

Selective X-ray induced NO-photodissociation in hemoglobin crystals: evidence from a Raman assisted-crystallographic study

SUPPLEMENTARY MATERIAL

Table S1. Data collection and refinement statistics of fully and partially nitrosylated HbTb.

Values in parentheses refer to the last resolution shell.

	NO-HbTb	NO_x1-HbTb	NO_x2-HbTb	NO_x3-HbTb
Data Collection statistics				
Space group	P2 ₁	P2 ₁	P2 ₁	P2 ₁
a (Å)	62.00	61.64	61.65	62.71
b (Å)	94.67	93.58	93.59	93.74
c (Å)	61.96	61.59	61.59	61.61
α (°)	90.00	90.00	90.00	90.00
β (°)	89.16	89.59	89.62	89.21
γ (°)	90.00	90.00	90.00	90.00
Resolution limits (Å)	50.0-2.30 (2.38-2.30)	50.0-2.15 (2.27-2.15)	50.0-2.17 (2.29-2.17)	50.0-2.24 (2.36-2.24)
No. of observations	104005	167905	164535	151865
No. of unique reflections	26655	37616	36709	33402
Mean I/σ(I)	15.6 (9.5)	19.6 (9.8)	19.6 (9.8)	19.5 (8.7)
Completeness (%)	83.6 (56.1)	98.9 (92.7)	99.2 (94.4)	99.1 (94.1)
Average multiplicity	3.9 (2.8)	4.5 (4.2)	4.5 (4.2)	4.5 (4.5)
Rmerge (%)	0.073 (0.113)	0.054 (0.110)	0.053 (0.120)	0.049 (0.136)
Refinement statistics				
Resolution limits	50.0-2.50	50.0-2.15	50.0-2.17	50.0-2.24
Number of reflections in working set	21249	35739	34878	31765
Number of reflections in test set	1095	1880	1836	1672
Rfactor/Rfree Fo > 4σ(Fo)	0.180/0.247	0.196/0.244	0.194/0.242	0.187/0.229
Rfactor/Rfree All data	0.181/0.249	0.199/0.249	0.198/0.247	0.193/0.237
No. of protein atoms	4488	4488	4488	4488
No. of heme atoms	172	172	172	172
No. of NO atoms	8	8	8	8
Geometry (%)				
Most favoured	99.2	99.0	99.0	98.0
Generously allowed	0.8	0.6	0.6	1.6
Disallowed	0	0.4	0.4	0.4

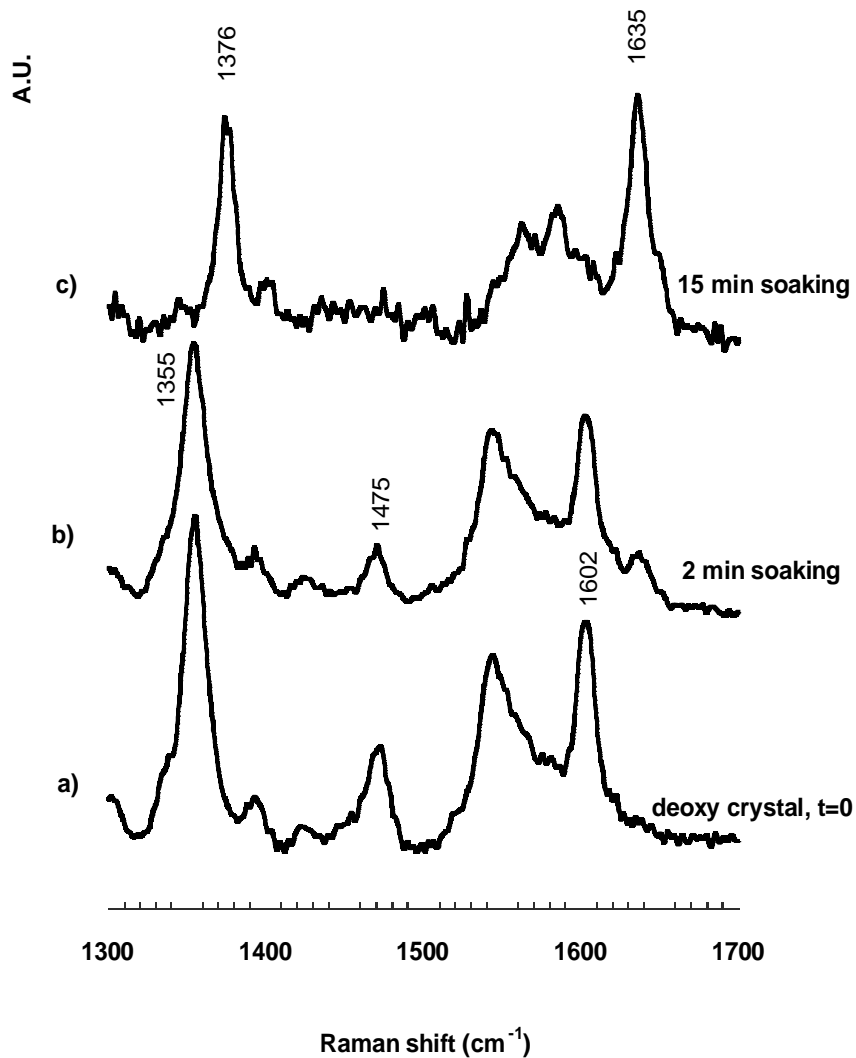


Figure S1. Resonance Raman spectra at several steps of NO soaking into HbTb deoxygenated crystals (a): after 2 minutes (b) and 15 minutes (c) from the NO in situ generation (see Methods). Excitation line is 514 nm (2mW at the sample), exposure time 300 s. Spectral resolution 4 cm^{-1} . In order to reduce the risk of local heating (absent in the experiments at 100 K performed at SLS), and so to reduce oxidative denitrosylation, the acquisition time for nitrosyl HbTb (after 15 minutes soaking) in house was kept as short as possible, causing a lower signal to noise ratio.