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1 **Crystallography of polysaccharides: Current state and challenges.**

2 Yu Ogawa*, Jean-Luc Putaux and Yoshiharu Nishiyama*

3 Univ. Grenoble Alpes, CNRS, CERMAV, F-38000 Grenoble, France.

4 * Corresponding authors:

5 Y.O. yu.ogawa@cermav.cnrs.fr

6 Y.N. yoshiharu.nishiyama@cermav.cnrs.fr

7

8 **Abstract**

9 Polysaccharides are the most abundant class of biopolymers, holding an important place in
10 biological systems and sustainable material development. Their spatial organization and
11 intra- and intermolecular interactions are thus of great interest. However, conventional
12 single crystal crystallography is not applicable since polysaccharides crystallize only into tiny
13 crystals. Several crystallographic methods have been developed to extract atomic-resolution
14 structural information from polysaccharide crystals. Small-probe single crystal
15 diffractometry, high-resolution fiber diffraction and powder diffraction combined with
16 molecular modeling brought new insights from various types of polysaccharide crystals, and
17 led to many high-resolution crystal structures over the past two decades. Current challenges
18 lie in the analysis of disorder and defects by further integrating molecular modeling
19 methods for low-resolution diffraction data.

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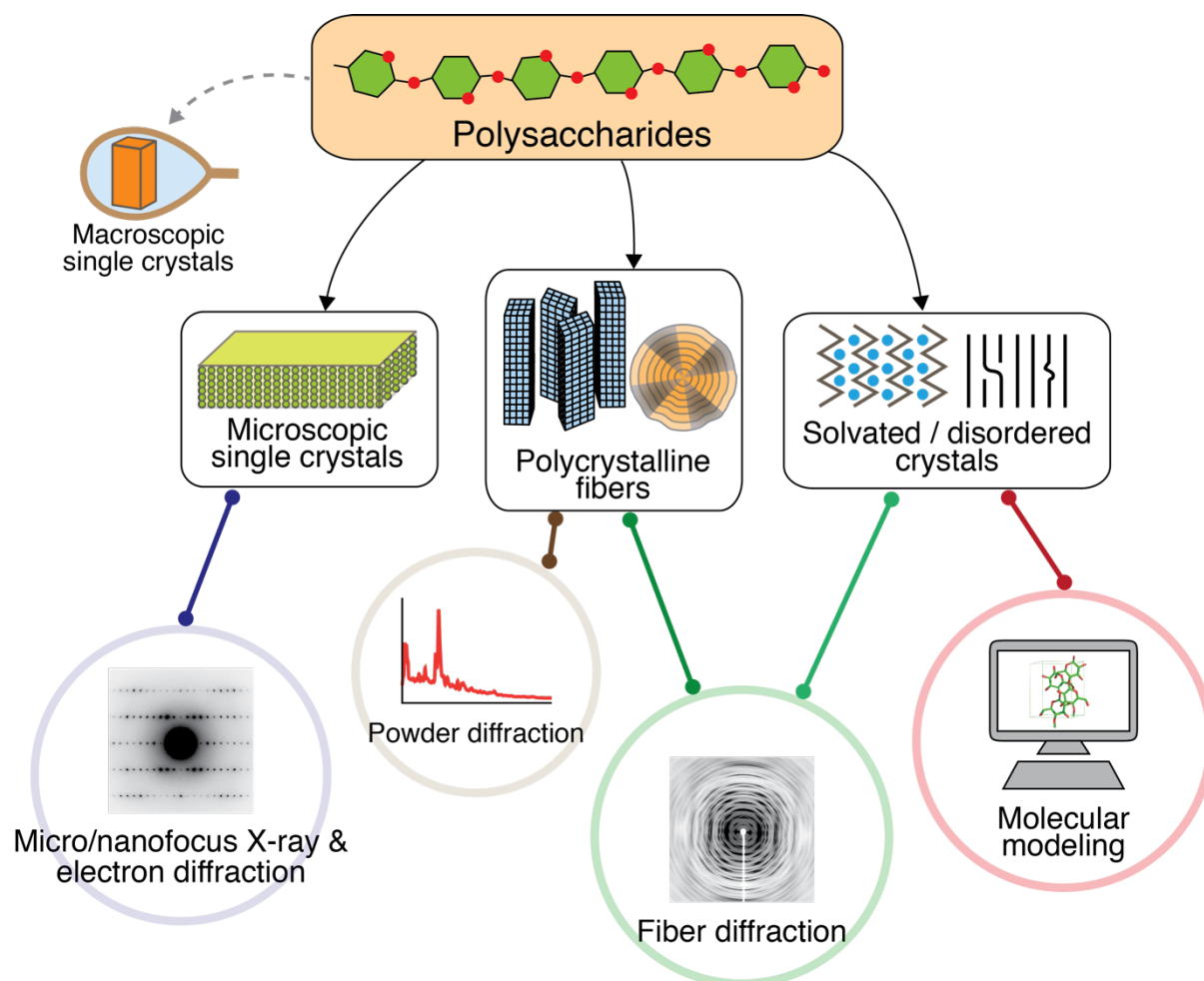
21 **Keywords:** Polysaccharides, Crystallography, Fiber diffraction, Electron diffraction

22

23 **Introduction**

24 The knowledge of the spatial arrangement of molecules is fundamental to a detail
25 understanding of the properties and molecular interactions in materials and biological
26 systems. Single crystal analysis using millimeter-sized crystallites is a standard tool to
27 analyze such structures at atomic or subatomic resolution. However, in many practical
28 systems, single crystals of the corresponding structure are not available. Polysaccharides,
29 the most abundant biopolymers on Earth, are one of such systems that do not yield
30 macroscopic single crystals but polycrystalline aggregates of smaller crystallites. This
31 polycrystalline nature has posed a challenge and led to methodological developments in the
32 structural determination of polysaccharide crystals. In this article, we review the recent

33 advances in the crystallography of polysaccharides in their native or recrystallized state (Fig.
 34 1). In particular, we will focus on the crystallographic methods for three different sample
 35 forms of polysaccharides, namely polycrystalline fibers, microscopic single crystals and
 36 crystalline powders. Finally, we will discuss the current challenges in the field of
 37 polysaccharide crystallography.



38

Figure 1. General scheme of polysaccharide crystallography. Polysaccharides are not crystallized into macroscopic single crystals but yield microscopic crystals with different geometries and features. Several crystallographic methods are available for structure determination of such small polysaccharide crystals.

39

40 Polysaccharides and their crystallinity

41 Polysaccharides are a class of molecules constituted of carbohydrate units (sugars) linked
 42 via glycosidic linkages. Potentially, the variety in molecular structures is huge, because each
 43 sugar can link at different positions, with two possible anomeric configurations, and it can
 44 contain different monomer sequences [1]. However, in terms of biomass quantity, relatively

45 simple homopolymers, such as cellulose and chitin, are dominant. Those are also the
46 molecules that tend to crystallize. Such carbohydrate crystals provide rich experimental
47 information about the details of molecular packing. In recent years, they have attracted
48 increasing attention as renewable resources for materials, thanks to their abundance,
49 renewability, biocompatibility, low toxicity, and interesting material properties.

50 Polysaccharides are traditionally categorized into two groups based on their *in vivo*
51 functionality, namely structural and storage polysaccharides. The former includes cellulose
52 [2] and chitin [3]. The structural polysaccharides confer the mechanical strength and
53 support to biological tissues. For instance, cellulose is the primary load-bearing material in
54 plant cell walls, and chitin is a structural component in crustacean/insect exoskeletons and
55 fungal cell walls. The storage polysaccharides, on the other hand, serve as the energy
56 storage in cells. Its representative examples are starch [4], β -1,3-glucan (paramylon) [5], and
57 inulin [6] in plants, and glycogen in animals and fungi [7]. Mannan has a dual nature as it can
58 be found both a structural and storage polysaccharide [8]. For instance,
59 galactoglucomannans are one of the main structural components in soft wood cell walls,
60 while linear mannans are found as energy storage in plant seeds such as ivory nuts. The
61 polysaccharides in the extracellular matrix, such as hyaluronic acid, possess specific
62 functionalities (e.g. lubricants in the joints) and are not categorized into either of the two
63 traditional groups [9].

64 In nature, structural polysaccharides often occur as fibrous objects in which the chains
65 are aligned along the long axis of the molecular assemblies. In particular, cellulose and chitin
66 are biosynthesized into crystalline nanofibrils. Other structural polysaccharides are found
67 naturally in amorphous matrices and sometimes associate with other fibrous components
68 (e.g. hemicelluloses) [10]. The native state of the storage polysaccharides can vary. For
69 instance, paramylon and starch are biosynthesized in plant cells as several micrometer-large
70 semicrystalline granules [4], while glycogen is an amorphous nanoparticle [7].

71 Regardless of the *in vivo* functionality, most linear polysaccharides have a strong
72 crystallization tendency. This characteristic has been exploited to prepare crystalline
73 specimens suitable for the crystallographic analysis as described below. Furthermore,
74 polysaccharides often exhibit polymorphism. Different crystalline allomorphs can be
75 obtained under different *in vitro* crystallization conditions. The polymorphism also occurs in
76 natural polysaccharide crystals (e.g. cellulose I α and I β , α - and β -chitin) [3,11].

77

78 **Crystallographic methods for polysaccharides in different geometries**

79 Conventional single crystal analysis is not applicable to the structural determination of
80 polysaccharides due to the lack of availability of their macroscopic crystals. Several other
81 crystallographic methods are available for microscopic polysaccharide crystals. Each of them
82 is applicable only to specific sample geometries as described below.

83

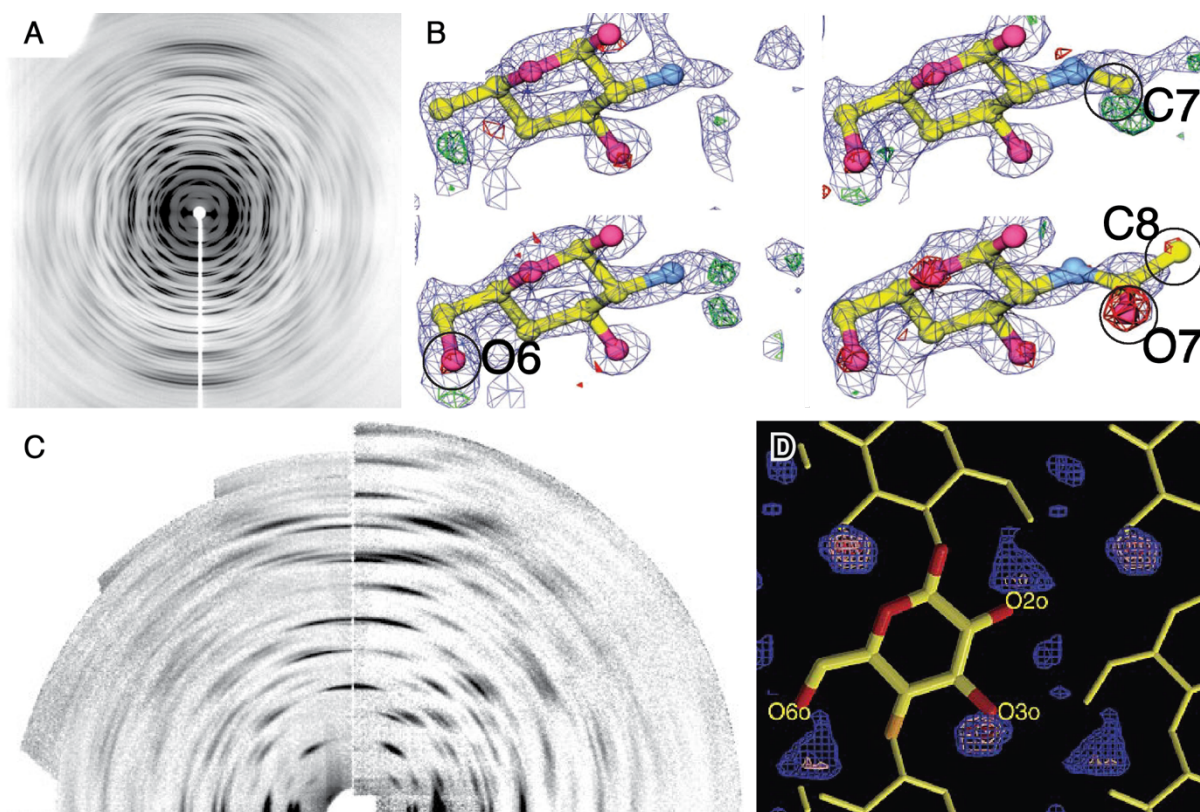
84 **Fibrous crystals**

85 Polycrystalline fibrous assemblies yield robust and reproducible fiber diffraction patterns
86 that contain invaluable structural information. One famous example is the X-ray fiber
87 diffraction pattern of DNA that led to the model of its helical structure (Nobel prize 1962)
88 through a trial-and-error model building. Fibrous polysaccharides have been providing such
89 fiber diffraction patterns since over a century [12]. The prerequisite of the fiber diffraction
90 analysis is the cylindrical symmetry of the crystallites along the fiber axis. While there are
91 some specimens that naturally possess individual crystallites in that symmetry (e.g.
92 cellulosic fibers), there are many different methods reported to prepare such fibrous
93 specimens from non-oriented polysaccharide crystallites. Examples include uniaxial
94 orientation of fibrous nanocrystals in a soft matrix [13,14] and recrystallization of stretched
95 films of polysaccharides [15]. In addition, crystalline domains in spherulites are also locally
96 arranged in a cylindrical symmetry. They thus provide fiber diffraction patterns when
97 probed at their periphery with small X-ray beams [16]. Fiber diffraction patterns were
98 successfully collected from large starch granules using microfocus X-ray beams [17,18].

99 In the 1960-80s, crystal structures were refined based on *a priori* constraints such as
100 interatomic distances and bond angles in the molecules (cf. linked-atom least square
101 refinement, or LALS). This led to a series of structural models of polysaccharide crystals with
102 limited data available at the time [19–22]. These early structures are still reliable as
103 approximations, largely due to the low degree of freedom of polysaccharides in the dry
104 unsolvated crystalline state, but sometimes fail to distinguish chain polarities, for example
105 [23].

106 In the late 1990s, highly resolved fiber diffraction patterns became available with
107 synchrotron X-ray sources becoming accessible, and more significantly large-area detection
108 systems with high-spatial resolution and dynamic range (Fig. 2A). They have resulted in a

109 series of atomic-resolution crystal structures of cellulose [24–28] and chitin [29–31]
110 allomorphs as well as chitosan [32,33]. Unlike the previous structures, they were refined
111 without strong geometric constraints. The positions of flexible side groups such as
112 hydroxymethyl groups were determined based directly on Fourier maps (Fig. 2B). In some
113 cases, hydroxy hydrogen positions were also refined based on neutron fiber diffraction
114 analyses coupled with intracrystalline deuteration (Fig. 2C,D) [14,25–27,33,34]. The
115 localization of hydrogen atoms allowed the unambiguous determination of the hydrogen
116 bonding networks in the crystal structures. These high-resolution fiber diffraction structures
117 serve as the structural basis in the various research fields of carbohydrate science, from
118 materials science to glycobiology.



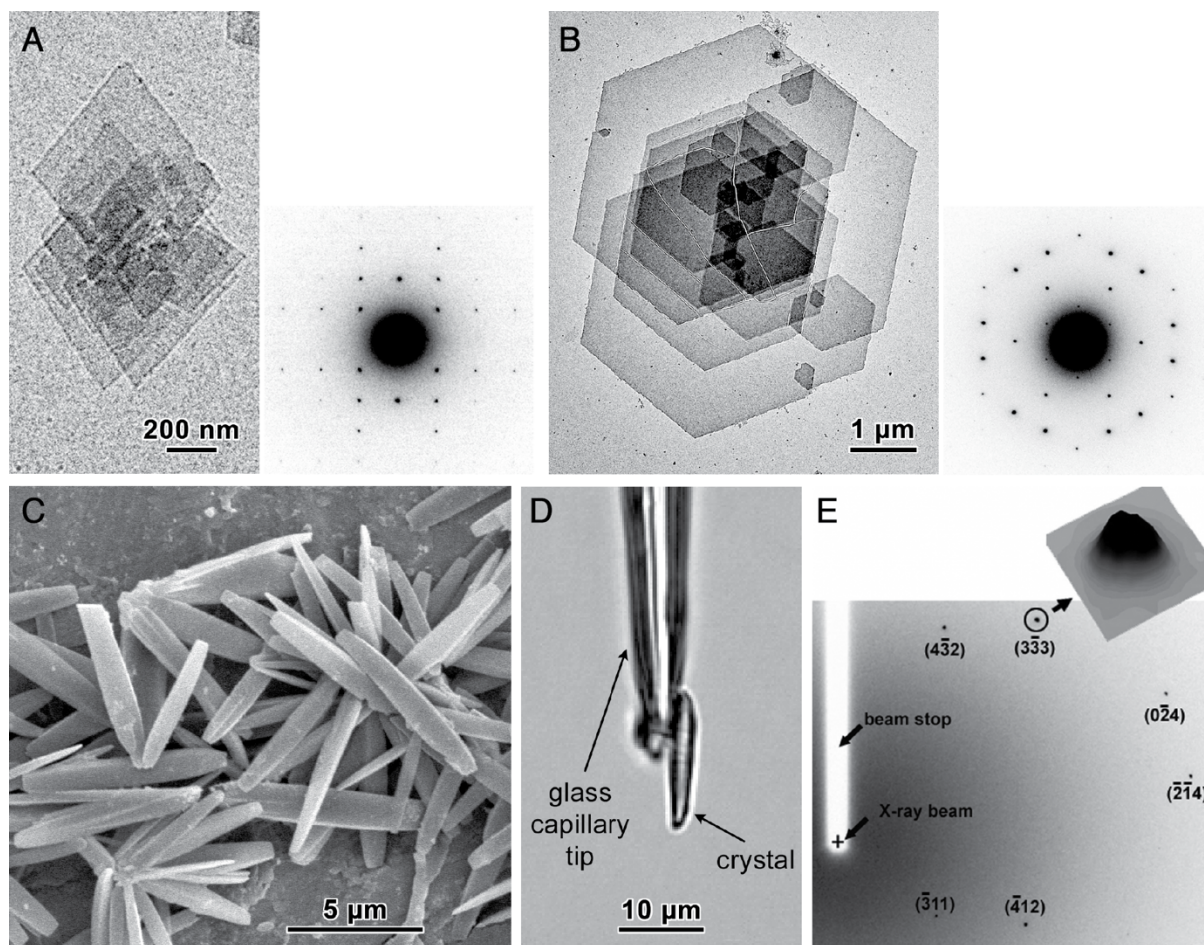
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Figure 2. Fiber diffraction analysis of polysaccharide crystals. (A) X-ray fiber diffraction pattern of anhydrous β -chitin with a resolution of 1 Å. (B) Fourier OMIT map analysis of anhydrous β -chitin based on the intensity data extracted from the diffraction pattern shown in (A). Acetamide side chain structure is refined based on the Fourier maps. Adapted with permission from Nishiyama et al. [28] (C) Neutron fiber diffraction pattern of hydrogenated (left) and deuterated (right) cellulose I β . (D) Fourier OMIT map analysis of cellulose I β based on the intensity data extracted from the diffraction patterns shown in (C). The density peaks indicate the positions of deuterium. Adapted with permission from Nishiyama et al. [24]

120

121 **Microscopic single crystals**

122 When polysaccharides are crystallized into microscopic single crystals, typically with at
123 least one dimension larger than a few hundreds of nanometers, single crystal
124 crystallography is applicable. Such crystals can be prepared by *in vitro* crystallization. In
125 many cases, *in vitro* crystallization of linear polysaccharides results in thin lamellar crystals
126 with a thickness of a few to tens of nanometers, suitable for electron diffraction
127 experiments (Fig. 3A,B) [35,36]. The acquisition of high-resolution electron diffraction data
128 of polysaccharide crystals is challenging due to their intrinsic radiation sensitivity. Despite
129 this challenge, several electron crystal structures of polysaccharides have been solved in the
130 1980-90s, including those of mannan [37], inulin [38], A-amylose[39] and anhydrous
131 chitosan [40]. More recently, the structure of polysaccharide-solvent complexes (e.g. V-
132 amylose) has been solved at near atomic resolution based on electron diffraction
133 experiments carried out under cryogenic condition [41–43]. These crystallographic analyses
134 were mostly based on single two-dimensional diffraction patterns recorded with the
135 electron beam perpendicular to the lamellar plane, *i.e.*, the crystals were thus treated as 2D
136 arrays of polysaccharide molecules. In some cases, three-dimensional diffraction data could
137 be collected by rotating the crystals along selected low-index crystallographic axes
138 [38,39,44]. Since the chain axes of polysaccharides are generally perpendicular to the
139 lamellar plane, the analysis of base-plane diffraction data can lead to inaccurate structural
140 refinement along the chain direction, as was demonstrated, for instance, by comparing X-
141 ray and electron structures of anhydrous chitosan [32]. The implementation of 3D electron
142 crystallography methods, such as microbeam electron diffraction (microED), is expected to
143 overcome this problem [45]. The 3D ED methods enable the collection of diffraction data
144 during sample rotation, thus covering a large reciprocal space volume.



145

Figure 3. Single crystal analysis of polysaccharides. (A,B) Transmission electron micrographs and corresponding electron diffraction patterns of lamellar crystals of mannan I (A) (adapted from Grimaud et al. [35] with permission) and V-amylose inclusion complex with myristic acid (B) [38]. (C) Scanning electron micrograph of A-amylose single crystals. (D) A single crystal mounted on a tip of glass capillary for microfocus X-ray diffraction measurement. (E) An example of diffraction diagram collected from one A-amylose single crystal using microfocused X-rays (reproduced from Popov et al. [45] with permission).

146

147 When polysaccharides grow into larger 3D crystals, X-ray single crystal analysis can be
 148 used based on micro- or nanofocus X-ray beams. To date, the only example is the crystal
 149 structure of A-amylose with 1.3 Å resolution data using acicular single crystals probed by a
 150 synchrotron X-ray microbeam [46]. Similarly to electron diffraction experiments, the
 151 radiation damage becomes significant when using microfocus X-rays [47]. In this analysis,
 152 the complete dataset was obtained by merging data from different crystals to limit the
 153 detrimental effects of radiation damage. Despite being powerful and robust, the method is

154 underused largely due to the lack of availability of micron-sized 3D crystals and the
155 accessibility to microdiffraction setups available only at synchrotron beamlines.

156

157 **Crystalline powders**

158 Fiber X-ray and single crystal electron diffraction techniques are not always available in
159 carbohydrate chemistry laboratories. Powder X-ray diffraction measurement using a
160 standard diffractometer is often the primary option for structural characterization. This
161 situation resulted in many powder diffraction patterns of polysaccharide crystals in the
162 literature whose structures are not determined so far [5,48]. Such powder diffraction
163 patterns contain useful structural information, but the analysis is not straightforward. For an
164 unknown structure, the unit cell determination, in particular indexing, is challenging partly
165 due to the limited crystallite size that contributes to the line broadening and peak overlaps.
166 Combining single-crystal or fiber methods is crucial at this step. For instance, electron
167 diffraction has played a key role in the unit cell determination of polysaccharide crystals as
168 demonstrated in the unambiguous identification of unit cells of two native cellulose
169 allomorphs [11]. A similar approach can be taken for further structural investigation of these
170 polysaccharide crystals.

171

172 **Molecular simulation as a complementary technique**

173 The use of molecular modelling, in particular force-field methods, is common practice in
174 the protein X-ray structure refinement process since the 1990s. Examples of such methods
175 include simulated annealing [49] and ensemble refinement (e.g. Phenix program) [50]. It
176 was used to overcome the problem of many-minima that hinders the search of the “most
177 likely” model. This approach has been extended to neutron and electron diffraction analyses
178 as well as cryogenic electron microscopy (cryoEM) structures. However, so far, it has not
179 been integrated into the atomic-resolution fiber diffraction analysis and thus hardly used in
180 the structure refinement of polysaccharide crystals.

181 In recent years, the force-field-based molecular dynamics (MD) simulations and
182 periodic density functional theory (DFT) calculations have been utilized to analyze the
183 hydrogen bonding patterns in cellulose allomorphs and anhydrous chitosan [33,51]. This
184 approach has provided useful insights into the geometry of hydrogen bonds in

185 polysaccharide structures and proved the effectiveness of computational methods in
186 complementing the neutron fiber diffraction analysis.

187

188 **Crystallographic analysis of biological systems**

189 In addition to information about the molecular arrangement in space, the
190 crystallographic information can provide insights into the biosynthesis mechanisms of
191 natural polysaccharide crystals. A texture analysis of *Valonia* cell wall based on fiber
192 diffraction revealed the existence of microfibrils running in opposite sense, suggesting a
193 movement of cellulose synthetic complex on the cell membrane [52]. A similar texture
194 analysis was performed on a housing tube of deep-sea tubeworm. The unipolar orientation
195 of chitin microfibrils in the tube implied the direct connection between the chitin secretion
196 and the motion of the worm [53].

197 Conversely, scientific discoveries regarding biosynthetic and/or biodegradation
198 mechanisms have advanced our understanding of polysaccharide crystal structure. For
199 instance, the directionality of cellulose and chitin molecules in the unit cells were
200 experimentally determined based on the electron diffraction measurements and the
201 knowledge of the chain elongation, by addition of monomers to the non-reducing end of the
202 existing chain [54,55].

203

204 **Current challenges and future directions in polysaccharide crystallography**

205 Over the last two decades, a series of high-resolution crystal structures of
206 polysaccharides were reported based on highly resolved X-ray/neutron fiber diffraction
207 data. This led to the detailed understanding of molecular interactions in cellulose
208 allomorphs and their analogues. However, these reliable crystal structures are mostly those
209 of crystals exhibiting a high-symmetry and a low degree of freedom. The samples are also
210 limited to the fibrous crystalline assemblies with large individual crystallite size. Many
211 crystalline polysaccharides do not fall into this specific category. Further methodological
212 developments are expected in the field of polysaccharide crystallography to tackle more
213 complex and yet relevant crystal structures.

214 One of such challenging structures is that of polysaccharide-solvent crystalline
215 complexes. These crystallosolvates are of great interest because they provide clues for the
216 understanding of solute-solvent interactions that govern the processing of materials. The

217 incorporation of guest molecules is also of interest to understand and design the
218 functionality of materials such as drug delivery or separation. Although several structures of
219 relatively simple crystallosolvates, such as cellulose-amine complexes, have been reported
220 [56,57], many crystallosolvates with larger and more complex guest molecules are yet to be
221 studied. Improved experimental data have become available for many such structures in
222 recent years [16,58,59]. They are, however, not yet producing reliable molecular models
223 due to the high degree of freedom and dynamic nature of the system and the lack of an
224 established method to explore all the potential molecular arrangements. In addition, some
225 parts of the structure such as guest molecules can adopt non-crystallographic positions, and
226 there are cases for which the crystal symmetry is only due to the spatial and time average.
227 For instance, in the chitin-hydrazine complex, the molecular dynamics simulation showed
228 that the positions of two nitrogen atoms were not fixed but interchangeable at room
229 temperature [60]. To investigate the large conformational space and the dynamic aspects of
230 the structures and the molecular interactions, it is necessary to fully integrate the molecular
231 modelling methods into the fiber diffraction analysis. Both force-field and *ab initio* methods
232 are useful for investigating different aspects of such dynamical structures as demonstrated
233 in the case of the chitin-hydrazine complex [60].

234 The full implementation of molecular modelling in the fiber diffraction method would
235 benefit structural analysis of polysaccharide crystals with defects and disorders. For
236 instance, many practical cellulosic materials are composed of crystallites much smaller than
237 model cellulose crystals used in the crystallographic analyses. These smaller crystals
238 generally contain more disorder and defects. While small or disordered crystals would not
239 yield high-resolution diffraction data, their fiber diffraction patterns provide rich structural
240 information [61]. Quantitative comparison of the experimental diffraction patterns and
241 those predicted from molecular simulations should shed light on the molecular details of
242 the disordered systems.

243

244 **Declaration of competing interest**

245 The authors declare that they have no competing financial interest or personal
246 relationships that influence the work reported in this paper.

247

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251

252 **References**

253 * of special interest

254 ** of outstanding interest

255

- 256 1. Aspinall GO: **Nature, occurrence and classification of polysaccharides**. In
257 "Polysaccharides", Aspinall GO ed., 1970, chap. 1, pp. 1–12.
258 <https://doi.org/10.1016/C2013-0-10317-0>
- 259 2. Nishiyama Y: **Structure and properties of the cellulose microfibril**. *J Wood Sci* 2009,
260 **55**:241–249. <https://doi.org/10.1007/s10086-009-1029-1>
- 261 3. Rinaudo M: **Chitin and chitosan: properties and applications**. *Prog Polym Sci* 2006,
262 **31**:603–632. <https://doi.org/10.1016/j.progpolymsci.2006.06.001>
- 263 4. Pérez S, Bertoft E: **The molecular structures of starch components and their**
264 **contribution to the architecture of starch granules: A comprehensive review**.
265 *Starch/Stärke* 2010, **62**: 389–420. <https://doi.org/10.1002/star.201000013>
- 266 5. Kiss JZ, Roberts EM, Brown RM Jr, Triemer RE: **X-ray and dissolution studies of**
267 **paramylon storage granules from *Euglena***. *Protoplasma* 1988, **146**:150–156.
268 <https://doi.org/10.1007/BF01405924>
- 269 6. Hendry G: **The ecological significance of fructan in a contemporary flora**. *New Phytol*
270 1987, **106**:201–216. <https://doi.org/10.1111/j.1469-8137.1987.tb04690.x>
- 271 7. Calder PC: **Glycogen structure and biogenesis**. *Int J Biochem* 1991, **23**:1335–1352.
272 [https://doi.org/10.1016/0020-711X\(91\)90274-Q](https://doi.org/10.1016/0020-711X(91)90274-Q)
- 273 8. Chanzy HD, Grosrenaud A, Vuong R, Mackie W: **The crystalline polymorphism of**
274 **mannan in plant cell walls and after recrystallisation**. *Planta* 1984, **161**:320–329.
275 <https://doi.org/10.1007/BF00398722>
- 276 9. Hargittai I, Hargittai M: **Molecular structure of hyaluronan: an introduction**. *Struct*
277 *Chem* 2008, **19**:697–717. <https://doi.org/10.1007/s11224-008-9370-3>
- 278 10. Park YB, Cosgrove DJ: **Xyloglucan and its interactions with other components of the**
279 **growing cell wall**. *Plant Cell Physiol* 2015, **56**:180–194.
280 <https://doi.org/10.1093/pcp/pcu204>
- 281 11. Sugiyama J, Vuong R, Chanzy H: **Electron diffraction study on the two crystalline**
282 **phases occurring in native cellulose from an algal cell wall**. *Macromolecules* 1991,
283 **24**:4168–4175. <https://doi.org/10.1021/ma00014a033>
- 284 12. Nishikawa S, Ono S: **Transmission of X-rays through fibrous, lamellar and granular**
285 **substances**. *Proc Tokyo Math-Phys Soc 2nd Ser* 1913, **7**:131–138.
- 286 13. Blackwell J, Parker KD, Rudall KM: **Chitin in pogonophore tubes**. *J Mar Biol* 1965,
287 **45**:659–661. <https://doi.org/10.1017/S0025315400016489>

- 288 14. Nishiyama Y, Johnson GP, French AD, Forsyth VT, Langan P: **Neutron crystallography,**
 289 **molecular dynamics, and quantum mechanics studies of the nature of hydrogen**
 290 **bonding in cellulose I β .** *Biomacromolecules* 2008, **9**:3133–3140.
 291 <https://doi.org/10.1021/bm800726v>
- 292 15. Wu H-CH, Sarko A: **The double-helical molecular structure of crystalline B-amylose.**
 293 *Carbohydr Res* 1978, **61**:7–25. [https://doi.org/10.1016/S0008-6215\(00\)84464-X](https://doi.org/10.1016/S0008-6215(00)84464-X)
- 294 16.* Song G, Zhang J, Nishiyama Y: **Twisted pseudo-tetragonal orthorhombic lamellar**
 295 **crystal in cellulose/ionic liquid spherulite.** *Cellulose* 2020, **27**:5449–5455.
 296 <https://doi.org/10.1007/s10570-020-03214-6>
- 297 This study shows that the spherulites of cellulose-ionic liquid complex provide high-
 298 resolution fiber diffraction patterns, suggesting possible new approach for structural
 299 characterization of polysaccharide crystallo-solvates.
- 300 17. Chanzy H, Putaux J-L, Dupeyre D, Davies R, Burghammer M, Montanar, S, Riekel C:
 301 **Morphological and structural aspects of the giant starch granules from *Phajus***
 302 ***grandifolius*.** *J Struct Biol* 2006, **154**:100–110.
 303 <https://doi.org/10.1016/j.jsb.2005.11.007>
- 304 18. Lemke H, Burghammer M, Flot D, Rössle M, Riekel C: **Structural processes during**
 305 **starch granule hydration by synchrotron radiation microdiffraction.**
 306 *Biomacromolecules* 2004, **5**:1316–1324. <https://doi.org/10.1021/bm0499536>
- 307 19. Chandrasekaran R, Millane RP, Arnott S, Atkin EDT: **The crystal structure of gellan.**
 308 *Carbohydr Res* 1988, **175**:1–15. [https://doi.org/10.1016/0008-6215\(88\)80151-4](https://doi.org/10.1016/0008-6215(88)80151-4)
- 309 20. Atkins EDT, Farnell S, Burden C, Mackie W, Sheldrick B: **Crystalline structure and**
 310 **packing of mannan I.** *Biopolymers* 1988, **27**:1097–1105.
 311 <https://doi.org/10.1002/bip.360270705>
- 312 21. Deslandes Y, Marchessault RH, Sarko A: **Triple-helical structure of (1->3)- β -D-glucan.**
 313 *Macromolecules* 1980, **13**: 1466–1471. <https://doi.org/10.1021/ma60078a020>
- 314 22. Nishimura H, Okano T, Sarko A: **Mercerization of cellulose. 5. Crystal and molecular**
 315 **structure of Na-cellulose I.** *Macromolecules* 1991, **24**:759–770.
 316 <https://doi.org/10.1021/ma00003a020>
- 317 23. French AD, Howley PS: **Comparisons of structures proposed for cellulose.** In
 318 “Cellulose and wood – chemistry and technology”, Schuerch C ed., 1989, pp. 159–167.
- 319 24. Langan P, Nishiyama Y, Chanzy H: **X-ray structure of mercerized cellulose II at 1 Å**
 320 **resolution.** *Biomacromolecules* 2001, **2**:410–416. <https://doi.org/10.1021/bm005612q>
- 321 25. **Nishiyama Y, Langan P, Chanzy H: **Crystal structure and hydrogen-bonding system in**
 322 **cellulose I β from synchrotron X-ray and neutron fiber diffraction.** *J Am Chem Soc*
 323 2002, **124**:9074–9082. <https://doi.org/10.1021/ja0257319>
- 324 This study set the current standard of structure determination of polysaccharide
 325 crystals and fibrous crystals in general.
- 326 26. Nishiyama Y, Sugiyama, J., Chanzy, H., Langan P: **Crystal structure and hydrogen**
 327 **bonding system in cellulose I α from synchrotron X-ray and neutron fiber diffraction.**
 328 *J Am Chem Soc* 2003, **125**:14300–14306. <https://doi.org/10.1021/ja037055w>

- 329 27. Wada M, Chanzy H, Nishiyama Y, Langan P: **Cellulose III_I crystal structure and**
 330 **hydrogen bonding by synchrotron X-ray and neutron fiber diffraction.**
 331 *Macromolecules* 2004, **37**:8548–8555. <https://doi.org/10.1021/ma0485585>
- 332 28. Wada M, Heux L, Nishiyama Y, Langan P: **X-ray crystallographic, scanning microprobe**
 333 **X-ray diffraction, and cross-polarized/magic angle spinning ¹³C NMR studies of the**
 334 **structure of cellulose III_{II}.** *Biomacromolecules* 2009, **10**:302–309.
 335 <https://doi.org/10.1021/bm8010227>
- 336 29.** Nishiyama Y, Noishiki, Y., Wada, M., 2011. **X-ray structure of anhydrous β-chitin at 1**
 337 **Å resolution.** *Macromolecules* **44**:950–957. <https://doi.org/10.1021/ma102240r>
- 338 This work demonstrates the high capability of high-resolution fiber diffraction analysis
 339 where the bulky side chain structure (acetamide group) is refined using Fourier maps
 340 of atomic-resolution X-ray fiber diffraction data.
- 341 30. Sawada D, Nishiyama Y, Langan P, Forsyth VT, Kimura S, Wada M: **Water in crystalline**
 342 **fibers of dihydrate β-chitin results in unexpected absence of intramolecular**
 343 **hydrogen bonding.** *PLOS ONE* 2012, **7**:e39376.
 344 <https://doi.org/10.1371/journal.pone.0039376>
- 345 31. Sikorski P, Hori R, Wada M: **Revisit of α-chitin crystal structure using high resolution**
 346 **X-ray diffraction data.** *Biomacromolecules* 2009, **10**:1100–1105.
 347 <https://doi.org/10.1021/bm801251e>
- 348 32. Naito P-K, Ogawa Y, Sawada D, Nishiyama Y, Iwata T, Wada, M: **X-ray crystal structure**
 349 **of anhydrous chitosan at atomic resolution.** *Biopolymers* 2016, **105**:361–368.
 350 <https://doi.org/10.1002/bip.22818>
- 351 33. Ogawa Y, Naito P-K, Nishiyama Y: **Hydrogen-bonding network in anhydrous chitosan**
 352 **from neutron crystallography and periodic density functional theory calculations.**
 353 *Carbohydr Polym* 2019, **207**: 211–217. <https://doi.org/10.1016/j.carbpol.2018.11.042>
- 354 34. Langan P, Nishiyama Y, Chanzy H: **A revised structure and hydrogen-bonding system**
 355 **in cellulose II from a neutron fiber diffraction analysis.** *J Am Chem Soc* 1999,
 356 **121**:9940–9946. <https://doi.org/10.1021/ja9916254>
- 357 35. Chanzy H, Vuong R: **Ultrastructure and morphology of crystalline polysaccharides**, in
 358 "Polysaccharides: Topics in Structure and Morphology", ed. E.D.T. Atkins (Macmillan
 359 Press Ltd, London), 1985, pp. 41–71.
- 360 36. Grimaud F, Pizzut-Serin S, Tarquis L, Ladevèze S, Morel S, Putaux J-L, Potocki-Veronese
 361 G: **In vitro synthesis and crystallization of β-1,4-mannan.** *Biomacromolecules* 2019,
 362 **20**:846–853. <https://doi.org/10.1021/acs.biomac.8b01457>
- 363 37. Chanzy H, Perez S, Miller DP, Paradossi G, Winter WT: **An electron diffraction study of**
 364 **the mannan I crystal and molecular structure.** *Macromolecules* 1987, **20**: 2407–2413.
 365 <https://doi.org/10.1021/ma00176a014>
- 366 38. André I, Mazeau K, Tvaroska I, Putaux J-L, Winter WT, Taravel FR, Chanzy H: **Molecular**
 367 **and crystal structures of inulin from electron diffraction data.** *Macromolecules* 1996,
 368 **29**:4626–4635. <https://doi.org/10.1021/ma951799f>
- 369 39. Imberty A, Chanzy H., Pérez S: **The double-helical nature of the crystalline part of A-**
 370 **starch.** *J Mol Biol* 1988, **201**: 365–378. [https://doi.org/10.1016/0022-2836\(88\)90144-1](https://doi.org/10.1016/0022-2836(88)90144-1)

- 371 40. Mazeau K, Winter WT, Chanz, H: **Molecular and crystal structure of a high-**
372 **temperature polymorph of chitosan from electron diffraction data.** *Macromolecules*
373 1994, **27**:7606–7612. <https://doi.org/10.1021/ma00104a015>
- 374 41. Nishiyama Y, Mazeau K, Morin M, Cardoso MB, Chanzy H, Putaux J-L: **Molecular and**
375 **crystal structure of 7-fold V-amylose complexed with 2-propanol.** *Macromolecules*
376 2010, **43**: 8628–8636. <https://doi.org/10.1021/ma101794w>
- 377 42. Le CAK, Choisnard L, Wouessidjewe D, Putaux J-L: **Polymorphism of crystalline**
378 **complexes of amylose with fatty acids.** *Int J Biol Macromol* 2018, **119**:555–564.
379 <https://doi.org/10.1016/j.ijbiomac.2018.07.163>
- 380 43. Le CAK, Ogawa Y, Dubreuil F, Grimaud F, Mazeau K, Ziegler GR, Tanwar S, Nishiyama Y,
381 Potocki-Veronese G, Choisnard L, Wouessidjewe D, Putaux J-L: **Crystal and molecular**
382 **structure of V-amylose complexed with ibuprofen.** *Carbohydr Polym* 2021,
383 **261**:117885. <https://doi.org/10.1016/j.carbpol.2021.117885>
- 384 44. Cardoso MB, Putaux J-L, Nishiyama Y, Helbert W, Hÿtch M, Silveira NP, Chanzy H:
385 **Single crystals of V-amylose complexed with α -naphthol.** *Biomacromolecules* 2007,
386 **8**:1319–1326. <https://doi.org/10.1021/bm0611174>
- 387 45. Gim S, Fittolani G, Nishiyama Y, Seeberger PH, Ogawa Y, Delbianco M: **Supramolecular**
388 **assembly and chirality of synthetic carbohydrate materials.** *Angew Chem* 2020,
389 **132**:22766–22772. <https://doi.org/10.1002/ange.202008153>
- 390 46.** Popov D, Buléon A, Burghammer M, Chanzy H, Montesanti N, Putaux J-L, Potocki-
391 Véronèse G, Riekkel C: **Crystal structure of A-amylose: A revisit from synchrotron**
392 **microdiffraction analysis of single crystals.** *Macromolecules* 2009, **42**:1167–1174.
393 <https://doi.org/10.1021/ma801789j>
- 394 This work reports the single crystal analysis of a polysaccharide crystal using
395 microfocus X-ray beam. So far, this is the only report of single crystal X-ray structure of
396 polysaccharide.
- 397 47. Riekkel C, Burghammer M. Davies RJ, DiCola E, Koenig C, Lemke HT, Putaux J-L, Schoeder
398 S: **Primary and secondary radiation damage in starch granules.** *J. Synchrotron Radiat*
399 2010, **17**:743–750. <https://doi.org/10.1107/S0909049510028335>
- 400 48. Rigby NM, MacDougall AJ, Ring SG, Cairns P, Morris VJ, Gunning PA, **Observations on**
401 **the crystallization of oligogalacturonates.** *Carbohydr Res* 2000, **328**:235–239.
402 [https://doi.org/10.1016/S0008-6215\(00\)00094-X](https://doi.org/10.1016/S0008-6215(00)00094-X)
- 403 49. Brünger AT, Adams PD, Clore GM, DeLano WL, Gros P, Grosse-Kunstleve RW, Jiang JS,
404 Kuszewski J, Nilges M, Pannu NS, et al.: **Crystallography & NMR system: A new**
405 **software suite for macromolecular structure determination.** *Acta Crystallogr D Biol*
406 *Crystallogr* 1998, **54**:905–921. <https://doi.org/10.1107/s09074444998003254>
- 407 50. Liebschner D, Afonine PV, Baker ML, Bunkóczi G, Chen VB, Croll TI, Hintze B, Hung L-
408 W, Jain S, McCoy AJ, et al.: **Macromolecular structure determination using X-rays,**
409 **neutrons and electrons: recent developments in Phenix.** *Acta Crystallogr D Struct Biol*
410 2019, **75**:861–877. <https://doi.org/10.1107/S2059798319011471>
- 411 51. Chen P, Ogawa Y, Nishiyama Y, Bergenstråhle-Wohlert M, Mazeau K: **Alternative**
412 **hydrogen bond models of cellulose II and III_I based on molecular force-fields and**

- 413 **density functional theory. *Cellulose* 2015, **22**: 1485–1493.
414 <https://doi.org/10.1007/s10570-015-0589-z>**
- 415 52. Sugiyama J, Chanzy H, Revol JF: **On the polarity of cellulose in the cell wall of *Valonia*.**
416 *Planta* 1994, **193**:260–265. <https://doi.org/10.1007/BF00192539>
- 417 53. Ogawa Y, Kobayashi K, Kimura S, Nishiyama Y, Wada M, Kuga S: **X-ray texture analysis**
418 **indicates downward spinning of chitin microfibrils in tubeworm tube.** *J Struct Biol*
419 2013, **184**:212–216. <https://doi.org/10.1016/j.jsb.2013.10.008>
- 420 54. Koyama M, Helbert W, Imai T, Sugiyama J, Henrissat B: **Parallel-up structure**
421 **evidences the molecular directionality during biosynthesis of bacterial cellulose.**
422 *Proc Natl Acad Sci USA* 1997, **94**:9091–9095. <https://doi.org/10.1073/pnas.94.17.9091>
- 423 55. Imai T, Watanabe T, Yui T, Sugiyama J: **The directionality of chitin biosynthesis: a**
424 **revisit.** *Biochem J* 2003, **374**:755–760. <https://doi.org/10.1042/bj20030145>
- 425 56. Wada M, Nishiyama Y, Langan P: **X-ray Structure of ammonia–cellulose I: New**
426 **insights into the conversion of cellulose I to cellulose III_I.** *Macromolecules* 2006,
427 **39**:2947–2952. <https://doi.org/10.1021/ma060228s>
- 428 57. Sawada D, Nishiyama Y, Petridis L, Parthasarathi R, Gnanakaran S, Forsyth VT, Wada
429 M, Langan P: **Structure and dynamics of a complex of cellulose with EDA: insights**
430 **into the action of amines on cellulose.** *Cellulose* 2013, **20**:1563–1571.
431 <https://doi.org/10.1007/s10570-013-9974-7>
- 432 58.** Li W, Ogawa Y, Perez J, Mazeau K, Morfin I, Nishiyama Y: **Fivefold helical cellulose**
433 **trapped in a sulfuric acid framework.** *Cryst Growth Des* 2022, **22**:20–25.
434 <https://doi.org/10.1021/acs.cgd.1c00664>
- 435 The study reports the first structure containing a five-fold helical conformation of non-
436 derivatized cellulose. It indicates the high conformational flexibility of polysaccharide
437 chain in crystallo-solvates.
- 438 59. Meng Z, Sawada D, Laine C, Ogawa Y, Virtanen T, Nishiyama Y, Tammelin T, Kontturi E:
439 **Bottom-up construction of xylan nanocrystals in dimethyl sulfoxide.**
440 *Biomacromolecules* 2021, **22**:898–906. <https://doi.org/10.1021/acs.biomac.0c01600>
- 441 60. Sawada D, Ogawa Y, Nishiyama Y, Togawa E, Kimura S, Langan P: **Molecular**
442 **interactions in an α -chitin/hydrazine complex: Dynamic hydrogen bonds and**
443 **improvement of polymeric crystallinity.** *Cryst Growth Design* 2016, **16**:3345–3352.
444 <https://doi.org/10.1021/acs.cgd.6b00315>
- 445 61. Müller M, Burghammer M, Sugiyama J: **Direct investigation of the structural**
446 **properties of tension wood cellulose microfibrils using microbeam X-ray fibre**
447 **diffraction.** *Holzforschung* 2006, **60**: 474–479. <https://doi.org/10.1515/HF.2006.078>
- 448