

Evaluation of novel Quartz Crystal Microbalance based technologies to quantify interactions of biological macromolecules with cell-bound markers

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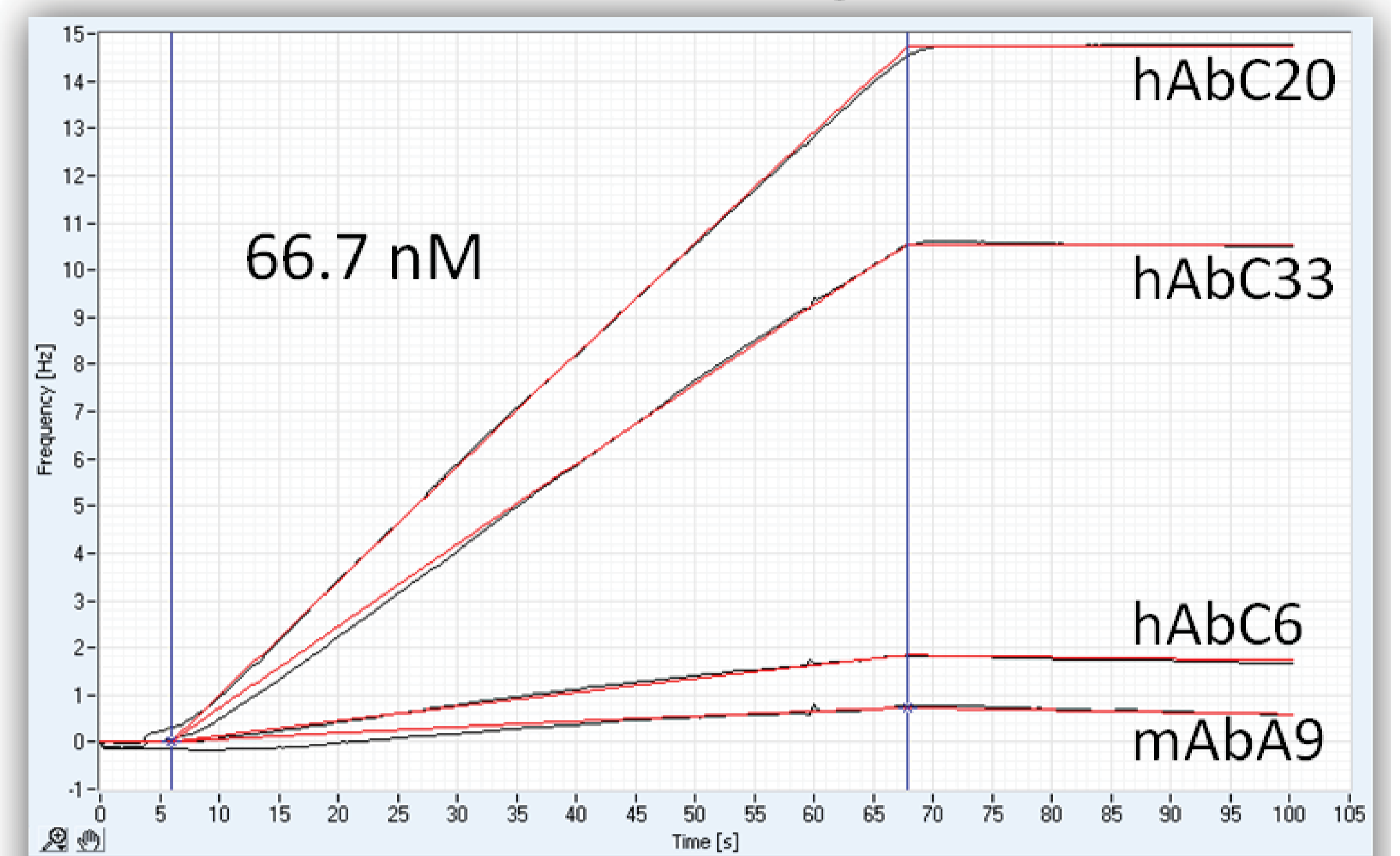
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2

Introduction

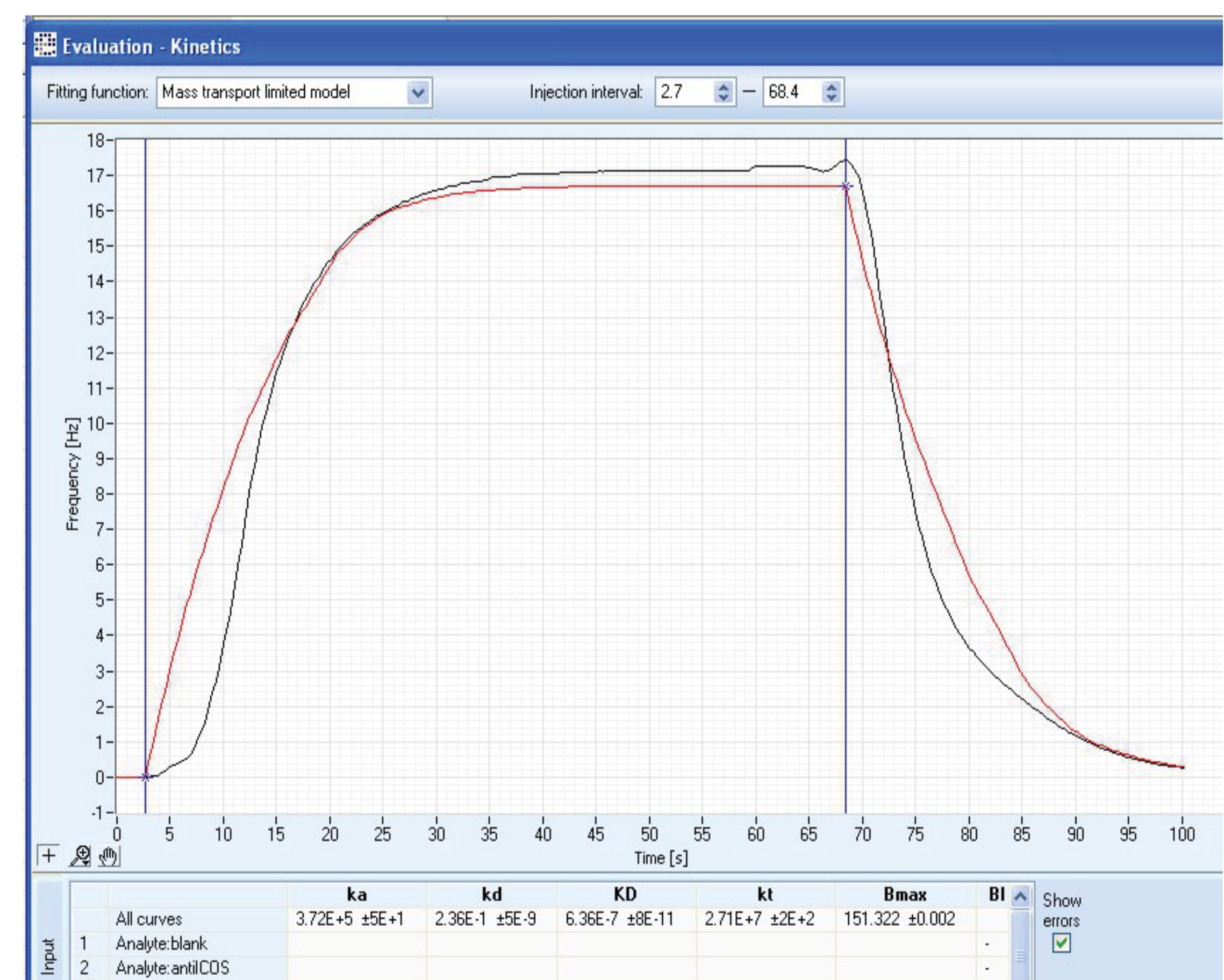
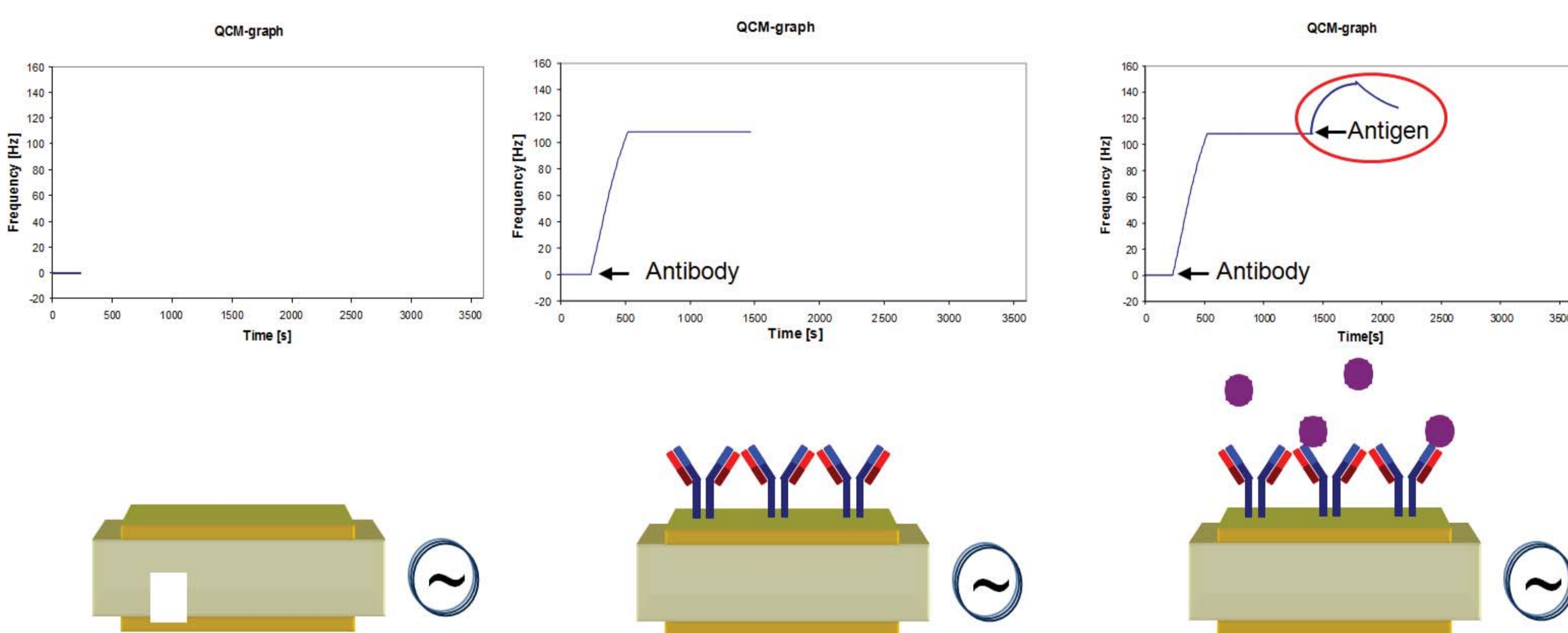
The homo- or heterotypic interaction of biomolecules, like e.g. proteins, peptides, nucleic acids and lipids are fundamental for cellular functions on all levels, including immune responses, regulation, metabolism, signaling, cellular and tissue architecture. Elucidation of these interactions by different tools is crucial to extend our knowledge aimed at understanding cellular functions, signaling pathways, and immune responses. Moreover a deep understanding of molecular interactions is essential for the development of novel diagnostic tools, for the discovery of new target molecules, and for the development of new therapeutic strategies. The data obtained from QCM analyses are both, qualitative delivering information about specificity and selectivity, and quantitative delivering exact thermodynamic and kinetic parameters of interactions. Here we report the evaluation of QCM based methods on the interaction of cells of the immune system (DCs, B cells, T cells) with specific ligands to cell surface molecules.

HuEMPD – IgG1



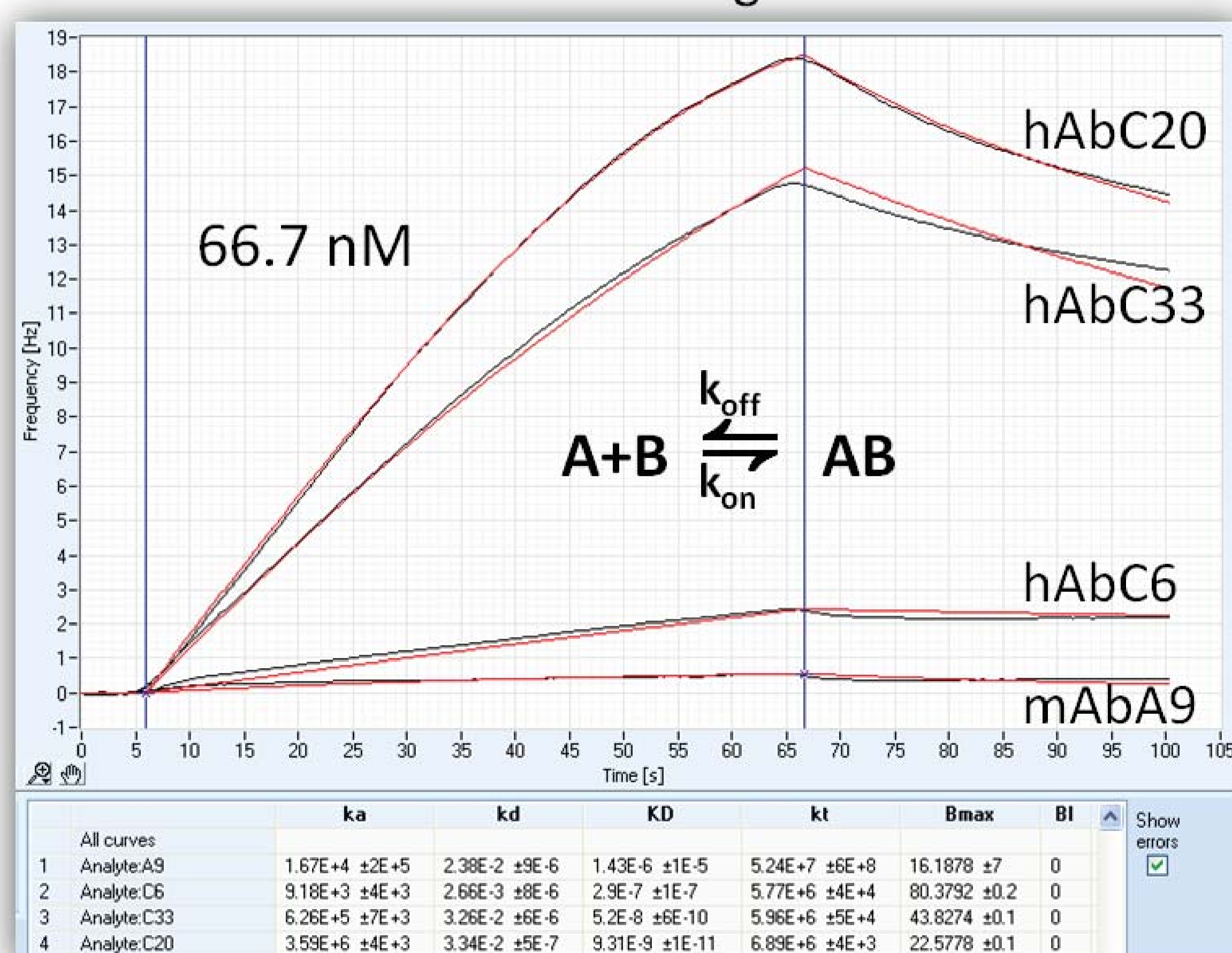
k_{on} : [M ⁻¹ s ⁻¹]	k_{off} : [s ⁻¹]	K_D : k_{off}/k_{on} [M]	k_{on} : [M ⁻¹ s ⁻¹]	k_{off} : [s ⁻¹]	K_D : k_{off}/k_{on} [M]
mAbA9 → 1.67×10^4	2.38×10^{-2}	1.43×10^{-6}	mAbA9 → 1.66×10^4	7.24×10^{-3}	4.35×10^{-8}
hAbC6 → 9.18×10^3	2.66×10^{-3}	2.90×10^{-7}	hAbC6 → 1.35×10^4	2.19×10^{-3}	1.63×10^{-7}
hAbC33 → 6.26×10^5	3.26×10^{-2}	5.20×10^{-8}	hAbC33 → 7.50×10^4	3.26×10^{-5}	4.35×10^{-10}
hAbC20 → 3.59×10^6	3.34×10^{-2}	9.31×10^{-9}	hAbC20 → 1.85×10^5	1.33×10^{-6}	7.17×10^{-12}

Affinity of a commercial anti-ICOS-L mAb to its ligand overexpressed on fibroblasts



Results

MuEMPD – IgG1



Conclusion

Currently we are using QCM to replace classical surface plasmon resonance experiments to quantify molecular interactions. We aim to establish this novel technology of measuring the interaction of biomolecules with molecules on entire cells, and to elucidate the binding properties of these interactions on defined system like e. g. affinity of antibody Fc parts to Fc receptors, affinity of therapeutic binders to their cellular target, and binding of antigens to APCs