Multiplexing on the ASF substrate

The ASF substrates were studied for multiplexed fluorescence-based array analysis. Three different proteins, a c-Myc-tagged protein containing bacterial cell lysate (top row Figure SF1), FLAG-tagged bacterial alkaline phosphatase (fBAP, middle row Figure SF1) and BAP (bottom row Figure SF1), were spotted on the same ASF substrate and probed with a mixture of two primary antibodies, one tagged with FITC (anti-c-Myc) and another with Cy3 (fAb-Cy3). Figure SF1 shows that the anti-c-Myc antibody binds its target specifically, whereas anti-FLAG antibody has affinity towards both the c-Myc protein containing cell lysate and its cognate partner, fBAP. The results were verified on a gel-based Western blot analysis (not shown). As expected, none of the antibodies bind to the control BAP protein. Once again, this indicates that the functional properties of immobilized proteins are preserved on the ASF substrate and the substrate is amenable to multiplexing.

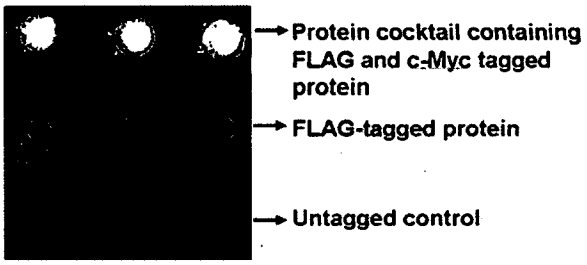

Figure SF1: Multiplexing on ASF substrate. Aliquots (2 μL) of bacterial cell lysate containing c-Myc-tagged protein, fBAP and BAP were manually deposited on the ASF substrate and probed with a mixture of antibodies tagged with different fluorophores. The color scheme adopted here indicates anti-c-Myc FITC-tagged antibody in red and fAb-Cy3-labeled antibody in green. The yellow color corresponds to the overlap of red and green, indicating affinity of the proteins in the spot for two antibodies. The substrate was imaged on a Typhoon 8600 (GE Healthcare).