Self-assembly of chlorosomes

Solid State NMR study

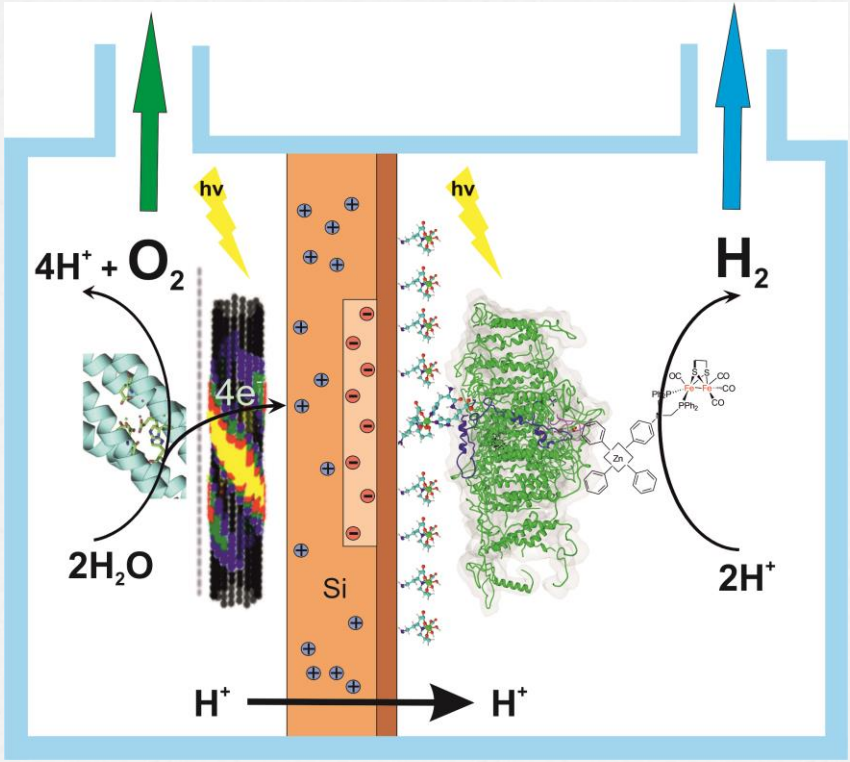
Universiteit Leiden

EuroSolarFuels

Our aims

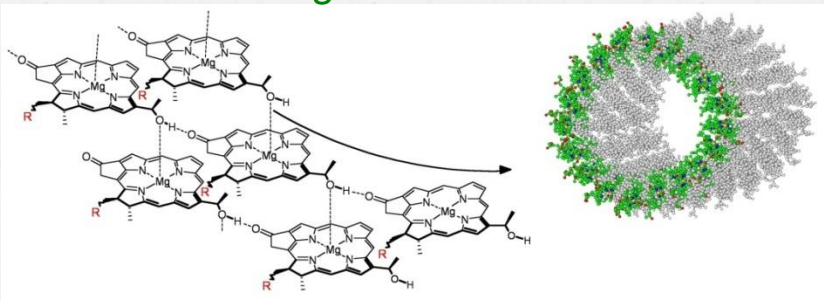
- Structure of chlorosomes
- To fill in the chlorosomes into alumina solid membrane
- Wide library of Zn-chlorines
- Strategy to change energy levels of semi-synthetic chlorines

- Immobilization of PSI onto electrode



EuroSolarFuels

Chlorosomes
Chlorobaculum tepidum
green sulfur bacterium



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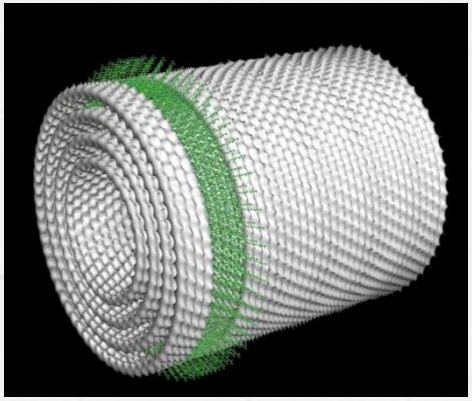
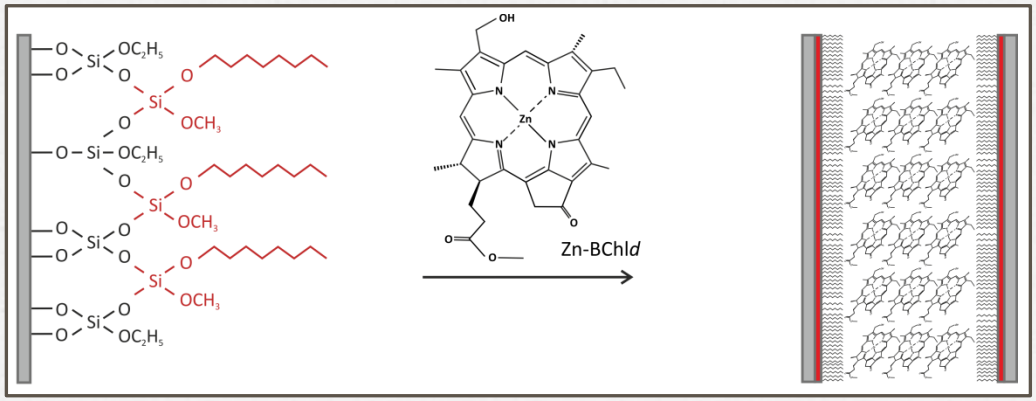
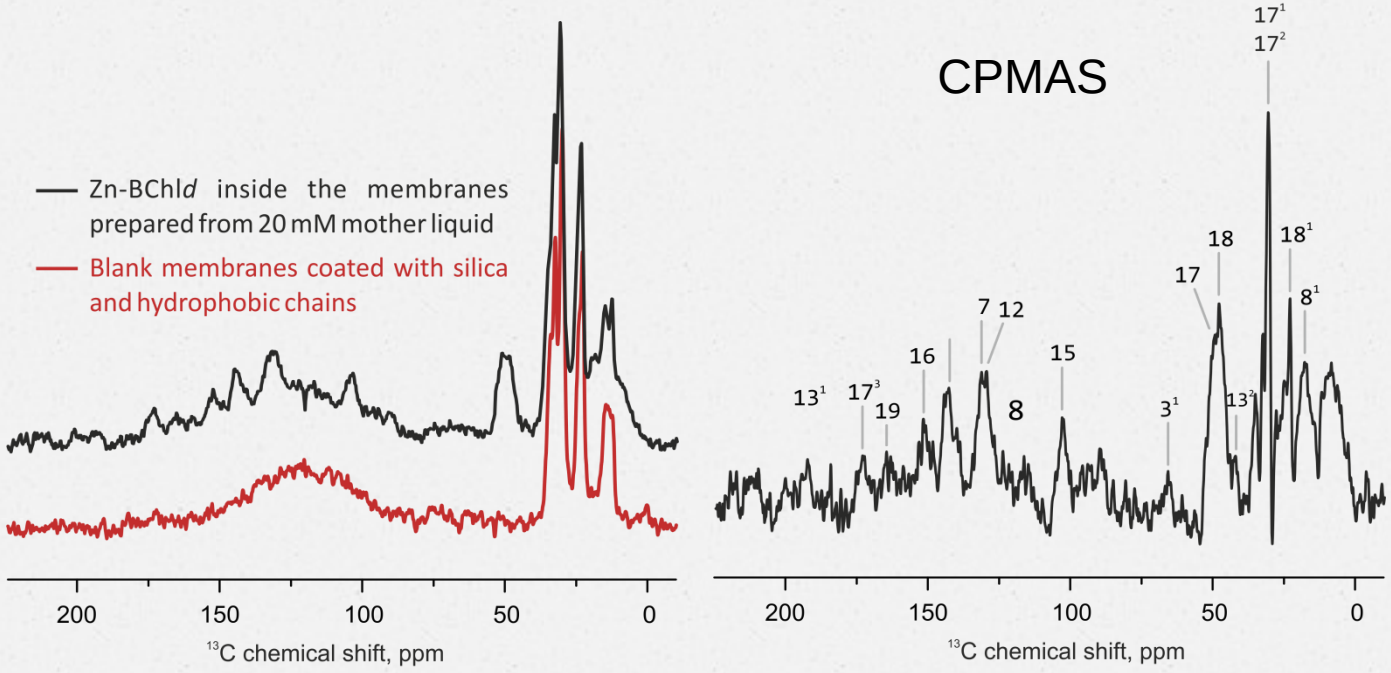


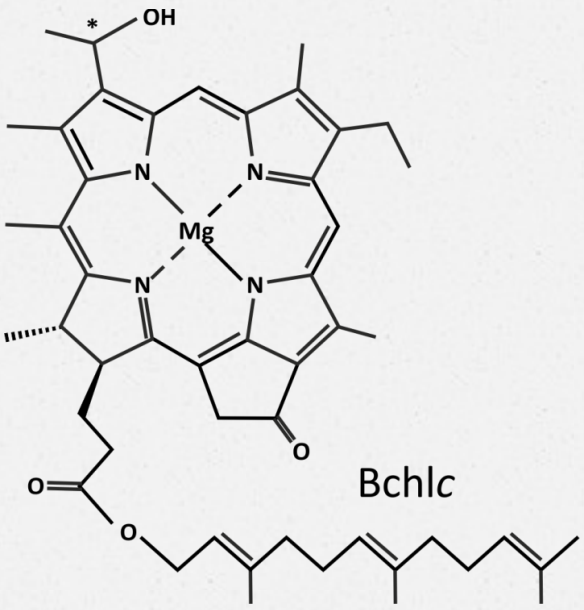
100-200 x 20-60 x 10-30 nm

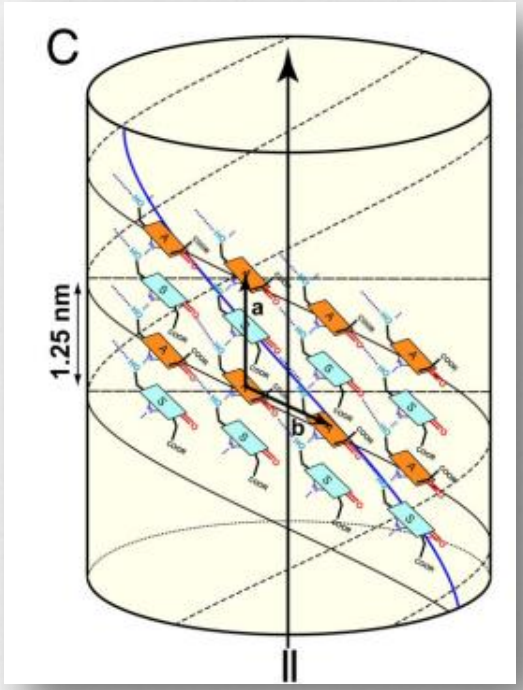


preparation of alumina membrane



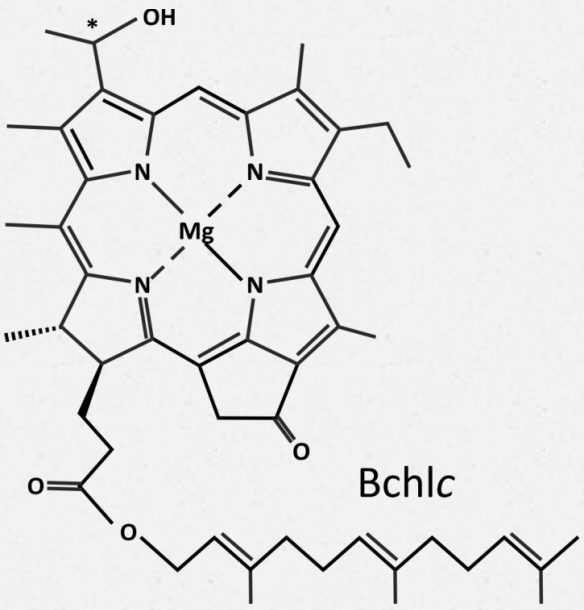
u-^{13}C-Bchl *c* - wt chlorosomes from *Chlorobaculum tepidum*





- Variable degrees of methylation at 8^{2}-C and 12^{1}-C
- Both R and S chirality at the 3^{1}C

u-^{13}C-Bchl *c* - wt chlorosomes from *Chlorobaculum tepidum*



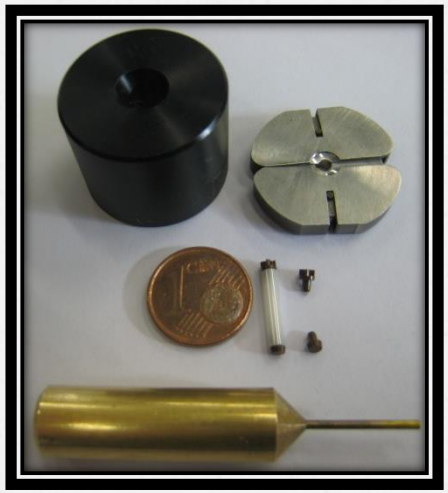
Problem

- NMR + EM: WT contains a complex mixture of BChlc molecules that are sterically crowded and heterogeneous in the side chain functionalities of the chlorin rings (Ganapathy et al. PNAS 2009)
- Single-chlorosome fluorescence polarization spectroscopy: all the chlorosomes are essentially identical in terms of their polarization properties (Tian et al, JACS 2011)

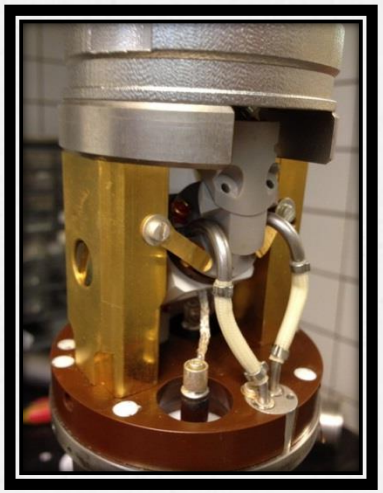
- Variable degrees of methylation at 8^2-C and 12^1-C
- Both R and S chirality at the 3^1C

Solid State NMR

2.5 mm rotor

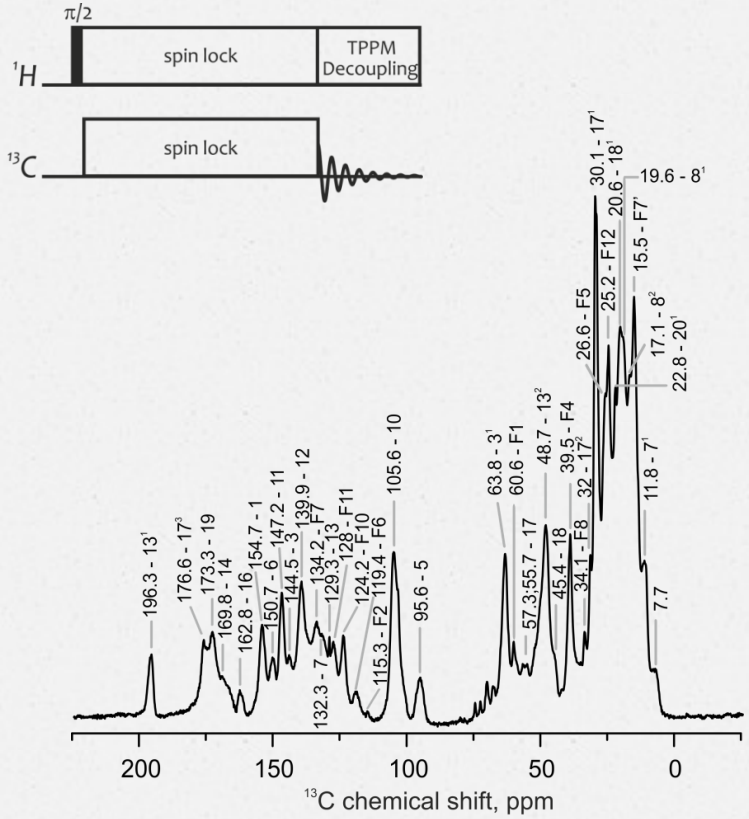


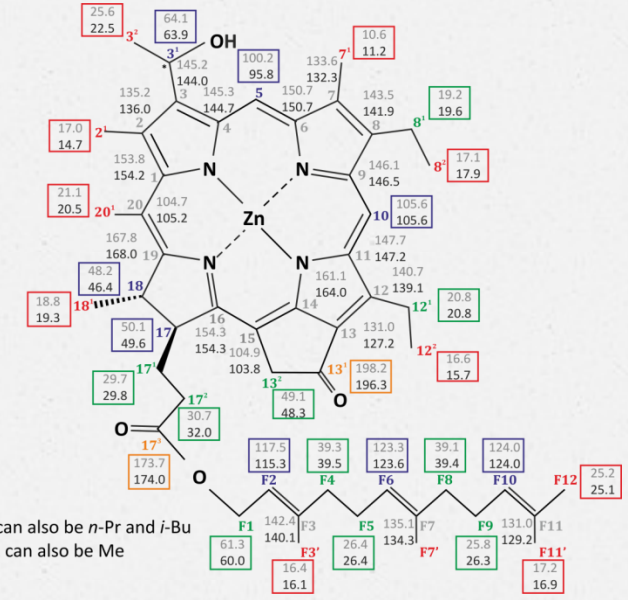
2.5 mm probe



Magnet 750 MHz, 17.6 T

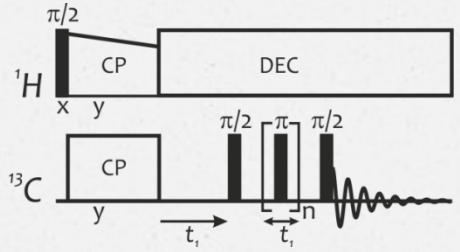
^{13}C CPMAS

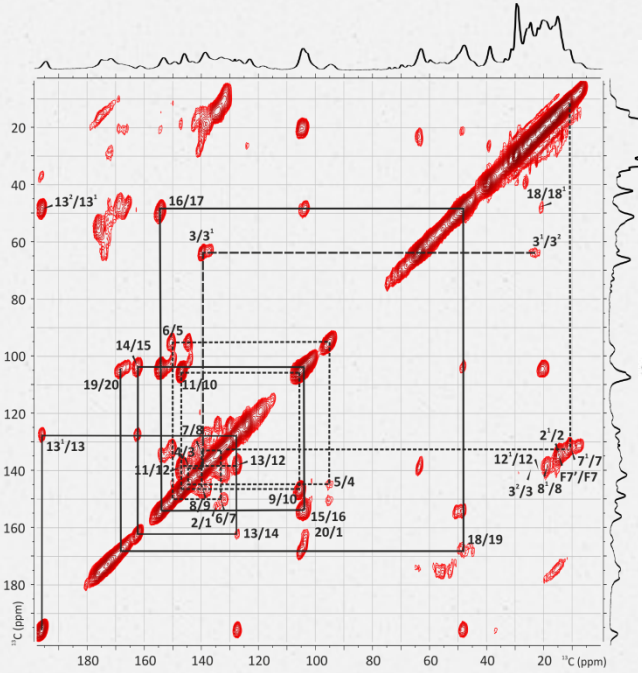




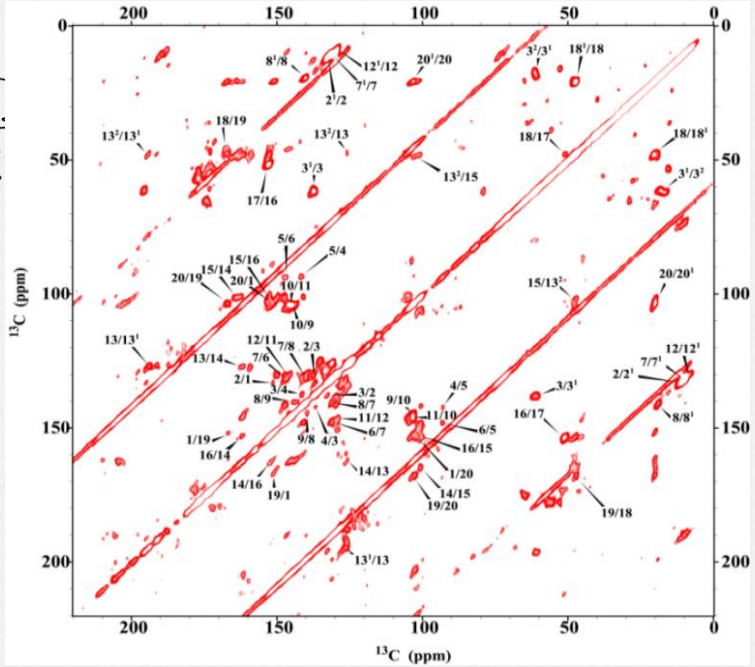
at C8 can also be *n*-Pr and *i*-Bu
at C12 can also be Me

RFDR



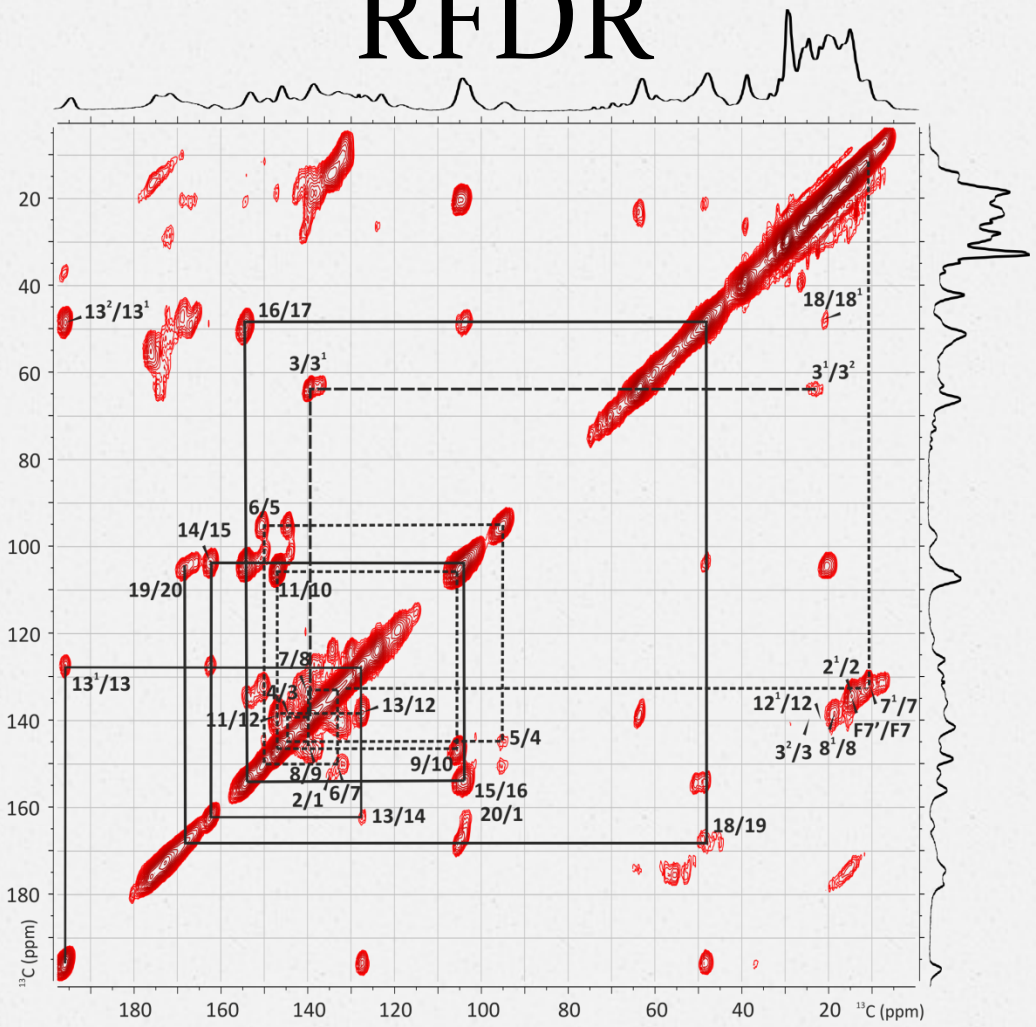


2.5 mm rotor, 30 kHz spinning
Artefacts are more outside!

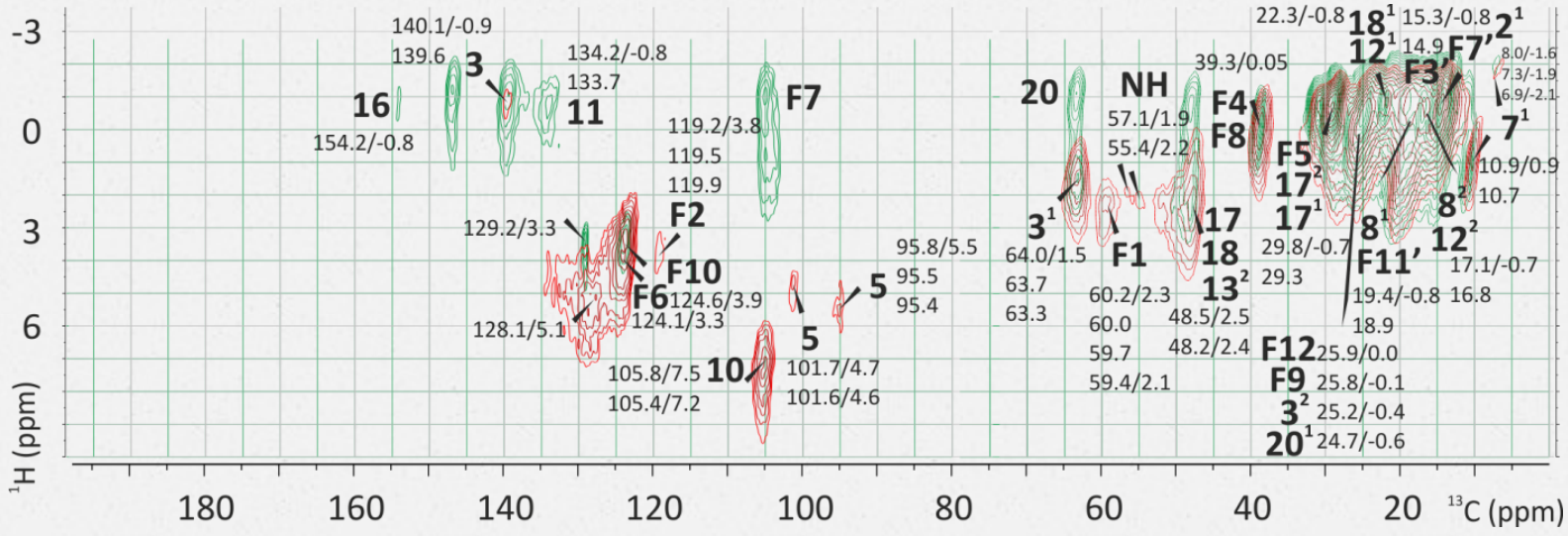


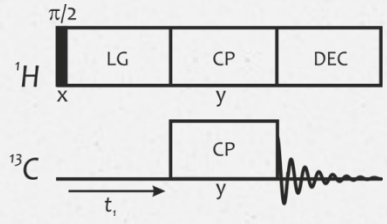
From Ganapathy et al. 2009:
4 mm rotor, 13 kHz spinning

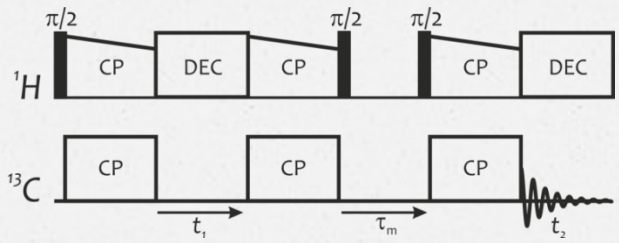
RFDR


13²/13¹
16/17
3/3¹
18/18¹
3¹/3²
6/5
14/15
19/20
11/10
7/8
13¹/13
11/12
13/12
5/4
9/10
8/9
6/7
2/1
15/16
20/1
13/14
18/19
2¹/2
12¹/12
7¹/7
F7'/F7
3²/3
8¹/8
180
160
140
120
100
80
60
40
20
¹³C (ppm)

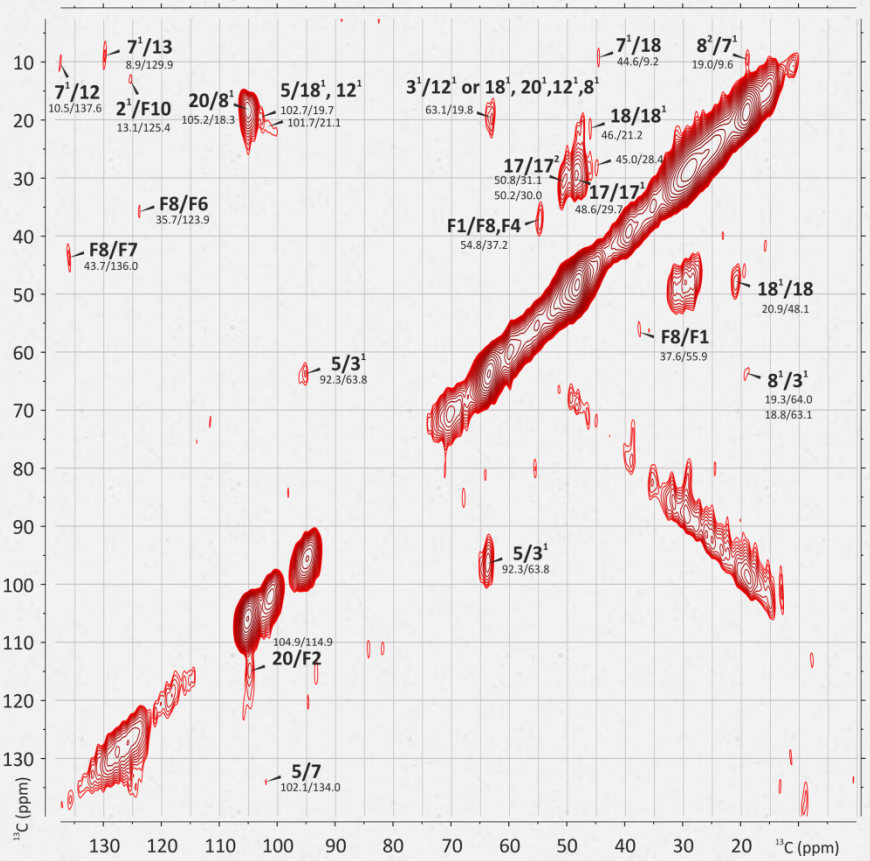
^{1}H-^{13}C Hetcor

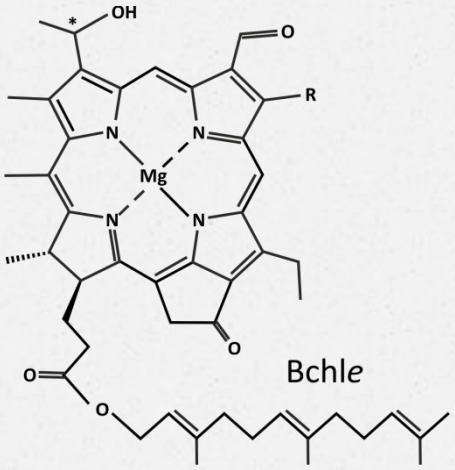





π/2
π/2
π/2
¹H
CP
DEC
CP
CP
DEC
¹³C
CP
CP
CP
t₁
τm
t₂

CHHC

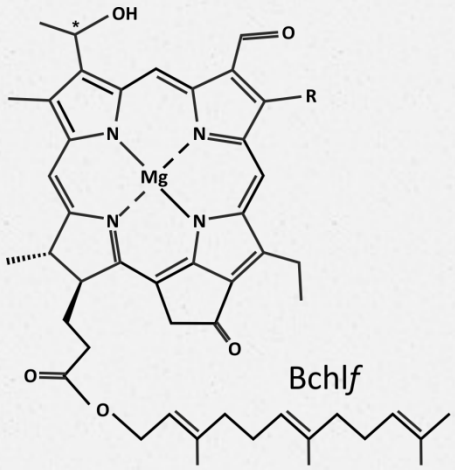

7¹/13
8.9/129.9
7¹/12
10.5/137.6
2¹/F10
13.1/125.4
20/8¹
105.2/18.3
5/18¹, 12¹
102.7/19.7
101.7/21.1
3¹/12¹ or 18¹, 20¹,12¹,8¹
63.1/19.8
7¹/18
44.6/9.2
8²/7¹
19.0/9.6
18/18¹
46./21.2
45.0/28.4
17/17²
50.8/31.1
50.2/30.0
17/17¹
48.6/29.7
F1/F8,F4
54.8/37.2
F8/F6
35.7/123.9
F8/F7
43.7/136.0
18¹/18
20.9/48.1
F8/F1
37.6/55.9
5/3¹
92.3/63.8
8¹/3¹
19.3/64.0
18.8/63.1
5/3¹
92.3/63.8
104.9/114.9
20/F2
5/7
102.1/134.0
¹³C (ppm)
¹³C (ppm)



Bchl*e*

$R=C_2H_5$, C_3H_7, *i*-C_4H_{10}, *n*-C_5H_{12}

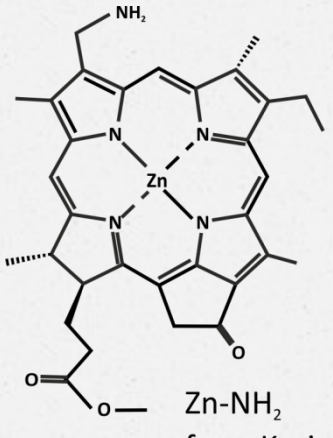
* - may have R or S sterochemistry



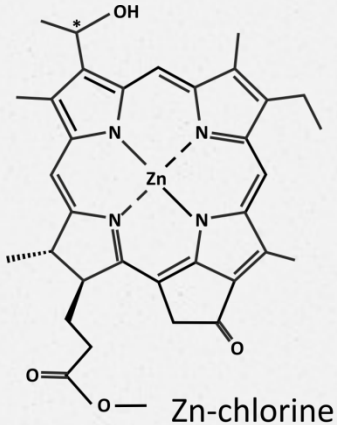
Bchl*f*

$R=C_2H_5$, C_3H_7, *i*-C_4H_{10}, *n*-C_5H_{12}

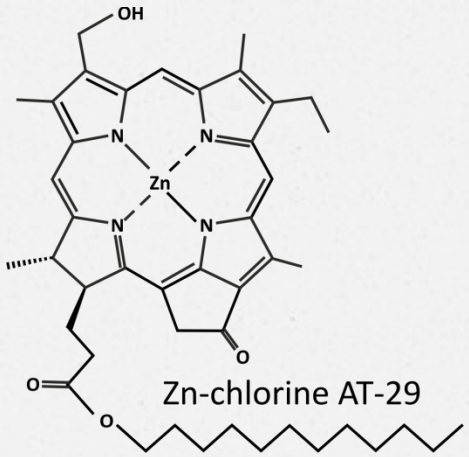
* - may have R or S sterochemistry



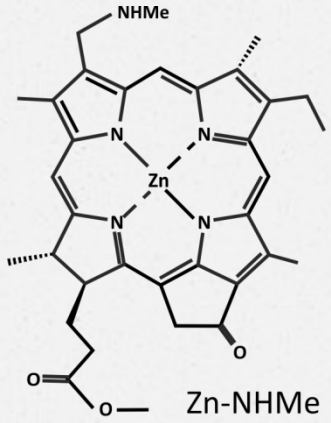
Zn-NH_2
from Kasim



Zn-chlorine OV-1



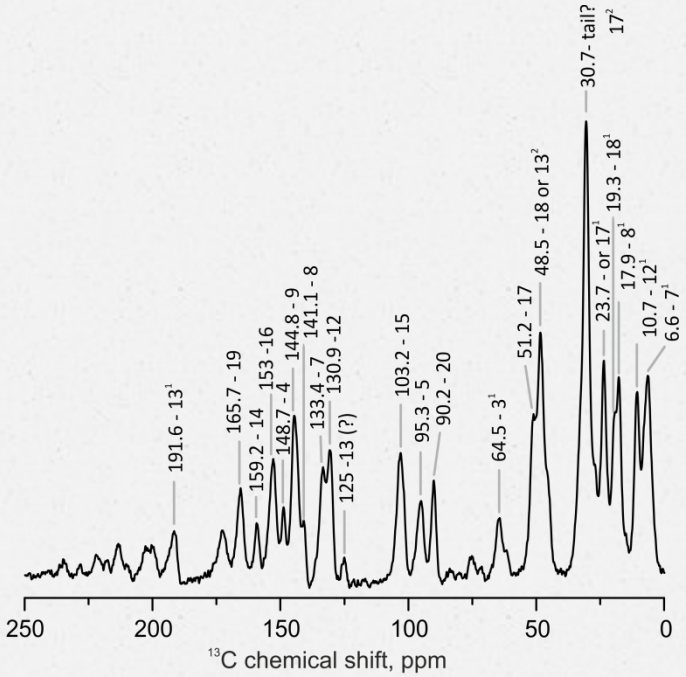
Zn-chlorine AT-29

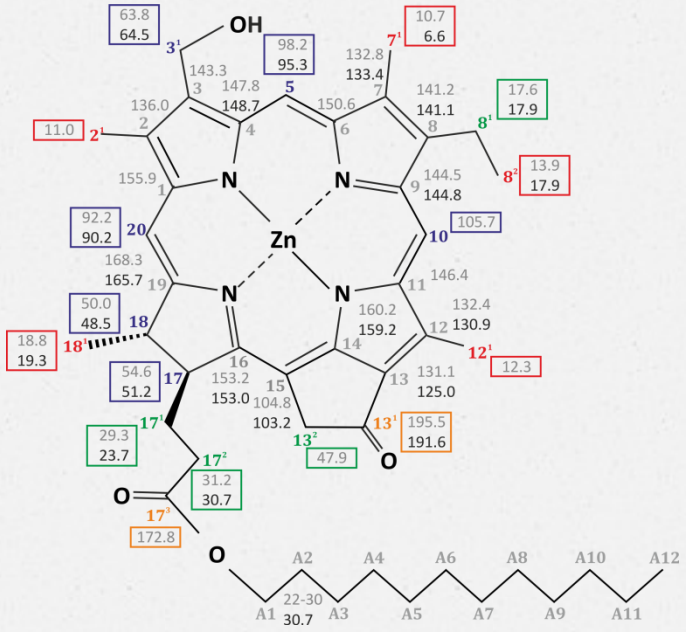


Zn-NHMe
from Kasim

CPMAS

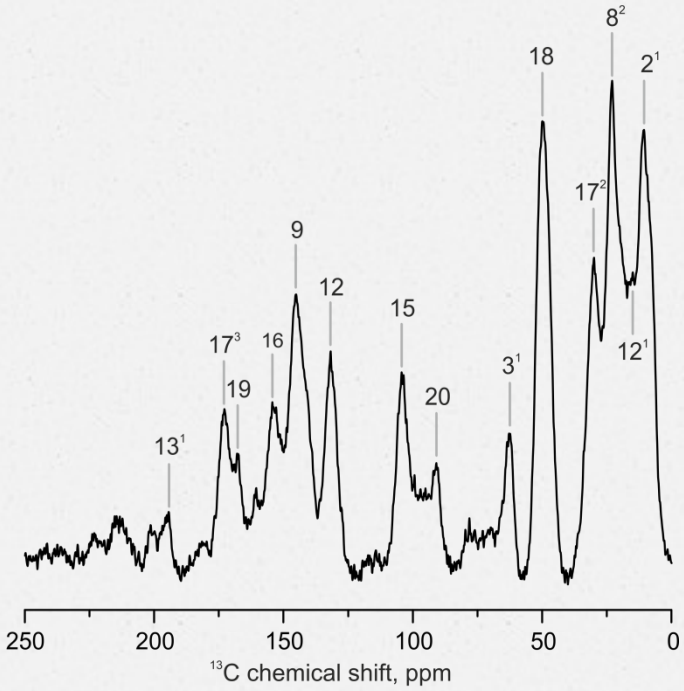
AT-29

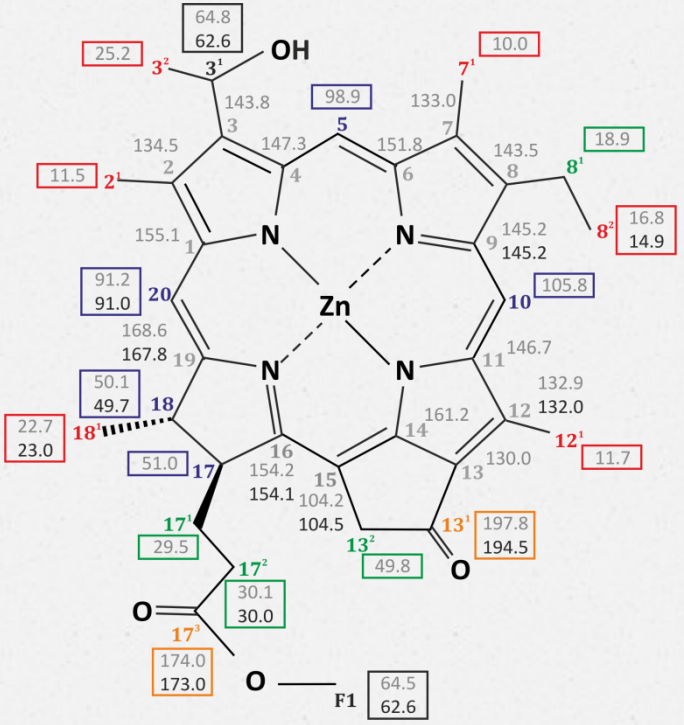

191.6 - 13¹
165.7 - 19
159.2 - 14
153 -16
148.7 - 4
144.8 - 9
141.1 - 8
133.4 - 7
130.9 -12
125 -13 (?)
103.2 - 15
95.3 - 5
90.2 - 20
64.5 - 3¹
51.2 - 17
48.5 - 18 or 13²
23.7 - or 17¹
19.3 - 18¹
17.9 - 8¹
10.7 - 12¹
6.6 - 7¹
30.7 - tail?
17²
250
200
150
100
50
0
¹³C chemical shift, ppm


OH
Zn
N
O
A1
A2
A3
A4
A5
A6
A7
A8
A9
A10
A11
A12
22-30
30.7

CPMAS

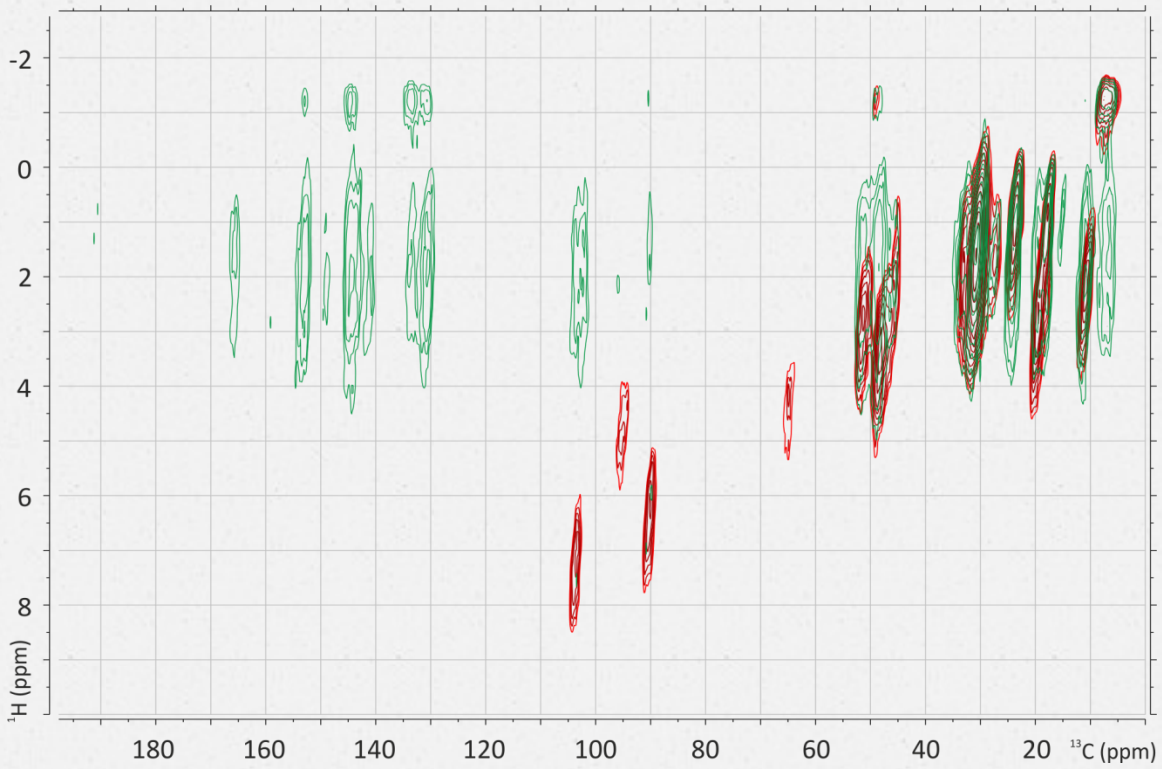
OV-1


13¹
17³
19
16
9
12
15
20
3¹
18
17²
8²
12¹
2¹
250
200
150
100
50
0
¹³C chemical shift, ppm


64.8
62.6
OH
25.2
3²
3¹
10.0
7¹
143.8
98.9
133.0
134.5
147.3
151.8
143.5
18.9
11.5
2¹
8¹
16.8
14.9
8²
155.1
145.2
145.2
91.2
91.0
105.8
Zn
168.6
167.8
146.7
50.1
49.7
132.9
132.0
22.7
23.0
161.2
18¹
12¹
11.7
51.0
154.2
154.1
130.0
104.2
104.5
197.8
194.5
29.5
49.8
30.1
30.0
174.0
173.0
64.5
62.6
F1
N
O

Hetcor

AT-29



mixing time is 256 µs & 4 ms

Conclusions

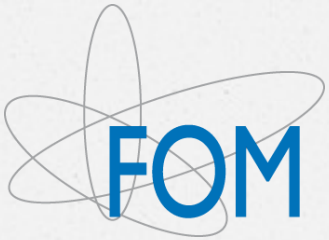
- U-13C labled chlorosomes were analysed by solid state NMR spectroscopy
- Rotation at 30 kHz speed in 2.5 mm rotor improved the resolution
- Ratio of two components of C-5 is 24:76%: heterogeneity comes not from line broadening but from these two components, which seems to be necessary for efficient energy transfer
- Samples from Poland are well structured, but we are puzzled that we don't have anti-parallel structure.
- Zn-chlorines inside the membranes – strong signal from the linkers

Plans

- EM on labeled chlorosomes
- Further analysis of the data + modeling
- Immobilization of PSI

Acknowledgment

- Karthick Babu Sai Sankar Gupta
- Huub J.M.de Groot



- Michael Reus
- Alfred R. Holzwarth
- Naoto Kuwamura

- Kasim Ocakoglu

- Anna Tarnowska
- Olena Vakulyuk
- Daniel Gryko