Chapter 13
Analysis of *Physcomitrella* Chloroplasts to Reveal Adaptation Principles Leading to Structural Stability at the Nano-Scale

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Abstract Proteins of the FtsZ (*filamentous temperature sensitive Z*) family establish complex polymeric spatial patterns in plastids of the moss *Physcomitrella patens*. These structures represent a "plastoskeleton" that might contribute to plastid shape and stability. Because additional forces, such as electrostatic interactions, are effective between molecules and atoms at the nano-scale, the applicability of the rules and principles of structural analysis to molecular structures (diameter of a moss plastid: 3–6 μm in the short axis and 4–8 μm in the long axis) is unknown.

The aim of this project is to develop mathematical models of FtsZ network connectivity and dynamics in order to investigate whether molecular structures of the plastoskeleton are evolutionarily optimised to withstand mechanical stresses. To our knowledge, this is the first study focused on the nano-scale characterisation of molecular features/ultrastructures found in plant organelles with the goal of developing new ideas and approaches for bioinspired architecture/building construction.

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13.1 Introduction

Eukaryotic cells are highly compartmentalised and can thus be compared with a building containing many different rooms. Cell compartments are separated from each other by lipid membranes and often show stability of shape, on the one hand, and movement and dynamic form changes, on the other (Osteryoung and Pyke 2014; Usami et al. 2012). Several protein families forming various types of cytoskeletons have been implicated in influencing cellular and organelle shape and dynamics. Currently, the underlying mechanisms of stability on the cellular scale are largely unclear (Ingber 2003a, b; Sultan et al. 2004), based on a lack of the ability to quantify, to monitor and to simulate the stability of the complex and dynamic cell system.

Plastids are endosymbiont-derived plant organelles that are surrounded by two membranes and that exhibit a characteristic lens-shape and a diameter of a few micrometers. Proteins of the FtsZ family have been implicated in chloroplast division and shaping (Martin et al. 2009a). This project utilises the plastid compartment of the model moss *Physcomitrella patens* to monitor stability-conferring proteins by fluorescent protein tags and confocal laser scanning microscopy and applies engineering-based algorithms to implement computational stability models. These models will be used in simulations (1) to derive hypotheses regarding the biological function of FtsZ protein isoforms for validation in the biological model generator (reverse biomimetics) and (2) to analyse and compare derived principles with standard structure networks and shell theory from architecture (in cooperation with the SFB-TRR141 project covered in Chap. 17).

The *Physcomitrella patens* genome (cf. Chap. 15, Zimmer et al. 2013) encodes five FtsZ isoforms in three different subfamilies. The *in vivo* interactions of these isoforms have been elucidated (Gremillon et al. 2007). Here, we generate separate models in order to reveal differences in function and polymer organization.

At the *in silico* level, the robustness of the mathematical models of FtsZ networks are tested (in cooperation with the SFB-TRR141 project covered in Chap. 17) with regard to the expected high robustness of complex biological multifunctional structures.

Analysed principles of adaptive stability at the nano-scale are then placed into an evolutionary (in cooperation with other SFB-TRR141 projects covered in Chap. 15) and architectural context and integrated with results from the micro-scale (in cooperation with other SFB-TRR141 projects covered in Chaps. 8 and 11) and macro-scale (in cooperation with the SFB-TRR141 project covered in Chap. 9). Thus, the scalability of structures will be characterised. Furthermore, the modelling results are constantly integrated into biological hypotheses of organelle evolution and FtsZ isoform functional diversification and are tested experimentally in the moss system.

Thus, the main aims of this project are to reveal functional and organisational properties of the plastoskeleton in moss by investigating the FtsZ polymer networks. The first aim is concerned with deriving from the FtsZ network the general nano-

network principles that can be used to derive conceptual biomimetic ideas. The second aim focuses on revealing the underlying adaptivity of FtsZ network. Fulfilment of both aims will eventually provide the basis for performing the biomimetic transfer of the biological nano-scale principles to architectural structures.

13.2 Research Rationale

The protein FtsZ (*filamentous temperature sensitive Z*) was discovered as a component of the "bacterial cytoskeleton" and provides a scaffold for bacterial cell division. In many eukaryotes, FtsZ genes have been introduced by the bacterial ancestors of endosymbiont-derived organelles, namely the plastids and mitochondria (TerBush et al. 2013). The presence of FtsZ proteins in the model plant *Physcomitrella patens* (Reski 1998a) has been demonstrated and visualised *in vivo* (Kiessling et al. 2000; Strepp et al. 1998). Further research on this protein family has revealed the presence of five FtsZ isoforms, which can be divided into three sub-families (Rensing et al. 2004; Martin et al. 2009b). The basic principles of FtsZ localisation, interaction and function have been established by fusing FtsZ proteins to fluorescent reporter proteins and by the targeted knockout of FtsZ genes by homologous recombination (Gremillon et al. 2007; Kiessling et al. 2004; Martin et al. 2009a, b; Suppanz et al. 2007). Proteins of all three subfamilies are necessary for plastid division, although differences in plastid size and shape between the distinct mutant lines indicate functional diversification (Martin et al. 2009a). Visualisation of FtsZ by fusion to the fluorescent reporter *Green Fluorescent Protein* (GFP) has shown complex and isoform-specific patterns of FtsZ localisation and interactions (Fig. 13.1 and Gremillon et al. 2007). Concerning the molecular structure of bacterial FtsZ polymers, Lu et al. (2000) have suggested a double helix that has a diameter of about 23 nm with two protofilaments.

As the confocal microscopy of fluorescence-labelled FtsZ in moss reveals complex geometrical patterns and as chloroplasts in mutant lines show distinct shape defects (see Fig. 13.2), polymers of FtsZ might provide scaffolds ensuring the stability and structural integrity of plastids (Reski 2002, 2009). Thus, the term

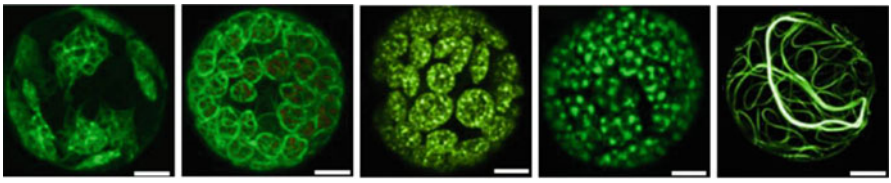

Fig. 13.1 FtsZ:GFP Network in Plastids (PhD thesis L. Gremillon, Universität Freiburg, modified). Localization of GFP-tagged FtsZ proteins in moss protoplasts. Protoplasts were transiently transfected with 35 *s::FtsZ::GFP* and imaged 3 days after transfection. Scale bars = 5 μm

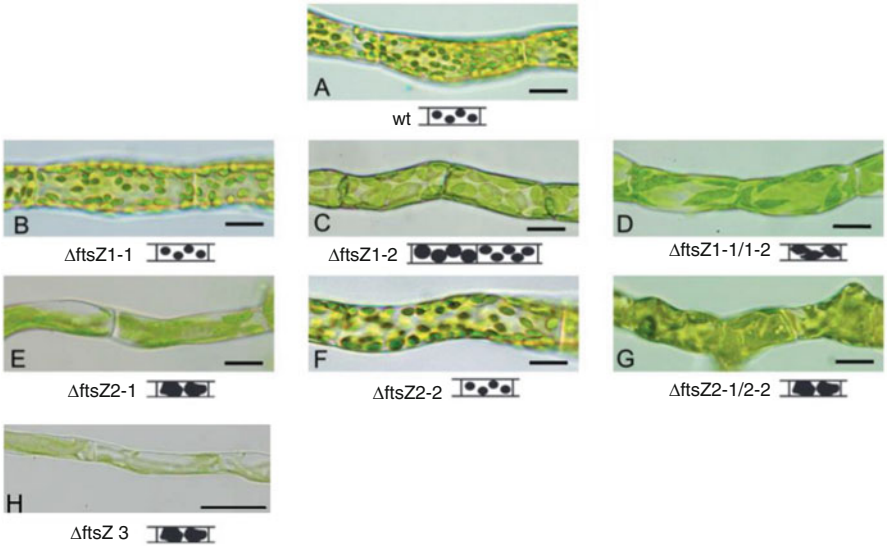

Fig. 13.2 Mutant macrochloroplasts (Martin et al. 2009a, modified). Knock-out of FtsZ genes affects chloroplast morphology. Moss protonema cells with mutated FtsZ isoforms show distinct defects in chloroplast division and shape. (**a**–**g**) scale bar = 25 μm, (**h**) scale bar = 50 μm

"plastoskeleton" has been coined for FtsZ polymers in plastids (Reski 2002). FtsZ networks are reminiscent of geodesic domes in architecture.

Although confocal or electron microscopy provides many new insights into geometrical patterns of various phenomena at the (sub)cellular scale, computational and geometrical models describing detailed functional and structural aspects at this scale remain rare. A few three-dimensional computational models exist in which the modelling focuses mainly on the cytoskeleton, cell morphogenesis or cell aggregates. Mechanical behaviour in these three-dimensional studies is modelled either by means of Tensegrity networks (Ingber 2003a, b; Sultan et al. 2004) or by three-dimensional continuum mechanical models either reduced to truss models (Muñoz et al. 2010) or discretised by the Finite Element method (Or-Tzadikario and Gefen 2011). Many of the three-dimensional structural or computational models focus on investigating a single cell through single image data sets or focus on determining material properties by simulating experimental setups, such as Atomic Force Microscopy (AFM) experiments. However, individual sets of images of particular cells or cell structures are not necessarily capable of revealing new insights into functional aspects. Further, such single-specimen image data sets do not allow the investigation of potential dynamic changes of structural or functional components caused by external influences. Confocal microscopy imaging is capable of investigating dynamic changes in living cells in three dimensions and over time. The challenge of acquiring the appropriate image data and automatically reconstructing computational models from image data

have prevented researchers from using three-dimensional computational models for investigating functional aspects of cell mechanics. The large amount of data and the specific characteristics of the biological specimen require tailored non-standard image registration and segmentation algorithms, e.g. the manual selection of adaptive thresholding algorithms, noise reduction by means of deconvolution algorithms and structure reconstruction in an iterative process including biological characteristics of FtsZ isoforms. This approach of extracting computer models from 3D images of biological environments, which proposes truncating the resulting intricate geometry of the segmented confocal images into a simpler form of structure through a specifically developed process for confocal microscopy data, can be seen, for example, in Röhrle et al. (2011) for the case of segmenting biological image data to provide geometrical input to finite element simulation. Furthermore, challenges based on performing image processing on small-scale data need to be taken into consideration e.g. the lack of resolution in the z-direction (perpendicular to the imaging planes) vs in-plane resolution and the greater impact of the noise on the segmented image. In addition, micromechanical aspects for an extensive finite element analysis of the biological structures, i.e. the FtsZ network, individuates itself from regular treatment of larger scale structures as mentioned in Röhrle et al. (2012).

13.3 The Process of Developing a Well-Grounded Computer Model of Plastoskeleton Leading to Reverse Biomimetics

As mentioned above, the aim of this research project is to analyse nano-structures, i.e. FtsZ isoforms in moss chloroplasts, in order to find new principles of structural integrity and adaptation that can be efficiently transferred to larger scales. To do so, the structural components of a single fluorescence-tagged FtsZ isoform are first analysed by using imaging data of single chloroplasts (see Sect. 13.3.1). The specifically designed image-processing algorithms for confocal microscopy images are further extended to include multiple FtsZ isoforms and dynamic changes of the protein networks (Sects. 13.3.1.4 and 13.3.2). This development of methods is only possible when data sets that contain information of all five isoforms of FtsZ within the different cell types of moss are available. Therefore, the acquisition of stably transformed transgenic moss lines is essential (Sect. 13.3.2). Fundamental principles behind the adaptive stability of plastoskeleton are driven and used to establish a framework that provides the necessary details to test and investigate the biological hypotheses and to derive methodologies for structural stability and adaptation on larger scales (Sect. 13.3.3). Furthermore, the application of image-processing algorithms to the image data set allows us to use computational techniques to investigate FtsZ redundancy and interdependence in a reverse biomimetics perspective (Sect. 13.3.3.4).

Analysed principles of adaptive stability at the nano-scale are then placed into an evolutionary and architectural context and integrated with results from the micro-scale (in collaboration with projects mentioned in Chaps. 8 and 11) and macro-scale (in collaboration with projects mentioned in Chap. 9). Thus, the scalability of structures will be characterised. Furthermore, the modelling results are constantly integrated into biological hypotheses of organelle evolution and FtsZ isoform functional diversification and tested experimentally in the moss system.

13.3.1 From Imaging to Models of FtsZ-Isoforms

13.3.1.1 Monitoring FtsZ Networks by Using Fluorescent Proteins

In molecular biology, the characterisation and mutagenesis of naturally occurring fluorescent proteins from jelly fish and corals has resulted in the emergence of a palette of genetically encoded fluorescent reporters that can be fused to proteins of interest (Shaner et al. 2005). DNA constructs for all five FtsZ isoforms fused to GFP (Gremillon et al. 2007) are available. These constructs are under the control of the constitutive cauliflower mosaic virus (CaMV) 35S promoter and can be used for the transient transfection of moss protoplasts. In addition to these existing constructs, new transient expression vectors have been generated by C-terminally labelling each FtsZ isoform with different fluorescent protein variants (e.g. EGFP, mCitrine, mCerulean and mCherry) and placing these fusion constructs under the control of the PpAct5 promoter (see Fig. 13.3). PpAct5 is a *P. patens*-specific constitutive promoter that has also been used for the production of human proteins in moss bioreactors, as shown by Weise et al. (2006). Transient transfection of moss protoplasts with these constructs and subsequent confocal microscopy analysis

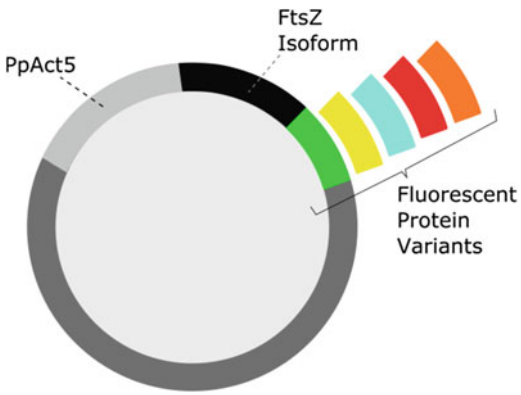

Fig. 13.3 Schematic illustration of the transient expression vectors: the plasmids were genetically engineered to carry the expression cassette that contains a fusion construct and a strong promoter (PpAct5) controlling its expression. The fusion construct consists of the coding sequence of a specific FtsZ isoform and the coding sequence of a specific fluorescent protein variant

enable the simultaneous imaging of two or more FtsZ isoforms within a single cell. This, in turn, permits a better understanding of the hierarchy and interplay of the different isoforms in the plastoskeleton structure.

In an initial phase, confocal microscopy of the networks formed by the various isoforms in single chloroplasts was employed to determine the necessary imaging set-up and data structure to provide a basis for generating computer models.

13.3.1.2 From Imaging to Model Geometry

Application-specific image analysis techniques for single FtsZ isoforms are being developed based on new image data sets that have been generated via the confocal microscopic analysis of moss protoplasts transfected with transient expression constructs (see Fig. 13.3). Within this work, the FtsZ1-2 isoform has been used to investigate and develop the basic mechanism for image analysis. Based on the images generated by confocal microscope, the diameter of the chloroplasts in moss is 3–6 μm in the short axis and 4–8 μm in the long axis. The resolution of the confocal microscopy of the preliminary data set is 0.5 μm between the single image slices (z-direction) and ∼0.1 μm in-plane (xy-plane). Based on the voxel data set, diffusion-based image segmentation techniques are employed to detect the isoform and the three-dimensional arrangements. Digital models based on rod-like structures are developed to analyse the structural arrangements of the investigated FtsZ isoform. In cases in which the out-of-plane resolution is too coarse, i.e. the FtsZ isoform connections are located in the space between two consecutive confocal planes, a novel post-processing algorithm is being developed to detect such interactions and to proceed with the structural reconstruction of the FtsZ isoforms. Since the development of the digital models is closely related to the image preparation and imaging itself, the feedback from the modelling tasks is needed to adjust the imaging protocols to improve the overall image quality.

Extraction of the structural information of FtsZ isoforms from the three-dimensional images starts with the decision as to whether a voxel contains material or not. This requires the application of different thresholding algorithms to the images. First, the parameters of the local adaptive thresholding algorithms are chosen in an interdisciplinary manner, i.e. based on expectations of the geometry of the structure such as continuity of the structure inside the chloroplasts and the localisation of certain isoforms in the chloroplasts. Then, a machine learning mechanism is utilised to decide on the selection of the thresholding process based on the previous results as part of the learning set.

A predictable problem in the segmentation procedure is the eradication of the noise produced in the process of imaging. To do so, deconvolution processes are used that will produce results whereby their quality will strongly depend on the parameters of the deconvolution algorithm. Again, a close interaction between the biologists and mechanical engineers is essential. Deconvolution, however, often leads to images that are smoothed to too great an extent. This causes problems in further steps of structure reconstruction. Therefore, thoughtful precautions should be considered when dealing with deconvolution algorithms.

13.3.1.3 Structural Analysis of the Spatial Arrangement of the Digitised FtsZ Isoform Arrangement

In order to obtain an understanding of the structural features of the plastoskeleton via an analysis of computer models based on three-dimensional images, a representative model (structure) is needed that provides essential parameters of any plastoskeleton that has been subjected to imaging. Building such a computer model is by no means an easy task. However, with a statistical analysis of the structural parameters of the individually extracted models from numerous image samples, one can come as close to building a representative model as possible. These structural parameters include a variety of characteristics, namely the different types of connections and their occurrence, the angles between the different members in a connection and the thickness in the different parts of the structure. In order to be able to extract the mentioned characteristics from each image data set, a skeleton building method is utilised founded on the images that have gone through the previous image processing steps. This skeleton formation method is based on edge detection techniques and a cellular automata algorithm that then helps to reconstruct the missing parts of the structure. The outcome of this technique is a graph consisting of connections, segments and points that are used for the statistical analysis of the mentioned structural features. An example of the initial image processing process and skeleton formation is shown in see Fig. 13.4. On consideration of the results produced by this statistical analysis, visual inspection of the digital models reveals further structural features that are needed to be investigated as part of this research project.

The resulting algorithm leads to image-informed digital models. The developed algorithm can be applied not only to the images of the plastoskeleton, but also to any other three-dimensional fluorescent images of biological structures such as the cytoskeleton.

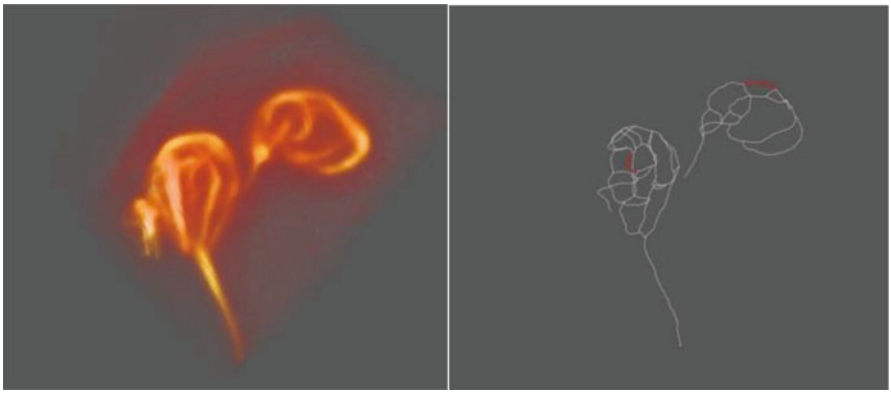

Fig. 13.4 The spatial graph as a result of applying the algorithm for extracting structural information from confocal images of FtsZ isoforms

13.3.1.4 Enhancing Image Analysis Techniques for Digital Model Creation Taking into Account Multiple FtsZ Isoforms

The methodologies and algorithms developed in Sects. 13.3.1.2 and 13.3.1.3 can be further extended to take into account the co-existence of multiple FtsZ isoforms. The image segmentation algorithms based on and reduced to two-colour images (fluorescence of the isoform and the background) are capable of being extended to a multi-colour FtsZ isoform image segmentation algorithm. Furthermore, the structural analysis algorithms need to include the ability to detect the interactions between various isoforms. The performance of a statistical analysis among the data sets between the different isoforms provides the basis for identifying concepts leading to structural stability and adaptation.

13.3.2 Integrating Dynamics: From Dynamics to Adaptivity

13.3.2.1 FtsZ Networks in Stably Transformed Transgenic Moss Lines

In order to reveal the structure and the dynamics of the plastoskeleton in distinct cell types and to visualise several FtsZ isoforms simultaneously, stable moss lines with tagged FtsZ proteins need to be established. The amenability of *Physcomitrella patens* to gene targeting (Strepp et al. 1998; Reski 1998b) can be used to integrate distinct fluorescent protein variants (e.g. EGFP, mCitrine, mCherry and mCerulean) into the coding sequence of the distinct FtsZ isoforms (a process called "knock-in", see Fig. 13.5). Under the control of the endogenous promoter, protein abundance is regulated as in wild-type plants and thus the differences between tissues and the influence of parameters such as the light conditions, growth medium and age become apparent, as evidenced by, for example, Mueller et al. (2014), Schuessele et al. (2016) and Horst et al. (2016). The use of different fluorescent protein variants enables us to perform the simultaneous imaging of the different FtsZ isoforms

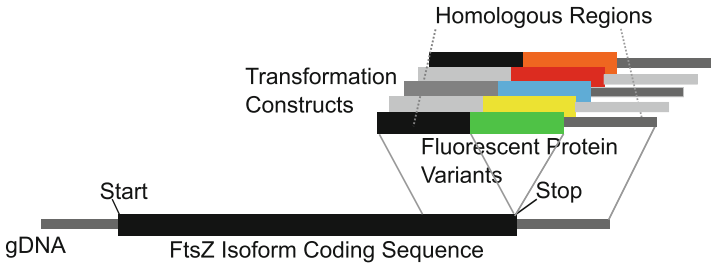

Fig. 13.5 The schematic illustration of the "knock-in" process. The coding sequence of various fluorescent protein variants are integrated in their target loci based on the homology between the flanking homologous regions and the corresponding sequences at the targeted locus. For each different FtsZ isoform, a specific fluorescent protein with a distinct colour was chosen

in a single cell. By stable transformation of the existing FtsZ mutant collection (Martin et al. 2009a) with the newly established knock-in constructs for fluorescent reporters, the effect of the lack of specific FtsZ isoforms can be characterised and integrated into the *in silico* network models. Hypotheses and predictions derived from the models can be tested *in vivo* in an iterative process. Confocal imaging of chloroplasts can be extended to various growth stages and conditions from which a subset of distinct plastoskeleton morphologies in well-defined cell types can be selected for modelling. By the simultaneous imaging of isoforms, problems such as the co-localisation of distinct isoforms and interactions (e.g. by Förster resonance energy transfer FRET, as shown in Gremillon et al. 2007) can be addressed.

13.3.2.2 Statistical Analysis of the Dynamics of the Plastoskeleton

In order to integrate dynamics into the analysis of FtsZ networks, time-lapse confocal imaging monitoring the dynamics of the plastoskeleton in various cell types and under diverse conditions will be assessed. Statistical distributions for specific measures, e.g. the number of connections or the angles at which the isoforms connect, will provide the basis for a statistical analysis of individual components and the way that these evolve over time.

Furthermore, to analyse the dynamic changes of the overall structure and for individual isoforms, a principal component analysis comparable with the statistical shape analysis methods frequently used for the segmentation of biological structures (Heimann and Meinzer 2009) will be employed to identify the most relevant variations within the time-lapse image data set. This information will then be used again in a statistical shape model representing FtsZ-isoform template structures.

The goal here is to use advanced imaging techniques and digital models qualitatively to identify the timing, reasons and manner of plastoskeleton changes. Thus, reverse biomimetics need to be employed to derive testable biological hypotheses concerning the plastoskeleton.

13.3.3 From Models to Principles

The resulting models of FtsZ networks enable us to investigate the robustness and underlying principles of FtsZ isoforms in cooperation with another SFB-TRR141 project (see Chap. 17). In order to use the methodologies developed in the project covered in Chap. 17, robust computational models of the overall structure need to be developed. A robust computational model in this context means that the model can be stably solved for a variety of input and material parameters.

13.3.3.1 Testing of Material Parameters

The establishment of realistic mechanical models requires either the approximation or the experimental determination of material parameters of the experimental system, i.e. the moss chloroplast.

Application of mechanical stress to an isolated chloroplast (Lang et al. 2011) with a micro-scale cantilever coupled to an Atomic Force Microscope yields force-deformation data describing the overall mechanical behaviour of the chloroplast. By replicating the experimental set-up with the help of computational models, one can estimate homogenised material parameters for the overall mechanical models of the chloroplast.

13.3.3.2 Mechanical Models for *In Silico* Testing

Complex biological systems are often more flexible and thus more resistant than simple optimised models suggest. The FtsZ models can be tested *in silico* as to whether they can adapt to and thus withstand mechanical stress and to what extent. For this purpose, the three-dimensional structural models are enhanced to three-dimensional continuum mechanical models. Based on these findings, a Finite Element modelling approach based on chain statistics as developed by Böl and Reese (2006) can be adopted. This modelling approach provides a methodology that takes into account, at the macro-level (the entire chloroplast), the statistical data of the various FtsZ isoforms at the micro-level (single FtsZ isoforms). The structural model obtained via image-processing-identified isoform structures will be utilised to construct the Finite Element mesh. The isoforms themselves will form hereby the element edges. This choice of mesh has the advantage that isoform-specific chain-associated parameters can be included as a reinforcement within the overall structure. Depending on the structural arrangements of the various FtsZ isoforms, the meshing or the modelling needs to be adapted, e.g. by considering volume fractions, as the different isoforms can be present within the same element.

The continuum-mechanical model will be implemented within the open-source software library OpenCMISS (www.opencmiss.org), which has specifically been designed as a mathematical modelling environment enabling the application of Finite Element analysis techniques to a variety of complex bioengineering problems. The research group of Oliver Röhrle is a key contributor to OpenCMISS. The advantage of OpenCMISS over other Finite Element simulation software is its markup-language-based data structures, such as CellML (www.cellml.org) or FieldML. In particular CellML, which is used to describe models in a system-biological and standardised way, will provide the potential for easy integration into the generic evolutionary tools developed in another SFB-TRR141 project (see

Chap. 15). The link between the use of a standardised description of processes at the subcellular level and the tools generated as part of the project covered in Chap. 15 needs to be further explored.

13.3.3.3 Abstracting Molecular Construction Concepts for Complex Structures

Following the extraction of the underlying principles from FtsZ network models, they can be investigated in simulations of structures in cooperation with another SFB-TRR141 project (see Chap. 17). Thus, the biological concept generator might provide the source for alternative construction options. However, the step from molecular structures to man-made structures is substantial. An intermediate step for analysing this potential of knowledge transfer will be the definition of general rules to generate generic but representative isoform network models. The rules are developed based on statistical analysis and shape analysis techniques (Sects. 13.3.1 and 13.3.2). In the same way as digital anatomical atlases are constructed, the principal component shape analysis of the overall chloroplast structure will reveal the geometrical structures (modes) that are the key contributors to their overall structure. To verify that the shape-analysis-identified modes are the key contributors to the mechanical stability of the overall chloroplast, the single modes will be mechanically tested by *in silico* experiments with the Finite Element modelling approach (Sect. 13.3.3.2) and will be compared with results from *in vivo* tests (Sect. 13.3.3.4).

A comparison of the results of single modes with the overall mechanical behaviour allows the separation of the essential modes from the redundant modes in an abstract way. Furthermore, whether the essential modes might directly translate to simplistic man-made structures in civil engineering and architecture can be analysed. If key structural modes can be identified, the remaining modes might be less essential.

13.3.3.4 Reverse Biomimetics: What Can Be Learned from *In Silico* Models

In silico predictions for FtsZ networks will be translated back into experiments in the moss system. The computational models of the distinct FtsZ isoforms allow predictions to be made regarding the behaviour of chloroplasts in the available loss-of-function mutant collection. The combination of the outputs enables us to design experiments that will provide insights into the biological meaning of FtsZ isoform functional diversification. Furthermore, specific perturbations of the plastoskeleton are feasible by synthetic biology approaches, e.g. by the fusion of a light-induced protease to an FtsZ isoform in the moss genome by homologous recombination. Thus, the spatial-temporal control of plastoskeleton perturbation can be achieved and used to verify digital-model-derived biological hypotheses.

Furthermore, results from transient transfection of protoplasts and stable moss lines expressing tagged FtsZ isoforms in specific mutant backgrounds will be used to integrate the functional diversification of FtsZ isoforms into the models. Changes in network architecture caused by the absence of a specific FtsZ isoform will give insights into the interdependence of the five isoforms. Differences between the distinct FtsZ isoforms, especially of the FtsZ1 and FtsZ2 subfamilies, are expected (TerBush et al. 2013), as FtsZ2 subfamily proteins are suggested to provide stability, whereas FtsZ1 subfamily proteins might enhance dynamics.

Moreover, the co-assembly of FtsZ isoforms into networks will be investigated in moss lines with several distinctly tagged FtsZ isoforms.

The key outcome is to obtain mechanical models of the chloroplast that can be analysed by using methodologies developed in another SFB-TRR141 project (see Chap. 17) in order for the computational results to be discussed in a molecular biological, an evolutionary biological and a constructional and design methodological context.

13.4 Outlook

Stability on a cellular scale (i.e. in the nanometer-micrometer range) is influenced by many forces and factors that do not play a role in building structures on the macro-scale. However, the investigation of cellular stability mechanisms by using molecular biology suggests a stability-conferring role of several protein networks. Chloroplasts as cell organelles with a defined lens shape and a reduced set of candidate proteins, namely the FtsZ family, can be used as a simplified cellular system to investigate dynamic stability mechanisms. The combination of engineering-based algorithms with fluorescence imaging will be key to unravelling the underlying principles.

In the future, this project will aim at the translation of any new insights gained from this methodological basic research project at the nano-scale to the building of structures. Hence, the extension of this research will see a shift from a purely methodologically driven approach to a more translational approach.

By analysing methodological principles of scalability, one can establish links between key structural features at the nano-scale and technical translations. Thus, the model principles define the technical limitations and hence provide the scope and potential for built structures. This can be achieved by integrating the findings of this project together with the results of some other projects in the SFB-TRR141 (see Chaps. 15 and 17) by investigating the boundaries of identified principles in relation to the scaling of the experimental system (nano-scale vs. micro- and macro-scale) and three-dimensional printing techniques in order to generate conceptual studies for demonstrators based on the computational models.

In addition, reverse biomimetics experiments can be taken to the next level by the overexpression and purification of FtsZ protein isoforms and *in vitro* studies on physical parameters, network assembly and self-organisation.

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