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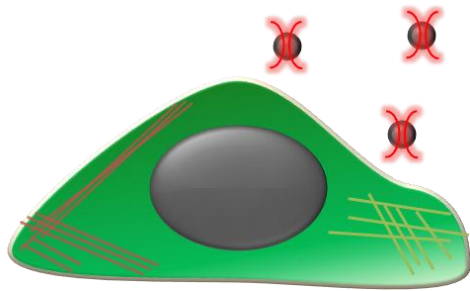


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**Graphical abstract**

Samples of cell membrane were non-destructively removed from individual, live cells using optically trapped beads, and deposited into a supported lipid bilayer mounted on an S-layer protein-coated substrate.

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## ARTICLE

# The Grab-and-Drop Protocol: A Novel Strategy for Membrane Protein Isolation and Reconstitution from Single Cells

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We present a rapid and robust technique for the sampling of membrane-associated proteins from the surface of a single, live cell and their subsequent deposition onto a solid-supported lipid bilayer. As a proof of principle, this method has been used to extract green fluorescent protein (EGFP) labelled K-ras proteins located at the inner leaflet of the plasma membrane of colon carcinoma cells and to transfer them to an S-layer supported lipid bilayer system. The technique is non-destructive, meaning that both the cell and proteins are intact after the sampling operation, offering the potential for repeated measurements of the same cell of interest. This system provides the ideal tool for the investigation of cellular heterogeneity, as well as a platform for the investigation of rare cell types such as circulating tumour cells.

## Introduction

Experiments performed upon large numbers of cells in culture form the mainstay of biochemical research and have provided many insights into the fundamental underpinnings of biology, but they have some major drawbacks that limit their application. Individual cells have characteristics such as the concentrations of critical metabolites or expressed proteins that differ significantly from the bulk mean, particularly in disease states.<sup>1-6</sup> Moreover, as biochemical processes proceed on very short timescales, signals averaged over a bulk cell population may present spurious and misleading information about real cellular biochemical behaviour. In order to avoid this problem, a number of bespoke microfluidic devices<sup>7</sup> and related techniques such as capillary electrophoresis<sup>8</sup> have been developed to provide versatile platforms for observing individual cell characteristics.<sup>9-12</sup> Mechanical manipulation methods such as scanning probe microscopy and field gradient traps provide practical and adaptable tools for investigation of different cell-based processes such as cell-cell interactions or cell-surface adhesion.<sup>13</sup> Optical tweezers in particular have been widely used in the field of single-cell manipulation for cell positioning, transportation, sorting, assembly, organisation, and even as a laser scalpel.<sup>14-18</sup>

In parallel with the development of single-cell manipulation tools, significant resources have been invested into the analysis and characterisation of membrane proteins. About 60% of all 430 drug targets presently known are membrane proteins,<sup>19</sup> while the human genome project suggests that more than 30% of all proteins contain one or more transmembrane helices or domains.<sup>20, 21</sup>

The complexity of biological membranes presents serious technical challenges in quantitative studies, and so scientists have been working for more than 30 years to create simplified, controllable model systems.<sup>22</sup> In particular, solid supported lipid bilayers (SSLB) have been widely used as analogues to mimic the cell membrane for biosensor applications.<sup>23</sup> SSLB fabrication techniques have been optimised by a number of researchers, but these are beyond the scope of this communication and are well-summarised elsewhere.<sup>22, 24</sup> However, the biggest challenge in the development of such platforms is the isolation and incorporation of functional target membrane proteins: a variety of techniques for this have been attempted, and are well summarised by Pullara *et al.*<sup>25</sup> However, each of the traditional biochemical techniques has significant drawbacks that limit their applicability in quantitative studies, particularly where cellular heterogeneity is of interest.

One common approach is to isolate whole cell membrane fragments in the form of vesicles for subsequent transfer to the SSLB.<sup>26, 27</sup> However, this technique introduces cell membrane fragments from a large number of cells to the system, introducing the same heterogeneity and averaging issues encountered in bulk cell assays. Alternatively, the isolation of the desired membrane protein may be attempted by specific disintegration techniques, then stabilising with (for example) detergent molecules and integrating them into the bilayer.<sup>26-28</sup> In general, proteins for SSLB-integration need to either be reconstituted into small vesicles which are then fused on the surface of interest, or *via* specific pull-down onto a previously protein-coated (or otherwise functionalised) surface to direct

bilayer formation.<sup>27, 29</sup> Consequently, the necessary preparation steps of these isolation/reconstitution protocols are very time consuming. Moreover, removing a protein from its natural and hydrophobic environment and reconstituting it into another is a challenging process that can damage the protein or result in less than optimal functionality.<sup>27</sup> A direct, physical technique for the introduction of small numbers of membrane proteins to a membrane has been demonstrated by Holden *et al.*,<sup>30</sup> introducing a measure of control to at least the delivery stage of the operation. However, this approach was designed with *E. Coli*-based protein synthesis in mind and as such provides no selectivity of sampling, preventing any meaningful study of cellular heterogeneity. Furthermore, it requires the complete lysis of donor cells, eliminating any possibility of repeated time-resolved measurements on individual targets of interest.

To bridge the gaps between existing manipulation and reconstitution technologies, we used modified optically trapped smart droplet microtools (SDMs) which previous experiments have demonstrated to be capable of extracting plasma membrane fragments and associated proteins from living cells.<sup>31</sup> These tools offer a new and highly selective technique for the extraction of specific proteins from the cell membrane, as well as presenting a range of new approaches for exogenously stimulating individual cells within a tissue. Previous studies have been based around SDMs generated from an oil emulsion, comprising a liquid hydrocarbon core encased in a monolayer of lipid or detergent; however, it was predicted that SDMs coated with lipid bilayers would offer substantial advantages over these tools, particularly in the extraction and analysis of transmembrane proteins. When compared to hydrocarbon-core emulsions, solid particles are much easier to prepare at a uniform size and to optically manipulate, as the refractive index of many suitable media are ideally suited to trapping. Furthermore, their density can be tuned by means of altering the core composition or porosity such that it is higher than that of aqueous media, so they sink towards the plane of the target cells. In previous studies, the coating of silica particles by lipids has been investigated via a number of techniques, each demonstrating that the affinity between the silica and lipid leads to the rapid formation of a single, even bilayer.<sup>32-35</sup> As a result of these studies and the material's wide availability, lipid-coated silica particles were investigated for their potential as membrane sampling probes.

In these experiments, we demonstrate for the first time that these SDMs can be precisely and reproducibly used not only to sample directly from the surface of a chosen target cell within a population but to then unload that cargo onto a defined region of interest, facilitating downstream characterisation. Although direct analysis of the unloaded protein was not possible in this instance due to the limitations of the experimental conditions, significant, reproducible and quantifiable differences in unloading kinetics were observed between SDMs attached to a SSLB *versus* untreated or functionalised glass surfaces. This strongly supports the conclusion that the membrane-to-SDM sampling process is reversible and that the technique may be extremely valuable, particularly when combined with surface analytical tools such as total internal reflection microscopy<sup>36</sup> or surface plasmon resonance measurement.<sup>37</sup>

With the current panoply of long term microfluidic cell culture devices<sup>7, 38</sup> and the widespread use and flexibility of micro-contact printing<sup>39</sup> a fully integrated chip that enabled repeated

grab-and-drop experiments on live, growing and dividing cells coupled to a single molecule readout system<sup>36</sup> would be an immensely powerful tool.

## Results and discussion

The bilayer around each particle was comprised 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine (DOPE) and 1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphocholine (DOPC) in the molar ratio of 3:1, as this lipid composition is known to be highly fusogenic.<sup>31</sup> Moreover, it possesses comparable extraction efficiency to the lipid or detergent-coated emulsions previously developed by Lanigan *et al.*<sup>40</sup> In these experiments, EGFP labelled K-ras protein<sup>41</sup> localised at the inner leaflet of the plasma membrane of a colon carcinoma cell was picked up and transferred by this tool to a SSLB.

The chosen bilayer support was based on 2D crystalline surface layer (S-layer) protein. Such proteins grow around many bacteria and almost all Archaea and, after specific isolation protocols, are able to recrystallise revealing the same structures on a number of substrates.<sup>42</sup> The fabrication and characterisation of lipid bilayers have recently been described on S-layer coated substrates such as glass or gold;<sup>43</sup> as in these protocols the protein SbpA was used, isolated from the organism *Lysinibacillus sphaericus* CCM 2177.<sup>44</sup> SbpA has a square lattice symmetry and shows great stability under a variety of conditions.<sup>45</sup> This approach was chosen to provide a space between the glass and lipid membrane, based on the hypothesis that this would assist any transferred protein to adopt its native conformation across the bilayer and thus retain as much activity as possible. This extra space should also improve the lateral mobility of such proteins, preventing ionic interactions between protruding domains and the silanol groups of the glass surface.

The bilayer formation was achieved by a new vesicle fusion technique recently described by Marchi-Artzner and co-workers,<sup>27, 28</sup> in which an amphiphilic  $\beta$ -diketone ligand was incorporated into vesicles causing the formation of an inter-vesicular complex with europium (III) ions.<sup>46, 47</sup> Utilizing this technique on a solid substrate, the creation of a near defect-free lipid bilayer can be accomplished.<sup>43</sup>

The efficiency of SDM coating was first evaluated by determining the zeta potential changes under different pH conditions. The outcomes of the zeta potential versus pH are shown in Figure 1.

Uncoated silica beads change their zeta potential value from ~15 to -80 mV during the pH titration from 2 to 9. In our findings the bare particles had an isoelectric point (pI) of 3.18 which is in good agreement with the literature.<sup>48, 49</sup> Silica particles have a large number of negative charges in this pH range due to free silanol groups on their surface.<sup>50</sup> In comparison, when the beads were coated with a lipid bilayer the pI value increased to 4.37, due to hydrogen bonding between the silica surface and the lipid head-groups. These results were in agreement with Katagiri *et al.* who have shown similar behaviour in zeta potential measurements,<sup>49</sup> suggesting strongly that the protocol leads to good coverage of the silica beads. Moreover, their research showed that controlled coating could be achieved by positive, negative and zwitterionic lipids, meaning that a wide range of tuneable SDM systems can be

rapidly produced. It is also of note that the zeta potential becomes more positive due to the coating process, increasing the colloidal stability of the suspension and decreasing aggregation relative to the uncoated beads. To investigate this, the SDMs were stored at 4 °C and their zeta potential was analysed repeatedly over time. No changes were observed over the course of a week, suggesting that the SDMs remain stable for extended periods (data not shown).

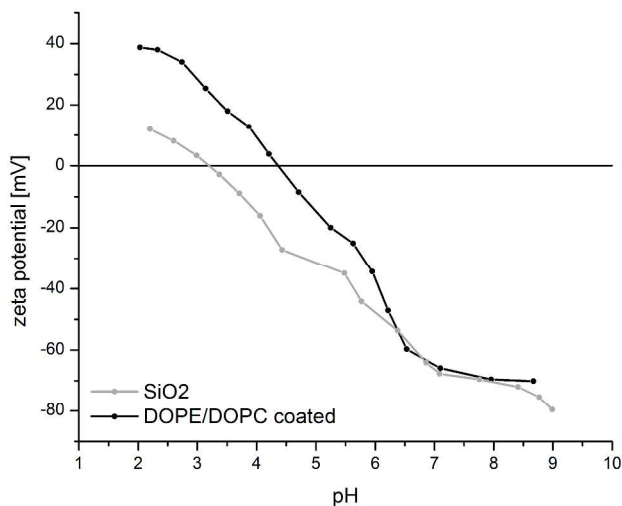


Figure 1. Plot of zeta potential of coated and uncoated SiO<sub>2</sub> beads against different pH values. The isoelectric point of the bare SiO<sub>2</sub> beads is 3.18 and for the lipid coated beads 4.37.

Additional coating verification was obtained through FACS analysis. Three sets of SiO<sub>2</sub> particles were tested: one uncoated set of particles, one set coated according to the procedure described above, and one set coated as above with the addition of 1% fluorescein DOPE (Sigma). The results (Figure 2) show that a low autofluorescence signal was measured from both sets of beads without fluorescent dye, but clear, strong fluorescent response is seen in the beads that had fluorescein DOPE added during the coating procedure. These results indicate that the coating procedure is effective in producing bilayers around the particles, while also suggesting that the predominant product is an SDM wrapped in a single bilayer. The presence of multiple distributions containing fewer detector events but with increasing median fluorescence intensities indicates that there are multiple populations within the particles, likely representing small clusters of SDMs passing through the analyser simultaneously, as similar clusters were commonly observed by microscopy.

The experimental set-up for sampling and delivery consisted of a holographic optical trapping system coupled to an epifluorescence microscope and the use of a three chamber PDMS-glass chip (full experimental details provided below). The first chamber contained the particles, the second the cells, and the third the S-layer supported lipid bilayer. For a successful membrane protein transfer, five steps had to be completed (Scheme 1): particle trapping (step 1), transport to cell (step 2), cell interaction and protein sampling (step 3), transport to the bilayer (step 4), and transfer to the SSLB (step

5). Trapping was achieved *via* a 20W holographic optical tweezer system, allowing up to 24 particles to be trapped and individually manipulated.<sup>51</sup> In most cases a maximum of three particles were trapped, to simplify handling and to maintain sufficient laser power in each trap for efficient control. The SDM reservoir chamber was also coated with S-layer proteins, in this case due to their anti-fouling properties,<sup>52, 53</sup> as interactions between the SDM beads and the glass surface meant that otherwise the majority of beads were irretrievably stuck to the glass surface before trapping could take place. The particles were then transported to the cell chamber (step 2) by repositioning the mechanical stage. Once there, they were brought in close contact to the cells so that surface interactions could occur leading to material transfer (step 3). Target cells were chosen from the population based upon their morphology and membrane integrity: cells exhibiting blebbing behaviour or similar signs of distress were often observed to capture SDMs irreversibly.

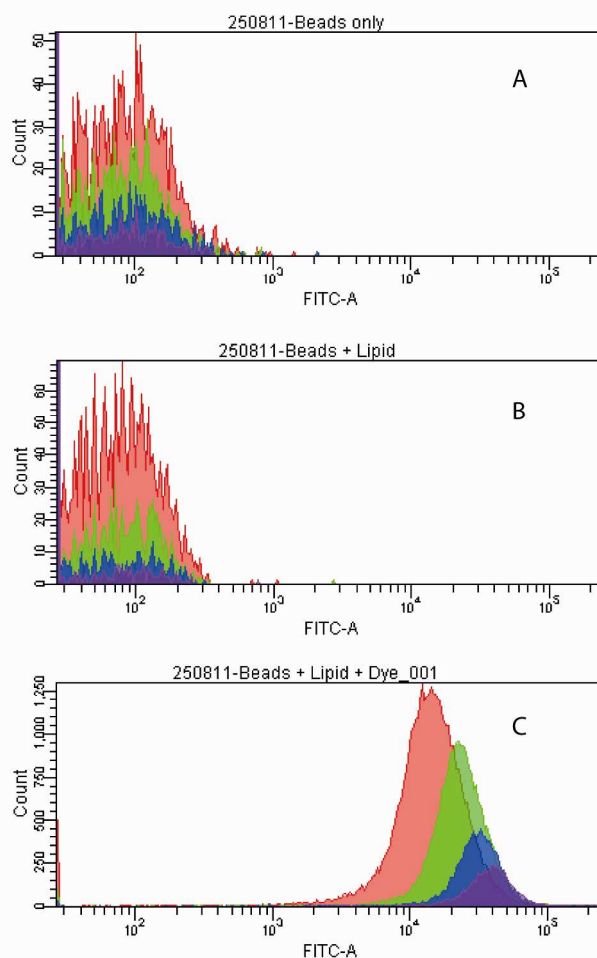
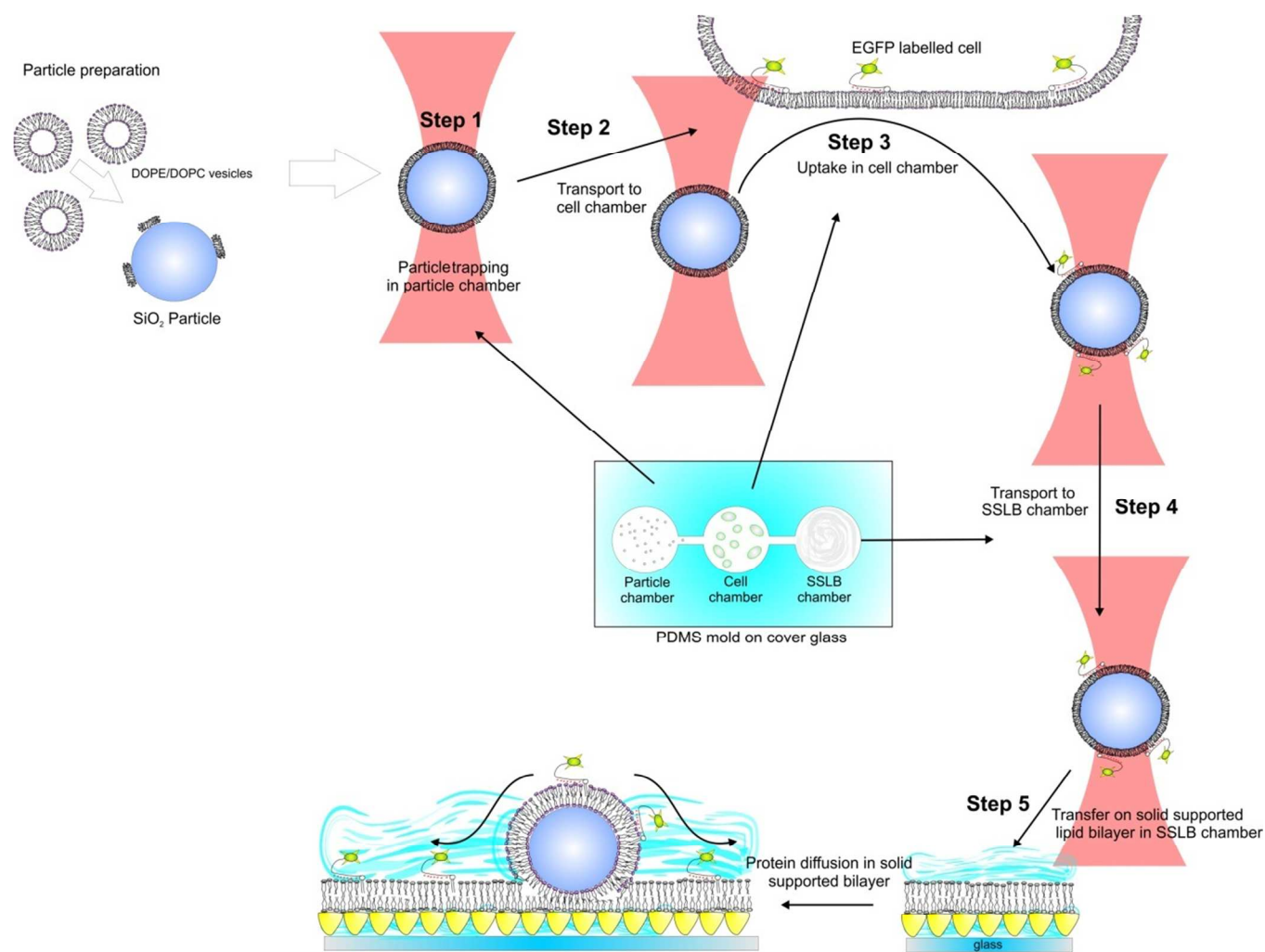


Figure 2. Results of FACS analysis of SiO<sub>2</sub> particles, shown as the counts of particles exhibiting a given fluorescence intensity in the FITC-A channel. (A): Uncoated SiO<sub>2</sub> particles. (B): SiO<sub>2</sub> particles coated as described. (C): SiO<sub>2</sub> particles coated as above, with the addition of 1% fluorescein DOPE. Colours indicate sub-populations of beads identified from side scatter analysis (data not shown).



Scheme 1. Illustration of the steps in the Grab-and-drop experiment. Si-based smart droplet microtools (SDMs) were prepared from a lipid vesicle suspension and optically trapped (step 1) to allow their manipulation to the surface of a cell of interest (2-3). The loaded SDMs were transferred to a solid-supported lipid bilayer (SSLB) in a third chamber (step 4) and the decrease in their fluorescence monitored over time (step 5).

A successful uptake was defined as a modification from a non-fluorescent to a fluorescent particle after interaction and extraction, indicating a transfer of fluorescent material from the target cell's membrane to the particle. Successful uptake from the cell to the particle occurs in around 20% of individual contacts, giving approximately a 50-60% success rate for at least one uptake from a cluster of three particles in contact simultaneously. It is important to clarify that besides the desired labelled protein, it is likely that other proteins and membrane-associated molecules are also transferred during the process. After sampling, the particles were transported to the membrane chamber (step 4), again with the use of the mechanical stage. Once in the membrane chamber, the trapped particles were lowered down in the z-direction onto the bilayer (step 5). Interactions with the surface meant that the particles' removal via optical trap was impossible after contact to the glass or SSLB-surface. However, this did not significantly impede the analysis of these proof-of-concept studies, and this protocol will be optimised in future studies.

Notwithstanding the preparation of the particles and the SSLB, the entire grab-and-drop experiment can be performed in approximately 40 minutes, from sample introduction into the chambers, trapping, uptake and transfer through to the deposition of the protein into the biomimetic membrane.

At the unloading stage as proteins are transferred to the SSLB, it is proposed that the lipid bilayer on the particle fuses or otherwise exchanges with the SSLB, resulting in a decrease of the fluorescence of the particle. For this reason, the time-dependent fluorescence decrease was monitored for 20 minutes to analyse the mixing and diffusion kinetics of the membrane protein in the SSLB. The transfer behaviour was then compared between glass and the SSLB. For the SSLB a stronger time-dependent decrease of fluorescence intensity was expected, due to this mixing and the much larger diffusion area (Scheme 1). In comparison, the transfer on glass was predicted to result in patch formation leading to an almost stable fluorescence intensity (see illustration in Figure 3C and Figure 4).

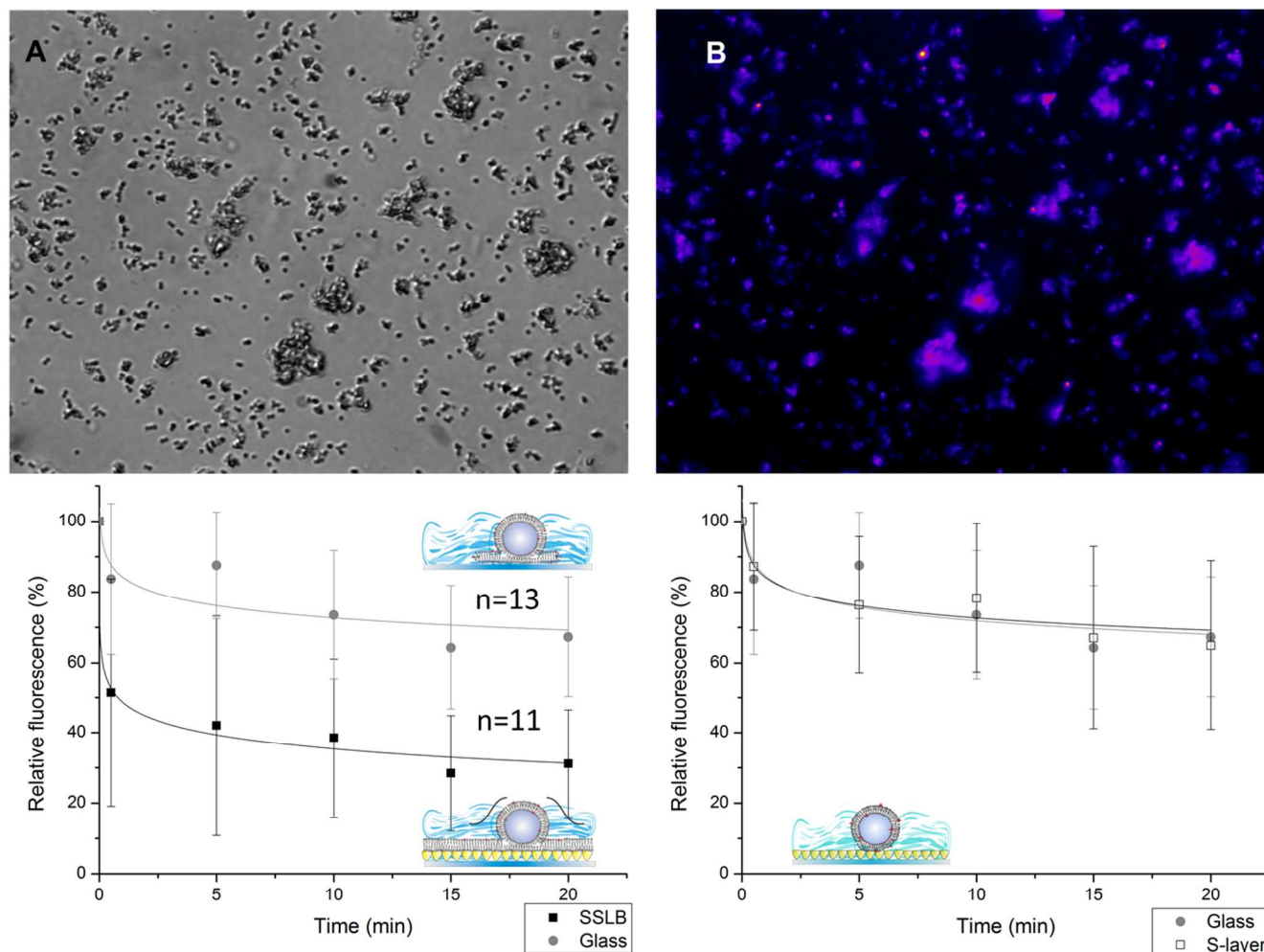


Figure 3. Controls for grab-and-drop experiment using fluorescently-labelled lipids. (A) and (B) show the bright-field and fluorescence images of lipid coated SiO<sub>2</sub> particles containing 0.5 mol% lissamine rhodamine DOPE lipids. Each bead has a diameter on  $1 \pm 0.05 \mu\text{m}$ ; field of view is  $\approx 200 \mu\text{m}$  wide. The transfer of material from the coated particle to the different surfaces results in a decrease of the relative fluorescence intensity on the particle over time. The decrease is compared between glass and the solid supported bilayer lipid membrane (SSLB) (C) and glass and an S-layer coated substrate (D). Fit lines are added to guide the eye.

Before the first complete grab-and-drop experiment was undertaken, step 5 was first assessed for suitability using fluorescently-labelled lipids. Here, the fluorescent lipid lissamine rhodamine DOPE (Avanti Polar Lipids, USA) was added to the membrane surrounding the SiO<sub>2</sub> beads. For this experiment, a PDMS chip with two chambers was utilised as cell sampling was not required. Bright field and fluorescence images were taken (Figure 3A and B) of lipid labelled beads, revealing almost all particles were coated.

The particle transfer of both the control and the complete grab-and-drop experiments were as follows: firstly, an image was taken above the surface, which corresponded to the maximal fluorescence intensity. The particle was then transferred onto the surface using the optical trap, which was turned off as soon as the particle settled into contact. Images were immediately recorded after the trap's removal (approximately 30 seconds after first contact) and repeated every 5 minutes for 20 minutes. In these steps, care was taken to keep the focus, background and exposure time constant. In the control experiments, plots of the relative fluorescence intensity against the reaction times showed the behaviour of each fitted well with the proposed mechanisms previously described (Figure 3C).

To isolate any potential effects due to direct interactions with any bare S-layer protein surface, fluorescent lipid transfer was also attempted with the functionalised surface in the absence of an SSLB. The results show similar behaviour to the bare glass surface (Figure 4D). It is also of note that due to the anti-fouling property of this specific S-layer protein,<sup>52-54</sup> the particles did not stick to the surface but rather slid over it without noticeable interactions. As a result, the trap was maintained while the particle was in contact with the surface in order to prevent loss of focus.

The remarkably fast decrease in fluorescence intensity in control experiments with the SSLB meant this technique was applied to the cell-sampling system without further modification. Interestingly, the successful grab-and-drop experiments on proteins showed similar kinetics as the control experiments (Figure 4), despite the proteins' much greater steric bulk and hydration sphere. These data suggest a much more rapid decrease in fluorescence intensity was observed for the protein in comparison to the labelled lipids, although it is likely that this is an artefact due to the smaller pool of labelled proteins relative to rhodamine-PE per SDM. This means that the migration of each protein constitutes a relatively greater

loss of fluorescence than does the equivalent transfer of a labelled lipid, rather than being indicative of a higher diffusion rate amongst the large proteins.

The apparatus developed during these experiments was highly specialised and was not easily amenable to the incorporation of analytical tools that could monitor the diffusion of unloaded cargo. The fluorescence of SDMs after sampling and extended periods of trapping and manipulation confirmed that these processes did not interfere with the functionality of the K-ras-bound EGFP; however, information about the eventual fate of the payload and the long-term functionality of the proteins thus transported was limited. Work is ongoing to integrate these tools with a single-molecule-detection total internal reflection microscopy platform developed elsewhere in the Proxomics collaboration<sup>36</sup> in order to further elucidate the stability of these protein payloads.

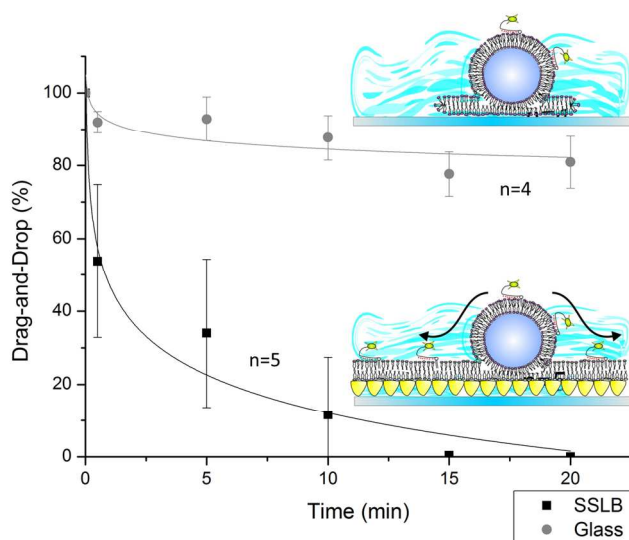


Figure 4. K-ras protein transfer experiment performed on glass and on the bilayer lipid membrane (SSLB). Fit lines added to guide the eye.

## Experimental Section

The experimental set-up used was an inverted epi-fluorescence microscope combined with a holographic optical trapping system. All trapping work was performed on a dual-carousel TE2000-U inverted microscope (Nikon), with a 20W Ytterbium fibre laser utilised to power the optical traps. The laser expanding factor was 2.5, achieved by a pair of IR-coated doublets of  $f=40$  mm and  $f=100$  mm respectively (Linos Photonics, Qioptiq), to fill the surface of the ferroelectric liquid crystal spatial light modulator (SLM) (CRL-Opto) and to maximize its usable area. Unwanted laser light was then removed from the system through two half-wave plates placed either side of the SLM and a polarizing beam-splitter (Edmund Optics), directing it into a commercial beam-dump (Thorlabs). The residual light was shrunk by a second pair of IR-coated doublets ( $f=200$  mm and  $f=160$  mm, Linos Photonics, Qioptiq), and reached the back aperture through an IR trapping dichroic (Chroma Corp), where it was focused by a 60x, 1.2 N.A. water immersion objective.

The traps were run by a home-built program written in a combination of LabVIEW and OpenGL. This system provided up to 24 independently-controllable optical traps, with additional controls for introducing aberration correction through Zernike polynomials to increase trapping efficiency. However, in this experiment the traps were generally stationary, with  $x$  and  $y$  movement controlled by a motorised stage (Prior, UK) and the  $z$  direction by the focus of the objective lens.<sup>51</sup>

## Cell culture

The cells used were adherent BE human colon carcinoma cells expressing EGFP-Tk (EGFP-labelled CAAX motive of K-Ras).<sup>41</sup> The cell culture and preparation for the experiments has been described previously.<sup>31, 40</sup> The only distinction here was the usage of the gentler Accutase solution (Innovative Cell Technologies, Inc) instead of trypsin for cell detachment, as experiments seemed to indicate that sampling success rates were higher when the membrane showed the greatest structural integrity. In the set-up described, cell viability was only necessary for a few hours in order to demonstrate proof of concept of selected protein transfer from a single cell membrane to an SSLB.

## Particle coating procedure

A silicon dioxide particle suspension in HEPES buffer was used for lipid bilayer coating. 100 nm vesicles composed of DOPE and DOPC in the molar ratio of 3:1 were prepared in HEPES buffer by the extrusion method through a polycarbonate membrane at room temperature. For the control experiments, 0.5 mol% Lissamine rhodamine DOPE (Avanti) were added. This concentration was chosen to provide a strong fluorescence signal whilst being some orders of magnitude outside rhodamine's self-quenching domain.<sup>55</sup> 10  $\mu$ l of a 5% SiO<sub>2</sub> particle dispersion (1  $\mu$ m diameter, Sigma Aldrich) was mixed together with 500  $\mu$ l of a vesicle suspension ( $c=2.08 \times 10^{-7}$  mol l<sup>-1</sup>) overnight under rotation. They were then washed twice with Milli-Q water (Millipore, Molsheim, France, resistivity: 18.2 M $\Omega$  cm<sup>-1</sup>) followed by HEPES buffer. Between each washing step, the suspension was centrifuged (4.5 rct, 1 min) and the pellet was vortexed with the new washing solution until complete dispersion was achieved. The lipid coating was verified by determining the pH dependence of the zeta potential by an electrophoretic light scattering device with a laser Doppler system (Malvern Instruments) and through FACS studies conducted at the Imperial College Flow Cytometry Facility using a BD LSRFortessa cell analyser.

## Solid supported S-layer stabilised lipid bilayer generation

The lipid bilayer generation was performed in the same manner as recently published.<sup>43</sup> Briefly, the isolated S-layer protein SbpA of *Lysinibacillus sphaericus* CCM 2177 was recrystallised on a cover glass surface at a concentration of 0.1 mg ml<sup>-1</sup> in recrystallisation buffer containing 0.5 mM Tris/HCl (Sigma), 10 mM CaCl<sub>2</sub> (98%, Sigma) at pH 9. This coating acted as layer between the glass surface and the bilayer. The isolation and recrystallisation procedure of this protein is published elsewhere.<sup>44</sup> The lipid bilayer was then formed *via* a modified vesicle fusion technique triggered by a  $\beta$ -diketone ligand (synthesised by Christian Stanetty, BOKU, Chemistry Department according to Marchi-Artzner *et al.*).<sup>47</sup> This ligand was incorporated with 1 mol% in a 1 mg ml<sup>-1</sup> lipid-CHCl<sub>3</sub>



1 solution composed of L- $\alpha$ -phosphatidylcholine (Egg-PC) and  
2 1,2-ditetradecanoyl-*sn*-glycero-3-phosphoethanolamine  
3 (DMPE) in the molar ratio of 4:1 (both from Avanti Polar  
4 Lipids). The chloroform was removed under nitrogen and then  
5 vacuum to leave a thin film, from which vesicles were formed  
6 by extrusion in 0.5 ml of 200 mM sucrose. The size of the  
7 vesicles was 100 nm in all experiments, controlled using a pair  
8 of polycarbonate membranes in a LIPEX extruder (Northern  
9 Lipids, Canada). The vesicles were then diluted with 200 mM  
10 glucose solution to a final concentration of 0.5 mg/ml and  
11 bound *via* 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide  
12 (15 mg/ml, Sigma Aldrich) coupling on the S-layer surface at  
13 pH 4.5 (adjusted with 0.1 M HCl). The addition of 1 mM EuCl<sub>3</sub>  
14 (Sigma Aldrich) causes immediate fusion of the vesicles to  
15 form a planar lipid bilayer. The surface was flushed afterwards  
16 with 200 mM glucose solution, followed with Milli-Q water  
17 and, in the last step, with HEPES buffer.

### 18 Preparation of chambers

19 The experimental chambers for the grab-and-drop experiment  
20 were comprised of a self-made polydimethylsiloxane (PDMS)  
21 (Sylgard 184 Elastomer Kit, VWR) chip which is illustrated  
22 schematically in Scheme 1. The PDMS was fabricated  
23 according to manufacturers' instructions and after drying was  
24 punched to form three chambers and mounted to a cover slip  
25 (1.5 thickness, VWR). The experimental chip consisted of three  
26 chambers, one for particle reservation, one for the cells and one  
27 for the lipid bilayer. The diameter of each chamber was 4 mm  
28 and the connections between them were made by cutting  
29 channels into the PDMS with a razor blade. Inter-chamber  
30 distances were kept to a minimum to reduce losses of SDMs in  
31 transit. The total trapping or grab-and-drop distance was in the  
32 order of 10 millimetres and future chip designs will need to  
33 reduce this distance significantly in order to both increase  
34 throughput and reduce losses in transit.

35 Prior to the experiments, 5  $\mu$ l of freshly split cells were  
36 transferred to the untreated cell chamber and 10  $\mu$ l of a 0.01%  
37 of SDM solution (see above) was added in the S-layer-coated  
38 particle chamber. After 5 min. resting time, the chambers were  
39 filled with pre-filtered 10 mM HEPES buffer (150 mM NaCl,  
40 pH 7.4, Gerbu). The SSLB membrane was prepared in its own  
41 chamber before all other steps, and care was taken to avoid  
42 mixing of the chamber contents. After all chambers had been  
43 filled with HEPES buffer, they were sealed with sealing film  
44 (Grace Bio Labs) to avoid evaporation during the experiment.  
45 All experiments were carried out at room temperature and cells  
46 remained stable for several hours to allow for multiple grab-  
47 and-drop attempts. However in future, longer-term live single  
48 cell based studies the entire procedure could be adapted to a  
49 more cell culture-friendly environment, incorporating  
50 microfluidics and an appropriate microscope incubation  
51 chamber.

### 52 Conclusions

53 We have conducted a proof-of-concept experiment showing  
54 that membrane fragments and proteins from a single cell can be  
55 transported to an S-layer supported lipid bilayer. The S-layer  
56 was chosen as supporting scaffold for the lipid bilayer due to  
57 the ease of coating without pre-activation of the surface. We  
58 used a lipid bilayer-coated silicon dioxide particle as transport  
59  
60

vehicle that could be manipulated and controlled by an optical  
trap combined with an inverted microscope. The entire grab-  
and-drop experiment can be performed in 30-40 minutes and  
represents a new tool for spatially-selective plasma membrane  
transfer from living cells.

The results showed a remarkably strong decrease in the relative  
fluorescence intensity of the bead when in contact with the  
bilayer compared to glass, indicating transfer of material from  
the bead to the bilayer. However, mechanistic information on  
how the membrane protein has been incorporated and released  
from the bilayer-coated particle remains unclear; the energetics  
and kinetics of membrane fusion and stalk formation are  
complex and poorly-understood, particularly when in protein-  
mediated systems.<sup>56</sup> Despite this limitation, the technique  
provides a valuable tool for the preparation of supported  
bilayers containing membrane-associated material from  
individual cells of interest, providing a powerful platform for  
the study of rare cells such as circulating tumour cells, and  
fields such as cancer in which cellular heterogeneity is a vital  
yet poorly understood contributor to overall therapeutic  
outcome.

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### Notes and references

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