Experimental methods to obtain the 3D structure of macromolecules

1. X-ray Crystallography
2. NMR

X-ray Crystallography

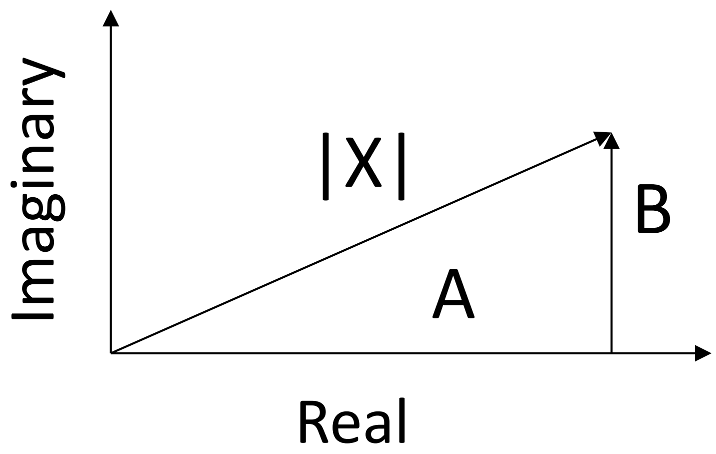
1. Basic concepts of Physics and Mathematics
    1. Complex Numbers
    2. Waves
    3. Fourier Transform
    4. Electromagnetic wave
2. Diffraction
    1. Bragg's law
3. Crystal
    1. Laue conditions
    2. Structure Factor
4. Phase problem
    1. Solutions

1. Basic concepts of Physics and Mathematics

1. Complex Numbers

Solving the equation $x^2=-1$, solution $x=i$

Generalization: X in C is X=A+iB, with A and B in R



$$X = A + iB = (A,B)$$

$$B = |X| Sen(\theta)$$

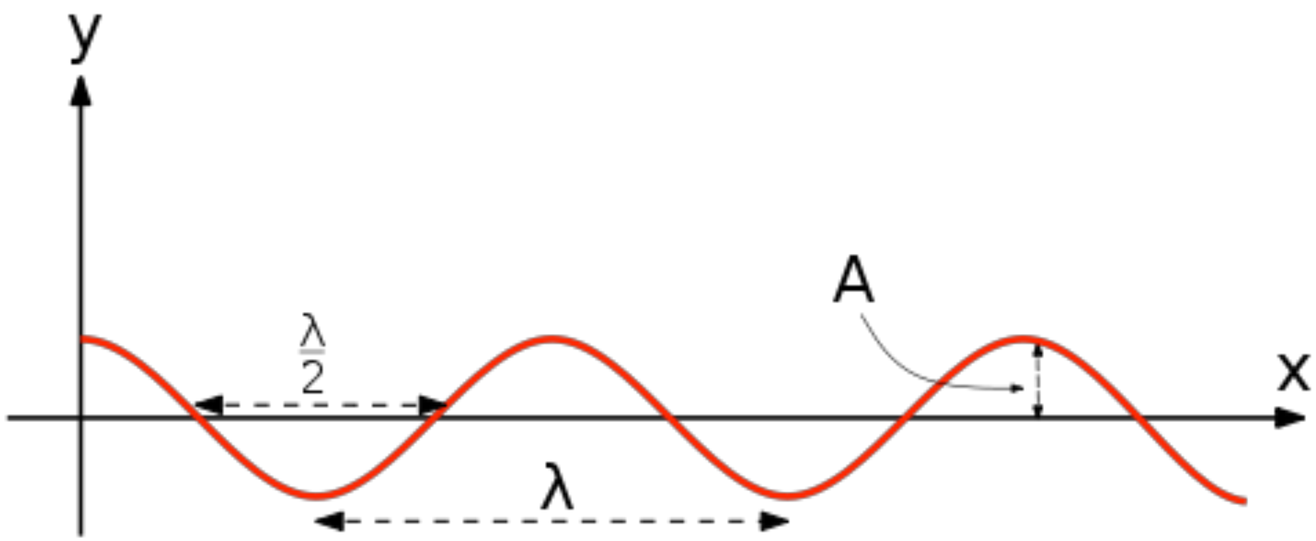
$$A = |X| Cos(\theta)$$

$$X = |X|\left(Cos(\theta) + iSen(\theta)\right)$$

$$X = |X| e^{i\theta}$$

1. Basic concepts of Physics and Mathematics

2. Waves (wavelength)

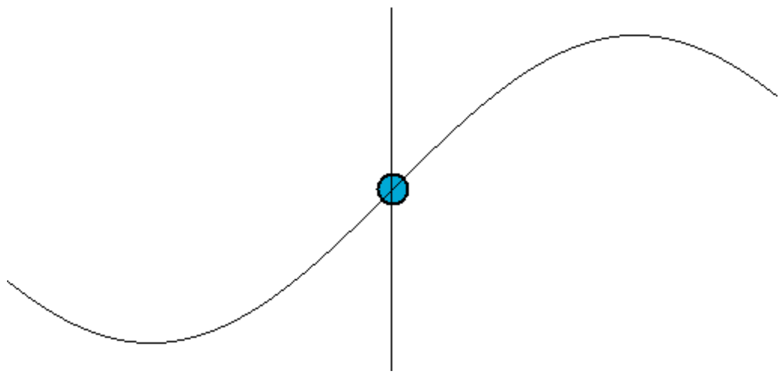


$$k = 2\pi / \lambda$$

$$y = f(x) = ASen(\kappa x)$$

$$y = f(x) = ACos(\kappa x + \delta)$$

1. Basic concepts of Physics and Mathematics
2. Waves (period)



$$\omega = 2\pi / T$$

$$y = f(t) = ASen(\omega t)$$

$$y = f(t) = ACos(\omega t + \rho)$$

1. Basic concepts of Physics and Mathematics

2. Waves (general equation)

Using complex numbers

$$y = f(x,t) = A\left(Cos(\kappa x + \omega t) + iSen(\kappa x + \omega t)\right)$$

$$y = f(x,t) = Ae^{i(\kappa x + \omega t)}$$

Using 3D coordinates

$$y = f(x,t) = Ae^{i(\vec{\kappa} \bullet \vec{x} + \omega t)}$$

1. Basic concepts of Physics and Mathematics

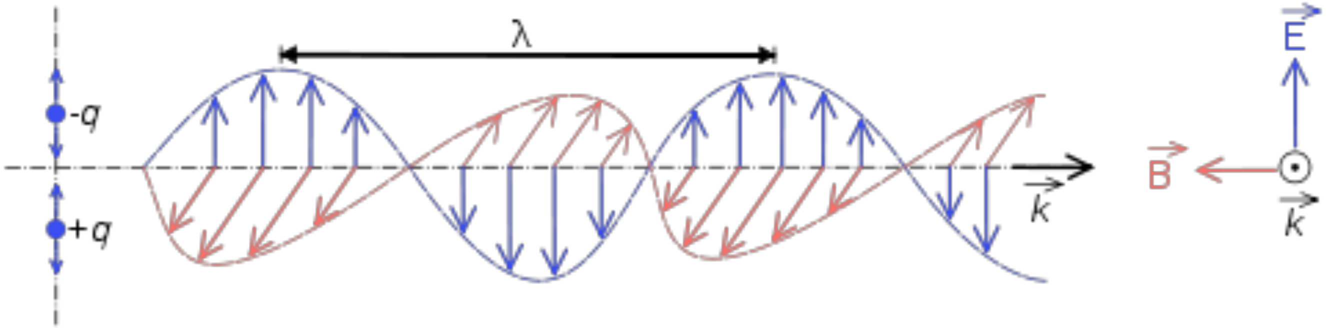
3. Fourier Transform

$$F(t) = \int_{-\infty}^{\infty} f(x) e^{i2\pi tx} \mathrm{dx}$$

$$f(x) = \int_{-\infty}^{\infty} F(t) e^{-i2\pi tx} \mathrm{dt}$$

1. Basic concepts of Physics and Mathematics

4. Electromagnetic wave



$$\vec{E} = (0, E_0 e^{i(\vec{\kappa} \bullet \vec{x} + \omega t)}, 0)$$

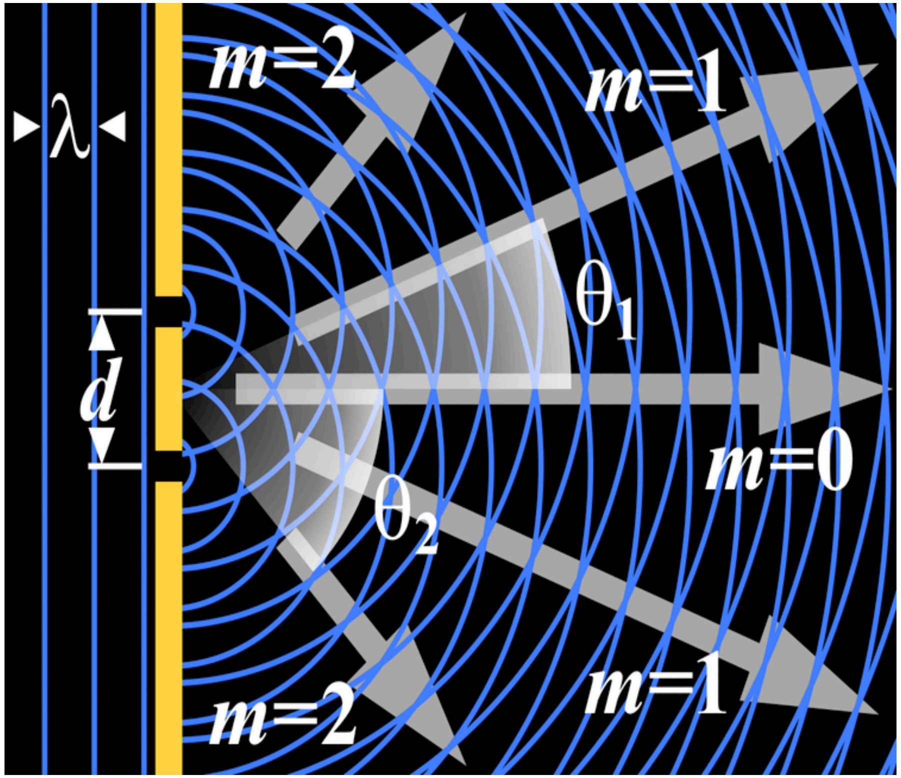
$$\vec{B} = (0, 0, B_0 e^{i(\vec{\kappa} \bullet \vec{x} + \omega t)})$$

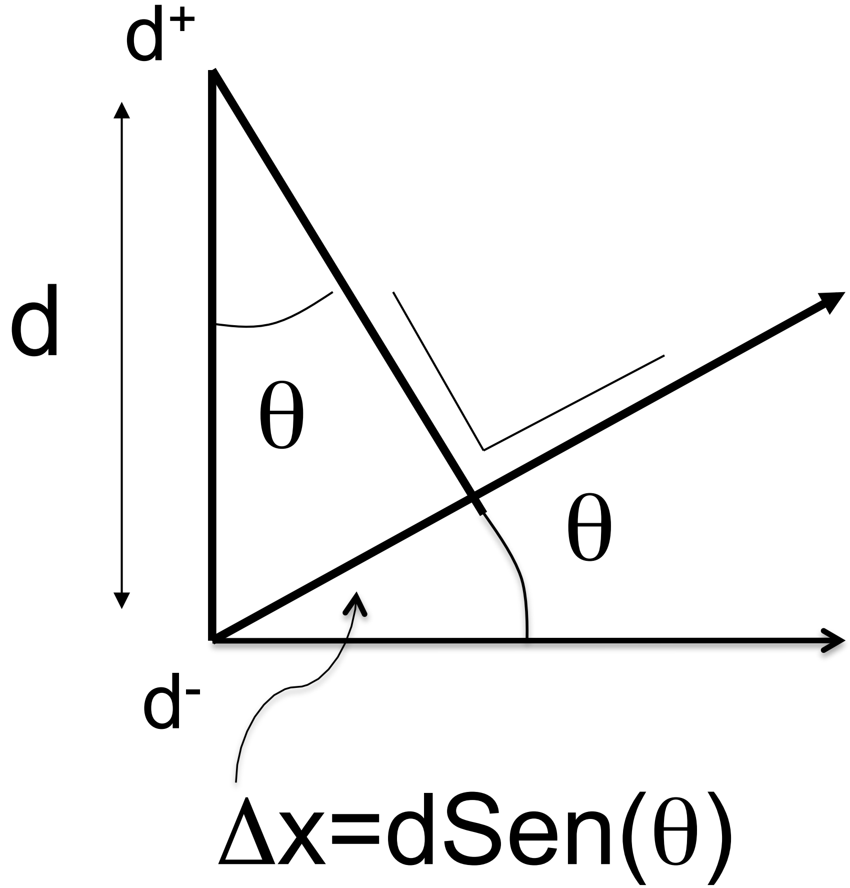
$$\vec{S} = (1,0,0)$$ Poynting vector

$$\vec{\kappa} = \frac{2\pi}{\lambda} \vec{S}$$

2. Diffraction

1. Bragg's law





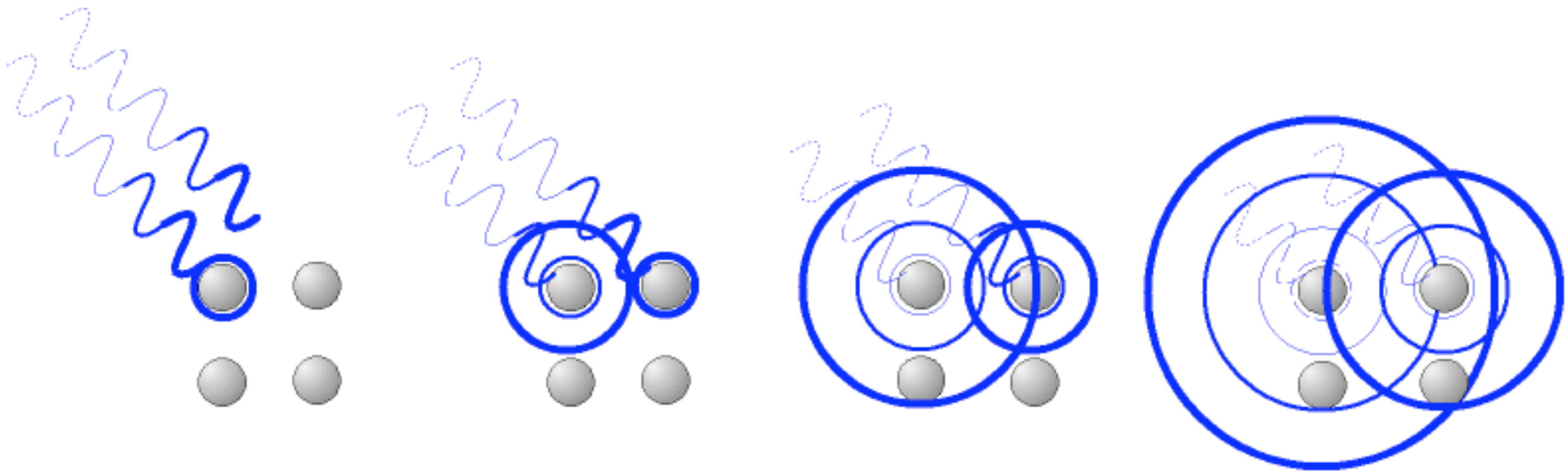
Wave from d^+ is $E=E_0e^{i(k^*x+\varpi t)}$

Wave from d^- is $E=E_0e^{i(k^*(x+\Delta x)+\varpi t)}$

On phase: $k^*\Delta x=m2\pi$ $(m=0,1,2,..)$

2. Diffraction

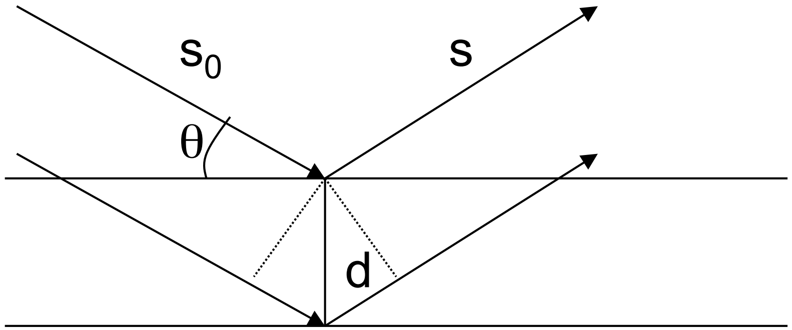
1. Bragg's law

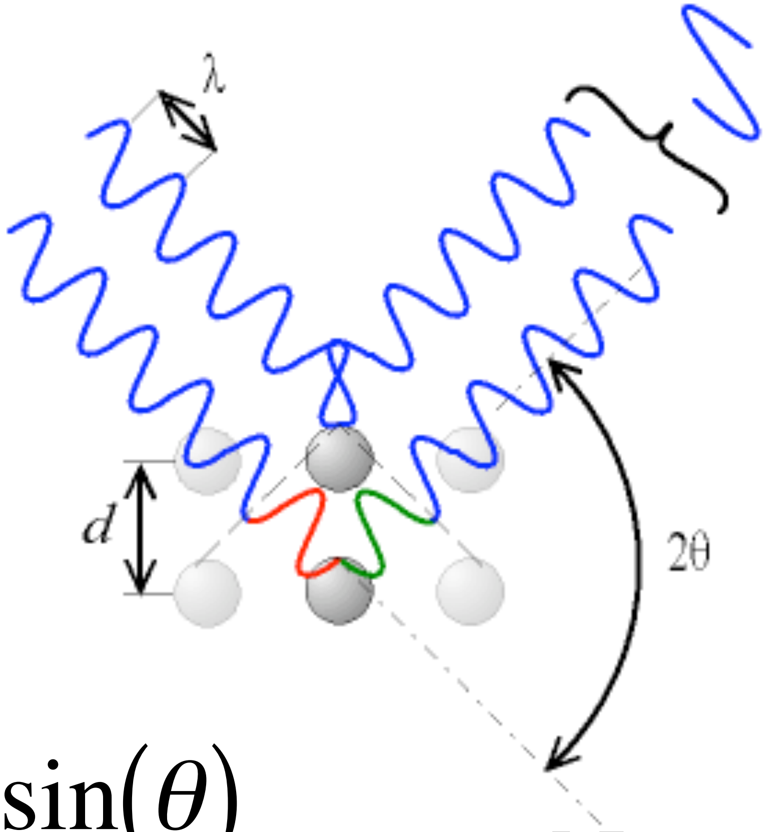


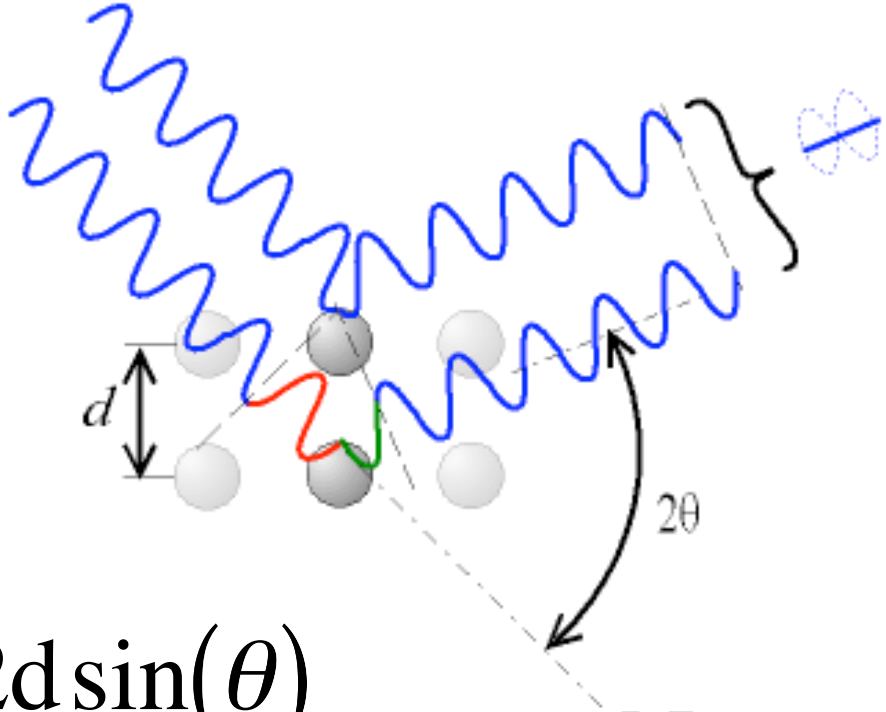
Ordered matter results in diffraction

2. Diffraction

1. Bragg's law



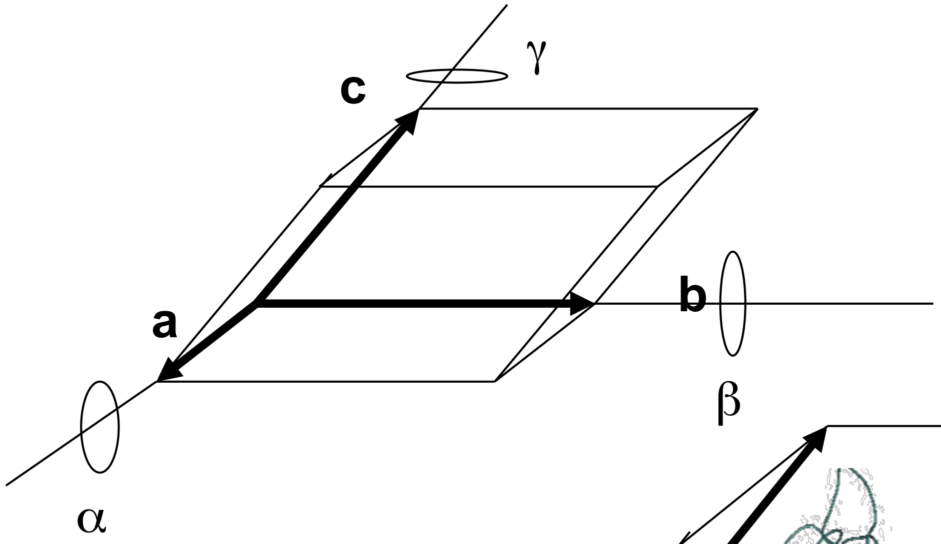


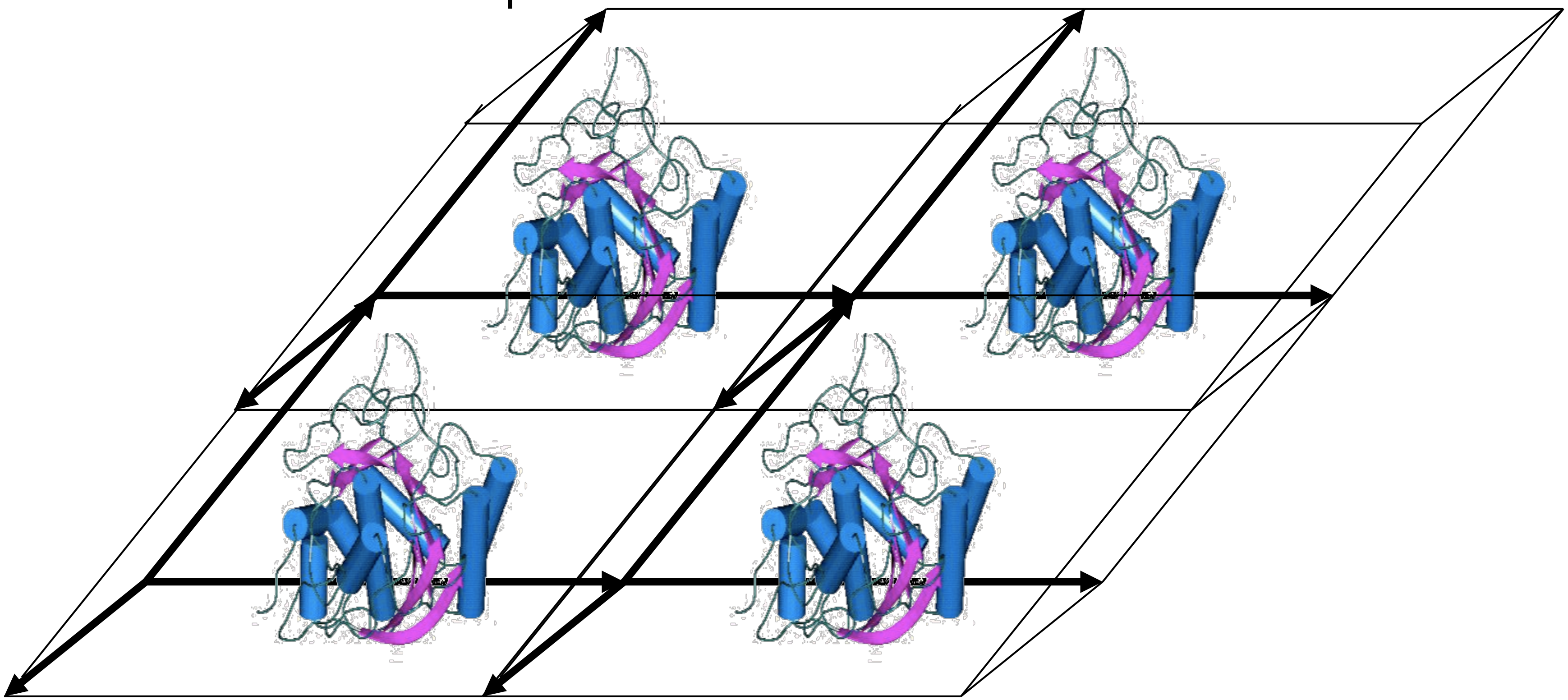


$$\frac{2d\sin(\theta)}{\lambda} = m \in \mathrm{N}$$

$$\frac{2d\sin(\theta)}{\lambda} \neq m \in \mathrm{N}$$

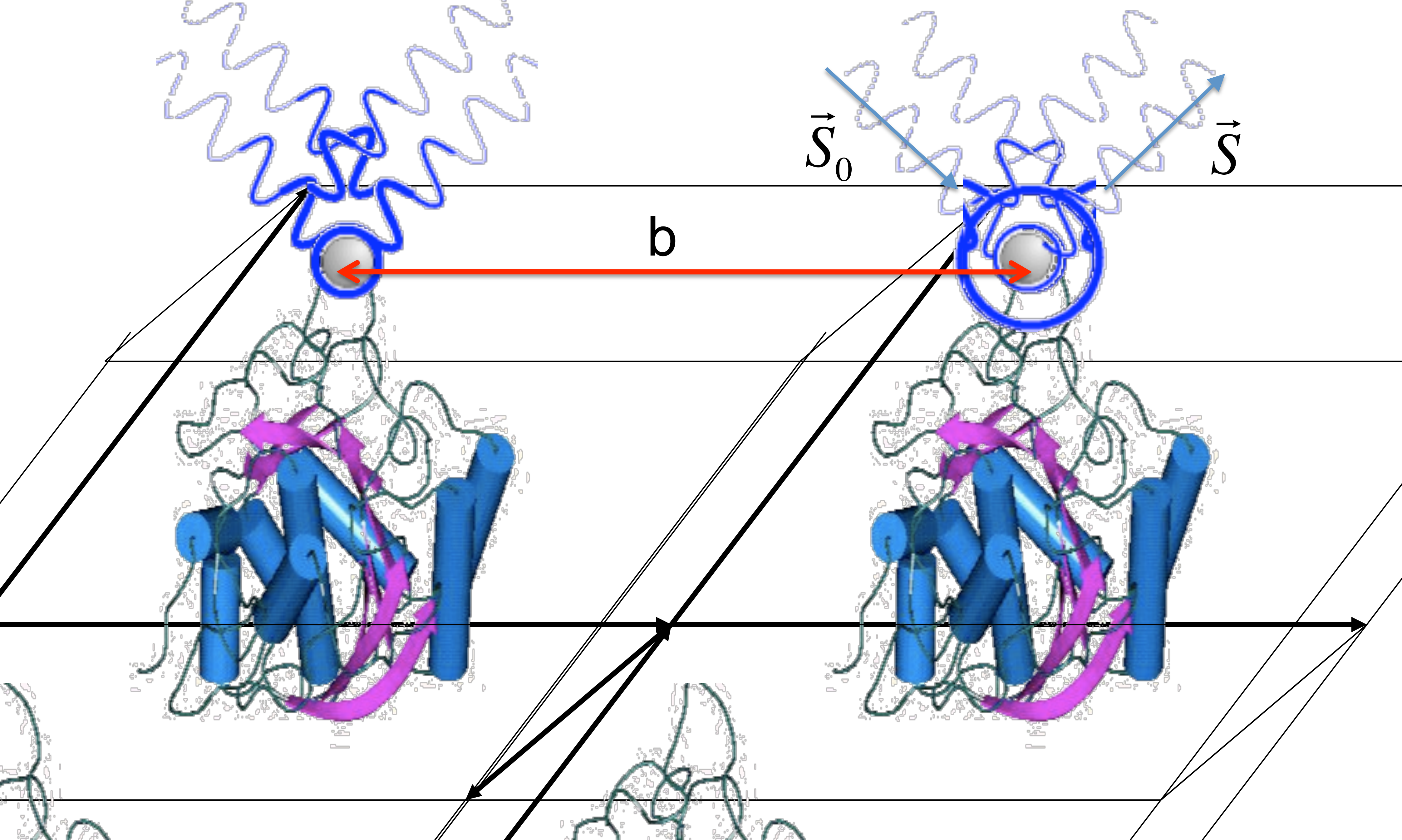
3. Crystal





3. Crystal

1. Laue conditions



3. Crystal

1. Laue conditions

$$\vec{a} * \left(\frac{\Delta\vec{S}}{\lambda}\right) = h$$

$$\vec{b} * \left(\frac{\Delta\vec{S}}{\lambda}\right) = k$$

$$\vec{c} * \left(\frac{\Delta\vec{S}}{\lambda}\right) = \ell$$

And for any position (r) in the crystal, using the directions of the unit cell:

$$\vec{r} = x\vec{a} + y\vec{b} + z\vec{c}$$

$$\vec{r} * \frac{\Delta\vec{S}}{\lambda} = xh + yk + z\ell$$

3. Crystal

1. Structure Factor

$$F(\Delta S) = \int_{Cell} \rho(\vec{r}) e^{i\, 2\pi \vec{r} \frac{\Delta\vec{S}}{\lambda}} dV$$

$$F(h,k,l) = V \iiint_{(0,1)} \rho(x,y,z)\, e^{i\, 2\pi(hx + ky + lz)} dxdydz$$

3. Crystal

1. Structure Factor

By Fourier Transform we can solve the electronic density of the macromolecule within the cell

$$\rho(\mathrm{x},\mathrm{y},\mathrm{z}) = \frac{1}{V}\sum_{h}\sum_{k}\sum_{l} F(\mathrm{h},\mathrm{k},\mathrm{l})\, e^{-i\ 2\pi(\mathrm{hx}+\mathrm{ky}+\mathrm{lz})}$$

4. Phase problem

Using the definitions for complex numbers, and being the structure factor a complex number, we have:

$$F(\mathrm{h,k,l}) = \left|F(\mathrm{h,k,l})\right| e^{\mathrm{i}\,\alpha(\mathrm{h,k,l})}$$

Therefore, we re-write the electron density map as:

$$\rho(\mathrm{x,y,z}) = \frac{1}{V}\sum_h \sum_k \sum_l \left|F(\mathrm{h,k,l})\right| e^{-2\pi i(\mathrm{hx+ky+lz}) + i\alpha(\mathrm{h,k,l})}$$

Where, |F| is the real part and can be known experimentally by the intensity of the reflection I:

$$I = \frac{\lambda^3 V_{cr}}{\omega V_{cell}^2}\left(\frac{\mathrm{e}^2}{mc^2}\right) I_0 \left|F(\mathrm{h,k,l})\right|^2$$

However, α(h,k,l) is not known. This is the phase problem.

4. Phase problem

1. Solutions

1. Direct determination
2. MIR: Multiple Isomorphous replacement
3. MAD: Multiple anomalous diffraction
4. MR: Molecular Replacement

4. Phase problem
1.1 Direct determination

For a small number of atoms, we teste all possible positions in the cristallographic cell, then we minimize the following function:

$$\Phi(\mathrm{h,k,l}) = \sum_{hkl} \left\{ \left|F_{obs}(\mathrm{h,k,l})\right| - \left|F_{calc}(\mathrm{h,k,l})\right| \right\}^2$$

obs, is the observed factor of structure
calc is the structure factor using the atomic predicted coordinates

4. Phase problem
1.2 MIR

We introduce a heavy metal in the protein by immersion of the cristal in a solution with a salt of the heavy metal.

The heavy metal gets into the protein, close to negatively charged residues.

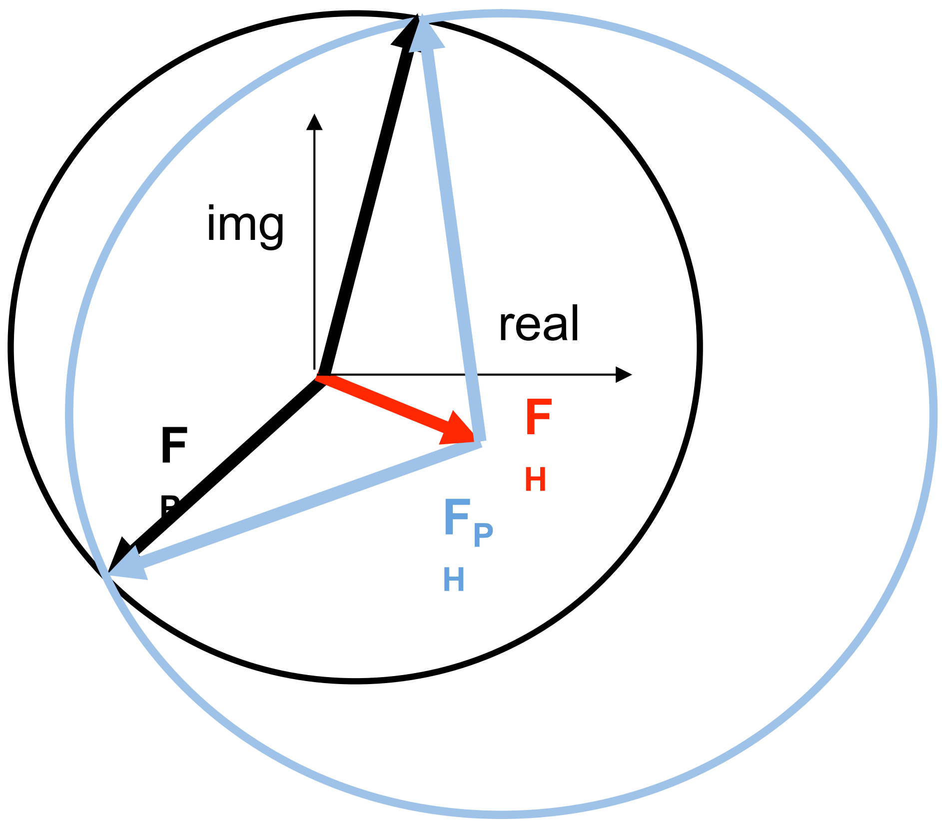
The inclusion of metals should not change the conformation of the protein.

After obtaining the factor of structure of the protein with the metal, we substitute the metal by another similar one (i.e. exchanging Ca^{+2} by Mg^{+2} or Se^{+2}). The substitution should not affect the conformation and the location of the metal should remain unchanged.

The result of this experiment is the Multiple Isomorphous Replacement.

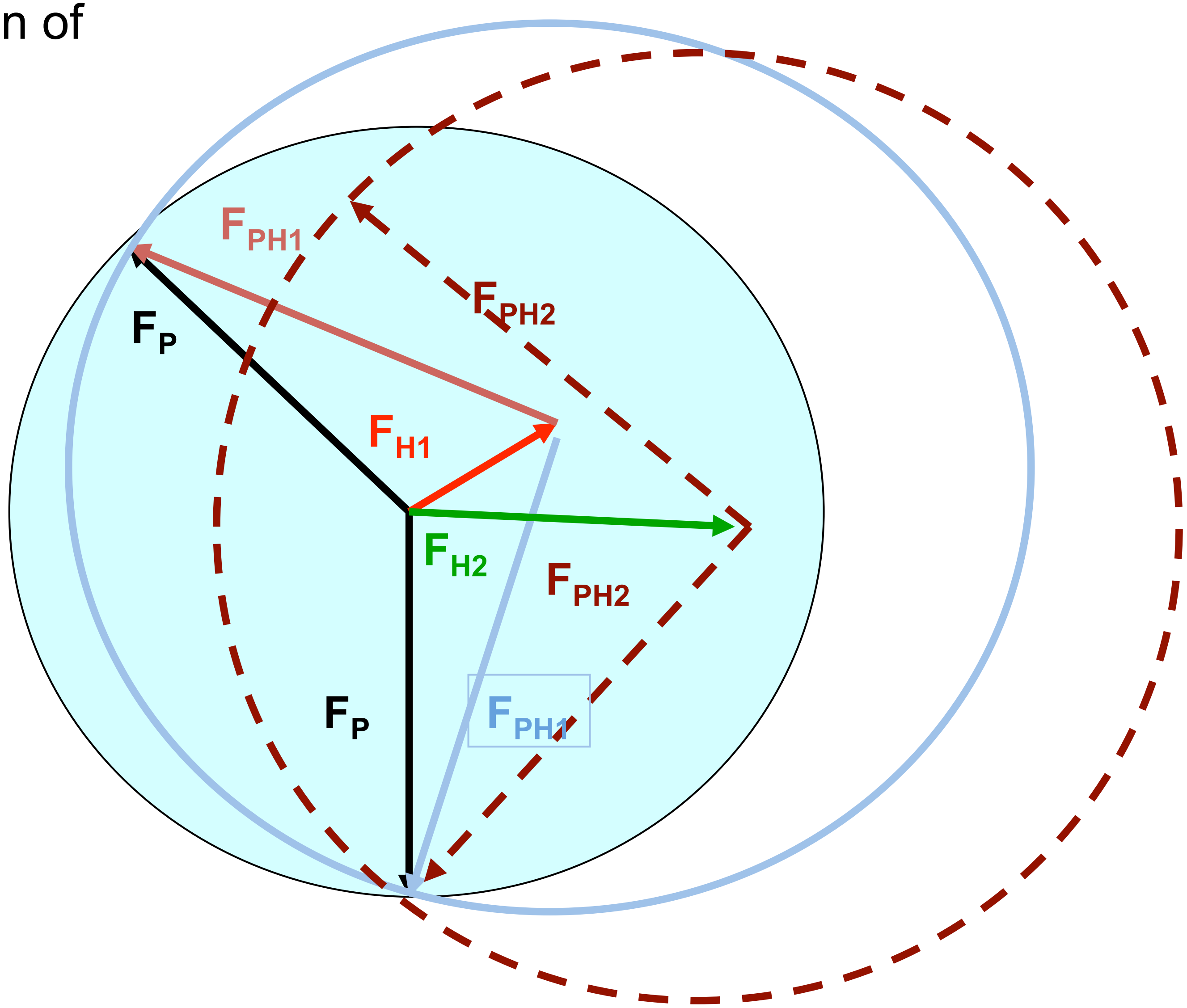
4. Phase problem
1.2 MIR

Let be F_P the factor of structure of the protein and F_{PH} the factor of structure of the protein with the first metal. The Factor of structure of only the metal is F_H. Then $F_{PH} = F_P + F_H$, where we know $|F_P|$, $|F_{PH}|$ and FH (by direct determination)



4. Phase problem
1.2 MIR

With the substitution of metal H1 by H2



4. Phase problem
1.3 MAD

The experiment is the same as for MIR. However, the first metal has already the property of diffracting different as a function of the wavelength of the Xray. Therefore, we don’t need a second metal, just two different diffractions using different wavelengths of the Xray beam.

4. Phase problem
1.4 MR

We use the known of structure of an homolog to calculate the factor of structure. Then we superpose both electronic maps by rotation and translation and we minimize the R factor, defined as:

$$R = \frac{\sum_{hkl} \left\| F(obs) \right| - k \left| F(calc) \right\|}{\sum_{hkl} \left| F(obs) \right|}$$

The conformation is modified until the R factor is minimum.

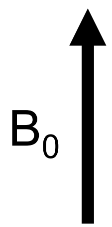
Nuclear Magnetic Resonance

1. Basic concepts of Physics
    1. Nuclear Spin
    2. Chemical displacement
    3. Nuclear Overhauser Effect
2. NMR bi-dimensional and multidimension
    1. Scalar coupling 2D
    2. COSY and TOCSY
    3. NOESY
3. Application on macromolecules

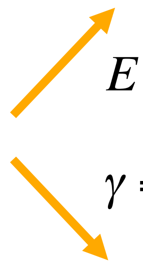
1. Basic concepts of Physics

1. Nuclear Spin

Some isotopes (i.e. ^{1}H, ^{13}C, ^{15}N, ^{31}P …) under a magnetic field have a nuclear spin (behaving like a little magnet). A small radiofrequency can change the spin state of a nucleus.



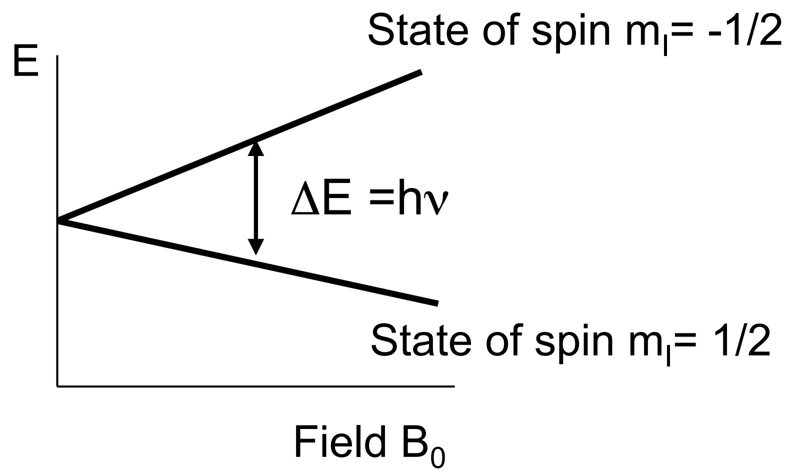
Magnetic field



$$E = -m_I \gamma \hbar B_0$$

$$\gamma = \frac{e}{2m}$$

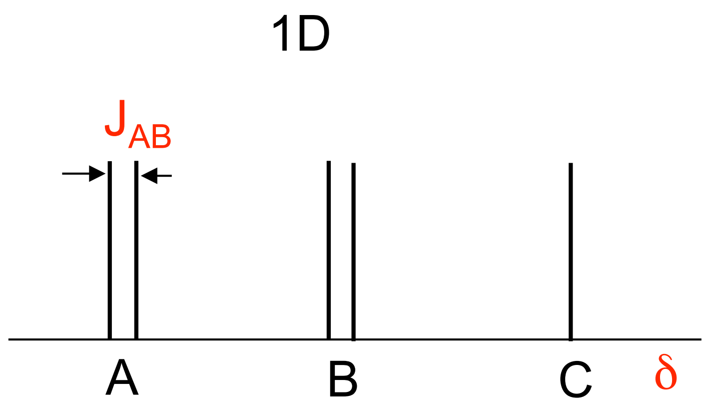
Oriented spins



1. Basic concepts of Physics

2. Chemical displacement

Nucleus with spin affect other neighboring magnetized nucleus. This is done through bonds transmission because of the electric field of the electrons of a bond. This implies a displacement of the original energy, detected as chemical displacement and scalar coupling.



1. Basic concepts of Physics

3. Nuclear Overhausser effect

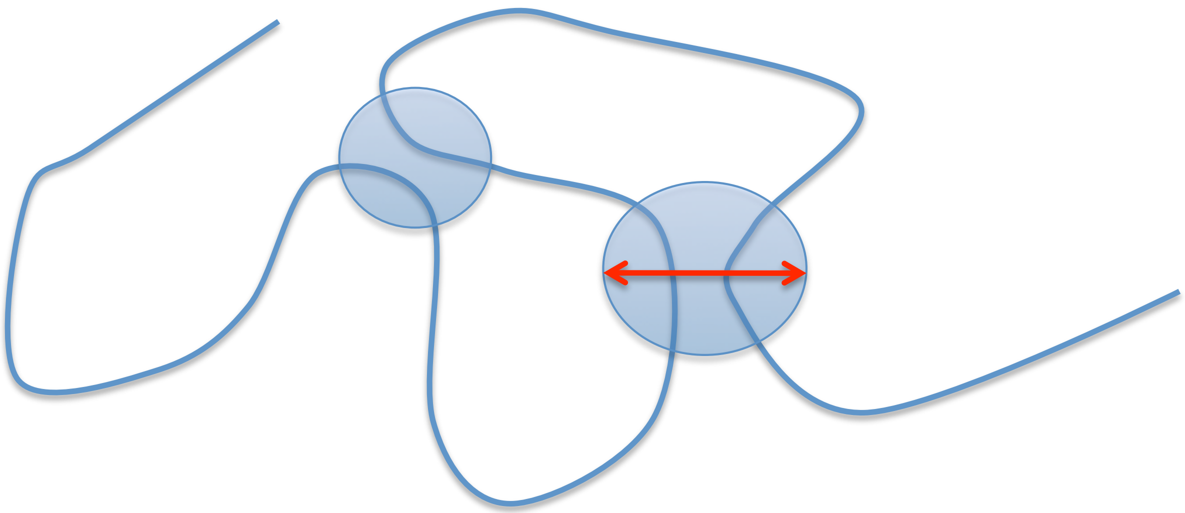
Nuclear spin can also be affected by those magnetized nucleus in close proximity in the three dimensional space. This is known as Overhausser effect.

NOE ⇒ distances $I_{NOE} \propto 1/r^6$

NOEs high: $1.8 < r_{HH} < 2.8$ Å

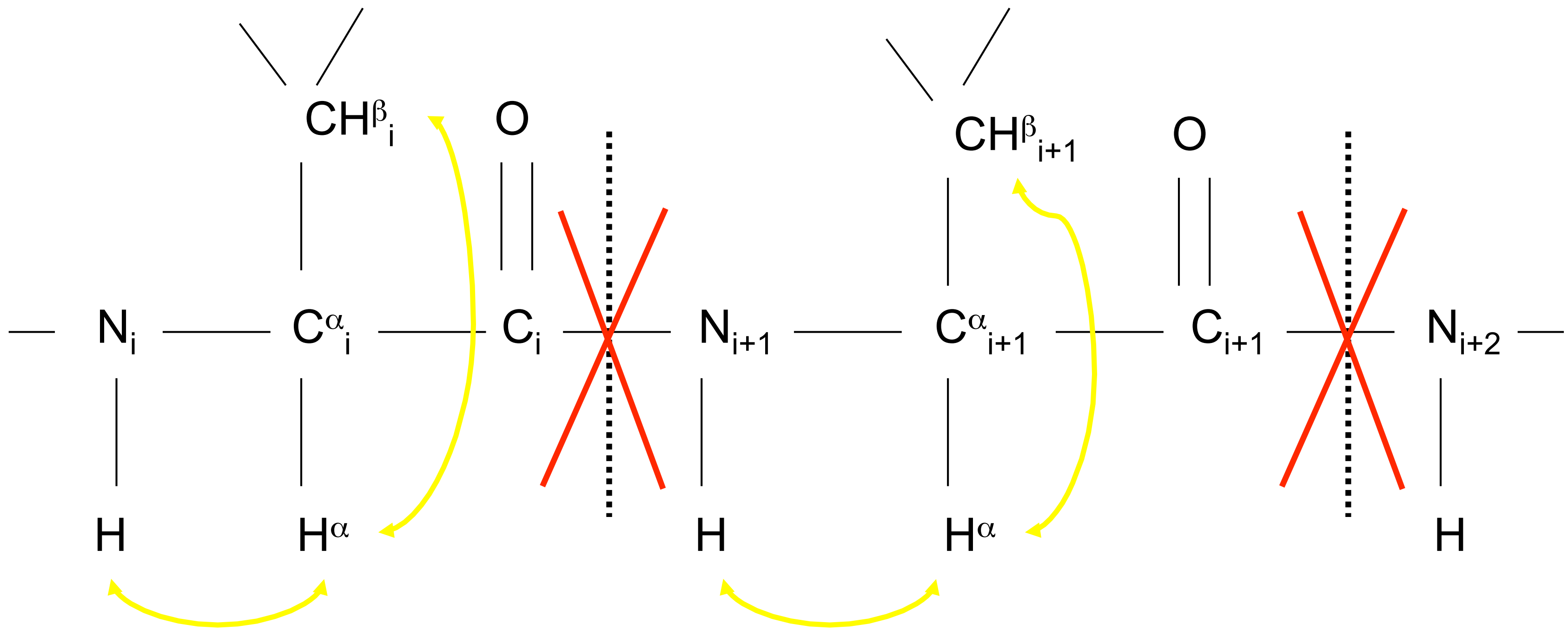
NOEs medium: $1.8 < r_{HH} < 3.5$ Å

NOEs weak: $1.8 < r_{HH} < 5$ Å



2. NMR bi-dimensional and multidimension

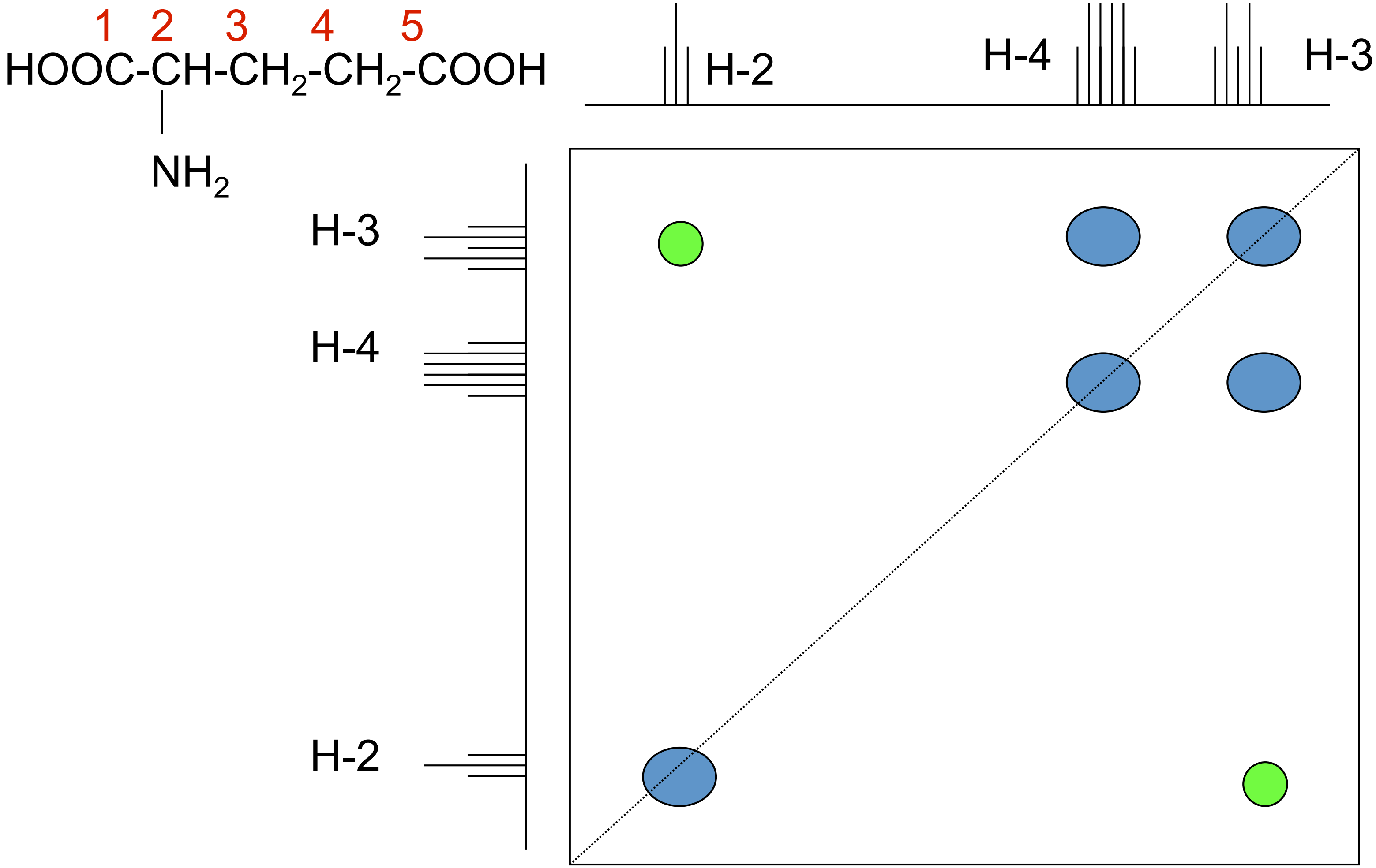
1. Scalar coupling



Scalar coupling between double bonds is forbidden

2. NMR bi-dimensional and multidimension

2. COSY-TOCSY

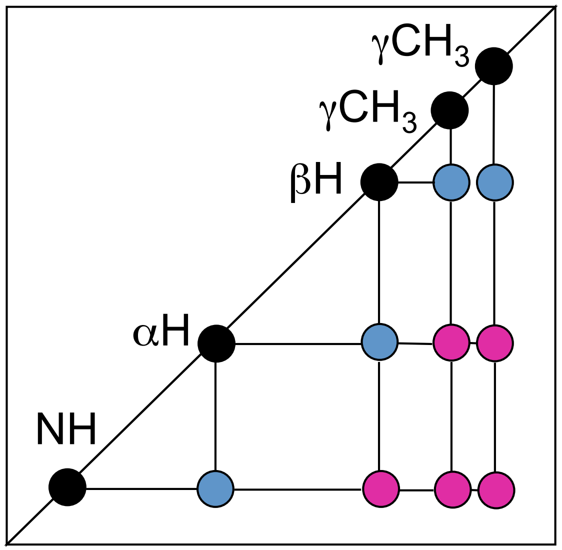


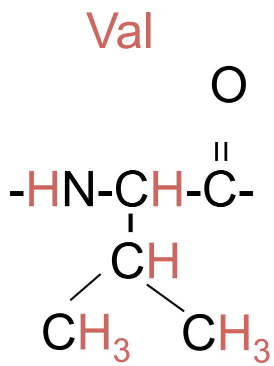
500MHz H,H COSY-90º de GLU

2. NMR bi-dimensional and multidimension
2. COSY-TOCSY

Experiment **2D COSY (COrrelation SpectroscopY):** correlation between ^1H scalar couplings, through $^3J_{HH}$

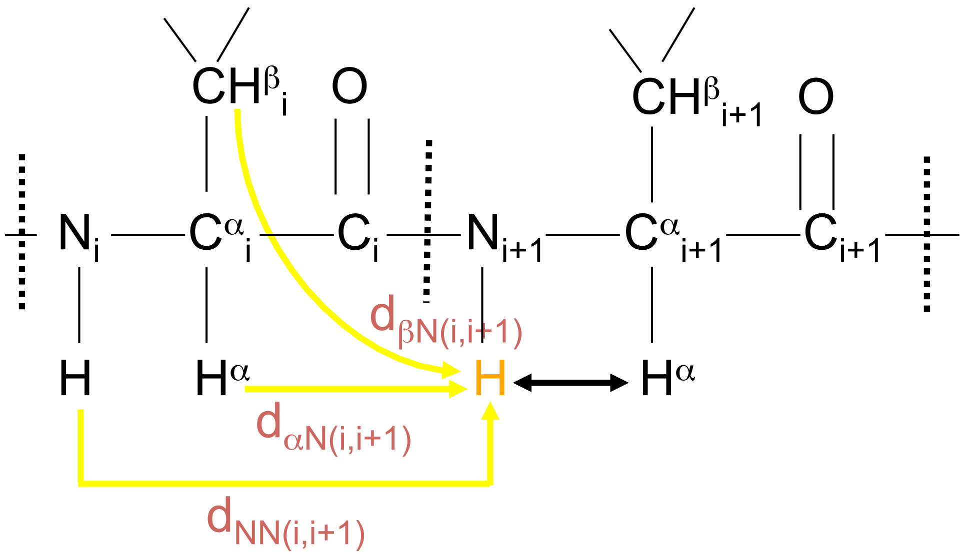
Experiment **2D TOCSY (TOtal Correlation SpectroscopY):** correlation between ^1H scalar couplings, through $^3J_{HH}$





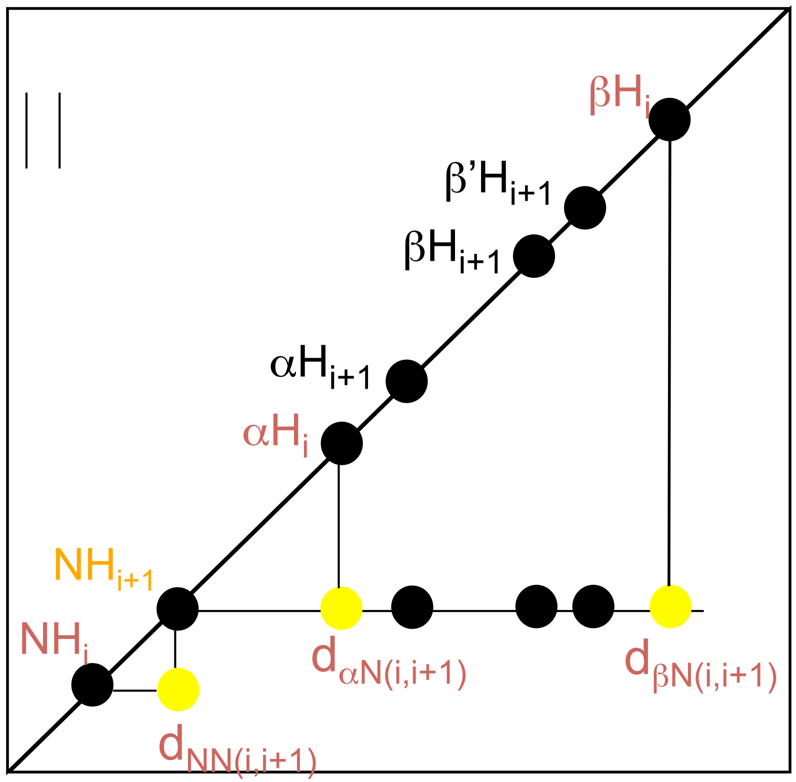
2. NMR bi-dimensional and multidimension

3. NOESY



COSY/TOCSY

NOESY



3. Application on macromolecules

(1) Assigning ^1H frequencies on the NMR spectrum to Aa

(2) Identify secondary structure elements

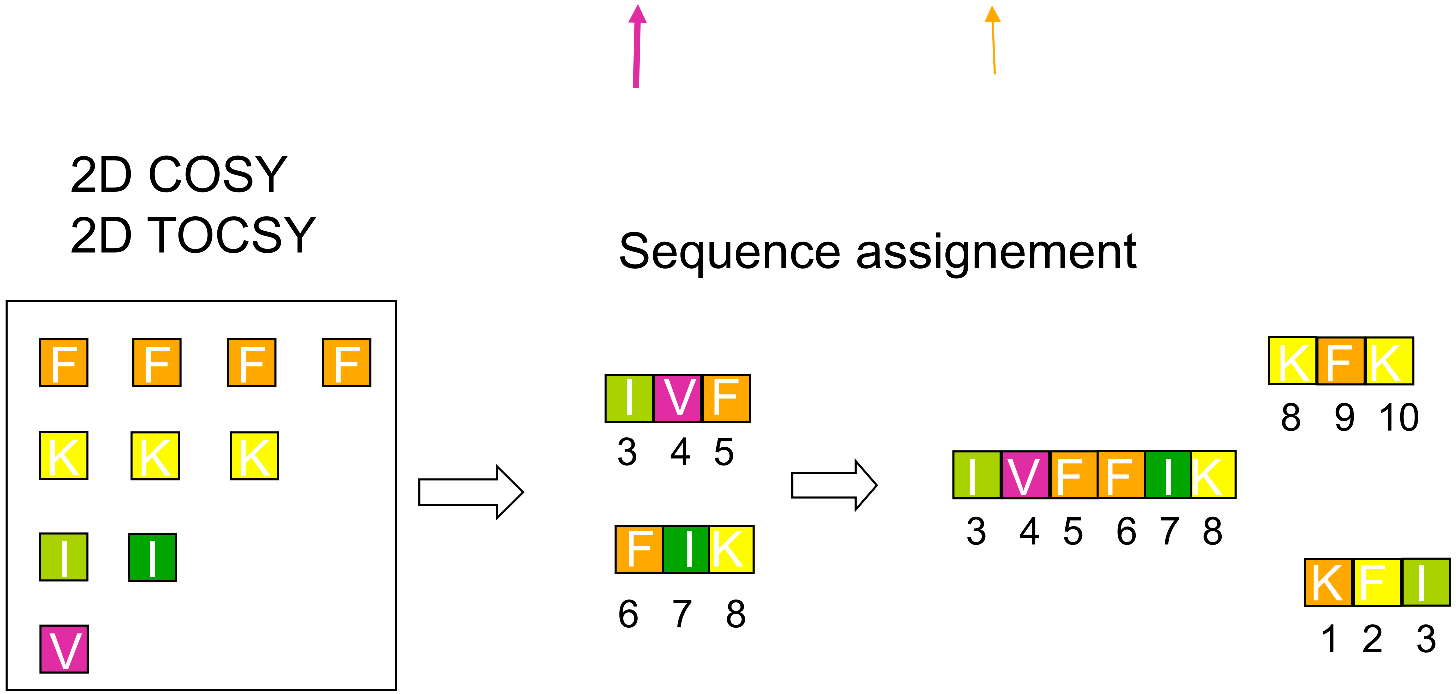
(3) Extract distance constraints and torsion angles.

(4) Obtain the 3D structure by distance geometry optimization

3. Application on macromolecules

1. Assigning ^{1}H frequencies on the NMR spectrum to Aa

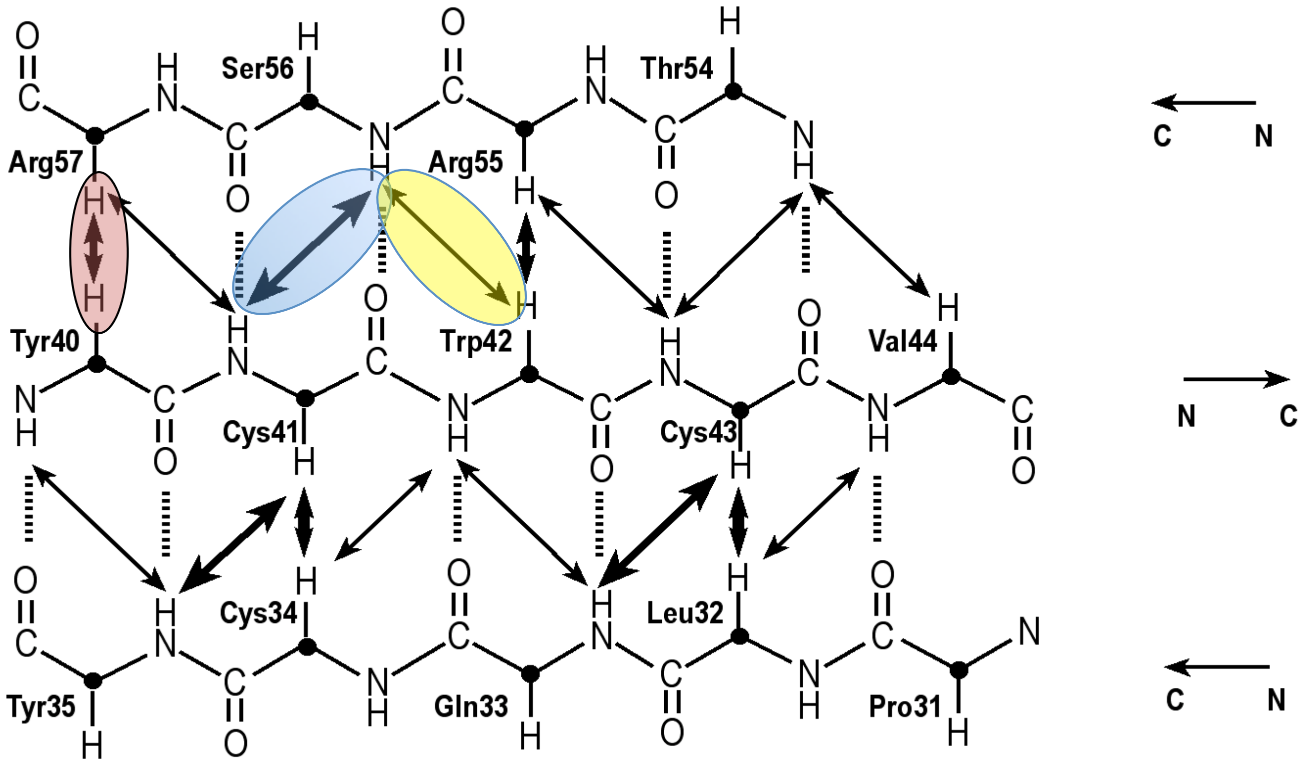
H-K1-F2-I3-V4-F5-F6-I7-K8-F9-K10-OH



3. Application on macromolecules

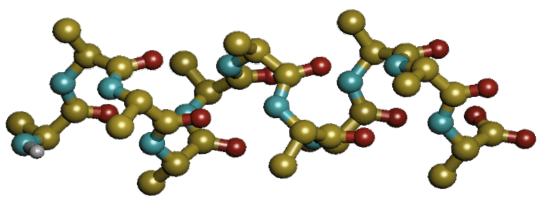
2. Identify secondary structure elements

d(Cα-Cα)
d(Cα-N)
d(N-N)



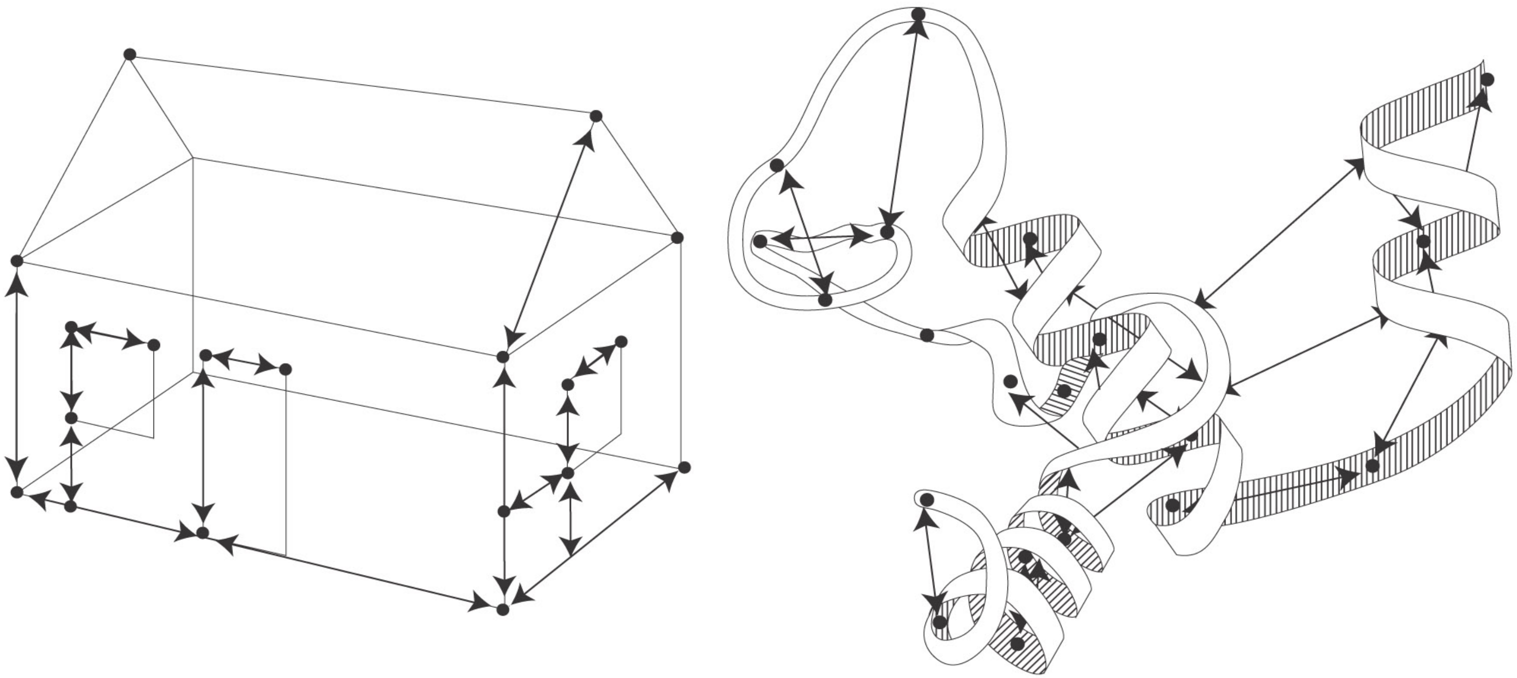
3. Application on macromolecules

2. Identify secondary structure elements



3. Application on macromolecules

3. Extract distance constraints and torsion angles.



3. Application on macromolecules

4. Obtain the 3D structure by distance geometry optimization

$$E_{bonding} = \sum_{bonds} \frac{1}{2} k_i \left(d_i - d_i^0\right)^2 + \sum_{angles} \frac{1}{2} k_j \left(\alpha_j - \alpha_j^0\right)^2 + \sum_{\substack{improper\\dihedral}} \frac{1}{2} k_n \left(\omega_n - \omega_n^0\right)^2 + \sum_{angles} E_m Cos\left(\omega_m \phi_m + \varphi_m\right)^2$$

$$E_{non-bonding} = \frac{1}{4\pi\varepsilon_0} \sum_i \sum_{j>i} \frac{q_i q_j}{r_{ij}} + \sum_i \sum_{j>i} \frac{C_6^{ij}}{r_{ij}^6} - \frac{C_{12}^{ij}}{r_{ij}^{12}}$$

$$E_{NMR} = \sum_{restrictions} \frac{1}{2} k_l \left(R_l - R_l^0\right)^2$$

$$E = E_{bonding} + E_{non-bonding} + E_{NMR}$$