# Diffraction <-> Structure

- First Born Approximation: (from quantum mechanics)
  Scattering is weak, so scattered amplitude is Fourier Transform (F.T.) of structure (spatial distribution of neutron scattering length density).
- In 1-dimension across membrane:

  $F(Q) = \text{F.T. } \{\rho(x)\}$ (scattering process)

  $\rho(x) = \text{F.T. } \{F(Q)\}$ (scientist's job)

  $Q = 4\pi \text{Sin}(\theta)/\lambda$ or $Q = 2\pi n/d$

  Bragg Equation: $n\lambda = 2d\text{Sin}(\theta)$

  d = repeat distance (across membrane).

  Q = dimension in reciprocal space.

# Fourier Transform for calculating diffraction from Molecular Model

(Centrosymmetric case of bilayer. Need Sine part as well if not centrosymmetric.)

$$F_n = \Sigma_j\, b_j \mathrm{Cos}(2\pi n x_j/d)\, \exp(-n^2 B_j/4d^2)$$

- $b_j$ = neutron coherent scattering length of nucleus j

  b(hydrogen) = $-3.74 \times 10^{-13}$ cm

  b(deuterium) = $6.67 \times 10^{-13}$ cm

  b(carbon) = $6.65 \times 10^{-13}$ cm

  b(oxygen) = $5.80 \times 10^{-13}$ cm

  b(nitrogen) = $9.40 \times 10^{-13}$ cm

  (compare X-ray scattering by electron: s.l. = $2.8 \times 10^{-13}$ cm)

- $B_j$ = Debye-Waller Temperature Factor: nucleus not fixed at $x_j$ but has Gaussian distribution at $x_j$.
- $B_j$ includes dynamic and static disorder.

# <u>Measured Intensities</u>: $I_n \sim |F_n|^2$

Phase relations of $F_n$ not included in measurement of intensity.

Must be recovered: **“the phase problem”**.

Geometric corrections:

$$|F_n|^2 = I_n \, \mathrm{Sin}(2\theta_n) \, C_{abs}(n)$$

$\mathrm{Sin}(2\theta_\nu)$ = Crystallographic Lorentz factor

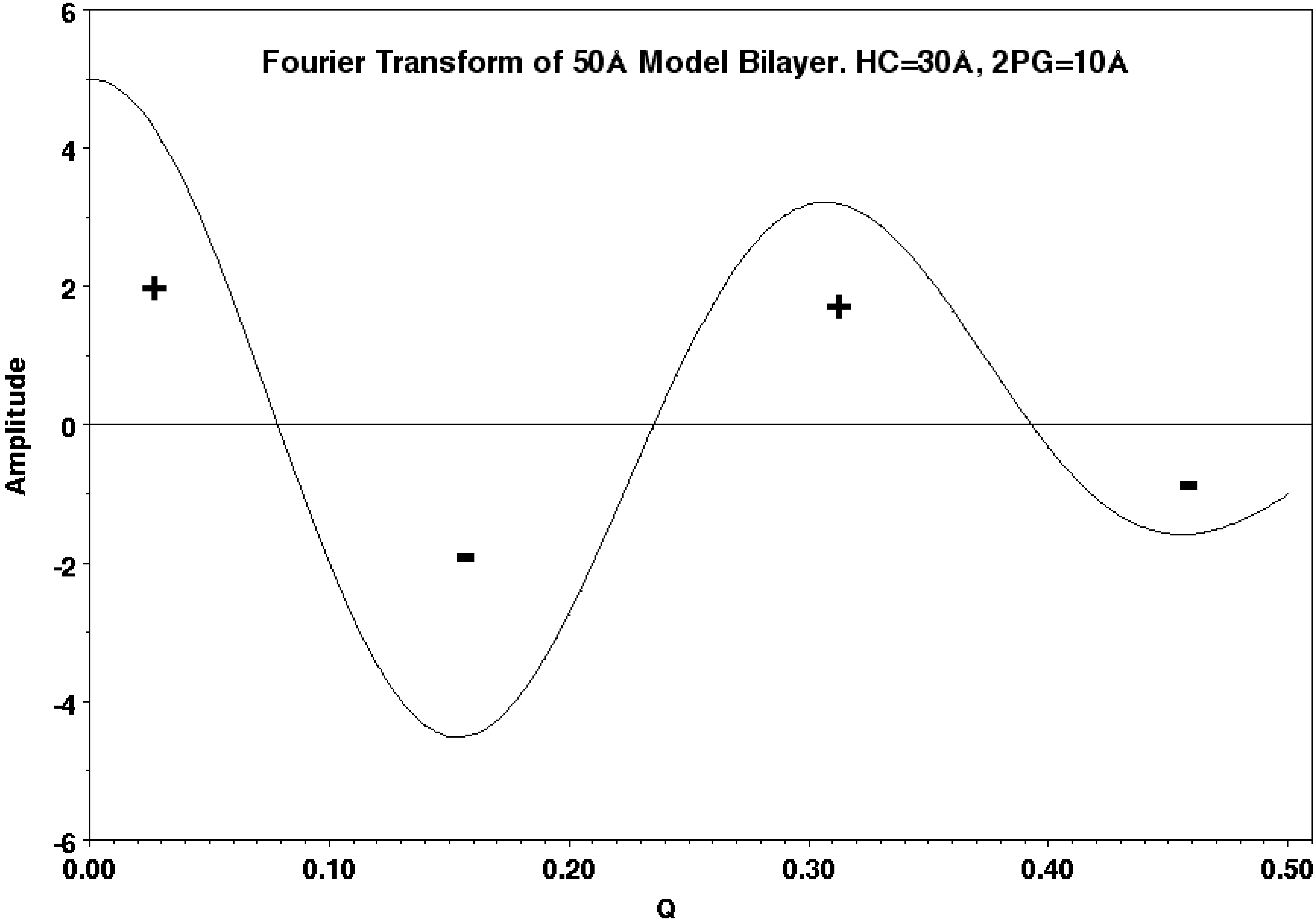
(for Bragg peaks integrated in $\theta$).

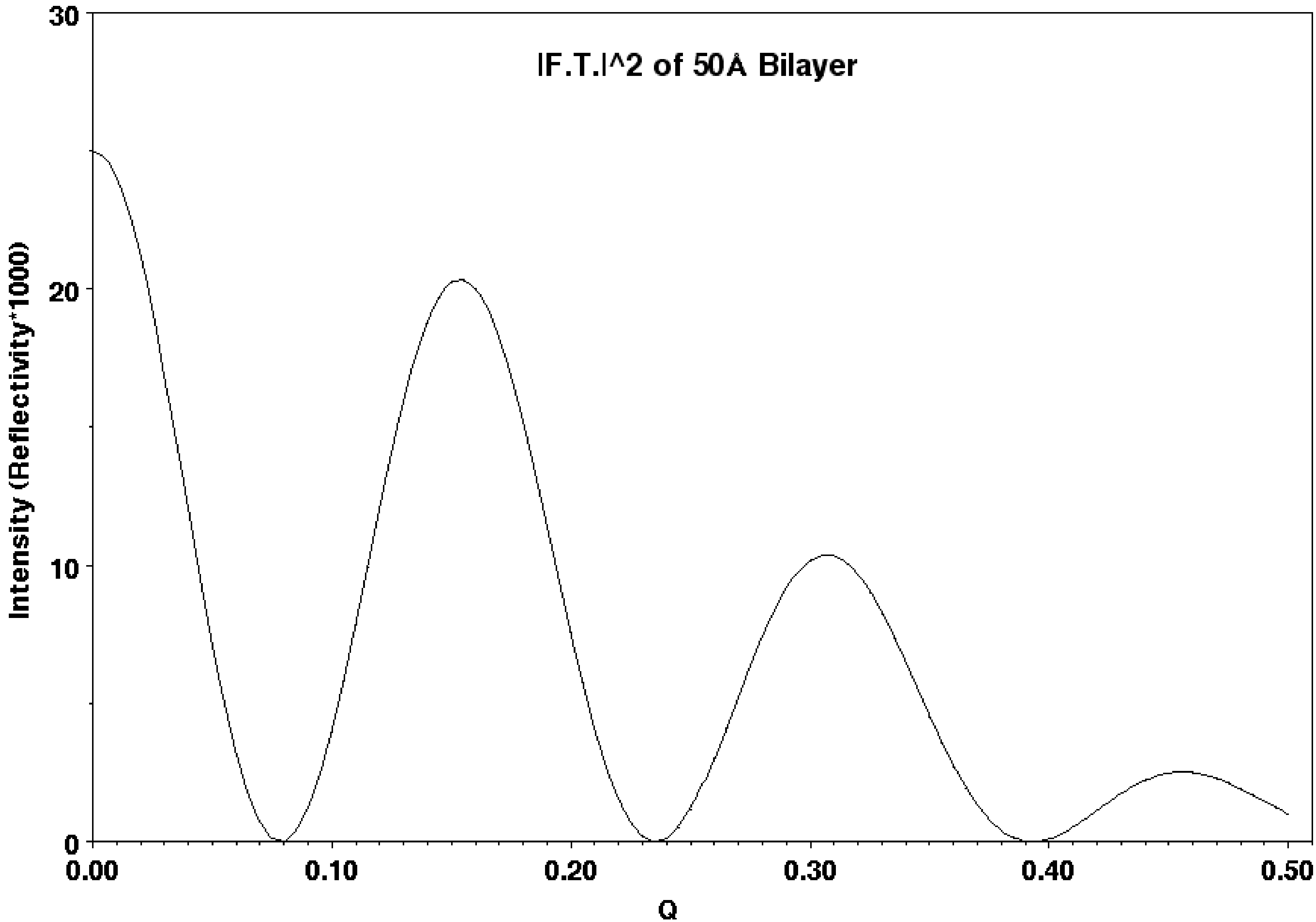
Lorentz factor is $\mathrm{Sin}(\theta_n)$ if integrated in Q.

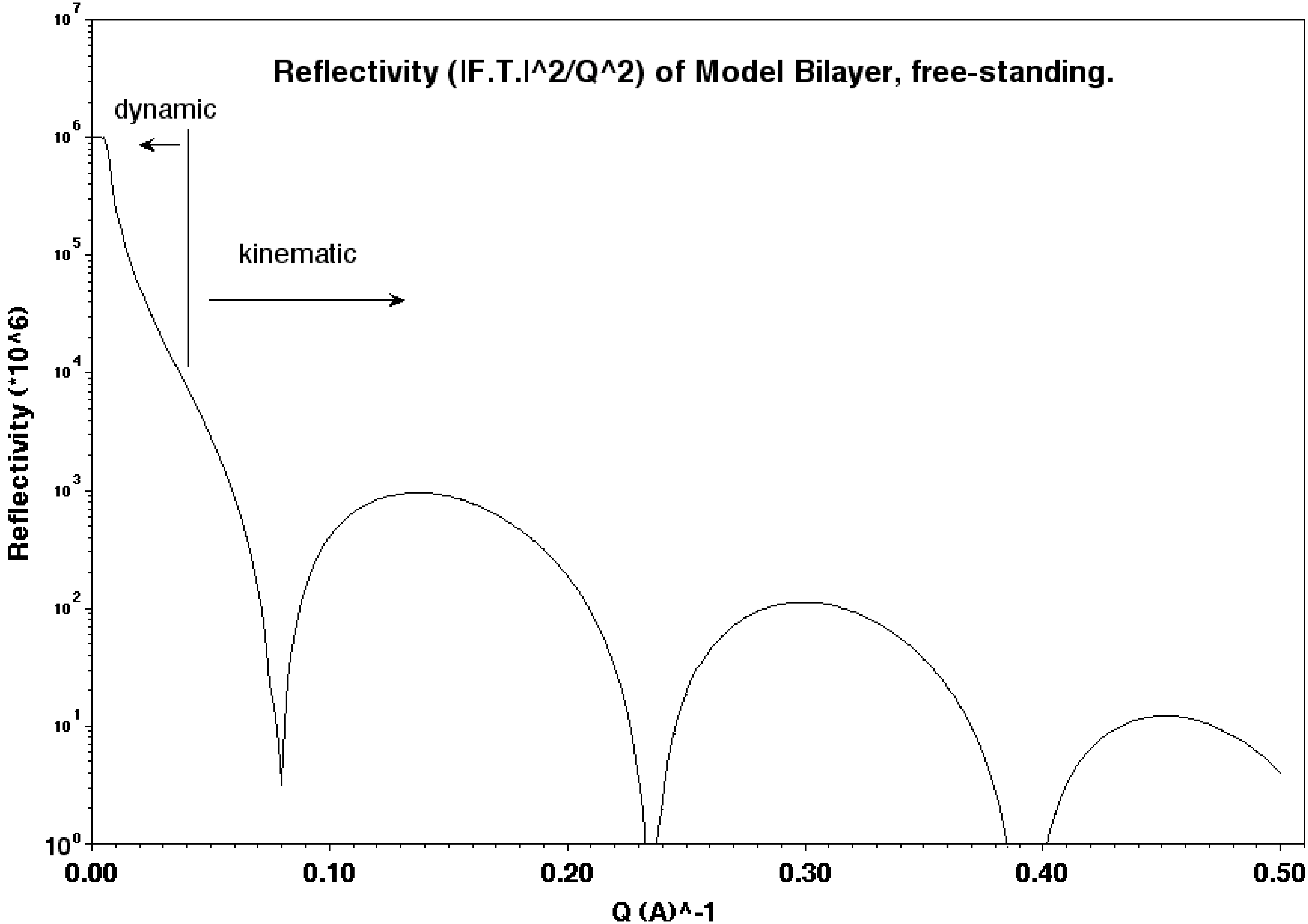
$I_n$ are corrected for absorption. A small correction for thin samples.

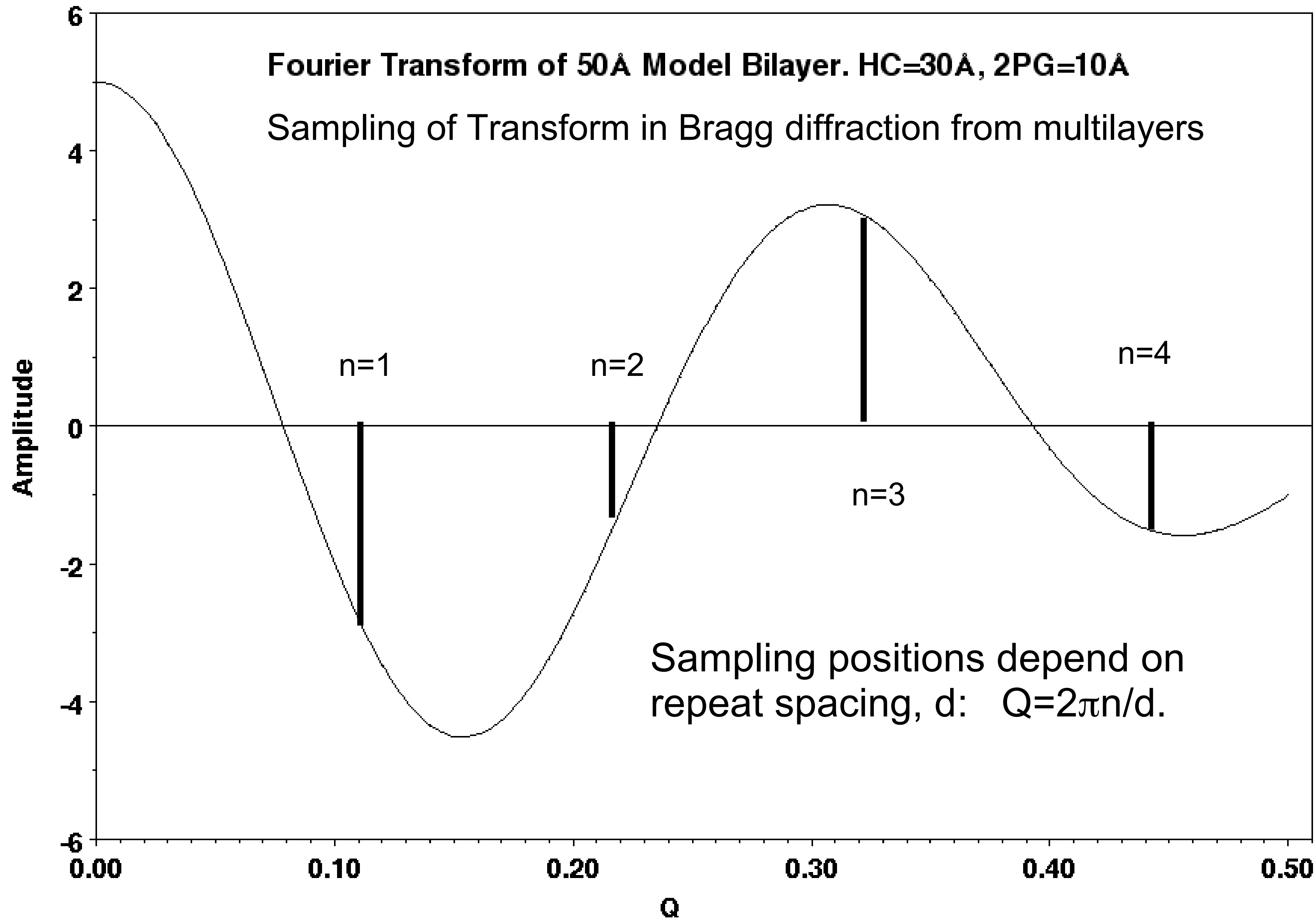
$\mathbf{C_{abs}(n) = z/(1-\exp(-z))}$ **where** $\mathbf{z = 2t/to\sin\theta}$

**to = 1/e absorption length**


Fourier Transform of 50Å Model Bilayer. HC=30Å, 2PG=10Å
Amplitude
Q
6
4
2
0
-2
-4
-6
0.00
0.10
0.20
0.30
0.40
0.50
+
-
+
-

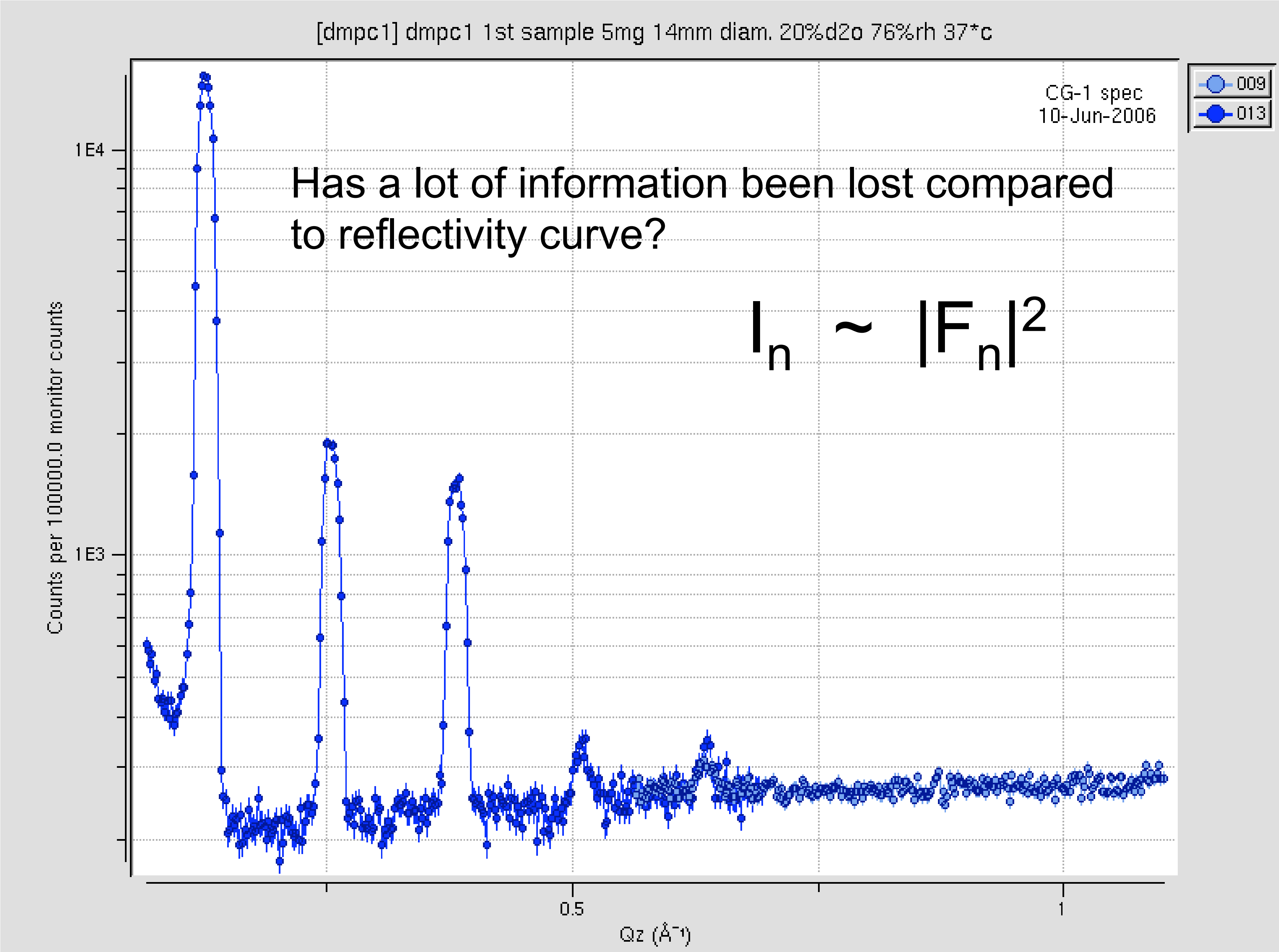

|F.T.|^2 of 50Å Bilayer
30
20
10
0
Intensity (Reflectivity*1000)
0.00
0.10
0.20
0.30
0.40
0.50
Q


Reflectivity (|F.T.|^2/Q^2) of Model Bilayer, free-standing.
dynamic
kinematic
Reflectivity (*10^6)
$10^7$
$10^6$
$10^5$
$10^4$
$10^3$
$10^2$
$10^1$
$10^0$
0.00
0.10
0.20
0.30
0.40
0.50
Q (A)^-1


Fourier Transform of 50A Model Bilayer. HC=30A, 2PG=10A
Sampling of Transform in Bragg diffraction from multilayers
n=1
n=2
n=3
n=4
Sampling positions depend on
repeat spacing, d: Q=2πn/d.
Amplitude
6
4
2
0
-2
-4
-6
0.00
0.10
0.20
0.30
0.40
0.50
Q

Has a lot of information been lost compared to reflectivity curve?

$$I_n \sim |F_n|^2$$



# The Shannon Sampling Theorem

Claude Shannon (1949) Proc. Inst. Radio Engrs. NY, **37**, 10
Shannon & Weaver (1949) The Mathematical Theory of Communication. Univ. of Illinois Press.

If a function $\rho(x)$ is non-zero only for $-a/2 \leq x \leq a/2$, then its Fourier Transform F(X) is completely specified by its values at X = 0, ±1/a, ±2/a,… etc.

The continuous F(X) can be obtained as a sum of $\sin(\pi aX)/\pi aX$, placed at each of the above points and weighted by the value of F(X) at each point.

Note that amplitude and phase of F(X) are needed.

Corollary: Data to $Q_{max}$ are completely specified by a small number of values, namely $aQ_{max}/\pi$.

# The Shannon Sampling Theorem can also apply to SANS.

Specifically, SANS data from solutions of objects of finite dimension. In this case, F.T. {I(Q)} yields the pair distribution function p(r). But since this is a truncated FT, better to generate p(r) as a series summation until its FT fits I(Q). The number of terms in the series is given by Shannon Theory.

See P.B. Moore, "Small-Angle Scattering. Information Content and Error Analysis", J. Appl. Cryst. (1980) **13**, 168-175.

Peter Moore's program for generating p(r) from I(Q) is extensively used in SANS, especially in biology.

**Calculate density of neutron scattering lengths:**

$$\rho(x) = \Sigma_n |F_n| \, \mathrm{Cos}(2\pi nx/d - \phi_n)$$

Bilayer membrane is centrosymmetric,
so use cosine F.T. and $\phi_n = 0, \pi$ .

Or, $$\rho(x) = \Sigma_n F_n \, \mathrm{Cos}(2\pi nx/d)$$

$$F_n = +|F_n| \;,\; \phi_n = 0$$
$$F_n = -|F_n| \;,\; \phi_n = \pi$$

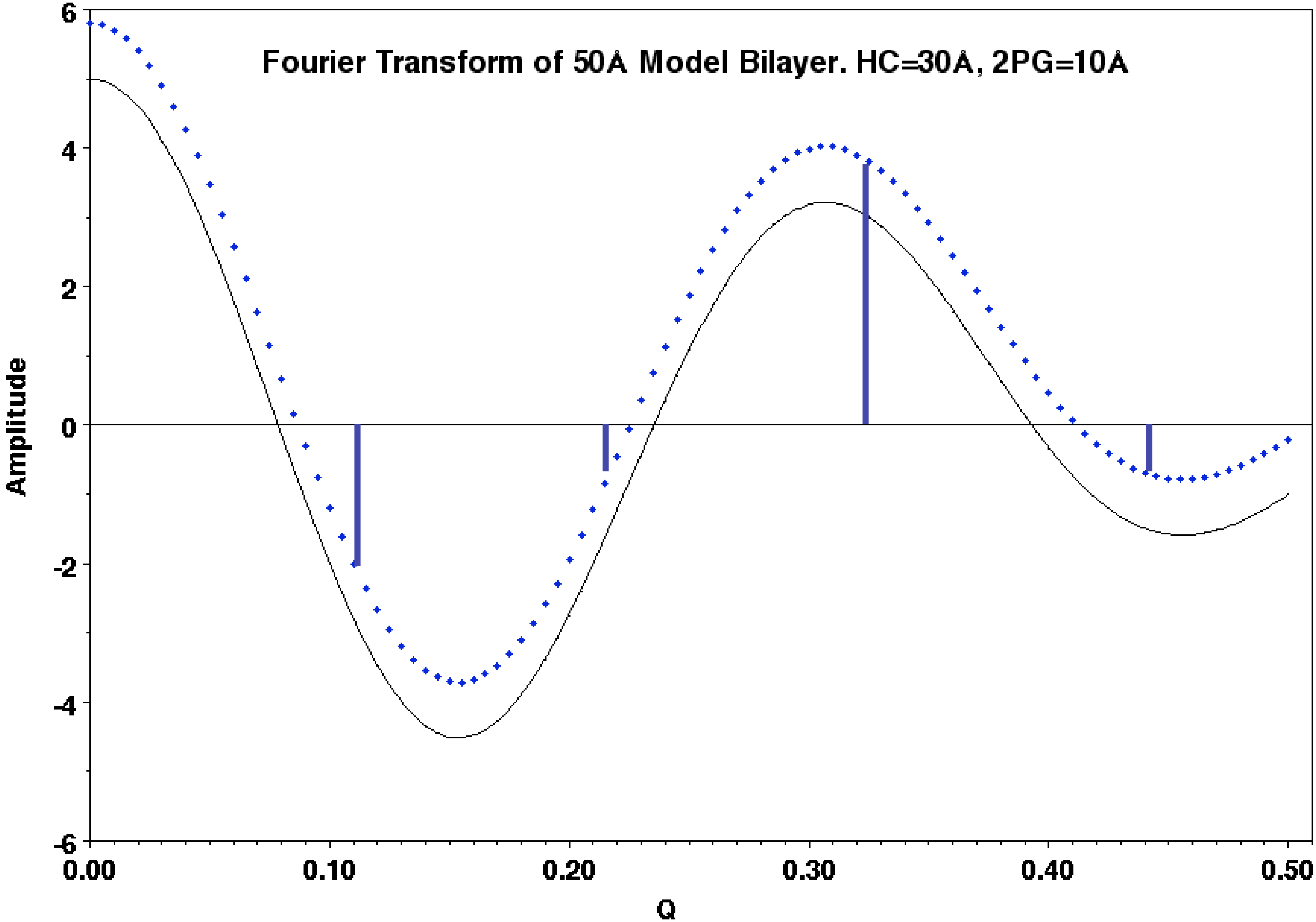
# Solving the Phase Problem

- Introduce heavy atom in the form of deuterium ($D_2O$) at $x = 0$, by diffusion from vapor.
- At low hydration (e.g. ~60%rh), $D_2O$ should be narrow Gaussian at $x = 0$. F.T.{$D_2O$} = <u>all positive values</u>.
  - Narrowest case: Dirac delta function. F.T.{$\delta(0)$} = positive constant.
- Fourier Transforms are linear operations:

$$\text{F.T.}\{\text{lipid}+D_2O\} = \text{F.T.}\{\text{lipid}\} + \text{F.T.}\{D_2O\}$$

- So, $D_2O$ at $x = 0$ increases $|F_n|$ for positive $F_n$ and decreases $|F_n|$ for negative $F_n$. Can simply observe if $I_n$ increase or decrease : gives phases.
- $F_n$ can change from negative to positive, but 3 measurements will show this ($H_2O$, 50%$D_2O$, $D_2O$) since $F_n$ is linear in %$D_2O$.

- **This is the membrane version of the multiple isomorphous heavy atom method of protein X-ray crystallography.**



Fourier Transform of 50Å Model Bilayer. HC=30Å, 2PG=10Å
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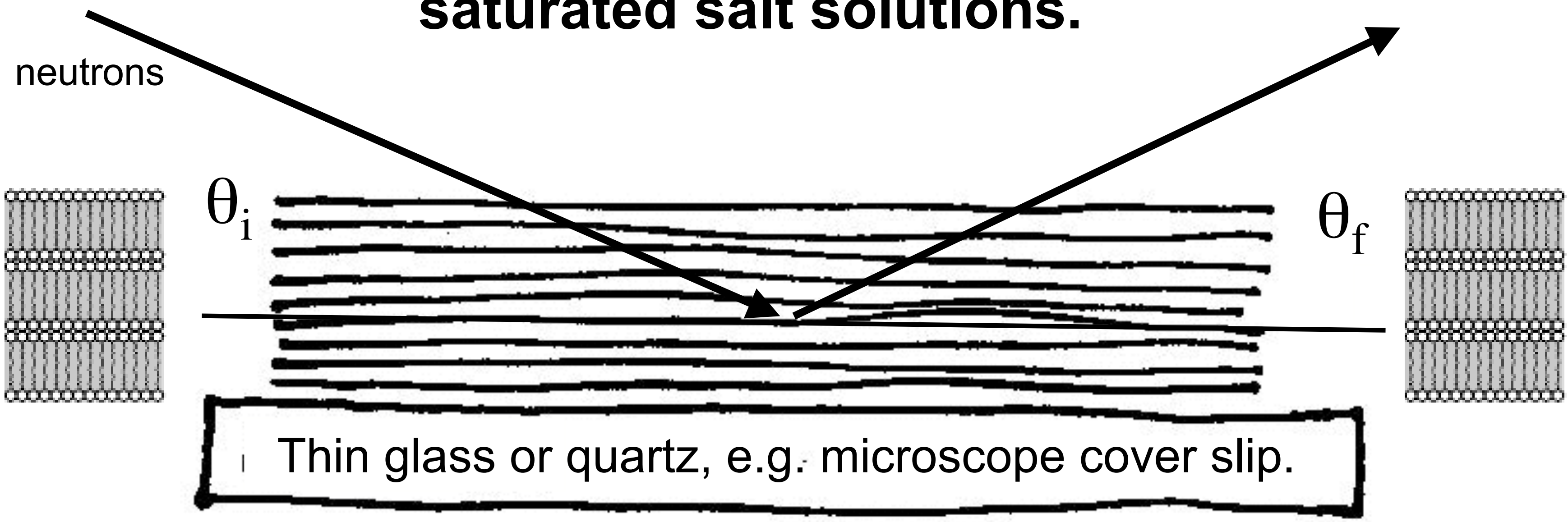
0.50

**Samples are multilayers on thin quartz or glass substrate,**
**usually 3-12mg of lipid covering ~6cm$^2$, so ~5-20μ thick.**
**This corresponds to ~1000 to 4000 bilayers.**

**Samples are hydrated from the vapor phase using saturated salt solutions.**



**Neutron Scattering Length Density Profile.**
**DMPC + Cholesterol (2/1). 86%rh.**