi.nlm.nih.gov/toolbox executables can be obtained from: https://www.ncbi.nlm.nih.gov/BLAST/download.shtml	RPS-BLAST stand-alone tool for searching databases of profile models, part of the NCBI toolkit distribution
https://www.ncbi.nlm.nih.gov/protfam	Entrez interface to the Protein Families collection, which includes SPARCLE domain architectures
https://ftp.ncbi.nlm.nih.gov/pub/mmdb/cdd/rpsbproc	Standalone utility for enriching and formatting RPS-BLAST results
https://ftp.ncbi.nlm.nih.gov/pub/mmdb/cdd/SparcleLabel/	Standalone utility for naming/labeling proteins using SPARCLE

significant hits to one or more domains that do not exceed a model-specific bitscore thresholds that labels them as high-confidence or 'specific'. Typically, they will be assigned more generic names, while specific domain architectures may group more closely releated proteins and be assigned more precise and informative names and functional descriptions. To date, CDD curators have reviewed and assigned names and functional labels to about 40,000 well-represented architectures, with a focus on those common in bacterial genomes and those providing coverage for the genomes of eukaryotes such as fungi, protists, and nematodes, in work aimed at supporting the NIH Comparative Genome Resource (CGR, https://www.ncbi.nlm.nih.gov/comparative-genomics-resource/). A publicly accessible Entrez database supports text queries into protein family models that include not only architectures, but also

HMMs, and NCBI BLAST rules, and points to summary information as well as links to other databases and sources of attribution.

For the past year, we added attribution to conserved domain architecture, which can be mapped to individual architecture member sequences. Attribution sources are (i) citations, recorded as PubMed IDs, (ii) E.C. numbers (9), (iii) GO terms (10), (iv) gene symbols, (v) TCDB identifiers (11), (vi) CAZy identifiers (12), (vii) MEROPS identifiers (13), (viii) SCOP (14) and/or CATH (15) identifiers. These are assigned and validated by curation staff and are now displayed on CD-Search and BATCH CD-Search pages if the user query matches the corresponding curated domain architecture (see Figure 1).

SPARCLE architecture curation supports the automated, evidence-based assignment of names to proteins in RefSeq and the Prokaryotic Genome Annotation Pipeline (PGAP) (6). At this time, about 60 million bacterial RefSeq proteins are named via SPARCLE (out of 195 million total bacterial proteins and 160 million proteins with naming evidence provided).

DATA AVAILABILITY

Table 3 lists URLs for major services, tools, and data collections provided by CDD. RPS-BLAST is a part of NCBI's BLAST software distribution, while CDD provides pre-formatted RPS-BLAST search databases, so that conserved domain searches can be run locally. The rpsbproc stand-alone utility uses additional data to format RPS-BLAST results so that they agree with reports generated by CD-Search (16) and BATCH CD-Search, including domain superfamily assignments and site annotations. The sparclbl (SparcleLabel) utility processes results from local RPS-BLAST searches and provides suggestions for protein names based on domain architecture and is used in NCBI's prokaryotic genome annotation pipeline (PGAP) (6).

CONCLUSION

The major focus of ongoing work at CDD is to ensure sustainability of the data flow and public services. We strive to keep up with the growth of the sequence collections and to provide a source of annotation that maintains relatively high coverage while improving accuracy. We continue to add novel domain family models to the collection and to establish hierarchical classifications of selected protein domain families where they will have a significant impact on protein naming by domain architecture. We are investigating whether we'll be able to provide meaningful filtering of search results by taxonomy, for cases where the taxonomic source of the user query sequence is known, and how this knowledge could be used to speed up searching.

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