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Cytochrome c as an electron acceptor of nanostructured titania and hematite semiconductors

作为纳米二氧化钛和赤铁矿半导体电子受体的细胞色素 C

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Abstract - **The use of hematite (α-Fe_2O_3) to promote water splitting for the generation of hydrogen and molecular oxygen has gained particular interest due to its characteristics of stability, low cost, abundant precursors and a 2.1 eV band gap that allows absorption of visible light. However, some unfavorable characteristics such as fast charge recombination and band positions impairing unassisted water splitting have challenged researchers for the improvement of hematite photoanodes. In the present study, it was demonstrated that an alkaline protein, respiratory cytochrome c, interacts with the nanostructured semiconductor materials, TiO_2 and α-Fe_2O_3 NPs. The association of cytochrome c with the nanostructured semiconductors was corroborated by zeta potential measurements and scanning electronic microscopy. Cytochrome c is a good acceptor of the electrons promoted to the conduction bands of the semiconductors exposed to simulated sunlight. The photoreduction of cytochrome c by TiO_2 and α-Fe_2O_3 NPs are pH-dependent and favored at the pH range from 9-11. Despite the short lifetime of charge separation, α-Fe_2O_3 was able to promote photo-reduction of bulk and adsorbed cytochrome c detected by electronic absorption spectroscopy. Therefore, this is a model for the development of systems able to bypass the short lifetime of α-Fe_2O_3 charge separation by using efficient electron collectors as cytochrome c.**

Keywords – **cytochrome c, iron oxide, titanium oxide, electron transfer, nanostructure**

I. INTRODUCTION

Sunlight is an abundant energy (~3×10^{24} J yr^{-1}) that directly and indirectly sustains the life on the Earth [1,2]. Living organisms have highly efficient apparatus to produce organic fuels by using solar energy. Photosynthesis is a redox process in which the thermodynamically unfavorable glucose synthesis by using water as the reducing agent is made feasible by collecting solar energy by chlorophyll and accessory pigments [3,4,5,6]. In the light step of photosynthesis, light energy is used for the non-spontaneous electron transport from water to reduce $NADP^+$ to NADPH. In the dark step, NADPH is recycled to NADP+ and the energy is stored in the glucose molecules [3,4]. The biological fuel produced by autotrophic organisms is distributed to the heterotrophic organisms in the alimentary chain. The mimicking of photosynthesis process for the production of fuels is a challenge for a green sustaining of the global production and development. Apparatus constructed with pure and doped semiconductors placed on the surface of a conductive substrate able to absorb visible light have been largely used for sunlight harvesting [7,8]. In these mimetic apparatus, the water splitting is promoted by photoactive semiconductor materials such as TiO_2, WO_3 and hematite. Hematite has gained particular interest due to the characteristics of high electrochemical stability, favorable band gap (2.1 eV) that allows absorption of visible light and low-cost due its abundance in Earth [9]. However some unfavorable characteristics such as short lifetime and low mobility of the photogenerated minority carriers (h+, hole) to

the liquid interface (hematite/water interface) increases the recombination rate and conduction band edge positions impairing unassisted water splitting have challenged researchers for the improvement of hematite photoanodes[9, 10 , 11 , 12 , 13 , 14 , 15]. Considering the application not only in light harvesting but also in biosensing and hydrogen generation, the photo-induced redox process involving heme proteins and semiconductors has gained the interest of the researchers [16 , 17 , 18 , 19 , 20]. Interesting mechanisms involving dyes and aromatic compounds as sensitizers for hemeproteins have been described. The aromatic imide, N,N'-bis(2-phosphonoethyl)-1,4,5,8-naphthalenetetracarboxylic diimide (PNDI) and the well-known dye methylene blue are able to promote photoreduction of hemeproteins such as cytochrome c and horseradish peroxidase [13,21]. The photochemistry of systems composed of cytochrome c and titanium dioxide (TiO_2), an n-type semiconductor material, has been studied using nanoporous TiO_2, TiO_2 nanoparticles (NP) and nanotubes [22,23,24,25,26,27,28,29,30]. In a previous study, we have demonstrated the photo-induced electron transfer from water to cytochrome c promoted by two TiO_2 structures: P25 TiO_2 nanoparticles (NPs) and titanate nanotubes. P25 has low affinity for the hemeprotein but efficiently photoreduced bulk cytochrome c differently of the titanate nanotubes that exhibits high affinity for cytochrome c but photoreduced poorly the remaining non-adsorbed protein. In that study, some basic aspects leading the interaction, functional changes and the photochemistry of TiO_2/cytochrome c systems were investigated by using two TiO_2 structures: TiO_2 NP and titanate nanotubes. It was demonstrated that TiO_2 NP and titanate nanotubes were able to promote the photo-reduction of cytochrome heme iron but with significant differences in molecular arrangements and efficiency [31]. However, due to the high costs, cheaper alternatives to the efficient semiconductors TiO_2 and WO_3 have been searched. This work describes a comparative study of the mechanisms underlying the photochemistry of cytochrome c associated to hematite and anatase.

II. Materials and Methods

The TiO_2 anatase used was the standard material Degussa P25 (Brunauer-Emmett-Teller (BET) surface area = 53.2 $m^2 g^{-1}$), and herein, referred to as TiO_2 NPs (nanoparticles). All other chemicals were purchased from Sigma-Aldrich Co.. For hematite synthesis the reagents $FeCl_3 \cdot 6H_2O$, $NaNO_3$, and HCl, were of analytical grade and were not submitted to further purification.

All aqueous suspensions and solutions were prepared with deionized water (mixed bed of ion exchanger, Millipore®), and the pH was measured using a combined glass electrode (Orion Glass pH SURE-FLOWTM). The reference electrode (ROSSTM, model 8102) was filled with OrionFilling Solutions (ROSSTM). The pH meter was calibrated using METREPAK pHydrion standard buffer solutions (Brooklyn, NY) Chemicals.

Rat liver mitochondria were isolated by conventional differential centrifugation from the livers of adult Wistar rats. The homogenate was prepared in 250 mM sucrose, 1.0 mM EGTA, and 5.0 mM HEPES buffer (pH 7.2). The mitochondrial suspension was washed twice in the same medium containing 0.1 mM EGTA, and the final pellet was diluted in 250mMsucrose to a protein concentration of 80-100 mg/mL.[32,33,34] Mitoplasts (mitochondria devoid of the outer membrane) were prepared exactly as described by Pedersen et al. [23, 24] For spectrophotometric assays, mitochondria were incubated at 30 °C with 2.5 μM rotenone in a standard incubation medium containing 125mM sucrose, 65m MKCl, 10mM HEPES-KOH, 0.5mM EGTA, and 10 mM K2HPO4 at pH 6.0 (maximal affinity of cytochrome c for cytochrome c-depleted mitoplasts.[31] Electronic absorption spectra of a mitoplast sample were obtained before and after the addition of 0.4 nmol of cytochrome c/mg of mitochondrial protein, determined according to ref. 31. The sample was placed in a bucket with a septum cap and purged with nitrogen before the first scanning and the addition of cytochrome c. After addition of cytochrome c new spectral acquisition was performed and the data loaded into the program Microcal Origin 8.5. The spectrum of cytochrome *c*-depleted mitoplasts was subtracted off the spectrum obtained after the hemeprotein addition.

Hematite nanoparticles were prepared by a chemical route using an aqueous solution controlled under hydrothermal conditions as previously reported.[35]

For adsorption of cytochrome c on TiO_2 NPs and hematite, a cytochrome c solution (10 μM (~0.125 mg mL^{-1}) was used for direct adsorption of the protein on TiO_2 NPs and hematite (0.05 mg mL^{-1}) suspension in 5 mM universal buffer, in the dark.

For irradiation of the samples, the sunlight conditions were simulated using a 450 W xenon lamp (Osram, ozone free) and an AM 1.5 filter. The light intensity was adjusted to the value of 100 $mWcm^{-2}$. Here, all experiments were conducted using this fixed light intensity and the samples containing TiO_2 NPs and hematite were all irradiated concomitantly.

The spectra of the samples were obtained at each irradiation interval were recorded using a Thermo Scientific™ Evolution™ Array UV-Visible spectrophotometer (MA, USA). The optical path length was 0.1 cm for all measurements.

The zeta potential (ζ) measurements of zeta potential were carried out in a Zetasizer Nano ZS, Malvern Instruments, Ltd. (London, UK), at the temperature of 25 °C. The ζ is determined by the electrophoretic mobility, μ_e, by the application of the Henry equation and calculated by using the Smoluchowski approximation. The ζ values are the average of 10 independent measurements that were calculated using a mono-modal model and time measurement was determined by the instrument.

The scanning electron microscopy (SEM) of the samples was carried out using a compact low vacuum electronic scanning microscopy JSM-6010LA, JEOL (Tokyo, Japan).

III. RESULTS

Previously, it was demonstrated that TiO_2 (anatase) NPs adsorbs cytochrome c and can promote efficient photoreduction of the protein that remained in solution [30]. Considering the crescent interest in hematite (α-Fe_2O_3) due to the advantages described before, here, it was performed a comparative study with α-Fe_2O_3 (hematite) and TiO_2 (anatase). To compare the properties of hematite and anatase associated to cytochrome c, we previously investigated the effect of pH on the zeta potential (ζ) of bare and cytochrome c-covered semiconductor nanostructures analyzed in a 2.5 mM universal buffer adjusted to each desired pH value. Figure 1 A and B show respectively, the ζ values of TiO_2 and α-Fe_2O_3 NPs, respectively. The experiments were carried out in the absence and presence of cytochrome c for both NPs.

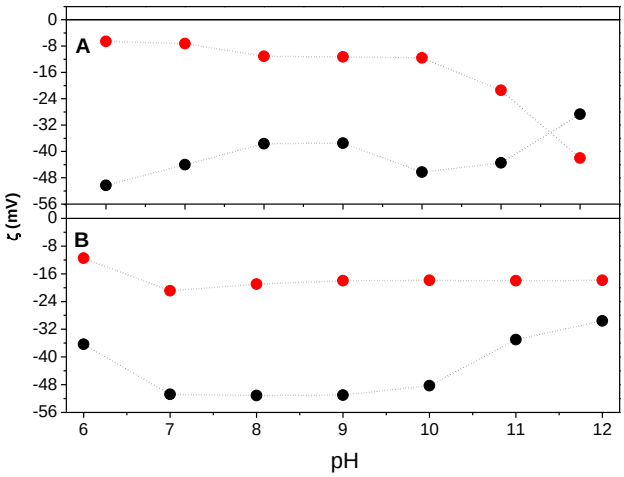
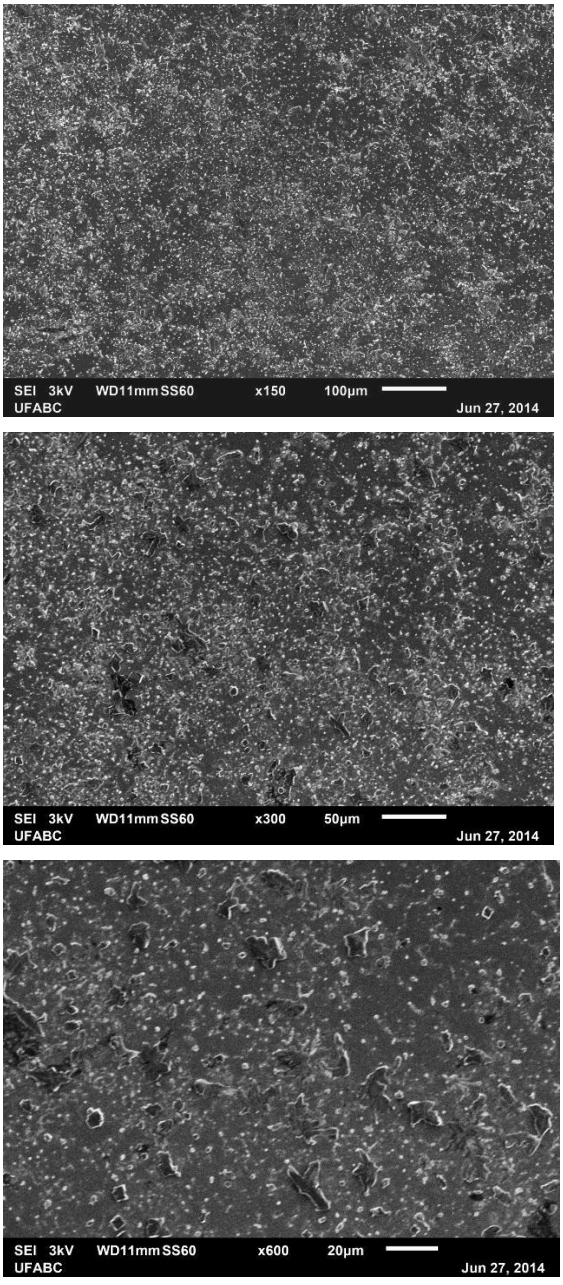


Fig. 1. The pH-dependent ζ values of bare (black closed circles) and cytochrome c-capped (red closed circles) TiO_2 NPs (A) and hematite (B). The samples were prepared in 2.5 mM universal buffer (acetate, phosphate and borate).

The pH-dependent curve of ζ values, obtained for the bare oxide nanoparticles in universal buffer reflects the effects of polyprotic phosphate and boric acids present in the composition of a universal buffer. In a similar manner to that described for the use of citric acid as a buffer [36], phosphate and borate led to a negative zeta potential at the analyzed pH range. The highly negative ζ obtained for bare TiO_2 at pH 6.0 suggests an effect of flocculation. Also, the significant increase of ζ observed for bare TiO_2 NPs at high pH values may be assigned to disaggregation and decrease of phosphate and borate adsorption.[33] For bare α-Fe_2O_3 the ζ values obtained at acidic pH values might reflect flocculation and even chemical modifications because the chemical instability of the material observed at pH < 7.0. Above pH 7.0, by increasing the pH, α-Fe_2O_3 gains stability and increasing ζ values are observed at pH values $\geq$ 10.0. The pH-dependent ζ values obtained for TiO_2 and α-Fe_2O_3 NPs incubated with cytochrome c are consistent with adsorption of the alkaline protein (pI = 10.2) leading to stabilization of the materials at all the analyzed pH range [37]. The decrease of cytochrome c-treated TiO_2 NPs ζ values at pH above 10 is consistent with decrease the adsorption of the hemeprotein above the pI. However, cytochrome c-treated α-Fe_2O_3 did not exhibit significant decrease of ζ values even at pH > 10.0. This result suggests a strong interaction between the negatively charged surface of α-Fe_2O_3 and cytochrome c leading to an increase of the protein pI at the interface. Furthermore, the occurrence of other types of interactions such as hydrogen bonds between cytochrome c and α-Fe_2O_3 could not be discarded and deserves future investigations.

A



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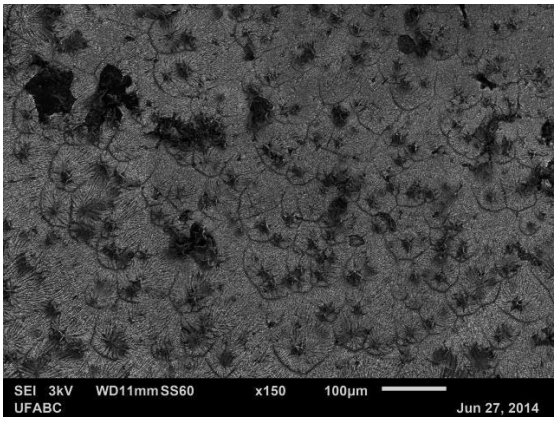
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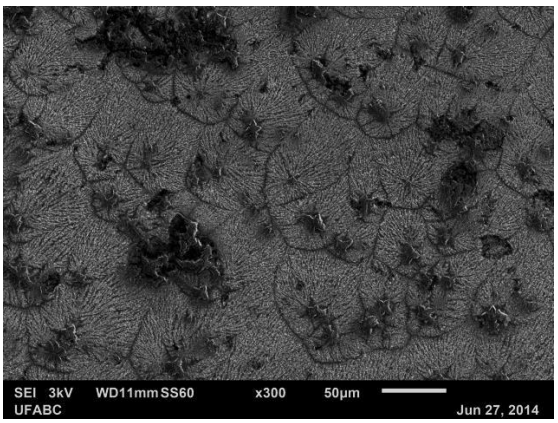
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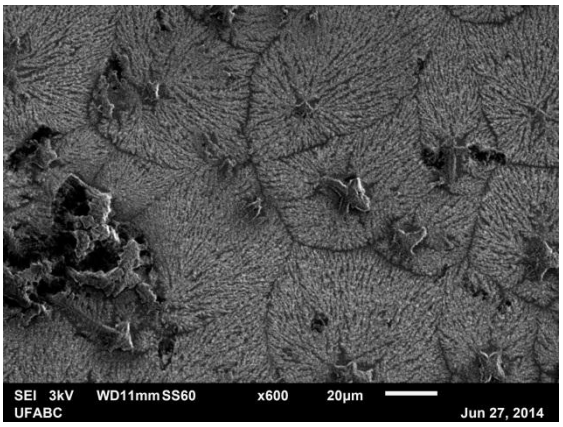
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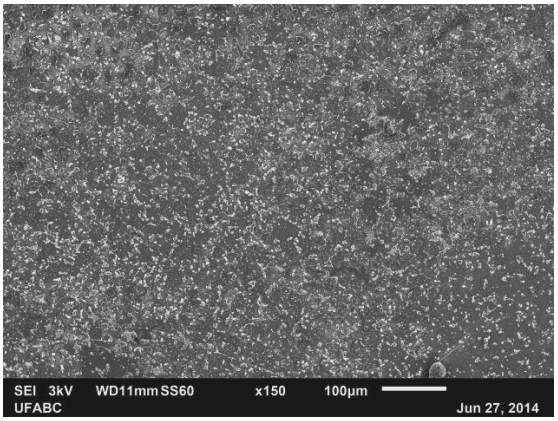
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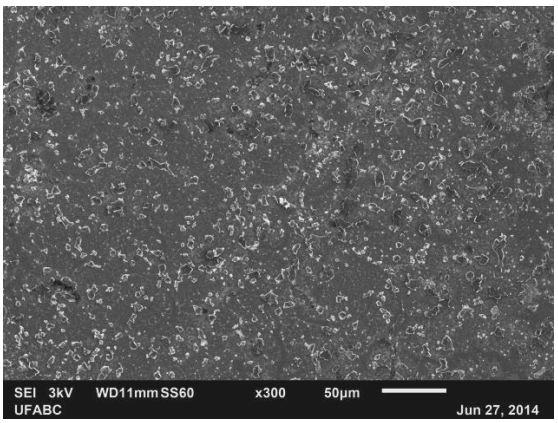
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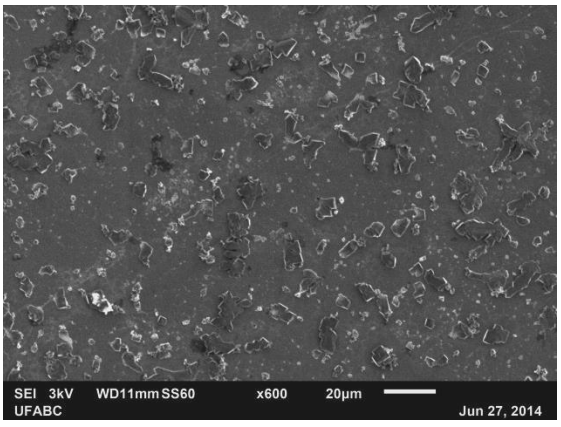
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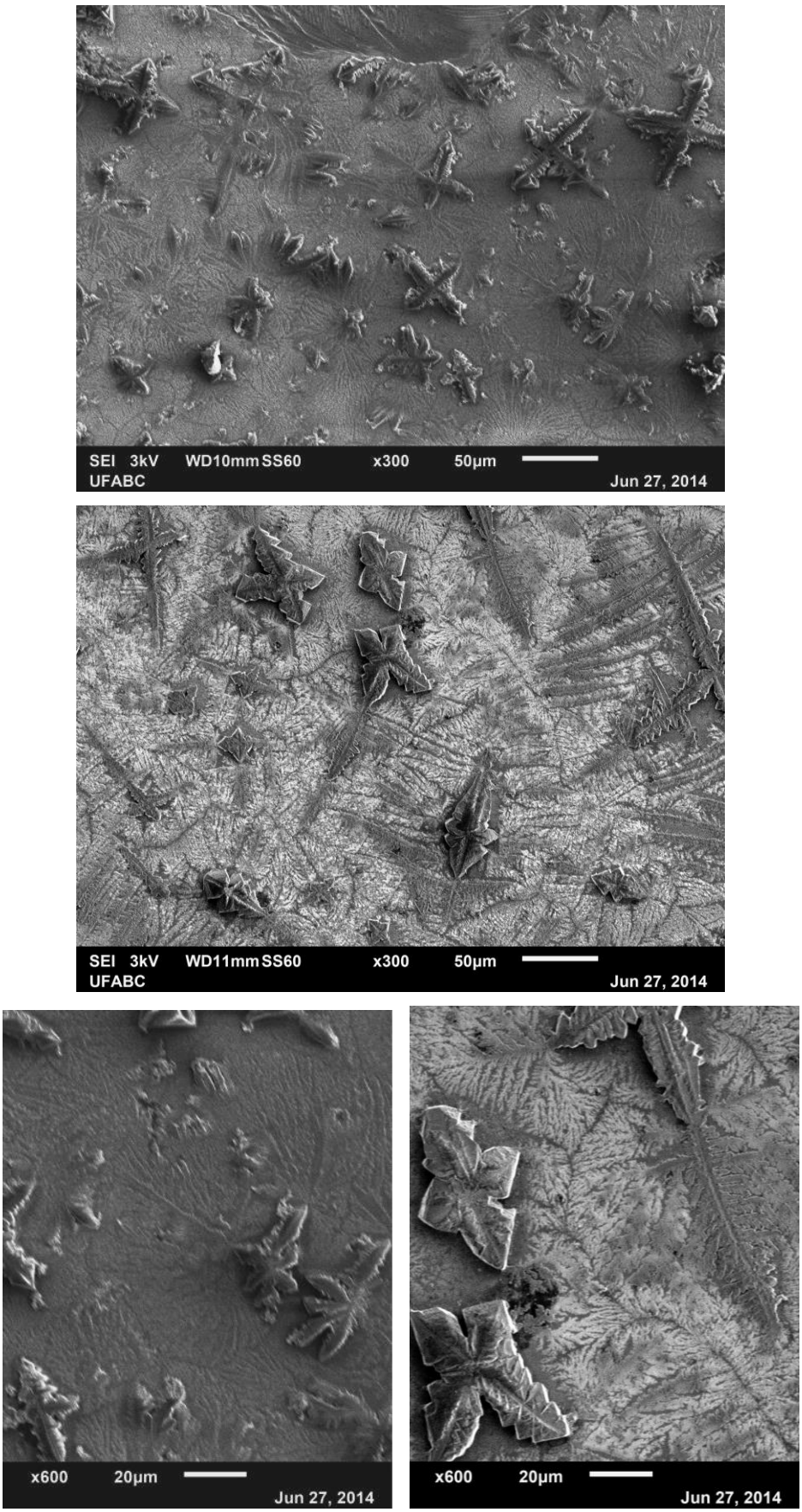

Fig. 2. SEM images of bare TiO_2 NPs (A) and cytochrome c-coated TiO_2 NPs; (B) and bare α-Fe_2O_3; (C) and cytochrome c-coated α-Fe_2O_3; (D). All the images are of the samples incubated at 2.5 mM universal buffer, pH 10.

The adsorption of cytochrome c on TiO_2 and α-Fe_2O_3 NPs was supported by SEM images obtained in the absence and in the presence of cytochrome c (Figure 2A, B, C and D).

SEM images of TiO_2 NPs, before and after incubation with cytochrome *c* in 5 mM universal buffer, pH 10 are shown in Fig. 2A and 2B, respectively. Figure 2A shows the presence of uneven aggregates of TiO_2 crystallites self-assembled and randomly distributed on the silicon surface. Fig. 2B shows that the adsorption of cytochrome c to TiO_2 NPs changes significantly the pattern of the material organization on the surface that may be related to the change of surface properties of the TiO_2 crystallites. In Fig. 2B it is shown a pattern of scales on which one can see structures with radial layout and ramification. Figure 2C shows the SEM images of hematite before incubation with cytochrome c, in 2.5 mM universal buffer, pH 10. In this condition, hematite exhibited also crystallite aggregates. Figure 2D shows that the interaction of cytochrome c on the surface of hematite particles led to extensive aggregation that creates images in relief that in some areas are similar to the branches of ferns. The ramified pattern obtained for TiO_2 and α-Fe_2O_3 NPs incubated with cytochrome c in 2.5 mM universal buffer at pH 10 was reproduced in the same buffer at pH 7 (not shown). However extensive aggregation and ramification were not observed for the cytochrome c-containing samples incubated in water titrated to the desired pH with HCl and NaOH (not shown). Therefore, these aggregated patterns are influenced by the ionic strength and the presence of polyprotic acids and deserves future investigations. Self-assembled aggregates of cytochrome c and titanate nanotubes had been previously demonstrated.[28] The anionic groups present on the surface of TiO_2 and α-Fe2O3 NPs promote the adsorption of the positively charged protonated amino groups of the alkaline cytochrome c [30, 38, 39, 40]. In the case of the amino group moieties, hydrogen bonds can also be established between deprotonated group and the OH^- groups on the surface of the semiconductor materials [28,37].

The effect of universal buffer (acetate. phosphate and borate) in promoting the extensive aggregation and ramified patterns for the cytochrome c TiO_2 and α-Fe_2O_3 aggregates is consistent with a study of Chusuei et al. [41] that described the adsorption of calcium ions on the surface of TiO_2 surface allowing subsequent adsorption of phosphate on the TiO_2-calcium layer. Complexes of the positively charged calcium ion and phosphate with TiO_2 have also been described by other authors [42,43].

Considering that the interaction of cytochrome c with the semiconductor materials studied here was characterized, the next step was to investigate the behavior of the photoreduction processes in the both systems.

The capacity to accept electrons from the conduction bands of photoexcited TiO_2 and α-Fe_2O_3 was compared by analysis of UV-visible spectra of cytochrome c before and after irradiation in the presence of the semiconductors. Cytochrome c irradiated in the presence of TiO_2 and α-Fe_2O_3 NPs exhibits spectral changes typical of heme iron reduction [18]. The reduction of cytochrome *c* heme iron from the oxidation state Fe^{3+} to Fe^{2+} is accompanied by Soret band red shift and increase of Qα band at 550 nm. Figure 3A shows a typical spectral change that accompanies cytochrome c reduction after 1h of exposure to UV-visible light in the presence of α-Fe_2O_3 NPs at pH 10. Similar spectral changes were obtained at similar conditions in the presence of the TiO_2 NPs (not shown). Figure 3B shows two differential spectra of cytochrome c obtained by subtraction of the spectrum after irradiation in the presence of α-Fe_2O_3 NPs of the initial spectrum before irradiation at pH values 10 and 11 (gray and light gray lines, respectively). Figure 3C shows the pH curve obtained from the differential spectra of cytochrome c submitted to irradiation in the presence of TiO_2 (blue balls) and α-Fe_2O_3 NPs (red balls) at pH 6, 7, 8, 9, 10, 11 and 12.

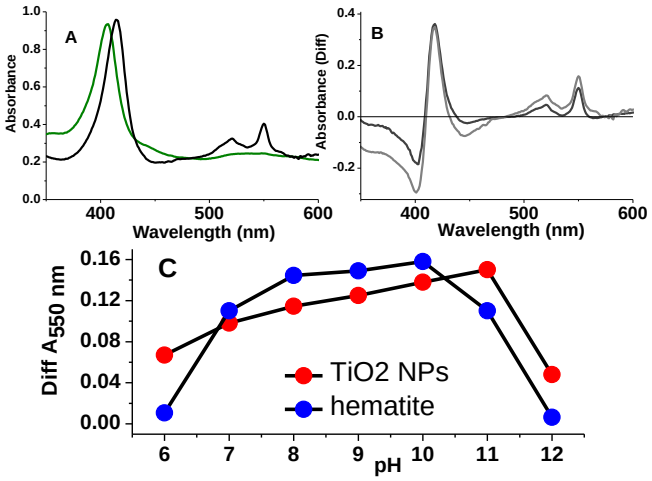

Fig. 3. Photoreduction of ferric cytochrome c by simulated sunlight simultaneous irradiation of TiO_2 NPs and hematite suspensions at different pH values. A- Spectra of ferric cytochrome c (olive line) and hematite-reduced cytochrome c (black line); B- Differential spectra of cytochrome c photoreduced by hematite at pH 10 (gray line) and at pH 11 (dark gray line); C- pH curves of cytochrome c photo-reduction by TiO_2 NPs (red balls) and α-Fe_2O_3 (blue balls).

It is observed that pH 10 is the optimal pH for cytochrome c reduction by α-Fe_2O_3 NPs and pH 11, for the TiO_2-promoted photoreduction. At the pH range from 8 to 10 α-Fe_2O_3 NPs was able to reduce a higher amount of cytochrome c than TiO_2 NPs.

Previously, we have demonstrated that ferrous cytochrome c produced by titania photochemistry could be recycled to the ferric form by a solution of hydrogen peroxide [28]. In the present study ferrous cytochrome c produced by excited α-Fe_2O_3 NPs was added to a nitrogen purged suspension of cytochrome c-depleted mitoplasts. The spectrum of mitoplasts was recorded before and after ferrous cytochrome c addition (Fig. 4).

Figure 4 shows the electronic absorption spectrum obtained by subtracting the spectrum of cytochrome c-depleted mitoplasts of the spectrum obtained after addition of ferrous cytochrome c (0.4 nmol/mg mitochondrial proteins) reduced by hematite (black line). Figure 4 shows also the Soret band of ferric and ferrous cytochrome c solutions overlapped on the cytochrome c spectrum in mitoplasts. The spectrum of

cytochrome c obtained after addition to the mitoplast suspension demonstrated that the hemeprotein was oxidized after association to mitoplasts. The cytochrome c-depleted mitoplasts was not supplemented with succinate and nitrogen was purged in the suspension to avoid reoxidation of ferrous cytochrome c by solved molecular oxygen. However, the possibility that ferrous cytochrome c was also oxidized by hydrogen peroxide produced by mitoplasts may not be discarded. However, even considering this possibility it was demonstrated the recycling of the hemeprotein by a biological system.

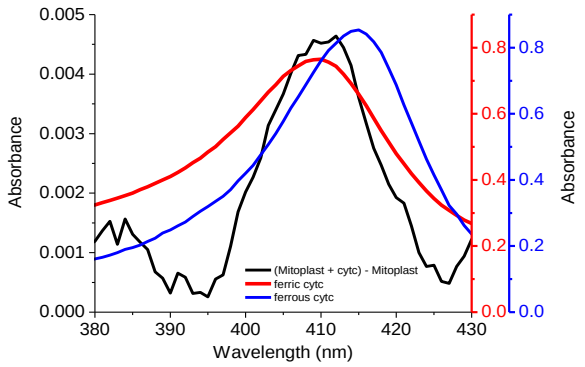

Fig. 4. Recycling of photoreduced cytochrome c. The black line is the spectrum of cytochrome c obtained after addition to a cytochrome c-depleted mitoplast suspension and obtained by subtracting the spectrum obtained before the addition of the hemeprotein. The red line corresponds to ferric cytochrome c before irradiation in the presence of α-Fe_2O_3 NPs (hematite) and blue line corresponds to the spectrum of cytochrome c after irradiation with simulated sunlight. One aliquot of the reduced cytochrome c peaking at 415 nm was added to mitoplast suspension for a final content of 0.4 nmol cytochrome c/mg mitochondrial protein. The experiments were done at pH 6.0 at 30 °C. The color of each y-axis refers to the line with the same color.

The data presented here show a piece of evidence supporting the capacity of TiO_2 and α-Fe_2O_3 NPs to reduce ferric cytochrome c in a process that is analogous to some steps of the light step of photosynthesis. According to the redox potentials presented in Fig. 5, [44] the light absorption by the semiconductors made feasible that water acted as the reducing agent for the heme iron.

The redox potential for TiO_2*cb* and α-Fe_2O_3*cb* are, respectively, −0.3V and -0.4V vs. SHE, which allows thermodynamically favorable electron transfer to ferric cytochrome c [45]. Therefore, the cytochrome/semiconductor systems are similar to the photosynthetic apparatus (left side of Fig. 4), because they use light energy to generate a high-energy electron donor for the heme iron of a cytochrome, the low energy electron acceptor. The potential values presented in Fig. 5 were extracted from literature data and may differ slightly according to the experimental conditions in which they were determined [46]. The capacity of TiO_2, to photoreduce cytochrome c heme iron had been previously reported by our research group. However, hematite has the inconvenience of rapid charge recombination after electronic excitation and the efficient capacity to reduce cytochrome c is an advance favourable for the applicability of hematite. The α-Fe_2O_3 used in the present study is nanostructured, interacted with cytochrome c as demonstrated by the ζ values and constitute a promisor system for photoelectrochemical applications.

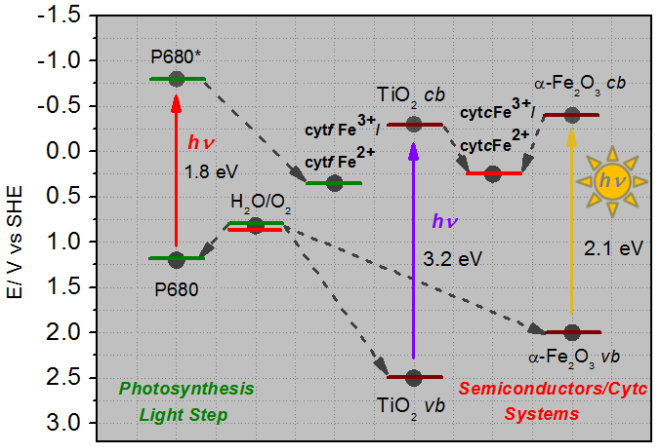

Fig. 5. Photo-reduction of cytochromes in biological and mimetic processes using semiconductors TiO_2 and α-Fe_2O_3 NPs. The intermediate steps of the photoreduction of cytochrome *f* were omitted for clarity. This Figure was adapted from ref. 28.

IV. Conclusion

In this paper, it was demonstrated that an alkaline protein, respiratory cytochrome c, interacts with the nanostructured semiconductor materials, TiO_2 and α-Fe_2O_3 NPs. Cytochrome c, in pH-dependent-manner, is a good acceptor of the electrons promoted to conduction bands of the semiconductors during exposure to simulated sunlight. Despite the short lifetime of charge separation, hematite could promote photo-reduction of bulk and adsorbed cytochrome c detected by electronic absorption spectroscopy. Therefore, this is a model for the development of systems able to bypass the short lifetime of α-Fe_2O_3 NPs (hematite) charge separation by using efficient electron collectors as cytochrome c.

Acknowledgments

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References

[1] M. Gratzel. "Solar Energy Conversion by Dye-Sensitized Photovoltaic Cells," *Inorg. Chem*., **44**, pp. 6841-6851, 2005.

[2] M. Gratzel. "Photoelectrochemical cells," *Nature*, **414**, pp. 338-344, 2001.

[3] R.E. Blankenship, D.M. Tiede, J. Barber, G.W. Brudvig, G. Fleming, M. Ghirardi, M.R. Gunner, W. Junge, D.M. Kramer, A. Melis, T.A. Moore, C.C. Moser, D.G. Nocera, A.J. Nozik, D.R. Ort, W.W. Parson, R.C. Prince and R.T. Sayre. "Comparing Photosynthetic and Photovoltaic Efficiencies and Recognizing the Potential for Improvementol," *Science*, **332**, pp. 805-809, 2011.

[4] P.M. Shih, J. Zarzycki, K.K. Niyogi and C.A. Kerfeld. "Introduction of a Synthetic CO2-fixing Photorespiratory Bypass into a Cyanobacterium," *J. Biol. Chem.*, **289**, pp. 9493-9500, 2014

[5] P.G. Falkowski and J.A. Raven. *Aquatic photosynthesis*. Princeton: University Press, 2007

[6] M. Graetzel, R.A.J. Janssen, D.B. Mitzi and E.H. Sargent. "Materials interface engineering for solution-processed photovoltaics," *Nature*, **488**, pp. 304-312, 2012.

[7] H. Tada, M. Fujishima and H. Kobayashi. "Photodeposition of metal sulfide quantum dots on titanium(IV) dioxide and the applications to solar energy conversion," *Chem. Soc. Rev.*, **40**, pp. 4232-4243, 2011.

[8] P.K. Santra and P.V. Kamat. "Mn-doped quantum dot sensitized solar cells: a strategy to boost efficiency over 5%," *J. Am. Chem. Soc.*, **134**, pp. 2508-2511, 2012.

[9] S.D. Tilley, M. Cornuz, K. Sivula and M. Gratzel. "Light -InducedWater Splitting with Hematite: Improved Nanostructure and Iridium Oxide Catalysis," *Angew. Chem.*, **122**, pp. 6549–6552, 2010.

[10] P. Dare-Edwards, J.B. Goodenough, A. Hamnett and P.R. Trevellick. "Electrochemistry and photoelectrochemistry of iron(III) oxide," *J. Chem. Soc. Faraday Trans. 1 Phys. Chem. Condensed Phases*, **79**, pp. 2027-2041, 1983.

[11] J.B. Goodenough. "Bond-length fluctuations in the copper-oxide superconductors," *Prog. Solid State Chem.*, **5**, pp. 145-399, 1971.

[12] C. Santato, M. Ulmann, and J. Augustynski. "Photoelectrochemical Properties of Nanostructured Tungsten Trioxide Films," *J. Phys. Chem. B.*, **105**, pp. 936-940, 2001.

[13] W.M. Carvalho Jr., F.L. Souza. "Recent advances on solar water splitting using hematite nanorod film produced by purpose built materials", *J. Materials Research*, **29**, pp. 16-28, 2014.

[14] V.A.N. Carvalho, R.A.S. Luz, B.H. Lima, F.N. Crespilho, E.R. Leite, and F.L. Souza. "Highly oriented hematite nanorod arrays for photoelectrochemical water splitting", *Journal of Power Sources*, **205**, pp.525-529, 2012.

[15] L.C.C. Ferraz, W.M. Carvalho Jr., D. Criado, and F.L. Souza. "Vertically oriented iron oxide films produced by hydrothermal process: effect of thermal treatment on the physical chemical properties", *ACS Appl. Materials and Interface*, **4**, pp.5515-5523, 2012.

[16] V.A. Soares, D. Severino, H.C. Junqueira, I.L.S. Tersariol, C.S. Shida, M.S. Baptista, O.R. Nascimento and I.L. Nantes. "Light-driven horseradish peroxidase cycle by using photo-activated methylene blue as the reducing agent," *Photochem. Photobiol.*, **83**, pp. 1254-1262, 2007.

[17] M.J.B. Hauser, A. Lunding and L.F. Olsen. "On the role of methylene blue in the oscillating peroxidase–oxidase reaction," *Phys. Chem. Chem. Phys.*, **2**, pp. 1685-1692, 2000.

[18] Carson and J. Walleczek. "Response of the peroxidase-oxidase oscillator to light is controlled by MBþ-NADH photochemistry," *J. Phys. Chem. B.*, **107**, pp. 8637-8642, 2003.

[19] I.B. Campos, I.L. Nantes, F.A. Rodrigues and S. Brochsztain. "Photoinduced electron transfer in silica-supported self-assembled thin films containing a 1, 4, 5, 8-naphthalenetetracarboxylic diimide and cytochrome c," *J. Mater. Chem.*, **14**, 54-60, 2004.

[20] K.M. Figueiredo, R.O. Marcon, I.B. Campos, I.L. Nantes and S. Brochsztain. "Photoinduced electron transfer between cytochrome *c* and a novel 1,4,5,8-naphthalenetetracarboxylic diimide with amphiphilic character," *J. Photochem. Photobiol. B*, **79**, pp. 1-9, 2005.

[21] M.L. Estevam, O.R. Nascimento, M.S. Baptista, P. Di Mascio, F.M. Prado, A. Faljoni-Alario, M.R. Zucchi and I.L Nantes. "Changes in the spin state and reactivity of cytochrome C induced by photochemically generated singlet oxygen and free radicals," *J. Biol. Chem.*, **279**, pp. 39214-39222, 2004.

[22] E. Topoglidis, T. Lutz, J.R. Durrant and E. Palomares. "Interfacial electron transfer on cytochrome-c sensitised conformally coated mesoporous TiO_2 films," *Bioelectrochemistry*, **74**, pp. 142-148, 2008.

[23] G.K. Mor, K. Shankar, M. Paulose, O.K. Varghese and C.A. Grimes. "Use of highly-ordered TiO2 nanotube arrays in dye-sensitized solar cells," *Nano Letters*, **6**, pp. 215-218, 2006.

[24] J. H. Park, S. Kim and A.J. Bard. "Novel Carbon-Doped TiO_2 Nanotube Arrays with High Aspect Ratios for Efficient Solar Water Splitting," *Nano Letters*, **6**, pp. 24-28, 2006.

[25] E.Topoglidis, Y. Astuti, F. Duriaux, M. Gratzel and J.R. Durrant. "Direct electrochemistry and nitric oxide interaction of heme proteins adsorbed on nanocrystalline tin oxide electrodes," *Langmuir*, **19**, pp. 6894-6900, 2003.

[26] Y. Astuti, E. Palomares, S.A. Haque and J.R. Durrant. "Triplet state photosensitization of nanocrystalline metal oxide electrodes by zinc-substituted cytochrome c: application to hydrogen evolution," *J. Am. Chem. Soc.*, **127**, pp. 15120-15126, 2005

[27] E. Topoglidis, C.J. Campbell, E. Palomares and J.R. Durrant. "Photoelectrochemical study of Zn cytochrome-c immobilised on a nanoporous metal oxide electrode" *Chem. Commun.*, **14**, pp. 1518-1519, 2002.

[28] E. Topoglidis, A.E.G. Cass, G. Gilardi, S. Sadeghi, N. Beaumont and J.R. Durrant. "Protein adsorption on nanocrystalline TiO films: a novel immobilisation

strategy for bioanalytical devices", *Anal. Chem*., **70**, pp. 5111-5113, 1998

[29] P. Cuendet and M. Gratzel. "Light-induced reduction of cytochrome c by colloidal TiO_2," *Bioelectrochem. Bioenerg.*, **16**, pp. 125-133, 1986.

[30] E. Topoglidis, T. Lutz, R.L. Willis, C.J. Barnett, A.E.G. Cass and J.R. Durrant. "Protein adsorption on nanoporous TiO2 films: a novel approach to studying photoinduced protein/electrode transfer reactions," *Faraday Discuss*., **116**, pp. 35-46, 2000.

[31] C.F.B. Dias, J.C. Araujo-Chaves, K.C.U. Mugnol, F.J. Trindade, O.L. Alves, A.C.F. Caires, S. Brochsztain, F.N. Crespilho, J.R. Matos, O.R. Nascimento and Iseli L Nantes. "Photo-induced electron transfer in supramolecular materials of titania nanostructures and cytochrome c," *RSC Advances*, **2**, pp. 7417-7426, 2012.

[32] P.L. Pedersen, J.W. Greenalwalt, B. Reynafarje, J. Hullihen, G,L. Decker, J.W. Soper and E. Bustamante. " Preparation and characterization of mitochondria and submitochondrial particles of rat liver and liver-derived tissues," *Methods Cell Biol*., **20**, pp. 411–481, 1978.

[33] R. Radi, K.M. Bush and B.A. Freeman. "The role of cytochrome c and mitochondrial catalase in hydroperoxide-induced heart mitochondrial lipid peroxidation," *Arch. Biochem. Biophys*., **300**, pp. 409–415, 1993

[34] C. Kawai, F.S. Pessoto, T. Rodrigues, K.C.U. Mugnol, V. Tortora, L. Castro, V.A. Milıcchio, I.L.S. Tersariol, P. Di Mascio, R. Radi, A.M. Carmona-Ribeiro and Iseli L. Nantes. "pH-Sensitive Binding of Cytochrome c to the Inner Mitochondrial Membrane. Implications for the Participation of the Protein in Cell Respiration and Apoptosis," *Biochemistry*, **48**, pp. 8335–8342, 2009.

[35] A.M. Xavier, F.F. Ferreira and F.L. Souza. "Morphological and structural evolution of one dimensional hematite nanorods" *RSC Advances*, **4**, pp. 17753-17759, 2014.

[36] D. Hanaor, M. Michelazzi, P. Veronesi, C. Leonelli, M. Romagnoli, C. Sorrell. "Anodic Aqueous Electrophoretic Deposition of Titanium Dioxide Using Carboxylic Acids as Dispersing Agents," *J. Eur. Ceram. Soc*., **31**, pp. 1041-1047, 2011.

[34] Moore and G.W. Pettigrew. *Cytochrome c*, Heidelberg: Springer-Verlag, 1990.

[38] H.-C. Chang. "Adsorption and Immobilization of Cytochrome c on Nanodiamonds," *Langmuir*, **20**, pp. 5879-5884, 2004.

[39] M. Meot-Ner. "The ionic hydrogen bond and ion solvation. 1. $NH^{+}\cdots O$, $NH^{+}\cdots N$, and $OH^{+}\cdots O$ bonds Correlations with proton affinity. Deriations due to structural effects," *J. Am. Chem. Soc*., **106**, pp. 1257-1264, 1984

[40] M. Meot-Ner, L.W. Sieck, J.F. Liebman and S. Scheiner. "Complexing of the Ammonium Ion by Polyethers. Comparative Complexing Thermochemistry of Ammonium, Hydronium, and Alkali Cations," *J. Phys. Chem*., **100**, pp. 6445–6450, 1996

[41] C.C. Chusuei, D.W. Goodman, M.J. Van Stipdonk, D.R. Justes, K.H. Loh and E.A. Schweikert. "Solid-Liquid Adsorption of Calcium Phosphate on TiO2," *Langmuir*, **15**, pp. 7355-7360, 1999.

[42] T.K. Ronson and A.J. McQuillan. "Infrared spectroscopic study of calcium and phosphate ion coadsorption and of brushite crystallization on TiO2," *Langmuir*, **18**, pp. 5019-5022, 2002.

[43] H. Jézéquel and K.H. Chu. "Enhanced adsorption of arsenate on titanium dioxide using Ca and Mg ions," *Environmental Chemistry Letters*, **3**, pp. 132–135, 2005.

[44] A. Grimes, O.K. Varghese and S. Ranjan. *Light, Water, Hydrogen: The Solar Generation of Hydrogen by Water Photoelectrolysis*, US, Springer, 2008.

[45] A. Fujishima, T.N. Rao and D.A. Tryk. "Titanium dioxide photocatalysis," *J. Photochem. Photobiol. C: Photochemistry Reviews*, **1**, pp. 1–21, 2000.

[46] P. Wardman. "Reduction Potentials of One-Electron Couples Involving Free Radicals in Aqueous Solution," *J. Phys. Chem. Ref. Data*, **18**, 1637-, 1989.