Research Summary

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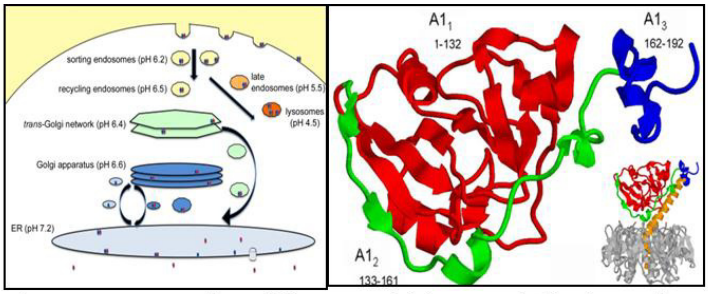
OBJECTIVE: The main goal of my research is to understand cellular and molecular mechanisms of pathogenicity of toxins like cholera toxin, pertussis toxin, heat-labile toxin and ricin using a combination of biochemical, biophysical and cell biological approaches. My research will also focus on examining the role of endoplasmic reticulum stress and unfolded protein response in host-pathogen interactions. Insights into these mechanisms will lead to a better understanding of important aspects of basic cellular processes and designing better therapeutic strategies. Another area of my research would be to investigate significant conformational transitions accompanying RNA-Protein interactions that occur as part of post-transcriptional gene regulation.

PROJECT 1: Use of chemical chaperones as a therapeutic measure to prevent cholera intoxication

Overall significance: Worldwide, *Vibrio cholerae* is estimated to cause 3-5 million illnesses and 120,000 deaths each year. Cholera toxin, one of the main virulence factors produced by *V. cholerae*, is responsible for inducing the profuse diarrhea. The recent outbreaks of cholera in Haiti, Asian countries, and Central Africa dramatically highlight the continued global presence of this disease. The overall aim of my research work is to elucidate the molecular details of the translocation of catalytic subunit of cholera toxin and other AB toxins, to identify key cellular host factors and new therapeutic agents that would prevent cholera intoxication.

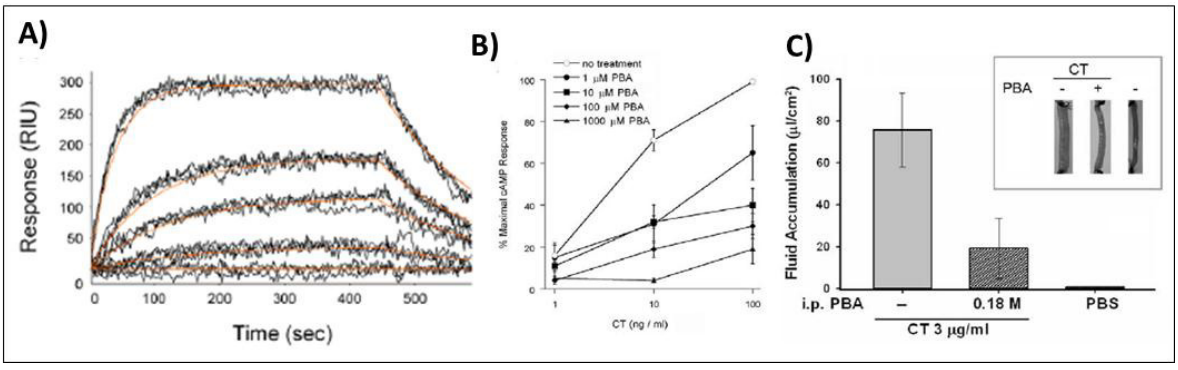


Schematic representation of intracellular transport of cholera toxin and ribbon diagram of CTA1

Background: Cholera toxin (CT) travels as an intact AB_5 protein toxin from the cell surface to the endoplasmic reticulum (ER) of an intoxicated cell. In the ER, the catalytic A1 subunit dissociates from the rest of the toxin. Translocation of CTA1 from the ER to the cytosol is then facilitated by the quality control mechanism of ER-associated degradation (ERAD). CTA1 thermal instability represents a promising target for anti-toxin therapeutics. Inhibition of CTA1 unfolding in the ER would prevent its recognition by the ERAD system, its translocation to the cytosol, and, thus, its cytopathic effect.

Important outcome from the above work

- PBA inhibits the thermal unfolding of CTA1 by direct binding, it also blocked ER-to-cytosol export of CTA1
- PBA represents a promising therapeutic to prevent or possibly treat cholera because (i) it is already approved for human use; (ii) it is effective against CT at concentrations that can be attained in patients; (iii) it exhibits low nM affinity for CT/CTA1; and (iv) it inhibits CT in the physiological ileal loop model of intoxication



(A)SPR trace showing direct binding of CTA1 with PBA (B) in-vitro toxicity assay showing maximal c-AMP response (C) rat-ileal loop results indicating decreased diarrheal response in presence of PBA.

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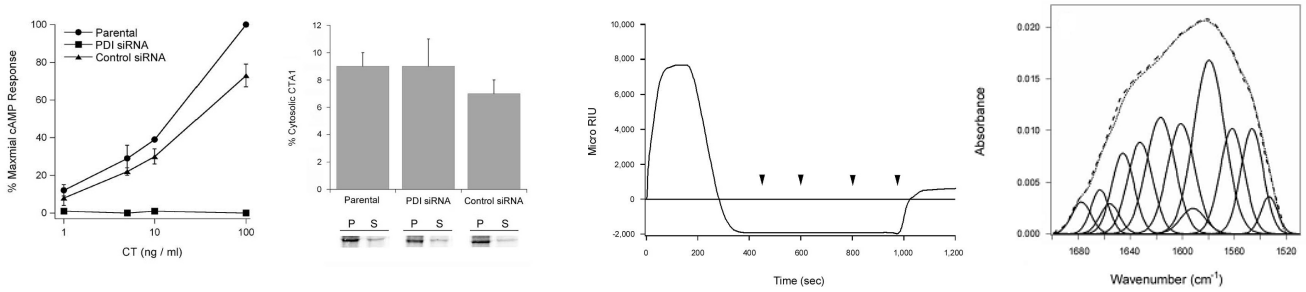
Journal of Molecular Biology. 2009, 393, 1083.
Biochemistry 2010, 49, 8839

PROJECT 2: Unraveling the molecular mechanism of Protein Disulfide Isomerase (PDI) mediated disassembly of Cholera toxin

Background: PDI was originally thought to unfold the holotoxin-associated CTA1 (catalytic subunit of cholera toxin) and thereby displace it from the rest of the toxin. To evaluate the role of PDI in CT disassembly and CTA1 unfolding, a real-time assay was employed to monitor the PDI-mediated separation of CTA1 from the CT holotoxin and the impact of PDI binding on CTA1 structure was directly measured by isotope-edited Fourier transform infrared spectroscopy.

Results: Cell based experiments (using siRNA) and biophysical assays (using SPR and Isotope edited protein FTIR) showed that PDI was essential for holotoxin disassembly, but is not needed for translocation of the isolated CTA1 subunit. As such, it appears that the only role of PDI in cholera intoxication is to displace CTA1 from the CT holotoxin.



From left: 1-2) In vivo role of PDI in CT intoxication using PDI siRNA. 3) Novel, SPR-based assay to monitor the PDI-mediated disassembly of CT in real time. 4) Structure of CTA1 evaluated by FTIR

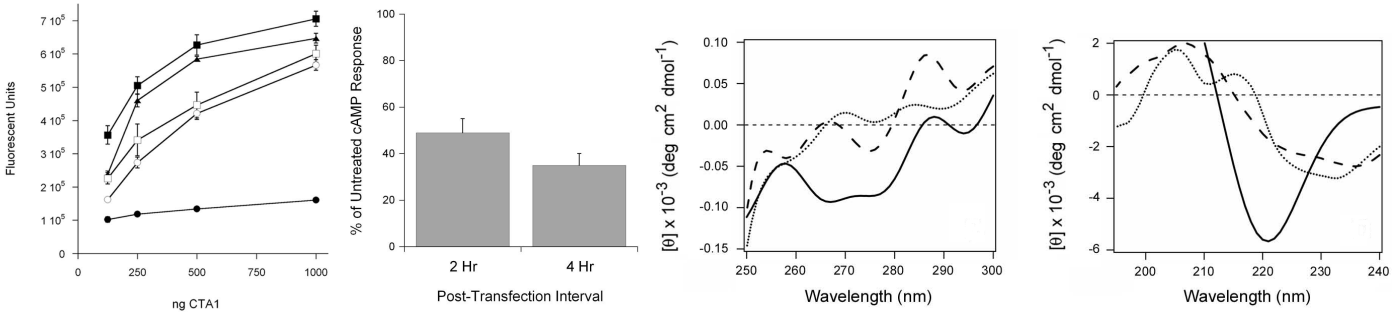
Published in Journal of Biological Chemistry, 2011, 286, 22090.

PROJECT 3: Lipid rafts alter the stability and activity of cholera toxin

Background: Cholera toxin enters the target cell in a disordered state and must attain a folded, active conformation to modify its G protein target which is located in lipid rafts at the cytoplasmic face of the plasma membrane.

Results:

- lipid rafts promotes a gain of structure in the disordered, 37°C conformation of cholera toxin
- gain of function: whereas CTA1 by itself exhibited minimal in vitro activity at 37°C, exposure to lipid rafts resulted in substantial toxin activity at 37°C
- In vivo, the disruption of lipid rafts with filipin substantially reduced the activity of cytosolic CTA1



From left: (1-2) CTA1 activity in the presence of lipid raft shown by activity assay using a synthetic substrate for the ADP-ribosyltransferase activity of cholera toxin. (3) In-vivo activity of cytosolic CTA1 in presence of lipid rafts measured by intracellular cAMP levels at 2 and 4 hr post-transfection (4) refolding of cholera toxin by lipid raft (shown by circular dichroism).

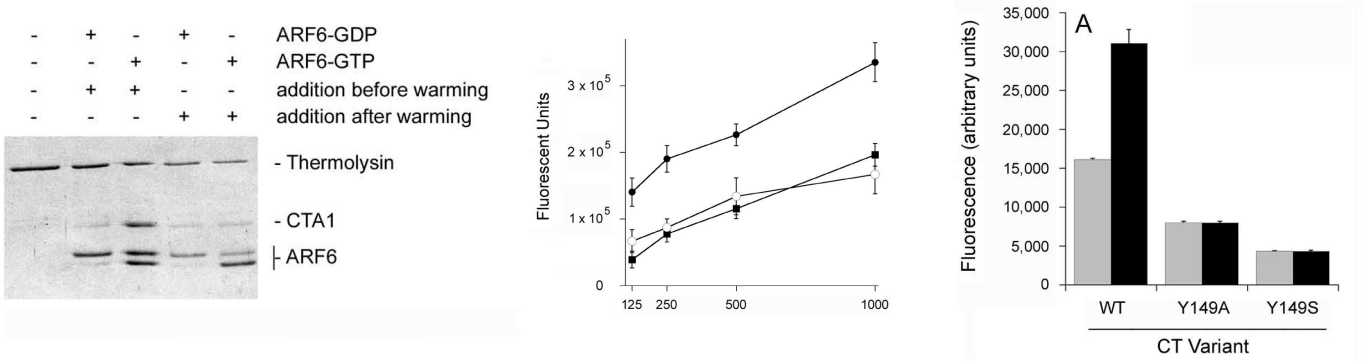
Published in Journal of Biological Chemistry, 2012, 287, 30395.

PROJECT 4: ADP-Ribosylation Factor 6 acts as an allosteric activator for the Folded but not Disordered Cholera Toxin A1 Polypeptide

Background: Cholera toxin (CTA1) enters the cytosol in an unfolded state and must regain a folded conformation in order to act upon its Gsα target which is located in lipid rafts at the cytoplasmic face of the plasma membrane. ARF proteins are small molecular weight GTPases that were originally isolated from cell extracts as *in vitro* allosteric activators of CTA1. The importance of CTA1-ARF interactions for *in vivo* toxin activity has yet to be established, however.

Results :

- Lipid rafts but not ARF6 shift disordered CTA1 from a protease-sensitive to protease-resistant conformation
- CTA1 is stabilized but not refolded by ARF6
- Folded but not disordered CTA1 is activated by ARF6



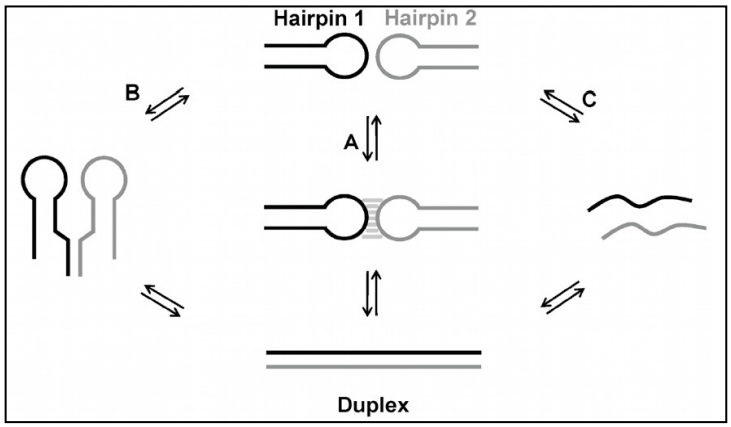
From left: (1) ARF6 induced alterations to the structure of CTA1 by thermolysin assay. (2) CTA1 activity in the absence or presence of ARF6. (3). CT Y149A and CT Y149S mutants are not activated by ARF6 and lack cytopathic activity.

Published in Molecular Microbiology (in press)

PROJECT 5: Thermodynamic and Kinetic Analysis of an RNA Kissing Interaction and Its resolution into an Extended Duplex

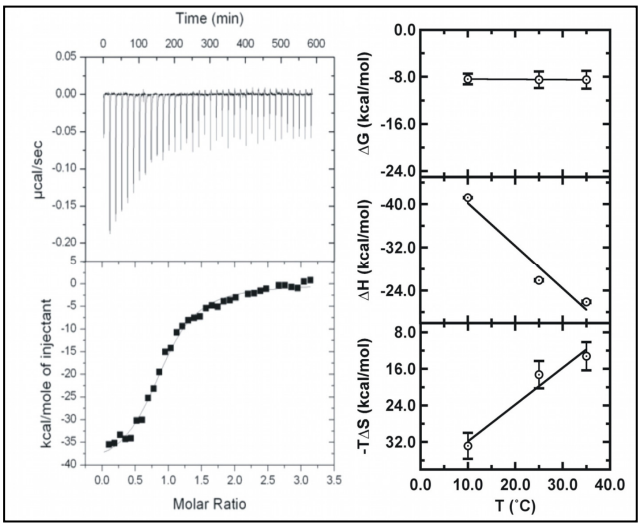
Background: In the case of bacterial and viral systems, kissing interactions between RNA hairpins are particularly prevalent in regulatory complexes, they are important for tertiary folding; provide a basis for cellular recognition and in some cases, kissing complexes can be a prelude to strand displacement reactions where the two hairpins resolve to form a stable extended intermolecular duplex. The kinetics and thermodynamics of kissing complex formation and the subsequent strand displacement reactions are poorly understood.

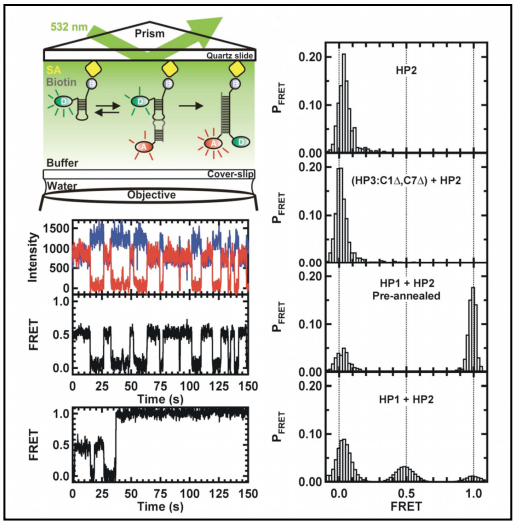
Schematic representation of possible pathways for strand displacement reaction via kissing interaction



Results:

- Recognition between two RNA' s is often under kinetic control at the level of the RNA-RNA intermediates
- Isothermal titration calorimetric (ITC) results on kissing interactions showed enthalpy contribution, enthalpy-entropy compensation was observed that led to little net ΔΔG within the temperature range studied for the kissing interaction.
- Single molecule spectroscopy FRET data showed that kissing interactions form and break many times at room temperature before becoming committed to a slow, irreversible forward transition to the strand-displaced form.





From left 1) ITC profile showing formation of kissing complex: thermodynamic energies as a function of temperature
2) smFRET analysis of kissing kinetics.

Published in Biophysical Journal, 2012,102, 1097.

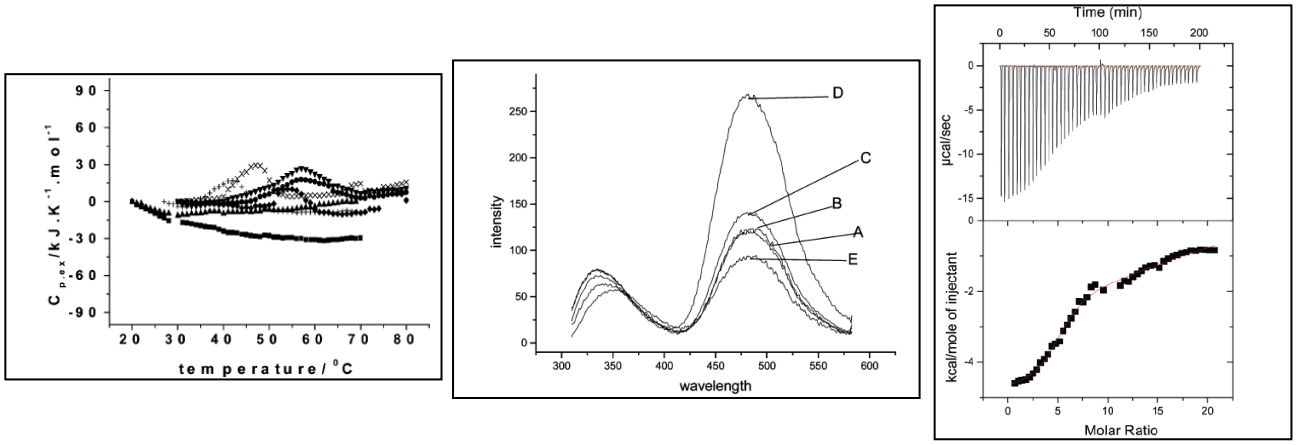
PROJECT 6: Calorimetric and spectroscopic studies on partially folded states of protein

Background: The study of partially folded conformations induced by extreme solvent conditions, such as low pH or addition of alcohols, is thought to provide a key to understanding the mechanism of protein folding. The structures of the observed partially folded states are believed to give information on early events in the folding process.

Results:

- Circular dichroism and fluorescence spectroscopic results corroborate the DSC observations and indicate protein conformation with a poorly defined tertiary structure and high content of secondary structure in the presence of 2,2,2 trifluoroethanol.

- Isothermal titration calorimetric measurements have demonstrated that ANS binding to the acid-induced state and the partially folded state of concanavalin A displays heterogeneity with two classes of non-interacting binding sites.



From left: (A) DSC scans of concanavalin A in the presence of TFE (B) Energy transfer plot monitored by ANS fluorescence (C) ITC profile of concanavalin A in the presence of TFE.

Published in Journal of Physical Chemistry B, 2005, 109, 22655.