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[1] *A new delivery date was approved by the Project Officer due to the delay caused by COVID-19 outbreak.*

Overview/Abstract

Novel polymers were designed and synthesized by ESRs 2, 3 and 4. These polymers are amphiphilic in nature and can form nanoparticles (NPs) in aqueous media. The NPs were loaded with Pd to perform biorthogonal reaction in aqueous media.

Explanation for large delay in submitting deliverable

N/A

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Document Control

Issue #	Date	Changed Pages	Cause of Change	Implemented by
1	18/02/20	3-5	Contribution of TAU to D3.2	Shreyas Wagle
2	18/02/20	7-9	Contribution of TUE to D3.2	Anjana Sathyan

Novel nanostructures loaded with Ru or Pd catalysts

1. Introduction

The use of abiotic catalysts in biological systems has received only recent attention. Biorthogonal chemistry largely makes use of abiotic metals to catalyze non-natural reactions in living systems.[1] Many transition metals (TMs) are viewed as good candidates for these reactions. Till now, the most extensively explored metals for biorthogonal catalysts are Pd, Cu, Ru, Ir, Au and Fe; mostly used for bond cleavage, cross-coupling, and cycloaddition reactions.[2,3] Previous research has established that these abiotic catalysts need hydrophobic environment to be efficient.[4,5]

In order to design a nano scaffold that can house an abiotic metal catalyst, there are three things we need to take into consideration. First, the scaffold should make the catalyst soluble in water whilst maintaining an active catalyst. Secondly, the scaffold should be biocompatible and finally, the nano scaffold should be able to reach the desired location for catalysis. Therefore, the rational design, construction, and optimization of metal catalytic centres and their ligands could be the key for translating the chemistry into applications.

Amphiphilic polymers (APs) can be used a building blocks to create a nano scaffolds for TMs. APs have a hydrophobic block and a hydrophilic block, which make them self-assemble into nanoparticle in aqueous environment. The formation of NPs can be attained using different approaches. The first is by using amphiphilic di-block copolymers (ABCs). In aqueous media, at certain concentration called critical micellar concentration (CMC), ABCs self-assemble into thermodynamically favoured polymeric micelles that are composed of a hydrophobic core and a hydrophilic shell.[6] The other way is by using single chain polymeric nanoparticles (SCNPs), which are also amphiphilic polymers, that can form structured nanoparticles in solution due to the optimal balance of hydrophobic and hydrophilic groups in the polymer backbone.[5]

2. Objective

The aim is to design and synthesize novel nanostructures that can be loaded with transition metal catalysts such as Pd, which can be used to perform biorthogonal reactions in aqueous media.

3. Results and discussion

3.1 Catalytic micellar systems for Pd(II) mediated O-Propargyl cleavage reactions in water (TAU-ESR2)

ESR2's project is focused on developing catalytic micelles based on amphiphilic polymers which are PEG dendron hybrids (PDHs). A set of 5 polymers with varying degree of hydrophobicity were synthesized (Figure 2A) and used for depropargylation experiments using 4-nitrophenyl propargyl ether (PNPPE) as the substrate under the following conditions: Polymer:$Pd(OAc)_2$:Substrate = 1:2:4; [substrate] = 160 µM.

The palladium acetate salt used for this reaction is a hydrophobic salt and we used the setup of the polymer + metal as it is to maintain the ratio between the metal and the substrate. We performed experiments with just the metal in PBS for PNPPE substrate with hardly any product formation observed as there were precipitation issues with the substrate and possibly the salt as well. Additionally, some of the substrates we work with are very hydrophobic, except the oligoethylene glycol substrate, and tend to get out of the solution after a few hours and thus causes discrepancies in the experiments.

To estimate where the palladium metal sits in the structure of the polymeric micelle, we performed a complexation experiment using a combination of $mPEG_{5k}$-D-$(C12)_4$ polymer and palladium (II) acetate salt in $CDCl_3$ with different molar fractions of metal to polymer, ranging between 0% to 200%, and monitored the complex formation by NMR (Figure1).

Upon the increasing percentage of the metal salt, the peaks of the protons near the thio-ether group were significantly broadened until entirely disappeared (marked in purple) (Figure1) after ~2 equivalents of $Pd(OAc)_2$ were added with the respect to the polymer. That can occur due to short relaxation times caused by decreased mobility, indicating a complex formation at or near these sites.

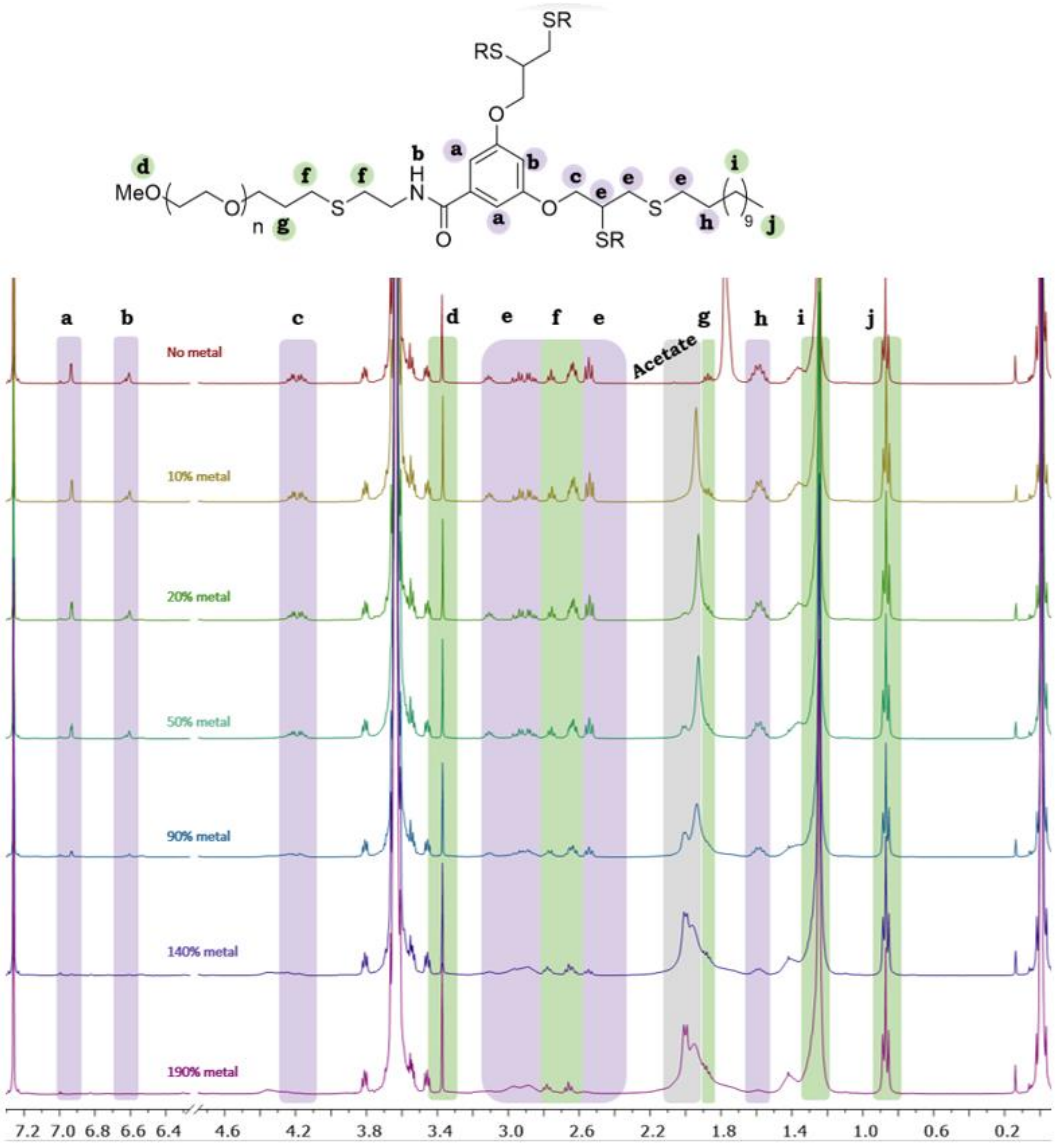

Figure 1: ^{1}H-NMR overlay spectra of mPEG$_{5k}$-D-(C12)$_4$ in the presence of increasing Pd(OAc)$_2$ ratios.

Nearly full conversion was achieved after approximately 48 hours for all the polymers (Figure 2B). Additionally, when plotting the rate constant (K) of substrate consumption and ClogP of the dendritic group of each polymer, a linear trend was observed (Figure 2C).

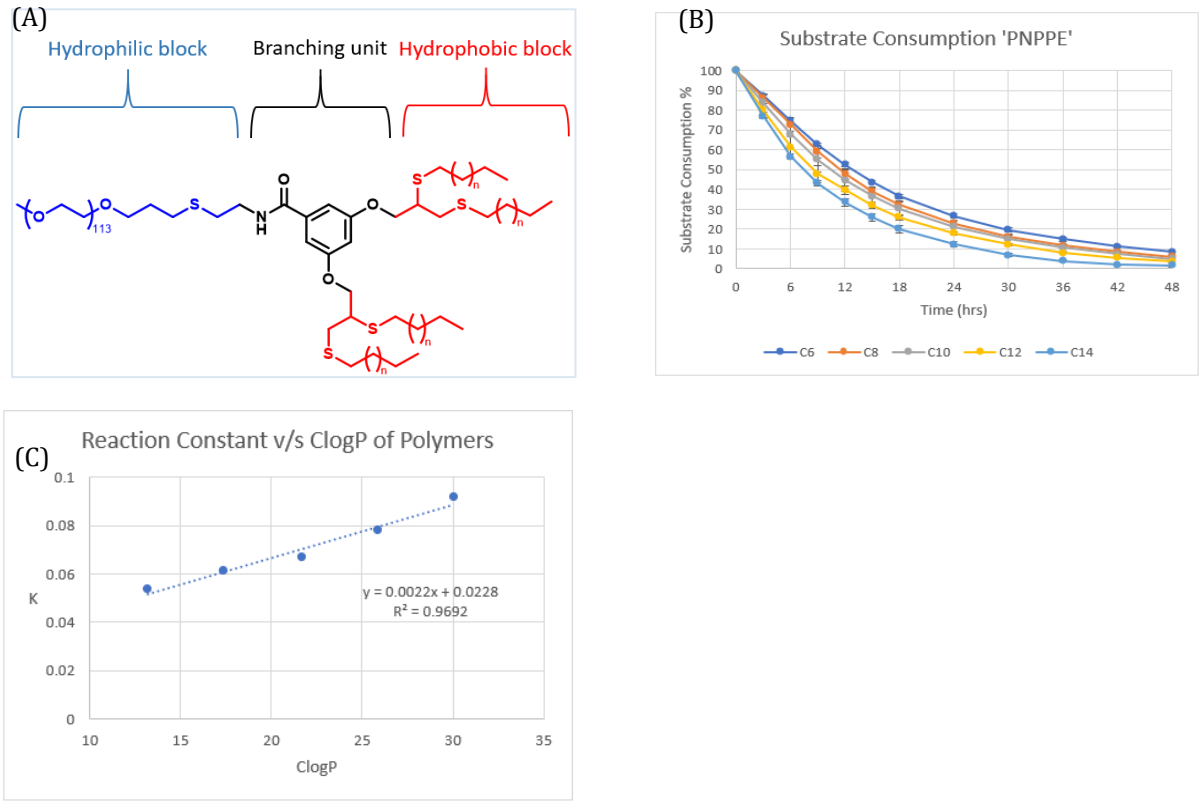

Figure 2: A. PEG-dendron based polymeric amphiphile, n = 3, 5, 7, 9 and 11. B. Consumption of 4-nitrophenol over time for five different polymers. C. K vs ClogP.

After obtaining these results, substrates with different degree of hydrophobicity were synthesized to understand the relationship between the logP of the substrates and the depropargylation kinetics. The substrates were based on 4-(prop-2-yn-1-yloxy)benzoic acid and depropargylation experiments were conducted using two polymers with 6 and 14 carbon chains in the hydrophobic dendron, respectively. These two polymers were the least and most hydrophobic polymers in the set of polymers that was synthesized. It was encouraging to see a clear trend when plotting a graph between 'K' values (substrate consumption rate with respect to hydrophobicity of substrate and polymer (Figure 3). The results are currently being drafted into a manuscript that is planned to be submitted it in the next few weeks.

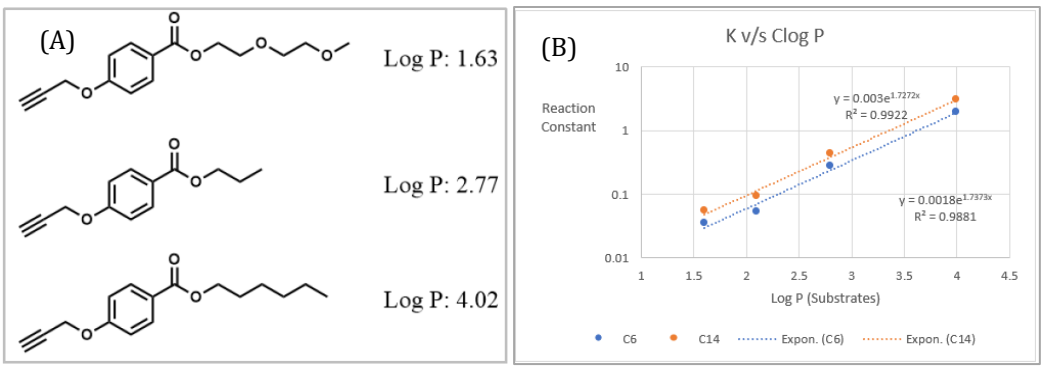

Figure 3: A. Structures of substrates with different log P values. B. k vs Log P of substrates.

3.2 Dendritic amphiphiles as a nano scaffold for abiotic catalysis (TAU, TEVA-ESR3)

The project is on using PDHs as amphiphiles to make nanoparticles that can be loaded with Pd and Cu. The combination of hydrophobic dendron and metal co-ordinating ligands in the hydrophobic core will aid in trapping hydrophobic transition metal salts in the micellar core. The synthetic steps follow similar protocol that our lab previously published till mPEG-dendron-2yne and mPEG-[dendron-(2yne)]$_2$.[7] Subsequently, they were reacted with azido alkanes by Cu-catalyzed azide-alkyne cycloaddition (CuAAC) reaction (Figure 4) to form PHDs. PHDs structures were characterized by ^{1}H NMR, and molecular weight and polydispersity were evaluated by gel permeation chromatography (GPC). Hydrodynamic radius of micelles formed by PDHs in phosphate-buffered saline (PBS) - pH 7.4 was measured using dynamic light scattering (DLS) and found to be less than 40nm.

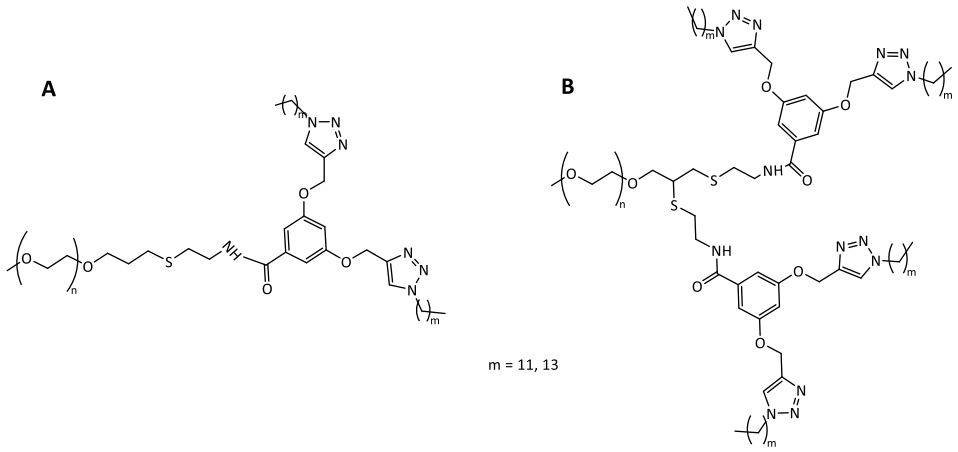

Figure 4: A. PDH with two branching dendrons. B. PDG with four branching dendrons.

To estimate the amount of metal PDHs can house as a micelle in aqueous media, a series of ^{1}H NMR experiments were conducted. PHDs and metal (either $Pd(OAc)_2$ or CuBr or $CuSO_4$) were dissolved in deuterated organic solvents with varying the amount of metal concentration and a constant PDH concentration. The concentration of metal at which the proton peaks next to sulphur atoms and the triazole ring disappeared in the spectra was used to complex with polymers for initial catalysis experiments.

3.2.1 Catalysis – depropargylation with PDH loaded with $Pd(OAc)_2$

PDH with four 12-carbon alkyl chains was used to perform catalysis experiments. For initial catalysis experiments, we used 100 mM and 50 uM polymer-$Pd(OAc)_2$ complex to form micelles and 50uM of 4-NPPE as a substrate. Formation of product (4-Nitrophenol) was monitored by measuring its absorbance in UV spectrophotometer.

From our initial experiments, depropargylation of 4-NPPE to para-nitro phenol (PNP) appears to be faster when the solution has polymer and metal in a 1:2 ratio rather than 1:1 ratio (Figure 5). The yields are detailed in Table 1. This might be because, in an

aqueous solution with micelles, a hydrophobic substrate will spend most of its time inside the micelle's hydrophobic core. If the micelle's core is densely packed with metal, then the probability of the substrate finding the metal and getting catalyzed into product increases.

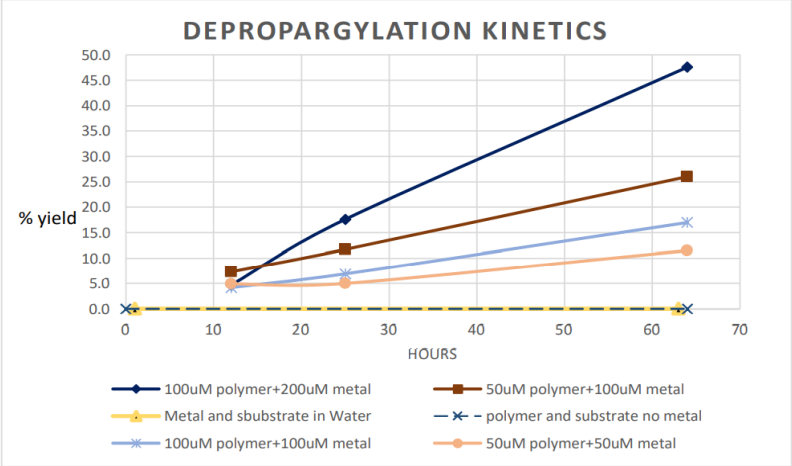

Figure 5: Reaction kinetics of depropargylation of 4-Nitrophenyl propargyl ether by catalytic micelles with $Pd(OAc)_2$ containing core.

Polymer uM	$Pd(OAc)_2$ uM	4-NPPE uM	Yield after 64h
100	200	50	47
100	100	50	17
50	100	50	26
50	50	50	12

Table 1: Depropargylation yields after 64h.

3.3 Pd(II) loaded single chain polymeric nanoparticles for pro-drug activation (TUE-ESR4)

ESR4's project is focused on creating SCNPs by synthesizing amphiphilic polymers that can form structured nanoparticles in aqueous solution due to the optimal balance of hydrophobic and hydrophilic groups in the polymer backbone. With the aim of developing SCNPs that can function efficiently in cells to perform prodrug activation in cancer therapy, amphiphilic polyacrylamide polymers were used as a backbone. The polymers were functionalized with contains palladium binding ligands, benzene-1,3,5-tricarboxamide (BTA), dodecyl units and jeffamine that were added by post functionalization approach.

Different amphiphilic polymers **P1-P6** containing Pd(II) binding ligands, BTA, dodecyl and water-soluble polyether side chains were synthesized from **pPFPA** (poly(pentafluorophenylacrylate) precursor (Figure 6, Table 2). The pPFPA backbone

was synthesized from pentafluorophenyl acrylate by reversible addition fragmentation chain-transfer (RAFT) polymerization. The thiocarbonyl end group was removed by reacting with excess of AIBN and lauroyl peroxide. **P1-P4** were equipped with triphenyl phosphine (TPP) ligands and **P5-P6** with 2,2'-bipyridine (Bipy) ligands for the comparison of their efficiency in catalyzing depropargylation reaction in different media. All polymers **P1-P6** were prepared by post-functionalization approach where different pendant groups with amine linker were sequentially added to the polymer backbone and the degree of incorporation was evaluated by NMR spectroscopy.

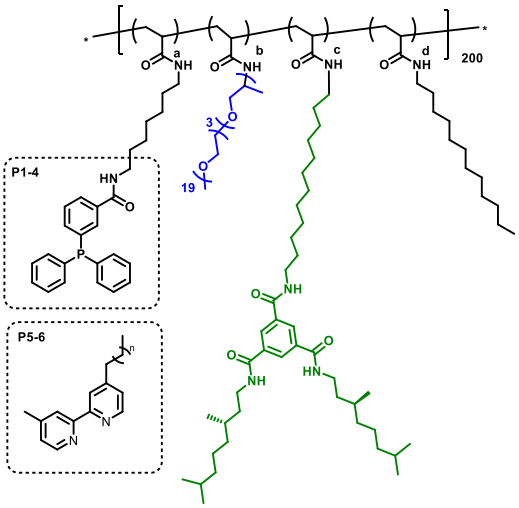

Figure 6: Structure of amphiphilic polymers P1-P6. P1-P4 are equipped with triphenylphosphine ligands, P5-P6 with bipyridine ligands.

Polymer	a	b	c	d	n	M_n (kD) SEC-DMF	M_n (kD) NMR	Đ
P1	10	80	10	-	-	46.8	183.6	1.28
P2	10	70	5	15	-	42.2	163.5	1.18
P3	10	70	-	20	-	62	158.7	1.43
P4	20	80	-	-	-	55.4	178.3	1.34
P5	5	75	5	15	6	46.9	179.1	1.16
P6	5	75	-	20	6	49.8	180.5	1.15

Table 2: Composition of pendant groups of polymers **P1-P6.**

3.3.1 Role of ligand in activity of SCPNs in different medium

First the activities of Pd(II) loaded SCPNs prepared from BiPy-based **P5** and TPP-based **P2** were compared in the deprotection of pro-dye propargyl-protected rhodamine in water, PBS and DMEM (Figure 7A). The formation of Mc-Rh110 was monitored using fluorescence spectroscopy over time (Figure 7B). In water and PBS, conversion after 60 min was found to be 48% and 37% for both polymers as quantified by from HPLC-UV. However, the initial rate of reaction suggests that phosphine-based polymer **P2** ensures catalytic and substrate accumulation in hydrophobic cavity owing to strong Pd(II)

complex. Slower rate of reaction of BiPy-based polymer **P5** suggests presence of free Pd(II) in solution owing to less stable complex, which is itself catalytically active. Phosphine-based **P2** was active in DMEM medium with a conversion of 7.8%.

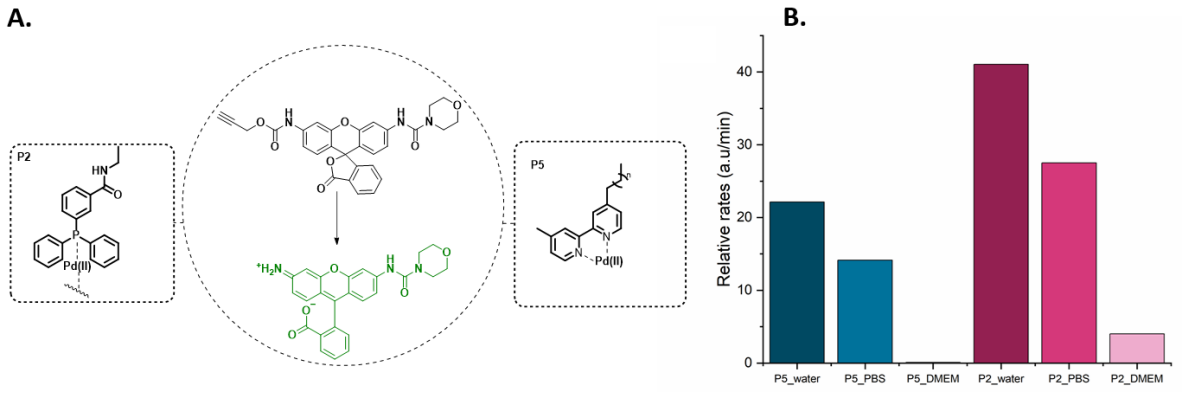

Figure 7: A. Comparison of catalytic activity of **P2@Pd(II)** and **P5@Pd(II)** on N-proc McRh110. B. Relative rates during depropargylation of pro-dye monitored using fluorescence kinetic measurements (Pd(II) = 10 µM, pro-dye = 50 µM).

4. Conclusion

All three ESRs successfully synthesized novel polymers that can form nanoparticles and can be loaded with Pd. They characterized the polymers with various analytical methods and validated the catalytic activity of nanoparticles by performing depropargylation reactions in aqueous media. Based on the obtained results, additional types of ligands and metals will be explored.

5. References

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