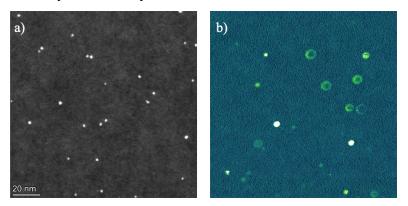
## Quantifying Nanoparticles via Single-Electron Tunneling in e-EFM

C. Boisvert, 1,# J. Bustamante<sup>2</sup>, L. Fairgrieve-Park<sup>1</sup>, O. Hmam<sup>3</sup>, A. Badia<sup>3</sup>, P. Grütter<sup>1</sup>

# Presenting author's e-mail: catherine.boisvert4@mail.mcgill.ca

Single-electron electrostatic force microscopy (e-EFM) has emerged as a powerful technique to probe single-electron tunneling events in nanoscale systems with discrete charge states. In this AFM-based method, an oscillating conductive cantilever acts as both a local gate and charge sensor, enabling the detection of tunneling events in quantum dots, nanoparticles, and molecules. These events appear as concentric "charging rings" in frequency shift and dissipation images, from which quantum properties such as Coulomb blockade energies, tunneling rates, and reorganization energies can be extracted via bias spectroscopy. However, unambiguously linking these charging rings to specific particles, particularly gold nanoparticles (AuNPs) as small as 1.6 nm, remains challenging, since the particles may not be clearly visible in the topography or may appear indistinguishable from other surface features. In this work, we present a methodology to extract charging ring radii directly from e-EFM dissipation images and establish a quantitative correlation with AuNP diameters. By comparing the distribution of ring radii to the nanoparticle size distribution obtained from transmission electron microscopy (TEM), which confirms a narrow range centered around 1.6 nm, we validate this approach for identifying particles of the appropriate size. In addition, this approach also provides insight into the surface density of 1.6 nm AuNPs, which will inform future estimates of enzyme coverage following nanoparticle functionalization. This is particularly important for upcoming experiments where 1.6 nm AuNPs will be attached to the metal center of metalloenzymes, requiring a precise size match with the enzyme's active site. By validating this ring-to-radius relationship, we build a foundation for targeted single-particle measurements in our future metalloenzyme-AuNP systems.



**Figure 1.** a) TEM image of synthesized AuNPs with a narrow size distribution centered around 1.6 nm, which will be compared to the ring radii found in the AFM dissipation channel in b).

## References

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<sup>&</sup>lt;sup>1</sup>Department of Physics, McGill University, Montréal, Québec, H3A 2T8, Canada

<sup>&</sup>lt;sup>2</sup>Departamento de Fisica, Universidad San Francisco de Quito, Quito, 170901, Ecuador

<sup>&</sup>lt;sup>4</sup>Department of Chemistry, Université de Montréal, Montréal, Québec, H2V 0B3, Canada