

Structural and functional proteomics of *Anopheles gambiae* salivary glands and saliva
V. Choumet¹, V. Jan¹, A. Carmi¹, A. Robbe-Vincent¹, A. Namane², C. Laurent², P. Lenormand², J.-C.
Rousselle², J. d'Alayer³ & P. Brey¹

¹ Biochemistry and Molecular Biology of Insects Unit, Institut Pasteur, 28 rue du Dr Roux, 75724 Paris cedex 15, France

² Proteomics Platform, Institut Pasteur, 28 rue du Dr Roux, 75724 Paris cedex 15, France

³ Protein microsequencing and analysis, 28 rue du Dr Roux, 75724 Paris cedex 15, France

The African mosquito *Anopheles gambiae* is the most important vector of human malaria, a parasitic disease that affects the 40% of the world population living in tropical and subtropical regions. The increasing resistance of parasite to inexpensive drugs and mosquitoes to insecticides points to the urgent need to find innovative methods to block parasite transmission at the insect stage and therefore prevent malaria. Mosquito saliva and salivary glands play a central role in the interaction between parasite, vector and host. The deciphering of vector arthropod saliva and salivary gland composition may lead to new vaccine targets against *Plasmodium* as well as to the development of new drugs such as anti-thrombotic, anti-inflammatory, or immunosuppressors. In the desire to better characterize saliva and salivary gland components implicated in saliva's major pharmacological activities as well as in the establishment of *Plasmodium* infection, we have developed a structural and functional proteomics-based approach of *Anopheles gambiae* saliva and salivary glands in non-infected and *Plasmodium berghei* infected mosquitoes. The structural proteomic analysis allowed the identification of 50 components from salivary glands of young blood-fed females, 46% of them being proteins of saliva (vasodilators, allergens, enzymes of digestion of sugars and proteins of unknown functions). The 2D-gel profiles of infected and non-infected salivary glands were also compared. With regard to the functional proteomic approach, we more particularly focused our studies on the characterization of molecules involved in blood feeding. The salivary gland extracts of females in various physiological conditions, young not blood-fed and blood fed, 21 day-old females infected or not by *Plasmodium berghei*, were separated by size exclusion chromatography and the fractions were tested for their pharmacological activity. The tests carried out on human plasma revealed that the delays of the time of coagulation resulted from the synergistic action from pro- and anticoagulant salivary gland components. The effects of the components of salivary glands on proteins of the cascade of coagulation showed the presence of inhibitors of kallikrein, FXa, thrombin, protein Ca, urokinase and plasmin. Some activities were shown to vary according to the age of the mosquito, to whether it took a blood meal or not and to whether the mosquito is infected by *Plasmodium* or not. Interestingly, salivary gland extracts from infected mosquitoes appeared to be the least anticoagulant among all the tested extracts. We expect this proteomic analysis to provide new and better information on the interaction between the *Plasmodium* parasite and the *Anopheles* mosquito and between the mosquito and the human.