Problem Session

Mar 10, 2018

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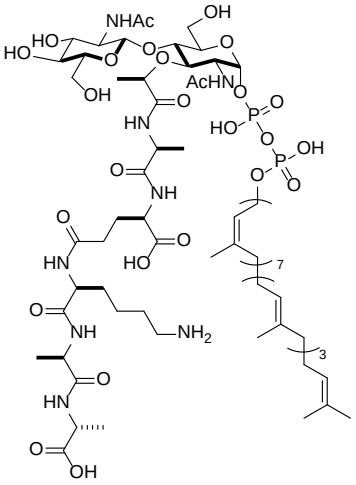
Please read the description below and answer the questions that follow.



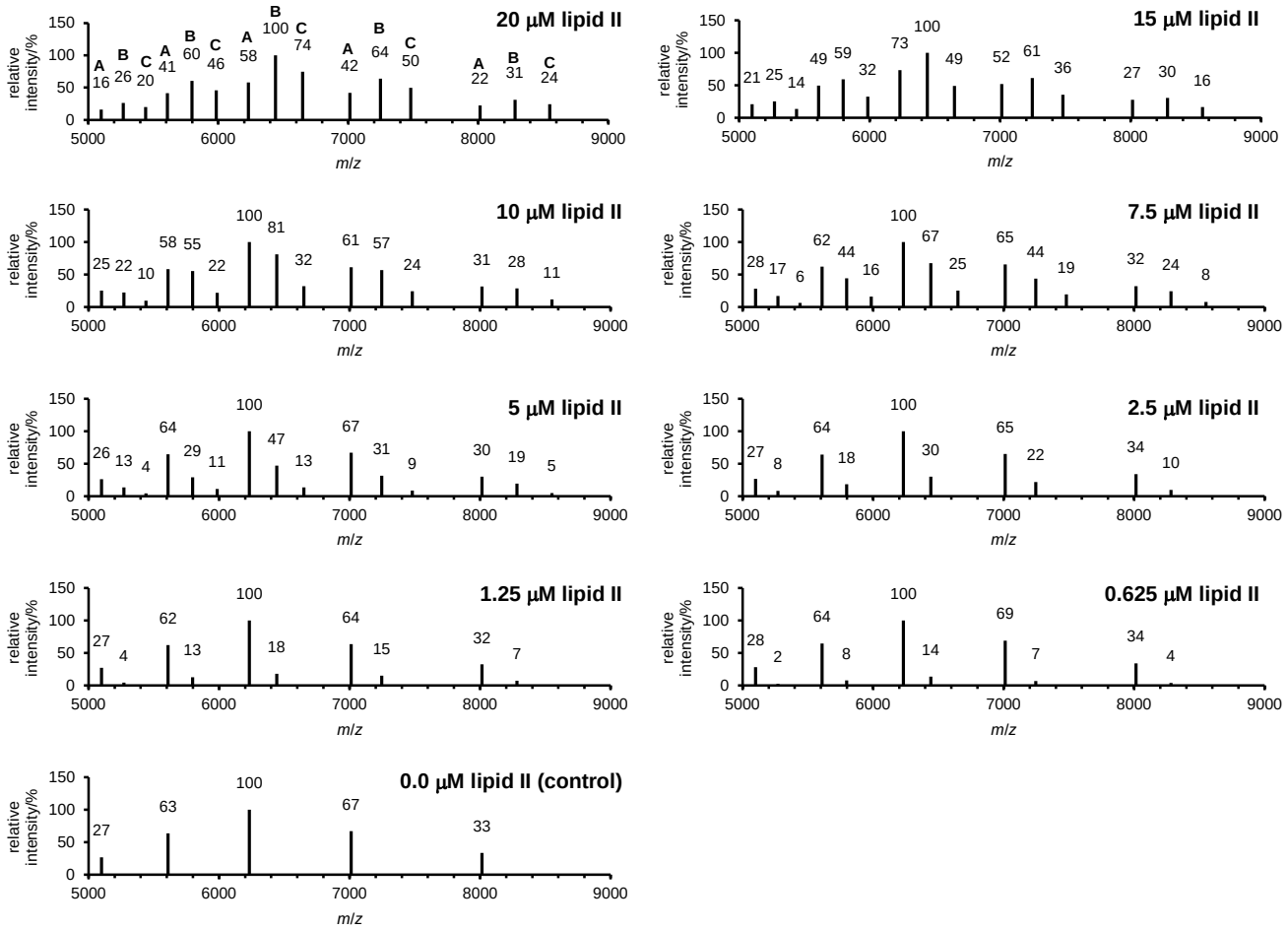
lipid II

Lipid II is a biosynthetic intermediate of peptidoglycan (Mw: 1,876 Da).

MurJ, which is a bacterial membrane-associated lipid II flippase, is involved with translocation of lipid II.

To investigate the interaction between MurJ and lipid II, ESI-MS analysis was carried out. A solution of MurJ with 6xHis tag (Mw: 56,090 Da) and lipid II (variable concentration: 0–20 μM) in buffer (200 mM ammonium acetate, 0.05% *n*-dodecylamine-*N*-oxide) was analyzed by ESI-MS.

The analysis provided MS spectra as follows:[a]



[a]Average mass signals are displayed. The number at the top of each signal represents the relative intensity of the peak (normalized against the maximum peak intensity of each spectrum).

Q1. Please calculate the dissociation constant (K_d) for the binding of lipid II to MurJ based on the two assumptions as follows:

(a) Signals of series B were derived from the 1:1 specific binding of lipid II to MurJ.

(b) The binding of lipid II to MurJ had no effect on the ionization and signal intensity of MurJ.

Q2. What kind of interaction is involved in generating signals of series C?

Topic: Native mass spectrometry and application for analysis of protein-ligand interaction

Q1 and **Q2** were created from:

Bolla, J. R.; Sauer, J. B.; Wu, D.; Mehmood, S.; Allison, T. M.; Robinson, C. V. Direct observation of the influence of cardiolipin and antibiotics on lipid II binding to MurJ. *Nat. Chem.* **2018**, DOI: 10.1038/NCHEM.2919.

(signal intensities were slightly changed for this problem session)

1. Introduction

1-1. What is the native MS?

- Particular approach of electrospray ionization (ESI) for analyzing noncovalent interaction of proteins
- The term "native" in native MS describes the biological status of the analytes in solution prior to the ionization.

The native MS-based analytical methods are applicable for the interaction of protein-lipid, protein-RNA, protein-DNA, protein-drug, and protein-protein complexes.

native MS analysis on protein-ligand complex → **physicochemical parameters:**
- **stoichiometry**
- **dissociation constant (K_d)**

etc.

To detect noncovalent interactions, gentle ionization process is required for Native MS. From this viewpoint, ESI is the preferred method for native MS.

advantages:
1. **applicable to samples that vary greatly in mass**
2. **multiple oligomeric states can be analyzed**
3. **highly sensitive**
4. **no chemical labelling or crosslinking is required**

limitations:
1. **detected complex in gas phase may deviate from that in solution (different ionization, transmission, and detection probabilities of each complex)**
2. **hydrophobic and electrostatic interactions become weaker and stronger, respectively**

Background:

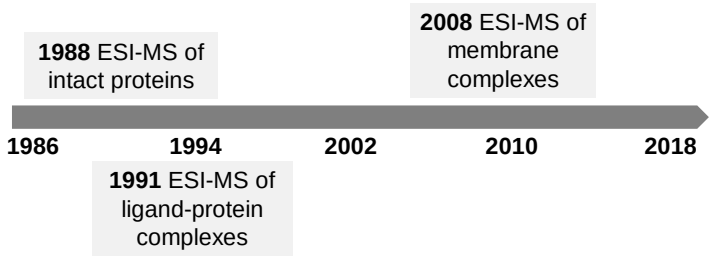
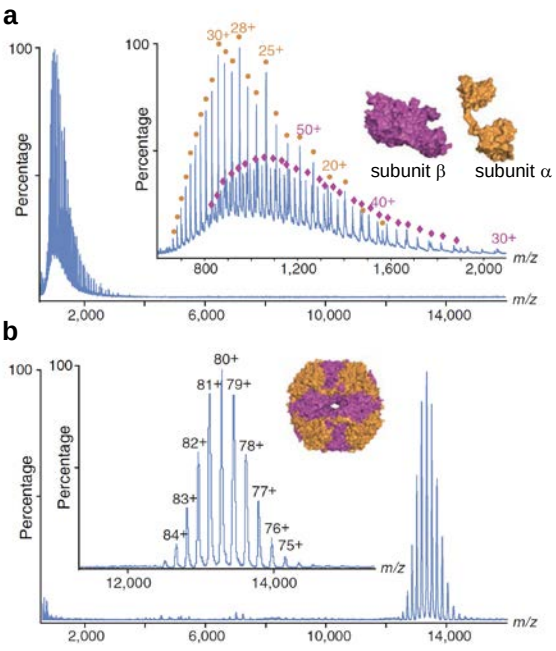
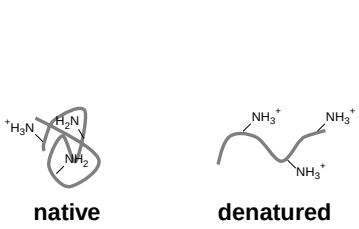


Figure 1. Timeline of mass spectrometry for analyzing biological macromolecules and their noncovalent interactions.[1]

Initial observation:

Proteins electrosprayed from aqueous solution exhibited narrower charge state distributions compared with proteins sprayed from organic solvents. → Protein structure could be partly maintained in the gas phase?

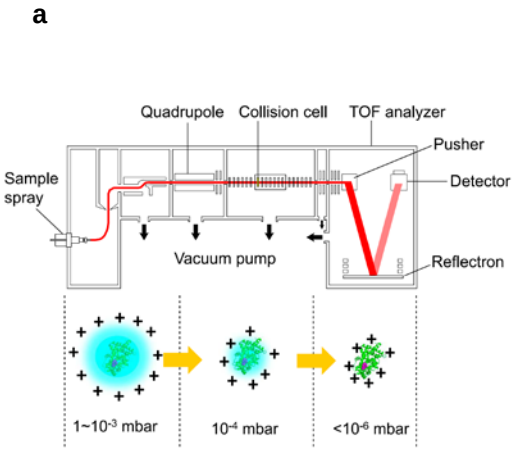


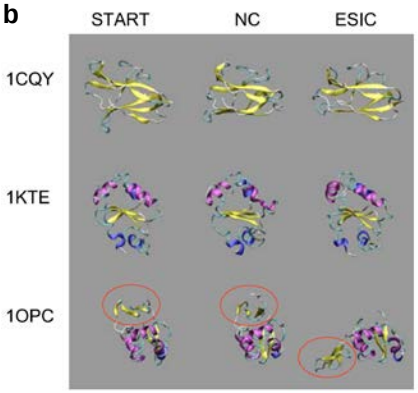


(a) denatured mass spectrum of *H. pylori* urease: denatured urease was electrosprayed from CH_3CN/H_2O (1:1) + 0.1% HCO_2H
subunits α (26.6 kDa) and β (61.7 kDa)
(b) native mass spectrum of *H. pylori* urease: native urease was electrosprayed from an aqueous ammonium acetate solution
intact protein complex ($\alpha_{12}\beta_{12}$, 1,063 kDa)

Figure 2. (a and b) Comparison of denatured MS spectrum and native MS spectrum.[2] (c) Schematic representation of "charged residue model" (solvent exposed basic residues could be charged/protonated)

Molecular dynamics (MD) simulations supported that gas phase experiments can be used to obtain structural information on solution structure (Figure 3b: folded structure is metastable conformation in the gas phase at least in the microsecond timescale).[3]





β-amylase from *Bacillus cereus*

thioltransferase from *Sus scrofa*

OmpR (transcriptional regulatory protein) from *Escherichia coli*

START: initial structure
NC: solution ionic state of protein residues was maintained
ESIC: ionizable residue was charged as typical total protein charge in ESI experiments

Figure 3. (a) General desolvation process in ESI-TOF MS.[4] (b) Comparison of the three-dimensional structure after MD simulations under vacuum.[3] The structures in NC and ESIC are similar to "START" structures expect for circled region of 1OPC (this region is the β-sheet structure of OmpR, which exposes the hydrophobic core during the ESIC simulation).

Although careful consideration is still required to avoid over-interpretation, native MS analysis could be useful methods to investigate noncovalent interactions of proteins.

1-2. Bacterial cell wall synthesis

Lipid II: biosynthetic intermediate for peptidoglycan synthesis (lipid II undergoes genus- and species-specific structural modifications. For example, see Figures 4 and 5)

MurJ: bacterial membrane-associated lipid II flippase

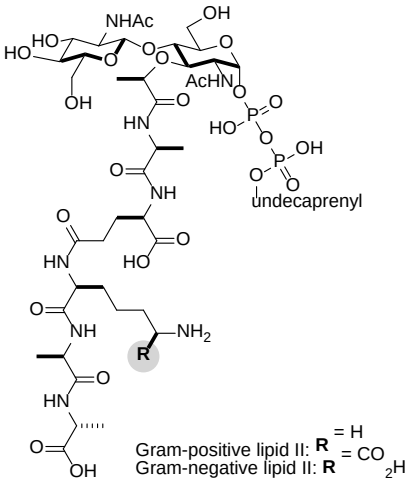


Figure 4. Structure of Gram-positive and Gram-negative lipid II.[5] In this study, gram-positive-type lipid II (R = H) was used.

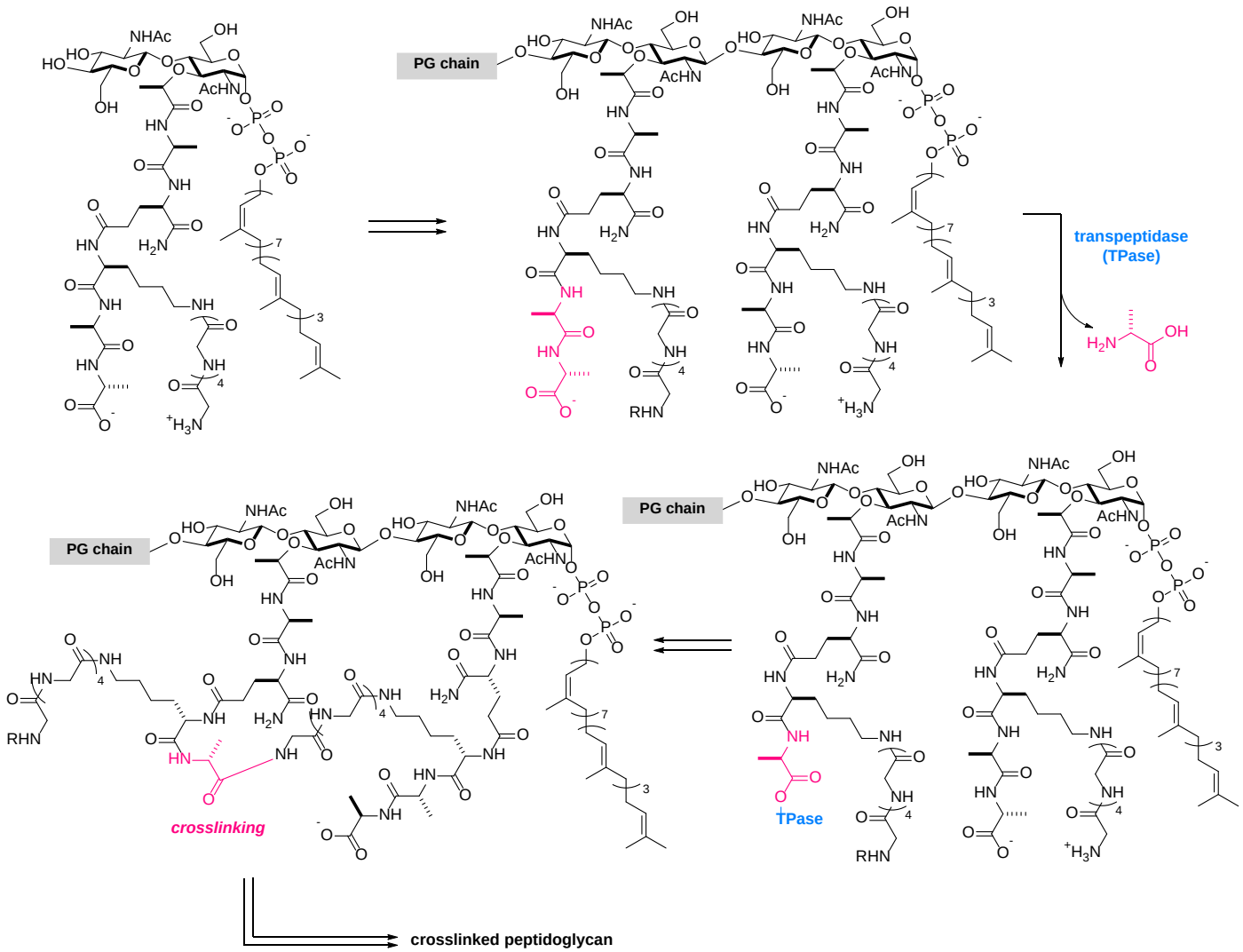


Figure 5. Biosynthesis of peptidoglycan in *S. aureus* (Gram-positive).[6] PG chain = peptidoglycan chain.

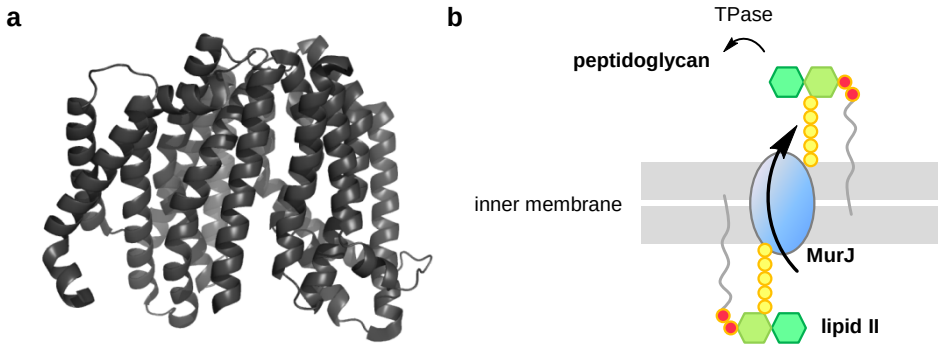


Figure 6. (a) Crystal structure of MurJ (PDB ID: 5T77).[7] (b) Translocation of lipid II via MurJ in inner membrane of *E. coli* (Gram-negative).

Several antimicrobial agents are known to target lipid II [vancomycin and ramoplanin (inhibition of cell wall synthesis); nisin (pore formation in the membrane) etc.] In the context of developing new antimicrobial agent for multi-drug resistant bacteria, understanding the interaction between lipid II and proteins is important.

2. Binding analysis of lipid II to MurJ by native MS

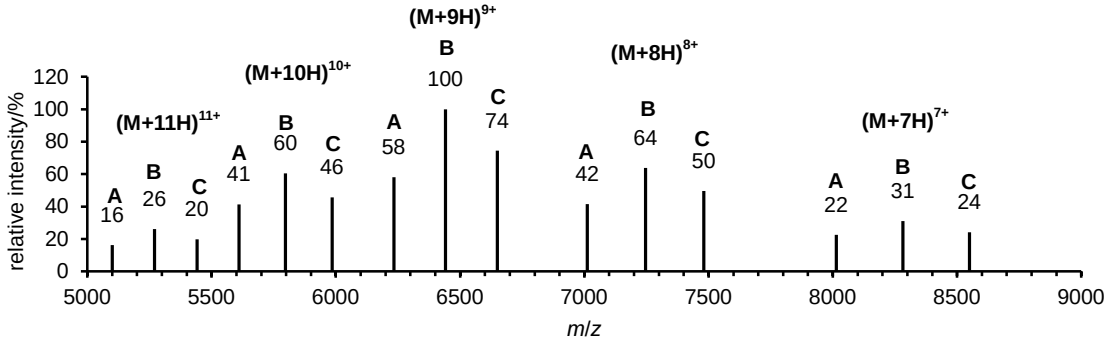
2-1. Conditions for native MS of MurJ

Proteins were solubilized in 200 mM ammonium acetate buffer [ESI compatible (volatile) buffer] with 0.05% *n*-dodecylamine-*N*-oxide [detergent to solubilize membrane protein into the micelle; critical micelle concentration (CMC): 0.023%]

2-2. Estimation of binding affinity from the native MS spectra

Several charge states could be observed in ESI-MS:

20 μM lipid II:



series A: unbound MurJ [*m*/*z* = 8014 $(M+7H)^{7+}$, 7012 $(M+8H)^{8+}$, 6233 $(M+9H)^{9+}$, 5610 $(M+10H)^{10+}$, 5100 $(M+11H)^{11+}$]

series B: MurJ + lipid II [*m*/*z* = 8282 $(M+7H)^{7+}$, 7247 $(M+8H)^{8+}$, 6442 $(M+9H)^{9+}$, 5798 $(M+10H)^{10+}$, 5271 $(M+11H)^{11+}$]

series C: MurJ + lipid II x2 [*m*/*z* =8550 $(M+7H)^{7+}$, 7481 $(M+8H)^{8+}$, 6650 $(M+9H)^{9+}$, 5985 $(M+10H)^{10+}$, 5441 $(M+11H)^{11+}$]

Figure 7. Interpretation of the MS spectra.

1. Calculation of ratio of the peak intensity against the total intensity of all observed species in one charge state
2. Calculation of the averaged ratio of all the charge states
3. Data plotting against the concentration of lipid II

conc. of lipid II	relative fractional intensity (%)		
	A	B	C
20	26.83842	41.42813	31.73345
15	34.9537	42.17801	22.86829
10	44.20289	39.50125	16.29586
7.5	51.71724	35.23395	13.04881
5	60.53519	30.32986	9.134945
2.5	76.82002	23.17998	
1.25	83.51463	16.48537	
0.625	90.04094	9.959064	
0	100	0	0

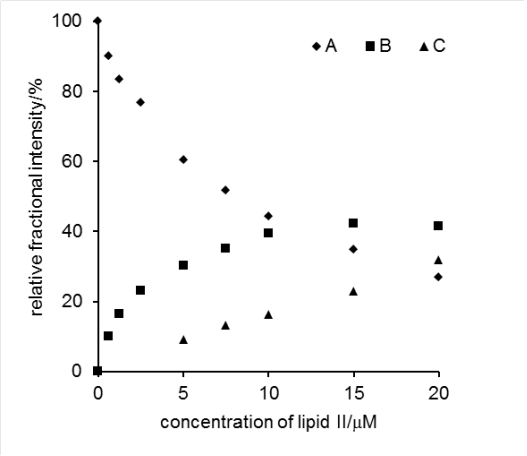


Figure 8. Plot of the relative fractional intensity against the concentration of lipid II.

- series B: saturable, used to calculate dissociation constant
- series C: non-saturable = non-specific binding (product of a constant and ligand concentration)[8]

$$P + L \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} PL$$

Concentration of protein (MurJ) = [P], concentration of ligand (lipid II) = [L]

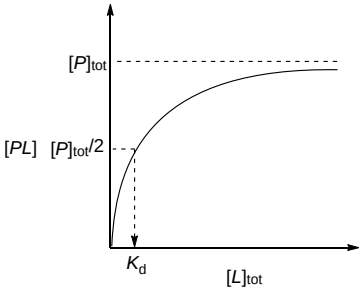
$$k_{on}[P][L]=k_{off}[PL]$$

$$\frac{k_{off}}{k_{on}}=K_d=\frac{[P][L]}{[PL]}$$

$$K_d=\frac{([P]_{tot}-[PL])[L]}{[PL]} \quad when \quad [P]=[P]_{tot}-[PL]$$

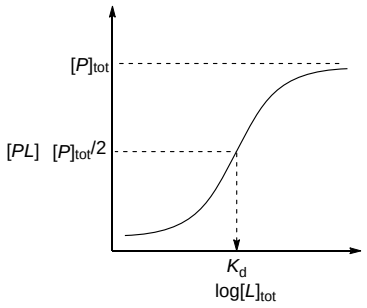
$$[PL]=\frac{[P]_{tot}[L]}{K_d+[L]}$$

$$[PL]=\frac{[P]_{tot}[L]_{tot}}{K_d+[L]_{tot}} \quad when \quad [L]\approx[L]+[PL]=[L]_{tot}$$



The above equation can be converted to log-logistic function:

$$[PL]=\frac{[P]_{tot}}{\frac{K_d}{[L]_{tot}}+1}=\frac{[P]_{tot}}{1+\exp(\ln(K_d)-\ln([L]_{tot}))}$$



Example: fitting by R[9] *with drc package*[10]

$$f(x,(b,c,d,e))=c+\frac{d-c}{1+\exp(b(\log(x)-\log(e)))}$$

b = relative slope around *e*, *c* = lower horizontal asymptote, *d* = upper horizontal asymptote, *e* = inflection point (= K_d)

In this case, *c* = 0 (LL.3)

- source code

```
library("drc")
data <- read.table("ps.csv", header=T, sep=",")#read a file in table format
conc <- data$x#x (csv file) is assigned to conc
res <- data$B#B (csv file) is assigned to res
ndf <- data.frame(x=conc, y=res)#create a new data frame, assigned to ndf
MurJL2 <- drm(res~conc, data=ndf, fct=LL.3())#three-parameter log-logistic fitting (c = 0), assigned to MurJL2
coef(MurJL2)#extract parameters b, d, and e
plot(MurJL2, xlim = c(0.5, 25), ylim = c(0, 50))#data plot
```

- data table of ps.csv

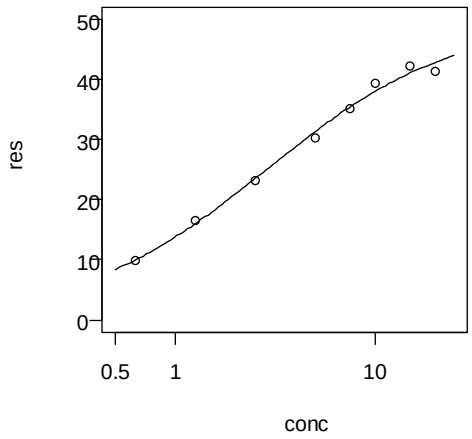
```
> data
      x       A        B        C
1 20.000 26.83842 41.428128 31.733449
2 15.000 34.95370 42.178011 22.868286
3 10.000 44.20289 39.501249 16.295865
4  7.500 51.71724 35.233948 13.048809
5  5.000 60.53519 30.329864  9.134945
6  2.500 76.82002 23.179983        NA
7  1.250 83.51463 16.485371        NA
8  0.625 90.04094  9.959064        NA
```

- extracted parameters and data plotting

```
> coef(MurJL2)
b:(Intercept) d:(Intercept) e:(Intercept)
   -0.9180789    50.0408347    2.8510751
```

→ K_d = 2.9 μM

```
plot(MurJL2, xlim = c(0.5, 25), ylim = c(0, 50))
```



References

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