On-Chip Biosynthesis

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On-Chip Protein Biosynthesis

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Microarray technology has evolved over the past almost 25 years to become a well-established approach for the fast and inexpensive analysis of multiple molecular interactions. While DNA microarrays are routinely used for transcription analysis, genotyping, and other applications in biomedical research,[1,2] protein microarrays are significantly less well developed because of the intrinsic complexity and instability of these biomacromolecules. DNA and many other nucleic acids can easily be prepared on a large scale by solid-phase syntheses and owing to their extraordinary high physicochemical stability these biopolymers can be tethered to solid supports without compromising their molecular-recognition properties. In contrast, proteins are delicate three-dimensional architectures whose function and interaction potential is strongly dependent on their correct native folding. Physicochemical manipulations, such as surface immobilization, can often lead to severe disturbance of protein function. Hence, special techniques are required for making protein microarrays, which include the development of mild chemical procedures for the directional immobilization of recombinant protein probes.[3,4] While these approaches usually employ purified proteins produced by heterologous expression in cellular host systems, a few methods have been delineated to generate protein biochips in situ by taking advantage of cell-free protein expression,[5] and recent work by Corn et al. emphasizes that this format holds great potential for on-line biosensing applications.[6] Cell-free protein expression is based on in vitro transcription and translation (IVTT) of a DNA template by means of extracts (lysates) from *Escherichia coli*, rabbit reticulocyte, wheat germ, or other cells, containing all necessary components for protein biosynthesis.[7]

IVTT is well established, reagents are commercially available, and it can offer advantages over cellular expression, for instance, to produce toxic proteins or introduce artificial amino acids. Moreover, the open and flexible nature of IVTT systems permits the use of readily available products of the polymerase chain reaction (PCR) as templates to generate small amounts of many different members of a protein library.[8] This approach can be downscaled by using nanowell plates with an individual cavity size of about 100 nL to facilitate the expression and characterization of large protein libraries.[9] To simplify the separation of proteins from IVTT components for subsequent characterization and binding studies, spontaneous immobilization of the newly synthesized proteins can be achieved when the nanowell surface or even planar substrates contain a specific capture reagent, such as a metal chelate which selectively binds an oligohistidine tag epitope genetically fused to the proteins of interest. This approach has been realized in the so-called PISA (protein in situ array) technique,[10] and it was further refined by multiple spotting of PCR templates and IVTT reagents to facilitate protein production and immobilization in individual spots.[11] With this method highly miniaturized protein arrays with up to 13000 spots per slide were generated in situ, and it was found that 35 fg of unpurified PCR product (approximately 22500 molecules) per spot were sufficient for expression of full-length GFP (green fluorescent protein).

Cell-free protein expression directly on a surface was already described in 2004 by LaBaer and co-workers.[12] Their "nucleic acid programmable protein array" (NAPPA) technique took advantage of a chemically reactive glass surface onto which was spotted a mixture of a DNA plasmid and a capture antibody directed against glutathion S-transferase (GST), a popular tag of many fusion proteins (Figure 1 A). Several different target plasmid DNAs encoding C-terminal GST fusion proteins were arrayed by microdeposition and the entire slide was incubated with rabbit reticulocyte lysate to generate the proteins, which were immediately captured inside the spots by the immobilized anti-GST antibodies. Immunostaining with target-specific antibodies verified that all proteins were properly expressed and no cross-talk occurred between individual spots of the slide. The method was then used to express human DNA replication proteins and to map their interaction with other proteins.[12]

A modification of the NAPPA technique was reported by Tao and Zhu, who fabricated protein chips in situ by capturing nascent polypeptides during ribosomal translation of RNA molecules (Figure 1 B).[13] To this end, surface-immobilized capture DNA oligonucleotides, complementary to the 3′-end of a messenger RNA (mRNA) molecule, were modified with the peptide antibiotic puromycin. During translation, the ribosome stalls on the captured mRNA when it encounters the double-stranded RNA–DNA region and this provides sufficient time for the puromycin to enter the ribosome's A site and to allow transfer of the newly synthesized peptide onto the puromycin-conjugated oligonucleotide. With this

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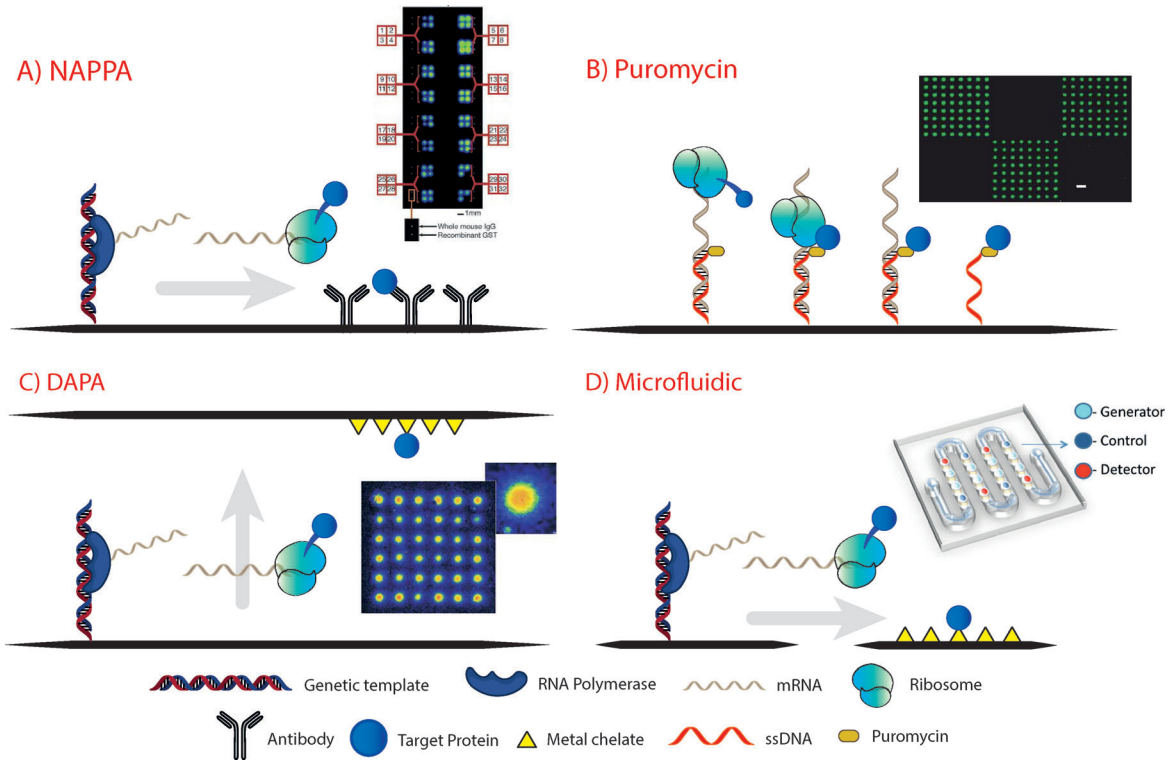

Figure 1. Different formats for on-chip protein synthesis. A)–D) show representative protein chips and their general principle of operation. A) Nucleic acid programmable protein array (NAPPA).[12] B) Puromycin-directed capture of proteins on a surface.[13] C) DNA array to protein array (DAPA).[14] D) Successive microfluidic generation and immobilization of proteins on separated spots in a microfluidic reactor. The control spots are blanks not containing metal chelate for the binding of newly generated proteins.[6]

method, approximately 0.8 fmol of protein could be synthesized per spot. This is comparable with the protein concentration on microarrays fabricated by the spotting of purified proteins.[13]

While NAPPA and puromycin capture are based on the co-immobilization of transcription template and a suitable protein-capture reagent within the same spot, transcription/translation and protein immobilization can also be achieved in different locations of a microstructure. This principle is realized in the DAPA (DNA array to protein array) technique developed by Taussig and co-workers.[14] A slide containing an array of spotted DNA template is assembled face-to-face with a second slide containing the protein capture reagent, for example the metal chelate nickel nitrilotriacetate (Ni-NTA). In a sandwichlike arrangement, the two slides are separated by a porous membrane soaked with IVTT reagent mix. Proteins synthesized on the spots of immobilized DNA template diffuse through the membrane and become immobilized on the surface of the capture slide (Figure 1C). Using hexahistidine-tagged green fluorescent protein and myc-tagged E2F6 model proteins, it was shown that the template slides could be translated repeatedly almost 20 times.[14]

Micro-compartmentalization of DNA template containing generator spots and detector spots bearing the capture reagent was also used in a recent publication by Corn et al.[6] The spots were connected with a microfluidic channel to provide IVTT reagents and facilitate transport of proteins from generator to detector spots (Figure 1D). Strikingly, the fluidically coupled spots were mounted on a biosensor surface enabling label-free, real-time observation of binding events by means of surface plasmon resonance imaging (SPRI). Although only two model proteins (GFP, Luciferase) were used in this initial demonstration, the results clearly indicated that IVTT processes on the generator spots as well as the binding of antibodies at newly synthesized proteins on the detector spots can be followed on-line in real time. Since the dual-element format reduces nonspecific adsorption processes, the authors suggest that this on-chip protein microarray fabrication method combined with SPRI read-out can be applied for multiplexed biosensing in both clinical and research settings.[6]

While the work described above illustrates innovative approaches for on-chip protein synthesis and implementation of sophisticated read-out, it seems particularly noteworthy that enormous power results from the combination of IVTT with high-end microfluidic devices. One extraordinary impressive example was reported by Quake et al. in 2007.[15] They developed an integrated microfluidic chip device for the mechanically induced trapping of molecular interactions (MITOMI) to analyze the low-affinity binding events between transcription factors (TFs) and DNA sequences. Their chip consisted of 2400 independent unit cells in which spotted DNA template was used for the in situ generation of four different TFs by IVTT. The soluble TFs were then microfluidically combined with fluorescently labeled target DNA fragments and the binding equilibria of free DNA and weakly

bound DNA–TF complexes were mechanically trapped by a pressure-activated plug inside the reaction chamber. This approach eliminated the off-rate problem of conventional surface array platforms and enabled a detailed description of the DNA binding energy landscapes for the eukaryotic TFs to experimentally test basic assumptions about TF binding and to predict their in vivo function.[15]

In conclusion, the summarized approaches clearly indicate that the implementation of cell-free protein expression in bioanalytical settings and lab-on-a-chip devices leads to novel opportunities. The microfluidic approach not only facilitates the tight control of reaction parameters like pressure and temperature for more reliable experiments but also offers high multiplexing capabilities with low requirements for reagent consumption and processing time. Therefore, microfluidic IVTT systems have a tremendous potential for applications in fundamental research as well as in economically relevant areas, such as drug development and more generally the assessment of the impact of substances on biological systems.

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[1] M. Schena, *DNA Microarrays* (*Methods Express*), Cold Spring Harbor Laboratory Press, New York, **2007**.
[2] P. Baldi, G. W. Hatfield, *DNA Microarrays and Gene Expression*: *From Experiments to Data Analysis and Modeling*, Cambridge University Press, Cambridge, **2011**.
[3] P. Jonkheijm, D. Weinrich, H. Schroeder, C. M. Niemeyer, H. Waldmann, *Angew. Chem.* **2008**, *120*, 9762; *Angew. Chem. Int. Ed.* **2008**, *47*, 9618.
[4] D. Weinrich, P. Jonkheijm, C. M. Niemeyer, H. Waldmann, *Angew. Chem.* **2009**, *121*, 7880; *Angew. Chem. Int. Ed.* **2009**, *48*, 7744.
[5] M. He, O. Stoevesandt, M. J. Taussig, *Curr. Opin. Biotechnol.* **2008**, *19*, 4.
[6] T. H. Seefeld, A. R. Halpern, R. M. Corn, *J. Am. Chem. Soc.* **2012**, *134*, 12358.
[7] Y. Shimizu, T. Kanamori, T. Ueda, *Methods* **2005**, *36*, 299.
[8] A. S. Spirin, *Trends Biotechnol.* **2004**, *22*, 538.
[9] P. Angenendt, L. Nyarsik, W. Szaflarski, J. Glokler, K. H. Nierhaus, H. Lehrach, D. J. Cahill, A. Lueking, *Anal. Chem.* **2004**, *76*, 1844.
[10] M. He, M. J. Taussig, *Nucleic Acids Res.* **2001**, *29*, e73.
[11] P. Angenendt, J. Kreutzberger, J. Glokler, J. D. Hoheisel, *Mol. Cell. Proteomics* **2006**, *5*, 1658.
[12] N. Ramachandran, E. Hainsworth, B. Bhullar, S. Eisenstein, B. Rosen, A. Y. Lau, J. C. Walter, J. LaBaer, *Science* **2004**, *305*, 86.
[13] S. C. Tao, H. Zhu, *Nat. Biotechnol.* **2006**, *24*, 1253.
[14] M. He, O. Stoevesandt, E. A. Palmer, F. Khan, O. Ericsson, M. J. Taussig, *Nat. Methods* **2008**, *5*, 175.
[15] S. J. Maerkl, S. R. Quake, *Science* **2007**, *315*, 233.

