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The Application of Biomolecules in the Preparation of Nanomaterials

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1. Introduction

1.1 Using ascorbic acid or amino acids as reducing agent to synthesize nanomaterials of special morphology

Recently, preparation of nanomaterial by the use of the special properties of biomolecules has gained more and more research interest. These strategies usually utilize the environmentally benign and "green" experimental condition, not the harsh condition used in the traditional chemical synthesis. And, preparation of nanomaterials with the use of biomolecules can often has some control on the morphology and size of the final products.

It is well-known that ascorbic acid has the mild reducing ability, which made it very appealing in the fabrication of nanomaterial with unusual morphology. Using the mild reducing ability of ascorbic acid, Murphy’s group and Huang’s group have synthesized silver nanowires and gold nanorods of controllable aspect ratio via seed-mediated growth approach (Jana et al., 2001; Gole et al., 2004; Wu et al., 2007). In the presence of trisodium citrate, nanoparticle seeds with the size about 4 nm were obtained via the reduction of the aqueous solution of AgNO₃ or HAuCl₄ by NaBH₄. The growth solution includes AgNO₃ (or HAuCl₄), the reducing agent ascorbic acid and surfactant cetyltrimethylammonium bromide (CTAB). After the seeds were added into the growth solution, silver nanowire or gold nanorod will be produced by reducing the corresponding metal salts with ascorbic acid for a period of time.

Some amino acids also have the reduction ability and can be used as reducing agent to prepare nanomaterials of special structure. For example, Shao and his coworkers have prepared the hexagonal single crystal gold nanoplate in one-step by aspartate reduction of HAuCl₄. The growth solution includes AgNO₃ (or HAuCl₄), the reducing agent ascorbic acid and surfactant cetyltrimethylammonium bromide (CTAB). After the seeds were added into the growth solution, silver nanowire or gold nanorod will be produced by reducing the corresponding metal salts with ascorbic acid for a period of time.

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1.2 Preparation of nanomaterials with amino acids as protecting agents

There are mainly two methods that nanoparticles can be synthesized by using amino acids as protecting agents. The first method is that: first prepare the nanoparticles, then conjugate amino acids onto the nanoparticles to protect the nanoparticles from aggregation. For example, using lysine as the capping agent, Sastry’s group has prepared gold nanoparticles with good dispersibility in water (Selvakannan et al., 2003). In their experiment, gold nanoparticles were first produced by reducing the aqueous solution of HAuCl$_4$ with NaBH$_4$. Lysine protected gold nanoparticles were obtained after mixing gold nanoparticles with lysine aqueous solution for 12 hours. The lysine capped gold nanoparticles show good dispersibility in water. Sastry et al. have also found that the dispersibility of lysine capped gold nanoparticles depends on the pH value of the aqueous solution. Under acidic condition (pH=3), the lysine capped gold nanoparticle are well dispersed from each other and the network of gold nanoparticles can be formed via the hydrogen bonding. In the basic environment, the gold nanoparticles aggregate into large superstructure in which the individual gold particles are difficult to be distinguished. The pH depended self-assembly of lysine capped gold nanoparticles was related to the formation of hydrogen bonding among the amino acids on the surfaces of the adjacent gold particles.
The second method for preparation of amino acid protected gold nanoparticles is that the amino acid protected gold nanostructures were produced in one step via reducing the corresponding noble metal salts directly by reducing agent in the presence of amino acids. For example, Zhong et al. have directly utilized lysine as protecting agent to prepare gold nanowires through reduction of HAuCl₄ by NaBH₄ (Zhong et al., 2004). Their preparation method is: First, mix the aqueous solution of HAuCl₄ and lysine at a certain molar ratio. Then, adjust the pH value of the mixture solution with aqueous NaOH to the suitable range. Finally, lysine protected gold nanowire structure was obtained directly by reducing HAuCl₄ with the addition of NaBH₄ under vigorous stirring. Lysine acts as capping and bridging agent in the formation process of the gold nanowire. There are two factors, the pH value and the molar ratio of lysine to gold, which influence the formation of the gold nanowire structure. Zhong et al. found that linear structures of gold, with the diameter of ca. 5 nm and length in the range of 80-200 nm, can be produced when the molar ratio of lysine to gold is 0.5 and the pH value is in the range of 8.4-9.5. At the low pH value, the main products of the reaction are some aggregates without uniform morphology. Under the basic condition (pH=11.1), the reaction products consist of the gold nanowire which are short and thickly bound with each other. Additionally, ultrasound also has influence on the formation of the gold nanowire structure. Gold nanoparticles with good dispersibility will be the products when heavy ultrasound was applied in the preparation process of the gold nanowire structure.

2. The application of biomacromolecules in the preparation of nanomaterials

2.1 The application of sugar in the synthesis of nanomaterials

Recently, the preparation of sugar modified metal nanoparticles has attracted a wide research interest. There are two methods that can be used to prepare sugar modified nanoparticles. The first method uses the biomolecules, such as glucose (Raveendran et al., 2003), chitosan (Huang et al., 2004), and amino-dextran (Ma et al., 2005), directly as reducing agent to reduce metal salts to produce metal nanoparticles. For example, Raveendran et al. have prepared the starch stabilized silver nanoparticles which were almost monodispersed by gentle heating the mixture aqueous solution of AgNO₃, glucose and starch (Raveendran et al., 2003). In their experiment, glucose was used as the “green” reducing agent and starch was used as the stabilizer of the nanoparticle. Additionally, Ma et al. have utilized a similar method to prepare the amino-dextran protected gold and silver nanoparticles via heating the mixture solution of amino-dextran and HAuCl₄ or AgNO₃. The amino-dextran was used directly as reducing and protecting agent. The size of the obtained nanoparticles can be adjusted by changing the molar ratio of amino-dextran to metal salts. These amino-dextran protected gold and silver nanoparticles can be used as biosensor for the detection of concanavalin A.

The second method utilizes the sugar, such as mannose and dextran, to modify the as-prepared nanoparticles (Zhang et al., 2004; Aslan et al., 2004; Lyu et al., 2008). These sugar modified nanoparticles can be used as biosensor for the detection of concanavalin A and glucose. Recently, Lyu and his co-workers have prepared the mannose-stabilized gold nanoparticles by the displacement self-assembly of citrate-capped gold nanoparticles solution with thiol-modified mannoside. The mannose-stabilized gold nanoparticles were also monodispersed as that of the original citrate-capped particles. These mannose-stabilized gold nanoparticles can be used as the signal amplifier in the determination of concanavalin A by quartz crystal microbalance (QCM).
2.2 The application of lipids in the preparation of nanomaterials

Recently, lipid bilayer modified nanomaterials, such as noble metal nanoparticles (He et al., 2005; Takahashi, 2006; Zhang et al., 2006, 2008), semiconductor quantum dots (Geissbuchler et al., 2005; Gopalakrishnan et al., 2006), silica nanoparticles (Mornet et al., 2005) and carbon nanotubes (Zhou et al., 2007; He et al., 2005; Artyuklin et al., 2005), have gained a wide research attention due to their water solubility, biocompatibility and their potential application in many fields. It is believed that the lipid bilayers capped on the nanomaterials act in the same way as biomembranes to a certain extent. This may lead people to use lipid bilayers as biomembrane models to study a wide variety of biological functions, such as membrane fusion, the interaction between protein and cell membranes, and other processes in the fields of biophysics, chemistry, and medicine (Mornet et al., 2005; Zhou et al., 2007).

The representative example of the lipid bilayer-coated nanomaterial is the didodecyldimethylammonium bromide (DDAB) lipid bilayer-protected gold nanoparticles prepared by Zhang et al (Zhang et al., 2006, 2008). Their preparation method is: in situ reduce HAuCl\(_4\) with NaBH\(_4\) in the aqueous solution of DDAB to directly form the DDAB lipid bilayer-capped gold nanoparticles. The scheme of the lipid bilayer-protected gold nanoparticle and its TEM image were shown in Figure 2. In addition, the research carried out by Li et al. has shown that capping of DDAB on the surface of gold nanoparticle can notably enhance the stability of the DDAB-DNA complex in the blood serum, which is crucial to the efficiency of gen delivery (Li et al., 2008). The other work of Li et al. further showed that lipid bilayer-capped gold nanoparticles can effectively transfer the plasmid DNA into human cells in the presence of blood serum (Li et al., 2008). Therefore, it can be seen that lipid bilayer-coated nanomaterials possess enormous application potential in biomedical field, especially the gen transfer area, due to their biocompatible surface.

![Fig. 2. The scheme of the DDAB lipid bilayer protected gold nanoparticle and its TEM image.](image)

2.3 The application of DNA in the fabrication of nanostructures

The biomacromolecule, DNA, demonstrates huge application potential in the fabrication of nanostructures and nanodevices (Nalwa, 2005). DNA molecules can be used in the assembly
of device as well as its connecting wires (Strohoff et al., 1999). Fabrication of nanostructure using DNA as building blocks has three advantages: (1) The intramolecule interaction of DNA can be programmably designed and controlled. This is a simple base-pairing theory between the components of DNA. Adeine (A) pairs thymine (T) via forming two hydrogen bonds. Guaine (G) pairs cytosine (C) via forming three hydrogen bonds. This unique property makes DNA not only an effective genetic substance but also a programmable building block in self-assembly. (2) The DNA sequence can be synthesized by chemical method. Many DNA derivatives, such as biotin or fluorescein labeled DNA fragments and some DNA linkers, have also been chemically synthesized due to the demand in biological science & technology. (3) DNA can be manipulated or modified by many enzymes, such as DNA polymerases, restriction endonucleases and kinases.

2.3.1 The application of oligonucleotides in the self-assembly of metal nanoparticles

In 1996, Mirkin’s research group in the Northwestern University of USA first reported that gold nanoparticles can be self-assembled into microscale aggregates by using DNA molecules as the linker (Mirkin et al., 1996). Two different thiol-derivated noncomplementary oligonucleotides were first used to modify the gold nanoparticles. Then, these two kinds of gold nanoparticles were mixed and the DNA linker was added. The fragments on the two ends of the DNA linker can complement with the oligonucleotides on the gold nanoparticles. The gold nanoparticles can self-assemble into aggregates when the hybridization process proceeds. This process is reversible. When the temperature was elevated, the DNA double strands would dissociate and the gold nanoparticles became monodispersed again. The DNA linkers possess special recognition property. By changing the composition of the DNA linker, the structure and property of the nanoparticle aggregates, such as the distance between the particles and the strength of the linking between the particles, can be effectively controlled. In the same year, Alivisators et al. also reported that gold nanoparticles can be self-assembled based on DNA hybridization (Alivisators et al., 1996). In their work, the 3’ or 5’ ends of a 19 nucleotides single strand DNA was first connected with gold nanoparticle by thiol. Then, a 37 nucleotides single strand DNA template was added into the gold nanoparticles solution. Gold nanoparticles were assembled onto the DNA template through hybridization into two kinds of dimer: parallel and antiparallel patterns.

2.3.2 DNA templated self-assembly of metal nanoparticles

The basic principle of DNA templated self-assembly of nanoparticle is: First, monodispersed nanoparticles with uniform size should be prepared. Then, the nanoparticles were assembled onto the DNA molecule via certain interactions between DNA and the particles. Based on the electrostatic interaction between the negatively charged DNA and the positively charged nanoparticles, gold nanoparticles (Nakao et al., 2003), silver nanoparticles (Wei et al., 2005; Sun et al., 2006), Fe₃O₄ nanoparticles (Nyamjav et al., 2005) have been self-assembled onto DNA. The representative work in this area is the self-assembly of aniline-capped gold nanoparticles on λ-DNA carried out by Nakao et al. Aniline was first used to reduce HAuCl₄ to produce the aniline-capped gold nanoparticles in one step. Two different assembly methods were used to prepare the highly ordered gold nanoparticles assemblies. In method (I), the DNA template was first stretched and fixed on the substrate, gold nanoparticles were then assembled on the DNA chains to form the
continuous linear structure of gold nanoparticles. In method (II), gold nanoparticles were first bound onto DNA in solution. The DNA-gold nanoparticle complex was then stretched and fixed onto the substrate. Gold nanoparticles assemblies prepared by the method (II) were loosely deposited, like the necklace.

Our group has also carried out some works on the DNA-templated self-assembly of metal nanoparticles and the in situ formation of metal nanostructures on the DNA assemblies. Based on the electrostatic interaction, 4-aminothiophenol capped silver nanoparticles have been successfully assembled on the predefined circular plasmid pBR322 DNA to form the silver nanoparticles ring (Sun et al., 2006), as shown in Figure 3. Another work from our group further showed that silver nanostructures can be generated on the DNA network by reduction of the silver ions that adsorbed on the DNA network with NaBH₄ solution (Wei et al., 2005). In this work, silver nanoparticles, nanorods, and nanowires can be formed by controlling the size of the DNA network. AFM images of the DNA network and the silver nanoparticles generated after different reducing time were shown in Figure 4.

![AFM images of the pBR322 DNA template (left) and the DNA templated silver nanoparticles assemblies (right).](image)

In addition to the electrostatic interaction, other interactions can also be used to assemble nanoparticles onto the DNA template. Harnack et al. reported the self-assembly of tris(hydroxymethyl)phosphine-capped gold nanoparticles on DNA template (Harnack et al., 2002). It should be noted that the tris(hydroxymethyl) phosphine-capped gold particles are negatively charged. So, the electrostatic interaction between DNA and the nanoparticle can be excluded. The reason that the negatively charged gold nanoparticles can still bind onto the DNA chain is because the formation of the DNA-nanoparticle conjugates. These DNA-gold nanoparticle conjugate can be used as precursors of the gold nanowires. Electroless plating the DNA-gold nanoparticle conjugates with gold leads to the formation of gold nanowires as narrow as ca.30-40 nm in width and longer than 2 μm showing ohmic behavior and resistivity of ca.10⁻⁵ Ωm.
2.3.3 DNA templated nanowire formation

DNA molecules can further be used as templates to fabricate metal or conducting polymer nanowires. For example, DNA has been used as templates for the fabrication of conductive silver nanowires (Braun et al., 1998). Positively charged Ag⁺ was first absorbed onto the negatively charged λ-DNA molecules. The absorbed Ag⁺ was then reduced on the DNA template and the silver nanoparticles coated DNA nanowire was formed. The as-formed silver nanowires, with the width of ca. 100 nm and length on the order of micrometer, connected two gold electrodes. The conductivity of the DNA-templated silver nanowire was also measured by Braun et al. The current-voltage (I-V) characteristic of the silver nanowire showed that no current was flowed through the nanowire when the bias voltage was low (10 V). These results suggested that the resistivity of silver wire was high. The silver nanowire can become conductive at very high bias voltage. Deposition of more silver onto the DNA-templated silver nanowire can produce thicker silver nanowire and the non-conducting area can be decreased from 10 V to 0.5 V, which showed that the electronic property of this system is controllable. Moreover, in the control experiment, no electrical current will be detected when any components of the system, such as DNA or silver, was removed. This showed that all the components are necessary to the conductivity of the DNA-templated silver nanowire.
The above DNA-templated nanowire formation method reported by Braum et al. can also be introduced to fabricate other metal nanowires. By using DNA molecule as template, many metal nanowires and polymer nanowires, such as Pd nanowires (Deng et al., 2003; Richter et al., 2000, 2001), Pt nanowires (Ford et al., 2001; Seidel et al., 2004), Cu nanowires (Monson et al., 2003) and polyaniline nanowires (Ma et al., 2004), have been produced by reducing the metal ions or polymerizing the monomers that bound on the DNA template. For example, Deng et al. have fabricated the parallel arrays or crossed arrays of Pd nanowire (Deng et al., 2003). Their experiment consisted of three steps: First, “molecule combing” method was used to stretch the DNA molecules into 1D parallel or 2D crossed patterns. Then, the Pd$^{2+}$ ions were quickly absorbed onto the negatively charged DNA back-bone. Finally, chemical reduction of the Pd$^{2+}$ ions on the DNA templates forms the Pd nanowires.

With the similar strategy, DNA-templated polyaniline nanowires have been fabricated (Ma et al., 2004). In the experiment, DNA molecules were first stretched and fixed on the silicon substrate via the “molecule combing” method. Then, the protonated aniline solution was incubated with the DNA templates for some time to let aniline absorb onto DNA. The aniline was finally polymerized on the DNA template to form the polyaniline nanowires. It was also found that the DNA-templated polyaniline nanowires can be used as the sensors of chemical gases.

Our group has also used a solution method to fabricate the DNA-aniline complex nanowire as well as the DNA templated polyaniline nanowire (Yang et al., 2006). With DNA as templates, linear aniline-DNA complex nanowires have been produced in solution. Gas flow was used to stretch the obtained aniline-DNA complex nanowires onto mica substrate. The ordered aniline-DNA complex nanowires can be directly observed from the AFM images. We propose that DNA molecular in solution were enwrapped by aniline monomers via a self-assembled process (Figure 5). Moreover, we obtained the polyaniline (PANI) nanowires based on the precursor of aniline-DNA complex nanowires through further chemical oxidative polymerization. The aniline-DNA complex and the PANI-DNA nanowires exhibit a low background on the unmodified mica substrates (Figure 6).

![Fig. 5. Schematic representing the formation of the aniline-DNA complex nanowire and the DNA-templated nanowire](image-url)
2.4 The application of peptides and proteins in the preparation of nanoparticles

In the past few years, the preparation of bioactive or biocompatible nanomaterials has attracted more and more research attention due to the demand of various practical applications. It was believed that bioactive or biocompatible nanomaterials have wide application potential in biomedical field and bioanalysis. There are mainly two strategies that can be used to prepare bioactive and biocompatible nanomaterials: (1) conjugate biomolecules with nanomaterials via a linker agent or the protecting agent on the nanomaterial; (2) conjugate the biomolecules directly onto nanomaterials by chemical interaction or biological method.

The conjugates of biomolecules and nanoparticles were usually prepared by mixing biomolecules and the modified nanoparticles in solution. Before mixing, the nanoparticles were usually functionalized by a linker agent, which can not only recognize the biomolecule but also stabilize the nanoparticles and prevent the nanoparticles from uncontrollable growth or aggregation (Niemeyer et al., 2001). For example, inorganic nanocrystals and nanoparticles have been bioconjugated by modification with various peptides (Dameron et al., 1989; Whaley et al., 2000) or proteins (Donglas et al., 1998; Chan et al., 1998; Mamedova et al., 2001). Protein transferrin and immunoglobulin G (IgG) have been conjugated onto the ZnS capped CdSe quantum dots by Chan et al. and the bioconjugated quantum dots were used in the ultrahigh sensitive biological detection. These quantum dot-protein conjugates are water soluble and biocompatible. The transferrin conjugated quantum dots can be transferred into Hela cells and IgG conjugated quantum dots can recognize certain antigen or antibody.

It can be proposed that direct conjugation of biomolecules onto nanomaterials will produce many advantages, but the direct conjugation was not usual. The reason is that usually the harsh experiment condition used in the chemical synethesis of nanomaterials was not suitable for the biological samples. Direct conjugation of biomolecules on nanomaterials can eliminate the use of the linker agents or the passivation of nanomaterial with capping agents. Therefore, the direct conjugation of biomolecule and
nanomaterial is more simple and effective for the preparation of bioactive and biocompatible nanomaterials than the first method. In the past, water soluble and biocompatible gold nanoparticles with the diameter less than 2 nm have been produced by using peptides, such as triopronin (Templeton et al., 1999) and glutathione (Schaaff et al., 1998), to directly stabilize the gold nanoparticles. Recently, biomacromolecules, such as proteins, have been used to conjugate nanoparticles directly. Sun’s group has prepared the bovine serum albumin (BSA) directly conjugated Ag$_2$S nanoparticles from rapid expansion of supercritical fluid solution into aqueous solution (Meziani et al., 2003). The Ag$_2$S nanoparticles produced by this method are monodispersed and well coated by BSA molecules. Because the protein BSA undergoes solution pH-dependent association and dissociation, the BSA-nanoparticle conjugates also assemble and disassemble with the change in solution in a reversible fashion.

Later, a more direct and convenient strategy for direct conjugation of BSA and gold nanoparticles was reported (Burt et al., 2004). With protein BSA directly as protecting agent, the BSA directly stabilized gold nanoparticles were prepared by reducing HAuCl$_4$ with NaBH$_4$ in the aqueous solution of BSA. TEM revealed that the obtained gold nanoparticles were well dispersed with an average diameter less than 2 nm. Infrared spectroscopy confirmed that the polypeptide backbone of BSA remained intact after BSA was conjugated with the gold particles. The conjugation of BSA onto gold nanoparticle was realized by the break of the disulfid bonds in the conjugated protein and thus available for interaction with the gold surface.

Enzymes have intrinsic ability to catalyze the formation of metal nanoparticles. It has been reported that $\alpha$-amylase can be used to synthesize and stabilize gold nanoparticles in aqueous solution via mixing the aqueous solution of $\alpha$-amylase and HAuCl$_4$ (Rangnekar et al., 2007). The activity of $\alpha$-amylase was retained in the enzyme-gold nanoparticle complex after the nanoparticles were synthesized. The $\alpha$-amylase in the enzyme-gold nanoparticle complex also showed its ability to digest starch.

We have also utilized proteins as stabilizer to prepare the protein-conjugated gold nanoparticles. Lysozyme monolayer-protected gold nanoparticles which are hydrophilic and biocompatible were synthesized in aqueous solution by chemical reduction of HAuCl$_4$ with NaBH$_4$ in the presence of lysozyme (Yang et al., 2007). The formation of a lysozyme monolayer on gold was achieved by the chemisorption of the free amino group or carboxylic group of lysozyme to the gold. The use of protein lysozyme as the capping agent gives the particles a biocompatible and hydrophilic surface, which allows their potential applications in biological and medical fields. The formation mechanism of the lysozyme monolayer-stabilized gold nanoparticles and the TEM image of obtained gold particles were shown in Figure 7.

Before aging under ambient conditions, the lysozyme-Au NPs aqueous solution is wine-colored and transparent, as shown in the photo 1 of Figure 8A. Interestingly, after the lysozyme-Au NPs aqueous solution was aged at room temperature for about 1 week, some red flocculent, fibrous material formed at the bottom of the solution, and could be seen with the naked eye (photo 2 of Figure 8A). Analysis of the red flocules by Field-emission scanning electron microscopy revealed that the wirelike products were tubular in nature, as observed by the clear contrast between the light periphery and the darker central part (Figure 8B). Hydrogen bonding between the carboxylic groups of lysozymes plays a key role in the self-assembly of lysozyme molecules and the formation of...
lysozyme microtubes. It is likely that partially unfolded lysozyme molecules on the Au NPs seed the formation of lysozyme microtubes. Moreover, the unusual formation of lysozyme microtubes in the lysozyme-Au NPs aqueous solution implies that bare Au NPs may be dangerous to organisms if they are present in organisms for a relative longer time because there is a possibility that they might induce the aggregation of proteins in the organisms, which is often associated with a range of human diseases including Parkinson’s disease, Alzheimer’s disease, and type 2 diabetes. So, special care should be taken when bare Au NPs are introduced in physiological environments. It is obvious that the spontaneous formation of lysozyme microtubes via the self-assembly process is a facile, effective, and economic method to produce protein microtube structure, which may not only have potential applications in biomedical fields but also provide new inspiration for protein study.

\[
\text{HAuCl}_4 + \text{NaBH}_4 \xrightarrow{\text{Reduction}} \text{Au} \\
\]

**Fig. 7.** The scheme of the formation of lysozyme-stabilized gold nanoparticle (up) and the TEM image of the as-formed gold particles (bottom).
Fig. 8. (A) The photographs of the lysozyme-Au NPs aqueous solution before aging (1) as well as after 1 week of aging under ambient conditions (2), some red fibrous materials formed at the bottom of sample 2; (B) Field-emission scanning electron microscopy images of the lysozyme microtubes on a silicon wafer.

3. References


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The Application of Biomolecules in the Preparation of Nanomaterials


In all different areas in biomedical engineering, the ultimate objectives in research and education are to improve the quality life, reduce the impact of disease on the everyday life of individuals, and provide an appropriate infrastructure to promote and enhance the interaction of biomedical engineering researchers. This book is prepared in two volumes to introduce recent advances in different areas of biomedical engineering such as biomaterials, cellular engineering, biomedical devices, nanotechnology, and biomechanics. It is hoped that both of the volumes will bring more awareness about the biomedical engineering field and help in completing or establishing new research areas in biomedical engineering.

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