H2020-ITN THERACAT (765497)

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Overview/Abstract

A library of amphiphilic polymers bearing BTA grafts, dodecyl groups and Jeffamine@1000 has been prepared via a post-functionalisation procedure. In aqueous solution, these polymers containing hydrophilic, hydrophobic and supramolecular units can fold into single-chain polymeric nanoparticles (SCPNs) with hydrophobic domains and are stable in complex media, which can be used as catalyst carriers.

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1. Introduction

Bio-orthogonal chemical reactions have been discovered and applied in the biological system due to their advantage of not interfering with other distant components in cells.[1,2] Catalysis-based local cancer chemotherapy using bio-orthogonal chemistries can reduce adverse pharmacological effects on healthy tissues, and thus is of great interest.

Single-chain polymeric nanoparticles (SCPNs), assembled from amphiphilic polymers in aqueous solution, are a new class of nanoassemblies capable of mimicking the function of enzymes. This set of SCPNs can create an adaptive reaction compartment to shield the catalyst from aqueous environment that allows for the catalytic reactions in the hydrophobic domain.[3] Therefore, SCPNs can serve as carriers to transport catalysts to the tumour site and to activate prodrugs in situ, limiting the side effects of anti-cancer drug diffusion to healthy cells. For instance, liu et al. reported that SCPNs comprising Cu(I) and Pd(II) can perform depropargylation in the presence of Hela cells and the nanoparticles proved to be nontoxic to the cells.[4] However, the catalytic efficiency inside the Hela Cell was lower compared to that in *in vitro* studies possibly because of the instability of SCPNs. Indeed, the stability of SCPNs plays an important role in achieving catalytic activities of SCPNs. To study SCPNs as catalyst carriers, we designed a modelling system by incorporating Nile Red into the polymer backbone to mimic the hydrophobicity of catalytic units and meanwhile study the stability of SCPNs in complex media.

2. Objectives

We aimed to prepare a library of amphiphilic polymers which are able to serve as catalysts carriers. In aqueous solution, the polymers bearing BTA grafts, dodecyl groups and Jeffamine@1000 as side chains are capable of folding into SCPNs with a hydrophobic domain that can carry catalysts and allow catalytic activities to occur. The SCPNs will be used by other THERACAT consortium partners to uncage prodrugs.

3. Results and Discussion

A set of amphiphilic polymers designed as catalyst carriers were synthesized (**Figure 1**). **P1** contains 5% benzene-1,3,5-tricarboxamide (BTA) grafts, 15% dodecyl groups and 80% Jeffamine@1000. **P2-P5** all bear Nile Red as the side chain to mimic the hydrophobicity of catalytic units. **P3** and **P4** are either with BTA grafts or with dodecyl grafts acting as the hydrophobic part. **P5** was synthesized with additional biotin to dock the particles on a glass-streptavidin substrate and therefore to study the catalytic activities of SCPNs at a single molecule level using single molecule fluorescence microscopy.

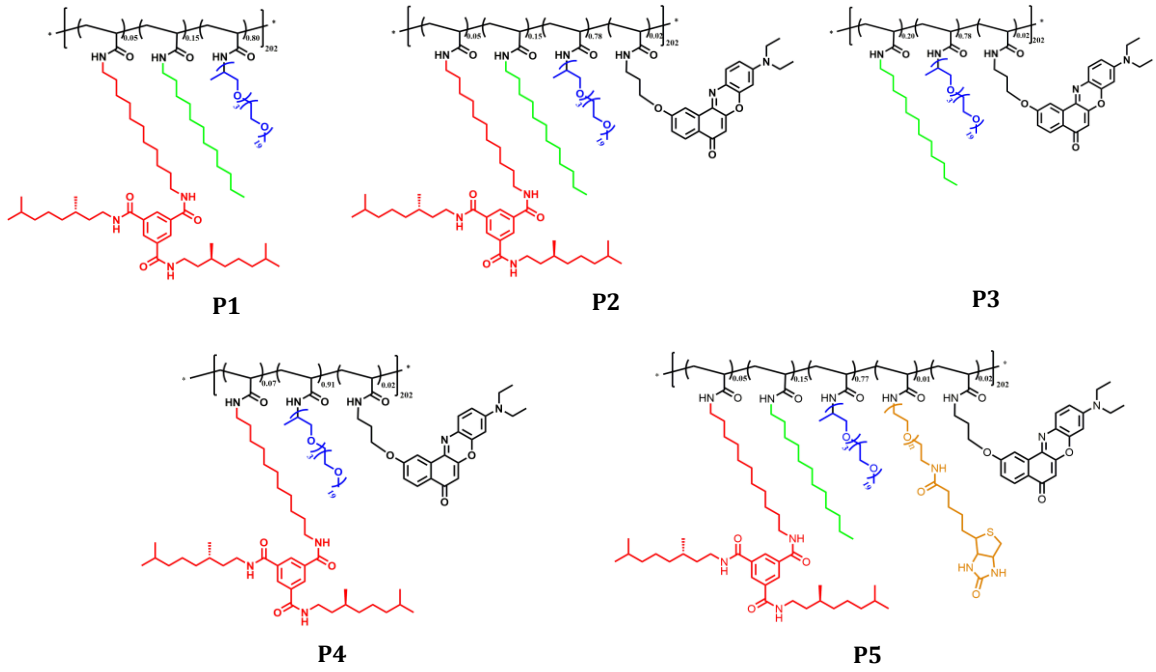

Figure 1. Structure of polymers as catalyst carriers **P1-P5** synthesized in task 5.1.

Synthesis of Nile Red derivative

In general, we prepared Nile Red derivative **5** with an amine group that can be covalently attached onto the polymer backbone via amide formation reaction. The synthesis of Nile Red derivative was achieved in four steps (**Figure 2**). Starting from 3-diethylaminophenol **1**, nitroso derivative **2** was synthesized by the reaction between **1** and $NaNO_2$. 2-hydroxy substituted Nile Red derivative was obtained by reacting 1,6-dihydroxynaphthalene with **2** following the literature.[5] The desired product **5** was next obtained in a moderate yield (43 %) by Williamson ether synthesis and de-protection of BOC-group.[6]

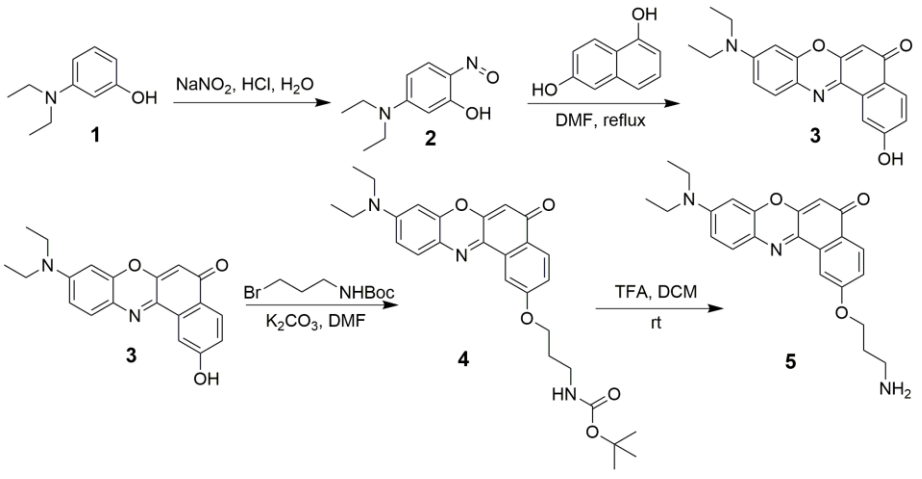

Figure 2. Total synthesis of Nile Red derivative **5** in task 5.1.

Synthesis of polymer precursor

The route of polymer precursor synthesis is shown in **Figure 3**. Monomer pentafluorophenyl acrylate was obtained by the reaction between pentafluorophenol and acryloyl chloride using triethylamine as base.

Reversible addition-fragmentation chain transfer (RAFT) polymerization was employed using 4-cyano-4 ((phenylcarbonothioyl) thio) pentanoic acid as the chain-transfer agent (CTA-agent) and azobisisobutyronitrile (AIBN) as the initiator following the reported protocol.[7] All the chemicals were dissolved in dry 1,4-dioxane and were placed in a Schlenk tube. Bubbling argon into the Schlenk tube for half an hour was an important step before doing polymerization. The Schlenk tube was then placed in an oil bath at 80℃. After about 2 hours, the conversion of polymerization was monitored using ^{19}F-NMR by taking samples from the reaction mixture. The chemical shifts of fluorine atoms between the free acrylate and the acrylate incorporated into the polymer backbone were different, so it can be used to calculate the degree of polymerization. When the conversion reached 70%, the polymerization was quenched using liquid nitrogen. ^{19}F-NMR showed that the degree of polymerization was around 202. Gel permeation chromatography (GPC) was used to measure the polydispersity and the value was 1.18. Total quantity synthesized was 1.4 g.

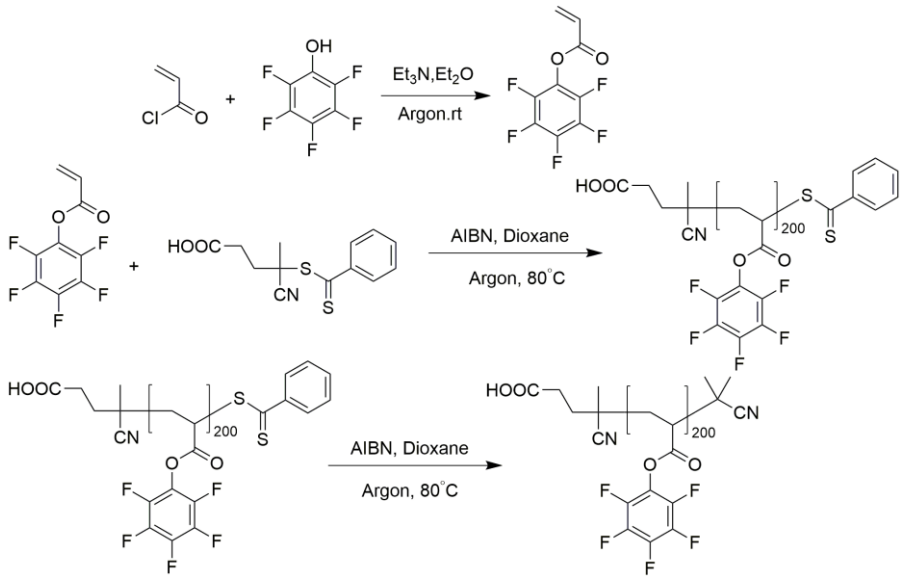

Figure 3. Synthesis of polymer precursor in task 5.1.

The end-group transformation was performed since thiocarbonylthio-containing CTA imparted the polymer ability to further polymerization, which can cause some problems in the next post-functionalization step. The end-group modification was done by addition of 20 equivalents of radical initiator AIBN with respect to the amount of CTA-agent used in the polymerization protocol. For acrylate-based polymers, which is in our case, lauroyl peroxide was added as well to help get rid of CTA-agent because lauroyl peroxide binds the CTA-agent more irreversibly compared to AIBN. The amount of lauroyl peroxide was

2 equivalents with respect to the amount of CTA-agent. The polydispersity after end-group modification was measured by GPC and the value was 1.16. Total quantity synthesized was 1.2 g.

Synthesis of polymers P1-P5

A post-functionalization approach was applied to prepare polymers **P1-P5**. The advantage of this approach is that while allowing changes in side chains, the average degree of polymerization (DP) and polydispersity ($Đ_M$) of the backbone are fixed. All polymer synthesis employed a similar protocol, which is described below. The general procedure of post-functionalization for polymer **P2** (**Figure 4**) started with introducing Nile Red derivative **5**, then adding BTA-C11-NH2 graft, followed by incorporating hydrophobic dodecyl groups and completed by adding an excess amount of hydrophilic Jeffamine M-1000. All reactions were performed in THF at 50 ℃. To monitor the conversions of different side-chain groups, ^{19}F-NMR spectroscopy was introduced. Due to the presence of the pentafluorophenol, the side-chain group conversion can be calculated by integrating the signal of released pentafluorophenol from the polymer precursor. To remove all unreacted reagents, a consecutive dialysis via a membrane with 6-8k Da molecular weight cut-off against THF and MeOH was performed, followed by precipitation of polymer in cold n-pentane. The final amphiphilic polymer **P2** with a DP of around 202, with side chain groups containing 2% Nile Red, 5% BTA, 15% dodecyl, 78% Jeffamine and $Đ_M$ of 1.2 (measured by SEC in DMF) was successfully prepared.

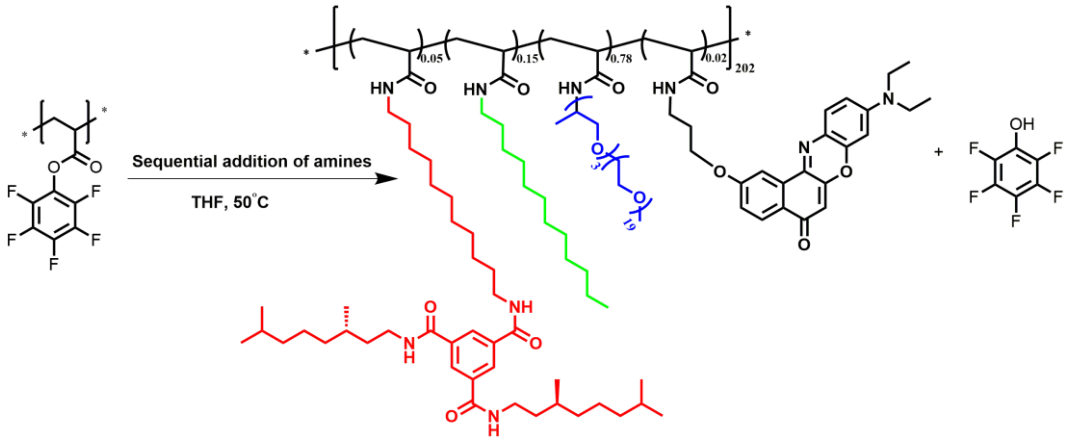

Figure 4. Sequential post-functionalization to prepare polymer **P2** in task 5.1.

Dynamic light scattering measurement

After the successful preparation of polymers **P1- P5**, we tested whether these polymers were able to form SCPNs in water. By performing dynamic light scattering (DLS) experiments, we obtained the hydrodynamic diameter (D_h) of polymers **P1- P5** in water with a standard protocol of sample preparation. Firstly, water was added to the polymer at a concentration of 1 mg/mL at room temperature. Next, sonication was applied for 45 minutes to fully dissolve the polymer. The polymer solution was subsequently heated at

80 ℃ for 45 minutes, and then allowed to cool down and aged overnight to reach equilibrium at room temperature. The DLS results (**Figure 5**) show that all the polymers formed nanoparticles in water with hydrodynamic diameter smaller than 12 nm.

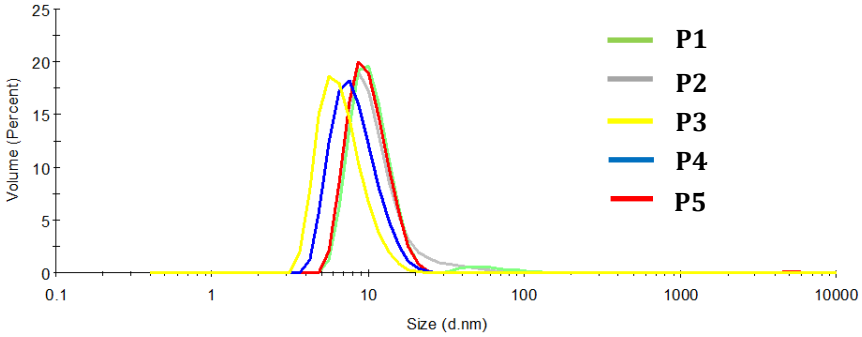

Figure 5. P1-P5 volume distribution of the hydrodynamic diameter

Fluorescence Spectroscopy

According to our previous study, **P2** can form more compact and globular single-chain polymeric nanoparticles[7] compared to other catalysts carriers that we prepared in task 5.1. We were firstly interested in studying the stability of SCPNs formed by **P2** in complex media, and the fluorescence spectra of Nile Red covalently attached to **P2** in various conditions were monitored. After forming SCPNs, we injected water, PBS buffer, DMEM buffer and bovine serum albumin solution into SCPNs to reach the final concentration of SCPNs at 2.5mg/3mL. The fluorescence intensity of SCPNs in complex media (**Figure 6**) did not change compared to that of SCPNs in water, which indicates that SCPNs in PBS buffer, DMEM buffer and bovine serum albumin solution are stable. The stability of other catalysts carriers which we synthesized in task 5.1 is under investigation.

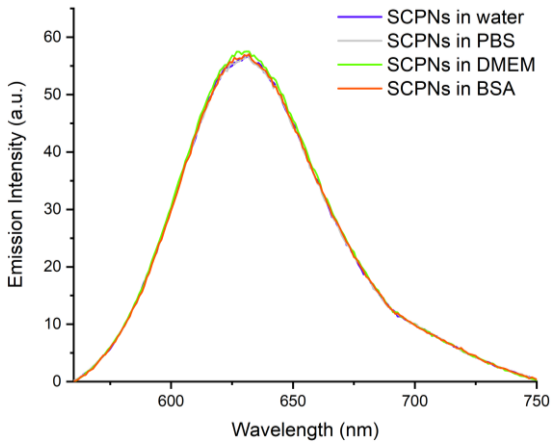

Figure 6. Fluorescence measurement of SCPNs in complex media

4. Conclusions

A set of amphiphilic polymers bearing BTA grafts, dodecyl groups and Jeffamine@1000 has been successfully prepared via a post-functionalisation procedure during T5.1. The five types of polymers are all present as nanoparticles in water and can be used as catalysts carriers. SCPNs made by polymer **P2** are stable in complex media, which are promising for biological application. This amphiphilic polymer library is now available for the consortium partners at request to do further study.

5. References

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