عنوان المشروع باللغة العربية - Title of the proposed project in Arabic	التعبير الجيني وتنقية والتوصيف البيو فيزيائي لإنزيم(Mpro) MERS-CoV main.
Title of the proposed project in English	Expression, purification and biophysical characterization of recombinant MERS-CoV main (Mpro) protease
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)	In last 15 years, the world has experienced two highly pathogenic outbreaks of coronaviruses. In 2002, SARS-CoV outbreak occurred in China and spread to 26 countries infecting more than 8000 people with 10% case fatality rate (CFR) (Cheng, Lau et al. 2007; Chan and Chan 2013). MERS-CoV, first reported 2012 in Saudi Arabia, rapidly spread across 27 counties with higher case fatality rate compared to SARS-CoV (38 % vs. 10 %), making it a deadlier virus (Hui, Memish et al. 2014; Xia, Liu et al. 2014; Modjarrad 2016; Chen, Chughtai et al. 2017), .

	Despite the frequent coronavirus outbreaks, no effective therapeutic or counter measure is available to control the viral epidemic spread(de Wit, van Doremalen et al. 2016). Therefore, it is an utmost need to develop coronavirus therapeutics to treat patents and to control its spread. Proteases are considered as potential durg targets. The main protease (Mpro) of MERS-CoV cleaved at 13 out of 16 sites on the polyprotein chain and its activity is must for its maturation. MERS-CoV Mpro is a dimeric protein and its overall topology is similar to SARS-CoV Mpro (Kilianski, Mielech et al. 2013; Yang, Chen et al. 2014). The biophysical properties (folding pathway, thermodynamic stability, aggregation kinetics etc.) of MERS-CoV Mpro is not characterized. In this proposal, we plan to recombinantly produce MERS-CoV Mpro and purify using different chromatographic techniques. Moreover, various techniques will be used for characterizing spectroscopic and thermodynamic properties of MERS-CoV Mpro.
Hypothesis of the proposal	There are two common strategies for anti-viral drug development: (A) designing high affinity inhibitor at the catalytic site. (B) Development of molecules to decrease the drug target stability by destabilizing its conformation (Szeltner and Polgar 1996; Todd, Semo et al. 1998). Destabilizing target proteins has been successfully used for development of Anti-HIV drug (Boggetto and Reboud-Ravaux 2002; De Clercq 2004).To develop destabilizer of MERS-CoV protease structure, detailed knowledge about their conformational structure and stability are required. In this proposal, we plan to express and purify MERS-CoV Mpro in large quantity. Structural conformation, themodynamic stability and folding pathway MERS-CoV Mpro will be evaluated at physiological pH using various techniques (Spectrophotometer, Spectroflurometer and Circular dichroism).
Specific objectives	1- Expression of MERS-CoV Mpro in E.coli 2-Purication of MERS-CoV Mpro using different chromatographic techniques. 3- Biophysical characterization of MERS-CoV Mpro.
Methodology & Major Techniques to be used	In this project, recombinant MERS-CoV Mpro will be overexpressed in E.coli. If required, important cultivation conditions (temperature, inducer concentration and post-induction incubation) will be optimized. MERS-CoV Mpro will be purified using affinity and gel filtration chromatography. The purity of MERS-CoV Mpro will by analyzed by SDS-PAGE and quantified spectrophotometrically using molar extinction coefficient.

	The biophysical properties of purified MERS-CoV Mpro will be characterized using following techniques. 1- Secondary structure determination: by Circular Dichroism CD spectropscopy in the far UV region. 2-Tertiary structure determination: by spectrofluorometer using intrinsic tryptophan fluorescence and/or CD spectropscopy in the near UV region. 3- Surface hydrophobicity: using extrinsic flurogenic probe such as ANS (1-Anilinonaphthalene-8-Sulfonic Acid). 4- Thermodynamic stability: by thermal shift assay using spectrofluorometer or Circular Dichroism CD spectopolarimeter using a dynamic multimode spectroscopic technique. 5- Biological activity: enzymatic assay using spectrophotometer.
Availability of Samples	Yes
Availability of Chemicals	Yes
Availability of Instruments	Yes
Ethical Approval	Not needed
Recent References	Boggetto, N. and M. Reboud-Ravaux (2002). "Dimerization inhibitors of HIV-1 protease." Biol Chem 383(9): 1321-1324. Chan, P. K. and M. C. Chan (2013). "Tracing the SARS-coronavirus." J Thorac Dis 5 Suppl 2: S118-121.

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