

CV Dr. Miguel A. De la Rosa

Miguel A. De la Rosa (Full Professor)
Instituto de Bioquímica Vegetal y Fotosíntesis
Universidad de Sevilla, Sevilla, España

Lines of Research

Molecular Recognition and Protein Engineering: Structure and Function of Redox Metalloproteins Involved in Plant Photosynthesis
Solar Energy Conversion and Mechanisms of Energy Transduction

Research and Expertise

Electron transfer processes are crucial for numerous important biological processes, as is photosynthesis. Even though a tremendous research effort has been made in this area, the understanding of molecular recognition and reaction mechanisms used by biological molecules is rather limited. The number of parameters is enormous and the synergistic effects are often complex and difficult to predict. It is becoming more and more obvious that in order to advance our understanding of these processes a very precise characterization of the molecules in question is necessary, putting together information obtained by a multitude of techniques as well as theoretical studies.

In order to investigate these questions, the group is studying the electron transfer between the membrane-embedded complexes cytochrome b6-f and photosystem I (PSI), a process that is mediated by the copper-protein plastocyanin or the haem-protein cytochrome c6. Whereas cytochrome c6 seems to be the only electron carrier in primitive organisms, there is a number of cyanobacteria and green alga that are able to synthesize both cytochrome c6 and plastocyanin; in higher plants, by contrast, the redox connection between the two membrane complexes is just mediated by the copper-protein. Special attention is thus being paid to the evolutionary aspects as this is an excellent case study of protein evolution along which the reaction mechanism has evolved from the primitive cyanobacteria to the highly evolved higher plants.

A novel aspect of the current research work is the application of a range of biological, biochemical and biophysical techniques to study in a comparative way the functional and structural features of a number of redox proteins, as well as the reaction mechanisms of energy transduction in photosynthesis. The techniques to be employed include site-specific mutagenesis and kinetic characterization of wild-type and mutant proteins, the latter involving mainly transient kinetic methods. NMR spectroscopy and molecular dynamics (MD) calculations are being used to provide useful information on the structures of these molecules to better explain their kinetic behaviour and physiological role.

Research Financial Support

Official Institutions:

Ministry of Education and Science ,
Andalusian Government ,
NATO,
European Union.

Private Companies:

INTEROX química, S.A. (c/o. SOLVAY & Cie, Belgium),
Agromedina SAT.

Facilities

The Institute of Plant Biochemistry and Photosynthesis is equipped with most advanced techniques in biochemistry, biophysics and molecular biology. It belongs to the Center for Scientific Research "Isla de la Cartuja", which comprises three others Institutes devoted to Chemistry, New Materials and Biomedicine (the latter is under construction). The Center thus provides the high scientific level, environment and technical facilities required for training of young scientists, thus warranting most fruitful and profitable stays of students from other countries.

Scientific Publications

- 1: Duran RV, Hervas M, De la Cerda B, De la Rosa MA, Navarro JA. A Laser Flash-Induced Kinetic Analysis of in Vivo Photosystem I Reduction by Site-Directed Mutants of Plastocyanin and Cytochrome c(6) in *Synechocystis* sp. PCC 6803. *Biochemistry*. 2006 Jan 24;45(3):1054-60.
- 2: Schmalzried TP, Silva M, de la Rosa MA, Choi ES, Fowble VA. Optimizing patient selection and outcomes with total hip resurfacing. *Clin Orthop Relat Res*. 2005 Dec;441:200-4.
- 3: Balme A, Hervas M, Campos LA, Sancho J, De la Rosa MA, Navarro JA. A comparative study of the thermal stability of plastocyanin, cytochrome c(6) and Photosystem I in thermophilic and mesophilic cyanobacteria. *Photosynth Res*. 2001;70(3):281-9.
- 4: Diaz-Quintana A, Navarro JA, Hervas M, Molina-Heredia FP, De la Cerda B, De la Rosa MA. A comparative structural and functional analysis of cyanobacterial plastocyanin and cytochrome c (6) as alternative electron donors to Photosystem I. *Photosynth Res*. 2003;75(2):97-110.
- 5: Diaz-Quintana A, De la Cerda B, Hervas M, Navarro JA, De la Rosa MA. Mutations in both leucine 12 and lysine 33 in plastocyanin from *Synechocystis* sp. PCC 6803 induce drastic changes in the hydrophobic interactions with Photosystem I. *Photosynth Res*. 2002;72(3):223-30.
- 6: Navarro JA, Hervas M, Sun J, De la Cerda B, Chitnis PR, De la Rosa MA. Negatively charged residues in the H loop of PsaB subunit in Photosystem I from *Synechocystis* sp. PCC 6803 appear to be responsible for electrostatic repulsions with plastocyanin*. *Photosynth Res*. 2000;65(1):63-8.
- 7: Hervas M, Diaz-Quintana A, Kerfeld CA, Krogmann DW, De la Rosa MA, Navarro JA. Cyanobacterial Photosystem I lacks specificity in its interaction with cytochrome c(6) electron donors. *Photosynth Res*. 2005;83(3):329-33.
- 8: Albarran C, Navarro JA, Molina-Heredia FP, Murdoch Pdel S, De la Rosa MA, Hervas M. Laser flash-induced kinetic analysis of cytochrome f oxidation by wild-type

and mutant plastocyanin from the cyanobacterium *Nostoc* sp. PCC 7119. *Biochemistry*. 2005 Aug 30;44(34):11601-7.

9: Duran RV, Hervas M, De la Rosa MA, Navarro JA. In vivo photosystem I reduction in thermophilic and mesophilic cyanobacteria: the thermal resistance of the process is limited by factors other than the unfolding of the partners. *Biochem Biophys Res Commun*. 2005 Aug 19;334(1):170-5.

10: Lange C, Luque I, Hervas M, Ruiz-Sanz J, Mateo PL, De la Rosa MA. Role of the surface charges D72 and K8 in the function and structural stability of the cytochrome c from *Nostoc* sp. PCC 7119. *FEBS J*. 2005 Jul;272(13):3317-27.

11: Navarro JA, Duran RV, De la Rosa MA, Hervas M. Respiratory cytochrome c oxidase can be efficiently reduced by the photosynthetic redox proteins cytochrome c6 and plastocyanin in cyanobacteria. *FEBS Lett*. 2005 Jul 4;579(17):3565-8.

12: Diaz-Moreno I, Diaz-Quintana A, Ubbink M, De la Rosa MA. An NMR-based docking model for the physiological transient complex between cytochrome f and cytochrome c6. *FEBS Lett*. 2005 May 23;579(13):2891-6.

13: Diaz-Moreno I, Diaz-Quintana A, De la Rosa MA, Crowley PB, Ubbink M. Different modes of interaction in cyanobacterial complexes of plastocyanin and cytochrome f. *Biochemistry*. 2005 Mar 8;44(9):3176-83.

14: Diaz-Moreno I, Diaz-Quintana A, De la Rosa MA, Ubbink M. Structure of the complex between plastocyanin and cytochrome f from the cyanobacterium *Nostoc* sp. PCC 7119 as determined by paramagnetic NMR. The balance between electrostatic and hydrophobic interactions within the transient complex determines the relative orientation of the two proteins. *J Biol Chem*. 2005 May 13;280(19):18908-15. Epub 2005 Feb 10. Erratum in: *J Biol Chem*. 2005 Oct 21;280(42):35784.

15: Nogues I, Hervas M, Peregrina JR, Navarro JA, de la Rosa MA, Gomez-Moreno C, Medina M. Anabaena flavodoxin as an electron carrier from photosystem I to ferredoxin-NADP⁺ reductase. Role of flavodoxin residues in protein-protein interaction and electron transfer. *Biochemistry*. 2005 Jan 11;44(1):97-104.

16: Diaz-Moreno I, Diaz-Quintana A, Molina-Heredia FP, Nieto PM, Hansson O, De la Rosa MA, Karlsson BG. NMR analysis of the transient complex between membrane photosystem I and soluble cytochrome c6. *J Biol Chem*. 2005 Mar 4;280(9):7925-31. Epub 2004 Dec 16.

17: Feio MJ, Navarro JA, Teixeira MS, Harrison D, Karlsson BG, De la Rosa MA. A thermal unfolding study of plastocyanin from the thermophilic cyanobacterium *Phormidium laminosum*. *Biochemistry*. 2004 Nov 23;43(46):14784-91.

18: Navarro JA, Lowe CE, Amons R, Kohzuma T, Canters GW, De la Rosa MA, Ubbink M, Hervas M. Functional characterization of the evolutionarily divergent fern plastocyanin. *Eur J Biochem*. 2004 Aug;271(16):3449-56.

- 19: Wastl J, Molina-Heredia FP, Hervas M, Navarro JA, De la Rosa MA, Bendall DS, Howe CJ. Redox properties of Arabidopsis cytochrome c6 are independent of the loop extension specific to higher plants. *Biochim Biophys Acta*. 2004 Jul 9;1657(2-3):115-20.
- 20: Navarro JA, Hervas M, De la Rosa MA. Purification of plastocyanin and cytochrome c6 from plants, green algae, and cyanobacteria. *Methods Mol Biol*. 2004;274:79-92.
- 21: Duran RV, Hervas M, De La Rosa MA, Navarro JA. The efficient functioning of photosynthesis and respiration in *Synechocystis* sp. PCC 6803 strictly requires the presence of either cytochrome c6 or plastocyanin. *J Biol Chem*. 2004 Feb 20;279(8):7229-33. Epub 2003 Dec 5.
- 22: Hervas M, Navarro JA, De La Rosa MA. Electron transfer between membrane complexes and soluble proteins in photosynthesis. *Acc Chem Res*. 2003 Oct;36(10):798-805. Review.
- 23: Lange C, Hervas M, De la Rosa MA. Analysis of the stability of cytochrome c(6) with an improved stopped-flow protocol. *Biochem Biophys Res Commun*. 2003 Oct 10;310(1):215-21.
- 24: Molina-Heredia FP, Wastl J, Navarro JA, Bendall DS, Hervas M, Howe CJ, De La Rosa MA. Photosynthesis: a new function for an old cytochrome? *Nature*. 2003 Jul 3;424(6944):33-4.
- 25: Nogues I, Martinez-Julvez M, Navarro JA, Hervas M, Armenteros L, de la Rosa MA, Brodie TB, Hurley JK, Tollin G, Gomez-Moreno C, Medina M. Role of hydrophobic interactions in the flavodoxin mediated electron transfer from photosystem I to ferredoxin-NADP+ reductase in *Anabaena* PCC 7119. *Biochemistry*. 2003 Feb 25;42(7):2036-45.
- 26: Hervas M, Myshkin E, Vintonenko N, De la Rosa MA, Bullerjahn GS, Navarro JA. Mutagenesis of prochlorothrix plastocyanin reveals additional features in photosystem I interactions. *J Biol Chem*. 2003 Mar 7;278(10):8179-83. Epub 2002 Dec 30.
- 27: Schlarb-Ridley BG, Navarro JA, Spencer M, Bendall DS, Hervas M, Howe CJ, De La Rosa MA. Role of electrostatics in the interaction between plastocyanin and photosystem I of the cyanobacterium *Phormidium laminosum*. *Eur J Biochem*. 2002 Dec;269(23):5893-902.
- 28: Crowley PB, Diaz-Quintana A, Molina-Heredia FP, Nieto P, Sutter M, Haehnel W, De La Rosa MA, Ubbink M. The interactions of cyanobacterial cytochrome c6 and cytochrome f, characterized by NMR. *J Biol Chem*. 2002 Dec 13;277(50):48685-9. Epub 2002 Sep 27.
- 29: Molina-Heredia FP, Balme A, Hervas M, Navarro JA, De la Rosa MA. A comparative structural and functional analysis of cytochrome cM cytochrome c6 and

plastocyanin from the cyanobacterium *Synechocystis* sp. PCC 6803. *FEBS Lett.* 2002 Apr 24;517(1-3):50-4.

30: Casaus JL, Navarro JA, Hervas M, Lostao A, De la Rosa MA, Gomez-Moreno C, Sancho J, Medina M. *Anabaena* sp. PCC 7119 flavodoxin as electron carrier from photosystem I to ferredoxin-NADP⁺ reductase. Role of Trp(57) and Tyr(94). *J Biol Chem.* 2002 Jun 21;277(25):22338-44. Epub 2002 Apr 11.

31: De la Rosa MA, Navarro JA, Diaz-Quintana A, De la Cerda B, Molina-Heredia FP, Balme A, Murdoch Pdel S, Diaz-Moreno I, Duran RV, Hervas M. An evolutionary analysis of the reaction mechanisms of photosystem I reduction by cytochrome c(6) and plastocyanin. *Bioelectrochemistry.* 2002 Jan;55(1-2):41-5.

32: Navarro JA, Myshkin E, De la Rosa MA, Bullerjahn GS, Hervas M. The unique proline of the *Prochlorothrix hollandica* plastocyanin hydrophobic patch impairs electron transfer to photosystem I. *J Biol Chem.* 2001 Oct 5;276(40):37501-5. Epub 2001 Jul 16.

33: Frazao C, Enguita FJ, Coelho R, Sheldrick GM, Navarro JA, Hervas M, De la Rosa MA, Carrondo MA. Crystal structure of low-potential cytochrome c549 from *Synechocystis* sp. PCC 6803 at 1.21 Å resolution. *J Biol Inorg Chem.* 2001 Mar;6(3):324-32.

34: Garcia-Sanchez MI, Diaz-Quintana A, Gotor C, Jacquot JP, De la Rosa MA, Vega JM. Homology predicted structure and functional interaction of ferredoxin from the eukaryotic alga *Chlamydomonas reinhardtii* with nitrite reductase and glutamate synthase. *J Biol Inorg Chem.* 2000 Dec;5(6):713-9.

35: Molina-Heredia FP, Hervas M, Navarro JA, De la Rosa MA. A single arginyl residue in plastocyanin and in cytochrome c(6) from the cyanobacterium *Anabaena* sp. PCC 7119 is required for efficient reduction of photosystem I. *J Biol Chem.* 2001 Jan 5;276(1):601-5.

36: Molina-Heredia FP, Diaz-Quintana A, Hervas M, Navarro JA, De La Rosa MA. Site-directed mutagenesis of cytochrome c(6) from *Anabaena* species PCC 7119. Identification of surface residues of the heme protein involved in photosystem I reduction. *J Biol Chem.* 1999 Nov 19;274(47):33565-70.

37: De la Cerda B, Diaz-Quintana A, Navarro JA, Hervas M, De la Rosa MA. Site-directed mutagenesis of cytochrome c6 from *Synechocystis* sp. PCC 6803. The heme protein possesses a negatively charged area that may be isofunctional with the acidic patch of plastocyanin. *J Biol Chem.* 1999 May 7;274(19):13292-7.

38: Banci L, Bertini I, De la Rosa MA, Koulougliotis D, Navarro JA, Walter O. Solution structure of oxidized cytochrome c6 from the green alga *Monoraphidium braunii*. *Biochemistry.* 1998 Apr 7;37(14):4831-43.

39: Molina-Heredia FP, Hervas M, Navarro JA, De la Rosa MA. Cloning and correct expression in *Escherichia coli* of the *petE* and *petJ* genes respectively encoding plastocyanin and cytochrome c6 from the cyanobacterium *Anabaena* sp. PCC 7119.

Biochem Biophys Res Commun. 1998 Feb 4;243(1):302-6.

40: Romero A, De la Cerda B, Varela PF, Navarro JA, Hervas M, De la Rosa MA. The 2.15 Å crystal structure of a triple mutant plastocyanin from the cyanobacterium *Synechocystis* sp. PCC 6803. *J Mol Biol.* 1998 Jan 16;275(2):327-36.

41: De la Cerda B, Navarro JA, Hervas M, De la Rosa MA. Changes in the reaction mechanism of electron transfer from plastocyanin to photosystem I in the cyanobacterium *Synechocystis* sp. PCC 6803 as induced by site-directed mutagenesis of the copper protein. *Biochemistry.* 1997 Aug 19;36(33):10125-30.

42: Hervas M, Navarro JA, Diaz A, De la Rosa MA. A comparative thermodynamic analysis by laser-flash absorption spectroscopy of photosystem I reduction by plastocyanin and cytochrome c6 in *Anabaena* PCC 7119, *Synechocystis* PCC 6803 and Spinach. *Biochemistry.* 1996 Feb 27;35(8):2693-8.

43: Frazao C, Soares CM, Carrondo MA, Pohl E, Dauter Z, Wilson KS, Hervas M, Navarro JA, De la Rosa MA, Sheldrick GM. Ab initio determination of the crystal structure of cytochrome c6 and comparison with plastocyanin. *Structure.* 1995 Nov 15;3(11):1159-69.

44: Hervas M, Navarro JA, Diaz A, Bottin H, De la Rosa MA. Laser-flash kinetic analysis of the fast electron transfer from plastocyanin and cytochrome c6 to photosystem I. Experimental evidence on the evolution of the reaction mechanism. *Biochemistry.* 1995 Sep 12;34(36):11321-6.

45: Navarro JA, Hervas M, Genzor CG, Cheddar G, Fillat MF, de la Rosa MA, Gomez-Moreno C, Cheng H, Xia B, Chae YK, et al. Site-specific mutagenesis demonstrates that the structural requirements for efficient electron transfer in *Anabaena* ferredoxin and flavodoxin are highly dependent on the reaction partner: kinetic studies with photosystem I, ferredoxin:NADP⁺ reductase, and cytochrome c. *Arch Biochem Biophys.* 1995 Aug 1;321(1):229-38.

46: Navarro JA, Hervas M, De la Cerda B, De la Rosa MA. Purification and physicochemical properties of the low-potential cytochrome C549 from the cyanobacterium *Synechocystis* sp. PCC 6803. *Arch Biochem Biophys.* 1995 Apr 1;318(1):46-52.

47: Navarro JA, Hervas M, Pueyo JJ, Medina M, Gomez-Moreno C, De la Rosa MA, Tollin G. Laser flash-induced photoreduction of photosynthetic ferredoxins and flavodoxin by 5-deazariboflavin and by a viologen analogue. *Photochem Photobiol.* 1994 Sep;60(3):231-6.

48: Diaz A, Navarro F, Hervas M, Navarro JA, Chavez S, Florencio FJ, De la Rosa MA. Cloning and correct expression in *E. coli* of the *petJ* gene encoding cytochrome c6 from *Synechocystis* 6803. *FEBS Lett.* 1994 Jun 27;347(2-3):173-7.

49: Diaz A, Hervas M, Navarro JA, De La Rosa MA, Tollin G. A thermodynamic study by laser-flash photolysis of plastocyanin and cytochrome c6 oxidation by

photosystem I from the green alga *Monoraphidium braunii*. *Eur J Biochem.* 1994 Jun 15;222(3):1001-7.

50: Campos AP, Aguiar AP, Hervas M, Regalla M, Navarro JA, Ortega JM, Xavier AV, De La Rosa MA, Teixeira M. Cytochrome c6 from *Monoraphidium braunii*. A cytochrome with an unusual heme axial coordination. *Eur J Biochem.* 1993 Aug 15;216(1):329-41.

51: Medina M, Diaz A, Hervas M, Navarro JA, Gomez-Moreno C, de la Rosa MA, Tollin G. A comparative laser-flash absorption spectroscopy study of *Anabaena* PCC 7119 plastocyanin and cytochrome c6 photooxidation by photosystem I particles. *Eur J Biochem.* 1993 May 1;213(3):1133-8.

52: Hervas M, Navarro F, Navarro JA, Chavez S, Diaz A, Florencio FJ, De la Rosa MA. *Synechocystis* 6803 plastocyanin isolated from both the cyanobacterium and *E. coli* transformed cells are identical. *FEBS Lett.* 1993 Mar 22;319(3):257-60.

53: Medina M, Hervas M, Navarro JA, De la Rosa MA, Gomez-Moreno C, Tollin G. A laser flash absorption spectroscopy study of *Anabaena* sp. PCC 7119 flavodoxin photoreduction by photosystem I particles from spinach. *FEBS Lett.* 1992 Nov 30;313(3):239-42.

54: Hervas M, De la Rosa MA, Tollin G. A comparative laser-flash absorption spectroscopy study of algal plastocyanin and cytochrome c552 photooxidation by photosystem I particles from spinach. *Eur J Biochem.* 1992 Jan 15;203(1-2):115-20.

55: Damian RT, de la Rosa MA, Murfin DJ, Rawlings CA, Weina PJ, Xue YP. Further development of the baboon as a model for acute schistosomiasis. *Mem Inst Oswaldo Cruz.* 1992;87 Suppl 4:261-9.

56: Navarro JA, De la Rosa MA, Tollin G. Transient kinetics of flavin-photosensitized oxidation of reduced redox proteins. Comparison of c-type cytochromes and plastocyanins. *Eur J Biochem.* 1991 Jul 1;199(1):239-43.

57: Roncel M, Hervas M, Navarro JA, De la Rosa MA, Tollin G. Related Flavin-photosensitized oxidation of reduced c-type cytochromes. Reaction mechanism and comparison with photoreduction of oxidized cytochromes by flavin semiquinones. *Eur J Biochem.* 1990 Aug 17;191(3):531-6.

58: Roncel M, Navarro JA, De la Rosa MA. Coupling of Solar Energy to Hydrogen Peroxide Production in the Cyanobacterium *Anacystis nidulans*. *Appl Environ Microbiol.* 1989 Feb;55(2):483-487.

59: Hervas M, de la Rosa FF, de la Rosa MA, Losada M. Coupling between redox and acid-base energy by cytochrome b-564 in Baker's yeast mitochondria. *Biochem Biophys Res Commun.* 1984 Nov 14;124(3):807-14.

60: De la Rosa MA. Assimilatory nitrate reductase from the green alga *Ankistrodesmus braunii*. *Mol Cell Biochem.* 1983;50(1):65-74.

61: De la Rosa MA, Vega JM, Zumft WG. Composition and structure of assimilatory nitrate reductase from *Ankistrodesmus braunii*. *J Biol Chem.* 1981 Jun 10;256(11):5814-9.

62: de la Rosa MA, Diez J, Vega JM. [Affinity chromatography of *Ankistrodesmus braunii* nitrate reductase using blue dextran-sepharose (author's transl)] *Rev Esp Fisiol.* 1980 Jun;36(2):177-82. Spanish.

63: de la Rosa MA, Diez J, Vega JM, Losada M. Purification and properties of assimilatory nitrate reductase [NAD(P)H] from *Ankistrodesmus braunii*. *Eur J Biochem.* 1980 May;106(1):249-56.