CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.201000808

Methods for the Functionalisation of Nanoparticles: New Insights and Perspectives

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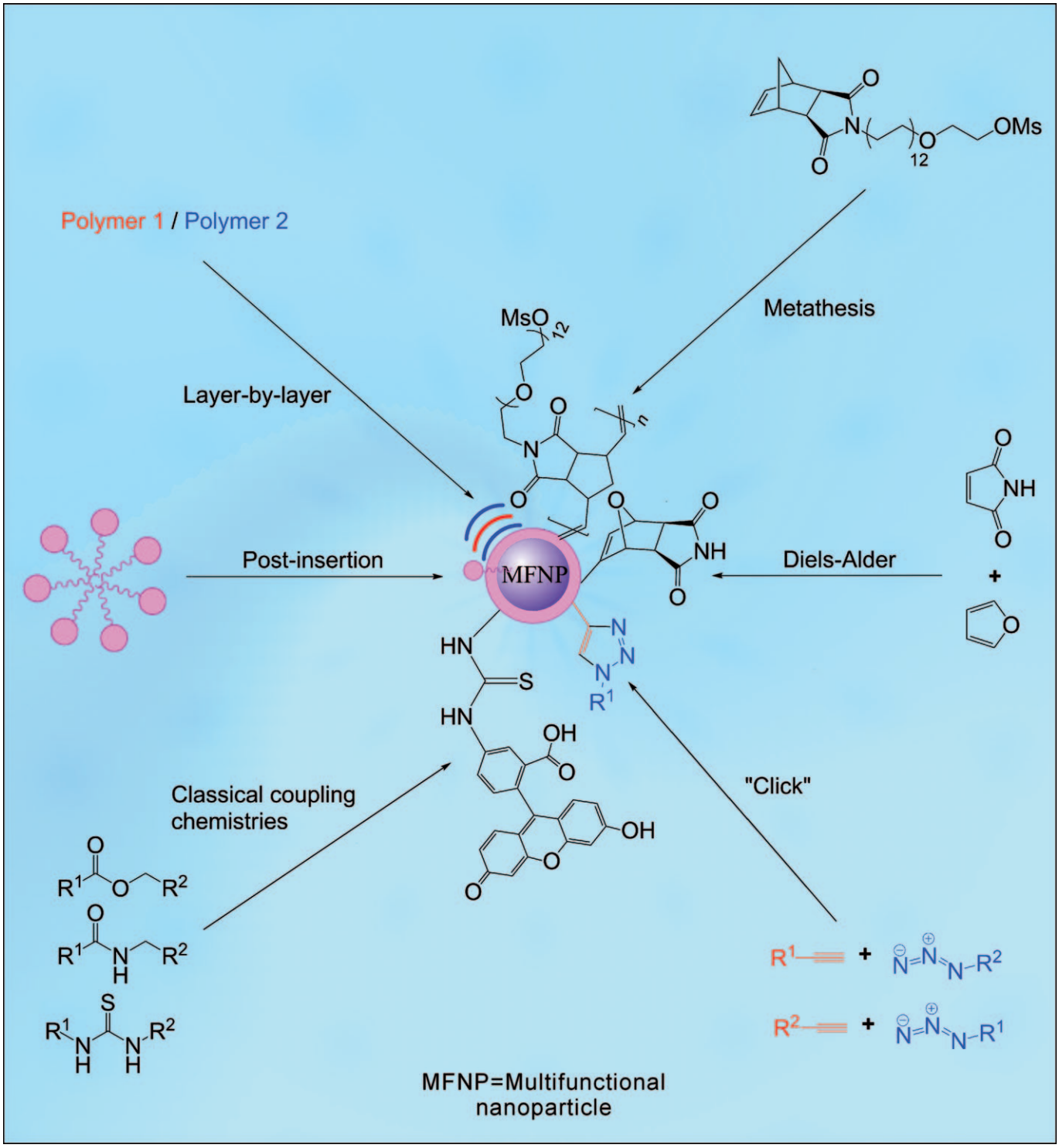

Abstract: In the field of nanometre-sized drug-delivery systems, a wide range of colloidal systems have been created in recent decades. All of the systems have a similar global structure, that is, an inner part and an external surface/interface. In several applications, the external interface is the support for desired properties and the basis of future developments. The engineering of the particle's surface is an emerging step in the design of systems at the nanometre scale. This review presents and summarises the available techniques with a particular attention to recent advances. It is also a base for future works in this expanding area of research.

Keywords: click chemistry · Diels–Alder reaction · metathesis · nanoparticles · self-assembly

Introduction

Nowadays, drug-delivery systems[1–4] are the centre of interest in many fields from chemistry to medicine.[5] Some of them, the organic nanoparticles, take advantage of particular molecular associations leading to colloidal objects that have been elaborated by many formulation protocols. However, few organic nanoparticles can be formulated directly with their final properties, such as correct assembly at the molecular level, specific drug and/or reporter loading, and an engineered interface. Modifications of the formulation protocols are difficult because production processes are relevant in narrow formulation windows, so called feasibility domains. For example, formulations based on phase-inversion temperature (PIT) methods[6] are affected by parameters as simple as salt concentration, that is, starting from the same amount of material, the PIT varies from 45 to almost 90 °C. The importance of such a variation is emphasised when nanoparticles have to be loaded with temperature-sensitive anti-cancer drugs.[7] Another example is the formulation of pegylated (peg = polyethylene glycol) liposomes: from a protocol designed from the production of first-generation liposomes, the introduction of pegylated amphiphiles requires the feasibility window to be completely rebuilt. Furthermore, these difficulties explain why few commercial liposome formulations are available on the market, despite the fact that liposomes have been developed for many decades. An important number of nanoparticles are designed for the vectorisation of hydrophobic substances and should be composed of a hydrophobic phase, such as triglycerides. Modifications in the triglyceride chains length can diverge from established protocols and work has to be re-done from the beginning, with evident additional financial cost. Figure 1 illustrates the use of nanoparticles in the nanomedicine field. If particles are used directly after formulation, their parameters (e.g., size or presence of electric charges at the surface) can dramatically decrease their supposed capabilities. Limited blood circulation and distribution of active drugs in the whole body brings problems.[8,9] On the other hand, if particles are modified through post-formulation strategies, problems can be solved without the need to set up the formulation process.

A new approach has emerged: instead of working to modify manufacturing processes in a complicated way, nanoparticles can be considered as nanometre-sized platforms on which one can perform modifications in a rational and controlled way. Thus, besides manufacturing processes, strategies for post-modified nanoparticles have arisen. Herein, we present methods, based on physics and chemistry, that are the most common ways to functionalise soft organic nanoparticles.

Methods to Functionalise Organic Nanoparticles

A key goal of nanoparticles is to interact with other systems, such as ligands, receptors, biopolymers or drugs. It supposes that nanoparticles should have a defined surface at the molecular level. Today, a number of practical approaches exist that allow molecular control of the surface of nanoparticles. All of these methods have a common goal: to insert molecules on the particles surface with control of their architecture and surface density.

Physical Methods

Physical methods are based on particular behaviour of surfactants or polymers. Thus, these methods rely on dynamic molecular transfer from an acceptor to a donor (post-insertion process) or molecular associations promoted by intermolecular interactions, such as electrostatic forces (layer-by-layer method).

Post-insertion: Generally speaking, post-insertion is the transfer of amphiphilic molecules from micellar assemblies to pre-formed nanoparticles. This process leads to the incorporation of all or a molar fraction of amphiphilic molecules into the external layer of the receiver. This technique was

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developed initially on liposomes[10,11] and has been adapted for nanoparticles of other types, in particular lipidic nanocapsules (LNCs).[12,13] Globally, post-insertion is used in two main ways to insert amphiphilic chemicals of high interest: the first way is the thermal incubation of functionalised micelles composed of the high-interest molecules with the nanoparticles. Subsequently, it leads directly to the functionalisation of nanoparticles with a tuneable amount of a specific amphiphile. Alternatively, post-insertion can be used to introduce a specific anchor ligand that can react later with a wide range of chemicals, depending on the nature of the ligand in a classical chemistry approach. In the literature, the amphiphile is very often from the family of pegylated phospholipids or from the family of pegylated sterols.[14–16] Not enough attention has been paid to the length of the hydrocarbon parts, the number of aliphatic chains required and whether the hydrophilic part could be something other than ethylene glycol polymers. As shown in Figure 2, post-insertion can lead to different nanoparticles systems.[17–19] Three cases are possible depending on the experimental conditions: incomplete insertion can be obtained if the amount of amphiphile is not adapted with regards to the number and the nature of the receiver, complete insertion can be reached if the ratio of amphiphile to nanoparticles is correct, with or without an excess of materials.

Nevertheless, few studies are available for the quantitative assessment of the amount of transferred materials. In fact, the yield of post-insertion techniques is difficult to evaluate. Indeed, as shown in Figure 2, one should be able to separate the nanoparticles obtained from micelles and waste. The yield could then be evaluated by means of additional experiments, such as spectrophotometric/fluorometric quantifications. In the same general concern, the chemical nature of the amphiphile required for post-insertion has not been fully investigated. To summarise, the post-insertion process leads to the manufacturing of functionalised nanoparticle suspensions without the use of a complete formulation process. In addition, the avoidance of the use of organic solvents is a strong argument towards pharmaceutical regulations and allows the development of nanodevices in an ecologically friendly way.

Layer-by-layer (LBL) approach: From a historical point of view, the deposition of polymers with oppositely charged groups into nanoassemblies has been developed for planar macroscopic surfaces.[20] This approach consists of the sequential soaking of a solid support into different polyelectrolyte solutions with washing steps to remove excess material. For many years, this technology has been transferred to the design of colloidal systems functionalised by polyelectrolyte brushes.[21–23] As described in Figure 3, polymers with opposite charges are added to nanoparticles with a charged surface. Electrostatic attraction leads to particles covered with additional layers. Purification is required and consists of centrifugation followed by supernatant exchange or dialysis.

The principal aim of the chemical modification of the particle's surface is to confer new properties at the same time. The LBL strategy has two mains goals: First to cover the nanoparticles to modify the colloidal stability and/or the kinetics of drug release.[24–25] Second, to bind and trap drug molecules (charged or uncharged) in polyelectrolyte layers.[26] Moreover, these layers could be the base for the chemical grafting of signalling molecules setting up an advance base for the development of multi-functional nanocarriers.[27] The polymers can be natural, such as homopolypep-

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Patrick Saulnier is a professor of Biophysics and Biostatistics teaching in the Department of Pharmaceutical Sciences of the Angers University, Angers, France. He obtained his Ph.D. after the development of a new methodology allowing the characterisation of electric charges at different water–air interfaces in University of Pau, France. He is now a group leader within the Inserm U646 team, Engineering of Drug Delivery Systems in Angers, France. His research activities are focused on the characterisation of molecular associations at different interfaces and the development and the characterisation of nanomedicine systems. He has published 60 papers and 6 patents.

Thomas Perrier studied organic chemistry and biochemistry at the University Claude Bernard in Lyon, France. After working on the chemical synthesis of amphiphilic macrocycles (calixarenes and cyclodextrins) as an engineer, he earned his Ph.D. in Pharmaceuticals Sciences from University of Angers, France, specialising in the design of multi-functional nanocarriers. Currently, he is a postdoctoral fellow with Inserm U646 at the University of Angers. His primary research interest is the setting up of chemically engineered nanoparticles as advanced drug-delivery systems.

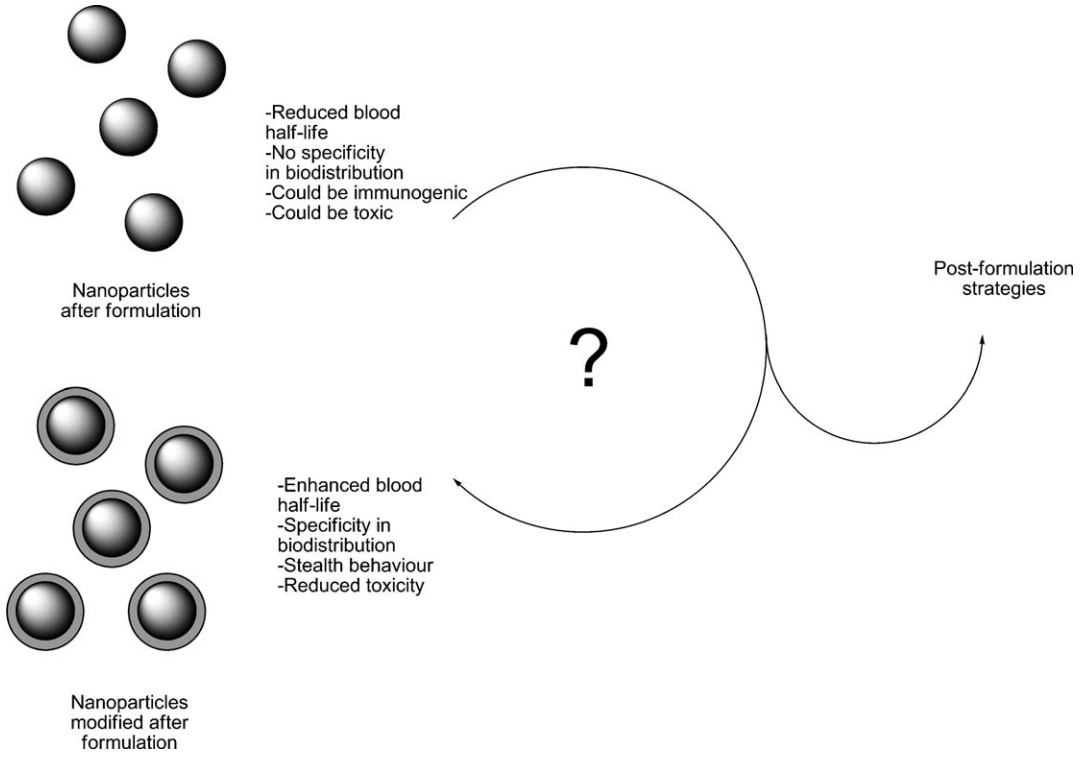


Figure 1. The dilemma of a nanoparticle from the perspective of nanomedicine.

tides and polyoses, or fully synthetic substances. It allows the scientist to adjust the degradation properties of the nanoparticles. Furthermore, this strategy put forward an alternative way to use cationic lipids in the encapsulation of natural or synthetic nucleic acids.[28]

From a wider point of view, the LBL approach promotes the development of nanoparticles in other fields. Another point must be underlined: the LBL approach can produce polyelectrolyte capsules starting from nanoparticles coated with alternate layers of polymers followed by a decomposition of the nanoparticle's core. The result is the formation of hollow polyelectrolyte capsules with a defined number of layers and controlled size.

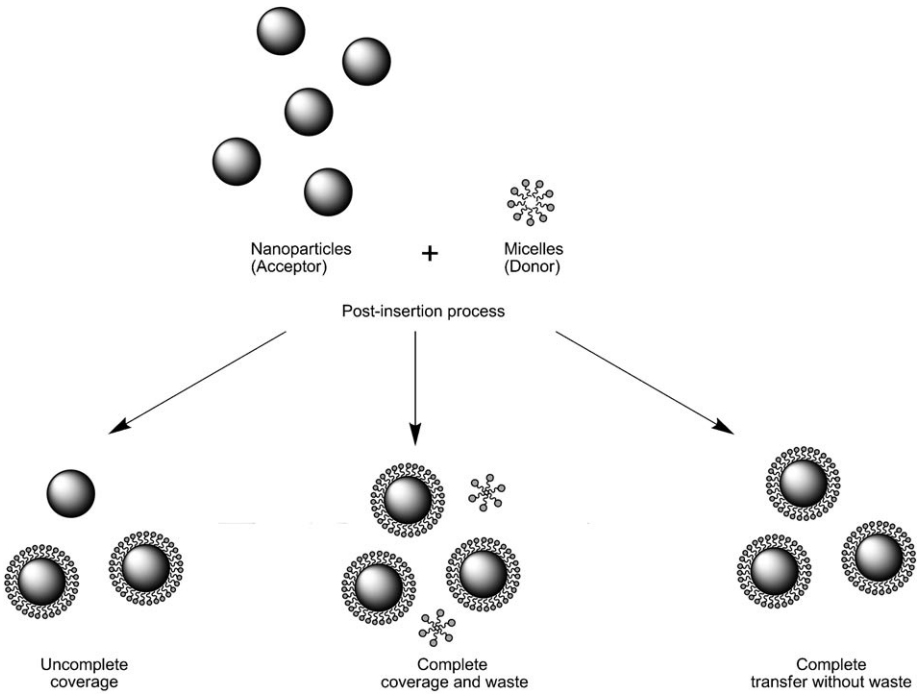


Figure 2. The possible results obtained through post-insertion methods.

Chemical Methods

Chemistry could be interesting to help towards the engineering of nanoparticle interfaces. But chemical reactions should be performed at room temperature under mild conditions because the stability and lifetime of the nanoparticles could be dramatically affected under harsher

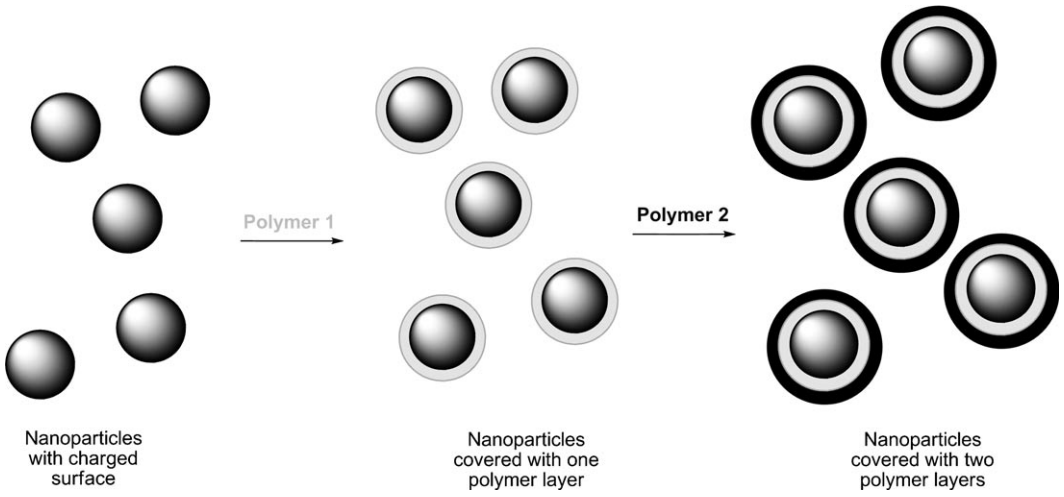
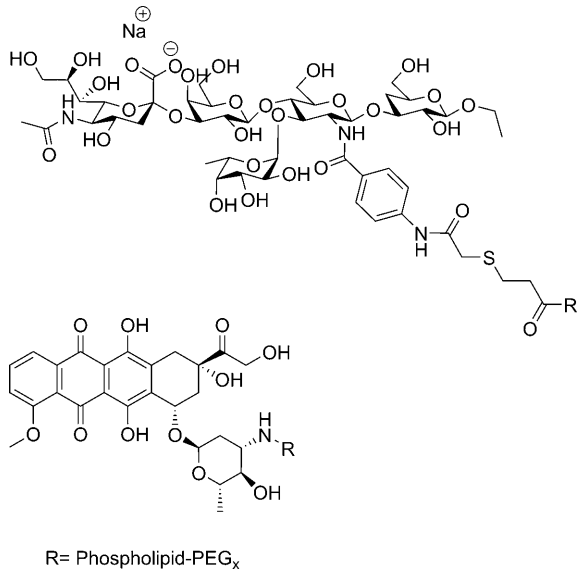


Figure 3. Sequential deposition of polymers onto nanoparticles by the LBL approach.

conditions. Thus, among the known chemical reactions, few of them can satisfy experimental conditions. As a reminder, most nanoparticles are produced in water or tend to be used in water-containing medium. In other words, water should be the solvent for the chemical modification of nanoparticles. Herein, we report reactions that could be carried out under these experimental conditions.

Classical coupling methods: Before presenting novel and attractive methods for the modification of nanoparticles, classical chemistry methods should be illustrated. In recent decades, a lot of work has been done on the synthesis of valuable molecules. First examples include the synthesis of phospholipid derivatives, which are further incorporated into formulations, bearing in mind that an important number of studies involved setting up feasible formulations, as explained in the Introduction. In this way, pegylated phospholipids were coupled to sialyl LewisX in 60% yield. Zalipsky et al. demonstrated that inhibition of cell adhesion increased when sialyl LewisX liposomes were used.[29] To the same extent, pegylated phospholipids have also been coupled to anti-cancer drugs, such as doxorubicin, by using carbodiimide chemistry.[30] The doxorubicin adduct was incorporated into liposome formulations and the obtained liposomes were shown to be able to bring doxorubicin into cells and inhibit tumour growth. Scheme 1 shows a phospholipid conjugated to sialyl LewisX (carbohydrates moiety) through a spacer (top) and the doxorubicin adduct (bottom). Alternatives to the use of carbodiimide chemistry exist and allow the introduction of peptides (such as TAT peptide) on phospholipids.[31] One more time, the modified liposomes show new properties such as good intracellular delivery even under unusual conditions.

Another well-established approach consists of the use of valuable molecules containing thiols that are designed to be grafted onto gold surfaces. For example, gold nanoparticles have been covered with sugar derivatives and peptides (both modified to contain thiol) to produce multi-functional



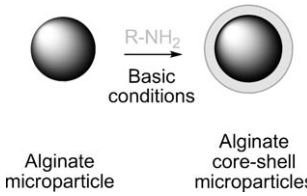
Scheme 1. Bioconjugates of sialyl LewisX and doxorubicin with pegylated phospholipids.

glyconanoparticles as a platform for potential carbohydrate-based anti-cancer vaccines.[32]

Today, conjugation between nanoparticles and molecules tends to be done after the formulation step. For example, green fluorescent protein (GFP) derivatives have been inserted into the outer layer of liposomes. This reaction has been made possible by the use of both modified liposomes and GFP derivatives. Other reactions, such as addition of thiols to a carbon double bond, have been intensively investigated and permit the fixation of various substrates as antibodies and peptides. Thiols are an interesting group of compounds because they are naturally present in proteins through the cysteine amino acids.[33]

Some reactions, such as transacylation, have been exploited to modify microparticles,[34] bringing new interesting

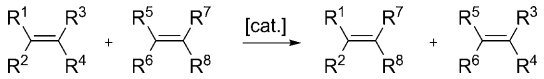
properties. In general, transacylation is the exchange of acyl chains. If acyl chains are immobilised into a particle shell (through ester bonds, for example), transacylation leads to the fixation of the reactant onto the particle shell. Thus, transacylation between alginate microparticles and a model protein, bovine serum albumin (BSA), produced capsule-like structures (Scheme 2). The capsule around the microparticles modifies the initial properties and shows promising results.[35–37] But, to date, even if a lot of surfactants contain ester groups, transacylation has not been used on nanoparticles.



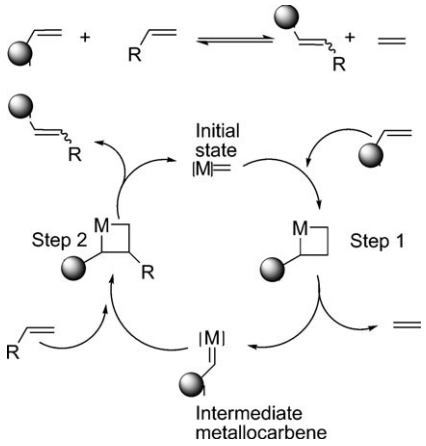
Scheme 2. Transacylation on microparticles produced core-shell microparticles.

Olefins metathesis: Olefin metathesis[38,39] allows the exchange of substituent between different olefins according to Scheme 3, in which [cat.] is the catalytic species based on transition metals.

Metathesis is catalysed by organometallic compounds that are based on transition metals. As shown in Scheme 4, the metathesis catalysts are metallocarbene species,[40,41] and metathesis starts when the olefin reacts with the metallocarbene to form metallacyclobutane (step 1). Thus intermediate metallocarbene reacts further (Scheme 4, step 2), forming the product and a new metallocarbene, which can be recycled to complete the catalytic cycle. The catalytic shown in Scheme 4 is a very general example (here the driven force is continual removal of ethylene).



Scheme 3. General scope of metathesis with two different alkenes. R^1, R^2, R^3, R^4, R^5, R^6, R^7 and R^8 are H atoms or organic radicals.



Scheme 4. Metathesis catalytic cycle. [M] represents organometallic catalyst with its ligands. The shaded sphere is the schematic representation of a nanoparticle.

The high potential of this reaction relies on the fact that it could be realised several ways. Indeed, intra- or intermolecular reactions can be carried out with cyclic or acyclic olefins. Scheme 5 summarises the different approaches through five examples.

As shown in Scheme 5, polymerisation reactions can be carried out both by cross-metathesis (ADMP) and by ring-opening metathesis (ROMP and ROCM). To date, EM has not been exploited on nanoparticles.

Concerning the use of these different reactions towards the functionalisation of nanoparticles, ring-opening metathesis (ROM), ROMP and ROCM have been used with success. Furthermore, starting from the first catalysts (Scheme 6), many generations have been developed. The actual catalysts are more stable towards experimental conditions (less sensitive to air and moisture) and more active (higher turnover number).

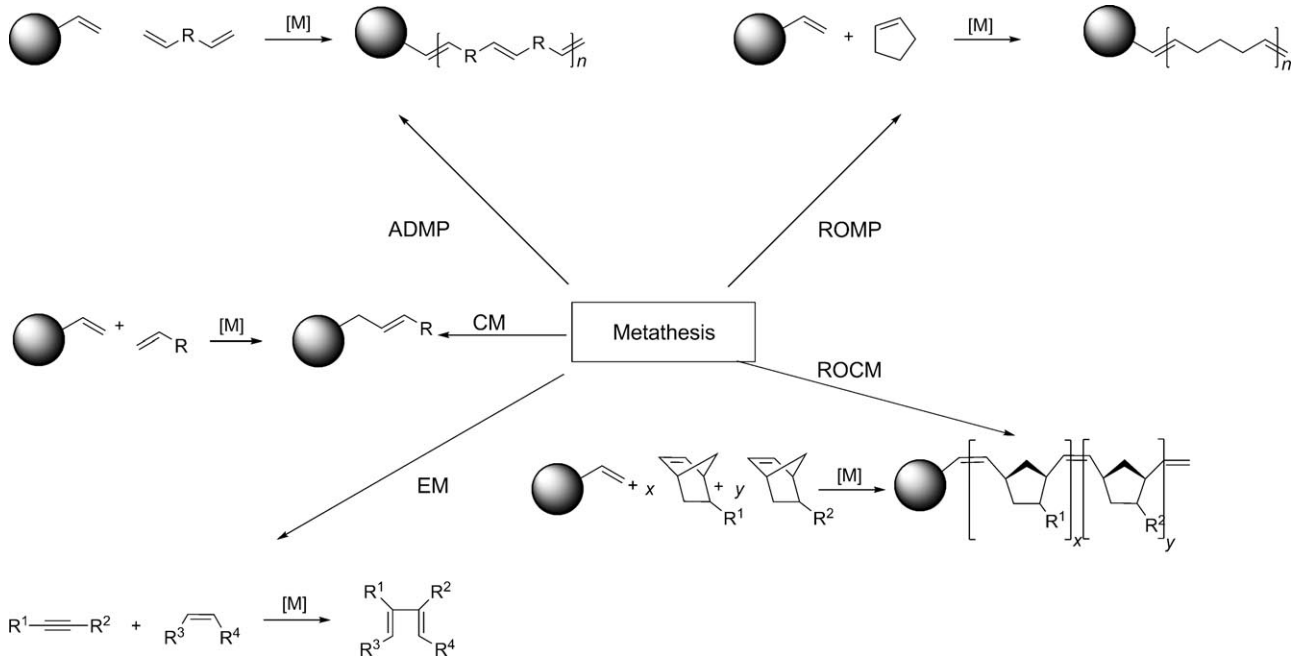
A major breakthrough has been the development of water-soluble catalysts, offering unexpected potential for chemistry on nanoparticles in water.[42–44] Indeed, since most drug-delivery systems are used in water and biological molecules such as proteins or nucleic acids are soluble into water, it is very important to have water-soluble catalysts.

The water-soluble metathesis catalysts shown in Scheme 7 can be synthesised by introducing electric charges or water-soluble polymers, in this case polyethylene oxide chains.

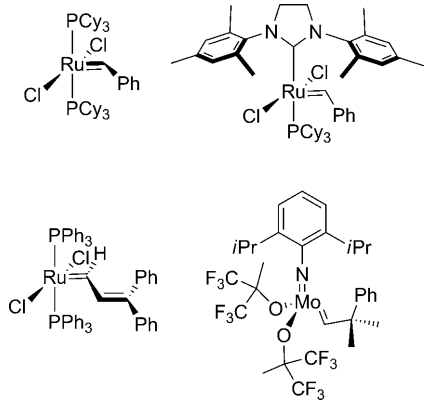
To introduce a classification, the use of olefin metathesis on nanostructures can be separated according the nature of the metathesis substrate(s) and the nature of the catalytic species.[45] In the first case, metathesis can be performed laterally at the surface of the particles producing a core–shell structure. Second, a radial approach can be performed. Both approaches are shown in Figure 4.

Lateral metathesis can operate in ring-closing or -opening polymerisation. In the case of ring-closing metathesis (RCM), the particles are covered with a multi-alkene substrate that can react by two processes: internal cross-linking if the alkene groups are from the same molecule or cross-linking between alkenes from different molecules. In both cases, the reaction leads to a cross-linked polymer shell around the particles.[46] This reaction has been carried out on gold nanoparticles covered by different alkyl dendrimers.[47] RCM on these particles (Scheme 8) enhances kinetic stability both to chemical (such as cyanide etching) and thermal processes (thermal ripening).

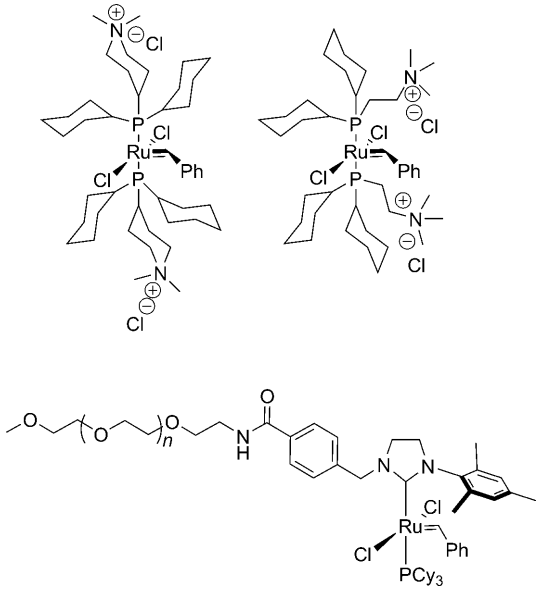
In a similar way, cross-linked glycerol-based dendrimers have been produced (Scheme 9).[48] Glycerol dendrimers have been allylated and then treated with RCM catalysts to produce cross-linked structures. The obtained carbon double bond could be further hydrogenated by using Adams catalyst or dihydroxylated by osmium tetroxide. This latter reac-



Scheme 5. The versatile possibilities of metathesis. ADMP = acyclic diene metathesis polymerisation, CM = cross-metathesis, EM = enyne metathesis, ROCM = ring-opening cross-metathesis, ROMP = ring-opening metathesis polymerisation.



Scheme 6. Examples of metathesis catalysts.



Scheme 7. Water-soluble metathesis catalysts.

tion leads to water-soluble dendrimers, starting from hydrophobic architectures.

In a similar way, radial metathesis is the orthogonal modification of the outer layer of the particles by using one of the available metathesis catalysts.[49] One example is the modification of the external part of gold nanoparticles by ROCM (Scheme 10). Different norbornene derivatives with ferrocenyl groups were polymerised from the particle surface to the external medium. The reaction was stopped by the addition of ethyl vinyl ether, a known ROCM termination agent. The sequential addition of the norbornene derivatives and the efficacy of the reaction lead to particles with a layered structure.[50]

A similar strategy was used to prepare cadmium selenide–polyolefin composites. Starting from CdSe quantum dots covered with trioctylphosphine (TOPO), ligand exchange was performed by thermal treatment of quantum dots with *p*-vinylbenzyldioctylphosphine (p-vinylbenzyl-DOPO). Hence, the metathesis catalyst (ruthenium-based catalyst, [RuL_n] in Scheme 11) was inserted into the particles, producing activated quantum dots. Finally, cyclooctene was added and ROCM took place. As described above, the reaction was quenched by the addition of ethyl vinyl ether.

In addition, these quantum dots were organised at the interface between water droplets and toluene. Polymerisation

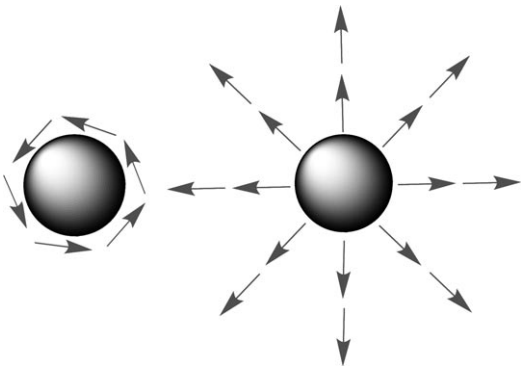
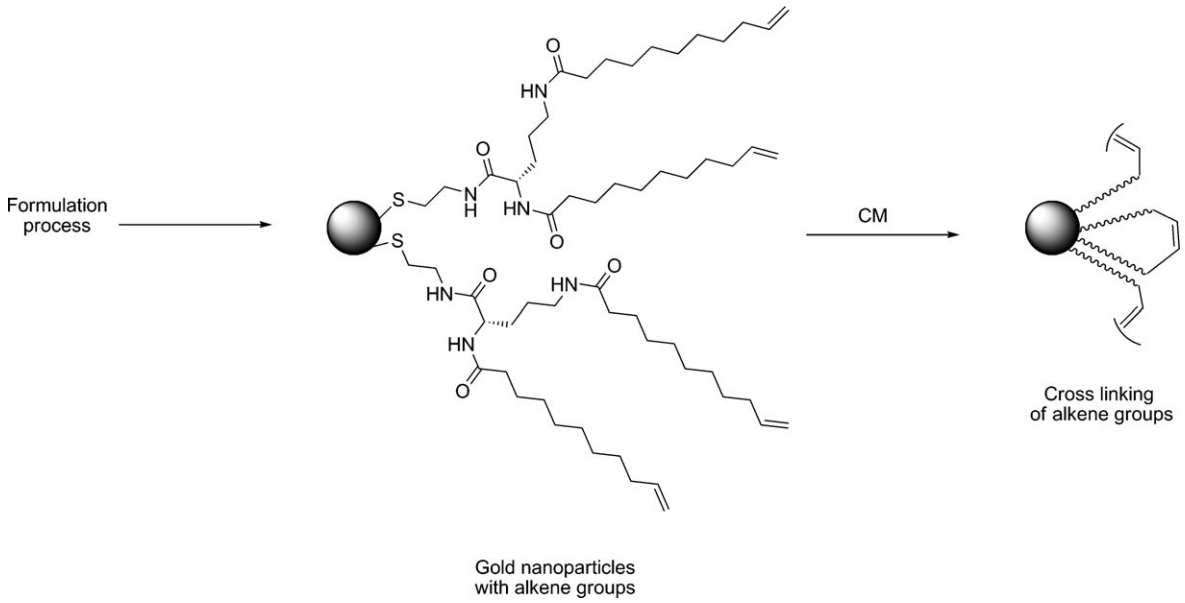


Figure 4. Approaches for the use of metathesis on nanostructures. Shaded spheres represent nanoparticles and the arrows mimic the direction of metathesis reaction.

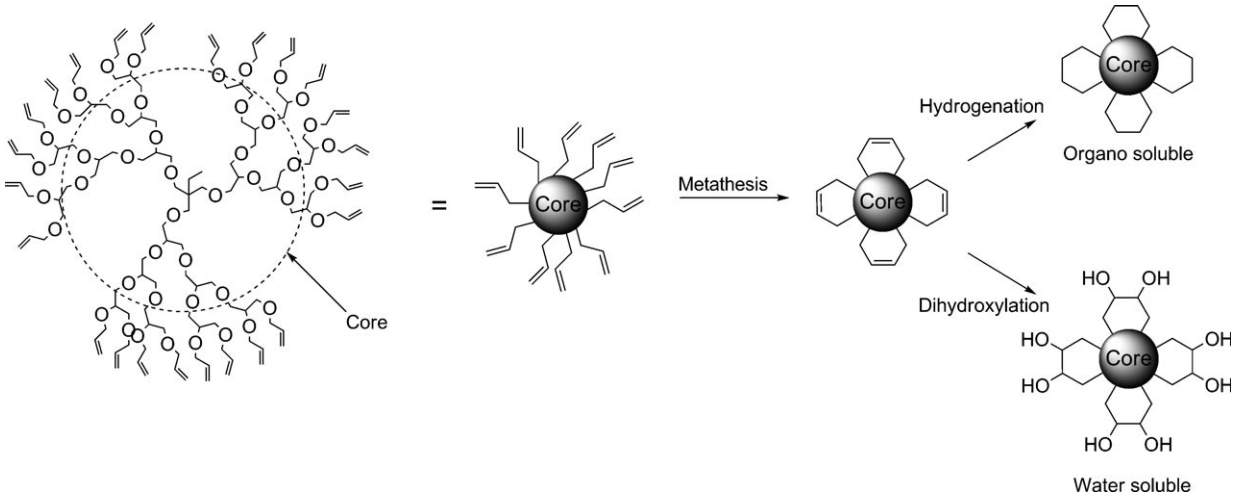
at the interface, by means of an amphiphilic catalyst, produces quantum dots coated water droplets, in the micrometre scale.

In the simplest way, radial metathesis could be used to introduce functional groups onto the nanoparticles.

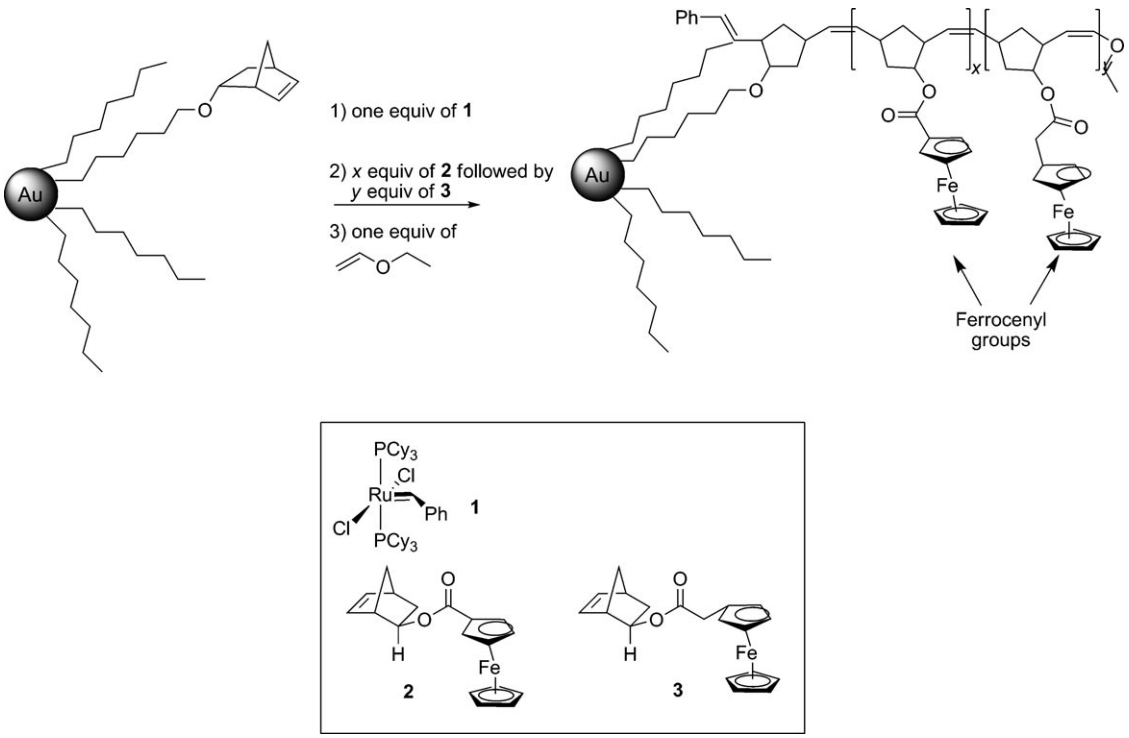
Copper(I)-catalysed azide–alkyne cycloaddition (CuAAC) and click chemistry: The azide–alkyne Huisgen cycloaddition is a 1,3-dipolar cycloaddition reaction between an azide and a terminal or internal alkynes to give a 1,2,3-triazole.[51,52] Huisgen was the first to understand the scope of this organic reaction. This reaction was improved, independently, by the groups of Sharpless and Meldal to give the well-known click-chemistry azide—alkyne coupling with several advantages, such as the use of non-toxic solvents (water) or the large favourable thermodynamic effect of the



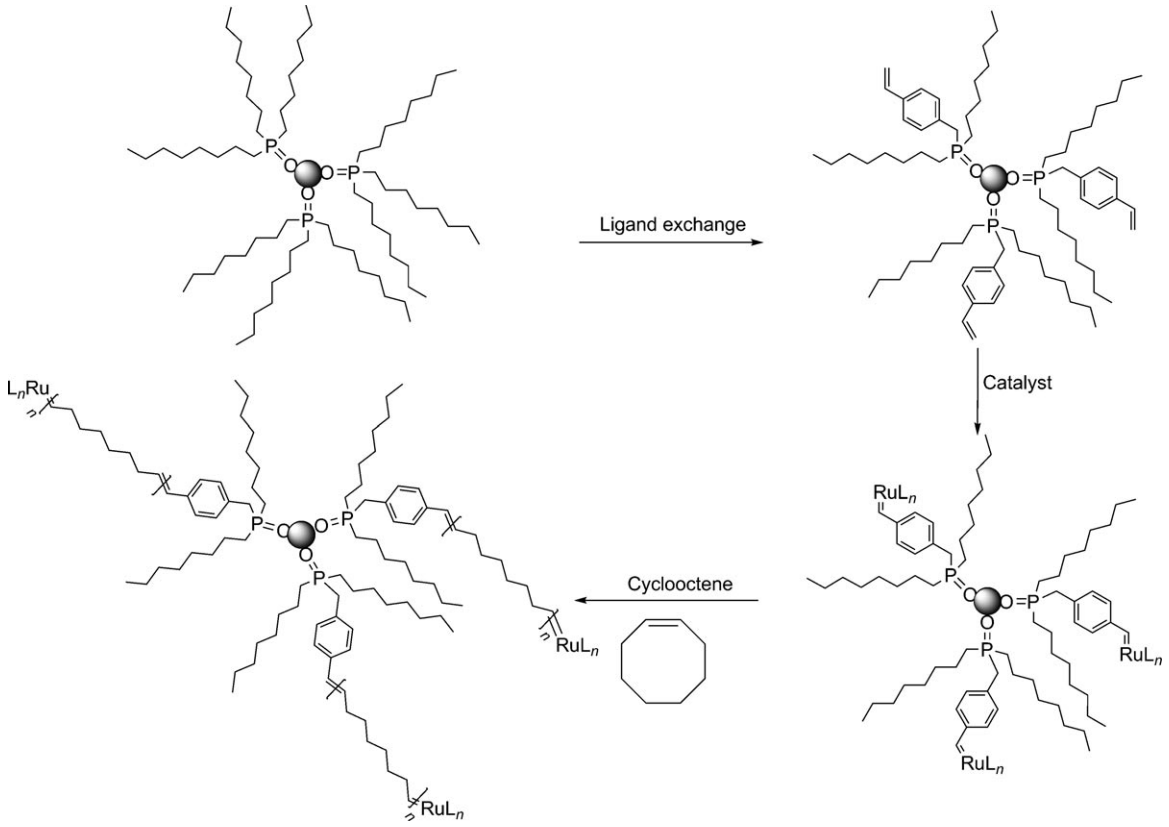
Scheme 8. RCM on gold nanoparticles, according to reference [46].



Scheme 9. RCM on glycerol dendrimers that are further modified, leading to nanoparticles with opposite solubility.

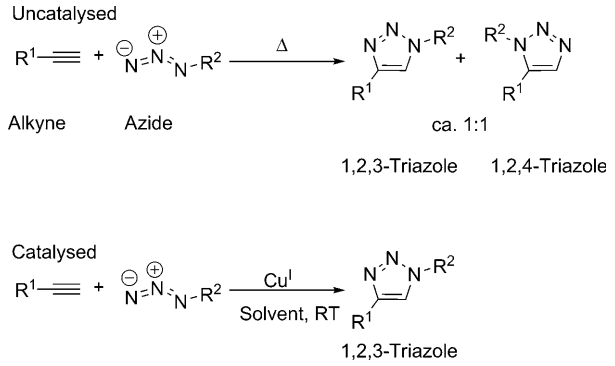


Scheme 10. Radial metathesis on gold nanoparticles. Layered particles are obtained.



Scheme 11. ROMP on quantum dots.

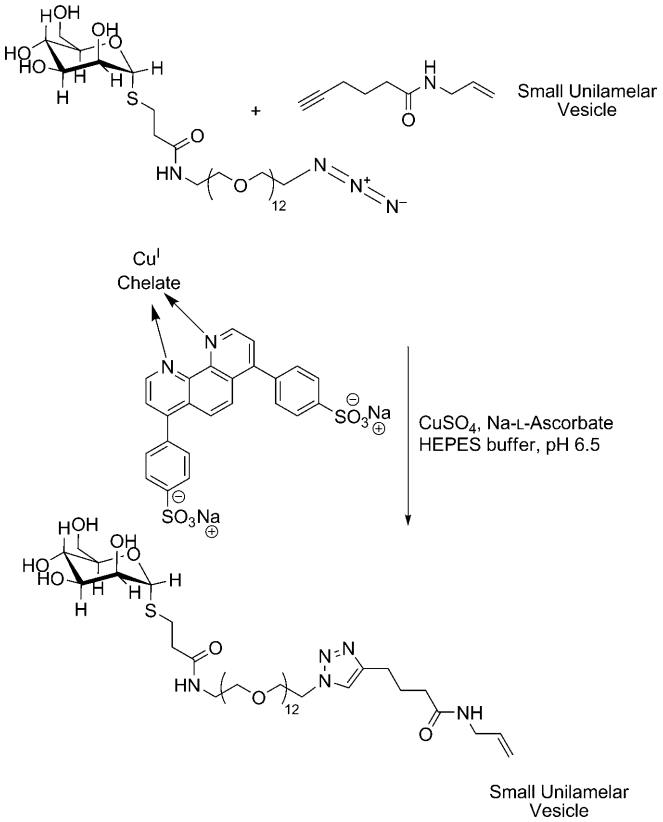
reaction. The use of copper sulfate and sodium ascorbate produces a nice way to functionalise soft nanoparticles under mild conditions. As shown in Scheme 12, the uncatalysed reaction requires heating (Δ) and produces a mixture of 1,2,3- and 1,2,4-triazoles. The copper-catalysed reaction takes place at room temperature and produces only 1,2,3-triazoles.



Scheme 12. Schematic representation of uncatalysed and catalysed Huisgen cycloaddition.

One example of the use of CuAAC towards functionalisation of nanoparticles was the introduction of mannose ligands onto liposomes.[53] A synthetic lipid, presenting a terminal alkyne group, was introduced into the liposome formulation process. The formulation process was based on the hydration of a lipid layer, producing monodisperse liposomes. Coupling with a mannosyl moiety, with a spacer with a reactive azido group, was achieved in Hepes buffer under catalysis by $CuSO_4$/sodium L-ascorbate (Scheme 13). The catalytic species (Cu^I complex) was stabilised by chelation of a water-soluble bathophenanthroline derivative. Under these conditions, the coupling yield is almost quantitative. Furthermore, as a model study, introduced mannosyl moieties have been shown to be accessible to concanavalin A; demonstrating that the liposome to be able to interact with other ligands. This experiment is the proof of concept for targeting tumours with cells expressing receptors for sugar, especially mannose.

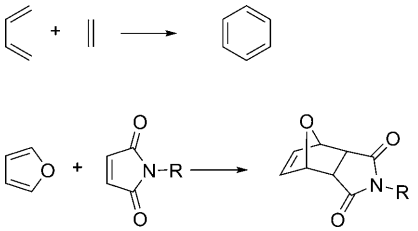
Another example is the reaction of poly(alkylcyanoacrylate) nanoparticles, made from modified cyanoacrylate polymers, with alkyne substrates like alkyne–dansyl or alkyne–PEG.[54] A similar methodology can be applied to hybrid organic/inorganic particles[55–63] for the introduction of biolinkers (biotin), fluorescent markers (indocyanin, fluorescein), steroid moieties (oestrogen) or anti-cancer drugs (paclitaxel). Alkyne or azide groups can easily be introduced into these kinds of molecules. To some extent, tumour-targeting inorganic nanoparticles have been covered with cyclic peptides and show extended blood circulation times and binding to the correct target within the tumours, such as p32 expressing cells.[64] In general, click chemistry refers to a family of reactions that can be carried out with effectiveness with regards to the number of atoms involved in those reactions. For complete definitions and aims, the reader is invited to see references [65–68].



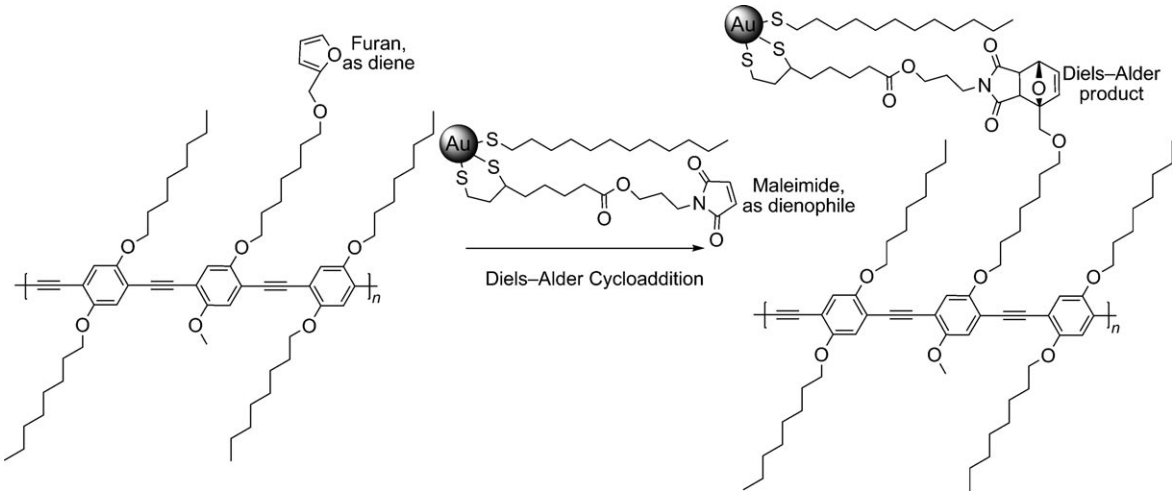
Scheme 13. Click liposomes obtained by means of CuAAC.

Diels–Alder reaction: The Diels–Alder reaction has been little investigated towards the functionalisation of nanoparticles (Scheme 14). Nevertheless, it could represent an interesting way to produce engineered nanoparticles. An interesting thing is that this reaction could be carried out in water if the diene and the dienophile are correctly chosen; even more the reaction could be accelerated in water with or without Lewis acid catalysis.[69–73]

Furthermore, Diels–Alder reactions can be catalysed by copper species.[74] An important point is the reversibility of this reaction, leading to degradable assemblies.[75] This last point must be underlined because the degradation capacity of multi-functional nanocarriers is a challenging issue in



Scheme 14. Examples of Diels–Alder reactions. Top: the production of benzene from butadiene (diene) and ethylene (dienophile). Bottom: the reaction between furan and maleimide moieties.



Scheme 15. Grafting of gold nanoparticles on polymer by the mean of Diels–Alder reaction.

many fields, such as nanomedicine. The Diels–Alder reaction represents a tuneable way to produce reversible assemblies on nanoparticles or between nanoparticles. To date, maleimide and furan are the most employed dienophile and diene, respectively.[76]

Rarely investigated reaction, such as the Diels–Alder cycloaddition, could find applications in materials science to create new hybrid organic/inorganic assemblies.[77] One example is the conjugation of gold nanoparticles to polymers, which permits the formation of uniform composites and allows efficient electronic interfacial interaction between the constituents (Scheme 15).

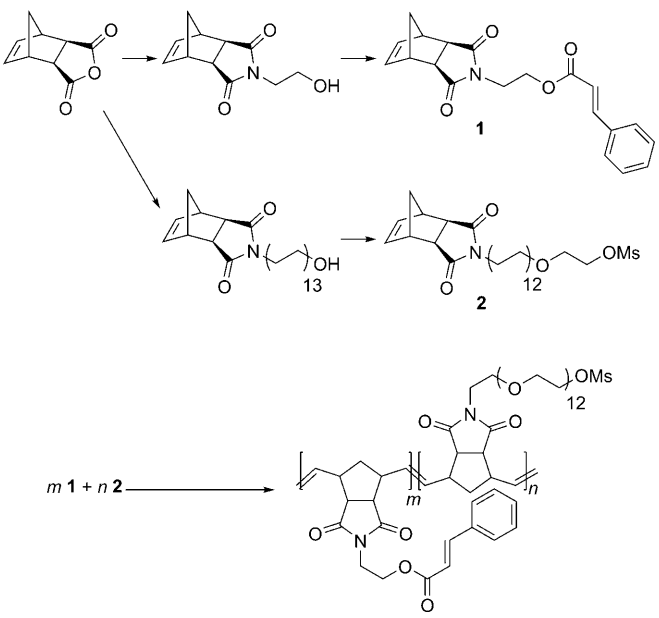
Selected Examples

Since post-insertion has been developed on liposomes, many of the applications are relevant in medicine. In these cases, post-insertion could provide help to introduce targeting ligands on the surface of liposomes.[78] Many examples could illustrate this fact; post-insertion allows the fast development of multi-functional carriers in combination of reliable encapsulation technique. The LBL approach has been used with success to create siRNA delivery systems, such as gold nanoparticles coated with poly(ethylene imine) (PEI)/siRNA mixed layers.[79] It demonstrates the feasibility of creating advanced nanoparticles in only a few steps. This example also establishes that siRNA organised into layers on the surface of nanoparticles are more effective in gene silencing than PEI/siRNA random conjugates.

The LBL approach is also a very promising technique for the use of nanoparticles in fields other than nanomedicine. One important field of research is the development of devices based on the LBL approach and nanoparticles. Such devices are developed for photovoltaic cells composed of fullerenes functionalised with the help of the Bingel reaction.[80] Another example is the building of layered systems in which enzymes are embedded, providing multi-enzyme reactors at the nanometre scale. The development of hybrid organic/inorganic nanoparticles could also be achieved to create a nanohybrid catalyst, for example.[81–82]

ROMP is employed for the production of nanoassemblies with defined properties. For example, starting from substituted norbornenes (with PEG for compound **2** or cinnamoyl groups for compound **1**, as shown in Scheme 16), ROMP is carried out to produce amphiphilic polymers.

Self-assembly of these polymers leads to micelles, which were further cross-linked by UV-light irradiation and radiolabelled with ^{18}F according to classical radiolabelling methods.[83] Thus, ROMP produces well-defined polymers containing several reactive groups. Micelles with high specific activity are produced in only three steps (assembly, polymer-

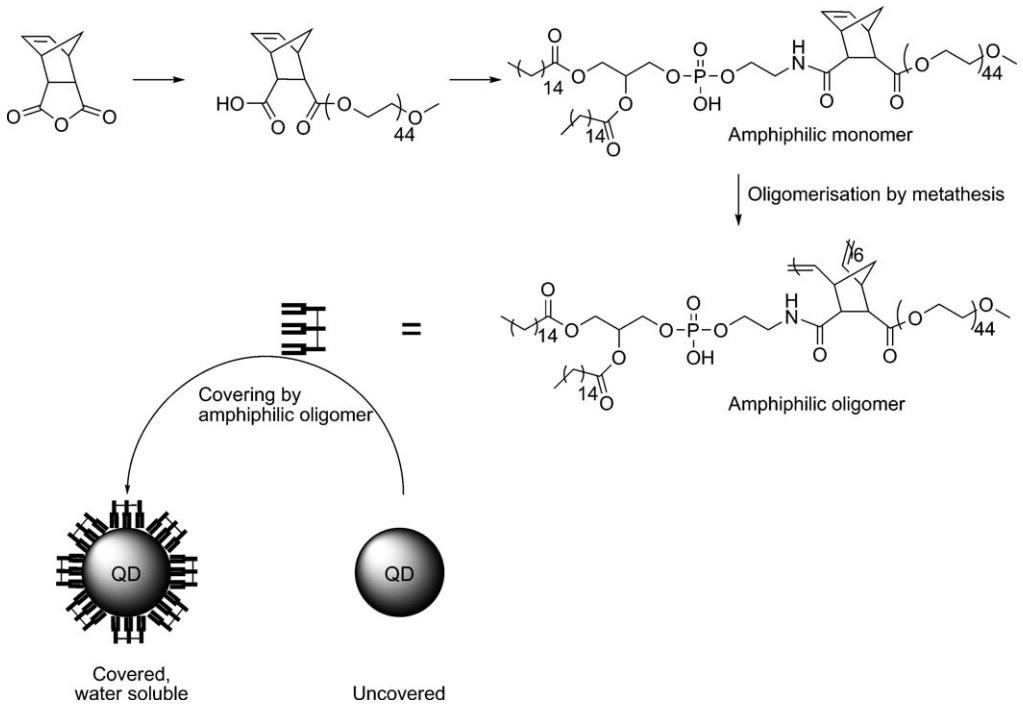


Scheme 16. Synthesis of amphiphilic polymers by ROMP starting from substituted norbornenes.

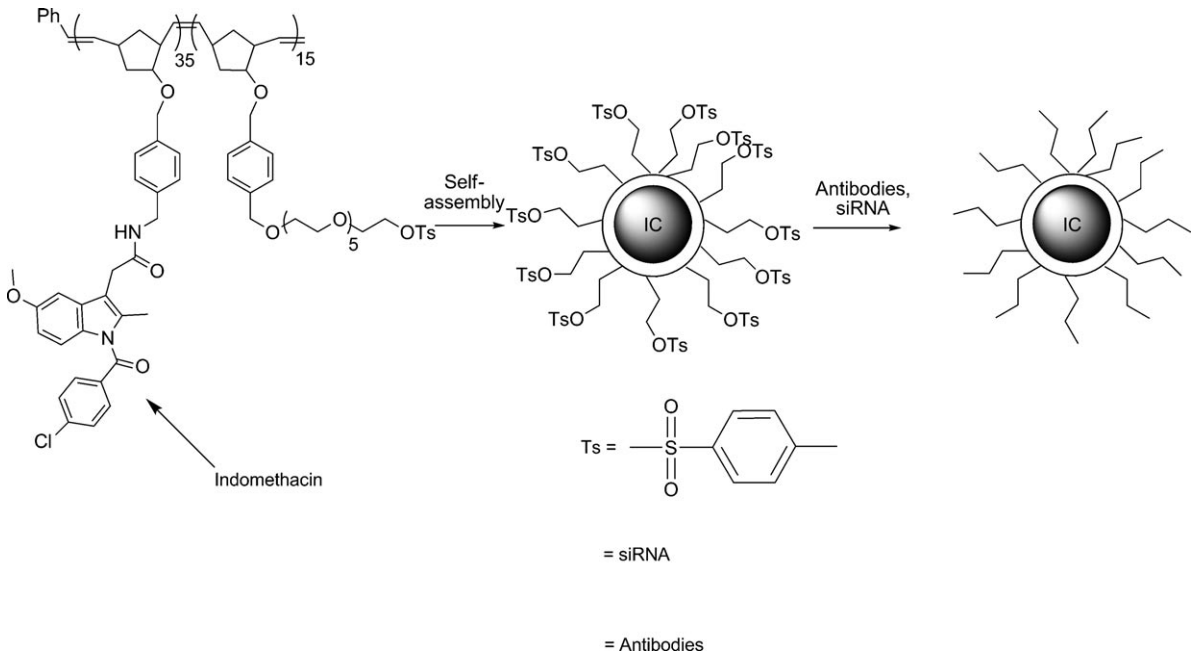
isation and radiolabelling). The production of engineered polymers by ROMP has been also employed to coat fluorescent nanocrystals in water. In this case, a norbornene derivative is conjugated with PEG and phospholipid to produce amphiphilic monomers. Subsequent oligomerisation by Grubbs 2nd generation catalyst produces biocompatible structures (Scheme 17) able to solubilise quantum dots in water without modifying fluorescence.[84]

A similar approach was used to yield multi-functional polymeric nanoparticles containing anti-HER-2 antibodies, Bcl-2 antisense oligonucleotides and an anti-cancer drug (indomethacin) in a straightforward way. Synergistic actions were demonstrated and make this approach very unique and promising (Scheme 18).[85–86]

In general, CuAAC allows the convenient synthesis of nanohybrid systems, such as multi-walled carbon nanotubes



Scheme 17. Covering quantum dots by nanostructures formed by ROMP.



Scheme 18. Synthesis of polymers by sequential ROMP and functionalisation of nanoparticles. Multi-functional carriers are created.

(MWNTs) covered with click magnetic nanoparticles. Furthermore, as CuAAC can be realised between distinct particles, the synthesis of supramolecular assemblies, such as hydrogels, can be achieved.

Summary and Outlook

It appears that a combination of techniques (formulation and post-modification) can create advanced colloidal systems. In this way, CuAAC performed on nanoparticles can bring out essential properties such as the capability to respond to external stimuli. For example, layered micelles have been created by chemical cross-linking and coverage by dendrimers with azido groups.[87] The cross-linked core is pH responsive and the dendrimers are thermo-responsive. The stability of the enhanced colloid at elevated temperatures, as well as the pH-responsive behaviour, make these layered micelles potential carriers for advanced drug delivery. In addition, nanoparticles with alkyne or azido groups can be assembled together by the combination of an emulsification process and an interfacial click reaction. Rotello et al. have produced stable magnetic colloidosomes by a convenient approach.[88] Furthermore, one can underline the fact that the properties of these assemblies can be tuned by the choice of the nature of starting nanoparticles, providing access to versatile applications.[89–90]

High-value nanoparticles should be produced by a combination of both formulation and post-formulation methods. Strategies are available and are under constant improvement. Integration of the strategies presented herein into production processes should lead to the discovery of groundbreaking nanoparticles, pushing the actual limits forward. The choice of the strategy depends on the nature of the nanoparticles and on other factors, such as the cost of the reactants and purification issues. One has to take into account the fact that physical interfacial modifications could be impossible if the particles are too small. In the same way, chemical interfacial modifications strongly depend on the accessibility of reactants. This accessibility is also strongly related to the curvature radius of the particles. In the same general concern, the ratio of grafted or modified molecules needs to be controlled to avoid any kind of colloidal instability, such as flocculation. After interfacial modifications, the control of electrical charge distribution is one of the parameters that needs to be evaluated, as well as steric interactions. We should also bear in mind that this breakthrough should be possible only if biodegradability/biocompatibility is imprinted into the nanoparticles at an early stage.

Acknowledgements

We would like to thank The Région Pays de La Loire and the European Union for financial support.

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Published online: August 27, 2010

