High Resolution Lithography for Nanofluidics

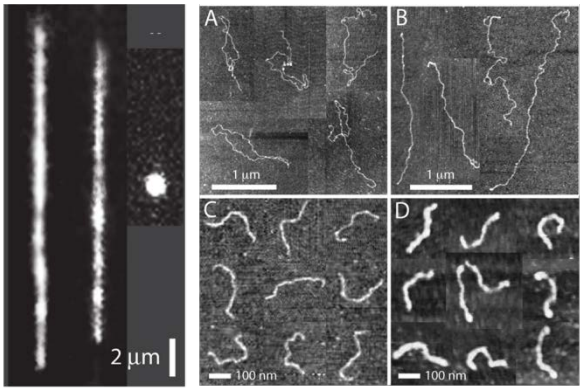

Figure 1: Condensation of DNA incubated with Hfq

Left: **Fluorescent microscopy of DNA confined in 150×250 nm² nanofluidics channels.** Hfq concentration increases left-to-right, where the right-hand image is for Hfq above the critical concentration for condensation.
Right: **Atomic Force Microscopy of long (10,000 base pairs, bp) and short (1000 bp) DNA molecules.** A+B: 10,000 bp; C+D: 1000 bp; A+C: Hfq/bp = 1/300; B+D: Hfq/bp = 1/6
Long DNA molecules show complicated folding behaviour; short DNA behave more simply as semi-flexible rods
From Figures 1 and 4 of Jiang et al, *Nucleic Acids Research* 2015

"***Hfq***" is an essential RNA "chaperone protein" identified nearly 50 years ago (in work on *Escherichia coli*) as "an ***H***ost ***f***actor for bacteriophage ***Q***β RNA replication" [E1], [E2].

The way DNA folds and unfolds is central to its function, and remains a difficult problem imperfectly understood. So-called "condensation" is an amazing part of this behaviour. As Bloomfield has explained in a valuable review (*Current Opinion in Structural Biology*, 1996 [1]):

> In dilute solution, the DNA of bacteriophage T4 has a radius of gyration of about 1000 nm, and a worm-like coil volume of 4.10^9 nm^3. When packaged inside the T4 phage head, the DNA has an outer radius of only 50 nm and a volume of 5.10^5 nm^3. Whereas phage have elaborate apparatuses for DNA packaging, a similar decrease in DNA volume to an orderly collapsed state can be produced *in vitro* simply by the addition of multivalent cations such as polyamines. It is this dramatic decrease in the volume occupied by a DNA molecule, provoked *in vitro* by chemical agents, that we define as *condensation*.

It turns out that the *environment* of the DNA is one of the determinants of its behaviour, and in particular, if the molecule is forced into a constrained space (in cell nuclei, for example) its condensation behaviour is different. This is of very significant current interest, and can be mathematically modelled as the "coil-globule transition of a single semi-flexible chain in slit-like confinement" (Dai et al, *Scientific Reports*, 2015 [2]).

But how to model it experimentally? ***Figure 1*** shows the condensation of DNA imaged with fluorescence microscopy, in a channel of a size comparable to the size of the molecule, stimulated by Hfq ("***h***ost ***f***actor for phage ***Q***β RNA replication"). Hfq is a widespread and phylogenetically

conserved protein found at high concentration in bacteria and is a bacterial pleiotropic regulator that mediates several aspects of nucleic acids metabolism: how it compacts DNA (that is, causes it to "condense") was studied recently by Jiang et al (*Nucleic Acids Research*, 2015) [3]. The Figure also shows AFM imaging of the molecules in various conditions; the study depends on small angle neutron scattering (SANS) to determine the structure of the nucleoprotein (hfq/DNS) complex.

This work is a very good example of the impact of a nanofluidics capability on cutting-edge research in structural biology. The nanofluidics devices were made in PDMS, stamped from a master made in HSQ resist by proton beam writing [4] (see van Kan et al, *NanoLetters*, 2006) [5]), and ***Figure 2*** shows a lab-on-a-chip [6] fabricated this way. The DNA condensation work was done using similar nanofluidic devices.

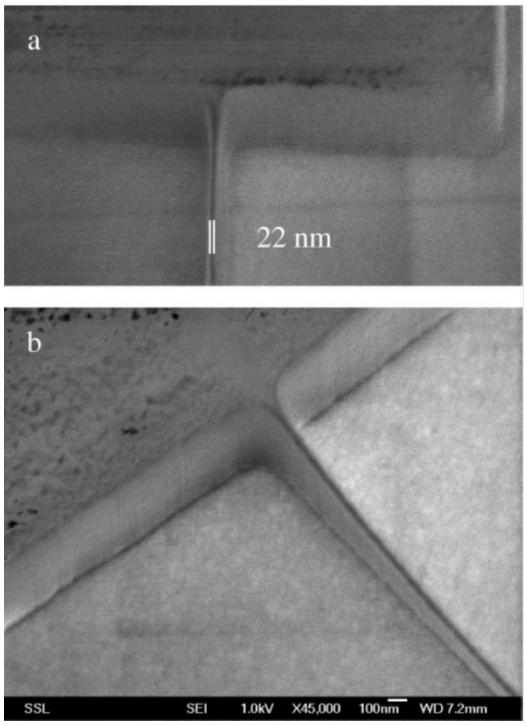

Figure 3: *22 nm line in 850 nm HSQ by Proton Beam Writing*

Two SEM views of the same structure formed by a focussed 1 MeV proton beam writing a line 3-pixels wide with a dose of 4.10^5 protons per pixel.

Figure 3 of van Kan, Bettiol & Watt et al, *Nano Letters* 2006

Proton beam writing uses a MeV proton beam, focussed deep sub-micron and scanned in a given pattern over a photoresist. ***Figure 3*** shows a structure written in a polymer by a beam with a nominal measured $\pm 2\sigma$ width of "100 nm", but using a "standard" known to overestimate beam size. For this application the damage response of the polymer probably sharpens the spatial resolution, and the focussed proton spot non-uniformity may accentuate this effect. In any case very sharply defined lines with an aspect ratio of almost 40 can be written. This performance is an order of magnitude better than is available with e-beam lithography.

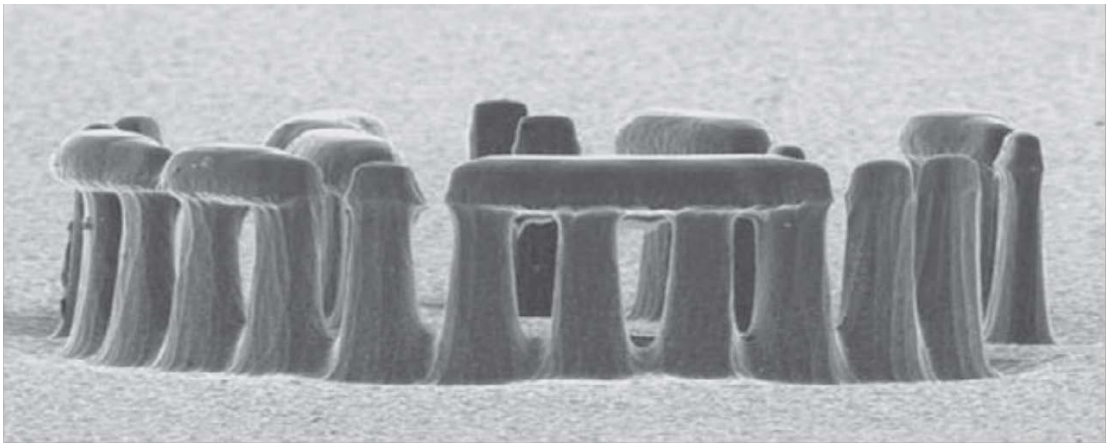

Figure 4: ***Stonehenge micromachined in silicon: 80 μm diameter***

Isotropic electrochemical etching of Si in HF creates porous silicon which is selectively etched by KOH. Proton irradiation inhibits porous silicon formation, so that 3-D structures can be formed. The horizontal "stones" are fabricated with 500 keV protons (with a range in Si of ~6 *μm*) and the vertical "stones" by 2 MeV protons (range ~48 *μm*).

Figure 11c of Watt et al, *Materials Today* 2007

Figure 4 shows a large 3-D structure written in silicon, showing how the damage density in a material can be directly written. Proton beam writing is thus also able to define 3-D structures in Si at a spatial resolution limited only by the proton spot size and which can approach 20 nm (see Figure 3). The mechanism for this process is well-understood (see Breese et al, *Physical Review B*, 2006 [7]), and has been used for MEMS (micro-electro-mechanical systems) devices (Azimi et al, *J.Micromech.Microeng.*, 2012 [8]), distributed Bragg reflectors (see ***Figure 5***, Mangaiyarkarasi et al, *NIMB* 2007 [9]), and 3-D beam splitters (Liang et al, *Optics Express*, 2015 [10]).

Figure 5: *Distributed Bragg Reflectors*

Arbitrary pattern formed from controlling the emission wavelength of silicon patterned with proton beam writing and processed as in Fig.2. White light illumination

Figure 4c of Mangaiyarkarasi et al, *NIMB* 2007

Proton beam writing has also been used to make optical waveguides and amplifiers in Nd-doped yttrium-aluminium-garnet (Nd:YAG, see ***Figure 6***, Tan et al, *Optics Express*, 2015 [11]). There are many other potential applications.

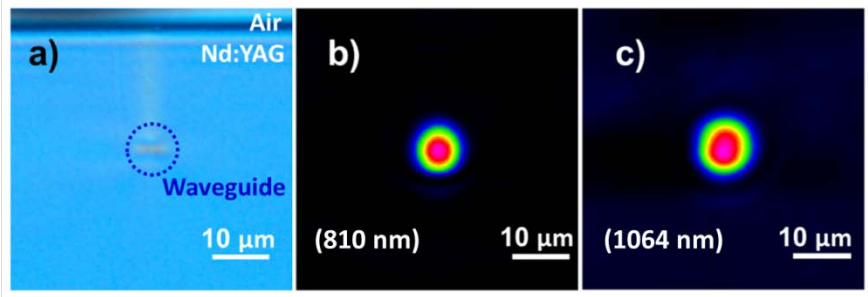

Figure 6: *Optical amplifiers in Nd:YAG*

2 MeV proton irradiation: (a) optical transmission microscope image; (b & c) measured intensity distribution of the propagation mode at the given wavelengths

Figure 2 of Tan et al, *Optics Express* 2015

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