4th International Workshop on

Expression, Structure and Function of Membrane Proteins

Florence, Italy
June 28- July 2, 2015

Book of Abstracts

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CASSA DI RISPARMIO
DI FIRENZE

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Membrane Proteins
FLORENCE

Interaction studies of HrpA proteins involved in the biosynthesis of Type Three Secretion System

Alessio Sacconi[1], Francesco Tadini-Buoninsegni[1], Maria Rosa Moncelli[1], Giancarlo Margheri[2], Matteo Cerboneschi [3], Stefania Tegli[3]
[1] Dipartimento di Chimica "Ugo Schiff", Università di Firenze, 50019 Sesto Fiorentino, Italy
[2] Institute for Complex Systems, National Research Council, 50019 Sesto Fiorentino, Italy
[3] Molecular Plant Pathology Lab, University of Florence, 50019 Sesto Fiorentino, Italy [3]
alessio.sacconi@unifi.it

Many Gram-negative bacteria pathogenic to plants and animals use Type III Secretion System (TTSS) to deliver virulence pathogenicity and proteic effectors into host cells [1]. In phytopathogenic bacteria, such as *Pseudomonas syringae*, proteins are translocated from bacterial cells to plant cytoplasm through the TTSS-associated Hrp-pilus, built up of HrpA subunits [2]. Previous studies have shown that HrpA can auto-assemble into filamentous structures *in vitro* [3] while *in vivo* pilus is assembled by HrpA addiction to its distal end [4]. The use of Virulence Inhibiting Peptides (VIPs) was demonstrated to be an effective strategy to avoid HrpA subunit interactions (*Cerboneschi et al.* unpublished data), that are crucial for pilus assembly [5].
We designed a series of synthesized peptides in order to study the HrpA-VIPs interactions in vitro and to show the ability of these peptides to prevent the assembly of TTSS-pilus and the activation of TTSS. In the present work we studied the affinity between C-terminal portion of HrpA, covalently immobilized on a gold sensor, and VIPs, using optical and electrochemical techniques combined *in situ* : Electrochemical Impedance Spectroscopy (EIS) and Surface Plasmon Resonance (SPR). EIS and SPR methods are specific, powerful and non-destructive tools that can be employed to measure biological interactions, allowing to analyze interfacial properties related to biorecognition. In particular MC16 peptide which correspond to a fragment of HrpA sequence has shown a very high interaction.

Acknowledgements: We gratefully acknowledge EU (project LIFE12 ENV/IT/336 "AFTER Cu"), and Ente Cassa di Risparmio di Firenze for their financial support

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