Fluorescence decay

Mathematical supplement S1: An exponential model of fluorescence decay

Let $P(i)$ be the “prompt” (instrumental response) and $Y(i)$ the “decay” (experimental response) for channel i. The times of measurement are δi, with δ the time interval between channels.
The decay is given by a mixture of exponentials with characteristic times τ_k and scaling parameters C_k ($\mathcal{H}$ is the Heavyside function):

$$G(t) = \mathcal{H}(t) \sum_k C_k e^{-t/\tau_k}$$

Convolving with the Prompt response $P(i)$ gives the model, with $B_k = \delta C_k$ scaling parameters:

$$F(i) = \sum_{j=0}^{\infty} P(j) \mathcal{H}(t) \sum_k B_k e^{-\delta(i-j)/\tau_k}$$

For the optimization procedures, we reparametrize the τ_k to $\theta_k = \log \tau_k$.

Listing S1: Final NLME models for both the inner and outer membranes (in the R statistical language)

```r
library(nlme)

# Shared coefficients between Hacd1-KO and WT
model_A <- nlme(
  Decay ~ ...,
  fixed = theta1 + B1 + theta2 + B2 + theta3 + B3 ~ 1,
  random = list(
    File = pdDiag(B1 + B2 + B3 + theta1 + theta2 + theta3 ~ 1)
  ),
  weights = varConstPower(fixed = c(power = 0.5), const = 1, form = ~ fitted(.))
)

# Only Bs (resolving to mixing proportions) allowed to be different
# in Hacd1-KO and WT populations
model_B1 <- update(
  model_A,
  fixed = list(
    theta1 + theta2 + theta3 ~ 1,
    B1 + B2 + B3 ~ Population
  )
)

# Only thetas (resolving to characteristic times) allowed to be different
model_B2 <- update(
  model_A,
  fixed = list(
    theta1 + theta2 + theta3 ~ Population,
    B1 + B2 + B3 ~ 1
  )
)

# All coefficients allowed to be different
model_C <- update(
  model_A,
  fixed = theta1 + B1 + theta2 + B2 + theta3 + B3 ~ Population
)
```

Table S1: Non-linear mixed-effects models of fluorescence decay for the inner membrane (mitoplasts)

	Dependent variable:			
	Experimental response (event count/ns)			
	A	B1	B2	C
	(1)	(2)	(3)	(4)
θ_1	0.455*** (0.153)	0.505*** (0.153)		
B_1	0.025*** (0.001)		0.025*** (0.001)	
θ_2	−2.852*** (0.242)	−2.631*** (0.257)		
B_2	0.0002* (0.0001)		0.0001* (0.0001)	
θ_3	−0.697*** (0.087)	−0.667*** (0.094)		
B_3	0.016*** (0.001)		0.016*** (0.001)	
B_1 (intercept)[a]		0.029*** (0.002)		0.029*** (0.002)
B_1 *(WT)*[a]		−0.007*** (0.002)		−0.007*** (0.002)
B_2 (intercept)		0.001*** (0.0002)		0.0005*** (0.0002)
B_2 *(WT)*		−0.0004 (0.0003)		−0.0004 (0.0003)
B_3 (intercept)		0.018*** (0.001)		0.018*** (0.001)
B_3 *(WT)*		−0.002 (0.002)		−0.002 (0.002)
θ_1 (intercept)			0.752*** (0.160)	0.846*** (0.172)
θ_1 *(WT)*			−0.647*** (0.226)	−0.708*** (0.243)
θ_2 (intercept)			−2.629*** (0.316)	−2.432*** (0.336)
θ_2 *(WT)*			−0.509 (0.448)	−0.549 (0.476)
θ_3 (intercept)			−0.727*** (0.121)	−0.692*** (0.126)
θ_3 *(WT)*			0.040 (0.171)	0.028 (0.178)
Observations	57,344	57,344	57,344	57,344
Log Likelihood	−136,223.600	−136,133.900	−136,268.900	−136,157.800
Akaike Inf. Crit.	272,475.200	272,301.900	272,571.700	272,355.600
Bayesian Inf. Crit.	272,600.600	272,454.100	272,724.000	272,534.800
Note:				*p<0.1; **p<0.05; ***p<0.01

[a] Whether the experiment concerns the *WT* or *Hacd1-KO* population is encoded in a categorical factor with the following contrast matrix:

$$L = \begin{array}{c} \\ \text{intercept} \\ \text{WT} \end{array} \begin{array}{c} \begin{array}{cc} \text{Hacd1-KO} & \text{WT} \end{array} \\ \begin{pmatrix} 1 & 0 \\ -1 & 1 \end{pmatrix} \end{array}$$

For instance, the mean θ_1 for population *Hacd1-KO* would be the intercept, and the mean θ_1 for population WT would be (intercept) + (WT).

Table S2: Non-linear mixed-effects models of fluorescence decay for the outer membrane (whole mitochondria)

	Dependent variable:			
	Experimental response (event count/ns)			
	A	B1	B2	C
	(1)	(2)	(3)	(4)
θ_1	0.371*** (0.043)	0.370*** (0.042)		
B_1	0.019*** (0.0004)		0.019*** (0.0003)	
θ_2	−1.767*** (0.030)	−1.764*** (0.029)		
B_2	0.001*** (0.0001)		0.001*** (0.0001)	
θ_3	−0.822*** (0.020)	−0.822*** (0.020)		
B_3	0.017*** (0.0003)		0.017*** (0.0003)	
B_1 (intercept)[a]		0.018*** (0.0004)		0.018*** (0.0004)
B_1 *(WT)*[a]		0.002** (0.001)		0.001** (0.001)
B_2 (intercept)		0.001*** (0.0001)		0.001*** (0.0001)
B_2 *(WT)*		−0.0003 (0.0002)		−0.0002 (0.0002)
B_3 (intercept)		0.017*** (0.0005)		0.016*** (0.0005)
B_3 *(WT)*		−0.0001 (0.001)		0.0001 (0.001)
θ_1 (intercept)			0.350*** (0.060)	0.363*** (0.060)
θ_1 *(WT)*			0.033 (0.085)	0.010 (0.084)
θ_2 (intercept)			−1.812*** (0.041)	−1.808*** (0.040)
θ_2 *(WT)*			0.094 (0.058)	0.083 (0.057)
θ_3 (intercept)			−0.877*** (0.025)	−0.869*** (0.025)
θ_3 *(WT)*			0.104*** (0.035)	0.092*** (0.035)
Observations	73,728	73,728	73,728	73,728
Log Likelihood	−162,949.900	−162,940.500	−162,954.600	−162,943.100
Akaike Inf. Crit.	325,927.700	325,915.000	325,943.200	325,926.100
Bayesian Inf. Crit.	326,056.600	326,071.600	326,099.700	326,110.300
Note:				*p<0.1; **p<0.05; ***p<0.01

[a] See note *a* of table S1.