Figure 1.9: Noise isolation and effect. A) The enclosure designed to contain the AFM setup. The AFM setup is mounted on a optical microscope and is isolated from mechanical isolation by a passive vibration isolation platform. **B)** PSD showing the effect of a precursor of the acoustic enclosure shown in A on the acoustic vibrations picked up by the AFM cantilever. In red a PSD without acoustic enclosure, in black with acoustic enclosure. The acoustic noise was provided by loudly talking researchers.

1.5 Outline thesis

The work in this thesis is focused on measuring and understanding the mechanical properties of small vesicles. As explained above, these mechanical properties might have a strong influence on *e.g.* the uptake of vesicles by cells. If we can measure and understand these properties, we can investigate if and how the cell responds to them for cellular uptake. The ultimate goal is to exploit the mechanical properties for design of drug delivery vehicles with beneficial properties for cellular uptake.

In **chapter 2** I describe the procedure that can be used for measurement of the geometry and bending modulus of vesicles. This procedure is based on reported nano-indentation studies of other nanoparticles, such as viruses. However, there are critical differences when performing experiments with vesicles, *e.g.* the deformation of vesicles onto the surface and bilayers adhering to the AFM tip. Also we introduce improvements in the data analysis.

In **chapter 3** I use the approach from chapter 2 for the mechanical characterization of unilamellar vesicles. A new model, based on Helfrich mechanics, is introduced here to describe the mechanical behavior of vesicles upon indentation. We show excellent agreement between measurements and this theory. Futhermore, we

show that previously observed non-linear behavior can be attributed to the size of the AFM tip. Also, this chapter reveals the critical role of pressure in providing stiffness to deformed vesicles. Finally, we measure the bending modulus of pressurized vesicles.

Chapter 4 addresses the case of multilamellar vesicles. Multilamellar vesicles might have beneficial properties for drug delivery purposes, such as increased volume for hydrophobic drugs. In this chapter, I show that we can determine the amount of lipid bilayers of a vesicle using AFM. We then show that multilamellar vesicles stay in a significantly more spherical shape and are stiffer, both potentially beneficial for uptake by cells.

Naturally excreted vesicles by red blood cells are the subject of **chapter 5.** Naturally excreted vesicles contain many proteins in the lumen and in the membrane. In this chapter we show that nevertheless they can be well described using our model introduced in chapter 3, meaning that they can be essentially described as just a fluid lipid bilayer. We show that mechanical properties of vesicles are altered for patients suffering from spherocytosis, who excrete vesicles with a lower bending modulus. Excretion of these softer vesicles could result into a stiffening of the red blood cells.

Tip wear is usually an unwanted effect in AFM. In **chapter 6** I show that tip wear can be turned into an advantage. I show that tip wear on high roughness surfaces results in a gradual increase in tip size, and that the geometry of the tip apex becomes increasingly rounded. This approach was used to create tips for investigating the effect of tip size on the indentation of vesicles in chapter 3. Furthermore, studying tip wear on high roughness surfaces allows direct tracking of tip shape and hence is potentially beneficial for fundamental studies of tip wear.

Finally, in **chapter 7** I move away from AFM and vesicles. Here we address the response of cell mechanics to a different, always present stimulus: gravity. On the cellular level, the force of gravity seems negligible (it is comparable to the force exerted by individual motor proteins), yet individual cells show altered behavior in microgravity conditions (in space or simulated). In this chapter I review current literature in search of the mechanism of the cellular sensing of gravity.

In **chapter 8** I provide an outlook on how the presented technique and model can be further developed. Here I discuss how the bending modulus of single vesicles can be measured, complementary measurement of the stretch modulus of small vesicles and further vesicles of interest. Finally, I explore some possibilities for testing the role of mechanics of small vesicles on cellular uptake.