Contact angle and active drop transport for time resolved macromolecular and serial femto-second crystallography

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ABSTRACT

This paper will detail the process of reviewing and benchmarking the static contact angle of a number of Macromolecular sample compatible microfluidic substrate by way of sessile droplet methods. These materials will then be patterned with the techniques investigated and the contact angles revisited. The work continues evaluating recently introduced techniques to capture micro crystal slurries, diffraction testing taking place at the Diamond Light Source, a third generation light source in the United Kingdom.

Introduction

Recent drive to examine functional proteins has led to a lot of interest in room temperature crystallography. Most previous work focused on cryogenic macromolecular (MX) crystallography. There was good reason for this as radiation damage presents differently in crystals held at cryogenic temperatures, typically greater X-ray dose may be absorbed [1], [2]. To achieve time resolved imaging (TRX) a multiple number of structures must be refined for the same protein[3]. Because of the greater number of samples and the need to activate them before imaging in some way, room temperature microfluidics is of significant interest. The following work addresses the need for an understanding of contact angle within protein handling systems as the potential for fouling of fluidic surfaces by mother liquors or sub optimal drop formation is very real and potentially very costly in terms of beam time, man hours and research opportunity. In support of this aim we conducted some preliminary experiments on contact angles of novel materials.

Potential sample environment microfluidic methodologies such as acoustophoresis[4]–[7], digital microfluidics[8], electrowetting, acoustic tweezers[9], [10], acoustic ejection [11], rely on the transport or support of a drop over a surface without degradation. A vast number of crystalisation

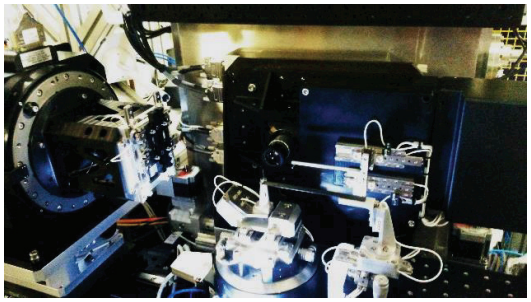


Figure 1 Current goniometer based apparatus for determining protein crystal structure.

solutions rely on the inclusion of some weight of polyethylene glycol (PEG). The following study takes a simple PEG solution and examines the resulting interaction with a range of surfaces.

Several of the surfaces trialled are re-entrant, this structure types refers to a surface that has significant local height variance, typically this sort of structure is represented by nanotube carpets and electrospun meshes.[12]

$$\gamma_{sv} - y_{sl} = \gamma_{lv} \cos\theta_Y$$

Equation 1 Youngs Equation for Contact Angle

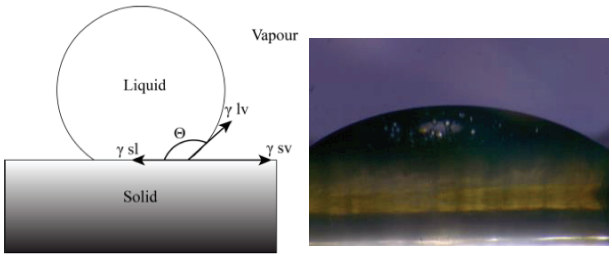


Figure 2 Diagram of Youngs contact angle and surface energies (left), Weakly birefringent lysozyme crystals in mother liquor on Kapton.

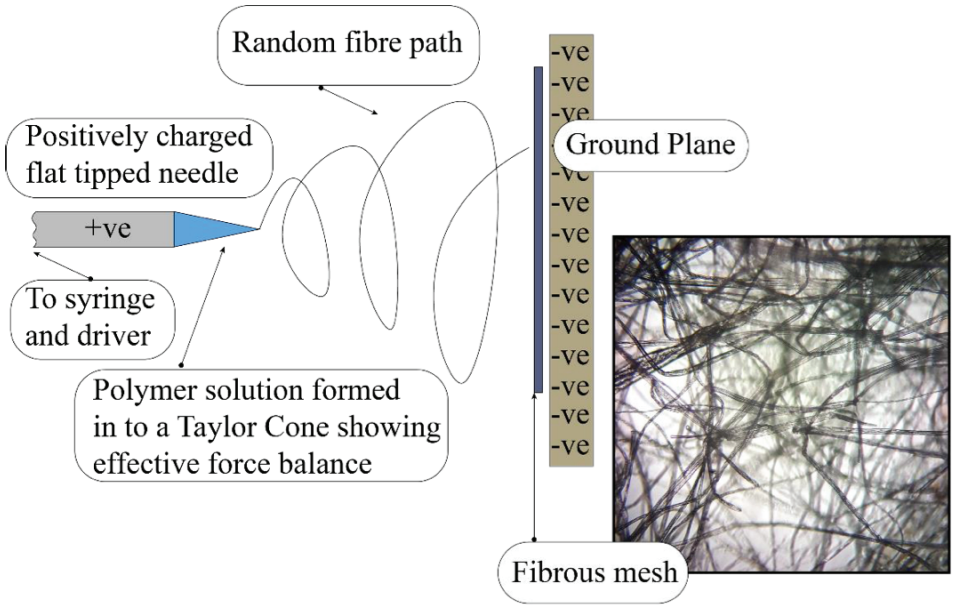


Figure 3 Electrospinning Apparatus, Cyclic olefin copolymer fibres used in the study shown on the right

Experimental Methods

The electrospinning apparatus consisted of a syringe driver, a high voltage power supply (0-30KV), a fume/safety cabinet, a flat/ground needle tip and a conductive ground plate. The positive side of the power supply is connected to the syringe needle with the negative side being attached to the ground plate collector. The syringe driver is then set to provide polymer solution at a rate that matches the solution lost to the spinning process. The spinning occurs as electrostatic forces cause the stretching of the drop at the tip of the needle, this continues until only a dry fibre remains and the solvent in the solution has evaporated.

Materials were prepared as follows

Polyethelyne glycol based precipitant solution was prepared in accordance with [13], a precipitant solution with pH 4.5 was prepared from a buffer solution of 50mM acetate, 30% PEG 4000 and 200mM NaCl.

Cyclic olefin copolymer (COC) (Supplied by Topas, US, free of charge to assist the investigation) it was dissolved in cyclohexane and stirred overnight to make a 20% (w/w) solution. The solution was then halved. Approximately 0.5 ml was coated on to a microscope slide using a 0.5 mil gated coater (Dyne Industries, UK). The remaining solution had 2% organosilane treated fumed silica added (Aerosil R805). The solution was vortexed for 30s to achieve distribution of the nanoparticles. The new fumed silica doped COC was then also coated on to a lab slide using the gated coater. The films were touch dry within 1 minute, however they were left for a further two hours to allow residual solvent reduction.

Kapton KJ was used as supplied by Goodfellow, UK.

The electrospinning solutions were prepared as follows:

COC 20% (w/w) in Tetrahydrofuran, ground plane distance 20cm voltage 17 KV

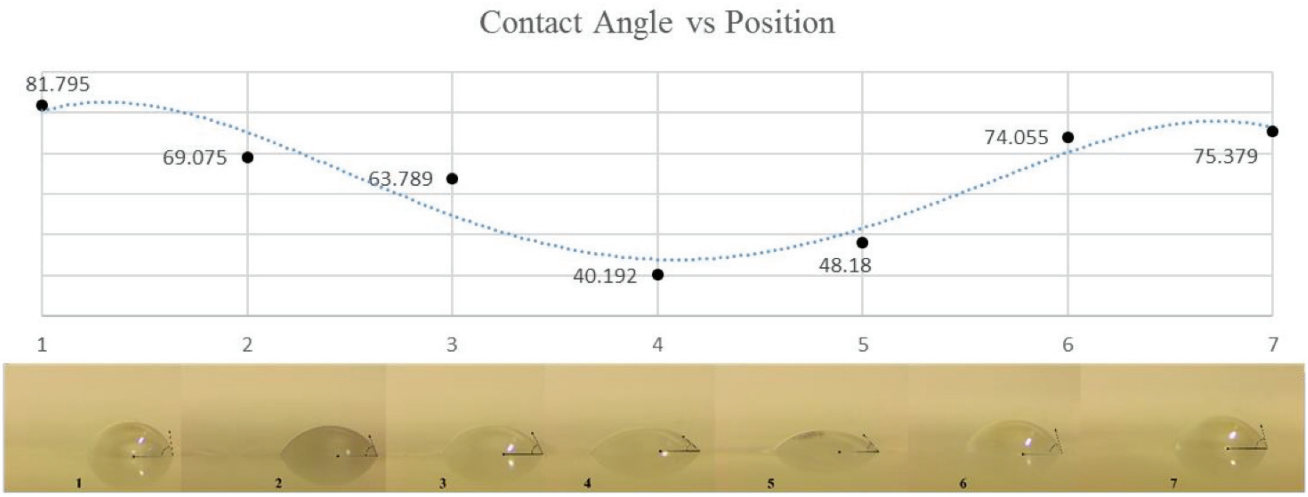
Custom PTFE formulation 23% (w/w) in Ethyl acetate, ground plane distance 20cm voltage 23 KV

The COC fibre mesh was then heated to 120 deg C for 1 hour while clamped between two weights to achieve a membrane.

Contact angles were determined using a telecentric imaging lens (Edmund Optic, UK) and a 6 axis kinematic mounting platform (Thor Labs, UK) to adjust for substrate angular and thickness deviation.

All droplets were added at approximately 10 µl volumes

For the fouling study a series of drops of precipitant solution were placed on a central point of the lab slide that had been treated with fumed silica and COC. The 10 µl drops were placed and removed after 5 minute for a total of 10 times. The removal was achieved by wicking away excess fluid (a common crystallography practice) then dry wiping with a lens cloth to remove any residual fluid. The image on the following page was constructed by stitching together 7 individual images. This can be done relatively easily as the telecentric imaging captures an orthographic image of the drop, meaning distance from the lens does not affect the size of the drop.



Results

Novel material testing

Kapton and COC, the common diffraction compatible materials performed as mild hydrophobes, both offering contact angles of around 60˚.

The re-entrant structures gave a sizeable increase. The electrospun COC membrane demonstrated a doubling of the contact angle with the precipitant achieving 131˚. Microscopy of the fibres showed that there was an apparent roughness to the fibres . This may allow them to operate as both a 'rough' surface and a re-entrant simultaneously. The fumed silica nano particles embedded in COC.

The fumed silica COC surface gave approximately a 50% increase over pure COC, this surface may possibly be considered chemically heterogenous an likely requires further study. The fumed silica by itself is an almost ideal hydrophobe so a more significant increase in the contact angle was expected.

Contact Angle results, all materials tested with precipitant solution based on PEG 4000

Image Number	Sample	Contact Angle
1	Kapton (polyimide – Dupont)	64.867
2	Solvent Cast Cyclic Olefin Copolymer	66.166
3	Electrospun (PTFE custom formulation)	151.553
4	Electrospun Cyclic Olefin Copolymer	131.602
5	Printed hydrophobic pattern on Kapton substrate	84.808

The spun PTFE variant was sufficiently hydrophobic to be classified as super hydrophobic, having a contact angle >150˚. While fluoropolymers frequently make good hydropobes, the electrospinning of the fibres produced sub micron fibre diameters, suggesting that the surface can also be classed as re-entrant. This would suggest that the material has performed at its optimum and fibre texturing would be required to achieve more.

Surface Fouling

The study capably demonstrated that a standard MX precipitant solution would foul a hydrophobic surface, reducing the contact angle by 50% from the peak recorded value.

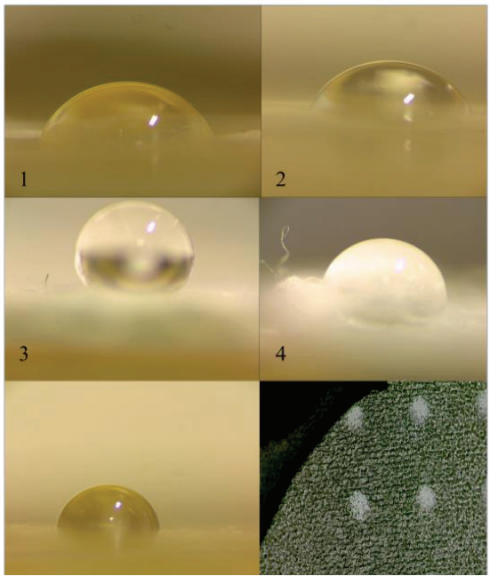


Figure 4 Contact angle study with novel materials patterned Kapton film shown in position 6

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Discussion and Conclusion

These preliminary studies were able to successfully demonstrate physical effects, both of fouling, and contact angle improvement. While both effects have been seen previously it is important to examine them as components of an active MX transport system.

In the work relating to contact angle it was seen that, in agreement with literature, fluoro-polymer surfaces achieved an excellent contact angle, improved by the randomly oriented re-entrant fibre structure. A similar improvement was seen in the electrospun COC membrane. With further work on fibre optimisation it is likely that a higher contact angle would be achievable in the diffraction compatible material.

In this light, the concentration of fouling materials leading to the degradation of contact angle ceases to be trivial, specifically as the fouling material, the PEG based precipitant solution, is ubiquitous within crystallisation trials. While the results should be confirmed by a further surface study, it is expected that some quantity of hydrophilic PEG and salt film remains on the surface despite bulk droplet removal.

Although this result is expected, it is non-trivial for sample environments handling crystals representing hundreds of man hours, at facilities with significant competition for beam time. A strong recommendation to examine contact angle degradation for precipitants and systems being trialled with high-throughput active surfaces.

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