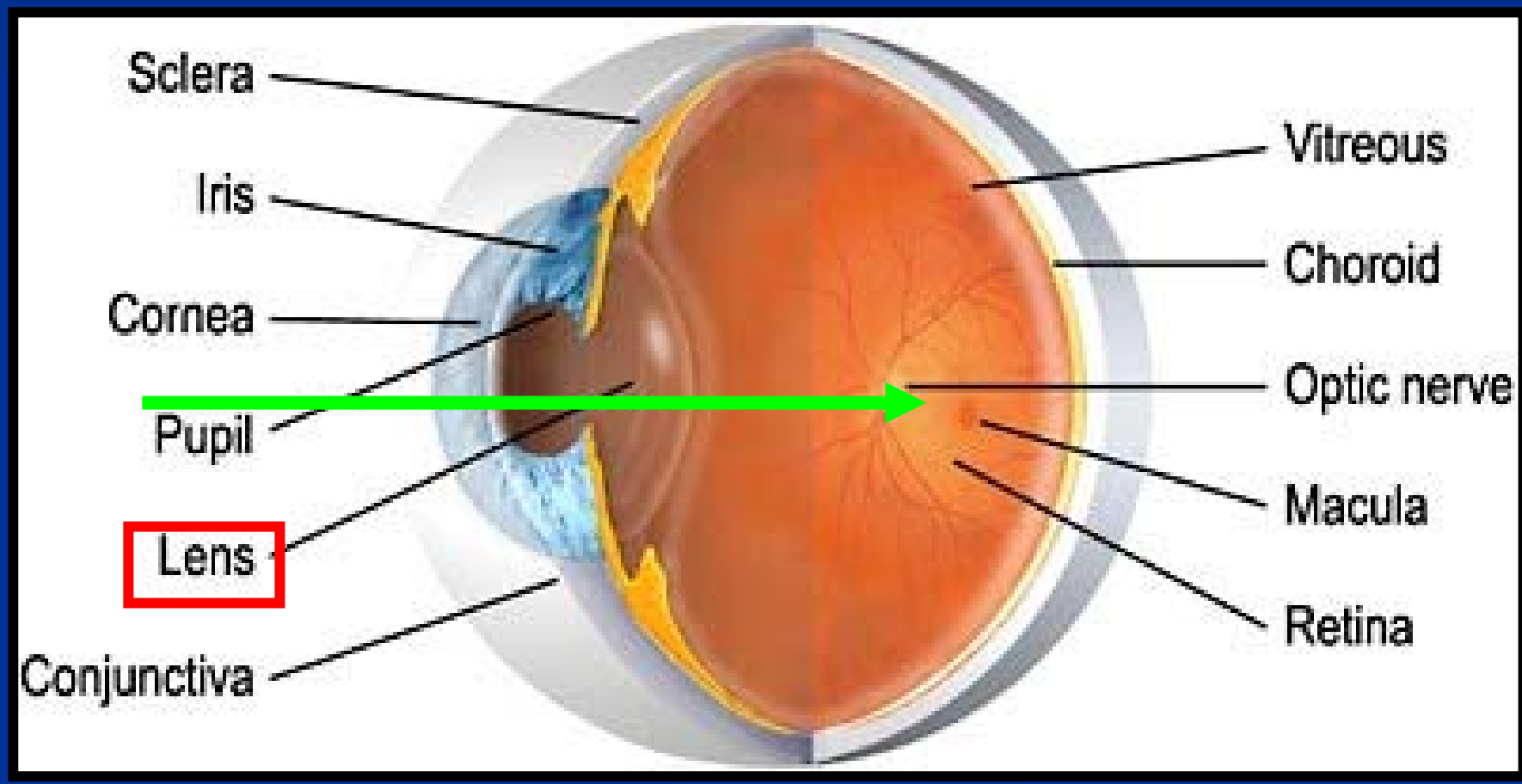
Physical Methods in Models of Cataract Disease

O. P. Srivastava
Department of Vision Science
University of Alabama at Birmingham

Function of the Lens:

Refraction



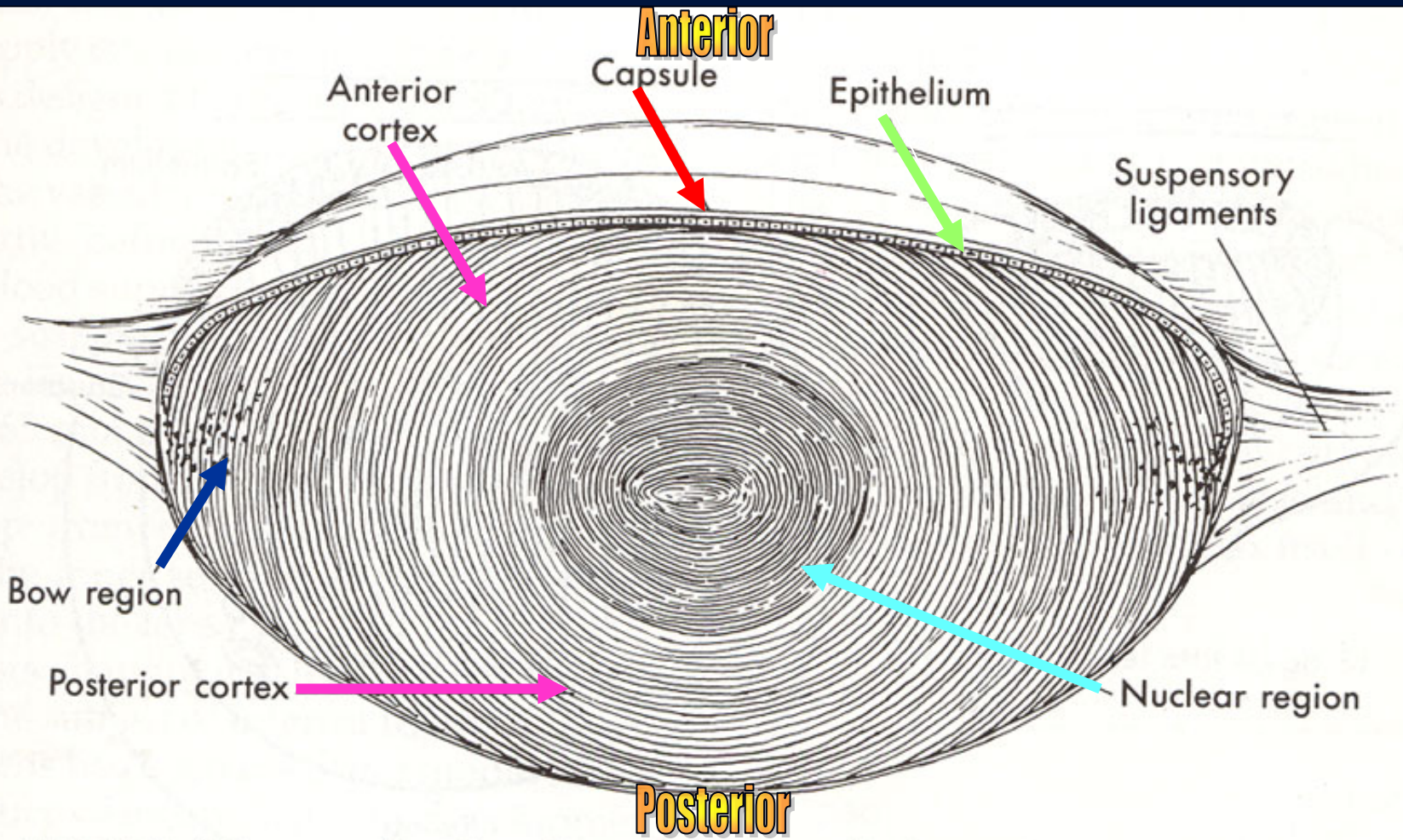


FIG. 10-2 Diagram of section through the lens. (Redrawn from Lerman,[150] p 72.)

Lens Specific Structural Proteins (α-, β- and γ-Crystallins)

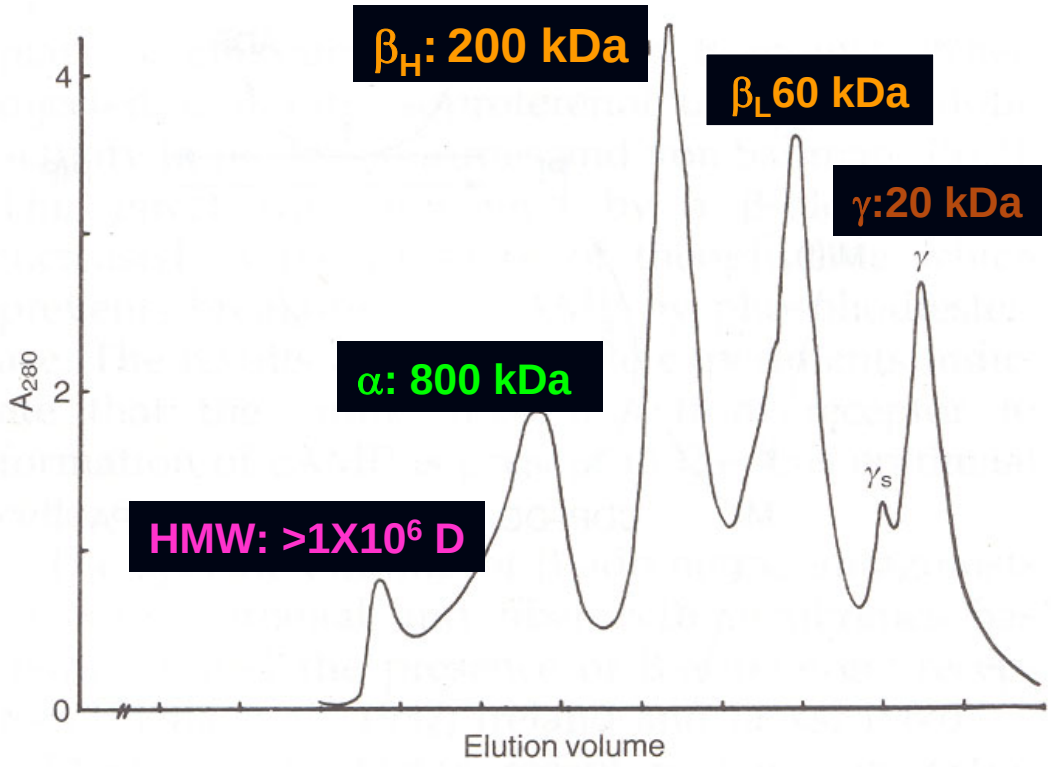
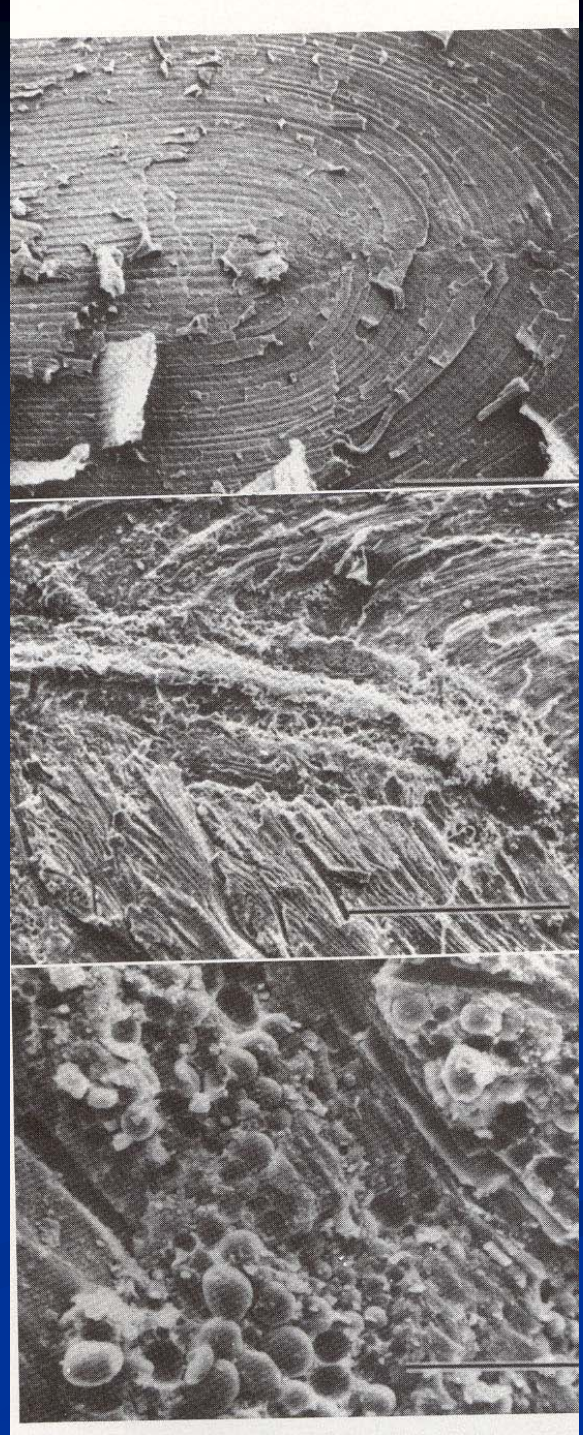


Fig. 4.9 Gel chromatography of bovine crystallins on TSK HW-55. After a small HM peak, α-crystallin is followed by β_H-, β_L- and γ-crystallins (Beswick and Harding, unpublished results).

- **α-Crystallin:**
  - Two primary gene products (αA and αB, both 20 kDa), 800 kDa oligomer (αA:αB, 3:1), Chaperone activity.
- **β-Crystallin:**
  - Eight primary gene products, 23-32 kDa (Acidic βA3/A1, βA3 and βA4, Basic βB1, βB2, βB3 and βB4), 50-200 kDa oligomer
- **γ-Crystallin:** Six primary gene products, 20 kDa (γA, γB, γC, γD, γE and γF), monomer.

Normal lens

Cataractous lenses

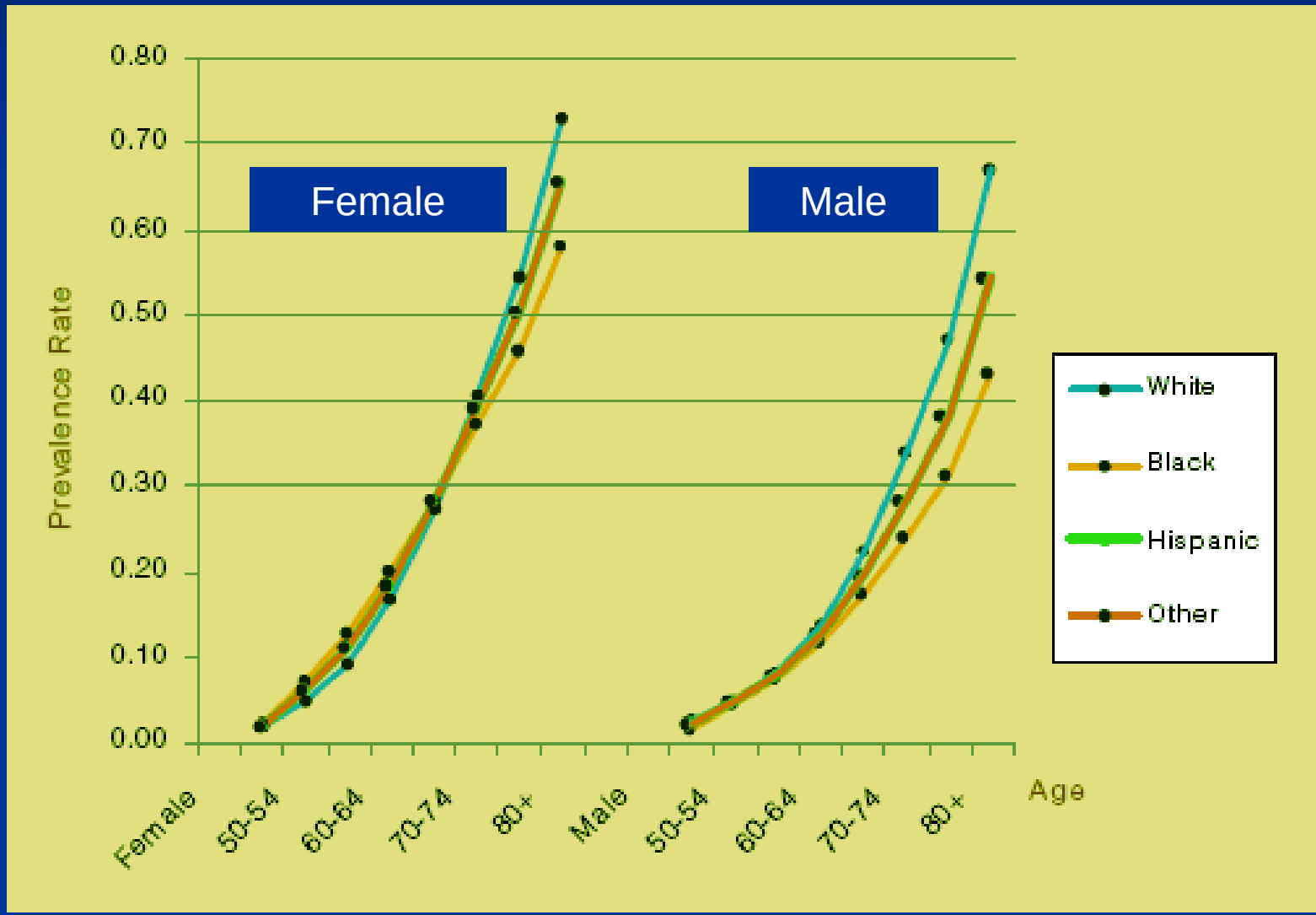


National Eye Institute (NIH)

- Most cataracts are related to aging.

- By age 80, more than half of all Americans either have a cataract or have had cataract surgery.

Estimated Specific Prevalence Rates for Cataract
Source: National Eye Institute



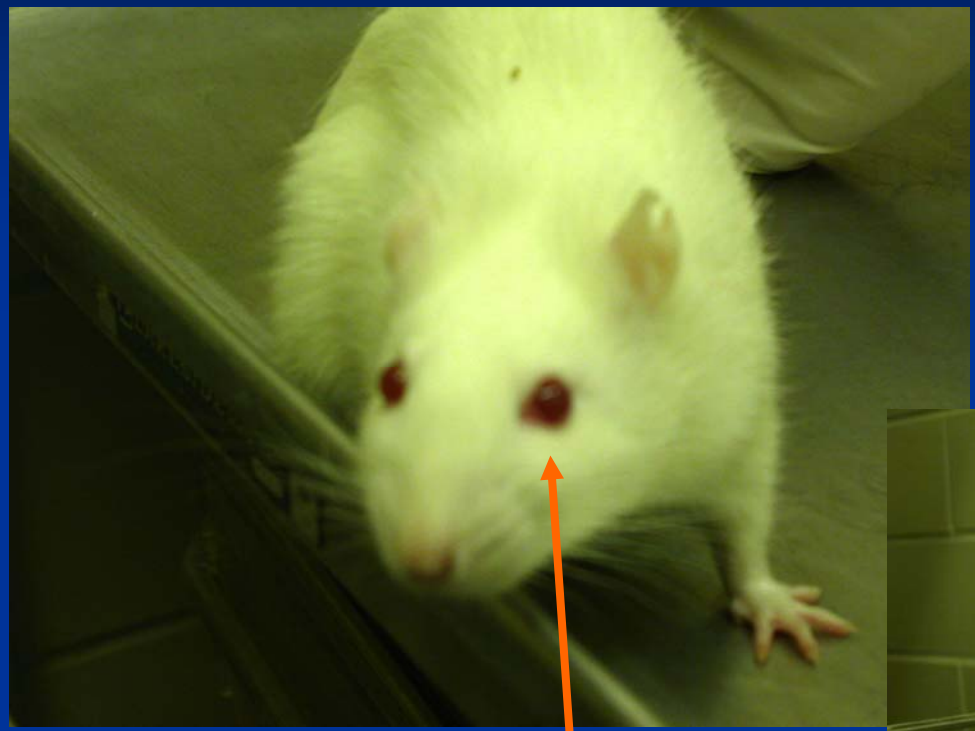
Summary of Eye Disease Prevalence Data
Source: National Eye Institute

Age,	Cataract		Advanced AMD		Intermediate AMD		Glaucoma	
Years	Persons	(%)	Persons	(%)	Persons	(%)	Persons	(%)
40-49	1,046,000	2.5%	20,000	0.1%	851,000	2.0%	290,000	0.7%
50-59	2,123,000	6.8%	113,000	0.4%	1,053,000	3.4%	318,000	1.0%
60-69	4,061,000	20.0%	147,000	0.7%	1,294,000	6.4%	369,000	1.8%
70-79	6,973,000	42.8%	388,000	2.4%	1,949,000	12.0%	530,000	3.9%
≥80	6,272,000	68.3%	1,081,000	11.8%	2,164,000	23.6%	711,000	7.7%
Total	20,475,000	17.2%	1,749,000	1.5%	7,311,000	6.1%	2,218,000	1.9%

ICR/f Rat Model: Study Effects of Botanicals on Mechanism of Age-Related Human Cataract

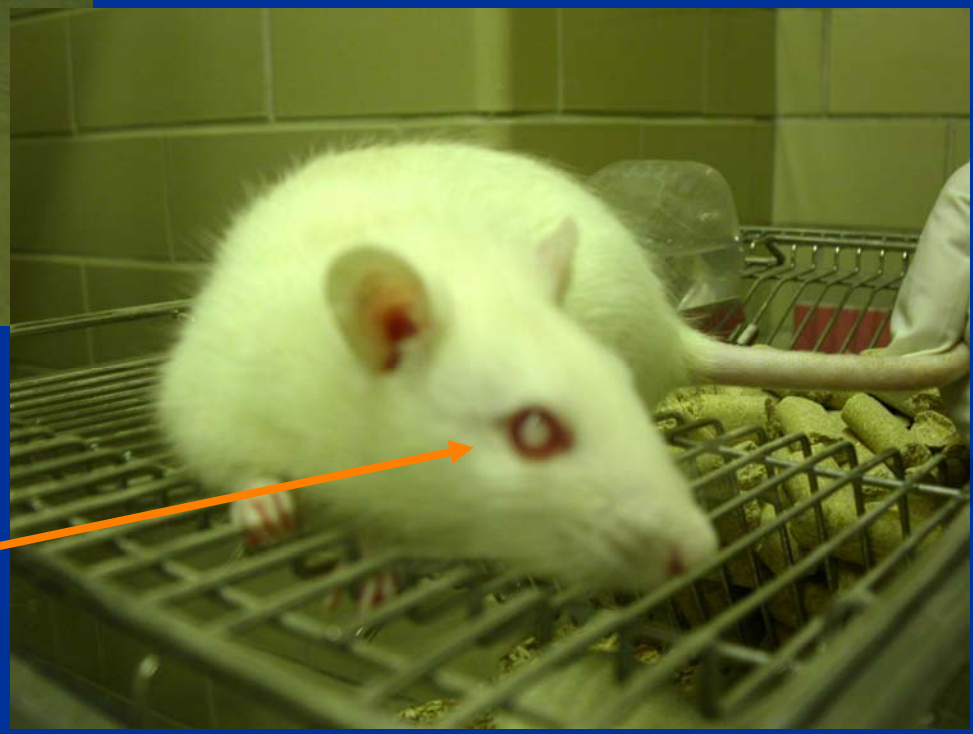
Cataract Disease in Rats

Sprague-Dawley Rat

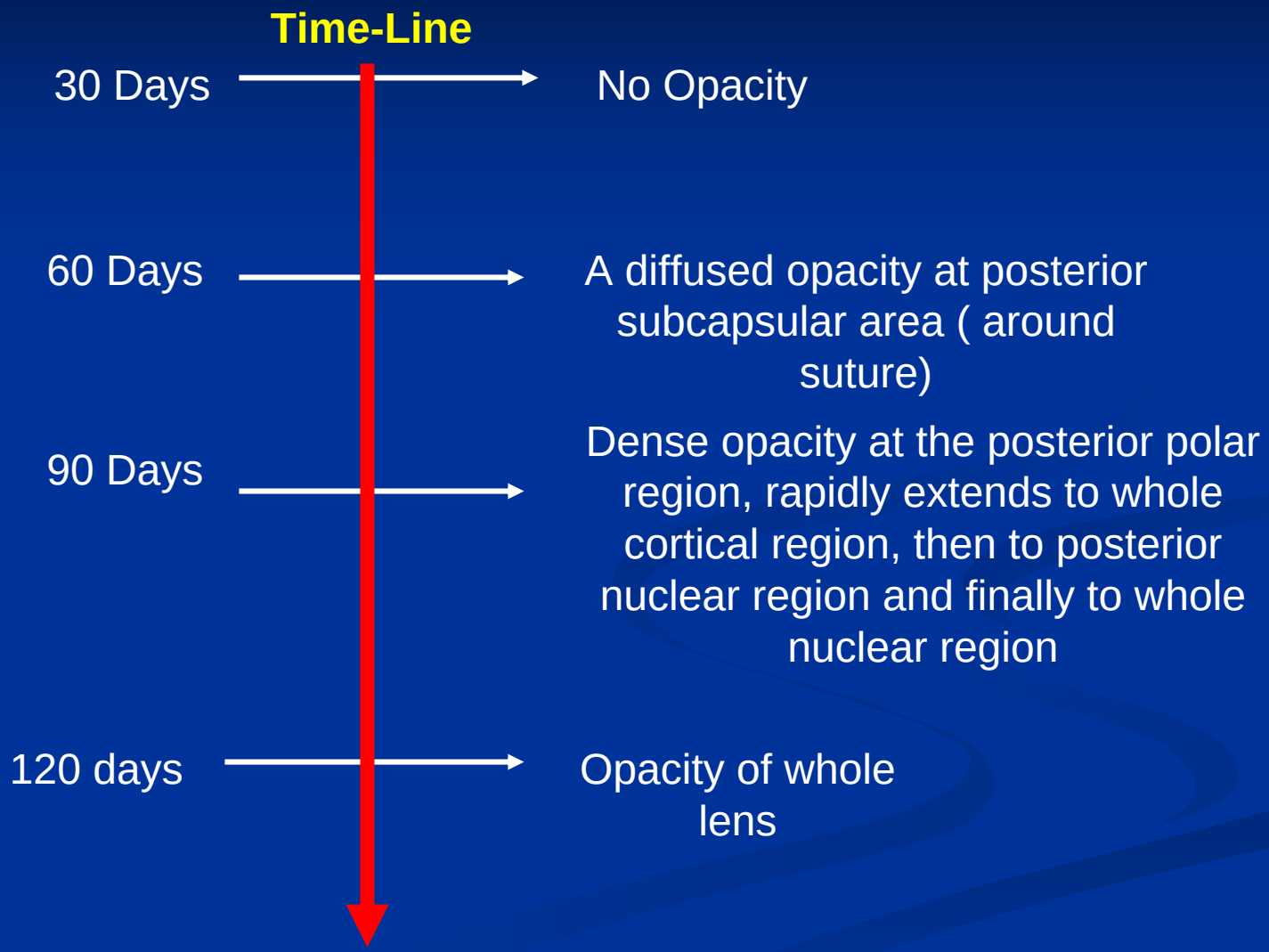


A colony of ICR/f rats has been established

ICR/f Rat



Development of Lens Opacity in ICR/f Rats



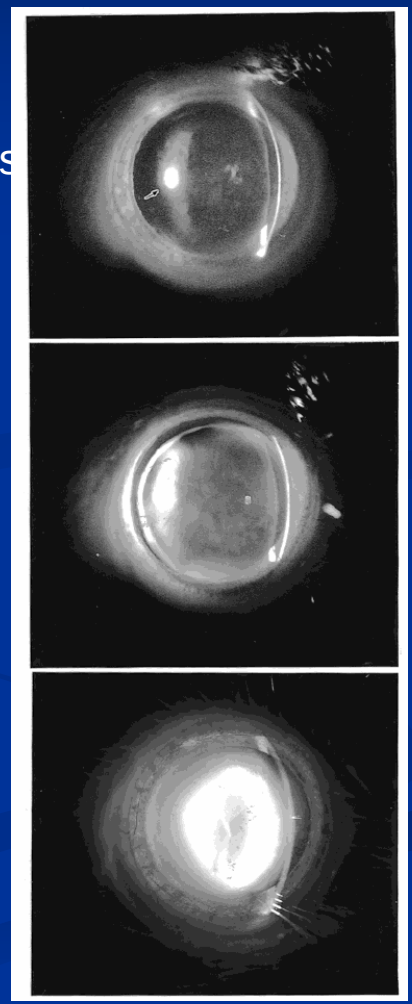
Grape Seed Extract Slows Onset of Cataract Disease in ICR/f Rats

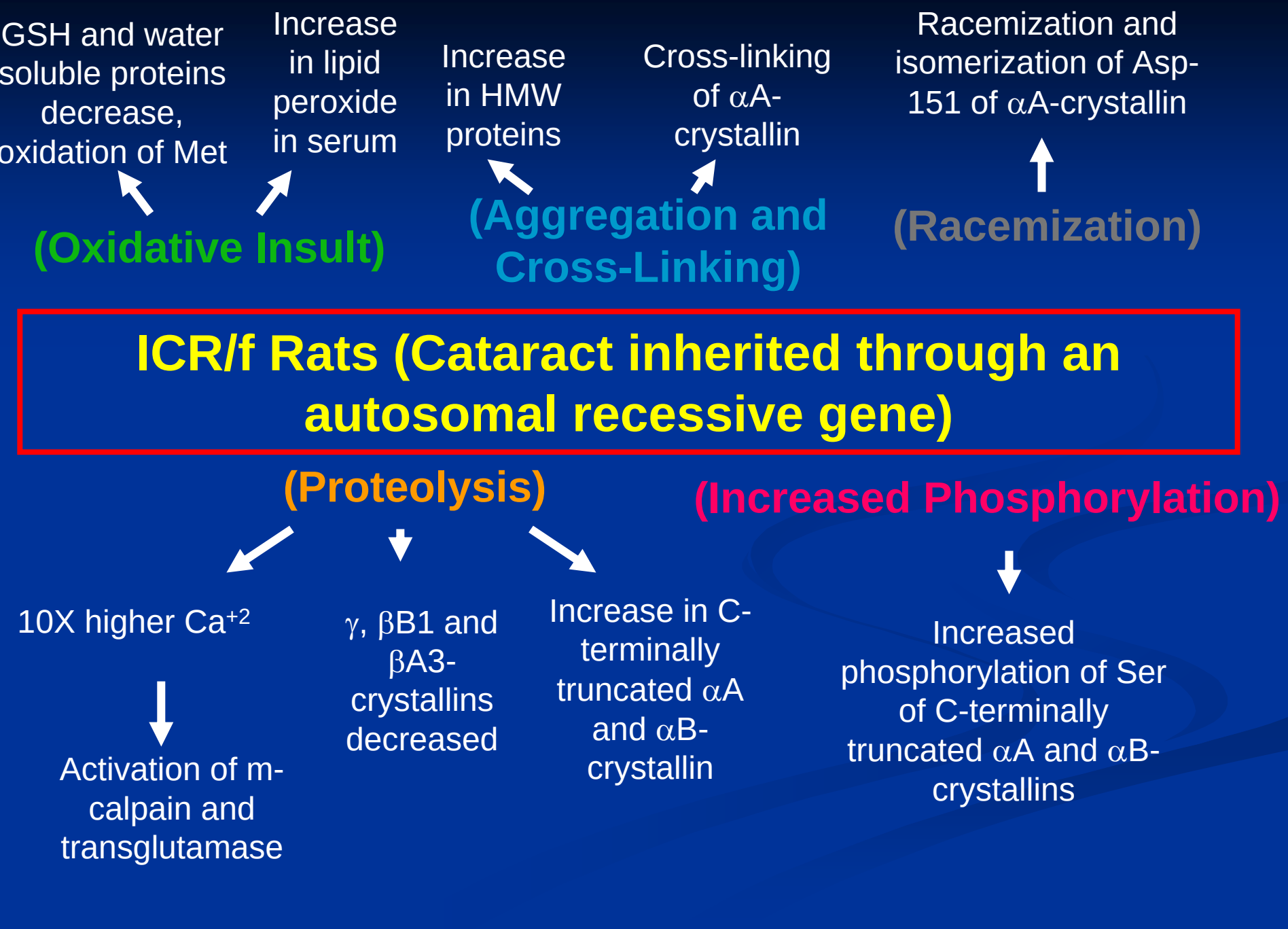
- **ICR/f rats imported from Meijo University in Japan to create a breeding colony at UAB**
- **Inbred strain derived from the original ICR rats**
- **Cataract formation significantly slowed by 0.2% grape seed extract in the diet (Yamakoshi et al., 2002)**
- **New experiments will evaluate a dose-response curve for PACNs (0.1-5%)**

Control at <30 days

GSE diet; 27 days later

Control diet; 27 days later




GSH and water soluble proteins decrease, oxidation of Met
Increase in lipid peroxide in serum
(Oxidative Insult)
Increase in HMW proteins
Cross-linking of αA-crystallin
(Aggregation and Cross-Linking)
Racemization and isomerization of Asp-151 of αA-crystallin
(Racemization)
ICR/f Rats (Cataract inherited through an autosomal recessive gene)
(Proteolysis)
10X higher Ca+2
Activation of m-calpain and transglutamase
γ, βB1 and βA3-crystallins decreased
Increase in C-terminally truncated αA and αB-crystallin
(Increased Phosphorylation)
Increased phosphorylation of Ser of C-terminally truncated αA and αB-crystallins

What is Molecular Mechanism of Cataract Development in ICR/f Rats?

How Do Age-Related Cataracts Develop?

Source: National Eye Institute

- **Clumps of protein accumulate in lens and become insoluble causing opacity.**
- **The clear lens slowly changes to a yellowish/brownish color, adding a brownish tint to vision.**

HYPOTHESIS

Lens α-, β- and γ-Crystallins [Water Soluble]

Post-translational modifications during Aging

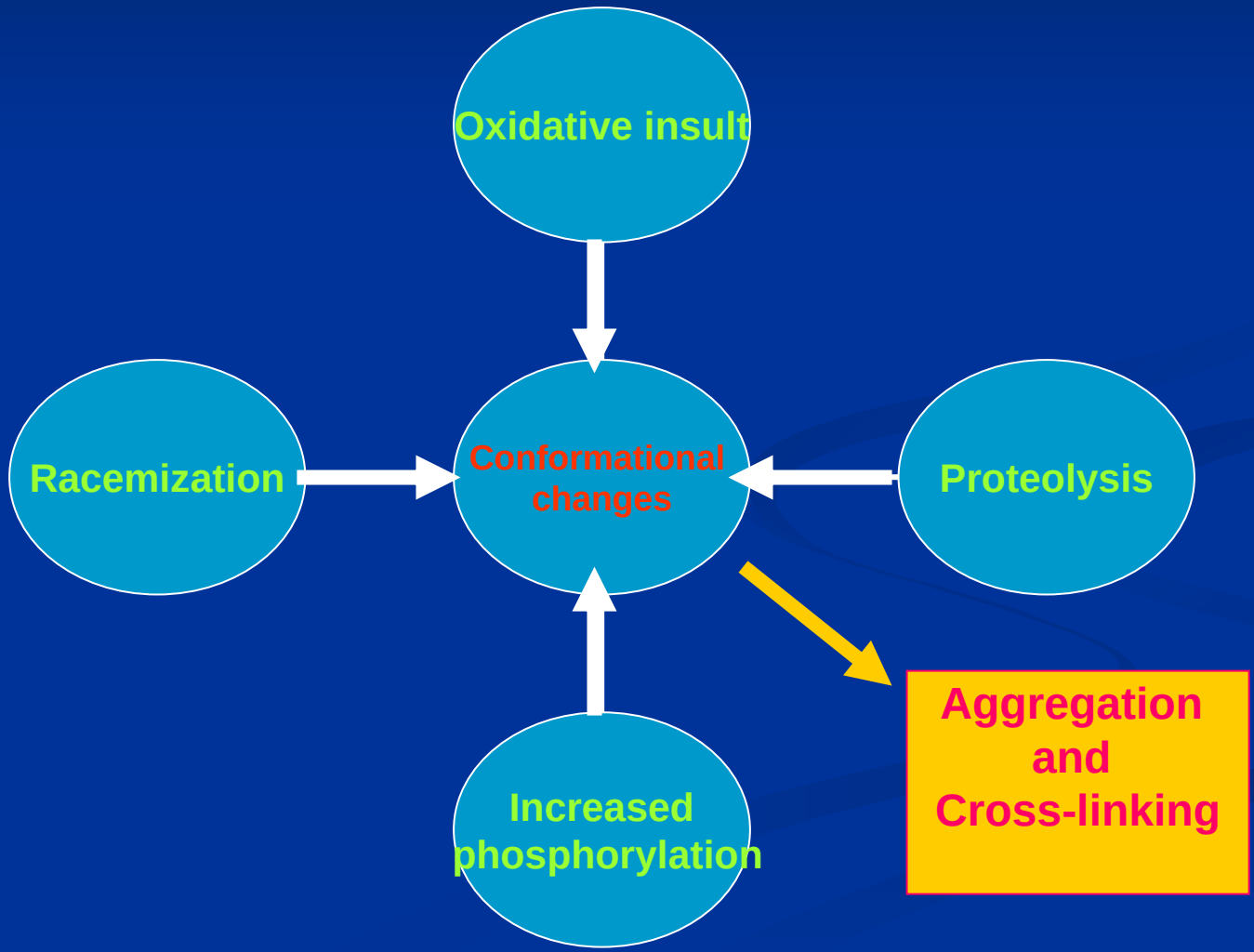
Conformation Changes

Aggregation (Hydrophobic interactions)
[Water Soluble-HMW- and Water Insoluble Proteins]

Covalent Cross-Linking
(Disulfide and non-disulfide types)
[Water Soluble and Water Insoluble]

Opacity [Water Insoluble]

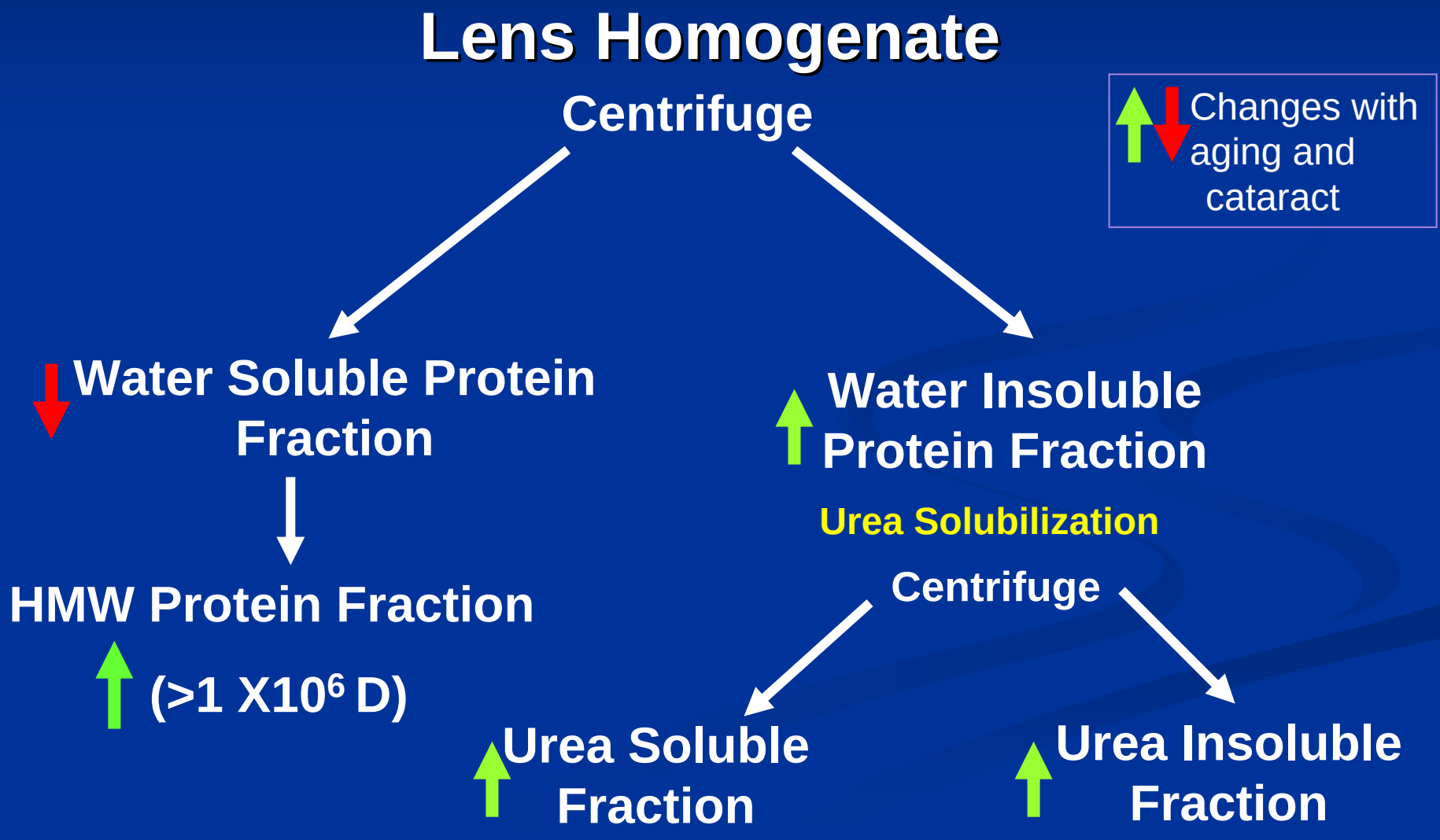
Conformational Changes Leading to Aggregation and Cross-Linking



Conformational Changes in Proteins and Protein Aggregates

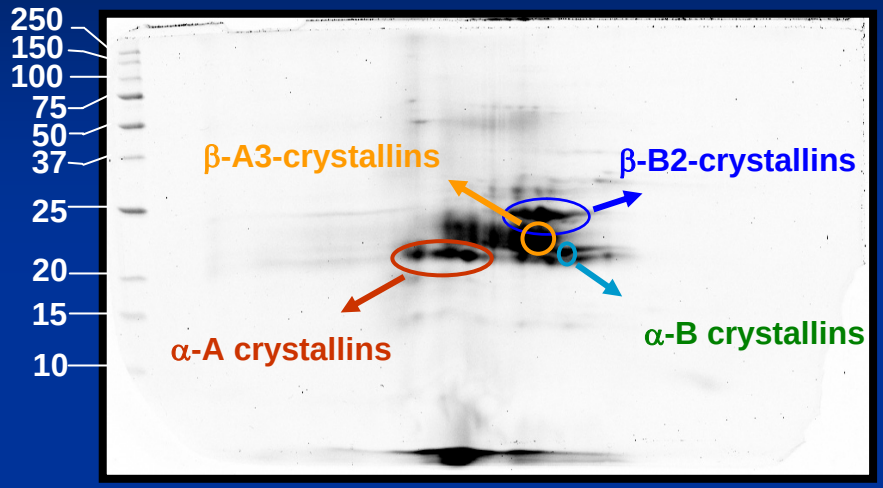
- Two-Dimensional gel Electrophoresis and Mass Spectrometric Analyses
- Molecular weights of Aggregates and Identification of their components (Dynamic Light-Scattering and Blue-native Gel Electrophoresis)
- Secondary and Tertiary Structures of Modified Proteins (CD Spectroscopy)
- Structural Changes in Modified Proteins (Determination of hydrophobicity and Trp fluorescence)

Fractionation of Human Lens Proteins

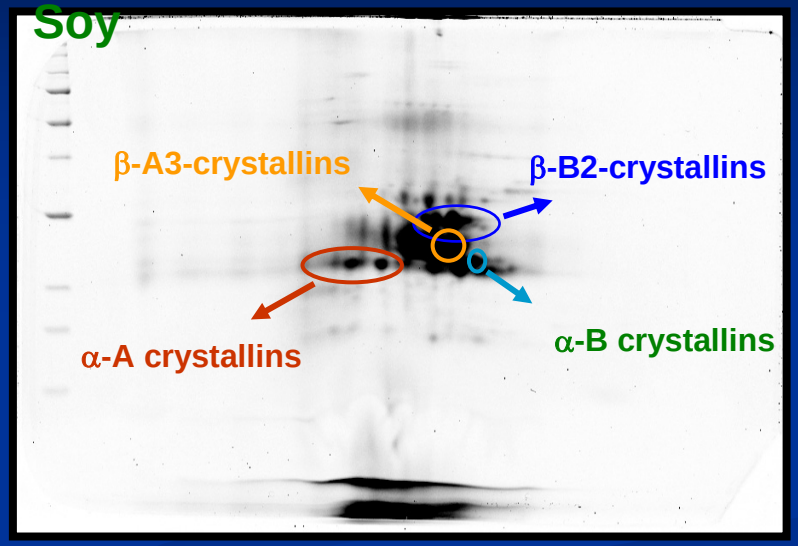


2DE-Profiles of Lens WS Proteins from Monkeys Fed Soy

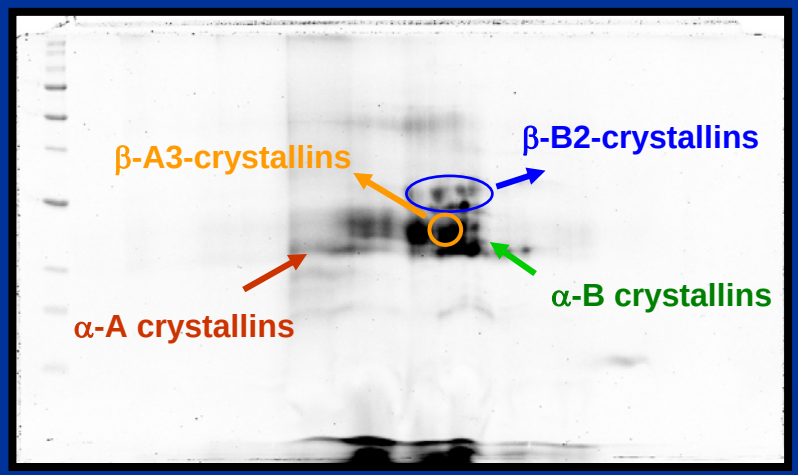
#89 Young_Control



#21 Old_high Soy



#57 Old_control



Molecular Mass of Protein Aggregates and Identification of their Components

(Dynamic Light-Scattering Method and Blue- native Gel Electrophoresis)

Determination of Molecular Weights and Hydrodynamic Radai of WS-HMW Proteins

WS-Proteins

↓

Size-exclusion HPLC using TSK G-5000 PW_{XL} Column

↓

Monitor protein absorbance, light scattering by QUELS (quasielastic light scattering device) and refractive index (DAWN HELEOS)

↓

Determine absolute molar mass and hydrodynamic radius

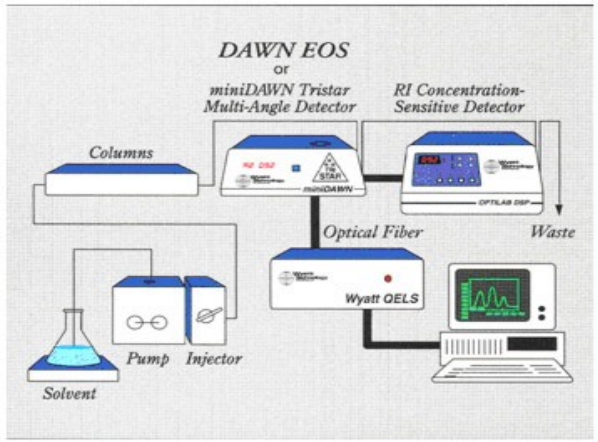
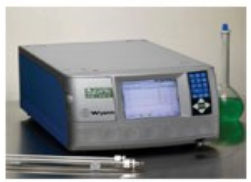


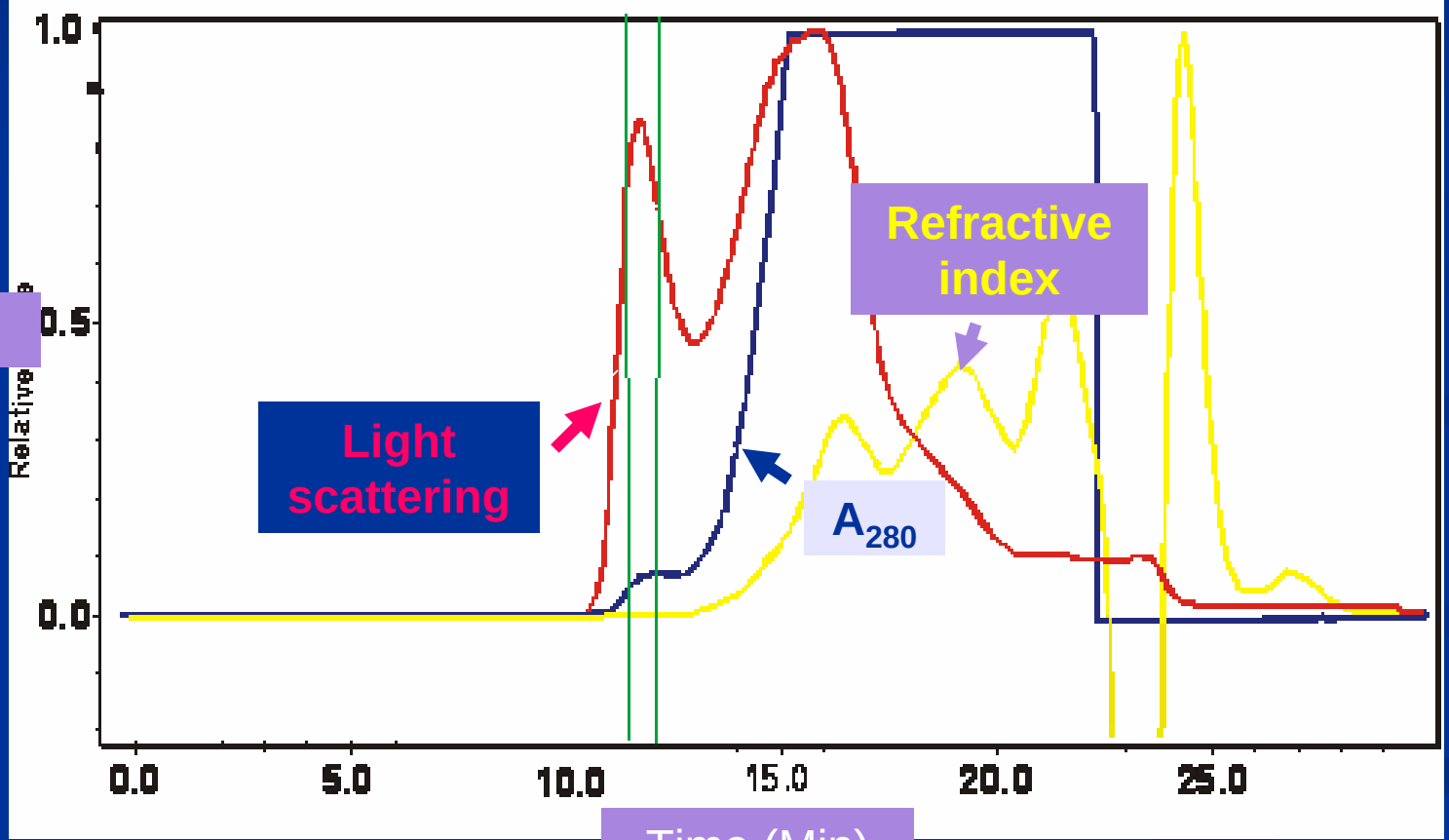
Figure 1: A typical configuration of HPLC system, multi-angle detector (DAWN-HELEOS [shown as an older model DAWN EOS], Wyatt QELS and RI-detector). We plan to add an HPLC system that includes a pump, a controller, and a UV-detector.



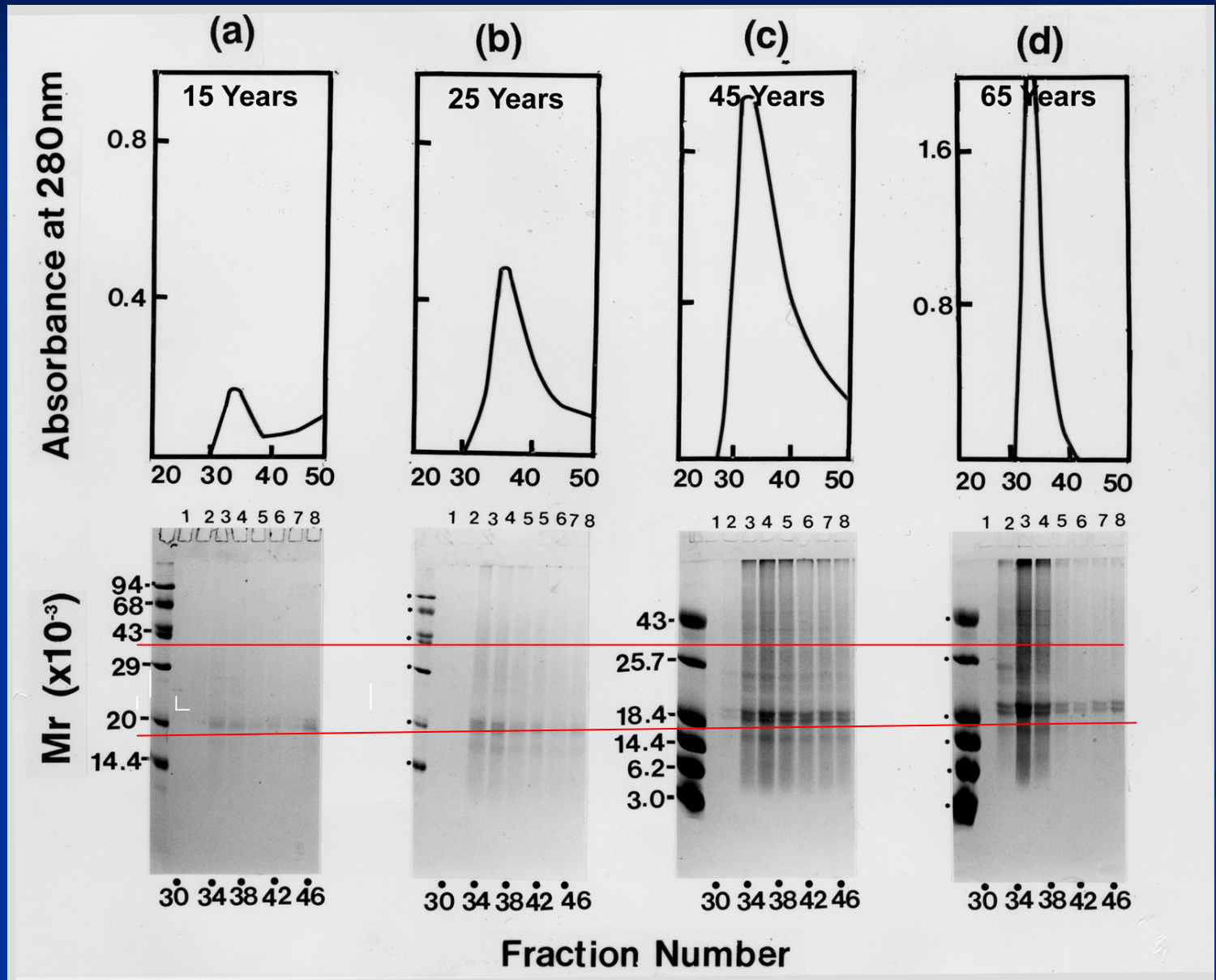
Dawn Heleos

Optilab (refractive index detector)

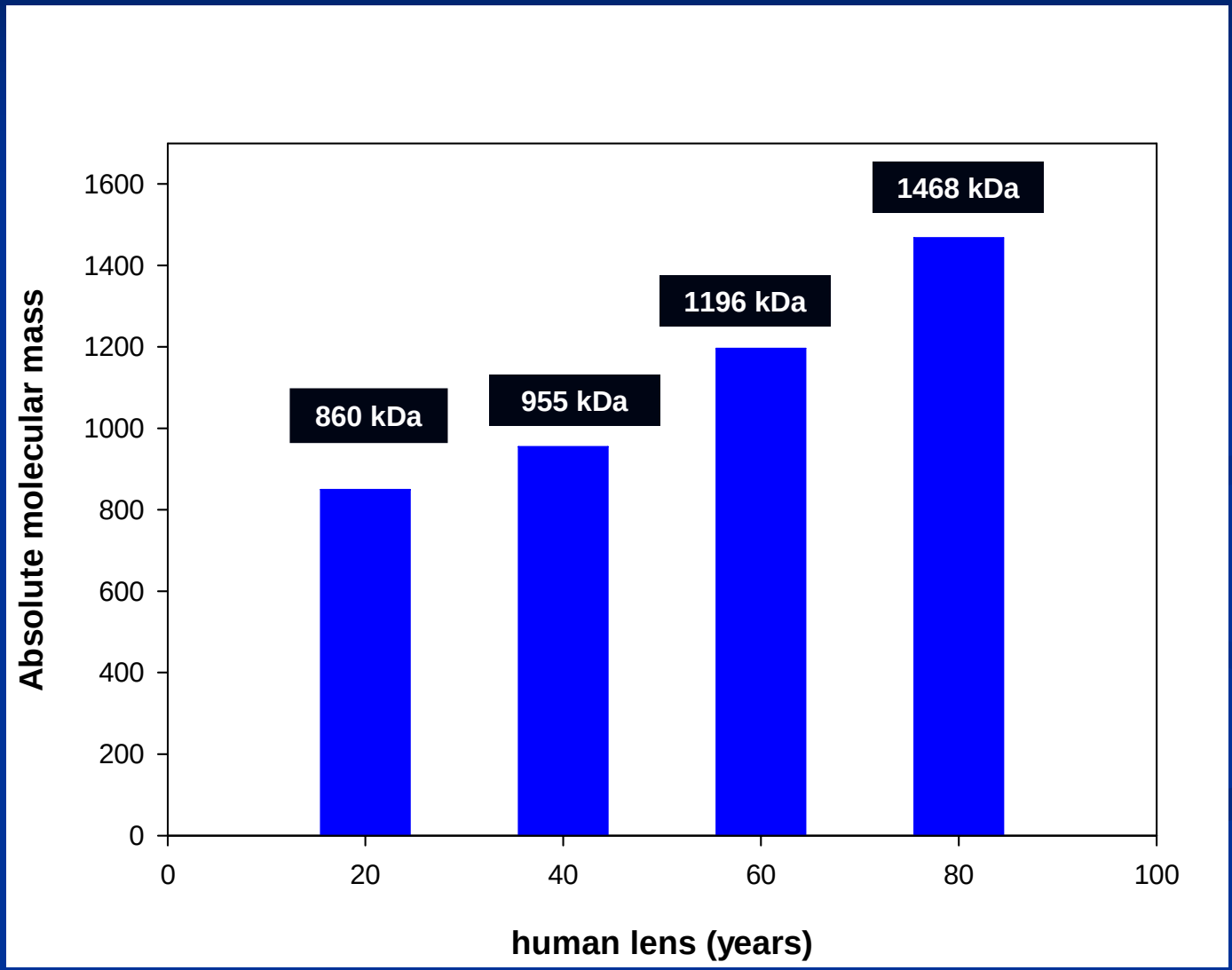
Protein Profile of WS-Proteins from a 60 Year-Old Human Donor



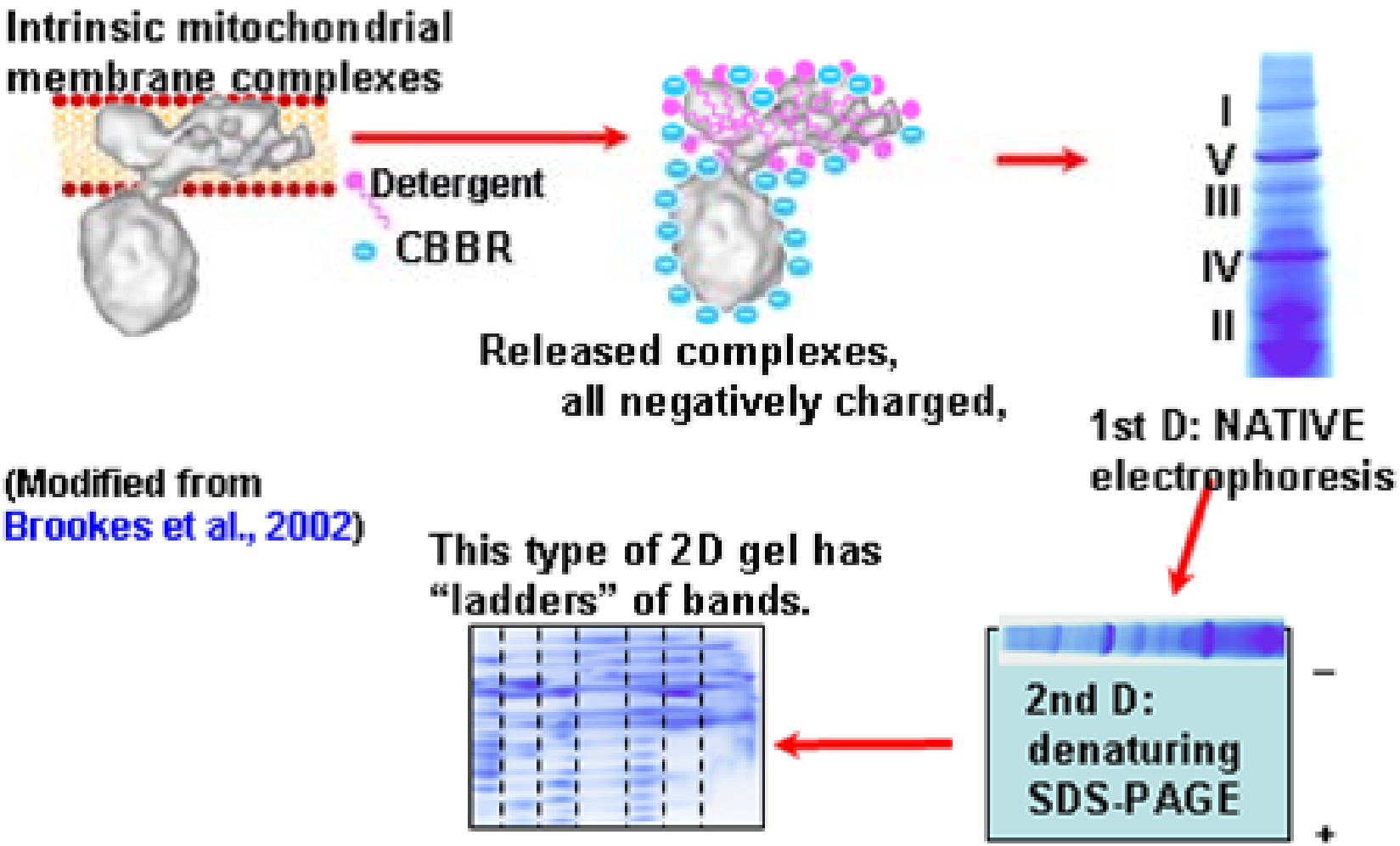
WS-HMW Proteins in Aging Human Lenses After Size-Exclusion Chromatography



Molecular Mass of HMW Proteins from Human Lenses



2D-Blue Native Gel Electrophoresis to analyze Protein Aggregates



(Modified from Brookes et al., 2002)

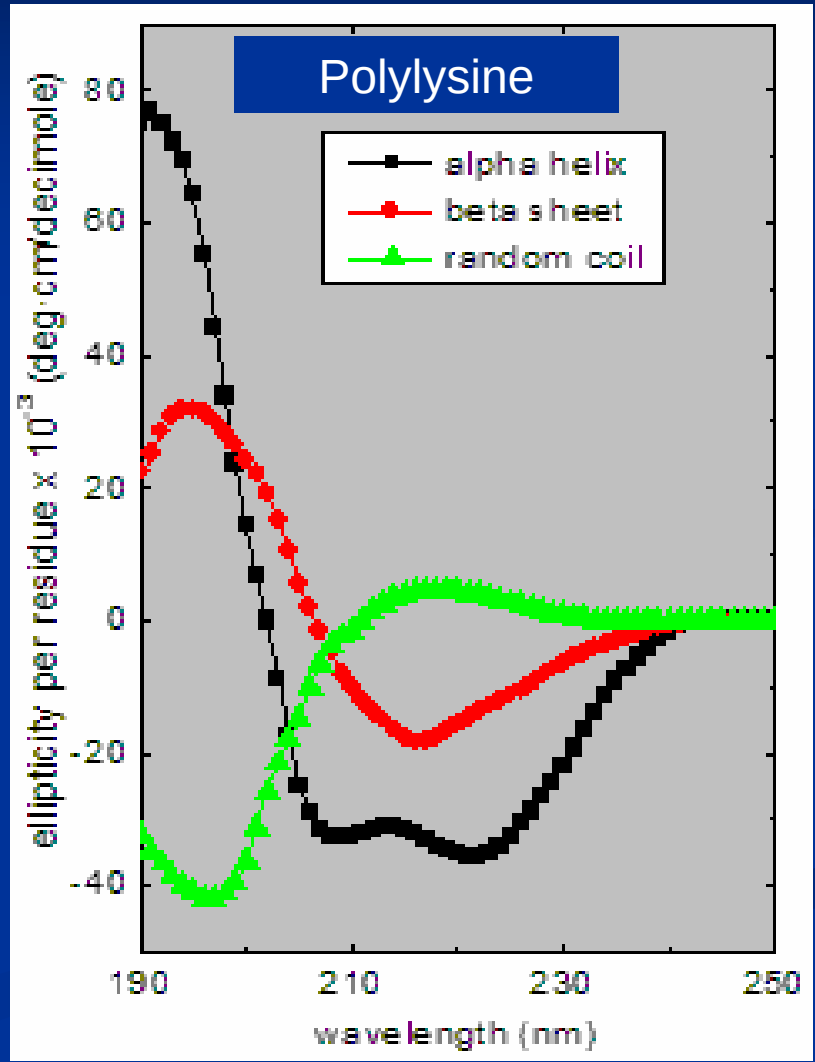
Secondary and Tertiary Structures of Modified Proteins (CD Spectroscopy)

Circular Dichroism Spectroscopy is Particularly Good for:

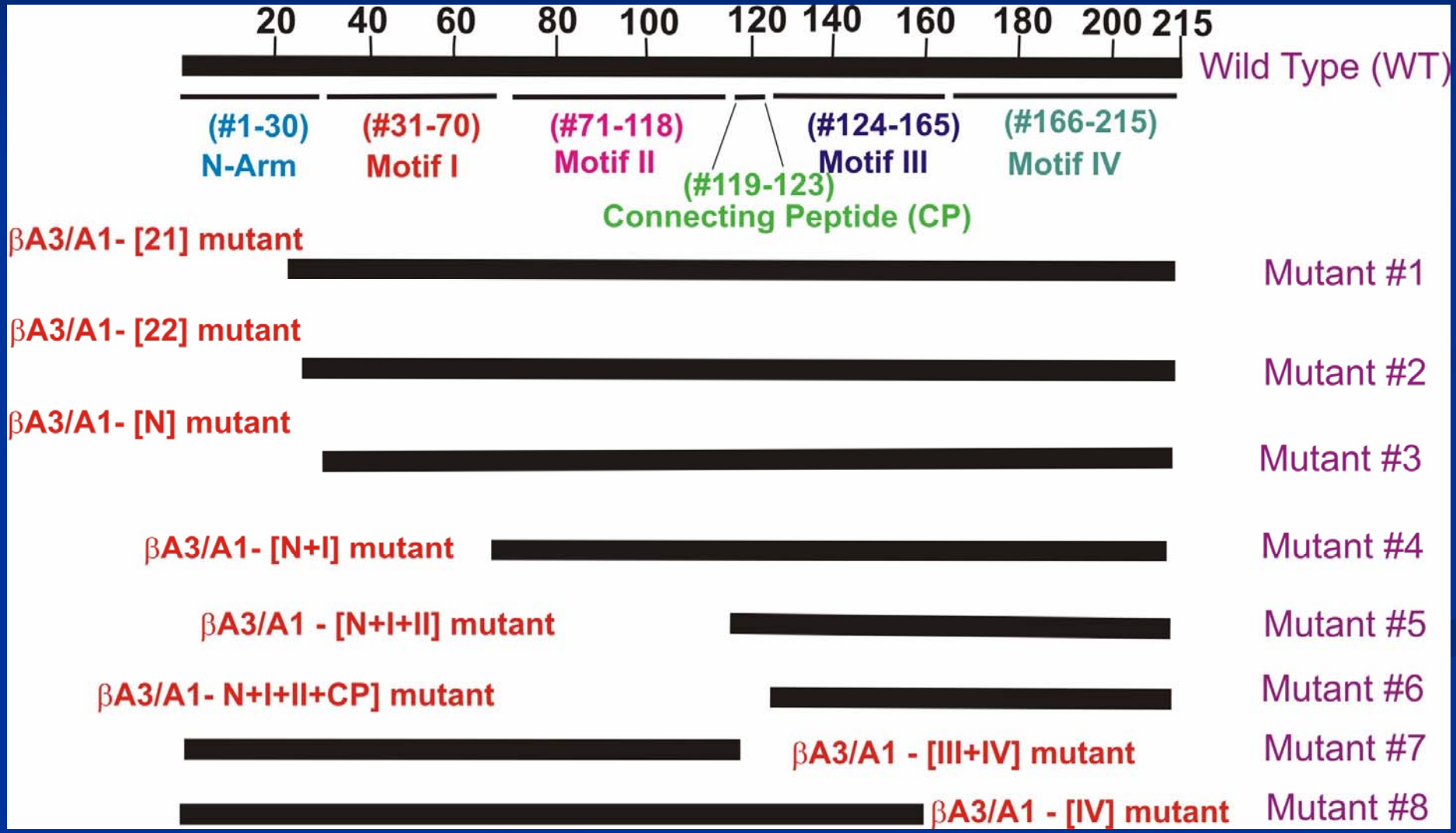
- Secondary and tertiary structures of proteins
- Comparing structures for different mutants of the same protein
- Studying the conformational stability of a protein under stress -- thermal stability, pH stability, and stability to denaturants
- For finding solvent conditions that increase the melting temperature and/or the reversibility of thermal unfolding conditions which generally enhance shelf life.
- Determining whether protein-protein interactions alter the conformation of protein.

Determination of Protein Secondary Structure by Circular Dichroism

- Secondary structure can be determined by CD spectroscopy in the "far-uv" spectral region (190-250 nm). At these wavelengths the chromophore is the peptide bond, and the signal arises when it is located in a regular, folded environment.



Mutants of Human Lens Beta A3-Crystallin



CD Spectra of Mutant Proteins of βA3-Crystallin

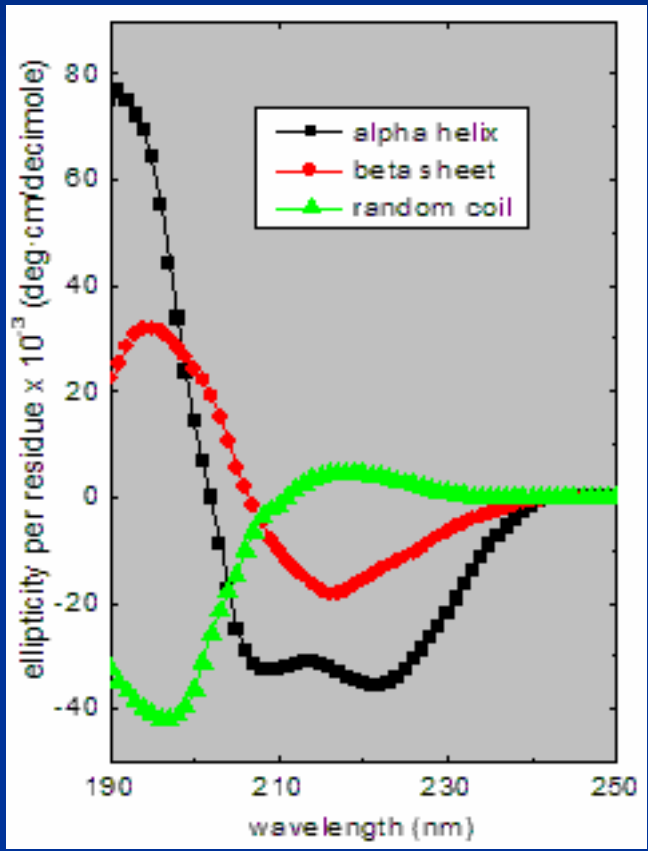
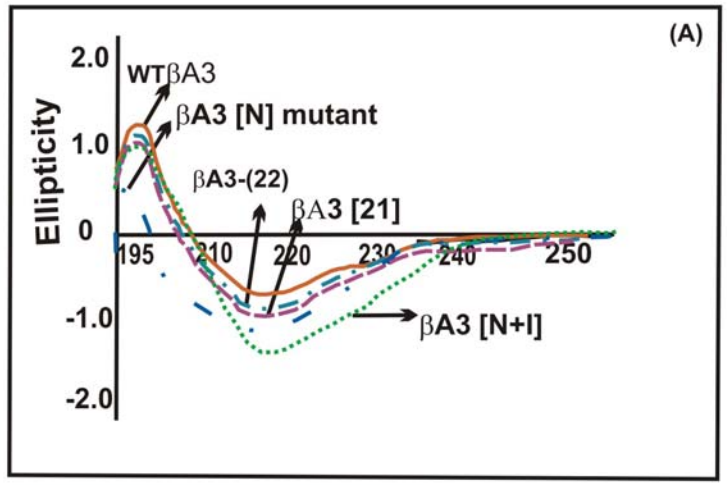
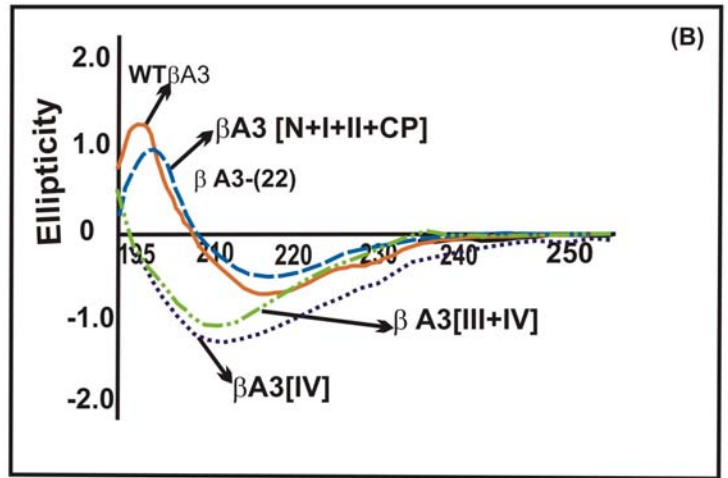
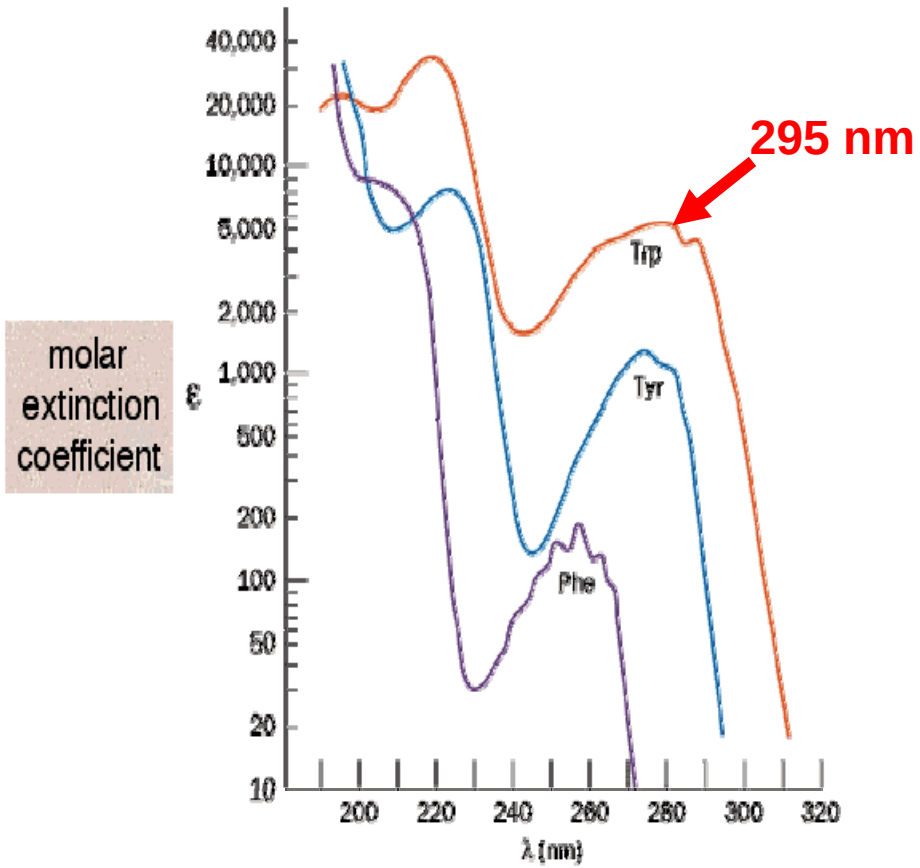


Figure 5





Absorption Spectra of Phe, Trp and Tyr

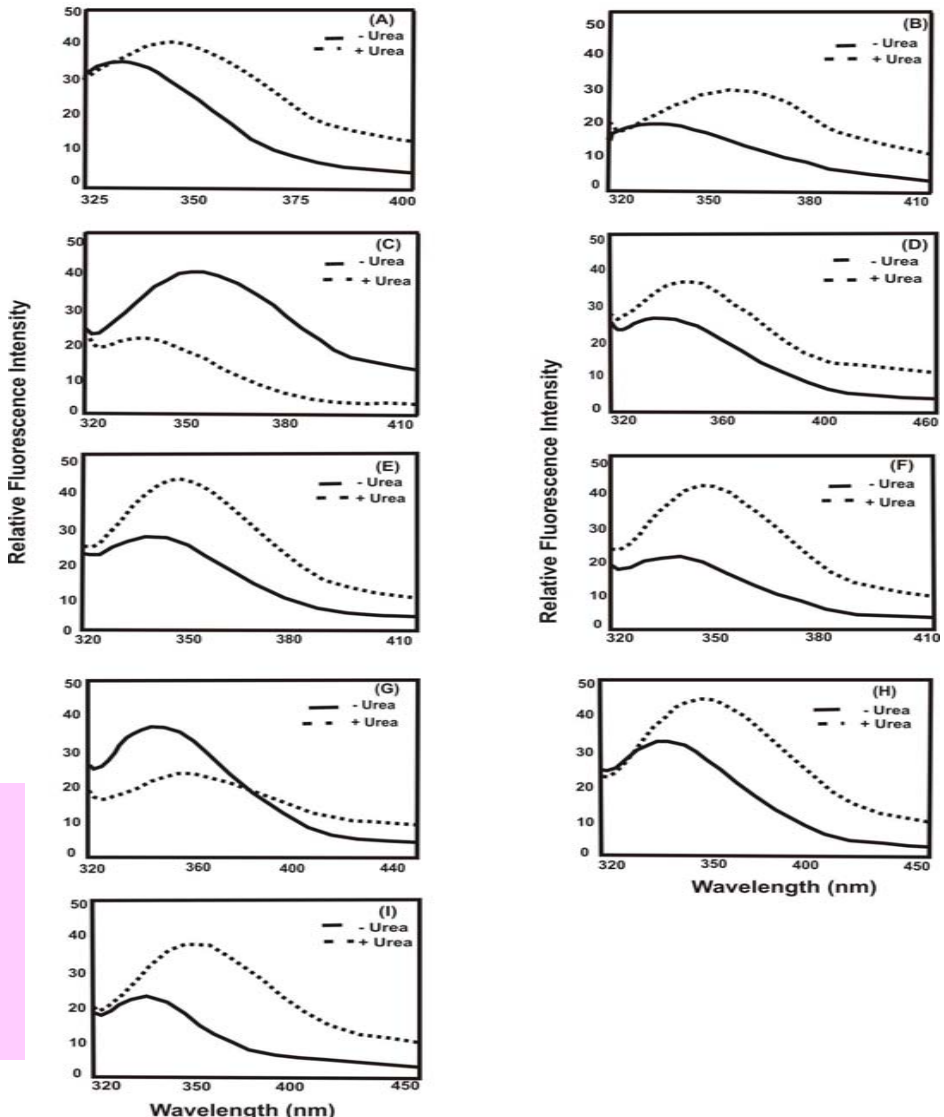


UV absorbance spectra of the three aromatic amino acids, phenylalanine, tryptophan, and tyrosine

Intrinsic Trp Fluorescence Spectra of Native and Denatured WT-βA3 and its eight Truncated Mutant Proteins.

Intrinsic Trp Fluorescence Spectra:

- **Excitation at 295 nm and emission between 300-400 nm.**
- **Quenching, red or blue shift suggest change in microenvironment of Trp residues.**



βA3-crystallin contains 9Trp residues; two are exposed (139, 153), four are buried (73, 99, 168, and 198) and three are partially buried (36, 96, 195).

Fluorescence Spectra of WT βA3 and its Eight Deletion Mutant Proteins After ANS Binding

ANS (8-amilino 1-naphthalenesulfate):

- **A hydrophobic probe**
- **Binding is assessed by fluorescence spectra (Excitation at 390 nm and emission between 400 to 600 nm)**
- **Quenching; Reduced binding**
- **Red Shift: Increased exposure of hydrophobic patches.**
- **Blue Shift: Decreased exposure of hydrophobic patches.**