Universität für Bodenkultur Wien
University of Natural Resources
and Applied Life Sciences, Vienna

Investigation of Polypeptoids and Iron Oxide Cores for Core-Shell Nanoparticles

Tanja Zwölfer

Institute for Biologically Inspired Materials

Department of Nanobiotechnology

University of Natural Resources and Life Sciences, Vienna

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KURZFASSUNG

Diese Masterarbeit ist in zwei Teile gegliedert: Die Erzeugung von superparamagnetischen Eisenoxid-Nanopartikeln (SPION) und die Untersuchung verschiedener Poymerisationsmethoden von Polypeptoiden mit komplexen stereochemischen Seitenketten als Grundlage zur zukünftigen Herstellung von Kern-Schalen-Nanopartikeln (CSNP). CSNP bestehen aus der Kombination von meist zwei Materialien, wie zum Beispiel einem anorganischen Kern (z.B. Eisenoxid-Nanopartikel (FeOx-Nps)) und einer organischen Polylmerhülle (z.B. Polypeptoide), wodurch ein System mit vereinten und komplexen Eigenschaften hergestellt werden kann.

In dieser Masterarbeit konnte die Möglichkeit zur Erzeugung von monodispersen größenkontrollierten FeOx-Nps (ca. 6 nm im Durchmesser) aufgezeigt werden, welche aufgrund ihrer biologischen Kompatibilität und ihres Superparamagnetismus interessant für die Verwendung als Kerne sind. Weiteres konnten catecholbasierte Initiatoren synthetisiert werden, um anschließend die Oberfläche der Partikel zu modifizieren. Besonders die Aufreinigung der Initiatorpartikel war hier eine Herausforderung und konnte im Rahmen dieser Arbeit optimiert werden.

Polypeptoide sind eine neuartige Form von Polymeren, die der Polymergruppe der Polypeptide nachempfunden sind. Der Hauptunterschied zu Polypeptiden ist die Bindung der Seitenkette an das Stickstoff- statt an das Kohlenstoffatom. Diese Kombination von Gemeinsamkeiten (z.B. biologische Kompatibilität) und Unterschieden (z.B. höhere Stabilität der Polypeptoide gegen Abbaumechanismen im Körper) macht Polypeptoide interessant für neuartige biomedizinische Anwendungen.

In dieser Masterthesis wurden Synthesen von Polypeptoiden mit komplexen stereochemischen Seitenketten wie Aromaten untersucht, die für die Bildung von Sekundärstrukturen verantwortlich sind. Für die Ringöffnungspolymerisation konnte gezeigt werden, wie erfolgreich Monomere in hoher Qualität und Reinheit erzeugt werden können und wie bereits bekannte Synthesewege auch für Monomere mit komplexen Seitenketten angewendet werden können. Jedoch stellte sich heraus, dass die Ringöffnungspolymerisation bei Monomeren, die für die Bildung sehr starrer Helices verantwortlich sind, schnell an ihre Grenzen stößt und es zu Kettenbrüchen und der Erzeugung von nur kurzen Polymeren kommt. Aufgrund dessen wurden erste Versuche mittels Schritt für Schritt Polymerisation mithilfe eines festen Stützmaterials unternommen. Diese Methode ist weitaus arbeitsintensiver und zeitaufwendiger, jedoch kann hier die Reihenfolge verschiedener Monomere bestimmt werden. Obwohl in dieser Masterarbeit vorrangig die Erzeugung von Homopolymeren untersucht wurde, bietet diese Methode zukünftig vor allem die Möglichkeit zur Erzeugung von sequenzspezifischen Polypeptoiden.

ABSTRACT

This master thesis is built of two parts: The synthesis of superparamagnetic iron oxide nanoparticles (SPIONS) and the investigation of methods for polymerisation of polypeptoids with complex stereo chemical side chains as a basis for the generation of core-shell nanoparticles (CSNP). CSNP are built of usually two different materials like an inorganic core (e.g. iron oxide nanoparticles (FeOx-Nps)) and an organic polymeric shell (e.g. polypeptoids) in order to generate a system with combined and complex properties.

In this master thesis, monodisperse and size-controlled FeOx-Nps (around 6 nm in diameter) could be synthesized, which are interesting for biomedical and biotechnological applications due to their biocompatibility and superparamagnetism. Furthermore, catechol-based initiators could be synthesized to subsequently modify the particles for surface-initiated polymerization. A major challenge that was overcome in this work was the purification of particles functionalized with initiators.

Polypeptoids represent a new generation of polymers, which are modelled based on polypeptides. The main difference to polypeptides is that the sidechain of polypeptoids is bound to the nitrogen instead of the carbon atom. Due to the combination of the similarities (e.g. biocompatibility) and differences (e.g. higher stability of polypeptoids against especially enzymatic degradation in the body and other biological environments) polypeptoids are interesting candidates for biomedical and biotechnological applications.

In this master thesis the synthesis of polypeptoids with complex stereo chemical side chains like aromatic ones was investigated using ring opening polymerisation (ROP). The side-chains are responsible for the formation of secondary structures that are analogous to secondary structures of polypeptides. Monomers in high quality and purity could be generated for the ROP and it could be shown that already known synthesis routes can also be used for monomers with more complex side chains. Nevertheless, experiments showed ROP seems to reach its limits with monomers that are known to form tight helices resulting in the formation of chain breaks and only short polymers. Therefore, a step-by-step polymerisation from a solid support was tested. This method may be more labour and time consuming, but additionally the sequence of each monomer can be controlled. Although in this thesis only the synthesis of homopolymers was investigated, this method shows its future potential in the generation of sequence specific polymers with polypeptide-like properties.

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CONTENTS

1. INTRODUCTION

1.1. Nanomaterials and Nanostructures

Nanotechnology is a relatively new scientific field, which investigates all kinds of materials and structures on a nanometre scale (1 nm = 10^{-9} m) in order to understand the relationship between physical properties or phenomena and material dimension [1]. Investigation of materials and structures reveal a wide range of new physical properties when their size drops under 100 nm. These properties differ significantly from those observed on a bigger scale and therefore open many possibilities of new applications and technology [2]. These materials between 1 and 100 nm with unique properties are often referred to as nanomaterials. In this size range quantum effects start to matter and the properties of the materials change. Examples are changes in melting point, mechanical properties, optical properties, electrical conductivity or magnetic properties [1].

1.2. Structure and Application of Core-Shell Nanoparticles (CSNP)

A major focus area of nanotechnology is research on nanoparticles. Nanoparticles have a large surface compared to their volume. Their properties are mainly defined by the surface and therefore can be changed drastically by modification of the surface [3]. CSNP are built up of a core (inner material) and a shell (outer material) and make it possible to combine advantages of the properties of both parts. Today a great diversity of these particles exists which vary in the core, the shell and the structure. The core as well as the shell can either consist of organic or inorganic material [4]. Furthermore, different shapes are possible like spherical or hexagonal CSNP and single or multi core CSNP where multiple cores are coated with a single shell [3]. In addition, the polymeric shell can be modified with for example anti-bodies to increase it functionality and to add extra properties (Figure 1) [5].

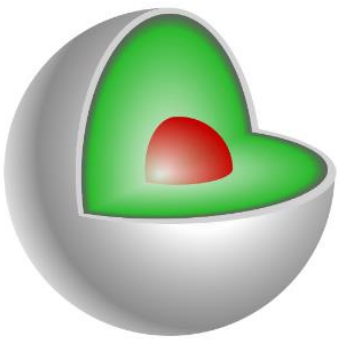


Figure 1: 3D model of a spherical single core CSNP. The red sphere represents the core, the green one the polymeric shell and the grey sphere potentially further modifications.

There are two classes of inorganic core/organic shell nanoparticles: Magnetic core/organic shell and nonmagnetic core/organic shell nanoparticles. Magnetic cores can be made of metals or metals oxides [3].

To link the core irreversibly with the shell additional molecules called anchors are often needed. For example to irreversibly link a polymer with a metal core anchor molecules like catechols and catechol derivates are used [6].

In this work the focus lies on magnetic inorganic/organic CSNP with FeOx-NPs as cores and polypeptoids as a shell. As an anchor the catechol derivate nitrodopamine (NDA) is used, which can be used as initiator for polymerization of organic shells with diverse properties. (Figure 2)

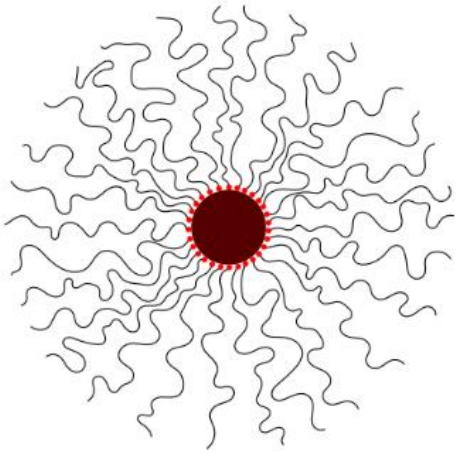


Figure 2: CSNP: The dark red sphere represents the core (e.g. iron oxide), the small light red spheres represent the anchors (e. g. NDA) and the black lines the polymer shell (e.g. polypeptoid).

CSNP are already used for biomedical applications, but their use has been limited until now with only a few examples of widespread use in vivo, like as contrast agents in magnetic resonance imaging (MRI). Magnetic nanoparticles with dextran or dextran derivates as coating agents are already commercially used as MRI contrast agents. For example the contrast agent Resovist® by Bayer Healthcare consists of carboxydextran coated FeOx-NPs with an overall hydrodynamic diameter of 62 nm and is used for liver imaging [8]. Furthermore starch coated FeOx-NPs as contrast agents were already investigated by targeting brain tumors in rats [9]. Also synthetic polymers like polyethylene glycol (PEG) are investigated for long term stabilisation of nanoparticles for MRI. Guo, et al., reported the usage of high crystalline and ultrasmall FeOx-Nps (advantage diameter of 5,4 nm) stabilised with PEG for a high-performance nanoparticulate MRI contrast reagent [10]. Despite problems in terms of controlling targeting of these FeOx-NP contrast agents, they are again a focus of interest, since competing polymer based contrast

agents, such as Gadolinium-complexing polymer contrast agents, got into discredit over the last years due to the accumulation of gadolinium in various tissues and therefore associated health risks so that alternatives gained more importance [7].

Nevertheless, commercially available medical applications of magnetic nanoparticles are still rare and at the beginning of their potential usage. The realisation of a so-called drug delivery system with functionalities like superparamagnetic, cell targeting, non-toxicity, controllability due to for example thermoresponsibility, controlled release of drugs and many more is still in a distant prospect. However, various promising polymers like polypeptoids have already been investigated as a first step for a new generation of CSNP.

1.3. Colloidal Stability of CSNP and Grafting of Polymers

1.3.1. Aggregation and Stabilization of Particles

A colloid is a mixture of two substances (gas, liquid or solid), where one substance is dispersed in the other, which forms a continuous phase. An example for a colloidal system with a solid dispersed in a liquid would be nanoparticles in a solvent. Nanoparticles, due to their small size, are not strongly affected by gravity and are kept dispersed throughout the solvent by Brownian motion. Colloidal stability means that the particles remain suspended in the liquid and do not aggregate by gravity or other forces. Aggregation is defined as the formation of clusters out of multiple particles due to attractive forces like the van der Waals force. Aggregation can lead to complete loss of colloidal properties as aggregation can proceed until complete phase separation has taken place, e.g. precipitation of all nanoparticles in a solution. Colloidal stabilisation can either be realised by electrostatic or steric effects or a combination of both (Figure 3).

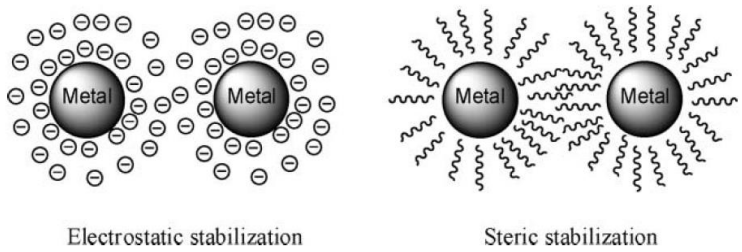


Figure 3: Electrostatic and steric stabilization of particles. Adapted from ref [11].

Electrostatic stabilisation is based on the DLVO-theory. If two particles with identical sign of the charge approach each other, the double-layer repulsive force increases but also the van der Waals attractive force starts to increase. The total energy potential is the sum of repulsive and attractive energies and defines whether the particles are stable or aggregate. If the surface charge of the colloids is high enough and thereby the double-layer repulsion strong enough, the particles will not aggregate. Nevertheless, stabilisation of inorganic nanoparticles in biological media cannot simply be achieved by following this theory, since high ionic strength and a multitude of differently charged polymer and particles in suspension decreases the power of electrostatic stabilisation tremendously.

Therefore, steric stabilisation is the better choice for solid nanoparticles in biological media. Stabilisers like polymers are used to gain steric stabilisation. Polymers can stabilise nanoparticles by encapsulating the particles without being bound to them or by being bound with anchors to the surface. The shell acts as a spacer that prevents the particles to get near each other through preventing the attractive forces to fully operate. Furthermore, when particles get near each other the osmotic pressure increases in the overlapping area of the shells due to a higher polymer concentration. Solvent is then transported to the overlapping areas to balance the osmotic pressure, resulting in a repulsion of the shells.

Furthermore, as has been previously mentioned, modification with a polymer is not only used to simply stabilise particles but also to change properties of or add properties to the particles. So called polymer brushes are defined as multiple polymers, which are end-attached covalently or by physisorption on a planar or curved surface. The grafting density has to be high enough, so that the polymers are forced to stretch away from the attachment surface.

An example of sterically stabilised inorganic core nanoparticles is FeOx-NPs with PEG on the surface. The stabilised particles are dispersible in aqueous media and therefore useable for biomedical applications like negative contrast agents MRI [12].

1.3.2. Grafting Methods for Polymers

Grafting of polymers describes how polymers can be linked to surfaces. There are two main approaches to generate a polymer brush on a solid surface (e.g. particle). The polymer can be synthesised first and then linked to the surface (grafting-to) or the polymer can be synthesised by starting to elongate from the surface (grafting-from) (Figure 4). In addition to the particles and the polymer an extra molecule is needed as a connection element of both parts, which is called an anchor.

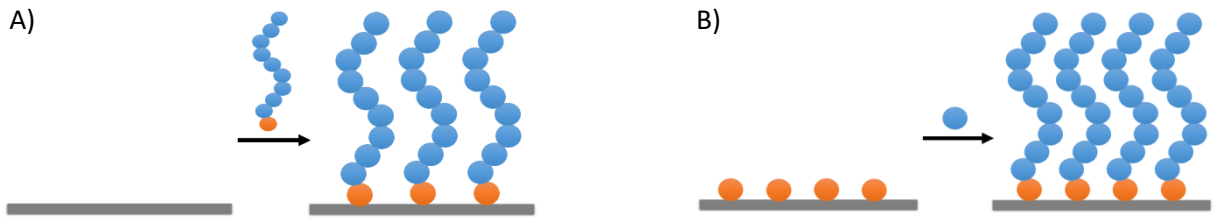


Figure 4: grafting to and grafting from: A) Grafting to: The prior synthesised polymer is linked to a surface. B) Grafting from: An initiator on the surface starts the polymerisation which leads to an elongation of the polymer.

For **grafting-to** (Figure 4A) a polymer must be synthesised with a suitable end functionality. This functional group is then linked to a surface using a reactive surface group in order to generate a polymer brush. The advantage of this method is that polymers can be synthesised separately with narrow molecular weight distributions [13]. The connection of surface and polymer can either be based on physical or chemical bonds. Chemical bonds are much stronger and therefore preferred. Systems with multiple reversibly adsorbing anchors may also have also a low desorption rate, however these anchors need more space on the particles' surface which results in a lower grafting density [14,15]. Depending on the surface, a compatible functional group has to be used. Suitable molecules which can be used to link the polymer with a surface are also called anchor.

For **grafting-from** (Figure 4B) the polymer is synthesised by starting the elongation from the surface [13]. The biggest advantage is the higher grafting density compared to grafting-to but polymerisation of polymers with a narrow size distribution becomes more complex. The anchor is linked to the surface first and serves as an initiator. The polymer is linked to the surface by the initiator after completed polymerization. Which initiator is suitable depends on the surface material and the polymerisation method.

1.3.3. Grafting Density

Grafting density describes the coverage of the surface with the polymer for polymer brushes. It is defined by the number of polymer chains per nm^2. Compared with grafting-to the advantage of grafting-from is that a higher grafting density can be reached [13]. A higher grafting density, which equals a denser coverage of the surface, therefore results in a stronger steric stabilisation. The grafting density has to be high enough to guarantee steric stability.

The thermodynamically favoured globular coil conformation of polymers in solutions leads to a steric limitation of the grafting density when using the grafting-to method, since the area per polymer chain will be defined by the size of the solvated coil in solution. The potential grafting density is highly defined by the type respectively the properties, the length and the concentration of the polymer.

When using the grafting-from method, this hindrance of steric limitation is evaded by first binding only the initiator followed by step by step elongation of the polymers. Therefore, the final grafting density is mainly defined by the grafting density of the initiator and its efficacy.

For example PEG (5 kDA) with NDA as an anchor can be bound to the surface of FeOx-NPs using the grafting-to method with an expected grafting density of around 1 molecule/nm^2 [16]. In comparison the maximum grafting density of only NDA on SPIONS and therefore the maximum grafting density for polymers initiated by it and synthesized by the grafting-from method is around 3 molecules/nm^2 [17,18].

1.4. Cores

1.4.1. Classes of Cores

As has been previously mentioned a broad spectrum of different organic and inorganic nanoparticles used as cores for CSNP exists. Inorganic cores can consist of metal (Ag, Au, Fe, …) , metal oxide (Fe_nO_n, ZrO_2, TiO_2, …), other inorganic compounds or silica. Silica is widely used for the synthesis of particles and also shells grown to encapsulate other inorganic cores. A specific property of some metal and metal oxide nanoparticles is the so called superparamagnetism, which means that the particles are only magnetic when a magnetic field is applied.

Organic cores mainly are made of polymers like polystyrene and are interesting due to their polymeric properties like optical properties, flexibility and toughness. They can be combined with an inorganic or an organic shell. Furthermore, the so called sacrificial organic cores can be used to produce inorganic hollow particles [3].

1.4.2. Iron oxide nanoparticles (FeOx-NPs)

FeOx-NPs have gained importance during recent years. There are many different methods to produce magnetic nanoparticles using solution techniques or obtaining them out of aerosol or vapour phases [19]. To prevent destabilization or agglomeration of the nanoparticles, coating with a shell, for example with polymers, is necessary. Only then it is possible to use them for example in aqueous or biological media and therefore also in the human body for medical diagnosis and treatment [20].

FeOx-NPs belong to the group of superparamagnetic nanoparticles and are superparamagnetic below the size of 25 nm [21]. The size of the particles can be controlled in the range of one nanometre with a high monodispersity (standard deviations $\sigma \leq 5\%$) [22]. Due to superparamagnetism external targeting to a required area with a magnetic field is possible, making the particles interesting for many applications

like drug delivery systems used for example in cancer treatment [23] or as contrast agents for MRI applications [24].

The particles have to be biocompatible to be used as a biomedical device. This means they have to be stable long enough in a biological system to be effective, reach the target destination and to achieve their purpose, however their toxicity has to be on a minimal and still tolerable level. Other ferromagnetic metals like cobalt may have the advantage of a stronger magnetic response but cannot be used due their toxicity [25]. In contrast 3-5 g iron naturally occurs in the human body bound in haemoglobin-form. Therefore the small additional amounts of iron used in medical applications have no toxic effects [26].

3-10 nm monodisperse oleic acid capped Fe_3O_4 nanoparticles can be synthesised by thermal decomposition of iron pentacarbonyl in the presence of oleic acid [16] by slightly modifying the synthesis already described by Hyeon et al [27]. The particles are capped with oleic acid during synthesis, which can then be replaced by a suitable anchor or ligand depending on further use [28].

1.5. Anchors and Initiators

1.5.1. Variety and Functionalities of Anchors Molecules for FeOx-NPs

To produce an inorganic/organic core-shell system, both parts have to be linked with each other. Rarely, this can be done directly and anchor molecules are necessary. Therefore, the basic requirement for an anchor is a functional group that can react with the polymer (grafting-to) or start initiation (grafting-from) and another functional group that binds to a reactive group of the surface. Only with anchors which bind irreversibly and with a high affinity, a high and long-term stability of the nanoparticles can be achieved.

For steric stabilisation of FeOx-NPs polymers with different anchors can be used, which are able to covalently bind to the hydroxyl group on the surface of the particles.

Molecules with a carboxylic acid moiety like oleic acid can be used for synthesis of the particles in organic solvents. Although these molecules can be used for stabilization, the bond is labile and therefore can easily be broken. Low affinity ligands like oleic acid can be exchanged with ligands that have a higher binding affinity. For example, oleic acid FeOx-NPs can be exchanged with PEG-NDA by dispersing in dimethylformamide (DMF) and sonication over night without the addition of

supplemental chemicals [16]. FeOx-NPs stabilised with the carboxylic acid citric acid are commercially used for the MRI reagent VSOP C184 [29].

Furthermore, alkoxysilan compounds can be covalently linked with the surface due to the Si-OCH_3 moiety. An advantage is that a wide range of already functionalised silane compounds is commercially available and well investigated. Commonly used silane compounds are for example (3-aminopropyl)triethoxysilane (APTES), (3-mercaptopropyl)trimethoxysilane (MPTMS) [30] or (3-glycidyloxypropyl)trimethoxysilane (GPTMS) [31], which differ in their functionalisation and can be used as initiators.

Exceptionally strong bonds with iron oxide are formed by compounds with bisphosphate moieties and by catechol derivates [32]. Our group focuses on catechol derivates. Particularly the catechol derivate NDA seems to be able to form an irreversible bond and to generate long term stabilisation of coatings using this moiety as the anchor group [33].

1.5.2. Catechols and Nitrodopamine (NDA)

To irreversibly bind polymers (grafting-to), to have a suitable initiator (grafting-from) or to make the system stable in aqueous media while using FeOx-NPs suitable anchors are needed. Catechols (Figure 5) especially catechol derivates like nitro-substituted catechols meet these requirements and therefore can be used as anchors for FeOx-NPs [6]. The FeOx-Nps synthesised in this master thesis are capped with oleic acid. Acids like oleic acid can be used to sterically stabilize the particles in organic solvents [33] but their bonds are labile and can be replaced with a ligand with a higher affinity to the surface (Figure 6) [17] .

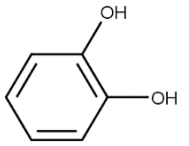


Figure 5: Chemical structure of catechol

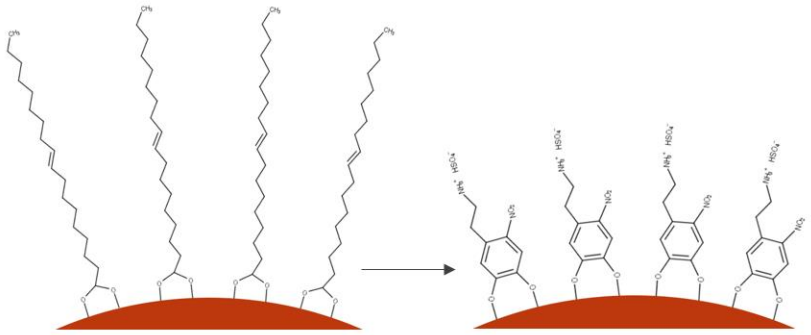


Figure 6: The scheme shows the ligand exchange. Oleic acid is displaced by NDA as a ligand on the surface of the particle.

Already in 1976, it was first claimed that the iron catechol bond could be strengthened to an irreversible bond by adding an electronegative substituent like a nitro substituent. As a reason for this strong bond it was presumed that nitro-substituted catechols (so called nitrocatechols) can act as oxidizing agents [34]. Our group focuses on nitro-substituted catechols like NDA as anchors for coatings of FeOx-Nps. With NDA as an anchor it is for example possible to connect a polymer brush shell of PEG irreversibly to the iron oxide core to achieve colloidal stability in aqueous solvents. NDA provide long-term stabilization due irreversible binding to the iron oxide of the bidentate catechol ligand. The PEG particles stabilized with NDA (dispersed in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) containing 150 mM NaCl) were stable for more than nine filtrations and could be repeatedly heated up to 90 °C while particles stabilized with for example only dopamine as an anchor formed aggregates and therefore indicates formation of only a reversible bond under relevant conditions for biotechnological and biomedical applications [28].

The NDA hemisulfate can be synthesised out of dopamine hydrochloride (Figure 7) [17]. To use NDA as an initiator, a base like N-methyl-2-pyrrolidone (NMP) is needed to remove the hemisulfate and enable the initiation through the amine moiety [35].

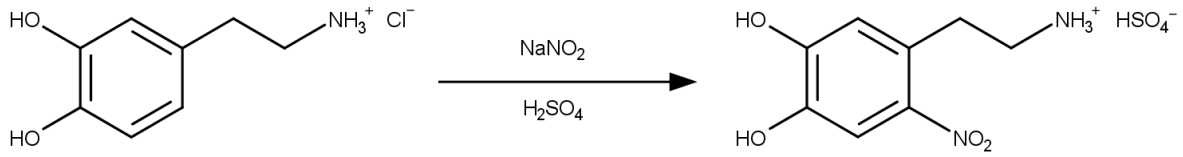


Figure 7: Synthesis of NDA hemisulfate out of dopamine hydrochloride.

Nevertheless, the complete removal of oleic acid from the surface of as-synthesized FeOx-Nps is still a problem. Incomplete removal of oleic acid can lead to a reduced grafting density of the wanted ligand and the ligand's effect on the system for example regarding the colloidal stability.

Furthermore, the removal of the excess, not bound, ligand after the ligand exchange is a big issue and is only possible by multiple washing steps. When the grafting density is measured by thermogravimetric analysis (TGA), unbound ligand cannot be distinguished from bound one and this fact leads to an overestimation of the grafting density. When grafting-from is used, unbound ligand furthermore can act as an initiator, which results in unbound polymer [17].

1.6. Polymer Shells

1.6.1. Polymers and their Application for Polymer Shells

A polymer is a macromolecule that consists of repeating subunits. It can be linear, branched or crosslinked and is made of a main chain (backbone) and side chains. Due to the diversity of different subunits and different structures polymers can have a broad range of properties making them an essential and unique tool in nature and synthetic chemistry. There are synthetic polymers like polypropylene (plastics), polystyrol (styropor®), polytetrafluorethylene (teflon®) or polyethylen (pharmacy, cosmetics …) and biopolymers with a biological origin such as starch, cellulose, polypeptides and DNA/RNA to name only a few examples [36].

Like already mentioned, magnetic particles are used for biomedical applications, especially FeOx-NPs due to their biocompatibility and biodegradability. In order to guarantee that the particles reach their target respectively that they are able to comply with their purpose, an adequate stabilisation has to be guaranteed and clearance of the particles by the immune system has to be avoided. This can be realised by polymeric modifications of the particles, which of course have to be nontoxic and biodegradable as well [37].

1.6.2. Polypeptoids - Structure and Properties

Like already mentioned the core and the shell of CSNP have to be biocompatible to be useable in biomedical applications, a fact which strongly limits the selection of suitable polymers. Often biopolymers are used because of their biological origin and therefore their biocompatibility. Biopolymers like polypeptides are chains of amino acids, which naturally occur in all creatures and plants to form for example macromolecules like proteins and are therefore suitable candidates for polymer brushes. But not only polypeptides are of interest but also less known polymers like polypeptoids have got more and more into focus [38]. Polypeptoids consist of repeating N-substituted glycine units and are very similar to polypeptides but also differ in many points.

They can be classified as synthetic molecules inspired by a biological polymers. Therefore, at the beginning polypeptoids like poly(N-methylglycine) were mainly used in research to better understand the mechanism of polypeptide synthesis [39]. The chemical structure of the backbone of polypeptoids are identical to the one of polypeptides. Therefore, polypeptoids as well are claimed to be biocompatible because their aliphatic backbones are degradable. Nevertheless enzymes facilitating proteolysis of peptides do not act on peptoids and therefore polypeptoids seem to be more stable against degradability than polypeptides, which makes them interesting for medical application [40]. Polypeptides have the side chain connected to the carbon of the backbone, polypeptoids to the

nitrogen atom (Figure 8). The bond of the sidechain to the nitrogen atom is the reason that the backbone has no H-donor anymore and consequently makes the backbone of the polypeptoids achiral and more flexible [41].

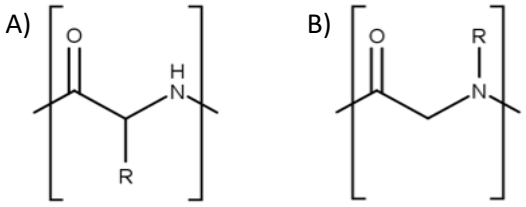


Figure 8: Structure of A) polypeptides and B) polypeptoids

Beside poly(N-methylglycine) other polypeptoids (Figure 9) are still rarely investigated and therefore also their properties like solubility, stability or melting point are not as well known. The potential high degree of functionality that can be obtained by polypeptoids such as thermoresponsibility or the formation of secondary structure is a reason that polypeptoids draw immense interest recently [39].

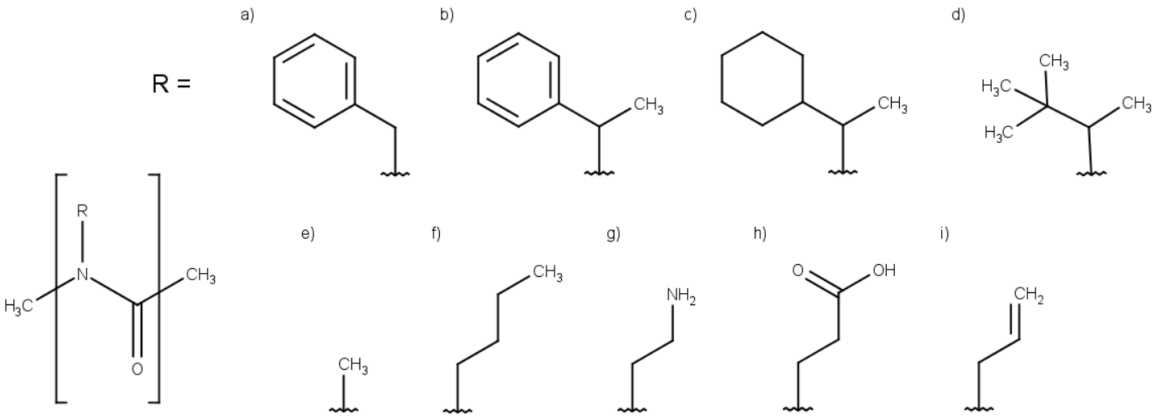


Figure 9: Structure of polypeptoids with different side groups like a) N-(1-phenylmethyl)glycine (Npe), b) N-(1-phenylethyl)glycine (2PE), c) N-(1-cyclohexylethyl)glycine, d) (3,3-dimethylbutan)glycine (tBu), e) N-methyglycine, f) N-butylglycine g) (2-aminoethyl)glycine (Nae) h) N-(2-carboxyethyl) (Nce) i) N-(prop-2-en-1)glycine

One of the best researched polypeptoids and simplest in its structure, consisting of a methylation of the nitrogen, is poly(N-methylglycine) (Figure 9e) also known as sarcosine. Poly(N-methylglycine) is soluble in a wide range of organic solvents and in aqueous media. Poly(N-methylglycine) forms random coils in water and belongs to the thermosresponsive polymers. This means that under a certain lower critical solution temperature (LCST) poly(N-methylglycine) is hydrophilic and soluble in water but above the LCST it loses part of its hydration and the polymer precipitates. The LCST depends on the molecular weight and the end group of the polymer. By mixing different monomers with different side

groups like methyl and butyl (Figure 9f) groups the LCST can be set and polypeptoids, with solubility and conformation contrallably by change of temperature, can be synthesized [35].

Polypeptides are known to form complex secondary structures in nature. For example, the secondary structure elements of proteins are determined by the sequence of different amino acids.

Like polypeptides, also some polypeptoids form secondary structures. Due to the lack of hydrogen bond forming moieties along their backbone, it is more flexible and the formation of secondary structures is mainly controlled by their sidechains. In contrast to peptides, the backbone of polypeptoids does not show a preference between formation of cis- or trans-conformations.

Homopolymers are polymers consisting of repeating units with identical sidechains. Polypeptoids with aromatic side chains are known to form α-helices in organic or aqueous solution. The side chains must have the same chirality to be able to induce a helical chain shape [42]. Already CD of short oligomers like pentamers of N-(1-phenylethyl)glycine (2PE) (Figure 9b, Figure 10) showed the formation of helices. Furthermore CD measurement displayed many similarities when compared to helices formed by peptides, raising hope for the development of peptide mimicking applications [43]. Another example for the formation of helices by a polypeptoid with an aromatic side chain is N-(1-phenylmethyl)glycine (Figure 9a) [44]. Nevertheless, the synthesis of longer chains resulting in tight helices like 2PE still seems to be problematic. Due to intramolecular transamination during the synthesis only short homopolymers (DP<100) were to date realised with ROP [44].

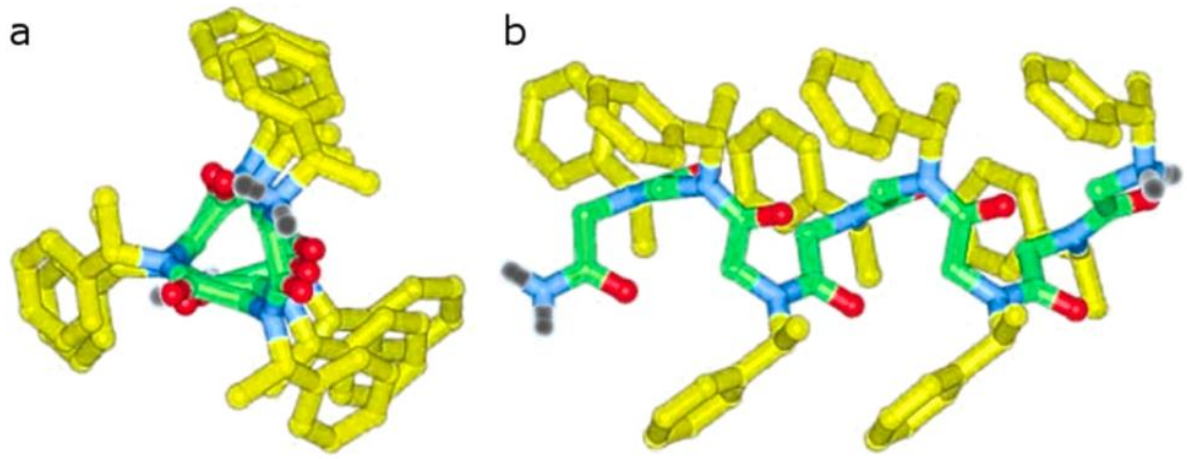


Figure 10: Predicted helical structure of 2PE viewed form a) a parallel and b) a perpendicular to the long axis perspective. The colour code for the backbone is green for carbon, grey for hydrogen, red for oxygen and blue for nitrogen. The sidechains are represented in yellow. The figure was adapted from the review ref [45] which adapted it original from ref [46]. Copyright 1997 Elsevier Ltd.

Research showed that not only aromatic but also α-chiral, aliphatic side chains lead to the formation of helical conformations. An example is N-(1-cyclohexylethyl)glycine (Figure 9c). These helices are very interesting because they closely mimic trans-membrane or membrane-disruptive polypeptides [47].

Due to the flexibility of the backbone, the formation of helices can also be generated by steric effects alone. An example therefore is polypeptoids containing a tert-butyl sidechains [48] raising hope for the synthesis of polypeptoids with other tert-butyl derivates like poly((3,3-dimethylbutan)glycine) (tBu) (Figure 9d) as side chains .

The formation of secondary structures by homopolymers is limited. However, recent publications show that heteropolymers containing diverse sidechains are more prolific formers of secondary structure. Not only longer polypeptoid chains, able to form alpha helices, but also more complex secondary structures can be realised with this new generation of polymers [45].

With heteropolypeptoids containing a variation of two or more different sidechains, the chain shape and stiffness and correlating properties can be controlled. Still, chiral side chains are responsible for the formation of helices. At least 50% of the polypeptoids have to consist of primarily bulky chiral side chains of the same chirality, like 2PE, to form a helix [42]. The synthesis of homopolymers such as 2PE seems to be troubled due to intramolecular transamination during the synthesis [44]. Sequence specific polymerisation of 2PE with non-helical forming side groups, seems to prevent these effect by minimising the stiffness of the helices [42].

Beside helices, also other secondary structures like sheets, turns or loops are present in proteins. Therefore, to be able to mimic protein like structures, built of polypeptoids, not only helices but also these other secondary structures are required.

Already in 2010 it was showed that amphiphilic peptoides containing polar and nonpolar residues are able of the formation of 2D nanosheets [49]. Next polypeptoids with hydroxyl side groups were polymerised and subsequently crystallized. Sheet-like structures, stabilized by intramolecular and intermolecular interactions caused by hydrogen bonding of the functional groups, could be measured with X-ray crystallography [50]. Since then more complex nanosheets like for example sheets built of identical composition of polypeptoids (N-(1-phenylmethyl)glycine (Npe), (2-aminoethyl)glycine (Nae) (Figure 9g) and N-(2-carboxyethyl) (Nce) (Figure 9h) but with different surface patterns and therefore defined surface chemistry were designed (Figure 11) [51].

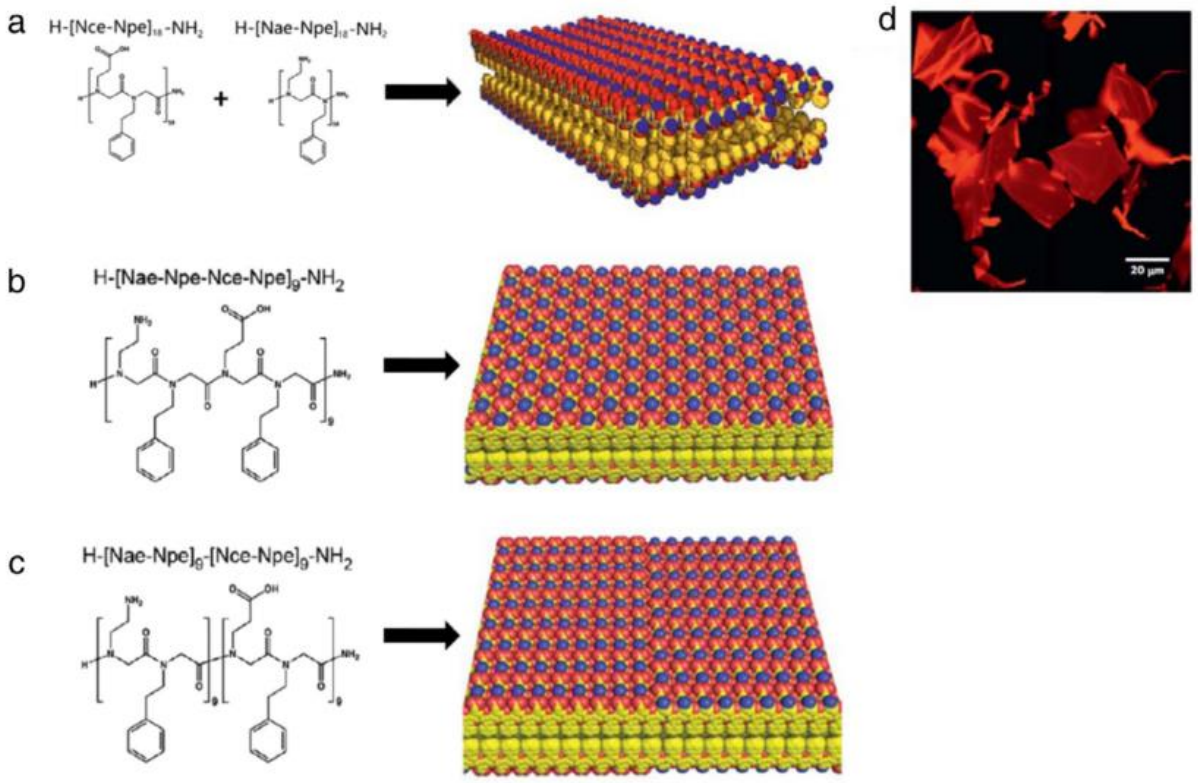


Figure 11: Polypeptoid nanosheets. The figure was adapted from the review [52]. Figure a) and d) were original adapted from ref [49] and b) and c) are original adapted from ref [51].

Furthermore, antibody-mimetic peptoid nanosheets for molecular recognition were synthesised, consisting of regions, responsible to form 2D nanosheets interspersed with sequences resulting in functional loop sequences [53]. The loops, exposed on the surface, mimic antibody recognition elements and can be used for diagnosis of diseases like Alzheimer [54].

Besides linear polymers, also the synthesis of cyclic, bottlebrush, branched and star-shaped polypeptoids could be realised [55].

Side groups, which are able to be functionalised, offer a possibility to generate even more complex polypeptoids. Therefore, polypeptoids containing monomers with reactive side chains were synthesised. For example alkenes and alkynes (Figure 9i) sidechains can react with molecules containing thiols, amides or phosphazine base groups in order to add supplemental properties to the polypeptoids [56,57].

These complex properties needed to form complex structures do not only motivate the importance of sequence specific polymerisation but also the importance of knowledge of detailed functions of individual side groups and their interplay with each other.

1.7. Polymerisation methods

1.7.1. Polymerisation of Polypeptoids

Polymerisations of polypeptoids with simpler structured side chains like poly(N-methylglycine) are well researched and polymers with a high molecular weight and narrow molecular weight distribution could be already realized. On the other hand polypeptoid with aromatic or branched sidechains, which result in the formation of secondary structures, pose new challenges [38]. There are two main approaches to synthesise polypeptoids: ROP and step-by-step polymerisation from a solid support.

1.7.2. Synthesis of Monomers and Ring opening polymerisation (ROP)

ROP is a chain-growth polymerisation method where cyclic monomers are used. The end of the chain terminates in a reactive center which attacks the ring of the monomer. The cyclic part of the monomer is broken open and in the following process added to the chain resulting in a new reactive center. This process is repeated multiple times and leads to an elongation of the chain. The reactive center can be radical, anionic or cationic. To activate the first monomer and to start the propagation additional molecules, so called initiators, are needed. One of the most known polymer produced by ROP is the polyamide. An example for polyamide used in industry is polyhexamethyleneadipamide also known under the trade name nylon [58].

Already in 1966 the synthesis of polypeptides and polypeptoids by anionic nucleophilic ROP could be realised. As initiators molecules with at least one mobile hydrogen atom, present in water, alcohols and primary amines, can be used. The free electron pair of the primary amine of the initiator or of the last added monomer attacks the carbon with the lowest electron density of the cyclic ring of the monomer which leads to decarboxylation, a new free amino group and therefore to an elongation of the polymer. The polymer contains the initiator with the corresponding amine on one side of the chain and a secondary amine on the other end of the chain (Figure 12) [59,60].

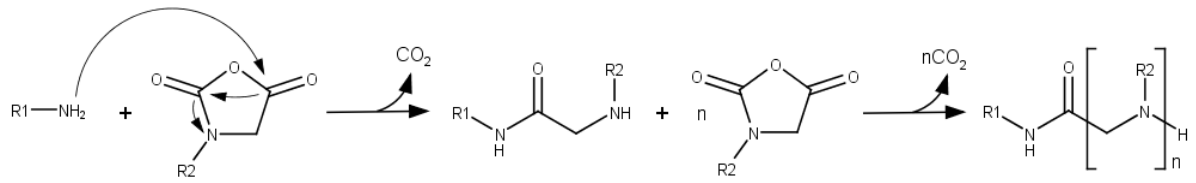


Figure 12: Reaction mechanism of ROP with a primary amine as initiator. R1 stands for different initiators, R2 for variations of side groups of NCAs.

Polymerisations of Methyl-N-carboxyanhydrides (NCA) showed that by using amine initiators like benzyl amine polymers with narrow molecular distribution (PDI < 1.1-1.3) can be synthesized and that the size of the polymers can be controlled by the ratio of monomer and initiator [61]. ROP is also possible using amine initiators bound to a surface. Luxenhofer et al. showed 2013 the possibility of polymerisation of polypeptoids from a Rink-type resins, connecting ROP and polymerisation from solid support [62]. In 2017 Kurzhals et al. managed polymerisation of *N*-Methylglycine-NCA and *N*-butylglycine-NCA, initiated by NDA bound to FeOx-NPs, to produce thermoresponsive polypeptoid-coated SPION [35].

Nevertheless, once the polymerisation is started the propagation is an autonomously ongoing process where the individual step cannot be controlled. Long polymers can be synthesised in a short time with little handling effort. Therefore only homopolymers, blockpolymers/craft polymers, where different sort of monomers are polymerised successively, or heteropolymers with a random sequence of monomers are possible [58].

For ROP, first monomers have to be synthesised. NCA are suitable monomers for this reactions because of their organic cyclic ring with a high electrophilic reactivity at the carbonyl group of the α-amino acid [13]. While always containing the identical cyclic structure, which is needed for the ROP, their diversity is defined by variation of their side chains. Polymers out of C-substituted NCAs are called polypeptides, N-substituted NCAs are called polypeptoids. Due to their high reactivity, a water and oxygen free atmosphere is necessary for the final steps of the synthesis and polymerisation of NCAs [55]. Typically, primary amines (-NH_2), consisting of the chemical structure that later results in the required side group of the polypeptoid, are used to generate an N-substituted glycine precursor. Next, a protective group like benzyl chloroformate, chloro(ethoxy)methanone or di-tert-butyldicarbonate is used for initial conversion of the N-substituted glycine precursor. For ring closing, various activating electrophiles are used like PCl_3, PBr_3, AcCl/Ac_2O or $SOCl_3$. It is also possible to react the N-substituted glycine precursor directly with phosgenes to generate the ring closed monomer but due to the high toxicity this method is not preferable (Figure 13) [55].

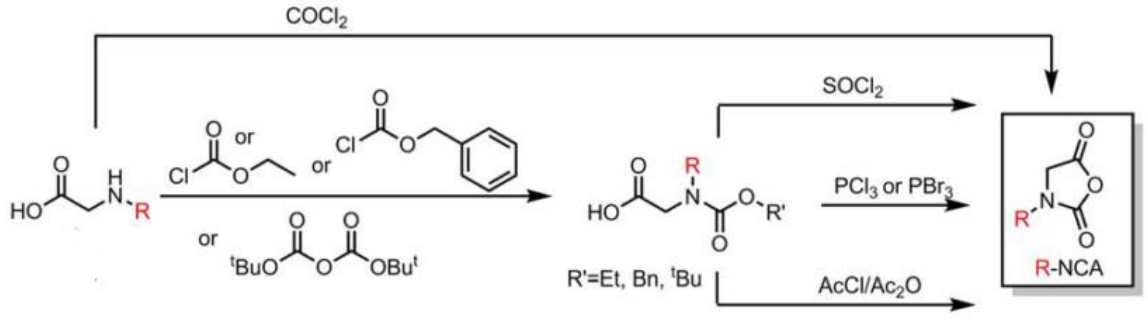


Figure 13: Synthesis of NCA, R stands for variations of side groups of NCAs. Adapted from ref. [55].

1.7.3. Step-by-step Polymerisation of Polypeptoids from a Solid Support

Step-by-step polymerisation from a solid support was originally introduced for the synthesis of sequence specific polypeptides but can also be used for the synthesis of polypeptoids. Polymerisation of polypeptoids from a solid support means that a solid support is first used to generate a polymer which then can be cleaved off. These polypeptoids can be used for example for the grafting-to method. The advantage of this method is that the polymer is elongated step by step with only one monomer per cycle. Therefore, the exact order of each monomer can be determined during the synthesis and the formation of sequence-specific polypeptoids is possible.

As a solid support for polymerisation for example Rink amide resin is used. The solid support serves as an anchorage during the synthesis and is kept in a column with a filter that it cannot pass. That enables several synthesis and washing steps with stable fixation of the generated polymer. By alternating acetylation of the secondary amine at the chain end with bromoacetic acid and nucleophilic displacement of the bromide with a primary amine the polymer can be synthesised. The polymerisation is started with the acetylation of the primary amine of the resin. (Figure 14)

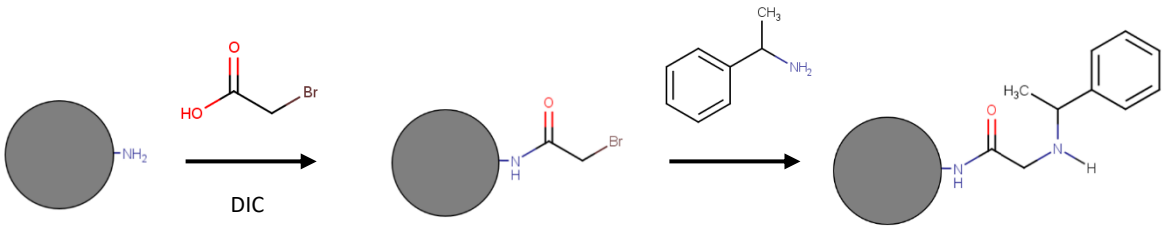


Figure 14: Synthesis mechanism of (sequence specific) polypeptoids from a solid support by step-by-step polymerisation, for example with 2PE like pictured.

The step-by-step method brings the advantage that each step can be controlled separately but at the same time also the disadvantage that the procedure is very time consuming (90 min per cycle). Therefore, for the research on polymers with high molecular weight or on multiple polymers with different combustion, helping equipment like a synthesis robotic is needed in order to reduce the handling time and the necessity to be present during the synthesis. Furthermore another disadvantage is that large excesses of reagents and high amount of solvents for washing steps are needed for every cycle [63,64].

Synthesis of polypeptoid-like homopolymers with faster polymerisation methods can be used to understand the unique functionalities of different side groups but their applications are limited. Therefore, methods like the step-by-step polymerisation are necessary to produce sequence specific polypeptoids with complex properties.

1.8. Characterisation of Cores and Polymers

1.8.1. Circular Dichroism Spectroscopy (CD)

CD measurements can be used to determine secondary structures of polymers and has its most important application in the study of large biological molecules like proteins. The existence of different secondary structures can be measured and furthermore their reaction to different temperatures, pH and so on. The method is based on measurement of the circular dichroism, which is a special property of optical active (chiral) molecules over a range of wavelengths. A laser with left- and right-circularly polarized light is shot at the sample. Chiral molecules absorb left- and right circularly polarized light differently. The resulting difference is measured as function of wavelength to generate a graph. This is compared to known absorption models in order to differ between common secondary structures (Figure 15).

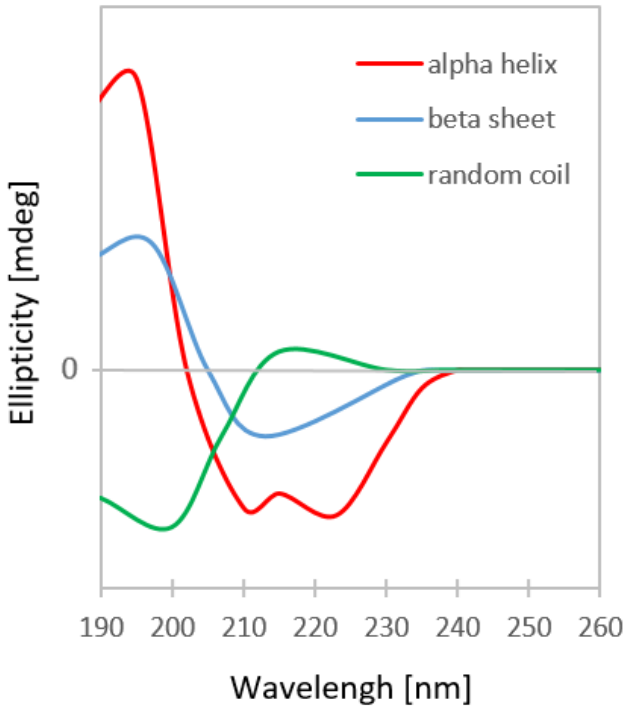


Figure 15: CD spectra of alpha helix, beta sheet and random coil

Like polypeptides also some polypeptoids are known for the formation of secondary structures. CD can be used to prove and investigate the formation of secondary structure of this new generation of polymers.

1.8.2. Matrix Assisted Laser Desorption/Ionisation - Time of Flight - Mass Spectroscopy (MALDI-TOF-MS)

MALDI-TOF-MS is a method to analyse especially bigger molecules like polymers and biopolymers (for example proteins) and based on the ionisation of the sample. For detection MALDI-TOF-MS is mostly connected with time of flight mass spectroscopy.

The sample is co-crystallised with a matrix and shot by an ultraviolet light laser. The matrix absorbs the light and converts it into heat energy which leads to vaporisation of a small part of the matrix and also of the sample. Furthermore the sample is ionised by addition of a proton or sodium ion. Charged ions of various sizes are generated which differ in mass-to-charge ratio. Ions with smaller molecular weight and higher charged ions are accelerated more strongly and therefore reach the detector faster. Therefore, the time of flight correlates with the mass-to-charge value and can be used for the measurement of it (Figure 16).

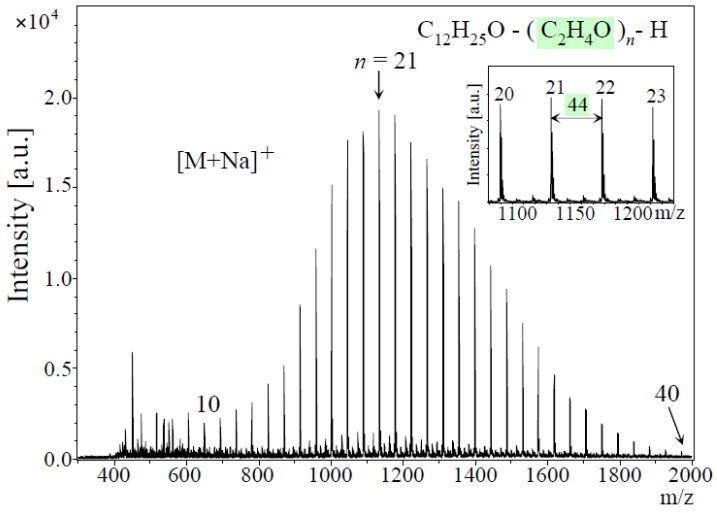


Figure 16: The Figure shows MALDI-TOF-MS of poly(ethylenglycol)monododecyl: The intensity describes the number of molecules measured. In this example most molecules are found at the molecular weight-to-charge value n=21. M/z. The measurement therefore shows that there are molecules with a typical chain length of 10-20 monomers. The distance between the peaks is 44 m/z. With z=1 that corresponds to the molecular mass of one monomer. Adapted from ref. [65].

1.8.3. Nuclear Magnetic Resonance Spectroscopy (NMR)

NMR is a spectroscopic technique to measure the composition of samples without destroying them, for determination of molecule structures and for the measurement of interactions between molecules. The method is based on measurement of nuclear magnetic resonance. The magnetic resonance is influenced by the interaction of the nuclear spin with an external magnetic field as well as the interaction of the nuclear spin with electrons surrounding the atom, electrons of other atoms and

neighbouring nucleus in the same molecule. Only isotopes with a magnetic moment like for example ^{1}H, ^{13}C, ^{15}N, ^{17}O can be measured.

One of most used method to analyse the composition and structure of a sample is ^{1}H-NMR. For example, it can be verified that a synthesis was successful and the desired molecule was generated, if the starting material is fully converted and weather side products or other impurities are present. This is for example important if monomers for ROP are synthesised because in this case impurities would strongly interfere with the polymerisation (Figure 17). Deuterated solvents of water, chloroform, DMF and so on have to be used for measurements.

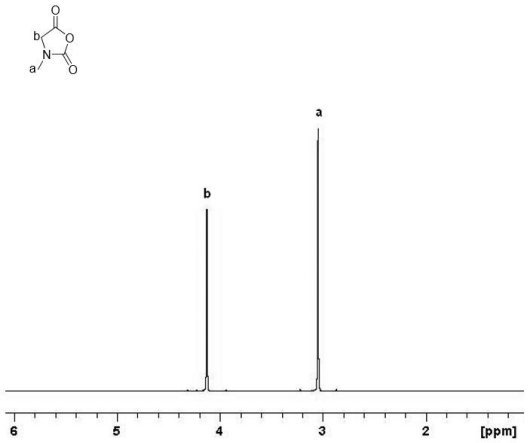


Figure 17: The ^{1}H-NMR shows the successful synthesis N-methyl-NCA and the high purity standard of NCA needed for ROP. Adapted from ref. [66].

1.8.4. Thermogravimetric Analysis (TGA)

TGA is an analytic method to measure the change of mass in dependence of time and temperature. The sample is put in an inert and temperature stable crucible and heated up to up to 2400 °C in an oven. The mass change during the heating process is measured by a microbalance. Modern equipment allows the regulation of heating rate, end temperature, gas flow and which kind of gas is used. Gases used for TGA are nitrogen, synthetic air or oxygen. During the decomposition the sample can take up substances from the environment for example due to oxidation (mass gaining) or emit substances to the environment due to vaporisation (mass loss). The mass change at certain temperature gives information of the composition of a sample. With TGA for example measurements of organic shell / inorganic core CSNP the content of organic material can be determined to gain information about the shell, like mass and grafting density, by combining the information with other data on polymer molecular weight and core morphology.

1.8.5. Transmission Electron Microscopy (TEM)

For characterisation of SPIONs transmission electron microscopy (TEM) can be used. TEM uses an electron beam to generate a picture. The electrons are first accelerated and directed to the sample. The electrons interact with the sample by scattering and absorption, and only a part of them are able to pass the sample. The intensity of the transmitted electrons on the detector provides and image with information on sample thickness and composition through the varying electron density of the sample.

Inorganic nanoparticles due to their high electron density are pictured as dark dots, from which even resolution of the crystal lattice of small nanoparticles can be obtained. Ligands like oleic acid or single polymer chains are too thin to absorb enough electrons to be seen. Nevertheless, their presence can be recognised indirectly due to the spacing and arrangement of the particles. Because of the steric stabilisation a free space between single particles is formed that is occupied by the spacer. (Figure 18) With a software like Pebbles [67] the contrast difference of the picture can be measured to determine the size and the size distribution of the particles.

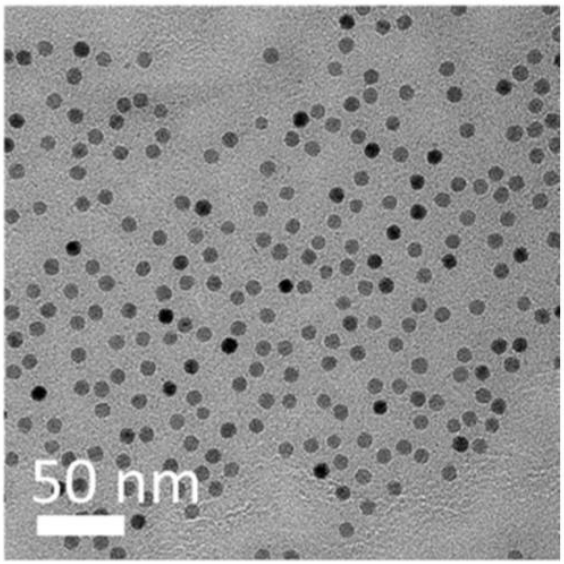


Figure 18: TEM picture of FeOx-NPs (9.6 nm) with a PEG (5kDa) shell. The dark spots represent the particles. The PEG can be recognized by the space between the particles. Adapted from ref. [16].

2. AIM OF THE THESIS

A major focus area of nanotechnology is the research on nanoparticles in particular on CSNP. CSNP include a broad field of various combinations of different cores and shells. Nevertheless, CSNP have only seen limited application in biomedicine until now. To realise new applications, it is important to enhance CSNP by optimising the quality and by taking a closer look on new components.

FeOx-NPs are already well investigated. Our group is one of many to produce FeOx-NPs with narrow size distribution and well-controlled morphology [16]. However, for applications, well-defined cores are just the first important step since they provide a good basis for grafting of an organic shell that controls the interactions with the environment. Polypeptoids are a new generation of polymers that can be more easily polymerized than polypeptides, but which possess a higher chain flexibility, no internal hydrogen bonding and the theoretical ability to just like polypeptides form sequence-specific 3D structures. Polypeptoid shells are therefore highly interesting for the creation of functionally diverse core-shell nanoparticles. Compared to simpler structured polypeptoids like poly(*N*-methylglycine), more complex polypeptoids like 2PE and tBu are still rarely investigated. The formation of helices by 2PE has already been described [44]. However, synthesis of these homopolymers with a high molecular weight and using different monomers are still a problem. The aim of this thesis is to investigate approaches to synthesize FeOx-NPs grafted with polypeptoids of 3-(1-phenylethyl)-1,3-oxazolidine-2,5-dione (2PE-NCA) and 3-(3,3-dimethylbutan-2-yl)-1,3-oxazolidine-2,5-dione (tBu-NCA).

The perhaps most important aspect of grafting a shell is to use an anchor chemistry that allows control over shell density and stability. Our group has shown that catechol derivates especially NDA is a highly advantageous anchor group for FeOx-Nps and also can serve as initiator for surface-initiated polymerization [16,17,35]. Still, modification with NDA raises a challenge. While ligand exchange with NDA has been demonstrated, purification of the NDA-functionalized cores from free NDA has shown to be a serious obstacle. A first goal of this thesis is therefore to modify FeOX-NPs with NDA and NDA-C_{11}-NH_2 and to obtain pure initiator-functionalized FeOx-NPs by establishing rigorous purification protocols, from which polypeptoid brush shells can be grown.

A second goal of the thesis is to investigate two different polymerisation methods, ROP and step-by-step polymerisation from a solid support, and their limitations for synthesis of polypeptoid brushes. ROP monomers in high purity are a requirement for a successful polymerisation and must be synthesised according to these high-quality aspects. The monomers in this master thesis were synthesised from *N*-substituted glycine precursors with a modified Fuchs-Farthing method, described by Guo et al., using di-tert-butyldicarbonat (Boc_2O) as a protection group and PCl_3 for ring closing [44].

Two monomers are investigated for ROMP: 3-(1-phenylethyl)-1,3-oxazolidine-2,5-dione (2PE-NCA) and 3-(3,3-dimethylbutan-2-yl)-1,3-oxazolidine-2,5-dione (tBu-NCA). The primary amines benzylamine and n-decylamine were used as initiators to investigate the ROP of linear polymers. A rink amine resin was used for the investigation of the step-by-step polymerisation from a solid support of 2PE and tBu. The addition of monomers was done by alternating acetylation of the secondary amine at the chain end with bromoacetic acid and nucleophilic displacement of the bromide with a primary amine [63,64]. Finally, the grafting-from polymerisation with 2PE from FeOx-NDA NPs is investigated to address the overall aim.

3. EXPERIMENTAL PART

3.1. Material and Methods

3.1.1. Chemicals

All chemicals used for this master thesis were purchased from Sigma Aldrich or Fluka and used without further purification unless noted otherwise.

3.1.2. Methods

Transmission Electron Microscopy (TEM):

TEM pictures were recorded by Martina Schroffenegger and Max Willinger on a FEI Tecnai G2 TEM with 160 kV acceleration voltage and carbon-coated grids where used.

Pebbles [67]

The size and the size distribution of the nanoparticles was calculated with the software Pebbles.

Thermogravimetric Analysis (TGA):

A Mettler Toledo TGA/DSC1 with the adjustment of 80 ml/min synthetic air as reactive gas, 20 ml/min nitrogen as protective gas, a heating rate of 20 K/min and a temperature range of 25-260 °C was used to determine the organic contend of the NDA nanoparticles.

Nuclear Magnetic Resonance (NMR):

All NMR ^{1}H measurements were measured by the Department of Chemistry, BOKU on a BRUKNER AV II 600 spectrometer. The Measurements were analysed with the software 2D NMR Processor.

Matrix Assisted Laser Desorption Ionization — Time of Flight Mass Spectrometry (MALDI-TOF MS):

Polypeptoids were measured by the Department of Chemistry BOKU on a Bruker Autoflex in linear positive mode. The samples were dissolved in tetrahydrofuran and mixed with lithium trifluoracetate. As matrix 2,5-dihydroxybenzoid acid was used as matrix.

Circular Dichroism (CD):

CD measurements were performed on the Department of Chemistry. A Chirascan CD spectrometer was used to determine the secondary structure of the polypeptoids.

Glovebox:

All polymerisations were executed in a GS glovebox (water level ≤1 ppm, oxygen level ≤45 ppm)

3.2. Synthesis of FeOx-NPs

Oleic acid capped FeOx-NPs were synthesised after the protocol of Andrea Lassenberger [16], based on the method of Park et. al. [22].

A three-neck flask was connected with a condenser and heated (heat gun, 140 °C) from top to bottom while purging with nitrogen. The flask was charged with dioctylether (50 ml) and oleic acid (4.5 ml, 14.18 mmol). The solution was heated to 100 °C with a heating rate of 10 °C/min while bubbling with N_2 (top open, condenser turned off). Iron pentacarbonyl (1 ml, 7.40 mmol) was added after filtration with a syringe filter (cellulose, 0.45 µm). After heating up to 290 °C (condenser turned on) with a heating rate of 3 °C/min the solution was left at this temperature for 1 h and then cooled down to room temperature.

The particles where precipitated with ethanol (EtOH) at room temperature. After the EtOH was removed the particles were dispersed in toluene (1 ml), precipitated with EtOH and then washed one more time with EtOH. Finally, the particles were centrifuged and the access of EtOH was disposed. EtOH was bubbled with N2 for 10 min before usage. A magnet was used to separate the particles from the solvent.

The dark brown particles (one batch) were stored wet with EtOH at 4°C. TEM pictures were taken and the size and size distribution calculated with Pebbles.

Batches: Iron oxide NPs 1: size: 6.2 nm (standard deviation: 6.9%)

Iron oxide NPs 2: size 5.9 nm (standard deviation: 11.6%)

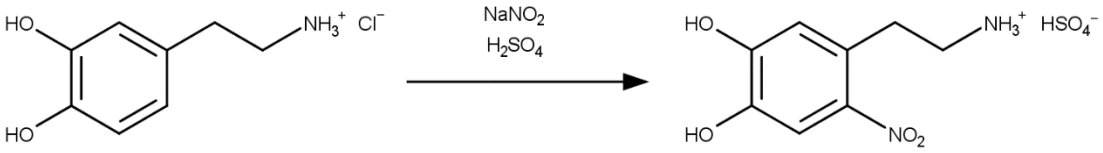
3.3. Synthesis of NDA

NDA (2) was synthesised after a modified protocol of our group [12,17] based on Napolitano et al. [68].

Dopamine hydrochloride (1) (5 g, 2.64 mmol) and sodium nitrite (6.3 g, 9.13 mmol) were dissolved in Milli-Q water (150 ml). Sulfuric acid (25 ml, 20 %) was added dropwise to the solution at 0 °C under N_2. The reaction was covered with aluminium foil and stirred overnight at room temperature.

The synthesis was cooled down (4 °C), the yellow solid was filtered off and washed with cold water (4 °C). Next the solid was dissolved in EtOH (200 ml) and stirred at 40 °C for 20 min. The product was precipitated with cold diethyl ether (4 °C), filtered off, washed tree times with diethyl ether (4 °C) and dried with the lyophilisator.

The dried yellow powdery **NDA (2**) (4.2 g, 14.18 mmol) was stored in a flask covered with aluminium foil at 4 °C. (Figure 19)



1 **2**

*Figure 19: Synthesis of **NDA (2)***

3.4. Preparation of FeOx-NPs with NDA (FeOx-NPs-NDA) (2)

Particles were prepared by a modification of a protocol developed by Olivier Bixner for palmityl NDA coated particles [17].

Iron oxide NPs 1 were used for the ligand exchange. The following table shows the washing procedure of the NDA-particles. The solvent respectively **NDA (2)** were added to the particle. After each repetition step the solvent was removed. For precipitation a magnet was used to collect the particles at the bottom of the flask and to remove the solvent. (Table 1)

Table 1: Washing and ligand exchange protocol for the preparation of FeOx-NPs-NDA

Number of repetition steps	**Washing step**	**Amount of solvents***
5	Dispersion in toluene and precipitation with EtOH	5 ml toluene 150 ml EtOH
4	1 mM oleic acid in methanol (MeOH), N_2, 60 °C for 5 min, precipitation at RT	50 ml oleic acid in MeOH
5	MeOH, N_2, 60 °C for 5 min, precipitation at RT	50 ml MeOH
1	**NDA (2)**, DMF, $CHCL_3$, MeOH**, sonication for 3 h***, N_2	400 mg (1.35 mmol) **NDA (2)** 15 mL DMF 15 mL $CHCL_3$ 18 mL MeOH
3	MeOH, N_2, 60 °C for 5 min, precipitation at RT	50 ml MeOH

2	MeOH, N_2	50 ml MeOH
1	**NDA (2)**, DMF, sonicated for 1 h, N_2	200 mg (0.68 mmol) **NDA (2)** 6 ml DMF
2	DMF, n-hexane, 60 °C for 5 min, N_2, precipitation at RT	3 mL DMF 80 mL n-hexane
2	acetone, 60 °C for 5 min, N_2, precipitation at RT	50 mL Acetone
2	MeOH, 60 °C for 5 min, N_2, precipitation at RT	80 mL MeOH
2	MeOH, 60 °C for 5 min, N_2, precipitation at RT	50 mL MeOH
20	MeOH, sonication (20 sec) at precipitation step 11, 14 and 16	50 mL MeOH
*Amounts needed for half a batch of FeOx-NPs **6 mL of the DMF was used to disperse the particles. The **NDA (2)** was dissolved in the rest of the DMF, the other solvent was added and the mixture was combined with the particles. ***The particles were sonicated for 1.5, left at RT overnight and sonicated another 1.5 h the next day.		

The particles were dissolved in Milli-Q water and lyophilised for 3 days. A brown powder (206 mg) could be gained and the product was stored in a glovebox.

Dispersion of the particles in different solvents was tested. A spatula tip of particles and the solvent were agitated (1 min), sonicated (1 min) and put on a magnet (10 min). List of solvents: acetonitrile, acrylate PEG, triethylamine (Et_3N), pentamethyldiethylenetriamine (PMDETA), pyridine, dimethyl sulfoxide (DMSO), N,N'-dimethylpropyleneurea (DMPU), DMF, dichloromethane (DCM) and N-methylformamide (MFP).

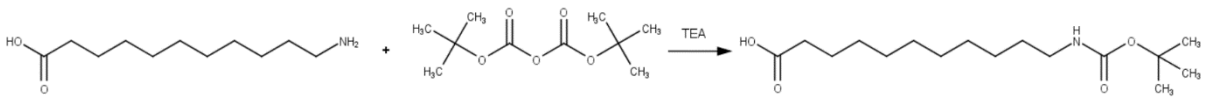
3.5. Synthesis of Nitrodopamin-C_{11}-NH_2 (NDA-C_{11}-NH_2 (7))

The Synthesis of **NDA-C_{11}-NH_2 (7)** was developed be Martina Schroffenegger, which has not been published until now.

1)

11-aminodecanoic acid (3) (1 g, 5.07 mmol) was dissolved in a mixture of H2O (50 ml) and THF (50 ml). First TEA (3.5 ml, 25.11 mmol) and then **Boc_2O (4)** (2.8 mL, 12.19 mmol) were added to the synthesis and stirred for 3 days at RT.

The solution was washed 2 times with hexane (115 mL) before HCl (4.6 mL) was added to the aqueous phase. The aqueous phase was than extracted 3 times with ethyl acetate (40 mL) and the combined ethyl acetate phases washed with brine (170 ml) before dried over $MgSO_4$. After evaporation of the solvent a white powder **(5)** (844.60 mg, 2.80 mmol) could be obtained. (Figure 20)



3 **4** **5**

*Figure 20: Synthesis of **5***

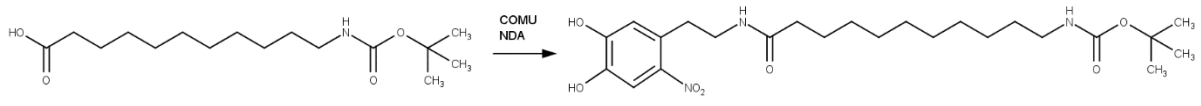
2)

5 (450 mg, 1.49 mmol) was dissolved in 6 mL anhydrous DMF (6 mL) und flushed with N_2 for 5 min. DIPEA (270 µL, 1.50 mmol) and COMU (642 mg, 1.50 mmol) were added and the synthesis was stirred for 30 min under N2 at 0 °C.

NDA (2) (355.5 mg, 1.20 mmol) was dissolved in anhydrous DMF (9 mL), DIPEA (270 µL, 1.50 mmol) was added and the synthesis stirred for 15 min under N_2.

The **NDA (2)** solution was added dropwise to the acid-COMU mixture and first stirred for 1 h at 0 °C and the overnight at RT.

Ethyl acetate (90 ml) was added and the phase then was tree times with HCl (1 M, 100 mL), tree times with $NaHCO_3$ (1 M, 100 mL) and 2 times with brine (100 mL). The organic phase was dried over $MgSO_4$. The ethyl acetate was evaporated and a yellow powder **(6)** (596 mg, 1.24 mmol) was obtained. (Figure 21)

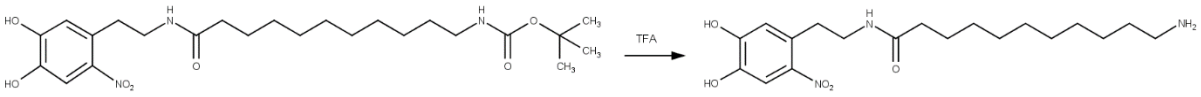


5 **6**

*Figure 21: Synthesis of **6***

3)

6 (596 mg, 1.24 mmol) was dissolved in DCM (45 mL), TFA (5 mL) was added and stirred overnight at RT. After evaporation of the solvent the solid was recrystallised two times in ethylacetat and two times in MeOH/diethyl ether to gain the yellow solid end product (**NDA-C_{11}-NH_2 (7)**). (Figure 22)



6 **7**

*Figure 22: Synthesis of **7***

3.6. Preparation of FeOx-NPs with NDA-C_{11}-NH_2 (7)

For the ligand exchange a magnet was always used to separate the particles from the solvent.

Ligand exchange:

A quarter batch (Iron oxide NPs 2) was dispersed in toluene and precipitated with EtOH (75 mL) six times. Next the particles where first washed 4 times with OA in MeOH (1 mM, 20 mL) and 4 times with only MeOH (20 mL). Therefore, the particles where agitated in the solvent at 60 °C for 3 min under N2 and let be cooled down afterwards.

The particles and **NDA-C_{11}-NH_2 (7)** (200 mg, 0.53 mmol) was dispersed/dissolved in a mixture of DMF (4 mL), $CHCl_3$ (7.5 mL) and MeOH (9 mL). This was then sonicated for 1 h, agitated overnight and again

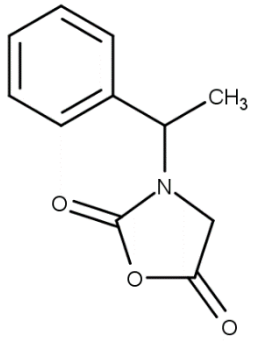
sonicated for 2 h and extracted 3 times with Hexane (20 mL). The solvents where evaporated except the DMF and the particles precipitated with diethyl ether (15 mL). Next the particles and **NDA-C_{11}-NH_2 (7)** (184 mg, 0.48 mmol) were again dispersed/dissolved but this time only in DMF (5 mL) and sonicated for 1 h under N_2. 25 ml of MeOH where added and washed with hexane (30 mL) three times. After evaporation of the solvents the particles were dried on the lyophiliser.

The dried particles where resuspended in EtOH and precipitated with petrolether. (EtOH/petrolether: 1x 40 mL/40 mL, 2x 30 mL/30 mL, 2x 20 mL/20 mL). The particles were then dispersed in ddH_2O and dried on the lyophiliser. A brown solid (75.5 mg) could be gained.

3.7. Synthesis of Monomers

3.7.1. Synthesis of (S)-3-(1-phenylethyl)oxazolidine-2,5-dione (2PE-NCA) (8)

Synthesis of the monomer **2PE-NCA (8)** (Figure 23).

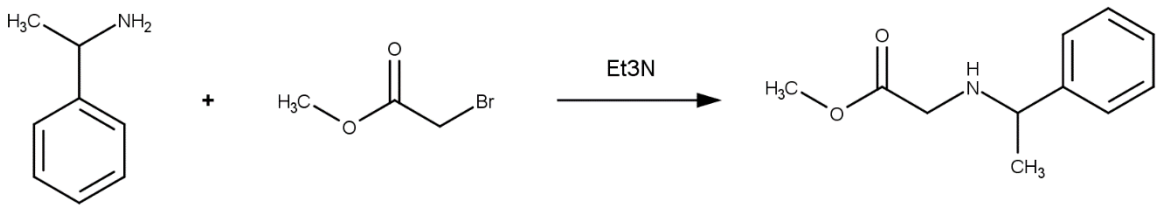


8

*Figure 23: Chemical structure of **2PE-NCA (8)***

1)

(S)-methylbenzylamine (9) (8.81 g, 72.70 mmol), **methyl bromacetate (10)** (13.34 g, 87.20 mmol) and trimethylamine (11.2 mL, 80.36 mmol) were added consecutively to ethylacetate (90 mL). The mixture was stirred overnight at 55 °C. The solution was cooled down to room temperature and washed two times with Milli-Q and one time with brine. $MgSO_4$ was then added to the organic phase, stirred for 5 min and filtered of. The solvent was removed with the rotavapor (40 °C) to get a clear oil **(11)** (12.62 g, 63.31 mmol). (Figure 24)

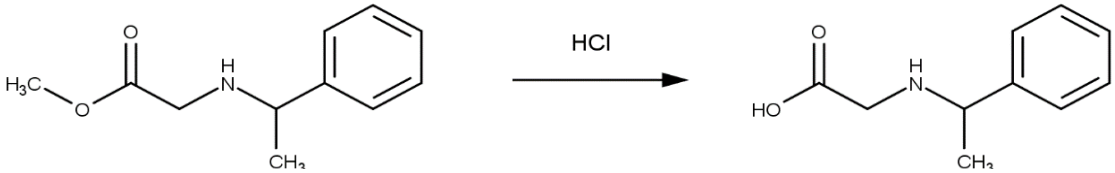


9 **10** **11**

*Figure 24: Synthesis of **11***

2)

11 (12.62 g, 65.31 mmol) was dissolved in HCl acid (4N, 325 ml, 1.25 mol) and stirred overnight at 55 °C. The HCl was removed with the rotavapor at high temperature (65 °C). The product was two times recrystallised in MeOH/diethyl ether to gain a brown-white solid **(12)** (12.18 g, 71.42 mmol). (Figure 25)

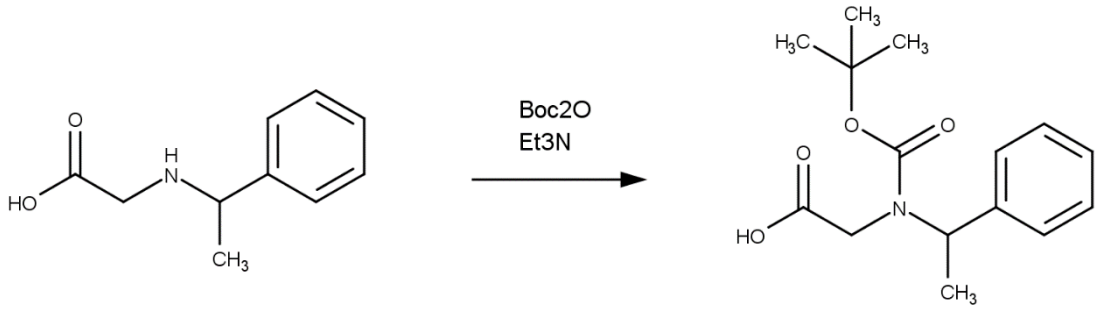


11 **12**

*Figure 25: Synthesis of **12***

3)

12 (12.18 g, 71.42 mmol), **Boc_2O (4)** (30.79 g, 141.08 mmol) and Et_3N (39.32 ml, 282.11 mmol) were added consecutively to Milli-Q H_20 (230 ml). The solution was stirred overnight at room temperature. The next day the solution was extracted two times with hexane (250 ml). HCl (4 N, 50 mL) was added to the aqueous phases. The phase was then extracted three times with ethyl acetate (60 ml) and the combined ethyl acetate phases washed with brine (250 mL). The organic phase was stirred with $MgSO_4$ for 10 min and filtered. The organic solvent was removed with the rotavapor (40 °C) and afforded a yellow oil **(13)** (5.22 g, 18.69 mmol). (Figure 26)

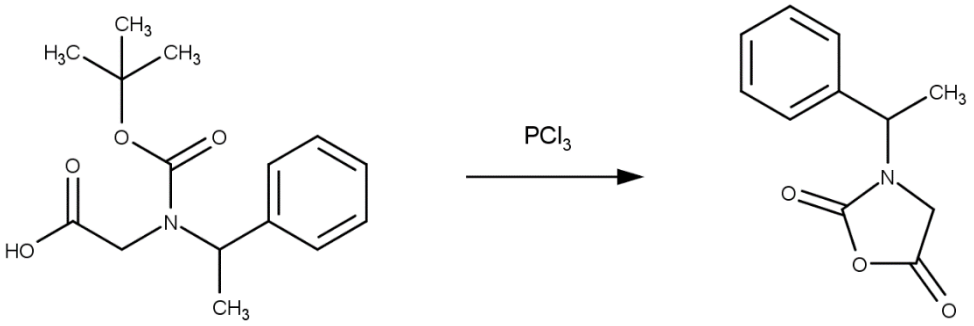


12 **13**

*Figure 26: Synthesis of **13***

4) End product/2PE-NCA (8)

13 (5.22 g, 18.69 mmol) was dissolved in 95 ml DCM under N_2. PCl_3 (1.97 ml, 22.52 mmol) was slowly added at 0 °C and the solution stirred for 2 h. After evaporation of the solvent, the product was dissolved again in DCM, sodium hydride was added and stirred for 30 min. After filtration the DCM was removed and the solid product recrystallised with DCM/hexane (-20 °C) and purified by sublimation. The end product were white crystals **(8)** (942 mg, 4.59 mmol). (Figure 27)

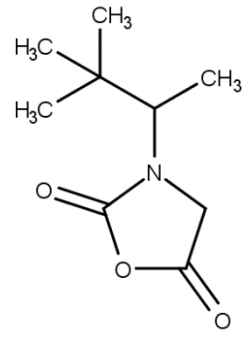


13 **8**

*Figure 27: Synthesis of **2PE (8)***

3.7.2. Synthesis of 1-(3,3-dimethylbutan-2-yl-2,4-dione) (tBu-NCA) (14)

Synthesis of the monomer **tBu-NCA (14)** (Figure 28).



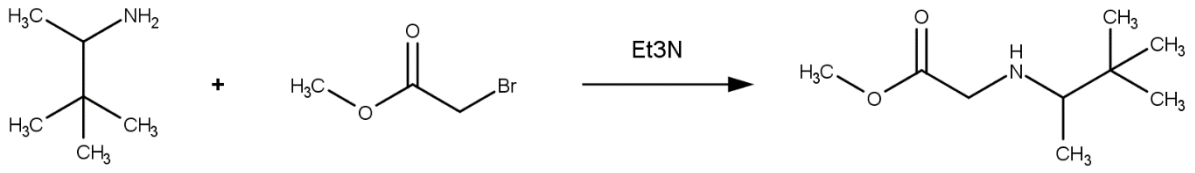
14

*Figure 28: Chemical structure of **tBu-NCA (14)***

1)

(S)-(+)-3,3-dimethyl-2-butylamine (15) (7.36 g, 72.70 mmol), **methyl bromacetate (10)** (13.34 g, 87.20 mmol) and Et_3N (11.2 mL, 80.36 mmol) were added consecutively to ethylacetate (90 mL). The mixture was stirred overnight at 55 °C.

The solution was cooled down to room temperature and washed two times with Milli-Q and one time with brine. $MgSO_4$ was then added to the organic phase, stirred for 5 min and filtered of. The solvent was removed with the rotavapor (40 °C) to gain a yellow oil **(16)** (11.97, 69.09 g). (Figure 29)



15 **10** **16**

Figure 29: Synthesis of 16

2)

16 (11.97, 69.09 g) was dissolved in HCl acid (4N, 337.5 ml) and stirred overnight at 55 °C. The HCl was removed with the rotavapor at high temperature (65 °C). The product was two times recrystallised in MeOH/diethyl ether to gain a white solid **(17)** (9.96 g, 58.16). (Figure 30)

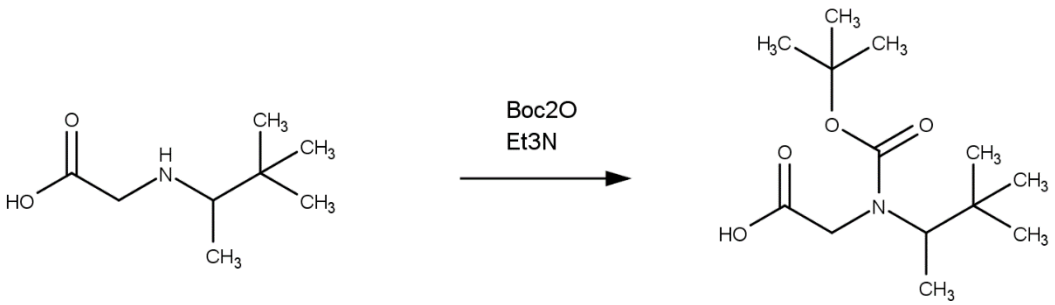
16 **17**

*Figure 30: Synthesis of **17***

3)

17 (9.96 g, 62.55), **Boc_2O (4)** (27.82 g, 127.47 mmol) and Et_3N (35 ml, 251.11) were added consecutively to Milli-Q (190 ml). The solution was stirred overnight at room temperature.

The next day the solution was extracted two times with hexane (260 ml). HCl (4 N, 50 mL) was added to the aqueous phases. The phase was then extracted three times with ethyl acetate (50 ml) and the combined ethyl acetate phases washed with brine (260 mL). The organic phase was stirred with MgSO4 for 10 min and filtered. The organic solvent was removed with the rotavapor (40 °C) and resulted in a yellow oil **(18)** (6.93, 26.72 mmol). (Figure 31)

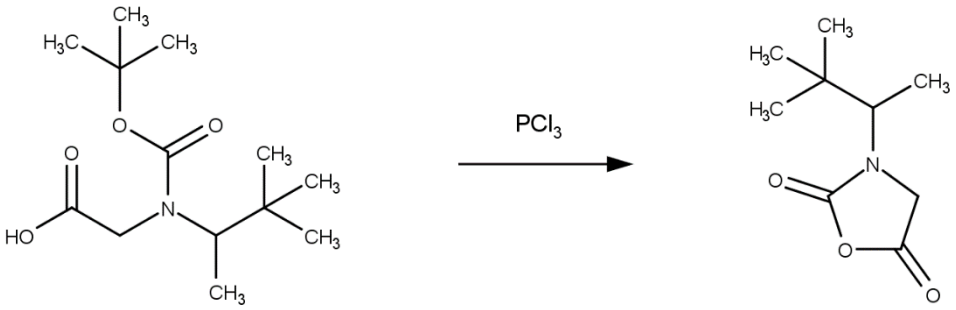


17 **18**

*Figure 31: Synthesis of **18***

4) End product/tBu-NCA (14)

18 (6.93, 26.72 mmol) was dissolved in DCM (95 ml) under nitrogen. PCl_3 (2.69 ml, 33.84 mmol) was slowly added at 0 °C and the solution stirred for 2 h. After evaporation of the solvent, the product was dissolved again in DCM, natriumhydrit was added and stirred for 30 min. After filtration the DCM was removed and the solid product recrystallised with DCM /hexane (-20 °C) and purified by sublimation, which resulted in white crystals (**tBu-NCA (14)**). (Figure 32)



18 **14**

*Figure 32: Synthesis of **tBu-NCA (14)***

3.8. Ring-opening Polymerisation with Benzyl- or Decylamine

All polymerisations where carried out in the glovebox. First the monomer was mixed with the solvent and warmed up until it was dissolved completely. Next a magnet stirrer and finally the initiator was added, and the polymerisation was stirred on a heating bloc under defined conditions (Table 2, Table 3). Polymerisation were carried out in sealable glass vials and the developed gas was released after 2 h, 6 h and thereafter once a day unless otherwise noted. Polymers were characterized by measuring with MALDI-TOF-MS.

Polymerisation of 2PE-NCA (8):

*Table 2: Overview of polymerisation with **2PE-NCA (8)***

	Monomer [mg]	Benzylamine [µl]	n-Decylamine [µl]	Toluene [mL]	Temperature [°C]	Time [day]
2PE-Poly-1	100	0.85		1.2	50	1
2PE-Poly-2	100	0.85	-	2	100	1
2PE-Poly-3	100	0.85		2	160	1
2PE-Poly-4	200	1.72	-	2.4	100	7, 11, 14*
2PE-Poly-5	200	-	3.13	2.4	100	7, 11, 14*
2PE-Poly-6**	100	-	1.56	1.15	100	1, 3, 6
*The gas was not released on day 5, 6, 12 and 13 **permeable cap (septum with needle), constant evaporation of the solvent						

Polymerisation of tBu-NCA (14):

*Table 3: Overview of polymerisations with **tBu-NCA (14)**, for both polymerisations 200 mg monomer and 2.7 mL toluene was used, a polymerisation temperature of 100 °C was used*

	benzylamine [µl]	n-decylamine [µl]	Time [day]
tBu-Poly-1	1.67		1, 7, 11, 14
tBu-Poly-2		3.04	7, 11, 14

3.9. Ring-opening Polymerisation on FeOx-NPs with NDA (2)

Glass vials with a septum were first charged with FeOx-NPs-NDA. Inside a glovebox the monomer was dissolved in the solvent (DMF or NMP) and added to the FeOx-NPs-NDA. NMP was added to the samples with DMF. Finally, a magnetic stirrer was put in each glass vial, a syringe was put through the septum in the cap and the samples were placed in a heating box at 100 °C for four days. (Table 4)

Table 4: Overview of polymerisation on FeOx-NPs-NDA, 8 mg FeOx-NPs-NDA were used for each polymerisation

Name	2PE-NCA (8) [mg]	tBu-NCA (14) [mg]	DMF [mL]	NMP [mL]
NP-PE-1	40	-	0.5	0.05
NP-PE-2	40	-		0.5
NP-tBu-1		40	0.5	0.05
NP-tBu-2		40		0.5

The particles were precipitated with diethyl ether and a magnet was used to collect the particles on the bottom. The particles were then dispersed in MeOH and precipitated in diethyl ether two times and dried on the lyophiliser.

The CSNP were characterised with TGA.

3.10. Stepwise Polymerisation from a solid support

The polymerisation was carried out like described by Figliozzi et al. [63] and Kirshenbaum et al. [64]. As a solid support rink amide resin (500 mg) was used and filled into a column with a filter.

The resin was swelled in DMF (10 ml) for 1-2 under agitating and drained. Piperidin (20% in DMF, 10 mL) was added, agitated for 1 min and drained. Again piperidin (20% in DMF, 10 mL) was added, this time agitated for 15 min and then drained and washed with DMF (6 x 5ml). Washing is defined by adding the solvent, agitating of 20 s and draining afterwards.

For the stepwise polymerisation several cycles had to be performed. For each cycle first **bromoacetic acid (10)** (1.2 M in DMF, 4.15 ml) was added and the column agitated at 37 °C for 40 min. After draining, the resin was washed with DMF (2x 5 mL) and DMSO (5 mL). Next **(S)-methylbenzylamine (9)** (1M in DMF, 4.25 mL) was added and again stirred at 37 °C for 40 min. After washing with DMF (4x 5 mL) the cycle is finished)

After polymerisation the polymer is split off the resin by stirring of the resin in trifluoroacetic (95 %, 25 mL). The resin was then washed again with trifluoroacetic (95 %, 5 mL). Both acid fractions where combined and the polymer precipitated by adding water (4°C). The polymer was centrifuged in a mixture of acetonitrile (20 mL) : water (20 mL) and the solvent was removed with a lyophiliser.

Resin polymerisation 1:

10 cycles were performed and the polymer was measured with MALDI-TOF-MS and CD.

Resin polymerisation 2:

DMF was treated with molecular sieve at least overnight and filtered shortly before usage.
(S)-methylbenzylamine (9) was treated with barium oxide for 3 days and purified with distillation at 52 °C.
62 cycles where performed and the polymer measured with MALDI-TOF-MS and CD.

4. RESULTS AND DISCUSSION

4.1. Synthesis of FeOx-NPs

The synthesis route of size-controlled monodisperse FeOx-NPs used in this thesis was established by Andrea Lassenberger [16] and based on the method of Park et al [22]. The synthesis is based on the thermal decomposition of iron pentacarbonyl. Thermal decomposition is the breakdown of metal carbonates when heated strongly. In the first phase an iron oleate complex is formed by displacement of the carbonyl group with oleic acid and a polyiron oxo precursor is formed by thermal decomposition supplying growth sides. A second heat-up lag phase results in burst nucleation at supersaturation and in the growth of the particles. The addition of monomers and therefore the growth is controlled by surfactants like oleic acid. Therefore the size of the particles is determined by the ratio of oleic acid and iron pentacarbonyl. The size can be controlled in a range of 1 nm between a minimum and maximum diameter of 3.5 and 11.5 nm and was calculated by using the software PEBBLES [67].

After the synthesis the particles were washed with EtOH and toluene/EtOH to remove the bigger part of excess of oleic acid. The particles were not dried but were stored wet with EtOH to prevent aggregation.

Iron oxide NPs 1: Size 6.2 nm (standard deviation: 6.9%) (Figure 33)

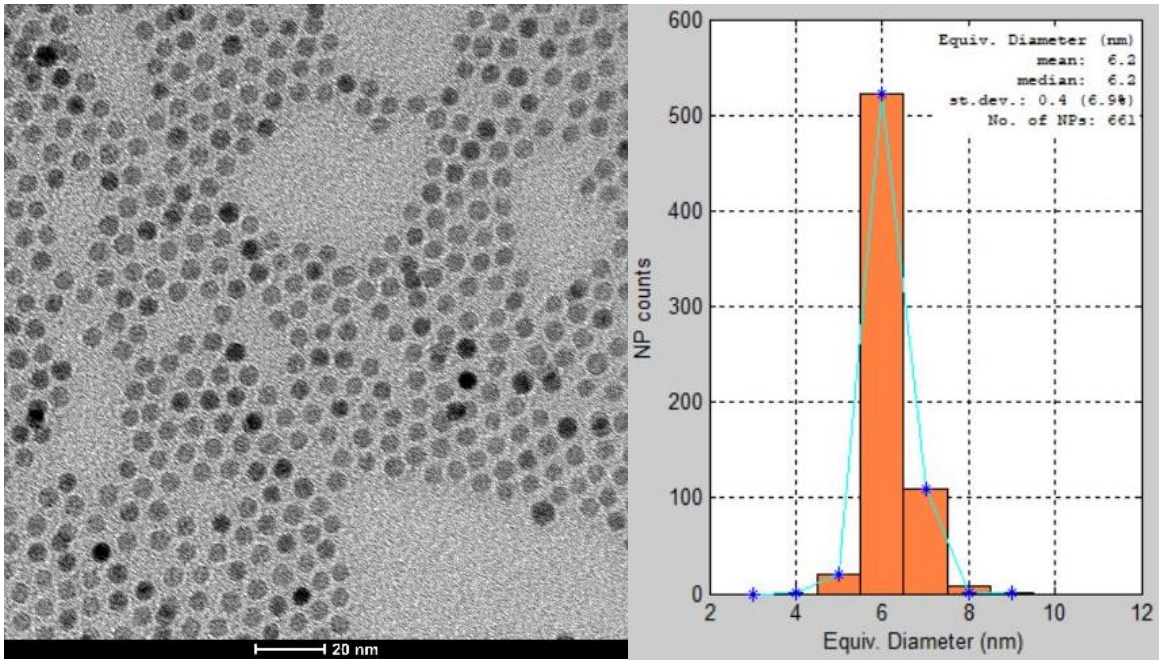


Figure 33: TEM picture and size histogram of iron oxide NPs 1

Iron oxide NPs 2: Size 5.9 nm (standard deviation: 11.6%) (Figure 34**)**

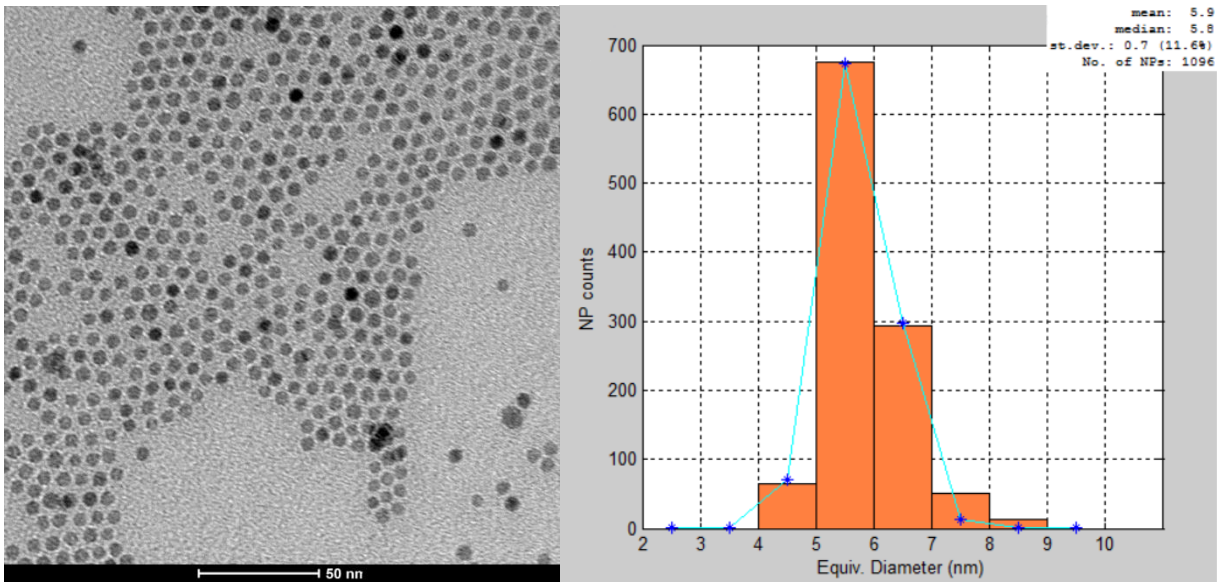


Figure 34: TEM picture and size histogram of iron oxide NPs 2

TEM pictures showed that monodisperse and monocrystalline OA stabilised FeOx-NPs could be synthesised.

4.2. Synthesis of NDA (2)

NDA (2) was synthesised after a modified protocol of our group [12,17] based on Napolitano et al. [68]. Napolitano et al. described a method for the nitration of dopamine HCl with the help of sodium nitrite under acidic conditions [68] based on a procedure for nitration of catecholic compounds [69].

The synthesis starts to foam when the acid is added. By cooling down the synthesis, adding the acid really slowly and by constantly flushing the synthesis with N_2 foaming can be minimised. A change of colour from brown to yellow and no brown vapour in the flask indicate a successful start of the synthesis and an oxygen free atmosphere. The synthesis and the finished product were covered with aluminium foil to protect them against damage from light. For purification the synthesis and the solvents were cooled down to 4 °C to reduce the solubility of **NDA (2)** in Milli-Qwater and diethyl ether.

NMR: (δ in DMSO-d6, ppm), 7.47 (s, 1H, Ph), 6.80 (s, 1H, Ph), 3.06 (s, 4H, CH_2)

4.3. Preparation of FeOx-NPs with NDA (2)

The goal was to produce particles in high quality that means particles with a high grafting density, low amount of remaining rest product from the synthesis and low amount of free ligand. A major problem is the not completely removed oleic acid that leads to an overestimation of the grafting density. A second problem is unbound **NDA (2)** that also leads to an overestimation of the grafting density and also acts as an initiator for polymerisation in the bulk.

A theoretical grafting density of 2 – 3 molecules/nm is realistic and therefore this range should be reached. The FeOx-NPs where stored wet in EtOH. Therefore the amount of particles could not be defined by weighting them. For the synthesis 1 ml of iron pentacarbonyl was used and the product defined as one batch. The theoretical amount of particles and their total surface area was calculated by the used amount of iron and their size measured to determine the needed amount of ligand.

For the ligand exchange of oleic acid with NDA or ligands using NDA as an anchor, the FeOx-NPs have to be mixed with the ligand in a suitable solvent. Only sonication and no further chemicals are required to exchange the oleic acid with NDA or NDA anchored ligands, which have a higher binding affinity to iron oxide than oleic acid [16,17].

To produce FeOx-NPs-NDA a washing protocol was implemented, based on an already existing protocol of our group. A similar protocol by Oliver Bixner had been developed for purification of palmityl-NDA grafted particles [17]. However, for **NDA (2)** the protocol had to be modified and the repetition of washing steps had to be increased drastically. Extensive washing steps with different solvents and a combination of solvents like toluene/EtOH, MeOH, DMF/$CHCL_3$/MeOH, DMF/n-Hexane and acetone were used to remove the oelic acid and unbound ligand. The washing step with acetone is problematic since the solution stays dark brown after the precipitation of the particles, which indicates that still a high amount of particles is dispersed and therefore disposed with the supernatant, leading to a lower yield. Since only small amounts of particles are needed but in a high quality this step was carried out nevertheless. The particles where washed with MeOH (60 °C and RT) until the solution in the end was only light yellow.

NDA (2) is only a small molecule and not able to act as a spacer to stabilise the particles. Dispersion in different solvents showed that the particles had aggregated.

Attempts to disperse FeOx-NDA NPs were tried to be dispersed in acetonitrile, acrylate PEG, Et_3N, PMDETA, pyridine and DMSO (Figure 35) and furthermore, also in DMPU, DMF, DCM and MFP. No solvent was able to disperse the particles and the particles could be collected on the bottom with a magnet. In DMSO the solution stayed light brown/orange. Nevertheless, a test with **NDA (2)** showed

that **NDA (2)** alone in DMF has a dark orange colour while in DMPU only a yellow one. This test shows that **NDA (2)** in DMSO has a more intense colour than in other solvents and maybe leads to an overestimation of the dispersity of FeOx-NDA NPs. (Figure 36)

Neither with sonication nor with heating up to 90 °C the particles could be dispersed.

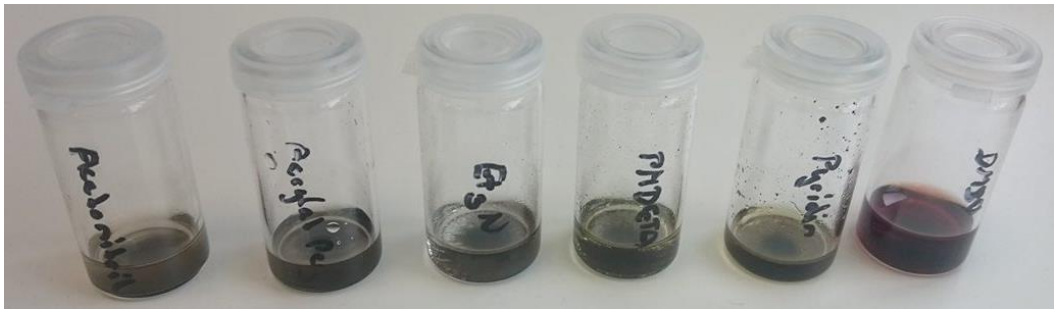


Figure 35: FeOx-NDA NPs in acetonitrile, acrylate PEG, Et3N, PMDETA, pyridinin, and DMSO.

*Figure 36: **NDA (2)** in DMPU, FeOx-NDA NPs in DMPU, **NDA (2)** in DMSO, FeOx-NDA NPs in DMSO.*

Iron oxide NPs 1 were used for the preparation of FeOx-NDA NPs. A grafting density of 2.6 molecules/nm^2 was measured (Figure 37), which meets the target range of 2-3 molecules/nm^2.

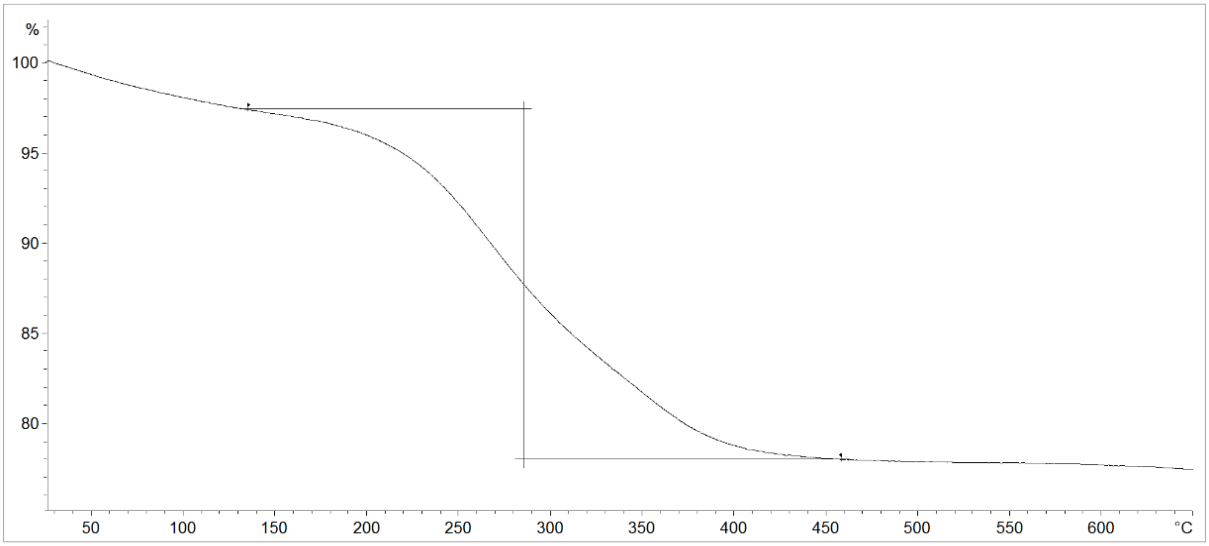


Figure 37: TGA analysis of FeOx-NDA NPs

Despite drying with lyophilisation the curve shows a mass loss between 25 and 130 °C that indicates that residues of solvents are still present. Also extended drying (1 week on the lyophiliser) did not lead to an improvement. Solvent residues of for example water or alcohols can serve as an initiator and lead to free polymer which means an additional consumption of monomer.

4.4. Synthesis of Nitrodopamin-C_{11}-NH_2 (7)

NDA-C_{11}-NH_2 (7) could be synthesised according to the protocol of Martina Schroffenegger like described in the experimental sections, which has not been published until now.

In this synthesis Boc2O is used as a protective group for the amine moiety of 11-aminodecanoic acid to link the 11-aminodecanoic acid with NDA. Recrystallization was used to gain an end product in good quality (Figure 38). **NDA-C_{11}-NH_2 (7)** was not completely dissolvable in ethyl acetate. Therefore, in addition to the original protocol, a recrystallization in MeOH/diethlyether was carried out.

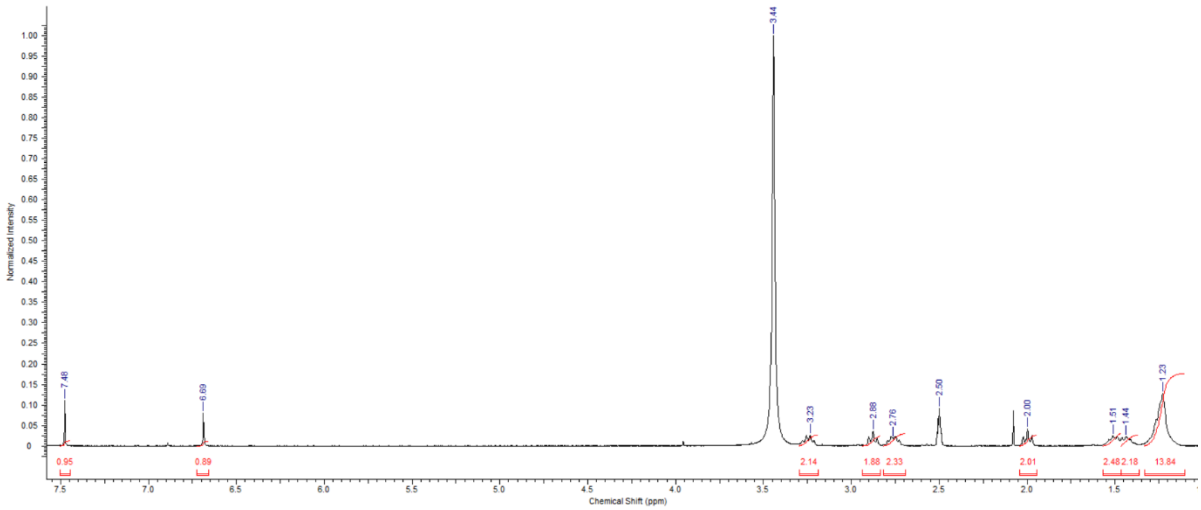


Figure 38: 1H NMR of NDA-C_{11}-NH_2 (7): (δ in DMSO-d6, ppm)

4.5. Preparation of FeOx-NPs with NDA-C_{11}-NH_2 (7)

First the particles were washed with toluene/EtOH to remove as much oleic acid as possible. The ligand exchange was then carried out similarly to the protocol for **NDA (2)** based on an already existing protocol of our group. A similar protocol by Oliver Bixner had been developed for purification of palmityl-NDA grafted particles [17]. Oleic acid was exchanged with the ligand NDA-C_{11}-NH_2, which has a

higher binding affinity to iron oxide than oleic acid, by mixing the FeOx-NPs with NDA-C_{11}-NH_2 and by using sonification.

For purification a method was used that was implemented by Andrea Lassenberger, PhD. for iron-oxide particles with PEG as a ligand. Therefore the particles were dispersed in EtOH and precipitated with petrol ether multiple times. Particles had a grafting density after purification of 1.9 molecules/cm^2. (Figure 39)

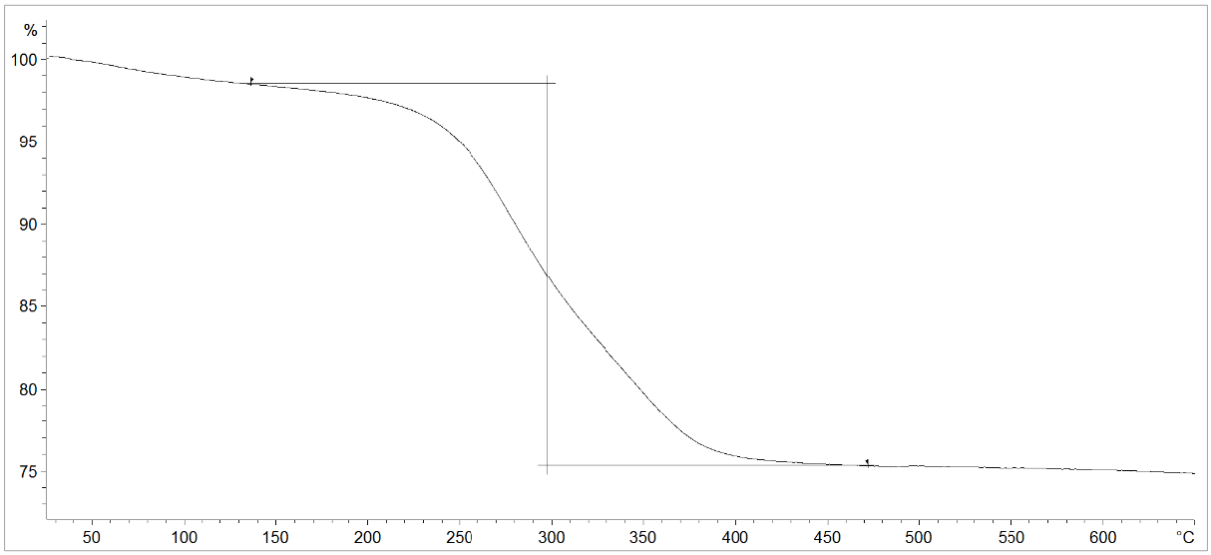


Figure 39: TGA of FeOx-NPs with NDA-C_{11}-NH_2

NDA-C_{11}-NH_2 (7) as a surface-bound initiator was used because of the longer carbon chain spacer compared to NDA. The idea was that in contrast to **NDA (2)** it could act as a spacer and stabilize the particles. However, the particles still agglomerated and could not be dispersed completely in DMSO.

NPs with **NDA-C_{11}-NH_2 (7)** are more time consuming to produce than NPs with **NDA (2)** and did not lead to a significant improvement in the ability to disperse the NPs. Therefore, polymerisations were later carried out with FeOx-NDA NPs.

Similarly to the FeOx-NDA also the TGA of these particles showed solvent impurities between 25 and 120 °C.

4.6. Synthesis of Monomers

The synthesis of the phenyl-NCA could be reproduced following the paper by Guo et al. [44]. The Monomers were synthesised from N-substituted glycine precursors using Boc_2O as a protection group and PCl_3 for ring closing (also see 1.7.2 Synthesis of Monomers and Ring opening polymerisation (ROP))

After addition of the protective group Boc_2O, HCl is added before the extraction with ethyl acetate to cause the product to pass over in the ethyl acetate phase. Following syntheses showed that it is important to add more acid than in the paper described until pH 1-2 is reached to get a higher yield. During ring closing of the monomer and further purification steps the synthesis must stay water and oxygen free the whole time to prevent polymerisation or destruction of the monomer.

Beside the already in the literature described **2PE-NCA (8)** (Figure 40) also the synthesis of **tBu-NCA (14)** (Figure 41) could be realised with the same method.

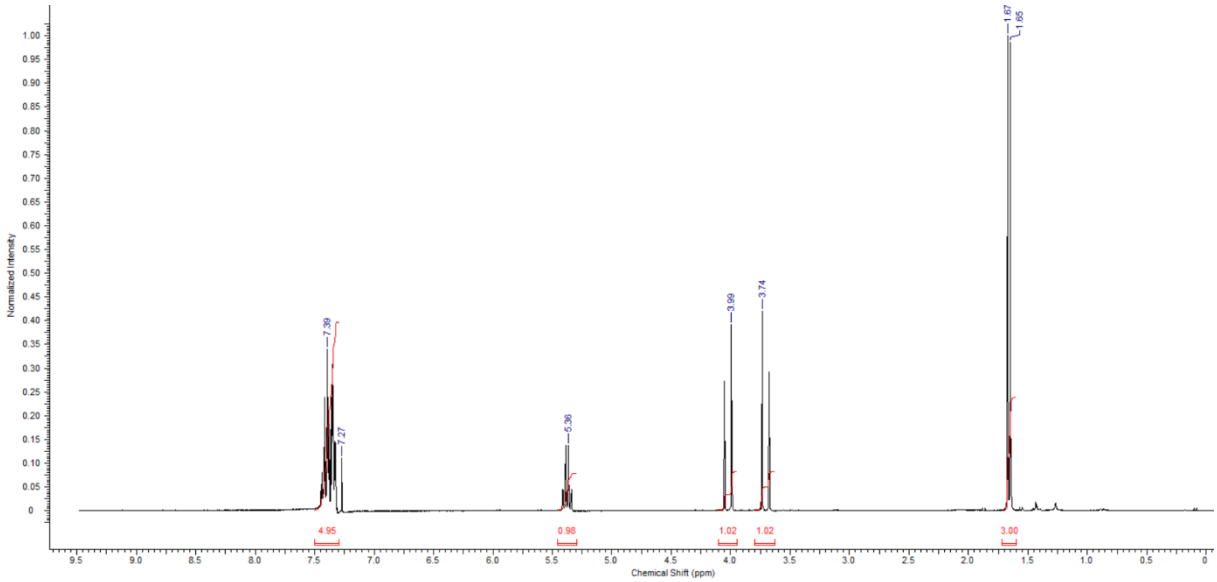


Figure 40: 1H NMR of 2PE-NCA (δ in CDCl3, ppm): 7.39 (m, 5H, Ph), 5.36 (q, 1H, CH), 3.99, 3.74 (dx2, 2H, CH_2), 1.65 (d, 3H, CH3)

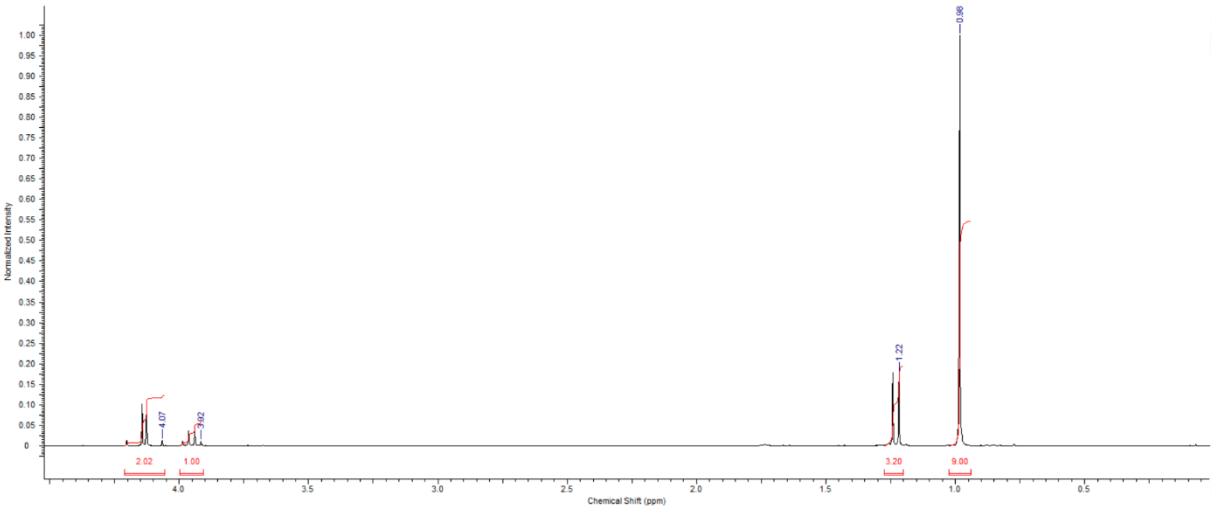


Figure 41: 1H NMR of tBu-NCA (δ in $CDCl_3$, ppm), 4.07 (dx2, 2H, CH_2), 3.92 (q, 1H, CH), 3.99, 1.22 (d, 3H, CH_3), 0.98 (s, 9H, 3xCH_3)

4.7. Ring-opening Polymerisation with Benzyl- and n-Decylamine as Initiator

First All polymers were synthesised as described in the experimental part and analysed with MALDI-TOF-MS. The polymerisation mechanism is based on the opening of the cyclic ring of the monomer, followed by its addition to the polymer chain (see 1.7.2 Synthesis of Monomers and Ring opening polymerisation (ROP)). Polymerisation of the polypeptoids in solutions with benzylamine and n-decylamine was investigated. Main series of MALDI-TOF-MS is defined as the series with the highest intensity. The code for the composition is given by BA for benzylamine and DA for n-decylamine, ()x-H for undefined number of monomers with NH as an end group and in the last position additional compounds like Na+, Li+ or H+ (example: BA-()x-H Li+).

4.7.1. Polymerisation of 2PE-NCA (8):

To find the best conditions for polymerisation of 2PE-NCA, different parameters (concentration of monomer in solution, initiator, temperature and reaction time) were investigated. An overview of the polymerisations are given in the following table (Table 5).

Table 5: Overview of polymerisation with 2PE-NCA (8)

	Monomer [mg]	Benzylamine [µl]	n-decylamine [µl]	Toluene [mL]	Temperature [°C]	MW (day of measurement) [kDA]
2PE-Poly-1	100	0.85		1.2	50	1 (1 d)
2PE-Poly-2	100	0.85	-	2	100	1 (1 d)
2PE-Poly-3	100	0.85		2	160	1 (1 d)
2PE-Poly-4	200	1.72	-	2.4	100	1.4 (7 d), 1.4 (11 d), 1.4 (14 d),
2PE-Poly-5	200	-	3.13	2.4	100	1.3 (7 d), 1.5 (11 d), 2.3 (14 d),
2PE-Poly-6	100	-	1.56	1.15	100	1.6 (1 d), 2.9 (3 d), 4.0 (6 d)

Influence of the temperature on ROP of 2 PE (2PE-Poly-1, 2PE-Poly-2 and 2PE-Poly-3)

MALDI-TOF-MS of 2PE-Poly-1 at 50 °C showed multiple series where no clear assignments were possible except BA()x-H H+ and a NMR conversion of the monomer to the polymer of 0 %. Also 2PE-Poly-3 under high temperature (160 °C) and pressure showed many series and no assignment was possible.

Therefore 100 °C was chosen as a suitable polymerisation temperature. MALDI-TOF-MS of 2PE-Poly-2 showed a main series of BA-()x-H Na+ and a molecular weight of 1 kDA after one day.

Initiator and Polymerisation Time (2PE-Poly-4, 2PE-Poly-5)

MALDI-TOF-MS of 2PE-Poly-4 and 2PE-Poly-5 showed a main series of BA-()x-H Li+ respectively DA-()x-H Li+. Decylamine seems to be the favourable initiator due to a higher molecular weight after 14 days (2.3 kDA) compared to the one initiated with benzylamine (1.4 kDA). In addition with benzylamine there is no further elongation of the polymer after 1 day.

Influence of the monomer concentration on ROP of 2 PE (2PE-Poly-6)

Solvent of 2PE-Poly-6 was evaporated during polymerisation until no solvent was left after 6 days. After 6 days a molecular weight of 4.0 kDa could be maintained (Figure 42), which shows that a high concentration of monomer in the solvent is important for a successful polymerisation. In comparison 2PE-Poly-5 only a molecular weight of 2.3 kDa after 14 days could be polymerised.

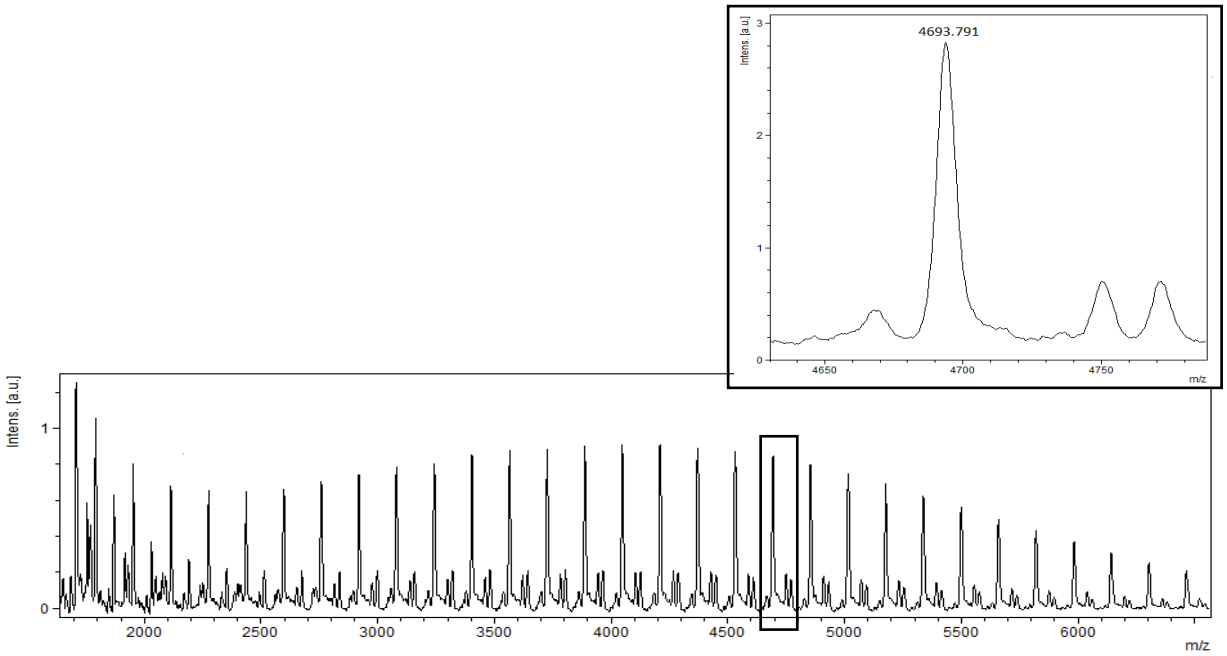


Figure 42: MALDI-TOF-MS of 2PE-Poly-6

Example 4693.791 m/z: Peak is part of the main series and shows the wanted polypeptoid. In this case the signal shows the polymer with n-decylamine as initiator, 28 units with NH as an end group and additional Na+ from the measurement.

Influence of the reaction time on ROP of 2 PE (2PE-Poly-1, 2PE-Poly-4, 2PE-Poly-5)

2PE-Poly-1 was carried out for 1 day and only a short polymer with 1 kDa could be gained compaired with 2PE-Poly-4 with 1.4 kDa after 7 days. Both reactions were initiated by benzyl amine and left under equal conditions. However, after 7 days no further elongation of the polymer could be detected. With n-decylamine as initiator like 2PE-Poly-5 an elongation of the polymer until at least 14 days could be monitored. Therefore, the reaction time should be scheduled for several days or even weeks.

For example, Kricheldorf et al. synthesised polypeptoids with methyl side groups with a molecular weight of ~4 kDa at 20 °C in 2 days [70]. Nevertheless, the monomers used in this thesis seemed to be more stable and more difficult to polymerise. Only polymers with low molecular weight (4 kDA) after a long reaction time (6 days) could be realised. A reason for this can be chain breaks due to transamination and the formation of cyclic polymers. Recently published papers indicate that the

stiffness of polypeptoids like 2PE seems to be problematic and that the formation of polymers with high molecular weight is not possible.

4.7.2. Polymerisation of tBu-NCA (14):

For the polymerisations of tBu-NCA two initiators and different reactions times were tested. An overview of the polymerisations are given in the following table (Table 6).

*Table 6: Overview of polymerisations with **tBu-NCA (14)**, for both polymerisations 200 mg monomer and 2.7 mL toluene was used, a polymerisation temperature of 100 °C was used.*

		benzylamine [µl]	n-decylamine [µl]	Time [day]
tBu-Poly-1	10	1.67		1, 7, 11, 14
tBu-Poly-2	11		3.04	7, 11, 14

Similar to 2PE-NCA (8) also for tBu-NCA (14) the reaction velocity is very slow. After 1 day no polymer could be precipitated out of a sample of tBu-Poly-1. Furthermore, no linear polymer could be gained either by benzylamine or n-decylamine. Main series of MALDI-TOF-MS of tBu-Poly-1 and tBu-Poly-2 indicates the possibility of the formation of macrocycles (Figure 43).

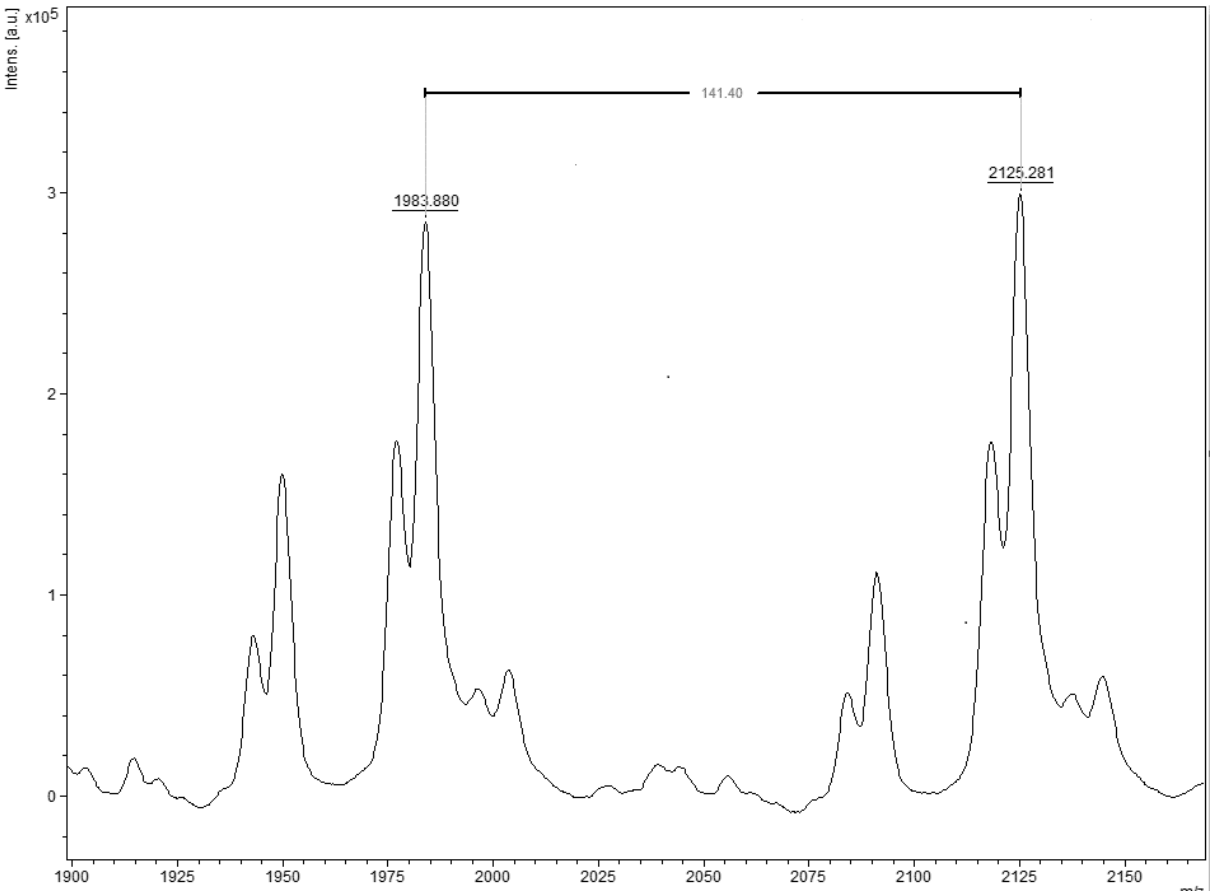


Figure 43: MALDI-TOF-MS of tBu-Poly-1

Example 1983.880 m/z: Peak is part of the main series in shows the possibility of macrocycles. In this case the signal shows the polymer with 14 units and additional Li+ from the measurement.

4.8. Ring-opening polymerisation on FeOx-Nps with NDA (2)

While the particles seemed to start to disperse during polymerisation in DMSO (already a visible change half an hour after the start of the polymerisation), the particles in NMP could still be easily precipitated with a magnet after polymerisation.

All four samples showed no significant increase of organic content in TGA compared to the initiator particles (max. increase of ~5 %). This indicated that the polymerisation was not successful. The reason could be general problems of polymerisation of 2PE with ROP combined with additional difficulties connected with using FeOx-NDA like limitation of solvents. In DMSO the particles are at least partly dispersible (light brown solution but still particles on the bottom) and the dispersibility seems to increase during polymerisation. Nevertheless, DMSO seems to be problematic when using ROP and seems to initiate side reactions. This was already showed with similar fabricated FeOx-NDA-NPs provided for previous research of our group, where particles grafted with polymethyl- and

polybutylglycin were generated. Only short polymers of a molecular weight of 2.1 – 2.5 kDa could be polymerised despite a calculated expected molecular weight of 20 kDA [35].

One reason therefore is that for polymerisation with benzyl and n-decylamine a wide range of solvents can be used like toluene, where at least polymers with low molecular weights can be polymerised. For dispersion of the initiator particles only a limited range of solvents like DMSO and MFD can be used.

4.9. Polymerisation from a Solid Support

Polymerisation from a solid support is a step-by-step polymerisation. Two syntheses were carried out with (S)-methylbenzylamine. One cycle consists of acetylation with bromoacetic acid followed by nucleophilic displacement of the bromide with a primary amine (≙ one monomer)

Resin Polymerisation 1:

MALDI-TOF-MS

MALDI-TOF-MS showed that nearly in each cycle one monomer was successfully added (peak 1630.039). Problematic is that it seems that in some steps ammonium was added instead of (S)-methylbenzylamine (peak 1422.067 and peak 1526.075). (Figure 44)

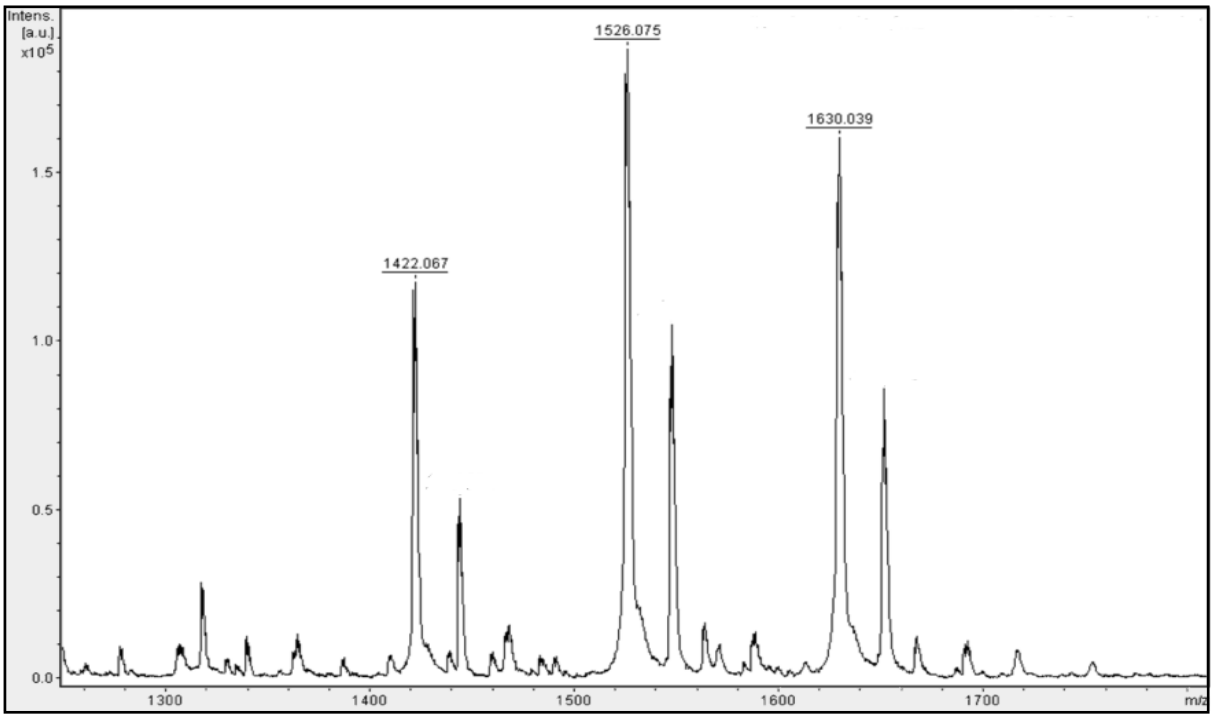


Figure 44: MALDI-TOF-MS of resin polymerisation 1

CD:

With CD measurements secondary structures can be distinguished due to their different absorption of left- and right-circular light. The differentiation results from the determination of the curve progression. The theoretical curve progressions of random coil, α-helix and β-sheet is shown in the introduction (Figure 15). In this case it was important to differ between random coils and the formation of α-helices. Measurement of the resin polymerisation 1 showed the successful formation of α-helices (Figure 45).

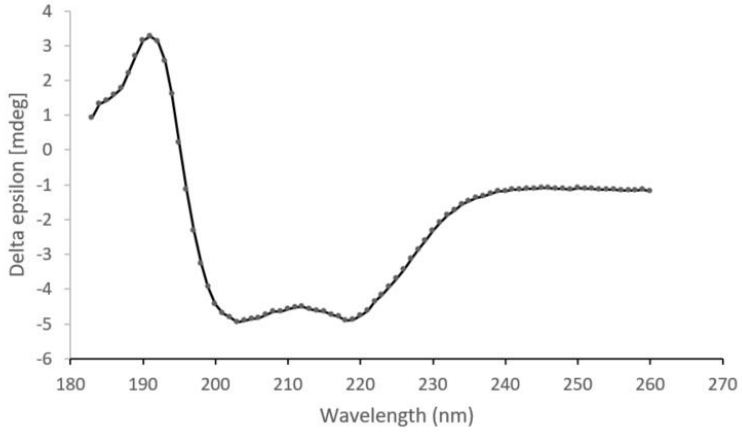


Figure 45: CD measurement of resin polymerisation 1.

Resin Polymerisation 2:

For the polymerisation (S)-methylbenzylamine was distilled and DMF was treated with molecular sieve to avoid insertion of ammonium instead of (S)-methylbenzylamine like in resin polymerisation 1. DMSO was not treated with molecular sieve because separation of solvent and molecular sieve with a syringe filter (cellulose, 0.2µm) was not possible anymore and the solvent stayed turbid.

MALDI-TOF-MS

MALDI-TOF-MS showed that one monomer was not added in every cycle. Like with ring-opening polymerisation it seemed to be problematic to synthesise longer polymers. Other reasons why in contrast to resin polymerisation 1 a monomer was not added in every cycle. (Figure 46)

It seems that compared to ROP also with the step-by-step polymerisation the polymerisation of polymers with high molecular weight is problematic. Again a reason for this could be chain breaks due

to transamination. Until now no successful synthesis of homopolymers of 2PE with high molecular weight have been described in literature.

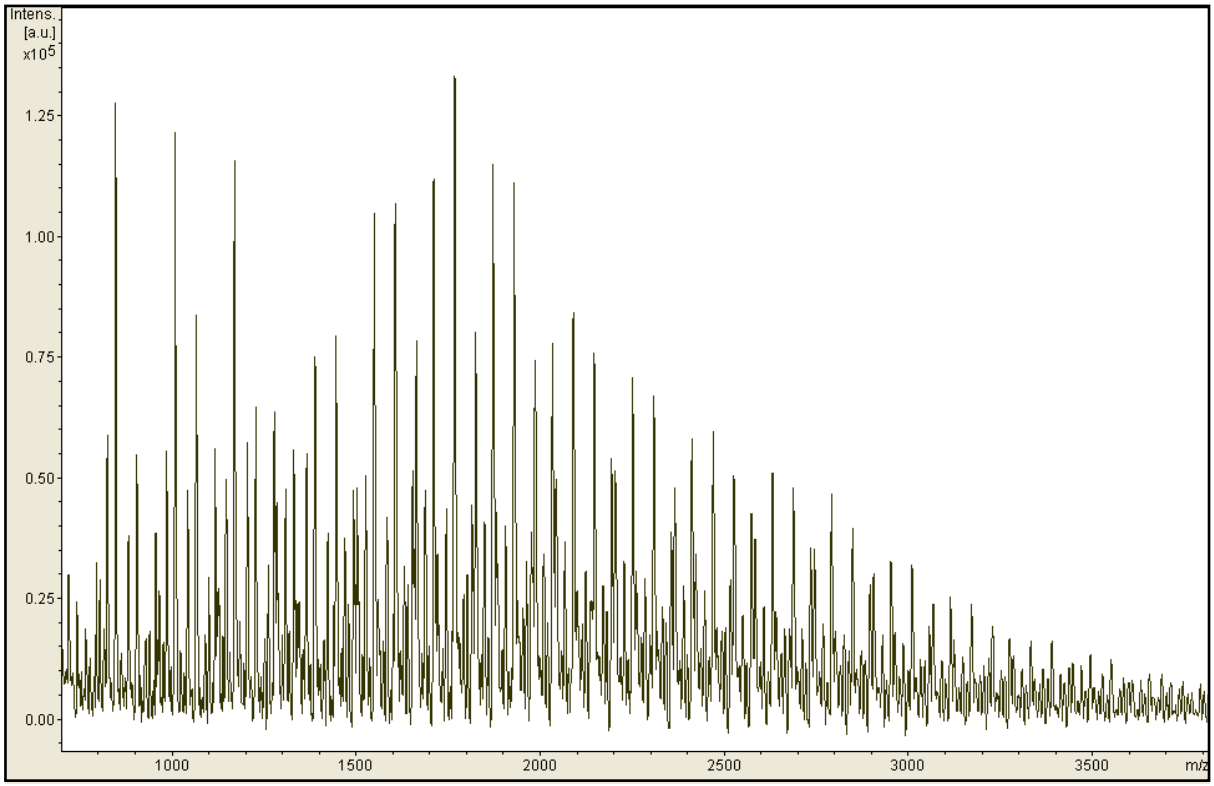


Figure 46: MALDI-TOF-MS of resin polymerisation 2

CD:

Measurement with CD showed that like resin polymerisation 1 also the curve progression of resin polymerisation 2 demonstrates the formation of α-helices (Figure 47).

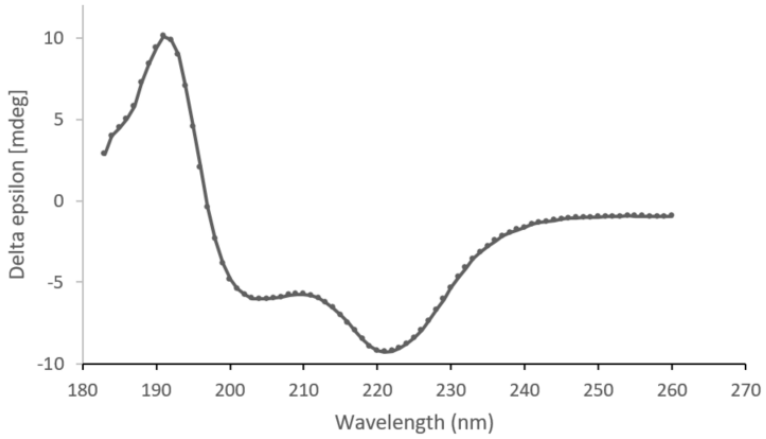


Figure 47: CD measurment of resin polymerisation 2

CONCLUSION AND OUTLOOK

Monodisperse size-controlled FeOx-NPs (~ 6 nm) stabilized by oleic acid could be synthesized. The well-researched dopamine derivate NDA was synthesised, the new derivate NDA-C_{11}-NH_2 could be realised and ligand exchange successfully performed for both ligands. Purification of FeOx-NPs modified with NDA was a known challenge critical to this work, since not fully removed oleic acid or unbound NDA is problematic in later polymerisation steps. By applying multiple washing steps, a grafting density of 2.6 molecules/nm^2, which meet the theoretically calculated target range of 2-3 molecules/nm^2, could be prepared. Therefore, the synthesis of monodisperse high-quality FeOx-NPs with initiator on the surface was realised.

Two methods were investigated for the polymerisation of polypeptoids: ROP and step-by-step polymerisation from a solid support.

First, suitable monomers were prepared for ROP. Although the yield was low, the syntheses of 2PE and tBu monomers with a high degree of purity were successfully realised. ROP proved itself to be a suitable polymerisation method for polypeptoids with simple sidechains. The minimum temperature at which a polymer could be synthesized was 90 °C. Furthermore, the polymerisation time had to be increased, compared to already in the literature described polymerisations of simple structured polypeptoids like *N*-methylglycine. Elongation of the polymer even after 11 days was observed. Furthermore, a higher concentration of the monomer in solution seems to promote an elongation of the polymer. Polymerisation of 2PE could be realised, although only with a low molecular weight (4 kDA).

The difficulty of synthesis of 2PE of higher molecular weight was also visible when using the step-by-step method. Polymerisations of 2PE of low molecular weight with only 10 circles showed that with each cycle one monomer could be added successfully. Nevertheless, also with this method the polymerisation of longer polymers seems to be problematic. The PDI is increasing with the number of cycles, indicating problems with the elongation of the polymer at high molecular weight or the breaks of the polymeric chains due to intramolecular transamination during the synthesis.

Despite the problematic polymerization with benzylamine and n-decylamine using ROP in the bulk, also polymerisation with FeOx-NPs-NDA particles was tested. Previous experiments with similar particles prepared for Barbara Pretzner showed that polypeptoids with a methyl- or butylgroup as a sidechain could be successfully polymerised from the surface, even if the low molecular weight, much lower than calculated, indicated difficulties [35]. As feared, the surface-initiated polymerisation of 2PE

and tBu could be not realised. One reason therefore is that for polymerisation with benzyl and n-decylamine a wide range of solvents can be used like toluene, where at least polymers with low molecular weights can be polymerised. For dispersion of the initiator particles only a limited range of solvents like DMSO and MFD can be used. However, these solvents seem to be problematic for ROP because they are suspected to initiate side reactions and to be responsible for the formation of cyclic polymers.

With ROP only homopolymers, blockpolymers/craft polymers, where different sorts of monomers are polymerised successively, or heteropolymers with a random sequence of monomers are possible. However, the synthesis of homopolymers with high molecular weight seems to be problematic with peptoids containing more complex stereochemical sidechains. Therefore the focus on heteropolymers seems to be more promising in the future. Sequence specific heteropolymers can be realised with the step-by-step polymerisation. Additional sequence-specific heteropolymers offer the possibility of more complex polymers with defined structures and properties, opening new perspectives in the application of polypeptoids.

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6. ABBREVIATIONS

2PE … N-(1-phenylethyl)glycine

2PE-NCA … 3-(1-phenylethyl)-1,3-oxazolidine-2,5-dione

APTES … (3-aminopropyl)triethoxysilane

CD … circular dichroism spectroscopy

CSNP … core-shell nanoparticles

DCM … dichloromethane

DMF … dimethylformamide

DMPU … N,N'-dimethylpropyleneurea

DMSO … dimethyl sulfoxide

Et_3N … triethylamine

EtOH … ethanol

FeOx-Nps … iron oxide nanoparticles

FeOx-NPs-NDA … iron oxide nanoparticles with nitrodopamine

GPTMS … (3-glycidyloxypropyl)trimethoxysilane

HEPES … 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

MALDI-TOF-MS … matrix assisted laser desorption/ionisation - time of flight - mass spectroscopy

MeOH … methanol

MFD … N-methylformamide

MPTMS … (3-mercaptopropyl)trimethoxysilane

MRI … magnetic resonance imaging

Nae … (2-aminoethyl)glycine

NCA … N-carboxyanhydrides

Nce … N-(2-carboxyethyl)

NDA … nitrodopamine

NMP … N-methyl-2-pyrrolidone

NMR … nuclear magnetic resonance spectroscopy

Npe … N-(1-phenylmethyl)glycine

PEG … polyethylene glycol

PMDETA … pentamethyldiethylenetriamine

ROP … ring opening polymerisation

SPION … superparamagnetic iron oxide nanoparticles

tBu … (3,3-dimethylbutan) glycine

tBu-NCA … 3-(3,3-dimethylbutan-2-yl)-1,3-oxazolidine-2,5-dione

TEM … transmission electron microscopy

TGA … thermogravimetric analysis