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Dielectrophoretic manipulation of DNA in microelectrode gaps for single-molecule constructs

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1. ABSTRACT

The construction with biomolecules and their manipulation represent a key step for developing new miniaturized structures. Such micro or nanometer systems promise a variety of novel features. Dielectrophoresis (DEP) is a powerful tool for trapping and orienting individual molecules in microelectrode arrangements, and was demonstrated to be applicable to DNA. This relatively rigid biomolecule could (after defined immobilization) act as template for further modifications and functionalizations, e.g. metallization. Parameters of the DEP process were adapted to the given electrode layout and for trapping a few or even a single DNA strand. Characterization with atomic force microscopy (AFM) extends the standard method of fluorescence imaging by resolving the resulting structures with single molecule resolution.

2. INTRODUCTION

The fabrication of novel miniaturized, functional structures for applications in micro electronic, computing or detection and biosensing are a key goal of modern nanotechnology. The realization of nanostructures using biological or biomimetic strategies is one possible approach with a huge potential regarding miniaturization and self-assembly. Promising new hybrid structures (containing e.g. nanoscale metal or magnetic structures) combine the desired functionality with molecular-scale units and offer novel electric or magnetic features with the prospect of smaller and faster circuits. Advantages are also smaller sample volumes and reduced costs.

Biomolecules like DNA, enzymes or antibodies are in the nm range and exhibit interesting properties regarding manipulation and construction. The capability to

self assemble into highly ordered structures is one of their decisive features (1,2).

So, it is possible to use them as building blocks for bottom up architectures together with nanoparticles or nanowires/-tubes (3-5). Such hybrid systems of nanoparticles and biomaterials provide new dimensions in the rapidly growing research field of bioelectronics and nano photonics.

A controlled handling of single molecules is essential for the fabrication and the investigation of highly defined devices based on molecules. Especially linearly stretched DNA is quite suitable for the demonstration of different applications that contain structures with nanometer addressability such as molecular electronics, real-time PCR, or single molecule sequencing.

There is a variety of approaches aimed at stretching and aligning of individual DNA molecules. Most of them utilize the hydrodynamic flow, e.g. molecular combing (6-8). Optical tweezers (9) and magnetic fields (10) are also suitable for such approaches, as well as scanning force microscopy methods.

These methods are usually developed for well-defined tasks and work well in the given environment. But problems of reproducibility, accommodation to different technical surroundings and, in particular, of addressing only a few or even single molecules simultaneously are still remaining.

In order to align DNA and beside the use of an external flow, dielectrophoresis represents another interesting approach with a high potential for the defined molecular manipulation (11). It allows the separation of cells, nanoparticles and DNA as well as their manipulation in an electrical environment. It uses the fact that a polarizable particle exhibit dielectrophoretic activity in the presence of an alternating electric field.

The induced dipole can cause electrokinetic effects like rotation, deformation and orientation. In a spatially inhomogeneous field, DEP leads to the lateral movement towards the highest field strength. The force does not require the particles to be charged and depends on the medium, the polarizability, shape and size of the objects as well as on the frequency and the electric field gradient (11,12).

Washizu and coworkers were the first to demonstrate a DEP method to stretch DNA along field lines and positioned it onto electrode edges (13). They described two effects, the dielectrophoretic attraction towards the electrode gaps and the electrostatic orientation of the DNA. So in ongoing studies it was possible to determine both DNA size distribution and activities of nuclease by measuring the apparent length of stretched DNA in the micrometer range. Therefore, they used a field of $1x10^6$ V/m and a frequency of about 1 MHz between aluminium microelectrodes (14).

Application of static as well as oscillating fields make it possible to move the trapped DNA strands from one edge of a gold film to the other (15). This is quite useful for the manipulation of small quantities or molecules in microdevices.

The design of the electrodes (especially the shape) is of key importance for a defined DEP. The influence of the electrode shape on the precision of positioning DNA molecules was demonstrated (16). The prevalent electrode material is gold, microstructured on glass or silicon substrates. Quadrupole electrode geometries were investigated with gaps ranging from 3 to 100 micrometer.,DNA and proteins could be trapped and their electrical resistance could be measured (17).

This paper describes the trapping of lambda DNA by DEP. Thereby key parameters (microelectrode geometry, DNA concentration, reaction time, voltage and frequency) were optimized semi-empirically using *in situ* and real-time fluorescence imaging during the trapping process by variation of these parameters.

Besides standard fluorescence imaging that shows DNA but does not allow for a single molecule resolution, it was important to characterize the gaps with high resolution techniques like scanning force microscopy (AFM) for a detailed view of the structure and the amount of the collected DNA. AFM allows for molecular resolution without the need for sample metallization, and is therefore a valuable tool for detailed characterization beyond optical resolution.

The intention of this work is to align single DNA strands over a given electrode gap in defined geometry in order to provide a scaffold for subsequent steps such as nanoparticle binding and/or metallization for potential applications in nanoelectronics and biosensing.

3. MATERIALS AND METHODS

Dielectrophoresis experiments were performed on microstructured chips with a size of 12.8 x 12.8 mm. The gold electrodes of 100 nm thickness were prepared on silicon oxide substrates by sputtering and standard photolithographic lift-off processes.

The designs of the 10-micrometer-gap-chip (a 42 electrode gap array) and of the 2-micrometer-gap-chip (a 4 electrode gap array) with tapered electrodes are shown in Figure 1. The microstructured chips were cleaned with acetone, water and ethanol for 10 min each and dried in a N_2 stream; a plasma cleaning step with 50 W for 10 min followed. No additionally adhesion layer for DNA immobilization was used.

The most commonly used DNA species for single molecule manipulations and trapping experiments is linearized lambda phage DNA (lambda DNA) which is double-stranded. This macromolecule has a size of 48500 base pairs with a contour length of 16.5 micrometer (B-conformation) when fully stretched. It combines a

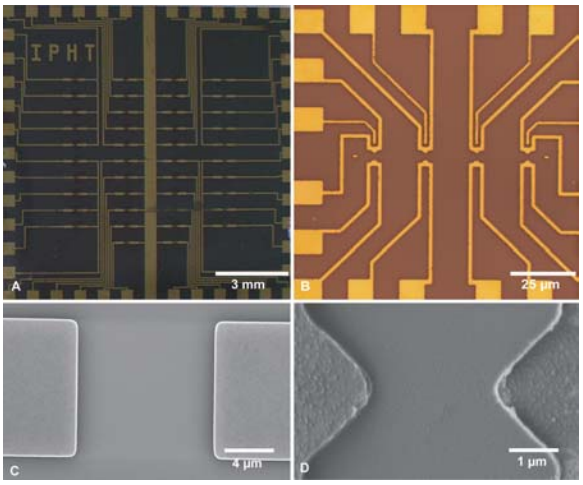

Figure 1. Light microscope images of the two different chip layouts used in the experiments: A) 10-micrometer-gap-chip with 42 electrode gaps and B) 2-micrometer-gap-chip, with arrangements of 4 smaller tapered electrode gaps. The SEM images show detailed views of one 10 micrometer electrode gap (C) and 2 micrometer electrode gap (D).

sufficient length with easy access due to its commercial availability and the comprehensive literature about its biophysical properties. The lambda DNA stock solution (300 ng/ micro liter; Fermentas, St. Leon-Rot, Germany) was diluted to working solutions of 0.02 ng/ micro liter or 0.004 ng/micro liter respectively.

For imaging the DNA during and/or after the dielectrophoresis it was stained using the fluorescence dye YOYO-1 (MoBiTec, Göttingen, Germany). The chips were covered with nearly 200 micro liter of the working solution without wetting the contact pads. The voltage was applied with a frequency generator Agilent 3320A (Agilent, Böblingen, Germany) in the range of 0.5 V up to 10 V. The used frequencies were between 1 kHz and 1 MHz for 1 to 20 min.

Fluorescence imaging was conducted using an Axiotech microscope (Carl Zeiss, Jena, Germany), equipped with a Sensicam CCD camera (PCO Computer Optics, Kehlheim, Germany) or an AxioImager A1m microscope with Axio Cam MRc5 (Carl Zeiss, Jena, Germany). All experiments were performed with an immersion objective under liquid phase conditions and at room temperature. Single images or a series of images were taken during the experiments or at the end of the experiment. High-resolution topographic images were obtained in tapping mode with the scanning force microscope NanoScopeIII in air using a Dimension 3100 measurement head (Digital Instruments, Santa Barbara, CA) and scanning electron microscope (SEM) Joel JSM 6700 (Joel Europe, Zaventem, Belgium).

4. RESULTS AND DISCUSSION

First experiments were conducted to determine optimal conditions for dielectrophoretic trapping of lambda DNA in microelectrode gaps. As shown in Figure 2, initial results exhibit the problem of the "all-or-none effect"; the yield of the DNA in the gaps differs considerably. A voltage of 6 V is collecting only a few, not stretched strands at the electrode edge (Figure 2A). On the other hand, voltages of 8 V or higher cause an assembly of thick bundles and also lead to trapping of impurities (Figure 2B and C). The characteristic funnel-like shape of the DNA bridges in Figure 2B is probably due to drying effects of the pinned DNA on the electrodes after the DEP process and before imaging with the fluorescence microscope. *In situ* real time fluorescence imaging during the process did not show such patterns. Further investigation regarding this problem are under way.

After this disappointing results a set of different frequencies as well as voltage series (data not shown) were studied. The effect of the applied frequency is given in Figure 3. At a low frequency of 10 kHz an alignment of DNA strands at the electrode edges in the gap is visible (Figure 3A), but no molecule is actually bridging the gap. At a frequency of 100 kHz the DNA was both collected at the edges and stretched over the gap (Figure 3B). A tremendeous amount of DNA between the electrodes results from high frequencies like 1 MHz as shown in Figure 3C. A bright spot of stained molecules is covering the entire electrode gap.

Studies using the 2-micrometer-gap layout confirm and extend these results. At a frequency of 10 kHz an alignment of DNA strands along the electrode wires is clearly visible and the gap remains empty (Figure 3D). However, at100 kHz DNA bundles are focused in the electrode gap and only a few molecules are attached to the electrode outside the gap (Figure 3E).

These results are in agreement with investigations of Namasivayam (18) and Asbury (15). It is supposed that inhomogeneities in the counterion cloud along the negatively charged backbone of the DNA contribute in different time responses to the polarization of a DNA strand. For trapping DNA in electrode gaps, 100 kHz proved to be the optimal frequency. It is also visible that the shape of the smaller 2-micrometer-gap arrangement is more suitable for DNA trapping applications. The increased field gradient at the tip of such a tapered electrode seems to enhance the accuracy of the positioning of single strands, so all further experiments were done with this layout.

For applications in the field of nano structuring and nano wires, single DNA molecules or thin bundles are preferred in the electrode gaps. Further experiments were aiming at this target. Therefore the gaps were more precisely characterized with an atomic force microscope in order to detect even single DNA molecules between the electrodes. DEP parameters from the first experiments were taken and adjusted to tune to achieve single DNA molecule trapping. Figure 4 shows a chip where voltages from 1 to 4 V were applied to four different gaps. A frequency of 100 kHz was taken as an optimum from previous experiments because higher frequencies yield an undesired excess of DNA molecules in the gap.

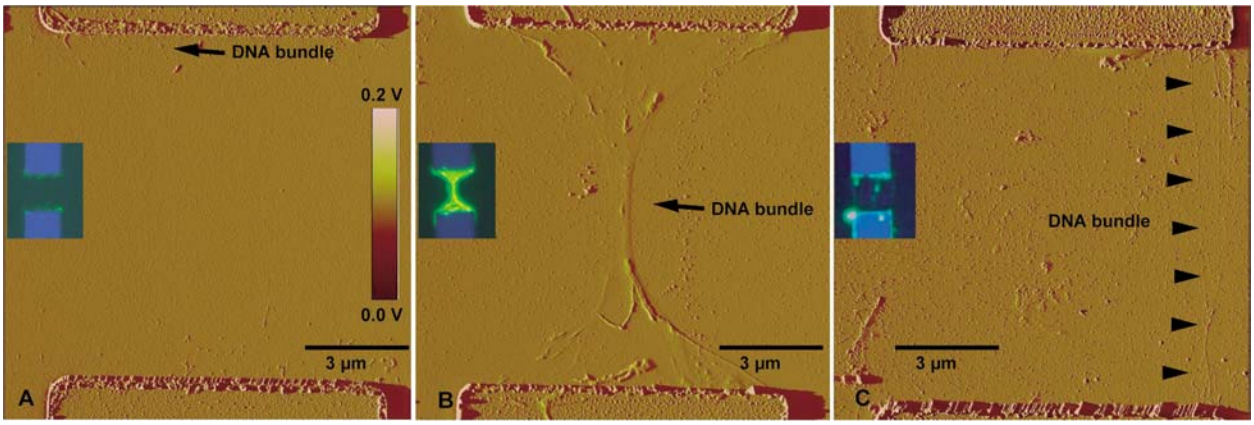

Figure 2. AFM images of the 10 micrometer gaps after applying different voltages with a frequency of 100 kHz. The insets show the corresponding fluorescence images. A) 6V: no fully stretched DNA is bridging the gap. B) 8V: a bulk of DNA fibers is bridging the gap. C) 10V: beside a couple of DNA structures, impurities are trapped in the electrode gap.

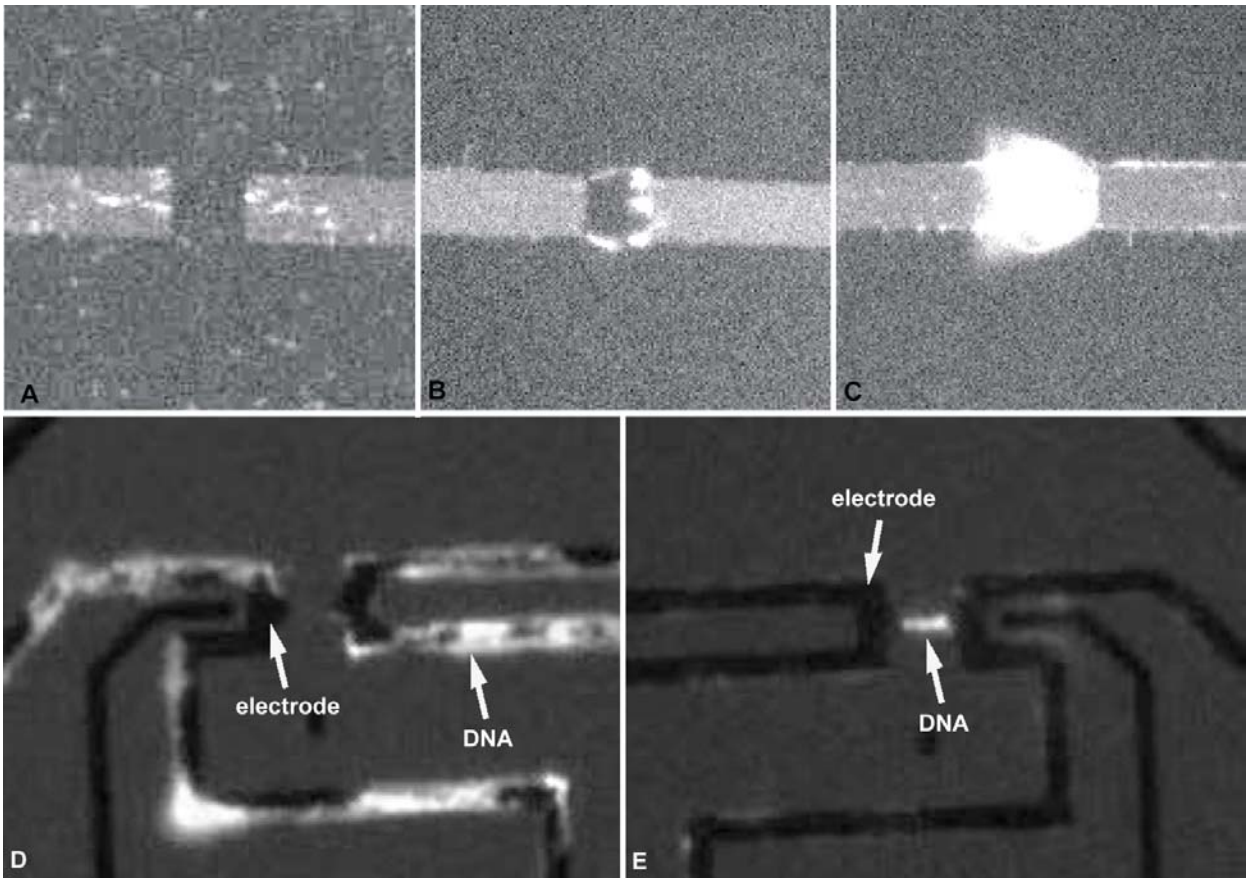

Figure 3. Fluorescence images (in solution) of trapped DNA in the 10 micrometer gap arrangement obtained by frequencies of: A) 10 kHz, B) 100 kHz, C) 1 MHz. D) 2 micrometer gap chip with DNA along the electrode structures using a frequency of 10 kHz. E) DNA trapped in the gap by a frequency of 100 kHz.

A detailed look at the gaps with the AFM exposes the single strands or bundles bridging the gap from the tip of the electrodes. It points out that with higher voltages more DNA is trapped and an assembly to bundles become apparent. In the gap at which 1V was applied single DNA strands or very thin bundles of a few DNA molecules could be found. At higher voltages the bundles become thicker especially from the tip of the electrode towards the gap. It is assumed that the DNA has contact with one end to the electrode and is pinned down there.

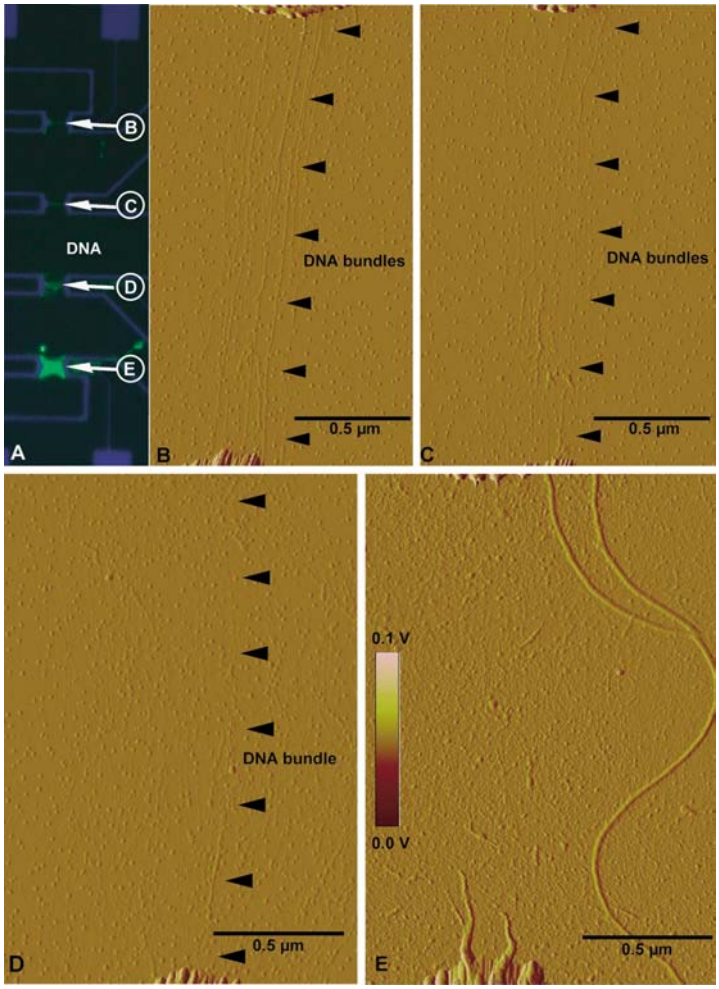

Figure 4. A) Overview of 4 electrode gaps taken with fluorescence microscope. Increasing voltages in 1V steps were applied top down. B-E) detail images of the gaps taken with AFM.

Starting from this point, the DNA is then spanning the gap. A terminal binding of DNA strands with a high affinity to gold electrodes is known (19, 20). Such binding can be enforced with an additional thiol group at the DNA strand end (18).

The applied voltage was further decreased in order to reduce the amount of DNA molecules in the gap. It turns out that the concentration of DNA in the applied solution (0.02 ng/ micro liter) appears to be too low in order to align molecules between the electrodes in the given time of 10 min. A satisfying result could be achieved by applying the electric field for 20 min (Figure 5). Then, two thin bundles as well as single molecules could be found.

We conclude that DEP is a useful tool for spatial manipulation of DNA in micrometer sized electrode gaps.

The combination of different imaging techniques like online *in situ* fluorescence imaging and atomic force microscopy allows the characterization and optimization of the DNA trapping process. Especially the investigation of electrode gaps with AFM after dielectrophoretic positioning and stretching steps improves the optimization processes regarding single molecule manipulation. So, single DNA strands can be resolved on microstructured substrates. Thus, DEP parameters derived from first experiments could be further defined.

It is important to understand that such optimized DEP conditions are valid for a given parameter complex. The interaction of focusing the electrical field (by increasing the frequency), enhanced field intensity (by rising the applied voltage and field radius), and the given DNA concentration results in a defined DNA structure, bridging the electrode gap. The tradeoff between efficiency and accuracy could be overcome with a frequency of 100 kHz, voltage of 0.5 V and reaction time of 20 min for the given parameter group (constant room temperature, 0.02 ng/microliter DNA and the 2-micrometer-gap-chip with the given geometry and surface roughness). So, it is possible to attach and stretch DNA strands from the one electrode to the other. The key parameters influencing the dielectrophoretic trapping are discussed in the following.

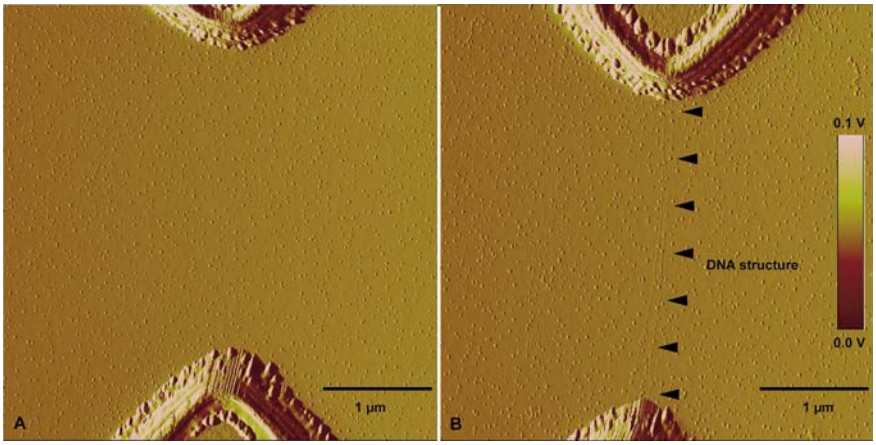

Figure 5. AFM images of A) an empty gap at a voltage of 0.5 V for 10 min. B) Two individual DNA structures spanned over the gap after applying a voltage for 20 min.

Higher voltages induce extended field intensities and radii. An increased action radius influences neighbor electrode gaps. This leads to additional amounts of DNA in the gap. Higher frequencies lead to better focusing effects (radii) during the trapping and also to defined DNA structures, like single molecule bridging the gap. This effect has also been observed by Tuukkanen (21). The advantage of lower frequencies is the possible application of higher voltages and therewith briefer trapping times (18), but for our conditions it results in unspecific accumulation of residues and thick DNA bundles. For single molecule applications low concentration DNA solutions, relatively low voltage and longer trapping times (20 min) represent favorable conditions with no observable electrolysis effects and sufficient DNA polarization. With these optimized parameters it is possible to bridge an electrode gap with single DNA molecules.

The difference in gap width also plays an enormous role on DEP experiments. The advantage of electrodes with a defined geometry could be shown and has been described in the literature as well (sinusoidal (22), trapezoidal (23) or triangular (16)). The change to the 2-micrometer-gap-chip with smaller electrode gaps led to better results concerning the localization of the DNA strands. An important point was the use of AFM that could resolve individual DNA structures and thereby clarify the local molecular arrangement.

The presented work demonstrates the successful utilization of DEP for single-molecular manipulation, thereby opening the way towards single molecule DNA constructs for future application in nanoelectronics or biosensorics.

5. ACKNOWLEDGMENTS

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Abbreviations: DEP: dielectrophoresis; AFM: atomic force microscopy

Key Words Atomic force microscopy, Dielectrophoresis, DNA, Microstructures, Nanotechnology

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