

Comprehensive characterization of the complex BAHD acyl transferase family from 25 plant species: phylogenetic analysis and identification of specificity determinant positions

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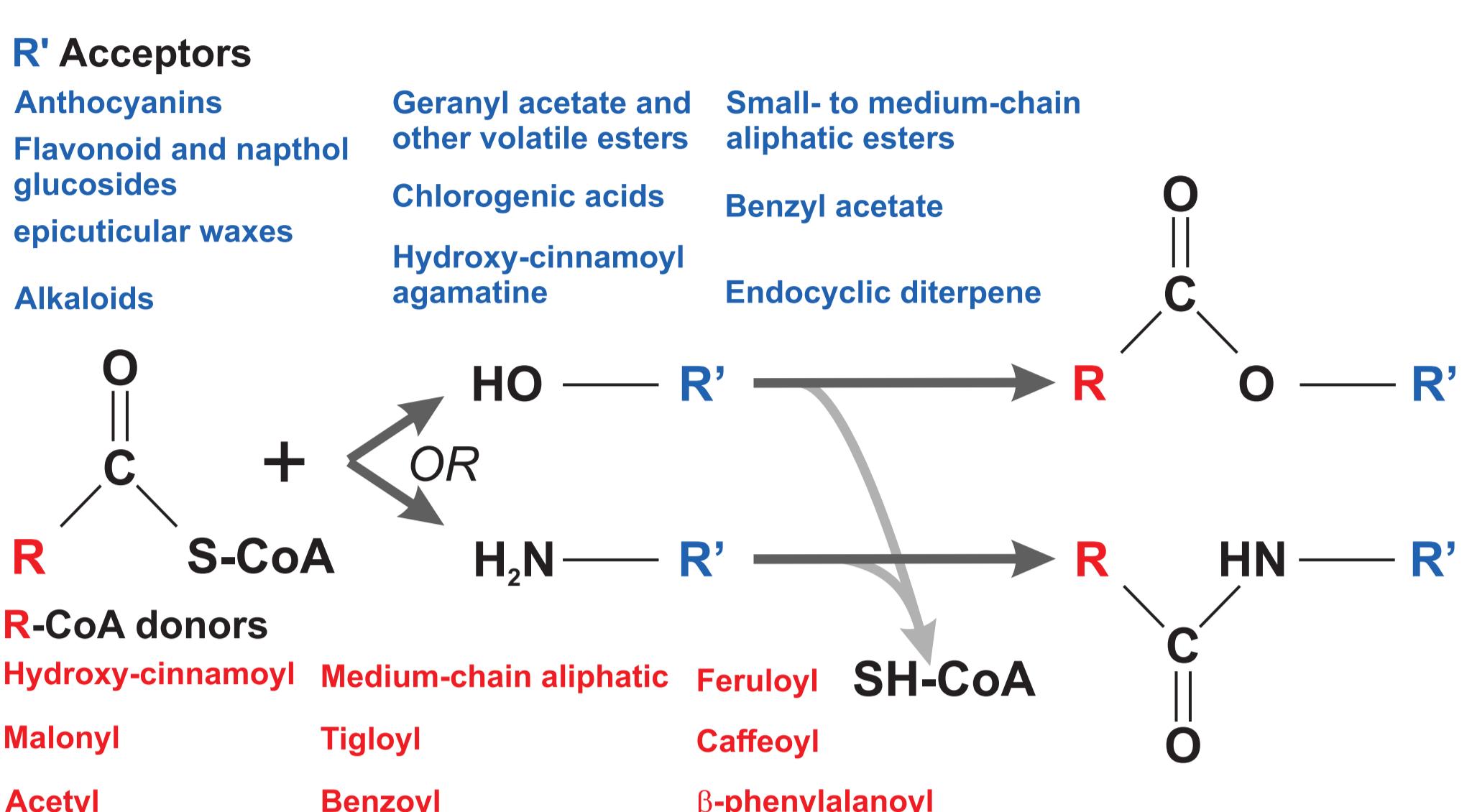
³Banco de Germoplasma, EEA INTA – Balcarce

Motivation

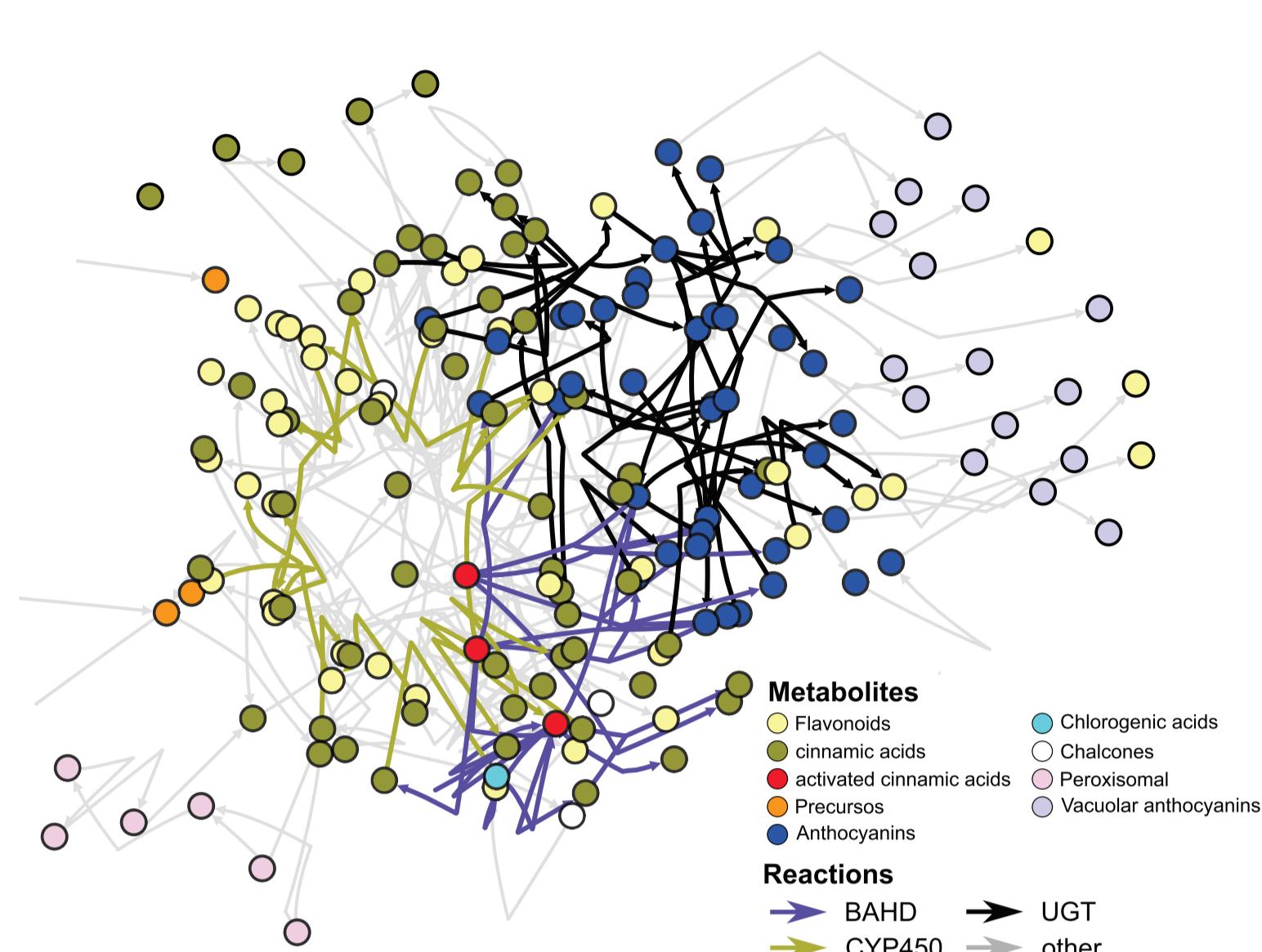
BAHD non-amino acyltransferases

These enzymes catalyze multiple reactions in land plants secondary metabolism (left). Together with Cytochrome P450 and UDP-glycosyl transferases (UGT), they are responsible for a large number of reactions in phenylpropanoid metabolism in potato tubers (center). At least 25 genes identified in potato tubers code for BAHD transferases (right), but how can we assign them proper function?

BAHD catalyzed reaction



Phenylpropanoid metabolism



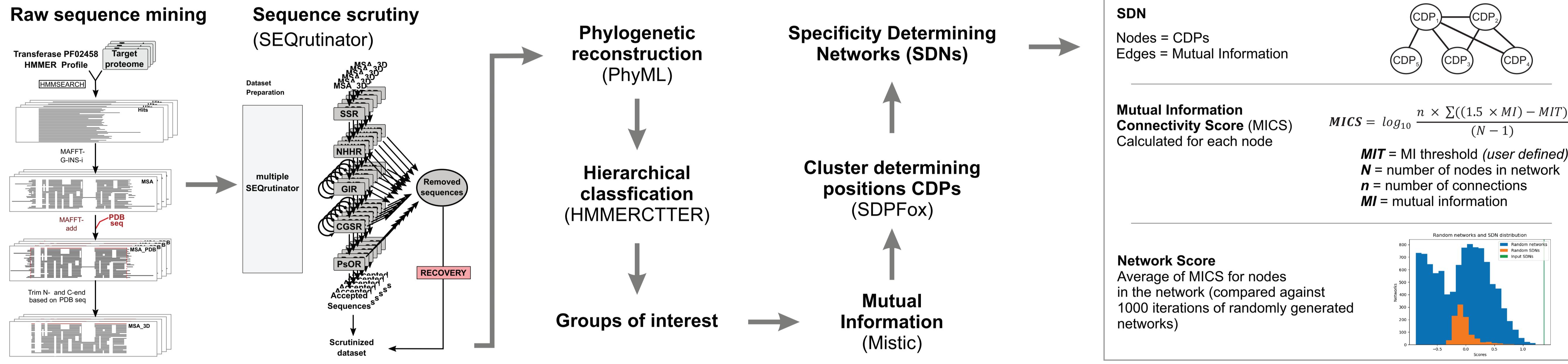
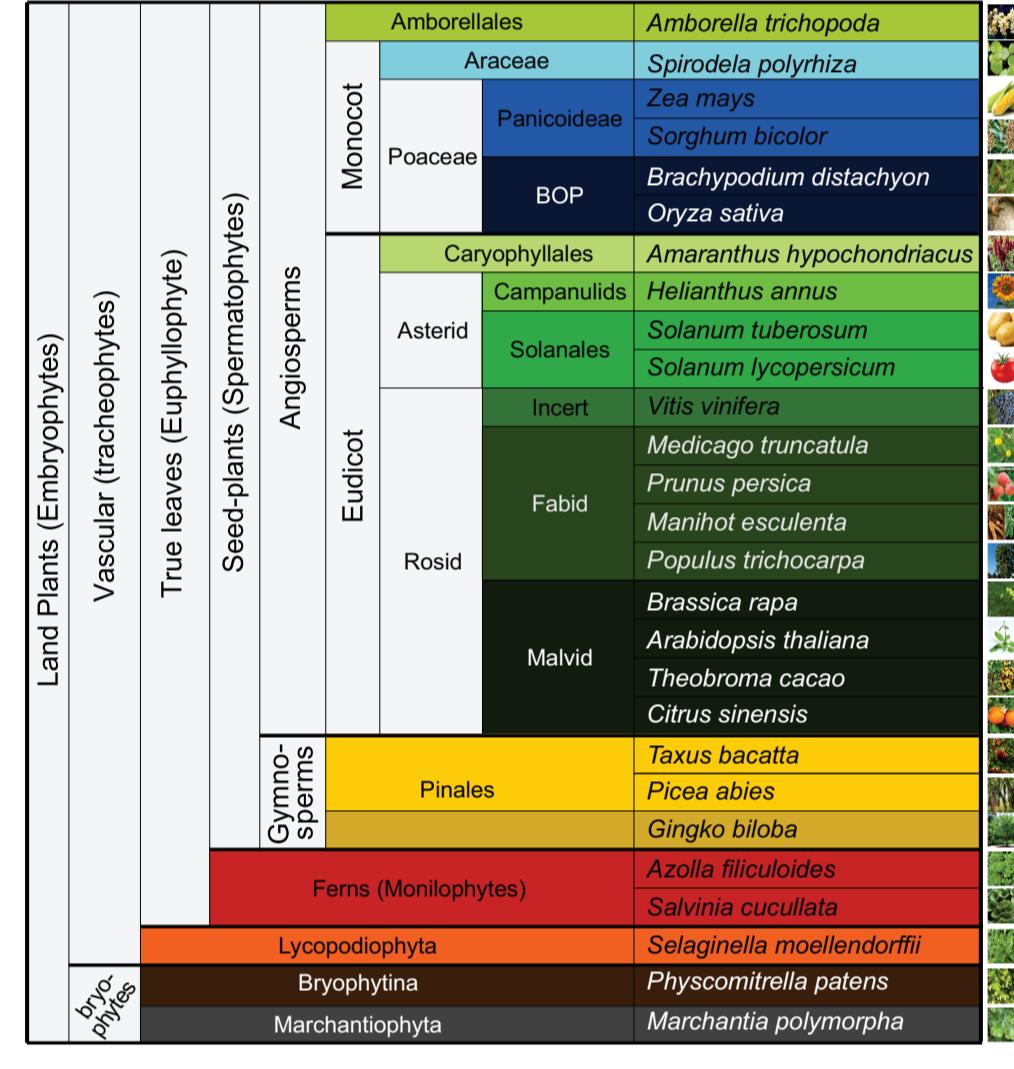
BAHD-genes in potato tuber

Potato tuber genes belonging to BAHD Family (Pfam PF02458)	
3'-N-debenzoyl-2'-deoxysaxol	PGSC0003DMT400032651 PGSC0003DMT400086558
N-benzoyltransferase	PGSC0003DMT400032637
Acetyltransferase	PGSC0003DMT400032637
Anthocyanin 5-aromatic acyltransferase	PGSC0003DMT400069897
Anthocyanin acyltransferase	PGSC0003DMT400011357 PGSC0003DMT400036371
PGSC0003DMT400020175 PGSC0003DMT400036374	
PGSC0003DMT400020180	
PGSC0003DMT40003107	
PGSC0003DMT400046332 PGSC0003DMT400041528	
PGSC0003DMT400046325 PGSC0003DMT400075205	
PGSC0003DMT400032641 PGSC0003DMT400075236	
Anthraniilate N-benzoyltransferase protein	PGSC0003DMT400001145 PGSC0003DMT400048126
PGSC0003DMT400018441 PGSC0003DMT400048131	
PGSC0003DMT400044934	
Anthocyanin-N-hydroxycinnamoyl benzoyltransferase	PGSC0003DMT400066505
Feruloyl transferase	PGSC0003DMT400081182
HQT	PGSC0003DMT400029078
Hydroxycinnamoyl CoA shikimate/quinate hydroxycinnamoyltransferase	PGSC0003DMT400087784
Hydroxycinnamoyl transferase	PGSC0003DMT400036695

Gene annotation from database SpudDB

Methods

27 land plants proteomes + Swissprot (functional annotation)

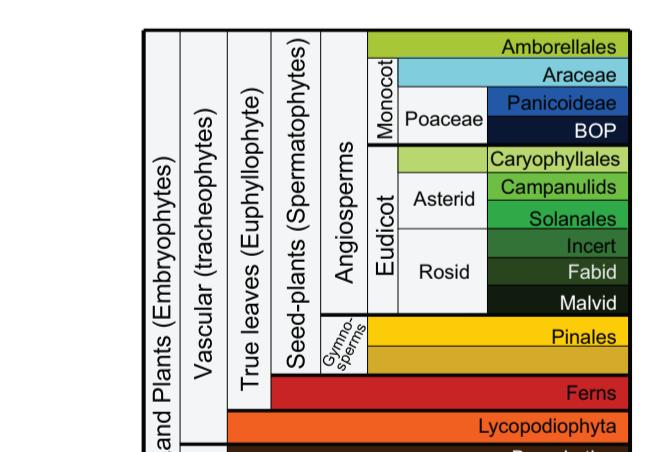


Results

Phylogeny and clustering

- Maximum likelihood tree with 1779 sequences Clusterized with HMMERCTTER in 20 groups 100% P&R-SD (only 8 sequences not clusterized)
* = group with genes in potato tubers

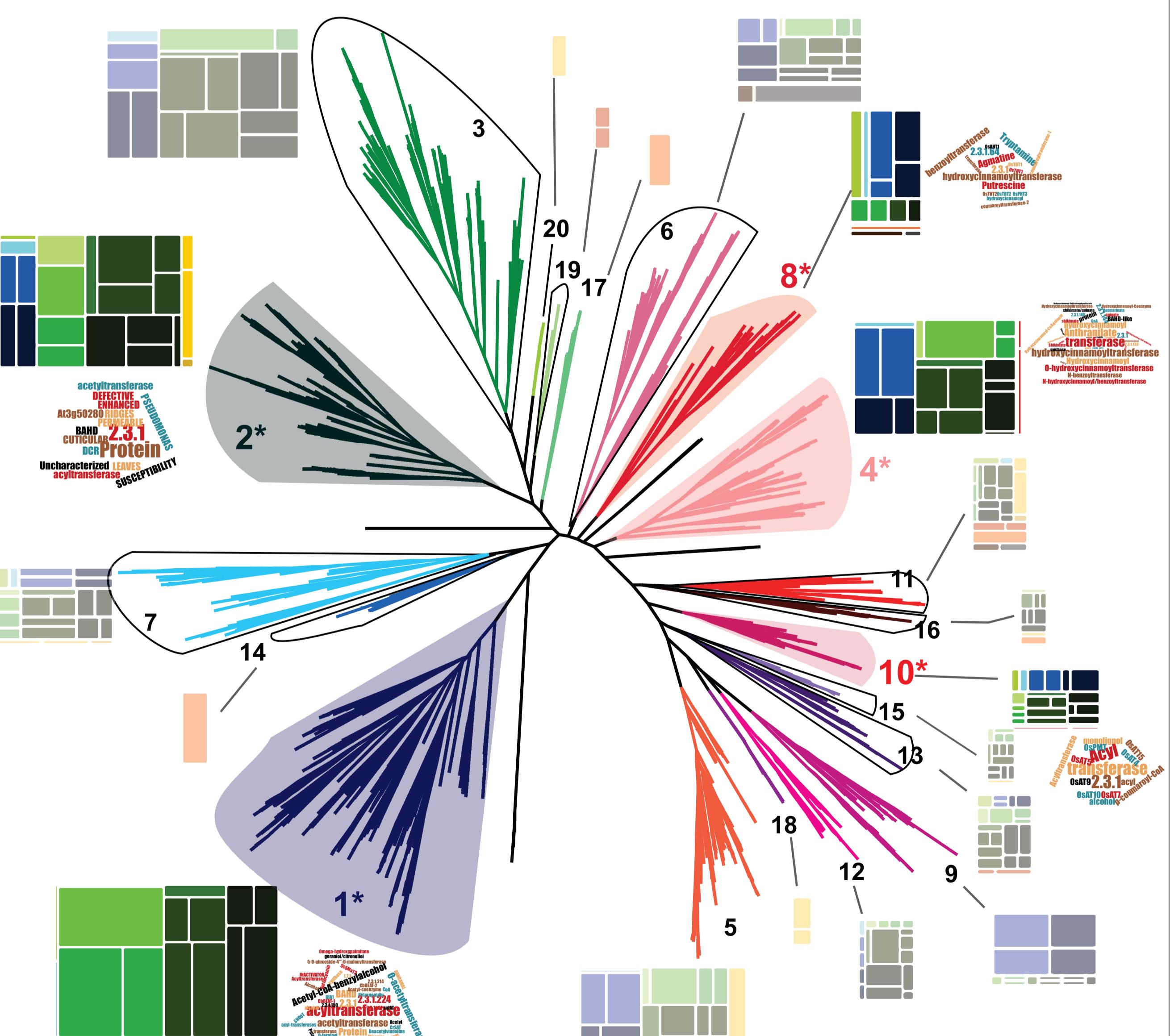
- Taxa represented by colored boxes (size relative to sequence number)



- Word clouds from Swissprot entries' annotations for selected group are shown

- We search for a representative PDB structure for each of the target target groups (hmmbuild + hmmsearch against PDB).

- Each group will be compared to related (close) groups in the phylogeny.

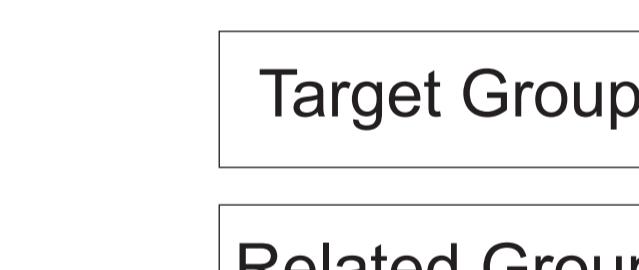


Group	Total sequences	Swissprot annotations	Representative PDB structure Annotation	Compared to related group
1	317	16	2BGH Vinorine Synthase	Rauvolfia serpentina 7
2	240	3	6DD2 Hydroxycinnamoyltransferase	Selaginella moellendorffii [3+19+20]
4	182	19	4G0B Hydroxycinnamoyltransferase	Coffea canephora 8
8	80	9	4G0B Hydroxycinnamoyltransferase	Coffea canephora 6
10	56	8	4G0B Hydroxycinnamoyltransferase	Coffea canephora [13+15]

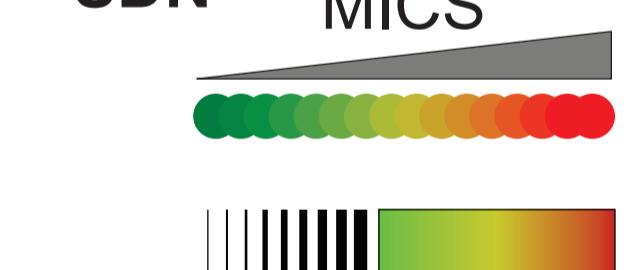
Contrasted to 5KJ7, Hydroxycinnamoyltransferase from *A. thaliana* complexed with p-coumaroyl-CoA

SDNs

LOGO



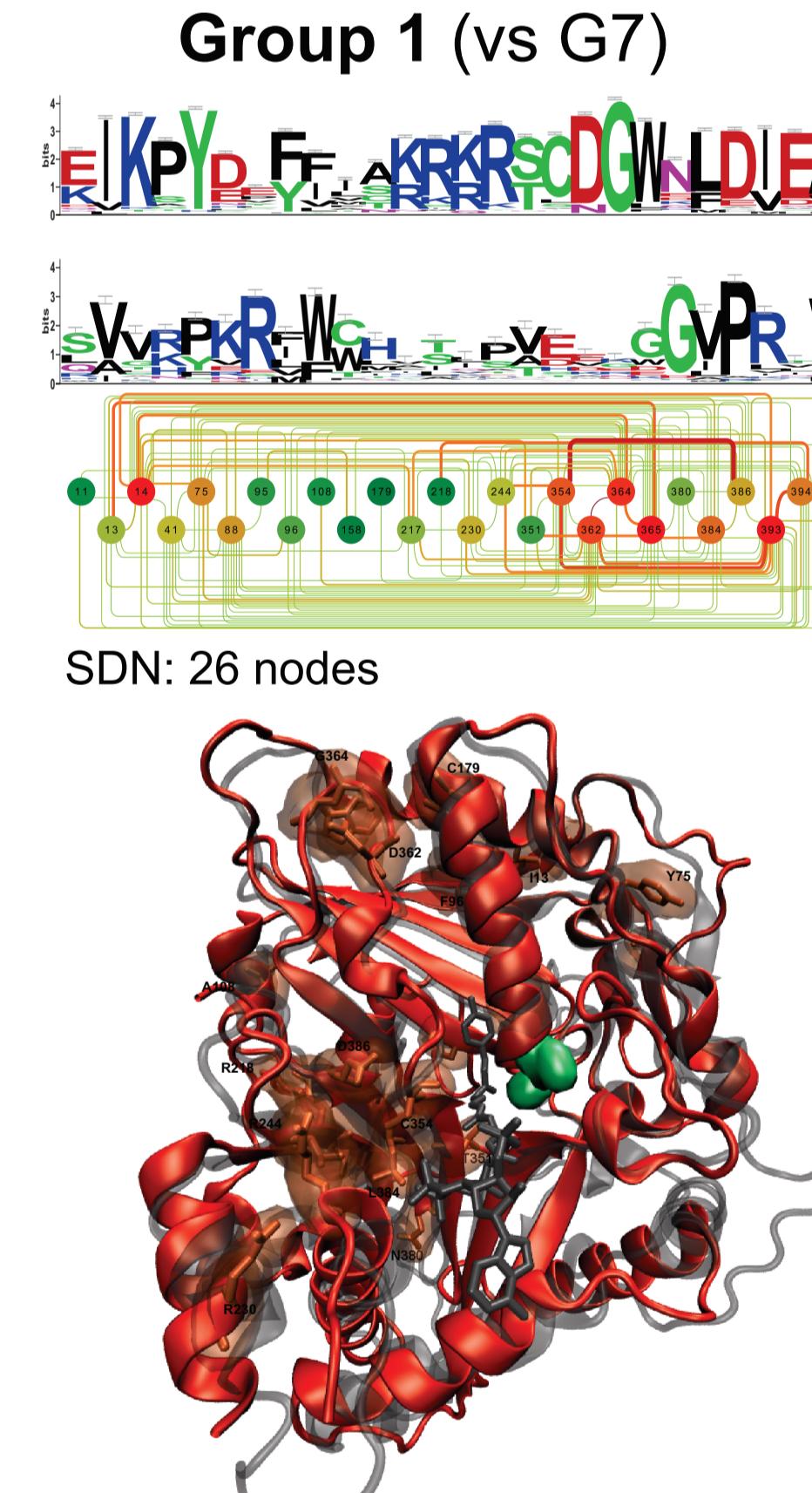
SDN



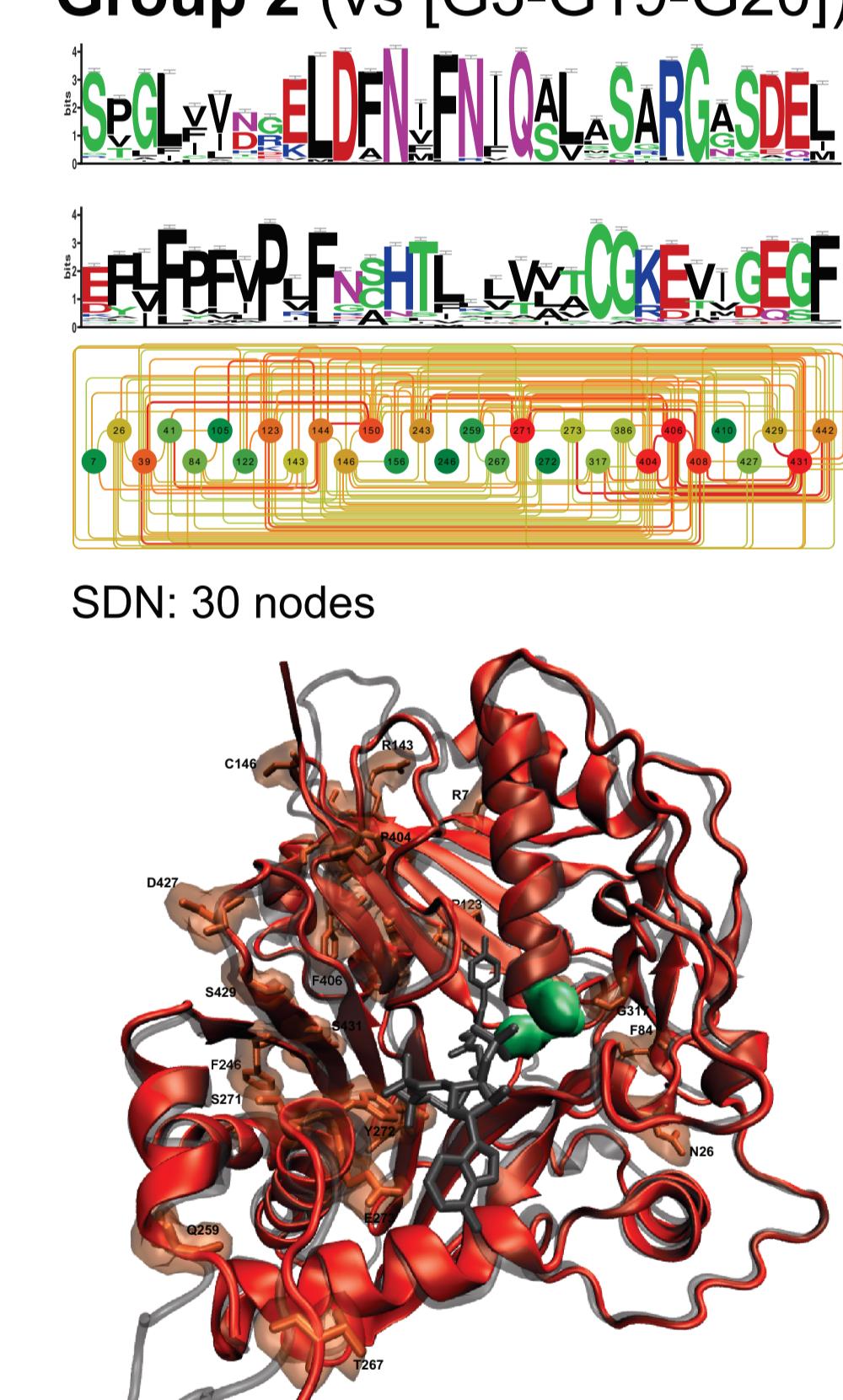
SDPs highlighted in reference

Orange = reference protein
Gray = contrasting 5KJ7 (with p-coumaroyl-CoA in gray)
Positions in green = catalytic residues (motif HxxxD)

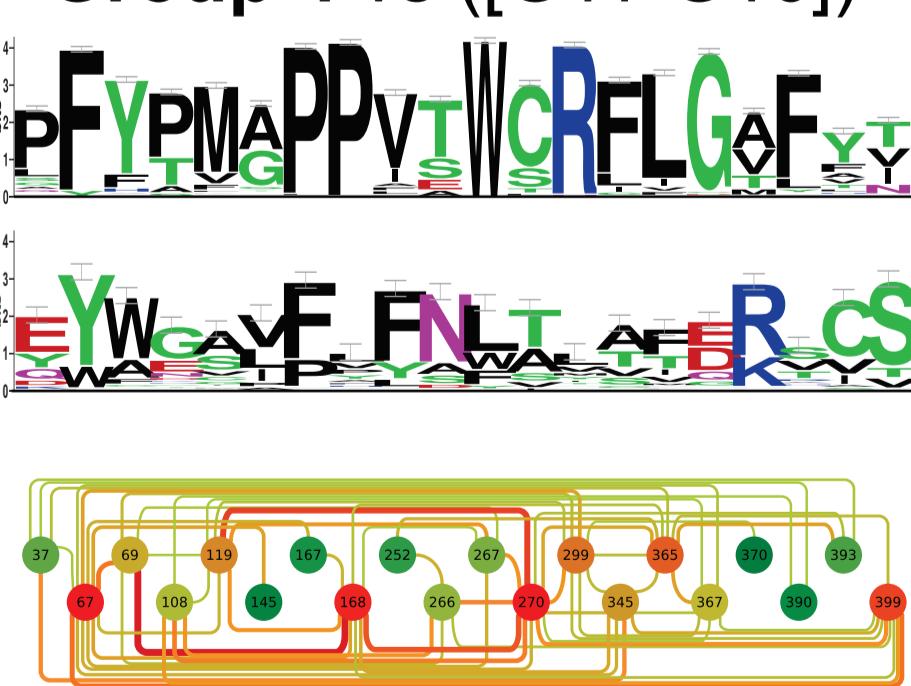
Group 1 (vs G7)



Group 2 (vs [G3-G19-G20])



Group 4 vs ([G11-G16])



Group 8 (vs G6)



Group 10 (vs [G13-G15])



Final remarks

- We find groups with apparent high functional diversification and others conserved across all land plants
- SDPs for each group are different, and mostly not associated to catalytic region
- Several positions seem to be surrounding the ligand cleft
- For highly diversified groups (i.e., Group 1), hierarchical clustering analysis is required