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SYNTHESIS AND FUNCTIONALIZATION OF HYPERBRANCHED POLYMERS FOR TARGETED DRUG DELIVERY

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Hyperbranched polymers (HBPs) have found use in a wide range of applications, such as optical, electronic and magnetic materials, coatings, additives, supramolecular chemistry, and biomedicine. HBPs have gained attention for the development of drug delivery systems due to the presence of internal cavities in their three-dimensional globular structure that can be used to encapsulate drugs and their facile synthesis as compared to dendrimers. The composition, topology, and functionality of HBPs have been tuned to design drug carriers with better efficacies. Recent advances have been reported to introduce functional groups to enhance targeting tumor cells. HBPs have been modified to promote passive and active targeting. This review article will describe the different routes to synthesize hyperbranched polymer, their use as drug carriers for targeted drug delivery, and their functionalization with ligands for active targeting through various synthesis strategies to give the reader an extended overview of the progresses accomplished in this field. The modification of HBPs with ligands such as peptides, oligonucleotides, and folic acid

have been demonstrated to enhance the accumulation of the drug selectively at the tumor sites. The potential uses and developments of HBPs as nanoobjects for theranostics for example are discussed as perspectives.

1. INTRODUCTION

Drug delivery systems have been developed considering various types of materials including mesoporous silica nanoparticles,[1] lipids,[2, 3] and polymers.[4-6] Inorganic nanoparticles have gained interest due to their optical, magnetic and plasmonic properties, but show limitations in clinic due to their cytotoxicity and limited drug loading.[7, 8] Lipids and polymers offer high biocompatibility and improved drug loading capacity. While lipids are still more prevalent than polymers in clinical applications, the ability to tune their composition, topology, and functionality makes polymers attractive candidates. The developments in macromolecular engineering have led to the expansion of polymer topologies available. Among them, dendritic macromolecules mimic the branching of trees and possess attractive features such as high degree of branching units, high density of terminal functional groups, and their nanometric size. Dendritic macromolecules can be subdivided into dendrimers, dendrimer-like star polymers, hyperbranched polymers, and dendronized polymers. Dendrimers are characterized by a perfect regular structure and unimolecularity.[9] While dendrimer-like star polymers have similar regularity in the structure as dendrimers but differ by the nature of the branches that are linear polymer chains in this case,[10] hyperbranched polymers are highly and randomly branched macromolecules,[11] and dendronized polymers consist in dendrons attached as side chains to a linear polymer backbone.[12] Hyperbranched polymers (HBPs) have like dendrimers a three-dimensional globular structure that have attracted the attention from both academia and industry. The advantages of HBPs (**Figure 1**) as compared to linear polymers are their low intrinsic viscosity, low tendency to chain

entanglements, smaller hydrodynamic radius, good solubility and high degree of branching (DB) leading to a high number of terminal functional groups. When compared to dendrimers, their structures are irregular with dendritic, linear and terminal units randomly distributed, and their synthesis leads to macromolecules with broad molecular weight distributions. However, HBPs can be easily synthesized in a one-pot reaction and thus are more cost efficient as compared to the multi-step approach for the dendrimers requiring a purification step after each coupling reaction. Furthermore, due to their higher steric hindrance, dendrimers may be more challenging to functionalize than HBPs.

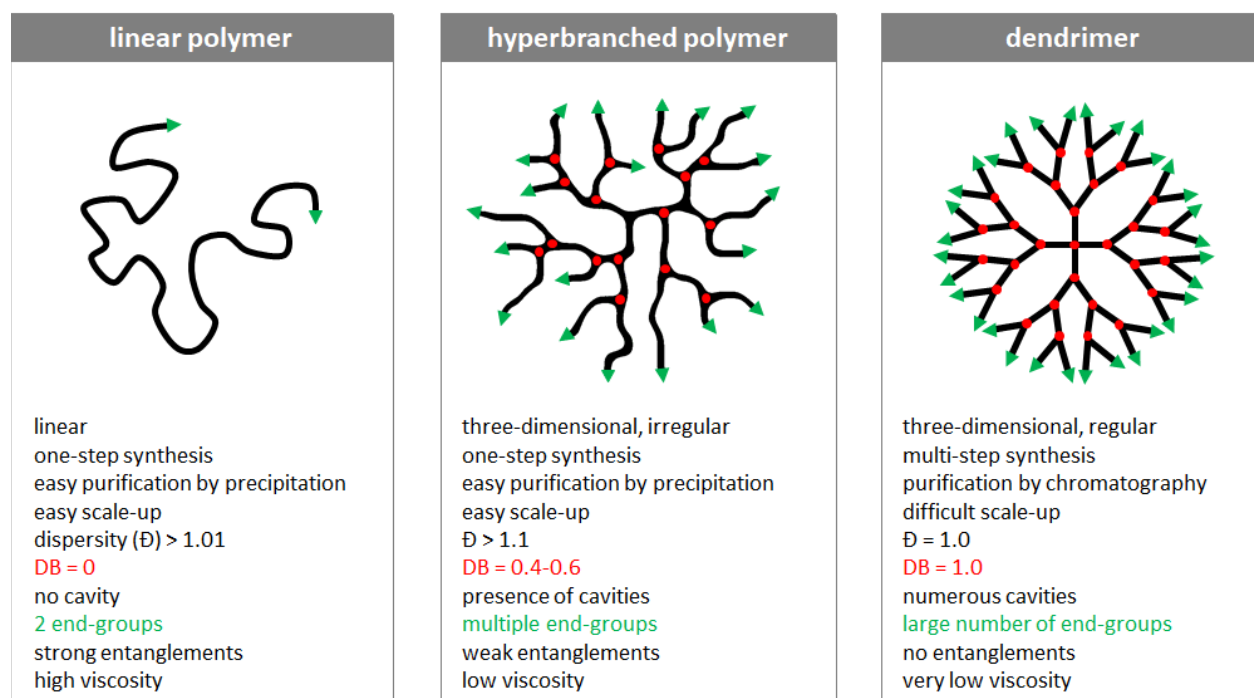


Figure 1. Comparison of HBPs with linear polymers and dendrimers.

HBPs have potential applications in optical, electronic and magnetic materials, coatings, additives, supramolecular chemistry, and biomedicine.[13-16] Their features are especially interesting for the development of nanocarriers in the field of drug delivery.[17, 18] The composition of HBPs (Figure 2) is tunable at the branching, linear, and terminal units offering a significant degree of

freedom in the design of nanocarriers for drug delivery. These units can be chosen to be responsive to one or multiple stimuli (*e.g.* pH,[19-23] temperature,[24-27] redox,[28-30] light,[31-35] enzyme[36, 37]) to induce a change in conformation of the polymer chain or its degradation to trigger drug release.[38] Their globular three-dimensional structures lead to the formation of internal cavities that can be used to encapsulate small-molecule drugs (less than 900 g mol⁻¹), *e.g.* doxorubicin (DOX) and paclitaxel (PTX) for cancer treatment, and radioisotopes, *e.g.* ^{99m}Tc, ¹³¹I, and ¹²⁵I, for diagnostic purposes. Furthermore, the high density of functional groups at the periphery of HBPs can be exploited to introduce functionalities on HBPs.[39] For biomedical applications, effective contrast agent probes for magnetic resonance imaging or targeting groups to promote the specific accumulation of drug carriers at the target site also known as targeted drug delivery have been considered. In this review, we propose to provide a comprehensive overview of the different types of targeting ligands used for targeted drug delivery and the strategies used to afford these HBP-based nanocarriers.

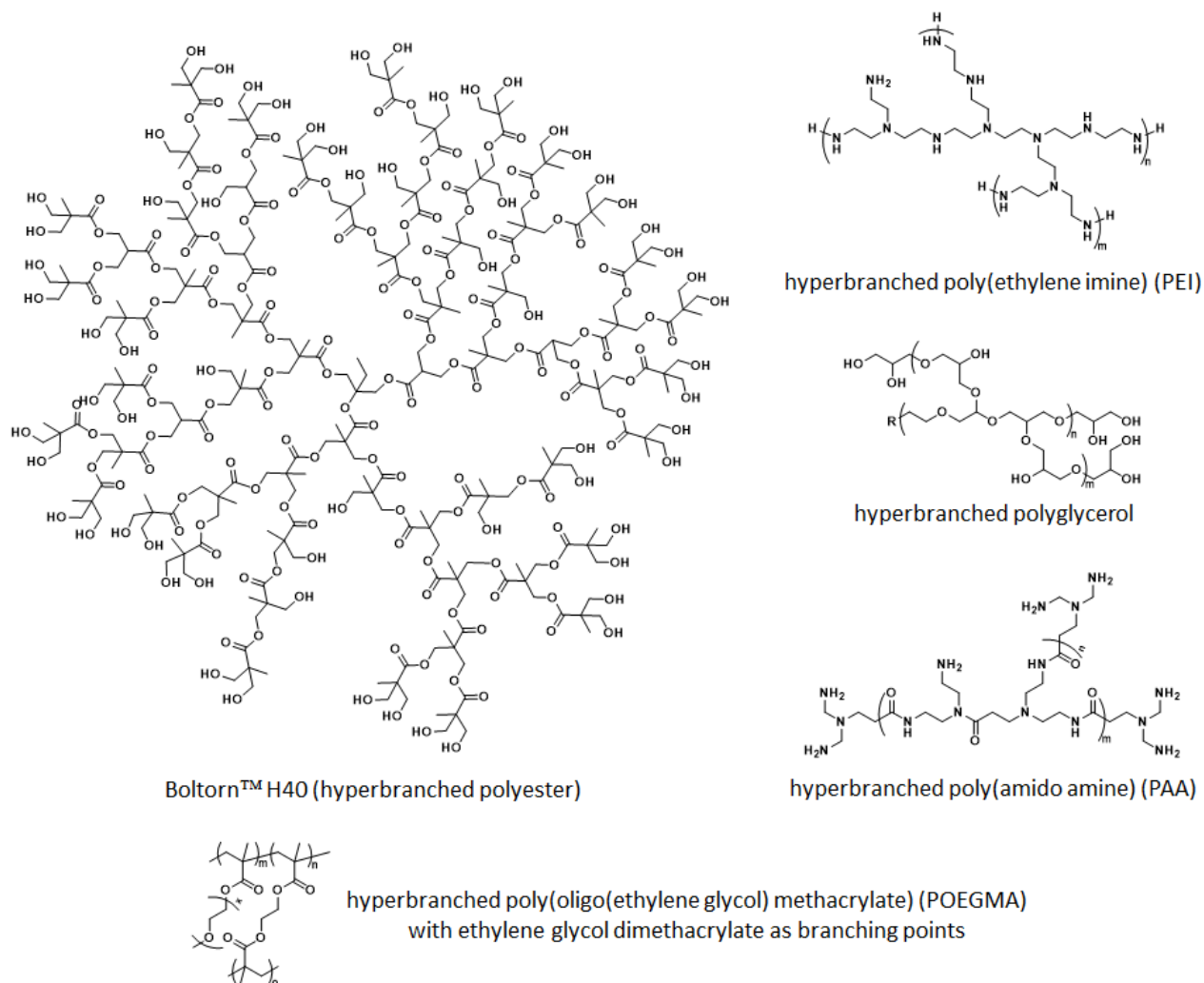


Figure 2. Structure of the most common HBPs used in drug delivery systems.

2. SYNTHESIS STRATEGIES TO PREPARE HYPERBRANCHED POLYMERS

Various synthesis strategies have been used to prepare HBPs and have been reviewed in details in previous reviews.[11, 13, 40, 41] This section aim at providing the reader a general overview of the main synthesis routes used to obtain HBPs. The two main strategies consider the use of either a pair of monomers or a single monomer with orthogonal functions to prepare HBPs (**Figure 3**). As compared to the single monomer route, the monomer-pair route has a stronger tendency to intramolecular cyclization leading to the formation of (multi)cyclic species.[42, 43] The degree of branching can be tuned for the polymerizations conducted through a chain-growth method by

changing the ratio between the monomers leading to linear units (monomers with one vinyl group) and those creating branching points (monomers possessing multiple vinyl groups in Section 2.1.2 and inimers in Section 2.2.2). These strategies have been extended to non-covalent interactions such as electrostatic interaction, hydrophobic interaction, and hydrogen-bonding interaction through both synthesis routes (monomer-pair and single monomer methodology).[44]

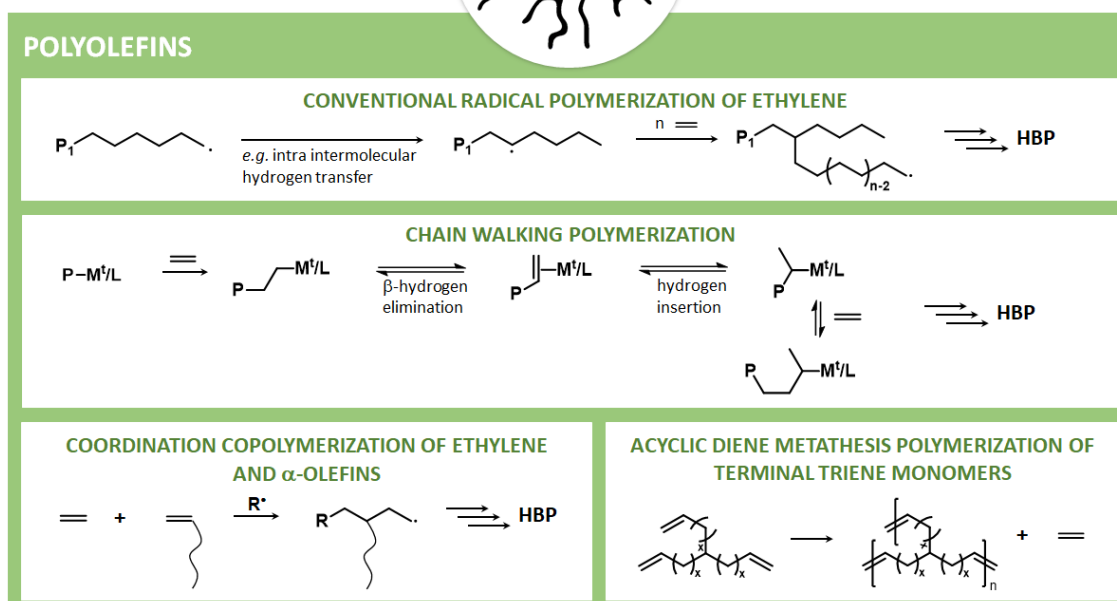
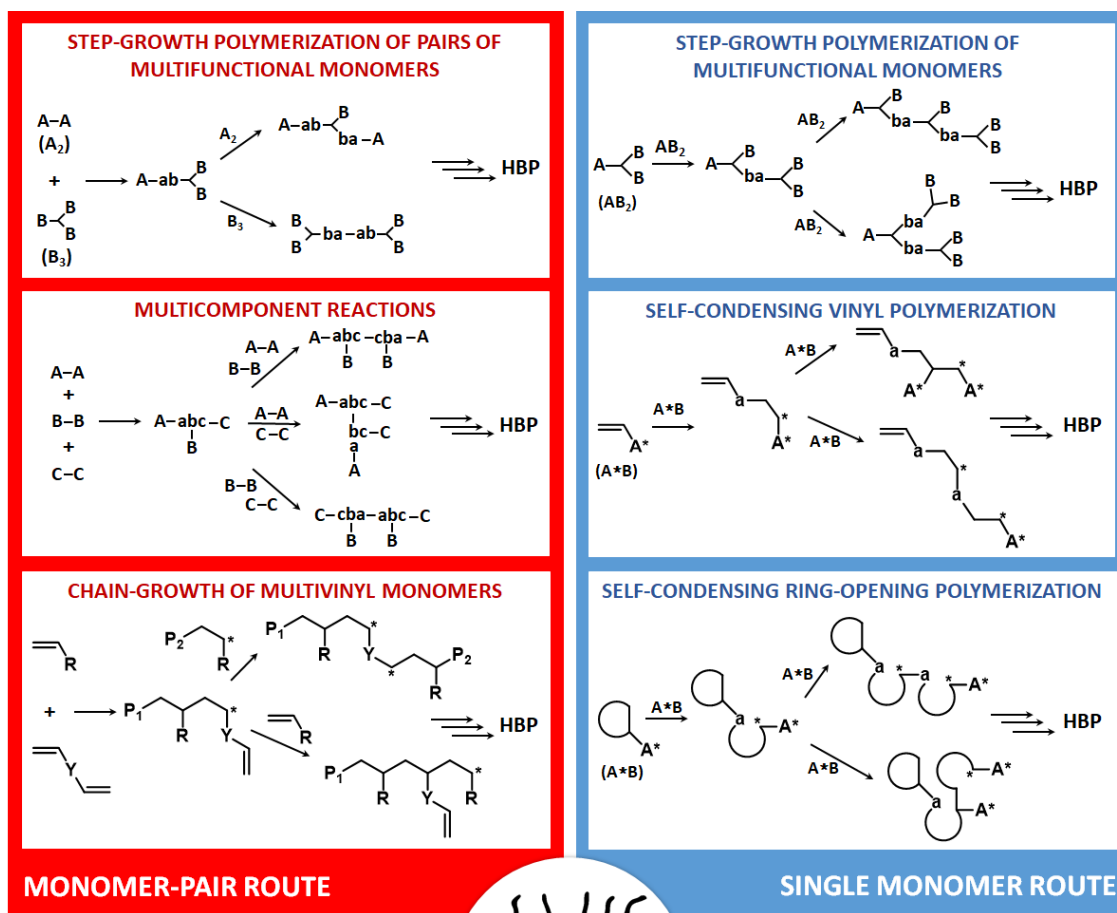


Figure 3. Main synthesis routes to prepare HBPs.

2.1. Monomer pair route

2.1.1. Step-growth copolymerization of A_2 and B_3 monomers

The $A_2 + B_3$ system (*i.e.* using two monomers with one bearing two identical functional groups A and the other one three identical functional groups B) is attractive as it can be used to produce HBPs in large scales through a one-pot synthesis. The choice of the groups A and B is dictated by the selective reactivity of the functional groups A with the functional groups B and their reactivity should be the same for the monomers and the functional groups present on the polymers. A large variety of A and B functional groups have been used, which includes those commonly used for step-growth polymerizations, such as hydroxyl groups with epoxides to prepare hyperbranched aliphatic polyethers,[45] and anhydrides with amines to prepare hyperbranched polyimides,[46, 47] but also click chemistry such as azide with alkyne groups involved in copper-assisted alkyne-azide cycloaddition (CuAAC) reactions.[48, 49] The control of the degree of branching is achieved by controlling the feed ratio and introducing a linear component. However, the $A_2 + B_3$ system generally suffers from a tendency to gelation and intramolecular cyclization.

The minimization of gelation can be afforded by quenching the polymerization before the gel point, conducting the polymerization in dilute solution, or introducing monofunctional end-capping reagents.[50] Another interesting approach, known as couple-monomer approach, is based on the use of monomer pairs with functional groups of non-equal reactivity.[51] B_3 is replaced by BB'_2 , for which the functional groups B and B' can both react with A, but do not have the same reactivity. An AB_2 intermediate is rapidly formed at the early stage of the polymerization, which then undergoes further propagation leading to the formation of HBPs. The first example of this approach was introduced by Yan *et al.* using 1-(2-aminoethyl)piperazine as BB'_2 in the presence of divinyl sulfone as A_2 to prepare hyperbranched poly(sulfone-amine)s.[51] This approach has

been extended to other functionalities (*e.g.* A₂ + CB₂ such as dithiols with propargyl acrylate forming as AB₂ intermediate a molecule bearing one thiol and one alkyne by thiol-ene reaction and leading to HBPs by thiol-yne reaction[52]) and the use of other asymmetric monomers (*e.g.* AD + CB₂ such as methacryloyl chloride with 2-amino-2-methyl-1,3-propanediol forming as AB₂ intermediate by the reaction of the acid chloride with the amine followed by Michael addition of the methacrylate on the hydroxyl groups[53]).

The stepwise reaction between the A and B functional groups is random and each step can lead to either the growth of the polymer or a reaction of intramolecular cyclization. The presence of this side reaction affects the structure of the polymer obtained leading to truncated polymer topologies with a more limited number of terminal functional groups. The choice of monomers used can influence the extent of intramolecular cyclization reactions. For example, Ban *et al.* have investigated A₂ monomers with different spacing units between the two terminal alkyne groups.[49] By increasing the rigidity (*i.e.* phenyl groups *vs.* alkyl chains) and decreasing the length (hexyl *vs.* dodecyl groups) of the spacing units, intramolecular cyclization reactions are diminished. Besides the choice of the monomers, the feed ratio between A₂ and B₃ strongly affect the intramolecular cyclization reactions. Using a feed ratio far from the stoichiometry in functional groups A and B limits the side reactions.[54] Furthermore, conducting the polymerization in dilute solution enhances the number of intramolecular cyclization reactions. Unal *et al.* have demonstrated that the melt polymerization of A₂ and B₃ monomers leads to highly branched polyesters without significant intramolecular cyclization reactions.[55]

With the recent developments in multi-component reactions such as Ugi and Passerini reactions,[56] the one-pot preparation of HBPs has been extended to the use of three or more monomers. Deng *et al.* have reported the synthesis of HBPs by ABC-type Passerini reaction using

hexanedioic acid (A_2), hexane-1,6-diol (B_2), 1,6-diisocyanohexane (C_2) and 10-undecenoic acid (A).[57] The control of the total amount of A groups used for the polymerization and the ratio between A_2 and A is critical to avoid gelation and prepare HBPs. Similarly, Zhang *et al.* have conducted a multi-component reaction using propargyl amine, *N*-acethomocysteine thiolactone, diethylenetriamine in the presence of CuCl and *p*-toluenesulfonyl azide to produce HBPs.[58]

2.1.2. Chain-growth polymerization of multivinyl monomers

Similarly, the $A_2 + B_x$ system has been adapted to chain growth polymerizations through the use of multivinyl monomers. Usually, multifunctional comonomers (B_x , *e.g.* divinylbenzene consisting in two difunctional groups corresponding to B_4) are used as crosslinking agents for the chain growth polymerization of vinyl monomers (A_2) allowing the formation of polymer networks using a small amount of this comonomer. Gelation can be retarded by using thiols as free radical chain transfer agents, but thiols need to be introduced at least in equimolar quantity relative to the multifunctional comonomer,[59] and the polymerization has to be conducted in dilute solution[60] to obtain HBPs. The degree of branching can be increased by increasing the polymerization temperature and the amount of multifunctional comonomer.[60, 61] The structure of the multifunctional comonomer affects the polymerization. For example, the two vinyl groups of divinylbenzene do not have the same reactivity (*i.e.* formation first of polymer chains with pendent vinyl groups followed by their reaction to form branching points) facilitating the formation of HBPs,[60] while polymer gelation is more challenging to inhibit using oligo(ethylene glycol) dimethacrylate, and ethylene glycol diacrylate is a poor branching agent.[62]

This strategy has been extended to polymerization in dispersed media (*i.e.* suspension[63] and emulsion[64] polymerizations) and controlled radical polymerization such as atom transfer radical polymerization[65] (ATRP) and reversible addition-fragmentation chain transfer (RAFT)

polymerization.[66] Among the controlled radical polymerization techniques catalytic chain transfer polymerization involving low-spin cobalt(II) complexes (usually cobaloximes) as chain transfer agents has attracted the attention to prepare HBPs using multifunctional monomers.[67] This technique permits the synthesis of HBPs with a minimal amount of chain transfer agent as compared to thiols and can be also performed in dispersed media.[68] Furthermore, HBPs with well-defined topology[69] (*i.e.* degree of branching and molecular weight) and functionalities (*i.e.* vinyl groups as terminal units that can be used for post-polymerization functionalization[70]) can be synthesized.

2.2. Single monomer route

2.2.1. Step-growth polymerization of AB_x monomers

The random polymerization of AB_x monomers bearing one reactive group A and multiple reactive groups B with $x \geq 2$ affording highly branched polymers without gelation considering the intramolecular reactions negligible has been predicted by Flory.[71] For AB₂ monomers, if both B groups have reacted with A groups of other AB₂ monomers a branching point is created, while a linear unit is obtained when only one of the two B groups is consumed. The resulting HBPs contain one A terminal group and (n+1) B terminal groups for n AB₂ monomers involved in the polymerization ((x-1)n+1 for AB_x monomers). AB_x monomers including not only AB₂, but also AB₃,[72-74] AB₄,[74, 75] AB₆,[74] and AB₈[75] have been used to prepare HBPs in an one-pot synthesis using different types of functionalities such as trimethylsiloxy groups with acid chlorides for the preparation of hyperbranched aromatic polyesters, protected isocyanates with hydroxyl groups to synthesize hyperbranched polyurethanes,[76] cyclopentadienones with alkyne groups affording hyperbranched polyphenylenes through Diels-Alder reaction,[77] and acrylate groups and one terminal olefin through acyclic diene metathesis.[73]

Extremely broad molecular weight distributions of these HBPs are expected at high conversions of A groups by enumeration of all the possible configurations.[78] The experimental dispersity of HBPs obtained from AB_x monomers is larger than the one of linear polymers from AB monomers, but smaller than the calculated ones. To obtain HBPs having narrower molecular weight distributions, few strategies have been proposed: use of multifunctional cores (B_x) for the polymerization of AB_2 monomers that can be enhanced by a slow addition of AB_2 into a dilute solution of B_x , [79, 80] but also the selection of monomers with functional groups having different reactivities if present on the monomer or polymer.[81-84]

This route has been combined with controlled radical polymerization to control the topology of HBPs. For example, Zhu *et al.* have reported the synthesis of V- and Y-type AB_2 monomers.[85] The V-type AB_2 monomer consists of an aromatic core with one alkyne and two bromides as A and B groups respectively, while the Y-type AB_2 monomer possesses one bromide and two alkynes. ATRP is performed from the bromo terminal groups followed by CuAAC reaction after modification of the bromides into azide groups to obtain HBPs with different branching patterns.

2.2.2. Self-condensing polymerization

Self-condensing vinyl polymerization (SCVP) was introduced by Fréchet using a vinyl monomer bearing a group able to initiate the polymerization of vinyl groups, known as inimer standing for *initiating monomer* (A^*B), that can be assimilated to the AB_2 system where the vinyl group behaves as a difunctional group equivalent to B_2 , and the initiating group A^* as the group A.[86] In this work, the inimer 3-(1-chloroethyl)ethenylbenzene is polymerized in the presence of $SnCl_4$ and tetrabutylammonium bromide. While the kinetics at the beginning of the polymerization is slow, the evolution of the molecular weight over time increases exponentially. The high dispersity of the obtained HBPs is attributed to the complex mechanism of polymer growth as each inimer can lead to the formation of different species. The A^* group of A^*B can initiate the polymerization

by attacking the B group on another A*B inimer leading to a dimer possessing a vinyl group (B), an initiating group (A*) and an active center (b*) resulting from the attack on the double bond. The addition of the next A*B can thus occur either through the addition of its A* group on the B group of another A*B or the attack of either its A* or b* group on the double bond of another A*B.

Besides cationic polymerization, SCVP has been extended to anionic and radical polymerizations with a preference for living and controlled polymerizations to minimize crosslinking reactions and thus gelation of the reaction mixture. Due to the high reactivity of carbanions, the preparation of inimers containing a vinyl group and an anionic initiator is difficult, requiring the formation of the inimer to be formed *in situ*.^[87] With the developments of group transfer polymerization inimers with a silylketene acetal group that can be activated by nucleophilic catalysts to initiate the polymerization have been synthesized and used for the preparation of HBPs.^[88, 89] In a similar manner than SCVP, A*B inimers have been developed for self-condensing anionic or cationic ring-opening polymerization of cyclic epoxides,^[90, 91] oxetanes,^[92] lactones,^[93] and phosphates.^[94] The inimer usually consists of a hydroxyl group as the initiating species (A*) and a ring (B) acting as the difunctional group. For example, hyperbranched polyethers have been prepared by addition of the hydroxyl (A*) group from glycidol onto the epoxide (B) of another one leading to the formation of an additional alkoxide (b*) that can also promote nucleophilic propagation. One of the potential side reactions is intramolecular cyclization.

The three main controlled radical polymerization techniques (*i.e.* nitroxide-mediated polymerization (NMP), ATRP, and RAFT polymerizations) have been investigated to synthesize HBPs by SCVP.^[95] Two approaches have been employed to prepare HBPs by NMP. Alkoxyamine-functionalized styrenes^[96] have been used as inimers affording HBPs with terminal

alkoxyamines, while polymerizable nitroxides (styrene and methacrylate bearing a nitroxide) lead to HBPs with alkoxyamines at the branching points.[97, 98] For the latter case, the branching points can be thermolytically degraded. Due to some limitations of NMP[99] such as slow polymerization kinetics, limited control over the homopolymerization of methacrylates and lower commercial availability of nitroxides and alkoxyamines, this controlled radical polymerization technique has been less extensively investigated as compared to ATRP and RAFT polymerizations. For ATRP, inimers derived from styrene and (meth)acrylates with an alkyl halide, either bromide or chloride, have been employed. Using a too high concentration in copper catalyst lead to gelation due to the formation of a high concentration in radicals promoting termination reactions by bimolecular couplings. The preparation of HBPs is strongly affected by the temperature and the choice of the ligand, which dictates the ability of radicals either to propagate or deactivate into the dormant species and consequently the topology of HBPs obtained, *i.e.* ratio between linear and branching units.[100, 101] Photoinduced ATRP SCVP has been recently described to prepare HBPs using perylene[102] or dimanganese decacarbonyl[103] as photocatalysts. RAFT polymerization uses A*B transmer (contraction of chain *transfer* agent and *monomer*) based on dithioester compounds, acting as chain transfer agents, functionalized with a vinyl group (styrene, (meth)acrylate, (meth)acrylamide, vinyl acetate).[104] The vinyl group introduced either on the R-group attached to the sulfur of the dithioester or on the Z-group next to the thioketone of the chain transfer agent leads to the positioning of the chain transfer agent either as terminal groups or at the branching points of HBPs respectively. Recently, organotellurium-mediated radical polymerization has been explored to prepare HBPs using a vinyl telluride possessing a hierarchical reactivity (*i.e.* the telluride cannot initiate by itself, but once the vinyl

group has been activated, it participates to the polymerization creating branching points) in the presence of acrylates and an organotellurium chain transfer agent.[105]

2.3. Case of branched polyolefins

Low-density polyethylene is commonly produced by radical polymerization under high temperature and high pressure leading to branched structures due to inter- and intramolecular chain transfer reactions,[106] while high-density polyethylene with a low content of branching is prepared by coordination polymerization. Late transition metal homogeneous catalysts such as $\text{Me}_2\text{Si}(\eta^5\text{-C}_5\text{Me}_4)(\eta^1\text{-N-tBu})\text{TiCl}_2$ have been used to copolymerize ethylene with a low amount of long α -olefins to prepare polyethylene with well-defined branches.[107, 108] The development of catalysts for coordination polymerization has been explored to synthesize branched polyethylenes. For example, Barnhart *et al.* have proposed the use of a tandem catalyst system consisting in $[(\eta^5\text{-C}_5\text{Me}_4)\text{SiMe}_2(\eta^1\text{-NCMe}_3)\text{TiCl}_2$ promoting the polymerization of ethylene and 1-alkenes and $[\text{C}_5\text{H}_5\text{B-Ph}]_2\text{ZrCl}_2$ producing 1-alkene *in situ*.[109] Guan *et al.* have introduced the concept of chain walking polymerization to prepare hyperbranched polyethylenes[110, 111] through the use of Pd-diimine catalysts, mechanism identified by Johnson *et al.*[112] The ethylene-dissociated state of the catalyst can yield either the trapping of new ethylene monomer leading to chain growth or β -hydride elimination and isomerization inducing chain migration and formation of branching units. Other catalysts such as catalysts based on nickel[112] and zirconium[113] can also induce *in situ* formation of olefin-terminated oligomers via β -hydride elimination.

More recently, the acyclic diene metathesis (ADMET) polymerization technique has been extended by the group of Meier replacing trienes by dienes to prepare HBPs.[114] Ren *et al.* have emphasized the importance of the choice of the monomers, metathesis catalysts, and reactions conditions to favor ADMET polymerization over intramolecular ring-closing metathesis.[115] The

hydrogenation of this hyperbranched polyolefins afforded hyperbranched polyethylene with a higher control of the spacing between branching points as compared to the approaches described previously in this section. Besides polyolefins, ADMET using trienes has been also explored for the preparation of hyperbranched unsaturated polyesters[116] and polyphosphates.[117]

3. HYPERBRANCHED POLYMERS AND DRUG DELIVERY

HBP as other materials designed for drug delivery combine two main functions: loading of the drug through various approaches (*i.e.* encapsulation or conjugation) and transport to tumor tissue promoted by passive targeting (*i.e.* importance of the size or modification with functional groups enhancing the circulation half-life).

3.1. Drug loading

Like dendrimers, HBPs form cavities that can be used to encapsulate cargos of different sizes[118] including small chemotherapeutic drugs such as DOX,[119, 120] camptothecin (CPT),[121, 122] cisplatin,[123-125] and 5-fluorouracil (5-FU).[126] Wu *et al.* have investigated hyperbranched polyglycerol and its ability to encapsulate and deliver a guest molecule.[126] HBP labeled with carboxyfluorescein (green light emission) entrapping chlorin e6 (red emission light) shows the co-localization of chlorin e6 and the HBP by confocal fluorescence microscopy in the cytoplasm of MGC-803 cells confirming the ability of the HBP to act as a carrier. The study of Rhodamine B-encapsulated in this HBP by nuclear magnetic resonance spectroscopy seems to indicate that Rhodamine B is entrapped by interactions between the xanthene ring of Rhodamine B and ether linkages of the hyperbranched polyglycerol. Larger drugs such as DNA[127-130] and siRNA[131-133] form complexes with unimolecular HBPs such as branched poly(ethylene imine) (PEI)[134] by electrostatic interactions. Tuning the structure of HBPs permits to control the strength of the interaction between gene and carrier by modulating the charge density at its surface, adjusting its

molecular weight, and preparing different molecular structures.[135] Besides, by controlling the functionality of HBPs multimolecular structures able to release its large cargo under a stimulus such as pH promoting demicellization have been prepared.[136]

Loading of the drug in polymer-based drug delivery systems has been explored through different types of non-covalent interactions (hydrophobic interaction, hydrogen bonding, ionic interaction, steric trapping in a crosslinked network), but also conjugation of the drug on the polymer.[137-139] The interactions between the drug and the polymer are primordial to enhance the stability of the drug, access high drug loading capacities, and tune the drug delivery profile. As drug delivery systems through a non-covalent approach are more sensitive to the physical forces involved in their environment, the conjugation of the drug covalently attached to the polymer through linkers that can be tuned to induce the release of the drug under conditions specifically encountered at the target site has been considered. Due to their high density in functional groups, HBPs provides access to high drug payload by conjugation of the drug to the terminal functionalities of HBPs.[140, 141] Kolhe *et al.* have conjugated ibuprofen as drug and fluorescein isothiocyanate (FITC) on the hydroxyl terminal groups present on hyperbranched polyglycerols using *N,N'*-dicyclohexylcarbodiimide (DCC) as coupling agent.[140] These conjugates have a high payload in ibuprofen (70%), enter A549 cells rapidly and are mainly distributed in the cytosol. The drug is released after cleavage of the ester bond by lysosomal enzymes present in the cell. Interestingly, the drug can also be one of the constituting units of HBPs. Liu *et al.* have synthesized HBPs with alternated hydrophobic diselenide and hydrophilic phosphate groups.[142] While the phosphate groups act as branching units, selenium compounds[143, 144] have been reported as anticancer agents affording HBP as a self-delivery anticancer agent.

3.2. Passive targeting

Targeted drug delivery systems have been developed to optimize their pharmacokinetics aiming at a targeted localization in the body. Nanosized carriers loaded with the drug can circulate in the bloodstream and accumulate preferentially at the tumor by the enhanced permeability and retention effect (EPR).[145-147] This passive targeting is promoted by a prolonged circulation in the bloodstream and the differences existing between tumoral and healthy tissues such as higher vasculature and larger gap junctions between endothelial cells of tumors (up to 1 μm). While very small carriers are rapidly cleared by the kidneys (*i.e.* threshold of renal clearance for nanoobjects with a hydrodynamic diameter of 6 nm)[148-150] and large ones accumulated mainly in the liver and spleen (greater than few hundreds of nanometers),[151] nanocarriers with a diameter between 20 and 200 nm can extravasate easily in tumor tissues.[152]

As the size of drug carriers has a critical role in promoting low accumulation in healthy tissue and high accumulation in tumor tissue via the EPR effect, this parameter should be considered when designing polymers as drug delivery systems. Polymers of various topologies including HBPs[153, 154] have been explored as drug carriers (**Figure 4**). Usually, HBPs of high molecular weight can be relatively easily synthesized reaching a reasonable size (>10 nm) to passively target tumors by EPR effect, while dendrimers with a number of generation higher than five are difficult to prepare due to steric hindrance affording nanostructures with a hydrodynamic diameter lower than 10 nm that are thus not suitable for passive targeting. However, unimolecular HBPs of low molecular weight have a small size and cannot be used for size-related passive targeting at the tumor via EPR effect as they can be easily removed by renal excretion or through bypassing filtration by the spleen.[155] Despite this limiting feature for drug delivery, their small size (less than 10 nm) has been exploited for other biomedical applications such as bioimaging reducing the toxicity of radioisotopes and facilitating their elimination through urine and feces.[156, 157] The

self-assembly of HBPs into multimolecular nanostructures has been considered to increase the size of the nanocarriers.[158-163] Son *et al.* have reported the synthesis of hyperbranched polyglycerol monofunctionalized with spiropyran.[164] As hydrophobic spiropyran is known to undergo reversible photochromism at 250-380 nm forming the corresponding water-soluble merocyanine species, these spiropyran-functionalized hyperbranched polyglycerol self-assemble into micelles and disassemble upon UV irradiation. Pyrene has been encapsulated into these micelles, released upon irradiation at 254 nm and partially reloaded into micelles upon irradiation at 620 nm. Besides controlling the size, drug loading can be increased as compared to unimolecular nanostructures,[162] but also the loading of large drugs such as enzymes and proteins is more efficient.[136]

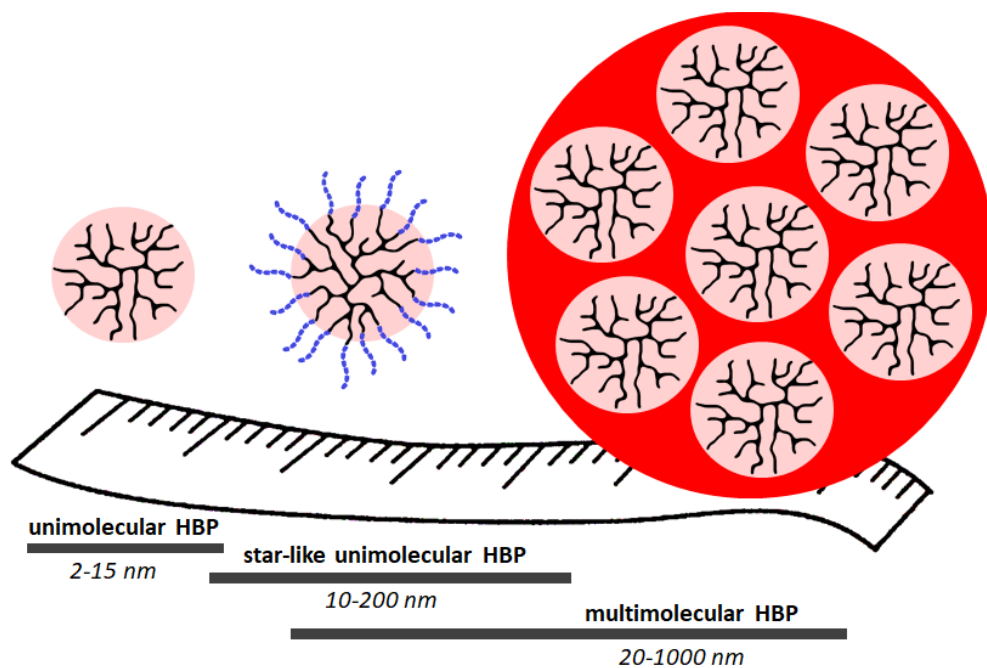


Figure 4. HBP nanostructures and their relative sizes.

Individual HBPs can be assimilated to unimolecular micelles formed from solely one HBP molecule. Due to their covalent nature with interconnected structures similar to nanogels, these individual HBPs have an excellent stability in diluted environments such as *in vitro* and *in vivo*

conditions as compared to micelles formed from the self-assembly of molecules, which can undergo demicellization at a concentration below its critical micelle concentration and are more prone to sustained drug release. Popeney *et al.* have developed hydrophilic hyperbranched polyglycerol grafted on a hydrophobic hyperbranched polyethylene.[165] The chain walking copolymerization of ethylene and a siloxy-functionalized comonomer followed by the removal of the protecting groups produce a hyperbranched polyethylene core terminated with hydroxyl groups used for the ring-opening polymerization of glycidol. This polymer under diluted conditions has been used to encapsulate hydrophobic fluorescent dyes such as Nile red. This core-shell hyperbranched copolymer permits the uptake of the dye into A549 cancer cells by endocytosis, while hyperbranched polyglycerol grafted on an aliphatic linear hydrocarbon shows poor cellular uptake. Donskyi *et al.* have prepared hyperbranched polyglycerol grafted on fullerene.[166] These nanostructures self-assemble with a decrease in their size by increasing the number of polyglycerol branches, *i.e.* multimolecular nanostructures of 19 nm with two branches per fullerene and unimolecular nanostructures of 8 nm for fullerene bearing five polyglycerol branches, as the higher number of branches on fullerene reduces their self-assembly. The loading of a hydrophobic dye decreases with the number of branches on fullerene as the interaction of the drug with the fullerene core is decreased. For unimolecular nanostructures the release profile of the dye depends solely on the interactions between the dye and the carrier, while its release is faster for multimolecular nanostructures where the dye is encapsulated in the aggregates that could be exhibiting dynamic equilibrium between unimolecular and multimolecular nanostructures.[167, 168]

The conjugation of poly(ethylene glycol) (PEG) directly to the drug or its carrier has been proposed to improve their shelf-life, solubility and circulation half-life, thus favoring their accumulation at the tumor sites through the EPR effect.[169] Various types of HBPs, including hyperbranched

polyether,[170-172] polyester,[173] and poly(amido amine) (PAA),[174] have been modified with PEG affording star-like HBPs. Xu *et al.* have reported the modification of hyperbranched polyglycerols[175] and PEIs[176] with tri-PEGylated benzaldehydes forming an imine group labile under acidic conditions. These tri-PEGylated HBPs leads to a higher encapsulation of dyes as compared to unmodified HBPs and even mono-PEGylated HBPs. Similarly, the higher the degree of functionalization of HBPs with tri-PEGylated benzaldehydes, the higher the encapsulation of the dye. The release of dyes and drugs can be triggered under acidic pH with shorter half-life for a pH of 5 as compared to physiological pH (7.4). Other neutral hydrophilic polymers have been also conjugated to HBPs. Kurniasih *et al.* have developed core-shell nanostructures based on hyperbranched polyglycerol functionalized at the periphery with PEG and core with hydrophobic biphenyl species.[177] Pyrene has been encapsulated in the core of HBPs forming unimolecular nanostructures (10-11 nm), while Nile red being located in the outer shell of HBPs and prone to self-assemble has induced the formation of aggregates of HBPs (100-200 nm). No release of pyrene and Nile red at pH 7.4 has been observed. However, at pH 5 pyrene has not been released within two weeks, while the complete release of Nile red has been observed after one week with a half-life of 38 h and decrease of the hydrodynamic diameter from 200 to 10 nm indicating the release of the dye by disassembly of the HBPs. Poly(*N*-isopropylacrylamide) (PNIPAM) undergoes a reversible phase transition at its lower critical solution temperature (32 °C) that has been exploited in the field of drug delivery.[178] While Luo *et al.* have synthesized unimolecular core-shell micelles based on hyperbranched polyglycerols with a shell based on PNIPAM that collapses on heating and expands on cooling,[179] Picco *et al.* have reported the synthesis of hyperbranched polyesters with a PNIPAM shell forming unimolecular nanostructures (20 nm) below the phase transition temperature that self-assemble into multimolecular

nanostructures (220 nm) above this temperature.[158] Zhao *et al.* have prepared a PEGylated thermo-responsive HBPs consisting in a PAA core modified with PEG and PNIPAM.[180] This HBP promotes the fast release of indomethacin used as model drug (90% of drug release in 12 h) at 30 °C, while at 37 °C a more sustained drug release (less than 30% in 12 h) is obtained.

4. FUNCTIONALIZATION OF HYPERBRANCHED POLYMERS FOR ACTIVE TARGETING IN DRUG DELIVERY

Although passive targeting is an effective strategy for targeted drug delivery, it has several limitations such as the inefficient diffusion of the nanocarrier into tumor cells due to its low interaction with the cell surface,[181] but also the extent of vascularization and porosity of the tumor depending on its type and status.[147, 182] The development of strategies to promote active targeting (**Figure 5**) aims at increasing the cellular uptake of the nanocarriers for efficient delivery of its cargo and enhancing cell specificity. Active targeting in drug delivery systems considers the insertion of targeting moieties directly attached at the surface of the nanocarriers. These targeting moieties interact specifically with receptors expressed on cancer or angiogenic endothelial cells enhancing the binding and internalization of nanocarriers. Active targeting moieties are particularly beneficial for cancer therapy due to the reduced delivery of potentially toxic drugs to healthy tissue. A wide variety of targeting moieties have been considered including aptamers that can be either peptides[183-186] or oligonucleotides,[187-189] and folic acid[190, 191] that have been conjugated on HBPs.

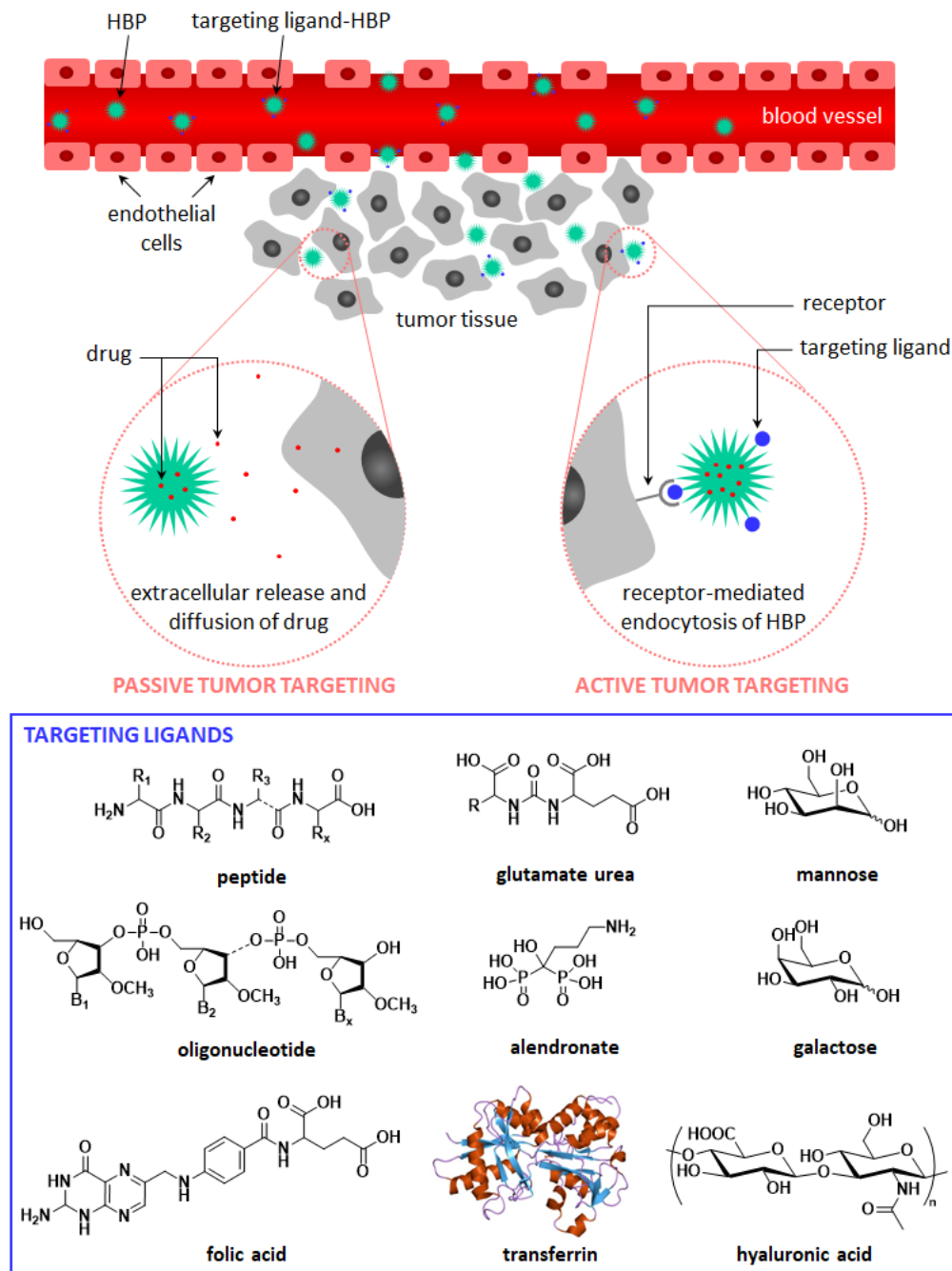


Figure 5. From passive to active targeting through functionalization of HBPs with targeting ligands to enhance recognition and cell uptake in cancer cells.

4.1. Peptides as active targeting groups

Peptides are good candidates as active targeting moieties for drug delivery systems due to their high avidity towards cell receptors and low immunogenicity, but also peptides are easy to

synthesize and conjugate onto nanocarriers.[192, 193] Peptides have been grafted onto the surface of different nanostructures such as gold,[194] quantum dots,[195] iron oxide,[196] and silica nanoparticles,[197] but also liposomes,[198] carbon nanotubes,[199] dendrimers,[200] and polymers of various topologies[201] including hyperbranched polymers.

4.1.1. Tumor targeting peptides

Tumor targeting peptides (TTPs), usually shorter than cell penetrating peptides (three to ten residues), interact more specifically with receptors overexpressed by tumor cells.[202-204] TTPs are designed to bind to cell surface receptors, intracellular receptors, and the extracellular matrix.

Peptides targeting cell surface receptors

Targeted cell surface receptors include $\alpha_v\beta_x$ integrins, somatostatin receptors, epidermal growth factor receptors, vascular endothelial growth factor receptors, and prostate-specific membrane antigen.[205]

Integrins are cell adhesion receptors[206] present on the cytoplasmic side of the lipid bilayer promoting the assembly of cytoskeletal polymers and signaling complexes, but also on the extracellular side of the lipid bilayer binding to the extracellular matrix or counter-receptors on adjacent cells. Various ligands have been identified to bind to integrins. The most common minimal peptide sequence used to target $\alpha_v\beta_3$ integrin overexpressed at the surface of endothelial tumor cells is Arg-Gly-Asp (RGD) that can be found as linear and cyclic (*e.g.* cyclic RGDdYK where dY stands for the D-isomer of tyrosine, and cyclic CRGDKGPDC known as iRGD) derivatives.[207]

Vascular endothelial adhesion molecules (VCAM) and intercellular adhesion molecule are counter-receptors of leukocyte $\alpha_4\beta_1$ and $\alpha_L\beta_2$ integrins expressed on endothelial cells. VHSPNKK is a homolog of the α -chain of very late antigen VLA-4, a known ligand for VCAM-1.[208] Somatostatin is a regulatory cyclic tetradecapeptide (sequence: AGCKNFFWKFTFTSC) that

affects neurotransmission and cell proliferation by interacting with guanidine nucleotide binding protein (known as G protein) coupled somatostatin receptors (SSTRs). However, the half-life of the wild type somatostatin is short (less than 3 min) due to enzymatic degradation. Analogs with higher potency and stability have been developed. Octreotide is a cyclic octapeptide (sequence: dFCFdWKTCT-ol where dF and dW stand for the D-isomer of phenylalanine and tryptophan and the C-terminus is an alcohol) and somatostatin analog that has a half-life of 1.5 h. Octreotide has been used for targeted neuroendocrine cancer therapy[209, 210] due to its high binding affinity to SSTR2 and moderate affinity to SSTR5.[211] Octreotide has been also radiolabeled by complexation with ^{111}In for targeted theranostics (*i.e.* imaging and therapy of tumors).[204, 212-214] KE108 (sequence: Y-cyclo(d-Dab-RFFdWKTF where d-Dab is the D-isomer of 2,4-diaminobutyric acid) is another somatostatin analog able to bind to all five SSTRs with high affinity.[215, 216]

Epidermal growth factor (EGF) regulates cell proliferation, survival, and differentiation by binding to EGF receptors (EGFRs).[217] These receptors are overexpressed or mutated for different cancer cells and play a crucial role in epithelial tumors enhancing tumor growth, invasion, and metastasis. Several EGFR inhibitors have been developed to disrupt the interaction between the EGF and its receptor and show a specific binding ability to tumors. GE11 (sequence: YHWYGYTPQNVI) is an EGF mimic of small size (twelve amino acids as compared to more than fifty for EGF) showing affinity to EGFRs.[218] GE11 has also been reported to enhance potentially nanoparticles endocytosis by EGFR-dependent actin-driven pathway.

Vascular endothelial growth factor (VEGF) plays an important role as a regulator of new blood vessel growth (angiogenesis) and inducer of vascular permeability.[219] Anti-VEGF approaches to treat cancers aim at inhibiting the interaction between VEGF and either tyrosine kinase receptors

or neuropilins. Various peptides affect the interaction of VEGF with its receptors such as HRH[220] (sequence: HRHTKQRHTALH) and K237[221] (sequence: HTMYYYHHYQHHL), while formin binding protein 21 (FBP21) alters the ratio of VEGF isoforms in favor of anti-angiogenic isoforms.[222]

Prostate-specific membrane antigen (PSMA) is expressed on the membrane of prostate epithelial cells and overexpressed in prostate cancer cells.[223] Peptides such as WQPDTAHHWATL[224] have been identified to specifically target PSMA and inhibit its enzymatic activity.[225] E'EAmc-Ahx-dEdEdEG (where 'E is Glu-OtBu where the gamma-carboxyl group is unprotected, dE the D-isomer of glutamic acid, Ahx 6-aminocaproic acid and Amc *N*-aminomethylcyclohexanoic acid) is a stable derivative of RBI-1033, known as an urea-based PSMA targeting ligand.[226]

Peptides targeting intracellular receptors

BCR-ABL fusion genes, cyclin-dependent kinases, and malignant mitochondria are intracellular receptors that have been also targeted.

BCR-ABL fusion gene, also known as Philadelphia chromosome, is formed by joining the ABL gene from chromosome 9 to the BCR gene on chromosome 22 and responsible for the chronic phase of chronic myelogenous leukemia. Peptides rich in serine and proline (*e.g.* YRAPWPP)[227] have a strong binding and specific affinity to BCR-ABL.

Mitosis is regulated by cyclins, cyclin-dependent kinases (CDKs) and complexes formed between cyclins and CDKs. In cancer cells, the CDK/cyclin complexes can be dysregulated inducing for example gene amplification, protein overexpression, and cyclin mutation. CDK/cyclin inhibitors include peptides such as NBI1 (sequence: dRdWdIdMdYdF, where all amino acids as D-isomers) binding to cyclin A.[228] The primary role of mitochondria is to produce adenosine triphosphate, but also to regulate apoptotic cell death.

Cancer cells can inhibit apoptosis by preventing mitochondrial outer membrane permeabilization necessary for caspase activation. Peptides mimicking protein regulating the release of apoptotic-inducing factors such as pore-forming Bax and Bak proteins, but also the cationic peptide (KLAKLAK)₂, are able to bind to tumor cells and induce apoptosis.[229]

Peptides targeting extracellular matrix

Preserving the integrity of the extracellular matrix is necessary to prevent the tumor cell migration and invasion through the targeting of fibronectins, fibroblast growth factors, matrix metalloproteinase, prostate-specific antigens, and cathepsins. Fibronectins are overexpressed in various tumors leading to the formation of fibronectin-fibrin complexes facilitating tumor proliferation, angiogenesis, and metastasis. The peptide CREKA binds strongly and selectively to fibronectin-fibrin complexes[230] and has been also used for tumor imaging.[231]

4.1.2. Peptide conjugation on HBPs

Peptide-conjugates are prepared through two main strategies: i) polymerization using peptide-containing macroinitiators or macromonomers and ii) post-polymerization modification with peptides.

The use of either macromonomers or macroinitiators bearing a peptide sequence permits to introduce the peptide sequence during the polymerization. The synthesis of macromonomers and macroinitiators bearing a peptide sequence has been described in the literature through different routes including coupling reactions in solution and on resin end-capping of the peptide sequence with a polymerizable or initiating group. Peptide-functionalized macroinitiators have been designed to prepare linear and star polymers bearing a peptide at the extremity of the polymer chain.[232] Different approaches and polymerization techniques have been explored including NMP,[233] ATRP,[234] and RAFT polymerization[235] from a peptide grafted on the resin, peptide-bearing initiator or chain transfer agent used under ATRP[236] and RAFT[237-239]

polymerization conditions, ring-opening polymerization of *N*-carboxyanhydrides from peptide-PEG macroinitiator.[240] The use of peptide-containing macromonomers afford polymers bearing peptides on the side chains of the polymer backbone. Depending on the polymerization technique used, the functional groups on the peptide may have to be protected during the polymerization. Various polymerization techniques such as ATRP,[241, 242] RAFT,[243-245] and ring-opening metathesis polymerization[246-248] have been used to (co)polymerize peptide-containing macromonomers.

Post-polymerization modification of polymers is a well-known strategy to prepare functional polymers through the introduction of further functionalities on polymers.[249, 250] The functional groups present on the polymer should be able to react chemoselectively with those of the molecules to be introduced. Various routes have been exploited to further functionalize polymers either by presenting chemoselective functional groups at one extremity of the polymer or on the side chains of the repeat units constituting the polymer chains. Activated esters[251] such as *N*-hydroxysuccinimide (NHS) and pentafluorophenyl (PFP) esters readily reacts with primary amines to form stable amide linkages. Thiols have been widely used to functionalize polymers through either disulfide exchange, Michael addition or radical mechanism reacting with disulfide bridges, epoxides, isocyanates, maleimides, vinyl groups (including (meth)acrylates), and alkynes.[252] Alkynes are involved in different coupling reactions such as CuAAC,[253] strain-promoted 1,3-cycloaddition reactions of cycloalkynes and azides,[254] and copper-catalyzed Glaser coupling reactions of terminal alkynes.[255] Other routes for post-polymerization modification include ring-opening reaction of azlactones,[256] atom transfer radical addition,[257, 258] nitroxide radical coupling,[259] and Diels-Alder reactions.[260] Various synthetic routes

have been considered for the conjugation of TTPs on HBPs by post-polymerization modification as depicted in **Figure 6** and summarized in **Table 1**.

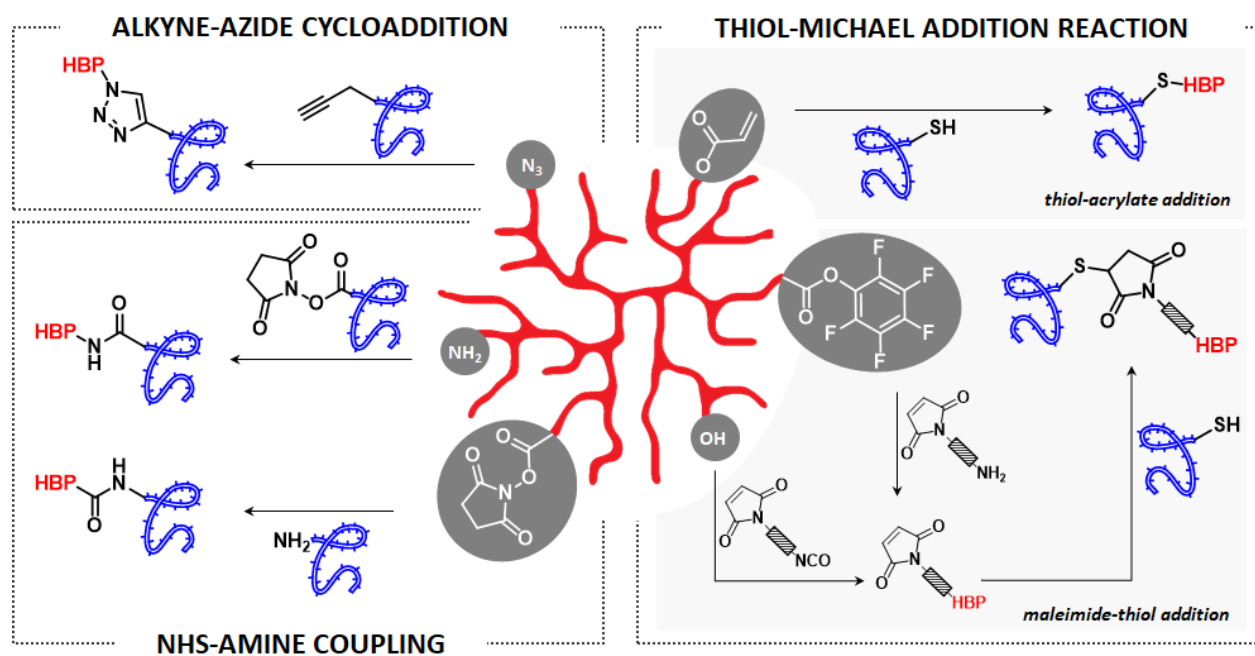


Figure 6. Post-polymerization functionalization routes of HBPs with peptides.

Table 1. HBPs conjugated with tumor targeting peptides

HBP•functional group ^a	tumor targeting peptide	receptor targeted ^b	drug loaded ^c	nanostructure (size, nm)	Ref.
<i>Copper-assisted alkyne-azide cycloaddition between HBP-N₃ and peptide-alkyne</i>					
POEGMA	SPWPRPTY	HSP70	-	unimolecular (6)	[261]
POEGMA	SPWPRPTY	HSP70	DOX (conjugated)	unimolecular (7)	[262]
POEGMA	YCAYYSPRHKTTF	HSP70	DOX (conjugated)	unimolecular (9)	[263]
<i>Coupling between HBP-NHS and peptide-NH₂</i>					
PAA	iRGD	$\alpha_v\beta_3$ integrin	siRNA	multimolecular (223)	[264]
H40•PLLA-PEG	octreotide	SSTR	thailandepsin-A	unimolecular (66)	[265]
polyglycerol	WPPPPRVPR	FBP21	-	unimolecular (-)	[266]
<i>Michael reaction between HBP-acrylate and peptide-SH</i>					
poly(β -thioester)	CGGG(KLAKLAK) ₂	mitochondria	DOX-HCl	multimolecular (80-120)	[267]
polyglycerol	VHSPNKK	VCAM	-	unimolecular (-)	[268]
<i>Coupling between HBP-maleimide and peptide-SH</i>					
PMMA•PHEMA	RGD	$\alpha_v\beta_3$ integrin	DOX	multimolecular (172)	[269]
PAE•PDLLA-DPPE	cyclic RGDfK	$\alpha_v\beta_3$ integrin	PTX	multimolecular (247-264)	[270]
H40•PLG- <i>b</i> -PEG	cyclic RGDfC	$\alpha_v\beta_3$ integrin	DOX (conjugated)	unimolecular (65)	[271]
H40•PBLA- <i>b</i> -PEG	GE11	EGFR	siRNA	unimolecular (63)	[272]

^aPAA, poly(amido amine); PEG, poly(ethylene glycol); POEGMA, poly(oligo(ethylene glycol) methacrylate); PBLA, poly(β -benzyl-L-aspartate); PLG, poly(L-glutamate); PMMA, poly(methyl methacrylate); PAE, poly(amine ester); PHEMA, poly(hydroxyethyl methacrylate); PLLA, poly(L-lactide); DPPE, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine; ^b HSP70, heat shock protein 70; EGFR, epidermal growth factor receptor; SSTR, somatostatin receptors; FBP21, formin-binding protein 21; VCAM, vascular endothelial adhesion molecule; ^c DOX, doxorubicin; PTX, paclitaxel; siRNA: small interfering ribonucleic acid.

The group of Thurecht has reported the synthesis of hyperbranched poly(oligo(ethylene glycol) methacrylate) (POEGMA) by copolymerization of oligo(ethylene glycol) methacrylate (OEGMA), trifluoroethyl acrylate and ethylene glycol dimethacrylate under RAFT conditions using an alkyne-terminated chain transfer agent.[261] The terminal alkyne is used to functionalize the HBP with an azide-terminated fluorophore (*i.e.* Rhodamine B) and targeting groups by CuAAC reaction. Peptides such as SPWPRPTY and YCAYYSPRHKTTF are used to target the heat shock protein 70 (HSP70) overexpressed on many tumors. Fluorescence imaging demonstrates the significant accumulation of peptide-conjugated HBPs at the tumor as compared to non-conjugated HBPs that are rapidly cleared by the renal system and folate-conjugated HBPs showing lower targeting efficiency (**Figure 7**). The authors have extended their work by conjugating DOX to HBPs containing hydrazine groups forming pH-sensitive hydrazone linkages for controlled drug release.[262] Under physiological conditions (*i.e.* pH 7.4, 37 °C) less than 10 % of DOX is released contrary to pH 5 exhibiting more than 80 % of the drug release. Replacing SPWPRPTY by YCAYYSPRHKTTF leads to the same level of targeting efficiency.[263]

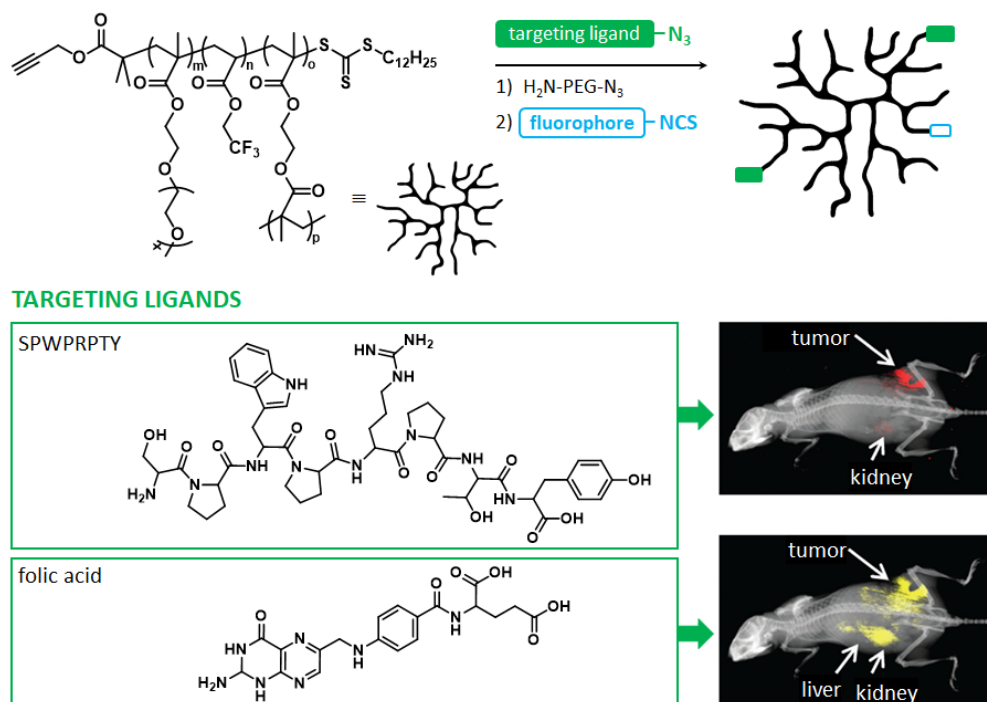


Figure 7. Conjugation of SPWPRPTY (peptide targeting HSP70) and folic acid as targeting ligands on HBP fluorescently labelled with Rhodamine B and NIR797 respectively: synthesis route and biodistribution in mouse by *in vivo* fluorescence 24 h post co-injections of HBPs. Adapted with permission.[261] 2013, Royal Society of Chemistry.

Conjugation of peptides on HBPs has been considered using the coupling reaction between NHS esters and primary amines. Guo *et al.* have conjugated iRGD targeting $\alpha_v\beta_3$ integrins on hyperbranched PAA that complexes with a siRNA specific to EGFR.[264] iRGD-conjugated PAA has a higher silencing ability as compared to PAA and PEI. Octreotide has been similarly conjugated on hyperbranched aliphatic polyester (Boltorn™ H40) with poly(L-lactide)-*b*-PEG (H40•PLLA-PEG)[15, 265] showing both higher affinity to STTR and enhanced anticancer activity. Henning *et al.* have prepared hyperbranched polyglycerol bearing terminal amines to which WPPPPRVPRGSG, a peptide with affinity to WW domains of FBP21, is conjugated by NHS-amine coupling reaction.[266] The multivalent HBP has a binding affinity to the WW domains of FBP21 ten times higher as compared to a monovalent ligand.

Michael addition reactions between thiols and acrylates have been exploited to conjugate peptides on HBPs. Jeong *et al.* have described the synthesis of functionalized hyperbranched polyglycerols obtained by reaction with octadecyl bromide and introduction of cysteine-terminated vasculature binding peptide VHSPNKK after modification of the terminal hydroxyl groups of the HBP with acryloyl chloride.[268] The presence of VHSPNKK enhances the affinity to VCAM proteins and targeting to inflamed endothelium. Cheng *et al.* have prepared amphiphilic acid-sensitive hyperbranched poly(β -thioester)s conjugated with cytotoxic peptide CGGG(KLAKLAK)₂ as proapoptotic peptide.[267] The peptide sequence terminated with cysteine and thiol-functionalized PEG has been conjugated on this HBP by thiol-acrylate Michael addition reaction (**Figure 8**). These HBPs have been loaded with DOX inducing their self-assembly into stable positively charged nanoparticles (42-100 nm) in aqueous solution. The release of DOX was faster at pH 5.0 as compared to pH 7.4, phenomenon associated to the acid-triggered degradation of the polymer. These nanoparticles show enhanced cellular uptake and higher cytotoxic activity as compared to the HBPs solely conjugated with PEG and the free CGGG(KLAKLAK)₂ peptide.

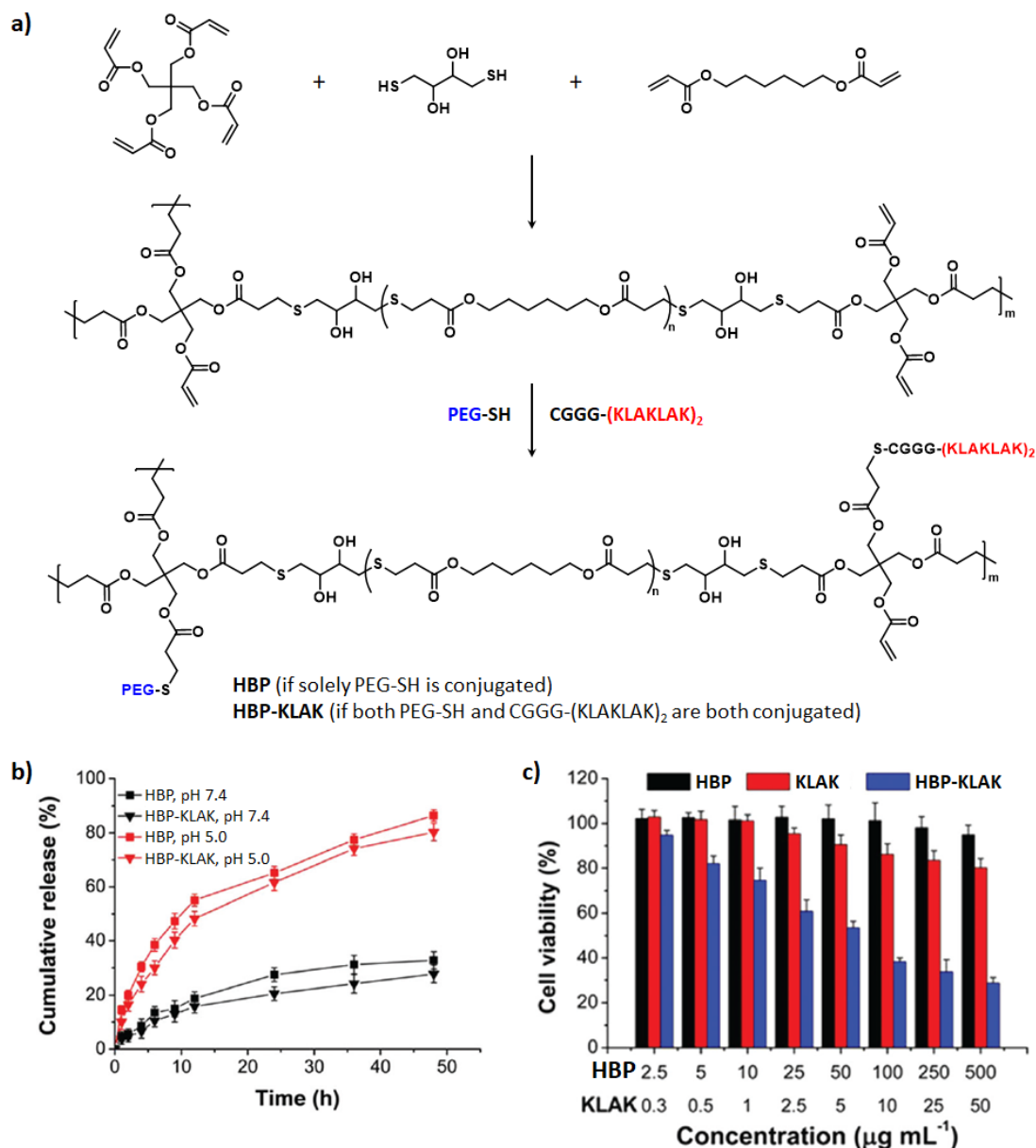


Figure 8. Conjugation of PEG and (KLAKLAK)₂ peptide (proapoptotic peptide) on hyperbranched poly(β-thioester)s: a) synthesis, b) DOX release at pH 5.0 and 7.4, and c) comparative cytotoxicities with CGGG-(KLAKLAK)₂ and PEGylated hyperbranched poly(β-thioester) measured by CCK-8 assay. Adapted with permission.[267] 2017, Royal Society of Chemistry.

Michael addition reactions have been considered between maleimides and thiols. Selegi *et al.* have synthesized amphiphilic star-like HBPs by light-induced self-condensing vinyl copolymerization of methyl methacrylate and 2-bromoethyl methacrylate followed by chain extension with 2-hydroxy ethyl methacrylate.[269] Maleimide groups are introduced on HBPs by reacting *N*-(4-

maleinimidophenyl) isocyanate with the hydroxyl groups present on the HBP permitting the conjugation with one cell penetrating peptide (TAT, sequence: CYGRKKRRQRRR) and one peptide targeting $\alpha_v\beta_3$ integrins (RGD). These HBPs loaded with DOX (4.3 %) form multimolecular nanostructures (diameter: 172 ± 23 nm) and exhibit low cytotoxicity and enhanced cellular uptake. Xu *et al.* have prepared hyperbranched poly(amine ester) (PAE) by adding *N,N*-diethylol-3-amine methylpropionate to 1,1,1-trimethylolpropane that is modified with *N*-(4-maleinimidophenyl) isocyanate to introduce maleimide groups for conjugation and chain extended with D,L-lactide to create a hydrophobic shell.[270] 1,2-Dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE), phospholipid introduced for better biocompatibility, has been reacted with the terminal carboxylic acid of the poly(D,L-lactide) (PDLLA), while thiol-terminated transferrin promoting cell entry through transferrin receptors and thiol-terminated RGDfK targeting $\alpha_v\beta_3$ integrins have been conjugated to the HBP through coupling to the maleimide group present on the HBP. This HBP loaded with PTX has a ten-fold improved efficiency in $\alpha_v\beta_3$ integrin overexpressing cells and twice in transferrin overexpressing cells. Xiao *et al.* have modified H40 with poly(γ -benzyl-L-glutamate)-*b*-PEG.[271] HBPs have been conjugated with DOX (16 wt%) as drug to glutamate units through hydrazone linkages using hydrazine along with cyclic RGDfC as TTP and a thiol-terminated macrocyclic chelator by reaction of the maleimide-terminated PEG present on the HBP. The drug release profile under simulated physiological conditions shows an initial burst release followed by a plateau (12 % released after 45 h), while the release rate increases at lower pH (pH = 5.3) reaching 93 % after 45 h due to the sensitivity of hydrazone linkage in acidic conditions. Higher cellular uptake and tumor targeting have been observed for the HBP with the targeting ligand as compared to the one without cyclic RGDfC. The conjugated HBP clears through the hepatobiliary pathway as their

hydrodynamic diameter (65 nm) is above the cut-off for renal filtration (5 nm). Similarly, Chen *et al.* have functionalized H40 with poly(β -benzyl-L-aspartate)-*b*-PEG introducing primary amines with redox-sensitive (*i.e.* disulfide) linkages on the aspartate units for complexation with siRNA and conjugating GE11 (an anti-EGFR peptide) on the maleimide-terminated PEG of the HBP.[272] The presence of GE11 on the polymer enhances cellular uptake in EGFR overexpressing cells promoting gene silencing.

4.2. Oligonucleotides as active targeting groups

Short strands of oligonucleotides (*i.e.* single-stranded DNA and RNA constituted of 15 to 40 bases) can specifically recognize a target molecule and have advantages such as their low molecular weight as compared to antibodies, simple modification, and remarkable affinity, but also high stability, non-immunogenicity and nontoxicity *in vivo*. [273, 274] Oligonucleotides have gained attention as targeting moieties grafted on the surface of various nanostructures in recent years,[275-278] including HBPs for targeted drug delivery.

Strain-promoted 1,3-cycloaddition reaction has been exploited to conjugate dibenzocyclooctyne-terminated oligonucleotides on azide-containing HBPs. Yang *et al.* have synthesized photoresponsive HBP-DNA conjugates by self-condensing ATRP of 2-(2-bromoisobutyryloxy)ethyl acrylate, *o*-nitrobenzyl acrylate and oligo(ethylene glycol) acrylate, modification of the bromines into azido groups and conjugation of dibenzocyclooctyne-functionalized sgc8 (*i.e.* ATCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGA, a DNA with selective binding affinity to cells overexpressing the protein tyrosine kinase 7[279]).[280] These HBPs self-assemble in aqueous medium into stable multimolecular nanostructures constituted of a core of HBP and a corona of DNA with a hydrodynamic diameter of 40 nm (**Figure 9**). *o*-Nitrobenzyl groups photodegrade under UV irradiation at 365 nm, which is used to control drug release investigated using Nile red as a model molecule. Besides the effect

of the photoresponsiveness of the HBP, the cytotoxicity study of these DOX-loaded nanostructures indicates that sgc8-conjugated HBP is more efficient than HBPs conjugates with DNA of random sequence.

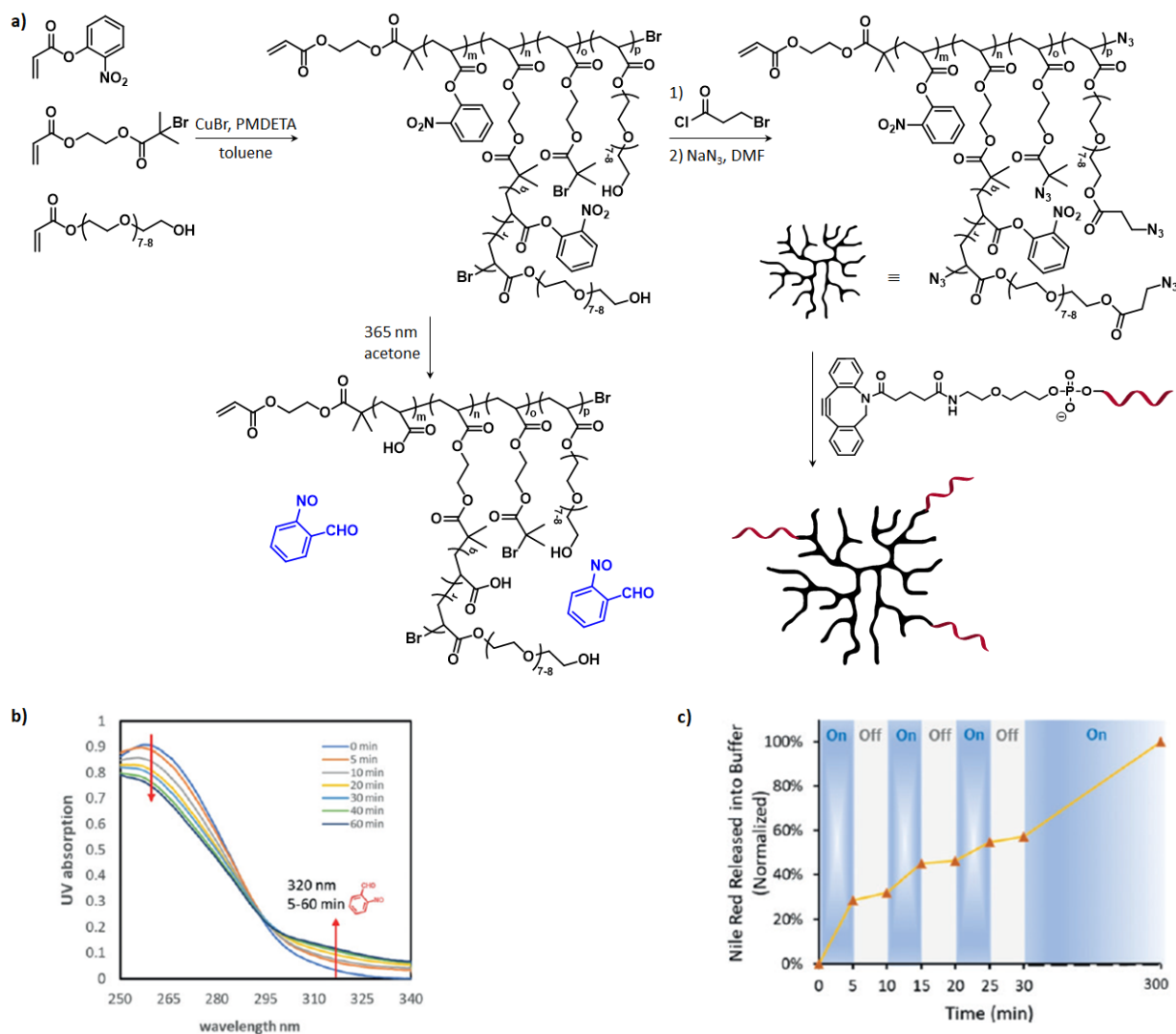


Figure 9. Photoresponsive HBP-DNA conjugates: a) synthesis of HBP by self-condensing ATRP, photodegradation, and conjugation with sgc8, b) UV spectra of multimolecular nanostructures based on HBP-DNA conjugates after UV irradiation, and c) light-triggered controlled release of Nile red loaded in multimolecular nanostructures. Reproduced with permission.[280] 2018, Wiley.

Addition reaction between amines and NHS esters has been also used to conjugate oligonucleotides on HBPs. Xu *et al.* have conjugated A10 (*i.e.* GGGAGGACGAUGCGGAUCAGCCAUGUUUACGUCACUCCUUGUCAAUCCUCAUCGG

CAGACGACUCGCCCCGA, RNA specifically targeting PMSA) on modified hyperbranched polyester H40 obtained by chain extension with L-lactide followed by coupling reaction with monomethoxy PEG and NHS-functionalized PEG.[281] The conjugation has been afforded by reaction of primary amines of A10 with the NHS ester groups present on the HBP. Micelle-like multimolecular nanostructures having a hydrodynamic diameter of 69 nm have been obtained by self-assembly of DOX-loaded A10-conjugated HBPs. Higher cellular uptake in tumor cells has been observed for A10-conjugated HBP as compared to the unconjugated one leading to more significant apoptosis in cancer cells. Biodistribution of DOX in different tissues 6 h post-treatment has been evaluated by measurement of DOX fluorescence intensity showing the strongest intensity in tumors for the A10-conjugated HBP.

Oligonucleotides have been conjugated on HBPs by Michael addition reaction between thiol-terminated oligonucleotides and acrylate-functionalized HBPs. Zhuang *et al.* have reported a redox-responsive DOX-loaded HBP-DNA conjugate prepared by self-condensing RAFT polymerization of OEGMA with a chain transfer agent bearing a disulfide linkage (**Figure 10**).[282] HBPs are modified with acryloyl chloride to permit the conjugation of thiol-containing AS1411 (*i.e.* GGTGGTGGTGGTTGTGGTGGTGGTGGTTT-C₃, DNA binding specifically to cells overexpressing nucleolin receptors) by Michael addition reaction. Drug release is enhanced due to the disulfide linkages on the backbone of these HBPs cleaving in the cytoplasm in the presence of glutathione. Confocal laser scanning microscopy shows a higher concentration of DOX (in red on Figure 10b) in the cytoplasm of MCF-7 breast cancer cells for AS1411-conjugated HBP as compared to unmodified HBPs, but also a higher accumulation of DOX in MCF-7 cells than in L929 healthy cells.

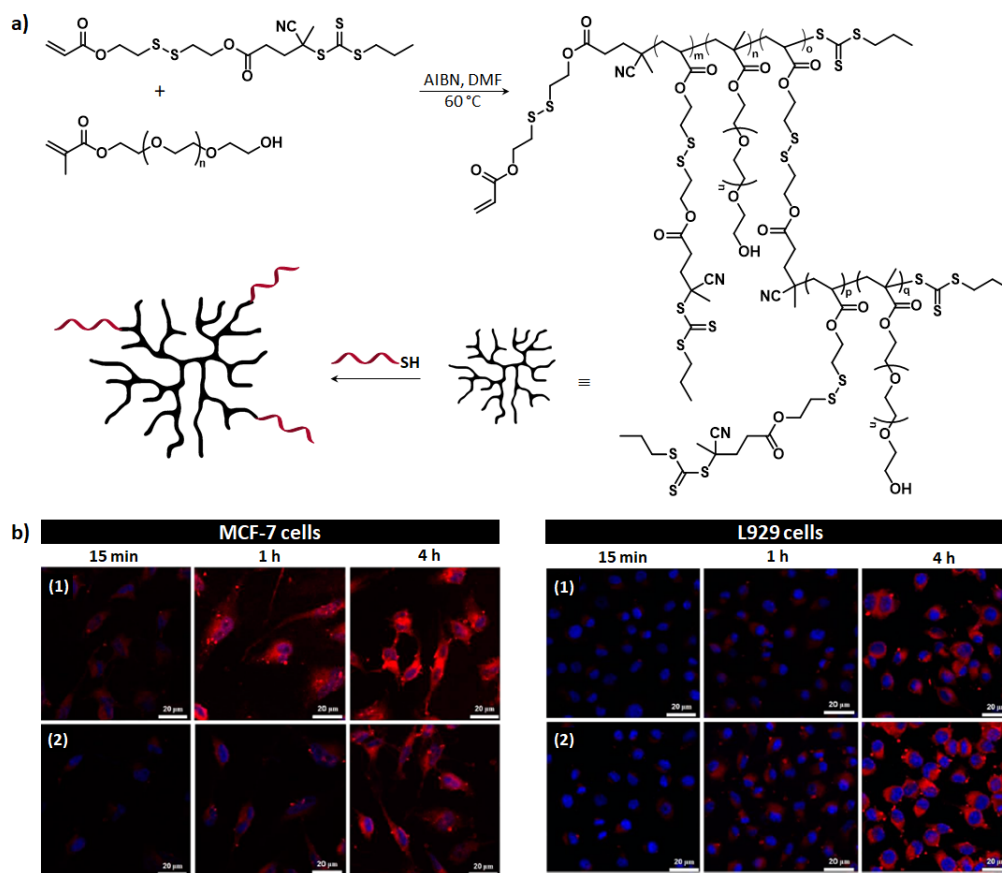


Figure 10. Redox-responsive HBP-DNA conjugates: a) synthesis of HBP by self-condensing RAFT polymerization and conjugation with AS1411, and b) confocal laser scanning microscopy merged images of MCF-7 and L929 cells incubated for 0.25, 1 and 4 h with (1) DOX-loaded AS1411-conjugated HBP and (2) DOX-loaded unmodified HBP (in blue cell nuclei stained with Hoechst 33342). Reproduced with permission.[282] 2016, American Chemical Society.

4.3. Folic acid as active targeting group

Small molecules have been also considered as targeting groups. Among them, vitamin B9 also known as folate when naturally occurring or folic acid in its synthetic form is the most investigated targeting ligand for tumor cells as folate receptors are highly overexpressed in epithelial, ovarian, cervical, breast, lung, kidney, colorectal, and brain tumors.[283] Folic acid has been conjugated to various materials[284] including HBPs due to their stability over a broad range of temperatures and pH values, non-immunogenicity, facile functionalization, inexpensiveness, and small size. Folic acid-conjugated HBPs prepared through different conjugation strategies (**Table 2**) show

higher targeting specificity as compared to unconjugated ones with a significant contrast between the tumor sites and healthy tissues, and a higher and faster accumulation at the tumor site.

Table 2. HBPs conjugated with folic acid

HBP•functional group	drug loaded	Nanostructure (size, nm)	Ref.
<i>carbodiimide coupling reaction between HBP-OH and FA-COOH</i>			
poly(citric acid)•PEG	quercetin	- (10-49)	[285]
polyglycerol	-	- (11)	[286]
H40•PCL-PEG	5-FU, PTX	unimolecular (100)	[287]
H40•PDLLA- <i>b</i> -PEG	DOX	unimolecular (97)	[288]
polyglycerol•PDLLA-PEG	PTX	multimolecular (100)	[289]
<i>carbodiimide coupling reaction between HBP-NH₂ and FA-COOH</i>			
PAA	siRNA	multimolecular (-)	[290]
poly(L-lysine)	-	unimolecular (-)	[291]
polyspermine	DNA	multimolecular (105-180)	[292]
poly(3-ethyl-3-(hydroxymethyl)oxetane)•poly(carboxybetaine)	DOX	unimolecular (40)	[293]
poly(ethylene imine)•PEG	5-fluorocytosine	-	[294]
POEGMA•PEG	-	unimolecular (11)	[295]
polyester•PEG-lysine	5-FU	multimolecular (177)	[296]
poly(dimethylaminoethyl methacrylate)•PEG	DNA	multimolecular (100-400)	[297]
<i>carbodiimide coupling reaction between HBP-COOH or HBPs-OH and FA-NH₂</i>			
H40•PEG	-	-	[298]
polyglycerol•PEG	tamoxifen	multimolecular (-)	[170]
polyester	-	multimolecular (63)	[299]
poly(2-(dimethylamino)ethyl methacrylate)	-	multimolecular (75-400)	[300]
<i>Copper-assisted alkyne-azide cycloaddition between HBP-N₃ and FA-alkyne</i>			
H40•PCL- <i>b</i> -POEGMA	PTX	unimolecular (20-100)	[301]
polyglycerol	-	unimolecular (-)	[302]
H40•PCL- <i>b</i> -poly(acrylic acid)- <i>b</i> -PEG	PTX	unimolecular (33)	[303]
<i>Copper-assisted alkyne-azide cycloaddition between HBP-alkyne and FA-N₃</i>			
polyester	PTX, azidothymidine	multimolecular (82-92)	[304]
H40•PCL- <i>b</i> -PVP- <i>b</i> -PEG	PTX	multimolecular (150)	[305]
POEGMA	-	unimolecular (8)	[306]
<i>Host-guest recognition</i>			
polyglycerol•PEG	DOX	multimolecular (80)	[307]
amylopectin•3-(dimethylamino)-1-propylamine	-	multimolecular (50-100)	[308]

Coupling reactions between carboxylic acid and amine groups have been exploited to conjugate folic acid on HBPs using a variety of carbodiimides, *e.g.* 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (EDC),[292, 296, 297] 1,1'-carbonyldiimidazole,[299] and DCC,[287-290, 293, 294, 298, 300, 309] but also aminium-based coupling agents such as 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium.[170] The carboxylic acid group next to the methylene on folic acid has been directly used for the conjugation on HBPs. Santra *et al.* have reported the synthesis of hyperbranched polyesters bearing terminal hydroxyl groups exploited to covalently attach folic acid and the encapsulation of fluorophores (*i.e.* 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate and indocyanine green) and cytochrome C (*i.e.* mitochondrial protein able to act as endogenous cellular apoptotic initiator).[299] Folic acid has been also conjugated using this conjugation route to H40 functionalized with poly(ϵ -caprolactone)-*b*-PEG (PCL-*b*-PEG) for the encapsulation of two drugs (*i.e.* 5-FU and PTX),[287] star-like hyperbranched polyglycerol with PEG arms loaded with pyrene and tamoxifen[170] or PDLLA-*b*-PEG arms studied with PTX as drug,[289] and star-like polyester with PEG arms loaded with 5-FU,[296] star-like hyperbranched poly(dimethylaminoethyl methacrylate) with PEG arms complexed with short linear DNA.[297] The coupling agent can also be used in the presence of NHS to prepare *in situ* activated esters to improve the efficiency of the amidation reaction to conjugate folic acid. This approach has been used on H40 functionalized with PLLA coupled to PEG loaded with DOX,[288] star-like hyperbranched poly(3-ethyl-3-(hydroxymethyl)oxetane) with poly(carboxybetaine) arms for the encapsulation and release of DOX,[293] redox-sensitive hyperbranched PAAs complexed with MMP-9 siRNA,[290] hyperbranched polyspermines used as vector of shAkt1 DNA,[292] and hyperbranched polyethylenimine modified with folic acid-functionalized PEG complexed with plasmid pCMVCD or pCMVTRAIL DNA.[294] For

example, Luo *et al.* synthesized hyperbranched polyspermine by condensation of spermine and citric acid followed by conjugation of folic acid using EDC as coupling agent in the presence of NHS (**Figure 11**).^[292] The HBPs have been complexed with shAkt1 DNA and investigated in HeLa cells. As compared to PEI 25k (branched PEI with a molecular weight of 25 kDa), the complex formed between folic acid-conjugated and unconjugated hyperbranched polyspermines and shAkt1 exhibits a lower cytotoxicity in HeLa, L02, and A549 cells. The cell uptake of these complexes determined by flow cytometry was superior to free shAkt1 especially the folic acid-conjugated hyperbranched polyspermine supporting the higher affinity of this polymer towards folate receptors.

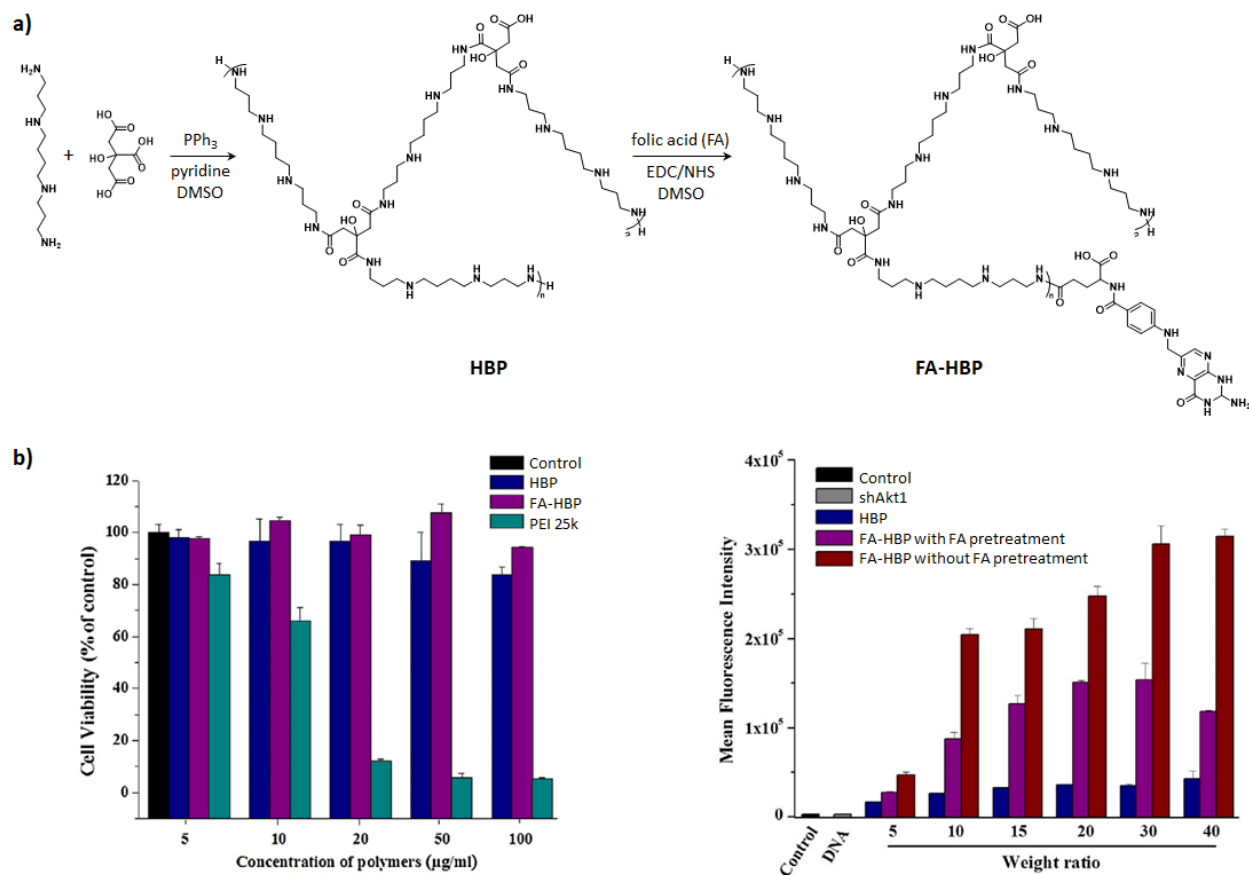


Figure 11. Hyperbranched polyspermine for the delivery of shAkt 1: a) synthesis of hyperbranched polyspermine and its functionalization with folic acid, and b) cell viability after 72 h of incubation and cell uptake in HeLa cells. Adapted with permission.[292] 2016, Elsevier.

CuAAC reactions of alkyne- or azide-functionalized polyesters with folic acid modified with azide or alkyne functional groups respectively have been reported.[301, 303-305, 310] Heckert *et al.* have prepared sulfur-containing hyperbranched polyesters used for the encapsulation of a fluorescent lipophilic cationic indocarbocyanine (DiI) dye for optical imaging, complex based on bismuth and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) for enhanced X-ray imaging and taxol as model drug (**Figure 12**) leading to nanoparticles of 82 nm in diameter.[304] These nanoformulations have been modified successively with α -amino- ω -carboxylic acid and propargylamine by EDC/NHS coupling reaction, and conjugated with azido-folate by CuAAC. The presence of sulfur atoms on the HBPs enhanced the encapsulation of Bi-

DOTA complexes due to its higher binding affinity for bismuth atoms towards the sulfur-based pendent groups. *In vitro* study showed folate-receptor mediated internalization of the loaded HBP and its optical and X-ray imaging. Li *et al.* have reported the synthesis of star-like HBPs based on H40 with PCL arms for which the terminal groups were modified to introduce an ATRP initiator to copolymerize OEGMA and 3-azidopropyl methacrylate.[301] Alkyne-modified folic acid and DOTA were conjugated to HBPs by CuAAC as targeting and gadolinium chelating ligands respectively. *In vitro* and *in vivo* studies of HBPs loaded with PTX showed selective cellular uptake with significantly higher cytotoxicity as compared to folate-free HBPs due to folic acid receptor-mediated endocytosis, good accumulation micelles in tumor cells, extended blood circulation and prominently positive contrast enhancement.

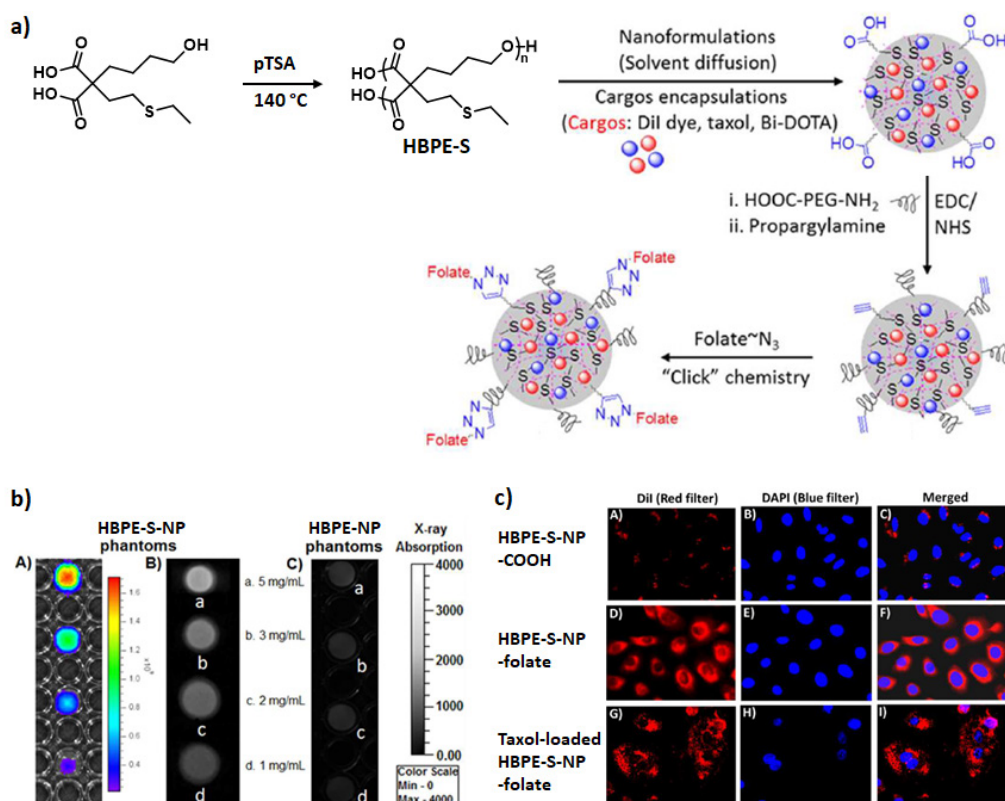


Figure 12. Nanoparticles based on sulfur-containing hyperbranched polyester (HBPE-S-NP): a) synthesis of HBPE-S by melt polymerization, loading with DiI dye, bismuth-based complex and taxol, and conjugation with folic acid by CuAAC, b) optical (A) and X-ray (B and C) imaging of HBPE-S-NP (A and B) and corresponding nanoparticles based on hyperbranched polyester without sulfur (HBPE-NP), and c) cellular uptake in A549 cells by fluorescence microscopy (nuclei stained in blue with DAPI dye). Adapted with permission.[304] 2017, American Chemical Society.

Besides covalent attachment of folic acid on HBPs, folic acid can be conjugated to HBPs by supramolecular assembly. Chen *et al.* have reported the synthesis of PEGylated hyperbranched polyglycerol modified with benzimidazole, a guest molecule able to interact with β -cyclodextrin.[307] This HBP was mixed with folic acid-functionalized β -cyclodextrin and DOX to obtain supramolecular nanoparticles (**Figure 13**) having a hydrodynamic diameter of 88 and 275 nm at pH 7.4 and 5.3 respectively attributed to the acid-responsiveness of benzimidazole. Benzimidazole exhibits hydrophobic properties at pH 7 and an increased hydrophilicity associated to the protonation of the aromatic amines under acidic conditions leading to stronger guest-host interactions under physiological conditions and disassembly at lower pH as in endosome-lysosome

compartments. The nanoassemblies showed a faster and higher drug release when lowering the pH, phenomenon that was not observed when benzimidazole was replaced by benzoic acid that cannot induce pH-responsiveness. The presence of folic acid enhanced the endocytosis of the nanoparticles loaded with DOX especially in HeLa cells due to their higher overexpression of folate receptors as compared to MCF-7 cells.

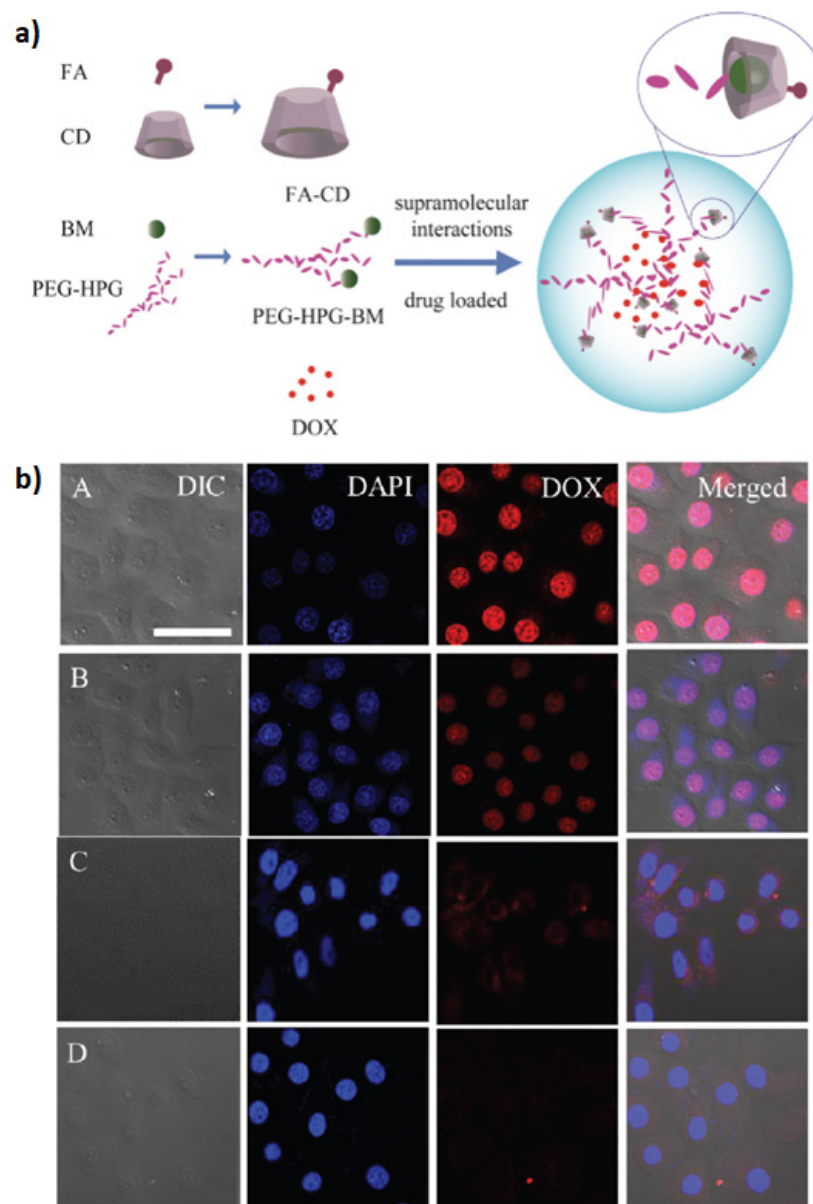


Figure 13. Supramolecular assembly of benzimidazole-functionalized PEGylated hyperbranched polyglycerol (PEG-HPG-BM) and folic acid-functionalized β -cyclodextrin (FA-CD) for targeted delivery of DOX: a) self-assembly by host-guest recognition and DOX loading, and b) cellular uptake in HeLa (A, C, and D) and HepG2 (B) cells after 3h of incubation of folate-conjugated DOX-loaded supramolecular assembly (A and B), DOX-loaded PEG-HPG-BM (C) and DOX-loaded PEG-HPG-benzoic acid (D) (nuclei stained in blue with DAPI dye, differential interference contrast (DIC) images, scale bar: 50 nm). Reproduced with permission.[307] 2015, Royal Society of Chemistry.

4.4. Other ligands as active targeting group

Glutamate urea is a small molecule that binds selectively to PSMA, that is overexpressed 10-fold higher in prostate cancer cells than in healthy prostate tissues.[311] Glutamate urea has been

conjugated on HBPs to treat and detect prostate cancer by the group of Thurecht.[312-314] Two approaches have been proposed either the coupling reaction of glutamate urea on the PFP ester present at one extremity of the HBP[312] or the functionalization of the chain transfer agent[313, 314] for RAFT polymerization. In their studies, OEGMA, ethylene glycol dimethacrylate, and Cy5-labelled methacrylamide were used as comonomers to prepare HBPs in the presence of either trifluoroethyl acrylate[312] or hydrazide-functionalized methacrylate[313, 314]. The hydrazide group was used to conjugate DOX[314] or fluorine-2-carboxaldehyde[313] as model drugs through a hydrolytically degradable linkage. *In vivo* studies showed the high specificity of glutamate urea on cells overexpressing PSMA (**Figure 14**).

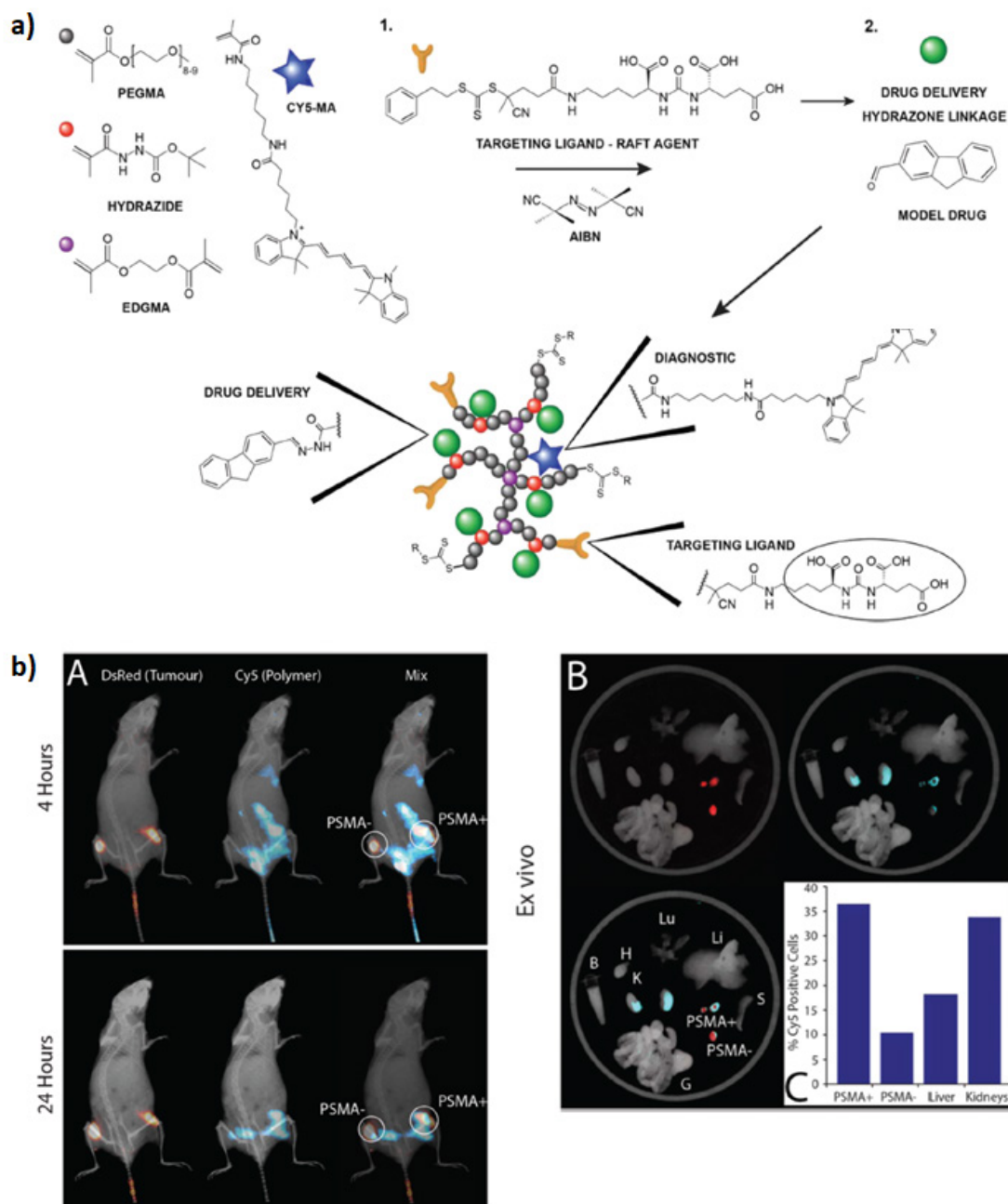


Figure 14. Glutamate-conjugated hyperbranched polymer for theranostic targeting prostate cancer: a) synthesis of POEMA-based HBP by RAFT polymerization labeled with Cy5 dye and conjugated with glutamate urea through modification of the chain transfer agent and fluorine-2-carboxaldehyde as a model drug through a hydrazone linkage, and b) targeting in mouse with subcutaneous PSMA+ and PSMA- tumors on contralateral flanks (A) imaging after 4 and 24 h post-injection, (B) imaging of excised organs (B: blood, H: heart, Lu: lung, Li: liver, K: kidneys, S: spleen, G: gut) after 24 h, and (C) flow cytometry of Cy5 positive cells in excised organs. Reproduced with permission.[313] 2014, Royal Society of Chemistry.

Alendronate is an amino bisphosphonate used to treat different bone diseases including osteoporosis and bone metastasis, but is also employed as bone-targeting ligand due to its high affinity for hydroxyapatite mineral composing human and animal bones.[315] Chen *et al.* have synthesized alendronate-conjugated amphiphilic HBPs consisting of a H40 core with PEG arms.[316] Alendronate was conjugated to the NHS-bearing HBP by coupling reaction. These HBPs self-assembled in aqueous solution forming uniform spherical nanostructures (14 nm in diameter) and encapsulating DOX. *In vitro* studies showed the good biocompatibility, cellular uptake and DOX release of these conjugates, while hydroxyapatite binding assay confirmed the favorable binding affinity of alendronate-conjugated amphiphilic HBPs to bone tissues.

Monosaccharides such as mannose and galactose are able to bind to carbohydrate-binding proteins known as lectins that are overexpressed in cancer cells.[317] Thurecht *et al.* have prepared HBPs based on 2-(dimethylamino)ethyl acrylate and trifluoroethyl acrylate using ethylene glycol dimethacrylate as branching agent by RAFT polymerization in the presence of an alkyne-bearing chain transfer agent for post-polymerization modification.[318] Mannose modified with an azide group was conjugated on the HBP by CuAAC. Binding assay to Concanavalin A showed the selective and targeted binding of the mannose-conjugated HBP to mannose-binding lectins. Sun *et al.* have introduced galactose on HBPs by introducing one galactose residue on an acrylate monomer that was copolymerized with a methacrylate bearing a fluorescent dye (*i.e.* 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene, known as BODIPY) in the presence of a transfer agent by RAFT SCVP.[319] These HBPs self-assembled into nanoparticles of 73 nm in diameter as determined by dynamic light scattering and showed specific internalization via galactose-ASGP receptors.

Hyaluronic acid (HA) is an anionic biopolymer which has several excellent properties such as biocompatible, biodegradable, non-toxic, and non-immunogenic.[320] HA can interact with

CD44, ICAM-1, and RHAMM receptors, which are overexpressed in many cancer cells, in particular in tumor-initiating cells.[321] Gu *et al.* have reported the synthesis of redox reducible hyperbranched PAA by Michael addition of *N,N'*-methylenebisacrylamide, *N,N'*-cystaminebisacrylamide, and 1-(2-aminoethyl)piperazine.[322] This HBP was complexed with plasmid DNA (pDNA) subsequently coated with HA by electrostatic interactions to obtain negatively charged spherical nanoassemblies (160-200 nm) for CD44-targeting gene delivery (**Figure 15**). The use of HA as coating significantly reduced the surface charges of nanoassemblies leading to higher stability in serum and longer circulation time. Fluorescence microscopy imaging demonstrated higher cellular uptake of HA/hyperbranched PAA/pDNA nanoassemblies as compared to nanoassemblies without HA into lung tissues of pulmonary tumor-bearing C57BL/6 mice and selectivity when compared to healthy C57BL/6 mice due to HA interaction with CD44 receptors.

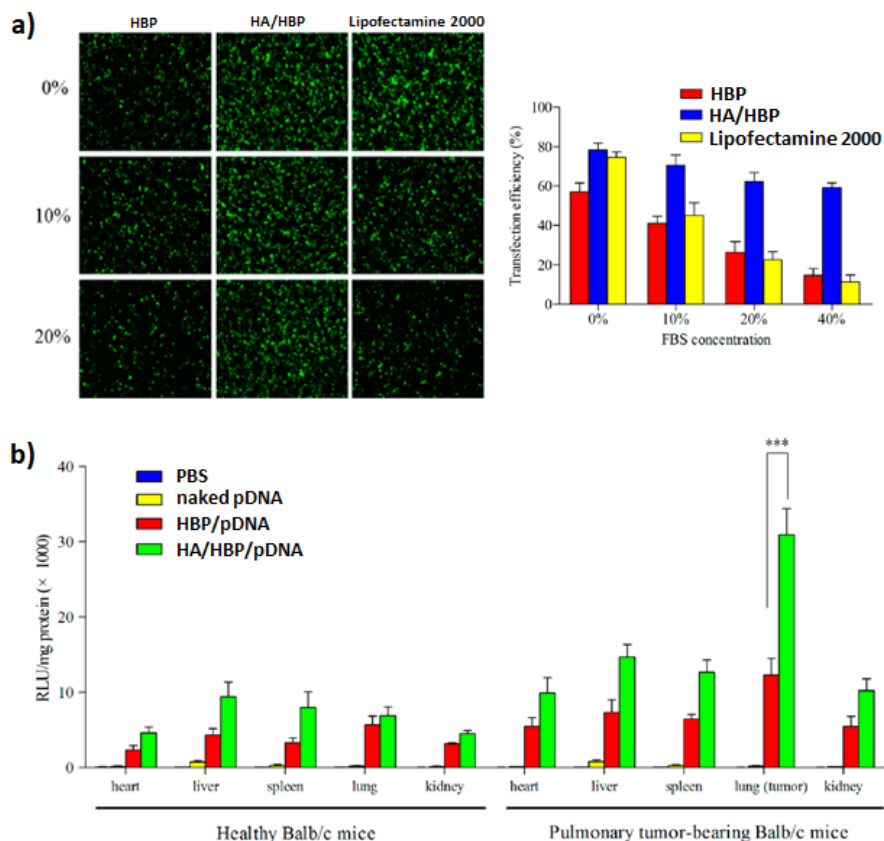


Figure 15. Nanoassemblies of cationic HBP with pDNA coated with hyaluronic acid as targeting ligand for CD44-targeted gene delivery in B16F10 cells: (a) serum-resistant transfection in the presence of fetal bovin serum by fluorescence imaging (left) and flow cytometry (right) of the complexes with pDNA, and (b) luciferase expression in healthy C57BL/6 and pulmonary tumor-bearing C57BL/6 mice. Reproduced with permission.[322] 2016, American Chemical Society.

Transferrin is an iron-binding glycoprotein promoting its transport into cells through transferrin receptors.[323] Transferrin receptors being overexpressed in cancer cells transferrin has been explored as active targeting ligand in drug delivery systems. Xu *et al.* have reported the modification of HBPs based on PAE having PDLLA and DPPE arms with cyclic RGDfK and transferrin by coupling reactions between the maleimide groups present on the HBP and thiol-functionalized ligands (**Figure 16**).[270] Spherical nanoparticles loaded with PTX having a diameter around 260 nm were prepared by emulsion/solvent evaporation. Modified HBPs modified and transferrin showed selective enhanced cellular uptake by HUVEC cells for RGDfK-

modified HBP by integrin-mediated endocytosis and HeLa cells for transferrin-modified HBP by transferrin receptor endocytosis.

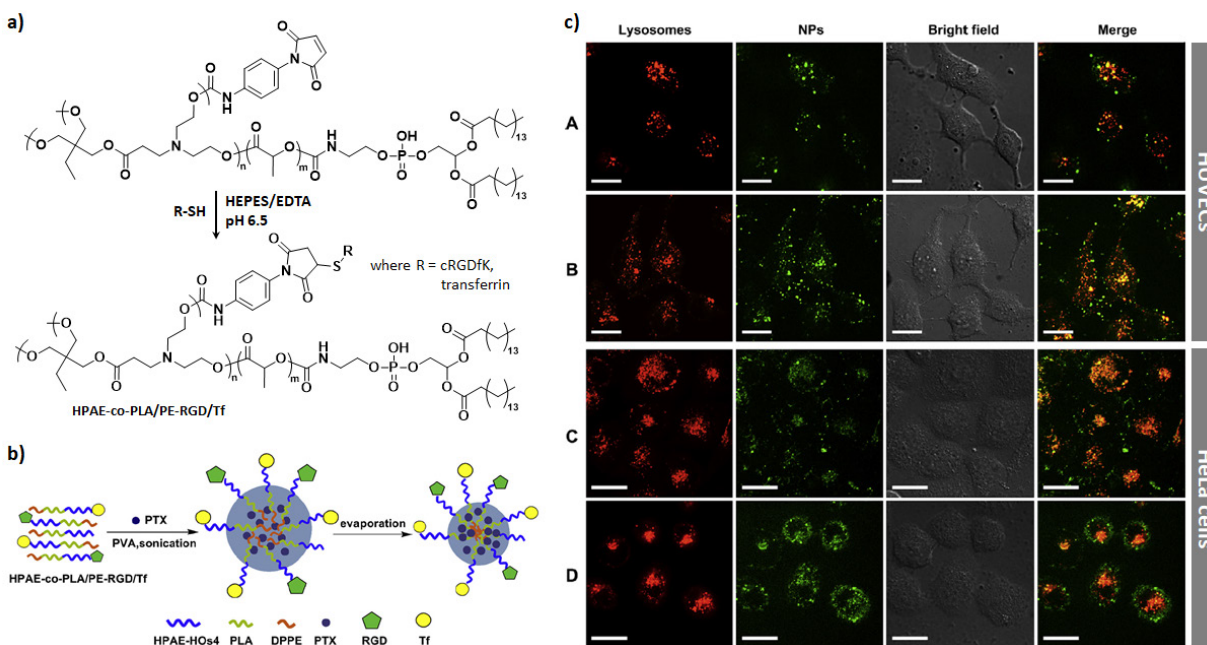


Figure 16. HBP conjugated with cyclic RGDfK and transferrin for dual targeting of integrins and transferrin receptors: a) synthesis of hyperbranched poly(amine ester) and conjugation of targeting ligands, b) preparation of PTX-loaded nanoparticles by emulsion/solvent evaporation, and c) intracellular localization of PTX-loaded HBP conjugated with targeting ligands after 4h of incubation in HUVECs and HeLa cells (lysosome stained with LysoTracker Red DND-99, scale bar: 20 μ m). Reproduced with permission.[270] 2012, Elsevier.

5. SUMMARY AND OUTLOOK

HBPs are highly branched three-dimensional macromolecules. HBPs are less regular than dendrimers and have a higher dispersity. However, their syntheses are easier and can be achieved in a one-pot polymerization process, which is advantageous when considering their scale up production. Various synthesis strategies have been developed to prepare HBPs including AB_x , “ $A_2 + B_n$ ”, self-condensing vinyl and self-condensing ring-opening polymerizations, click chemistry and multicomponent reactions. The unique properties of HBPs, such as low intrinsic viscosity, low glass transition temperature, presence of internal cavities, and a large number of functional groups at the periphery due to their globular and dendritic structures, are attractive for applications in a

large variety of fields such as coatings, modifier additives, light-emitting materials, and drug delivery systems.

Regarding the development of drug delivery systems, some examples of dendritic structures, *e.g.* DEP[®] (Starpharma) and SuperFect[®] (Qiagen) are dendrimers based on polylysine and poly(amido amine) respectively, have been tested in clinical trials and are commercially available. The high dispersity of HBPs could be perceived as an obstacle in their potential clinical uses as the fractions with the lowest and highest molecular weights could have different pharmacokinetical behaviors. However, hyperbranched polymers combine simplicity in synthesis when compared to dendrimers with three-dimensional globular structures and a high number of terminal functional groups as for dendrimers, which have been demonstrated to enhance drug encapsulation and functionalization due to their internal cavities and large number of termini respectively. HBPs have been used as carriers of drugs ranging from small molecules (*e.g.* DOX and CPT) to large nucleic acids (*e.g.* DNA and siRNA). Passive and active targeting can enhance the accumulation of the drug at the tumor sites, which can be achieved by modification of HBPs at their periphery using different synthetic routes (*e.g.* amide bond formation using carbodiimides, CuAAC, thiol-ene reactions). For passive targeting, functional groups promoting more prolonged circulation in the bloodstream, and thus higher accumulation at the tumor sites, such as PEG, have been covalently attached to HBPs. Active targeting of tumor sites has been first investigated by conjugating folic acid and extended to specific ligands, including peptides and oligonucleotides, on HBPs to target specific receptors overexpressed on cancer cells. The conjugation of such ligands has been proven to be an efficient approach to enhance the delivery of the drug in cancer cells. Antibodies have been successfully explored as ligands on HBPs for targeted bioimaging[312, 324] and could be also of interest for targeted drug delivery.

The conjugation of targeting ligands is mostly achieved through post-polymerization modification. This strategy is efficient, but shows some limitations especially in the control of the number of ligands covalently attached at the periphery of HBPs. The copolymerization of imers or transmers with a monomer bearing a targeting ligand by SCVP, especially under RAFT polymerization conditions, has more rarely been explored,[313] but seems an interesting approach to better control the insertion of ligands in terms of number of ligands but also their localization on HBPs.

The field is evolving towards the development of HBPs for theranostics providing a dual role as drug carrier for targeted drug delivery and imaging probe (*i.e.* optical or magnetic resonance imaging) for diagnostic purposes. Regarding magnetic resonance imaging, different approaches have been reported: i) incorporation of a comonomer containing fluoride in the HBP by copolymerization,[318, 325, 326] ii) conjugation to HBPs of chelating ligands able to complex with copper[295, 327] or gadolinium,[291, 301, 302, 328-331] and iii) grafting of HBP on Fe₃O₄ magnetic nanoparticles by either growth of the HBP from the surface of the nanoparticle[332] or coupling reaction between HBPs and nanoparticles by thiol-ene reaction[333] or reduction of imine bond.[334] Furthermore, fluorophores such as BODIPY[319] and cyanide dyes[312] have been conjugated to HBPs for optical imaging using conventional conjugation approaches affording HBPs decorated with targeting ligands and fluorophores for theranostics. Few recent reports are also describing HBPs with intrinsic fluorescence such as hyperbranched polysiloxane[335] and conjugated HBPs.[336] More recently, luminescent nanoparticles have been investigated as imaging probes. For example, hyperbranched polyglycerol has been prepared from the surface of red fluorescent silicon nanoparticles and modified with cyclic RGDfK to target $\alpha_v\beta_3$ integrins and afford optical imaging.[337] The recent advances in nanomaterials for optical imaging[338] pave

the way to the development of novel nanoobjects for theranostics combining the potential of HBPs due to their high number of functional groups at their periphery to introduce various functionalities such as targeting ligands, and luminescent nanoparticles (*e.g.* gold nanoparticles, silicon nanoparticles, quantum dots and upconverting nanoparticles) due to their higher photostability, tunable emission wavelength and brightness as compared to organic dyes.

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DATA AVAILABILITY

Not applicable

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