

**FORMULAIRE STAGE Recherche-M2 BBSG
(période de stage : du 5 janvier 2017 au 3 juillet 2017)**

Titre du stage : Unravelling the structural and functional basis of calmodulin-regulated protein kinases regulation

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Descriptif du stage :

Already almost 60 years ago, the first kinase was identified and described; yet kinases are still not a boring topic and not all is about drugging their ATP-binding pocket! We still have so much to understand about these hub proteins! In particular, the mechanisms behind the fine regulation of kinase activation and their substrate specificity keep intriguing researchers.

One of the long-term interests of the Wilmanns group resides in the activity regulation of calcium/calmodulin (Ca²⁺/CaM)-dependent protein kinases. In the last five years, we have contributed to unravel the structure of the pro-apoptotic death-associated protein kinase 1 (DAPK1), in the presence of the regulatory protein Ca²⁺/CaM (de Diego *et al.*, *Sci Signal.* 2010; 3(106):ra6). This structure provided insight into how Ca²⁺/CaM binding and other structural changes lead to DAPK activation (Temmerman *et al.*, *FEBS J.* 2013; Temmerman *et al.*, *Chem Biol.* 2014). More recently, a dimeric DAPK2 crystal structure has also revealed the importance of DAPK2 dimerisation for Ca²⁺/CaM binding regulation (Simon *et al.*, *Structure* 2016).

Together with previous and ongoing publications, those data have allowed us to propose a new model for DAPKs regulation. The current focus lies in phosphorylation events within the auto-regulatory domain of DAPKs. Based on the structures of DAPK1 and DAPK2 in complex with Ca²⁺/CaM, we aim to mechanistically understand the conformational implications of these post-translational modifications in the release of auto-inhibition. We also want to solve the structure of other kinases in complex with Ca²⁺/CaM or to trap the known complexes in alternative conformations.

The master student will be supervised by Anne-Sophie Huart, a postdoc in the Wilmanns group since nearly 3 years (https://www.researchgate.net/profile/Anne-Sophie_Huart). English is the used language at EMBL and Hamburg is an international city. The candidate, who would already have some related experience, will mainly focus on cloning (restriction-based and SLiCE cloning, mutagenesis), expression (*E.coli* cultures, eventually use of insect cell pellets) and purification of different DAPK variants (affinity-based techniques, size exclusion columns, on the bench and using AEKTAs). Using successfully purified proteins, the student would assist in their biophysical characterisations (thermo-fluor analysis, circular dichroism, dynamic light scattering) and binding assays with Ca²⁺/CaM by isothermal titration calorimetry and fluorescence anisotropy. Our goal is to complement the insights gained from our recently obtained structures with the results from these *in vitro* experiments to translate them into a publishable paper.