Matrix Metalloproteinase inhibition and specificity

International Innovation speaks to Professor Steven Van Doren, Director of Graduate Studies, Biochemistry Department, University of Missouri, Columbia

In broad strokes, what are you doing in your research?

Protein molecules represent many of the actors on the cellular and molecular stages of life. We have been especially interested in proteins active in inflammatory diseases of arteries, lungs, joints, and cancer. We are interested in mechanisms of how proteins recognise their partners, such as other proteins and smaller molecules. We want to know what they look like, where and how they fit together, and how they move. Our NIH-funded project has been focusing on metallated protein enzymes called MMPs that catalyse cutting of other proteins outside of cells. These events affect the integrity of the connective tissue matrix and signalling that profoundly influences cell behaviours such as their proliferation and migration.

What are the main interests of your NIH-funded project?

Our NIH grant has sought to learn why certain MMPs fit well together with inhibitor molecules that block them. The project is also investigating why one MMP is so good at attacking protein fibrils, inflicting damage upon lungs and arteries (especially in smokers).

What findings or concepts have emerged from that research?

Scientists studying proteins tend to focus on their parts that are best preserved through evolutionary history, especially at the main functional surface of the protein molecule. We began noticing in some interactions of MMPs, with either molecules that inhibit them or proteins they attack, some interesting cases where interactions and “mechanical” changes occur farther away from the working end. This has suggested to me that the workings of MMPs and their inhibitors are more complicated than the field has recognised so far. Thus, the molecular recognition and activity of MMPs may not depend solely on the locale of the catalytic site, but also on more distant processes (on the submicroscopic distance scale of tens of Ångstroms). The concept that molecular recognition can run deep and wide has also turned up in our measurements on other, diverse protein complexes as well. I am curious about how the entirety of protein structural scaffolds may participate in recognition events that other labs study as well.

Does your basic research have any practical implications?

In development of therapeutic agents targeting MMPs, selectivity among MMPs is sought-after for minimising potential side effects. Our observations are suggesting to us potential new strategies to bind or block MMPs more selectively in diagnosing and treating disease. As a practical spin-off of our esoteric, basic research, Mark Palmier (recent PhD graduate from my lab) and I have an idea for developing medical tests for monitoring diseases common in smokers.

What hurdles have you faced?

Difficulties in preparing enough of protein molecules and smaller molecules. We want to know what they look like, where and how they fit together, and how they move. Our NIH-funded project has been focusing on metallated protein enzymes called MMPs that catalyse cutting of other proteins outside of cells. These events affect the integrity of the connective tissue matrix and signalling that profoundly influences cell behaviours such as their proliferation and migration.

How suited are you to collaborate internationally?

I have collaborated and published well with investigators far away in Florida, New York, Michigan, and California. In each case, our group contributed near atomic-resolution structural studies using NMR spectroscopy. We are launching a collaboration with a group in the UK out of enthusiasm to test the PI’s hypothesis of novel molecular interactions. As to trans-Atlantic communication, I have enjoyed my business trips to Europe, and video conferencing is easier than ever.

What scientific talents do you bring to collaborations?

We are best known for diverse structural studies using NMR spectroscopy, mainly with protein molecules and their complexes, but also on RNA. More recently, we have complemented this with some interesting enzymology, mutagenesis, and thermodynamic studies of associations with enzymes.

Tell us a little about yourself...

Life outside of work for me and my wife Kelli revolves largely around our four children: three teenage girls and a seven year-old boy. We enjoy outdoor recreations such as hiking, cycling, swimming, tennis and camping. Kelli and I and our two older girls have enjoyed visits to Europe: the UK, France, Germany, Switzerland, and Italy. We’ve been gripped by European art, architecture, and history.

Tell us a little about diversity among your research group members...

I have enjoyed employing young scientists from China, India, Ukraine, South Korea, and Iran, three of whom worked in science in Europe. Three African-Americans have done fine undergraduate research in my lab. Among the graduate and undergraduate students currently in the lab, three are men and four are women.
Exploring molecular recognition by MMPs, enzymes active in inflammatory disease

The interactions of proteins with other proteins and nucleic acids represent much of the molecular machinery of life. Steven Van Doren’s research mission is to bring to molecular medicine the rigor and detailed insight into molecular workings available from Nuclear Magnetic Resonance (NMR) spectroscopy and molecular biophysics. His group has been exploring mechanisms of how proteins fit together to provide biological control in inflammatory diseases, innate immunity, and response to the environment. The disease contexts for the protein interactions have included cancer, cardiovascular and pulmonary diseases, stroke, and arthritis.

NIH has supported his group’s research into MMP-12, MMP-3, and TIMP-1. MMP-12 fosters artery damage in atherosclerosis and aortic aneurysms and lung damage in emphysema. MMP-3 causes brain damage after stroke and damage of arthritic joints. Their physiological inhibitor protein TIMP-1 can impede the spread of tumors and progression of arthritis. His research group has contributed 3D atomic structures of MMP-3\(^1\), MMP-12\(^2\), TIMP-1\(^1\), and the complex of MMP-3 and N-TIMP-1\(^4\) in solution using NMR.

Emerging concept: Molecular recognition can run deep and wide

An intriguing theme from the research of Van Doren’s laboratory regarding how these proteins interact with other proteins or smaller molecules is that remote parts of each protein couple to the interaction at the main binding site. The group’s favorite approach is NMR spectroscopy. NMR has been instrumental in their discoveries because it is sensitive to subtle effects throughout a molecule and beyond the anticipated regions of interest. His first example of the concept of remote parts of a protein molecule participating in molecular recognition regards the coupling of the core of TIMP-1 to its tight binding to MMP-3\(^4\). Though proteins are normally expected to become more rigid upon association, TIMP-1 starts out as mostly rigid by itself. Its core, well away from its inhibitory ridge where MMPs fit, is enhanced in motions once MMP-3 is bound (see fig. 1), thereby contributing affinity by a surprising mechanism\(^4\). A second case of subtle changes in a protein core has been the unanticipated structural adjustment within MMP-12, and very possibly other MMPs, that may be linked to binding of inhibitors (drug lead compounds)\(^2\). Though MMP-3 and MMP-12 share highly similar 3D atomic structures, the latter is more active, more rigid, and less stable\(^6\). These subtle differences in their global properties fit with the general hypothesis of tradeoffs of stability for activity in enzymes, as Shoichet and coworkers have asserted.

The molecular recognition and activity of MMPs may not depend solely on the locale of the catalytic site, but also on more distant processes.
Remote, unexpected surfaces are emerging as important in molecular recognition events by MMP-12. For example, remote exosites of MMP-12 contribute to its interaction with a collagen-derived, triple helical peptide substrate (see fig.2, supported by the American Heart Association)\(^1\) with other protein fibrils from lungs and arteries\(^2\), and with a cancer-preventative compound from oriental diets (Liang et al., unpublished).

These anecdotes, and behaviours of other protein recognition events studied in his lab and other biological labs, suggest that not only the functional site but also much more of a protein or enzyme can participate in how it works. For example, though the notion of exosites may seem odd, exosites have long been thoroughly characterised in thrombin\(^3\). Intensive investigations of thrombin inhibition via exosites represent basic science underlying the multi-billion dollar per year medical industry to limit blood coagulation.

**INNOVATIVE NMR AND ENZYMOLOGY APPROACHES FOR UNDERSTANDING MOLECULAR RECOGNITION**

NIH support for research on MMPs and TIMP-1 has enabled his group to develop widely useful and innovative methods for investigating how proteins and enzymes recognize macromolecular partners, using NMR spectroscopy and enzymology.

- His group developed an NMR method of mapping macromolecular interfaces that is consistently more accurate than the NMR method used most commonly. The higher accuracy results from measuring coverage and protection of interfacial surfaces from a small molecule probe\(^4,5\).
- By combining NMR identification of recognition surfaces with their sequence properties, his group has been successful in identifying residues important to the functions of proteins\(^6,9\).
- To support enzyme engineering studies, he and a coworker developed a faster and cheaper method to obtain enzyme kinetics parameters from just a few measurements\(^10\). The method is also more accurate since it avoids the non-linear distortions that normally plague fluorescence detection.
- His group has combined these three innovations into a general strategy to identify key residues that confer to an enzyme or protein its selectivity for preferred partners or substrates to act upon. The group will be reporting how well the approach worked for elucidating features of MMP specificity for protein fibrils\(^4\) (as illustrated in fig. 3).

Van Doren would like to entertain new collaborative investigations of mechanisms of protein-protein and protein-nucleic acid interactions that influence inflammatory diseases. He would not only like to continue work relevant to cancer, cardiovascular and pulmonary conditions, but also to branch into molecular recognition studies pertinent to other common inflammatory disease states such as acute respiratory distress syndrome (ARDS), inflammatory bowel disease, and neurodegenerative disease. The partnership of the University of Missouri, National Institutes of Health, and National Science Foundation has provided a strong research environment. Together, they have supported excellence and growth in NMR infrastructure, biophysical research, life sciences research, and training at Missouri’s flagship campus in Columbia.

**References**