カチオン性脂質とDNAからなる遺伝子導入剤の構造と機能の相関

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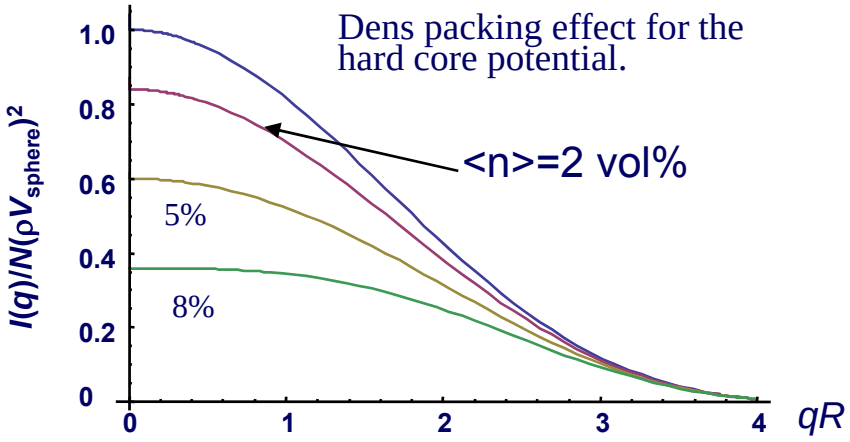
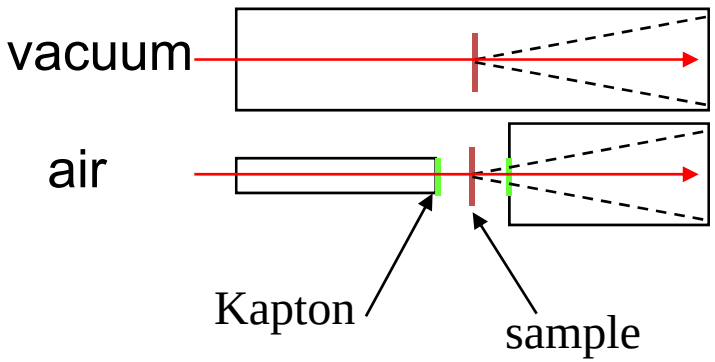
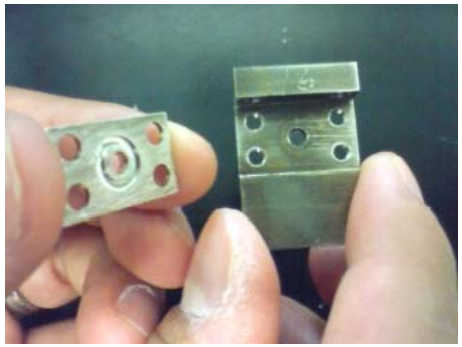
Background & Aims

- Millions of biologically functional molecules have been synthesized or discovered from nature. Most of them turns out useless as drugs in practical sense, because of high toxicity.
- Necessary for a good vehicle to transport the molecules to their target, which is called Drug Delivery System (DDS) .
- In-situ structural characterization of DDS particles is a challenging issue, owing to ultra-dilution and small amount.
  - Generally, 0.1-10 mg/mL (0.01-1 %)
- Conventional SAXS set-up:
  - Need of high concentration ➔ Dens Packing effect
  - Low concentration ➔Low S/N
- We go to Synchrotron SAXS with a newly designed vacuum chamber.



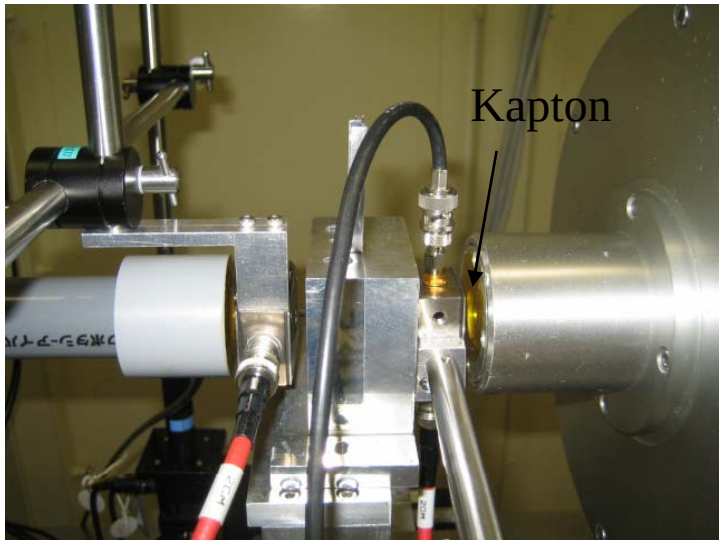
Vacuum chamber and cells



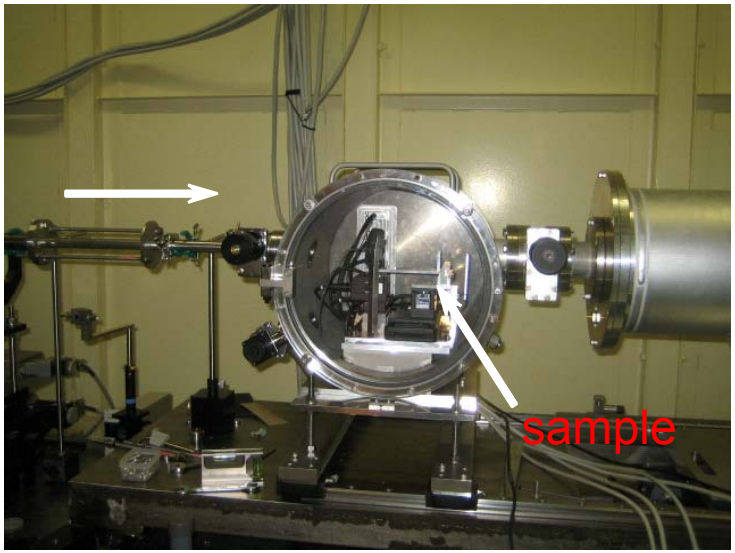


Open-able to wipe the inside taint of glass.

Conventional set-up



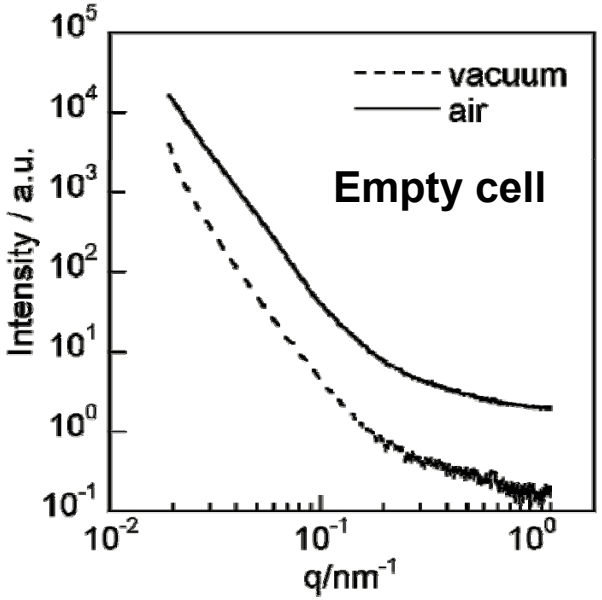
Vacuum chamber

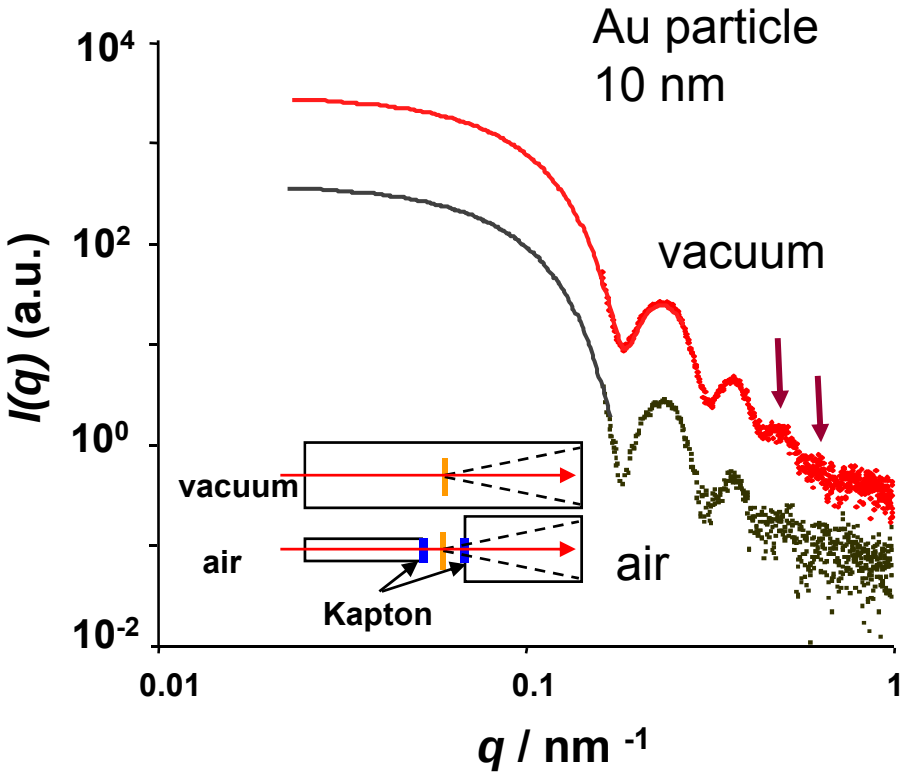


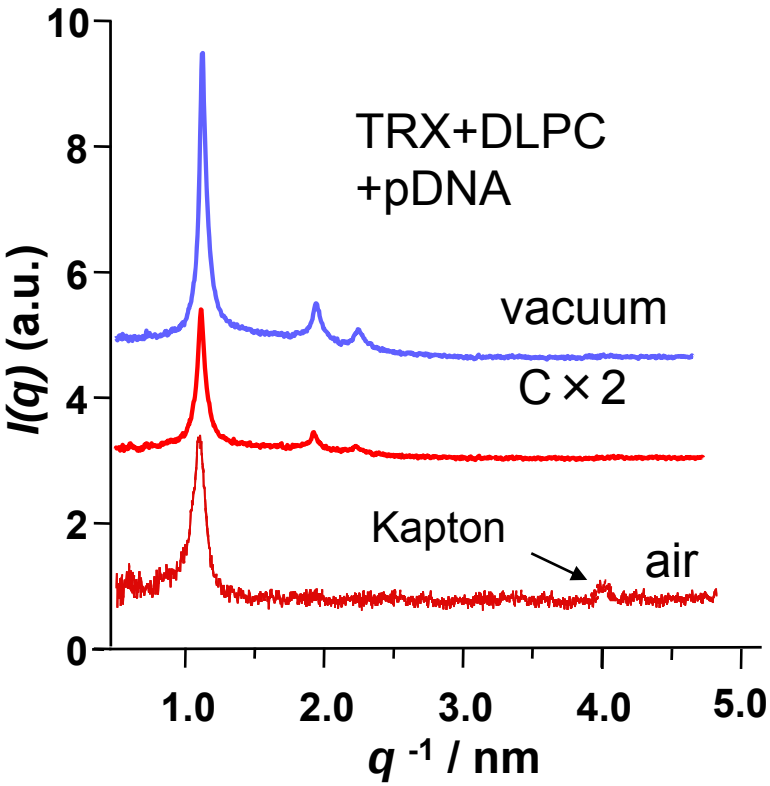
Collaborated with Dr. Masunaga in SP8

Performance of the new set-up

-S/N 10-100 times.
-Low BG at high *q*.
-No peaks from the kapton window
-Low noise around beam stopper







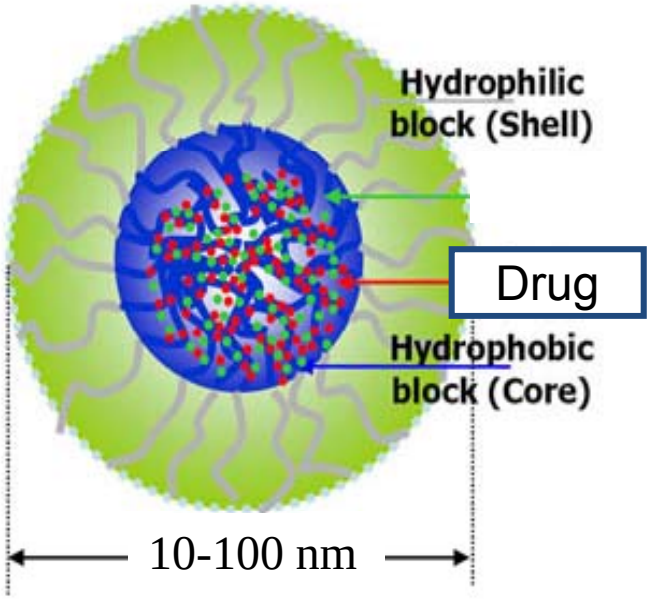
Materials: DDS vehicles

Polymeric micelle DDS for hydrophobic drug delivery

Amphipathic blockcopolymer

+

Hydrophobic Drug



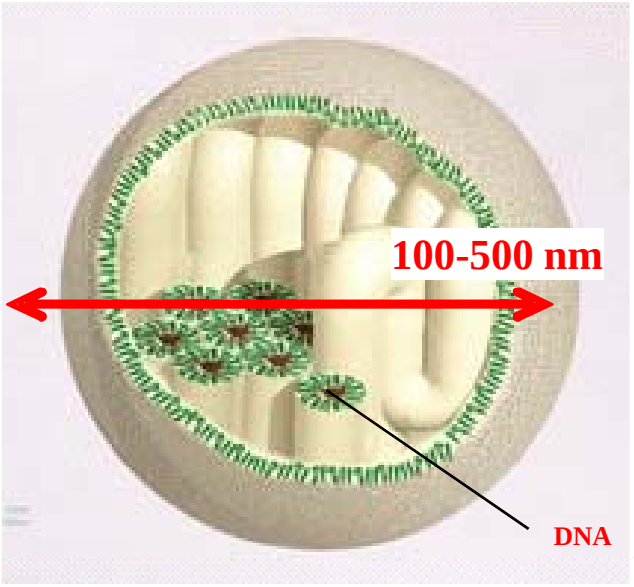
Core-shell

Lipoplex DDS for gene therapy

Cationic lipids + amphisbaena lipids

+

*p*DNA

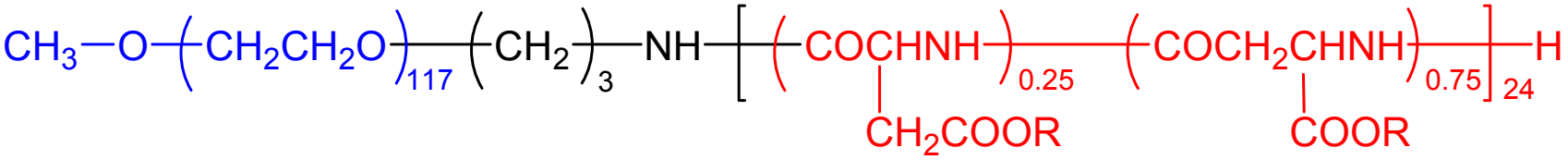


DNA-templated highly ordered structure

PEG-Poly(Asp,Bzl) Micelle

Collaborating with Prof. Yokoyama
KIST

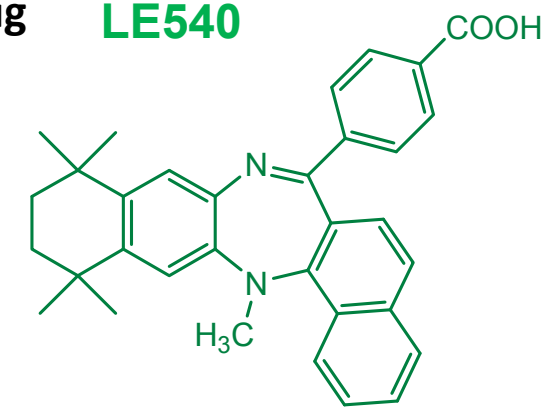
Di-Block Copolymer



PEG—P(Asp Bzl)

R = H (27.4 %) or —CH2— (82.6 %)

Drug **LE540**

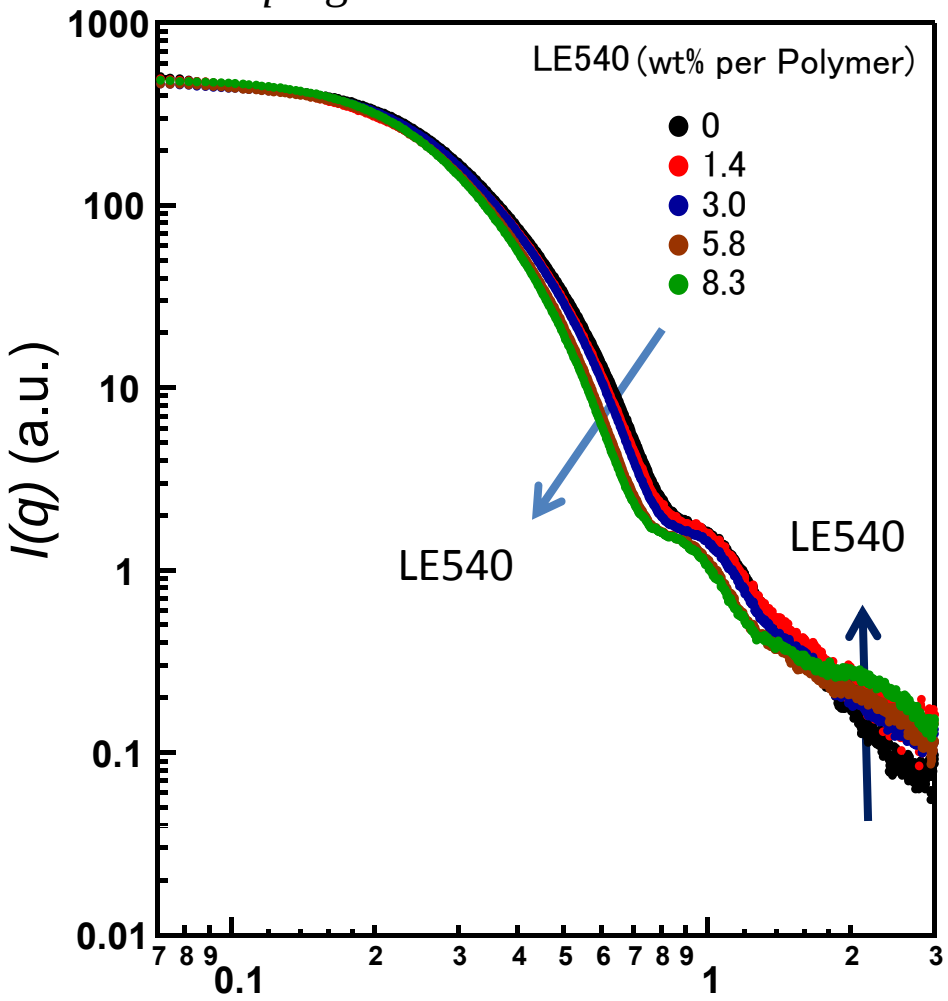


Neuroblastoma: Pre-incubation of SH-SY5Y human neuroblastoma cells with either RAR-pan-antagonist LE540 or MAP kinase kinase 1 (MEK-1) inhibitor PD98059.
Breast Neoplasms (Breast Cancer) In ZR-75-1 human breast cancer cells, cotreatment of LE135 and LE540 with all-trans-RA inhibited all-trans-RA-induced apoptosis.

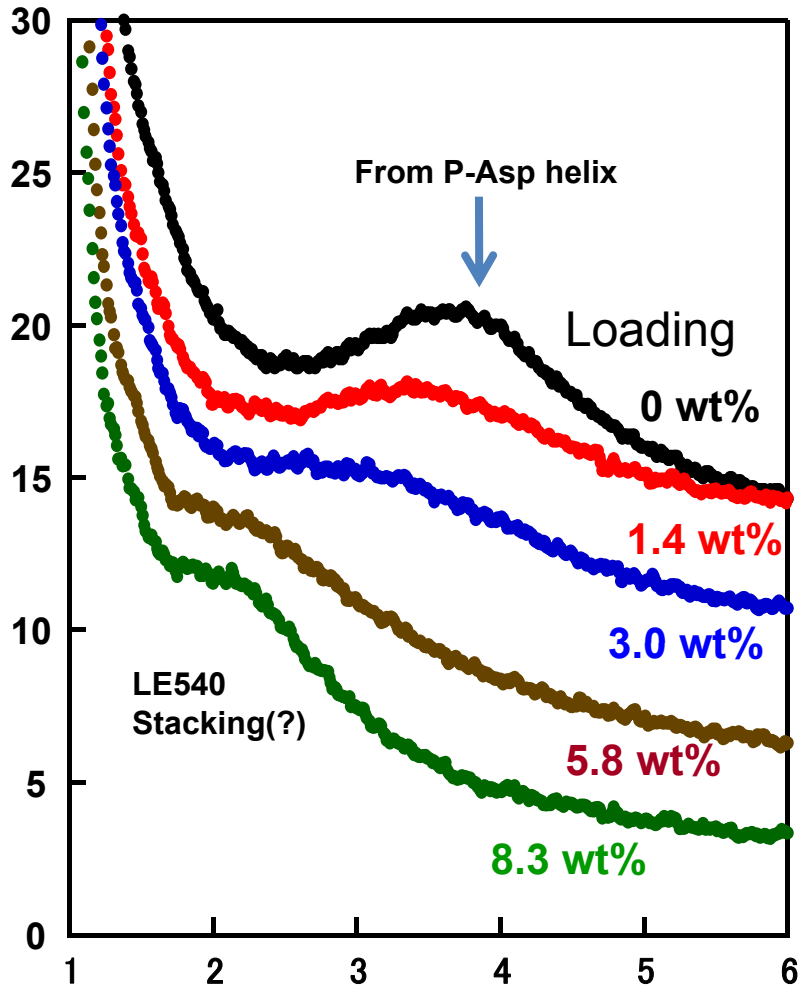
SAXS from LE540 loaded PEG-b-P(Asp,Bzl)

SPring-8, 40 B2

Low *q* region



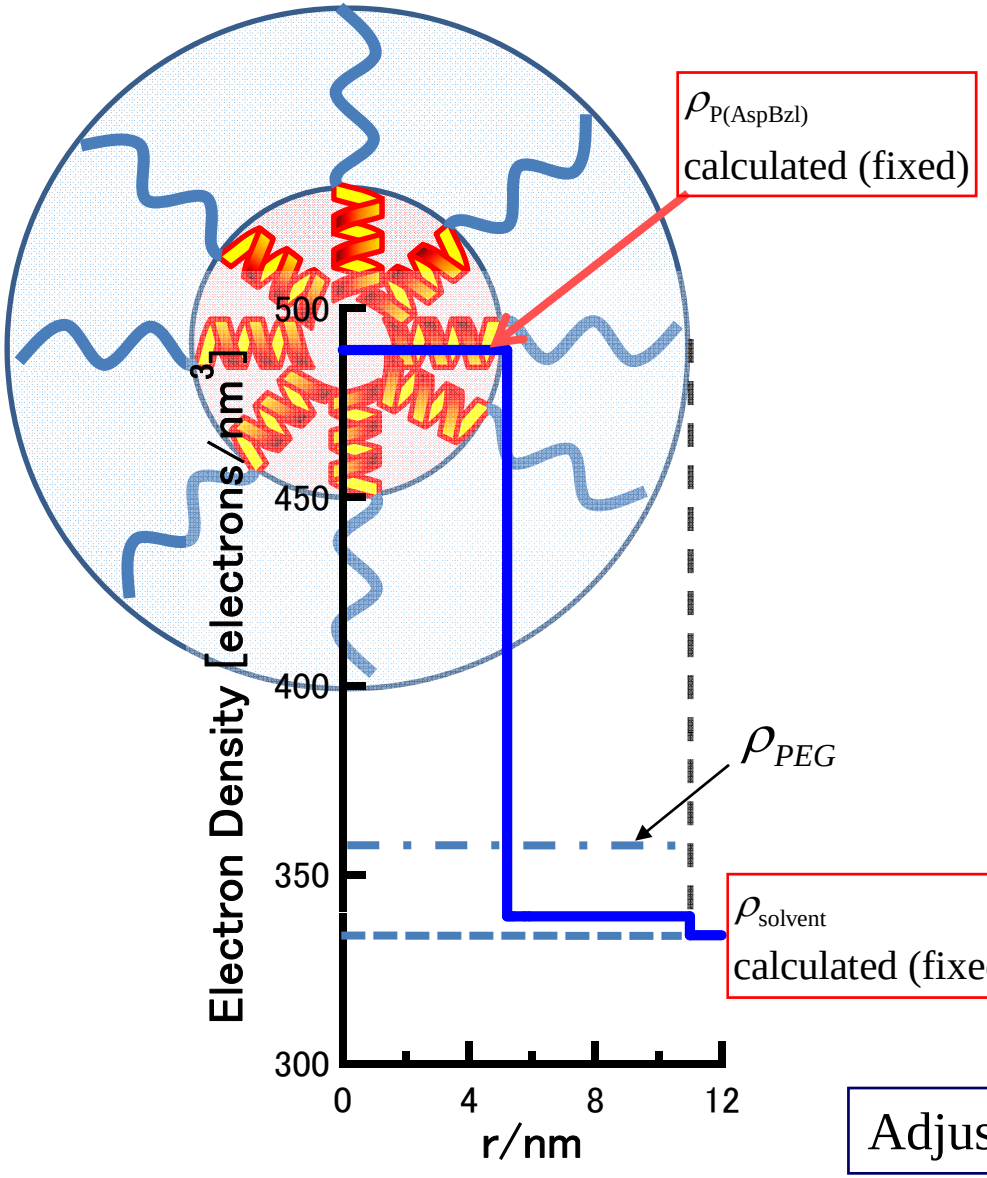
Middle&high *q* region



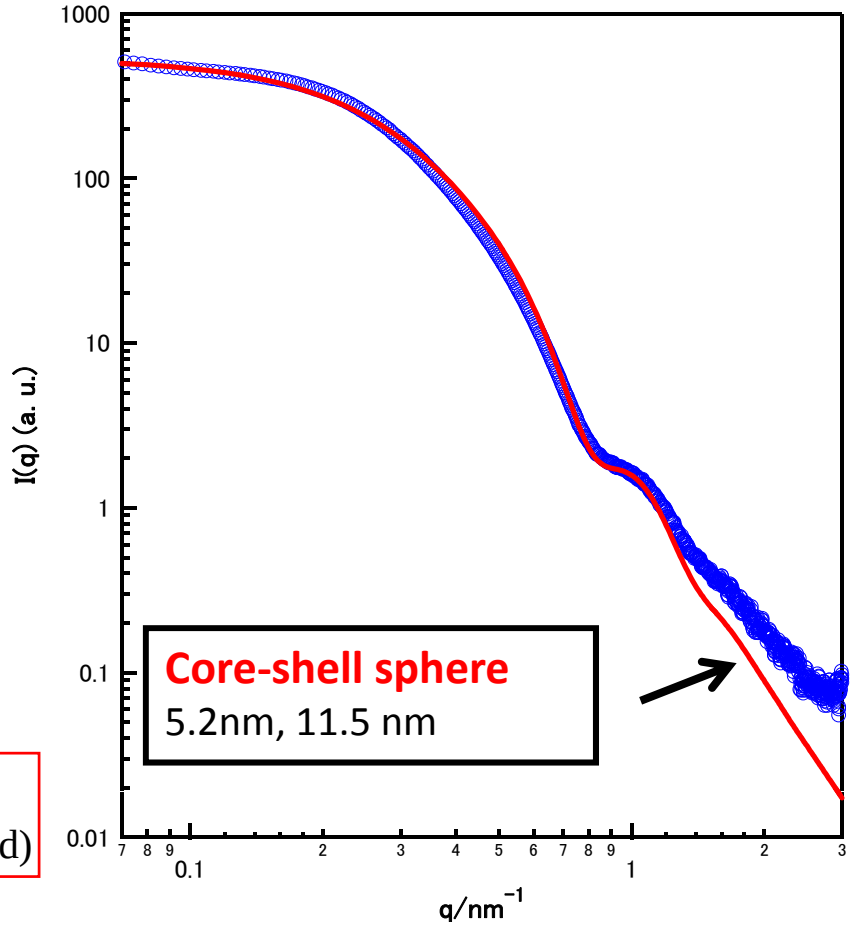
40B2, 6.25 mg/mL (0.625 wt%)
Exposure 10 min, camera length: 1.5m

q / nm^{-1}

Fitting with core-shell sphere



Loading rate = 0



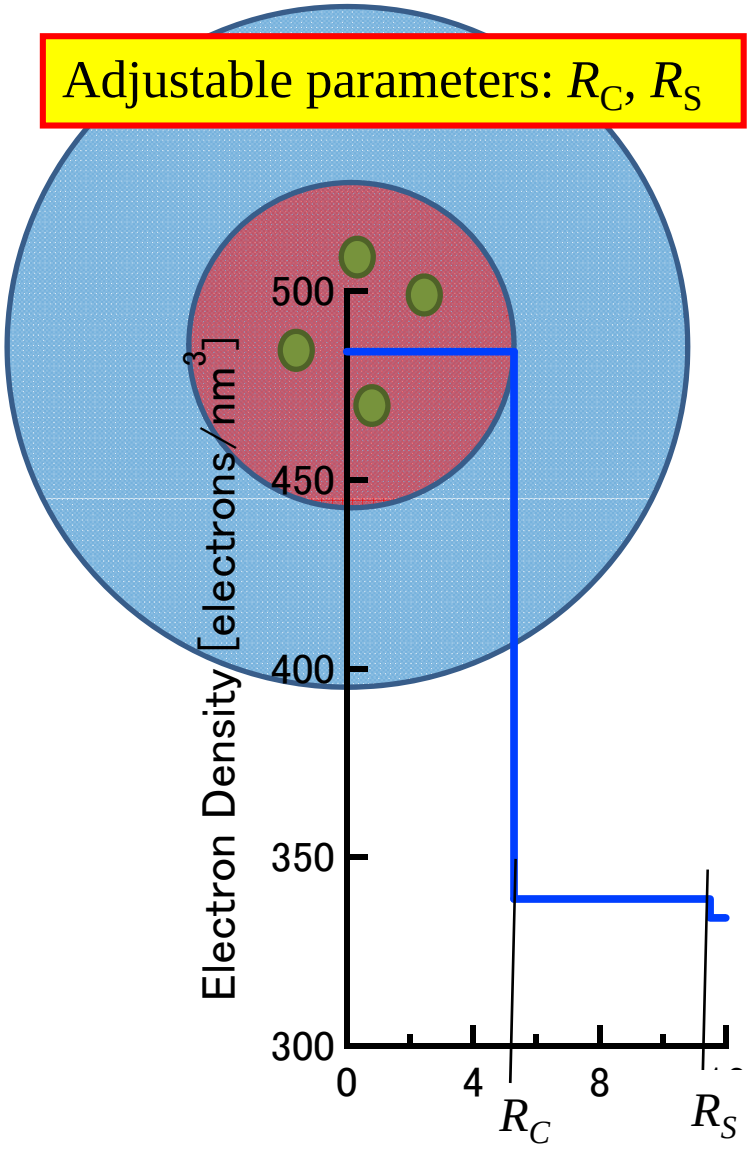
Adjustable parameters: ρ_{shell} R_C, R_S

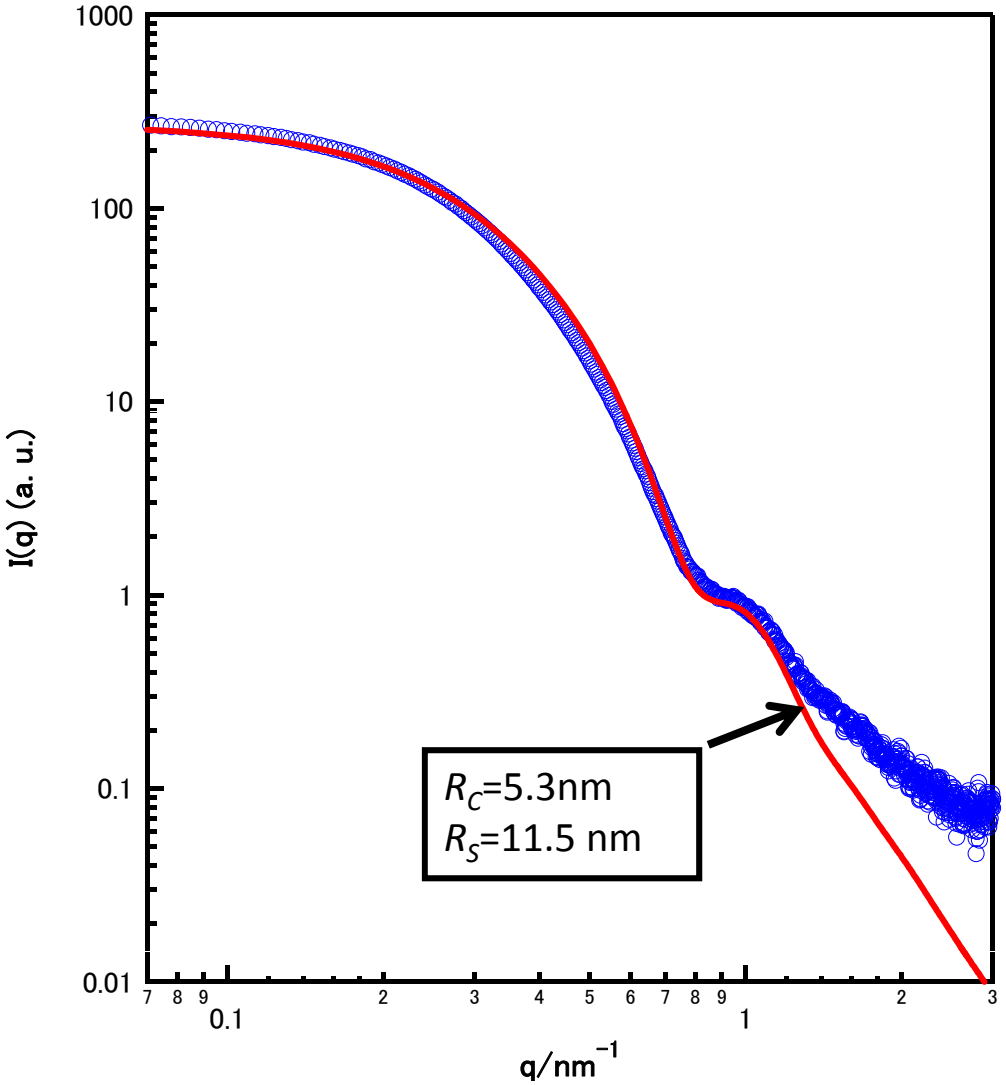
LE540 loading 1.4 wt%

$$\rho_{\text{core}} = \phi\rho_{\text{P(AspBzl)}} + (1-\phi)\rho_{\text{LE540}}$$

ϕ: volume fraction of P(Asp Bzl)
[density $_{\text{P(Asp Bzl)}}$ =1.3 g/cm^3]

Adjustable parameters: R_C, R_S



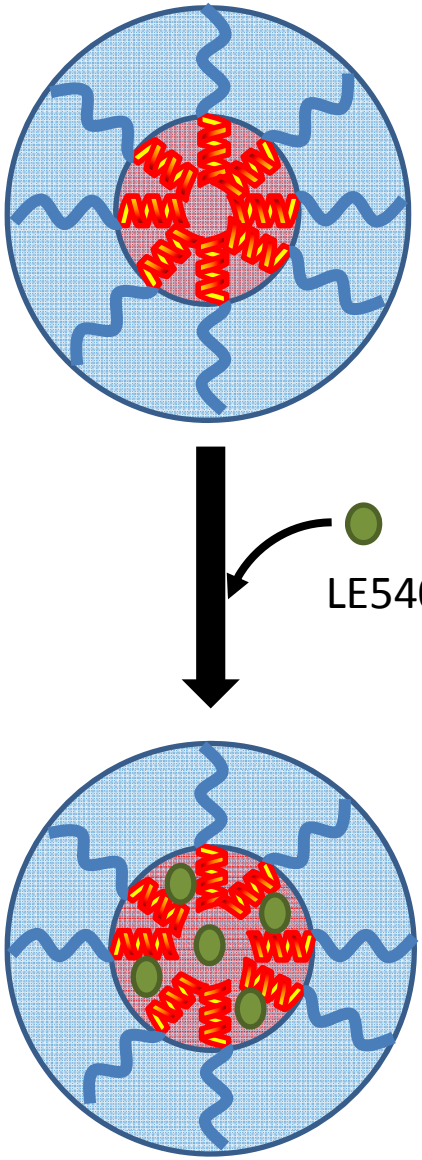


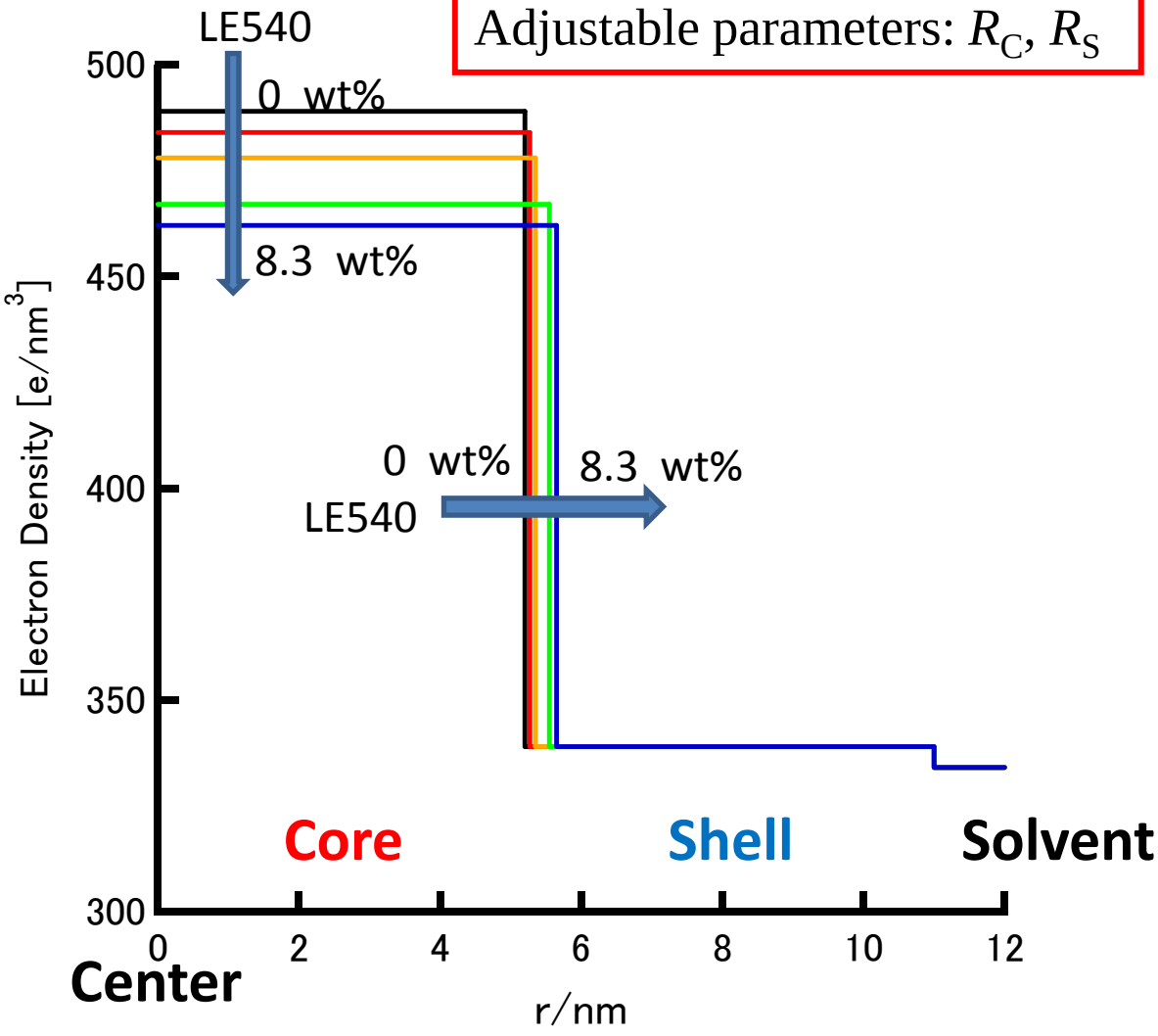
Fitting with a confined condition

$$\rho_{\text{core}} = \phi\rho_{\text{P(AspBzl)}} + (1-\phi)\rho_{\text{LE540}}$$

ϕ: volume fraction of P(Asp Bzl)
[density $_{\text{P(Asp Bzl)}}$ =1.3 g/cm^3]

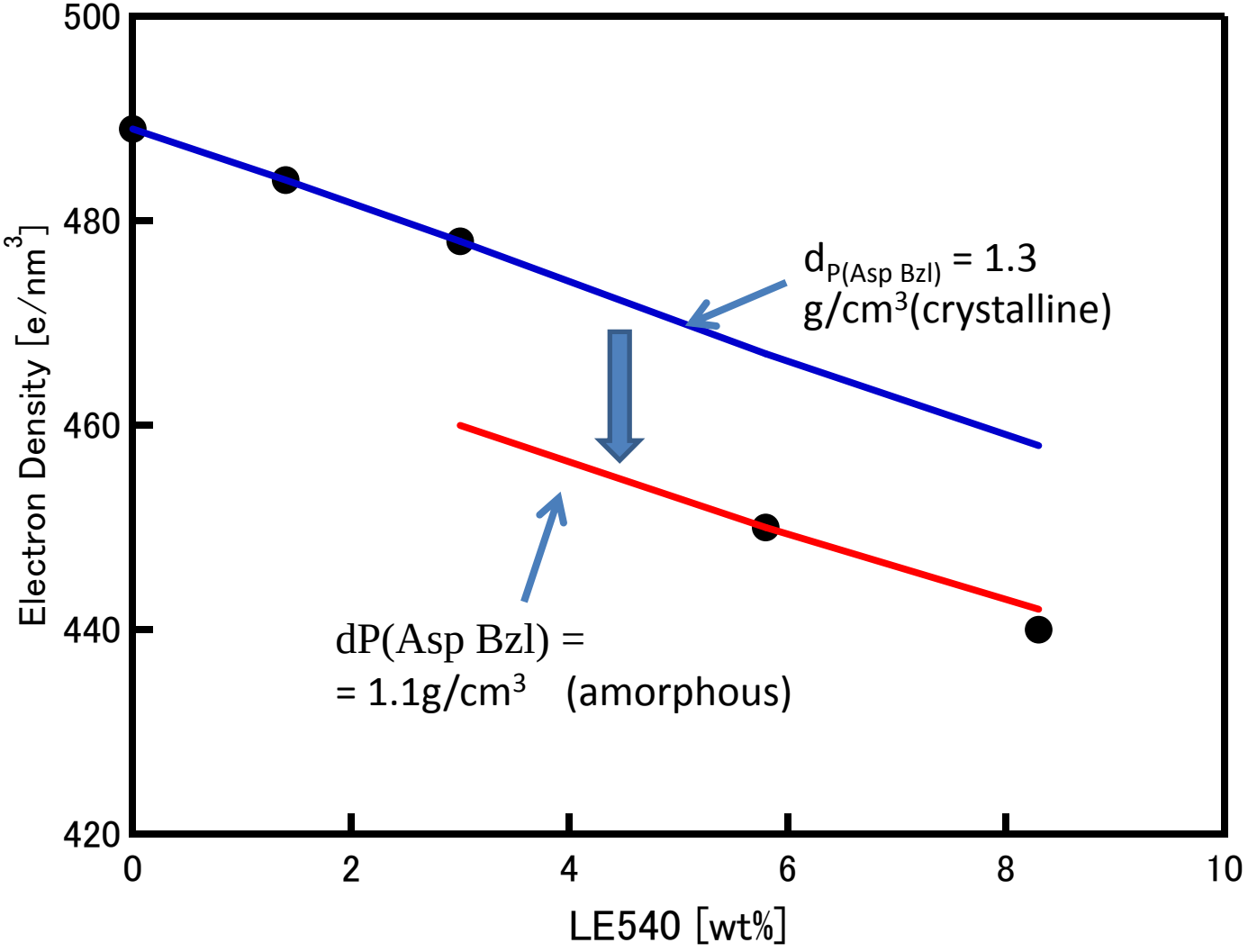
Adjustable parameters: R_C, R_S

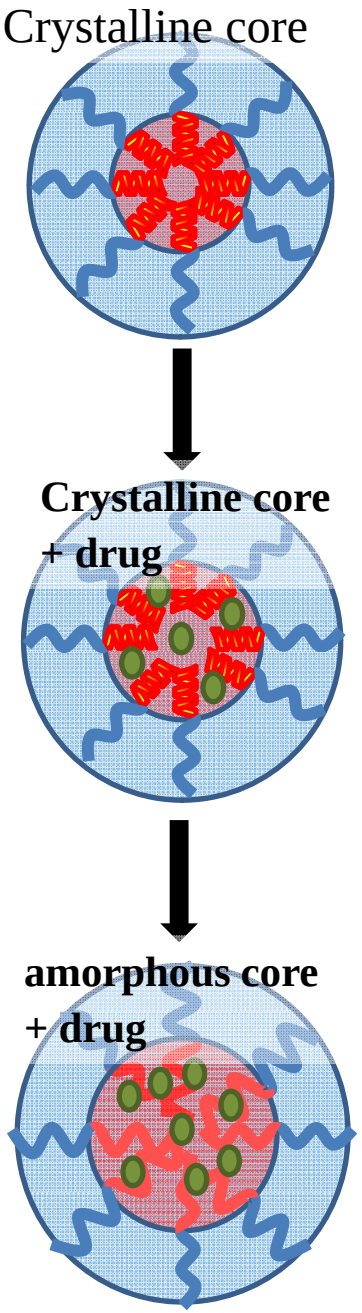




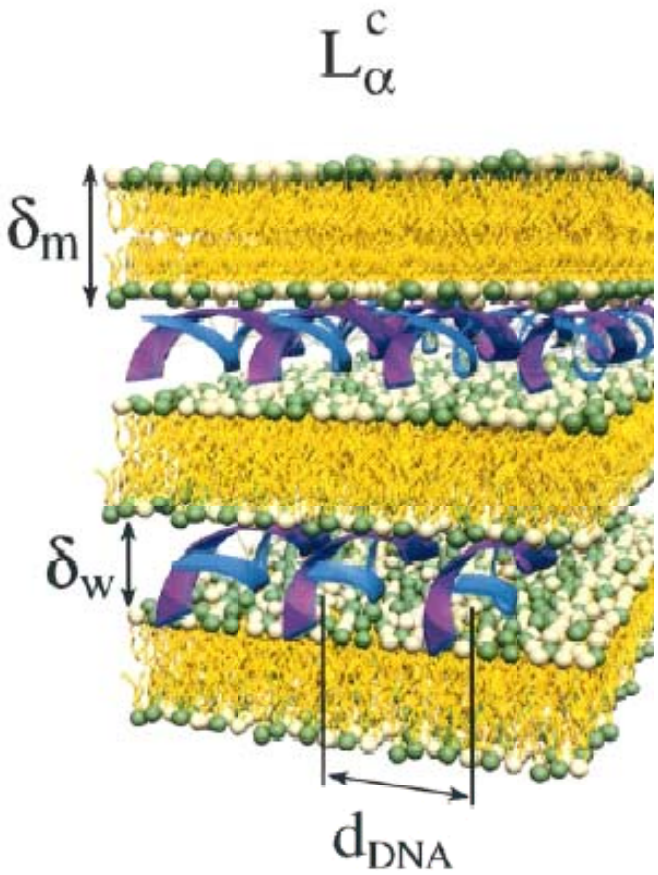
Density change upon loading

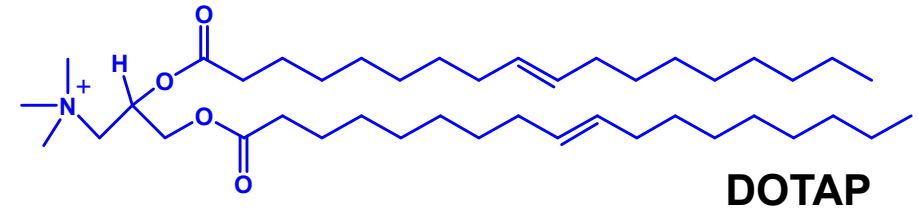
$$\rho_{core} = \phi\rho_{P(AspBzl)} + (1-\phi)\rho_{LE540}$$



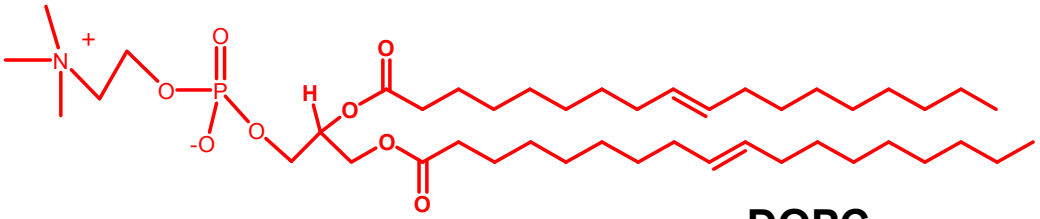


Gene Carrier: pDNA/cationic lipid complex





DOTAP

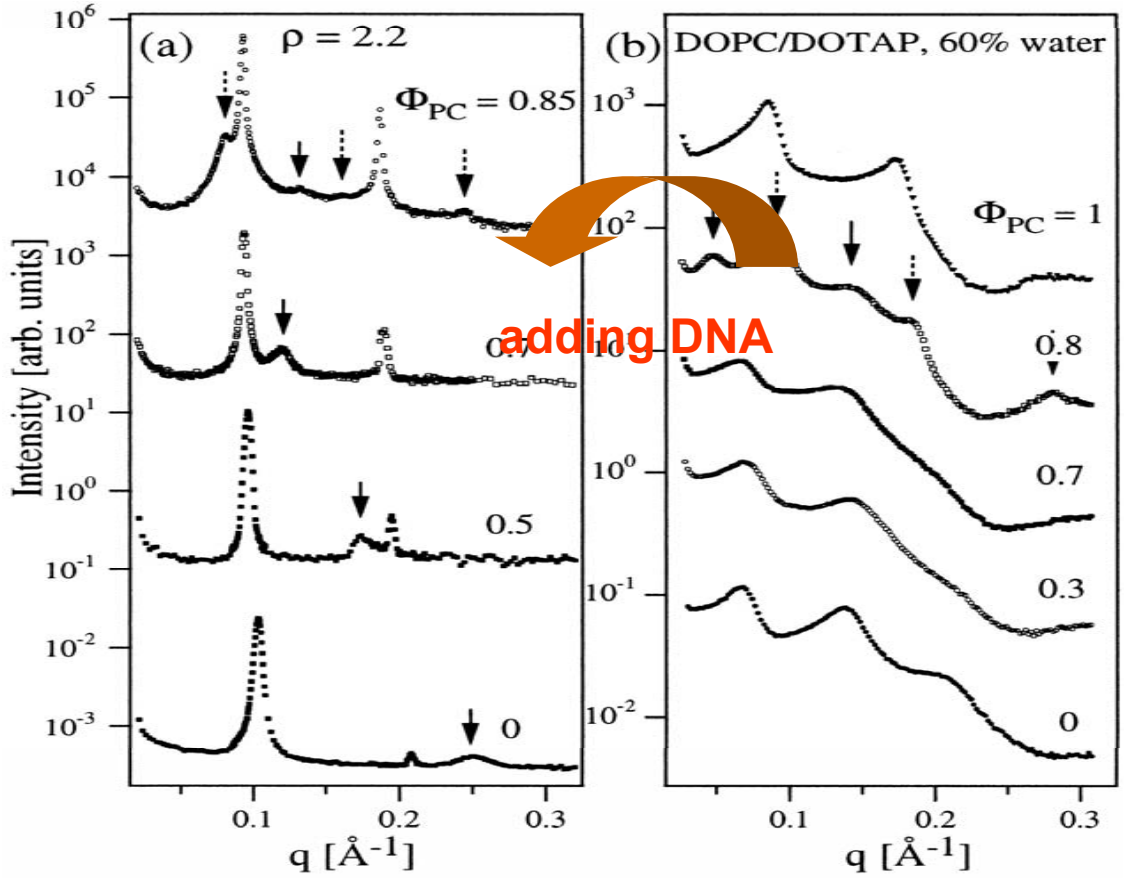


DOPC

Safinya, Science 2000 288, 2035-2039

Previous work

micelle



adding DNA

Lamellar to lamellar transition

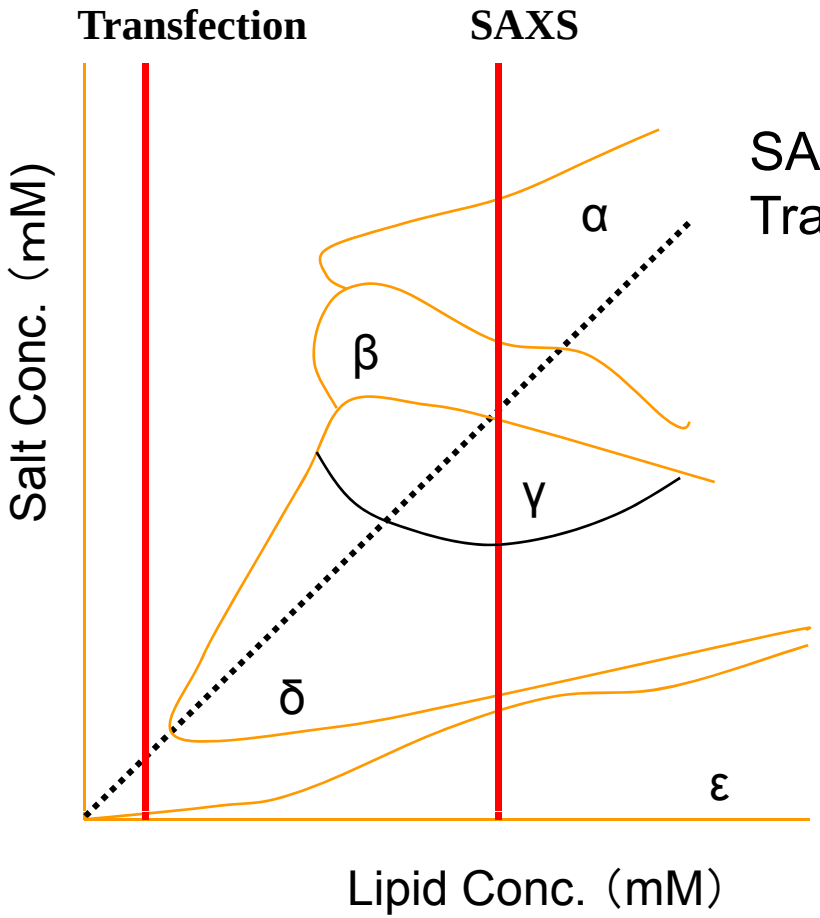
SAXS profiles DOTAP/DOPC/pGL3 lipoplexes

Biophysical Journal 77 1999 915–924
Science 1997 275, 810-814
Phys. Rev. Lett. 1997 79, 2582-2585
Phys. Rev. E. 1998) 58, 889-904
Science 1998 281, 78-81
Science 2000 288, 2035-2039

Safinya (UCLA), Stanford Synchrotron Radiation Laboratory
Their SAXS concentration (600 mM) is much hither than normal transfection concentrations (ca., 0.08-0.004 mM).

Concentration and structure

In order to measure SAXS from ultra-dilute solutions (less than 1 wt%, hopefully 0.1 wt%), we need a strong synchrotron source and a cell & set-up with low BG and high S/N.

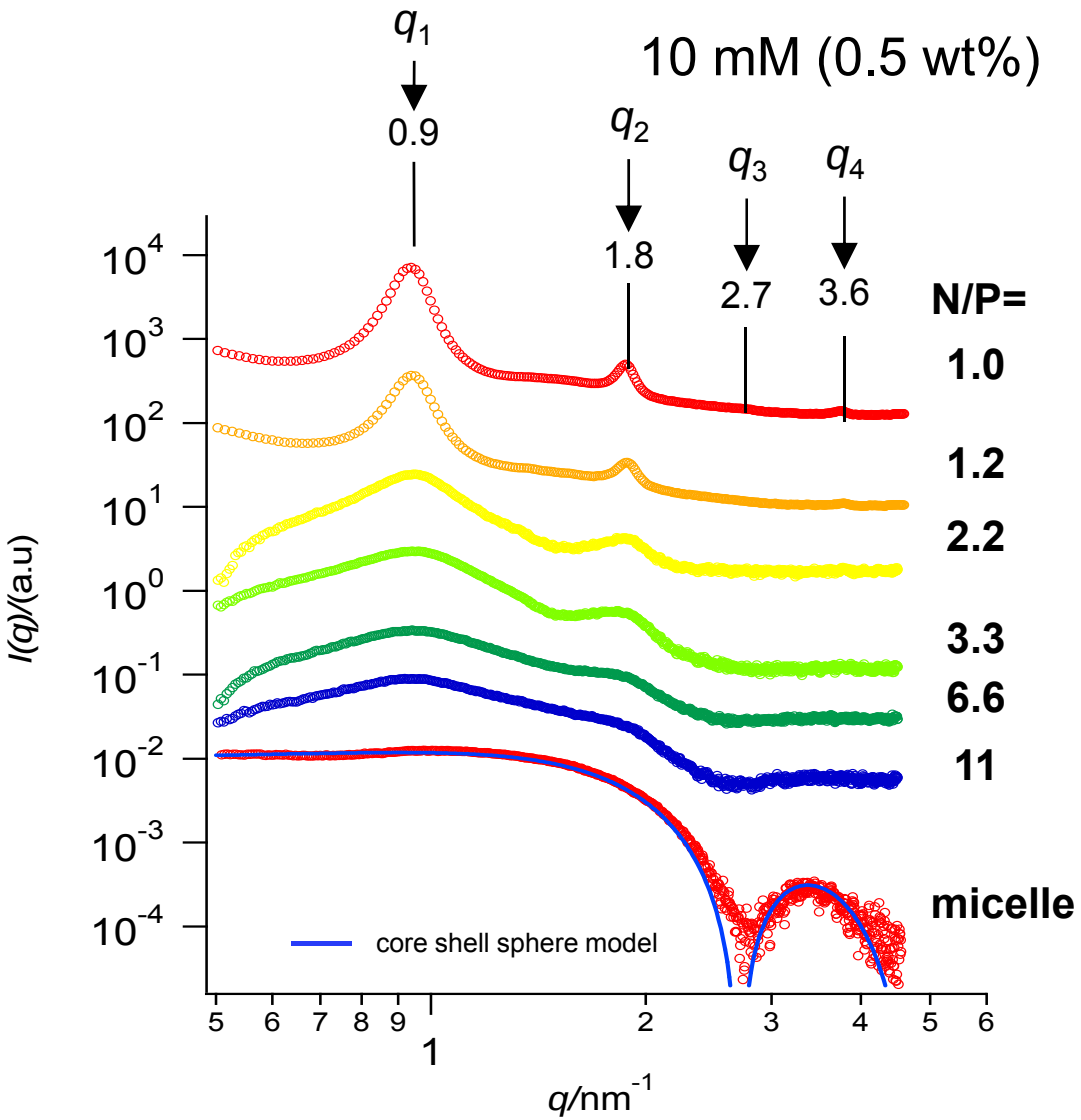


SAXS：15 - 30 mM

Transfection：1-10 × 10^{-3} mM

Typical phase diagram for lipids.

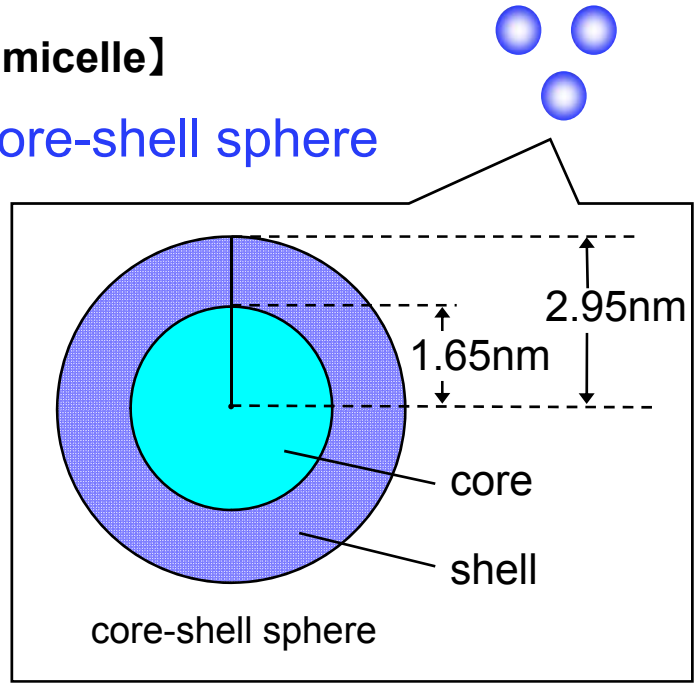
Re-examination with the vacuum chamber



SAXS profiles of DOTAP/DOPC micelle and DOTAP/DOPC/pGL3 (5300 bp) lipoplex (10mM) as a function of N/P ratio. BL40B2, wavelength = 1Å, sample to detector = 0.7m, 3000 × 3000 IP, N/P ratio = nitrogen of DOTAP/ phosphate of DNA

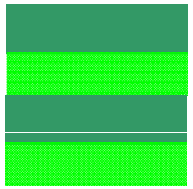
【micelle】

core-shell sphere



【Lipoplex】

lamellar structure with D = 7.0 nm



spherical micelle

↓

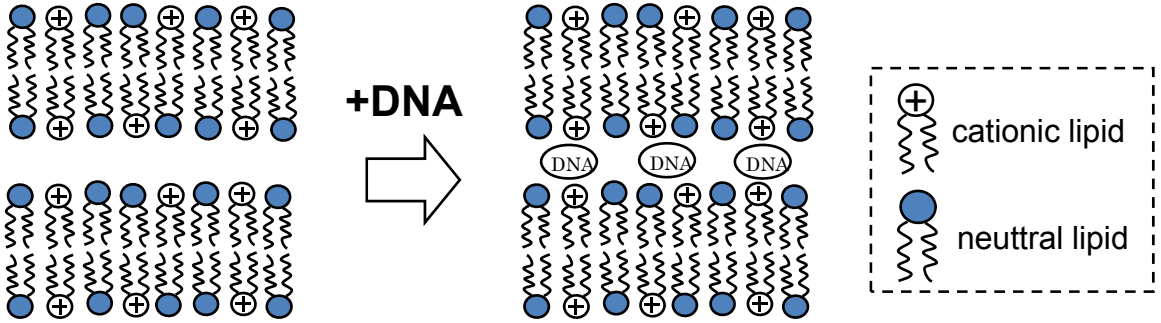
lamellar structure lipoplex

Two possible structures

sandwiched between two bilayers

< Safinya's condition >

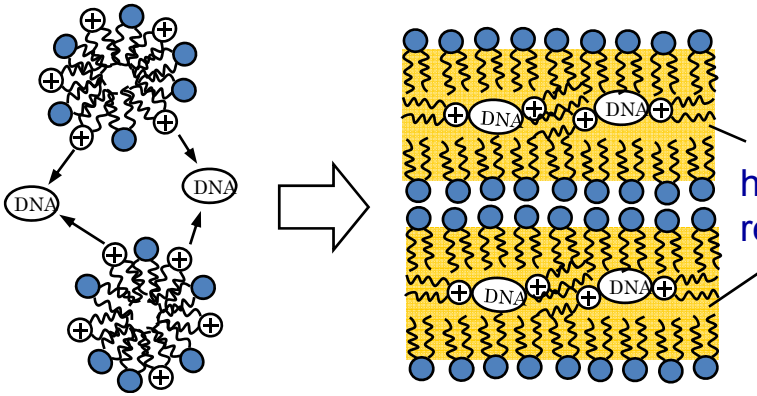
<micelle> <lipoplex>
lamella → lamella



< more dilute >

<micelle> <lipoplex>
sphere → lamella

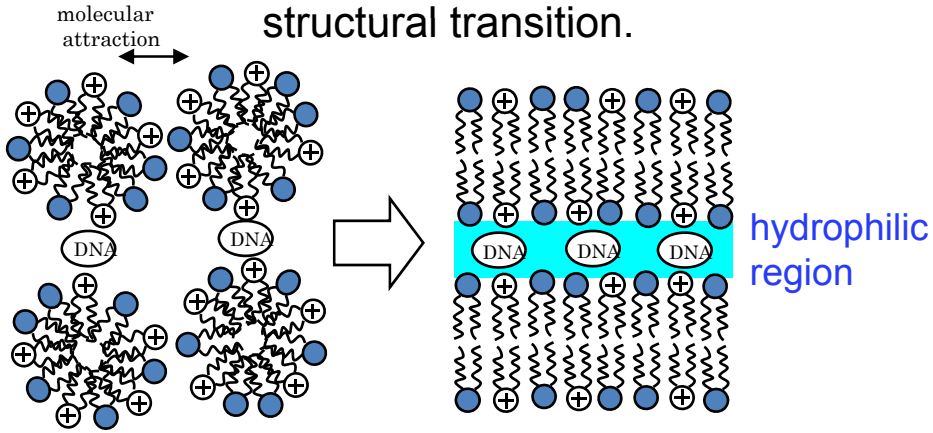
< rearrangement of micelles >



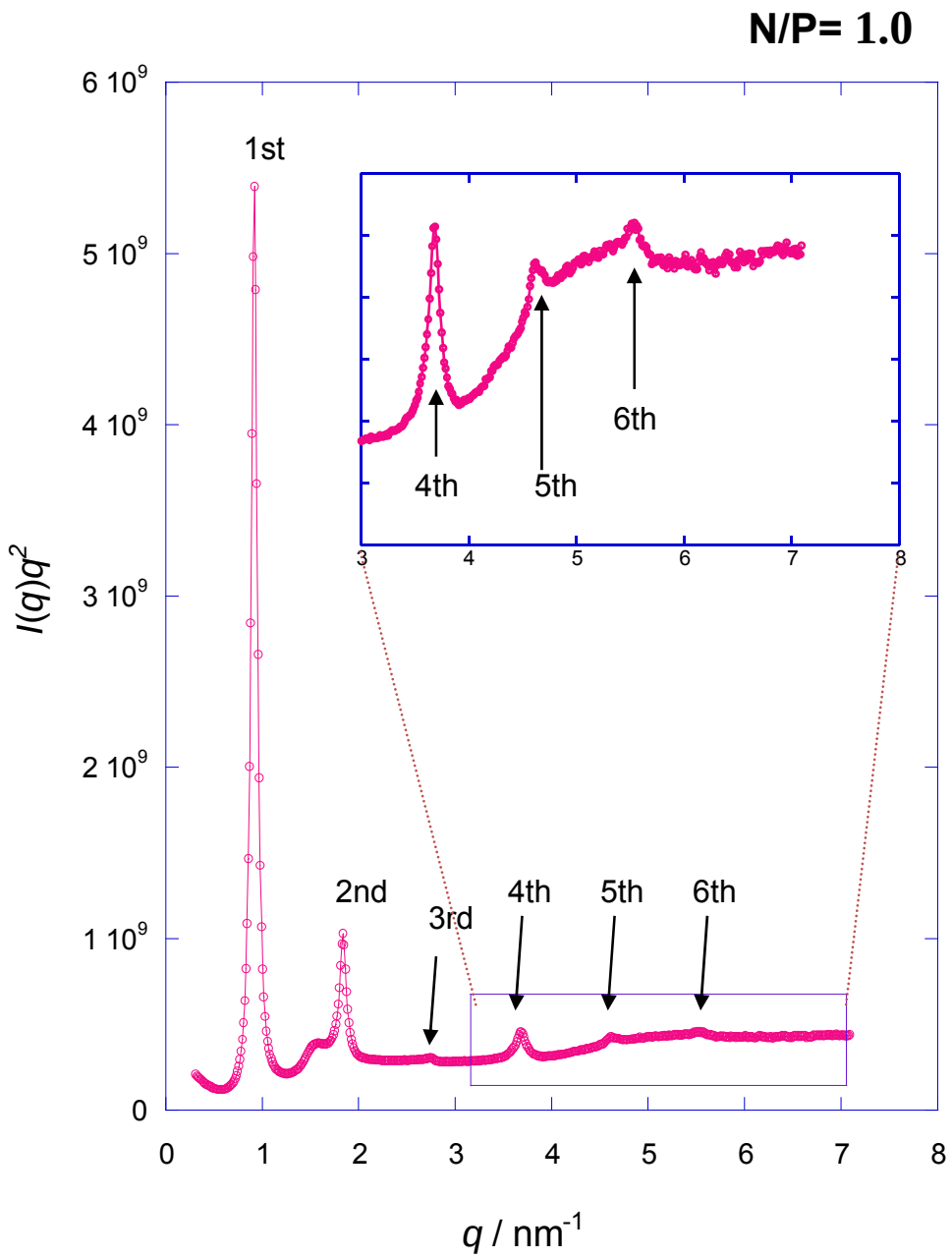
The hydrophobic ion pairs go to the hydrophobic domain. This model can explain the N/P =1.

< deformation of micelles >

Attachment of DNA to the micelle surface induces structural transition.



Direct Inv. Fourier Transform



Density profile

$$\rho(x) = \frac{A_0}{2} + \sum_{n=1}^{N} A_n \cos n\frac{2\pi x}{T}$$

Fourier Transform

$$I(q) = \frac{A_0^2}{4}\delta(q) + \sum_{n=1}^{N} A_n^2 \delta\left(q - \frac{2\pi}{T}n\right)^2$$

Scattering profile

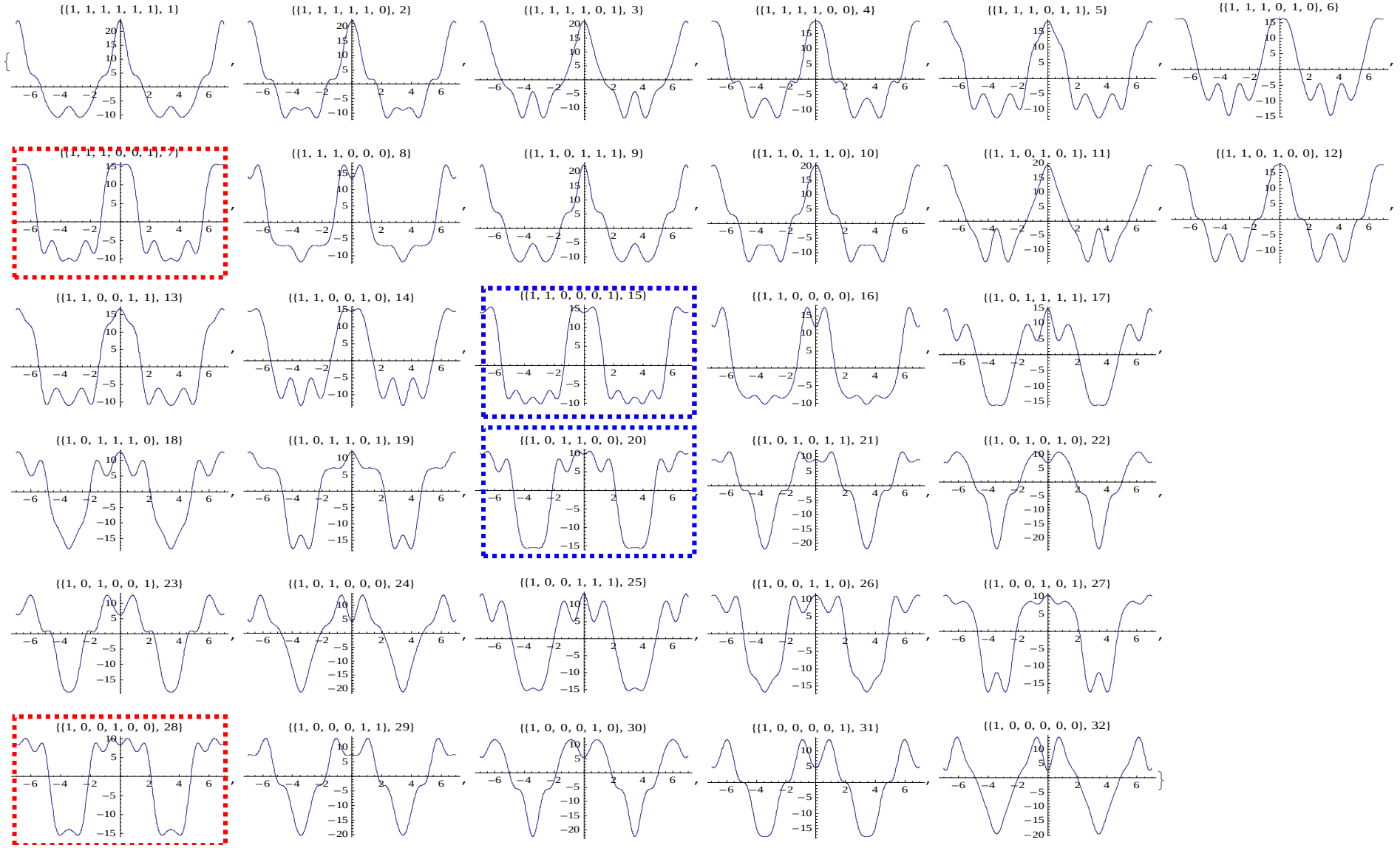
Ai^2: peak area

Svergun et al, Chem. Mater. 2000

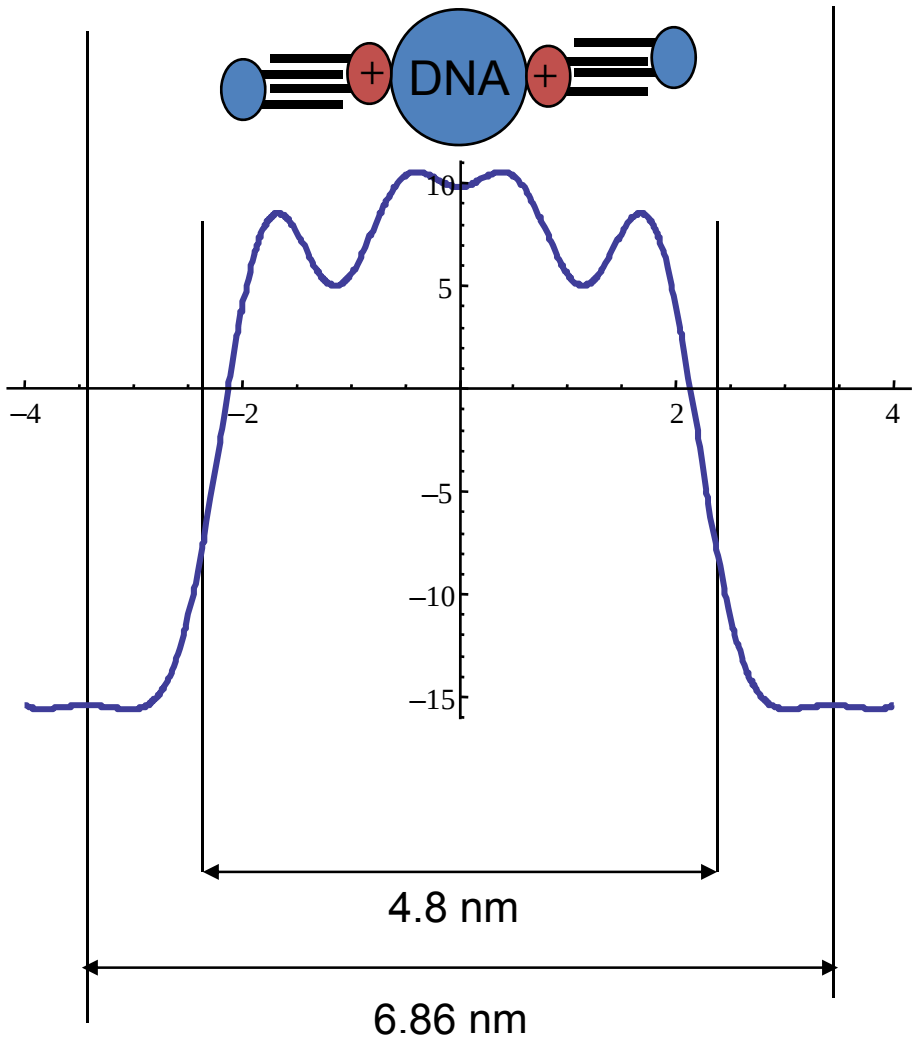
All possible combination

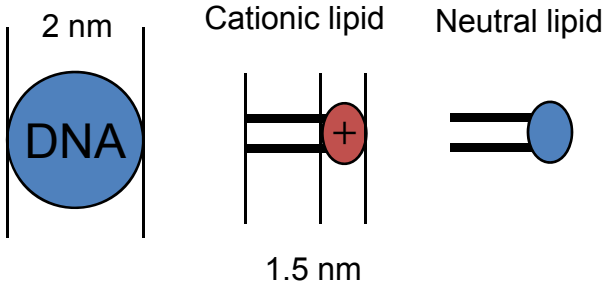
Combination $N=2^6=64$

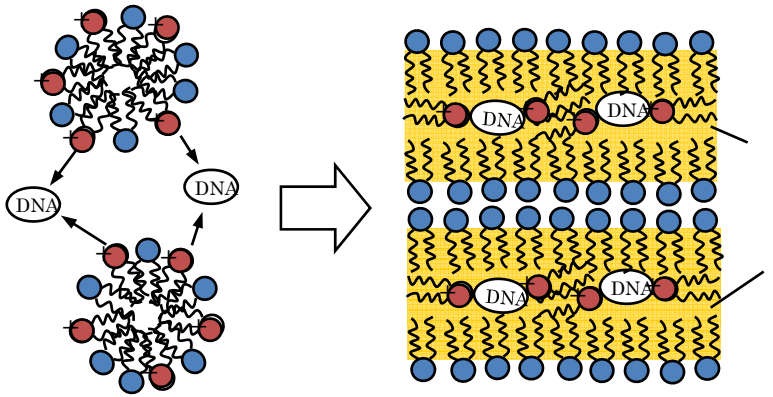
```
ClearAll[T, r, i, An];
T = 6.866;
An = {13.237, 4.594, 0.764, 2.829, 1.386, 1.0233};
comb = Table[IntegerDigits[2^6 - i, 2], {i, 1, 2^5}];
den[s_] := Sum[(-1)^(comb[[s, n]] + 1) An[[n]] Cos[2 π n/T × r], {n, 1, 6}];
Table[Plot[den[i], {r, -7, 7}, PlotLabel → {comb[[i]], i}], {i, 1, 32}]
```



The most possible structure



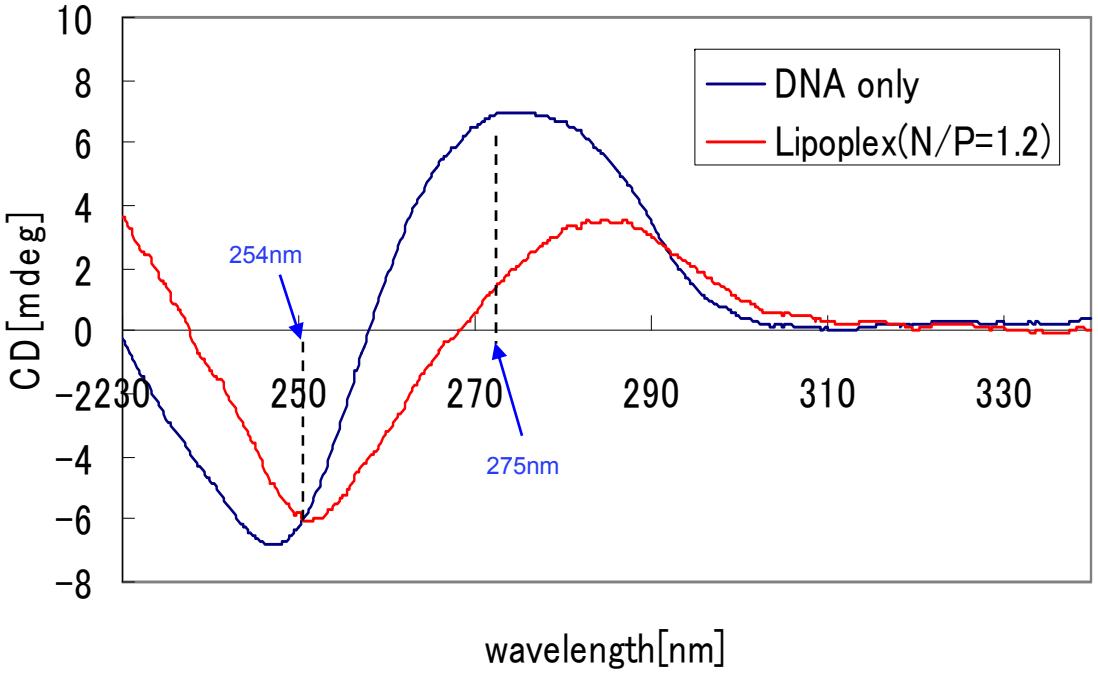




Anther evidence with CD

Circular dichroic (CD) spectra is related DNA conformation

DNA changes the conformation by changing surrounding environment.



CD spectra DNA solution and DOTAP/DOPC/pGL3 lipoplex
(Lipoplex = 0.3mM, N/P=12)

【DNA solution】

→ typical B-form DNA
water-rich

【Lipoplex】

→disappearing the positive 275 nm band and appearing the negative 254nm band

Structual transition from B- to C-form DNA

Böttcher et al.
J. Am. Chem. Soc., 1998
Vol. 120, No. 1 12-17

This result suggests that the atmosphere surrounding DNA was changed to water poor condition by complexation.

Bilayer Fusion

DNA/cationic lipid complex: lipoplex

Supramolecular drug

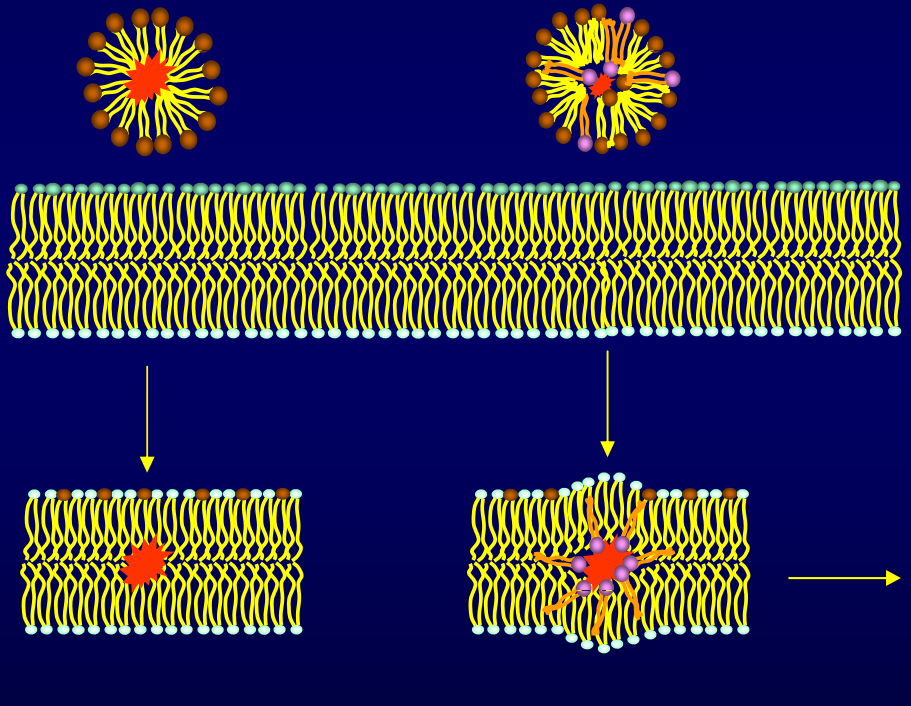
1. Cellular Up-Take: electrostatic
2. Endosomal Escape: →fusion ?
3. Nucleus Ingestion : diffusion

How the DNA is included in the micelle is essential for the transportation between cellular vesicles.

Micelle including a drug in the hydrophobic domain

Micelle including a drug/lipid complex in the hydrophobic domain

Or , an inverted micelle covered with normal layer

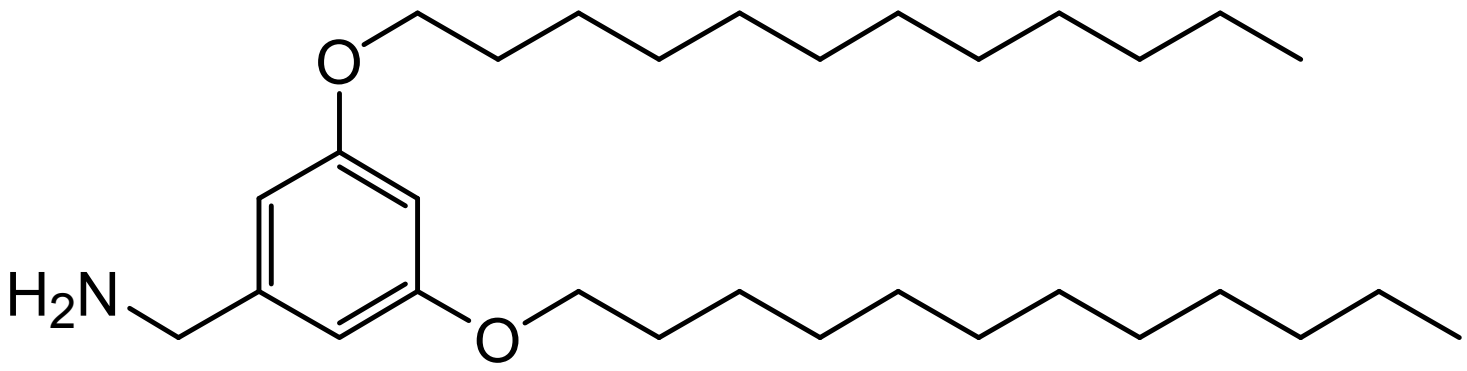


Vesicle bilayer

Escape from endosomal vesicle

Drug is just transported to the inside of bilayer.

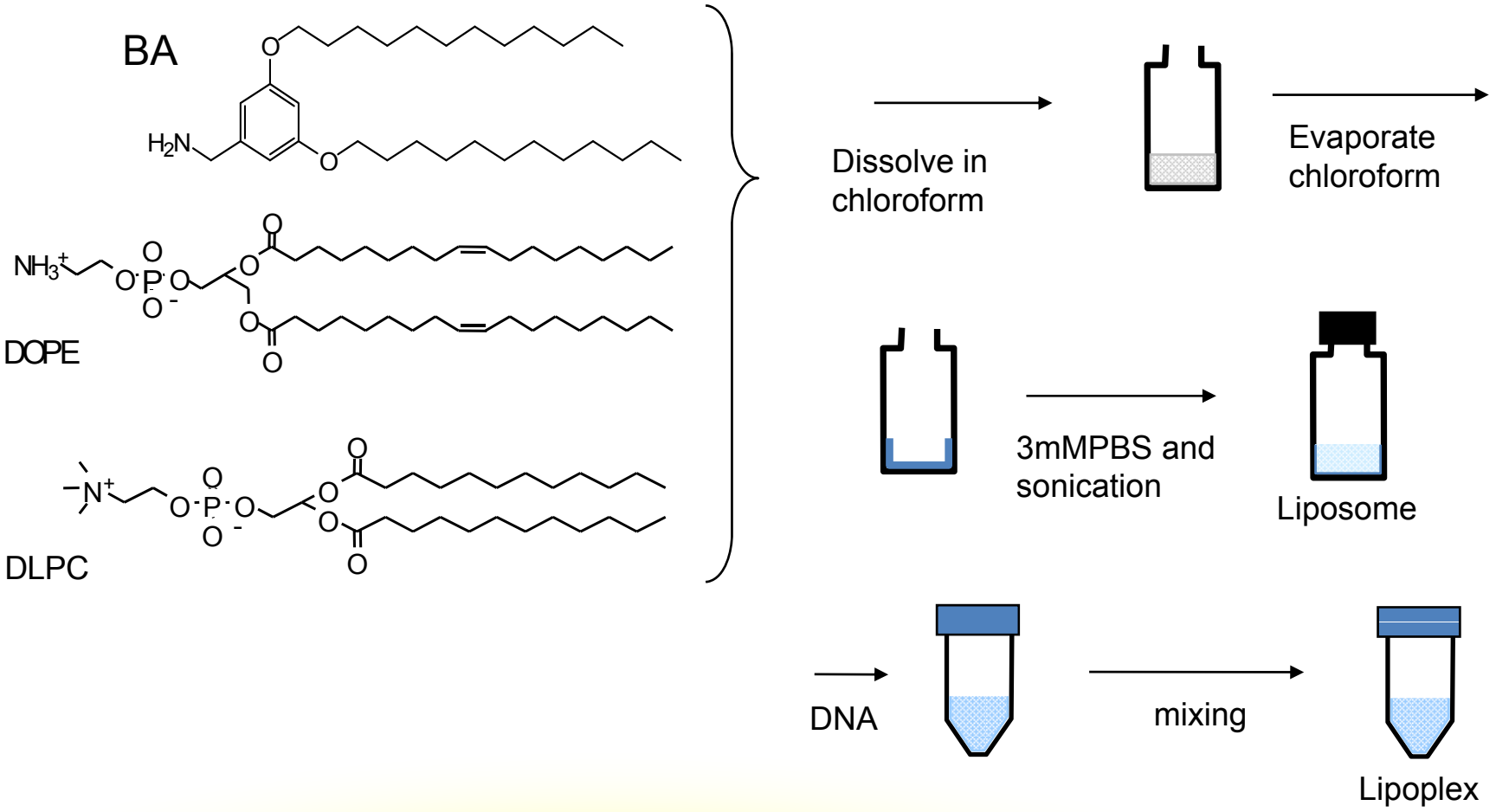
Our new cationic lipid

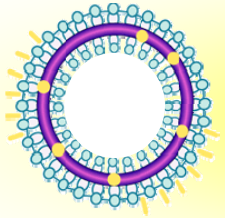


Benzyl amine (BA)

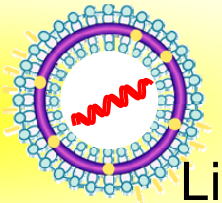
JPA2006-287855

Preparation of micelle



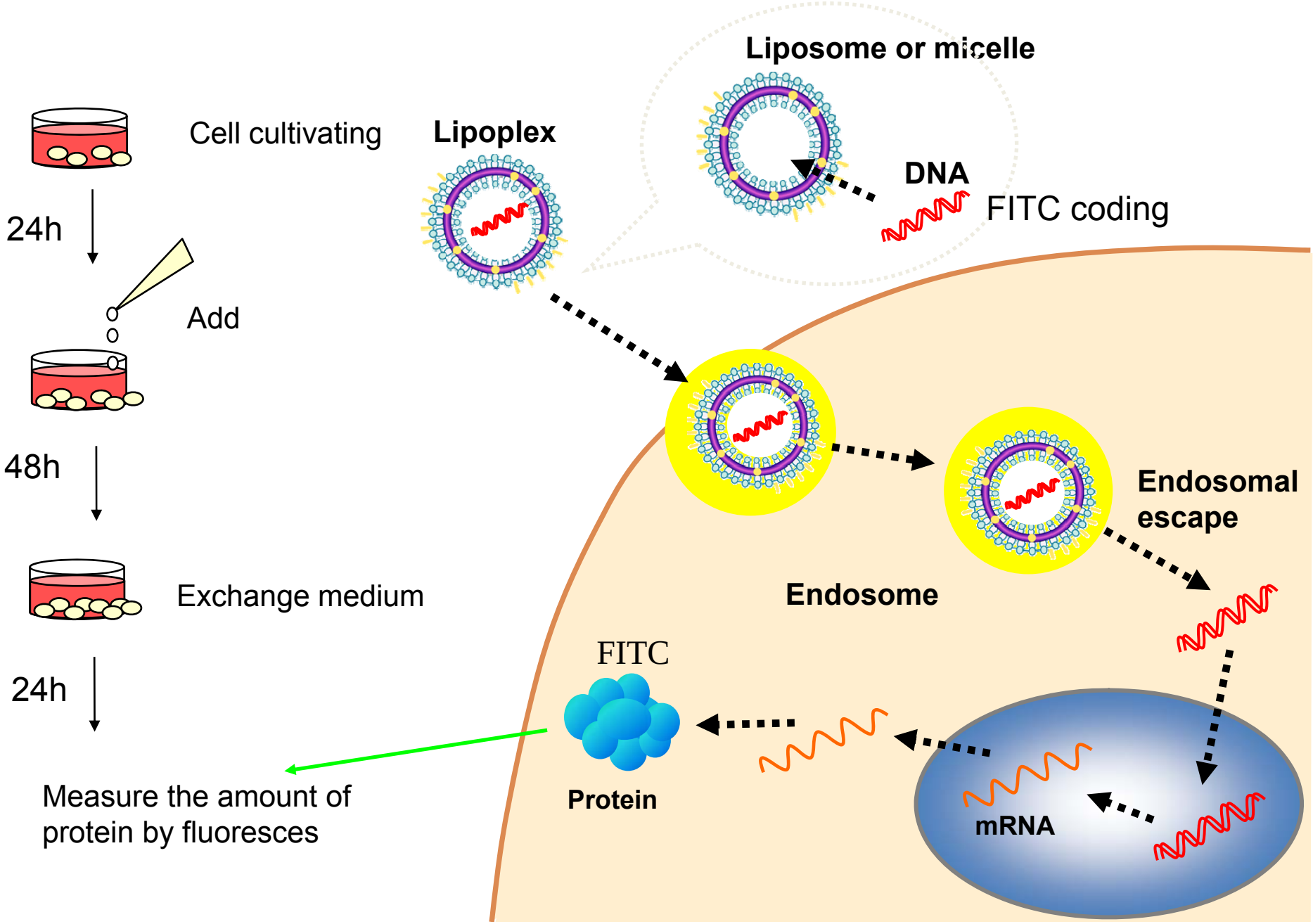


micelle

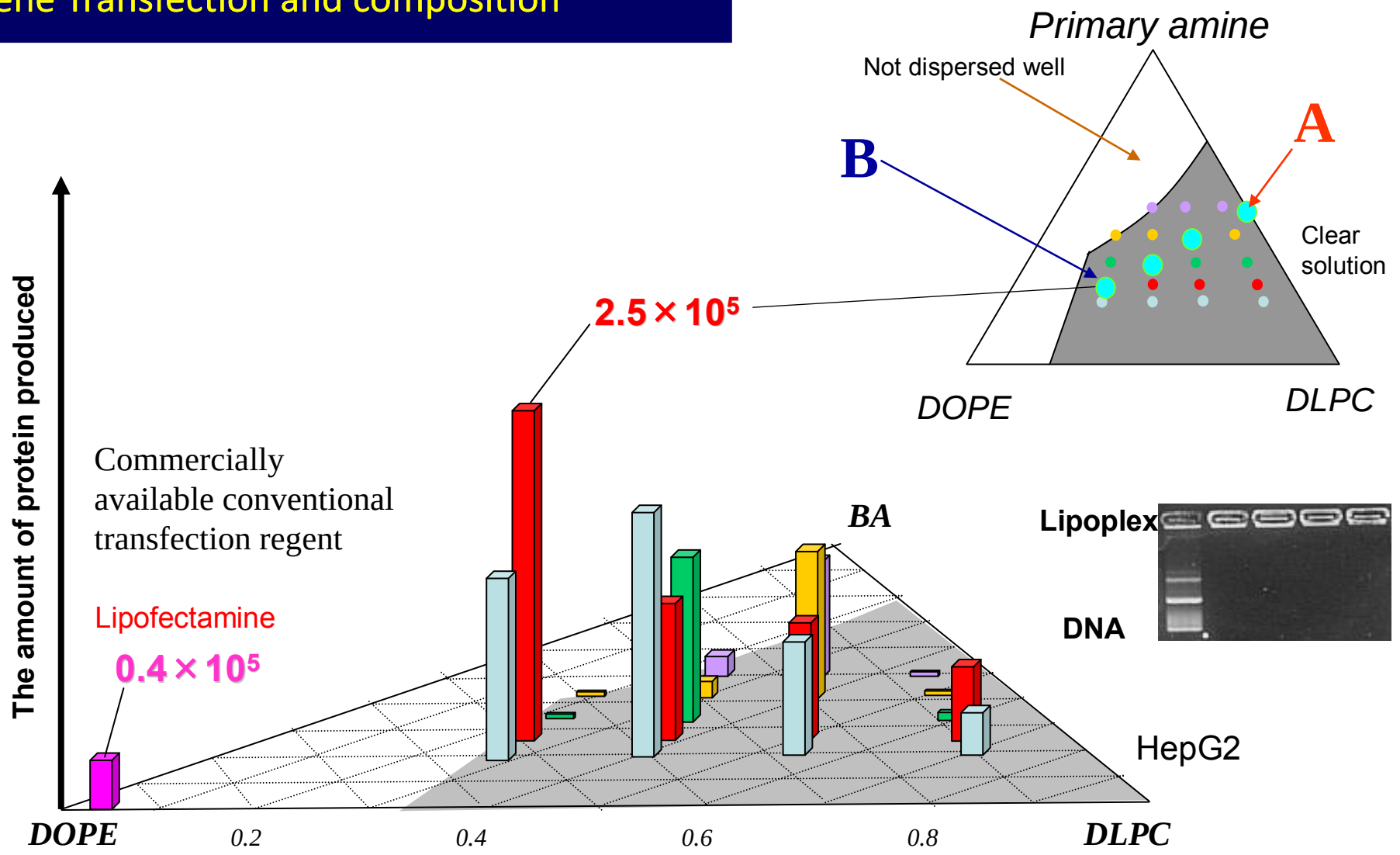


Lipoplex (after complexed with DNA)

How to evaluate DNA transfection

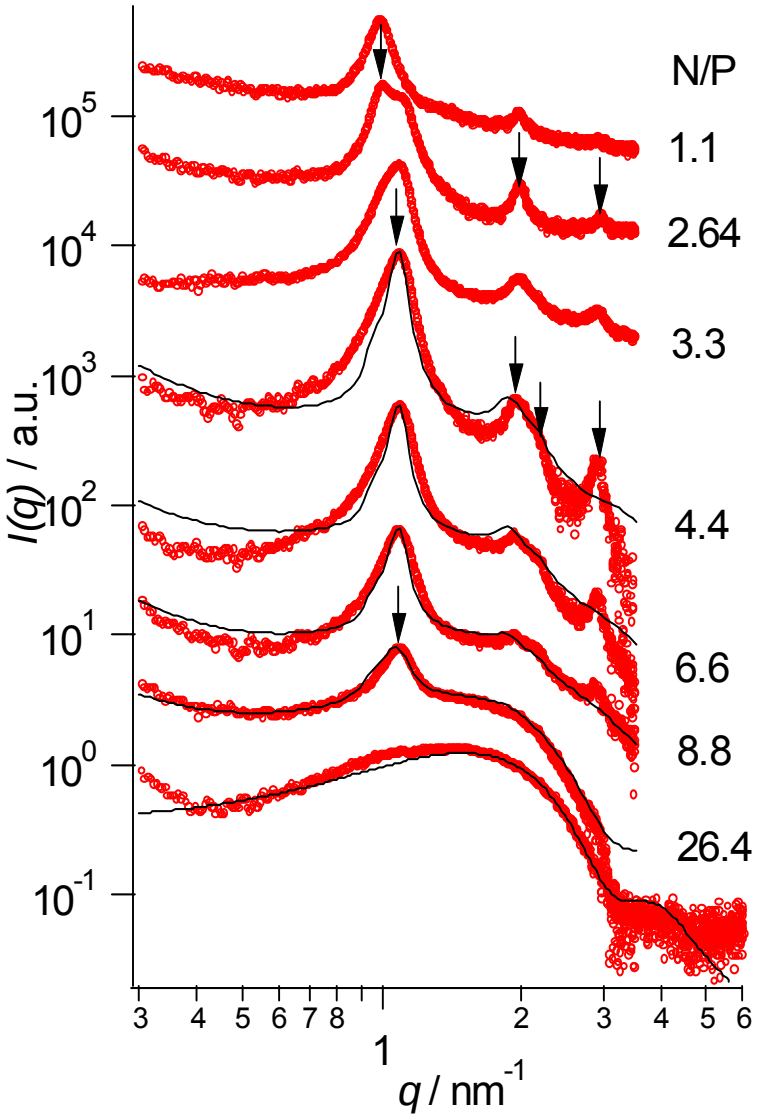


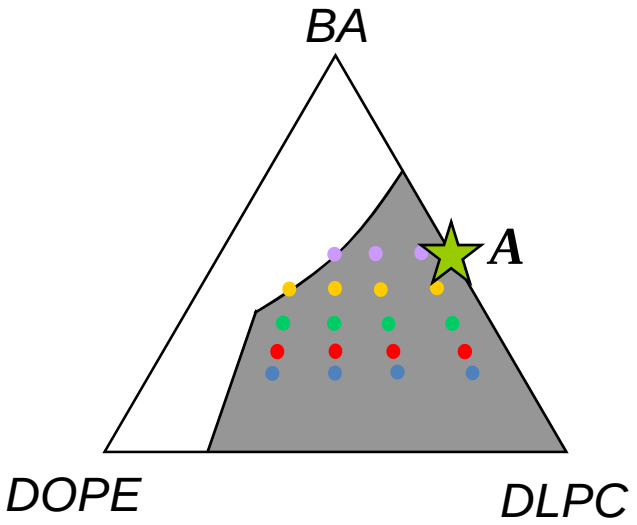
Gene Transfection and composition



The efficiency strongly depends on the composition

SAXS from A (poor transfection)





Sphere -> Cylinder -> Lamella

Fig.2 SAXS of A-lipoplex

Fitting the data

Lipid micelle: $I(q) = P_s(q)$

from factor of core-shell sphere

Mixture of lipid micelle and hexagonally packed cylinder $I(q) = P_C(q)S_h(q) + conP_s(q)$

structural factor for hexagonal packing with the second kind imperfection.

from factor of three layer cylinder

Fitting

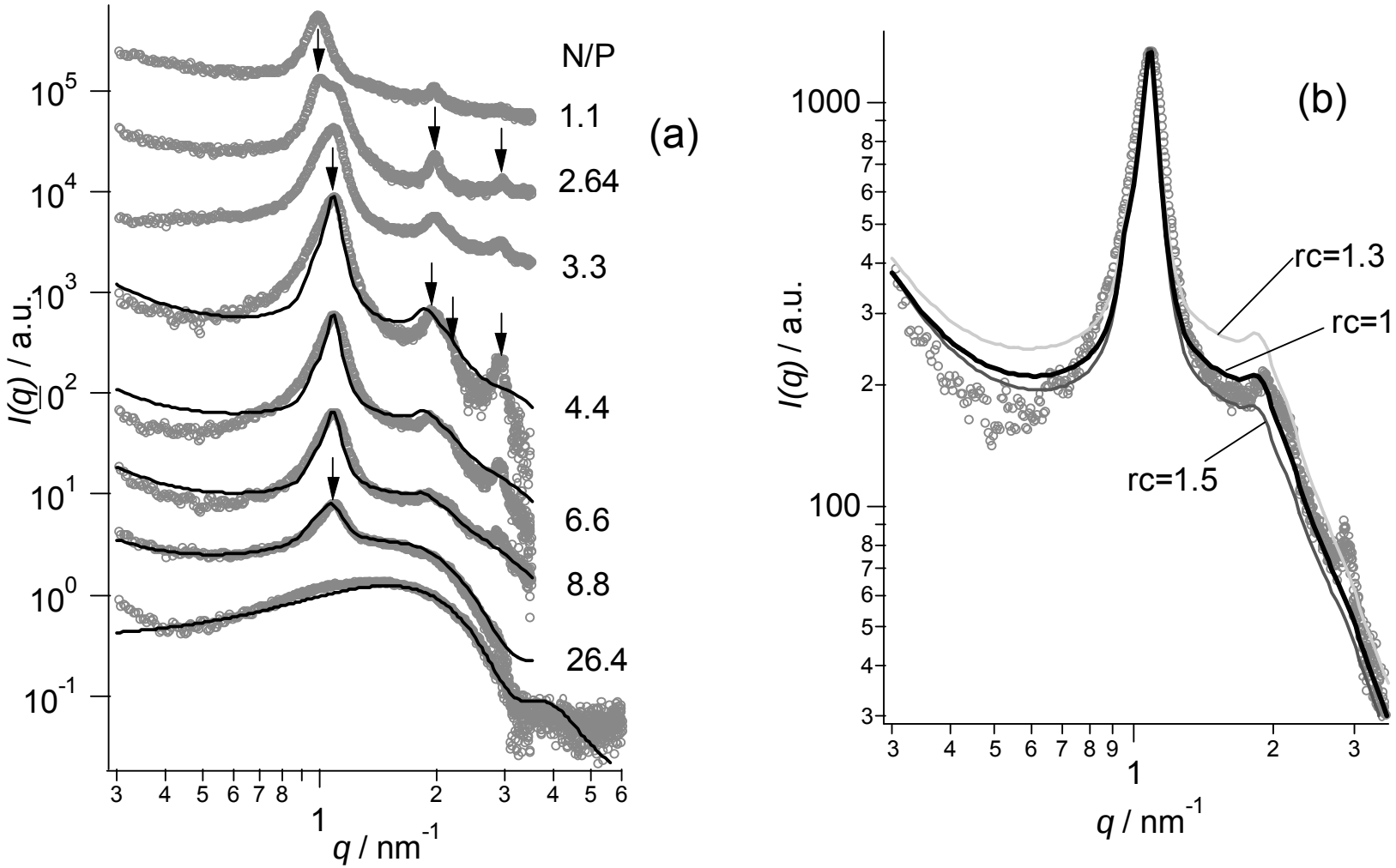
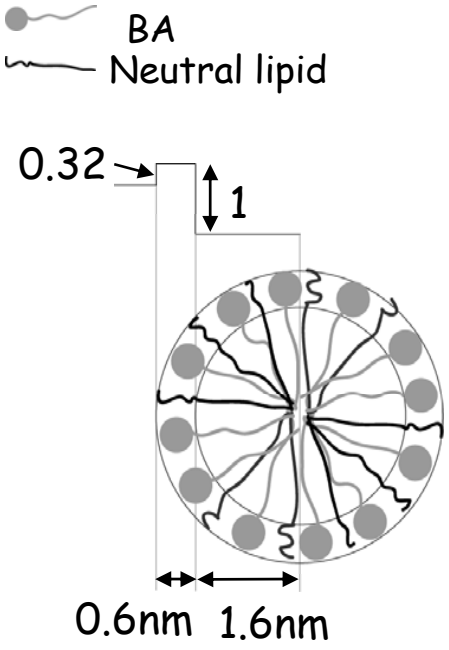
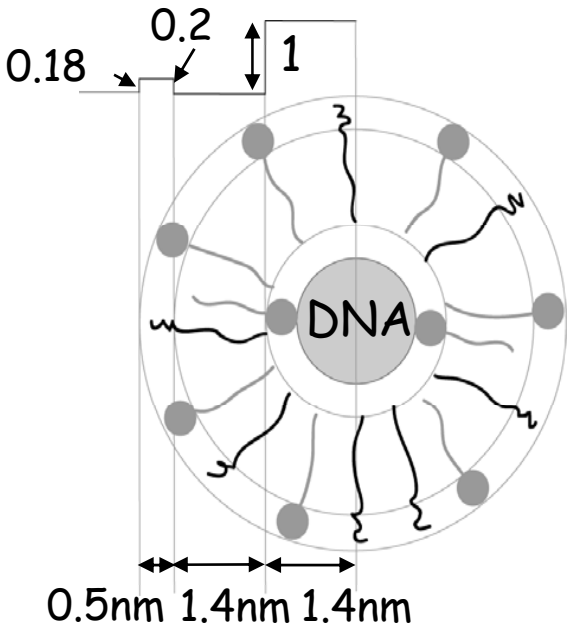


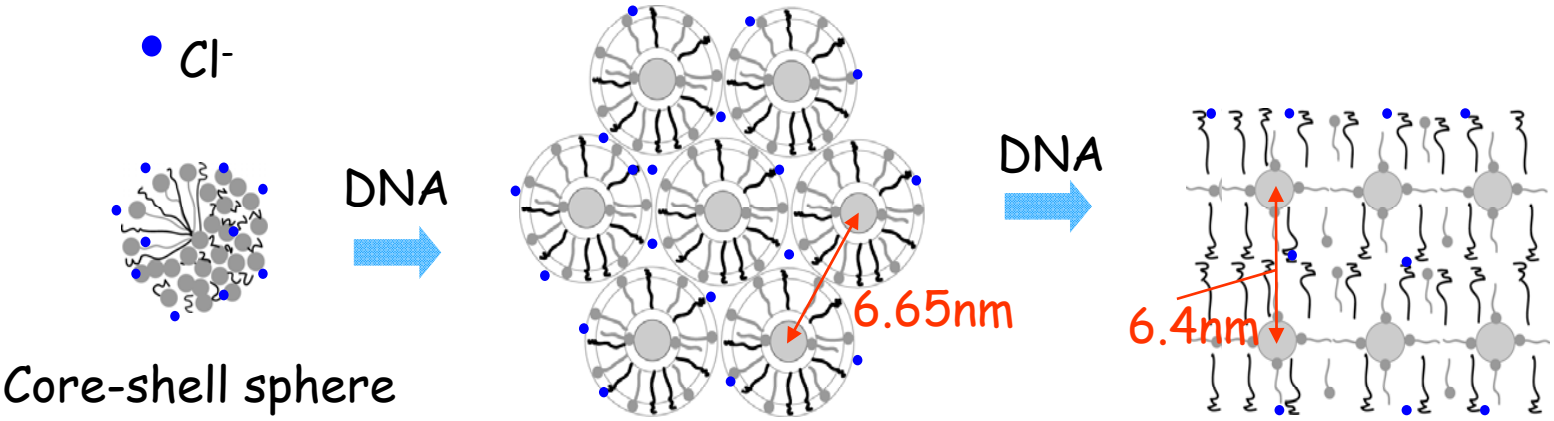
Figure 3. SAXS profile of A-lipoplex

Model for formation of the complex





The complex SAXS can be fitted by a three layer cylinder that contains a high electron density domain at the core. The core may be DNA.



Core-shell sphere

Double layer inverted cylinder

Lamella including DNA in the hydrophobic domain

SAXS at the best transfection: point B

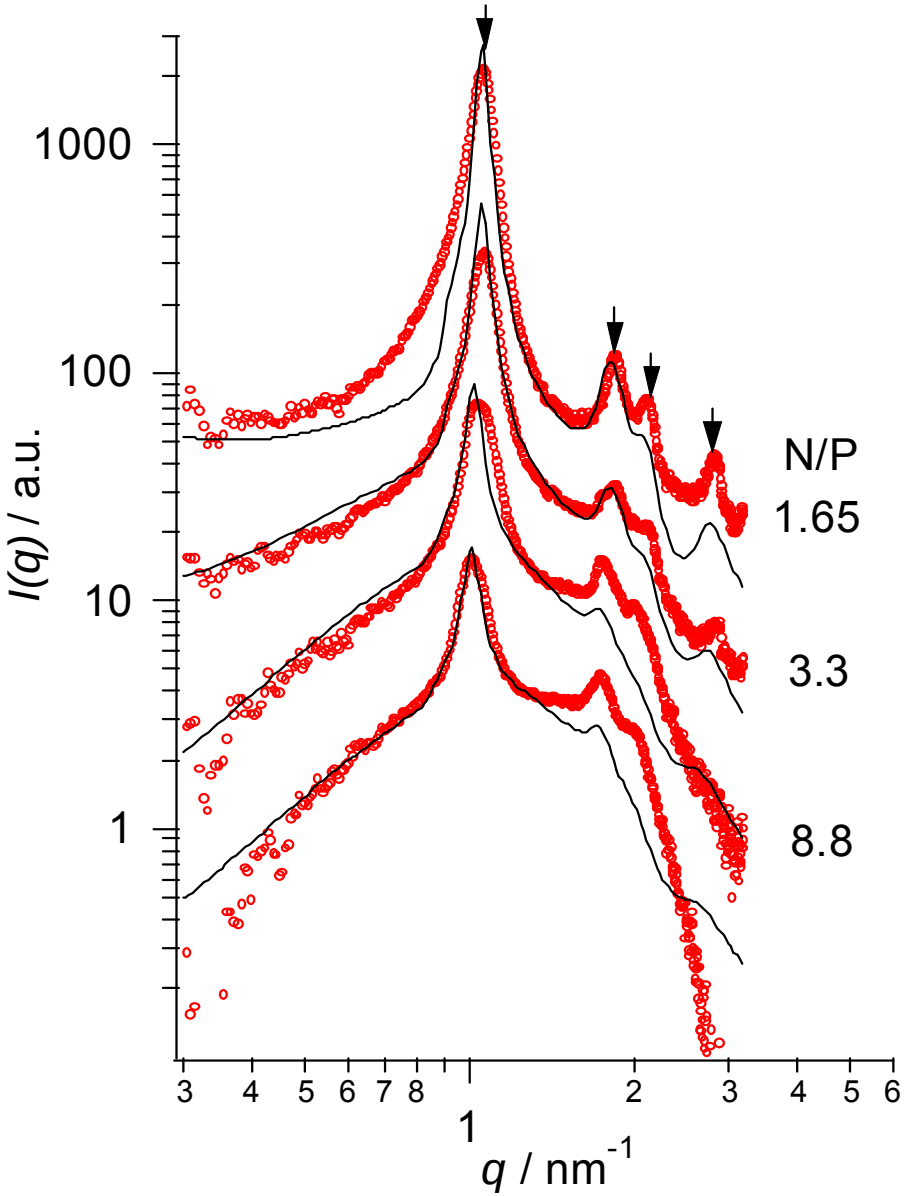
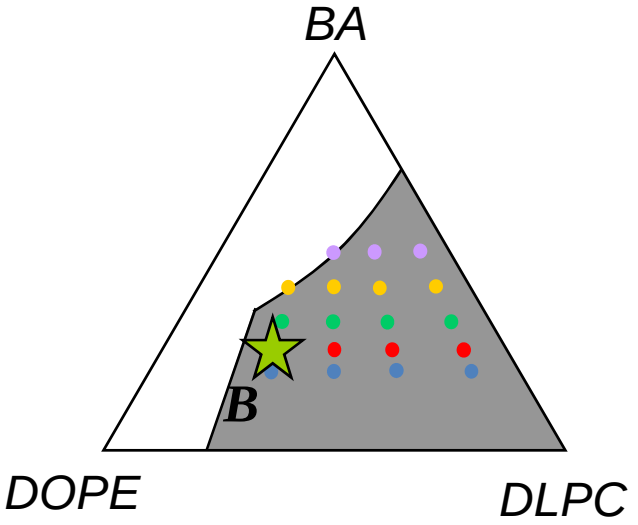


Fig.4 SAXS at B-lipoplex



The lipid already form a cylinder before adding DNA. Upon addition of DNA, the cylinder peak becomes more sharp and the higher order peaks appear. The peak position essentially is same. This indicates that the cylinder structure does not change. The order of the structure is enhanced by addition of DNA

Model for the formation of complex