Supporting Information

Analysis of Gold Nanoparticle Mixtures: A Comparison of Hydrodynamic Chromatography (HDC) and Asymmetrical Flow Field-flow Fractionation (AF4) Coupled to ICP-MS

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Data Processing

AF4 data was baseline corrected by averaging the first 10 minutes of each run and subtracting this value from each time point measurement. The first 10 minutes of each run was focusing time, and thus any analyte detected during this time represented the Au background in the carrier solution. Recovery was determined by integrating the peak area of each run and comparing it to the peak area of an identical run with no cross flow. Recovery was split into total and resolved recovery. Resolved recovery is defined here as the total mass of Au detected between the void peak and the time point of removal of the separation field. All data were smoothed by averaging every data point with the fourteen previous ones to minimize the impact of short-term instrumental noise.

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Table S1. Relevant instrument specifications and flow conditions for both AF4 and HDC

AF 4 Instrumentation and Separation Methods (AsFlFFF)	
Nominal Void Volume	1.41 ml
Spacer Height	500 µm
Membrane Type	Nova PES, 10 kDa MWCT
Channel Nominal Volume	
Carrier Liquid Formula	0.025% Fl-70, 0.01% NaN3
Injection volume/flow	100 µL 0.1 ml/min
Detector Flow	1.0 ml/min
Focusing time	10-15 min
Rinse Cross Flow	ml/min – 10 to 15 min
4 Particle Mixture Separation Conditions	
Cross Flow Step 1	2.0 ml/min – 5 min
Cross Flow Step 2	2.0-0.5 ml/min – 10 min
Cross Flow Step 3	0.5 ml/min – 45 min
Decay Power	0.4
2 Particle Mixture Separation Conditions	
Cross Flow Step 1	1.4 ml/min – 2 min
Cross Flow Step 2	1.4-0.4 ml/min – 5 min
Cross Flow Step 3	0.4 ml/min – 30 min
Decay Power	0.3

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HDC Instrumentation and Flow Conditions	
Column Type	PL-PSDA Type-1
Channel Flow	1.7 ml/min
Injection Volume	20 µL
Mobile Phase Formula	0.002 M Na2HPO4, 0.2 Triton-X100, 0.05% SDS, 0.2% formaldehyde

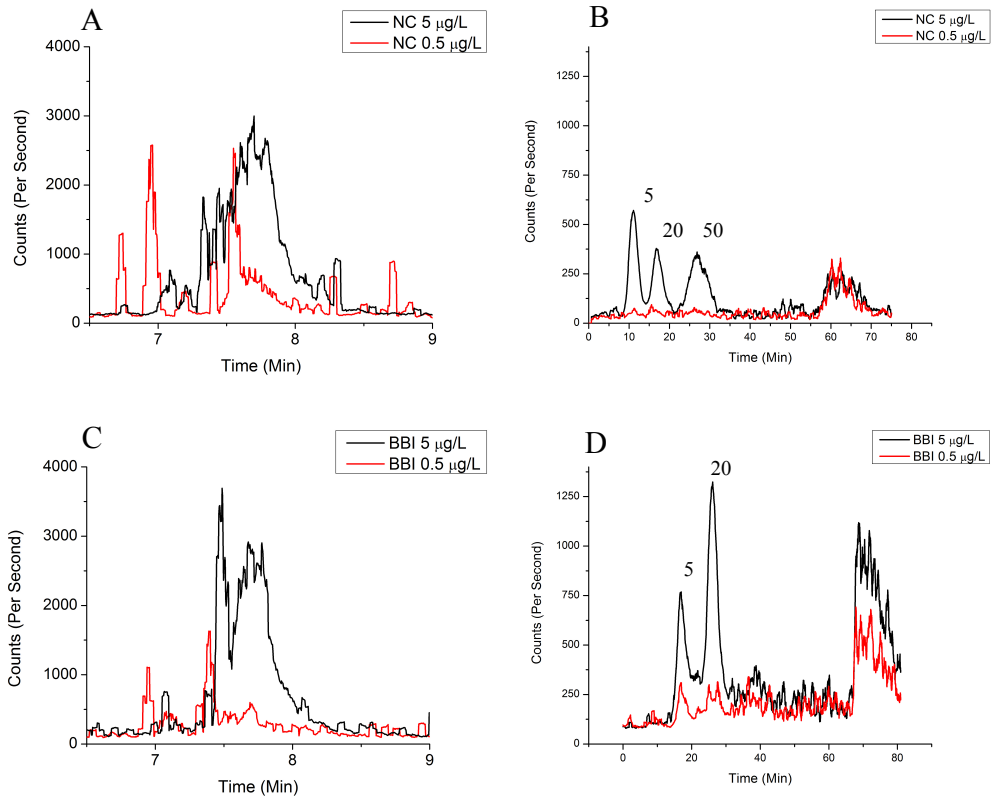

Figure S1. Detection limit of mixtures containing 5, 20, 50 and 100 nm NC and BBI particles separated by HDC (panels A and C) and AF4 (panels B and D). Injection concentrations were 500 ng/L and 50 µg/L for all runs.

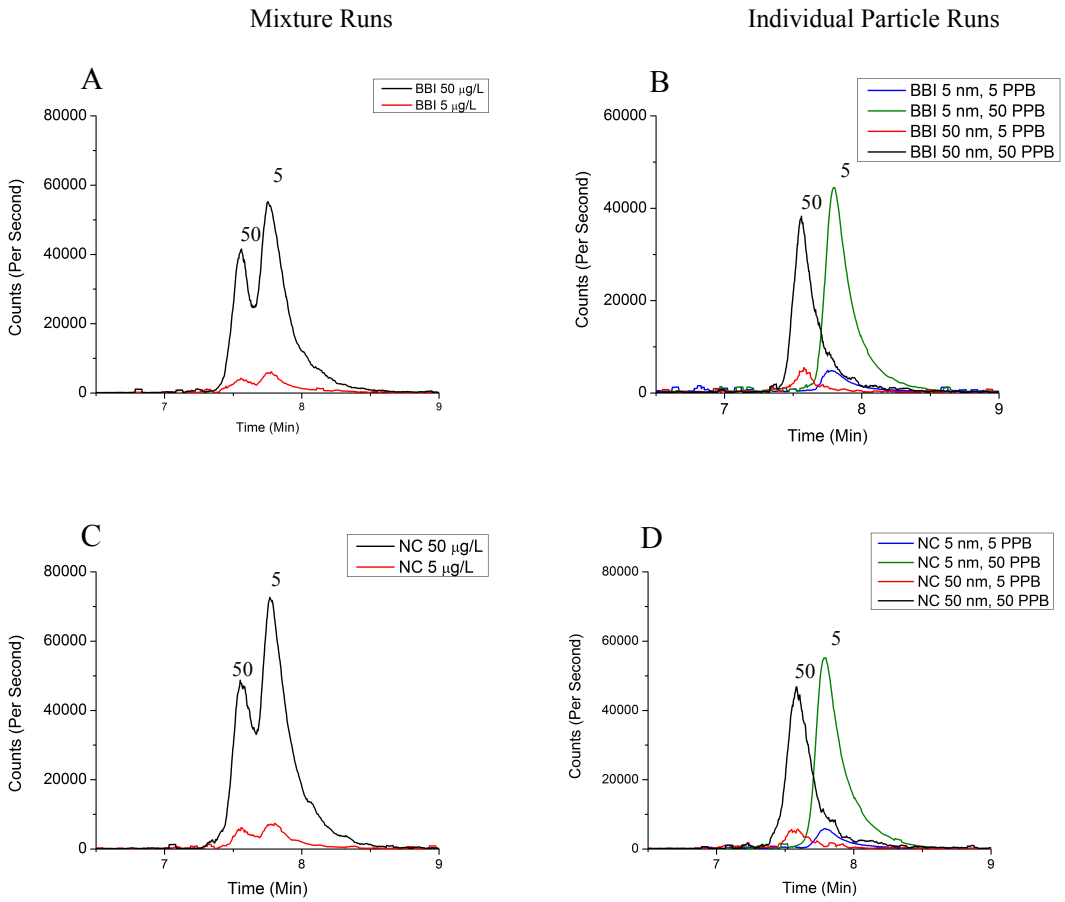

Figure S2. Resolution of a HDC separated mixture containing 5 and 50 nm NPs compared to individual runs of 5 and 50 nm particles. Injection concentrations were 5 and 50 µg/L and injection volume was 20 µL. (A) Mixture run of 5 and 50 nm NC particles. (B) Overlaid runs of 5 and 50 nm NC particles run individually. (C) Mixture run of 5 and 50 nm BBI particles. (D) Overlaid runs of 5 and 50 nm BBI particles run individually.

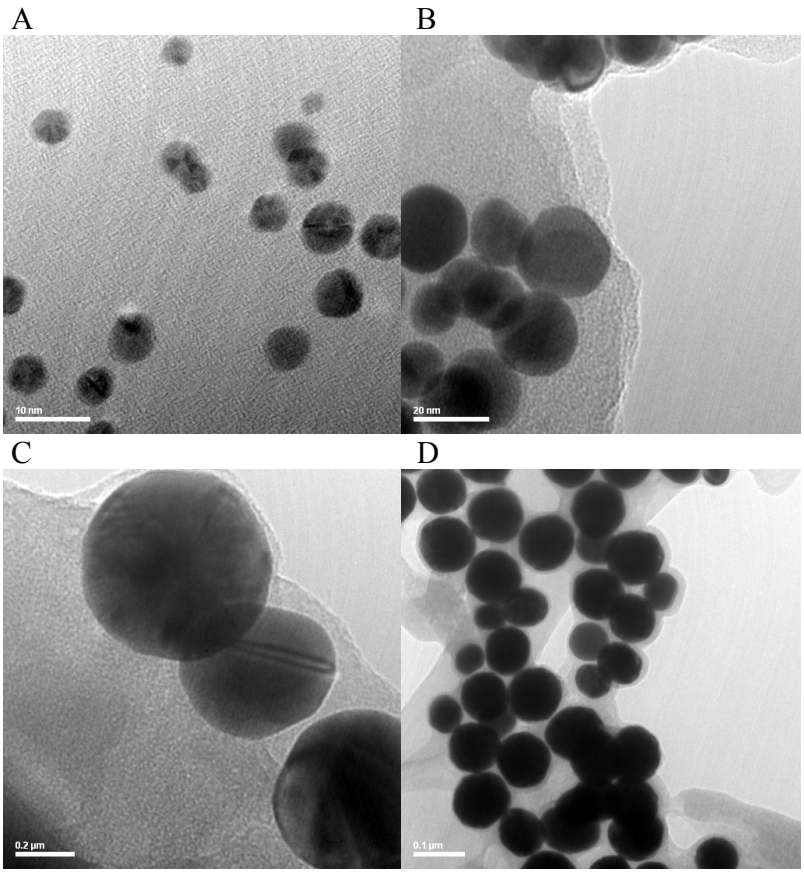

Figure S3. TEM images of BBI nanoparticles analyzed in this study. (A) 5 nm particles. (B) 20 nm particles. (C) 50 nm particles. (D) 100 nm particles. The manufacturer reported a coefficient of less than 8% around the reported mean size

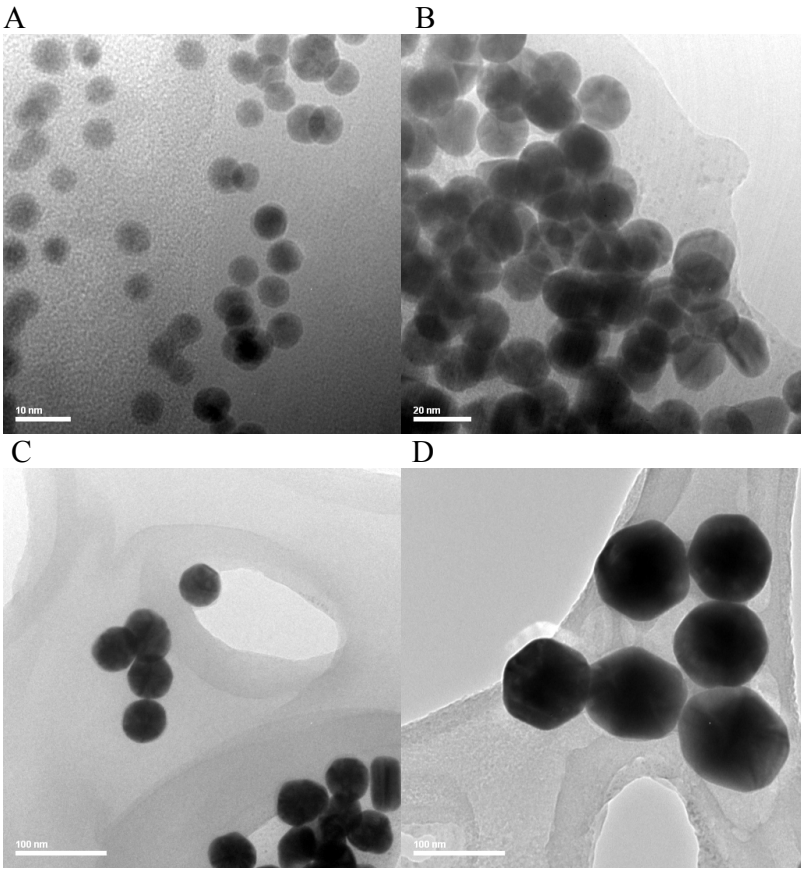

Figure S4. TEM images of NC nanoparticles analyzed in this study. (A) 5 nm particles. (B) 20 nm particles. (C) 50 nm particles. (D) 100 nm particles. The manufacturer reported a coefficient of less than 15% around the reported mean size

Table S2. AF4 recovery trends.

Particle Type	Particles per Mixture	Mass Concentration of Each Particle (µg/L)	Total Recovery (%)
BBI	4 (5, 20, 50, 100 nm)	50	88
BBI	2 (5, 50 nm)	50	62
BBI	1 (5 nm)	50	44
NC	4 (5, 20, 50, 100 nm)	50	89
NC	2 (5, 50 nm)	50	66