Centre for Research in Molecular Modeling
CERMM
Centre de recherche en modélisation moléculaire

UNIVERSITÉ Concordia UNIVERSITY

Seminar Series of the
CENTRE FOR RESEARCH IN MOLECULAR MODELLING

February 28th, 2011, 3:00 pm – SP-S110

Generation of Longer Emission Wavelength Red Fluorescent Proteins Using Computationally Designed Libraries

Roberto A. Chica
Department of Chemistry, University of Ottawa

The longer emission wavelengths of red fluorescent proteins (RFPs) make them attractive for whole-body imaging because cells are more transparent to red light. Although several useful RFPs have been developed, the quest for further red-shifted and improved RFPs continues. Herein, we report a structure-based rational design approach to red shift the fluorescence emission of RFPs. We applied a combined computational and experimental approach that uses computational protein design as an in silico pre-screen to generate focused combinatorial libraries of mCherry mutants. The computational procedure helped us identify residues that could fulfill interactions hypothesized to cause red shifts without destabilizing the protein fold. These interactions include stabilization of the excited state through H-bonding to the acylimine oxygen atom, destabilization of the ground state by hydrophobic packing around the charged phenolate, and stabilization of the excited state by a π-stacking interaction. Our methodology allowed us to identify three mCherry mutants (mRojoA, mRojoB, and mRouge) that display emission wavelengths >630 nm, representing red shifts of 20 to 26 nm. Moreover, our approach required the experimental screening of a total of 5000 clones, a number several orders of magnitude smaller than those previously used to achieve comparable red shifts. Additionally, crystal structures of mRojoA and mRouge allowed us to verify fulfillment of the interactions hypothesized to cause red shifts, supporting their contribution to the observed red shifts.

Roberto Chica is an Assistant Professor in the Department of Chemistry at the University of Ottawa. He received a Ph.D. degree in chemistry in 2007 from the Université de Montréal. There, he worked under Profs. Joelle Pelletier and Jeffrey Keillor and used a semi-rational approach to engineer the substrate specificity of transglutaminase in order to develop a new biocatalyst for peptide synthesis. Following his Ph.D., he undertook a FQRNT-funded postdoctoral fellowship in computational protein design at the California Institute of Technology, working under Prof. Stephen Mayo. His postdoctoral research exploited high-performance calculations to reduce experimental screening efforts by several orders of magnitude while rapidly generating mutant proteins with desired properties. This work was selected as a must-read article by the post-peer review site Faculty of 1000, placing it among the top 2% of published articles in biology and medicine. Since January 2010, he is an Assistant Professor at the University of Ottawa His research interests involve using computational tools to engineer new proteins for their application in chemistry and biology. In particular, he is interested in developing new biocatalysts for organic synthesis as well as improved red fluorescent proteins for whole-body imaging. His multidisciplinary research program involves a combination of both experimental and computational work, and uses techniques from fields such as experimental protein chemistry, enzymology, molecular biology, and molecular modelling.