A nitric oxide synthase-like protein from *Synechococcus* produces NO/NO_3^- from L-arginine and NAPDH in a tetrahydrobiopterin- and Ca^{2+}-dependent manner

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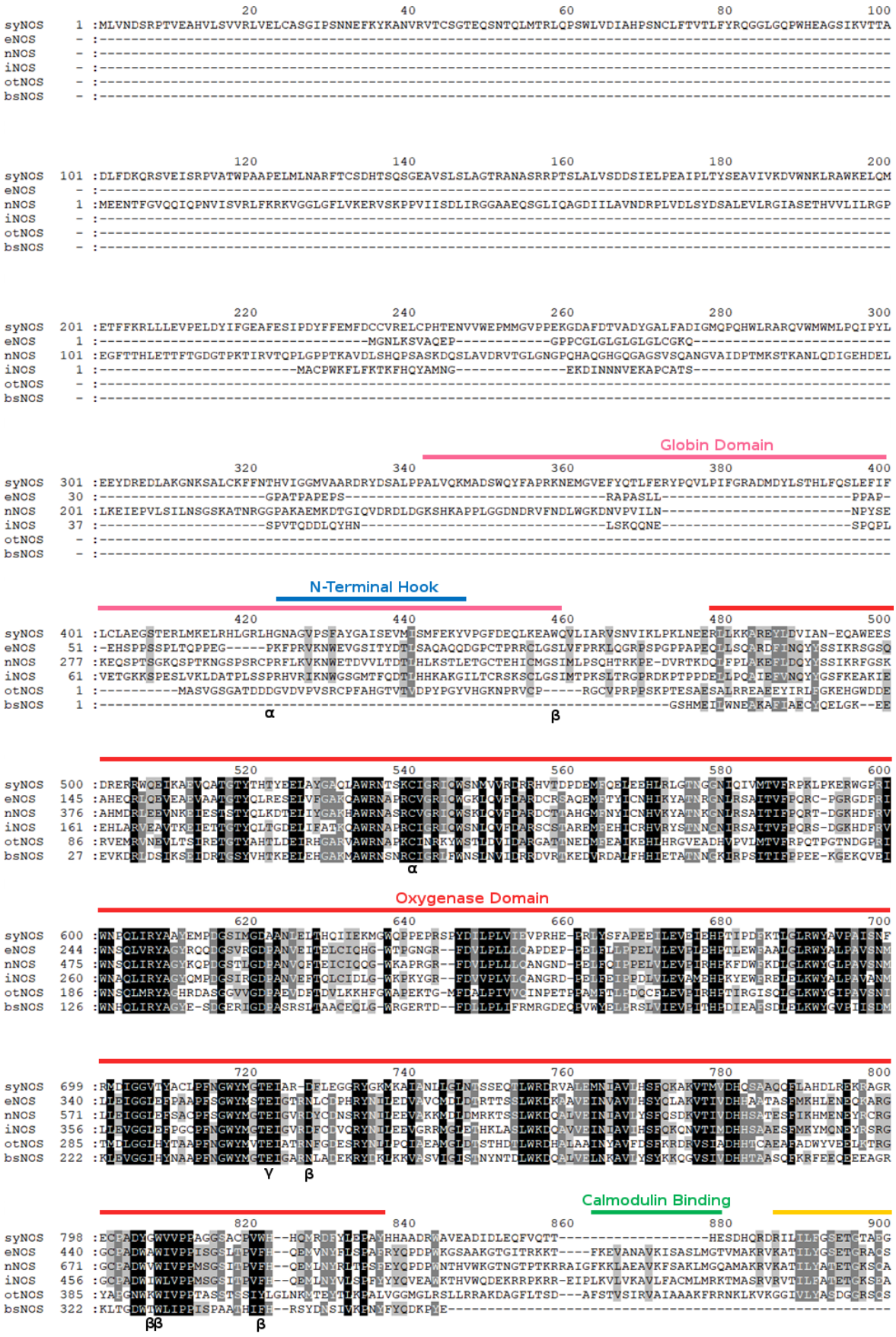
	nmol NO_2^- + NO_3^-
syNOS	
+ Fe-MGD	1.0 ± 1.0
- Fe-MGD	35 ± 4
NOC – 7	78 ± 2

Table S1: NO_2^- + NO_3^- production from syNOS measured by the Griess assay, in the presence or absence of the spin-trap Fe-MGD.

	Hemin & δ-ala	δ-ala
syNOS	1.00 ± 0.10	0.77 ± 0.10
C539A	0.72 ± 0.07	0.42 ± 0.02
H422A	0.39 ± 0.08	0.20 ± 0.03
H422A/C539A	0.10 ± 0.02	0.036 ± 0.009

Table S2: Heme incorporation (μM heme per μM protein) of syNOS constructs expressed in the presence of excess hemin and δ-ala, or just δ-ala.


Globin Domain
N-Terminal Hook
α
β
Oxygenase Domain
α
γ
β
Calmodulin Binding
ββ
β

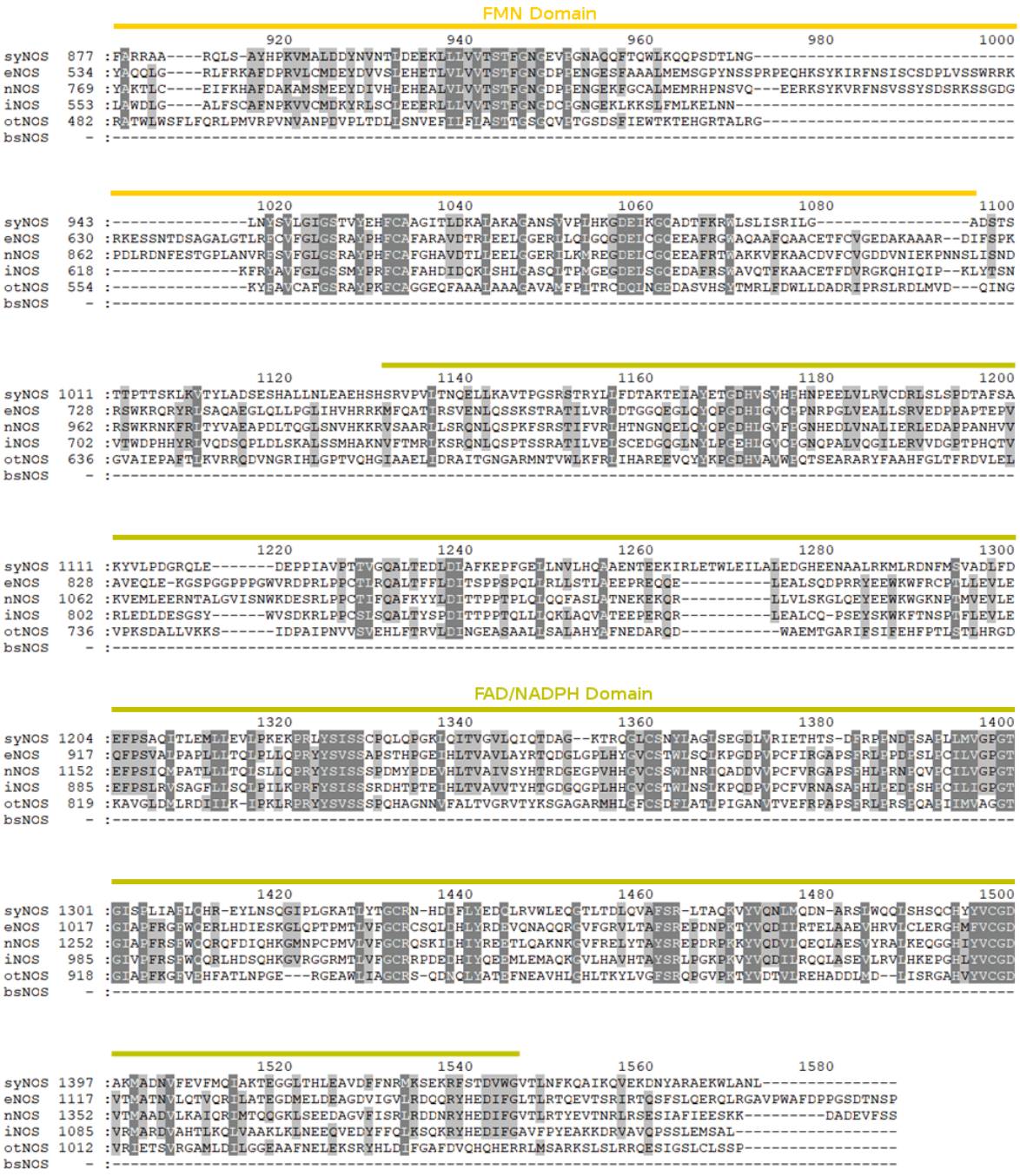


Figure S1: Protein sequence alignment of syNOS to other NOS proteins. The alignment was performed using ClustalX2 and visualized with GeneDoc; proximal heme binding residues (α), pterin binding residues (β), arginine coordinating glutamate (γ), *Homo sapien* endothelial NOS (eNOS), *Rattus norvegicus* neuronal NOS (nNOS), *Homo sapien* inducible NOS (iNOS), *Ostreococcus tauri* NOS (otNOS), *Bacillus subtilis* (bsNOS).

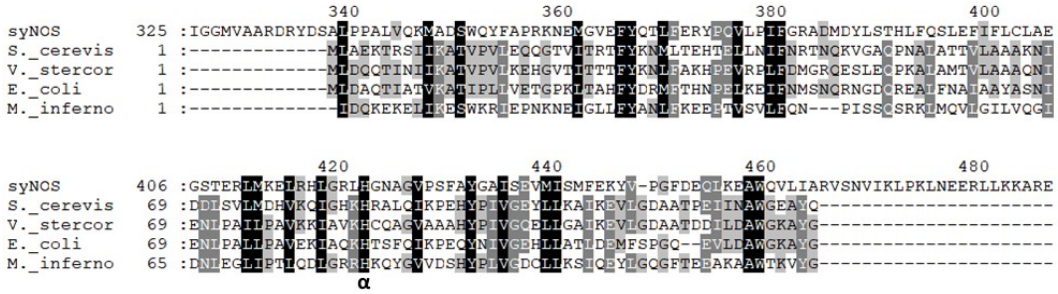


Figure S2: Protein sequence alignment of $syNOS_g$ to other globins and flavohemoglobins of known structures (*Saccharomyces cerevisiae* 4G1V, *Vitreoscilla stercoraria* 1VHB, *Escherichia coli* 1GVH, *Methylokorus infernorum* 3UBC). The alignment was performed using ClustalX2 and visualized with GeneDoc; proximal heme binding residue (α).

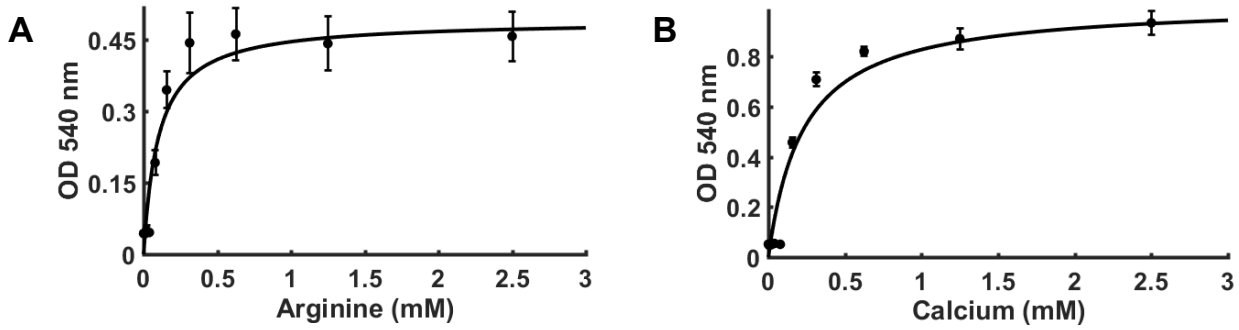


Figure S3: Michaelis-Menten plots for syNOS activity as a function of L-arg concentration (A) and calcium concentration (B). K_M values for arginine and calcium were calculated to be 101 ± 12 μM and 228 ± 9 μM, respectively.

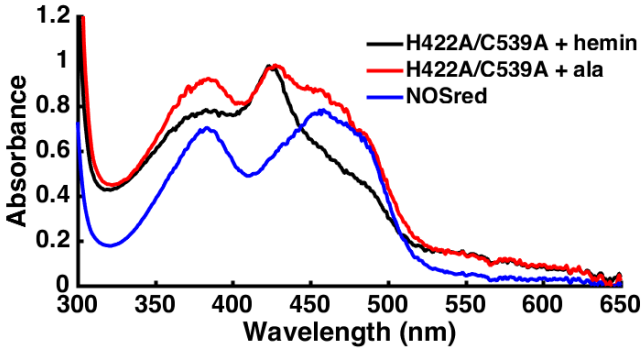


Figure S4: UV-vis spectra of the heme double mutant H422A/C539A compared to the syNOS reductase domain reveals minimal heme is bound when the proximal heme ligands are mutated.