Supporting Information

Small Molecule Migration in *Methanosarcina Acetivorans* Protoglobin: Effect of Ligand Binding and Quaternary Structure

Flavio Forti,[1] Leonardo Boechi,[2] Damian Bikiel,[2] Marcelo A. Martí,[2,3] Marco Nardini,[4] Martino Bolognesi,[4] Cristiano Viappiani,[5,6] Darío Estrin[2]* and F. Javier Luque[1]*

[1] Departament de Fisicoquímica and Institut de Biomedicina, Facultat de Farmàcia, Universitat de Barcelona, Av. Diagonal 643, E-08028, Barcelona, Spain

[2] Departamento de Química Inorgánica, Analítica, y Química Física, INQUIMAE-CONICET, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Buenos Aires, Argentina

[3] Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Buenos Aires, Argentina

[4] Dipartimento di Scienze Biomolecolari e Biotecnologie and CIMAINA, Università degli Studi di Milano, I-20131 Milano, Italy

[5] Dipartimento di Fisica, Università degli Studi di Parma, Parma, Italy

[6] NEST, Istituto Nanoscienze-CNR, Italy

* Send correspondence to fjluque@ub.edu or dario@qi.fcen.uba.ar

Table S1. Specific bond angle and torsional parameters (degrees) introduced in the heme unit in order to account for the structural distortion observed in the heme in *Ma*Pgb.

NP-FE-NP	172.0
NO-FE-NO	172.0
X-NO-FE-X	0.0
X-NP-FE-X	0.0
NP-FE-NO-CC	150.0
NO-FE-NP-CC	210.0
HD-CD-CC-NP	150.0
CC-CD-CC-NP	150.0
HD-CD-CC-NO	210.0
CC-CD-CC-NO	210.0

Figure S1. Time (ns) evolution of the rmsd (Å) of the backbone atoms for the core residues (23-189) in the (*left*) deoxygenated and (*right*) oxygenated forms of the protein determined relative to the X-ray crystallographic structures (PDB entries 2VEB and 2VEE). Monomer (MPgb) and dimer (DPgb) species are shown in black and grey, respectively.

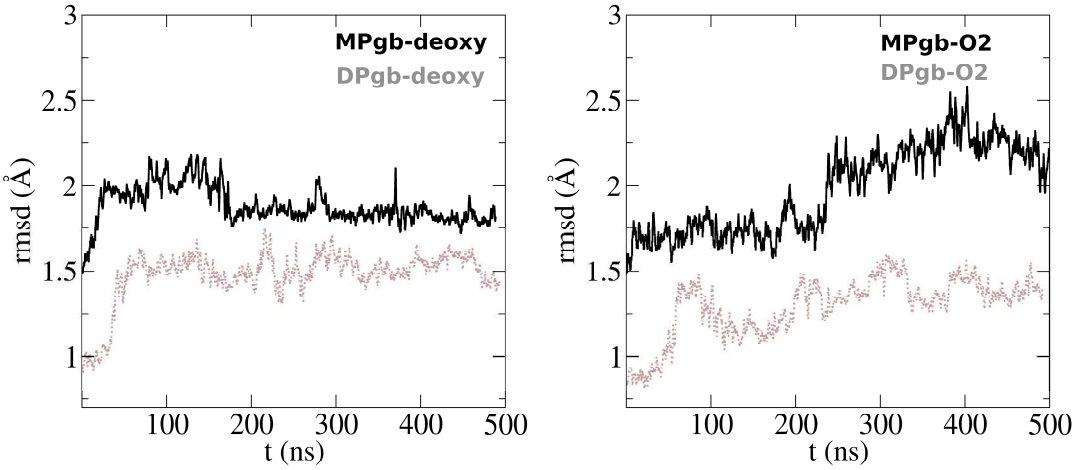


Figure S2. Representation of residues Phe(145)G8 (magenta) and Phe(93)E11 (cyan) in the heme cavity of the oxygenated *Ma*Pgb (PDB code 2VEB). Heme is represented using atom-coloured sticks. O_2 is shown in red spheres.

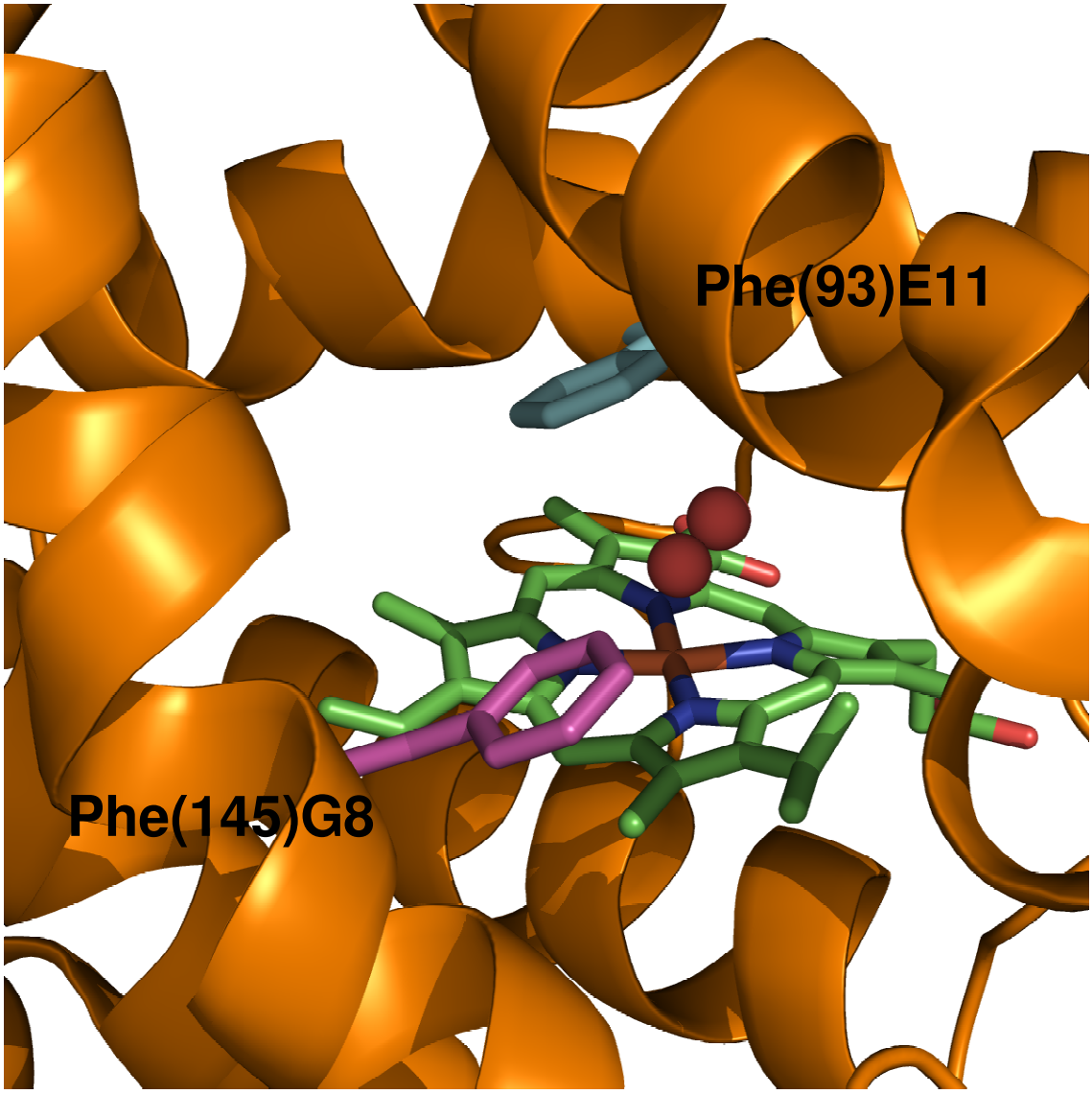


Figure S3. Free energy isosurfaces used to delineate tunnels 1 and 2 for (*top*) MPgb-deoxy and (*bottom*) DPgb-O_2. For MPgb-deoxy the closed conformation of Phe(145)G8, which is the main conformational state, reduces the accessibility through tunnel 1. In contrast, tunnel 1 (enclosed in solid line) is found in both open (left) and closed (right) states depending on the conformation of Phe(145)G8 (shown in yellow) in DPgb-O_2. Tunnel 2 (enclosed in dashed line) is open in the two cases. Heme is shown as atom-coloured sticks.

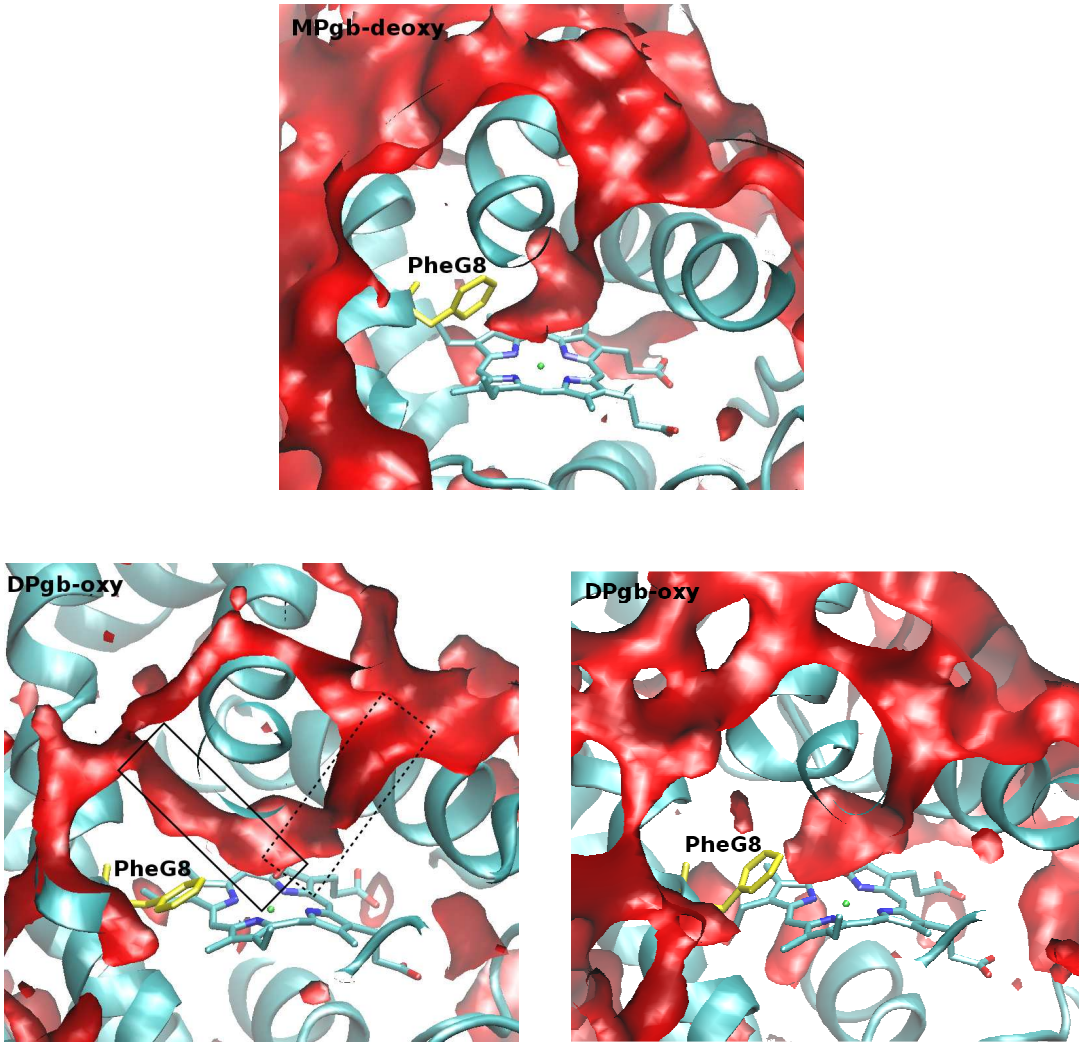


Figure S4. Normalized distribution of the dihedral angle (degrees) of Phe(145)G8 side chain in (*top*) the deoxygenated (left) and oxygenated (right) monomer, (*middle*) the two units of the deoxygenated dimer, and (*bottom*) the two units of the oxygenated dimer.

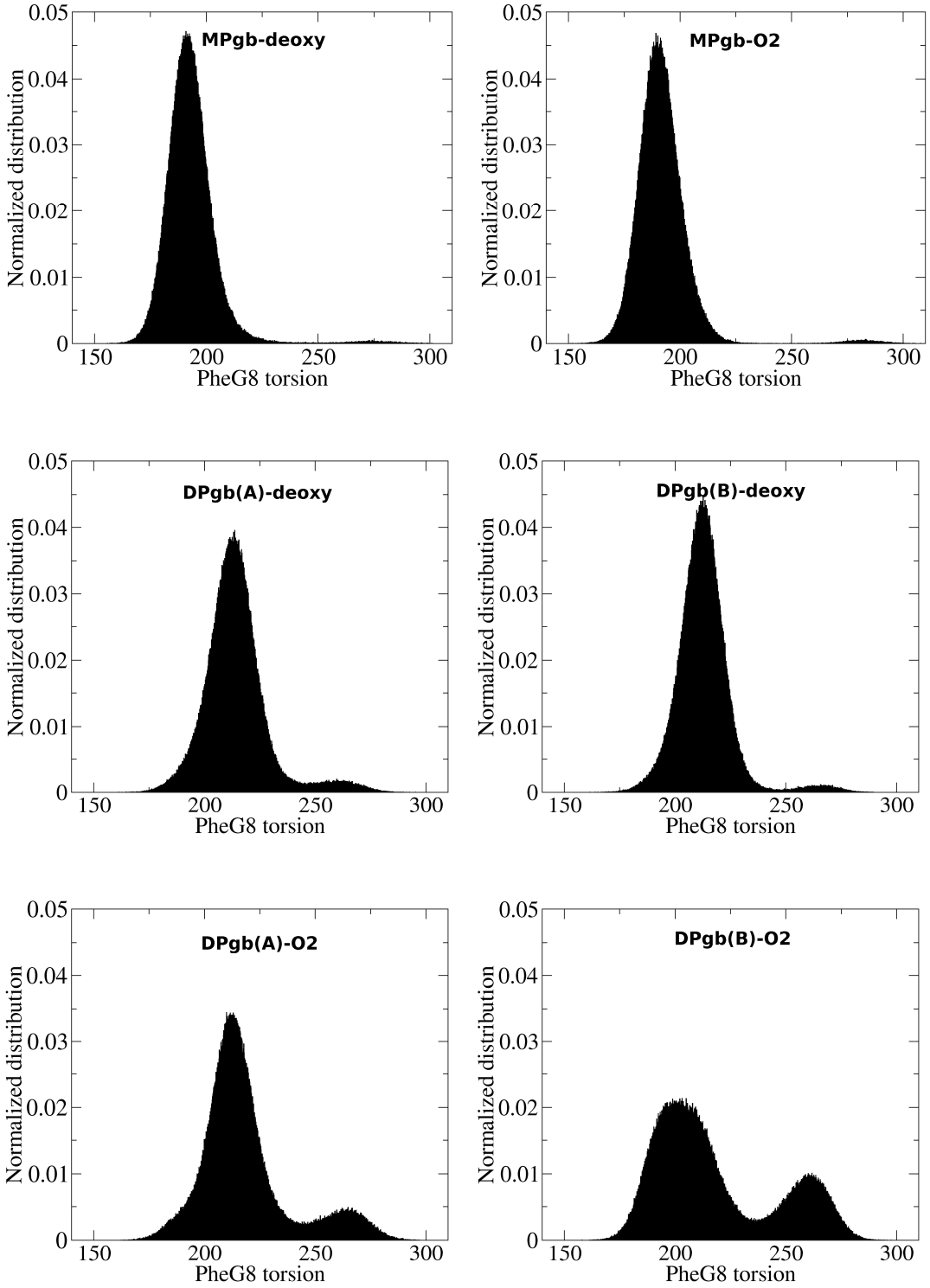
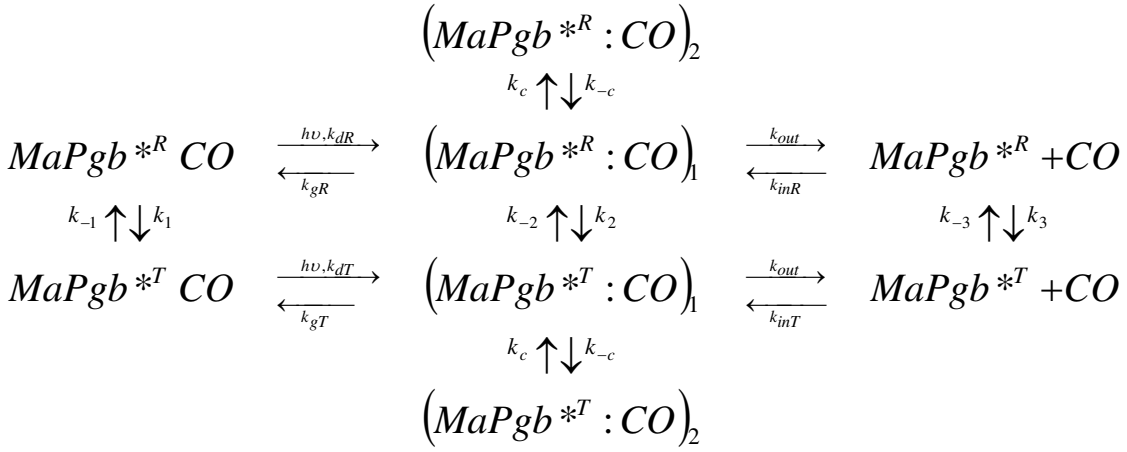


Figure S5. Reaction scheme proposed for the CO rebinding kinetics to *Ma*Pgb (taken from ref. 40), where two distinct conformational forms ($MaPgb^R$ and $MaPgb^T$) mediate the fast and slow rebinding kinetics. The bound state consists of a mixture of $MaPgb^RCO$ and $MaPgb^TCO$. After photodissociation, CO can migrate from the primary docking site $(MaPgb^R{:}CO)_1/(MaPgb^T{:}CO)_1$ to a secondary docking site $(MaPgb^R{:}CO)_2/(MaPgb^T{:}CO)_2$ or exit to the solvent $(MaPgb^R + CO)/(MaPgb^T + CO)$. Rebinding occurs through two distinct pathways involving either $MaPgb^T$ or $MaPgb^R$.



Ligation shifts the equilibrium towards a highly reactive species ($MaPgb^R$) with high binding kinetics. In the absence of ligand, the protein adopts preferentially a lower reactive conformation ($MaPgb^T$), with low binding kinetics. Thus, whereas the bound state consists of a mixture of $MaPgb^RCO$ (~77%) and $MaPgb^TCO$ (~23%), as derived from the kinetic constants k_1= 1.35 10^5 s^{-1} and k_{-1}= 4.55 10^5 s^{-1}, the reverse situation is found for the unbound state, where $MaPgb^R$ and $MaPgb^T$ populate around 25% and 75%, as derived from the kinetic constants k_3= 6.0 10^4 s^{-1} and k_{-3}= 2.0 10^4 s^{-1} (data taken from ref. 40).