Miniaturization for drug discovery applications

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When combinatorial chemistry emerged in the early nineties, miniaturization was a prerequisite for many breakthrough approaches. A wave of scale-up development ensued, driven by the need to interface this technology with classical medicinal chemistry. A renaissance of the microscopic perspective is now taking place, motivated by the implementation of increased throughput screening and the application of concepts and hardware from the diagnostics and genomics sectors. The author describes the different approaches of wet-chip technology with reference to the generation of molecular diversity.

Small is beautiful, and the marriage of biotechnology and electronics on the submilimeter scale is fascinating[1–3]. Research and development in solid-state physics have created highly miniaturized and complex architectures of dry matter capable of storing and processing digital information. Now it appears that these technologies, invented decades ago, have found another application by providing the framework for the circulation of fluids rather than currents. Typical semiconductor chips are massive and dry, whereas chips for biochemical processes contain cavities or channels with wettable surfaces to be contacted and filled by liquids. In the same way that computer chips are predesigned for a certain task by the layout written in the three dimensional multilayer structure, wet chips are pre-equipped with biochemical agents bound to channel walls or held in containments. This definition discriminates wet chips from miniaturized sample holders, such as the so-called micro- or nanotitre plates, which simply present physical boundaries to separate liquids and provide a means for their manipulation. Capillaries, columns or plates can be transformed into wet chips by purposeful arrangement of biochemical agents within the sample carrier. The layout of reagents may have various forms. It can, for instance, be gridwise as in high-density arrays, or be tortuous as in capillary tubes. Adapting laboratory procedures to the wet-chip format requires standardization and automation facilitating enhanced reproducibility and advanced process control. The wet-chip format prohibits manual interference and so purposefully reaches beyond ergonomics. Highly optimized and time-critical routines can reign only in a fully automated environment where effort and responsibility are shifted away from the end user.

Shrinking laboratory

The advantages of a shrinking laboratory appear obvious – one can expect tremendous savings on reagents – but miniaturization does not necessarily shorten the time taken for individual processes. For example, in micro-HPLC the use of capillary columns and micropumps drastically reduces solvent consumption, but a standard gradient run will still take 30–45 min. A substantial time reduction can only be achieved if processes are carried out in parallel. The true promise of miniaturization lies hidden in the power of massive parallelization.

Parallelization is inherent to the questions raised in drug discovery research, where comprehensive structure–activity relationships (SARs) are at the focus of interest. Ideally, an SAR table would be generated in a single fully automated run, starting from one target cell or protein preparation and encompassing a vast number of potential drug candidates in

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a parallel fashion. Wet chips promise to develop such synthesis and screening efforts into an art of biochemical information processing.

Whoever addresses the issues of chip development and production will have to make allowances for predesigned sample collections or at least the facilitated import of large numbers of compounds in a preformatted fashion. It is the number and the diversity of biological or chemical reagents that determines the information content of wet chips. The main focus of this article is on the generation of molecular diversity in miniaturized formats.

Learning from diagnostics

A decade ago, miniaturization in the life science arena was driven by research in diagnostics and bioanalytical chemistry. There have been several technological advances. Test strips and dip sticks for diagnostics were and still are routinely being manufactured using submicrolitre spotting devices. Capillary electrophoresis has evolved as a powerful tool for rapid low volume separations and now plays a dominant role in the analysis of trace samples. More recently, advances in biosensors and electronics have helped to position miniaturized blood or DNA processing systems at the forefront of integrated chip development[4]. The discussion of such diagnostic devices is beyond the scope of this article, but it serves as a starting point for the illustration and classification of different chip approaches.

The terminology used above defines wet chips as preequipped with biological or chemical information content, such as oligonucleotide probes or biosensor coatings. These well-defined molecular chip-based structures can be termed 'reagents'. A wet chip is 'fabricated' by reagents being deposited or synthesized on the supporting structure of the carrier. The chip may contain several different reagents. 'Samples' are added from an external source to the prearranged layout of reagents. The number of samples processed on a single chip is equal to or smaller than the number of given reagents. 'Activation' of a wet chip occurs at the moment of contact between a sample and a reagent, and the chip may be activated several times during assembly and even during the read-out stage. If repetitive activation of the chip is desired, assembly and read-out can occur at the same time, even using the same sample manipulation techniques and biochemical protocols. Activation at the level of chip assembly introduces, for instance, a chemical agent sample to interact with a prearranged layout of reaction sites. Multiple addition of various chemical agents can generate the completed reagent array to perform the diagnostic or pharmacological test it was designed for. At the final read-out stage, the sample will be, perhaps, a few drops of blood, and the reagents, a panel of electrodes – such as in the i-STAT (Princeton, NJ, USA) system[5]. These 25 mm × 44 mm disposable devices are probably among the most highly developed miniature diagnostic chips available; they can measure up to a dozen different characteristic blood indices, including pH, CO_2 concentration, glucose and Ca^{2+} levels. For each value to be determined, a solid-state potentiometric or amperometric sensor is integrated into a linear array along a capillary transporting the blood. The interaction of blood with the reagent coating on each electrode is read out automatically and determines the test result.

Wet-chip devices

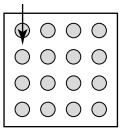
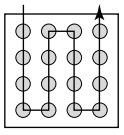
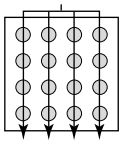
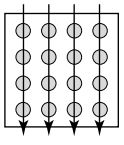
A classification of wet-chip devices according to the different sampling processes is suggested below. The complexity of a wet-chip design relates to the variety of sample and reagent combinations it provides for. The classification applies to both the read-out and the assembly stages and is rank ordered according to complexity. For an overview see Table 1.

- Arbitrary chips: one sample, one activation step using one reagent.
- Linear chips: one sample, a sequence of activations employing individual reagents for each step.
- Branched chips: one sample, one activation step with a multitude of arrayed reagents.
- Combinatorial chips: a multitude of samples, performing parallel activations on a multitude of reaction partners.

In the case of chips to be 'interrogated' in a branched design, such as cDNA collections on glass slides, the source of reagent diversity may be external and imported through a parallelized linear process. PCR products layed out in 96-well microtiter plates need to be aliquoted and applied to the chip's array. Automation to facilitate this process has been developed at Brown's laboratory (Stanford University, CA, USA) and commercialized through Synteni (Palo Alto, CA, USA)[6,7]. A contact printing method with capillary-action rubber stamps transfers submicroliter amounts of cDNA from microplate source racks to flat glass slides. DNA molecules bind by ionic interaction with the polylysine coating of the glass slides.

In a slightly more macroscopic approach, Lehrach's group (Max Planck Institute of Molecular Genetics, Berlin, Germany) developed robots for rapid generation of high-density filters

Table 1. Different wet chip designs

Wet chip design	Combinations of reagents and samples	Examples
Arbitrary 	Of a variety of reagents on a wet chip, any individual reagent can be addressed by the sample	Nanogen
Linear 	Of a variety of reagents on a wet chip, the sample will address all reagents in a sequential manner	i-STAT Biacore Caliper Soane Nanogen
Branched 	Of a variety of reagents on a wet chip, the sample will address all the reagents at the same time	Affymax Affymetrix Synteni Pharmacopeia
Combinatorial 	Of a variety of reagents on a wet chip, several different samples address given subsets of the reagent collection	Orchid

with thousands of cDNA spots[8]. Here, small pins are dipped into microplates and the adhering liquid is spotted onto filter paper. These automated systems are commercialized through Genetix (Christchurch, UK).

Import of DNA samples and subsequent transport to a specific location within the wet chip's architecture is achieved by the Nanogen (San Diego, CA, USA) approach[9]. Charged molecules within a homogeneous sample are attracted to spots laid out in an array. As each spot constititutes the surface of an electrode, its net charge can be altered in a freely programmable fashion, allowing for automated sample attraction, concentration and hybridization. This approach is highly flexible as it allows switching between arbitrary, linear or branched designs.

If collections of reagents are to be synthesized directly on the chip, the synthetic process can either be 'branched' or 'combinatorial'. An example of a branched process is provided by a photolithographic assembly of oligomeric DNA probes (Affymetrix, Santa Clara, CA, USA). Samples are introduced individually through a flow chamber to react with the synthesis sites on the glass support[10]. Branching occurs through spatially resolved photolytic deprotection. A combinatorial design is being used by Southern's group (University of Oxford, UK), with an arrangement of linear channels bringing several samples in contact with the glass slide's reagent array[11].

Wet chips for drug discovery

The same classification as outlined for genomic devices may be used to review miniaturization efforts in a preclinical pharmaceutical context. Here, the application is high-throughput screening (HTS), which is inherently a branched process: one sample is used in one reaction step on a multitude of reaction partners. The sample is usually the target cell or protein, the reaction is the established binding or competition assay and the multitude of reaction partners constitutes the chemical diversity to be screened.

Wet chips with arbitrary synthesis design

A high-density oligopeptide synthesis approach was developed by Frank (Gesellschaft für Biotechnologische Forschung, Braunschweig, Germany) and instrumentation commercialized by Abimed (Langenfeld, Germany)[12]. A single needle on a rapid xyz-arm aspirates amino acid reagents and dispenses submicrolitre aliquots by contacting cellulose filter paper. After termination of synthesis, the filter paper, containing the deprotected peptide libraries, is brought in contact with, for instance, an antibody and antibody-detection reagents. Read-out of the precipitative colour formation can be performed by the naked eye or densitometry. Filter spots can also be excised and distributed to assay wells with orthogonal linker chemistry allowing for subsequent direct release of products into the assay environment[13].

Sequential oligopeptide synthesis in silicon wafers has been explored in the context of evolutionary biotechnology (Institute of Physical High Technology, Jena, Germany). Miniaturized wells fabricated from photolithographically structured, wet-etched-silicon, single-crystal wafers are manually filled with one bead per well. Each well provides a volume of 125 nl, typically permitting a 100-fold excess of liquid reagent. Waste is removed by vacuum filtration through a microsieve well bottom. Non-contact, piezoelectric liquid-jet technology allows for accurate dispensing of submicrolitre volumes. A

motorized x–y table affords spatial resolution by controlling the wafer coordinates, and computer control automatically synchronizes wafer movement and drop-on-demand dispenser action. With this set-up, the solid-phase assembly on individual synthesis beads has been achieved for the first time. Oligopeptide products were cleaved photolytically and subjected to a homogeneous functional bioassay based on chemifluorescence and laser light scanning detection.

Wet chips with linear synthesis and screening design

Biacore's (Uppsala, Sweden) biosensor apparatus provides an example of miniaturized screening instrumentation in a linear sequential outline. The device uses optical detection of refractive-index changes at the interface between aqueous sample and reagents immobilized on the chip surface. Automated sample injection from 96-well plates and integrated microfluidics direct compounds through 0.5 mm × 2 mm flow chambers to interact competitively with a biomacromolecular system such as an antibody–target-protein complex[14]. The system can include up to four flow cells that are addressed individually or in line[15]. Therefore a linear sequential arrangement can be envisioned where four different ELISA-type assays are set up on one chip and samples from the microplate-based compound collection are sequentially injected.

Other linear arrangements are being investigated by the Manz group (Imperial College, London, UK) and Harrison's group (University of Alberta, Canada)[16]. Commercialization of their capillary electrophoresis-derived systems is taking place at Caliper (Palo Alto, CA, USA)[17], and a similar approach is being pursued at Soane BioSciences (Hayward, CA, USA)[18]. The investigators use micromachined and bonded glass slides to hold layouts of 100 μm channels. A chip is typically fitted with three to five inlets and one or two outlets on a 20 mm × 55 mm layout. Samples are loaded manually by conventional pipetting. A variety of immunoassay systems and single-cell manipulation assays has been demonstrated[19]. It is likely that sample import will be facilitated by automated liquid delivery in the near future, enabling an HTS adaptation similar to that outlined for the Biacore system. However, in contrast to the refractive-index sensor, assays based on capillary electrophoresis are not limited to the solid-phase ELISA scheme; the approach permits fully homogeneous and functional detection of bioactivity in solution by integrating binding and separation features in one layout. Future developments may involve even more complex sequential arrangements, such as synthesis linked to on-chip purification.

A miniaturized HPLC system was prototyped by researchers at Purdue University (West Lafayette, IN, USA) in collaboration with PerSeptive Biosystems (Framingham, MA, USA). It will be interesting to see how stacking and integration of these devices can result in increased throughput systems.

Wet chips with branched screening design

If external sources of compound diversity are to be used on a screening chip, the collections must be reformatted to the chip's footprint using a liquid handling equivalent of 'pick-and-place' technology to provide the necessary macro-to-micro conversion. In conventional HTS, compounds that are to be subjected to a particular bioassay are obtained from compound collections laid out in the robot-compatible 96-well microplate format. To obtain 96-well formatted libraries, a substantial effort goes into the transfer of compounds from storage flasks and containers to the wells of a mother microplate. Such mother microplates are then used to generate daughter plates of various dilutions.

The process of loading 1536-, 3456- or 9600-well plates with a chemical library involves aspiration of 10–100 microlitre volumes from 96-well mother plates and subsequent parallel dispensing of submicrolitre aliquots to the recipient chip plates. In this way the contents of one 96-well plate are mapped to a multitude (several hundred) of miniaturized sample holder plates by sequential transport. The process is continued by aspiration of contents from the next mother plate and repeating the mapping process, but dispensing to a different set of 96 wells on the same multitude of chip plates. Companies that offer fluorescence-based ultra-HTS systems, such as Aurora (San Diego, CA, USA), and those involved in encoded combinatorial synthesis, such as Pharmacopeia (Princeton, NJ, USA), are currently setting up mapping stations for this purpose[20] (see www.aurorabio.com).

Instead of distributing chemical collections in solution, there are cases where the manipulation of small polystyrene beads is of interest. Very often, bead technology is used to generate chemical diversity. Mix-and-split synthesis is currently used to access synthetic libraries of millions of compounds, but it cannot be adapted to the chip format. For plates or chips to hold millions of beads in a two-dimensional arrangement they would require dimensions measurable in terms of square metres. Hence, the 100 μm-sized heterogeneous microcarriers must be delivered from a macroscopic sample, diversified by split-synthesis protocols, to either a micro- or a nanoplate layout. As the beads carry a chemical code to allow for prediction of the associated

compound's structure, the particles must remain physically associated with their products, even if the products are released into the assay medium. Schullek and colleagues at Affymax (Palo Alto, CA, USA) used 2025-well plates filled with a slurry of agarose, 1000 statistically distributed encoded beads and assay reagents[21]. A photolabile linker allowed for biocompatible direct release of synthesis products from single beads into the surrounding 0.37 μl well[22]. The preloaded chip was readily screened for leads by homogeneous incubation with a chemifluorescent substrate, xenon-fibre illumination and CCD imaging.

Wet chips with branched synthesis design

A branched synthesis process was used at Affymax and in Schultz' group (University of California, Berkeley, CA, USA) for the fabrication of peptide and carbamate libraries by spatially resolved photolytic deprotection[23]. The branched design was effective for the production of long oligomeric structures from only a few building blocks, but it was not well suited for the synthesis of small non-oligomeric compounds – the flow chamber delivering the reaction partners for the solid-phase assembly holds only a single compound at a time.

Wet chips with combinatorial synthesis design

For truly combinatorial syntheses, a multitude of reagents for a given reaction step must interact with a multitude of reaction partners in a parallel fashion. Technology suitable for this task is under development at Sarnoff (Princeton, NJ, USA) and being commercialized by Orchid Biocomputing (Princeton, NJ, USA)[24–26]. The approach is based on the electrokinetic control of the properties of liquids to effect valveless aliquoting and mixing, and pumpless transport. In a recent paper Harrison and coworkers described how two organic reagents are directed via electro-osmotic pumping to yield a coloured product that would be detected by absorption[27]. This technology is a prerequisite for the generation of chemical diversity inside miniature channel structures.

In the approach taken by Sarnoff/Orchid a much more complex liquid-management system is employed. The device consists of two parts – a liquid-delivery top plate and a liquid-recipient bottom plate. The bottom plate carries a 12 × 12 well array and can be integrated to hold 6 × 144 wells on a standard microplate footprint. Wells are equipped with a drain for waste removal and filled with beads as the synthesis support. The top plate is an elaborate multilayer structure of a three dimensional network of channels, reservoirs and electric contacts. Organic liquids and reagents are moved by means of an electrohydrodynamic pump and an electro-osmotic valve.

A similar but less compact format is under development at Glaxo Wellcome (Research Triangle Park, NC, USA)[28]. Instead of glass microstructures, moulded propylene sheets combined both solid-phase support and sample carrier functions. Liquid delivery is performed in a contact-free mode by multiple solenoid valve-driven dispensers, linearly aligned to fill rows or columns in a combinatorial fashion.

Merging diagnostics with pharmaceuticals

Miniaturization in the biology and chemistry arena is highly interdisciplinary. Synthesis and analysis will be integrated by a common technology base. As diagnostics and pharmacological screening increasingly use the same chemical and biological tools for test development, microstructure engineering will provide a basic hardware environment for those needs. The never-ending demand for high throughput will foster development of miniaturized formats with the capability to handle many (patient) samples in the same way as many (chemical) reagents are currently processed.

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