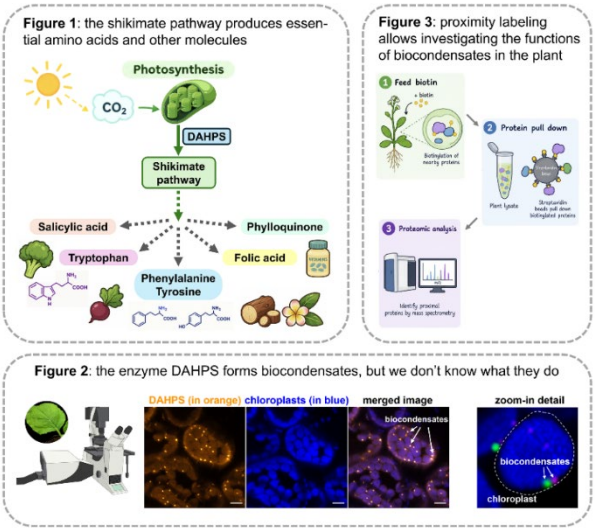
Project: Regulation of chloroplast metabolism by enzyme biocondensates
Investigator: Jorge El-Azaz



Plant chloroplasts can transform CO2 into hundreds of essential molecules, such as amino acids, fatty acids, or vitamins. Hundreds of different enzymes coexist and work within this tiny compartment. How do plants regulate this complex metabolism to produce the molecules they need, when they need them? Although transcriptional regulation in plants has been studied quite well, we don't know that much about how plants regulate these enzymes after expressing them.

Over the last decade, many studies have investigated a new cellular mechanism called liquid phase separation, which allows proteins to separate into droplets named biocondensates. Inside these biocondensates, the activity of the proteins can change, and they can even recruit other biomolecules to regulate many cell functions. Very interestingly, many enzymes of the chloroplast seem to form biocondensates, but we don't know what they do. Could these biocondensates be a new mechanism to organize and regulate the metabolism of the chloroplast?

In this project, you will investigate how biocondensates regulate one of the most important pathways of plants, the shikimate pathway (Figure 1). This pathway produces the three aromatic amino acids (phenylalanine, tyrosine and tryptophan), as well as many other key molecules like folic acid, salicylic acid, or phylloquinones (Figure 1).

We will focus on the enzyme DAHPS, which forms biocondensates (Figure 2). You will apply a powerful technique known as proximity labeling to investigate how these condensates work inside of the chloroplast (Figure 3). This technique consists in fusing a biotin ligase domain to the DAHPS enzyme. After adding biotin, the biotin ligase domain will mark other proteins that are next to the DAHPS. Then, we will purify these marked proteins from the plant, and we will analyze them by proteomics (Figure 3). This will reveal a list of proteins that were around the DAHPS, which we will then study in more detail to understand how the biocondensates regulate plant metabolism.

You will work with Arabidopsis thaliana and Nicotiana benthamiana plants and will learn a comprehensive set of tools, related to making and handling transgenic plants, molecular biology (DNA genotyping, RNA extraction, and quantitative PCR), laser confocal microscopy, and various protein and proteomic techniques (biotin feeding, purification of proteins, and preparation of samples for mass spectrometry).

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Contact email: jorge.el-azaz@upv.es