

# Modulator Combination Improves In Vitro the Microrheological Properties of the Airway Surface Liquid of Cystic Fibrosis Airway Epithelia

## Supplementary Materials

**Alessandra Ludovico, Oscar Moran, Debora Baroni\***

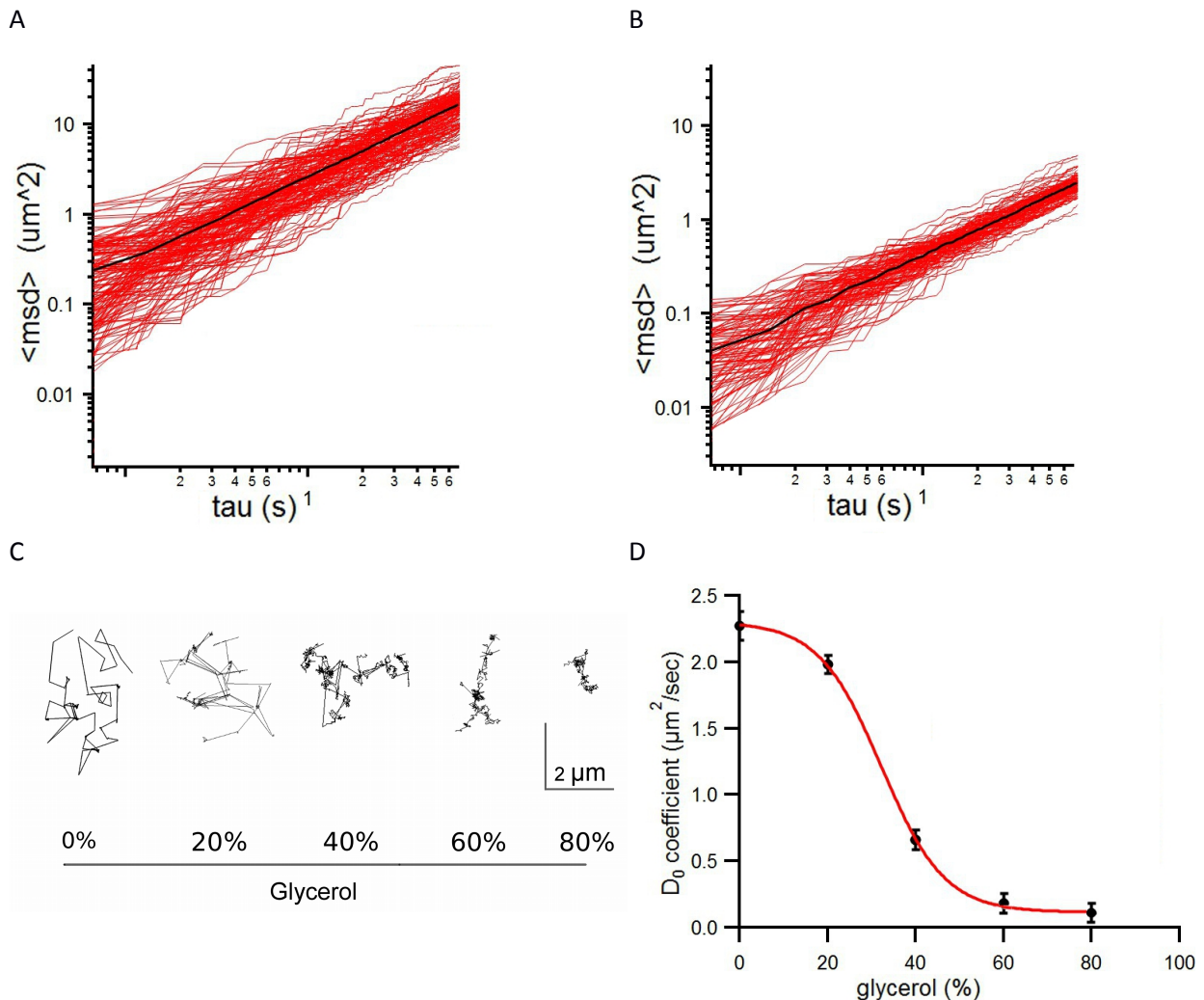
Istituto di biofisica, Consiglio Nazionale delle Ricerche (CNR), Via De Marini 6, 16149, Genova, Italy

Micro-rheology of glycerol

\*To whom correspondence should be addressed: [debora.baroni@ibf.cnr.it](mailto:debora.baroni@ibf.cnr.it);  
Tel.: +39-0106475559; fax: +39-010647550

## Micro-rheology of glycerol

To verify the ability of MPT to provide the parameters characterizing mucus viscosity, we first prepared aqueous solutions containing glycerol at 5 different concentrations: 0%, 20%, 40%, 60% and 80% (V/V), respectively. We added 1  $\mu\text{l}$  of a solution containing the fluorescent beads at 25  $\mu\text{l}$  to the different glycerol samples, and recorded the time course of the position of the beads for 45 seconds for at least fivefold. Images were captured at 1280 $\times$ 960 pixels, at a rate of 5 frames/s. The temperature of the samples was maintained at 22  $^{\circ}\text{C}$ . Figures S1A and S1B show the plots of the squared displacement,  $\langle\text{msd}\rangle$ , versus time interval of  $\sim 100$  beads in 40% and 80% glycerol. The displacement of the beads showed a clear dependence on the glycerol concentration (Figure S1C); figure S1D shows the plot of the apparent diffusion coefficient,  $D_0$ , calculated from the  $\langle\text{msd}\rangle$  of the beads, versus glycerol concentration. As expected, the values of  $D_0$  depended on the glycerol concentration and this dependence could be fitted with equation (2). Table S1 reports the values of the diffusion coefficient,  $D_0$ , the elastic modulus,  $\alpha$ , and the viscosity,  $\eta$ , of the different glycerol solutions used to calibrate our MPT system.



**Figure S1.** (A, B) Plot of the squared displacement versus time interval of  $\sim 100$  beads in 40% and 80% glycerol. The mean square displacement,  $\langle\text{msd}\rangle$ , is shown as a black solid line. (C) Displacement of fluorescent beads in solutions containing 0%, 20%, 40%, 40%, 60% and 80% (V/V) glycerol, respectively. (D) Dependence of  $D_0$  on glycerol concentration in aqueous solutions.

**Table S1.** Values of the diffusion coefficient,  $D_0$ , the elastic modulus,  $\alpha$ , and of the viscosity,  $\eta$ , obtained from different glycerol solutions. Data are expressed as mean  $\pm$  sem

<b>Glycerol %(V/V)</b>	<b><math>D_0</math> (<math>\mu\text{m}^2/\text{sec}</math>)</b>	<b><math>\alpha</math></b>	<b><math>\eta</math> (cPoise)</b>
0	$2.274 \pm 0.011$	$0.978 \pm 0.003$	$0.950 \pm 0.004$
20	$1.098 \pm 0.007$	$0.957 \pm 0.003$	$1.968 \pm 0.012$
40	$0.660 \pm 0.001$	$0.964 \pm 0.001$	$3.272 \pm 0.005$
60	$0.220 \pm 0.001$	$0.996 \pm 0.002$	$9.833 \pm 0.034$
80	$0.066 \pm 0.002$	$0.988 \pm 0.002$	$32.981 \pm 0.134$