عنوان المشروع باللغة العربية - Title of the proposed project in Arabic	دراسة الفعالية الكيميائية لمركبات (sulfobetaines) على تركيب وذوبانية انزيمlysozyme
Title of the proposed project in English	Elucidation of the chemical chaperone (sulfobetaines) on the folding and solubility of reduced lysozyme
المشرف الرئيس - PI	Dr. Ajamaluddin Malik
التخصص الدقيق للمشرف الرئيس - Specialty of PI	Protein Folding and engineering
المشرف المساعد - Co-PI	Dr. Mona Al-Onazi
المدة المتوقعة لإنجاز البحث منذ الحصول على موافقة عمادة الدراسات العليا (بالشهور) - Expected time in month to finish	8
Abstract of the proposal (No more than 200 words)	Both unfolding and misfolding of proteins resulted into aggregates (Santucci, Sinibaldi et al. 2008; Wang and Kaufman 2016). The protein aggregates caused a large number of neurodegenerative diseases. Some aggregates are pathological due to (i) gain of function (Alzheimer's, Huntington's, Parkinson's and Prion's disease) or (ii) loss of function (cystic fibrosis and alpha1-antitrypsin deficiency) (Stefani 2004; Chiti and Dobson 2006). These aggregated proteins could either lead to the formation of harmful amyloids and become cytotoxic or

	recognized and degraded by the cellular protein quality control system. Previous studies indicated that some low-molecular-weight compounds named as chemical chaperones could reverse the unfolding and/or aggregation of proteins associated with human conformational diseases (Chaudhuri and Paul 2006; Loo and Clarke 2007; Nicoll, Trevitt et al. 2010). Moreover, chemical chaperones are recommended to use as stabilizing agents during cell lysis, protein purification, protein storage and therapeutic and diagnostic protein formulations (Leibly, Nguyen et al. 2012; Singh, Upadhyay et al. 2015). Although chemical chaperones mimic the molecular chaperone and improve the protein solubility but the mechanism is still not fully understood (Papp and Csermely 2006; Winter, Staszewska et al. 2014). In a screening experiment, we found two chemical chaperones of the sulfobetain family (Sulfobetain10 and sulfobetaine-201) efficiently suppress the lysozyme aggregation. Sulfobetaine-201 as a chemical chaperone is reported in the literature (Wangkanont, Forest et al. 2015), but sulfobetaine-10 is a novel finding in our lab. In this study, we planned to investigate the role of Sulfobetaine-10 and sulfobetaine-201 on the denaturation and renaturation of lysozyme in detail using different techniques.
Hypothesis of the proposal	The primary cause of several neurodegenerative diseases in humans is believed to be Protein misfolding, such as Alzheimer's disease, Huntington's disease, Creutzfeldt-Jakob disease, Parkinson's disease etc(Stefani 2004; Chiti and Dobson 2006). In most of the cases, an undesirable mutation in the polypeptide or altered physiological environment or in a few cases, some less known reasons caused protein Misfolding (Tosato, Zamboni et al. 2007; Gasser, Saloheimo et al. 2008). More than twenty proteins in humans are involved in aberrant aggregation, include amyloid beta peptide, PolyQ, PABPN1, prion, b2-microglobulin, tau, a-synuclein, etc. (Stefani 2004; Tuite and Melki 2007). Aggregation can also occur in proteins with native conformations due to change in physiological condition such as improper use of antioxidants in extracellular environment and/or intracellular reducing agents can break proteins disulfide bonds making them unstable and prone to misfolding and aggregation (Jahn and Radford 2008; Yang, Dutta et al. 2015; Weids, Ibstedt et al. 2016). Disulfide-bond scrambling promotes protein aggregation which may leads to severe pathological condition (Toichi, Yamanaka et al. 2013; Yang, Dutta et al. 2015). The challenging question is how to improve folding and solubility of disulfide scrambled proteins? In a large screening experiment in our lab, we identified two low molecular weight compound of sulfobetaine family which suppress reducing agent (DTT) induced aggregation of lysozyme. Sulfobetain-201 is reported in the literature as chemical chaperone However Sulfobetain-10 is novel finding in our lab. In this study, we plan to investigate roles of both Sulfobetaines (Sulfobetain-201 and Sulfobetain-10) in the denaturation and renaturation of reduced lysozyme.

	We will characterize the mode of action of Sulfobetain-201 and Sulfobetain-10 on the structural, thermodynamic and functional properties of reduced lysozyme using various techniques.
Specific objectives	1- Effect of Sulfobetaines on the conformation and stability of native lysozyme. 2- Evaluation of the extent of aggregate suppression of disulfide scrambled lysozyme by sulfobetaines. 3- Role of Sulfobetaines in the renaturation of disulfide scrambled lysozyme.
Methodology & Major Techniques to be used	Commercial lysozyme will be used in this study. The purity of commercial lysozymes will be analyzed on SDS-PAGE. If required, lysozyme will be further purified using the chromatographic technique. The methodology to achieve different objectives is described below: Objective 1: Lysozyme will be treated with different concentration of sufobetaines and allowed to equilibrate. The aggregation will be monitored by (absorption and Rayleigh scattering); secondary and tertiary structure by CD and fluorescence; stability by dynamic multi-mode spectroscopy or thermal shift assay; function by enzymatic assay. Objective 2: Lysozyme in the presence of different concentrations of Sulfobetaines will be treated with DTT to reduce disulfide bonds and induce unfolding and aggregation. The rate and extent of aggregate suppression, protection of secondary and tertiary structures, the effect on stability and biological activity will be studied by above mentioned techniques. Objective 3: Fully reduced and denatured Lysozyme will be subjected to refolding in the presence of different concentrations of sulfobetaines. The sulfobetaine assisted refolding of lysozyme will be monitored by enzymatic assay and quantified by measuring the soluble concentration of lysozyme. The gain of secondary and tertiary structures, stability and biological activity will be studied by above mentioned techniques.
Availability of Samples	Yes

Availability of Chemicals	Yes
Availability of Instruments	Yes
Ethical Approval	Not needed
Recent References	Chaudhuri, T. K. and S. Paul (2006). "Protein-misfolding diseases and chaperone-based therapeutic approaches." FEBS J 273(7): 1331-1349. Chiti, F. and C. M. Dobson (2006). "Protein misfolding, functional amyloid, and human disease." Annu Rev Biochem 75: 333-366. Gasser, B., M. Saloheimo, et al. (2008). "Protein folding and conformational stress in microbial cells producing recombinant proteins: a host comparative overview." Microb Cell Fact 7: 11. Jahn, T. R. and S. E. Radford (2008). "Folding versus aggregation: polypeptide conformations on competing pathways." Arch Biochem Biophys 469(1): 100-117. Leibly, D. J., T. N. Nguyen, et al. (2012). "Stabilizing additives added during cell lysis aid in the solubilization of recombinant proteins." PLoS One 7(12): e52482. Loo, T. W. and D. M. Clarke (2007). "Chemical and pharmacological chaperones as new therapeutic agents." Expert Rev Mol Med 9(16): 1-18. Nicoll, A. J., C. R. Trevitt, et al. (2010). "Pharmacological chaperone for the structured domain of human prion protein." Proc Natl Acad Sci U S A 107(41): 17610-17615. Papp, E. and P. Csermely (2006). "Chemical chaperones: mechanisms of action and potential use." Handb Exp Pharmacol(172): 405-416. Santucci, R., F. Sinibaldi, et al. (2008). "Protein folding, unfolding and misfolding: role played by intermediate States." Mini Rev Med Chem 8(1): 57-62. Singh, A., V. Upadhyay, et al. (2015). "Solubilization and refolding of inclusion body proteins." Methods Mol Biol 1258: 283-291. Stefani, M. (2004). "Protein misfolding and aggregation: new examples in medicine and biology of the dark side of the protein world." Biochim Biophys Acta 1739(1): 5-25. Toichi, K., K. Yamanaka, et al. (2013). "Disulfide scrambling describes the oligomer formation of superoxide dismutase (SOD1) proteins in the familial form of amyotrophic lateral sclerosis." J Biol Chem 288(7): 4970-4980. Tosato, M., V. Zamboni, et al. (2007). "The aging process and potential

interventions to extend life expectancy." Clin Interv Aging 2(3): 401-412.
Tuite, M. F. and R. Melki (2007). "Protein misfolding and aggregation in ageing and disease: molecular processes and therapeutic perspectives." Prion 1(2): 116-120.
Wang, M. and R. J. Kaufman (2016). "Protein misfolding in the endoplasmic reticulum as a conduit to human disease." Nature 529(7586): 326-335.
Wangkanont, K., K. T. Forest, et al. (2015). "The non-detergent sulfobetaine-201 acts as a pharmacological chaperone to promote folding and crystallization of the type II TGF-beta receptor extracellular domain." Protein Expr Purif 115: 19-25.
Weids, A. J., S. Ibstedt, et al. (2016). "Distinct stress conditions result in aggregation of proteins with similar properties." Sci Rep 6: 24554.
Winter, L., I. Staszewska, et al. (2014). "Chemical chaperone ameliorates pathological protein aggregation in plectin-deficient muscle." J Clin Invest 124(3): 1144-1157.
Yang, M., C. Dutta, et al. (2015). "Disulfide-bond scrambling promotes amorphous aggregates in lysozyme and bovine serum albumin." J Phys Chem B 119(10): 3969-3981.