Comparative characterisation of non-monodisperse gold nanoparticle populations by X-ray scattering and electron microscopy

Supporting information

Ye Yang,[a] Suiyang Liao,[b] Zhi Luo,[b] Runzhang Qi,[a] Niamh Mac Fhionnlaoich,[a] Francesco Stellacci,[bc] and Stefan Guldin*[a]

[a] *Department of Chemical Engineering, University College London, Torrington Place,London, WC1E 7JE, U.K.; E-mail: s.guldin@ucl.ac.uk*

[b] *Institute of Materials, École Polytechnique Fédérale de Lausanne (EPFL), Station 12, 1015 Lausanne, Switzerland*

[c] *Interfaculty Bioengineering Institute, École Polytechnique Fédérale de Lausanne (EPFL), Station 12, 1015 Lausanne, Switzerland*

1 AuNP synthesis

In a two-step procedures, AuNPs were prepared with oleylamine (OAm) as intermediate stabilising agent before subsequent thiol ligand exchange. The reaction started with preparing the precursor at room temperature (∼22 °C) by dissolving 0.5 mmol hydrogen tetrachloroaurate (III) hydrate ($HAuCl_4{\cdot}3H_2O$, Reagent ACS, Acros Organics) in a 40 ml solvent mixture of OAm (C18 content: 80% – 90%, Acros Organics) and *n*-octane (99%, Acros Organics) ($1{:}1_{v:v}$). The solution was mixed and sonicated under Ar flow for 10 min before stirring at the reaction temperature. The temperature was controlled by employing a 100 ml jacketed flask with a temperature-controlled GR150-R2 circulating bath (Grant Instruments) The reducing solution was prepared by dissolving 0.5 mmol tert-butylamine borane (tBAB, 97%, Sigma-Aldrich) in 1 ml OAm and 1 ml octane. Intensive mixing was required before the injection into the precursor solution under vigorous stirring. The mixture was left reacting in Ar atmosphere at the designated reaction temperature for 2 h before 30 ml of ethanol (EtOH) was added to quench the reaction. The AuNPs were collected by centrifugation at 5000 g for 10 min and then re-dispersed in dichloromethane (DCM).

The obtained samples were dried in a vacuum desiccator after repeated centrifugal washing in EtOH and methanol (MeOH). The reaction temperatures of 40, 25, 15, 10 °C were used in four syntheses and the obtained MUS-AuNP samples were labelled as MUS-NP1 – NP4, respectively. A biphasic solution of DCM-H_2O ($1{:}1_{v:v}$) was used for the MUS-for-OAm ligand-exchange reaction. 0.1 mmol MUS was dissolved in 12 ml of the biphasic solution mixture by vigorous stirring for 10 min. Subsequently, 30 mg of the OAm-capped AuNPs in 6 ml DCM solution were injected and the reaction solution was allowed to react at room temperature for 20 h. To quench the ligand-exchange reaction, acetone was added and the AuNPs were collected by centrifugation at 5000 g for 10 min. To remove unbound water-soluble MUS ligands, repetitive washing in H_2O were implemented with Amicon Ultra centrifugal filters (15 ml, 10,000 NMWL, Merck Millipore) at 5000 g for 10 min before final washing in acetone and subsequent vacuum drying.

2 TEM characterisation

2.1 TEM imaging

Table 1: Sample information of employed single-type MUS-AuNPs.

Sample label	**OAm-AuNP synthesis temperature** °C	**Core diameter by TEM** nm
MUS-NP1	40	2.6±0.3
MUS-NP2	20	3.8±0.6
MUS-NP3	10	5.1±0.6
MUS-NP4	5	5.6±0.8

Table 2: Sample information of employed binary mixed MUS-AuNPs.

Sample	**single-type sample**	**Mixing ratio**
MUS-B1	MUS-NP1:MUS-NP4	$1{:}1_{wt}$
MUS-B2	MUS-NP1:MUS-NP4	$1{:}5_{wt}$

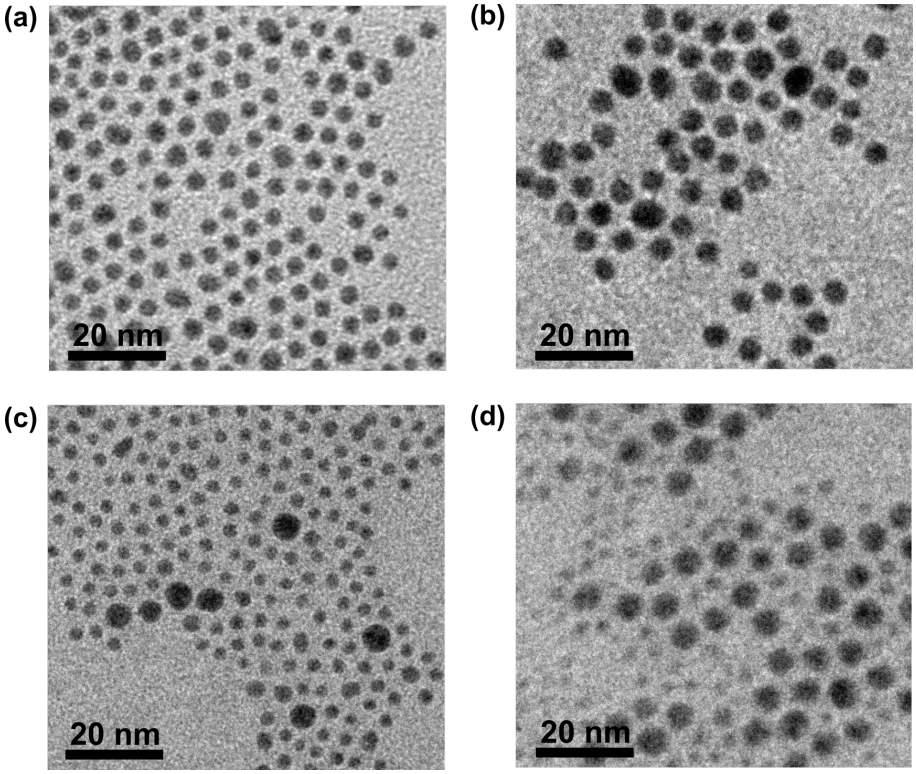

Figure 1: Representative TEM image of MUS-AuNPs: (a) MUS-NP2, (b) MUS-NP3, (c) MUS-B1 and (d) MUS-B2.

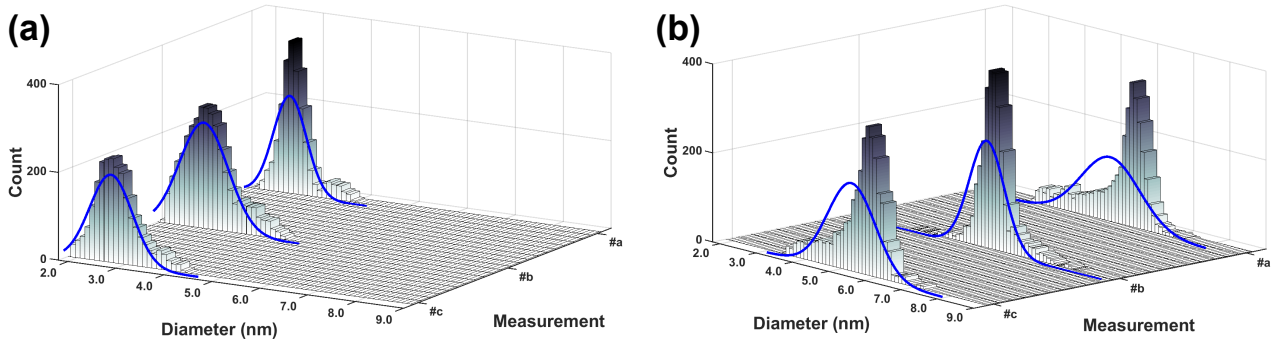

Figure 2: Diameter distribution histograms of (a) MUS-NP1 and (b) MUS-NP4. The histograms for each batch of AuNPs were obtained from three separate experiments of different sample grids prepared from an identical sample solution. Here, the corresponding normal distribution based on the mean diameter and standard deviation obtained by statistical analysis of each histogram is plotted alongside.

2.2 Statistical comparisons of histograms

2.2.1 t-Test

The t-test assumes that both populations follow a normal distribution (Tab 6) with equal but unknown variance and determines if the means of the two populations are the same.[1] The two-sample t-test was performed for each pair of measurements. For almost all cases, the null hypothesis was rejected at a 5% significance level; the corresponding p-values are shown in Tab 4. It is important to note the large p-value from the a-c pair. While the histograms for MUS-NP1-a and MUS-NP1-c were dissimilar as they do not following normal distribution patterns, the values of the actual mean obtained from the

TEM analysis were very similar as were the standard deviations.

Table 3: The statistical information obtained from the normal distribution analysis.

Measurement	**Mean, nm**	**Standard deviation, nm**
MUS-NP1-a	2.8	0.3
MUS-NP1-b	2.9	0.5
MUS-NP1-c	2.8	0.4
MUS-NP4-a	5.6	0.9
MUS-NP4-b	5.7	0.5
MUS-NP4-c	5.4	0.7

Table 4: The p-values obtained by two-sample t-test with equal variance.

	MUS-NP1	**MUS-NP4**
a-b	$1.78 * 10^{-9}$	$5.95 * 10^{-7}$
a-c	0.2265	$2.02 * 10^{-17}$
b-c	$1.80 * 10^{-6}$	$2.41 * 10^{-60}$

2.2.2 Kolmogorov-Smirnov test

The two-sample Kolmogorov-Smirnov test was used to determine if each pair of populations follow the same distribution.[2,3] This is a non-parametric technique which can be applied to any arbitrary, continuous distribution and therefore does not require the populations to follow a normal distribution. By pairwise examination of the three populations obtained from samples MUS-NP1 and MUS-NP4, it was found that, statistically, no pair came from the same parental distribution at the 5% significance level; the resulting p-values are reported in Tab 5.

Table 5: The p-values obtained by two-sample Kolmogorov-Smirnov test.

	MUS-NP1	**MUS-NP4**
a-b	$1.10 * 10^{-28}$	$5.73 * 10^{-111}$
a-c	$3.27 * 10^{-9}$	$3.76 * 10^{-105}$
b-c	$1.30 * 10^{-6}$	$2.56 * 10^{-46}$

In summary, the non-parametric two-sample Kolmogorov-Smirnov test, rejected the null hypothesis that each TEM analysis followed the same distribution. Furthermore, the t-test largely rejected the null hypothesis that each sample had the same mean, with exception of MUS-NP-a and MUS-NP-c. Overall this provides evidence that the variation in TEM sample preparation and analysis is statistically significant.

2.2.3 Information entropy test

This analysis was inplemented using our recently published NP dispersity tool based on information entropy.[4] In each analysis, 50 repetitions were used for the calculation to converge on the true mean and avoid the error related to the random sampling. The bin width was set 0.1 nm and 0.072 nm for TEM and SAXS data, respectively.

Table 6: The statistical information obtained from the information entropy test.

Measurement	**Absolute Entropy**		**Normalised Entropy**		**Reliability Index**
	Mean	**SD**	**Mean**	**SD**	
MUS-NP1-a	1.27	0.58	0.46	0.0208	3.5E-06
MUS-NP1-b	1.96	0.47	0.69	0.0166	2.0E-06
MUS-NP1-c	1.76	0.54	0.63	0.0195	2.5E-06
MUS-NP4-a	2.71	0.10	0.49	0.0181	4.7E-06
MUS-NP4-b	1.72	0.07	0.30	0.0117	2.8E-06
MUS-NP4-c	2.40	0.09	0.44	0.0172	3.3E-06

3 MC-SAXS characterisation

Table 7: Discrepancies among 10 number-weighted output distributions generated from independent MC fitting repetitions for each sample.

Sample	**Mean, nm**	**Deviation of mean**	**Variance$^{1/2}$, nm**	**Deviation of variance**
MUS-NP1	3.1	1.0%	0.5	5.6%
MUS-NP2	4.0	0.4%	0.4	9.3%
MUS-NP3	5.2	0.5%	0.3	3.7%
MUS-NP4	5.6	1.6%	0.8	9.7%
MUS-B1	3.5	2.4%	0.9	5.3%
MUS-B2	4.4	1.5%	1.1	2.4%

4 Comparative studies

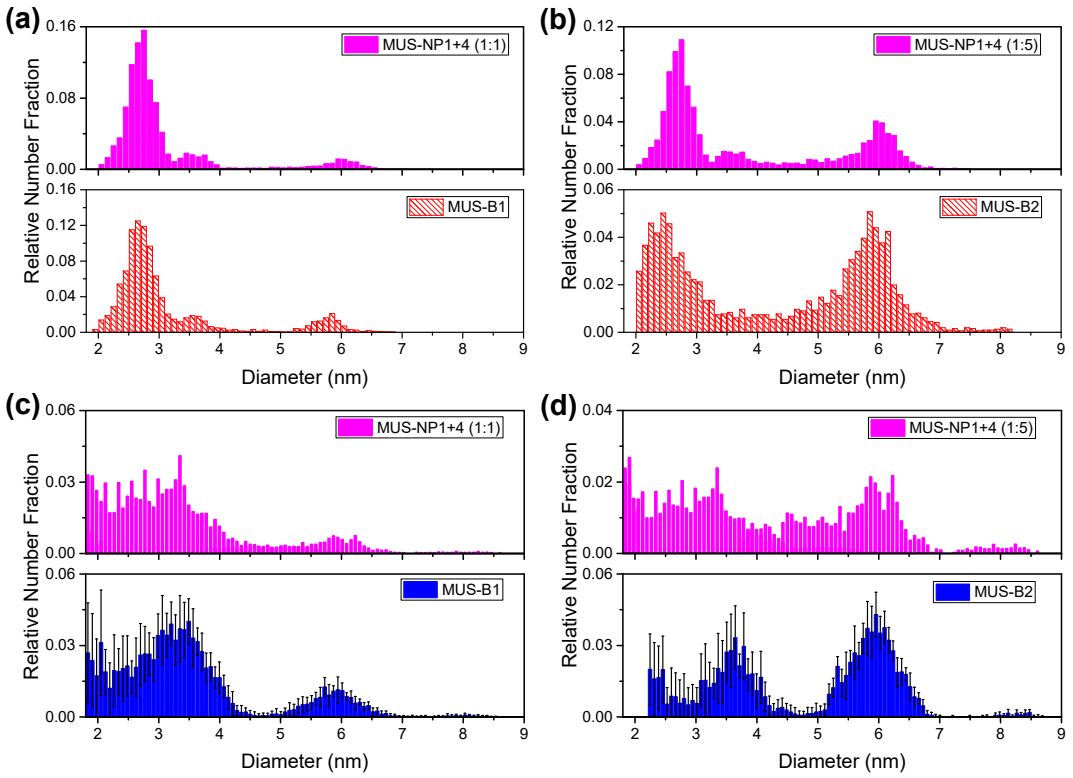

Figure 3: Diameter distribution analysis of binary mixtures by TEM (red) and SAXS (blue): (a),(c) MUS-B1 ($1{:}1_{\mathrm{wt}}$), (b),(d) MUS-B2 ($1{:}5_{\mathrm{wt}}$), with corresponding simulated distribution histograms (pink) calculated from single-type results.

References

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[3] J. Durbin, *Distribution Theory for Tests Based on the Sample Distribution Function*, Society for Industrial and Applied Mathematics, Philadelphia, 1st edn., 1973.

[4] N. M. Fhionnlaoich and S. Guldin, *Chem. Mater.*, 2020, 10.1021/acs.chemmater.0c00539.