Exploring structural diversity across the protein universe with The Encyclopedia of Domains

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Introduction

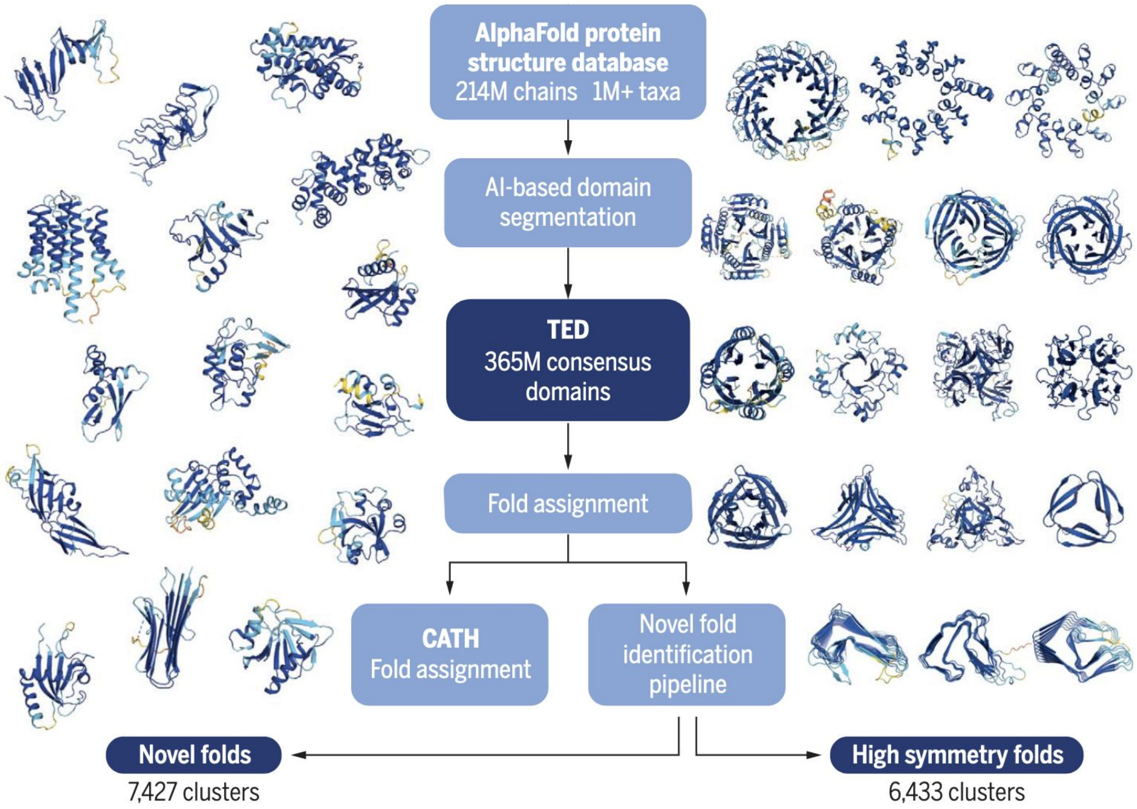
Analysis of domain composition for the entirety of the AFDB (version 4).

365 million putative domains derived from >214 million protein sequences across >1 million taxa.

Consensus of three automated parsing methodologies: Merizo, Chainsaw, and UniDoc.

Structural comparison methods such as Foldseek and an in-house deep learning method called Merizo-search

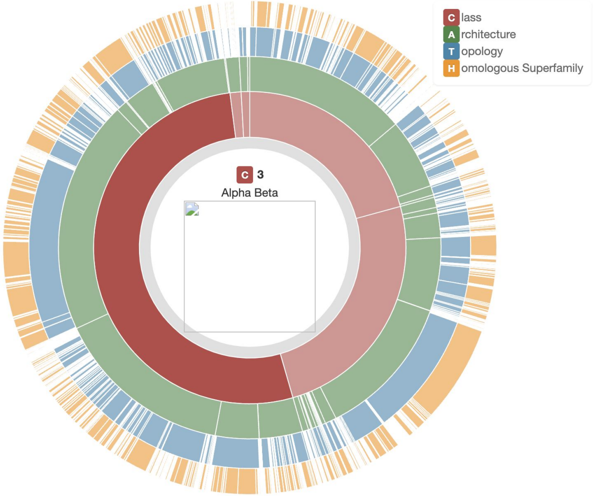
>251 million domai nsplaced on the CATH hierarchy.



TED workflow. TED consists of 365 million domains identified from the AFDB by taking a consensus from three state-of-the-art domain segmentation methods. Nonredundant domains are assigned with CATH labels, and unassigned domains are subjected to a novel fold identification pipeline. Overall, we found several thousand novel folds, as well as novel high-symmetry fold clusters.

What is CATH?

The CATH (Class, Architecture, Topology, Homology) database stands as one of the most important resources in structural biology, providing a hierarchical classification system for protein domains.



CATH Hierarchy

The name CATH reflects its four main levels of classification:

- **Class (C):** The most basic level, describing the protein domain's secondary structure composition
  - Mainly Alpha (α), Mainly Beta (β), Alpha/Beta (α/β), Few Secondary Structures
- **Architecture (A):** The general shape formed by the secondary structures
  - Barrel shapes, Sandwiches, Roll structures, Irregular structures
- **Topology (T):** The specific folding pattern of the secondary structures
- **Homology (H):** Groups of proteins with evidence of evolutionary relationship

CATH Examples

Example 1: Hemoglobin (CATH ID: 1.10.490.10)

- Class (1): Mainly Alpha - The protein is dominated by α-helices
- Architecture (10): Orthogonal Bundle - α-helices arranged at roughly right angles
- Topology (490): Globin-like - Specific arrangement of helices characteristic of globins
- Homology (10): Globin family - Shows clear evolutionary relationship to other globins

This classification tells us that hemoglobin belongs to a well-characterized family of proteins that share not just their structural features but also their evolutionary history and function of oxygen transport.

CATH Examples

Example 2: Triose Phosphate Isomerase (CATH ID: 3.20.20.70)

- Class (3): Alpha/Beta - Contains both α-helices and β-sheets
- Architecture (20): TIM Barrel - Named after this very enzyme
- Topology (20): TIM Barrel - Specific arrangement of 8 α-helices and 8 β-strands
- Homology (70): Aldolase superfamily - Related to other enzymes that catalyze similar reactions

This example shows how a protein's structure can become the archetypal representative of an entire architectural class (the TIM barrel).

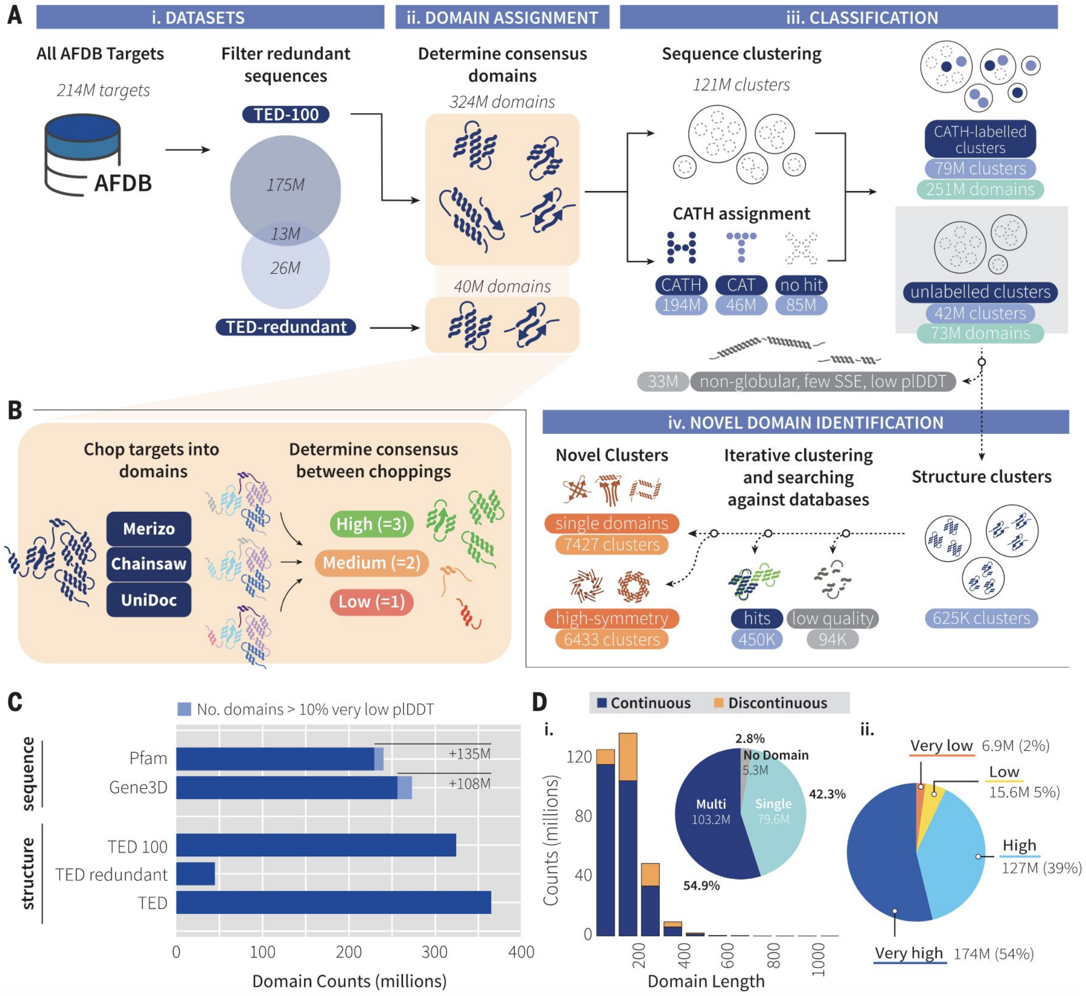
CATH Examples

Example 3: Immunoglobulin (CATH ID: 2.60.40.10)

- Class (2): Mainly Beta - Dominated by β-sheets
- Architecture (60): Sandwich - Two β-sheets packed face-to-face
- Topology (40): Immunoglobulin-like - Specific connectivity pattern of β-strands
- Homology (10): Immunoglobulin - Part of the antibody protein family

This classification demonstrates how structural arrangements can be optimized for specific functions, in this case, antigen recognition.

Workflow Of TED



Deriving a unified assignment of domains in the AFDB

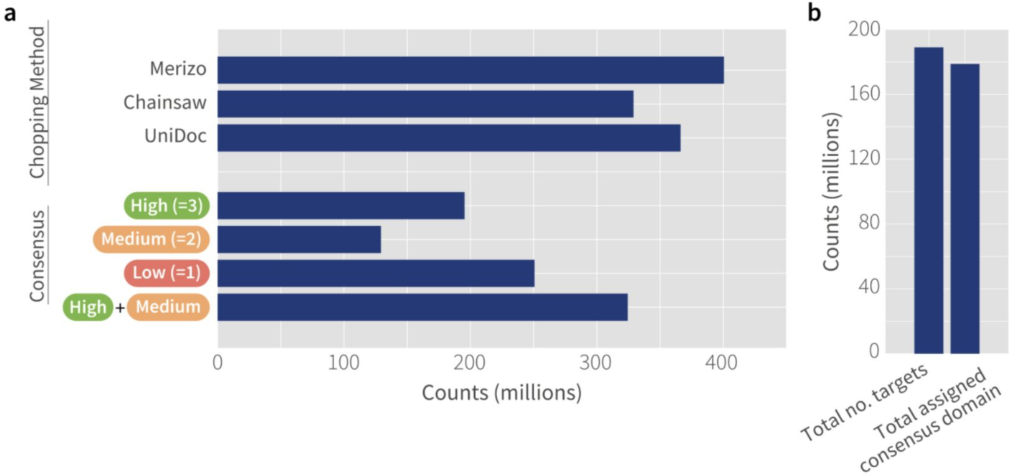


Fig. S2. Domain counts identified from consensus chopping methods in TED-100. (a)

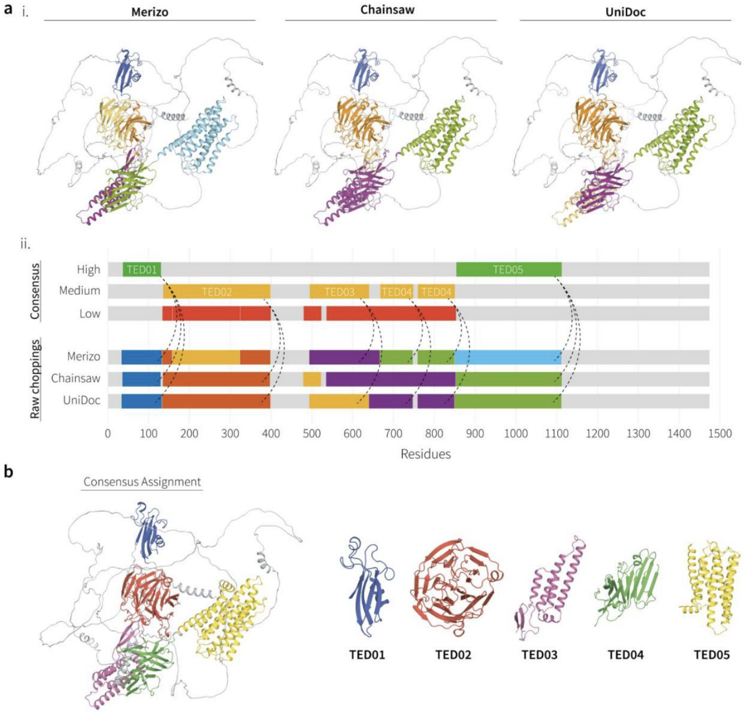


Fig. S14. Example of consensus domain derivation. **(a)** i. Example of predicted domain choppings from Merizo, Chainsaw and UniDoc for target AF-O94910-F1-model_v4 (Adhesion

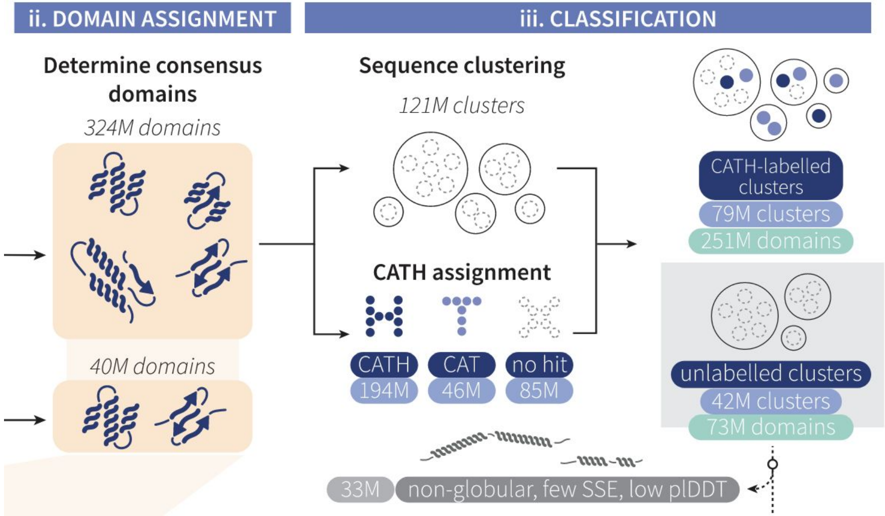
Domain classification

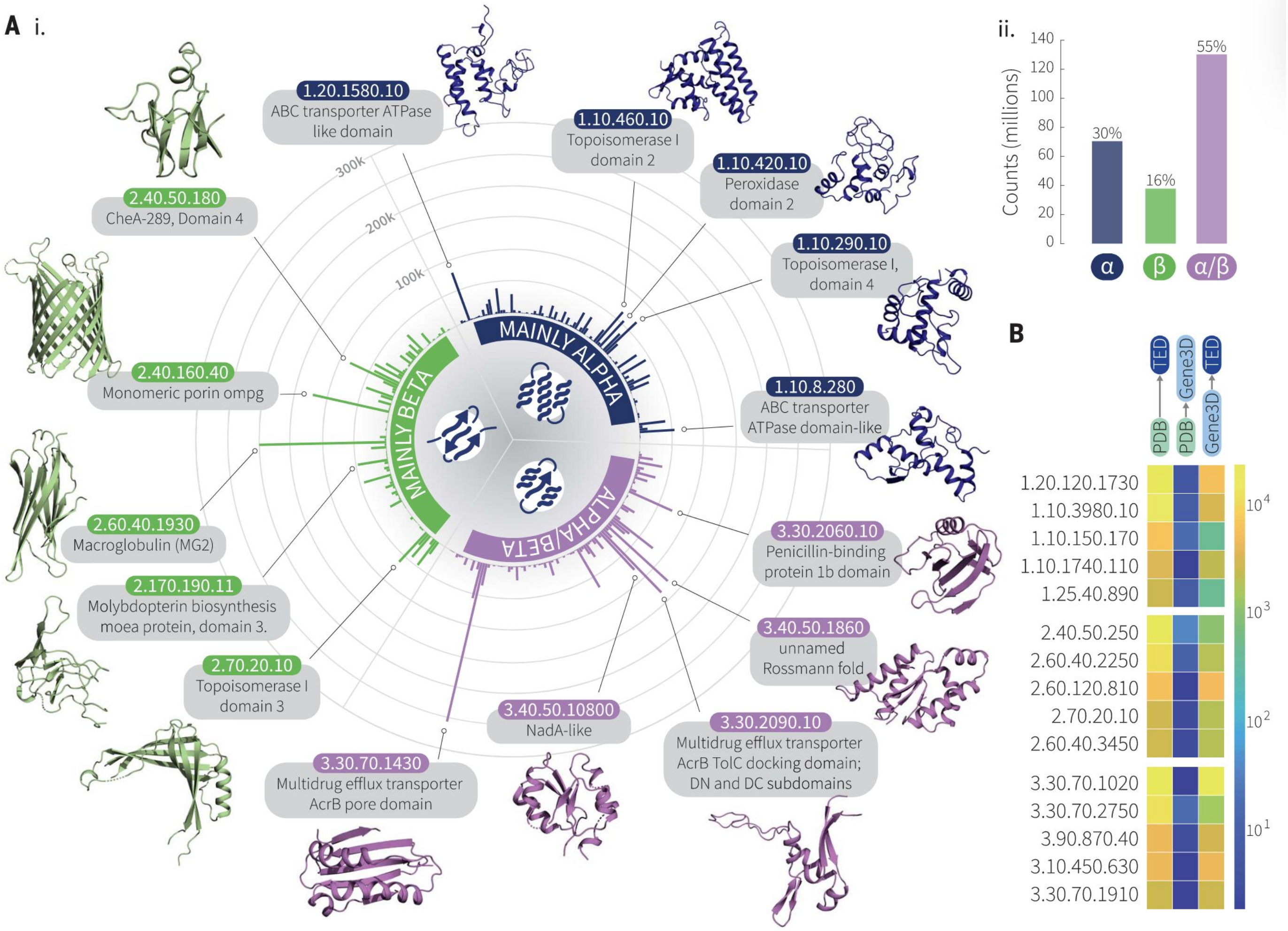
1. TED-100 domains are processed by MMseqs2, creating >121 million clusters at 50% identity.

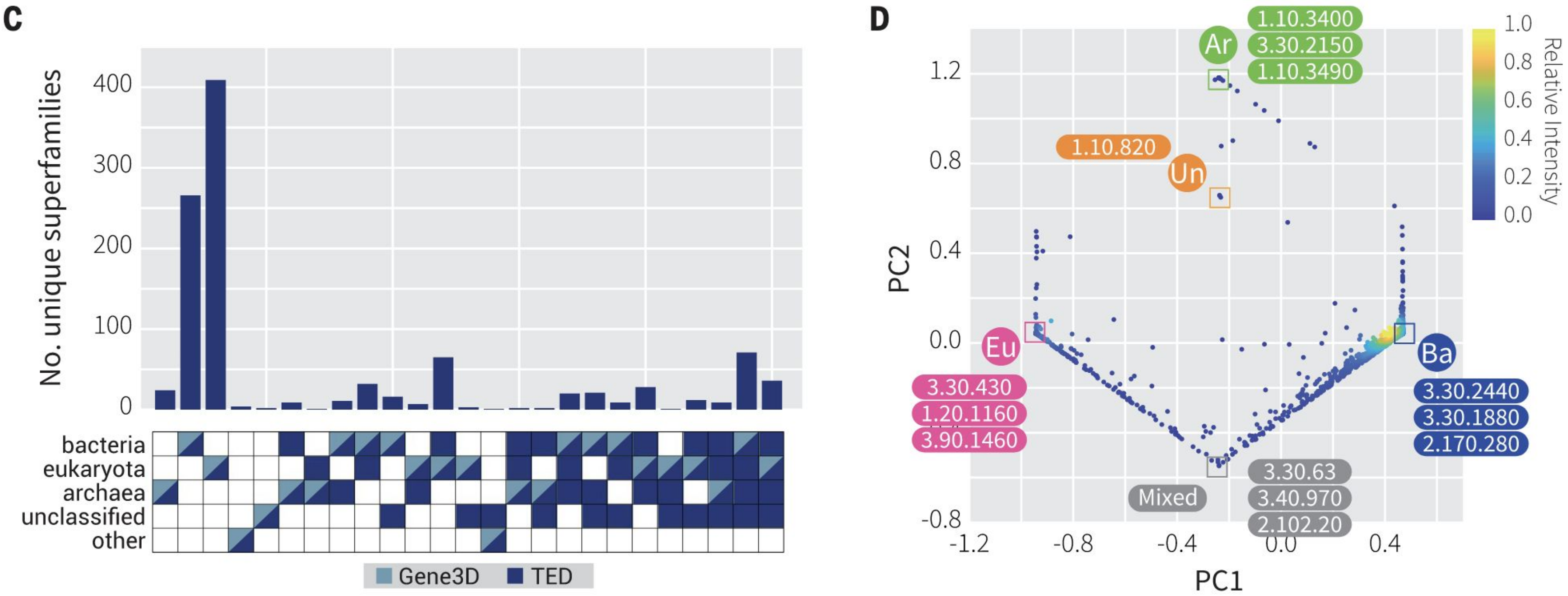
2. Foldseek was optimized using a curated CATH dataset (3186 domains) to establish thresholds for identifying superfamily (H-level) and fold (T-level) matches with 98% precision.

3. Foldseek scanned 324,389,697 domains against a CATH SSG5 library, identifying 193,939,494 H-level and 16,026,530 T-level matches, leaving 114,423,673 domains unmatched.

4. Remaining unmatched domains were analyzed using Merizo-search, which leverages Foldclass (an equivariant graph neural network) and TM-align to assign domains based on structure-embedding similarity (matches are considered as topology-level matches).




A
i.
300k
200k
100k
MAINLY ALPHA
MAINLY BETA
ALPHA/BETA
1.20.1580.10
ABC transporter ATPase like domain
1.10.460.10
Topoisomerase I domain 2
1.10.420.10
Peroxidase domain 2
1.10.290.10
Topoisomerase I, domain 4
1.10.8.280
ABC transporter ATPase domain-like
3.30.2060.10
Penicillin-binding protein 1b domain
3.40.50.1860
unnamed Rossmann fold
3.30.2090.10
Multidrug efflux transporter AcrB TolC docking domain; DN and DC subdomains
3.40.50.10800
NadA-like
3.30.70.1430
Multidrug efflux transporter AcrB pore domain
2.70.20.10
Topoisomerase I domain 3
2.170.190.11
Molybdopterin biosynthesis moea protein, domain 3.
2.60.40.1930
Macroglobulin (MG2)
2.40.160.40
Monomeric porin ompg
2.40.50.180
CheA-289, Domain 4
ii.
Counts (millions)
140
120
100
80
60
40
20
0
30%
16%
55%
α
β
α/β
B
PDB → TED
PDB → Gene3D
Gene3D → TED
1.20.120.1730
1.10.3980.10
1.10.150.170
1.10.1740.110
1.25.40.890
2.40.50.250
2.60.40.2250
2.60.120.810
2.70.20.10
2.60.40.3450
3.30.70.1020
3.30.70.2750
3.90.870.40
3.10.450.630
3.30.70.1910
10^4
10^3
10^2
10^1


C
No. unique superfamilies
400
300
200
100
0
bacteria
eukaryota
archaea
unclassified
other
Gene3D
TED
D
Ar
1.10.3400
3.30.2150
1.10.3490
1.10.820
Un
Eu
3.30.430
1.20.1160
3.90.1460
Ba
3.30.2440
3.30.1880
2.170.280
Mixed
3.30.63
3.40.970
2.102.20
PC2
1.2
0.8
0.4
0.0
-0.8
PC1
-1.2
-0.8
-0.4
0.0
0.4
Relative Intensity
1.0
0.8
0.6
0.4
0.2
0.0

Novel Domain Identification Workflow

Initial Filtering:

- 41.8M domain clusters were assessed using normalized radius of gyration and packing density metrics.
- Secondary structure element (SSE) filtering excluded domains with fewer than six SSEs.
- The plDDT80 metric ensured retention of well-folded domains while excluding clusters with unfolded tail regions.

Library Comparisons: Iterative searches were performed against PDB, CATH, ECOD, and SCOPe libraries using Foldseek to identify novel domains.

Core Subset Selection: High-symmetry domains were separated to identify potential novel folds, accounting for repetitive units like WD40 repeats.

Chopping Quality Assessment: Retrained Foldclass networks evaluated domain segmentation, distinguishing between well-chopped domains and over/under-chopped segments.

Novelty Ranking: Final domains were ranked using Foldclass embeddings, calculating Euclidean distances to known domain neighbors in established libraries.

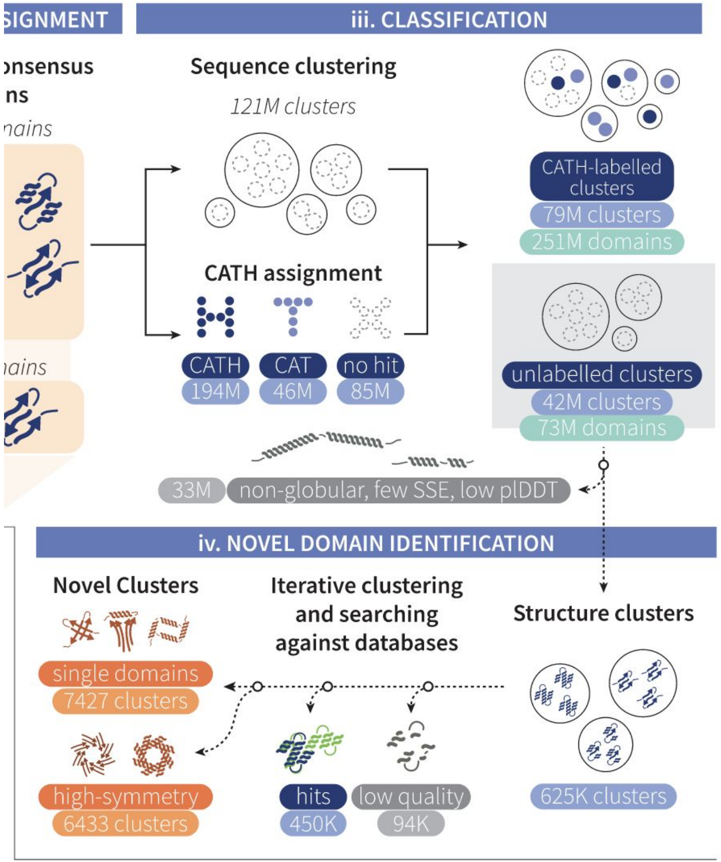
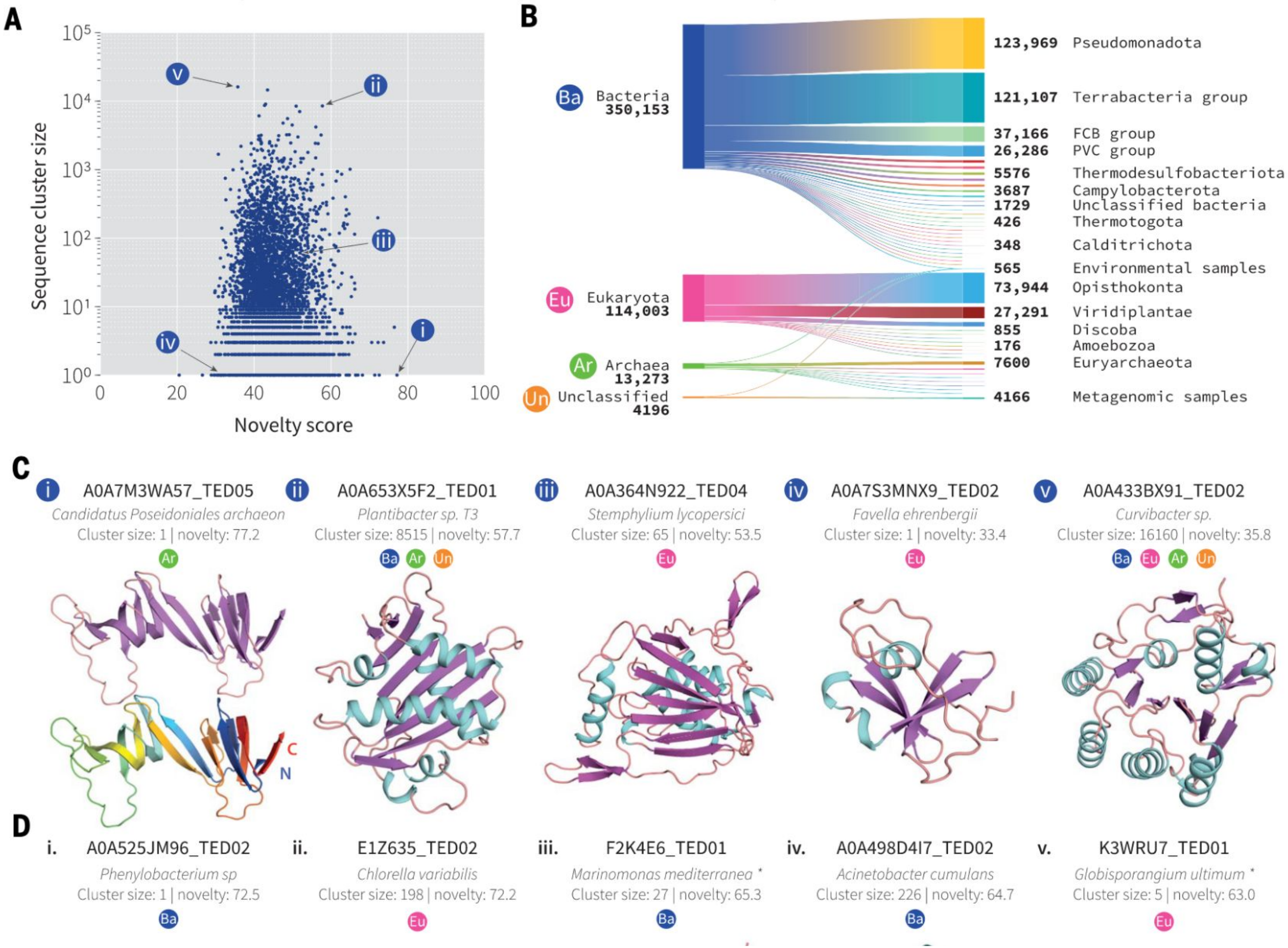


Fig. 4. Examples of novel domain clusters identified in TED. (**A**) Comparison of domain novelty score versus sequence cluster size (*n* = 7427). Novelty scores are predicted by the Foldclass algorithm where novel domains are ranked with a score close to 100. (**B**) Taxonomic distribution of novel domain clusters (for all sequence cluster members; *n* = 483,732). Largest common phyla are shown across superkingdoms, along with the number of domains in sequence clusters assigned to each level of the hierarchy. (**C**) (i) to (v) correspond to labels shown in (A). In (i), the bottom subpanel shows the arrangement of strands that form the coiled hairpin loop from the N terminus (blue)

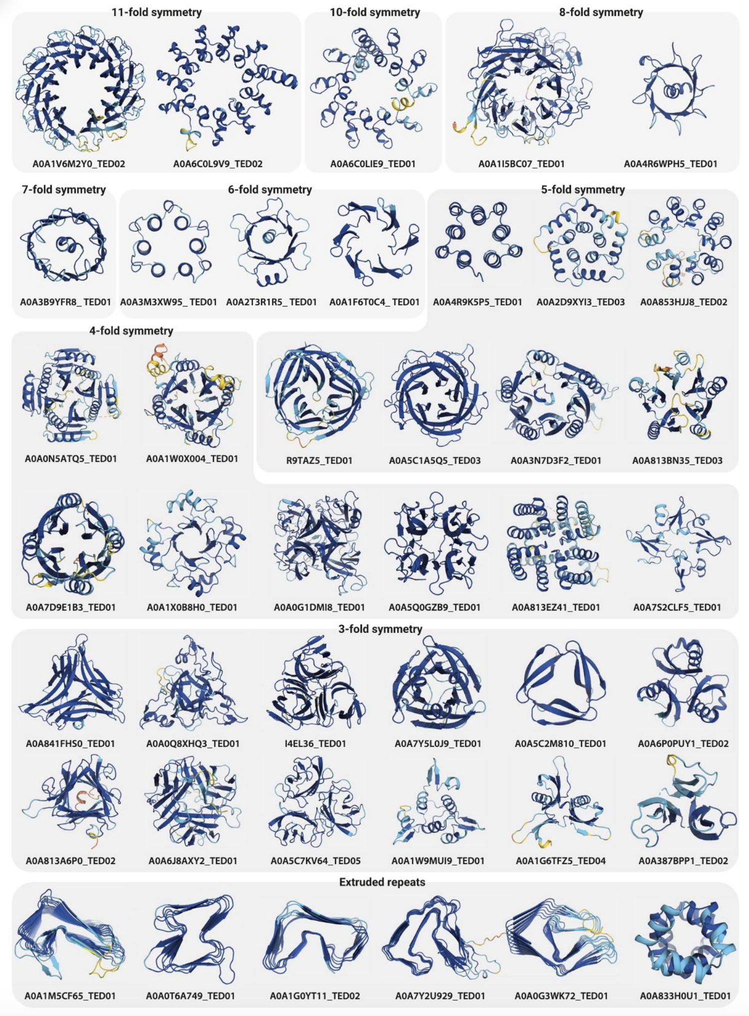


High symmetry domains and extruded repeats:

Symmetric Domain Clusters: Domains with high internal symmetry, such as WD40 beta-propellers, require special categorization. Using the SymD program, clusters with a symmetry Z score >9 were grouped into 6433 highly symmetric novel fold clusters. These include unique architectures like an 11-bladed beta-propeller and various other propeller arrangements.

Extruded Repeat Architectures: A category of cyclic repeats, termed "extruded repeats," extruded along an axis to form highly repetitive and symmetric structures. Examples include alpha- and beta-solenoids and horseshoes with diverse, unstructured loops.

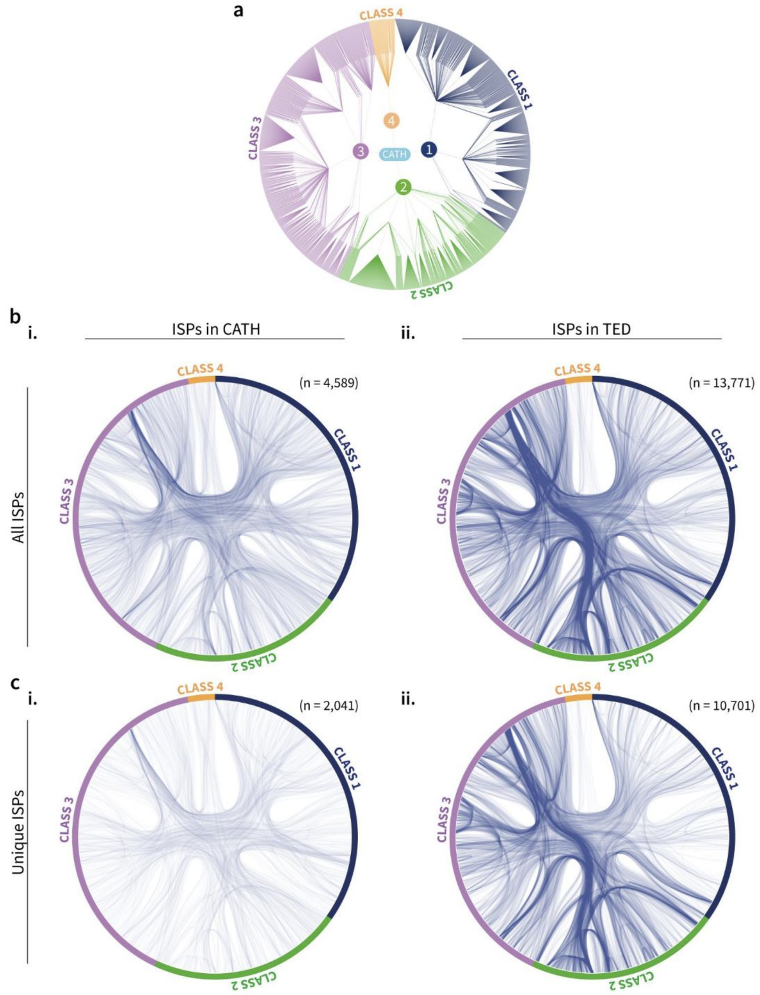
Systematic Studies and Databases: Repeat architectures have been curated by studies and databases (e.g., DbStRiPs), revealing connections to known solenoid folds, including HEAT, ankyrin, and armadillo repeats.



Interactions between domain pairs

Domain Interaction Identification: Domains are defined as interacting if at least three Cβ (or Cα for Gly) atoms are within 8 Å. An interacting Superfamily Pairs (ISP) is the collection of all instances of such contacting pairs of domains belonging to specific superfamilies.

Comparison of TED and CATH ISPs: TED includes 27,280,057 domain interaction instances across 13,771 ISPs, significantly outnumbering CATH’s 196,234 instances across 5111 ISPs. Most ISPs in TED are unique, while 2041 are exclusive to CATH.

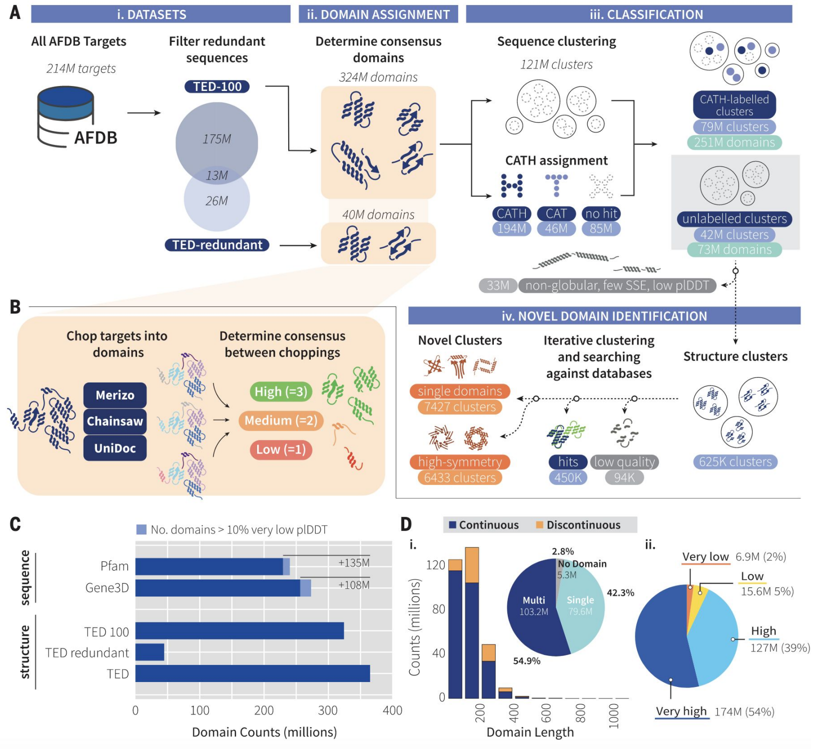


Conclusion

- TED fills critical gaps left by sequence-based methods, offering a comprehensive resource for identifying and classifying protein domains within the AFDB.
- By uncovering over 100 million previously undetectable domains and revealing thousands of new folds, TED substantially expands our understanding of protein structure diversity.
- TED highlights evolutionary trends, including conserved and lineage-specific folds, providing insights into protein evolution across the Tree of Life.
- The resource uncovers more than 10,000 new structural interactions and enriches knowledge of domain-packing geometries, tripling known superfamily interactions.
- With ongoing updates aligned with AFDB releases, TED facilitates functional analyses, from drug discovery to evolutionary studies, setting the stage for future breakthroughs in structural biology.

Thanks for listening!

Questions-Comments?


A
i. DATASETS
ii. DOMAIN ASSIGNMENT
iii. CLASSIFICATION
All AFDB Targets
214M targets
AFDB
Filter redundant sequences
TED-100
175M
13M
26M
TED-redundant
Determine consensus domains
324M domains
40M domains
Sequence clustering
121M clusters
CATH assignment
CATH
194M
CAT
46M
no hit
85M
CATH-labelled clusters
79M clusters
251M domains
unlabelled clusters
42M clusters
73M domains
33M
non-globular, few SSE, low pIDDT
B
Chop targets into domains
Merizo
Chainsaw
UniDoc
Determine consensus between choppings
High (=3)
Medium (=2)
Low (=1)
iv. NOVEL DOMAIN IDENTIFICATION
Novel Clusters
single domains
7427 clusters
high-symmetry
6433 clusters
Iterative clustering and searching against databases
hits
450K
low quality
94K
Structure clusters
625K clusters
C
No. domains > 10% very low pIDDT
sequence
structure
Pfam
Gene3D
TED 100
TED redundant
TED
+135M
+108M
0
100
200
300
400
Domain Counts (millions)
D
Continuous
Discontinuous
i.
120
80
40
0
Counts (millions)
200
400
600
800
1000
Domain Length
2.8%
No Domain
5.3M
Multi
103.2M
Single
79.6M
42.3%
54.9%
ii.
Very low
6.9M (2%)
Low
15.6M 5%)
High
127M (39%)
Very high
174M (54%)