Rotational disorder and sulfide binding in neuroglobin: a spectroscopic view

*Federico Sebastiani [a,b], Mirco Meglioli [c], Laura Cesati [a], Maurizio Becucci [a,b], Gianantonio Battistuzzi [c], Marco Borsari [c], Giulietta Smulevich [a]

[a] Department of Chemistry (DICUS), University of Florence

[b] The European Laboratory for Non-Linear Spectroscopy (LENS), Sesto Fiorentino

[c] Department of Chemical and Geological Science, University of Modena and Reggio Emilia

* federico.sebastiani@unifi.it

A remarkable feature of native neuroglobin (Ngb), a neuroprotective protein present in the nervous system of both vertebrates and invertebrates [1,2], is the presence of the heme rotational disorder. Unlike the majority of globins, in addition to the canonical heme *b* conformation, a second conformer, which differs in its orientation by a 180° rotation about the α,γ-meso axis of the porphyrin in the protein pocket, has been observed [3]. The reversed conformer has a 70% occupancy in both human and murine Ngbs as identified in crystals, by the X-ray diffraction, and in solution by spectroscopic techniques, such as circular dichroism, NMR, and more recently by resonance Raman spectroscopy [4, 5].

Here we present our results on Ngbs in single crystals and in solution, proving resonance Raman spectroscopy to be a diagnostic tool that could be extended to other proteins to detect heme rotational disorder, even in absence of structural data.

Interestingly, the heme rotational disorder has been proposed to affect not only the functional properties of the proteins, but also to be responsible for the effective mechanism of controlling ligand binding [6]. Ngbs, despite their bis-histidine coordination of the heme iron, bind a number of ligands with specific physiological functions [7]. In particular, this protein is reactive toward H_2S, an endogenously-synthesized signaling molecule which, being toxic at high concentration, is detoxified via different pathways [8]. H_2S binds not only to the sixth coordination position of the heme iron, but in presence of oxidants, it can react resulting in a covalent modification of one of the heme pyrrole rings, generating the so-called sulfheme derivatives [8]. The effect of sulfide binding in native and mutated Ngbs, including its effects on both the heme rotational disorder and the formation of adducts, will be discussed in relation to the protein function and its active site structure.

References

[1] Vallone, Nienhaus, Brunori, Nienhaus, *Protein Sci.* **2004**, *56, 85-92.*
[2] De Simone, Sbardella, Oddone, Pesce, Coletta, Ascenzi, *Cells* **2021**, *10, 3366.*
[3] Milazzo, Exertier, Becucci, Freda, Montemiglio, Savino, Vallone, Smulevich, *FEBS J.* **2020**, *287, 4082-4097.*
[4] Sebastiani, Milazzo, Exertier, Becucci, Smulevich, *J. Raman Spectrosc.* **2021**, *52, 1-14.*
[5] Meglioli, Sebastiani, Bellei, Di Rocco, Ranieri, Bortolotti, Sola, Borsari, Smulevich, Battistuzzi, *Inorg. Chem.* **2025**, *64*, 9066-9083.
[6] Exertier, Sebastiani, Gugole, Cerutti, Parisi, Montemiglio, Becucci, Viappiani, Bruno, Savino, Zamparelli, Anselmi, Abbruzzetti, Smulevich, Vallone, *ACS Chem. Biol.* **2022**, *17, 2099-2108.*
[7] Dewilde, Kiger, Burmester, Hankeln, Baudin-Creuza, Aerts, Marden, Caubergs, Moens, *J. Biol. Chem.* **2001**, *276, 38949-38955.*
[8] Rios-Gonzalez, Roman-Morales, Pietri, Lopez-Garriga, *J. Inorg. Biochem.*, **2014**, *133, 78-86.*