Project Description – Project Proposals

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Interfacial processes at plant internal surfaces: microbubble dynamics at flexible fibril networks

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Project Description

1 State of the art and preliminary work

Land plants evaporate huge amounts of water into the air resulting in the transfer of about 40% of the global incident precipitation from the soil back to the atmosphere (Schlesinger and Jasechko, 2014). The total water flow is realized by a special transport tissue of plants, the xylem, which consists of a network of dead cells representing the conduits. The water flow within the xylem follows the gradient of the chemical potential caused by the transpirational water loss from the leaves, and is thus not “pumped” but rather “pulled” upwards. Accordingly, the water inside the conduits is under tension, i.e. negative pressure. There is ample evidence for the existence of tensile water within xylem conduits, and the principal mechanism of plant water transport is termed “cohesion-tension theory” (Brown, 2013; Venturas et al. 2017). Land plants, therefore, rely on a transport principle in which water is in a (thermodynamically) metastable state, particularly prone to become dysfunctional due to cavitation and the subsequent formation of gas bubbles (embolism). To avoid embolism and/or to repair dysfunctional conduits, various mechanisms have been shown to exist comprising interactions between gas bubbles, water and flexible conduit wall structures (Konrad and Roth-Nebelsick, 2009; Konrad et al., 2018). We aim at analysing these dynamic interactions at the interface to understand the underlying physical processes crucial to maintain the functionality of this unique metastable transport system that has no technical equivalent so far. The project thus pursues two objectives: 1) To address problems related to the physics of three-phase-interactions (two fluids – gas and water – forming interfaces which are attached to flexible, solid parts of the conduit) and 2) to contribute to the understanding of the physical processes enabling the unique water transport system of plants with consequences beyond biology.

The xylem conduits, termed vessels, show typical diameters of about 20 µm to 250 µm and lengths varying from a few centimeters to some meters. The vessels are interconnected by pores of a few micrometers in diameter, termed pits, which are of vital importance in maintaining the water transport functionality (see Figure 1). Firstly, they represent trans-conduit water pathways, allowing the water to change from one conduit to the next resulting in a three-dimensional transport network. Secondly, they represent valves, by forming a stable three phase interface that fixes air bubbles within the pore (Figure 3) and as a consequence isolates embolized conduits. Pits connecting adjacent vessels show a characteristic structure, resembling two funnels connected at their wide ends and separated by a pit membrane. When a conduit becomes dysfunctional, the embolism results in a pressure difference between the dysfunctional element and its still functioning neighbor conduit, which can amount to 1 MPa and more. Without special control features provided by pits, embolism would spread uncontrolled over the whole xylem posing a life-threatening situation for the tree (and which is a typical failure in artificial conduits intended to conduct water under tension). The ability of pits to trap a certain gas volume inside a conduit by interfacial effects and to support embolism repair is a fascinating property of plants since a transport mechanism able to lift water up to 100 m is maintained by physical processes occurring at the scale of nanometers and micrometers. The functionnality of pits has therefore been in the focus of research for several decades (Venturas et al., 2017) but is still not reasonably understood.

At least two components of the pits interact with gas-water interfaces (Figure 3): the pit membrane and the pit channel. The basic scaffold of the pit membrane (Figure 1) is a flexible cellulosic nanofibril network containing various other components, and whose hydraulic conductance can be adapted by the concentration of ions (Pereira et al., 2018; Schenk et al., 2018). Pectic substances can be present (Plavcová and Hacke, 2011), and for some species there is evidence for a hydrogel-like material covering and/or filling the pit membrane (Pesacreta et al., 2005; Lee et al., 2012). Due to its flexibility, the pit membrane interacts dynamically with microbubbles. Additionally, the curved geometry of pit channel and chamber affects the formation and stability of gas bubbles as was shown by theoretical analyses (Konrad and Roth-Nebelsick, 2003, 2005). Bubble dynamics at pits as derived from these theoretical studies was supported recently by observations via X-ray tomography (Brodersen et al., 2018). The curved pit chamber walls are expected to be flexible, as suggested by their special architecture (there will be – as is the usual case for organisms – species-specific differences), and therefore to interact with gas-water interfaces as well.

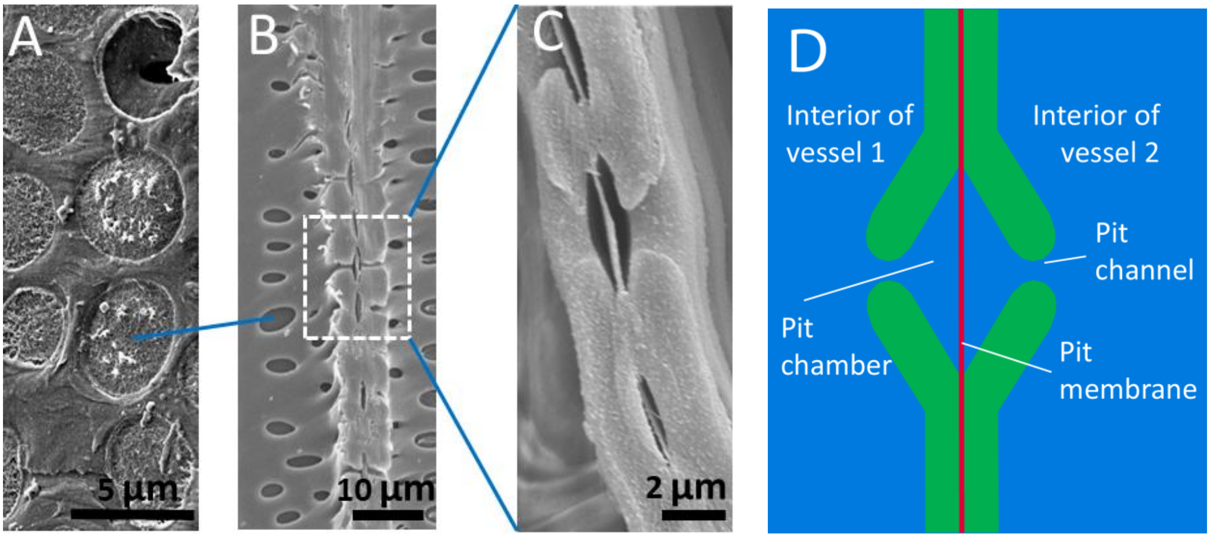

Figure 1. A: Conduits in wooden stems of the shrub *Mallotus japonicus* (cryo-SEM). Top view on pit membranes at the conduits. B: Conduits of laurel (SEM, material dehydrated in absolute ethanol). Longitudinal section through two adjacent conduits. C: Section through pit (detail of B). D: Sketch showing pit structure. The blue color indicates the presence of liquid water.

Until now, theoretical analyses of interactions between bubbles and pit structures neglected the flexibility of some of its parts although the tissue must be able to support pressure differences of more than one Megapascal across the gas-water interface in case of embolism. Including the flexible (visco-)elastic and anisotropic properties of pit wall and pit membrane increases the number of “interaction modes”, forming the basis of the capabilities of this biological system to manipulate interfaces and microbubbles. Considering the high biological relevance of this apparatus, its reliability and versatility, a thorough knowledge of its performance and the underlying physical principles is of high interest also to applied sciences. To unravel the interdependencies between structure and interfaces, mathematical methods previously applied by the team (e.g., differential geometry (Konrad et al., 2009), or visco-elasticity (Konrad et al., 2013)) will be applied and improved, in combination with novel methods of interface and microstructure analytics to clarify structural details of the pit apparatus.

In summary, the aim of the presented project is to evaluate the "interaction modes" of the xylem pit apparatus with gas-water interfaces while acting as microvalve, by 1) putting together and solving an exhaustive set of equations from physics describing the biological situation, 2) clarifying structural details by novel methods of interface and microstructure analytics (e.g. by FIB-SEM and cryoFIB-SEM) and 3) including artificial systems to assess theoretical results and methods of transfer.

1.1 Project-related publications

Konrad W, Apeltauer C, Frauendiener J, Barthlott W, Roth-Nebelsick A. 2009. Applying methods from Differential Geometry to devise stable and persistent air layers attached to objects immersed in water. Journal of Bionic Engineering 6: 350-356.

Konrad W, Katul G, Roth-Nebelsick A, Jensen KH. 2018. Xylem functioning, dysfunction and repair: A physical perspective and implications for phloem transport. Tree physiology, DOI: 10.1093/treephys/tpy097.

Konrad W, Roth-Nebelsick A. 2003. The dynamics of gas bubbles in conduits of vascular plants and implications for embolism repair. J. Theor. Biol. 224: 43-61.

Konrad W, Roth-Nebelsick A. 2005. The significance of pit shape for hydraulic isolation of embolized conduits of vascular plants during novel refilling. Journal of Biological Physics 31: 57-71.

Roth-Nebelsick A, Voigt D, Gorb S. 2009. Cryo-scanning electron microscopy studies of pits in *Pinus wallichiana* and *Mallotus japonicus*. IAWA Journal 31: 257-267.

Helbig, R., Nickerl, J., Neinhuis, C., and Werner, C. (2011) Smart Skin Patterns Protect Springtails. PLoS one, 6(9): e25105. https://doi.org/10.1371/journal.pone.0025105.

Hensel R, Helbig R, Aland S, Braun H-G, Voigt A, Neinhuis C, Werner C. 2013. Wetting resistance at its topographical limit: the benefit of mushroom and Serif T structures. Langmuir 29: 1100-1112.

Joens, M. S., Huynh, C., Kasuboski, J. M., Ferranti, D., Sigal, Y. J., Zeitvogel, F., Obst, M., Burkhardt, C. J., Curran, K. P., Chalasani, S. H., Stern, L. A., Goetze, B., Fitzpatrick, J. A. (2013). Helium Ion Microscopy (HIM) for the imaging of biological samples at sub-nanometer resolution Sci Rep. 2013 Dec 17;3:3514. doi: 10.1038/srep03514.

1.1.1 Articles published by outlets with scientific quality assurance, book publications, and works accepted for publication but not yet published.

Konrad W, Roth-Nebelsick A, Neinhuis C. 2018. Epicuticular Wax Formation and Regeneration—A Remarkable Diffusion Phenomenon for Maintaining Surface Integrity and Functionality in Plant Surfaces. *Diffusive Spreading in Nature, Technology and Society*. Springer, Cham, 71-91

1.1.2 Other publications

Konrad W., Roth-Nebelsick A. 2009. The influence of the wall contact angle on gas bubble behaviour in xylem conduits under tension and possible consequences for embolism. Proceedings of the Sixth Plant Biomechanics Conference. Institut für Angewandte Physik, Vienna.

1.1.3 Patents

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1.1.3.1 Pending

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1.1.3.2 Issued

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2 Objectives and work programme

2.1 Anticipated total duration of the project

We anticipate a duration of 6 years and apply for the first funding period of 36 months:
1.10.2019 – 31.9.2022 (First phase)

2.2 Objectives

The proposed project intends to clarify and understand the interfacial processes acting at flexible pit membranes and other components of the pit apparatus upon contact with gas/water interfaces. The aims will be achieved based on three interrelated work packages:

1. A thorough analysis of structural details of pits by advanced methods (e.g., by FIB combined with cryoSEM and tomography).
2. A comprehensive theoretical analysis of the possible “interaction modes” of the apparatus with interfaces enabling the entire structure to act as a microvalve. This requires equations to describe and analyze the biological situation analytically (resulting in an at least qualitative understanding of the interaction between interfaces and pit membrane or pit fabric, respectively) as well as numerically (leading to quantitatively more accurate results).
3. Additionally, artificial systems will be included to evaluate theoretical results and methods of transfer.

2.3 Work programme incl. proposed research methods

2.3.1 Project part I:
Structural analysis of the pit membrane
State Museum of Natural History Stuttgart (SMNS) and The Natural and Medical Sciences Institute (NMI) at the University of Tübingen/Reutlingen

Although the principal pit structure is known (Jansen et al., 2009; Lee et al., 2012), a number of details still need to be explained, such as the precise arrangement of fibrils and other components relevant for interfacial effects and the adaptability of this compound membrane. For instance, the structure of the fibrillary network changes upon dehydration (Pesacreta et al., 2005) causing problems of analyzing pit membranes since many preparation methods (including conventional TEM or SEM preparation) involve sample drying. Since details in pit membrane structure (and of the pit apparatus as a whole) are crucial for interactions with gas/water interfaces, it is necessary to know its precise anatomy in the native state. Therefore, fully hydrated pit membranes have to be observed (Schenk et al., 2018). We will apply Cryo-SEM, combined with the focused ion beam (FIB) technique to detect the detailed arrangement and nature of the cellulosic network and its hydrogel components, as well as water-gas bubble appearance and impact at the interface. In addition, further suitable state of the art techniques will be explored, also in collaboration with partners from the network of SPP 2171, as explained further below (see also section 2.8).

Cryo-SEM studies on pit membrane structure are rare. The suitability of cryo-techniques for the study of pit membranes was already demonstrated by members of the team (Roth-Nebelsick et al. 2009) and to our knowledge these are so far the only existing cryo-SEM studies on pit membranes. Combining cryo-SEM and FIB, together with sophisticated preparation techniques are essential to provide insight into the native state of the pit apparatus. These methods comprise High Pressure Freezing (HPF) and established methods like cryo transfer, plunge freezing, freeze fracture (also in situ with a dedicated micro manipulator) and local FIB milling. These methods will further allow us to prepare cross sections of membranes and of pits in contact with water films and microbubbles. In addition, microfluidic measurement and imaging techniques will be applied in order to understand the natural system in context with theoretical approximations.

2.3.1.1 Selected plant species

We will concentrate on species for which various results on pit membrane structure and/or composition are already available and which can be easily obtained from Botanical Gardens (Tübingen, Hohenheim, Dresden) (Table 1).

List of species which will be considered in this project.

Plant Species	**Plant Family**	**Data/Results available**	**References**
Acer monspessulanum	Sapindaceae	Chemical composition, structure	Klepsch et al. 2016, Lens et al. 2011
Acer pseudoplatanus	Sapindaceae	Chemical composition, structure	Klepsch et al. 2016, Lens et al. 2011
Laurus nobilis	Lauraceae	External structure	Jansen et al. 2009
Mallotus japonicus[1]	Euphorbiaceae	External structure	Roth-Nebelsick et al. 2009
Salix alba	Salicaceae	External structure	Jansen et al. 2009
Populus nigra	Salicaceae	Chemical composition	Pereira et al. 2018
Liriodendron tulipifera	Magnoliaceae	Chemical composition	Schenk et al. 2018

Table 1: Citations as in the reference list.[1]For another tree from the Euphorbiaceae, *Sapium sebiferum*, the presence of a hydrogel-like substance was demonstrated by Pesecreta (2005) using AFM. Furthermore, data for chemical composition of *S. sebiferum* were provided by Schenk et al. (2018). This species is not readily available. However, first SEM observations indicated a similar appearance for *M. japonicus*.

It is planned to focus on mature plants, and not on seedlings. After cutting, the twigs will be stored in water-tight containers or bags, equipped with humidifying material to keep the internal air moist to prevent drying and then transported immediately to the NMI (this is standard routine for transporting twig material for anatomical and physiological investigations of the xylem).

2.3.1.2 Analysis of pit structure

Preparation

To obtain consistent results, only material from the outermost growth ring (containing the youngest vessels) of twigs will be studied. Care must be taken to exclude immature vessels because their pit membrane structure differs fundamentally from those of mature vessels. Young but mature pit membranes may differ to a certain extent from pit membranes that were already fully functional and “working” for one season. Therefore, aging effects have to be considered as well. Two different approaches to prepare wood will be followed to study pit membranes in the SEM. First, by splitting the sample in longitudinal direction (Figure 2A) to separate adjacent vessels, allowing to look directly onto the pit membrane: outer wall perspective (Figure 2). This preparation is readily made. However, it means forceful separation of two connected walls and might affect the pit membrane. Therefore, a second method will be tested during which the xylem will be cut longitudinally prior to freezing. This cut “opens” the vessels and exposes the openings of the pit channels into the lumen (Figure 2B). Care must be taken to avoid sample dehydration because it is essential to maintain the native state as far as possible. The sample will subsequently be frozen, via plunge freezing and/or high pressure freezing. For cryo-preparation and vacuum cryo-transfer, a Leica system is available. After freezing and freeze-fracturing the pit membranes remain undisturbed but are “hidden” behind the pit channel openings (Figure 2). To access the pit membrane, the covering wall material will be removed via FIB milling resulting in the exposed native membrane. For cryo-FIB/SEM, a crossbeam workstation, LEO 1540 XB cryo, is available at the NMI. The complete structure will then be characterized in detail by repeated removal of ultrathin layers of the pit membrane by FIB and subsequent generation of tomographic images, allowing for a detailed representation of the 3D-structure.

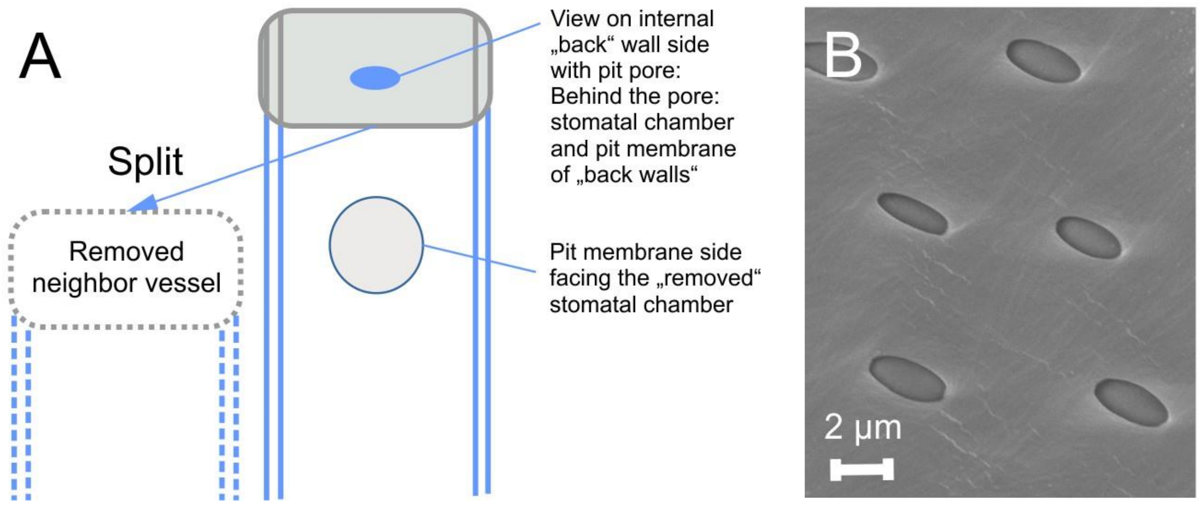

Figure 2: **A**. Orientations of conduits and position of pits. A longitudinal split reveals the outer wall of the remaining conduit and exposes the pit membrane which separates the pit channel connecting the lumen of the remaining conduit to the lumen of the removed neighbor conduit. Dotted lines show outline of removed vessel after splitting, solid lines indicate outline of remaining sample observed in cryo-SEM. **B**. A longitudinal cut through a conduit allows for a top view on to the openings of pits into the lumen. Behind these openings, the pit membrane is visible (material was dehydrated in absolute ethanol).

Cross-sections allow to obtain a detailed picture of the pit structure from another spatial perspective (Figure 1 C). Cross-sectioning of the native material with a knife will be carried out prior to freezing. High precision FIB cuts will then be performed on the frozen pit apparatus, including the membrane. By combining these different approaches, it will be possible to obtain – for the first time – a complete picture of the detailed 3D structure of a native, operating pit membrane.

2.3.1.3 Observation of “embolized conduits”

Preparation

This work package aims at obtaining images from embolized conduits, i.e. vessels that contain air. In embolized conduits, the pit apparatus is expected to be (at least partly) filled with gas bodies and therefore exhibits gas-water interfaces. Two common methods will be applied to realize gas filled pits. In the first approach the sample will be dehydrated to a certain degree simply by air drying (“bench drying”). This mimics the “natural” case of water stress leading to embolism by high negative water potential. In the second approach, the sample will be subjected to positive air pressure. After inducing gas bodies in the conduits according to either method the sample will be frozen. In the first case of high-tension conditions, the freezing process might produce artefacts since gas spaces might form spontaneously upon freezing. For both methods, cuts are necessary after the freezing to expose the internal surfaces. Splitting is the easiest method but may damage structure and arrangement of pit apparatus components and gas bodies. Therefore, additional post-freezing trimming may be necessary. For embolized conduits, similar analyses as described in the preceding section will be conducted by performing FIB sectioning on cross-sections and longitudinal cuts. Particularly FIB-SEM tomography will be applied to obtain a complete image of gas-water interfaces in contact with the pit apparatus.

In complementation to cryo-SEM techniques we will investigate the dynamics of fluid transport as well as the in-situ structure of pits with high temporal and spatial resolution using super-resolution as well as high-speed fluorescence microscopy. These studies will be carried out in collaboration with Prof. Biesalski and PD Dr. Tobias Meckel (Macromolecular Chemistry & Paper Chemistry, Technical University Darmstadt). Additional attempts aim at observing internal interfaces by applying methods and equipment (e.g. Confocal Laser Scanning Microscopy) provided by our cooperation partners within the frame of the SPP 2171, i.e. AG Stannarius (Otto-von-Guericke University) and AG Gambaryan-Roisman (Technical University Darmstadt) to explore the interaction between deflection and surface behavior, contact line dynamics and contact angles,.

Furthermore, topical overlap exists with the proposed project of AG Ionov (University of Bayreuth) focusing on reconfigural surfaces and wetting behavior towards synthetic model systems. Here, the cooperation is envisaged to consider mainly observations and results with respect to interrelationships between material deformability and interfacial interactions (and possibly also the influence of material wettability) which are to be expected of mutual relevance for both project groups. We expect valuable insights from comparing and discussing results from both project groups with respect to crucial influence of structural details on surface interactions, particularly effects of flexibility. Also, mutual overlap with respect to methods and results are anticipated with AG Gorb (University of Kiel) whose proposed project focuses on cellulose nanofiber materials, their structure, mechanics and also interaction with liquids.

It is planned to establish methods during the first 12 months of the project (see Working plan).

2.3.1.4 Procedure

As already mentioned, three-dimensional structural data of the native state of pit membranes are not available so far. At least three pit membranes from different vessels will be studied for each species, because some individual variation can be expected. We intend to study as many of the species shown in Table 1 as possible, depending on difficulties arising during preparation and image analysis. Priority will be given to *Acer* and *Mallotus japonicus*. Criteria for priority are the amount of already available data (for *Acer,* data for both pit membrane structure and chemical composition) and unsolved questions (hydrogel cover of pit membranes of *M. japonicus*).

The structural analysis will be carried out by a doctoral researcher and in tight cooperation between the SMNS and the NMI. Therefore, the PhD researcher will be employed jointly by both institutions (see section 4.1.1.).

Theoretical and structural analyses shall proceed interactively and complementary from the very start of the project. Once structural information has been obtained and implemented into the theoretical approach, concepts for artificial materials with defined switchable and adaptable properties are expected to emerge and will be further explored experimentally and theoretically. In a later stage, first trials of devising such materials, particularly artificial hydrogel matrices, will be carried out in coordination with our cooperation partners in the SPP2171 network. The working plan is listed in section 2.3.3.

2.3.2 Project part II: Theoretical analysis of interfacial effects at the pit membrane
Technical University of Dresden (TUD)

The pit apparatus primarily serves as pathway for water exchange between adjacent vessels and, secondly, provides protection against uncontrolled spread of gas following an embolism event in a vessel. To analyse and understand interfacial effects at the gas-water-cell wall boundary that determine the functionality of the pit apparatus, the events and conditions of an embolism event have to be considered first.

2.3.2.1 Embolism scenario

Prior to an embolism, water flows at moderate velocity (about 0.5 mm/s) from vessel 1 to vessel 2 through the pit membrane through tiny openings in the pit membrane (Figure 3). An embolism produces a sudden and huge pressure difference between an affected and a still functioning vessel causing the water in the embolized vessel to rush with much higher (but hitherto unknown) velocity through the pits. Gas pockets created by the embolism event are swept along with the xylem water, become trapped in the pit chamber, and eventually form the gas/water-interfaces shown in Figure 3. Since these interfaces have to cope with considerable pressure differences (amounting to roughly 0.1 MPa in the pit chamber and about 1 MPa at the membrane), both the pit membrane and the material forming the pit chamber most probably react by elastic or viscoelastic deformation.

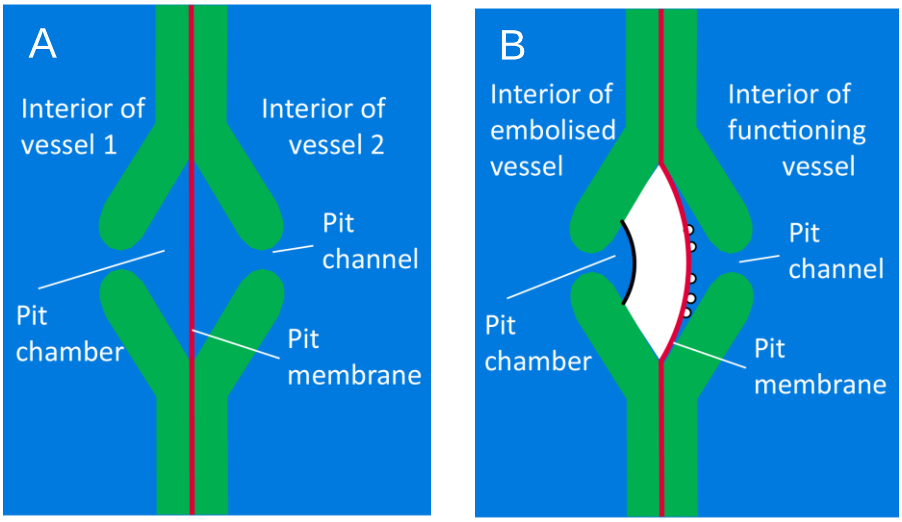

Figure 3: Role of gas/water-interfaces (black) in the repair scenario in case of embolism. **A**. Prior to embolism, water flows from vessel 1 to vessel 2 through tiny openings (of the order of 100 nm) in the pit membrane (red). The pit membrane diameter is in the range of several µm. **B**. After embolism, gas bubbles (white) have formed (i) in the pit chamber and, (ii) in the openings of the pit membrane. They cause hydraulic isolation of the embolised vessel. Due to the pressure difference between embolised ($p > 0$) and still functioning vessel ($p << 0$) the membrane bulges towards the latter. The pressure differences across the two species of gas/water-interfaces differ by one order of magnitude: the small ones forming at the membrane have to sustain 1 MPa or more (corresponding to a curvature radius of 0.15 µm or smaller) the one in the pit chamber has probably to withstand roughly 0.1 MPa (that is, a curvature radius of about 1.5 µm).

Due to the vital importance of a functional water pathway, it is reasonable to assume that evolution led to pit and membrane systems which deform in such a way that gas/water-interfaces with favorable properties, such as the quick establishment of a stable and lasting equilibrium, are formed. Such an evolutionary driven natural system is a suitable model for the study of smart interfacial dynamic wetting effects on flexible, adaptive and switchable substrates.

Once stable interfaces have formed in all pits of an embolized vessel, the vessel is hydraulically isolated and the spreading of the embolism to other vessels is inhibited. If favorable circumstances prevail, a process to repair the dysfunctional vessel may start. At the end of the refilling process the gas/water-interfaces have to break down at the right moment in order to restore vessel conductivity. We assume that the timing of this breakdown is triggered by the interaction between pit material and the pressure within the fluid components on both sides of the pit membrane (Konrad and Roth-Nebelsick, 2003).

2.3.2.2 General approach

Our approach rests on the embolism scenario outlined above. The salient features are:

(A) The valve function of the pits is accomplished by the formation of gas/water-interfaces, which are able to withstand the pressure difference between functional and embolised vessel (Konrad and Roth-Nebelsick, 2003, 2005). [Similarly, within the framework of embolism repair scenarios these interfaces have to break down at the “right” moment in order to restore vessel conductivity.]

(B) Whether a gas/water-interface can form and persist in a stable equilibrium position depends on both the shape and the surface properties of the solid to which the interface is attached.

(C) If the solid is flexible (rather than rigid) the formation of an interface is subject to the elastic (or visco-elastic) properties of this solid as well.

Our working hypothesis is therefore the following:

(A) A strong interaction between pit structures and gas/water-interfaces, with crucial consequences for the formation and behavior of the interfaces exists.

(B) Depending on (i) the pressure difference across the interfaces and (ii) the structural properties, shape and surface properties of pit and membrane, the dynamics between solid pit material and interface may result in various qualitative distinct “interaction modes”.

(C) We expect a wide span of “interaction modes”. Potential end members are stationary and stable interfaces which are “insensitive” against perturbations if the system defining parameters remain within certain ranges, and “sensitive” interfaces which break down very rapidly if parameter values are driven out of these ranges.

2.3.2.3 Procedure

As mentioned above, theoretical and structural analyses shall proceed interactively and complementary from the very start of the project.

Regarding theoretical considerations, our concept is to combine the equations governing (visco-)elastic solids and the equations relevant for existence and dynamic behaviour of gas/liquid-interfaces (equations of (visco-)elasticity, Young-Laplace-equation, Rayleigh-Plesset-equation, Bernoulli-equation, perhaps Navier-Stokes-equation) to form an exhaustive description of our problem.

Because analytic expressions are much easier examined than their numeric counterparts (for instance with respect to existence of solutions and their properties, such as bifurcations) we will - as a first step - simplify (and perhaps oversimplify) these basic equations, for example by exploiting symmetries or linearising parts of the system of equations, and similar instruments from the toolbox of mathematical physics. Playing around in this spirit with the equations will probably (at least hopefully) allow us to identify the permissible maximum degree of simplification which guarantees the minimum complexity of the equations that yields a sufficient number of interaction modes compatible with the observed behavior of the biological system. The interpretation of these results in terms of the biological background may serve as an indicator which properties of pit material and shape are pivotal for the observed biological effects and should be experimentally explored more closely. This first step represents a basic link between structural analysis and theory.

As second step, the hitherto obtained theoretical and experimental results will be merged and used to modify and/or expand the theoretical framework. At this point we might be forced to switch to numeric methods to obtain solutions that are closer to reality than in the first step. Here, we may benefit from the first step: experience suggests that the application of numerical solution methods is less intricate if basic properties of the solutions have already been established by analytic methods. Cooperation with partners from the SPP 2171 will be important. Both the analytical and numerical approaches will be conducted in cooperation with AG Voigt (Institute of Scientific Computing, TUD) where expertise exists both in analytic and numerical methods (for the latter AMDiS, the user-friendly adaptive finite element toolbox for high performance computing, will be used). The AMDiS package will be complemented by the package COMSOL which is an established tool at the AG Stannarius (OvGU) that will cooperate with us also in this respect (see also section 2.8).

2.3.3 Hands-on tutorial

Within the proposed project, a hands-on tutorial will be created consisting of two parts, considering the topics “Surface effects in plants” and “Cryo-SEM and FIB analysis”. All two parts will refer to each other, exploiting the integrative character of the project to produce an “interdisciplinary” tutorial. Nonetheless, each part can be used independently from the others. All project partners will contribute to the tutorials, supported by student assistants. This tutorial can valuably contribute to common knowledge base and overall network activities, such as workshops, training schools, and transdisciplinary exchange within the SPP 2171.

2.4 Data handling

Various data types will be obtained during the proposed project. Besides publishing in international journals, these data will be made available for the public via a generally accessible data base. Due to the interdisciplinary character of the project, two basic types of data are to be expected: 1) anatomical/structural data, together with the underlying methods, and 2) theoretical results on interface effects. The first data type will comprise mainly biological results considering pit structure which should be reproducible according to the description of material and methods. For this data type, the guidelines for biodiversity data are applicable and will be applied as far as possible (http://www.dfg.de/download/pdf/foerderung/antragstellung/forschungsdaten/richtlinien_forschungsdaten_biodiversitaetsforschung.pdf). Accordingly, the following data will be summarized: 1) species name, 2) site and accession number (Botanical Garden information and inventory number of the specimen), 3) description of preparation, 4) images and structural results derived from image processing (also as downloadable files), 4) description of image production and processing, 5) reference to publication (if available). Data 1 - 3 represent text or string data while data 4 will consist of text, numbers and image files. The latter can be shown as thumbnails and may be also available as downloadable image file. There should be no conflict with third parties since data and images will be produced within the project, and possible copyright problems due to publication will be checked beforehand. With respect to the specimen samples, all finally used samples will be stored permanently at the SMNS.

Besides providing access to the biological/structural data, it is also planned to make available the basic theoretical results, as separate entries. In a first step, the basic physics will be summarized, followed by the obtained results referring to the biological system. With respect to this data type we cannot be more specific at the moment because the final processing for the data base depends on the detailed nature of the results. For example, short animations of working principles may be appropriate or downloadable Maple sheets or Matlab sheets.

Additionally, the hands-on tutorials (see preceding section) could be made available together with the project data.

2.3.3 Working plan:

<table>
<tr><th colspan="2">1st Year</th><th colspan="2">2nd Year</th><th colspan="2">3rd Year</th></tr>
<tr><td>Establishing and testing methods and basic protocol</td><td>Pit structure analysis, improvement and adaptation of methods</td><td>Structural analysis of pits containing gas-water interfaces, establishing and testing methods and basic protocol</td><td>Structural analysis of pits containing gas-water interfaces, integrating theoretical results</td><td>Final analysis of images, considering new aspects of theoretical results</td><td rowspan="5">Final discussion and evaluation of combined data, preparation of publications, finishing of hands-on tutorials, creation of the data base

All cooperation partners</td></tr>
<tr><td>Pit structure analysis of first species (Table 1)</td><td>Additional structural analyses according to theoretical results</td><td colspan="2">Continuation of pit structure analysis</td><td>Additional analyses, combining aspects from artificial material results and theory</td></tr>
<tr><td>Setting up of the basic equations (assuming homogenous and isotropic tissue and exploiting symmetries of pit shape)</td><td>Inspection of this system of equations, characterisation and stability analysis of its solutions</td><td>Modification of the basic equations to encompass first experimental results regarding tissue structure</td><td>Solution and analysis of extended equations.

Application of numerical packages (AMDiS, COMSOL) to equations</td><td>Extension of equations to describe realistic tissue, according to experimental results.

Analysis of solutions.</td></tr>
<tr><td colspan="5">Joint discussion of structural and theoretical results</td></tr>
<tr><td>Discussion of structure and theory with external partners, planning of joint approaches</td><td>Testing methods available at external partners</td><td>Analyses performed with external partners</td><td>Analyses performed with external partners</td><td>First concepts and trials of artificial materials with external partners</td></tr>
</table>

SMNS, NMI
TUD
SMNS, NMI, TUD (Botany)
SMNS, NMI, TUD (Botany), OvGU, UB, TUD (Mathematics)

For setting up the data base, equipment and expertise at the SMNS is available. One of the applicants, A. R.-N. was already involved in devising a public scientific data base, “Morphyll” (https://www.re3data.org/repository/r3d1000122, http://www.morphyll.naturkundemuseum-bw.de/index.php, FKZ RO 3250/21), funded within the LIS (Scientific Library Services and Information Systems) program of the German Science Foundation. The data base for the proposed project can be stored and managed in the same manner via a server hosted at the SMNS. At the SMNS, various data bases are operated for longer-term use. For creation of this data base, we apply for limited funding (6 months, 50 %) for a PostDoc who is experienced in setting up scientific data bases, including processing and transfer of data. We suggest that this project part will be conducted during the final 6 months of the first funding phase (see Working programme).

2.5 Other information

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2.6 Descriptions of proposed investigations involving experiments on humans, human materials or animals as well as dual use research of concern

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2.7 Information on scientific and financial involvement of international cooperation partners

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2.8 Information on scientific cooperation within SPP 2171

The integration of the proposed project into the SPP 2171 represents a unique opportunity for joint research and application of complementary methods to the considered problem. In particular, cooperation plans with AG Voigt (Institute of Scientific Computing, TU Dresden), AG Biesalski/Meckel (TU Darmstadt), AG Stannarius (University of Magdeburg), AG Gambaryan-Roisman (TU Darmstadt), and AG Ionov (University of Bayreuth) were devised with respect to both experimental and theoretical methods (see also preceding sections in this chapter describing the working program). Besides their crucial role for the project, the cooperation activities will be valuable for the doctoral researchers with respect to obtaining expertise and skills in different methods and for collecting experience in conducting joint and interdisciplinary research.

Cooperation with AG Voigt (Institute of Scientific Computing, *Technical University Dresden):* As described in the theoretical section above, we will try to explore the structural properties (e.g. stability) of the basic equations from their (perhaps simplified) analytic versions. To investigate more realistic pit-interface layouts it will probably be necessary to employ numerical methods. These tasks will be carried out in cooperation with the AG Voigt providing expertise both in analytic and numerical methods (for the latter AMDiS, the adaptive finite element toolbox for high performance computing, will be used).

Cooperation with AG Biesalski/Meckel (Technical University Darmstadt): In complementation to cryoSEM techniques we will also attempt to investigate the dynamics of fluid transport as well as the in-situ structure of pits with high temporal and spatial resolution using super-resolution as well as high-speed fluorescence microscopy methods provided by and in cooperation with AG Biesalski/Meckel.

Cooperation with AG Gambaryan-Roisman (Technical University Darmstadt): Topical overlap exists with respect to modelling of spreading, imbibition and evaporation of liquids on deformable porous substrates. Aspects of coupling of wetting, transport and deformation of the porous material as considered by AG Gambaryan-Roisman will be relevant. Besides methods for modeling interactions of the various processes, visualization methods for wetting behavior and deformation are also available at AG Gambaryan-Roisman.

Cooperation with AG Stannarius (Otto-von-Guericke University Magdeburg): Methods and equipment available at AG Stannarius may be applied for determination of surface effects to explore interactions between deflection and surface behavior, contact line dynamics and contact angles, for example by Confocal Laser Scanning Microscopy. Furthermore, cooperation may also include theory and modeling, in particular with respect to numerical methods. Methods based on Finite Elements as provided by the COMSOL package used by AG Stannarius may be appropriate.

Cooperation with AG Ionov (University of Bayreuth): Topical overlaps exist with the project of AG Ionov focusing on reconfigural surfaces and wetting behavior towards synthetic model systems. Here, the cooperation is envisaged to consider mainly observations and results with respect to interrelationships between material deformability and interfacial interactions (and possibly also the influence of material wettability) which are to be expected of mutual relevance for both project groups. We anticipate valuable insights from comparing and discussing results from both project groups with respect to crucial influence of structural details on surface interactions, particularly effects of flexibility.

Cooperation with AG Gorb (University of Kiel): Since the project proposed by Gorb and cooperation partners (Lieleg and Zollfrank, Technical University München) focuses on cellulose nanofiber materials, their structure, mechanics and also interaction with liquids, mutual overlap with respect to methods and results are anticipated

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4 Requested modules/funds

4.1 Basic Module

4.1.1 Funding for Staff

SMNS and NMI

One doctoral researcher 3 years 75% E13 Level 2 – E14 Level 1
The PhD researcher will perform the structural analysis. Since this work will be carried out in tight cooperation between the SMNS and the NMI, the PhD researcher will be employed jointly by both institutions.

TUD
One doctoral researcher 3 years 75% E13 Level 2 – E14 Level 1
The PhD researcher will perform the theoretical analysis.

SMNS

Student assistant, 3 years, 6 hrs/week, hourly rate 9,58 €
3 x 46 x 8 x 9,58 7932 Euro
Student assistants will support the structural analysis work, by, for example, collecting and preparing material and by assisting the image analysis. Furthermore, student assistants will support the production of the tutorial and the data handling.

NMI

Student assistant, 3 years, 8 hrs/week, hourly rate 9,58 €
3 x 46 x 10 x 9,58 10 576 Euro
Student assistants will support the technological procedures necessary for image analysis. Furthermore, the student assistant will support the processing of images towards the overall data analyses. Furthermore, the student assistant will support the production of the tutorial and the data handling.

TUD
Student assistant, 3 years, 15 hrs/week, hourly rate 9,58 €
3 x 46 x 15 x 9,58 19 830 Euro
Student assistants will support the theoretical analysis. Furthermore, student assistants will support the production of the tutorial and the data handling.

In total: 38338 Euro

4.1.2 Direct Project Costs

4.1.2.1 Equipment up to Euro 10,000, Software and Consumables

SMNS & NMI

Consumables for material storage and transport:		
Vessels, bags, knifes, blades, tweezers	3 (years) x 1500	4500 Euro
Software SigmaScan Pro		800 Euro
Support of SEM image analysis		

TUD

Data transfer storage	3 (years) x 800	2400 Euro
Software Maple, Einzelplatzlizenz		3000 Euro

In total 10700 Euro

4.1.2.2 Travel Expenses

(SNMS and NMI: 3 persons: PI´s and one doctoral researcher):
Travel expenses for participation in SPP 2171 events

Workshop 1st and 2nd year (2 x persons x days x daily costs, plus travel expenses):

SMNS	(2 x 2 x 4 x 80)+2x2x200	2080 Euro
NMI	(2 x 1 x 4 x 80)+2x1x200	1040 Euro
TUD	(2 x 3 x 4 x 80)+2x3x200	3120 Euro

Advanced school 1st year (year x person x days x daily costs, plus travel expenses):

SMNS	(1 x 1 x 5x80)+1x200	600 Euro
TUD	(1 x 1 x 5x80)+1x200	600 Euro

PhD candidate workshop 2nd year (year x person x days x daily costs, plus travel expenses):

SMNS	(1 x 1 x 4x80)+1x200	520 Euro
TUD	(1 x 1 x 4x80)+1x200	520 Euro

International conference of the SPP 2171 3rd year (year x person x days x daily costs, plus travel expenses):

SMNS	(1 x 2 x 5 x 80)+1x2x200	1200 Euro
NMI	(1 x 1 x 5 x 80)+1x1x200	600 Euro
TUD	(1 x 3 x 5 x 80)+1x3x200	1800 Euro

Meetings of project partners (4 each year in total)
(2 x year x person x costs)

SMNS/NMI	2 x 3 x 2 x 200	2400 Euro
TUD	2 x 3 x 2 x 200	2400 Euro

Participation in international conferences
(year x person x costs)

SMNS/NMI	3 x 1 x 1000	3000 Euro
TUD	3 x 1 x 1000	3000 Euro

For doctoral researchers working at the facilities of external partners
Envisaged: in total 5 x 10 days
Doctoral researcher SMNS and NMI
SMNS/NMI 5 x 10 x 80 4000 Euro

Doctoral researcher TUD
TUD 5 x 10 x 80 4000 Euro
In total **30880 Euro**

4.1.2.3 **Visiting Researchers** (excluding Mercator Fellows)

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4.1.2.4 Expenses for Laboratory Animals

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4.1.2.5 Other Costs

Project specific excess costs will occur with respect to cryo-FIB/SEM, due to

- Instruction and initial supervising the PhD student (first project months) by staff.
- Devising, establishing and improving special novel methods, requiring intensive participation of staff. This applies particularly to work program section 2.3.1.3 (Observation of “embolized conduits”).

The costs will comprise hourly rates for application (not service) of 80 Euro/hr.

We envisage the following costs:

Instruction and supervising during initial project months:
25 hours x 80 Euro 2000 Euro

Support of establishing novel methods:
18 months x 5 hours/month x 80 Euro 7200 Euro

In total **9200 Euro**

4.1.2.6. Project-related publication expenses

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4.1.3 Instrumentation

4.1.3.1 Equipment exceeding Euro 10,000

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4.1.3.2 Major Instrumentation exceeding Euro 50,000

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4.2 Module Temporary Position for Principle Investigator

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4.3 Module Replacement Funding

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4.4 Module Temporary Clinician Substitute

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4.5 Module Mercator Fellows

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4.6 Module Workshop Funding

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4.7 Module Public Relations Funding

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5 Project requirements

5.1 Employment status information

PD Dr. Roth-Nebelsick, Anita, curator at the State Museum of Natural History Stuttgart (permanent contract)
Dr. Burkhardt, Claus J., team leader at NMI Natural and Medical Sciences Institute at the University of Tübingen (permanent contract)
Professor Dr. Christoph Neinhuis, Chair of Botany, Institute of Botany, Technical University of Dresden (permanent contract)
PD Dr. Dr. Wilfried Konrad, Institute of Botany, Technical University of Dresden: Depending on the approval of a project proposal by the Federal Ministry of Economy and Energy, WK will be from November 2018 on a three-year contract with the Institute of Botany, Technical University of Dresden.

5.2 First-time proposal data

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5.3 Composition of the project group

SMNS
Cristina Gascó Martín, technical assistance Electron Microscopy (basic funding)
Dieter Hagmann, EDV (basic funding)
Matthias Krause, laboratory technician (basic funding)
Monika Pfeffer, administration (basic funding)

NMI
Dr. Birgit Schröppel, scientist (basic funding)

TUD
Markus Günter, technical assistant for microscopy, including Cryo SEM
Sylvi Malcher, secretary and administration

5.4 Cooperation with other researchers

5.4.1 Researchers with whom you have agreed to cooperate on this project

Dagmar Voigt, Technical University of Dresden

Cooperation will focus on experimental Cryo-SEM methods and structure analysis: screening of cryo preparation techniques and *exploration of alternative, suitable cryo techniques, freeze fracturing, cryo planning, cryo-SEM in situ wetting experiments, bioimaging including morphometrics, topography, artefact determination, structural identification

5.4.2 Researchers with whom you have collaborated scientifically within the past three years

ARN: PD Dr. Jürgen Burkhardt (University of Bonn, DE); Prof. Gabriel Katul (Duke University, US); Dr. Lutz Kunzmann (Head of Section Palaeobotany, Senckenberg Naturhistorische Sammlungen Dresden); Prof. Ralf Reski (University of Freiburg, DE); Prof. Dana Royer (Wesleyan University, US)

WK: PD Dr. Jürgen Burkhardt (University of Bonn, DE); Prof. Kaare H. Jensen (Department of Physics, Technical University of Denmark, Denmark); Prof. Gabriel Katul (Duke University,US); Prof. Dana Royer (Wesleyan University, US)

CN: Prof. Wilhelm Barthlott (Nees-Institut für Biodiversität der Pflanzen, Universität Bonn); Prof. Ingo, Burgert (ETH Zürich, Schweiz); Prof. Darren Crayn, (Tropical Herbarium, James Cook University, Cairns, Queensland, Australia); Dr. Truong Van Do (Vietnam National Museum of Nature, 18 Hoang Quoc Viet, Hanoi, Vietnam); Prof. Stefan Dötterl, (AG Ökologie, Biodiversität & Evolution der Pflanzen, Universität Salzburg); Prof. Maria Fatima (Maria de Universidade Kimpa Vita, Uíge, Angola); Prof. Claude dePamphilis (Department of Biology, Penn State University, 405B Life Sciences, University Park, PA 16802, USA); Dr. Nick Rowe (Directeur de Recherche CNRS (UMR 5120), Co-director Equipe "Individu", Botanique et Modélisation de l'Architecture des Plantes et des végétations (AMAP) Boulevard de la Lironde, Montpellier) ; Prof. Thomas Speck (Botanischer Garten, Universität Freiburg); Prof. Axel Voigt (Institute of

Scientific Computing, TU Dresden, DE); Prof. Carsten Werner (IPF Dresden, DE),

5.5 Scientific equipment

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5.6 Project-relevant cooperation with commercial enterprises

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5.7 Project-relevant participation in commercial enterprises

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6 Additional information

Current projects of A. R.-N.:
Project “Transport of heat and mass in natural porous materials with graded structure: from functional properties of plant tissues towards customized construction materials” within the TRR 141 “Biological design and integrative structures – Analysis, simulation and implementation in architecture” (Project A01) (https://www.trr141.de/) (German Research Foundation). This project deals with extracellular ice formation in plant tissues and is topically independent of the project described in the present proposal.

Current projects of C. N.:
„Bestäubung und Blütenduft bei Aristolochia-Täuschpflanzen“ (D-A-CH Lead-Agency) (WA 2461/9-1), together with Stefan Wanke (TU Dresden) (German Research Foundation. This project deals with pollination and flower scent and is topically independent of the project described in the present proposal.