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Microfluidic free-flow electrophoresis for the discovery and characterisation of calmodulin binding partners THERESE HERLING, University of Cambridge, SARA LINSE, Lund University and University of Cambridge, TUOMAS KNOWLES, University of Cambridge — Non-covalent and transient protein-ligand interactions are integral to cellular function and malfunction. Key steps in signalling and regulatory pathways rely on reversible non-covalent protein-protein binding or ion chelation. Here we present a microfluidic free-flow electrophoresis method for detecting and characterising protein-ligand interactions in solution. We apply this method to probe the binding equilibria of calmodulin, a central protein to calcium signalling pathways. In this study we characterise the specific binding of calmodulin to phosphorylase kinase, a known target, and creatine kinase, which we identify as a putative binding partner through a protein array screen and surface plasmon resonance experiments. We verify the interaction between calmodulin and creatine kinase in solution using free-flow electrophoresis and investigate the effect of calcium and sodium chloride on the calmodulin-ligand binding affinity in free solution without the presence of a potentially interfering surface. Our results demonstrate the general applicability of quantitative microfluidic electrophoresis to characterise binding equilibria between biomolecules in solution.

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