Legends of supplemental data

Figure S1

Low-frequency region of the resonance Raman spectra of the $Fe^{III}NO$ complexes of H_4B-saturated bsNOS in the presence of NOHA and citrulline. The spectra were obtained with NOHA and H_4B with $^{14}N^{16}O$ (a) and $^{15}N^{16}O$ (b). The $^{14}N^{16}O$ minus $^{15}N^{16}O$ difference spectrum (a minus b) is shown in e. The spectra of the citrulline- and H_4B-saturated bsNOS were obtained with $^{14}N^{16}O$ (c) and $^{15}N^{16}O$ (d). The $^{14}N^{16}O$ minus $^{15}N^{16}O$ difference spectrum (c minus d) is shown in f.

Figure S2

Determination of the affinity of the ferric form of saNOS for L-arginine. Ferric saNOS (0.5 μM) was titrated with 0.6 μM to 300 μM L-arginine. The fraction of L-arginine-bound protein was calculated from the variations of the absorbance at 421 nm and 380 nm. The solid line represents the fit of the data to equation 1. K_d was found to be 4.2 ± 0.2 μM. The inset shows the titration over the complete set of L-arginine concentrations.

Figure S3

Kinetics of NO recombination to saNOS determined by flash photolysis. The rates of NO association to saNOS (solid squares) and to saNOS saturated with L-arginine (solid circles) are plotted as a function of the NO concentration. The solid lines represent the linear fit to these data. The goodness of the fits is shown by an r^2 of 0.998 for the saNOS data and 0.995 for the saNOS/Arg data. From these fits, the calculated k_{on} for NO were 17 and 2 $\mu M^{-1}s^{-1}$ for saNOS and saNOS/Arg, respectively. The calculated k_{off} were 1266 s^{-1} and 34 s^{-1} for saNOS and saNOS/Arg, respectively.

Figure S4

High-frequency region of the resonance Raman spectra of $Fe^{II}NO$ complexes of bsNOS. The L-arginine sample (a) and the substrate-free and pterin-free sample (b) are shown. The inset shows the optical absorption spectra of the L-arginine sample (dashed line) and the substrate-free and pterin-free sample (solid line).

Figure S1

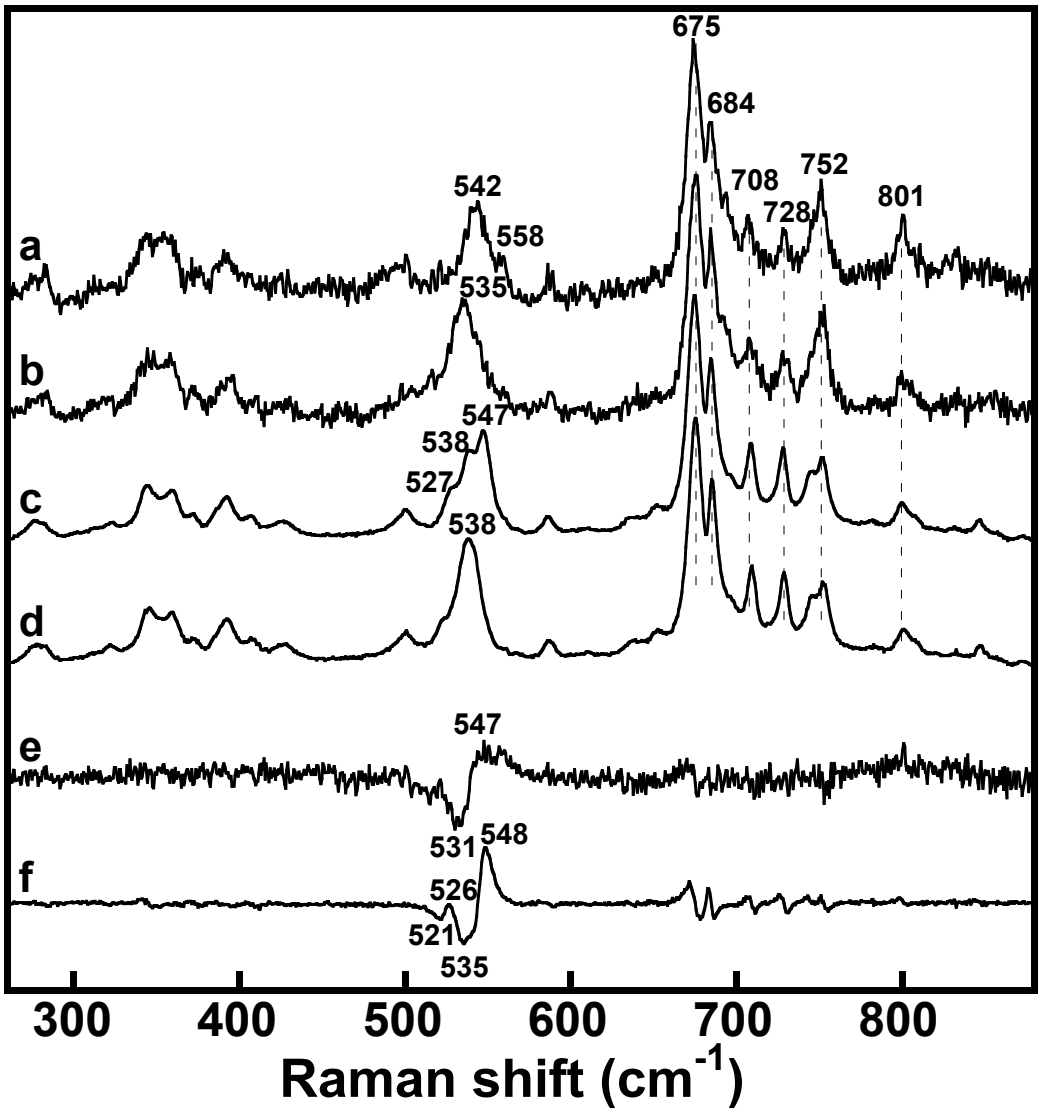

675
684
752
708
728
801
542
558
535
a
b
547
538
527
c
538
d
547
e
531
548
f
526
521
535
300
400
500
600
700
800
Raman shift (cm-1)

Figure S2

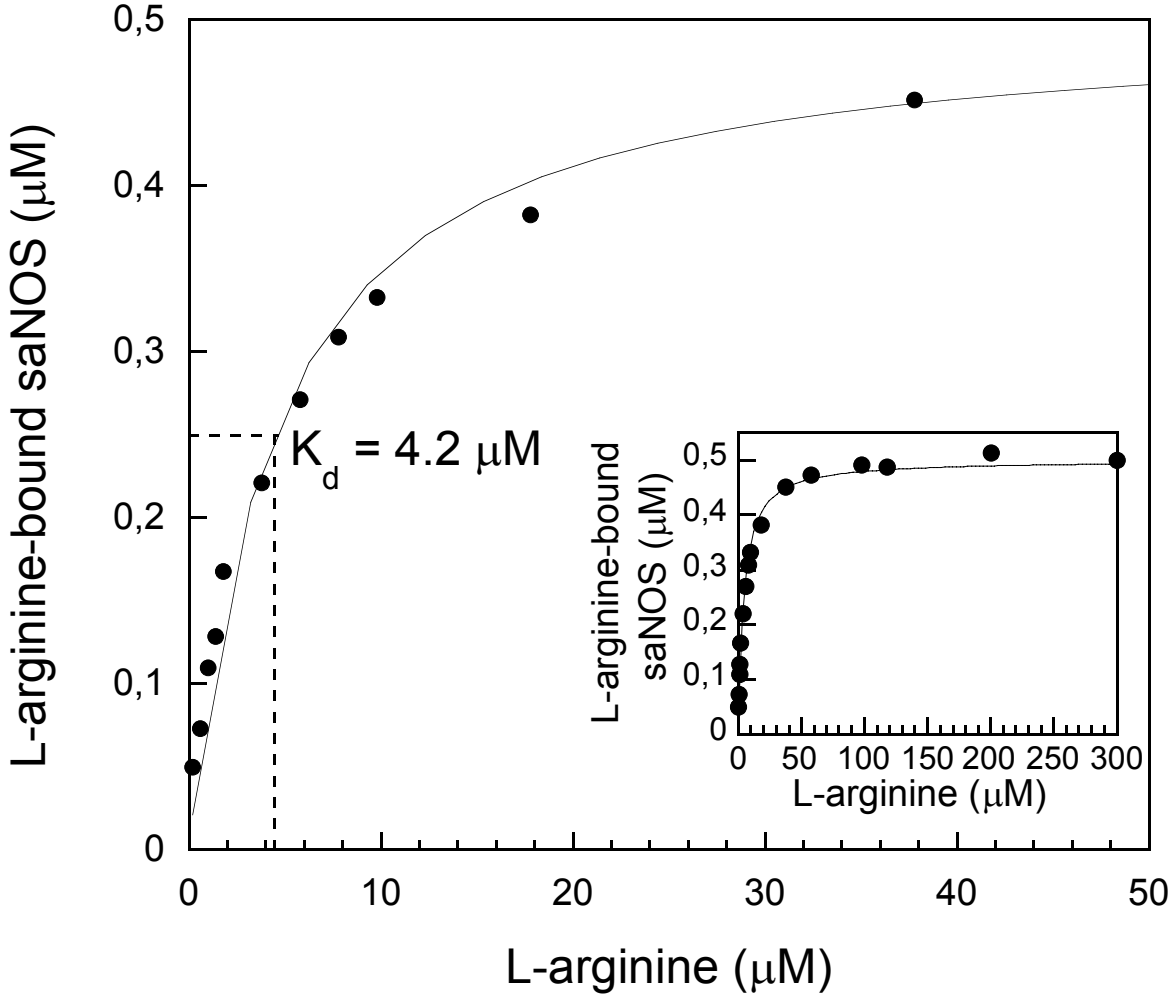

0,5
0,4
0,3
0,2
0,1
0
L-arginine-bound saNOS (μM)
K_d = 4.2 μM
0
10
20
30
40
50
L-arginine (μM)
L-arginine-bound saNOS (μM)
0,5
0,4
0,3
0,2
0,1
0
0
50
100
150
200
250
300
L-arginine (μM)

Figure S3

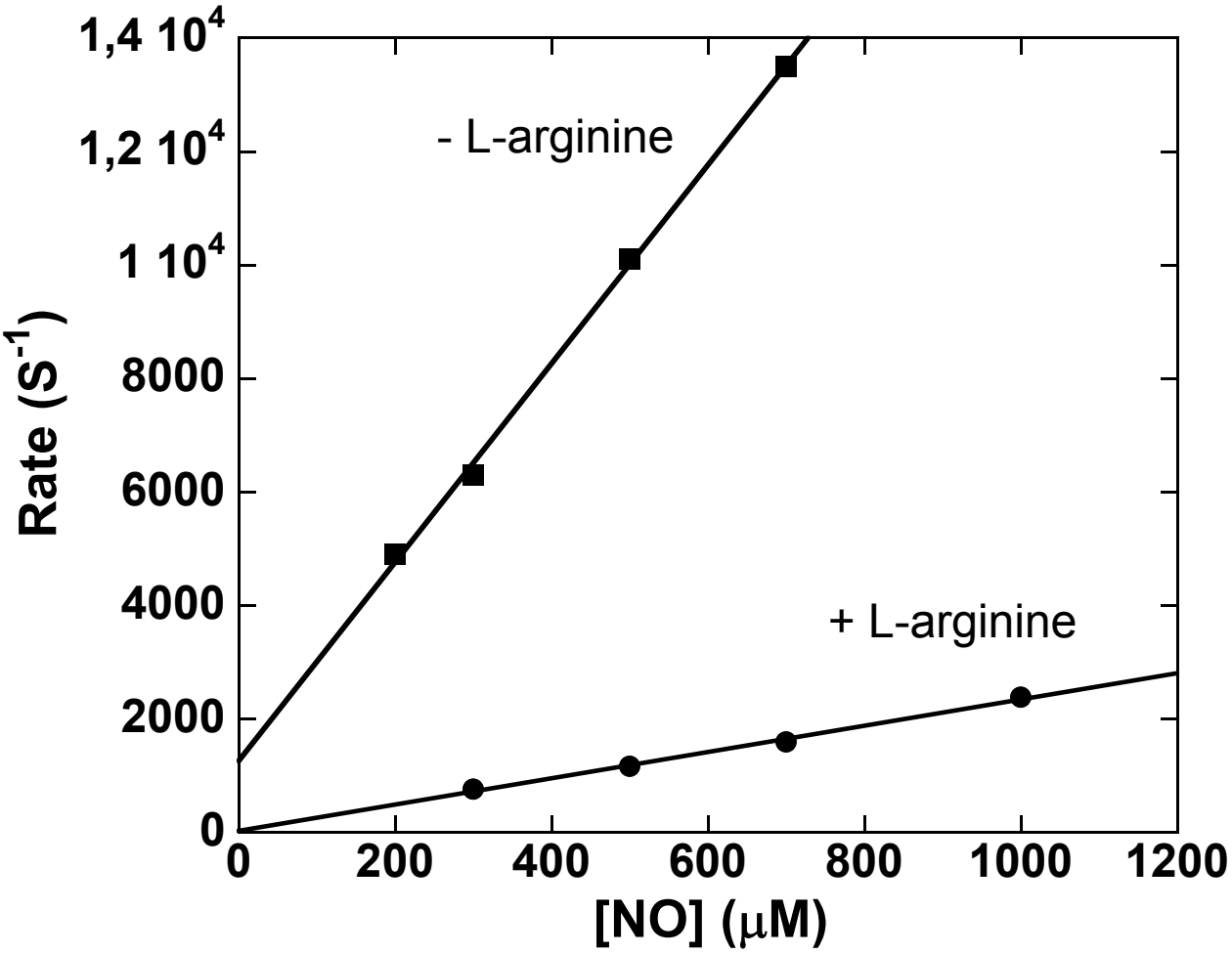
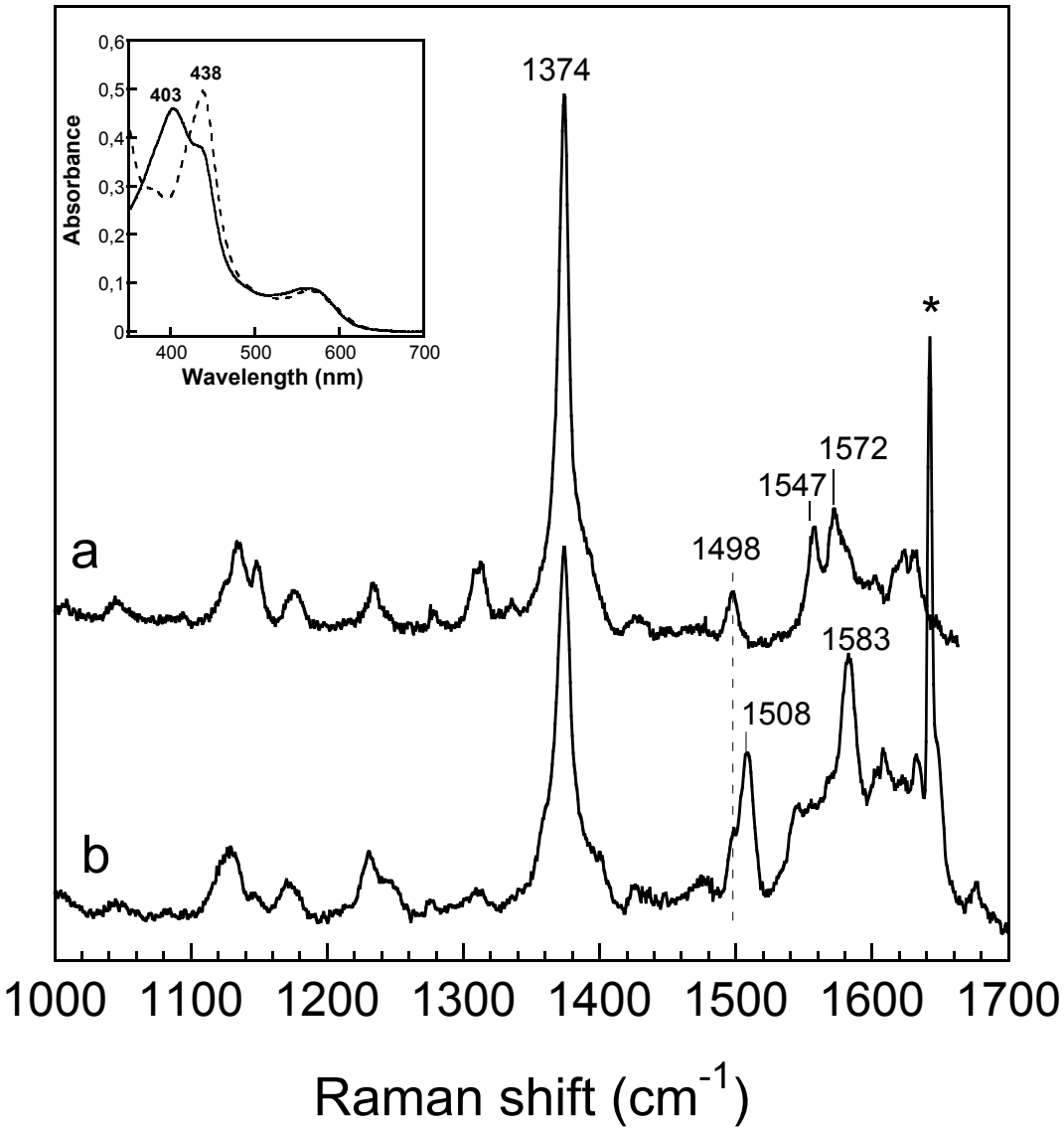

1,4 10^4
1,2 10^4
1 10^4
8000
6000
4000
2000
0
Rate (S^-1)
- L-arginine
+ L-arginine
0
200
400
600
800
1000
1200
[NO] (μM)

Figure S4


Absorbance
Wavelength (nm)
403
438
1374
1572
1547
1498
1583
1508
a
b
*
1000 1100 1200 1300 1400 1500 1600 1700
Raman shift (cm-1)