SCIENCES & BIOENGINEERING SCIENCES

The Research Group

Structural Biology Brussels (SBB)

has the honor to invite you to the public defense of the PhD thesis of

Imke VAN DEN BROECK

to obtain the degree of Doctor of Bioengineering Sciences

Title of the PhD thesis:

The assembly and biotechnological application of the bacterial functional amyloid curli

Promotor:

Prof. Dr. Han Remaut

The defense will take place on

Thursday 11 January 2018 at 17:00 h

in Auditorium E.0.12 at the campus Humanities, Sciences and Engineering of the Vrije Universiteit Brussel, Pleinlaan 2 - 1050 Elsene, and will be followed by a reception.

Members of the jury:

Prof. Dr. Eveline Peeters (chairman)
Prof. Dr. Peter Tompa (secretary)
Prof. Dr. ir. Guido Verniest
Prof. Louise Serpell (Univ. Sussex)
Prof. Dr. Joost Schymkowitz (KUL)

Curriculum vitae

Imke Van den Broeck obtained a Master in Bio-engineering sciences at the VUB in 2013. Subsequently she was granted a IWT doctoral fellowship to perform her PhD in the Structural and Molecular Microbiology lab of Prof. Han Remaut. Her work resulted in a first-author publication in a peer reviewed journal, several oral and poster presentations at national and international scientific conferences. In addition she supervised one master thesis project.

Abstract of the PhD research

Curli are proteinaceous fibers found on the extracellular surface of many Gram-negative bacteria such as *Escherichia coli* and *Salmonella enterica*, where they mediate biofilm formation and bacterial adherence to both biological and non-biological surfaces. Apart from their physiological importance to bacteria curli fibers share many biochemical and structural characteristics with amyloid fibers including an extremely robust cross-β spine quaternary structure, and a self-assembling nature. Curli formation requires a dedicated protein secretion machinery comprised of the outer membrane lipoprotein, CsgG, responsible for the transport of pro-amyloidogenic curli CsgA subunits across the outer membrane and two accessory proteins CsgE and CsgF. Once secreted, CsgA subunits then aggregate into linear fibers on the extracellular surface in a process that is templated by the minor subunit, CsgB. Numerous fundamental questions about the curli biogenesis pathway remain unsolved. Recently the X-ray structure of the CsgG translocator protein was solved within our group, revealing a nonameric complex embedded in the outer membrane by a 36-stranded β-barrel. Based on this structure and preliminary single-channel results we observe that CsgG is an ungated, non-selective protein secretion channel. We show that the secretion adaptor CsgE temporarily blocks the secretion channel by formation of a dynamic complex with CsgG. Here we propose a mechanism of "targeted diffusion" as a working model for the CsgG translocator, a novel concept in protein secretion and protein transport as a whole.

Also the molecular events during curli nucleation and fiber extension remain largely unknown. In this thesis the *in vitro* CsgA polymerization at single fiber level was studied by combining established biophysical methods with real-time *in situ* nanoscopic imaging using high speed atomic force microscopy. Our findings support a model of direct, one-step nucleation where monomeric species contemporaneously fold and oligomerize into minimal fiber units that have growth characteristics identical to the mature fibrils that emanate from these structures. We show that curli fibers display polar growth, and we detect two kinetic regimes of fiber elongation.

In a synthetic biology context, the curli biogenesis pathway shows large potential for the recombinant formation of highly robust, multivalent nanowires that are attractive in a range of biotechnological applications. Using the curli subunit CsgA as a carrier protein, affinity reagents can be recombinantly introduced resulting in functionalized nanowires displayed on the bacterial cell surface. Unfortunately, this first attempt was unsuccessful, as secretion of larger polypeptide sequences through the CsgG transporter seemed to be inefficient. Based on insights from the curli assembly pathway we developed and optimized the display of purified CsgA fusions on an abiotic surface. In this proof-of-principle study, we show our curli-based biosorbent platform provides robust materials with a great potential of functionality.