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DEPARTMENT OF BIOCHEMISTRY & MOLECULAR BIOLOGY

MILLER SCHOOL OF MEDICINE
UNIVERSITY OF MIAMI

Presents

PhD DISSERTATION SEMINAR

Biophysical Insights into the Oligomerization of BclXL Apoptotic Repressor

By

Vikas Bhat

Tuesday, May 7th, 2013
12:00pm-1:00pm
Gautier #118

Thesis Committee

Thomas Harris PhD, *Committee Chair*
Sapna Deo PhD, *Committee Member*
Vincent Moy PhD, *External Examiner*
Amjad Farooq PhD DIC, *Research Mentor*

BIOGRAPHY

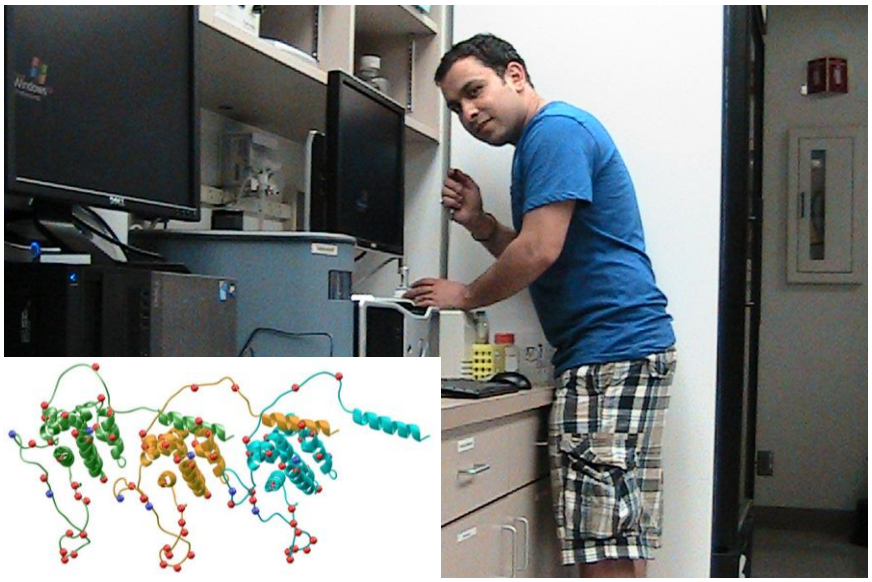
Born in Anantnag in 1984, Vikas's early childhood bore the hallmarks of a life in hell instead of the luxury of growing up in the beautiful valley of Kashmir that has often been described as "paradise on earth" for the most part of its history. Amidst the rising insurgency and terrorism that broke out during the late 1980s in Kashmir, his family was forced to relocate to New Delhi—about 500 miles south of Anantnag—in 1990.

In stark contrast to his early life in the foothills of Himalayas, his newly adopted home in the Indian capital offered Vikas a much needed respite. Assured by a renewed sense of shaanti (peace) and security, Vikas began to flourish in school and quickly developed a keen interest in science—a talent that was recognized with the award of the Best Student in Chemistry & Biology at the Guru Harkishan Public High School in 2002. To fulfill his scientific dreams, Vikas next pursued his baccalaureate studies in Biotechnology at the Guru Gobind Singh Indraprastha University (New Delhi)—one of the most prestigious institutions of higher education in India. As a part of his broad curriculum at Indraprastha, Vikas was handed the opportunity to become acquainted with laboratory research early on in his undergraduate studies. This not only helped him to quickly understand the nuance of research but also paved the way for him to discover that his true passion lay in the biological sciences. Importantly, classes in areas such as protein engineering not only further captivated his scientific passion but also challenged his innermost conscience to explore the mechanistic aspects of proteins at atomic level. Accordingly, his desire to undertake doctoral studies in biochemistry was a natural progression of his scientific interests amalgamated during his undergraduate studies.

After graduating with a Bachelor of Technology (BTech) degree from the Indraprastha University in 2008, Vikas was admitted into the PhD Program in the Department of Biochemistry & Molecular Biology at the Miller School of Medicine of the University of Miami. With the full support of his beloved parents and under the gaze of Bhagwaan, Vikas set out to embark on a journey in untreaded waters. With a vision to pursue his doctoral studies at the interface of physics, chemistry and biology with an emphasis on understanding the basic mechanisms of life, the Farooq Laboratory not only provided him with an ideal opportunity to fulfill such aspirations but also laid the foundations for him to broaden his scientific horizons coupled with high scientific productivity. On May 7th, 2013—within five years of having been admitted into the PhD Program—Vikas will earn his PhD degree from the University of Miami with outstanding credentials that students of only the very highest caliber can match.

Outside science, Vikas leads an active social and sporting life. He is a keen cricketer and an ardent follower of the game, particularly the Indian national team—Chakde India! He enjoys going places with a particular interest in discovering new cultures and meeting new people. In his spare time, Vikas loves to cook the quintessential Indian dishes, including the Chicken Tikka Masaala and Gobhi Paraatha! With those essential housewife skills and a PhD degree under his belt, few dare bet against his bachelor days quickly drying up and with that his personal freedom! Despite his outrageous success in his professional life, Vikas gives all the credit to his parents, mentors, and above all, the Almighty Bhagwaan. That is the true virtue of a very humble man whose life began with a struggle and a future of uncertainty.

ABSTRACT



The BclXL apoptotic repressor—a member of the B-cell lymphoma 2 family of proteins—plays a central role in determining the fate of cells to live or die during physiological processes such as embryonic development and tissue homeostasis.

Herein, using a wide array of biophysical methods, I investigate the molecular basis of action of BclXL. Briefly, I provide evidence that BclXL bears intrinsic propensity to oligomerize in solution. Importantly, such oligomerization of BclXL is driven by the intermolecular binding of its C-terminal transmembrane (TM) domain to the canonical hydrophobic groove in a domain-swapped trans fashion, whereby the TM domain of one monomer occupies the hydrophobic groove within the other monomer and vice versa. Of particular interest is the observation that acidic pH promotes the assembly of BclXL into a higher-order megadalton aggregate with a plume-like appearance and harboring structural features characteristic of a molten globule. Moreover, BclXL undergoes irreversible aggregation and assembles into highly-ordered rope-like homogeneous fibrils at elevated temperatures. Remarkably, the formation of such fibrils correlates with the decay of a largely α-helical fold into a predominantly β-sheet architecture of BclXL in a manner akin to the formation of amyloid fibrils. Further interrogation reveals that while BclXL aggregates in solution display diminished affinity toward BH3 ligands, they appear to be optimally primed for insertion into cardiolipin bicelles. This salient observation strongly argues that BclXL aggregates likely represent an on-pathway intermediate for insertion into mitochondrial outer membrane during the onset of apoptosis.

Collectively, my study sheds light on the propensity of BclXL to aggregate in solution, particularly under acidic conditions and at elevated temperatures—the physical factors that mimic cellular stress—thus bearing important consequences on its mechanism of action in gauging the apoptotic fate of cells in human health and disease.