

Working Group 1: RNAi technology

Minutes of the working group meeting on “Advanced delivery strategies for dsRNA into cells” –Ghent (Belgium) 5th September 2019

The workgroup meeting organized at the faculty of Bioscience Engineering (Ghent University) by the WG1 consisted of three parts: 1) a scientific session in the framework of advanced delivery strategies for dsRNA into cells, 2) a working group brainstorm and discussion on the deliverables of the WG1 and 3) a visit to the Syngenta Innovation Center (Ghent).

The meeting was attended by ca 20 people and was opened with three invited lectures. The presentation by Dr. Olivier Christians (UGhent, Belgium) provided an overview of the potential barriers related to cellular uptake and endosomal release of dsRNA and the potential of nanocarriers to overcome these barriers. Prof. Stefan De Smedt (UGhent, Belgium) elaborated on this topic focusing on the extracellular barriers present in mammalian cells. In the first part of his lecture he discussed the interactions of the nucleic acids and their carriers with the extracellular environment. Further he presented novel delivery techniques for nucleic acids into cells in cell culture. Complementary to the talk by Prof. De Smedt, the lecture by Prof. Koen Raemdonck (UGhent, Belgium) focused on the cytosolic delivery and endosomal release, showing that localization in the lysosomes is not necessarily the endpoint.

The scientific session was concluded by presentations selected from submitted abstracts. Dr. Michel Ravelonandro (INRA, France) presented the work on RNAi-mediated pest control of *Macrolophus*. Dr. Silvia Sabbadini (Marche Polytechnic University, Italy) and Dr. Elena Baraldi (University of Bologna, Italy) presented on RNAi for pathogen resistance in major woody fruit species.

The second part of the meeting was a workgroup meeting where the WG1 members discussed on the tasks and deliverables of WG1. In particular the generation of a dsRNA sequence database focusing on target and off-target genes was discussed.

In the last part of the scientific agenda, the Syngenta Innovation Center (Ghent) was visited. With the motto ‘bringing plant potential to life’, Syngenta aims to help humanity face its toughest challenge: how to feed a rising population, sustainably. In its facilities in Ghent the focus is on RNAi-based crop protection. After an overview of their research lines, the labs were visited with demonstrations of the different insect rearing techniques.

A walking tour through the historical city of Ghent and a social nextworking dinner in the former monastery ‘het pand’ concluded the workgroup meeting.

Minutes of the WG1 workgroup meeting

The meeting was opened by Prof. Guy Smagghe with an overview of the objectives, tasks and deliverables of the WG1

Before discussion on the deliveries there was an announcement concerning projects for STSM grants and the funding for conferences to present data obtained within the iPlanta Cost action.

- As the COST action will end in 2020, all projects should end, and reports submitted, by 29 February 2020

Deliverable 1: Develop a database of references and relevant documents

- Suggested to follow a similar format as the EFSA database
- Excel
- Suggestion to limit the data to invertebrates, this would only require to update the EFSA database with the recent publications

With the addition of plant, bacteria, fungi and viruses, the database will become too large

- Michel will create entries concerning plant viruses and will write a review on this
This review will contribute in deliverable 2

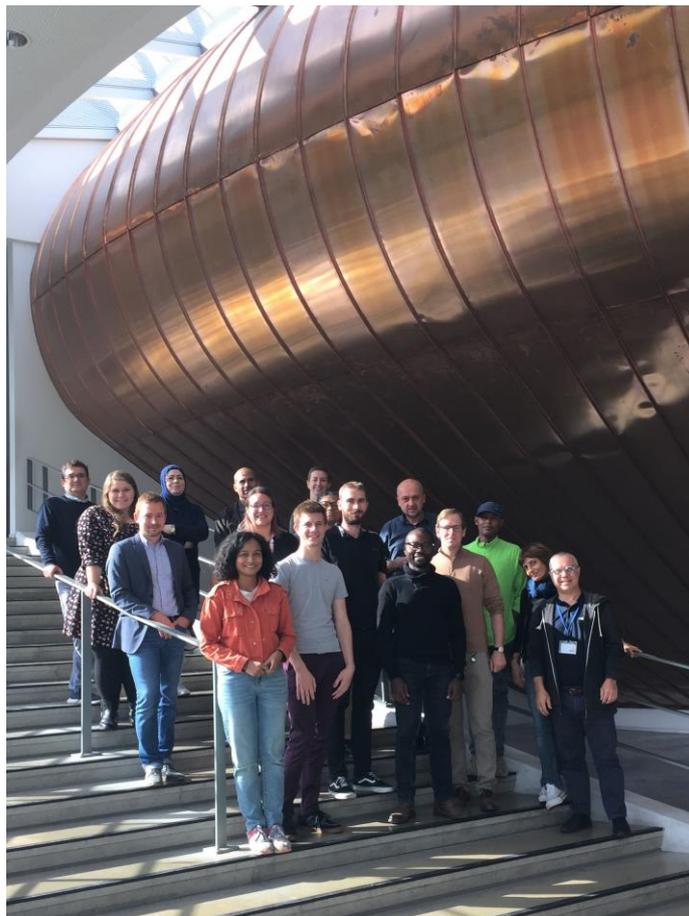
Deliverable 2: a review of the applications of plant siRNA and miRNA systems

- A review will be written concerning the use of RNAi against plant viruses

Deliverable 4: Bioinformatics database on target and off-target genes of characterized dsRNA and miRNA sequences

- As a holistic database is not feasible, it is suggested to work with cases
- Partly this database would overlap with deliverable 1
Per species a list of dsRNAs with the effects and way of introduction (deliverable 1)
With the addition of target and off-target effects
- how far should we check for off target effects
 - o search is limited to publically available datasets
- how to approach for this search
 - o if needed to search for all possible 21bp fragments in these genomes, this will generate long lists
- will we explore what is published or analyze potential off-targets ourself

- when analyzed, data will be hypothetical and not confirmed
- there will always be 21 bp matches, but this homology will not guaranty actual activity
- Data on off-targets will be sensitive data
 - As the data is generated by expert groups, the data will be considered as truth
- Database is already available: genbank
 - Focus on the tools available to screen this database
 - Design a pipeline to screen for off-targets in beneficial insects and human
- Collection of tools will indeed be a safe strategy
- Because of the overlap with deliverable 1 and deliverable 3 (review on existing and future applications of RNAi), it would be best to combine these
- What is the overlap with WG3: biosafety
- Limit to cases published and generate no new data as this might be too sensitive for registration dossiers of products



Picture 1: Participants in the WG1 meeting



Picture 2: Visit to Syngenta innovation center (Ghent) focused in RNAi-based crop protection