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Poly(ampholytes) as antifreeze protein mimetics

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Abstract

Cryopreservation can be a life-saving tool that could save millions of people. The limited life-time of the cryopreserved tissues or organs, formation of ice crystals and the damage caused by thawing are challenging issues that have to be tackled. Different research related to this area has been going on since the 1950s. Dimethylsulphoxide (DMSO) can be used as a cryoprotectant but is not ideal due to its potential toxicity. The isolation of naturally occurring antifreeze (glycol)proteins as well as synthetic biomimetics were attempted but they were proved to be difficult. In spite of the problems that currently exist, novel or alternative methods were proposed in order to improve the challenges.

Herein, previous research about cryoprotective and antifreeze materials as well as the inspirations those lead to this project will be fully discussed. A library of poly(ampholytes) will be constructed *via* RAFT, post-modification polymerization and it is aiming to observe whether they can be applied as potential antifreeze materials using Splat Cooling Assay. In addition, it is aiming to see whether changing the structures can alter their ability as antifreeze materials.

Table of Abbreviations

ATRP: Atom transfer radical polymerisation

Boc-Lys/Boc-Lys-OH: Boc-protected lysine

$CDCl_3$: Deuterated chloroform

$CHCl_3$: Chloroform

D_2O: Deuterated water

DCM: Dichloromethane

DMF: Dimethylformamide

DMSO: Dimethyl sulphoxide

GPC: Gel permeation chromatography

IR: Infrared

IRI: Ice recrystallization inhibition

Lys: lysine

$\overline{M_n}$: Average number molecular weight

NEt_3: Triethylamine

NMR: Nuclear magnetic resonance

PFMA: Pentafluorophenyl methacrylate

PPFMA: Poly(pentafluorophenyl methacrylate)

RAFT: Reversible addition-fragmentation chain transfer

SEC: Size exclusion chromatography

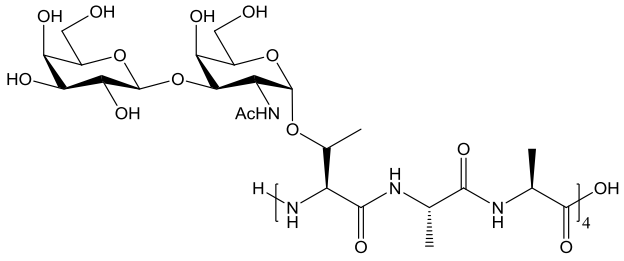
THF: Tetrahydrofuran

TFA: Trifluoroacetic acid

1. Introduction

1.1 Cryopreservation - Cryopreservation of biological tissues, organs,[1] and embryos[2] is vital to tissue and organ banking[3] for organ transplantations[4] but it is indeed a very challenging aspect in biomedicine. Due to the fast growing population, the demands of regenerative medicine and organ transplantation are getting higher than the supply.[5] One major reason is the fact that cell tissues, organs cannot be stored easily. For instance, 6000 units of blood are required in the United Kingdom each day but blood can only be stored for 42 days without cryopreservation. However, complex isotonic solutions with high rates of haemolysis are required.[6] When biological material is stored at sub-zero degrees Celsius, formation and growth of ice crystals can lead to mechanical damage on a cellular level. In addition, osmotic shock occurs when the concentration of extracellular solutes increases with decreasing liquid water volume fraction.[7] Thus, this technique has proved to be difficult because of the complexity and intrinsic variations between isolated cells and tissue.

1.2 The history of cryoprotectant and antifreeze materials - On the applications on frozen systems, antifreeze do not prevent freezing, but controlling the size, shape and aggregation of ice crystals.[8] Scientific studies regarding cryoprotectants started in the early 1950s. It was reported that glycerol has cryoprotective properties to preserve living cells at very low temperatures.[9] Approximately 10 years later, it was discovered that dimethyl sulfoxide, DMSO can be used as a cryoprotectant to preserve red blood cells.[10] Glycerol and DMSO can prevent cells from lethal damage due to formation of intracellular ice and ice recrystallization which is caused by Ostwald ripening during freezing and thawing.[11] However, glycerol is a relatively weak antifreeze compared to DMSO[12] but DMSO has potential toxicity[13] and ought to be removed immediately after thawing.[14] In the 1970s, research focus shifted to naturally existing cryoprotectants inspired by the ability of Antarctic and Arctic fish to survive in sub-zero-environments. DeVries *et. al*. had identified poly(peptides) capable of depressing the freezing point in Antarctic fish's blood serum. Isolations of these materials were succeeded and confirmed that their structures mainly consist of a peptide chain that linked to a carbohydrate (Figure 1) and names as antifreeze (glycol)proteins, AF(G)P.[15]



Native AF(G)P **1**

Figure 1. Structures of native AF(G)P **1**.

Unfortunately, the abundance of naturally occurring antifreeze (glyco)proteins is low and extraction of these materials is prohibitively expensive.[16] The native antifreeze (glyco)proteins are very sensitive to pH cleavage which undergoes bond cleavage under acidic or basic conditions;[17] they can induce cellular damage by the needle-like ice crystal morphology due to the dynamic ice shaping (Figure 2).[18] It was also reported that some of them even lead to the death of human liver and kidney cells.[19]

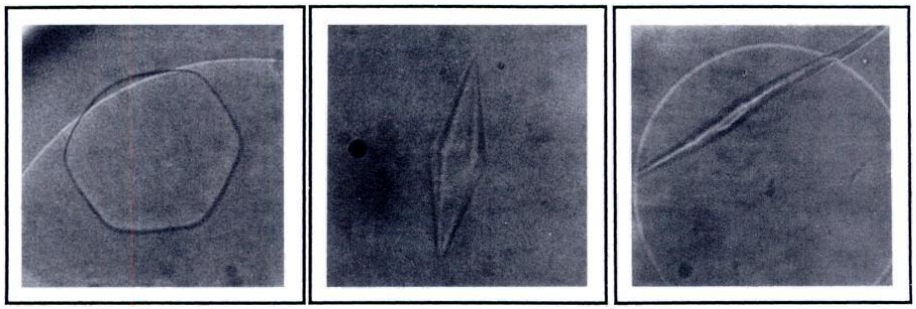
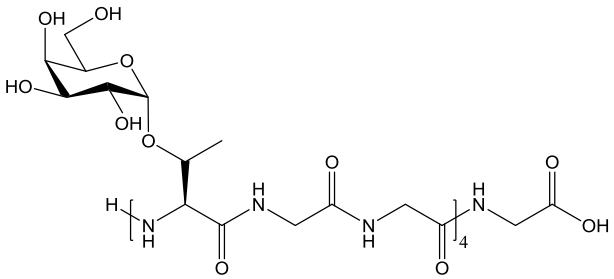


Figure 2. Hexagonal ice crystal growth in the absence of AF(G)P (left), bipyramidal shape ice crystal with the presence of AF(G)P (middle), needle-like ice crystal when concentration of AF(G)P increased (right)[58]

1.3 Modern approach for new development - In order to overcome the issues relating to native antifreeze (glyco)proteins, research-activity is growing to deliver compounds that are non-cytotoxic and effectively inhibit ice crystal growth. Early research into synthesis of antifreeze materials tended to focus on synthesizing analogues of native antifreeze (glyco)proteins containing poly(peptides) and carbohydrate (Figure 3).[20,21,22]



Analogue AF(G)P **2**

Figure 3. An example of analogue AF(G)P **2**[19]

It was believed that the carbohydrate linked to the backbone, the distance between the carbohydrate moieties[20], the values of the repeating unit and conformation all contribute to their performances as antifreeze materials.[23] As a result, artificial AF(G)Ps are synthetically challenging and difficult to purify, leading to high costs and low yeilds.[20] Therefore, different strategies were proposed to seek for simpler solutions to synthesize alternative materials that also have cryoprotective properties.

1.3.1 Alternative antifreeze materials - The trend in the researching antifreeze (glyco)proteins over the past 10 years has slightly changed. Focus has moved away from synthesis of antifreeze (glyco)protein analogues and towards chemical compounds with simpler structures, which can also be applied as potential antifreeze materials. For instance, Gibson *et. al*. tested a selection of water soluble polymers with advantages including scalable syntheses and highly variable structures. Amongst the polymers tested, several showed significant ice recrystallization inhibition, IRI, especially poly(vinyl alcohol), PVA (**3**).[24] The activity of ice recrystallization inhibition can be measured using a simple technique known as Splat Cooling Assay in which a wafer of ice made of polymer solution is observed on a cryostage with a microscope for 30 minutes. The sizes of ice-crystal growth can be compared to its relative blank solution.[25] When PVA (**3**) was used as an additive, ice crystal growth was effectively inhibited even in micromolar concentrations. Such efficiency is greatly affected by the molecular weight and degree of polymerisation of PVA (**3**) (Figure 4).[26,24]

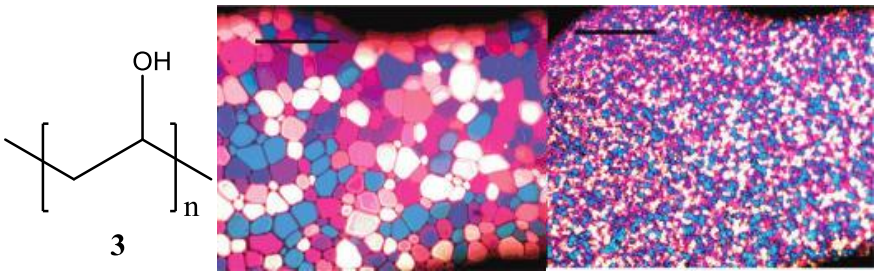


Figure 4. Structure of PVA **3** (left), ice crystals in PBS blank solution (middle) & in PVA **3** PBS (right)[24]

The Gibson group continues to do research in this field to explore the properties required in antifreeze materials. Their studies include aiming to understand the mechanism of ice recrystallization inhibition, the role of hydrophobicity/amphiphlicity and chemical modification of monosaccharides on IRI. Apart from PVA (**3**), poly(ethylene glycol) PEG (**4**), Dextran (**5**) and (Per-7-acetyl)-B-cyclodetrin (**6**) (Figure 5) were used and their quantitative concentration-dependant ice recrystallization inhibition activities were observed using the Splat Cooling Assay. Results indicated that PVA **3** achieved a much better inhibiting effect but there was lack of understanding about the importance of the hydroxyl groups and the mechanism involved.

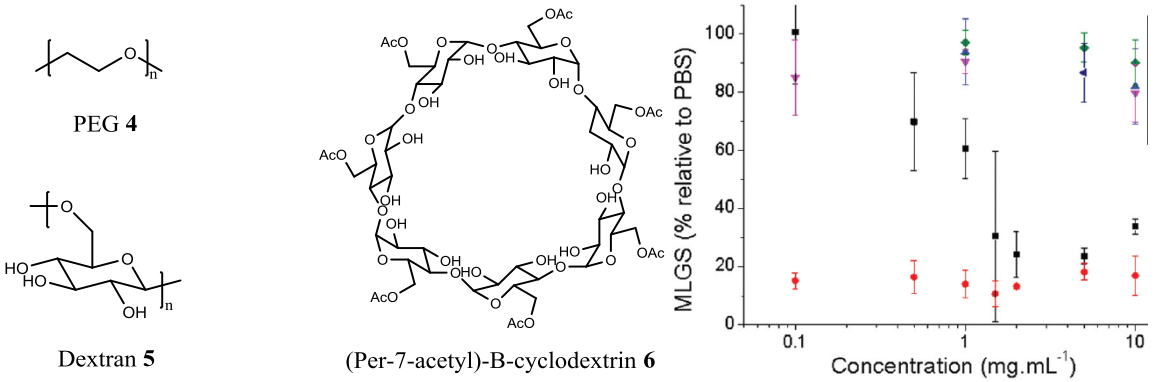
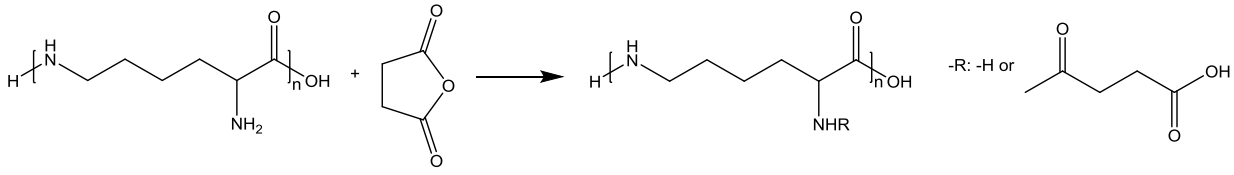


Figure 5. Structures of PEG **4**, Dextran (**5**) & cyclodextrin **6** (left), graph showing PVA (**3**) (red dots) is a stronger ice recrystallization inhibitor compared to others (right)

Then, a selection of low-molecular weight poly-hydroxylated compounds was tested which showed that replacement of anomeric hydroxyl groups with hydrophobic alkyl units can inhibit the ice recrystallization further. Although PVA (**3**) does not contain hydrophobic units, dye incorporation assays and dynamic light scattering, DLS showed that PVA (**3**) can arrange into a conformation which presents a hydrophobic surface. Thus, the data showed that introducing hydrophobic moieties was also important for preparing antifreeze materials.[27] Meanwhile, Balcerzak *et al*. investigated a library of small molecules as ice recrystallization inhibitors. They noticed that inhibition was related to the presence of long alkyl chains and increased hydrophobicity which is in agreement with the study done by the Gibson group. The importance of having hydrophobic groups was highlighted and it was also suggested that the best ice recrystallization inhibitors should be amphiphilic and consist of well-tuned balance between hydrophobic and hydrophilic components.[28]

1.3.2 Poly(ampholytes) as potential cryoprotectants and antifreeze - Work by Matsumura *et. al*. focuses on using poly(ampholytes) as cryoprotective agents. He showed that *ε*-poly-*L*-lysine, *ε*PLL **7** with more than 50 mol% of amino groups carboxylated exhibited good cryoprotective properties. It involved introducing a non-toxic homopolymer,[29] poly(*L*-lysine) (**7**) with carboxyl groups using succinic anhydride (**8**) to produce COOH- *ε*PLL **9** (Scheme 1). It was discovered that COOH- *ε*PLL **9** solutions showed lower osmotic pressure as well as lower cytotoxicity compared to DMSO. During the ice recrystallization assay, polymer **9** exhibited a high specific activity which is carboxyl group ratio dependant. Then, a polycarboxylic acid, NH_2-PAAc with 5.3 mol% of –amine groups was synthesized but cryoprotective properties were not observed. Therefore, it was suggested that not all poly(*L*-lysine) derivatives can be used as cryoprotectant. However, poly(ampholytes) with appropriate amount of both amine and carboxyl groups should play and important role as antifreeze materials.[30] By applying these polymers, rat mesenchymal stem cells were cryopreserved without altering their phenotype characteristics. Their viability and proliferative ability were preserved even after thawing.[31] However, their results were obtained using fetal bovine serum, FBS which can act as a buffer of osmotic pressure as well as a cell membrane protector, reducing the risks of damage by ice recrystallization during freezing and thawing.[32]



7 **8** **9**

Scheme 1. Synthesis of COOH- εPLL **9** via succinylation of εPLL **7**

Gibson *et. al*. had prepared poly(ampholytes) and relationship between the structure of poly(ampholytes) and antifreeze properties was investigated. Polymers with different chain lengths and ratios of cationic and anionic groups were tested. It was observed that longer polymers can enhance the IRI activity. It was also observed that polymers with 1:1 ratio of cationic and anionic groups can provide maximal IRI activity compared to other ratios.[33]

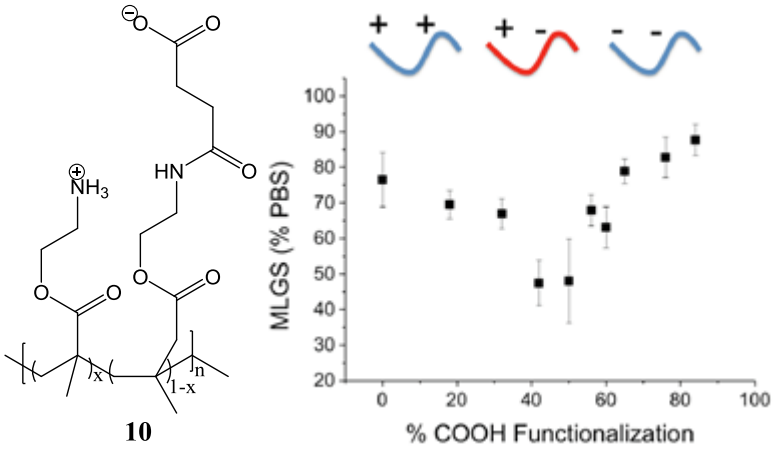
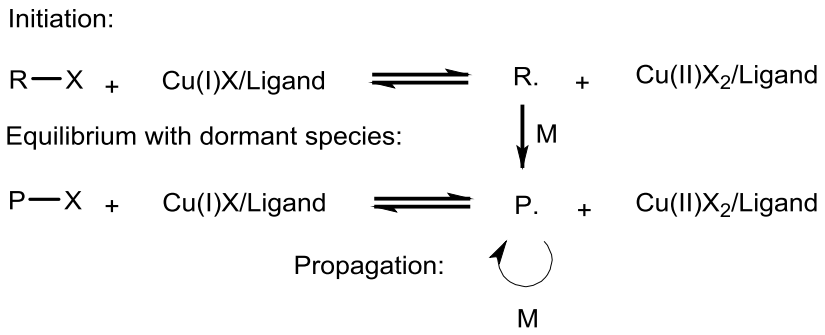


Figure 6. Structure of poly(ampholyte) used (left) and effect of degree of carboxylation on activity[33]

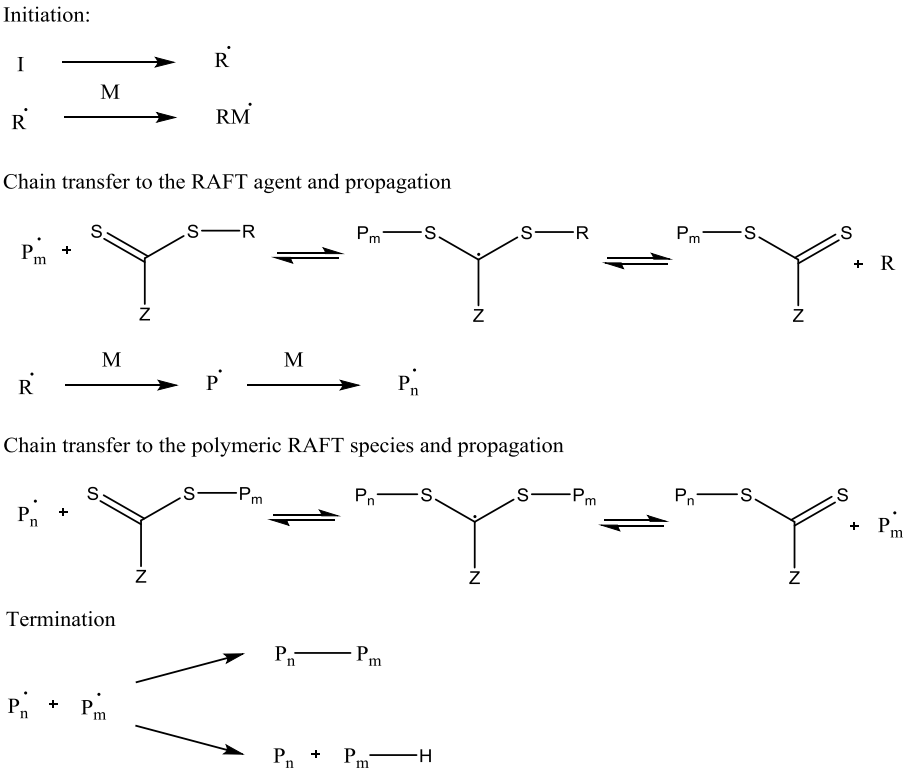
1.4 Controlled radical polymerisation - Hence, it is intended to combine all these ideas to make a library of polymers which have antifreeze properties. It was well understood that degree of polymerisation and dispersity, Đ are vital to the efficiency of antifreeze polymers. Therefore, polymers ought to be synthesized *via* controlled radical polymerisation instead of simple free radical polymerisation. Amongst all controlled radical polymerisations, atom transfer radical polymerisation, ATRP[34] and reversible addition-fragmentation chain-transfer, RAFT polymerisation[35] are two of the most common methods that can be used to obtain polymers with low values of dispersity and controlled molecular weights

1.4.1 Atom transfer radical polymerisation - ATRP requires alkyl halides as initiators and catalysts which are available for two oxidation states with metal centres (e.g. copper) consist of affinity for halogens. The metal catalyst, Cu(I)X/Ligand abstracts halogen from halide, RX to from radical $R^{\bullet}$ and oxidised species. Propagation of the chain radical is intercepted by the reverse process in which oxidised metal species donates a halogen atom back to the propagating radical resulting in deactivation of the chain radical through formation of a new C-X bond at the chain end and regeneration of the catalyst, Cu(I)X/Ligand (Scheme 2).[34] The chain grow *via* a series of activation-propagation-deactivation cycles; equilibrium can be controlled by great choices of reagents and temperature; most chains exist in the dormant state, so concentration of monomer radical is low which leads to decrease of bimolecular termination events as well as reducing the rate of polymerisation to achieve a narrow molecular weight distribution.[36]



Scheme 2. General reaction scheme for ATRP (X=halide)

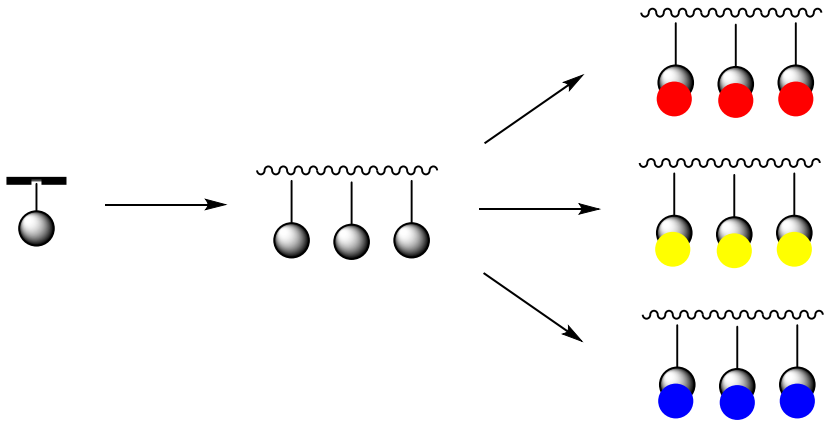
1.4.2 Reversible addition-fragmentation chain transfer polymerisation - Unlike ATRP, metal catalysts are not required but RAFT agents are required which play important role to control the polymerisation and have to be chosen carefully. A RAFT agent consists of a C=S bond as well as R and Z groups. Ideally, R group of a RAFT agent should be a good leaving group than the propagating radical which usually has a similar structure to monomer. Z group has an effect on altering its reactivity and its effectiveness at mediating polymerisation. The whole process involves initiation to generate oligomeric radicals; growth of polymer chains and rapid exchange between existing growing radicals and the thiocarbonylthio group capped species; finally, termination via combination or disproportionation (Scheme 3).[35] In which, the rapid exchange can ensure the concentration of growing radical chains is kept lower than that of the stabilised radical intermediates. Hence, rate of polymerisation can be controlled in order to achieve low dispersity.



Scheme 3. General structure of a RAFT agent and main equilibrium during RAFT polymerisation

Both techniques are very useful and a wide range of monomers can be polymerised using these methods.[37] However, the fact that ATRP involves the use of metal catalysts, especially cytotoxic copper, is a concern when the resulting polymers are used for biomedical applications. High uptake of copper can cause increased oxidative damage to lipids, proteins and DNA that lead to neurodegenerative disorders[38] and so, removal of copper is essential.[39] In contrast, RAFT agents have low cytotoxicity[40] and so this should be a more suitable method to produce the polymers.

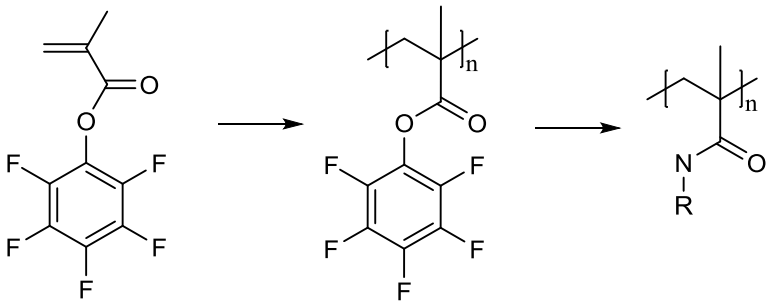
1.5 Post-modification polymerisation - As mentioned above, control of chain length and dispersity of polymers are crucial when studying anti-freeze inhibition. RAFT polymerisation is ideal for constructing a library of antifreeze polymers with different chain lengths, properties and functional groups. Instead of polymerisations of functionalised monomers, using post-polymerisation modification would be used. The idea is about synthesis of one single monomer and the resultant polymer precursor can then be modified to produce a selection of polymers with different functional groups. Using this method, polymers with different properties but uniform chain length and dispersity can be generated from a single batch of polymer precursor.[41]



Scheme 4. Representation of post-polymerisation modification

It was reported that poly(methacrylamides) **13** were synthesized using this method.[42,42] Firstly, it involved synthesis of monomer, pentafluorophenyl methacrylate (**11**) which will then be polymerised using RAFT to produce a precursor **12**. Secondly, poly(methacrylamides) **13** can be produced upon reacting precursor **12** with amines (Scheme 5). The efficiency of ester conversion depends upon the sterics and nucleophilicity of amines which can be measured using simple techniques such as ^{19}F NMR and FTIR. Advantages include the preservation of controlled chain lengths as well as dispersities and poly(methacrylamides) **13** can be obtained using mild conditions (e.g. temperature of 50 ^{0}C and reaction time as short as 2 hours), high ester conversion (maximum of 100%) as well as

yield up to 89% were achieved.[41] In addition, the Product **13** contains chemically orthogonal thiol end-group (-SH).[43]



11 **12** **13**

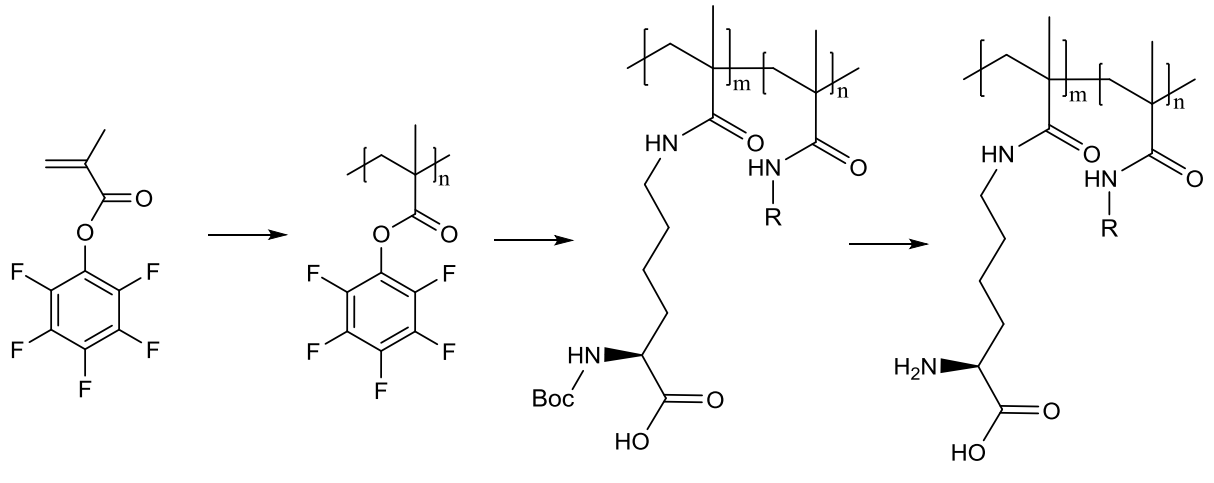
Scheme 5. Polymerisation of monomer **10**

1.6 The aim of the project

The aim of this project is to develop the synthetic methodology to enable a structure-property investigation into the ability of poly(ampholyte) to inhibit the growth of ice crystals. This will enable a new paradigm in antifreeze-mimetic materials, by eliminating the need for poly-ols, which are currently the only family of materials which have been widely studied.

The key aims are:

1. Synthesis of reactive precursors monomer, pentafluorophenyl methacrylate PFMA **10** and RAFT agent
2. RAFT polymerization of PFMA **10** to prepare precursor **11**
3. Modification of precursor 11 with Boc-protected lysine as well as various amines
4. Deprotection of polymers **13** to produce poly(lysine) **14** (Scheme 6)
5. Splat cooling assay of polymers in phosphate buffer saline, PBS solution



10 **11** **13** **14**

Scheme 6. General reaction schemes in this project

2. Experimental

2.1 General Directions:

Solvents and reagents: pentafluorophenol, methacryloyl chloride, 2,6-lutidine, dichloromethane DCM, magnesium sulphate, silica gel petroleum ether, ethyl acetate, dioxane, 4-cyanopentanoic acid dithiobenzoate, 4,4-azobis(4-cyanovaleric acid), pentane, tetrahydrofuran THF, mesitylene, potassium phosphate, 1-dodecanethiol, carbon disulphide, 2-bromo-1-methylpropionic acid, magnesium sulphate, N_{α}-(*tert*-Butoxycarbonyl)-L-lysine, dimethylformamide DMF, triethylamine, ethanolamine, trifluoroacetic acid TFA, propylamine, pentylamine, hexylamine, benzylamine, snakeskin/cellulose dialysis tubes were used as received.

^{1}H, ^{19}F and ^{13}C NMR spectra: Depend upon the samples, they were recorded in $CDCl_3$ or D_2O on a Bruker instruments and chemical shifts (δ) are quoted in ppm.

Infra-red, IR spectra: All samples were recorded on a Bruker Vector 22 GI003097 IR machine and characteristic peaks are quoted in cm^{-1}

GPC: Depend upon the samples, they were recorded using eluent, DMF with 5 mM NH_4BF_4 and 1 x PLgel Guard + 2 x PLgel Mixed D columns with flow rate of 1 mL/min; or aqueous eluent with 0.1 M $NaNO_3$ and 1 x PL aquagel-OH Guard + 1 x PL aquagel-OH 30 + 1 x PL aquagel-OH 40 columns with flow rate of 1 mL/min

2.2 Synthesis of Pentafluorophenylmethacrylate (11)

In a 100 mL round-bottom-flask, pentafluorophenol (**16**, 5.4 g, 29.3 mmol) and 2,6-lutidine (**18**, 3.5 mL 30.0 mmol) were dissolved in 50 mL DCM and cooled on an ice-bath. Methacryloyl chloride (**17**, 3.0 mL, 32.7 mmol) were added to the mixture drop-wise. The mixture were continued to stir at 0 ^{0}C for 3 h. After that, they were stirred at room temperature for overnight. Then, precipitate was removed with filtration and the filtrate was washed with distilled water (30 mL × 2) and dried over magnesium sulphate. Solvent was then removed and the crude product was purified with column chromatography using petroleum ether only (R_f: 0.31) to give pentafluoophenylmethacrylate (**11**) as a colour-less liquid (3.77 g, 51%); **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 2.09 (3H, s, –C=CH_3–), 5.91 (1H, s, cis –CH=C–), 6.45 (1H, s, trans –CH=C–); **^{19}F NMR** ($CDCl_3$, 300 MHz) δ -163.16 (2F, t, *m*-Ar), -158.87 (1F, t, *p*-Ar), -153.17 (2F, d, *o*-Ar); **IR** ν_{max} 802, 858, 991 (m-s, =C–H), 1086 (s, C–O), 1147, 1302 (m, C–F), 1516 (s, aromatic C=C), 1759 (s, C=O) cm^{-1}

2.3 Synthesis of 2-(Dodecylthiocarbonothioylthio)-2-methylpropanoic acid (24)

In a 100 mL round-bottom-flask, tripotassium phosphate (**23**, 4.20 g, 19.76 mmol) was stirred in 60 mL acetone at room temperature. 1-Dodecanethiol (**20**, 4.00 g, 19.76 mmol) was added to this suspension drop-wise. After stirring for 10 minutes, carbon disulphide (**22**, 3.3 mL, 53.85 mmol) were added to the mixture drop-wise and they were stirred for further another 10 minutes. 2-Bromo-1-methylpropionic acid (**21**, 3.00 g, 17.96 mmol) was then added to the yellow mixture and stirred for overnight. The precipitate produced was filtered off followed by removal of solvent. The residue was extracted with DCM (100 mL X 2) from 1M HCl (100 mL) which was then washed with distilled water (100 mL), brine (100 mL) then dried over magnesium sulphate. Solvent was removed and the crude product was recrystallized from hexane to give **24** as a bright yellow powder (1.0015 g, 15 %): **^{1}H NMR** ($CDCl_3$, 300 MHz) 0.88 (t, 3H, $–CH_3$), 1.18 – 1.43 (m, 16H, $–CH_2–$), 1.65 (s, 2H, $–CH_2–$), 1.73 (s, 6H, $–C–CH_3$), 1.94 (s, 2H, $–CH_2–$), 3.28 (t, 2H, $S–CH_2–$); **^{13}C NMR** ($CDCl_3$, 300 MHz) 14.12 ($–CH_3$), 22.68 ($–\underline{CH_2}–CH_3$), 23.94, 25.20 [$–S–C–(\underline{CH_3})_2$], 27.78, 28.95, 29.09, 29.33, 29.43, 29.62, 30.55, 31.90 [$–S–CH_2–(\underline{CH_2})_8–$], 37.06 ($–S–CH_2–$), 55.53 [$–S–\underline{C}–(CH_3)_2$], 177.74 (–COOH), 178.53 (–C=S); **IR** ν_{max} 514, 575, 610, 659, 694, 721, 814, 912 (m, C-S), 1068, 1105, 1149 (s, C=S), 1283, 1373 (s, C–O), 1457 (m, –C–H), 1713 (s, C=O), 2849 (s, C–H), 2917 (s, O–H)

2.3.1 Determination of kinetics of 2-(dodecylthiocarbonothioylthio)-2-methylpropanoic acid (24)

In a medium vial, pentafluorophenylmethacrylate (**11**, 1.0 g, 3.97 mmol) was dissolved in 1 mL dioxane. 2-(Dodecylthiocarbonothioylthio)-2-methylpropanoic acid (**24**, 0.013 g, 0.040 mmol) and 4,4-azobis(4-cyanovaleric acid) (**27**, 0.0056 g, 0.020 mmol) were dissolved in 1 mL dioxane and added to the vial. 200 μL Mesitylene was added to the mixture and it was stirred for 15 minutes. 25 μL of this mixture was taken as t = 0 for calculating the monomer conversion. The vial was sealed with a sub-a-seal and the mixture was degassed with nitrogen for 15 minutes. They were then heated at 90^0C in an oil-bath for 90 minutes. In the meantime, *ca*. 0.01 mL sample was collected at a 15-minute interval. Then, the remaining mixture was cooled and precipitated into cold 50 mL pentane. The crude product was then reprecipitated from 0.5 mL THF into 50 mL pentane to give poly(pentafluoophenylmethacrylate) as a very pale yellow solid

2.4 Synthesis of poly(pentafluorophenylmethacrylate) (12)

General procedure: In a medium vial, pentafluoophenylmethacrylate (**11**, 2.0 g, 7.93 mmol), 2-(dodecylthiocarbonothioylthio)-2-methylpropanoic acid (**24**) and 4,4-azobis(4-cyanovaleric acid) (**27**) were dissolved in 4 mL dioxane. 200 μL Mesitylene was added and they were

stirred at room temperature for 15 minutes or until all solids were dissolved. 25 *μ*L of this mixture was taken for NMR as t = 0 for calculating the monomer conversion. The vial was sealed with a sub-a-seal and the mixture was degassed with nitrogen for 15 minutes. It was immersed in a 90^0C oil-bath for 90 minutes. After that, the vial was unsealed and quenched in N_2(l) for 30s. 25 *μ*L of sample was taken for NMR as t = 90 and the remaining mixture was precipitated into cold 50 mL pentane. Crude product was then reprecipitated for twice from *ca*. 3 mL THF into 50 mL pentane to give poly(pentafluoophenylmethacrylate) (**12**) as a very pale yellow solid

Poly(pentafluoophenylmethacrylate) (12a, DP 27): RAFT agent (**24**, 0.053 g, 0.16 mmol) and initiator (**27**, 0.022 g, 0.079 mmol) were used to give product **12a** (1.10 g, 54%; conversion = 55%): **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 1.15 – 1.79 (br, 3H, backbone –CH_3), 1.93 – 2.73 (br, 2H, backbone –CH_2–); **^{19}F NMR** ($CDCl_3$, 300 MHz) δ -162.72 (s, 2F, *m*-Ar), -157.52 (s, 1F, *p*-Ar), -151.43 (d, 2F, *o*-Ar); **IR** ν_{max} 992, 1058 (C–F), 1518 (s, aromatic C=C), 1778 (s, C=O); **GPC** (DMF) $\overline{M_n}$: 5307, Đ: 1.45

Poly(pentafluoophenylmethacrylate) (12b, DP 54): RAFT agent (**24**, 0.027 g, 0.079 mmol) and initiator (**27**, 0.011 g, 0.040 mmol) were used to give product **12b** (1.12 g, 54%; conversion = 54%): **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 1.18 – 1.77 (br, 3H, backbone –CH_3), 1.96 – 2.71 (br, 2H, backbone –CH_2–); **^{19}F NMR** ($CDCl_3$, 300 MHz) δ -162.73 (s, 2F, *m*-Ar), -157.56 (s, 1F, *p*-Ar), -151.38 (d, 2F, *o*-Ar); **IR** ν_{max} 988, 1044 (C–F), 1515 (s, aromatic C=C), 1776 (s, C=O); **GPC** (DMF) $\overline{M_n}$: 6893, Đ: 1.63

Poly(pentafluoophenylmethacrylate) (12c, DP 88): RAFT agent (**24**, 0.013 g, 0.040 mmol) and initiator (**27**, 0.0056 g, 0.020 mmol) were used to give product (0.93 g, 46%; conversion = 47%): **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 1.21 – 1.66 (br, 3H, backbone –CH_3), 1.98 – 2.67 (br, 2H, backbone –CH_2–); **^{19}F NMR** ($CDCl_3$, 300 MHz) δ -162.73 (s, 2F, *m*-Ar), -157.60 (s, 1F, *p*-Ar), -151.41 (d, 2F, *o*-Ar); **IR** ν_{max} 991, 1052 (C–F), 1517 (s, aromatic C=C), 1777 (s, C=O); **GPC** (DMF) $\overline{M_n}$: 10216, Đ: 1.45

Poly(pentafluoophenylmethacrylate) (12d, DP 94): RAFT agent (**24**, 0.013 g, 0.040 mmol) and initiator (**27**, 0.0056 g, 0.020 mmol) were used and reaction time was 100 mins instead of 90 mins to give product (0.79 g, 40%; conversion = 44%): **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 1.11 – 1.69 (br, 3H, backbone –CH_3), 1.78 – 2.68 (br, 2H, backbone –CH_2–); **^{19}F NMR** ($CDCl_3$, 300 MHz) δ -162.73 (s, 2F, *m*-Ar), -157.59 (s, 1F, *p*-Ar), -151.45 (d, 2F, *o*-Ar); **IR** ν_{max} 991, 1053 (C–F), 1517 (s, aromatic C=C), 1776 (s, C=O); **GPC** (DMF) $\overline{M_n}$: 12485, Đ: 1.17

2.5 Modification of poly(pentafluorophenylmethacrylate) (12)

General procedure: In a large vial, poly(pentafluorophenyl-methacrylate) (**12**, 0.2 g, *ca*. 0.79 mmol PFMA groups) and N_{α}-(*tert*-Butoxycarbonyl)-L-lysine (28) were mixed with 3 mL DMF. The vial was sealed and they were degassed with nitrogen for 10 minutes. The vial was immersed into 70^0C oil-bath and the mixture was continue to stir under N_2(g) followed by addition of triethylamine (29) in 3 mL DMF slowly. They were then heated at **70^0**C for overnight. 0.15 mL Hydrophilic/hydrophobic amine was added to the vial drop-wise and stirred for further 2 hours. After that, the mixture was cooled to room temperature and 75 µL of sampled were taken for ^{19}F NMR to calculate ester conversion. The remaining was added into cold diethyl ether drop-wise for precipitation. The residue was reprecipitated twice from *ca*. 3 mL methanol into 50 mL diethyl ether. Product was dried in desiccator with vacuum for overnight to give white solid.

Poly(Boc-Lys methacrylamide-*co*-hydroxyethyl methacrylamide) (14a, *ca*. 20%-Lys, DP 27): Boc-Lys-OH (**28**, 0.039 g, 0.16 mmol), triethylamine (**29**, 0.032 g, 0.32 mmol) and ethanolamine (**30**, 0.15 mL) were used to give white product **14a** (0.15 g, ester conversion: 100%): **^{1}H NMR** (D_2O, 400 MHz) δ 069. – 2.14 (br, backbone + Boc), 2.93 [br, 2H, –N–$\underline{CH_2}$–(Lys)], 3.17 – 3.38 [br, 2H, –$\underline{CH_2}$–OH (ethylhydroxy)], 3.49 – 3.72 [br, 2H, –N–$\underline{CH_2}$–(ethylhydroxy)], 3.83 – 3.98 [br, 1H, –N–$\underline{CH}$– (Lys)]; **IR** ν_{max} 1064 (C-C), 1165 (C-O), 1204, 1365 (w-m, C-N), 1387(m, C-H), 1526 (m, N-H), 1634 (s, C=O), 2361 (w, C-H), 2932 (m, C-H), 3305 (br, O-H); **GPC** (DMF) $\overline{M_n}$: 20186, Đ: 1.44

Poly(Boc-Lys methacrylamide-*co*-hydroxyethyl methacrylamide) (14b, *ca*. 40%-Lys, DP 27): Boc-Lys-OH (**28**, 0.078 g, 0.32 mmol), triethylamine (**29**, 0.064 g, 0.63 mmol) and ethanolamine (**30**, 0.15 mL) were used to give white product **14b** (0.19 g, 84%, ester conversion: 100%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.70 – 2.14 (br, backbone + Boc), 2.94 [br, 2H, –N–$\underline{CH_2}$– (Lys)], 3.16 – 3.39 [br, 2H, –$\underline{CH_2}$–OH (ethylhydroxy)], 3.58 – 3.72 [br, 2H, –N–$\underline{CH_2}$– (ethylhydroxy)], 3.73 – 4.01 [br, 1H, –N–$\underline{CH}$– (Lys)]; **IR** ν_{max} 1019, 1066 (C-C), 1164 (C-O), 1205, 1249, 1365 (w-m, C-N), 1391(m, C-H), 1525 (m, N-H), 1652 (s, C=O), 2341, 2361 (w, C-H), 2928 (m, C-H), 3305 (br, O-H); **GPC** (DMF) $\overline{M_n}$: 21425, Đ: 1.41

Poly(Boc-Lys methacrylamide-*co*-hydroxyethyl methacrylamide) (14c, *ca*. 60%-Lys, DP 27): Boc-Lys-OH (**28**, 0.12 g, 0.48 mmol), triethylamine (**29**, 0.096 g, 0.95 mmol) and ethanolamine (**30**, 0.15 mL) were used to give white product **14c** (0.23 g, 85%, ester conversion: 100%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.70 – 2.16 (br, backbone + Boc), 2.94 [br, 2H, –N–$\underline{CH_2}$– (Lys)], 3.15 – 3.34 [br, 2H, –$\underline{CH_2}$–OH (ethylhydroxy)], 3.56 – 3.74 [br, 2H, –N–$\underline{CH_2}$– (ethylhydroxy)], 3.75 – 3.94 [br, 1H, –N–$\underline{CH}$– (Lys)]; **IR** ν_{max} 1066, (w, C-C), 1164 (w,

C-O), 1249, 1365 (w-m, C-N), 1520 (w, N-H), 1645 (w, C=O), 2341, 2361 (m, C-H), 2972 (br, C-H); **GPC** (DMF) $\overline{M_n}$: 21814, Đ: 1.41

Poly(Boc-Lys methacrylamide-*co*-hydroxyethyl methacrylamide) (14d, *ca*. 80%-Lys, DP 27): Boc-Lys-OH (**28**, 0.16 g, 0.63 mmol), triethylamine (**29**, 0.13 g, 1.27 mmol) and ethanolamine (**30**, 0.15 mL) were used to give white product **14d** (0.26 g, 86%, ester conversion: 100%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.74 – 1.80 (br, backbone + Boc), 2.95 [br, 2H, –N–$\underline{CH_2}$– (Lys)], 3.04 – 3.26 [br, 2H, –$\underline{CH_2}$–OH (ethylhydroxy)], 3.46 – 3.63 [br, 2H, –N–$\underline{CH_2}$– (ethylhydroxy)], 3.75 – 3.97 [br, 1H, –N–$\underline{CH}$– (Lys)]; **IR** ν_{max} 1020, 1066 (C-C), 1164 (C-O), 1249, 1365 (w-m, C-N), 1392, 1455 (m, C-H), 1520 (m, N-H), 1660 (s, C=O), 2341, 2361 (m, C-H), 2972 (m, C-H): **GPC** (DMF) $\overline{M_n}$: 22905, Đ: 1.41

Poly(Boc-Lys methacrylamide-*co*-hydroxyethyl methacrylamide) (14e, *ca*. 20%-Lys, DP 54): Boc-Lys-OH (**28**, 0.039 g, 0.16 mmol), triethylamine (**29**, 0.032 g, 0.32 mmol) and ethanolamine (**30**, 0.15 mL) were used to give white product **14e** (0.16 g, ester conversion: 100%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.71 – 2.24 (br, backbone + Boc), 2.93 [br, 2H, –N–$\underline{CH_2}$– (Lys)], 3.16 – 3.42 [br, 2H, –$\underline{CH_2}$–OH (ethylhydroxy)], 3.56 – 3.72 [br, 2H, –N–$\underline{CH_2}$– (ethylhydroxy)], 3.78 – 4.02 [br, 1H, –N–$\underline{CH}$– (Lys)]; **IR** ν_{max} 1062 (C-C), 1165 (C-O), 1206, 1252, 1365 (w-m, C-N), 1387, 1438 (m, C-H), 1529 (m, N-H), 1652 (s, C=O), 2930 (m, C-H), 3335 (br, O-H); **GPC** (DMF) $\overline{M_n}$: 25574, Đ: 1.78

Poly(Boc-Lys methacrylamide-*co*-hydroxyethyl methacrylamide) (14f, *ca*. 40%-Lys, DP 54): Boc-Lys-OH (**28**, 0.078 g, 0.32 mmol), triethylamine (**29**, 0.064 g, 0.63 mmol) and ethanolamine (**30**, 0.15 mL) were used to give white product **14f** (0.19 g, 85%, ester conversion: 100%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.77 – 2.28 (br, backbone + Boc), 2.97 [br, 2H, –N–$\underline{CH_2}$– (Lys)], 3.18 – 3.45 [br, 2H, –$\underline{CH_2}$–OH (ethylhydroxy)], 3.60 – 3.76 [br, 2H, –N–$\underline{CH_2}$– (ethylhydroxy)], 3.80 – 4.06 [br, 1H, –N–$\underline{CH}$– (Lys)]; **IR** ν_{max} 1019, 1062 (C-C), 1165 (C-O), 1206, 1250, 1365 (w-m, C-N), 1388, 1455 (m, C-H), 1526 (m, N-H), 1653 (s, C=O), 2932 (m, C-H), 3303 (br, O-H); **GPC** (DMF) $\overline{M_n}$: 28273, Đ: 1.91

Poly(Boc-Lys methacrylamide-*co*-hydroxyethyl methacrylamide) (14g, *ca*. 60%-Lys, DP 54): Boc-Lys-OH (**28**, 0.12 g, 0.48 mmol), triethylamine (**29**, 0.096 g, 0.95 mmol) and ethanolamine (**30**, 0.15 mL) were used to give white product **14g** (0.24 g, 80%, ester conversion: 100%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.78 – 2.28 (br, backbone + Boc), 2.97 [br, 2H, –N–$\underline{CH_2}$– (Lys)], 3.09 – 3.39 [br, 2H, –$\underline{CH_2}$–OH (ethylhydroxy)], 3.62 – 3.79 [br, 2H, –N–$\underline{CH_2}$– (ethylhydroxy)], 3.80 – 4.02 [br, 1H, –N–$\underline{CH}$– (Lys)]; **IR** ν_{max} 1019, 1065, 1095 (C-C), 1165 (C-O), 1207, 1250, 1365 (w-m, C-N), 1388, 1455 (m, C-H), 1528 (m, N-H), 1654 (s, C=O), 2931 (m, C-H), 3304 (br, O-H); **GPC** (DMF) $\overline{M_n}$: 29810, Đ: 1.76

Poly(Boc-Lys methacrylamide-*co*-hydroxyethyl methacrylamide) (14h, *ca*. 80%-Lys, DP 54): Boc-Lys-OH (**28**, 0.16 g, 0.63 mmol), triethylamine (**29**, 0.13 g, 1.27 mmol) and ethanolamine (**30**, 0.15 mL) were used to give white product **14h** (0.26 g, 85%, ester conversion: 100%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.60 – 2.19 (br, backbone + Boc), 2.88 [br, 2H, –N–$\underline{CH_2}$– (Lys)], 2.98 – 3.26 [br, 2H, –$\underline{CH_2}$–OH (ethylhydroxy)], 3.51 – 3.67 [br, 2H, –N–$\underline{CH_2}$– (ethylhydroxy)], 3.70 – 3.87 [br, 1H, –N–$\underline{CH}$– (Lys)]; **IR** ν_{max} 1019, 1066, 1097 (C-C), 1164 (C-O), 1207, 1250, 1365 (w-m, C-N), 1389, 1455 (m, C-H), 1526 (m, N-H), 1655 (s, C=O), 2930 (m, C-H), 3294 (br, O-H); **GPC** (DMF) $\overline{M_n}$: 31419, Đ: 1.78

Poly(Boc-Lys methacrylamide-*co*-hydroxyethyl methacrylamide) (14i, *ca*. 20%-Lys, DP 88): Boc-Lys-OH (**28**, 0.039 g, 0.16 mmol), triethylamine (**29**, 0.032 g, 0.32 mmol) and ethanolamine (**30**, 0.15 mL) were used to give white product **14i** (0.17 g, ester conversion: 100%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.76 – 2.52 (br, backbone + Boc), 2.98 [br, 2H, –N–$\underline{CH_2}$– (Lys)], 3.22 – 3.43 [br, 2H, –$\underline{CH_2}$–OH (ethylhydroxy)], 3.61 – 3.78 [br, 2H, –N–$\underline{CH_2}$– (ethylhydroxy)], 3.79 – 4.08 [br, 1H, –N–$\underline{CH}$– (Lys)]; **IR** ν_{max} 1065 (C-C), 1166 (C-O), 1205, 1251, 1365 (w-m, C-N), 1389, 1458 (m, C-H), 1529 (m, N-H), 1637, 1654 (s, C=O), 2936 (m, C-H), 3322 (br, O-H); **GPC** (DMF) $\overline{M_n}$: 34508, Đ: 1.64

Poly(Boc-Lys methacrylamide-*co*-hydroxyethyl methacrylamide) (14j, *ca*. 40%-Lys, DP 88): Boc-Lys-OH (28, 0.078 g, 0.32 mmol), triethylamine (29, 0.064 g, 0.63 mmol) and ethanolamine (30, 0.15 mL) were used to give white product 14j (0.19 g, 84%, ester conversion: 100%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.75 – 2.43 (br, backbone + Boc), 2.97 [br, 2H, –N–$\underline{CH_2}$– (Lys)], 3.20 – 3.41 [br, 2H, –$\underline{CH_2}$–OH (ethylhydroxy)], 3.60 – 3.77 [br, 2H, –N–$\underline{CH_2}$– (ethylhydroxy)], 3.78 – 4.03 [br, 1H, –N–$\underline{CH}$– (Lys)]; **IR** ν_{max} 1021, 1066 (C-C), 1165 (C-O), 1206, 1250, 1365 (w-m, C-N), 1392, 1457 (m, C-H), 1530 (m, N-H), 1655 (s, C=O), 2341, 2359 (w, C-H), 2931 (m, C-H), 3272 (br, O-H); **GPC** (DMF) $\overline{M_n}$: 34970, Đ: 1.73

Poly(Boc-Lys methacrylamide-*co*-hydroxyethyl methacrylamide) (14k, *ca*. 60%-Lys, DP 88): Boc-Lys-OH (**28**, 0.12 g, 0.48 mmol), triethylamine (**29**, 0.096 g, 0.95 mmol) and ethanolamine (**30**, 0.15 mL) were used to give white product **14k** (0.24 g, 80%, ester conversion: 100%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.75 – 2.46 (br, backbone + Boc), 2.98 [br, 2H, –N–$\underline{CH_2}$– (Lys)], 3.09 – 3.42 [br, 2H, –$\underline{CH_2}$–OH (ethylhydroxy)], 3.61 – 3.78 [br, 2H, –N–$\underline{CH_2}$– (ethylhydroxy)], 3.79 – 4.07 [br, 1H, –N–$\underline{CH}$– (Lys)]; **IR** ν_{max} 1020, 1066 (C-C), 1164 (C-O), 1206, 1249, 1365 (w-m, C-N), 1393, 1457 (m, C-H), 1522 (m, N-H), 1654 (s, C=O), 2343, 2359 (w, C-H), 2972 (br, C-H), 3271 (br, O-H); **GPC** (DMF) $\overline{M_n}$: 40247, Đ: 1.63

Poly(Boc-Lys methacrylamide-*co*-hydroxyethyl methacrylamide) (14l, *ca*. 80%-Lys, DP 88): Boc-Lys-OH (**28**, 0.16 g, 0.63 mmol), triethylamine (**29**, 0.13 g, 1.27 mmol) and

ethanolamine (**30**, 0.15 mL) were used to give white product **14l** (0.23 g, 87%, ester conversion: 100%): ^{1}H NMR (D_2O, 400 MHz) δ 0.71 – 2.25 (br, backbone + Boc), 2.98 [br, 2H, –N–$\underline{CH_2}$– (Lys)], 3.07 – 3.34 [br, 2H, –$\underline{CH_2}$–OH (ethylhydroxy)], 3.59 [br, 2H, –N–$\underline{CH_2}$– (ethylhydroxy)], 3.80 – 4.00 [br, 1H, –N–$\underline{CH}$– (Lys)]; IR ν_{max} 1019, 1066 (m, C-C), 1164 (m, C-O), 1207, 1249, 1364 (w-m, C-N), 1392, 1456 (m, C-H), 1522 (m, N-H), 1654 (s, C=O), 2343, 2359 (w, C-H), 2931. 2972 (m, C-H), 3276 (br, O-H); GPC (DMF) $\overline{M_n}$: 42295, Đ: 1.56

Poly(Boc-Lys methacrylamide-*co*-propyl methacrylamide) (14m, *ca*. 20%-Lys, DP 94): Boc-Lys-OH (**28**, 0.16 g, 0.63 mmol), triethylamine (**29**, 0.13 g, 1.27 mmol) and propylamine (**32**, 0.15 mL) were used to give white product **14m** (0.23 g, ester conversion: 100%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.80 – 2.26 (br, backbone + Boc), 2.97 [br, 2H, –N–$\underline{CH_2}$–(propylamide)], 3.16 [br, 2H, –N–$\underline{CH_2}$–(Lys)], 3.87 [br, 1H, –N–$\underline{CH}$– (Lys)]; **IR** ν_{max} 1021, 1049 (C-C), 1165 (m, C-O), 1248, 1365 (w-m, C-N), 1390 (m, C-H), 1523 (m, N-H), 1655 (s, C=O), 2361 (w, C-H), 2933 (br, C-H); **GPC** (DMF) $\overline{M_n}$: 38860, Đ: 1.90

Poly(Boc-Lys methacrylamide-*co*-pentyl methacrylamide) (14n, *ca*. 20%-Lys, DP 94): Boc-Lys-OH (**28**, 0. 16 g, 0.63 mmol), triethylamine (**29**, 0.13 g, 1.27 mmol) and pentylamine (**33**, 0.15 mL) were used to give white product **14m** (0.23 g, 87%, ester conversion: 100%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.63 – 2.37 (br, backbone + Boc), 2.96 [br, 2H, –N–$\underline{CH_2}$–(propylamide)], 3.15 [br, 2H, –N–$\underline{CH_2}$–(Lys)], 3.88 [br, 1H, –N–$\underline{CH}$– (Lys)]; **IR** ν_{max} 1021, 1050 (C-C), 1164 (m, C-O), 1248, 1365 (w-m, C-N), 1390 (m, C-H), 1521 (m, N-H), 1661 (s, C=O), 2342, 2361 (w, C-H), 2931 (br, C-H); **GPC** (DMF) $\overline{M_n}$: 39439, Đ: 1.79

Poly(Boc-Lys methacrylamide-*co*-hexyl methacrylamide) (14o, *ca*. 20%-Lys, DP 94): Boc-Lys-OH (**28**, 0. 16 g, 0.63 mmol), triethylamine (**29**, 0.13 g, 1.27 mmol) and hexylamine (**34**, 0.15 mL) were used to give white product **14o** (0.24 g, 87%, ester conversion: 100%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.69 – 2.20 (br, backbone + Boc), 2.98 [br, 2H, –N–$\underline{CH_2}$–(propylamide)], 3.14 [br, 2H, –N–$\underline{CH_2}$–(Lys)], 3.86 [br, 1H, –N–$\underline{CH}$– (Lys)]; **IR** ν_{max} 1020, 1052 (w, C-C), 1163 (m, C-O), 1248, 1365 (w-m, C-N), 1389 (m, C-H), 1521 (m, N-H), 1655 (s, C=O), 2342, 2360 (w, C-H), 2931, 2980 (br, C-H); **GPC** (DMF) $\overline{M_n}$: 40013, Đ: 1.87

Poly(Boc-Lys methacrylamide-*co*-benzyl methacrylamide) (14p, *ca*. 20%-Lys, DP 94): Boc-Lys-OH (**28**, 0. 16 g, 0.63 mmol), triethylamine (**29**, 0.13 g, 1.27 mmol) and benzylamine (**35**, 0.15 mL) were used to give white product **14p** (0.23 g, 88%, ester conversion: 100%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.75 – 2.20 (br, backbone + Boc), 3.12 [br, 2H, –N–$\underline{CH_2}$–(Lys)], 3.84 [br, 1H, –N–$\underline{CH}$– (Lys)], 4.16 [br, 2H, –N–$\underline{CH_2}$– (benzylamide)], 7.44 [br, 5H, aromatic (benzylamide)]; **IR** ν_{max} 1021, 1060 (w-m, C-C), 1162 (m, C-O), 1248, 1365 (w-m, C-N),

1390 (m, C-H), 1456, 1520 (w-m, N-H), 1661 (s, C=O), 2341, 2360 (w, C-H), 2980 (br, C-H); **GPC** (DMF) $\overline{M_n}$: 43347, Đ: 1.77

Poly(Boc-Lys methacrylamide-*co*-propyl methacrylamide) (14q, *ca*. 50%-Lys, DP 94): Boc-Lys-OH (**28**, 0.088 g, 0.36 mmol), triethylamine (**29**, 0.13 g, 0.71 mmol) and propylamine (**32**, 0.20 mL) were used to give white product **14q** (0.20 g, ester conversion: 100%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.74 – 1.91 (br, backbone + Boc), 2.98 [br, 2H, –N–$\underline{CH_2}$– (propylamide)], 3.18 [br, 2H, –N–$\underline{CH_2}$–(Lys)], 3.89 [br, 1H, –N–$\underline{CH}$– (Lys)]; **IR** ν_{max} 1048 (C-C), 1165 (m, C-O), 1248, 1365 (w-m, C-N), 1390 (m, C-H), 1520 (m, N-H), 1660 (s, C=O), 2341, 2360 (w, C-H), 2936 (br, C-H); **GPC** (DMF) $\overline{M_n}$: 58004, Đ: 1.85

Poly(Boc-Lys methacrylamide-*co*-pentyl methacrylamide) (14r, *ca*. 50%-Lys, DP 94): Boc-Lys-OH (**28**, 0.088 g, 0.36 mmol), triethylamine (**29**, 0.13 g, 0.71 mmol) and pentylamine (**33**, 0.25 mL) were used to give white product **14r** (0.20 g, ester conversion: 100%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.68 – 2.20 (br, backbone + Boc), 3.00 [br, 2H, –N–$\underline{CH_2}$– (propylamide)], 3.18 [br, 2H, –N–$\underline{CH_2}$–(Lys)], 3.88 [br, 1H, –N–$\underline{CH}$– (Lys)]; **IR** ν_{max} 1017, 1033, 1053 (C-C), 1164 (s, C-O), 1247, 1365 (w-m, C-N), 1389 (m, C-H), 1520 (m, N-H), 1660 (s, C=O), 2342, 2360 (w, C-H), 2865, 2934 (br, C-H); **GPC** (DMF) $\overline{M_n}$: 60523, Đ: 1.83

Poly(Boc-Lys methacrylamide-*co*-hexyl methacrylamide) (14s, *ca*. 50%-Lys, DP 94): Boc-Lys-OH (**28**, 0.088 g, 0.36 mmol), triethylamine (**29**, 0.13 g, 0.71 mmol) and hexylamine (**34**, 0.25 mL) were used to give white product **14s** (0.21 g, ester conversion: 100%): **^{1}H NMR** [$(CD_3)_2CO$, 400 MHz] δ 0.75 – 2.01 (br, backbone + Boc), 2.97 [br, 2H, –N–$\underline{CH_2}$–(propylamide)], 3.13 [br, 2H, –N–$\underline{CH_2}$–(Lys)], 3.88 [br, 1H, –N–$\underline{CH}$– (Lys)]; **IR** ν_{max} 1016, 1033, 1054 (w, C-C), 1165 (m, C-O), 1248, 1365 (w-m, C-N), 1389 (m, C-H), 1520 (m, N-H), 1659 (s, C=O), 2342, 2360 (w, C-H), 2862, 2933 (br, C-H); **GPC** (DMF) $\overline{M_n}$: 60700, Đ: 1.80

Poly(Boc-Lys methacrylamide-*co*-benzyl methacrylamide) (14t, *ca*. 50%-Lys, DP 94): Boc-Lys-OH (**28**, 0.088 g, 0.36 mmol), triethylamine (**29**, 0.13 g, 0.71 mmol) and benzylamine (**35**, 0.20 mL) were used to give white product **14t** (0.21 g, ester conversion: 100%): **^{1}H NMR** [$(CD_3)_2CO$, 400 MHz] δ 0.56 – 2.20 (br, backbone + Boc), 3.09 [br, 2H, –N–$\underline{CH_2}$–(Lys)], 3.85 [br, 1H, –N–$\underline{CH}$– (Lys)], 4.13 [br, 2H, –N–$\underline{CH_2}$– (benzylamide)], 7.21 [br, 5H, aromatic (benzylamide)]; **IR** ν_{max} 1008, 1033, 1054 (w-m, C-C), 1163 (m, C-O), 1247, 1365 (w-m, C-N), 1390 (m, C-H), 1455, 1519 (w-m, N-H), 1660 (s, C=O), 2341, 2360 (w, C-H), 2937, 2973 (br, C-H); **GPC** (DMF) $\overline{M_n}$: 61122, Đ: 1.87

2.6 Deprotection of poly(Boc-Lys methacrylamide-*co*-alkyl methacrylamide) (14)

General procedure: In a medium vial, poly(Boc-Lys methacrylate-*co*-hydroxyethyl methacrylate) (**14**) was mixed with trifluoroacetic acid (**31**) at room temperature. After all the solids were completely dissolved, it was stirred for 1 hour. The solution was then added into diethyl ether drop-wise for precipitation. The crude product was reprecipitated for twice from methanol into diethyl ether. The isolated crude product was dried in desiccator with vacuum for overnight. It was then further purified with dialysis for 1 day followed by freeze-drying to give white solid.

Poly(Lys methacrylamide-*co*-hydroxyethyl methacrylamide) (15a, *ca*. 20%-Lys, DP 27): Modified polymer (**14a**, 0.15 g) was reacted with 1.80 mL TFA (**31**) and dialysed using dialysis tubing with molecular weight cut off of 100 – 500 Da to give a white product **15a** (0.085 g, 54%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.73 – 2.49 [br, backbone + –CH_2– (Lys)], 3.30 [br, 2H, –$\underline{CH_2}$–OH (ethylhydroxy)], 3.68 [br, 2H, –N–$\underline{CH_2}$– (ethylhydroxy)], 3.76 [br, 2H, –N–$\underline{CH_2}$– (Lys)], 3.98 [br, 1H, –N–$\underline{CH}$– (Lys)]; **IR** ν_{max} 1059 (C-C), 1201, 1341 (m, C-N), 1388, 1459 (m, C-H), 1529 (m, N-H), 1654 (s, C=O), 2942 (br, C-H), 3342 (br, O-H); **GPC** (Aqueous) $\overline{M_n}$: 5211, Đ: 1.93

Poly(Lys methacrylamide-*co*-hydroxyethyl methacrylamide) (15b, *ca*. 40%-Lys, DP 27): Modified polymer (**14b**, 0.19 g) was reacted with 2.30 mL TFA (**31**) and dialysed using dialysis tubing with molecular weight cut off of 100 – 500 Da to give a white product **15b** (0.085 g, 55%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.71 – 2.64 [br, backbone + –CH_2– (Lys)], 3.29 [br, 2H, –$\underline{CH_2}$–OH (ethylhydroxy)], 3.68 [br, 2H, –N–$\underline{CH_2}$– (ethylhydroxy)], 3.76 [br, 2H, –N–$\underline{CH_2}$– (Lys)], 3.97 [br, 1H, –N–$\underline{CH}$– (Lys)]; **IR** ν_{max} 1058 (C-C), 1201, 1343 (m, C-N), 1393, 1456 (m, C-H), 1532 (m, N-H), 1652 (s, C=O), 2342, 2360 (w, C-H), 2943 (br, C-H), 3247 (br, O-H); **GPC** (Aqueous) $\overline{M_n}$: 5682, Đ: 2.14

Poly(Lys methacrylamide-*co*-hydroxyethyl methacrylamide) (15c, *ca*. 60%-Lys, DP 27): Modified polymer (**14c**, 0.23 g) was reacted with 2.60 mL TFA (**31**) and dialysed using dialysis tubing with molecular weight cut off of 1000 Da to give a white product **15c** (0.072 g, 42%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.65– 2.48 [br, backbone + –CH_2– (Lys)], 3.29 [br, 2H, –$\underline{CH_2}$–OH (ethylhydroxy)], 3.68 [br, 2H, –N–$\underline{CH_2}$– (ethylhydroxy)], 3.76 [br, 2H, –N–$\underline{CH_2}$– (Lys)], 3.96 [br, 1H, –N–$\underline{CH}$– (Lys)]; **IR** ν_{max} 1066 (C-C), 1204, 1341 (m, C-N), 1394 (m, C-H), 1534, 1560 (m, N-H), 1637, 1654 (s, C=O), 2341, 2360 (w, C-H), 2942 (br, C-H), 3246 (br, O-H); **GPC** (Aqueous) $\overline{M_n}$: 9586, Đ: 1.51

Poly(Lys methacrylamide-*co*-hydroxyethyl methacrylamide) (15d, *ca*. 80%-Lys, DP 27): Modified polymer (**14d**, 0.26 g) was reacted with 3.00 mL TFA (**31**) and dialysed using

dialysis tubing with molecular weight cut off of 1000 Da to give a white product **15d** (0.087 g, 47%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.71– 2.44 [br, backbone + –CH_2– (Lys)], 3.29 [br, 2H, –$\underline{CH_2}$–OH (ethylhydroxy)], 3.67 [br, 2H, –N–$\underline{CH_2}$– (ethylhydroxy)], 3.76 [br, 2H, –N–$\underline{CH_2}$– (Lys)], 3.96 [br, 1H, –N–$\underline{CH}$– (Lys)]; **IR** ν_{max} 1066 (C-C), 1203, 1341 (m, C-N), 1395 (m, C-H), 1534, 1560 (m, N-H), 1624, 1654 (s, C=O), 2341, 2360 (w, C-H), 2943 (br, C-H), 3246 (br, O-H); **GPC** (Aqueous) $\overline{M_n}$: 9945, Đ: 1.53

Poly(Lys methacrylamide-*co*-hydroxyethyl methacrylamide) (15e, *ca*. 20%-Lys, DP 54): Modified polymer (**14e**, 0.16 g) was reacted with 1.90 mL TFA (**31**) and dialysed using dialysis tubing with molecular weight cut off of 3500 Da to give a white product **15e** (0.034 g, 24%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.57 – 2.26 [br, backbone + –CH_2– (Lys)], 3.20 [br, 2H, –$\underline{CH_2}$–OH (ethylhydroxy)], 3.58 [br, 2H, –N–$\underline{CH_2}$– (ethylhydroxy)], 3.68 [br, 2H, –N–$\underline{CH_2}$– (Lys)], 3.90 [br, 1H, –N–$\underline{CH}$– (Lys)]; **IR** ν_{max} 1059 (C-C), 1203, 1336 (m, C-N), 1387, 1462 (m, C-H), 1531 (m, N-H), 1651, 1713(s, C=O), 2937 (br, C-H), 3349 (br, O-H); **GPC** (Aqueous) $\overline{M_n}$: 11026, Đ: 1.87

Poly(Lys methacrylamide-*co*-hydroxyethyl methacrylamide) (15f, *ca*. 40%-Lys, DP 54): Modified Deprotected polymer (**14f**, 0.19 g) was reacted with 2.20 mL TFA (**31**) and dialysed using dialysis tubing with molecular weight cut off of 3500 Da to give a white product **15f** (0.030 g, 20%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.63 – 2.39 [br, backbone + –CH_2– (Lys)], 3.21 [br, 2H, –$\underline{CH_2}$–OH (ethylhydroxy)], 3.59 [br, 2H, –N–$\underline{CH_2}$– (ethylhydroxy)], 3.67 [br, 2H, –N–$\underline{CH_2}$– (Lys)], 3.89 [br, 1H, –N–$\underline{CH}$– (Lys)]; **IR** ν_{max} 1061 (C-C), 1203, 1339 (m, C-N), 1391, 1462 (m, C-H), 1531 (m, N-H), 1651 (s, C=O), 2340, 2361 (w, C-H), 2937 (br, C-H), 3245 (br, O-H); **GPC** (Aqueous) $\overline{M_n}$: 11432, Đ: 1.52

Poly(Lys methacrylamide-*co*-hydroxyethyl methacrylamide) (15g, *ca*. 60%-Lys, DP 54): Modified polymer (**14g**, 0.24 g) was reacted with 2.80 mL TFA (**31**) and dialysed using dialysis tubing with molecular weight cut off of 3500 Da to give a white product **15g** (0.050 g, 27%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.61– 2.38 [br, backbone + –CH_2– (Lys)], 3.19 [br, 2H, –$\underline{CH_2}$–OH (ethylhydroxy)], 3.58 [br, 2H, –N–$\underline{CH_2}$– (ethylhydroxy)], 3.66 [br, 2H, –N–$\underline{CH_2}$– (Lys)], 3.88 [br, 1H, –N–$\underline{CH}$– (Lys)]; **IR** ν_{max} 1065 (C-C), 1204, 1339 (m, C-N), 1393 (m, C-H), 1531 (m, N-H), 1633 (s, C=O), 2341, 2361 (w, C-H), 2935 (br, C-H), 3246 (br, O-H); **GPC** (Aqueous) $\overline{M_n}$: 14579, Đ: 1.53

Poly(Lys methacrylamide-*co*-hydroxyethyl methacrylamide) (15h, *ca*. 80%-Lys, DP 54): Modified polymer (**14h**, 0.26 g) was reacted with 3.00 mL TFA (**31**) and dialysed using dialysis tubing with molecular weight cut off of 3500 Da to give a white product **15h** (0.062 g, 34%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.60– 2.33 [br, backbone + –CH_2– (Lys)], 3.19 [br, 2H, –

$\underline{CH_2}$–OH (ethylhydroxy)], 3.57 [br, 2H, –N–$\underline{CH_2}$– (ethylhydroxy)], 3.66 [br, 2H, –N–$\underline{CH_2}$– (Lys)], 3.87 [br, 1H, –N–$\underline{CH}$– (Lys)]; **IR** ν_{max} 1059 (C-C), 1155, 1210, 1341 (m, C-N), 1396 (m, C-H), 1460, 1528 (m, N-H), 1629, 1714 (s, C=O), 2358 (w, C-H), 2933 (br, C-H), 3215 (br, O-H); **GPC** (Aqueous) $\overline{M_n}$: 17290, Đ: 1.58

Poly(Lys methacrylamide-*co*-hydroxyethyl methacrylamide) (15i, *ca*. 20%-Lys, DP 88): Modified polymer (**14i**, 0.17 g) was reacted with 1.90 mL TFA (**31**) and dialysed using dialysis tubing with molecular weight cut off of 1000 Da to give a white product **15i** (0.067 g, 46%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.65 – 2.44 [br, backbone + –CH_2– (Lys)], 3.31 [br, 2H, –$\underline{CH_2}$–OH (ethylhydroxy)], 3.68 [br, 2H, –N–$\underline{CH_2}$– (ethylhydroxy)], 3.76 [br, 2H, –N–$\underline{CH_2}$– (Lys)], 3.99 [br, 1H, –N–$\underline{CH}$– (Lys)]; **IR** ν_{max} 1058 (C-C), 1203, 1337 (m, C-N), 1388, 1458 (m, C-H), 1531 (m, N-H), 1651 (s, C=O), 2342, 2360 (w, C-H), 2943 (br, C-H), 3272 (br, O-H); **GPC** (Aqueous) $\overline{M_n}$: 19278, Đ: 1.69

Poly(Lys methacrylamide-*co*-hydroxyethyl methacrylamide) (15j, *ca*. 40%-Lys, DP 88): Modified polymer (**14j**, 0.18 g) was reacted with 2.10 mL TFA (**31**) and dialysed using dialysis tubing with molecular weight cut off of 1000 Da to give a white product **15j** (0.084 g, 49%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.74 – 2.50 [br, backbone + –CH_2– (Lys)], 3.30 [br, 2H, –$\underline{CH_2}$–OH (ethylhydroxy)], 3.68 [br, 2H, –N–$\underline{CH_2}$– (ethylhydroxy)], 3.76 [br, 2H, –N–$\underline{CH_2}$– (Lys)], 3.97 [br, 1H, –N–$\underline{CH}$– (Lys)]; **IR** ν_{max} 1060 (C-C), 1202, 1338 (m, C-N), 1390, 1462 (m, C-H), 1531 (m, N-H), 1651, 1712 (s, C=O), 2942 (br, C-H), 3273 (br, O-H); **GPC** (Aqueous) $\overline{M_n}$: 19961, Đ: 1.72

Poly(Lys methacrylamide-*co*-hydroxyethyl methacrylamide) (15k, *ca*. 60%-Lys, DP 88): Modified polymer (**14k**, 0.23 g) was reacted with 2.60 mL TFA (**31**) and dialysed using dialysis tubing with molecular weight cut off of 3500 Da to give a white product **15k** (0.084 g, 49%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.75– 2.50 [br, backbone + –CH_2– (Lys)], 3.30 [br, 2H, –$\underline{CH_2}$–OH (ethylhydroxy)], 3.68 [br, 2H, –N–$\underline{CH_2}$– (ethylhydroxy)], 3.77 [br, 2H, –N–$\underline{CH_2}$– (Lys)], 3.97 [br, 1H, –N–$\underline{CH}$– (Lys)]; **IR** ν_{max} 1058 (C-C), 1201, 1338 (m, C-N), 1393 (m, C-H), 1455, 1538 (m, N-H), 1633, 1651 (s, C=O), 2341, 2360 (w, C-H), 2942 (br, C-H), 3275 (br, O-H); **GPC** (Aqueous) $\overline{M_n}$: 23568, Đ: 1.70

Poly(Lys methacrylamide-*co*-hydroxyethyl methacrylamide) (15l, *ca*. 80%-Lys, DP 88): Modified polymer (**14l**, 0.23 g) was reacted with 2.70 mL TFA (**31**) and dialysed using dialysis tubing with molecular weight cut off of 3500 Da to give a white product **15l** (0.10 g, 64%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.77– 2.49 [br, backbone + –CH_2– (Lys)], 3.29 [br, 2H, –$\underline{CH_2}$–OH (ethylhydroxy)], 3.68 [br, 2H, –N–$\underline{CH_2}$– (ethylhydroxy)], 3.76 [br, 2H, –N–$\underline{CH_2}$– (Lys)], 3.97 [br, 1H, –N–$\underline{CH}$– (Lys)]; **IR** ν_{max} 1057 (C-C), 1201, 1343 (m, C-N), 1395 (m, C-H),

1528 (m, N-H), 1654, (s, C=O), 2341, 2360 (w, C-H), 2949 (br, C-H); **GPC** (Aqueous) $\overline{M_n}$: 25049, Đ: 1.77

Poly(Lys methacrylamide-*co*-propyl methacrylamide) (15m, *ca*. 80%-Lys, DP 94): Modified polymer (**14m**, 0.23 g) was reacted with 2.60 mL TFA (**31**) and dialysed using dialysis tubing with molecular weight cut off of 3500 Da to give a white product **15m** (0.12 g, 72%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.65 – 2.44 (br, backbone), 2.99 – 3.38 (br, backbone), 3.59 [br, 2H, –N–$\underline{CH_2}$– (propylamide)], 3.77 [br, 2H, –N–$\underline{CH_2}$–(Lys)], 3.97 [br, 1H, –N–$\underline{CH}$–(Lys)]; **IR** ν_{max} 1202 (C-C), 1342, 1394, 1460 (m, C-H), 1524 (m, N-H), 1625 (s, C=O), 2361 (w, C-H), 2936 (br, C-H)

Poly(Lys methacrylamide-*co*-pentyl methacrylamide (15n, *ca*. 80%-Lys, DP 94): Modified polymer (**14n**, 0.24 g) was reacted with 2.70 mL TFA (**31**) and dialysed using dialysis tubing with molecular weight cut off of 3500 Da to give a white product **15n** (0.11 g, 66%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.75 – 2.28 (br, backbone), 2.97 – 3.38 (br, backbone), 3.57 [br, 2H, –N–$\underline{CH_2}$– (pentylamide)], 3.76 [br, 2H, –N–$\underline{CH_2}$–(Lys)], 3.97 [br, 1H, –N–$\underline{CH}$–(Lys)]; **IR** ν_{max} 1015, 1033, 1055, 1202 (w-m, C-C), 1343, 1394, (m, C-H), 1524 (m, N-H), 1625 (s, C=O), 2342, 2361, (w, C-H), 2867, 2937 (br, C-H)

Poly(Lys methacrylamide-*co*-hexyl methacrylamide) (15o, *ca*. 80%-Lys, DP 94): Modified: polymer (**14o**, 0.24 g) was reacted with 2.70 mL TFA (**31**) and dialysed using dialysis tubing with molecular weight cut off of 3500 Da to give a white product **15o** (0.085 g, 51%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.71 – 2.26 (br, backbone), 2.95 – 3.38 (br, backbone), 3.59 [br, 2H, –N–$\underline{CH_2}$– (hexylamide)], 3.76 [br, 2H, –N–$\underline{CH_2}$–(Lys)], 3.97 [br, 1H, –N–$\underline{CH}$–(Lys)]; **IR** ν_{max} 1060, 1138, 1201 (w-m, C-C), 1339, 1393, (m, C-H), 1520, 1538 (m, N-H), 1622, 1644 (s, C=O), 2342, 2360, (w, C-H), 2869, 2933 (br, C-H)

Poly(Lys methacrylamide-*co*-benzyl methacrylamide) (15p, *ca*. 80%-Lys, DP 94): Modified polymer (**14p**, 0.23 g) was reacted with 2.70 mL TFA (**31**) and dialysed using dialysis tubing with molecular weight cut off of 3500 Da to give a white product **15p** (0.12 g, 72%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.67 – 2.49 (br, backbone + Boc), 2.98 – 3.38 (br, backbone), 3.61 [br, 2H, –N–$\underline{CH_2}$– (benzylamide)], 3.77 [br, 2H, –N–$\underline{CH_2}$–(Lys)], 3.97 [br, 1H, –N–$\underline{CH}$– (Lys)], 7.39 [br, 5H, aromatic (benzylamide)]; **IR** ν_{max} 1010, 1033, 1055 (w-m, C-C), 1345 (w-m, C-N), 1394 (m, C-H), 1456, 1520 (w-m, N-H), 1622 (s, C=O), 2341, 2360 (w, C-H), 2844, 2866, 2938 (br, C-H)

Poly(Lys methacrylamide-*co*-propyl methacrylamide) (15q, *ca*. 50%-Lys, DP 94): Modified polymer (**15q**, 0.20 g) was reacted with 2.30 mL TFA (**31**) and dialysed using dialysis tubing with molecular weight cut off of 3500 Da to give a white product **15q** (0.11 g,

72%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.65 – 2.25 (br, backbone), 2.95 – 3.36 (br, backbone), 3.59 [br, 2H, –N–$\underline{CH_2}$– (propylamide)], 3.76 [br, 2H, –N–$\underline{CH_2}$–(Lys)], 3.96 [br, 1H, –N–$\underline{CH}$–(Lys)]; **IR** v_{max} 1136 (m, C-C), 1342, 1389 (m, C-H), 1524 (m, N-H), 1655 (s, C=O), 2935 (br, C-H)

Poly(Lys methacrylamide-*co*-pentyl methacrylamide) (15r, *ca*. 50%-Lys, DP 94): Modified polymer (**14r**, 0.20 g) was reacted with 2.40 mL TFA (**31**) and dialysed using dialysis tubing with molecular weight cut off of 3500 Da to give a white product **15r** (0.11 g, 68%): **^{1}H NMR** (D_2O, 400 MHz) δ 0.66 – 2.24 (br, backbone), 2.92 – 3.36 (br, backbone), 3.61 [br, 2H, –N–$\underline{CH_2}$– (pentylamide)], 3.75 [br, 2H, –N–$\underline{CH_2}$–(Lys)], 3.95 [br, 1H, –N–$\underline{CH}$–(Lys)]; **IR** v_{max} 1136, 1200 (w-m, C-C), 1342, 1392, (m, C-H), 1523 (m, N-H), 1630 (s, C=O), 2360, (w, C-H), 2931 (br, C-H)

Poly(Lys methacrylamide-*co*-hexyl methacrylamide) (15s, *ca*. 50%-Lys, DP 94): Modified polymer (**14s**, 0.21 g) was reacted with 2.50 mL TFA (**31**) and dialysed using dialysis tubing with molecular weight cut off of 3500 Da to give a white product **15s** (0.076 g, 45%): **^{1}H NMR** [(D_2O + $(CD_3)_2CO$, 400 MHz)] δ 0.80 – 2.27 (br, backbone), 3.14 – 3.61 (br, backbone), 3.75 [br, 2H, –N–$\underline{CH_2}$– (hexylamide)], 3.94 [br, 2H, –N–$\underline{CH_2}$–(Lys)], 4.20 [br, 1H, –N–$\underline{CH}$– (Lys)]; **IR** v_{max} 1056, 1135, 1200 (w-m, C-C), 1340, 1393, (m, C-H), 1456, 1520 (m, N-H), 1633 (s, C=O), 2341, 2360, (w, C-H), 2929 (br, C-H)

Poly(Lys methacrylamide-*co*-propyl methacrylamide) (15t, *ca*. 50%-Lys, DP 94): Modified polymer (**14t**, 0.22 g) was reacted with 2.50 mL TFA (**31**) and dialysed using dialysis tubing with molecular weight cut off of 3500 Da to give a white product **15t** (0.11 g, 62%): **^{1}H NMR** [(D_2O + $(CD_3)_2CO$, 400 MHz)] δ 0.77 – 2.18 (br, backbone + Boc), 3.01 – 3.37 (br, backbone), 3.66 [br, 2H, –N–$\underline{CH_2}$– (benzylamide)], 3.83 [br, 2H, –N–$\underline{CH_2}$–(Lys)], 4.07 [br, 1H, –N–$\underline{CH}$– (Lys)], 7.43 [br, 5H, aromatic (benzylamide)]; **IR** v_{max} 1055 1136, 1201 (w-m, C-C), 1340 (w-m, C-N), 1394 (m, C-H), 1456, 1520 (w-m, N-H), 1651 (s, C=O), 2341, 2359 (w, C-H), 2944 (br, C-H)

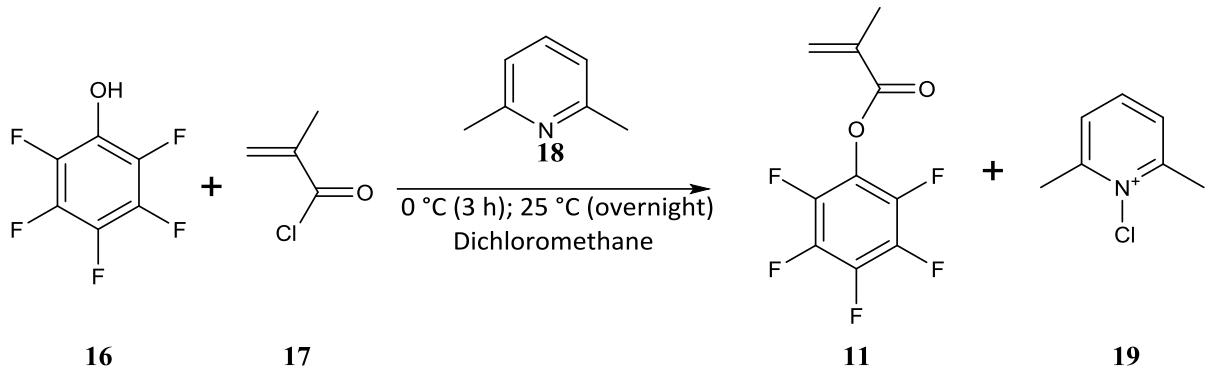
2.7 Splat cooling assay

1 mg of polymer was dissolved in 0.5 mL PBS solution to prepare polymer solutions with concentration of 20 mg/mL in PBS. Approximately 10 μL of such solution was dropped from 1.80 m onto a glass coverslip which was chilled on an aluminium plate with dry ice. The glass coverslip was quickly placed onto the Linkam cryostage and held at -8^{0}C for 30 minutes. Pictures of ice-crystals were taken at t=0 and 30 minutes using an Olympus CX 41 microscope with a 10x objective lens. The average sizes of ice-crystal were obtained using ImageJ by calculating 10 of the largest ice-crystals. Minimum of 3 repeats were performed.

3. Results & Discussion

3.1 Synthesis of monomer, pentafluorophenyl methacrylate (11)

PFMA **11** was synthesized using a modified procedure to that reported in the literature.[44] Pentafluorophenol (**16**) was reacted with acryloyl chloride (**17**) in the presence of a base, 2,6-lutidine (**18**) at 0 ^{0}C for 3 hours then allowed to stir at room temperature overnight to give monomer **11** and by-product **19** (Scheme 7). By-product **19** was precipitated and filtered-off. Product **11** can be further extracted and purified using liquid-liquid extraction (water, DCM) and followed by column chromatography with petroleum ether (R_f: 0.31).



Scheme 7. Synthesis of PFMA **11** using Pentafluorophenol (**16**), acryloyl chloride (**17**) & 2,6-lutidine (**18**)

Monomer **11** can be characterised using ^{1}H & ^{19}F NMR effectively. By comparing ^{1}H NMR spectra of starting material **17** and product **11**, peaks corresponding to the C=C double bond from 6.03 and 6.49 ppm shifted to 5.92 and 6.46 ppm. In the ^{19}F NMR of starting material **16**, peaks at -169.12, -164.39, -164.09, pm correspond to the fluorine atoms and after the reaction, they were shifted to -163.16, -158.87 and -153.87 ppm (Figure 7).

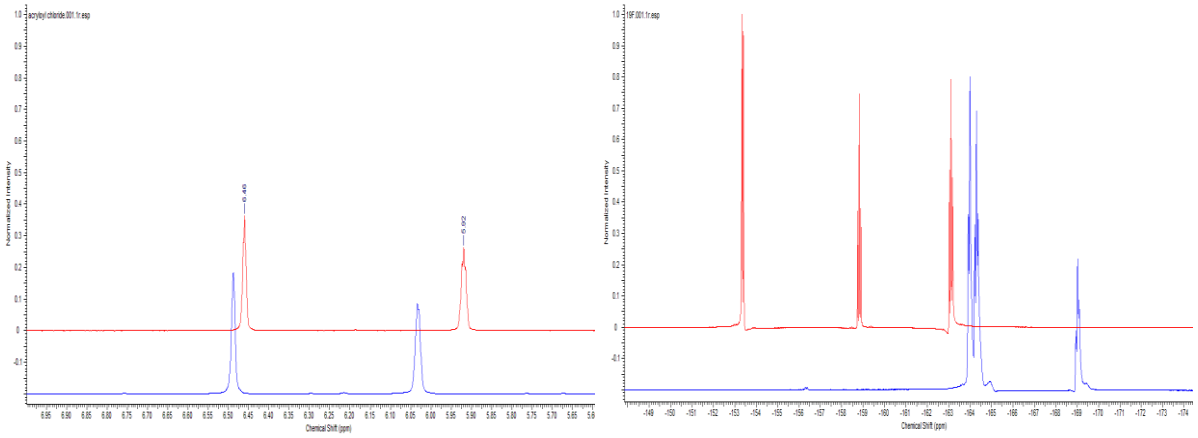
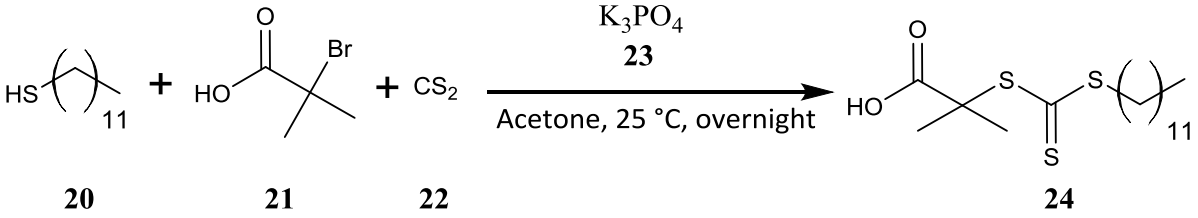


Figure 7. ^{1}H NMR of starting material **17** (blue) & product **11** (red) (left); ^{19}F NMR of starting material **16** (blue) & product **11** (red) (right)

3.2 Synthesis of 2-(Dodecylthiocarbonothioylthio)-2-methylpropanoic acid (24)

RAFT agent 2-(Dodecylthiocarbonothioylthio)-2-methylpropanoic acid (**24**) was synthesized using a reported protocol[45] by reacting 1-dodecanethiol (**20**), 2-bromo-1-methylpropionic acid (**21**), carbon disulphide (**22**) in the presence of tribasic potassium phosphate (**23**) in acetone at 25 ^{0}C overnight. RAFT agent **24** can simply be isolated using liquid-liquid extraction and recrystallization from hexane and can be characterised with ^{1}H NMR. RAFT agent 24 is capable to polymerise monomers such as styrenes, methacrylates and methacrylamides.[46] Therefore, it is chosen to polymerise PFMA **11**.

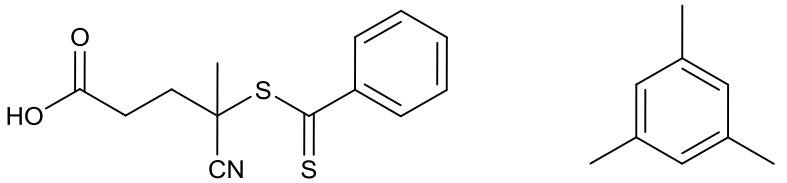


Scheme 8. Synthesis of RAFT agent **24**

3.2.1 Kinetic study of PFMA 10 polymerisation using 2-(Dodecylthiocarbonothioylthio)-2-methylpropanoic acid (24)

Kinetics of PFMA **10** polymerisation with RAFT **24** were measured using ^{1}H NMR. Gibson *et. al*. used RAFT agent, 4-cyanopentanoic acid dithiobenzoate (**25**) (Figure 8) to synthesize PFMA **11** and it was noticed that deviations from linearity were observed a longer polymerisation times. Deviation from first-order kinetics started at 80% conversion for [monomer]/[RAFT] = 50, 71% conversion for [monomer]/[RAFT] = 100 and at 68% conversion for [monomer]/[RAFT] = 200. It was suggested that polymerisation of monomer **11** should be stopped at 70% conversion.[42] During this measurement of kinetics, the polymerisation was performed for 90 minutes only and samples were collected in 15-minute intervals to calculate conversions using ^{1}H NMR. Mesitylene (**26**) (Figure 8) was added as an internal NMR standard as it gives a sharp singlet at 6.8 ppm which can be used to intergrate the monomer peak against before and after the reaction then conversions can be calculated:

$$1 - \frac{Monomer_{t=90}}{Monomer_{t=0}} \times 100\%$$



4-cyanopentanoic acid dithiobenzoate (**25**)

Mesitylene (**26**)

Figure 8. Structures of RAFT agent **25** and mesitylene (**26**)

Then, -ln(1-conversion) versus time was plotted and it was calculated that rate constant in the linear region is 0.0156 min^{-1} and the overall rate constant is 0.0126 min^{-1} (Figure 9, left). Samples were also taken for collecting GPC data. All GPC curves have almost identical shape and the peak shifted towards higher molar masses region with increasing reaction time (Figure 9, right).

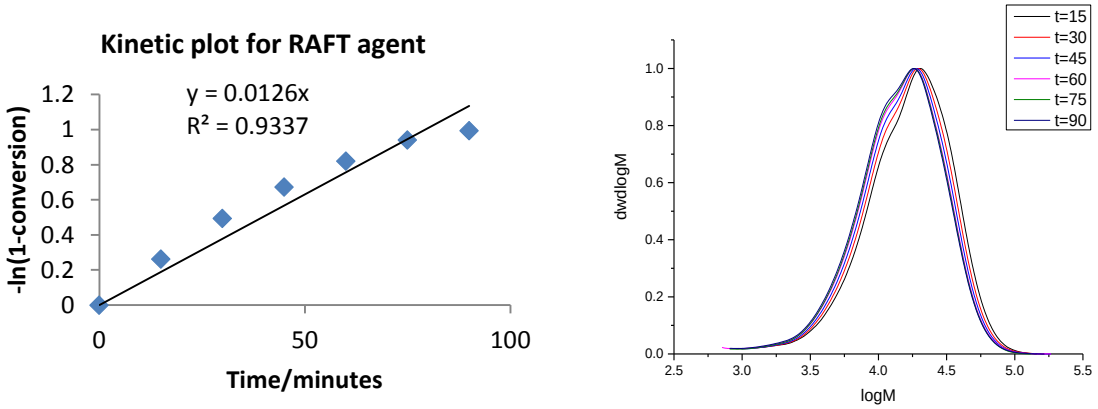


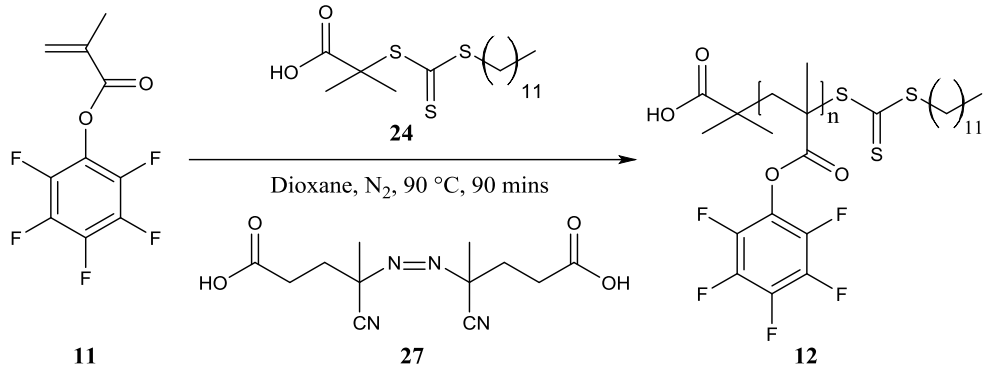
Figure 9. Kinetic plot (left) and GPC curves during the kinetics measurement of RAFT agent **23**

3.3 Polymerisation of pentafluorophenyl methacrylate (11)

Having established the kinetics of the reaction, a library of polymers was synthesis. Using the reported procedure,[42] PFMA **11** was then polymerised with RAFT agent **24** and 4,4-azobis(4-cyanovaleric acid) (**27**) as initiator under N_2 atmosphere at 90 ^{0}C for 90 minutes in dioxane to produce polymer **12** as precursor (Table 1, Scheme 9).

Polymer	[Monomer]/[RAFT]	Monomer coversion	$\overline{M_n}$ (GPC)	Actual DP (NMR)	Đ	Yield
11a	50	55%	5307	27	1.45	54%
11b	100	54%	6893	54	1.63	54%
11c	200	44%	10216	88	1.45	46%
11d	200	47%	12485	94	1.17	40%

Table 1. Results of polymerisation of PFMA (**11**)



Scheme 9. General reaction scheme of polymerisation of PFMA **11** using RAFT **24** and initiator **27**

4 polymers with degree of polymerisation, 27, 54, 88 and 94 were prepared. ^{1}H NMR is used to confirm the structure of the polymers. The disappearance of the peaks at 5.92 and 6.46 ppm (C=C double bond) indicates consumption of monomers. In the ^{19}F NMR, peaks correspond to the aromatic fluorine atoms at -163.16, -158.87 and -153.87 ppm (monomer) shifted to -162.7, -157.5 and -151.4 ppm (all polymers) and shapes become broader (Figure 8). The monomer conversions were calculated using ^{1}H NMR with internal standard, mesitylene. The actual degree of polymerisation were then calculated by monomer conversion $\times \frac{[Monomer]}{[RAFT]}$

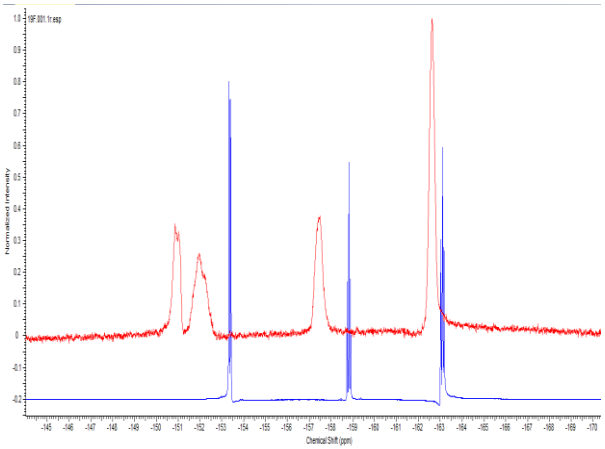


Figure 10. ^{19}F NMR of PFMA (**11**) (blue) and polymer **12**

Gel permeation chromatography, GPC, a type of size exclusion chromatography was used to analyse the polymers. Polymers **12** can be dissolved in both $CHCl_3$ and THF within seconds but minimum of 8 hours are required for DMF to dissolve the polymers at room temperature. However, distinctive peaks cannot be seen using $CHCl_3$ even when concentrations of polymer solutions increased. This is likely due to the polymers and $CHCl_3$ having similar refractive indices.[47] When THF was used as the eluent, values of Đ obtained were usually higher than 1.7. In contrast, although it took a much longer period of time to dissolve polymers **12** in DMF, values of Đ obtained were as low as 1.17. Different columns or eluents could have an impact on GPC results[48] but there is lack of information to understand the actual reason. Another issue observed from the GPC data is the inaccuracy of the values of $\overline{M_n}$. In GPC, $\overline{M_n}$, $\overline{M_w}$ as well as Đ of polymers were calculated based upon PMMA/PS calibration and the Mark-Houwink equation:[49]

$$K_{sample} \times M_{sample}^{\propto_{sample}+1} = K_{standard} \times M_{standard}^{\propto_{standard}+1}$$

(K and α are Mark-Houwink parameter; M= molecular weight)

$$M_{sample} = \left(\frac{K_{standard} \times M_{standard}^{\propto_{standard}+1}}{K_{sample}}\right)^{\frac{1}{\propto_{sample}+1}}$$

Since polymers **12** are not the same polymers used for calibration, values of $\overline{M_n}$ will not be accurate. Hence, the monomer conversions and the actual DPs were calculated using ^{1}H NMR only.

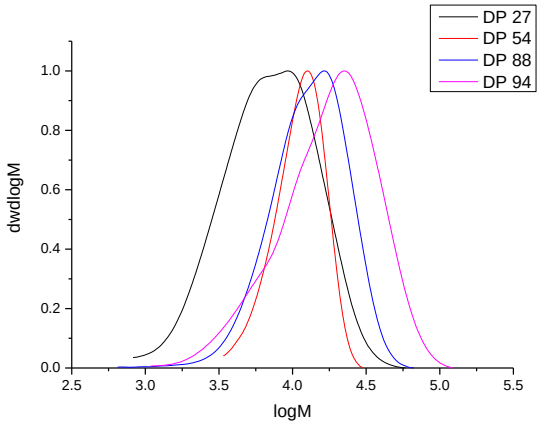


Figure 11. GPC curves of PPFMA **12**

3.4 Modifcation of PPFMA (12) using Boc-lysine (28) and ethanolamine (30)

Polymers **12** were then modifed to remove the pentafluorophenyl ring Boc-Lys-OH (**28**) and ethanolamine (**30**). The reason main reason to use Boc-Lys-OH (**28**) is to generate poly(ampholytes) with 1:1 ratio of amine and carboxyl group after the removal of boc-protecting group. Lysine itself is an essential amino acid for human body[50] and poly(lysine) can be used as a food additive[29] and so, it is expecting that the target polymers should be non-toxic. In addition, the resulting polymers composed of repeating units with amine and carboxyl groups adjacent to each other generate both cationic and anionic charges via conjugation. Therefore, Boc-Lys-OH (**28**) should be the good choice to synthesize poly(ampholytes).[51] Ethanolamine (**30**) was used to remove unreacted pentafluorophenyl group and quench the reaction as it was reported that very high ester conversion can easily be achieved in the modification of PPFMA **12** due to its good nucleophilicity and size.[43] Moreover, it can alter the hydrophilicity of the polymers, giving a route to modifying IRI activity.

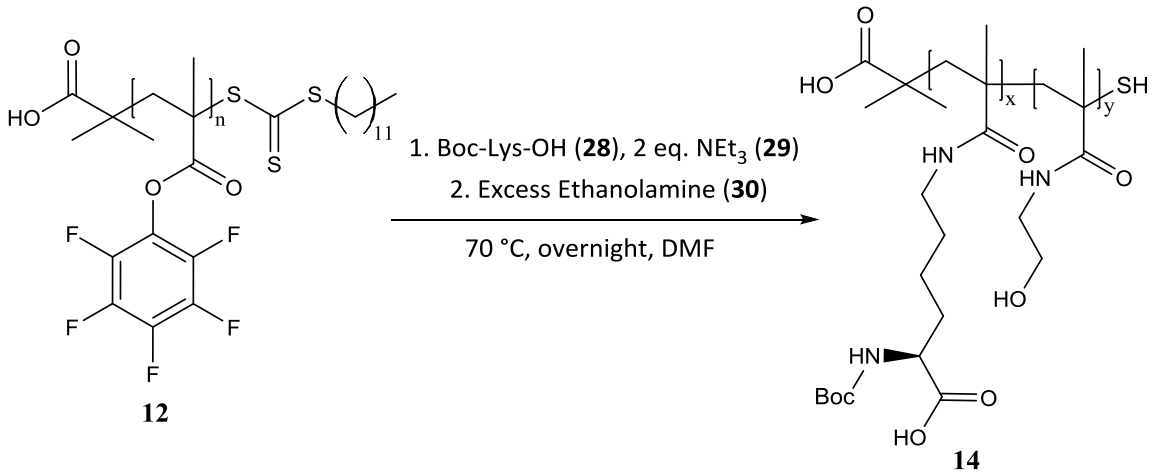
3.4.1 Modification of PPFMA (DP = 27, 12a)

Modification of polymers **12** using Boc-Lys-OH (**28**) and ethanolamine (**30**) were performed in a slightly different manner as reported in the literature.[42,43] The reported modifications of PPFMA (**12**) with amines were done at 50 ^{0}C and 1 equivalent of triethylamine (**29**) relative to amines. However, it was noticed that Boc-Lys-OH (**28**) cannot be dissolved in a wide range of common solvents including THF, $CHCl_3$, acetone, acetonitrile and DMF etc. Therefore, the reaction was performed at 70 ^{0}C and 2 equivalent of

triethylamine (**29**) relative to Boc-Lys-OH (**28**) were used instead (Scheme 10). Results are shown below.

Polymer	Ester conversion	Approx. % of Boc-Lys (28)	Approx. % of amine 30	$\overline{M_n}$ (GPC)	Đ	Yield (%)
14a	100%	20	80	20186	1.44	85
14b	100%	40	60	21425	1.41	84
14c	100%	60	40	21814	1.41	85
14d	100%	80	20	22905	1.41	86

Table 2. Results of modification of polymer **12a**



Scheme 10. General reaction scheme for the modification of polymers **12** with Boc-Lys-OH (**28**), base **29**, ethanolamine (**30**)

All ester conversions confirmed simply using ^{19}F NMR. Firstly, 75 μL of unpurified reaction mixture after modification was taken for running ^{19}F NMR. The spectrum can then be compared to ^{19}F NMR of PFMA (**11**) and PPFMA (**12**) . In the ^{19}F NMR spectra of all 4 reaction mixtures, peaks correspond to the fluorine atoms in PPFMA **12** were not seen. These ^{19}F NMR spectra are indeed identical to that of PFMA (**11**). This indicates that all pentafluorophenyl groups were removed and 100% ester conversions were achieved. In addition, peaks were not seen in all ^{19}F NMR of polymers **14a-d** also indicates the achievement of very high ester conversions (Figure 12).

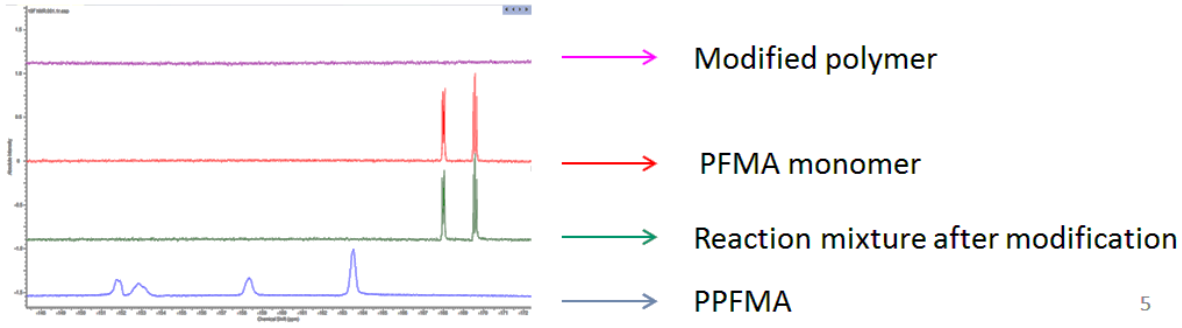


Figure 12. ^{19}F NMR spectra that were used for obtaining the ester conversions.

All samples **14a-d** can then be further characterised using ^{1}H NMR and FTIR. In all ^{1}H NMR spectra, very distinctive singlet at 1.41 ppm (9 protons in total) indicate the presence of the Boc-protecting group. As the percentage of Boc-Lys increased from polymer **14a** to **14d**, peaks were getting bigger to confirm increasing % of Boc-Lys from polymers **14a** to **14d** (Figure 2, left). In the comparison of IR spectra for polymer **12a**, **14a**-**d**, the peak at *ca*. 1780 cm^{-1} which corresponds to the C=O ester bond in PPFMA (**12a**) were not seen in the IR spectra for **14a**-**d**. Instead, peaks were seen at 1630-1660 cm^{-1} which are correspond to the C=O amide bonds (Figure 12). This also indicates complete ester conversions were achieved.

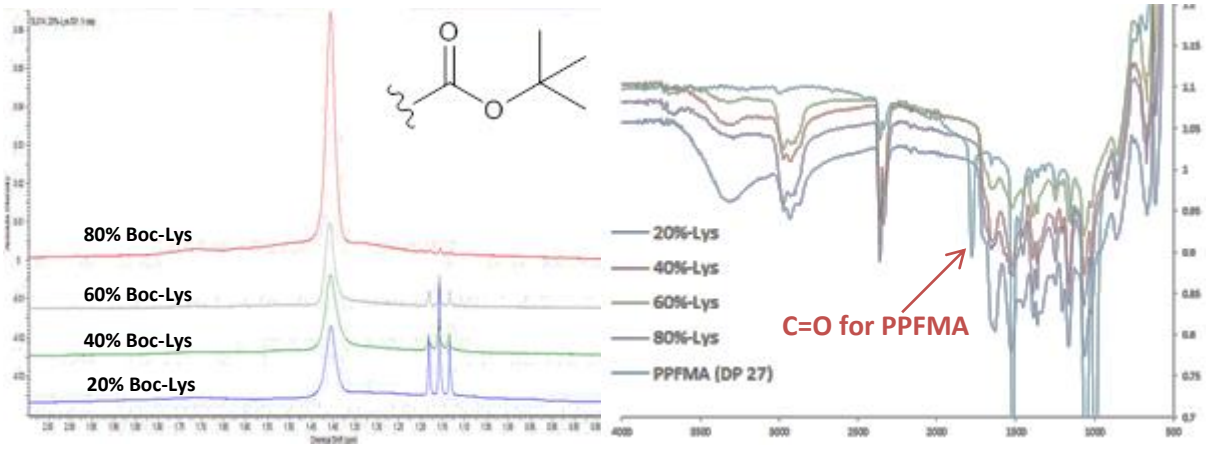


Figure 13. Partial ^{1}H NMR to indicate the Boc-group (left) and IR spectra for polymer **12**, **14a**-**d** (right)

Finally, GPC data for polymers **14a**-**d** were collected using DMF as eluent. As it was mentioned, values of $\overline{M_n}$ obtained were not accurate but the trend did indicate that all polymers **14a**-**d** have higher $\overline{M_n}$ than polymer **12a** (Figure 13). This indicates the changes of the repeating units of polymer **12a** were converted.

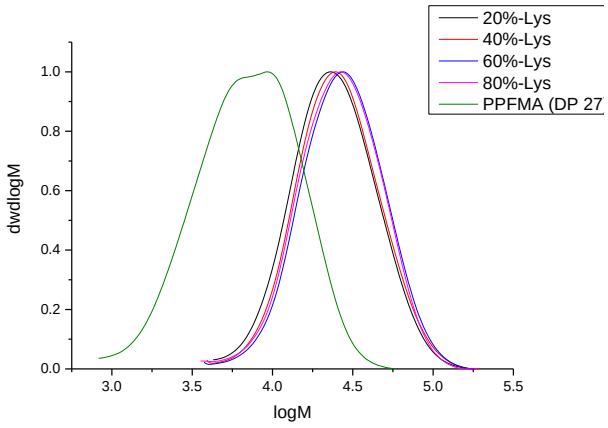


Figure 13. GPC curves for polymer **12**, **14a**-**d**

3.4.2 Modification of PPFMA (DP = 54, 12b)

PPFMA (**12b**) were modified with Boc-Lys-OH (**28**) and ethanolamine (**30**) in exactly the way as described in section 4.4.1 (Scheme 10). ^{19}F NMR spectra of the unpurified

reaction mixtures only consist of two peaks; distinctive peaks (Boc-group) can be seen at 1.41 ppm with increasing integrals as more Boc-Lys presence (Figure 16). It was also noticed that Đ increased for this series of polymers. It is likely that polymers have became more complex which have a different solubility in the eluent. Beside, interactions between the polymers can the column material have also changed which lead to higher values of Đ.[52]

Polymer	Ester conversion	Approx. % of Boc-Lys (28)	Approx. % of amine 30	$\overline{M_n}$ (GPC)	Đ	Yield (%)
14e	100%	20	80	25574	1.78	84
14f	100%	40	60	28273	1.91	85
14g	100%	60	40	29810	1.76	80
14h	100%	80	20	31419	1.78	85

Table 3. Results of modification of polymer **11b**

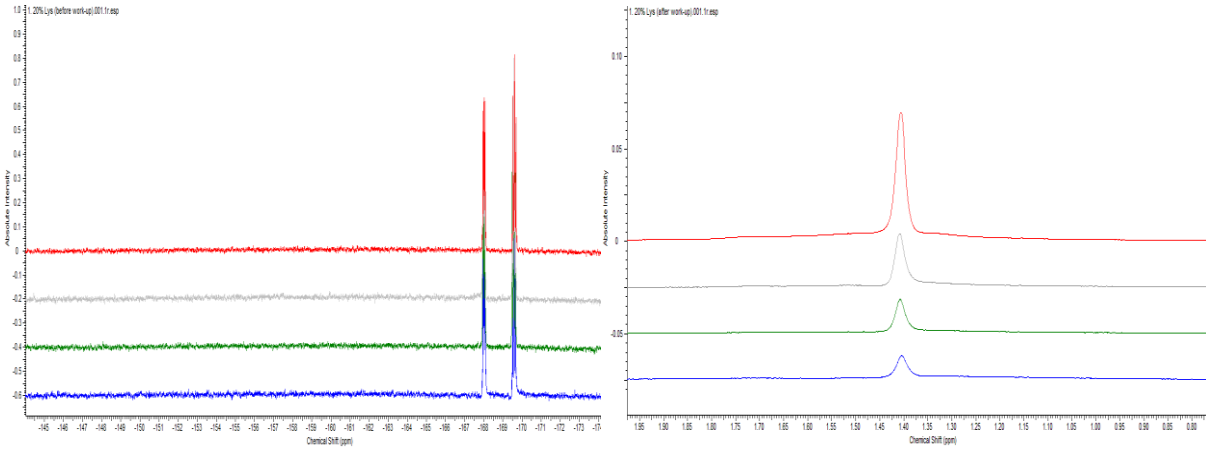


Figure 15. From bottom to top represent spectra for polymers **14e-h**: ^{19}F NMR for crude mixtures; ^{1}H NMR to indicate the presence of Boc-group

Similarly, the IR spectra of polymers **14e-h** were compared to that of PPFMA (**12b**). Peak at 1776 cm^{-1} (C=O for ester bond) were shifted to *ca*. 1650cm^{-1} (C=O bonds for amide) also indicate complete ester conversions (Figure 16).

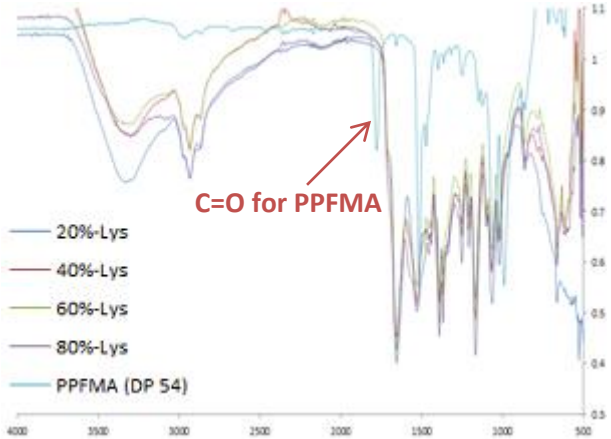


Figure 16. IR spectra for polymers **12b**, **14e-h**

GPC data was also collected using DMF (Figure 17). $\overline{M_n}$ increased after the modification.

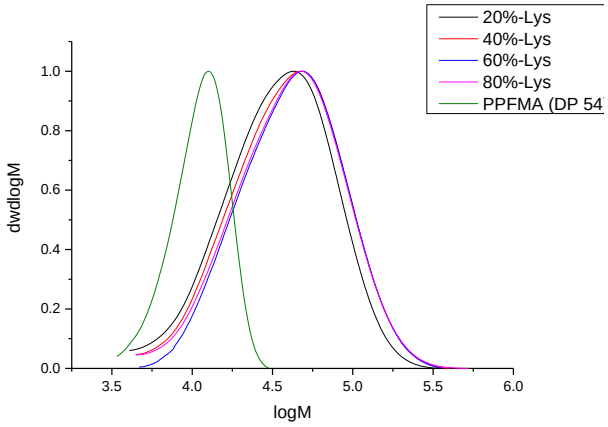


Figure 17. GPC curves for polymers **12b**, **14e**-**h**

3.4.3 Modification of PPFMA (DP = 88, 12c)

PPFMA (**12c**) were modified with Boc-Lys-OH (**28**) and ethanolamine (**30**) in the way as described in section 4.4.1 and 4.4.2 (Scheme 10). ^{19}F NMR spectra of the unpurified reaction mixtures only consist of two peaks; distinctive peaks (Boc-group) can be seen at 1.45 ppm with increasing integrals as more Boc-Lys presence (Figure 18).

Polymer	Ester conversion	Approx. % of Boc-Lys (28)	Approx. % of amine 30	$\overline{M_n}$ (GPC)	Đ	Yield (%)
14i	100%	20	80	34508	1.64	82
14j	100%	40	60	34970	1.73	84
14k	100%	60	40	40247	1.63	80
14l	100%	80	20	42295	1.56	87

Table 4. Results of modification of polymer **11b**

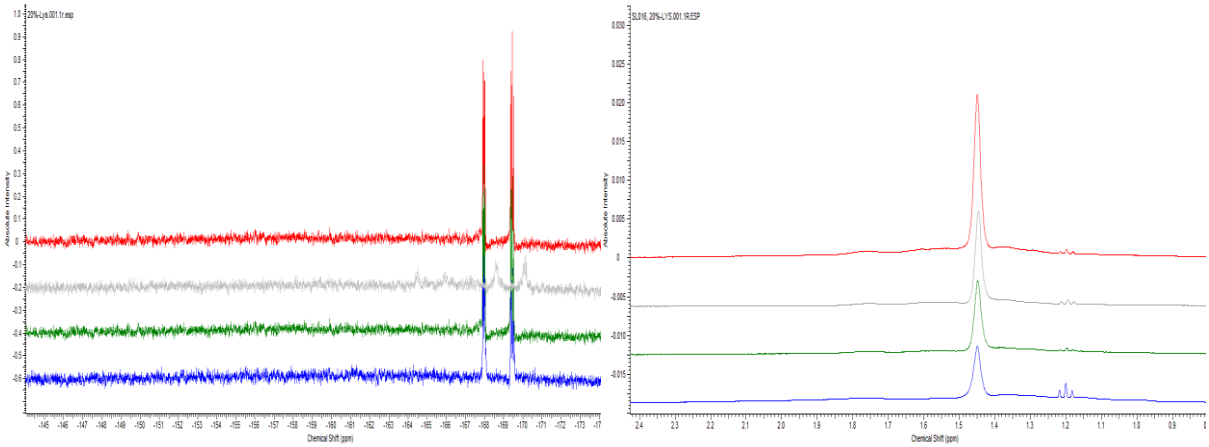


Figure 18. From bottom to top represent spectra for polymers **14i**-**l**: ^{19}F NMR for crude mixtures; ^{1}H NMR to indicate the presence of Boc-group

Again, IR spectra of polymers **14i-l** were compared to that of PPFMA (**12c**). Peak at 1777 cm^{-1} (C=O for ester bond) was note seen but a peak appears at *ca*. 1650cm^{-1} (C=O bonds for amide) shows complete ester conversions (Figure 19).

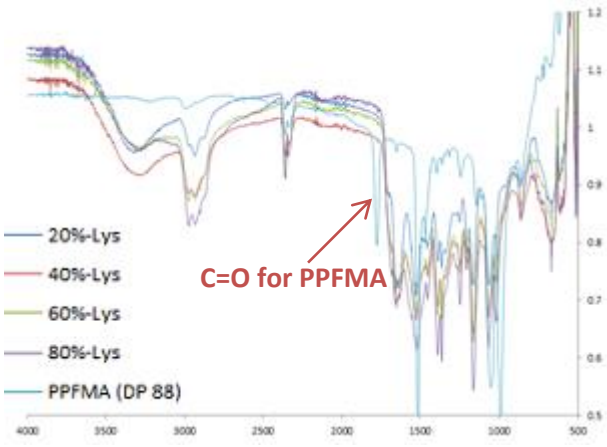


Figure 19. IR spectra for polymers **12c**, **14i-l**

GPC data was also collected using DMF (Figure 20). $\overline{M_n}$ increased after the modification.

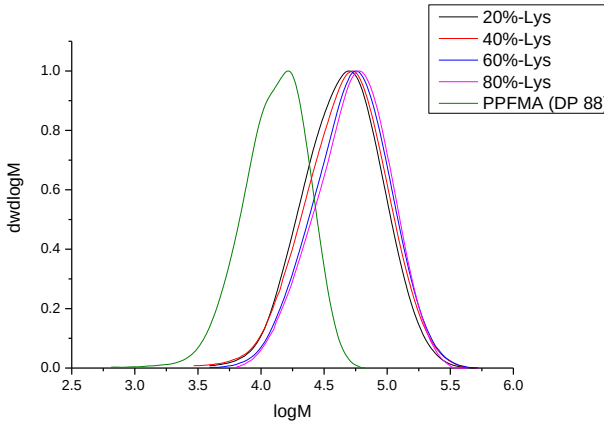
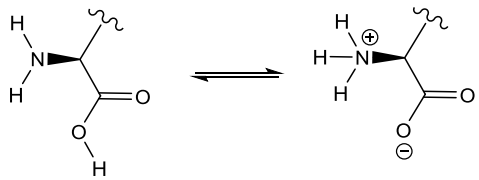


Figure 20. GPC curves for polymers **12c**, **14i-l**

3.5 Deprotection of polymers 14

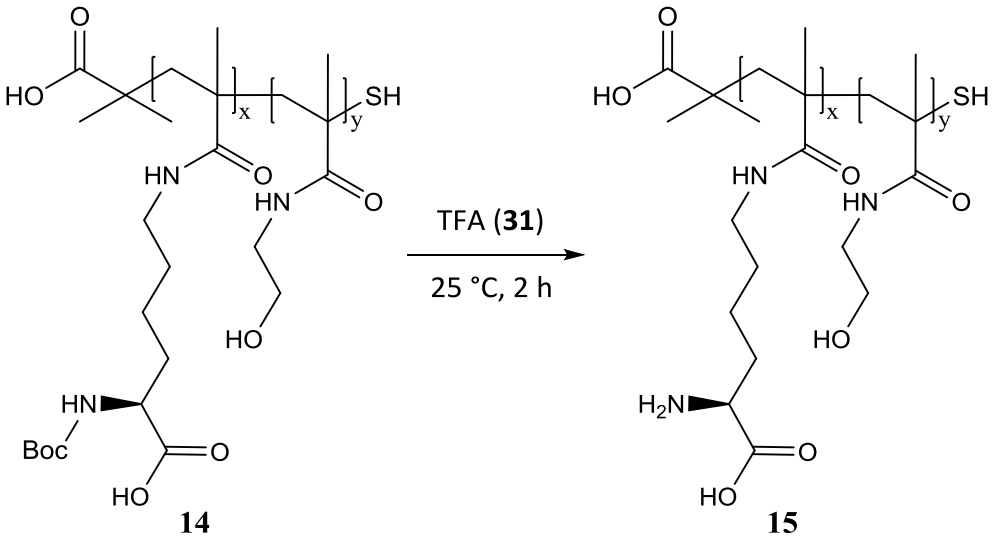
After the deprotection, each lysine group in polymers **14** consist of 1:1 ratio of amine and carboxyl group. They regarded as poly(ampholytes) because of the proton exchange between the amine and carboxyl groups in the lysine repeating unit. This can induce cationic and anionic charges (Scheme 11).



Scheme 11. Proton exchange between the amine and carboxyl group

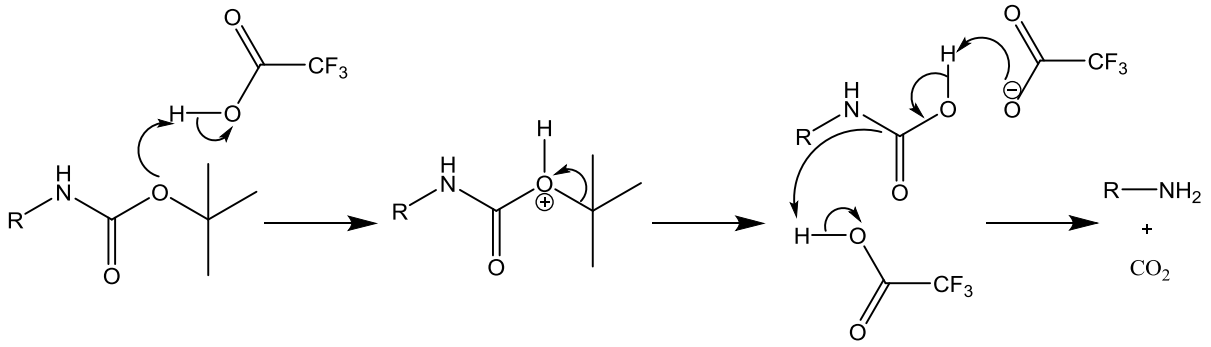
3.5.1 Deprotection of polymers 14a-d (DP 27)

All deprotections were performed according to the reported procedure.[53] This involved using TFA (**31**) as the only solvent as well as the reagent to remove the Boc-protecting group at 25 ^{0}C for 2 hours (Scheme 12).



Scheme 12. General reaction scheme for the deprotection of polymers **14**

The Boc-group was removed via (i) protonation of tert-butyl carbamate, (ii) loss of tert-butyl cation to give a carbamic acid, (ii) decarboxylation of carbamic acid to give free amine and carbon dixode (Scheme 13).[54] Therefore, it was observed that gas was produced during the deprotection of all polymers **14**. On the other hand, it is vital to further purify the products after re-precipitation to remove remaining TFA (**31**), its salts and any other impurities. Dialysis with appropriate tubing was applied to purify products **15** as this method can also help for eliminating some short chained-polymers to in order to narrow the Đ.



Scheme 13. Mechanism for deprotection of Boc-group using TFA (**31**)[54]

^{1}H NMR was used to characterise the deprotected polymers **15**. Unlike the ^{1}H NMR spectra for polymers **14**, the sharp and distinctive at 1.41 ppm that represents the Boc-group were not seen in the spectra for polymers **15**. Instead, board peaks ranged from *ca*. 0.60 – 2.40 ppm correspond to the polymer backbones can be seen clearly (Figure 21, left). Peaks

ranged from *ca*. 2.85 – 4.05 ppm can indicate the different % of Lys/ethylhydroxyl amide groups that present in the polymers. Peaks at 3.29, 3.68 ppm correspond to –NH-$\underline{CH_2}$-$\underline{CH_2}$-OH in the ethylhydroxyl amide groups can it can be seen that these peaks became smaller when % of ethylhydroxyl amide groups drcreased. Similary, peaks at 3.76, 3.96 ppm correspond to the protons next to the amide bond and amine/carboxyl groups respectively became larger when % of Lys groups increase (Figure 21, right). Although high resolution NMR was used, peaks are not distinctive in order to determine the exact ratios of lysine and ethylhydroxyl amide groups. IR spectra were also collected for all polymers **15** but they do not contain of any significant features for chracterisations.

Polymer	Approx. % of Lys	Approx. % of amine 30	$\overline{M_n}$ (GPC)	Đ	Yield (%)
15a	20	80	5211	1.93	54
15b	40	60	5682	2.14	55
15c	60	40	9586	1.51	42
15d	80	20	9945	1.53	47

Table 5. Results of deprotection of polymer **14a-d**

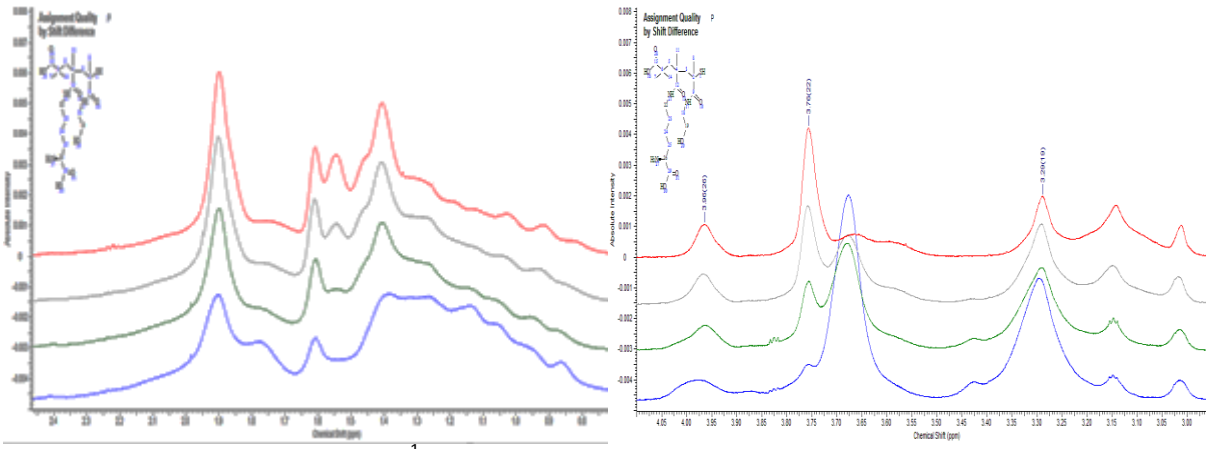


Figure 21. From bottom to top are ^{1}H spectra for **15a**-**d** in the backbone regions (left); regions showing how the peaks changed when % of lys/ethylhydroxyl amide groups changed.

Polymers **15** were then analysed with GPC. However, only aqueous eluent can be used as they cannot be dissolved Other eluents (THF, $CHCl_3$, DMF). It was also noticed that some values of Đ increased as well as tailing toward lower molar masses which could be caused by interactions between the polymers and column materials (Figure 22).[52] Another major disadvantage of switching the eluent from DMF to aqueous solution, is the fact that GPC data cannot be compared together.

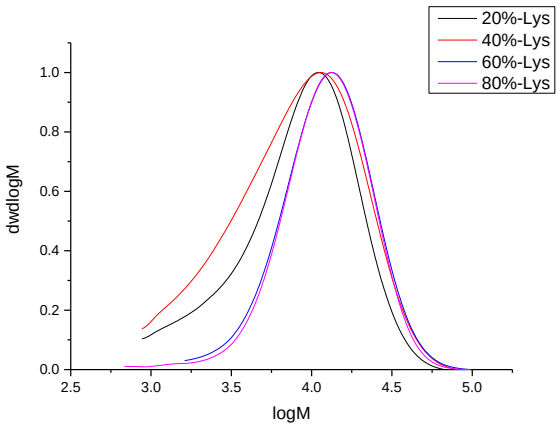


Figure 22. GPC curves for polymers **15a**-**d**

3.5.2 Deprotection of polymers 14e-h (DP 54) for synthesis of polymers 15e-h

^{1}H NMR of polymer **15e**-**h** consist of the same pattern compared to polymers **15a**-**d**. Broad peaks could be seen between *ca*. 0.55 – 2.40 ppm and peak at 1.41 ppm correspond to the Boc-disappeard (Figure 23, left). Peaks at *ca*. 3.20, 3.60, 3.66 and 3.86 ppm confirmed that all four polymers consist of different & of lysine and ethylhydroxyl amide groups (Figure 23, right).

Polymer	Approx. % of Lys	Approx. % of amine 30	$\overline{M_n}$ (GPC)	Đ	Yield (%)
15e	20	80	11026	1.87	24
15f	40	60	11432	1.52	20
15g	60	40	14579	1.53	27
15h	80	20	17290	1.58	34

Table 6. Results of deprotection of polymer **14e-h** to give polymers **15e**-**h**

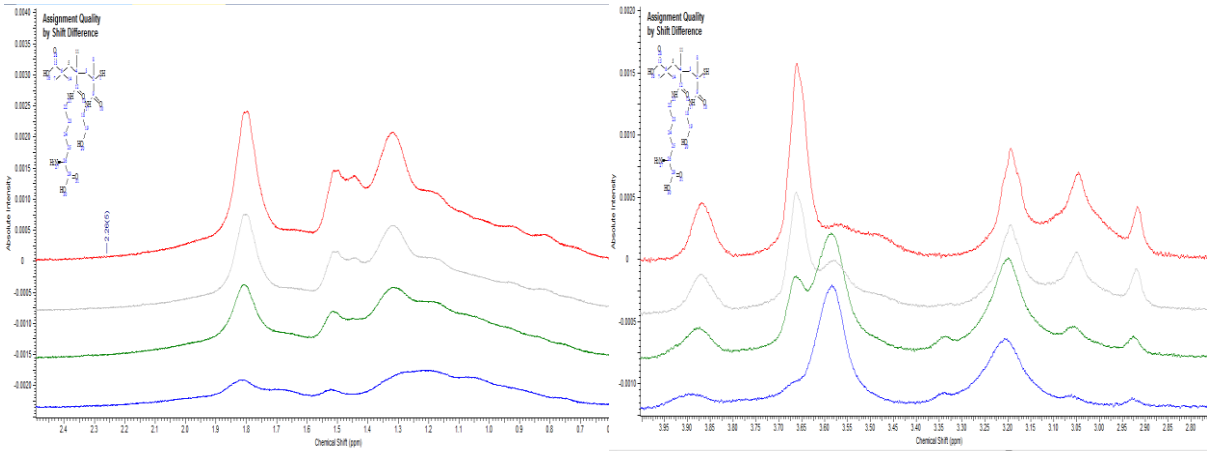


Figure 23. From bottom to top are ^{1}H spectra for **15e**-**h** in the backbone regions (left); regions showing how the peaks changed when % of lys/ethylhydroxyl amide groups changed.

Increased values of Đ and tailing towards lower molar masses was also observed in the GPC data for polymers **15e**-**h** (Figure 24).

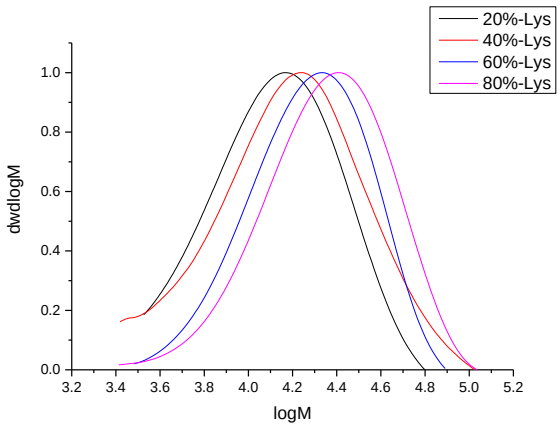


Figure 24. GPC curves for polymers **15e**-**h**

3.5.3 Deprotection of polymers 14i-l (DP 88) for the synthesis of polymers 15i-l

In the characterisations of polymers **15i**-**l**, peak at 1.41 ppm correspond to Boc-group had vanished (Figure 25, left). Peaks at *ca*. 3.29, 3.68, 3.76 and 3.97 ppm indicate different ratios of lysine and ethylhydroxy amide groups that present in this series (Figure 25, right).

Polymer	Approx. % of Lys	Approx. % of amine 30	$\overline{M_n}$ (GPC)	Đ	Yield (%)
15i	20	80	19278	1.69	46
15j	40	60	19961	1.72	49
15k	60	40	23568	1.70	49
15l	80	20	25049	1.77	64

Table 7. Results of deprotection of polymer **14e-h** to give polymers **15i-l**

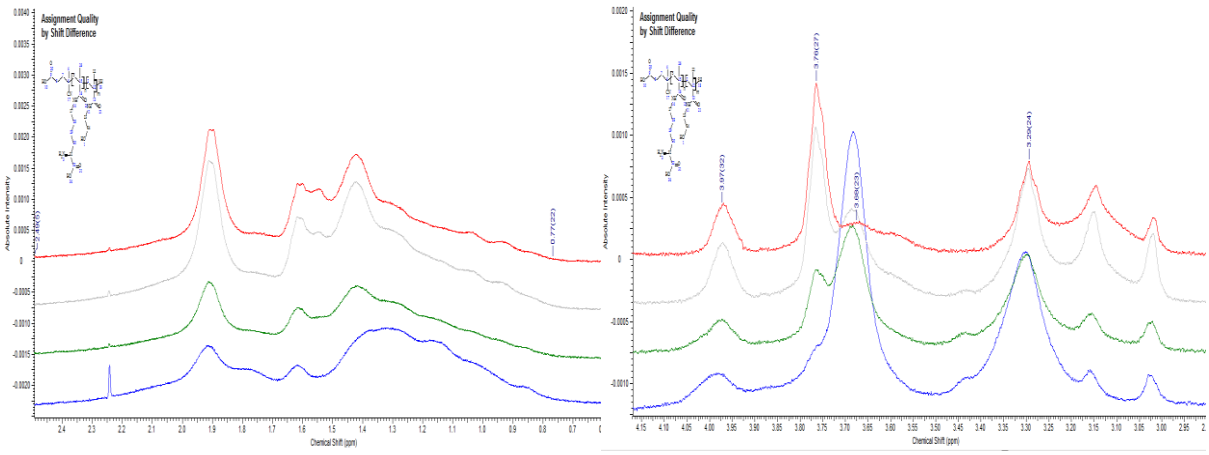


Figure 25. From bottom to top are ^{1}H spectra for **15i**-**l** in the backbone regions (left); regions showing how the peaks changed when % of lys/ethylhydroxyl amide groups changed.

GPC data was also collected for polymers **15i-l** (Figure 26). It can be see that but once again, the data obtained cannot provide signicant features of the products.

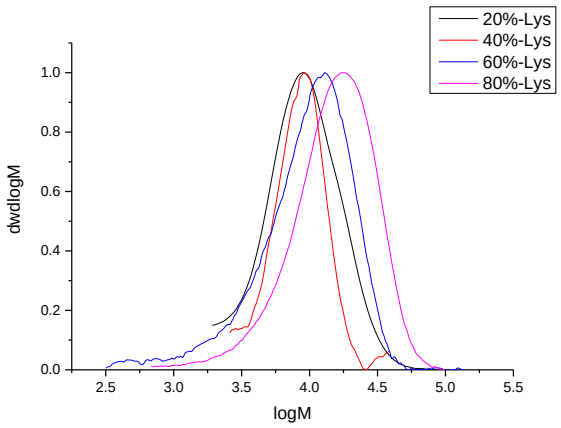


Figure 26. GPC curves for polymers **15i-l**

3.6 Splat cooling assay

The "splat test" is the method used for monitoring whether polymers **15** have ice-crystals recrystallisation inhibiting/antifreeze properties. This firstly involves dissolving 20 mg of polymer in 1 mL of PBS solution, a drop of this solution then dropped from *ca*. 2 meters onto a glass cover slip that is chilled on an aluminium plate with dry ice. The glass slip was then quickly transferred to the cryostage attached to a microscope for 30 mins at -8 ^{0}C (Figure 27). The reason for using PBS solution because it consists of colligative solutes such as sodium chloride, NaCl to increase the sensitivity of the splat test.[11,55] In addition, PBS can be used for mimicking biological environments.[56]

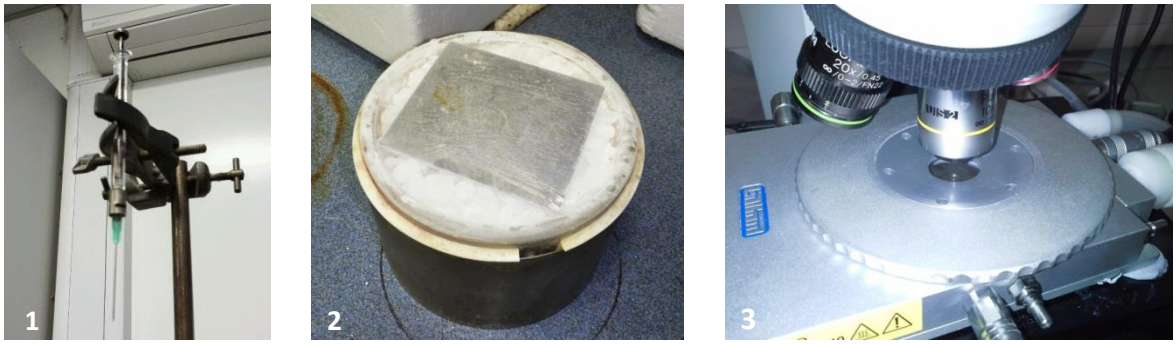


Figure 27. Equipment used for splat cooling assay

In order to see whether a trend such as degree of polymerisation, ratios of lysine and ethylhydroxyl amide groups can be observed by performing the splat tests. Size of ice crystals were compared to that of PBS control solution at t=30 (Figure 28).

Using imageJ, sizes of the ice crystals were calculated and the % of crystals growth:

$$\% = \frac{Size\ of\ crystal_{Polymer}}{Size\ of\ crystal_{PBS}} \times 100\%$$

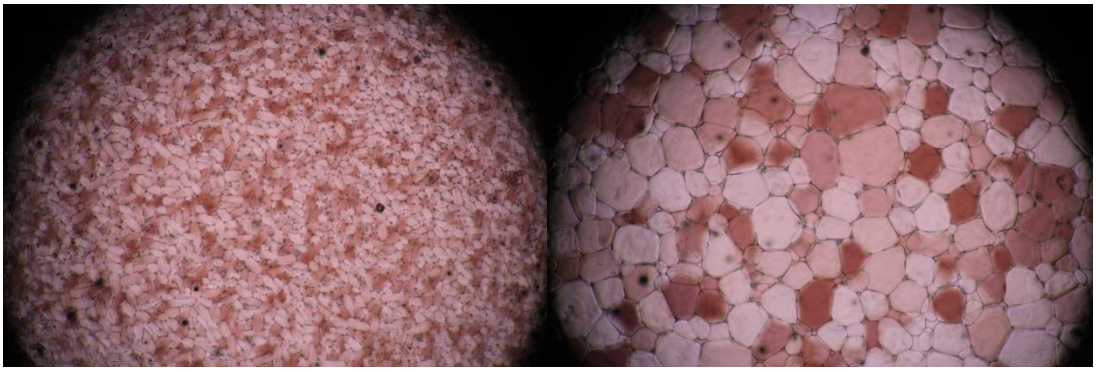


Figure 28. Ice crystals of PBS solution at t=0 (left) and t=30 (right)

3.6.1 Poly(lys methacrylamide-*co*-hydroxyethyl methacrylamide) (15a-d, DP 27)

When polymers **15a**-**d** were used as additives, an average of 75% ice-crystals growth was observed. It was noticed that polymers **15b**, **c** seemed to perform better but not very significantly (Table 8, Figure 29).

Polymer	**15a**	**15b**	**15c**	**15d**
Average % of ice-crystal growth	77	73	74	76
Standard deviation: $\frac{\sum_{i=1}^{N}(x_i-\bar{x})^2}{N-1}$	2.06	2.10	2.36	2.12

Table 8. Results of splat test for polymers 15a-d

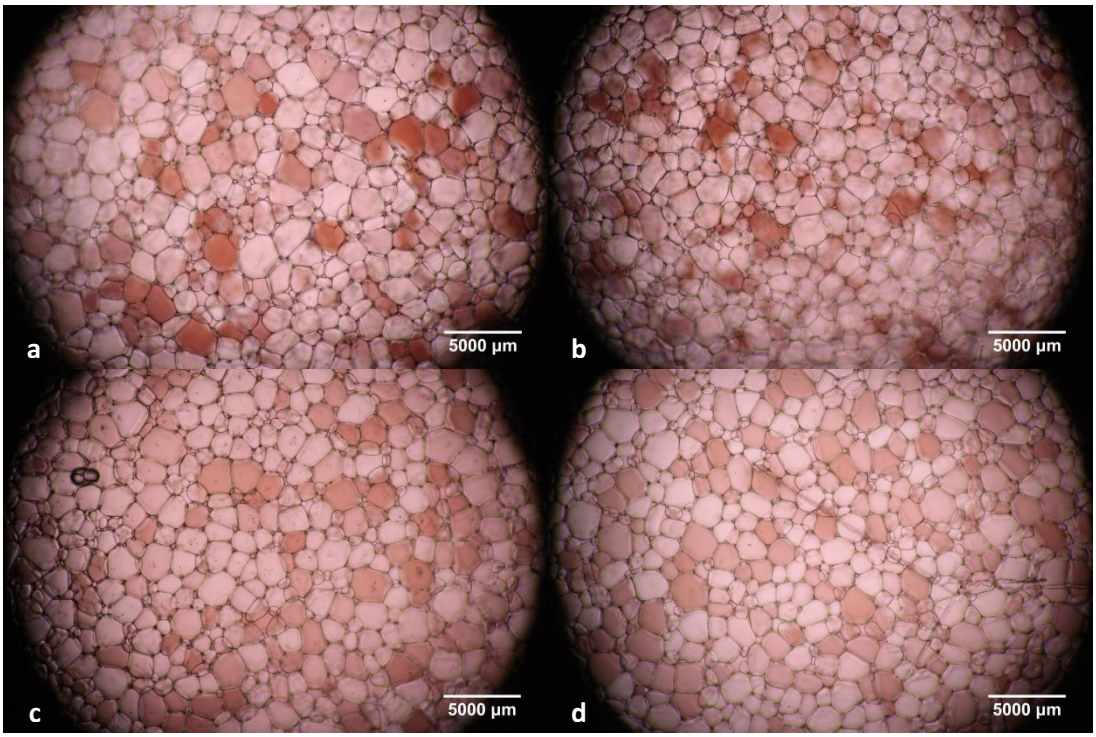


Figure 29. Pictures of ice crystals at t=30 for polymer **15a**-**d**

3.6.2 Poly(lys methacrylamide-*co*-hydroxyethyl methacrylamide) (15e-h, DP 54)

For polymers **15e**-**h** with doubled chain-length, an average of 72% ice-crystal growth was observed. Polymers **15f**, **h** can supress thw growths slightly better (Table 9, Figure 30).

Polymer	15e	15f	15g	15h
Average % of ice-crystal growth	74	71	76	69
Standard deviation: $\frac{\sum_{i=1}^{N}(x_i-\bar{x})^2}{N-1}$	2.34	0.45	2.10	2.10

Table 9. Results of splat test for polymers **15e**-**h**

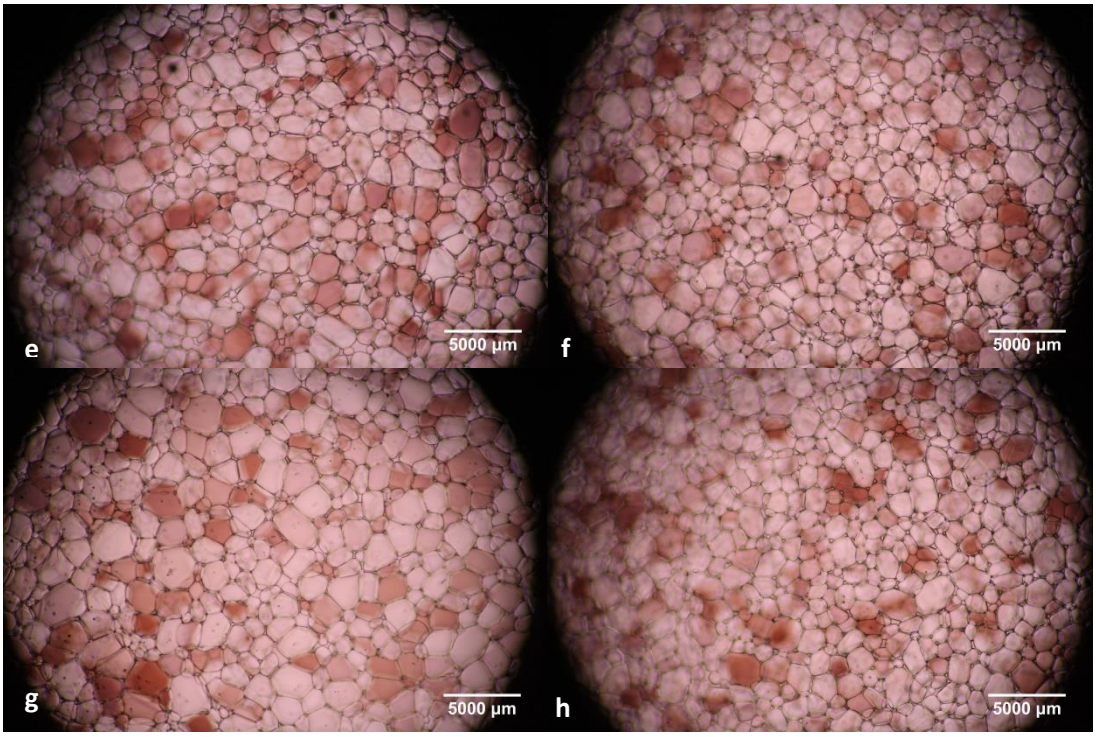


Figure 30. Ice crystals picture for polymers **15e**-**h**

3.6.3 Poly(lys methacrylamide-*co*-hydroxyethyl methacrylamide) (15i-l, DP 88)

Polymers **15i**-**l** which consist of even higher degree of polymerisation allowed 68% of ice crystal growth on average. In this series, polymers **15i**, **j**, **l** achieved better performance (Table 10, Figure 31).

Polymer	15i	15j	15k	15l
Average % of ice-crystal growth	68	68	71	66
Standard deviation: $\frac{\sum_{i=1}^{N}(x_i-\bar{x})^2}{N-1}$	1.58	3.38	0.96	3.15

Table 10. Results of splat test for polymers **15i**-**l**

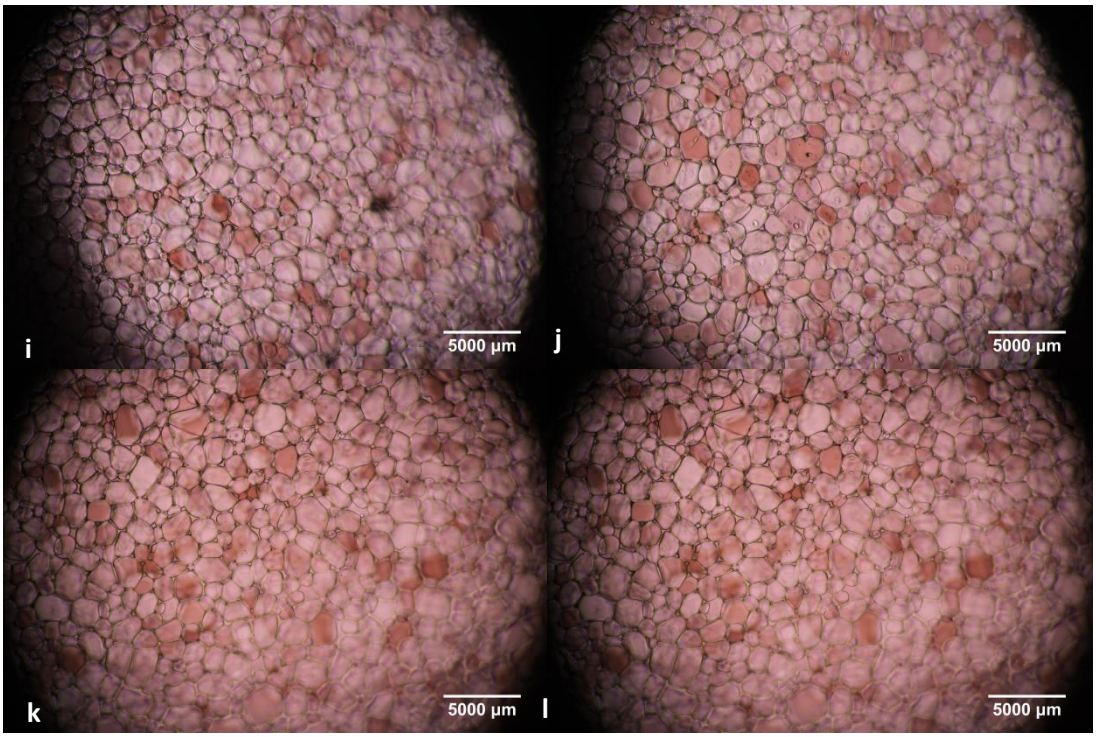
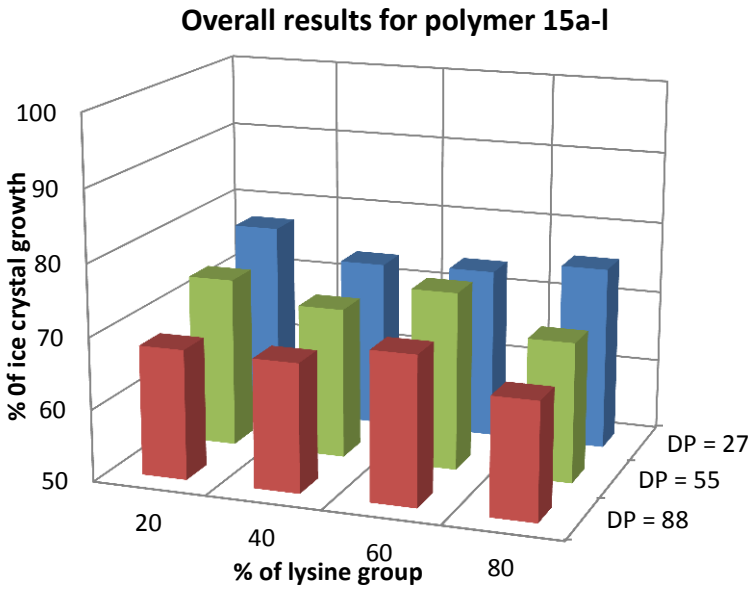


Figure 31. Ice crystal picture for polymers **15i-l**

The overall all results were displayed in a bar chart (Figure 32) and the average values of ice crystal growth for each series of polymer were summaried in Table 11. According to the results of these 12 polymers, ratios of lysine or ethylhydroxyl groups did not affect the performance. Fortunately, it can be seen that when the chain length increases, polymers can inhibit the ice crystal growth further. The strategy of the modification of PPFMA **12** has then changed. Boc-Lys-OH (**28**) will continued to be used as the main componet but ethanolamine will be replaced by hydrophobic compounds.



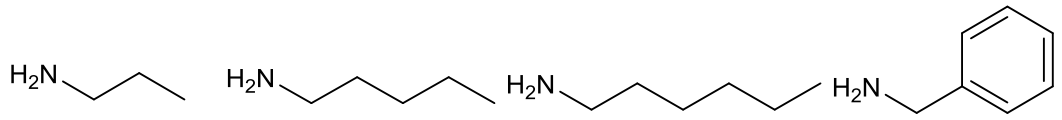
DP	% of ice crystal growth
27	75
54	72
88	68

Table 11. Average performance for each set of polymer

Figure 32. Combined data of the splat tests for polymers **15a-l**

3.7 Investigations of Poly(ampholytes) with hydrophobic moieties

It already was mentioned that introducing hydrophobic moieties to small molecules can enhance their IRI ability.[28] However, it was only reported that introducing hydrophobic moieties to polymers can enhance their cryopreservative ability[30] and it is unsure whether this can also benefit them to inhibit ice crystal growth as antifreeze materials. In addition, it is concerning that the hydrophobic moieties can reduce the polymers' solubility in aqueous solution,[57] especially in PBS solution for the Splat test. While performing the Splat test for polymer **15a**-**l**, it was observed that polymers with higher percentage of lysine groups can be dissolved more easily. Therefore, it was then attempt to produce the poly(lysine) with only ca. 20% hydrophobic moieties first by using PPFMA **12d** (DP 94), propylamine (**32**), pentylamine (**33**), hexylamine (**34**) and benzylamine (**35**) (Figure 33). The synthetic methods, characterisations discussed earlier would be applied again.

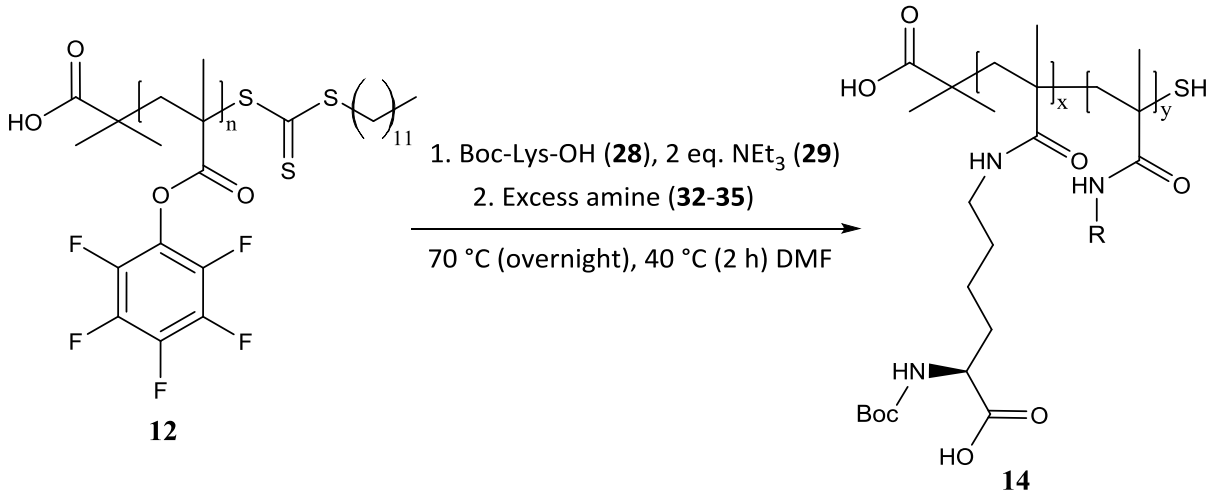


Propylamine (**32**) Pentylamine (**33**) Hexylamine (**34**) Benzylamine (**35**)

Figure 33. Compounds that were used for increasing the hydrophobicity of poly(ampholytes)

3.7.1 Poly(ampholytes) (14m-p, DP 94) with *ca*. 20% of hydrophobic moieties

Firstly, PPFMA **12d** was modified with Boc-Lys-OH (**28**) and amine (**32**-**35**) in a very similar protocol that was used to produce **14a**-**l**. Same procedure was followed but due to the lower boiling points of amine (**32**-**35**), temperature was reduced to 40 ^{0}C after addition of these amines (Scheme 14).



12 **14**

Scheme 14. General reaction scheme used for modifying PPFMA **12** with hydrophobic amine (**32**-**35**)

Results of preparing polymer **14m**-**p** were summarised in Table 12; ^{19}F NMR and IR spectra used for confirming the ester conversions are shown in Figure 33.

Polymer	Ester conversion	Approx. % of Lys	Approx. % of amine 32-35	$\overline{M_n}$ (GPC)	Đ	Yield (%)
14m	100%	80	20	38860	1.90	86
14n	100%	80	20	39439	1.79	87
14o	100%	80	20	40013	1.87	87
14p	100%	80	20	43347	1.77	88

Table 12. Results of modification of PPFMA **12d** to give polymers **14m**-**p**

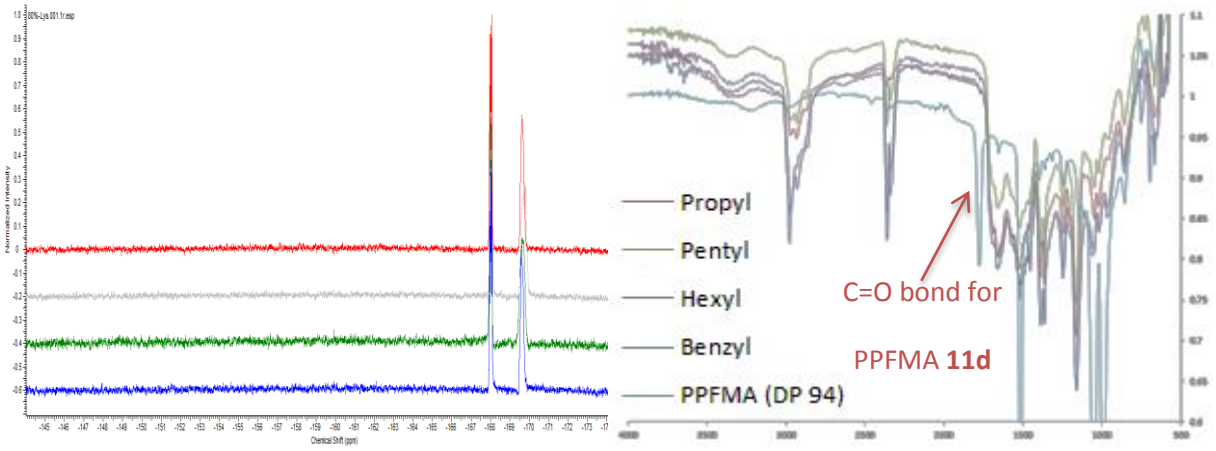


Figure 34. ^{19}F NMR and IR spectra that were used to confirm the ester conversions

Polymers **14m**-**p** were then treated with TFA (**31**) as shown in Scheme 12 to give polymer **15m**-**n**. The results are tabulated in Table 13 and the deprotections were also confirmed using ^{1}H NMR peaks correspond to the Boc-group were not seen (Figure 34). Since they both contain same amount of lysine and hydrophobic amines, their ^{1}H NMR indeed look similar except for polymer **15p** which consists of a broad peak at 7.44 ppm because of its benzyl group.

Polymer	Approx. % of Lys	Approx. % of amine 32-35	Yield (%)
15m	80	20	86
15n	80	20	87
15o	80	20	87
15p	80	20	88

Table 13. Results of deprotection of polymers **14m-p** to give polymers **15m**-**p**

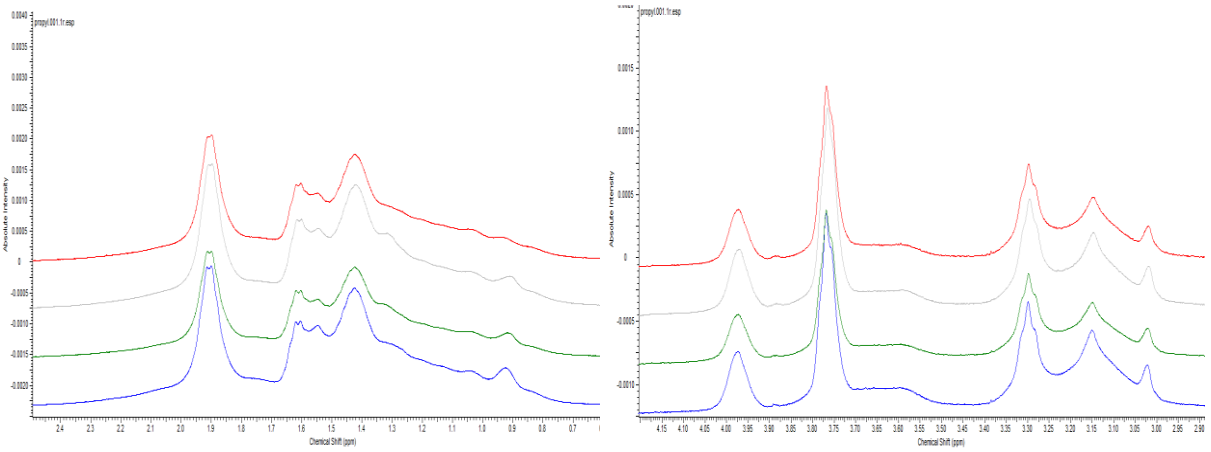


Figure 35. From bottom to top: ^{1}H NMR for polymer **15m**-**p** showing the backbone (left) and protons adjacent to amide bonds or nitrogen atoms (right)

The Splat tests revealed that polymers **15m**-**o** allowed *ca*. 80% of ice crystals to grow whereas polymer **15p** can further inhibit the ice crystals growth for 10% (Table 14, Figure 36). Overall, polymers **15m**-**o** cannot improve the ability to suppress ice crystals growth. Although polymer **15p** has a better result but it is not very significant. Therefore, the next was to synthesize the same set of polymers but % of hydrophobic moieties would increase to 50%.

Polymer	15m	15n	15o	15p
Average % of ice-crystal growth	79	78	79	68
Standard deviation: $\frac{\sum_{i=1}^{N}(x_i-\bar{x})^2}{N-1}$	2.28	2.90	2.64	0.44

Table 14. Results of Splat test for polymer **15m**-**p**

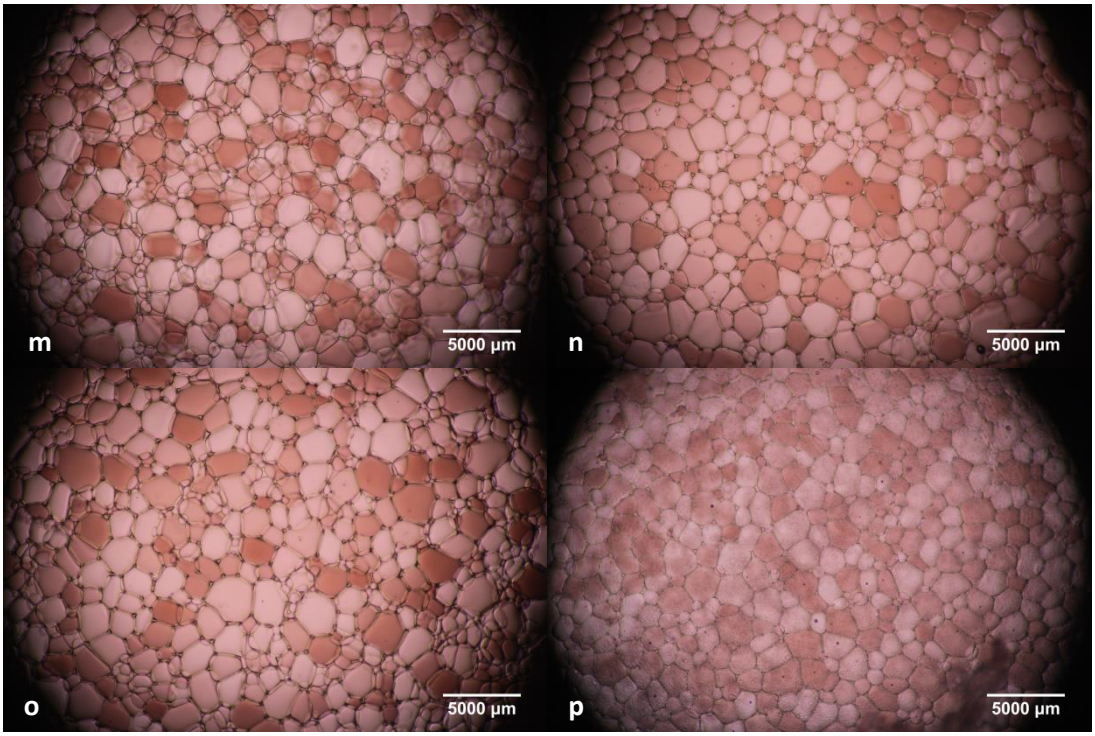


Figure 36. Pictures of ice crystals when polymers **15m**-**p** were used as additives in PBS solution

3.7.2 Poly(ampholytes) (15q-t, DP 94) with *ca*. 50% of hydrophobic moieties

PPFMA (**12d**, DP 94) was modified to another 4 polymers and major results and data are shown below (Table 15, Figure 34).

Polymer	Ester conversion	Approx. % of Lys	Approx. % of amine 32-35	$\overline{M_n}$ (GPC)	Đ	Yield (%)
14q	100%	50	50	58004	1.85	84
14r	100%	50	50	60523	1.83	85
14s	100%	50	50	60700	1.80	84
14t	100%	50	50	61122	1.87	83

Table 15. Results of modification PPFMA **12d** to give polymers **14q**-**t**

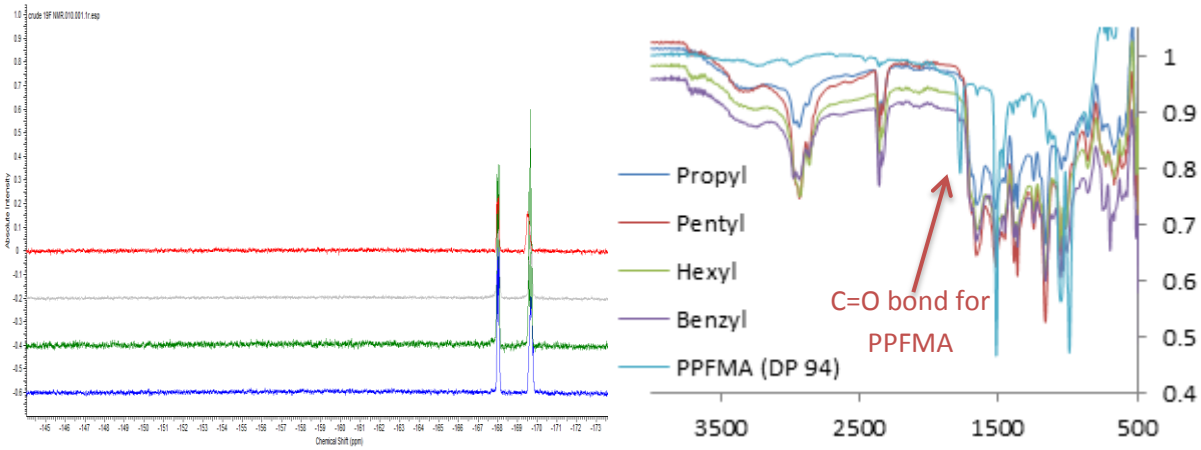


Figure 37. From bottom to top: ^{19}F NMR for unpurified mixture for polymer **14q**-**t** (left); IR for PPFMA **12d** and polymers **14q**-**t**

Polymers **14q**-**t** were then treated with TFA (**31**) to give polymers **15q**-**t** with information shown in Table 15. Their ^{1}H NMR spectra look similar to that of polymers **15m**-**p** but it can be seen that the peak at 0.92, 0.90. 1.06 and 7.43 ppm correspond to the propyl (**15m**), pentyl (**15n**), hexyl terminal protons (**15o**) and benzyl proton (**15p**) are larger indicating higher percentage of hydrophobic groups (Figure 37, left).

Polymer	Approx. % of Lys	Approx. % of amine 32-35	Yield (%)
15q	50	50	72
15r	50	50	68
15s	50	50	45
15t	50	50	62

Table 16. Results of deprotection of polymers **14q**-**t** to give polymers **15q**-**t**

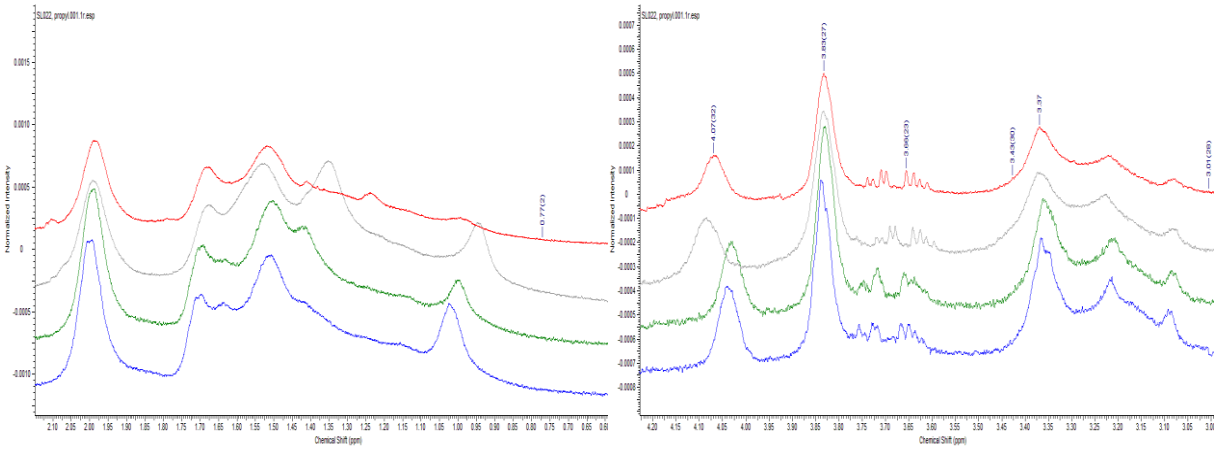


Figure 37. From bottom to top: ^{1}H NMR for polymers **15q**-**t** of backbone (left) and peaks for protons adjacent to amide bonds or nitrogen atoms

Polymers **15q**-**t** were then dissolved in PBS for Splat test. However, solubility of polymers **15r**-**t** in PBS becomes very poor and their Splat tests were performed in much lower concentrations. Overall, polymer **15q** allows almost 60% of ice crystal growth, whereas polymer **15r**-**t** cannot exhibit any ice crystals recrystallization (Table 17, Figure 37).

Polymer	**15q**	**15r**	**15s**	**15t**
Conc. in PBS (mg/mL)	20	2	1	1
Average % of ice-crystal growth	62	100	100	100
Standard deviation: $\frac{\sum_{i=1}^{N}(x_i-\bar{x})^2}{N-1}$	1.57	0.40	0.90	2.52

Table 17. Results of Splat test for polymer **15q**-**t**

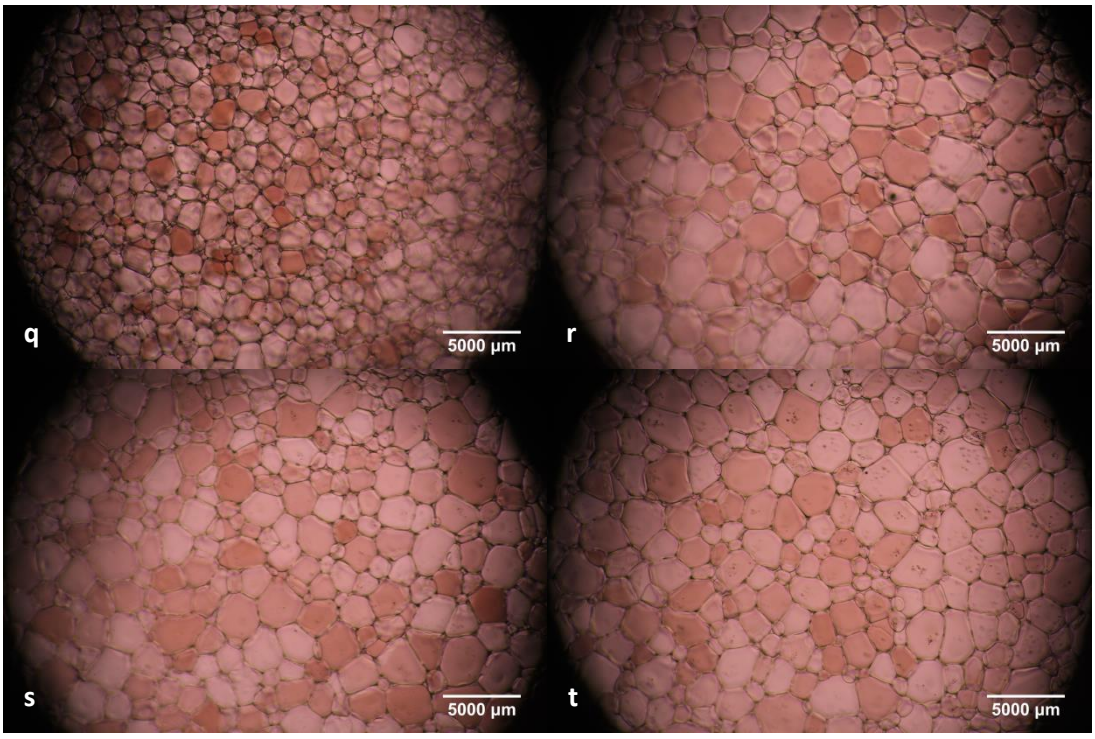


Figure 38. Pictures of ice crystals when polymers **15q**-**t** were used as additives in PBS solution

As polymer **15q** can inhibit ice crystal growth better than other polymers, it was aiming to see its IRI ability in different concentration. In addition, the growths of ice crystals over a period of hour in different concentrations were compared with PBS solution (Figure 39). It was noticed that when concentration of polymer-PBS solution was below 5 mg/mL, no IRI ability was observed.

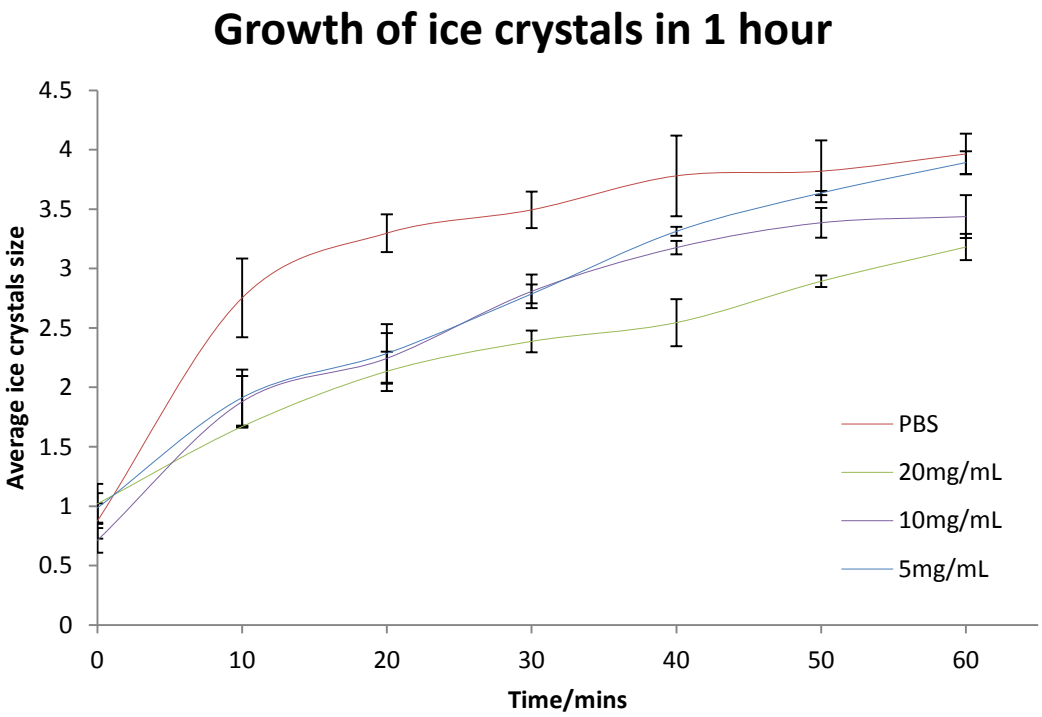


Figure 39. Growth of ice crystals in PBS and different concentration of **15q**-PBS solution

4. Conclusion

4.1 Overview of the project

In this project, 20 polymers were synthesized to build a small library of poly(ampholytes) which were tested using Splat Cooling Assay to investigate the relationship between the structures and antifreeze properties.

Pentafluorophenyl methacrylate (**11**) was firstly synthesized and polymerized to four polymers with degree of polymerization of 27, 54, 88, 94 *via* RAFT polymerization. Polymers with DP of 27, 54, 88 were each modified to four different polymers containing different ratios of lysine and ethylhydroxy groups. Ratios between the two components did not seem to place an effect to inhibit ice crystal growth but it could be observed that increasing the overall polymer chain length can indeed enhance IRI activity.

Polymer with DP of 94 was modified to 8 different polymers. 4 of them contained *ca*. 80% of lysine and *ca*. 20% of hydrophobic moieties; the rest contained *ca*. 50% of lysine and *ca*. 50% of hydrophobic moieties. The former series did not have a dramatic effect to inihibit ice growth. In the latter series, polymer contained *ca*. 50% propyl amide group can inhibit almost 40% of ice crystals growth but the rest of the polymers had poor solubility in most common solvents.

This project has demonstrated the use of RAFT, post-modification polymerization to build a small library of polymers with different sizes and functional groups. Reactions and products can be monitored and characterized using simple techniques such as NMR and IR easily.

4.2 Proposal of further studies

In this project, only one RAFT agent was used to polymerise PFMA **11**. It is suggested to attempt polymerizing monomer **11** with different RAFT agents in order to see whether a more optimal yield and higher monomer conversions can be achieved along with low values of Đ. Then, DPs of 100, 200 can be synthesized and modified with Boc-Lys-OH (**28**) and ethanolamine (**30**) again to see whether the IRI activity can be enhanced even further.

It is also crucial to find an alternative way perform the Splat test for polymers **15q**-**t**. For instance, Splat tests can be performed using sucrose solution as test results can be more sensitive even at much lower concentrations.[11] Changing the pH, introducing different ions to the polymers is also a possible way to dissolve the polymers in PBS.

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