1

From Antisense Oligonucleotide Degradation to Ocular mRNA Delivery: How the Journal of Controlled Release Shaped My Academic Journey

Katrien Remaut

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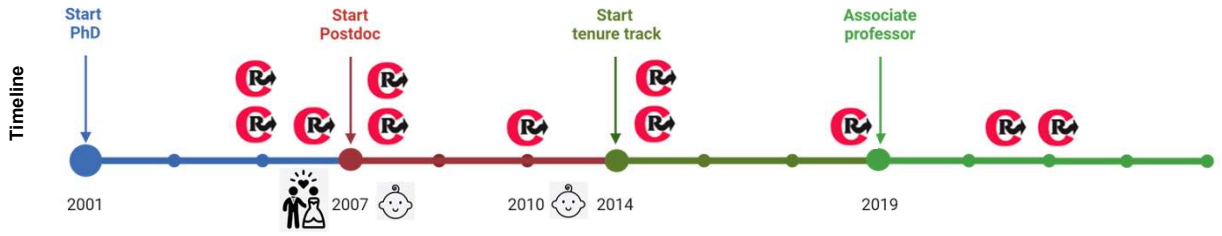
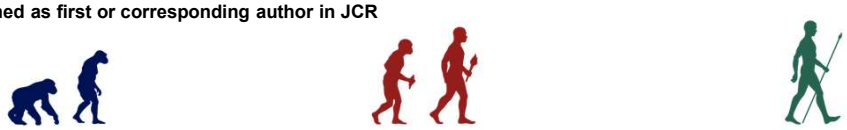
My academic journey from PhD student to postdoc



Keywords			
Antisense oligonucleotides	Plasmid DNA	Messenger RNA	
Degradation	Nuclear entry	Degradation	Degradation	Ocular delivery
Nanoparticles	Nanoparticles	Autophagy	Lipid Nanoparticles	Immunogenicity
FCS	FCS	FCS	Peritoneal carcinomatosis

Paper published as first or corresponding author in JCR



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Phase 1: Antisense oligonucleotides – degradation – FCS - nanoparticles

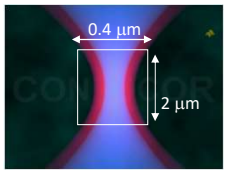
Journal of Controlled Release
Volume 103, Issue 1, 2 March 2005, Pages 259-271

FRET-FCS as a tool to evaluate the stability of oligonucleotide drugs after intracellular delivery

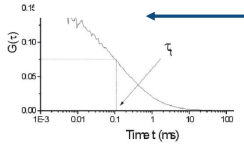
K. Remaut, B. Lucas, K. Braeckmans, N.N. Sanders, S.C. De Smedt, J. Demeester

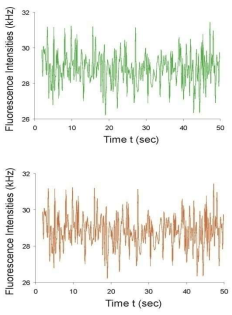
FCS: Fluorescence Correlation Spectroscopy

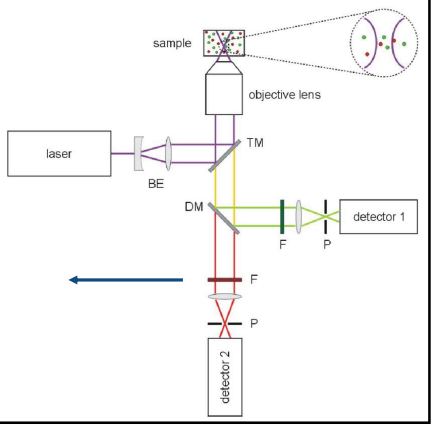
Experimental setup



- **Concentration** of fluorescently labeled molecules
- **Diffusion** coefficient
- **Encapsulation** efficiency
- **Release** ifo time or ≠ environment
- **Degradation** of nucleic acids







SINGLE COLOR DUAL COLOR

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Phase 1: Antisense oligonucleotides – degradation – FCS - nanoparticles

Journal of Controlled Release
Volume 103, Issue 1, 2 March 2005, Pages 259-271

FRET-FCS as a tool to evaluate the stability of oligonucleotide drugs after intracellular delivery

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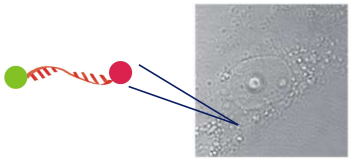

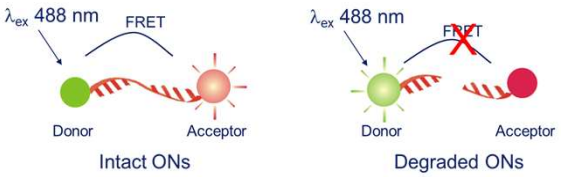

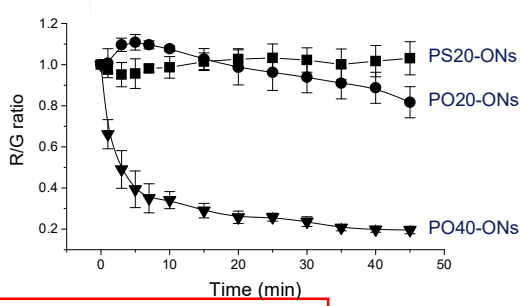

R/G ratio ~ amount of intact ONs → measure degradation ifo time

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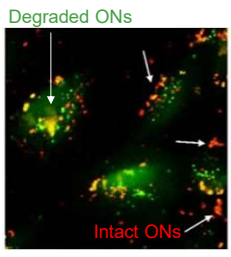
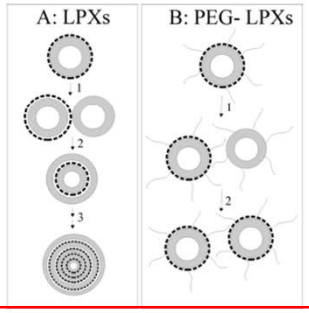
Phase 1: Antisense oligonucleotides – degradation – FCS - nanoparticles

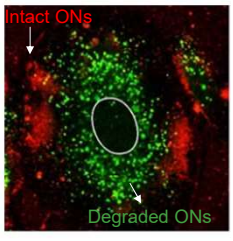
Journal of Controlled Release
Volume 110, Issue 1, 10 December 2005, Pages 212-226

Protection of oligonucleotides against nucleases by pegylated and non-pegylated liposomes as studied by fluorescence correlation spectroscopy

K. Remaut, B. Lucas, K. Braeckmans, N.N. Sanders, J. Demeester, S.C. De Smedt







Journal of Controlled Release
Volume 117, Issue 2, 12 February 2007, Pages 256-266

Pegylation of liposomes favours the endosomal degradation of the delivered phosphodiester oligonucleotides

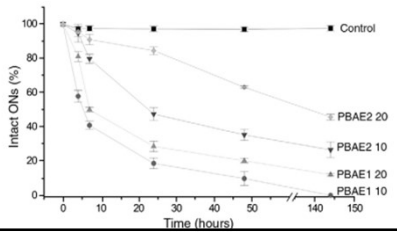
K. Remaut, B. Lucas, K. Braeckmans, J. Demeester, S.C. De Smedt

Encapsulation efficiency and protection against degradation by lipid- and polymer based nanoparticles

Journal of Controlled Release
Volume 144, Issue 1, 21 May 2010, Pages 65-74

Efficient delivery of intact phosphodiester oligonucleotides by poly-β-amino esters

K. Remaut, N. Symens, B. Lucas, J. Demeester, S.C. De Smedt



Yes!

Journal of Controlled Release
Volume 121, Issues 1–2, 16 August 2007, Pages 49-63

Can we better understand the intracellular behavior of DNA nanoparticles by fluorescence correlation spectroscopy?

K. Remaut, B. Lucas, K. Raemdonck, K. Braeckmans, J. Demeester, S.C. De Smedt

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Phase 2: Plasmid DNA – nuclear entry – autophagy - degradation – FCS - nanoparticles

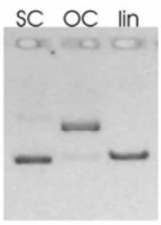
Start Postdoc — Start tenure track

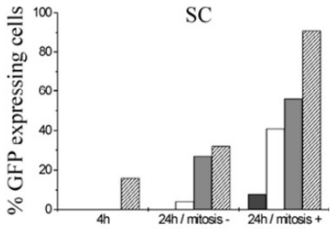
2007 — 2010 — 2014

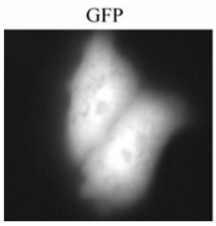
Journal of Controlled Release
Volume 115, Issue 3, 27 October 2006, Pages 335-343

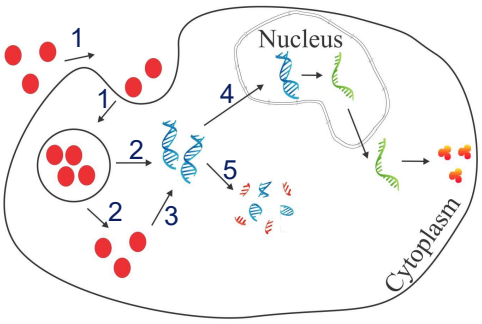
Influence of plasmid DNA topology on the transfection properties of DOTAP/DOPE lipoplexes

Katrien Remaut, Niek N. Sanders, Farzaneh Fayazpour, Jozef Demeester, Stefaan C. De Smedt









1. Internalization
2. Endosomal escape
3. Dissociation
4. Nuclear entry
5. Degradation

Nuclear delivery of plasmid DNA most efficient for SC pDNA and during cell division

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Phase 2: Plasmid DNA – nuclear entry – autophagy - degradation – FCS - nanoparticles

Start Postdoc — Start tenure track

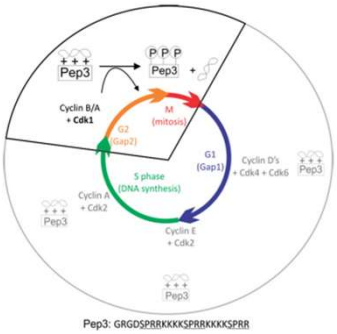
2007 — 2010 — 2014

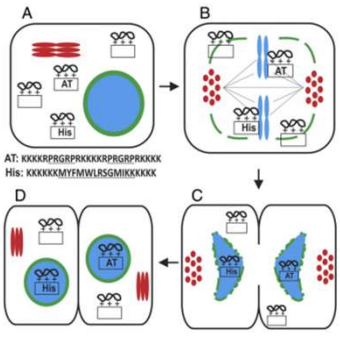
Journal of Controlled Release
Volume 179, 10 April 2014, Pages 1-9

Cell division responsive peptides for optimized plasmid DNA delivery: The mitotic window of opportunity?

K. Remaut, N. Symens, B. Lucas, J. Demeester, S.C. De Smedt

No, but…





Journal of Controlled Release
Volume 179, 10 April 2014, Page 76

Cover story

The mitotic window of opportunity for plasmid DNA delivery

Kinam Park

The difficulties described in the study by Remaut et al. provide valuable lessons. Although the approach is sound, the results do not match the expectations. This means that there are many other factors that the authors did not consider, or the assumptions taken may not be the right ones. While the experiments need to be done by simplifying complex problems into an experimentally manageable one, such a process may unintentionally delete yet-unknown critical factors. Advancing science takes time, and requires unraveling the complexity of the intracellular environment step by step. The result of the study by Remaut et al. made one small step forward in knowledge, but the authors' attempt to describe the difficulties and problems made one large leap forward. It is time for many of us to stop highlighting only a marginal improvement, and discussing the real challenges to define the problems more clearly so that they can be solved.

Two strategies tested to enhance pDNA delivery during cell division

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Phase 2: Plasmid DNA – nuclear entry – autophagy - degradation – FCS - nanoparticles

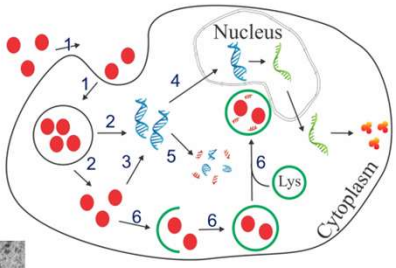
Start Postdoc — Start tenure track

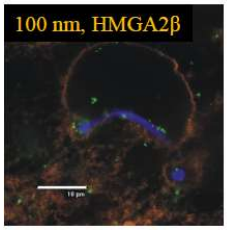
2007 — 2010 — 2014

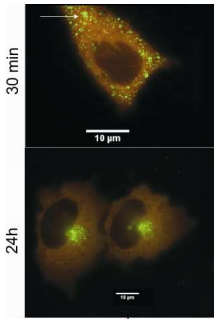
Journal of Controlled Release
Volume 195, 10 December 2014, Pages 29-36

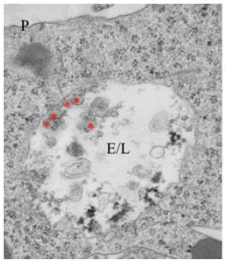
Lysosomal capturing of cytoplasmic injected nanoparticles by autophagy: An additional barrier to non viral gene delivery

Katrien Remaut [a], Viola Oorschot [b], Kevin Braeckmans [a], Judith Klumperman [b], Stefaan C. De Smedt [a]









1. Internalization
2. Endosomal escape
3. Dissociation
4. Nuclear entry
5. Degradation
6. **Autophagy**

Chromatin targeting in articifal nuclei, but not upon microinjection in living cells → autophagy!

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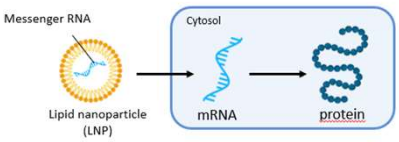
Phase 3: mRNA – degradation – FCS – lipid nanoparticles – ocular delivery - immunogenicity

Journal of Controlled Release
Volume 307, 10 August 2019, Pages 315-330

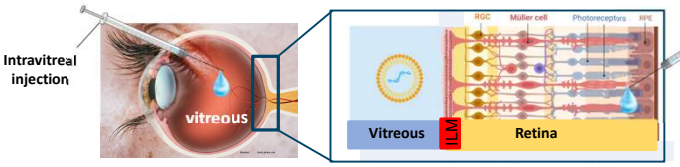
Non-viral delivery of chemically modified mRNA to the retina: Subretinal *versus* intravitreal administration

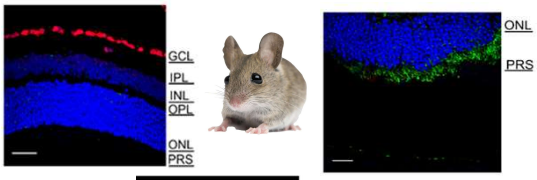
Jake Devoldere [a], Karen Peynshaert [a], Heleen Dewitte [a b c], Christian Vanhove [d], Lies De Groef [e], Lieve Moons [e], Sinem Yilmaz Özcan [f], Deniz Dalkara [g], Stefaan C. De Smedt [a c], Katrien Remaut [a c]

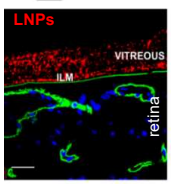


Start tenure track — Associate professor

2014 — 2019







Title: Non-viral delivery of chemically modified mRNA to induce protein expression in the retina
Journal of Controlled Release

Dear Dr Remaut,

Thank you for your interest in the Journal of Controlled Release. We have recently received two reviews of your manuscript.

Unfortunately, both of the reviewers felt that the manuscript was unsuitable for publication. Because of this ruling, and the large amount of manuscripts the journal receives, we regret to inform you that the Journal of Controlled Release is unable to publish your manuscript.

You may, however, consider resubmitting the work after full revision.

Inner limiting membrane is large barrier for intravitreal injected nanoparticles
Initial JCR rejection helped bringing the science to a better level

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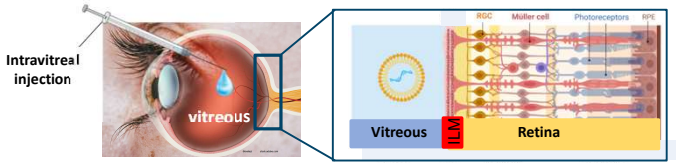
Phase 3: mRNA – degradation – FCS – lipid nanoparticles – ocular delivery - immunogenicity

Journal of Controlled Release
Volume 349, September 2022, Pages 315-326

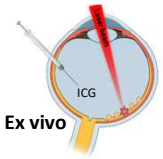
ICG-mediated photodisruption of the inner limiting membrane enhances retinal drug delivery

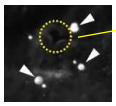
Karen Peynshaert [a,b], Helena Vanluchene [a,b], Koat De Clerck [a,b], An-Katrien Minnaert [a,b], Morgane Verhoeven [b], Noémie Gouspillou [c], Nezahat Bostan [d], Toshio Hisatomi [e], Geraldine Accou [f], Félix Sauvage [a,b], Kevin Braeckmans [a,b], Stefaan De Smedt [a,b], Katrien Remaut [a,b]

Start tenure track 2014 — Associate professor 2019

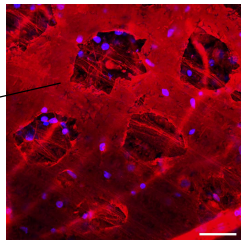


ILM photoablation

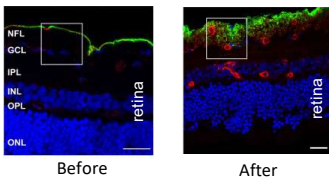




ILM pore formation



LNP
ILM



Before — After

Laser based strategy can make pores in the ILM to improve drug delivery

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Conclusion

- ☑ JCR has shaped my contributions to science from PhD student to associate professor
- ☑ Reference journal for drug delivery and related technology
- ☑ I'm happy to contribute as
  - author (21% of my manuscripts are in JCR, responsible for 24% of my citations)
  - reviewer (60 completed assignments so far)
  - editorial board member

Katrien F. Remaut, PhD
Ghent University, Gent, Belgium
Non-viral gene delivery, mRNA, ocular administration, peritoneal carcinomatosis

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Acknowledgements

GHENT UNIVERSITY

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Stefaan De Smedt
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Ragna Haegebaert

Jo Demeester

Karen Peynshaert

Lab. General Biochemistry & Physical Pharmacy

www.drugdelivery.be

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