Advancing RNAi Delivery in Plants through Guanidinylated Polymer Systems

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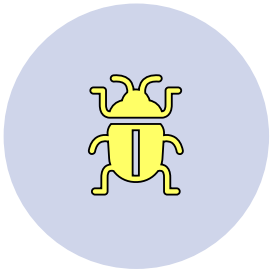
Plant GLYCO Biology

GHENT UNIVERSITY

RNAi for pest control

INCREASING FOOD DEMAND

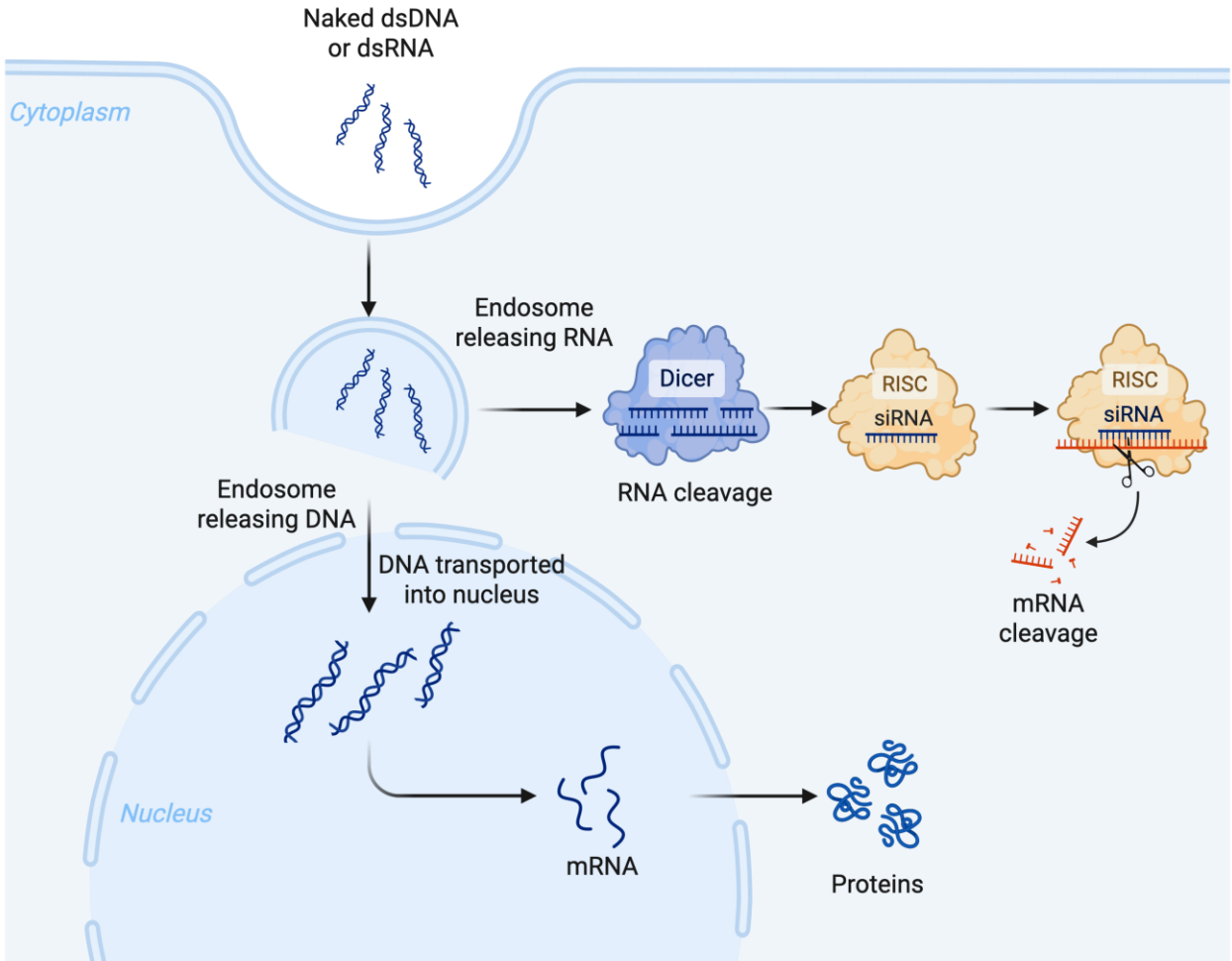


INVASIVE SPECIES

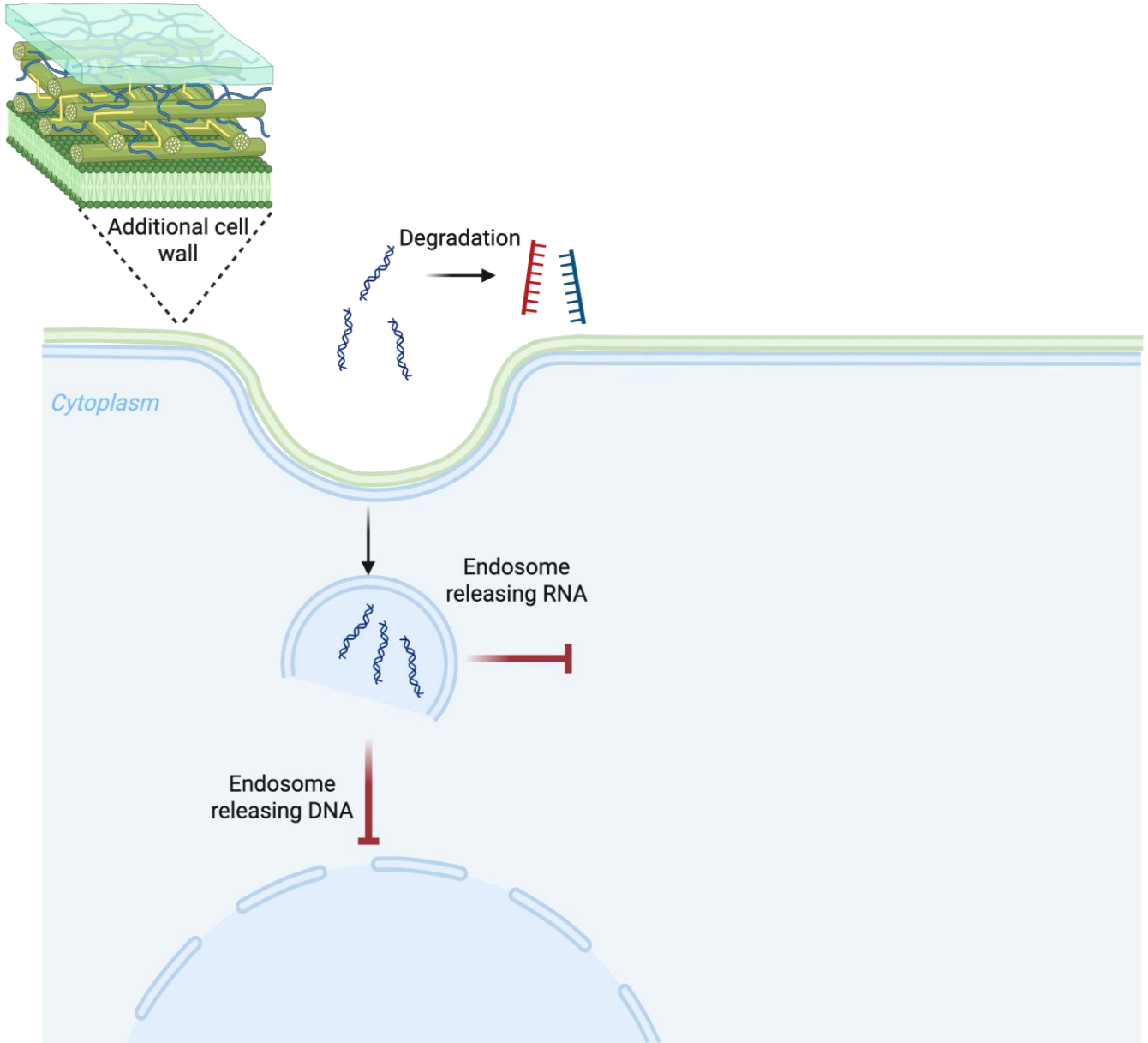
USE OF CHEMICAL PESTICIDES

BIOLOGICAL ALTERNATIVES

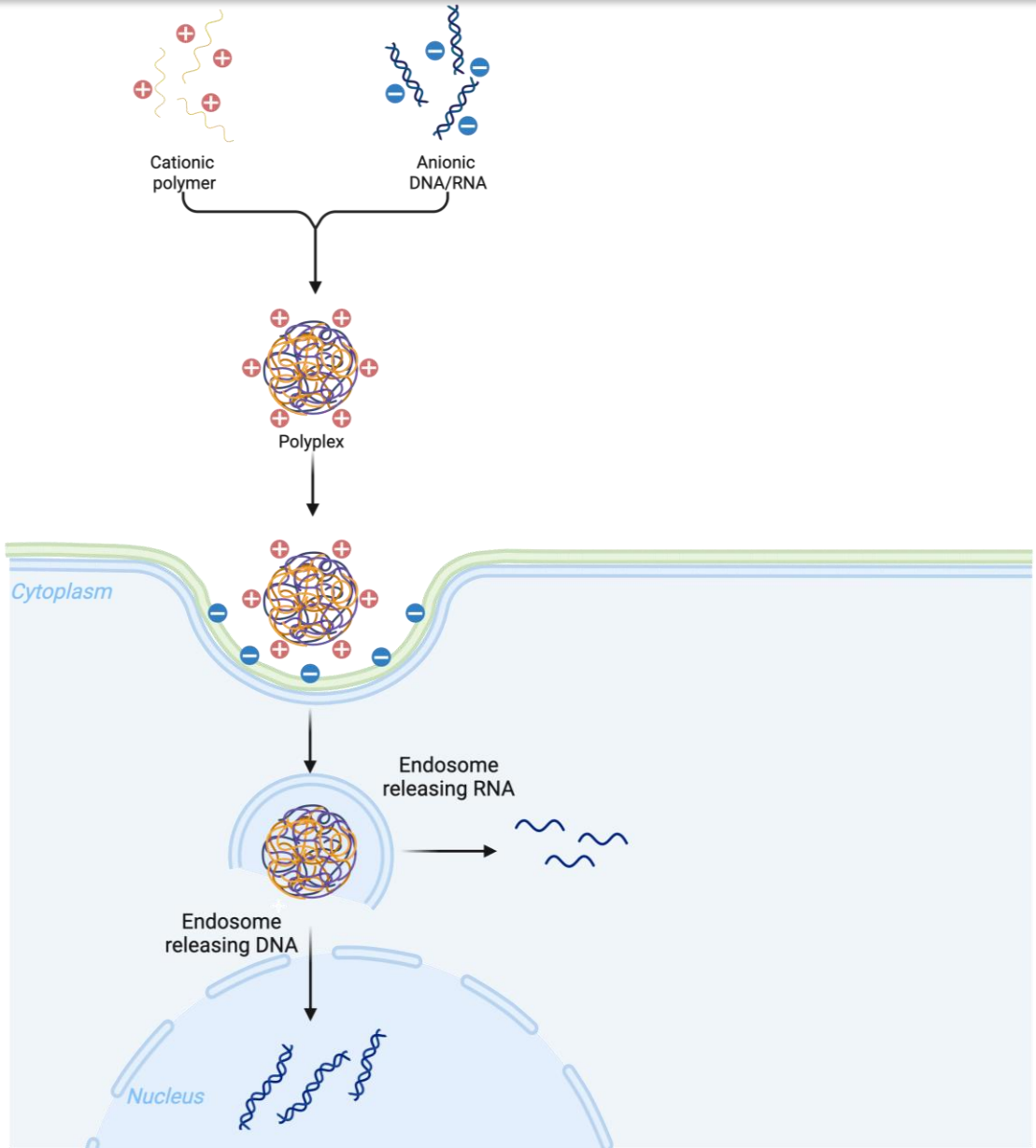
RNAi for pest control



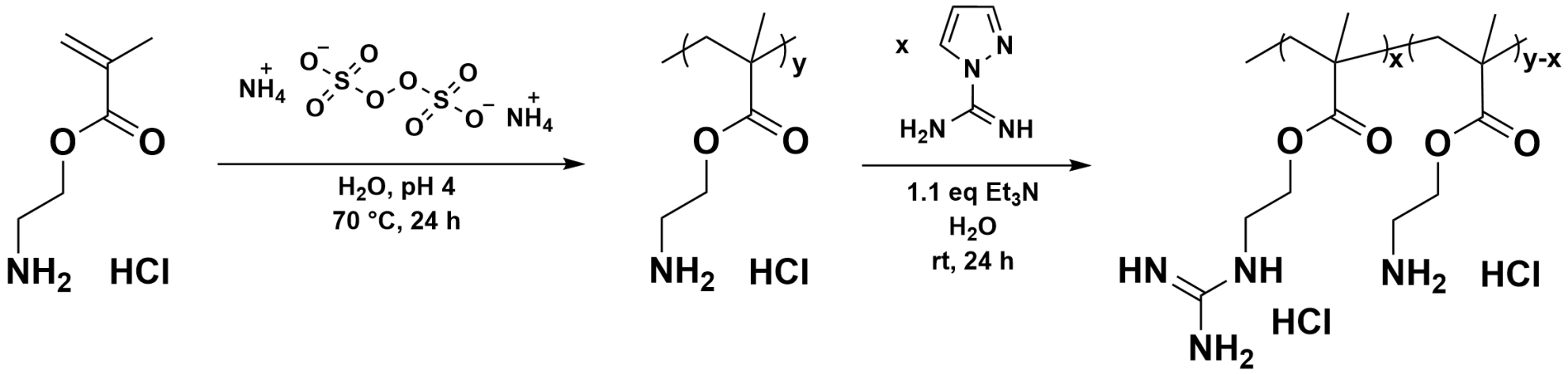
Challenges in RNAi for pest control



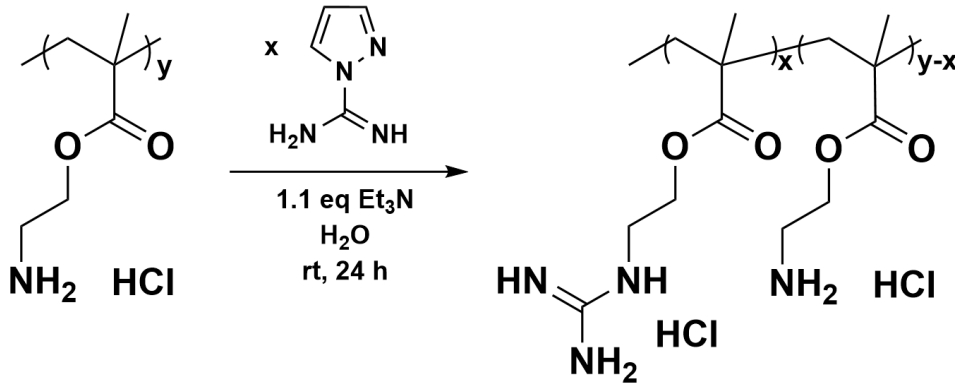
Tackling the challenges using cationic polymers

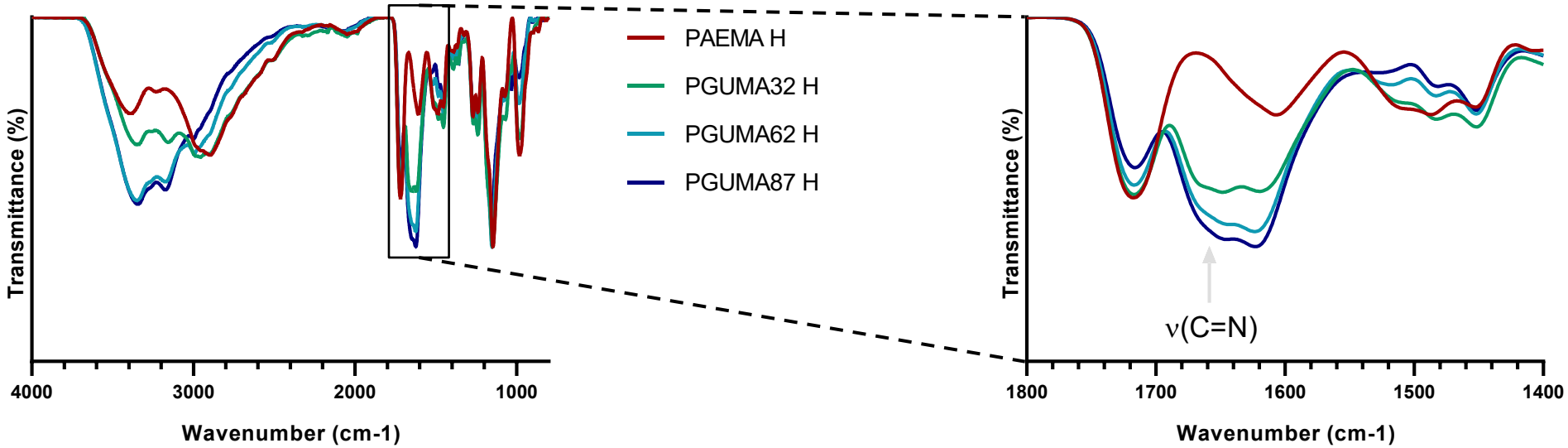


Synthesis of polymer library



Synthesis of polymer library – Guanidinylation





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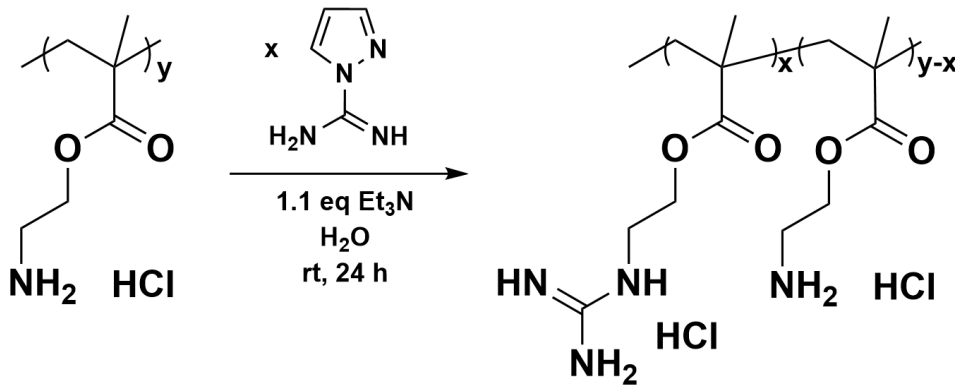
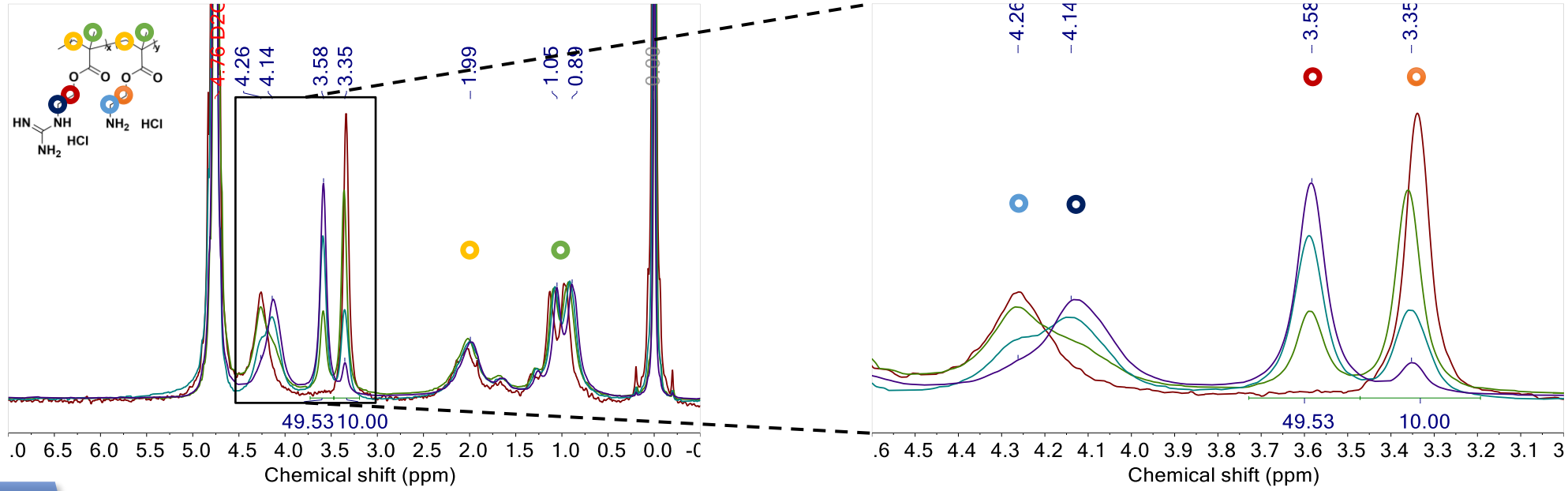
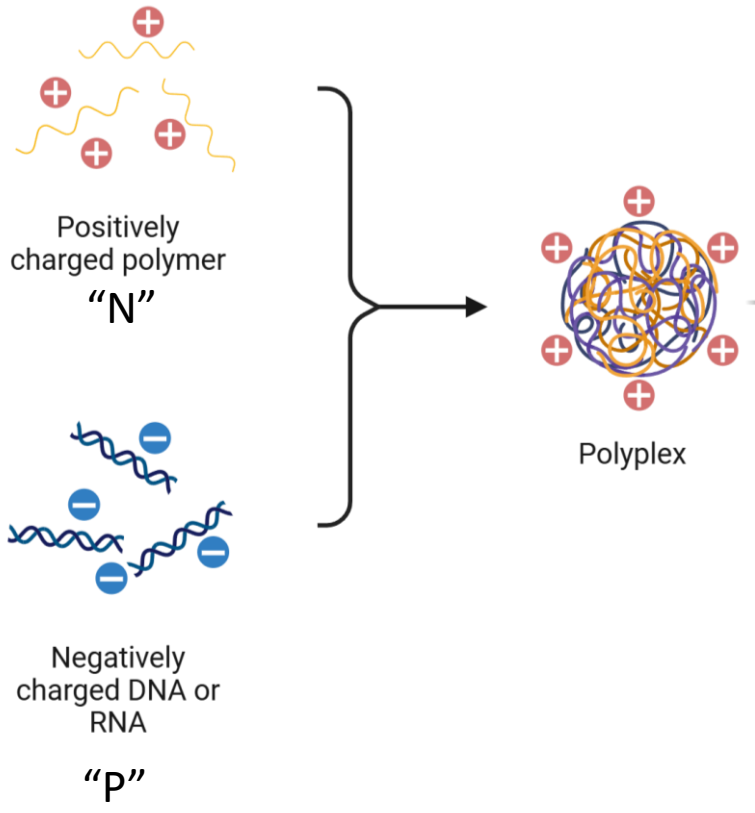


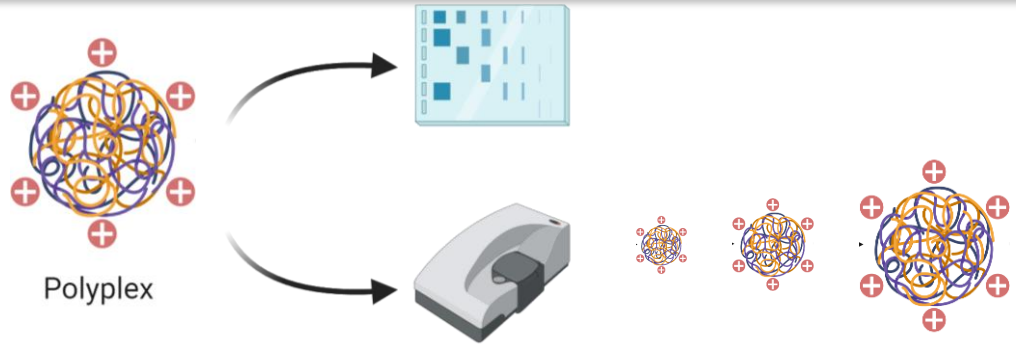


Creation of small polymer library of twelve polymers with different MM and DS

Polyplex formation

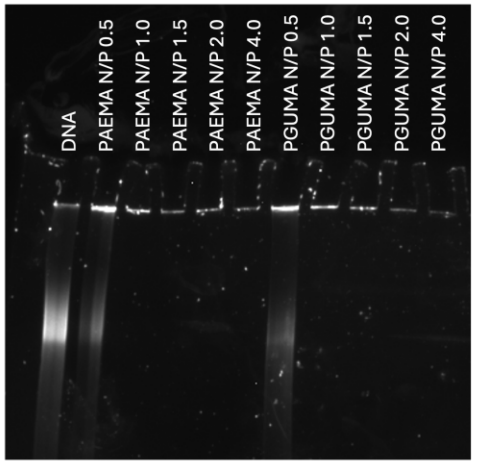


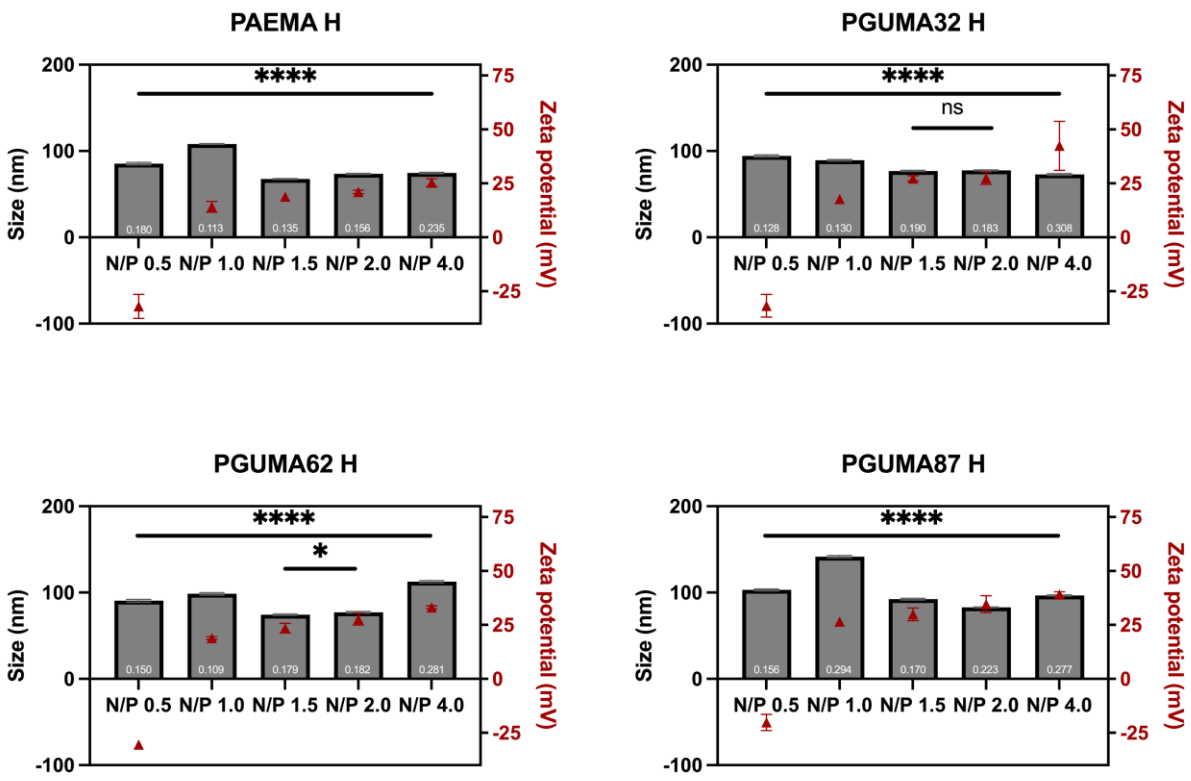
Polyplex characterization



Condensation of DNA/RNA

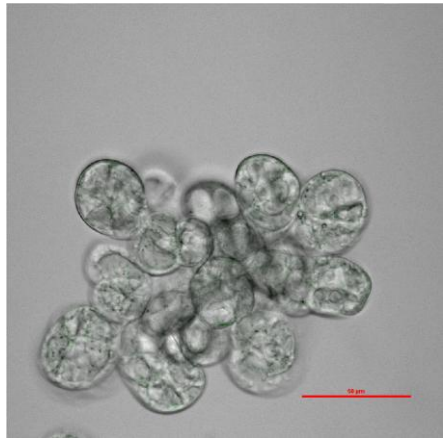
Size (< 200 nm)
Charge (+ at N/P ≥ 1.0)



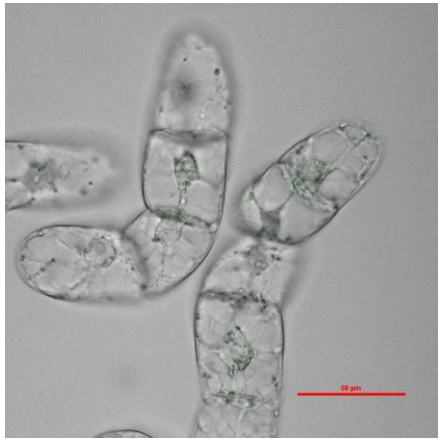


Cytotoxicity evaluation of polymers – polyplexes

- 2 model systems
  - ***Arabidopsis thaliana* (Thale cress, PSB-D)**
  - *Nicotiana tabacum* (Tobacco, BY-2)



PSB-D

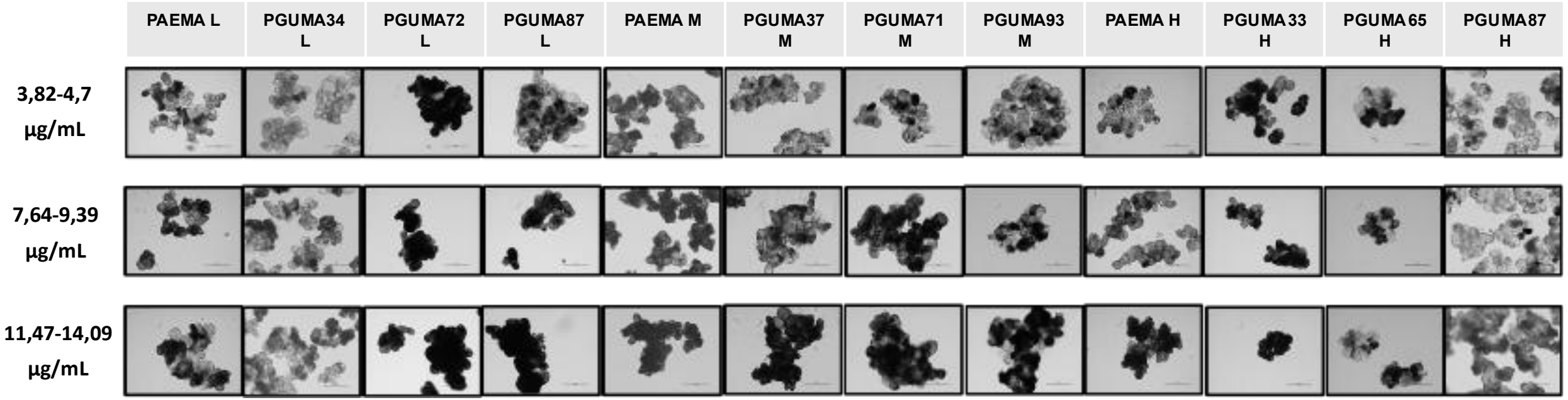


BY-2

- Conditions
  - 33 333 cells/cm^2
  - Increasing polymer concentrations corresponding to N/P ratios 2.0, 4.0 and 6.0

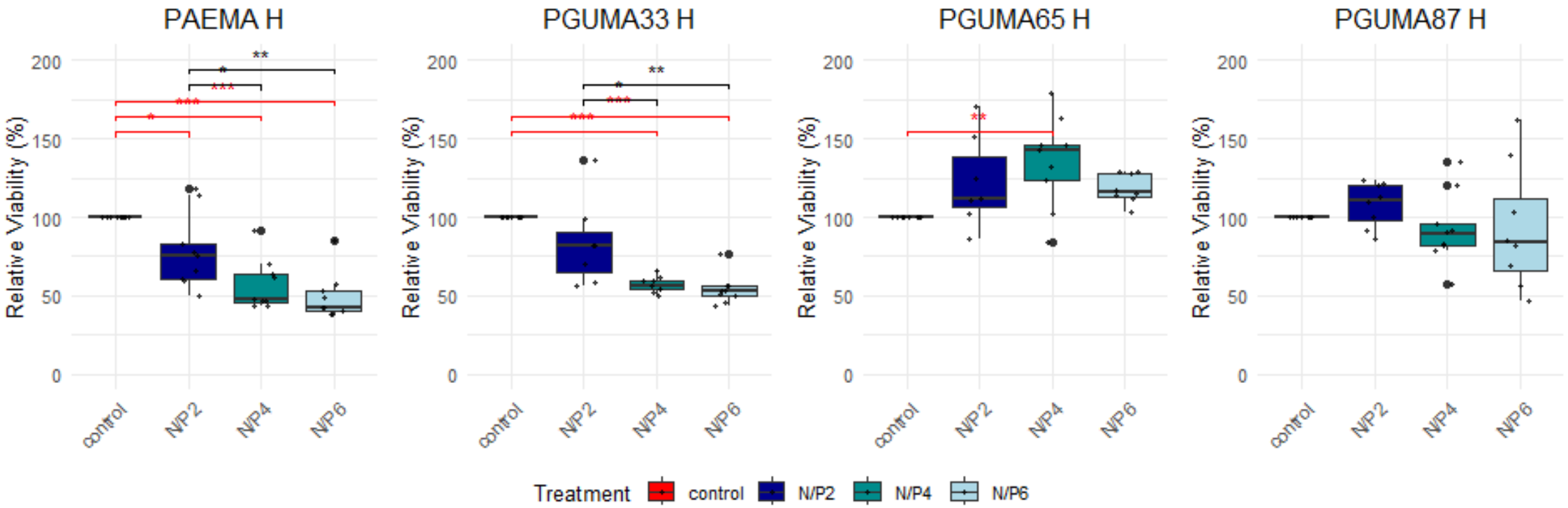
Cytotoxicity evaluation of polymers – PSB-D

- Dead cells stained with Trypan Blue



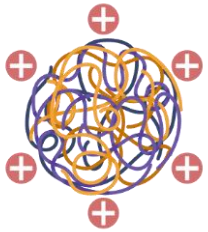
- Aggregation visualized under BF microscopy

Cytotoxicity evaluation of polymers – LD_{50}

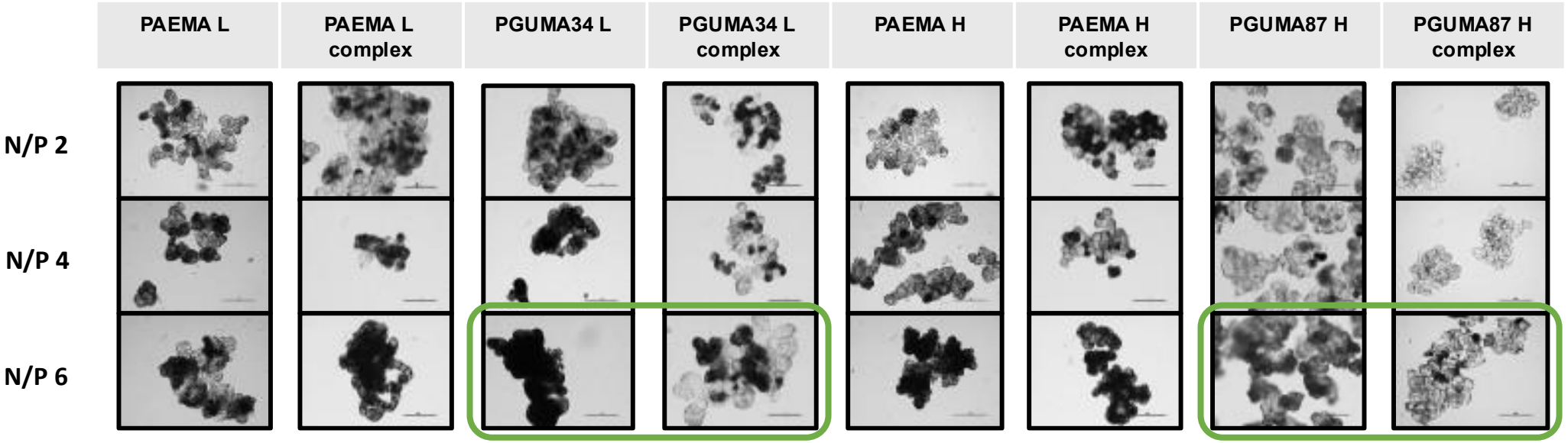


- Higher guanidinylation degree results in higher LD_{50} value

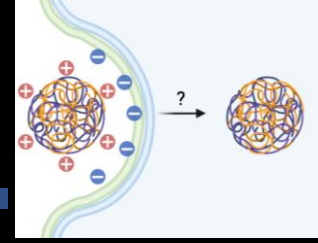
Cytotoxicity evaluation of polyplexes



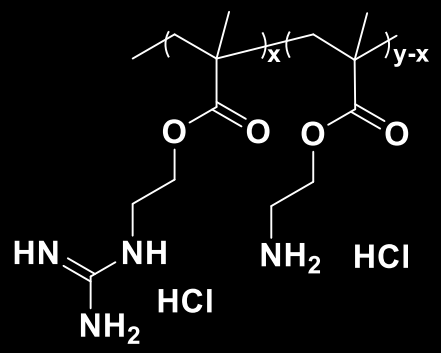
- Polymers combined with 300 bp GFP fragments
  - Increased LD_{50} values compared to naked polymers
  - High DS beneficial

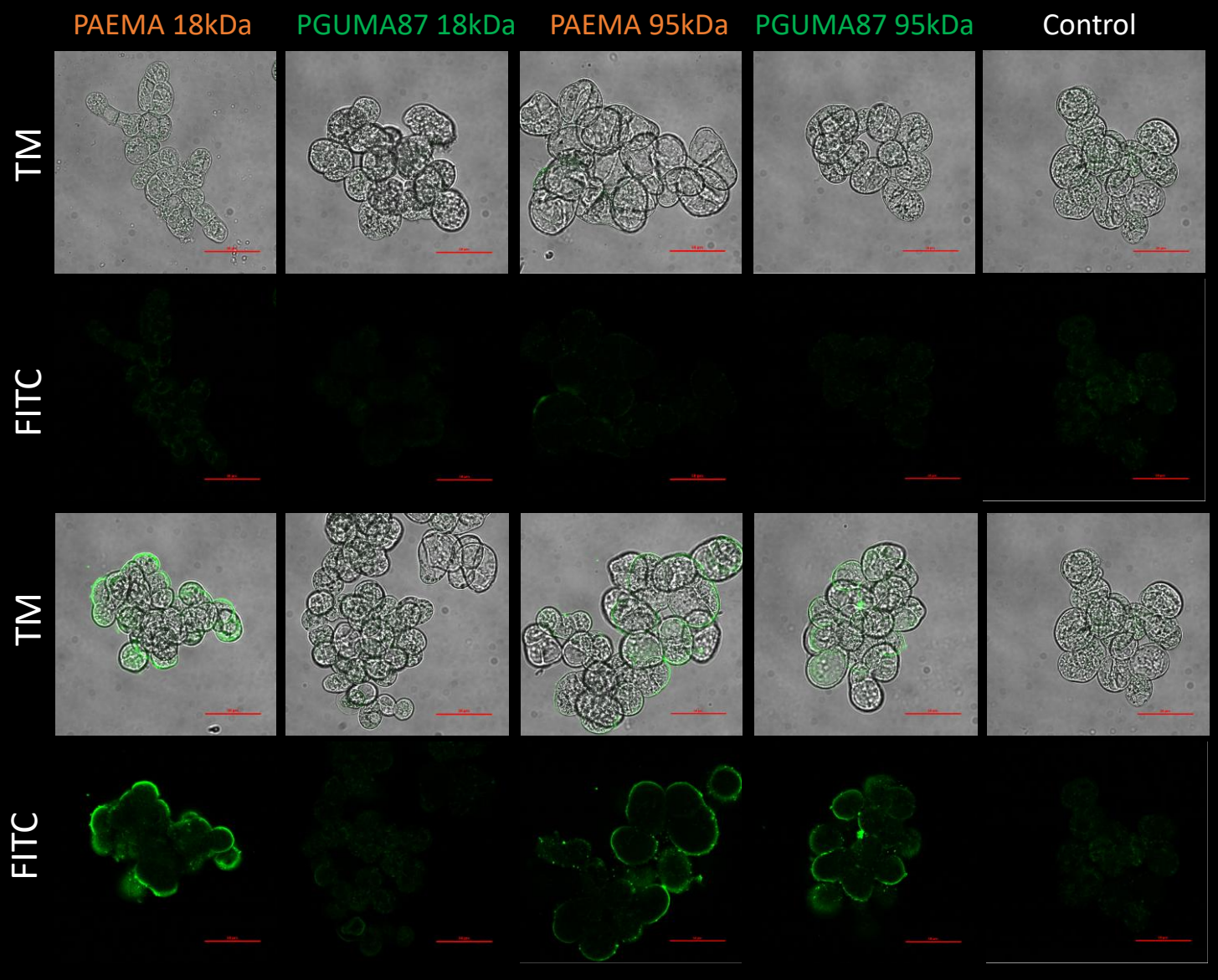


Cellular uptake – PSB-D



33 333 cells/cm^2
1h incubation time
Polymers labelled with FITC





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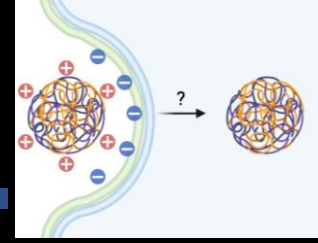
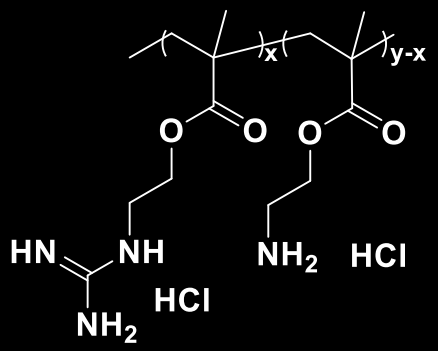
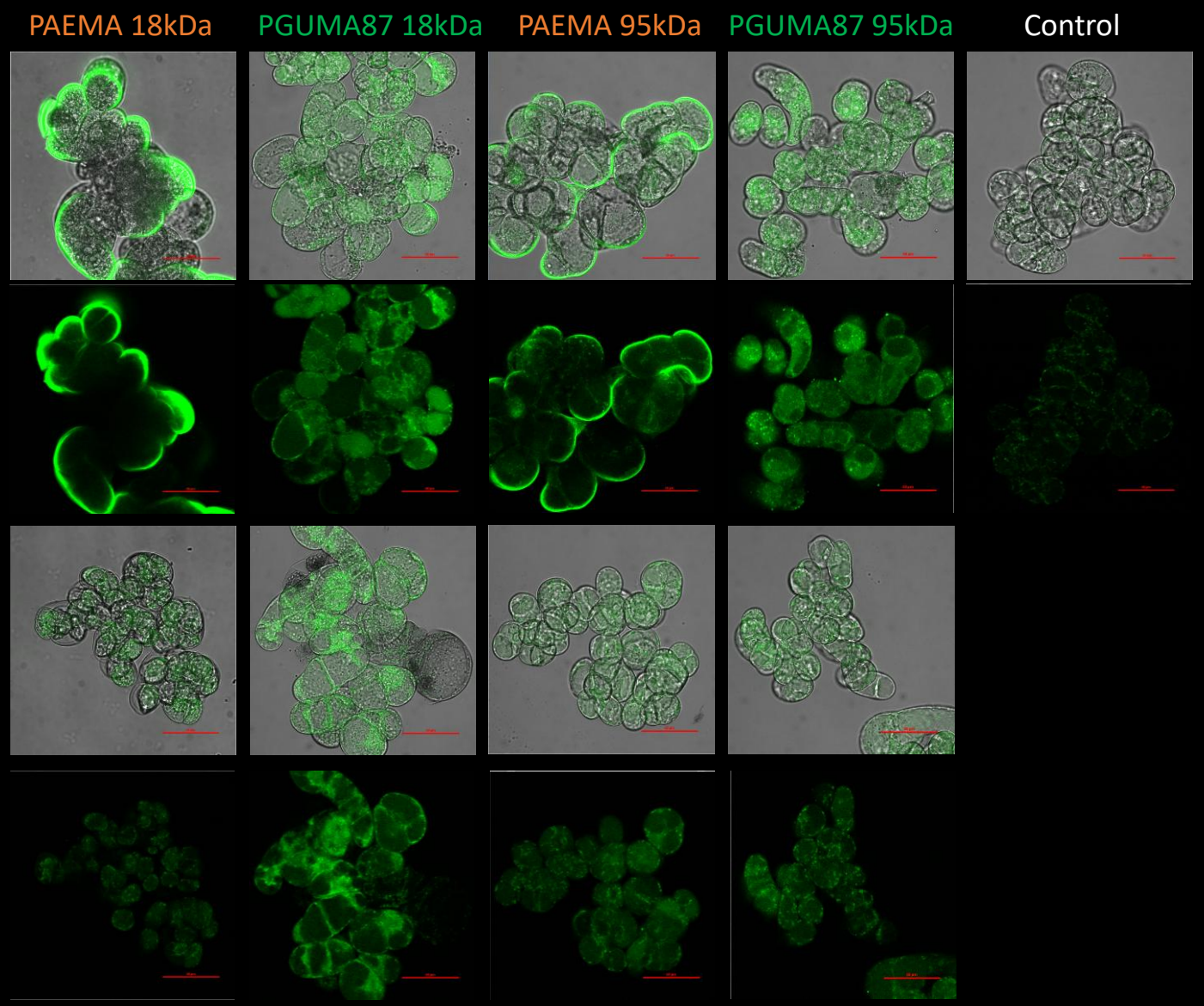
Improving cellular uptake using Silwet L-77



33 333 cells/cm^2
1h incubation time
Polymers labelled with FITC
N/P of 4.0



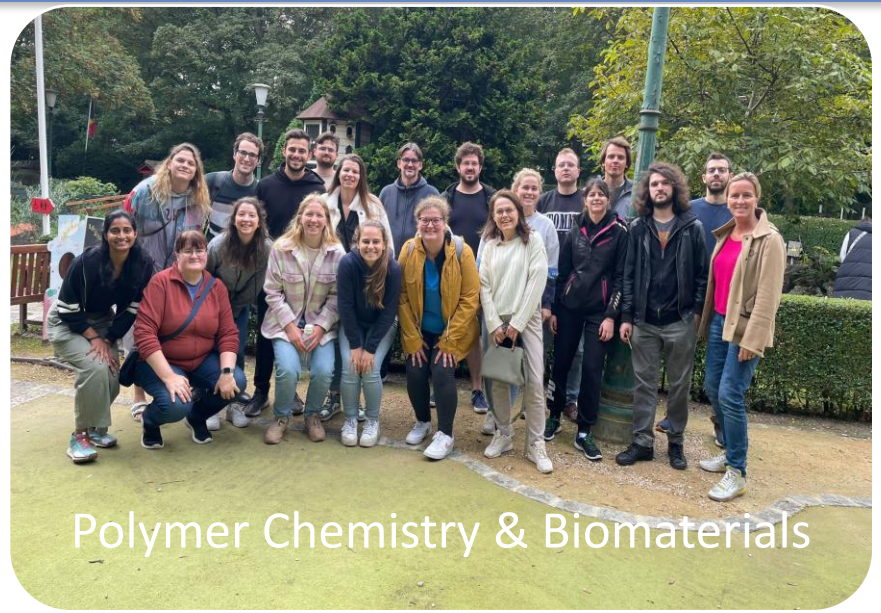


Conclusion and future perspectives

- ✓First steps taken towards sustainable pest control delivery system
  - ✓Small polymer library was built to investigate influence of MM and DS
  - ✓Higher guanidinylation results in decreased cytotoxicity and increased uptake
  - ✓Polyplexes exhibit lower toxicity compared to polymers as such
  - ✓Polyplexes are efficiently taken up by combination with Silwet L-77

- Screening of undesired effects to beneficial insects
- Investigate RNAi response in plant cells
- Development of biodegradable alternatives

Acknowledgements



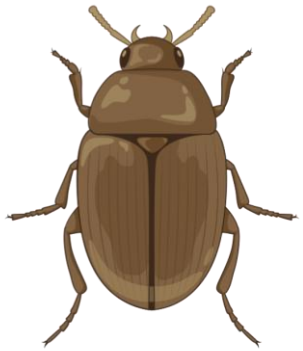
Biochemistry & Glycobiology

GOA consortium

Prof. Dr. Peter Dubruel

Prof. Dr. Els Van Damme
Ellen Vandenbussche

Prof. Dr. Kristof De Schutter



Molecular Entomology

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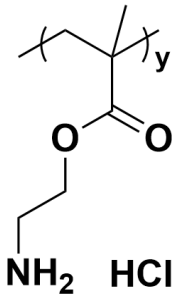

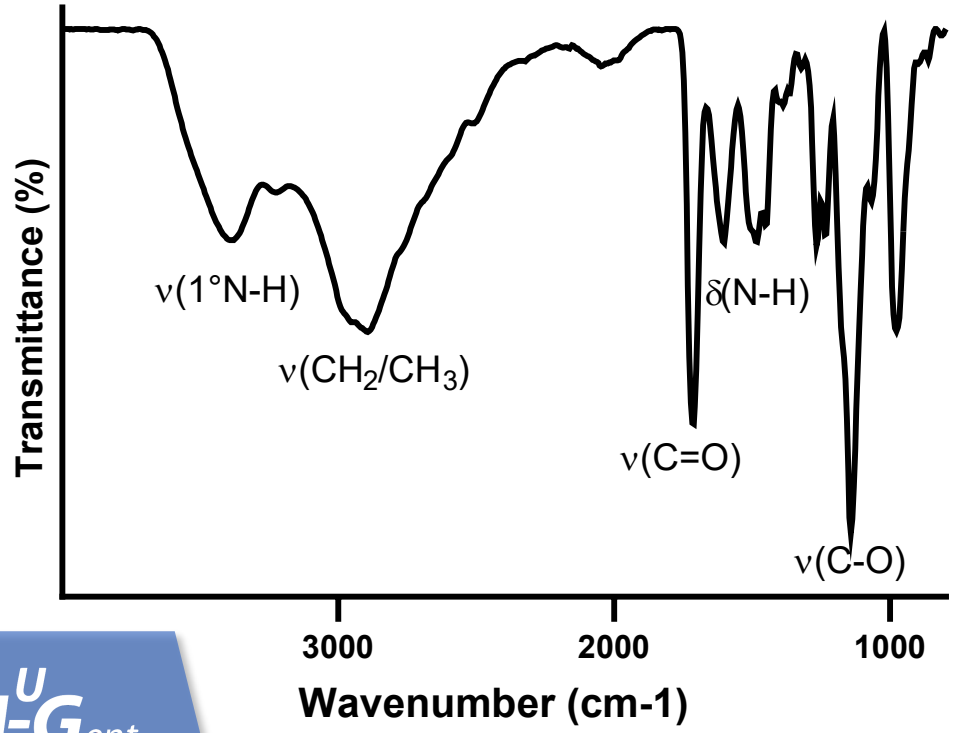

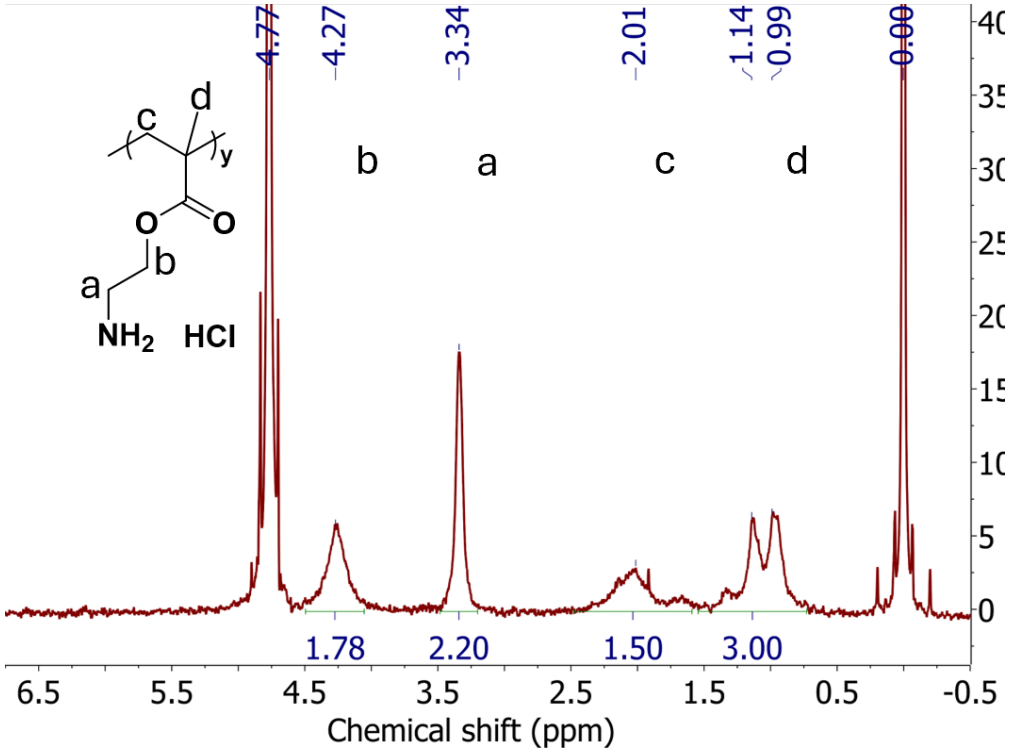

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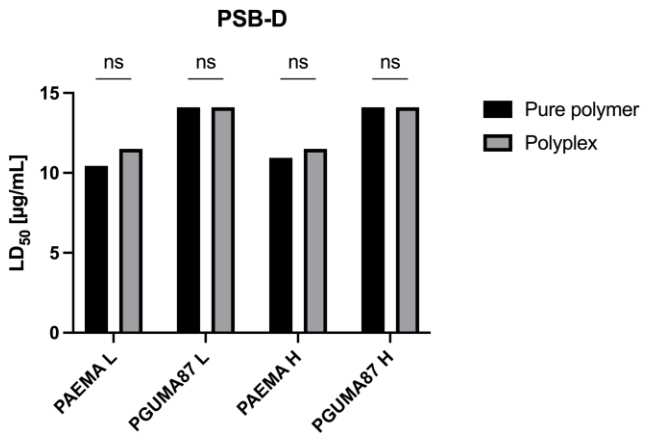
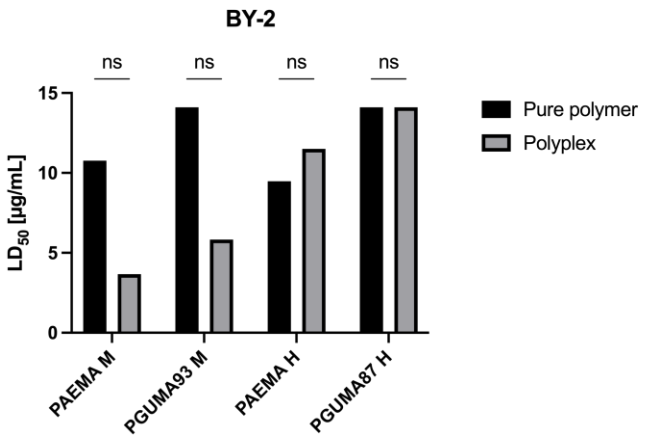
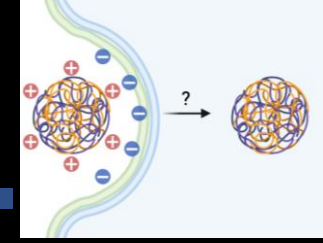
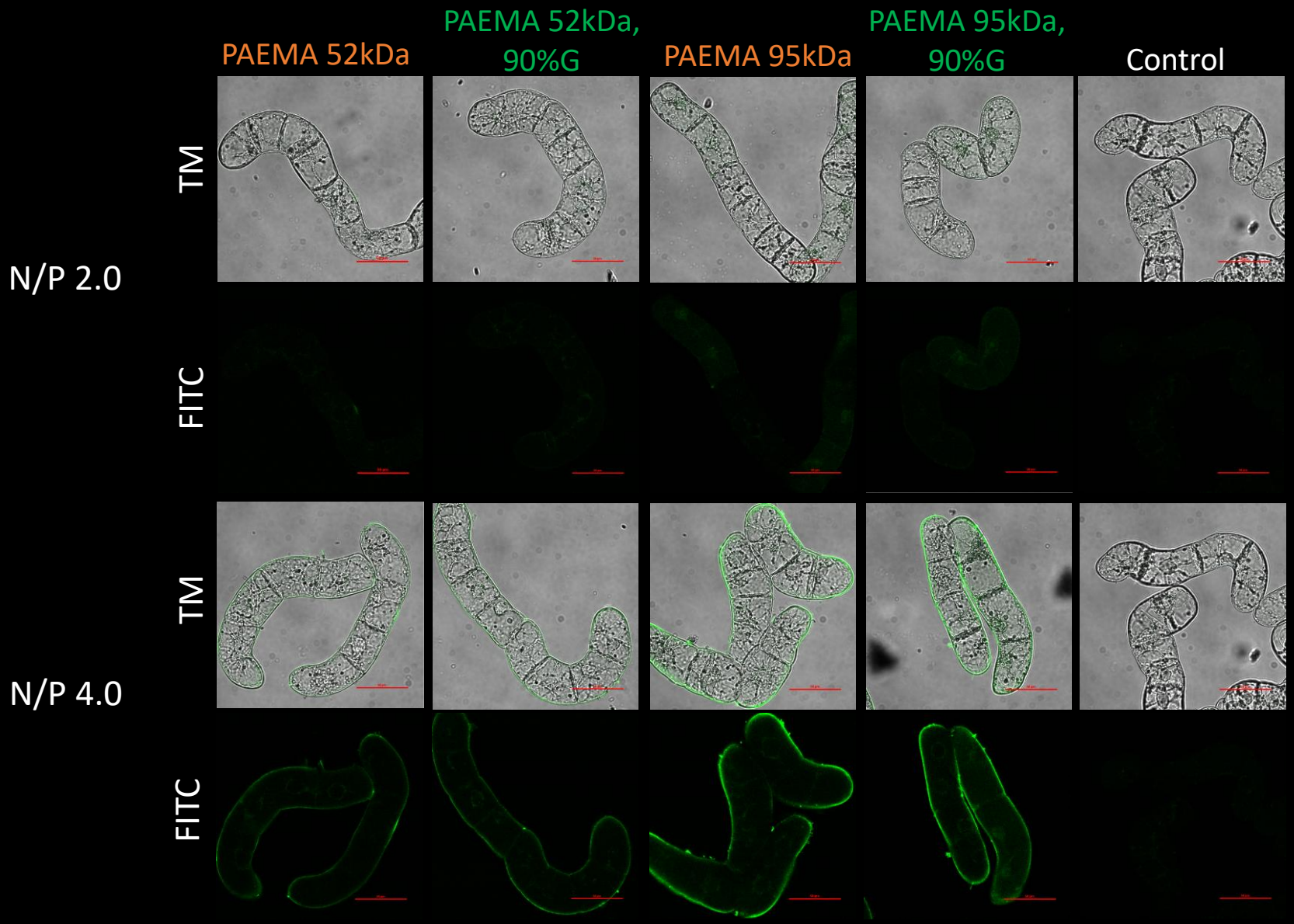
Cytotoxicity evaluation of polyplexes – LD_{50}





Cellular uptake – BY-2





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1h incubation time

Polymers labelled with FITC

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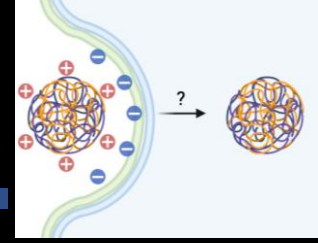

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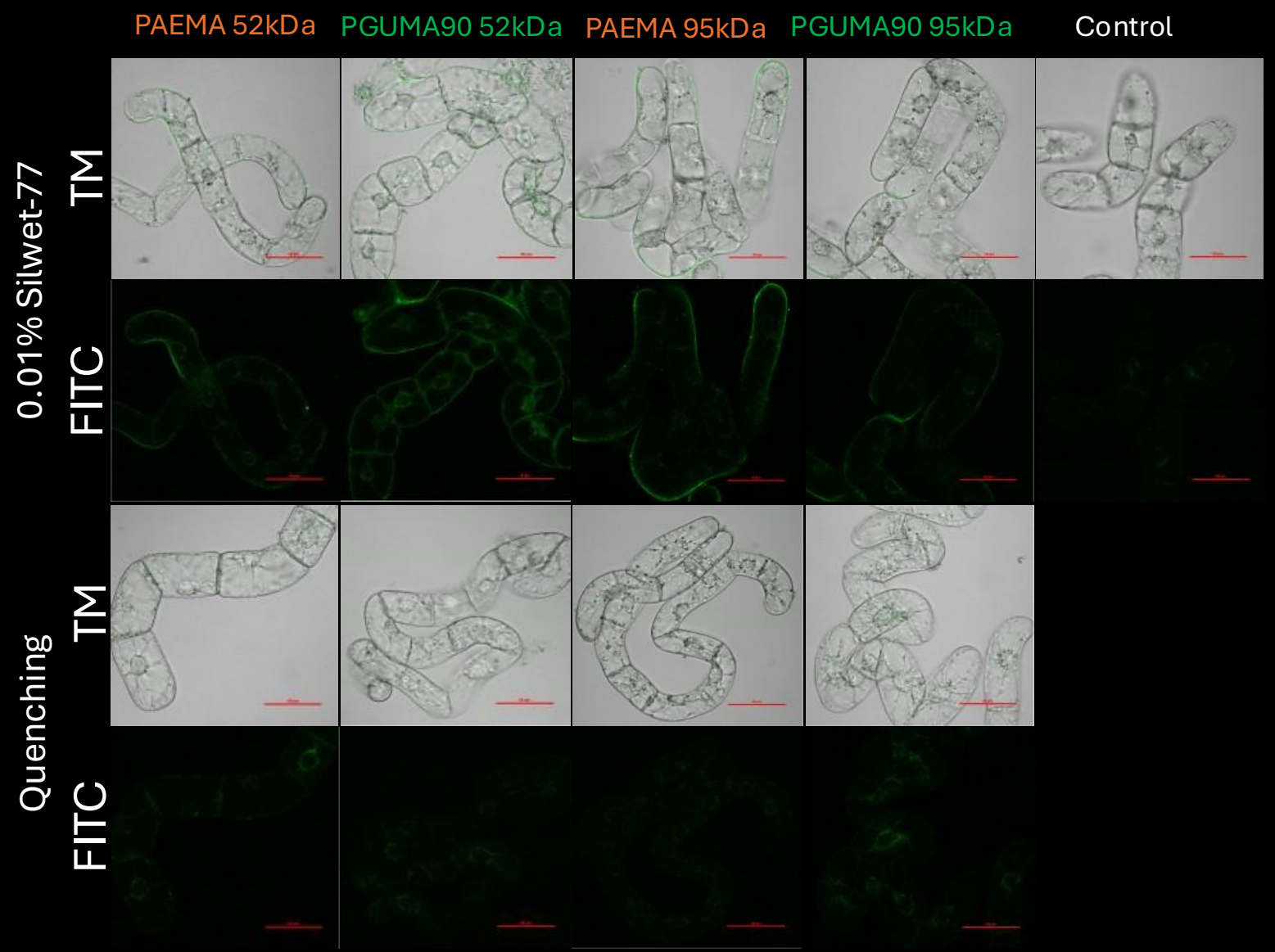

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X