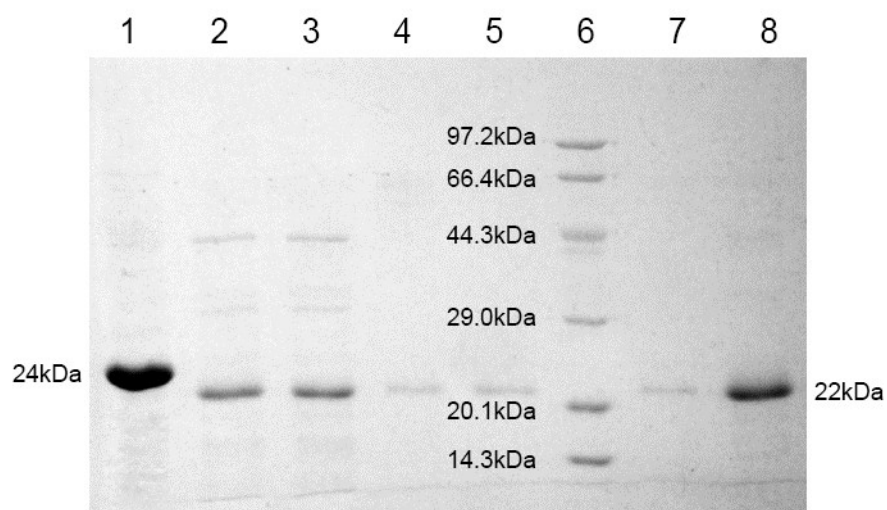
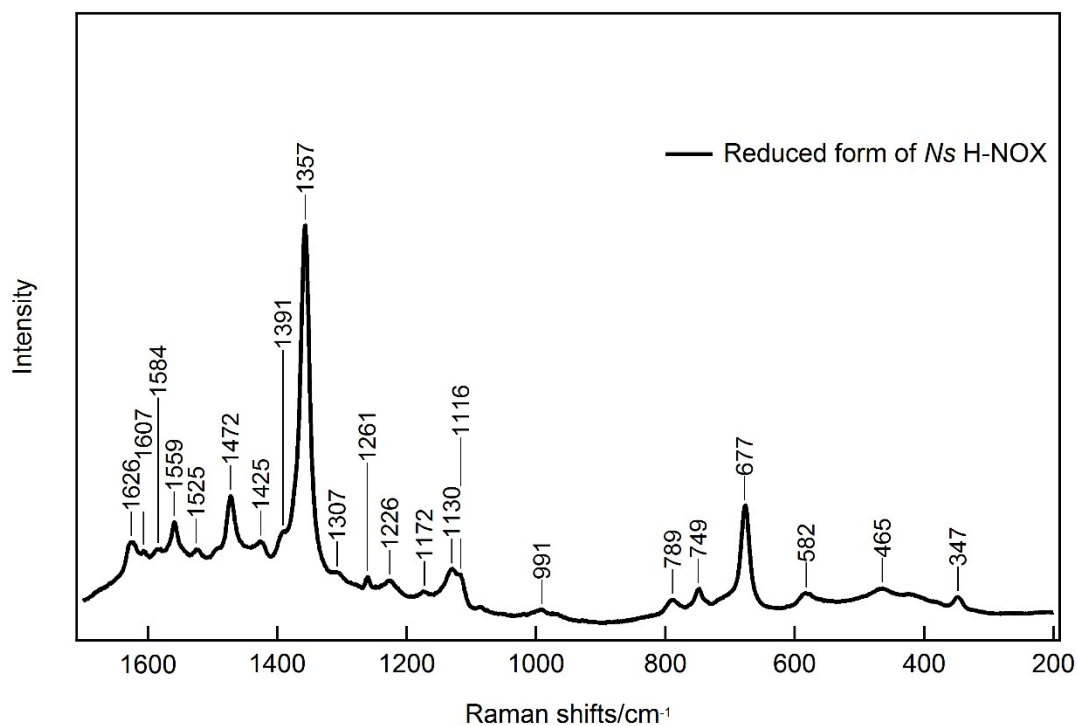


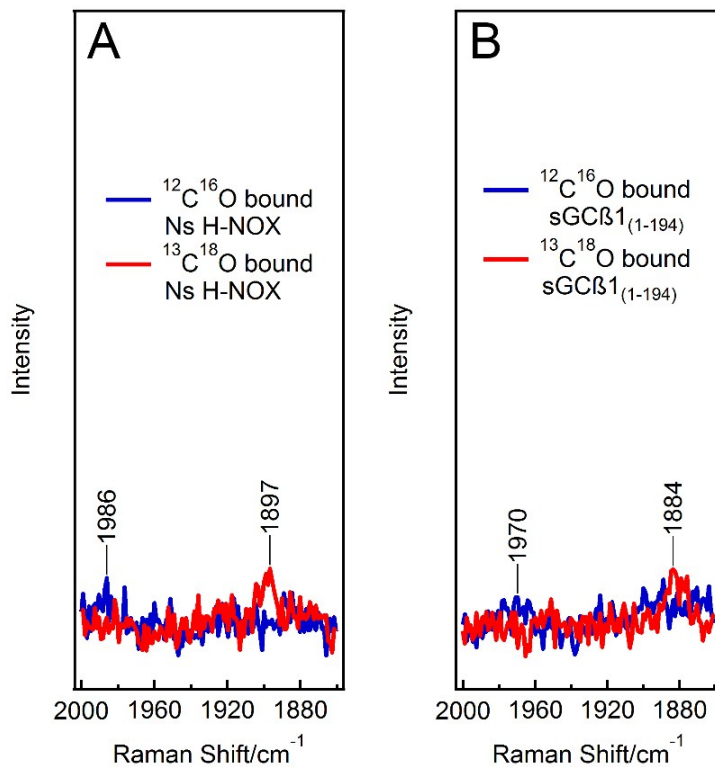
## Electronic Supplementary Information (ESI)



**Fig. S1** The 15% SDS-PAGE of sGC  $\beta 1_{(1-194)}$  (Lane 1), Marker (Lane 6) and *Ns* H-NOX (Lane 8)

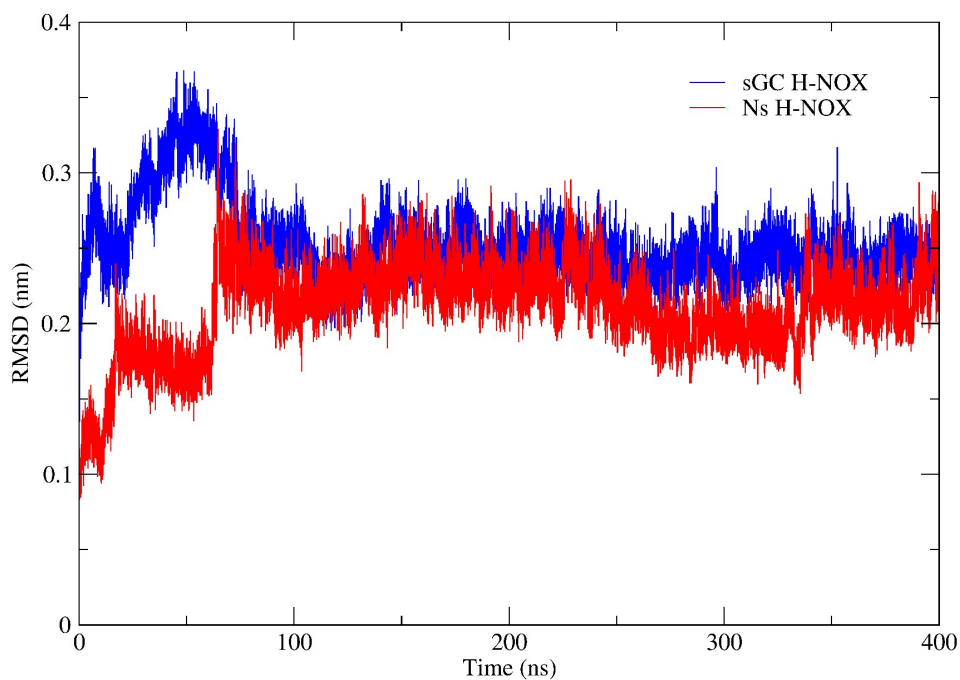


**Fig. S2** Resonance Raman spectrum of the reduced form of *Ns* H-NOX using an excitation wavelength of 413.1 nm.

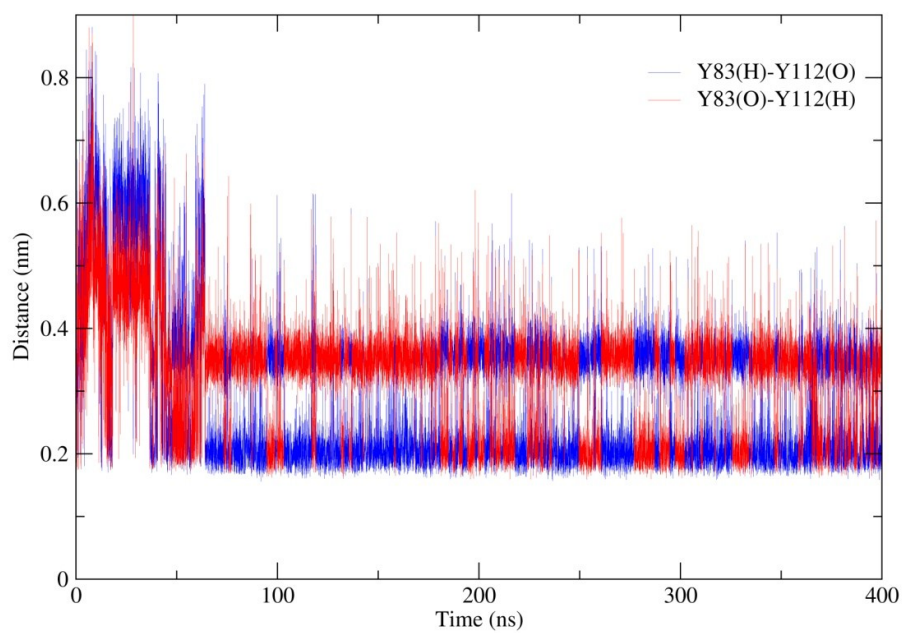


**Fig. S3** High-frequency Raman spectra of *Ns* H-NOX, with <sup>12</sup>C<sup>16</sup>O complex colored blue and <sup>13</sup>C<sup>18</sup>O complex colored red. (A) *Ns* H-NOX; (B) sGC β1<sub>(1-194)</sub>.

#### Backbone RMSD



**Fig. S4** Backbone root-mean-square deviation (RMSD) of the two H-NOX proteins during MD simulations (blue for sGC H-NOX; red for *Ns* H-NOX).



**Fig. S5** Hydrogen bond formed between Y112 and Y83 in sGC H-NOX, which leads to the bending of  $\alpha$ F that subsequently affects the heme position.

**Table S1** Comparison of the UV-visual spectra of the *Ns* H-NOX and sGC  $\beta 1$  (1-194) proteins before and after the Raman measurement.

nm	<i>Ns</i> H-NOX		sGC $\beta 1$ (1-194)	
	before	after	before	after
Soret	423	423	422	422
$\beta$	538	538	540	540
$\alpha$	566	566	570	570

**Table S2.** Selected DFT-optimized parameters of (ImH)FeP(CO) in the absence or presence of water.

	Absence of water	Presence of water
$R_{\text{H bonding}} (\text{\AA})$		2.237
$R_{\text{C=O}} (\text{\AA})$	1.151	1.154
$R_{\text{Fe-CO}} (\text{\AA})$	1.797	1.784
$R_{\text{Fe-N}} (\text{\AA})$	2.079	2.078

### Sequence alignment file used for homology modelling

>P1;2o09

structureX:2o09\_A: 1 :A:+184 :A::-1.00:-1.00

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 TYTSEEGYGELLASAGDSLPEFMENLDNLHARVGLSFPQLRPPAFECQHTSS-KSMELHYQSTRCGLAPMVLGLL  
 HGLGKRFQTKVEVTQTAFRETGEDHDIFSIKYEhb\*

>P1;rat\_sgc

sequence:rat\_sgc: : : : 0.00: 0.00

MYGFVNHALELLVIRNYGPEVWEDIKKEAQLDEEGQFLVRIIYDDSKTYDLVAAASKVLNLNAGEILQMFQKMF  
 VFCQESGYDTILRVLGSNREFLHNLDALHDHLATIYPGMRAPFRCTDAEKGKGLILHYYSEREGLDIVIGII  
 KTVAAQQIHGTEIDMKVIQRSEECDHTQFLIEEhb\*