# Transcript

[Slide 1]

Good morning everyone. I’m Vivek Rai, a rising senior student studying biotechnology in Department of Biotechnology at Indian Institute of Technology Kharagpur, and today it is my privilege to present my work done as a BEST student.

During the past ten weeks, I worked with an experienced Integrated PhD student Mr. Pradeep and under the eminent guidance of Prof. Sandhya, before concluding into the report title ‘Fluorescent labelling and Lipid Dependent Kinetics of Cytolysin a Pore Forming Toxin’. It is said that a perfect title is nothing but a one line summary of your story. Following this approach, I’ll dissect this title into its constitutive components and try to explain how my work follows from them and conversely how the title follows from my work.

The most important components of the title are – Pore forming Toxin Cytolysin a, Fluorescent labelling and lipid phase dependent kinetic study.

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Beginning with the subject of the title, Pore forming toxins represent the largest class of the toxins having a bacterial origin. This figure goes up as high as 30% and is also ubiquitously spread across organisms like sea anemones, earthworm, plants and even mammals. Although they derive from disparate sources with no apparent homology, they seem to serve similar function via a similar structural mechanism i.e., their ability to exist in bi-stable states, one corresponding to hydrophilic aqueous phase and other corresponding to hydrophobic lipid phase. Their secretion in soluble form which then oligomerizes on the membrane surface leads to an unregulated efflux of ions, biomolecules, and entry of other toxic material or even induce apoptosis in some scenarios. However, even though these proteins can be deadly in cases, they are stunning pieces of design as can be seen in the following examples.

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This is an example of alpha-hemolysin from Staph. Aureus, as it appears in an oligomeric structure after pore formation.

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The next example is a cryo-electron microscopy of mammalian protein perforin, which is a key protein in complement system and is also released by lymphocytes. These showcase the degree of symmetry and structural characteristic exhibited by these PFTs.

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The next protein, Cytolysin A, is derived from strains of *E. coli* and a member of the alpha class of PFTs (as evident from the structure image). It is, however, of particular interest because it is the only alpha PFT for which high resolution structures were available for both the monomeric and oligomeric fraction. The oligomer structure was found to contain twelve monomer units, creating a 2.8 nm cation selective lumen. However, very low amount of detectable levels in most strains indicated that the toxin’s expression might be induced under stress conditions and may also help in pathogenicity.

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A curious observation that partly explains the soluble to membrane bound state is the structural rearrangement of around 60% of the total residues between the two states. And not to mention, it looks equally stunning as its counterparts.

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One of the obvious questions that stumps scientists is that how this transition does from monomer to oligomer occurs. We have