عنوان المشروع باللغة العربية	تنقية و دراسة خصائص إنزيم زيتا كريستالين في الجمل
عنوان المشروع باللغة الإنجليزية	**Purification and Characterization of Recombinant Camel zeta-Crystallin**
المشرف الرئيس	Dr. Abdulrahman M. Al-Senaidy
التخصص الدقيق للمشرف الرئيس	Protein chemistry
المشرف المساعد	1- Dr. Ajamaluddin Malik 2-Dr. Abeer Mohammad Al-Dbass
المدة المتوقعة لإنجاز البحث منذ الحصول على موافقة عمادة الدراسات العليا	**8 months**
Abstract or synopsis of the proposal (200 words or less):	Camels eye lens possess unique quinone oxidoreductase enzyme in abundance which is known as zeta-crystallin (Rao, Krishna et al. 1992; Duhaiman, Rabbani et al. 1995; Fujii, Kimoto et al. 2001). In human and other animal lenses zeta- crystallin is present but at very low levels (Rao, Gonzalez et al. 1997). In the eye lens, the role of zeta-crystallin is not well understood. In this study, we will purify recombinant camel zeta crystallin to homogeneity using affinity, ion-exhange and/or size exclusion chromatographic techniques. Subsequently, functional, spectroscopic and thermodynamic properties zeta crystallin will be characterized using different techniques.
Hypothesis or scientific justification of the proposal	Nature designed eye lenses as a specialized tissue for the single known purpose of allowing for clarity of vision. The eye lenses are filled with highly concentrated, water soluble proteins, called crystallins, which constitutes about 90% of all proteins in the lens (Jaenicke 1996). The high concentration of soluble crystallins (~500 mg/ml) increases the refractive index and maintains transparency. Matured eye lenses neither have nuclei nor cellular organelles in order to avoid light scattering (Ivanov, Dvoriantchikova et al.

	2005; Gong, Cheng et al. 2007), therefore no new protein can be synthesized. As a result the eye lenses proteins that are made embryonically need to be able to last the whole life of an organism, withstanding high levels of UV light, and possibly temperature changes and dehydration, depending on the organism. Misfolding and aggregation of crystallins increases light scattering and makes lenses opaque (Sharma and Santhoshkumar 2009), a condition known as cataract formation. Camel eye lens are exposed to very high ambient temperatures, dehydration and high light levels. Like other mammals, camel eye lenses contain three ubiquitous crystallins (α, β and γ). In addition, camels also possess zeta-crystallin (EC 1.6.5.5) (Rao, Krishna et al. 1992; Duhaiman, Rabbani et al. 1995; Fujii, Kimoto et al. 2001) which makes up to nearly 10 % of the total eye lens protein in camel (Garland, Rao et al. 1991; Gonzalez, Hernandez-Calzadilla et al. 1994; Bazzi 2001). In human and other animal lenses zeta- crystallin is present but at lower levels (Rao, Gonzalez et al. 1997). In the eye lens, the role of zeta-crystallin is not well understood. Here, we will purify and characterize functional, spectroscopic and thermodynamic properties of zeta crystallin.
Specific objectives	1- To purify recombinant camel zeta crystallin from *E.coli*. 2- To characterize functional, spectroscopic and thermodynamic properties of zeta crystallin.
Methodology & Major Techniques to be used	**Purification:** To obtain biomass of *Escherichia coli* expressing zeta-crystallin in gram quantities, liter-scale shake flask expression experiment will be performed. Soluble proteins from biomass of *Escherichia coli* will be extracted. To remove nucleic acids and reduce viscosity, soluble extract will be treated with DNase. Subsequently, protein extract will be subjected to centrifugation at high speed and supernatant will be passed through 0.25 micron filter. His-tagged zeta-crystallin variants will bind on pre-equilibrated Ni-NTA column and eluted with linear gradient of Imidazole. Purity of the fractions will be analyzed by SDS-PAGE and activity will be monitored by

	NADPH:quinone oxidoreductase assay. Fractions containing zeta-crystallin proteins will be pooled and dialysed for another affinity chromatography (ADP-Sepharose). Bound proteins on ADP-sepharose will be eluted by NaCl gradient. Again purity and quinone oxidoreductase activity will be checked in different fractions. As per requirement, Gel filtration and/or ion exchange chromatography will be done to get highly pure zeta-crystallin. Using this method we will obtain homogenous population of zeta- crystallin. Pure fractions of zeta- crystallin will be pooled and concentrated to achieve highly concentrated stock solution of zeta- crystallin protein **Characterization:** The purified zeta- crystallin variants will be characterized using following techniques. **1- Secondary structure determination:** By CD spectropscopy in the far UV region **2- Tertiary structure determination:** By CD spectropscopy in the near UV region and by spectrofluorometer using intrinsic tryptophan fluorescence. **3- Surface hydrophobicity:** By spectrofluorometer using extrinsic flurogenic probe such as ANS (1-Anilinonaphthalene-8-Sulfonic Acid) **4- Thermal stability:** By circular dichroism spectopolarimeter using dynamic multimode spectroscopic technique. **5- Biological activity:** Enzymatic assay using spectrophotometer
Availability of Samples	Yes
Availability of Chemicals	Yes
Availability of Instruments	Yes
Availability of Ethical Approval (if needed)	Under process
Recent References	Bazzi, M. D. (2001). "Interaction of camel lens zeta-crystallin with quinones: portrait of a substrate by fluorescence spectroscopy." Arch Biochem Biophys **395**(2): 185-190. Duhaiman, A. S., N. Rabbani, et al. (1995).

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