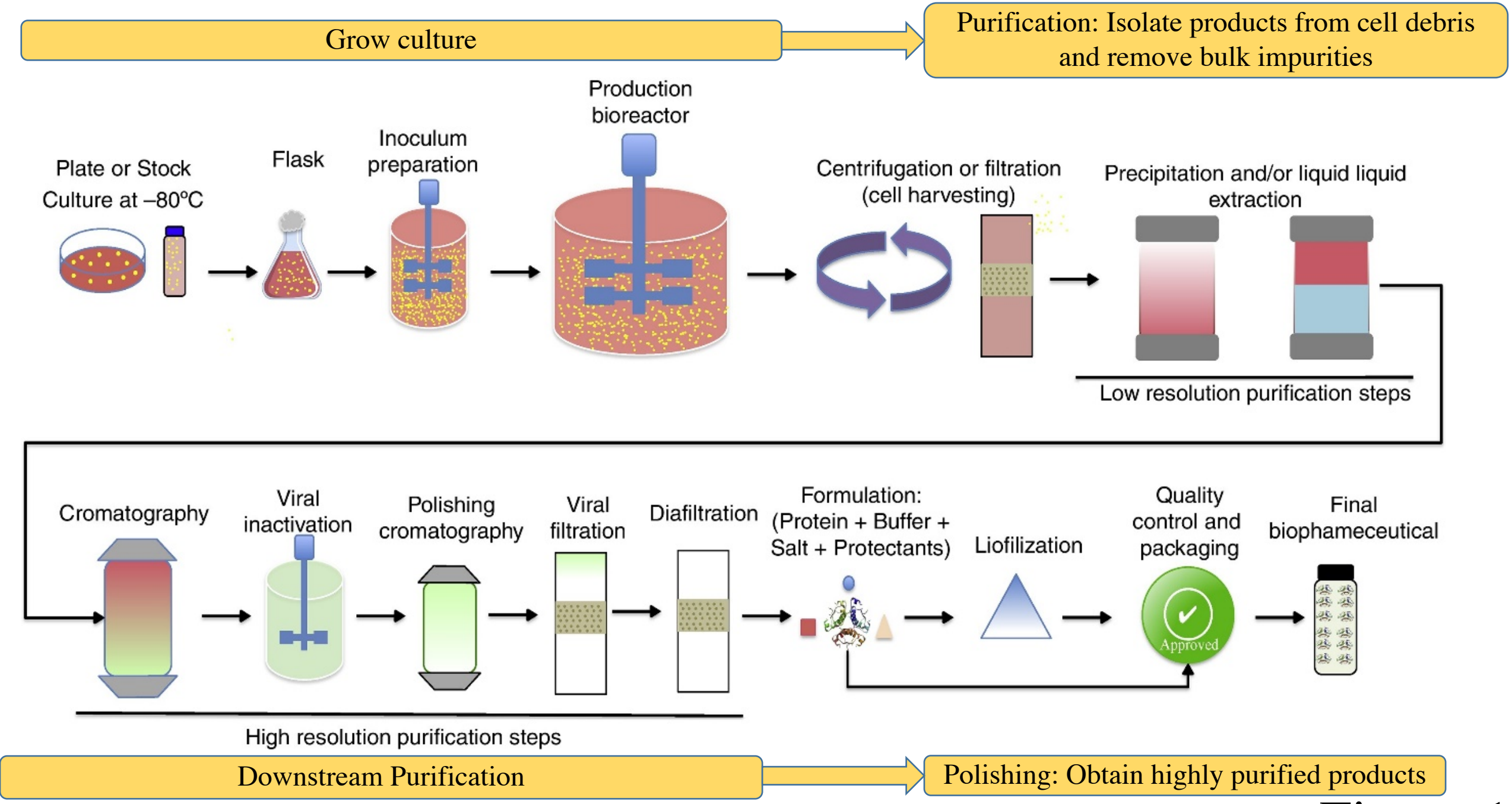
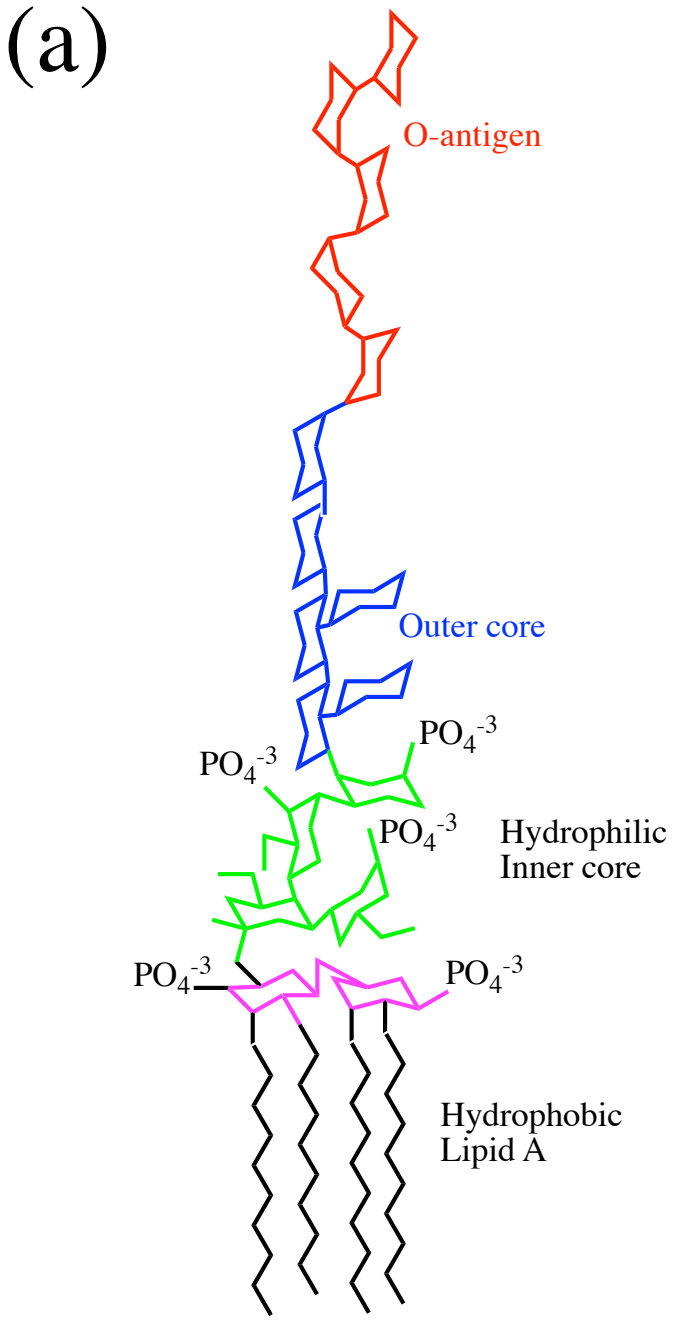
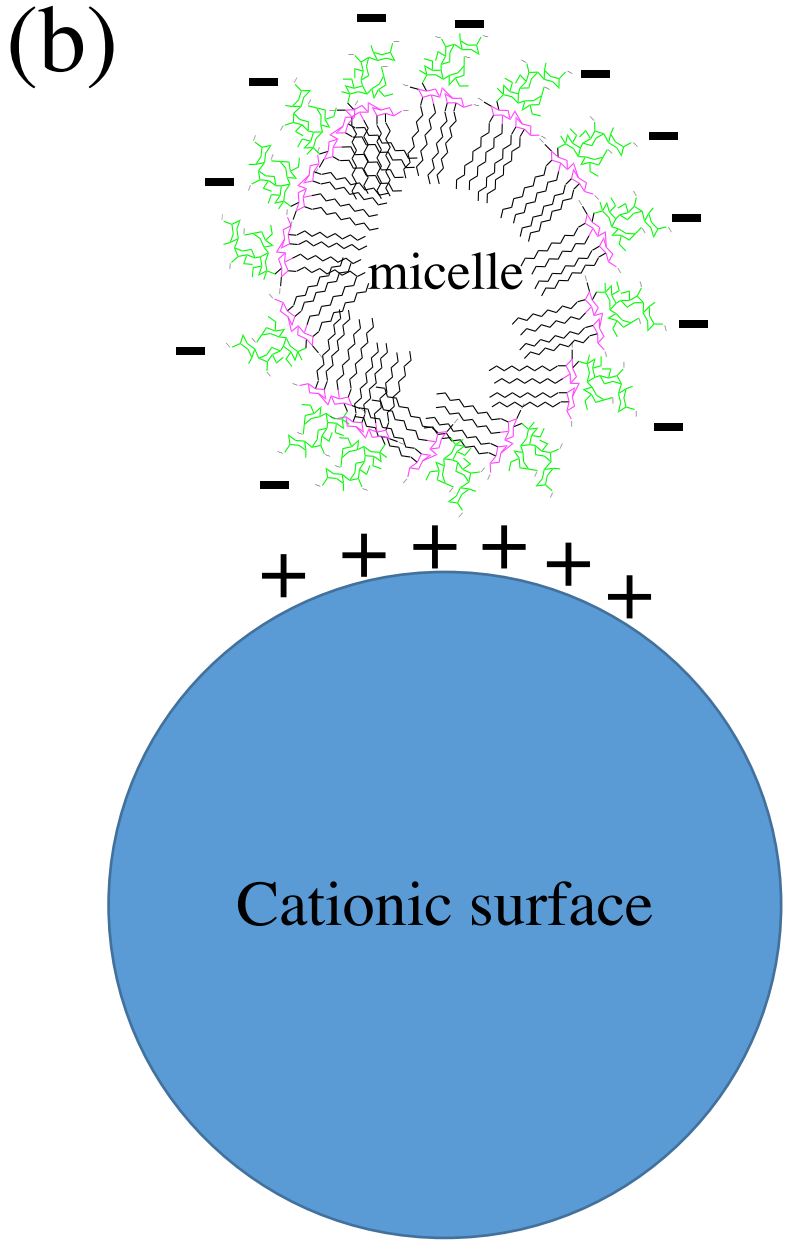

Grow culture
Purification: Isolate products from cell debris and remove bulk impurities
Plate or Stock Culture at –80°C
Flask
Inoculum preparation
Production bioreactor
Centrifugation or filtration (cell harvesting)
Precipitation and/or liquid liquid extraction
Low resolution purification steps
Cromatography
Viral inactivation
Polishing cromatography
Viral filtration
Diafiltration
Formulation: (Protein + Buffer + Salt + Protectants)
Liofilization
Quality control and packaging
Approved
Final biophameceutical
High resolution purification steps
Downstream Purification
Polishing: Obtain highly purified products

Figure 1


(a)
O-antigen
Outer core
$PO_4^{-3}$
$PO_4^{-3}$
$PO_4^{-3}$
Hydrophilic
Inner core
$PO_4^{-3}$
$PO_4^{-3}$
Hydrophobic
Lipid A


(b)
micelle
Cationic surface

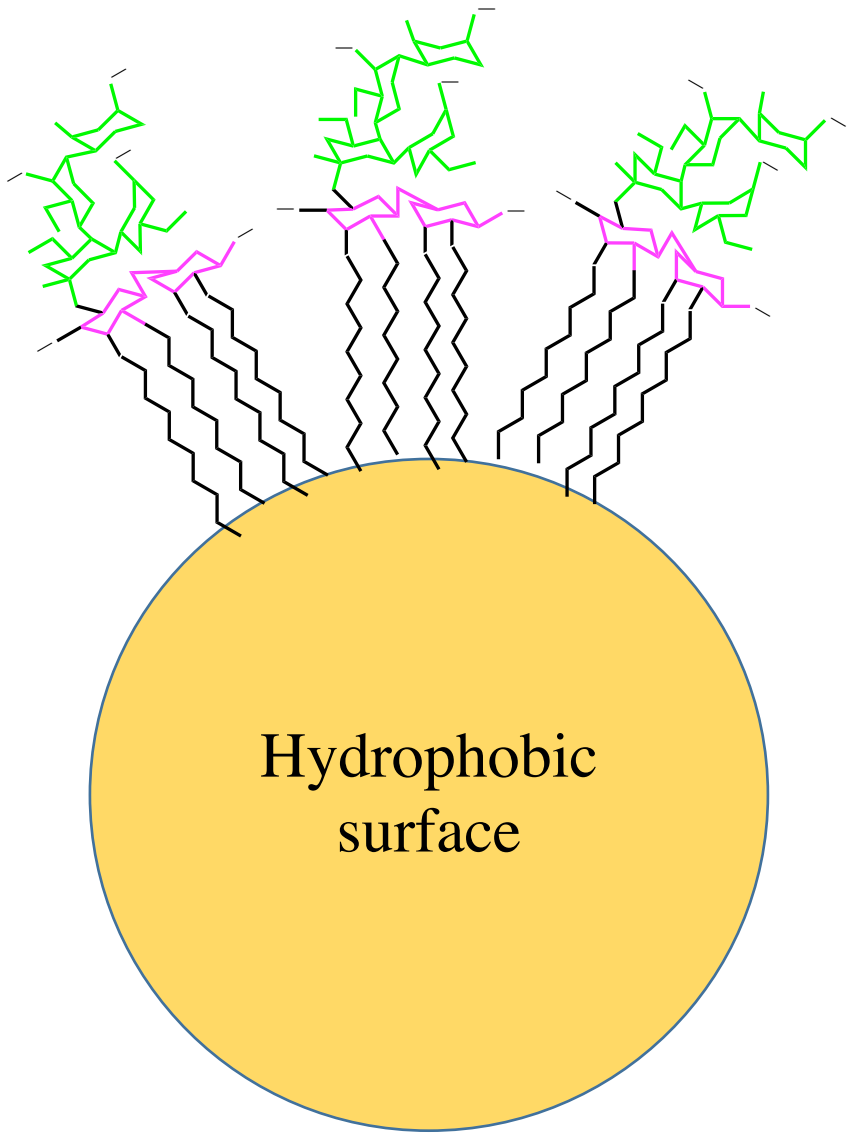

Hydrophobic
surface

Figure 2

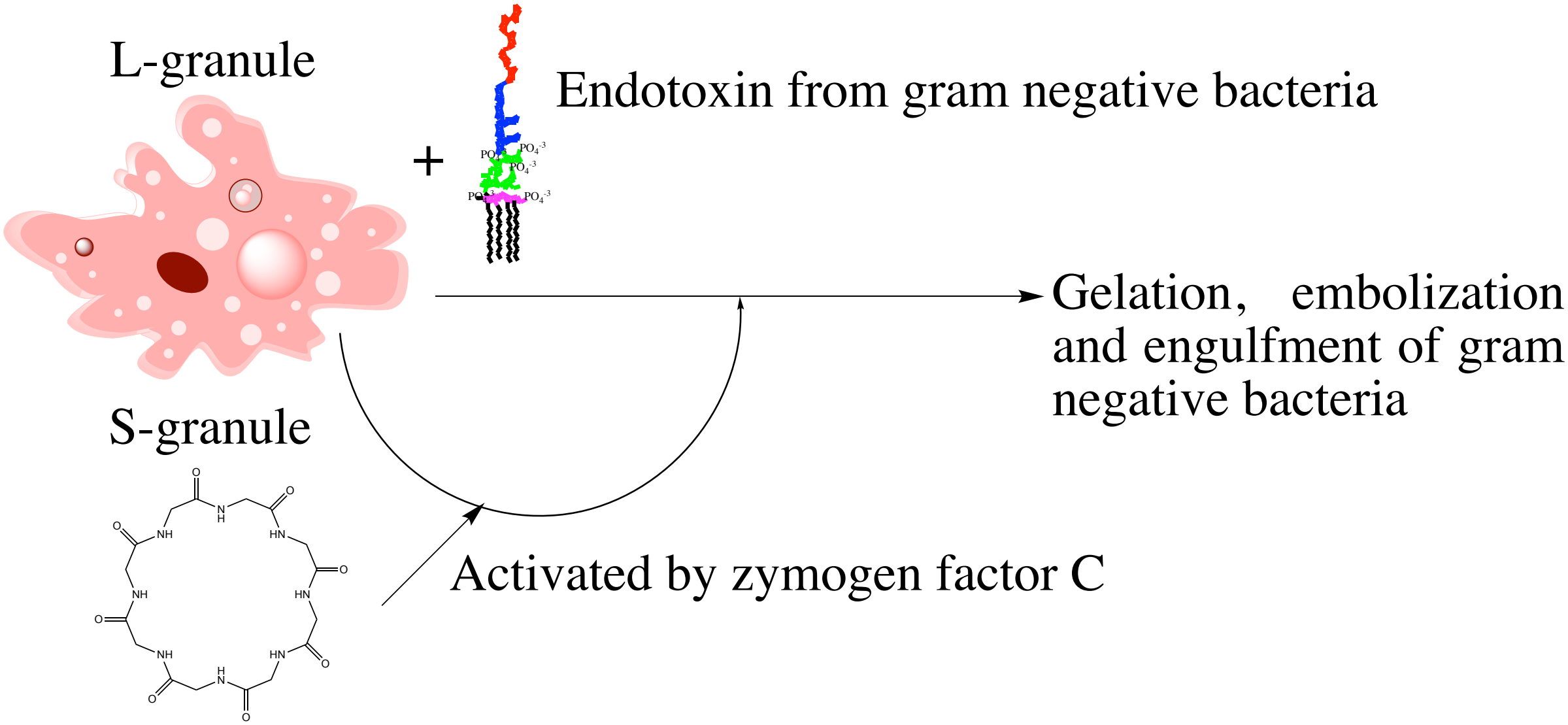

L-granule
+
Endotoxin from gram negative bacteria
Gelation, embolization and engulfment of gram negative bacteria
S-granule
Activated by zymogen factor C

Figure 3 (a)

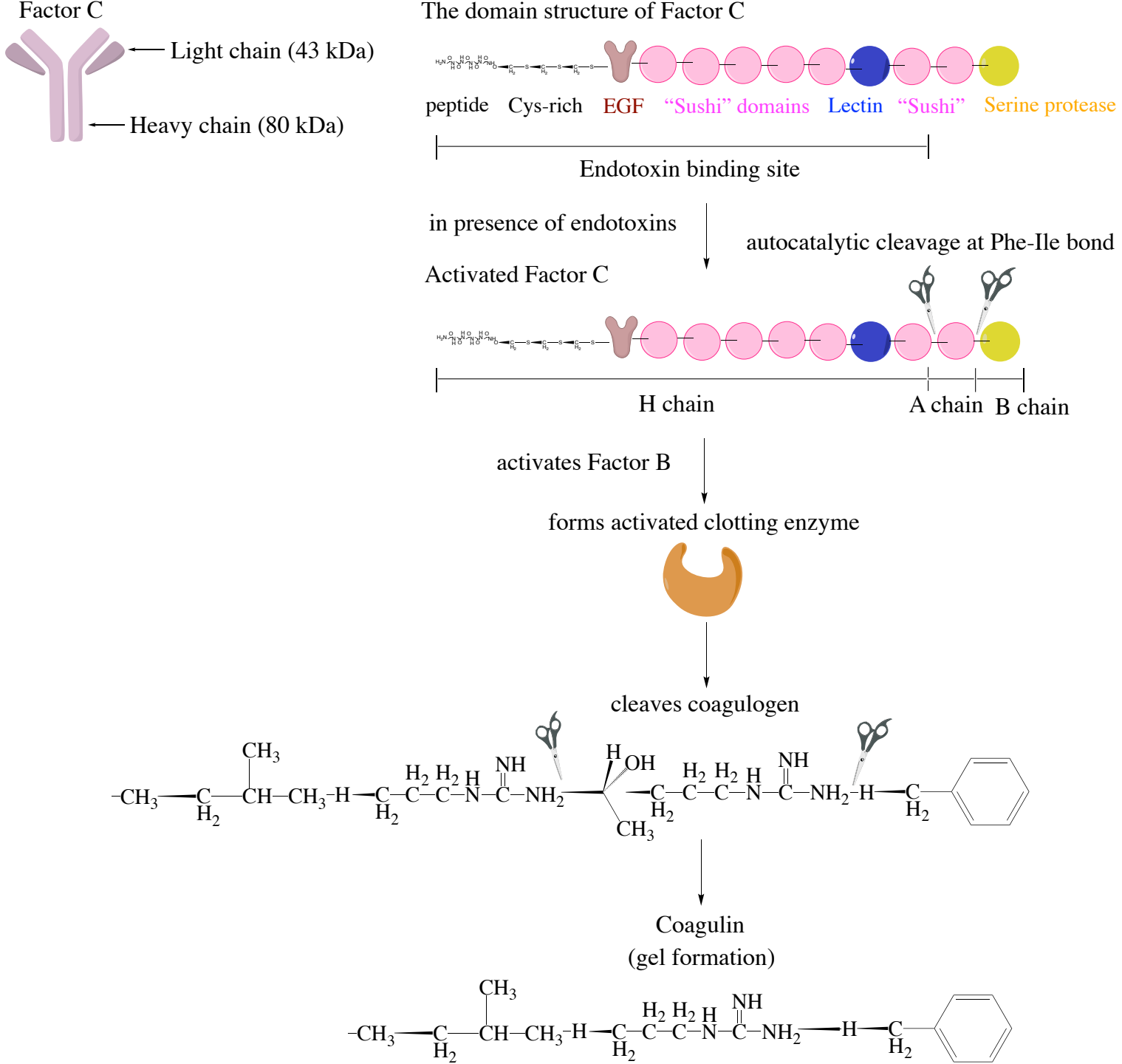

Factor C
Light chain (43 kDa)
Heavy chain (80 kDa)
The domain structure of Factor C
peptide
Cys-rich
EGF
"Sushi" domains
Lectin
"Sushi"
Serine protease
Endotoxin binding site
in presence of endotoxins
autocatalytic cleavage at Phe-Ile bond
Activated Factor C
H chain
A chain
B chain
activates Factor B
forms activated clotting enzyme
cleaves coagulogen
Coagulin
(gel formation)

Figure 3 (b)

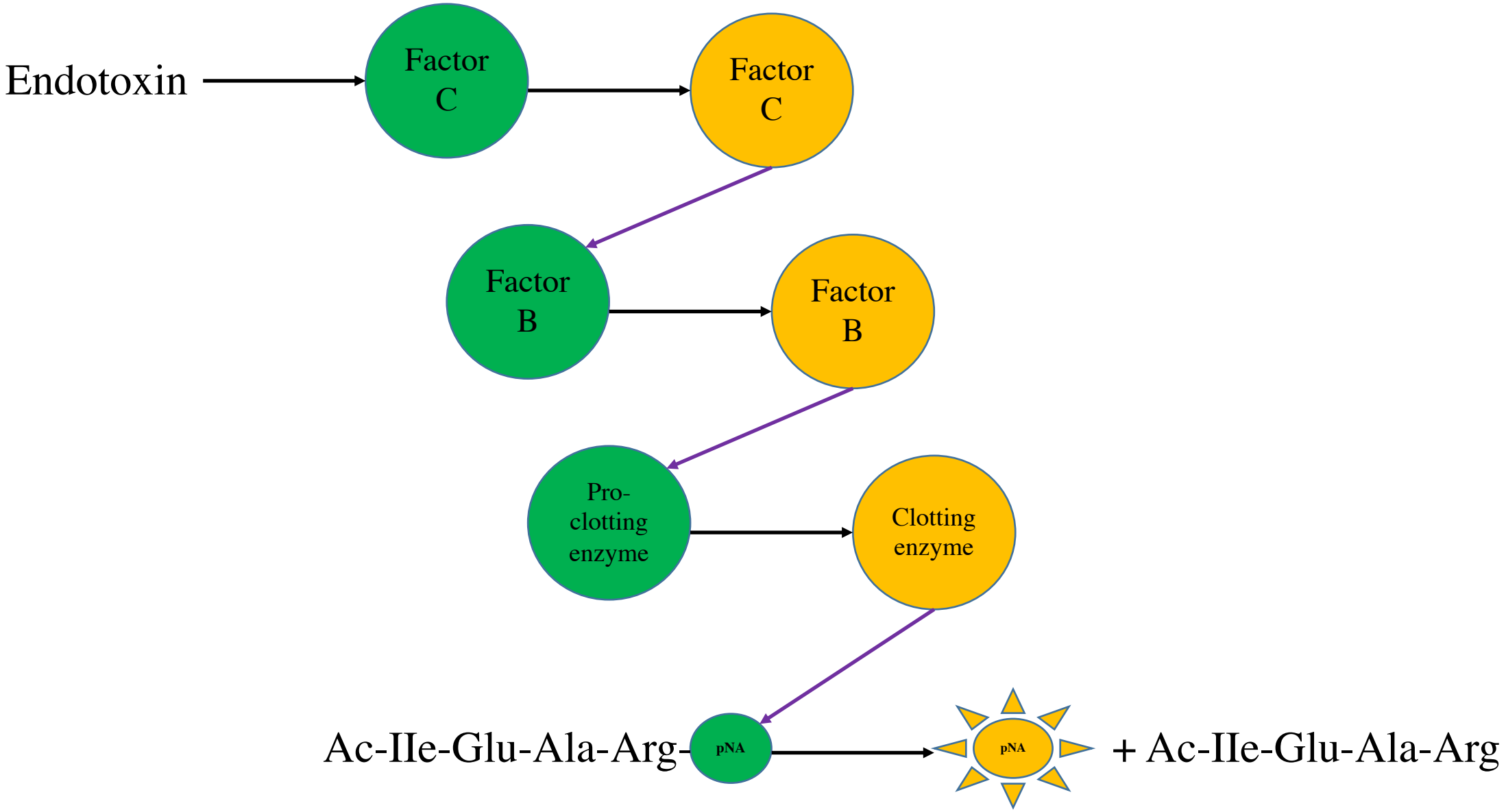

Endotoxin
Factor C
Factor C
Factor B
Factor B
Pro-clotting enzyme
Clotting enzyme
Ac-IIe-Glu-Ala-Arg-
pNA
pNA
+ Ac-IIe-Glu-Ala-Arg

Figure 3 (c)

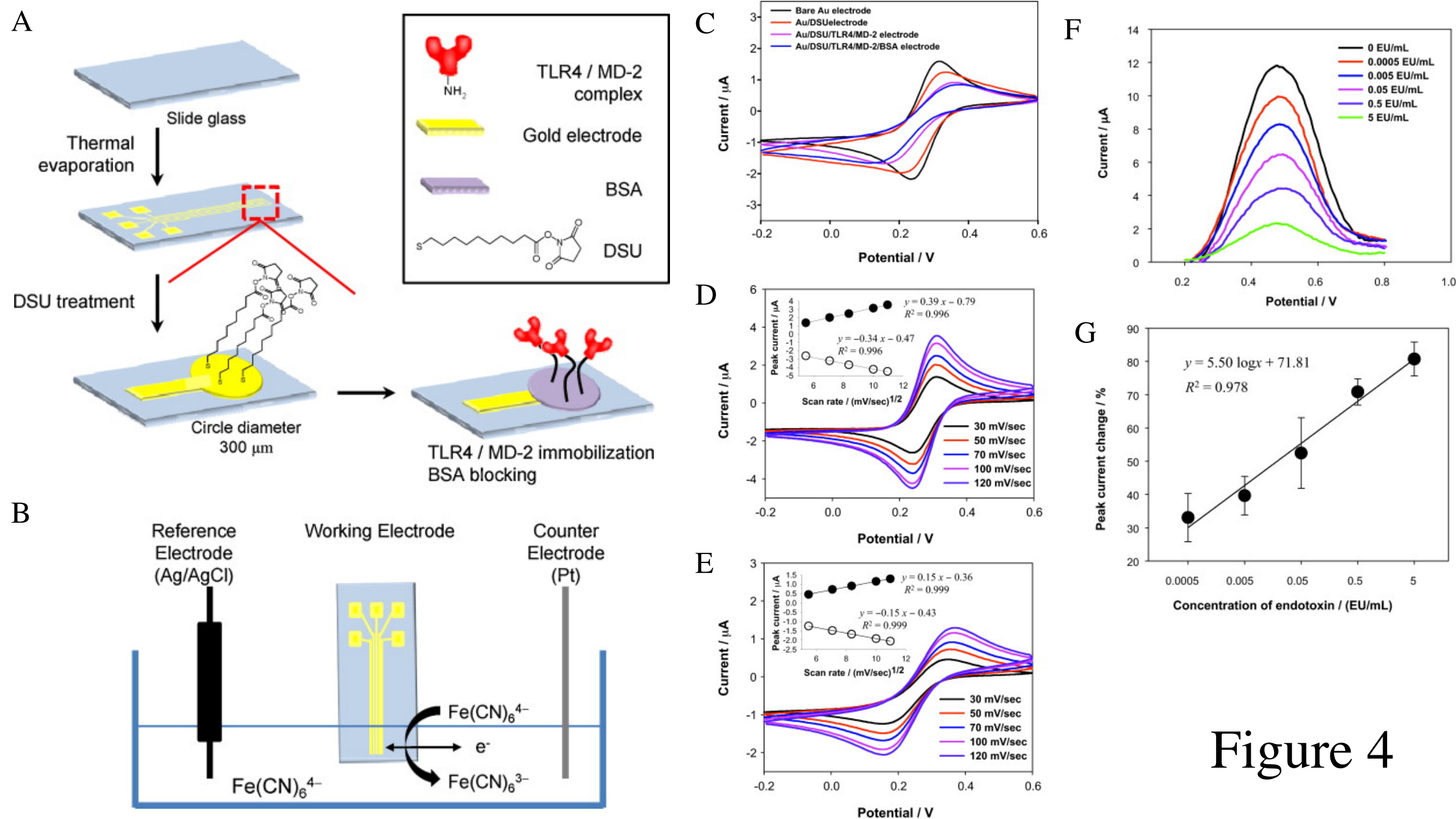

A
Slide glass
Thermal evaporation
DSU treatment
Circle diameter 300 μm
TLR4 / MD-2 immobilization
BSA blocking
TLR4 / MD-2 complex
Gold electrode
BSA
DSU
B
Reference Electrode (Ag/AgCl)
Working Electrode
Counter Electrode (Pt)
Fe(CN)6 4−
e-
Fe(CN)6 3−
Fe(CN)6 4−
C
Bare Au electrode
Au/DSUelectrode
Au/DSU/TLR4/MD-2 electrode
Au/DSU/TLR4/MD-2/BSA electrode
Current / μA
Potential / V
D
y = 0.39 x − 0.79
R² = 0.996
y = −0.34 x − 0.47
R² = 0.996
Peak current / μA
Scan rate / (mV/sec)1/2
30 mV/sec
50 mV/sec
70 mV/sec
100 mV/sec
120 mV/sec
Current / μA
Potential / V
E
y = 0.15 x − 0.36
R² = 0.999
y = −0.15 x − 0.43
R² = 0.999
Peak current / μA
Scan rate / (mV/sec)1/2
30 mV/sec
50 mV/sec
70 mV/sec
100 mV/sec
120 mV/sec
Current / μA
Potential / V
F
0 EU/mL
0.0005 EU/mL
0.005 EU/mL
0.05 EU/mL
0.5 EU/mL
5 EU/mL
Current / μA
Potential / V
G
y = 5.50 logx + 71.81
R² = 0.978
Peak current change / %
Concentration of endotoxin / (EU/mL)

Figure 4

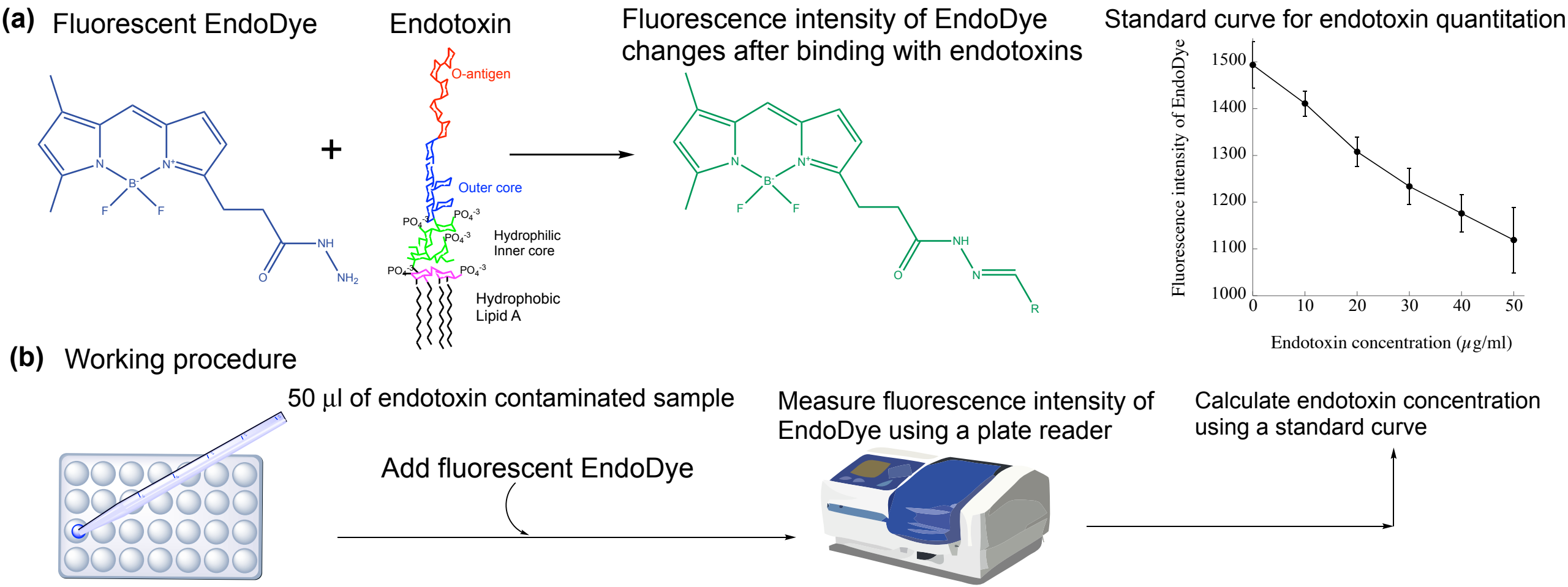
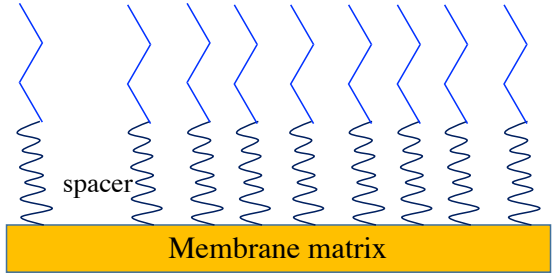

(a)
Fluorescent EndoDye
Endotoxin
O-antigen
Outer core
Hydrophilic
Inner core
Hydrophobic
Lipid A
Fluorescence intensity of EndoDye
changes after binding with endotoxins
Standard curve for endotoxin quantitation
Fluorescence intensity of EndoDye
Endotoxin concentration (μg/ml)
(b)
Working procedure
50 μl of endotoxin contaminated sample
Add fluorescent EndoDye
Measure fluorescence intensity of
EndoDye using a plate reader
Calculate endotoxin concentration
using a standard curve

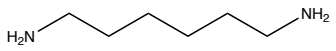
Figure 5

## Endotoxin binding ligands

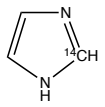



Spacer

Diaminohexane

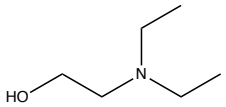



Imidazole

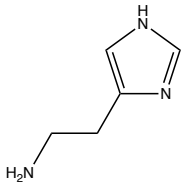



## Low molecular weight ligands

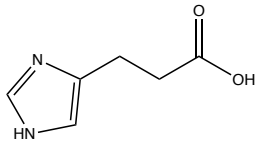
Diethylaminoethane (DEAE)



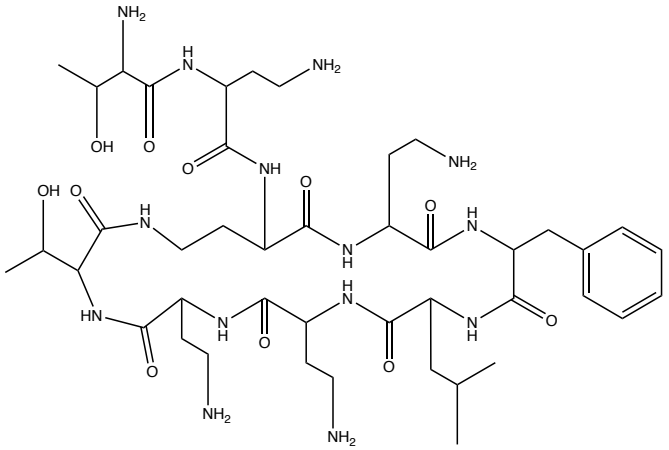

Histamine




Diaminohistidine

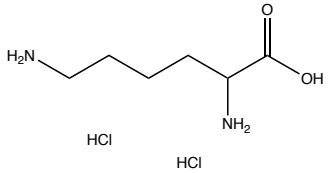



Polymyxin B



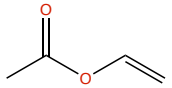

## High molecular weight ligands

Poly-l-lysine




Poly(ethyleneimine) (pEI)

Polyvinyl alcohol (PVA)




Chitosan

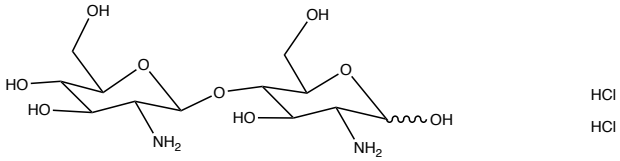



Figure 6

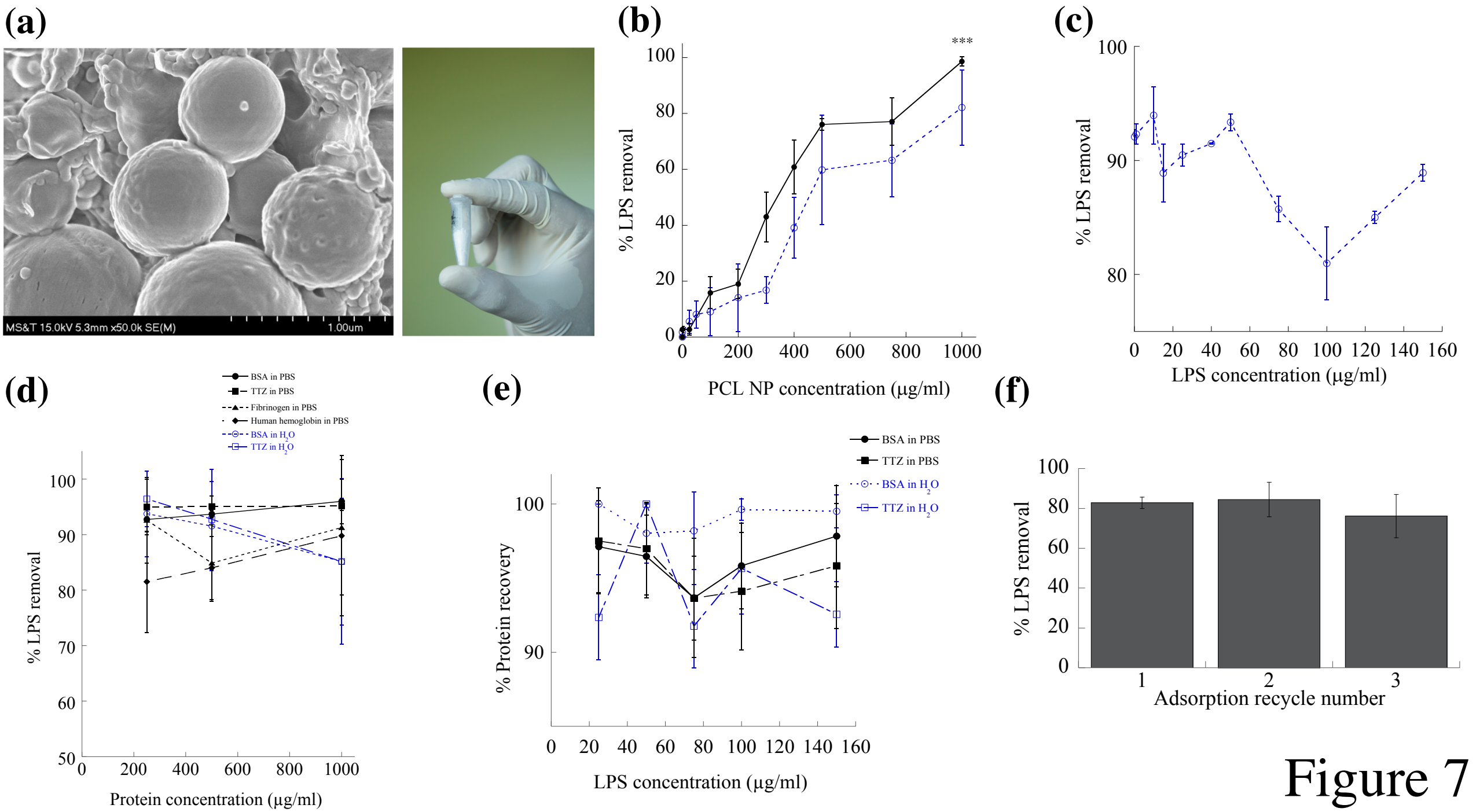

(a)
MS&T 15.0kV 5.3mm x50.0k SE(M)
1.00um
(b)
***
% LPS removal
PCL NP concentration (μg/ml)
(c)
% LPS removal
LPS concentration (μg/ml)
(d)
BSA in PBS
TTZ in PBS
Fibrinogen in PBS
Human hemoglobin in PBS
BSA in H2O
TTZ in H2O
% LPS removal
Protein concentration (μg/ml)
(e)
BSA in PBS
TTZ in PBS
BSA in H2O
TTZ in H2O
% Protein recovery
LPS concentration (μg/ml)
(f)
% LPS removal
Adsorption recycle number

Figure 7

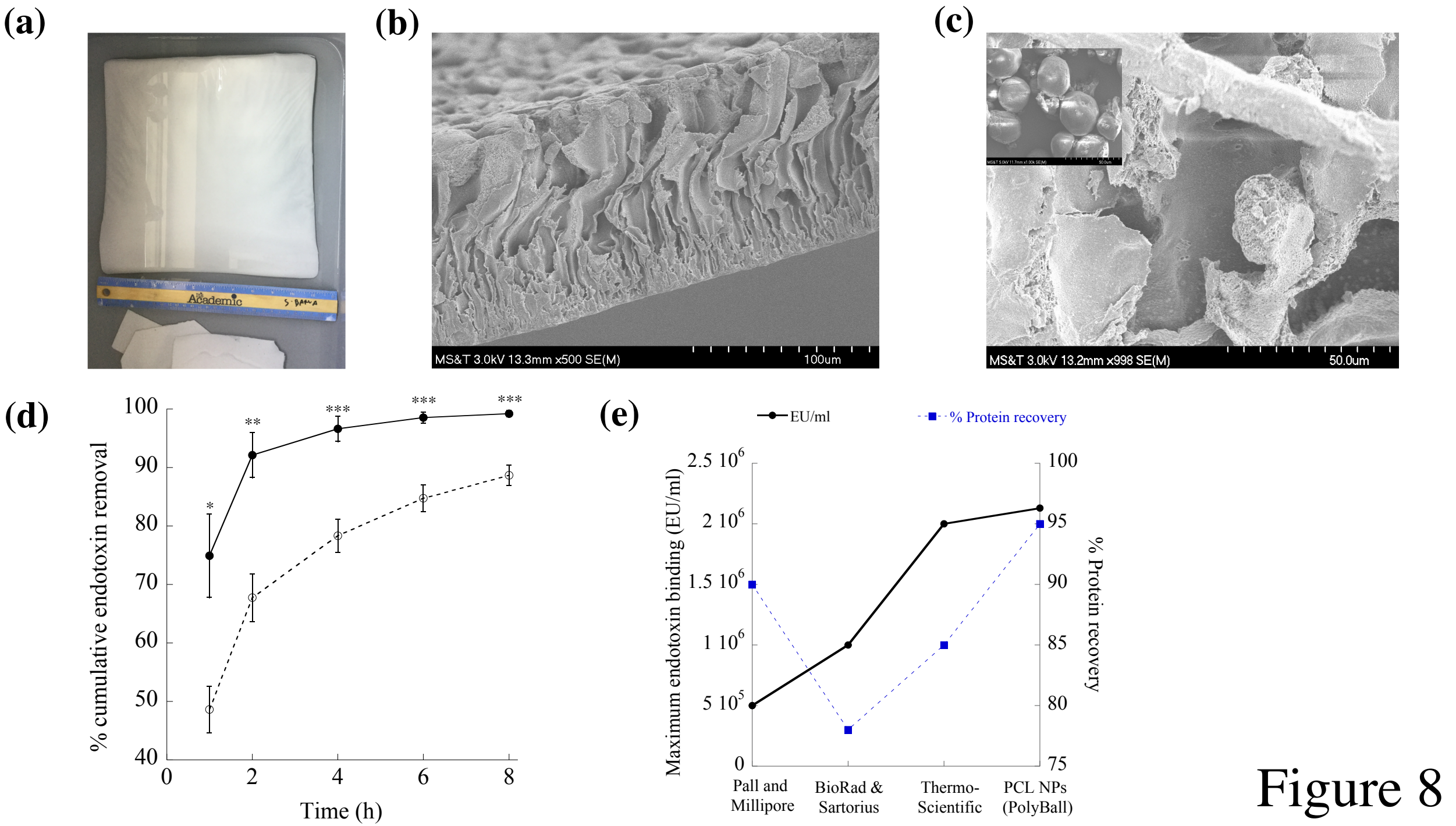

(a)
(b)
MS&T 3.0kV 13.3mm x500 SE(M)
100um
(c)
MS&T 3.0kV 13.2mm x998 SE(M)
50.0um
(d)
% cumulative endotoxin removal
Time (h)
(e)
EU/ml
% Protein recovery
Maximum endotoxin binding (EU/ml)
% Protein recovery
Pall and Millipore
BioRad & Sartorius
Thermo-Scientific
PCL NPs (PolyBall)

Figure 8

| Product Name | Maximum Endotoxin Binding capacity (EU/ml) | Cost ($) | Reusability |
|---|---|---|---|
| Pall Acrodisc Unit with Mustang E membrane | $5.0 \times 10^5$ | $ 9.2 per 1 $cm^2$ membrane area | Yes |
| Millipore charged Durapore cartridge membrane filters | $>5.0 \times 10^5$ | $ 2.7 per 1 $cm^2$ membrane area | Yes |
| BioRad Proteus Endotoxin Removal Kits (Membrane based) | $5.0 \times 10^5 – 10^6$ | $ 12.4 per 1 $cm^2$ membrane area | Yes |
| Sartobind Q100 membrane adsorbers (Sartorius) | $1.0 \times 10^6$ | NA | Yes |
| Thermo scientific Pierce High capacity endotoxin removal resins | $2 \times 10^6$ | $ 20.2 per ml of resin slurry | Yes |
| PCL nanoparticles | $1.45 \times 10^6$ | $2.4 per 1 g | Yes |
| PCL nanoparticles incorporated membrane | $2.8 \times 10^6$ | $ 0.05 per 1 $cm^2$ membrane area | Not tested yet |

Table 1