Using platinum nanoparticles on ferritin to form hydrogen gas from methyl viologen and organic acids

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A senior thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Bachelor of Science

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ABSTRACT

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Hydrogen (H_2) gas is a possible alternative fuel to help meet increasing worldwide energy needs, but a major obstacle in the use of H_2 for green, environmentally-friendly fuel is the energetic and chemical requirements to synthesize the gas. I am studying the use of photocatalytic reactions to produce H_2, where a light-absorbing substance acts as a catalyst in shuttling electrons from a donor to protons that are reduced into H_2. Previous research conducted at BYU showed that platinum nanoparticles bound to ferritin catalyzed the photoreaction of methyl viologen to reduce protons in an organic acid; this catalytic system offered an increase in hydrogen production efficiency by up to 100 times over platinum black (a commonly available platinum-based catalyst). I am reporting on our efforts to optimize the synthesis of the platinum nanoparticles bound to ferritin that are used in this photocatalytic system and how I characterize these nanoparticles, as well as how these characteristics affect H_2 production.

Keywords: platinum nanoparticles, methyl viologen, ferritin, photochemistry, photocatalyst, hydrogen evolution, hydrogen catalyst

ACKNOWLEDGMENTS

Writing a thesis likes this represents the culmination of almost four years of classes, research, and balancing life with them. I never really thought I'd get this far, but I owe the fact that I did to my parents for some much-needed pep talks at all hours of the day and night, to Leslie Howe for some classic late-night lab work solidarity, to John Colton for giving me a chance, to Paul Minson for endless questions about the electron microscope, and to Dalton Galke for doing everything else.

I also owe the other members of my research group, particularly Matthew Richards and Chapman Lindsay. Thanks for the memes and the collaboration, guys.

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Chapter 1

Introduction

1.1 Motivation/Green Energy

As energy needs grow ever greater in today's high-tech world, many scientists are considering alternative energy sources to the traditionally-used source of fossil fuels. There is a significant amount of interest in the viability of vehicles and electric power generators fueled by hydrogen H_2 gas, in academia, industry, and the general public. A major obstacle in the use of H_2 for green, environmentally-friendly fuel is the energetic and chemical requirement to synthesize the gas. Currently, fossil fuels are the primary sources of H_2 production, but these resources will likely be depleted by the end of the twenty-first century and are also contributing to climate change [1].

A possibility in satisfying current and future hydrogen production needs is the use of photocatalytic reactions, where a light-absorbing substance either acts a catalyst in shuttling electrons from a donor to protons, or the substance itself acts as an electron shuttle between a donor and protons that are reduced to form H_2 gas [2, 3]. Such photocatalytic systems have been under scientific investigation for a number of years [4] and at BYU Oscar D. Petrucci investigated a reaction involving methyl viologen (MV), various organic acids, and a platinum catalyst. The platinum catalyst

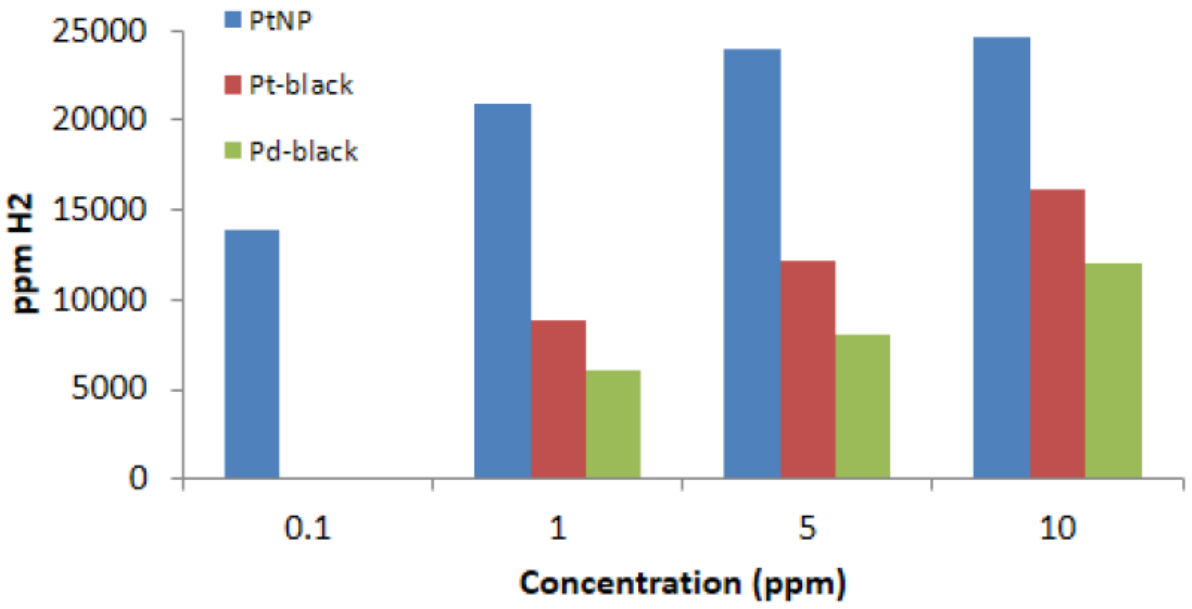

Figure 1.1 This histogram compares the relative efficiency in H_2 production between the use of platinum nanoparticles on ferritin, platinum black (a commercially-available catalyst), and palladium black (another commercially-available catalyst). Reproduced from Ref. [4].

in Petrucci's system is bound to ferritin in nanoparticles, as these nanoparticles have been found to have greater catalytic activity and lower cost than bulk platinum [4]. Petrucci's system offers greater efficiency than other photocatalytic H_2 production systems because of a simplification of the electron shuttling process, and the use of platinum nanoparticles offered an increase in catalytic activity over more traditional bulk platinum catalysts by a factor of 2-5. Figure 1.1 illustrates this increased catalytic activity.

In this report we set out to describe our efforts and progress in replicating and verifying Petrucci's work. It should be noted, however, that the work presented here only represents one part of an ongoing effort; the work here by no means represents the end of our group's work towards the goal of successfully synthesizing H_2 using platinum nanoparticles but instead we will here discuss methods that have worked well and will provide some direction for possible avenues of future

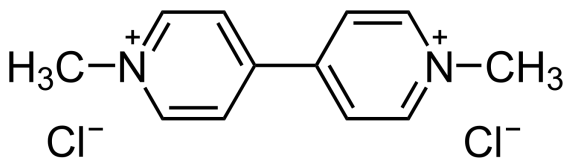

Figure 1.2 This diagram shows the structure of methyl viologen, a relatively simple organic molecule.

research. I would also recommend that the interested reader obtains a copy of Matthew Richard's thesis, submitted in April 2019 [5]. Richards has collaborated with me on many aspects of this work, but focuses more on hydrogen synthesis in his thesis.

1.2 A Simple Explanation of Petrucci's Photocatalytic Reaction

Methyl viologen, an organic acid (normally citric acid), and platinum nanoparticles are placed in a sealed glass vial with an inert atmosphere. The vial is irradiated with UV light. The light activates the methyl viologen, which is reduced by taking an electron from a sacrificial organic electron donor (normally citrate). The platinum nanoparticles, bound to the protein ferritin, catalyze the reduction of protons in the organic acid by the extra electrons carried by methyl viologen, which then form H_2. The ferritin is sometimes also activated by the UV and acts as a go-between for electrons between the organic electron donor and the methyl viologen [4].

As a side note, the structure of methyl viologen is shown in Figure 1.2 and a schematic of the reaction described in this section is shown in Figure 1.3.

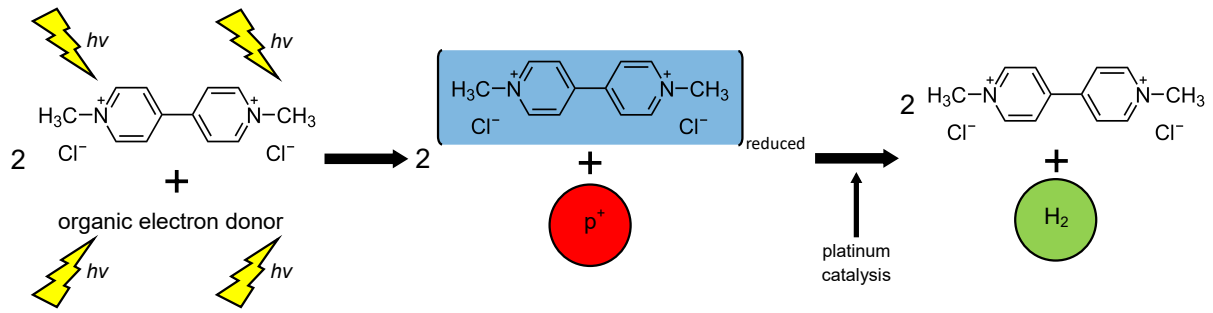

Figure 1.3 This schematic summarizes the photoreaction methyl viologen undergoes in our study; the reaction is described in detail in Section 1.2.

1.3 The Work Done So Far

Our experimental process has several main phases: synthesis of the nanoparticles, verification of the presence of the particles and measuring their size, reacting the nanoparticles to form H_2, and verifying the amount of hydrogen that was formed.

Prior to June 2018, our group had successfully synthesized over a dozen different samples of nanoparticles, following the procedure that was determined to be optimal in Petrucci's dissertation [4], varying only the amount of time the nanoparticle mixture was exposed to UV light during synthesis. After synthesizing the nanoparticles, we ran their solutions through a gel column that divided the samples into fractions based on particle size, meant to isolate nanoparticles bound to ferritin from leftover reactants. These fractions were analyzed using UV/visible spectrophotometry and the Lowry protein assay to determine protein concentrations. We also analyzed several of the fractions' metal content using inductively-coupled plasma mass spectrometry.

1.4 Intention for this Work

Our primary goals for the course of this work were to finish analyzing the metal and protein content of our previously-synthesized nanoparticles, characterize nanoparticle structure using electron

microscopy, and quantify hydrogen production efficiency using gas chromatography. While we met with only limited success in characterizing metal and protein content and with synthesizing hydrogen gas, we were able to glean a good deal of information on nanoparticle structure and composition using electron microscopy methods.

Chapter 2

Methods

In this chapter I discuss the methods we used to synthesize our platinum nanoparticles; characterize their size, structure, and composition; and use them to evolve hydrogen gas from an organic acid.

2.1 Nanoparticle synthesis

Our platinum nanoparticles began as a solution of holoferritin, platinum salts, and TRIS buffer solution. Each solution had 150 μg/mL of ferritin, 30 mM of TRIS (pH 7.4), 2 mM of Pt^{2+} salt, and 50 mM of NaCl; Petrucci found these to be the optimal concentrations for nanoparticle synthesis [4].

The solution was then irradiated under a full-spectrum Hg-halide floodlight (Integrated Dispensing Solutions, Inc.) for a variety of times; we varied the synthesis time between different samples in increments of five minutes from fifteen minutes up to one hour and fifteen minutes. A darkening of the solution showed a successful synthesis.

2.2 Nanoparticle characterization

After synthesis we filtered nanoparticle samples using size-exclusion gel columns to isolate nanoparticles from leftover reactants. The columns were made by mixing a solution of Sephadex G-100 gel beads with distilled water according to manufacturer instructions. After the gel set, it was flushed with TRIS buffer (pH 7.4) and then we poured our samples through it. The larger particles fell through the gel fastest, and we diluted our samples (that were originally 1.5 mL in volume) into ten different 1-1.5 mL fractions, with the largest particles in the fractions that came earliest. We presumed that ferritin with platinum nanoparticles bound to the outside would come out near the beginning of the filtration as ferritin was the largest particle in our initial reaction solution. The platinum nanoparticles did not add much to the size of the ferritin, so we anticipated a few random ferritins without nanoparticles bound to the surface would come out near the beginning of the filtration, as well. (More detailed instructions on the use of gel columns can be found in Hansen [6].)

Following nanoparticle filtration we wished to first identify the filtered fraction(s) where the nanoparticles landed after gel filtration and then quantify the relative masses of the platinum nanoparticles and protein substrate. UV/visible spectrophotometry was particularly useful in identifying the filtered fractions with the greatest amounts of protein and platinum and where further quantitative analysis could best be applied. To perform the spectrophotometry we placed filtered fractions of the original sample in quartz cuvettes and measured absorbance using a spectrophotometer in the 270-290 nm band. Greater absorbance in this band suggested greater amounts of nanoparticles bound to ferritin [6], so using this method to identify the filtered fractions where nanoparticles were present and the fractions that were mostly leftover reactants, we were able to continue with quantitative protein and platinum analysis.

We used the Lowry protein assay to quantify protein concentration among our filtered fractions of each sample. The Lowry protein assay was developed in 1951 by Oliver H. Lowry and is well-known

as a quick and accurate way to measure protein concentration. After reacting the Lowry reagent with protein in the sample and in solutions with known protein concentrations, then measuring the absorbance of the samples in the peak wavelength of the dye using a spectrophotometer, one can with reasonable accuracy measure the protein concentration in a sample [7].

We used inductively-coupled plasma mass spectrometry (ICP-MS) to quantify metal content; samples for ICP-MS were prepared from gel-fractioned samples and prepared using a strong solution of HCl. The ICP-MS analysis itself, however, was performed by a technician in the chemistry department, Jonathan Lynch.

The combination of ICP-MS and the Lowry protein assay provided a quantitative estimate of the relative masses of platinum and protein on our nanoparticles, but we also wanted to have a more concrete idea of the microscopic structure of the nanoparticles.

We used transmission electron microscopy (TEM) and energy-dispersive X-ray spectroscopy (EDS) to image nanoscale structure of the platinum nanoparticles and verify chemical content of the nanoparticles. The electron microscope was Tecnai F20 operating at 200 kV either in scanning transmission electron microscopy (STEM) mode or EDS mode. The performance of accurate TEM analysis requires approximately twenty hours of training and the preparation of samples is a separate process described in Hansen [6]. It should be noted here that TEM was performed on samples largely to view contrast between the platinum nanoparticles and the background (the protein was not readily visible), so structure of the platinum nanoparticles on the ferritin is largely inferred.

While performing electron microscopy, energy-dispersive X-ray spectroscopy (EDS) was also performed. This form of spectroscopy provided an elemental analysis of each sample by bombarding it with a high-energy beam of electrons that caused the sample to release characteristic X-rays. A schematic of the function of EDS is shown in Figure 2.1. It should also be noted that preparation for EDS was no different than preparation for TEM, though special training is required to perform EDS independently (above that which is required to operate the TEM).

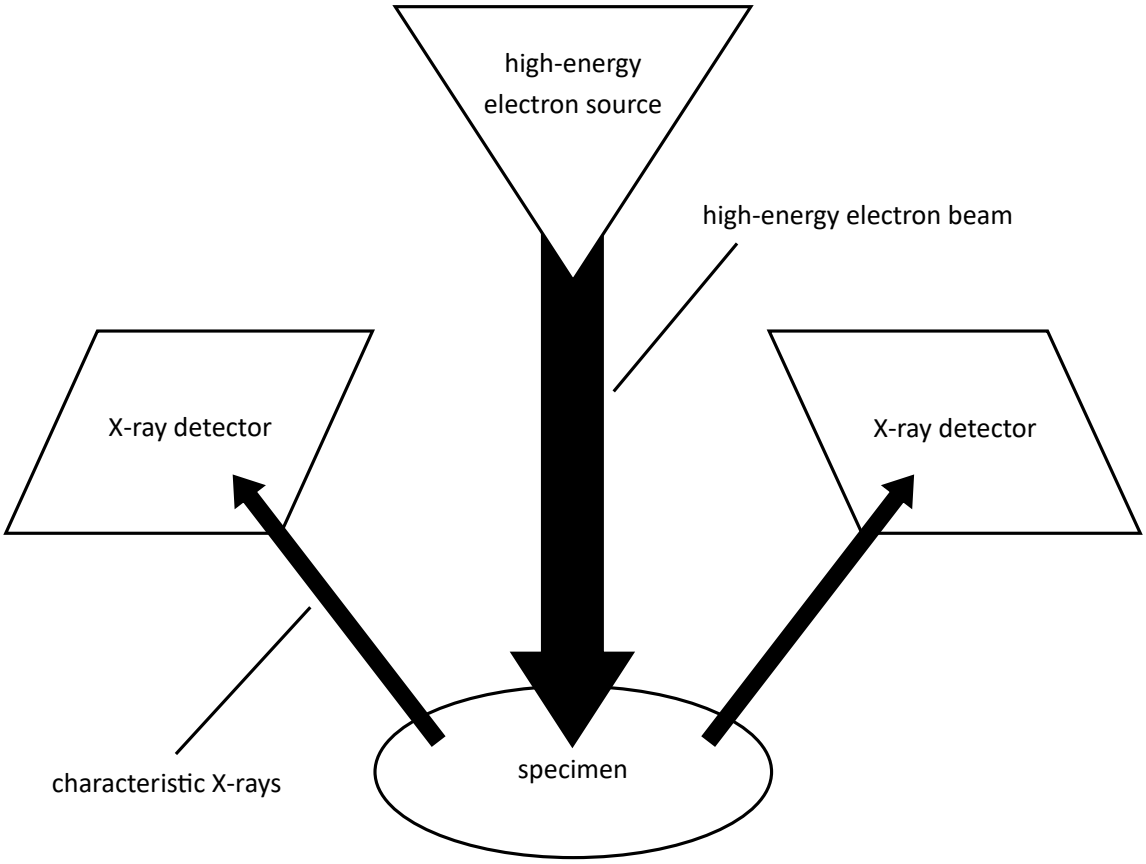

Figure 2.1 This diagram shows how energy-dispersive X-ray spectroscopy (EDS) takes place inside a transmission electron microscope (TEM). High-energy electrons bombard the specimen, which energize it and cause it to emit X-rays characteristic of the elemental compostion of the specimen. These X-rays are measured by detectors, which allows us to analyze the characteristic X-ray spectrum of the sample.

2.3 Hydrogen synthesis and measurement

To synthesize H_2 gas and to see how nanoparticle structure affected catalytic activity of the photochemical reaction of methyl viologen, reaction solutions were mixed such that they contained 133 mM citrate, 13 mM methyl viologen, 33 mM NaCl, about 10 ppm platinum nanoparticles, and a pH of 4.5 (these concentrations were reported to be optimal by Petrucci [4]). This solution was placed in a vial that could be sealed with a septum cap. After the vial was sealed, it was prepared with an inert atmosphere.

Preparation of the sample in an inert atmosphere was essential because because it prevents unexpected reactions before, during, or after H_2 synthesis. The sample is prepared with an inert atmosphere using a vacuum manifold system, which pumps out air left in the vial and replaces it with an inert gas (normally N_2, or occasionally Ar). Detailed instructions on using a vacuum manifold system are provided by Hansen [6].

The sealed vial prepared with an inert atmosphere was exposed to the same Hg-halide floodlight as mentioned in Section 2.1 to trigger the reaction of methyl viologen with citrate and protons in the acid to form hydrogen gas. We allowed the reaction to occur for a variety of times to fully explore how nanoparticle structure related to catalytic activity.

It was essential that we immediately analyzed the atmosphere of the sample vial after synthesis to avoid leakage of the H_2. We measured gas content using gas chromatography with a thermal conductivity detector (GC-TCD, operated by Jiping Zou in the BYU Nutrition, Dietetics and Food Science Department), which identifies gas content by measuring characteristic differences in the flow rate and thermal conductivities of gases.

Chapter 3

Results/Discussion

3.1 Analysis of nanoparticle structure

3.1.1 Analysis based off chemical assays and mass spectrometry

As discussed in Section 1.4, we met only limited success in using chemical assays, spectrophotometry, and mass spectrometry to characterize our nanoparticles. The success we did have was in using them to confirm that nanoparticles had been synthesized. Use of these instruments proved to be somewhat less than helpful due to wide variance in results, particularly with the Lowry assay and mass spectrometry. Despite repeating experiments many times with the Lowry assay and ICP-MS, we received widely varying results, sometimes nonsensical, that caused us to question the integrity of these methods. For example, results from ICP-MS in several instances indicated that samples had far more platinum than we had inserted in the first, and similarly results from using the Lowry protein assay often indicated that there was far more protein in samples than what we had put in.

We have a number of theories that may explain why these methods are producing inconsistent results. The Lowry assay has its basis in a sensitive chemical reaction; the Lowry assay functions by binding a dye to protein groups in a sample, but we suspect that the Lowry reagent may be

binding to other salts or pH buffers left over in our sample or that those leftover products may be inhibiting the Lowry reagent from binding to the protein, distorting our results. ICP-MS functions using very sensitive calibration curves and in addition we have been attempting to use it to measure concentrations of platinum outside the ideal operating range. By using samples that are too small we are essentially only reading statistical noise in our results.

Beyond the basic visual confirmation that our samples had changed color after synthesis, as mentioned in Section 2.1, we were able to use ICP-MS and the Lowry assay to qualitatively show that nanoparticles had formed on the surface of ferritin. After performing these analyses on samples that were divided into fractions by particle mass, we were able to see that the larger particles in each of our samples had higher concentrations of both platinum and protein.

3.1.2 Analysis based off electron microscopy

The use of energy-dispersive X-ray spectroscopy (EDS) and transmission electron microscopy (TEM) allowed us to both characterize elemental content and analyze nanoparticle structure in our samples. EDS analysis, such as that shown in Figures 3.1 and 3.2, confirmed that platinum nanoparticles formed on the outside ferritin nanoparticles. Figure 3.2 in particular conclusively shows the platinum content of our nanoparticles. Electron microscopy gave us a broader view of how the nanoparticles assembled themselves after synthesis and how varying synthesis time varied (or did not vary) structure of the nanoparticles.

In Figure 3.3 we get a good look at the nanoparticles themselves. This image comes from a sample of nanoparticles that was irradiated for 60 minutes during synthesis, but is characteristic of what was observed with most of our samples. We see that nanoparticles were regularly 1-2 nm in diameter and bound to ferritin in clusters of as few as 10 nanoparticles per ferritin, but as many as 35 nanoparticles binding to a single ferritin was also observed. Greater detail of nanoparticle/ferritin complexes is shown in Figure 3.4, where we are able to see the smaller

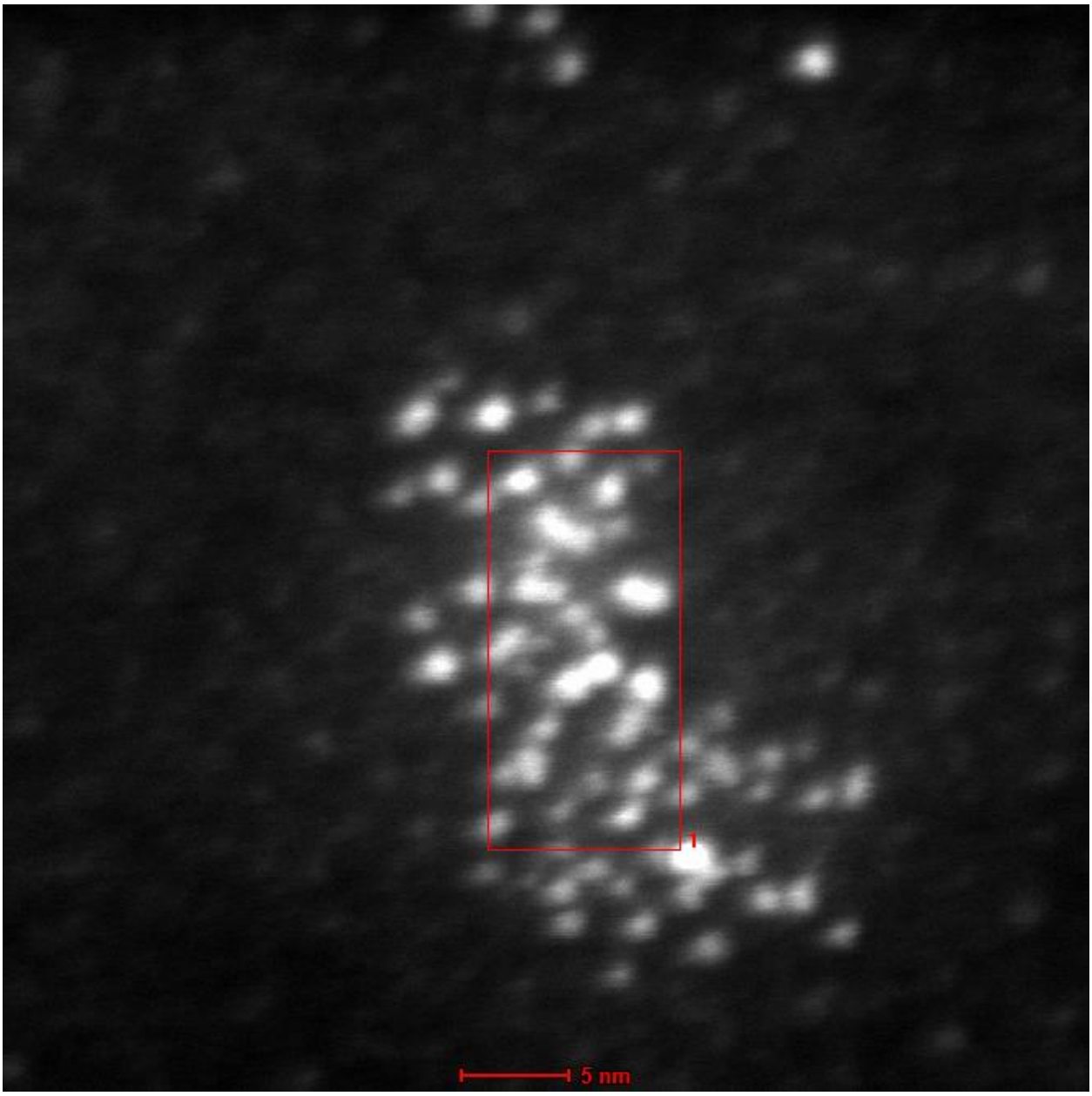

Figure 3.1 This image shows detail on ferritins with nanoparticles bound to the outside. The red rectangle shows the area where energy-dispersive X-ray spectroscopy (EDS) was performed. The results from this spectroscopy are shown in Figure 3.2.

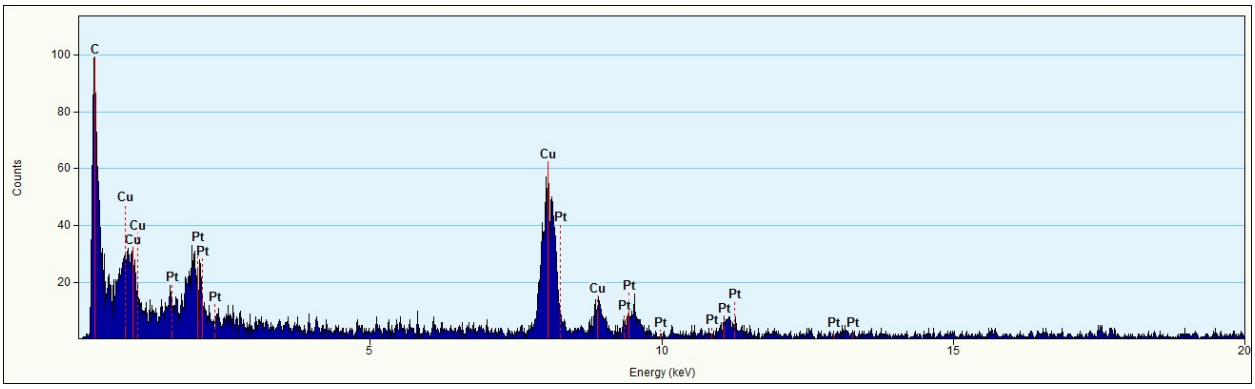

Figure 3.2 This image shows results from energy-dispersive X-ray spectroscopy (EDS) performed on the nanoparticles shown in Figure 3.1. The *x*-axis shows the energy of characteristic X-rays detected in keV and the *y*-axis shows counts of these detected X-rays. The locations of X-rays typically emitted by carbon, copper, and platinum are shown in an overlay. Carbon and copper are shown to be present in great quantities as well because the sample substrate is made from a carbon film and copper grid.

nanoparticles clustered around a larger (8-9 nm) sphere in the center of the image, some loose nanoparticles, and nanoparticles clustered together without a central sphere. The sphere in the central complex in the image is the ferrous mineral core common to the type of equine ferritin we used in synthesis, but we also observed that nanoparticles had formed without binding to ferritin or had bound to ferritins that had lost their mineral core (becoming apoferritins). We were surprised to observe that so many ferritins (as many as 70 percent) had lost their mineral core, as during synthesis we used a solution that had a relatively high concentration of ferritin with cores. We suspect that some process during synthesis or possibly deposition of samples onto electron microscopy grids may have ejected the cores, but determining the exact cause of the observed ejection requires more investigation.

Overall, Figures 3.3 and 3.4 represented very typical nanoparticle/ferritin structures observed across samples that were irradiated with UV light during synthesis for a wide variety of times. Structures like this were observed in samples that were irradiated for as little as 15 minutes or as much as 75 minutes during synthesis. Once the threshold of sixty minutes of exposure was crossed,

however, nanoparticle structures began to be far more irregular. Figure 3.5 shows a TEM image of a collection of nanoparticles that were exposed to UV light for 75 minutes during synthesis. The nanoparticle shapes and sizes here are very irregular, with diameters ranging from 4 nm to approximately 10 nm. The mechanism causing this irregularity after a specific time is unknown, but it may simply be a natural by-product of the nanoparticles growing larger and larger. Due to issues with measuring concentrations of H_2 gas we have been unable to ascertain the effect this change in nanoparticle structure has on catalytic activity.

3.2 Analysis of nanoparticle catalytic activity in hydrogen evolution

Gas chromatography also proved to be a problematic method in quantifying H_2 content that was synthesized. We discovered that hydrogen gas which formed in our vials tended to leak out very quickly, in some cases before we could perform chromatography measurements. This obviously diminished the accuracy of the readings taken with gas chromatography. We have attempted to minimize the potential for error by running our samples through gas chromatography directly after H_2 synthesis, but it is still unknown how much gas might be leaking out of the sample container before measurement. After reducing time between synthesis and gas chromatography we were able to positively detect the presence of H_2. This detection, however, presented its own issues and uncertainties on top of what has been previously discussed.

Gas chromatography returns concentrations of H_2 in units of gas parts per million, but we have not devised an accurate way to find the pressure of the sample gas in its container after H_2 synthesis. Consequently we have been unable to calculate absolute amounts of hydrogen produced, which would be critical for forming a quantitative model of hydrogen formation.

It should also be noted that H_2 production was only recorded in relative units in Petrucci [4],

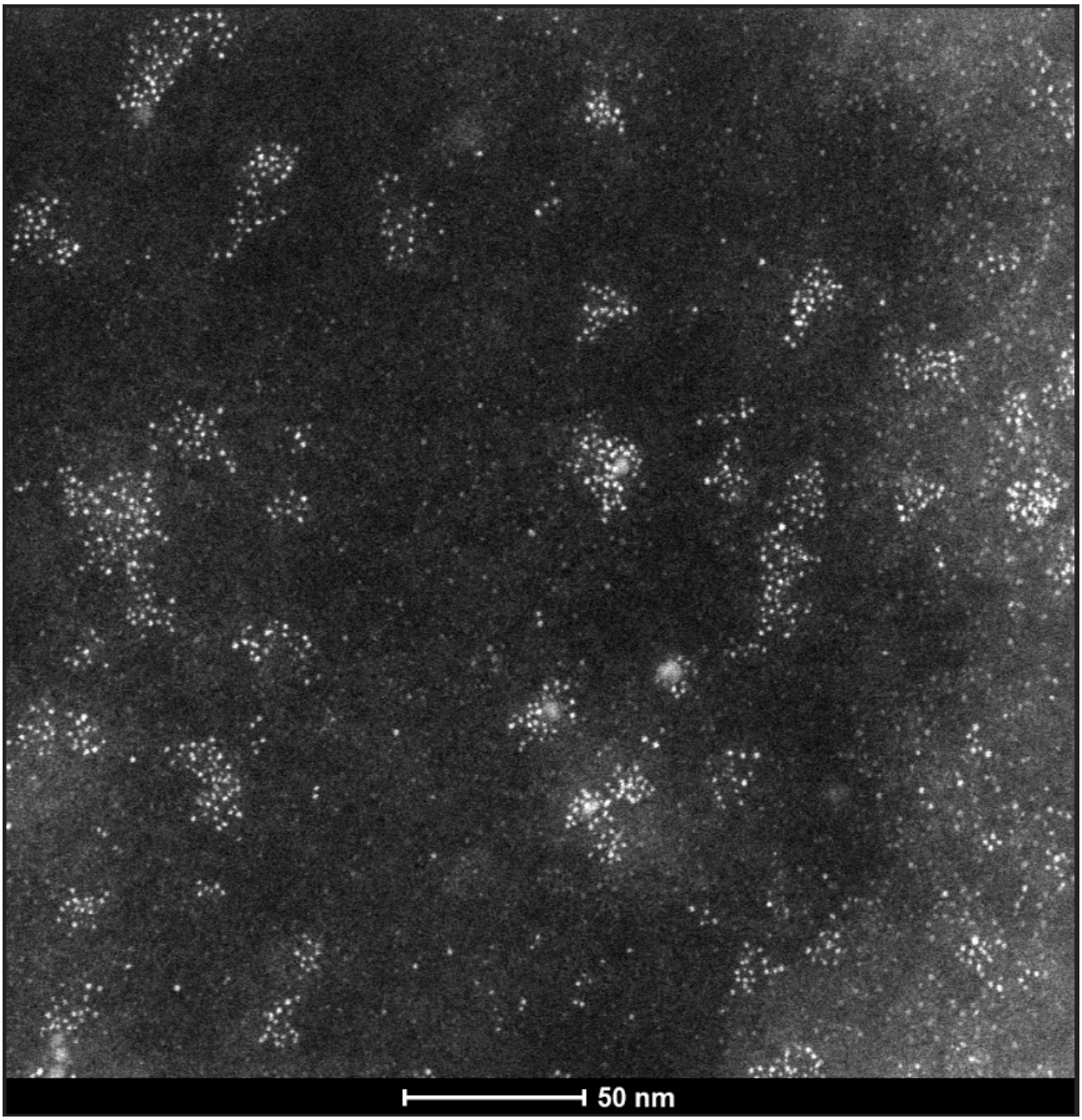

Figure 3.3 This image shows a zoomed-out image of over twenty ferritin protein complexes with platinum nanoparticles bound to the outside. The bright white dots that are approximately 1-2 nm in diameter are the platinum nanoparticles and the fainter smudges approximately 8 nm in the centers of clusters of nanoparticles are the iron mineral core of the ferritin. This image was taken at 580 kx magnification with the TEM set to 200 kV STEM mode. The nanoparticles here were irradiated for 60 minutes during synthesis.

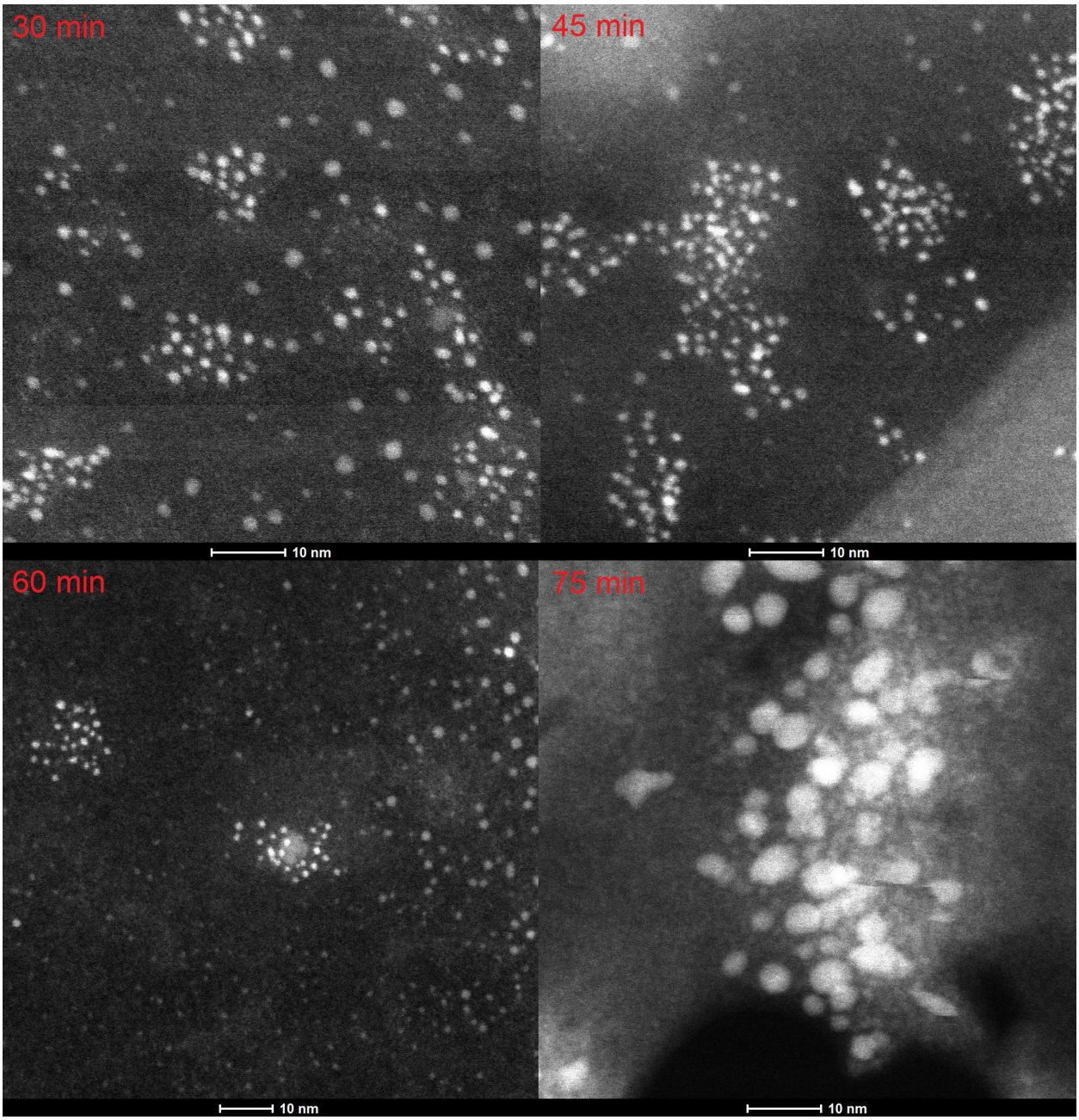

Figure 3.4 This image shows greater detail on a variety of samples. The caption in the upper corner of each image correspond to the amount of time that sample was exposed to UV light during synthesis. As before, the bright white dots that are approximately 1-2 nm in diameter are the platinum nanoparticles and the fainter smudges approximately 8 nm in the centers of clusters of nanoparticles are the iron mineral core of the ferritin. It should be noted not all of the clusters of nanoparticles appear to surround a ferritin that has a mineral core. Though we can not see the ferritin's iron core because it has been lost, the nanoparticles do surround a ferritin shell. These images were taken with the TEM set to 200 kV STEM mode.

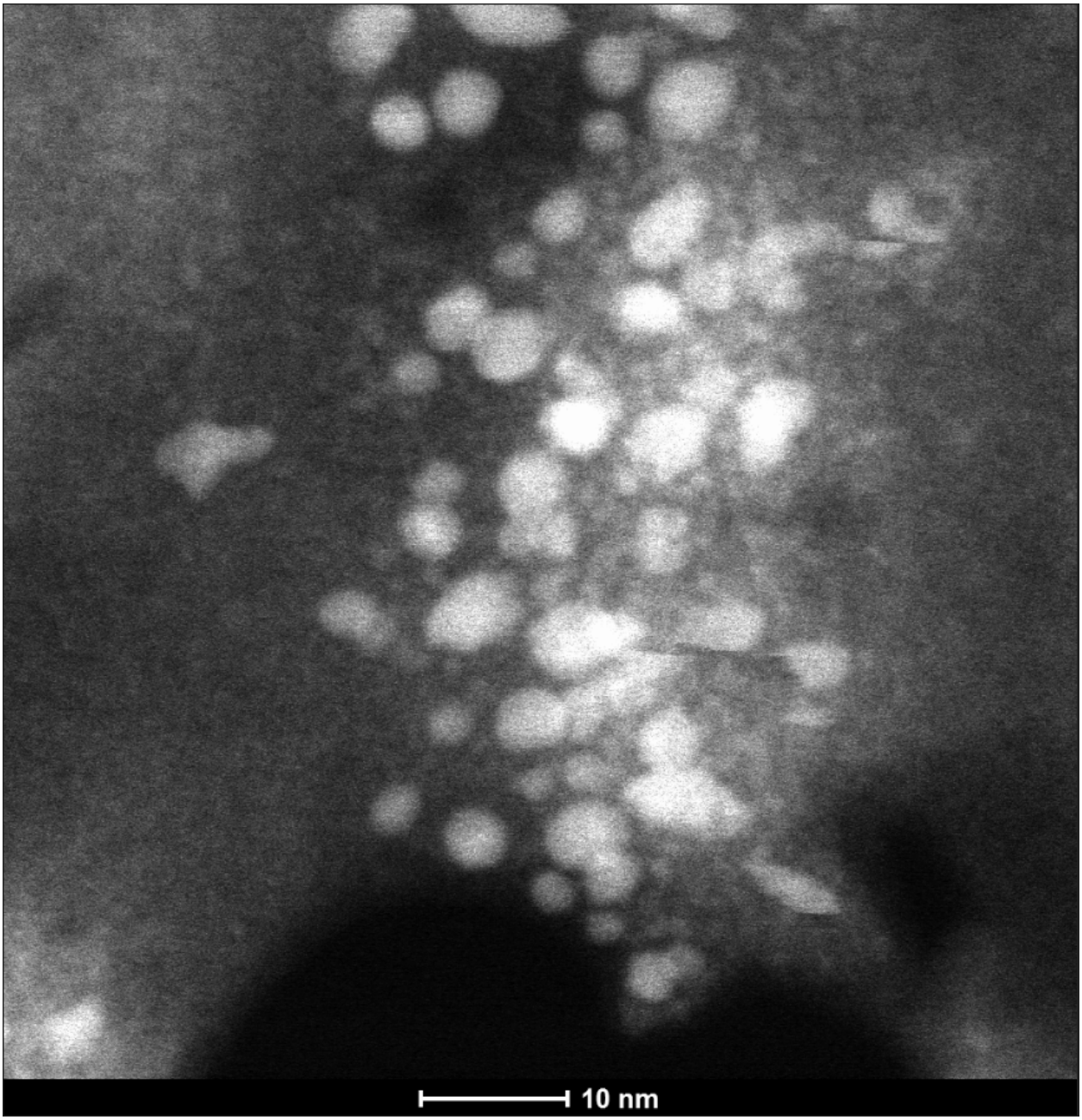

Figure 3.5 This image shows detail on the irregularly-formed platinum nanoparticles that formed after 75 minutes of exposure during synthesis. These nanoparticles are irregularly shaped and at their largest point seem to be as much as 8 nm in diameter. There are no ferritin cores apparently visible in this image, though they may simply be obscured. This image was taken at 2.3 Mx magnification with the TEM set to 200 kV STEM mode. The nanoparticles here were irradiated for 75 minutes during synthesis.

limiting our ability to fulfill our goal of comparing our results to that in Petrucci, unless we were somehow able to perfectly reproduce the container setup for hydrogen synthesis used there, but details on configuration were not recorded in any detail.

3.3 Conclusion and future outlook

Through the course of this work we have been able to image nanoparticle structure using electron microscopy and determine that nanoparticle structure remains uniform through a wide range of synthesis times. We have also positively verified the synthesis of hydrogen gas, though we do not have a reliable quantitative measurement of hydrogen gas produced for any samples. To meet our final goal of verifying the work of Petrucci, we require more accurate methods for metal and protein content and have several technical challenges to overcome in measuring the content of H_2 after synthesis. The lack of absolute units used by Petrucci in reporting H_2 concentrations also presents a setback in comparing our work to his.

Moving forward, I recommend that our group focuses on reevaluating possible methods for measuring metal and protein content, that we investigate methods to reduce leakage of H_2 from reaction vials after synthesis, and devise a method to measure the absolute amount of hydrogen produced after synthesis.

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