# Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XXXVI

# Iron oxide nanoparticles: From purification to characterization.

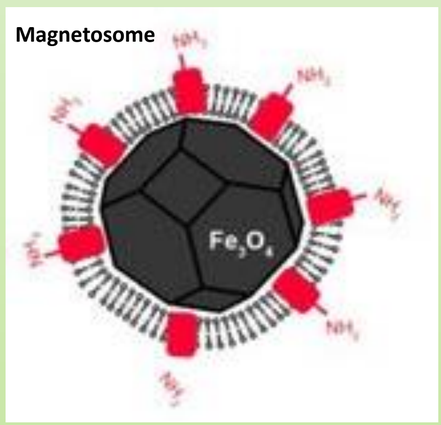
*Laboratorio Biofisica Molecolare, DiSVA*

**PHD student: Adnan Khan** **Tutor: Prof. Francesco Spinozzi**

## INTRODUCTION

In today's world, **nanoparticles** (NPs) are widely used in the treatment of many diseases and drug delivery processes. The dimensions, the morphology, the characteristics of the surface and the type of material used are important aspects to consider for a tailored functionalization of NPs on the basis of their use. The process of synthesizing pure and uniform sized NPs is a quite expensive and time-consuming. An innovative approach to overcome these problems is the use of naturally synthesized NPs, which are isolated from bacteria. In the PhD project, we propose to use iron-NPs naturally produced by **Magnetotactic bacteria**. These iron-NPs, also called **Magnetosomes**, are formed by a core of iron oxide (Fe3O4) coated with a lipid bilayer filled with membrane proteins. This protein-rich lipid bilayer could provide a large surface area for the attachment of desirable drugs or bioactive molecules.

Currently we are characterizing magnetosomes purified from Magnetospirillum gryphiswaldense by means of Small Angle X-Ray Scattering (SAXS), Dynamic Light Scattering (DLS) and Atomic Force Microscopy (AFM).

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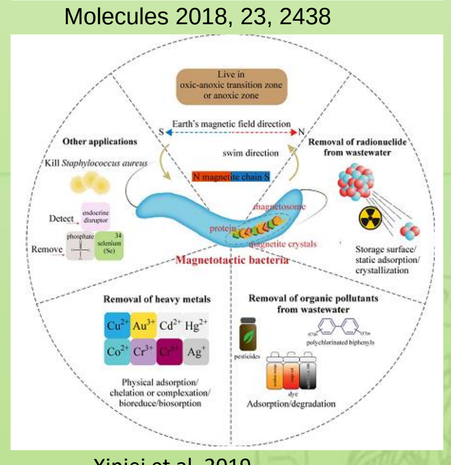
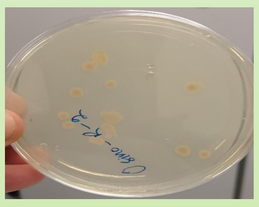
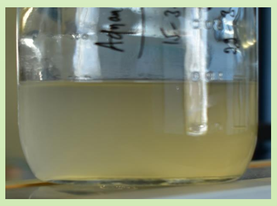



Xinjei et al.,2019

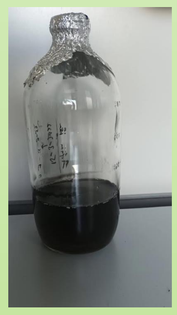
## MAGNETOSOME PURIFICATION

- Culturing of the strain Magnetospirillum gryphiswaldense in anaerobic conditions

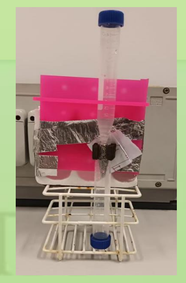




- Lysis of cells through centrifugation and sonication to obtain magnetosomes



- Purification of magnetic nanoparticles (magnetosomes) by Magnetic separation column



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## SMALL ANGLE X RAY SCATTERING

- SAXS curve recorded at ESRF, The European Synchrotron (Grenoble, France).
- Beam-line ID2.
- Magnetosomes prepared in 70 mM PBS solution and kept at 4° C.
- The curves were recorded at 25° C
- SAXS data analysis in progress.

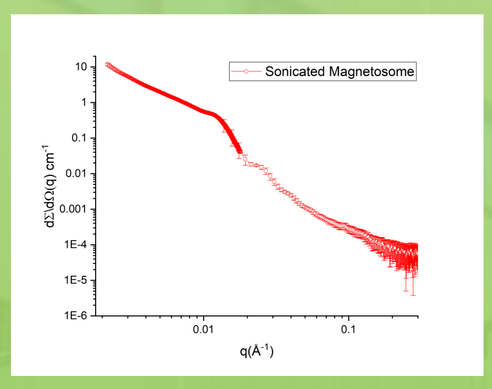



**Fig.1**

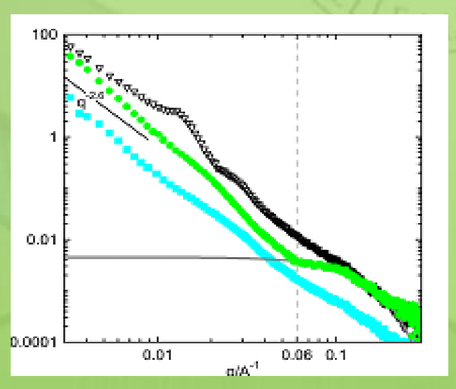
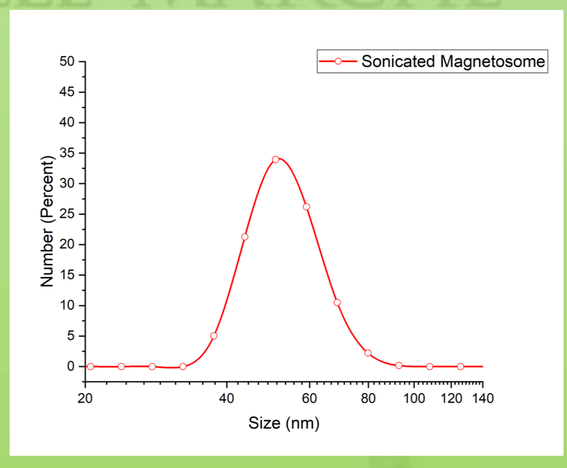



**Fig.2**

Comparison of the SAXS curve of our sample (Figure 1) with the ones published by Sabine et al. (2021) (Figure 2)

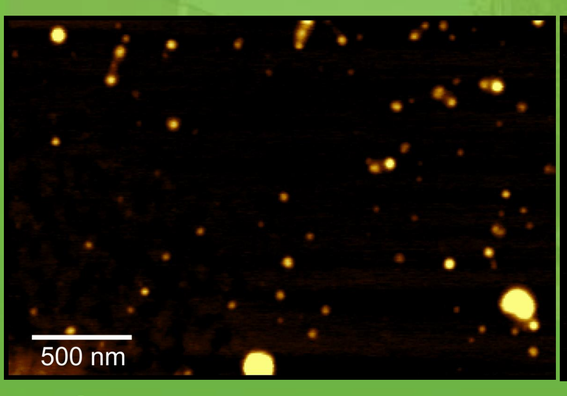
## DYNAMIC LIGHT SCATTERING

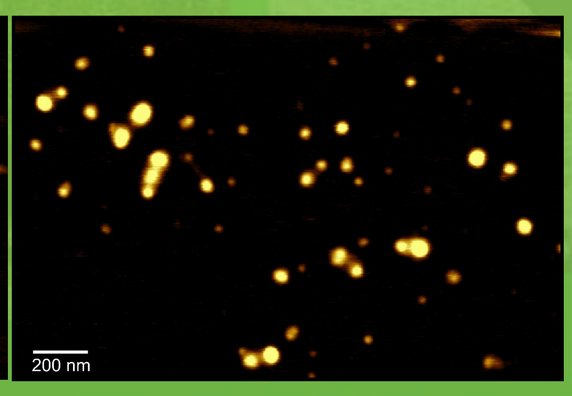
DLS measurements were done using a Malvern Zetasizer PRO operating with a fixed angle of 173°. The temperature was at 25° C.
The diameter of the magnetosomes results around 35-55 nm, with a poly dispersion index (PDI) of 20%.

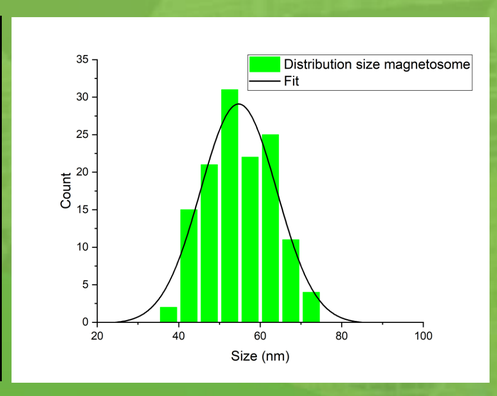



## ATOMIC FORCE MICROSCOPY

AFM measurements were carried out on an AIST-NT's Scanning Probe Microscopy, (Horiba Scientific). Images were generated in non-contact mode, with a pyramidal silicon tip with radius of 8 nm. Samples were measured at the original concentration. 5 mL of solution were deposited on a freshly cleaved mica surface and then dried by nitrogen blow down.
All images were acquired at resolution of 512×512 pixels, with a scan rate of 1 Hz and were analyzed with the Gwyddion and ImageJ (version 1.8.0) software. A statistical measurement of the average particle diameter was performed on a large
enough number of particles (>80). The average diameter resulted 54.7 nm with PDI 18.6.




## PERSPECTIVE

- ❑ Transmission Electron Microscopy (TEM) will be performed in order to find out the internal structure and crystal properties of magnetosomes chain inside the bacteria.
- ❑ Scanning Electron Microscopy (SEM) will be performed to find out surface composition.
- ❑ X-Ray Diffraction (XRD) will provide information on the crystalline structure of the magnetosomes.
- ❑ After the characterization, the drug delivery properties of our purified magnetosomes will be investigated.

### Future Applications of Magnetosomes

- Drug delivery
- Wastewater treatment
- Bio mineralization
- Magnetic resonance imaging
- Magnetic Hyperthermia

## References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- M. Du, Y. Chen, J. Tu, C. Liufu, J. Yu, Z. Yuan, X. Gong, ZhiYi Chen, Ultrasound Responsive Magnetic Mesoporous Silica Nanoparticle-Loaded Microbubbles for Efficient Gene Delivery, ACS Biomater. Sci. Eng. 6 (5) (2020) 2904–2912, https://doi.org/10.1021/acsbiomaterials.0c00014.
- Global Cancer Observatory, see https://gco.iarc.fr for information on global cancer statistic