

CATH: expanding the horizons of structure-based functional annotations for genome sequences

Ian Sillitoe^{1,*}, Natalie Dawson¹, Tony E. Lewis¹, Sayoni Das¹, Jonathan G. Lees¹, Paul Ashford¹, Adeyelu Tolulope¹, Harry M. Scholes¹, Ilya Senatorov¹, Andra Bujan¹, Fatima Ceballos Rodriguez-Conde¹, Benjamin Dowling¹, Janet Thornton² and Christine A. Orengo¹

¹Structural and Molecular Biology, University College London WC1E 6BT, UK and ²European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridgeshire CB10 1SD, UK

Received September 15, 2018; Revised October 16, 2018; Editorial Decision October 18, 2018; Accepted November 02, 2018

ABSTRACT

This article provides an update of the latest data and developments within the CATH protein structure classification database (<http://www.cathdb.info>). The resource provides two levels of release: CATH-B, a daily snapshot of the latest structural domain boundaries and superfamily assignments, and CATH+, which adds layers of derived data, such as predicted sequence domains, functional annotations and functional clustering (known as Functional Families or FunFams). The most recent CATH+ release (version 4.2) provides a huge update in the coverage of structural data. This release increases the number of fully-classified domains by over 40% (from 308 999 to 434 857 structural domains), corresponding to an almost two-fold increase in sequence data (from 53 million to over 95 million predicted domains) organised into 6119 superfamilies. The coverage of high-resolution, protein PDB chains that contain at least one assigned CATH domain is now 90.2% (increased from 82.3% in the previous release). A number of highly requested features have also been implemented in our web pages: allowing the user to view an alignment between their query sequence and a representative FunFam structure and providing tools that make it easier to view the full structural context (multi-domain architecture) of domains and chains.

INTRODUCTION

CATH is an online, publicly accessible resource that provides a classification of protein domains based on structural data deposited into the worldwide Protein Data Bank (wwPDB) (1). The resource splits 3D structures into con-

stituent domains (considered as semi-independently folding globular units) and clusters these domains into homologous superfamilies where there is sufficient evidence of evolutionary similarity (2). CATH uses a number of sensitive structure-comparison and sequence comparison tools (including SSAP (3), HMMER3 hmmmer.org, PRC (4)) to assist the manual curation of these remote evolutionary relationships. Homologous superfamilies are organised into a hierarchy of Class, Architecture and Topology according to an increasing degree of structural similarity.

Knowledge of 3D protein structure is hugely valuable when investigating remote evolutionary relationships and detailed functional mechanisms, however the number of solved 3D structures is relatively small compared to the number of protein sequences. In order to harness the information available in the sequence databases, CATH uses a set of representative structural domains to ‘seed’ a set of sequence alignments (HMMER, hmmmer.org). These alignments are converted into hidden Markov models (HMMs) and the resulting library of sequence-based ‘fingerprints’ is used to identify the location of closely related domains within protein sequences taken from the genome databases UniProtKB (5) and Ensembl (6).

By combining protein structure and sequence, the CATH resource provides comprehensive structure-based domain superfamily assignments to over 95 million protein sequences (7). In many cases, these annotated proteins are already associated with a considerable amount of additional functional annotation (e.g. UniProtKB provides annotations such Gene Ontology (GO) (8), Enzyme Commission (EC) terms (9) and Catalytic Site Atlas (10)).

The Homologous Superfamily (H) level is useful for investigating distant evolutionary relationships as it groups domains that have the same structural core but may have structural embellishments to that core that result in diverse functions. Therefore, to investigate more finely tuned features, such as the evolution of functional sites, superfam-

*To whom correspondence should be addressed. Tel: +44 20 7679 2171; Fax: +44 20 7679 2000; Email: i.sillitoe@ucl.ac.uk

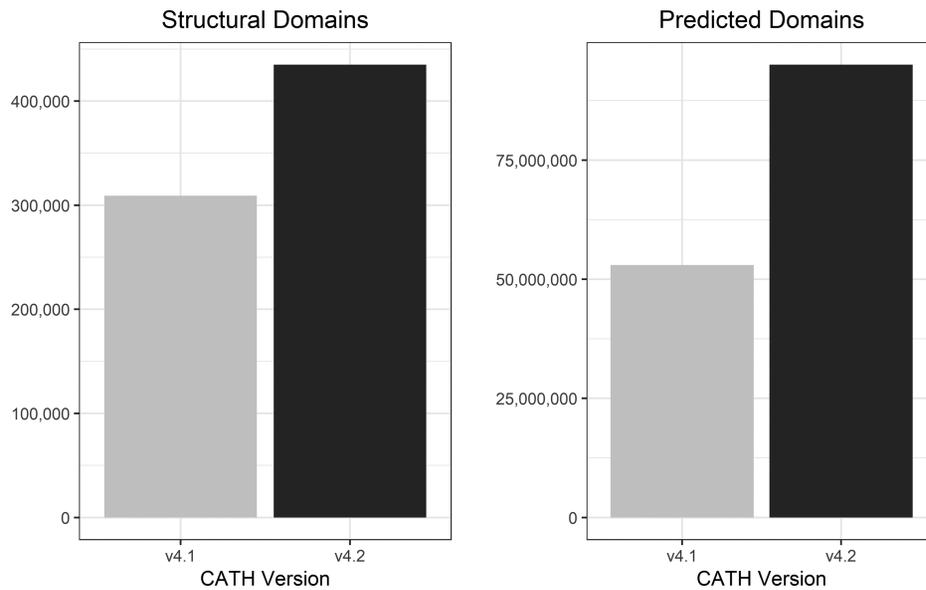


Figure 1. Comparison of the structural domains and predicted (sequence) domains between CATH+ releases 4.1 and 4.2.

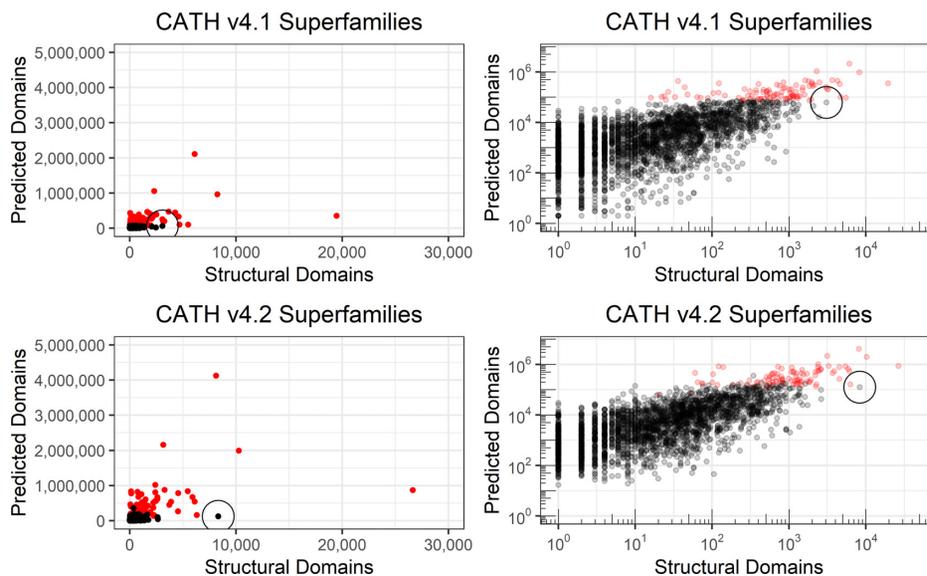


Figure 2. Superfamilies in CATH v4.1 and v4.2 highlighting the number of structural domains and predicted domains (shown with a linear and logarithmic scale). Each dot represents a superfamily and the largest 100 superfamilies (according to number of predicted domains in CATH v4.1) have been highlighted in red. These superfamilies contain more than half of all known protein domains. To help illustrate the growth of data, an example superfamily (3.60.20.10) has been circled in each plot. The number of structural domains in this superfamily increased 2.7-fold from v4.1 to v4.2 (3074 to 8328 domains), however this corresponded to only a small increase in the number of predicted domains. On investigation, this superfamily contains domains from a number of large proteasomes, which contain many copies of identical (or very similar) structural domains.

ilies are also broken into smaller, more focussed groups of domains called Functional Families (FunFams). FunFams aim to group domain sequences that perform a similar function and are created via a functional classification protocol based on distinguishing sequence patterns (11,12). The resulting clusters of domains have been found to be more functionally coherent than other domain-based resources (11). While this is a non-trivial task (13–16), it is extremely useful when attempting to correlate functional annotations with sequence patterns (11). Function predic-

tion pipelines based on the FunFams have been consistently ranked among the top 10 function prediction methods by the international CAFA competition (17,18). Since structures within FunFams tend to be highly conserved (even at low sequence identity), these clusters have also been found to be useful when suggesting templates for 3D modelling (19).

The resource allows users to search for matching FunFams by submitting a query protein sequence to the web pages (cathdb.info/search/by_sequence), or through the

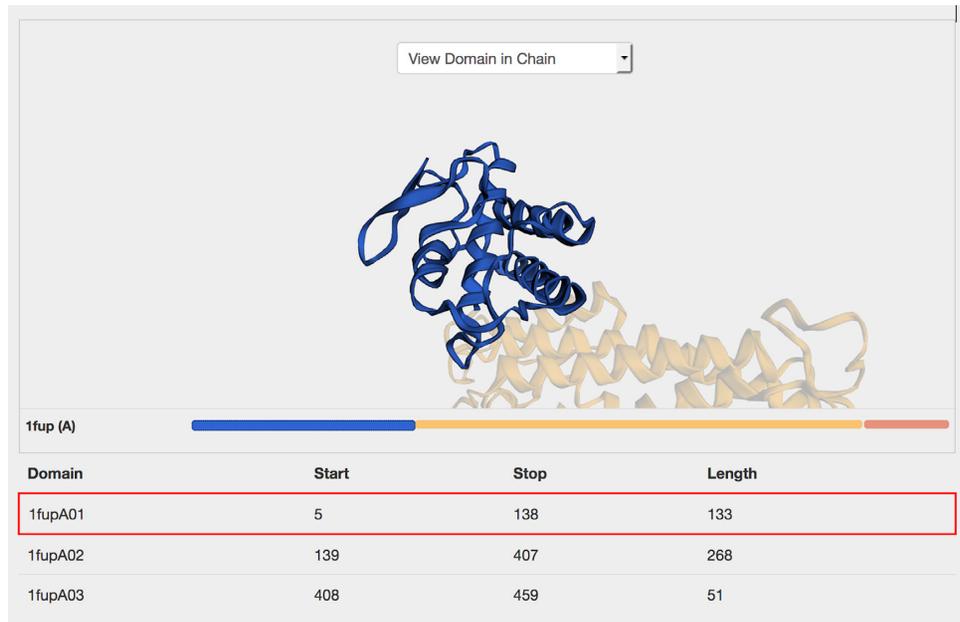


Figure 4. Interactive links between 3D structure (3DMol.js) and multi-domain architecture (MDA) allow the user to view each domain in the context of the full chain.

guaranteed that no two domains have more than 20%/40% sequence identity (at 60% overlap). If groups are interested in using CATH domains for benchmarking or training their own algorithms, it is strongly recommended that they take advantage of these datasets (see Availability).

CATH+ adds many layers of information on top of the classification of domain boundaries and Homologous superfamily, and the process of creating a CATH+ release takes approximately 12–18 months. The first step in the process of generating a CATH+ release is to ‘freeze’ the incoming data from the wwPDB. While this is important to ensure consistency when calculating additional layers of information, it also means that the latest PDB structures may be missing from a CATH+ release. To address this, we offer a daily snapshot containing the very latest domain boundary and superfamily annotations (CATH-B). At the time of writing (Sep, 2018), CATH-B contained a further 46 906 structural domains since CATH+ 4.2 was ‘frozen’ (July 2017).

ADDITIONAL FEATURES IN CATH

Improved superfamily names and descriptions

Since the last release, there has been considerable effort to review existing superfamily names and provide names for unnamed superfamilies. Superfamilies in CATH were mapped to a number of related resources (InterPro, Pfam, SCOP, UniProtKB) as a means to provide evidence for names that were unique and informative while still representing the potentially diverse members in each cluster. This manual curation work resulted in 1,255 superfamily names being reviewed and standardised with respect to equivalent clusters in InterPro. It also generated names and detailed descriptions for an additional 1215 CATH superfamilies that had not yet been annotated.

Alignment of query sequences against FunFams

The FunFHMMER server is a web tool that allows users to search a query protein sequence against a library of FunFam HMMs (11). A search typically takes a few seconds and then the user is provided with a list of significant FunFam matches with a visual indication of the location of each match on the original query sequence. In previous releases, the user was able to click through to web pages describing these FunFams in more detail (e.g. to view a multiple sequence alignment with highly conserved positions highlighted on a representative 3D structure), however they were not able to see their own sequence in that alignment. A highly requested feature has been to provide users with the opportunity to view their own query sequence aligned to this structure. This feature has been added and the query sequence is automatically inserted as the first sequence in the FunFam alignment when following links from the search page (Figure 3).

Visualising structural context of domains

While it is important to consider CATH domains as semi-independent globular units, it is also important to consider the context in which each domain appears in the native protein, i.e. the multi-domain architecture. This is crucial when considering functional annotations (such as GO terms) since these are made at the protein level, rather than the domain level. To highlight the structural context of domains within the full protein, a new interactive component has been added to the pages that combines a 3D structural viewer (3DMol.js (24)) with a simple 1D schematic of the multi-domain architecture (MDA) of the protein chain (Figure 4). This enables users to view the domain within the context of the PDB chain (‘View Domain in Chain’) or in the context of the full PDB (‘View Domain in PDB’). In

these modes, clicking a domain in the MDA adds focus to the domain in the 3D viewer while ghosting the surrounding domain partners.

CONCLUSION

This article describes the latest release of CATH version 4.2 that contains over 430,000 structural domains and over 95 million predicted domain sequences. This represents a huge increase in coverage both in terms of coverage of the PDB and of sequences in UniProtKB.

A number of highly requested features have also been implemented in our web pages: allowing the user to view an alignment between their query sequence and a representative FunFam structure and providing tools that make it easier to view the full structural context (multi-domain architecture) of domains and chains.

DATA AVAILABILITY

Daily snapshot of latest annotations (CATH-B) is available to download at <ftp://orengoftp.biochem.ucl.ac.uk/cath/releases/daily-release>.

CATH+ data is available to download at ftp://orengoftp.biochem.ucl.ac.uk/cath/releases/all-releases/v4_2_0/ and can be browsed through the web site at <http://www.cathdb.info>

Datasets of non-redundant domains are available to download at: <ftp://orengoftp.biochem.ucl.ac.uk/cath/releases/latest-release/non-redundant-data-sets/>

FUNDING

Wellcome Trust [104960/Z/14/Z to N.D., 203780/Z/16/A to H.S]; Biotechnology and Biological Sciences Research Council [BB/K020013/1 to I.S., BB/N019253/1 to S.D.]. Funding for open access charge: University College London [BB/K020013/1].

Conflict of interest statement. None declared.

REFERENCES

- Berman, H., Henrick, K., Nakamura, H. and Markley, J.L. (2007) The worldwide Protein Data Bank (wwPDB): ensuring a single, uniform archive of PDB data. *Nucleic Acids Res.*, **35**, D301–D303.
- Dawson, N.L., Lewis, T.E., Das, S., Lees, J.G., Lee, D., Ashford, P., Orengo, C.A. and Sillitoe, I. (2017) CATH: an expanded resource to predict protein function through structure and sequence. *Nucleic Acids Res.*, **45**, D289–D295.
- Orengo, C.A. and Taylor, W.R. (1996) SSAP: sequential structure alignment program for protein structure comparison. *Methods Enzymol.*, **266**, 617–635.
- Brandt, B.W. and Heringa, J. (2009) webPRC: the Profile Comparer for alignment-based searching of public domain databases. *Nucleic Acids Res.*, **37**, W48–W52.
- UniProt Consortium, T. (2018) UniProt: the universal protein knowledgebase. *Nucleic Acids Res.*, **46**, 2699.
- Aken, B.L., Achuthan, P., Akanni, W., Amode, M.R., Bernsdorff, F., Bhai, J., Billis, K., Carvalho-Silva, D., Cummins, C., Clapham, P. *et al.* (2017) Ensembl 2017. *Nucleic Acids Res.*, **45**, D635–D642.
- Lewis, T.E., Sillitoe, I., Dawson, N., Lam, S.D., Clarke, T., Lee, D., Orengo, C. and Lees, J. (2018) Gene3D: extensive prediction of globular domains in proteins. *Nucleic Acids Res.*, **46**, D435–D439.
- The Gene Ontology Consortium. (2017) Expansion of the Gene Ontology knowledgebase and resources. *Nucleic Acids Res.*, **45**, D331–D338.
- Tipton, K.F. (1992) Enzyme nomenclature 1992. Recommendations of the nomenclature committee of the international union of biochemistry and molecular biology on the nomenclature and classification of enzymes. *Acad. Press*, **223**, 1–5.
- Ribeiro, A.J.M., Holliday, G.L., Furnham, N., Tyzack, J.D., Ferris, K. and Thornton, J.M. (2018) Mechanism and Catalytic Site Atlas (M-CSA): a database of enzyme reaction mechanisms and active sites. *Nucleic Acids Res.*, **46**, D618–D623.
- Das, S., Lee, D., Sillitoe, I., Dawson, N.L., Lees, J.G. and Orengo, C.A. (2015) Functional classification of CATH superfamilies: a domain-based approach for protein function annotation. *Bioinformatics*, **31**, 3460–3467.
- Sillitoe, I., Cuff, A.L., Dessailly, B.H., Dawson, N.L., Furnham, N., Lee, D., Lees, J.G., Lewis, T.E., Studer, R.A., Rentzsch, R. *et al.* (2013) New functional families (FunFams) in CATH to improve the mapping of conserved functional sites to 3D structures. *Nucleic Acids Res.*, **41**, D490–D498.
- Brown, S.D. and Babbitt, P.C. (2014) New insights about enzyme evolution from large scale studies of sequence and structure relationships. *J. Biol. Chem.*, **289**, 30221–30228.
- Martinez Cuesta, S., Furnham, N., Rahman, S.A., Sillitoe, I. and Thornton, J.M. (2014) The evolution of enzyme function in the isomerases. *Curr. Opin. Struct. Biol.*, **26**, 121–130.
- Das, S. and Orengo, C.A. (2016) Protein function annotation using protein domain family resources. *Methods*, **93**, 24–34.
- Dessailly, B.H., Dawson, N.L., Mizuguchi, K. and Orengo, C.A. (2013) Functional site plasticity in domain superfamilies. *Biochim. Biophys. Acta*, **1834**, 874–889.
- Jiang, Y., Oron, T.R., Clark, W.T., Bankapur, A.R., D'Andrea, D., Lepore, R., Funk, C.S., Kahanda, I., Verspoor, K.M., Ben-Hur, A. *et al.* (2016) An expanded evaluation of protein function prediction methods shows an improvement in accuracy. *Genome Biol.*, **17**, 184.
- Radivojac, P., Clark, W.T., Oron, T.R., Schnoes, A.M., Wittkop, T., Sokolov, A., Graim, K., Funk, C., Verspoor, K., Ben-Hur, A., Pandey, G. *et al.* (2013) A large-scale evaluation of computational protein function prediction. *Nat. Methods*, **10**, 221–227.
- Lam, S.D., Dawson, N.L., Das, S., Sillitoe, I., Ashford, P., Lee, D., Lehtinen, S., Orengo, C.A. and Lees, J.G. (2016) Gene3D: expanding the utility of domain assignments. *Nucleic Acids Res.*, **44**, D404–D409.
- Dawson, N.L., Sillitoe, I., Lees, J.G., Lam, S.D. and Orengo, C.A. (2017) CATH-Gene3D: generation of the resource and its use in obtaining structural and functional annotations for protein sequences. *Methods Mol. Biol.*, **1558**, 79–110.
- Blomberg, N., Oliveira, A., Mons, B., Persson, B. and Jonassen, I. (2016) The ELIXIR channel in F1000Research [version 2; referees: not peer reviewed]. *F1000Research*, **4**, ELIXIR-1471.
- Dawson, N., Sillitoe, I., Marsden, R.L. and Orengo, C.A. (2017) The classification of protein domains. *Methods Mol. Biol.*, **1525**, 137–164.
- Valdar, W.S.J. (2002) Scoring residue conservation. *Proteins Struct. Funct. Genet.*, **48**, 227–241.
- Rego, N. and Koes, D. (2015) 3Dmol.js: molecular visualization with WebGL. *Bioinformatics*, **31**, 1322–1324.