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Assessing DNA-binding specificity of MADS-domain transcription factors

The specific molecular interaction between DNA and transcription factors (TFs) is of utmost importance to control gene expression and govern cell differentiation and organ development. DNA-binding specificity of different TF families is largely determined by a highly conserved DNA-binding domain. Owing to the high conservation of the DNA-binding domain, different members of a TF family often bind very similar DNA sequences, yet they may perform highly specific and distinct functions by binding different target genes. This poses a conundrum that is far from being resolved. To address this issue, MADS-domain proteins will be used as a model system. They are found in almost all eukaryotes, but have especially prominent developmental functions in seed plants. Within this project the thermodynamics and kinetics of the molecular interactions of MADS-domain TFs with DNA will be studied, using a variety of biophysical, bioinformatics and molecular biology tools. It will be studied to which extent the primary DNA sequence ("base readout") and also the shape of the DNA ("shape readout") contribute to binding specificity. Also, the structural characteristics of the proteins, the DNA and the protein-DNA-complexes will be determined using e.g. circular dichroism (CD) spectroscopy. Sequence analyses will reveal candidate amino acid residues that may be involved in determining binding specificity. Based on these results, proteins with amino acid substitutions will be generated to gain additional insights into how different residues contribute to binding specificity. Finally, *in planta* complementation experiments will demonstrate to which extent the previously identified determinants of binding specificity contribute to protein function *in vivo*.

Publications

Käppel S, Eggeling R, Rümpler F, Groth M, Melzer R, Theißen G (2021) DNA-binding properties of the MADS-domain transcription factor SEPALLATA3 and mutant variants characterized by SELEX-seq. *Plant Mol Biol* 105(4-5), 543-557. [Details](#) [PubMed](#)

Käppel S, Melzer R, Rümpler F, Gafert C, Theißen G (2018) The floral homeotic protein SEPALLATA3 recognizes target DNA sequences by shape readout involving a conserved arginine residue in the MADS-domain. *Plant J* 95(2), 341-357. [Details](#) [PubMed](#)

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