

INTERNSHIP PROPOSAL

Institute and Group: BIG/Integrated Structural Biology and Plant Development

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Research project title: Structural Studies of Transcription Factor-Remodeller Complexes

5 Keywords to describe the project: chromatin remodelling, gene regulation, flower development, MADS transcription factors, SWI/SNF ATPase

Description of the project (aims, experimental techniques, recommended background):

10 to 15 lines:

The proposed project addresses a central question in biology- **how are genes properly regulated during development?** In plants and animals, gene regulation is a critical process with misregulation resulting in decreased fitness, diseases such as cancer or mortality of the organism. The genetic code is densely packed into chromatin and transcribing the genetic code requires the opening and closing of specific regions in a highly orchestrated manner, allowing access to different genes during different developmental processes. Certain transcription factors are able to bind as “pioneers” to closed regions of chromatin, recruit chromatin remodeling complexes and facilitate access of RNA polymerase, allowing the successful transcription of target genes.

The molecular mechanism of recruitment of chromatin remodelers by different TF families, an evolutionary conserved process common to plants and animals, remains a black box. The project will directly address this using an integrated structural and *in vivo* approach. The student will focus on a transcription factor from Arabidopsis called SEPALLATA3 (SEP3) and the chromatin remodeler, BRAHMA. We have performed initial yeast 2-hybrid mapping experiments and shown robust interactions for different domains of SEP3 and BRM. The student will **(I)** refine the mapping of interaction domains, **(II)** clone the domains for recombinant expression in *E. coli*, **(III)** purify the proteins of interest and **(IV)** perform crystallization trials. Based on these structural studies, **(V)** mutants with altered capacity to form complexes will be designed for transgenic experiments. Experience in molecular, structural and/or plant biology is desired.

Justification that the internship’s subject fits with the general theme of GRAL (3 lines):

The proposed project will integrate structural studies with *in vivo* experiments in order to determine how transcription factors are able to recruit chromatin remodellers and how this recruitment affects the chromatin landscape. The project elegantly combines biochemistry and protein crystallography with structure-based mutagenesis, *in vitro* assays and *in vivo*

transgenics. This integration of structure and plant biology experiments fulfils the essential theme of GRAL by directly addressing the molecular determinants of complex developmental processes, in this case flowering and plant reproduction.

Relevant publications of the team (3 max): (current and former team members in **bold**)

1. **Conn, V. M., Hugouvieux, V., Nayak, A.,** Conos, S. A., Capovilla, G., Cildir, G., **Jourdain, A.,** Tergaonkar, V., Schmid, M., **Zubieta, C.,** and **Conn, S. J.** (2017) A circRNA from SEPALLATA3 regulates splicing of its cognate mRNA through R-loop formation, *Nat Plants* 3, 17053.
2. **Silva, C. S., Puranik, S.,** Round, A., Brennich, M., **Jourdain, A.,** Parcy, F., **Hugouvieux, V. & Zubieta, C.*** (2016). Evolution of the Plant Reproduction Master Regulators LFY and the MADS Transcription Factors: The Role of Protein Structure in the Evolutionary Development of the Flower. *Front Plant Sci* 6, 1193.
3. **Puranik S, Acajjaoui S, Conn S,** Costa L, **Conn V,** Vial A, Marcellin R, Melzer R, Brown E, Hart D, Theissen G, **Silva CS,** Parcy F, Dumas R, Nanao M, **Zubieta C.** (2014) Structural basis for the oligomerization of the MADS domain transcription factor SEPALLATA3 in Arabidopsis, *Plant Cell*, 26(9):3603-15 doi: 10.1105/tpc.114.127910.