

Eye lens proteomics

Review Article

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Summary. The eye lens is a fascinating organ as it is in essence living transparent matter. Lenticular transparency is achieved through the peculiarities of lens morphology, a semi-apoptotic process where cells elongate and loose their organelles and the precise molecular arrangement of the bulk of soluble lenticular proteins, the crystallins. The 16 crystallins ubiquitous in mammals and their modifications have been extensively characterized by 2-DE, liquid chromatography, mass spectrometry and other protein analysis techniques. The various solubility dependant fractions as well as subproteomes of lenticular morphological sections have also been explored in detail. Extensive post translational modification of the crystallins is encountered throughout the lens as a result of ageing and disease resulting in a vast number of protein species. Proteomics methodology is therefore ideal to further comprehensive understanding of this organ and the factors involved in cataractogenesis.

Keywords: Eye lens – Proteomics – Crystallins – 2-DE – Liquid chromatography – Mass spectrometry

Introduction

Proteomics research on the eye lens has a long history. The lens is an avascular cellular conglomerate whose function is to guarantee proper light refraction and visual acuity (Bloemendal, 1977). Following the development of the lens nucleus from the posterior epithelial cells of the lens vesicle, lenticular cells cease mitosis with the exception of the cuboidal cells of the anterior epithelium. These cells are eventually passed from the central region of the anterior epithelium through the germinative region to the lens equator, where they undergo differentiation into fiber cells. The cuboidal cells elongate and synthesize large amounts of proteins of the crystallin protein super family (Mörner, 1894) and at the terminal stage of differentiation loose their organelles in a process reminiscent of

apoptosis (Papaconstantinou, 1967; Bassnett, 2002). These so called secondary fiber cells are layered onto the lens nucleus and successively onto each other forming the lens cortex. Lenticular development is essentially complete a few days after birth but continues at a slowed rate throughout life.

The lack of organelles, high protein concentrations and the short range order of the soluble proteins are necessary for lens transparency (Delaye and Tardieu, 1983). Naturally, aspects of lenticular biology vary greatly between species, reflecting optical specialization and environmental demands. The human and fish lens represent two extremes, the former, like diurnal animals having a softer more uniform mould, while the latter is clearly divided into a hard nucleus and a much softer cortical region (Koretz and Handelsmann, 1988; Fernald and Wright, 1983). The rodent lens is very hard with evidence from Raman spectroscopy suggesting the proteins in its core are denatured (Yu et al., 1985). Concurrently, refractive indices also vary greatly between species (Kröger et al., 1994).

Thirty to thirty-five percent of bovine lens wet weight is protein the other 65% is virtually water. The crystallin protein super family comprises 90% of lenticular protein mass (Bloemendal, 1977). The 16 crystallins ubiquitous in mammals undergo extensive post translational modification so that a vast number of protein species are in constant flux in various degrees of dynamic interaction both physically and physiologically (Bhat, 2004) maintaining the soluble basis for lenticular transparency. Serious perturbations of this balance leads to improper protein association,

aggregation and insolubilization. Incident light is diffracted by the protein aggregates resulting in lens opacification and a more or less grievous loss of visual acuity clinically termed cataract (Lambert, 1994).

Proteomics evidence characterizing various aspects of lens proteins has accumulated over the years. The term proteome was initially introduced in 1995 (Wasinger et al., 1995) and defines a samples full protein complement. One must bear in mind that the proteome is highly dynamic as transcription, translation and protein modification activity to name just three primary cellular processes are constantly changing. While methods such as *in situ* labeling that realize the protein constituents at a precise point in an organism's life are available, the core proteomics techniques, 2-DE and LC for protein and/or peptide separation and MS for identification, applied by themselves realize a samples history up to the point of analysis. Both applications are valid and should be considered for the problem of interest. Also, many prefractionation techniques can be implemented reducing proteome complexity or for the enrichment of specific subproteomes (Jungblut and Klose, 1985). Most importantly, advances in proteomics have shown the high degree of modification undergone by many primary translation products (Scheler et al., 1997) as well as individual heterogeneity (Klose et al., 2002) thus demanding an increased consciousness for the protein species concept (Jungblut et al., 1996; Jungblut and Thiede, 1997), i.e. the understanding of the proteome on a molecular level.

The eye lens, consisting almost exclusively of protein and water is ideal for proteomics research, which in turn has proven invaluable for characterizing the healthy lenticular protein constituents both of the whole lens and various morphological sections in various species and in the elucidation of some of the complex factors involved in cataractogenesis. In the course of this review with a few important exceptions we will limit ourselves to discussing work based on 2-DE, LC and MS arbitrarily drawing the line between proteomics and protein analysis and techniques such as SDS-PAGE, Edman degradation and Western blot.

The lenticular proteome

The crystallins are the primary soluble lenticular proteins in all vertebrate species comprising about 90% of total lenticular protein mass. They are divided into three major classes, alpha (α), beta (β) and gamma (γ) crystallins. Evolutionary constraints have imparted a high degree of stability on this protein super family, as lenticular

cells devoid of cellular machinery are unable to turnover proteins.

Native alpha crystallin is an oligomer with a Mw in excess of 300 kDa consisting of up to three types of monomeric subunits in dynamic exchange (van der Ouderaa et al., 1973; Puri et al., 1983; van den Oetelaar et al., 1990; Gesierich and Pfeil, 1996; Bova et al., 1997). The two major alpha crystallin monomers are the products of two genes, alpha A and alpha B and share this terminology. They are 60% identical in primary structure (Schoenmakers et al., 1969; Bloemendal and de Jong, 1991). The third alpha crystallin monomer is an alternative splicing product of the alpha A crystallin gene and is termed alpha A insert crystallin (Hendriks et al., 1990). The alpha A chain has a Mw around 20 kDa, alpha B around 22 kDa and alpha A insert around 24 kDa. The monomers have a global, hydrophobic N-terminal domain characterized by α -helical structure function regions (Smith et al., 1996; Pasta et al., 2003), a β -sheet structured C-terminal region containing the so called "alpha crystallin domain" (Caspers et al., 1995) and a C-terminal extension. The alpha crystallin domain is implicit to all small heat shock proteins and it was shown that alpha B crystallin is indeed a member of this family (Klemenz et al., 1991) and that the alpha A crystallin chain can function as a molecular chaperone (Horwitz, 1992).

Both native and recombinant alpha crystallin has not been successfully crystallized due to its polydisperse nature, however models of the monomers tertiary structures (Farnsworth et al., 1997) and the functional oligomers quaternary structure have been proposed (Tardieu et al., 1986; Groenen et al., 1994; Haley et al., 1998; Horwitz et al., 1998; Abgar et al., 2000). In a typical mammalian lens alpha crystallin constitutes about 35% of proteins and is synthesized throughout lens development and is thus evenly distributed throughout the organ (Aarts et al., 1989) with trace amounts being detected in other tissues (Iwaki et al., 1990; Srinivasan et al., 1992). Apparently alpha crystallin is not only a major structural component of transparent lenticular architecture but its properties as a molecular chaperone are an important factor in maintaining lenticular homeostasis.

The beta and gamma crystallins are organized in two structural domains with a short connecting peptide. The domains are in turn made up of two consecutive greek key motifs each consisting of four β -strands which intercalate and arrange the domain in anti-parallel β -sheet conformation (Driessen et al., 1981; Wistow et al., 1981; Wistow and Piatigorsky, 1988; Slingsby et al., 1996; Carver, 1999). Native beta crystallins assemble in homo- or heterodimers

which can dissociate into monomers or associate in higher Mw oligomers probably up to octamers while gamma crystallins generally do not associate (Berbers et al., 1984; Bateman et al., 2001; Hejtmancik et al., 1997, 2004; Slingsby and Bateman, 1990; Zarina et al., 1994). These similarities in protein secondary and tertiary structure suggest a common ancestral gene encoding a single motif and a series of ancient gene duplication and recruitment events resulting in the genetic diversification of beta and gamma crystallins in today's vertebrate species (Wistow and Piatigorsky, 1988; Lubsen et al., 1988; Norledge et al., 1996; Clout et al., 2000).

There are six mammalian beta crystallin genes and similar genes detected in amphibian and fish expressing three basic (B1–B3) and four acidic (A1–A4) beta crystallin proteins. Beta A1 and A3 crystallin are encoded by the same gene from distinct transcription start codons, the beta A3 start codon being upstream to A1. The beta crystallin monomers have molecular weights ranging from 22 kDa (beta A4) to 28 kDa (beta B1) (Berbers et al., 1984). They are characterized by N- and C-terminal extensions reaching beyond the molecular domains and a short connecting peptide between the domains. It is an accepted hypothesis that these extensions and the connecting peptide are instrumental in dimer- and oligomerization of beta crystallins (Carver et al., 1993; Cooper et al., 1993a, 1993b) initiated by 3D domain swapping (Bennett et al., 1994, 1995) with the extensions promoting higher order association beyond the dimer (Lampi et al., 2001; Bateman et al., 2001, 2003). The beta B1 and B3 crystallin genes are expressed early in lenticular development, consequently their proteins are found primarily in the lens nucleus. Beta B2 is often seen as the main beta crystallin and the acidic beta crystallins are found throughout the lens in the nucleus and cortex (Aarts et al., 1989; Chambers and Russell, 1991; Lampi et al., 1998; Ueda et al., 2002a).

The six gamma crystallin proteins common to mammals, gamma crystallins A through F, are linked to a gene cluster of highly homologous genes (Shinohara et al., 1982; Breitman et al., 1984; Lok et al., 1984; Goring et al., 1992; Graw et al., 1993). They are among the earliest gene products in lenticular development in fiber cells and like the beta crystallins they are fiber cell specific. It has been suggested that the mammalian gamma crystallins constitute the tightest molecular arrangement among crystallins and exclude water to the highest degree being constituents of the harder lens nucleus especially in rodents (Slingsby, 1985). Gamma S crystallin, the only gamma crystallin detected in all vertebrate species and

present in numerous tissues outside the lens is particularly suited to water rich tissue regions and can thus be regarded as a separate branch in vertebrate gamma crystallin gene evolution (van Rens et al., 1989). Not all beta and gamma crystallin polypeptides are detected in all mammalian species reflecting the proteins diverse nature and ideal suitability to environmental demands.

Crystallins are extensively modified in the course of healthy lenticular development beginning at an early onset. Unhealthy protein modification eventually results in an organism developing a cataract or other disease phenotype if a certain threshold is surpassed and perpetuated. The concept is emerging that the crystallins in high concentration are more than physical structural lens components, but that their protein species possess additional manifold catalytical or regulatory functions also essential for lens transparency (Bhat, 2004). Thus the dynamics of this protein super family and indeed of the lens itself are only beginning to be understood. A standard and two newer comprehensive crystallin reviews are cited for further reading (Graw, 1997; Bhat, 2003; Bloemendal et al., 2004).

Another central concern in guaranteeing light transmission without diffraction is proper lens architecture (Kuszak et al., 1994). Fiber cell shape is maintained by an extensive cytoskeleton whose protein components are synthesized and finalized prior to terminal differentiation (Kuwabara, 1975; FitzGerald, 1988). Actin, synthesized in the epithelial cells is polymerized from globular actin (Ramaekers et al., 1981) while the intermediate filament protein vimentin also synthesized throughout the epithelium and the elongating fiber cells is eliminated (Bradley et al., 1979; Sandilands et al., 1995). The major component of the differentiated fiber cell cytoskeleton, the lens unique beaded filament structure is established last (Maisel and Perry, 1972). The beaded filament is made up of two proteins, phakinin and filensin also termed CP49 and CP95, and has a 5–7 nm backbone with 20 nm beads evenly spaced throughout. It associates with alpha crystallin and is itself membrane associated and has a primary function in lens architecture (Ireland and Maisel, 1984, 1989; Carter, 1995).

Membrane proteins such as gap junctions constituents also contribute to cellular stability and arrangement. The maintenance of homeostasis of the interior fiber cells in the lens is attributed to a network connecting the cells to each other, to the differentiating cells at the lens equator and to the mitotically active cells of the anterior epithelium (Robinson and Patterson, 1982–1983; Mathias et al., 1997). Gap junctions consisting of two hemichannels each

made up of six connexin proteins are a large part of this network (Goodenough, 1992; Rae et al., 1996). The $\alpha 1$, $\alpha 3$ and $\alpha 8$ connexin proteins have been detected in the lens and so naturally contribute to stability and proper cellular arrangement (Gong et al., 1997).

Sample treatment and prefractionation

Proper sample handling and sensible prefractionation are prerequisites for any successful proteomics experiment. Following excision the lens can be decapsulated either removing the mitotically active cells of the anterior epithelium (Toyofuku and Bentley, 1970) or leaving them attached to the underlying cortical fibers (Dewey et al., 1995). The decapsulated organ can be dissected further into the major morphological regions termed the lens cortex and nucleus which in turn can arbitrarily be subdivided into distinct zones such as various cortical layers and the embryonic-, fetal- or adult nucleus (David and Shearer, 1984; Garland et al., 1996). These regions can be analyzed individually or the organ can be analyzed in its entirety foregoing dissection.

Prior to protein extraction lenticular material is often frozen in liquid nitrogen and ground to a powder or homogenized directly. The material can then be resuspended in distilled water or an aqueous buffer yielding the water-soluble fraction (WS) upon excitation and centrifugation (Clark et al., 1969; Kibbelaar and Bloemendal, 1975). The resulting pellet can be solubilized by sonication in aqueous buffer, pH manipulation or most common in buffer containing 6 M urea yielding the water-insoluble fraction (WI) in the supernatant upon further centrifugation (Ortwerth et al., 1986; Ortwerth and Olesen 1989, 1992; Kibbelaar and Bloemendal, 1975). The remaining pellet is termed the urea-insoluble fraction (UI) and can be solubilized in buffer containing high SDS concentrations (Kibbelaar and Bloemendal, 1975). Alternatively, the starting material can be resuspended directly in a buffer containing 6 M urea resulting in the urea-soluble fraction (US) in the supernatant and the urea-insoluble fraction in the pellet upon centrifugation. The urea-soluble fraction termed here constitutes the water-soluble and water-insoluble protein fractions described by Kibbelaar and Bloemendal (Kibbelaar and Bloemendal, 1975).

In many cases size exclusion chromatography is employed for the further fractionation of the raw protein extracts. The water-soluble (WS) protein fraction of the total lens or of morphological sections is generally separated by size exclusion chromatography into the high molecular weight (HMW), alpha (α), beta high (β_H) and

beta low (β_L), and gamma (γ) fractions (Kibbelaar and Bloemendal, 1975; Berbers et al., 1982; Ringens et al., 1982; Kleiman et al., 1988; Kelley et al., 1993) with slight variations depending on the material and age. The HMW fraction consists of protein aggregates considered to be on the verge of insolubilization and precipitation (Jedziniak et al., 1978) and contains mainly alpha crystallin (Messmer and Chakrabarti, 1988; Chiou and Azari, 1989) and is isolated in the chromatographic void volume. The α fraction contains the native alpha crystallin oligomers with a Mw in excess of 200 kDa. The β_H fraction contains all seven beta crystallin polypeptides (David et al., 1993a) in diverse oligomeric arrangement probably consisting of 7 or 8 subunits (Bindels et al., 1981) with Mw around 160 kDa whereas the β_L fraction contains dimers and trimers (Bindels et al., 1981) with a Mw around 40 kDa. The beta fractions can be separated further into six or more β_H and two β_L fractions, β_{L1} and β_{L2} (Asselbergs et al., 1979), as well as a monomeric crystallin containing fraction, β_s , today termed γ_s (Bindels et al., 1982; van Rens et al., 1989). The γ fraction with a molecular weight around 20 kDa contains the gamma crystallin monomers and can be subdivided into two fractions (Bindels et al., 1982).

The water-insoluble (WI) raw protein fraction can also be subjected to size exclusion chromatography on a column equilibrated with 6 M urea and was shown to result in four distinct chromatographic peaks. Fraction I contained actin while both cytoskeletal proteins actin and vimentin were recovered from fraction II. Fraction III contained the monomeric alpha crystallin subunits while fraction IV contained two low molecular weight proteins (Kibbelaar and Bloemendal, 1979). However other investigations separated the adult human WI fraction into three and two chromatographic peaks (Lund et al., 1996; Hanson et al., 2000).

The size exclusion chromatography fractions or the unfractionated water-soluble (WS), water-insoluble (WI) and urea-soluble (US) fractions can subsequently be applied to 2-DE or liquid chromatography steps for further separation and analysis prior to protein identification. Finally, the urea-insoluble (UI) raw protein fraction was applied to SDS-PAGE and shown to contain the membrane protein MP26 (Kibbelaar and Bloemendal, 1979). As a final note, the results of the described fractionation procedures apply to the healthy adult mammalian lens and may vary depending on species and age as well as on the analysis of whole lens or morphological sections (Li et al., 1986; Bours et al., 1990). An overview of the procedures is given in Fig. 1.

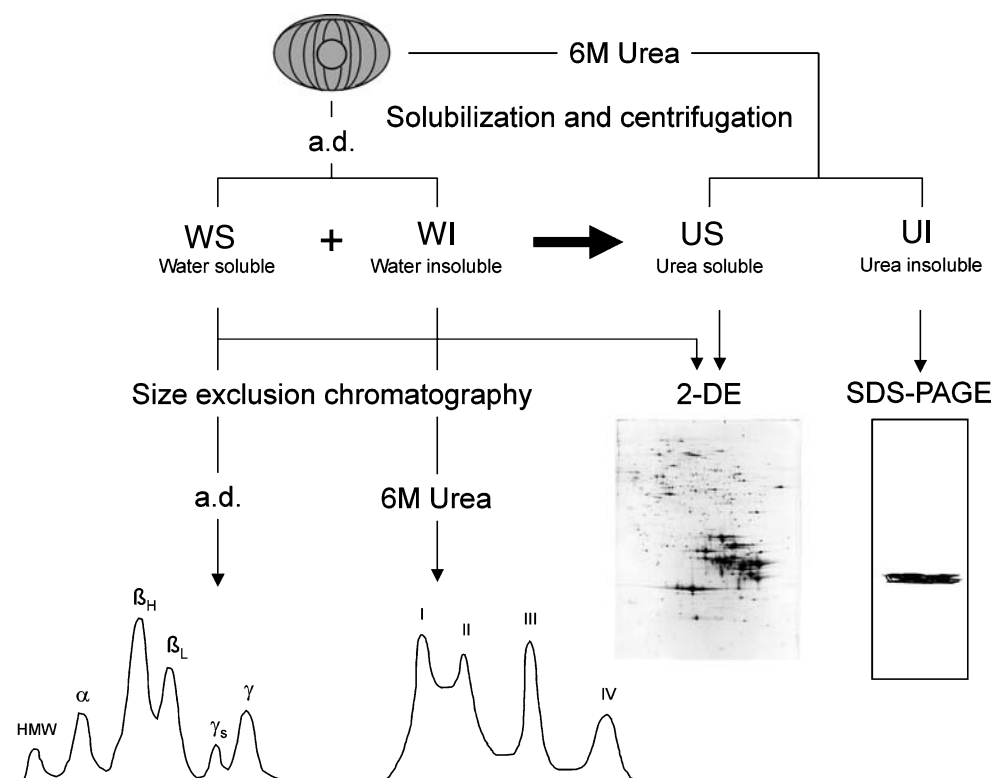


Fig. 1. Overview of lens solubilization and prefractionation procedures and common separation steps in a proteomics study

Two-dimensional electrophoresis and lenticular proteomics: a long relationship

Two-dimensional electrophoresis (2-DE) is probably the core proteomics technique for protein separation. It was developed independently by O'Farrell and Klose in 1975 and combines SDS-PAGE and isoelectric focusing for protein separation in two dimensions (O'Farrell, 1975; Klose, 1975). Proteins migrate to positions termed spots on the 2-DE gel as dictated by the relation of their chemical parameters to the applied 2-DE parameters. Generally the proteins are then stained with a dye and the spots excised from the gel. The proteins are digested in the gel spots with enzymes or reagents and the resulting peptides are analyzed by mass spectrometry, the essential proteomics technique for polypeptide characterization measuring the masses of ionized molecules or in the past by Edman degradation (Edman, 1949). The key improvement in mass spectrometry for the analysis of peptides and proteins was the introduction of soft ionization techniques such as matrix assisted laser ionization/desorption (MALDI) (Karas and Hillenkamp, 1988; Tanaka et al., 1988) and electrospray ionization (ESI) (Fenn et al., 1989). Today's generation of mass spectrometers yield both peptide

masses and *de facto* peptide sequence information by fragmenting analyzed peptides in the mass spectrometer and applying a second round of mass spectrometry to the fragments. This data can then be used to identify peptides and proteins. Alternatively, proteins can be analyzed directly by mass spectrometry without prior digestion yielding masses of the entire polypeptide (Mann et al., 2001).

High quality 2-DE has remarkable resolution capability separating and concomitantly visualizing up to 10000 sample constituents distinguished by a single amino acid or PTM (Klose and Kobalz, 1995). The ability to detect protein modifications by themselves is offered by a number of techniques, the visualization of the polypeptides however is a premier advantage of 2-DE as it allows the correct assignment of these modifications to the respective protein species. At this analytical level modified and unmodified polypeptides should not be termed protein but protein species as this describes translation products at the covalent molecular level (Jungblut and Thiede, 1997). An experienced user can thus readily pinpoint differences between proteomes and truly characterize and understand them in detail expediting detection of physiologically or clinically relevant molecules such as genetic

variants or disease markers (Klose et al., 2002; Krah et al., 2004).

Two-dimensional electrophoresis and lens proteomics go together from the outset. Already in 1975 Kibbelaar and Bloemendal applied whole water soluble, size exclusion chromatography α , β_H , β_L and γ fractions and water insoluble as well as the urea solubilized pellet from calf lens to urea PAGE combined with SDS-PAGE producing a first rudimentary spot pattern of the major lenticular protein components, the unmodified crystallins and suggested alpha crystallin interaction with membrane components (Kibbelaar and Bloemendal, 1975). With the improvement of 2-DE a definitive pattern or reference

map for the crystallins and other lenticular components was achieved in 1982 for the water soluble proteins of the bovine lens cortex with a final nomenclature for beta crystallins added in 1984 (Berbers et al., 1982, 1984). Similar investigations were performed in chicken, mouse, rat and human yielding comparable crystallin reference patterns and alpha crystallin characterization with nomenclature as well as demonstrating the presence of vimentin, actin and GAPDH in the urea soluble fraction presumably from the anterior epithelial cell layer and outer cortex (Garadi et al., 1983; Garber et al., 1984; de Vries et al., 1991; Datiles et al., 1992; David and Shearer, 1993b). Morphological regions of human lenses were analyzed

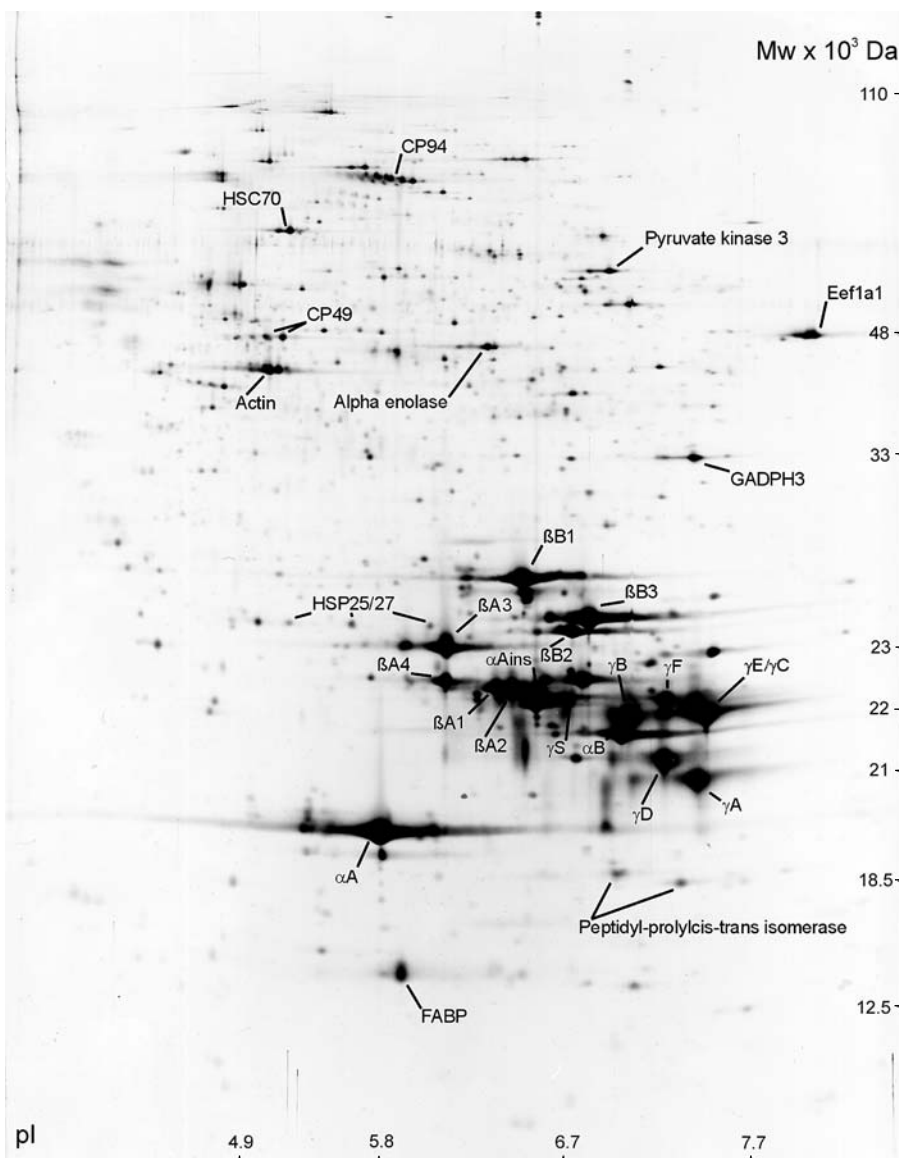


Fig. 2. 2-DE protein pattern of whole urea soluble mouse C57BL/6J 10 day old eye lens fraction with some identified proteins. The crystallins are named as token only

separately following dissection revealing a nuclear 2-DE pattern which is quite distinct from the previously described cortical or whole lens pattern (Garland et al., 1996). This implies a high degree of post translational modification as the nuclear protein species must be derived from the primary crystallin proteins as well as highlighting the differences in protein complement between lenticular regions. 2-DE patterns of human epithelial lens cells were also published recently (Wang-Su et al., 2003; Paron et al., 2004).

Newer investigations have become increasingly comprehensive, often further characterizing primary crystallin proteins, confirming amino acid sequences predicted from cDNA or detecting discrepancies and annotating sequences and creating higher quality reference maps as well as detecting numerous non crystallin lenticular proteins for various species and ages (Shih et al., 1998; Lampi et al., 2002; Wang-Su et al., 2003; Wilmarth et al., 2004; Hoehenwarter et al., 2005). A detailed reference map of the entire adult murine lenticular proteome using high resolution large scale 2-DE which separated the urea soluble fraction into 1940 spots was produced (Jungblut et al., 1998) ultimately followed by an in depth analysis of the water soluble and insoluble protein fractions at different ages (Ueda et al., 2002a). A 2-DE image of the young urea soluble whole lens murine lenticular proteome with the characteristic crystallin 2-DE pattern produced in our laboratory is shown in Fig. 2. An accessible repository for mouse lens and other proteomics data is urgently required and under preparation in the form of our 2-DE database at 2-DPAGE at <http://www.mpiib-berlin.mpg.de/2D-PAGE/>.

Endeavors towards an inclusive map and database of the water soluble proteins of the human lens culminated in the attainment of the infant, young, mature and old whole human lenticular proteomes as well as of the α , β_H , β_L and γ size exclusion chromatography fractions (Datiles et al., 1992; David et al., 1996; Bloemendal et al., 1997; Lampi et al., 1997, 1998). Considering 90% of the lenticular proteome is made up of twelve primary translation products these investigations have impressively demonstrated the extent of post translational modification in the lens beginning in the prenatal organism and increasing with age.

Recent advances in technology have firmly established 2-DE in the analytical laboratory allowing directed implementation as part of larger experimental designs. The water soluble and insoluble fractions of the lens nucleus and cortex have been comparatively analyzed by 2-DE with emphasis on the beta crystallins (Werten et al.,

1999), the beaded filament constituents phakinin and filensin (Carter et al., 1995) and covalent crystallin multimers in the water insoluble fraction (Srivastava et al., 2004). The water soluble size exclusion chromatography fractions HMW and α (Spector et al., 1985; Harrington et al., 2004), β_H (Srivastava et al., 1999; Kamei et al., 2003), β_L (Kleiman et al., 1988) and γ (Srivastava and Srivastava, 1998) as well as the α crystallin A and B chains in the urea soluble fractions (Colvis et al., 2000; Colvis and Garland, 2002; Schaefer et al., 2003) have been analyzed independently with specific questions in mind.

In conclusion, 2-DE has not only been a pillar in the exploration of the lenticular proteome from the beginning, shedding light on the primary constituents, the unmodified crystallins and cell architecture components and marking changes as a result of aging and characterizing morphological sections but has also seen specialized applications. Most importantly the technique has been invaluable in the elucidation of post translational modifications in the healthy and cataractous lens which will be discussed below. Lenticular proteomics and 2-DE can thus be seen as a vanguard for functional proteomics at the protein species level.

Liquid chromatography and mass spectrometry for lens proteome analysis

Liquid chromatography is one of the oldest and best techniques for molecular separation in biochemical analysis earning Archer Martin and Richard Synge the Nobel Prize for Chemistry in 1952. It is based on the individual behavior of soluble analytes in a mobile phase passed over and interacting differentially with a solid phase or matrix. Various combinations of liquid and solid phases featuring distinct molecular interaction and separation properties have been developed and tried over the years leading to several popular liquid chromatography approaches, affinity chromatography, ion exchange chromatography, reverse phase chromatography and size exclusion chromatography to name a few.

Initially reverse phase chromatography was the method of choice for separating proteins and peptides according to hydrophobic interaction with C4, C8 or C18 alkyl chains in many proteomics experiments. By means of a connecting apparatus the molecules were eluted directly into an ESI mass spectrometer for analysis of proteins by exact measurement of protein mass and peptides by MS/MS analysis and measurement of fragment masses. This marriage of reverse phase liquid chromatography and ESI mass spectrometry proved ideal as the organic

composition and liquid state of aggregation of the reverse phase eluent is highly compatible with the electrospray ionization process (Dole et al., 1968; Yamashita and Fenn, 1984; Fenn et al., 1989) and was originally termed shotgun proteomics (McCormack et al., 1997; Yates 3rd, 1998).

Early experiments quickly showed that one chromatographic step alone could not adequately separate complex protein or peptide mixtures for reasonable detection and characterization by mass spectrometry due to molecular abundance in chromatographic retention time exceeding the mass spectrometers specificity. Therefore a second chromatographic step, in many cases on- or offline ion exchange chromatography was added to improve separation and alleviate this problem. This procedure became known as multidimensional protein identification technology or MudPIT (Washburn et al., 2001). The primary advantage offered by liquid chromatography techniques coupled to mass spectrometers is the relative speed at which complex protein mixtures can be characterized at the protein level. However, should more directed applications or the detection of post translational modifications be aspired to, higher degrees of separation and consequently often more than two chromatographic steps are required. Ultimately, true high-end multidimensional chromatography for the analysis of complex proteomes includes up to 15 or more chromatography steps with reverse phase chromatography directly preceding mass spectrometry as most other chromatographic buffers and solutions are incompatible with ionization (Link et al., 1999; Washburn et al., 2001; Wolters et al., 2001). Experiments of this type with peptides generate between 10 and 100 thousand distinct mass spectra or scans, making comprehensive evaluation equally laborious as the complete analysis of spots from 2-DE (Hoehenwarter et al., 2006; Swanson and Washburn, 2005).

Recently MALDI mass spectrometry has also become available for shotgun proteomics experiments with automated spotting techniques depositing liquid chromatography eluents onto MALDI templates (Fung et al., 2004; Nagele and Vollmer, 2004; Zhen et al., 2004).

Major multi-dimensional liquid chromatography investigations have characterized the infant to adult water soluble and the adult water insoluble human lens proteome. Following size exclusion chromatography water soluble α , β_H , β_L and γ fractions and two water insoluble fractions were applied to reverse phase chromatography on C4 columns for further fractionation. Whole proteins were then analyzed offline with ESI mass spectrometry. The 11 human crystallin proteins known then, alpha A and

B, beta B1, B2 and B3, A1, A3 and A4 and gamma C, D and S were detected in the water soluble fraction and changes in their abundance as well as post translational modifications with age were characterized (Ma et al., 1998). The adult water insoluble fraction contained little beta B2 crystallin, a major component of the water soluble fraction and numerous crystallin degradation products. Nevertheless, the major components were shown to be intact crystallins (Hanson et al., 2000).

Another investigation subjected the water insoluble monomeric alpha crystallin subunit containing size exclusion chromatography fraction to cation exchange chromatography before reverse phase chromatography and offline ESI mass spectrometry. A high degree of separation with three liquid chromatography steps was achieved and it was shown that alpha crystallin is the major component of the water insoluble fraction constituting about half of protein abundance and numerous alpha crystallin protein species were identified and distinguished from the water soluble fraction (Lund et al., 1996). Also, a high end MudPit analysis of young human cataractous lens combining 18 chromatographic steps was carried out and in addition to detecting the 11 human crystallin proteins and modifications detected 270 non-crystallin proteins, an impressive number by any means (MacCoss et al., 2002).

As mentioned above a first step in lenticular proteomics is often size exclusion chromatography. Water soluble high molecular weight and α fractions were analyzed by reverse phase chromatography and MALDI-MS as well as further separated into alpha A and alpha B crystallin subunit containing fractions using ion exchange chromatography prior to mass spectrometry, implicating conformational changes effected by spontaneous asparagine racemization and isomerization in alpha crystallin aggregation and insolubilization (Fujii et al., 2003, 2004). A reverse phase chromatography ESI-MS analysis of the HMW and α fractions in conjunction with other data suggested that alpha crystallin subunit C-terminal truncation and phosphorylation do not adversely affect protein chaperone activity (Carver et al., 1996). All of the investigations confirmed alpha crystallin as the main component of the HMW size exclusion chromatography fraction while another investigation identified chaperone sites by analyzing hydrogen-deuterium exchange in the presence of ATP with ESI-MS (Hasan et al., 2002). Major C-terminal truncation sites on the alpha A crystallin chain were characterized using MALDI-MS (Takemoto, 1995) and cleavage in the N-terminal region was shown using fast atom bombardment (FAB) MS (Kamei et al., 1997). Finally, a novel modification on the C-terminal lysine of the alpha B

crystallin chain was detected by ESI/TOF-MS after purification of the alpha B crystallin subunit from the α size exclusion chromatography fraction with reverse phase LC (Lin et al., 1997).

The human beta crystallins were purified using anion exchange chromatography and reverse phase chromatography following size exclusion chromatography and analyzed with ESI-MS resulting in the detection of numerous post translational modifications and of beta A2 crystallin in humans (Ajaz et al., 1997; Lapko et al., 2003a). The solubility properties of beta B2 crystallin, the major beta crystallin throughout the lens, were also analyzed with reverse phase LC and ESI-MS (Feng et al., 2000).

The human size exclusion γ fraction was analyzed in detail corroborating the known sequences for the human gamma crystallins, gamma C, D and S as well as identifying deamidation, disulfide bonding, methylation and carbamylation sites. Truncated low molecular mass fragments were also analyzed (Hanson et al., 1998; Abbasi et al., 1998; Lapko et al., 2003b) and the bovine gamma E crystallin sequence was realized following additional purification of the γ fraction with cation exchange chromatography (Kilby et al., 1997).

A smaller LMW fraction containing proteins under 10kDa was produced by 2 subsequent size exclusion chromatography steps and was shown to contain ubiquitin by MALDI-MS as well as its C-terminal degradation products in cataractous lenses (Stiuso et al., 2002). Finally, the major intrinsic membrane protein (MIP) comprising 50% of membrane protein content was extracted with urea and analyzed with reverse phase ESI- and MALDI-MS (Schey et al., 2000).

In conclusion, liquid chromatography techniques coupled with mass spectrometry for protein separation and analysis have characterized most of the lenticular proteome and detected large numbers of post translational modifications. For a low complexity sample such as the lens where the individual components can be purified to a high degree using several chromatography steps the protein species level can be investigated and has been extensively realized.

Crystallin post-translational modifications: important molecules in lenticular ageing and implicated in cataractogenesis

It is a known fact that the primary crystallin translation products are extensively modified beginning at an early age. Also, evidence is abundant that crystallin post translational modifications increase as the lens ages both in the

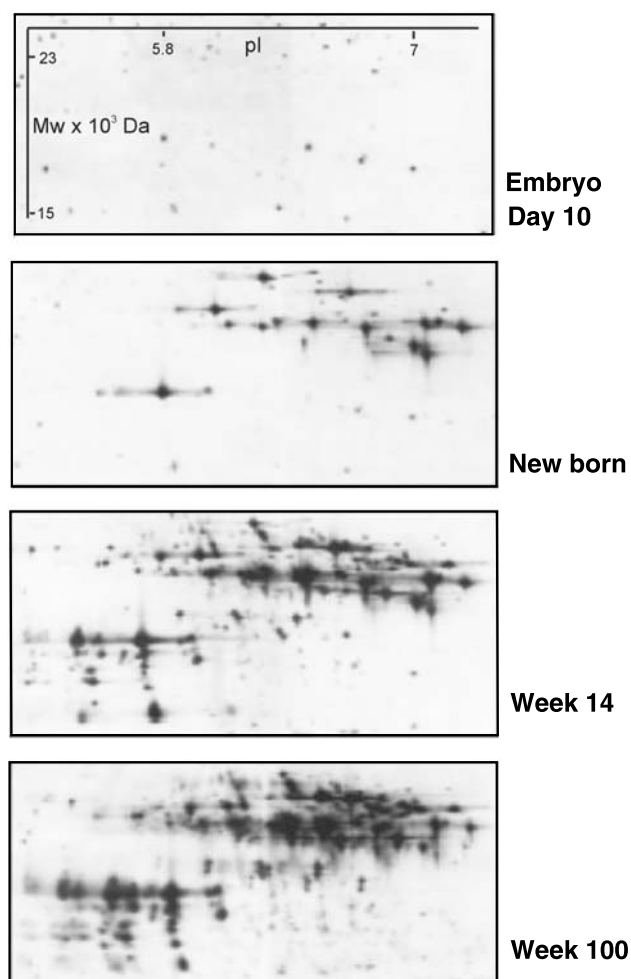


Fig. 3. The crystallin protein super family. Post translational modifications and the effects of development and ageing

appearance of new modification types and in the continued increase of earlier onset modifications. This is illustrated in Fig. 3 which shows the crystallin area of 2-DE gels prepared in our laboratory of the urea soluble fraction of the whole mouse lens proteome at different ages.

Probably the most common post translational modification is truncation, primarily at the alpha crystallin C-terminal as well as N- and C-terminal beta crystallin extensions. It seems alpha crystallin C-terminal truncation is a result of calpain protease activity (Ueda et al., 2001, 2002b). C-terminal cleavage by m-calpain adversely affects alpha crystallin chaperone activity as well as subunit assembly while there is evidence that cleavage at the major C-terminal lens specific protease Lp82 sites does not (Carver et al., 1996; Thampi and Abraham, 2003). Beta crystallin N- and C-terminal truncation may be a result of calpain activity and effect subunit organization and higher order assembly (David et al., 1993a, 1996;

Table 1. Crystallin modifications in the lens detected by proteomics techniques

Protein	Modification ^a	Lenticular region ^b	Species ^c	Detection method ^d	Citation
alpha A crystallin	acetylation K70	WL: WS, α	H, H*	P LC/ESI-MS p LC/ESI-MS/MS p MudPIT/ESI-MS/MS	Lin et al., 1998 MacCoss et al., 2002
alpha A crystallin	acetylation K78	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
alpha A crystallin	acetylation K88	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
alpha A crystallin	acetylation K145	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
alpha A crystallin	acetylation M1	WL: WS; WI	B, C, H	P LC/ESI-MS p 2-DE ESI-MS/MS	Smith et al., 1991 Srivastava et al., 2004 Wilmarth et al., 2004
alpha A crystallin	deamidation N101	WL: WS; WI, mF	H	p LC/ESI-MS p LC/ESI-MS/MS p LC/FAB-MS	Lund et al., 1996 Miesbauer et al., 1994
alpha A crystallin	deamidation Q6	WL: WS; WI, mF	H	P LC/ESI-MS p LC/ESI-MS p LC/ESI-MS/MS p LC/FAB-MS	Lund et al., 1996 Hanson et al., 2000 Miesbauer et al., 1994
alpha A crystallin	deamidation Q50	WL: WS; WI, mF	H	p LC/ESI-MS p LC/ESI-MS/MS p LC/FAB-MS	Lund et al., 1996 Miesbauer et al., 1994
alpha A crystallin	deamidation Q90	WL: WI, mF	H	p LC/ESI-MS p LC/ESI-MS/MS	Hanson et al., 2000 Lund et al., 1996
alpha A crystallin	deamidation Q104	WL: WI	H	p LC/ESI-MS p LC/ESI-MS/MS	Hanson et al., 2000
alpha A crystallin	deamidation Q147	WL: WS; WI, mF	H	P LC/ESI-MS p LC/ESI-MS p LC/ESI-MS/MS p LC/FAB-MS	Lund et al., 1996 Hanson et al., 2000 Miesbauer et al., 1994
alpha A crystallin	disulfide bond C131–142	WL: WS; WI, mF	H	p LC/ESI-MS p LC/ESI-MS/MS p LC/FAB-MS	Lund et al., 1996 Miesbauer et al., 1994
alpha A crystallin	formylation H79	WL: WI	H	p 2-DE ESI-MS/MS	Srivastava et al., 2004
alpha A crystallin	methylation K88	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
alpha A crystallin	methylation R21	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
alpha A crystallin	oxidation M1	WL: WI, mF	H	p LC/ESI-MS p LC/ESI-MS/MS p 2-DE ESI-MS/MS	Hanson et al., 2000 Lund et al., 1996 Srivastava et al., 2004
alpha A crystallin	oxidation M138	WL: WI, mF	H	p LC/ESI-MS p LC/ESI-MS/MS	Hanson et al., 2000 Lund et al., 1996
alpha A crystallin	oxidation W9	WL: WI	H	p 2-DE ESI-MS/MS	Srivastava et al., 2004
alpha A crystallin	oxidation Y18	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
alpha A crystallin	oxidation Y34	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
alpha A crystallin	phosphorylation S45	WL: WS; WI, mF	H, H*	P LC/ESI-MS p LC/ESI-MS/MS p MudPIT/ESI-MS/MS	Lund et al., 1996 MacCoss et al., 2002
alpha A crystallin	phosphorylation S122	WL: WS; WI, mF; US	B, H, H*	P LC/ESI-MS p LC/ESI-MS p LC/ESI-MS/MS p MudPIT/ESI-MS/MS p LC/FAB-MS p LC/FAB-MS/MS p LC/MALDI-MS	Lund et al., 1996 MacCoss et al., 2002 Miesbauer et al., 1994 Schaefer et al., 2003 Smith et al., 1991 Takemoto, 1996
alpha A crystallin	phosphorylation S148	WL: US	M	p LC/ESI-MS/MS	Schaefer et al., 2003
alpha A crystallin	phosphorylation T13	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
alpha A crystallin	phosphorylation T140	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
alpha A crystallin	racemization D151	WL: WS, HMW, α	H, R, R*	p LC/MALDI-MS p LC Edman	Fujii et al., 1994, 1999, 2003, 2004
alpha A crystallin	racemization D58	WL: WS, HMW, α	H, R, R*	p LC/MALDI-MS p LC Edman	Fujii et al., 2001, 2003, 2004
alpha A crystallin	S59 to dehydroalanine	WL: WI	H	p 2-DE ESI-MS/MS	Srivastava et al., 2004
alpha A crystallin	truncation 3/4	WL: WS	H	p LC/FAB-MS p LC Edman	Kamei et al., 1997
alpha A crystallin	truncation 41/42	WL: US	M	p 2-DE ESI-MS/MS	Hoehenwarter et al., 2006
alpha A crystallin	truncation 50/51	WL: WI, mF	H	P LC/ESI-MS p LC/ESI-MS/MS	Lund et al., 1996
alpha A crystallin	truncation 65/66	WL: WI	H	P LC/ESI-MS p LC/ESI-MS/MS	Hanson et al., 2000

(continued)

Table 1 (continued)

Protein	Modification ^a	Lenticular region ^b	Species ^c	Detection method ^d	Citation
alpha A crystallin	truncation 80/81	WL: WI	H	P LC/ESI-MS p LC/ESI-MS/MS	Hanson et al., 2000
alpha A crystallin	truncation 101/102	WL: WS, HMW; WI, mF	B, H	P LC/ESI-MS p LC/ESI-MS/MS	Carver et al., 1996 Kilby, 1995a Lund et al., 1996
alpha A crystallin	truncation 118/119	N: WI	R*	P LC/ESI-MS	Ueda et al., 2002a
alpha A crystallin	truncation 119/120	N: WI	R, R*	P LC/ESI-MS	Ueda et al., 2002a
alpha A crystallin	truncation 126/127	N: WI	R, R*	P LC/ESI-MS	Ueda et al., 2002a
alpha A crystallin	truncation 130/131	N: WI	R, R*	P LC/ESI-MS	Ueda et al., 2002a
alpha A crystallin	truncation 147/148	N: WI	R, R*	P LC/ESI-MS	Ueda et al., 2002a
alpha A crystallin	truncation 151/152	WL: WS, HMW, α ; WI N: WI	B, M, R, R+, R*	P LC/ESI-MS P 2-DE ESI-MS p 2-DE ESI-MS/MS	Smith et al., 1991 Thampi et al., 2002 Ueda et al., 2002a, 2002b
alpha A crystallin	truncation 154/155	WL: WS, α	B	P LC/ESI-MS	Smith et al., 1991
alpha A crystallin	truncation 156/157	N: WI	R, R*	P LC/ESI-MS	Ueda et al., 2002a
alpha A crystallin	truncation 157/158	WL: WS, HMW, α N: WI	R, R+, R*	P LC/ESI-MS	Thampi et al., 2002 Ueda et al., 2002a
alpha A crystallin	truncation 162/163	WL: WS, HMW, α ; US N: WI	H, R, R+	P LC/ESI-MS p 2-DE MALDI-MS	Colvis et al., 2002 Thampi et al., 2002 Ueda et al., 2002a
alpha A crystallin	truncation 163/164	WL: WS, HMW, α N: WI	R, R+, R*	P LC/ESI-MS	Thampi et al., 2002 Ueda et al., 2002a
alpha A crystallin	truncation 165/166	N: WI	R, R*	P LC/ESI-MS	Ueda et al., 2002a
alpha A crystallin	truncation 168/169	WL: WS, HMW, α ; WI, mF; US N: WI	B, H, M, R, R+, R*	P LC/ESI-MS P LC/MALDI-MS P 2-DE ESI-MS p LC/ESI-MS/MS p 2-DE ESI-MS/MS	Lund et al., 1996 Takemoto, 1995 Thampi et al., 2002 Ueda et al., 2001 Ueda et al., 2002a, 2002b
alpha A crystallin	truncation 172/173	WL: WS, HMW, α ; WI, mF; US	B, H, R+	P LC/ESI-MS p LC/ESI-MS/MS P LC/MALDI-MS p 2-DE MALDI-MS	Carver et al., 1996 Colvis et al., 2002 Emmons and Takemoto, 1992 Hanson et al., 2000 Kilby et al., 1995a Lund et al., 1996 Miesbauer et al., 1994 Smith et al., 1991 Takemoto, 1995 Thampi et al., 2002 Ueda et al., 2001
alpha B crystallin	acetylation K92	WL: WS, α	H*	P LC/ESI-MS p LC/ESI-MS/MS p MudPIT/ESI-MS/MS	Lapko et al., 2001 MacCoss et al., 2002
alpha B crystallin	acetylation M1	WL: WS	C	p 2-DE ESI-MS/MS	Wilmarth et al., 2004
alpha B crystallin	carbamylation K92	WL: WS, α	H*	P LC/ESI-MS p LC/ESI-MS/MS	Lapko et al., 2001
alpha B crystallin	deamidation N78	WL: WI	H	p LC/ESI-MS/MS	Hanson et al., 2000
alpha B crystallin	deamidation N146	WL: WI, mF	H	p LC/ESI-MS/MS	Lund et al., 1996
alpha B crystallin	deamidation Q108	WL: WI, mF	H	p LC/ESI-MS/MS	Hanson et al., 2000 Lund et al., 1996
alpha B crystallin	methylation R22	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
alpha B crystallin	methylation R50	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
alpha B crystallin	novel modification K175	WL: WS C: US	H, HSr	P LC/ESI-MS p LC/ESI-MS/MS p 2-DE MALDI-MS	Colvis et al., 2000 Lin et al., 1997
alpha B crystallin	oxidation M1	WL: WI, mF	H	p LC/ESI-MS/MS	Hanson et al., 2000 Lund et al., 1996
alpha B crystallin	oxidation M68	WL: WS; WI	H, H*	p 2-DE ESI-MS/MS p MudPIT/ESI-MS/MS	MacCoss et al., 2002 Srivastava et al., 2004
alpha B crystallin	oxidation M168	WL: WI, mF	H	p LC/ESI-MS/MS	Hanson et al., 2000 Lund et al., 1996
alpha B crystallin	oxidation W60	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
alpha B crystallin	oxidation Y48	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002

(continued)

Table 1 (continued)

Protein	Modification ^a	Lenticular region ^b	Species ^c	Detection method ^d	Citation
alpha B crystallin	phosphorylation S19	WL: WS, α ; WI, mF C: US	B, H, H*, Hsr	P LC/ESI-MS p LC/ESI-MS/MS p MudPIT/ESI-MS/MS p LC/FAB-MS p 2-DE MALDI-MS p LC Edman	Colvis et al., 2000 Lund et al., 1996 MacCoss et al., 2002 Miesbauer et al., 1994 Smith et al., 1992 Voorter et al., 1989
alpha B crystallin	phosphorylation S21	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
alpha B crystallin	phosphorylation S43	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
alpha B crystallin	phosphorylation S45	WL: WS, α ; WI, mF	B, H, H*	P LC/ESI-MS p LC/ESI-MS/MS p MudPIT/ESI-MS/MS p LC/FAB-MS p LC Edman	Lund et al., 1996 MacCoss et al., 2002 Miesbauer et al., 1994 Smith et al., 1992 Voorter et al., 1989
alpha B crystallin	phosphorylation S53	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
alpha B crystallin	phosphorylation S59	WL: WS, α ; WI, mF	B, H, H*	P LC/ESI-MS p LC/ESI-MS/MS p MudPIT/ESI-MS/MS p LC/FAB-MS	Lund et al., 1996 MacCoss et al., 2002 Miesbauer et al., 1994 Smith et al., 1992
alpha B crystallin	phosphorylation S76	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
alpha B crystallin	truncation 1/2	WL: WS	H	p LC/FAB-MS p LC Edman	Kamei et al., 1997
alpha B crystallin	truncation 6/7	WL: WS	H	p LC/FAB-MS p LC Edman	Kamei et al., 1997
alpha B crystallin	truncation 40/41	C: WS	H, H*	p 2-DE MALDI-MS	Jimenez-Asensio et al., 1999
alpha B crystallin	truncation 42/43	C: WS	H, H*	p 2-DE MALDI-MS	Jimenez-Asensio et al., 1999
alpha B crystallin	truncation 44/45	C: WS	H, H*	p 2-DE MALDI-MS	Jimenez-Asensio et al., 1999
alpha B crystallin	truncation 150/151	C: US	H, Hsr	p 2-DE MALDI-MS	Colvis et al., 2000
alpha B crystallin	truncation 151/152	C: US	H, Hsr	p 2-DE MALDI-MS	Colvis et al., 2000
alpha B crystallin	truncation 152/153	C: US	H, Hsr	p 2-DE MALDI-MS	Colvis et al., 2000
alpha B crystallin	truncation 153/154	C: US	H, Hsr	p 2-DE MALDI-MS	Colvis et al., 2000
alpha B crystallin	truncation 154/155	C: US	H, Hsr	p 2-DE MALDI-MS	Colvis et al., 2000
alpha B crystallin	truncation 161/162	C: US	Hsr	p 2-DE MALDI-MS	Colvis et al., 2000
alpha B crystallin	truncation 162/163	C: US	H, Hsr	p 2-DE MALDI-MS	Colvis et al., 2000
alpha B crystallin	truncation 163/164	N: WI	R	P LC/ESI-MS	Ueda et al., 2002a
alpha B crystallin	truncation 170/171	WL: WS, HMW, α . C: US N: WI	H, Hsr, R, R+, R*	P LC/ESI-MS p 2-DE MALDI-MS	Colvis et al., 2000 Thampi et al., 2002 Ueda et al., 2002a
alpha B crystallin	truncation 174/175	WL: WI; mF C: WS; US	H, H*, Hsr	P LC/ESI-MS p LC/ESI-MS p LC/ESI-MS/MS p 2-DE MALDI-MS	Jimenez-Asensio et al., 1999 Colvis et al., 2000 Lund et al., 1996
beta A1 crystallin	acetylation, M1 removed	WL: WS	C	P LC/ESI-MS p 2-DE ESI-MS/MS	Wilmarth et al., 2004
beta A1/A3 crystallin	acetylation K122	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
beta A1/A3 crystallin	acetylation K125	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
beta A1/A3 crystallin	acetylation K131	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
beta A1/A3 crystallin	deamidation N22	N: WI	R*	p 2-DE Edman	David et al., 1993a
beta A1/A3 crystallin	deamidation Q38	WL: WI	H	p LC/ESI-MS p LC/ESI-MS/MS	Hanson et al., 2000
beta A1/A3 crystallin	deamidation Q42	WL: WI	H	p LC/ESI-MS p LC/ESI-MS/MS	Hanson et al., 2000
beta A1/A3 crystallin	methylation R137	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
beta A1/A3 crystallin	oxidation M126	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
beta A1/A3 crystallin	phosphorylation S160	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
beta A1/A3 crystallin	phosphorylation T34	WL: WS, β_H	B	p 2-DE MALDI-MS	Kamei et al., 2003
beta A1/A3 crystallin	phosphorylation T127	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
beta A1/A3 crystallin	truncation 20/21	N: WI	R*	p 2-DE Edman	David et al., 1993a
beta A1/A3 crystallin	truncation 21/22	WL: WS; WI N: WI	M*, R*	p 2-DE Edman	David et al., 1993a, 1994
beta A1/A3 crystallin	truncation 22/23	WL: WS, β_H , β_L ; WI C: WS, WI N: WS, WI	B, C, H, M	P LC/ESI-MS P 2-DE ESI-MS p 2-DE ESI-MS p 2-DE ESI-MS/MS p 2-DE Edman p 2-DE FAB-MS	Lampi et al., 1997, 1998 Ma et al., 1998 Shih et al., 1998 Ueda et al., 2002b Werten et al., 1999 Wilmarth et al., 2004

(continued)

Table 1 (continued)

Protein	Modification ^a	Lenticular region ^b	Species ^c	Detection method ^d	Citation
beta A1/A3 crystallin	truncation 26/27	WL: WS	H	p 2-DE ESI-MS	Lampi et al., 1998
beta A1/A3 crystallin	truncation 39/40	WL: WS, β_H	H	p 2-DE Edman	Srivastava et al., 1999
beta A2 crystallin	acetylation, M1 removed	WL: WS	C	P LC/ESI-MS	Wilmarth et al., 2004
beta A2 crystallin	acetylation, M1 removed	WL: WS	C	p 2-DE ESI-MS/MS	Wilmarth et al., 2004
beta A2 crystallin	alternate start codon M5	WL: WS	H	P LC/ESI-MS	Lapko et al., 2003a
beta A2 crystallin	phosphorylation S30	WL: WS	H	p 2-DE ESI-MS/MS	David et al., 1993a,
beta A3 crystallin	truncation 11/12	WL: WS, β_H ; WI	B, M, M*, R, R*	P LC/ESI-MS	1993b, 1994
		N: WS; WI		P 2-DE ESI-MS	Lampi et al., 2002
				p 2-DE ESI-MS/MS	Shih et al. 1998
				p 2-DE Edman	Ueda et al., 2002b
					Werten et al., 1999
beta A4 crystallin	oxidation M13	WL: WI	H	p 2-DE ESI-MS/MS	Srivastava et al., 2004
beta A4 crystallin	oxidation W149	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
beta A4 crystallin	phosphorylation S35	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
beta A4 crystallin	phosphorylation T43	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
beta A4 crystallin	truncation 18/19	WL: WS; WI	M*, R*	p 2-DE Edman	David et al., 1993a, 1994
		N: WI			
beta B1 crystallin	acetylation K6	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
beta B1 crystallin	acetylation K160	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
beta B1 crystallin	deamidation N157	WL: WI	H	p LC/ESI-MS	Hanson et al., 2000
				p LC/ESI-MS/MS	
beta B1 crystallin	methylation K235	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
beta B1 crystallin	methylation R230	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
beta B1 crystallin	methylation R231	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
beta B1 crystallin	oxidation M112	WL: WI	H	p 2-DE ESI-MS/MS	Srivastava et al., 2004
beta B1 crystallin	oxidation M226	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
beta B1 crystallin	oxidation W126	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
beta B1 crystallin	phosphorylation S10	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
beta B1 crystallin	phosphorylation T12	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
beta B1 crystallin	truncation 4/5	WL: WS	C	p 2-DE Edman	Wilmarth et al., 2004
beta B1 crystallin	truncation 11/12	WL: WS; WI	M*	p 2-DE Edman	David et al., 1994
beta B1 crystallin	truncation 15/16	WL: WS, β_H , β_L	B, H	P LC/ESI-MS	Ajaz et al., 1997
				p LC ESI MS	David et al., 1996
				p LC Edman	Lampi et al., 1997, 1998
				p 2-DE ESI-MS	Ma et al., 1998
				p 2-DE Edman	Shih et al., 1998
				p 2-DE FAB-MS	
beta B1 crystallin	truncation 26/27	WL: WS; WI	M*	p 2-DE Edman	David et al., 1994
beta B1 crystallin	truncation 27/28	N: WI	R*	p 2-DE Edman	David et al., 1993a
beta B1 crystallin	truncation 34/35	WL: WS, β_H , β_L ; WI	H	P LC/ESI-MS	Ajaz et al., 1997
				p LC/ESI-MS	David et al., 1996
				p LC Edman	Ma et al., 1998
				p LC/ESI-MS/MS	Hanson et al., 2000
beta B1 crystallin	truncation 35/36	WL: WS, β_H , β_L	H	P LC/ESI-MS	Ajaz et al., 1997
				p LC/ESI-MS	David et al., 1996
				p LC Edman	Ma et al., 1998
beta B1 crystallin	truncation 36/37	WL: WS, β_H , β_L	H	P LC/ESI-MS	Ajaz et al., 1997
				p LC/ESI-MS	David et al., 1996
				p LC Edman	Ma et al., 1998
beta B1 crystallin	truncation 39/40	WL: WS, β_H , β_L	H	P LC/ESI-MS	Ajaz et al., 1997
				p LC/ESI-MS	David et al., 1996
				p LC Edman	Lampi et al., 1997
				p 2-DE FAB-MS	Ma et al., 1998
beta B1 crystallin	truncation 40/41	WL: WS, β_H , β_L	H	P LC/ESI-MS	Ajaz et al., 1997
				p LC/ESI-MS	David et al., 1996
				p LC Edman	Ma et al., 1998
beta B1 crystallin	truncation 41/42	WL: WS, β_H , β_L	H	P LC/ESI-MS	Ajaz et al., 1997
				p LC/ESI-MS	David et al., 1996
				p LC Edman	Ma et al., 1998
beta B1 crystallin	truncation 49/50	WL: WS; WI	M*, R*	p 2-DE Edman	David et al., 1993a, 1994
		N: WI			
beta B1 crystallin	truncation 72/73	WL: WI	H	P LC/ESI-MS	Hanson et al., 2000
				p LC/ESI-MS/MS	
beta B2 crystallin	acetylation K76	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
beta B2 crystallin	acetylation K121	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002

(continued)

Table 1 (continued)

Protein	Modification ^a	Lenticular region ^b	Species ^c	Detection method ^d	Citation
beta B2 crystallin	acetylation, M1 removed	WL: WS, β_H	B	p 2-DE MALDI-MS	Kamei et al., 2003
beta B2 crystallin	acetylation, M1 removed	WL: WS	C	p 2-DE ESI-MS/MS	Wilmarth et al., 2004
beta B2 crystallin	alternative splicing				
beta B2 crystallin	disulfide bond C37–66	WL: US	H*	p LC/MALDI-MS	Takemoto, 1997
beta B2 crystallin	glutathione adduct C66	WL: WS	H	p LC Edman	
beta B2 crystallin				P LC/ESI-MS	Feng et al., 2000
beta B2 crystallin				p LC/ESI-MS/MS	
beta B2 crystallin	methylation K42	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
beta B2 crystallin	methylation K68	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
beta B2 crystallin	methylation K121	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
beta B2 crystallin	oxidation M122	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
beta B2 crystallin	oxidation W59	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
beta B2 crystallin	oxidation W151	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
beta B2 crystallin	phosphorylation T118	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
beta B2 crystallin	truncation 7/8	WL: WS; WI	M	P 2-DE ESI-MS	Ueda et al., 2002b
beta B2 crystallin				p 2-DE ESI-MS/MS	
beta B2 crystallin	truncation 8/9	WL: WS	B	p 2-DE Edman	Shih et al., 1998
beta B2 crystallin	truncation 203/204	WL: WS	B	P LC/ESI-MS	Kilby et al., 1995b
beta B2 crystallin				p LC/ESI-MS/MS	
beta B2 crystallin				p MudPIT/ESI-MS/MS	
beta B3 crystallin	acetylation K128	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
beta B3 crystallin	acetylation, M1 removed	WL: WS, β_H	C	P LC/ESI-MS	Wilmarth et al., 2004
beta B3 crystallin				p 2-DE ESI-MS/MS	
beta B3 crystallin	methylation K128	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
beta B3 crystallin	oxidation M129	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
beta B3 crystallin	phosphorylation S6	WL: WS, β_H	B	p 2-DE MALDI-MS	Kamei et al., 2003
beta B3 crystallin	phosphorylation T7	WL: WS, β_H	B	p 2-DE MALDI-MS	Kamei et al., 2003
beta B3 crystallin	phosphorylation Y29	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
beta B3 crystallin	truncation 5/6	WL: WS; WI	M*, R*	p 2-DE Edman	David et al., 1993a, 1994
beta B3 crystallin		N: WI			
beta B3 crystallin	truncation 17/18	WL: WS; WI	M, M*, R*	P 2-DE ESI-MS	David et al., 1993a, 1993b, 1994
beta B3 crystallin				p 2-DE ESI-MS/MS	Ueda et al., 2002b
beta B3 crystallin				p 2-DE Edman	
beta B3 crystallin	truncation 22/23	WL: WS	B	p 2-DE Edman	Shih et al., 1998
gamma B crystallin	acetylation, M1 removed	C: WS, γ	H	P LC/ESI-MS	Lapko et al., 2003b
gamma B crystallin		N: WS, γ		p LC/ESI-MS/MS	
gamma B crystallin	carbamylation, M1 removed	C: WS, γ	H	p LC/MALDI-MS	
gamma B crystallin		N: WS, γ		P LC/ESI-MS	Lapko et al., 2003b
gamma B crystallin				p LC/ESI-MS/MS	
gamma B crystallin				p LC/MALDI-MS	
gamma B crystallin	kynurenine W158	WL: US	M	p 2-DE MALDI-MS	Jungblut et al., 1998
gamma B crystallin	methylation C22	C: WS, γ	H	P LC/ESI-MS	Lapko et al., 2003b
gamma B crystallin		N: WS, γ		p LC/ESI-MS/MS	
gamma B crystallin				p LC/MALDI-MS	
gamma B crystallin	methylation C79	C: WS, γ	H	P LC/ESI-MS	Lapko et al., 2003b
gamma B crystallin		N: WS, γ		p LC/ESI-MS/MS	
gamma B crystallin				p LC/MALDI-MS	
gamma B crystallin	oxidation M70	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
gamma B crystallin	oxidation W69	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
gamma B crystallin	phosphorylation Y63	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
gamma B crystallin	phosphorylation Y66	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
gamma C crystallin	acetylation, M1 removed	C: WS, γ	H	P LC/ESI-MS	Lapko et al., 2003b
gamma C crystallin		N: WS, γ		p LC/ESI-MS/MS	
gamma C crystallin				p LC/MALDI-MS	
gamma C crystallin	carbamylation, M1 removed	C: WS, γ	H	P LC/ESI-MS	Lapko et al., 2003b
gamma C crystallin		N: WS, γ		p LC/ESI-MS/MS	
gamma C crystallin				p LC/MALDI-MS	
gamma C crystallin	deamidation N137	WL: WS, γ	H	P LC/ESI-MS	Hanson et al., 1998
gamma C crystallin	disulfide bond C22–32	WL: WS, γ	H	P LC/ESI-MS	Hanson et al., 1998
gamma C crystallin	disulfide bond C78–79	WL: WS, γ	H	P LC/ESI-MS	Hanson et al., 1998
gamma C crystallin	kynurenine W157	WL: US	M	p 2-DE MALDI-MS	Jungblut et al., 1998
gamma C crystallin	methylation C22	C: WS, γ	H	P LC/ESI-MS	Lapko et al., 2003b
gamma C crystallin		N: WS, γ		p LC/ESI-MS/MS	
gamma C crystallin				p LC/MALDI-MS	
gamma C crystallin	methylation C79	C: WS, γ	H	P LC/ESI-MS	Lapko et al., 2003b
gamma C crystallin		N: WS, γ		p LC/ESI-MS/MS	
gamma C crystallin				p LC/MALDI-MS	

(continued)

Table 1 (continued)

Protein	Modification ^a	Lenticular region ^b	Species ^c	Detection method ^d	Citation
gamma C crystallin	oxidation M70	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
gamma C crystallin	oxidation W69	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
gamma C crystallin	oxidation W131	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
gamma C crystallin	oxidation Y56	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
gamma C crystallin	phosphorylation Y63	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
gamma C crystallin	phosphorylation Y66	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
gamma D crystallin	acetylation, M1 removed	C: WS, γ N: WS, γ	H	P LC/ESI-MS p LC/ESI-MS/MS p LC/MALDI-MS	Lapko et al., 2003b
gamma D crystallin	carbamylation, M1 removed	C: WS, γ N: WS, γ	H	P LC/ESI-MS p LC/ESI-MS/MS p LC/MALDI-MS	Lapko et al., 2003b
gamma D crystallin	disulfide bond C18–32	WL: WS, γ	H	P LC/ESI-MS	Hanson et al., 1998
gamma D crystallin	disulfide bond C108–110	WL: WS, γ	H	P LC/ESI-MS	Hanson et al., 1998
gamma D crystallin	methylation C110	C: WS, γ N: WS, γ	H	P LC/ESI-MS p LC/ESI-MS/MS p LC/MALDI-MS	Lapko et al., 2003b
gamma D crystallin	oxidation Y46	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
gamma D crystallin	truncation 73/74	WL: WS, γ	H	P 2-DE Edman	Srivastava et al., 1998
gamma D crystallin	truncation 86/87	WL: WS, γ	H	P LC/ESI-MS P 2-DE Edman	Abbasi et al., 1998 Srivastava et al., 1998
gamma D crystallin	truncation 172/173	WL: WS, γ C: WS, γ N: WS, γ	H	P LC/ESI-MS p LC/ESI-MS/MS p LC/MALDI-MS	Abbasi et al., 1998 Lapko et al., 2003b
gamma S crystallin	deamidation N14	N: WS; WI	H*	p LC/ESI-MS/MS	Lapko et al., 2002
gamma S crystallin	deamidation N37	N: WS; WI	H*	p LC/ESI-MS/MS	Lapko et al., 2002
gamma S crystallin	deamidation N53	N: WS; WI	H*	p LC/ESI-MS/MS	Lapko et al., 2002
gamma S crystallin	deamidation N76	WL: WI N: WS; WI	H, H*	P LC/ESI-MS p LC/ESI-MS/MS	Hanson et al., 2000 Lapko et al., 2002
gamma S crystallin	deamidation N143	N: WS; WI	H*	p LC/ESI-MS/MS	Lapko et al., 2002
gamma S crystallin	deamidation Q16	N: WS; WI	H*	p LC/ESI-MS/MS	Lapko et al., 2002
gamma S crystallin	deamidation Q63	N: WS; WI	H*	p LC/ESI-MS/MS	Lapko et al., 2002
gamma S crystallin	deamidation Q70	N: WS; WI	H*	p LC/ESI-MS/MS	Lapko et al., 2002
gamma S crystallin	deamidation Q92	WL: WS, γ ; WI N: WS; WI	H, H*	P LC/ESI-MS p LC/ESI-MS p LC/ESI-MS/MS	Hanson et al., 1998, 2000 Lapko et al., 2002
gamma S crystallin	deamidation Q106	N: WS; WI	H*	p LC/ESI-MS/MS	Lapko et al., 2002
gamma S crystallin	deamidation Q120	N: WS; WI	H*	p LC/ESI-MS/MS	Lapko et al., 2002
gamma S crystallin	deamidation Q148	N: WS; WI	H*	p LC/ESI-MS/MS	Lapko et al., 2002
gamma S crystallin	deamidation Q170	WL: WS, γ ; WI N: WS; WI	H, H*	P LC/ESI-MS p LC/ESI-MS p LC/ESI-MS/MS	Hanson et al., 1998, 2000 Lapko et al., 2002
gamma S crystallin	disulfide bond C22–24	WL: WS, γ	H	p LC/ESI-MS	Hanson et al., 1998
gamma S crystallin	disulfide bond C114–129	WL: WS, γ	H	p LC/ESI-MS	Hanson et al., 1998
gamma S crystallin	methylation K6	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
gamma S crystallin	oxidation M41	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
gamma S crystallin	oxidation M73	WL: WI	H	P LC/ESI-MS p LC/ESI-MS/MS	Hanson et al., 2000
gamma S crystallin	oxidation M101	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
gamma S crystallin	oxidation M106	WL: WS	H*	p MudPIT/ESI-MS/MS	MacCoss et al., 2002
gamma S crystallin	truncation 61/62	WL: WS, γ ; WI	H	P LC/ESI-MS p LC/ESI-MS/MS	Abbasi et al., 1998 Hanson et al., 2000
gamma S crystallin	truncation 83/84	WL: WS, γ	H	P 2-DE Edman	Srivastava et al., 1998
gamma S crystallin	truncation 84/85	WL: WS, γ	H	P 2-DE Edman	Srivastava et al., 1998
gamma S crystallin	truncation 86/87	WL: WS, γ	H	P 2-DE Edman	Srivastava et al., 1998
gamma S crystallin	truncation 89/90	WL: WS, γ	H	P LC/ESI-MS	Abbasi et al., 1998
gamma S crystallin	truncation 90/91	WL: WS, γ	H	P LC/ESI-MS P 2-DE Edman	Abbasi et al., 1998 Srivastava et al., 1998
gamma S crystallin	truncation 174/175	WL: WS, γ	H	P LC/ESI-MS	Abbasi et al., 1998
gamma S crystallin	truncation 176/177	WL: WS, γ	H	P LC/ESI-MS	Abbasi et al., 1998

^a Modification sites are listed as modification type followed by the modified amino acid residue in one-letter code and the residue number

^b Lenticular regions are listed as morphological sections followed by solubility fractions followed by size exclusion chromatography fractions. Abbreviations are: WL, whole lens; C, cortex; N, nucleus; WS, water-soluble fraction; WI, water-insoluble fraction; US, urea-soluble fraction; HMW, high molecular weight fraction; α , alpha fraction; β_H , beta high fraction; β_L , beta low fraction; γ , gamma fraction; mF, monomeric fraction

^c Species are abbreviated as follows: B, bovine; C, chicken; H, human; HSr, human Soemmerring's ring; H*, human cataract; M, mouse; M*, mouse cataract; R, rat; R+, rat diabetic; R*, rat cataract

^d Non-standard detection method abbreviations are: P, protein; p peptide; denoting whether the analysis was carried out on the entire protein or on cleaved peptides

David and Shearer, 1993b; Ajaz et al., 1997). It has also been suggested that beta crystallin truncation leads to a tighter molecular arrangement and therefore heightened water exclusion increasing from cortex to nucleus (Werten et al., 1999). Other important modifications are phosphorylation, deamidation, which has been shown to have an impact on molecular structure and subunit assembly (Lampi et al., 2001; Lapko et al., 2002), methylation, oxidation and tryptophan oxidation to kynurenine (Jungblut et al., 1998) as well as covalent kynurenine attachment (Garner et al., 2000). Numerous post-translational modifications have been detected in the healthy lens and are therefore a part of the natural ageing process (Miesbauer et al., 1994). We have attempted a more or less comprehensive list of post-translational modifications detected by proteomics techniques in Table 1. Many of the major post-translational modifications in the table were previously characterized by techniques such as peptide mapping. These citations are not included as we have limited ourselves to proteomics techniques in the actual sense.

Appearance of post-translational crystallin modification is also correlated to the onset of lenticular opacification or cataract. Cataract is the leading cause of blindness worldwide. Twenty million children under the age of sixteen suffer from congenital cataract appearing early in life (Graw, 2003) and four million people are rendered blind yearly in India alone as a result of increasing lenticular opacification (Minassian and Mehra, 1990). It seems the unfolding, misfolding, insolubilization and eventual aggregation of proteins leads to diffraction of incident light classifying cataract as a conformational disease (Harding, 1972; Carrell and Lomas, 1997). Chemical changes in a molecule's primary structure alter its conformation. Therefore many of the protein species resulting from post-translational modification of the crystallins can be implicated in cataractogenesis. Indeed, major proteomics studies on cataractous lenses with varying degrees of opacification have identified large numbers of post translationally modified crystallins using 2-DE (Garber et al., 1984; Li et al., 2002; Calvin et al., 1996; Tumminia et al., 2001; David et al., 1994). For a more complete collection of work on cataractous lenses with the full proteomics methods repertoire see Table 1.

Many of the modifications detected in the healthy lens are also inherent to cataractous lenses. This suggests that while in some cases there may be unique modifications that compulsorily lead to cataract, in many cases the onset of lenticular opacification may be a question of degrees. Protein species that are mandatory for establishing a healthy refractive index and lenticular milieu may be

deleterious when their abundance exceeds a certain threshold which can no longer be checked by other factors. For an illustration of some of the pathways involved in cataractogenesis see a review by Harding (Harding, 2002).

Conclusion

The eye lens is a unique tissue whose facile simplicity disguises an exquisite dynamics we are beginning to understand. Proteomics methods are ideal for exploring this tissue as it is composed almost exclusively of proteins, the proteins are mainly the result of 10 to 20 primary translation products and the proteins can be fractionated and purified with relative ease allowing the realization of most of the protein species with today's proteomics technology. A possible equilibrium between healthy and disease states and the evident importance of post translationally modified proteins may prove a valuable model for a deeper understanding of biological concepts. The availability of a simple and complete proteome for both congenital and age related cataract can be valuable in the analysis of congenital disease. The large number of cataract patients gives the work immanent clinical relevance.

Note added in proof

Recently a new crystallin, gamma N crystallin was detected. This seems to be the link in beta and gamma crystallin gene evolution (Wistow et al., 2005).

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References

- Aarts HJ, Lubsen NH, Schoenmakers JG (1989) Crystallin gene expression during rat lens development. *Eur J Biochem* 183: 31–36
- Abbasi A, Smith DL, Smith JB (1998) Characterization of low molecular mass gamma-crystallin fragments from human lenses. *Exp Eye Res* 67: 485–488
- Abgar S, Backmann J, Aerts T, Vanhoudt J, Clauwaert J (2000) The structural differences between bovine lens alpha A- and alpha B-crystallin. *Eur J Biochem* 267: 5916–5925
- Ajaz MS, Ma Z, Smith DL, Smith JB (1997) Size of human lens beta-crystallin aggregates are distinguished by N-terminal truncation of beta B1. *J Biol Chem* 272: 11250–11255
- Asselbergs FA, Koopmans M, van Venrooij WJ, Bloemendal H (1979) Improved resolution of calf lens beta-crystallins. *Exp Eye Res* 28: 223–228
- Bassnett S (2002) Lens organelle degradation. *Exp Eye Res* 74: 639–649
- Bateman OA, Lubsen NH, Slingsby C (2001) Association behavior of human betaB1-crystallin and its truncated forms. *Exp Eye Res* 73: 321–331

- Bateman OA, Sarra R, van Genesen ST, Kappe G, Lubsen NH, Slingsby C (2003) The stability of human acidic beta-crystallin oligomers and hetero-oligomers. *Exp Eye Res* 77: 409–422
- Bennett MJ, Choe S, Eisenberg D (1994) Domain swapping: entangling alliances between proteins. *Proc Natl Acad Sci USA* 91: 3127–3131
- Bennett MJ, Schlunegger MP, Eisenberg D (1995) 3D domain swapping: a mechanism for oligomer assembly. *Protein Sci* 4: 2455–2468
- Berbers GA, Boerman OC, Bloemendal H, de Jong WW (1982) Primary gene products of bovine beta-crystallin and reassociation behavior of its aggregates. *Eur J Biochem* 128: 495–502
- Berbers GA, Hoekman WA, Bloemendal H, de Jong WW, Kleinschmidt T, Braunitzer G (1984) Homology between the primary structures of the major bovine beta-crystallin chains. *Eur J Biochem* 139: 467–479
- Bhat SP (2003) Crystallins, genes and cataract. *Prog Drug Res* 60: 205–262
- Bhat SP (2004) Transparency and non-refractive functions of crystallins—a proposal. *Exp Eye Res* 79: 809–816
- Bindels JG, de Man BM, Hoenders HJ (1982) High-performance gel permeation chromatography of bovine eye lens proteins in combination with low-angle laser light scattering. Superior resolution of the oligomeric beta-crystallins. *J Chromatogr* 252: 255–267
- Bindels JG, Koppers A, Hoenders HJ (1981) Structural aspects of bovine beta-crystallins: physical characterization including dissociation-association behavior. *Exp Eye Res* 33: 333–343
- Bloemendal H (1977) The vertebrate eye lens. *Science* 197: 127–138
- Bloemendal H, de Jong W, Jaenicke R, Lubsen NH, Slingsby C, Tardieu A (2004) Ageing and vision: structure, stability and function of lens crystallins. *Prog Biophys Mol Biol* 86: 407–485
- Bloemendal H, de Jong WW (1991) Lens proteins and their genes. *Prog Nucleic Acid Res Mol Biol* 41: 259–281
- Bloemendal H, Van de Gaer K, Benedetti EL, Dunia I, Steely HT (1997) Toward a human crystallin map. Two-dimensional gel electrophoresis and computer analysis of water-soluble crystallins from normal and cataractous human lenses. *Ophthalmic Res* 29: 177–190
- Bours J, Wegener A, Hofmann D, Fodisch HJ, Hockwin O (1990) Protein profiles of microsections of the fetal and adult human lens during development and ageing. *Mech Ageing Dev* 54: 13–27
- Bova MP, Ding LL, Horwitz J, Fung BK (1997) Subunit exchange of alpha A-crystallin. *J Biol Chem* 272: 29511–29517
- Bradley RH, Ireland M, Maisel H (1979) The cytoskeleton of chick lens cells. *Exp Eye Res* 28: 441–453
- Breitman ML, Lok S, Wistow G, Piatigorsky J, Treton JA, Gold RJ, Tsui LC (1984) Gamma-crystallin family of the mouse lens: structural and evolutionary relationships. *Proc Natl Acad Sci USA* 81: 7762–7766
- Calvin HI, Wu JX, Viswanadhan K, Fu SC (1996) Modifications in lens protein biosynthesis signal the initiation of cataracts induced by buthionine sulfoximine in mice. *Exp Eye Res* 63: 357–368
- Carrell RW, Lomas DA (1997) Conformational disease. *Lancet* 350: 134–138
- Carter JM, Hutcheson AM, Quinlan RA (1995) In vitro studies on the assembly properties of the lens proteins CP49, CP115: coassembly with alpha-crystallin but not with vimentin. *Exp Eye Res* 60: 181–192
- Carver JA (1999) Probing the structure and interactions of crystallin proteins by NMR spectroscopy. *Prog Retin Eye Res* 18: 431–462
- Carver JA, Cooper PG, Truscott RJ (1993) 1H-NMR spectroscopy of beta B2-crystallin from bovine eye lens. *Eur J Biochem* 213: 313–320
- Carver JA, Nicholls KA, Aquilina JA, Truscott RJ (1996) Age-related changes in bovine alpha-crystallin and high-molecular-weight protein. *Exp Eye Res* 63: 639–647
- Caspers GJ, Leunissen JA, de Jong WW (1995) The expanding small heat-shock protein family, and structure predictions of the conserved “alpha-crystallin domain”. *J Mol Evol* 40: 238–248
- Chambers C, Russell P (1991) Deletion mutation in an eye lens beta-crystallin. An animal model for inherited cataracts. *J Biol Chem* 266: 6742–6746
- Chiou SH, Azari P (1989) Physicochemical characterization of alpha-crystallins from bovine lenses: hydrodynamic and conformational properties. *J Protein Chem* 8: 1–17
- Clark R, Zigman S, Lerman S (1969) Studies on the structural proteins of the human lens. *Exp Eye Res* 8: 172–182
- Clout NJ, Basak A, Wieligman K, Bateman OA, Jaenicke R, Slingsby C (2000) The N-terminal domain of betaB2-crystallin resembles the putative ancestral homodimer. *J Mol Biol* 304: 253–257
- Colvis C, Garland D (2002) Posttranslational modification of human alpha A-crystallin: correlation with electrophoretic migration. *Arch Biochem Biophys* 397: 319–323
- Colvis CM, Douglas-Tabor Y, Werth KB, Vieira NE, Kowalak JA, Janjani A, Yergey AL, Garland DL (2000) Tracking pathology with proteomics: identification of in vivo degradation products of alpha B-crystallin. *Electrophoresis* 21: 2219–2227
- Cooper PG, Aquilina JA, Truscott RJ, Carver JA (1993a) Supramolecular order within the lens: 1H NMR spectroscopic evidence for specific crystallin-crystallin interactions. *Exp Eye Res* 59: 607–616
- Cooper PG, Carver JA, Truscott RJ (1993b) 1H-NMR spectroscopy of bovine lens beta-crystallin. The role of the beta B2-crystallin C-terminal extension in aggregation. *Eur J Biochem* 213: 321–328
- Datiles MB, Schumert DJ, Zigler JS Jr, Russell P, Anderson L, Garland D (1992) Two-dimensional gel electrophoretic analysis of human lens proteins. *Curr Eye Res* 11: 669–677
- David LL, Calvin HI, Fu SC (1994) Buthionine sulfoximine induced cataracts in mice contain insolubilized crystallins with calpain II cleavage sites. *Exp Eye Res* 59: 501–504
- David LL, Lampi KJ, Lund AL, Smith JB (1996) The sequence of human betaB1-crystallin cDNA allows mass spectrometric detection of betaB1 protein missing portions of its N-terminal extension. *J Biol Chem* 271: 4273–4279
- David LL, Shearer TR (1984) Calcium-activated proteolysis in the lens nucleus during selenite cataractogenesis. *Invest Ophthalmol Vis Sci* 25: 1275–1283
- David LL, Shearer TR (1993b) Beta-crystallin insolubilized by calpain II in vitro contain cleavage sites similar to beta-crystallins insolubilized during cataract. *FEBS Lett* 324: 265–270
- David LL, Shearer TR, Shih M (1993a) Sequence analysis of lens beta-crystallins suggests involvement of calpain in cataract formation. *J Biol Chem* 268: 1937–1940
- de Vries AC, Vermeer MA, Hendriks AL, Bloemendal H, Cohen LH (1991) Biosynthetic capacity of the human lens upon aging. *Exp Eye Res* 53: 519–524
- Delaye M, Tardieu A (1983) Short-range order of crystallin proteins accounts for eye lens transparency. *Nature* 302: 415–417
- Dewey J, Bartling C, Rae JL (1995) A non-enzymatic method for lens decapsulation which leaves the epithelial cells attached to the fibers. *Curr Eye Res* 14: 357–362
- Dole M, Mach LL, Hines RL, Mobley RC, Ferguson LD, Alice MB (1968) Molecular beams of macroions. *J Chem Phys* 49: 2240–2247
- Driessen HP, Herbrink P, Bloemendal H, de Jong WW (1981) Primary structure of the bovine beta-crystallin Bp chain. Internal duplication and homology with gamma-crystallin. *Eur J Biochem* 121: 83–91
- Edman P (1949) A method for the determination of the amino acid sequence in peptides. *Arch Biochem* 22: 475–476
- Emmons T, Takemoto L (1992) Age-dependant loss of the C-terminal amino acid from alpha crystallin. *Exp Eye Res* 55: 551–554
- Farnsworth PN, Groth-Vasselli B, Greenfield NJ, Singh K (1997) Effects of temperature and concentration on bovine lens alpha-crystallin secondary structure: a circular dichroism spectroscopic study. *Int J Biol Macromol* 20: 283–291
- Feng J, Smith DL, Smith JB (2000) Human lens beta-crystallin solubility. *J Biol Chem* 275: 11585–11590

- Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM (1989) Electrospray ionization for mass spectrometry of large biomolecules. *Science* 246: 64–71
- Fernald RD, Wright SE (1983) Maintenance of optical quality during crystalline lens growth. *Nature* 301: 618–620
- FitzGerald PG (1988) Age-related changes in a fiber cell-specific extrinsic membrane protein. *Curr Eye Res* 7: 1255–1262
- Fujii N, Awakura M, Takemoto L, Inomata M, Takata T, Fujii N, Saito T (2003) Characterization of alpha A-crystallin from high molecular weight aggregates in the normal human lens. *Mol Vis* 9: 315–322
- Fujii N, Matsumoto S, Hiroki K, Takemoto L (2001) Inversion and isomerization of Asp-58 residue in human alpha A-crystallin from normal aged lenses and cataractous lenses. *Biochim Biophys Acta* 1549: 179–187
- Fujii N, Satoh K, Harada K, Ishibashi Y (1994) Simultaneous stereo-inversion and isomerization at specific aspartic acid residues in alpha A-crystallin from human lens. *J Biochem (Tokyo)* 116: 663–669
- Fujii N, Takemoto LJ, Momose Y, Matsumoto S, Hiroki K, Akaboshi M (1999) Formation of four isomers at the asp- 151 residue of aged human alpha A-crystallin by natural aging. *Biochem Biophys Res Commun* 265: 746–751
- Fujii N, Takeuchi N, Fujii N, Tezuka T, Kuge K, Takata T, Kamei A, Saito T (2004) Comparison of post-translational modifications of alpha A-crystallin from normal and hereditary cataract rats. *Amino Acids* 26: 147–152
- Fung KY, Askovic S, Basile F, Duncan MW (2004) A simple and inexpensive approach to interfacing high-performance liquid chromatography and matrix-assisted laser desorption/ionization-time of flight-mass spectrometry. *Proteomics* 4: 3121–3127
- Garadi R, Katar M, Maisel H (1983) Two-dimensional gel analysis of chick lens proteins. *Exp Eye Res* 36: 859–869
- Garber AT, Goring D, Gold RJ (1984) Characterization of abnormal proteins in the soluble lens proteins of CatFraser mice. *J Biol Chem* 259: 10376–10379
- Garland DL, Douglas-Tabor Y, Yimenez-Asensio J, Datiles MB, Magno B (1996) The nucleus of the human lens: demonstration of a highly characteristic protein pattern by two-dimensional electrophoresis and introduction of a new method of lens dissection. *Exp Eye Res* 62: 285–291
- Garner B, Shaw DC, Lindner RA, Carver JA, Truscott RJ (2000) Non-oxidative modification of lens crystallins by kynurenine: a novel post-translational protein modification with possible relevance to ageing and cataract. *Biochim Biophys Acta* 1476: 265–278
- Gesierich U, Pfeil W (1996) The conformational stability of alpha-crystallin is rather low: calorimetric results. *FEBS Lett* 393: 151–154
- Gong X, Li E, Klier G, Huang Q, Wu Y, Lei H, Kumar NM, Horwitz J, Gilula NB (1997) Disruption of alpha3 connexin gene leads to proteolysis and cataractogenesis in mice. *Cell* 91: 833–843
- Goodenough DA (1992) The crystalline lens. A system networked by gap junctional intercellular communication. *Semin Cell Biol* 3: 49–58
- Goring DR, Breitman ML, Tsui LC (1992) Temporal regulation of six crystallin transcripts during mouse lens development. *Exp Eye Res* 54: 785–795
- Graw J (1997) The crystallins: genes, proteins and diseases. *Biol Chem* 278: 1331–1348
- Graw J (2003) The genetic and molecular basis of congenital eye defects. *Nat Rev Genet* 4: 876–888
- Graw J, Liebshtein A, Pietrowski D, Schmitt-John T, Werner T (1993) Genomic sequences of murine gamma B- and gamma C-crystallin-encoding genes: promoter analysis and complete evolutionary pattern of mouse, rat and human gamma-crystallins. *Gene* 136: 145–156
- Groenen PJ, Merck KB, de Jong WW, Bloemendal H (1994) Structure and modifications of the junior chaperone alpha-crystallin. From lens transparency to molecular pathology. *Eur J Biochem* 225: 1–19
- Haley DA, Horwitz J, Stewart PL (1998) The small heat-shock protein, alpha B-crystallin, has a variable quaternary structure. *J Mol Biol* 277: 27–35
- Hanson SR, Hasan A, Smith DL, Smith JB (2000) The major in vivo modifications of the human water-insoluble lens crystallins are disulfide bonds, deamidation, methionine oxidation and backbone cleavage. *Exp Eye Res* 71: 195–207
- Hanson SR, Smith DL, Smith JB (1998) Deamidation and disulfide bonding in human lens gamma-crystallins. *Exp Eye Res* 67: 301–312
- Harding JJ (1972) Conformational changes in human lens proteins in cataract. *Biochem J* 129: 97–100
- Harding JJ (2002) Viewing molecular mechanisms of ageing through a lens. *Ageing Res Rev* 1: 465–479
- Harrington V, McCall S, Huynh S, Srivastava K, Srivastava OP (2004) Crystallins in water soluble-high molecular weight protein fractions and water insoluble protein fractions in aging and cataractous human lenses. *Mol Vis* 10: 476–489
- Hasan A, Smith DL, Smith JB (2002) Alpha-crystallin regions affected by adenosine 5'-triphosphate identified by hydrogen-deuterium exchange. *Biochemistry* 41: 15875–15882
- Hejtmancik JF, Wingfield PT, Chambers C, Russel P, Chen HC, Sergeev YV, Hope JN (1997) Association properties of betaB2- and betaA3-crystallin: ability to form dimers. *Protein Eng* 10: 1347–1352
- Hejtmancik JF, Wingfield PT, Sergeev YV (2004) Beta-crystallin association. *Exp Eye Res* 79: 377–383
- Hendriks W, Weetnik H, Voorter CE, Sanders J, Bloemendal H, de Jong WW (1990) The alternative splicing product alpha Ains-crystallin is structurally equivalent to alpha A and alpha B subunits in the rat alpha crystallin aggregate. *Biochim Biophys Acta* 1037: 58–65
- Hoehenwarter W, Ackermann R, Zimny-Arndt U, Kumar NM, Jungblut PR (2006) The necessity of functional proteomics: protein species and molecular function elucidation exemplified by in vivo alpha A crystallin N-terminal truncation. *Amino Acids* (in press)
- Hoehenwarter W, Kumar NM, Wacker M, Zimny-Arndt U, Klose J, Jungblut PR (2005) Eye lens proteomics: from global approach to detailed information about phakinin and gamma E and F crystallin genes. *Proteomics* 5: 245–257
- Horwitz J (1992) Alpha-crystallin can function as a molecular chaperone. *Proc Natl Acad Sci USA* 89: 10449–10453
- Horwitz J, Huang QL, Ding L, Bova MP (1998) Lens alpha-crystallin: chaperone like properties. *Methods Enzymol* 290: 365–383
- Ireland M, Maisel H (1984) A cytoskeletal protein unique to lens fiber cell differentiation. *Exp Eye Res* 38: 637–645
- Ireland M, Maisel H (1989) A family of lens fiber cell specific proteins. *Lens Eye Toxic Res* 6: 623–638
- Iwaki T, Kume-Iwaki A, Goldman JE (1990) Cellular distribution of alpha B-crystallin in non-lenticular tissues. *J Histochem Cytochem* 38: 31–39
- Jedziniak JA, Nicoli DF, Baram H, Benedek GB (1978) Quantative verification of the existence of high molecular weight protein aggregates in the intact normal human lens by light-scattering spectroscopy. *Invest Ophthalmol Vis Sci* 17: 51–57
- Jimenez-Asensio J, Colvis CM, Kowalak JA, Douglas-Tabor Y, Datiles MB, Moroni M, Mura U, Rao CM, Balasubramanian D, Janjani A, Garland D (1999) An atypical form of alpha B-crystallin is present in high concentration in some human cataractous lenses. Identification and characterization of aberrant N- and C-terminal processing. *J Biol Chem* 274: 32287–32294
- Jungblut P, Klose J (1985) Genetic variability of proteins from mitochondria and mitochondrial fractions of mouse organs. *Biochem Genet* 23: 227–245
- Jungblut P, Thiede B (1997) Protein identification from 2-DE gels by MALDI mass spectrometry. *Mass Spectrom Rev* 16: 145–162

- Jungblut P, Thiede B, Zimny-Arndt U, Müller EC, Scheler C, Wittmann-Liebold B, Otto A (1996) Resolution power of two-dimensional electrophoresis and identification of proteins from gels. *Electrophoresis* 17: 839–847
- Jungblut PR, Otto A, Favor J, Lowe M, Muller EC, Kastner M, Sperling K, Klose J (1998) Identification of mouse crystallins in 2D protein patterns by sequencing and mass spectrometry. Application to cataract mutants. *FEBS Lett* 435: 131–137
- Kamei A, Iwase H, Masuda K (1997) Cleavage of amino acid residue(s) from the N-terminal region of alpha A- and alpha B-crystallins in human crystalline lens during aging. *Biochem Biophys Res Commun* 231: 373–378
- Kamei A, Takeuchi N, Nagai M, Mori S (2003) Post-translational modification of beta H-crystallin of bovine lens with aging. *Biol Pharm Bull* 26: 1715–1720
- Karas M, Hillenkamp F (1988) Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons. *Anal Chem* 60: 2299–2301
- Kelley MJ, David LL, Iwasaki N, Wright J, Shearer TR (1993) alpha-Crystallin chaperone activity is reduced by calpain II in vitro and in selenite cataract. *J Biol Chem* 268: 18844–18849
- Kibbelaar M, Bloemendal H (1975) The topography of lens proteins based on chromatography and two-dimensional gel electrophoresis. *Exp Eye Res* 21: 25–36
- Kibbelaar MA, Bloemendal H (1979) Fractionation of the water-insoluble proteins from calf lens. *Exp Eye Res* 29: 679–688
- Kilby GW (1995a) Electrospray mass spectrometry of peptides and lens proteins. Ph.D. Thesis, The University of Wollongong, Australia
- Kilby GW, Carver JA, Zhu JL, Sheil MM, Truscott RJ (1995b) Loss of the C-terminal serine residue from bovine beta B2-crystallin. *Exp Eye Res* 60: 465–469
- Kilby GW, Sheil MM, Shaw D, Harding JJ, Truscott RJ (1997) Amino acid sequence of bovine gamma E (IVa) lens crystallin. *Protein Sci* 6: 909–912
- Kleiman NJ, Chiesa R, Kolks MA, Spector A (1988) Phosphorylation of beta-crystallin B2 (beta Bp) in the bovine lens. *J Biol Chem* 263: 14978–14983
- Klemen R, Frohli E, Steiger RH, Schafer R, Aoyama A (1991) Alpha B-crystallin is a small heat shock protein. *Proc Natl Acad Sci USA* 88: 3652–3655
- Klose J (1975) Protein mapping by combined isoelectric focusing and electrophoresis of mouse tissues. A novel approach to testing for induced point mutations in mammals. *Humangenetik* 26: 231–243
- Klose J, Kobalz U (1995) Two-dimensional electrophoresis of proteins: an updated protocol and implications for a functional analysis of the genome. *Electrophoresis* 16: 1034–1059
- Klose J, Nock C, Herrmann M, Stühler K, Marcus K, Blüggel M, Krause E, Schalwyk LC, Rastan S, Brown SDM, Büssow K, Himmelbauer H, Lehrach H (2002) Genetic analysis of the mouse brain proteome. *Nat Genet* 30: 385–393
- Koretz JF, Handelman GH (1988) How the human eye focuses. *Sci Am* 259: 92–99
- Krah A, Miehke S, Pleissner KP, Zimny-Arndt U, Kirsch C, Lehn N, Meyer TF, Jungblut PR, Aebischer T (2004) Identification of candidate antigens for serologic detection of *Helicobacter pylori*-infected patients with gastric carcinoma. *Int J Cancer* 108: 456–463
- Kröger RH, Campbell MC, Munger R, Fernald RD (1994) Refractive index distribution and spherical aberration in the crystalline lens of the African cichlid fish *Haplochromis burtoni*. *Vis Res* 34: 1815–1822
- Kuszak JR, Peterson KL, Sivak JG, Herbert KL (1994) The interrelationship of lens anatomy and optical quality. II. Primate lenses. *Exp Eye Res* 59: 521–535
- Kuwabara T (1975) The maturation of the lens cell: a morphologic study. *Exp Eye Res* 20: 427–443
- Lambert S (1994) Lens. In: Albert D, Jakobiec F (eds) *Principles and practices of ophthalmology*. Saunders, Philadelphia
- Lampi K, Shih M, Ueda Y, Shearer TR, David LL (2002) Lens proteomics: analysis of rat crystallin sequences and two-dimensional electrophoresis map. *Invest Ophthalmol Vis Sci* 43: 216–224
- Lampi KJ, Ma Z, Hanson SR, Azuma M, Shih M, Shearer TR, Smith DL, Smith JB, David LL (1998) Age-related changes in human lens crystallins identified by two-dimensional electrophoresis and mass spectrometry. *Exp Eye Res* 67: 31–43
- Lampi KJ, Ma Z, Shih M, Shearer TR, Smith JB, Smith DL, David LL (1997) Sequence analysis of betaA3, betaB3, and betaA4 crystallins completes the identification of the major proteins in young human lens. *J Biol Chem* 272: 2268–2275
- Lampi KJ, Oxford JT, Bachinger HP, Shearer TR, David LL, Kapfer DM (2001) Deamidation of human beta B1 alters the elongated structure of the dimer. *Exp Eye Res* 72: 279–288
- Lapko VN, Pukiss AG, Smith DL, Smith JB (2002) Deamidation in human gamma S-crystallin from cataractous lenses is influenced by surface exposure. *Biochemistry* 41: 8638–8648
- Lapko VN, Smith DL, Smith JB (2001) In vivo carbamylation and acetylation of water-soluble human lens alpha B-crystallin lysine 92. *Protein Sci* 10: 1130–1136
- Lapko VN, Smith DL, Smith JB (2003a) Expression of beta A2 crystallin in human lenses. *Exp Eye Res* 77: 383–385
- Lapko VN, Smith DL, Smith JB (2003b) Methylation and carbamylation of human gamma-crystallins. *Protein Sci* 12: 1762–1774
- Li LK, Roy D, Spector A (1986) Changes in lens protein in concentric fractions from individual normal human lenses. *Curr Eye Res* 5: 127–135
- Li W, Calvin HI, David LL, Wu K, McCormack AL, Zhu GP, Fu SC (2002) Altered patterns of phosphorylation in cultured mouse lenses during development of buthionine sulfoximine cataracts. *Exp Eye Res* 75: 335–346
- Lin P, Smith DL, Smith JB (1997) In vivo modification of the C-terminal lysine of human lens alpha B-crystallin. *Exp Eye Res* 65: 673–680
- Lin PP, Barry RC, Smith DL, Smith JB (1998) In vivo acetylation identified at lysine 70 of human lens alpha A-crystallin. *Protein Sci* 7: 1451–1457
- Link AJ, Eng J, Schieltz DM, Carmack E, Mize GJ, Morris DR, Garvik BM, Yates JR 3rd (1999) Direct analysis of protein complexes using mass spectrometry. *Nat Biotechnol* 17: 676–682
- Lok S, Tsui LC, Shinohara T, Piatigorsky J, Gold R, Breitman M (1984) Analysis of the mouse gamma-crystallin gene family: assignment of multiple cDNAs to discrete genomic sequences and characterization of a representative gene. *Nucleic Acids Res* 12: 4517–4529
- Lubsen NH, Aarts HJ, Schoenmakers JG (1988) The evolution of lenticular proteins: the beta- and gamma-crystallin super gene family. *Prog Biophys Mol Biol* 51: 47–76
- Lund AL, Smith JB, Smith DL (1996) Modifications of the water-insoluble human lens alpha-crystallins. *Exp Eye Res* 63: 661–672
- Ma Z, Hanson SR, Lampi KJ, David LL, Smith DL, Smith JB (1998) Age-related changes in human lens crystallins identified by HPLC and mass spectrometry. *Exp Eye Res* 67: 21–30
- MacCoss MJ, McDonald WH, Saraf A, Sadygov R, Clark JM, Tasto JJ, Gould KL, Wolters D, Washburn M, Weiss A, Clark JI, Yates JR 3rd (2002) Shotgun identification of protein modifications from protein complexes and lens tissue. *Proc Natl Acad Sci USA* 99: 7900–7905
- Maisel H, Perry MM (1972) Electron microscope observations on some structural proteins of the chick lens. *Exp Eye Res* 14: 7–12
- Mann M, Hendrickson RC, Pandey A (2001) Analysis of proteins and proteomes by mass spectrometry. *Annu Rev Biochem* 70: 437–473
- Mathias RT, Rae JL, Baldo GJ (1997) Physiological properties of the normal lens. *Physiol Rev* 77: 21–50
- McCormack AL, Schieltz DM, Goode B, Yang S, Barnes G, Drubin D, Yates JR 3rd (1997) Direct analysis and identification of proteins in

- mixtures by LC/MS/MS and database searching at the low-femtomole level. *Anal Chem* 69: 767–776
- Messmer M, Chakrabarti B (1988) High-molecular-weight protein aggregates of calf and cow lens: spectroscopic evaluation. *Exp Eye Res* 47: 173–183
- Miesbauer LR, Zhou X, Yang Z, Yang Z, Sun Y, Smith DL, Smith JB (1994) Post-translational modifications of water-soluble human lens crystallins from young adults. *J Biol Chem* 269: 12494–12502
- Minassian DC, Mehra V (1990) 3.8 million blinded by cataract each year: projections from the first epidemiological study of incidence of cataract blindness in India. *Br J Ophthalmol* 74: 341–343
- Mörner CT (1894) Untersuchung der Proteinsubstanzen in den leichtbrechenden Medien des Auges. *Z Physiol Chem* 18: 61–106
- Nagele E, Vollmer M (2004) Coupling of nanoflow liquid chromatography to matrix-assisted laser desorption/ionization mass spectrometry: real-time liquid chromatography run mapping on a MALDI plate. *Rapid Commun Mass Spectrom* 18: 3008–3014
- Norledge BV, Mayr EM, Glockshuber R, Bateman OA, Slingsby C, Jaenicke R, Driessen HP (1996) The X-ray structures of two mutant crystallin domains shed light on the evolution of multi-domain proteins. *Nat Struct Biol* 3: 267–274
- O'Farrell PH (1975) High resolution two-dimensional electrophoresis of proteins. *J Biol Chem* 250: 4007–4021
- Ortwerth BJ, Olesen PR, Sharma KK (1986) Solubilization of the lens water-insoluble fraction by sonication. *Exp Eye Res* 43: 955–963
- Ortwerth BJ, Olesen PR (1989) Studies on the nature of the water-insoluble fraction from aged bovine lenses. *Exp Eye Res* 48: 605–619
- Ortwerth BJ, Olesen PR (1992) Studies on the solubilization of the water-insoluble fraction from human lens and cataract. *Exp Eye Res* 55: 777–783
- Papaconstantinou J (1967) Molecular aspects of lens cell differentiation. *Science* 156: 338–346
- Paron I, D'Elia A, D'Ambrosio C, Scaloni A, D'Aurizio F, Prescott A, Damante G, Tell G (2004) A proteomic approach to identify early molecular targets of oxidative stress in human epithelial lens cells. *Biochem J* 378: 929–937
- Pasta SY, Raman B, Ramakrishna T, Rao ChM (2003) Role of the conserved SRLFDQFFG region of alpha-crystallin, a small heat shock protein. Effect on oligomeric size, subunit exchange and chaperone-like activity. *J Biol Chem* 278: 51159–51166
- Puri N, Augusteyn RC, Owen EA, Siezen RJ (1983) Immunochemical properties of vertebrate alpha-crystallins. *Eur J Biochem* 134: 321–326
- Rae JL, Bartling C, Rae J, Mathias RT (1996) Dye transfer between cells of the lens. *J Membr Biol* 150: 89–103
- Ramaekers FC, Boomkens TR, Bloemendal H (1981) Cytoskeletal and contractile structures in bovine lens cell differentiation. *Exp Cell Res* 35: 454–461
- Ringens PJ, Hoenders HJ, Bloemendal H (1982) Protein distribution and characterization in the prenatal and postnatal human lens. *Exp Eye Res* 34: 815–823
- Robinson KR, Patterson JW (1982–83) Localization of steady currents in the lens. *Curr Eye Res* 2: 843–847
- Sandilands A, Prescott AR, Carter JM, Hutcheson AM, Quinlan RA, Richards J, FitzGerald PG (1995) Vimentin and CP49/filensin form distinct networks in the lens which are independently modulated during lens fiber cell differentiation. *J Cell Sci* 108: 1397–1406
- Schaefer H, Marcus K, Sickmann A, Herrmann M, Klose J, Meyer HE (2003) Identification of phosphorylation and acetylation sites in alpha A-crystallin of the eye lens (*mus musculus*) after two-dimensional gel electrophoresis. *Anal Bioanal Chem* 376: 966–972
- Scheler C, Müller EC, Stahl J, Müller-Werdan U, Salnikow J, Jungblut P (1997) Identification and characterization of heat shock protein 27 protein species in human myocardial 2-DE patterns. *Electrophoresis* 18: 2823–2831
- Schey KL, Little M, Fowler JG, Crouch RK (2000) Characterization of human lens major intrinsic protein structure. *Invest Ophthalmol Vis Sci* 41: 175–182
- Schoenmakers JG, Gerding JJ, Bloemendal H (1969) The subunit structure of alpha-crystallin. Isolation and characterization of the S-carboxymethylated acidic subunits from adult and embryonic origin. *Eur J Biochem* 11: 472–481
- Shih M, Lampi KJ, Shearer TR, David LL (1998) Cleavage of beta crystallins during maturation of bovine lens. *Mol Vis* 4: 4–11
- Shinohara T, Robinson EA, Appella E, Piatigorsky J (1982) Multiple gamma-crystallins of the mouse lens: fractionation of mRNAs by cDNA cloning. *Proc Natl Acad Sci USA* 79: 2783–2787
- Slingsby C (1985) Structural variation in lens crystallins. *Trends Biochem Sci* 10: 281–284
- Slingsby C, Bateman OA (1990) Quaternary interactions in eye lens beta-crystallins: basic and acidic subunits of beta-crystallins favor heterologous association. *Biochemistry* 29: 6592–6599
- Slingsby C, Norledge B, Simpson A, Bateman OA, Wright G, Driessen HPC, Lindley PF, Moss DS, Bax B (1996) X-ray diffraction and structure of crystallins. *Prog Retin Eye Res* 16: 3–29
- Smith JB, Liu Y, Smith DL (1996) Identification of possible regions of chaperone activity in lens alpha-crystallin. *Exp Eye Res* 63: 125–128
- Smith JB, Sun Y, Smith DL, Green B (1992) Identification of the posttranslational modifications of bovine lens alpha B-crystallins by mass spectrometry. *Protein Sci* 1: 601–608
- Smith JB, Thevenon-Emeric G, Smith DL, Green B (1991) Elucidation of the primary structures of proteins by mass spectrometry. *Anal Biochem* 193: 118–124
- Spector A, Chiesa R, Sredy J, Garner W (1985) cAMP-dependent phosphorylation of bovine lens alpha-crystallin. *Proc Natl Acad Sci USA* 82: 4712–4716
- Srinivasan AN, Nagineni CN, Bhat SP (1992) alpha A-crystallin is expressed in non-ocular tissues. *J Biol Chem* 267: 23337–23341
- Srivastava OP, Kirk MC, Srivastava K (2004) Characterization of covalent multimers of crystallins in aging human lenses. *J Biol Chem* 279: 10901–10909
- Srivastava OP, Srivastava K (1998) Degradation of gamma D- and gamma S-crystallins in human lenses. *Biochem Biophys Res Commun* 253: 288–294
- Srivastava OP, Srivastava K, Harrington V (1999) Age-related degradation of beta A3/A1-crystallin in human lenses. *Biochem Biophys Res Commun* 258: 632–638
- Stiuso P, Libondi T, Facchiano AM, Colicchio P, Ferranti P, Lilla S, Colonna G (2002) Alteration in the ubiquitin structure and function in the human lens: a possible mechanism of senile cataractogenesis. *FEBS Lett* 531: 162–167
- Swanson SK, Washburn MP (2005) The continuing evolution of shotgun proteomics. *Drug Discov Today* 10: 719–725
- Takemoto LJ (1995) Identification of the in vivo truncation sites at the C-terminal region of alpha-A crystallin from aged bovine and human lens. *Curr Eye Res* 14: 837–841
- Takemoto LJ (1996) Differential phosphorylation of alpha-A crystallin in human lens of different age. *Exp Eye Res* 62: 499–504
- Takemoto LJ (1997) Disulfide bond formation of cysteine-37 and cysteine-66 of beta B2 crystallin during cataractogenesis of the human lens. *Exp Eye Res* 64: 609–614
- Tanaka K, Waki H, Ido Y, Akita S, Yoshida Y, Yoshida T (1988) Protein and polymer analysis up to m/z 100,000 by laser ionization *time-of-flight mass spectrometry*. *Rapid Commun Mass Spectrom* 2: 151–153
- Tardieu A, Laporte D, Licinio P, Krop B, Delaye M (1986) Calf lens alpha-crystallin quaternary structure. A three-layer tetrahedral model. *J Mol Biol* 192: 711–724
- Thampi P, Abraham EC (2003) Influence of the C-terminal residues on oligomerization of alpha A-crystallin. *Biochemistry* 42: 11857–11863

- Thampi P, Hassan A, Smith JB, Abraham EC (2002) Enhanced C-terminal truncation of alpha A- and alpha B-crystallins in diabetic lenses. *Invest Ophthalmol Vis Sci* 43: 3265–3272
- Toyofuku H, Bentley PJ (1970) The effects of decapsulation on ion movements across the lens of the toad, *Bufo marinus*. Influence on drug actions. *Invest Ophthalmol* 9: 959–965
- Tumminia SJ, Clark JI, Richiert DM, Mitton KP, Douglas-Tabor Y, Kowalak JA, Garland DL, Russell P (2001) Three distinct stages of lens opacification in transgenic mice expressing the HIV-1 protease. *Exp Eye Res* 72: 115–121
- Ueda Y, Duncan MK, David LL (2002a) Lens proteomics: the accumulation of crystallin modifications in the mouse lens with age. *Invest Ophthalmol Vis Sci* 43: 205–215
- Ueda Y, Fukiage C, Shih M, Shearer TR, David LL (2002b) Mass measurements of C-terminally truncated alpha-crystallins from two-dimensional gels identify Lp82 as a major endopeptidase in rat lens. *Mol Cell Proteomics* 1: 357–365
- Ueda Y, McCormack AL, Shearer TR, David LL (2001) Purification and characterization of lens specific calpain (Lp82) from bovine lens. *Exp Eye Res* 73: 625–637
- van den Oetelaar PJ, van Someren PF, Thomson JA, Siezen RJ, Hoenders HJ (1990) A dynamic quaternary structure of bovine alpha-crystallin as indicated from intermolecular exchange of subunits. *Biochemistry* 29: 3488–3493
- van der Ouderaa FJ, de Jong WW, Bloemendal H (1973) The amino-acid sequence of the alpha A2 chain of bovine alpha-crystallin. *Eur J Biochem* 39: 207–222
- van Rens GL, Raats JM, Driessen HP, Oldenburg M, Wijnen JT, Khan PM, de Jong WW, Bloemendal H (1989) Structure of the bovine eye lens gamma S-crystallin gene (formerly beta S). *Gene* 78: 225–233
- Voorter CE, de Haard-Hoekman WA, Roersma ES, Meyer HE, Bloemendal H, de Jong WW (1989) The in vivo phosphorylation sites of bovine alpha B-crystallin. *FEBS Lett* 259: 50–52
- Wang-Su ST, McCormack AL, Yang S, Hosler MR, Mixon A, Riviere MA, Wilmarth PA, Andley UP, Garland D, Li H, David LL, Wagner BJ (2003) Proteome analysis of lens epithelia, fibers, and the HLE B-3 cell line. *Invest Ophthalmol Vis Sci* 44: 4829–4836
- Washburn MP, Wolters D, Yates JR 3rd (2001) Large-scale analysis of the yeast proteome by multidimensional protein identification technology. *Nat Biotechnol* 19: 242–247
- Wasinger VC, Cordwell SJ, Cerpa-Poljak A, Yan JX, Gooley AA, Wilkins MR, Duncan MW, Harris R, Williams KL, Humphrey-Smith I (1995) Progress with gene-product mapping of the Mollicutes: mycoplasma genitalium. *Electrophoresis* 16: 1090–1094
- Werten PJ, Vos E, De Jong WW (1999) Truncation of beta A3/A1-crystallin during aging of the bovine lens; possible implications for lens optical quality. *Exp Eye Res* 68: 99–103
- Wilmarth P, Taube JR, Riviere MA, Duncan MK, David LL (2004) Proteomic and sequence analysis of chicken lens crystallins reveals alternate splicing and translational forms of beta B2 and beta A2 crystallins. *Invest Ophthalmol Vis Sci* 45: 2705–2715
- Wistow G, Slingsby C, Blundell T, Driessen H, DeJong W, Bloemendal H (1981) Eye-lens proteins: the three-dimensional structure of beta-crystallin predicted from monomeric gamma-crystallin. *FEBS Lett* 133: 9–16
- Wistow GJ, Piatigorsky J (1988) Lens crystallins: the evolution and expression of proteins for a highly specialized tissue. *Annu Rev Biochem* 57: 479–504
- Wistow G, Wyatt K, David L, Gao C, Bateman O, Bernstein S, Tomarev S, Segovia L, Slingsby C, Vihtelic T (2005) gamma N-crystallin and the evolution of the betagamma-crystallin superfamily in vertebrates. *FEBS J* 272: 2276–2291
- Wolters DA, Washburn MP, Yates JR 3rd (2001) An automated multi-dimensional protein identification technology for shotgun proteomics. *Anal Chem* 73: 5683–5690
- Yamashita M, Fenn JB (1984) Electrospray ion source. Another variation on the free jet theme. *J Phys Chem* 88: 4451–4459
- Yates JR 3rd (1998) Mass spectrometry and the age of the proteome. *J Mass Spectrom* 33: 1–19
- Yu NT, DeNagel DC, Pruett PL, Kuck JFR Jr (1985) Disulphide bond formation in the eye lens. *Proc Natl Acad Sci USA* 82: 207–214
- Zarina S, Slingsby C, Jaenicke R, Zaidi ZH, Driessen H, Srinivasan N (1994) Three-dimensional model and quaternary structure of the human eye lens protein gamma S-crystallin based on beta- and gamma-crystallin X-ray coordinates and ultracentrifugation. *Protein Sci* 3: 1840–1846
- Zhen Y, Xu N, Richardson B, Becklin R, Savage JR, Blake K, Peltier JM (2004) Development of an LC-MALDI method for the analysis of protein complexes. *J Am Soc Mass Spectrom* 15: 803–822

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