

**The Five Near-Iron Transporter (NEAT) Domain Anthrax Hemophore, IsdX2,
Scavenges Heme from Hemoglobin and Transfers Heme to the Surface Protein
IsdC.**

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Supplemental Fig. S1. Alignments of *B. anthracis* and *S. aureus* NEAT domains. ClustalW2 alignments of the IsdX2 NEAT domains with NEAT domains from *B. anthracis* and *S. aureus* indicate two key areas of homology, namely the lip (*underlined residues*) and the YXXXY signature sequence located on the β 8-sheet (*grey shaded residue to arrowhead residue*). Of note is a glutamine in the lip region of NEATs one and five (*bold, underlined residue*), which is absent from NEATs two, three, and four, which may facilitate heme extraction from Hb. In the YXXXY signature sequence, the second tyrosine of NEAT two is substituted with a histidine, suggesting this tyrosine is necessary for heme stabilization, as observed for IsdB NEAT one and IsdH NEATs one and two (*highlighted black*) (8,12,17,40,42,50,64). The first tyrosine is conserved in all NEAT domains. * = fully conserved residue, : = conserved substitution, . = semi-conserved substitution.

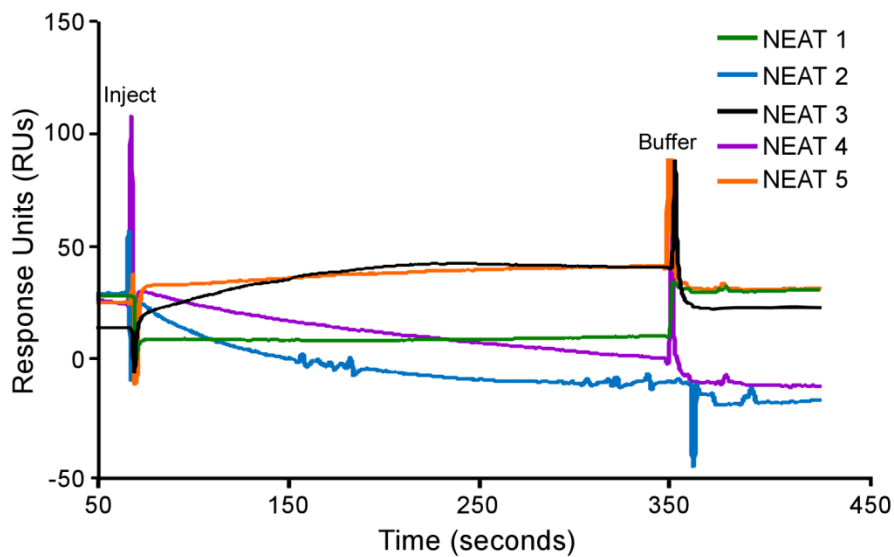
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IsdX2 NEAT 1  --AATKLADGEYSIGFKVLKDTSD EESMNNQY SVSPGTLKVKDGGKKVVSFTLTNSSWITKFETEKA---GK 94
IsdX2 NEAT 2  --NSNTIKDGEYSIPFKVLKNQTD EESKMNTYMVNPGVLKIENGKKKAIIVTLKSSSLIKNFQTEKD---GA 257
IsdX2 NEAT 3  --DAETIKDGEYSINFKALKDQTD EISMNNYTKSPGLLKVKDGGKYYVSFTLTNSSWITKFEFEKN---GS 416
IsdX2 NEAT 4  --DPNALKDGEYSIGFKVLKDKTEEI SMNNTYTKNPGVLKVKDGGKYYVSFTLTNSSWITKFEFEKN---GA 569
IsdX2 NEAT 5  --DPKNLKDQYDIAFKVLKDKTEEI SMNQYVVS PARLTVKVDGKKYIAMTLKNSEWITKFQTEKN---GG 740
IsdX1
BslK NEAT
BAS0520 NEAT --MAVQSPKKHVFDAVIKAYKDNSDEESYATVYIKDP-KLTIENGRKRIITATLKSDDFDYLKVEDSKEPGV 96
IsdC B.a
IsdC S.a
IsdA
IsdB NEAT 1  --DKDHSAPNSRPIDFEMKKKDGTOQFYHYASSVKPARVIFTDSKPEIELGLQSGQFWRKFEVYEG---DK 205
IsdB NEAT 2  --PTNEKMTDLQDTKYVVYVESVENNESMMDTFVKHPIKTKGMLNGKKYVMETTNDDYWKDFMVEGQ----- 400
IsdH NEAT 1  --NKEHDIGPREQVNFQLLDKNNETQYHYHFFSIKDPADVYYTKKAEVELDINTASTWKKFEVYEN---NQ 166
IsdH NEAT 2  --DKEHTADNWRPIDFQMKNDKGERQFYHYASTVEPATVIFTKTGPIIELGLKTA STWKKFEVYEG---DK 406
IsdH NEAT 3  --PTNDQLTDVQEAHFVVFSEENSESVMDFVEHPFYATATLNGQKYVVMKTKDDSYWKDLIVEGK----- 602

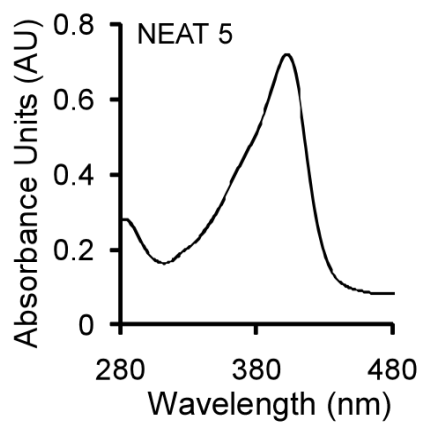
IsdX2 NEAT 1  LVATNVI SEDKEKD-TRVVEFDVEDVEKVLNAKVVDIDF-----LNYHHEYDVRIAFDQNSIT----- 152
IsdX2 NEAT 2  FVDAKVVSENKEKD-TRVVEFEVADLSKKLNTKVFIEMAS-----RNYKQTEDVQLLFEQDKLE----- 315
IsdX2 NEAT 3  FVDANVISEDKAD-TRVVEVAVDDLSKKLNAKVVDIDS-----MNYHFFYDIQFAFDKGSIK----- 474
IsdX2 NEAT 4  FVDAKVLGTNKEQD-TRVVEFEVADLSKKLNAKVVDIDS-----MNYHFFYDIQFAFDKGSIK----- 627
IsdX2 NEAT 5  FADAKVSEDKAAN-TRVVEFEANDLFAKLNAAKVVDIDS-----MNYHFFYDVQIQFDPTKI----- 797
IsdX1
BslK NEAT
BAS0520 NEAT FHDVKVISEDKANNGTKVVQFEIDFSSKKNMQMHILIPA-----IKYDHKYQVQFEIDASAI----- 165
IsdC B.a
IsdC S.a
IsdA
IsdB NEAT 1  KLPIKLVSYDTV-KDYAIRFSVNGTKAVKIVSSTHF-NNKEEK-YDYTLMYFAQPIYNSADKFKT---- 269
IsdB NEAT 2  --RVRTISKDAKNN-TRTIIIPYVEGKTLYDAIVKVHVKT-----IDYDGQYHVRIVDKEAFTKANTDKS 462
IsdH NEAT 1  KLPVRLVSYSPVPEDHAYIRFPVSDGTQELKIVSSTQIDDGEETN-YDYTKLVFAKPIYNDPSLVKS---- 229
IsdH NEAT 2  KLPVELVSYDSD-KDYAIRFPVSNGTREVKIVSSIEYGENIHED-YDYTLMYFAQPIITNPDYVD---- 468
IsdH NEAT 3  --RVTTVSKDPKNN-SRTLIFPIPDKAVYNAIVKVVAN-----IGYEGQYHVRIINQDINTKDDDTSQ 664

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Supplemental Fig. S2. IsdX2 NEAT domains do not interact with apo-Hb. Recombinant NEATs one (20 μM , *green*); NEAT two (15 μM , *blue*); NEAT three (20 μM , *black*); NEAT four (14 μM , *purple*) or NEAT five (12 μM , *orange*) were injected at a constant flow rate of 20 $\mu\text{L}/\text{min}$ over 8000 RU of immobilized apo-Hb. Association and dissociation phases were monitored for 300 seconds by observing changes in the response units (RUs) with time. Each curve is a representation of two independent determinations and had a χ^2 value of less than two.



Supplemental Fig. S3. Spectral scan of NEAT five for heme transfer to IsdC. NEAT five was purified and heme-loaded as described in “Experimental Procedures.” A preliminary spectroscopic scan from 280 – 480 nm demonstrated the presence of an intense Soret band, indicating the NEAT five protein preparation was holo before incubation with GST-IsdC.



Supplemental Table S1. Primers for the amplification of each IsdX2 NEAT domain. Underlined nucleotides in forward and reverse primers are BamH1 and EcoR1 restriction sites, respectively. Bold nucleotides in the reverse primers represent stop codons.

NEAT domain	Forward Primer 5' - 3'	Reverse Primer 5' - 3'
One	GATCGATC <u>GGATCC</u> GCTACCAAGTTGGCTGACGG	GATCGATC <u>GAATTC</u> CAATT GTAATACTATTTTGATCAAATGC
Two	GATCGATC <u>GGATCC</u> AATTCTAACACAATTAAGATGGAG	GATCGATC <u>GAATTC</u> CAATT CAAGTTTATCTTGTCAAATAAAAG
Three	GATCGATC <u>GGATCC</u> GATGCTGAAACAATTAAGATGG	GATCGATC <u>GAATTC</u> CAATT TTAATGCTACCTTTATCAAATGC
Four	GATCGATC <u>GGATCC</u> GATCCCAATGCTCTTAAAGACG	GATCGATC <u>GAATTC</u> CAATT TTAATACTTCCTTTATCAAATGC
Five	GATCGATC <u>GGATCC</u> GATCCGAAAAATTTAAAGGATGG	GATCGATC <u>GAATTC</u> CAATA ATCTTCGTCGGATCAAATTG