Electronic Supplementary Material (ESI) for Nanoscale Advances.
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# Electronic Supplementary Information (ESI)

## Impurities in Polyvinylpyrrolidone: the key factor in the synthesis of gold nanostars

Patricia Taladriz-Blanco[1,3], Miguel Spuch-Calvar[1,#], Anselmo del Prado[1], Christoph Weder[1], Barbara Rother-Rutishauser[1], Alke Petri-Fink[1,2,]* and Laura Rodriguez-Lorenzo[1,3,]*

[1]Adolphe Merkle Institute, University of Fribourg, Chemin des Verdiers 4, Fribourg, CH-1700, Switzerland.

[2] Chemistry Department, University of Fribourg, Chemin du Musée 9, Fribourg, CH-1700, Switzerland

[3] International Iberian Nanotechnology Laboratory (INL), Water Quality group, Av. Mestre José Veiga s/n, 4715-330 Braga, Portugal.

**Present Addresses**

† Departamento de Química Orgánica, Facultad de Ciencias, Universidad Autónoma de Madrid, 28049 Madrid, Spain.

# Department of Applied Physics, Department of Physical Chemistry, Singular Center for Biomedical Research (CINBIO), Southern Galicia Institute of Health Research (IISGS) and Biomedical Research Networking Center for Mental Health (CIBERSAM), Universidade de Vigo, 36310 Vigo, Spain.

E-mail: alke.fink@unifr.ch, laura.rodriguez-lorenzo@inl.int

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## Materials and Methods

Gold (III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$, ≥99.9%), sodium citrate tribasic dihydrate (NaCit, $C_6H_5Na_3O_7$ $2H_2O$, ≥98%), N,N-dimethylformamide (DMF, anhydrous, 99.8%), sodium borohydride ($NaBH_4$, ReagentPlus®, 99%), polyvinylpyrrolidone (PVP, $M_w$ = 10k, Lot BCBM0097V and Lot BCBG5331V; $M_w$= 40 kDa, Lot WXBC7054V), diethyl ether (ACS reagent, ≥ 98.0%), 1,4-dioxane (ACS, ISO), 2,2′-azobis(isobutyronitrile) (AIBN, $(CH_3)_2C(CN)N{=}NC(CH_3)_2CN$, 98%), and hydrazine (35 wt.% solution in water) were purchased from Sigma-Aldrich. Polyvinylpyrrolidone (PVP, $(C_6H_9NO)_n$, $M_w$ = 10 kDa, Lot 48NUD-DE) was supplied by TCI chemicals and Polyvinylpyrrolidone (PVP, $(C_6H_9NO)_n$, $M_w$ = 8 kDa, Lot 41626) from Alfa Aesar. Deuterium oxide ($D_2O$, 99.9 atom % D) and chloroform deuterated (chloroform-d, 99.8 atom % D) were supplied by Sigma-Aldrich. Ethanol (EtOH) was obtained from VWR Chemicals. AIBN was used after recrystallization in methanol, and Milli-Q grade water was used for all preparations.

**Synthesis of PVP-AIBN.** PVP-AIBN was prepared by free radical polymerization in 1,4-dioxane at 60 °C using azobisisobutyronitrile (AIBN) as an initiator. The total concentration of monomer and AIBN was 1 M and 0.015 M, respectively. Reactions were carried out in the absence of oxygen by gently bubbling nitrogen for 20 – 30 min before sealing the reaction flask and heating the reaction mixture to 60 °C. After stirring for 24 hours, the reaction mixture was poured into diethyl ether, and the resulting precipitate was collected by filtration and dried under vacuum overnight.

**Purification of PVP.** PVP from TCI (PVP-TCI-10k, $M_w$ = 10 kDa, Lot 48NUD-DE) and PVP from Sigma (PVP-Sigma-40k, $M_w$= 40 kDa, Lot WXBC7054V) were purified by ether precipitation. Briefly, 3 g of PVP were dissolved in 10 mL of chloroform. Then, diethyl ether was added to the mixture (≈ 600 mL) observing the formation of a precipitated. The precipitated PVP was vacuum-filtrated and dried using a vacuum oven at 60°C. The soluble fraction was concentrated by removing all the solvent using a rotavap, and the white precipitate was freeze-dried.

**Modification of purified PVP-TCI-10k with a hydroxyl end group (PVP-TCI-OH).** 200 mg (5.3 mmol) of $NaBH_4$ were added to 1 g of commercial PVP from TCI (PVP-TCI-10k, $M_w$ = 10 kDa, Lot 48NUD-DE) previously dissolved in 10 mL of MeOH. After 15 hours of stirring at 25 °C, the reaction mixture was poured into diethyl ether and a white powdered precipitate was collected by filtration. The resulting polymer was dissolved in chloroform and reprecipitated in diethyl ether in order to eliminate residual $NaBH_4$.

**Modification of purified PVP-TCI-10k with a pyridine end group (PVP-TCI-Pyridine).** 0.1 g commercial PVP from TCI (PVP-TCI-10k, $M_w$ = 10 kDa, Lot 48NUD-DE) were dissolved in $CHCl_3$ and 20 mg (0.2 mmol) of 4-(aminomethyl) pyridine were added to the PVP solution and stirred overnight at 50 °C. Then, the crude was cooled down and poured into diethyl ether yielding the product as a white powder. The polymer was purified by precipitation–solution–precipitation cycles using diethylether–chloroform–diethylether.

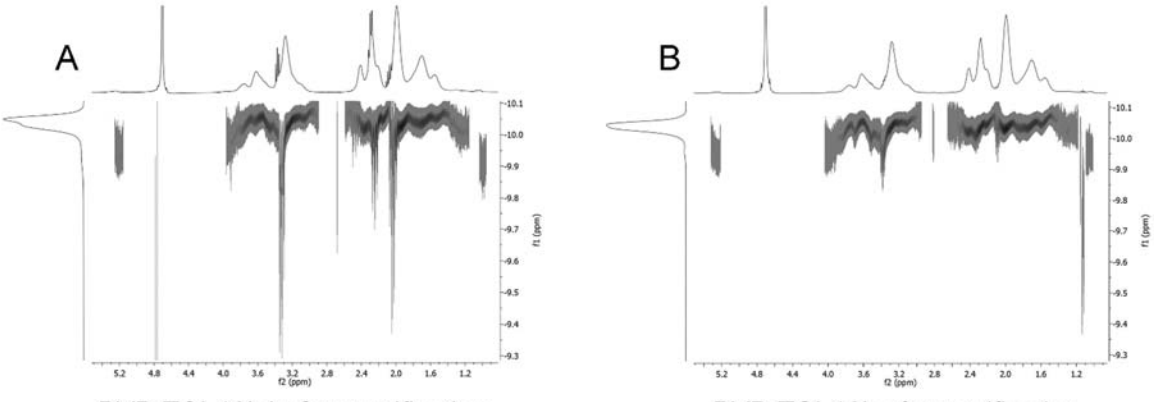
**Synthesis and coating of gold seeds.** Gold nanoparticles with a diameter of 15 nm were prepared by the well-known Turkevich method.[1,2] 1.82 mL of sodium citrate ([NaCit] = 0.47 M) were added in a single step to 200 mL of a boiling aqueous gold salt solution ([Au] = 0.5 mM) under magnetic stirring. Immediately after the addition of NaCit, the heater was turned off and the dispersion was left under magnetic stirring for 15 minutes. After that, the dispersion was cooled down to room temperature and stored a refrigerator until further use. Seeds were coated with PVP (Mw = 8 kDa) by mixing 200 mL of as-prepared gold 15 nm seeds ([Au] = 0.5 mM) with 5 mL of an aqueous PVP solution ([PVP] = 60 mg/mL). The mixture was left under magnetic stirring for 24 hours. The excess of PVP was removed by centrifugation at 7000 g for 1 hour, and the particles re-suspended in EtOH.

**Synthesis of gold nanostars (Au NSTs) using PVP.** The synthesis of Au NSTs was carried out following the method described by Liz-Marzán et.al.[3] For commercial PVP-TCI-10k ($M_w$ = 10 kDa, Lot 48NUD-DE), PVP-Sigma-10k-1v ($M_w$ = 10 kDa, Lot BCBG5331V) and PVP-Sigma-10k-7v ($M_w$ = 10 kDa, Lot BCBM0097V), the synthetic procedure was carried out by adding 22.4 µL of gold salt ([Au] = 111 mM) to 0.5 g of PVP ($M_w$ = 10 kDa) previously dissolved in 5 mL of DMF under magnetic stirring. After two minutes, 33 µL of 3.5 mM of as-prepared gold seed were added to the mixture. The dispersion was left under magnetic stirring for 15 minutes. The excess of PVP was removed by centrifugation three times at 4500 g for 30 minutes, and the particles resuspended in Milli-Q water. For purified PVP-TCI-10K, PVP-TCI-OH and PVP-TCI-Pyridine imine, the synthesis was carried out in a volume of 0.5 mL and in absence of stirring. Briefly, 50 mg of PVP were dissolved in 0.5 mL of DMF and subsequently 2.24 µL of $Au^{+3}$ solution were added ([Au] = 111 mM). After 2 minutes reaction, 3.3 µL of the seed suspension was added and the mixture was then left to react for 20 h (1200 min). The particles were centrifuged 3-times at 4500 g for 30 min and resuspended in Milli-Q water. Nanostars synthesized using PVP-Sigma-40k were prepared as follows: 1 g of PVP ($M_w$= 40 kDa, Lot WXBC7054V) were dissolved in 5 mL of DMF and subsequently 21 µL of $Au^{+3}$ solution was added ([Au] = 117 mM) to the mixture under magnetic stirring. After 3 minutes, 29.87 µL of the seeds ([Au] = 3.85 mM) was added and the mixture was left to react for 15 minutes. The particles were cleaned by centrifugation at 2800 g for 35 minutes and redispersed in Milli-Q water.

**Synthesis of gold nanostars in the presence of hydrazine.** 21 µL of a gold solution ([Au] = 117 mM) were added to 1 g of PVP-Sigma-40k ($M_w$ = 40 kDa, Lot WXBC7054V) or PVP-TCI-10k ($M_w$ = 10 kDa, Lot 48NUD-DE) dissolved in 5 mL of DMF followed by the addition of 11.3, 22.6, 50 or 100 µL of hydrazine 3.5 wt. % in water. After 3 minutes, 29.87 µL of as-prepared gold seeds ([Au] = 3.85 mM) was added to the mixture. Excess of PVP was removed by centrifugation at 2800 g for 35 minutes. Particles were re-dispersed in 5 mL of water.

**Nanoparticles characterization.** Gold nanoparticles were visualized with a transmission electron microscope (TEM, 120 kV Tecnai Spirit transmission electron microscope equipped with a 2048×2048 Veleta CCD camera, ThermoFisher, Waltham, Massachusetts, United States). Ten µL of the nanoparticle dispersions were drop casted onto a copper grid for their characterization. The Ultraviolet-Visible-Near Infrared (UV-Vis-NIR) extinction spectra of the particles were recorded at room temperature with a Jasco V-670 spectrophotometer using a 10 mm path length quartz suprasil cuvette (Hellma Analytics).

**PVP characterization.** $^1$H NMR spectra (≈ 40 mg in 0.8 mL deuterium oxide) were recorded on a JEOL JNM-ECX 400 MHz spectrometer and all chemical shifts, were quoted on the $\delta$-scale in parts per million (ppm) relative to the signal of tetramethylsilane. The signal of residual solvent ($D_2O$ at 4.79 ppm) was employed as an internal standard. The matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrum of the soluble fraction in 2,5-dihydroxy benzoic acid (DHB) + cationization agent sodium trifluoroacetate (NaTFA) was obtained on a BRUKER ultrafleXtreme. DOSY spectra were acquired using the ledbpgp2s pulse sequence on a Bruker Avance 500 MHz AVANCE III HD spectrometer running TopSpin 3.2 and equipped with a 5 mm PABBO BB/19F-1H/D Z-GRD Z116098/0361 probe in $D_2O$. The $^1$H DOSY spectra were collected using the Bruker pulse program ledbpgp2s at a frequency of 400.13 MHz with a spectral width of 4801.5 Hz and a relaxation delay of 3 s.



PVP-TCI-10k before purification PVP-TCI-10k after purification

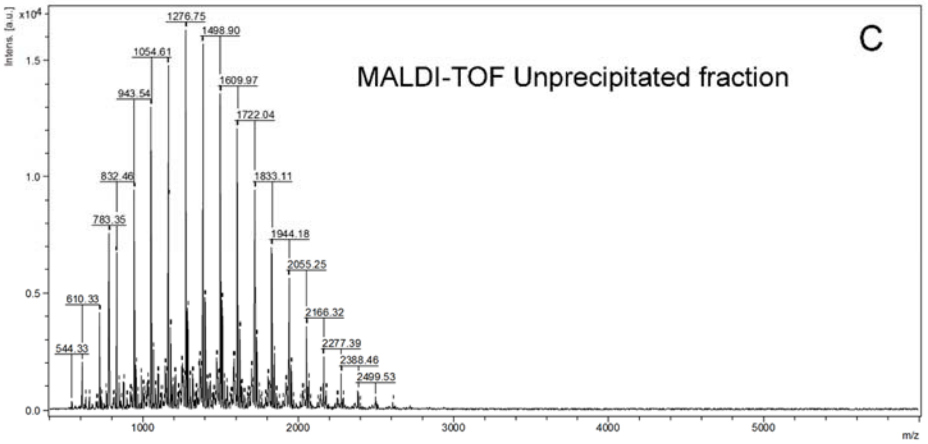


**Figure S1.** DOSY-NMR spectra of PVP-TCI-10k before (A) and after (B) purification confirming that the aldehyde group belongs to the PVP polymer (aldehyde group band at 5.2 ppm). C) The broad distribution of masses detected by MALDI-TOF confirms the presence of oligomers on the un-precipitated fraction.

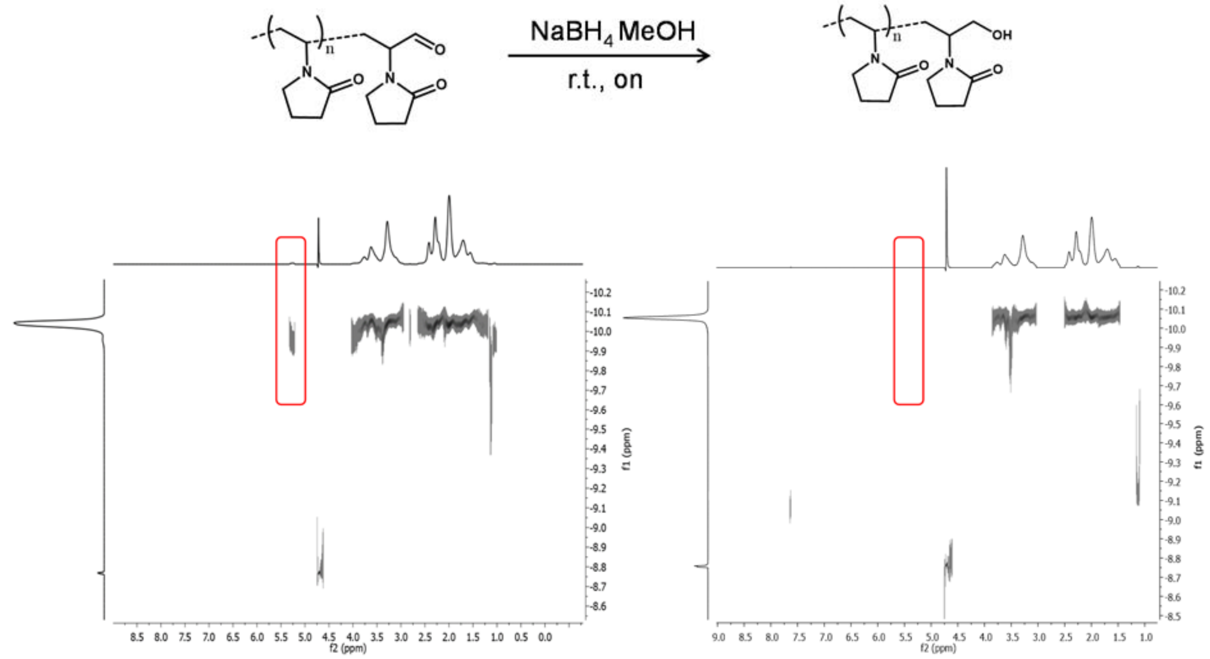


**Figure S2**. $^{1}$H-DOSY confirming the presence of aldehyde groups in the purified PVP-TCI backbone. Reduction of purified PVP-TCI lead to the disappearance of the band at 5.2 ppm.

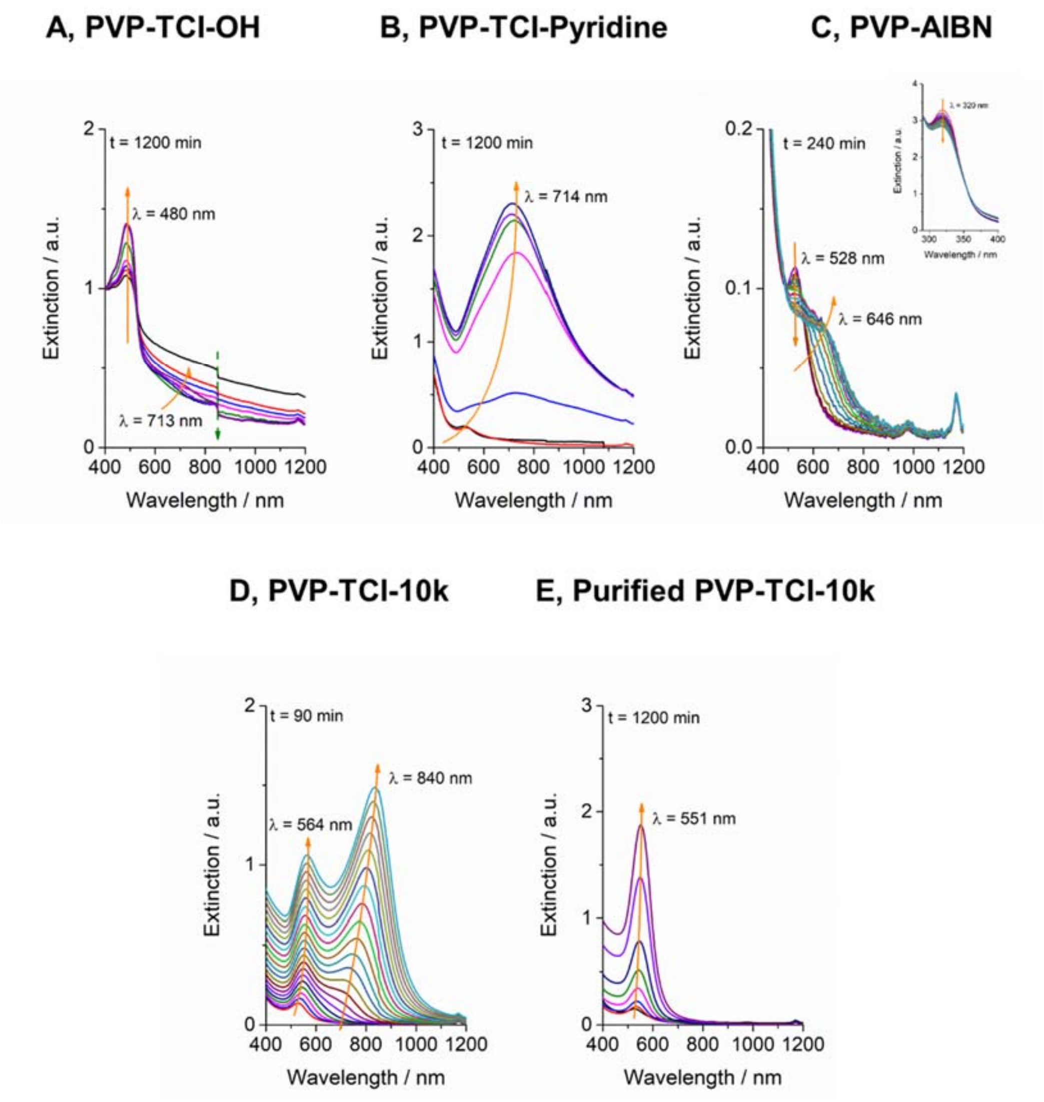


**Figure S3.** Evolution of the UV-Vis-NIR extinction spectra of gold particles in the presence of PVP in which the end groups had been reduced to hydroxyl groups (A, PVP-TCI-OH), PVP pyridine end-terminated (B, PVP-TCI-Pyridine), PVP synthesized using AIBN as an initiator (C, PVP-AIBN), impure PVP-TCI-10k (D), and purified PVP-TCI-10k (E). No Au NSTs are formed in the presence of PVP-TCI-OH, and PVP-AIBN. For PVP-TCI-OH an increase in the absorbance was observed at 480 nm and 713 nm after 1200 min (orange arrows). However, the spectra decay over time (green dash line), which indicates that seeds sediment due to their aggregation. PVP-AIBN induces a decrease in absorbance at 528 nm while the absorbance at 646 nm increases over time (orange arrows). The insert in Figure S4C represents the reduction of $Au^{3+}$ to $Au^{+}$ in the presence of PVP-AIBN over 240 min. Minor anisotropic growth is confirmed by the appearance of a band at 714 nm in the presence of PVP-TCI-Pyridine. PVP-TCI-10k leads to the formation of Au NSTs with two bands centred at 564 nm and 840 nm whereas rough spheres were obtained with purified PVP-TCI-10k.

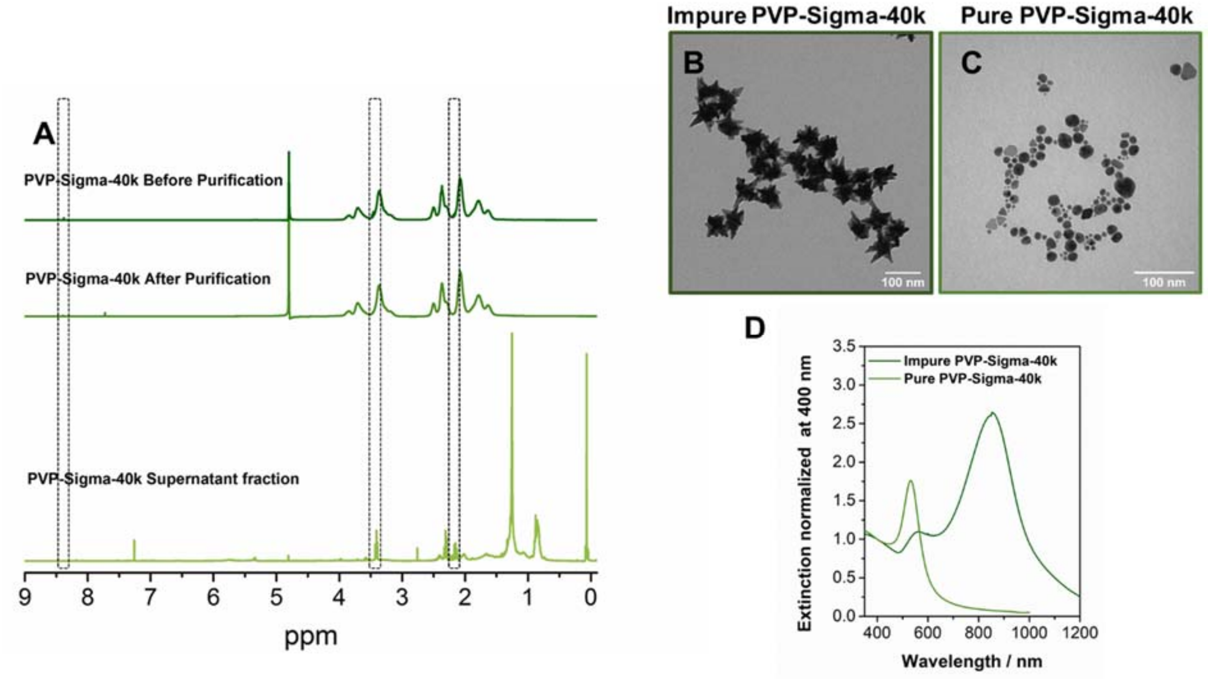


**Figure S4.** $^1$H-NMR spectra of commercial PVP-Sigma-40k (Mw= 40k, Lot WXBC7054V) in $D_2O$ before (A) and after (B) purification by ether precipitation. $^1$H-NMR spectra of unprecipitated fraction after purification of PVP-Sigma-40k in $CDCl_3$ (C). Dashed vertical black lines represent the presence of oligomers and the presence of an aldehyde end group. TEM micrographs of nanoparticles formed in the presence of impure (B) and pure (C) PVP-Sigma-40k demonstrating that purification of PVP inhibits the formation of gold nanostars. UV-Vis-NIR extinction spectra of particles formed in the presence of impure and pure PVP-Sigma-40k (D).

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3. P.S. Kumar, I. Pastoriza-Santos, B. Rodríguez-González, F.J. García de Abajo and L. M. Liz-Marzán, *Nanotechnol.,* 2008, **19**, 15606.