Click'n ROPISA: Functional Polypeptidic Nanoparticles

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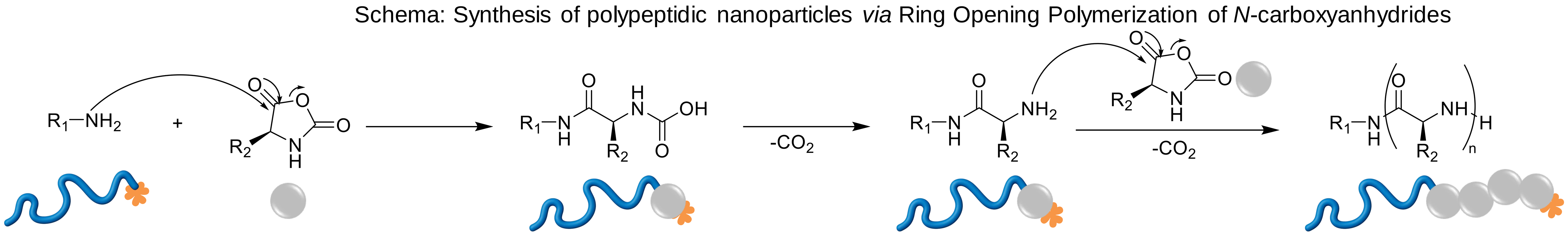
Introduction Polypeptide-based nanoparticles have a wide range of applications, such as drug delivery, biosensing and bioimaging, due to their inherent biocompatibility and biodegradability.[1] These nanoparticles are typically synthesized from amphiphilic copolymers *via* the ring-opening polymerization (ROP) of *N*-carboxyanhydrides (NCAs).[2]

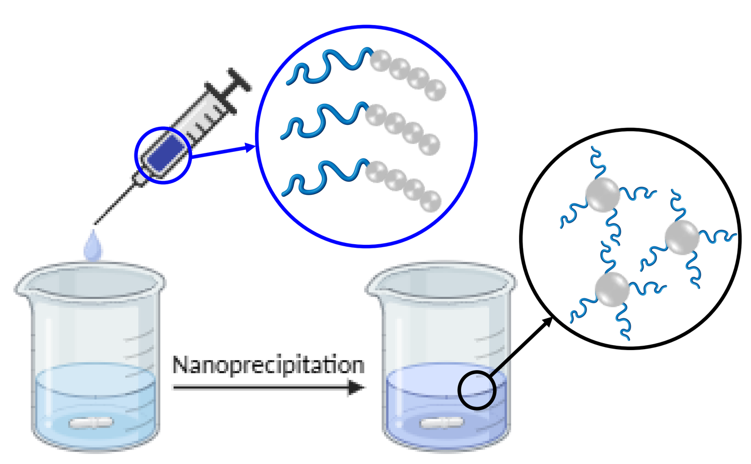
Schema: Synthesis of polypeptidic nanoparticles *via* Ring Opening Polymerization of *N*-carboxyanhydrides

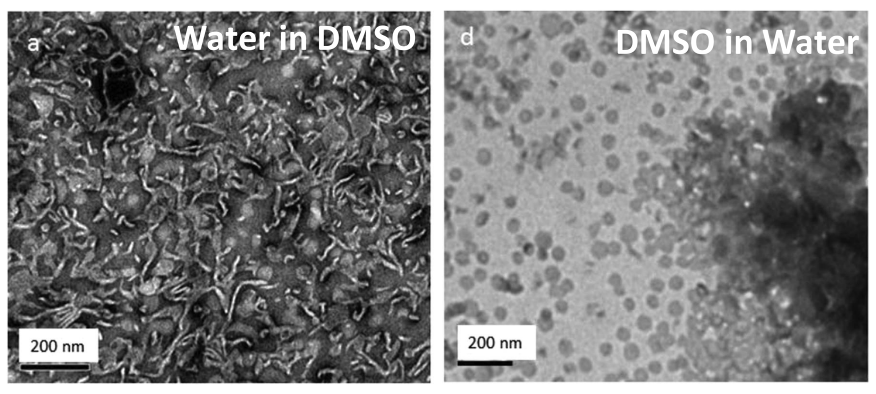


R_1-Hydrophilic
R_2-Hydrophobic

- ✓ Scalable
- ✓ Economical method
- ✓ High molecular weight
- ✓ Polypeptide backbone
- ✓ Biodegradable and biocompatible

These nanoparticles are typically synthesized from amphiphilic copolymers via the **ring-opening polymerization (ROP) of *N*-carboxyanhydrides (NCAs)** in organic solvents, followed by a **nanoprecipitation** in water.[3]

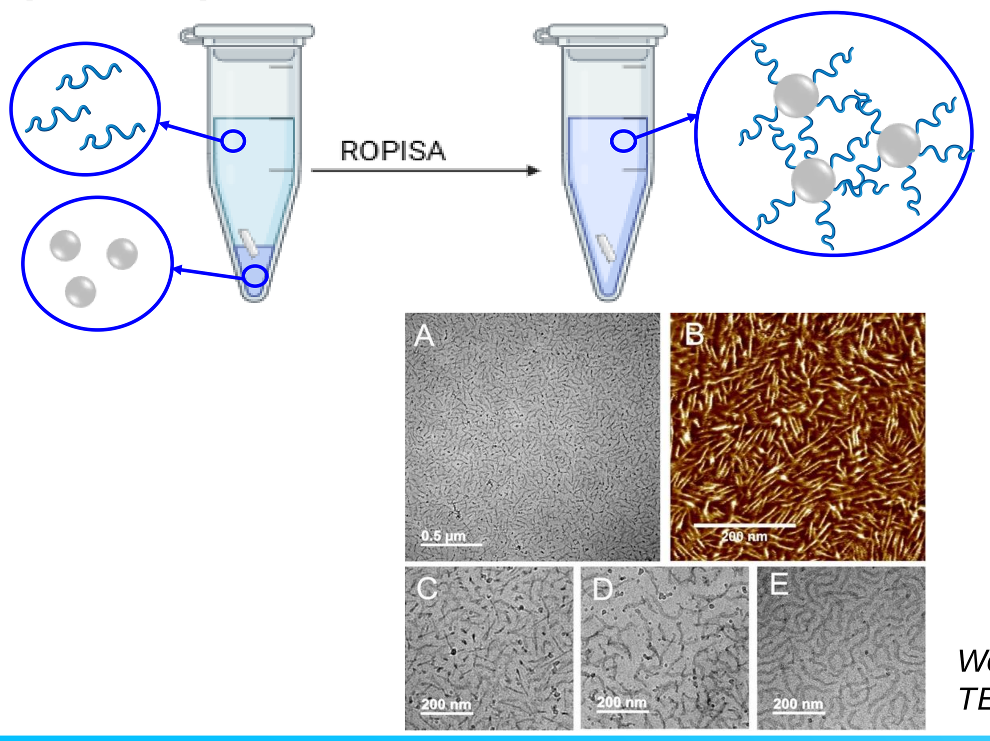




$PBLG_{20}$-*b*-PGG_{18} TEM images of samples obtained by instantaneously adding (a) water in DMSO and (d) DMSO in water[3]

- ✗ Use of organic solvents
- ✗ Hazardous up-scaling
- ✗ Stability of nanoparticles

A more promising approach involves the use of **Polymerization Induced by Self-Assembly (PISA)** combined with **Ring Opening Polymerization (ROP) of *N*-Carboxyanhydrides (ROPISA).**[4]



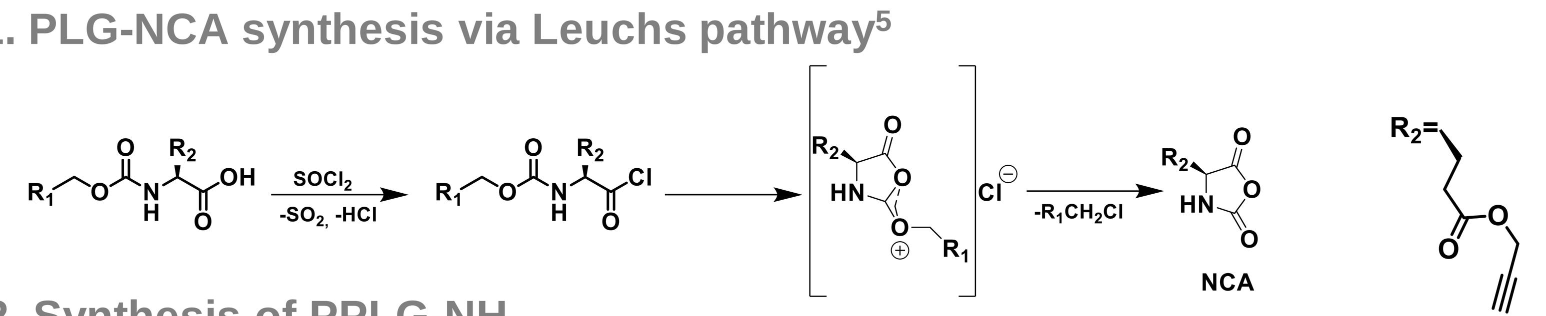
Worm-like morphologies characterized by Cryo-TEM (A, C, D, E) and AFM (B).[3]

- ✓ Reduces preparation steps
- ✓ Simplifies purification
- ✓ Increases the yield
- ✓ Formation of nanoobjects:
  - with a high solid content
  - with a biodegradable core
  - In H2O, no solvent
- ✗ No surface functionalization
- ✗ No biodegradable shell

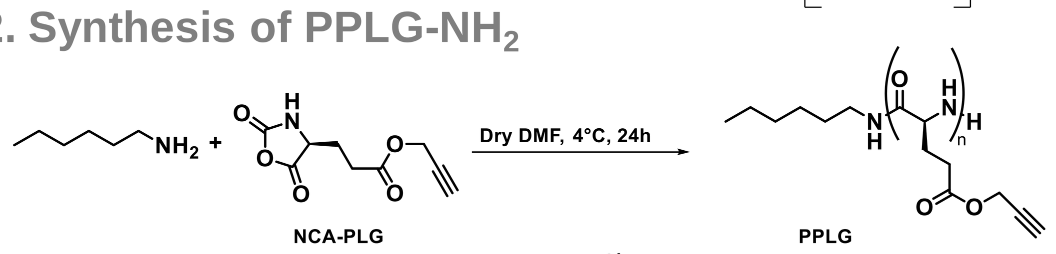
ROPISA with a hydrophobic clickable monomers: PLG-NCA

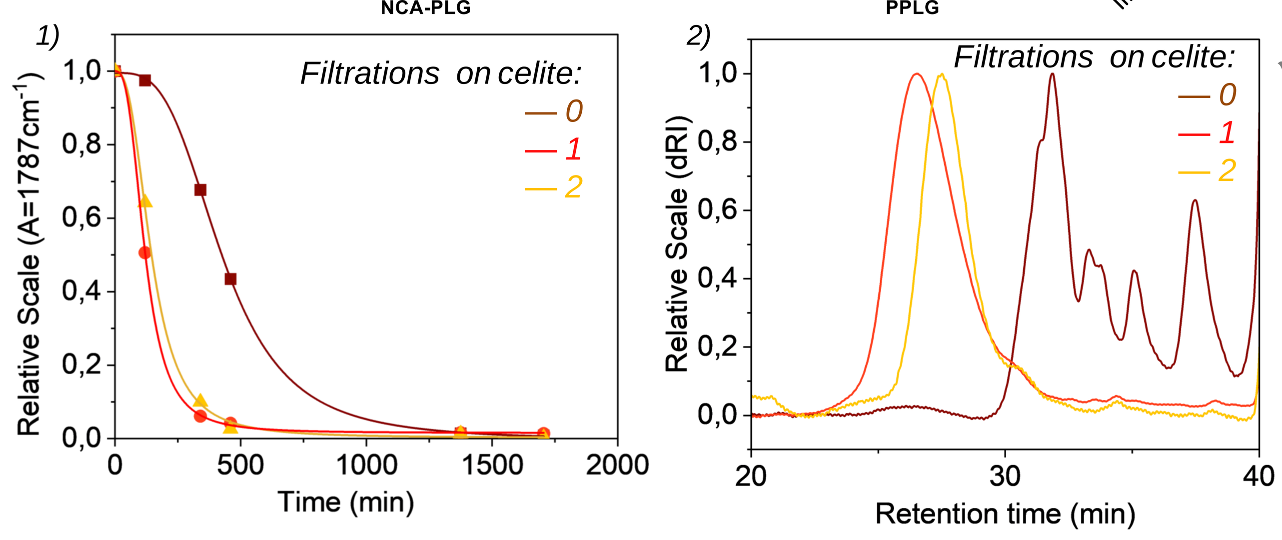
*NCA clickable monomers offer a great **versatility** thanks to the dandling **alkyne groups** on the backbone which are availables for further **click chemistry**.*

1. PLG-NCA synthesis via Leuchs pathway[5]



2. Synthesis of PPLG-NH_2





✓ Succesful polymerization of PPLG via ROP of PLG-NCA in anhydrous conditions.

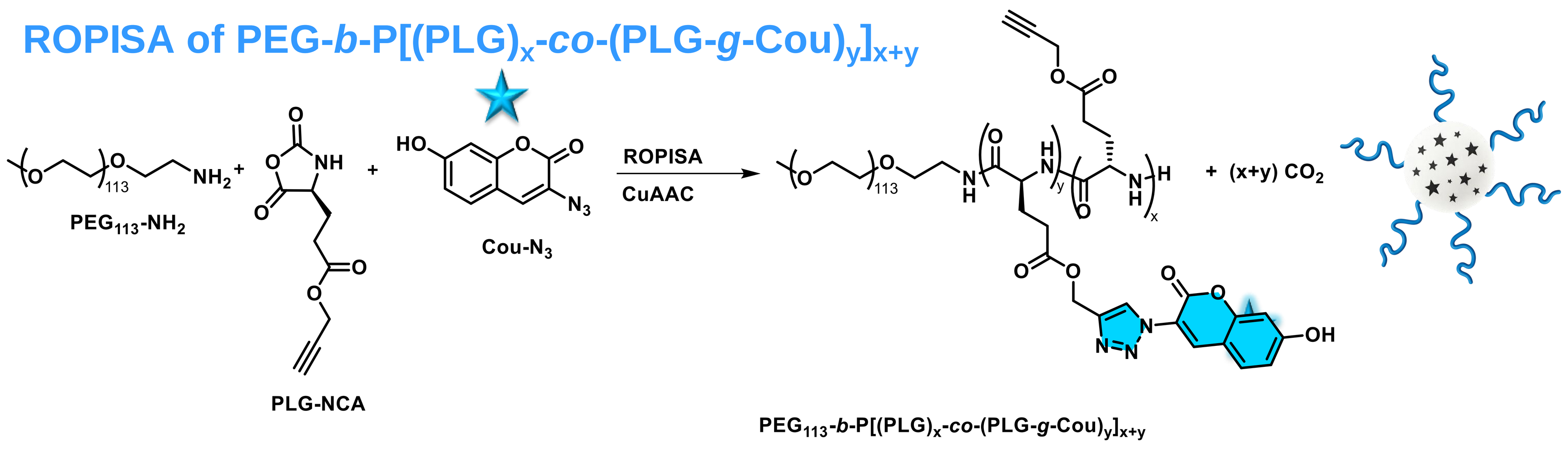
1) Kinetics by FITR at A=1787cm-1 2) Size-exclusion chromatograms in DMF (+LiBr 1mg.ml-1), RI detection, calibration using polystyrene as a standard.

- **Synthesis of PLG-NCA** was done using 3 successive filtrations on celite, allowing to obtain a faster polymerization rates and better control of the polymerization.

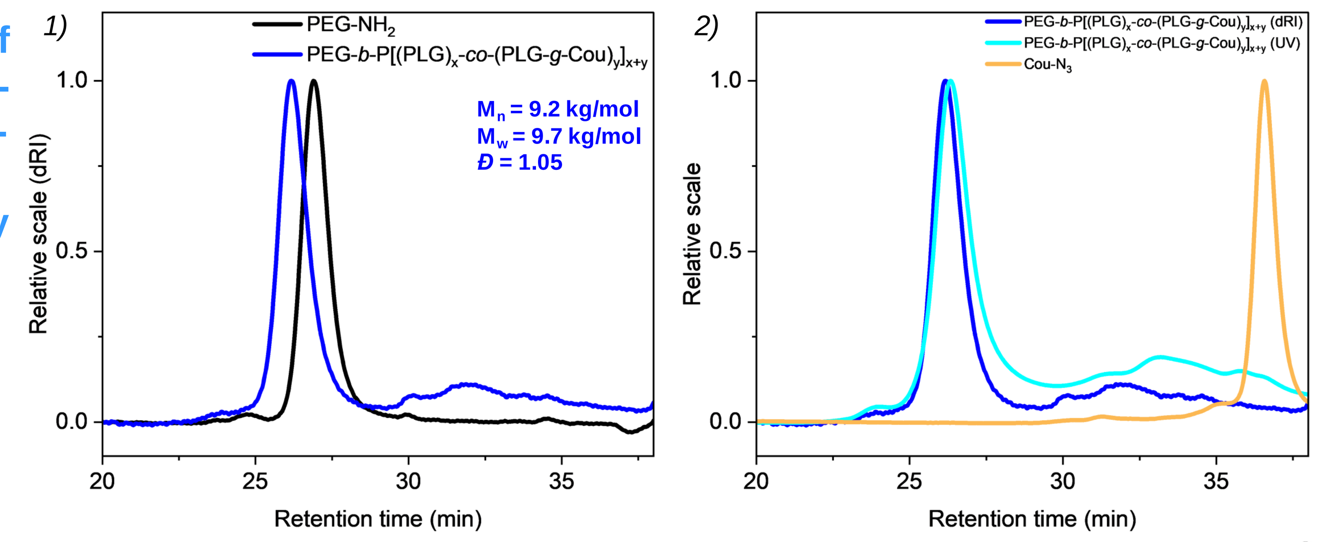
Click'n ROPISA: Synthesis of PEG-*b*-P[(PLG)$_x$-*co*-(PLG-*g*-Cou)$_y$]$_{x+y}$

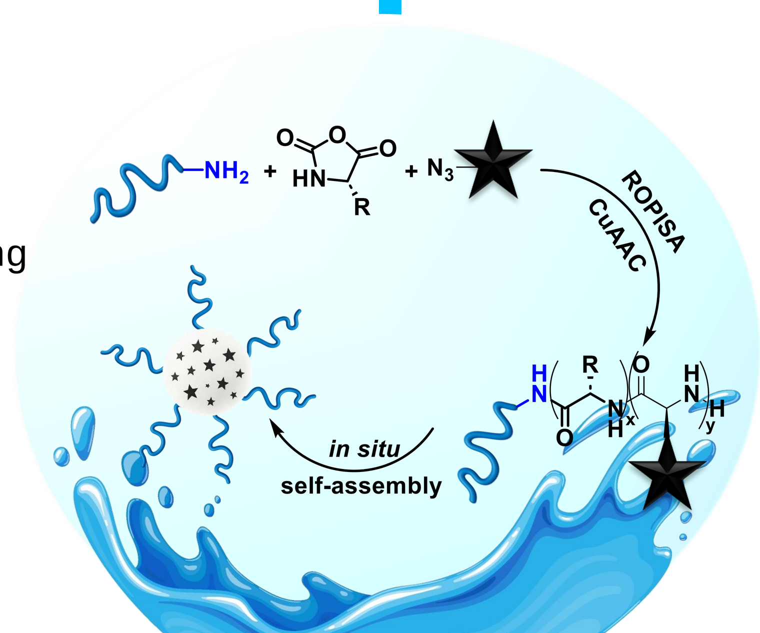
***Ring Opening Polymerization induced by Self Assembly** of PLG-NCA using PEG-NH_2 as initiator and **CuAAC**[6] of coumarine azide.*

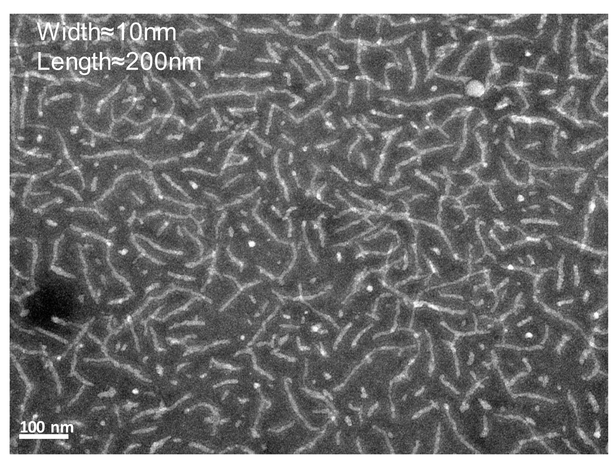
ROPISA of PEG-*b*-P[(PLG)$_x$-*co*-(PLG-*g*-Cou)$_y$]$_{x+y}$

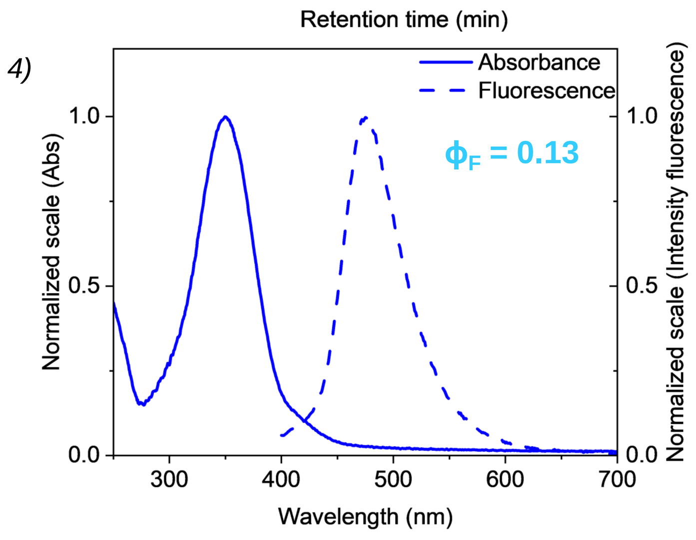


- ✓ Succesful synthesis of PEG-*b*-P[(PLG)$_x$-*co*-(PLG-*g*-Cou)$_y$]$_{x+y}$ using ROPISA-CuAAC process.
- ✓ Nanoparticles morphology (Wormlike or spherics).
- ✓ Fluorescent.





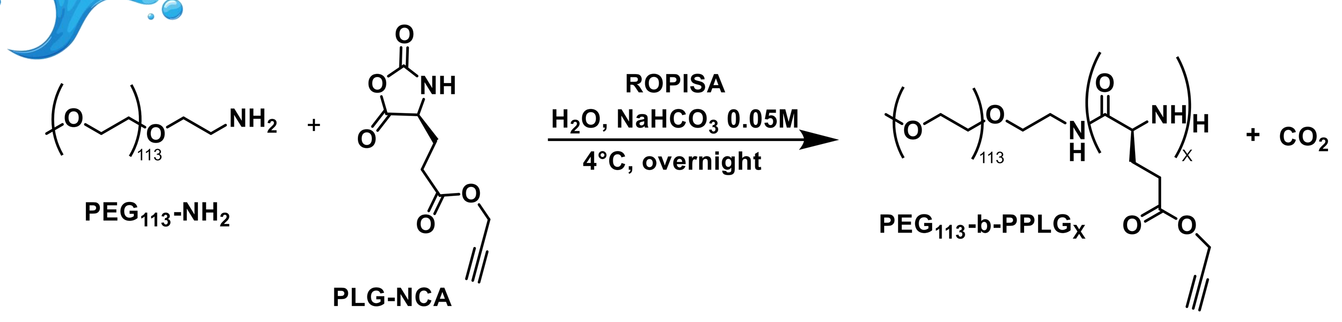


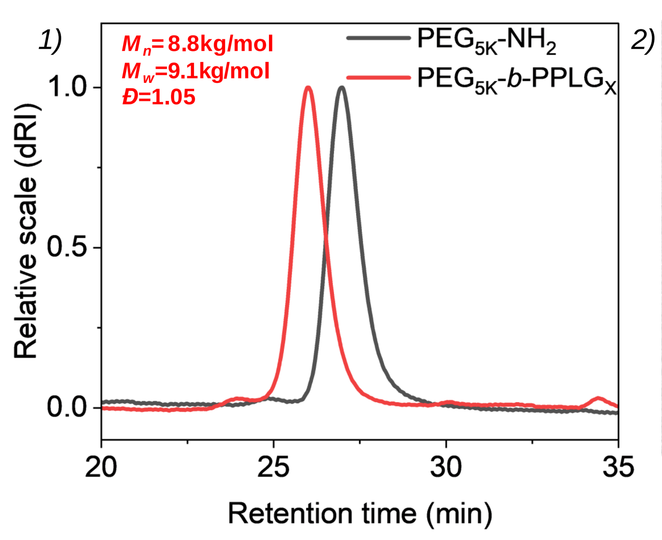


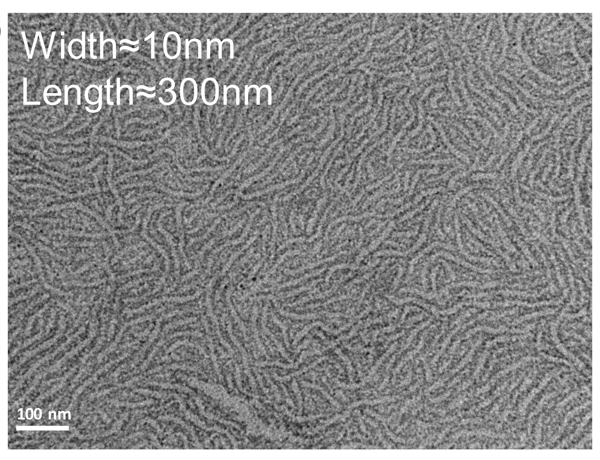
1) Size-exclusion chromatograms in DMF (+LiBr 1mg.ml-1), RI detection, calibration using polystyrene as a standard. 2) Size-exclusion chromatograms in DMF (+LiBr 1mg.ml-1), RI detection, UV detection calibration using polystyrene as a standard. 3) Worm-like and spheres (~15nm) nanoparticles characterized by TEM. 4) Absorbance and fluorescence emission spectra of PEG-b-P[(PLG)$_x$-co-(PLG-g-Cou)$_y$]$_{x+y}$ in water at λ_{ex} = 350 nm.

Aqueous ROPISA of PLG-NCA using PEG-NH_2 as initiators

ROPISA of PLG-NCA







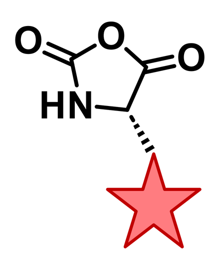
✓ succesful synthesis of PPLG using ROPISA process.
✓ core-shell NPs of PEG-*b*-PPLG
→ use of click chemistry to functionalize the PPLG.

1) Size-exclusion chromatograms in DMF (+LiBr 1mg.ml-1), RI detection, calibration using polystyrene as a standard. 2) Worm-like morphologies characterized by TEM

Conclusions and perspectives

- ✓ Synthesis and polymerization of PLG-NCA
- ✓ ROPISA of PLG-NCA to obtain nanoparticles of PEG-*b*-PPLG
- ✓ ROPISA-CuAAC synthesis to achieve fluorescent core-shell nanoparticles in water
- ✓ Anisotropic objects

→ Improvement of ROPISA and CuAAC synthesis conditions
→ Synthesis of a library of nanoparticles using others dye-N_3
→ Direct polymerization of new dye-NCA



Dye-NCA

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