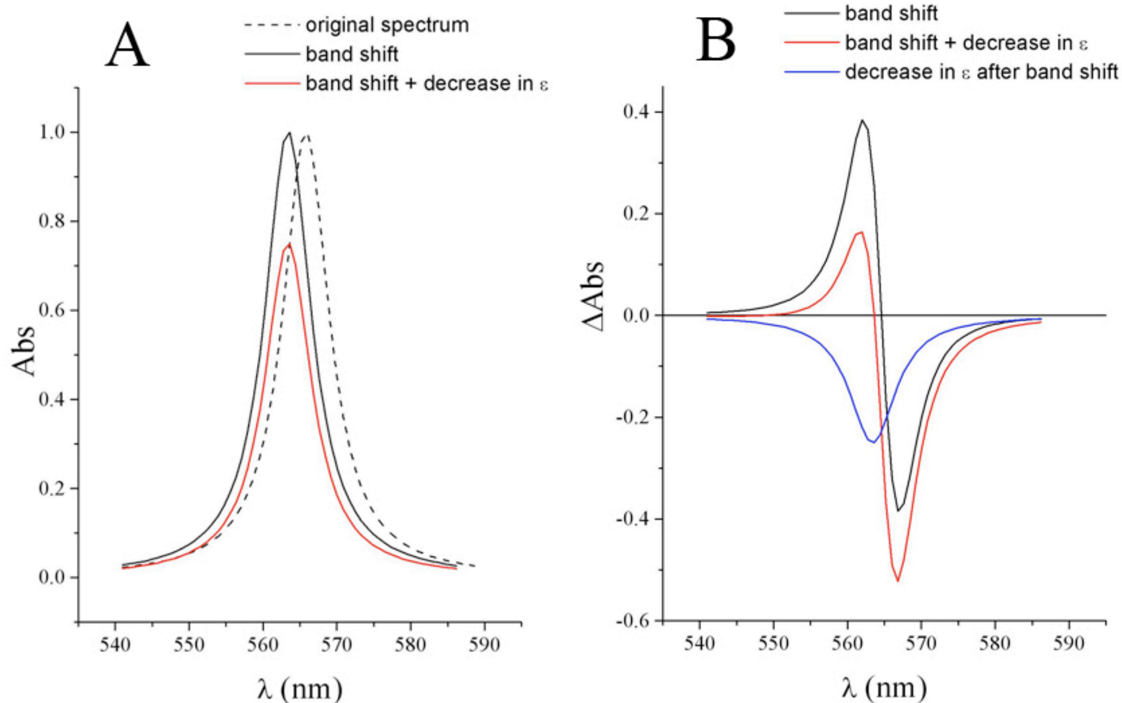
**SUPPLEMENTAL DATA**

**1. Figure S1. Spectral change expected from a decrease in the extinction coefficient of the $b_L$ hemes occurring after an initial symmetrical shift generated by homogeneous stigmatellin binding to all center P sites.** Panel A: A Lorentzian function was used to simulate an absorbance peak centered at 566 nm (dotted black curve). A blue shift of 3 nm generates a peak (solid black curve) centered at 563 nm. A 25% decrease in the absorbance of this shifted spectrum generates the red curve. Panel B: Subtracting the original spectrum from the shifted one generates a symmetrical blue shift (solid black curve) with a peak and trough of equal amplitude. Subtracting the original spectrum from the shifted one that has a decreased absorbance results in an asymmetrical peak (red solid curve), in which the trough is larger in amplitude than the peak. This spectrum has a similar shape to that shown in Fig. 3B after stigmatellin binding. However, subtraction of the original symmetrical shift (black) from the asymmetrical one (red) results in a trough centered close to the peak of both spectral shifts (~563 nm), and not at the trough. This contrast with the deconvolution shown in Figs 5B and 6B, which show that the absorbance at the shifted peak (562 nm) remains almost constant during the slow kinetic phase of stigmatellin binding, while the trough (569) decreases in absorbance markedly during that same slow phase. This analysis supports our conclusion that the asymmetry and biphasicity of stigmatellin binding is the product of heterogeneous binding of stigmatellin to the two center P sites in the $bc_1$ complex dimer, and is not a consequence of homogeneous binding to all center P sites resulting in a band shift along with a simultaneous or subsequent loss of $b_L$ absorbance.



**2. Dynafit script used for fitting and simulations of pre-steady state reduction of cytochrome c1 and b (Fig. 1).**

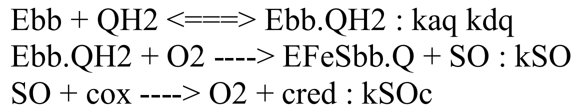
```
[task]

  task  = simulate ; or fit when optimizing parameters marked with "?"
  data  = progress

  ; E = oxidized bc1 complex
  ; EFeSb = Rieske protein and one b heme reduced
  ; Ecb = cyt c1 and one b heme reduced
  ; EFeSbb = Rieske protein and two b hemes reduced
  ; Ecbb = cyt c1 and two b hemes reduced
  ; QH2 = decylubiquinol
  ; Q = decylubiquinone
  ; units in s-1 and microM


[mechanism]

 ; first turnover

  E + QH2 <===>  E.QH2  :  kaq    kdq
  E.QH2 <===> EFeSb.Q    :  k1   k_1
  EFeSb.Q <===> Ecb.Q  :  kFeS     k_FeS
  EFeSb.Q <===> EFeSb + Q  :  kdq2   kaq
  EFeSb <===> Ecb  :  kFeS     k_FeS
  Ecb.Q <===> Ecb + Q  :  kdq2   kaq

  ; second turnover

  Ecb + QH2 <===> Ecb.QH2  :  kaq    kdq
  Ecb.QH2 <===> EFeScbb.Q  :  k1   k_1'
  EFeScbb.Q <===> EFeScbb + Q  :  kdq2   kaq
  EFeSb + QH2 <===> EFeSb.QH2  :  kaq   kdq
  EFeSb.QH2 <===> Ecb.QH2  :  kFeS     k_FeS
```

```
[constants]

   kaq = 0.13 ?
   kdq = 2  ; to yield a Km = 15 microM
   kdq2 = 1.3 ; to yield a Ki = 10 microM
   k1 = 43
   k_1 = 9.1
   k_1' = 39.8; or 9.1 when assuming crossover between cyt b subunits
   kFeS = 70000
   k_FeS = 30000


[responses]

   ; extinction coefficients x2, due to 2 cm pathlength
   ; when measuring cyt c1 (ext. coeff = 23.2 mM-1 cm-1):

   Ecb = 0.046
   Ecb.QH2 = 0.0464
   Ecb.Q = 0.0464
   EFeScbb = 0.0464
   EFeScbb.Q = 0.0464


[progress]

   directory ./Paracoccus/anti/data

   file Pdantc1
   concentration QH2 = 12 , E = 1.1; or 0.55 when half-of-the-sites was assumed


[output]

   directory ./Paracoccus/anti/output


[end]
```

**3. Dynafit script used for fitting and simulations of pre-steady state reduction of exogenous cytochrome (Fig. 2).**

```
[task]

   task  = simulate ; or fit when optimizing parameters marked with "?"
   data  = progress

   ; E = oxidized bc1 complex
   ; EFeSb = Rieske protein and one b heme reduced
   ; Ecb = cyt c1 and one b heme reduced
   ; EFeSbb = Rieske protein and two b hemes reduced
   ; Ecbb = cyt c1 and two b hemes reduced
   ; QH2 = decylubiquinol
   ; Q = decylubiquinone
   ; cox = oxidized cytochrome c
   ; cred = reduced cytochrome c
   ; O2 = oxygen
   ; SO = superoxide
   ; units in s-1 and microM


[mechanism]

   ; first turnover

   E + QH2 <===>  E.QH2  :  kaq    kdq
   E.QH2 <===> EFeSb.Q    :  k1   k_1
   EFeSb.Q <===> EFeSb + Q  :  kdq2   kaq
   EFeSb <===> Ecb  :  kFeS     k_FeS
   Ecb + cox <===> Ecb.cox  :  kac    kdc
   Ecb.cox <===> Eb.cred  :  kc    k_c
   Eb.cred <===> Eb + cred  :  kdc   kac

   ; second turnover

   Eb + QH2 <===> Eb.QH2  :  kaq    kdq
   Eb.QH2 <===> EFeSbb.Q  :  k1   k_1'
   EFeSbb.Q <===> EFeSbb + Q  :  kdq2    kaq
   EFeSbb <===> Ecbb  :  kFeS     k_FeS
   Ecbb + cox <===> Ecbb.cox  :  kac    kdc
   Ecbb.cox <===> Ebb.cred  :  kc    k_c
   Ebb.cred <===> Ebb + cred  :  kdc   kac
```

```
; antimycin insensitive rate
```



```
Ebb + QH2 <===> Ebb.QH2 : kaq kdq
Ebb.QH2 + O2 ----> EFeSbb.Q + SO : kSO
SO + cox ----> O2 + cred : kSOc
```

```
[constants]

   kaq = 3.8 ?
   kdq = 57 ; to yield a Km = 15 microM
   kdq2 = 38 ; to yield a Ki = 10 microM
   kac = 60
   kdc = 600
   k1 = 43
   k_1 = 9.15
   k_1' = 39.8 ; or 9.15 assuming crossover between cyt b subunits
   kFeS = 70000
   k_FeS = 30000
   kc = 10000
   k_c = 10000
   kSO = 0.0079 ?
   kSOc = 1.1


[responses]

   ; extinction coefficients x2, due to 2 cm pathlength
   ; when measuring cyt c (ext. coeff= 21.5 mM-1 cm-1):

   Eb.cred = 0.043
   Ebb.cred = 0.043
   cred = 0.043

[progress]

   directory ./Paracoccus/anti/data

   file Pdantcex
   concentration QH2 = 12 , cox = 7.5, O2 = 400, E = 1.1; or 0.55 for half-of-the-sites

[output]

   directory ./Paracoccus/anticex/output

[end]
```