Controlled radical polymerisation in dispersed systems for biological applications

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Keywords:

Reversible deactivation radical polymerisation, dispersed phase polymerisation, polymerisation-induced self-assembly, emulsion polymerisation, miniemulsion polymerisation, biocompatible nanoparticles

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Abbreviations

AGET	Activators generated by electron transfer
AMPS	2-acrylamido-2-methylpropane sodium sulfonate
ASNS	L-asparaginase
ATRP	Atom transfer radical polymerisation
bFGF	Basic fibroblast growth factor
BMA	Butyl methacrylate
BODIPY	Boron dipyrromethene
BSA	Bovine serum albumin
CMP	Cobalt mediated polymerisation
ConA	Concanavalin A
CPT	Camptothecin
DAAm	Diacetone acrylamide
DEAm	Diethyl acrylamide
DEGMA	Di(ethylene glycol) methyl ether methacrylate
DLS	Dynamic light scattering
DMB	3,3'-dimethoxybenzidine
DNA	Deoxyribonucleic acid
DOX	Doxorubicin
DP	Degree of polymerisation
DTT	Dithiothreitol
EGDMA	Ethylene glycol dimethacrylate
EPR	Enhanced permeability and retention
Fluc	Firefly Luciferase
FRP	Free radical polymerisation
Gd-DOTA	Gd-tetra-azacyclododecatetraacetic acid
GFP	Green fluorescent protein
GMA	Glycidyl methacrylate
GOx	Glucose oxidase
GSH	Glutathione
HEA	2-Hydroxyethyl acrylate
HPMA	2-Hydroxy propyl methacrylate
HPMAm	*N*-(2-hydroxypropyl)methacrylamide
HRP	Horseradish peroxidase
IC50	Half maximal inhibitory concentration
MAA	Methacrylic acid
MEA	2-methoxyethyl acrylate
MMA	Methyl methacrylate
MPC	2-Methacryloyloxyethyl phosphoryl choline

MRI	Magnetic Resonance Imaging
NAM	*N*-acryloyl morpholine
NAT	*N*-acryloyl thiomorpholine
n-BA	*n*-butyl acrylate
NBMA	2-Nitrobenzyl methacrylate
NIPAM	*N*-isopropyl acrylamide
NMP	Nitroxide mediated polymerisation
PAA	Poly(acrylic acid)
PDi	Polydispersity index
PEG	Poly(ethylene glycol)
PEGMA	Poly(ethylene glycol) methacrylate
PEO	Poly(ethylene oxide)
PISA	Polymerisation-induced self-assembly
PS	Polystyrene
PVA	Poly(vinyl alcohol)
qDMAEMA	Quaternised dimethyl amino ethyl methacrylate
RAFT	Reversible-addition fragmentation chain-transfer polymerisation
RDRP	Reversible deactivation radical polymerisation
RITC-Dx	Rhodamine isothiocyanate dextran
Rluc	Renilla Luciferase
SAXS	Small angle X-ray scattering
SDS	Sodium dodecyl sulfate
SET-LRP	Single electron transfer- living radical polymerisation
siRNA	Small interfering RNA
t-BMA	*tert*-butyl methacrylate acrylate
TEM	Transmission electron microscopy
TERP	Organotellerium -mediated radical polymerisation
TFEA	Trifluoroethyl acrylate
VBA	Vinylbenzyl aldehyde

Abstract:

Polymeric nanoparticles show great promise in a range of biomedical applications, improving pharmacokinetic properties, dose requirements and immune response in drug delivery and bioimaging. Common synthesis techniques such as self-assembly, while prevalent, are unscalable and require the use of organic solvents, or extensive purification. In contrast, recent developments in dispersed state reversible deactivation radical polymerisation allow the preparation of well-defined nanomaterials in fully aqueous environments often achieving full monomer conversion, and thus direct use in biological environments without purification in high quantities. These techniques have allowed the preparation of a variety of nanoparticle architectures (nanogel, latex, micelle, nanoworms, vesicles), using ATRP, RAFT and NMP, which in many cases perform significantly better than free radical alternatives. This review focusses on the biological relevance of RDRP in dispersed systems, *covering miniemulsion, dispersion, suspension and emulsion polymerisations.*

1 Introduction

Over the past few decades, nanomaterials, specifically nanoparticles have become ubiquitous in modern biomedical research. There are now many examples of organic (polymeric, liposomal, protein)[1-3] and inorganic (metallic, quantum dots, silica)[4-6] nanoparticles which excel in applications such as cargo (drug/protein/nucleic acid)[7-9] delivery, bio-imaging[10] and diagnostics.[11] While to date their clinical use has been limited,[12, 13] 'nanomedicine' is an active field of research within the academic and industrial world, largely due to the versatility of materials available, and the advantageous properties they display. Their large volumes (relative to molecular drugs) are known to significantly increase circulation time[14] and encapsulate/protect a tremendous amount of pharmaceutic payloads.[15] Whereas their large surface area has been exploited to improve biocompatibility and disease targeting through conjugation of 'stealthy' polymers[16] and bio-active moieties respectively.[17] In particular nanoparticles have been studied for their potential use in cancer therapy, mostly driven by their ability to passively accumulate in leaky malignant tumour tissue, known as the enhanced permeability and retention (EPR) effect.[18, 19] Overall these properties can improve therapeutic efficacy, minimise clinical side effects while reducing the maximum dose requirements.

Out of the plethora of reported biomedical targeted nanoparticle systems, polymeric systems have shown great promise due to their chemical versatility.[20] For example, factors such as chemical functionality, charge, stimuli-responsivity and degradability can be easily introduced by tuning the monomer composition. Furthermore, a variety of polymeric nanoparticle architectures (self-assembled micelles,[21] nanoworms,[22] polymersomes,[23] latex particles,[24] branched polymers (hyperbranched[25] and dendrimers[26]) and nanogels[27]) can now be prepared with relative ease.

Perhaps the most prominent advancements in polymer nanoparticle design have arisen from developments surrounding reversible deactivation radical polymerisations (RDRP), either based on reversible deactivation (e.g atom transfer radical polymerisation (ATRP),[28] nitroxide mediated polymerisation (NMP)[29, 30] and single electron transfer-living radical polymerisation (SET-LRP)[31]) or degenerative chain transfer (e.g reversible addition fragmentation chain transfer (RAFT)) (**Figure 1**).[32] These methods have allowed researchers to prepare materials with controlled molecular weight distributions,[33-35] versatility in terms of block

architecture,[36] [37] and functional end-groups.[38] Since RDRP is well suited for preparing block copolymers, self-assembly has been the most popular method to prepare biologically relevant nanoparticles, as the ability to transform intricate polymer design into supramolecular structures is appealing from a design perspective.[39-41] Furthermore, the ability to control dispersity of a polymer chain population using these techniques is vital, as this is known to have a profound effect on the self-assembly properties of these materials. [42-44] These processes however suffer from scalability limitations, with suspensions above a few weight percent being difficult to achieve.[45] Furthermore, these nanomaterials may disassemble at low concentrations (such as those found endogenously), thus releasing their cargo prematurely.

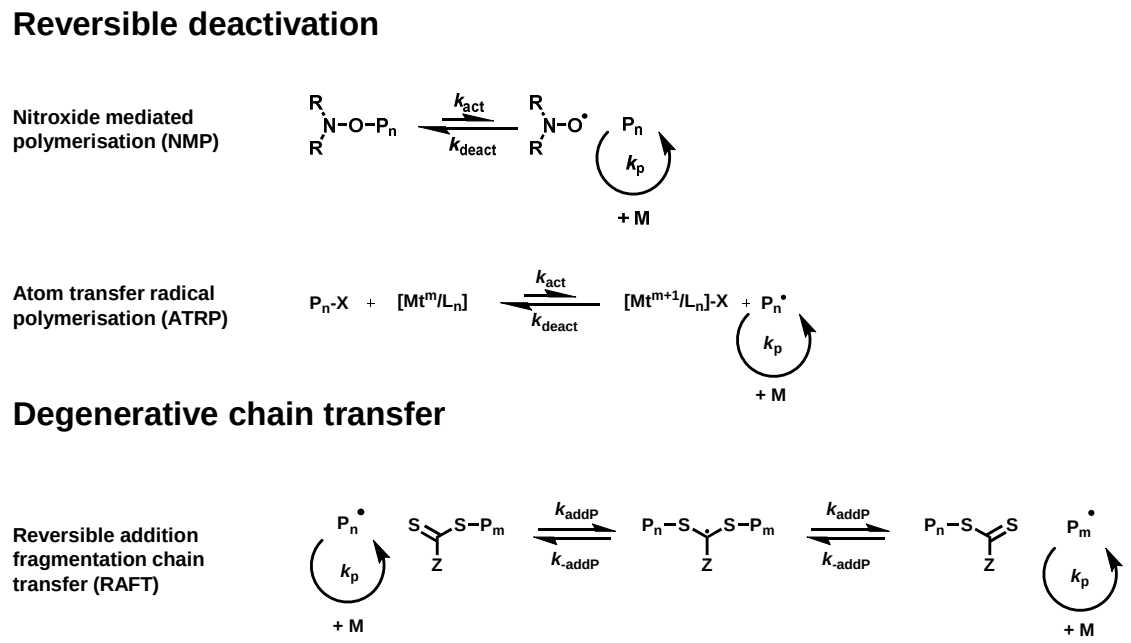

Figure 1 Mechanisms of nitroxide mediated polymerisation (NMP), atom transfer radical polymerisation (ATRP) and reversible addition-fragmentation chain transfer (RAFT).

Heterogeneous polymerisations (emulsion,[46] dispersion,[47] suspension,[48] miniemulsion[49] and inverse emulsion)[50] are now heavily exploited to generate polymer colloids reproducibly at large scales and with environmentally friendly conditions. In many, but not all of these systems, compartmentalisation effects (i.e segregation of propagating radicals) lead to fast propagation rates, full monomer conversion, low termination and therefore high molecular weight materials. However the multicomponent nature of many RDRP techniques means that these processes cannot be directly translated to heterogeneous systems.[51, 52] Nonetheless, this approach was initially achieved by Bon *et al.* who described the NMP of styrene in emulsion conditions in 1996.[53] However, until 2007, research on RDRP techniques in dispersed states remained fairly fundamental, of which there are many reviews on this topic.[52-61] Since overcoming many of the obstacles initially presented, much of the present work points to applying these to synthesise nanomaterials geared towards a range of applications.

With the increased use biomedical nanoparticles, and RDRP in dispersed states there are now many examples using these approaches to generate nanoparticles for these applications. In this review, different heterogeneous polymerisations will be critically analysed but from the perspective of preparing biologically relevant

nanomaterials. In each section a description of the heterogeneous system, examples from the literature, and analysis of the advantages and disadvantages each brings for biological applications will be given. Within this review we will focus solely reports which explicitly show to have been designed for biological applications, due to the already available reviews on their synthesis.

2 Miniemulsion polymerisation

In conventional miniemulsion polymerisations, a water immiscible monomer is emulsified with surfactant in an aqueous continuous phase using external shearing forces such as ultrasonication, resulting in kinetically stable, but thermodynamically unstable droplets (**Figure 2a**).[49] This can also be applied to a water in oil system - inverse miniemulsion polymerisation, in which the polymerisation occurs within aqueous monomer droplets dispersed in an oil continuous phase (**Figure 2b**).[50] In an ideal system, each monomer droplet is converted into a polymeric particle, and as these are effectively a mini-bulk/solution polymerisations, this removes the necessity for monomer diffusion through the aqueous phase (as in normal emulsion polymerisation). As such many RDRP (RAFT,[62-65] ATRP,[66-68] and NMP[69, 70]) miniemulsion polymerisations, either conventional or inverse, have been reported.

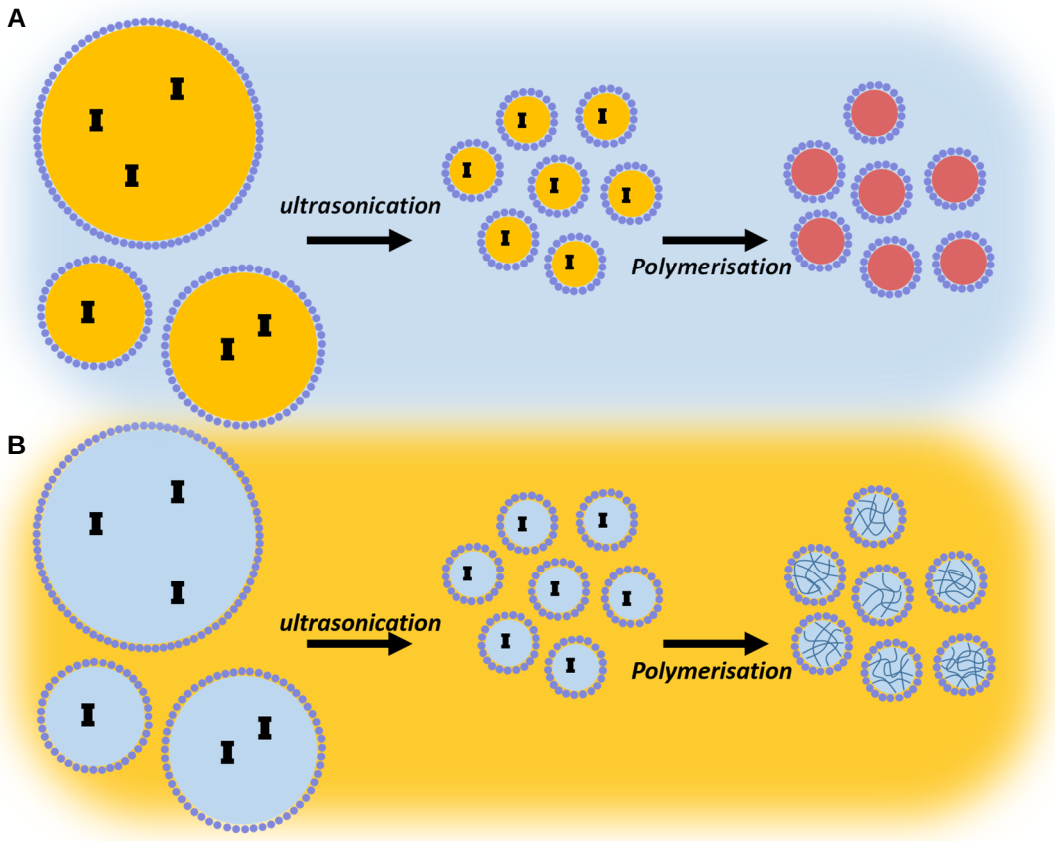

Figure 2 Schematic representation of (A) miniemulsion polymerisation and (B) inverse miniemulsion polymerisation. I = initiator species, yellow = oil/monomer phase, blue = aqueous phase, red = polymer phase.

2.1 Conventional Miniemulsion polymerisation

Conventional miniemulsion polymerisation occurs within sub-500 nm monomer droplets, stabilised by surfactant. This results in the formation of solid hydrophobic particles potentially capable of encapsulating hydrophobic drugs for controlled delivery. While some examples of ATRP and NMP miniemulsion polymerisation have been reported,[66, 67, 69-72] none are for biological purposes, with RAFT being the most favoured option for this application due to its compatibility with many monomer families, and is easily translated from traditional free radical systems, simply by addition of a suitable RAFT agent.

Introduction of glycosylated moieties at the particle surface has been shown to significantly improve disease targeting and cellular uptake, especially in immune cells.[73, 74] Yaacoub and co-workers reported the RAFT miniemulsion polymerisation of protected glucose and fructose methacrylates, with a subsequent chain extension with either methyl methacrylate (MMA) or butyl methacrylate (BMA).[75] Polymerisations were attempted with three different RAFT agents, with one yielding significantly narrow dispersities, as low as 1.10 and particle diameters between 150 and 350 nm. Nonetheless no biological characterisation (lectin binding, toxicity etc) were reported, possibly due to the large quantities of surfactant present in the polymerisation mixture.[75]

It is desirable to avoid conventional surfactants during nanoparticle synthesis, and such 'surfactant free' systems, which use amphiphilic macro-RAFT agent stabilisers are prevalent within this field. While not specifically for biological applications, Clavier and co-workers report on the RAFT miniemulsion copolymerisation of styrene and various boron-dipyrromethene (BODIPY) monomer types (styrenic, acrylate, methacrylate) to produce fluorescently labelled nanoparticles.[76, 77] This approach used a poly(ethylene oxide)-*b*-(acrylic acid) (PEO-*b*-PAA) macro-RAFT agent as the stabiliser and therefore avoids the use of conventional surfactants. Depending on the analogue of BODIPY monomer used in the polymerisations, some particles were up to 2000 times brighter than typical quantum dots, and as such could be useful in bioimaging applications.[76] It should be noted however that quantum dots are significantly smaller than the nanoparticles synthesised here (> 60 nm diameters), which would impact their circulation time.

An elegant approach which combines miniemulsion polymerisation with nanogel formation was described by Stenzel and co-workers using a P(PEGMA)-*b*-P(DMAEMA)-*b*-P(*t*-BMA) macro-RAFT agent stabiliser.[78] This was chain extended with miniemulsion polymerisation using *t*-BMA and crosslinked with different ethylene glycol dimethacrylate (EGDMA) amounts (10, 20 or 30%) resulting in nanoparticles with roughly 200 nm diameters. Hydrolysis of the tert-butyl methacrylate cores with trifluoroacetic acid yielded swollen P(MAA) nanogels with varying cross-linking densities. The anionic core was then used to encapsulate large quantities (50 wt%) of anticancer therapeutic doxorubicin with electrostatic interactions, also enabling almost quantitative drug loading efficiencies. In release studies, they found that addition of acid shrunk the P(MAA) core and swelled the P(DMAEMA) shell resulting in a gated squeezing effect releasing over 90% of the encapsulated drug. This translated into a lower IC_{50} concentration for the drug loaded nanoparticles compared to free doxorubicin, which is exceptionally difficult to achieve (**Figure 3**).[78]

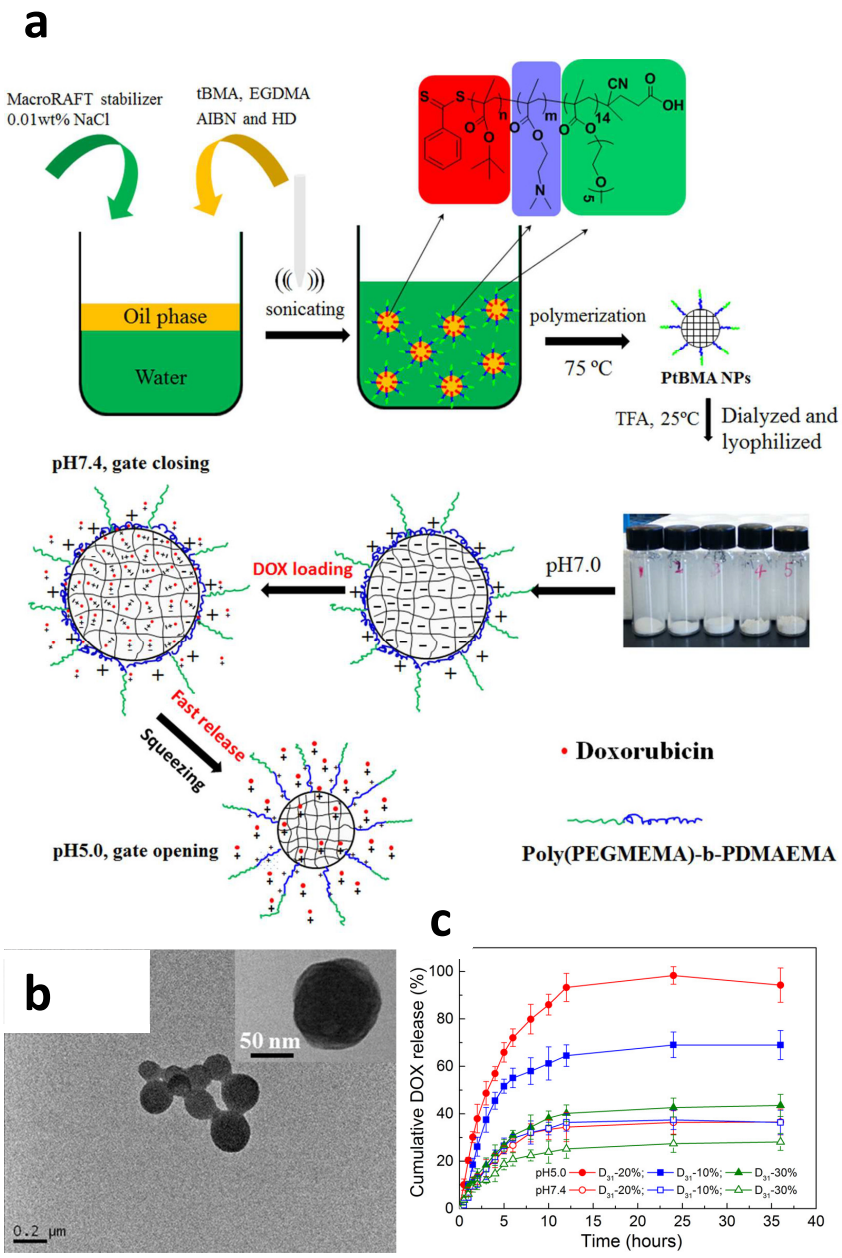

Figure 3 (A) Conventional miniemulsion polymerisation of *tert*-butyl methacrylate stabilised by a P(PEGMA)-*b*-P(DMAEMA)-*b*-P(*t*-BMA) macro-RAFT agent and subsequent hydrolysis with trifluoroacetic acid to produce pH gated nanogels. (B) TEM images of P(*t*-BMA) latexes and (C) release of loaded doxorubicin at pH 5.0 and 7.4.[78] Copyright 2010. Adapted with permission from the American Chemical Society.

The lack of RDRP miniemulsion polymerisation systems for biologically relevant nanoparticles may arise from rising popularity of other techniques yielding similar core-shell structures without the limitations of miniemulsion polymerisation. For instance, the requirement of high shearing limits the scalability of this process in comparison to polymerisation-induced self-assembly or conventional emulsion procedures (*vide infra*). Furthermore, the effect of molecular weight control may not influence any biological activity, as particle size and surface functionality are controlled by shearing and surfactant composition. This may then render FRP and RDRP latexes identical when using surfactant stabilised miniemulsion polymerisation, and therefore the added complexity of RDRP agents may be redundant. However, a key advantage of RDRP techniques here is the introduction of particular functionality at the particle surface, and the evasion of conventional surfactant use to improve potential biocompatibility.

2.2 Inverse miniemulsion polymerisation

Inverse miniemulsion polymerisation enables the preparation of fully hydrophilic nanoparticles, in which the polymer chains are cross-linked (covalent or non-covalent) in a network like structure giving them the term 'nanogels'. These are typically swollen when suspended in aqueous solutions, making their chemical structure accessible for potential degradation triggered by endogenous biochemical stimuli (e.g glutathione,[79] enzymes,[80] pH changes[81]). It should be noted that although these are conventionally called 'inverse emulsion polymerisation' in reality they are closer to an 'inverse miniemulsion polymerisation' as the monomer does not diffuse from large droplets to growing.

Many examples of nanogels synthesised with free-radical polymerisation exist in the literature, and are used mainly in from drug delivery,[82] diagnostics[83] and imaging[84]. However, their network-like structure in particular lends them towards encapsulation of biomacromolecules, such as proteins and nucleic acids by physical entrapment. RDRP techniques even allow for the distribution of cross-linking points thus a more homogenous gel structure directly impacting their biological performance. This has been extensively reported for ATRP and RAFT based nanogels.[85] Notably there are no NMP inverse miniemulsion polymerisations reported, and is likely a consequence of commercially available nitroxides being relatively water insoluble.

2.2.1 ATRP nanogels

ATRP relies on an external catalyst (typically a transition metal complex) to reversibly deactivate propagating radicals to a dormant state.[28] This reliance on a secondary species means that for efficient ATRP, the two components must be present in the same phases, without significant partitioning. For these reasons inverse miniemulsion polymerisations, which are simply compartmentalised aqueous polymerisations, are the most commonly used for heterogenous ATRP.

This was first reported by Matyjaszewski and co-workers who prepared 200 nm poly(poly(ethylene glycol) methyl ether methacrylate) (P(PEGMA)) nanogels cross-linked with a redox responsive disulphide functional dimethacrylate monomer, and were synthesised using a cyclohexane continuous phase.[68] They highlight that the sonication process intended for homogenisation also results in oxidation of the active Cu(I) species back to the Cu(II) complex. For that reason, the authors successfully implemented the activators generated by electron transfer (AGET) ATRP mechanism, using ascorbic acid as a water soluble reducing agent, alongside a PEG macromolecular ATRP (macro-ATRP) initiator to limit partitioning into the oil phase. They found that their ATRP 220 nm nanogels had better colloidal stability, almost double swelling ratios, and were degradable into individual polymer chains ($Đ < 1.5$), when compared to analogous nanogels prepared with free radical polymerisation.

In a follow up study, these nanogels were evaluated as potential anticancer agents with the encapsulation of up to 16.4 wt % doxorubicin, which released steadily after biodegradation with tripeptide glutathione.[86] The drug loaded nanogels gave reduced viability in *in vitro* studies against a cervical cancer cell line (HeLa). Further to this, they exploited the RDRP approach, and chain extended the initial nanogel with 2-hydroxyethyl acrylate

(HEA) to introduce hydroxyl functionality to the nanogel surface, and subsequently conjugated with bioactive moiety biotin.

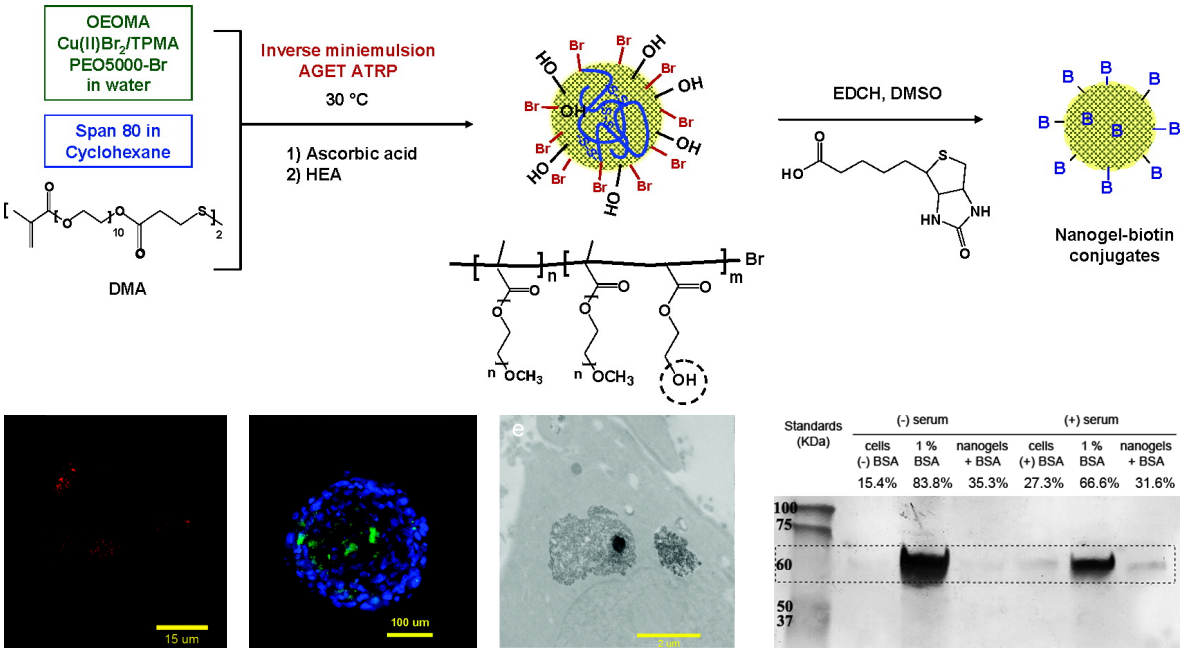

Figure 4 Typical ATRP inverse miniemulsion polymerisation to produce disulphide cross-linked nanogels. [68, 86] Copyright 2007 and 2009. Adapted with permission from the American Chemical Society.

As stated above, the ability to entrap hydrophilic objects within a nanogel matrix remains a major advantage. Matyjaszewski and co-workers have thoroughly exploited this property with their ATRP nanogel system. Initially they reported encapsulation of rhodamine modified dextran (RITC-Dx), as a model for carbohydrate therapeutics.[87] These were entrapped with over 80% efficiency, with a maximum loading of 6.4 wt %. Upon degradation with glutathione, the released dextran was able to bind strongly to Concanavalin A (ConA), and cause aggregation monitored by an increase in turbidity with UV-Vis spectroscopy. Analogous non-degradable RITC-Dx loaded nanogels were found to accumulate in clathrin coated pits (indicating uptake *via* clathrin mediated endocytosis) in murine osteoblast cells (MC3T3).[88] This was then further confirmed with TEM by encapsulating gold nanoparticles. By copolymerising PEGMA and an RGD (integrin binding peptide) functionalised monomer, an increased cellular internalisation of the nanogels was observed.[88] To evaluate if protein encapsulation was viable during such harsh preparation conditions (sonication, heating high radical flux), bovine serum albumin (BSA) was added into the aqueous polymerisation phase. Western-blot analysis of lysed cells which were treated with BSA-nanogels indicated that a large amount of the protein remained native after nanogel preparation (**Figure 4**).[88]

Instead of physical entrapment, an alternative approach to generate protein-nanogel hybrids was reported, whereby a green fluorescent protein (GFP) was genetically engineered such that a phenyl alanine bearing an ATRP initiator was introduced in a site specific manner.[89] This was used in conjunction with the traditional PEG-ATRP initiator described above to produce nanogels with covalently bound GFP. Confocal microscopy and fluorescence spectroscopy confirmed the incorporation of 2.1 wt% GFP into the nanogels. When compared to physical entrapment, the authors found that no GFP was retained after purification, highlighting the importance

of covalent attachment. This is somewhat contradictory to the identical BSA nanogels above which were readily retained after physical entrapment.[88, 89]

More recently this AGET ATRP inverse miniemulsion polymerisation approach was exploited to produce nanogels capable of encapsulating of nucleic acids. Similar systems have been shown to protect these macromolecules from nucleases, potentially enhancing gene delivery applications.[90] PEGMA was copolymerised with quarternised 2-(dimethylamino) ethyl methacrylate (qDMAEMA) as a cationic comonomer following the same inverse miniemulsion approach. The resulting nanogels had a larger hydrodynamic diameter (275 nm) than those without qDMAEMA and a strongly positive zetapotential (+43.7 mV) thus making it capable of complexing plasmid DNA. They found w/w ratios greater than 5:1 nanogel:pDNA was sufficient to bind plasmids, and heparin sulfate could be used to displace it below 10:1. In contrast, siRNA required a ratio of at least 15:1 nanogel:siRNA for full complexation and could be displaced up to 25:1. Nanogel polyplexes with pDNA encoding for firefly luciferase (FLuc), and silencing siRNA for Renilla luciferase (RLuc) showed comparable transfection and knockdown efficiencies to commercially available FuGENE-HD (**Figure 5**).[90]

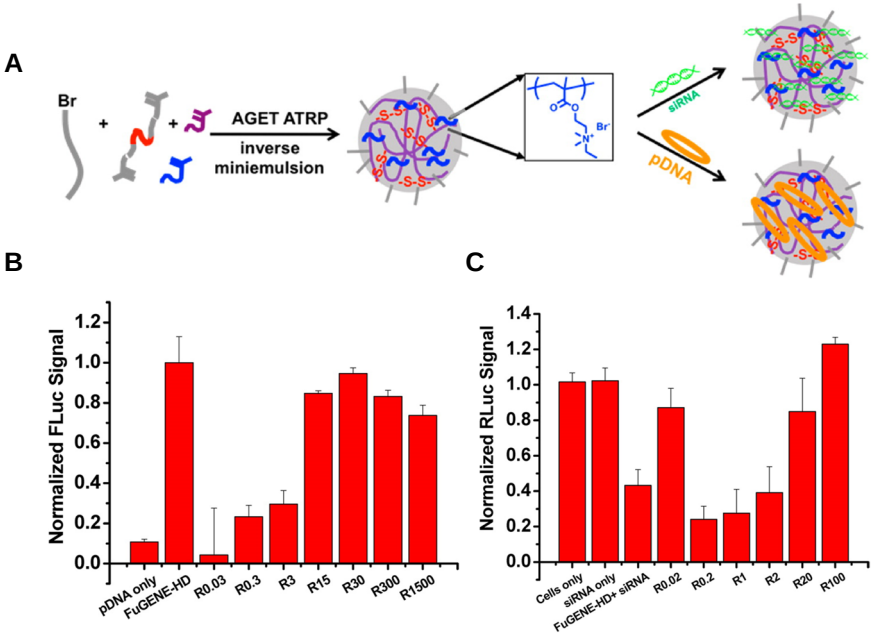

Figure 5 (A) ATRP inverse miniemulsion polymerisation of qDMAEMA to produce cationic nanogels capable of complex both siRNA and plasmid DNA. Relative transfection efficiency of a (B) plasmid containing a firefly luciferase reporter gene and (C) knockdown of Renilla luciferase with siRNA.[90] Copyright 2012. Adapted with permission from the American Chemical Society.

Hollinger and co-workers later used these cationic nanogels to complex siRNA which silences *Runx2* and *Osx*, key regulators of osteogenetic differentiation, as a potential treatment of heterotopic ossification (abnormal bone growth).[91] In general, similar silencing capabilities (40-60% reduction in gene expression) were observed between nanogels and commercial transfection agent lipofectamine, however no cytotoxic effects were evident after nanogel treatment whereas lipofectamine yielded 60% cell viability.[91]

2.2.2 RAFT nanogels

In contrast to ATRP, RAFT is a degenerative chain transfer process which does not typically require a catalyst, and involves a chain transfer agent (thiocarbonylthio compounds) and an external radical source. From a biological perspective, nanogels synthesised using RAFT may offer significant advantages over ATRP as the synthetic process is simpler, and the presence of excess copper complexes (the most common ATRP catalysts) can be cytotoxic.[92] While most biologically relevant RAFT nanogels are synthesised by precipitation/dispersion polymerisation of thermoresponsive polymers (e.g P(NIPAM)),[85] or soluble branched structures, there are a few notable examples using inverse miniemulsion procedures for biomedical applications.

As common RAFT agents are fairly hydrophobic, their applicability in fully aqueous polymerisation can be limited. This has been overcome by preparation of hydrophilic macro-RAFT agents to enhance water solubility. For instance, Davis and co-workers report on the synthesis of cationic DMAEMA based nanogels from a hydrophilic P(PEGMA) macro-RAFT agent, previously prepared in acetonitrile, for gene delivery applications.[93] Inverse miniemulsions were performed with and without macro-RAFT agent at 60°C, and cross-linked with a similar disulphide dimethacrylate. Interestingly only the RAFT inverse miniemulsion achieved over 98% monomer conversion in 6 h, whereas the analogous free radical analogous polymerisation was limited to 90% within the same time frame. Narrow dispersity polymers ($Đ < 1.3$), and consistent average molar masses were achieved with the RAFT system, however a small proportion of the macro-RAFT agent remained unconsumed. In contrast, the free radical polymerisation (FRP) nanogels consisted of much higher molecular weight polymers, however the particles had smaller diameters (~100 nm), compared to the RAFT nanogels (~250 nm), both with zetapotentials of +42 mV which is in agreement with the ATRP nanogels described above. This difference in size depending on polymerisation technique may have significant implications on their biological activity.[93]

Klok and co-workers have reported the synthesis of poly(*N*-(2-hydroxypropyl)methacrylamide) P((HPMAm)) nanogels using a similar approach.[94] P(HPMAm) is widely used in polymer therapeutics as it shows high biocompatibility, tuneable functionality, and is included in materials which have entered clinical trials after FDA approval.[95] In contrast to the above study, the authors use a solvent mixture of 9:1 H_2O:Methanol to aid solubility of their RAFT agent. A variety of RAFT P(HPMAm) nanogels were synthesised using different experimental conditions (DP, surfactant, initiator concentration, cross-linker concentration) as well as a free radical analogue cross-linked with a disulphide bridge. With high concentrations of initiator, quantitative monomer conversions were attained, yielding P(HPMAm) RAFT nanogels between 150-200 nm in diameter and dispersities around 1.4 for the component polymers. As a proof of concept protein encapsulation experiment, cytochrome C (5 wt%) was added to the aqueous phase with 73% incorporation. Release studies showed rapid protein release (100% within 1 h) only after disulphide reduction with a phosphine, with none observed without nanogel degradation (**Figure 6**).[94]

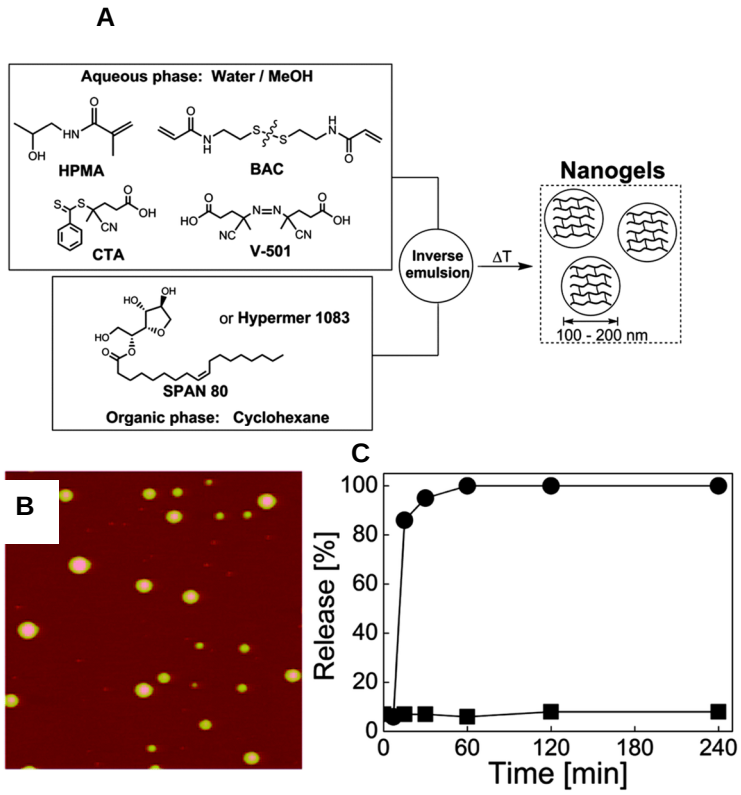

Figure 6 (A) Inverse RAFT miniemulsion polymerisation of HPMA to produce disulphide cross-linked nanogels. (B) Atomic force micrographs of the synthesised nanogels and (C) release of encapsulated cytochrome C with (cirlces) and without (squares) addition of Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) as a reducing agent.[94] Copyright 2013. Adapted with permission from the Royal Society of Chemistry.

2.2.3 General remarks on RDRP nanogels

As mentioned previously, inverse emulsion polymerisations are a highly favourable route to design fully hydrophilic nanoparticles for loading and transport of biomacromolecular cargo. However excess surfactant and the continuous phase (typically cyclohexane) must be removed prior to biological use due to inherent cytotoxicity or aqueous incompatibility. Surfactant use can be circumvented by utilising amphiphilic RDRP control agents (inistab),[96, 97] however with this it is impossible to independently tune the colloidal stability and the molecular weight of resulting polymers. The sonication procedures used are unfeasible in large volume reactors and the low monomer concentrations used limit their scale up potential. Nanoparticle size is a key parameter in a variety of biomedical applications, with nanoparticles with diameters below 200 nm reported being most effective.[98] From the above studies, it seems that RDRP inverse miniemulsion procedures are rarely able to produce nanoparticles below this threshold, potentially limiting clinical translation. Additionally the above RDRP nanogel assemblies use proteins such as BSA and cytochrome C, which are relatively tolerant to extreme conditions such as sonication, heating and solvents during purification. Therapeutic proteins under these conditions could easily denature thus rendering them inactive thus reducing efficacy. In general RDRP techniques have been shown to improve release rate and targeting potential through chain extension with functional moieties, however as of yet has not improved synthetic issues limiting their biological use.

3 Dispersion polymerisation

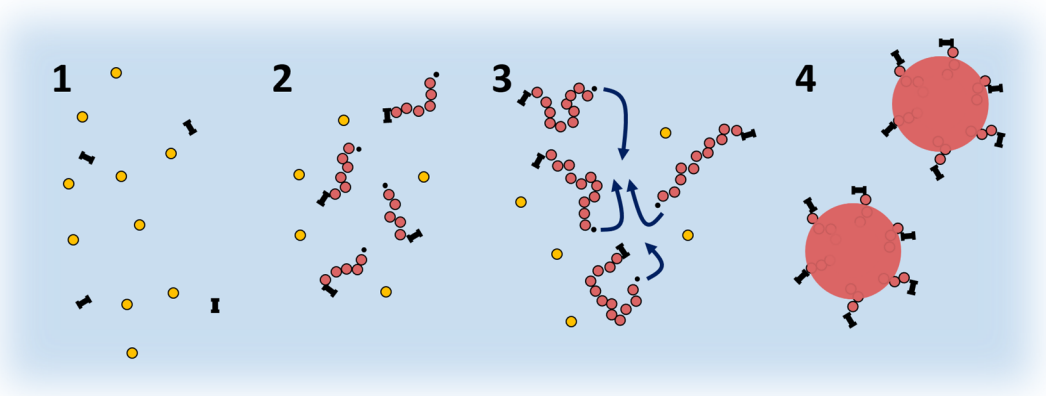

Figure 7 Mechanism of aqueous dispersion polymerisation. I = initiator species, yellow = monomer, blue = aqueous phase, red = polymer phase.

In contrast to other dispersed phase systems, in dispersion polymerisations all of the components (monomer, chain transfer agents, initiator) are soluble in the continuous phase at the start of the reaction.[47] The phase separation then occurs during the polymerisation, where the polymer chains reach a critical chain length then become insoluble in the continuous phase and precipitate resulting in particle formation (**Figure 7**). Common RDRP dispersion polymerisations have manifested themselves as a type of polymerisation-induced self-assembly (PISA), which is where a solvophilic unit is chain extended with a block which selectively precipitates leading to self-assembled nano-objects coated in the initial solvophilic block (**Figure 8**).[99] The resulting morphologies are then dependent on the solvophobic/solvophilic volume ratio, and are often similar to those found using conventional selective solvent self-assembly procedures.[100, 101] Typically PISA is performed by taking a single solvophilic homopolymer and throughout the elongation of the second solvophobic block, the morphology reorganises from spheres, to worms, to vesicles and in some cases to lamellae as the polymerisation ensues. The *in situ* self-assembly allows for a much higher solids contents (up to 40%) in comparison to conventional self-assembly however can be limited, as not all monomers display this phenomenon. Many RDRP dispersion polymerisations have been reported including NMP,[102] ATRP,[103] cobalt mediated polymerisation (CMP),[104] reversible complexation mediated polymerisation (RCMP)[105], organotellerium-mediated radical polymerisation (TERP),[106] and *via* 'non-living' addition fragmentation chain transfer (AFCT)[107, 108] polymerisation, however RAFT polymerisation remains the most popular approach and is the only method used for biologically relevant particles. Aspects such as particle morphology, particle size and core/shell compositions can be easily tuned using RAFT, and as such PISA is highly attractive from a biological standpoint either for fundamental studies or specific applications.[109]

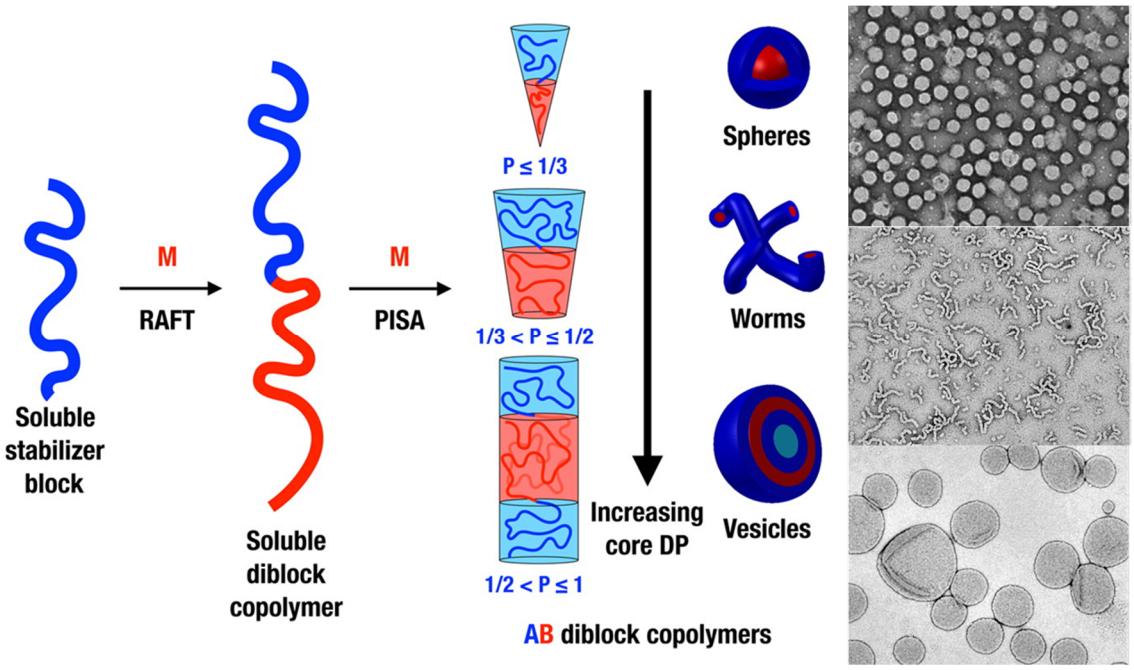

Figure 8 Generalised representation of RAFT polymerisation-induced self-assembly from a solvophilic macro-RAFT agent by chain extending with a monomer which upon polymerisation becomes solvophobic inducing self-assembly into spherical, worm and vesicle morphologies. [110] Copyright 2014. Adapted with permission from the American Chemical Society.

In particular, the aqueous compatibility of RAFT polymerisation is likely to be a key factor in its successful translation to dispersion polymerisation. Early developments of aqueous RAFT dispersion polymerisation were reported by Hawker and co-workers who described polymerisation of *N*-isopropylacrylamide to generate block copolymer micelles *in situ*.[111] Following this study, a number of different monomers, such as diethyl acrylamide (DEAm),[112] 2-methoxyethyl acrylate (MEA)[113, 114] and di(ethylene glycol) methyl ether methacrylate (DEGMA)[115] were also shown to be capable of aqueous dispersion polymerisations, usually utilising the thermoresponsive behaviour of their respective polymers (**Figure 10**). Nonetheless, the above systems were only capable of producing spherical systems, however over the past 8 years it is has been established that morphological controlled assemblies can easily be using other core forming monomers such as 2-hydroxy propyl methacrylate (HPMA),[101, 116] diacetone acrylamide (DAAm)[117-119] and NIPAM (**Figure 9**).[120] This is potentially useful for biomedical applications, as the formed nano-objects could be injected directly for therapy, bolstered by compartmentalisation effects resulting in a rapid increase in polymerisation rate and high monomer conversions. Furthermore the cost, abundance and high boiling point of water make this an attractive solvent from an industrial perspective potentially enabling scale up of aqueous dispersion polymerisations. Given this, a number of PISA formulations have been employed to generate biologically relevant nanoparticles.

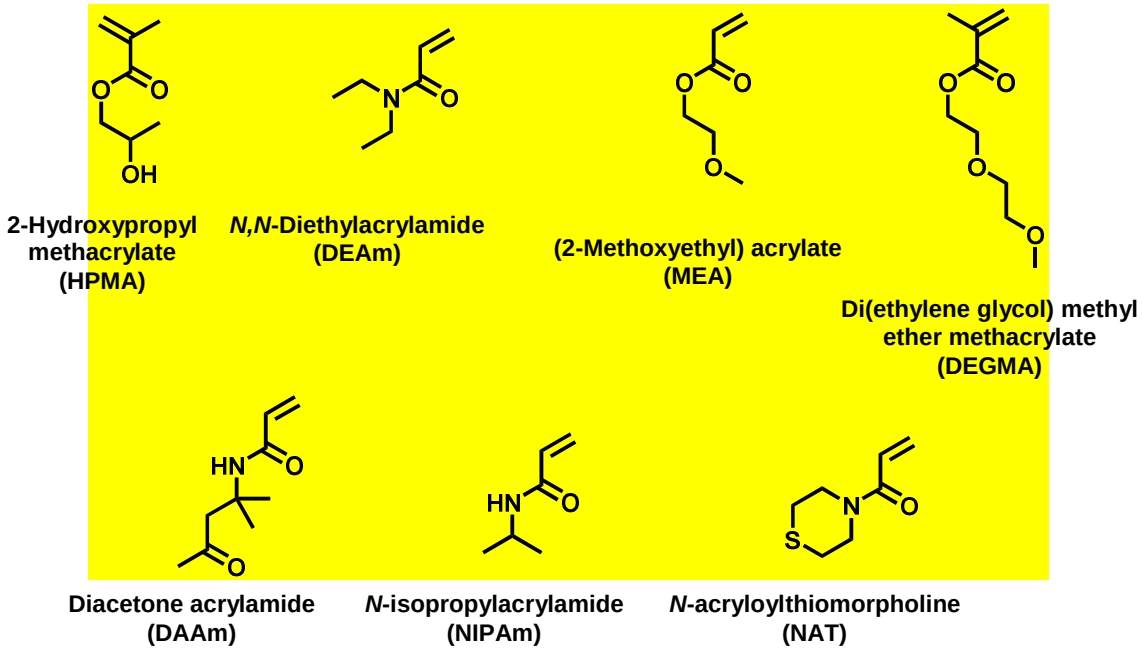

Figure 9 Monomers used for the core-forming block of aqueous dispersion polymerisation-induced self-assembly.

Aqueous dispersions of HPMA have been widely used due to its robust control over individual morphologies.[101, 116, 121-126] Chain extension of a poly(glycerol methacrylate) (P(GMA) macro-RAFT agent with HPMA of three different chain lengths (90, 140 and 220) yielded the conventional sphere, worm, vesicle morphologies.[121] Importantly quantitative monomer conversions were observed within 2 h. Upon cooling to room temperature, the $P(GMA)_{54}$-$P(HPMA)_{140}$ worm-like assemblies formed a soft malleable gel at 10 wt% due to worm-worm entanglements. Interestingly further cooling to 4°C resulted in a free flowing solution, which was later found be due to a worm to sphere transformation. The authors postulated that such a system could be used as a thermoresponsive biological storage medium, which can be sterilised by degelation and passing through a typical 0.45 µm filter upon degelation. To probe this the free flowing dispersion was contaminated with fluorescently labelled *S. aureus* and after sterilisation negligible contamination was observed. Furthermore the worm-gels were found to be fully biocompatible in cell viability assays.[121]

In two follow-up publications, this system was shown to be versatile in a number of biological applications as a potential replacement for cryopreservation. In one study, the authors used the worm-gels as mucin mimics which are known to induce stasis of mammalian embryos consisting of pluripotent stem cells *in utero*.[122] Storage of human embryos within these worm gels showed significantly greater stability than those in a commercially available alternative (Matrigel). Remarkably a high degree of a nuclear envelope statin was observed through immunostaining experiments, suggesting cell stasis within these conditions. When repeated with only pluripotent stem cell colonies, the majority of cells continued to express pluripotency markers rather than those for differentiation. The promising results observed are clearly related to the close mucin-mimicry available (worm-like and hydroxyl rich) through PISA and RAFT dispersion polymerisation.[122]

Gibson and co-workers have previously shown that polyalcohols are capable of mimicking anti-freeze glycoproteins in solvent-free cryopreservation of biologics.[127] This was recently expanded to use the above HPMA worm-gel system.[124] Ice recrystallization inhibition studies with worm-gels indicated limited activity,

and neither ice growth nor nucleation was promoted in these conditions. Interestingly, the worm-gels alone only resulted in 20% red blood cell recovery, however a cooperative effect between the worms and poly(vinyl alcohol) (PVA) was apparent resulting in over 60% erythrocyte recovery with no indication of haemaglutination or abnormal cell shape.

In the above cases, control over the surface monomer composition was critical to their performance in the desired applications. This approach has been employed to introduce biologically active moieties. Armes and co-workers described simultaneous co-PISA from the previous P(GMA) macro-RAFT agent, and a novel glycosylated poly(galactose methacrylate) macro-RAFT agent using HPMA as the core-forming block forming assemblies containing different degrees of galactose at the surface.[123] Similar to other studies, the dispersions display reversible thermoresponsive gelation between worm and spherical assemblies albeit with less rheological hysteresis than the previous example. Lectin binding studies indicated a much faster and greater response from worms and vesicles than the respective spherical nanoparticles, which likely contributed to the strong cellular uptake observed in live HDF cells.[123]

In an inspiring strategy, Huang and co-workers describe protein coated block copolymer assemblies *via* PISA by modifying BSA with an *N*-hydroxysuccinimide functionalised RAFT agent capable of reacting with lysine residues.[127] This was then used as the solvophilic block and chain extended with HPMA using a mild low temperature light activated RAFT to avoid denaturation of the protein during polymerisation. In this case however only spherical nanoparticles, albeit with increasing size, were obtained as the P(HPMA) chain length increased. Importantly by monitoring the hydrolysis of 4-nitrophenyl acetate, it was observed that the esterase activity of BSA was retained, and the hydrophobic nature of the core was used for encapsulation of a hydrophobic drug model, pyrene.[127]

3.1 Fundamental biological studies

The design of biomedical nanoparticles is often led by fundamental studies identifying the effect of individual physico-chemical properties (size, shape and surface functionality)[98, 128] on cellular uptake, biodistribution and pharmacokinetics. Non-spherical nanoparticles have attracted significant interest as many reports suggest they have improved cellular uptake and circulation time, and therefore greater therapeutic efficacy compared to spherical counterparts.[22] As such, PISA represents a promising method to probe this feature as morphology can be easily tuned without significantly affecting their surface chemistry which is also known to heavily influence biological performance.

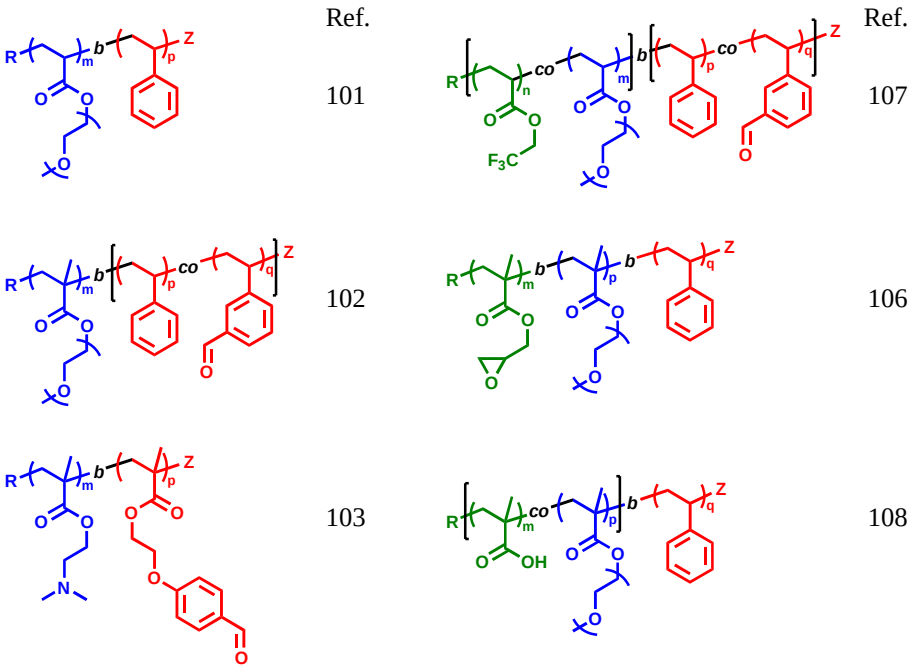

Figure 10 Block copolymers produced using polymerisation-induced self-assembly in alcoholic medium for biological applications. Green = functional group used for specific application, blue = solvophilic block, red = solvophobic block.

Boyer and Davis reported the RAFT PISA of styrene from a P(PEGMA) macro-RAFT in methanol, where styrene is soluble but polystyrene is insoluble.[129] As expected nanoparticle morphology evolved from spheres through to polymersomes with increasing monomer conversion. When relating the DP of styrene to the resulting morphology, it became apparent that isolating individual morphologies, particularly high aspect ratio particles was non-trivial as mixed morphologies could be seen at various chain lengths. To probe the *in situ* encapsulation of a model hydrophobic drug Nile Red was added at the start of the polymerisation. UV-Vis analysis showed higher encapsulation with longer PS chain length (i.e. higher conversion), and Nile Red concentrations over 10 fold of that found in the methanol highlighting their potential use in drug delivery.[129]

In a subsequent study, styrene was copolymerised with vinyl benzaldehyde (VBA) during the PISA process to produce pH-responsive pro-drug imine containing nanoparticles with doxorubicin to evaluate the effect of morphology on drug delivery efficiency.[130] Narrow particle size distributions and the expected morphologies were produced depending on styrene chain length. Perhaps one of the major disadvantages in this approach is that large DP's e.g. 5000 were targeted, and therefore specific morphologies were formed at very low monomer conversion (< 20%). This meant that extensive purification was necessary to fully remove monomeric styrene and

methanol before use in biological studies. Nonetheless, cellular studies with nanoparticles carrying a 5 wt% drug load indicated significantly higher cellular uptake for rod and worm-like micelles, which yielded the lowest IC_{50} values (0.796 and 0.302 μM for rod and worm-like nanoparticles respectively).[130]

In a similar approach, Pan and co-workers performed PISA with an aldehyde functional methacrylate from a P(DMAEMA) macro-RAFT agent in ethanol for post-functionalisation.[131] In contrast to the above studies, the particle morphology was controlled by targeting different solvophobic chain lengths (50, 100, 150, 200 and 250), each reaching full monomer conversions in 18 h. Although this eliminated the need to remove unconsumed monomer, purification was still necessary to remove the ethanol continuous phase. All of the resulting nanoparticles, regardless of morphology, had a smaller total diameter, as measured by dynamic light scattering (DLS), below 200 nm, which is the cited upper limit for endocytosis.[132] Doxorubicin loading was achieved using Schiff base formation, which naturally increased with longer aldehyde chain lengths reaching up to 16.2 wt%. Similar to the studies described above, DOX-loaded nanorods displayed the greatest cytotoxic effects against HeLa cells in comparison to spheres and vesicles, however longer nanowires showed minimal activity. This was rationalised with cellular uptake studies which indicated that nanorods had greater lysosomal (low pH compartment) residence time, thus improving drug release potential.[131]

The study of doxorubicin release was further investigated by Gooding and Gaus using pair correlation microscopy to probe the intracellular trafficking and site of drug release for different shapes nano-objects.[133] In this work, identical nanoparticles to those reported by Davis and co-workers were prepared instead with a PS-*co*-P(VBA) core. Their findings indicated that worm and rod-like materials passively enter the nucleus in a five-fold higher amount than spherical and vesicular systems. Furthermore attachment of nuclear localising peptides resulted in much greater doxorubicin release within the nucleus and enhanced cytotoxic effects.[133]

Many of the above studies use relatively simple block copolymer compositions (P(PEGMA)-*b*-PS) for nano-objects synthesis (**Figure 10**). As dispersion polymerisations begins in solution, complex functionality can be introduced through the copolymerisation of functional monomers when preparing the solvophilic block which can be further utilised for specific biological applications. Kaminskas and Whittaker reported the first fundamental *in vivo* study on particle morphology using nano-objects decorated with functional groups generated via PISA.[134] In this work a hydrophilic diblock copolymer of poly(glycidyl methacrylate-*b*-P(PEGMA)) was synthesised to introduce amine reactive functionality to the particle surface. Dispersion polymerisation of styrene in methanol was then used to generate various nanoparticle morphologies with an amine reactive epoxide surface, which were ring-opened with a tritium labelled (^3H) ethanolamine for radiolabelling. Biodistribution studies indicated significantly higher accumulation in reticuloendothelial organs (liver, spleen) for worms and rods, however small 21 nm micelles showed much greater association with tumour tissue.[134]

Drug delivery and cancer therapy are not the only applications in which nanomaterials can excel within biomedicine. The long circulation times and non-specific biodistribution exhibited by these structures makes them ideal imaging agents. In particular there are now a number of reports describing dispersion polymerisation to produce nano-objects for magnetic resonance imaging (MRI)[135] and the effect of morphology on their contrast.

Whittaker and co-workers report the synthesis of block copolymer nano-objects with a fluorine containing monomer as potential ^{19}F MRI contrast agents.[136] The authors describe that fluorine density is a major

parameter in MRI performance and therefore different morphologies may yield different contrasts. Initially a range of PEGA and trifluoroethyl acrylate (TFEA) copolymers were synthesised with different feed monomer compositions, with relaxivity studies indicating 2.68 wt% TFEA as optimum to avoid ^{19}F association. Dispersion polymerisations of styrene and VBA were then performed in isopropanol, targeting a total DP of 5000 stopping at 4.4 (spheres), 11.3 (worms), 17.2 (vesicles) and 22.5% (compound vesicles) styrene conversion to isolate individual morphologies. Relaxivity studies of block copolymer nanoassemblies suggested that T_1 and T_2 relaxation was independent of morphology.[136]

In a different approach, Davis and co-workers adapted their epoxide functional macro-RAFT agents to introduce gadolinium chelates into their nano-objects as potential MRI contrast agents.[137] Polystyrene nano-assemblies were prepared in methanol as described above. Prior to gadolinium functionalisation, the epoxide functionality was modified with thiols and to generate azide functional particles, highlighting the tuneable functionality possible with RAFT dispersion. Amine functional Gd-tetra-azacyclododecatetraacetic acid (Gd-DOTA) was used to ring open the surface functional epoxides on spherical, worm and vesicle nanoparticles. Similarly to Whittaker and co-workers, little difference in relaxivity was evident between morphologies.[137]

In an earlier study, Davis and Boyer used dispersion polymerisation to generate nanoparticles containing iron-oxide nanoparticles which are well-known MRI contrast agents.[138] In an versatile approach, Fe(II) and Fe(III) salts were fed to aqueous solutions of spheres, worms and vesicles which bound electrostatically to methacrylic acid moieties introduced in the macro-RAFT agents. Subsequent addition of ammonia resulted in precipitation of iron oxide on the surface of the nanoparticles. Interestingly the size of the resulting iron oxide nanoparticles could be tuned by increasing the MAA content of the solvophilic sections. In contrast to the above studies, micellar morphologies gave the highest transverse relaxivity (r_2 = 582 mM^{-1} s^{-1}), several times higher than commercially available contrast agent Feridex (r_2 = 100 mM^{-1} s^{-1}) .[138]

3.2 Stimuli responsive nano-assemblies

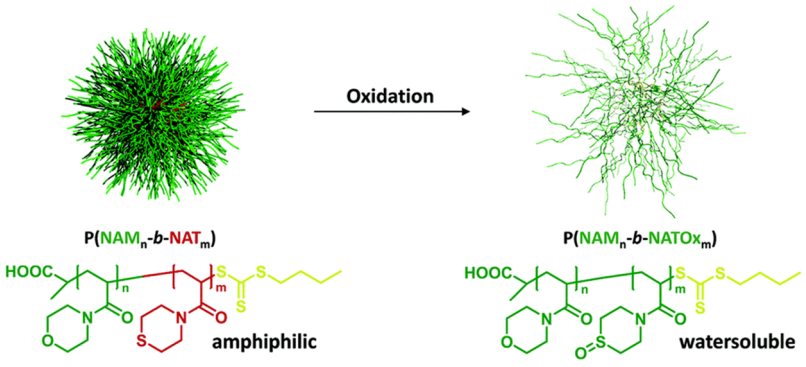

Figure 11 Oxidation responsive micelles synthesised by polymerisation-induced self-assembly of *N*-acryloylthiomorpholine from a poly(*N*-acryloylmorpholine) macro-RAFT agent.[139] Copyright 2018. Adapted with permission from the Royal Society of Chemistry.

One of the major problems in nanoparticle drug delivery is that most therapeutics can be cytotoxic in any area of the body, causing adverse side effects once release from its carrier. This has typically been overcome through introduction of functionalities which have either a chemical or physical transition after treatment with a particular stimulus.[140] This can either be an external stimulus, such as temperature or light, which can give spatial control over the site of drug release, or using endogenous biochemical stimuli such as the low pH and high glutathione content found in cancer tissue. Such a stimuli-responsive nature has been incorporated into dispersion polymerisations through copolymerisation of functional monomers.

With this in mind, Pan and co-workers report a redox-responsive system using PISA for delivery of a topoisomerase inhibitor camptothecin (CPT).[141] A disulphide functional prodrug containing solvophilic block copolymer was chain extended with benzyl methacrylate and a disulphide cross linker as the core-forming block to form 35 nm spherical micelles. All of the polymerisations reached full monomer conversion negating the need for extensive purification. The covalent drug-monomer approach allowed for high (12.8 wt%) drug content, and when incubated with 10 mM GSH (cytosolic concentration), 40% drug release occurred over 48 h. Although high loading was obtained, IC_{50} values of the micelles against HeLa cells were found to be between 6-7 μg mL^{-1} which is significantly higher than values reported for free CPT (0.08 μg mL^{-1}).[141, 142]

In a second study, a photo-sensitive system using 2-nitrobenzyl methacrylate (NBMA) and a coumarin methacrylate was developed.[143] In this work the above monomers were chain extended from a P(HPMA) macro-RAFT agent to form spherical, worm-like and vesicle nano-objects in methanol as previously described. Upon photoirradiation (UV 365 nm) it was expected that the nitrobenzyl unit of NBMA would cleave and become a carboxylate group, and subsequently cause disassembly of the nano-objects. However, inclusion of the coumarin groups resulted in their dimerization as subsequent chain-chain cross-linking producing anionic nanogels. This strong transition from hydrophobic to hydrophilic was exploited for fast and efficient release of doxorubicin from each of the morphologies generated. The vesicle assemblies had the greatest cytotoxicity in comparison to worm-like and spherical morphologies.[143]

Brendel and co-workers, report an oxidation responsive system as a possible vector to inflammatory disorders which have increased levels of reactive oxygen species.[139, 144] By chain extending an *N*-acryloylmorpholine (NAM) macro-RAFT agent with *N*-acryloylthiomorpholine (NAT), a PISA process ensued resulting in 25-75 nm

spherical micelles depending on the comonomer composition. After incubation with 10 mM hydrogen peroxide the thiomorpholine solvophobic block oxidised into a hydrophilic polysulfoxide, resulting in particle disassembly. This was further probed by the reduction of fluorescence intensity of a model hydrophobic drug, Nile Red, which was released after treatment with hydrogen peroxide, highlighting this systems potential use for drug delivery (**Figure 11**).[139]

3.3 Biomacromolecule encapsulation

As discussed in the previous section, delivery and transportation of biomacromolecules, such as therapeutic proteins are a potential alternative to small molecule drug delivery. Polymeric vesicles (polymersomes) have been shown as promising carriers for enzymes, proteins and antibodies due to their large hydrophilic compartment.[145] Typically polymersomes are prepared at low polymer concentrations and with organic solvents, using traditional self-assembly methods[100] or thin-film rehydration[146] of block copolymers which becomes problematic at large scales. In the above sections, we have highlighted how PISA can be used to produce a variety of morphologies including polymersomal vesicles, which may be able to encapsulate cargo in high quantities. Traditional PISA protocols are an adaptation of conventional RAFT polymerisations, and as such are performed at high temperatures (e.g 70°C), which could lead to denaturation of biological materials. This has been overcome by low temperature PISA protocols, such as redox initiation,[113] however many of these examples only resulted in spherical morphology. Boyer and co-workers reported on the low temperature photoinduced PISA of HPMA from a PEGylated macro-RAFT agent able to produce spheres worms and vesicles. This approach has been extended in three separate studies for protein encapsulation and subsequent biological applications.

Zhang and co-workers describe an adaptation of Boyer and co-workers HPMA photo-PISA, where at HPMA concentrations > 15 wt%, higher order morphologies, including vesicles could be prepared.[147] Using this system full monomer conversions were achieved in under 30 mins irradiation. As a proof of concept for encapsulation, fluorescein labelled BSA was added into the PISA formulations, which remained 90% active as determined by the hydrolysis of 4-nitrophenyl acetate. Interestingly the BSA encapsulated within the polymersomes was 65% more active than BSA heated to 70°C, similar to that if the polymerisation was conducted with thermally initiation.[147]

Such polymersome-protein hybrids can also be used as enzymatic nanoreactors assuming that the membrane is permeable to small molecule substrates. This approach was studied by Gibson and O'Reilly who utilised the above HPMA photo-PISA technique to generate separate glucose oxidase (GOx) and horseradish peroxidase (HRP) loaded vesicles with encapsulation efficiencies of around 25%.[148] First proof of concept experiments, showed that HRP vesicles could catalyse the oxidation of 3,3'-dimethoxybenzidine (DMB) despite the substrates residence in the external medium, indicating the vesicle membranes were permeable to small molecules. Furthermore, using the GOx and HRP loaded vesicles, D-glucose was metabolised into d-glucono-1,5'-lactone, producing hydrogen peroxide as a by-product which facilitated the catalytic oxidation of DMB by HRP within a separate vesicle, highlighting how multiple enzymes can be used in a cascade even though they are physically seperated.[148]

The above strategy was further utilised in which L-asparaginase (ASNS) was encapsulated into the permeable vesicles as a possible treatment for lymphoblastic leukaemia. Importantly, the ASNS loaded vesicles showed excellent stability towards proteolylsis from α-chymotrypsin, whereas free ASNS and PEGylated ASNS had no activity after the same treatment(**Figure 12**). To test this effect *in vitro*, human lung cancer fibroblasts were silenced to inhibit ASNS activity, thus rendering the cells reliant on the treated ASNS loaded vesicles for proliferation. *In vivo* biodistribution studies of the ASNS loaded vesicles showed accumulation within the typical organs for nanoparticle systems (spleen, liver, kidneys) and slower clearance in comparison to the free enzyme.[149] This approach was extended by exploiting poly(sarcosine) macro-RAFT agents to produce enzymatic nanoreactors *via* photoinduced PISA of HPMA. The resulting polymersomes showed high stability against multiple proteases compared to PEG counterparts. [150] The authors were subsequently able to utilise surfactants within these PISA reactions to incorporate a functional channel-forming membrane protein (OmpF Porin). Colorimetric assays revealed successful incorporation and membrane channel function of these OmpF Porin vesicles, in comparison to analogous polymersomes without OmpF Porin which showed negligible colour changes.[151]

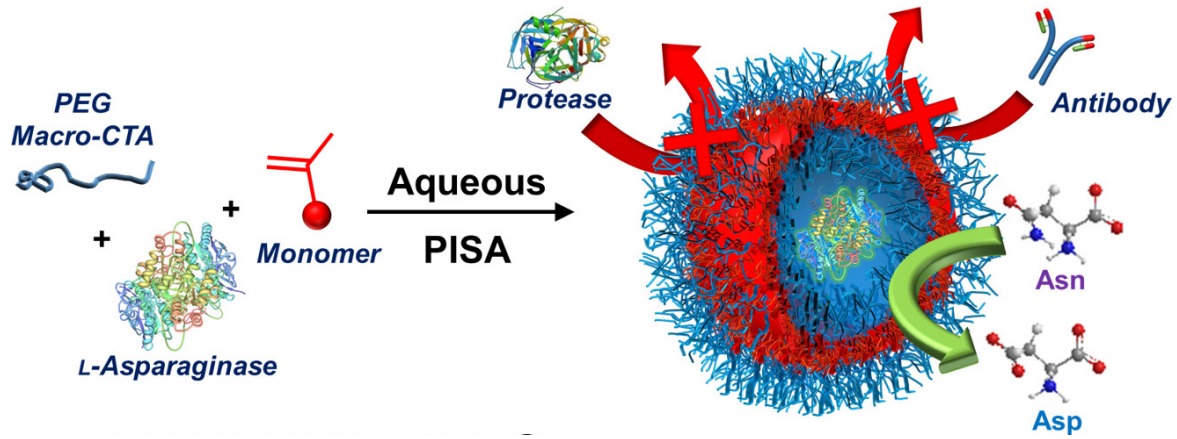

Figure 12 Encapsulation of *L*-Asparaginase using photo-PISA of HPMA to produce permeable catalytic polymersomes.[149] Copyright 2017. Reproduced with permission from the American Chemical Society.

Over the last 5-7 years RAFT dispersion polymerisations in the form of PISA has made nanoparticle formation more accessible than ever before. The ability to modulate surface and core chemistries, as well as particle morphology in one-step has made this particularly attractive for fundamental or application driven research at the biological interface. Recent synthetic advances made now mean that these systems can easily be generated in aqueous conditions with no by-products (100% monomer conversion), in low temperature and oxygenated conditions.[152, 153] Nonetheless, the limited number of core-forming monomers capable of aqueous PISA mean that introduction of stimuli-responsivity is a challenging prospect, unlike in traditional self-assembly processes. Furthermore, what is clear from PISA oriented literature is that the resulting spherical particles are relatively small (< 30 nm diameter)[99] and unlike in emulsion systems (*vide infra*), altering the molecular weight of the core forming block typically does not modify particle size without a morphological transformation, which is not suitable for all applications. Even still, if worm-like or polymersomal morphologies are required from

novel/unreported block copolymer compositions, one must perform the arduous task of producing a pseudo-phase diagram by mapping the morphologies of the resulting nano-objects by solvophobe chain length and total solids content.

4 Suspension polymerisation

Suspension polymerisations take place within micron sized monomer droplets, using a monomer soluble initiator stabilised by conventional surfactants.[48] They are similar to miniemulsion polymerisations in the sense that they are essentially compartmentalised bulk polymerisations, however are dispersed with agitation only and not with sonication (**Figure 13**). This typically results in the formation of microparticles (> 1 µm diameter) decorated with the surfactants present during the polymerisation. While this review is focussed on the synthesis of biologically relevant nanoparticles (not micron sized) using RDRP, an interesting approach to generate non-spherical nanoparticles has arisen using a post-polymerisation strategy pioneered by Monteiro and co-workers using RAFT suspension polymerisation.[154] As mentioned in the previous section, the formation of non-spherical, elongated nanoparticles is becoming increasingly important due to their longer circulation time and thus greater therapeutic efficacy compared to spherical analogues. This approach operates on the use of a thermoresponsive macro-RAFT agents which collapse and become hydrophobic during the high temperatures (70°C) of suspension polymerisations. Upon cooling the macro-RAFT agent swells within the aqueous phase, and after addition of small amount of plasticiser, the 1-2 µm spheres transform into long worm-like fibrils stabilised by the thermoresponsive shell. Notably in many of the works which use this technique, it is mistakenly called a dispersion or emulsion polymerisation whereas it is actually a suspension polymerisation.

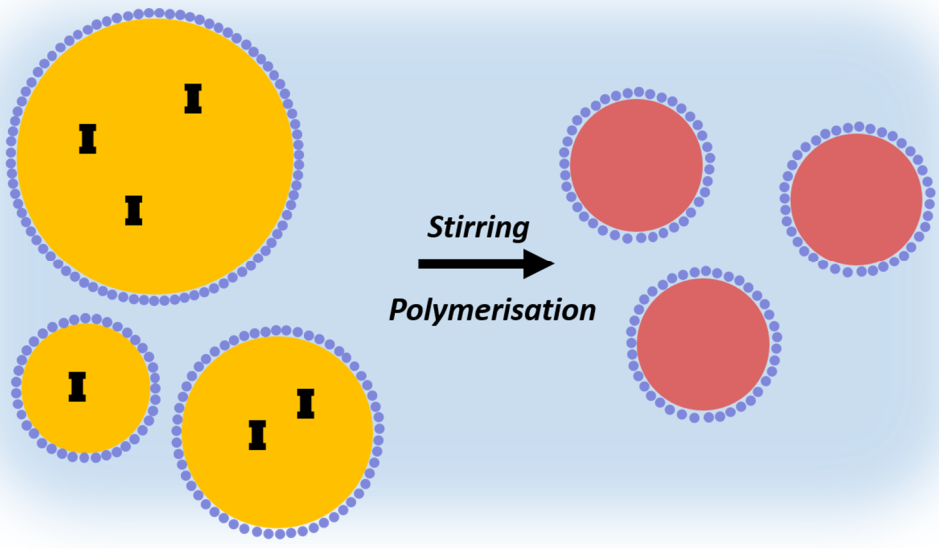

Figure 13 Mechanism of suspension polymerisation. I = initiator species, yellow = oil/monomer phase, blue = aqueous phase, red = polymer phase.

Davis and co-workers have expanded on this approach using poly((diethyleneglycol) methyl ether methacrylate)-*co*-(HPMA) (P(DEGMA)-*co*-(HPMA)) macro-RAFT agents with P(DEGMA) being the thermoresponsive component.[155] Following suspension polymerisation of styrene droplets stabilised by sodium dodecyl sulfate (SDS), 1 µm polystyrene block copolymer spheres were generated, and after cooling and addition of different volumes of toluene (20-160 µL mL^{-1}) polymeric nanowires, vesicles and lamellae were produced (**Figure 14**). Interestingly, as seen in other supramolecular systems, sonication could be used to cut the nanowires into much shorter rod-like structures. To expand the scope of this approach Davis and co-workers have now shown it is possible to achieve these nanobjects with a variety of polymer cores in multigram quantities, and showing high biocompatibility in humane endothelial (HUVEC) and fibrosarcoma (HT1080) cells regardless of morphology.[155, 156] In a further study, Davis and co-workers report the morphological stability in the presence of surfactants of the above nanoparticle system, via NOESY NMR spectroscopy. This aspect is vital in understand the fate of complex nanomaterials once exposed to biological environments. [157]

Although this technique allows for control over nanoparticle morphology, the specific thermoresponsive shell composition required to achieve this phenomenon severely limits surface functionality available for further modification. Relatively little biological work (only cell viability) has been reported using nanoparticles prepared using this particular technique thus far. However, it is easy to see that molecular drug delivery, bioimaging and other applications not requiring biologics (which may denature in the presence of surfactant and organic solvent), could be achieved using this approach. Aqueous dispersion polymerisations (as discussed above) can achieve many of the same properties (morphology control, tuneable surface and core composition), but can be performed in the absence of surfactant and at low temperatures, possibly making this suspension polymerisation method less useful.

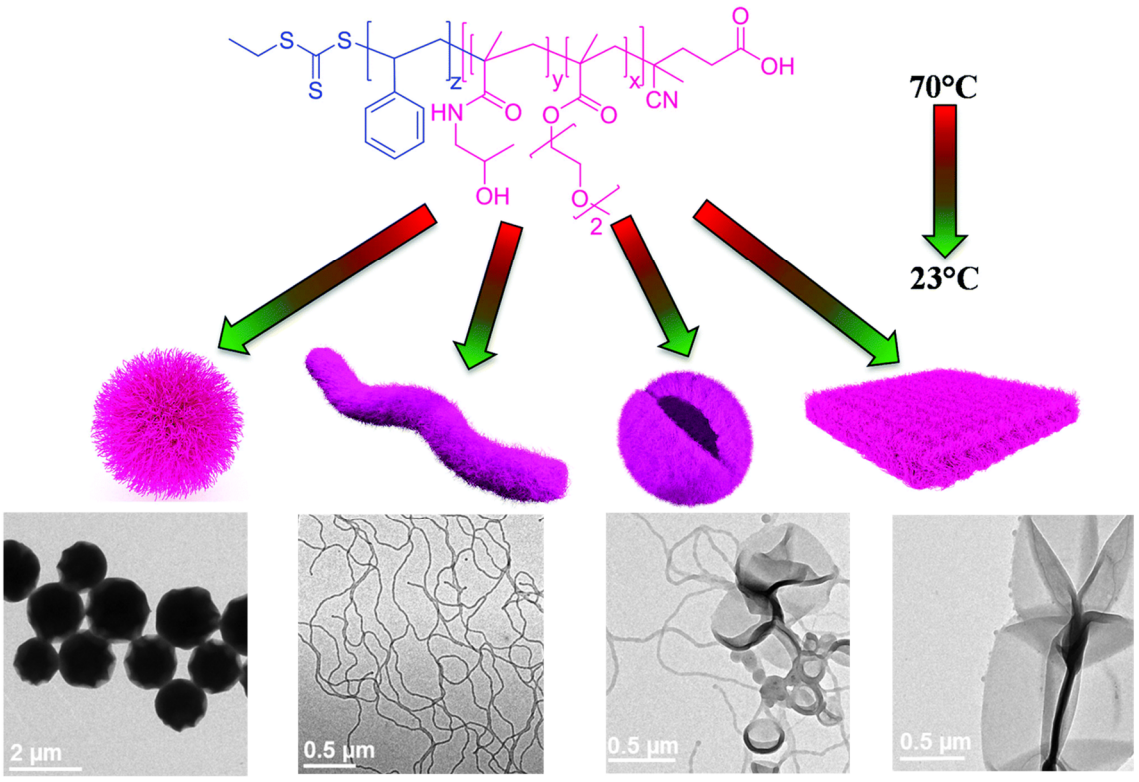

Figure 14 Suspension polymerisation of styrene from a hydrophilic thermoresponsive macro-RAFT agent. Upon addition of different volumes of toluene (0, 20, 40, 80 and 160 μL mL^{-1}) and cooling to room temperature various morphologies were formed.[155] Copyright 2015. Adapted with permission from the Royal Society of Chemistry.

5 Emulsion polymerisation

In a typical emulsion polymerisation, the initial reaction mixture is comprised of micrometre sized monomer droplets which are immiscible in the aqueous continuous phase, surfactant micelles and a water soluble initiator.[46] Particles are generated first through monomer propagation in the aqueous phase, forming hydrophobic Z_{mers} which subsequently nucleate micelles (or self-nucleate in surfactant free emulsion polymerisations). Particle growth ensues during polymerisation of monomer which is continuously diffusing from monomer droplets into the surfactant micelles, which when complete, results in surfactant coated solid latex nanoparticles. This process has been implemented using free-radical polymerisation for decades to generate vinyl polymers at large scales and in environmentally friendly conditions. The compartmentalisation effect enables fast propagation rates, high monomer conversions and importantly low termination rates resulting in high molecular products.[46] Due to these key advantages, especially for industrial applications, much work has gone into translating RDRP techniques to emulsion polymerisation. However the complex monomer and radical transfer mechanisms mean that RDRP methods cannot be implemented in *ab initio* emulsion polymerisations by simply adding in a control agent.

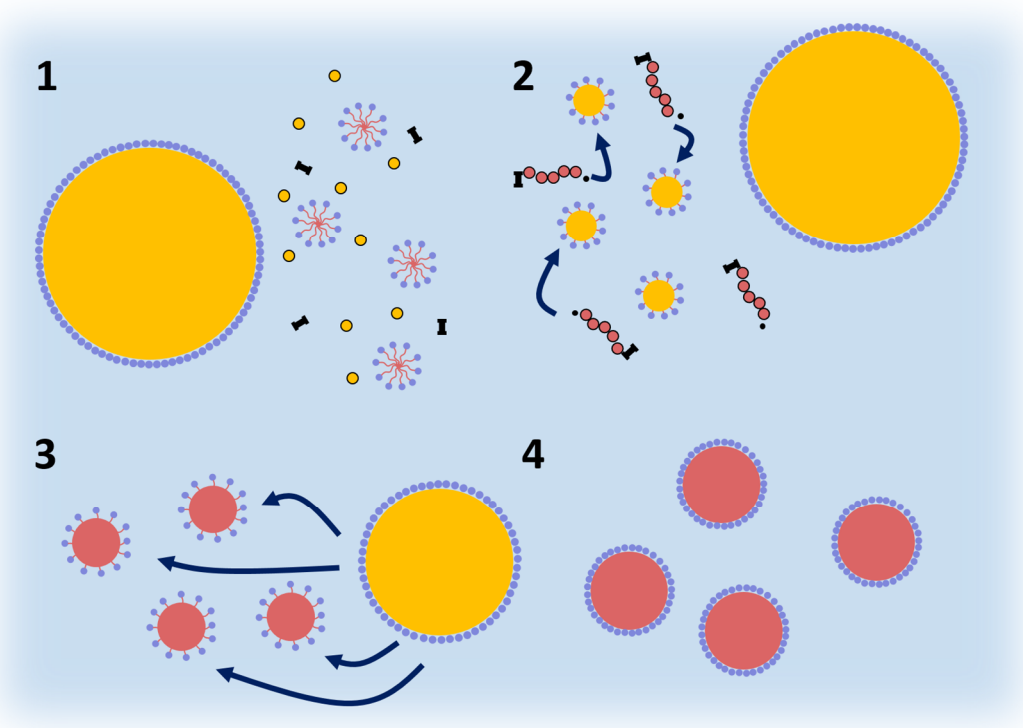

Figure 15 Mechanism of emulsion polymerisation (1) reaction mixture, (2) initiation phase, (3) propagation and diffusion of monomer from monomer droplets to growing particles, (4) final latex particles. I = initiator species, yellow = oil/monomer phase, blue = aqueous phase, red = polymer phase.

In general, early studies to implement RDRP methods in emulsion processes resulted in one or more of the following drawbacks: (1) poor colloidal stability of the resulting latex; (2) lack of control over the molar mass, and (3) high M_w/M_n values.[51] This was overcome by implementing stabilising amphiphilic macroinitiators which are chain extended in the emulsion polymerisation and avoid the use of external surfactant. This was first pioneered by Hawkett and co-workers who used poly(acrylic acid)-*b*-poly(*n*-butyl acrylate) macro-RAFT agent micelles to seed a RAFT emulsion polymerisation of *n*-butyl acrylate (*n*-BA) leading to colloidally stable uniform block copolymer nanoparticles (D_h = 60 nm, PDi < 0.01) which are coated with P(AA) at their surface (**Figure 16**).[158, 159] After dissolution in organic solvent, the block copolymers displayed narrow molecular weight distributions ($Đ$ < 1.3) and excellent molecular weight control. This tactic was further implemented using NMP (poly(acrylic acid) based macroalkoxyamines) however little progress has been made on this topic since 2008, possibly due to its incompatibility with methacrylates.[160-163] In contrast, ATRP emulsion procedures have mainly focussed on inistabs (amphiphilic surfactant-like ATRP initiators) and water soluble initiators using either conventional ATRP or ARGET ATRP.[164-167] Many examples utilising these approaches unfortunately led to broad particle size distributions and unsatisfactory molecular weight control likely due to phase separation of the transition metal catalyst from the ATRP initiator.[60] As such the degenerative chain transfer mechanism of RAFT, and its compatibility with most monomer families, have led to this technique being the most popular RDRP emulsion polymerisation method and has now been expanded to a large range of monomers and stabilisers (cationic, anionic and neutral).[168] Many of these studies focus on synthetic aspects (kinetics and morphology control)[169-171], however some features could be useful in designing nanoparticles for biological applications.[172]

5.1 RAFT emulsion polymerisation

Particle size has been shown to drastically influence biological performance, affecting nanoparticle cellular uptake, biodistribution and thus potential therapeutic efficacy.[98] Even though most reports on RAFT emulsion polymerisations yields only spherical morphologies the resulting nanoparticles are highly uniform and in over different size ranges (30-200 nm diameters).[98] Davis and co-workers have previously been shown that particle volume has a linear relationship with the molecular weight of the polymeric core and as such can be tuned during the core-forming step.[173] The particle sizes produced by RAFT emulsion polymerisation are significantly larger than those produced by dispersion polymerisations (<30 nm diameters), which may make the former more useful *in vivo* by reducing their susceptibility to renal clearance.[174]

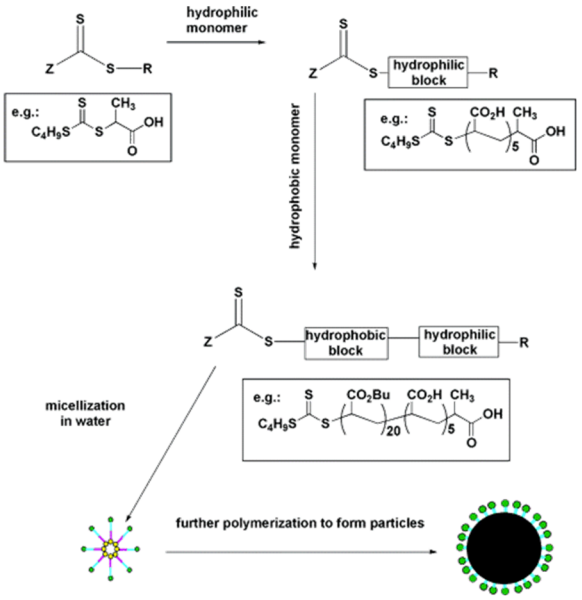

Figure 16 Schematic representation of RAFT emulsion polymerisation using an amphiphilic macro-RAFT agent stabiliser.[159] Copyright 2005. Reproduced with permission from the American Chemical Society.

As with the dispersion PISA formulations previously described, the stabilising macro-RAFT agents in RAFT emulsion processes are imparted at the particle surface. Stenzel and co-workers were the first to exploit this approach for biological applications, through the introduction of glycosylated moieties, which are known to improve disease targeting and bacterial interactions *in vivo*, when present at the particle surface.[175] To achieve this poly(glucose methacrylamide)-*b*-PS was used to stabilise the emulsion cross-linking copolymerisation of styrene and a redox responsive disulphide diacrylate monomer. The resulting nanoparticles were produced within 6 h, were fairly uniform (*PDi* < 0.2) and within a biologically relevant size range (50-80 nm diameters). While the particles did disassemble after dithiothreitol addition (DTT), this did not occur in an aqueous environment but had to be pre-swollen in organic media making this response unsuitable for redox responsivity *in vivo*. Nonetheless, turbidimetric binding assays revealed a strong binding event to lectin Con A, with similar performances evident in bacterial adhesion studies. Importantly the RAFT emulsion polymerisation approach yields a particle whereby the polymeric corona is covalently attached to the nanoparticle core, thus cannot desorb *in vivo* potentially inducing toxicity or other negative side effects.[175]

More recently, Lansalot and co-workers reported RAFT emulsion polymerisation using glycopolymer stabilisers. In this work alginate derived polysaccharide macromonomers were copolymerised with NAM and further chain extended with styrene emulsion polymerisation to generate polysaccharide coated nanoparticles.[176] Surprisingly, when emulsion polymerisations were performed with polysaccharide macromonomers severely decreased particle uniformity ($D_w/D_n > 1.07$) and broadened molecular weight distributions ($Đ > 1.50$) were seen in comparison to pure P(NAM) stabilisers ($D_w/D_n < 1.05$; $Đ < 1.3$).[176]

Wang and co-workers report the synthesis of shell cross-linked pH responsive nanoparticles using RAFT emulsion polymerisation.[177] Emulsion polymerisation of styrene was initiated from a poly(2-(dimethylamino)ethyl methacrylate) (P(DMAEMA)) and cross-linked during the early phase of the polymerisation with a disulphide diacrylate monomer yielding 95 nm particles. Similarly to the above, degradation studies were performed in DMF, with particles completely disassembling after addition of DTT. In proof of concept drug release studies,

indomethacin was encapsulated within the nanoparticles, and due to the pH responsive P(DMAEMA) shell, a fast release (70% with 24 h) was evident at pH 5.4 with the addition of DTT. Finally, although high cell viability was reported against human cervical cancer (HeLa) cells, this was only measured with concentrations up to 50 μg mL^{-1} yielding 70% viability which is relatively cytotoxic for such a low concentration.[177]

In a recent report by Armes and co-workers, they were able to elegantly combined RAFT emulsion polymerisation, and RAFT dispersion polymerisation to produce framboidal hollow pH responsive nanoparticles. Chain extension of a preformed P(Gly)-*b*-P(HPMA) polymersome or 3% P(MPC)-*b*-P(HPMA) mixed polymersome with pH responsive monomer diisopropylaminoethyl methacrylate under emulsion conditions leds to the formation of ~300 nm framboidal vesicles. Upon addition of acid the DPA block becomes hydrophilic reverting the structure into small ABC triblock nano-objects with no defined morphology determined via TEM and SAXS analysis. Interestingly these peculiar vesicles closely mimic the structure of the dengue virus which are renowned for their endosomal escape properties. Furthermore the phosphoryl-choline decorated derivatives displayed preferential uptake into triple-negative breast cancer cells (MDA-MB-231) in comparison to normal MCF-7 breast cancer cells. The endosomal escape properties of these materials were exploited to deliver an EGFP encoding plasmid using electroporation to load into the hollow core.

One of the major advantages of RDRP methods is the possibility of introducing functional groups at both ends of the polymer chain typically imparted by the respective control agent(s).[178] These can then be used to introduce biologically relevant functional molecules (e.g. nucleic acids, proteins, targeting peptides etc.). This approach was applied to RAFT emulsion polymerisation by Poon *et al*. who described the post-modification of carboxylated particles with microRNA.[179] First a poly(acrylamide)-*b*-PS stabiliser was synthesised with a RAFT agent exhibiting a functional carboxylic acid on the reinitiating group. This was then chain extended in an *ab initio* RAFT emulsion polymerisation of styrene yielding very small 11 nm nanoparticles. The carboxylic functionality was first modified with an amino functional pyridyldisulfide as a redox-responsive linker for the microRNA, which was further conjugated at the particle surface in a typical thiol exchange reaction. Addition of 10 mM GSH resulted in 60% microRNA release from the particle surface over 72 h. They explain that the particular microRNA used (miR-200b) can supress the epithelial-mesenchymal cell transition.[179]

The high functional group density that RAFT emulsion polymerisation imparts on the particle corona was further used for attachment of a rhodamine dye to produce nanoparticles capable in *in vitro* and *in vivo* imaging applications.[180] In this case, Poon *et al* synthesised both 11 and 22 nm polystyrene particles and were subsequently conjugated with rhodamine amine at 10 and 12% efficiencies respectively. After incubation with HK2 cells, the punctuated structure in fluorescence microscopy images indicated endosomal or lysosomal colocalisation, consistent with many other studies. Finally the 22 nm rhodamine labelled nanoparticles were injected intravenously in healthy mice, with the highest accumulation occurring in the liver and kidneys. While, this was the first *in vivo* study of latex nanoparticles synthesised *via* RAFT emulsion.[180]

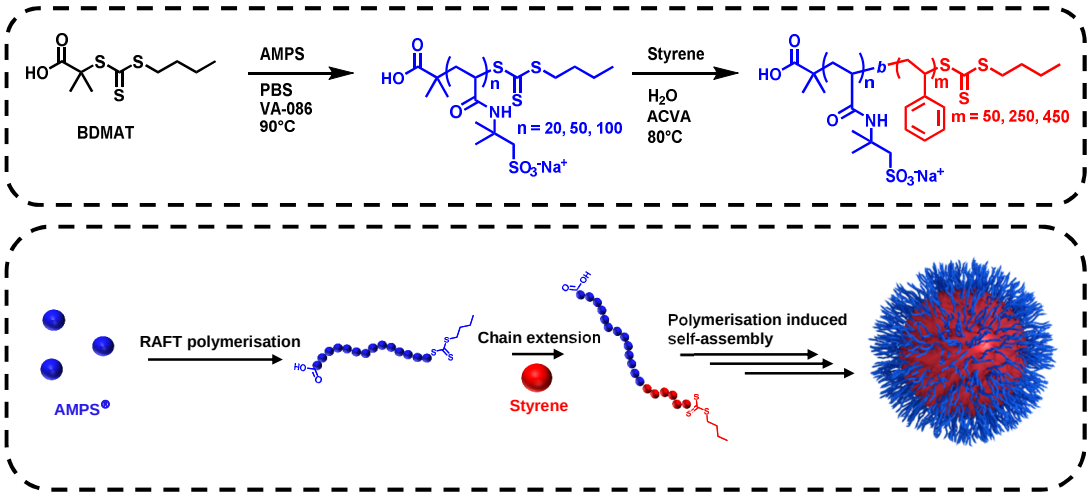

Figure 17 Synthesis of polysulfonate decorated polystyrene nanoparticles synthesised using RAFT emulsion polymerisation-induced self-assembly as potential wound healing agents.[181] Copyright 2018. Adapted with permission from Wiley-VCH Verlag GmbH & Co.

Our group have also utilised RAFT emulsion polymerisation to produce the first heparin mimicking, polysulfonated nanoparticles, able to stabilise the basic growth factor (bFGF), imperative in wound-healing applications. [182] In this work, poly(2-acrylamido-2-methylpropane sodium sulfonate) (P(AMPS)) macro-RAFT agents were prepared using aqueous RAFT polymerisation, then were directly chain extended with styrene *via* emulsion polymerisation without prior purification (**Figure 17**). A systematic parameter screening study revealed that stirring speed and P(AMPS) chain length had the greatest effect on particle size and polydispersity. Proliferation studies indicated that the polysulfonated nanoparticles had three fold and two fold greater bFGF stabilisation compared to heparin and bFGF alone respectively. It was postulated that this effect was due to a higher valency of growth factor being transported to the bFGF receptor at the cell surface.[181]

Additionally, we have reported the *in vivo* and *in vitro* tolerability of different sized PEGylated nanoparticles prepared *via* RAFT emulsion polymerisation. Diblock macro-RAFT agents of P(PEGA)-*b*-((*t*-BA) or (*n*-BA)) were prepared *via* conventional RAFT polymerisation, then further assembled into micelles which were chain extended during the subsequent RAFT emulsion polymerisations. Particles with diameters between 30 and 130 nm were prepared, and were found to be non-toxic against colorectal carcinoma, and tolerated in acute and sub-acute *in vivo* studies after intraperitoneal injection. Finally, after loading with a near infra-red fluorescent probe, the, organ accumulation and pharmacokinetic studies revealed significant deposition in the liver and intestine, with clearance rates similar to other intraperitoneally administered formulations.[183]

Due to their non-degradable nature, highly uniform polydispersity and dense surface chemistry, nanoparticles prepared by RAFT emulsion polymerisation can be excellent models when studying nanomaterials in biological systems. In a recent study, Davis and co-workers utilised polystyrene core nanoparticles, copolymerised with Cy5 maleimide for fluorescent labelling to investigate the effect of particle size and surface chemistry on cellular association under flow conditions. Nanoparticle suspensions with diameters of 40, 70 and 130 nm, each with a variety of surface chemistries were incubated with HUVEC cells cultured in a model microfluidic vascular network, and compared to HUVEC cells under static conditions. In general it was found that flow conditions significantly reduced the cellular association, however it was noted that the larger positively charged particles had

significantly higher association than the smaller derivatives under flow conditions. In constrast, when studying the negatively charged particles, he opposite effect was observed.[184]

Not all biological applications need to be biomedical. For instance, Carlmark and co-workers have recently reported the preparation of both low and high T_g latexes with RAFT emulsion polymerisation for cellulose modification. Emulsion polymerisations of BMA and MMA were initiated from a P[(DMAEMA)-*co*-(MAA)] macro-RAFT agent, resulting in uniform nanoparticles (PDi < 0.1). The aqueous system allowed for direct adsorption onto cellulose fibres monitored using a quartz crystal microbalance. Contact angle measurements indicated that P(BMA) latexes induced hydrophobicity before and after annealing, whereas with high T_g P(MMA) latexes, this only occurred after annealing. It was found that these latexes significantly improved the mechanical strength of these biocomposites after annealing.[185]

From a biological perspective, RAFT emulsion polymerisation is a relatively simple method to produce uniform nanoparticles with tuneable (monomer composition) cores and corona, specific surface functionality (reinitiating group on RAFT agent) with a variable size range (target molar mass) all in an aqueous environment without external surfactants. Many of the advantages seen here are comparable in RAFT aqueous dispersion polymerisations, which can also produce non-spherical species (notwithstanding the emulsion systems also show this ability)[186] using similar conditions. However, there are very few monomers capable of the soluble monomer to insoluble polymer transition required for aqueous dispersion polymerisation (*vide supra*).[99] In contrast, the number of monomers capable of emulsion polymerisation (i.e water immiscible molecules e.g MMA, styrene, BA) is far greater, which may be useful when tailoring core properties. Furthermore, the most common use of core-shell nanoparticles is controlled drug delivery, powered by the high loading efficiencies of insoluble therapeutics.[187] However aqueous dispersion polymerisations typically use much more hydrophilic components than analogous emulsion polymerisations, potentially limiting this application.

Nonetheless similar core-shell structures with higher order morphologies can also be prepared using popular self-assembly procedures such as solvent-switch protocols.[100] To date, these are the most heavily used methods and have been used to prepare nanoparticles far more complex (stimuli-responsiveness,[188] degradability[189] and specific disease targeting[190]) than any heterogenous RDRP technique. However, as stated above, these efforts can only be realised at low concentrations and require organic solvents, thus must be purified before biological use. Moreover the resulting particles are generally dynamic and may disassemble at low concentration possibly leading to premature clearance *in vivo*.

There have been some efforts to avoid the use of surfactants without RDRP methods (i.e surfactant free emulsion polymerisations using conventional free radical polymerisation) which can be achieved in one step from monomer directly to nanoparticle.[191-193] However these typically lead to a large proportion of high molar mass hydrophilic polymers contaminating the continuous phase, which must be removed through dialysis or centrifugation. Furthermore unless specific monomer combinations are used with this approach, it is non-trivial to produce sub 200 nm particles which are the most efficacious in biological studies.[194]

6 Conclusions and Future Perspectives

Overall the field of RDRP in dispersed states is undergoing a clear revolution from primarily synthetic work towards understanding how it can be implemented in modern applications, including biomedicine. It is now well understood how to translate popular RDRP techniques with many heterogeneous polymerisations and their advantages realised to prepare systems applicable for delivery (gene and drug), and fundamental studies both *in vitro* and *in vivo*. The degenerative chain transfer mechanism of RAFT, and its synthetic versatility (monomer compatibility, aqueous and low temperature polymerisations) have resulted in this approach being applied in all heterogeneous polymerisations discussed above, with NMP and ATRP being largely forgotten. An important factor in this, has been the development of RAFT aqueous dispersion polymerisations to produce non-spherical nanoparticles, such as rods and vesicles in low temperature environments which show enhanced cellular uptake and can be used to entrap hydrophilic cargo. Nonetheless the limited number of core-forming monomers severely reduces the flexibility of this system. In contrast, RAFT emulsion procedures can be performed with practically any water immiscible monomer, and by modifying the monomer and stabilising macro-RAFT agents involved, the polymer shell, core, particle size and surface functionality can be tuned. The original motivation to implement heterogeneous techniques was to enhance scalability, and improve the industrial applicability of polymers and block copolymers with narrow molar mass distributions. What is clear however, is that the facile nature of many of these techniques has led to monomer functionality and block copolymer composition being the more important features compared to polymer dispersity.

Nonetheless, a number of challenges still remain if such systems are to be implemented in commercial therapeutic applications. At present, very few of these formulations have been studied *in vivo*, and very little information is available regarding biodistribution, clearance rate and long-term tolerability. A potential reason for this may be that while responsive materials which disassemble under heat, light or chemical stimuli have been developed, there are almost no examples of heterogeneous RDRP techniques to produce degradable materials such as polyesters, or containing such subunits. Such systems such as polymerisation of cyclic ketene acetal monomers, are relatively easy methods to produce such materials using radical chemistry. As such, focus should shift to these milestones over the next few years if heterogeneous RDRP is to reach its full potential in medical applications.

References

[1] Elsabahy M, Wooley KL. Design of polymeric nanoparticles for biomedical delivery applications. Chemical Society Reviews. 2012;41:2545-61.
[2] Samad A, Sultana Y, Aqil M. Liposomal Drug Delivery Systems: An Update Review. Current Drug Delivery. 2007;4:297-305.
[3] Lohcharoenkal W, Wang L, Chen YC, Rojanasakul Y. Protein Nanoparticles as Drug Delivery Carriers for Cancer Therapy. BioMed Research International. 2014;2014:12.
[4] Liao H, Nehl CL, Hafner JH. Biomedical applications of plasmon resonant metal nanoparticles. Nanomedicine. 2006;1:201-8.
[5] Michalet X, Pinaud FF, Bentolila LA, Tsay JM, Doose S, Li JJ, Sundaresan G, Wu AM, Gambhir SS, Weiss S. Quantum Dots for Live Cells, in Vivo Imaging, and Diagnostics. Science. 2005;307:538-44.
[6] Slowing II, Vivero-Escoto JL, Wu C-W, Lin VSY. Mesoporous silica nanoparticles as controlled release drug delivery and gene transfection carriers. Advanced Drug Delivery Reviews. 2008;60:1278-88.
[7] De Jong WH, Borm PJA. Drug delivery and nanoparticles: Applications and hazards. International Journal of Nanomedicine. 2008;3:133-49.
[8] Gu Z, Biswas A, Zhao M, Tang Y. Tailoring nanocarriers for intracellular protein delivery. Chemical Society Reviews. 2011;40:3638-55.
[9] Panyam J, Labhasetwar V. Biodegradable nanoparticles for drug and gene delivery to cells and tissue. Advanced Drug Delivery Reviews. 2003;55:329-47.
[10] Nune SK, Gunda P, Thallapally PK, Lin Y-Y, Forrest ML, Berkland CJ. Nanoparticles for biomedical imaging. Expert opinion on drug delivery. 2009;6:1175-94.
[11] Chen G, Roy I, Yang C, Prasad PN. Nanochemistry and Nanomedicine for Nanoparticle-based Diagnostics and Therapy. Chemical Reviews. 2016;116:2826-85.
[12] Wilhelm S, Tavares AJ, Dai Q, Ohta S, Audet J, Dvorak HF, Chan WCW. Analysis of nanoparticle delivery to tumours. Nature Reviews Materials. 2016;1:16014.
[13] Bobo D, Robinson KJ, Islam J, Thurecht KJ, Corrie SR. Nanoparticle-Based Medicines: A Review of FDA-Approved Materials and Clinical Trials to Date. Pharmaceutical Research. 2016;33:2373-87.
[14] Jin-Wook Y, Elizabeth C, Samir M. Factors that Control the Circulation Time of Nanoparticles in Blood: Challenges, Solutions and Future Prospects. Current Pharmaceutical Design. 2010;16:2298-307.
[15] Pinto Reis C, Neufeld RJ, Ribeiro AJ, Veiga F. Nanoencapsulation I. Methods for preparation of drug-loaded polymeric nanoparticles. Nanomedicine: Nanotechnology, Biology and Medicine. 2006;2:8-21.
[16] Suk JS, Xu Q, Kim N, Hanes J, Ensign LM. PEGylation as a strategy for improving nanoparticle-based drug and gene delivery. Advanced Drug Delivery Reviews. 2016;99:28-51.
[17] Steichen SD, Caldorera-Moore M, Peppas NA. A review of current nanoparticle and targeting moieties for the delivery of cancer therapeutics. European Journal of Pharmaceutical Sciences. 2013;48:416-27.
[18] Fang J, Nakamura H, Maeda H. The EPR effect: Unique features of tumor blood vessels for drug delivery, factors involved, and limitations and augmentation of the effect. Advanced Drug Delivery Reviews. 2011;63:136-51.
[19] Maeda H, Wu J, Sawa T, Matsumura Y, Hori K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. Journal of Controlled Release. 2000;65:271-84.
[20] Rao JP, Geckeler KE. Polymer nanoparticles: Preparation techniques and size-control parameters. Progress in Polymer Science. 2011;36:887-913.
[21] Croy SR, Kwon GS. Polymeric Micelles for Drug Delivery. Current Pharmaceutical Design. 2006;12:4669-84.
[22] Truong NP, Quinn JF, Whittaker MR, Davis TP. Polymeric filomicelles and nanoworms: two decades of synthesis and application. Polymer Chemistry. 2016;7:4295-312.
[23] Brinkhuis RP, Rutjes FPJT, van Hest JCM. Polymeric vesicles in biomedical applications. Polymer Chemistry. 2011;2:1449-62.
[24] Anton N, Benoit J-P, Saulnier P. Design and production of nanoparticles formulated from nano-emulsion templates—A review. Journal of Controlled Release. 2008;128:185-99.
[25] Zheng Y, Li S, Weng Z, Gao C. Hyperbranched polymers: advances from synthesis to applications. Chemical Society Reviews. 2015;44:4091-130.
[26] Abbasi E, Aval SF, Akbarzadeh A, Milani M, Nasrabadi HT, Joo SW, Hanifehpour Y, Nejati-Koshki K, Pashaei-Asl R. Dendrimers: synthesis, applications, and properties. Nanoscale Research Letters. 2014;9:247-.
[27] Raemdonck K, Demeester J, De Smedt S. Advanced nanogel engineering for drug delivery. Soft Matter. 2009;5:707-15.

[28] Matyjaszewski K, Xia J. Atom Transfer Radical Polymerization. Chemical Reviews. 2001;101:2921-90.
[29] Hawker CJ, Bosman AW, Harth E. New Polymer Synthesis by Nitroxide Mediated Living Radical Polymerizations. Chemical Reviews. 2001;101:3661-88.
[30] Nicolas J, Guillaneuf Y, Lefay C, Bertin D, Gigmes D, Charleux B. Nitroxide-mediated polymerization. Progress in Polymer Science. 2013;38:63-235.
[31] Anastasaki A, Nikolaou V, Haddleton DM. Cu(0)-mediated living radical polymerization: recent highlights and applications; a perspective. Polymer Chemistry. 2016;7:1002-26.
[32] Perrier S. 50th Anniversary Perspective: RAFT Polymerization—A User Guide. Macromolecules. 2017;50:7433-47.
[33] Matyjaszewski K, Spanswick J. Controlled/living radical polymerization. Materials Today. 2005;8:26-33.
[34] Whitfield R, Parkatzidis K, Rolland M, Truong NP, Anastasaki A. Tuning Dispersity by Photoinduced Atom Transfer Radical Polymerisation: Monomodal Distributions with ppm Copper Concentration. Angewandte Chemie International Edition. 2019.
[35] Gentekos DT, Dupuis LN, Fors BP. Beyond Dispersity: Deterministic Control of Polymer Molecular Weight Distribution. Journal of the American Chemical Society. 2016;138:1848-51.
[36] Chong YK, Le TPT, Moad G, Rizzardo E, Thang SH. A More Versatile Route to Block Copolymers and Other Polymers of Complex Architecture by Living Radical Polymerization: The RAFT Process. Macromolecules. 1999;32:2071-4.
[37] Tanaka J, Gurnani P, Cook AB, Häkkinen S, Zhang J, Yang J, Kerr A, Haddleton DM, Perrier S, Wilson P. Microscale synthesis of multiblock copolymers using ultrafast RAFT polymerisation. Polymer Chemistry. 2019;10:1186-91.
[38] Mansfeld U, Pietsch C, Hoogenboom R, Becer CR, Schubert US. Clickable initiators, monomers and polymers in controlled radical polymerizations - a prospective combination in polymer science. Polymer Chemistry. 2010;1:1560-98.
[39] Klok HA, Lecommandoux S. Supramolecular Materials via Block Copolymer Self-Assembly. Advanced Materials. 2001;13:1217-29.
[40] Rösler A, Vandermeulen GWM, Klok H-A. Advanced drug delivery devices via self-assembly of amphiphilic block copolymers. Advanced Drug Delivery Reviews. 2001;53:95-108.
[41] Martin L, Gurnani P, Zhang J, Hartlieb M, Cameron NR, Eissa AM, Perrier S. Polydimethylsiloxane-Based Giant Glycosylated Polymersomes with Tunable Bacterial Affinity. Biomacromolecules. 2019;20:1297-307.
[42] Terreau O, Bartels C, Eisenberg A. Effect of Poly(acrylic acid) Block Length Distribution on Polystyrene- b -poly(acrylic acid) Block Copolymer Aggregates in Solution. 2. A Partial Phase Diagram. Langmuir. 2004;20:637-45.
[43] Terreau O, Luo L, Eisenberg A. Effect of Poly(acrylic acid) Block Length Distribution on Polystyrene- b -Poly(acrylic acid) Aggregates in Solution. 1. Vesicles. Langmuir. 2003;19:5601-7.
[44] Jiang Y, Chen T, Ye F, Liang H, Shi A-C. Effect of Polydispersity on the Formation of Vesicles from Amphiphilic Diblock Copolymers. Macromolecules. 2005;38:6710-7.
[45] Paliwal R, Babu RJ, Palakurthi S. Nanomedicine Scale-up Technologies: Feasibilities and Challenges. AAPS PharmSciTech. 2014;15:1527-34.
[46] Thickett SC, Gilbert RG. Emulsion polymerization: State of the art in kinetics and mechanisms. Polymer. 2007;48:6965-91.
[47] Kawaguchi S, Ito K. Dispersion Polymerization. In: Okubo M, editor. Polymer Particles. Berlin, Heidelberg: Springer Berlin Heidelberg; 2005. p. 299-328.
[48] Yuan HG, Kalfas G, Ray WH. SUSPENSION POLYMERIZATION. Journal of Macromolecular Science, Part C. 1991;31:215-99.
[49] Asua JM. Miniemulsion polymerization. Progress in Polymer Science. 2002;27:1283-346.
[50] Vanderhoff JW, Bradford EB, Tarkowski HL, Shaffer JB, Wiley RM. Inverse Emulsion Polymerization. Polymerisation and Polycondensation Processes: American Chemical Society; 1962. p. 32-51.
[51] Prescott SW, Ballard MJ, Rizzardo E, Gilbert RG. RAFT in Emulsion Polymerization: What Makes it Different? Australian Journal of Chemistry. 2002;55:415-24.
[52] Zetterlund PB, Kagawa Y, Okubo M. Controlled/Living Radical Polymerization in Dispersed Systems. Chemical Reviews. 2008;108:3747-94.
[53] Bon SAF, Bosveld M, Klumperman B, German AL. Controlled Radical Polymerization in Emulsion. Macromolecules. 1997;30:324-6.
[54] Cunningham MF. Controlled/living radical polymerization in aqueous dispersed systems. Progress in Polymer Science. 2008;33:365-98.
[55] Save M, Guillaneuf Y, Gilbert RG. Controlled Radical Polymerization in Aqueous Dispersed Media. Australian Journal of Chemistry. 2006;59:693-711.
[56] McLeary JB, Klumperman B. RAFT mediated polymerisation in heterogeneous media. Soft Matter. 2006;2:45-53.

[57] Cunningham MF. Recent progress in nitroxide-mediated polymerizations in miniemulsion. Comptes Rendus Chimie. 2003;6:1351-74.
[58] Cunningham MF. Living/controlled radical polymerizations in dispersed phase systems. Progress in Polymer Science. 2002;27:1039-67.
[59] Qiu J, Charleux B, Matyjaszewski K. Controlled/living radical polymerization in aqueous media: homogeneous and heterogeneous systems. Progress in Polymer Science. 2001;26:2083-134.
[60] Min K, Matyjaszewski K. Atom transfer radical polymerization in aqueous dispersed media. Open Chemistry2009. p. 657.
[61] Kwon OJ. Recent advances in controlled/living radical polymerization in emulsion and dispersion. Journal of Polymer Science Part A: Polymer Chemistry. 2008;46:6983-7001.
[62] Boursier T, Chaduc I, Rieger J, D'Agosto F, Lansalot M, Charleux B. Controlled radical polymerization of styrene in miniemulsion mediated by PEO-based trithiocarbonate macromolecular RAFT agents. Polymer Chemistry. 2011;2:355-62.
[63] Yitao Y, Qinghua Z, Xiaoli Z, Fengqiu C. Interfacially confined RAFT miniemulsion copolymerization of styrene and butadiene. Journal of Applied Polymer Science. 2013;127:2557-65.
[64] Wang Y, Jiang G, Sun X, Ding M, Hu H, Chen W. Preparation of shell cross-linked nanoparticles via miniemulsion RAFT polymerization. Polymer Chemistry. 2010;1:1638-43.
[65] Pham BTT, Nguyen D, Ferguson CJ, Hawkett BS, Serelis AK, Such CH. Miniemulsion Polymerization Stabilized by Amphipathic Macro RAFT Agents. Macromolecules. 2003;36:8907-9.
[66] Dong H, Matyjaszewski K. Thermally Responsive P(M(EO)2MA-co-OEOMA) Copolymers via AGET ATRP in Miniemulsion. Macromolecules. 2010;43:4623-8.
[67] Min K, Gao H, Matyjaszewski K. Preparation of Homopolymers and Block Copolymers in Miniemulsion by ATRP Using Activators Generated by Electron Transfer (AGET). Journal of the American Chemical Society. 2005;127:3825-30.
[68] Oh J, Tang C, Gao H, Tsarevsky NV, Matyjaszewski K. Inverse Miniemulsion ATRP: A New Method for Synthesis and Functionalization of Well-Defined Water-Soluble/Cross-Linked Polymeric Particles. Journal of the American Chemical Society. 2006;128:5578-84.
[69] B. ZP, Nur AM, Hideto M, Masayoshi O. Nitroxide-Mediated Controlled/Living Free Radical Copolymerization of Styrene and Divinylbenzene in Aqueous Miniemulsion. Macromolecular Rapid Communications. 2005;26:955-60.
[70] E. ET, F. CM, Barkev K. Nitroxide-Mediated Bulk and Miniemulsion Polymerization in a Continuous Tubular Reactor: Synthesis of Homo-, Di- and Triblock Copolymers. Macromolecular Reaction Engineering. 2010;4:186-96.
[71] Nicolas J, Charleux B, Guerret O, Magnet S. Novel SG1-Based Water-Soluble Alkoxyamine for Nitroxide-Mediated Controlled Free-Radical Polymerization of Styrene and n-Butyl Acrylate in Miniemulsion. Macromolecules. 2004;37:4453-63.
[72] Céline F, Julien N, Bernadette C. Kinetic study of the nitroxide-mediated controlled free-radical polymerization of n-butyl acrylate in aqueous miniemulsions. Journal of Polymer Science Part A: Polymer Chemistry. 2002;40:4410-20.
[73] de la Fuente JM, Penadés S. Glyconanoparticles: Types, synthesis and applications in glycoscience, biomedicine and material science. Biochimica et Biophysica Acta (BBA) - General Subjects. 2006;1760:636-51.
[74] Ladmiral V, Melia E, Haddleton DM. Synthetic glycopolymers: an overview. European Polymer Journal. 2004;40:431-49.
[75] Al-Bagoury M, Buchholz K, Yaacoub EJ. Synthesis of well-designed polymers carrying saccharide moieties via RAFT miniemulsion polymerization. Polymers for Advanced Technologies. 2007;18:313-22.
[76] Grazon C, Rieger J, Méallet-Renault R, Charleux B, Clavier G. Ultrabright Fluorescent Polymeric Nanoparticles Made from a New Family of BODIPY Monomers. Macromolecules. 2013;46:5167-76.
[77] Grazon C, Rieger J, Méallet-Renault R, Clavier G, Charleux B. One-Pot Synthesis of Pegylated Fluorescent Nanoparticles by RAFT Miniemulsion Polymerization Using a Phase Inversion Process. Macromolecular Rapid Communications. 2011;32:699-705.
[78] Qu J-B, Chapman R, Chen F, Lu H, Stenzel MH. Swollen Micelles for the Preparation of Gated, Squeezable, pH-Responsive Drug Carriers. ACS applied materials & interfaces. 2017;9:13865-74.
[79] Pan Y-J, Chen Y-Y, Wang D-R, Wei C, Guo J, Lu D-R, Chu C-C, Wang C-C. Redox/pH dual stimuli-responsive biodegradable nanohydrogels with varying responses to dithiothreitol and glutathione for controlled drug release. Biomaterials. 2012;33:6570-9.
[80] Wang Y, Luo Y, Zhao Q, Wang Z, Xu Z, Jia X. An Enzyme-Responsive Nanogel Carrier Based on PAMAM Dendrimers for Drug Delivery. ACS Applied Materials & Interfaces. 2016;8:19899-906.
[81] Yusa S-i, Sugahara M, Endo T, Morishima Y. Preparation and Characterization of a pH-Responsive Nanogel Based on a Photo-Cross-Linked Micelle Formed From Block Copolymers with Controlled Structure. Langmuir. 2009;25:5258-65.

[82] Oh JK, Drumright R, Siegwart DJ, Matyjaszewski K. The development of microgels/nanogels for drug delivery applications. Progress in Polymer Science. 2008;33:448-77.
[83] Oishi M, Nagasaki Y. Stimuli-responsive smart nanogels for cancer diagnostics and therapy. Nanomedicine. 2010;5:451-68.
[84] Hasegawa U, Nomura S-iM, Kaul SC, Hirano T, Akiyoshi K. Nanogel-quantum dot hybrid nanoparticles for live cell imaging. Biochemical and Biophysical Research Communications. 2005;331:917-21.
[85] Sanson N, Rieger J. Synthesis of nanogels/microgels by conventional and controlled radical crosslinking copolymerization. Polymer Chemistry. 2010;1:965-77.
[86] Oh J, Siegwart DJ, Lee H-i, Sherwood G, Peteanu L, Hollinger JO, Kataoka K, Matyjaszewski K. Biodegradable Nanogels Prepared by Atom Transfer Radical Polymerization as Potential Drug Delivery Carriers: Synthesis, Biodegradation, in Vitro Release, and Bioconjugation. Journal of the American Chemical Society. 2007;129:5939-45.
[87] Oh JK, Siegwart DJ, Matyjaszewski K. Synthesis and biodegradation of nanogels as delivery carriers for carbohydrate drugs. Biomacromolecules. 2007;8:3326-31.
[88] Siegwart DJ, Srinivasan A, Bencherif SA, Karunanidhi A, Oh J, Vaidya S, Jin R, Hollinger JO, Matyjaszewski K. Cellular Uptake of Functional Nanogels Prepared by Inverse Miniemulsion ATRP with Encapsulated Proteins, Carbohydrates, and Gold Nanoparticles. Biomacromolecules. 2009;10:2300-9.
[89] Averick SE, Magenau AJD, Simakova A, Woodman BF, Seong A, Mehl RA, Matyjaszewski K. Covalently incorporated protein –nanogels using AGET ATRP in an inverse miniemulsion. Polymer Chemistry. 2011;2:1476-8.
[90] Averick SE, Paredes E, Irastorza A, Shrivats AR, Srinivasan A, Siegwart DJ, Magenau AJ, Cho HY, Hsu E, Averick AA, Kim J, Liu S, Hollinger JO, Das SR, Matyjaszewski K. Preparation of Cationic Nanogels for Nucleic Acid Delivery. Biomacromolecules. 2012;13:3445-9.
[91] Shrivats AR, Hsu E, Averick S, Klimak M, Watt ACS, DeMaio M, Matyjaszewski K, Hollinger JO. Cationic Nanogel-mediated Runx2 and Osterix siRNA Delivery Decreases Mineralization in MC3T3 Cells. Clinical Orthopaedics and Related Research®. 2015;473:2139-49.
[92] Cao B, Zheng Y, Xi T, Zhang C, Song W, Burugapalli K, Yang H, Ma Y. Concentration-dependent cytotoxicity of copper ions on mouse fibroblasts in vitro: effects of copper ion release from TCu380A vs TCu220C intra-uterine devices. Biomedical Microdevices. 2012;14:709-20.
[93] Oliveira MM, Boyer C, Nele M, Pinto J, Zetterlund PB, Davis TP. Synthesis of Biodegradable Hydrogel Nanoparticles for Bioapplications Using Inverse Miniemulsion RAFT Polymerization. Macromolecules. 2011;44:7167-75.
[94] Wutzel H, Richter FH, Li Y, Sheiko SS, Klok H-A. Poly[N -(2-hydroxypropyl)methacrylamide] nanogels by RAFT polymerization in inverse emulsion. Polymer Chemistry. 2013;5:1711-9.
[95] Vasey PA, Kaye SB, Morrison R, Twelves C, Wilson P, Duncan R, Thomson AH, Murray LS, Hilditch TE, Murray T, Burtles S, Fraier D, Frigerio E, Cassidy J. Phase I Clinical and Pharmacokinetic Study of PK1 [N-(2-Hydroxypropyl)methacrylamide Copolymer Doxorubicin]: First Member of a New Class of Chemotherapeutic Agents—Drug-Polymer Conjugates. Clinical Cancer Research. 1999;5:83-94.
[96] Li W, Matyjaszewski K, Albrecht K, Möller M. Reactive Surfactants for Polymeric Nanocapsules via Interfacially Confined Miniemulsion ATRP. Macromolecules. 2009;42:8228-33.
[97] Ryan J, Aldabbagh F, Zetterlund PB, Okubo M. First nitroxide-mediated free radical dispersion polymerizations of styrene in supercritical carbon dioxide. Polymer. 2005;46:9769-77.
[98] Albanese A, Tang PS, Chan WCW. The Effect of Nanoparticle Size, Shape, and Surface Chemistry on Biological Systems. Annual Review of Biomedical Engineering. 2012;14:1-16.
[99] Warren NJ, Armes SP. Polymerization-Induced Self-Assembly of Block Copolymer Nano-objects via RAFT Aqueous Dispersion Polymerization. Journal of the American Chemical Society. 2014;136:10174-85.
[100] Mai Y, Eisenberg A. Self-assembly of block copolymers. Chemical Society Reviews. 2012;41:5969-85.
[101] Blanazs A, Ryan AJ, Armes SP. Predictive Phase Diagrams for RAFT Aqueous Dispersion Polymerization: Effect of Block Copolymer Composition, Molecular Weight, and Copolymer Concentration. Macromolecules. 2012;45:5099-107.
[102] Qiao XG, Lansalot M, Bourgeat-Lami E, Charleux B. Nitroxide-Mediated Polymerization-Induced Self-Assembly of Poly(poly(ethylene oxide) methyl ether methacrylate-co-styrene)-b-poly(n-butyl methacrylate-co-styrene) Amphiphilic Block Copolymers. Macromolecules. 2013;46:4285-95.
[103] Wang G, Schmitt M, Wang Z, Lee B, Pan X, Fu L, Yan J, Li S, Xie G, Bockstaller MR, Matyjaszewski K. Polymerization-Induced Self-Assembly (PISA) Using ICAR ATRP at Low Catalyst Concentration. Macromolecules. 2016;49:8605-15.
[104] Cordella D, Ouhib F, Aqil A, Defize T, Jérôme C, Serghei A, Drockenmuller E, Aissou K, Taton D, Detrembleur C. Fluorinated Poly(ionic liquid) Diblock Copolymers Obtained by Cobalt-Mediated Radical Polymerization-Induced Self-Assembly. ACS Macro Letters. 2017;6:121-6.

[105] Sarkar J, Xiao L, Jackson AW, van Herk AM, Goto A. Synthesis of transition-metal-free and sulfur-free nanoparticles and nanocapsules via reversible complexation mediated polymerization (RCMP) and polymerization induced self-assembly (PISA). Polymer Chemistry. 2018;9:4900-7.
[106] Yukiya K, Kazuya K, Hideto M, Masayoshi O. Preparation of poly(n-butyl acrylate)-b-polystyrene particles by emulsifier-free, organotellurium-mediated living radical emulsion polymerization (emulsion TERP). Journal of Polymer Science Part A: Polymer Chemistry. 2012;50:1991-6.
[107] Zhou D, Kuchel RP, Zetterlund PB. A new paradigm in polymerization induced self-assembly (PISA): Exploitation of “non-living” addition–fragmentation chain transfer (AFCT) polymerization. Polymer Chemistry. 2017;8:4177-81.
[108] Lotierzo A, Schofield RM, Bon SAF. Toward Sulfur-Free RAFT Polymerization Induced Self-Assembly. ACS Macro Letters. 2017:1438-43.
[109] Khor S, Quinn JF, Whittaker MR, Truong NP, Davis TP. Controlling Nanomaterial Size and Shape for Biomedical Applications via Polymerization-Induced Self-Assembly. Macromolecular Rapid Communications. 2019;40.
[110] Canning SL, Smith GN, Armes SP. A Critical Appraisal of RAFT-Mediated Polymerization-Induced Self-Assembly. Macromolecules. 2016;49:1985-2001.
[111] An Z, Shi Q, Tang W, Tsung C-K, Hawker CJ, Stucky GD. Facile RAFT Precipitation Polymerization for the Microwave-Assisted Synthesis of Well-Defined, Double Hydrophilic Block Copolymers and Nanostructured Hydrogels. Journal of the American Chemical Society. 2007;129:14493-9.
[112] Grazon C, Rieger J, Sanson N, Charleux B. Study of poly(N,N-diethylacrylamide) nanogel formation by aqueous dispersion polymerization of N,N-diethylacrylamide in the presence of poly(ethylene oxide)-b-poly(N,N-dimethylacrylamide) amphiphilic macromolecular RAFT agents. Soft Matter. 2011;7:3482-90.
[113] Liu G, Qiu Q, Shen W, An Z. Aqueous Dispersion Polymerization of 2-Methoxyethyl Acrylate for the Synthesis of Biocompatible Nanoparticles Using a Hydrophilic RAFT Polymer and a Redox Initiator. Macromolecules. 2011;44:5237-45.
[114] Liu G, Qiu Q, An Z. Development of thermosensitive copolymers of poly(2-methoxyethyl acrylate-co-poly(ethylene glycol) methyl ether acrylate) and their nanogels synthesized by RAFT dispersion polymerization in water. Polymer Chemistry. 2012;3:504-13.
[115] Shen W, Chang Y, Liu G, Wang H, Cao A, An Z. Biocompatible, Antifouling, and Thermosensitive Core−Shell Nanogels Synthesized by RAFT Aqueous Dispersion Polymerization. Macromolecules. 2011;44:2524-30.
[116] Blanazs A, Madsen J, Battaglia G, Ryan AJ, Armes SP. Mechanistic Insights for Block Copolymer Morphologies: How Do Worms Form Vesicles? Journal of the American Chemical Society. 2011;133:16581-7.
[117] Byard SJ, Williams M, McKenzie BE, Blanazs A, Armes SP. Preparation and Cross-Linking of All-Acrylamide Diblock Copolymer Nano-Objects via Polymerization-Induced Self-Assembly in Aqueous Solution. Macromolecules. 2017;50:1482-93.
[118] Wang X, Zhou J, Lv X, Zhang B, An Z. Temperature-Induced Morphological Transitions of Poly(dimethylacrylamide)–Poly(diacetone acrylamide) Block Copolymer Lamellae Synthesized via Aqueous Polymerization-Induced Self-Assembly. Macromolecules. 2017;50:7222-32.
[119] Zhou W, Qu Q, Xu Y, An Z. Aqueous Polymerization-Induced Self-Assembly for the Synthesis of Ketone-Functionalized Nano-Objects with Low Polydispersity. ACS Macro Letters. 2015;4:495-9.
[120] Figg CA, Simula A, Gebre KA, Tucker BS, Haddleton DM, Sumerlin BS. Polymerization-induced thermal self-assembly (PITSA). Chemical Science. 2015;6:1230-6.
[121] Blanazs A, Verber R, Mykhaylyk OO, Ryan AJ, Heath JZ, Douglas CWI, Armes SP. Sterilizable Gels from Thermoresponsive Block Copolymer Worms. Journal of the American Chemical Society. 2012;134:9741-8.
[122] Canton I, Warren NJ, Chahal A, Amps K, Wood A, Weightman R, Wang E, Moore H, Armes SP. Mucin-Inspired Thermoresponsive Synthetic Hydrogels Induce Stasis in Human Pluripotent Stem Cells and Human Embryos. ACS Central Science. 2016;2:65-74.
[123] Ladmiral V, Semsarilar M, Canton I, Armes SP. Polymerization-Induced Self-Assembly of Galactose-Functionalized Biocompatible Diblock Copolymers for Intracellular Delivery. Journal of the American Chemical Society. 2013;135:13574-81.
[124] Mitchell DE, Lovett JR, Armes SP, Gibson MI. Combining Biomimetic Block Copolymer Worms with an Ice-Inhibiting Polymer for the Solvent-Free Cryopreservation of Red Blood Cells. Angewandte Chemie International Edition. 2016;55:2801-4.
[125] Jonathan Y, Cyrille B. Photoinitiated Polymerization-Induced Self-Assembly (Photo-PISA): New Insights and Opportunities. Advanced Science. 2017;4:1700137.
[126] Blackman LD, Doncom KEB, Gibson MI, O'Reilly RK. Comparison of photo- and thermally initiated polymerization-induced self-assembly: a lack of end group fidelity drives the formation of higher order morphologies. Polymer Chemistry. 2017;8:2860-71.

[127] Biggs CI, Bailey TL, Ben G, Stubbs C, Fayter A, Gibson MI. Polymer mimics of biomacromolecular antifreezes. Nature Communications. 2017;8:1546.
[128] He C, Hu Y, Yin L, Tang C, Yin C. Effects of particle size and surface charge on cellular uptake and biodistribution of polymeric nanoparticles. Biomaterials. 2010;31:3657-66.
[129] Karagoz B, Boyer C, Davis TP. Simultaneous Polymerization-Induced Self-Assembly (PISA) and Guest Molecule Encapsulation. Macromolecular Rapid Communications. 2014;35:417-21.
[130] Karagoz B, Esser L, Duong HT, Basuki JS, Boyer C, Davis TP. Polymerization-Induced Self-Assembly (PISA) – control over the morphology of nanoparticles for drug delivery applications. Polymer Chemistry. 2013;5:350-5.
[131] Qiu L, Xu C-R, Zhong F, Hong C-Y, Pan C-Y. Fabrication of Functional Nano-objects through RAFT Dispersion Polymerization and Influences of Morphology on Drug Delivery. ACS Applied Materials & Interfaces. 2016;8:18347-59.
[132] Zhang S, Li J, Lykotrafitis G, Bao G, Suresh S. Size-Dependent Endocytosis of Nanoparticles. Advanced materials (Deerfield Beach, Fla). 2009;21:419-24.
[133] Hinde E, Thammasiraphop K, Duong HTT, Yeow J, Karagoz B, Boyer C, Gooding JJ, Gaus K. Pair correlation microscopy reveals the role of nanoparticle shape in intracellular transport and site of drug release. Nature Nanotechnology. 2016;12:nnano.2016.160.
[134] Kaga S, Truong NP, Esser L, Senyschyn D, Sanyal A, Sanyal R, Quinn JF, Davis TP, Kaminskas LM, Whittaker MR. Influence of Size and Shape on the Biodistribution of Nanoparticles Prepared by Polymerization-Induced Self-Assembly (PISA). Biomacromolecules. 2017;18:3963-70.
[135] Ding Y, Zhang X, Xu Y, Cheng T, Ou H, Li Z, An Y, Shen W, Liu Y, Shi L. Polymerization-induced self-assembly of large-scale iohexol nanoparticles as contrast agents for X-ray computed tomography imaging. Polymer Chemistry. 2018;9:2926-35.
[136] Zhao W, Ta HT, Zhang C, Whittaker AK. Polymerization-Induced Self-Assembly (PISA) - Control over the Morphology of (19)F-Containing Polymeric Nano-objects for Cell Uptake and Tracking. Biomacromolecules. 2017;18:1145-56.
[137] Esser L, Truong NP, Karagoz B, Moffat BA, Boyer C, Quinn JF, Whittaker MR, Davis TP. Gadolinium-functionalized nanoparticles for application as magnetic resonance imaging contrast agents via polymerization-induced self-assembly. Polymer Chemistry. 2016;7:7325-37.
[138] Karagoz B, Yeow J, Esser L, Prakash SM, Kuchel RP, Davis TP, Boyer C. An efficient and highly versatile synthetic route to prepare iron oxide nanoparticles/nanocomposites with tunable morphologies. Langmuir : the ACS journal of surfaces and colloids. 2014;30:10493-502.
[139] Sobotta FH, Hausig F, Harz DO, Hoeppener S, Schubert US, Brendel JC. Oxidation-responsive micelles by a one-pot polymerization-induced self-assembly approach. Polymer Chemistry. 2018;9:1593-602.
[140] Alarcon CdlH, Pennadam S, Alexander C. Stimuli responsive polymers for biomedical applications. Chemical Society Reviews. 2005;34:276-85.
[141] Zhang W-J, Hong C-Y, Pan C-Y. Fabrication of Reductive-Responsive Prodrug Nanoparticles with Superior Structural Stability by Polymerization-Induced Self-Assembly and Functional Nanoscopic Platform for Drug Delivery. Biomacromolecules. 2016;17:2992-9.
[142] Zare-Mirakabadi A, Sarzaeem A, Moradhaseli S, Sayad A, Negahdary M. Necrotic Effect versus Apoptotic Nature of Camptothecin in Human Cervical Cancer Cells. Iranian Journal of Cancer Prevention. 2012;5:109-16.
[143] Zhang W-J, Hong C-Y, Pan C-Y. Efficient Fabrication of Photosensitive Polymeric Nano-objects via an Ingenious Formulation of RAFT Dispersion Polymerization and Their Application for Drug Delivery. Biomacromolecules. 2017;18:1210-7.
[144] Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. Reactive Oxygen Species in Inflammation and Tissue Injury. Antioxidants & Redox Signaling. 2014;20:1126-67.
[145] Lee JS, Feijen J. Polymersomes for drug delivery: Design, formation and characterization. Journal of Controlled Release. 2012;161:473-83.
[146] O'Neil CP, Suzuki T, Demurtas D, Finka A, Hubbell JA. A Novel Method for the Encapsulation of Biomolecules into Polymersomes via Direct Hydration. Langmuir. 2009;25:9025-9.
[147] Tan J, Sun H, Yu M, Sumerlin BS, Zhang L. Photo-PISA: Shedding Light on Polymerization-Induced Self-Assembly. ACS Macro Letters. 2015;4:1249-53.
[148] Blackman LD, Varlas S, Arno MC, Fayter A, Gibson MI, O'Reilly RK. Permeable Protein-Loaded Polymersome Cascade Nanoreactors by Polymerization-Induced Self-Assembly. ACS Macro Letters. 2017;6:1263-7.
[149] Blackman LD, Varlas S, Arno MC, Houston ZH, Fletcher NL, Thurecht KJ, Hasan M, Gibson MI, O'Reilly RK. Confinement of Therapeutic Enzymes in Selectively Permeable Polymer Vesicles by Polymerization-Induced Self-Assembly (PISA) Reduces Antibody Binding and Proteolytic Susceptibility. ACS Central Science. 2018;4:718-23.

[150] Varlas S, Georgiou PG, Bilalis P, Jones JR, Hadjichristidis N, O'Reilly RK. Poly(sarcosine)-Based Nano-Objects with Multi-Protease Resistance by Aqueous Photoinitiated Polymerization-Induced Self-Assembly (Photo-PISA). Biomacromolecules. 2018;19:4453-62.
[151] Varlas S, Blackman LD, Findlay HE, Reading E, Booth PJ, Gibson MI, O'Reilly RK. Photoinitiated Polymerization-Induced Self-Assembly in the Presence of Surfactants Enables Membrane Protein Incorporation into Vesicles. Macromolecules. 2018;51:6190-201.
[152] Ng G, Yeow J, Xu J, Boyer C. Application of oxygen tolerant PET-RAFT to polymerization-induced self-assembly. Polymer Chemistry. 2017;8:2841-51.
[153] Tan J, Liu D, Bai Y, Huang C, Li X, He J, Xu Q, Zhang L. Enzyme-Assisted Photoinitiated Polymerization-Induced Self-Assembly: An Oxygen-Tolerant Method for Preparing Block Copolymer Nano-Objects in Open Vessels and Multiwell Plates. Macromolecules. 2017;50:5798-806.
[154] Holdsworth CI, Jia Z, Monteiro MJ. RAFT-mediated emulsion polymerization of styrene with a thermoresponsive MacroCTA. Polymer. 2016;106:200-7.
[155] Truong NP, Whittaker MR, Anastasaki A, Haddleton DM, Quinn JF, Davis TP. Facile production of nanoaggregates with tuneable morphologies from thermoresponsive P(DEGMA- co -HPMA). Polymer Chemistry. 2015;7:430-40.
[156] Truong NP, Quinn JF, Anastasaki A, Rolland M, Vu MN, Haddleton DM, Whittaker MR, Davis TP. Surfactant-free RAFT emulsion polymerization using a novel biocompatible thermoresponsive polymer. Polymer Chemistry. 2017;8:1353-63.
[157] Truong NP, Zhang C, Nguyen TAH, Anastasaki A, Schulze MW, Quinn JF, Whittaker AK, Hawker CJ, Whittaker MR, Davis TP. Overcoming Surfactant-Induced Morphology Instability of Noncrosslinked Diblock Copolymer Nano-Objects Obtained by RAFT Emulsion Polymerization. ACS Macro Letters. 2018;7:159-65.
[158] Ferguson CJ, Hughes RJ, Pham BT, Hawkett BS, Gilbert RG, Serelis AK, Such CH. Effective ab initio emulsion polymerization under RAFT control. Macromolecules. 2002;35:9243-5.
[159] Ferguson CJ, Hughes RJ, Nguyen D, Pham BT, Gilbert RG, Serelis AK, Such CH, Hawkett BS. Ab Initio Emulsion Polymerization by RAFT-Controlled Self-Assembly §. Macromolecules. 2005;38:2191-204.
[160] Charleux B, Nicolas J. Water-soluble SG1-based alkoxyamines: A breakthrough in controlled/living free-radical polymerization in aqueous dispersed media. Polymer. 2007;48:5813-33.
[161] Delaittre G, Nicolas J, Lefay C, Save M, Charleux B. Aqueous suspension of amphiphilic diblock copolymer nanoparticles prepared in situ from a water-soluble poly(sodium acrylate) alkoxyamine macroinitiator. Soft Matter. 2006;2:223-31.
[162] Nicolas J, Charleux B, Guerret O, Magnet S. Nitroxide-Mediated Controlled Free-Radical Emulsion Polymerization Using a Difunctional Water-Soluble Alkoxyamine Initiator. Toward the Control of Particle Size, Particle Size Distribution, and the Synthesis of Triblock Copolymers. Macromolecules. 2005;38:9963-73.
[163] Delaittre G, Nicolas J, Lefay C, Save M, Charleux B. Surfactant-free synthesis of amphiphilic diblock copolymer nanoparticles via nitroxide-mediated emulsion polymerization. Chemical Communications. 2005;0:614-6.
[164] S. Z, B. X, Y. X. PMMA-co-PHFBA-co-PHPA acrylic fluoropolymers via an ab initio emulsion atom transfer radical polymerisation with activators regenerated by electron transfer. Pigment & Resin Technology. 2012;41:95-9.
[165] Cheng C, Gong S, Fu Q, Shen L, Liu Z, Qiao Y, Fu C. Hexamethylenetetramine as both a ligand and a reducing agent in AGET atom transfer radical batch emulsion polymerization. Polymer Bulletin. 2011;66:735-46.
[166] Shu J, Cheng C, Zheng Y, Shen L, Qiao Y, Fu C. "One pot" synthesis of fluorinated block copolymers using a surface-active ATRP initiator under emulsion polymerization conditions. Polymer Bulletin. 2011;67:1185-200.
[167] Cheng C, Shu J, Gong S, Shen L, Qiao Y, Fu C. Synthesis and use of a surface-active initiator in emulsion polymerization under AGET and ARGET ATRP conditions. New Journal of Chemistry. 2010;34:163-70.
[168] Zhou J, Yao H, Ma J. Recent advances in RAFT-mediated surfactant-free emulsion polymerization. Polymer Chemistry. 2018;9:2532-61.
[169] Zhang W, D'Agosto F, Boyron O, Rieger J, Charleux B. Toward a Better Understanding of the Parameters that Lead to the Formation of Nonspherical Polystyrene Particles via RAFT-Mediated One-Pot Aqueous Emulsion Polymerization. Macromolecules. 2012;45:4075-84.
[170] Rieger J, Zhang W, Stoffelbach Fo, Charleux B. Surfactant-free RAFT emulsion polymerization using poly (N, N-dimethylacrylamide) trithiocarbonate macromolecular chain transfer agents. Macromolecules. 2010;43:6302-10.
[171] Manguian M, Save M, Charleux B. Batch Emulsion Polymerization of Styrene Stabilized by a Hydrophilic Macro-RAFT Agent. Macromolecular rapid communications. 2006;27:399-404.
[172] Gurnani P, Lunn AM, Perrier S. Synthesis of mannosylated and PEGylated nanoparticles via RAFT emulsion polymerisation, and investigation of particle-lectin aggregation using turbidimetric and DLS techniques. Polymer. 2016;106:229-37.

[173] Davis TP, Truong Phuoc N, Dussert M, Whittaker MR, Quinn JF. Rapid Synthesis of Ultrahigh Molecular Weight and Low Polydispersity Polystyrene Diblock Copolymers by RAFT-mediated Emulsion Polymerization. Polymer Chemistry. 2015;6:3865-74.
[174] Alexis F, Pridgen E, Molnar LK, Farokhzad OC. Factors Affecting the Clearance and Biodistribution of Polymeric Nanoparticles. Molecular Pharmaceutics. 2008;5:505-15.
[175] Ting SSR, Min E, Zetterlund PB, Stenzel MH. Controlled/Living ab Initio Emulsion Polymerization via a Glucose RAFTstab: Degradable Cross-Linked Glyco-Particles for Concanavalin A/FimH Conjugations to Cluster E. coli Bacteria. Macromolecules. 2010;43:5211-21.
[176] Chaduc I, Reynaud E, Dumas L, Albertin L, D'Agosto F, Lansalot M. From well-defined poly(N-acryloylmorpholine)-stabilized nanospheres to uniform mannuronan- and guluronan-decorated nanoparticles by RAFT polymerization-induced self-assembly. Polymer. 2016;106:218-28.
[177] Jiang G, Wang Y, Zhang R, Wang R, Wang X, Zhang M, Sun X, Bao S, Wang T, Wang S. Preparation of Redox-Sensitive Shell Cross-Linked Nanoparticles for Controlled Release of Bioactive Agents. ACS Macro Letters. 2012;1:489-93.
[178] Gurnani P, Cook AB, Richardson RAE, Perrier S. A study on the preparation of alkyne functional nanoparticles via RAFT emulsion polymerisation. Polymer Chemistry. 2019;10:1452-9.
[179] Poon C, Tang O, Chen X-M, Pham BTT, Gody G, Pollock CA, Hawkett BS, Perrier S. Preparation of Inert Polystyrene Latex Particles as MicroRNA Delivery Vectors by Surfactant-Free RAFT Emulsion Polymerization. Biomacromolecules. 2016;17:965-73.
[180] Poon C, Tang O, Chen XM, Kim B, Hartlieb M, Pollock CA, Hawkett BS, Perrier S. Fluorescent Labeling and Biodistribution of Latex Nanoparticles Formed by Surfactant-Free RAFT Emulsion Polymerization. Macromolecular Bioscience. 2017;17:1600366.
[181] Gurnani P, Bray CP, Richardson RAE, Peltier R, Perrier S. Heparin-Mimicking Sulfonated Polymer Nanoparticles via RAFT Polymerization-Induced Self-Assembly. Macromolecular Rapid Communications. 2018.
[182] Bray C, Gurnani P, Mansfield EDH, Peltier R, Perrier S. Sulfonated Copolymers as Heparin-Mimicking Stabilizer of Fibroblast Growth Factor: Size, Architecture, and Monomer Distribution Effects. Biomacromolecules. 2019;20:285-93.
[183] Gurnani P, Sanchez-Cano C, Abraham K, Xandri-Monje H, Cook AB, Hartlieb M, Lévi F, Dallmann R, Perrier S. RAFT Emulsion Polymerization as a Platform to Generate Well-Defined Biocompatible Latex Nanoparticles. Macromolecular Bioscience. 2018.
[184] Khor SY, Vu MN, Pilkington EH, Johnston APR, Whittaker MR, Quinn JF, Truong NP, Davis TP. Elucidating the Influences of Size, Surface Chemistry, and Dynamic Flow on Cellular Association of Nanoparticles Made by Polymerization-Induced Self-Assembly. Small. 2018;14:1801702.
[185] Engström J, Hatton FL, Wågberg L, D'Agosto F, Lansalot M, Malmström E, Carlmark A. Soft and rigid core latex nanoparticles prepared by RAFT-mediated surfactant-free emulsion polymerization for cellulose modification – a comparative study. Polymer Chemistry. 2017;8:1061-73.
[186] Lesage de la Haye J, Zhang X, Chaduc I, Brunel F, Lansalot M, D'Agosto F. The Effect of Hydrophile Topology in RAFT-Mediated Polymerization-Induced Self-Assembly. Angewandte Chemie. 2016;128:3803-7.
[187] Rocca JD, Liu D, Lin W. Are high drug loading nanoparticles the next step forward for chemotherapy? Nanomedicine. 2012;7:303-5.
[188] Mura S, Nicolas J, Couvreur P. Stimuli-responsive nanocarriers for drug delivery. Nature Materials. 2013;12:991.
[189] Hans ML, Lowman AM. Biodegradable nanoparticles for drug delivery and targeting. Current Opinion in Solid State and Materials Science. 2002;6:319-27.
[190] Wang M, Thanou M. Targeting nanoparticles to cancer. Pharmacological Research. 2010;62:90-9.
[191] Camli ST, Buyukserin F, Balci O, Budak GG. Size controlled synthesis of sub-100nm monodisperse poly(methylmethacrylate) nanoparticles using surfactant-free emulsion polymerization. Journal of Colloid and Interface Science. 2010;344:528-32.
[192] Marc E, Rudolf Z. Surfactant-Free Emulsion Polymerization of Various Methacrylates: Towards Monodisperse Colloids for Polymer Opals. Macromolecular Chemistry and Physics. 2004;205:1479-88.
[193] Tauer K, Deckwer R, Kühn I, Schellenberg C. A comprehensive experimental study of surfactant-free emulsion polymerization of styrene. Colloid and Polymer Science. 1999;277:607-26.
[194] M. LA, Sébastien P. Synthesis of Sub-100 nm Glycosylated Nanoparticles via a One Step, Free Radical, and Surfactant Free Emulsion Polymerization. Macromolecular Rapid Communications.0:1800122.