Development of a tRNA-Synthetase Microarray for Protein Analysis

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Proteins are composed of 20 different amino acids. In the translation process, each of these 20 amino acids is specifically recognized by their cognate aminoacyl-tRNA synthetase. The fidelity of this recognition system is essential if translation is to function properly. The development of an in vitro system based on this recognition scheme would make a powerful analytical tool with which to analyse translation, as well as providing an additional biomimetic scheme for protein analysis. Aminoacyl-tRNA synthetases microarrays could be applied to protein fingerprinting and sequence analysis. The fabrication of aminoacyl-tRNA synthetase arrays requires the use of advanced protein arraying technology that has only recently become available. In order to demonstrate the feasibility of this scheme, glutamyl-tRNA synthetase (GluRS) was immobilized on the streptavidin-based XNA on Gold™ biochip platform. The streptavidin layer provides a simple, efficient immobilization scheme that reduces nonspecific binding and improves the biocompatibility of the surface. Here, we demonstrate that biotinylated GluRS can be successfully immobilized on XNA on Gold™. The immobilization efficiency was determined by double labelling GluRS with biotin and the fluorescent label Cy5. The CCD fluorescent microscopy images revealed that the GluRS was efficiently immobilized and evenly distributed over the surface. Control experiments indicate a very low degree of nonspecific binding which is essential if detection of these multicomponent, low-affinity interactions is to be realized. Furthermore, we show that immobilization does not significantly reduce the function of the enzyme. In addition to the specific aims of this study, this technology would provide valuable insights into the biomechanics of translation as well as being a tool for studying tRNA modifications and subclasses. Moreover, the implications for developing coupled transcription and translation systems should not be overlooked. Protein analysis schemes based on this approach would provide an urgently needed compliment to traditional methods. Finally, these arrays might also be useful tools in our efforts to understand the regulatory functions that small RNAs, i.e., iRNA, have been shown to play.

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(The color pictures can be obtained from the author.)