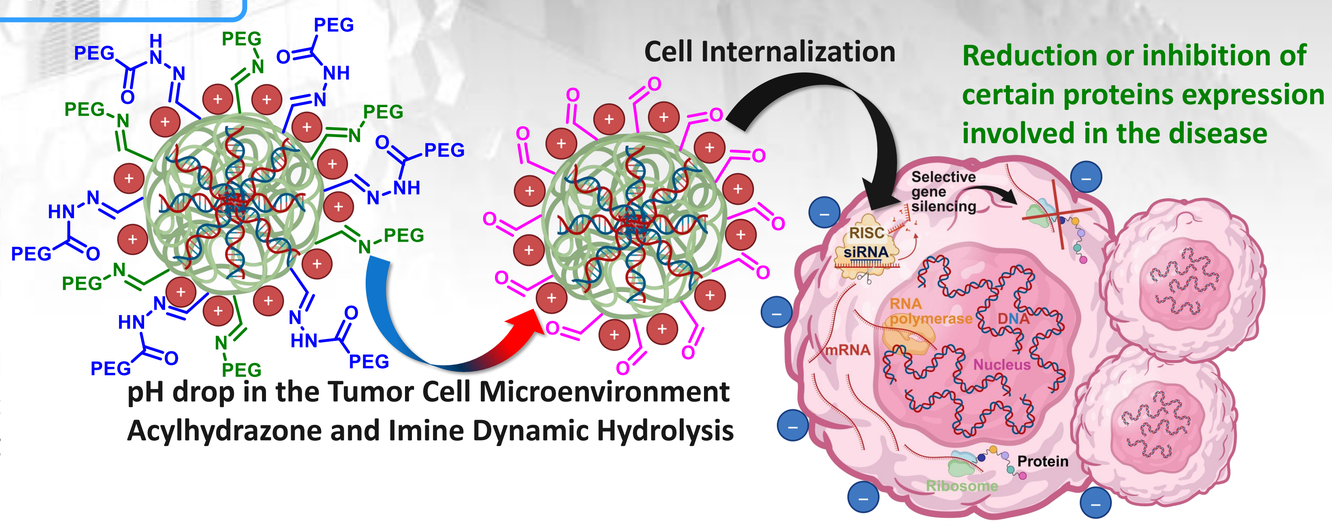
Dynamic PEGylation of Peptide-based Nanoparticles for siRNA Delivery

Jules Vastra[a], **Thania Hammoum**[b], **Prisca Boisguérin**[b], **Sébastien Ulrich**[a]
[a] *IBMM, Université de Montpellier, CNRS, ENSCM, Montpellier, France*
[b] *PhyMedExp, Université de Montpellier, INSERM, CNRS, Montpellier, France*
E-mail : jules.vastra@etu.umontpellier.fr
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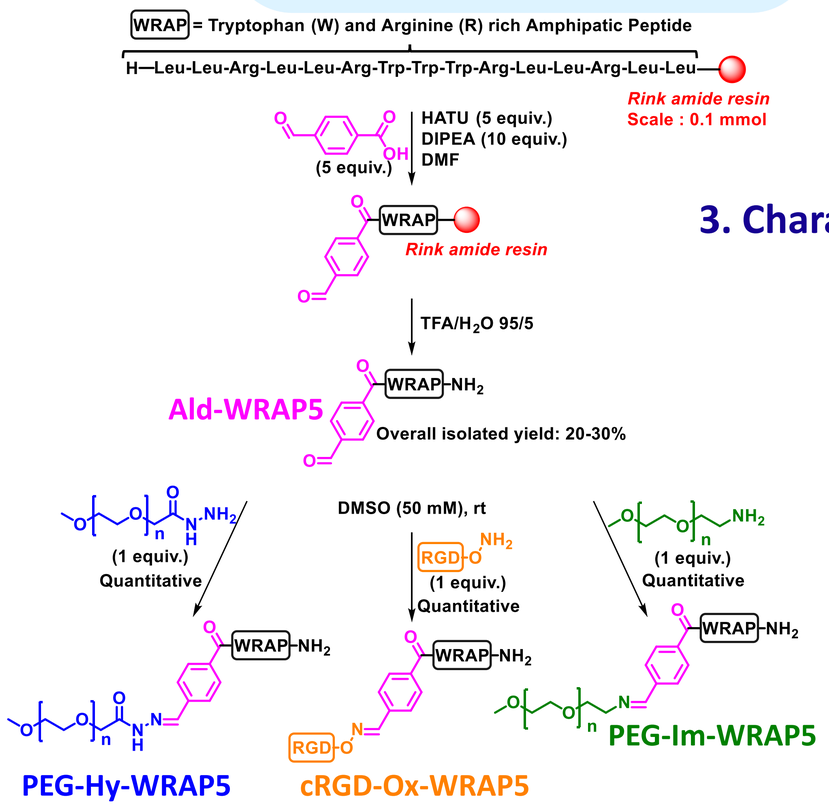
Project Motivation

Despite recent successes in the design of effective nucleic acid vectors enabling to reach therapeutic applications,[1,2] there are still huge opportunities for improvement since only less than 5% of the RNA payload actually ends up being released by the nanoparticles.[3] Although PEGylation of nanoparticles is a general strategy to improve the circulation time *in vivo*, this shielding is often detrimental to cell uptake and nucleic acid release, which calls for a dynamic PEGylation approach.[4] In this context, we recently reported on a Tryptophan and Arginine rich Amphipatic Peptide (WRAP), previously shown to effectively complex and deliver siRNA,[5] coupled to a PEG 2000 via an acylhydrazone linkage, and undergoing a pH-controlled dePEGylation.[6] Although this work set a nice proof-of-concept, the kinetics and efficacy of dePEGylation were unfortunately not fitted for a real application, with half-lives at pH 4 of 2 to 8 hours. In this presentation, we will describe a **new approach combining multiple dynamic linkages in order to better tune the dePEGylation process**.

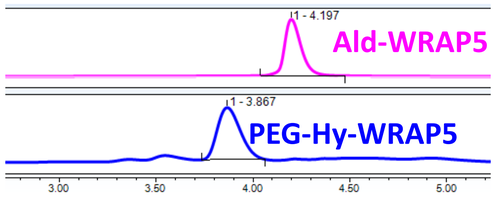


Design, Synthesis and Characterization of Acylhydrazone and Imine PEG-WRAP5 Conjugates

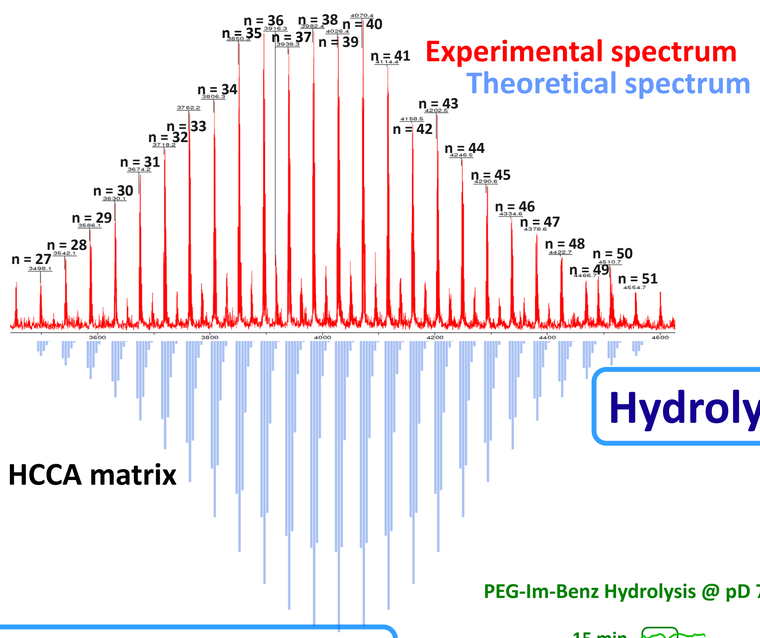
1. Design and Synthesis



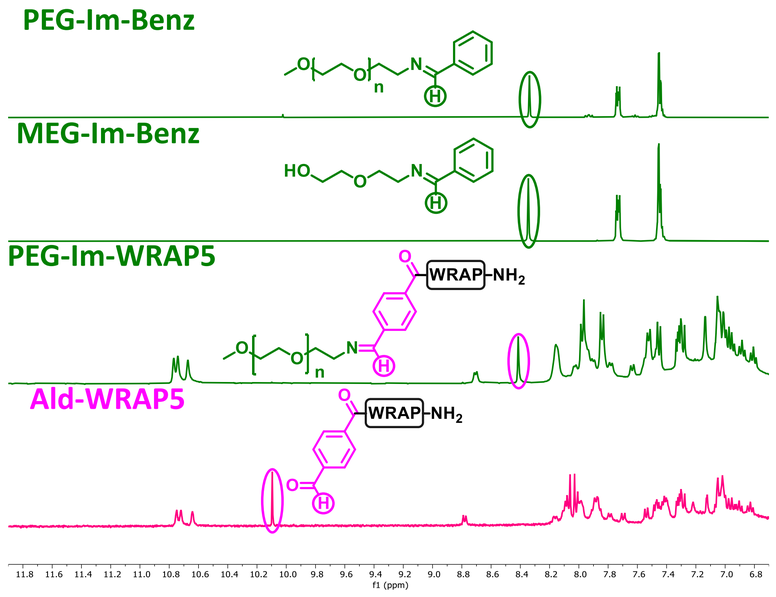
2. HPLC Reaction Monitoring



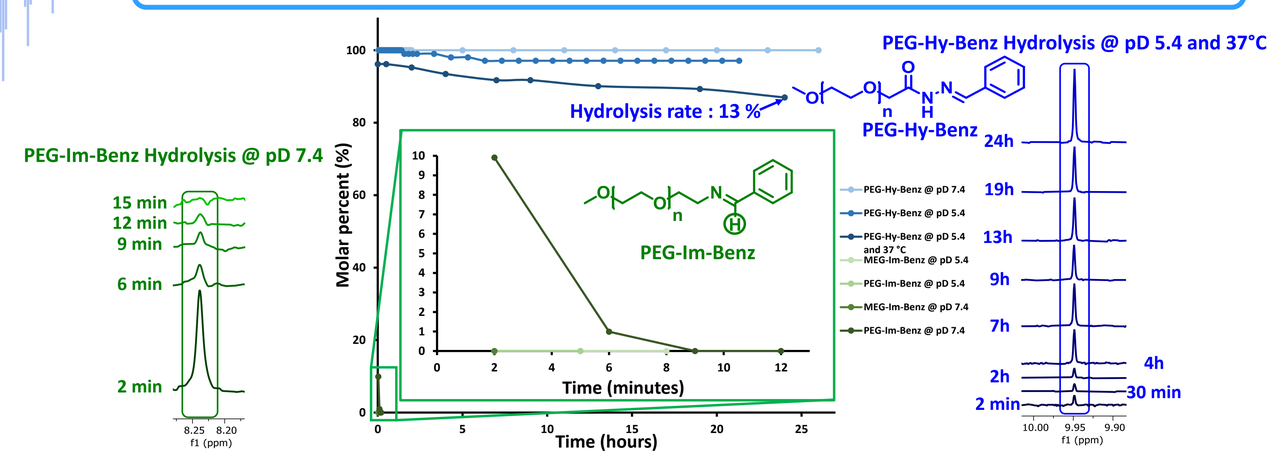
3. Characterization of PEG-Hy-WRAP5 by MALDI-MS



4. ^{1}H NMR of Imine Conjugates



Hydrolysis Kinetics of Acylhydrazone and Imine Bonds

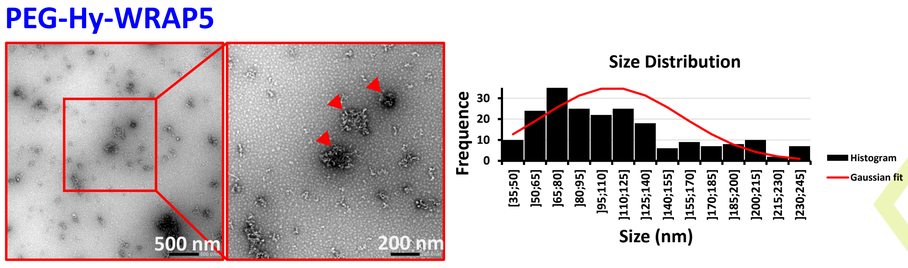


Nanoparticle Characterization by TEM, DLS and ζ Potential

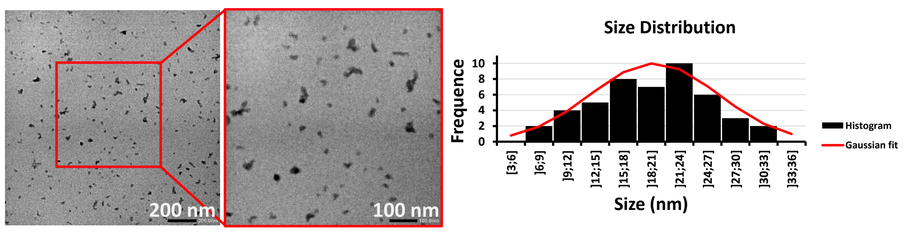
Formulation conditions:
Conjugate/siRNA 20/1 in H_2O/EtOH 80/20
5% glucose (m/V) for DLS and ζ potential and NaCl 1 mM for ζ potential

Nanoparticles	Ald-WRAP5	PEG-Hy-WRAP5	PEG-Im-WRAP5
Size Intensity (nm)	124.9 ± 1.3	162.1 ± 2.3	164.5 ± 4.4
PdI	0.244 ± 0.012	0.303 ± 0.038	0.179 ± 0.010
Size Number (nm)	44.2 ± 22.6	80.9 ± 12.6	29.3 ± 5.5
ζ potential (mV)	+ 20.8 ± 2.1	+ 25.1 ± 5.7	+ 43.3 ± 2.5

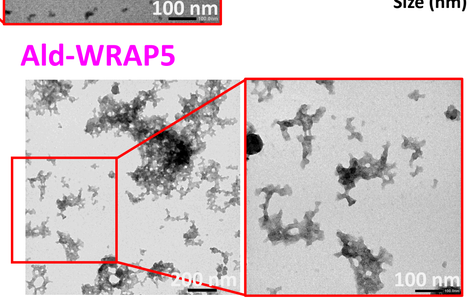
PEG-Hy-WRAP5



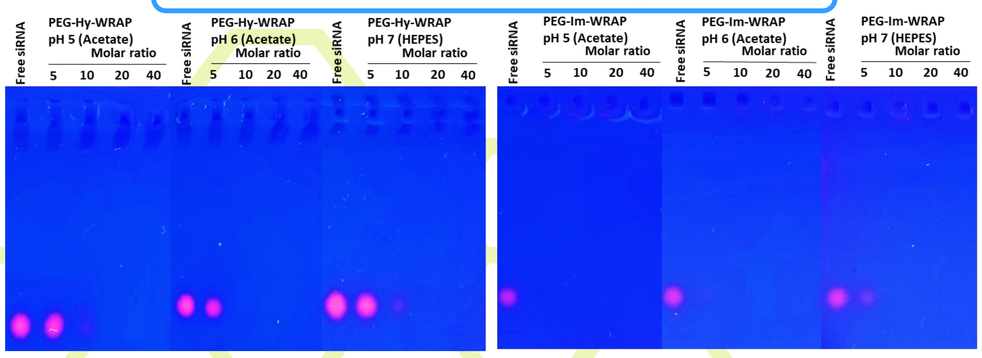
PEG-Im-WRAP5



Ald-WRAP5

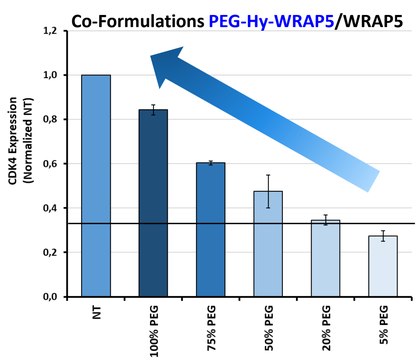


Evidence of siRNA Complexation

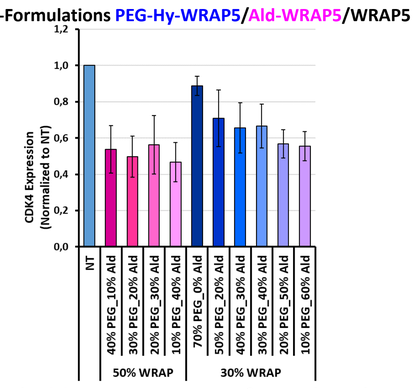


Biological Activity in GIST-T1 Cells

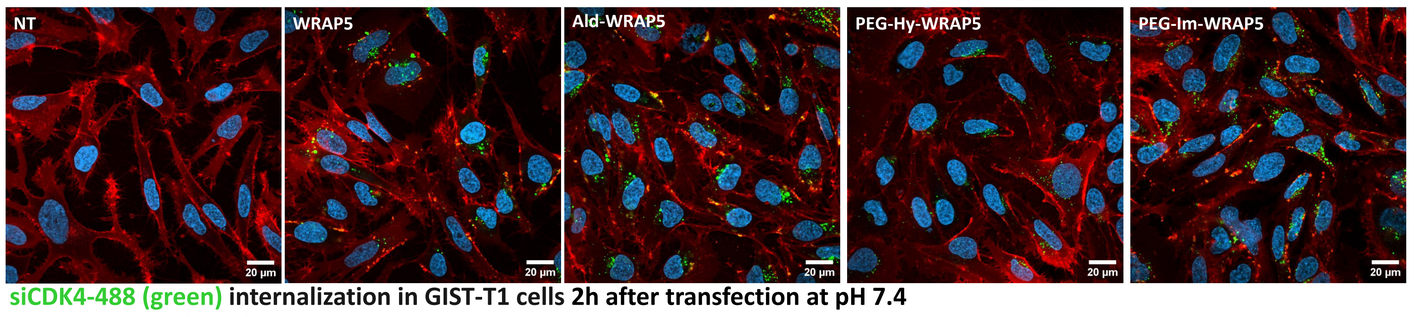
Shielding Effect of PEGylation



Potential of dePEGylation



Retention of activity when WRAP5 and Ald-WRAP5 are co-formulated



siCDK4-488 (green) internalization in GIST-T1 cells 2h after transfection at pH 7.4
Nuclei Hoechst (cyan)
Cytoskeletons phalloidin (red)

Conclusions and Outlook

- Effective **diversification** of Ald-WRAP5 using unique conditions to synthesize conjugates featuring distinct **acylhydrazone**, **imine** and **oxime** linkages, exhibiting **different hydrolysis stabilities**
- Significant **shielding effect** observed when nanoparticles are PEGylated and **retention of activity** when **WRAP5** and Ald-WRAP5 are **co-formulated**

Perspectives:

- New co-formulations containing **PEG-Hy-WRAP5** and **PEG-Im-WRAP5**

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