# MICROSTRUCTURE AND PROPERTIES OF ALLOYED SILVER-GOLD NANOPARTICLES

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## Abstract

*Alloyed silver-gold nanoparticles recently raised an interest in biomedicine as potential antibacterial and surface-functionalized agents for imaging, drug-delivery, and tumor thermo-therapy [1,2]. The here synthesized alloyed AgAu nanoparticles with different compositions of silver and gold, as determined by atomic absorption spectroscopy (AAS), were prepared by reduction with citrate and tannic acid in aqueous media and subsequently functionalized by the addition of polyvinylpyrrolidone (PVP) [3]. UV spectroscopy confirmed that the particles consisted of alloyed Ag:Au and are not of a separate core-shell structure. The resulting nanoparticles were monodisperse and had a uniform size of ~6 nm, except pure Ag and Ag:Au-90:10, as shown by differential centrifugal sedimentation (DCS) and transmission electron microscopy (TEM). By means of X-ray powder diffraction (XRD) and use of Rietveld refinement [4], the precise lattice parameters, crystallite size and microstrain were determined. Based on the results by XRD, DCS and TEM it was shown, that the nanoparticles were not twinned, except pure Ag and Ag:Au-90:10. Additionally, a distinct deviation from Vegard's linear rule of alloy mixtures for the lattice parameter was found for the nanoparticles. This effect was also found for AgAu bulk materials, but was much more pronounced in the nanostate. Further investigations of the crystal structure of the alloyed nanoparticles by means of synchrotron radiation might be helpful to gain more information about the interactions of silver and gold atoms.*

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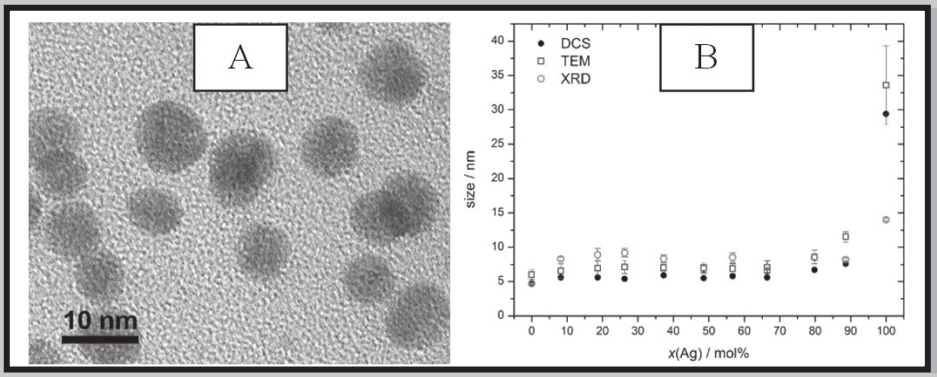


**FIG. 1. (A) Representative TEM image of Ag:Au-40:60 nanoparticles, stabilized with PVP and (B) average particle size from DCS (hydrodynamic diameter), TEM (metallic core), and XRD (crystallite size).**

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# CALCIUM PHOSPHATE NANO-PARTICLES FOR DELIVERING SYNTHETIC DRUG MOLECULES ACROSS THE CELL MEMBRANE

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## Introduction

Many synthetic drug molecules have their targets sites inside the cells. Typically, large molecules are not able to cross the cell membrane on their own and in order to bring them across it, an efficient carrier is needed [1]. We have loaded calcium phosphate nanoparticles with different synthetic drug molecules, i.e. a polyfunctional anionic polymer, a cationic calixarene dimer and a molecular tweezers. A polyfunctional anionic polymer was developed for selective inhibition of lysozyme as a model of enzyme inhibition [2]. A calixarene dimer due to its chemical and topological characteristics has the ability to specifically bind to the major groove of the DNA molecule that result in cell death [3]. Molecular tweezers inhibit the specific protein-protein interactions that lead to the formation of amyloidogenic aggregates inside the cells[4]. These aggregates are the cause of multiple incurable diseases, for example, Alzheimer's disease, Parkinson's disease and type-2 diabetes [5].

## Materials and methods

Calcium phosphate nanoparticles were prepared by rapid precipitation, followed by functionalization with drug molecules. The polyfunctional anionic polymer and the cationic calixarene dimer were highly charged and therefore able to colloidally stabilize the nanoparticles. In the case of the molecular tweezers, first the cationic polymer polyethyleneimine (PEI) was adsorbed onto the nanoparticle surface, and then the molecular tweezers themselves. Afterwards, all particles were ultracentrifuged to separate them from dissolved counter-ions and non-adsorbed molecules and subsequently redispersed in pure water.

All nanoparticle dispersions were characterized by dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), and scanning electron microscopy (SEM). By means of quantitative UV spectroscopy the amount of the fluorescing synthetic molecules on the nanoparticles was estimated.

The cell experiments were carried out on the HeLa cell line. The cells were incubated with the drug-loaded nanoparticles as well as with controls (the same concentration of the drug molecules dissolved in water, but without calcium phosphate nanoparticles). For quantifying the viability of the cells after incubation the MTT test was performed. Light and fluorescence microscopy along with confocal laser scanning microscopy were used to determine the uptake efficiency.

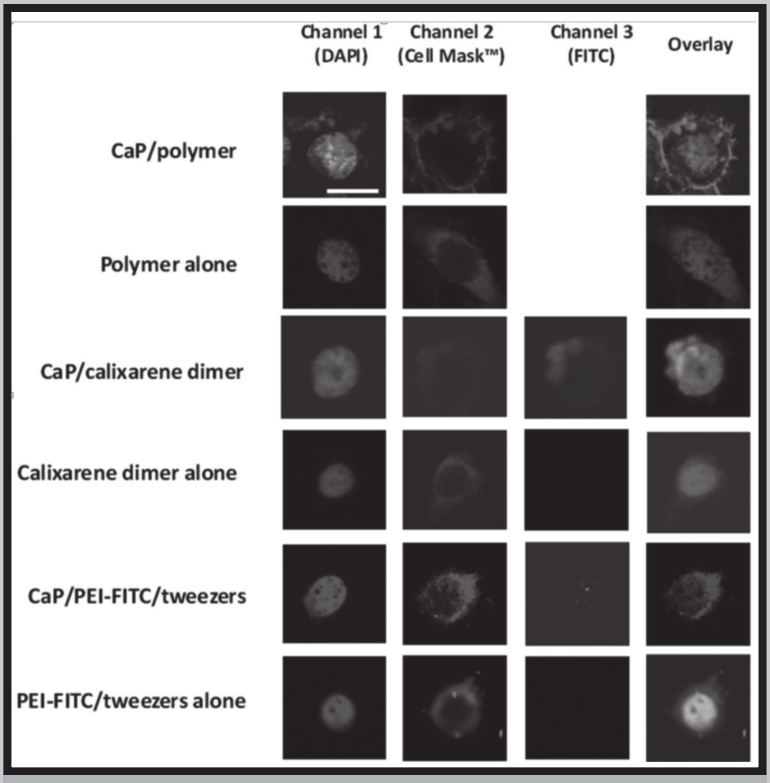


**FIG. 1. Confocal laser scanning microscopy micrographs of HeLa cells after 3 h incubation with functionalized calcium phosphate nanoparticles and with the dissolved drug molecules. The blue channel represents the cell nucleus (DAPI) and the polymer, the red channel represents the cell membrane stained with Cell MaskTM, and the green channel represents the calixarene dimer and FITC-PEI/tweezers. Scale bar is 5 μm.**

## Results

The functionalized nanoparticles had spherical morphology with the size of 150-200 nm. The UV-spectroscopy data showed that, the amount of adsorbed drug molecules on the nanoparticles was between 22 and 51% of the initially present amount of drug molecules. The MTT test showed no toxic effects for the cells after interaction with the drug-loaded nanoparticles as well as with dissolved molecules, except for the ones with calixarene dimer. These results are in accordance to the purpose of the used molecules. The results of the uptake investigations (FIG. 1) had shown that together with calcium phosphate nanoparticles, all three drug molecules were easily detectable inside the cells, whereas the synthetic molecules alone were not taken up by cells [6].

## Conclusions

We have shown the loading of calcium phosphate nanoparticles with three chemically different synthetic drug molecules. Fluorescence microscopy and confocal laser scanning microscopy showed that the functionalized calcium phosphate nanoparticles were easily taken up by HeLa cells after three hours incubation, whereas the dissolved drug molecules were not able to penetrate the cell membrane. We conclude that drug-loaded calcium phosphate nanoparticles represent a suitable carriage system for such molecules into the cell where they can exert their therapeutic action.

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