Study of Glycine/Cysteine-Rich proteins from Megavirinae Giant Viruses by in vitro chemical Fe-S reconstitution experiments

In 2003, mimivirus, initially mistaken for an intracellular parasitic bacterium, was identified as the first giant virus with a large particle size of around 700 nm-diameter and a complex DNA-genome (1.2Mb) of about 1000 proteins[1]. Mimivirus is the first representative and prototype member of the *Mimiviridae* family. In *Megavirinae*, a sub-family of *Mimiviridae* [2], we recently identified a new family of small proteins (about 60 amino-acids) with a high glycine, cysteine, and aromatic residues content, and endowed with very unusual Fe-S cluster binding properties[3]. This family was coined **GciS** for **G**lycine/**C**ysteine-rich **I**ron-**S**ulfur proteins[3]. This protein is found in all *Megavirinae* members. The GciS protein, of unknown function, is very abundant in the viral particles.

Combining biochemical, structural, and spectroscopic methods, we showed that GciS proteins possess the inherent ability to accommodate several types of Fe-S clusters, as a mixture of a $[2Fe\text{-}2S]^{2+}$ and a linear $[3Fe\text{-}4S]^{1+}$, the latter geometry being very uncommon in proteins,[3] and to perform Fe-S cluster conversions. *In vitro* chemical Fe-S reconstitutions performed on both recombinant as purified (partially metalated) and demetalated GciS proteins from all clades of *Megavirinae*, demonstrated that GciS protein is able to bind a $[4Fe\text{-}4S]^{2+}$ cluster in anaerobic conditions. Upon exposure to air, the $[4Fe\text{-}4S]^{2+}$ cluster was converted into a mixture of linear $[3Fe\text{-}4S]^{1+}$ and $[2Fe\text{-}2S]^{2+}$ clusters (Figure 1).

This unusual Fe-S binding behavior was shared between all clades, suggesting that it is an intrinsic property of the family and a valuable clue to identify the function of these Fe-S viral proteins.

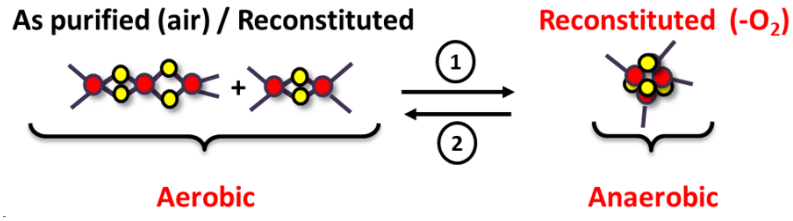

Figure 1: Fe-S clusters interconversion in GciS proteins

References

[1] Scola, B.; Audic S. *et al.* A Giant Virus in Amoebae. *Science*. 2003. 299, 2033–2033.

[2] Speciale, I.; Notaro, A. *et al.* The Astounding World of Glycans from Giant Viruses. *Chem. Rev.* 2022. 122, 15717–15766.

[3] Villalta, A.; Srour, B. *et al.* Evidence for [2Fe-2S]2+ and Linear [3Fe-4S]1+ Clusters in a Unique Family of Glycine/Cysteine-Rich Fe-S Proteins from Megavirinae Giant Viruses. *J. Am. Chem. Soc.* 2023. 145, 2733–2738.