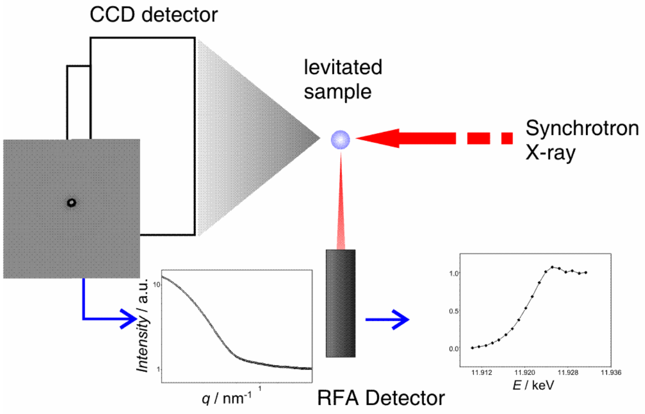
S1

Small-Angle X-ray Scattering (SAXS)/X-ray Absorption Near Edge Spectroscopy (XANES).

The combined SAXS/XANES measurements were carried out at the µSpot beamline at BESSY II (Berlin, Germany). The beamline with the focusing scheme is designed to provide divergence < 1 mrad (horizontally and vertically) and beam diameter of about 100 µm at a photon flux of 5×10^9 s^{-1} at a ring current of 100 mA.

The SAXS experiment was conducted continuously parallel to the XANES measurements, whereas the small shift of the wavelength necessary for the XANES measurement is negligible concerning the evaluation of the SAXS data. The SAXS data were processed and converted from 2d scattering images into 1d diagrams of scattered intensities versus scattering vector *q* employing the computer program FIT2D. The scattering vector is defined using the scattering angle θ and the wavelength λ of the radiation: thus $q = 4\pi / \lambda \sin(\theta / 2)$. The obtained data were corrected for background scattering. For the curve fitting SANS Analysis 3_v3.00 implemented in the software suite IGOR was used.

The parallel XANES measurement was performed by fine tuning the energy of the incoming photons in steps of 1.2 eV over the region around the Au LIII-edge (11.919 keV) between 11909 eV to 11933 eV using a high-resolution double crystal monochromator and measuring the fluorescence radiation by means of Si drift detector (KETEK, model AXAS) at a working distance of 30 mm. The measurement time per XANES scan was chosen due to the rate of the observed reaction and the needed statistics (relative error less than 1%). To achieve the desired time resolution only a small energy range was scanned. The fluorescence radiation emitted from the sample was detected at an angle of ca. 90° with respect to the X-ray beam. All XANES spectra were normalized to 0 at the lowest and to 1 at the highest used excitation energy. The oxidation state of the gold atoms during the process was modelled as superposition of the start and the final state. The curve fitting was performed using an routine written in IDL (Interactive Data Language, ITT VIS) which based on the downhill simplex method of Nelder and Mead.



S 1 Schematic of the experimental setup employed for SAXS and XANES analysis on droplets of the reaction solution during GNP formation.

SAXS is the common method to determine the shape and size of particles in solution. The scattering signal derives from the contrast between the growing nanoparticles and the solvent. When evaluating SAXS curves it is common to use model curves to fit the experimental scattering curves. A detailed review is given by Pedersen[39]. In our case the experimental scattering curves were fitted with a model which describes the scattered intensity of hard spheres having a Schultz-Zimm distribution. This approach is choosen since for high polydispersities a asymmetrical size distribution is reasonable and since any electron microscope image show spherical particles.

The Schultz-Zimm distribution is given by

$$f(r) = (z+1)^{z+1} x^2 \frac{\exp[-(z+1)x]}{R_{Avg}\Gamma(z+1)} \qquad (1)$$

where R_{Avg} is the mean radius, $x = r / R_{Avg}$, z is related to the polydispersity ($p = \sigma / R_{Avg}$) by $z = 1/p^2 - 1$m where σ^2 is the variance of the distribution.

The scattering intensity of non aggregated particles can be assumed to be proportional to the form factor of a single particle. Thus the scattering intensity of monodisperse hard spheres with volume V is given by

$$I(q,r) = scale \cdot P(qr) = scale \cdot \left[\frac{3V(\Delta\rho)(\sin(qr) - qr\cos(qr))}{(qr)^3} \right]^2 \quad (2)$$

where *P*(*qr*) is the form factor of a single hard sphere and $\Delta\rho$ the scattering length density. In case of polydisperse spherical particles one has to sum the scattering intensities over all particle sizes weighted by their frequency or to integrate using a size distribution function, respectively. It is common to use the Schulz-Zimm distribution for polydisperse particles[40]. Hence the scattering intensity is given by

$$I(q) = scale \cdot \int_0^\infty f(r)P(r)dr \quad (3)$$

An analytical solution of that integral can be found in Kotlarchyk et al.[41].

In order to analyze the growth mechanism of nanoparticles the number of particles is important information. This can be obtained by using a general relation of *I*(*q* = 0) for a single particle which is independent of its shape and size, i.e. $I_s(q=0) = (\Delta\rho)^2 V^2$. Thus the scattered intensity *I*(*q* = 0) of polydisperse particles can be written as

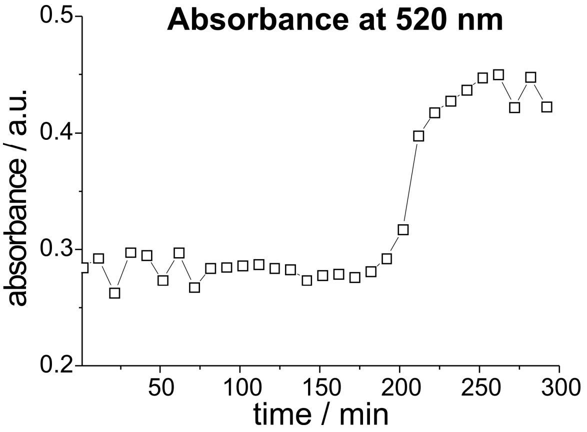
$$I(q=0) = N\left\langle V^2 \right\rangle (\Delta\rho)^2 \quad (4)$$

where *N* is the number of particles and $\left\langle V^2 \right\rangle$ the mean value of V^2. *I*(*q* = 0) can not be measured directly due to the overlapping of the scattering intensity with the primary beam, thus I(q = 0) is only accessible via the extrapolation of I(q) for q $\rightarrow$ 0.

S2

UV-vis results of the synthesis of gold nanoparticles at lower $HAuCl_4$ concentration

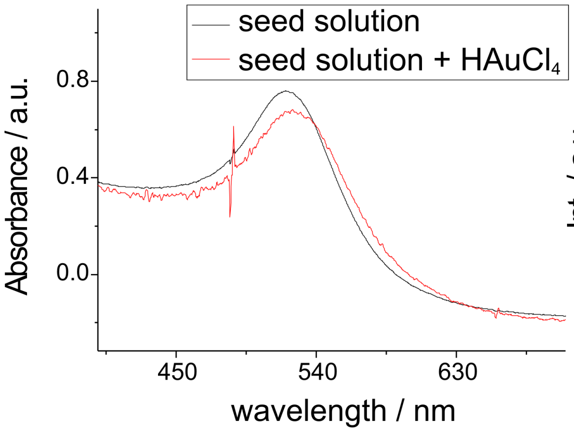
For the synthesis of GNP at four times lower concentration of gold precursor, 35 ml of aqueous sodium citrate solution (5 mM) and 35 ml of aqueous $HAuCl_4$ solution (0.125 mM) were preheated to 65°C, mixed, and the reaction progress followed by UV/vis.



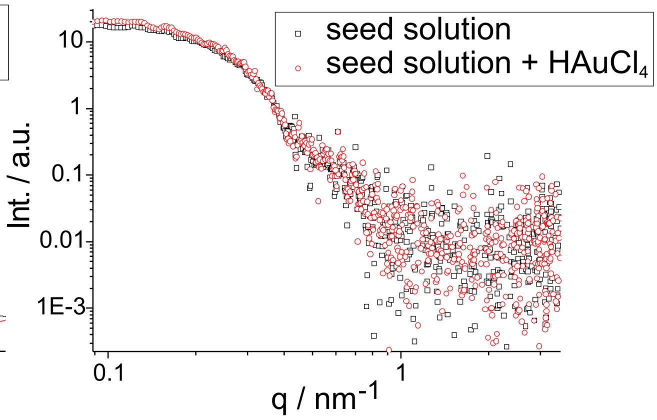
S3

Addition of further precursor solution to the colloid carried out at room temperature, and analyzed by UV-vis and SAXS before and after adding aqueous $HAuCl_4$ solution

UV-vis spectra

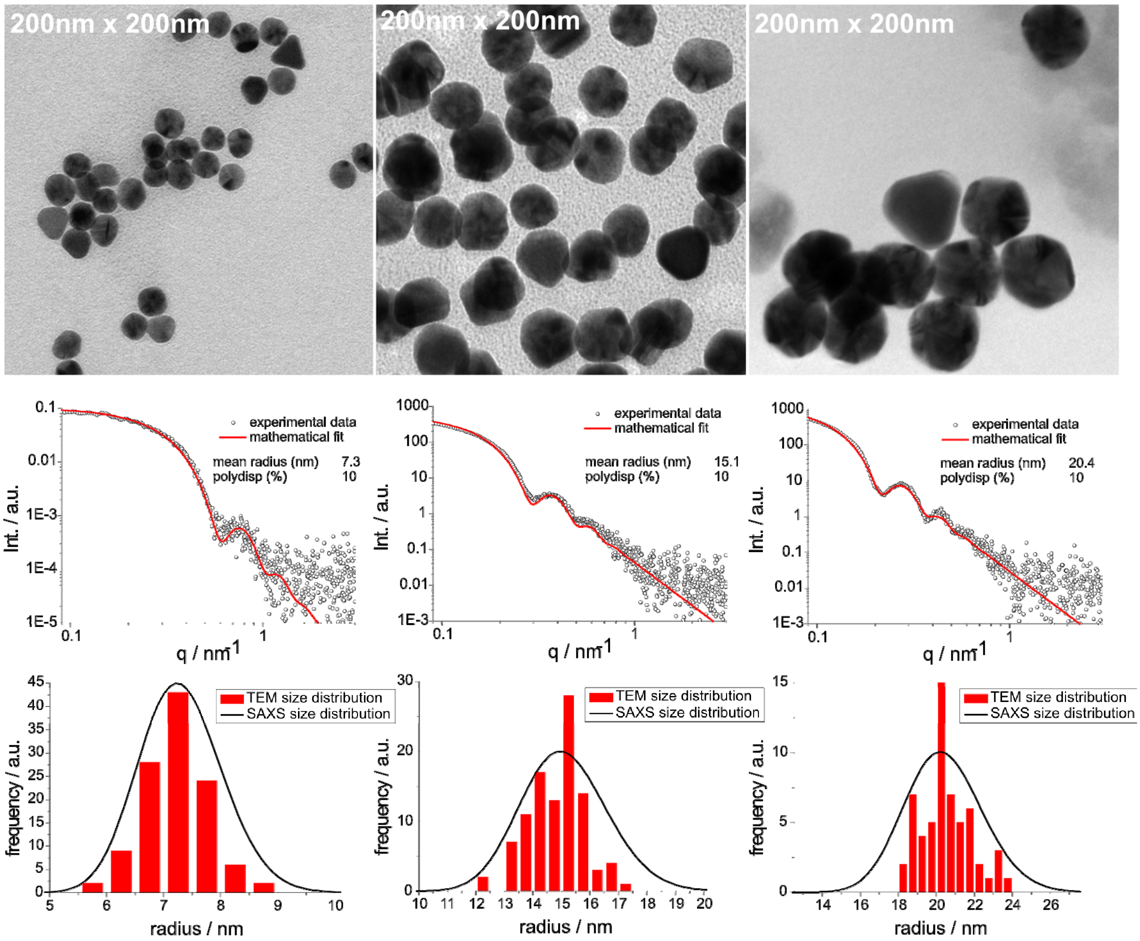


SAXS scattering curves



TEM

Transmission electron measurements (TEM) were acquired on a Zeiss EM Omega 912X at an acceleration voltage of 120 kV. For the sample preparation a drop of a colloidal solution was deposited on a carbon coated copper grid and let to dry. Typically the size of 100 particles were measured for each growth step.



Upper row: TEM images of three different steps of the seeded growth process in comparison with the respective SAX data (middle row). Lower row: comparison of the size distribution derived from TEM and SAXS measurements.