MAX-PLANCK-GESELLSCHAFT

On the development of dually-responsive PNIPAM copolymers

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To Carolina, and my family who made this possible.

I also dedicate this thesis to those who
challenge themselves beyond what they already know

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Abstract

In this research work we studied the development of dually responsive smart materials based on PNIPAM by incorporating thioethers functionalities alternatingly.
Thioethers at first glance may seem rather unreactive and unspectacular particularly compared with their analogous thiol and disulfide; however in contrast to regular ether, the nature of sulfur provides the possibility to functionalize via oxidation, alkylation, alkoxylation, and even, as we will see later along the evolution of this thesis, to form coordinating bonds with metal complexes.

As a result, we proved a new coordination bond between thioethers and a triphenolphosphate polypyridyl-Ruthenium complex and the successful employment of this to coordinate a PNIPAM copolymer; however, although the coordination bond was formed, because of its dynamic properties and instability at high temperatures, it was found impossible isolate the Ru-coordinated polymer in order to measure the influence in a physical property of such as the LCST of PNIPAM.
Moreover, we investigated the influence of oxidizing the thioethers functional group of PNIPAM in solution, and measure the influence in the LCST. We observed a huge change, even of 23 ºC, and a 100% efficient and selective oxidation to sulfoxide achieved. This could open up a new research work of oxidizing *in-vivo* a PNIPAM copolymer for controlled drug delivery and imaging, since oxidation Reactive Oxygen Species (ROS) are formed as result of inflammation processes and which causes endothelial dysfunction and tissue injury.

Introduction

For decades the advancement of biomedical research have demanded the development of new smart polymer materials for applications such as controlled drug deliver, cell patterning, DNA separation and sequencing, among others, which have led to the development of novel smart materials which have one or more properties such as chain dimension, solubility, intermolecular association, etc. which can response to an external stimuli such as pH, temperature, light irradiation, etc., imitating the behavior of the most important substances in living systems which allow us to work as the way we do.
It has been proved in the last decades that synthetic (co)polymers can gain such similar adaptive behavior by incorporating multiple copies of functional groups readily amenable to change in character (e.g. charge, polarity, solvency) along a polymer backbone leading to transformations at macroscopic material properties. [1]

1.Thermo-responsive materials

Among all smart materials, temperature-responsive polymers probably belong to the one of the largest classes, presenting the unique characteristic of a volume-phase transition at a certain temperature which cause a sudden change in the solvation state; among these, polymers which become insoluble upon heating, have a so called LCST (lower critical solution temperature), while systems which become soluble upon heating have an UCST (upper critical solution temperature) where the change in hydration state causes the volume phase transition, reflecting hydrogen bonding properties, where intra- and intermolecular hydrogen bonding of the polymer molecules are favored compared to a solubilization by water [1]. In Figure 1 a phase diagram of a LCST and UCST phenomenon is presented.

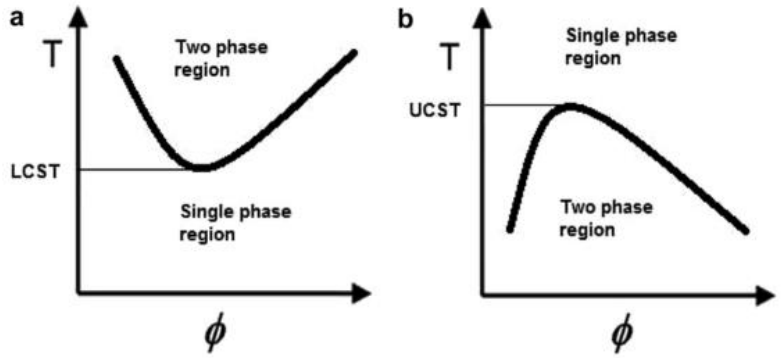


Figure 1. Schematic Illustration of Temperature vs. polymer volume fraction (ϕ), representing the phase diagrams for polymer solution a) LCST and b) UCST behavior[1]

Although LCST and UCST systems are not restricted to aqueous solution environment, it's only the aqueous systems which are of interest for biomedical applications. As it is further shown in Table 1, typical LCST polymers are based on NIPAM, DEAM, MVE and NVCl as monomers, while typical UCST systems are based on a combination of AAm and AAC.

Table 1. Polymers with LCST or UCST behavior in the temperature region for biomedical applications (in aqueous solution) [1]

Water soluble polymer	Phase transition temperature
LCST behavior:	
Poly(N-isopropylacrylamide)	30 – 34 ºC
Poly(N,N-diethylacrylamide)	32 – 34 ºC
Poly(methyl vinyl ether)	37 ºC
Poly(N-vinylcaprolactam)	30 – 50 ºC
PEO-b-PPO	20 – 85 ºC
Poly(pentapeptide) of elastin	28 – 30 ºC
UCST behavior:	
Polyacrylamide and PAA	25 ºC

Among these, particularly exciting for medical applications is the LCST of some water soluble polymers, which tend to phase-separate from solution upon heating at around the

body temperature of 32ºC, therefore in this research work we will focus specially in Poly(N-isopropylacrylamide) PNIPAM, whose LCST oscillates around 33.5 ºC.

1.1. PNIPAM: General features

As we have mentioned before PNIPAM is the most promising thermo-responsive material nowadays, actually it is already being employed in high-tech applications such as in membrane technology, etc., so before we go further in the development of this research work I will explain some details about this outstanding polymer. Figure 2 shows the chemical structure of PNIPAM and its hydrophilic/hydrophobic segments. [2]

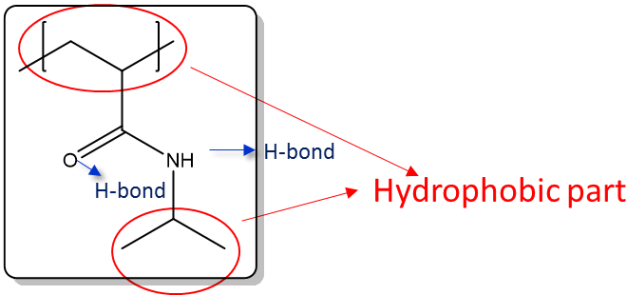


Figure 2. Schematic Illustration of PNIPAM chemical structure and its hydrophilic/hydrophobic segments in its composition

So as every LCST thermo-responsive polymer, when PNIPAM it's in aqueous dilute solution, undergoes a coil-to-globule transition at 33.5 ºC, which means that possess an inverse solubility upon heating. This phenomenon occurs due to the fine balance between hydrophobicity and hydrophilicity in its chemical structure, for which at lower temperatures PNIPAM manage to order itself in order to undergo hydrogen bonding with the already arranged water molecules, i.e. the negative enthalpy term from hydrogen bonding effects dominates the Gibbs free energy (*G(p, T) = H – TS, where H is the enthalpy term (Joules) and S is the entropy (Joule per Kelvin))* causing the PNIPAM to absorb water and dissolve in solution. However by increasing the temperature over 33 ºC, the water molecules must reorient themselves around the nonpolar regions of the polymer structure resulting in a decreased entropy, for which the entropy term dominates causing PNIPAM to release water and phase separate.

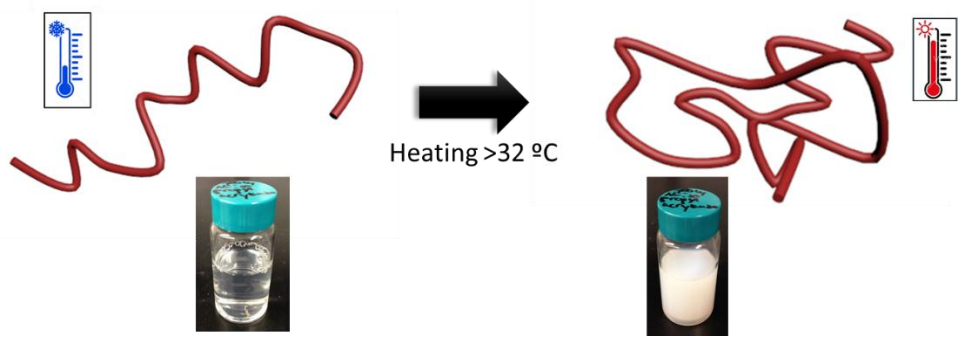


Figure 3. Schematic Illustration of the coil-to-globule phase transition of PNIPAM at 32 ºC

2. Light-responsive polymers

As you might imagine, shining light really represents a very straightforward and attractive way to trigger molecular or supramolecular events because it doesn't modify concentrations and it doesn't involve the addition of secondary components which might lead to collateral reactions. We could even say it can be precisely localized in time and space, which as we know is a very attractive feature for applications such as drug delivery, controlled enzymatic activation, etc. Considering this, within the last few decades several types of photo-responsive polymers have been developed by incorporation of several types of chromophores such as azobenzenes and spyropyrans which can undergo reversible light-triggered reactions, or o-nitrobenzyl esters which irreversibly transform themselves into a more polar species upon light irradiation.

Another strategy has been addressed by incorporation of photo-cleavable ruthenium complexes of N-heterocyclic chelating ligands, which photo cleavage is similar to those well-known o-nitrobenzyl photocage, however being the fact that the photo cleavage of Ru-bipyridine complexes can be induced by long-wave length light since they exhibit metal-to-ligand charge transfer (MLCT) bands in the visible or NIR regions. For this reason in this research we will study further the employment of these complexes as strategy to modify the solubilization of PNIPAM In a reversible way upon light irradiation. [3]

2.1.Ruthenium polypyridyne coordination complexes

Ruthenium polypyridyne coordination complexes are comprised by the central metal atom which is surrounded by ligands (or "complexing agents") which donates most of the electronic density in the coordinating bond with Ruthenium, being this the essential requirement in order to form a new bond with it. [4]

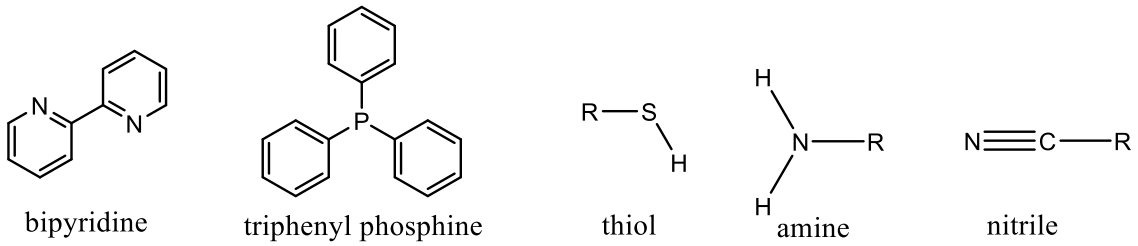


Figure 4. Some of the most common ligands in Ruthenium coordination complexes

Moreover, the bipyridines are molecules without any absorption in the visible light range, with σ-donor properties in the atoms of nitrogen, and π-donor and π*-acceptor properties from the aromatic rings. After stablishing the coordination bond, the Ruthenium accepts the electronic density from bipyridine σ-bond, while the bipyridines on the other hand accept the electronic density coming from the metal in its empty π*-bonds.

Among the photo-physical properties of the complex Ruthenium-polypyridyn complex we find its long lived excited states (usually attributed in part to a large crystal field splitting and to the metal-ligand charge transfer nature of the first excited state (See Figure 3 below), its huge absorption in the range of visible light, and its high stability in terms of thermal decomposition and photochemistry. It is worth to mention that this photochemical stability is not provided by the absence of photo-substitution reactions, but by the efficient re-capture of the leaving groups which suffer the ligands with two points of attachment to the metal atom, such as the bipyridine group.

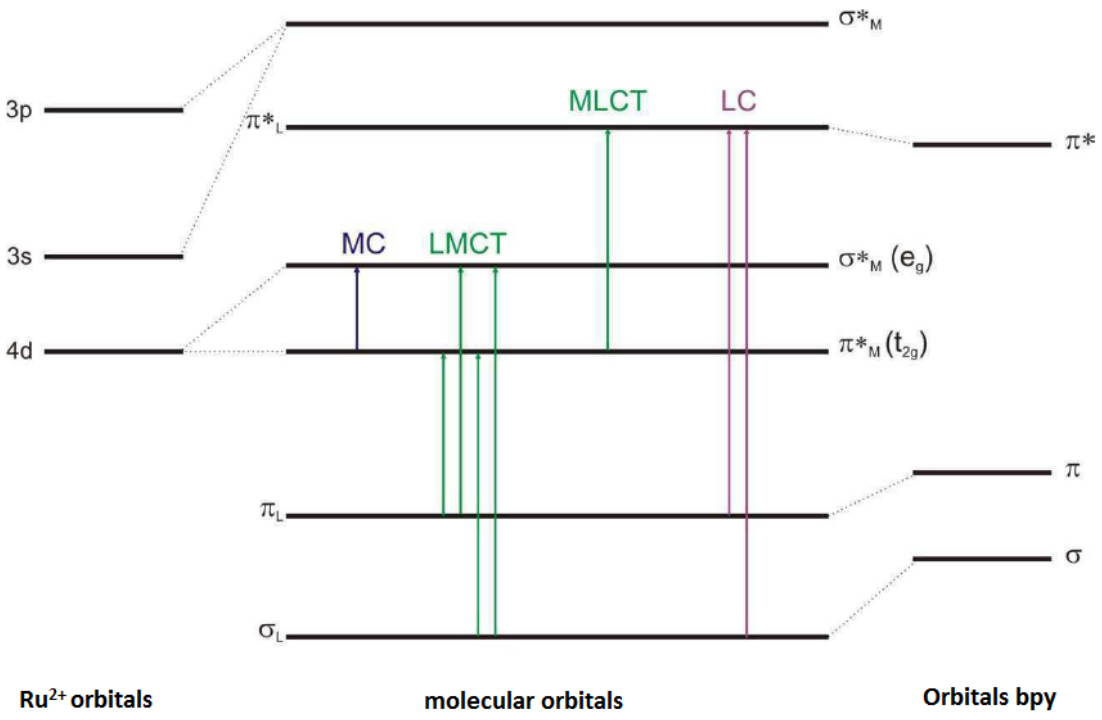
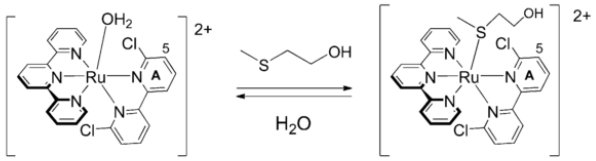


Figure 5. Simplified scheme of the energy levels corresponding to $[Ru(bpy)_3]^{2+}$

As the main previous background in this field for this research work, Roberto Etchenique et al., (5) in 2009 introduced a new ruthenium-based system comprised of ruthenium-bipyridine-triphenylphosphine which could provide repeatable temporally and spatially controlled liberation of GABA by means of Visible-light irradiation, which proved to be as efficient as the existent UV-sensitive caged GABA compounds, since can be uncaged using visible light which enables the employment of long wavelength, resulting in less scattering and allowing the light source to penetrate deeper into living tissue

In addition, in 2002, Jean-Pierre Sauvage et al. (6) reported the light-responsive supramolecular interaction based on coordination between $Ru(terpy)(dcbpy)(OH_2))^{2+}$ (dcbpy = 6,6'-dichloro-2,2'bipyridine) with 2-methylthioethanol in pure water, which bond was formed spontaneously in aqueous solution and in the dark, but was selectively broken under visible light irradiation, to afford aqua complexes. They also showed that the blue light induced shifting of the equilibrium between $RuOH_2$ and RuHmte in water for the N – N = dcbpy system, which besides could be repeated up to four cycles at room temperature without any sign of degradation.



[1]$^{2+}$ [2]$^{2+}$

Scheme 1. Equilibrium between [**1**]$^{2+}$, Hmte, and [**2**]$^{2+}$ in water.

Figure 6. Equilibrium between a methylthiol molecule and a Ruthenium complex

It is worth to mention that Ru complexes are analogs to the anticancer metallodrug cisplatin, even currently two Ru complexes are in clinical Phase II trial. However similar to platinum drugs, Ru complexes exhibit toxic side effects and it is believed, as reported by Wu et al., (wens paper) that photoactivated Ru complexes may reduce these toxic side effects, and provide an alternative to deliver the Ru complexes to a target site, since besides the uptake of Ru complexes by cancer cells can be problematic due to not passing through intact cell membranes. However incorporation of Ru into self-assembly structures could mean longer circulation times in blood and enhanced cellular internalization [3]

Redox-responsive polymers

Redox-responsive polymers are another sub-class of smart materials which have arose within the last years, and which seems very promising for drug-delivery applications among others. They consist in the incorporation of a redox-sensitive functional group into a polymer, being sulfide groups the most studied for this application due to their outstanding oxidation reactivity. [7]

At the early stage of development, redox-sensitive polymers have been mainly used to modulate permeability of vesicle membranes, for example triblock copolymer of PEG-b-poly(propylene sulfide) upon oxidation of the PPS block for vesicle disruption triggered by the increase on hydrophilicity. It is clear, although this is a very straightforward method to trigger a supramolecular interaction, very few reports have been made on this field, and even less of those not involving the employment of disulfide bridges oxidation.

The interest on this topic for this research work arise from the fact that, as it is well known, an enhanced Reactive Oxygen Species (ROS) generation is encountered at the site of inflammation processes, induced by another pathological problems such as tumors,

which causes endothelial dysfunction and tissue injury. The concept of chronic or prolonged ROS production is considered central to the progression of inflammatory disease.

ROS are partially reduced metabolites of oxygen that possess strong oxidizing capabilities, therefore deleterious to cells at high concentrations because they oxidize protein and lipid cellular constituents and damage DNA. A breakthrough would be to develop a ultra-Redox sensitive polymer, and achieve disruption of micelles upon oxidation by the ROS, changing drastically the polarity of the micelles, and release antioxidants stored in them, or dyes for imaging applications. [8]

1.2. Synthesis of Responsive Polymer Systems

In contrast to traditional polymers, in order to incorporate responsive components, it is necessary to copolymerize responsive blocks into a polymer or copolymer backbone. For this reason the preparation of well-defined polymers is essential such as employing controlled radical polymerization techniques (CRP's) which minimizes the disadvantage of FRP thus permitting the synthesis of well-defined copolymer structures, furthermore, the growing demand for well-defined and functional soft materials in a nanoscale has led to a significant increase of procedures that combine architectural control with the flexibility of incorporating functional groups. In view of these considerations, there has arose a variety of controlled polymerization strategies, such as nitroxide-mediated radical polymerization (NMRP), atom transfer radical polymerization (ATRP) and reversible addition fragmentation chain transfer (RAFT) procedures, being the latter suitable for water-soluble polymers such as PNIPAM, therefore being the one which is utilized in this research work. Details for this synthetic route are readily available in the literature and Figure summarized the principle governing this technique.

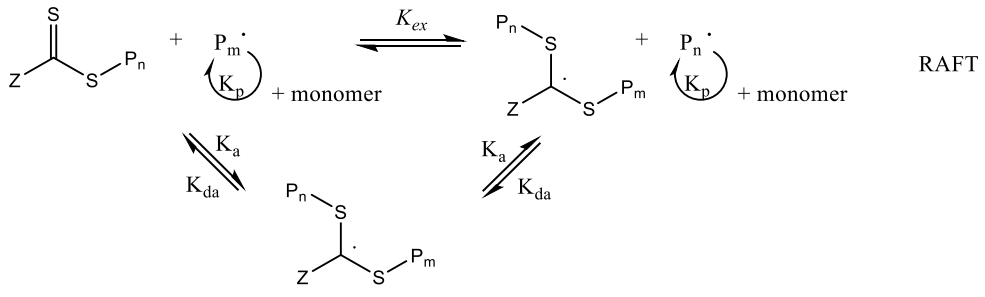


Figure 7. General mechanisms for RAFT polymerization

RAFT process involves conventional radical polymerization in the presence of a suitable chain transfer agent (CTA). The degenerative transfer between the growing radicals and the CTAs provide controlled chain growth. A wide range of structurally diverse CTAs has been reported including dithioesters, trithiocarbonates, dithiocarbonates, and xanthates. The mechanism of the RAFT process is composed of the same three main strps as that of conventional free-radical polymerization, initiation, propagation and termination, but additionally the propagation step in RAFT consist in two stages:, the RAFT pre-equilibrium and the main RAFT equilibrium. The first stage involves the activation of all added CTA along with some degree of propagation, while the second stage consists of chain equilibrium and propagation. [10]

Objective

The general objective of this research work is to develop dually responsive polymer systems based on PNIPAM by incorporation of thioethers alternatively, which are sensitive to oxidation and coordination of photocleavable Ruthenium-polypyridyl complexes, and utilize this for modifying the inherent LCST property of PNIPAM at 33.5 ^{o}C.

Specific objectives

1. Copolymerize PNIPAM with thioethers-containign monomers via RAFT polymerization
2. Attempt to form a coordinating bond with Ruthenium complex
3. Study the oxidation sensitivity of this thioethers groups.
4. Measure the influence on the LCST of these PNIPAM copolymers

Hypothesis

1. We believe that the hydrophobicity of the methylthiol segment groups will decrease the LCST of PNIPAM itself prior oxidation/Ru coordination.
2. We believe that a stable coordinating bond will be formed between our Ruthenium-polypirdyl complex, and which could be photocleaved by employing light of appropriate wavelength. The cationic nature of the Ru-complex itself should increase the hydrophilicity of PNIPAM, and therefore increase the LCST value,
3. We believe that upon oxidation, the polarity of the thiol group will increase in a great amount and increase the LCST of PNIPAM close to its original value of 33 ^{o}C.

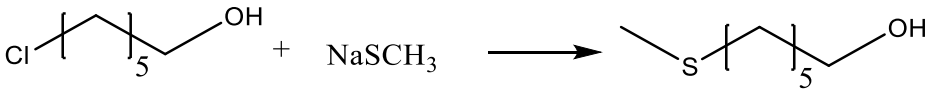
Methodology

For the development of this thesis we synthesized different novel PNIPAM copolymers containing segments of thioethers alternated, with different length of alkyl chain and at different molar ratios (5 & 10%). Besides we synthesized two different Ruthenium-polypyridine complexes according to the previously reported in the literature, and we investigated the sensitivity of this copolymers upon oxidation and Ruthenium complex coordination in order to modify the LCST of PNIPAM for future drug/antioxidant delivery applications

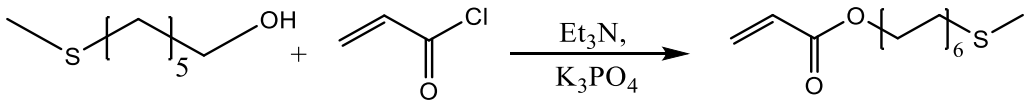
1. Synthesis of Reagents

All reagents were obtained commercially and used without prior purification. All synthesis which are presented below were following the reported in the literature for similar systems. All synthesis were carried out by using Schlenk techniques and under nitrogen atmosphere. All NMR spectra and GPC traces can be found in the Supporting Information.

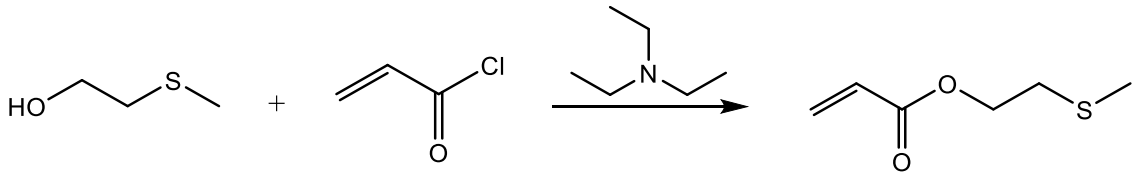
Synthesis of 6-methylthio)hexan-1-ol. To a stirred aqueous solution of sodium mercaptide (111.1mmol) was added to 6-clorohexanol (37.2mmol) and let stirring at room temperature for 48h and then extracted with chloroform. The organic phase was collected and dried over anhydrous K_2CO_3. Following filtration, the filtrate was concentrated under reduced pressure yielding a yellow oil (90%)



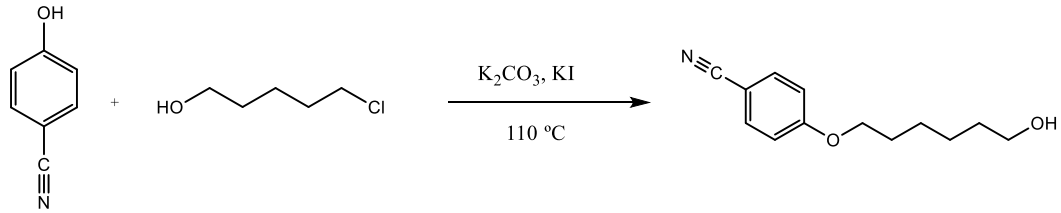
Synthesis of 6-(methylthio)hexyl acrylate: A mixture of 6-(methylthio)hexan-1-ol (27.3mmol) and triethylamine (54.6mmol) was dissolved in dichloromethane (50mL) and cooled to 0ºC. To this mixture, a solution of methacryloyl chloride (32.7 mmol) in diclorometh ane was added dropwise within a period of 20 min. The reaction mixture was then stirred for 48h at room temperature. Subsequently, 30 mL of cold water were added to the flask and the organic phase was washed with aqueous solution at $NaHCO_3$ (3 x 20 mL). After drying over $MgSO_4$ the raw product was concentrated under reduced pressure and purified by column chromatography on silica gel using dichloromethane as eluent to give a yellow oily liquid (1.5g, 50%)



Synthesis of 2-(methylthio)ethyl acrylate (Figure): 2-(methylthio)ethanol (2.38g, 27.3mmol) and triethylamine (4.57ml, 54mmol) were dissolved in 100 ml of dry dichloromethane. The mixture was stirred at room temperature. After that acryloyl chrloride (3.42g, 30mmol) was added dropwise to the flask among 30 min. The mixture was kept stirring at room temperature for 48 h. Subsequently, 30 mL of cold water were added to the flask and the organic phase was washed with aqueous solution at NaHCO3 (3 x 20 mL), and finally dried with $MgSO_4$, filtered and concentrated in the rotating evaporator.

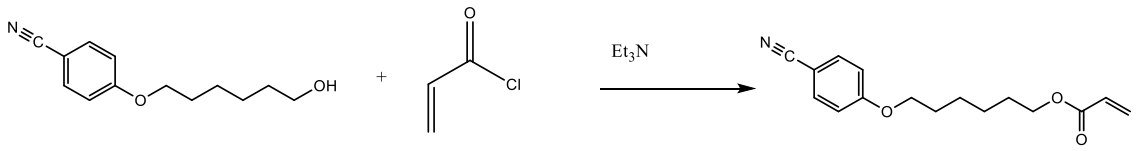


Synthesis of 4-((6-hydroxyhexyl)oxy)benzonitrile (Figure S3): 4-Hydroxybenzonitrile (7.14 g, 60.0 mmol), K_2CO_3 (8.28 g, 60.0 mmol), KI (0.05 g) were dissolved in N,N-dimethylformamid (DMF) (40 mL). The mixture was heated to 110 ℃. After that, DMF (8 mL) containing 6-chlorohexanol (10 mL, 0.07 mol) was added dropwise to the flask. The mixture was kept 110℃ for 24 h, cooled to room temperature and filtered. The solvent was evaporated under reduced pressure and the obtained crude product was purified through column chromatography (eluent: ethyl acetate/ petroleum ether = 1/1 to 2/1) to get a white solid (12.0 g, 91%)

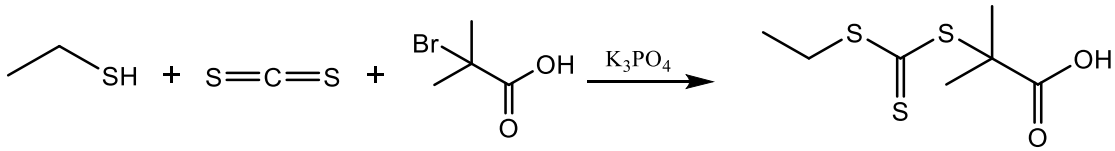


Synthesis of 6-(4-cyanophenoxy) hexyl acrylate (CPH, Figure S3): 4-((6-Hydroxyhexyl)oxy)benzonitrile (9.60 g, 43.8 mmol) and triethylamine (10 mL) into THF (200 mL) under an ice-bath condition. Then, a solution of acryloyl chloride (4.3 mL, 46.1 mmol) in 150 mL THF was added into the mixture dropwise. After that, the solution was stirred over night at room temperature. The solvent was evaporated under reduced

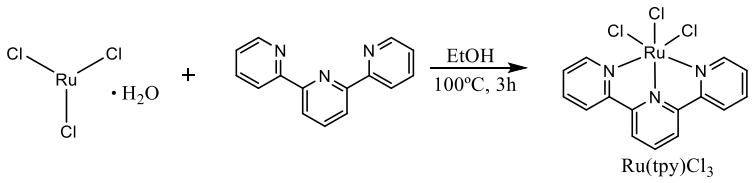
pressure. Then, the crude product was purified by column chromatography (eluent: ethyl acetate/petroleum ether = 1/15 to 1/5) to get a white waxy solid (10.6 g, 85%).



Synthesis of *S*-Ethyl-*S'*-(*α,α'*-dimethyl-*α''*-acetic acid)trithiocarbonate: A flame-dried flask was charged with K_3PO_4 (0.13mol) and dry acetone (22 mL). Ethanethiol (0.13mol) was added ant the mixture was stirred for 10 mn. Carbon disulfide (28.77mol) was then added dropwise and the mixture was stirred for 10 min. After the solution turned bright yellow, 2-bromo isobutyric acid (0.031mol) was added and the mixture was stirred for 48 h. The mixture was then concentrated and the crude material was dissolved in CH_2Cl_2. The organic layer was washed with 1N HCl, water and brine, dried with sodium sulfate and concentrated. The residue was purified by column chromatography using a mixture of Ethyl acetate/Hexane (3:1) as eluent to afford a yellow solid.

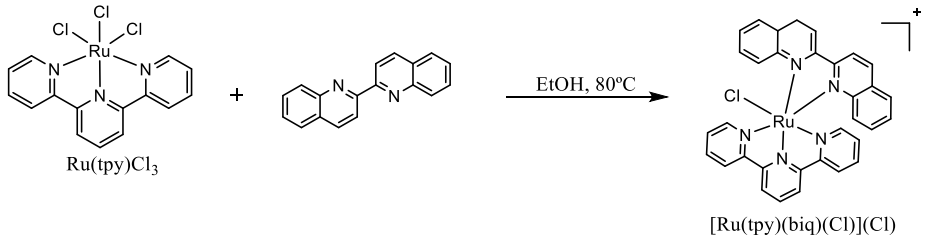


Synthesis of Ru(tpy)Cl$_3$: $RuCl_3 3H_2O$ (262 mg, 1.0 mmol) and 2,2′:6′,2″-terpyridine (tpy, 233 mg, 1.0 mmol) was mixed in absolute ethanol (150 mL). The mixture was heated at reflux for 3 h with vigorous magnetic stirring. The mixture was then cooled to room temperature. Fine brown powders appeared and were filtered from the reddish yellow solution. The product was washed with ethanol and diethyl ether, and air-dried (850 mg, 85%).



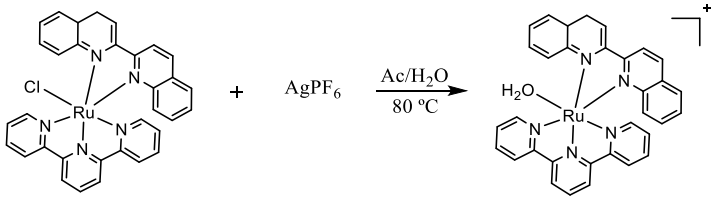
Synthesis of R1: [Ru(tpy)(biq)(Cl)]Cl: [Ru(tpy)(biq)(Cl)]Cl was synthesized according to the literature.[1] $Ru(tpy)Cl_3$ (173 mg, 0.39 mmol) and 2,2'-biquinoline (biq, 100 mg, 0.39 mmol) were mixed in 3:1 ethanol/H_2O mixture (20 mL) and the solution was bubbled with argon for 5 min. Then, trimethylamine (0.094 mL, 0.68 mmol) was added to the mixture. The reaction mixture was refluxed under argon for 7 h in the dark. After that, the mixture was filtered hot and the filtrate was evaporated under reduced pressure. The

product was purified by column chromatography with silica gel (eluent: methanol/dichloromethane =1:8 to 1:5). The solvent was evaporated and the product was obtained as violet powders (90 mg, 35%).



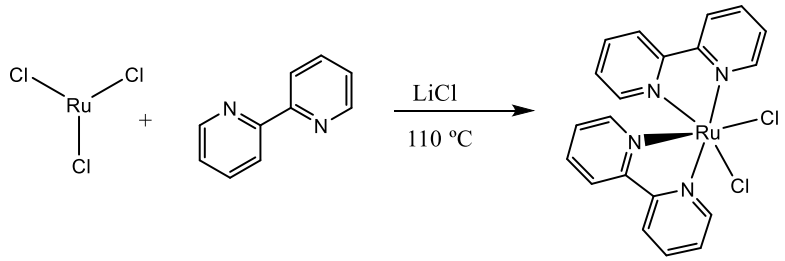
$Ru(tpy)Cl_3$ [Ru(tpy)(biq)(Cl)](Cl)

Synthesis of $[Ru(tpy)(biq)(H_2O)](PF_6)_2$: $[Ru(tpy)(biq)(H_2O)](PF_6)_2$ was synthesized according to the literature.[2] [Ru(tpy)(biq)(Cl)]Cl (62 mg, 0.094 mmol) and $AgPF_6$ (53 mg, 0.201 mmol) were dissolved in 3:1 acetone/H_2O mixture (8 mL). The solution was degassed and heated under reflux in an argon atmosphere for 2 h. The solution was cooled and filtered to remove AgCl. The solvent of the reaction was reduced to ~2 mL. Then, an aqueous solution of KPF_6 was added. The precipitate was filtered, washed with H_2O, and dried to give a purple solid (62 mg, 73%).

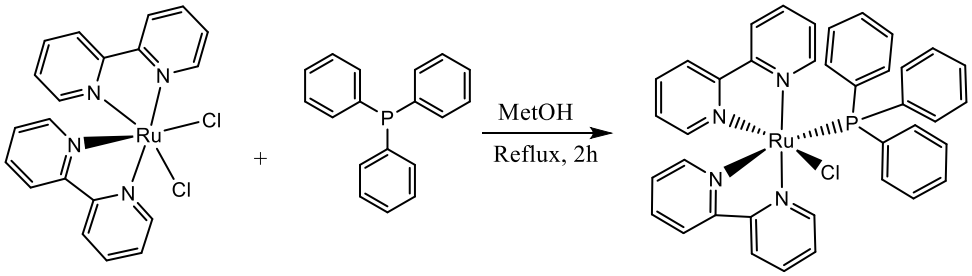


[Ru(tpy)(biq)(Cl)](Cl) [Ru(tpy)(biq)(H_2O)](PF_6)

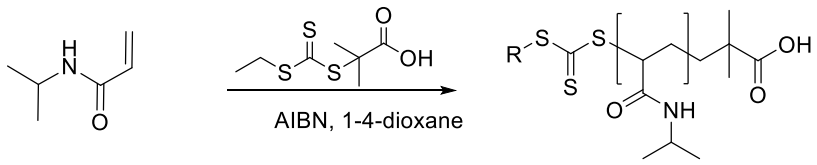
Synthesis of $[Ru(bpy)_2Cl_2]$: The synthesis following the reported in the literature [17]. To a solution of LiCl (0.93g, 22 mmol) in degassed ethanediol-water (4 mL. 3:1) was added $RuCl_3$ and heat to 110 ºC (1 g, 4,1 mmol) under argon atmosphere. To a resulting slurry was added after 15 min, 2,2' bipyridine (1.3 g, 8.3 mmol), then after 15 min, glucose (0.15 g, 8 mmol), then after 15 min L-ascorbic acid (0.37 g, 2.2 mmol) was added. The mixture was kept at 110 ºC for 30 minutes and cooled down before addition of brine, then kept at 0 ºC for 1 h. A crude complex was recovered by filtration, washed twice with brine and with a mixture of toluene/ether/acetone at a ratio of 70:25:5. After filtration the black solid was sonicated in DCM and filtrated. This procedure was repeated until the filtrate was almost colorless. Furthermore the solvent was removed and obtained the compound as a microcrystalline black-purple powder.



Synthesis of R2: [Ru(bpy)$_2$PPh$_3$Cl]Cl: The synthesis was carried out following the reported in the literature [18]. Ru(bpy)$_2$Cl$_2$ (682 mg, 1.3mmol) was dissolved in methanol (45 mL). Triphenylphosphine (410 mg, 1.5 mmol) was added, and the mixture was stirred until complete dissolution. After this, water (20 mL) was added, and the mixture was heated at reflux for 2 h. Once the reaction was complete the solution was concentration by solvent evaporation. The solid was suspended in acetone (50 mL), which first produced the dissolution of [Ru(bpy)$_2$PPh$_3$Cl]$^+$ and then precipitation of [Ru(bpy)$_2$PPh$_3$Cl]Cl. The solution was kept at 0 ºC for one hour before filtration. The red solid was washed with acetone and diethyl ether.

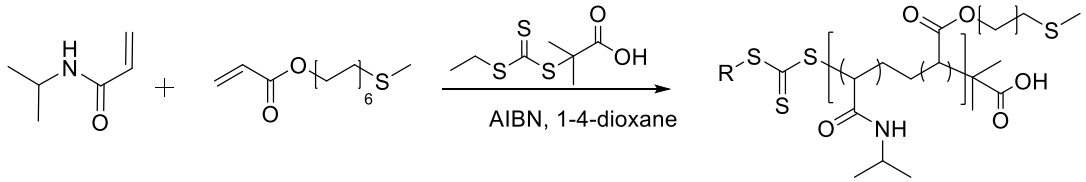


Synthesis of P1: Homopolmer of Poly(N-isopropylacrylamide): The synthesis of an homoPNIPAM was carried out to use a Reference material. The polymerization was carried out using standard Schelnk techniques. N-isopropyl acrylamide (7.5mmol), 0.129 mmol of *S*-Ethyl-*S'*-(*α,α'*-dimethyl-*α*''-acetic acid)trithiocarbonate, 7mg of AIBN and 15 mL of pre-distilled 1,4-dioxane were added to the Schlenk tube. The solution was degassed by three cycles of freeze-drying and later saturated by Argon. The schlenk flask was placed in a 70 ºC bath to initiate the polymerization. After 24h the reactions were stopped by exposure to oxygen. The solvent was evaporated under reduced pressure and precipitated from THF in an excess of Hexane. The product was dried in a vacuum overnight at room temperature (950 mg, 80%) The molecular weight (Mn) and molecular weight distribution (Mw/Mn) was determined by GPC. The degree of polymerization (DP) was determined by 1H NMR (See Supporting information)



Synthesis of P2 and P3 (10%/5% NIPA/thiol respectively): PNIPAM-co-Poly(6-[methylthio]hexyl acrylate): Polymerizations were carried out using standard Schlenk techniques. 6-(methylthio) hexyl acrylate (0.84mmol (10%) or 0.42mmol (5%)), N-isopropyl acrylamide (7.5mmol), 0.129 mmol of *S*-Ethyl-*S'*-(*a,a'*-dimethyl-*a''*-acetic acid)trithiocarbonate, 7 mg of AIBN and 15 mL of pre-distilled 1,4-dioxane were added to the Schlenk tube. The solution was degassed by three cycles of freeze-drying and later saturated by Argon. The Schlenk flask was placed in a 70 ºC bath to initiate the polymerization. After 24h the reactions were stopped by exposure to oxygen. The solvent was evaporated under reduced pressure and precipitated from THF in an excess of Hexane. The product was dried in a vacuum overnight at room temperature (950 mg, 80%) The molecular weight (Mn) and molecular weight distribution (Mw/Mn) was determined by GPC. The degree of polymerization (DP) was determined by 1H NMR (See Supporting information)

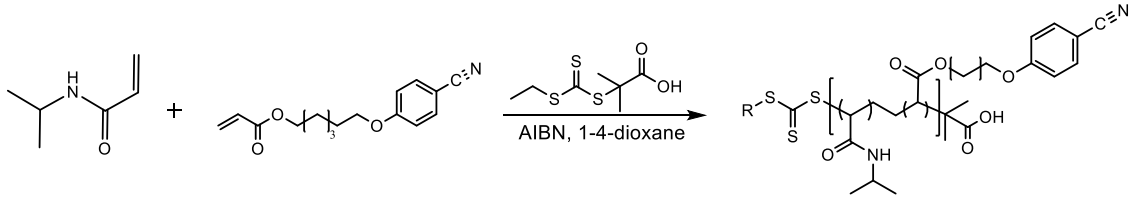
The NIPAM/6-(methylthio) hexyl acetate ratio were calculated by comparing the integrals of the methyl proton peaks of the 6-methylthio hexyl acetate (2.0 ppm, 3H) and those of the methane proton on the PNIPAM (4.0 ppm, 1H). Polymers were purified by dialysis against H_2O for 72 h.



Synthesis of P4: Synthesis of PNIPAM-co-(6-[4-cyanophenoxy] hexyl acrylate): Polymerizations were carried out using standard Schlenk techniques. 6-(4-cyanophenoxy) hexyl acrylate (0.84mmol), N-isopropyl acrylamide (7.5mmol), 0.129 mmol of *S*-Ethyl-*S'*-(*a,a'*-dimethyl-*a''*-acetic acid)trithiocarbonate, 7mg of AIBN and 15 mL of pre-distilled 1,4-dioxane were added to the Schlenk tube. The solution was degassed by three cycles of freeze-drying and later saturated by Argon. The schlenk flask was placed in a 70 ºC bath to initiate the polymerization. After 24h the reactions were stopped by exposure to oxygen. The solvent was evaporated under reduced pressure and

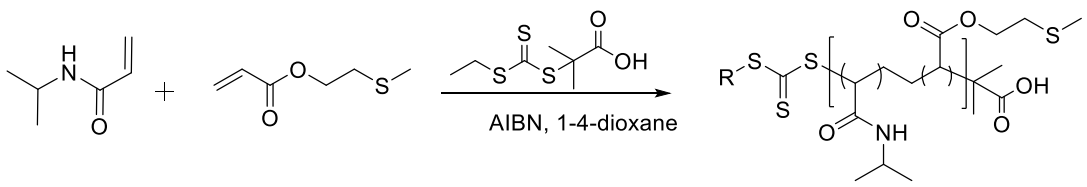
precipitated from THF in an excess of Hexane. The product was dried in a vacuum overnight at room temperature (900 mg, 75%) The molecular weight (Mn) and molecular weight distribution (Mw/Mn) was determined by GPC. The degree of polymerization (DP) was determined by 1H NMR (See Supporting information)

The NIPAM/6-(methylthio) hexyl acetate ratio were calculated by comparing the integrals of the benzylic proton peaks (7.5 ppm, 2H) and those of the methane proton on the PNIPAM (4.0 ppm, 1H). Spectra is shown in supporting information. Polymers were purified by dialysis against H_2O for 72 h.

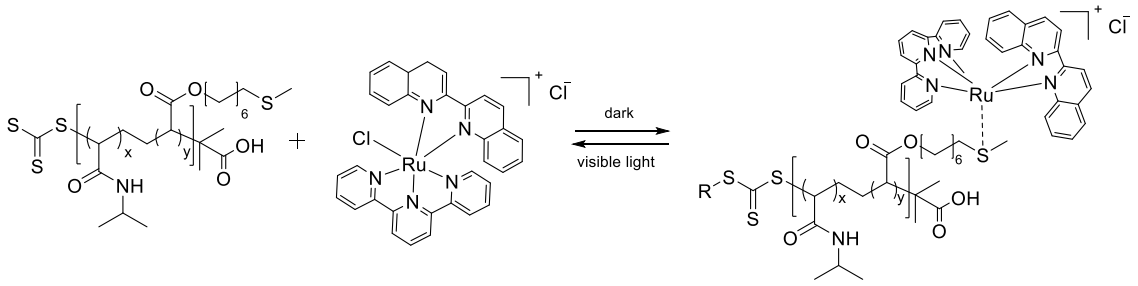


Synthesis of P5 and P6: PNIPAM-co-Poly(2-[methylthio]ethyl acrylate): Polymerizations were carried out using standard Schlenk techniques. 6-(methylthio) hexyl acrylate (0.84mmol (10%) or 0.42mmol (5%)), N-isopropyl acrylamide (7.5mmol), 0.129 mmol of *S*-Ethyl-*S'*-(*a,a'*-dimethyl-*a*''-acetic acid)trithiocarbonate, 7 mg of AIBN and 15 mL of pre-distilled 1,4-dioxane were added to the Schlenk tube. The solution was degassed by three cycles of freeze-drying and later saturated by Argon. The Schlenk flask was placed in a 70 ºC bath to initiate the polymerization. After 24h the reactions were stopped by exposure to oxygen. The solvent was evaporated under reduced pressure and precipitated from THF in an excess of Hexane. The product was dried in a vacuum overnight at room temperature (950 mg, 80%) The molecular weight (Mn) and molecular weight distribution (Mw/Mn) was determined by GPC. The degree of polymerization (DP) was determined by 1H NMR (See Supporting information)

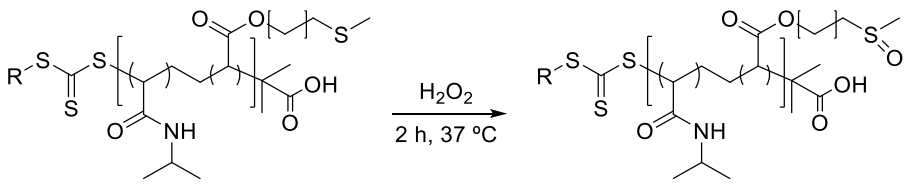
The NIPAM/methylthio ratio were calculated by comparing the integrals of the methyl proton peaks of the 6-methylthio hexyl acetate (2.0 ppm, 3H) and those of the methane proton on the PNIPAM (4.0 ppm, 1H). Polymers were purified by dialysis against H_2O for 72 h.



Ruthenium coordination to methylthiol containing PNIPAM: The corresponding copolymers and Ruthenium compounds were mixed in a mixture of acetone/water 1:3 (20 mL). in the presence of $AgPF_6$ in 1:1 ratios. The solution was degassed for 5 min. The mixture was stirred under argon for 48h in the dark at 80 ºC. After that the solvent was evaporated under reduced pressure, and the precipitated AgCl was removed via filtration.



Oxidation of PNIPAM copolymer: The oxidation the copolymers was carried out by means of H_2O_2 at 1-wt% in distilled water at 37.5 ºC for 30 minutes. Furthermore In order to gain insight of the oxidation efficiency we carried out the same conditions in an NMR tube and using deuterium oxide as solvent.



2. Characterization

The theoretical molecular weight Mn_{th} at conversion χ was calculated by eq 1

$$Mn_{Th} = \frac{M_{MW}[CTA]_0\chi}{[CTA]_0} + CTA_{MW} \quad (1)$$

In which M_{Mw} and CTA_{MW} are the formula weights of monomer and CTA; $[CTA]_0$ and $[M]_0$ are the initial CTA and monomer concentrations.

NMR spectra were recorded on a Bruker AV250 NMR spectrometer operated in the Fourier transform mode. The molecular weight of the resultant copolymers, and the ratio between NIPA/methylthiol were calculated by comparing the integral areas of the characteristic peaks.

The molecular weight characteristics were determined using a PSS-WinGPC (PSS) (pump: alliance GPC 2000) equipped with UV and RI detectors running in THF at 30 ºC and a PLgel MIXED-B column (particle size: 10 mm, dimension: 0.8 x 30 cm) calibrated using PS standards.

The UV-vis-NIR absorption spectra were measured on a Lambda 900 spectrometer (Perkin Elmer). The fluorescence spectra were recorded on a TIDAS II spectrometer (J&M).

Chapter V: Findings and Discussion

In the following part of this research work we will address the most relevant results obtained, and a discussion of them. This will be divided in two parts, being as follows:

1. Part I: Development of a light-responsive PNIPAM via Ru complex functionalities
2. Part II: Development of a Redox-responsive PNIPAM via a selective thioethers oxidation.

Part I. Developing a photoresponsive PNIPAM via Ru complex functionalities

As it has been mentioned along the thesis, the objective was to develop a PNIPAM which could coordinate with Ru-polypyridyne complexes in order to utilize their cationic properties to enhance the solubility of the polymers, and which behavior could be cleaved simply by irradiating light of appropriate wavelength. In the Figure 8 below, a schematic representation of this is presented.

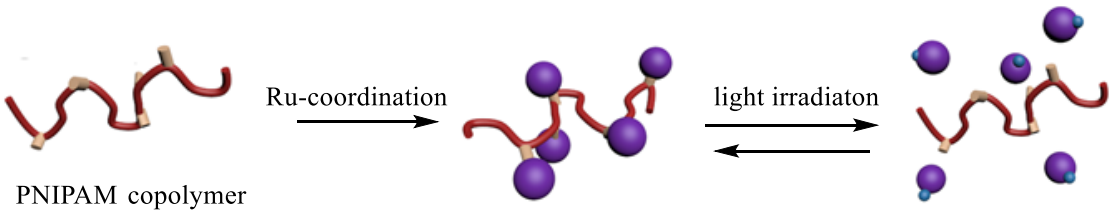


Figure 8. Schematic representation of the light- thermoresponsive PNIPAM copolymers

A potential application attempted for this project dealing with developing a light-thermoresponsive polymer which hydrophilicity increased considerably by coordinating the Ruthenium complexes due to their cationic nature, and which after light irradiation lose this hydrophilicity; was to, after copolymerizing with a hydrophobic polymer, could be to form micelles which could eventually be collapsed by light irradiation, useful for

specific targeting in drug delivery applications. Figure 9 shows an schematic representation of the expected phenomena.

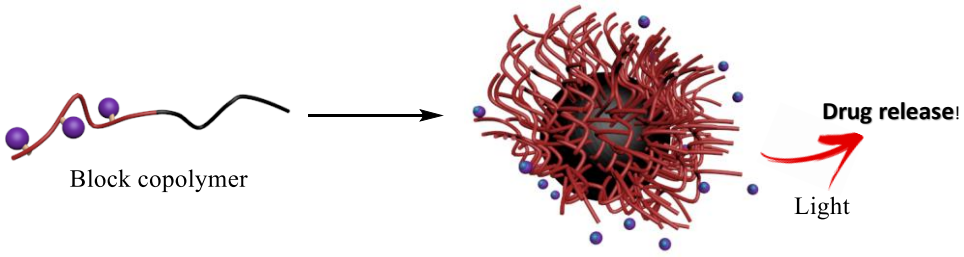
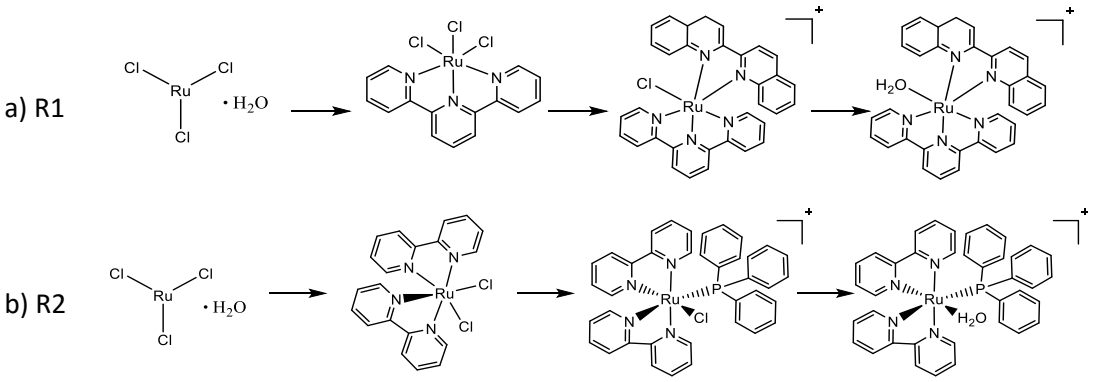


Figure 9. Potential applicaton in drug delviery as photo-controlled micellation

On the other hand, as it is known in order to achieve a successful coordination from Ruthenium complexes, it is necessary to have ligands which are able to donate most of the electronic density in the coordinating bond with Ruthenium, therefore in a very first stage of this project, we investigated two different Ruthenium complex functionalities with different absorption spectrum in the range between 400-600 nm, corresponding both to the Visible-light range (see Figure 10) and their coordination with two small organic molecules containing cyano and thiol group respectively in order to elucidate which functional group could be more suitable for the attempted applications we were looking forward to.



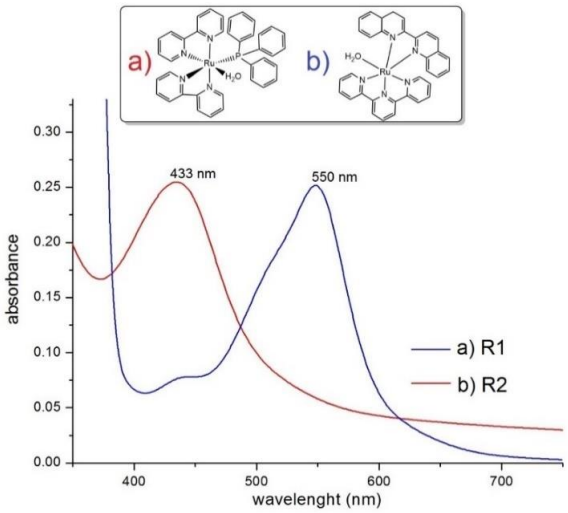


Figure 10. Different synthesis of Ruthenium complex and their corresponding absorption spectrum. A) R1: $[Ru(tpy)(biq)(Cl)](PF_6)$, b) $[Ru(tpy)(biq)(H_2O)](PF_6)_2$

It was rapidly that we realized that for small organic molecules both Ruthenium complexes could form robust coordination bonds, as suggested in the literature, which they could form even at room temperature, but was promoted at reflux temperatures and in absence of oxygen which could oxidize the Ruthenium complexes themselves.

Considering this we proceeded to synthesize acrylates monomers containing each functional group, both cyano and thiol (See Figure 11, a) 6-(methylthio)hexyl acrylate and b) 6-(methylthio)hexyl acrylate) which both contained an alkylic chain of 6 carbons, in order to prevent the steric hindrance of NIPA which could block the coordination of the Ruthenium complex; for later polymerize them with NIPA by means of RAFT polymerization, employing *S*-Ethyl-*S'*-(*a*,*a*'-dimethyl-*a*''-acetic acid)trithiocarbonate as RAFT agent, which is compatible with both acrylate and acrylamide functional group.

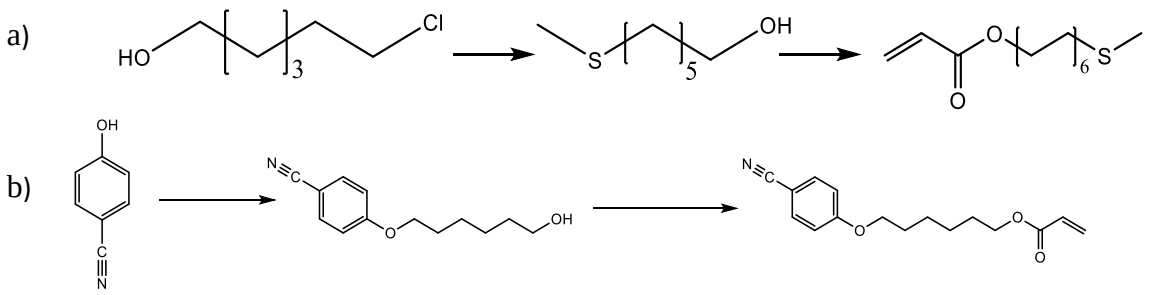


Figure 11. Synthesis of the functional monomers, a) 6-(methylthio)hexyl acrylate b) 6-(4-cyanophenoxy) hexyl acrylate

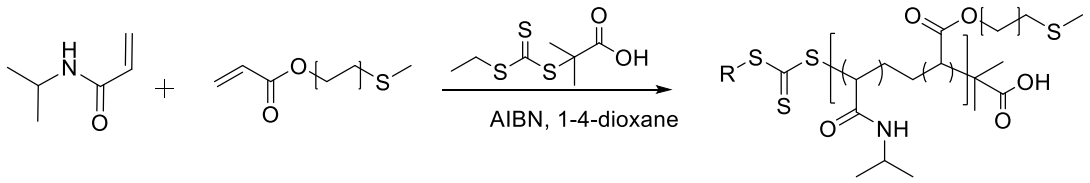


Figure 12. Co-polymerization of PNIPAM with 6-(methylthio)hexyl acrylate

The general features of the synthesized polymers are summarized in Table 2. In general the obtained polymers presented a molar mass distribution of around 10,000 g/mol in all cases, and a rather low polydispersity lower to 2.0 in all cases, characteristic of controlled radical polymerization. Furthermore this narrow polydispersity suggested a quasi-equal reactivity of NIPA in comparison to the synthesized functional monomers, implying that alternating copolymers could be expected, as desired. A method to be sure of this would be to calculate the reactivity ratio by means of the Finemann-Ross equation, however was out of the scope of this research work.

Table 2. General features of the synthesized polymers

Run[a]	Composition[b]	M_n (10^{-4})[c]	PDI[d]	LCST
P1	Homo-PNIPAM	1.01	1,16	--
P2	$NIPA_{127}MTHA_8$	1.86	1,13	3 ºC
P3	$NIPA_{125}CPHA_6$	1.13	1,67	Below 0ºC

[a]Polymer 1: Homo-PNIPAM; Polymer 2: PNIPAM-co-poly(6-[methylthio]hexyl acetate; Polymer 3: PNIPAM-co-(6-[4-cyanophenoxy] hexyl acrylate); [b]Composition determined by means of NMR; [c]Number molar mass determined by NMR; [d]Polydispersity index measured by SEC

Furthermore, the polymers were dissolved in a mixture of Acetone/H_2O (acetone is required since the copolymers are not soluble at room temperature in water, see LCST value) and mixed with the Ruthenium complexes in presence of $AgPF_6$, in order to exchange the counter ion from Cl^- to PF_6 in situ, and later the coordination was studied by means of UV-spectrometer, see Figure 13.

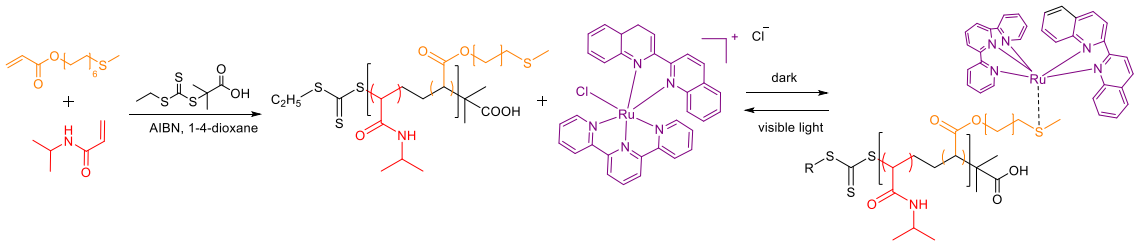


Figure 14. Schematic illustration of the synthesis of the PNIPAM copolymer, proceeded by the coordination with one of the Ruthenium complexes

As result, we observed that in the case of the R1:$[Ru(tpy)(biq)(H_2O)](PF_6)_2$ no coordination was established with any of the polymers, but R2: $[Ru(bpy)_2PPh_3Cl]Cl$ did coordinate with both, thiol and cyano functionalized PNIPAM, however in the case of cyano group, the yield of coordination was pretty low, as it can be observed in Figure 15, while in the case of the thio-containing PNIPAM a roughly 100% efficient coordination was observed.

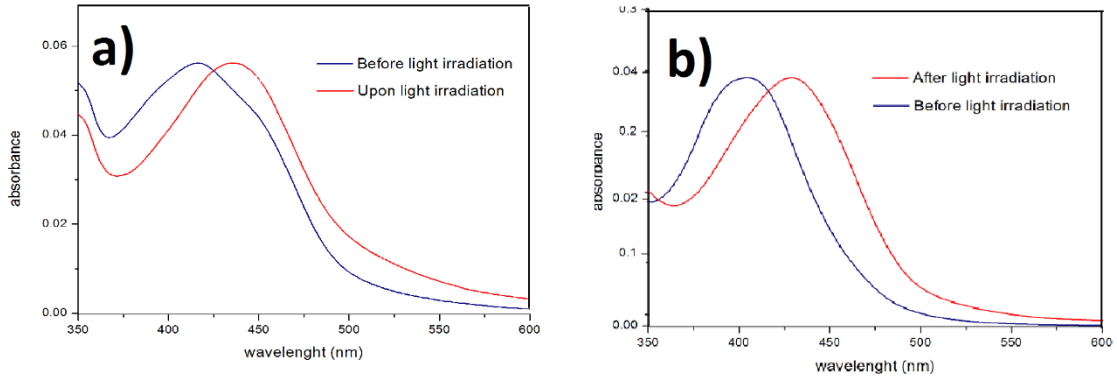


Figure 15. Coordination of R2 with both polymers; a); Polymer 3: PNIPAM-co-(6-[4-cyanophenoxy] hexyl acrylate); b) Polymer 2: PNIPAM-co-poly(6-[methylthio]hexyl acetate

The results implied that although our polymerization had been successful, and the functional groups were readily available for coordination as we expected, the hydrophobicity of the cyanophenoxy group, a) in Figure 15, proved that the coordination wasn't efficient at all, therefore we consider to take the methylthiol containing copolymer for the further investigations.

Later, in order to gain insight about how efficient was this coordinating bond between our Ruthenium complex and the PNIPAM copolymer, we made a comparison with a small organic molecule (see Figure 16), to gain feedback of the influence of the great steric hindrance in the polymer.

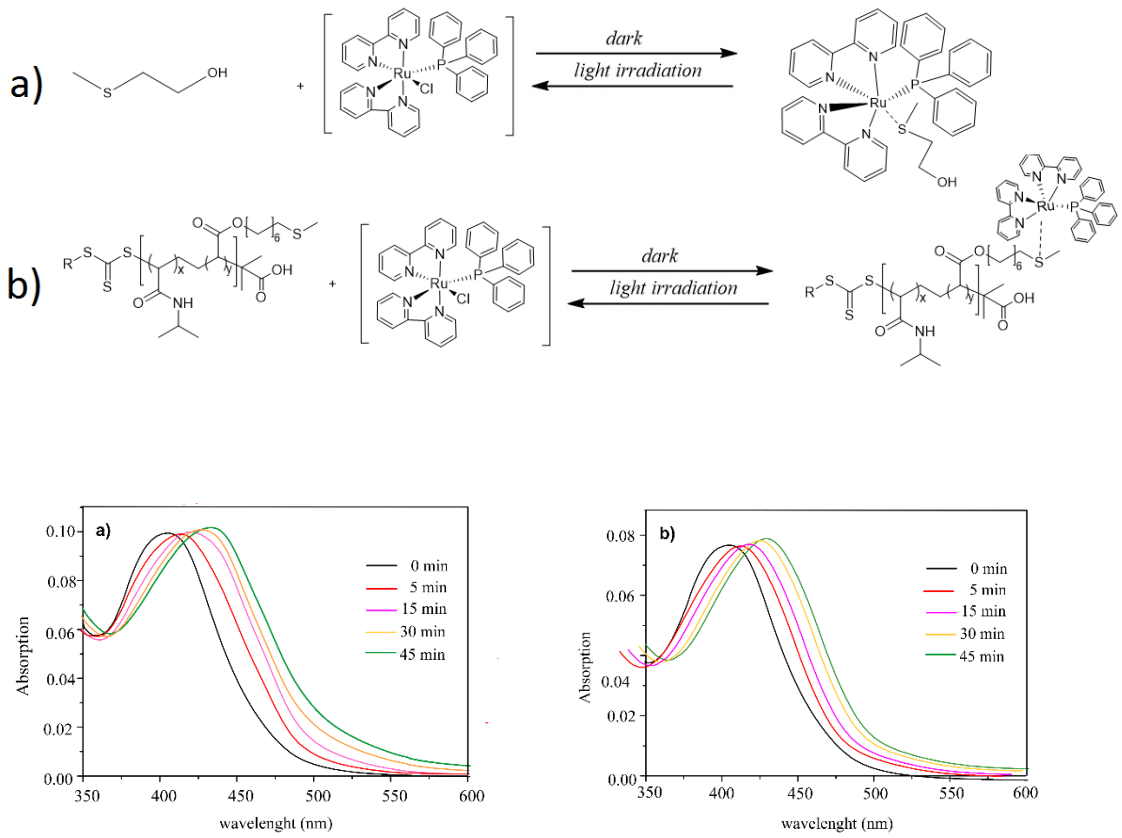


Figure 16. Cleavage of the coordination bond after blue light irradiation at different times. A comparison between a small organic molecule and the synthesized copolymer.

We found that at this conditions, the coordination with both the organic molecule and polymer itself yielded almost 100% of efficiency, and as we observe in Figure 16, by irradiation of blue light, we could observe total cleavage after 45 minutes, when we reached an equilibrium. This implied us that at Room temperature and using Acetone/H_2O mixture as solvent, the steric hindrance of the polymer was not significant, probably because the conformation of the incorporated methylthio chains was extended.

After cleavage we left the polymers in the dark for 2 hours, in order to investigate how reversible this bond was in each system. In Figure 17 a deconvolution of the UV-spectra Is shown, which was was carried out in order to gain an approximate notion of the

percentage of Ru-complex which coordinated again after cleavage. This is based on the assumption of that the observed spectra is a superposition of both populations, and that the maximum absorption peak for a pure Ruthenium complex is at 433 nm, as observed in Figure 10.

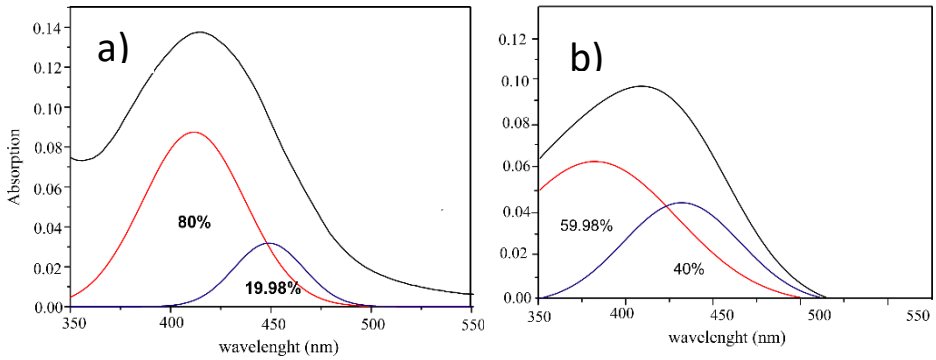


Figure 17. Deconvolution of the corresponding UV-spectra. A) Ru-MTE B) Ru-PNIPAM

As a result we observed that after 2 hours in the dark, the small organic molecule was coordinated again in a 80% approximately, while the Polymer in only 60%, implying that the rate of re-coordination after cleavage is much lower for the polymer.

Later, we proceeded to investigate the LCST of the polymers, to observe it there was any influence in the solubility of the polymer by incorporating this cationic Ruthenium complexes. However. unfortunately, it was found impossible to isolate the Ru-coordinated polymer for further characterization since the coordination should be done in a mixture of Acetone/H2O at reflux temperatures, and the LCST its 3 ºC, which means that in pure water the polymer would collapse and the coordination would be impossible; and since after coordination the acetone had to be removed, however even though the temperature was kept at room temperature, it was found that by removing it the coordination Ru-Polymer was cleaved, which was also promoted significantly by increasing the temperature, which well, it indicates that the rate of substitution of the aqua ligand by the polymer was increased with more sterically hindered complexing agents, in this case the PNIPAM copolymer, but also that by removing the acetone and thus decreasing the polarity of the solvent, the hydrophobic interaction to the methylthiol segments in PNIPAM was proved to be stronger, making them collapse and go into the

core of the polymer backbone, therefore cleaving any coordination bond with the Ruthenium.

Part II. Development of Redox-responsive PNIPAM

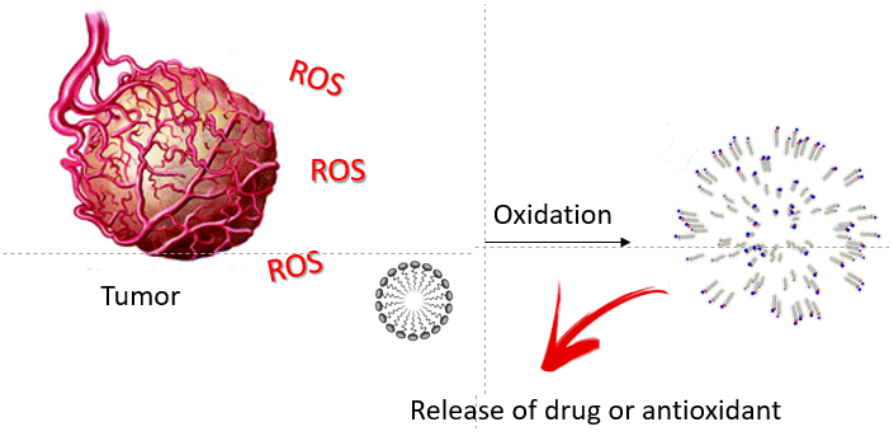


Figure. Possible application as antioxidant carrier in order to

Thioether can selectively be oxidized to sulfoxide or sulfone moieties, which leads to a strong increase in their dipole moment. Consequently a significant change in the polarity of the PNIPAM copolymers upon oxidation is expected, which should be reflected in their solubility properties in aqueous solution, as in their LCST.

We employed two kinds of monomers for this purpose, which contained, as all along this research work, a methylthiol group in their chemical structure, but vary in their alkylic chain length, (see Figure), being one with an alkyl chain of 6 carbons, and other with 2 carbons, This we did it in order to measure the extent of influence over solubility properties upon oxidation, since by increasing the alkyl chain the hydrophobicity increases, and which effect is offset upon oxidation.

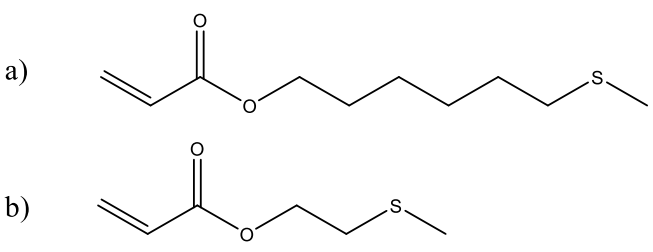


Figure. Different synthesized methylthiol acrylate monomers

Later we copolymerized this monomers into PNIPAM at a molar ratio of 10 and 5% NIPA/monomer respectively by means of RAFT polymerization, using *S*-Ethyl-*S'*-(*a,a'*-dimethyl-*a*''-acetic acid) trithiocarbonate as RAFT agent, which was synthesized according to the previously reported in the literature, and we proceeded to oxidize them using H_2O_2 as oxidizing agent at 37 ºC, simulating the most encountered ROS near a tumor and at the body temperature during 2 hours, and measure the solubility prior and upon oxidation. In figure this process is schematized.

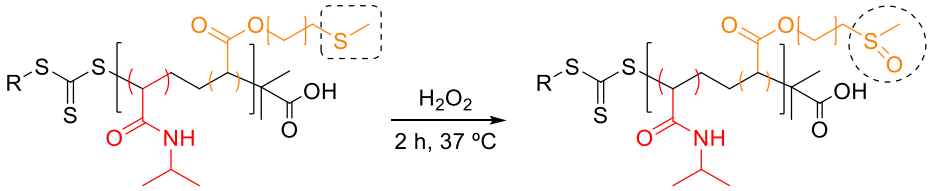


Figure. Schematic Illustration of the oxidation process of the synthesized copolymers

At first, in order to gain insight into the redox-sensitivity of the PNIPAM copolymers we first monitored the oxidation with H_2O_2 in an NMR tube. Experiments were performed in D_2O at 37ºC with 3%-wt H_2O_2 solution during 10 minutes.

In Figure X it is shown an enlargement of the important section of ^1H NMR spectra, in which first, the singlet of the methyl group ($-SCH_3$) at 2.25 ppm shows a split until complete disappearance, while a new singlet appeared at 2.78 ppm, representing the methyl group next to the sulfoxide group ($-SOCH_3$). On the other hand, the methylene group ($-CH_2SCH_3$) at 2.74 ppm shifted downfield to 3.07 – 3.27 ppm.. By comparing the integral areas it was observed that oxidation at 37 ºC showed full conversion after these 10 min, which is incredibly flast.. besides of showing the preservation of the polymer backbone, since monomer ratios remained constant after oxidation. It is worth to mention that only sulfoxide moieties were detected, without track of sulfone groups, implying the selective oxidation to sulfoxide.

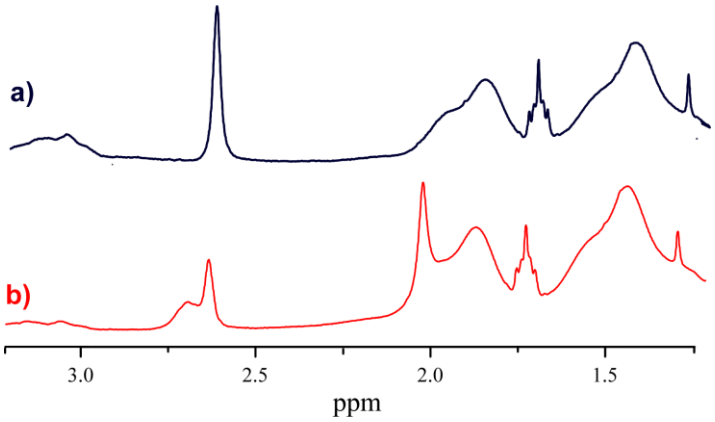


Figure. NMR spectra of P2 before and after oxidation process

The same procedure of oxidation was done for all copolymers, and the LCST was measured before and after oxidation by means of a UV-Spectrometer Lambda 900, by measuring the %-Transmittance as a function of temperature at a fixed wavelength of 800 nm. The obtained results are presented in Table 3.

Table 3. General features of the synthesized copolymers, before and upon oxidation

Sample[a]	Composition[b]	Mn (10^{-4})[c]	PDI[d]	Initial LCST[e]	LCST upon oxidation[e]
P1	Homo-PNIPAM	1.01	1.14	31.4	31.4
P2	$NIPA_{127}MTHA_8$	1.86	1.12	3	26
P3	$NIPA_{168}MTHA_5$	1.37	1.55	9.8	24.7
P5	$NIPA_{109}MTEA_9$	2.01	1.84	22.9	29.3
P6	$NIPA_{156}MTEA_6$	1.60	1.86	25.8	29.3

[a]P1: PNIPAM homopolymer; P2 and P3: PNIPAM-co-Poly(6-[methylthio]hexyl acrylate) at 10 and 5% respectively; P5 and P6: PNIPAM-co-Poly(2-[methylthio]ethyl acrylate) at 10 and 5% respectively; [b]Composition determined by means of NMR [c]Number molar mass determined by NMR; [d]Number molar mass determined by SEC; [e]Polydispersity index measured by SEC; [f]LCST determined by measuring %-transmittance as a function of temperature at a fixed wavelength of 800 nm.

As we can observe in Table 1, the the LCST could be fine-tuned by incorporation of the hydrophobic methyl thiol segments into the polymer backbone, which hydrophobicity was in order of the length of the alkylic chain, and the NIPA/methyl thiol molar ratio as expected. Furthermore upon oxidation, a big chain in the LCST was observed, especially when having 6 carbons in the alkylic chain (P2 and P3), being specially interesting P2,

where an increase of 23 ºC in the LCST was observed, which imply us that this method, if its finely fixed to be in order of the temperature in the human body, and as it has proved to be extremely sensitive, by reaching a 100% of oxidation after 10 minutes with only 3 %-wt, it could be utilized for screening, or releasing of drugs/antioxidants; since as it was mentioned previously, elevated rates of reactive oxygen species (ROS) have been detected in almost all cancers, where they promote many aspects of tumor development and progression. Furthermore, tumor cells also express increased levels of antioxidant proteins to detoxify from ROS, suggesting a delicate balance of intracellular ROS levels is required for cancer cell function, therefore it is considered that a breakthrough would be to employ this system, containing antioxidants in order to break this "delicate balance", and thus prevent a fast progression and development of the tumor.

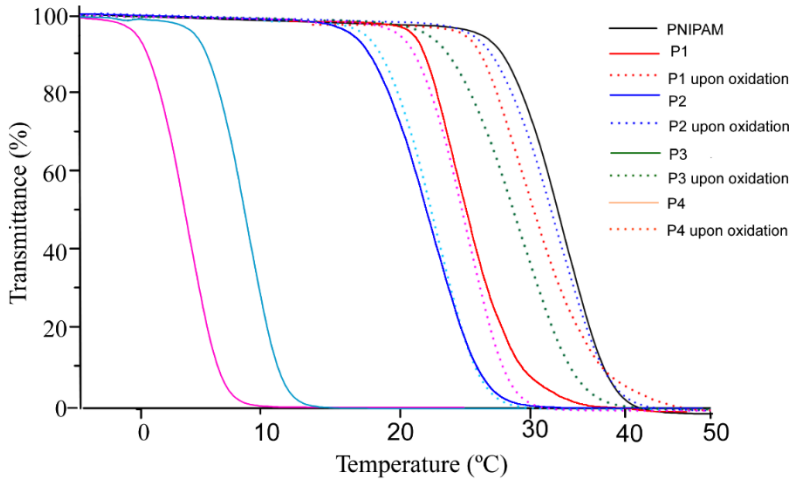


Figure. LCST of the synthesized copolymers before and upon oxidation

Conclusion

As matter of summary, by the development of this thesis we reported a successful RAFT copolymerization of PNIPAM with novel methylthiol containing functional monomers.

Furthermore, we reported, as far as our concern, the first case of a Ru-coordinated PNIPAM. The coordination was found to be roughly 100% efficient, which could be fully cleaved after 45 minutes, and 60% reversible after 2 hours in the dark. Unfortunately we didn't manage to observe any influence in a physical property of the polymers, such as LCST, since the coordination bond wasn't stable when in pure aqueous solution.

On the other hand we introduce a new concept of Redox-responsive PNIPAM by means of oxidizing *in-situ* the thioethers functionalities by means of H_2O_2 yielding 100% of oxidation after 10 minutes at 37 ºC and only 3-%wt. This demonstrated that this high-redox sensitive copolymers could be employed for biomedical applications, being activated by the high concentration of Reactive Oxygen species.

Bibliography

1. Gandhi, A., Paul, A., Sen, S. O., & Sen, K. K. (2015). Studies on thermoresponsive polymers: Phase behaviour, drug delivery and biomedical applications. *Asian Journal of Pharmaceutical Sciences*, *10*(2), 99–107. http://doi.org/10.1016/j.ajps.2014.08.010

2. Wei, K., Su, L., Chen, G., & Jiang, M. (2011). Does PNIPAM block really retard the micelle-to-vesicle transition of its copolymer ? *Polymer*, *52*, 3647–3654. http://doi.org/10.1016/j.polymer.2011.06.005

3. Sun, W., Parowatkin, M., Steffen, W., Butt, H. J., Mailänder, V., & Wu, S. (2016). Ruthenium-Containing Block Copolymer Assemblies: Red-Light-Responsive Metallopolymers with Tunable Nanostructures for Enhanced Cellular Uptake and Anticancer Phototherapy. *Advanced Healthcare Materials*, *5*(4), 467–473. http://doi.org/10.1002/adhm.201500827

4. Viala, C., & Coudret, C. (2006). An expeditious route to cis-Ru(bpy)2C12 (bpy = 2,2′-bipyridine) using carbohydrates as reducers. *Inorganica Chimica Acta*, *359*(3), 984–989. http://doi.org/10.1016/j.ica.2005.07.019

5. Zayat, L., Noval, M. G., Campi, J., Calero, C. I., Calvo, D. J., & Etchenique, R. (2007). A new inorganic photolabile protecting group for highly efficient visible light GABA uncaging. *ChemBioChem*, *8*(17), 2035–2038. http://doi.org/10.1002/cbic.200700354

6. Schofield, E. R., Collin, J., Sauvage, J., Organo-minérale, L. D. C., Cnrs, U. M. R., Pasteur, U. L., … Chimie, F. De. (2003). Photochemical and thermal ligand exchange in a ruthenium (II) complex based on a scorpionate terpyridine ligand benzonitrile group has been synthesised ; coordination and thermally and photochemically respectively , in an acetone – . *The Royal Society of Chemistry*, *6*, 188–189.

7. a) Ward, M. A., & Georgiou, T. K. (2011). Thermoresponsive polymers for biomedical applications. *Polymers*, *3*(3), 1215–1242. http://doi.org/10.3390/polym3031215. b) Hrsic, E., Zografou, I., Schulte, B., Pich, A., Keul, H., & Möller, M. (2013). Amphiphilic block copolymers with pendant thiol groups in side chains by RAFT polymerization. *Polymer (United Kingdom)*, *54*(2), 495–504

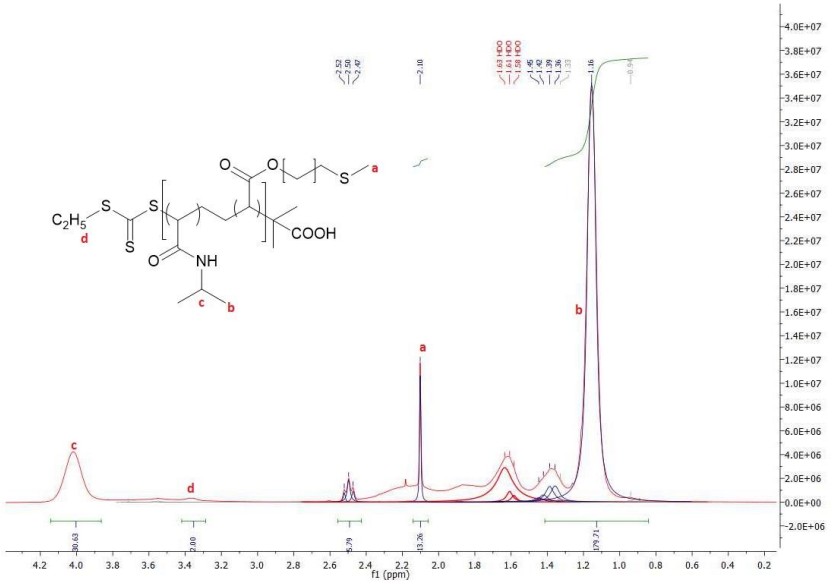
8. Liou, M.-Y., & Storz, P. (2010). *Reactive oxygen species in cancer*. *Free Radic Res.* (Vol. 44). http://doi.org/10.3109/10715761003667554.Reactive

9. Skey, J., & Reilly, R. K. O. (2008). Facile one pot synthesis of a range of reversible addition – fragmentation chain transfer (RAFT) agents w. *The Royal Society of Chemistry*, 4183–4185. http://doi.org/10.1039/b804260h

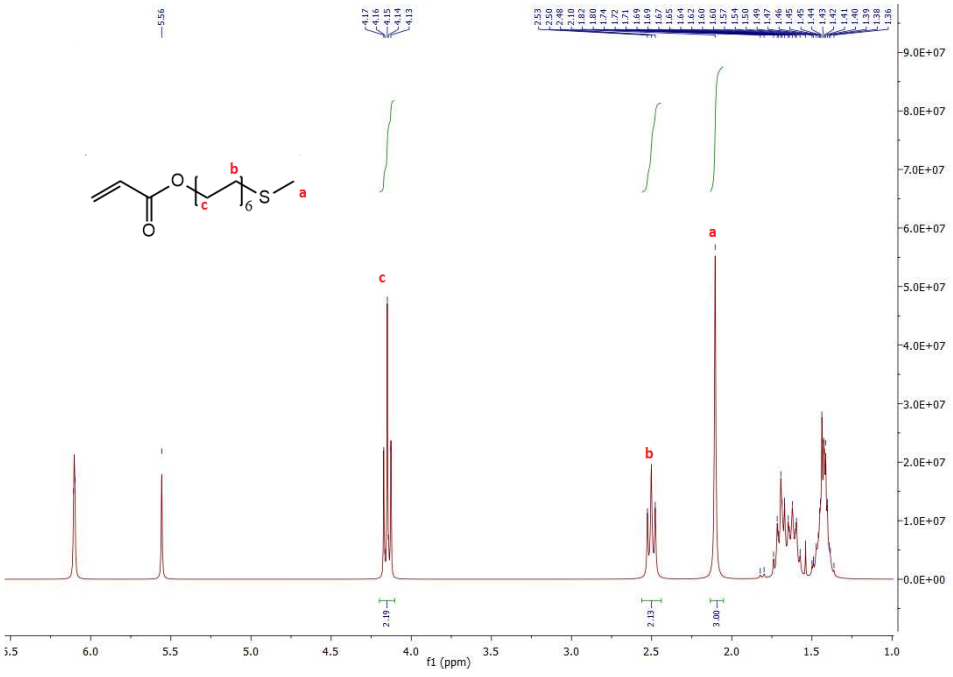
10. Viala, C., & Coudret, C. (2006). An expeditious route to cis-Ru(bpy)2C12 (bpy = 2,2′-bipyridine) using carbohydrates as reducers. *Inorganica Chimica Acta*, *359*(3), 984–989.

11. Zayat, L., Noval, M. G., Campi, J., Calero, C. I., Calvo, D. J., & Etchenique, R. (2007). A new inorganic photolabile protecting group for highly efficient visible light GABA uncaging. *ChemBioChem*, *8*(17), 2035–2038.

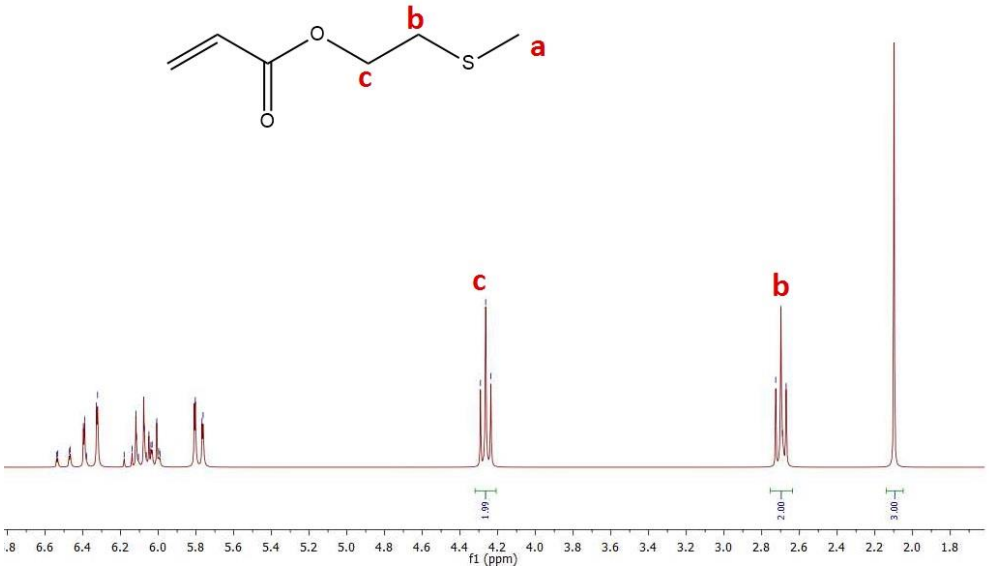
12. Mittal M, Rizwan M, Tran K, Antioxid Redox Signal, 2014, 20(7), 1126-1167



1H NMR spectra of P2: PNIPAM-co-poly(6-methylthio)hexyl acrylate



^{1}H NMR spectra of 6-(methylthio)hexyl acrylate



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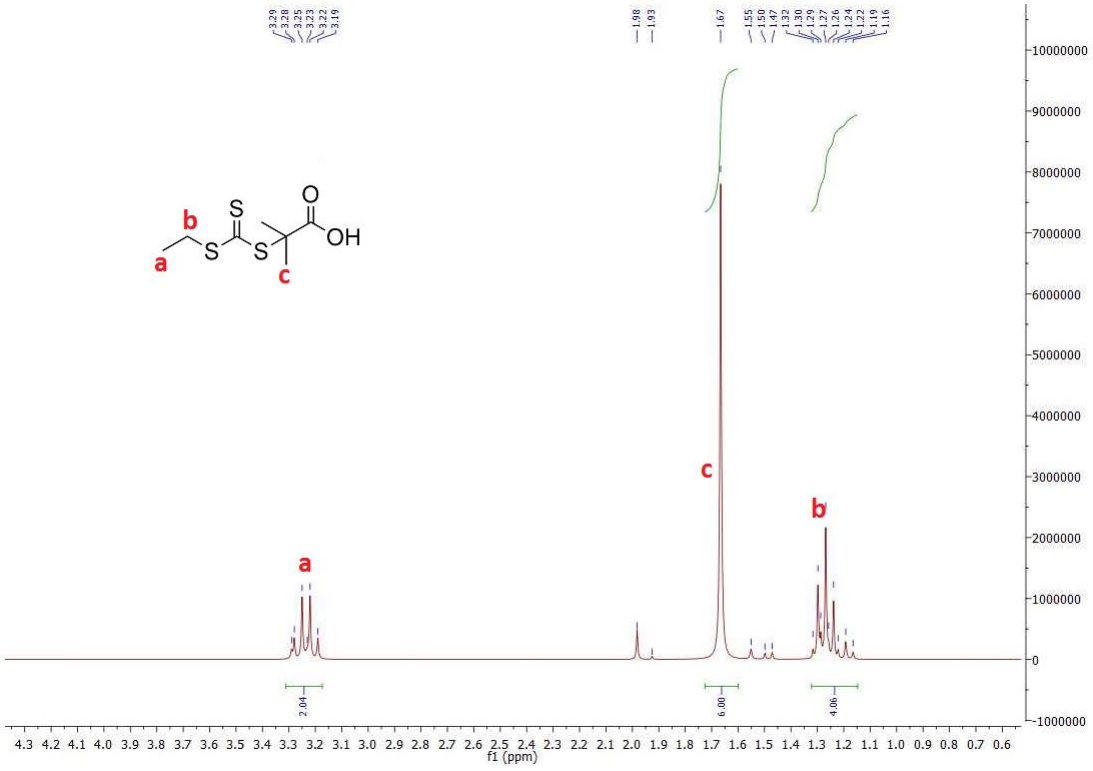
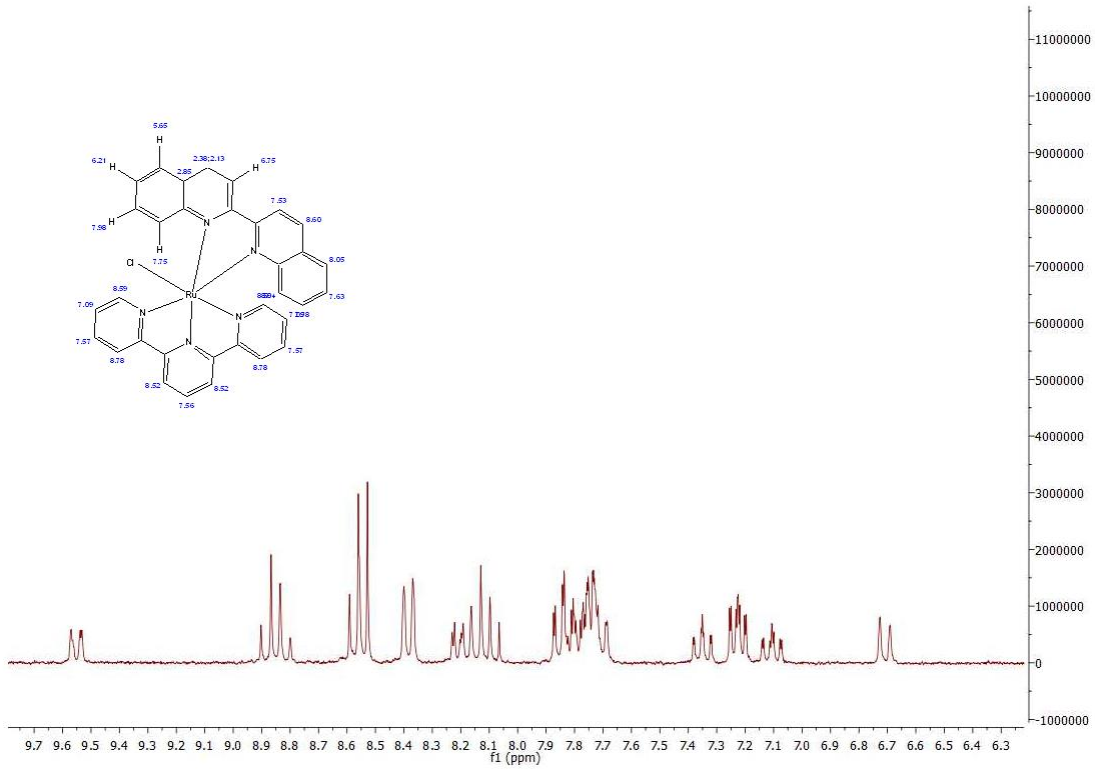
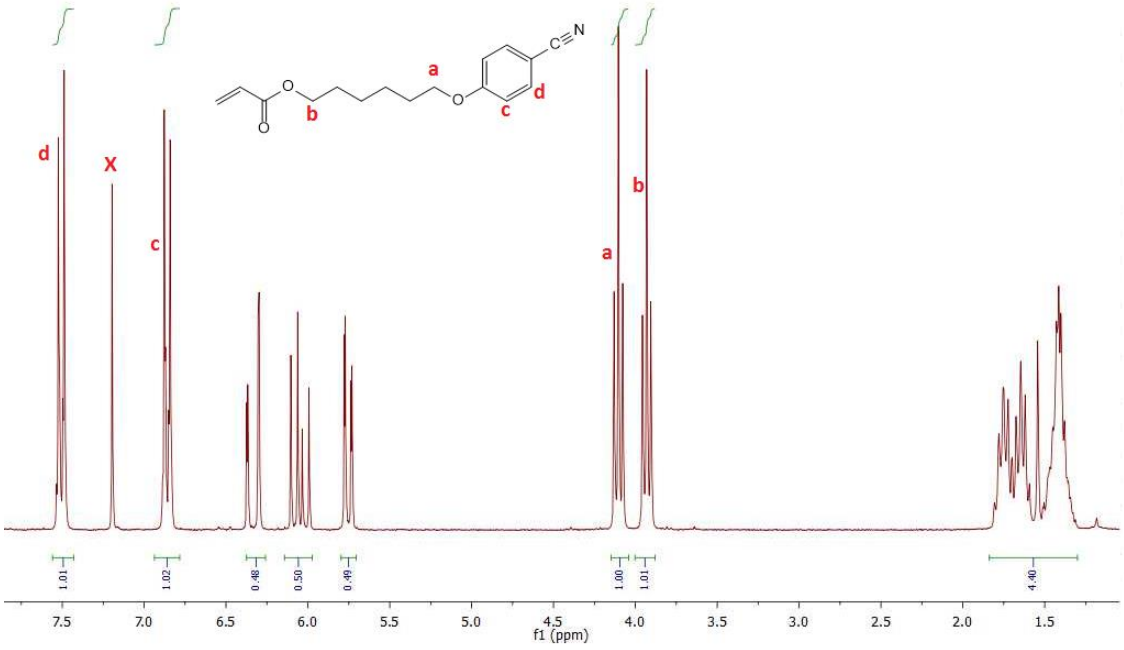


1H NMR spectra of *S*-Ethyl-*S'*-(*a*,*a*'-dimethyl-*a*''-acetic acid)trithiocarbonate



^{1}H NMR NMR spectra of [Ru(tpy)(biq)(Cl)]Cl



1H NMR spectra of *6-[4-cyanophenoxy] hexyl acrylate)*