Seeing is believing

Scientists at the Center for Integrated Protein Science Munich have taken advantage of the 3D scanning capabilities of Tecan's Infinite® M1000 microplate reader.

Heinrich Leonhardt, Professor of Molecular Human Biology at LMU

Dr Carina Frauer, a postdoctoral researcher in Professor Leonhardt's group

The Center for Integrated Protein Science Munich (CIPSM), based at Ludwig-Maximilians University Munich (LMU), Germany, focuses on the study of epigenetics using a number of different technologies. In addition to using live cell imaging, live microscopy, dynamic imaging techniques and super-resolution microscopy, CIPSM scientists have been capitalizing on the 3D scanning capabilities[1] of the premium Quad4 Monochromators™ -based Infinite M1000 microplate reader to investigate nanobodies, as well as its flexibility and sensitivity for multicolor interaction studies.

Professor Heinrich Leonhardt, Professor of Molecular Human Biology at LMU, explained: "Conventional antibodies are large molecules consisting of two heavy chain and two light chain proteins and are not ideally suited for studies in living cells. Nanobodies are relatively simple proteins about a tenth the size of human antibodies, and consist of a single heavy chain. They are effective alternatives to conventional antibodies, possessing similar antigen-binding characteristics but with enhanced stability and reduced size, and have the advantage that they are functional inside living cells. This allows them to be fused with fluorescent marker proteins and used to trace antigens within the cell. Using reverse-transcription PCR (RT-PCR), we can amplify the antigen binding domain, make recombinant libraries and isolate specific binders. These binders are then expressed in *E. coli*, purified and used for live-cell studies, or coupled to matrices for affinity purification applications."

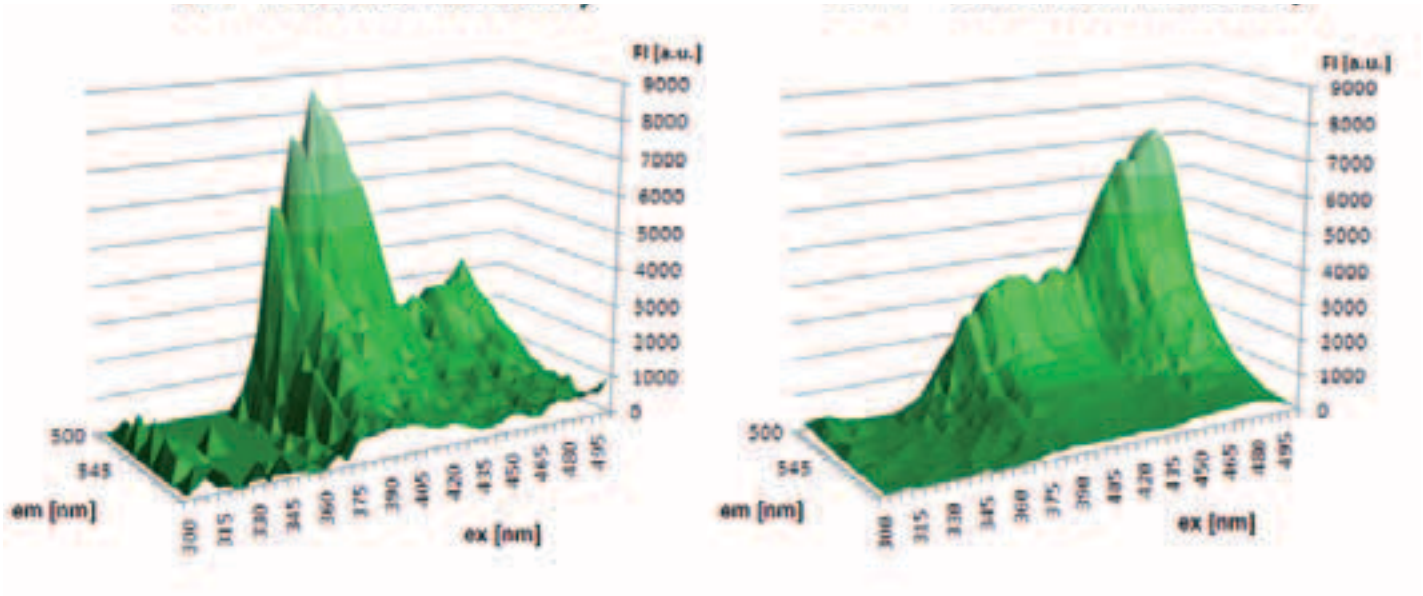
Dr Carina Frauer, a scientist at LMU, described the process: "We use nanobodies for a number of different applications, including various binding assays, and we try to combine the *in vivo* studies with the biochemical assays. GFP-binding nanobodies coupled to sepharose beads (GFP-Trap®, ChromoTek) are used to capture GFP fusion proteins for purification and biochemical characterization. The immobilized GFP fusions are then incubated with fluorescently labeled DNA substrates or peptides and any unbound substrate is washed away."

"We then measure the fluorescence using the Infinite M1000 reader, which gives us the sensitivity we need and allows us to distinguish between the various fluorescent labels by using very specific settings. The labels have been carefully chosen to enable us to simultaneously compare different DNA substrates that are in direct competition with each other, as this allows us to directly determine sequence specificity. The fluorescence of the DNA or peptide labels and the immobilized GFP fusion are measured, and their ratio calculated, to quantify specific DNA or peptide binding. We also use DNA substrates containing a mechanism-based inhibitor for methyltransferases, and determine the formation of irreversible covalent complexes with active methyltransferases. This provides a measure of catalytic activity, so we have essentially established a non-radioactive methyltransferase activity assay."

Professor Leonhardt added: "An important aspect of this assay is that we can actually quantify the protein. There are many binding assays available, but they don't usually quantify the protein itself. Our assay enables quantification of the protein; the input, bound and unbound fractions. We have also been able to use the 3D scanning capability

GFP + control nanobody

GFP + enhancer nanobody



3D scanning makes it easy to study nanobody-induced changes to the spectral properties of GFP

of the M1000 to study nanobody-induced changes of the spectral properties of GFP in living cells. The results are really beautiful."

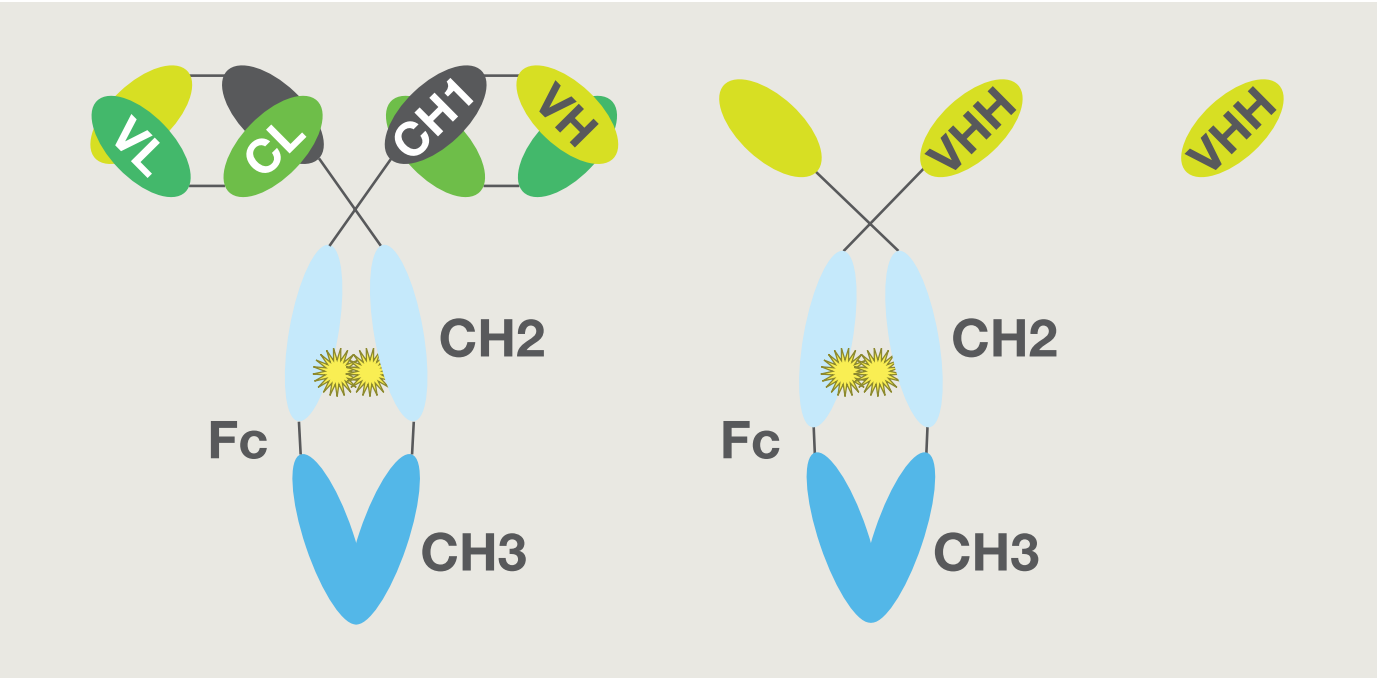
Carina continued: "We co-expressed GFP in cells with specific nanobodies which modulate the spectral properties of GFP. 3D scanning shows excitation and emission on the same plot, very nicely illustrating the shift in GFP fluorescence excitation and emission wavelengths induced by nanobody binding."

Professor Leonhardt concluded: "The instrument is flexible, easy to use and very fast, which has increased the speed of the assay. The software is simple to understand and, if we need any additional advice, Tecan is happy to help. We focus on developments in technology and the application of new methods, and the instrument's versatility really helps in this."

1. Kirchhofer, A., Helma, J., Schmidthals, K., Frauer, C., Cui, S., Karcher, A., Pellis, M., Muyldermans, S., Casas-Delucchi, C.S., Cardoso, M.C., Leonhardt, H., Hopfner, K.P., Rothbauer, U. (2010). Modulation of protein properties in living cells using nanobodies. *Nat Struct Mol Biol*. Jan;17(1):133-8. Epub 2009 Dec 13.

For more information on Tecan's Infinite M1000, visit **www.tecan.com/infinitem1000**

For more information on CIPSM, visit **www.cipsm.de**



Schematic comparison of conventional and camelid antibodies to nanobodies (left to right)