ChemComm

COMMUNICATION

Electronic Supplementary Information (ESI):

Using the dendritic polymer PAMAM to form gold nanoparticles in the protein cage thermosome

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Experimental details:

Materials:

A thermosome (THS) mutant carrying one cysteine residue on the inside surface of each beta-subunit and no other surface-exposed cysteines was recombinantly expressed and purified as reported elsewhere.[1] Poly(amidoamine) (PAMAM; ethylenediamine core, generation 4.0, 10 wt.% solution in methanol) and gold(III) chloride ($HAuCl_4$; 30 wt.% solution, 99.99% purity) were obtained from Sigma Aldrich. The heterobifunctional linkers succinimidyl-6-hydrazinonicotinamide acetone hydrazone (S-HyNic) and maleimido trioxa-6-formylbenzamide (MTFB), needed for the conjugation of THS and PAMAM, were purchased from Solulink (San Diego, CA, USA). MilliQ water was used for all experiments. All other chemicals were purchased from Sigma-Aldrich. Regents were used as received unless otherwise stated.

THS-PAMAM conjugation:

Conjugates of THS and PAMAM were prepared and purified according to our previously published protocol.[2] For the modification of THS with MTFB, 10 µM THS was reacted with 2mM of MTFB in 100 mM sodium phosphate pH 7.5, 150 mM NaCl for 2.5 h at RT under shaking. Subsequently, the buffer was exchanged to 100 mM sodium phosphate pH 6.5, 150 mM NaCl. This was done by at least five concentration–dilution cycle with Amicon centrifugal filters (MWCO: 100 kDa). 500 µM PAMAM was reacted with a tenfold excess of S-HyNic (5mM) in 100 mM sodium phosphate pH 6.5, 150 mM NaCl at RT for 2 h under shaking. The unreacted excess of S-HyNic was separated with Amicon centrifugal filters (MWCO: 10 kDa). The conjugation of protein and polymer was done by reacting 10 µM THS-MTFB (10µM) with 500 µM HyNic-PAMAM. This reaction was done in 100 mM sodium phosphate pH 6.5, 150 mM NaCl at RT overnight under shaking. The excess of HyNic-PAMAM was seperated from THS-PAMAM by size exclusion chromatography (HiPrep 16/60 Sephacryl S-200 column) in SEC buffer (20 mM Tris/HCl pH 7.5, 100 mM NaCl, 1 mM EDTA, 0.02% NaN_3). Finally, Amicon centrifugal filters (MWCO: 100 kDa) were used to exchange the buffer to 20 mM Tris/HCl pH 9.0, 200 mM NaCl, in which the AuNP formation was performed. On average, about 3.8 PAMAM dendrimers per THS were found. This was determined by UV/Vis spectroscopy.

AuNP formation in THS-PAMAM:
All reactions were conducted at room temperature. Low gold concentration (110 µm $HAuCl_4$) protocol to form AuNPs: 10 µl of a 550 µM (= 55 eq.) $HAuCl_4$ solution in 20 mM Tris/HCl buffer (pH 9.0, 200 mM NaCl) were added to 30 µl of a 3.3 µM (= 1 eq.) PAMAM, THS or THSPAMAM solution in the same buffer. The mixture was incubated for 45 min under gentle shaking. Afterwards, 10 µl of a 2.75 mM (= 275 eq.) $NaBH_4$ solution in 20 mM Tris/HCl buffer (pH 9.0, 200 mM NaCl) was added (final concentration: 2.0 µM THS-PAMAM, 550 µM $NaBH_4$ and 110 µM $HAuCl_4$). The reaction mixture was incubated for another 45 min under gentle shaking. $NaBH_4$ solution was always prepared minutes before the experiment. To stop the reaction, the buffer was exchanged to PBS with centrifugal filters (MWCO: 3 kDa for PAMAM, MWCO: 100 kDa for THS-PAMAM; Amicon Ultra, Merck Millipore, USA). To remove Au aggregates, the samples were filtered with syringe filters (0.22 µm, Millex PVDF; Merck Millipore, USA). For the high gold concentration (1.1 mM $HAuCl_4$) protocol, ten-fold higher $HAuCl_4$ and $NaBH_4$ concentrations were used. In the repetitive protocol, the low gold concentration protocol was repeated 5 times including the filtration steps, but with an incubation time of the sample with $HAuCl_4$ of 15 min.

Methods:
UV/Vis spectra were measured on a NanoDrop 2000c (Thermo Scientific, USA) and smoothened with Prism 6 (GraphPad Software, USA). The smoothing was done with the 15 neighbors on each size and a 6^{th} order polynomial. For TEM imaging 5 µl of 20 nM protein or 80 nM PAMAM solutions were deposited for 60 s on a glow-discharged grid, washed twice with ddH_2O and negatively stained twice with 1% uranyl acetate solution. The samples were imaged with a FEI Morgagni 268 D TEM at an accelerating voltage of 80 kV. TEM images of AuNP formed in THS-PAMAM were analyzed using the software ImageJ 1.48v (National Institutes of Health, USA). The diameters of AuNP were manually measured. For the low gold concentration protocol 486 THS-PAMAM and 11 AuNP particles were analyzed, for the high gold concentration protocol 481 THS-PAMAM and 75 AuNP particles were analyzed and for the repetitive protocol 70 THS-PAMAM and 53 AuNP particles were analyzed. For Figure 1a, the cryo-electron microscopy structure of the wild-type group II chaperonin from *Methanococcus maripaludis* (Mn-Cpn; PDB: 3IZH)[3] in an open conformation was rendered using UCSF Chimera 1.8.1.[4] No structural data of the *Thermoplasma acidophilum* THS in open conformations is available in the databases. The structure of Mn-Cpn is, however, in good agreement with published cyro-EM data of the THS.[5]

Supplementary data:

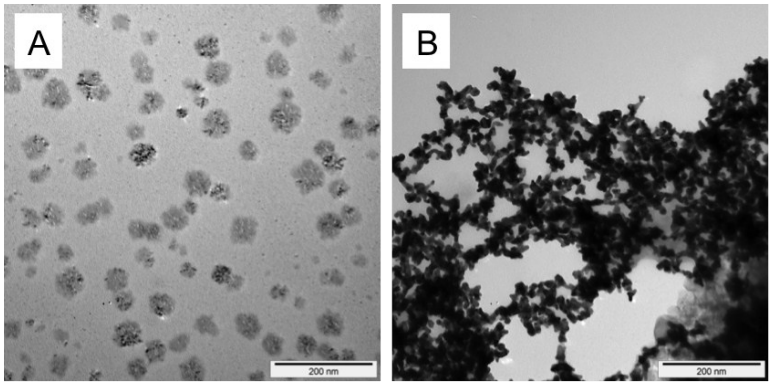

Fig. S1: TEM image of AuNPs formed in presence of PAMAM with low gold concentration (110 µM $HAuCl_4$) protocol (A) and AuNP formed without PAMAM or THS-PAMAM (not filtered) (B).

References:

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