Electrical and magnetic properties of ferritin: electron transport phenomena and electron paramagnetic resonance

Labra Muñoz, J.A.

1

INTRODUCTION

In this chapter, a brief description of the role of proteins in bioelectronics and magnetic applications is introduced. In particular, the relevance of metalloproteins is provided, along with some of their most prominent applications. After that, ferritin is introduced as a promising metalloprotein that offers the possibility of employing it as a central component to fabricate single-electron bio-transistors while empathizing the ferritin connection to iron-related neurodegenerative diseases. In this context, the dissertation covers two main topics: the electrical description of ferritin, and its magnetic characterization.

1

1.1. PROTEIN-BASED BIOELECTRONICS AND MAGNETISM

The interest in using proteins as the main bricks to generate bio-electronic devices has been increasing over the past decade, considering proteins as ideal prospects in a wide range of applications [1–6]. On one hand, their high specificity makes them ideal candidates for sensor usage. Additionally, proteins allow flexibility to be included in an electronic circuit, due to the various forms in which proteins can be used (scaffolds, films, single particles, fibers, among others). Another valuable aspect is their biocompatibility, a property that makes them unique materials for the fabrication of biomedical devices. [1] Some examples of possible uses of proteins in the design of bioelectronic devices include, bio-molecular circuits [2], creation of artificial retinas [3], early disease [4, 7] and environmental toxin [5, 6] detections, etc.

In terms of possible magnetic applications, the combination of proteins and magnetic materials is also getting a growing attention. Protein material has been widely used for creating magnetic composites based on the combination of proteins (e.g. keratin, elastin, collagen, soy, etc.) [8–13] and polysaccharides with magnetic materials [14, 15]. These materials are easy to isolate and incorporate into devices, being harmless to human health since they degrade in the body. [1]

However, above what is mentioned before, a specific type of protein is in vogue, the so-called metalloproteins [16]. Metalloproteins are proteins that have a metal-ion cofactor [17]. Approximately half of all proteins have metal content (magnesium, iron, zinc, copper, and manganese) [18], being associated with a variety of functions, most importantly for human health, catalyzing photosynthesis, respiration, nitrogen fixation, molecular oxygen reduction, and water oxidation [19]. Many metalloproteins have unpaired electrons, behaving as paramagnets, which can be detected and manipulated by magnetic techniques, for example, acting as contrast agents in magnetic resonance imaging (MRI) of biological specimens [16]. In addition to their relevance from a medical point of view, metalloproteins are promising elements in bioelectronics for different reasons. First, they can be used to create logical systems (e.g. based on myoglobin) [20] and biomolecular transistors for data storage [21]. Second, a protein with redox centers has been proposed to be the "island" in single-electron transistors (SET), although, only one SET based on protein has been reported so far [22] (based on myoglobin) as a proof of concept.

1.2. FERRITIN: A PROMISING CANDIDATE

Ferritin is a spherical metalloprotein, capable of storing and releasing iron in a controllable way. It is composed of a protein shell of about 12 nm and within its cavity, iron is stored in a mineral form [23]. The ferritin core resembles an iron-based nanoparticle, which is isolated from the environment by the ferritin shell, which makes ferritin the perfect candidate for the fabrication of a single-electron transistor (SET). In a SET, "the island" is the ferritin core, and the tunnel barriers are defined by the ferritin shell. Another intriguing aspect of ferritin is its potential relation to neurodegenerative diseases, such as Alzheimer's and Parkinson's. The relation is not yet well understood, but the studies indicate that dysfunctional ferritin appears to play an important role [24–27].

Having these two motivations in mind, the need to obtain further information about the physical properties of ferritin is clear; in particular, the magnetic properties can supply information about the ferritin core, while the electrical properties can help to understand the charge transport through ferritin.

1.3. BRIDGING THE SINGLE-PARTICLE AND MULTI-PARTICLE-ENSEMBLE DESCRIPTIONS OF FERRITIN

This dissertation aims to gather information about the electric and magnetic properties of ferritin, to provide insights into the application of ferritin to bioelectronics, and as a methodology to study the role of ferritin in neurodegenerative diseases, as well as its possible use as a diagnostic tool. To achieve this goal, a combination of two approaches is applied, comprising of the study of single-ferritin particles and of multiple-ferritin particles. On one hand, the study of the current vs. voltage characteristics of single-ferritin particles constitutes a source of information for understanding electron transport through them. On the other hand, the magnetic characterization through the analysis and modeling of the electron paramagnetic resonance (EPR) spectra provides information about the electron-spin structure of the ferritin core based on the measurement of multiple ferritins. To combine both approaches, the magnetic information gathered by EPR on multiple ferritin particles can be compared with the information from one single ferritin subjected to an externally applied varying magnetic field (B_0) while three-terminal electrical measurements are performed. Figure 1.1 displays a first result, in which the differential conductance ($\mathrm{d}I/\mathrm{d}V$) of a single ferritin particle is measured as a function of B_0 while varying the bias voltage between source and drain electrodes (V_{SD}). Whilst sweeping B_0, some transitions between different ground or excited states can be observed as variations in the horizontal lines, e.g., the ones indicated by the arrows. The vertical axis can be considered as an energy axis, and therefore, Fig. 1.1 shows that some transitions have a magnetic origin, since their energy changes as a function of B_0. Unfortunately, the magnetic effect can not be studied in detail, since small switches in the $\mathrm{d}I/\mathrm{d}V$ hinder the detection of the magnetic transitions. These switches are also observed in the absence of magnetic field and gate voltage and are described in chapter 4.

The current vs. voltage (IV) characteristics that comprise the data displayed in Figure 1.1 present Coulomb-blockade behavior, which is characteristic of a single-electron transistor. Therefore, one way to enhance the magnetic transitions is to tune, with a gate electrode and at $B_0 = 0$ T, the chemical potential of the ferritin core to the value in which the Coulomb blockade is lifted. This occurs at the so-called degeneracy points, in which changing the number of electrons from N to N+1 does not cost any energy [28]. In these points, the blockade region is minimized making the detection of small $\mathrm{d}I/\mathrm{d}V$ variations more sensitive to magnetic field variations. For the device whose results are shown in Fig. 1.1, finding the degeneracy point was not possible, since the silicon back gate of a standard silicon substrate was used, which had a low gate coupling, inducing an insufficient shift in the electrochemical potential of the ferritin to properly identify the degeneracy point. In this dissertation, we address this issue, by fabricating devices that

1

have a local gate, resulting in a gate coupling that is at least one order of magnitude larger than the gate coupling that results from using the standard silicon back gate.

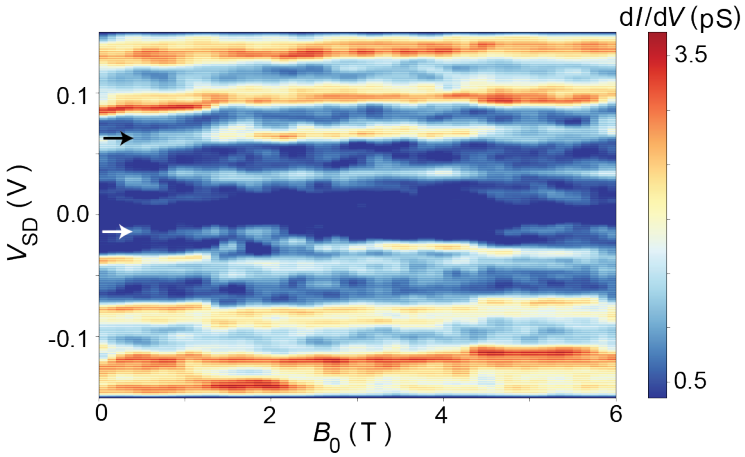

Figure 1.1: Differential conductance of a single ferritin particle in the presence of a magnetic field, recorded at ~ 850 mK and zero gate voltage. Variations in the horizontal lines indicate transitions between different ground or excited states. Two examples are indicated by the arrows. The vertical axis can be viewed as an energy axis and the experiment thus shows that some transitions are magnetic (since their energy changes as a function of the magnetic field).

On the other hand, EPR enables to correlate magnetic phenomena with specific physical events, since it is a technique for studying unpaired electrons in materials, by detecting the transitions between their spin states [29]. EPR allows the determination of the material-characteristic energy that is required for those transitions to occur at a given magnetic field strength. Additionally, EPR can help to establish the intrinsic properties of the unpaired electrons in the material under study [30]. Some examples of these properties are the g factor, which results from the electron gaining or losing angular momentum; the zero-field splitting, which originates from the presence of more than one unpaired electron in the material; dipole-dipole interactions; among others [30]. Due to the high sensitivity of EPR, this technique is used in a broad range of applications. Some very recent examples include studying chemical reactions (electronic structures, reactivity, etc.) [31, 32], dating characterization of materials in geology and archaeology [33, 34], medical and biological systems (e.g. spin labels) [35, 36], to quantum computing studies (pulsed-EPR electron-spin qbit control) [37, 38]. In our particular case, we use continuous-wave (CW) EPR to study the electron-spin structure of the ferritin core, attempting the quantification of the magnetic properties that result from ~ 10^{12} ferritin particles. Published EPR studies on the interpretation of the magnetic properties of ferritin are based on different models that consider the lineshape dependence on frequency [39], anisotropy [40], temperature [41], among other characteristics [42–44]. However, these studies do not reproduce all the features that are visible in the EPR spectra. Instead, we described the ferritin magnetic system based on the Giant spin model [45], a model that treats the total magnetic moment of the ferritin core as com-

posed of all the individual magnetic moments within the core. This approach reveals multiple EPR spectral components that result from the presence of the magnetic moment and the anisotropy-field distributions. Finally, on its own, EPR is a powerful technique that we propose as a potential tool for investigating iron-related diseases, due to its particular sensitivity to the surface spins of the ferritin core.

In the next step, it would be interesting to examine, if the magnetic characterization obtained from a ferritin single-electron transistor in the presence of a varying magnetic field can be related to the EPR description that resulted from the analysis of multiple ferritin particles.

1.4. DISSERTATION OUTLINE

The outline of this thesis is as follows: After this introduction, the implementation of a local gate in wide self-aligned nanogap devices is described in chapter 2. We then, in chapter 3, electrically characterize single-ferritin particles that are trapped in self-aligned nanodevices that did not possess a local gate. In chapter 4, we use the devices that were described in chapter 2 to perform three-terminal electrical measurements on single ferritin, resulting in the fabrication of the first ferritin single-electron transistor and the proposition of two possible scenarios that are consistent with our data, which indicate the involvement of one or more ferritin particles (depending on the case) in the electron transport across them. Following, chapter 5 presents an EPR characterization of ferritin, in which a model of the electron-spin structure of the ferritin core is proposed. Finally, in chapter 6, we extend the model that we proposed in chapter 5, to lyophilized ferritin samples from human liver and post-mortem brain tissues.

1

REFERENCES

[1] M. Torculas, J. Medina, W. Xue, and X. Hu, *Protein-based bioelectronics,* ACS Biomaterials Science & Engineering **2**, 1211 (2016).

[2] V. M. Aguilella and A. Alcaraz, *All-optical logic gates using bacteriorhodopsin films,* Nature Nanotechnology **4**, 403 (2009).

[3] Y. Saga, T. Watanabe, K. Koyama, and T. Miyasaka, *Mechanism of photocurrent generation from bacteriorhodopsin on gold electrodes,* The Journal of Physical Chemistry B **103**, 234 (1999).

[4] S. Carrara, V. V. Shumyantseva, A. I. Archakov, and B. Samorì, *Screen-printed electrodes based on carbon nanotubes and cytochrome p450scc for highly sensitive cholesterol biosensors,* Biosensors and Bioelectronics **24**, 148 (2008).

[5] I. Palchetti and M. ascini, *Electroanalytical biosensors and their potential for food pathogen and toxin detection,* Analytical and Bioanalytical Chemistry **391**, 455 (2008).

[6] F. S. Ligler, C. R. Taitt, L. C. Shriver-Lake, K. E. Sapsford, Y. Shubin, and J. P. Golden, *Array biosensor for detection of toxins,* Analytical and Bioanalytical Chemistry **377**, 469 (2003).

[7] C. Baj-Rossi, G. D. Micheli, and S. Carrara, *Electrochemical detection of anti-breast-cancer agents in human serum by cytochrome p450-coated carbon nanotubes,* Sensors **12**, 6520 (2012).

[8] K. G. DeFrates, R. Moore, J. Borgesi, G. Lin, T. Mulderig, V. Beachley, and X. Hu, *Protein-based fiber materials in medicine: A review,* Nanomaterials **8**, 457 (2018).

[9] B. D. Ulery, L. S. Nair, and C. T. Laurencin, *Biomedical applications of biodegradable polymers,* Journal of Polymer Science Part B: Polymer Physics **49**, 832 (2011).

[10] A. Vasconcelos, G. Freddi, and A. Cavaco-Paulo, *Biodegradable materials based on silk fibroin and keratin,* Biomacromolecules **9**, 1299 (2008).

[11] U. R. Rodgers and A. S. Weiss, *Cellular interactions with elastin,* Pathologie Biologie **53**, 390 (2005).

[12] J. Rnjak-Kovacina, S. G. Wise, Z. Li, P. K. Maitz, C. J. Young, Y. Wang, and A. S. Weiss, *Electrospun synthetic human elastin:collagen composite scaffolds for dermal tissue engineering,* Acta Biomaterialia **8**, 3714 (2012).

[13] V. A. Kumar, J. M. Caves, C. A. Haller, E. Dai, L. Liu, S. Grainger, and E. L. Chaikof, *Acellular vascular grafts generated from collagen and elastin analogs,* Acta Biomaterialia **9**, 8067 (2013).

[14] N. B. Shelke, R. James, C. T. Laurencin, and S. G. Kumbar, *Polysaccharide biomaterials for drug delivery and regenerative engineering,* Polymers for Advanced Technologies **25**, 448 (2014).

[15] S. L. McArthur, K. M. McLean, P. Kingshott, H. A. St John, R. C. Chatelier, and H. J. Griesser, *Effect of polysaccharide structure on protein adsorption,* Colloids and Surfaces B: Biointerfaces **17**, 37 (2000).

[16] Y. Matsumoto and A. Jasanoff, *Metalloprotein-based mri probes,* FEBS Letters **587**, 1021 (2013).

[17] L. Banci and I. Bertini, *Metallomics and the cell: Some definitions and general comments,* in *Metallomics and the Cell*, edited by L. Banci (Springer Netherlands, Dordrecht, 2013) pp. 1–13.

[18] A. J. Thomson and H. B Gray, *Bio-inorganic chemistry,* Current Opinion in Chemical Biology **2**, 155 (1998).

[19] Y. Lu, N. Yeung, N. Sieracki, and N. M. Marshall, *Design of functional metalloproteins,* Nature **460**, 855 (2009).

[20] Y.-H. Chung, T. Lee, S.-Y. Yoo, J. Min, and J.-W. Choi, *Electrochemical bioelectronic device consisting of metalloprotein for analog decision making,* Scientific reports **5**, 14501 (2015).

[21] T. Zhang, C. Zhang, G. Fu, Y.-D. Li, L. Gu, G. Zhang, Q. Wang, Song, B. P. Parsons, and R. R. Birge, *All-optical logic gates using bacteriorhodopsin films,* Optical Engineering **39**, 527 (2000).

[22] D. Li, P. Gannett, and D. Lederman, *An investigation into the feasibility of myoglobin-based single-electron transistors.* Nanotechnology **23**, 395705 (2012).

[23] N. Chasteen and P. M. Harrison, *Mineralization in ferritin: An efficient means of iron storage,* Journal of Structural Biology **126**, 182 (1999).

[24] A. Friedman, P. Arosio, D. Finazzi, D. Koziorowski, and J. Galazka-Friedman, *Ferritin as an important player in neurodegeneration,* Parkinsonism and Related Disorders **17**, 423 (2011).

[25] P. Arosio, L. Elia, and M. Poli, *Ferritin, cellular iron storage and regulation,* IUBMB Life **69**, 414 (2017).

[26] C. Quintana, S. Bellefqih, J. Laval, J.-L. Guerquin-Kern, W. Ting-Di, J. Avila, I. Ferrer, R. Arranz, and C. Patiño, *Study of the localization of iron, ferritin, and hemosiderin in alzheimer's disease hippocampus by analytical microscopy at the subcellular level,* Journal of structural biology **153**, 42 (2006).

[27] C. Quintana and L. Gutiérrez, *Could a dysfunction of ferritin be a determinant factor in the aetiology of some neurodegenerative diseases?* Biochimica et Biophysica Acta (BBA) - General Subjects **1800**, 770 (2010).

[28] I. Aleiner, P. Brouwer, and L. Glazman, *Quantum effects in coulomb blockade,* Physics Reports **358**, 309 (2002).

[29] J. A. Bau, A.-H. Emwas, and M. Rueping, *An overview of solid-state electron paramagnetic resonance spectroscopy for artificial fuel reactions,* iScience **25**, 105360 (2022).

[30] J. A. Weil and J. R. Bolton, *Electron Paramagnetic Resonance. Elementary Theory and Practical Applications* (John Wiley and Sons, Inc., Hoboken, New Jersey, 2007) p. 546.

[31] M. M. Roessler and E. Salvadori, *Principles and applications of epr spectroscopy in the chemical sciences,* Chem. Soc. Rev. **47**, 2534 (2018).

[32] S. Suzen, H. Gurer-Orhan, and L. Saso, *Detection of reactive oxygen and nitrogen species by electron paramagnetic resonance (epr) technique,* Molecules **22**, 28117726 (2017).

[33] V. Guilarte, *Esr dating in archaeology,* in *Spectroscopic and Microscopy Techniques for Archaeological and Cultural Heritage Research (Second Edition)*, 2053-2563 (IOP Publishing, 2023) pp. 5–1 to 5–29.

[34] C. Bortolussi, A. Zoleo, L. Maritan, A. Collauto, M. Brustolon, M. Marrale, A. Parlato, and D. Usai, *Electron paramagnetic resonance and petrographic analysis for dating mesolithic and neolithic pottery from al khiday (sudan),* Radiation Measurements **89**, 89 (2016).

[35] E. Zurlo, L. Passerini, P. Kumar, and M. Huber, *In situ continuous wave electron paramagnetic resonance investigation of the amyloid aggregation of parkinson's protein alpha-synuclein—the second spin-label position,* Applied Magnetic Resonance **53**, 1133 (2022).

[36] F. Torricella, A. Pierro, E. Mileo, V. Belle, and A. Bonucci, *Nitroxide spin labels and epr spectroscopy: A powerful association for protein dynamics studies,* Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics **1869**, 140653 (2021).

[37] S. Moreno-Da Silva, J. I. Martínez, A. Develioglu, B. Nieto-Ortega, L. de Juan-Fernández, L. Ruiz-Gonzalez, A. Picón, S. Oberli, P. J. Alonso, D. Moonshiram, E. M. Pérez, and E. Burzurí, *Magnetic, mechanically interlocked porphyrin–carbon nanotubes for quantum computation and spintronics,* Journal of the American Chemical Society **143**, 21286 (2021).

[38] L. E. Rosaleny, S. Cardona-Serra, L. Escalera-Moreno, J. J. Baldoví, V. Gołbiewska, K. Wlazło, P. Casino, H. Prima-García, A. Gaita-Ariño, and E. Coronado, *Peptides as versatile platforms for quantum computing,* The Journal of Physical Chemistry Letters **9**, 4522 (2018).

[39] R. J. Usselman, M. T. Klem, M. Allen, E. D. Walter, K. Gilmore, T. Douglas, M. Young, Y. Idzerda, and D. J. Singel, *Electron magnetic resonance of iron oxide nanoparticles mineralized in protein cages,* Journal of Applied Physics **97**, 10M523 (2005).

[40] Y. L. Raikher and V. I. Stepanov, *Ferromagnetic resonance in a suspension of single-domain particles,* Phys. Rev. B **50**, 6250 (1994).

[41] R. Berger, J.-C. Bissey, J. Kliava, H. Daubric, and C. Estournès, *Temperature dependence of superparamagnetic resonance of iron oxide nanoparticles,* Journal of Magnetism and Magnetic Materials **234**, 535 (2001).

[42] R. Berger, J. Kliava, J.-C. Bissey, and V. Baïetto, *Magnetic resonance of superparamagnetic iron-containing nanoparticles in annealed glass,* Journal of Applied Physics **87**, 7389 (2000).

[43] R. Berger, J.-C. Bissey, J. Kliava, R. Berger, and J.-C. Bissey, *Lineshapes in magnetic resonance spectra,* Journal of Physics: Condensed Matter **12**, 9347 (2000).

[44] M. M. Yulikov and P. A. Purtov, *Fmr study of superparamagnetic ni particles with weak and strong magnetic anisotropy,* Applied Magnetic Resonance **29**, 1613 (2005).

[45] M. Fittipaldi, R. Mercatelli, S. Sottini, P. Ceci, E. Falvo, and D. Gatteschi, *Sensing the quantum behaviour of magnetic nanoparticles by electron magnetic resonance,* Phys. Chem. Chem. Phys. **18**, 3591 (2016).