The Unit provides a broad range of instrumentation for the biophysical characterisation of biomolecules and their interactions, including spectrophotometers, fluorometer, a nanoDSF (Differential Scanning Fluorimetry) device, isothermal titration and differential scanning calorimeters, a circular dichrograph, dynamic and multi-angle static light scattering (MALS) equipments, 2 biosensor instruments — surface plasmon resonance (SPR) and biolayer interferometry (BLI) — and a multiple-well microplate reader with numerous technologies. Research groups mostly from but not limited to (i.e., DNA Replication Group, Metabolism and Cell Signalling Group, Experimental Oncology Group) the Structural Biology Programme used these technologies throughout the year. For example, in collaboration with the Protein Production and Molecular Imaging Core Units, using nanoDSF and MALS, we validated that 2 anti-MT1-MMP nanobodies are well-folded, stable and monomeric proteins (FIGURE 1, panels A and B). In addition, we used SPR to characterise the affinity and kinetics of the interaction of each antibody with human MT1-MMP protein (FIGURE 1, panels C and D). This research is useful to further develop labelled nanobodies as PET probes for triple negative breast cancer imaging.

The Unit hosts a 700 MHz NMR spectrometer that is equipped with probes and a sample changer to run up to 120 samples automatically. This provides medium throughput for the screening of small molecule protein binders (together with the Experimental Therapeutics Programme), as well as for metabolite quantification that in 2022 was done in collaboration with the Growth Factors, Nutrients and Cancer, and Transformation and Metastasis Groups (Molecular Oncology Programme) and the Hereditary Endocrine Cancer Group (Human Cancer Genetics Programme). Collectively with our client groups, we will continue implementing sample preparation protocols and developing spectroscopic and analytical tools to characterise metabolites present in different biological samples.

**FIGURE 1** Characterisation of 2 nanobodies targeting a matrix metalloproteinase. (A) Superimposed chromatographic traces of light scattering (solid lines), refractive index (dashed lines) and the calculated molecular weight from each nanobody. (B) NanoDSF thermal unfolding profiles of the 2 nanobodies. Infection temperatures (T) are shown. (C, D) Overlay of the SPR sensorgrams and corresponding kinetic fits to a 1:1 binding model (solid lines) for the interaction of each nanobody with MT1-MMP. Nanobody concentrations and the calculated dissociation constants ($K_D$) of the complexes are indicated.