Mitochondria and peroxisomes are ubiquitous subcellular organelles that fulfill essential metabolic functions, rendering them indispensable for human development and health. Both are highly dynamic organelles that can undergo remarkable changes in morphology and number to accomplish cellular needs. While mitochondrial dynamics are also regulated by frequent fusion events, the fusion of mature peroxisomes in mammalian cells remained a matter of debate. In our recent study, we clarified systematically that there is no complete fusion of mature peroxisomes analogous to mitochondria. Moreover, in contrast to key division components such as DLP1, Fis1 or Mff, mitochondrial fusion proteins were not localized to peroxisomes. However, we discovered and characterized novel transient, complex interactions between individual peroxisomes which may contribute to the homogenization of the often heterogeneous peroxisomal compartment, e.g., by distribution of metabolites, signals or other “molecular information” via interperoxisomal contact sites.

Mitochondria and peroxisomes are ubiquitous subcellular organelles that fulfill essential metabolic functions, rendering them indispensable for human development and health. Although mitochondria are often reduced to being “the powerhouse of the cell,” other essential mitochondrial metabolic processes are performed in close co-operation with peroxisomes in mammalian cells (e.g., the β-oxidation of fatty acids, metabolism of reactive oxygen species (ROS), anti-viral signaling). Moreover, mitochondria and peroxisomes are highly dynamic organelles that can undergo drastic changes in morphology and number to accomplish cellular needs (Fig. 1). Mitochondrial dynamics are regulated by a combination of frequent fusion and fission events which serve to homogenize the compartment in regard to lipid, protein and mtDNA composition. Furthermore, proper distribution of mitochondria throughout the cell is mediated by microtubule-dependent movements, which are important for axonal development and neuronal survival. Peroxisomes are similarly regulated by organelle fission and microtubule-dependent distribution. Growth and division of the peroxisomal compartment follow morphologically well-defined steps of membrane deformation/elongation, constriction and final fission (Fig. 1). Interestingly, peroxisomes and mitochondria share key components of their division machinery: DLP1, a large GTPase mediating final membrane scission is recruited to both organelles via its membrane receptors Fis1 and Mff, which as well localize to peroxisomes and mitochondria (Fig. 1). However, the question whether peroxisome fusion also contributes to peroxisome dynamics in mammalian cells remained a matter of debate. Fusion of pre-peroxisomal vesicular structures has however been implicated in the biogenesis of peroxisomes in yeast.

We recently addressed this question by employing an in vivo fusion assay based on the co-cultivation of mammalian CHO cells stably expressing either red or green fluorescent peroxisomal (matrix or membrane) proteins. Subsequently, hybridoma cells were generated and screened for an intermixing of red and green marker proteins (potentially resulting in the appearance of “yellow” peroxisomes due to fluorescent marker overlay) after further incubation at 37°C (to guarantee optimal cellular conditions/peroxisome motility) by a combination of epifluorescence microscopy (in fixed cells) as well as spinning disk confocal microscopy and live cell imaging. Successful shut-down of protein biosynthesis in the course of the experiment was verified by pulse-chase labeling with S-methionine excluding false positives by protein import into peroxisomes. Successful fusion of mitochondria served as a positive control. In line with previous observations in yeast, plant and mammalian cells, a complete peroxisomal fusion mechanism analogous to mitochondria could be excluded. Moreover, evidence was provided by combining overexpression studies, epifluorescence microscopy and immunoblotting that although peroxisomes and mitochondria share
components of their division machinery, mitochondrial fusion proteins (e.g., Mfn1, Mfn2, Opa1) do not contribute to peroxisome dynamics (Fig. 1).

However, in depth-analyses of CHO hybridoma cells by deconvolution microscopy and live cell imaging revealed for the first time that individual red and green peroxisomes (a subpopulation of around 4% at all time points examined) were engaged in several transient, but vivid and long-term contacts, some of which extended over the total observation time (~20 min) (Fig. 2). Detailed mathematical analysis of the duration of those interaction events suggested that the distribution of long-term contacts displays so-called power law behavior,\(^22-27\) as the number of long-term peroxisomal contacts is substantially larger than could be expected from an exponential distribution which would in turn indicate random events. Interestingly, power law distributions in biological processes are indicative of the existence of intricate dynamics originating from diverse, and yet specific mechanisms, suggesting that peroxosome interactions are more complex than previously assumed. Thus, a new dynamic behavior of peroxisomes was characterized in our study.

Still, how may interactions between only a subpopulation of peroxisomes potentially contribute to peroxisome dynamics? Using a simple computational model, we demonstrated that a combination of ATP-driven peroxisome movement (performed by ~15% of the peroxisome population)\(^10,28,29\) and subsequent formation of inter-peroxisomal contacts may potentially contribute to e.g., the homogenization of the often heterogeneous peroxisomal compartment (e.g., by distribution of metabolites, signals or other “molecular information”) in the course of 1–3 h (Fig. 1). Interestingly, our model points to a relationship between the percentage of fast moving peroxisomes, energy consumption and the mixing time of different peroxisome populations within a cell. Hence, our studies indicate for the first time that around 15% of peroxisomes engage in long-range microtubule-dependent movements due to a potential evolutionary optimization process aiming at the homogenization of the peroxisomal compartment at low energy costs. Strikingly, our model is consistent with the experimental observation shortly after hybridoma formation that previously separated red and green peroxisome populations acquire a uniform, interspersed distribution within the cell. Microtubule-driven fast movements of peroxisomes and subsequent transient contacts might thus contribute to equilibrate peroxisome pools throughout the cell.

But in what respect may peroxisomal populations require homogenization and which components might be exchanged? As peroxisomes are very heterogeneous in terms of density, protein composition and import competence in different species, organs and cells as well as within the same cell,\(^30-35\) an exchange of metabolic information might be required. Our initial experiments do not support the exchange of protein markers, but a close apposition of peroxisomes...
might favor an exchange of certain metabolites between hetero-
geneous organelles. Nonetheless, an increase in heterogeneity
among different peroxisome populations by manipulating ROS
and fatty acid levels did not result in an increase of peroxisome
interactions, thus questioning the exchange of metabolites by
inter-peroxisomal contacts. Alternatively, the transient complex
peroxisomal interactions might be part of a “signaling system”
to sense the state and/or distribution of the peroxisomal popula-
tions within the cell. Although the physiological significance of
the inter-peroxisomal contacts requires further investigation,
the identification of those complex interactions adds a novel, interest-
ting twist to peroxisome dynamics.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
This work was supported by grants from CRUP/DAAD
(A-20/08) and FCT (PTDC/SAU-OSM/103647/2008, SFRH/
BD/37647/2007 to N.A.B).

References
1. Bonekamp NA, Volkl A, Fahimi HD, Schrader M. Reactive oxygen species and peroxisomes: strug-
gling for balance. Biofactors 2009; 35:354-65; PMID:19459143; http://dx.doi.org/10.1002/biof.48.
2. Dintz E, Bouland S, Zhang Y, Lee AS, Odendall C, Shum B, et al. Peroxisomes are signaling platforms
for antiviral innate immunity. Cell 2010; 141:668-81; PMID:20451243; http://dx.doi.org/10.1016/j.
cell.2010.04.018.
3. Fransen M, Noordgren M, Wang B, Apanasets O. Role of peroxisomes in ROS/RNS-metabolism: Implications
for human disease. Biochim Biophys Acta 2012; 1822:1363-73; PMID:22178243; http://dx.doi.
org/10.1016/j.bbadis.2011.12.001.
4. Schrader M, Yoon Y. Mitochondria and peroxisomes: are the ‘big brother’ and the ‘little sister’ closer than
assumed? Bioessays 2007; 29:1105-14; PMID:17935214; http://dx.doi.org/10.1002/bies.20059.
5. Bereiter-Hahn J, Jendrach M. Mitochondrial dynamics. Int Rev Cell Mol Biol 2010; 284:1-65; PMID:20878528; http://dx.doi.org/10.1016/S0010-7517(10)38301-3.
6. Westermann B. Mitochondrial fusion and fission in mammalian cells. Mol Biol Cell 2010; 19:2402-12; PMID:20353969; http://dx.doi.org/10.1090/mbc.E07-12-1287.
7. Ishihara N, Nomura M, Jofuku A, Kato H, Suzuki SO, Nakatsuka T. Mff is an essential factor for mito-
chondrial and peroxisomal fission in mammalian cells. Mol Biol Cell 2010; 21:3263-75; PMID:20966229; http://dx.doi.org/10.1091/mbc.E08-10-0970.x.
8. van der Zand A, Gent J, Fulco UL, Havlin S, et al. Levy flight random search-as models of animal foraging. J R Soc Interface 2011; 8:233-43; PMID:21683159; http://dx.doi.org/10.1098/rsif.2010.0706.
9. van der Zand A, Gent J, Beakman I, Tabak HF. Biochemically distinct vesicles from the endoplas-
mic reticulum fuse to form peroxisomes. Cell 2012; 149:397-409; PMID:22508805; http://dx.doi.
org/10.1016/j.cell.2012.01.054.
10. Arimura S, Yamamoto J, Aida GP, Nakazono M, Tsutsui N. Frequent fusion and fission of plant mito-
chondria with unequal nucleoid distribution. Proc Natl Acad Sci U S A 2004; 101:7809-8; PMID:15136720; http://dx.doi.org/10.1073/pnas.0401077101.
11. Huybrechts SJ, Van Veldhoven PP, Bree C, Mannerts GP, Los GV, Franzen M. Peroxisome dynamics in
cultured mammalian cells. Traffic 2009; 10:1722-33; PMID:19794777; http://dx.doi.org/10.1111/j.1600.
12. Motley AM, Hetteham EH. Yeast peroxisomes multiply by growth and division. J Cell Biol 2007; 178:399-
410; PMID:17646399; http://dx.doi.org/10.1083/jcb.200702167.
13. Clauset A, Shalizi CR, Newman MEJ. Power-Law Distributions in Empirical Data. SIAM Rev 2009;
51:661-703; http://dx.doi.org/10.1137/070710111.
14. James A, Plank MJ, Edwards AM. Assessing Levy walks as models of animal foraging. J R Soc Interface
2011; 8:1233-47; PMID:21625699; http://dx.doi.org/10.1098/rsif.2011.0208.
15. Newman MEJ. Power laws, Pareto distributions and Zipf’s law. Contemp Phys 2005; 46:323-51; http://
dx.doi.org/10.1080/0010751042000201882.
16. Rhodes CJ, Anderson RM. Power laws governing epidemics in isolated populations. Nature 1996;
381:600-2; PMID:8637594; http://dx.doi.org/10.1038/381600a0.
17. Sornette D. Critical Phenomena in Natural Sciences, Chaos, Fractals, Self-organization and Disorder:
Concepts and Tools. Springer, Berlin, 2006.
18. Viewanathan GM, Bartoszewska F, Bukdyner SV, Catalan J, Falco UL, Havlin S, et al. Levy flight random search-in biological phenomena. Physica A 2002; 314:208-13; http://dx.doi.org/10.1016/S0378-4371(02)01157-3.
36. Delille HK, Agricola B, Guimaraes SC, Borda H, Lüers GH, Fransen M, et al. Pex11pbeta-mediated growth and division of mammalian peroxisomes follows a maturation pathway. J Cell Sci 2010; 123:2750-62; PMID:20647371; http://dx.doi.org/10.1242/jcs.062109.

37. Bonekamp NA, Sampaio P , de Abreu FV , Lüers GH, Schrader M. Transient complex interactions of mammalian peroxisomes without exchange of matrix or membrane marker proteins. Traffic 2012; 13:960-78.

33. Lüers G, Hashimoto T , Fahimi HD, Völkl A. Biogenesis of peroxisomes: isolation and characterization of two distinct peroxisomal populations from normal and regenerating rat liver. J Cell Biol 1993; 121:1271-80; PMID:8509448; http://dx.doi.org/10.1083/jcb.121.6.1271.

34. Wiese S, Gronemeyer T , Ofman R, Kunze M, Grou CP , Almeida JA, et al. Proteomics characterization of mouse kidney peroxisomes by tandem mass spectrometry and protein correlation profiling. Mol Cell Proteomics 2007; 6:2045-57; PMID:17768142; http://dx.doi.org/10.1074/mcp.M700169-MCP200.

35. Angermüller S, Fahimi HD. Heterogenous staining of D-amino acid oxidase in peroxisomes of rat liver and kidney. A light and electron microscopic study. Histochemistry 1988; 88:277-85; PMID:2896644.