

# Endosymbiont Legacy Organelles: a tantalizing hypothesis on the long-lasting impacts of endosymbiosis

Dayana E. Salas-Leiva<sup>1</sup>, Elin Einarsson<sup>1</sup>, Konstantin Barylyuk<sup>1</sup>, Victor Flores<sup>1</sup>, Arnab Pain<sup>2</sup>, Sebastian G. Gornik<sup>3</sup>, and Ross F. Waller<sup>1</sup>

<sup>1</sup>Department of Biochemistry, University of Cambridge, Cambridge CB2 1QW, UK, <sup>2</sup>DBiological and Environmental Science and Engineering Division, King Abdullah University of Science and Technology, Thuwal, Saudi Arabia, <sup>3</sup>Centre for Organismal Studies, University of Heidelberg, Heidelberg, Germany

## Introduction

- One of the most intimate achievements of endosymbiosis is the integration of an endosymbiont's genome into its host genome while maintaining much of the symbiont's structural compartments and functions.
- Most plastid endosymbionts retain some genes on a relict genome (i.e., plastome), and the site of its maintenance, expression and interactions with nucleus-encoded plastid-targeted proteins define this plastid space as a direct inheritance from the endosymbiont.
- Perkinsus* and *Eleutheris* have been proposed to have plastid relics that lack plastomes. Protein homology predictions from genomic/transcriptomic data indicate that nuclear-encoded-proteins for the plastid-derived pathways for isoprenoids and Fe-S clusters are targeted to membrane-bound symbiont relics.

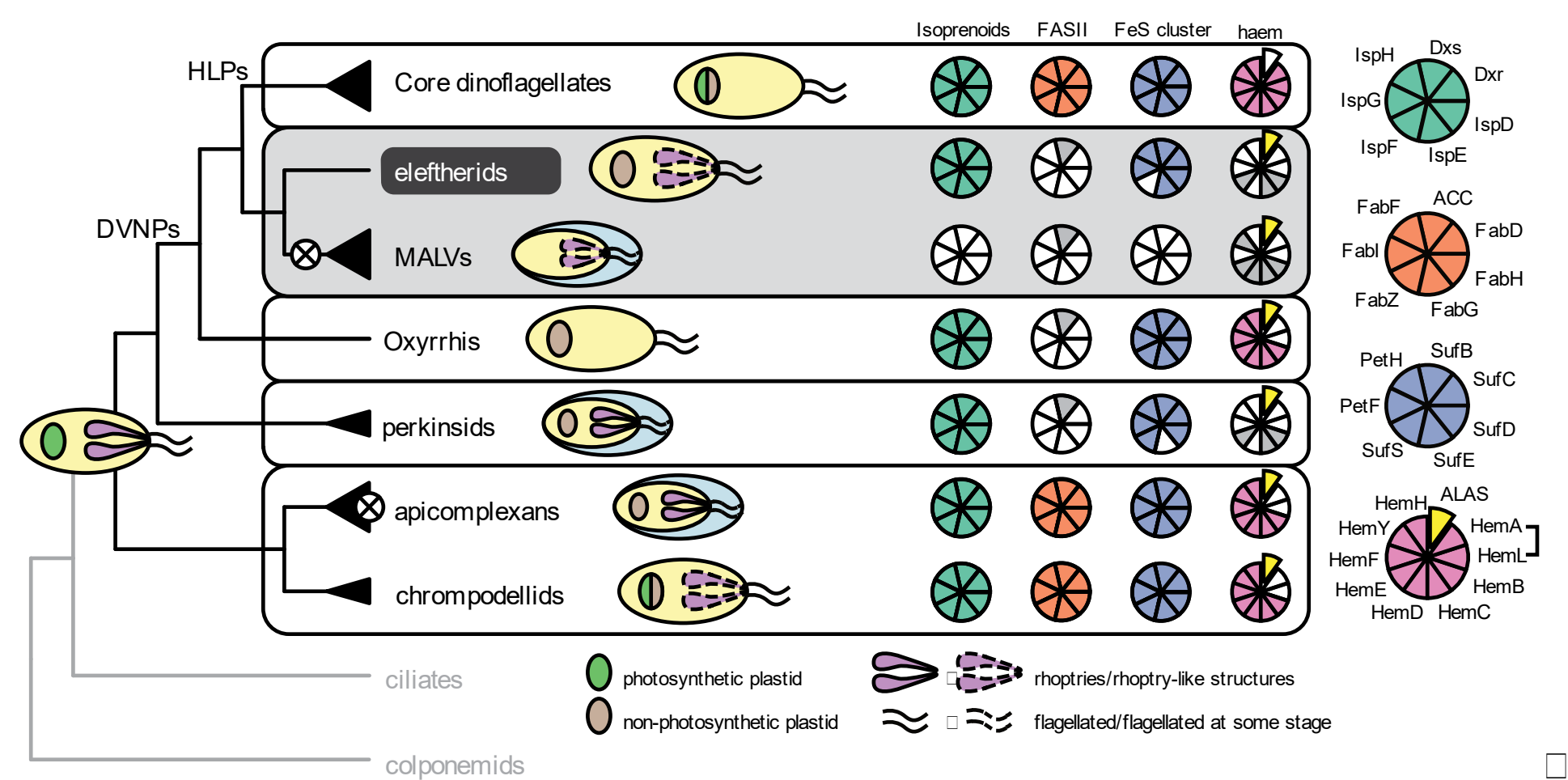


Figure 1. Character and plastid metabolic pathway evolution in dinoflagellates, apicomplexans and related lineages. From: Hehenberger et al. preprint 10.21203/rs.3.rs-1472581/v1

- However, the sites of these pathways have not been unambiguously identified and defined in these organisms, and the question remains:

## What is the impact of the loss of a symbiont's genome on the structure and biogenesis of the derived organelle?

### Aims

- To sequence *Perkinsus marinus* genomes (nuclear, organellar)
- To analyze the predicted nuclear proteome for plastid-derived genes
- To apply global spatial proteomics (LOPIT) for determining the destinations of these genes' products and their proteomic context.

### Background

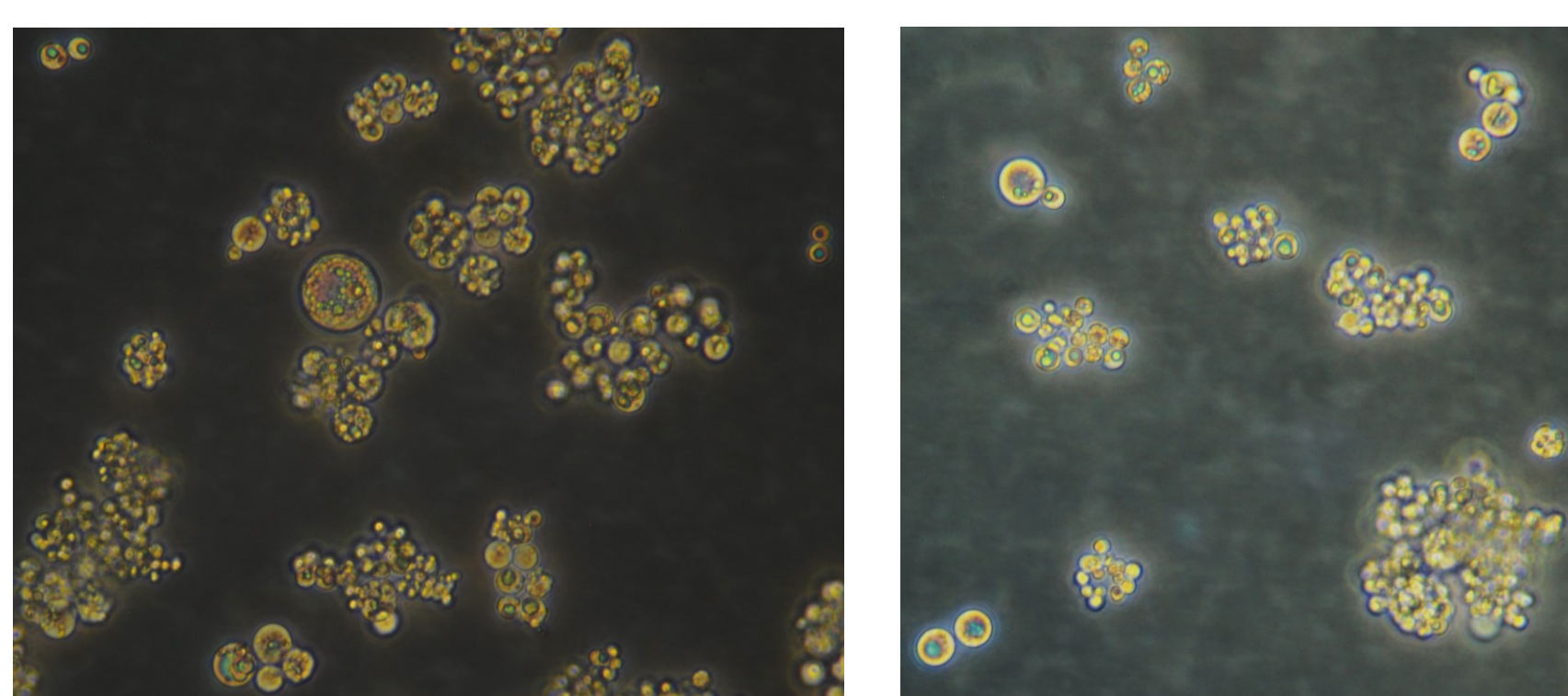


Figure 2. Oyster's parasite *Perkinsus marinus*

- Plastome has been elusive in *P. marinus*
- No unambiguous microscopic evidence of a plastid with controversial evidence for multimembrane organelles
- Plastid-targeted proteins with possible bipartite SP-TP signal

## Results

### Genomics

- 42X coverage (Pacbio long-reads)
- 129X coverage (Illumina paired-end reads)
- Genome size 54.7 Mb (83 scaffolds, N50: ~2.4 Mb)
- 16732 predicted proteins (11596 with HMM annotations)
- There is no evidence of a plastome but there are plastid-derived genes in the host nucleus.
- We found 35 plastid orthologs from a previously identified from Barylyuk et al 2020, among these are those shown in Fig 3.

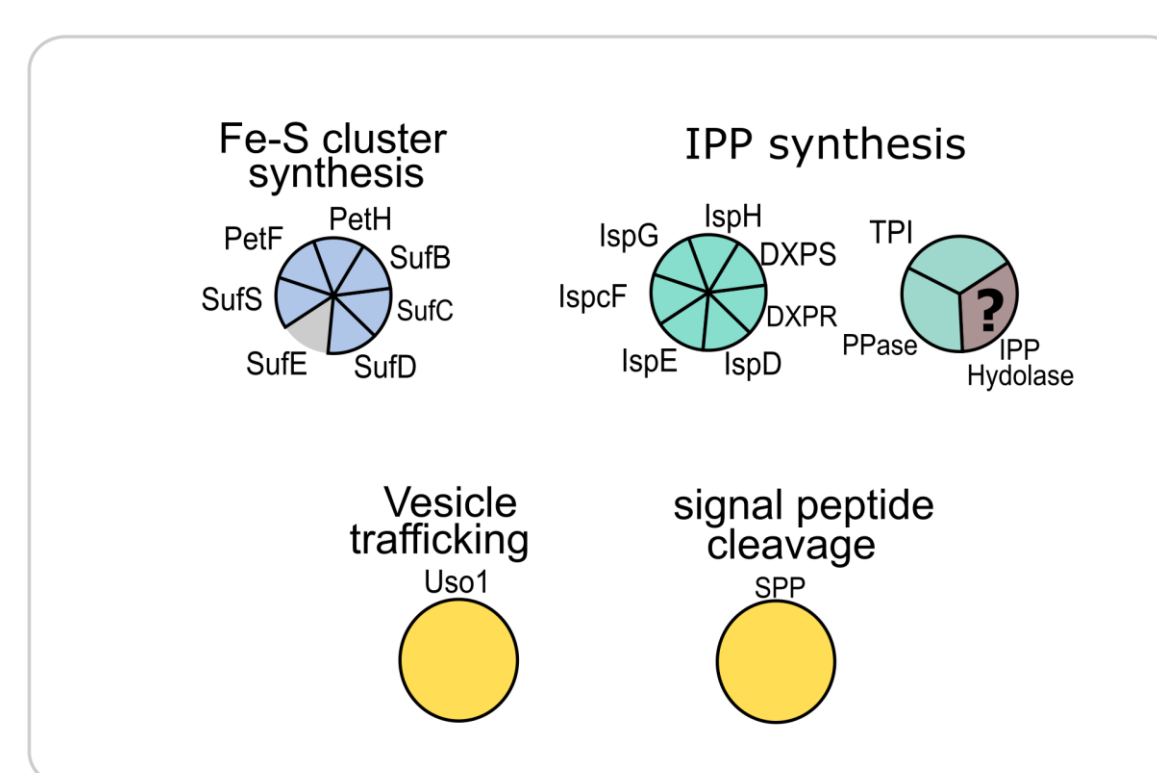


Figure 3. Plastid-predicted (in silico) proteins in *P. marinus*

- The plastid proteome lacks any plastome maintenance or expression machinery providing strong corroborating evidence for the loss of the plastome in *Perkinsus* plastids.
- Lack of the typical protein import machinery for a plastid compartment suggests remodeling of its membranes and/or import routes after the complete loss of genetic autonomy of the symbiont.
- LOPIT spatial proteomic analysis resolves multiple subcellular protein niches, one of which is consistent with a plastid-like organelle capable of isoprenoid and Fe-S cluster synthesis.

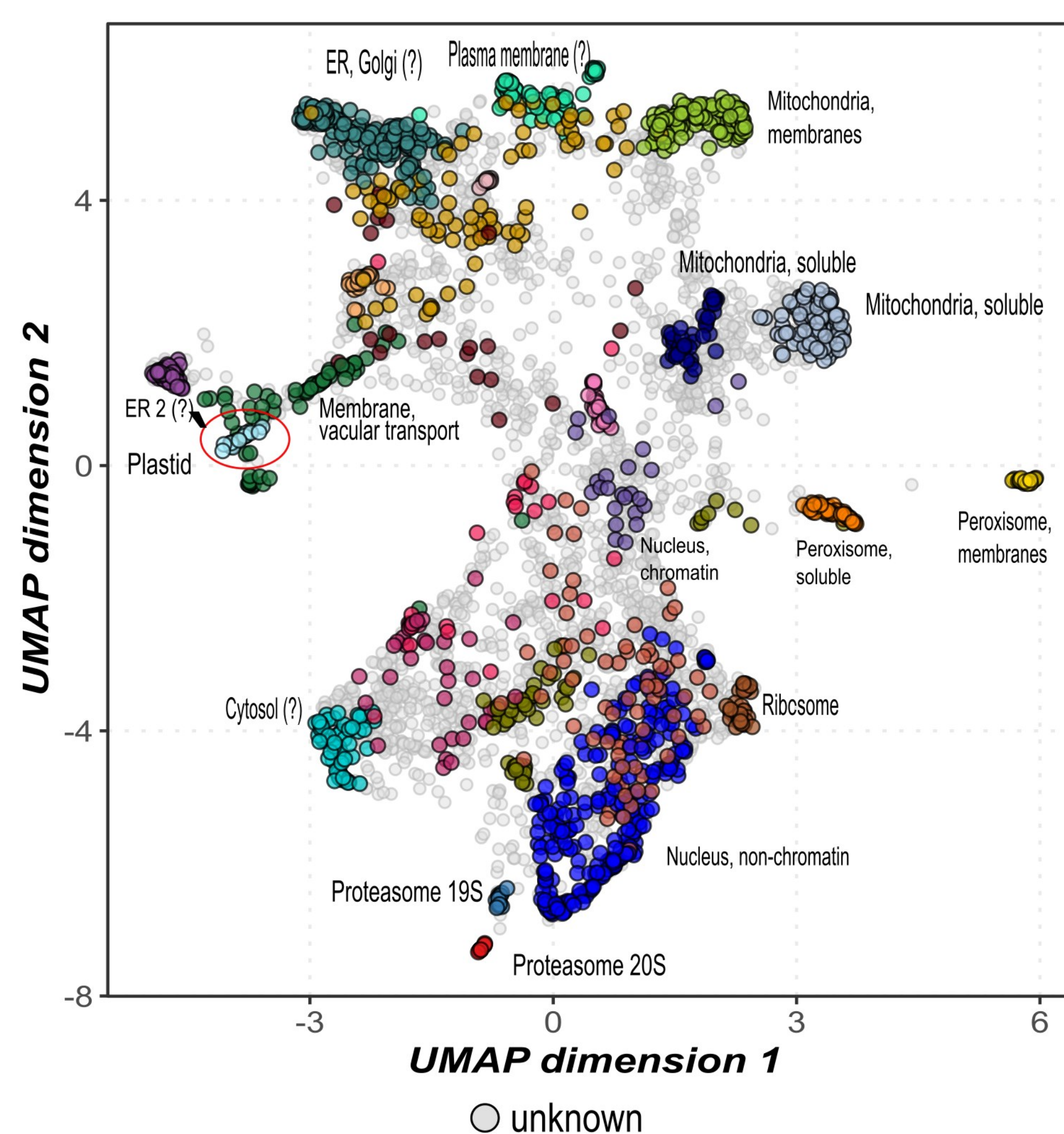


Figure 4. Preliminary novelty TAGM prediction result from two LOPIT dataset for *P. marinus*. Red circle indicates the plastid proteome.

- Isoprenoid IspD-mCherry fusion protein reveals multiple small discrete compartments as the sites of these plastid proteomes.

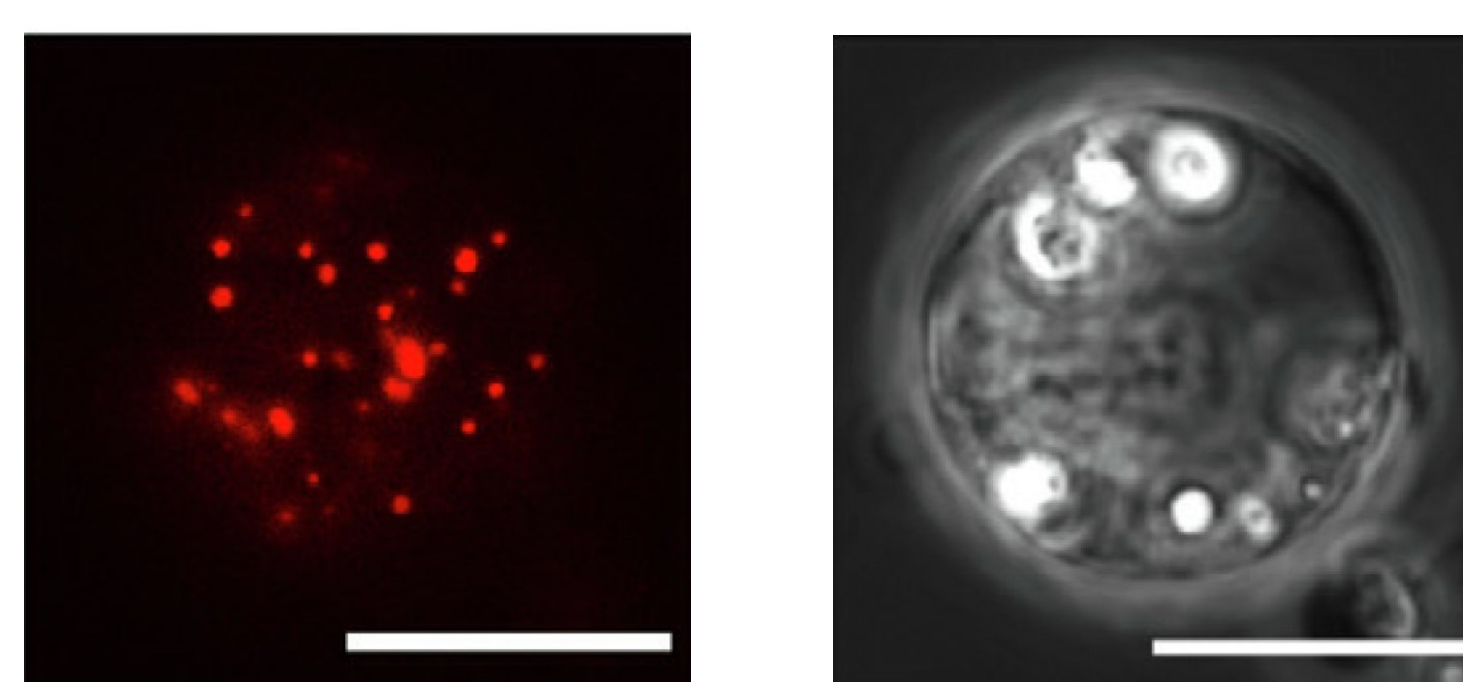


Figure 5. IspD-mCherry expression in *P. marinus* (Einarsson et al 2021)

## Endosymbiont Legacy Organelles

- Perkinsus* 'plastids' share similarities with experimentally induced plastid and plastome loss of the apicoplast in the apicomplexan *Plasmodium falciparum* where such mutant cells can be rescued by exogenously supplied isoprenoid precursor molecules.
- P. falciparum* plastid loss results in the formation of multiple single-membrane vesicles that can still perform select plastid functions.
- These compartments are interpreted as the protein sorting vesicles that fail to reach the target apicoplast destination.

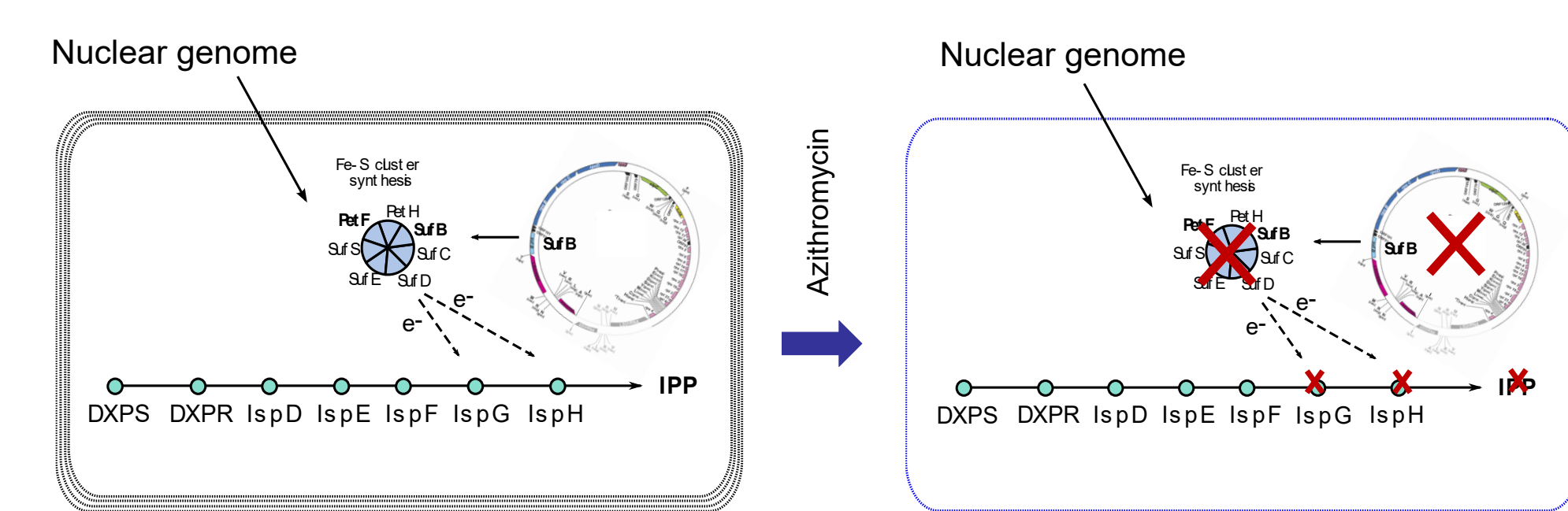


Figure 6. *P. falciparum* apicoplast loss after Azytromycin treatment. Adapted from: Sean Prigge: <https://www.youtube.com/watch?v=B0QVp8gfXQA&t=2s> Swift et al 2022 mBio 2022 Pages e0302321

- Observations in *P. marinus* are consistent with similar but naturally derived compartments and raise the question, are they highly reduced plastid relics, or are they in fact a novel type of organelle: an Endosymbiont Legacy Organelle.

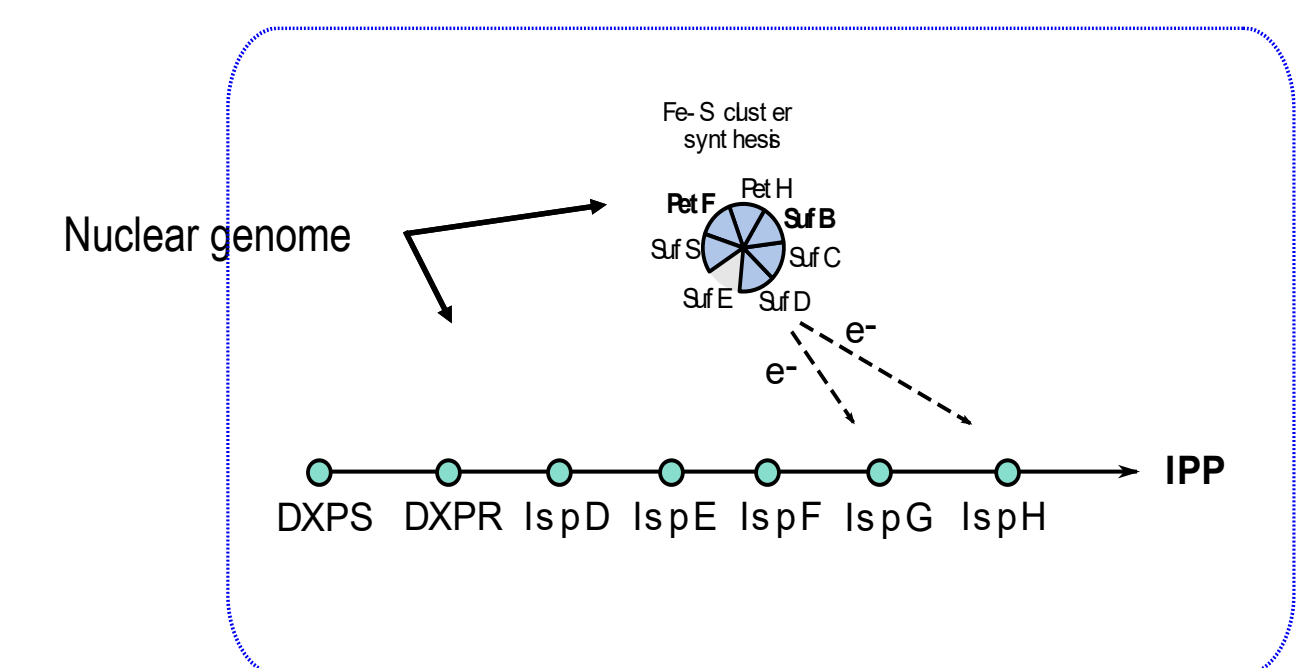


Figure 7. Endosymbiotic Legacy Organelle in *P. marinus*

- An **Endosymbiont Legacy Organelle** is a predicted subcellular compartment that is not directly derived from the original endosymbiont, as this space could be lost after plastome loss. Instead, it would be a new host-synthesized compartment capable of carry out the functions that were acquired via endosymbiont gene transfer.

## Methods

- Genomics:**
  - Pacbio long-reads and Illumina paired-end reads
  - Assembly Canu, Pilon, Flye, Racon, WTDBG2, + manual curation
  - RNAseq for gene prediction done in several rounds using: Genemark + Augustus + exonerate + read mapping + Interproscan
  - Annotation: blast, PANTHER and PFAM homology searches
  - Orthologs identification: direct and reverse homologous searches
- Spatial proteomics:**

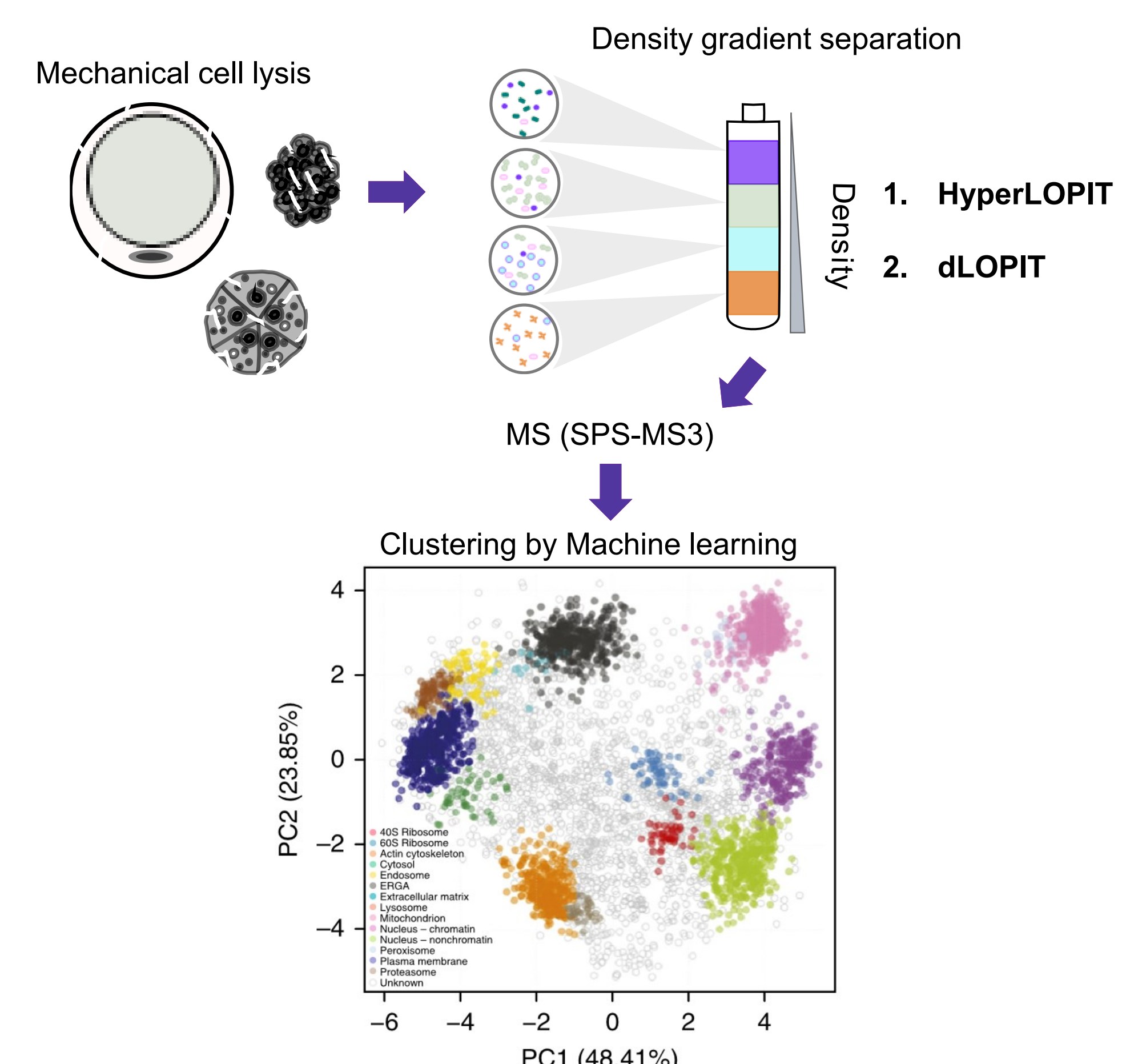


Figure 8 Methodology workflow. Adapted from Mulvey et al 2017 and Elzek et al 2021