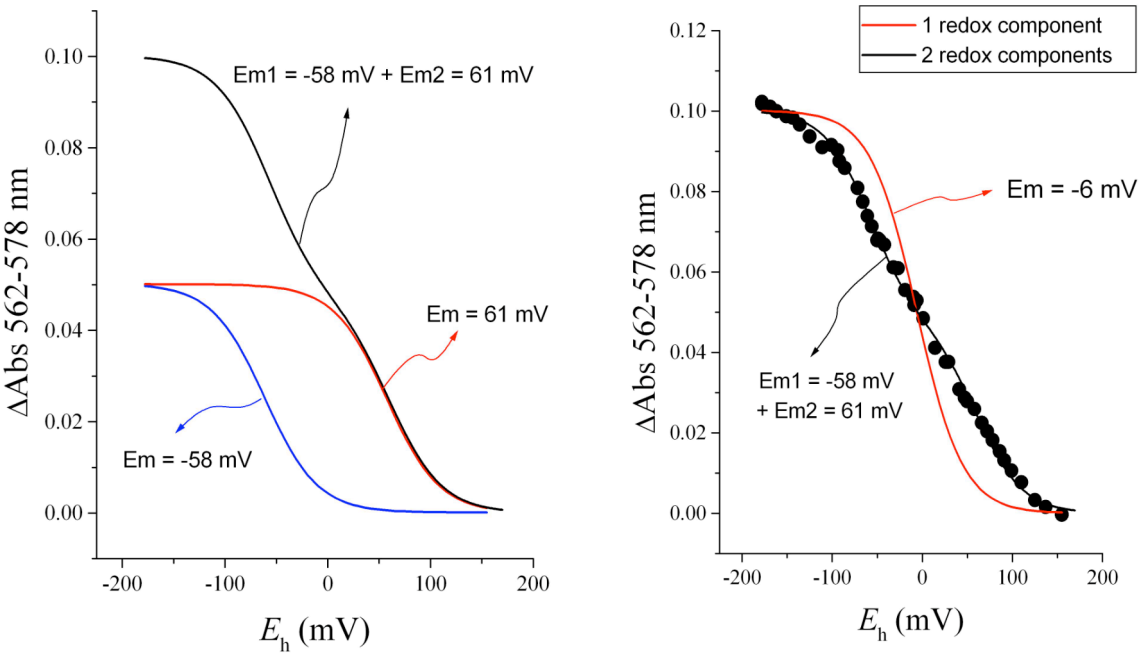
SUPPLEMENTAL DATA

1. Fig. S1. Deconvolution of the fitting of cytochrome *b* absorbance (Fig. 2) to two Nernst (n=1) redox groups (Eq. 1) into its individual redox components (left) and comparison of the fits with one or two redox groups (right).



2. Dynafit Files used for fitting of cytochrome *b* reduction by DBH_2 in the presence of myxothiazol (Fig. 7) or stigmatellin (Fig. 8).

All concentrations and parameter units are expressed in μM, $\mu M^{-1}s^{-1}$, or s^{-1}

A. Model assuming simultaneous binding to both center N sites in the dimer, each one having the same kinetic properties. This model was used for the fitting shown in Figs. 7A (myxothiazol present) and 8A (stigmatellin present).

TASK

Fit of progress curves

CONCENTRATIONS

E = 0.75 (dimer)
Q = 1.5 (1 equivalent per monomer)
DBH2 = 48

REACTION MECHANISM

DBH2 + E <===> EbH.DSQ : k1 k_1
DBH2 + E <===> DSQ.bHE : k1 k_1
EbH.DSQ <===> bHE.DSQ : kid kid
DSQ.bHE <===> DSQ.EbH : kid kid
DSQ.EbH <===> bHEbH + DBQ : k2 k_2
bHE.DSQ <===> bHEbH + DBQ : k2 k_2
Q + bHE.DSQ <===> SQ.E.DSQ : k_2' k2'
Q + DSQ.EbH <===> DSQ.E.SQ : k_2' k2'
SQ.E.DSQ <===> DBQ + SQ.EbH : k2 k_2
DSQ.E.SQ <===> DBQ + bHE.SQ : k2 k_2
bHEbH + Q <===> bHE.SQ : k_2' k2'
bHEbH + Q <===> SQ.EbH : k_2' k2'
SQ.EbH <===> SQ.bHE : kid kid
EbH.SQ <===> bHE.SQ : kid kid
SQ.bHE <===> E + QH2 : k_1' k1'
EbH.SQ <===> E + QH2 : k_1' k1'
Q + bHE.SQ <===> SQ.E.SQ : k_2' k2'
Q + SQ.EbH <===> SQ.E.SQ : k_2' k2'
bHE.DSQ + DBQ <===> DSQ.E.DSQ : k_2 k2
DSQ.EbH + DBQ <===> DSQ.E.DSQ : k_2 k2
DBH2 + DSQ.bHE <===> DSQ.bHEbH.DSQ : k1 k_1
DBH2 + EbH.DSQ <===> DSQ.bHEbH.DSQ : k1 k_1
DBH2 + SQ.bHE <===> SQ.bHEbH.DSQ : k1 k_1
DBH2 + EbH.SQ <===> DSQ.bHEbH.SQ : k1 k_1
DSQ.bHE + QH2 <===> DSQ.bHEbH.SQ : k1' k_1'
EbH.DSQ + QH2 <===> SQ.bHEbH.DSQ : k1' k_1'
SQ.bHE + QH2 <===> SQ.bHEbH.SQ : k1' k_1'
EbH.SQ + QH2 <===> SQ.bHEbH.SQ : k1' k_1'

RESPONSES (molar extinction coefficients x 2 for 2 cm pathlength)

EbH.DSQ = 0.072
bHE.DSQ = 0.072
DSQ.EbH = 0.072
DSQ.bHE = 0.072

EbH.SQ = 0.072
bHE.SQ = 0.072
SQ.EbH = 0.072
SQ.bHE = 0.072

bHEbH = 0.144
DSQ.bHEbH.DSQ = 0.144
SQ.bHEbH.SQ = 0.144
DSQ.bHEbH.SQ = 0.144
SQ.bHEbH.DSQ = 0.144

OUTPUT

For Fig. 7A (myxothiazol present):

LEAST-SQUARES FIT

mean square 1.81e-6
standard deviation 0.0013
datapoints 11598
parameters 9

PARAMETERS & STANDARD ERRORS

Parameter	Fitted	Error	%Error
k1	0.75	0.007	1
k_1	0.219	0.003	1.64
k1'	4.2e-6	3.3	7.8e+7
k_1'	10.5	1.23	11.8
k2	1.569	0.03	2
k_2	4.5	0.07	1.6
k2'	0.002	0.014	789
k_2'	32.89	0.42	1.3
kid	138.24	1.7	1.2

For Fig. 8A (stigmatellin present):

LEAST-SQUARES FIT

mean square 2.75e-6
standard deviation 0.0017
datapoints 11586
parameters 9

PARAMETERS & STANDARD ERRORS

Parameter	Fitted	Error	%Error
k1	0.687	0.007	1.07
k_1	0.074	0.0012	1.59
k1'	1.9e-5	0.0016	8301.5
k_1'	5.7	0.69	12.1
k2	9.8	0.502	5.12
k_2	55.36	3.08	5.6
k_2'	58.11	1.09	1.9

k2'	0.6	0.15	9.58
kid	48.08	0.54	1.12

B. Model assuming formation of SQ in only one monomer, followed by a conformational change (k*) that allows SQ to be formed in the second center N site, with the same kinetic properties as the first. This model was used for fitting shown in Fig. 7B (myxothiazol present).

TASK

Fit of progress curves

CONCENTRATIONS

*E = 0.75 (dimer with one inactive center N)
Q = 1.5 (1 equivalent per monomer)
DBH2 = 48

REACTION MECHANISM

DBH2 + *E <===> *EbH.DSQ : k1 k_1
*E + QH2 <===> *EbH.SQ : k1' k_1'
EbH.DSQ ---> EbH.DSQ : k
EbH.SQ ---> EbH.SQ : k
DBH2 + E <===> EbH.DSQ : k1 k_1
DBH2 + E <===> DSQ.bHE : k1 k_1
*EbH.DSQ <===> *bHE.DSQ : kid kid
*bHE.DSQ <===> *bHEbH + DBQ : k2 k_2
bHE.DSQ ---> bHE.DSQ : k
*EbH.SQ <===> *bHE.SQ : kid kid
*bHEbH + Q <===> *bHE.SQ : k_2' k2'
bHE.SQ ---> bHE.SQ : k
EbH.DSQ <===> bHE.DSQ : kid kid
DSQ.bHE <===> DSQ.EbH : kid kid
DSQ.EbH <===> DBQ + bHEbH : k2 k_2
bHE.DSQ <===> DBQ + bHEbH : k2 k_2
bHE.DSQ + Q <===> SQ.E.DSQ : k_2' k2'
Q + DSQ.EbH <===> DSQ.E.SQ : k_2' k2'
SQ.E.DSQ <===> DBQ + SQ.EbH : k2 k_2
DSQ.E.SQ <===> DBQ + bHE.SQ : k2 k_2
Q + bHEbH <===> bHE.SQ : k_2' k2'
Q + bHEbH <===> SQ.EbH : k_2' k2'
SQ.EbH <===> SQ.bHE : kid kid
EbH.SQ <===> bHE.SQ : kid kid
SQ.bHE <===> E + QH2 : k_1' k1'
EbH.SQ <===> E + QH2 : k_1' k1'
Q + bHE.SQ <===> SQ.E.SQ : k_2' k2'
Q + SQ.EbH <===> SQ.E.SQ : k_2' k2'
DBQ + bHE.DSQ <===> DSQ.E.DSQ : k_2 k2
DBQ + DSQ.EbH <===> DSQ.E.DSQ : k_2 k2
DBH2 + DSQ.bHE <===> DSQ.bHEbH.DSQ : k1 k_1

DBH2 + EbH.DSQ <===> DSQ.bHEbH.DSQ : k1 k_1
DBH2 + SQ.bHE <===> SQ.bHEbH.DSQ : k1 k_1
DBH2 + EbH.SQ <===> DSQ.bHEbH.SQ : k1 k_1
QH2 + DSQ.bHE <===> DSQ.bHEbH.SQ : k1' k_1'
QH2 + EbH.DSQ <===> SQ.bHEbH.DSQ : k1' k_1'
QH2 + SQ.bHE <===> SQ.bHEbH.SQ : k1' k_1'
QH2 + EbH.SQ <===> SQ.bHEbH.SQ : k1' k_1'

RESPONSES (molar extinction coefficients x 2 for 2 cm pathlength)

*EbH.DSQ = 0.072
*bHE.DSQ = 0.072
EbH.DSQ = 0.072
bHE.DSQ = 0.072
DSQ.EbH = 0.072
DSQ.bHE = 0.072

*EbH.SQ = 0.072
*bHE.SQ = 0.072
EbH.SQ = 0.072
bHE.SQ = 0.072
SQ.EbH = 0.072
SQ.bHE = 0.072

*bHEbH = 0.144
bHEbH = 0.144
DSQ.bHEbH.DSQ = 0.144
SQ.bHEbH.SQ = 0.144
DSQ.bHEbH.SQ = 0.144
SQ.bHEbH.DSQ = 0.144

OUTPUT

LEAST-SQUARES FIT

mean square 1.56e-6
standard deviation 0.0012
datapoints 11598
parameters 10

PARAMETERS & STANDARD ERRORS

Parameter	Fitted	Error	%Error
k1	1.92	0.022	1.2
k_1	0.14	0.003	2.2
k1'	1.6e-9	4.8e-10	29.7
k_1'	11.51	0.003	11.4
k2	1.014	0.025	2.5

k_2	9.2	0.17	1.8
k2'	0.038	0.006	16
k_2'	76.3	1.06	1.4
kid	340.4	19	1.4
k*	12040	2023	16.8

C. Model assuming that SQ forms with different kinetic parameters in each monomer, with a conformational change ($k^{\#}$) that allows both center N sites to have the same kinetic properties in the end. This model was used for fitting shown in Fig. 8B (stigmatellin present).

TASK

Fit of progress curves

CONCENTRATIONS

$^{\#}$E = 0.75 (dimer with two kinetically different center N sites)
Q = 1.5 (1 equivalent per monomer)
DBH2 = 48

REACTION MECHANISM

DBH2 + $^{\#}$E <===> $^{\#}$EbH.DSQ : k1 k_1
QH2 + $^{\#}$E <===> $^{\#}$EbH.SQ : k1' k_1'
DBH2 + $^{\#}$E <===> DSQ.$^{\#}$bHE : k3 k_3
QH2 + $^{\#}$E <===> SQ.$^{\#}$bHE : k3' k_3'
$^{\#}$EbH.DSQ ----> EbH.DSQ : $k^{\#}$
$^{\#}$EbH.SQ ----> EbH.SQ : $k^{\#}$
DBH2 + E <===> EbH.DSQ : k1 k_1
DBH2 + E <===> DSQ.bHE : k1 k_1
$^{\#}$EbH.DSQ <===> $^{\#}$bHE.DSQ : kid kid
DSQ.$^{\#}$bHE <===> DSQ.$^{\#}$EbH : kid kid
$^{\#}$bHE.DSQ <===> $^{\#}$bHEbH + DBQ : k2 k_2
DSQ.$^{\#}$EbH <===> $^{\#}$bHEbH + DBQ : k4 k_4
$^{\#}$bHE.DSQ ----> bHE.DSQ : $k^{\#}$
$^{\#}$EbH.SQ <===> $^{\#}$bHE.SQ : kid kid
SQ.$^{\#}$bHE <===> SQ.$^{\#}$EbH : kid kid
Q + $^{\#}$bHEbH <===> $^{\#}$bHE.SQ : k_2' k2'
SQ.$^{\#}$EbH <===> $^{\#}$bHEbH + Q : k4' k_4'
$^{\#}$bHE.SQ ----> bHE.SQ : $k^{\#}$
EbH.DSQ <===> bHE.DSQ : kid kid
DSQ.bHE <===> DSQ.EbH : kid kid
DSQ.EbH <===> bHEbH + DBQ : k2 k_2
bHE.DSQ <===> bHEbH + DBQ : k2 k_2
Q + bHE.DSQ <===> SQ.E.DSQ : k_2' k2'
Q + DSQ.EbH <===> DSQ.E.SQ : k_2' k2'
SQ.E.DSQ <===> SQ.EbH + DBQ : k2 k_2
DSQ.E.SQ <===> bHE.SQ + DBQ : k2 k_2
Q + bHEbH <===> bHE.SQ : k_2' k2'

Q + bHEbH <===> SQ.EbH : k_2' k2'
SQ.EbH <===> SQ.bHE : kid kid
EbH.SQ <===> bHE.SQ : kid kid
SQ.bHE <===> E + QH2 : k_1' k1'
EbH.SQ <===> E + QH2 : k_1' k1'
Q + bHE.SQ <===> SQ.E.SQ : k_2' k2'
Q + $^{\#}$bHE.SQ <===> SQ.$^{\#}$E.SQ : k_4' k4'
Q + SQ.EbH <===> SQ.E.SQ : k_2' k2'
Q + SQ.$^{\#}$EbH <===> SQ.$^{\#}$E.SQ : k_2' k2'
SQ.$^{\#}$bHE.SQ ----> SQ.bHE.SQ : k$^{\#}$
DBQ + bHE.DSQ <===> DSQ.E.DSQ : k_2 k2
DBQ + $^{\#}$bHE.DSQ <===> DSQ.$^{\#}$E.DSQ : k_4 k4
DBQ + DSQ.EbH <===> DSQ.E.DSQ : k_2 k2
DBQ + DSQ.$^{\#}$EbH <===> DSQ.$^{\#}$E.DSQ : k_2' k2'
DSQ.$^{\#}$bHE.SQ ----> DSQ.bHE.SQ : k$^{\#}$
Q + $^{\#}$bHE.DSQ <===> SQ.$^{\#}$E.DSQ : k_4' k4'
Q + DSQ.$^{\#}$EbH <===> DSQ.$^{\#}$E.SQ : k_2' k2'
SQ.$^{\#}$E.DSQ <===> SQ.$^{\#}$EbH + DBQ : k2 k_2
DSQ.$^{\#}$E.SQ <===> $^{\#}$bHE.SQ + DBQ : k4 k_4
SQ.$^{\#}$bHE.DSQ ----> SQ.bHE.DSQ : k$^{\#}$
DSQ.bHE + DBH2 <===> DSQ.bHEbH.DSQ : k1 k_1
DSQ.$^{\#}$bHE + DBH2 <===> DSQ.bHEbH.DSQ : k1 k_1
EbH.DSQ + DBH2 <===> DSQ.bHEbH.DSQ : k1 k_1
$^{\#}$EbH.DSQ + DBH2 <===> DSQ.$^{\#}$bHEbH.DSQ : k3 k_3
SQ.bHE + DBH2 <===> SQ.bHEbH.DSQ : k1 k_1
SQ.$^{\#}$bHE + DBH2 <===> SQ.$^{\#}$bHEbH.DSQ : k1 k_1
EbH.SQ + DBH2 <===> DSQ.bHEbH.SQ : k1 k_1
$^{\#}$EbH.SQ + DBH2 <===> DSQ.$^{\#}$bHEbH.SQ : k3 k_3
DSQ.bHE + QH2 <===> DSQ.bHEbH.SQ : k1' k_1'
DSQ.$^{\#}$bHE + QH2 <===> DSQ.$^{\#}$bHEbH.SQ : k1' k_1'
EbH.DSQ + QH2 <===> SQ.bHEbH.DSQ : k1' k_1'
$^{\#}$EbH.DSQ + QH2 <===> SQ.$^{\#}$bHEbH.DSQ : k3' k_3'
SQ.bHE + QH2 <===> SQ.bHEbH.SQ : k1' k_1'
SQ.$^{\#}$bHE + QH2 <===> SQ.$^{\#}$bHEbH.SQ : k1' k_1'
EbH.SQ + QH2 <===> SQ.bHEbH.SQ : k1' k_1'
$^{\#}$EbH.SQ + QH2 <===> SQ.$^{\#}$bHEbH.SQ : k3' k_3'

RESPONSES (molar extinction coefficients x 2 for 2 cm pathlength)

$^{\#}$EbH.DSQ = 0.072
$^{\#}$bHE.DSQ = 0.072
EbH.DSQ = 0.072
bHE.DSQ = 0.072
DSQ.$^{\#}$EbH = 0.072
DSQ.$^{\#}$bHE = 0.072
DSQ.EbH = 0.072
DSQ.bHE = 0.072

$^{\#}$EbH.SQ = 0.072
$^{\#}$bHE.SQ = 0.072
EbH.SQ = 0.072

bHE.SQ = 0.072
SQ.#EbH = 0.072
SQ.#bHE = 0.072
SQ.EbH = 0.072
SQ.bHE = 0.072

#bHEbH = 0.144
bHEbH = 0.144

DSQ.#bHEbH.DSQ = 0.144
SQ.#bHEbH.SQ = 0.144
DSQ.#bHEbH.SQ = 0.144
SQ.#bHEbH.DSQ = 0.144
DSQ.bHEbH.DSQ = 0.144
SQ.bHEbH.SQ = 0.144
DSQ.bHEbH.SQ = 0.144
SQ.bHEbH.DSQ = 0.144

OUTPUT

LEAST-SQUARES FIT

mean square 1.25e-6
standard deviation 0.0011
datapoints 11586
parameters 17

PARAMETERS & STANDARD ERRORS

Parameter	Fitted	Error	%Error
k1	0.746	0.01	1.35
k_1	0.219	0.061	7.2
k3	0.679	0.01	1.53
k_3	9.563	0.84	8.75
k1’	1.42e-7	4.2e-8	29.7
k_1’	3.7e-5	6.2e-6	16.6
k3’	2.4e-6	7.8e-7	32.9
k_3’	2.7	0.457	16.9
k2	0.517	0.016	3.15
k_2	1.063	0.103	9.73
k4	4.04	0.13	3.2
k_4	0.044	0.003	5.83
k2’	6.9e-7	1.5e-7	22.1
k_2’	6.33	0.146	2.31
k4’	4.81	0.25	5.1
k_4’	24.2	0.49	2.1
kid	2.5e+9	5.9e+8	24
k#	0.15 fixed		