

## Electronic Supplementary Material

# Direct detection and measurement of wall shear stress using a filamentous bio-nanoparticle

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## Production of M13 bacteriophage

M13 bacteriophage (M13) has a mono-dispersed, fibre morphology, with a well-defined semi-rigid shape, being 7 nm wide by 900 nm long. It has five structural proteins: 1 major coat protein, the pVIII, capped with 4 minor coat proteins, pIII and pVI, pVII and pIX, on the proximal and distal ends, respectively. These proteins can be chemically modified in a site-specific fashion, without disrupting its functionality [S1]. M13 was produced as per the method of Pacheco-Gomez et al. [S2] which involved the following.

A 250 mL flask containing 50 mL autoclaved NB2 (Oxoid) medium with tetracycline at 5 µg/mL (added to ensure the *Escherichia coli* used for propagating M13 maintain the F' plasmid encoding the pilus structure, since it is required for the infection to occur) was inoculated with 50 µL of One Shot Top10F' (Invitrogen). The culture was incubated overnight at 37 °C and 200 rpm. A volume of 40 mL of the overnight *Escherichia coli* culture was transferred into 400 mL autoclaved NB2 medium and allowed to equilibrate at 37 °C. The culture was then inoculated with M13, and incubated overnight at 37 °C and 200 rpm. The next day, the samples were centrifuged at 8,000g. The supernatant containing M13 was mixed with PEG6000 and NaCl (2.5 M) to precipitate the bacteriophage particles. These were then collected by centrifugation of the samples at 8,000g for 20 min. The supernatant was removed, and the pellet was re-suspended in 50 mM phosphate buffer, pH 8.

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## Caesium chloride purification of M13 bacteriophage

To purify the M13, the supernatant was mixed with caesium chloride powder to give a final concentration of 0.4 mg/mL of CsCl. The samples were centrifuged for 24 h at 35,000 rpm in 70.1 Ti rotor at 15 °C. After extraction, the samples were dialysed using 3500 MWCO dialysis tubing (Sigma-Aldrich) in 50 mM potassium phosphate buffer pH 8 for 24 h.

## Measurement of M13 bacteriophage concentration

M13 concentration was determined from its absorption spectrum measured using a JASCO V550 UV/Vis spectrometer. The concentration of M13 (in mg/cm<sup>3</sup>) was then calculated based on its absorbance at 269 nm (using  $\epsilon = 3.84 \text{ cm}^2/\text{mg}$ ) using the Beer-Lambert law.

## Bioconjugation of M13 bacteriophage pIII protein with anti-collagen IV antibody

Anti-collagen IV antibodies (aCol) were selectively conjugated to the 5 copies of pIII, at one end of the M13, by first using mild conditions to reduce partially the disulfide bonds of the pIII protein. A 2,000 molar tris(2-carboxyethyl)phosphine (TCEP Sigma-Aldrich) excess in phosphate buffer pH 7.5 (0.21 mg) was added to an initial mass of M13 (1.24 mg,  $7.25 \times 10^{-8}$  mmoles) in order to originate partially reduced pIII proteins ( $3.63 \times 10^{-8}$  mmoles). The sample was incubated for 5 min at room temperature. The partially reduced M13 was separated from low molecular mass byproducts using a PD-10 column (Sephadex G-25, GE Healthcare), with a conjugation buffer (50 mM potassium phosphate buffer, 150 mM NaCl, 5 mM EDTA buffer, pH 7.0) as eluent. The aCol was then derivatised with maleimide. 0.05 mL of aCol (abcam, ab19808) were required in order to provide a 1:1 ratio ( $3.63 \times 10^{-7}$  mmoles) over the pIII protein. Succinimidyl-4-(N-maleimidomethyl cyclohexane-1-carboxylate) (0.0012 mg, SMCC, Thermo Fisher Scientific) was added to the aCol in a 10:1 molar excess (0.0012 mg). The sample was incubated for 1 h and eluted in the conjugation buffer to remove low molecular mass byproducts using a PD-10 column (Sephadex G-25, GE Healthcare). Finally, the maleimide derivatised aCol and the partially reduced M13 were mixed and left to react for 1 h before gently stirring overnight at 4 °C. N-ethylmaleimide (NEM, Sigma-Aldrich) was then added to the mixture (0.90 mg) in order to produce a 20,000 molar excess over the pIII protein. The sample was left to react at room temperature (RT) for 15 min and was later purified using PD-10 columns (Sephadex G-25, GE Healthcare) in conjugation buffer.

## Bioconjugation of M13 bacteriophage pIII protein with wheat germ agglutinin (WGA)

The disulfide bonds of the pIII protein ( $3.75 \times 10^{-7}$  mmoles) were reduced using 0.5 M TCEP in phosphate buffer pH 7.5, added to an initial mass of M13 (1.28 mg,  $7.5 \times 10^{-8}$  mmoles). The sample was incubated for 5 min at RT. The partially reduced M13 was separated from low molecular mass byproducts using a PD-10 column, with a conjugation buffer (50 mM potassium phosphate buffer, 150 mM NaCl, 5 mM EDTA buffer, pH 7.0) as eluent. The WGA was then derivatised with maleimide. 2.5 mg of WGA (lectin from *Triticum vulgaris*, lyophilized powder, Sigma-Aldrich) in PBS pH 7 was used at molar equivalents to the pIII proteins. A 10× excess 1.5 mM SMCC (2.5 µL) in phosphate buffer was added. The sample was incubated for 1 h and eluted in the conjugation buffer to remove low molecular mass byproducts using a PD-10 column. These products were then mixed and left to react for 1 h before gently stirring overnight at 4 °C. 940 µL of a 1 mg/mL solution of NEM in conjugation buffer was then added to the mixture. The sample was left to react at RT for 15 minutes and was later purified using PD-10 columns (Sephadex G-25, GE Healthcare) in conjugation buffer.

## Fluorescent labelling of M13 bacteriophage pVIII protein with TRITC

The pVIII proteins of the protein-derivatised M13 were then labeled with tetramethylrhodamine-5-(and-6)-isothiocyanate (TRITC, Life Technologies). TRITC is an amine-reactive dye that produced a bright orange-red fluorescent bioconjugate with excitation/emission maxima at 555/580 nm. TRITC (9.73  $\mu\text{L}$  of a 10 mg/mL solution) in dimethyl sulfoxide (DMSO, Thermo Fisher Scientific) was slowly added to the M13 in 50 mM potassium phosphate buffer, pH 8.0 in order to provide a 1:1 ratio over the amine groups of the pVIII proteins (it is assumed that there are 2,700 copies of this protein per M13 particle) of which there are 2 per pVIII protein ( $2.43 \times 10^{-4}$  mmoles), the N-terminus and lysine residues. The sample was then incubated at RT, with continuous stirring for 20 h. Both constructs, M13-aCol-TRITC and M13-WGA-TRITC, were separated from unreacted TRITC using PD-10 columns, eluting the samples in conjugation buffer.

## Purification and quantification of the constructs M13-antibody-TRITC and M13-WGA-TRITC

Both constructs, the M13- aCol-TRITC and the M13-WGA-TRITC, were purified using a ÄKTA Explorer 10 purification system (GE Healthcare), fitted with a Superdex 200 (120 mL column volume) HiLoad 16/60 prepacked column. The column was equilibrated with 1 column volume of water and 1.2 column volumes of elution buffer (50 mM potassium phosphate buffer, 150 mM NaCl, pH 8) before sample injection. Three wavelengths were recorded simultaneously (269, 280, and 556 nm). 2 mL fractions were collected using a Frac-950 fraction collector (Amersham Pharmacia Biotech).

## Growth of endothelial cells

Primary culture human glomerular endothelial cells were conditionally immortalized using a temperature-sensitive simian virus 40 large tumor antigen (SV40LT) construct which allows proliferation at a permissive temperature of 33 °C, and it is switched off by transfer to a non-permissive temperature of 37 °C. At this temperature, cells take on a mature phenotype [S3]. GEnC were used for experiments after they were maintained at the non-permissive temperature for 4/5 days. Cells were grown in endothelial growth medium (EGM-2MV, Lonza). For experiments cells were seeded into  $\mu$ -Slide I Luer<sup>0.8</sup> collagen coated slides (ibidi).

## Imaging collagen-coated slides

The images were acquired at 10 frames per second using an Ultraview spinning disk confocal microscope with a 1.4 NA, 100 $\times$  oil immersion objective, 561 nm laser, TRITC filter sets (Croma) and an ORCA R<sup>2</sup> camera (Hamamatsu) under control of Volocity 6.3 (Perkin-Elmer).

## Imaging endothelial cell-coated slides

The M13 on the cells were visualised using a Nikon Ti Eclipse inverted microscope through 60 $\times$  oil immersion objective 1.4 (Nikon Plan Apo VC 60 $\times$  Oil DIC N2). Cells within the imaging window were illuminated at 535 nm using a widefield fluorescent light emitting diode (Precise Excite Cool LED) and emitted light was filtered through a 620 nm filter block and detected on a high resolution 14-bit monochromatic camera (Photometric's CoolSnap HQ2). The imaging window yielded  $1,392 \times 1,040$  pixels with a pixel size manufacturer-calibrated to be 107.5 nm. The camera exposure was set to 10 ms and images captured at 11.05 focusing using Nikon's NIS Elements software.

## Collagen-coated slides

A chamber flow slide with a collagen IV coating (ibidi,  $\mu$ -Slide I Luer<sup>0.8</sup>) was used. The channel was carefully filled with the M13-aCol-TRITC construct sample and left to incubate for 1 h at room temperature. The slide was linked to the tubing ('white' perfusion set, #10963) of the pump system. Reservoirs were filled with PBS. The software allows varying the pressure/flow rate/shear stress to the desired testing values. A defined nominal shear stress (WSS on a flat surface due to the bulk flow rate) profile was applied, ranging from  $-3.5$  to  $+3.5$   $\text{dyn}\cdot\text{cm}^{-2}$ , lasting 20 s each cycle. The experiments were performed at  $35^\circ\text{C}$ , on the temperature-controlled system box of the microscope. The reservoirs and tubing left inside the box for 1 h to equilibrate the temperature of the buffer.

## Endothelial-coated slides

The apparatus used for the collagen antibody constructs was also used for the cellular experiments. GEnCs were seeded onto the same type of flow collagen-coated slide (ibidi,  $\mu$ -Slide I Luer<sup>0.8</sup>) and the M13-WGA-TRITC construct was added to the chamber and left to react for 1 h in an incubator at  $37^\circ\text{C}$ . The slide was linked to the pressure pump and the reservoir of the fluidic unit was filled with PBS buffer. A defined nominal wall shear stress profile was applied, ranging from  $-3.5$  to  $+3.5$   $\text{dyn}\cdot\text{cm}^{-2}$ , lasting 20 s each cycle.

## Probability model of M13 bacteriophage orientation in flow

The tethered fibre motion under Brownian effects and flow is modelled phenomenologically by a random walk with spatially homogeneous jump probability, but a spatially-varying difference in the probability of left or right jumps, modelling the biasing effect of the viscous restoring force. The partial differential function (PDF)  $p(\varphi, t)$  was modeled on a one-dimensional grid  $\{0, \pm\Delta\varphi, \pm2\Delta\varphi, \dots, \pm n\Delta\varphi\}$  with jump probabilities  $k_-(\varphi)$  and  $k_+(\varphi)$  having constant sum ( $k_+ + k_-$ ) and their difference ( $k_+ - k_-$ ) varying with angle  $\varphi$ , corresponding to the increase in restoring viscous stress with deviation from the flow direction.

The discrete random walk model can be written down by considering respectively the probabilities that a fibre will jump from the left, will stay where it is, or will jump from the right, over a time period  $[t, t + \Delta t]$

$$p(\varphi, t + \Delta t) = p(\varphi - \Delta\varphi, t)k_+(\varphi - \Delta\varphi)\Delta t + p(\varphi, t)\left(1 - [k_+(\varphi) + k_-(\varphi)](\Delta t)\right) + p(\varphi + \Delta\varphi, t)k_-(\varphi + \Delta\varphi)(\Delta t) \quad (\text{S1})$$

A constant diffusion coefficient and a bias function  $U(\varphi)$  in terms of the jump probabilities were defined as follows

$$D = (\Delta\varphi)^2 \frac{(k_+ - k_-)}{2} \quad (\text{S2})$$

$$U(\varphi) = (\Delta\varphi)(k_+ - k_-) \quad (\text{S3})$$

Substituting for  $k_+$  and  $k_-$  and taking the limits as  $\Delta\varphi \rightarrow 0$  and  $\Delta t \rightarrow 0$  yields the partial differential equation

$$\frac{\partial p}{\partial t} = D \frac{\partial^2 p}{\partial \varphi^2} - \frac{\partial}{\partial \varphi} U p \quad (\text{S4})$$

A steady state solution is expected to be valid because of the over-damped very low Reynolds number nature of the flow. Thus

$$\frac{\partial p}{\partial t} = 0 \quad (\text{S5})$$

and hence

$$p = p(\varphi) \quad (\text{S6})$$

For situations physically symmetric about the flow direction  $\varphi = 0$ , the PDF must also be symmetric about  $\varphi = 0$  yielding the boundary condition  $\partial p / \partial \varphi(0) = 0$ .  $p$  is also required to integrate to 1 and is  $2\pi$  periodic. An approximate solution valid for small  $\varphi$  will give an easily-interpretable closed-form solution.

While we do not have an explicit form for  $U(\varphi) = 0$ , without detailed fluid dynamical modeling, it is clear that  $U(0) = 0$ , i.e. there is no restoring viscous force when the fibre is aligned with the flow direction, and that the fibre is aligned with the flow direction and that  $U'(0) < 0$  so that the force does indeed bias the fibre towards alignment. At leading order therefore  $U \approx \beta\varphi$  for some positive constant  $\beta$  which depends on shear rate. These assumptions lead to the Ordinary Differential Equation

$$0 = D \frac{d^2 p}{d\varphi^2} + \frac{d(\beta\varphi p)}{d\varphi} \quad (\text{S7})$$

which has solution

$$p = A \exp\left(\frac{-\beta\varphi^2}{2D}\right) \quad (\text{S8})$$

i.e. a normal distribution with mean 0 and variance  $D/\beta$ . Given that viscous drag at very low Reynolds number is linear in fluid velocity, it follows that the standard deviation of  $p$ ,  $\sigma$ , will be proportional to the inverse square root of the shear rate.

$$\sigma^2 \propto \frac{1}{\text{WSS} \cdot M} \quad (\text{S9})$$

where  $M$  is the material time constant which is essentially the inverse rotational diffusion constant so proportional to  $L^3$  for a slender rod of length  $L$  [S4]. Thus

$$\sigma^2 \propto \frac{1}{\text{WSS} \cdot L^3} \quad (\text{S10})$$

so

$$\ln(\sigma) = -0.5 \ln(\text{WSS}) - 1.5 \ln(L) + k \quad (\text{S11})$$

where  $k$  is a constant. The full data plot for the collagen-coated slides is given in Fig. S1.

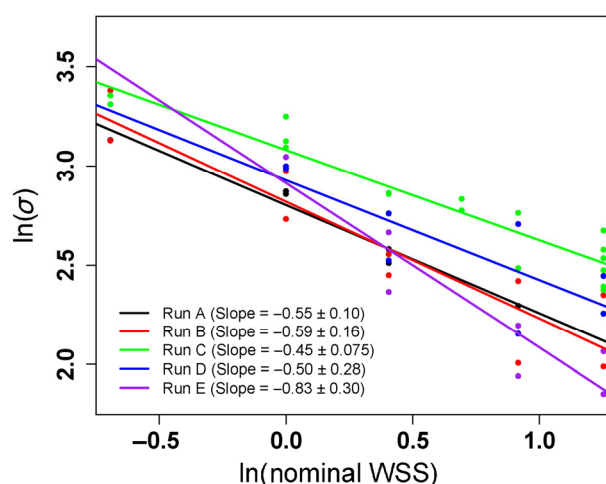


**Figure S1**  $\ln(\sigma)$  versus  $\ln(\text{nominal WSS})$  5 measured ColIV-M13-TRITC movies, for  $\sigma$  the standard deviation of the angle at each flow rate.

## Movies of M13 bacteriophage in flow

Movie S1: Movie of behaviour of M13- aCol-TRITC bound to a collagen IV coated flow slide (Fig. 3 Run A). The movie is approximately 4× real-time speed.

Movie S2: Movie of behaviour of M13-WGA-TRITC bound to the surface of a cultured endothelial cell (Figs. 2(b) and 4). The angles derived by our detection method are drawn over the M13 on the left and plotted in the chart. The same M13 with the angles drawn over measured by the directionality plugin (Fiji/ImageJ) is on the right. The movie is approximately 6x real-time.

## References

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