# Structural and Functional studies on Nitric Oxide Synthase complexed with $Re(CO)_3$-compounds

Leisico F[1], Correia MAS[1], Morais M[2], Oliveira BL[2], Macedo AL[1], Romão MJ[1], Santos-Silva T[1], Correia JDG[2]

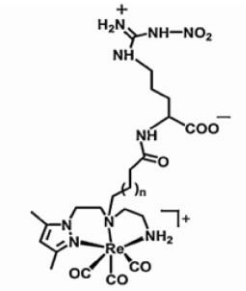
[1]UCIBIO@REQUIMTE, Dep. de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, FCT-UNL, 2829-516 Caparica, Portugal; [2]Unidade de Ciências Químicas e Radiofarmacêuticas, IST/ITN, Instituto Superior Técnico, Universidade Técnica de Lisboa, Estrada Nacional 10, 2686-953 Sacavém, Portugal

E-mail: franciscoleisico@fct.unl.pt

Nitric oxide synthase (NOS) catalyzes the $O_2$-dependent conversion of L-arginine to L-citruline and nitric oxide (NO). The enzyme presents three isoforms, namely neuronal (nNOS), endothelial (eNOS) and inducible (iNOS), in which nNOS and eNOS are constitutively expressed and $Ca^{2+}$-dependent while iNOS is inducible expressed and $Ca^{2+}$-independent. NO is a key physiological modulator in mammals which, among other functions, stimulates vasodilatation in the cardiovascular system and serves as a neurotransmitter in the central nervous system. Besides mediating several physiological functions, NO overproduction by nNOS and iNOS has been associated to several clinical disorders including stroke, Alzheimer's and Parkinson's disease and cancer [1].

Monitoring NOS expression *in vivo* represents a promising approach to early detection of diverse pathologies which represents a potential advantage for the treatment of such disorders. Aiming the design of innovative radioactive probes for *in vivo* targeting of NOS, a set of $^{99m}Tc(CO)_3$-complexes containing NOS-recognizing units (e.g. L-Arginine derivatives, guanidine or S-methylisothiourea moieties) were established and $Re(CO)_3$-complex analogs were studied as "non-radioactive" surrogates [2]. Interestingly, previous results have showed that some of the rhenium complexes have high inhibitory activity towards iNOS, namely Re1 and Re2 (Figure 1) which were characterized by KI's of 84 μM and 6 μM, respectively. Additionally, both Re complexes permeate through macrophage cell membranes and interact with the cytosolic target enzyme, inhibiting NO biosynthesis in LPS-induced macrophages [3].

Aiming to structurally characterize the interaction of the synthesized metal compounds with the proteins of interest, the oxygenase domain of human NOS of each isoform was produced. The expression system of iNOS has been optimized and pure active enzyme has been obtained. More than a thousand crystallization conditions have already been tested to try to crystallize the protein-metal complexes and some positive hits were obtained and multiple crystals are under optimization using soaking and co-crystallization approaches. Several crystallization trials involving eNOS and nNOS are being performed in order to find suitable conditions to crystallize all isoforms of NOS and to carry out structural studies regarding the complexes of NOS proteins and the organometallic compounds.



Re1 (n=1) and Re2 (n=4)

Figure 1- Organometallic $Re(CO)_3$ complexes which showed higest inibitory action towards NOS isoforms [2].

Complementary studies have also been conducted. Isothermal Titration Calorimetry (ITC) was used to test the activity of the produced isoforms showing that iNOS and eNOS are active in the presence of tetrahydrobiopterin (BH4) and L-arginine. The interaction of the protein with the two Re compounds is going to be characterized by this technique in the near future. Furthermore, interaction of iNOS and Re1 and Re2 compounds was studied through NMR saturation-transfer difference (STD). We identified the nearest interacting protons in binding moieties for each compound towards to the protein, confirming previous molecular docking studies [4]. The results will allow to shed light on selectivity and affinity properties of these compounds toward NOS, which constitute important interaction evidences for the design of new specific compounds to target NOS proteins. Further crystallography and ITC analysis will complement the all spectra of structural and functional results regarding the three isoforms of NOS and the most interesting organometallic complexes.

*References*

1. Alderton WK & Knowles, *Biochemistry Journal* **357**, 593-615 (2001);
2. Oliveira BL, et al., *Journal of Organometallic Chemistry* **696**, 1057-1065 (2011);
3. Oliveira BL, et al., *Bioconjugate Chemistry* **21**, 2168-2172 (2010);
4. Oliveira BL, et al., *Journal of Molecular Graphics and Modelling* **45**, 13-25 (2013).