matters

# Rapid one-step synthesis of gold nanoparticles using the ubiquitous coenzyme NADH

**Maxwell Baymiller, Frank Huang, Snezna Rogelj PhD**

Biochemistry, University of Illinois at Urbana-Champaign; Civil and Environmental Engineering, New Mexico Institute of Mining and Technology; Biology, New Mexico Tech

**Correspondence**
maxbaymiller@gmail.com

**Disciplines**
Biomaterials

**Keywords**
Coenzyme
Biosynthesis
Gold Nanoparticles

**Type of Observation**
Standalone

**Type of Link**
Orphan Data

**Submitted** Feb 14, 2017
**Published** Jul 21, 2017

**Triple Blind Peer Review**
The handling editor, the reviewers, and the authors are all blinded during the review process.

**Full Open Access**
Supported by the Velux Foundation, the University of Zurich, and the EPFL School of Life Sciences.



## Abstract

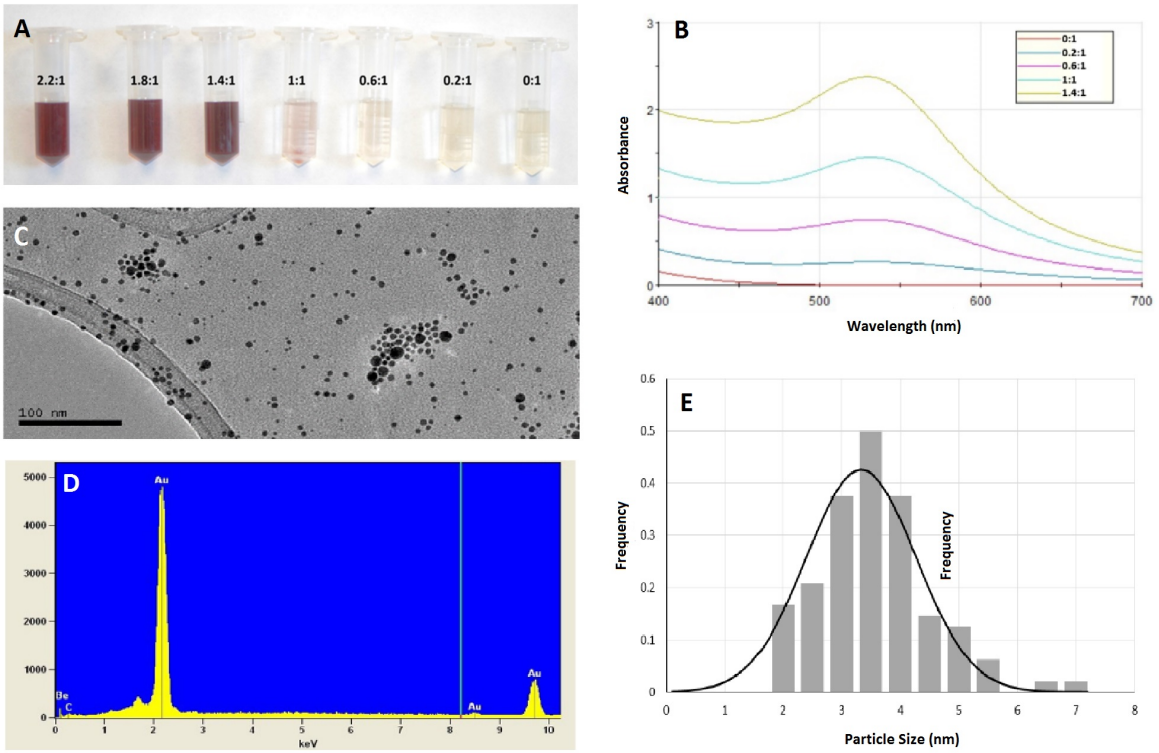
Recent efforts to develop biocompatible and environmentally-friendly nanomaterials have yielded many biosynthetic methods for producing metallic nanoparticles which employ organisms from almost every branch of life. However, little progress has yet been made regarding the underlying mechanisms of most of these biosynthetic methods. In an attempt to address this gap in a knowledge, we have investigated the nanoparticle-producing ability of the ubiquitous biomolecule nicotinamide adenine dinucleotide (NADH), and have found that this coenzyme alone is sufficient to reduce $Au^{3+}$ ion to gold nanoparticles (GNPs) *in vitro*. Synthesis using this method occurs nearly instantaneously at room temperature and produces uniformly spherical plasmonic nanoparticles with small sizes (<10 nm diameter). Both the speed of synthesis and the monodispersity of the produced GNPs are advantages over many other biosynthetic methods. As NADH is a universal component of all living things, our finding also suggests that this coenzyme may contribute to - or be wholly responsible for - some of the many previously- reported syntheses of GNPs by biological systems.

## Objective

Determine whether NADH alone is sufficient to synthesize GNPs from $Au^{3+}$.

## Introduction

The study of gold and other metallic nanoparticles is a rapidly growing area of research in the materials science, and the products of these investigations have been applied to disciplines as diverse as medicine, sensing, and catalysis [1]. The properties of these nanoparticles such as their size, shape, and surface chemistry greatly influences their potential applications, especially in the case of their usage in living organisms [2]. To achieve these desirable properties a range of reducing agents and stabilizers have been explored, which has led to further concerns about the bio-compatibility of the nanoparticles produced with these synthetic methods [3]. A profusion of biosynthetic methods intended to be more environmentally-friendly and bio-compatible have been developed to assuage these concerns, and live cells or preparations of diverse bacteria, fungi, and plants are now known to be capable of carrying out reduction of $Au^{3+}$ in a way leading to gold nanoparticle (GNP) formation [4]. While these GNPs are certainly more bio-compatible, the mechanism of nanoparticle production is unknown for the majority of these biosynthetic methods. In the few cases that a mechanism is well described, nanoparticle formation either occurs as a mechanism of gold toxicity resistance through a species-specific microbial metallophore [5], or is mediated by reducing sugars and terpenoids present only in certain plants [6]. In other instances individual enzymes have been used for GNP synthesis, but the possibility that enzyme cofactors employed in these reactions are sufficient for GNP production has yet to be excluded [7]. To address these gaps in knowledge, we set out to investigate the GNP-forming capabilities of the ubiquitous coenzyme nicotinamide adenine dinucleotide (NADH). In biological systems, NADH is used as a reducing agent for many enzyme-catalyzed reactions where it transfers two electrons to a substrate in the form of a hydride ion. We chose to investigate NADH specifically because- while it has no known chemical interaction with $Au^{3+}$- it is known that intact GNPs catalyze its oxidation [8]. Furthermore, it has often been speculated that NADH-dependent enzymes are responsible for some observations of GNP biosynthesis by cellular extracts [9], and it has been previously reported that addition of NADH restores the nanoparticle-forming capabilities of various enzyme extracts [10].




i

## Figure Legend

**Figure 1:**

**A)** Photograph of GNP preparations with increasing NADH:$Au^{3+}$ molar ratios (inset labels), the dark red color is characteristic of plasmonic GNPs.

**B)** Visible light absorption spectra of selected preparations from Figure A showing increase in surface plasmon resonance (SPR) peak absorbance with increasing NADH:$Au^{3+}$.

**C)** Transmission electron micrograph of a 2:1 molar ratio of NADH:$Au^{3+}$ GNP preparation at 40,000 times magnification with inset 100 nm scale bar.

**D)** Energy-dispersive X-ray spectograph of the same particles shown in C, with elemental peak assignments from NSS Thermo software.

**E)** Histogram of particle diameters from 2:1 molar ratio GNP preparation shown in Figure C. Overlain curve is a normal distribution with the same mean value.

**Supplementary Figures:**

**A)** Surface plasmon resonance (SPR) peak absorbance at 530 nm of various GNP solutions with increasing NADH:$Au^{3+}$ molar ratios.

**B)** Average diameters of GNP preparations with varying NADH:$Au^{3+}$ molar ratio, with linear line of best fit and associated $R^2$ value.

## Results & Discussion

Synthesis of GNPs with NADH was accomplished by mixing small (100 µl) equal volumes of NADH and $HAuCl_4$ solutions at room temperature (see Materials and Methods). When the NADH added was either equivalent or in excess of the molarity of the Au in solution, a pink to deep red color characteristic of plasmonic GNPs resulted within several seconds (Figure 1A).

Spectra which corroborate these visual observations are presented in Figure 1B, as the surface plasmon resonance (SPR) absorbance peak characteristic of GNPs (at approximately 530 nm) was found to increase in intensity with increasing concentration of

NADH. This likely indicates that greater quantities of GNPs were produced as molar ratio increased. Supplementary Figure A shows that this effect plateaued above a 4:1 molar ratio of NADH:$Au^{3+}$, suggesting that complete reduction of available $Au^{3+}$ occurred at this point. At molar ratios above 4:1 the location of the SPR peak did however, shift from its initial location, potentially indicating changes in the sizes or size distributions of the produced nanoparticles [11].

To further confirm the presence of GNPs in these solutions, several preparations were observed by transmission electron microscopy (TEM). In the micrograph displayed in Figure 1C and throughout the data collected- it was found that all particles were spherical in shape- in contrast to other biosynthetic methods where there is high variability in shape [12]. This relative regularity in shape may be due to the absence of confounding factors such as peptides, sugars, and salts that would inevitably be present in the crude extracts or cultures used in other methods. Energy-dispersive X-ray spectrometry of these same nanoparticles showed that they were indeed composed of Au (Figure 1D).

Supplementary Figure B shows that the size of GNPs as determined by TEM was found to be from 3 nm to 7.5 nm on average, but that average size was not found to correlate significantly with the molar of NADH:$Au^{3+}$ ($R^2$=0.4722). The lack of predictability in diameter observed is surprising, given that one would expect molar ratio to influence the relative rates of particle nucleation and growth [13], however others have also reported the absence of clear relationship between particle nucleation rates and final size [14]. Particle size for the majority of GNP preparations was found to follow an approximately normal distribution at the 95% confidence level using the Anderson-Darling goodness of fit test. An example of such a distribution is shown in Figure 1E (p=0.251 for normality). The presence of larger outlier particles in each preparation- for instance the 6.5-7 nm particles in Supplementary Figure B- was also found to be typical among those imaged. The fact that all imaged preparations contained GNPs with small (<10 nm) average diameters agrees with the possibility that NADH is involved in other biosynthetic methods, as many of these also produce particles with average diameters under 15 nm [9] [12].

Here, we have shown that the ubiquitous biomolecule NADH is capable of reducing $Au^{3+}$ to form GNPs. Nanoparticle synthesis using this method occurs very rapidly, without heating, and produces uniformly spherical particles, all of which are advantages over many of the current biologically- and environmentally-friendly methods available. The ubiquity of the coenzyme NADH also ensures that these particles would be highly biocompatible. However, the price of pure NADH may make the widespread adoption of this method unlikely, as other non-biological reductants are far more economical. Furthermore, the size of particles was not defined by the amount of NADH added, and other factors such as pH need to be explored to obtain a size-controllable synthesis using NADH. The fact that NADH is capable of forming GNPs *in vitro* without any other input reveals the possibility that some - if not many - of the previously reported biosyntheses of gold and other metallic nanoparticles are in fact due to the action of this coenzyme. The only exceptions to this possibility are those cases in which biological extracts are extensively heated, as NADH is heat labile. With this information future investigations of nanoparticle biosynthesis should take into consideration, the GNP-forming ability of NADH when aiming to determine the mechanism of nanoparticle production, and control for its effects.

## Additional Information

### Methods and Supplementary Material

Please see https://sciencematters.io/articles/201705000007.

This work was supported by National Science Foundation award #IIA-1301346 to NM EPSCoR and its Osmotic Power Research Group led by F.H. and S.R., National Institute of General Medical Sciences award #P20GM103451 to S.R, and New Mexico Water Resources Research Institute Student Research Grant #Q01706 to M.B.

The authors would like to acknowledge Dr. Ying-Bing Jian and Robert Johnston for assistance with TEM imaging, as well as Gary Chandler for assistance with SEM operation. Much gratitude goes to the students who worked on early stages of this project:

Casper Brady, Alexandra Gobble, Chantelle LeTourneau, and Tyler Pratt.

## Ethics Statement

Not applicable.

## Citations


[1] Daniel Marie-Christine and Astruc Didier. "Gold Nanoparticles: Assembly, Supramolecular Chemistry, Quantum-Size-Related Properties, and Applications toward Biology, Catalysis, and Nanotechnology". In: *Chemical Reviews* (). DOI: 10.1021/cr030698. URL: https://doi.org/10.1021/cr030698.

[2] Pan Yu et al. "Size-Dependent Cytotoxicity of Gold Nanoparticles". In: *Small* 3.11 (Nov. 2007), pp. 1941–1949. DOI: 10.1002/smll.200700378. URL: http://dx.doi.org/10.1002/smll.200700378.

[3] Khlebtsov Nikolai and Dykman Lev. "Biodistribution and toxicity of engineered gold nanoparticles: a review of in vitro and in vivo studies". In: *Chem. Soc. Rev.* 40.3 (2011), pp. 1647–1671. DOI: 10.1039/c0cs00018c. URL: http://dx.doi.org/10.1039/C0CS00018C.

[4] Thakkar Kaushik N., Mhatre Snehit S., and Parikh Rasesh Y. "Biological synthesis of metallic nanoparticles". In: *Nanomedicine: Nanotechnology and Biology and Medicine* 6.2 (Apr. 2010), pp. 257–262. DOI: 10.1016/j.nano.2009.07.002. URL: http://dx.doi.org/10.1016/j.nano.2009.07.002.

[5] Johnston Chad W et al. "Gold biomineralization by a metallophore from a gold-associated microbe". In: *Nature Chemical Biology* 9.4 (Feb. 2013), pp. 241–243. DOI: 10.1038/nchembio.1179. URL: http://dx.doi.org/10.1038/nchembio.1179.

[6] Shankar S.Shiv et al. "Rapid synthesis of Au, Ag, and bimetallic Au core-Ag shell nanoparticles using Neem (Azadirachta indica) leaf broth". In: *Journal of Colloid and Interface Science* 275.2 (July 2004), pp. 496–502. DOI: 10.1016/j.jcis.2004.03.003. URL: http://dx.doi.org/10.1016/j.jcis.2004.03.003.

[7] Khan Shadab Ali and Ahmad Absar. "Enzyme mediated synthesis of water-dispersible, naturally protein capped, monodispersed gold nanoparticles; their characterization and mechanistic aspects". In: *RSC Advances* 4.15 (2014), p. 7729. DOI: 10.1039/c3ra43888k. URL: http://dx.doi.org/10.1039/C3RA43888K.

[8] Pingarrón José M., Yáñez-Sedeño Paloma, and González-Cortés Araceli. "Gold nanoparticle-based electrochemical biosensors". In: *Electrochimica Acta* 53.19 (Aug. 2008), pp. 5848–5866. DOI: 10.1016/j.electacta.2008.03.005. URL: https://doi.org/10.1016/j.electacta.2008.03.005.

[9] Ahmad Absar et al. "Extracellular Biosynthesis of Monodisperse Gold Nanoparticles by a Novel Extremophilic Actinomycete,Thermomonosporasp." In: *Langmuir* 19.8 (Apr. 2003), pp. 3550–3553. DOI: 10.1021/la026772l. URL: https://doi.org/10.1021/la026772l.

[10] Khan Shadab Ali and Ahmad Absar. "Enzyme mediated synthesis of water-dispersible, naturally protein capped, monodispersed gold nanoparticles; their characterization and mechanistic aspects". In: *RSC Advances* 4.15 (2014), p. 7729. DOI: 10.1039/c3ra43888k. URL: https://doi.org/10.1039/c3ra43888k.

[11] Njoki Peter N. et al. "Size Correlation of Optical and Spectroscopic Properties for Gold Nanoparticles". In: *The Journal of Physical Chemistry C* 111.40 (Oct. 2007), pp. 14664–14669. DOI: 10.1021/jp074902z. URL: https://doi.org/10.1021/jp074902z.

[12] Song Jae Yong, Jang Hyeon-Kyeong, and Kim Beom Soo. "Biological synthesis of gold nanoparticles using Magnolia kobus and Diopyros kaki leaf extracts". In: *Process Biochemistry* 44.10 (Oct. 2009), pp. 1133–1138. DOI: 10.1016/j.procbio.2009.06.005. URL: https://doi.org/10.1016/j.procbio.2009.06.005.

[13] Polte Jörg et al. "Mechanism of Gold Nanoparticle Formation in the Classical Citrate Synthesis Method Derived from Coupled In Situ XANES and SAXS Evaluation". In: *Journal of the American Chemical Society* 132.4 (Feb. 2010), pp. 1296–1301. DOI: 10.1021/ja906506j. URL: https://doi.org/10.1021/ja906506j.

[14] WAGNER J, TSHIKHUDO T, and KOHLER J. "Microfluidic generation of metal nanoparticles by borohydride reduction". In: *Chemical Engineering Journal* 135 (Jan. 2008), S104–S109. DOI: 10.1016/j.cej.2007.07.046. URL: https://doi.org/10.1016/j.cej.2007.07.046.

[15] Polte Jörg et al. "Mechanism of Gold Nanoparticle Formation in the Classical Citrate Synthesis Method Derived from Coupled In Situ XANES and SAXS Evaluation". In: *Journal of the American Chemical Society* 132.4 (Feb. 2010), pp. 1296–1301. DOI: 10.1021/ja906506j. URL: http://dx.doi.org/10.1021/ja906506j.