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Influence of Microplate Mixing on Binding Assays

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Introduction: Microplate-based analytical assays represent one of the most commonly used tools for the sensitive and reproducible detection of bioactive molecules. However, despite the widespread use of microplate assays, the degree of optimisation of the experimental procedures is questionable [1]. Effective mixing is expected to shorten incubation periods, increase reproducibility and sensitivity. To investigate this, the association of cytoadhesive lectins and lectin-modified nanoparticles to epithelial cell monolayers was determined under stationary and mixing conditions.
Materials and Methods: Fluorescent-labelled wheat germ agglutinin (f-WGA, MW~36 kDa) and bovine serum albumin (f-BSA, MW~68kDa) were used as soluble ligands. Fluorescent-labelled nanoparticles with a mean particle size of 130 ± 65 nm were prepared by a solvent evaporation procedure and conjugated with WGA (138 ± 66 nm) [2]. The analytes were transferred to the Caco-2 monolayers in 96-well plates and incubated for up to 240 min at 4°C. For the experiments involving mixing of the assay a PlateBooster96 (Olympus Life Science Research Europa GmbH, Germany). In regular intervals the cell-associated fluorescence was determined with a microplate reader (Tecan Infinite200i, Tecan GmbH, Austria) after three washing steps.
Results and Discussion: In the study presented, the effect of mixing on the adsorption kinetics in 96-well microplate assays was studied using analytes with different diffusion coefficients, but identical targets for binding. Within 240 min reaction time, saturation of the monolayer was neither reached in the case of free lectin, nor in the case of lectin-conjugated nanoparticles when incubated under stationary conditions. In contrast, mixing of the assay resulted in accelerated reaction kinetics not only in the case of nanoparticle binding, but surprisingly also for studies using free protein which bears clearly higher molecular diffusivity. Thus, not only particle binding studies, but also solid phase immunoassays (ELISA, RIA) could highly benefit from mixing regarding plate coating efficiency, incubation time as well as reproducibility.

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[2] Fillafer C, Friedl DS, Wirth M, Gabor F. Fluorescent bionanoprobes to characterize cytoadhesion and cytoinvasion. Small. 2008; 4: 627–633. doi:10.1002/smll.200701086