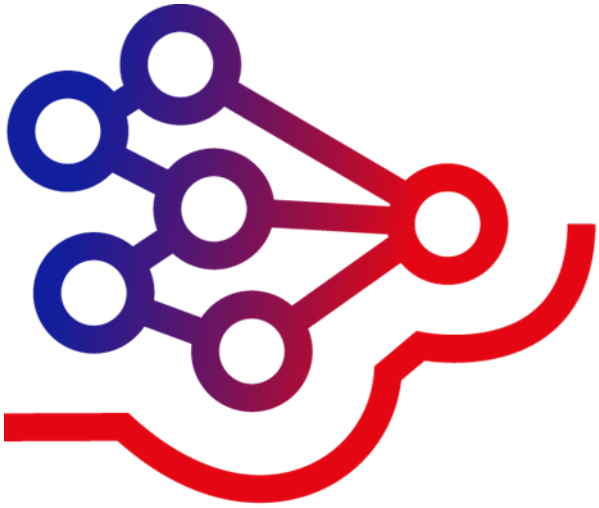

ML4NGP

MACHINE LEARNING FOR NON GLOBULAR PROTEINS

ABSTRACT BOOK

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Group 4 Late Embryogenesis Abundant (LEA) proteins as a model to study propensity for oligomerisation

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Late Embryogenesis Abundant Proteins (LEAP), are intrinsically disordered proteins essential in tolerance to desiccation (up to 95% of water loss). Only about 130 plant species, such as resurrection plants are able to survive a long desiccation period and fully recover metabolic functions one day upon watering. To investigate their role in desiccation tolerance we structurally characterised and classified LEAPs in hydrated and desiccated leaves of Ramonda serbica an ancient resurrection plant.

Differential transcriptome and proteome analyses revealed that members of the LEA4 protein family represent the majority of desiccation-inducible LEAPs. To evaluate their structural properties in vitro and their potential functions in vivo, a representative RsLEA4 protein, predicted to be highly disordered and localised in chloroplasts, was obtained in Escherichia coli by recombinant DNA technology. However, electrophoresis, gel filtration, circular dichroism spectroscopy, and dynamic light scattering showed that this protein aggregates in different media (polar, non-polar, broad pH range). To decipher the nature of the oligomerisation/aggregation pathway, the conserved domain (CD) characteristic for LEA4 proteins and the domain with the highest dimerisation probability (DD) were analysed by molecular dynamics simulation (MDS) under physiological and desiccation conditions.

Under both conditions, the structures of CD and DD domains retained their α-helical structure. In the case of DD, dimers were formed via five salt bridges under simulated physiological conditions. Under desiccation, the MDS showed a tendency of the DD dimer to roll into a partially globular structure through hydrophobic interactions, retaining the α-helices as the most favourable conformations, stabilised by intramolecular salt bridges. Similarly, the α-helical structures of the CD dimer were preserved under both conditions. In contrast to DD dimer, the CD dimer was composed of two α-helices held by hydrophobic interactions at physiological conditions, and by electrostatic interactions under desiccation.

In conclusion, due to the amphipathic nature of the selected domains, both dimer structures are adaptable to different environments, which explains the high LEA4 protein aggregation propensity. The information derived from the representative structural model is key to identifying the endogenous partners of LEAPs and their targets in the cell, providing further insight into the protective mechanisms of desiccation tolerance.

Keywords: Aggregation, desiccation, intrinsically disordered proteins, late embryogenesis abundant proteins, Ramonda serbica, resurrection plants.

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