

Target Classification in the 14th Round of the Critical Assessment of Protein Structure Prediction (CASP14)

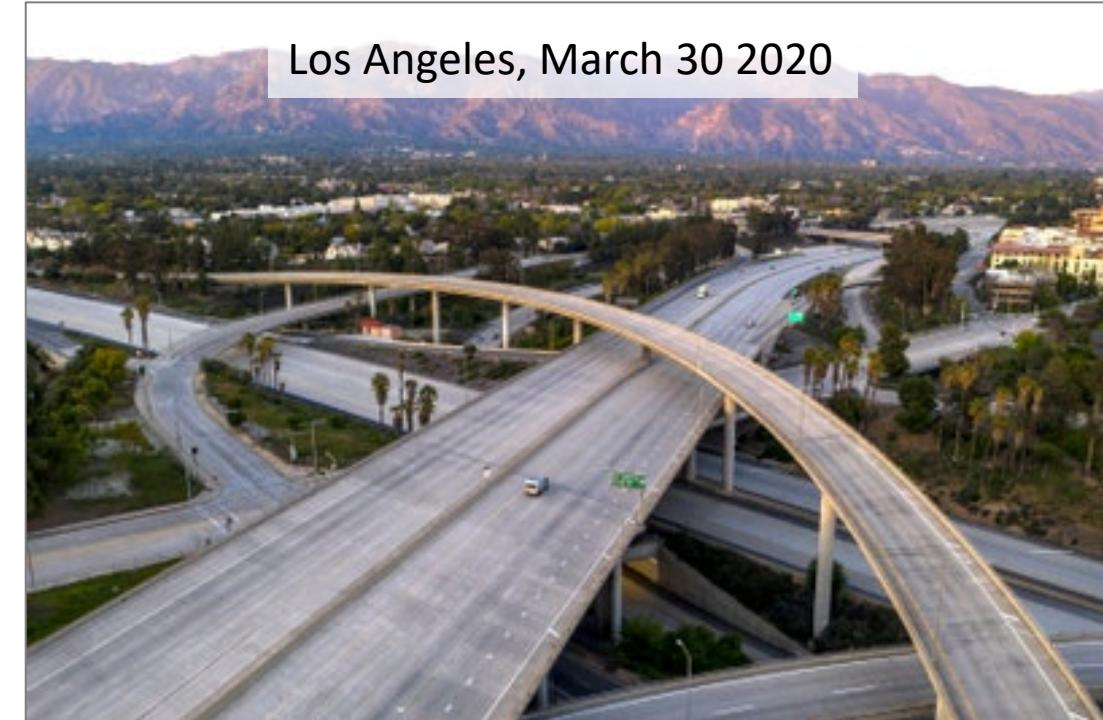
Lisa Kinch, Andriy Kryshtafovych and Nick Grishin

CASP14 Target Classification during COVID-19

What used to be



Start of Registration

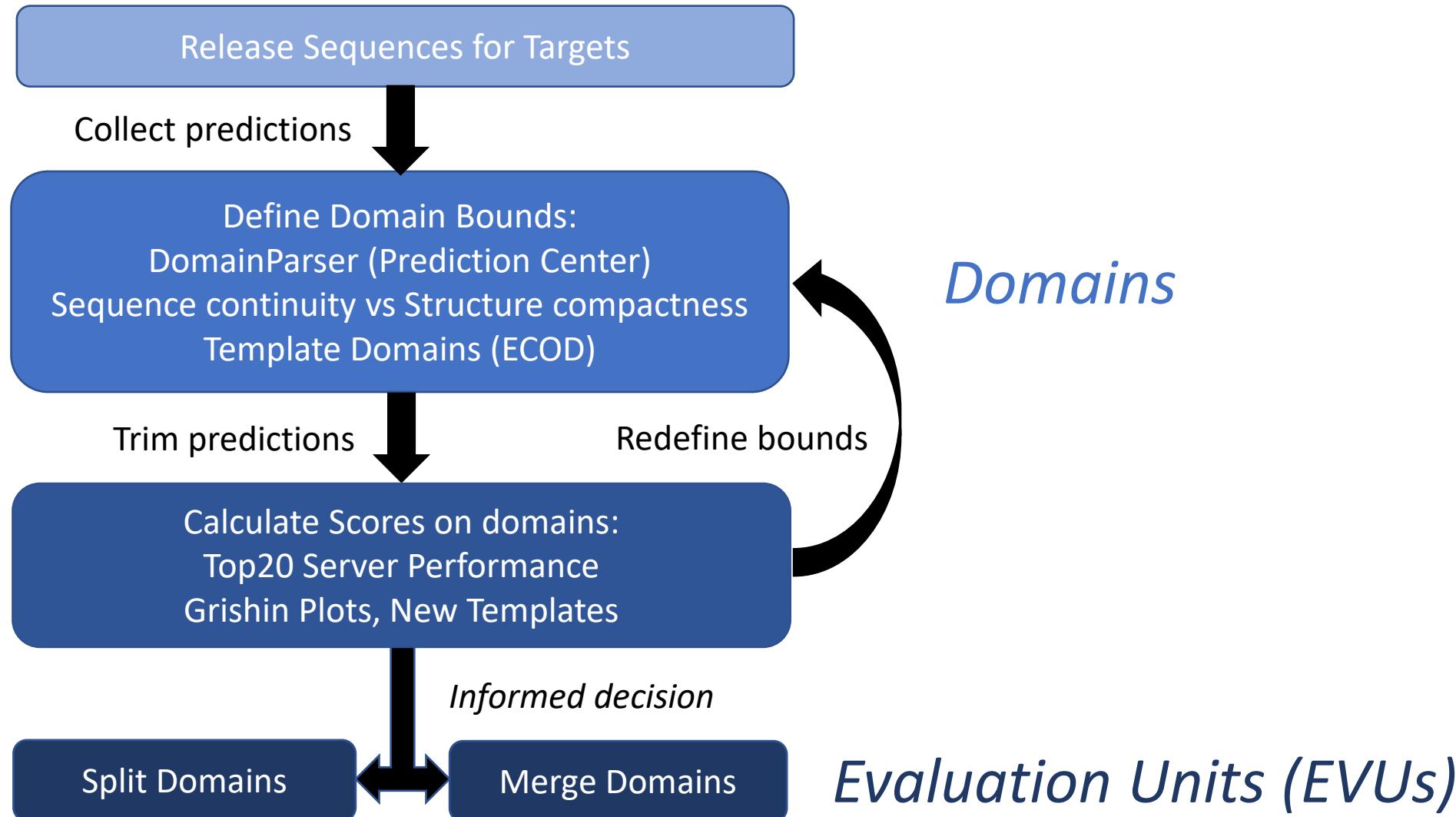


Registration for CASP14 opened,
March 9, 2020

Can we get enough targets?

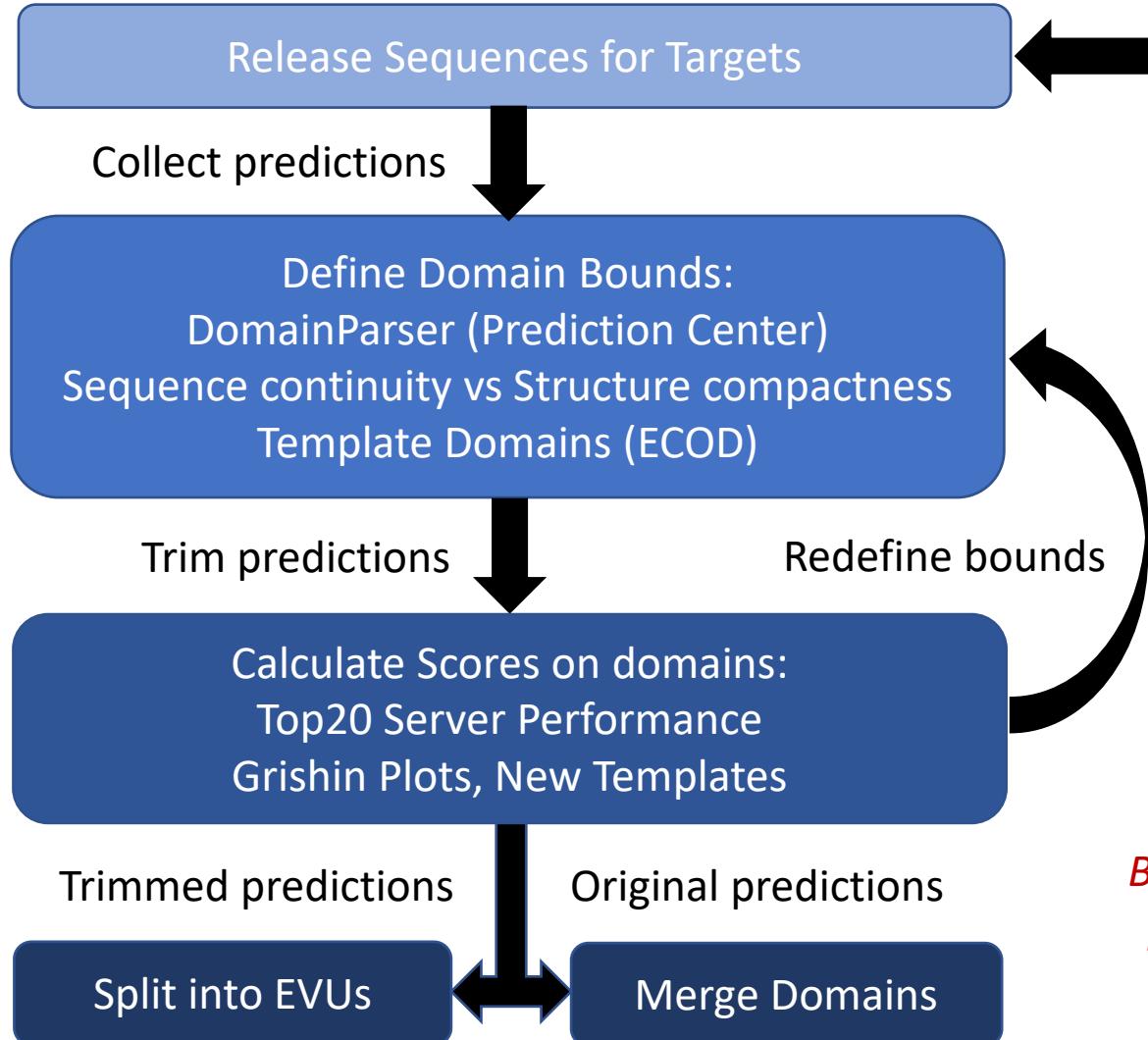
CASP14 Domain Definition and Evaluation Units

What used to be



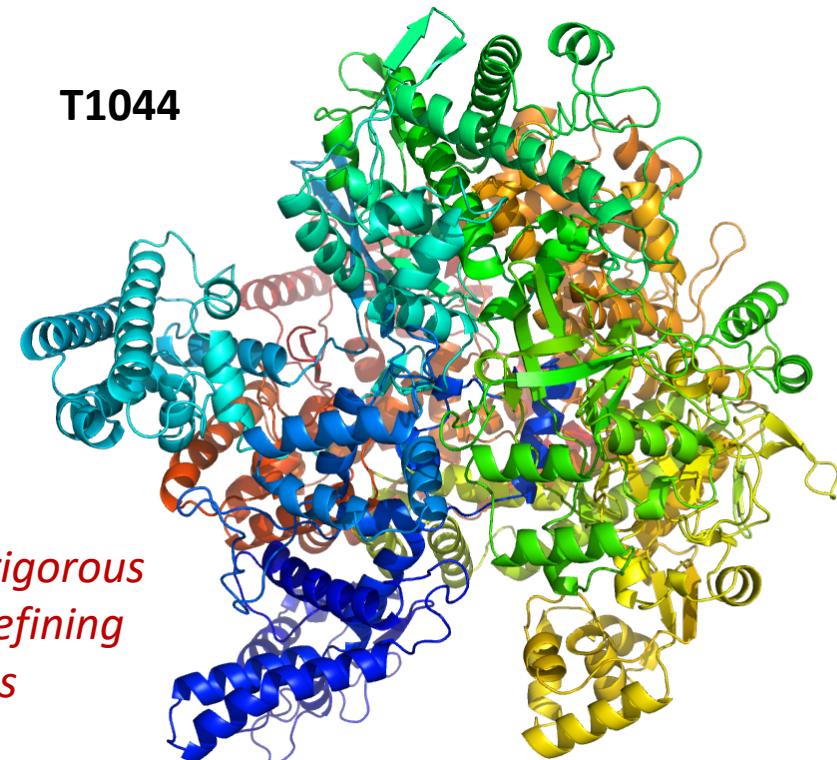
CASP14 *Pre-Evaluation* Domain Definition

What used to be



CASP14 adaptation

T1044 Define Domain Bounds:
Suggestion from Experimentalist
Sequence continuity vs Structure compactness
Template Information

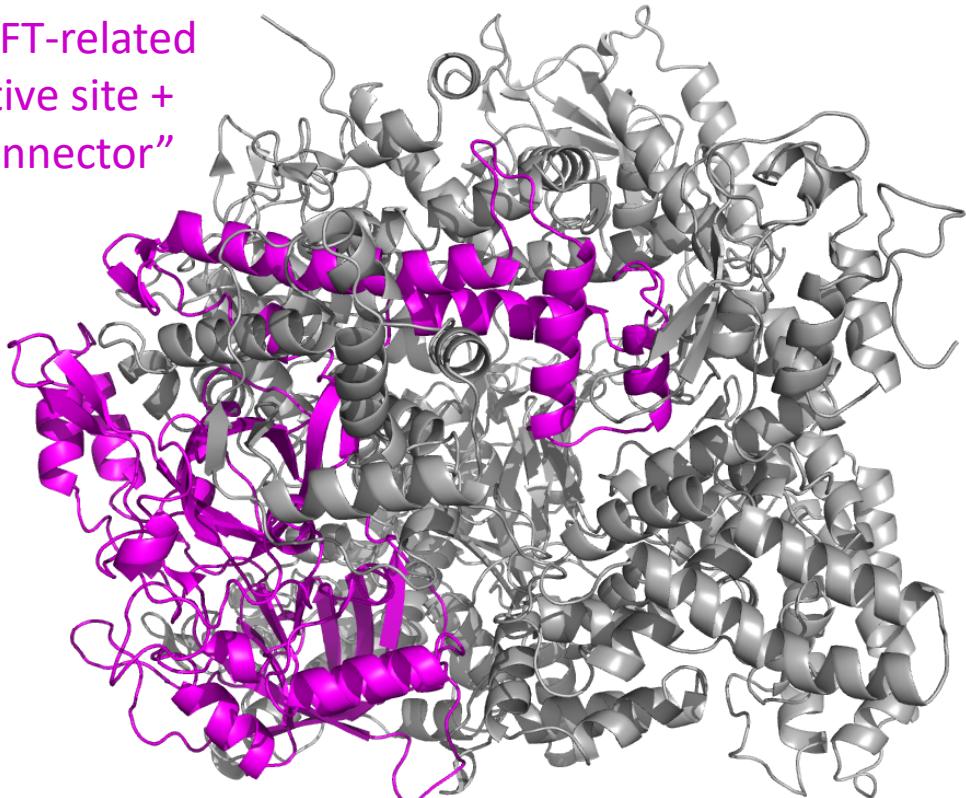


*Bypasses the rigorous
process for defining
domains*

CASP14 *Pre-Evaluation* Leak of Information

T1044: Phage DNA-dependent RNA polymerase

2xRIFT-related
Active site +
“connector”



THE PREPRINT SERVER FOR BIOLOGY

New Results

Structure and function of virion RNA polymerase

Arina V. Drobysheva, Sofia A. Panafidina, Matvei V. Kolesnik, Evgeny I. K Sergei Borukhov, Emelie Nilsson, Karin Holmfeldt, Natalya Yutin, Kira Konstantin V. Severinov, Petr G. Leiman, Maria L. Sokolova

doi: <https://doi.org/10.1101/2020.03.07.982082>

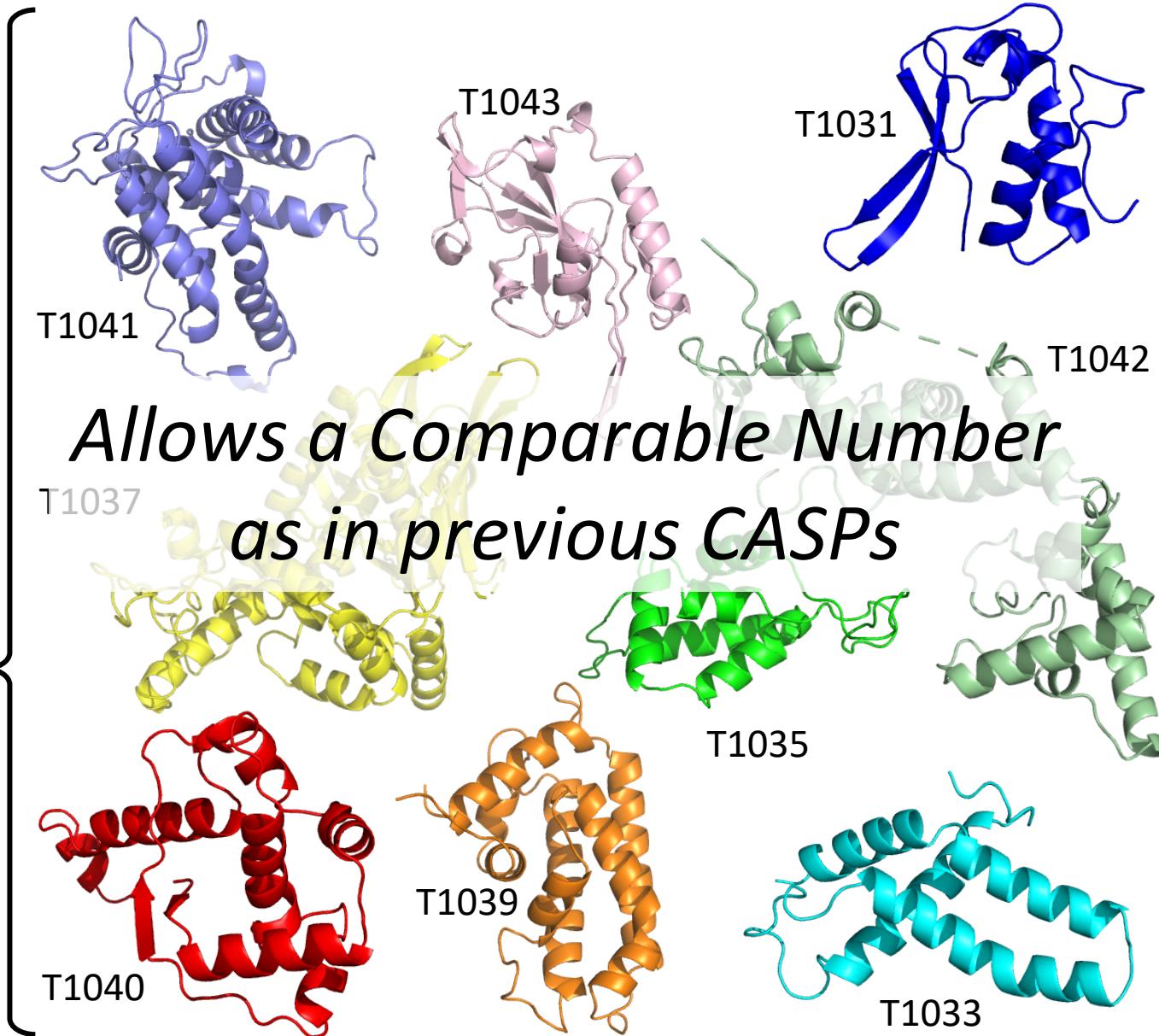
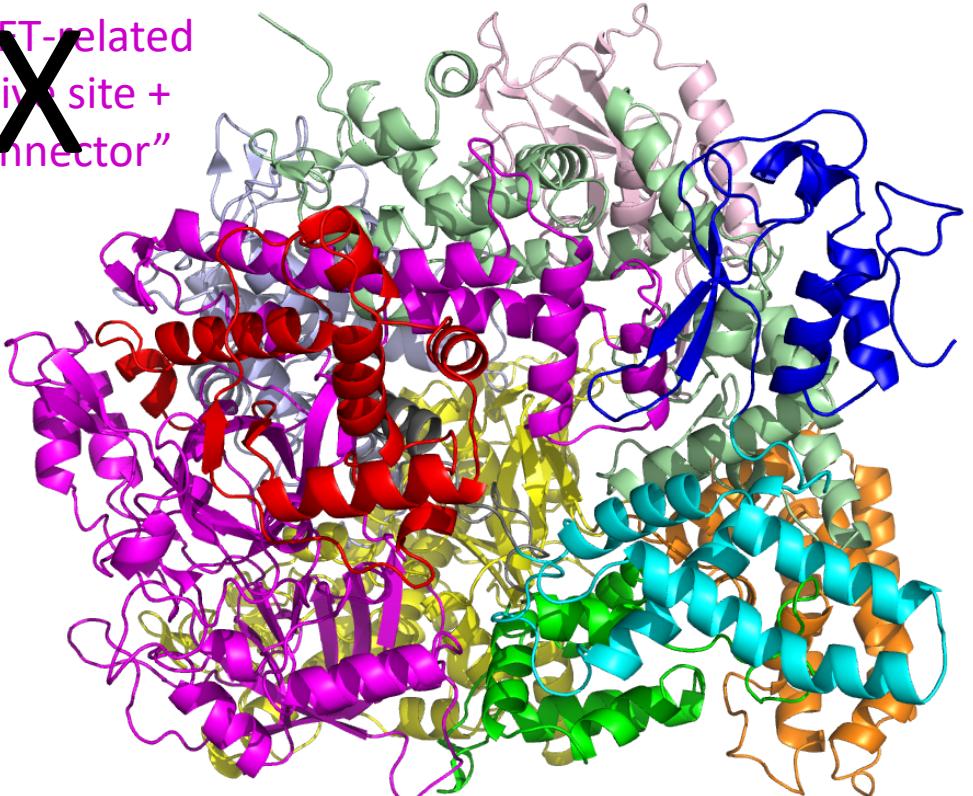
DPBB-A Connector DPBB-B Bridge Helix Trigger Loop

Preprint Statistics (Andriy): CASP boosts Interest 5-fold in 1 week

T1044 *Pre-Evaluation* split into 9 Targets

T1044: Phage DNA-dependent RNA polymerase

exclude
2xRIET-related
Active site +
“connector”



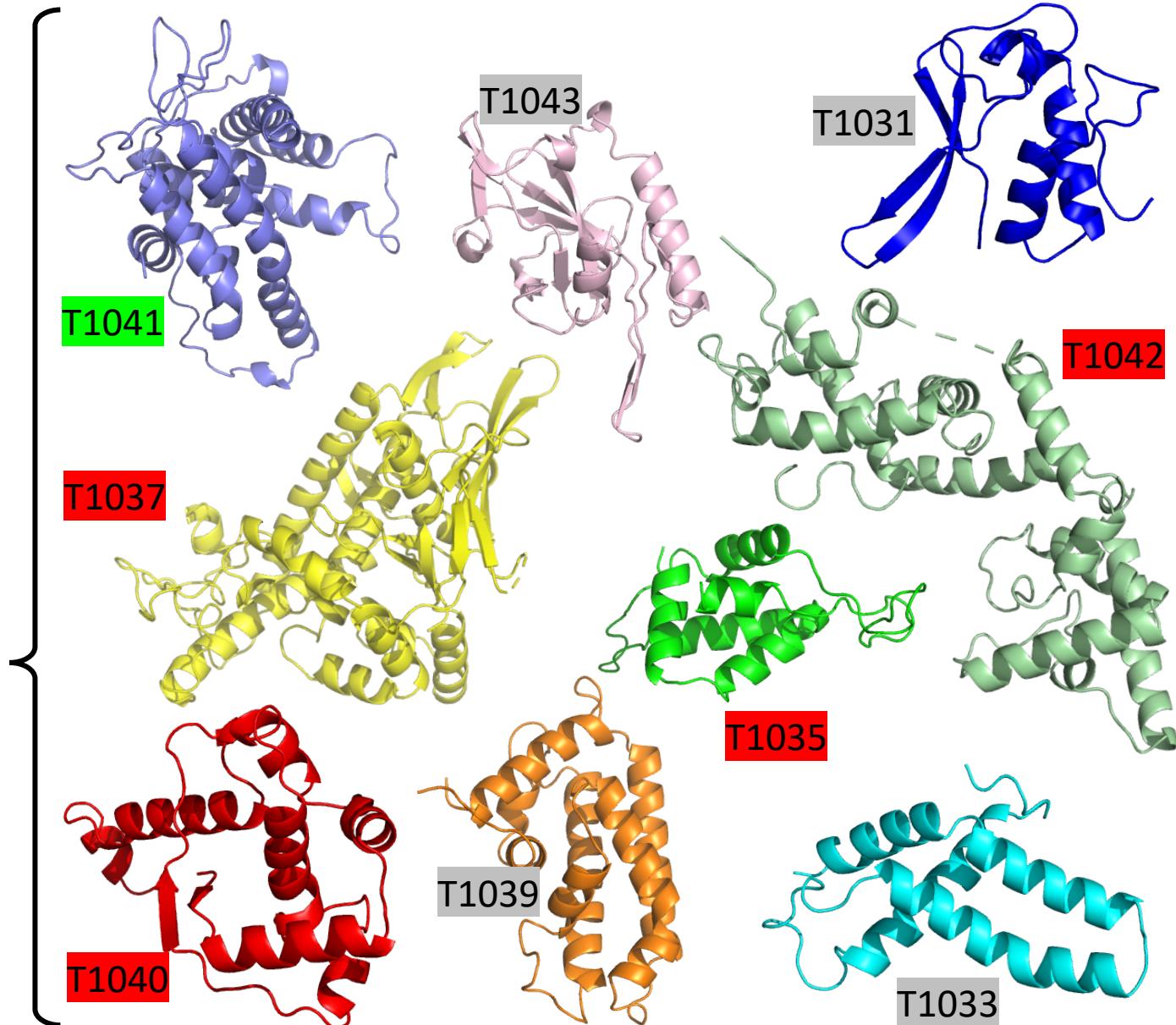
Template Information was Lacking

T1044: Phage DNA-dependent RNA polymerase

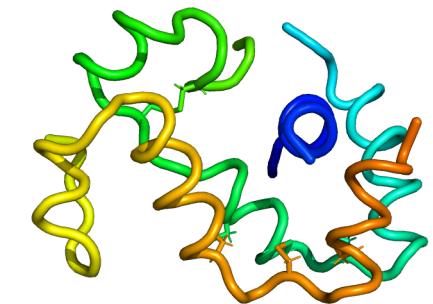
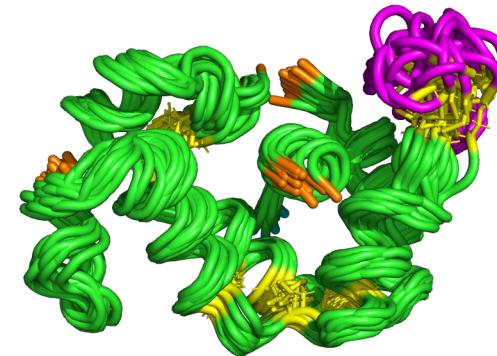
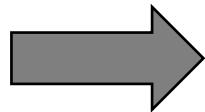
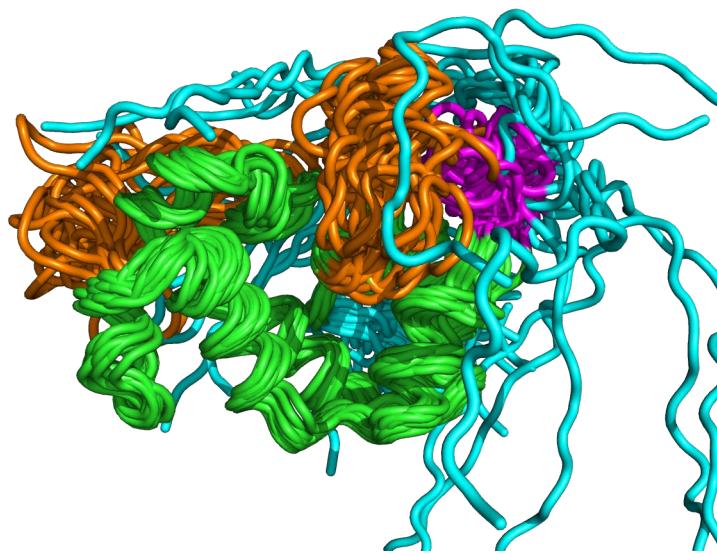
Evolutionary Relationships

- █ New Fold (4)
- █ Topology-level (4)
- █ Distant Homolog (1)

Lack of Templates and extensive domain interactions mean Domains might not be independent folding units



Technical Considerations for Evaluation Units



T1027: Gaussia luciferase

- NMR structure with **high flexibility**
- Loose ensemble
- 5 **disulfide** bond pairs

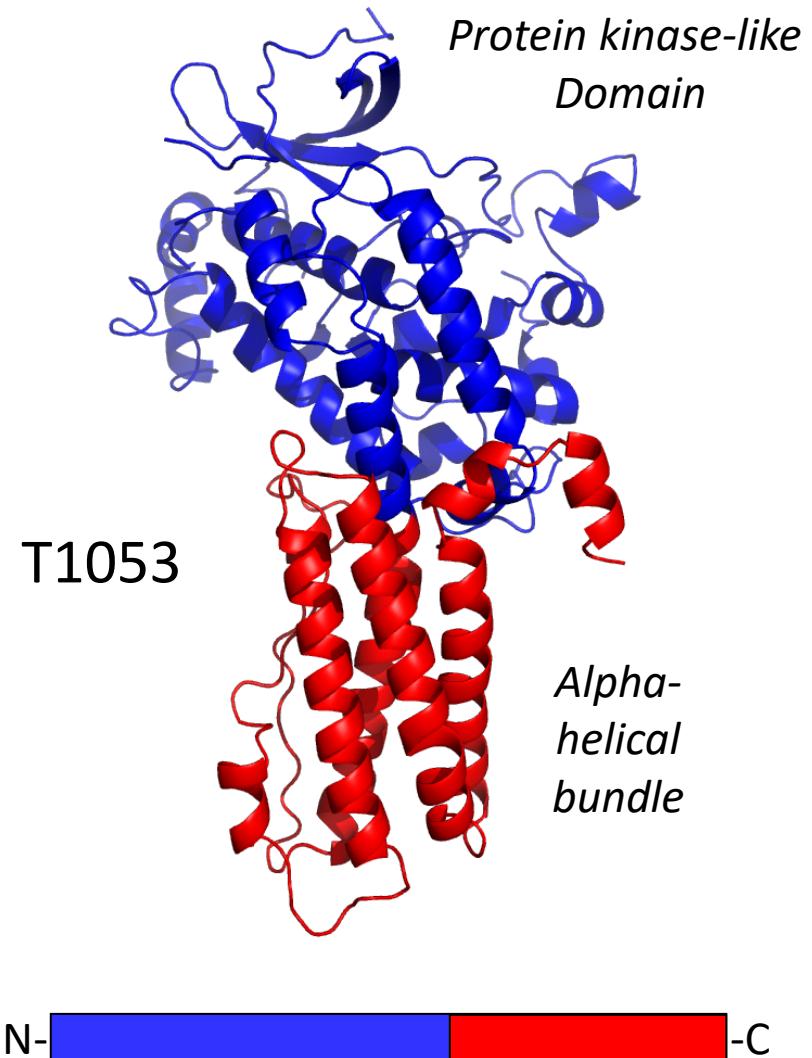
- Keep **overlapping** parts of the structure
- Trim **last disulfide pair**

Keep trimmed model1 as the T1027 EVU

Domains Have Many Different Definitions

What is a Domain?

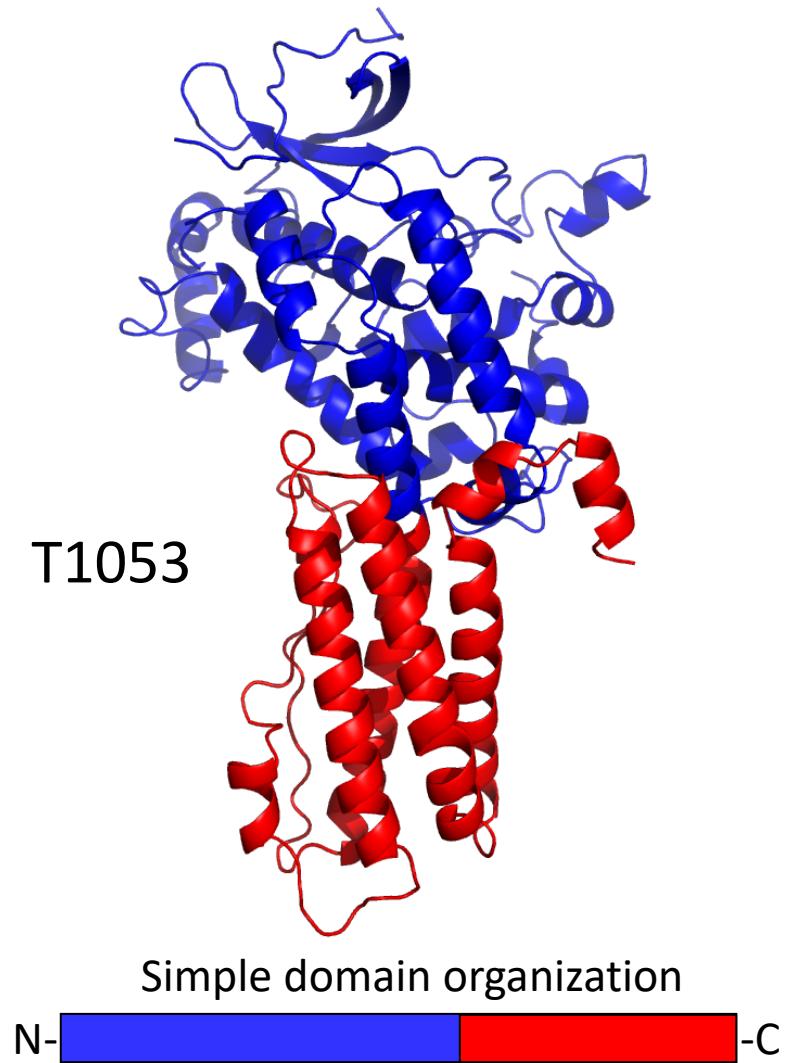
- Compact, globular substructures that have more interactions within them than with the rest of the structure



Domains are More than Compact Substructures

What is a Domain?

- Compact, globular substructures that have more interactions within them than with the rest of the structure
- Conserved, Independent folding unit that can exist in multiple contexts, i.e. serve as building blocks of evolution
- Evolution tends to preserve sequence continuity in domains

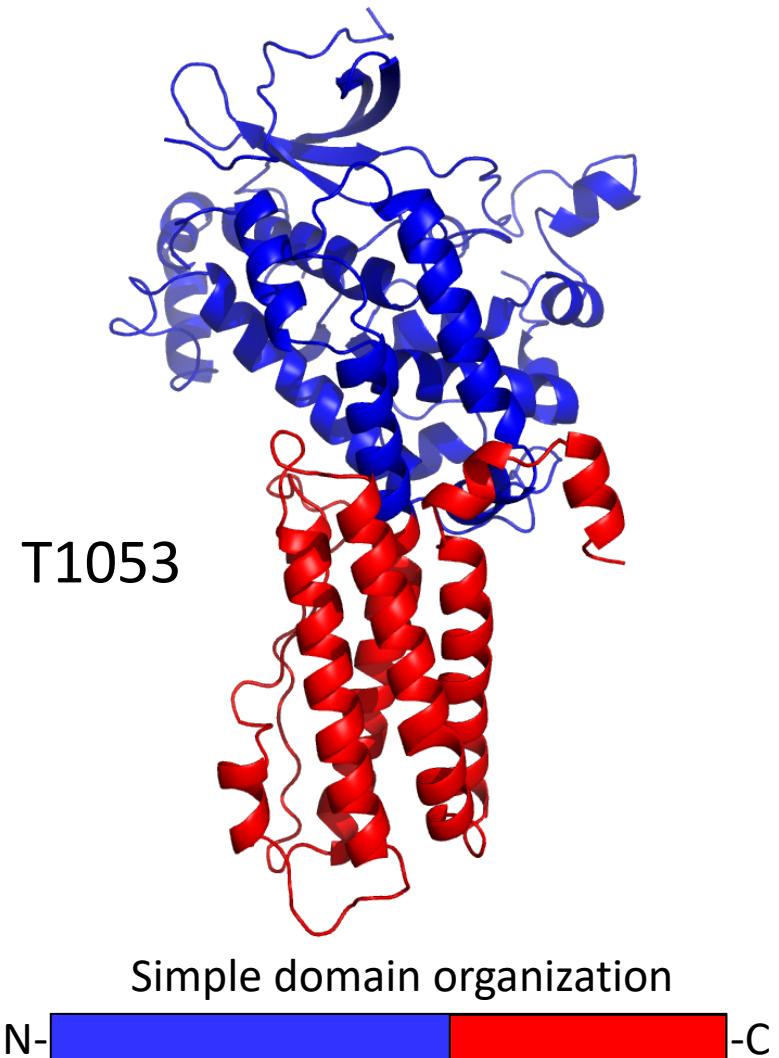


ECOD Database as a Resource for Definition

What is a Domain?

- Compact, globular substructures that have more interactions within them than with the rest of the structure
- Conserved, Independent folding unit that can exist in multiple contexts i.e. serve as building blocks of evolution
- Evolution tends to preserve sequence continuity in domains
- Evolutionary Classification of Protein Domains (ECOD) database was *an essential resource* for defining domains:
prodata.swmed.edu/ecod/ (thanks Dustin!)

ECOD PMID: 25474468

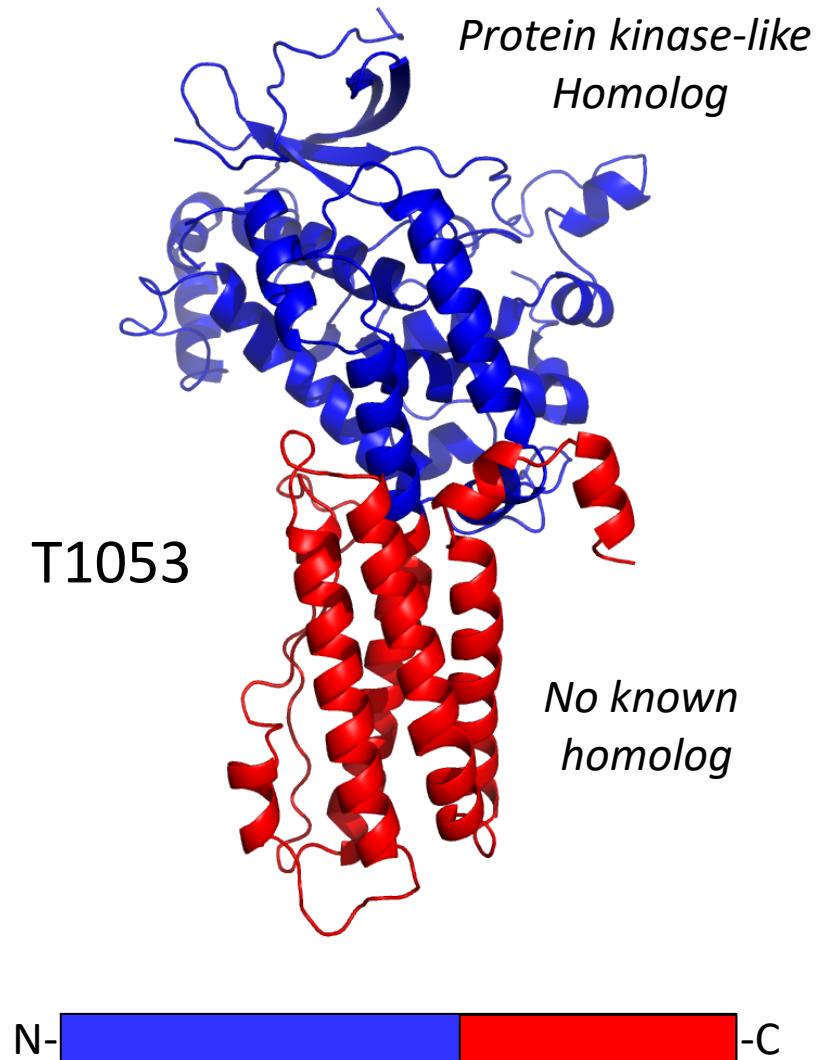


Turning Domains into Evaluation Units

Domains = Evaluation Units

- Using split domains as EVUs are required when templates have known conformation changes (example to follow)
- Using split domains as EVUs are required when they have different difficulty levels (perhaps not in the future)

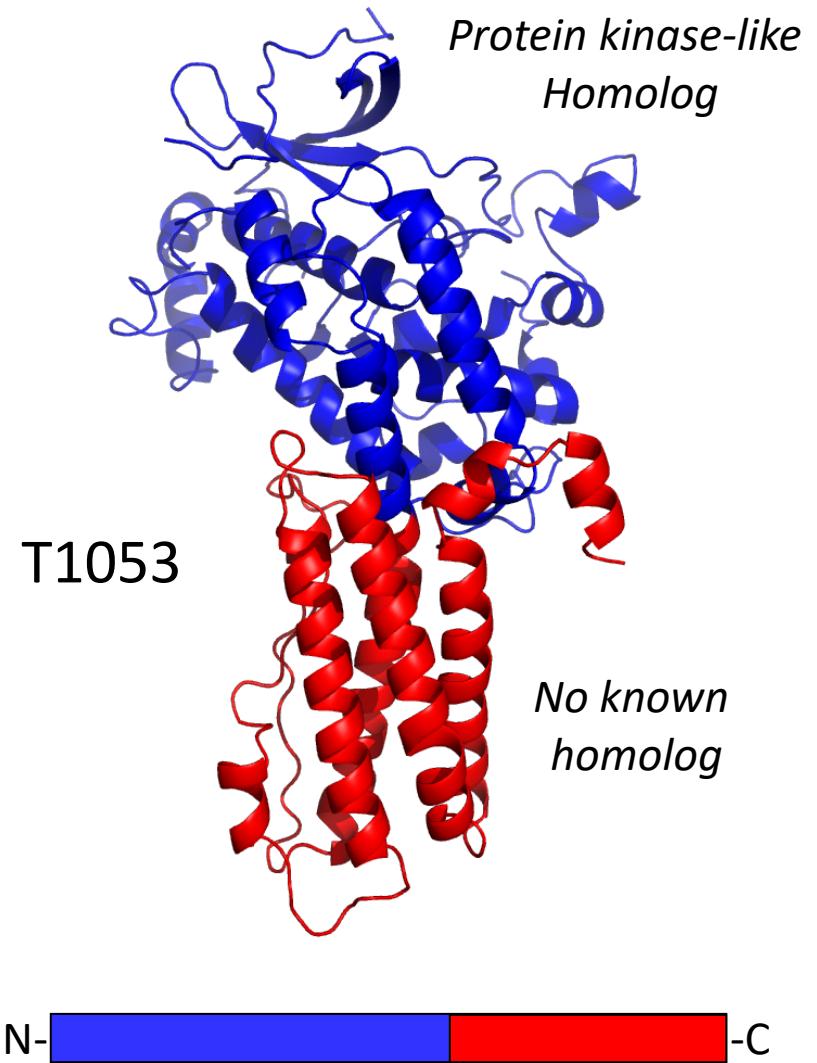
*For CASP14 we tried to **keep domains together**;
If not, we evaluated domain interactions in a separate
assessment*



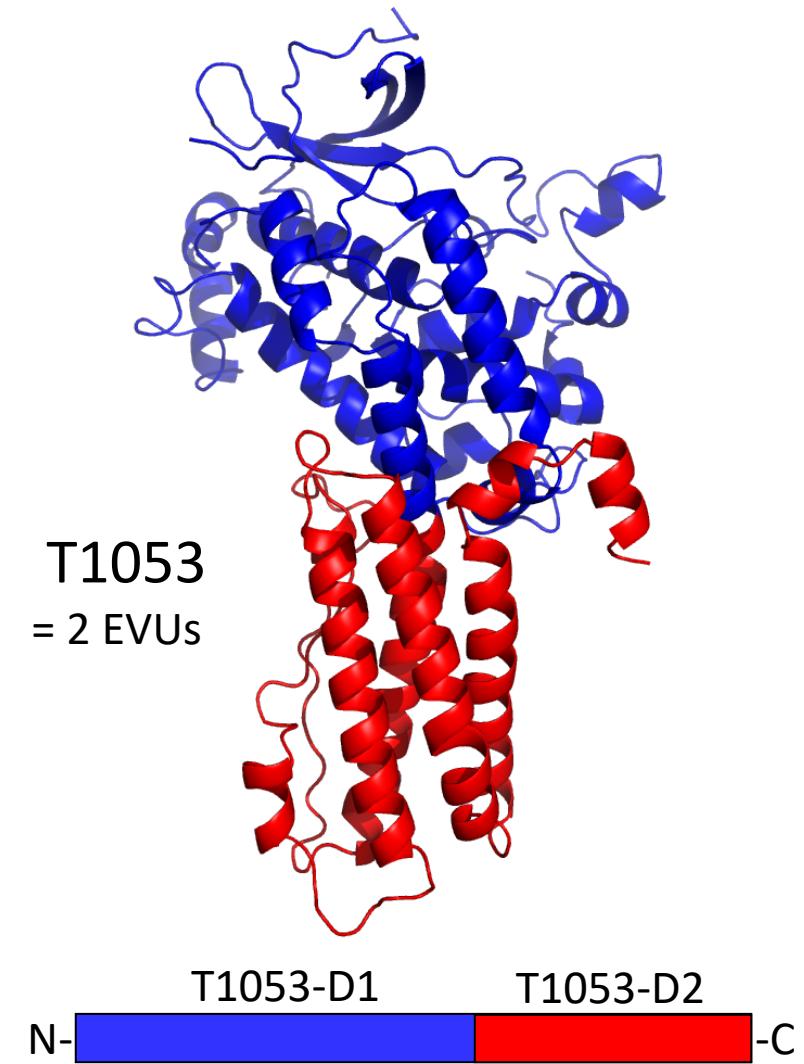
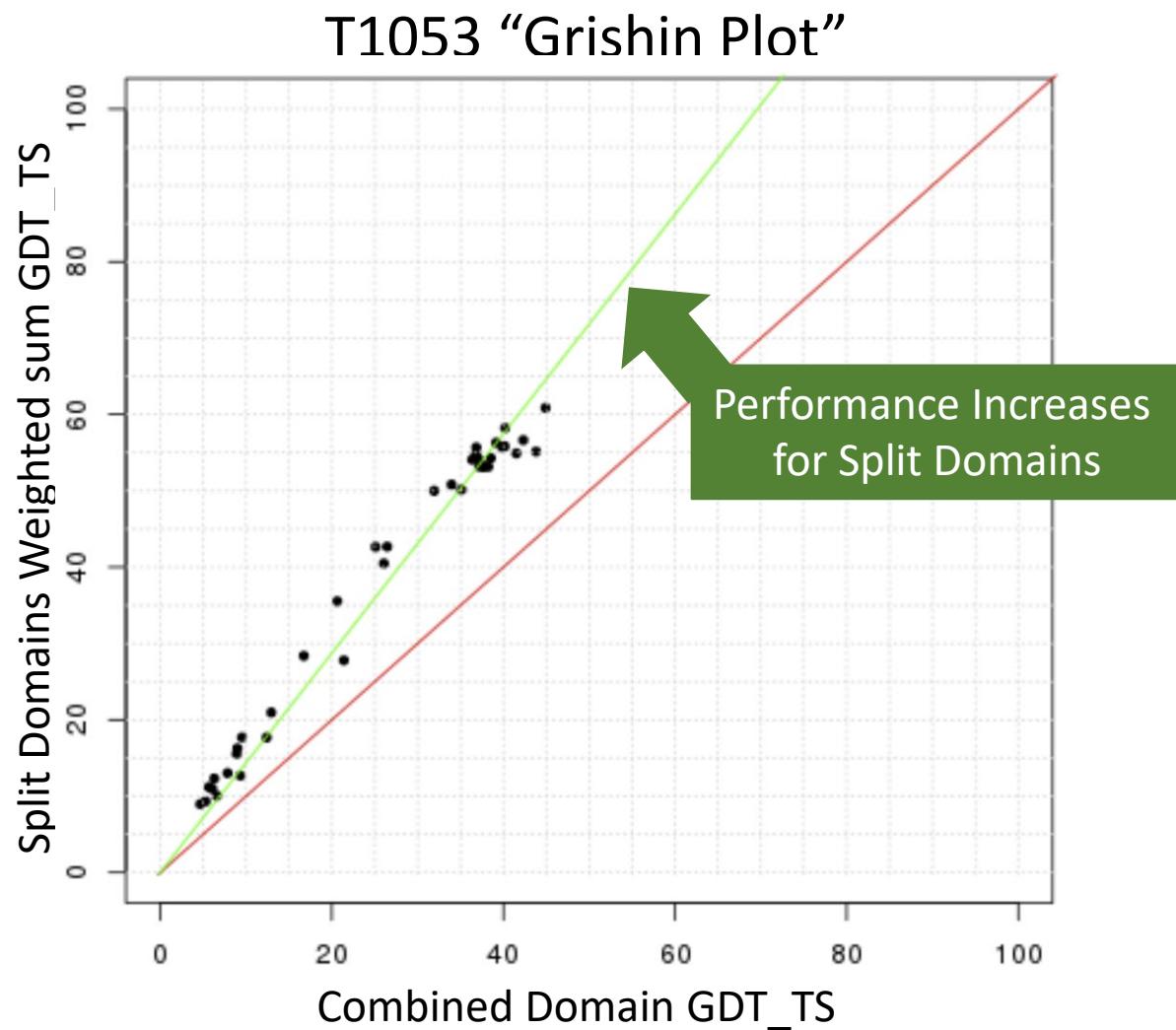
Turning Domains into Evaluation Units

Domains = Evaluation Units

- Using split domains as EVUs are required when templates have known conformation changes (example to follow)
- Using split domains as EVUs are required when they have different difficulty levels
- Decisions to split or merge are based on group performance: traditionally evaluated using “Grishin Plots”

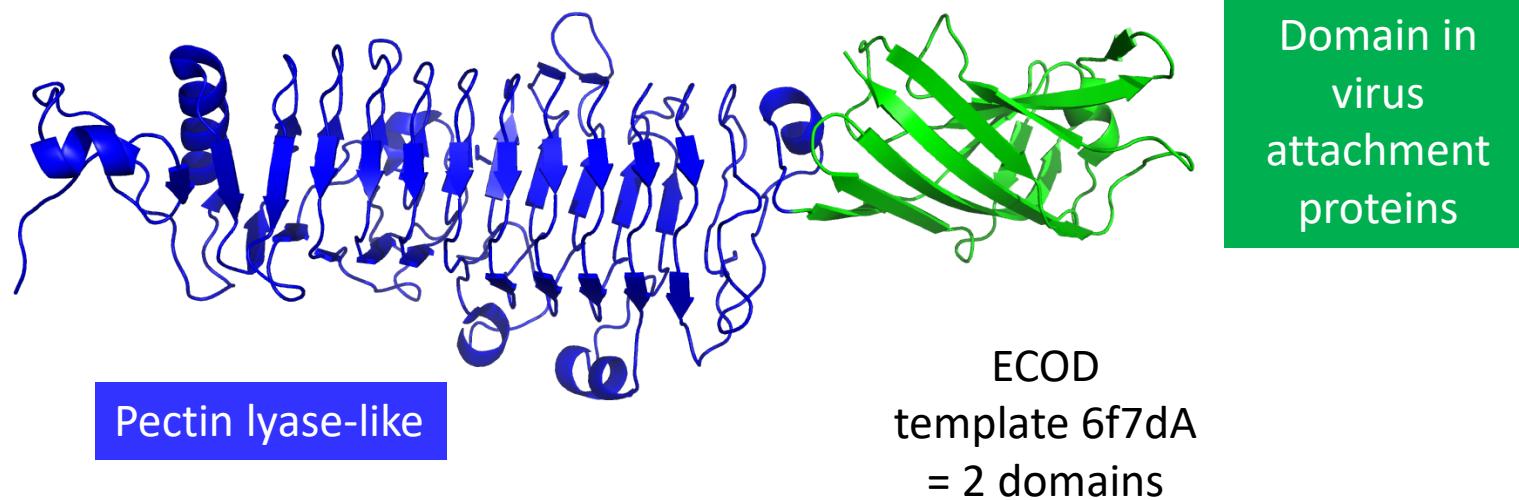
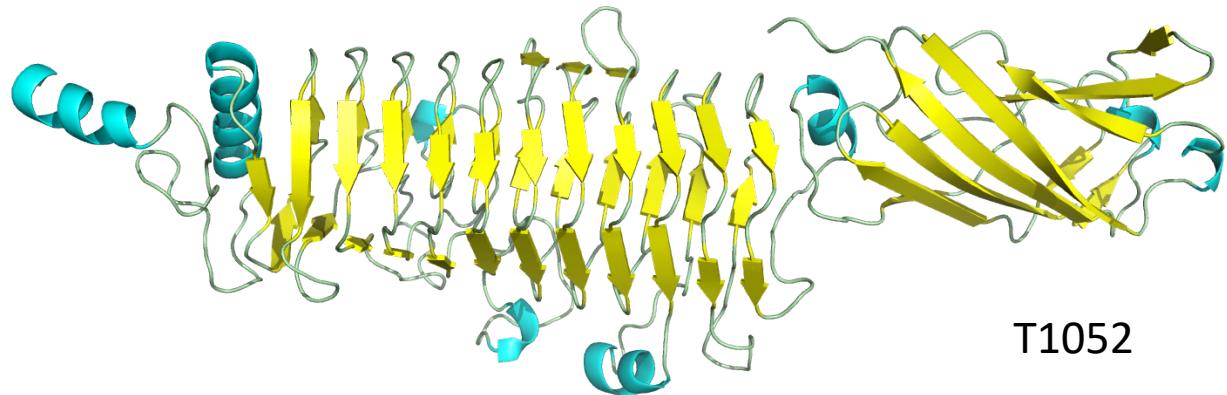


Grishin Plots Inform Decisions to Split Targets



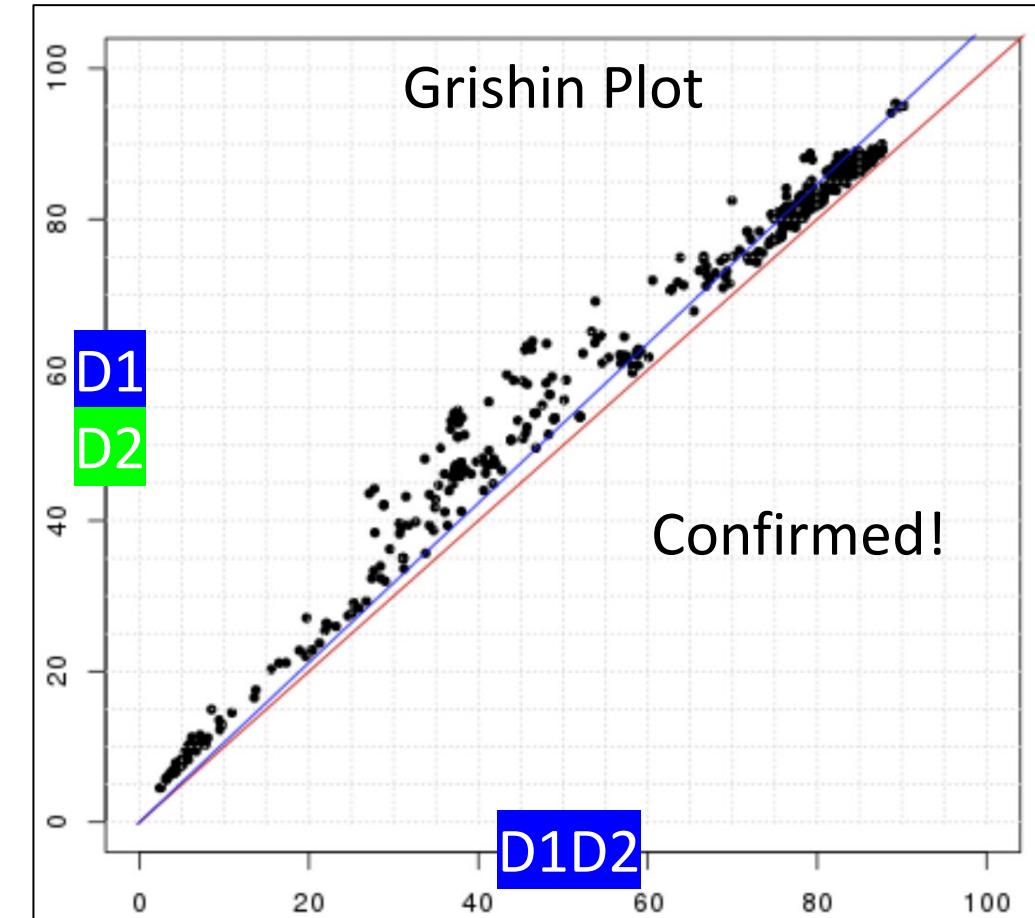
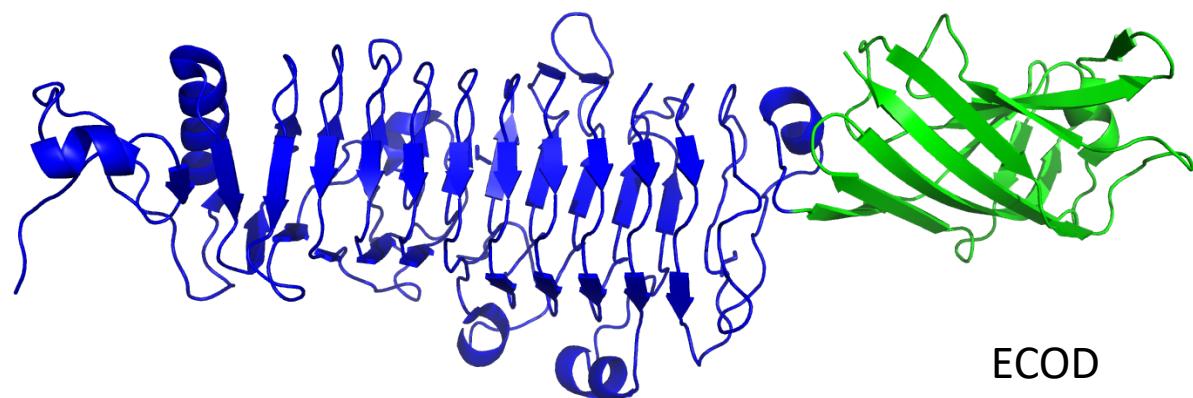
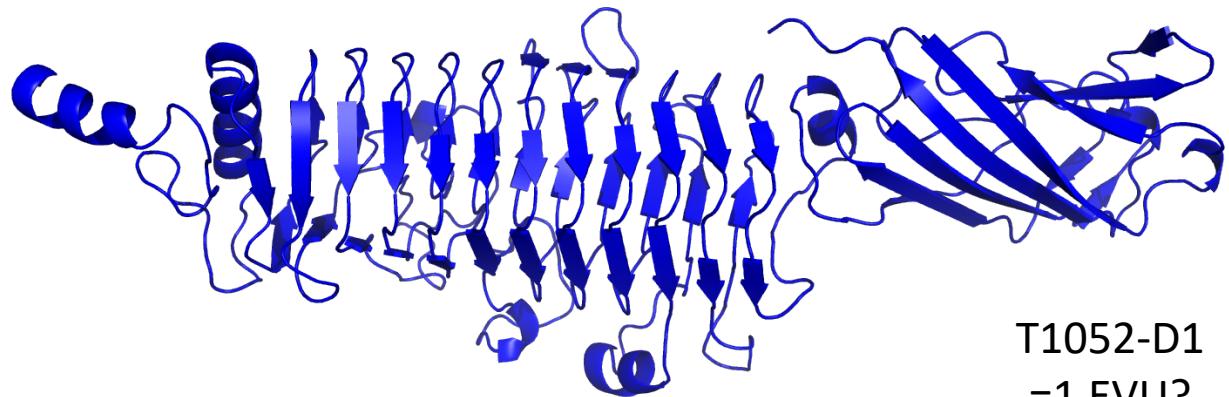
Merging Target Domains as Evaluation Units

No Need to Split when Good Templates Exist

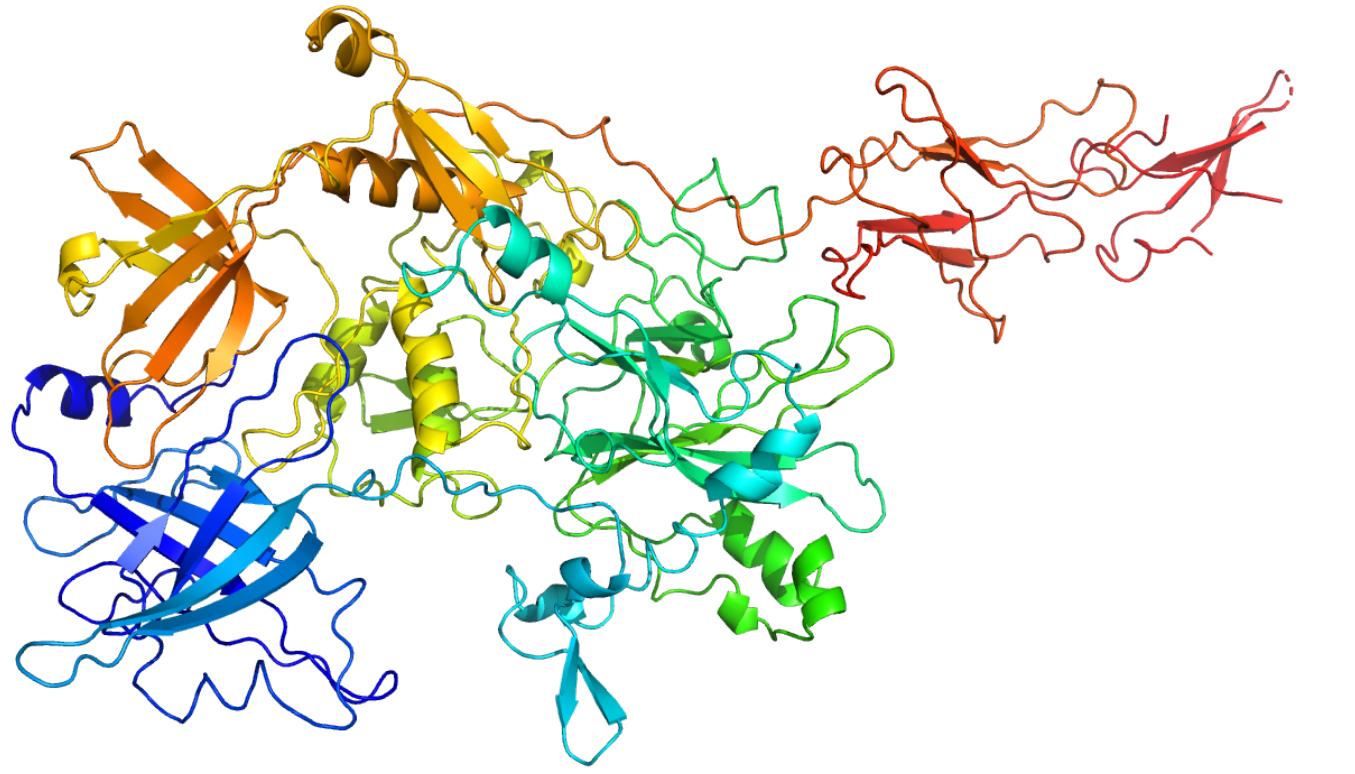


Merging Target Domains as Evaluation Units

No Need to Split when Good Templates Exist



Some Domain Definitions are Difficult

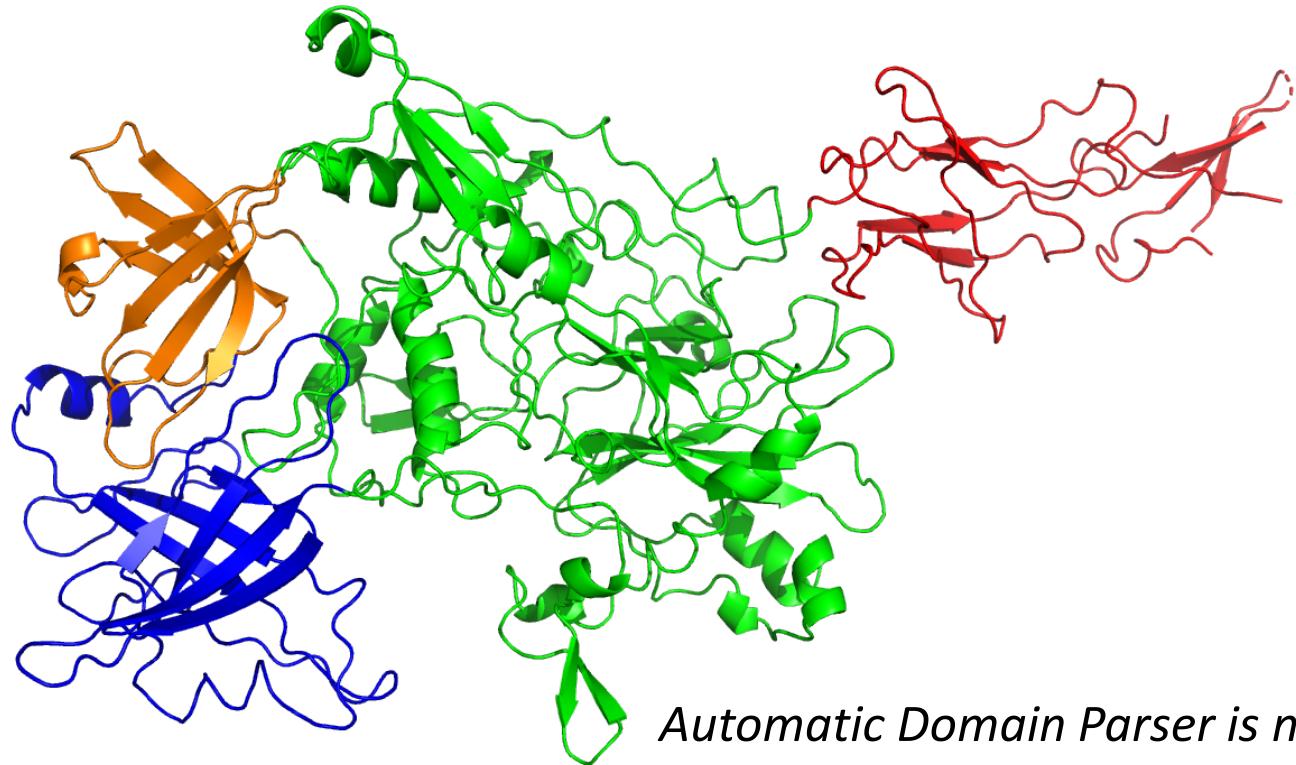


N- -C

T1061: *E.coli* phage tail

- Complex domain organization

Some Domain Definitions are Difficult



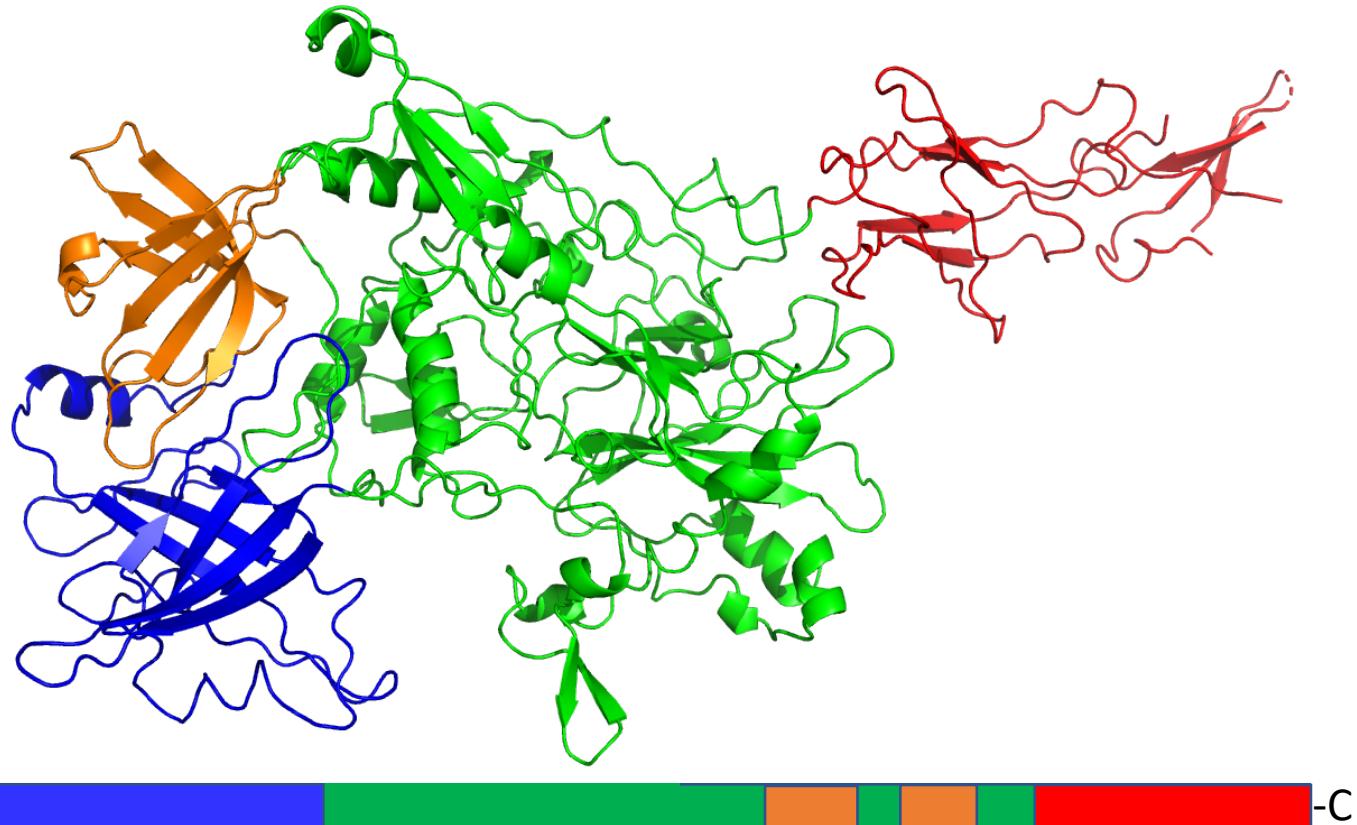
Automatic Domain Parser is non continuous



T1061: *E.coli* phage tail

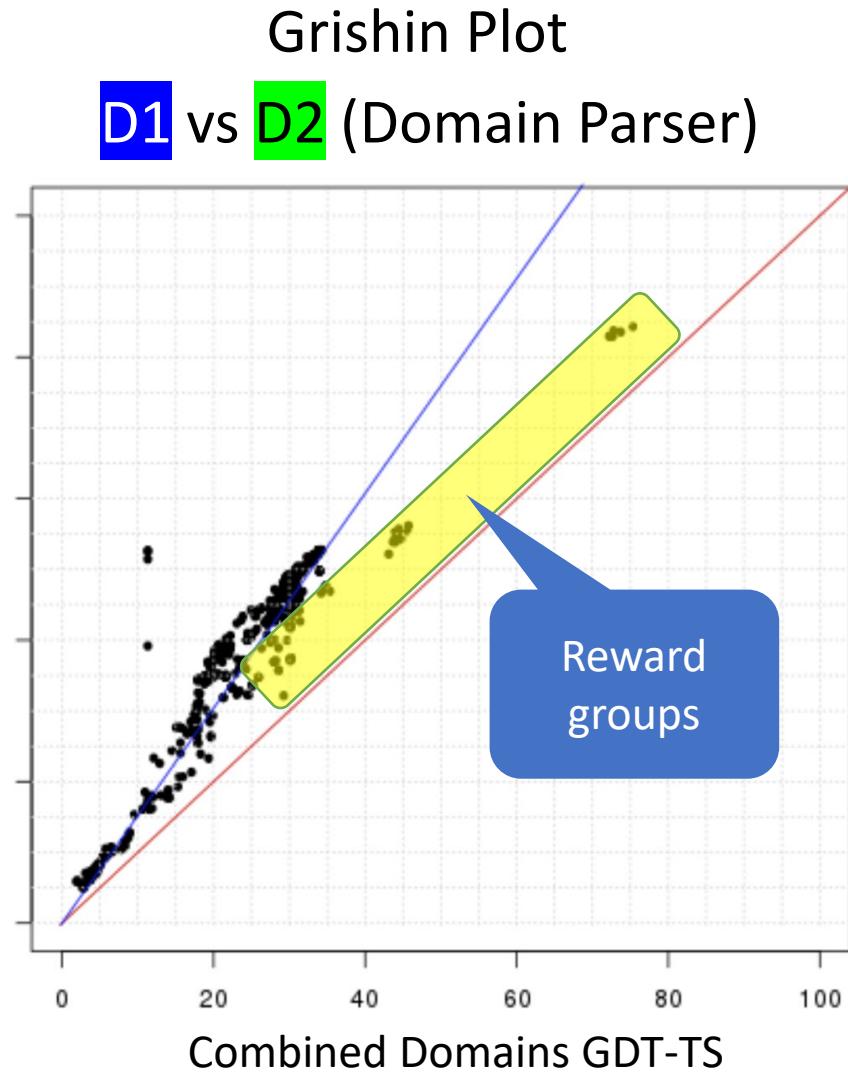
- Complex domain organization
- Domain parser and Ddomain split differently (4 vs 5)

Some Domain Definitions are Difficult

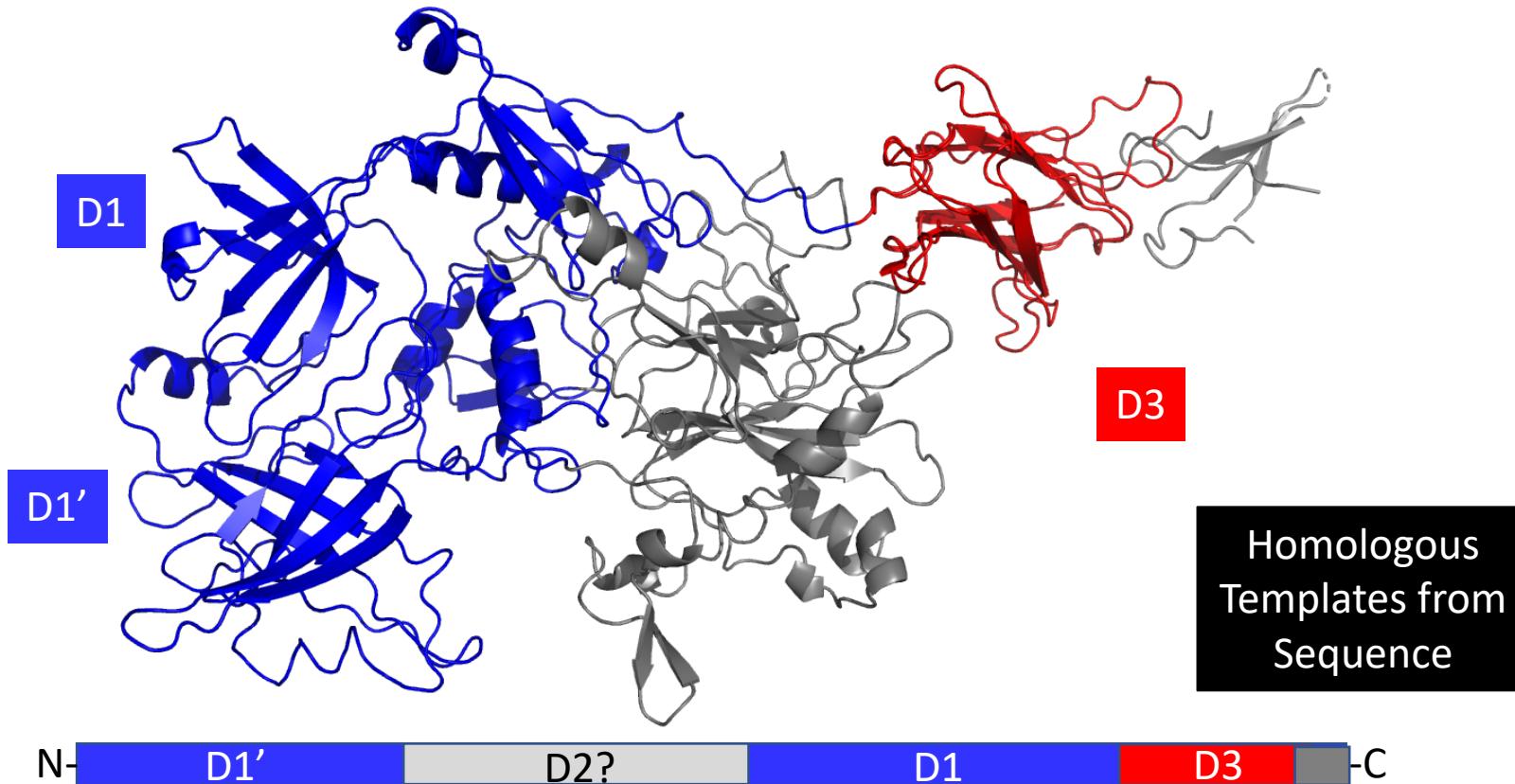


T1061: *E.coli* phage tail

- Complex domain organization
- Domain parser and Ddomain split differently (4 vs 5)
- Grishin Plot has multiple clouds



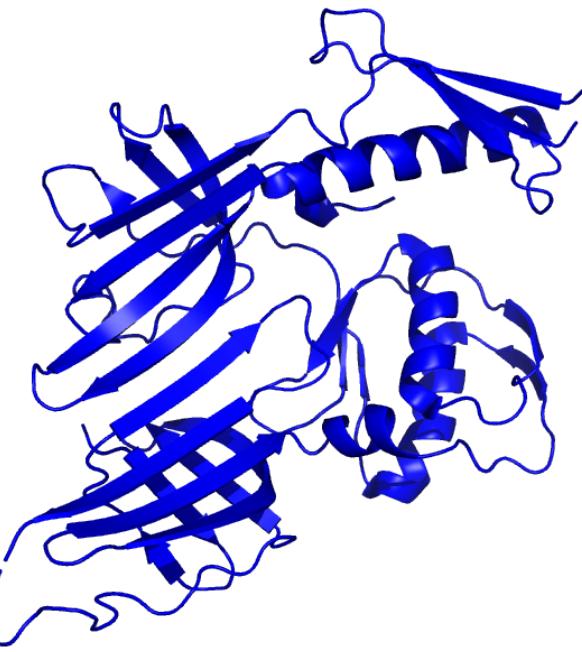
Homologous Templates Suggest Domain Bounds



T1061: *E.coli* phage tail

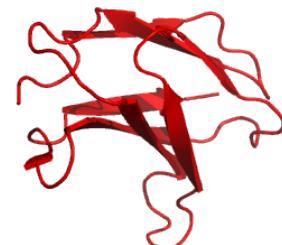
- Complex domain organization
- Domain parser and Ddomain split differently (4 vs 5)
- Grishin Plots have multiple clouds
- Templates for blue and red domains

Homologous
Templates from
Sequence



D1' D1 3cddF Template

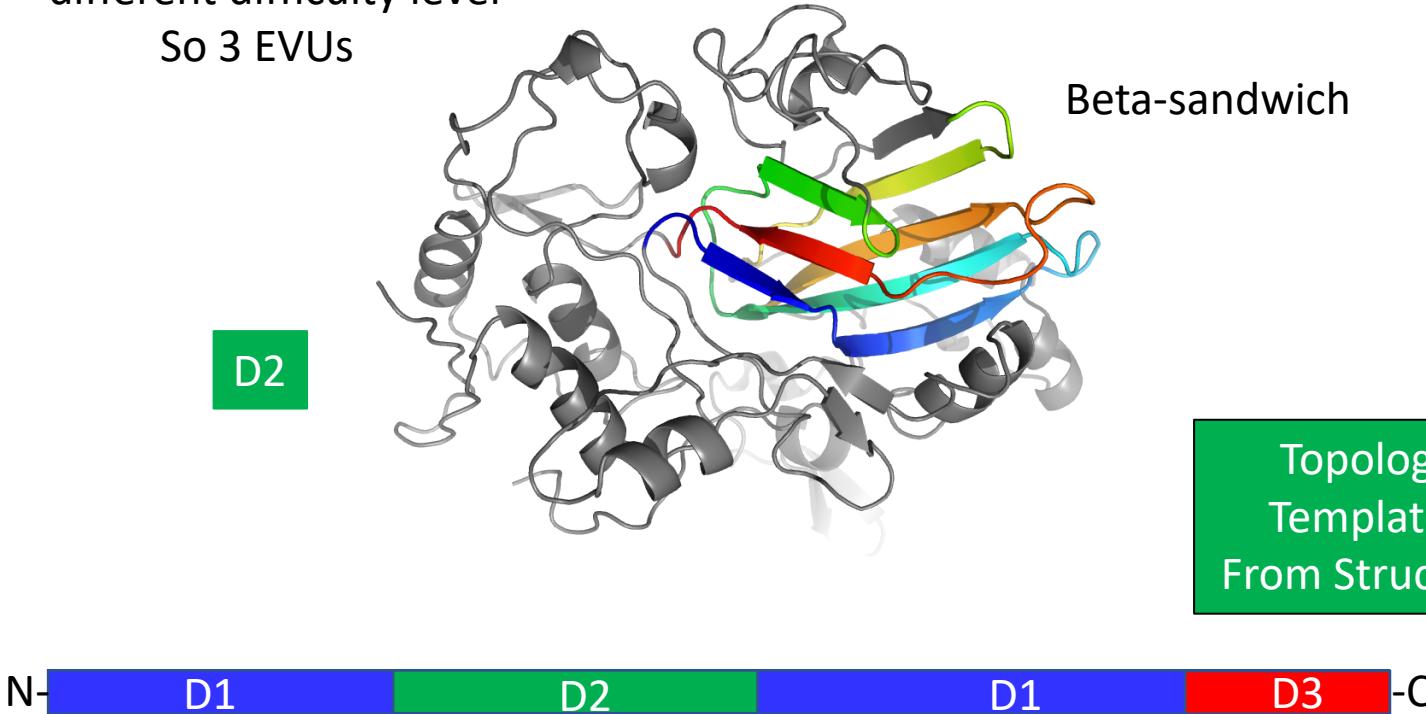
4 domains: RIFT-related, N0 domain, insert, and RIFT-related **but 1EVU**



D3 1ten Top LGA_S Template
Immunoglobulin-related

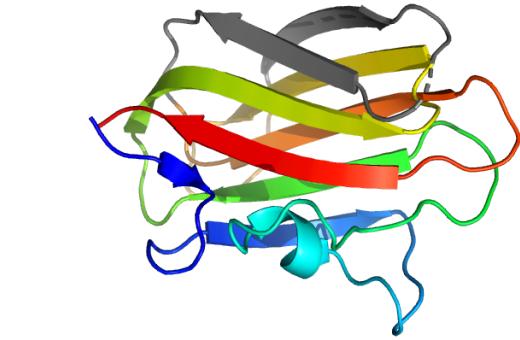
Topology-level Insert is More Difficult: Suggests a Split

= different difficulty level
So 3 EVUs

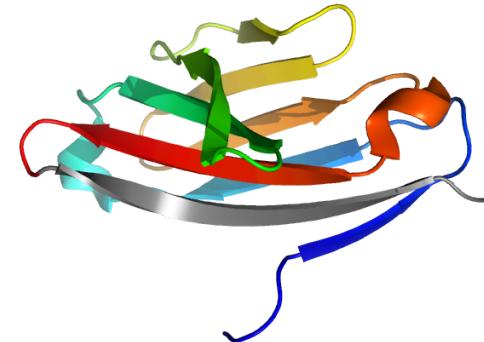


T1061: *E.coli* phage tail

- Complex domain organization
- Domain parser and Ddomain split differently (4 vs 5)
- Grishin Plots have multiple clouds
- Templates for blue and red domains

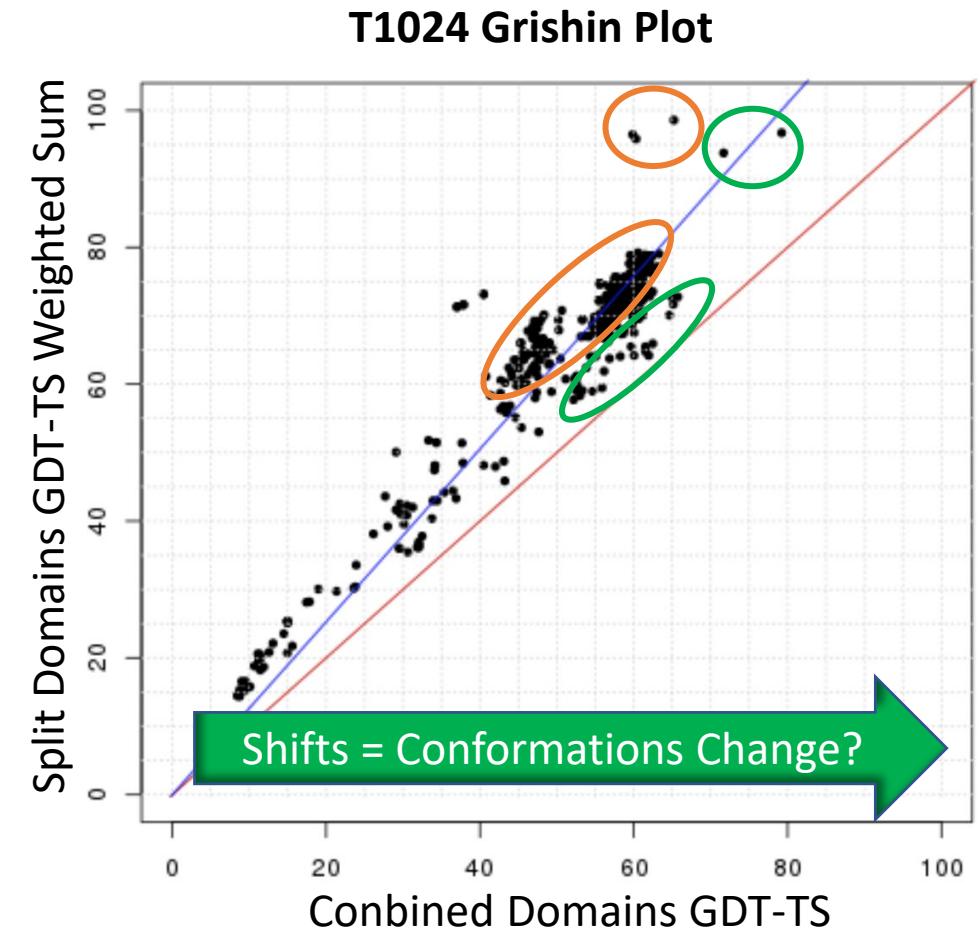
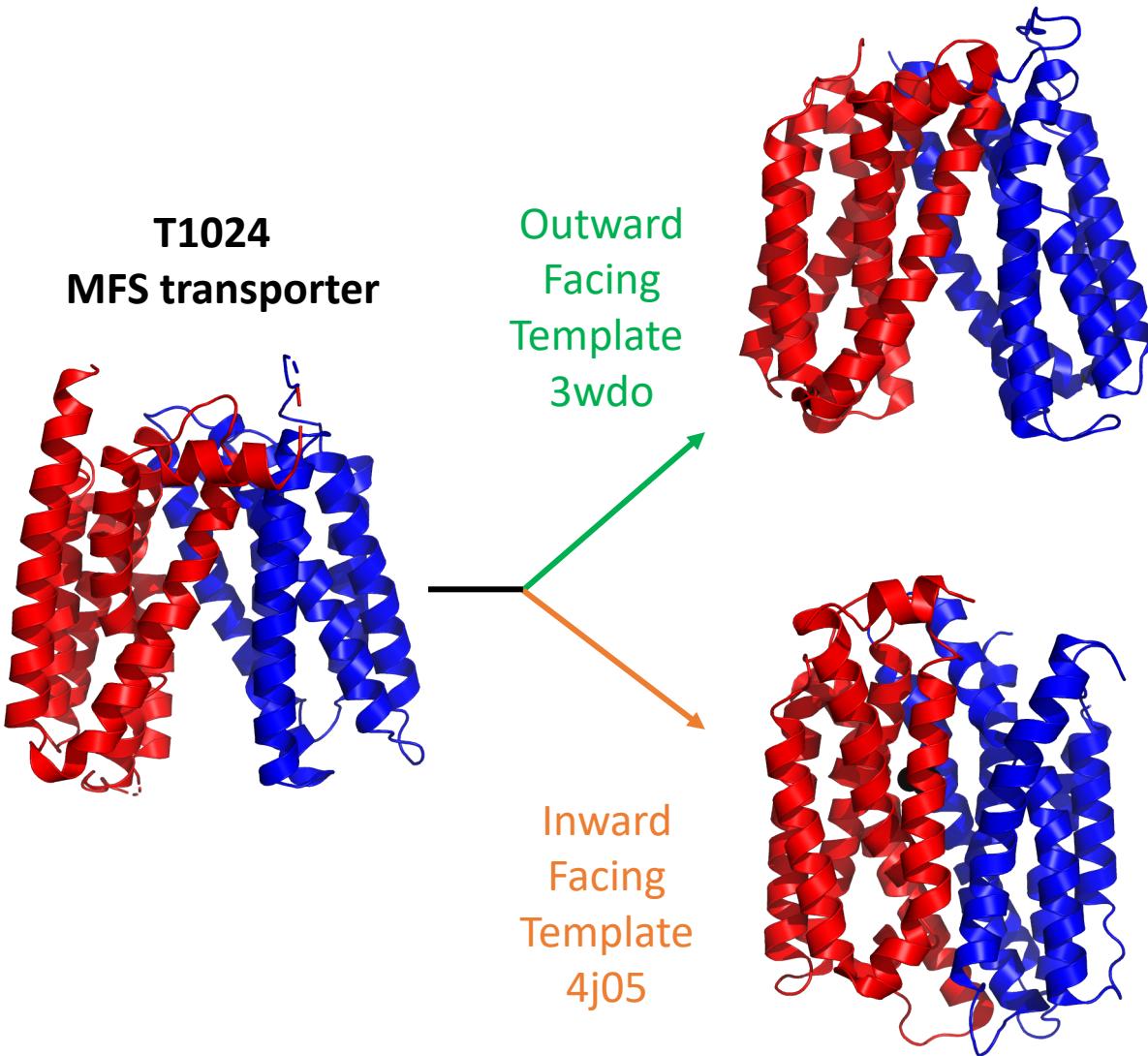


2yc2 Top LGA_S Template
Intraflagella Transport Protein 25
jelly-roll

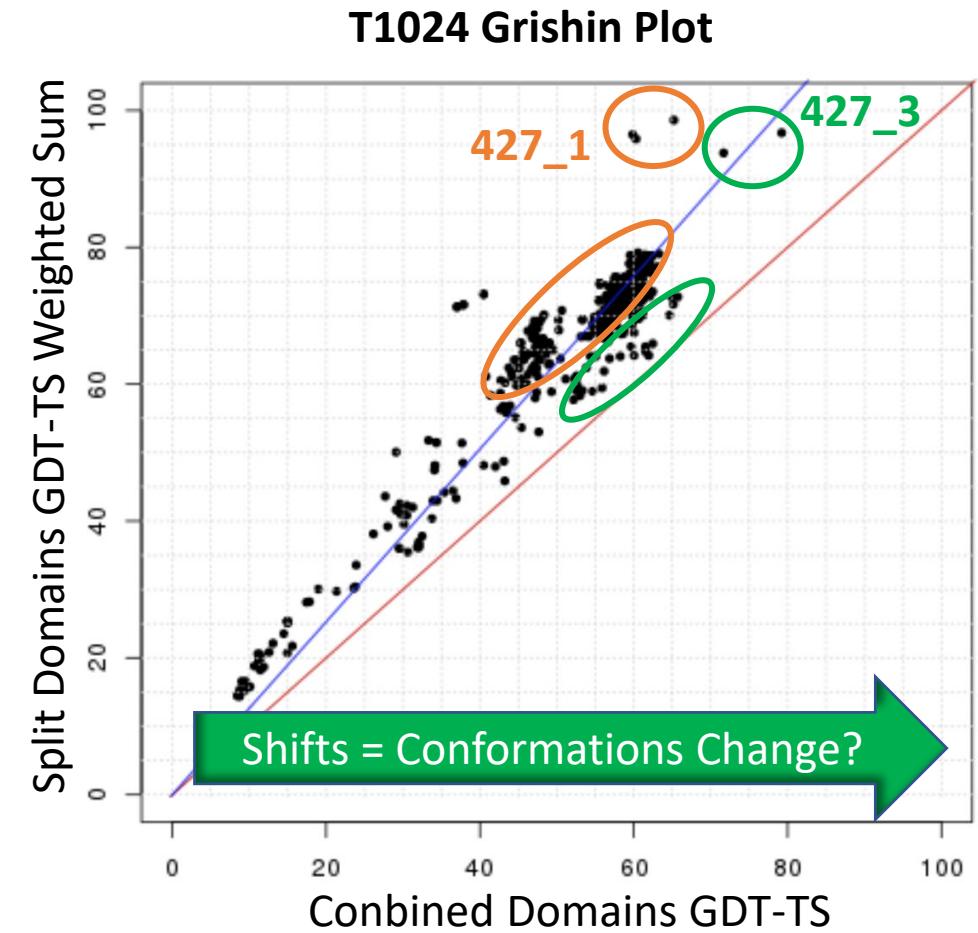
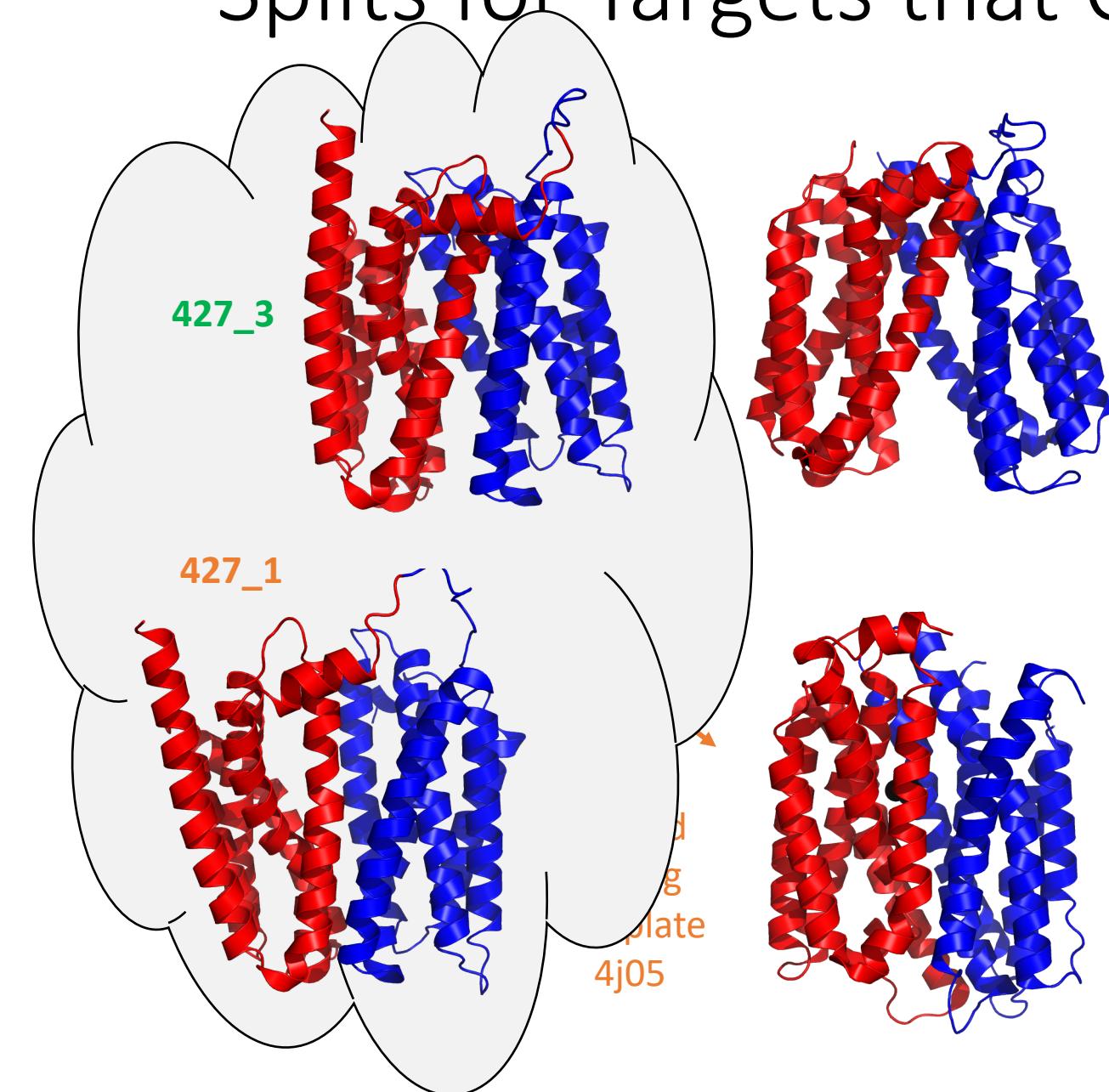


2frg Top Dali Template
human TLT1
Immunoglobulin-like β-sandwich

Splits for Targets that Change Conformation

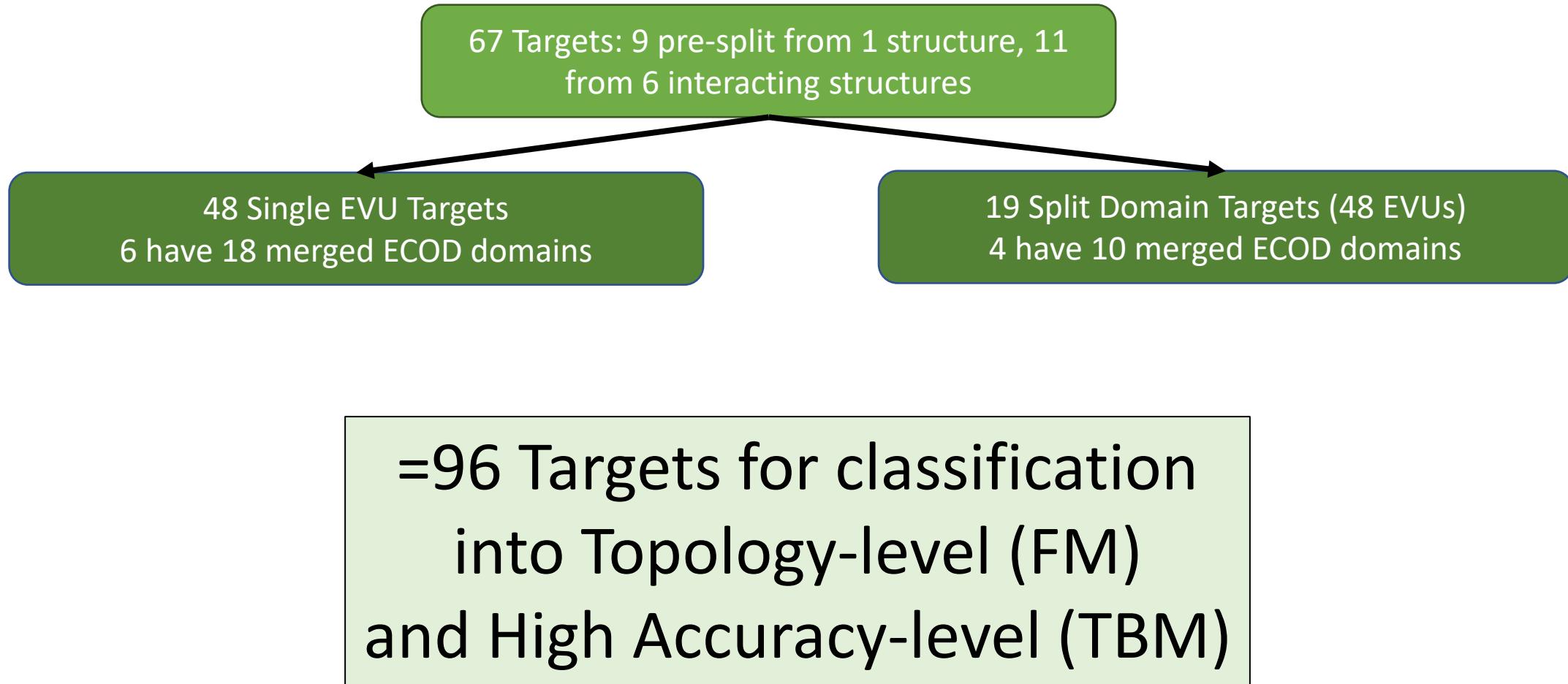


Splits for Targets that Change Conformation



4 Similar Targets: T1024, T1050, T1100, T1101

CASP14 Domains and EVUs in Numbers



Evolutionary Relationships to known Templates help Classification

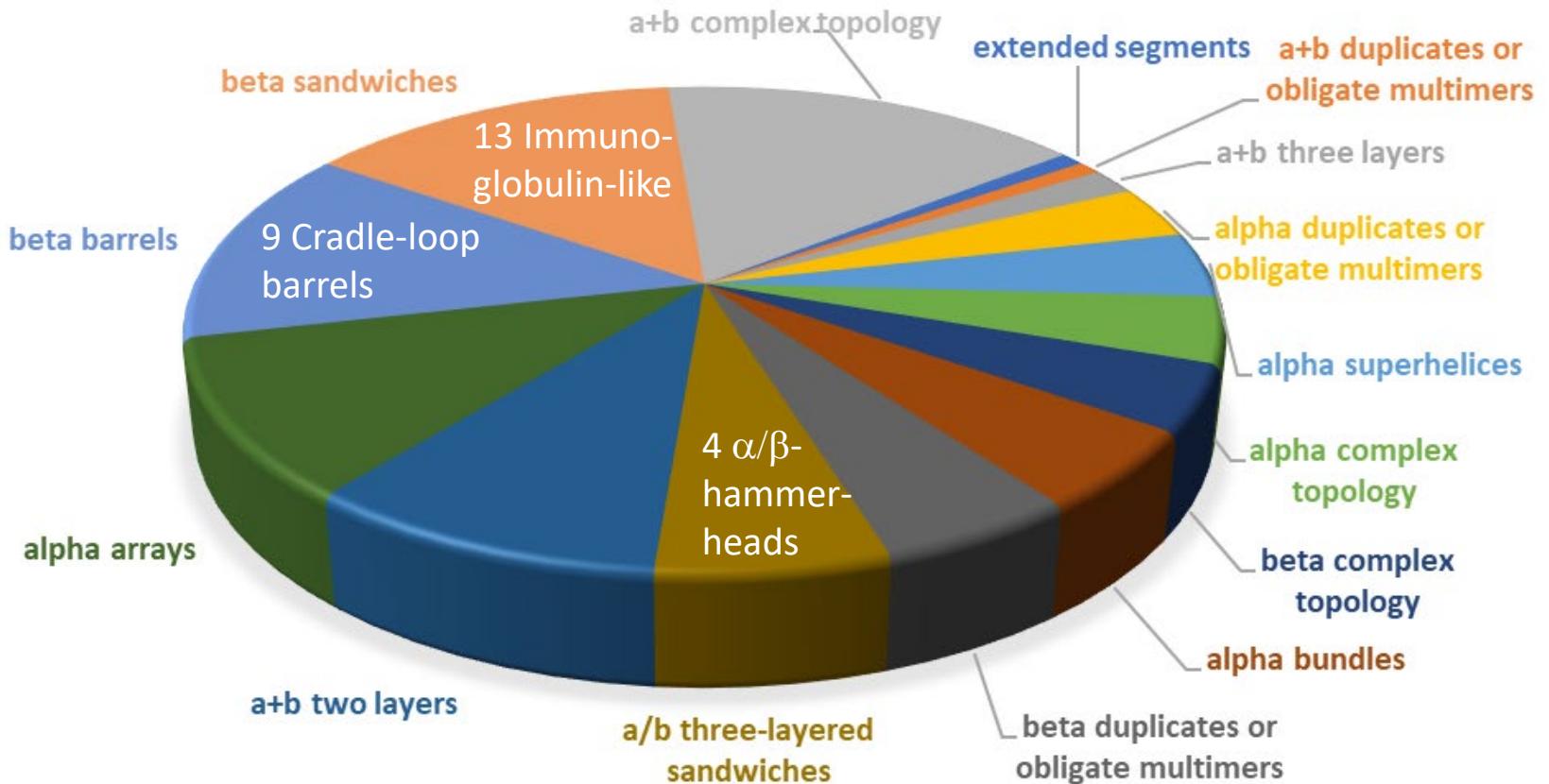
Evolution-Based Classification of CASP14 EVUs

67 Targets: 9 pre-split from 1 structure, 11 from 6 interacting structures

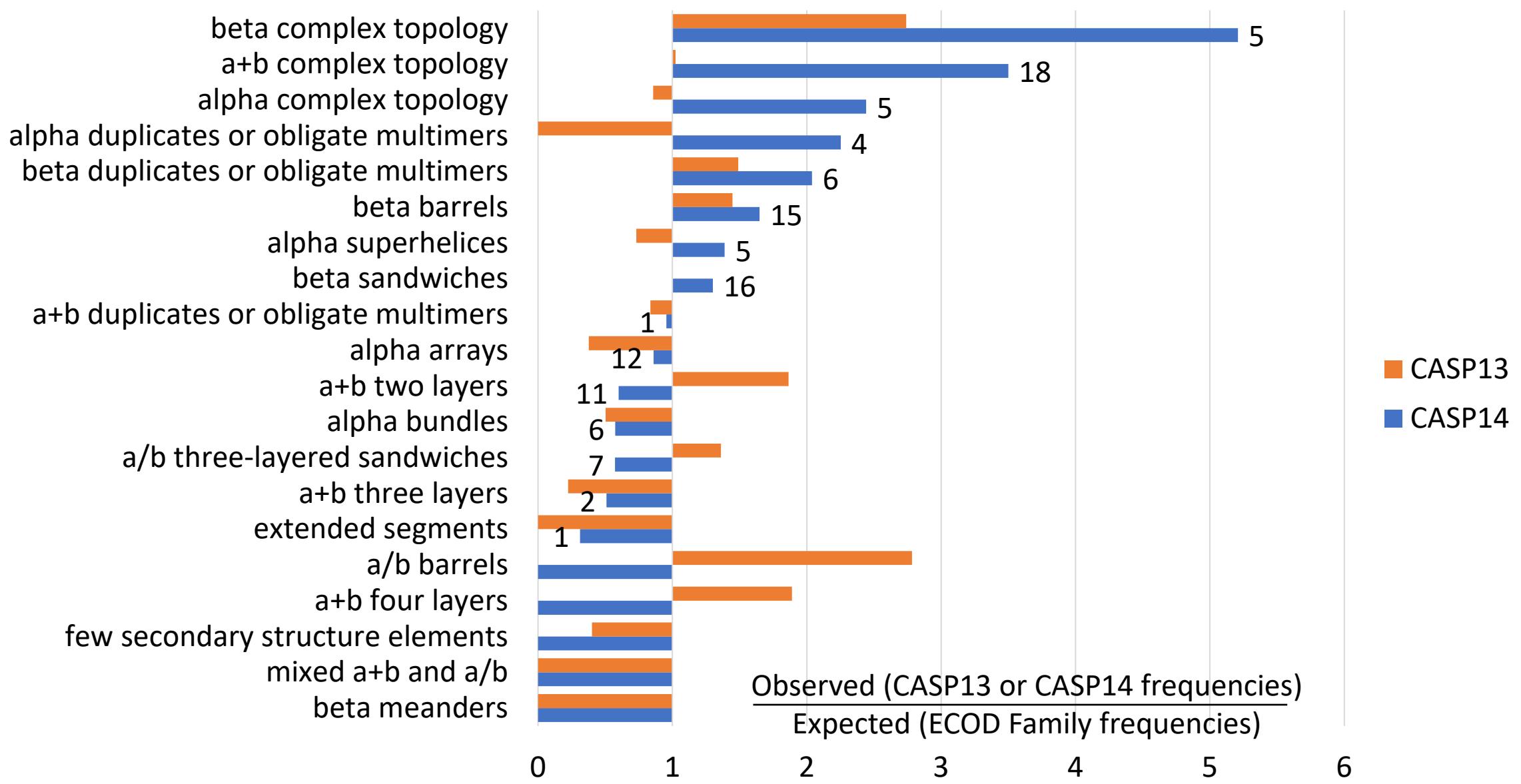
48 Single EVU Targets
6 have 18 merged ECOD domains

19 Split Domain Targets (48 EVUs)
4 have 10 merged ECOD domains

ECOD Classification based on distance to template:	
Class	Definition
Family (24EVU)	Template is in the same cdd
H-group (50 EVU)	Template is homologous
X-group (12 EVU)	Topological similarities
New (10 EVU)	Unique combination of SSEs

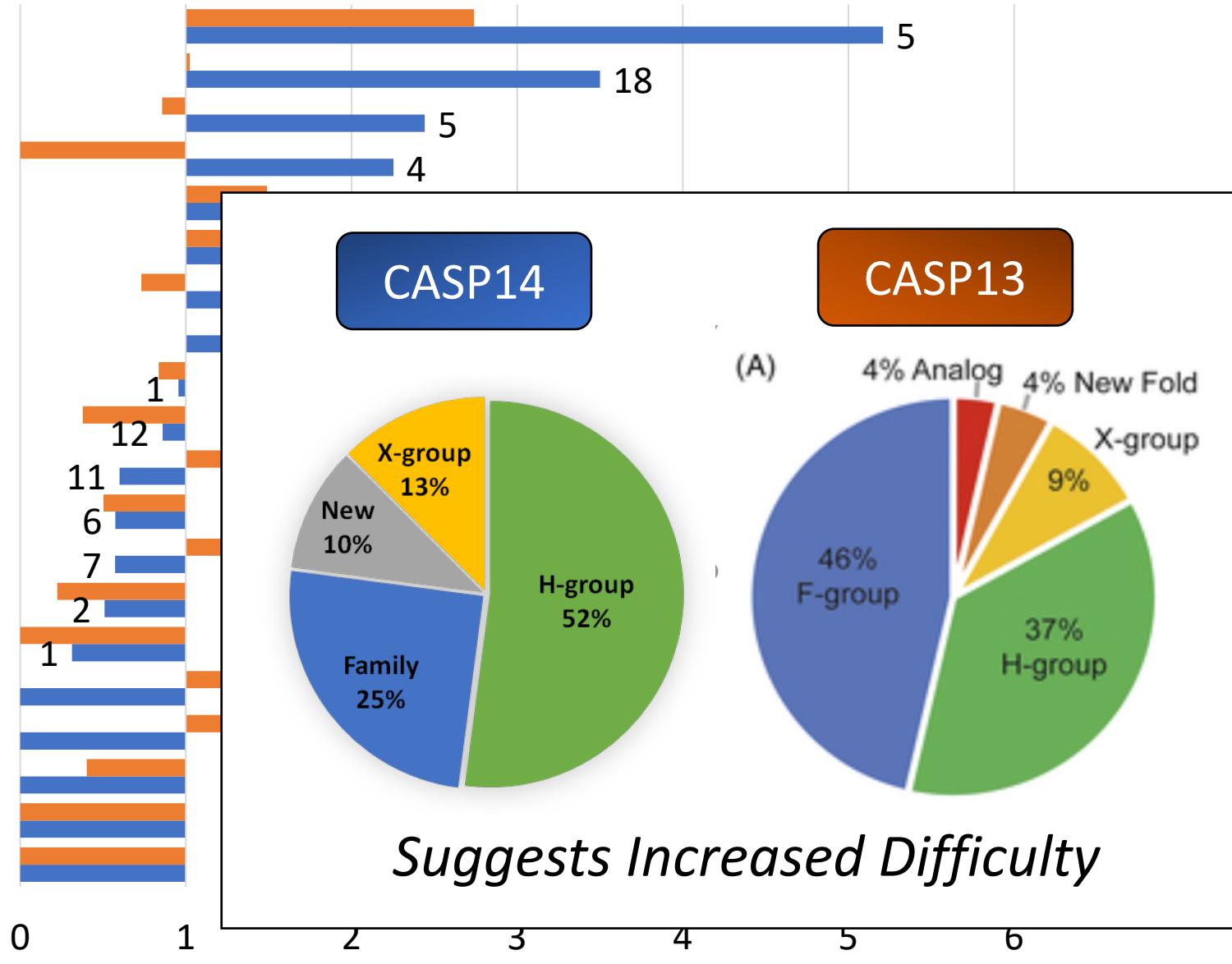


ECOD Architectures: CASP14 compared to CASP13



ECOD Relationships: CASP14 compared to CASP13

beta complex topology
a+b complex topology
alpha complex topology
alpha duplicates or obligate multimers
beta duplicates or obligate multimers
beta barrels
alpha superhelices
beta sandwiches
a+b duplicates or obligate multimers
alpha arrays
a+b two layers
alpha bundles
a/b three-layered sandwiches
a+b three layers
extended segments
a/b barrels
a+b four layers
few secondary structure elements
mixed a+b and a/b
beta meanders

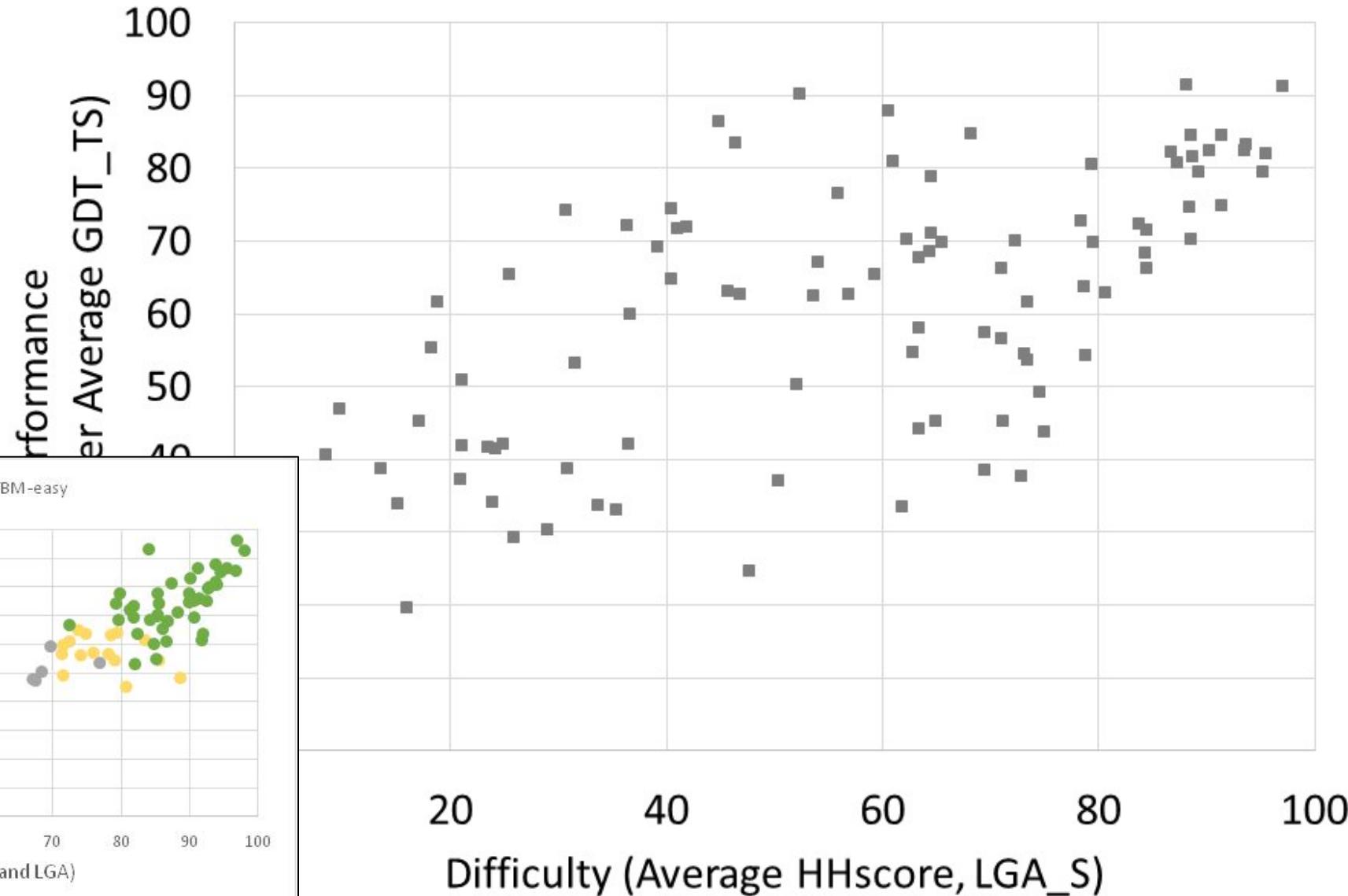
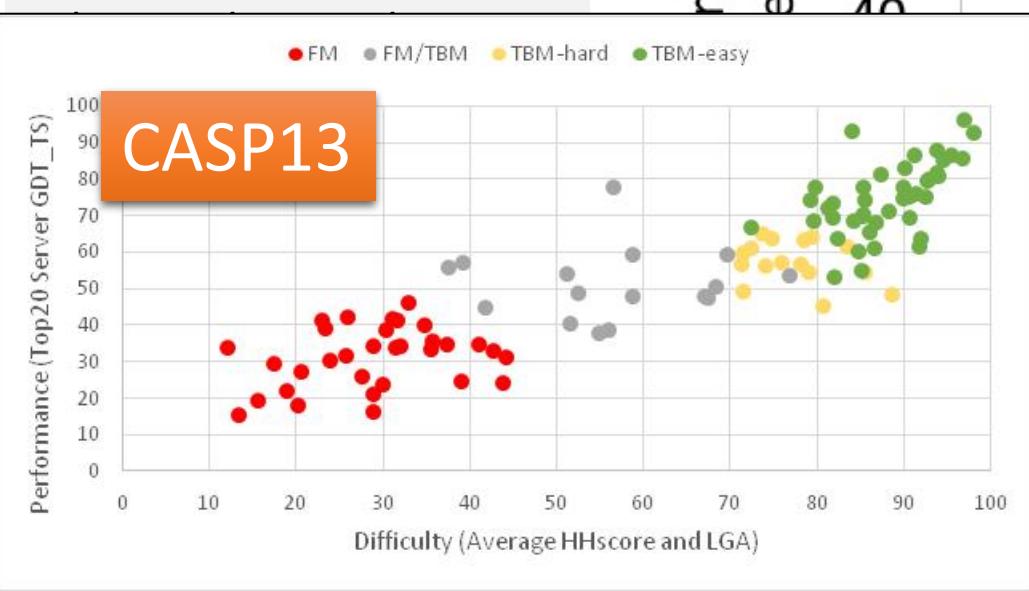


Traditional CASP Classification Plot: Scatter is Broad

EVU Classification Scores

Hhscore = HHprobability x
Coverage for Chosen
Template

Use higher Hhscore from 2
methods: Uniprot100 or
PDB70 for query profile



Traditional CASP Classification Plot: Scatter is Broad

EVU Classification Scores

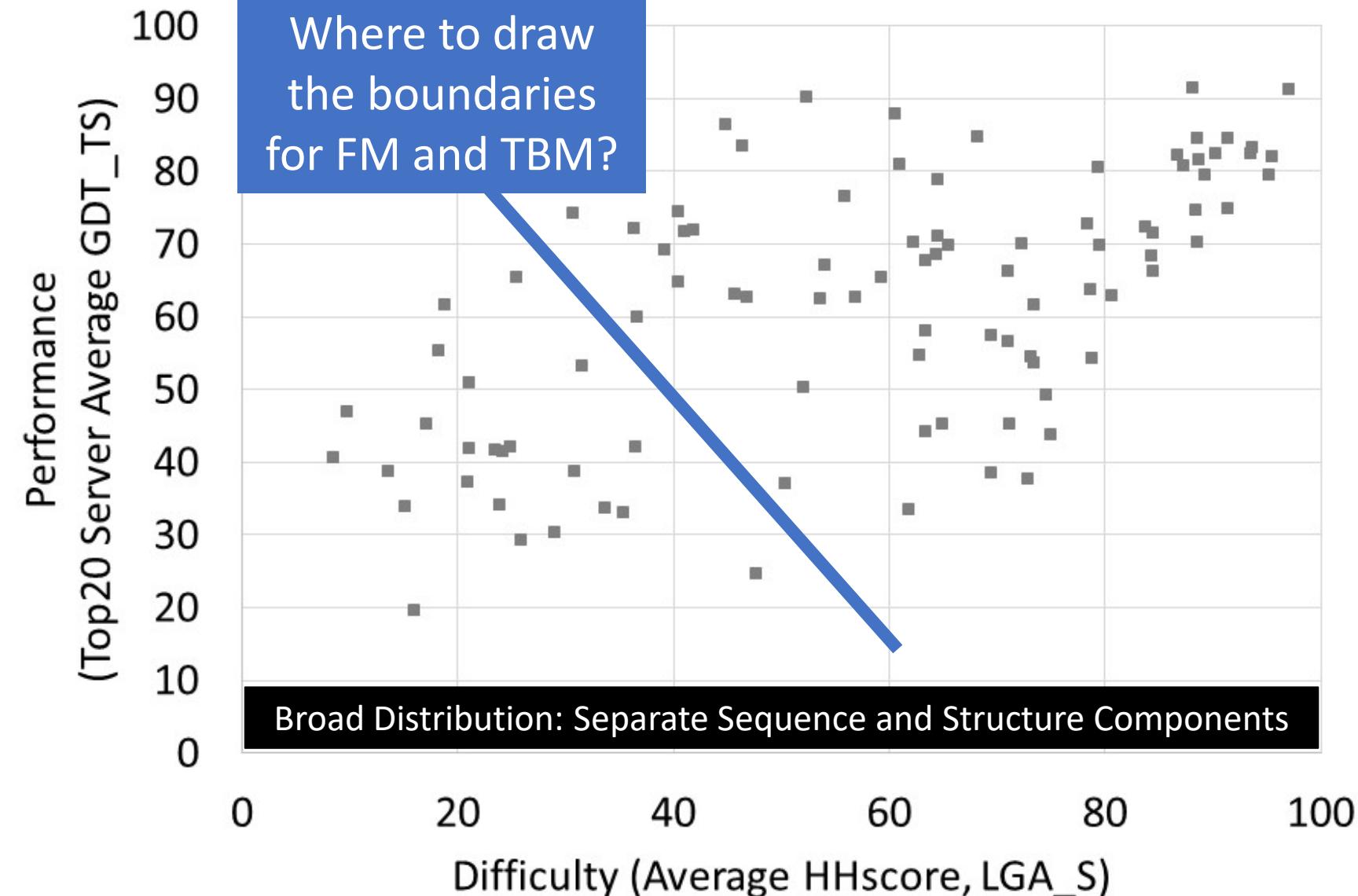
Hhscore = HHprobability x Coverage for Chosen Template

Use higher Hhscore from 2 methods: Uniprot100 or PDB70 for query profile

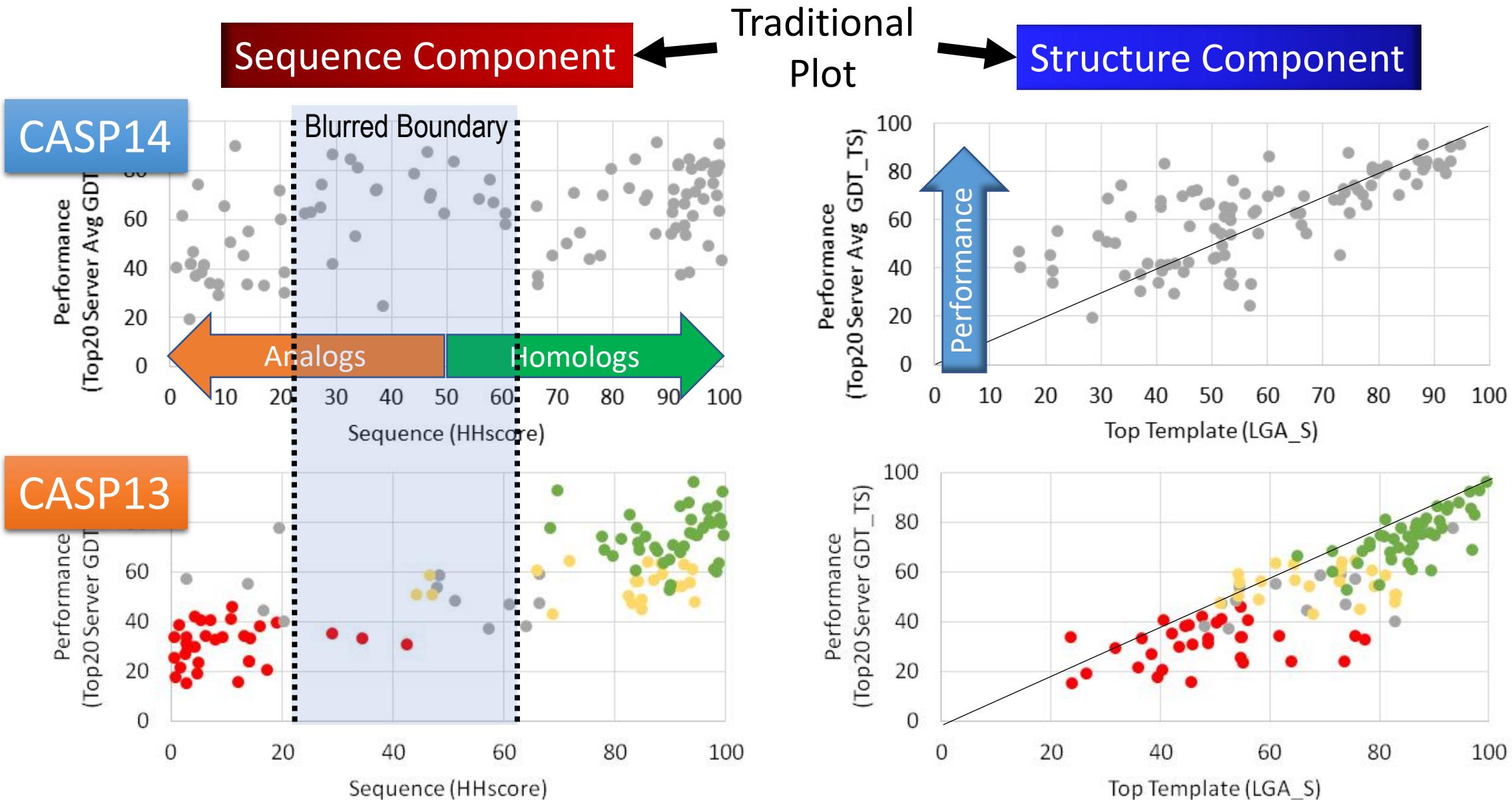
Select Rank1 template unless

- Max Hhscore > for alternate homolog
- Lower rank homolog replaces analog

Top LGA_S from homolog or analogous fragment

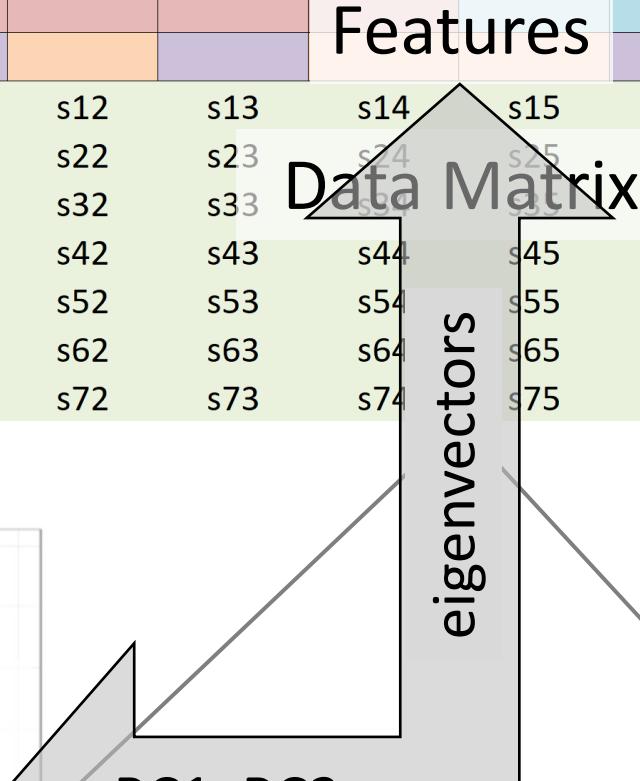
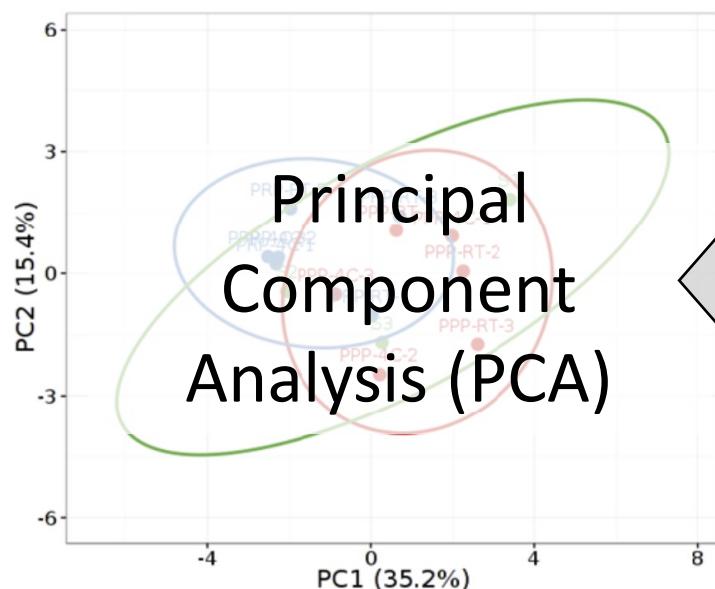


What Contributors to Broadened Scatter?

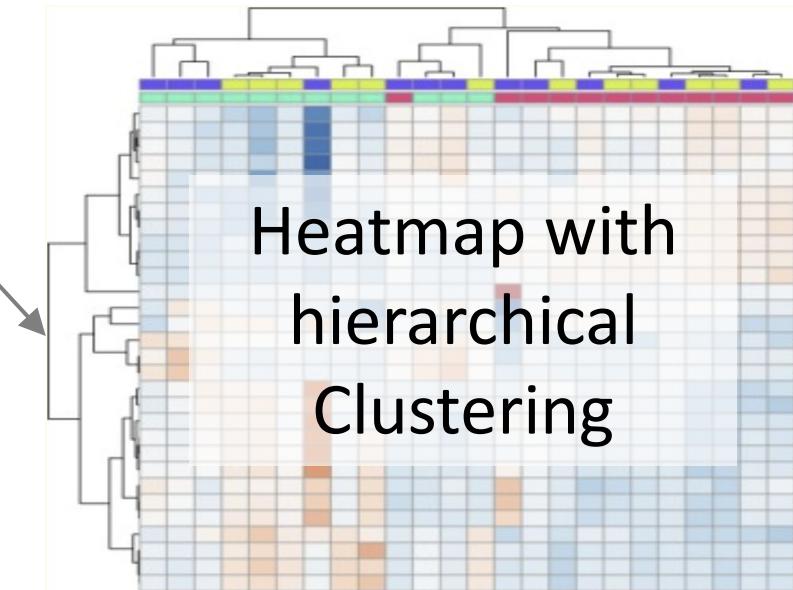


Cluster Data to Help Confirm Classification Bounds

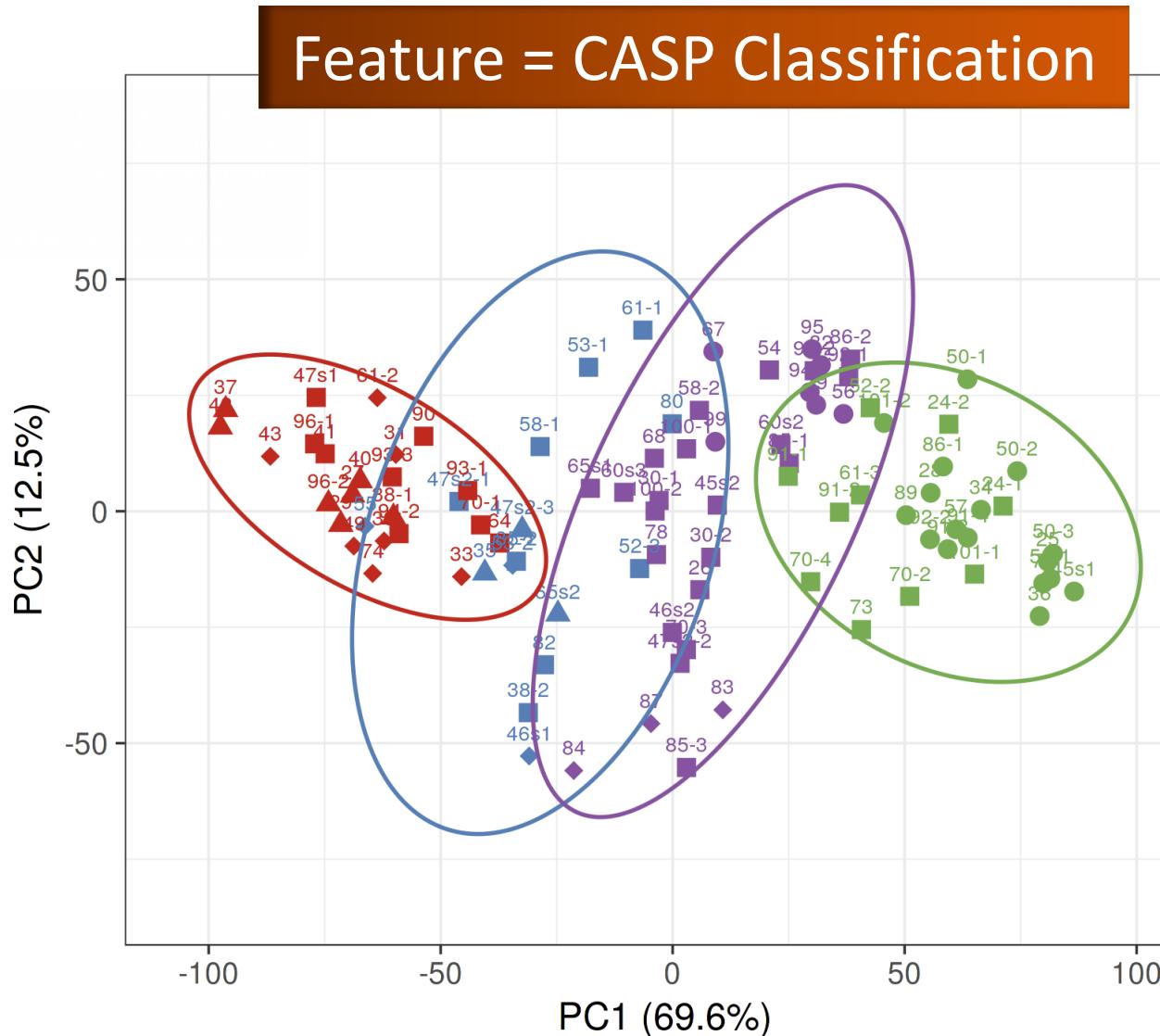
	Target1	Target2	Target3	Target4	Target5	Target6	Target7	Target8	Target96	<i>Classification ECOD Level</i>
feature1										
feature2										
Hhscore	score1	s11	s12	s13	s14	s15	s16	s17	s18	s196
LGA_S	score2	s21	s22	s23	s24	s25	s26	s27	s28	s296
Top20server	score3	s31	s32	s33	s34	s35	s36	s37	s38	s396
Dali%self	score4	s41	s42	s43	s44	s45	s46	s47	s48	s496
DaliCoverage	score5	s51	s52	s53	s54	s55	s56	s57	s58	s596
Neff (%max)	score6	s61	s62	s63	s64	s65	s66	s67	s68	s696
%parent Template	score7	s71	s72	s73	s74	s75	s76	s77	s78	s796



PMID: 25969447



PCA Plot of Targets Roughly Separates Classes



Scores Used:

- HHscore
- %parentTBM
- Neff%max
- performance
- TopLGA
- Dali%self
- DaliCvg

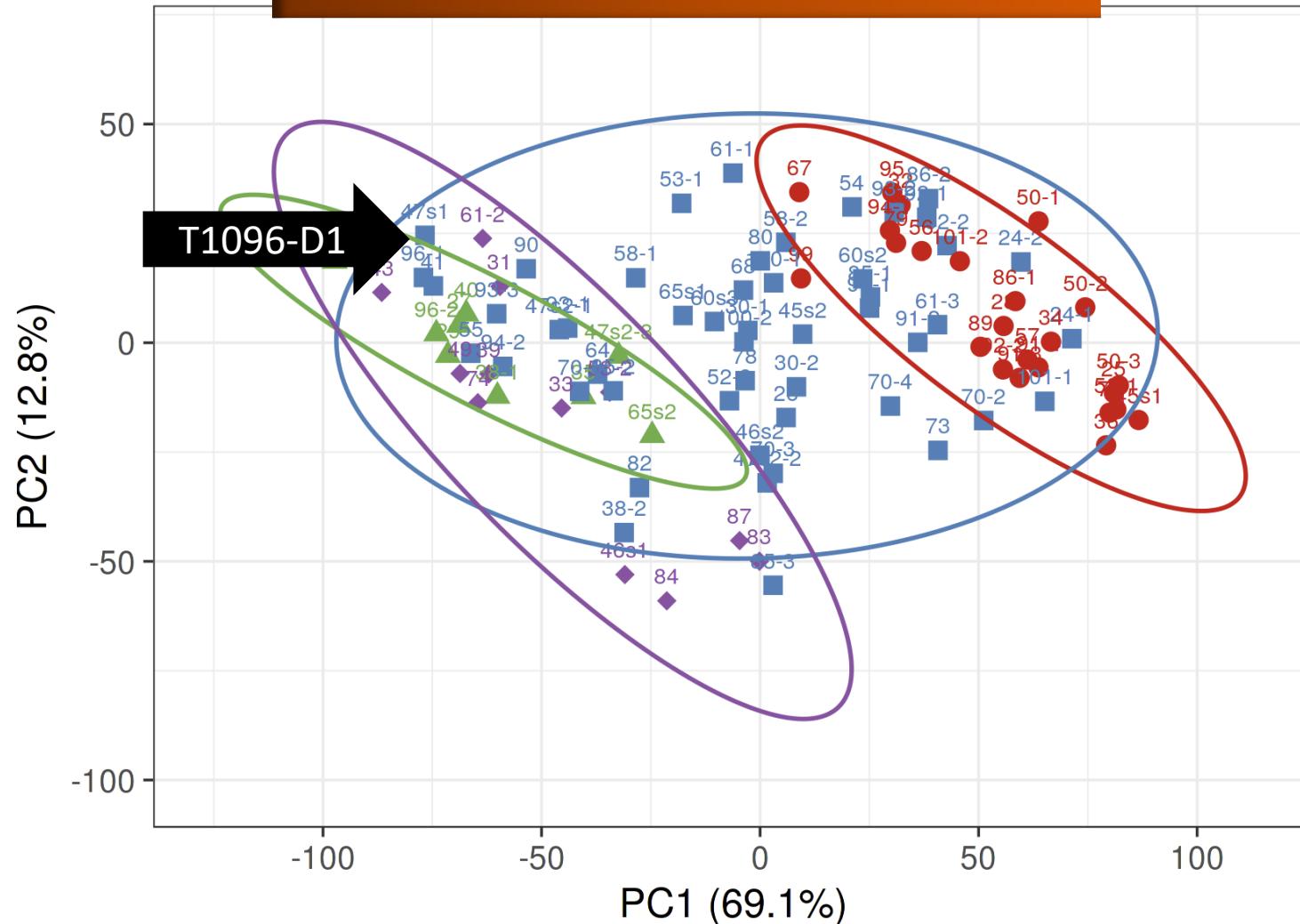
Data Preprocessing:
No scaling, rows centered

PCA Method:
SVD with Imputation

Prediction ellipses:
Probability 0.95

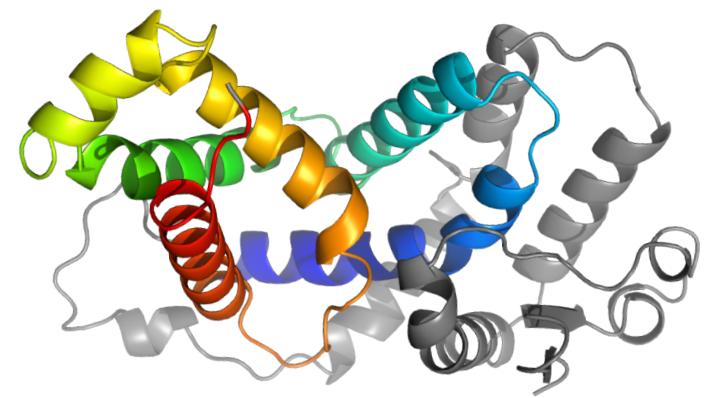
PCA Plot of Targets Roughly Separates ECOD Groups

Feature = ECOD Classification

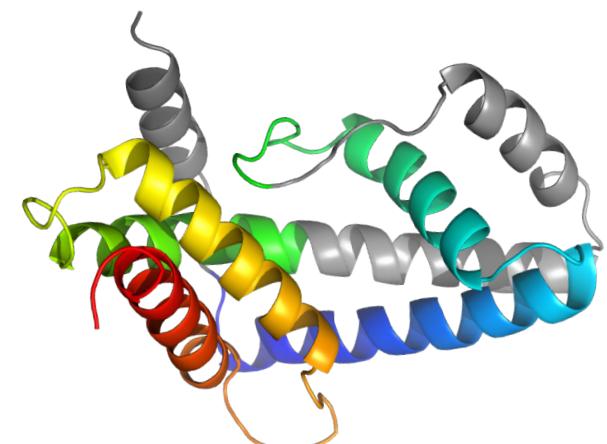


Level

- Family
- H-group
- New
- X-group



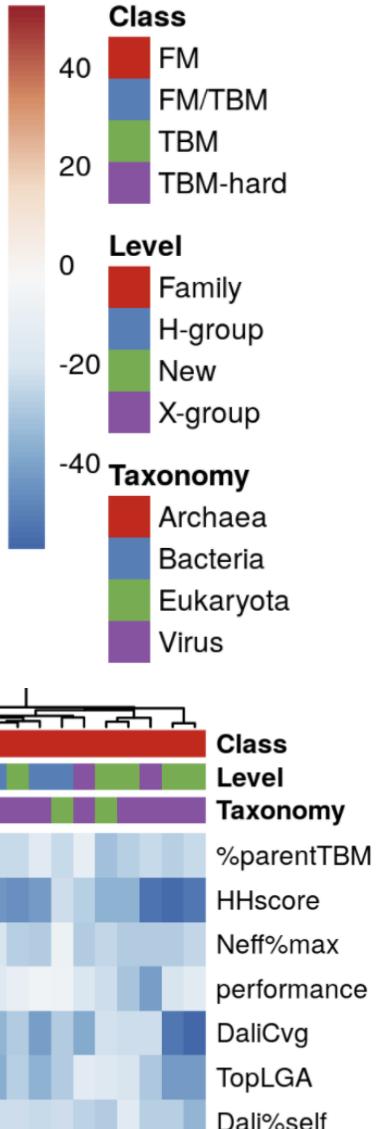
T1096-D1
Phage RNA Pol Subunit



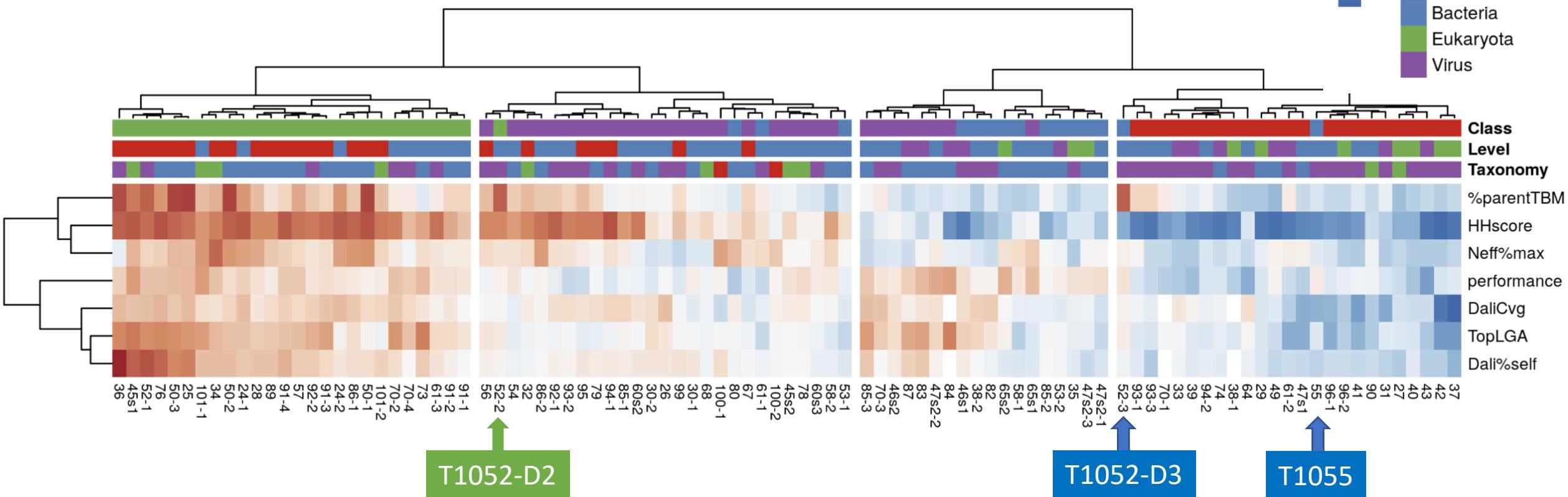
3les RNA Pol
Sigma Factor

Heatmap Clusters Targets by Classes

No scaling is applied to rows. Imputation is used for missing value estimation. Rows are clustered using correlation distance and Ward linkage. Columns are clustered using Euclidean distance and Ward linkage. 7 rows, 96 columns.

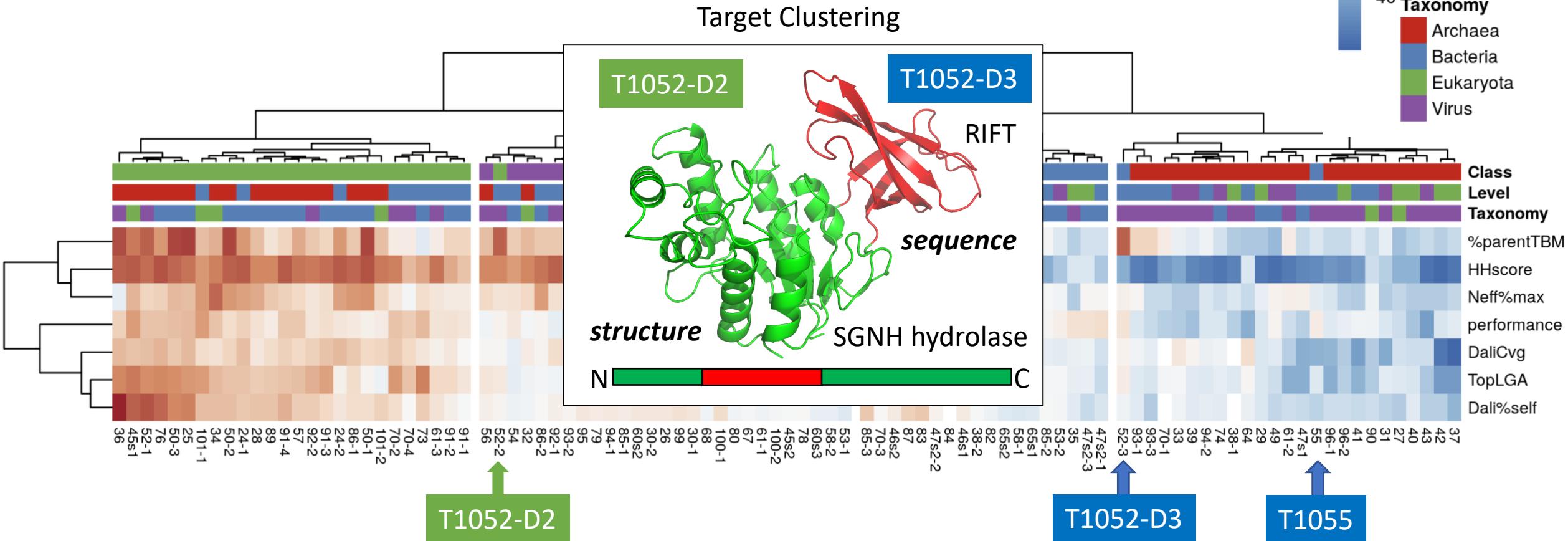
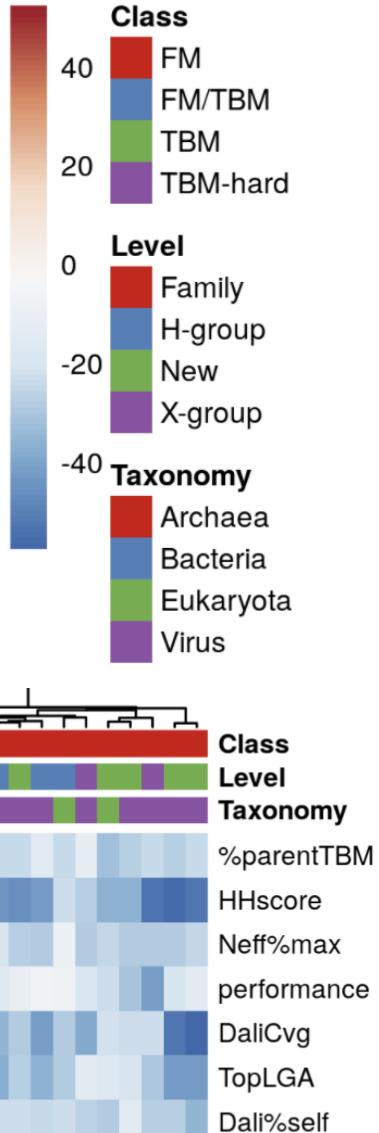


Target Clustering



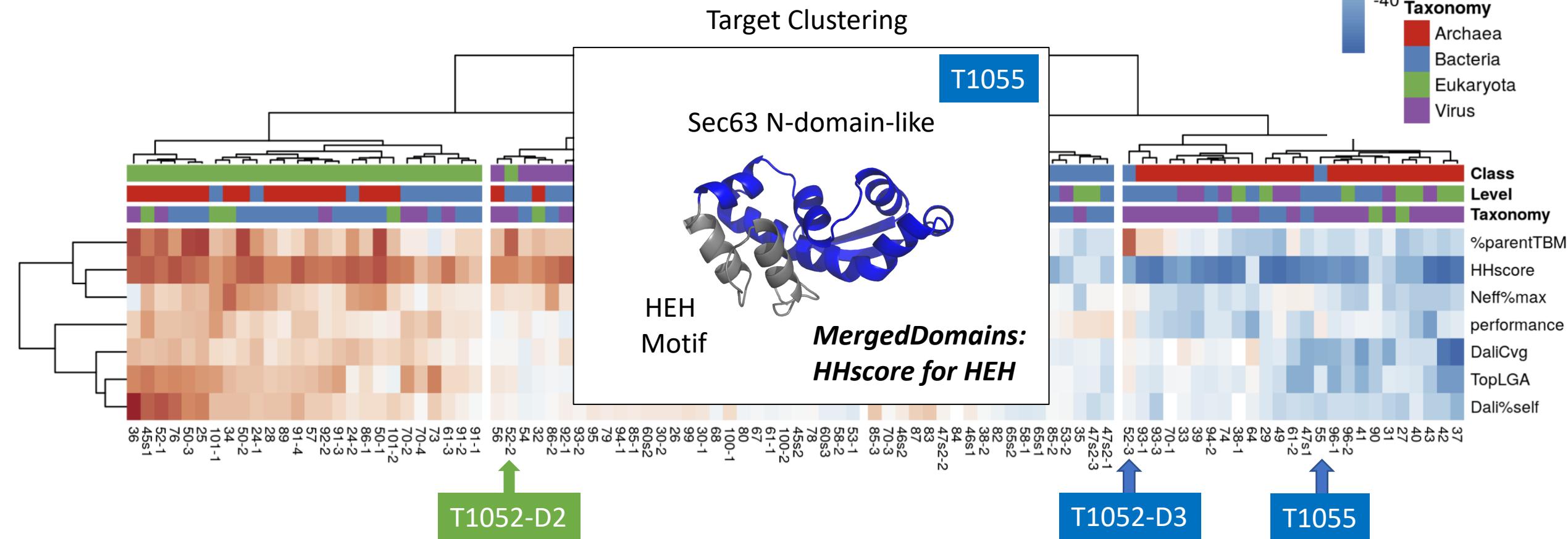
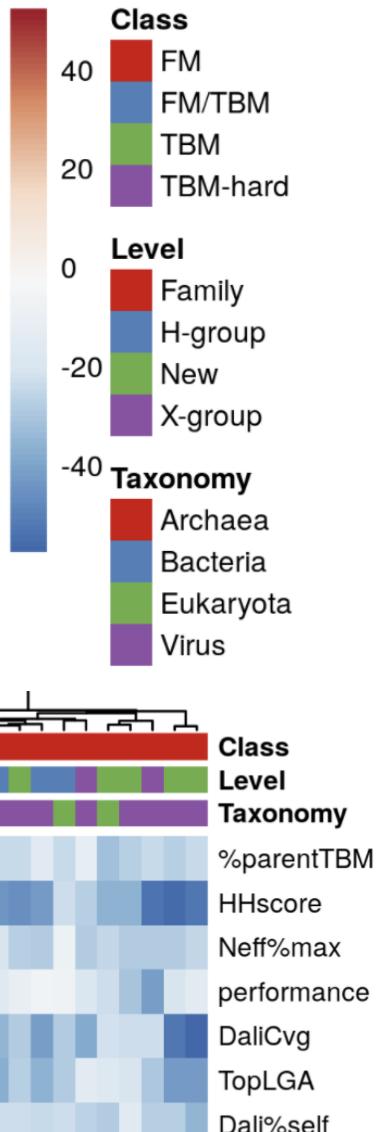
Heatmap Clusters Targets by Classes

No scaling is applied to rows. Imputation is used for missing value estimation. Rows are clustered using correlation distance and Ward linkage. Columns are clustered using Euclidean distance and Ward linkage. 7 rows, 96 columns.



Heatmap Clusters Targets by Classes

No scaling is applied to rows. Imputation is used for missing value estimation. Rows are clustered using correlation distance and Ward linkage. Columns are clustered using Euclidean distance and Ward linkage. 7 rows, 96 columns.

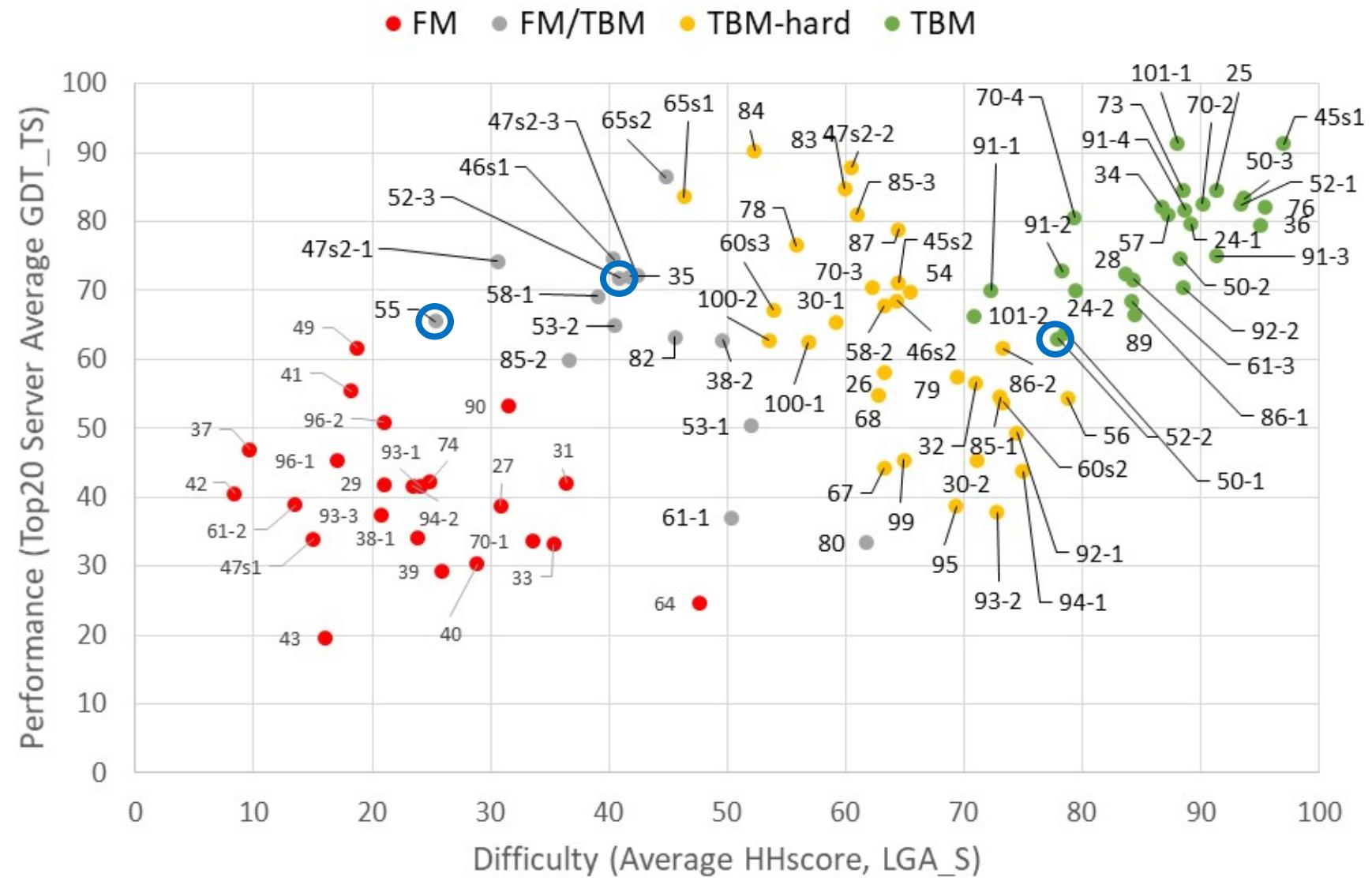


Traditional CASP Classification Plot: Outliers

Domains at the edge:
i.e. near the boundary in
the traditional
classification

Most domains were
classified by the traditional
scatter (to be consistent
with CASP13)

T1055, T1052-D2 and
T1052-D3 cluster differently
by heatmaps, but are
classified by the scatter



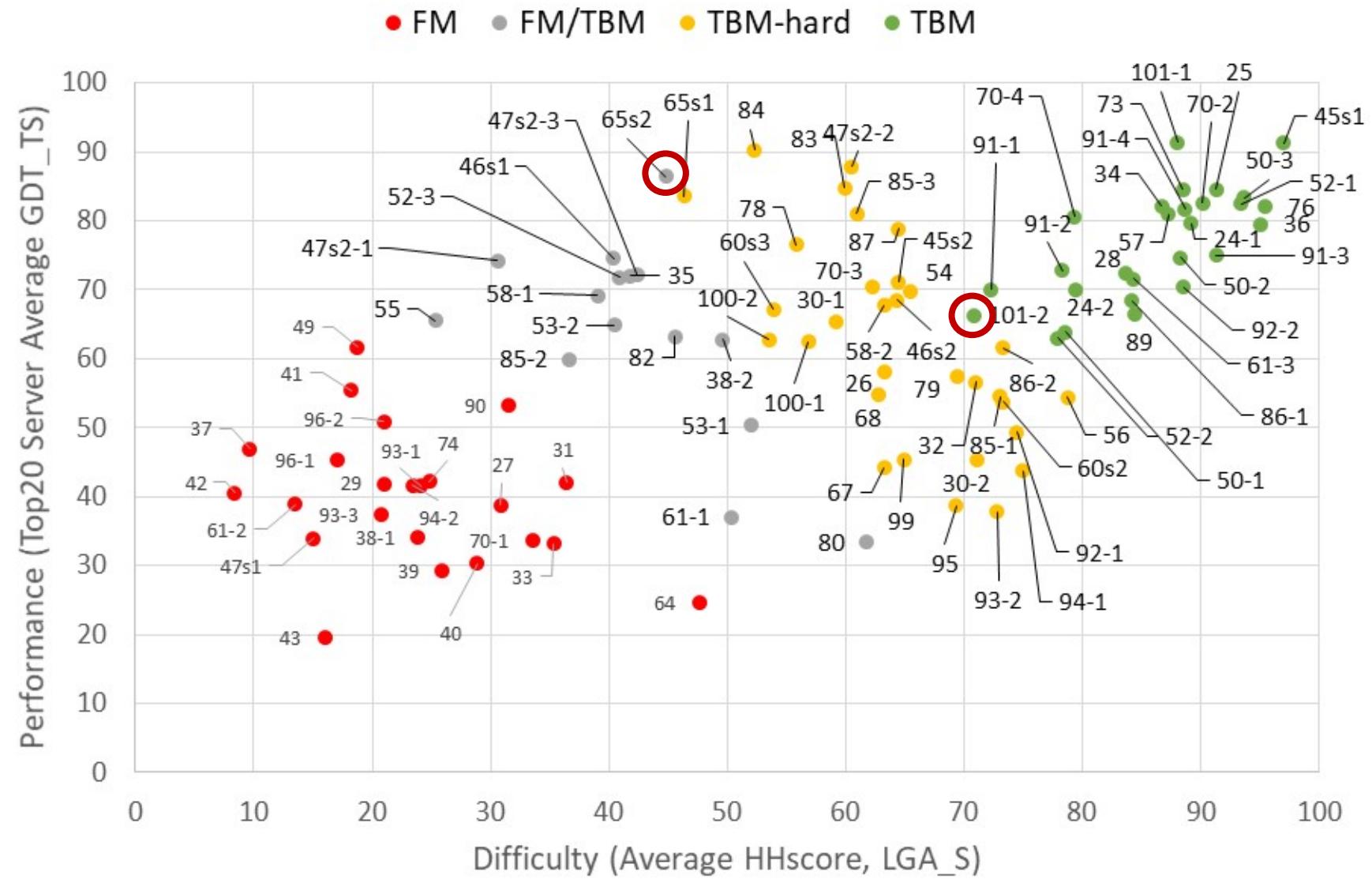
Traditional CASP Classification Plot: Outliers

Domains at the edge:
i.e. near the boundary in
the traditional
classification

Most domains were
classified by the traditional
scatter (to be consistent
with CASP13)

T1055, T1052-D2 and
T1052-D3 cluster differently
by heatmaps, but are
classified by the scatter

T101-2 and T1065s2 cluster
differently by the scatter,
but are classified by the
heatmap groups



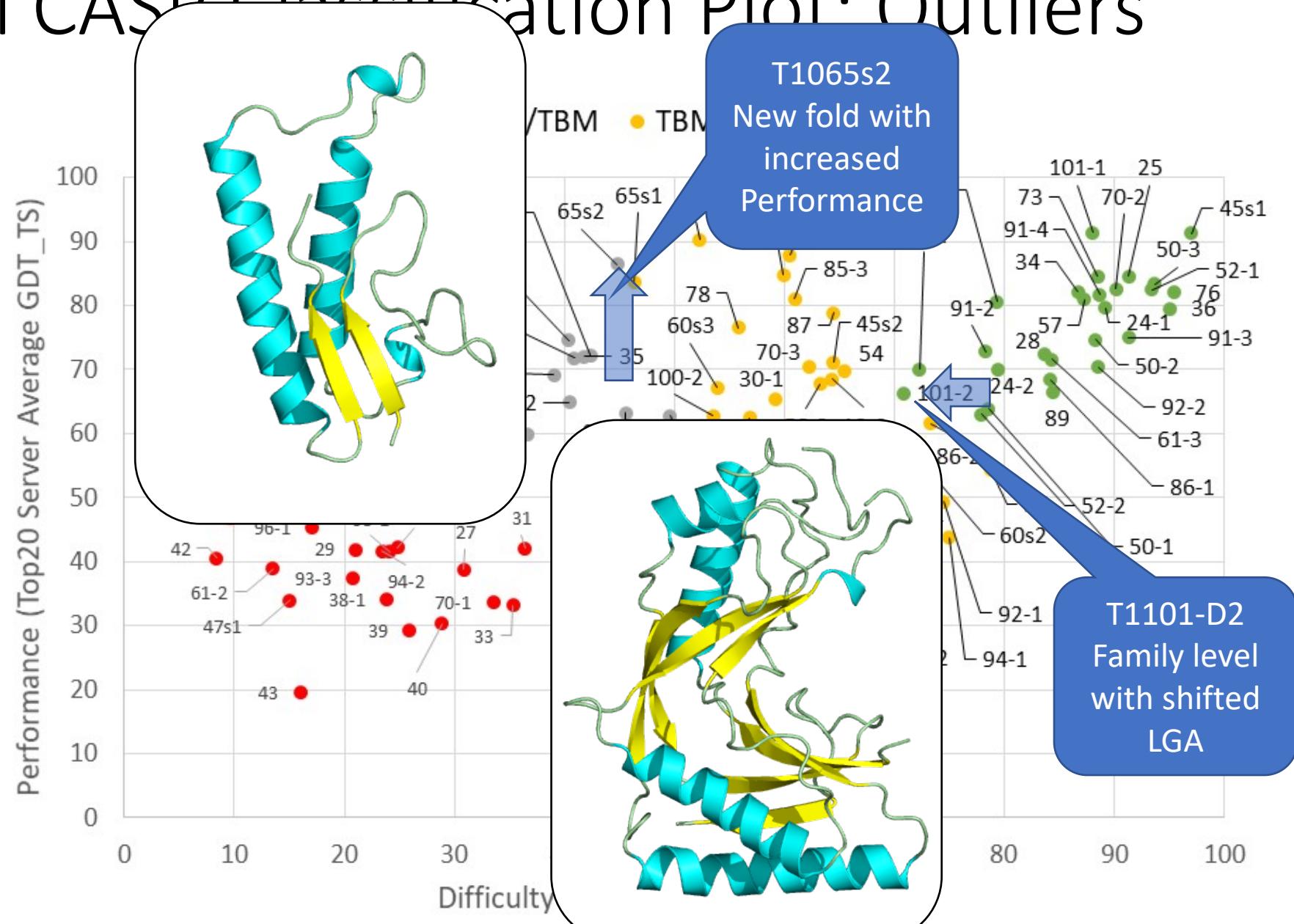
Traditional CASP Classification Plot: Outliers

**Domains at the edge:
i.e. near the boundary in
the traditional
classification**

Most domains were
classified by the traditional
scatter (to be consistent
with CASP13)

T1055, T1052-D2 and
T1052-D3 cluster differently
by heatmaps, but are
classified by the scatter

T101-2 and T1065s2 cluster differently by the scatter, but are classified by the heatmap groups



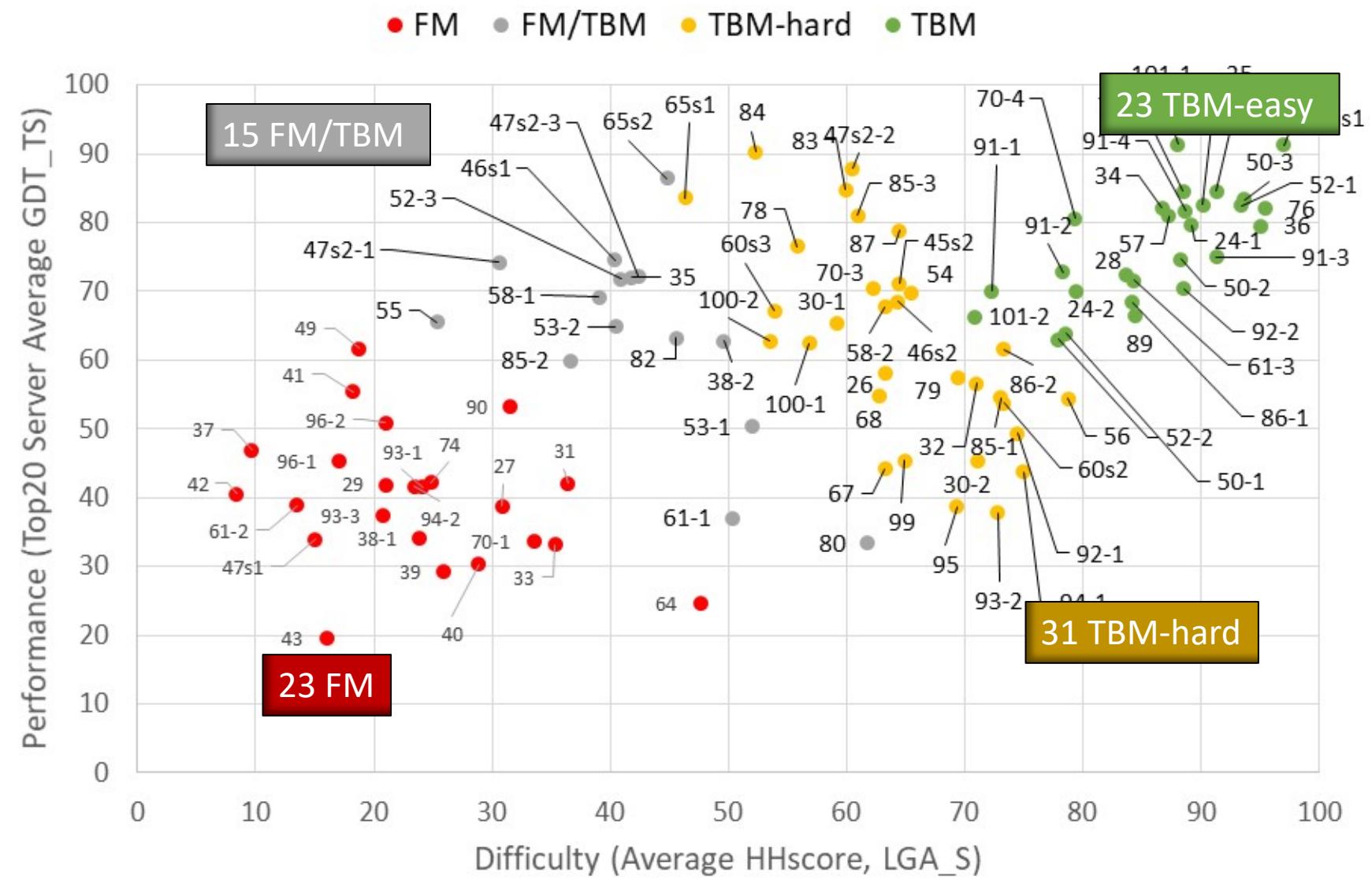
Final Traditional CASP Classification Plot

Domains at the edge:
i.e. near the boundary in
the traditional
classification

Most domains were
classified by the traditional
scatter (to be consistent
with CASP13)

T1055, T1052-D2 and
T1052-D3 cluster differently
by heatmaps, but are
classified by the scatter

T101-2 and T1065s2 cluster
differently by the scatter,
but are classified by the
heatmap groups



Thank You!



Collaborators
Nick Grishin (UTSW)
Dustin Schaeffer (UTSW)
Jimin Pei (UTSW)
Andriy Kryshtafovych (Prediction Center)

CASP Assessors

Andrei Lupas (High Accuracy Models)
Alfonso Valencia (Contacts)
Daniel Rigden (Refinement)
Ezgi Karaca (Assembly)
Chaok Seok (Model Accuracy)
Sandor Vajda (Function)

CASP Organizing Committee

John Moult, CASP chair and founder; IBBR, University of Maryland, USA

Krzysztof Fidelis, founder, University of California, Davis, USA

Andriy Kryshtafovych, University of California, Davis, USA

Torsten Schwede, University of Basel, Switzerland

Maya Topf, Birkbeck, University of London, UK