Supporting material

Modeling of Mitochondrial Donut Formation

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Donut formation induced by FCCP treatment

There are few donut mitochondria in MEF cells in normal culture condition. However, most mitochondria turn into donut after FCCP treatment (Fig S1A). The donut mitochondria could be divided into two groups: the tailed one and the no tail one. The percentage of no tail one group increased, while that of the tailed one group decreased during FCCP treatment, indicating the tailed ones transformed into donut without tail (Fig S1B). This is in accordance with the Eq. 12, which shows that the forming of perfect donut releases much more potential energy.

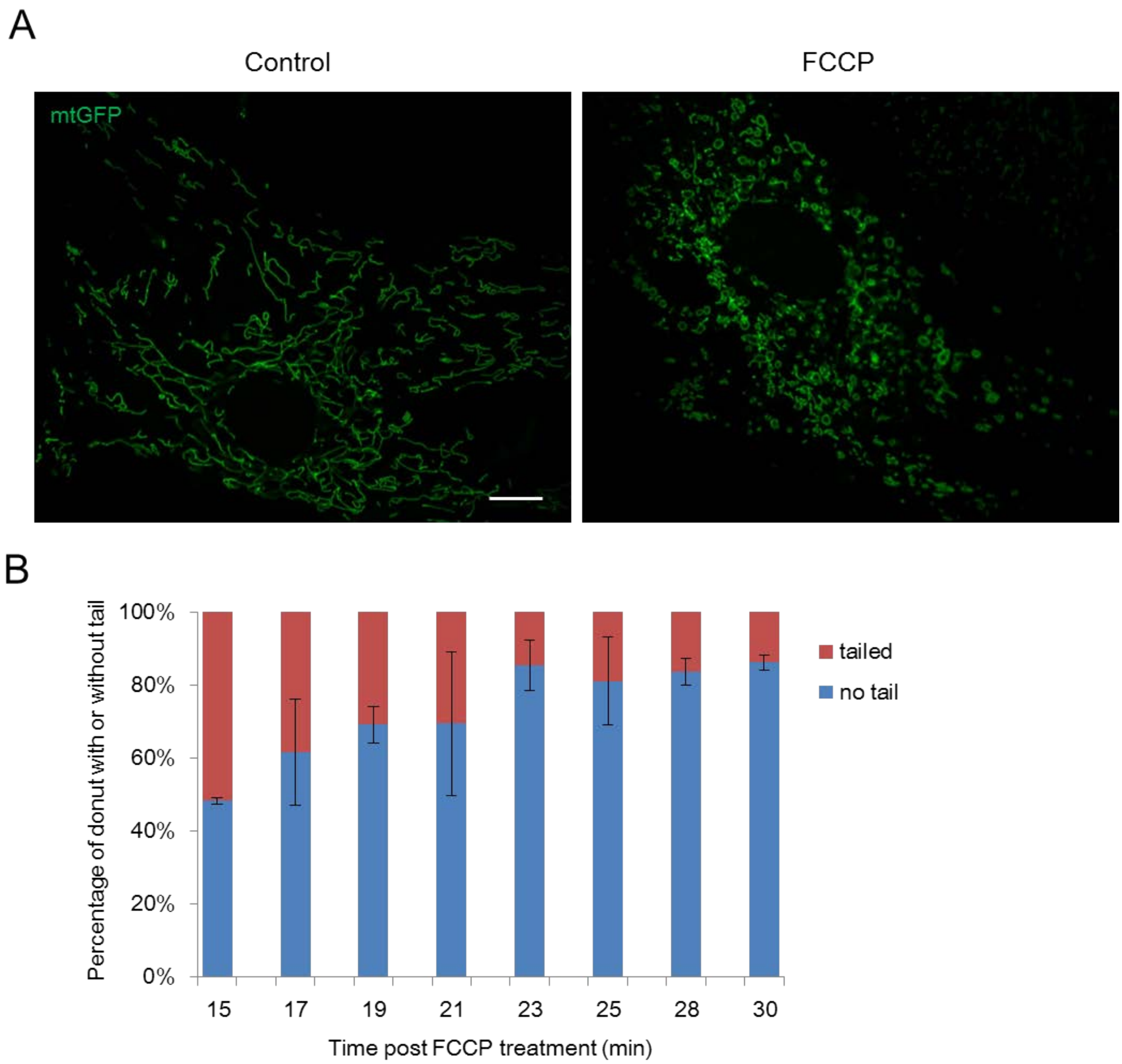

FIGURE S1. (A) Donut formation following FCCP treatment. MEF cells were marked with mtGFP and imaged after treatment with or without 5 μM FCCP for 15 min. Bar = 10 μm. (B) The percentage of tailed donuts and donuts without tail was recorded at different time points after FCCP treatment.

The surface area and volume of mitochondria

The surface area and volume of mitochondrion before swelling are:

$$S_0 = 4\pi r^2 + 2\pi r * L \qquad [14]$$

$$V_0 = \pi r^2 * L + \pi r^3 \qquad [15]$$

In model A, the donut has a tail and the surface area and the volume of mitochondrion are

$$S^a = 2\pi r\, L' + 2\pi r^2 + 4\pi^2 rR \qquad [16]$$

$$V^a = V(\text{tubular}) + V(\text{hemisphere}) + V(\text{donut}) = \pi r^2 L' + 2/3\pi r^3 + 2\pi^2 r^2 R \qquad [17]$$

As $S^a=S_0$, thus $R=\frac{L-L'+r}{2\pi}$, and the volume change is

$$\Delta V^a = V^a - V_0 = \frac{1}{3}\pi r^3 \qquad [18]$$

For model B the surface area and the volume of mitochondrion are:

$$S^b = 4\pi^2 R*r;\ V^b = 2\pi^2 r^2 R \qquad [19]$$

As $S^b=S_0$, thus $R=\frac{L+2r}{2\pi}$ [20]

The volume change in model B is:

$$\Delta V^b = V^b - V_0 = \frac{2}{3}\pi r^3 \qquad [21]$$

The bending energy of donut mitochondria

The bending energy of the closed membrane could be described in the equation as:

$$E_b = \oiint \frac{K_b}{2}(C_1 + C_2 - C_0)^2 dA + \oiint K_g * C_1 * C_2 dA \qquad [2]$$

So, we can get the bending energy of the cap of mitochondria

$$E_{cap} = \int \frac{K_b}{2}\frac{4}{r^2}dA + \int K_g * \frac{1}{r^2}dA = (\frac{2K_b}{r^2} + \frac{K_g}{r^2})4\pi r^2 = 8\pi K_b + 4\pi K_g \qquad [22]$$

The bending energy of tubular mitochondria is

$$E_{tub} = \int \frac{K_b}{2}\frac{1}{r^2}dA_{tub} + E_{cap} = \frac{K_b}{2r^2} * 2\pi rL + 8\pi K_b + 4\pi K_g = \frac{\pi K_b L}{r} + 8\pi K_b + 4\pi K_g \qquad [23]$$

For a donut with a tail in model A, the tail part has no morphological change in donut formation. Therefore, there is no bending energy change, and we ignored this part during calculation.

For a random point on the donut surface, angle φ and angle θ are defined as shown in Fig. 2D. For an infinitesimal surface on a donut, dA can be decomposed to two infinitesimal elements du and dv: du is an infinitesimal arc of the donut circumference and dv is an infinitesimal arc of the mitochondrial circumference.

So that

$$dv = (R \pm r*\sin\varphi)*d\theta;\ \theta \in [0, \omega]$$

$$du = r * d\varphi; \quad \varphi \in [0, 2\pi]$$

$$dA = du * dv = (R \pm r * \sin\varphi) * r * d\theta d\varphi \qquad [24]$$

C_1, C_2 are the curvature of longitude and latitude, respectively.

$$C1 = \frac{1}{\frac{R}{\sin\varphi} \pm r}; \; C2 = \frac{1}{r} \qquad [25]$$

So, the bending energy of a forming donut in radius of R_ω ($R_\omega > \frac{L+2r}{2\pi}$) is

$$E_b = \frac{K_b}{2} \oiint (C_1 + C_2)^2 * dA + K_g \oiint C_1 * C_2 * dA$$

$$= \frac{K_b}{2} \int_0^{\omega} \int_0^{\pi} \left(\frac{1}{\frac{R_\omega}{\sin\varphi} \pm r} + \frac{1}{r}\right)^2 * (R_\omega \pm r * \sin\varphi) * r * d\theta d\varphi$$

$$+ K_g \int_0^{L/R_\omega} \int_0^{\pi} \frac{1}{\frac{R_\omega}{\sin\varphi} \pm r} * \frac{1}{r} * (R_\omega \pm r * \sin\varphi) * r * d\theta d\varphi + E_{cap} \qquad [26]$$

$$= \frac{K_b L}{2R_\omega r} \int_0^{\pi} \left(\frac{R_\omega^{\ 2}}{R_\omega - r\sin\varphi} - \frac{R_\omega r \sin\varphi}{R_\omega + r\sin\varphi}\right) d\varphi + \frac{4K_b L}{R_\omega}$$

$$+ \frac{\pi K_b L}{2r} + \frac{4LK_g}{R_\omega} + 8\pi K_b + 4\pi K_g$$

For a completed donut ($R_\omega = \frac{L+2r}{2\pi}$, $R = \frac{L}{2\pi}$) without caps, the bending energy is

$$E_b = \frac{K_b}{2} \int_0^{2\pi} \int_0^{\pi} \left(\frac{1}{\frac{R}{\sin\varphi} \pm r} + \frac{1}{r}\right)^2 * (R \pm r * \sin\varphi) * r * d\theta d\varphi$$

$$+ K_g \int_0^{2\pi} \int_0^{\pi} \frac{1}{\frac{R_\omega}{\sin\varphi} \pm r} * \frac{1}{r} * (R_\omega \pm r * \sin\varphi) * r * d\theta d\varphi + E_{cap} \qquad [27]$$

$$= \frac{\pi K_b}{r} \int_0^{\pi} \left(\frac{R^2}{R - r\sin\varphi} - \frac{Rr\sin\varphi}{R + r\sin\varphi}\right) d\varphi + \frac{\pi K_b L}{2r} + 8\pi K_b + 8\pi K_g$$

$$= \frac{\pi K_b}{r} \int_0^{\pi} \left(\frac{R^2}{R - r\sin\varphi} - \frac{Rr\sin\varphi}{R + r\sin\varphi}\right) d\varphi + \left(\pi^2 \frac{R}{r} + 8\pi\right) K_b + 8\pi K_g$$

The change of bending energy from tubular to a forming donut is

$$\Delta E_b = \frac{K_b L}{2Rr} \int_0^{\pi} \left(\frac{R_\omega^{\ 2}}{R_\omega - r\sin\varphi} - \frac{R_\omega r \sin\varphi}{R_\omega + r\sin\varphi}\right) d\varphi + \frac{4K_b L}{R_\omega} + \frac{4LK_g}{R_\omega} - \frac{\pi K_b L}{2r} \qquad [28]$$

The change of bending energy between tubular mitochondrion to perfect donut is

$$\Delta E_b = \frac{\pi K_b}{r}\int_0^{\pi}\left(\frac{R^2}{R - r\sin\varphi} - \frac{Rr\sin\varphi}{R + r\sin\varphi}\right)d\varphi$$
$$+(\pi^2\frac{R}{r} + 8\pi)K_b + 8\pi K_g - (\frac{\pi K_b L}{r} + 8\pi K_b + 4\pi K_g) \qquad [29]$$
$$= \frac{\pi K_b}{r}\int_0^{\pi}\left(\frac{R^2}{R - r\sin\varphi} - \frac{Rr\sin\varphi}{R + r\sin\varphi}\right)d\varphi + 4\pi K_g - \frac{\pi^2 R K_b}{r}$$

MATLAB files

1. Figure 3a. ΔE_b/R/L

```
x=2e-1:1e-3:10; %x=R
y=0.2:1e-3:10;      %y=L
r=1e-1;
% x=2e-7:1e-8:2e-6;
% y=2e-6:1e-8:2e-5;
%r=1e-7;
lx=length(x);
ly=length(y);
z=zeros(lx,ly);
for i=1:lx
   for j=1:ly
          if y(j)>6.3*x(i)-0.2
             z(i,j)=nan;
           continue;
         end

z(i,j)=1e18*(1e-19*y(j).*(3.14159*x(i)./(0.1*sqrt(x(i).*x(i)-0.01))+4./x(i)-31.4159)+8e-20*4*y(j)./x(i));
          if isreal(z(i,j))==0
              z(i,j)=nan;
          end
    end
end

% [xx,yy]=meshgrid(x,y);
% z=1e18*{Kb*L*[Pi*R/(r*sqrt(R^2-r^2))+4/R-Pi/r]+Kg*4*L/R};
% z(imag(z)~=0)=nan;
% z(z>1e-10)=nan;
fig=figure(1);
clf;
surface(y,x,z);
view([-125 15]);
```

```
shading interp;
grid on;
ylabel(['R/\mu', 'm']);
xlabel(['L/\mu', 'm']);
zlabel(['Bending Energy \Delta', 'E_b/10^{-18}J']);
set(findall(fig,'-property','FontSize'),'FontSize',14);
print(fig,'fig2.bmp','-dbmp');
```

2. Figure 3C. E_b/R/r

```
x=1e-2:5e-3:5e-1; %x=r
y=2e-1:5e-3:5; %x=R

lx=length(x);
ly=length(y);
z=zeros(lx,ly);
for i=1:lx
        for j=1:ly
                if y(j)<2*x(i)
                      z(i,j)=nan;
                    continue;
                end

z(i,j)=1e16*(3.1416*1e-19*2*3.1416*y(j).*y(j)./(x(i)*sqrt(y(j).*y(j)-x(i).*x(i)))+8*3.1416*1e-19+
8*3.1416*0.8e-19);
                if isreal(z(i,j))==0
                        z(i,j)=nan;
                end
        end
end

% [xx,yy]=meshgrid(x,y);
% Z=1e16*{Kb*[ Kb*2*Pi^2*R^2/(r*sqrt(R^2-r^2))+8*Pi*Kb+8*Pi*Kg
% z(imag(z)~=0)=nan;
% z(z>1e-10)=nan;
fig=figure(1);
clf;
surface(y,x,z);
view([-105 15]);
shading interp;
grid on;
ylabel(['r/\mu', 'm']);
xlabel(['R/\mu', 'm']);
zlabel(['Bending Energy \Delta', 'E_b/10^{-16}J']);
set(findall(fig,'-property','FontSize'),'FontSize',14);
```

```
print(fig,'fig.bmp','-dbmp');
```

3. Figure 3D. E_bdS/R/r

```
x=1e-8:1e-9:4e-7; %x=r
y=1e-7:1e-9:3e-6; %x=R

lx=length(x);
ly=length(y);
z=zeros(lx,ly);
for i=1:lx
    for j=1:ly
      if y(j)<2*x(i)
          z(i,j)=nan;
        continue;
        end

z(i,j)=1e17*(1e-19*0.5.*y(j)./(x(i).*x(i).*sqrt(y(j).*y(j)-x(i).*x(i)))+3.61*1e-19./(3.1416.*y(j).*x(i)
));
        if isreal(z(i,j))==0
            z(i,j)=nan;
        end
    end
end

% [xx,yy]=meshgrid(x,y);
% z=1e17*{Kb*R/[2*r^2* sqrt(R^2-r^2)]+3.6*Kb./(Pi*R*r)
% z(imag(z)~=0)=nan;
% z(z>1e-10)=nan;
fig=figure(1);
clf;
surface(y,x,z);
view([-105 15]);
shading interp;
grid on;
ylabel(['r/\mu', 'm']);
xlabel(['R/\mu', 'm']);
zlabel(['Bending Energy \Delta', 'E_b/10^{-17}J']);
set(findall(fig,'-property','FontSize'),'FontSize',14);
print(fig,'figN1.bmp','-dbmp');
```