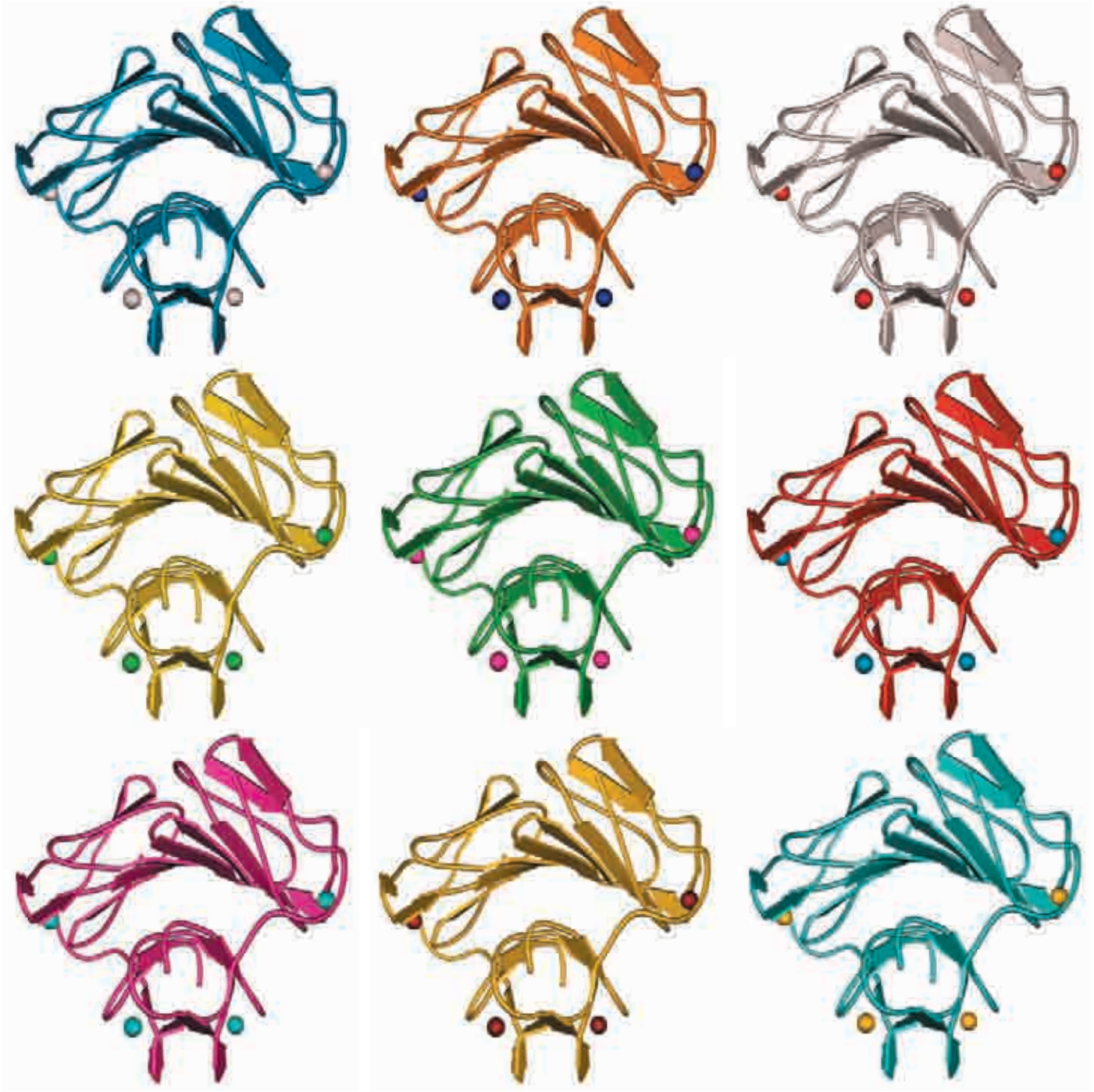

Proteins we love

www.fct.unl.pt

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Prefácio

Proteins we love é uma exposição na forma de arte molecular. Como o nome indica, esta mostra pretende revelar a beleza fundamental da estrutura 3D de proteínas de que "gostamos". Uma relação íntima entre ciência e arte, entre cientistas e proteínas, quase um "love affair"!

As proteínas escolhidas pertencem à classe de metaloproteínas e têm sido objecto, desde longa data, de estudos de caracterização bioquímica e espectroscópica efectuados pelos grupos de Bioinorgânica e Biofísica de Proteínas no Departamento de Química, FCT-UNL.

O revelar da estrutura é uma etapa fundamental para a compreensão da função (e vice-versa). As estruturas tridimensionais apresentadas foram obtidas por diferentes grupos cristalográficos.

Destes estudos, novas metaloproteínas foram isoladas, novas estruturas reveladas e muitas implicações mecanísticas estabelecidas.

No espaço de exposição da Biblioteca da UNL, no *Campus* de Caparica, estas estruturas são expostas na forma de painéis de dimensões variáveis, utilizando metodologias correntes de grafismo molecular e alguma imaginação.

Março 2007

Índice

Exposição

Proteínas de Transferência Electrónica e Sistemas Enzimáticos

Sala de exposições da Biblioteca do *Campus* de Caparica

Plano da exposição

Painéis 1 (4,5x1,5 m), 8 (1,5x1,5 m) e 9 (1x1 m) e uma instalação.

O painel (4,5x1,5) compara proteínas hémicas com diferentes massas moleculares. Os painéis 1x1 colocados no pavimento relembram algumas das montagens de Andy Wahrol (por exemplo Marylin Monroe). Os painéis de 1.5x1.5 mostram diferentes aspectos moleculares (com mais ou menos detalhe) de estruturas de metaloproteínas, cobrindo uma larga gama de centros metálicos de interesse em biocatálise.

A instalação cria uma visão espacial de uma estrutura.

Em cada caso, no catálogo que acompanha a exposição, é fornecido um breve texto de descrição da proteína e o resumo científico que pode fornecer detalhes ou proporcionar leituras mais completas para os mais interessados.

Desulfoferrodoxina (Capa - Cartaz de exposição - Pavimento)

Desulfoferrodoxin (Cover - Poster - Floor)

Uma proteína simples contendo dois átomos de ferro. Um dos átomos de ferro é coordenado por 4 cisteínas. O outro, rodeado por quatro histidinas, uma cisteína e um ácido glutâmico (ou água), foi demonstrado mais tarde estar envolvido na redução do ião superóxido (ver Redutases de superóxido).

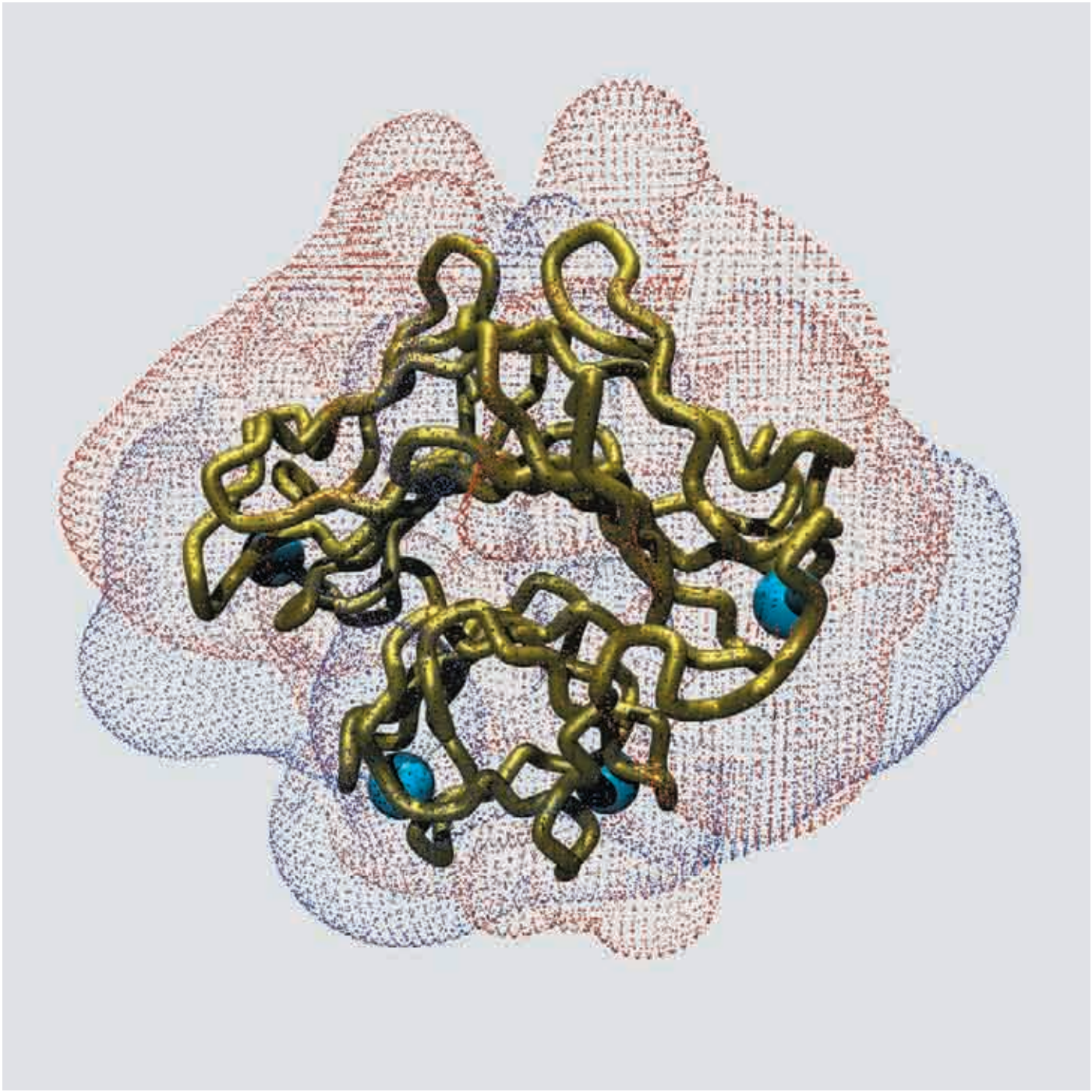
Preliminary crystallographic analysis of the oxidized form of a two mono-nuclear iron centers protein from *Desulfovibrio desulfuricans* ATCC 27774.
Coelho AV, Matias PM, Carrondo A, Tavares P, Moura JJG, Moura I, Fulop V, Hajdu J, Le Gall J.
Protein Sci. 5, 1189-1191 (1996)

Crystals of the fully oxidized form of desulfoferrodoxin were obtained by vapor diffusion from a solution containing 20% PEG 4000, 0.1 M HEPES buffer, pH 7.5, and 0.2 M $CaCl_2$. Trigonal and/or rectangular prisms could be obtained, depending on the temperature used for the crystal growth. Trigonal prisms belong to the rhombohedral space group R32, with a = 112.5 Å and c = 63.2 Å; rectangular prisms belong to the monoclinic space group C2, with a = 77.7 Å, b = 80.9 A, c = 53.9 A, and beta = 98.1 degrees.

The crystallographic asymmetric unit of the rhombohedral crystal form contains one molecule. There are two molecules in the asymmetric unit of the monoclinic form, in agreement with the self-rotation function.

Other relevant references:

Purification and characterization of desulfoferrodoxin – a novel protein from *Desulfovibrio desulfuricans (*ATCC 27774) and from *Desulfovibrio vulgaris* that contains a distorted rubredoxin center and a mononuclear ferrous center
Moura I, Tavares P, Moura JJG, Ravi N, Huynh BH, Liu MY, LeGall J.
J. Biol. Chem. 265, 21596-21602 (1990)



Desulforredoxina (Cubo - Sala de exposições)

Desulforedoxin (Cube - Exibition Hall)

A metaloproteína mais simples contendo um átomo de ferro coordenado por 4 cisteínas. Está envolvida em transferência electrónica. A primeira estrutura 3D de uma metaloproteína que foi resolvida em Portugal por RMN.

Crystal structure of desulforedoxin from *Desulfovibrio gigas* determined at 1.8 Å resolution: a novel non-heme iron protein structure.
Archer M, Huber R, Tavares P, Moura I, Moura JJG, Carrondo MA, Sieker LC, LeGall J, Romao MJ.
J Mol Biol. 251, 690-702 (1995)

The crystal structure of desulforedoxin from Desulfovibrio gigas, a new homo-dimeric (2 x 36 amino acids) non-heme iron protein, has been solved by the SIRAS method using the indium-substituted protein as the single derivative. The structure was refined to a crystallographic R-factor of 16.9% at 1.8 Å resolution. Native desulforedoxin crystals were grown from either PEG 4K or lithium sulfate, with cell constants a = b = 42.18 Å, c = 72.22 Å (for crystals grown from PEG 4K), and they belong to space group P3(2)21. The indium-substituted protein crystallized isomorphously under the same conditions. The 2-fold symmetric dimer is firmly hydrogen bonded and folds as an incomplete beta-barrel with the two iron centers placed on opposite poles of the molecule. Each iron atom is coordinated to four cysteinyl residues in a distorted tetrahedral arrangement. Both iron atoms are 16 Å apart but connected across the 2-fold axis by 14 covalent bonds along the polypeptide chain plus two hydrogen bonds. Desulforedoxin and rubredoxin share some structural features but show significant differences in terms of metal environment and water structure, which account for the known spectroscopic differences between rubredoxin and desulforedoxin.

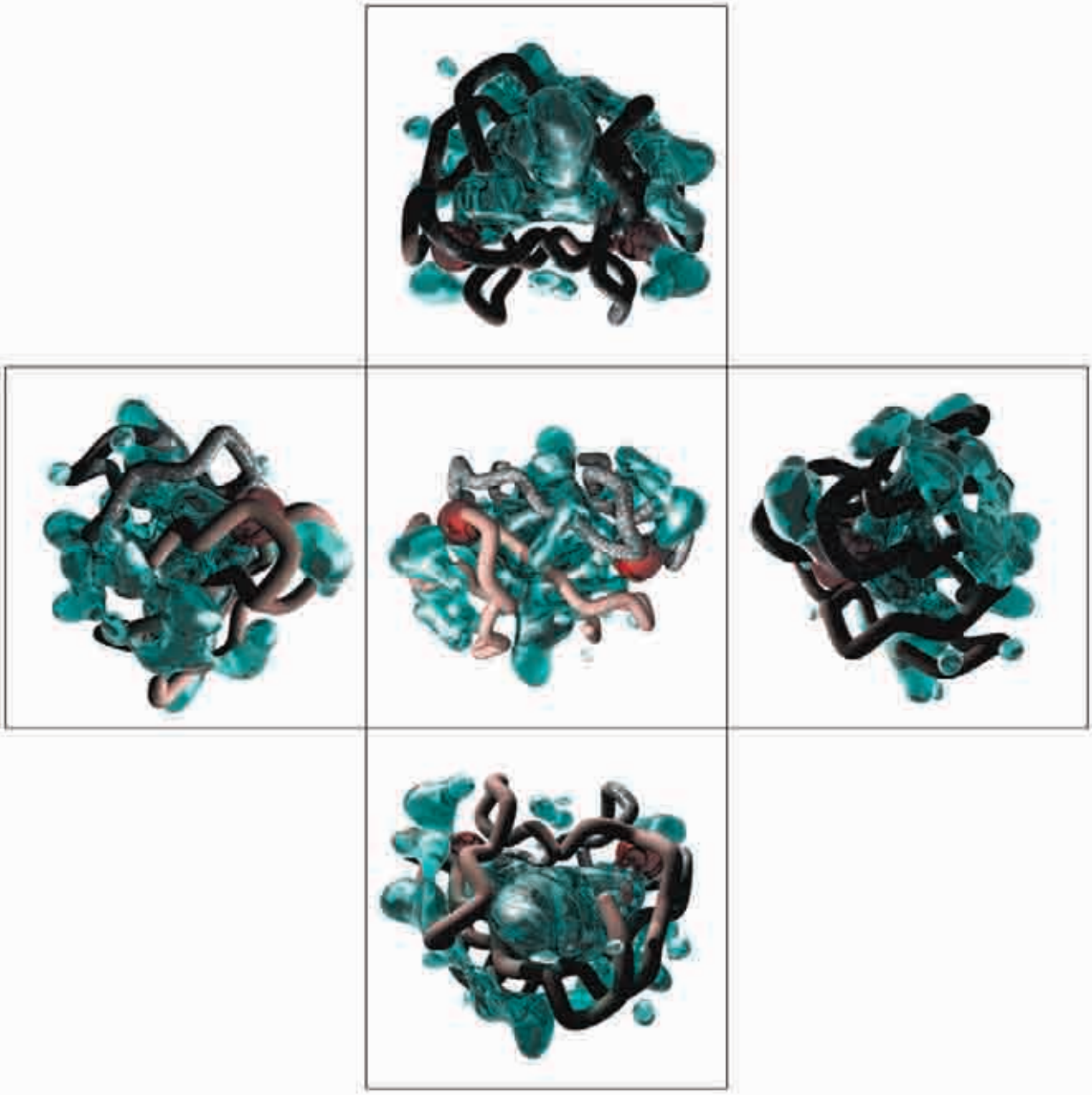
Other relevant references:
Isolation and characterization of desulforedoxin, a new type of non-heme iron protein from *Desulfovibrio gigas*
Moura I, Bruschi M, LeGall J, Moura JJG, Xavier AV
Biochem. Biophys. Res. Commun. 75, 1037-1044 (1977)

The solution structure of Desulforedoxin, a simple iron-sulfur protein – A NMR study of a Zn derivative
Goodfellow BJ, Tavares P, Romão MJ, Czaja C, Rusnak F, LeGall J, Moura I, Moura JJG.
J Biol Inorg Chem 1, 341-353 (1996)

NMR solution structures of two mutants of desulforedoxin.
Goodfellow BJ, Rusnak F, Moura I, Ascenso CS, Moura JJG.
J Inorg Biochem. 93, 100-8 (2003)

Zinc-substituted *Desulfovibrio gigas* desulforedoxins: resolving subunit degeneracy with nonsymmetric pseudocontact shifts.
Goodfellow BJ, Nunes SG, Rusnak F, Moura I, Ascenso C, Moura JJG, Volkman BF, Markley JL.
Protein Sci. 11, 2464-70 (2002)

NMR determination of the global structure of the 113Cd derivative of desulforedoxin: investigation of the hydrogen bonding pattern at the metal center.
Goodfellow BJ, Rusnak F, Moura I, Domke T, Moura JJG.
Protein Sci. 17, 928-37 (1998)



Cubo - Instalação

Citocromo c_{552}
Redutase do nitrito
Proteína hexadecahémica

O grande painel compara, numa só escala, três proteínas: uma proteína monohémica (citocromo $c_{552,}$ dímero, 2x9,5 kDa), uma enzima pentahémica (redutase do nitrito, dímero, 2x66 kDa) e um citocromo hexadecahémico (aproximadamente 70 kDa) de forma a realçar a dimensão molecular relativa.

Cytochrome c_{552}

MAD structure of *Pseudomonas nautica* dimeric cytochrome c552

Brown K, Nurizzo D, Besson S, Shepard W, Moura J, Moura I, Tegoni M, Cambillau C.

J Mol Biol. 289, 1017-1028 (1999).

The monohemic cytochrome c552 from *Pseudomonas nautica* (c552-Pn) is thought to be the electron donor to cytochrome cd1, the so-called nitrite reductase (NiR). It shows as high levels of activity and affinity for the *P. nautica* NiR (NiR-Pn), as the Pseudomonas aeruginosa enzyme (NiR-Pa). Since cytochrome c552 is by far the most abundant electron carrier in the periplasm, it is probably involved in numerous other reactions. Its sequence is related to that of the c type cytochromes, but resembles that of the dihemic c4 cytochromes even more closely. The three-dimensional structure of P. nautica cytochrome c552 has been solved to 2.2 Å resolution using the multiple wavelength anomalous dispersion (MAD) technique, taking advantage of the presence of the eight Fe heme ions in the asymmetric unit. Density modification procedures involving 4-fold non-crystallographic averaging yielded a model with an R -factor value of 17.8 % (Rfree=20.8 %). Cytochrome c552 forms a tight dimer in the crystal, and the dimer interface area amounts to 19% of the total cytochrome surface area. Four tighly packed dimers form the eight molecules of the asymmetric unit.The c552 dimer is superimposable on each domain of the monomeric cytochrome c4 from *Pseudomomas stutzeri* (c4-Ps), a dihemic cytochrome, and on the dihemic c domain of flavocytochrome *c* of Chromatium vinosum (Fcd-Cv). (...) The dimer observed in the crystal also exists in solution. It has been hypothesised that the dihemic c4-Ps may have evolved via monohemic cytochrome *c* gene duplication followed by evolutionary divergence and the adjunction of a connecting linker. In this process, our dimeric c552 structure might be said to constitute a “living fossile” occurring in the course of evolution between the formation of the dimer and the gene duplication and fusion. The availability of the structure of the cytochrome c552-Pn and that of NiR from *P. aeruginosa* made it possible to identify putative surface patches at which the docking of c552 to NiR-Pn may occur.

Nitrite reductase

Cytochrome *c* nitrite reductase from *Desulfovibrio desulfuricans* ATCC 27774. The relevance of the two calcium sites in the structure of the catalytic subunit (NrfA).

Cunha CA, Macieira S, Dias JM, Almeida G, Goncalves LL, Costa C, Lampreia J, Huber R, Moura JJG, Moura I, Romao MJ.

J Biol Chem. 278, 17455-17465 (2003).

Cytochrome *c*552
Nitrite reductase
Hexadecaheme protein

(Large panel - three structures)

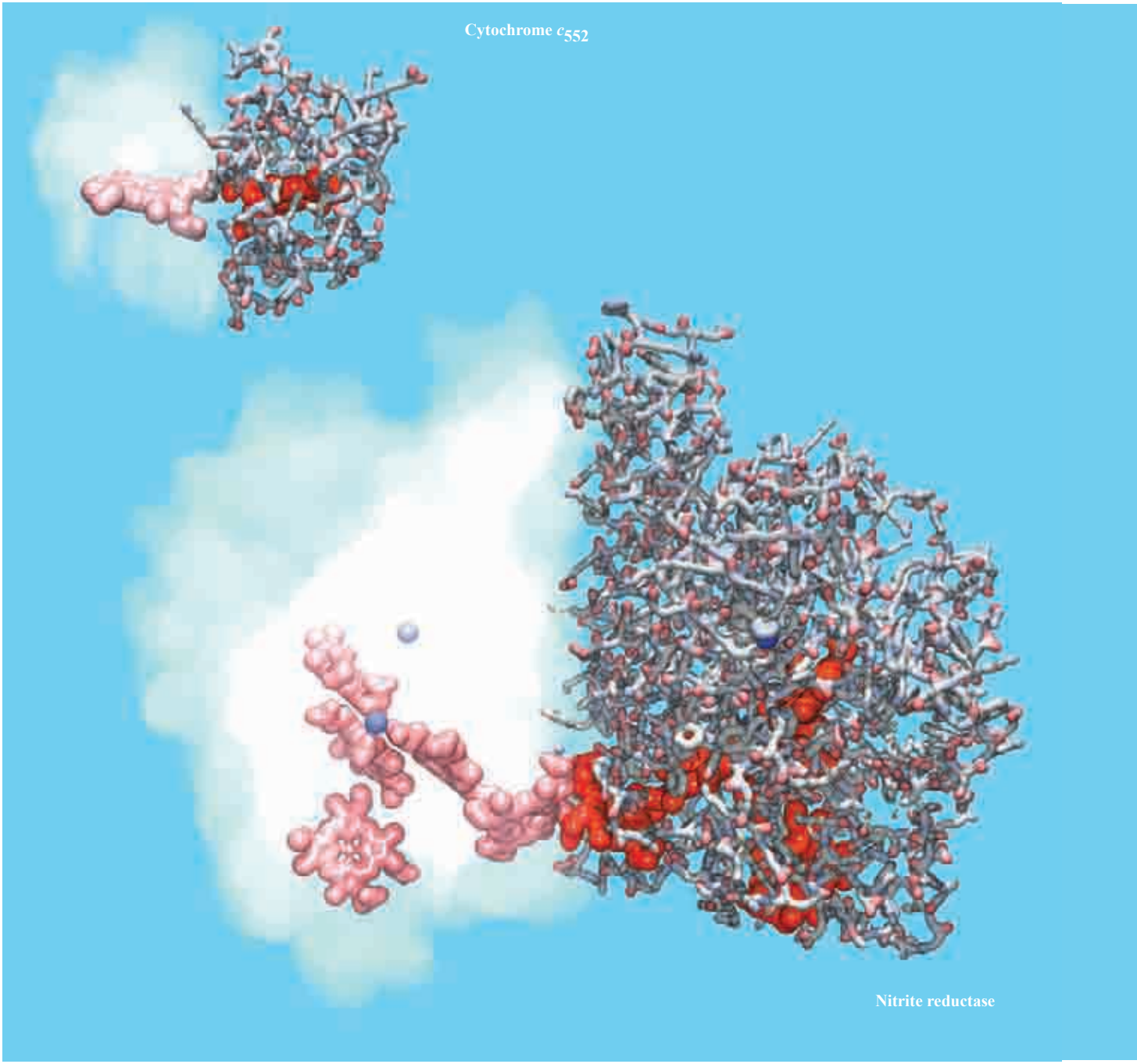
The gene encoding cytochrome c nitrite reductase (NrfA) from *Desulfovibrio desulfuricans* ATCC 27774 was sequenced and the crystal structure of the enzyme was determined to 2.3-Å resolution. In comparison with homologous structures, it presents structural differences mainly located at the regions surrounding the putative substrate inlet and product outlet, and includes a well defined second calcium site with octahedral geometry, coordinated to propionates of hemes 3 and 4, and caged by a loop non-existent in the previous structures. The highly negative electrostatic potential in the environment around hemes 3 and 4 suggests that the main role of this calcium ion may not be electrostatic but structural, namely in the stabilization of the conformation of the additional loop that cages it and influences the solvent accessibility of heme 4. The NrfA active site is similar to that of peroxidases with a nearby calcium site at the heme distal side nearly in the same location as occurs in the class II and class III peroxidases. This fact suggests that the calcium ion at the distal side of the active site in the NrfA enzymes may have a similar physiological role to that reported for the peroxidases.

Other relevant references:

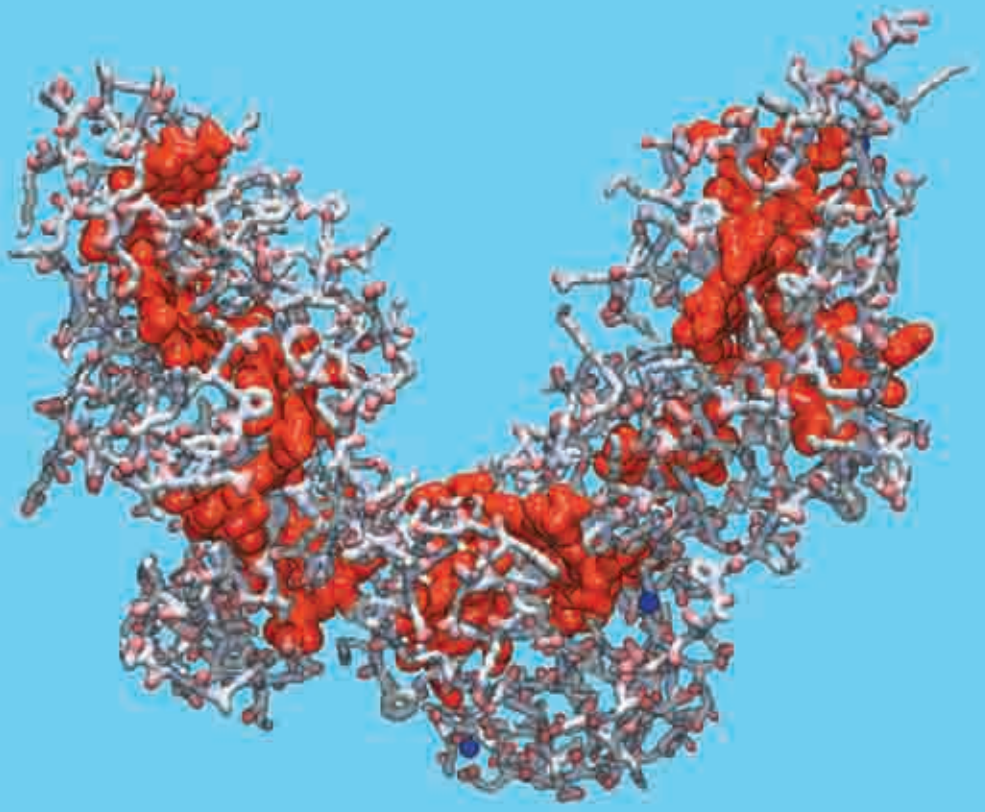
Crystal Structure of the sixteenheme cytochrome from Desulfovibrio gigas: a glycosylated protein in a sulphate reducing bacterium
Santos-Silva T, Dias JM, Dolla A, Durand M-C, Gonçalves LL, Lampreia J, Moura I, Romão MJ
J.Mol.Biol. (2007) *in press*

Hexadecaheme Protein
Czjzek M, ElAntak L, Zamboni V, Morelli X, Dolla A, Guerlesquin F, Bruschi M.
The crystal structure of the hexadeca-heme cytochrome Hmc and a structural model of its complex with cytochrome c(3).
Structure 10, 1677-1686 (2002)

Sulfate-reducing bacteria contain a variety of multi-heme c-type cytochromes. The cytochrome of highest molecular weight (Hmc) contains 16 heme groups and is part of a transmembrane complex involved in the sulfate respiration pathway. We present the 2.42 Å resolution crystal structure of the *Desulfovibrio vulgaris* Hildenborough cytochrome Hmc and a structural model of the complex with its physiological electron transfer partner, cytochrome c(3), obtained by NMR restrained soft-docking calculations. The Hmc is composed of three domains, which exist independently in different sulfate-reducing species, namely cytochrome c(3), cytochrome c(7), and Hcc. The complex involves the last heme at the C-terminal region of the V-shaped Hmc and heme 4 of cytochrome c(3), and represents an example for specific cytochrome-cytochrome interaction.


Cytochrome c_{552}
Nitrite reductase

Hexadecaheme Protein



[Escala 200.000.000:1] Se o tamanho real das proteínas fosse o tamanho representado neste painel, poderiamos jogar ténis com o planeta Terra.

[Scale 200,000,000:1] If the real size of a protein was the one portraited in this panel, we would be able to play tennis with planet Earth.

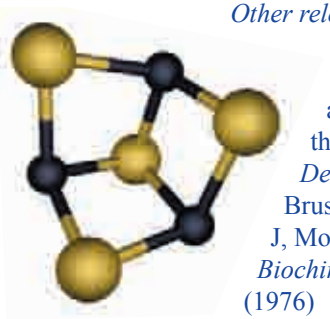
Ferredoxina II

Ferredoxin II

A ferredoxina II contém um centro único com 3 átomos de ferros e 4 átomos de enxofre. Quando isolada pela primeira vez, representou uma adição importante ao reconhecimento de novas estruturas em biologia e permitiu estudar processos de interconversão entre agregados Ferro-Enxofre.

The solution structure of a [3Fe-4S] ferredoxin: oxidized ferredoxin II from *Desulfovibrio gigas*.
Goodfellow BJ, Macedo AL, Rodrigues P, Moura I, Wray V, Moura JJG
J Biol Inorg Chem. 4, 421-30 (1999)

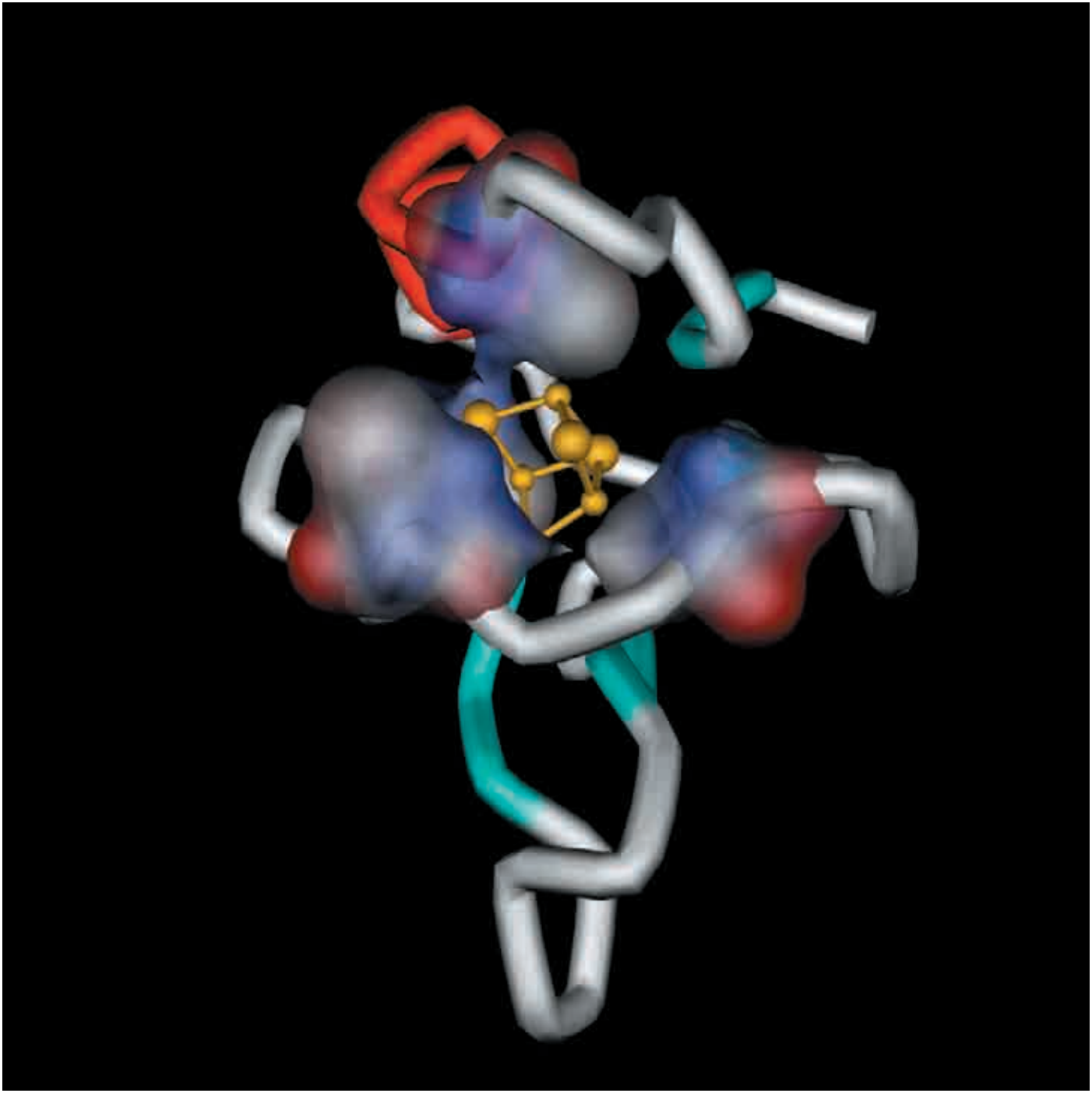
The use of standard 2D NMR experiments in combination with 1D NOE experiments allowed the assignment of 51 of the 58 spin systems of oxidised [3Fe4S] ferredoxin isolated from Desulfovibrio gigas. The NMR solution structure was determined using data from 1D NOE and 2D NOESY spectra, as distance constraints, and information from the X-ray structure for the spin systems not detected by NMR in torsion angle dynamics calculations to produce a family of 15 low target function structures. The quality of the NMR family, as judged by the backbone r.m.s.d. values, was good (0.80 Å), with the majority of phi/psi angles falling within the allowed region of the Ramachandran plot. A comparison with the X-ray structure indicated that the overall global fold is very similar in solution and in the solid state. The determination of the solution structure of ferredoxin II (FdII) in the oxidised state (FdIIox) opens the way for the determination of the solution structure of the redox intermediate state of FdII (FdII(int)), for which no X-ray structure is available.



Other relevant references:

Purification, characterization and biological studies of threee forms of ferredoxin from *Desulfovibrio gigas*
Bruschi M, Hatchikian EC, LeGall J, Moura JJG, Xavier AV
Biochim. Biophys. Acta, 449, 275-284 (1976)

Interconversions of [3Fe-3S] and [4Fe-4S] Clusters – Mössbauer and EPR studies of *Desuslfovibrio gigas* ferredoxin II
Moura JJG, Moura I, Kent TA, Lipscomb JD, Huynh BH, LeGall J, Xavier AV, Münck E
J. Biol. Chem. 257, 6259-6267 (1982)



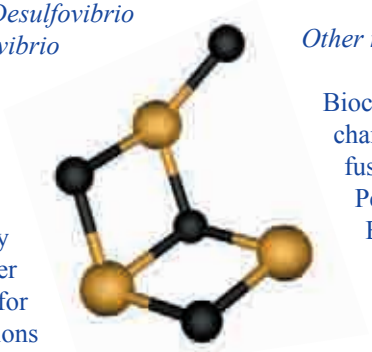
Fuscoredoxina

Fuscoredoxin

A descoberta deste centro catalítico permitiu uma visão mais alargada de estruturas de centros contendo Ferro e Enxofre em que a coordenação adicional por átomos de oxigénio está presente. Porque o solvente biológico é a água optou-se, neste painel, por representar a vermelho as moléculas de água que envolvem (solvatam) a Fuscoredoxina

Hybrid cluster proteins (HCPs) from *Desulfovibrio desulfuricans* ATCC 27774 and *Desulfovibrio vulgaris* (Hildenborough): X-ray structures at 1.25 Å resolution using synchrotron radiation.
Macedo S, Mitchell EP, Romao CV, Cooper SJ, Coelho R, Liu MY, Xavier AV, LeGall J, Bailey S, Garner DC, Hagen WR, Teixeira M, Carrondo MA, Lindley P.
J Biol Inorg Chem. 7, 514-525 (2002)

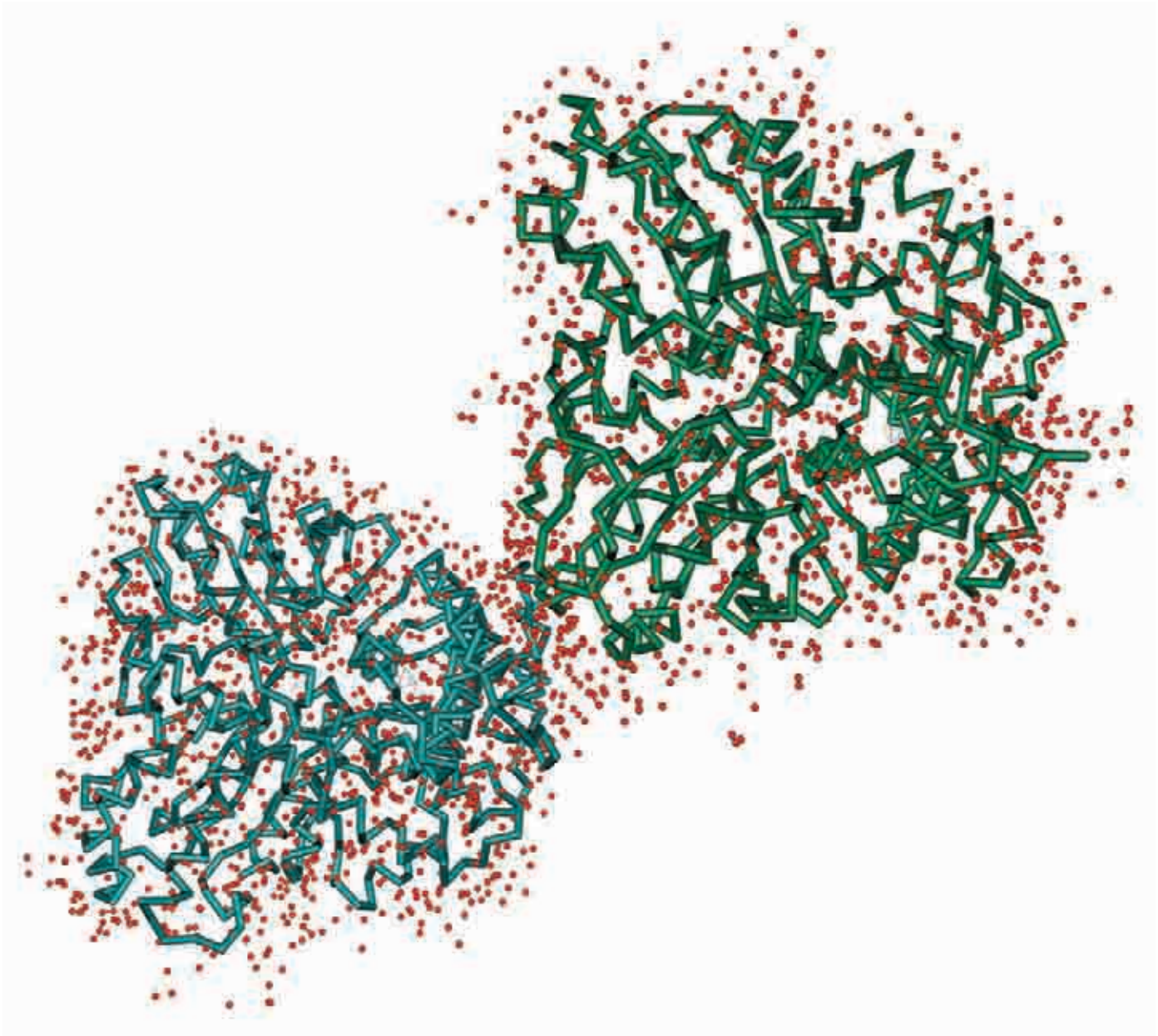
The structures of the hybrid cluster proteins (HCPs) from the sulfate-reducing bacteria *Desulfovibrio desulfuricans* (ATCC 27774) and *Desulfovibrio vulgaris* (Hildenborough) have been elucidated at a resolution of 1.25 Å using X-ray synchrotron radiation techniques. In the case of the *D. desulfuricans* protein, protein isolation, purification, crystallization and X-ray data collection were carried out under strict anaerobic conditions, whereas for the *D. vulgaris* protein the conditions were aerobic. However, both structures are essentially the same, comprising three domains and two iron-sulfur centres. One of these centres situated near the exterior of the molecules in domain 1 is a cubane [4Fe-4S] cluster, whereas the other, located at the interface of the three domains, contains the unusual four-iron cluster initially found in the *D. vulgaris* protein. Details of the structures and the associated EPR spectroscopy of the *D. desulfuricans* protein are reported herein. These structures show that the nature of the hybrid cluster, containing both oxygen and sulfur bridges, is independent of the presence of oxygen in the isolation and crystallization procedure and also does not vary significantly with changes in the oxidation state. The structures and amino acid sequences of the HCP are compared with the recently elucidated structure of the catalytic subunit of a carbon monoxide dehydrogenase from *Carboxydothermus hydrogenoformans* and related dehydrogenases.



Other relevant references:

Biochemical and spectroscopic characterization of overexpressed fuscoredoxin from *Escherichia coli.*
Pereira AS, Tavares P, Krebs C, Huynh BH, Rusnak F, Moura I, Moura JJG.
Biochem Biophys Res Commun. 260, 209-215 (1999)

Spectroscopic characterization of a novel tetranuclear Fe cluster in an iron-sulfur protein isolated from *Desulfovibrio desulfuricans.*
Tavares P, Pereira AS, Krebs C, Ravi N, Moura JJG, Moura I, Huynh BH.
Biochemistry 37, 2830-2842 (1998)



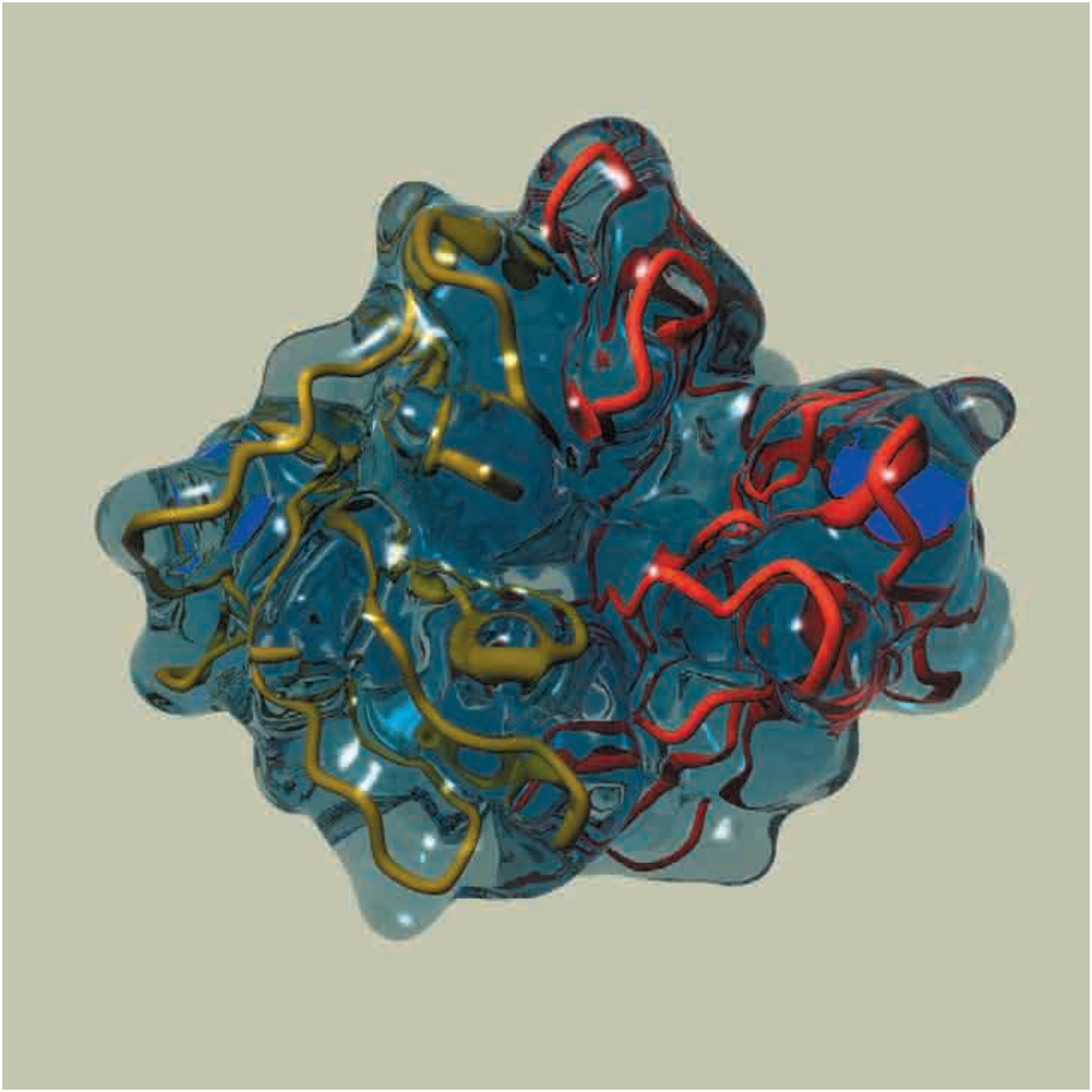
Redutase do superóxido

Superoxide reductase

As redutases de superóxido (incluindo a Desulfoferrodoxin) representam uma nova classe de enzimas capazes de reduzir superóxido em mecanismos de defesa e eliminação de radicais nocivos. O centro activo contém ferro coordenado por quatro histidinas, uma cisteína e um resíduo de ácido glutâmico (ou água).

Superoxide reductase from the syphilis spirochete *Treponema pallidum*: crystallization and structure determination using soft X-rays.
Santos-Silva T, Trincao J, Carvalho AL, Bonifacio C, Auchere F, Moura I, Moura JJG, Romao MJ.
Acta Crystallograph Sect F Struct Biol Cryst Commun. 61, 967-70 (2005)

Superoxide reductase is a 14 kDa metalloprotein containing a catalytic non-haem iron centre [$Fe(His)_4Cys$]. It is involved in defense mechanisms against oxygen toxicity, scavenging superoxide radicals from the cell. The oxidized form of *Treponema pallidum* superoxide reductase was crystallized in the presence of polyethylene glycol and magnesium chloride. Two crystal forms were obtained depending on the oxidizing agents used after purification: crystals grown in the presence of $K_3Fe(CN)_6$ belonged to space group P2(1) (unit-cell parameters a = 60.3, b = 59.9, c = 64.8 Å, beta = 106.9 degrees) and diffracted beyond 1.60 Å resolution, while crystals grown in the presence of Na2IrCl6 belonged to space group C2 (a = 119.4, b = 60.1, c = 65.6 Å, beta = 104.9 degrees) and diffracted beyond 1.55 Å. A highly redundant X-ray diffraction data set from the C2 crystal form collected on a copper rotating-anode generator (lambda = 1.542 Å) clearly defined the positions of the four Fe atoms present in the asymmetric unit by SAD methods. A MAD experiment at the iron absorption edge confirmed the positions of the previously determined iron sites and provided better phases for model building and refinement. Molecular replacement using the P2(1) data set was successful using a preliminary trace as a search model. A similar arrangement of the four protein molecules could be observed.



Oxido-redutase do aldeído

Aldehyde oxido-reductase

Os estudos cristalográficos da Oxido-redutase do aldeído de *D. gigas* revelou a primeira estrutura 3D de uma proteína contendo Mo mononuclear e foi modelo, durante anos, das enzimas da família da xantina oxidase. A enzima tinha sido isolada em 1976.

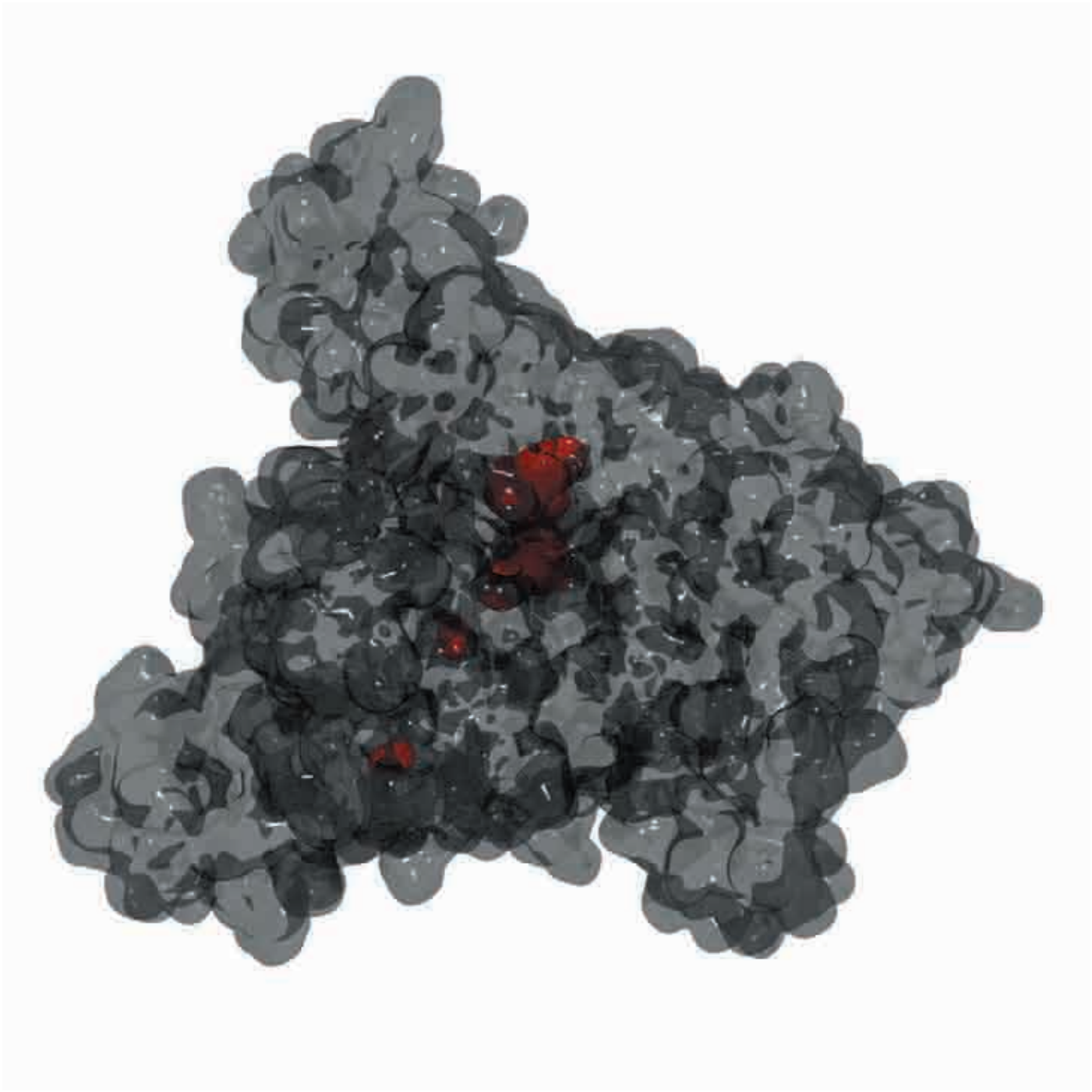
Crystal structure of the xanthine oxidase-related aldehyde oxido-reductase from *D.gigas*. Romao MJ, Archer M, Moura I, Moura JJG, LeGall J, Engh R, Schneider M, Hof P, Huber R. *Science* 270,1170-1176 (1995).

The crystal structure of the aldehyde oxido-reductase (Mop) from the sulfate reducing anaerobic Gram-negative bacterium *Desulfovibrio gigas* has been determined at 2.25 Å resolution by multiple isomorphous replacement and refined. The protein, a homodimer of 907 amino acid residues subunits, is a member of the xanthine oxidase family. The protein contains a molybdopterin cofactor (Mo-co) and two different [2Fe-2S] centers. It is folded into four domains of which the first two bind the iron sulfur centers and the last two are involved in Mo-co binding. Mo-co is a molybdenum molybdopterin cytosine dinucleotide. Molybdopterin forms a tricyclic system with the pterin bicycle annealed to a pyran ring. The molybdopterin dinucleotide is deeply buried in the protein. The cis-dithiolene group of the pyran ring binds the molybdenum, which is coordinated by three more (oxygen) ligands.

Other relevant references:

Structure refinement of the aldehyde oxidoreductase from Desulfovibrio gigas (MOP) - 1.28 Å. Rebelo JM, Dias JM, Huber R, Moura JJG, Romao MJ.
J Biol Inorg Chem. 6, 791-800 (2001).

A Molydbenum containing [2Fe,2S] protein from *Desulfovibrio gigas*
Moura JJG, Xavier AV, Bruschi M, LeGall J, Cabral JMP
J. Less-Common Metals 54, 555-562 (1976).



Peroxidase do citocromo *c*

Cytochrome *c* peroxidase

As peroxidases catalizam a redução do peróxido de hidrogénio (água oxigenada) a água e têm um papel importante em mecanismos de desintoxicação celular. A estrutura das peroxidases dihémicas permitiu pôr em evidência alterações conformacionais que conduzem à activação da enzima.

Structural basis for the mechanism of Ca(2+) activation of the di-heme cytochrome c peroxidase from Pseudomonas nautica 617.
Dias JM, Alves T, Bonifacio C, Pereira AS, Trincao J, Bourgeois D, Moura I, Romao MJ. *Structure* 12, 961-73 (2004)

Cytochrome c peroxidase (CCP) catalyses the reduction of H(2)O(2) to H(2)O, an important step in the cellular detoxification process. The crystal structure of the di-heme CCP from Pseudomonas nautica 617 was obtained in two different conformations in a redox state with the electron transfer heme reduced. Form IN, obtained at pH 4.0, does not contain Ca(2+) and was refined at 2.2 Å resolution. This inactive form presents a closed conformation where the peroxidatic heme adopts a six-ligand coordination, hindering the peroxidatic reaction from taking place. Form OUT is Ca(2+) dependent and was crystallized at pH 5.3 and refined at 2.4 Å resolution. This active form shows an open conformation, with release of the distal histidine (His71) ligand, providing peroxide access to the active site. This is the first time that the active and inactive states are reported for a di-heme peroxidase.

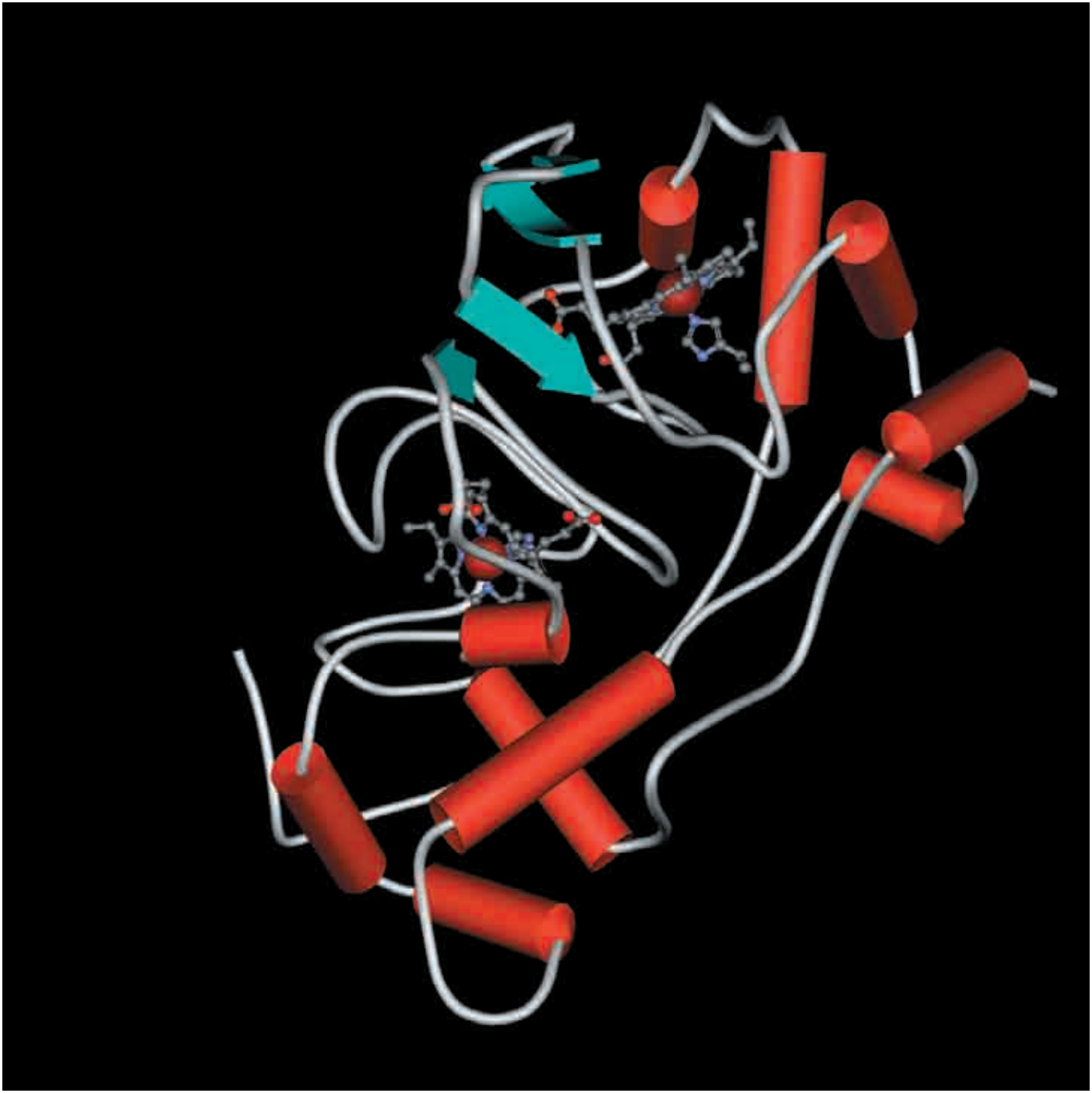
Other relevant references:

The kinetics of the oxidation of cytochrome c by *Paracoccus* cytochrome *c* peroxidase.
Gilmour R, Goodhew CF, Pettigrew GW, Prazeres S, Moura JJG, Moura I.
Biochem J. 300, 907-14 (1994).

Spectroscopic characterization of cytochrome *c* peroxidase from *Paracoccus denitrificans.*
Gilmour R, Goodhew CF, Pettigrew GW, Prazeres S, Moura I, Moura JJG
Biochem J. 15, 745-52 (1993).

Ca^{2+} and the bacterial peroxidases: the cytochrome *c* peroxidase from *Pseudomonas stutzeri.*
Timoteo CG, Tavares P, Goodhew CF, Duarte LC, Jumel K, Girio FM, Harding S, Pettigrew GW, Moura I.
J Biol Inorg Chem. 8, 29-37 (2003).

Electron transfer complexes of cytochrome *c* peroxidase from *Paracoccus denitrificans* containing more than one cytochrome.
Pettigrew GW, Pauleta SR, Goodhew CF, Cooper A, Nutley M, Jumel K, Harding SE, Costa C, Krippahl L, Moura I, Moura J.
Biochemistry 42, 11968-81 (2003).



Ferritina

Ferritin

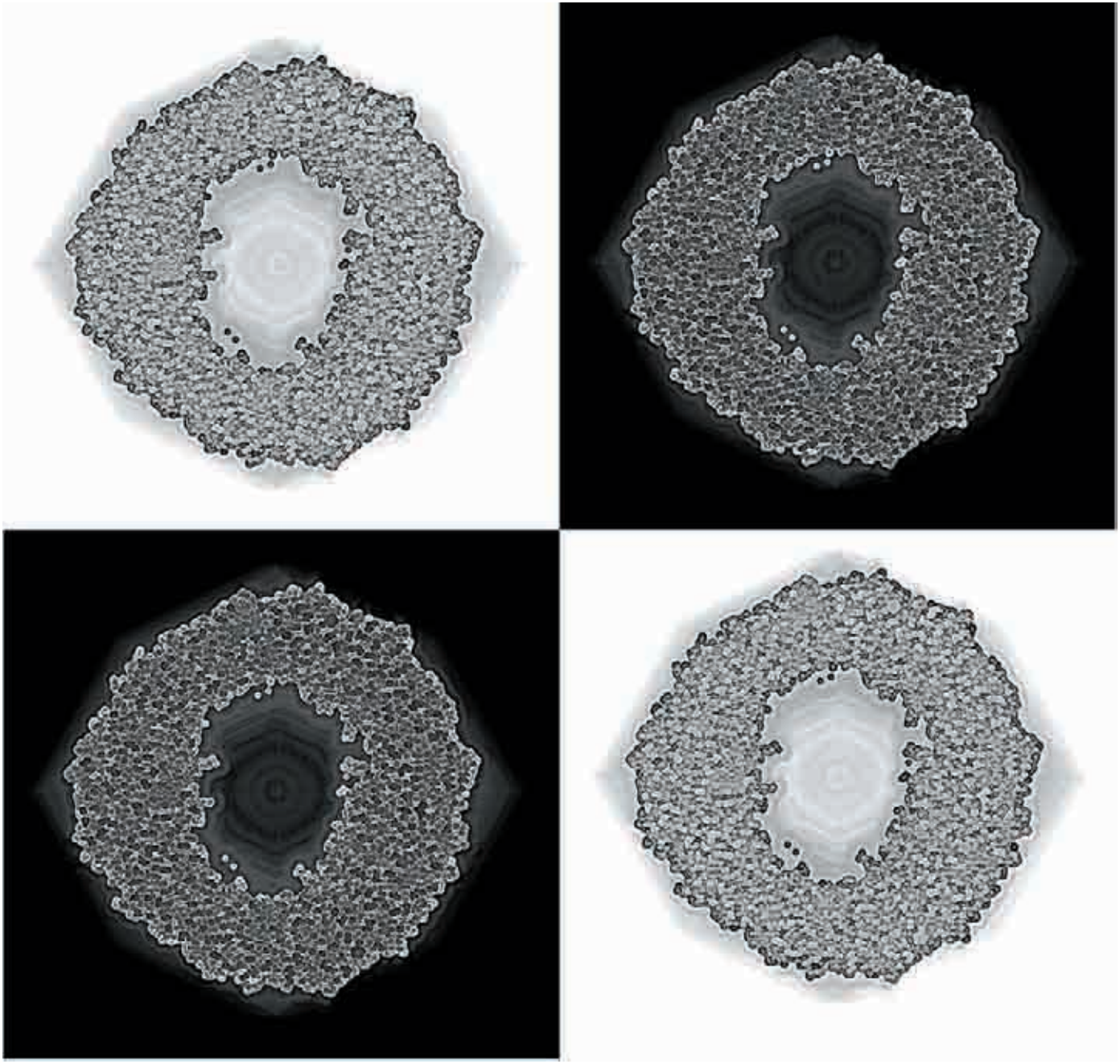
O principal papel da ferritina nas células é concentrar e armazenar o ferro, pois este metal é usado em quantidades cerca de 100 biliões de vezes superiores à solubilidade do ferro livre. A ferritina é a única proteína que dirige uma transição de fase reversível de um ião metálico em solução para um ião metálico numa matriz sólida através de um processo análogo à formação dos ossos e dos dentes.

Crystal structure of bullfrog M ferritin at 2.8 Å resolution: analysis of subunit interactions and the binuclear metal center.
Ha Y, Shi D, Small W, Theil EC, Allewell NM
J. Biol. Inorg. Chem. 4, 243-256 (1999).

Ferritins concentrate and store iron as a mineral in all bacterial, plant, and animal cells. The two ferritin subunit types, H or M (fast) and L (slow), differ in rates of iron uptake and mineralization and assemble in vivo to form heteropolymeric protein shells made up of 24 subunits; H/L subunit ratios reflect cell specificity of H and L subunit gene expression. A diferric peroxo species that is the initial reaction product of Fe(II) in H-type ferritins, as well as in ribonucleotide reductase (R2) and methane monooxygenase hydroxylase (MMOH), has recently been characterized, exploiting the relatively high accumulation of the peroxo intermediate in frog H-subunit type recombinant ferritin with the M sequence. The stability of the diferric reaction centers in R2 and MMOH contrasts with the instability of diferric centers in ferritin, which are precursors of the ferric mineral. We have determined the crystal structure of the homopolymer of recombinant frog M ferritin in two crystal forms: P4(1)2(1)2, a = b = 170.0 Å and c = 481.5 Å; and P3(1)21, a = b = 210.8 Å and c = 328.1 Å. The structural model for the trigonal form was refined to a crystallographic R value of 19.0% (Rfree = 19.4%); the two structures have an r.m.s.d. of approximately 0.22 A for all C alpha atoms. Comparison with the previously determined crystal structure of frog L ferritin indicates that the subunit interface at the molecular twofold axes is most variable, which may relate to the presence of the ferroxidase site in H-type ferritin subunits. Two metal ions (Mg) from the crystallization buffer were found in the ferroxidase site of the M ferritin crystals and interact with Glu23, Glu58, His61, Glu103, Gln137 and, unique to the M subunit, Asp140. The data suggest that Gln137 and Asp140 are a vestige of the second GluxxHis site, resulting from single nucleotide mutations of Glu and His codons and giving rise to Ala140 or Ser140 present in other eukaryotic H-type ferritins, by additional single nucleotide mutations. The observation of the Gln137xxAsp140 site in the frog M ferritin accounts for both the instability of the diferric oxy complexes in ferritin compared to MMOH and R2 and the observed kinetic variability of the diferric peroxo species in different H-type ferritin sequences.

Other relevant references:

Direct spectroscopic and kinetic evidence for the involvement of a peroxodiferric intermediate during the ferroxidase reaction in fast ferritin mineralization.
Pereira AS, Small W, Krebs C,, Tavares P, Edmondson DE, Theil EC, Huynh BH.
Biochemistry 37, 9871-9876 (1998).



Redutase do óxido nitroso

Nitrous oxide reductase

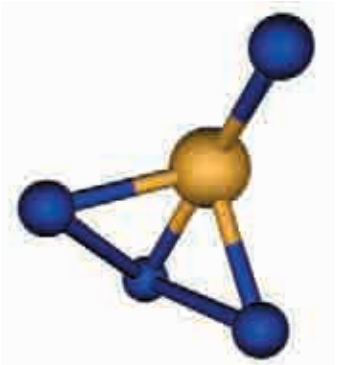
A redutase do N_2O é a enzima terminal da cadeia de desnitrificação transformando N_2O em N_2. A estrutura revelou a presença de um centro contendo quatro átomos de cobre (e um ligando S em ponte) sem precedentes em Química e Biologia.

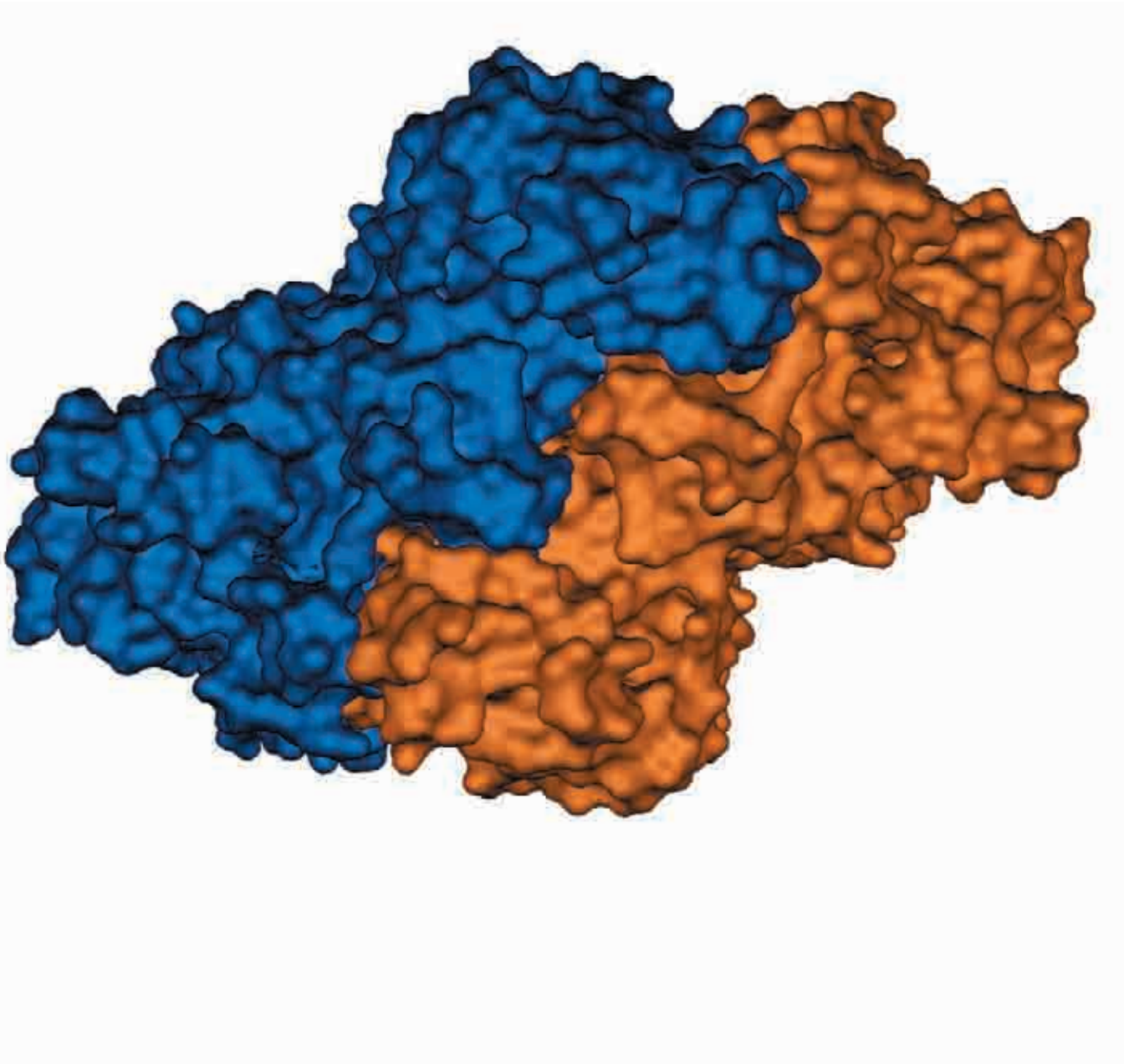
A novel type of catalytic copper cluster in nitrous oxide reductase.
Brown K, Tegoni M, Prudencio M, Pereira AS, Besson S, Moura JJG, Moura I, Cambillau C.
Nat Struct Biol. 7, 191-5 (2000)

Nitrous oxide (N20) is a greenhouse gas, the third most significant contributor to global warming. As a key process for N20 elimination from the biosphere, N20 reductases catalyze the two-electron reduction of N20 to N2. These 2 x 65 kDa copper enzymes are thought to contain a CuA electron entry site, similar to that of cytochrome c oxidase, and a CuZ catalytic center. The copper anomalous signal was used to solve the crystal structure of N20 reductase from Pseudomonas nautica by multiwavelength anomalous dispersion, to a resolution of 2.4 Å. The structure reveals that the CuZ center belongs to a new type of metal cluster, in which four copper ions are liganded by seven histidine residues. N20 binds to this center via a single copper ion. The remaining copper ions might act as an electron reservoir, assuring a fast electron transfer and avoiding the formation of dead-end products.

Other relevant references:

Revisiting the catalytic CuZ cluster of nitrous oxide (N2O) reductase. Evidence of a bridging inorganic sulfur.
Brown K, Djinovic-Carugo K, Haltia T, Cabrito I, Saraste M, Moura JJG, Moura I, Tegoni M, Cambillau C.
J Biol Chem. 275, 41133-6 (2000)





Redutase do nitrato

Nitrate reductase

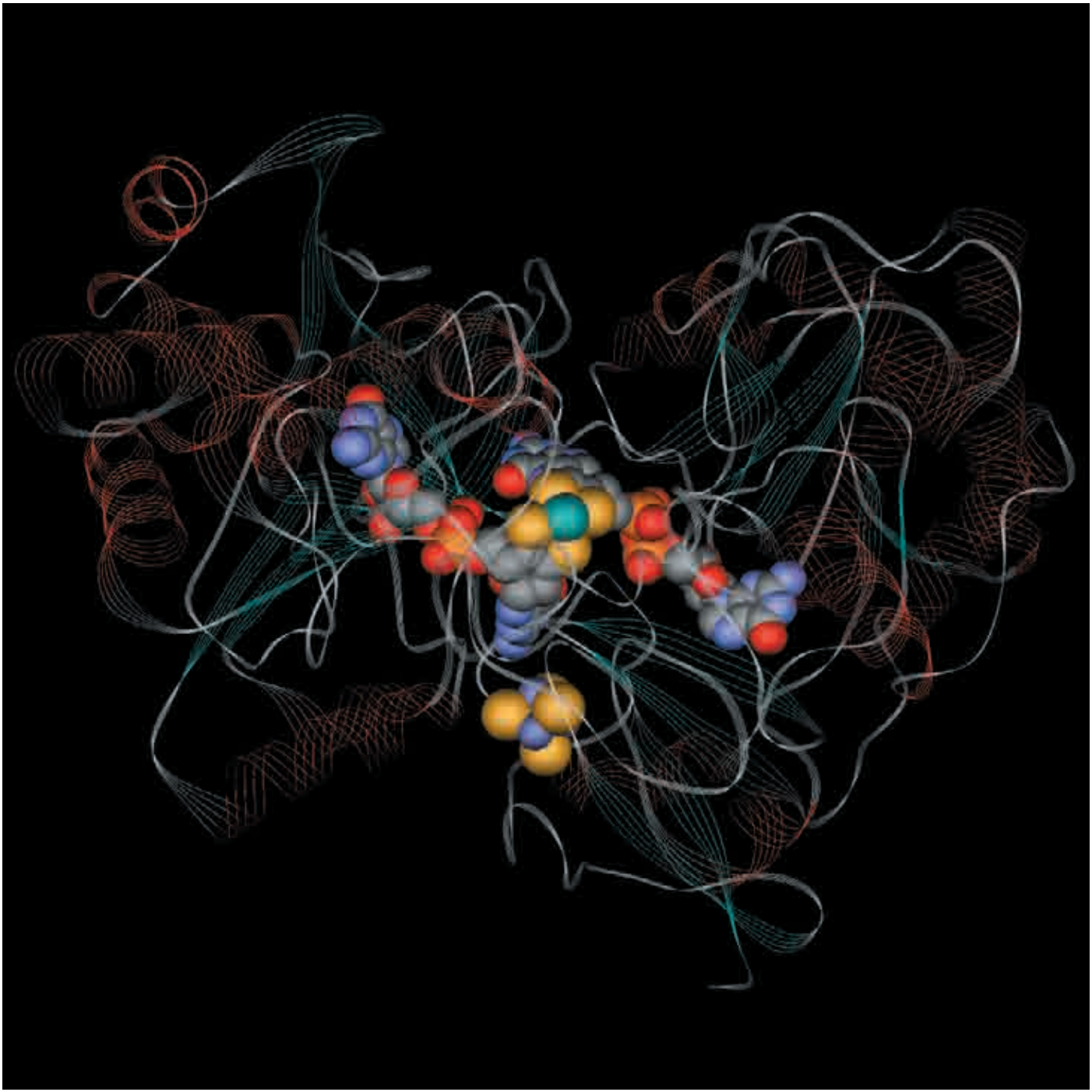
A redução de nitrato a nitrito (uma reacção relevante do ciclo do Azoto) é efectuada por uma enzima que contem molibdénio mononuclear no centro activo. A estrutura 3D da redutase do nitrato aqui apresentada foi a primeira estrutura resolvida para esta classe de enzimas.

Crystal structure of the first dissimilatory nitrate reductase at 1.9 Å solved by MAD methods. Dias JM, Than ME, Humm A, Huber R, Bourenkov GP, Bartunik HD, Bursakov S, Calvete J, Caldeira J, Carneiro C, Moura JJG, Moura I, Romao MJ
Structure 7, 65-79 (1999).

The periplasmic nitrate reductase (NAP) from the sulphate reducing bacterium Desulfovibrio desulfuricans ATCC 27774 is induced by growth on nitrate and catalyses the reduction of nitrate to nitrite for respiration. NAP is a molybdenum-containing enzyme with one bis-molybdopterin guanine dinucleotide (MGD) cofactor and one [4Fe-4S] cluster in a single polypeptide chain of 723 amino acid residues. To date, there is no crystal structure of a nitrate reductase. RESULTS: The first crystal structure of a dissimilatory (respiratory) nitrate reductase was determined at 1.9 Å resolution by multiwavelength anomalous diffraction (MAD) methods. The structure is folded into four domains with an alpha/beta-type topology and all four domains are involved in cofactor binding. The [4Fe-4S] centre is located near the periphery of the molecule, whereas the MGD cofactor extends across the interior of the molecule interacting with residues from all four domains. The molybdenum atom is located at the bottom of a 15 Å deep crevice, and is positioned 12 Å from the [4Fe-4S] cluster. The structure of NAP reveals the details of the catalytic molybdenum site, which is coordinated to two MGD cofactors, Cys140, and a water/hydroxo ligand. A facile electron-transfer pathway through bonds connects the molybdenum and the [4Fe-4S] cluster. CONCLUSIONS: The polypeptide fold of NAP and the arrangement of the cofactors is related to that of Escherichia coli formate dehydrogenase (FDH) and distantly resembles dimethylsulphoxide reductase. The close structural homology of NAP and FDH shows how small changes in the vicinity of the molybdenum catalytic site are sufficient for the substrate specificity.

Other relevant references:

Isolation and preliminary characterization of a soluble Nitrate reductase from the sulphate reducing *Desulfovibrio desulfuricans* ATCC 27774
Bursakov S, Liu MY, Payne WJ, LeGall J, Moura I, Moura JJG
Anaerobe 1, 55-60 (1995).



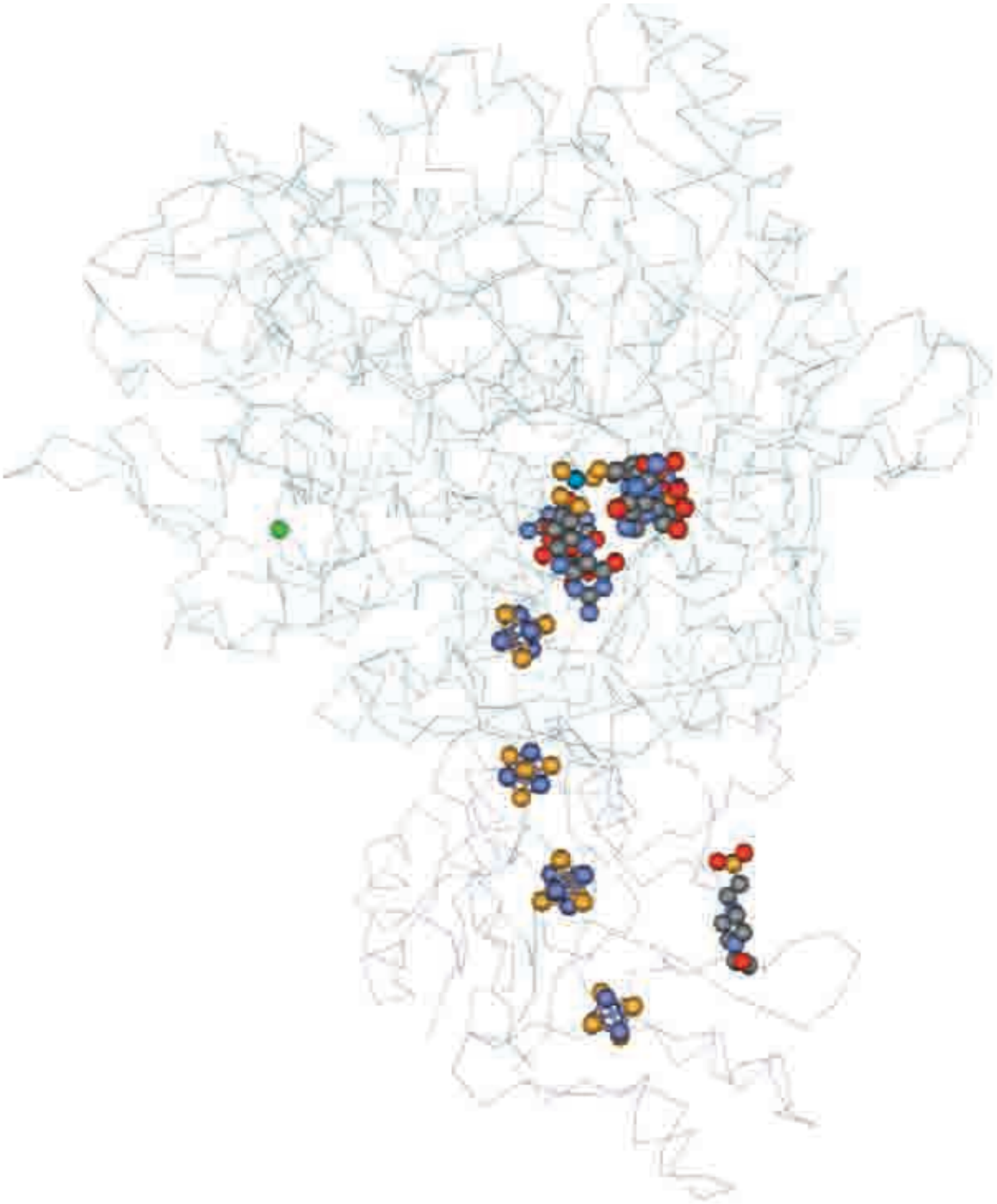
Desidrogenase do formato

Formate dehydrogenase

Tungsténio (W) é um metal biologicamente relevante. A estrutura da Formato desidrogenase (que cataliza a transformação de formato em CO_2) revelou pela primeira vez o ambiente químico deste tipo de centros catalíticos com muitas semelhanças com o grupo das Redutases de nitrato.

Tungsten-containing formate dehydrogenase from Desulfovibrio gigas: metal identification and preliminary structural data by multi-wavelength crystallography.
Raaijmakers H, Teixeira S, Dias JM, Almendra MJ, Brondino CD, Moura I, Moura JJG, Romao MJ.
J Biol Inorg Chem. 6, 398-404 (2001).

The tungsten-containing formate dehydrogenase (W-FDH) isolated from Desulfovibrio gigas has been crystallized in space group P2(1), with cell parameters a = 73.8 Å, b = 111.3 Å, c = 156.6 Å and beta = 93.7 degrees. These crystals diffract to beyond 2.0 Å on a synchrotron radiation source. W-FDH is a heterodimer (92 kDa and 29 kDa subunits) and two W-FDH molecules are present in the asymmetric unit. Although a molecular replacement solution was found using the periplasmic nitrate reductase as a search model, additional phasing information was needed. A multiple-wavelength anomalous dispersion (MAD) dataset was collected at the W- and Fe-edges, at four different wavelengths. Anomalous and dispersive difference data allowed us to unambiguously identify the metal atoms bound to W-FDH as one W atom with a Se-cysteine ligand as well as one [4Fe-4S] cluster in the 92 kDa subunit, and three additional [4Fe-4S] centers in the smaller 29 kDa subunit. The *D. gigas* W-FDH was previously characterized based on metal analysis and spectroscopic data. One W atom was predicted to be bound to two molybdopterin guanine dinucleotide (MGD) pterin cofactors and two [4Fe-4S] centers were proposed to be present. The crystallographic data now reported reveal a selenium atom (as a Se-cysteine) coordinating to the W site, as well as two extra [4Fe-4S] clusters not anticipated before. The EPR data were re-evaluated in the light of these new results.



Programas utilizados na execução das imagens

Chemera

http://www.cqfb.fct.unl.pt/bioin/chemera/

Weblab ViewerPro 3.7

Molecular Simulations Inc., http://www.accelrys.com/

POV-Ray for Windows 3.6

Persistence of Vision Team, http://www.povray.org/

Photoshop CS

Adobe, http://www.adobe.com/

Maqueta da exposição

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