

Title: Structural basis of antibody affinity maturation

PI: Claudia Scotti

Laboratory: Protein engineering in physiology and disease

Research theme / topic: Immunology / Antibody structure and function

Main Abstract:

Affinity maturation is a process that leads to the emergence of more efficient antibodies following initial antigen encounter and represents a key strategy of the adaptive immunity of vertebrate organisms. A model system to investigate this feature of the immune system is represented by the mouse response to the hapten 2-phenyl-5-oxazolone (phOx). Earlier and detailed sequence studies of the antibody response to phOx performed by the Nobel Prize Cesar Milstein defined three different classes of antibodies. Class I antibodies use the VHOx1/VκOx1 gene pair and dominate the early stages of the anti-phOx response, class II antibodies use the VκOx1 gene but a different VH segment and are common in the intermediate stages, and class III antibodies use the TEPC15/Vκ45.1 gene combination and play the greatest role in the late stages. Only the crystal structures of some anti-phOx antibodies are available so far and more are needed to have a full picture and to be able to translate this knowledge to “real-life” systems.

The aim of the project is to determine the structure of at least 4 anti-phOx antibodies of the anti-phOx model system. The main structural techniques used will be X-ray crystallography, NMR and cryo-EM. Both natural and recombinant sources of the proteins will be used.

Techniques: Antibody fragments will be produced in recombinant form in bacterial, yeast and mammalian cell systems, purified, characterized from the biochemical point of view (Surface Plasmon Resonance) and crystallized for X-ray studies to be performed at European synchrotrons (ESRF, Diamond, EMBL). Alternative techniques will involve NMR or the study of antibody-antigen complexes by cryoEM. Site directed mutagenesis and structural modelling will also be applied to investigate specific features of the molecules involved in affinity maturation.

