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Review

Rahmad Syah, Marziah Zahar and Ehsan Kianfar*

Nanoreactors: properties, applications and characterization

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Abstract: Nanoreactors are a type of chemical reactor that is used mostly in nanotechnology and nanobiotechnology. These unique reactors are critical to the operation of a nano foundry, which is essentially a foundry that produces goods on a nanoscale. Active sites, such as transitional metal species, can also be added to nanoreactors. In this situation, the NR's limited area might impact reaction rate and mechanism by increasing the contacts between reactants and active sites and changing the concentration of the reactant at the active site. Immobilization of chiral active centers inside porous materials has received a lot of interest in this context, and there have been a lot of publications proving the benefits of nano space confinement in chemical processes. The specific mechanism in which enantioselectivities are strengthened has been clarified using molecular dynamics simulations. Nanoreactors are nanometer-sized chambers with the potential to improve chemical conversions by shielding catalysts from external effects and encapsulating reactors and catalysts in a tiny space for an extended period of time. Natural and synthetic nanoreactors are the two types of nanoreactors that can be found in general. The first group has a more selective function while also having a more complicated structure, whereas the second group has more variation and a simpler structure. Synthetic nanoreactors have so far been made with a variety of molecules and large types of molecules. The space inside the nanoreactors is a good environment for the production of various nanostructures, in addition to a wide range of chemical reactions. When chemical reactions are carried out in confined spaces with nanometer dimensions and micrometer volumes, the kinetics and the entire process path are altered. Nanoreactors are restricted areas used to execute specialized chemical processes. In the cells of living organisms, numerous simultaneous reactions are based on the same concept. As a result, various biological and chemical structures with nanoreactor characteristics are used in this strategy.

Keywords: biological; catalysts; chemical; environmental; micrometer volume; nanometer.

1 Introduction

Nanostructured materials with specific shape size and geometry have unique and different properties from bulk materials. Nanomaterials have attracted a lot of interest in various fields of technology. Nowadays, researchers are well aware that by using reaction environments with nanometer and micrometer dimensions (nanoreactors) they can produce new nanomaterials with interesting and remarkable properties (Möhwald et al. 1999; Nardin et al. 2000). Nanoreactors are, in general, nanometer-sized chambers in which chemical reactions can occur. Of course, nanoreactors are somehow part of the reaction, and this is their main difference from micro-reactors (Kianfar et al. 2018a, 2018b, 2018c; Liu and Kianfar 2020). By looking closely at nature, many examples of natural nanoreactors can be seen. These include cellular organs or secretory and intracellular cavities (in which a cascade of reactions occurs) (Kianfar 2019d; Kianfar and Ali 2020; Kianfar and Salimi 2020). These include the mitochondrial nucleus, the Golgi apparatus, lysosomes, and cavities of channel and carrier proteins (Kianfar 2020a; Kianfar et al. 2020c). The kinetics and mechanism of reaction in small media such as micelles, vesicles and cells have been well studied (Couvreur et al. 2002; Dawkins 1989). It has been observed that the reaction kinetics in a small closed environment is different from the kinetics of the same reaction in bulk solutions. A small closed environment containing a

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certain number of molecules has the property of intense molecular accumulation (Flocculation) and causes a change in the reaction rate and in some cases a change in the type and direction of the reaction (Kianfar 2018; Kianfar 2019a; Salimi et al. 2017a). Also, since nanoreactors contain a limited number of reactants, the reaction efficiency is different from that in which the reaction takes place in a mass solution (Kianfar 2019b; Kianfar et al. 2019; Kianfar et al. 2018d). Specific statistical methods are used to model the probabilities of molecular aggregation properties and reactions between molecules (Bechthold et al. 2000). Different factors affecting the reaction rate and reaction mechanism in nanoreactors have been stated, which is beyond the scope of this discussion; In short, however, the confinement of a liquid in a nanoreactor, sometimes called a miniemulsion, has provided new processes and possibilities for the production of nanoparticles and nanocapsules (Kianfar and Kianfar 2019; Salimi et al. 2017b). We can refer to the synthetic process of various nanomaterials, including semiconductor metal and polymer nanomaterials (Kianfar 2019c; Kianfar et al. 2020a), which are prepared through nanoreactor systems such as micelles (Samuelson et al. 2001), reverse micelles (Ingert and Pileni 2001), trees (Zhao et al. 1998), liposomes (Graff, Winterhalter, and Meier 2001), and so on.

At the macroscopic scale, a chemical reactor is a chamber that allows a reaction to be performed in a certain volume. One of the advantages of using a reactor is the possibility of precise control of reaction conditions such as solvent, temperature and stirring speed. At the micro and nano scales, chambers can also be created that separate a certain volume of the reaction mixture from the bulk medium. If a chemical reaction is trapped inside such a chamber, then the chamber is considered a nanoreactor. The advantages of using nanoreactors can be more control over the reaction, selectivity, separation of toxic and unstable substances from the bulk environment, followed by reducing the toxicity of the system or increasing the stability of the catalyst and being ideal in processes such as drug delivery (due to size Their small) pointed out (Möhwald et al. 1999). Porous materials with a dimension of less than 100 nm (Nardin et al. 2000) or chambers with a diameter of less than 500 nm are usually considered as nanoreactors. But in the more general case, the diameter of the nanoreactor is considered 1 μm ≥. In a general category, nanoreactors are classified into two groups: natural nanoreactors and synthetic nanoreactors. Natural nanoreactors include Protein-based Bacterial Microcompartment, Protein Cages, and Viruses (Möhwald et al. 1999). Synthetic nanoreactors are more diverse and include molecules, macromolecules, nanostructures and porous solids.

2 Organic molecular nanoreactors

In general, organic nanoreactors are aggregates of molecules or macromolecules that create a unique shape. The cavity formed inside this structure is permeable to one or more molecules, and the molecules inserted inside it are subjected to certain chemical changes. According to this definition, a wide range of molecules are also classified as nanoreactors. In chemistry, such structures are called molecular baskets. The reaction performed in organic nanoreactors depends on the structural nature of the reactants enclosed in them. For example, increasing the decomposition capacity of methane in molecular baskets has the ability of the basket to collect ethanol from the peripheral solution, or the efficiency of self-assembled nanocages in the cycloaddition reaction of aromatic compounds has a similar property (Nishioka et al. 2007; Ryu, Cho, and Zhao 2007). A very interesting example of these organic nanoreactors is a very small molecular nanocapsule with a diameter of 4 nm called Rumbibicubooctahedral (Figure 1) (Liu, Liu, and Warmuth 2007). A capsule made from the aromatic compound pyrocalol is another interesting example (Avram and Cohen 2006).

3 Large molecule nanoreactors

Large molecular nanoreactors are nanoreactor structures with iterative units, and this definition covers a wide range of compounds. Organic polymer nanoreactors such as polymerosomes, dendrimers and hydrogels are examples of this. Organic polymeric materials have been used as micrometer reaction chambers (Dähne et al. 2001) of enzymes (Neumann, Haupt, and Ballauff 2004) (Figure 2) and photochromic dyes (Jang and Oh 2003). Proteins have many functions as natural nanoreactors in living cells. One of these proteins is alpha-hemolysin (Figure 3). The cavity of this protein has been used as a biosensor based on covalent and non-covalent interactions with various metabolites (Mirkin 2004). Cellulose fibers (He, Kunitake, and Nakao 2003) and other proteins (Shi, Shen, and Möhwald 2004) can also be used as nanoreactors to synthesize metal nanoparticles. Polymerosomes, which some researchers say are the first step in synthesizing cell synthesis, are one of the most important nanoreactor structures. These structures are made of different copolymer blocks and have the ability to encapsulate different materials in their central part. The very large hydrophilic part of di-block polymers is a vital requirement for polymerosome aggregation (Vo-Dinh 2006). The polymerosome is stable in physiological serum for up to

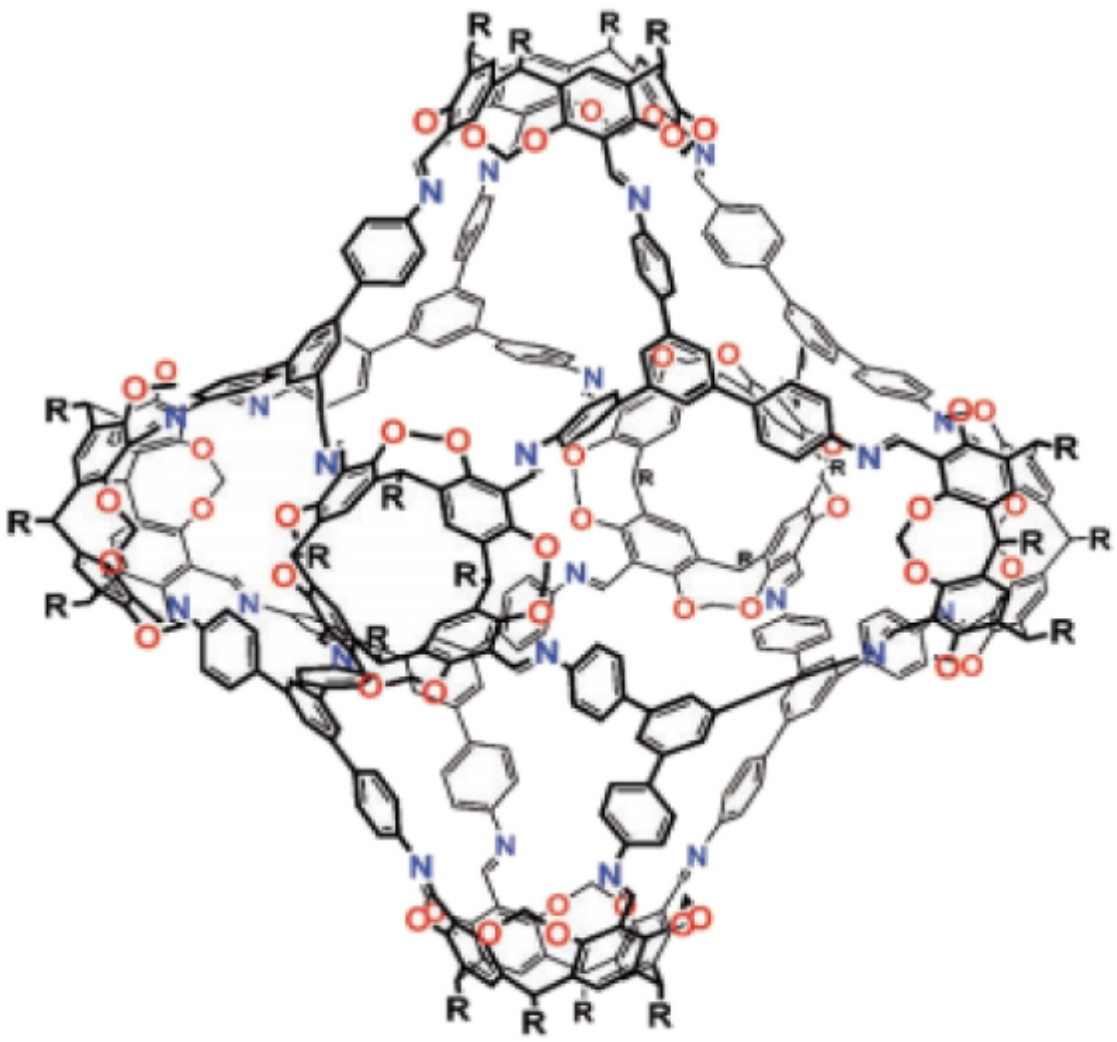


Figure 1: Nanocapsules (Rum bibi cubooctahedral (R = Phenyl)) (Liu, Liu, and Warmuth 2007).

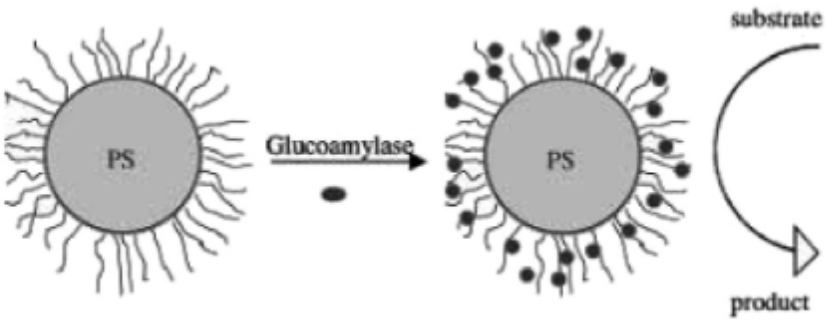


Figure 2: The polymer substrate is used as a site for the acting enzyme. This nanoreactor contains a polystyrene core from which polyacrylic acid branches have been removed. The enzyme binds spontaneously to these charged branches (Neumann, Haupt, and Ballauff 2004).

one month and water infiltration into it is much less compared to other nanocapsules such as liposomes (Vo-Dinh 2006). These materials can be various solvents such as water, soluble materials, metals (Bronstein et al. 1999), semiconductors (Joly et al. 2000) or magnetic nanoparticles (San Choi et al. 2005). Polymerosomes can encapsulate enzymes within themselves and carry out a chain of enzymatic reactions within themselves (Figure 4) (Nallani et al. 2007). For example, polyelectrolyte and hydrophilic polymers can be used to make heat-sensitive and pH-sensitive polymerosomal nanoreactors (Chen et al. 2005). Some of these described complexes have been used to synthesize gold and silver nanoparticles and other nanoparticles (Carrot et al. 1998). Some of these nanoparticles can be used as reaction catalysts, for example cobalt metal has the ability to hydrolyze epoxides with high efficiency (Rossbach et al. 2006). Dendrimers are very large molecules with a regular structure and monodispersed. Dendrimers have three main structural components in their structure, which include a core, branches, and functional groups. Their synthesis is controlled and hierarchical. For example, in the bottom-up method, successive branches are added generation by generation on the primary core (Vo-Dinh 2006). The ability of arboreals to be nanoreactors has been identified as very high and they are very suitable for use as enzymatic nanoreactors (Lee and Kim 2003) or in the synthesis of nanoparticles (Zhao, Sun, and Crooks 1998). Hydrogels are water-saturated polymers that have

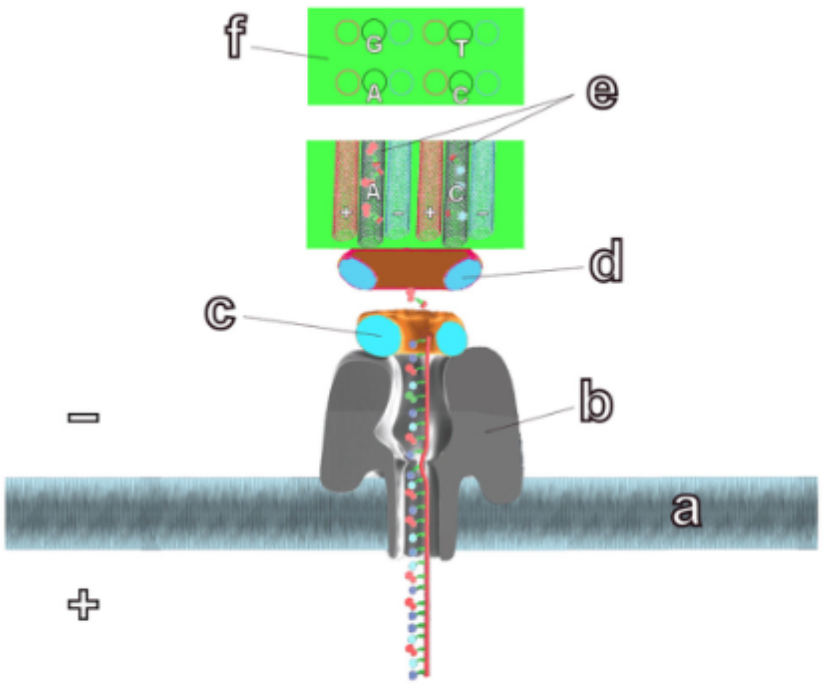


Figure 3: A. a – membrane, b – α-hemolysin nanopore, single nucleotide according to the program, c – ssDNA ligase, d – protein as a funnel, directing the nucleotide to the active center of ssDNA ligase, e – nanotube controlled by a computer with an electromagnetic field, f – bottom view of the nanotube (Wells et al. 2012).

significant biocompatibility properties. The lifespan of a hydrogel depends on the nature of the crosslinking of the hydrogel and changes its porosity and elasticity over time. Because of this property, hydrogels are widely used in tissue engineering. In this way, the hydrogel is designed in such a way that the substances obtained from tissue metabolism cause destructive reactions of the hydrogel. Of course, this feature is also useful in drug delivery applications (Vo-Dinh 2006). The cavity space inside the hydrogel is considered as a nanoreactor. In these cavities, like other nanoreactors mentioned, it is possible to synthesize metal nanoparticles of metal oxide (Murali Mohan et al. 2007). The antibacterial properties of silver nanoparticles are well known and the hydrogels in which these nanoparticles are formed can be used as a coating and bandage.

4 Micelles, liposomes and emulsions

These substances are usually made from surfactants, lipid molecules or other similar substances. Their synthesis methods are usually such that they distribute the appropriate particle size. Micelles are usually composed of droplets of

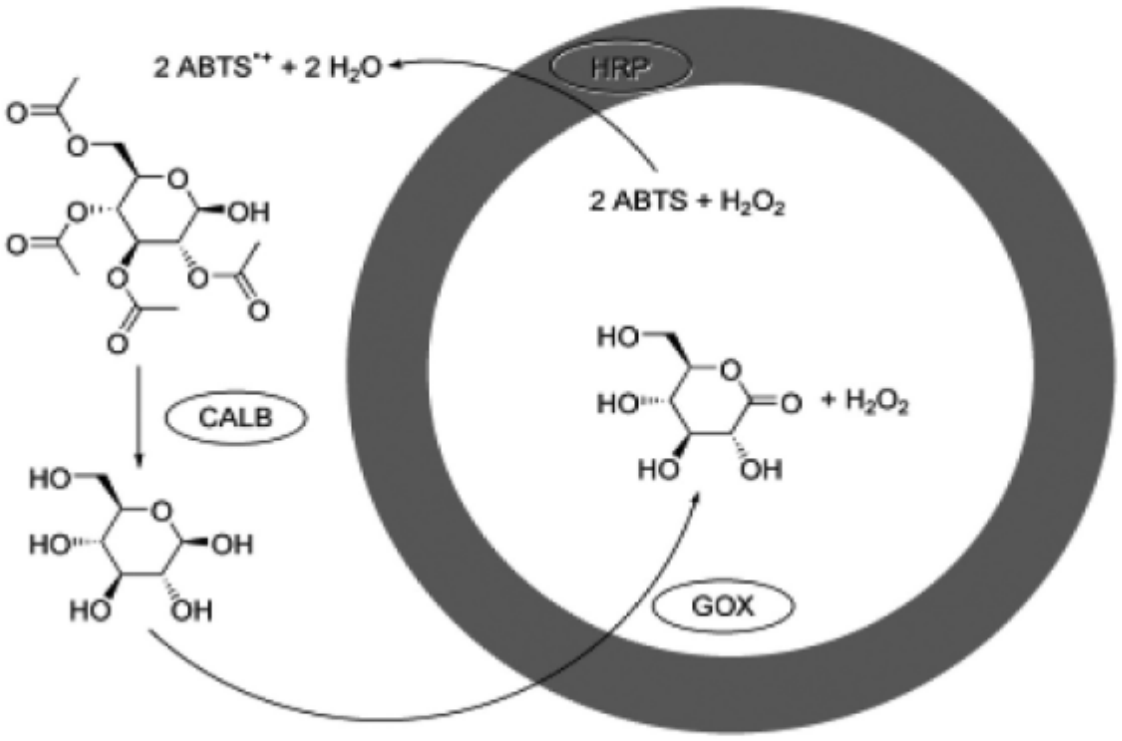


Figure 4: Schematic of a multi-step reaction performed in a three-enzyme polymersome system (Vriezema et al. 2007).

water-soluble oil (Normal) or vice versa (Reverse). Oil-core micellar nanoreactors have been used for condensation processes (O'Reilly 2007). Reverse microwave nanoreactors with blue cores are commonly used to synthesize magnetic nanoparticles (Lee et al. 2005) and other metal nanoparticles as well as enzyme reaction medium (Carvalho and Cabral 2000).Liposomes and vesicles are blue-nucleated nanoreactors coated with two layers of lipids. The water in the core can be filled with different materials or the space between different layers of multilayer liposomes can be used to synthesize nanostructured layers in between. Emulsions have been used to synthesize various metal nanoparticles (Landfester 2006) and silicate shell structures of magnetic nanoparticles (Xu et al. 2006) and other nanoparticles. Through silica and micro emulsion processes, pure silica nanoparticles are prepared or contaminated with fluorescent dyes. Figure 5 shows the formation steps of these nanoparticles (Naumann 2005).

5 Carbon nanotubes

It should be noted that some researchers consider carbon nanotubes to be a large organic molecule because they are composed of a large number of carbon atoms. The inside diameter of these nanotubes is usually small and about a few angstroms. This space can be used as a nanoreactor to produce nanowires and nanoparticles (Hu, Bando, and Golberg 2008). The outer part of carbon nanotubes can be functionalized with charged groups (Lee, Kim, and Tománek 1997). Depending on the specific properties of carbon, carbon atoms can take on different shapes and structures of different sizes. These include single-walled and multi-walled nanotubes, each of which imparts specific properties and morphology to carbon-based nanotubes (Vo-Dinh 2006). They can also create single-layer and multi-layer tubular nanoreactors. Some of these nanoreactors contain metal oxides or lanthanides (Saito et al. 1993).

6 Porous solid structures

Clear examples of this group include the porous structures of silicate and zeolite. The empty space inside these particles can be from a few angstroms to a few nanometers. The cavities inside them can be used as nanoreactors for various purposes (Alauzun et al. 2006; Ding et al. 2010; Fan et al. 2005; Karimi and Zareyee 2008; Tretyakov et al. 2004). These structures have been used to produce nanoparticles (Ding et al. 2010), as a surface for enzyme placement (Hartmann and Jung 2009), and other applications. Their pore structure can be manipulated by adding molecules such as cyclodextrin (Han et al. 2003). It should be noted that the reaction occurring inside the cavities of these structures, like other nanoreactors, is different from the reaction occurring in the surrounding environment (bulk solvent) (Ostafin and Chen 1999).

7 Enzymatic nanoreactors

Figure 6 shows a schematic of the synthesis of an enzymatic bioreactor. The polycarbonate membrane is first sputtered on a thin layer of gold (A). The gold film is used as an electrode for the electrical polymerization of a Poly-Pyrrole film. Some polypyrrole is deposited inside the cavity as a plaque (B). The added polypyrrole then covers the cavity walls by chemical polymerization (C). The thickness of the polypyrrole layer is determined by controlling the reaction conditions. The thickness of the factor is very important and determines the permeability of the

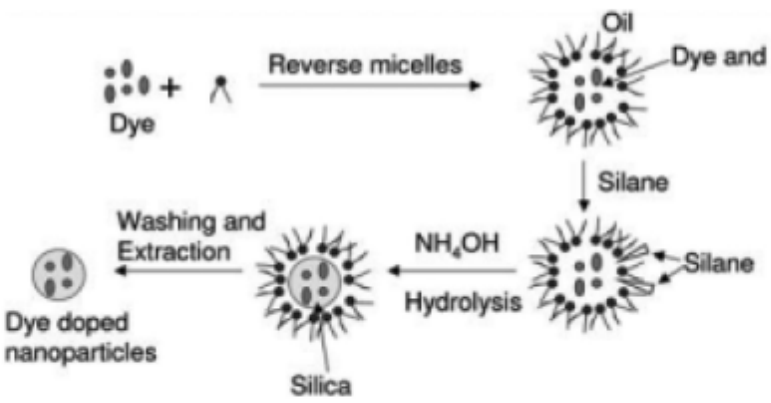


Figure 5: Schematic of how dye-impregnated silica nanoparticles are produced by the mini-emulsion process: by adding a surfactant to an unmixable mixture of oil and water, the water droplets created in the micro emulsion system act as nanoreactors to synthesize nanoparticles (Naumann 2005).

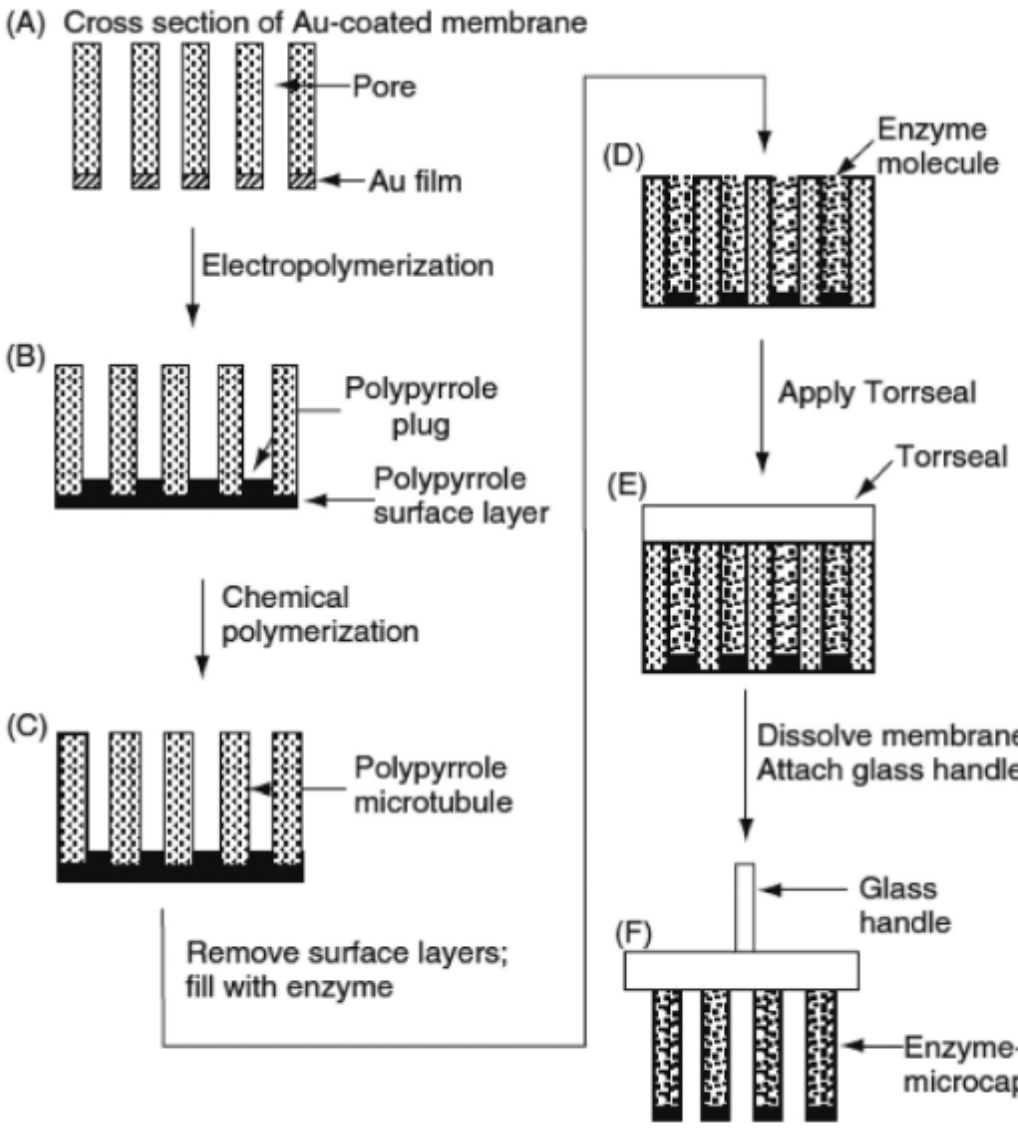


Figure 6: Schematic of how synthetic enzyme nanoreactors are synthesized.

system. The enzyme is then loaded into the system by washing the unreacted material (D). The system is then covered with an epoxy torpedo (E) and the entire system is placed in dichloromethane solvent to dissolve the membrane between the nanoreactor arrays. The time required for a strong connection of the system to the epoxy coating is given. Glucose oxidase, catalase, alcohol hydrogenase and several other enzymes have been successfully encapsulated using this method. It should be noted that the solvent has the ability to pass through the polymer coating, but the enzyme remains inside the nanoreactor due to its large size (Vo-Dinh 2006).

8 Characterization of nanoreactors

Characterization methods vary depending on whether the nanoreactor is part of a larger system or a separate structure. Some methods further consider the size and shape of the nanoreactor cavity and the wall that makes them up. For soluble nanoparticles, their size can be obtained from DLS analysis and suitable microscopic methods such as TEM, AFM, and SEM (Zhang et al. 2019; Zhang et al. 2020a, 2020b, 2020c). Of course, TEM is more important because of its ability to show the cavity and wall space of nanoreactors (Figure 7). Permeability can be measured by placing a probe

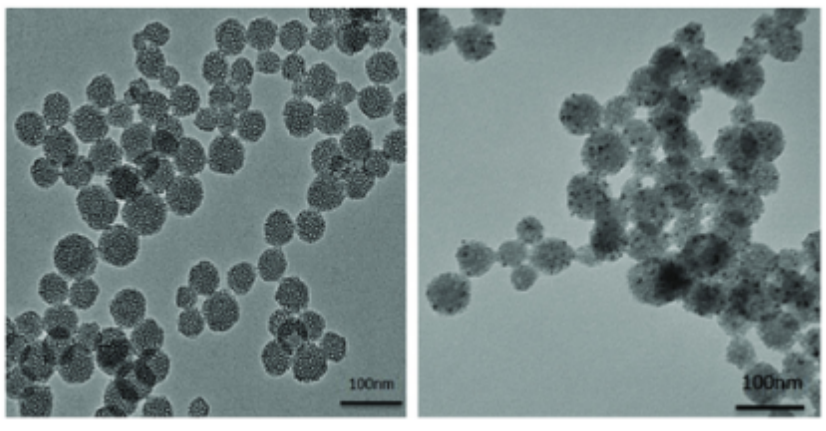


Figure 7: TEM image sample of porous solid structures (Fan et al. 2005).

molecule inside the nanoreactor space and observing the response of the molecule and molecules in the surrounding area through spectroscopic methods. For nanoreactors inside larger macroscopic solids, microscopic methods are of great interest. Using microtomes, thin sections of the sample are prepared and examined with electron microscopes. XRD study (Zhang et al. 2020d, 2020e) also provides useful information on the size and distribution of nanoreactors in these structures (Ostafin and Chen).

9 Natural nanoreactors

Cells and cell organs, which are the most ideal nanoreactors, have lipid membranes. These nanoreactors are selectable, meaning that they are able to differentiate between different molecules and allow only specific molecules to enter their internal cavity. In addition to selectivity, by providing pores in the membrane that open and close with external stimuli such as pH changes, cells are receptive. Selectivity and sensitivity are characteristic of all natural nanoreactors (Möhwald et al. 1999). Recently, protein containers have attracted a lot of attention. In the general classification, proteins belong to the branch of macromolecules, but since these compounds are considered biomolecules, they will be discussed in the discussion of natural nanoreactors.

9.1 Biomacromolecular nanoreactors

The first examples of natural nanoreactors are bacterial microparticles that are present as protein organelles in bacterial cells (Chen et al. 2020b; Wang and Chen 2020; Xu et al. 2019; Zhao et al. 2019). In these nanoreactors, several thousand proteins form a shell with a multifaceted structure that is between 80 and 150 nm in diameter and contains several different enzymes (Li et al. 2018a; Wang et al. 2017; Xia et al. 2017). carboxy zoom are these types of microparticles that are found in all cyanobacteria and encapsulate metabolic enzymes to increase carbon dioxide fixation (CO_2 fixation) (Figure 8) (Möhwald et al. 1999).

9.2 Protein cages

In nature, there are different types of proteins that act as transporters or stores of metal ions and minerals. Among these, Apoferritin, which is an iron storage protein, has been studied more than others, and its use and other storage proteins as nanoreactors have been widely developed. Apoferritin is found in animals, plants and microbial organisms. The compound consists of a hydrated iron (III) oxide core encapsulated by a protein shell. Apoferritin are powerful proteins that can withstand high temperatures (85 °C) and high pH (8.5–9) for a long time without significant disruption to their fourth structure. Iron-free Apoferritin molecules (Apoferritin) are composed of 24 polypeptide subunits. The outer diameter of the protein is 12 nm and the inner diameter is 8 nm. About 4500 iron atoms can fit into the protein cavity. Ferritin has been used extensively, including the production of ferromagnetic nanocrystals within its cavity and the fabrication of a magnetic protein, the use of Apoferritin iron oxide nucleus as a catalyst in photoreduction reactions, and the modification of protein levels by Alkalizing it noted. The latter case has caused Apoferritin to be soluble in most organic solvents, thereby providing the alkylated protein as a nanoreactor for the compression reactions of metal alkoxides and other similar organometallic compounds that are only possible in a non-aqueous medium. Apart from Apoferritin, other proteins such as bacterial enzymes have also been used as nanoreactors. Apoferritin is a spherical

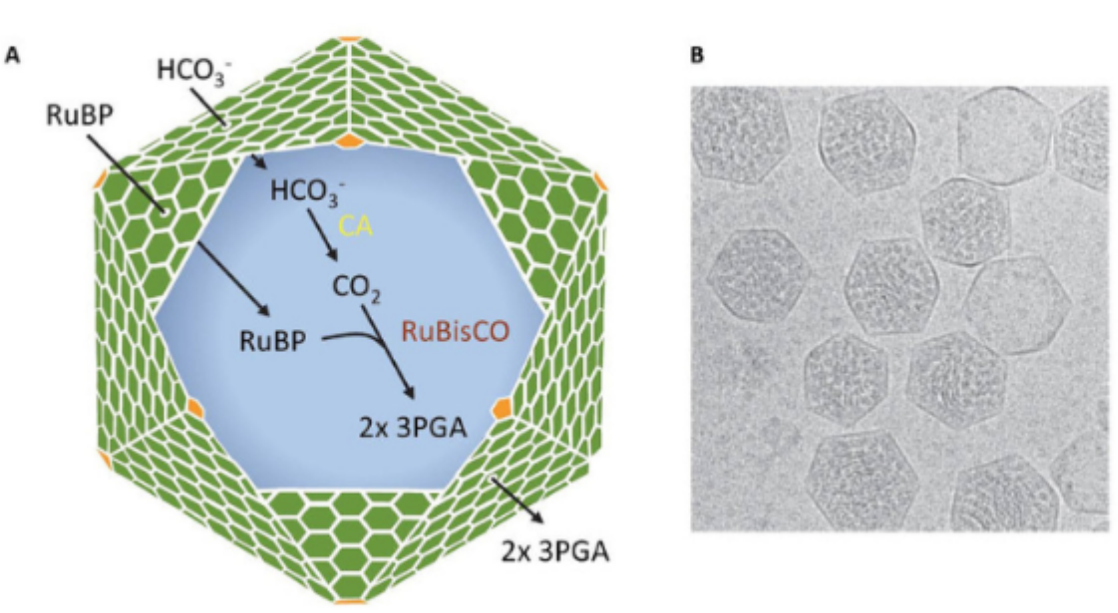


Figure 8: (A) Proposed model for carboxy zoom, (B) electron micrograph of purified carboxysomes from *Halothiobacillus neapolitanus* (Möhwald et al. 1999).

cage with an outer diameter of 12 nm and a cavity of 8 nm (Figure 9). This compound is widely used to make mineral nanoparticles. Apoferritin has also been used as a catalyst in reactions such as optical reduction of some organic compounds and reduction of metal ions such as chromium (VI) and copper (II) in aqueous solution. In addition to Apoferritin, other proteins such as DNA-binding proteins that protect it from oxidative damage have been studied as protein cages (Figure 10) (Möhwald et al. 1999).

9.3 Viruses

Virus particles contain several hundred to several thousand protein molecules that, by self-assembly, form a hollow structure that contains nucleic acid. This self-assembled protein cage, called a capsid, is a powerful structure that comes in a variety of sizes and shapes. When preparing nanomaterials, viruses are used as a blueprint. They offer a rare opportunity for synthetic and catalytic applications since certain forms of viruses are readily available. For example, the Tobacco Mosaic Virus, a rod-shaped virus, is the most well-known structure of biological self-assembly. The virus is able to withstand temperatures of 60 °C and pH between 2 and 10 and consists of 2130 units of the same protein that form an empty half tube with a size of 18 × 300 nm and an inner diameter of 4 nm. By modifying the outer and inner surface of the virus's protein cage, it has been used in reactions such as nucleation of mineral solids. Also, by chemically controlling the load on the virus surface, the outer or inner surface of the capsid can be selectively metallized. The protein cage of viruses that contain nucleic acid is called a capsid. Despite the variety of known capsids, so far only one capsid has been used as an *in vitro* nanoreactor. The capsid, which belongs to the Cowpea Chlorotic Mottle Virus (CCMV), the disease-causing virus in the bean plant, has an outer diameter of 28 nm and is composed of 180 protein layers that enclose an 18 nm-diameter cavity (Figure 11). Using the enzyme peroxidase as a catalyst, oxidation of a non-fluorescent compound and its conversion into fluorescent products in this nanoreactor has been done (Chen et al. 2016; Couvreur et al. 2002; Hu et al. 2015; Shen et al. 2016).

10 Synthetic nanoreactors

Although protein shells are unique natural structures for catalyzing reactions at the nanometer scale, these compounds are highly complex. Synthetic molecules are simpler nanoreactors that are easier to control than natural types.

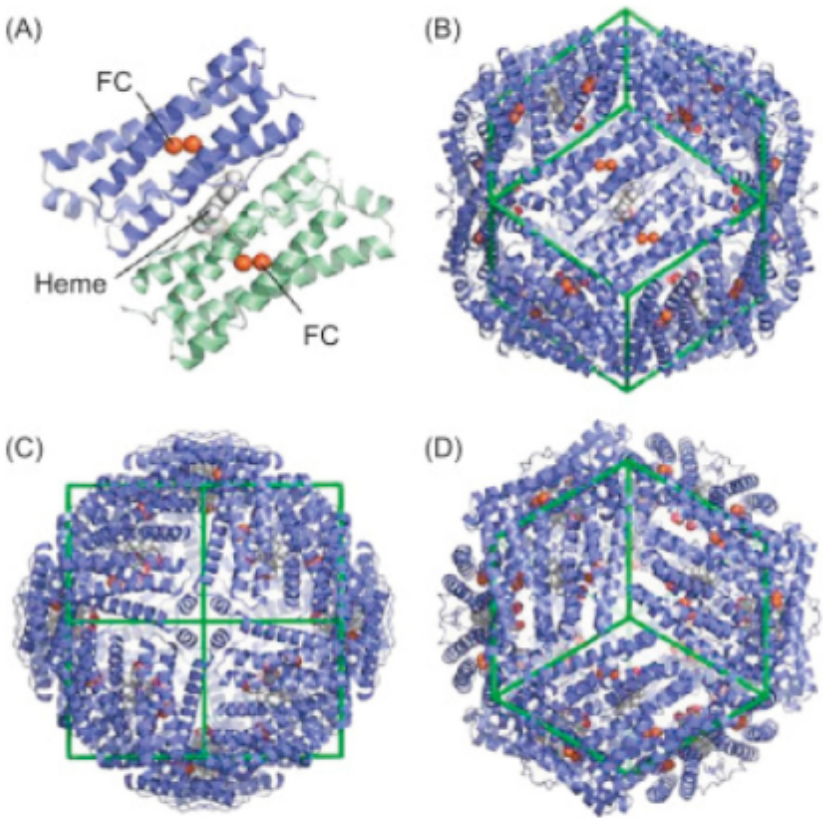


Figure 9: Structure of BFR. Cartoon representations of A, the *Escherichia coli* BFR (pdb 3E1M) subunit dimer peptide backbone showing the position of the intra-subunit dinuclear ferroxidase center and inter-subunit heme site, B–D, the overall structure of 24meric *E. coli* BFR looking down a twofold (B), fourfold (C) and threefold (D) symmetry axis (Möhwald et al. 1999).

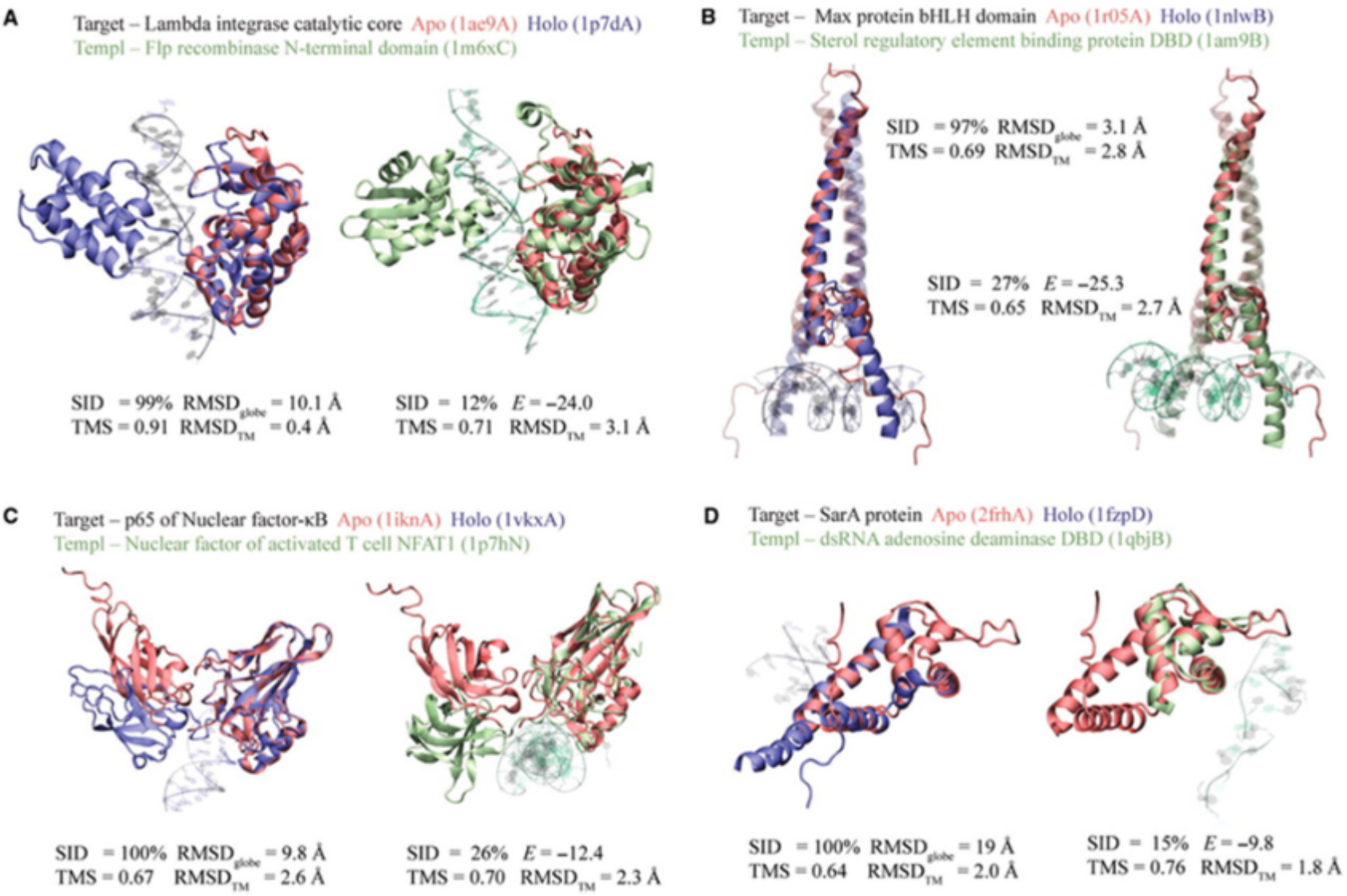


Figure 10: Examples of DNA-binding protein predictions on DB179. (A–F) Structural alignment of the target structure and the template in cartoon representations. In each panel, the left snapshot shows the overall alignment, together with the cocrystallized DNA molecules. The color codes for protein and DNA representations are red and purple for the target, and green and cyan for the template, respectively. The right snapshot highlights DNA-binding residues of both the target and the template in the same color code as the left snapshot. Non-DNA-binding residues of the target were dimmed in gray. For a clear view of the binding interface, the two snapshots were taken from different orientations. In parentheses, each structure was labeled in the format of xxxxX, where xxxx is the four-digit PDB code and X is the chain identifier of the protein. If the PDB record contains no chain identifier, X is replaced with an underscore. Sequence identity (SID), TM-score (TMS), RMSD and the statistical potential energy E are provided at the bottom of each panel. Graphic images were made with the program VMD (Zhang et al. 2020c).

10.1 Molecular nanoreactors

The accumulation of a number of molecules together and the formation of a cavity for a chemical reaction form a molecular nanoreactor. Although a wide range of molecules fall into this category, they can all be structurally classified into one of three categories: Capsules, Micelles, and Vesicles. The interactions that lead to the formation of a capsule are divided into two categories: covalent and non-covalent interactions. Cyclodextrins (CDs), which are natural porous molecules, covalently bind to other molecules to form nanoreactors that can selectively perform different reactions (Figure 12) (Dawkins 1989). Calixarenes, which have cup-like hydrophobic cavities, are also in this category. Surrounded by hydrophilic groups, these molecules are molecular nanoreactors in which various reactions, including metal reduction, can be performed (Figure 12) (Bechthold et al. 2000). Due to the increasing quantity and complexity of products, the design and production of nanoreactors based on covalent interactions becomes more difficult, in recent years, the production of structures with non-covalent interactions has developed a lot. The most important non-covalent interactions are hydrogen bonding and metal-ligand interactions (Figure 13).

The idea for such nanoreactors is derived from cell membranes. Most cell membranes are phospholipids, which are amphiphilic molecules with a hydrophilic charged phosphate head and a hydrophobic hydrocarbon terminus. Types of these dual-friendly species have been synthesized that self-assemble in an aqueous medium that accompanies the aggregation process, resulting in the formation of micelles (single-layer structure) or vesicles (two-layer

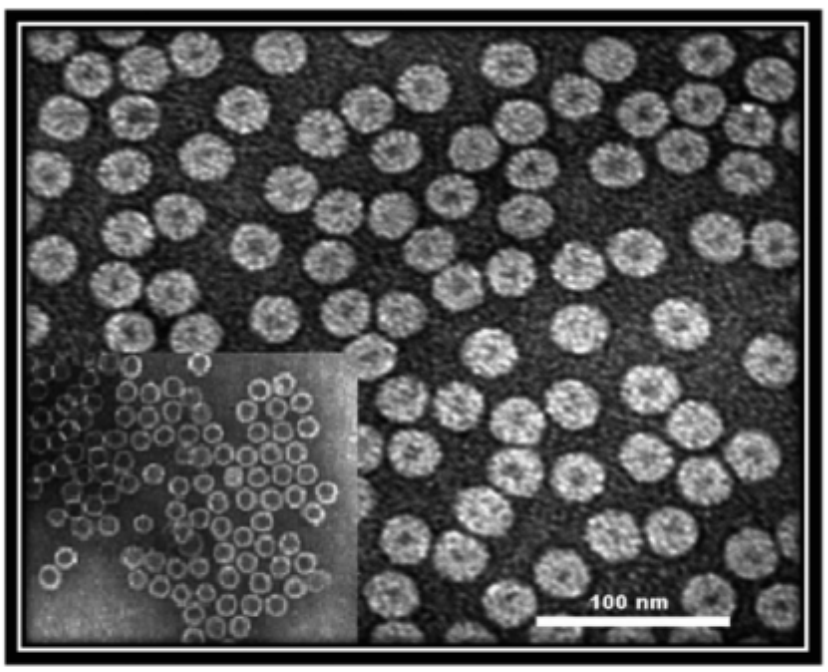


Figure 11: Image TEM (transmission electron microscopy) of CCMV [large image] and its empty capsid (thumbnail) (Couvreur et al. 2002).

structure) (Table 1, Figure 14) (Kianfar 2020b; Kianfar, Baghernejad, and Rahimdashti 2015a; Kianfar, Pirouzfar, and Sakhaeinia 2017; Kianfar and Viet 2021; Mousavian et al. 2020). Dual molecules have a hydrophilic head and a hydrophobic end. One of the best known examples is phospholipids, which make up the bulk of cell membranes (Gao et al. 2020; Kianfar, Salimi, and Koohestani 2020d; Yang et al. 2020b). Depending on the value of an indicator called the Packing Parameter, denoted by p and defined as ($p = v/l.a$) (a is the effective level of the group located at the phospholipid head and v and l to the order of volume and length of the hydrocarbon chain are phospholipids), the accumulation of phospholipid molecules leads to the formation of micelles, reverse micelles or vesicles (Figure 14). The vesicle is actually a bilayer structure that has a more regular surface compared to micelles (Dawkins 1989). It should be noted that vesicles with a phospholipid membrane are called liposomes (Kianfar 2020c, 2020d, Kianfar and Mafi, 2020e). The term enzyme nanoreactors also refers to nanoreactors that contain free enzymes and the most common type are lipid vesicles in which enzymes are encapsulated (Samuelson et al. 2001). All of these structures have dimensions in the nanometer range (Hajimirzaee, Soleimani Mehr, and Kianfar 2020; Kianfar 2015; Kianfar 2016; Kianfar 2019e; Kianfar 2021a, 2021b; Kianfar, Moghadam, and Kianfar 2015b, 2015c).

11 Macromolecular nanoreactors

Structural diversity of polymers in terms of constituent monomers, molecular mass, functional groups and their shape has led to their use in many fields. In the field of nanoreactors, polymers are used in the form of large single molecules with empty internals or in the form of self-assembled structures with one or more cavities (such as polymer micelles).

11.1 Polymersomes

Polymers have many applications due to their structure, molecular mass, functional groups and various shapes, and their use as nanoreactors, either as single-molecular macro with empty interiors or as self-assembled structures with one or more cavities. Block copolymers can be used to make vesicles, in which case they are called polymersomes. The advantage of polymersome over conventional vesicles is its greater stability and membrane strength, which increases its lifespan. The amplitude of monomers and the possibility of changing the ratio of two groups in batch co-fractions make it possible to change the properties of the polymer, such as its size, polarity, stability and toxicity. Due to the greater thickness of the membrane in these structures, its permeability is reduced and their internal cavity is often used to encapsulate molecules such as proteins. Because the membrane thickness of polymerosomes is greater than that of liposomes, the penetration of water into their membranes is slower and protein channels within the membranes of these structures are used to solve this problem (Figure 15). In addition to single-enzyme reactions, multi-step reactions can also be performed within polymers. For example, by encapsulating the enzyme glucose oxidase into the polymer's cavity, placing the lipase enzyme in its membrane, and covalently binding a

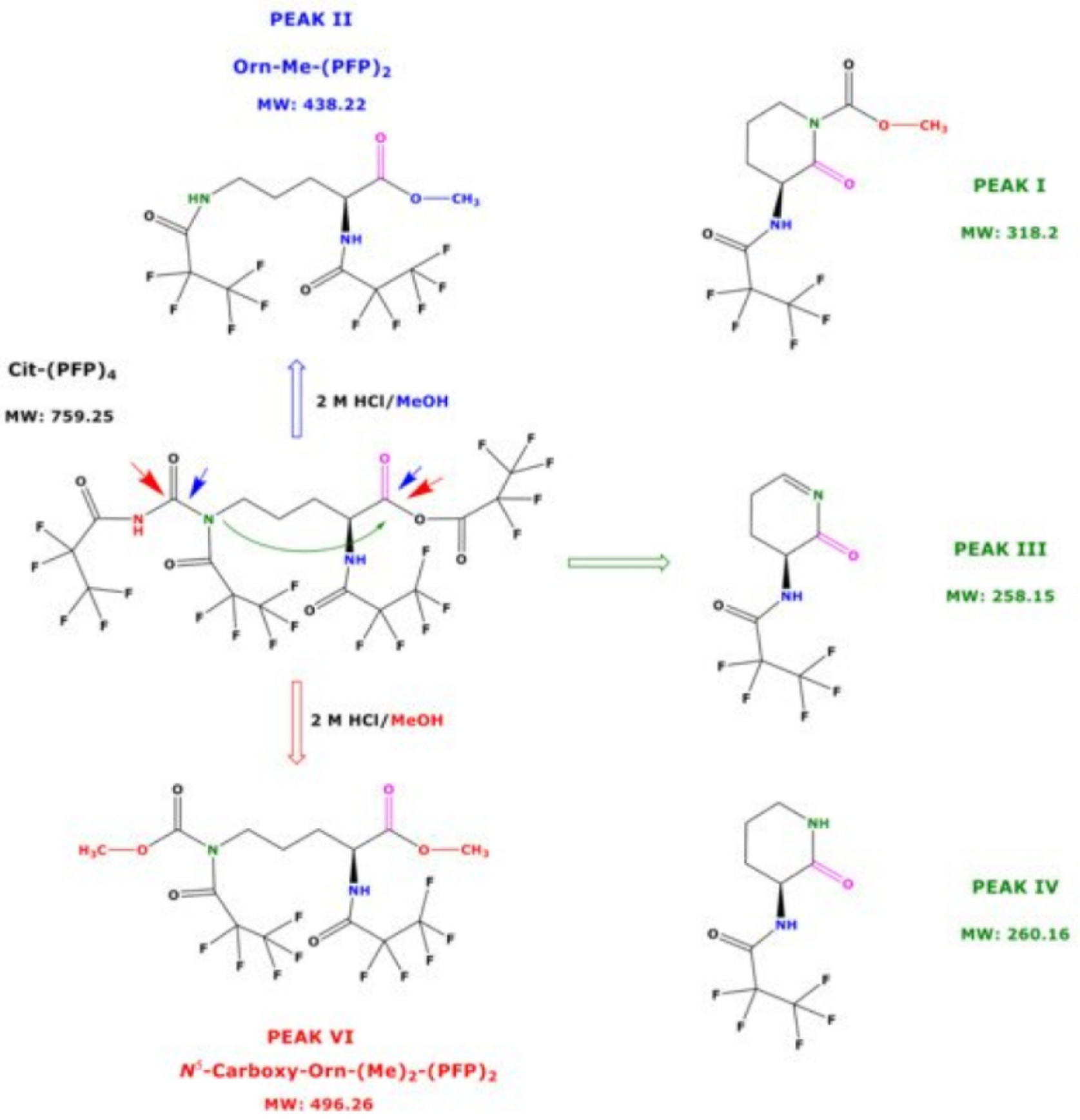


Figure 12: The structure of cyclodextrins (above) and the hydrolysis reaction of cyclic phosphodiesters esters catalyzed by a β-cyclodextrin functionalized (bottom) (Dawkins 1989) and Pd (II) ion reduction reaction in the cavity of a polycalciazrene (Bechthold et al. 2000).

peroxidase enzyme to its outer surface, a three-enzyme nanoreactor is obtained from which derivatives are oxidized in a multi-step reaction. Glucose is used (Graff, Winterhalter, and Meier 2001). So far, copolymer two-batch (Triblock) and three-batch (Triblock) have been used to prepare polymers with different membrane sizes and thicknesses (Table 2).

11.2 Polymeric micelles

Microns can also be made from polymers and used as nanoreactors. In general, the shape that all batch polymers take is the same as that of micelles, that is, the polar handle on the outside and the non-polar handle on the inside, or vice versa, depending on the polarity or non-polarity of the

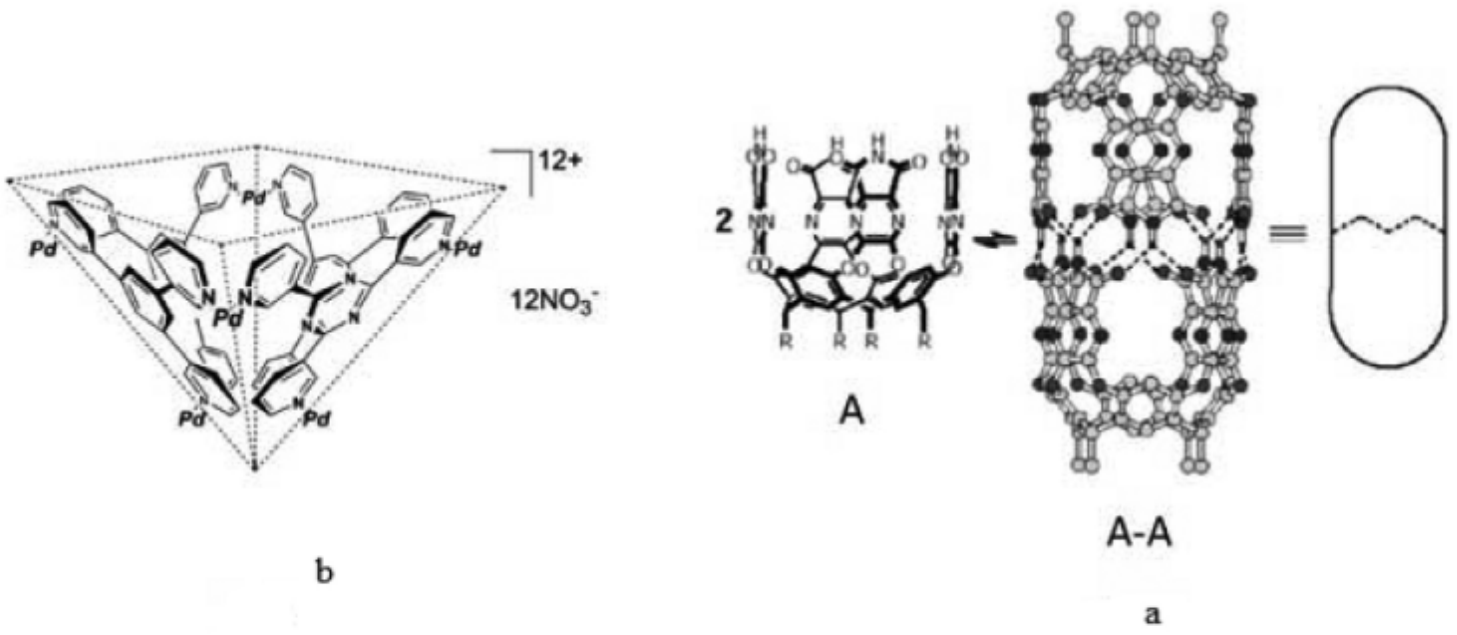


Figure 13: (a) Capsule A–A, which is stabilized by 12 hydrogen bonds. (b) A semi-open cage formed by metal-ligand interactions (Dawkins 1989).

solvent. Such micelles, which have shapes such as spherical, rod-shaped, and hexagonal, have a section that is capable of adapting to the solvent and are used to stabilize metal nanoparticles. The presence of a polymer shell around the metal particles prevents them from agglomerating. In fact, micelles of all batch polymers are considered nanoreactors because metal nanoparticles are made inside them (Vriezema et al. 2005).

11.3 Hydrogels

Hydrogels are water-saturated polymers that have a large gap between their cross-links. These voids can be used as nonreactors to form nuclei and grow nanoparticles (Lee and Kim 2003; Xu and Chen 2014; Y. Zhang et al. 2020a, 2020b; Zhao et al. 2020). For example, these compounds have been used to prepare silver nanoparticles, and due to the antibacterial properties of silver nanoparticles, hydrogels containing these nanoparticles are used as coatings and bandages (Figure 16) (Nishioka et al. 2007; Ryu, Cho, and Zhao 2007).

12 Unimolecular nanoreactors

Dendrimers are single molecules that have a central nucleus with regular radial branches (Figure 17a). The application of these compounds in catalyzing reactions has been well studied (Shan et al. 2021; Tu 2021; Yu 2021). The covalent link between the catalytic species and the tree can be on the perimeter, in the middle or in the center of the tree. In recent years, selective and sensitive trees have been developed (Hu et al. 2020; Yu et al. 2020; Zhao et al. 2014). An example of these nanoreactors that have selectable and temperature sensitive branches has been used as a homogeneous catalyst in the oxidation of thiols (Möhwald et al. 1999).

Hyperbranched polymers and star polymers also fall into the category of single-molecule nanoreactors (Figure 17b and c). These large molecule nanoreactors are the inexpensive correspondents of dendrites and are able to encapsulate metal complexes with catalytic activity and nanoparticles through their cores (Dawkins 1989).

12.1 Miniemulsion

A miniemulsion, also known as a nanoemulsion, is a method of producing stable microparticles that act as nanoreactors. In this method, a mixture of two immiscible liquids such as water and oil with one or more surfactants is stirred at high speed using various techniques such as ultrasonication. In this case, macro-droplets with a large size distribution are broken down into nanoparticles with a limited size distribution (Figure 18). The size range of these droplets is usually between 30 and 500 nm and each droplet acts as an independent nanoreactor (Liu, Liu, and Warmuth 2007). The application of this method is expanding in the field of pharmaceutical sciences, because with these nanoreactors, compounds with smaller particle sizes can be prepared that will have a greater capacity to carry drugs. For example, nanoreactors with a droplet size of 7 ± 27 nm have been used to produce Solid Lipid Nanoparticles (SLNs) (Avram and Cohen 2006).

Table 1: Types of dual-friendly molecules and abbreviated name (Faghih and Kianfar 2018; Kianfar 2020f, 2020g, 2020h; Kianfar and Mafi 2020; Kianfar and Mazaheri 2020a, 2020b; Kianfar, Salimi, and Koohestani 2020e; Vriezema et al. 2005).

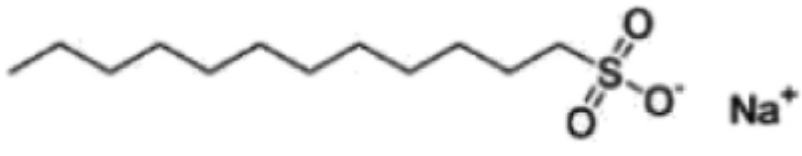
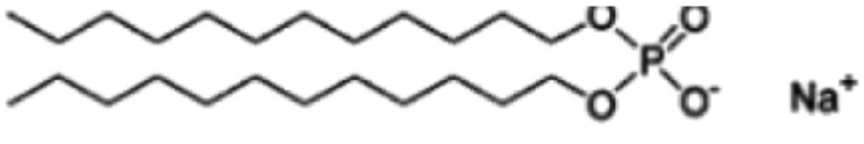
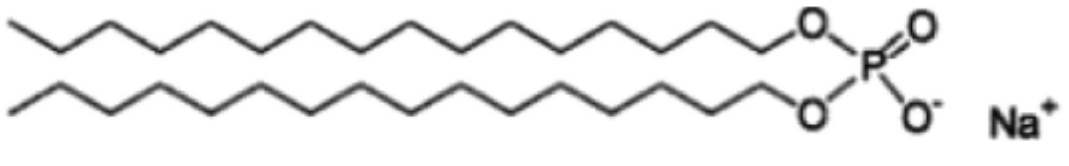
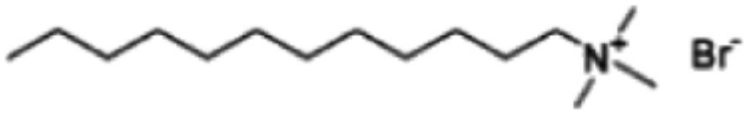
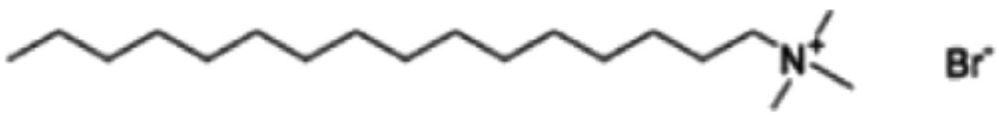
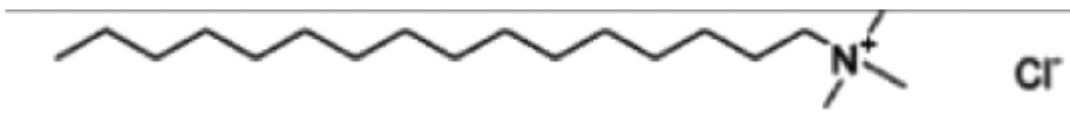
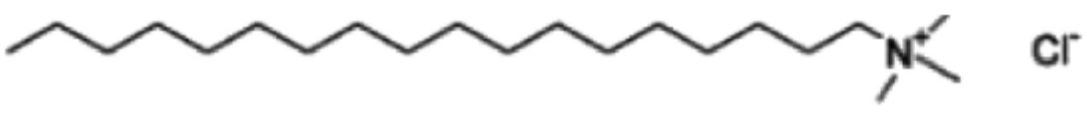
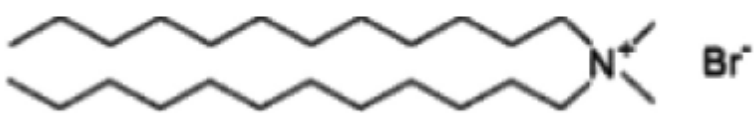
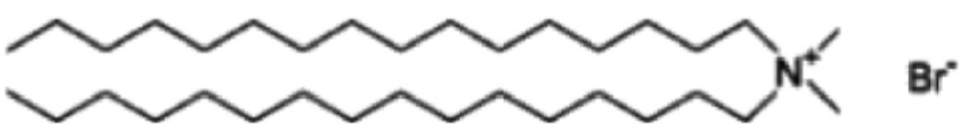
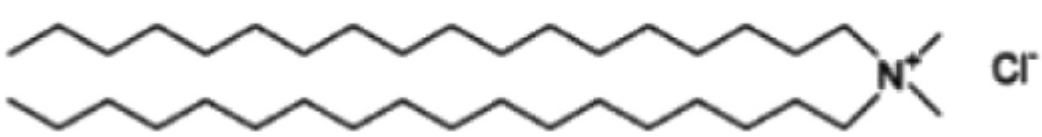
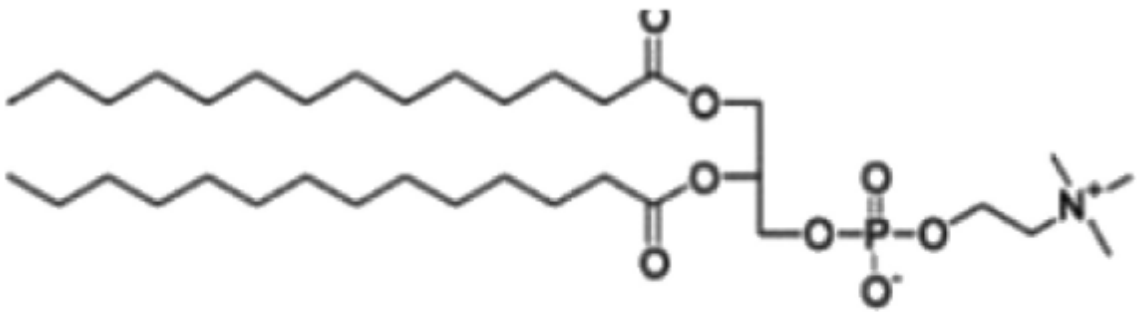
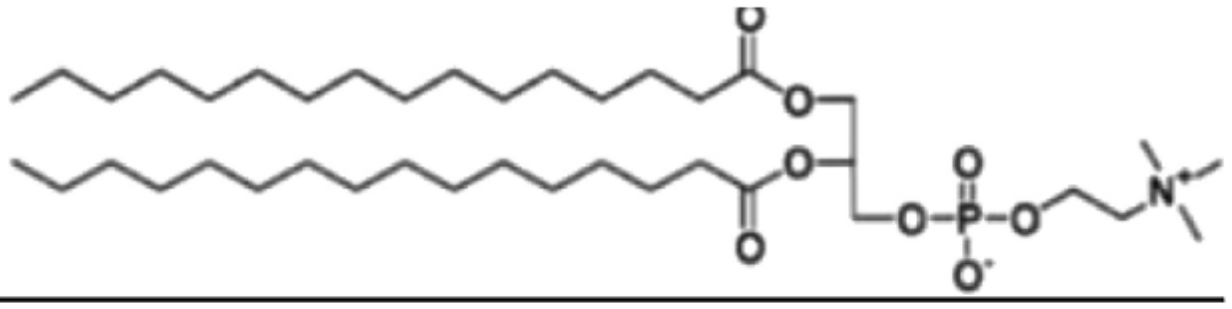
Abbreviated name	Molecular structure
SDS	
DDP	
DHP	
DTAB	
CTAB	
CTACl	
OTACl	
DDDAB	
DHDAB	
DODAC	
DMPC	
DPPC	

Figure 14: Formation of micelles, reverse micelles and vesicles according to the Packing Parameter (Dawkins 1989).

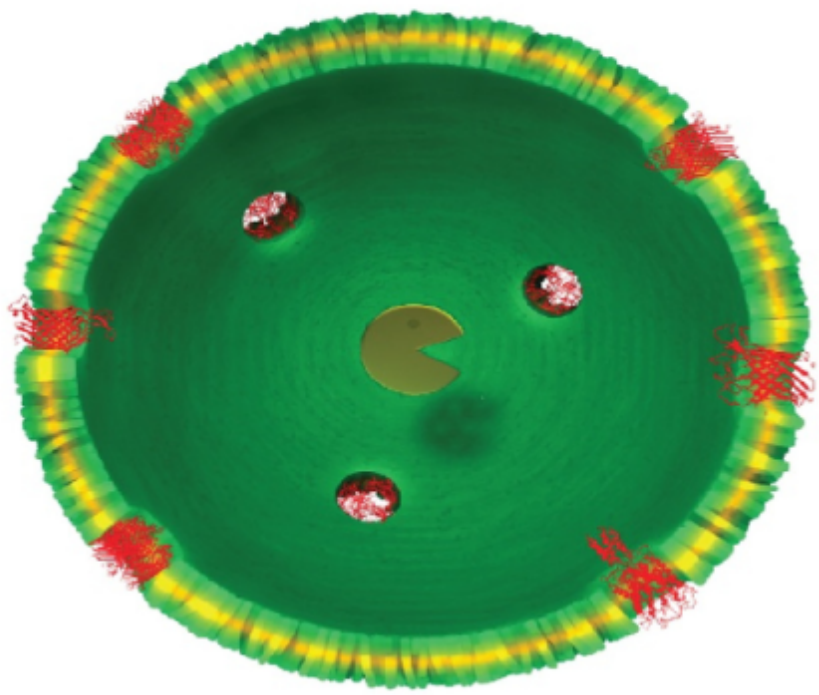


Figure 15: Cross-section of a polymer with a protein channel inside its membrane (Möhwald et al. 1999).

12.2 Shell-core nanostructures

Shell-core structures are highly diverse, ranging from multiphase semiconductors to metal-metal oxide nanocomposites. In recent years, the development of structures with variable cores embedded in a hollow shell has been greatly developed. If the shell is permeable to reactants, these shell-core nanostructures become nanoreactors with variable catalytic cores. Shell-core nanoreactors include Pt-CoO nanostructures used to hydrogenate ethylene. Another example is the Au-TiO_2 nanocomposite with photocatalytic activity, the activity of which has been demonstrated by desorption or decomposition of stabilized organic molecules on metal cores under ultraviolet radiation (Figure 19a) (Dähne et al. 2001). The core–shell structure of Au-SiO_2 has also been used as a nanoreactor for the catalytic reduction of 4-nitrophenol (Figure 19b) (Neumann, Haupt, and Ballauff 2004).

12.3 Porous solids

The most prominent and commonly used compounds in this group are the porous structures of silicate and zeolite. Zeolites are porous compounds and are primarily composed of aluminosilicate (Kianfar 2020i, 2020j, 2020k, 2020l, 2020m; Kianfar et al. 2020b; Kianfar, Kianfar, and Kianfar 2016). The

Table 2: Structure and abbreviation of a number of copolymer batch used in the formation of polymerosomes (hydrophobic bundles are shown in red) (Vriezema et al. 2005).

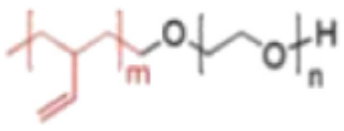
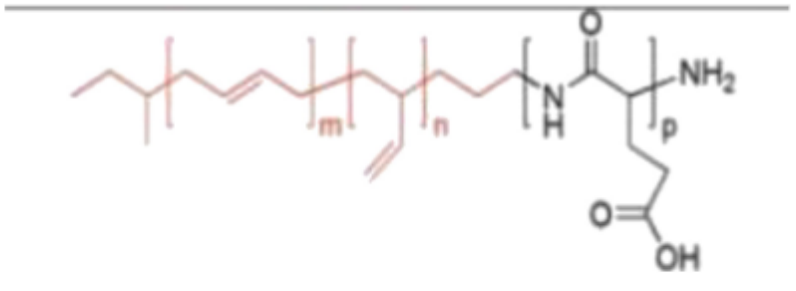
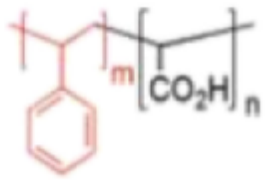
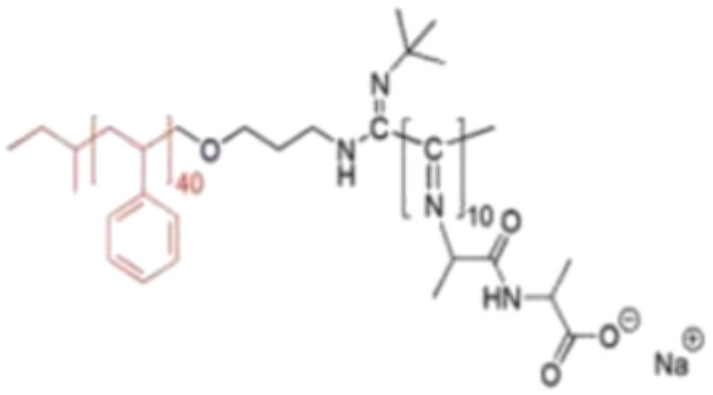
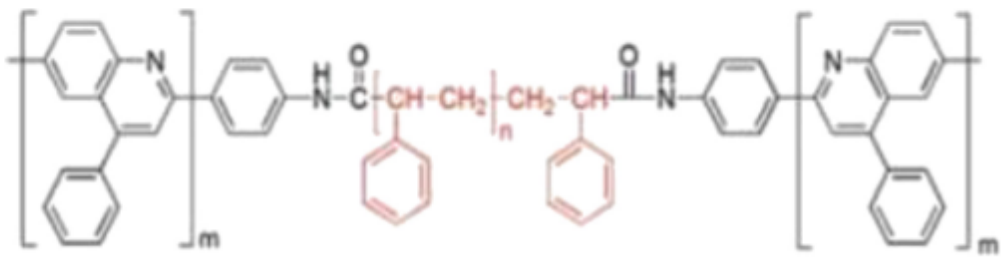
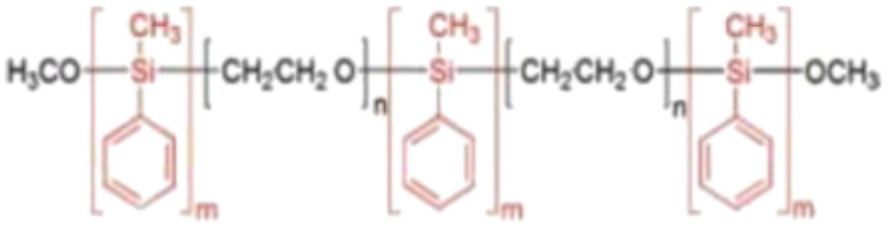
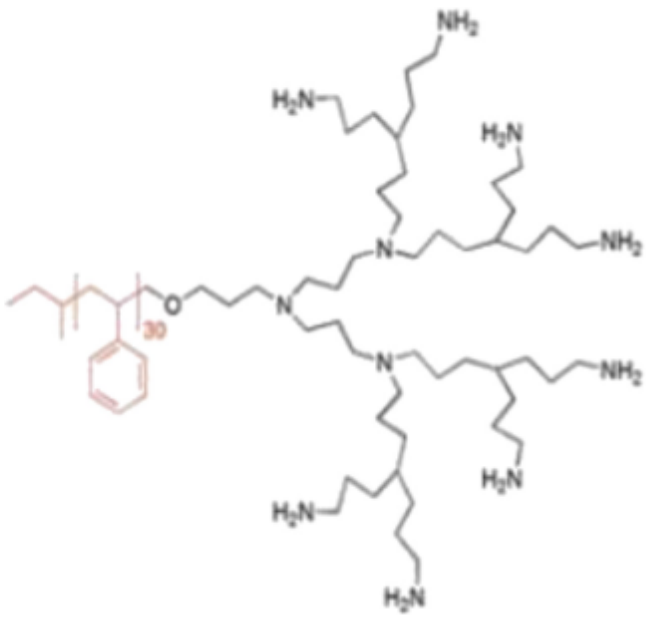
Abbreviated name	Structure copolymer batch
PB-PEO	
PB-PGA	
PS-PAA	
PS-PIAA	
PPQ-PS-PPQ	
PMOXA-PDMS-PMOXA	
PMPS-PEO-PMPS-PEO-PMPS	

Table 2: (continued)

Abbreviated name	Structure copolymer batch
PS-dendr-$(NH_2)_{32}$	

dimensions of the cavities inside silicates and zeolites vary from a few angstroms to a few nanometers. These structures have been used as nanoreactors in various processes. For example, cyano-functionalized porous silicate with an average pore diameter of 18 nm has been used for the proteolysis process (Jang and Oh 2003). Also, zeolites that have metal complexes with catalytic activity inside their cavities have been used in oxidation reactions, hydrogenation, acid-catalyzed isomeric conversion and disproportionate partition reactions (Dawkins 1989). In addition, these structures have been used to produce nanoparticles (Figure 20) (Mirkin 2004).

12.4 Nanotubes

Nanotubes are sheets of atoms organized into tubes. These compounds are structurally composed of organic or mineral materials and can be prepared in the form of single-walled or multi-walled. Nanotubes have a large internal volume and their outer surface is easily functionalized. One of the most important compounds in this group is carbon nanotubes, which were first discovered in 1991. The interior of carbon nanotubes has been used as nanoreactors to produce a variety of nanostructures, including nanowires and nanoparticles (Figure 21) (He, Kunitake, and Nakao 2003; Yang et al. 2020a; Zhang et al. 2021; L. Zhang et al. 2020; M. Zhang et al. 2020).

13 Reaction kinetics inside nanoreactors

Rate-equation for a simple bimolecular reaction in which reactants A and B lead

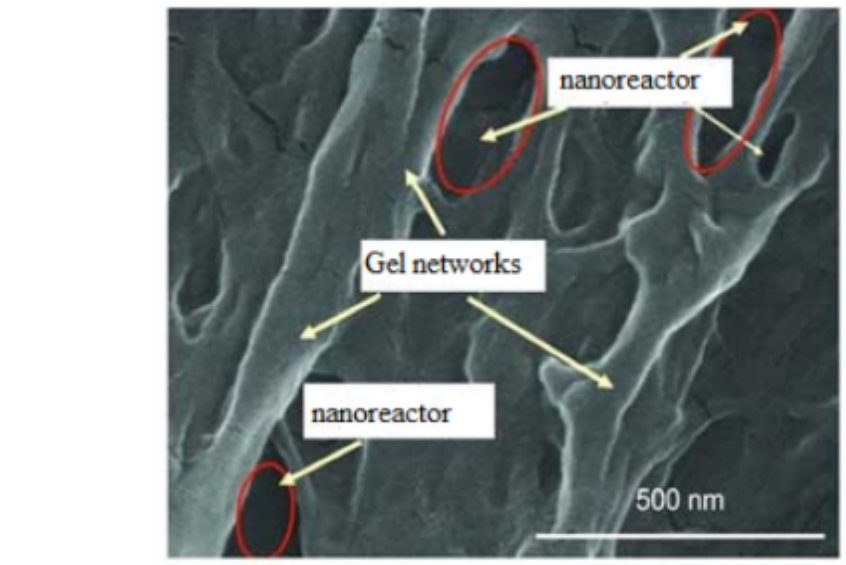
To product C can be calculated using Eq. (1) (Yang et al. 2021; Yang et al. 2017; Zhang et al. 2017).

$$v = \mathrm{d}[\mathrm{C}]/\mathrm{dt}\, k[\mathrm{A}][\mathrm{B}] \tag{1}$$

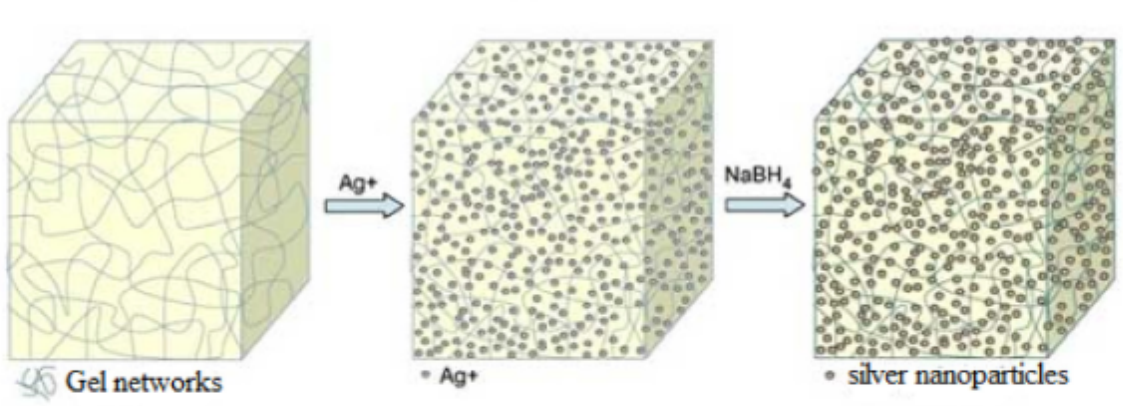
For the reaction inside the NR, a more complicated rate equation is expected due to encapsulation effects as well as product release in-and-out of the NR (Koblenz, Wassenaar, and Reek 2008). When the reaction between A and B within the NR is the rate-determining step, the rate equation simplifies to Michaelis–Menten kinetics (Wang et al. 2016; Zhang et al. 2015a, 2015b) and depends solely on the rate-constant of this step, that is, kb, and on the NR concentration with the encapsulated substrates (NR⊃A.B) (Eq. (2)) (Koblenz, Wassenaar, and Reek 2008). In Figure 22a, energy diagrams of these equations have been represented.

$$v = \mathrm{d}[\mathrm{C}]/\mathrm{dt}\, k[(\mathrm{NR} \supset \mathrm{A.B})] \tag{2}$$

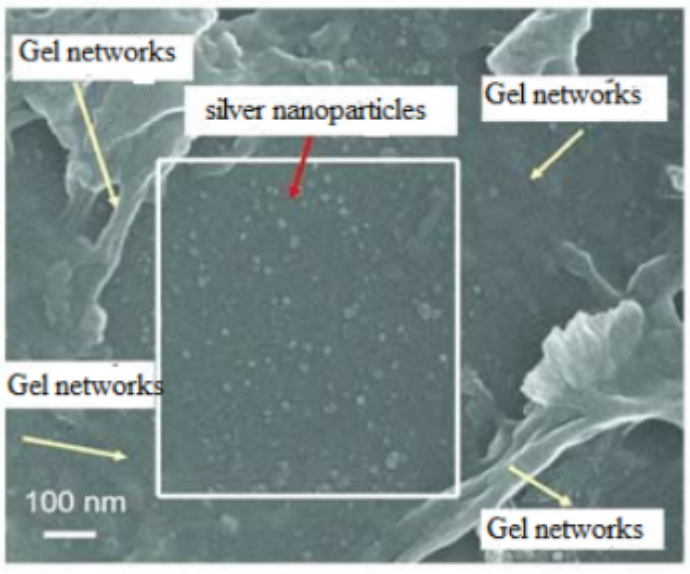
The rate-constant k is a function of the thermodynamic activation parameters, that is, the Gibbs free energy of activation ($\Delta G\#$), and hence to the activation enthalpy ($\Delta H\#$) and the activation entropy ($\Delta S\#$) via the Eyring and



(a)



(b)



(c)

Figure 16: (a) Image SEM (Scanning Electron Microscopy) of pure hydrogel, (b) formation of silver nanoparticles in the hydrogel network and (c) image of SEM growth of silver nanoparticles in hydrogel nanoreactor (Ryu, Cho, and Zhao 2007).

Arrhenius equations: $\Delta G\# = \Delta H\# - T.\Delta S\# = -RT(\ln k) + c$ (T, temperature; R, gas constant; c, a constant) (Koblenz, Wassenaar, and Reek 2008). This observation can be attributed to the changes in the reaction activation energy barrier $\Delta G\#$ of products D and E compared to the bulk. Encapsulation can stabilize the transition state, which leads to product E, thereby favoring its formation. In contrast, formation of product E can become unfavorable through encapsulation and destabilization of the transition state that lead to it (Figure 22b) (Brinker 2010).

14 Applications of nanoreactors

The application of nanoreactors is a relatively new area in which, much progress has been made during the last

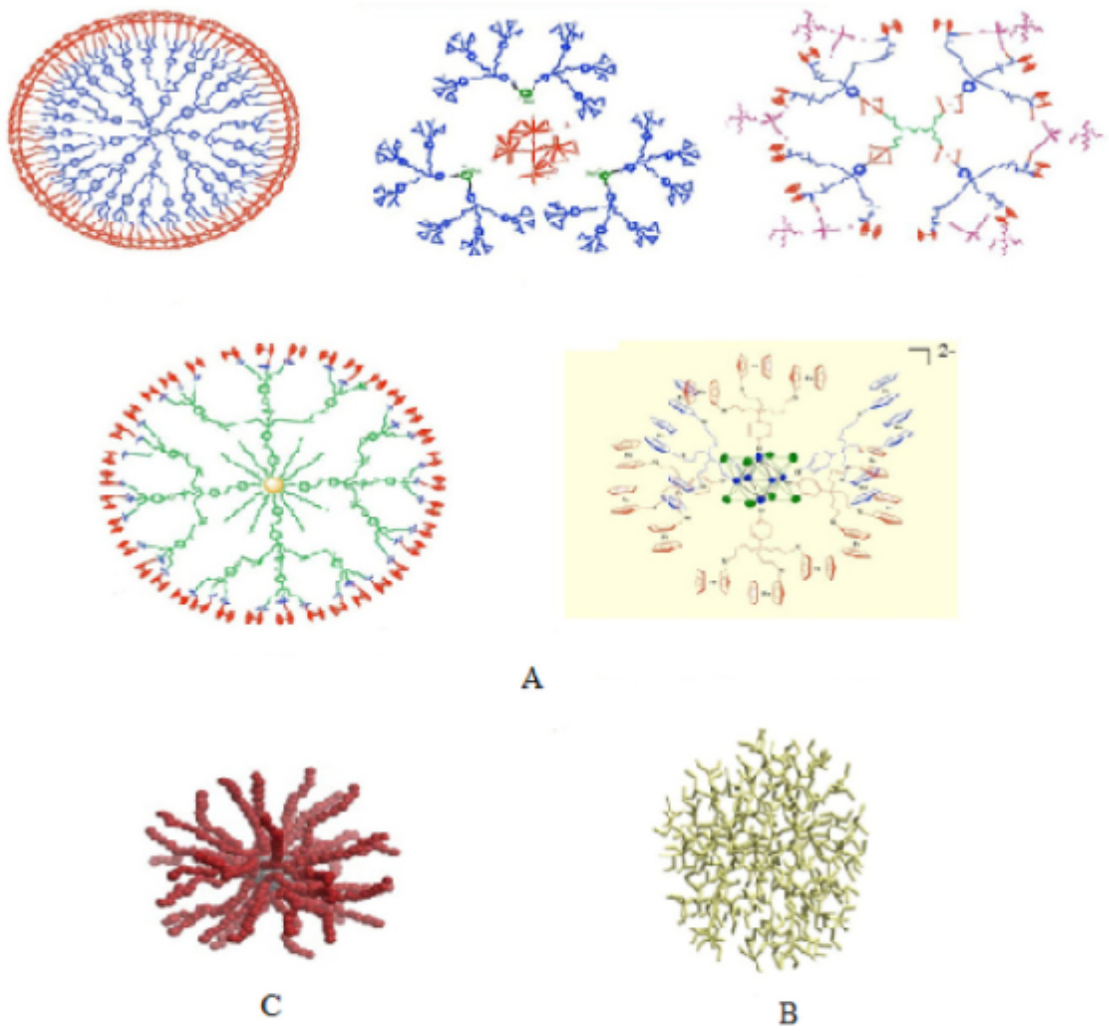


Figure 17: (a) Dendrimers (b) hyperbranched polymers and (c) star polymers (Dawkins 1989).

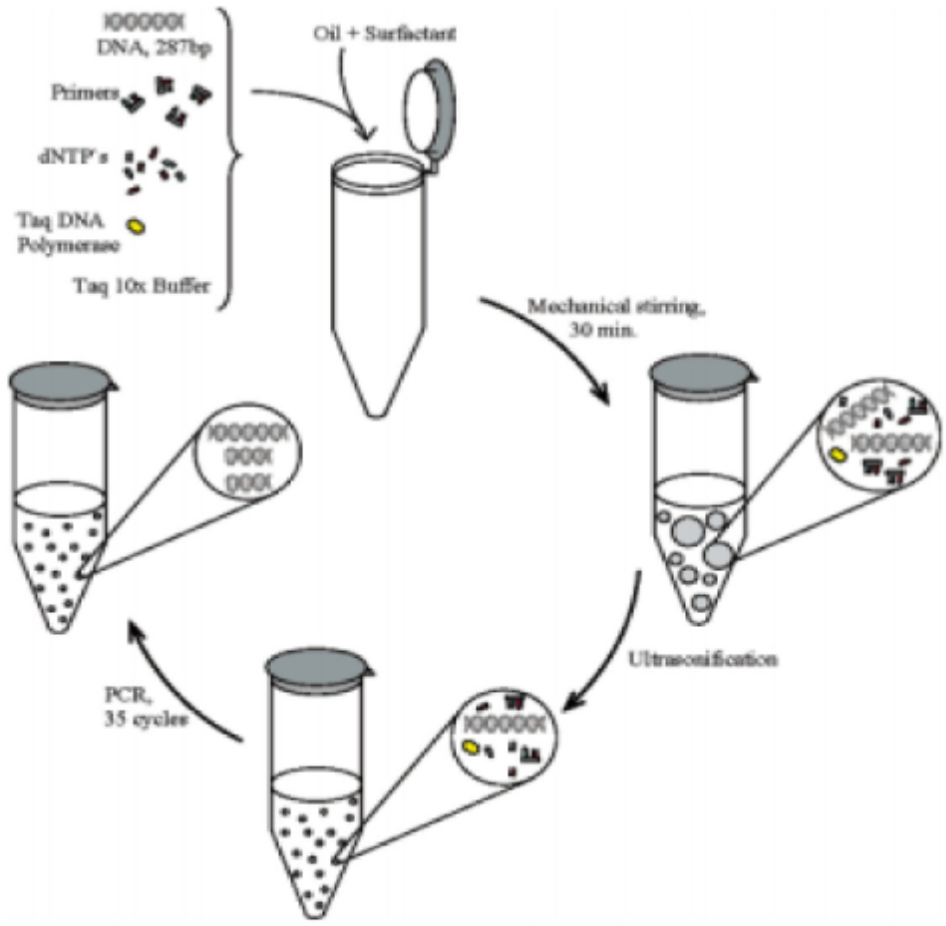
decade. This review highlights the most important application of such polymeric nanoreactors in several domains including medicine, ecology, biotechnology, and material science and pay attention to the advantages and drawbacks related with them. While in most cases the active components of nanoreactors are enzymes, there are also some examples where the nanostructure itself serves as a template, for example, to produce gold nanoparticles (Caruthers, Wickline, and Lanza 2007; Deraedt et al. 2013). Nanoreactors are able to provide more precise and early diagnostics, can support surgery through fine localization of tumors, decrease side effects while increasing efficiency in drug therapy and show the way to development of new medical devices due to its sensitivity, specificity and rapid response (Ostafin and Chen). However, a complex situation of requirements must be fulfilled by nanoreactors if medical applications are to be realized (a) the chemical nature and properties of polymers and their assemblies must be modified to avoid impairing the required biological activity; (b) non-toxicity with low immunogenicity; (c) biodistribution, bioaccumulation, and degradation in the human body should be controllable in the lab before clinical trials.

14.1 Diagnostic applications

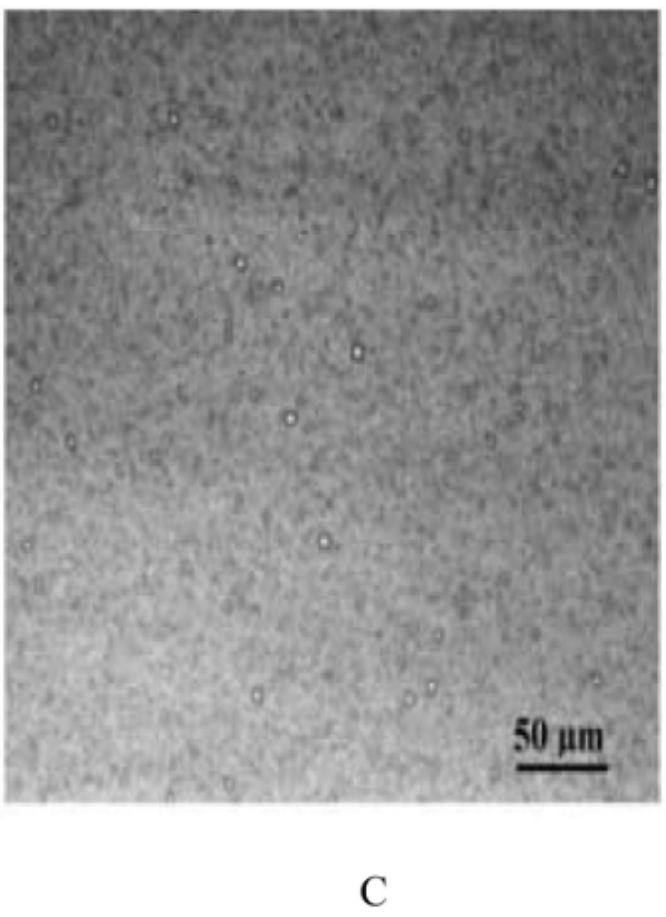
Accurate and non-invasive diagnosis is necessary to prevent disease progression. Various physical, chemical, and biochemical techniques have been developed for rapid estimation of biochemical status in biological fluids like blood, urine, stool, or saliva, for measurements of various physical properties in the body for example temperature and electrical currents and for imaging techniques like optical, ultrasound, magnetic, and nuclear resonance (Han et al. 2003; Wang, Zhao, and Qiu 2007; Zhang 2003). Nanoreactors were able to detect small changes in physical properties of the surface or the presence of biological molecules with high precision. Polymeric nanoreactors containing acid phosphate immobilized on a glass surface were shown to convert a nonfluorescent substrate to a fluorescent product (Vriezema et al. 2005).

14.2 Theragnostics

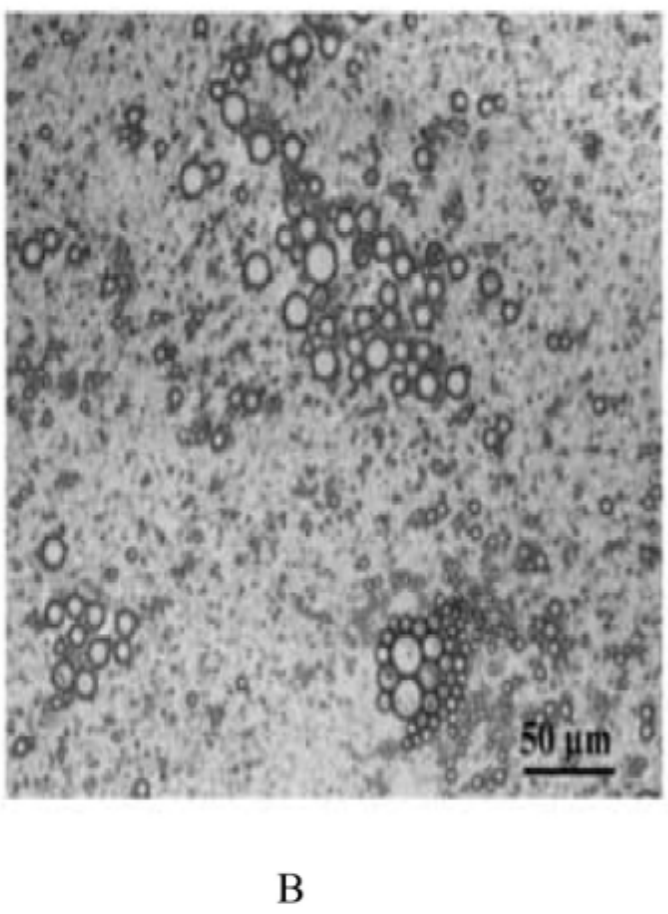
Nanoreactors that contain various hydrophilic and hydrophobic compounds are potential candidates for theragnostic



A



C



B

Figure 18: (a) Stages of preparation of miniemulsion, (b) light microscope image of macroemulsion and (c) mini-emulsion (Liu, Liu, and Warmuth 2007).

applications, providing a possible step toward modified medicine. By combining diagnostic techniques like ultrasound and MRI together with enzymes in one nanoreactor, overall patient treatment will become more actives and appropriate (Koblenz, Wassenaar, and Reek 2008). For example, a nanoreactor proposed for theragnostic

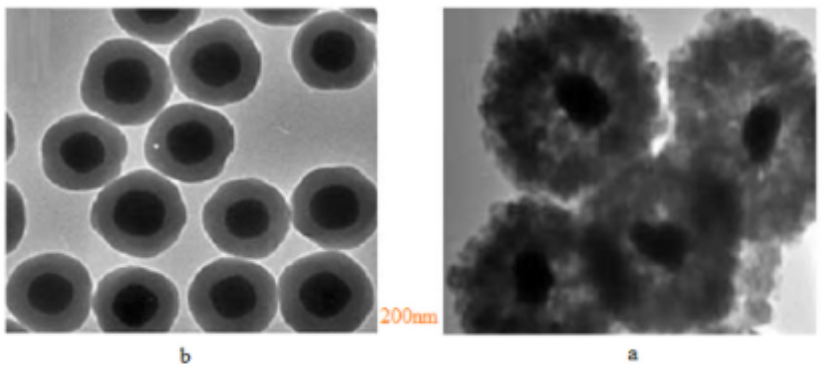


Figure 19: (a) TEM image (Chen et al. 2020a; Wang, Li, and Xie 2021; Zuo et al. 2015) of Au-TiO_2 core–shell nanoreactor after gold nucleation growth (Dähne et al. 2001), (b) TEM image (Chen et al. 2019; Li et al. 2018b; H. Zhang et al. 2019) of Au-SiO_2 core–shell nanoreactor and (Neumann, Haupt, and Ballauff 2004).

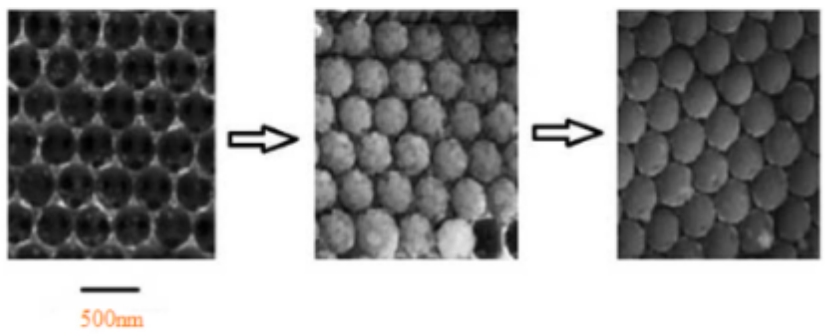


Figure 20: SEM images (Sun et al. 2019; Yang et al. 2019; K. Zhang et al. 2019; K. Zhang et al. 2020) of nanoparticle growth in a zeolite nanoreactor (Mirkin 2004).

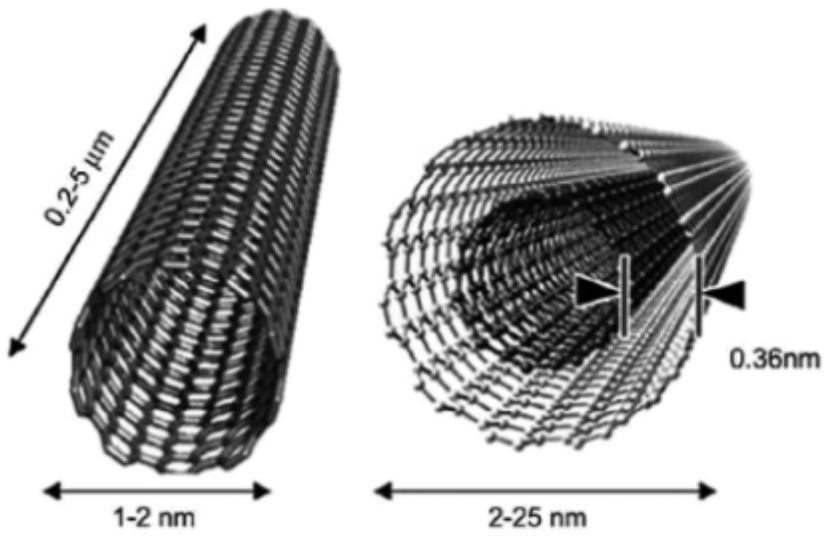


Figure 21: Single-walled and multi-walled carbon nanotubes (He, Kunitake, and Nakao 2003).

application is based on the concept of an enzymatic cascade reaction taking place inside the cavity of polymeric vesicles, to simultaneously detect and combat oxidative stress as shown in Figure 6. In this, combination of enzymes SOD (C) and lactoperoxidase (E) act inside polymeric vesicles in a cascade reaction with substrate Ample-Red (D) and product resorufin (F) to detoxify reactive oxygen species (A) (Brinker 2010). Firstly, SOD detoxified superoxide radicals to hydrogen peroxide then lactoperoxidase converted the substrate (Amplex-Red), together with hydrogen peroxide, to a fluorescent product (resorufin). The final products of the cascade reaction were water and oxygen. The process of detoxification of oxygen species was monitored in real time, and the system behaved like an artificial organelle in die rent cell lines.

14.3 Sensors

Nanoreactors can be used as a platform for developing sensing and stimuliresponsive systems. For example, an NR based on a biocompatible calcium phosphate cage has been synthesized that responds to reactive oxygen species

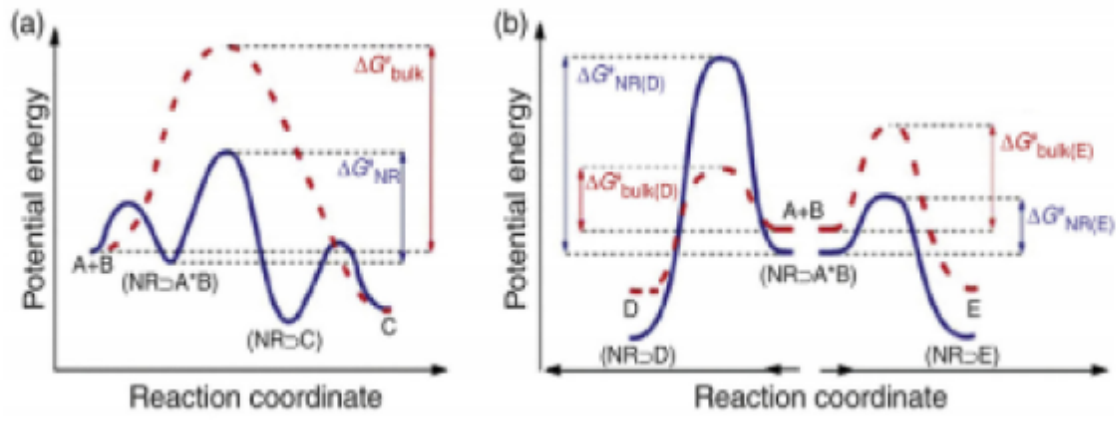


Figure 22: (a) Simplified reaction profiles of a reaction in the bulk solution (dashed red line) and of a reaction within an NR (blue line). (b) Simplified reaction profiles of a reaction leading to product D that is destabilized by the NR (blue line), compared to the bulk solution (dashed red line); and of a reaction leading to product E that is stabilized by the NR (blue line), compared to the bulk solution (dashed red line) (Brinker 2010; Koblenz, Wassenaar, and Reek 2008).

and emits fluorescence at near-infrared region (Ostafin and Landfester 2009). Stimuli-responsive NRs such as polymer vesicles, which also refers to smart polymer vesicles, responds to stimulus such as changes of pH, temperature, and light, and can be considered as useful carriers for encapsulation and controlled delivery of drug and other biological compounds (Cabane, Malinova, and Meier 2010; Kim et al. 2010).

14.4 Environmental applications of nanoreactors

Today's major problems are the depletion of natural resources and excessive levels of environmental pollutants. Nanoreactor technology is the exploratory phase of research in terms of presenting innovative concepts for alternative sources of energy. They will certainly provide help to the problems like reduction in pollution, and development of "green industry". The nanoreactors can be used for decontamination processes. But the problem related with the use of enzymes for decontamination purpose is the presence of water, which is not suitable for some applications such as use in the cleaning of electronic equipment's. Encapsulation of lysozyme in reverse micelles will allowed distribution between a water phase and an organic phase that contains surfactant (Bermudez and Forciniti 2004; Kishimura et al. 2009). Reverse micelles are used to increase the confined concentration of lysozyme and reduce the water usage. Reverse micelles containing lysozyme are used to decontaminate various surfaces, contaminated with Gram-positive and Gram-negative bacteria. The encapsulated enzyme can be used directly for the electronic devices. Nanoreactors can selectively detect and recover compounds in complex mixtures like contaminated soil and mine slimes, should be ideal candidates for downstream processing.

Tanner et al. conducted a nanoreactor that could combat oxidative stress (Kuiper et al. 2008). They verified that in poly (2-methyloxazoline)-poly-(dimethylsiloxane)-poly(2-methyloxazoline) (PMOXA-PDMSPMOXA) polymer somes the coen-capsulation of copper-zinc superoxide (Cu, Zn-SOD) and lactoperoxidase (LPO), provided an active device to detoxify reactive oxygen species (ROS). Firstly, the superoxide radical ion was converted into hydrogen peroxide by Cu, Zn-SOD which subsequently acts as substrate for LPO to convert amplex red into resorufin. Confocal fluorescence measurements revealed that the system could be used for both the complete detoxification of ROS under the conditions of oxidative stress.

Yang et al. employed yolk-shell nanoparticles (YSNs) in a deacetylation-Henry cascade reaction (Yang et al. 2012). The YSNs consisted of a silica core, functionalized with primary amines, and a permeable silica shell, functionalized with sulfonic acid groups. The core and shell could be separated from each other because of organosilane-assisted etching procedure. The YSNs gave a maximal conversion to the nitrostyrene product (>99%) when applied on deacetylation – Henry cascade reaction of benzaldehyde dimethyl acetyl.

15 Conclusion

– Nanoreactors are nanometer containers that can be made from a variety of compounds, including molecules, synthetic macromolecules, and biomolecules. In these structures, raw materials and products are

exchanged between the mass solution and the nanoreactor cavity, and therefore, membrane permeability plays an important role in their selective performance. Due to many advantages such as the possibility of controlling the reaction and protecting the catalysts against environmental influences, various types of catalytic and enzymatic reactions as well as the preparation of various nanostructures within nanoreactors are performed.

- Many biological and chemical (synthetic) structures can be used as nanoreactors. The bond between the components of these structures is covalent or non-covalent. Mini-emulsions, micelles, cell organs, polymerosomes, liposomes, vesicles, hydrogels, Dendrimers, zeolites, and carbon nanotubes are examples of nanoreactors. Each of the mentioned cases has its own characteristics and can be used as a mold or synthesis medium in the preparation of nanomaterials.
- Nature has put the nanoparticle of life inside the cells. Although there is a long way to go before a complete cell can be built, cell similarities consisting of the self-assembly of synthetic and biological building blocks are a significant improvement. Molecules, macromolecules, and biomolecules each show significant advantages in forming self-assembled nanoreactors that can insert a species into their capsule. The important point is that despite the complexities, the more natural building blocks are used, such as enzyme-based dual-friendly species and virus capsids, the closer we get to the goal of modeling natural systems.
- Many novel approaches have been applied to develop more and more efficient nanoreactors. One was to use of simple surfactants for the construction of micellar or emulsion reactions in chronological order. In the second half of the 20th century, the application of lipidbased assemblies as potential cell membrane mimics and subsequently as enzyme and catalyst-containing nanoreactors received considerable attention. The concept of the nanoreactor has been successfully brought to completion by combining supramolecular assemblies (dendrimers, micelles, vesicles, and capsules), natural (enzymes, proteins) and synthetic (mimics of enzymes) compounds. Properly designed, nanoreactors allow the encapsulation/insertion of both hydrophobic and hydrophilic compounds, thereby supporting the development of the multifunctional systems that are in focus today in various fields. In this respect because of morphology, size, and general properties-polymersomes are currently show the way especially for therapeutic applications. Introducing multifunctionality through cascade reactions, targeting distinct locations in biocompartments, and immobilization on surfaces are the various directions of research being taken today. This will provide a diversity of technical approaches that should yield nanoreactors that are simple, robust solutions, as required today in medicine, catalysis, environmental or food science.

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