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Novel Analytical Techniques for Studying the Milk Fat Globule Membrane

A thesis presented in partial fulfilment of the requirements for the degree of

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Abstract

Fat in milk and cream is present as tiny droplets, which are each enveloped in a thin membrane, called the milk fat globule membrane (MFGM). The MFGM can easily be damaged by factors such as pumping the milk and applying other forms of agitation. MFGM damage is believed to reduce processing efficiency and compromise the quality of manufactured products.

A comprehensive review of the literature showed that our understanding of changes occurring in the MFGM post secretion of the fat globule by the mammary secretory cell is still rudimentary. Furthermore, it was found that a fundamental understanding of MFGM damage in raw milk is lacking. Hence, this study sought to develop analytical techniques for studying the MFGM.

Fluorescent probes were identified that associated with the MFGM (bovine, ovine, human) in one of two ways: either by embedding in the phospholipid bilayer (lipophilic probe) or by binding to carbohydrate moieties of glycosylated chains in the glycocalyx (lectin probes). The use of these probes, in combination with either conventional fluorescence microscopy or confocal laser scanning microscopy, allowed 2-D images and 3-D images of fat globules to be made. Application of water-soluble lipophilic probes and the lectin wheat germ agglutinin (WGA) directly to milk allowed the staining of the MFGM in its native environment. Variable distribution patterns of the probes in the MFGM were observed, which suggests that the MFGM of fat globules in harvested milk is structurally and chemically heterogeneous both within and among globules from the same species and between species, and even among fat globules within the milk of an individual animal. Furthermore, the binding behaviour of WGA to the MFGM of native fat globules (in bovine milk) and washed fat globules (in model systems) following heat treatment implicated β-lactoglobulin, α-lactalbumin, immunoglobulin M and/or the glycosylated proteins Periodic acid Schiff 6/7 in the disappearance of fat globule aggregation upon elevated heat treatment of milk. The results of the current study showed that the use of membrane-specific fluorescent probes, particularly in combination with confocal laser scanning microscopy, has significant potential for providing real time structural and chemical information about the MFGM in matrices such as harvested milk and milk products.

In addition to the fluorescence microscopy techniques, development of other techniques was also conducted. Flow cytometry was shown to have significant potential for the quantitative determination of various properties of fat globules and their membranes. Although no suitable sample preparation technique could be developed in this study, atomic force microscopy is believed to have significant potential for studying structural and physical properties of the MFGM. Selective harvesting of individual fat globules was shown to be possible by using a micromanipulator. In future work, this technique is expected to be used in combination with fluorescence microscopy, or atomic force microscopy.

The present study has shown that the development and application of novel analytical techniques has advanced, and in the future will further advance, understanding of the MFGM.
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The great Creator of the universe of which I was privileged to study a tiny, tiny part. Delving into the microscopic world I found this to be true, “For the invisible things of him from the creation of the world are clearly seen, being understood by the things that are made, even his eternal power and Godhead” (Romans 1:20). Marvellous are thy works; and that my soul knoweth right well (Psalm 139.14b).

Take my life, and let it be consecrated, Lord, to thee; take my intellect, and use every power as thou shalt choose.¹

¹ Abridged from Frances Ridley Havergal (1874).
# Table of Contents

Abstract ......................................................................................................................................... iii  
Acknowledgements ......................................................................................................................... iv  
Table of Contents ............................................................................................................................ v  
List of Tables .................................................................................................................................... x  
List of Figures ................................................................................................................................... xi  
List of Video Clips and Movie ......................................................................................................... xv  
List of Abbreviations ..................................................................................................................... xvii

## CHAPTER 1

**General introduction, objectives and format of the thesis** .............................................................. 1  
1.1 General introduction .................................................................................................................. 1  
1.2 Objectives .................................................................................................................................. 1  
1.3 Format of the thesis ................................................................................................................... 2

## CHAPTER 2

**The MFGM — compositional and structural changes post secretion by the mammary secretory cell** ......................................................................................................................... 3  
2.1 Introduction ............................................................................................................................... 3  
2.2 Formation and secretion of milk fat globules by the mammary secretory cell ......................... 4  
\hspace{1cm} 2.2.1 Origin of milk fat globules ....................................................................................... 4  
\hspace{1cm} 2.2.2 Changes in the MFGM during and after secretion by the mammary secretory cell ........... 7  
2.3 Changes in the MFGM and fat globule during and after milk harvesting .................................. 8  
\hspace{1cm} 2.3.1 Some physical and mechanical factors affecting the stability of fat globules ............... 9  
\hspace{1cm} 2.3.2 Bacteriological quality and mastitis ............................................................................ 16  
\hspace{1cm} 2.3.3 Stage of lactation and season ................................................................................... 17  
2.4 Conclusions ............................................................................................................................. 19

## CHAPTER 3

**The MFGM — methodologies for measuring milk fat globule (membrane) damage** .............. 21  
3.1 Introduction .............................................................................................................................. 21  
3.2 Principles and application of methodology for measuring MFGM damage .............................. 21  
\hspace{1cm} 3.2.1 Definitions of milk fat globule damage and MFGM damage .................................... 21  
\hspace{1cm} 3.2.2 Methodologies used for measuring milk fat globule (membrane) damage .................. 25  
3.3 General discussion ................................................................................................................... 37

## CHAPTER 4

**Development of fluorescence microscopy techniques for studying the MFGM** ....................... 39  
4.1 Theoretical assessment of the potential suitability of fluorescent probes for staining the MFGM ............................................................................................................................ 41
CHAPTER 5

WGA-staining of the MFGM

5.1 Introduction

5.2 Control experiments
  
5.2.1 Verification that stained objects are fat globules
  
5.2.2 Degree of non-specific binding of WGA488 to MFGM
  
5.2.3 WGA binding to MFGM — Effect of Alexa Fluor® conjugate
  
5.2.4 Heat stability of WGA
  
5.2.5 Heat treatment of milk at 55°C and 90°C
  
5.2.6 Heat treatment of WGA488 stock
  
5.2.7 Heat treatment of WGA488-stained milk followed by addition of WGA488
  
5.2.8 Discussion & conclusions

5.3 Reconstitution, heat treatment and staining of washed fat globules
5.4 Effect of milk plasma proteins on fluorescence intensity of WGA-stained MFGM
5.4.1 Caseins..........................88
5.4.2 Whey protein isolates..................90
5.4.3 β-Lactoglobulin, α-lactalbumin, bovine serum albumin and immunoglobulin G .....92

5.5 Effect of the protein concentration on the fluorescence intensity of WGA488-stained fat globules
5.5.1 β-Lactoglobulin..........................95
5.5.2 IgG and BSA..........................97

5.6 Fat globule aggregation — What promotes it?
5.6.1 Effect of PBS and WGA488..................99
5.6.2 Effect of pH..........................100
5.6.3 Effect of NaCl concentration..............100

5.7 Effect of WGA concentration on fat globule staining efficacy
5.7.1 Effect of different concentrations of WGA488 on the staining of fat globules........102
5.7.2 Addition of washed cream to WGA488 stock..........................105
5.7.3 Effect of Alexa Fluor® conjugate...............106

5.8 General discussion
5.8.1 Assessing fat globules in WGA-stained milk using fluorescence microscopy........106
5.8.2 Conventional fluorescence microscopy allows accurate detection of gross differences in fluorescence intensity of WGA-stained fat globules...............108
5.8.3 Fat globule aggregation..................109
5.8.4 Factors influencing the degree of fluorescence of stained fat globules in model systems..........................117
5.8.5 Effect of WGA concentration on the staining of fat globules in milk...............118

5.9 General discussion and conclusions ..................119

CHAPTER 6
Structural and compositional features of the MFGM as revealed by fluorescence microscopy — a preliminary evaluation
6.1 Introduction..................................121
6.2 Double fluorescent rings..................121
6.2.1 Microscope objective..........................122
6.2.2 Fluorophore..........................124
6.2.3 Pinhole size..........................124
6.2.4 Sample matrix..........................125
6.2.5 Effect of heating..........................126
6.2.6 Multiple rings..........................126
6.2.7 Discussion..........................127

6.3 Heterogeneity within fat globules and between fat globules within species...............130
6.4 Similarities and differences between fat globules from different species...............134
<table>
<thead>
<tr>
<th>Chapter Section</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.5</td>
<td>Heat treatment of milk</td>
<td>137</td>
</tr>
<tr>
<td>6.6</td>
<td>Cytoplasmic crescents</td>
<td>139</td>
</tr>
<tr>
<td>6.7</td>
<td>General discussion and conclusions</td>
<td>144</td>
</tr>
<tr>
<td>6.7.1</td>
<td>Background interference and photobleaching</td>
<td>144</td>
</tr>
<tr>
<td>6.7.2</td>
<td>3-D imaging</td>
<td>145</td>
</tr>
<tr>
<td>6.7.3</td>
<td>Do the probes perturb the membrane?</td>
<td>146</td>
</tr>
<tr>
<td>6.7.4</td>
<td>Final considerations</td>
<td>147</td>
</tr>
<tr>
<td>7</td>
<td>Other techniques — the potential</td>
<td>149</td>
</tr>
<tr>
<td>7.1</td>
<td>Atomic force microscopy</td>
<td>149</td>
</tr>
<tr>
<td>7.1.1</td>
<td>Introduction</td>
<td>149</td>
</tr>
<tr>
<td>7.1.2</td>
<td>Principle of operation of the AFM</td>
<td>151</td>
</tr>
<tr>
<td>7.1.3</td>
<td>Operating modes</td>
<td>152</td>
</tr>
<tr>
<td>7.1.4</td>
<td>Cantilevers/tips</td>
<td>153</td>
</tr>
<tr>
<td>7.1.5</td>
<td>Experimental techniques and results</td>
<td>153</td>
</tr>
<tr>
<td>7.1.6</td>
<td>General discussion, conclusions and future work</td>
<td>159</td>
</tr>
<tr>
<td>7.2</td>
<td>Micromanipulator</td>
<td>161</td>
</tr>
<tr>
<td>7.2.1</td>
<td>Introduction</td>
<td>161</td>
</tr>
<tr>
<td>7.2.2</td>
<td>Materials and equipment</td>
<td>161</td>
</tr>
<tr>
<td>7.2.3</td>
<td>Experimental techniques and results</td>
<td>163</td>
</tr>
<tr>
<td>7.2.4</td>
<td>Discussion</td>
<td>166</td>
</tr>
<tr>
<td>7.2.5</td>
<td>Conclusions</td>
<td>169</td>
</tr>
<tr>
<td>7.3</td>
<td>Flow cytometry</td>
<td>169</td>
</tr>
<tr>
<td>7.3.1</td>
<td>Introduction</td>
<td>169</td>
</tr>
<tr>
<td>7.3.2</td>
<td>Principle of flow cytometry</td>
<td>169</td>
</tr>
<tr>
<td>7.3.3</td>
<td>Experimental and results</td>
<td>170</td>
</tr>
<tr>
<td>7.3.4</td>
<td>Discussion</td>
<td>171</td>
</tr>
<tr>
<td>7.3.5</td>
<td>Conclusions</td>
<td>173</td>
</tr>
<tr>
<td>8</td>
<td>General discussion, conclusions and future work</td>
<td>175</td>
</tr>
<tr>
<td>8.1</td>
<td>Fluorescence microscopy</td>
<td>176</td>
</tr>
<tr>
<td>8.1.1</td>
<td>Location of fluorescent probes in the MFGM</td>
<td>176</td>
</tr>
<tr>
<td>8.1.2</td>
<td>Potential of fluorescent probes</td>
<td>177</td>
</tr>
<tr>
<td>8.1.3</td>
<td>Key conclusions</td>
<td>183</td>
</tr>
<tr>
<td>8.2</td>
<td>Other techniques</td>
<td>183</td>
</tr>
<tr>
<td>8.2.1</td>
<td>AFM</td>
<td>183</td>
</tr>
<tr>
<td>8.2.2</td>
<td>Micromanipulator</td>
<td>184</td>
</tr>
<tr>
<td>8.2.3</td>
<td>Flow cytometry</td>
<td>184</td>
</tr>
</tbody>
</table>
8.3 Final conclusions and future work ................................................................. 185

BIBLIOGRAPHY .................................................................................................... 187

APPENDICES
Appendix 1 List of fluorescent probes that were deemed to have potential as MFGM probes .............................................................................................................. 205
Appendix 2 Principle of operation of the confocal laser scanning microscope (CLSM) .......... 211
Appendix 3 Sample staining and microscopy imaging protocol ........................................ 213
Appendix 4 CLSM filter settings for selected fluorescent probes .................................... 215
Appendix 5 List of lectins that have been shown to bind to intact MFGM or isolated MFGM ........................................................................................................ 217
Appendix 6 Fat globule washing procedure ..................................................................... 221
Appendix 7 Image capturing protocol for conventional fluorescence microscopy ............... 223
Appendix 8 NMR spectroscopy .................................................................................. 225
Appendix 9 Summary of recommendations made in Chapters 2–7 ................................. 229
Appendix 10 Assessment of ethics of handling human milk .......................................... 233
List of Tables

CHAPTER 2
2.1 Summary of various factors and their effects on the bovine MFGM after the milk leaves the udder .................................................................................................................. 6

CHAPTER 3
3.1 Summary of the main techniques reported in the literature for measuring MFG(M) damage .................................................................................................................. 22

CHAPTER 4
4.1 Selected data for fluorescent probes that were purchased for initial screening ................. 43
4.2 Product codes, product/chemical names and structures (where available) for the screened fluorescent probes .................................................................................... 44
4.3 Summary of initial screening results for a range of fluorescent probes as observed by conventional fluorescence microscopy ................................................................. 49

CHAPTER 5
5.1 Experimental conditions of the heat stability experiment for WGA488 ............................. 85
5.2 Summary of results obtained for various model systems and heat treatments investigated for studying the effects of protein and buffer on WGA binding to washed fat globules .................................................................................................................. 93
5.3 Model system, protein concentration and treatment of washed fat globules ..................... 98
5.4 Composition of buffers (molar concentrations) ................................................................. 100
5.5 Volume of HCL (0.1 M) added to 30 mL of buffer stock, calculated chloride concentration and final pH ........................................................................................................ 101
5.6 Effects of several factors relating to on-slide fat globule aggregation ............................. 120

CHAPTER 7
7.1 Examples of dairy-related studies using AFM, as reported in the literature .................... 150
List of Figures

CHAPTER 2
2.1 Schematic of the fluid mosaic membrane according to Singer and Nicolson (1972) ............... 5
2.2 Overviews of the structure of the MFGM ........................................................................ 10
2.3 Schematic overview of fat globule membrane damage by air ....................................... 11

CHAPTER 3
3.1 Confocal laser scanning micrograph of a milk sample that was damaged by blending ....... 36

CHAPTER 4
4.1 Human milk fat globules stained with Nile Red and imaged using CLSM ..................... 39
4.2 Molecular structures of selected fluorescent probes reported in the literature as having been used for staining milk fat globules ................................................................. 40
4.3 Flow diagram of planned experimental approach for developing microscopy techniques ... 41
4.4 Molecular structure of D109 ......................................................................................... 44
4.5 Molecular structure of DiOC_{18}(3) ........................................................................... 44
4.6 Molecular structure of DiIC_{18}(3) ............................................................................ 44
4.7 Molecular structure of DiA ....................................................................................... 45
4.8 Molecular structure of DiIC_{16}(3)-DS ..................................................................... 45
4.9 Molecular structure of SP-DiIC_{16}(3) ...................................................................... 45
4.10 Molecular structure of SP-DiOC_{16}(3) .................................................................... 46
4.11 Molecular structure of 5,5'-Ph2-DiIC_{18}(3) ............................................................. 46
4.12 Molecular structure of N316 .................................................................................. 46
4.13 Molecular structure of FM4-64 .............................................................................. 46
4.14 Molecular structure of FM1-84 .............................................................................. 47
4.15 Conventional microscopy image (combined phase contrast and fluorescence) of a bovine milk sample stained with ConA488 ................................................................. 51
4.16 Conventional fluorescence microscopy image of DiA-stained (40 μg/mL milk) bovine fat globules ..................................................................................................................... 53
4.17 Human fat globules in milk stained using DiIC_{18}(3)-DS (aqueous stock) and imaged using conventional fluorescence microscopy ...................................................... 58
4.18 Conventional microscopy image of bovine milk stained with ConA488 showing the presence of a significant number of fat globule clusters .............................................. 61
4.19 CLSM image of a bovine fat globule the membrane of which was stained using DiIC_{18}(3)-DS (ethanolic stock) ............................................................... 63
4.20 2-D images of human fat globules .......................................................................... 64
4.21 3-D images of human fat globules stained by using DiOC_{18}(3), DiA or DiIC_{18}(3) ........ 66
4.22 WGA488-stained bovine fat globule in milk that was heat treated (75°C, 5 min) and shaken (30 s) ......................................................................................... 67
4.23 Conventional microscopy image of human milk fat globules to which DiA stock (ethanolic) and DiOC<sub>16</sub>(3) stock (ethanolic) had been added sequentially ................................................................. 68
4.24 Dual stained human fat globules .................................................................................................................................................... 70
4.25 Expected location and orientation of different types of fluorescent membrane probes in a phospholipid bilayer ......................................................................................................................... 74
4.26 Conventional fluorescence microscopy images of a fat globule dual-stained with DiIC<sub>18</sub>(3)-DS and DiOC<sub>16</sub>(3) using a combined probe stock .......................................................................................................................... 75
4.27 Chemical structure of tocopherol (vitamin E).............................................................................................................................................. 76

CHAPTER 5

5.1 Chemical structures of sugars specifically bound by WGA ............................................................................................................. 82
5.2 Testing the degree of non-specific binding of WGA488 to MFGM ........................................................................................................... 83
5.3 Milk dual stained with WGA488 (A) and WGA594 (B) ................................................................................................................................. 84
5.4 Washed fat globules re-suspended in Na-caseinate solution and stained with WGA488... 90
5.5 Illustration of typical shapes of dark patches observed by conventional fluorescence microscopy of WGA488-stained washed fat globules re-suspended in β-lactoglobulin (globular) solution .................................................................................................................. 97
5.6 Image showing absence or presence of aggregation of washed fat globules re-suspended in PBS in the absence or presence of WGA48 ................................................................................................................. 100
5.7 Heat treated (75°C, 5 min) bovine milk stained with WGA488 (final concentration 28 μg/mL) and viewed by conventional fluorescence microscopy ......................................................................................................................... 103
5.8 Heat treated (75°C, 5 min) bovine milk stained with WGA488 (final concentration 28 μg/mL) and viewed by simultaneous bright field and fluorescence microscopy .............. 104
5.9 Fluorescence image of WGA488-stained fat globules upon adding washed cream to WGA488 stock and reducing the microscope camera exposure time from 148 ms to 6 ms ........................................................................................................................................ 105
5.10 Possible mechanism for on-slide fat globule aggregation in model systems consisting of washed fat globules re-suspended in an aqueous environment................................................... 111
5.11 Possible mechanisms for the loss of fat globule aggregation properties, in the presence of WGA as a result of elevated heat treatment of bovine milk ........................................................................ 116

CHAPTER 6

6.1 Optical CLSM section of a human milk fat globule stained with DiIC<sub>18</sub>(3)-DS (ethanolic stock), showing the double ring phenomenon ......................................................................................................................... 123
6.2 Human milk fat globules imaged using low electronic zoom (< 2). Double rings are observed for the larger fat globules........................................................................................................................................ 123
6.3 3-D images of a human fat globule stained with DiIC<sub>18</sub>(3)-DS at low and high zoom .... 124
6.4 Dual stained milk fat globule. (A) WGA488. (B) FM4-64. Both probes show a double ring ........................................................................................................................................................................................................ 125
6.5 WGA488-stained human fat globules. Double rings are observed irrespective of pinhole size .......................................................... 126
6.6 Human fat globule stained with DilC16(3)-DS (ethanolic stock), showing four rings in some places ........................................................................ 127
6.7 2-D CLSM images of the surfaces of fat globules showing a variety of probe staining patterns ........................................................................ 131
6.8 Dual staining of human fat globules .................................................................................. 133
6.9 CLSM optical section showing part of the WGA488-stained membrane of an ovine fat globule .......................................................... 135
6.10 3-D images of bovine fat globules stained with WGA488 after heat treatment ............. 140
6.11 Conventional fluorescence microscopy image of heat-treated (75°C, 5 min) human fat globules stained with DilC16(3)-DS (aqueous stock; added prior to heat treatment) and counterstained with Nile Blue (added after heat treatment) ........................................................................ 143
6.12 Heat treated human fat globule stained with DilC16(3)-DS (aq) .................................. 143
6.13 Heat treated (75°C, 5 min) human fat globules dual stained with Nile Red and WGA488 and imaged using CLSM .................................................................................. 143
6.14 3-D images of heat treated human fat globules stained with DilC16(3)-DS. The crescents are of a rounded shape and sit on the fat globule like a large blister or sac .... 143

CHAPTER 7
7.1 The principle of the atomic force microscope (adapted from Morris et al., 1999a and Dufrène, 2004) .................................................................................. 151
7.2 Photo of the AFM-2 ........................................................................................................ 152
7.3 AFM deflection image of air-dried 10x diluted (aq) milk showing the footprint of a cluster of fat globules ........................................................................ 155
7.4 Differential interference contrast microscopy image of an air-dried 10x diluted (aq) milk sample ........................................................................ 156
7.5 3-D AFM deflection image of air-dried fat globules, cooled to 8°C prior to scanning in tapping mode in air ........................................................................ 157
7.6 Effect of drying temperature of washed fat globules as examined by light microscopy .... 158
7.7 Image of cold air-dried fat globules on mica. Scan performed at room temperature using the AMF-2. Only footprints remained after scanning ........................................................................ 159
7.8 Photo of the complete set of equipment used to manipulate individual fat globules .... 162
7.9 Close-up photo of the motorised device which can be moved with micron precision in the x, y, and z planes ........................................................................ 162
7.10 CustomTips® produced by Eppendorf. Dashed circle denotes inserts showing the different types of tip available ........................................................................ 167
7.11 Use of the micromanipulator for precise location of individual fat globules .......... 168
7.12 Schematic of a flow cytometer .................................................................................. 170
7.13 Flow cytometer plot of fluorescence as measured by the two detectors (D3:FL1 (green) and D4:FL2 (red)). (A) Unstained washed fat globules. (B) Washed fat globules stained with Nile Blue .......................................................................................................................... 172

7.14 Flow cytometer plots of fluorescence as measured by the two detectors (D3:FL1 (green) and D4:FL2 (red)). (A) Background signal for unstained washed fat globules. (B) 10x diluted milk stained with DiA .................................................................................................................... 172

CHAPTER 8
8.1 Orientation of DilC16(3)-DS, FM4-64 (adapted from, and used with permission by, Molecular Probes), and Alexa Fluor® conjugates of WGA on the MFGM ................................................. 177

8.2 CLSM images showing two partially coalesced fat globules. (A) Reflectance image. (B) Combined reflectance and fluorescence image ............................................................... 181

8.3 Outline of proposed future work ......................................................................................................................... 186
List of video clips and movie

CHAPTER 7
4um Tip 2 Globules.wmv .............................................................. On accompanying CD
4um Tip Cluster.wmv ................................................................. On accompanying CD
4um Tip Globule expel.wmv ....................................................... On accompanying CD
15um Tip 2 Glob 1 harvest.wmv .................................................. On accompanying CD
15um Tip 2 Glob 2 harvest.wmv .................................................. On accompanying CD
15um Tip 2 Glob 3 harvest.wmv .................................................. On accompanying CD
15um Tip 2 Glob 4 harvest.wmv .................................................. On accompanying CD
15um Tip 2 Glob 5 expel.wmv ...................................................... On accompanying CD

CHAPTER 8
ShM 3% 6 WGA cav 63x zoom6_chan00.avi .................................. On accompanying CD
List of abbreviations

5,5'-PhrDiIC_{18}(3) 1,1'-Dioc-tadecyl-5,5'diphenyl-3,3',3'-tetramethylin-docarbocyanine chloride
AFM Atomic force microscope/microscopy (depending on context)
BSA Bovine serum albumin
BTN Butyrophilin
CD36 Cluster of differentiation 36
CLSM Confocal laser scanning microscope/microscopy (depending on context)
ConA Concanavalin A
DiA 4-(4-(Dihexadecylamino)steryl)-N-methylpyridinium iodide
DiIC_{18}(3) 1,1'-Dioc-tadecyl-3,3,3',3'-tetramethylin-docarbocyanine perchlorate
DiIC_{18}(3)-DS 1,1'-Dioc-tadecyl-3,3,3',3'-tetramethylindocarbocyanine-5,5'-disulfonic acid
DiOC_{18}(3) 3,3'-Dioc-tadecyl-xocarbocyanine perchlorate
DMF Dimethylformamide
DMSO Dimethylsulfoxide
D109 5-Dodecanoylaminoisofluorescein
FFA(s) Free fatty acid(s)
FFMR Free frozen milk fat for recombining
FITC Fluorescein isothiocyanate
FM1-43 N-(3-Triethylammoniumpropyl)-4-(4-(dibutylaminosteryl)pyridinium dibromide
FM1-84 N-(3-Triethylammoniumpropyl)-4-(4-(dipentylaminosteryl)pyridinium dibromide
FM4-64 N-(3-Triethylammoniumpropyl)-4-(6-(4-(diethyramino)phenyl)hexatrienyl)-pyridinium dibromide
GlcNac N-Acetyl-D-glucosamine
GMP Glycomacropeptide
Ig(G,M) Immunoglobulin (G,M)
MDCK cells Madin-Darby canine kidney cells
MFGM Milk fat globule membrane
MUC1 Mucin 1
NANA N-Acetylneuraminic acid
NMR Nuclear magnetic resonance
N316 6-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)amino)hexanoic acid
PAS 6/7 Periodic acid Schiff 6/7
PBS Phosphate buffered saline
SDS-PAGE Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SEM Scanning electron microscopy
SP- DiIC_{18}(3) 1,1'-Dioc-tadecyl-6,6'-di(4-sulfophenyl)-3,3',3'-tetramethylin-docarbocyanine
SP- DiOC_{18}(3) 3,3'-Dioc-tadecyl-5,5'-di(4-sulfophenyl)-oxocarbocyanine, sodium salt
TEM Transmission electron microscopy
UHT Ultra-high temperature
WGA Wheat germ agglutinin
WPI Whey protein isolate
XO Xanthine oxidase