



University of Groningen

## Unraveling structure and dynamics by confocal microscopy

Manca, Marianna

**IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.**

*Document Version*

Publisher's PDF, also known as Version of record

*Publication date:*

2015

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Manca, M. (2015). Unraveling structure and dynamics by confocal microscopy: From starch to organic semiconductors. [Groningen]: University of Groningen.

**Copyright**

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

**Take-down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

# *Chapter 1*

## **Introduction**

*This Chapter offers a brief introduction to the properties and structure of the systems which have been investigated in this thesis: the formation of inclusion complexes in starch granules and organic bulk–heterojunctions.*

## **1.1 Introduction**

Confocal Laser Scanning Microscopy (CLSM) has been showing in the recent years a high potential as experimental tool in very different research fields, due to several advantages over conventional widefield optical microscopy. The ability to produce high quality images in a relative short time and ease, and the possibility to investigate also living tissues, make CLSM a very popular technique in biology and other fields, which are far removed from the traditional fields of application.

In this thesis we present two different applications of CLSM, exploiting the high spatial resolution and the high sensitivity of the photoluminescence detection.

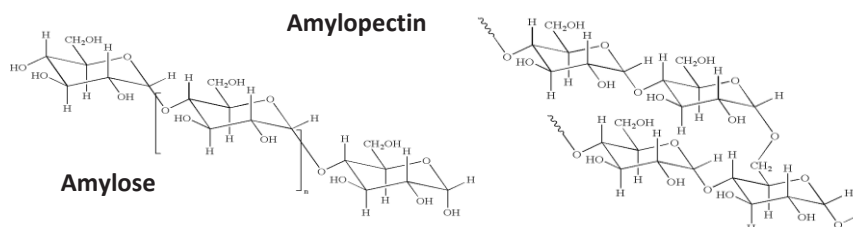
The first application we present regards the unravelling of inclusion complex formation in starch granules, a process which has many implications in both food and non-food industry; the second application is related to the investigation of photophysical properties of organic semiconductor heterostructures and the strategies to improve the efficiency of organic bulk-heterojunctions solar cells. In the following paragraphs an overview of the sample studied in this thesis, and an outline of the thesis are presented.

## **1.2 Revealing amylose-lipid inclusion complex formation**

Starch is the main storage reserve of carbohydrates for humans <sup>[1]</sup> and in amount the second most pervasive biopolymer present on earth after cellulose. <sup>[2]</sup> In the past few decades, starch and its functional properties have been the object of a broad research interest due to its very important role in the food and non-food industries. <sup>[3, 4]</sup>

Starch can be found in several botanical sources, such as potato, maize, wheat, rice, sorghum, cassava; these plants use starch as an energy reservoir to survive from one growing season to the other.

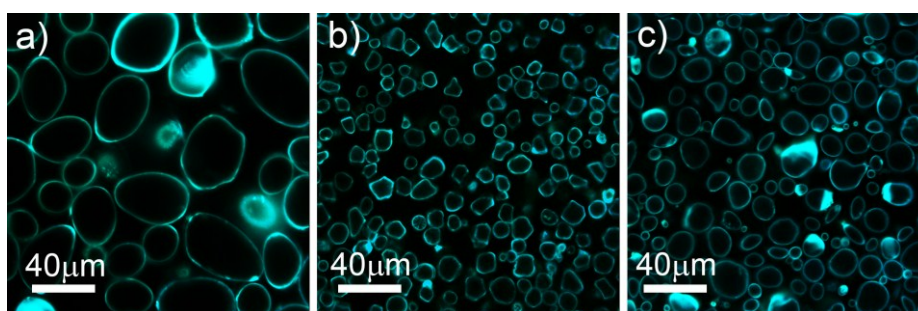
Native starch, which forms water-insoluble semi-crystalline granules, is mainly constituted by two polymers of D-glucose, amylopectin and amylose, with varying relative ratios - high amylose starch can present an amylose content up to 85%, while so-called waxy starch may reach up to 100% amylopectin content. <sup>[5]</sup> Amylose is essentially a linear  $\alpha[1\rightarrow4]$  linked glucan, while amylopectin is a branched polymer consisting of a linear  $\alpha[1\rightarrow4]$  linked glucan with a number of  $\alpha[1\rightarrow6]$  branching points. <sup>[6, 7]</sup>



**Figure 1.1:** Chemical structures of amylose and amylopectin.

**Figure 1.1** shows the chemical structures of amylose and amylopectin polymers. Depending on the botanical source, amylose and amylopectin are arranged in semi-crystalline granules of specific size, shape and morphology. For example, root and tuber starch granules are generally large with smooth surfaces, and round or elliptical in shape. Cereal starches often contain small pores, maize starch presents an irregular polygonal shape that tends to be smoother as the content of amylose increases, while wheat starch mainly consists of disk shaped granules. The different characteristics of potato, maize and wheat starch granules are evident in the CLSM micrographs shown in **Figure 1.2**.

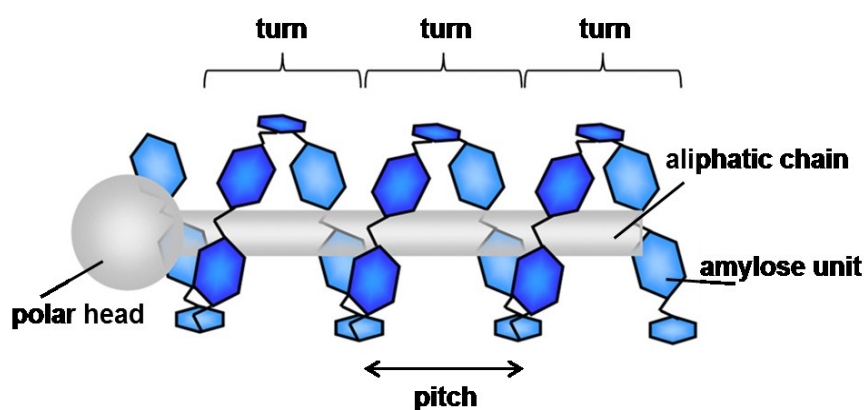
The common set of characteristics of starch granules belonging to the same botanical source can be related to the particular types and amounts of synthetic enzymes involved in the growth of the granules.<sup>[8]</sup>



**Figure 1.2:** CLSM measurements (field of view  $210 \mu\text{m} \times 210 \mu\text{m}$ ) of potato (a), maize (b) and wheat (c) starch granules in water suspension.

It has been demonstrated that the amylose contained in starch, in the presence of a ligand like iodine or linear alcohols, tends to assume a compact helical conformation that can complex with the ligand<sup>[9,10]</sup>, giving rise to the so called “V-amylose”.

V-amylose is structured as a left-handed single helix with a hydrophobic cavity, which is able to host the apolar aliphatic chain of a ligand, while the polar head tends to stay outside the amylose coil<sup>[10-12]</sup>, as depicted in **Figure 1.3**.



**Figure 1.3:** Complex formation between a left-handed single amylose helix and a ligand [based on Ref. 10].

The driving forces of this complex formation have been identified as determined by weak intramolecular bonds (van der Waals forces and hydrogen bonds) which take place between the turns of the amylose helix,<sup>[13,14]</sup> and intermolecular forces that stabilize the complex between the amylose chain and the ligand. Regarding the inclusion complex formation, many factors are still under debate; in particular, many studies are devoted to the understanding of the hydrophobic effect, which seems to be the leading force between the guest and the host.<sup>[11]</sup>

Optical Microscopy is one of the most exploited techniques for the visualization of starch granules.<sup>[15]</sup> Chapters 3 and 4 are devoted to the investigation of the localization of inclusion complex between amylose as a component of different types of starches from various botanical sources and a fatty acid functionalized with a chromophore. Chapter 5 is devoted to the study of the investigation of the dynamic of the inclusion complex formation. Live experiments were performed with the CLSM to estimate the time of interaction between the amylose chains at

the surface of the granules and the chromophore-functionalized fatty acids in a water based solution.

### 1.3 Charge transfer state in bulk-heterojunction

In recent years, organic bulk heterojunction (BHJ) solar cells<sup>[16,17]</sup> have garnered increased attention in the scientific community for their potential as ultra-low-cost photovoltaic technology.<sup>[18]</sup> Due to the latest progress, the result of a broad and international multidisciplinary effort, the maximum efficiency reported for organic solar cells (>10%) is approaching the performances of devices based on amorphous silicon.<sup>[19]</sup>

Currently, fundamental research on organic solar cells is focused on understanding the factors that affect their performance and on strategies for further improvement.

Despite the fundamental importance of the charge generation process for the optimization of organic solar cells, this aspect of their functioning is still not fully understood, and the debate is still open.<sup>[20,21]</sup> Recently, the formation of *charge transfer states* (CTSs) has been observed in several bulk-heterojunctions<sup>[22]</sup> and references therein. In the CTS state, the exciton is weakly bounded by Coulombic force, with the hole and the electron localized in the donor and acceptor material, respectively.<sup>[23]</sup> CTS recombination has been demonstrated to be detrimental for the overall efficiency of the device.<sup>[24,25]</sup> Therefore, a better understanding of the physics of the CTS in polymer: fullerene blends is crucial for the improvement of organic solar cells.

Previous studies indicate that the donor: acceptor interfacial area and the phase separation of the materials and their domain size play a crucial role in the formation of the CTS. Therefore, identifying the position of the CTS recombination in the BHJ will allow for addressing some of the relevant properties of this state, which have an impact for the charge generation and recombination in solar cells.

In chapter 6 we use CLSM and spatially resolved PL to correlate the blend morphology with the optical fingerprints of the CTS excited state, thus enabling the detection of the circumstances under which the CTS state population is formed. For the study we chose a prototypical BHJ composed by poly((2,7-(9,9-(di-n-octyl)fluorene)-alt-5,5-(4',7'-di-thienyl-2',1',3'-benzothiadiazole))) (APFO3) and [6,6]-phenyl-C61-butyric acid methyl ester (PCBM), which was reported by several authors to display CTS recombination.<sup>[26-28]</sup>

## **1.4 Outline of the thesis**

The aim of this thesis is to unravel the physical-chemical processes occurring in different types of samples (from starch to organic semiconductors thin films) by using Confocal Laser Scanning Microscopy to study their spatial localization (*microstructure*) and their dynamics.

In *Chapter 2* an overview of confocal microscopy and the other experimental techniques used in this thesis is presented.

In *Chapter 3* we present an investigation of the complex inclusion formation occurring between lipophilic molecules and the amylose polymers present in starch granules from different botanical sources. In natural starch granules we reveal complexation of lipophilic molecules labeled with fluorescein with amylose located at the periphery of the granules, appearing as a bright well-defined luminescent rim. This phenomenon occurs below the gelatinization point for very low concentrations of aliphatic chains. Control experiments performed with the fluorescein dye show staining of the whole granule, evidencing a non-specific interaction. Similarly, experiments performed with waxy granules (starch granules containing >98% amylopectin) show a broader luminescent rim when interacting with the lipid-dye molecules.

In *Chapter 4* we show that by combining Confocal Laser Scanning Microscopy with spatial resolved photoluminescence spectroscopy we are able to discriminate the presence of amylose in the peripheral region of regular and waxy granules from potato and corn starch, associating a clear optical fingerprint of the interaction between starch granules and lipophilic dye molecules. We show in particular that in the case of regular starch the polar head of the lipophilic dye molecules remains outside the amylose helix experiencing a water-based environment. Measurements performed on samples that have been extensively washed provide strong proof of the specific interaction between lipid dye molecules and amylose chains in regular starch. These measurements also confirm the tendency of longer amylopectin chains, located in the hilum of waxy starch granules, to form inclusion complexes with ligands. Through real-time recording of CLSM micrographs, a time frame within tens of seconds is measured for the occurrence of the inclusion process between lipids and amylose located at the periphery of starch granules.

In *Chapter 5* we report for the first time the study in real time of the dynamics of inclusion with the peripheral starch of the granules. This measurement demonstrates that the process is completed in about 10 seconds, giving a first upper

value for the dynamic of the process. This is further proof of the efficiency and specificity of the inclusion process between the amylose located in the periphery of the starch granules and lipid molecules.

In *Chapter 6* organic semiconductors thin films are investigated. Charge transfer state emission in organic bulk heterojunctions has been demonstrated to be an important loss mechanism; for this reason a better understanding of charge transfer state recombination is fundamental for the improvement of organic solar cells. Here, the relation between photophysical and morphological features of a prototypical organic bulk heterojunction is investigated in blends with different donor acceptor ratios. By correlating imaging with photoluminescence spectra measured in different areas of the blends, the charge transfer state emission is unambiguously assigned to microscopical regions in which the intermixing of the two organic semiconductors is higher.



## References

- [1] J. Juansang, C. Puttanlek, V. Rungsardthong, S. Pucha-arnon, D. Uttapap, *Food Chem.* **2012**, *131*, 500-507.
- [2] A.-C. Eliasson, *Starch in Food: Structure, Function and Applications*; CRC Press, **2004**.
- [3] M. M. Burrell, *J. Exp. Bot.* **2003**, *54*, 451-456.
- [4] L. Copeland, J. Blazek, H. Salman, M. C. Tang, *Food Hydrocoll.* **2009**, *23*, 1527-1534.
- [5] B. Zhang, Y. Zhao, X. Li, L. Li, F. Xie, L. Chen, *Food Hydrocoll.* **2014**, *35*, 700-709.
- [6] H. F. Zobel, *Starch - Stärke* **1988**, *40*, 44-50.
- [7] Q. Jiang, W. Gao, X. Li, J. Zhang, *Food Hydrocoll.* **2011**, *25*, 521-528.
- [8] J.-L. Jane, T. Kasemsuwan, S. Leas, H. Zobel, J. F. Robyt, *Starch - Stärke* **1994**, *46*, 121-129.
- [9] W. Saenger, *Naturwissenschaften* **1984**, *71*, 31-36.
- [10] J. A. Putseys, L. Lamberts, J. A. Delcour, *J. Cereal Sci.* **2010**, *51*, 238-247.
- [11] S. Immel, F. W. Lichtenthaler, *Starch - Stärke* **2000**, *52*, 1-8.
- [12] M. Tusch, J. Krüger, G. Fels, *J. Chem. Theory Comput.* **2011**, *7*, 2919-2928.
- [13] W. Banks, C. T. Greenwood, *Polymer* **1971**, *12*, 141-145.
- [14] J. Karkalas, S. Ma, W. R. Morrison, R. A. Pethrick, *Carbohydr. Res.* **1995**, *268*, 233-247.
- [15] G. E. Moss, In *Examination and Analysis of Starch and Starch Products*; Radley, J. A., Ed.; Springer Netherlands, **1976**; pp. 1-32.
- [16] C. J. Brabec, N. S. Sariciftci, J. C. Hummelen, *Adv. Funct. Mater.* **2001**, *11*, 15-26.
- [17] G. Yu, J. Gao, J. C. Hummelen, F. Wudl, A. J. Heeger, *Science* **1995**, *270*, 1789-1791.
- [18] G. Li, R. Zhu, Y. Yang, *Nat. Photonics* **2012**, *6*, 153-161.
- [19] M. A. Green, K. Emery, Y. Hishikawa, W. Warta, E. D. Dunlop, *Prog. Photovolt. Res. Appl.* **2012**, *20*, 606-614.
- [20] G. Grancini, M. Maiuri, D. Fazzi, A. Petrozza, H.-J. Egelhaaf, D. Brida, G. Cerullo, G. Lanzani, *Nat. Mater.* **2013**, *12*, 29-33.
- [21] B. Kraabel, D. McBranch, N. S. Sariciftci, D. Moses, A. J. Heeger, *Phys. Rev. B* **1994**, *50*, 18543-18552.
- [22] C. Piliago, M. A. Loi, *J. Mater. Chem.* **2012**, *22*, 4141-4150.
- [23] M. Pope, C.E. Swenberg, *Electronic Process in Organic Crystals*; 2nd ed.; Oxford University Press, Clarendon Press: Oxford; **1982**, Vol. ch.1.

- [24] M. C. Scharber, C. Lungenschmied, H.-J. Egelhaaf, G. Matt, M. Bednorz, T. Fromherz, J. Gao, D. Jarzab, M. A. Loi, *Energy Environ. Sci.* **2011**, *4*, 5077-5083.
- [25] M. C. Scharber, M. Koppe, J. Gao, F. Cordella, M. A. Loi, P. Denk, M. Morana, H. Egelhaaf, K. Forberich, G. Dennler, R. Gaudiana, D. Waller, Z. Zhu, X. Shi, C. J. Brabec, *Adv. Mater.* **2010**, *22*, 367-370.
- [26] M. A. Loi, S. Toffanin, M. Muccini, M. Forster, U. Scherf, M. Scharber, *Adv. Funct. Mater.* **2007**, *17*, 2111-2116.
- [27] D. Veldman, O. İpek, S. C. J. Meskers, J. Sweelssen, M. M. Koetse, S. C. Veenstra, J. M. Kroon, S. S. van Bavel, J. Loos, R. A. J. Janssen, *J. Am. Chem. Soc.* **2008**, *130*, 7721-7735.
- [28] K. Tvingstedt, K. Vandewal, A. Gadisa, F. Zhang, J. Manca, O. Inganäs, *J. Am. Chem. Soc.* **2009**, *131*, 11819-11824.

