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# Searching for interactions at the membrane interface that confer antagonistic activities on floral regulators FT and TFL1.

Mathieu Pottier<sup>1</sup>, Beata Orman-Ligeza<sup>1</sup>, Gaëlle Redouté<sup>2</sup>, Geert De Jaeger<sup>3</sup>, Yoann Laurin<sup>2</sup>, Magali Deleu<sup>2</sup>, Laurence Lins<sup>2</sup>, Marc Boutry<sup>4</sup>, Claire Périlleux<sup>1</sup> (Contact: mathieu.pottier@uliege.be)

<sup>1</sup>InBioS-PhytoSYSTEMS, Laboratory of Plant Physiology, ULiège, Belgium, <sup>2</sup>TERRA, Laboratory of Molecular Biophysics at Interfaces, Gembloux Agro-Bio Tech, ULiège, Belgium, <sup>3</sup>Department of Plant Biotechnology and Bioinformatics, Ghent University & VIB Center for Plant Systems Biology, Belgium, <sup>4</sup>LIBST, UCLouvain, Belgium

**Introduction:** The switch from leaf- to flower-producing meristems is controlled by FLOWERING LOCUS T (FT) which is the major component of the "florigen", a systemic signal that moves from leaves to shoot meristems and triggers the floral transition. In *Arabidopsis*, its effect on flowering is repressed by *TFL1* which is expressed in the shoot meristems. Despite their opposite functions, FT and TFL1 are members of the same family, namely PHOSPHATIDYLETHANOLAMINE BINDING PROTEINS (PEBPs). Although their binding to phosphatidylethanolamines are still unclear, they were shown to bind phosphatidylcholines that are major phospholipids of plant membranes and whose concentration impacts flowering time (Nakamura *et al.*, 2014). In this context, we are investigating the ability of *Arabidopsis* FT and TFL1 to interact with membrane proteins and lipids by biochemical and biophysical approaches.



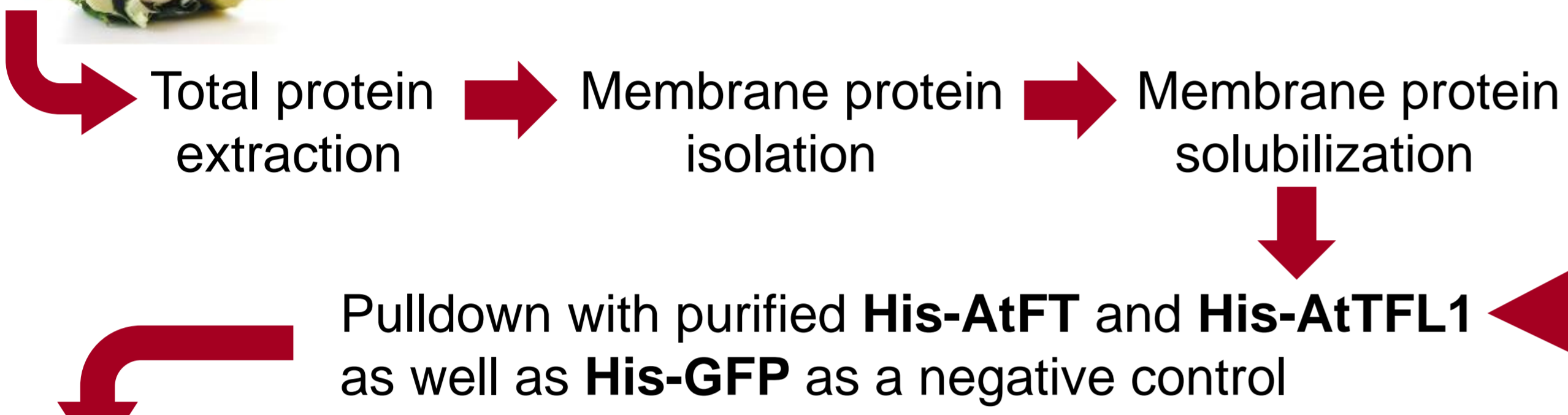
## 1 AtFT and AtTFL1 interactomes:

### in vitro pull-down assays

**Prey:** solubilized membrane proteins from Cauliflower meristems

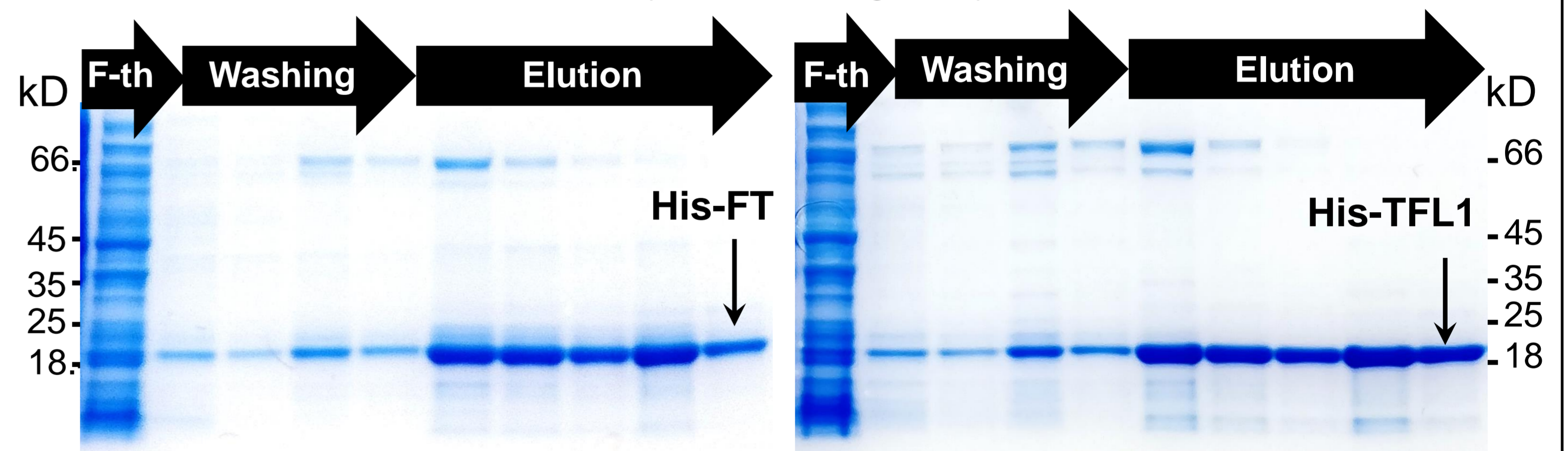
#### Advantages:

- A Brassicaceae like *Arabidopsis*
- Easy to get high amount of proteins from meristem cells



Purification of His-tagged complexes -> Protein identification through label free MS *in progress*

**Bait:** His-AtFT and His-AtTFL1 produced in *E. coli* and purified through Immobilized Metal Affinity Chromatography (IMAC)



### in vivo pull-down assays

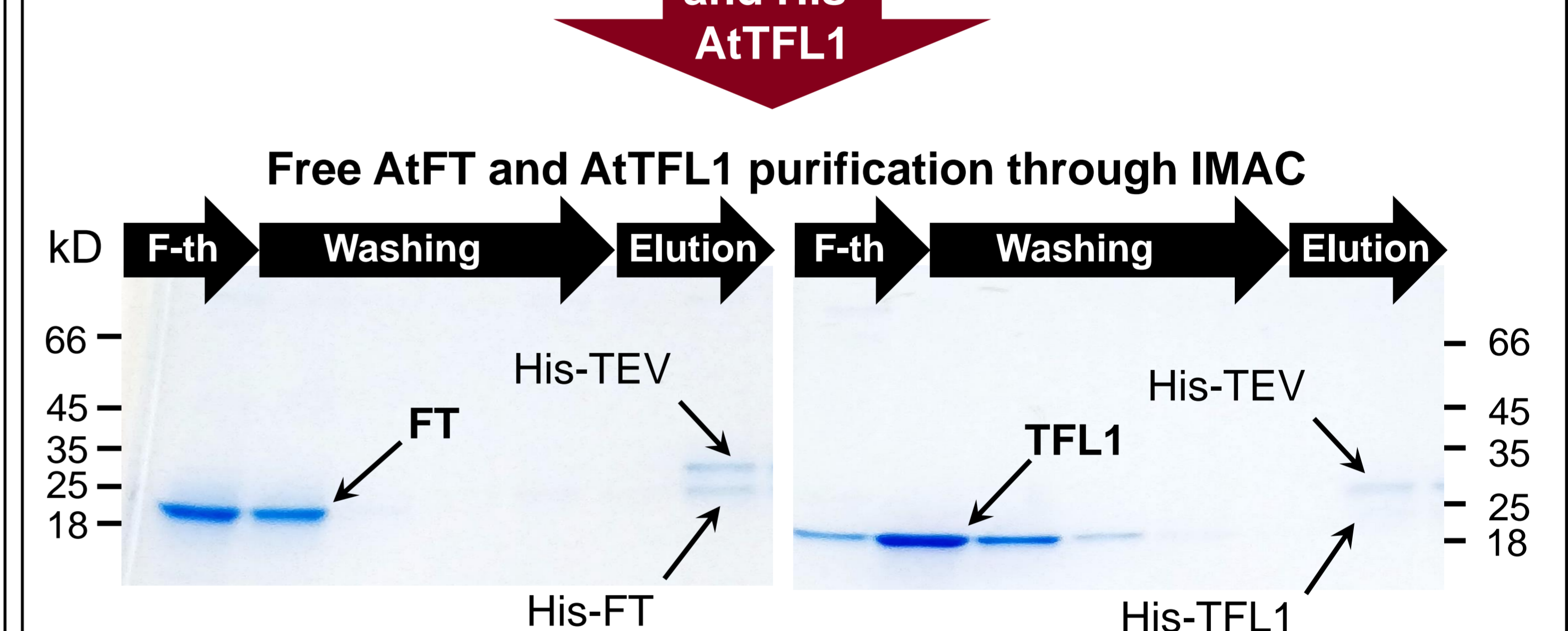
Overexpression of AtFT and AtTFL1 tagged with a protein G/ streptavidin-binding peptide (GS) double tag



AtFT-GS and AtTFL1-GS complement *ft* and *tfl1* mutants, respectively

Total protein extraction and complex isolation according to Jelle Van Leene, Nature Plants (2019) *in progress*

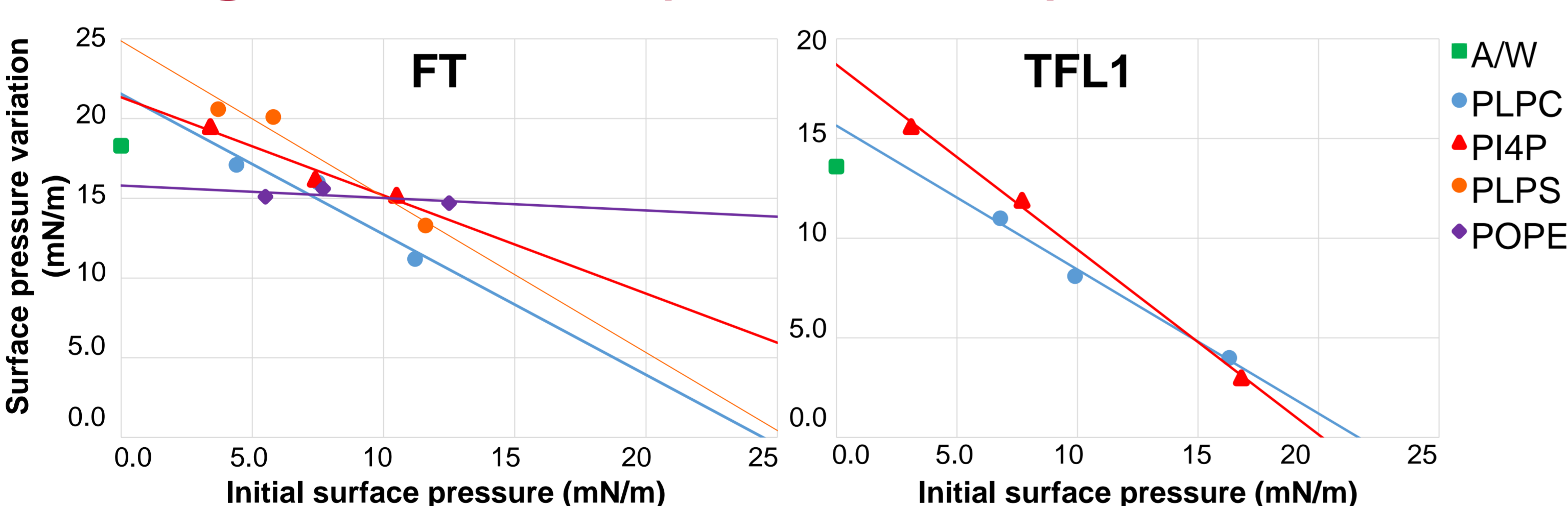
His-AtFT and His-AtTFL1 TEV digestion (16h, 4°C)



Free AtFT and AtTFL1 For Langmuir and Circular dichroism methods

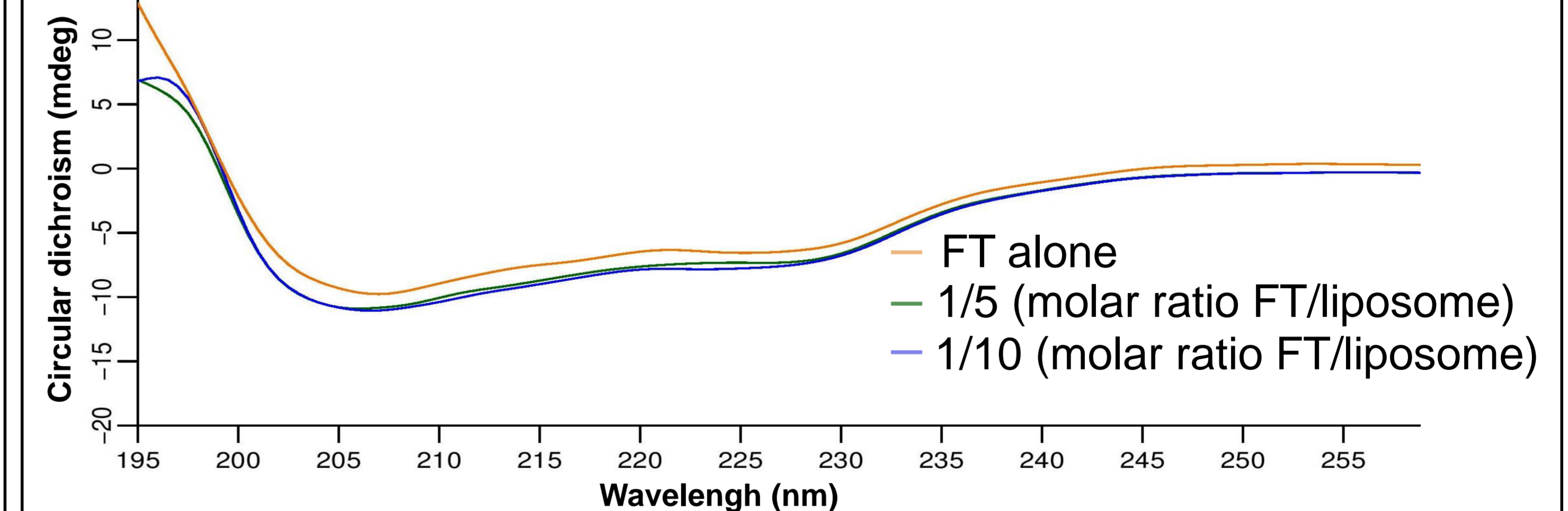
## 2 FT and TFL1 interactions with membrane lipids:

### Langmuir balance (FT and TFL1)

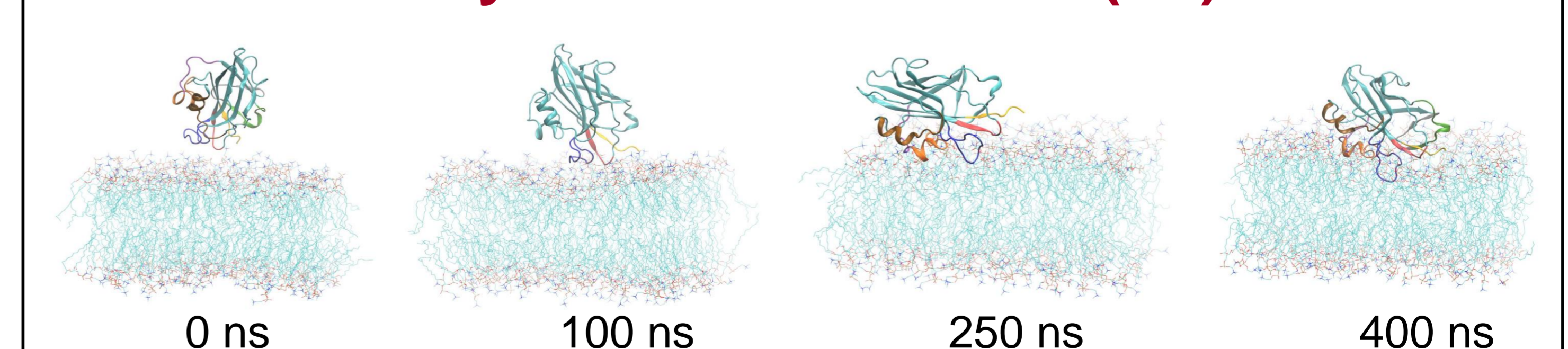


- FT and TFL1 interact with membranes mainly at the surface
- FT and TFL1 interact at least with PLPC and PI4P
- FT interactions with PLPC are mainly due to hydrogen bonds and salt bridges
- FT does not show major conformational modifications when interacting with membranes
- The binding pocket of FT is moving towards the membrane and can interact with PLPC

### Circular dichroism (FT)



### Molecular dynamics simulation (FT)



### Perspectives:

- Validate the protein interactions through BIFC and split-ubiquitin Yeast Two-Hybrid
- Investigate the roles of identified proteins by phenotyping the corresponding mutants
- Investigate through experimental and *in silico* mutagenesis the involvement of FT residues putatively involved in hydrogen bonds and salt bridges with membrane lipids