

Thematic Enzyme Microarrays (TEMA): high-throughput and high-content platform of the future?

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The potential of protein microarrays in high-throughput screening (HTS) still remains largely unfulfilled, essentially because of the difficulty of extracting meaningful, quantitative data from such experiments [1]. Overcoming these shortfalls, we developed enzyme microarrays [2],[3], by using low-molecular-weight fluorescent affinity labels (FALs) that function as activity probes of the microarrayed enzymes. Because FALs form covalent bonds with enzymes in an activity-dependent manner they can be used to characterize enzyme activity at each enzyme's address, as predetermined by the microarraying process. Relying on this principle, we describe herein thematic enzyme microarrays (TEMA), Figure 1, and their validation in the case of cathepsins and caspases. In a kinetic setup we used TEMAs to determine the full set of kinetic constants and the reaction mechanism between the microarrayed enzymes (the theme of the microarray) and a family-wide FAL. Based on this kinetic understanding, in an HTS setup we established the practical and theoretical methodology for quantitative, multiplexed determination of the inhibition profile of compounds from a chemical library against each microarrayed enzyme. Finally, in a validation setup, Kiapp values and inhibitor profiles were confirmed and refined.

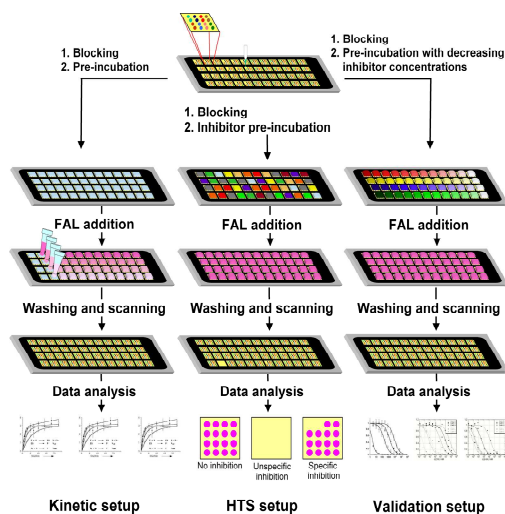


Figure 1. Explanation of TEMA technology. Members of an enzyme family are microarrayed within identical subarrays on a functionalized glass slide. **(a)** In the kinetic setup, four different concentrations of FAL (one row of subarrays for each concentration) are reacted for 12 **different** reaction times with each subarray. Analysis of the data from this setup confirms the enzymatic nature of the reaction between the microarray enzymes and the FAL and the kinetic constants that characterize the activity of each enzyme are extracted. This kinetic characterization is used in defining a set of experimental conditions under which one can run a meaningful HTS experiment. **(b)** In the HTS setup, each of the subarrays is preincubated with a potential inhibitor (colored) or a blank (light blue) and treated with FAL at a concentration and for a reaction time defined by the kinetic constants derived in **a**. Data analysis provides each inhibitor's activity profile against all enzymes of the subarray, under exactly the same experimental conditions. **(c)** In the validation setup, the subarrays within one row of subarrays are preincubated with different concentrations of an inhibitor (including a blank) and subsequently treated with FAL in a manner similar to **b**. Analysis of the data provides an inhibitor titration curve, from which refined K_i^{app} values can be calculated.

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- [2] Eppinger, J., Funeriu, D.P., Miyake, M., Denizot, L. & Miyake, J. *Angew. Chem. Int. Ed.* 43, 3806–3810 (2004).
- [3] Funeriu, D.P., Eppinger, J., Denizot, L., Miyake, M., & Miyake, J. *Nature Biotechnology*, 23, 622–627, (2005).