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Mechanisms governing subcompartmentalization of biological membranes

Julien Gronnier², Anthony Legrand^{1,3}, Antoine Loquet³,
Birgit Habenstein³, Véronique Germain¹ and
Sébastien Mongrand¹

Membranes show a tremendous variety of lipids and proteins operating biochemistry, transport and signalling. The dynamics and the organization of membrane constituents are regulated in space and time to execute precise functions. Our understanding of the molecular mechanisms that shape and govern membrane subcompartmentalization and inter-organellar contact sites still remains limited. Here, we review some reported mechanisms implicated in regulating plant membrane domains including those of plasma membrane, plastids, mitochondria and endoplasmic reticulum. Finally, we discuss several state-of-the-art methods that allow nowadays researchers to decipher the architecture of these structures at the molecular and atomic level.

Addresses

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Introduction

Spatiotemporal organization of the cellular biomolecules is critical to coordinate the numerous activities simultaneously carried out by cells. Biological membranes delimit cells and organelles and constitute specialized subunits that are constantly reshaped to adapt to ever-changing environmental conditions and to operate cell functions effectively (Special issue on cell biology edited by Ref. [1]). Cell membranes are composed of a specific

set of biomolecules defining their identity. For example, phosphoinositide lipids and small GTPases proteins are major contributors to endosome identity [2,3]. A tremendous body of evidence shows that the motion and the organization of membrane constituents are dynamically regulated on the level of the membrane to form functional domains and this conversely throughout the tree of life [4^{*},5,6]. Thus, it appears that membrane subcompartmentalization into domains is universal and may represent an essential characteristic. Taking into account the knowledge acquired in various model organisms and model systems over the past decades, membrane domains can be defined as membrane regions in which the local composition, lateral organization, and/or dynamics differ in some way from the average membrane properties [7–9]. Such local specificity is dictated by preferential intermolecular interactions, including intra-membrane interactions (i.e lipid–protein, lipid–lipid and protein–protein) and associations with structures peripheral to the membrane for example cortical cytoskeleton and the cell wall in the case of plasma membrane. This also leads to the formation of inter-membrane interaction through Membrane Contact Sites (MCS), important functional platforms for the exchange of lipids and signalling proteins [10,11], see [Figure 1](#). Yet, membranes being constituted of several thousands of molecules surrounded by variable and complex environments, a tremendous mechanistic complexity remains to be uncovered. Here, we review some described mechanisms regulating membrane architecture in plants and discuss recent technological advancements allowing researchers to study membrane organization with molecular and atomic resolution.

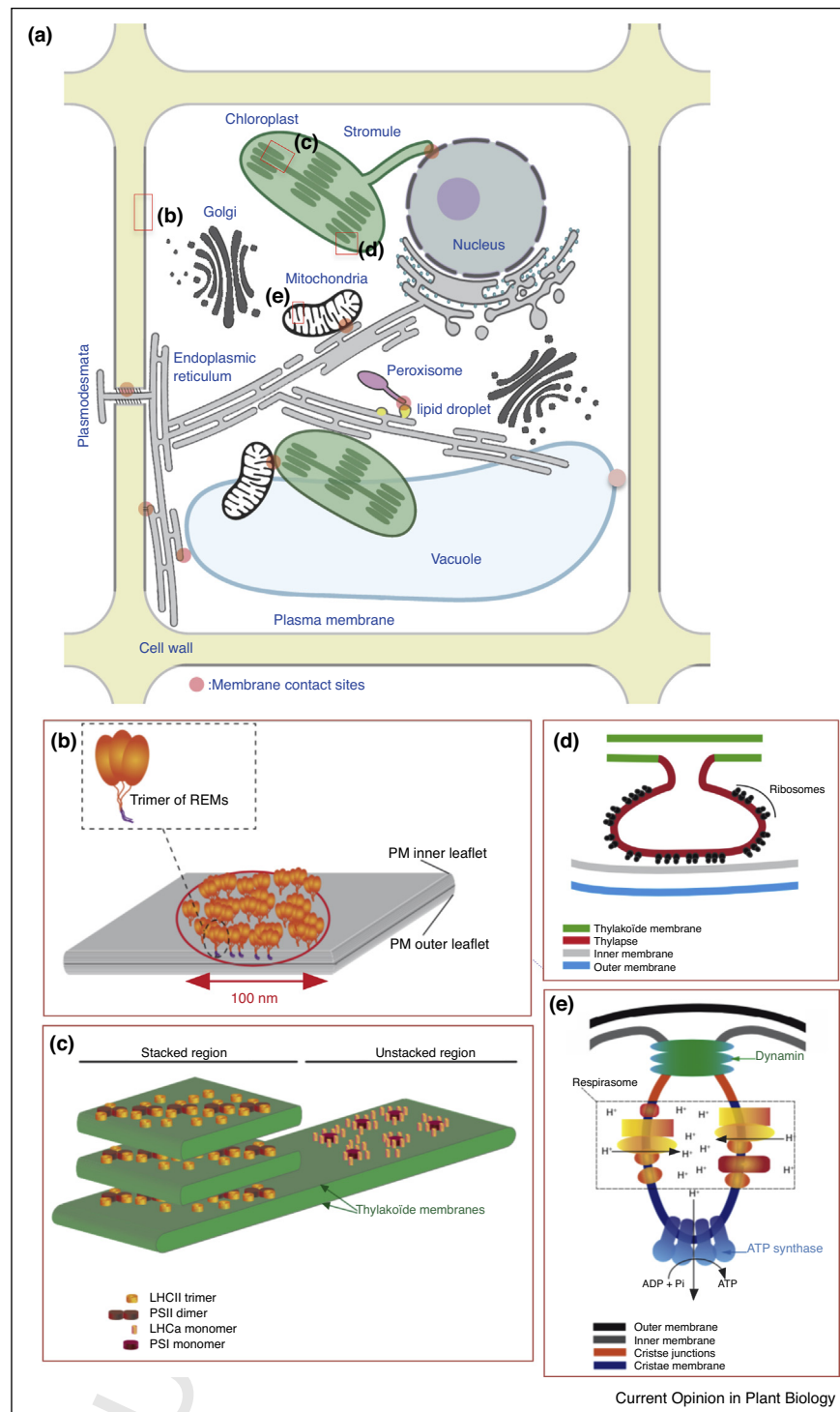
Examples of subcompartmentalization of plant membranes

Plasma membrane domains

The plasma membrane (PM) is the outermost boundary of the cell, acting as a communication headquarter integrating signal from the environment to the cell interior and *vice-versa*. The PM is asymmetric, as exemplified by the enrichment of sphingolipids in the outer leaflet and phospholipids in the inner leaflet [12,13]. The PM associates with the cortical cytoskeleton network and the cell wall creating a continuum at the cell surface [14]. PM establishes MCS with organelles, notably with ER at the level of PD, see [Figures 1a](#) and [2](#). The lipid and protein composition of the domains formed at these MCS is very

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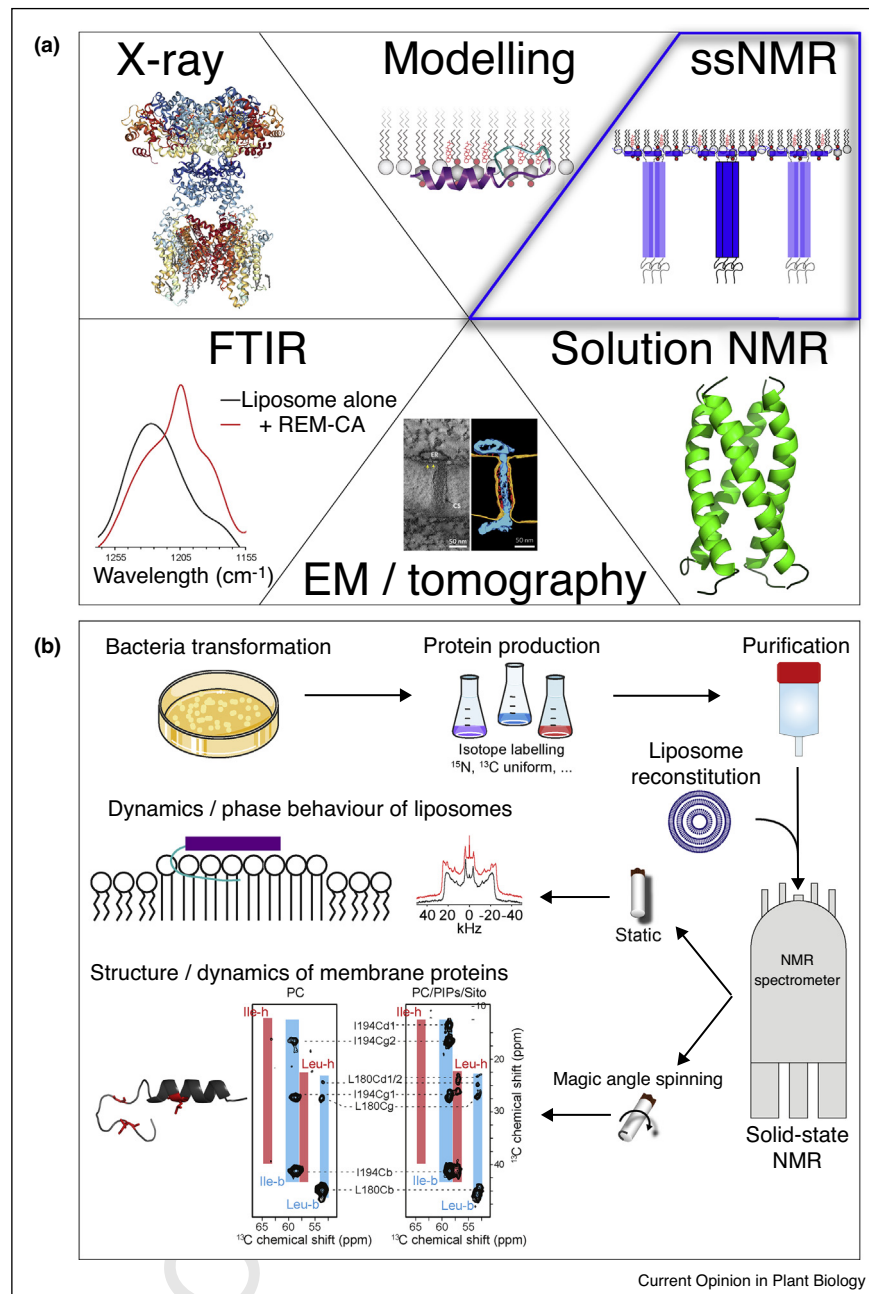
Figure 1



Examples of subcompartmentalization of membranes and membrane contact sites (MCS) in different organelles of plant cells.

(a) Scheme of a plant cell showing the membrane contact sites (MCS) between organelles shown by a red dot. Few examples of subcompartmentalization of biological membranes are emphasized in the plasma membrane **(b)**, thylakoids **(c)**, chloroplast envelope **(d)**, mitochondria cristae **(e)**.

Figure 2



Biophysical techniques to study membrane domains.

(a) Examples of major biophysical tools to analyse membrane domains and membrane-associated proteins, subsequently deciphering the molecular mechanisms at play in nanodomain organization. X-ray: shaker family voltage-dependent potassium channel Kv represented in the cartoon, lipids in stick (PDB: 2R9R, [111]). Modelling: C-terminal anchor of StREM1.3 interacting with membranes enriched in phosphoinositol-4-phosphate and sitosterol. ssNMR: model of StREM1.3 nanodomains. FTIR: insertion of C-terminal anchor of StREM1.3 in nanodomain-like membranes [16**]. EM/tomography: observation of ER-PM membrane contact sites at plasmodesmata [89**]. Solution NMR: membrane-embedded domain of the Influenza B BM2 integral protein (PDB: 2KIX, [112]). **(b)** ssNMR workflow to study membrane domain-associated proteins. Bacterial expression cells (e.g. *E. coli* BL21-DE3) are transformed with a high expression level plasmid coding for the protein of interest. Protein production is achieved in minimal culture media supplemented with isotope labelled metabolites depending on the desired isotopic labelling scheme of the protein (e.g. ^{13}C -glucose, 1,3- ^{13}C -glycerol, 2- ^{13}C -glycerol . . .). Proteins are purified and reconstituted into liposomes of a chosen lipid composition. ssNMR allows obtaining two types of structural data: magic angle spinning (MAS) ssNMR is used to analyze the structure and dynamics of the membrane protein [16**], and ^2H (unpublished typical data) and ^{31}P (not shown) ssNMR to decipher the dynamics and phase behavior of the membranes of interest comprising deuterated lipids.

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specific [11]. Organization of the PM can be rationalized into two types: microdomains and nanodomains. Microdomains are site-specific enrichment of membrane compounds at the cellular level usually referred to as polar domains that control localized cell activities. Nanodomains represent submicrometric heterogeneity of the PM whose visualization often requires the use of high or superresolution microscopy techniques [15,9]. Nanodomains have been proposed to act as dedicated platforms regulating cell signalling notably [4**,16**,9,17*,15]. Mechanisms regulating the organization of PM domains being recently reviewed [9,15,13,18–22], we present here only three case studies to illustrate the molecular mechanisms at play in the organization and dynamics of PM domains.

REMORINs are plant-specific proteins regulating notably immunity [23–26], symbiosis [27,28,29*] and development [30] possibly by modulating nanodomain-associated complexes [16**,31,32,17*,29*]. REMORINs predominantly associate with the PM [33,23,31,34,35]. In addition, isoforms from group 1 and group 6 REMORINs have been shown to be associated with plasmodesmata (PD) in Rice and in *Solanaceae* [23,36,30,31]. Electron microscopy immunolocalization, stimulated emission depletion microscopy (STED) and photoactivated localization microscopy (PALM) studies showed group 1 REMORINs are organized into nanodomains of about 70–90 nm in diameter that are sensitive to sterol composition [23,37,16**] and cytoskeleton integrity [38]. Molecular mechanisms at the basis of REMORIN domain organization are being discovered: REMORINs are targeted from the cytosol to the cytosolic leaflet of the PM *via* a short unconventional sequence at the extremity of the C-terminus of the protein, called REM-CA (REMORIN C-terminal Anchor) [39,34,40], see Figures 1b and 2; REM-CA undergoes conformational changes upon binding of conserved positively charged residues to phosphoinositides and provides to REMORINs biochemical properties indistinguishable from integral proteins [23,39,16**]; REM-CA-sterol-phosphoinositide interactions are required for Group 1 REMORINs supra-molecular organization into functional domains involved in plant response to the *Potato Virus X* (PVX). Numerous REMORINs present cysteine residues that can be S-acylated [41,34,42,43]. While S-acylation of *Arabidopsis* REMORINs seems to regulate PM affinity but not primarily nanodomain organization [34], the substitution of an S-acylated cysteine of *Nicotiana benthamiana* REM alters nanodomain organization [43], suggesting functional divergence of REMORIN S-acylation. Oligomerization of group 1 REMORINs into homotrimers is required for PM localization [39,44], suggesting that REMORINs' self-assembly constitute an early step of PM targeting. Furthermore, REMORIN organization seems regulated by intermolecular protein association. Indeed, in *Medicago*, FLOT4 scaffolds SYMBIOTIC REM1 to recruit the Nod factor co-receptor LYSINE MOTIF KINASE 3 (LYK3) to specific nanodomains

controlling root hair infection by *Sinorhizobium meliloti* and the establishment of symbiosis [29*]. Interaction of AtREM1.3 with AtHIR1 in *Arabidopsis* suggests that association of SPFH (Stomatin, Prohibitin, Flotillin, HflK/C) proteins with REMORINs may represent a conserved core module shaping PM organization [45]. Finally, phosphorylation of group 1 REMORINs upon infection of *N. benthamiana* by the PVX modulates REM1.3 organization and function [31], probably through the modulation of protein–protein interactions. Thus the genesis and regulation of REMORIN nanodomains appear to rely on several molecular mechanisms such as post-translational modifications, and protein–lipid and protein–protein interactions.

Rho of Plants (ROPs) are the plant-specific subfamily of Rho/Rac small GTP binding proteins, regulating numerous cellular processes such as signalling, trafficking and cytoskeleton dynamics [46,47]. Reversible switch from a GDP-bound state to a GTP-bound state mediated by ROP-GEFs and ROP-GAPs regulates ROPs activity [48]. Polarization of the growth machinery to a predefined root hair initiation domain (RHID) pledges root hair formation in trichoblast cells. ROP2, 4 and 6, are recruited to the RHID before any detectable cell bulging and serve as a landmark for the recruitment of downstream effectors [49,50*]. Strikingly, guanine nucleotide exchange factor 3 (GEF3) defines the RHID by guiding ROPs polarization *via* direct protein–protein binding [50*]. At the bulging stage, phosphatidylinositol-4-phosphate 5-kinase 3 (PIP5K3), the AGCVIII kinase D6 PROTEIN KINASE (D6PK) and sterol composition modulate ROPs association to the RHID [51,52]. Here, co-regulation of ROP, phosphoinositides and phosphoinositide kinases has been proposed to form a self-organizing system amplifying ROP recruitment and activation [3]. In addition, ROPs associate with the PM *via* post-translational lipid modifications and direct interaction with membrane lipids mediated by the carboxy-terminal tail [53,47,4**]. For example, ROP6 interacts with phosphatidylserine (PS) via its polybasic tail, a process likely at the basis of nanodomain organization. Recently, using live superresolution microscopy, Platre *et al.* elegantly showed that variation in PS level during root development stabilized ROP6 into nanodomains to regulate auxin signaling [4**]. In metaxylem vessel cells, ROP-GEF4 locally activate ROP11 to recruit MICROTUBULE DEPLETION DOMAIN 1 scaffold protein which in turn recruits microtubule-depolymerizing kinesin-13A enabling the formation of pits in secondary cell walls [54,55]. IQD13 associates with cortical microtubules (cMTs) and the PM to laterally restrict the localization of ROP GTPase domains, establishing a lateral fence for ROP GTPase [56]. In contrary, CORTICAL MICROTUBULE DISORDERING1-induced disorganization of cortical microtubules impairs the boundaries of PM domains of active ROP11 GTPase [57].

Cellulose microfibrils are synthesized by the PM-embedded cellulose synthase (CESA) complexes (CSCs)

196 which are composed of 18–36 cellulose synthase subunits
197 [58]. Cortical microtubules recruit CESA-containing vesicles and guide the trajectory of CSCs at the PM [59–61]. In
198 addition, S-acylation of CESAs influences its immediate
199 membrane environment and conditions their location to
200 the PM [62]. CSCs are tethered to cortical microtubules via
201 two integral components, CELLULOSE SYNTHASE
202 INTERACTING 1 [63–65] which determines the trajec-
203 tory of CSCs along the cMTs [59] and COMPANION OF
204 CELLULOSE SYNTHASE 1 (CC1), which sustains cel-
205 lulose synthesis by promoting the formation of a stress-
206 tolerant microtubule array during salt stress [66].
207

208 These examples emphasized that regulation of plasma
209 membrane subcompartmentalization is regulated as part
210 of developmental program, is modulated to respond to
211 environmental clues, relies on the cooperation of multiple
212 factors and is fundamental for function.

213 Chloroplastic membrane domains

214 Chloroplasts are organelles composed of a double mem-
215 brane envelope and thylakoids found in plant cells and
216 algae that conduct photosynthesis. Little is known about
217 how the photosynthetic membrane machinery is arranged
218 in time and space. Microscopy and biophysical shreds of
219 evidence showed the coexistence of domains where lipids
220 are organized in lamellar or hexagonal phases. For exam-
221 ple, hexagonal phases have been described in etioplasts of
222 prolamellar bodies or during the transfer of lipids between
223 the envelope and thylakoids, such hexagonal phase
224 domains may be of importance for localizing metabolic
225 activities, for example the violaxanthine-epoxidase in
226 thylakoid domains [67].

227 Biochemical, 3D reconstruction, *in vivo* spectroscopy and
228 immunolocalization data, reveal that thylakoids display a
229 heterogeneous subcompartmentalization of photosyn-
230 thetic complexes in domains which redistribute during
231 state transitions in *Chlamydomonas* [68] and diatoms [69],
232 see Figure 1c. These domains are interconnected, ensur-
233 ing fast equilibration of electron carriers for efficient and
234 optimal photosynthesis. Underlying molecular events at
235 the basis of thylakoid subcompartmentalization remain
236 unclear. Thylakoids possess a special fatty acid namely
237 trans- Δ 3-hexadecenoic acid (trans-16:1) esterified in
238 phosphatidylglycerol (PG) which may play a role in
239 cementing thylakoids during granum formation and con-
240 trol of light reactions of photosynthesis [70]. Recent 3D
241 cryo-electron tomography showed the thylakoid network
242 of cyanobacteria is organized in domains and forms a
243 synapse-like MCS decorated by ribosomes (but not by
244 phycobilisomes) in tight association with the PM of
245 cyanobacteria. This MCS was named the ‘thylapse’, for
246 ‘thyl(akoid syn)apse’, and likely serves for compart-
247 mentalization of the different functions of the thylakoids that
248 is photosynthesis or protein synthesis [71**], see
249 Figure 1d. Because PM of cyanobacteria represents the

250 inner membrane of eukaryotic plastids, thylapses most
251 likely also exist in higher plants.

252 Chloroplast envelope establishes numerous MCS with other
253 organelles [10]. For example, plastid and mitochondrion
254 envelopes establish membrane connection during phos-
255 phate deprivation. The molecular content of this MCS
256 has been recently identified by biochemical and proteomic
257 approaches and showed a big complex of hundred proteins
258 enriched in specific lipids. AtMic60, a conserved protein of
259 the mitochondria inner membrane, plays a crucial role in the
260 lipid transport process by regulating the proximity between
261 mitochondrial membranes via its interaction with the outer
262 membrane protein Tom40 and by destabilizing membranes,
263 likely to promote lipid desorption [72**]. Plastids can also
264 undergo drastic changes in shape under stress, through
265 specialized protrusive membrane domains called stromules
266 (stroma-filled tubules, see Figure 1a) which link plastid
267 envelope with other organelles such as ER, Golgi and
268 nucleus [73,74]. The molecular mechanisms governing stro-
269 mule formation are not established, but the involvement of
270 cytoskeleton motors has been proposed [75]. Similarly,
271 peroxules, peroxisomal protrusions tethering chloroplasts
272 or mitochondria through specialized membrane microdo-
273 mains have been evidenced [76,77]. Peroxules also for
274 example link with lipid droplets, see section ‘Endoplasmic
275 reticulum domains’. These studies reveal the importance of
276 physical connections through plant membrane domains for
277 establishing complex metabolic pathways.

278 Mitochondrial membrane domains

279 Mitochondria are double-membrane-bound organelles.
280 The outer mitochondrial membrane encloses the entire
281 organelle and can be in contact with other organelles for
282 example during phosphate starvation, see above [10]. The
283 inner membrane separates the mitochondrial matrix from
284 the intermembrane space. The structure of the inner
285 mitochondrial membrane is extensively folded. These
286 invaginations are separated from the inner membrane
287 by dynamin proteins to form three domains namely,
288 the inner boundary membrane, the cristae junctions
289 and the cristae membranes [78], see Figure 1e. The latter
290 contains enzymes of the mitochondrial respiratory chain
291 that, instead of being dispersed in the membrane, are
292 organized into a functional supramolecular respiratory
293 domain called respirasome, see Figure 1. ATP synthase
294 dimers sit at the edge of the cristae. Mitochondria inner
295 membrane is rich in cardiolipin (CL), a key phospholipid
296 playing important roles in maintaining the functional
297 integrity and dynamics of mitochondria. Arabidopsis
298 CL localizes to mitochondria and is enriched at specific
299 domains and CARDIOLIPIN SYNTHASE targets to the
300 inner membrane of mitochondria with its C-terminus in
301 the intermembrane space [79]. Mitochondria of *cls*
302 mutants exhibit altered structural integrity and morpho-
303 genesis. In contrast to animal and yeast, plant CL plays a
304 dominant role in mitochondrial fission and exerts this

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function through stabilizing the protein complex of DYNAMIN-RELATED PROTEIN3 [79]. In addition, CL induces membrane invaginations which are stabilized by dimers of ATP synthase. In reconstituted systems, bovine ATP synthase is sufficient to deform a lipid bilayer, which is likely the driving force triggering cristae curvature [80]. Recently, dimers of mitochondrial ATP synthase from the green algae *Polytomella* were shown to be required for cristae formation and constitute the main factor in mitochondrial morphogenesis to induce membrane curvature and self-assembly into rows [81^{*}]. Finally, mitochondria-associated ER membrane (MAM) is another structural element that is increasingly recognized for its critical role in cellular physiology and homeostasis of mitochondria [10].

Endoplasmic reticulum domains

The endoplasmic reticulum forms a membrane network virtually in contact with all cell organelles, see Figure 1a. Thus, the ER is actively engaged in organizing membrane domains to perform various functions. For example, ER is known to be organized into smooth and rough domains, the latter being enriched in ribosomes involved in protein production, protein folding, quality control and dispatch. Formation of these domains is regulated by syntaxin proteins [82]. Lipid droplets (LD) are lipid-rich cellular organelles regulating storage and hydrolysis of neutral lipids. LD biogenesis takes place at ER subdomains which are regulated by lipodystrophy proteins called SEIPINs in human, yeast, and plants. SEIPINs reorganize the normal, reticulated ER structure into discrete ER domains that colocalize with LD. In plants, SEIPINs modulate the number and sizes of LD [83^{*},84]. Recent work in plants showed that peroxisome extensions deliver the major TAG lipase Sugar-Dependent 1 (SDP1) to the LD. At early stages of seedling development, SDP1 localizes to a peroxisome membrane domain and then possibly moves to the LD surface through peroxisome tubulations [85]. This constitutes an interesting case of inter-organelle communication and protein transport that is reminiscent of stromule.

In the next chapter, we will briefly describe state-of-the-art biophysical methods that have provided access to the structural basis membrane domain organization.

How to study the molecular mechanisms shaping biological membrane domains in plants?

Membrane subcompartmentalization is intimately linked to the preferential association of membrane constituents. Therefore, establishing the structure-function relationship between the membrane subcompartment components is an essential piece of the puzzle towards understanding the complex interplay of the cells with the extracellular environment. Yet, the intrinsic soft matter state of membrane-related systems in their native environment, such as

peripheral or membrane-embedded proteins, hampers the application of numerous techniques in structural biology to visualize molecular association at the atomic level. To provide an overview on a promising route towards understanding the molecular basis underlying membrane subcompartmentalization, we can list tools such as X-ray, crystallography and solution NMR [86–88]. The recent developments of superresolution microscopy (eg. STED, PALM), cryo-electron microscopy (EM) and tomography methods allowed the study of the organization of proteins and lipids and the characterization of membrane subcompartmentalization and MCS [89^{**},16^{**},90^{*}] with unprecedented resolution. The complementary biophysical tools to investigate lipid/protein interactions such as Langmuir monolayer, Fourier-Transform InfraRed spectroscopy (FTIR), NMR or modelling are reviewed in [91,11,13]. Figure 2a shows several examples of diverse contributions, including solid-state nuclear magnetic resonance (ssNMR), X-ray crystallography, modelling, FTIR, tomography by EM and solution NMR. The development of lipid and protein imagery by isotope-labeled high-resolution secondary ion mass spectrometry (nano-SIMS) would allow the study of molecular events at play in domain formation and dynamics [92,93]. In plants, nano-SIMS was used to localize elements such as manganese, arsenic, iron, zinc, and cadmium at the nanoscale level [94], but this approach could also be used for lipids and proteins in internal organelles.

Here, we further describe the powerful technique ssNMR that emerges as a tool to understand domain assembly. SsNMR is a versatile technology reporting on membrane and protein structure, sensitive to dynamics and protein–lipid interactions. A major advantage relies in its application on systems in the native bilayer environment, that is reconstituted liposomes that can represent membranes of a chosen lipid composition. The flowchart in Figure 2b illustrates the overall procedure applied to inquire on the previously mentioned aspects of membrane-associated proteins. Reporting membrane biophysical and structural parameters are achieved by well-established membrane-focused ssNMR, mainly recorded on ²H and ³¹P nuclei [95,96]. The quadrupolar ²H signal in static ssNMR encodes for the overall lipid mobility and, importantly, the local dynamics along the acyl chain. Upon varying the membrane components (lipid composition, presence or absence of protein) and environment (temperature, pH), ²H ssNMR reveals detailed insights on phase, phase transitions, acyl chain dynamics and membrane thickness and curvature depending on the precise lipid composition and on the presence of a potential interaction partner. The chemical shift of ³¹P nuclei complements and corroborates the data reporting on phase behavior and the impact of potential partner molecules on the lipid head groups. Tackling membrane proteins is based on Magic-Angle spinning (MAS) ssNMR, a method which has seen tremendous advances in elucidating insoluble protein structures, dynamics and interactions in soft matter states such as assemblies, aggregates [97^{*},98,99] or

415 membrane-association [100^{••},101,102]. Since 2002, when the
 416 first structure of a microcrystalline protein has been solved by
 417 MAS ssNMR [103], the technology has proven very powerful
 418 to elucidate protein assemblies such as the first amyloid
 419 protein structure [104], bacterial filaments [105,106] and
 420 protein–membrane complexes [107^{*}]. A considerable knowl-
 421 edge has already been derived from ssNMR on protein–lipid,
 422 lipid–lipid interactions and membrane dynamics and func-
 423 tioning [108–110,96,102]. Most recent technological devel-
 424 opments achieving ultra-fast MAS frequencies (≥ 100 kHz)
 425 MAS ssNMR allow for observing proton nuclei in protonated
 426 protein samples (~ 500 μ g) and should facilitate ssNMR to
 427 serve as a common tool for structural biology on membrane/
 428 protein related questions. Because of its striking technologi-
 429 cal evolution, MAS ssNMR has recently been applied in few
 430 cases to shed light on protein structures, dynamics and
 431 protein–lipid interactions promoted by lipid-dependent
 432 membrane features [100^{••}]. Membrane domain formation
 433 in plants, relying on the plant protein and lipid interplay (see
 434 below the example of REMORIN in PM [16^{••},44]) remains a
 435 field to explore by MAS ssNMR.

436 **Conclusions**

437 Virtually all membranes are organized in functional
 438 domains that coordinate cell functions. Recent break-
 439 through in biochemistry, biophysic and microscopy
 440 approaches allow nowadays the study of the mechanisms
 441 regulating the formation of membrane domains, particu-
 442 larly the interplay between lipids and proteins. The next
 443 decade will likely open a vast area of research to under-
 444 stand the roles of membrane organization during plant
 445 development and adaptation.

446 **Conflict of interest statement**

447 Nothing declared.

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458 **References and recommended reading**

459 Papers of particular interest, published within the period of review,
 460 have been highlighted as:

- of special interest
- of outstanding interest

461 1. Russinova E, Schumacher K: **Editorial overview: cell biology:
 462 membrane dynamics - being at the right place at the right time.**
 463 *Curr Opin Plant Biol* 2017, **40**:iii-iv.

464 2. Jean S, Kiger AA: **Coordination between RAB GTPase and
 465 phosphoinositide regulation and functions.** *Nat Rev Mol Cell
 466 Biol* 2012, **13**:463-470.

3. Noack LC, Jaillais Y: **Precision targeting by phosphoinositides:
 467 how Pls direct endomembrane trafficking in plants.** *Curr Opin
 468 Plant Biol* 2017, **40**:22-33. 469

4. Platre MP, Bayle V, Armengot L, Bareille J, Marques-Bueno MDM,
 •• Creff A, Maneta-Peyret L, Fiche JB, Nollmann M, Miede C *et al.*:
**Developmental control of plant Rho GTPase nano-
 470 organization by the lipid phosphatidyserine.** *Science* 2019,
 471 **364**:57-62. 472

In this study, the authors elegantly showed that variation in phosphati-
 473 dyserine during root development stabilized Rho protein ROP6 into
 474 nanodomains to regulate auxin signaling. 475

5. Garcia-Fernandez E, Koch G, Wagner RM, Fekete A, Stengel ST,
 476 Schneider J, Mielich-Suss B, Geibel S, Markert SM, Stigloher C
et al.: **Membrane microdomain disassembly inhibits MRSA
 477 antibiotic resistance.** *Cell* 2017, **171**:1354-1367 e1320. 478

6. Zhou Y, Prakash P, Liang H, Cho KJ, Gorfe AA, Hancock JF: **Lipid-
 479 sorting specificity encoded in K-ras membrane anchor
 480 regulates signal output.** *Cell* 2017, **168**:239-251 e216. 481

7. Jacobson K, Liu P, Lagerholm BC: **The lateral organization and
 482 mobility of plasma membrane components.** *Cell* 2019, **177**:806-819. 483

8. Sezgin E, Levental I, Mayor S, Eggeling C: **The mystery of
 484 membrane organization: composition, regulation and roles of
 485 lipid rafts.** *Nat Rev Mol Cell Biol* 2017, **18**:361-374. 486

9. Gronnier J, Gerbeau-Pissot P, Germain V, Mongrand S, Simon-
 487 Plas F: **Divide and rule: plant plasma membrane organization.**
Trends Plant Sci 2018, **23**:899-917. 488

10. Michaud M, Jouhet J: **Lipid trafficking at membrane contact
 489 sites during plant development and stress response.** *Front
 490 Plant Sci* 2019, **10**:2. 491

11. Petit JD, Immel F, Lins L, Bayer EM: **Lipids or proteins: who is
 492 leading the dance at membrane contact sites?** *Front Plant Sci*
 493 2019, **10**:198. 494

12. Cacas JL, Bure C, Grosjean K, Gerbeau-Pissot P, Lherminier J,
 495 Rombouts Y, Maes E, Bossard C, Gronnier J, Furt F *et al.*: **Re-
 496 visiting plant plasma membrane lipids in tobacco: a focus on
 sphingolipids.** *Plant Physiol* 2015, **170**:367-384. 497

13. Mamode Cassim A, Gouguet P, Gronnier J, Laurent N, Germain V,
 498 Grison M, Boutte Y, Gerbeau-Pissot P, Simon-Plas F, Mongrand S:
**Plant lipids: key players of plasma membrane organization
 499 and function.** *Prog Lipid Res* 2019, **73**:1-27. 500

14. McKenna JF, Tolmie AF, Runions J: **Across the great divide: the
 501 plant cell surface continuum.** *Curr Opin Plant Biol* 2014, **22**:132-140. 502

15. Ott T: **Membrane nanodomains and microdomains in plant-
 503 microbe interactions.** *Curr Opin Plant Biol* 2017, **40**:82-88. 504

16. Gronnier J, Crowet JM, Habenstein B, Nasir MN, Bayle V, Hosity E,
 •• Platre MP, Gouguet P, Raffaele S, Martinez D *et al.*: **Structural
 505 basis for plant plasma membrane protein dynamics and
 506 organization into functional nanodomains.** *eLife* 2017, **6**. 507

Combining super resolution microscopy, modelling and biophysical
 508 experiments, the authors decipher a molecular mechanism regulating
 509 the formation of functional nanodomains involved in plant immunity
 against a virus. 510

17. Bucherl CA, Jarsch IK, Schudoma C, Segonzac C, Mbengue M,
 • Robotzcek S, MacLean D, Ott T, Zipfel C: **Plant immune and
 511 growth receptors share common signalling components but
 512 localise to distinct plasma membrane nanodomains.** *eLife*
 2017, **6**. 513

Using quantitative image analysis the authors propose that spatial
 514 separation support functional specification of immune and growth recep-
 515 tors kinase. 516

18. Faulkner C: **A cellular backline: specialization of host
 517 membranes for defence.** *J Exp Bot* 2015, **66**:1565-1571. 518

19. Burkart RC, Stahl Y: **Dynamic complexity: plant receptor
 519 complexes at the plasma membrane.** *Curr Opin Plant Biol* 2017,
 520 **40**:15-21. 521

20. Tilsner J, Nicolas W, Rosado A, Bayer EM: **Staying tight:
 522 plasmodesmal membrane contact sites and the control of
 523 cell-to-cell connectivity in plants.** *Annu Rev Plant Biol* 2016,
 524 **67**:337-364. 525

8 Cell biology

- 525 21. Naramoto S: **Polar transport in plants mediated by membrane**
526 **transporters: focus on mechanisms of polar auxin transport.**
527 *Curr Opin Plant Biol* 2017, **40**:8-14.
- 528 22. Nakamura M, Grebe M: **Outer, inner and planar polarity in the**
529 **Arabidopsis root.** *Curr Opin Plant Biol* 2018, **41**:46-53.
- 530 23. Raffaele S, Bayer E, Lafarge D, Cluzet S, German Retana S,
531 Boubekour T, Leborgne-Castel N, Carde JP, Lherminier J, Noïrot E
532 *et al.*: **Remorin, a solanaceae protein resident in membrane**
533 **rafts and plasmodesmata, impairs potato virus X movement.**
534 *Plant Cell* 2009, **21**:1541-1555.
- 535 24. Bozkurt TO, Richardson A, Dagdas YF, Mongrand S, Kamoun S,
536 Raffaele S: **The plant membrane-associated REMORIN1.3**
537 **accumulates in discrete periahaustorial domains and enhances**
538 **susceptibility to phytophthora infestans.** *Plant Physiol* 2014,
539 **165**:1005-1018.
- 540 25. Son S, Oh CJ, An CS: **Arabidopsis thaliana remorins interact**
541 **with SnRK1 and Play a role in susceptibility to beet curly top**
542 **virus and beet severe curly top virus.** *Plant Pathol J* 2014,
543 **30**:269-278.
- 544 26. Jamann TM, Luo X, Morales L, Kolkman JM, Chung CL, Nelson RJ:
545 **A remorin gene is implicated in quantitative disease**
546 **resistance in maize.** *Theor Appl Genet* 2016, **129**:591-602.
- 547 27. Lefebvre B, Timmers T, Mbengue M, Moreau S, Herve C, Toth K,
548 Bittencourt-Silvestre J, Klaus D, Deslandes L, Godiard L *et al.*: **A**
549 **remorin protein interacts with symbiotic receptors and**
550 **regulates bacterial infection.** *Proc Natl Acad Sci U S A* 2010,
551 **107**:2343-2348.
- 552 28. Toth K, Stratil TF, Madsen EB, Ye J, Popp C, Antolin-Llovera M,
553 Grossmann C, Jensen ON, Schussler A, Parniske M *et al.*:
554 **Functional domain analysis of the Remorin protein**
555 **LjSYMREM1 in Lotus japonicas.** *PLoS One* 2012, **7**:e30817.
- 556 29. Liang P, Stratil TF, Popp C, Marin M, Folgmann J, Mysore KS,
557 • Wen J, Ott T: **Symbiotic root infections in Medicago truncatula**
558 **require remorin-mediated receptor stabilization in membrane**
559 **nanodomains.** *Proc Natl Acad Sci U S A* 2018, **115**:5289-5294.
560 The authors proposed that immobilization of symbiotic cell entry receptor
561 LYK3 is mediated by two molecular scaffold proteins, FLOT4 and SYM-
562 REM1 and ensures progression of the primary infection thread into root
563 cortical cells.
- 564 30. Gui J, Liu C, Shen J, Li L: **Grain setting defect1, encoding a**
565 **remorin protein, affects the grain setting in rice through**
566 **regulating plasmodesmatal conductance.** *Plant Physiol* 2014,
567 **166**:1463-1478.
- 568 31. Perraki A, Gronnier J, Gouguet P, Boudsocq M, Deroubaix AF,
569 Simon V, German-Retana S, Legrand A, Habenstein B, Zipfel C
570 *et al.*: **REM1.3's phospho-status defines its plasma membrane**
571 **nanodomain organization and activity in restricting PVX cell-**
572 **to-cell movement.** *PLoS Pathog* 2018, **14**:e1007378.
- 573 32. Jarsch IK, Ott T: **Perspectives on remorin proteins, membrane**
574 **rafts, and their role during plant-microbe interactions.** *Mol*
575 *Plant Microbe Interact* 2011, **24**:7-12.
- 576 33. Jarsch IK, Konrad SS, Stratil TF, Urbanus SL, Szymanski W,
577 Braun P, Braun KH, Ott T: **Plasma membranes are**
578 **subcompartmentalized into a plethora of coexisting and**
579 **diverse microdomains in arabidopsis and Nicotiana**
580 **benthamiana.** *Plant Cell* 2014, **26**:1698-1711.
- 581 34. Konrad SS, Popp C, Stratil TF, Jarsch IK, Thallmair V, Folgmann J,
582 Marin M, Ott T: **S-acylation anchors remorin proteins to the**
583 **plasma membrane but does not primarily determine their**
584 **localization in membrane microdomains.** *New Phytol* 2014,
585 **203**:758-769.
- 586 35. Marin M, Thallmair V, Ott T: **The intrinsically disordered N-terminal**
587 **region of AtREM1.3 remorin protein mediates protein-protein**
588 **interactions.** *J Biol Chem* 2012, **287**:39982-39991.
- 589 36. Fernandez-Calvino L, Faulkner C, Walshaw J, Saalbach G,
590 Bayer E, Benitez-Alfonso Y, Maule A: **Arabidopsis**
591 **plasmodesmal proteome.** *PLoS One* 2011, **6**:e18880.
- 592 37. Demir F, Horntich C, Blachutzik JO, Scherz S, Reinders Y,
593 Kierszniowska S, Schulze WX, Harms GS, Hedrich R, Geiger D
594 *et al.*: **Arabidopsis nanodomain-delimited ABA signaling**
595 **pathway regulates the anion channel SLAH3.** *Proc Natl Acad*
596 *Sci U S A* 2013, **110**:8296-8301.
- 597 38. Szymanski WG, Zauber H, Erban A, Gorka M, Wu XN,
598 Schulze WX: **Cytoskeletal components define protein location**
599 **to membrane microdomains.** *Mol Cell Proteomics* 2015,
600 **14**:2493-2509.
- 601 39. Perraki A, Cacas JL, Crowet JM, Lins L, Castroviejo M, German-
602 Retana S, Mongrand S, Raffaele S: **Plasma membrane**
603 **localization of Solanum tuberosum remorin from group 1,**
604 **homolog 3 is mediated by conformational changes in a novel**
605 **C-terminal anchor and required for the restriction of potato**
606 **virus X movement.** *Plant Physiol* 2012, **160**:624-637.
- 607 40. Raffaele S, Perraki A, Mongrand S: **The remorin C-terminal**
608 **anchor was shaped by convergent evolution among**
609 **membrane binding domains.** *Plant Signal Behav* 2013, **8**:e23207.
- 610 41. Hemsley PA: **Assaying protein S-acylation in plants.** *Methods*
611 *Mol Biol* 2013, **1043**:141-146.
- 612 42. Gui J, Zheng S, Shen J, Li L: **Grain setting defect1 (GSD1)**
613 **function in rice depends on S-acylation and interacts with**
614 **actin 1 (OsACT1) at its C-terminal.** *Front Plant Sci* 2015, **6**:804.
- 615 43. Fu S, Xu Y, Li C, Li Y, Wu J, Zhou X: **Rice stripe virus interferes**
616 **with S-acylation of remorin and induces its autophagic**
617 **degradation to facilitate virus infection.** *Mol Plant* 2018,
618 **11**:269-287.
- 619 44. Martinez D, Legrand A, Gronnier J, Decossas M, Gouguet P,
620 Lambert O, Berbon M, Verron L, Grelard A, Germain V *et al.*:
621 **Coiled-coil oligomerization controls localization of the plasma**
622 **membrane REMORINS.** *J Struct Biol* 2018.
- 623 45. Lv X, Jing Y, Xiao J, Zhang Y, Zhu Y, Julian R, Lin J: **Membrane**
624 **microdomains and the cytoskeleton constrain AthIR1**
625 **dynamics and facilitate the formation of an AthIR1-associated**
626 **immune complex.** *Plant J* 2017, **90**:3-16.
- 627 46. Christensen TM, Vejrupkova Z, Sharma YK, Arthur KM,
628 Spatafora JW, Albright CA, Meeley RB, Duvick JP, Quatrano RS,
629 Fowler JE: **Conserved subgroups and developmental**
630 **regulation in the monocot rop gene family.** *Plant Physiol* 2003,
631 **133**:1791-1808.
- 632 47. Feiguelman G, Fu Y, Yalovsky S: **ROP GTPases structure-**
633 **function and signaling pathways.** *Plant Physiol* 2018, **176**:57-79.
- 634 48. Berken A, Wittinghofer A: **Structure and function of rho-type**
635 **molecular switches in plants.** *Plant Physiol Biochem* 2008,
636 **46**:380-393.
- 637 49. Molendijk AJ, Bischoff F, Rajendrakumar CS, Friml J, Braun M,
638 Gilroy S, Palme K: **Arabidopsis thaliana Rop GTPases are**
639 **localized to tips of root hairs and control polar growth.** *EMBO J*
640 2001, **20**:2779-2788.
- 641 50. Denninger P, Reichelt A, Schmidt VAF, Mehlhorn DG, Asseck LY,
642 • Stanley CE, Keinath NF, Evers JF, Grefen C, Grossmann G:
643 **Distinct RopGEFs successively drive polarization and**
644 **outgrowth of root hairs.** *Curr Biol* 2019.
645 Using microfluidics and live cell imaging, Denninger *et al.* dissect the
646 timing of the growth machinery assembly in polarizing hair cells and show
647 that GEF3 serves as a membrane landmark recruiting growth machinery to
648 specialized membrane domains.
- 649 51. Stanislas T, Huser A, Barbosa IC, Kiefer CS, Brackmann K,
650 Pietra S, Gustavsson A, Zourelidou M, Schwechheimer C,
651 Grebe M: **Arabidopsis D6PK is a lipid domain-dependent**
652 **mediator of root epidermal planar polarity.** *Nat Plants* 2015,
653 **1**:15162.
- 654 52. Barbosa IC, Zourelidou M, Willige BC, Weller B,
655 Schwechheimer C: **D6 PROTEIN KINASE activates auxin**
656 **transport-dependent growth and PIN-FORMED**
657 **phosphorylation at the plasma membrane.** *Dev Cell* 2014,
658 **29**:674-685.
- 659 53. Bloch D, Yalovsky S: **Cell polarity signaling.** *Curr Opin Plant Biol*
660 2013, **16**:734-742.
- 661 54. Oda Y, Fukuda H: **Initiation of cell wall pattern by a Rho- and**
662 **microtubule-driven symmetry breaking.** *Science* 2012,
663 **337**:1333-1336.

639 55. Oda Y, Fukuda H: **Rho of plant GTPase signaling regulates the behavior of Arabidopsis kinesin-13A to establish secondary cell wall patterns.** *Plant Cell* 2013, **25**:4439–4450.
640
641

642 56. Sugiyama Y, Wakazaki M, Toyooka K, Fukuda H, Oda Y: **A novel plasma membrane-anchored protein regulates xylem cell-wall deposition through microtubule-dependent lateral inhibition of rho GTPase domains.** *Curr Biol* 2017, **27**:2522–2528 e2524.
643
644
645

646 57. Sasaki T, Fukuda H, Oda Y: **Cortical microtubule disordering1 is required for secondary cell wall patterning in xylem vessels.** *Plant Cell* 2017, **29**:3123–3139.
647
648

649 58. McFarlane HE, Doring A, Persson S: **The cell biology of cellulose synthesis.** *Annu Rev Plant Biol* 2014, **65**:69–94.
650

651 59. Paredes AR, Somerville CR, Ehrhardt DW: **Visualization of cellulose synthase demonstrates functional association with microtubules.** *Science* 2006, **312**:1491–1495.
652
653

654 60. Crowell EF, Bischoff V, Desprez T, Rolland A, Stierhof YD, Schumacher K, Gonneau M, Hofte H, Vernhettes S: **Pausing of Golgi bodies on microtubules regulates secretion of cellulose synthase complexes in Arabidopsis.** *Plant Cell* 2009, **21**:1141–1154.
655
656
657

658 61. Gutierrez R, Lindeboom JJ, Paredes AR, Emons AM, Ehrhardt DW: **Arabidopsis cortical microtubules position cellulose synthase delivery to the plasma membrane and interact with cellulose synthase trafficking compartments.** *Nat Cell Biol* 2009, **11**:797–806.
659
660
661

662 62. Kumar M, Wightman R, Atanassov I, Gupta A, Hurst CH, Hemsley PA, Turner S: **S-acylation of the cellulose synthase complex is essential for its plasma membrane localization.** *Science* 2016, **353**:166–169.
663
664

665 63. Bringmann M, Landrein B, Schudoma C, Hamant O, Hauser MT, Persson S: **Cracking the elusive alignment hypothesis: the microtubule-cellulose synthase nexus unraveled.** *Trends Plant Sci* 2012, **17**:666–674.
666
667

668 64. Gu Y, Somerville C: **Cellulose synthase interacting protein: a new factor in cellulose synthesis.** *Plant Signal Behav* 2010, **5**:1571–1574.
669
670

671 65. Li S, Lei L, Somerville CR, Gu Y: **Cellulose synthase interactive protein 1 (CSH1) links microtubules and cellulose synthase complexes.** *Proc Natl Acad Sci U S A* 2012, **109**:185–190.
672
673

674 66. Endler A, Kesten C, Schneider R, Zhang Y, Ivakov A, Froehlich A, Funke N, Persson S: **A mechanism for sustained cellulose synthesis during salt stress.** *Cell* 2015, **162**:1353–1364.
675

676 67. Jouhet J: **Importance of the hexagonal lipid phase in biological membrane organization.** *Front Plant Sci* 2013, **4**:494.
677

678 68. Nawrocki WJ, Santabarbara S, Mosebach L, Wollman FA, Rappaport F: **State transitions redistribute rather than dissipate energy between the two photosystems in chlamydomonas.** *Nat Plants* 2016, **2**:16031.
679
680

681 69. Flori S, Jouneau PH, Bailleul B, Gallet B, Estrozi LF, Moriscot C, Bastien O, Eicke S, Schober A, Bartulos CR et al.: **Plastid thylakoid architecture optimizes photosynthesis in diatoms.** *Nat Commun* 2017, **8**:15885.
682
683

684 70. Furse S: **Is phosphatidylglycerol essential for terrestrial life?** *J Chem Biol* 2017, **10**:1–9.
685

686 71. Rast A, Schaffer M, Albert S, Wan W, Pfeffer S, Beck F, Plitzko JM, Nickelsen J, Engel BD: **Biogenic regions of cyanobacterial thylakoids form contact sites with the plasma membrane.** *Nat Plants* 2019, **5**:436–446.
687
688
689
690
691

692 72. Michaud M, Gros V, Tardif M, Brugiere S, Ferro M, Prinz WA, Toulmay A, Mathur J, Wozny M, Falconet D et al.: **AtMic60 is involved in plant mitochondria lipid trafficking and is part of a large complex.** *Curr Biol* 2016, **26**:627–639.
693
694
695
696

697 73. Park E, Caplan JL, Dinesh-Kumar SP: **Dynamic coordination of plastid morphological change by cytoskeleton for chloroplast-nucleus communication during plant immune responses.** *Plant Signal Behav* 2018, **13**:e1500064.
698
699
700

701 74. Erickson JL, Kanteck M, Schattat MH: **Plastid-nucleus distance alters the behavior of stromules.** *Front Plant Sci* 2017, **8**:1135.
702

703 75. Erickson JL, Schattat MH: **Shaping plastid stromules-principles of in vitro membrane tubulation applied in planta.** *Curr Opin Plant Biol* 2018, **46**:48–54.
704
705

706 76. Gao H, Metz J, Teanby NA, Ward AD, Botchway SW, Coles B, Pollard MR, Sparkes I: **In vivo quantification of peroxisome tethering to chloroplasts in tobacco epidermal cells using optical tweezers.** *Plant Physiol* 2016, **170**:263–272.
707
708

709 77. Jaipargas EA, Mathur N, Bou Daher F, Wasteneys GO, Mathur J: **High light intensity leads to increased peroxule-mitochondria interactions in plants.** *Front Cell Dev Biol* 2016, **4**:6.
710
711

712 78. Quintana-Cabrera R, Mehrotra A, Rigoni G, Soriano ME: **Who and how in the regulation of mitochondrial cristae shape and function.** *Biochem Biophys Res Commun* 2018, **500**:94–101.
713
714

715 79. Pan R, Jones AD, Hu J: **Cardiolipin-mediated mitochondrial dynamics and stress response in Arabidopsis.** *Plant Cell* 2014, **26**:391–409.
716
717

718 80. Jiko C, Davies KM, Shinzawa-Itoh K, Tani K, Maeda S, Mills DJ, Tsukihara T, Fujiyoshi Y, Kuhlbrandt W, Gerle C: **Bovine F1Fo ATP synthase monomers bend the lipid bilayer in 2D membrane crystals.** *eLife* 2015, **4**:e06119.
719
720

721 81. Blum TB, Hahn A, Meier T, Davies KM, Kuhlbrandt W: **Dimers of mitochondrial ATP synthase induce membrane curvature and self-assemble into rows.** *Proc Natl Acad Sci U S A* 2019.
722
723
724
725
726

727 82. Iinuma T, Aoki T, Arasaki K, Hirose H, Yamamoto A, Samata R, Hauri HP, Arimitsu N, Tagaya M, Tani K: **Role of syntaxin 18 in the organization of endoplasmic reticulum subdomains.** *J Cell Sci* 2009, **122**:1680–1690.
728
729

730 83. Cai Y, Goodman JM, Pyc M, Mullen RT, Dyer JM, Chapman KD: **Arabidopsis SEIPIN proteins modulate triacylglycerol accumulation and influence lipid droplet proliferation.** *Plant Cell* 2015, **27**:2616–2636.
731
732
733

734 84. Taurino M, Costantini S, De Domenico S, Stefanelli F, Ruano G, Delgadillo MO, Sanchez-Serrano JJ, Sanmartin M, Santino A, Rojo E: **SEIPIN proteins mediate lipid droplet biogenesis to promote pollen transmission and reduce seed dormancy.** *Plant Physiol* 2018, **176**:1531–1546.
735
736

737 85. Thazar-Poulot N, Miquel M, Fobis-Loisy I, Gaude T: **Peroxisome extensions deliver the Arabidopsis SDF1 lipase to oil bodies.** *Proc Natl Acad Sci U S A* 2015, **112**:4158–4163.
738
739

740 86. Hunte C, Richers S: **Lipids and membrane protein structures.** *Curr Opin Struct Biol* 2008, **18**:406–411.
741

742 87. Maslennikov I, Choe S: **Advances in NMR structures of integral membrane proteins.** *Curr Opin Struct Biol* 2013, **23**:555–562.
743

744 88. Oxenoid K, Chou JJ: **The present and future of solution NMR in investigating the structure and dynamics of channels and transporters.** *Curr Opin Struct Biol* 2013, **23**:547–554.
745
746

747 89. Nicolas WJ, Grison MS, Trepout S, Gaston A, Fouche M, Cordelieres FP, Oparka K, Tilsner J, Brocard L, Bayer EM: **Architecture and permeability of post-cytokinesis plasmodesmata lacking cytoplasmic sleeves.** *Nat Plants* 2017, **3**:17082.
748
749

750 Using electron tomography the authors shows that within plasmodesmata ER–PM contact sites undergo substantial remodelling events during cell differentiation to regulate cell–cell communication.
751
752

753 90. Hosi E, Martiniere A, Choquet D, Maurel C, Luu DT: **Super-resolved and dynamic imaging of membrane proteins in plant cells reveal contrasting kinetic profiles and multiple confinement mechanisms.** *Mol Plant* 2015, **8**:339–342.
754
755

10 Cell biology

- 756 Proof of concept for the use of spt-PALM in plant PM proteins. 795
- 757 91. Deleu M, Crowet JM, Nasir MN, Lins L: **Complementary** 796
- 758 **biophysical tools to investigate lipid specificity in the** 797
- 759 **interaction between bioactive molecules and the plasma** 798
- 760 **membrane: a review.** *Biochim Biophys Acta* 2014, 799
- 761 **1838:3171-3190.** 800
- 762 92. Frisz JF, Klitzing HA, Lou K, Hutcheon ID, Weber PK, 801
- 763 Zimmerberg J, Kraft ML: **Sphingolipid domains in the plasma** 802
- 764 **membranes of fibroblasts are not enriched with cholesterol.** *J* 803
- 765 *Biol Chem* 2013, **288:16855-16861.** 804
- 766 93. Frisz JF, Lou K, Klitzing HA, Hanafin WP, Lizunov V, Wilson RL, 805
- 767 Carpenter KJ, Kim R, Hutcheon ID, Zimmerberg J *et al.*: **Direct** 806
- 768 **chemical evidence for sphingolipid domains in the plasma** 807
- 769 **membranes of fibroblasts.** *Proc Natl Acad Sci U S A* 2013, **110:** 808
- 770 **E613-E622.** 809
- 771 94. Ondrasek G, Rengel Z, Clode PL, Kilburn MR, Guagliardo P, 810
- 772 Romic D: **Zinc and cadmium mapping by NanoSIMS within the** 811
- 773 **root apex after short-term exposure to metal contamination.** *Ecotoxicol Environ Saf* 2019, **171:571-578.** 812
- 774 95. Epand RM, D'Souza K, Berno B, Schlame M: **Membrane** 813
- 775 **curvature modulation of protein activity determined by NMR.** *Biochim Biophys Acta* 2015, **1848:220-228.** 814
- 776 96. Molugu TR, Lee S, Brown MF: **Concepts and methods of solid-** 815
- 777 **state NMR spectroscopy applied to biomembranes.** *Chem Rev* 816
- 778 2017, **117:12087-12132.** 817
- 779 97. Loquet A, El Mammeri N, Stanek J, Berbon M, Bardiaux B, 818
- 780 • Pintacuda G, Habenstein B: **3D structure determination of** 819
- 781 **amyloid fibrils using solid-state NMR spectroscopy.** *Methods* 820
- 782 2018, **138-139:26-38.** 821
- 783 Structural studies of molecular assemblies such as amyloids are one of 822
- 784 the major application of solid-state NMR. This review concisely describes 823
- 785 state-of-the-art ssNMR methods to investigate structures of protein 824
- 786 assemblies. The explained methods can generally be applied to elucidate 825
- 787 structures of molecular assemblies. 826
- 788 98. Habenstein B, Loquet A: **Solid-state NMR: an emerging** 827
- 789 **technique in structural biology of self-assemblies.** *Biophys* 828
- 790 *Chem* 2016, **210:14-26.** 829
- 791 99. Weingarh M, Baldus M: **Solid-state NMR-based approaches for** 830
- 792 **supramolecular structure elucidation.** *Acc Chem Res* 2013, 831
- 793 **46:2037-2046.** 832
- 794 100. Mandala VS, Williams JK, Hong M: **Structure and dynamics of** 833
- 795 **membrane proteins from solid-state NMR.** *Annu Rev Biophys* 834
- 796 2018, **47:201-222.** 835
- 797 The authors review ssNMR studies that ingeniously combine different 836
- 798 ssNMR approaches to understand membrane protein structure and 837
- 799 functional mechanisms. Protein structures, as well as protein–lipid inter- 838
- 800 actions, are discussed from an ssNMR point of view. 839
- 801 101. Opella SJ, Marassi FM: **Applications of NMR to membrane** 840
- 802 **proteins.** *Arch Biochem Biophys* 2017, **628:92-101.** 841
- 803 102. Ladizhansky V: **Applications of solid-state NMR to membrane** 842
- 804 **proteins.** *Biochim Biophys Acta Proteins Proteom* 2017, 843
- 805 **1865:1577-1586.** 844
- 806 103. Castellani F, van Rossum B, Diehl A, Schubert M, Rehbein K, 845
- 807 Oschkinat H: **Structure of a protein determined by solid-state** 846
- 808 **magic-angle-spinning NMR spectroscopy.** *Nature* 2002, 847
- 809 **420:98-102.** 848
- 810 104. Wasmer C, Soragni A, Sabate R, Lange A, Riek R, Meier BH: 849
- 811 **Infectious and noninfectious amyloids of the HET-s(218-289)** 850
- 812 **prion have different NMR spectra.** *Angew Chem Int Ed Engl* 851
- 813 2008, **47:5839-5841.** 852
- 814 105. Habenstein B, Loquet A, Hwang S, Giller K, Vasa SK, Becker S, 853
- 815 Habbeck M, Lange A: **Hybrid structure of the type 1 pilus of** 854
- 816 **uropathogenic Escherichia coli.** *Angew Chem Int Ed Engl* 2015, 855
- 817 **54:11691-11695.** 856
- 818 106. Loquet A, Sgourakis NG, Gupta R, Giller K, Riedel D, Goosmann C, 857
- 819 Griesinger C, Kolbe M, Baker D, Becker S *et al.*: **Atomic model of** 858
- 820 **the type III secretion system needle.** *Nature* 2012, **486:276-279.** 859
- 821 107. Retel JS, Nieuwkoop AJ, Hiller M, Higman VA, Barbet-Massin E, 860
- 822 • Stanek J, Andreas LB, Franks WT, van Rossum BJ, 861
- 823 Vinothkumar KR *et al.*: **Structure of outer membrane protein G in** 862
- 824 **lipid bilayers.** *Nat Commun* 2017, **8:2073.** 863
- 825 Recent MAS ssNMR approaches have been used to determine the 864
- 826 structure of an integral membrane protein. This study is a well-timed 865
- 827 example of the potential of MAS ssNMR in structural investigations on 866
- 828 membrane proteins. 867
- 829 108. Huster D: **Solid-state NMR spectroscopy to study protein-lipid** 868
- 830 **interactions.** *Biochim Biophys Acta* 2014, **1841:1146-1160.** 869
- 831 109. Brown LS, Ladizhansky V: **Membrane proteins in their native** 870
- 832 **habitat as seen by solid-state NMR spectroscopy.** *Protein Sci* 871
- 833 2015, **24:1333-1346.** 872
- 834 110. Elkins MR, Hong M: **Elucidating ligand-bound structures of** 873
- 835 **membrane proteins using solid-state NMR spectroscopy.** *Curr* 874
- 836 *Opin Struct Biol* 2019, **57:103-109.** 875
- 837 111. Long SB, Tao X, Campbell EB, MacKinnon R: **Atomic structure of** 876
- 838 **a voltage-dependent K⁺ channel in a lipid membrane-like** 877
- 839 **environment.** *Nature* 2007, **450:376-382.** 878
- 840 112. Wang J, Pielak RM, McClintock MA, Chou JJ: **Solution structure** 879
- 841 **and functional analysis of the influenza B proton channel.** *Nat* 880
- 842 *Struct Mol Biol* 2009, **16:1267-1271.** 881