

ENHANCED DYNAMIC RANGE AND SENSITIVITY OF PROTEIN ARRAYS USING GOLD PROBES AND SCANNING ELECTRON MICROSCOPY

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Detection of low-abundance proteins, such as hormones, cytokines, small G-proteins, and DNA binding proteins, is an unanswered challenge, especially with techniques that can be applied to protein chips. These proteins often serve as key mediators in response to various physiological stimuli, but their detection at the single molecule level requires painstaking techniques with tedious sample preparation and imaging, which are hard to apply with high throughput methods.

Here we present a rigorous, quantitative method that reaches single molecule detection levels, gives high signal to noise ratios, and demonstrates a broad dynamic range, while retaining easy sample preparation and potential for high throughput abilities. Our method is based upon target-coated gold particles followed by scanning electron microscopy to probe proteins or their ligands, arrayed on a microscope slide.

As model systems we quantified the interactions of biotin-streptavidin and of an anti-hapten antibody with its cognate antigen. Our results demonstrate an increased sensitivity and a highly enhanced dynamic range over other detection methods such as fluorescence. In addition, our technique offers important advantages such as non-bleaching of the signal, high reproducibility and reduction of non-specific binding.