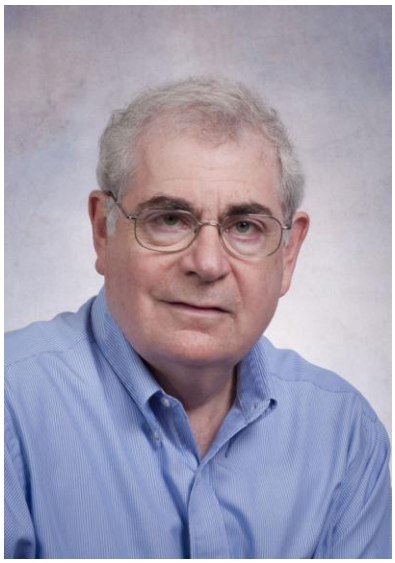

Charles Deber, PhD, FRSC

- Research Institute, Molecular Structure & Function, Senior Scientist
- University of Toronto, Biochemistry, Professor
- Cystic Fibrosis Centre, Centre Advisory Committee

The Cystic fibrosis transmembrane conductance regulator (CFTR) is the main focus of our work. Our CF research is divided into two complementary approaches: (1) to use CFTR constructs to determine, in structural terms, how or why mutations in CFTR cause cystic fibrosis; and (2) to address the bacterial infections that are so devastating to CF patients by developing new types of antimicrobial peptides that are highly active against organisms such as *Pseudomonas aeruginosa*.

With respect to CFTR, if one wants to develop a drug or therapeutic, we must first know what the nature of the target is. One must have a systematic understanding of how various mutations compromise the function, or alter the size and shape, of the target protein CFTR. This systematic understanding involves looking at the normal and mutant segments of CFTR to see how they compare and how CFTR may become changed structurally. These are first steps towards correlating mutant-induced defects ultimately with phenotypic manifestations of CF disease in the clinic. To approach these questions, we are studying segments of the CFTR membrane domain – the business centre of the molecule through which transported chloride ions must pass.

In the case of developing new antibiotics, my understanding is that clinicians may soon be running out of options as to how to treat CF patients that have recurring or chronic lung infections, which often have *P. aeruginosa* colonized in their lungs in the form of biofilms. Sometimes with prolonged treatment, the bacteria develop resistance, and as a result, most conventional antibiotics may become relatively less effective. We have discovered a new category of antibiotics – called cationic antimicrobial peptides (CAPs) - that are very active against these biofilms but work by a novel mechanism of disrupting bacterial membranes. Utilizing these compounds by themselves or in combination with other antibiotics can be an effective strategy towards treating *Pseudomonas* infections.

As early as in my high school and college days in the New York area, when the Russian orbiting satellite Sputnik went up, students who were seen by their teachers as being good at math and science were funneled in those directions. There was little introspection in those days, because there was no Internet for easily accessible information and communication, so as young students we were influenced mostly by our peers and instructors. I was one of these students who was good at math and science, and by the time I began graduate school at MIT, I knew that I enjoyed science very much and that I would strive for a career as a professor.

Once I became a scientist, with the opportunity to work at SickKids, and to witness first-hand the patients and the healthcare that takes place here day in and day out, it did not take long for me to be inspired by its environment. It soon became very natural to apply my training in biological chemistry research to paediatric disease. I find the SickKids Research Institute to be an ideal atmosphere for application of basic biomedical research to clinical situations.

My research led into cystic fibrosis because it is a focus of a major patient population at SickKids, and the research on CF was catalyzed once the gene for CFTR was discovered in this very building in 1989. CFTR is a membrane protein, and the focus in my lab at the time was dealing with membrane interactive peptides proteins, so it was a very natural transition into my interest in CF research.

Click here for a complete list of Dr. Charles Deber's publications at NCBI PubMed.

Trainees:

Vincent Nadeau (PhD student) – Nadeau's project involves looking at the extracellular loops (ECLs) that are part of the membrane domain of CFTR. His work focuses on 'helical hairpins' – two-transmembrane helix strands connected by the intervening ECL – to determine how the loops affect the folding of the CFTR protein as a whole, and more specifically, how they affect the two helices that are immediately adjacent to both sides of the loops. This focus is especially important because the ECLs are likely the most externally accessible segments of the CFTR protein in terms of incoming correctors and potentiators. As well, there are a significant number of CF phenotypic mutations that occur in these loops. Nadeau is studying how to induce the loops to fold more tightly and to observe what happens to the folding to the overall neighbouring segments of the CFTR protein. His results thus far indicate that the exact folding of the loop is a major factor in determining the normal structure of the CFTR protein.

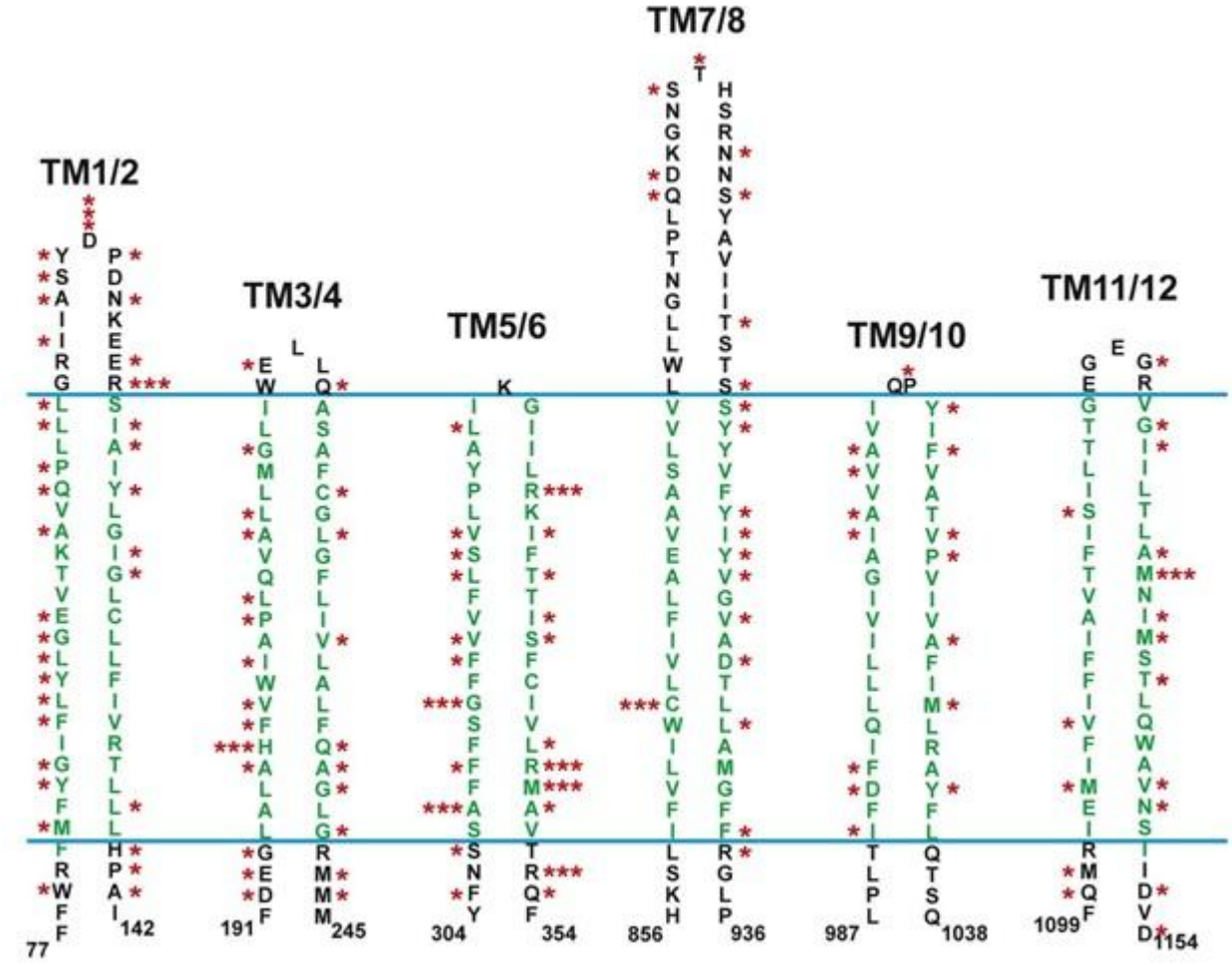

Figure 1. The six 'helical hairpins' and inclusive extracellular loops derived from the two portions (TMD1 and TMD2) of the transmembrane domain of wild type CFTR. Residues in green are predicted to constitute the membrane-spanning TM segments of CFTR, with membrane entry and exit points estimated. Blue lines delineate the approximate bilayer cross-section. Red stars (*) indicate sites of CF-phenotypic mutations in TMD1 + TMD2 among ca. 660 total missense mutations reported for full-length CFTR. "Hot spots" at which three or more mutations occur are indicated (***) in TMD1 at D110, R117, H199, A309, G314, R334, M335, R347, and R352; and in TMD2 at C866 and M1137. Diagram adapted from Cheung & Deber, Biochemistry (2008).

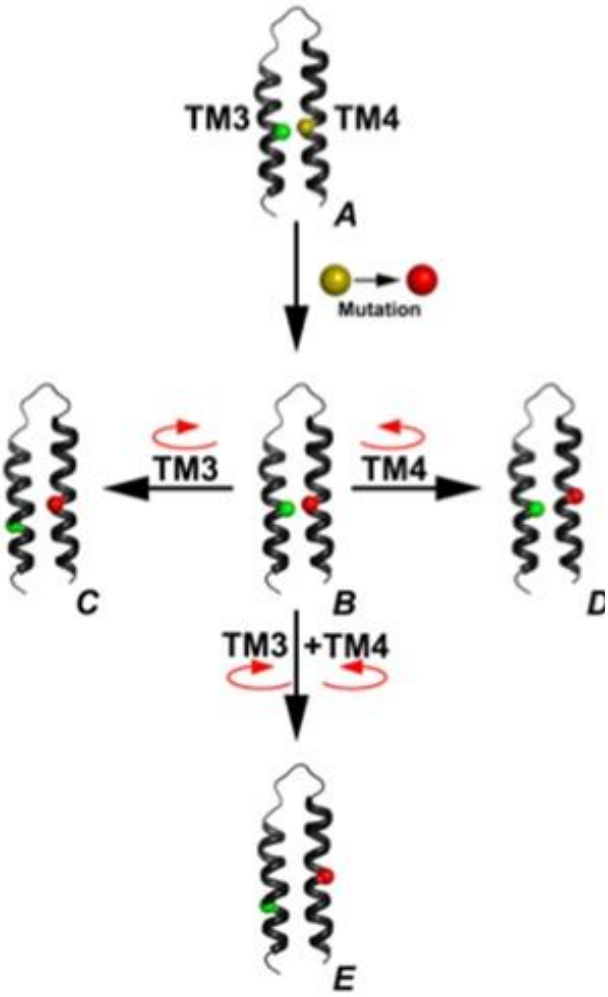

Figure 2. Schematic representation of the potential effects of CF-phenotypic mutations that occur in the transmembrane domains of CFTR. (**A**) Depiction of a given initial hairpin construct, shown as folded, with reference residues in CFTR TM3 (green) and in TM4 (gold) initially in the helix-helix interface. Upon introduction of a CF-phenotypic mutation of the TM4 reference residue (rendered as gold to red), the resulting mutant hairpin (**B**) may remain similarly folded, with the green and red residues persisting in the native helix-helix interface. In contrast, the mutation may instead propagate misfolding of local CFTR structure, to produce a new helix-helix interface, such as (**C**), where rotation about the TM3 major axis (depicted by a red arrow) now exposes the face containing the green residue to a neighboring helix or to lipid; (**D**) where rotation about the TM4 major axis similarly exposes the face containing the red residue; or (**E**) where rotation about both axes exposes both residues. The diagram emphasizes the several ways through which a single point mutation in the CFTR membrane domain can drastically alter native CFTR folding and impair function. Diagram adapted from Nadeau, Rath & Deber, Biochemistry (2012).

Lois Yin (graduate student) – Yin has been working on the design, chemical synthesis, characterization, and detailed investigation of the antimicrobial properties of a new category of peptides. These peptides are typified by sequences containing six lysine residues at the N-terminal end of the peptide, followed by 11 hydrophobic residues consisting mostly of alanine, leucine and phenylalanine. By having their positive charges separated from the hydrophobic core, the peptides are able to attach to – and then penetrate – bacterial membranes and biofilms. Yin studies all aspects of this problem, including properties of peptide sequence that would be required in a good antibiotic such as harmlessness to the host, while maintaining killing activity (termed minimum inhibitory concentration (MIC)) against bacteria that is competitive or better than existing conventional antibiotics. The lab has successfully developed lead compounds that are generating considerable interest for further development.

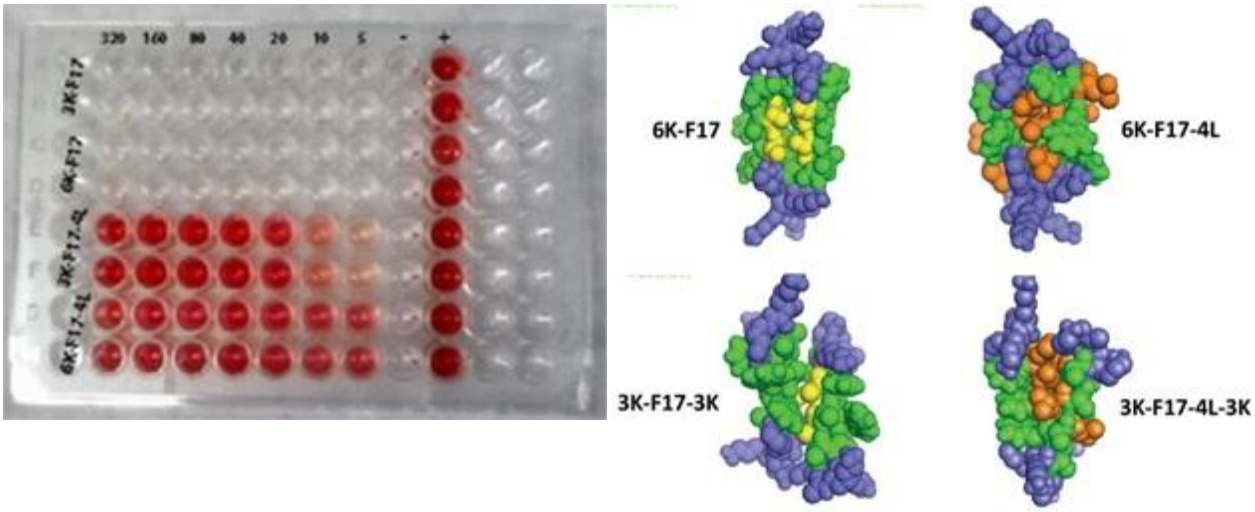

Figure 3. Hemolytic analysis and molecular structure of de novo designed cationic antimicrobial peptides (CAPs). (Left) Hemolysis tests against human red blood cells (RBCs). The colourless wells indicate the red cells are stable to the peptides; the red wells indicate that the cells have been lysed by the peptides. (Right) Representative structural models of antiparallel dimers of the four CAPs studied in this work. Lys residues are rendered in blue; interfacial Ala residues are yellow; Leu residues are orange; other core hydrophobic residues are shown in green. These are the structures proposed as the active species in the experiments. The fact that the peptides become hemolytic only when the four Leu residues are present guides our development of peptide sequences that are active against bacteria but harmless toward human red cells. Diagram adapted from Yin, Edwards, Li, Yip, & Deber, J. Biol. Chem. (2012).

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