Nanoparticles

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Electron Transfer in Peptides: On the Formation of Silver Nanoparticles**

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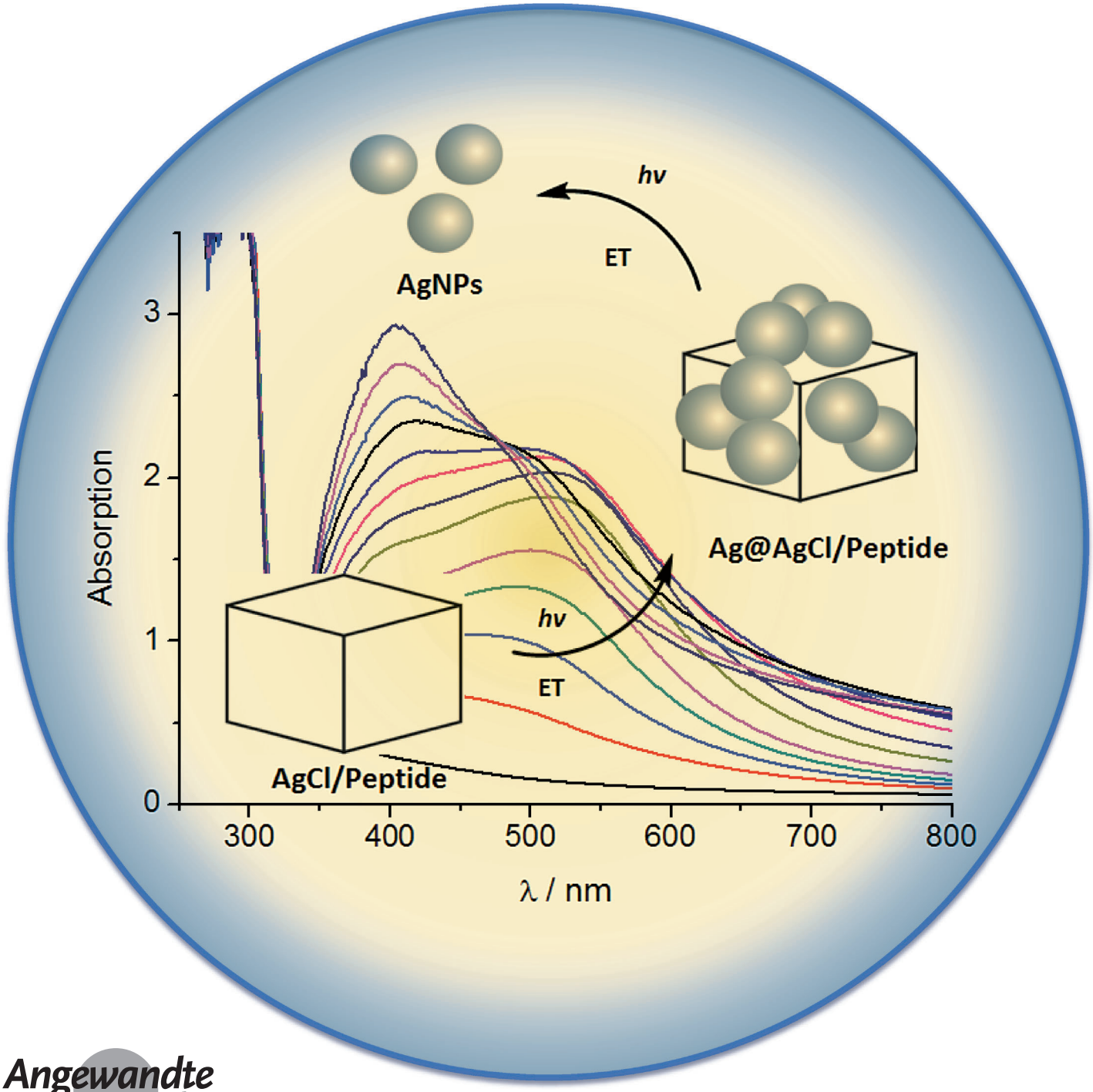
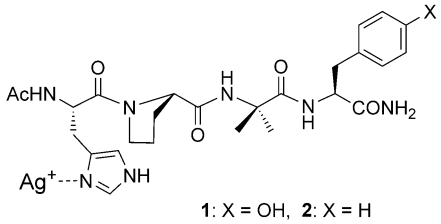


Abstract: *Some microorganisms perform anaerobic mineral respiration by reducing metal ions to metal nanoparticles, using peptide aggregates as medium for electron transfer (ET). Such a reaction type is investigated here with model peptides and silver as the metal. Surprisingly, Ag^+ ions bound by peptides with histidine as the Ag^+-binding amino acid and tyrosine as photoinducible electron donor cannot be reduced to Ag nanoparticles (AgNPs) under ET conditions because the peptide prevents the aggregation of Ag atoms to form AgNPs. Only in the presence of chloride ions, which generate AgCl microcrystals in the peptide matrix, does the synthesis of AgNPs occur. The reaction starts with the formation of 100 nm Ag@AgCl/peptide nanocomposites which are cleaved into 15 nm AgNPs. This defined transformation from large nanoparticles into small ones is in contrast to the usually observed Ostwald ripening processes and can be followed in detail by studying time-resolved UV/Vis spectra which exhibit an isosbestic point.*

Anaerobic microorganisms are able to reduce extracellular metal ions and generate nanoparticles (NPs) or precipitates outside of the cell and far away (μm distance) from the outer cell membrane.[1,2] Such redox reactions between electron-accepting metal ions outside of the cell and metabolic processes in the inner cell membrane can be enabled by peptide filaments (pili). These are assemblies of peptides[1–3] that bind metal ions[4] and assist electron transfer (ET) over long distances.[5] Studies on the mechanism of ET through peptides is a topic of current research.[5–7] The reduction of the peptide-bound metal ions to NPs is the next step of this biochemical process, and represents also a major challenge because redox potentials of metals at the atomic level are quite different from those of bulk material.[8] Even for a noble metal like silver, the reduction of a metal ion to a single silver atom is an endergonic reaction. Silver converts into a metallic state only after the aggregation of several atoms, hence when a minimum number of metal atoms within a cluster is reached.[8,9] Herein, we describe our findings that such aggregations of silver atoms and ions can be prevented by peptides. In these cases Ag^+-peptide complexes are not reduced to Ag^0-nanoparticles (AgNPs) in ET processes. After addition of chloride ions, however, the formation of AgNPs occurs in sequential reaction processes.

Based on earlier work of the Wennemers and Fromm groups[10] the Ag^+-peptide complex **1** was used (Scheme 1). The peptide contains histidine as a strong Ag^+-binding ligand



1: X = OH, **2**: X = H

Scheme 1. Silver-bound peptides Ac-L-His-L-Pro-Aib-L-Aaa-NH_2 **1** and **2**.

at the N-terminus,[4,11] and tyrosine as electron donor at the C-terminus.[12] NMR titration showed that the Ag^+/peptide ratio in DMSO is 3:1 and the changes of chemical shifts indicate that Ag^+ is bound to imidazole (Figure S1). Tyrosine was used as the electron donor because its irradiation generates electrons, which are then available for the ET process.

Laser irradiation of **1** at 308 nm gave rise to the transient UV/Vis spectra shown in Figure 1 A.[13] Already the first 20 ns laser flash generated tyrosyl radicals with their typical

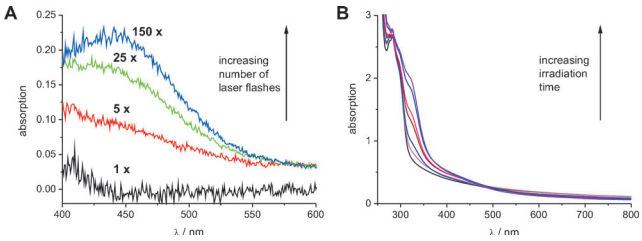


Figure 1. A) Laser flash photolysis of the Ag^+-peptide **1**; UV/Vis spectra were recorded 80 ns after the laser flash. The number of the 20 ns laser flashes is indicated. B) Irradiation of the Ag^+-peptide **1** with a continuous light source (Hg lamp) for up to 2 h.

absorption at 410 nm demonstrating that tyrosine has functioned as electron donor.[12,14] Additional laser flashes on the same sample led to spectra with a band of $\lambda_{max} = 430$ nm that grew with increasing number of laser flashes. This absorption can be misinterpreted as a plasmon resonance of AgNPs,[15] but laser irradiation of the peptide in the absence of silver ions, and even irradiation of tyrosine alone, generated nearly the same transient UV/Vis spectrum (Figure S2). This clearly demonstrates that the absorption at 430 nm is not caused by AgNPs!

Continuous irradiation of **1**, of the peptide without silver ions, and of tyrosine alone with a Hg lamp for two hours led to UV/Vis spectra showing the evolution of a shoulder next to the tyrosine absorption band (Figure 1 B),[16] which corresponds to tyrosyl radical dimers.[17] Indeed, laser irradiation experiments of the synthetized dimers generated a similar 430 nm absorption, which could be quenched within 2 μs by

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O_2. This is typical for triplet–triplet transitions of biaryl systems.[18] Also the irradiation of Ag^+-peptide complex **2**, in which the peptide carries phenylalanine instead of tyrosine, mixed with additional tyrosine, yielded only the dimerization products of tyrosyl radicals. In all these experiments, no AgNPs were observed during the two hours of irradiation.

Our experiments clearly demonstrate that intramolecular and intermolecular ET from photoexcited tyrosine to Ag^+-peptide complexes **1** and **2** does not lead to AgNPs! In order to understand these experiments one has to bear in mind that the redox potential of Ag atoms in water is −1.8 V, and thus Ag atoms are unstable compared to the Ag^+/e^- couple.[8,9] Only if the silver atoms aggregate, does the reaction become exergonic (+0.8 V).[8,9] Apparently, the shielding effect of the peptides can inhibit this aggregation. Hence, for the synthesis of AgNPs these aggregation steps have to be facilitated and enabled. This turns out to be possible if the solutions either contain preformed AgNPs or chloride ions.

In the presence of minor amounts of AgNPs induced by, for example, partial chemical reduction of silver ions with $NaBH_4$ (Figure 2A), irradiation of **1** led to AgNPs with a characteristic plasmon resonance at 415 nm.[19] In this case, already the first aggregation step, the addition of an Ag^0 atom to the AgNP, is exergonic as large silver aggregates are formed. An alternative method leading to AgNP generation from Ag^+-peptide complex **1** by ET is the addition of chloride ions.[20] Figure 2B–D demonstrate that under these conditions the formation of NPs follows a completely different mechanism; two distinct particle types, **NP-3** and **NP-4**, are formed which are characterized by their respective plasmon resonances at about 520 and 410 nm. The maximum of the absorption band of **NP-3** shifted slightly to longer wavelengths when the pH value (Figure 2B,D) or the Cl^-/Ag^+ ratio (Figure 2C,D, pH 12.5) was increased.

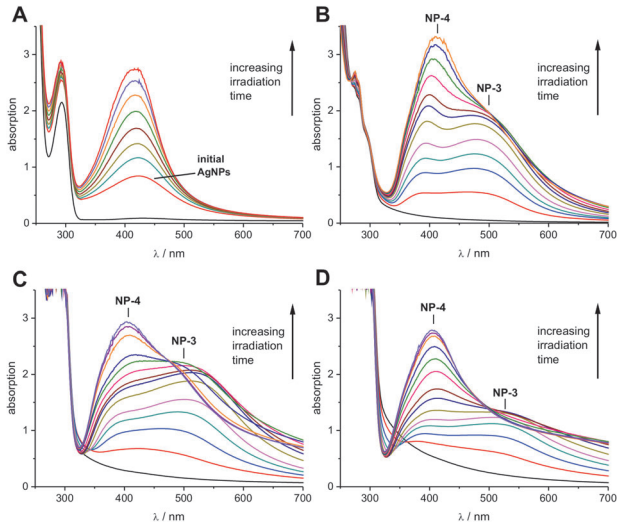


Figure 2. Irradiation of Ag^+-peptide complex **1** with a Hg lamp (cutoff filter 360 nm; lowest, black trace at $t = 0$ min; the colored traces are the UV/Vis spectra at different times): A) Irradiation after addition of 2 mol% $NaBH_4$ that produced an initial concentration of AgNPs at pH 12.5; UV/Vis spectra recorded at time intervals of 30 min; B) irradiation after addition of 12 equiv NaCl at pH 8.5; C) irradiation after addition of 1 equiv NaCl at pH 12.5; D) irradiation after addition of 12 equiv NaCl at pH 12.5. The UV/Vis spectra for (B–D) were recorded after 0.5, 1, 1.5, 2, 3, 4, 5, 7.5, 10, 15, 30, 45, and 60 min.

At the beginning of the ET process, both **NP-3** and **NP-4** were formed simultaneously. At the isosbestic point the formation of **NP-3** was complete, and the conversion of **NP-3** into **NP-4** was the only remaining reaction. The generation of **NP-3** took 5–10 min whereas its transformation into **NP-4** was much slower (50–60 min, Figure 2C,D). Both reactions could be fitted by first-order kinetics. The corresponding data from Figure 2C showed that the formation of **NP-3** is about 8 times faster than its transformation into **NP-4** (Figure S3). In order to understand this consecutive reaction process we analyzed the particles by transmission electron microscopy (TEM), static light scattering (SLS) and powder X-ray diffraction. TEM pictures at $t = 0$ min, and after 0.5, 4, and 30 min irradiation demonstrated a surprising decrease of the particle sizes and narrowing of their size distribution (Figure 3A–D).

Powder X-ray experiments confirmed that the addition of chloride ions to **1** generated AgCl microcrystals, which were imbedded in the peptide matrix (Figure 3A). The polydispersity of the AgCl-peptide particles depended on the conditions of their formation. At pH 8.5 they showed a narrow size distribution with an average diameter of 110 nm (Figure S4), whereas at pH 12.5 a much higher polydispersity was observed with particles up to 400 nm in diameter (Figure 3A).[20] The differences in the size distributions influenced the light scattering patterns of the AgCl-peptide microcrystals, which could be detected by SLS experiments (Figure S4) and UV/Vis spectroscopy (Figure 2B–D, $t = 0$ min).[21] A size increase of the AgCl-peptide particles increased the UV/Vis absorption intensity between 300 and 600 nm. In some cases this scattering effect was so pronounced that the ET-induced decrease of the AgCl-microcrystals could be detected by UV/Vis spectroscopy together with the increase of **NP-3**, and the transformation of **NP-3** to **NP-4** (Figure 2D).

TEM analysis showed that after 30 s of irradiation **NP-3** with an average diameter of about 100 nm were formed (Figure 3B). Further irradiation converted **NP-3** into the smaller **NP-4** with a size of roughly 15 nm Figure 3C), which were the final products (Figure 3D). Powder X-ray diffraction proved that **NP-4** are AgNPs where the silver ions had been completely reduced to Ag^0. A similar consecutive reaction sequence was observed in photoreactions of Ag^+-peptide complex **2** after addition of tyrosine and chloride ions. But in the absence of tyrosine no photoreduction occurred although AgCl was present (Figure S5). This proves that AgCl is not the electron donor. Furthermore, irradiation experiments with the tyrosine-containing Ag^+-peptide complex **1** in the presence of NaCl yielded its dimers, demonstrating that tyrosine acts as an electron donor also in the presence of AgCl. The composition of **NP-3** was elucidated by TEM EDX-mapping and revealed oxygen (from the peptide), silver, and chlorine (Figure S6). Powder X-ray diffraction determined that **NP-3** consist of only crystalline Ag^0 as well as AgCl (Figure S7).

Irradiation of independently synthesized cubic AgCl-peptide microcrystals (Figure 4A) led also to **NP-3**.[22] Within 30 s of ET the surface of AgCl-peptide microcrystals was

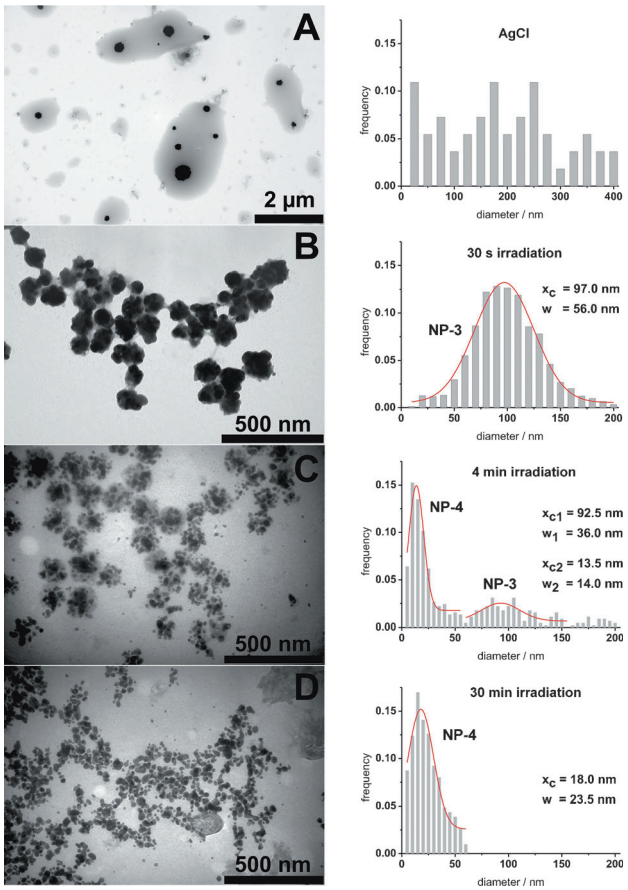


Figure 3. TEM pictures (Ag^+-peptide complex **1**/Cl^- = 1:1, pH 12.5, see Figure 2C) after different irradiation times and the corresponding NP size distribution (analysis of 55–2100 particles; x_c = mean average diameter, w = half width). A) t = 0 s; B) t = 30 s; C) t = 4 min; D) t = 30 min.

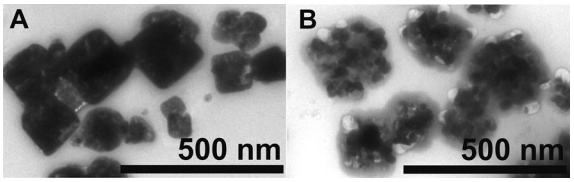
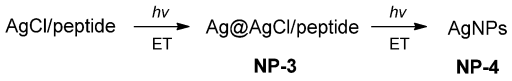


Figure 4. TEM pictures. A) Cubic AgCl-peptide microcrystals. B) Reduction of the cubic AgCl-peptide microcrystals (Hg lamp, 100 mW, 30 s irradiation) to Ag@AgCl-peptide nanocomposites.

covered by small, roundish AgNPs, which is typical for Ag@AgCl nanocomposites (Figure 4B).[23] Apparently, ET reduces Ag^+ ions at the surface of AgCl-peptide microcrystals to Ag^0 atoms that are long-lived enough to recombine with adjacent silver atoms and yield AgNPs. Even if the Ag atoms are generated far away from each other, ET through the crystal can lead to adjacent Ag atoms and hence cluster growth.[24] SLS measurements showed that during this partial reduction of AgCl-peptide NPs into Ag@AgCl/peptide nanocomposites **NP-3** the overall size decreased by 10% (Figure S8). This might be caused by the release of chloride from AgCl-peptide particles into water during the formation of **NP-3**. Such a chloride migration from the NP to the surrounding water should be slowed down in the subsequent reduction step **NP-3** to **NP-4**, as AgCl in the composites **NP-3** is covered by AgNPs. This could explain why the formation of **NP-3** is faster than its conversion into **NP-4.**

Another interesting feature of the Ag@AgCl nanocomposites **NP-3** is the narrow size distribution shown in Figure 3B although their precursors, the AgCl microcrystals, are highly polydisperse (Figure 3A). Molecular orbital calculations had demonstrated that AgNPs are stabilized by AgCl surfaces.[25] This allows aggregations of AgNPs in the composites **NP-3** although the peptide matrix favors smaller NPs (**NP-4**). These opposing interactions might lead to an equilibrium size of about 100 nm for the nanocomposites **NP-3** (Figure 3B). The sharp, reproducible isosbestic points in Figure 2C,D and the kinetic analysis provide evidence for the consecutive reactions shown in Scheme 2 where large NPs are converted into small ones. This is a unique experimental case for a hypsochromic shift of plasmon resonances with a sharp isosbestic point.



Scheme 2. ET-induced consecutive reaction of AgCl-peptides via Ag@AgCl/peptide nanocomposites **NP-3** into their AgNPs components **NP-4**.

Our experiments demonstrate that the silver ions of an Ag^+-peptide complex, where histidine is the metal-binding amino acid, cannot be transformed into AgNPs by long-distance ET because the aggregation of Ag^0 atoms is prevented by the peptide.[26] Chloride ions, however, which are ubiquitous in biological systems, can facilitate the ET-induced synthesis of AgNPs from Ag^+-peptides by assembling silver ions into AgCl microcrystals.[27] In addition, small amounts of AgNPs generated by reducing molecules[28] might also be at the start of the microbiological formation of AgNPs.

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[20] A 1.25 mM solution of Ac-L-His-L-Pro-Aib-L-$TyrNH_2$ with 0.33 equiv $AgNO_3$ and NaCl in MiliQ water was irradiated with an Osram Hg lamp HBO 500 W/2 on a Thermo Oriel irradiation equipment in the presence of a 360 nm cut-off filter. The experiments were carried out at a pH-value of 6.5, 7.5, 8.5, 9.5, 10.5, 11.5, and 12.5. Due to the deprotonation of tyrosine with increasing pH the conformation of the tetrapeptide changes (shown in CD spectra). Therefore changes in the size and reactivity of the particles are expected.
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