Supplementary Data for

THE HEME BINDING PROPERTIES OF GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE

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Fig. S1. Gel filtration of the rGAPDH-heme complex. Panel A: Chromatogram of the GAPDH-heme complex. Fractions were collected between 8 and 65 min and analyzed by UV-vis spectroscopy. Panel B: UV-visible analysis of the fractions. The ratio A_{280}/A_{415} of the complex prior to injection was 3.5. The ratio A_{280}/A_{415} of the eluted fractions containing the highest concentration of heme ranged from 5 to 8.

Fig. S2. In-gel heme staining of apo-and heme-bound GAPDH using the o-dianisidine/H_2O_2 peroxidase assay. Panel A shows the heme-staining bands corresponding to rGAPDH-heme (5:1) and apo-GAPDH (negative control). Panel B shows different doses of GAPDH-heme, and iNOSoxy as a positive control for specific heme staining.

Fig. S3. Far-UV CD spectra of apo- and heme-bound GAPDH. Addition of heme to apo-GAPDH produced mild changes in the far-UV CD spectrum, suggesting that heme binding is accompanied by modest conformational changes.

Fig. S4. Spectrophotometric titrations of rabbit GAPDH with increasing doses of hemin. Panel A. Titration of 10 μM GAPDH with hemin (0-10 μM). Panel B: UV-visible spectra of free hemin in buffer.

Fig. S5. Transfer of ferrous heme from GAPDH to apomyoglobin at 25 ^{o}C. Heme transfer to apomyoglobin is accompanied by a decrease in absorption at 425 nm (GAPDH-heme) and a concomitant increase at 434 nm (reduced myoglobin).The inset shows the initial and final spectra.

Fig. S6. Reaction of native, anaerobic GAPDH with ferrous heme (Panels A and B), and reaction of GAPDH pre-treated with dithionite and ferrous heme (Panel C). The reaction of GAPDH with ferrous heme (5:1) was slow (k_{obs} = 0.0091 s^{-1} at 10 ^{o}C) and incomplete; only 19% of the total available ferrous heme formed a complex with GAPDH after 10 min. No measurable spectral changes were detected for the reaction between dithionite-treated GAPDH and ferrous heme.

Fig. S7. Kinetics of CO binding to two major subpopulations of heme-bound to rGAPDH (2:1, GAPDH:heme, 10 ^{o}C). Formation of a Fe(II)-CO complex with a Soret absorption maximum at 410 nm occurs ~4-fold faster than the formation of the 6-coordinate Fe(II)-CO-GAPDH with a characteristic band at 420 nm. The nature of the species absorbing at 410 nm is currently unknown.

Fig. S8. UV-visible spectra of purified human GAPDH (Panel A) or *S. suis* (Panel B) GAPDHs from cultures grown in the presence or absence of the heme synthesis precursor δ-amino levulinic acid.

Fig. S9. Amino acid sequence alignment of *H. sapiens* (Accession P04406.3), *O. cuniculus* (Accession P46406.3) and *S. suis* (Accession AAN86058.1) GAPDHs. The alignment was generated with ClustalW 2.1 software (http://www.ebi.ac.uk/Tools/msa/clustalw2/)

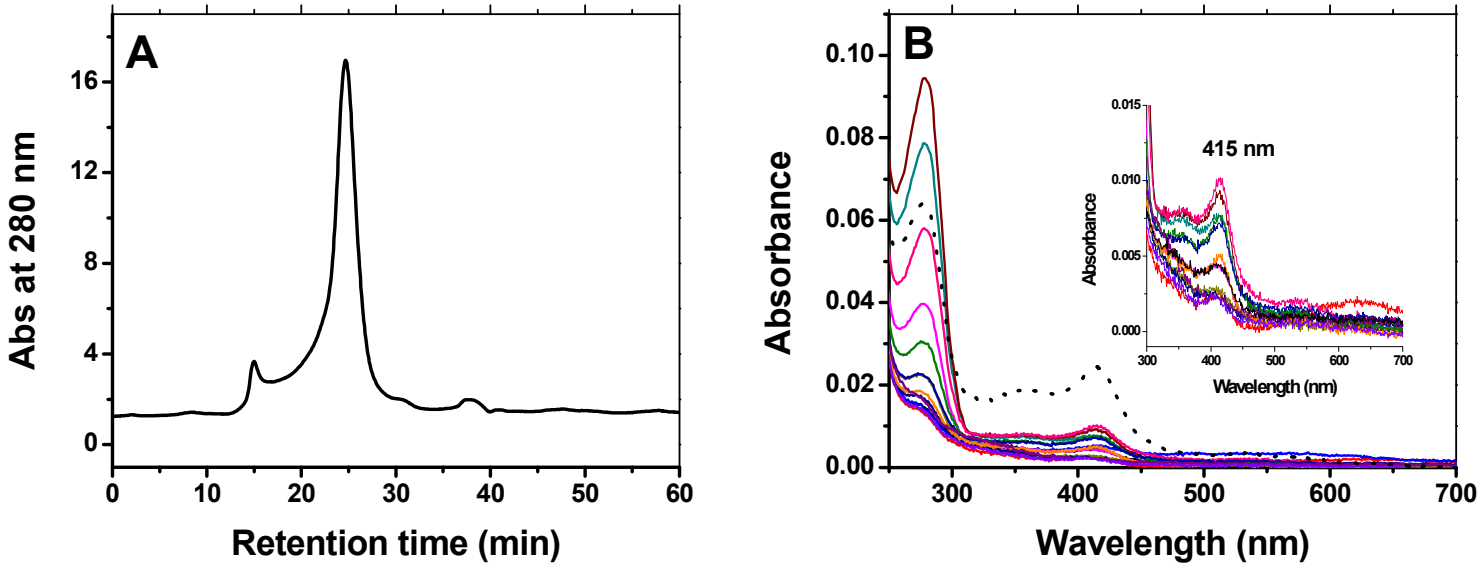
A
Abs at 280 nm
Retention time (min)
B
Absorbance
Wavelength (nm)
415 nm
Absorbance
Wavelength (nm)

Figure S1

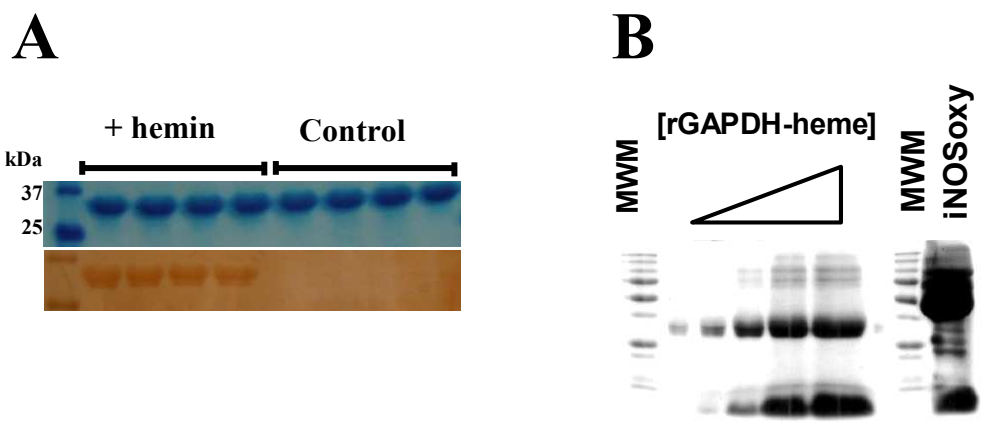

Figure S2

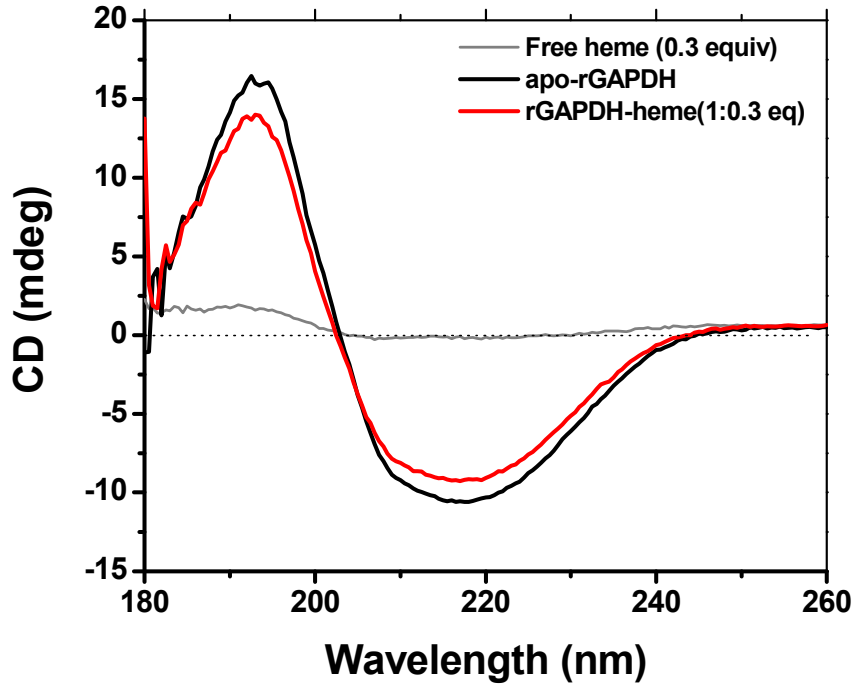
Free heme (0.3 equiv)
apo-rGAPDH
rGAPDH-heme(1:0.3 eq)
CD (mdeg)
Wavelength (nm)
20
15
10
5
0
-5
-10
-15
180
200
220
240
260

Figure S3

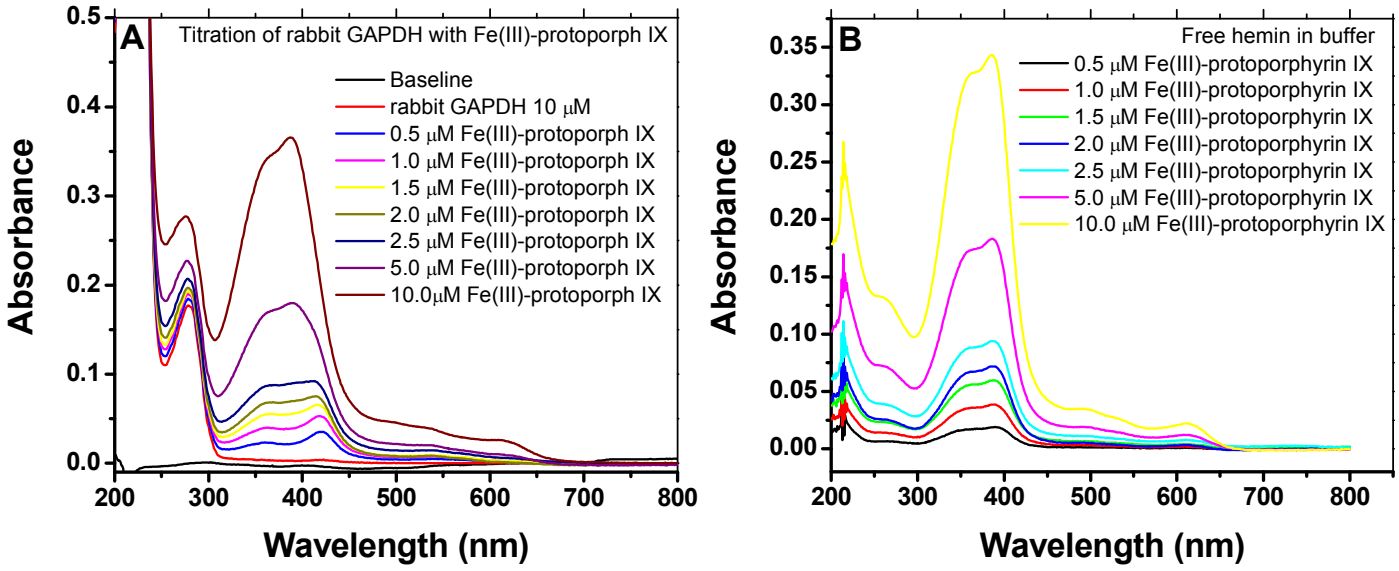
A
Titration of rabbit GAPDH with Fe(III)-protoporph IX
Baseline
rabbit GAPDH 10 μM
0.5 μM Fe(III)-protoporph IX
1.0 μM Fe(III)-protoporph IX
1.5 μM Fe(III)-protoporph IX
2.0 μM Fe(III)-protoporph IX
2.5 μM Fe(III)-protoporph IX
5.0 μM Fe(III)-protoporph IX
10.0μM Fe(III)-protoporph IX
Absorbance
Wavelength (nm)
B
Free hemin in buffer
0.5 μM Fe(III)-protoporphyrin IX
1.0 μM Fe(III)-protoporphyrin IX
1.5 μM Fe(III)-protoporphyrin IX
2.0 μM Fe(III)-protoporphyrin IX
2.5 μM Fe(III)-protoporphyrin IX
5.0 μM Fe(III)-protoporphyrin IX
10.0 μM Fe(III)-protoporphyrin IX
Absorbance
Wavelength (nm)

Figure S4

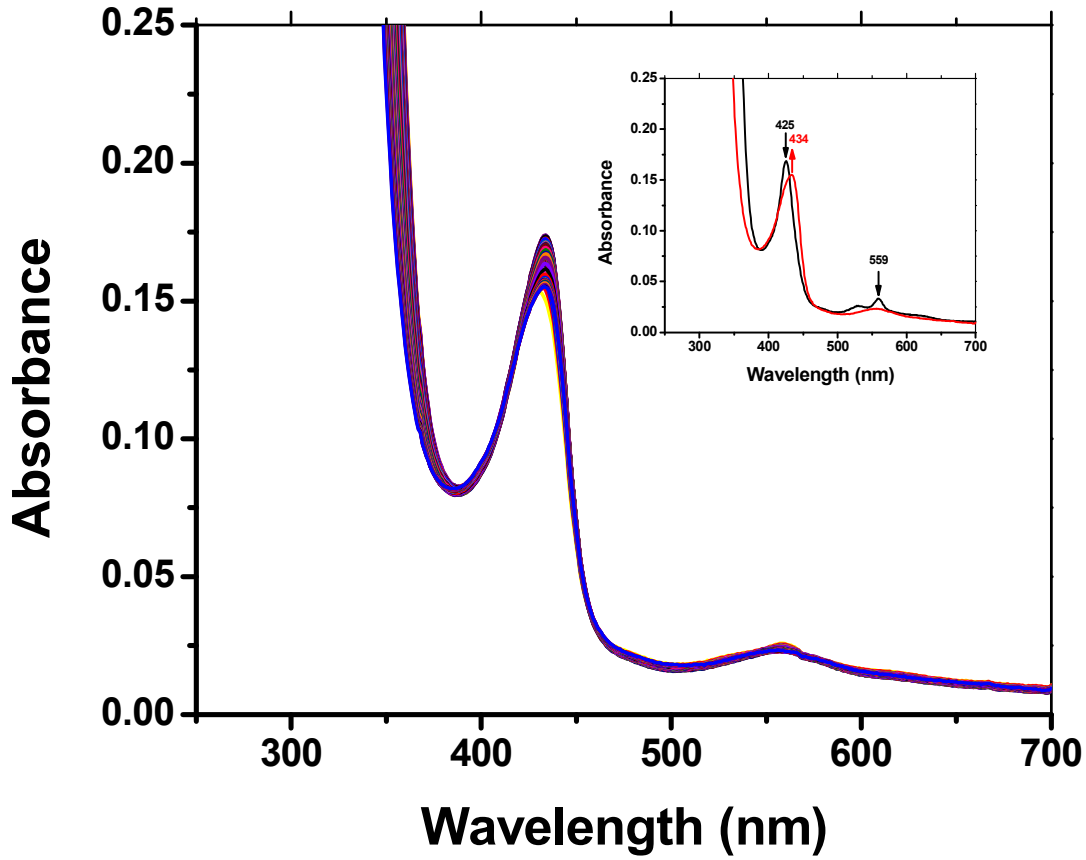
Absorbance
Wavelength (nm)
0.25
0.20
0.15
0.10
0.05
0.00
300
400
500
600
700
425
434
559

Figure S5

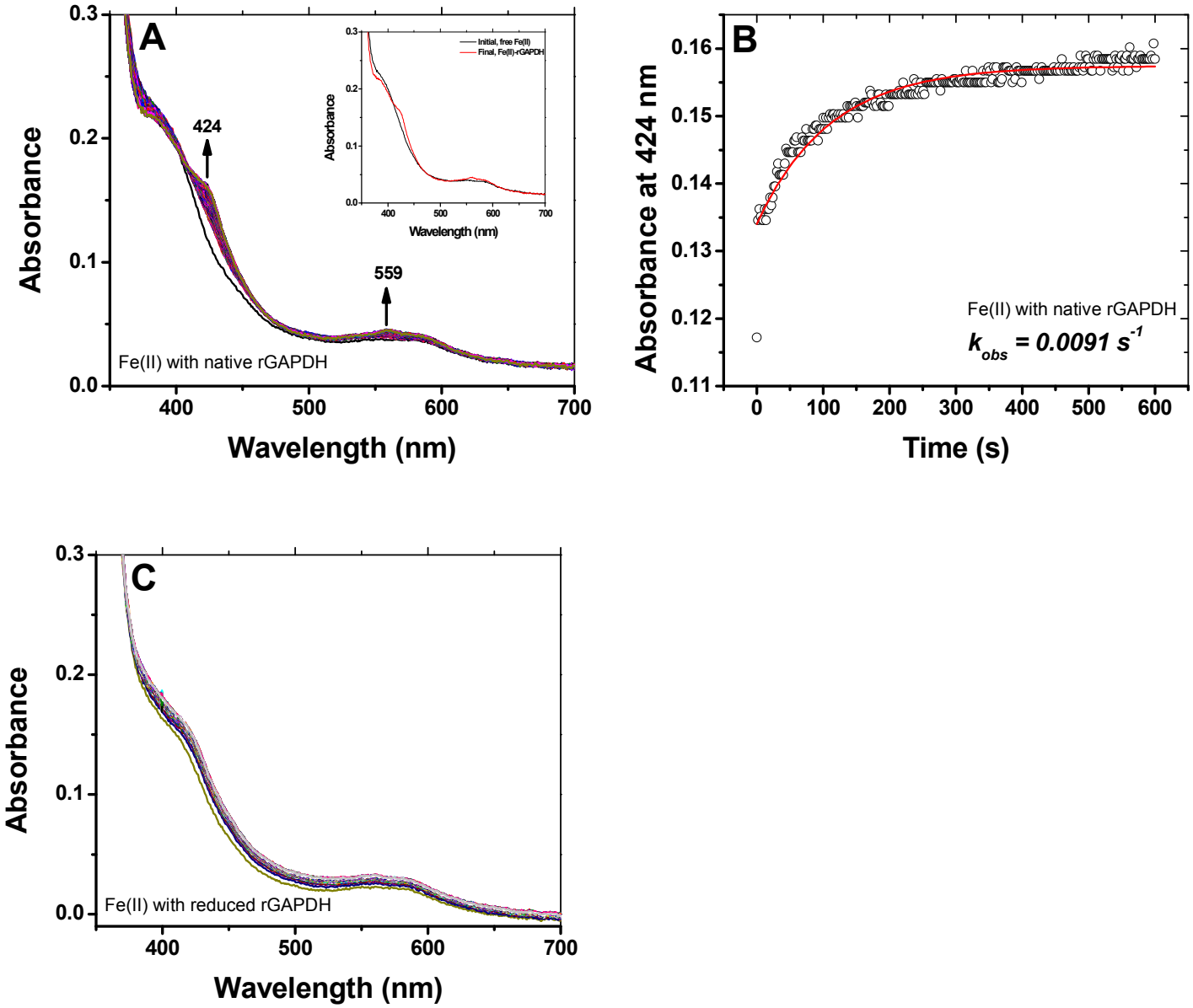
A
424
559
Fe(II) with native rGAPDH
Absorbance
Wavelength (nm)
Initial, free Fe(II)
Final, Fe(II)-rGAPDH
B
Absorbance at 424 nm
Time (s)
Fe(II) with native rGAPDH
k_{obs} = 0.0091 s^{-1}
C
Fe(II) with reduced rGAPDH

Figure S6

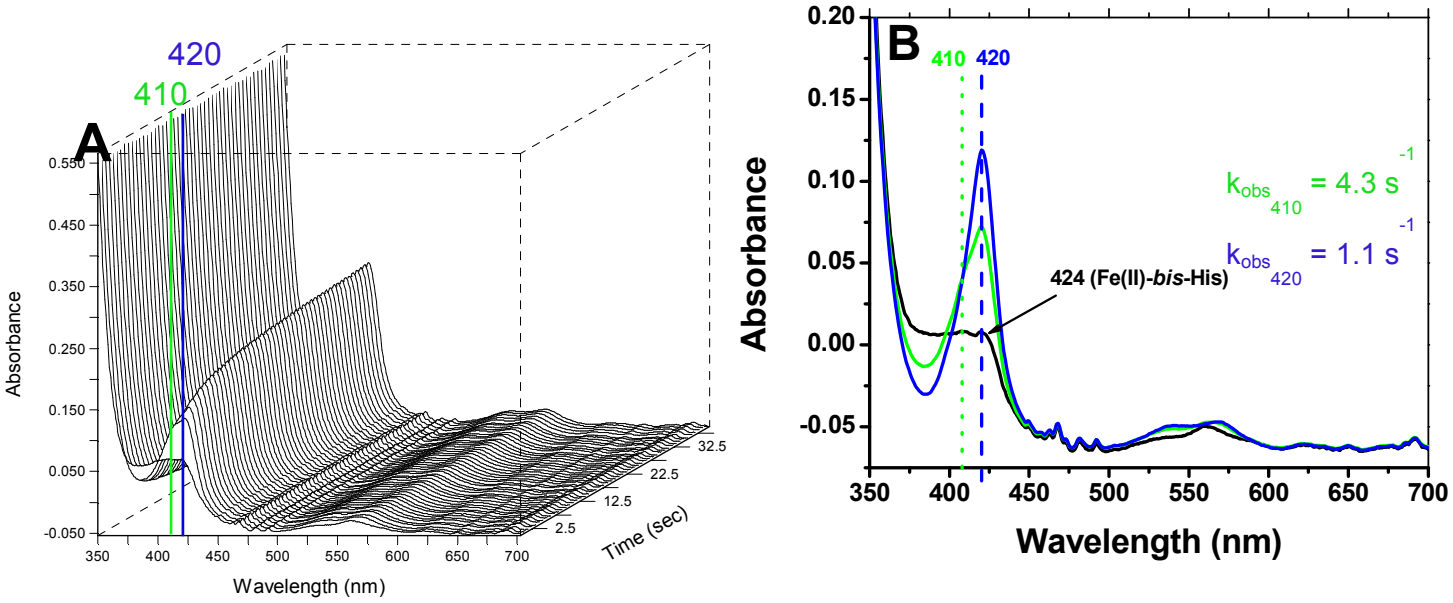
A
420
410
Absorbance
0.550
0.450
0.350
0.250
0.150
0.050
-0.050
350
400
450
500
550
600
650
700
Wavelength (nm)
32.5
22.5
12.5
2.5
Time (sec)
B
410
420
Absorbance
0.20
0.15
0.10
0.05
0.00
-0.05
350
400
450
500
550
600
650
700
Wavelength (nm)
k_{obs} 410 = 4.3 s^{-1}
k_{obs} 420 = 1.1 s^{-1}
424 (Fe(II)-*bis*-His)

Figure S7

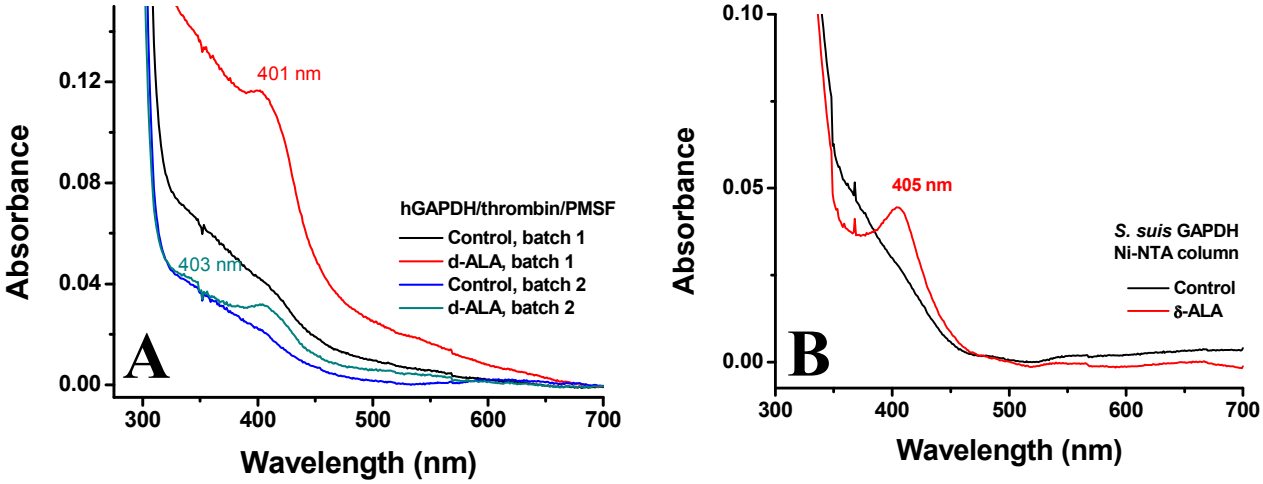
A
Absorbance
Wavelength (nm)
401 nm
403 nm
hGAPDH/thrombin/PMSF
Control, batch 1
d-ALA, batch 1
Control, batch 2
d-ALA, batch 2
B
405 nm
S. suis GAPDH
Ni-NTA column
Control
δ-ALA

Figure S8

CLUSTAL 2.1 multiple sequence alignment for *H. sapiens*, *O. cuniculus* and *S. suis* GAPDHs (in this order).

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MGKVKVGVNGFGRIGRLVTRAAFNSGKVDI      GNPITIFQERDPSKIKWGDAGAEYVVESTGVFTTMEKAGAHLQG-GAKRV 49
MVKVGVNGFGRIGRLVTRAAFNSGKVDVVA      --AITIFQERDPANIKWGDAGAEYVVESTGVFTTMEKAGAHLKG-GAKRV 47
MVVKVGINGFGRIGRLAFRRIQNVEGVEVT      --FVKVSAEREPGNIDWATDGVDIVLEATGFFASKEKAEQHIHANGAKKV 48
                                       :.:  **:*.:*.*.  *.: *:*:**.*:: ***  *::. ***:*

MGKVKVGVNGFGRIGRLVTRAAFNSGKVDI      IISAP-SADAPMFVMGVNHEKYDNSLKIISNASCTTNCLAPLAKVIHDNF 98
MVKVGVNGFGRIGRLVTRAAFNSGKVDVVA      IISAP-SADAPMFVMGVNHEKYDNSLKIVSNASCTTNCLAPLAKVIHDHF 96
MVVKVGINGFGRIGRLAFRRIQNVEGVEVT      VITAPGGNDVKTVVFNTNHDILDGTETVISGASCTTNCLAPMAKALHDAF 98
                                     :*:** . *.  .*:..**:  *.: .::*.**********:**.:** *

MGKVKVGVNGFGRIGRLVTRAAFNSGKVDI      GIVEGLMTTVHAITATQKTVDGPS-GKLWRDGRGALQNIIPASTGAAKAV 147
MVKVGVNGFGRIGRLVTRAAFNSGKVDVVA      GIVEGLMTTVHAITATQKTVDGPS-GKLWRDGRGAAQNIIPASTGAAKAV 145
MVVKVGINGFGRIGRLAFRRIQNVEGVEVT      GVQKGLMTTIHGYTGDQMVLDGPHRGGDLRRARAAAANIVPNSTGAAKAI 148
                                     *: :*****:*. *. * .:***  *   * .*.*  **:* *******:

MGKVKVGVNGFGRIGRLVTRAAFNSGKVDI      GKVIPELNGKLTGMAFRVPTANVSVVDLTCRLEKPAKYDDIKKVVKQASE 197
MVKVGVNGFGRIGRLVTRAAFNSGKVDVVA      GKVIPELNGKLTGMAFRVPTPNVSVVDLTCRLEKAAKYDDIKKVVKQASE 195
MVVKVGINGFGRIGRLAFRRIQNVEGVEVT      GLVIPELNGKLDGAAQRVPVPTGSVTELVATLDKKVTAEEVNAAMKAAAT 198
                                     * ********* * * ***... **.:*.. *:* .. :::: .:* *:

MGKVKVGVNGFGRIGRLVTRAAFNSGKVDI      GPLKGILGYTEHQVVSSDFNSDTHSSTFDAGAG--IALN-DHFVKLISWY 244
MVKVGVNGFGRIGRLVTRAAFNSGKVDVVA      GPLKGILGYTEDQVVSCDFNSATHSSTFDAGAG--IALN-DHFVKLISWY 242
MVVKVGINGFGRIGRLAFRRIQNVEGVEVT      ----ESFGYTEDQLVSSDIVGISFGSLFDATQTKVIEVDGEQLVKVVSWY 244
                                           :****.*:**.*: . :..* ***     * :: :::**::***

MGKVKVGVNGFGRIGRLVTRAAFNSGKVDI      DNEFGYSNRVVDLMAHMASKE- 265
MVKVGVNGFGRIGRLVTRAAFNSGKVDVVA      DNEFGYSNRVVDLMVHMASKE- 263
MVVKVGINGFGRIGRLAFRRIQNVEGVEVT      DNEMSYTAQLVRTLEYFAKIAK 266
                                    ***:.*: ::*  : ::*.
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Figure S9