
linker
hyaluronic acid
binding motif
Arg-Gly-Glu
(128-130)
JEN14
epitope

FIGURE 1

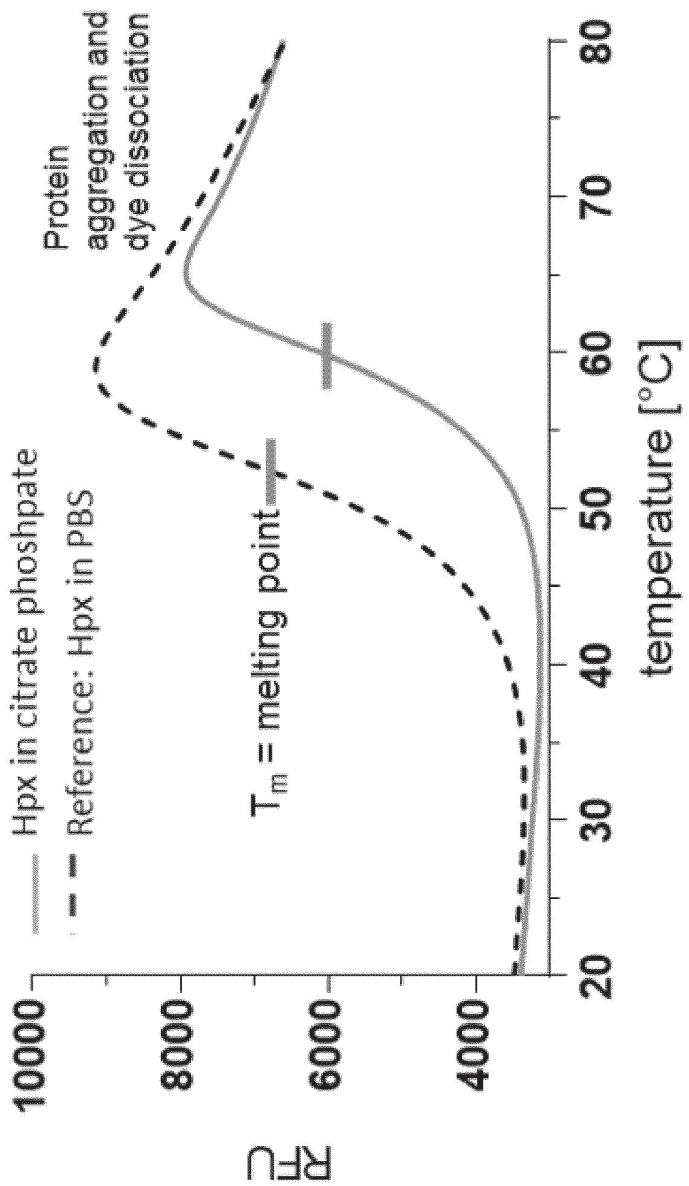
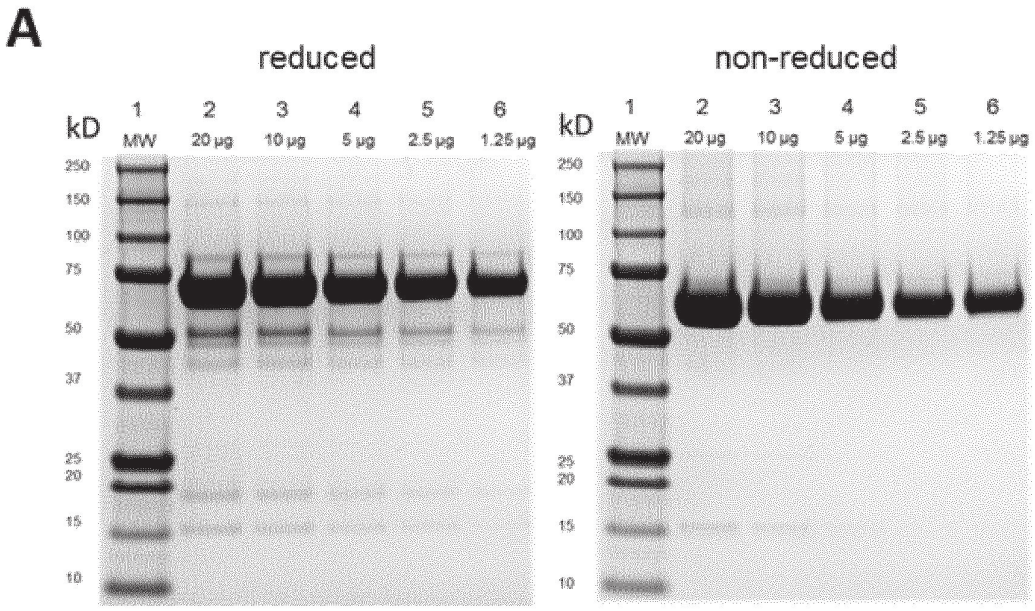

Hpx in citrate phoshpate
Reference: Hpx in PBS
Protein aggregation and dye dissociation
T_m = melting point
RFU
10000
8000
6000
4000
20
30
40
50
60
70
80
temperature [°C]

FIGURE 2

FIGURE 3


A
reduced
non-reduced
kD
1 MW
2 20 µg
3 10 µg
4 5 µg
5 2.5 µg
6 1.25 µg
250
150
100
75
50
37
25
20
15
10

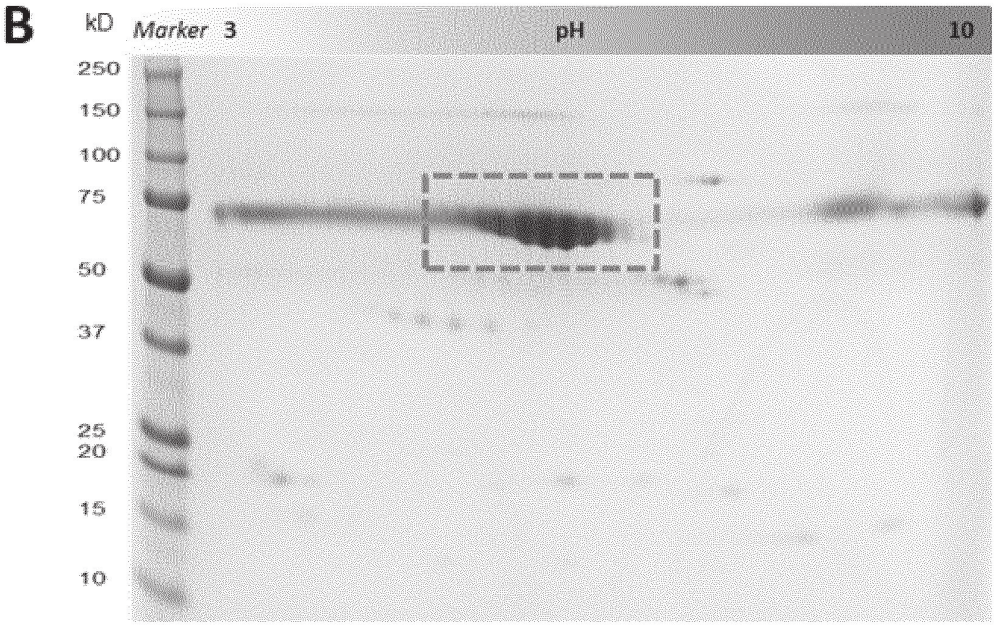
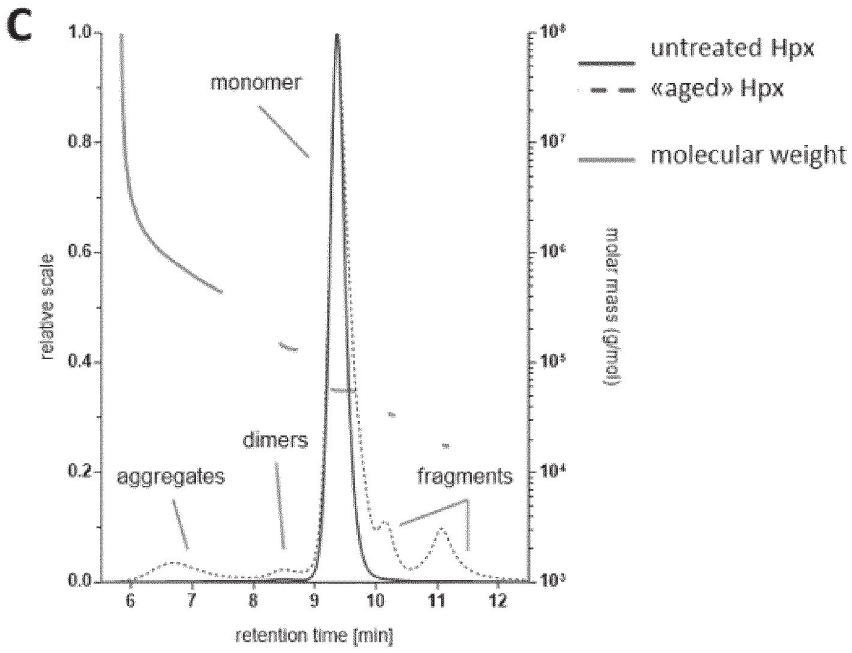

B
kD
Marker
3
pH
10
250
150
100
75
50
37
25
20
15
10

FIGURE 3


C
untreated Hpx
«aged» Hpx
molecular weight
monomer
dimers
aggregates
fragments
relative scale
molar mass (g/mol)
retention time [min]

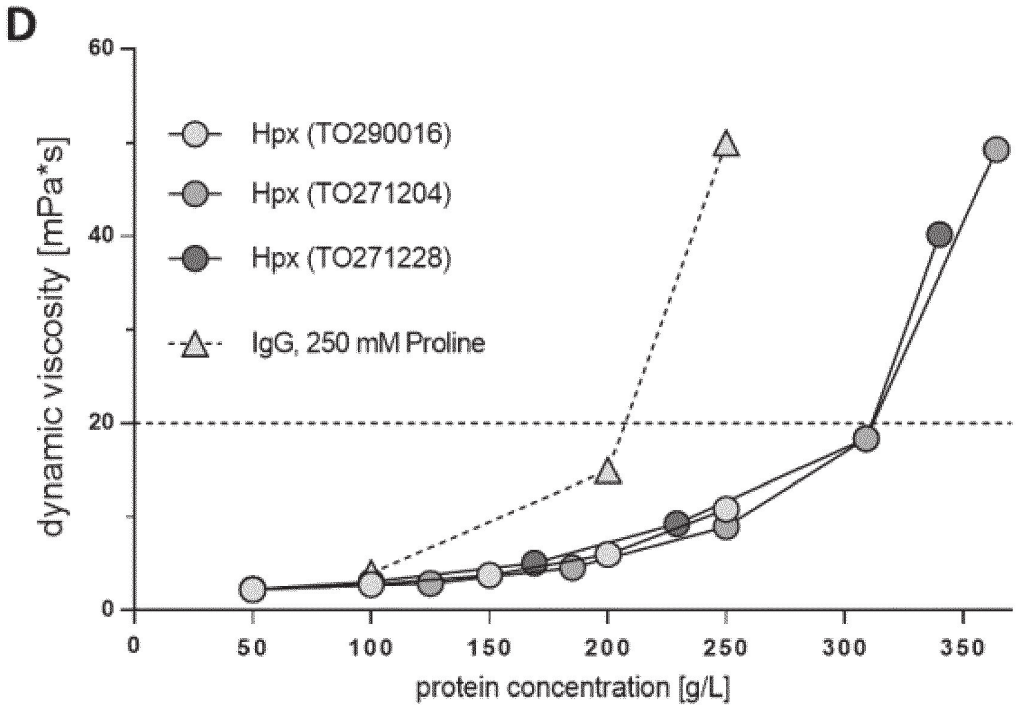
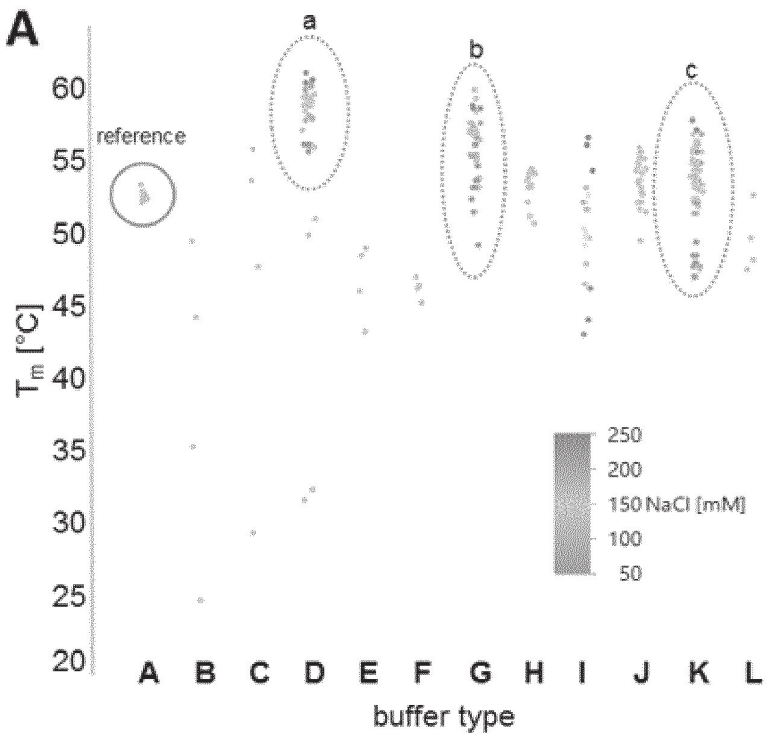

D
Hpx (TO290016)
Hpx (TO271204)
Hpx (TO271228)
IgG, 250 mM Proline
dynamic viscosity [mPa*s]
protein concentration [g/L]

FIGURE 4


A
a
b
c
reference
60
55
50
45
40
35
30
25
20
T_m [°C]
250
200
150 NaCl [mM]
100
50
A B C D E F G H I J K L
buffer type

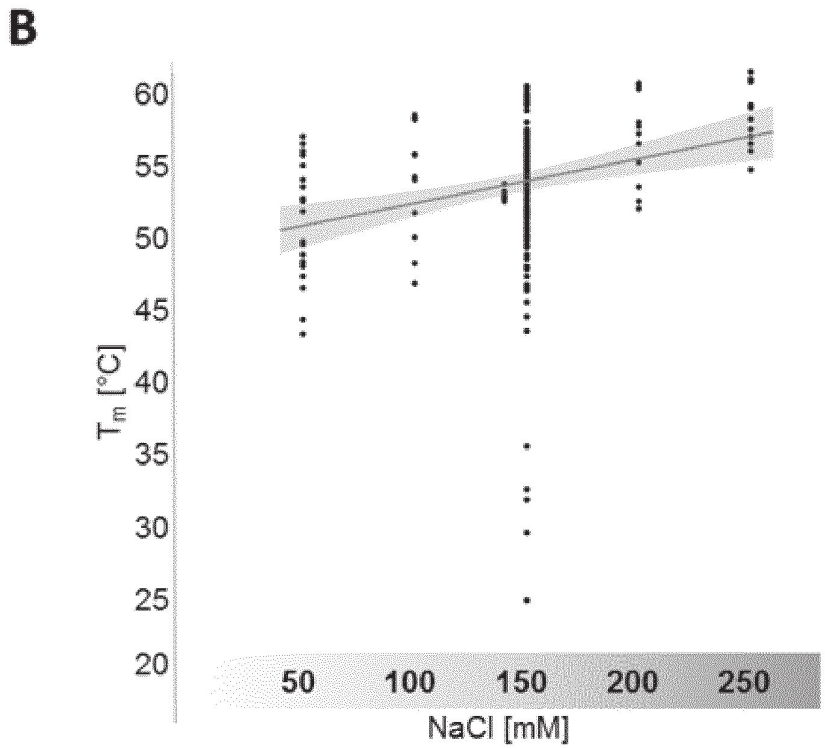
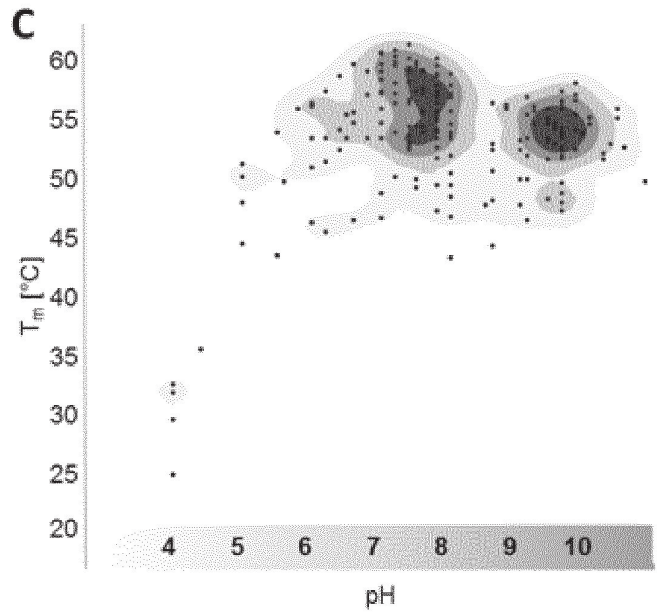

B
60
55
50
45
40
35
30
25
20
T_m [°C]
50 100 150 200 250
NaCl [mM]

FIGURE 4


C
60
55
50
45
40
35
30
25
20
T_m [°C]
4
5
6
7
8
9
10
pH

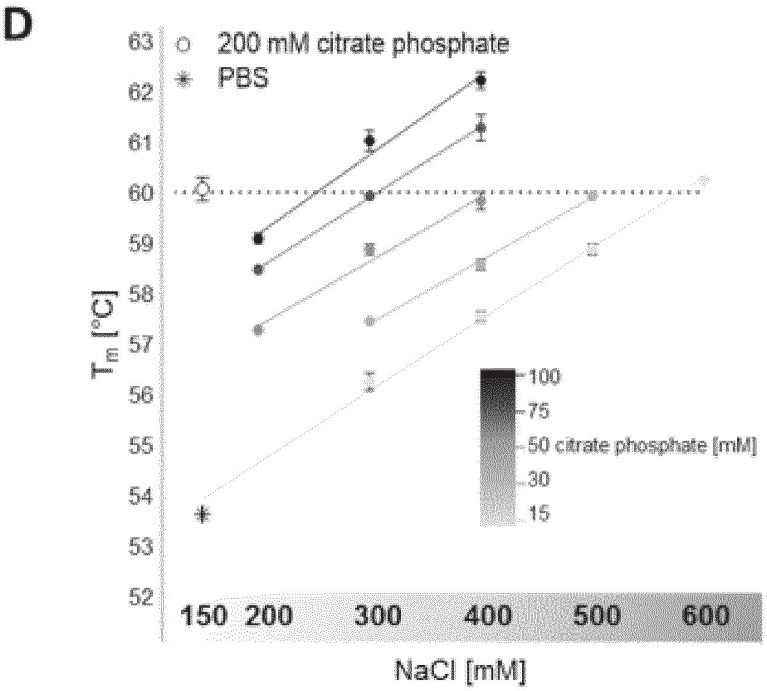
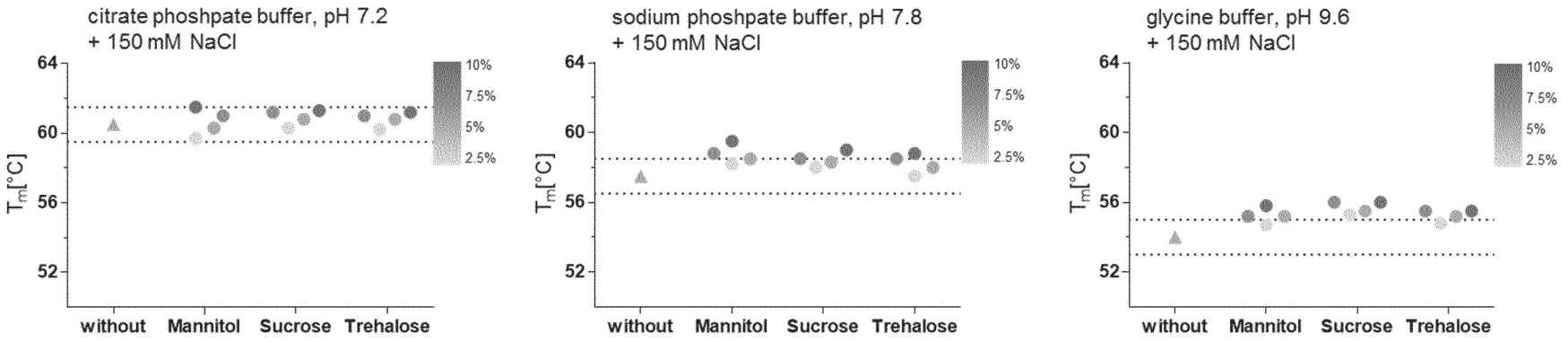

D
63
62
61
60
59
58
57
56
55
54
53
52
T_m [°C]
200 mM citrate phosphate
PBS
100
75
50 citrate phosphate [mM]
30
15
150
200
300
400
500
600
NaCl [mM]

FIGURE 5


citrate phoshpate buffer, pH 7.2
+ 150 mM NaCl
sodium phoshpate buffer, pH 7.8
+ 150 mM NaCl
glycine buffer, pH 9.6
+ 150 mM NaCl
T_m[°C]
64
60
56
52
10%
7.5%
5%
2.5%
without
Mannitol
Sucrose
Trehalose

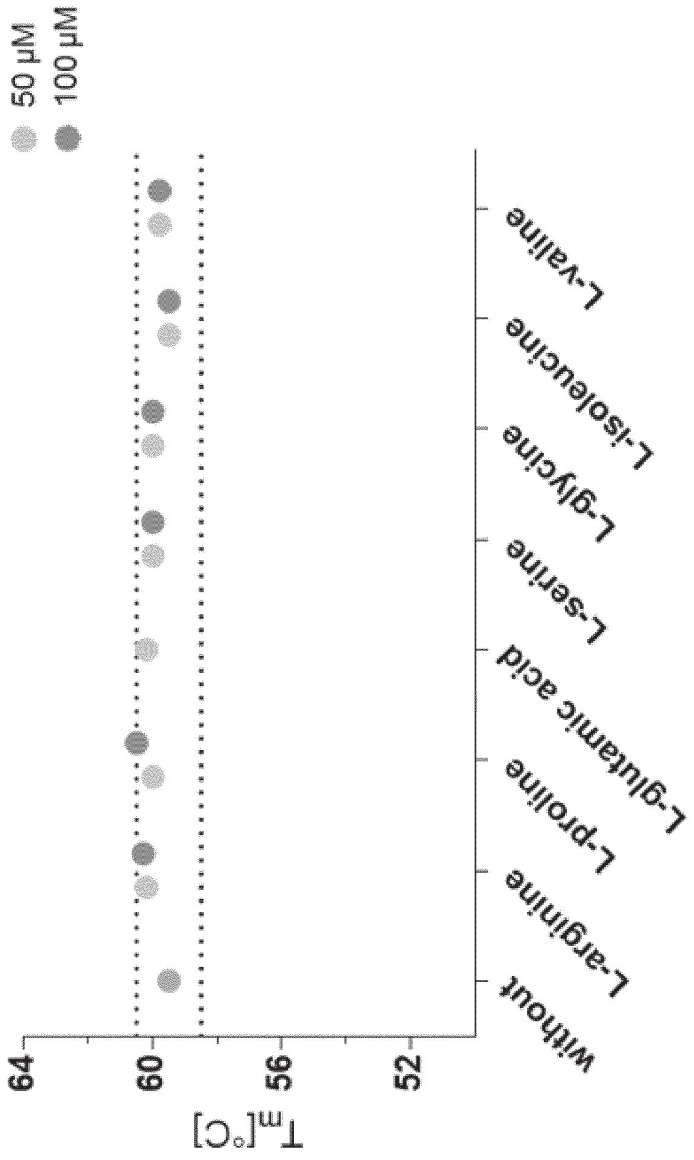
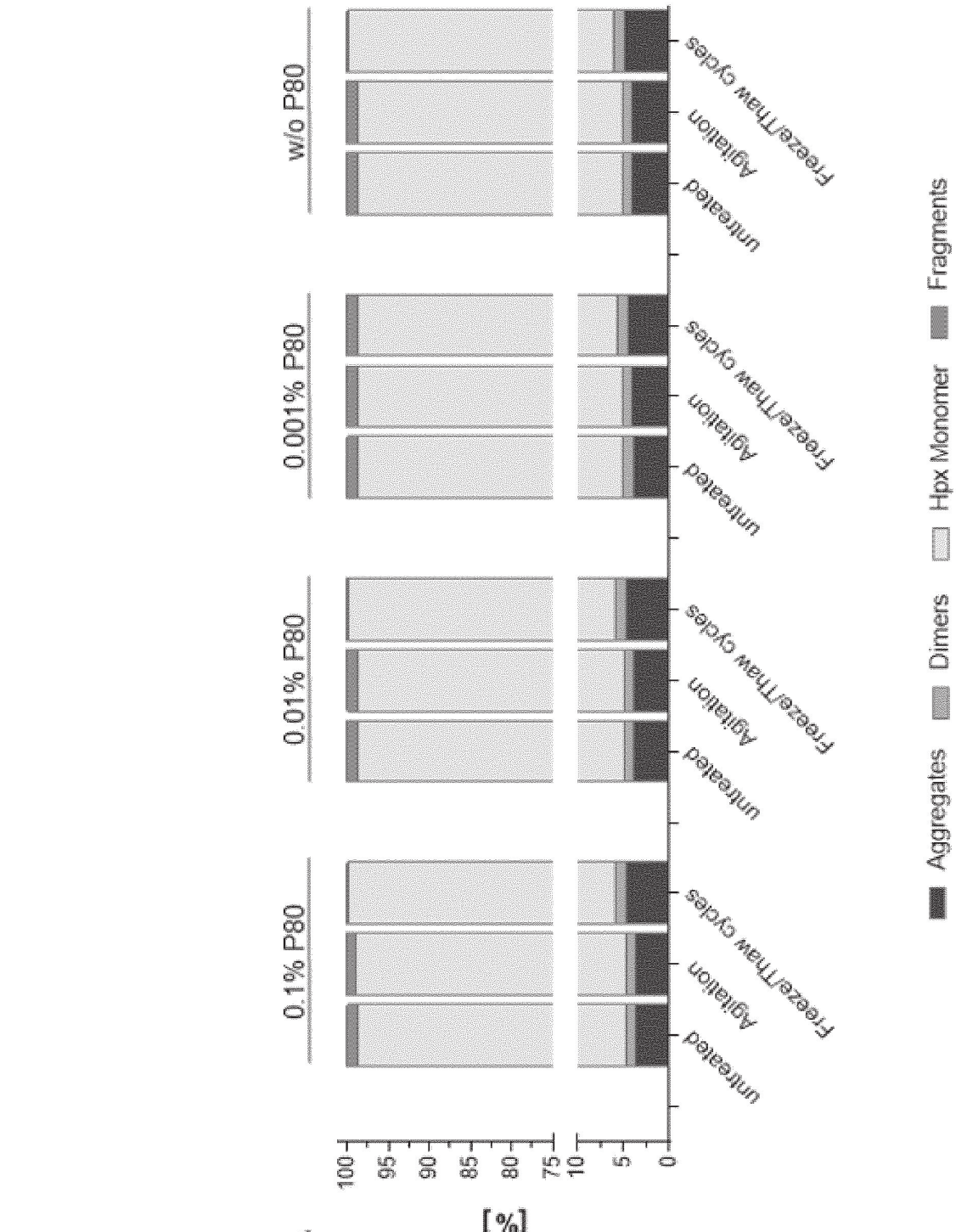

50 µM
100 µM
64
60
56
52
T_m[°C]
without
L-arginine
L-proline
L-glutamic acid
L-serine
L-glycine
L-isoleucine
L-valine

FIGURE 6

FIGURE 7


A
[%]
100
95
90
85
80
75
10
5
0
0.1% P80
0.01% P80
0.001% P80
w/o P80
unteated
Agitation
Freeze/Thaw cycles
Aggregates
Dimers
Hpx Monomer
Fragments

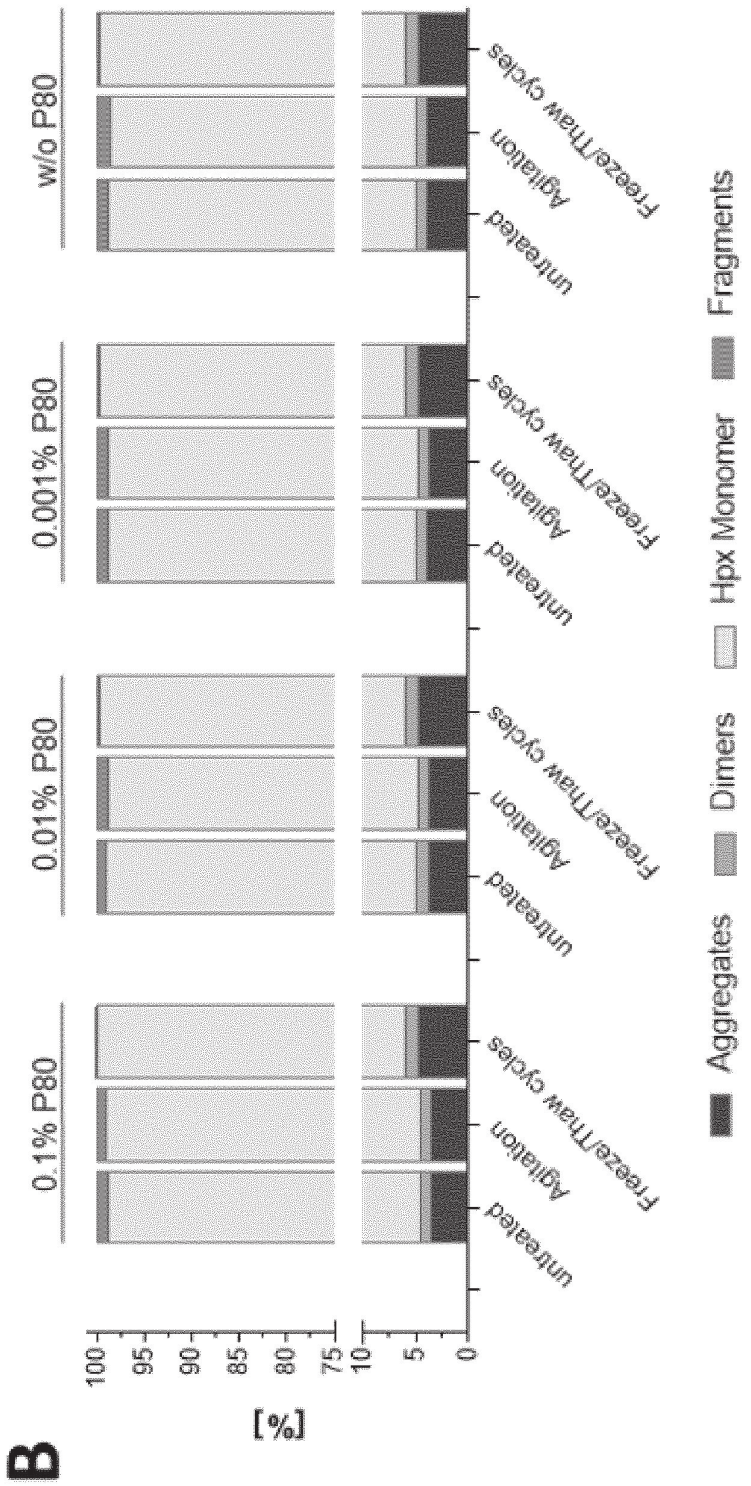
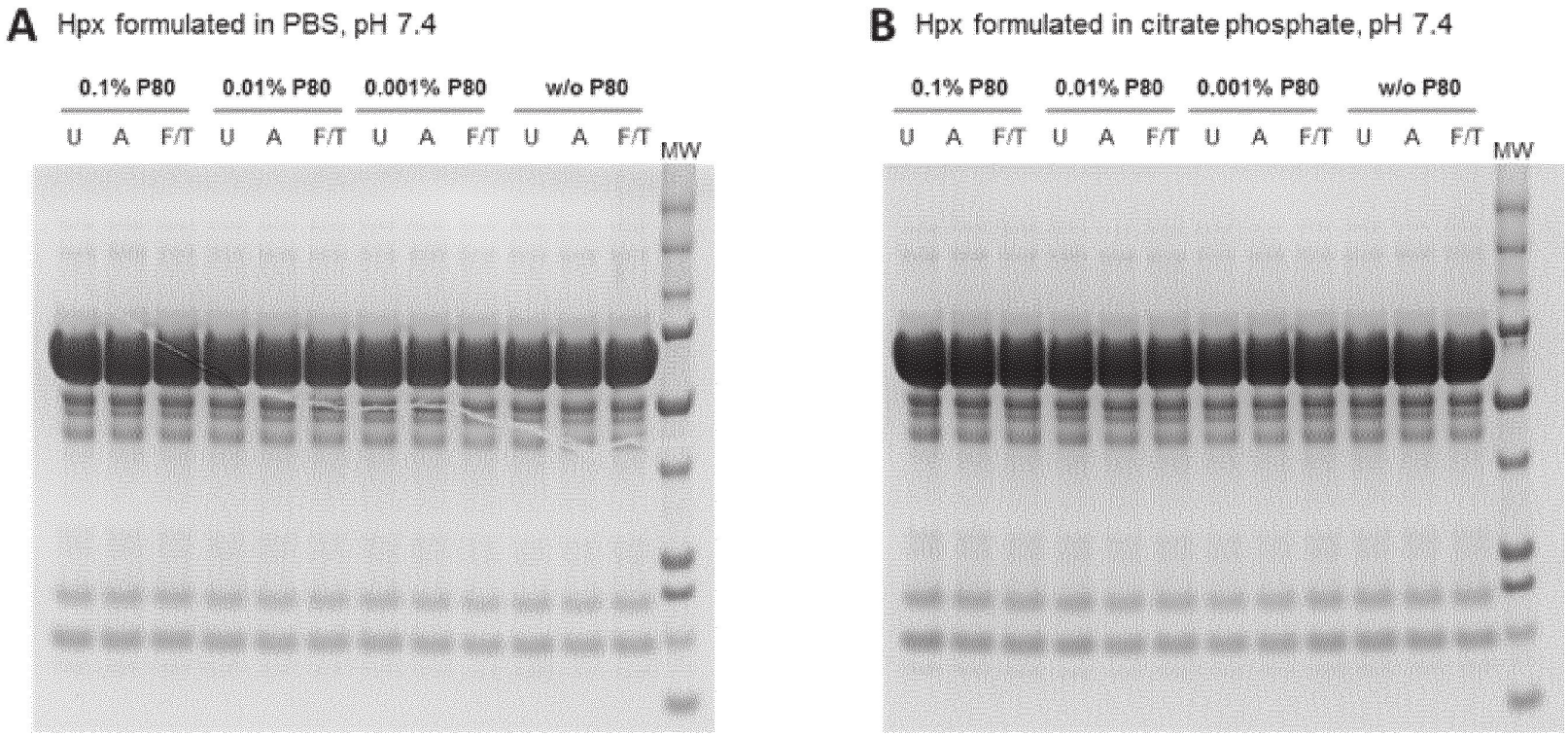

B
0.1% P80
0.01% P80
0.001% P80
w/o P80
unstressed
Agitation
Freeze/Thaw cycles
[%]
100
95
90
85
80
75
10
5
0
Aggregates
Dimers
Hpx Monomer
Fragments

FIGURE 7

FIGURE 8



REDUCING

FIGURE 8

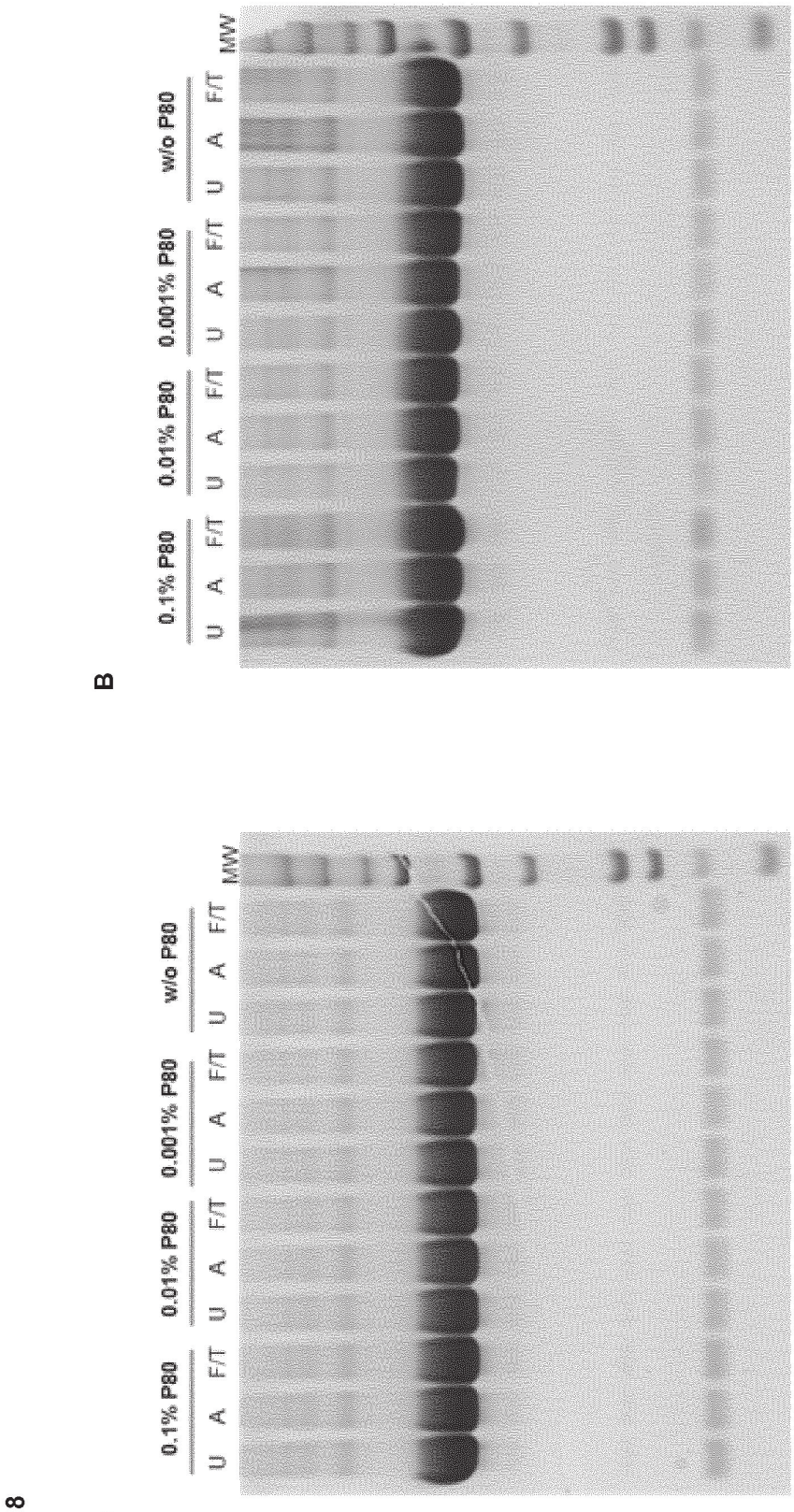


FIGURE 9

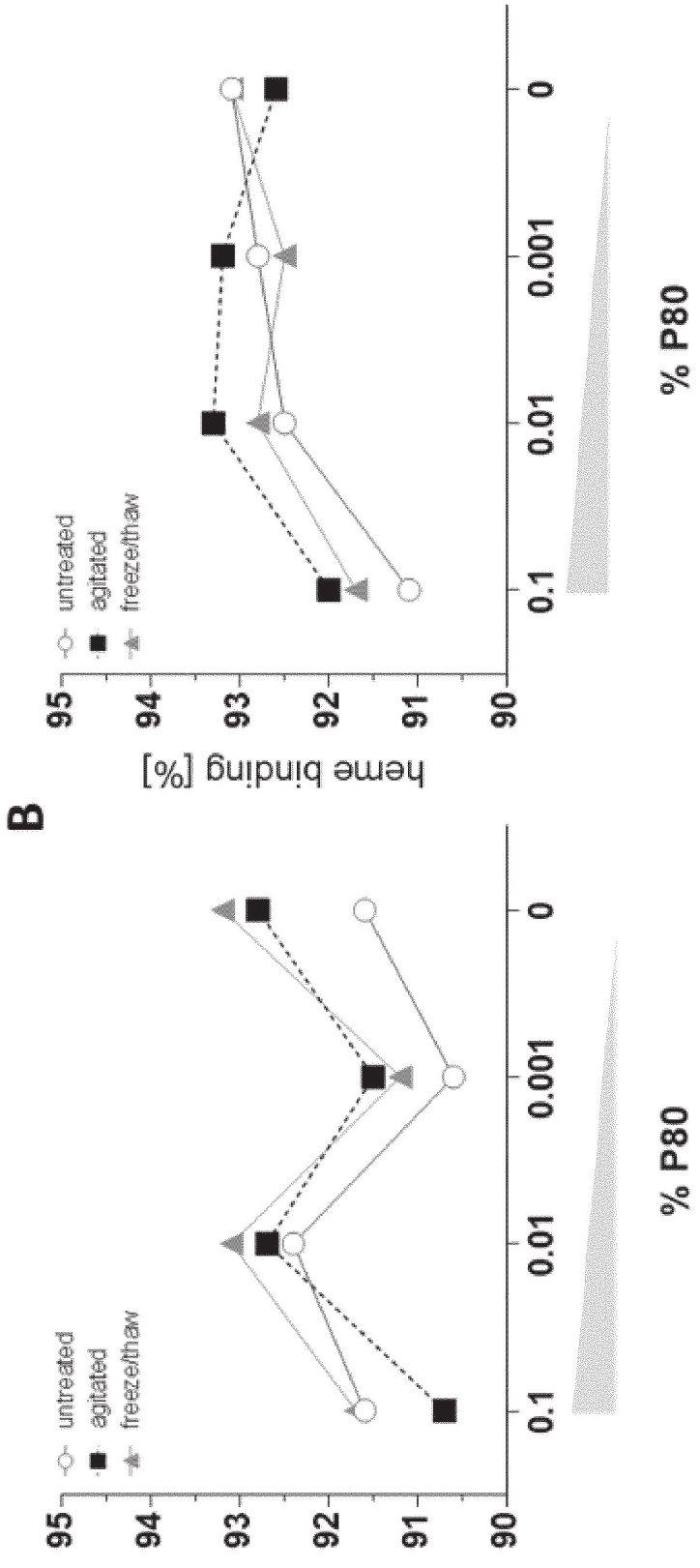

A
B
heme binding [%]
95
94
93
92
91
90
untreated
agitated
freeze/thaw
0.1
0.01
0.001
0
% P80

FIGURE 10

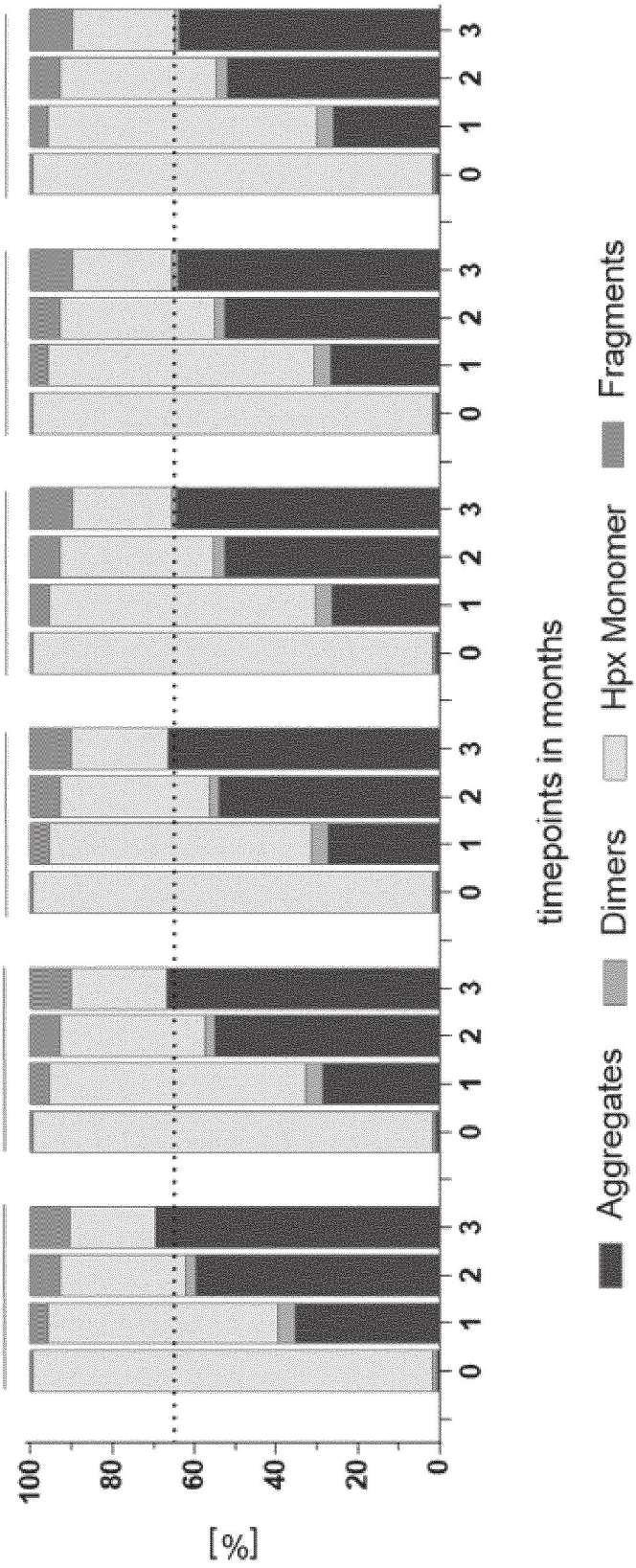
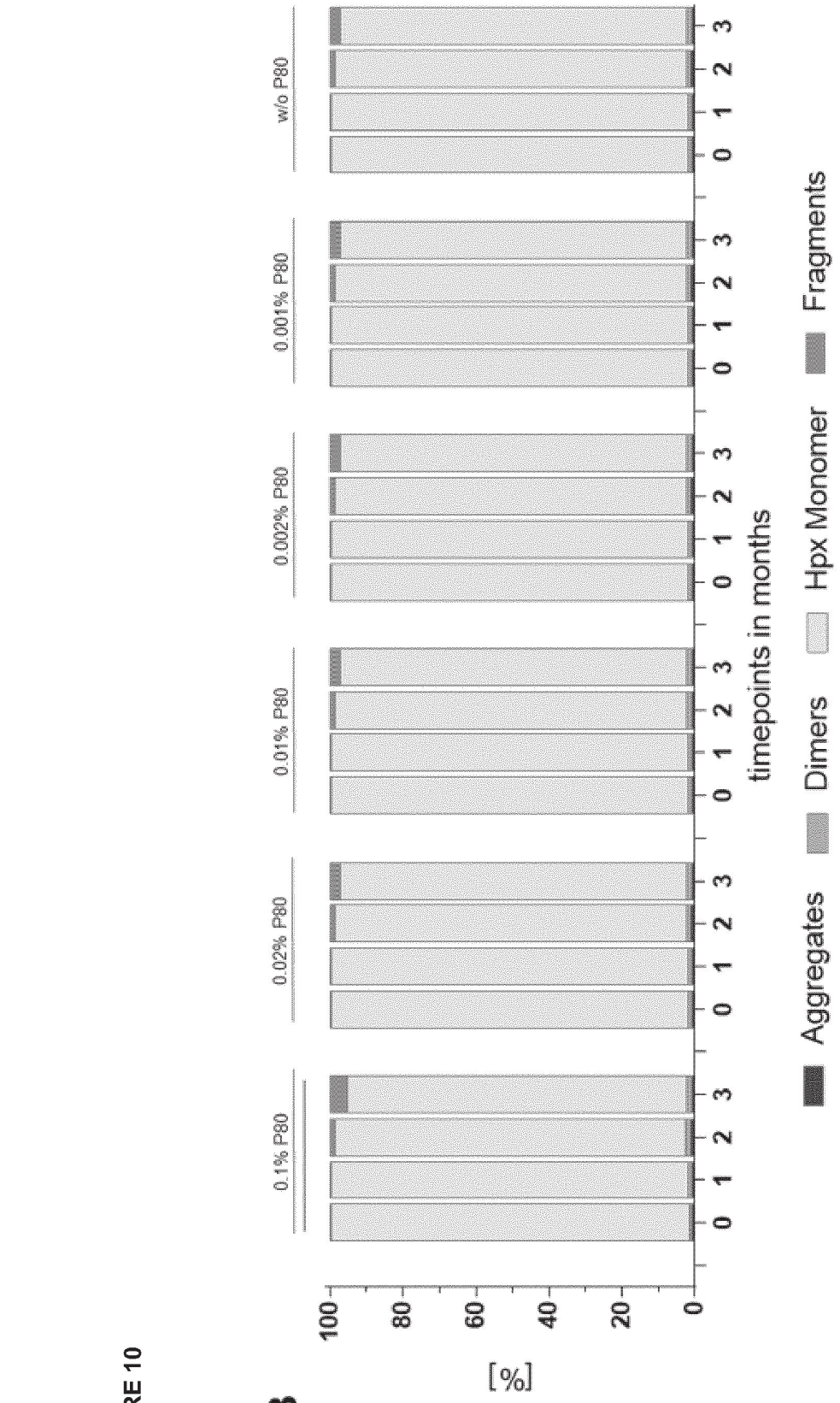

A
[%]
100
80
60
40
20
0
0.1% P80
0.02% P80
0.01% P80
0.002% P80
0.001% P80
w/o P80
0 1 2 3
timepoints in months
Aggregates
Dimers
Hpx Monomer
Fragments

FIGURE 10


B
[%]
100
80
60
40
20
0
0.1% P80
0.02% P80
0.01% P80
0.002% P80
0.001% P80
w/o P80
0 1 2 3
timepoints in months
Aggregates
Dimers
Hpx Monomer
Fragments

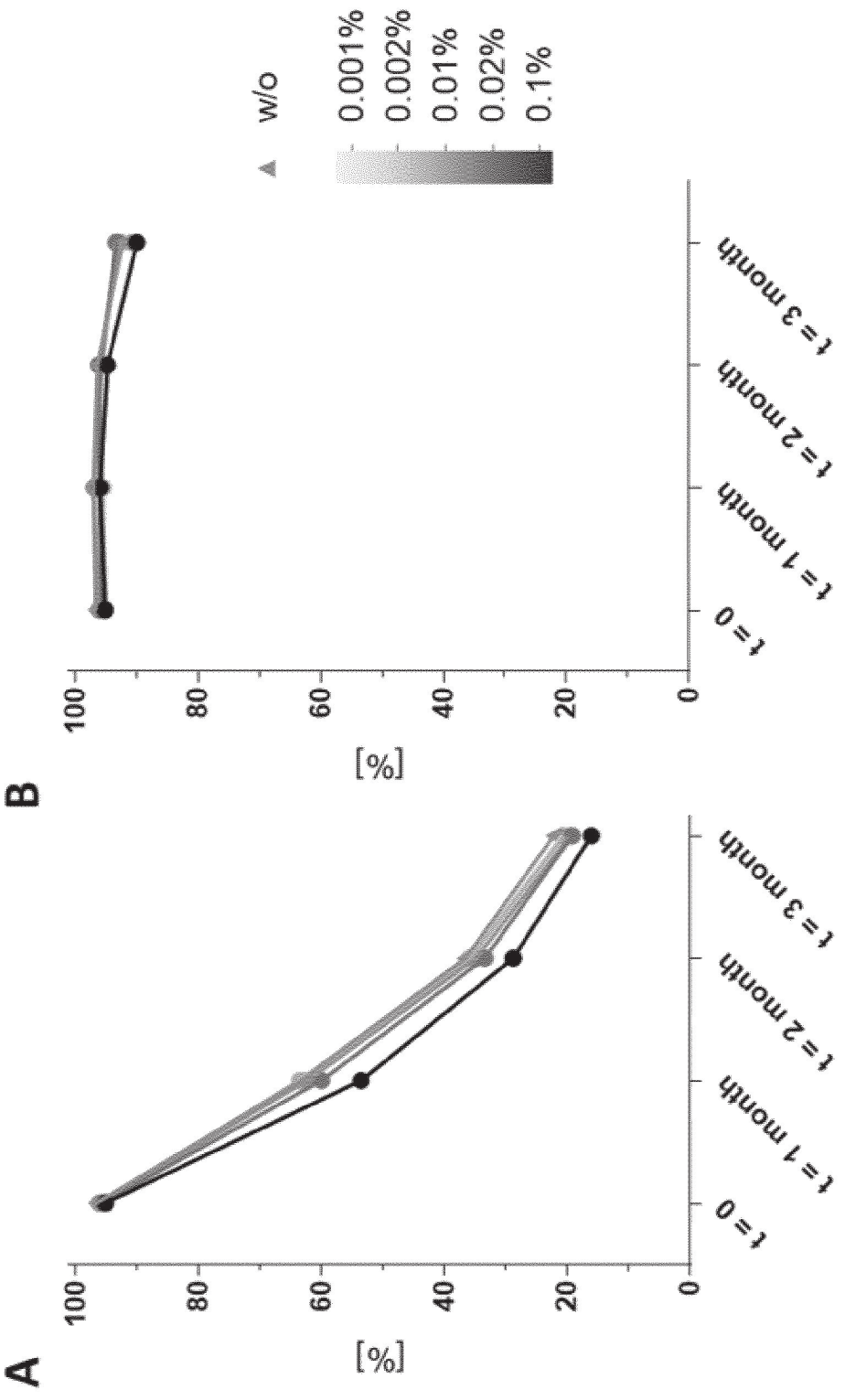

A
B
[%]
100
80
60
40
20
0
t = 0
t = 1 month
t = 2 month
t = 3 month
w/o
0.001%
0.002%
0.01%
0.02%
0.1%

FIGURE 11

FIGURE 12

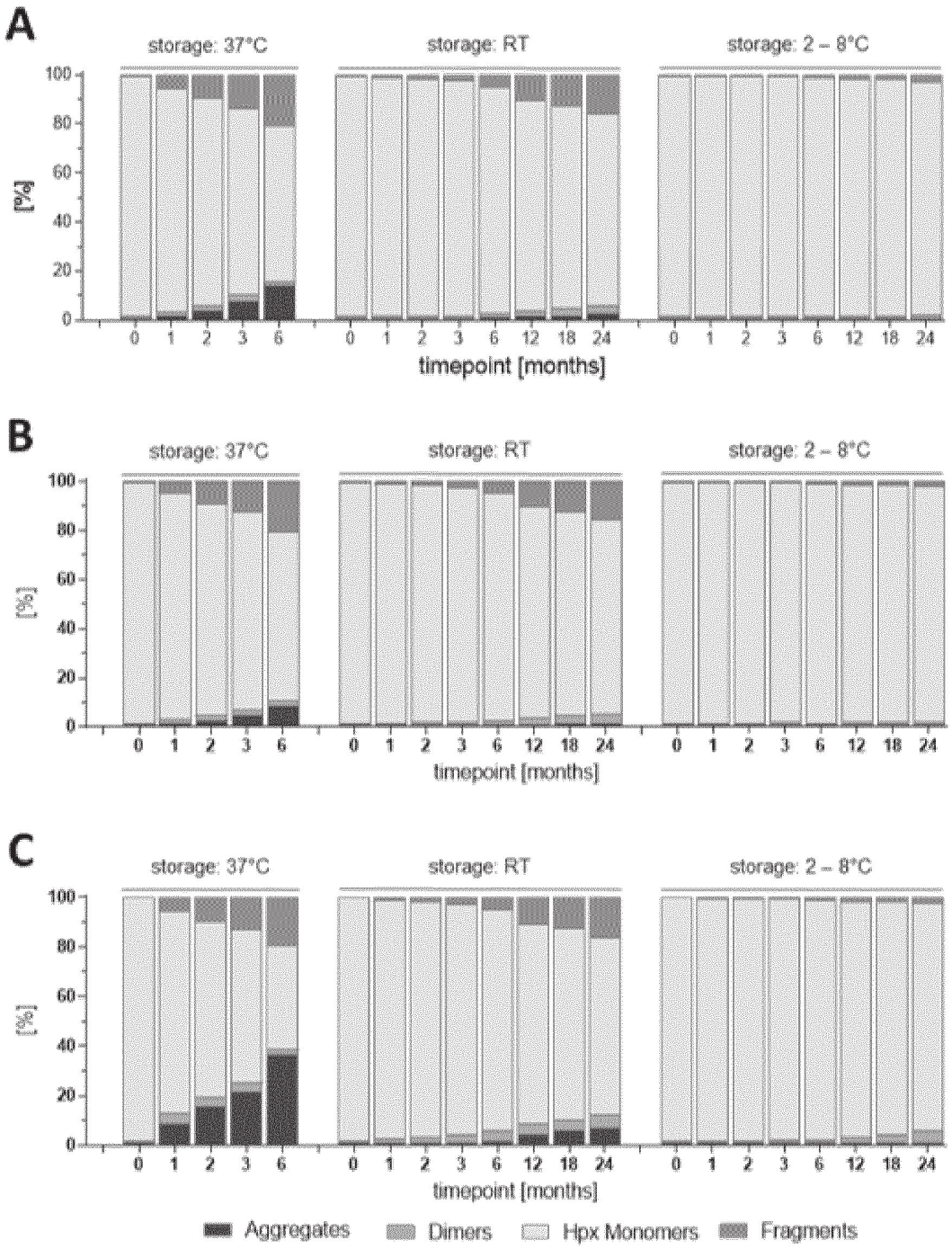

A
storage: 37°C
storage: RT
storage: 2 – 8°C
[%]
timepoint [months]
B
storage: 37°C
storage: RT
storage: 2 – 8°C
[%]
timepoint [months]
C
storage: 37°C
storage: RT
storage: 2 – 8°C
[%]
timepoint [months]
Aggregates
Dimers
Hpx Monomers
Fragments

FIGURE 12

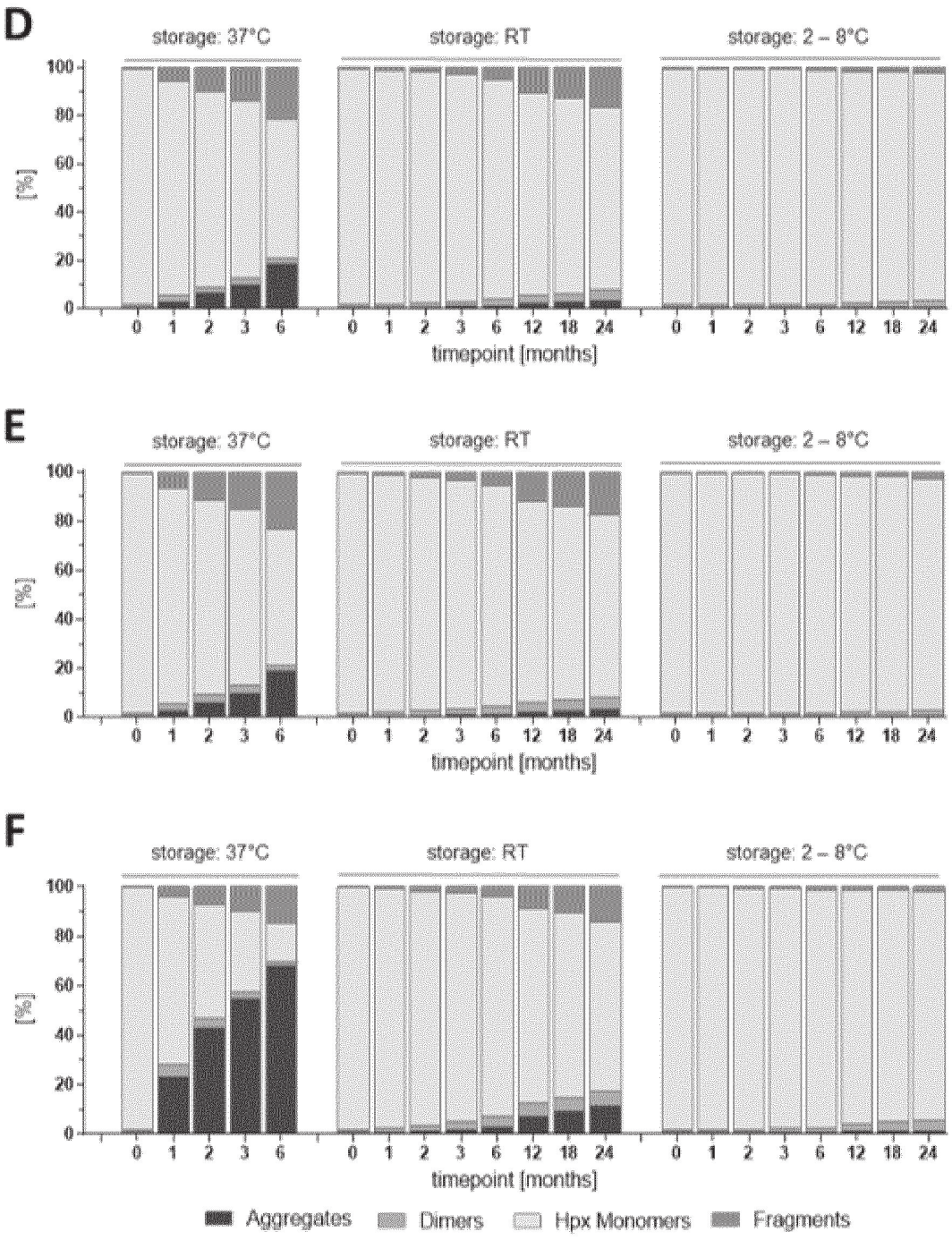
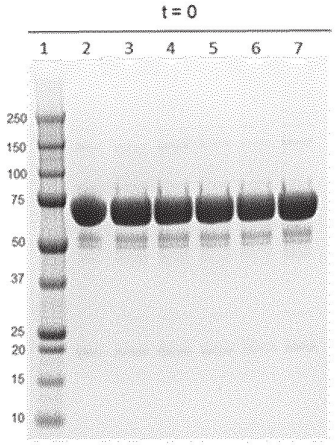

D
storage: 37°C
storage: RT
storage: 2 – 8°C
[%]
timepoint [months]
E
storage: 37°C
storage: RT
storage: 2 – 8°C
[%]
timepoint [months]
F
storage: 37°C
storage: RT
storage: 2 – 8°C
[%]
timepoint [months]
Aggregates
Dimers
Hpx Monomers
Fragments

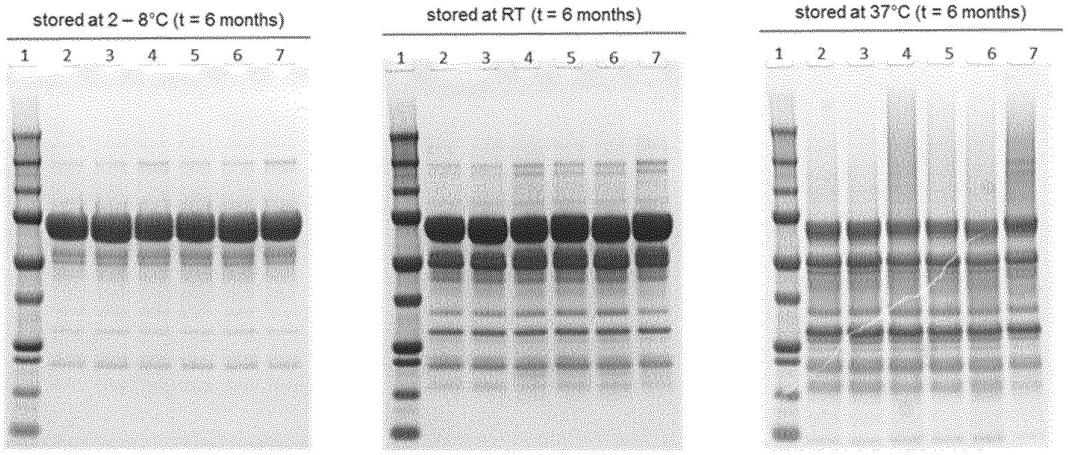
FIGURE 13

A

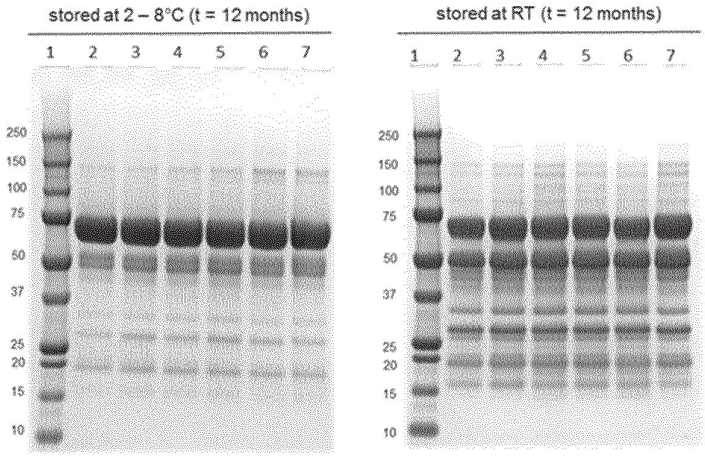


Lane #	Formulation
1	molecular weight marker
2	200 mM citrate phosphate, 150 mM NaCl, 0.002% P80, pH 7.2
3	200 mM citrate phosphate, 300 mM NaCl, 0.002% P80, pH 7.2
4	100 mM sodium phosphate, 150 mM NaCl, 0.002% P80, pH 7.6
5	100 mM sodium phosphate, 300 mM NaCl, 0.002% P80, pH 7.6
6	300 mM sodium phosphate, 150 mM NaCl, 0.002% P80, pH 7.6
7	PBS, pH 7.4

B



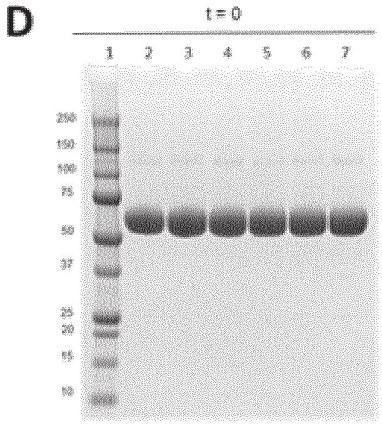
C



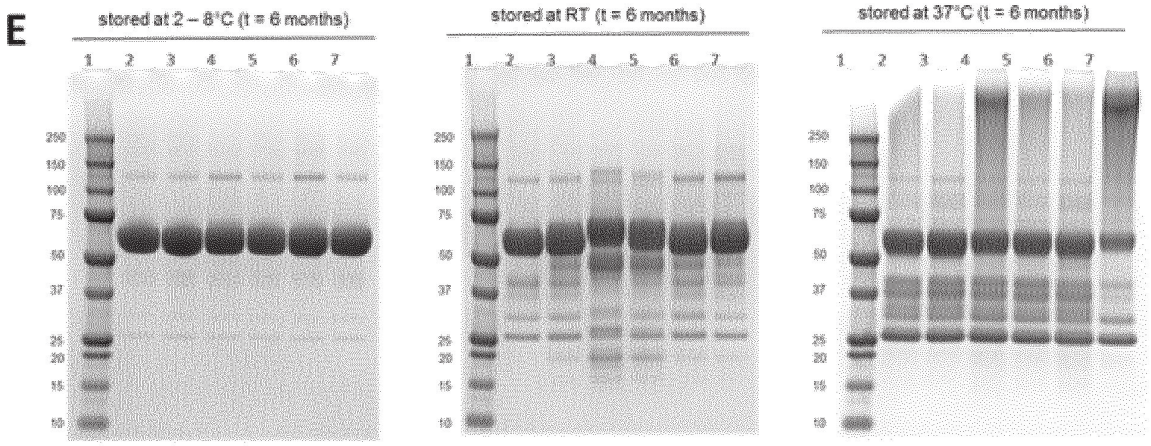
stored at 37°C (t = 12 months)

not done

FIGURE 13



Lane #	Formulation
1	molecular weight marker
2	200 mM citrate phosphate, 150 mM NaCl, 0.002% P80, pH 7.2
3	200 mM citrate phosphate, 300 mM NaCl, 0.002% P80, pH 7.2
4	100 mM sodium phosphate, 150 mM NaCl, 0.002% P80, pH 7.6
5	100 mM sodium phosphate, 300 mM NaCl, 0.002% P80, pH 7.6
6	300 mM sodium phosphate, 150 mM NaCl, 0.002% P80, pH 7.6
7	PBS, pH 7.4



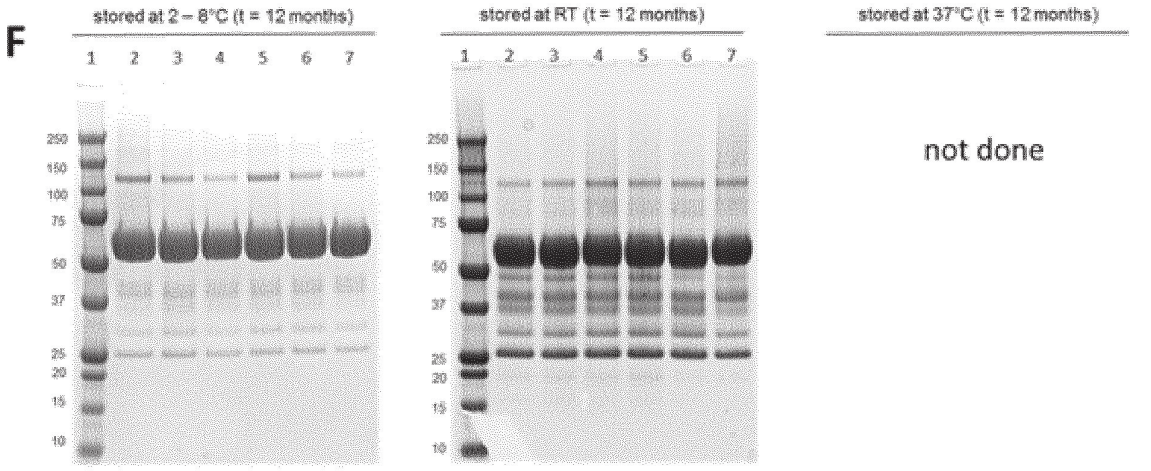


FIGURE 14

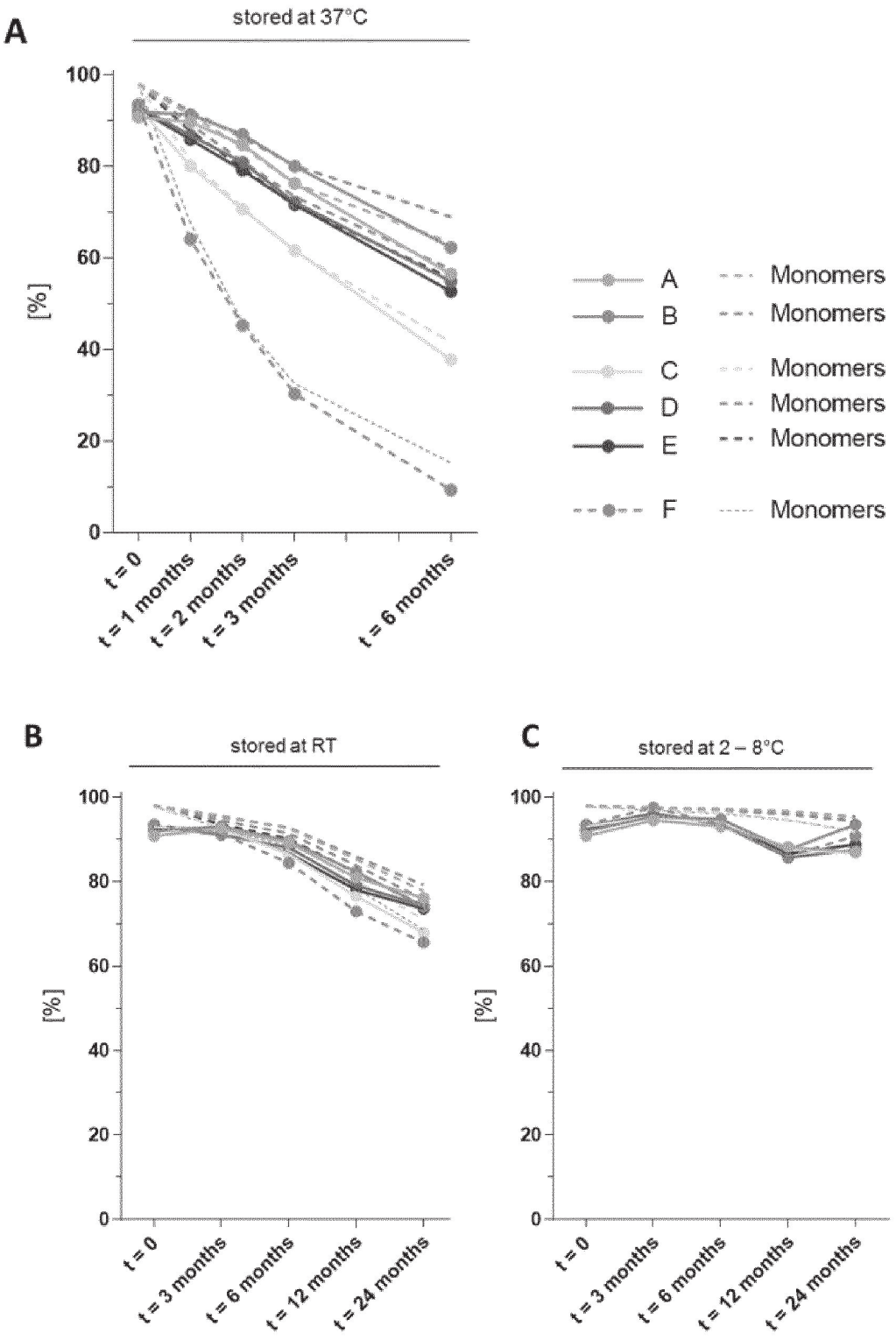

A
stored at 37°C
100
80
60
40
20
0
[%]
t = 0
t = 1 months
t = 2 months
t = 3 months
t = 6 months
A
Monomers
B
Monomers
C
Monomers
D
Monomers
E
Monomers
F
Monomers
B
stored at RT
100
80
60
40
20
0
[%]
t = 0
t = 3 months
t = 6 months
t = 12 months
t = 24 months
C
stored at 2 – 8°C
100
80
60
40
20
0
[%]
t = 0
t = 3 months
t = 6 months
t = 12 months
t = 24 months

FIGURE 14

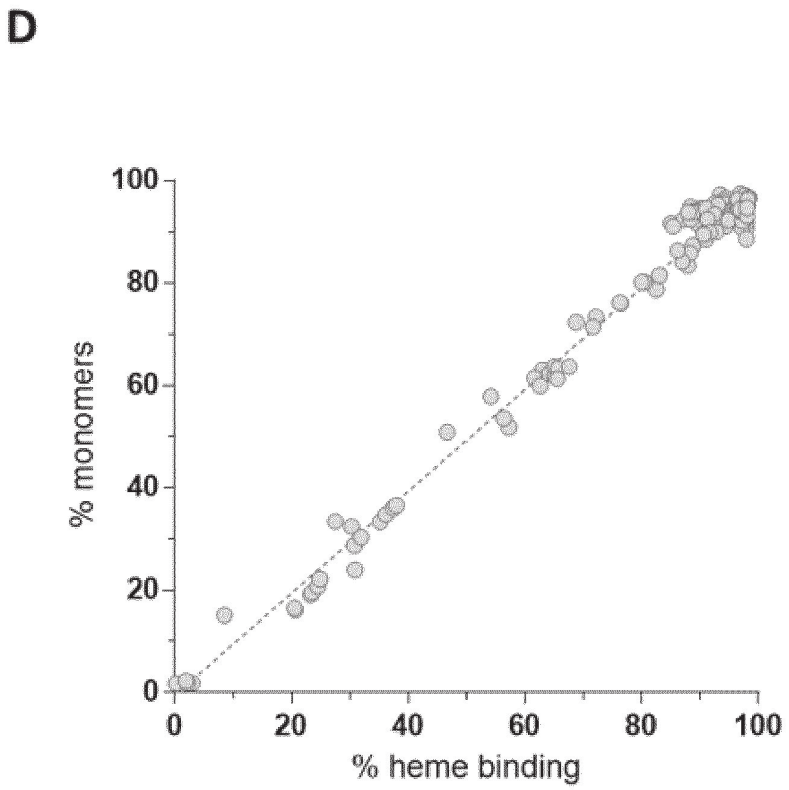


FIGURE 15

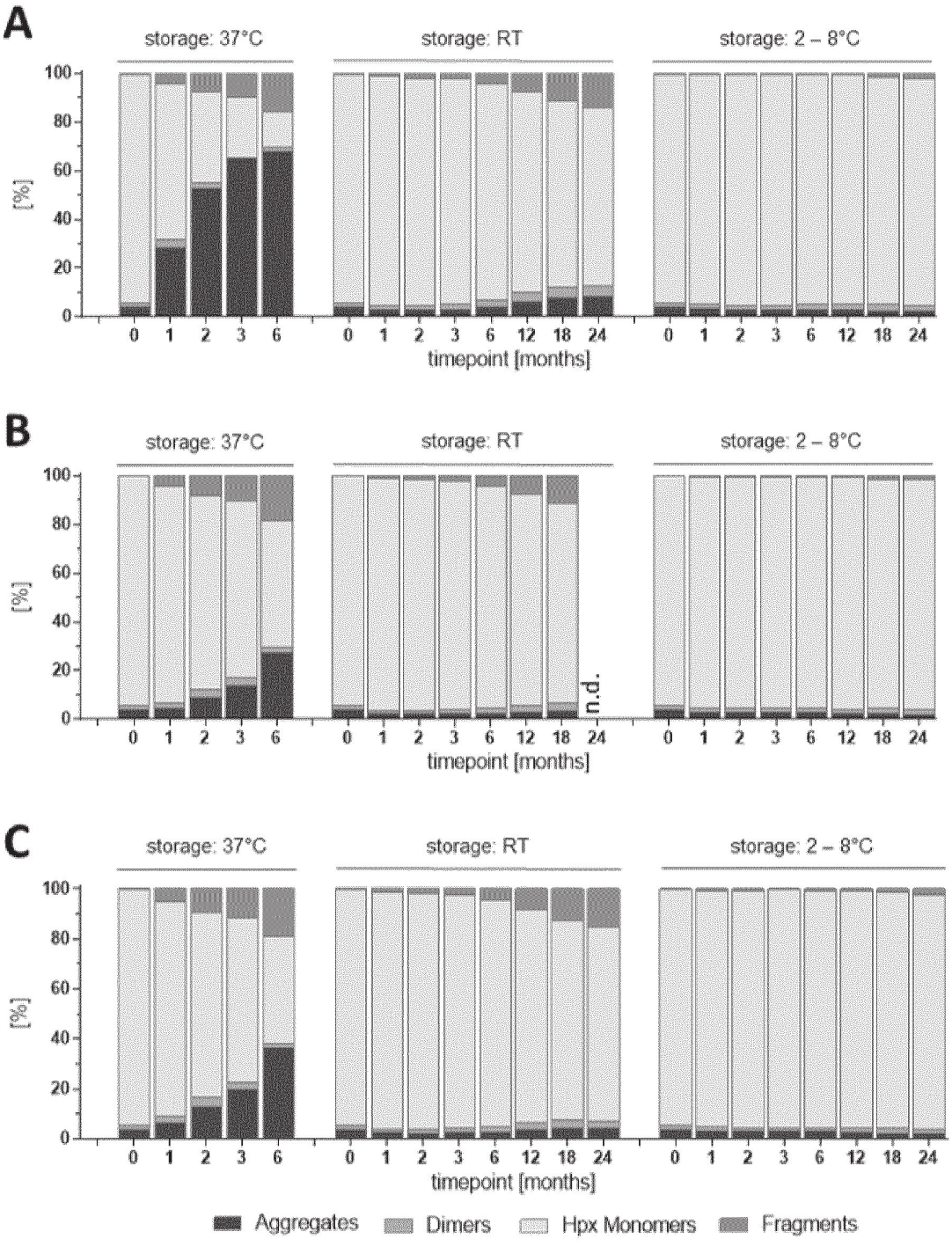

A
storage: 37°C
storage: RT
storage: 2 – 8°C
[%]
timepoint [months]
B
n.d.
C
Aggregates
Dimers
Hpx Monomers
Fragments

FIGURE 15

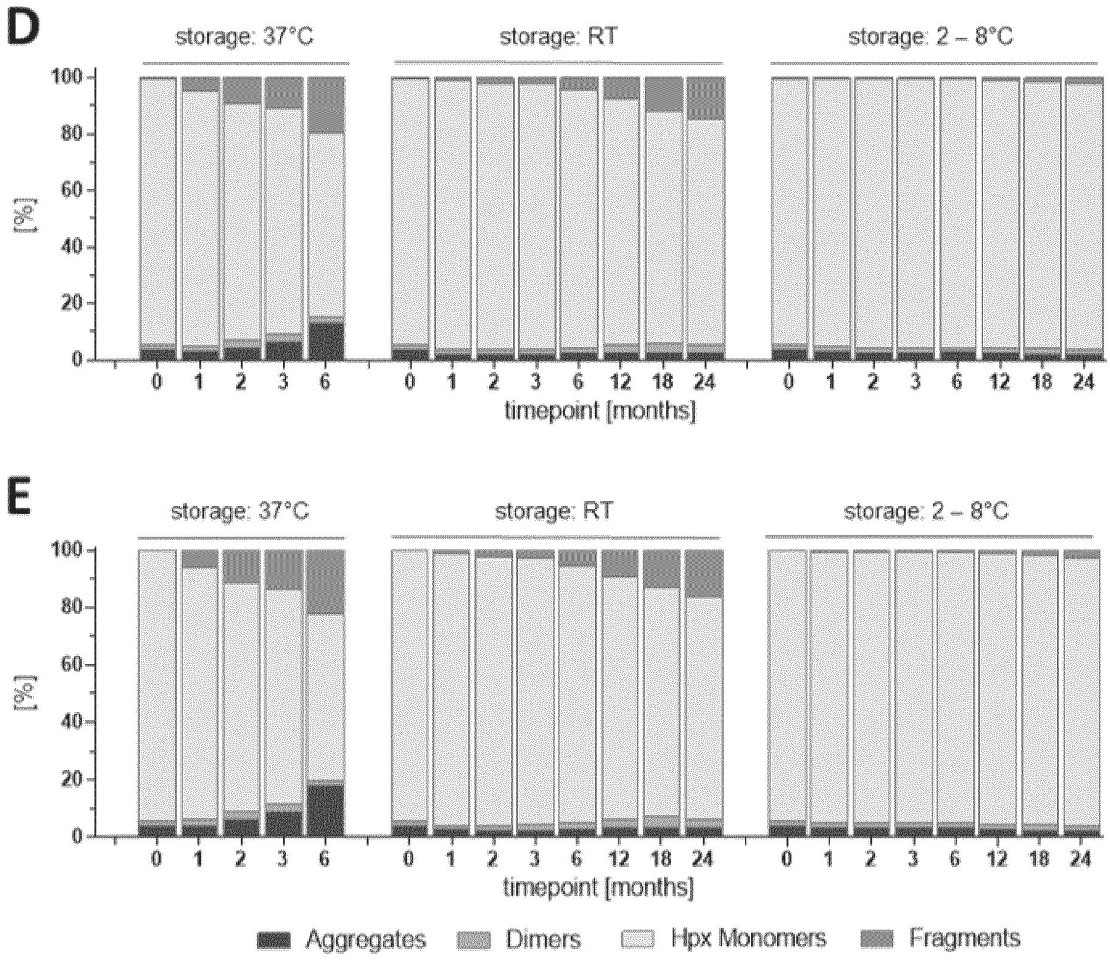

D
storage: 37°C
storage: RT
storage: 2 – 8°C
[%]
100
80
60
40
20
0
0 1 2 3 6 0 1 2 3 6 12 18 24 0 1 2 3 6 12 18 24
timepoint [months]
E
storage: 37°C
storage: RT
storage: 2 – 8°C
[%]
100
80
60
40
20
0
0 1 2 3 6 0 1 2 3 6 12 18 24 0 1 2 3 6 12 18 24
timepoint [months]
Aggregates
Dimers
Hpx Monomers
Fragments

FIGURE 16

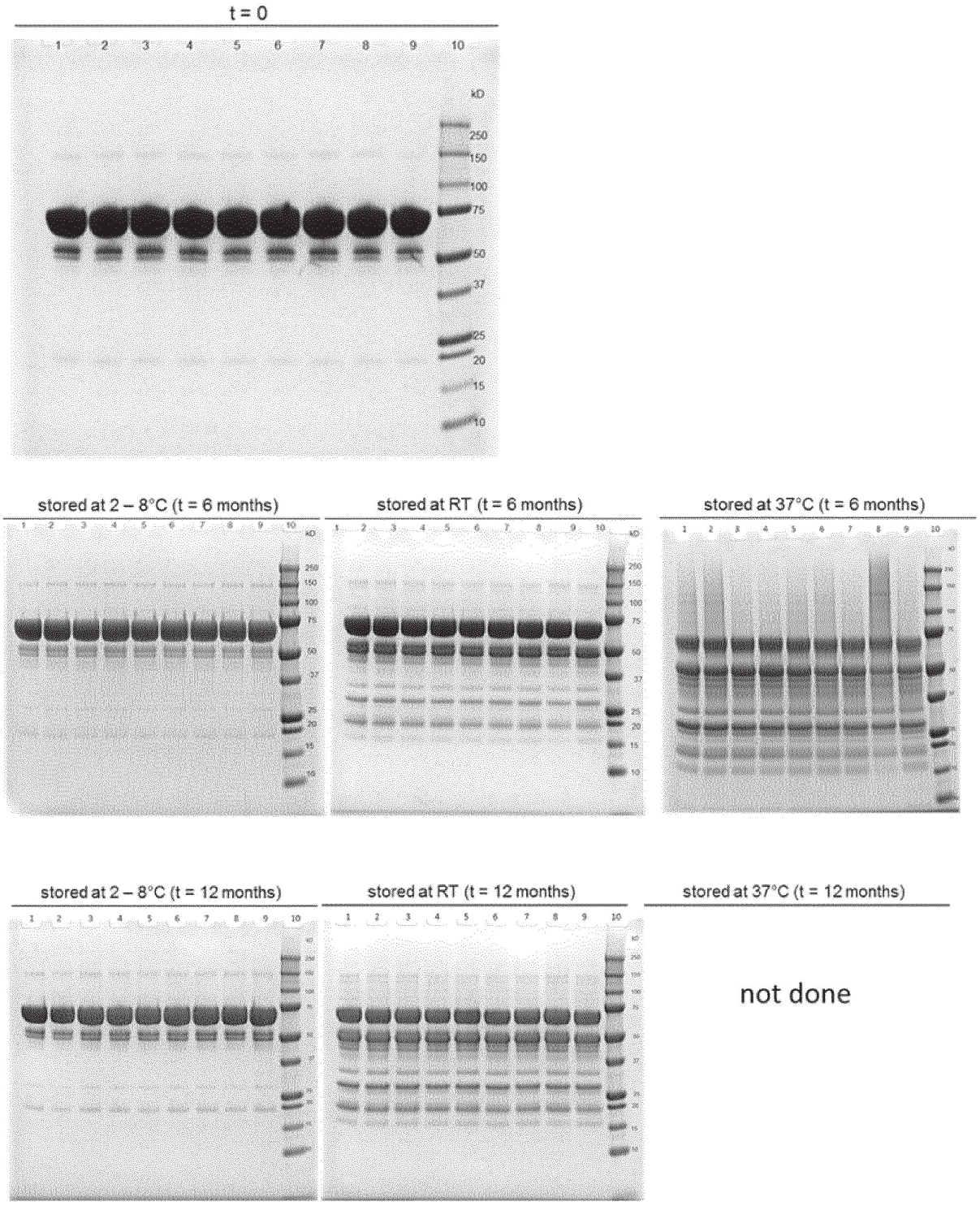

t = 0
1 2 3 4 5 6 7 8 9 10
kD
250
150
100
75
50
37
25
20
15
10
stored at 2 – 8°C (t = 6 months)
stored at RT (t = 6 months)
stored at 37°C (t = 6 months)
stored at 2 – 8°C (t = 12 months)
stored at RT (t = 12 months)
stored at 37°C (t = 12 months)
not done

FIGURE 17

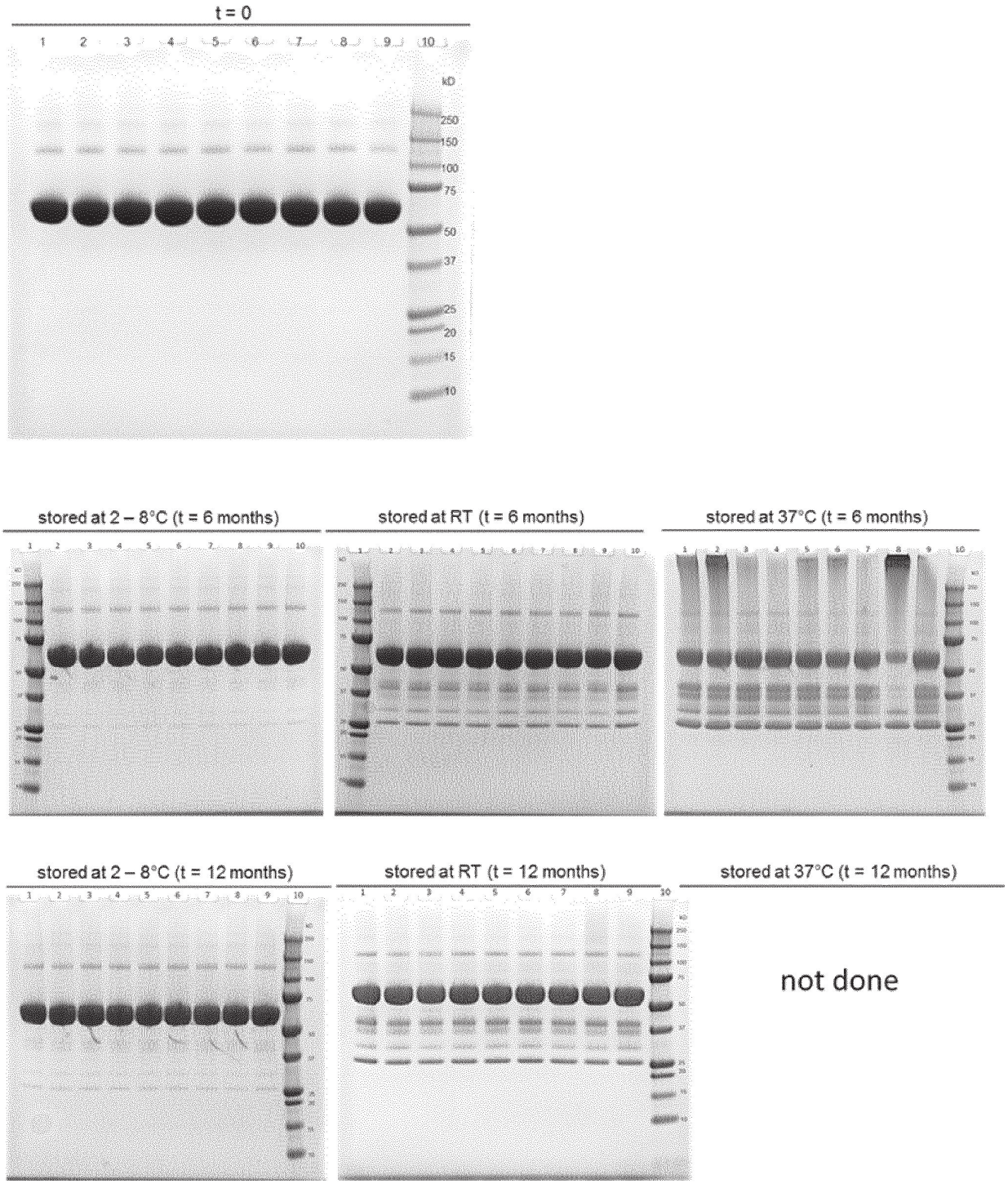

t = 0
1 2 3 4 5 6 7 8 9 10
kD
250
150
100
75
50
37
25
20
15
10
stored at 2 – 8°C (t = 6 months)
stored at RT (t = 6 months)
stored at 37°C (t = 6 months)
stored at 2 – 8°C (t = 12 months)
stored at RT (t = 12 months)
stored at 37°C (t = 12 months)
not done

FIGURE 18

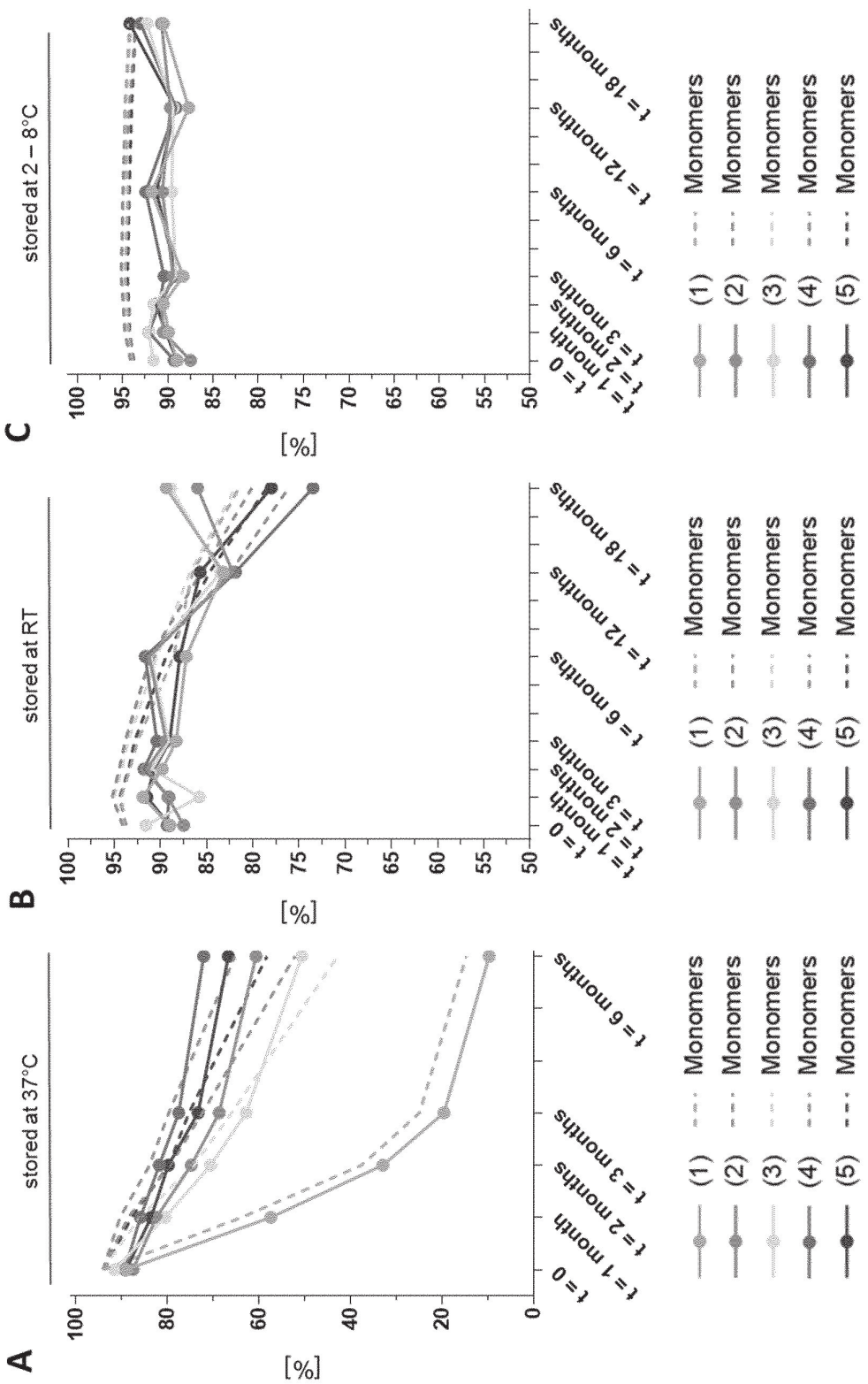

A
stored at 37°C
[%]
100
80
60
40
20
0
t = 0
t = 1 month
t = 2 months
t = 3 months
t = 6 months
B
stored at RT
[%]
100
95
90
85
80
75
70
65
60
55
50
t = 0
t = 1 month
t = 2 months
t = 3 months
t = 6 months
t = 12 months
t = 18 months
C
stored at 2 – 8°C
[%]
100
95
90
85
80
75
70
65
60
55
50
t = 0
t = 1 month
t = 2 months
t = 3 months
t = 6 months
t = 12 months
t = 18 months
(1) Monomers
(2) Monomers
(3) Monomers
(4) Monomers
(5) Monomers
(1) Monomers
(2) Monomers
(3) Monomers
(4) Monomers
(5) Monomers

FIGURE 19

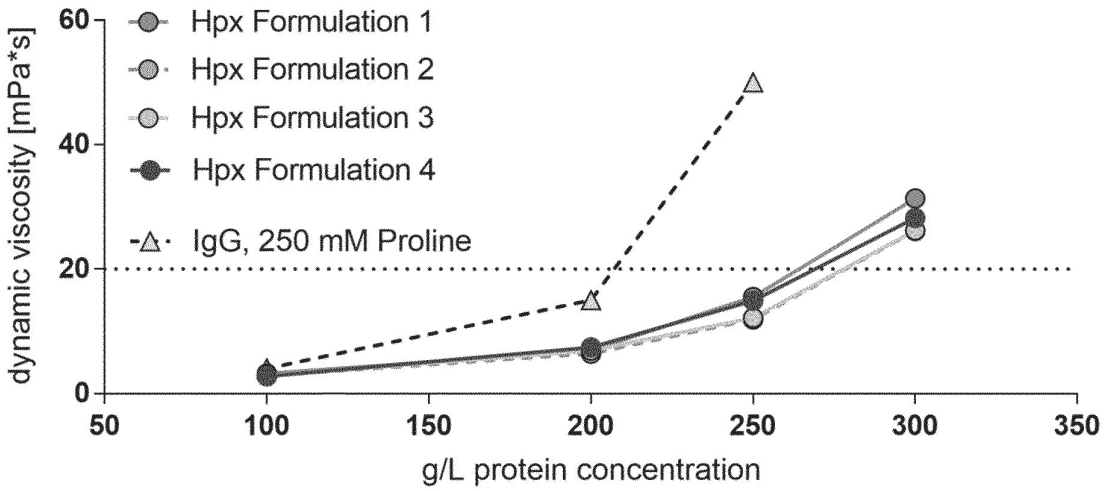


FIGURE 20

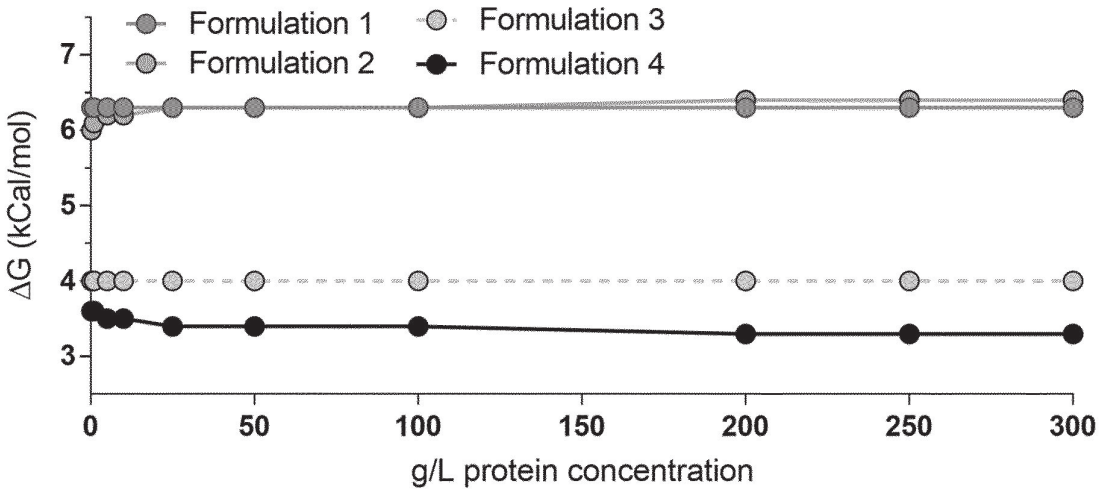


FIGURE 21

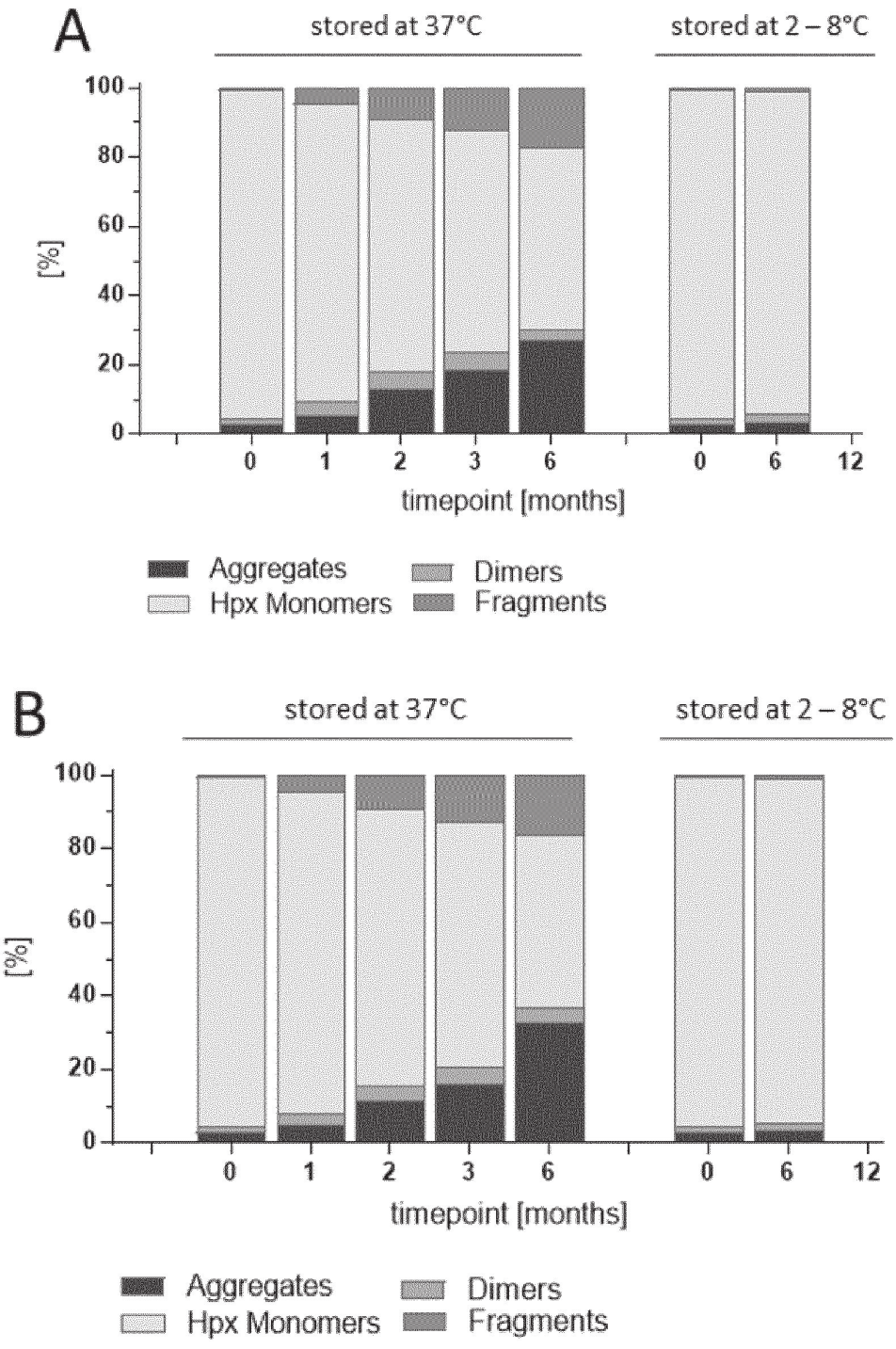

A
stored at 37°C
stored at 2 – 8°C
100
80
60
40
20
0
[%]
0
1
2
3
6
0
6
12
timepoint [months]
Aggregates
Hpx Monomers
Dimers
Fragments
B
stored at 37°C
stored at 2 – 8°C
100
80
60
40
20
0
[%]
0
1
2
3
6
0
6
12
timepoint [months]
Aggregates
Hpx Monomers
Dimers
Fragments

FIGURE 21

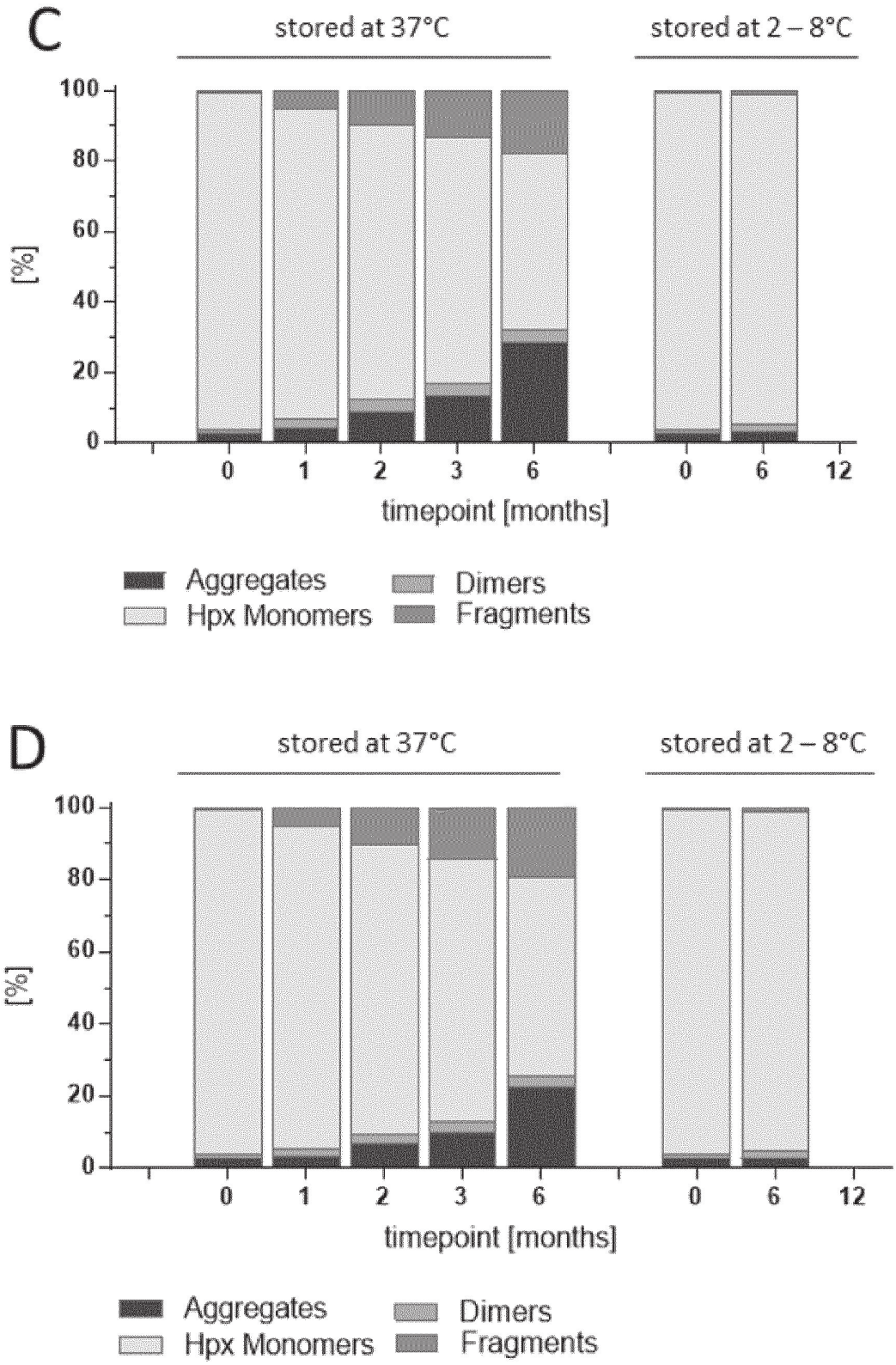
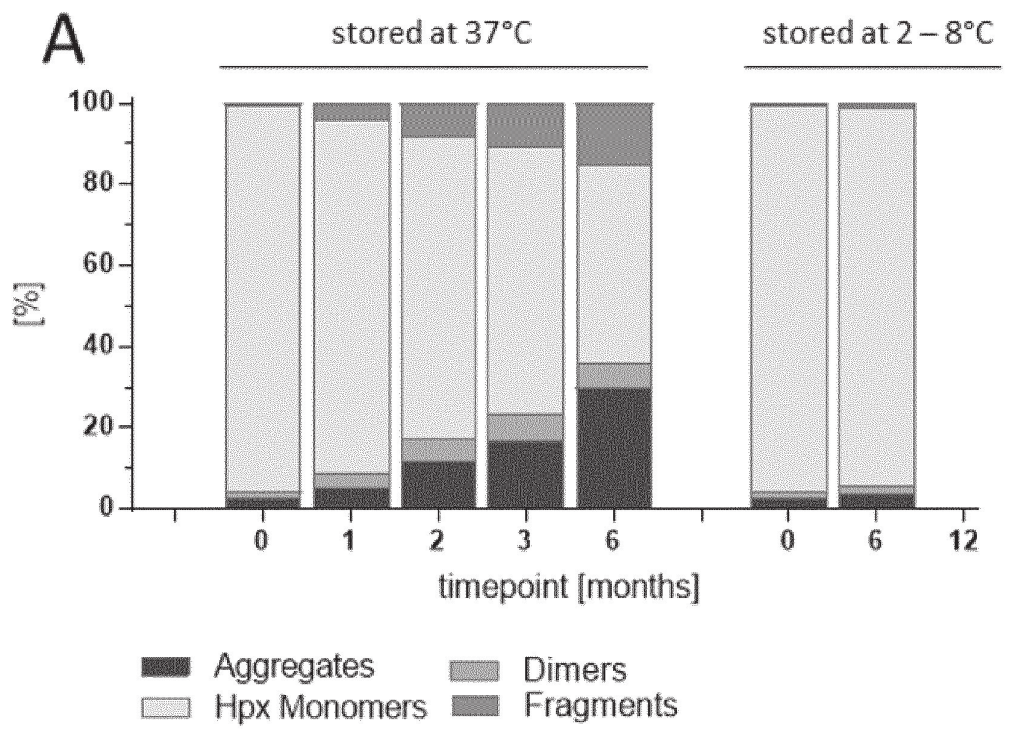

C
stored at 37°C
stored at 2 – 8°C
100
80
60
40
20
0
[%]
0
1
2
3
6
0
6
12
timepoint [months]
Aggregates
Hpx Monomers
Dimers
Fragments
D
stored at 37°C
stored at 2 – 8°C
100
80
60
40
20
0
[%]
0
1
2
3
6
0
6
12
timepoint [months]
Aggregates
Hpx Monomers
Dimers
Fragments

FIGURE 22


A
stored at 37°C
stored at 2 – 8°C
100
80
60
40
20
0
[%]
0
1
2
3
6
0
6
12
timepoint [months]
Aggregates
Hpx Monomers
Dimers
Fragments

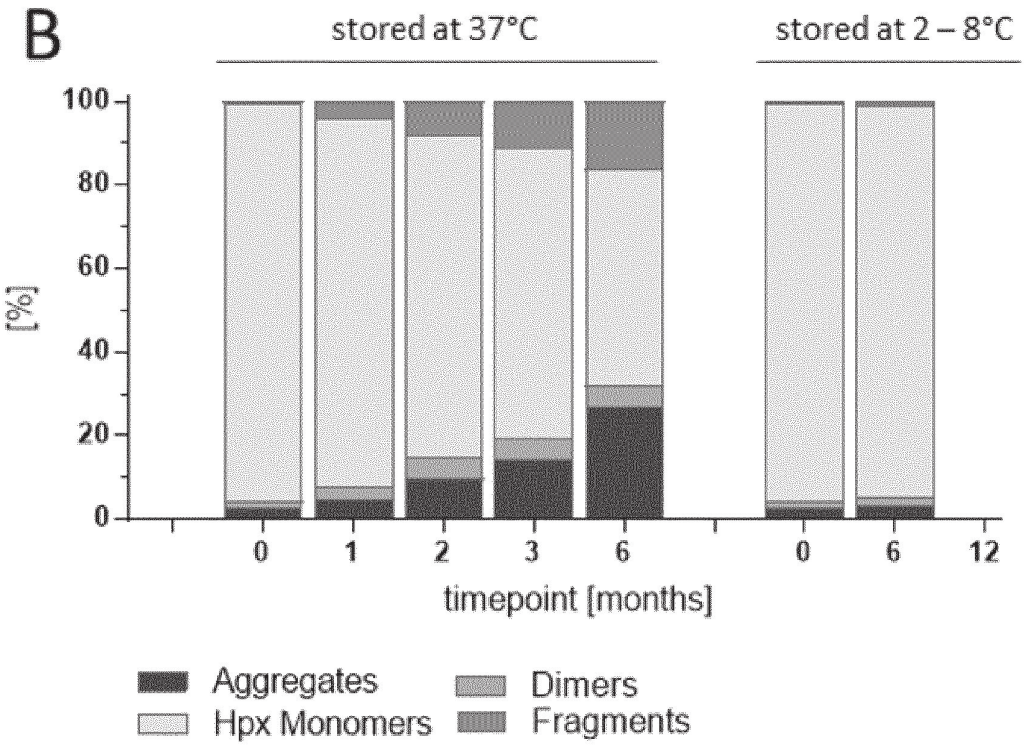

B
stored at 37°C
stored at 2 – 8°C
100
80
60
40
20
0
[%]
0
1
2
3
6
0
6
12
timepoint [months]
Aggregates
Hpx Monomers
Dimers
Fragments

FIGURE 22

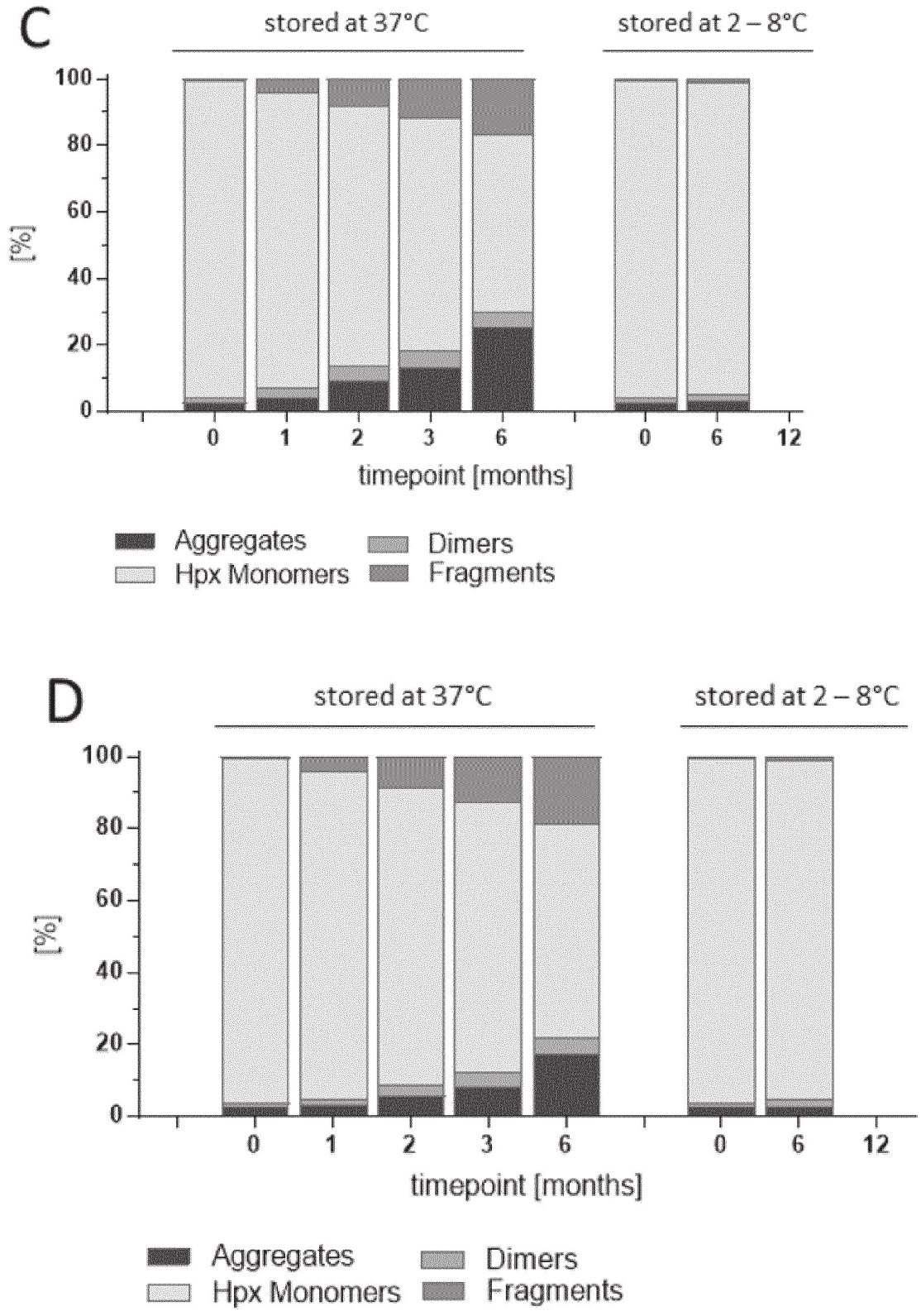

C
stored at 37°C
stored at 2 – 8°C
100
80
60
40
20
0
[%]
0
1
2
3
6
0
6
12
timepoint [months]
Aggregates
Hpx Monomers
Dimers
Fragments
D
stored at 37°C
stored at 2 – 8°C
100
80
60
40
20
0
[%]
0
1
2
3
6
0
6
12
timepoint [months]
Aggregates
Hpx Monomers
Dimers
Fragments

FIGURE 23

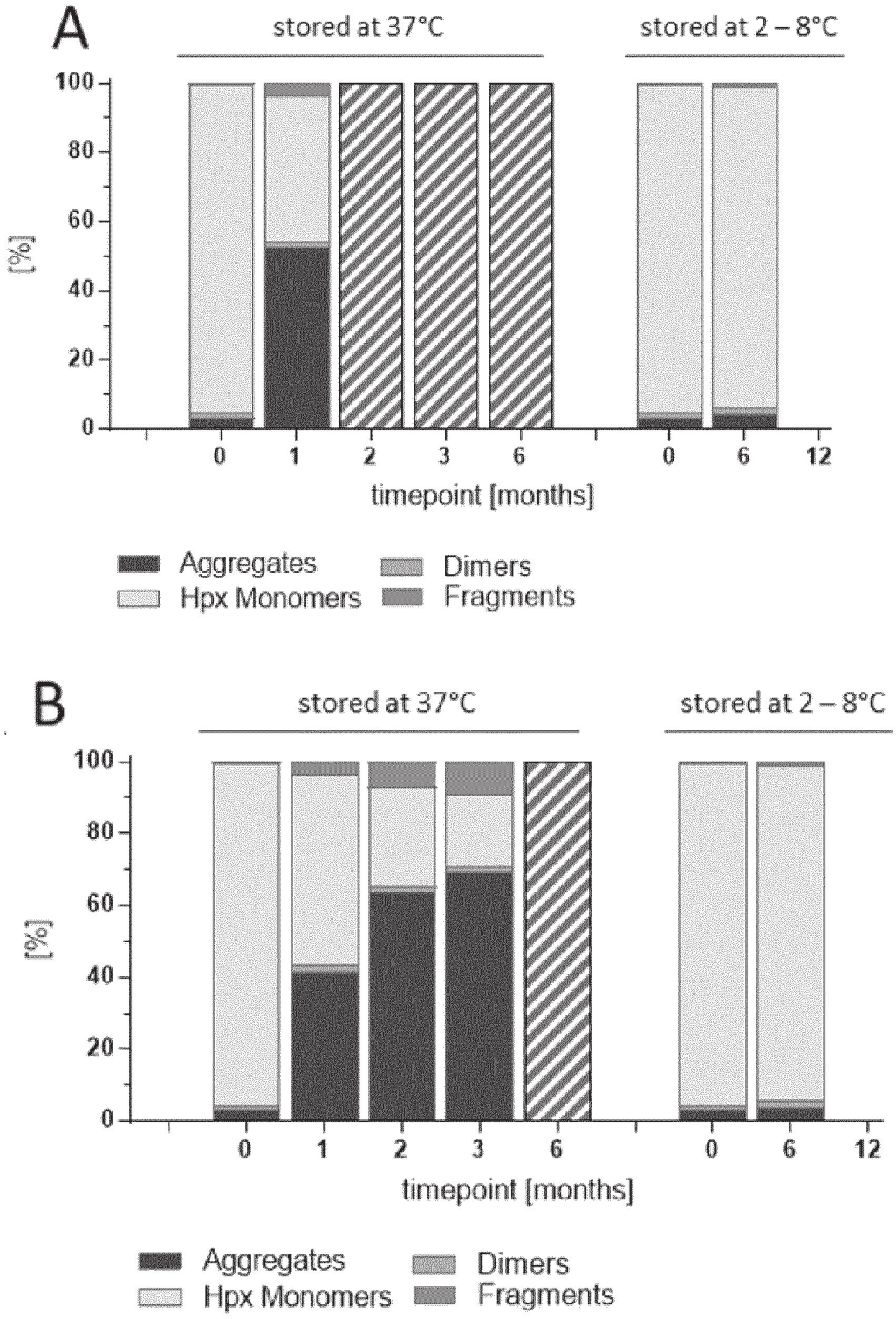

A
stored at 37°C
stored at 2 – 8°C
100
80
60
40
20
0
[%]
0
1
2
3
6
0
6
12
timepoint [months]
Aggregates
Hpx Monomers
Dimers
Fragments
B
stored at 37°C
stored at 2 – 8°C
100
80
60
40
20
0
[%]
0
1
2
3
6
0
6
12
timepoint [months]
Aggregates
Hpx Monomers
Dimers
Fragments

FIGURE 23

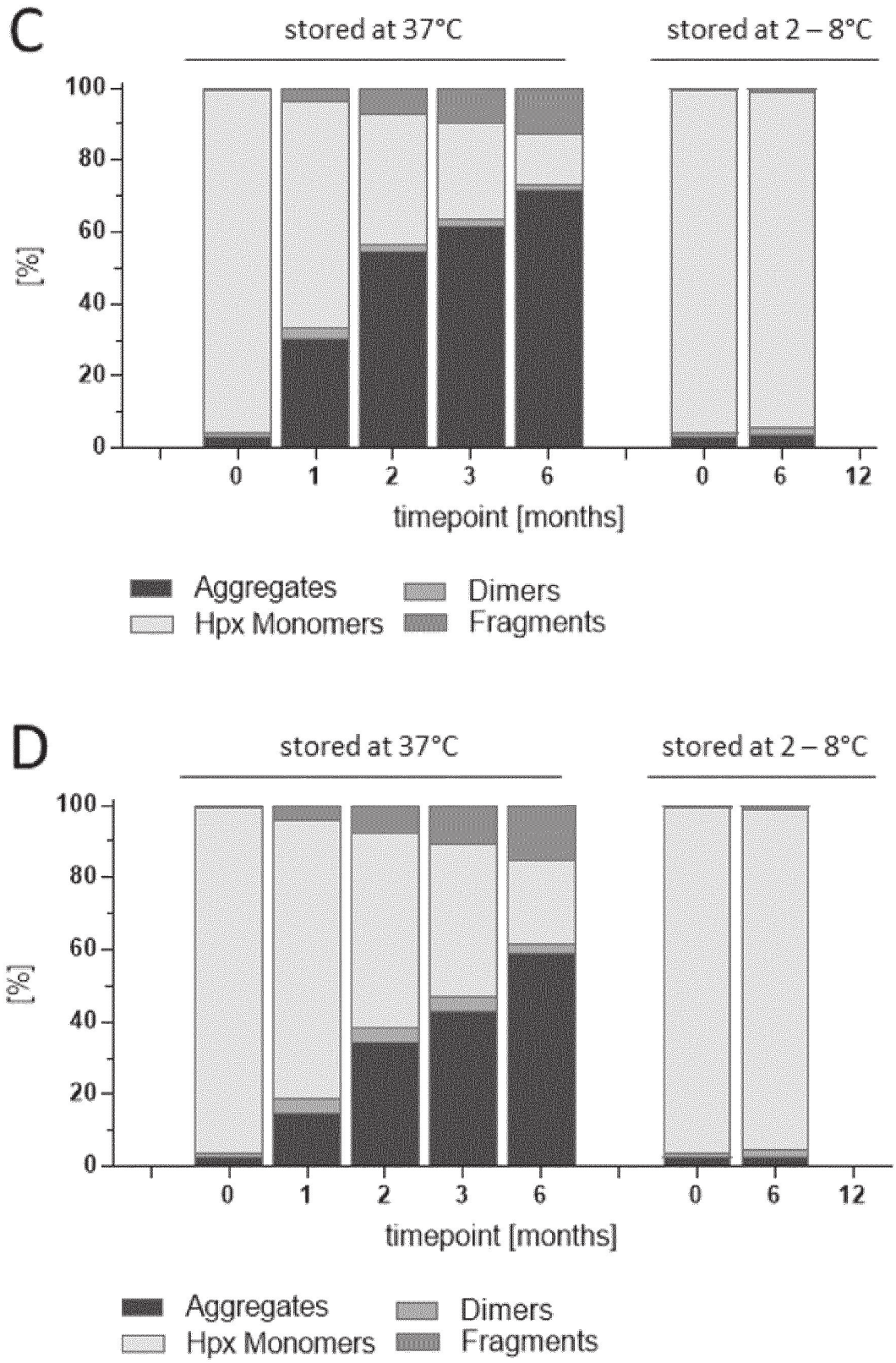

C
stored at 37°C
stored at 2 – 8°C
100
80
60
40
20
0
[%]
0
1
2
3
6
0
6
12
timepoint [months]
Aggregates
Hpx Monomers
Dimers
Fragments
D
stored at 37°C
stored at 2 – 8°C
100
80
60
40
20
0
[%]
0
1
2
3
6
0
6
12
timepoint [months]
Aggregates
Hpx Monomers
Dimers
Fragments

FIGURE 24

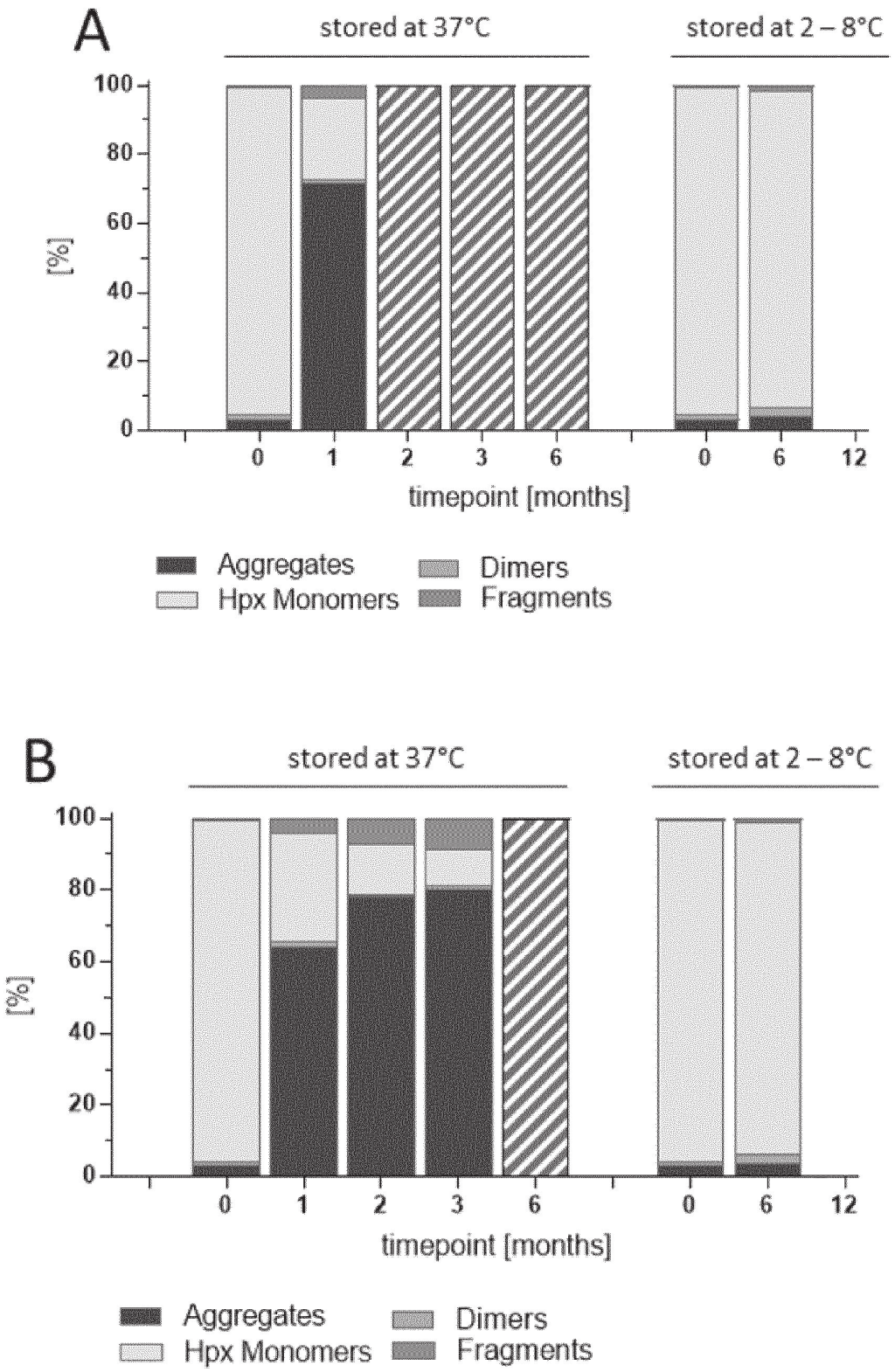
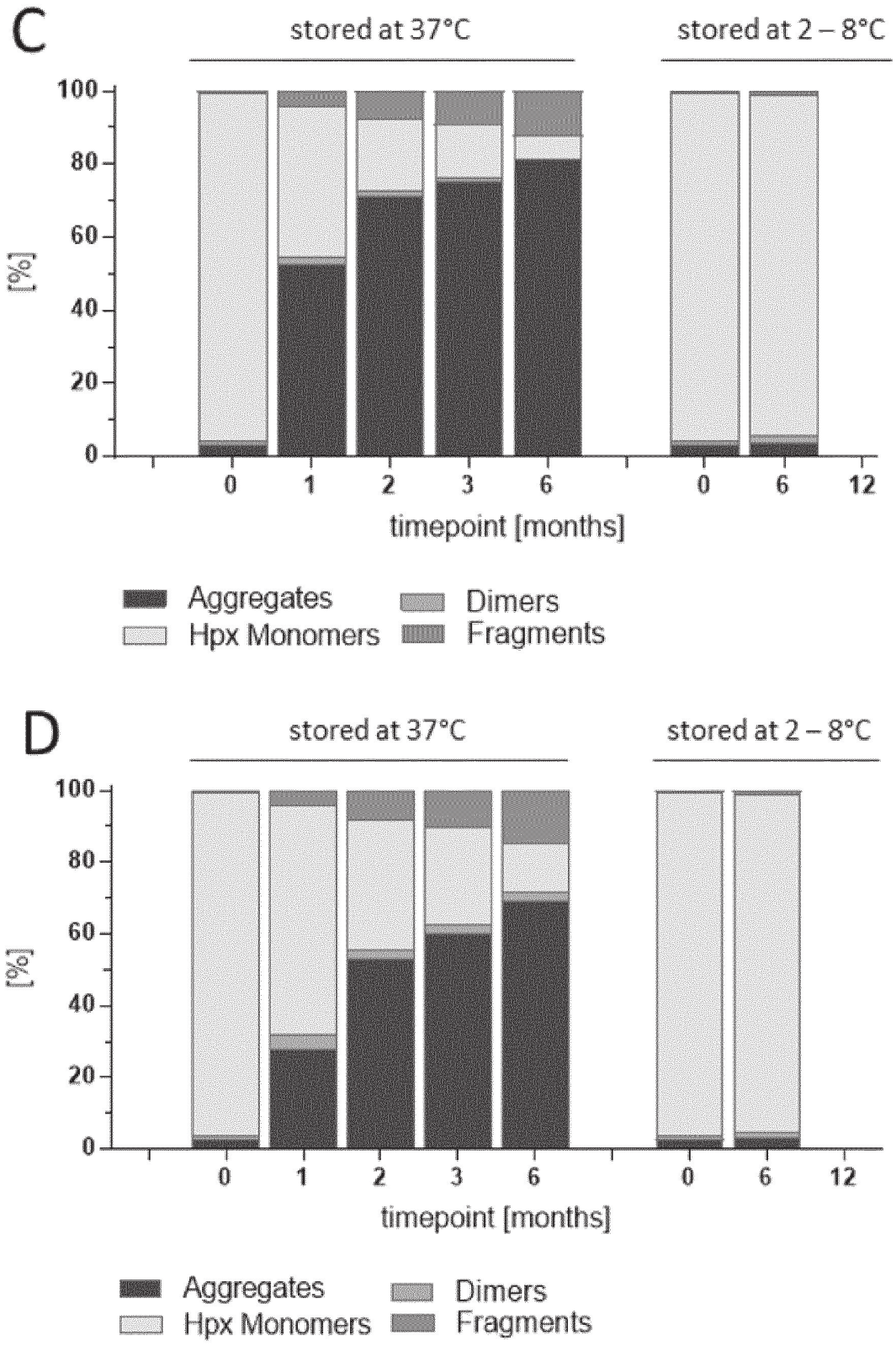

A
stored at 37°C
stored at 2 – 8°C
100
80
60
40
20
0
[%]
0
1
2
3
6
0
6
12
timepoint [months]
Aggregates
Hpx Monomers
Dimers
Fragments
B
stored at 37°C
stored at 2 – 8°C
100
80
60
40
20
0
[%]
0
1
2
3
6
0
6
12
timepoint [months]
Aggregates
Hpx Monomers
Dimers
Fragments

FIGURE 24


C
stored at 37°C
stored at 2 – 8°C
100
80
60
40
20
0
[%]
0
1
2
3
6
0
6
12
timepoint [months]
Aggregates
Hpx Monomers
Dimers
Fragments
D
stored at 37°C
stored at 2 – 8°C
100
80
60
40
20
0
[%]
0
1
2
3
6
0
6
12
timepoint [months]
Aggregates
Hpx Monomers
Dimers
Fragments