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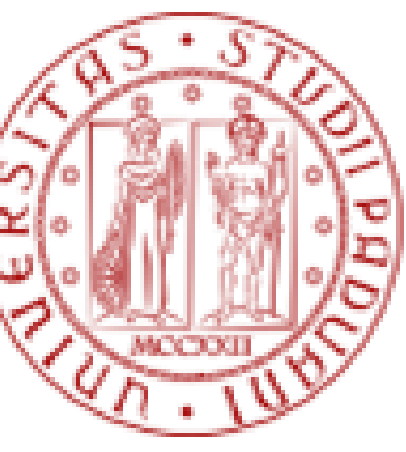
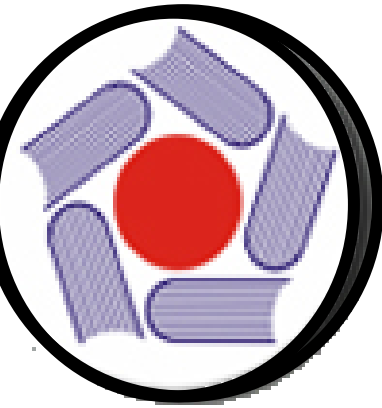
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**Introduction:** Resurrection plant *Ramonda serbica* Panc. survives desiccation for a long period and fully recovers metabolic functions already within one day upon watering. Besides osmotic stress, desiccation provokes the accelerated generation of reactive oxygen species.

**Aim:** to obtain more insight into the mechanisms of desiccation tolerance in *R. serbica* by TMT labelled comparative quantitative proteomics of hydrated (HL) and desiccated leaves (DL).

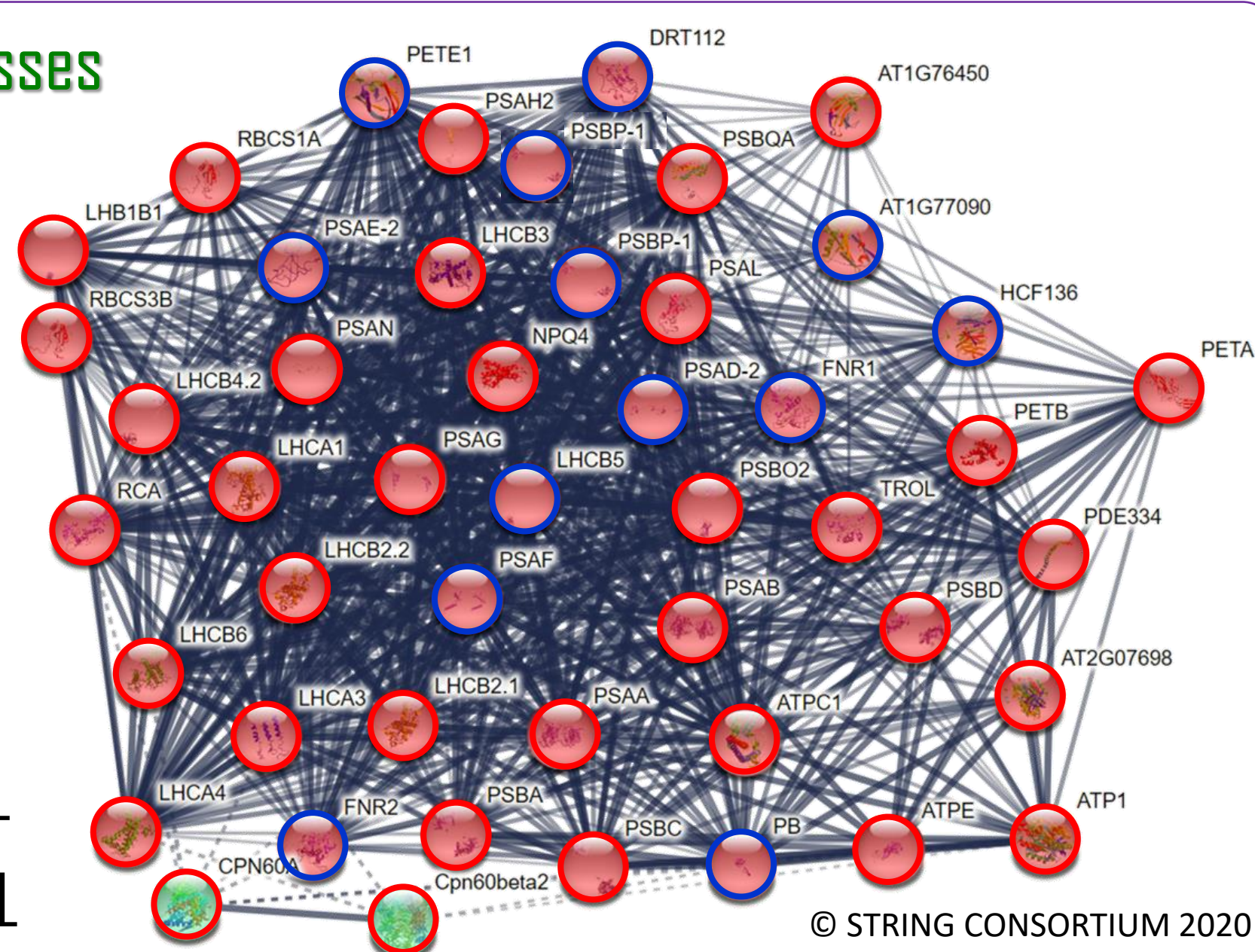


## Results:

After *de novo* transcriptome analysis, 189456 transcripts with 189003 unigenes were annotated with seven common databases. Proteomic analysis allowed for the relative quantification of 895 different protein groups, 314 with a statistically significant difference ( $p < 0.05$ ,  $F_c > 1.3$ ) in abundance between HL and DL.

## Photosynthetic processes

Almost all differentially abundant proteins related to photosynthetic processes were down-regulated in DL

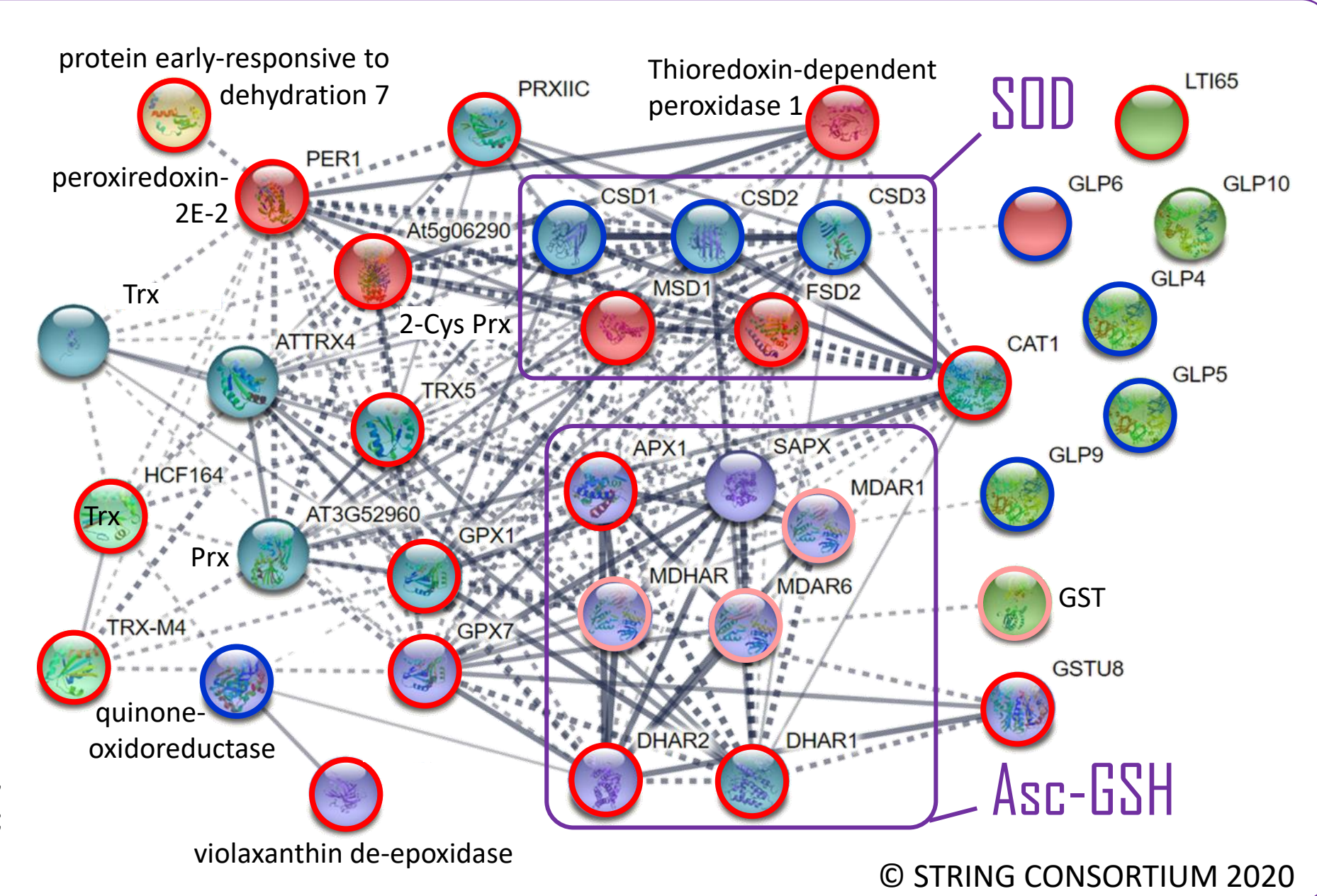


○ More abundant in HL  
○ More abundant in DL

## Antioxidative enzymes

Within differentially abundant proteins between HL and DL, 25% were associated with desiccation and oxidative stress. Among them, the levels of enzymes involved in ascorbate-glutathione (Asc-GSH) cycle, peroxiredoxins, superoxide dismutases (SOD): Fe-SOD (FSD2) and MnSOD (MSD1) were all reduced in DL, while germin-like proteins (GLPs), three Cu/Zn SOD (CSD) isoforms and polyphenol oxidases (PPOs) were more abundant in DL compared with HL.

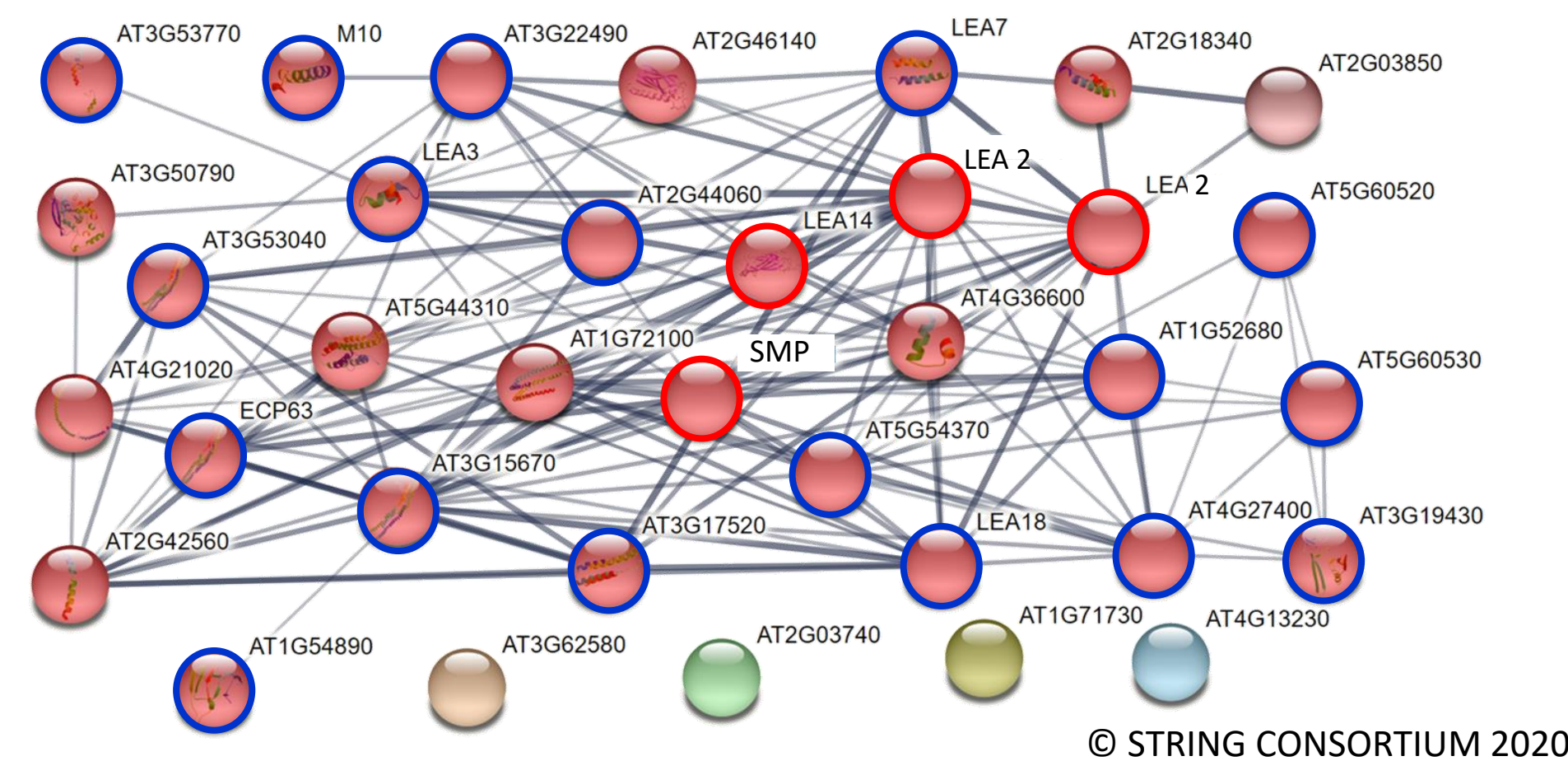
GPX, glutathione peroxidase; GST, glutathione S-transferase; MDAR, monodehydroascorbate reductase; DHAR, dehydro-ascorbate reductase; LTI65, low-temperature-induced 65 kDa protein.



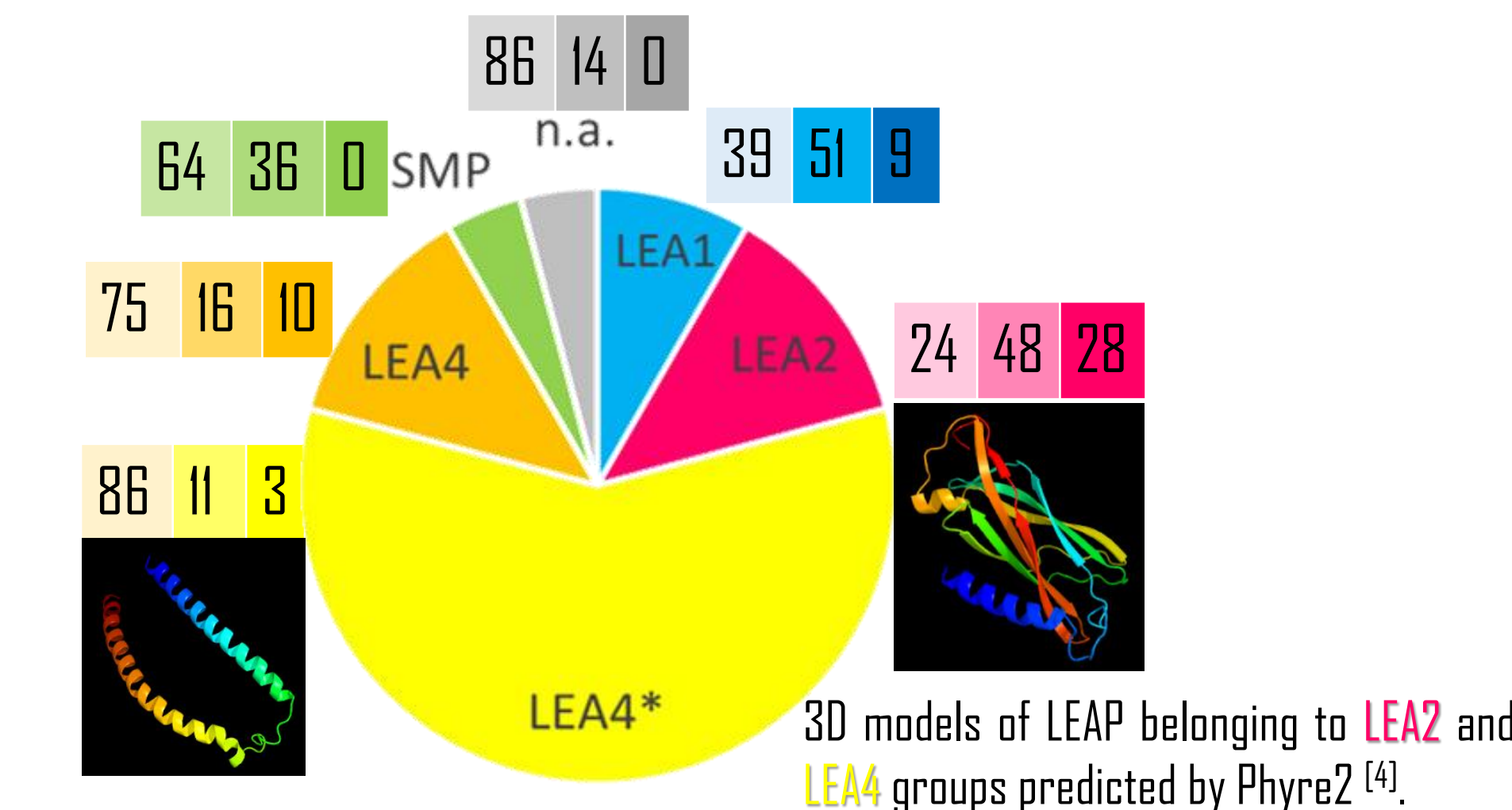
**Our results imply an important role of LEAPs, PPOs, GLPs and Cu/Zn SODs in protective mechanism against desiccation in *R. serbica*.**

## Late embryogenesis abundant proteins (LEAPs)

The protein family with the highest number of members showing the greatest accumulation upon desiccation comprised 29 different LEAPs (18 statistically significant):

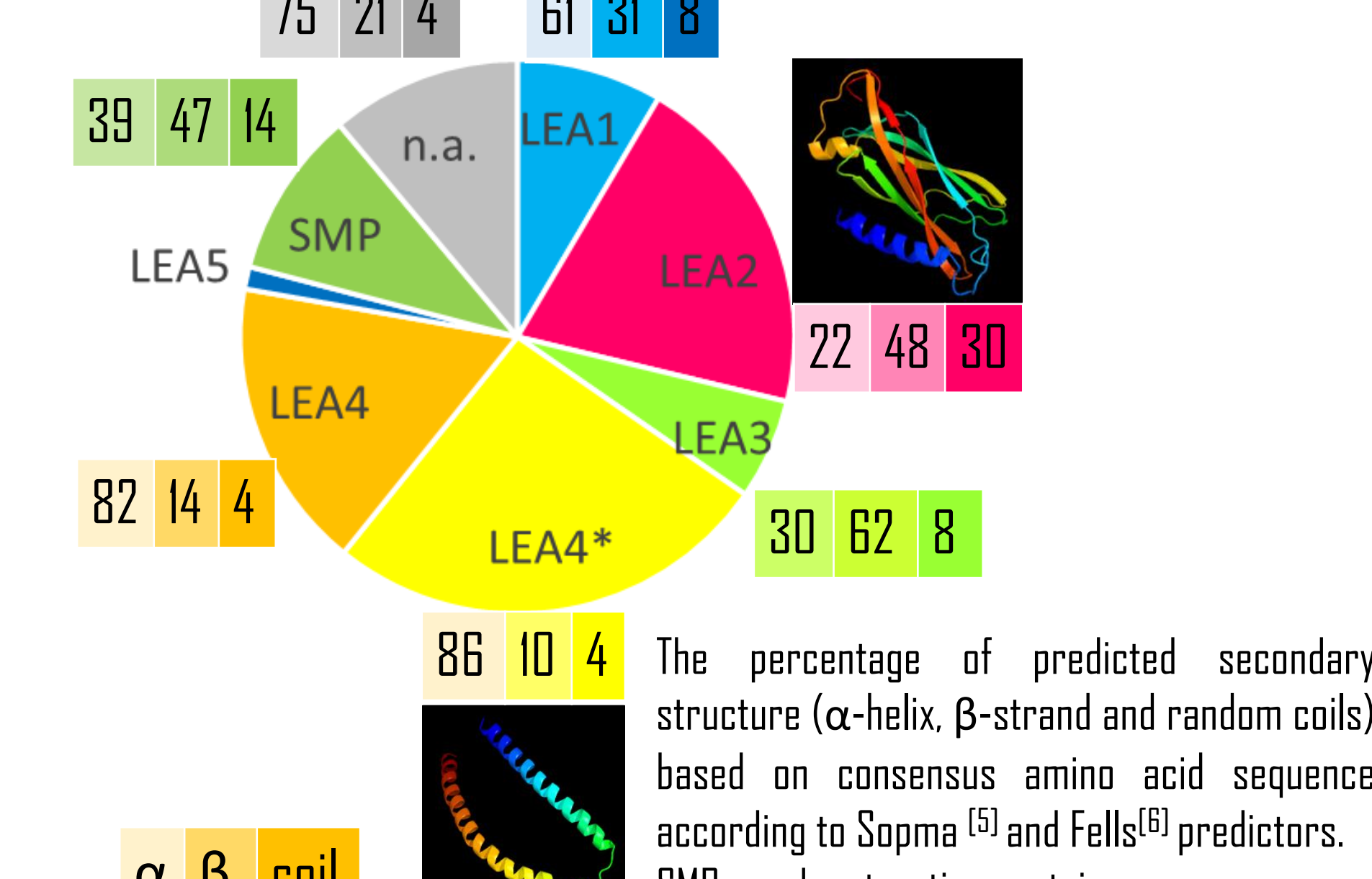


22 LEAPs differentially abundant in DL vs HL of *R. serbica*

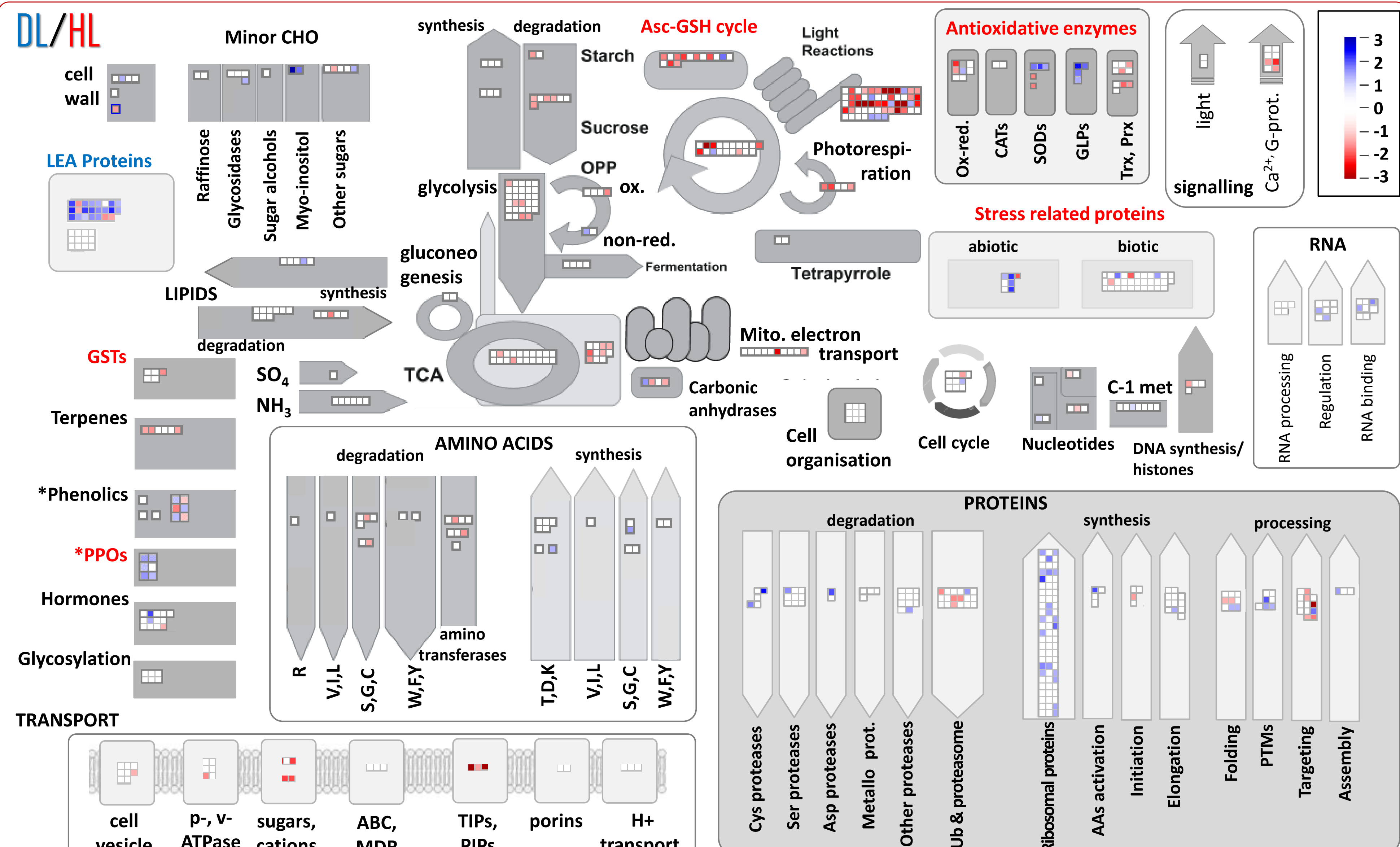


3D models of LEAP belonging to LEA2 and LEA4 groups predicted by Phyre2<sup>[4]</sup>. Peptide chain presented by rainbow color pattern: blue, N-terminus; red, C-terminus

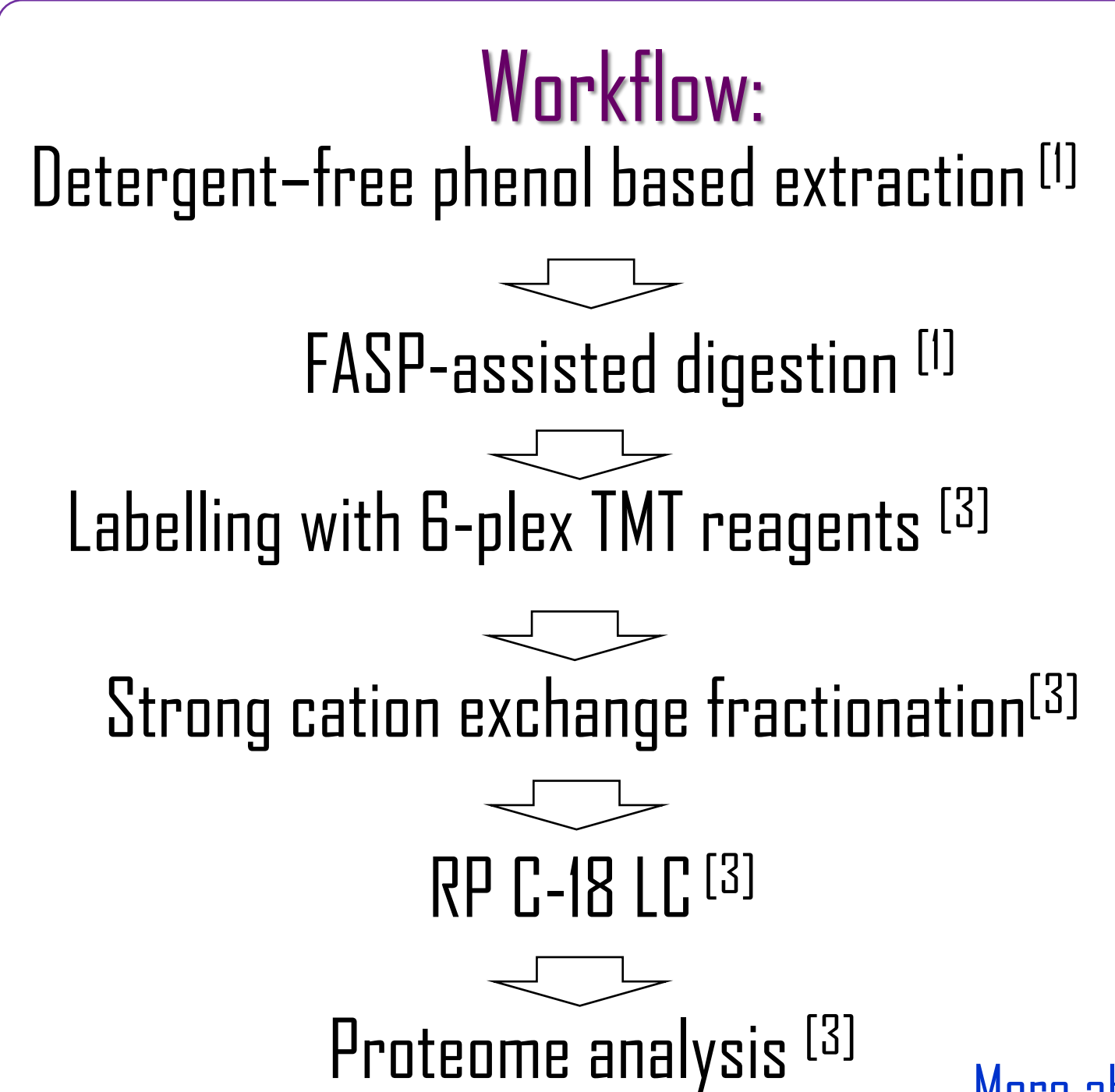
## 153 LEAPs in *R. serbica* annotated by transcriptomics



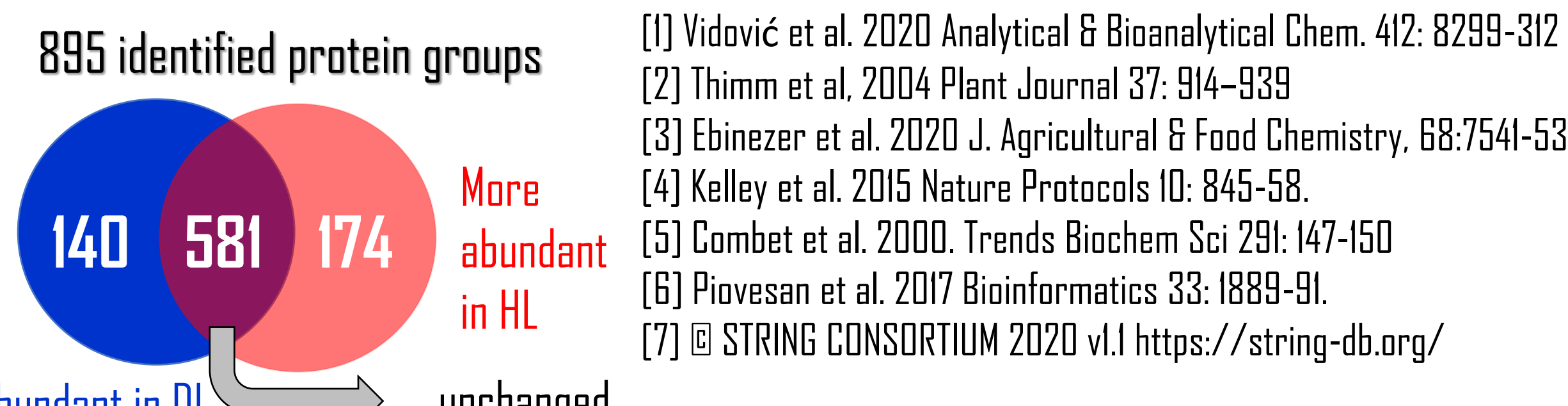
The percentage of predicted secondary structure ( $\alpha$ -helix,  $\beta$ -strand and random coils) based on consensus amino acid sequence according to Sopma<sup>[5]</sup> and Fells<sup>[6]</sup> predictors. SMP: seed maturation proteins



The metabolic overview obtained by MapMan<sup>2</sup> with additional pathways\* showing the abundance (heatmap) of the proteins associated with major metabolic pathways in DL compared with HL. A Z-test was performed to identify proteins with a significantly different abundance ( $p \leq 0.05$ ), and significant hits further included proteins showing a fold change (FC)  $\geq 1.3$  and  $\leq -1.3$ . AAs, amino acids; Asc, ascorbate; CAT, catalase; GLPs, germin-like proteins; GSH, reduced glutathione; GSTs, glutathione-S-transferases; PPOs, polyphenol oxidases; Prx, peroxiredoxins; PTMs, post-translational modifications; ox-red, oxidoreductases; OPP, oxidative pentose phosphate pathway; TCA, tricarboxylic acid cycle; Trx, thioredoxins; Ub, ubiquitin;



Protein extracts of desiccated and hydrated *R. serbica* leaves were analysed with an LTQ-Orbitrap XL mass spectrometer coupled online with an Ultimate 3000 nano-HPLC. Peptides were eluted according to the method described in [3]. The instrument performed a full scan at high resolution (60000) on the Orbitrap, followed by MS/MS scans on the three most intense ions with both CID and HCD fragmentation. Raw MS/MS files were analysed using Proteome Discoverer 1.4 connected to a Mascot Search Engine server version 2.2.4 using a MudPIT protocol. Mean protein ratios (DL/HL) were calculated dividing the obtained quantification value of each protein in treated samples to that of control samples.



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NGP-net  
Non-globular proteins in  
molecular physiopathology

