Master 2 research internship in Integrated Structural & Cell Biology in Grenoble

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Supervisor(s):
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Host laboratory:
Lab : IBS

Host group/team:
Membrane Group /Nury Team

Title of the M2 research internship:
De novo design of antithrombotic peptides

Project summary:
This internship will focus on the biophysical and structural characterization of *de novo* designed proteins as potential therapeutics in the field of thrombo cardiovascular diseases (CVDs). CVDs, encompassing venous thromboembolism, myocardial infarction, and ischemic stroke, remain a crucial threat to global health (over 20 million deaths worldwide per year). Thromboinflammation, a maladaptive crosstalk between platelets and the innate immune system represents a critical checkpoint in the progression of these pathologies. This process is characterized by aberrant platelet activation and the release of Neutrophil Extracellular Traps (NETs), which serve as pro-coagulant scaffolds that exacerbate tissue injury. Traditionally characterized as a specialized collagen chaperone within the endoplasmic reticulum, Heat Shock Protein 47 (HSP47) has recently been identified as a regulator of NET formation and thrombosis when displayed at the platelet surface by the groups of Thienel and Petzold (Thienel et al., 2023). Using a cross-species approach, this article demonstrated that HSP47 downregulation has a conserved thromboprotective mechanism in long-term immobilized, hibernating brown bears and spinal cord injured (SCI) patients. Building on these findings, we initiated a collaboration a year ago with the Petzold group (German Heart Center, Berlin) to exploit HSP47 as a high-value therapeutic target. In the last couple of years, we have been building a computational and experimental workflow for the *de novo* design of proteins, and have started to apply it to the generation and screening of HSP47 targeting proteins. Deep learning-based methods including RFdiffusion (Watson et al., 2023) and BindCraft (Pacesa et al., 2025) enabled our collaborator (F. Dehez, LPCT, Nancy) to provide libraries of putative binders. Those were either expressed and screened through a yeast surface display approach or directly produced and tested in an *in vitro* functional assay, in Berlin, to assess their capacity to interfere with platelet activation and platelet–neutrophil interactions. So far, we have identified two promising hits. On the one hand Yeast surface display screening revealed a binder with a 100 nM affinity for HSP47 (Figure A), on the other we identified a binder that markedly reduced platelet aggregation following thrombin stimulation (Figure B). Building on those unpublished promising results we propose an M2 project to further analyse those hits. Their interaction with their target will be biophysically characterised by SPR or BLI. The high-resolution structure of the binders in complex with HSP47 will be solved by X-Ray crystallography (HSP47 has already been crystallised). This knowledge will be critical to undertake a rational optimisation of the binders by site directed mutagenesis and computational methods. The refined binders will thus be evaluated *in vitro* by our German collaborator in platelet activation, aggregation, and NET formation assays. It will be possible for the student to travel and participate to these experiments. This internship should pave the way to a PhD focused on the identication and validation of optimized protein binders in murine model of thromboinflammation (venous/arterial thrombosis,

myocardial ischemia-reperfusion, stroke) without bleeding risks compared to conventional molecules targeting the coagulation cascade.

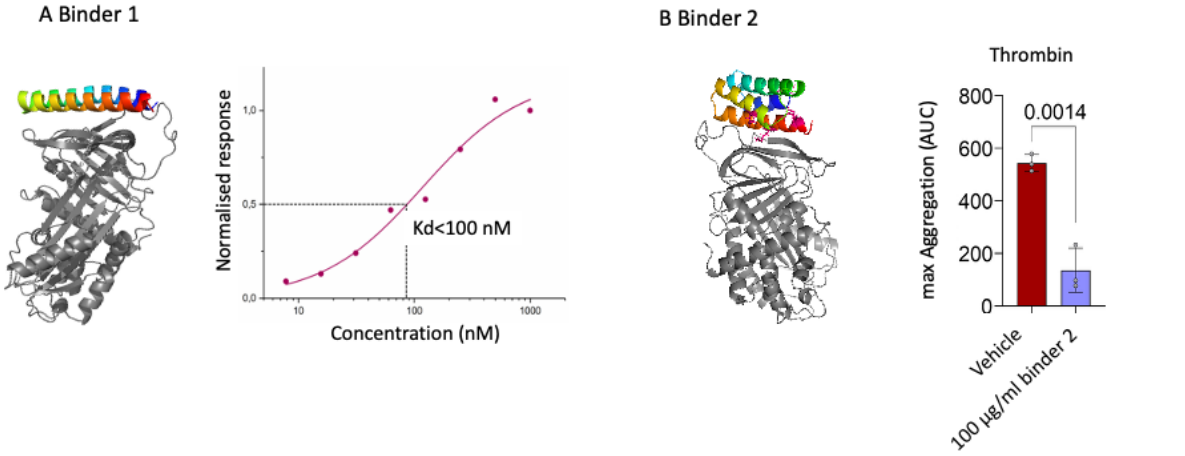

Figure A: Design model of binder 1 against Hsp47 and dose response curve of Hsp47 binding to binder 1 expressing yeast.
Figure B: Design model of binder 2 against Hsp47 and platelet aggregation after thrombin stimulation.

Pacesa, M., et al. (2025). Nature 646, 483–492 DOI: 10.1038/s41586-025-09429-6
Thienel, M., et al. (2023). Science 380(6641): 178-187 DOI: 10.1126/science.abo5044.
Watson, J.L., et al. (2023) Nature 620, 1089–1100 DOI: 10.1038/s41586-023-06415-8.

Keywords:
Thomboinflammation, *in silico* design, yeast surface display, BLI, SPR, X-Ray crystallography

Relevant publications of the team:
This project started just a year ago, nothing has been published so far.