SUPPLEMENTARY MATERIAL

The Role of Intestinal Fatty Acid Binding Proteins in Protecting Cells from Fatty Acid Induced Impairment of Mitochondrial Dynamics and Apoptosis

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Table S1: Primers used for sub-cloning

Constructs	Forward primer (5'à 3')	Forward primer (5'à 3')
pEGFP-N1	GCTCGAGATGGCATTTGATGGC	CGGGATCCCGTTCCTTCTTAAAGATC

Table S2: D of IFABP-GFP inside HeLa cells as measured by FCS

Locations	D, μm^2 sec^{-1}
Cytoplasm	8.3 ± 0.1
Peri-nucleuar region (fast component)	3.3 ± 0.3
Peri-nucleuar region (slow component)	0.8 ± 1.1

Fluorescence correlation spectroscopy data analysis:

The autocorrelations G(τ) obtained from IFABP's free cytosolic species in the FCS experiment, were fitted using single component diffusion model (without any triplet contribution), as shown below:

$$G(\tau) = 1 + \frac{1}{N} \cdot \frac{1}{(1+\frac{\tau}{\tau_D})} \cdot \frac{1}{(1+S^2\frac{\tau}{\tau_D})^{1/2}} \quad (1)$$

where, τ_D is the translational diffusion time of the fluorescent species, N is the average number of fluorescent molecules present in the confocal volume, S is the structural parameter that defines the ratio between the radius and the height of the confocal volume.

Considering the cellular compartments to be crowded, our data were fit to an anomalous diffusion model with the power law $<r(t)^2> \sim t^{dw}$ for the mean-square displacement [1, 2],

$$G(\tau) = \frac{1/N}{\left(1+\left(\frac{\tau}{\tau_D}\right)^{2/d_W}\right)\left(1+\frac{1}{S^2}\left(\frac{\tau}{\tau_D}\right)^{2/d_W}\right)^{1/2}} \quad (2)$$

where, d_w is the degree of anomaly. d_w with a value of 2 is considered as the free Brownian diffusion, while with a value greater than 2 shows obstructed diffusion and value less than 2 implies facilitated diffusion.

The bright dynamic clustered species around the nucleus were analyzed using two diffusional fitting model (without any triplet contributions) that could be defined as:

$$G(\tau) = 1 + \frac{1}{N}\sum_i \frac{A_i}{\left(1+\left(\frac{\tau}{\tau D_i}\right)\right)} \times \frac{1}{\left(1+ S^2\left(\frac{\tau}{\tau D_i}\right)\right)^{1/2}} \quad (3)$$

Here, τ_{Di} is the translational diffusion time of the i^{th} fluorescently labeled diffusing species in the cell and A_i is the relative amplitude of the diffusing species. It should be noted that,

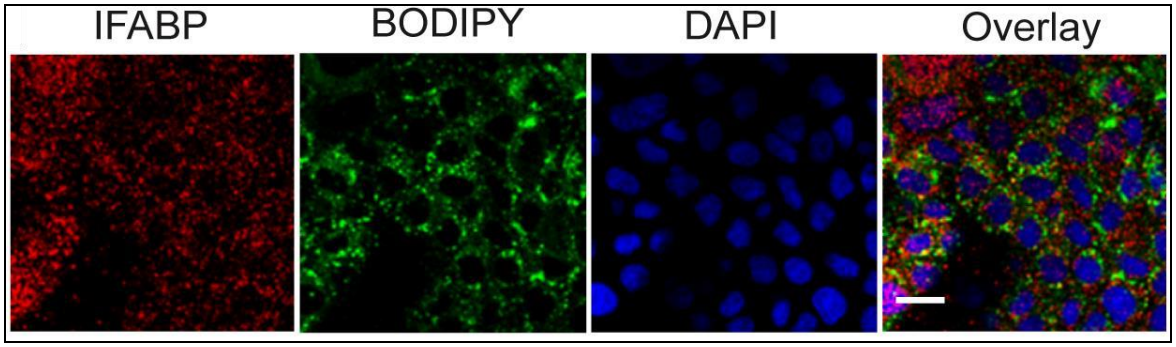
$$\sum_i A_i = 1 \quad (4)$$

We have typically used a two component diffusion model, since our autocorrelation curves could not fit well into a single component one diffusion model. The fast diffusion component corresponds to the unhindered diffusion of the protein clusters, while the slow component corresponds to the diffusion of the protein clusters inside the heterogeneous crowded cellular environment. The two diffusion model was used earlier to explain protein diffusion inside live cells [3].

The relation between the values of τ_D obtained by fitting the correlation function and the diffusion coefficient (D) of a molecule is given by the following equation:

$$\tau_D = \frac{\omega^2}{4D} \quad (5)$$

SUPPLEMENTARY FIGURE



Supplementary Fig. S1: Immunocytochemistry of Caco-2 cells showing expression of basal IFABP (red) along with the uptake and distribution of BODIPY FL C16 (green), scale bar 25 µm.

REFERENCES

1 Schwille P, Korlach J, Webb WW: Fluorescence correlation spectroscopy with singlemolecule sensitivity on cell and model membranes. Cytometry 1999;36:176-182.

2 Banks DS, Fradin C: Anomalous diffusion of proteins due to molecular crowding. Biophys J 2005;89:2960-2971.

3 Schmick M, Vartak N, Papke B, Kovacevic M, Truxius DC, Rossmannek L, Bastiaens PI: KRas localizes to the plasma membrane by spatial cycles of solubilization, trapping and vesicular transport. Cell 2014;157:459-471.