Neutron and X-ray studies of γ-crystallins, structural proteins of the eye lens.

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Thesis directors:

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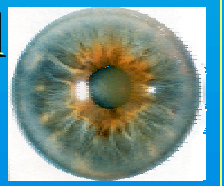
Importance of hydrogen atoms in biology

- Biological process
- Enzyme catalysis
- Molecular functions
- Folding-Unfolding
- Physico-chemical properties :

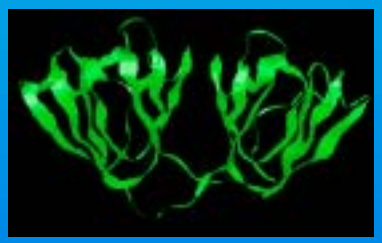


Phase Separation

→ Opacification

3D structure

Structure-function relationship

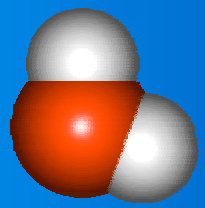


→ γ-crystallins

Hydrogen location

Hydrogen exchange

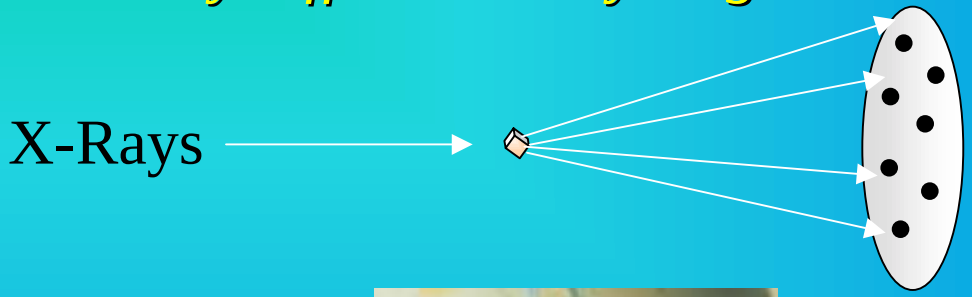
Solvent content



→ Water molecules

Limitations of protein crystallography for hydrogen determination

•*X-ray diffraction: Hydrogenated / Deuterated sample*



Schoenborn,, 2000

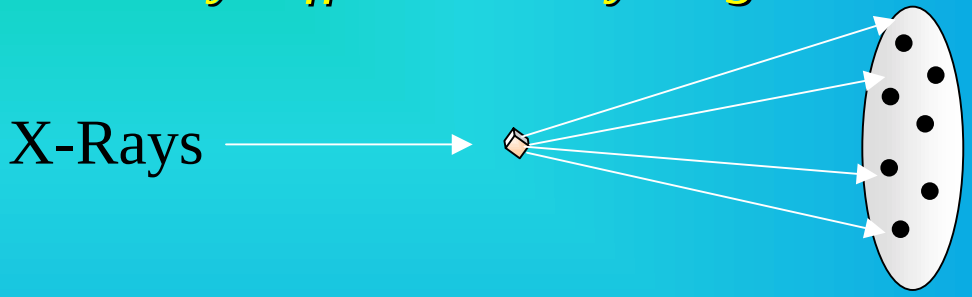
Small Crystals of gamma E crystallin

➜X rays interact with the electronic clouds of atoms of a protein crystal

➜ No hydrogen atoms

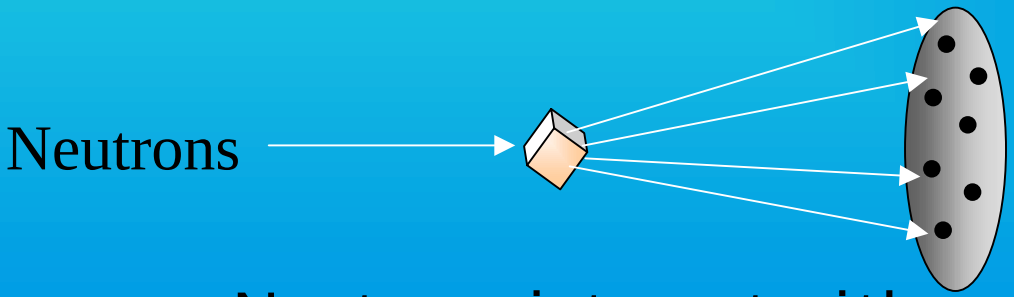
Limitations of protein crystallography for hydrogen determination

•*X-ray diffraction: Hydrogenated / Deuterated sample*



Schoenborn,, 2000

• *Neutron diffraction : Hydrogenated sample*

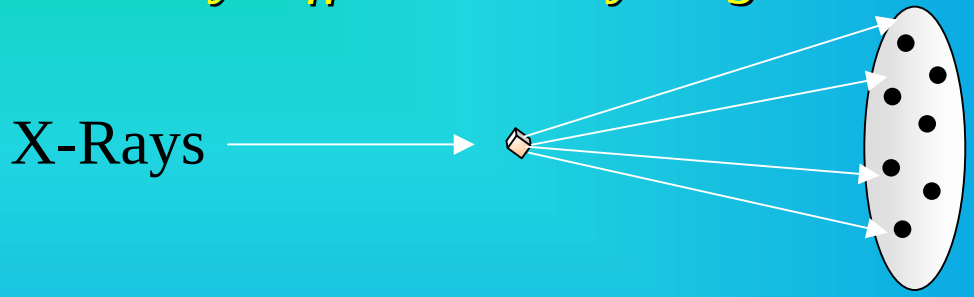


- Neutrons interact with nuclei of atoms
- Hydrogens produce background due to their large incoherent scattering
- Coherent scattering length : H is **-** and C, N, O are **+**

→ cancellation in density map

Limitations of protein crystallography for hydrogen determination

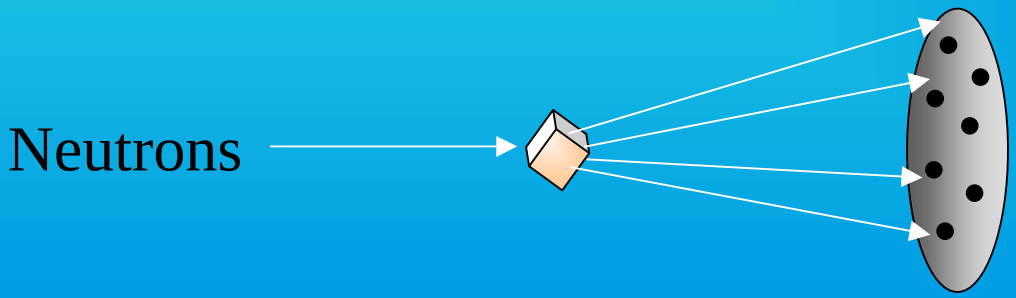
- *X-ray diffraction: Hydrogenated / Deuterated sample*



No hydrogen atoms

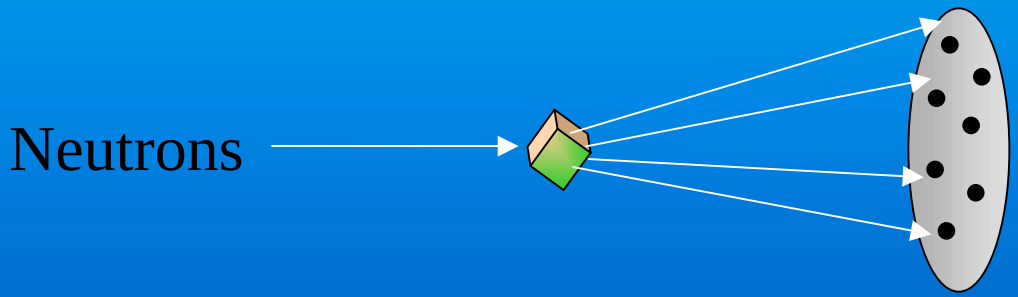
Schoenborn,, 2000

- *Neutron diffraction : Hydrogenated sample*



Very difficult

- *Neutron diffraction : Deuterated sample*



Less background

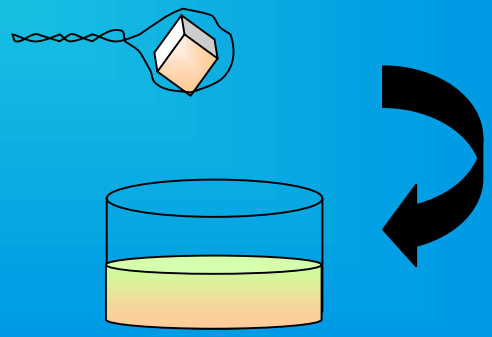
No cancellation

➜ Easier

Deuteration of samples

Ways to deuterate samples :

- *Soaking hydrogenated crystal in mother liquor containing D_2O*



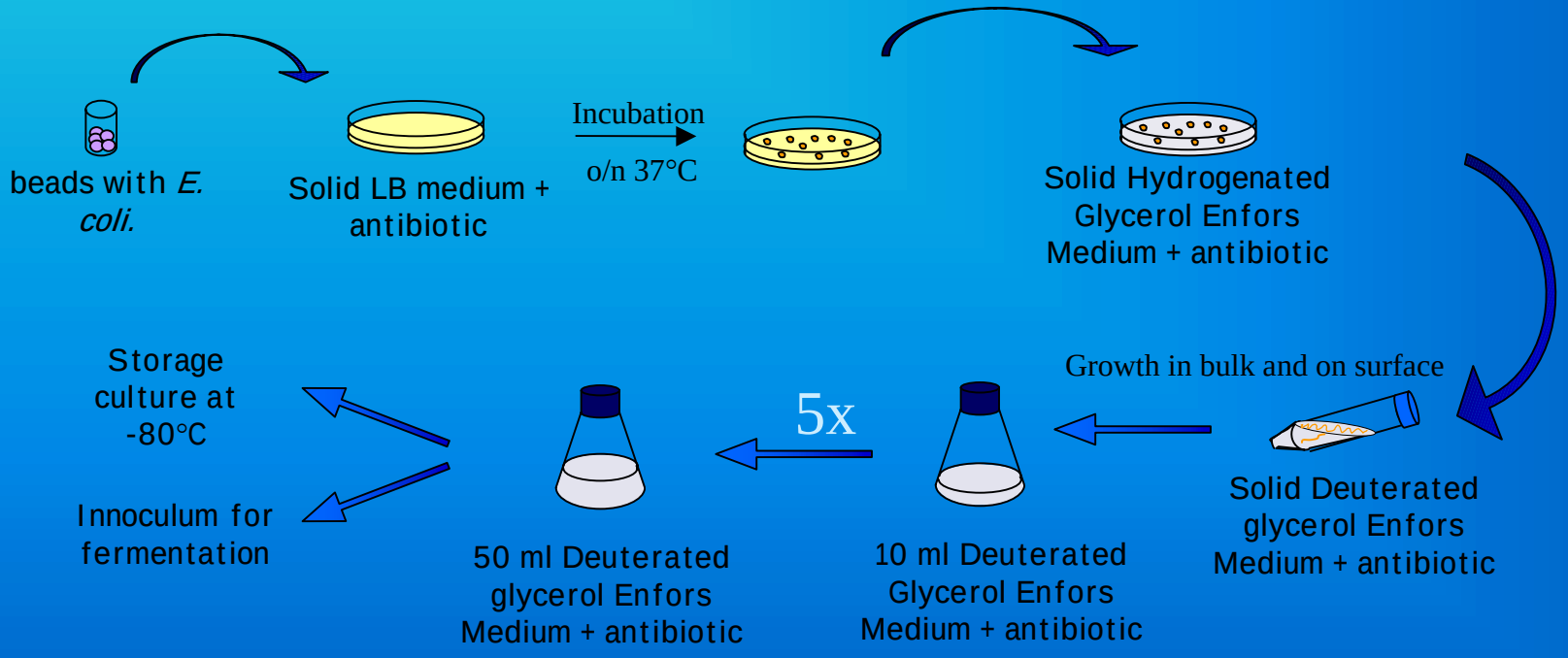
➜ Exchangeable H is just 25 % of total H atoms

Deuteration of samples

Ways to deuterate samples :

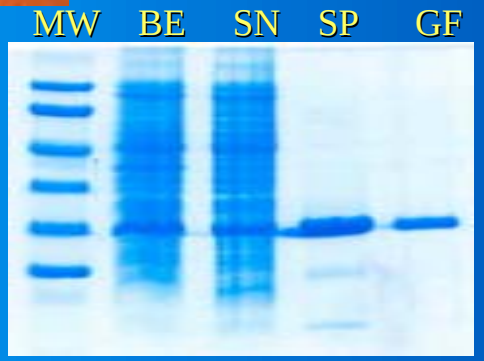
- *Soaking hydrogenated crystal in mother liquor containing D_2O*
- *In vivo synthesis of fully deuterated protein in D_2O medium.*

Adaptation of *E. coli* cells to deuterium



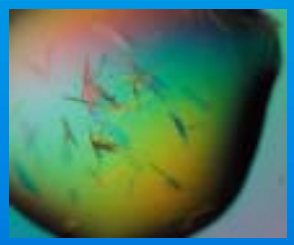
Work already done

- Cloning of genes from rat and human eye lens in bacterial expression system



- Over-expression and purification of hydrogenated and deuterated forms
- Crystallization of hydrogenated crystallin protein and an X-ray structure at 1.5 Å






- Crystallization tests on deuterated protein for experiments on LADI