SUPPLEMENTAL DATA

Dynafit Files

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

1. Models used for simulation of cytochrome *b* reduction kinetics by QH_2 in the presence of stigmatellin (Fig. 1A, 2 and S1).

A. Assuming intermonomeric electron equilibration (Fig. 1A).

REACTION MECHANISM

```
E + I <===> E.I : kaI kdI
E + I <===> I.E : kaI kdI
I + I.E <===> I.E.I : kaI kdI
I + E.I <===> I.E.I : kaI kdI
E + QH2 <===> E.QH2 : kaQH kdQH
E + QH2 <===> QH2.E : kaQH kdQH
QH2 + E.QH2 <===> QH2.E.QH2 : kaQH kdQH
QH2 + QH2.E <===> QH2.E.QH2 : kaQH kdQH
E.QH2 <===> EbH.SQ : k1 k_1
QH2.E <===> SQ.bHE : k1 k_1
EbH.SQ <===> bHE.SQ : k3 k3
SQ.bHE <===> SQ.EbH : k3 k3
bHE.SQ <===> bHEbH.Q : k2 k_2
SQ.EbH <===> Q.bHEbH : k2 k_2
QH2.E.QH2 <===> QH2.EbH.SQ : k1 k_1
QH2.E.QH2 <===> SQ.bHE.QH2 : k1 k_1
QH2 + EbH.SQ <===> QH2.EbH.SQ : kaQH kdQH
QH2 + SQ.bHE <===> SQ.bHE.QH2 : kaQH kdQH
QH2 + bHE.SQ <===> QH2.bHE.SQ : kaQH kdQH
QH2 + SQ.EbH <===> SQ.EbH.QH2 : kaQH kdQH
QH2.EbH.SQ <===> QH2.bHE.SQ : k3 k3
SQ.bHE.QH2 <===> SQ.EbH.QH2 : k3 k3
QH2.bHE.SQ <===> QH2.bHEbH.Q : k2 k_2
SQ.EbH.QH2 <===> Q.bHEbH.QH2 : k2 k_2
QH2.EbH.SQ <===> SQ.bHEbH.SQ : k1 k_1
SQ.bHE.QH2 <===> SQ.bHEbH.SQ : k1 k_1
QH2 + E.I <===> QH2.E.I : kaQH kdQH
QH2 + I.E <===> I.E.QH2 : kaQH kdQH
I.E.QH2 <===> I.EbH.SQ : k1 k_1
QH2.E.I <===> SQ.bHE.I : k1 k_1
I.EbH.SQ <===> I.bHE.SQ : k3 k3
SQ.bHE.I <===> SQ.EbH.I : k3 k3
I.bHE.SQ <===> I.bHEbH.Q : k2 k_2
SQ.EbH.I <===> Q.bHEbH.I : k2 k_2
```

CONSTANTS

kaQH = 1.5 , kaI = 0.15 (association rate constants for quinol and inhibitor)
kdQH = 30 , kdI = 0.001 (dissociation rate constants for quinol and inhibitor)
k1 = 200, k_1 = 400 (forward and reverse rates for semiquinone formation from quinol)
k2 = 400, k_2 = 150 (forward and reverse rates for quinone formation from semiquinone)
k3 = 500 (intermonomeric electron transfer)

RESPONSES (molar extinction coefficients x 2 to account for a 2 cm pathlength)

EbH.SQ = 0.072
SQ.bHE = 0.072
bHE.SQ = 0.072
SQ.EbH = 0.072
Q.bHEbH = 0.144
bHEbH.Q = 0.144
QH2.EbH.SQ = 0.072
SQ.bHE.QH2 = 0.072
QH2.bHE.SQ = 0.072
SQ.EbH.QH2 = 0.072
SQ.bHEbH.SQ = 0.144
QH2.bHEbH.Q = 0.144
Q.bHEbH.QH2 = 0.144
I.EbH.SQ = 0.072
SQ.bHE.I = 0.072
I.bHE.SQ = 0.072
SQ.EbH.I = 0.072
Q.bHEbH.I = 0.144
I.bHEbH.Q = 0.144

B. Assuming movement of the inhibitor between center N sites (Fig. 2) or fast inhibitor dissociation rate (Fig. S1) without intermonomeric electron equilibration.

E + QH2 <===> E.QH2 : kaQH kdQH
E + QH2 <===> QH2.E : kaQH kdQH
QH2 + E.QH2 <===> QH2.E.QH2 : kaQH kdQH
QH2 + QH2.E <===> QH2.E.QH2 : kaQH kdQH
E.QH2 <===> EbH.SQ : k1 k_1
QH2.E.QH2 <===> QH2.EbH.SQ : k1 k_1
QH2.E <===> SQ.bHE : k1 k_1
QH2.E.QH2 <===> SQ.bHE.QH2 : k1 k_1
QH2 + EbH.SQ <===> QH2.EbH.SQ : kaQH kdQH
QH2 + SQ.bHE <===> SQ.bHE.QH2 : kaQH kdQH
SQ.bHE.QH2 <===> SQ.bHEbH.SQ : k1 k_1
QH2.EbH.SQ <===> SQ.bHEbH.SQ : k1 k_1
E + I <===> E.I : kaI kdI
E + I <===> I.E : kaI kdI
E.I + I <===> I.E.I : kaI kdI
I.E + I <===> I.E.I : kaI kdI
I + E.QH2 <===> I.E.QH2 : kaI kdI
I + QH2.E <===> QH2.E.I : kaI kdI
QH2 + E.I <===> QH2.E.I : kaQH kdQH
QH2 + I.E <===> I.E.QH2 : kaQH kdQH
I + EbH.SQ <===> I.EbH.SQ : kaI kdI
I + SQ.bHE <===> SQ.bHE.I : kaI kdI
I.E.QH2 <===> I.EbH.SQ : k1 k_1
QH2.E.I <===> SQ.bHE.I : k1 k_1
I.E <===> E.I : kid kid

CONSTANTS

kaQH = 1.5, kaI = 0.15 (1500 in Fig. S1)
kdQH = 30 , kdI = 0.001 (10 in Fig. S1)
k1 = 270, k_1 = 100
kid = 500 (intermonomeric movement of the inhibitor; all values between 0 and 50000 yielded identical curves)

RESPONSES (molar extinction coefficients x 2 to account for a 2 cm pathlength)

EbH.SQ = 0.072
SQ.bHE = 0.072
QH2.EbH.SQ = 0.072
SQ.bHE.QH2 = 0.072
SQ.bHEbH.SQ = 0.144
I.EbH.SQ = 0.072
SQ.bHE.I = 0.072

CONCENTRATIONS

E = 0.75 (dimer)
QH2 = 15

PROGRESS

equilibrate E = 0.75, I = 0, 0.13, 0.26, 0.39, 0.52, 0.65, 0.78, 0.91, 1.04, 1.15, 1.28, 1.4, 1.53, and 1.82, dilute 1:1 (indicates pre-equilibration with I before mixing with QH2)

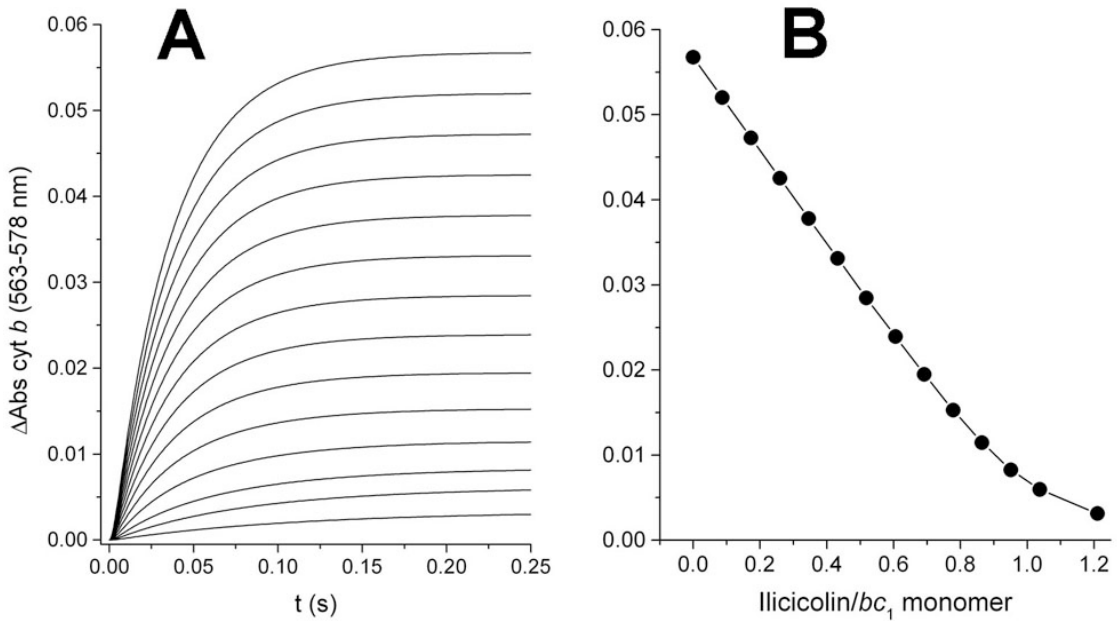

Fig. S1. **Simulation of the inhibition of pre-steady state reduction by an inhibitor with a high dissociation rate.** Simulated kinetic traces at the same concentrations of ilicicolin as in Fig. 1 were obtained assuming a dissociation rate of 10 s^{-1} for the inhibitor according to model B (panel A). Panel B shows the extent of cytochrome *b* reduction obtained at 0.25 s for each of the simulated reduction kinetic curves, resulting in a linear inhibition pattern.

2. Model used for calculating the relative concentrations of dimers with zero (E) and/or one (E.I and I.E) inhibitor molecules (Fig. 1B and 3B).

REACTION MECHANISM

E + I <===> E.I : kaI kdI
E + I <===> I.E : kaI kdI
I + I.E <===> I.E.I : kaI kdI
I + E.I <===> I.E.I : kaI kdI

CONSTANTS

kaI = 0.150
kdI = 0.001

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

For Fig. 1B:

E = 0.053
E.I = 0.053
I.E = 0.053

For Fig. 3B:

E.I = 0.0051
I.E = 0.0051

CONCENTRATIONS

E = 0.5 (dimer), I variable from 0 to 1.2, step 0.001