Marathon MSC Marathon A2 Amberlite IRC748 Amberlite IRA458

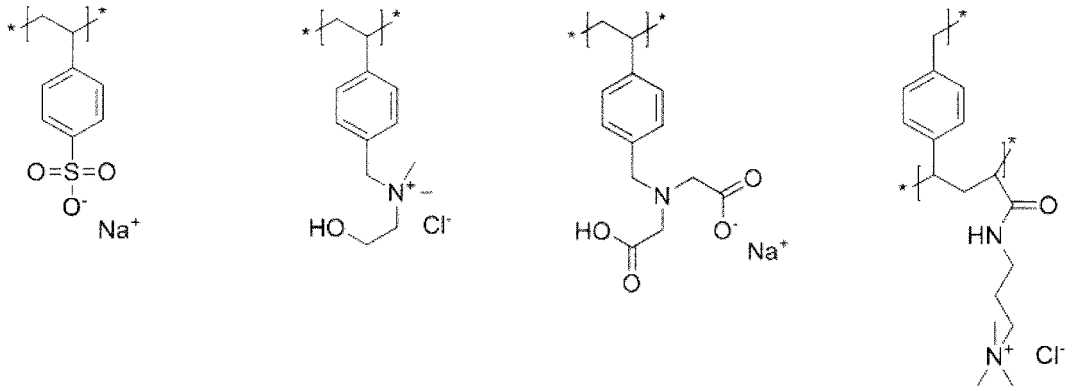


Fig. 1

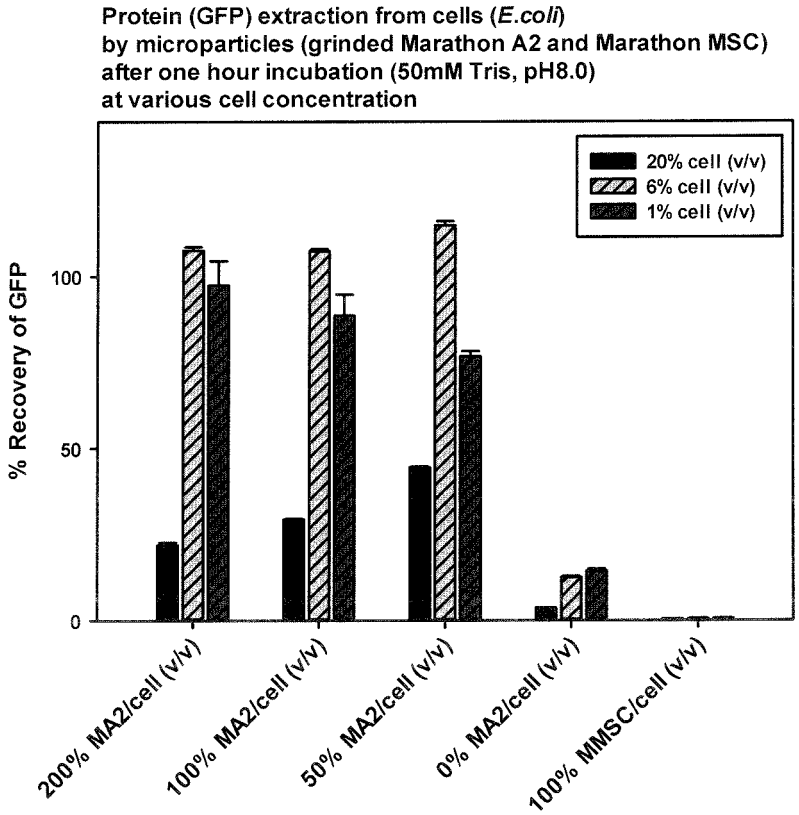


Fig. 2

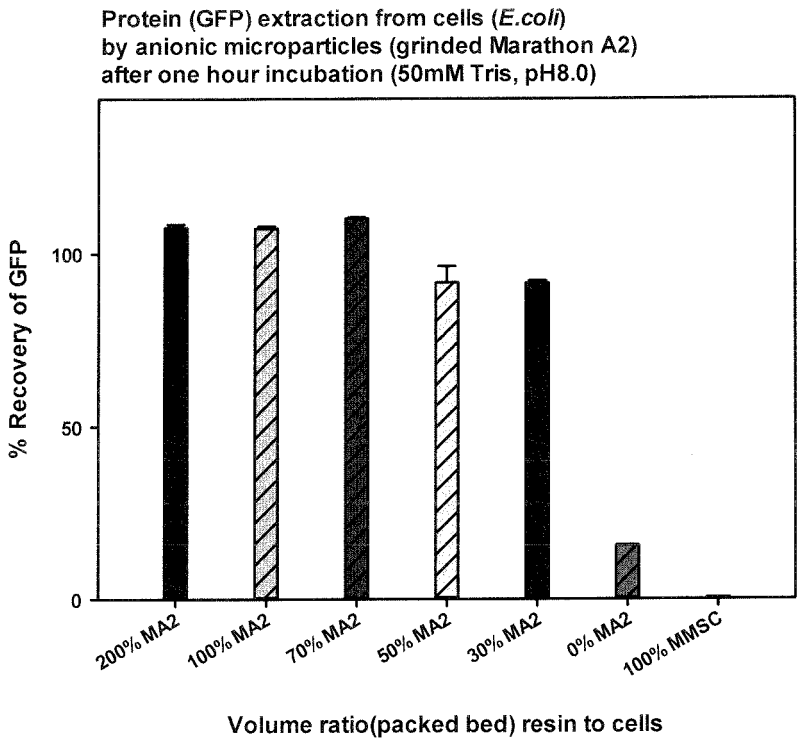

Protein (GFP) extraction from cells (E.coli)
by anionic microparticles (grinded Marathon A2)
after one hour incubation (50mM Tris, pH8.0)
% Recovery of GFP
100
50
0
200% MA2
100% MA2
70% MA2
50% MA2
30% MA2
0% MA2
100% MMSC
Volume ratio(packed bed) resin to cells

Fig. 3

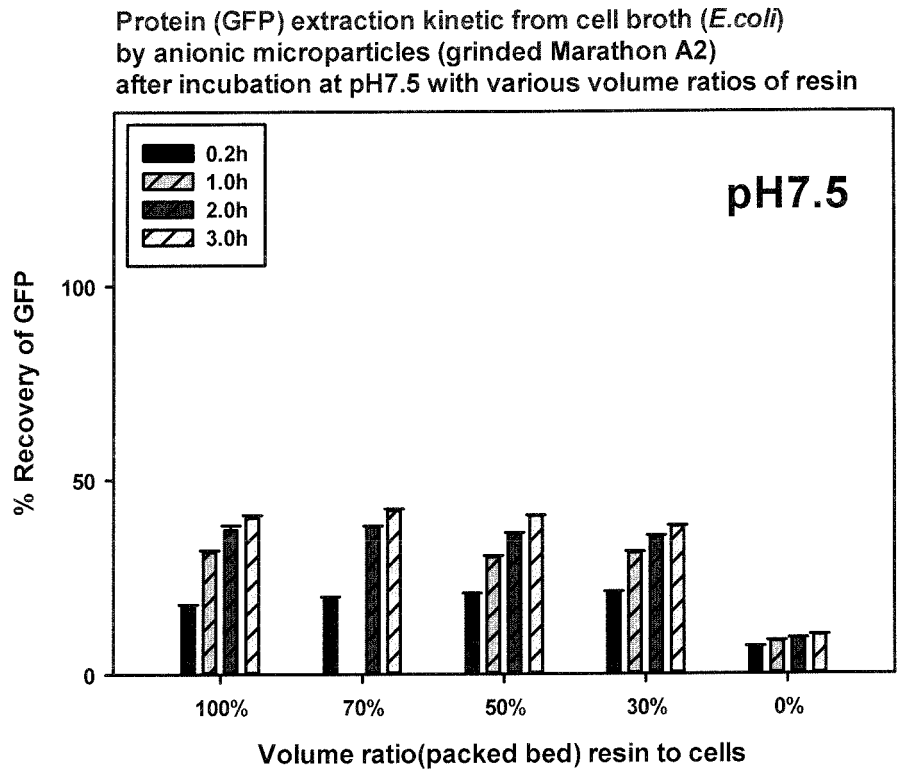

Protein (GFP) extraction kinetic from cell broth (E.coli)
by anionic microparticles (grinded Marathon A2)
after incubation at pH7.5 with various volume ratios of resin
0.2h
1.0h
2.0h
3.0h
pH7.5
% Recovery of GFP
100
50
0
100%
70%
50%
30%
0%
Volume ratio(packed bed) resin to cells

Fig. 4a

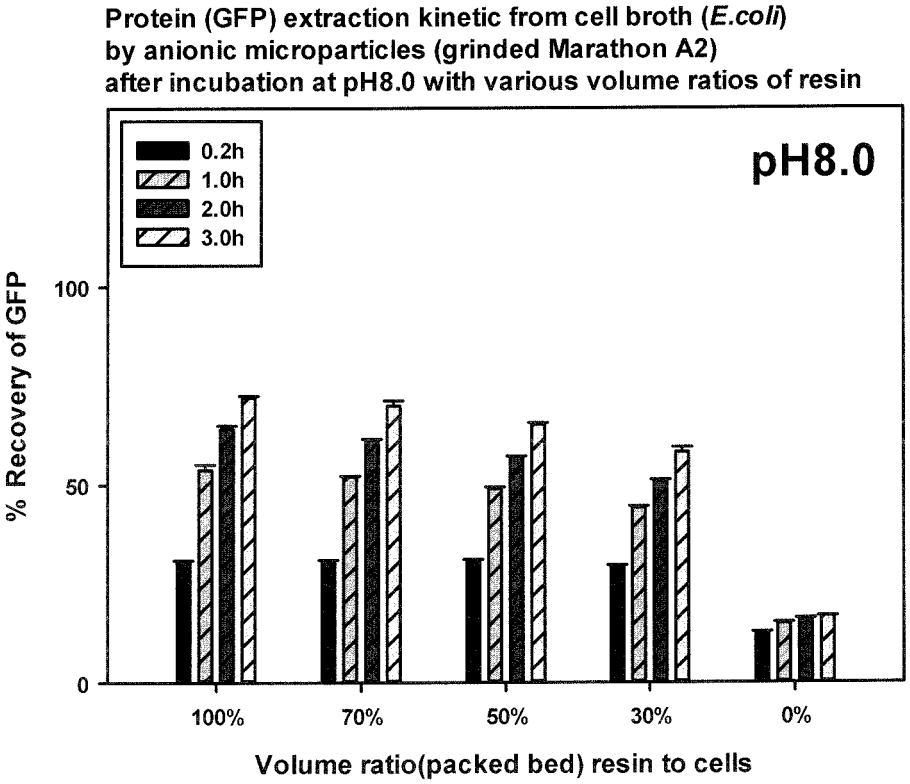

Protein (GFP) extraction kinetic from cell broth (E.coli)
by anionic microparticles (grinded Marathon A2)
after incubation at pH8.0 with various volume ratios of resin
0.2h
1.0h
2.0h
3.0h
pH8.0
% Recovery of GFP
100
50
0
100%
70%
50%
30%
0%
Volume ratio(packed bed) resin to cells

Fig. 4b

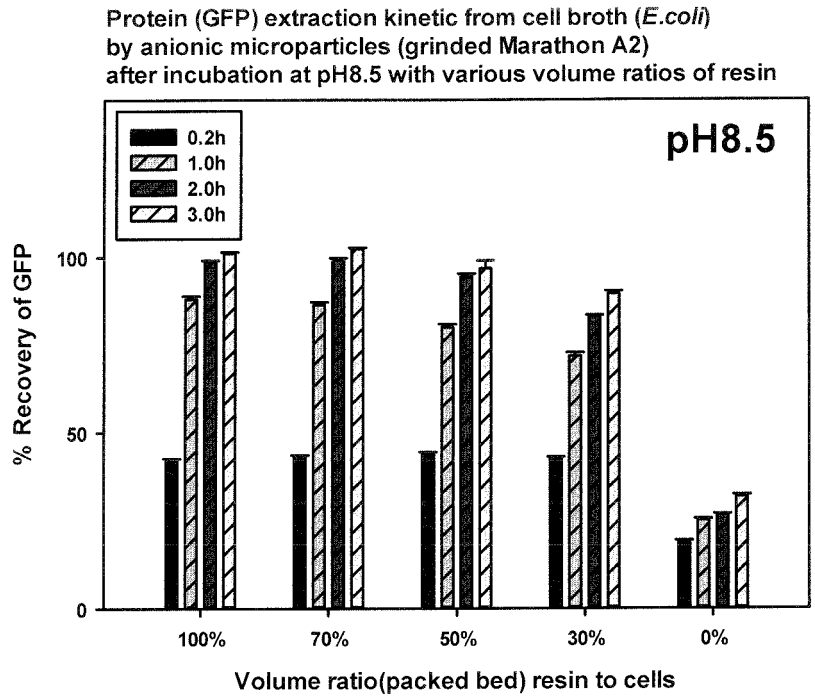

Protein (GFP) extraction kinetic from cell broth (E.coli)
by anionic microparticles (grinded Marathon A2)
after incubation at pH8.5 with various volume ratios of resin
pH8.5
0.2h
1.0h
2.0h
3.0h
% Recovery of GFP
100
50
0
100%
70%
50%
30%
0%
Volume ratio(packed bed) resin to cells

Fig. 4c

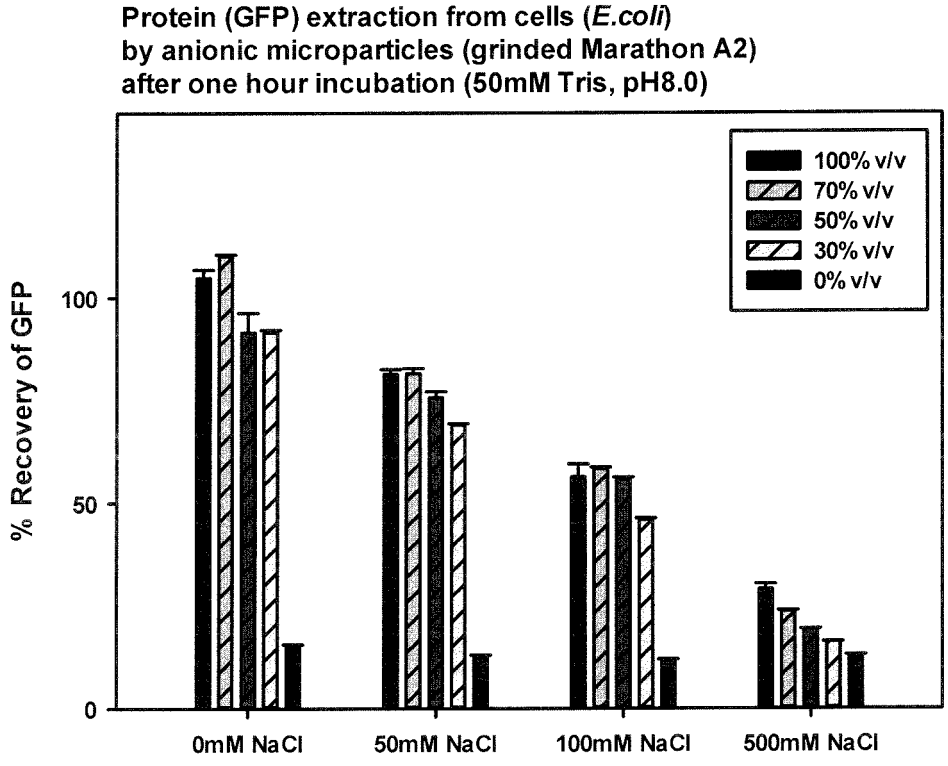

Protein (GFP) extraction from cells (E.coli)
by anionic microparticles (grinded Marathon A2)
after one hour incubation (50mM Tris, pH8.0)
100% v/v
70% v/v
50% v/v
30% v/v
0% v/v
% Recovery of GFP
100
50
0
0mM NaCl
50mM NaCl
100mM NaCl
500mM NaCl

Fig. 5

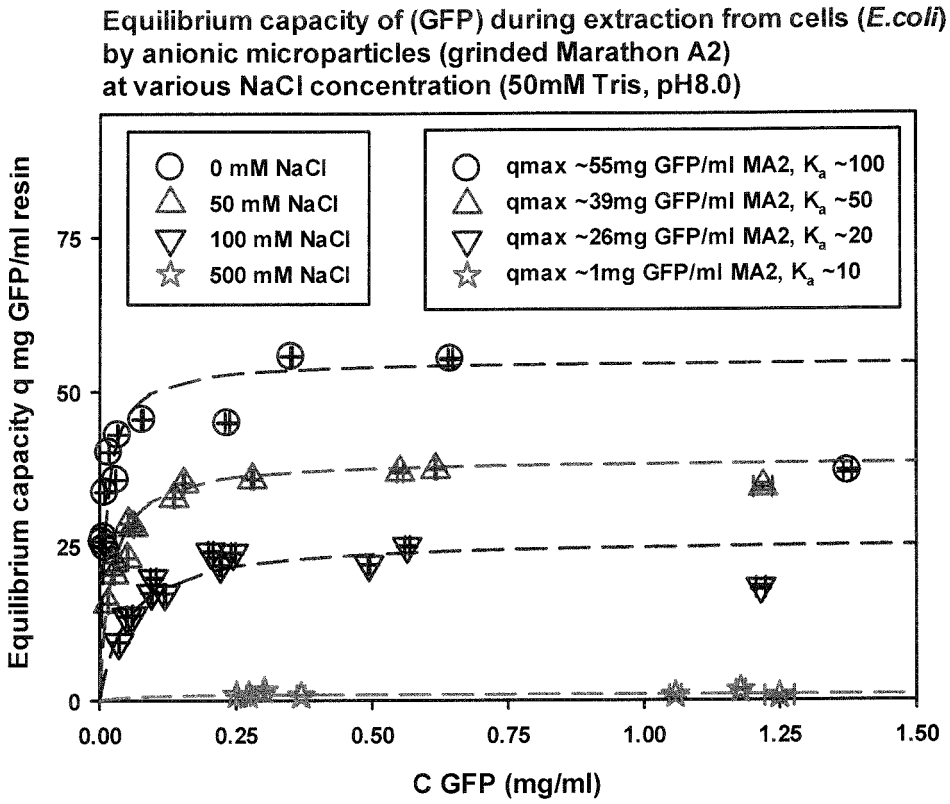

Equilibrium capacity of (GFP) during extraction from cells (E.coli)
by anionic microparticles (grinded Marathon A2)
at various NaCl concentration (50mM Tris, pH8.0)
0 mM NaCl
50 mM NaCl
100 mM NaCl
500 mM NaCl
qmax ~55mg GFP/ml MA2, Ka ~100
qmax ~39mg GFP/ml MA2, Ka ~50
qmax ~26mg GFP/ml MA2, Ka ~20
qmax ~1mg GFP/ml MA2, Ka ~10
Equilibrium capacity q mg GFP/ml resin
0
25
50
75
0.00
0.25
0.50
0.75
1.00
1.25
1.50
C GFP (mg/ml)

Fig. 6

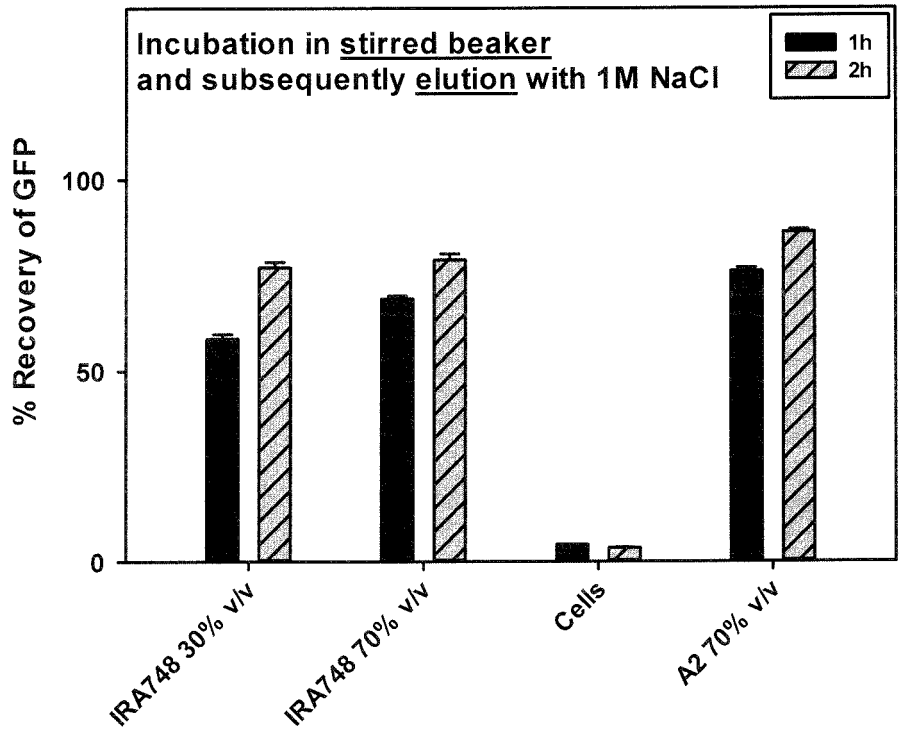

Incubation in stirred beaker
and subsequently elution with 1M NaCl
1h
2h
% Recovery of GFP
0
50
100
IRA748 30% v/v
IRA748 70% v/v
Cells
A2 70% v/v

Fig. 7a

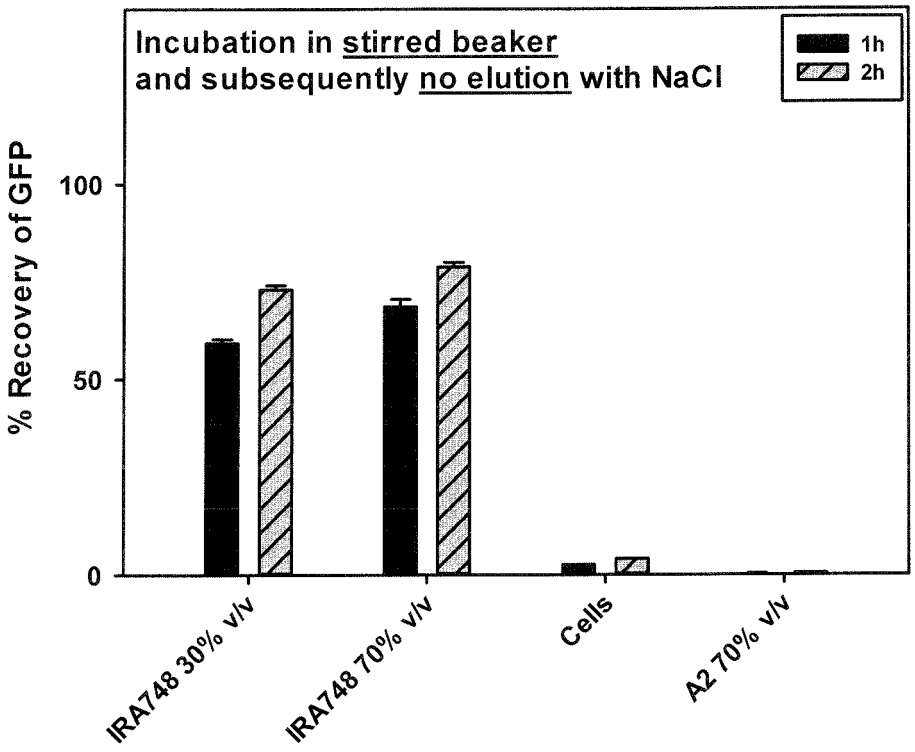

Incubation in stirred beaker
and subsequently no elution with NaCl
1h
2h
% Recovery of GFP
100
50
0
IRA748 30% v/v
IRA748 70% v/v
Cells
A2 70% v/v

Fig. 7b

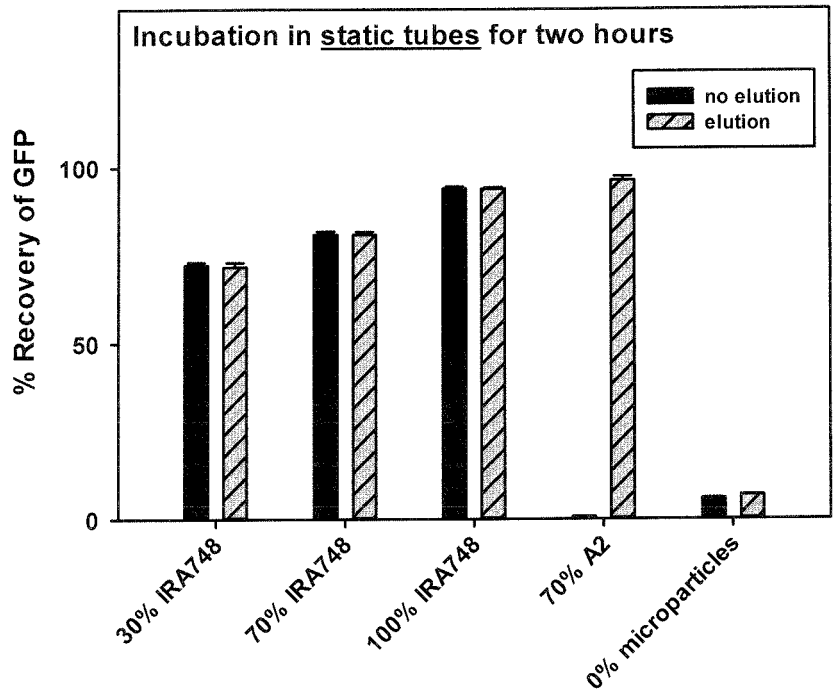

Incubation in static tubes for two hours
no elution
elution
% Recovery of GFP
100
50
0
30% IRA748
70% IRA748
100% IRA748
70% A2
0% microparticles

Fig. 8

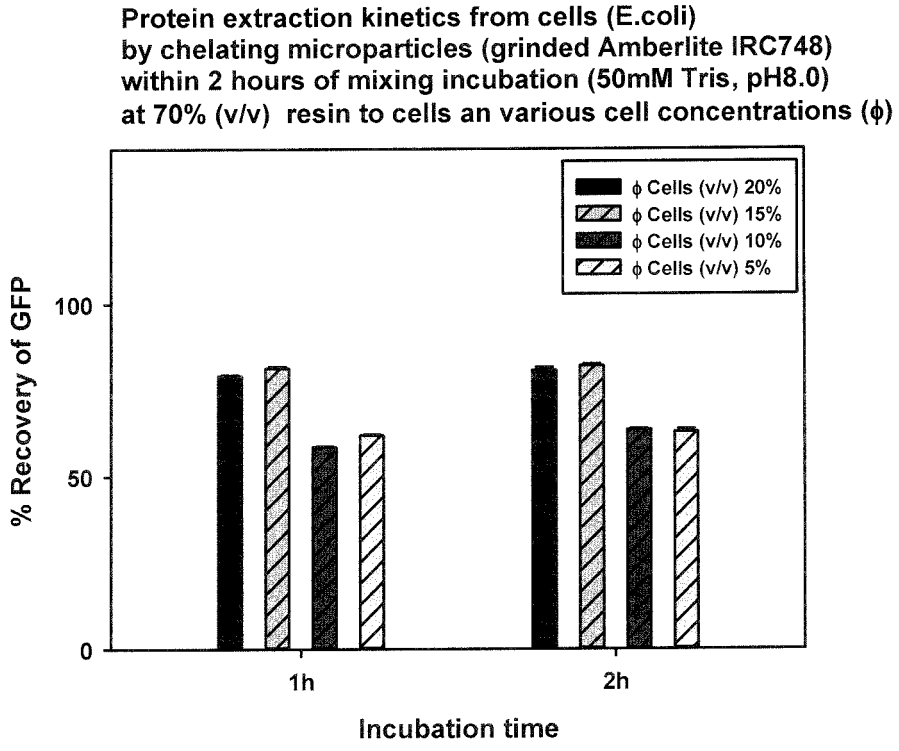

Protein extraction kinetics from cells (E.coli)
by chelating microparticles (grinded Amberlite IRC748)
within 2 hours of mixing incubation (50mM Tris, pH8.0)
at 70% (v/v) resin to cells an various cell concentrations (ϕ)
ϕ Cells (v/v) 20%
ϕ Cells (v/v) 15%
ϕ Cells (v/v) 10%
ϕ Cells (v/v) 5%
% Recovery of GFP
100
50
0
1h
2h
Incubation time

Fig. 9

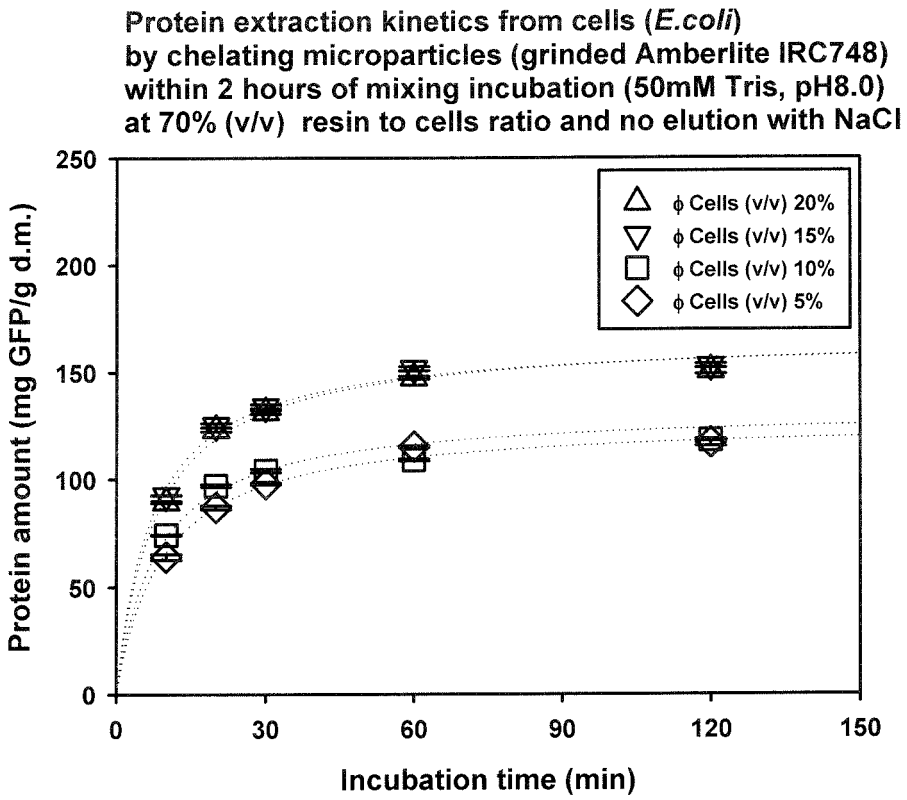

Protein extraction kinetics from cells (E.coli)
by chelating microparticles (grinded Amberlite IRC748)
within 2 hours of mixing incubation (50mM Tris, pH8.0)
at 70% (v/v) resin to cells ratio and no elution with NaCl
Protein amount (mg GFP/g d.m.)
Incubation time (min)
ϕ Cells (v/v) 20%
ϕ Cells (v/v) 15%
ϕ Cells (v/v) 10%
ϕ Cells (v/v) 5%
0
50
100
150
200
250
0
30
60
90
120
150

Fig. 10

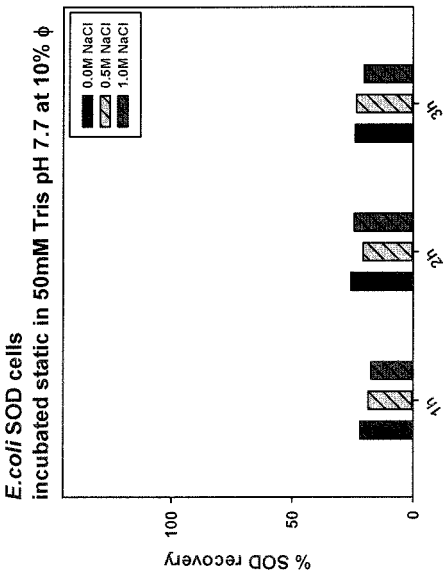


Fig. 11a

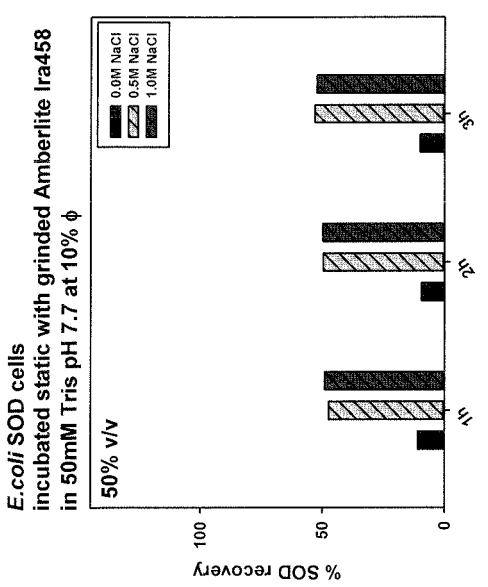


Fig. 11b

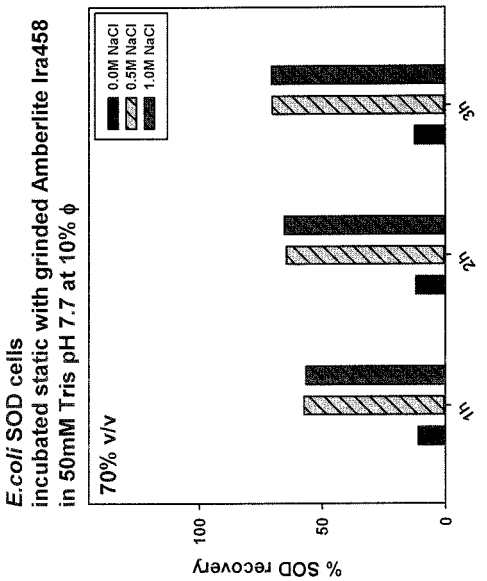


Fig. 11c

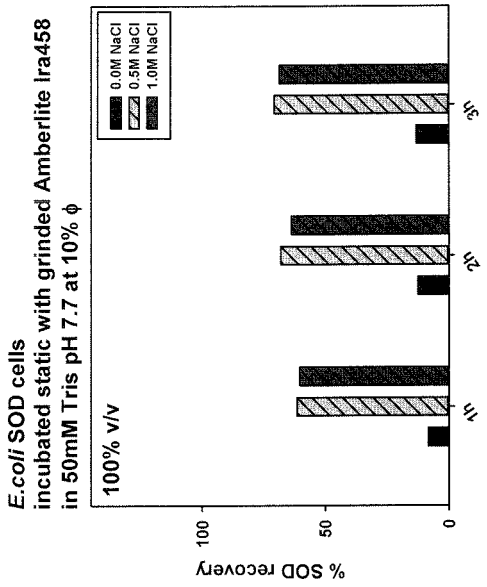


Fig. 11d

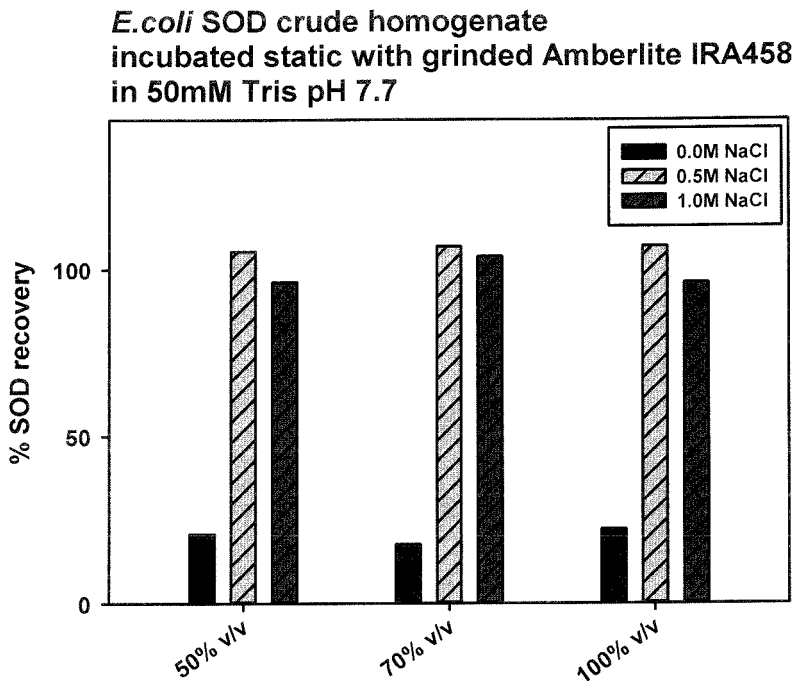


Fig. 12

GFPmut3.1 extraction from E.coli HMS174 by grinded Marathon A2 adsorption at pH8.0 in 50mM Tris (3h) and elution with NaCl

		Dilution	Reduced	Vol. (µl)
1	MW Standard SeeBLU2plus			3
2	Cell debris fraction		X	20
3	Homogenate	1:4	X	20
4	Homogenate (supernatant)	1:4	X	20
5	Eluate 200mM NaCl (supernatat)	1:4	X	20
6	Eluate 250mM NaCl (supernatat)	1:4	X	20
7	Eluate 300mM NaCl (supernatat)	1:4	X	20
8	Eluate 350mM NaCl (supernatat)	1:4	X	20
9	Eluate 400mM NaCl (supernatat)	1:4	X	20
10	Eluate 450mM NaCl (supernatat)	1:4	X	20
11	Eluate 500mM NaCl (supernatat)	1:4	X	20
12	Eluate 1000mM NaCl(supernatat)	1:4	X	20

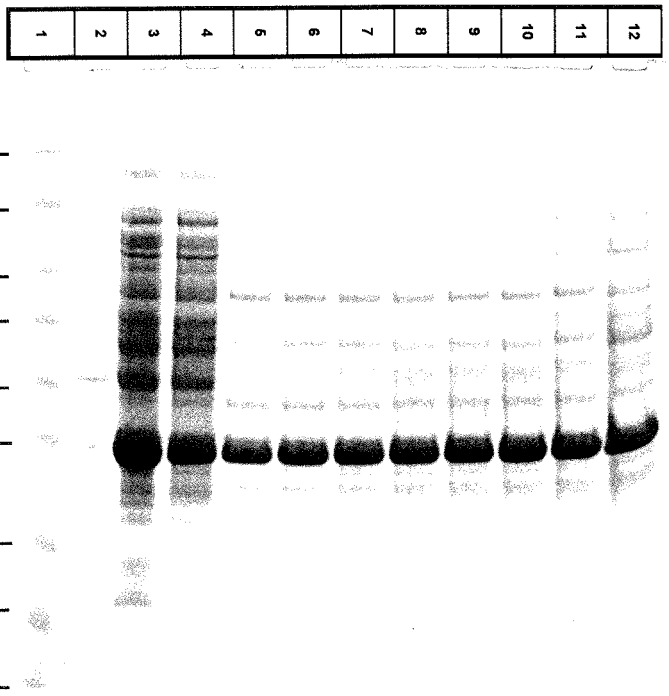


Fig. 13

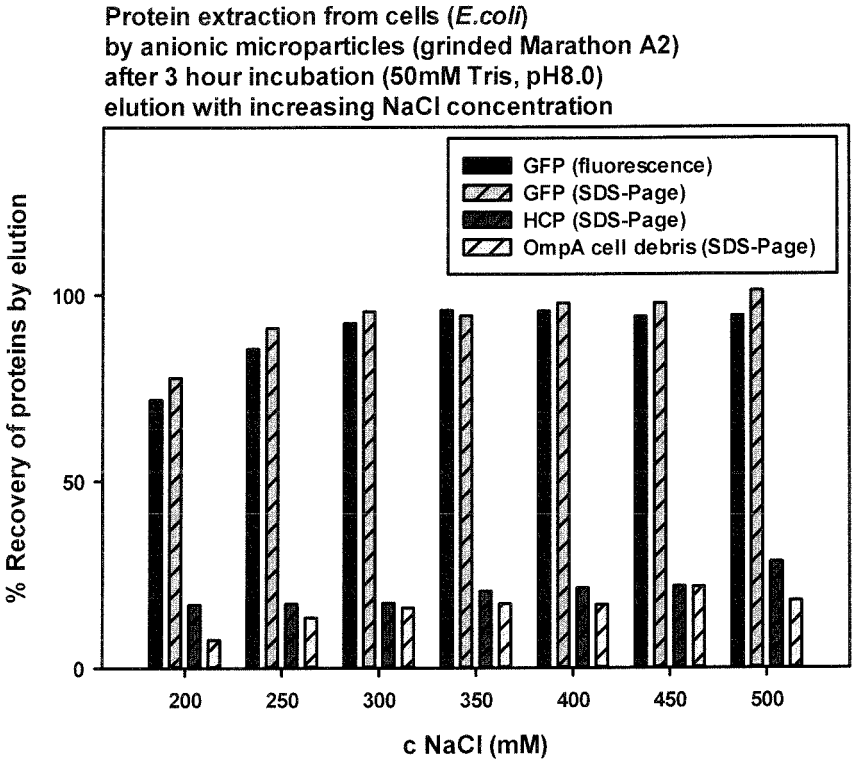

Protein extraction from cells (E.coli)
by anionic microparticles (grinded Marathon A2)
after 3 hour incubation (50mM Tris, pH8.0)
elution with increasing NaCl concentration
GFP (fluorescence)
GFP (SDS-Page)
HCP (SDS-Page)
OmpA cell debris (SDS-Page)
% Recovery of proteins by elution
0
50
100
200
250
300
350
400
450
500
c NaCl (mM)

Fig. 14

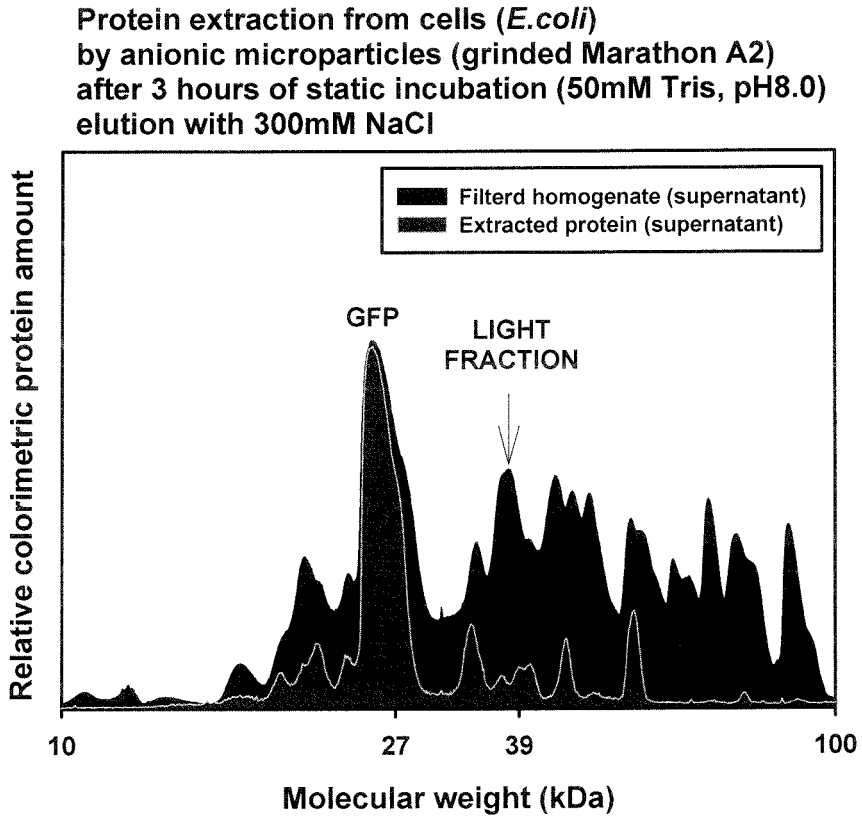

Protein extraction from cells (E.coli)
by anionic microparticles (grinded Marathon A2)
after 3 hours of static incubation (50mM Tris, pH8.0)
elution with 300mM NaCl
Filterd homogenate (supernatant)
Extracted protein (supernatant)
GFP
LIGHT
FRACTION
Relative colorimetric protein amount
10
27
39
100
Molecular weight (kDa)

Fig. 15

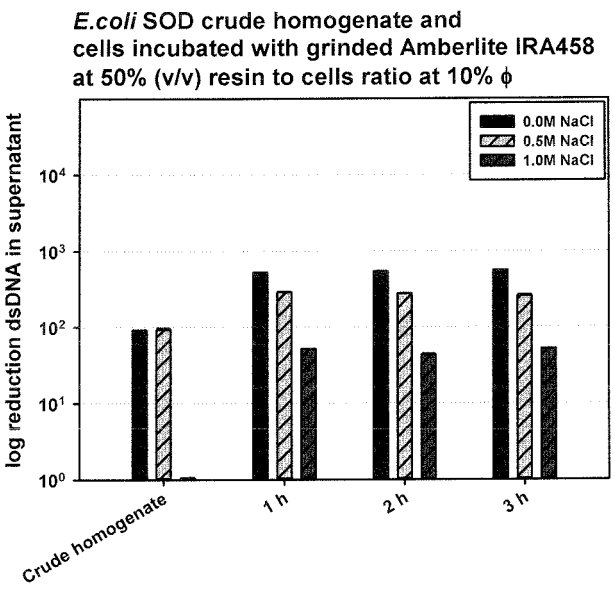


Fig. 16a

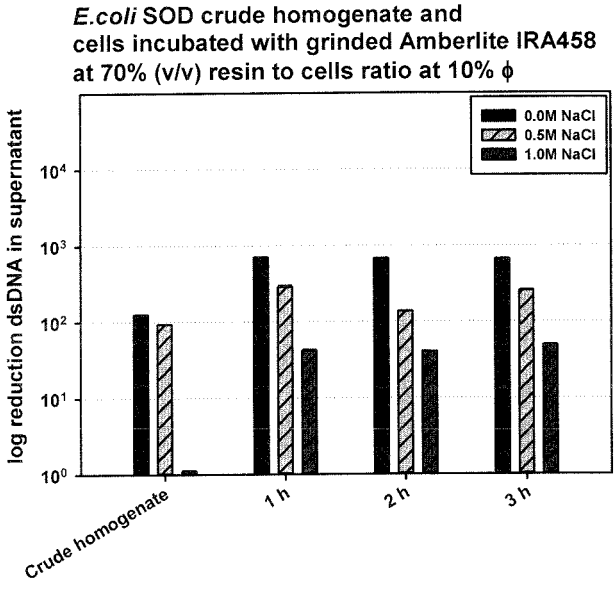


Fig. 16b

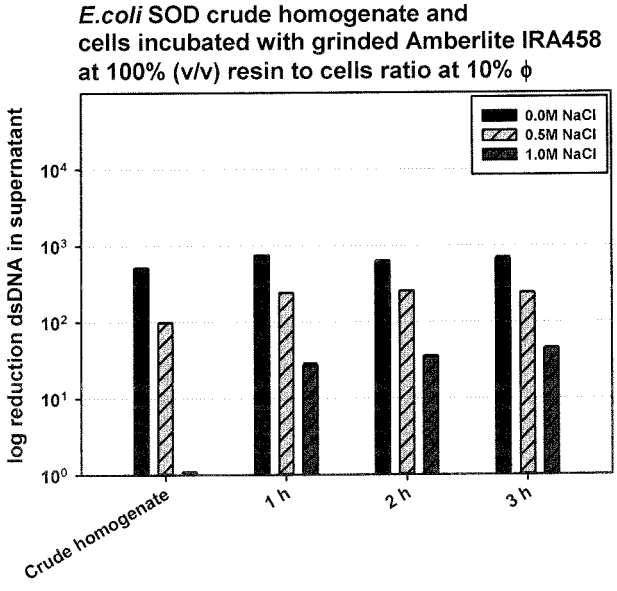


Fig. 16c

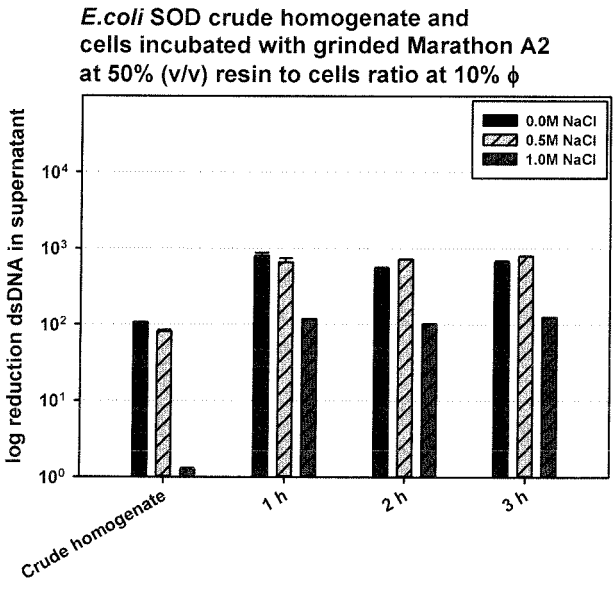


Fig. 16d

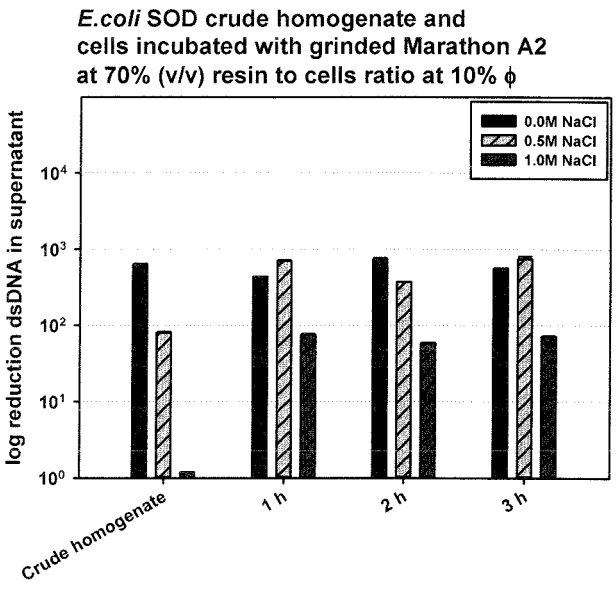


Fig. 16e

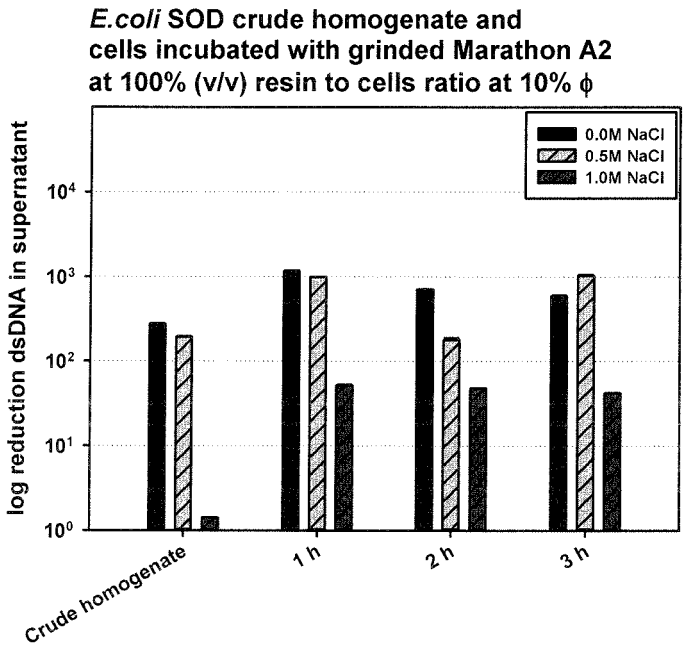


Fig. 16f

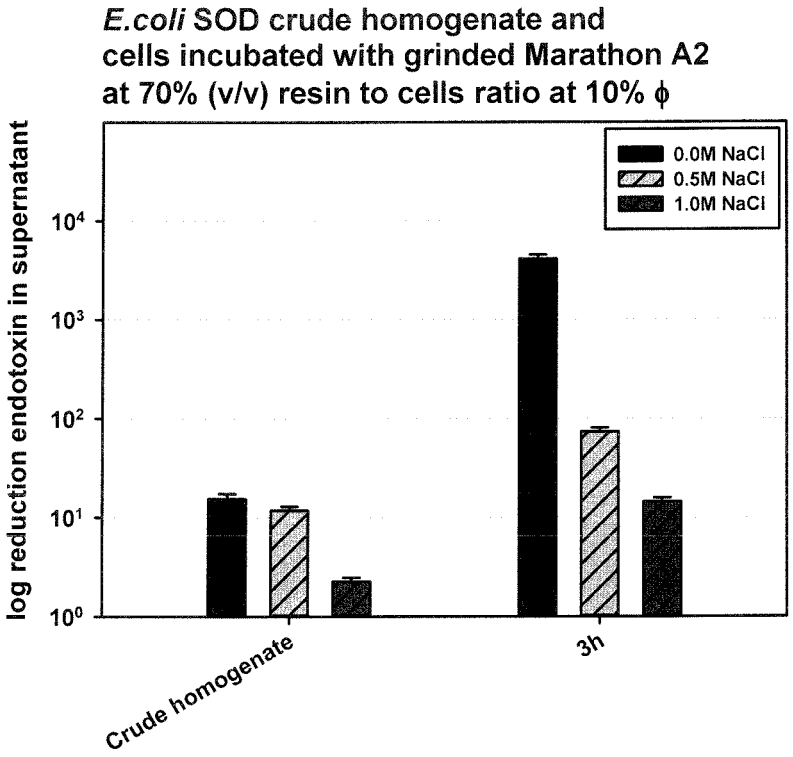

E.coli SOD crude homogenate and
cells incubated with grinded Marathon A2
at 70% (v/v) resin to cells ratio at 10% ϕ
log reduction endotoxin in supernatant
10⁴
10³
10²
10¹
10⁰
0.0M NaCl
0.5M NaCl
1.0M NaCl
Crude homogenate
3h

Fig. 17

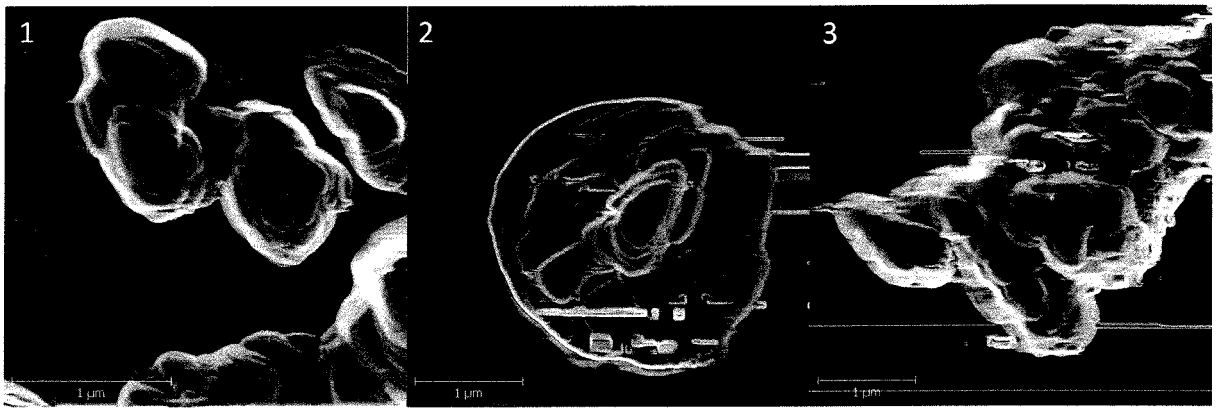

1
2
3
1 µm
1 µm
1 µm

Fig. 18

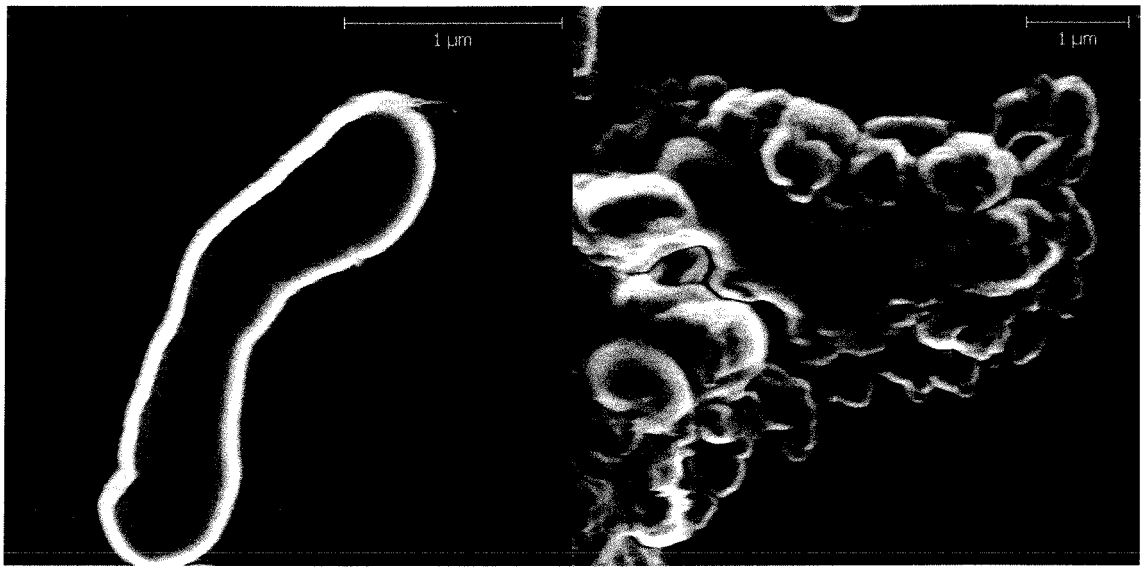


Fig. 19

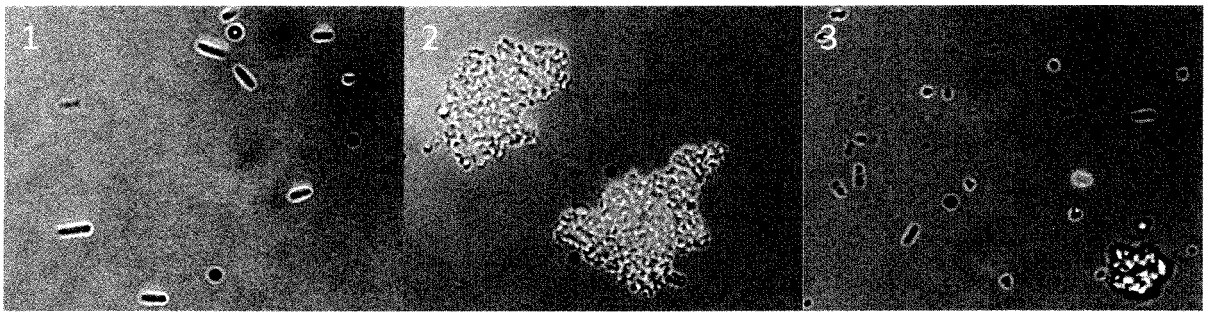


Fig. 20

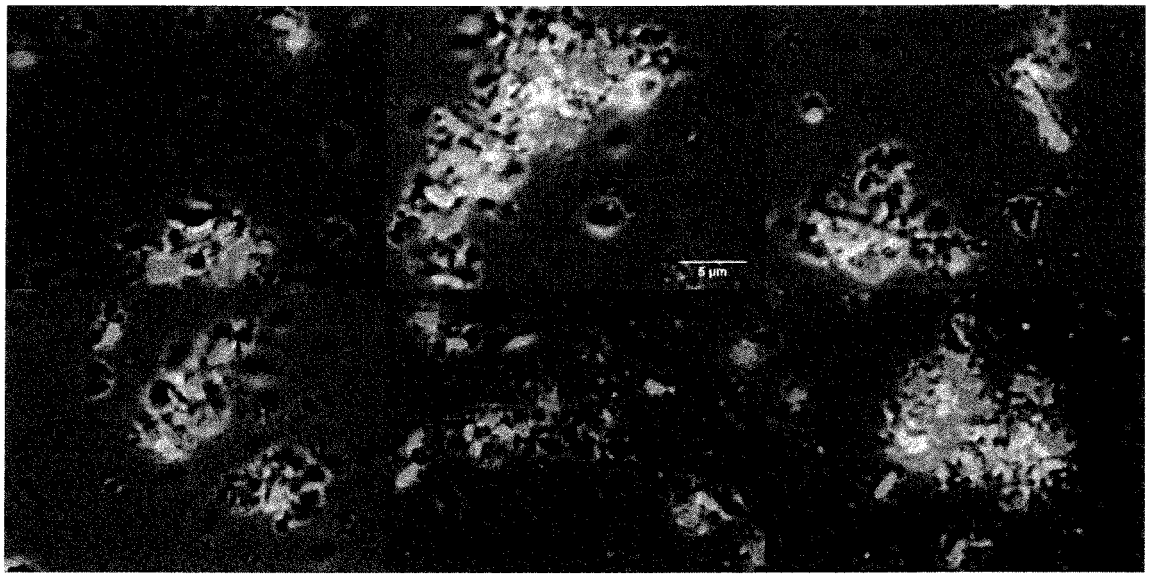


Fig. 21

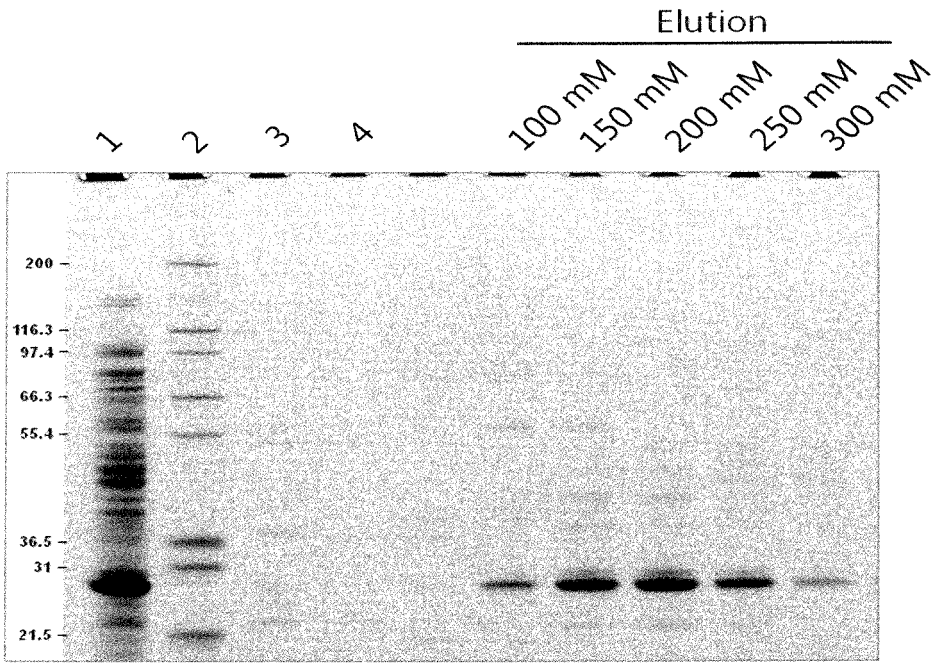

Elution
100 mM
150 mM
200 mM
250 mM
300 mM
1
2
3
4
200
116.3
97.4
66.3
55.4
36.5
31
21.5

Fig. 22

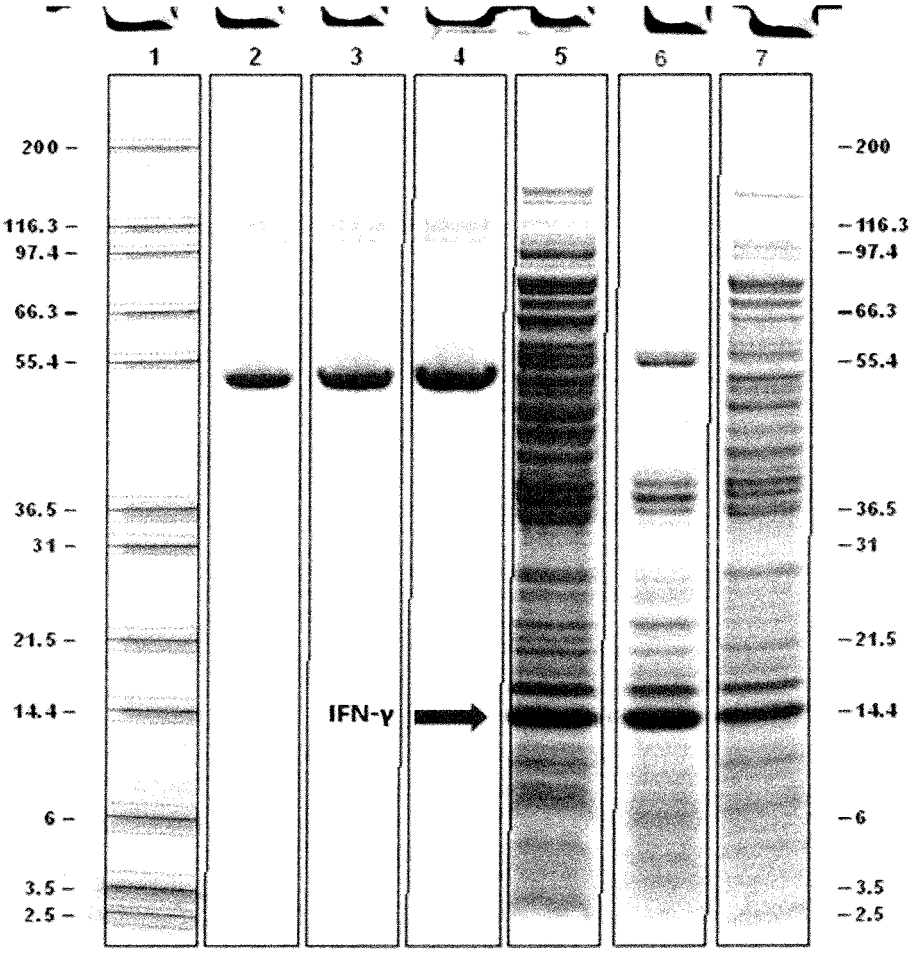

1
2
3
4
5
6
7
200
116.3
97.4
66.3
55.4
36.5
31
21.5
14.4
6
3.5
2.5
IFN-γ

Fig. 23

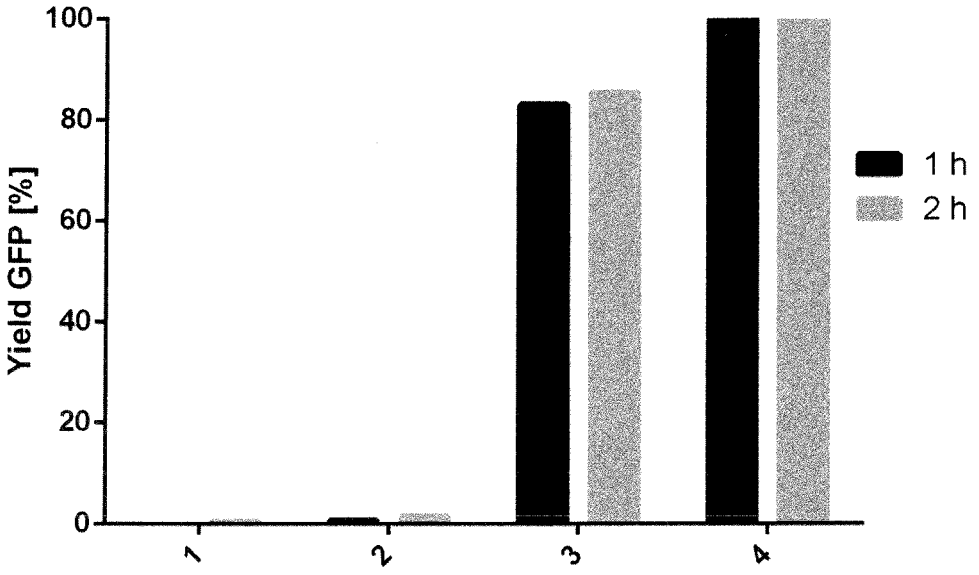


Fig. 24