Year of publication 2020

Emilie Mathieu, Anne-Sophie Bernard, H Y Vincent Ching, Andrea Somogyi, Kadda Medjoubi, Jennifer Rodon Fores, Hélène C Bertrand, Amandine Vincent, Sylvain Trépout, Jean-Luc Guerquin-Kern, Andreas Scheitler, Ivana Ivanović-Burmazović, Philippe Seksik, Nicolas Delsuc, Clotilde Policar (2020 Feb 6)

**Anti-inflammatory activity of superoxide dismutase mimics functionalized with cell-penetrating peptides.**


**Summary**

A superoxide dismutase mimic (Mn1) was functionalized with three positively charged-peptides: RRRRRRRR (Mn1-R9), RRWWRRRWRR (Mn1-RW9) or F-r-F-K (Mn1-MPP). Characterization of the physico-chemical properties of the complexes show that they share similar binding affinity for Mn, apparent reduction potential and intrinsic superoxide dismutase activity. However, their accumulation in cells is different (Mn1-R9 < Mn1-MPP < Mn1-RW9 < Mn1), as well as their subcellular distribution. In addition, the three functionalized-complexes display a better anti-inflammatory activity than Mn1 when assayed at 10 μM. This improvement is due to a combination of an anti-inflammatory effect of the peptidyl moiety itself, and of the SOD mimic for Mn1-RW9 and Mn1-MPP. In contrast, the enhanced anti-inflammatory activity of Mn1-R9 is solely due to the SOD mimic.

Year of publication 2019

Tao X., Chen H., Trepout S., Cen J., Ling J., Li M.H. (2019 Oct 15)

**Polymersomes with Aggregation-Induced Emission Based on Amphiphilic Block Copolypeptoids**


**Summary**

Biocompatible polymersomes are prepared from amphiphilic block copolypeptoids with aggregation-induced emission, where the hydrophobic block P(TPE-NAG) is a tetraphenylethylene (TPE)-modified poly(N-allylglycine) and the hydrophilic block is polysarcosine. These nanoparticles are non-cytotoxic and show strong fluorescence emission in aqueous solution.

Sylvain Trépout (2019 Jul 19)

**Tomographic Collection of Block-Based Sparse STEM Images: Practical Implementation and Impact on the Quality of the 3D Reconstructed Volume.**

_Materials (Basel, Switzerland)_ : [DOI: E2281]
Summary

The reduction of the electron dose in electron tomography of biological samples is of high significance to diminish radiation damages. Simulations have shown that sparse data collection can perform efficient electron dose reduction. Frameworks based on compressive-sensing or inpainting algorithms have been proposed to accurately reconstruct missing information in sparse data. The present work proposes a practical implementation to perform tomographic collection of block-based sparse images in scanning transmission electron microscopy. The method has been applied on sections of chemically-fixed and resin-embedded cells. There are 3D reconstructions obtained from various amounts of downsampling, which are compared and eventually the limits of electron dose reduction using this method are explored.

David Partouche, Jérémie Mathurin, Antoine Malabirade, Sergio Marco, Christophe Sandt, Véronique Arluison, Ariane Deniset-Besseau, Sylvain Trépout (2019 Apr 1)

Correlative infrared nanospectroscopy and transmission electron microscopy to investigate nanometric amyloid fibrils: prospects and challenges.


Summary

Propagation of structural information through conformational changes in host-encoded amyloid proteins is at the root of many neurodegenerative disorders. Although important breakthroughs have been made in the field, fundamental issues like the 3D-structures of the fibrils involved in some of those disorders are still to be elucidated. To better characterise those nanometric fibrils, a broad range of techniques is currently available. Nevertheless none of them is able to perform direct chemical characterisation of single protein fibrils. In this work, we propose to investigate the structure of the C-terminal region of a bacterial protein called Hfq as a model amyloidogenic protein, using a correlative approach. The complementary techniques used are transmission electron microscopy and a newly developed infrared nanospectroscopy technique called AFM-IR. We introduce and discuss the strategy that we have implemented as well as the protocol, challenges and difficulties encountered during this study to characterise amyloid assemblies at the nearly single-molecule level. LAY DESCRIPTION: Propagation of structural information through conformational changes in amyloid proteins is at the root of many neurodegenerative disorders. Amyloids are nanostructures originating from the aggregation of multiple copies of peptide or protein monomers that eventually form fibrils. Often described as being the cause for the development of various diseases, amyloid fibrils are of major significance in the public health domain. While important breakthroughs have been made in the field, fundamental issues like the 3D-structures of the fibrils implied in some of those disorders are still to be elucidated. To better characterise these fibrils, a broad range of techniques is currently available for the detection and visualisation of amyloid nanostructures. Nevertheless none of them is able to perform direct chemical characterisation of single protein fibrils. In this work, we propose to investigate the structure of model amyloidogenic fibrils using a correlative approach. The complementary techniques used are transmission electron microscopy and a newly developed infrared nanospectroscopy technique called
AFM-IR that allows chemical characterisation at the nanometric scale. The strategy, protocol, challenges and difficulties encountered in this approach are introduced and discussed herein.

**Summary**

Genetically inheritable pigmentation defects provide a unique opportunity to reveal the function of proteins contributing to melanogenesis. Dyschromatosis universalis hereditaria (DUH) is a rare pigmentary genodermatosis associated with mutations in the ABCB6 gene. Here we use optical and electron microscopy imaging combined with biochemical tools to investigate the localization and function of ABCB6 in pigment cells. We show that ABCB6 localizes to the membrane of early melanosomes and lysosomes of the human melanocytic cell line MNT-1. Depletion of ABCB6 by siRNA impaired PMEL amyloidogenesis in early melanosomes and induced aberrant accumulation of multilamellar aggregates in pigmented melanosomes. PMEL fibril formation and normal maturation of pigmented melanosomes could be restored by the overexpression of wild-type ABCB6 but not by variants containing an inactivating catalytic mutation (K629M) or the G579E DUH mutation. In line with the impairment of PMEL matrix formation in the absence of ABCB6, morphological analysis of the retinal pigment epithelium of ABCB6 knockout mice revealed a significant decrease of melanosome numbers. Our study extends the localization of ABCB6 to melanosomes, suggesting a potential link between the function of ABCB6 and the etiology of DUH to amyloid formation in pigment cells.

**Summary**

The protist Trypanosoma brucei is an emerging model for the study of cilia and flagella. Here, we used scanning transmission electron microscopy (STEM) tomography to describe the structure of the trypanosome transition zone (TZ). At the base of the TZ, nine transition fibres irradiate from the B microtubule of each doublet towards the membrane. The TZ adopts a 9 + 0 structure throughout its length of ~300 nm and its lumen contains an electron-dense structure. The proximal portion of the TZ has an invariant length of 150 nm...
and is characterised by a collarette surrounding the membrane and the presence of electron-dense material between the membrane and the doublets. The distal portion exhibits more length variation (from 55 to 235 nm) and contains typical Y-links. STEM analysis revealed a more complex organisation of the Y-links compared to what was reported by conventional transmission electron microscopy. Observation of the very early phase of flagellum assembly demonstrated that the proximal portion and the collarette are assembled early during construction. The presence of the flagella connector that maintains the tip of the new flagellum to the side of the old was confirmed and additional filamentous structures making contact with the membrane of the flagellar pocket were also detected. The structure and potential functions of the TZ in trypanosomes are discussed, as well as its mode of assembly.

Sylvain Trépout, Anne Marie Wehenkel (2017 Sep 5)
**Bacterial Tubulins: A Eukaryotic-Like Microtubule Cytoskeleton.**
*Trends in microbiology*: 782-784 : [DOI : S0966-842X(17)30194-4](https://doi.org/S0966-842X(17)30194-4)

**Summary**

Ever since their discovery, bacterial tubulins, found in several Prosthecobacter species, have raised curiosity as they are closely related to eukaryotic tubulin. Deng and colleagues now present new evidence for the functional homology of the two cytoskeletal systems where in vitro reconstituted Btub-microtubules display eukaryote-like biochemical and dynamic properties.

Ilse Hurbain, Maryse Romao, Ptissam Bergam, Xavier Heiligenstein, Graça Raposo (2017 May 1)
**Analyzing Lysosome-Related Organelles by Electron Microscopy.**

**Summary**

Intracellular organelles have a particular morphological signature that can only be appreciated by ultrastructural analysis at the electron microscopy level. Optical imaging and associated methodologies allow to explore organelle localization and their dynamics at the cellular level. Deciphering the biogenesis and functions of lysosomes and lysosome-related organelles (LROs) and their dysfunctions requires their visualization and detailed characterization at high resolution by electron microscopy. Here, we provide detailed protocols for studying LROs by transmission electron microscopy. While conventional electron microscopy and its recent improvements is the method of choice to investigate organelle morphology, immunoelectron microscopy allows to localize organelle components and description of their molecular make up qualitatively and quantitatively.

Year of publication 2014

Guillaume van Niel, Ptissam Bergam, Aurelie Di Cicco, Ilse Hurbain, Alessandra Lo Cicero, Florent
Dingli, Roberta Palmulli, Cecile Fort, Marie Claude Potier, Leon J Schurgers, Damarys Loew, Daniel Levy, Graça Raposo (2014 Nov 13)

**Apolipoprotein E Regulates Amyloid Formation within Endosomes of Pigment Cells.**

*Cell reports*: 43-51 : [DOI : 10.1016/j.celrep.2015.08.057](https://doi.org/10.1016/j.celrep.2015.08.057)

**Summary**

Accumulation of toxic amyloid oligomers is a key feature in the pathogenesis of amyloid-related diseases. Formation of mature amyloid fibrils is one defense mechanism to neutralize toxic pre-fibrillar oligomers. This mechanism is notably influenced by apolipoprotein E variants. Cells that produce mature amyloid fibrils to serve physiological functions must exploit specific mechanisms to avoid potential accumulation of toxic species. Pigment cells have tuned their endosomes to maximize the formation of functional amyloid from the protein PMEL. Here, we show that ApoE is associated with intraluminal vesicles (ILV) within endosomes and remain associated with ILVs when they are secreted as exosomes. ApoE functions in the ESCRT-independent sorting mechanism of PMEL onto ILVs and regulates the endosomal formation of PMEL amyloid fibrils in vitro and in vivo. This process secures the physiological formation of amyloid fibrils by exploiting ILVs as amyloid nucleating platforms.