Affinity-Based Mass Photometry for Targeted Label-Free Detection of Individual Proteins in Complex Mixtures

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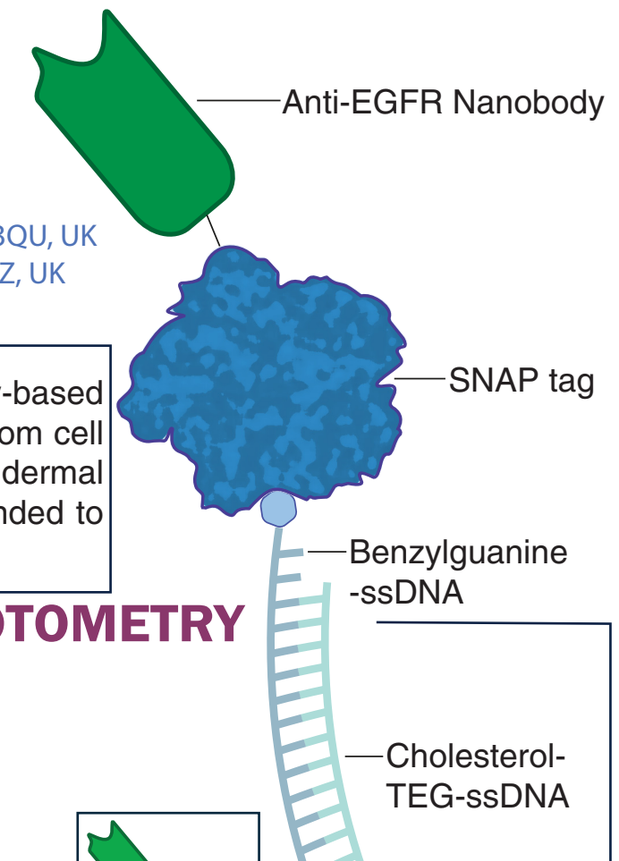
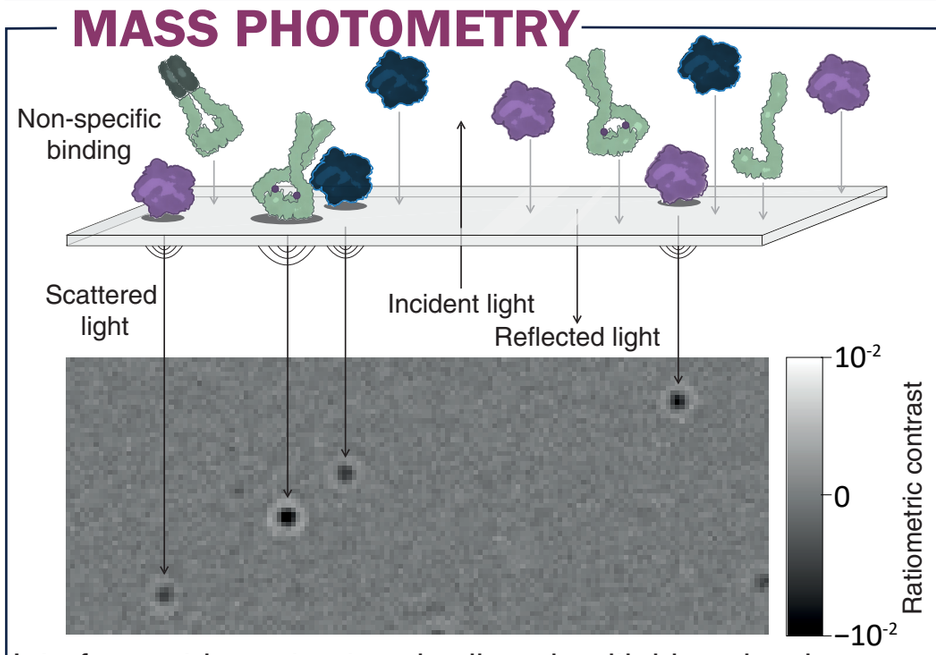


INTRODUCTION

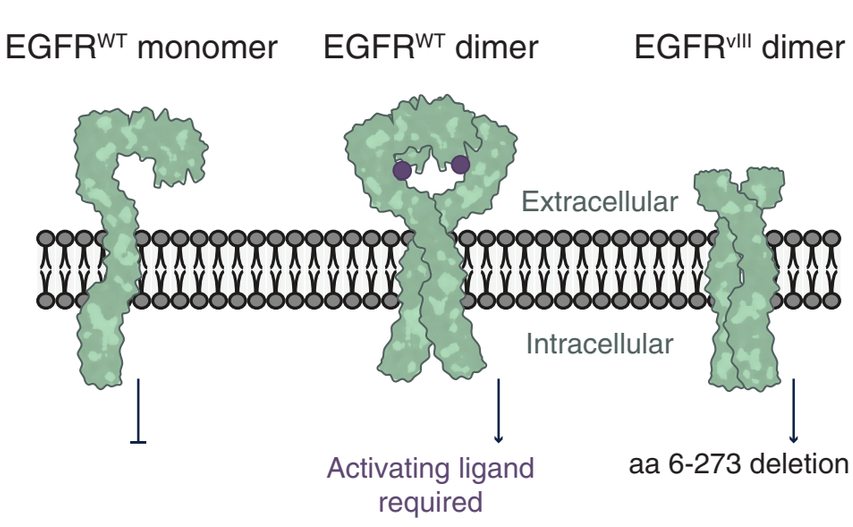
Mass Photometry (MP) is a label-free, optical method for detection and mass-measurement of single biomolecules in solution. Affinity-based Mass Photometry (AffinMP) is an implementation of MP that enables targeted protein detection and mass measurement directly from cell lysates and tissue homogenates. Here, we begin to develop an AffinMP assay to characterise the abundance and assembly of Epidermal Growth Factor Receptor (EGFR) in glioblastoma mulftiforme (GBM) patient-derived cell lines. Our approach can be broadly extended to study a variety of protein interactions under physiologically or pathologically relevant conditions.

MASS PHOTOMETRY



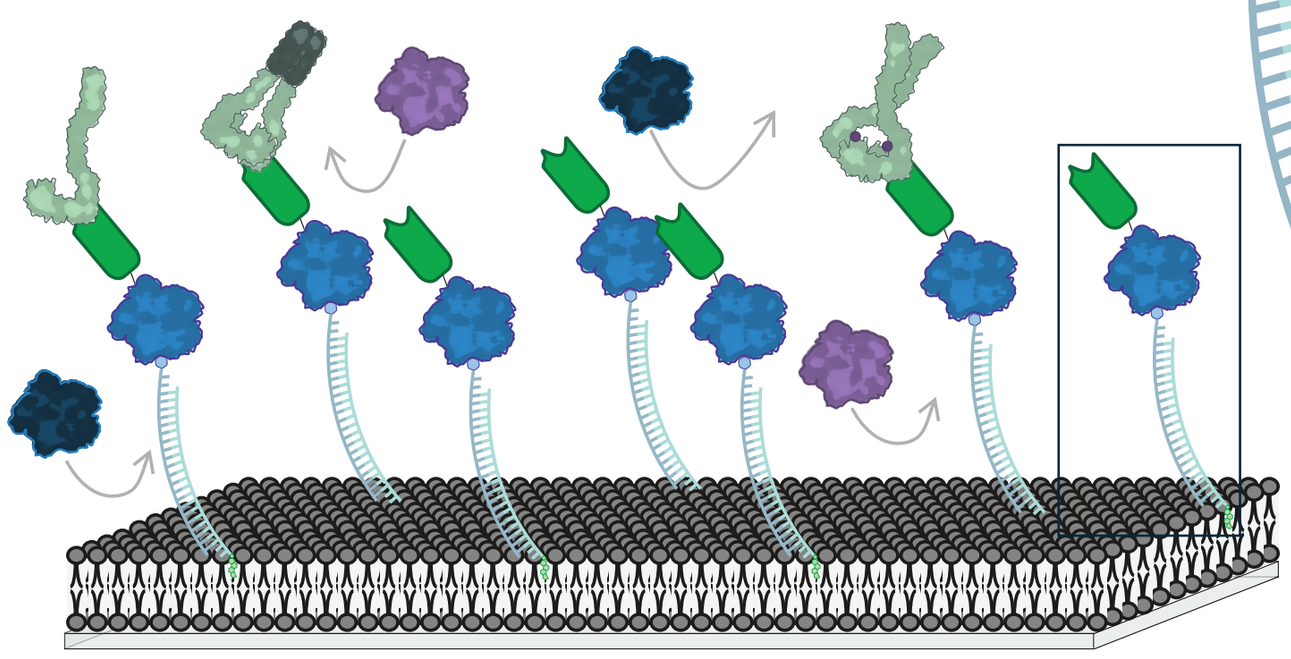
Interferometric contrast scales linearly with biomolecular mass.[1]

EPIDERMAL GROWTH FACTOR RECEPTOR

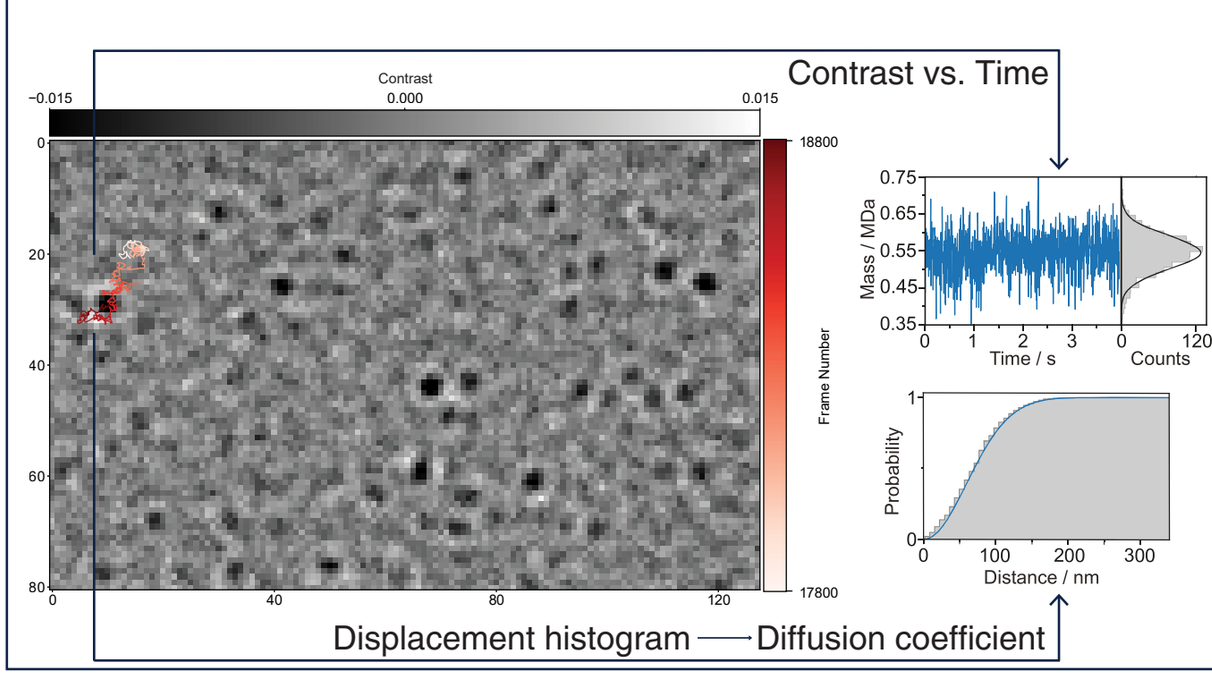


EGFR dimers affect downstream cell-signalling pathways, leading to tumour proliferation. The EGFR^{vIII} mutant is frequently expressed in GBM tumours.

AFFINITY-BASED MASS PHOTOMETRY

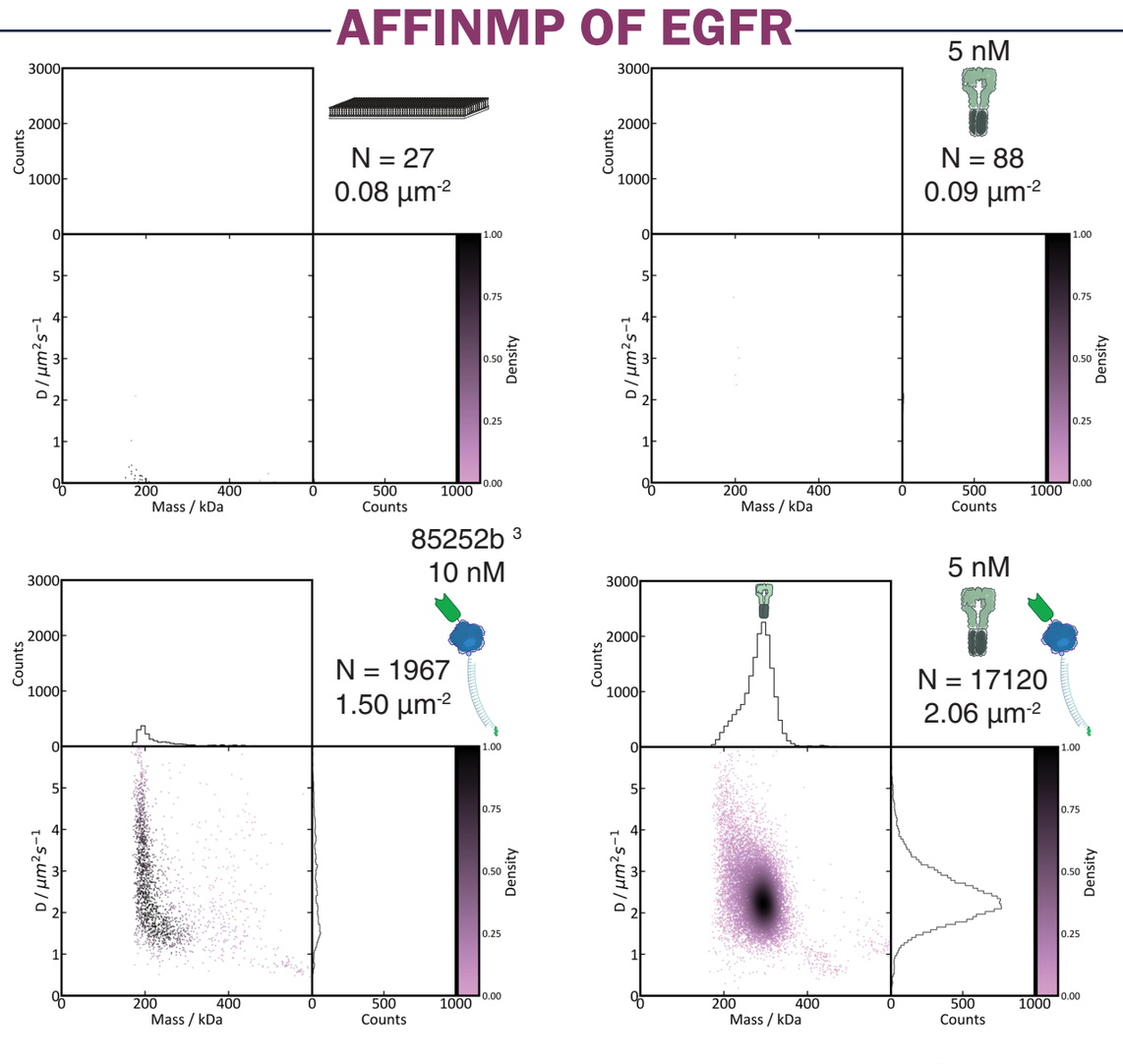


A supported lipid bilayer passivates all non-specific protein detection.
Insertion of nanobody-based protein capture complexes facilitates targeted protein detection.



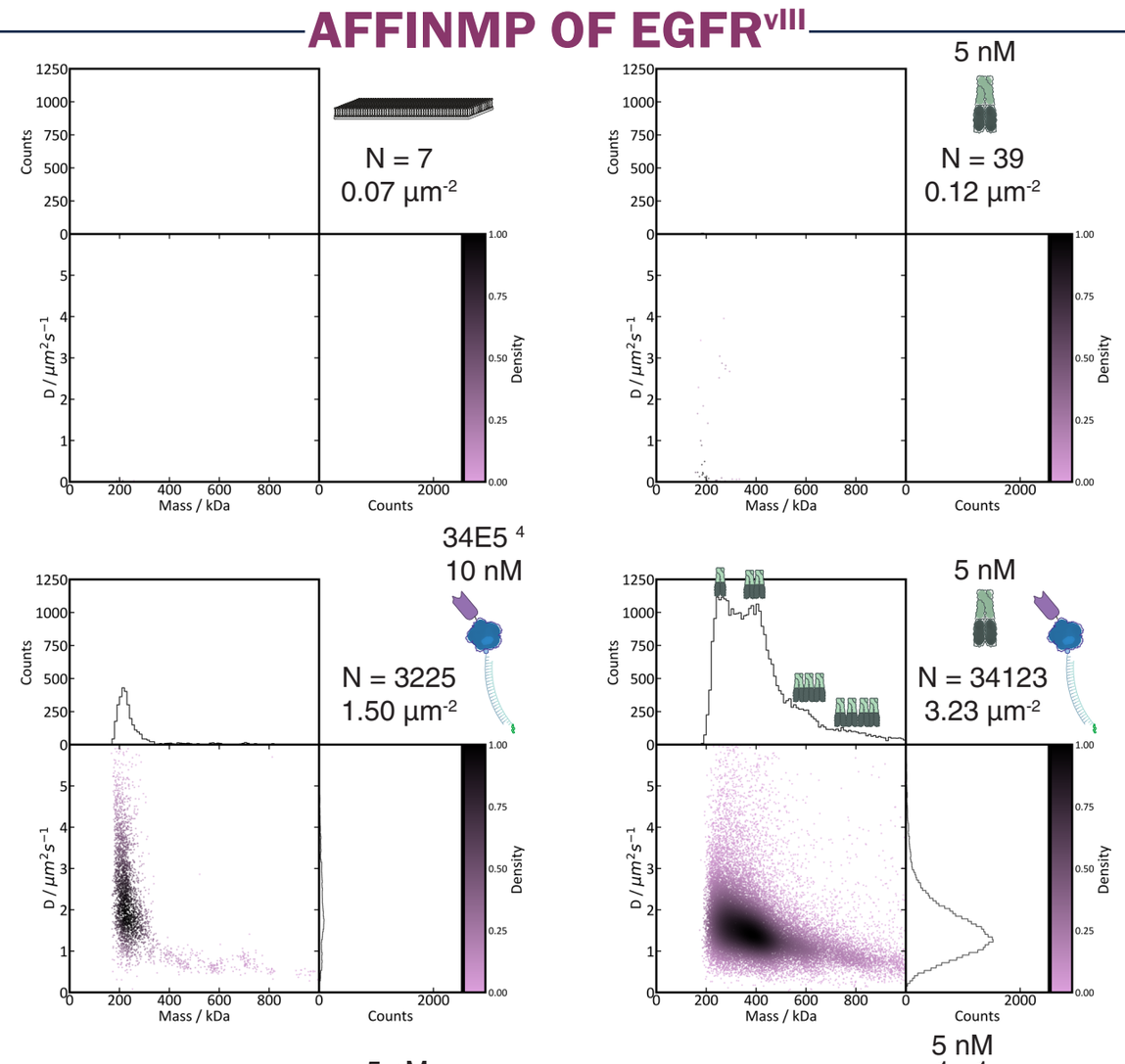
Example of single particle tracking, mass and diffusion measurement. A single particle trajectory is shown on an example median ratiometric frame. From this, we extract the mass trace and the cumulative probability distribution of the distance travelled within a single frame. Figure adapted from Asor et al. (2024), with permission.[2]

AFFINMP OF EGFR



An EGFR-Fc chimera protein was chosen to test our EGFR AffinMP assay. AffinMP of EGFR-Fc without capture complex (top) reveals passivation of non-specific protein binding. AffinMP of EGFR-Fc with the 85252b capture complex (middle) reveals strong specific binding and detection of the EGFR-Fc dimer, using as little as 500 pM EGFR-Fc (bottom).[3]

AFFINMP OF EGFR^{vIII}



An EGFR^{vIII}-Fc chimera protein was chosen to test our EGFR^{vIII} AffinMP assay. AffinMP of EGFR^{vIII}-Fc without capture complex (top) reveals passivation of non-specific protein binding. AffinMP of EGFR^{vIII}-Fc with the 34E5 capture complex (middle) reveals strong specific binding and detection of EGFR^{vIII}-Fc oligomers. The 34E5 nanobody preferentially binds to EGFR^{vIII}-Fc over EGFR-Fc (bottom).[4]

CONCLUSIONS

We have developed an AffinMP assay for the targeted characterisation of EGFR and EGFR^{vIII}. Next, we plan to use AffinMP to characterise EGFR directly from GBM patient-derived cell lines. Future work could combine AffinMP with mass spectrometry-based proteomics to identify interaction partners and assign stoichiometries. This assay can be adapted to allow targeted, label-free detection and mass measurement of a wide range of proteins in physiologically or pathologically relevant systems.

ACKNOWLEDGEMENTS

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