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# Optimised 'on demand' protein arraying from DNA by cell free expression with the 'DNA to Protein Array' (DAPA) technology☆

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ABSTRACT

We have previously described a protein arraying process based on cell free expression from DNA template arrays (DNA Array to Protein Array, DAPA). Here, we have investigated the influence of different array support coatings (Ni-NTA, Epoxy, 3D-Epoxy and Polyethylene glycol methacrylate (PEGMA)). Their optimal combination yields an increased amount of detected protein and an optimised spot morphology on the resulting protein array compared to the previously published protocol. The specificity of protein capture was improved using a tag-specific capture antibody on a protein repellent surface coating. The conditions for protein expression were optimised to yield the maximum amount of protein or the best detection results using specific monoclonal antibodies or a scaffold binder against the expressed targets. The optimised DAPA system was able to increase by threefold the expression of a representative model protein while conserving recognition by a specific antibody. The amount of expressed protein in DAPA was comparable to those of classically spotted protein arrays. Reaction conditions can be tailored to suit the application of interest.

Biological significance
DAPA represents a cost effective, easy and convenient way of producing protein arrays on demand. The reported work is expected to facilitate the application of DAPA for personalized medicine and screening purposes.
This article is part of a Special Issue entitled: New Horizons and Applications for Proteomics [EuPA 2012].



## 1. Introduction

Protein and antibody arrays provide versatile methods of screening for biomarkers, protein–protein interactions, and protein-small molecule interactions, and for evaluation of protein-binding reagents employed in research and in the clinic, reaching single molecule detection sensitivity [1–6]. Nevertheless, there are several technical issues to overcome before protein arrays can fulfil all requirements for routine use. A central problem is the availability of purified,

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functional proteins for immobilisation on the arrays. Another is the tendency of immobilised proteins to deteriorate while stored over long periods of time, limiting reproducibility of different experimental runs.

Classically, protein arrays have been made by large scale *in vivo* protein expression, followed by purification and immobilisation on an array support material—a complicated, expensive, and time-consuming process, which many laboratories are unable to carry out themselves or afford. It is also restricted by protein insolubility in, or toxicity for, the expression host cell, rendering certain proteins unavailable by cell based expression. To solve these issues, in recent years, methods for *in situ* generation of proteins by cell-free transcription and translation have been presented, based on *Escherichia coli*, rabbit reticulocyte, wheat germ and mammalian cell lysates [7–9]. A general challenge for *E. coli-based* cell free expression systems is the incorporation of post-translational modifications, such as glycosylation. This issue can be overcome by using insect based or mammalian lysate systems. Membrane proteins have been a difficult area for cell free expression, but by addition of detergents which do not interfere with the lysate function, it has been shown that proteins such as GPCRs can be expressed in cell free systems [15].

The DAPA ('DNA Array to Protein Array') method is a technique in which protein arrays are 'printed' directly and quickly from DNA array template slides by means of cell free protein expression [10–13]. The expressed protein is deposited on a protein capture slide via diffusion through a narrow gap between the DNA and protein capture surfaces [13], yielding regular and reproducible protein spots [10,12]. Compared to conventional arraying of pre-expressed proteins, the DAPA process is available 'on demand', as and when required, by simple introduction of the expression lysate between the preformed DNA array and the protein capture surface, with protein expression achieved in a few hours without further purification steps. This reduces production time significantly, maintains reproducible quality, renders storage of previously produced slides unnecessary and results in significant cost saving. Moreover, as a DNA technology, DAPA protein arrays can link next generation sequencing to functional proteomics, by displaying as arrays the proteomes corresponding to the data output of DNA sequencing of individuals, tissues or single cells. The DNA template array is also re-useable, allowing several successive copies of the corresponding protein array to be created [10] in a convenient and economical way.

The optimal array support coating is crucial for any array based application. The surface modifications compared here for DAPA (both the DNA and protein capture surfaces) were Ni-NTA, Epoxy, 3D-Epoxy and PEGMA. Ni-NTA was investigated because it was previously used and reported [10–13]. The others were chosen for their versatility in different assay formats and detection methods. Epoxy slides are the most common 2D surfaces used for protein arrays. The 3D PEGMA (100–150 nm) and to a lower extent also 3D-Epoxy (20 nm) surfaces were chosen because of their properties of protein repellence, high density of functional groups and chemical inertness [3,16]. These characteristics allow a higher loading with proteins and require less extensive washing and surface blocking steps, thereby simplifying and shortening handling compared to conventional 2D-Epoxy surfaces. Nitrocellulose and 3D-Hydrogels were not investigated because they are incompatible with through-the-slide scanners and total internal reflection detection devices. Another common surface, aminosilane, was also omitted because of its similar behaviour to epoxy (data not shown).

Here we present results of DAPA optimisation, comparing array support coatings and different cell-free expression systems to optimise expression conditions, and taking these conditions forward to screen a small set of antibodies. Defining optimal conditions and array support materials greatly enhances the performance of the DAPA technology and can enable new applications.

## 2. Materials and methods

### 2.1. Chemicals

All chemicals, unless otherwise stated, were from Sigma-Aldrich (Gillingham, United Kingdom) in the highest purity available and were used as received.

### 2.2. Array slides

Slides used were 3D-Epoxy (PolyAn, Berlin, Germany), Polyethylene glycol methacrylate (PEGMA) (Pepperprint, Heidelberg, Germany), Nexterion Slide E (Schott, Germany) and nickel-nitrilotriacetic acid (Ni-NTA) (Xenopore, Boulder, US).

### 2.3. DNA constructs

DNA constructs for DAPA cell-free expression were generated by assembly PCR as previously reported [10–13]. They included an N-terminal triple c-myc tag for generic detection by anti-myc antibodies and a C-terminal human κ light chain constant domain [14] (Cκ) for immobilisation by anti-κ antibody.

### 2.4. Spotting of DNA arrays

Purified PCR products in spotting buffer (50 mM sodium phosphate, pH 8.5) were arrayed on 3D-Epoxy microarray glass slides, PEGMA slides or Slide E using a GeSiM NP2.1 non-contact nanoplotter. DNA arrays were dried at 30 °C for 30 min and stored at 4 °C until used. Prior to use, the DNA slides were washed with PBS containing 0.05% Tween-20, 3% BSA and 1% ethanolamine.

### 2.5. Protein capture slides

Protein capture slides for DAPA were prepared by coating with 33 μg/mL of anti-κ antibody (Rockland Inc., Gilbertsville, PA, USA) in 0.2 M $NaHCO_3$ buffer (pH 8.2) for 30 min, washed with water and spun dry.

### 2.6. Cell free expression kits

Two different kits were used, RTS 100 *E. coli* HY protein synthesis kit (RTS) or EasyXpress protein synthesis kit (both from RiNA GmbH, Berlin, Germany).