

In Vitro Effects of rhDNase on Sputum Rheology in Cystic Fibrosis Patients

Étienne Ghiringhelli,¹ Matthieu Robert de Saint Vincent,¹ Jérémy Patarin,¹ Stéphane Mazur,² Marie Perceval,² and Isabelle Durieu²

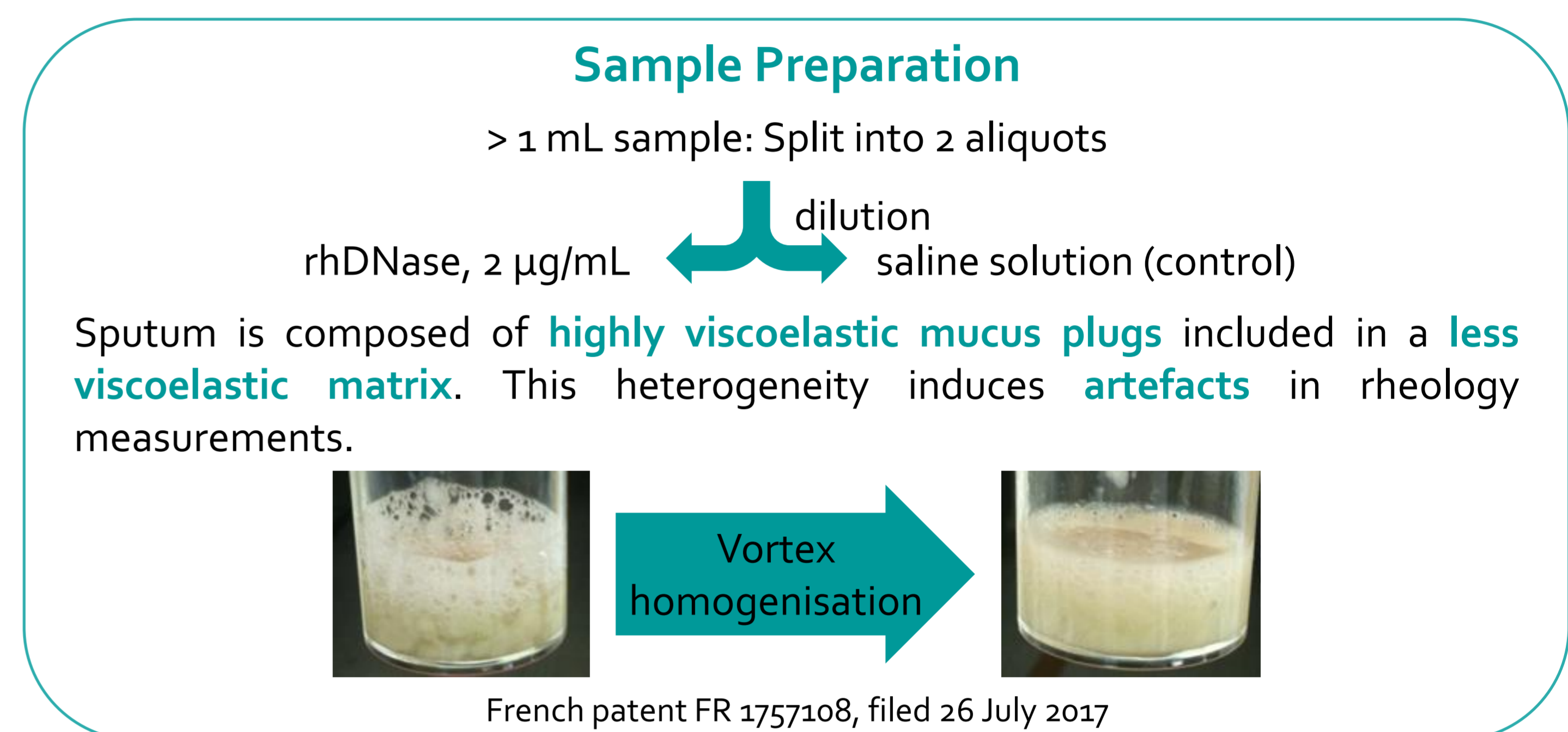
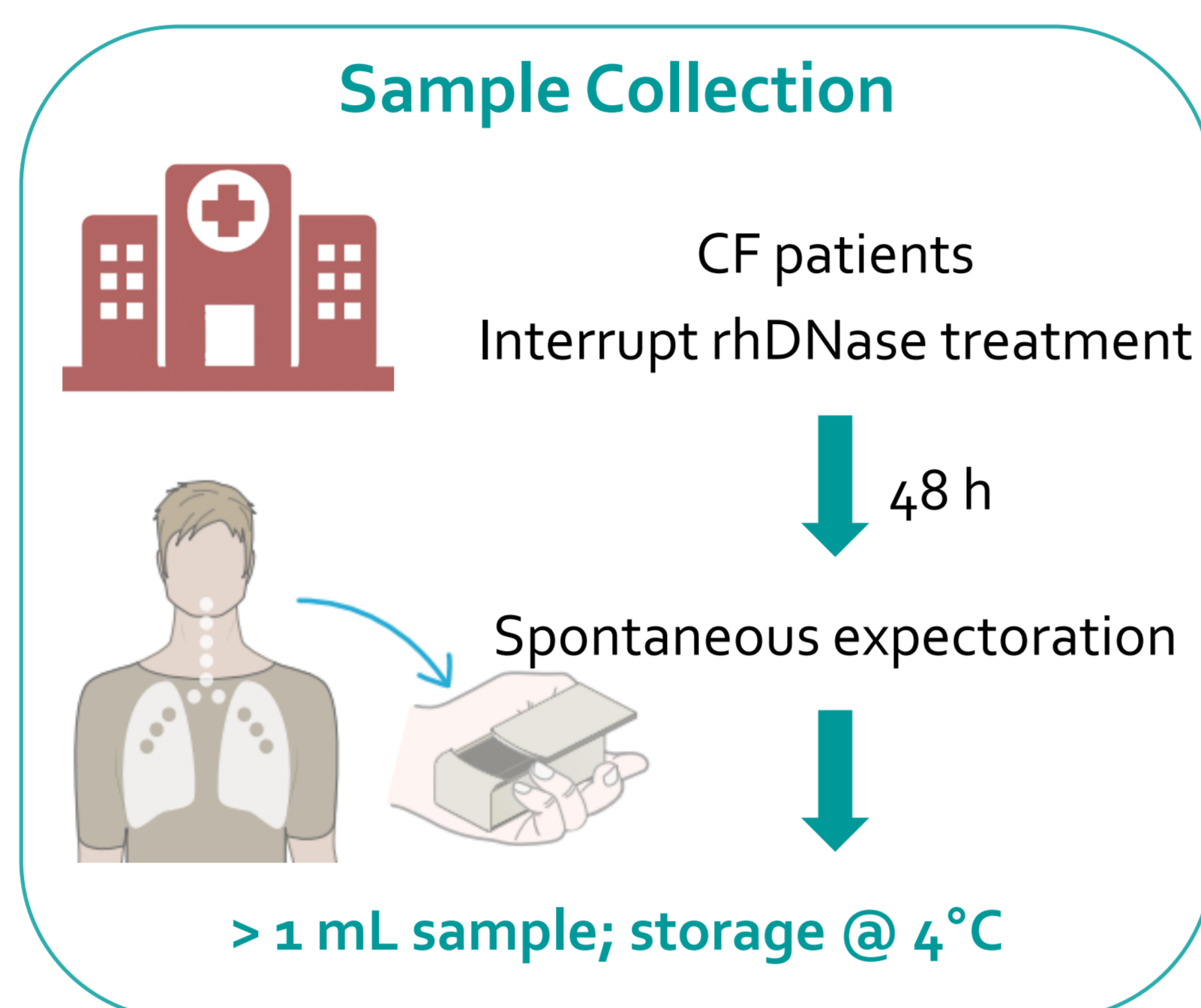
¹ Rheonova, Grenoble, France, contact@rheonova.fr; ² CRML, Hospices Civils de Lyon, Hôpital Femme Mère Enfant, Lyon, France

Context

Sputum abnormal viscoelasticity causes cystic fibrosis (CF) patients difficulties to expectorate. To promote clearance, recombinant human deoxyribonuclease I (rhDNase) is often prescribed in its purified form (Dornase alpha) as it is expected to fluidise the mucus by selectively cleaving DNA. The mucolytic efficiency of rhDNase has been assessed,¹⁻³ but neither **sputum heterogeneity** nor **sample history** were taken into consideration in previous investigations. The present study establishes a **unified rheological protocol** to validate *in vitro* the rheological effect of rhDNase on sputum.

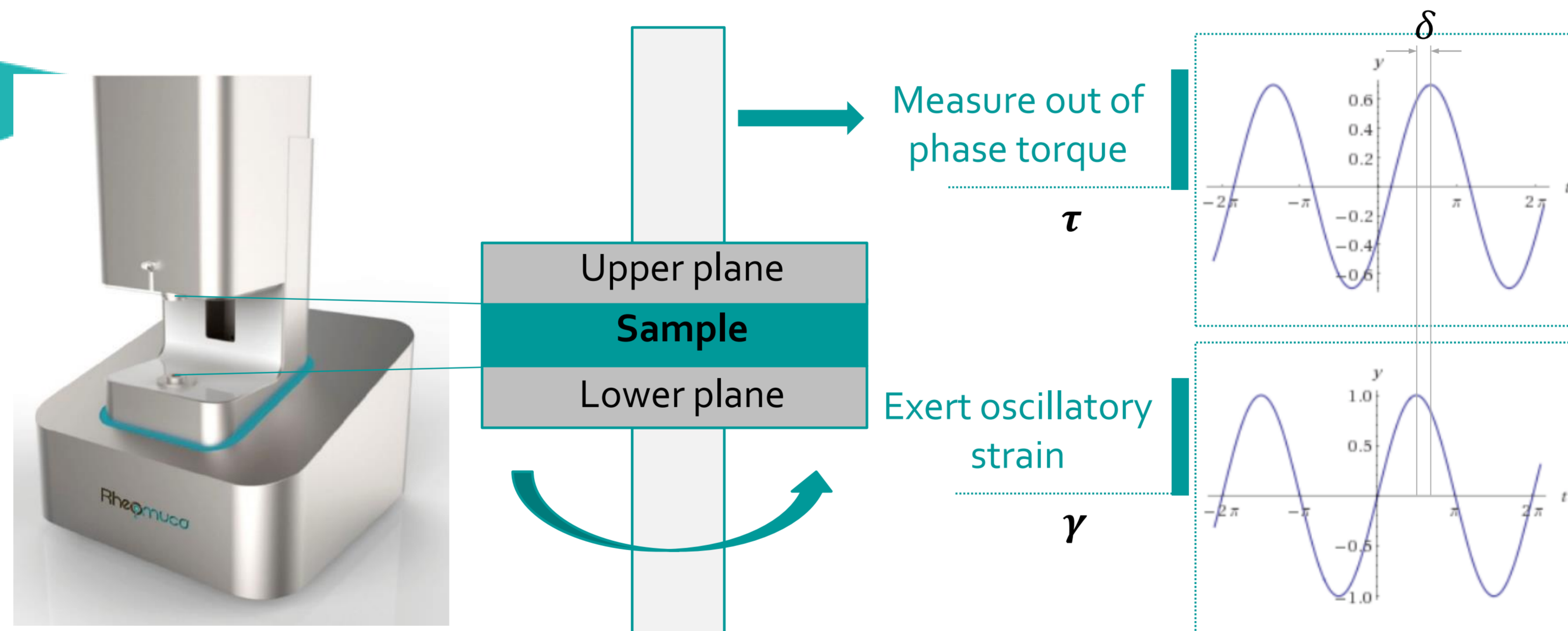
1. Shak *et al.* Recombinant human DNase I reduces the viscosity of cystic fibrosis sputum, *PNAS* **87**, 9188 (1990)
2. Zahm *et al.* Dose-dependent *in vitro* effect of recombinant human DNase on rheological and transport properties of cystic fibrosis respiratory mucus, *Eur. Respir. J.* **8**, 381 (1995)
3. King *et al.* Rheology of Cystic Fibrosis Sputum after *in vitro* Treatment with Hypertonic Saline Alone and in Combination with Recombinant Human Deoxyribonuclease I, *Am. J. Respir. Crit. Care Med.* **156**, 173 (1997)

Protocol



Rheological Measurements

Rheology is the study of the flow of matter that exhibits a combination of **elastic, viscous and plastic behaviours** by combining elasticity and fluid mechanics.



In phase contribution:

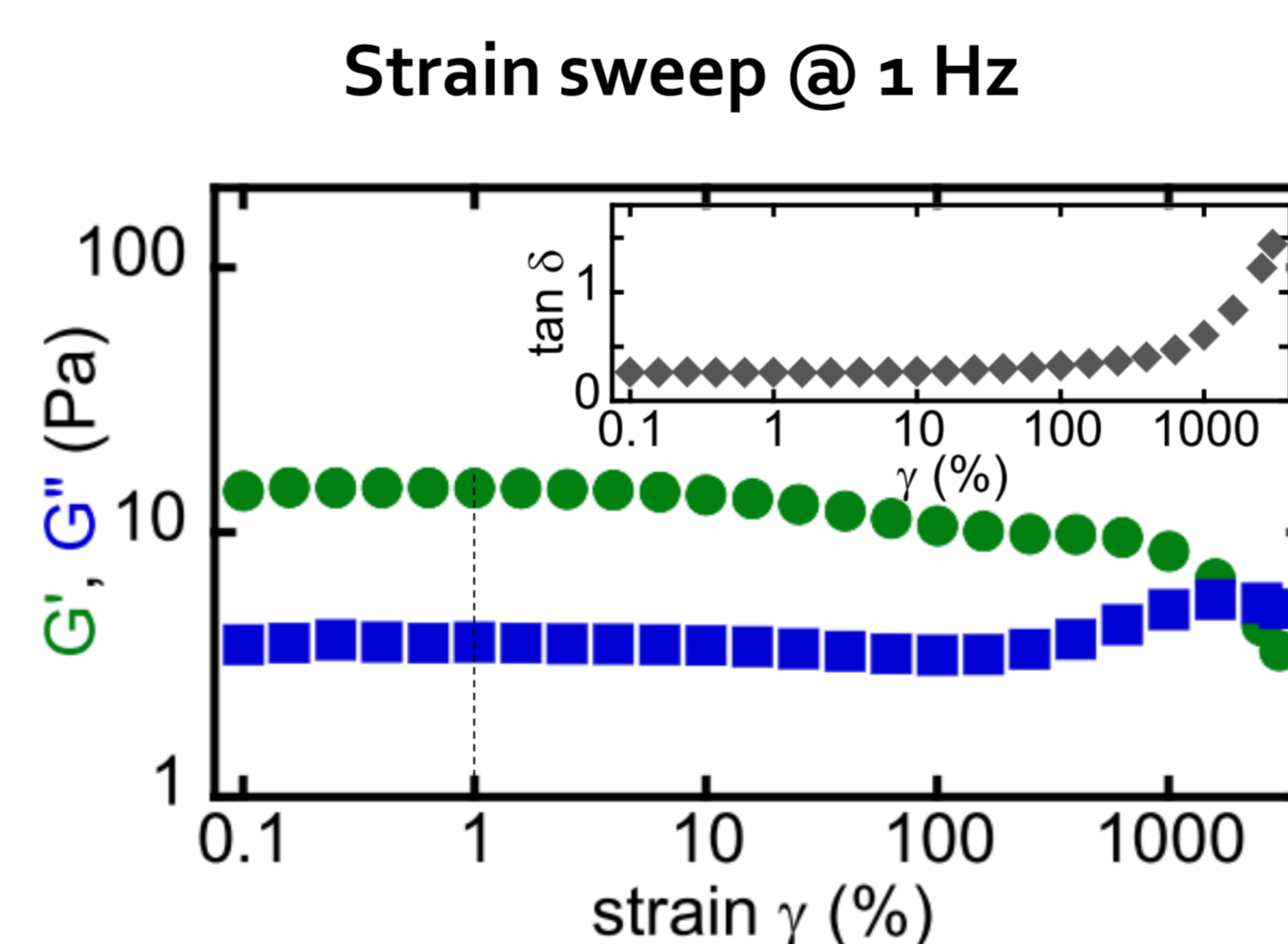
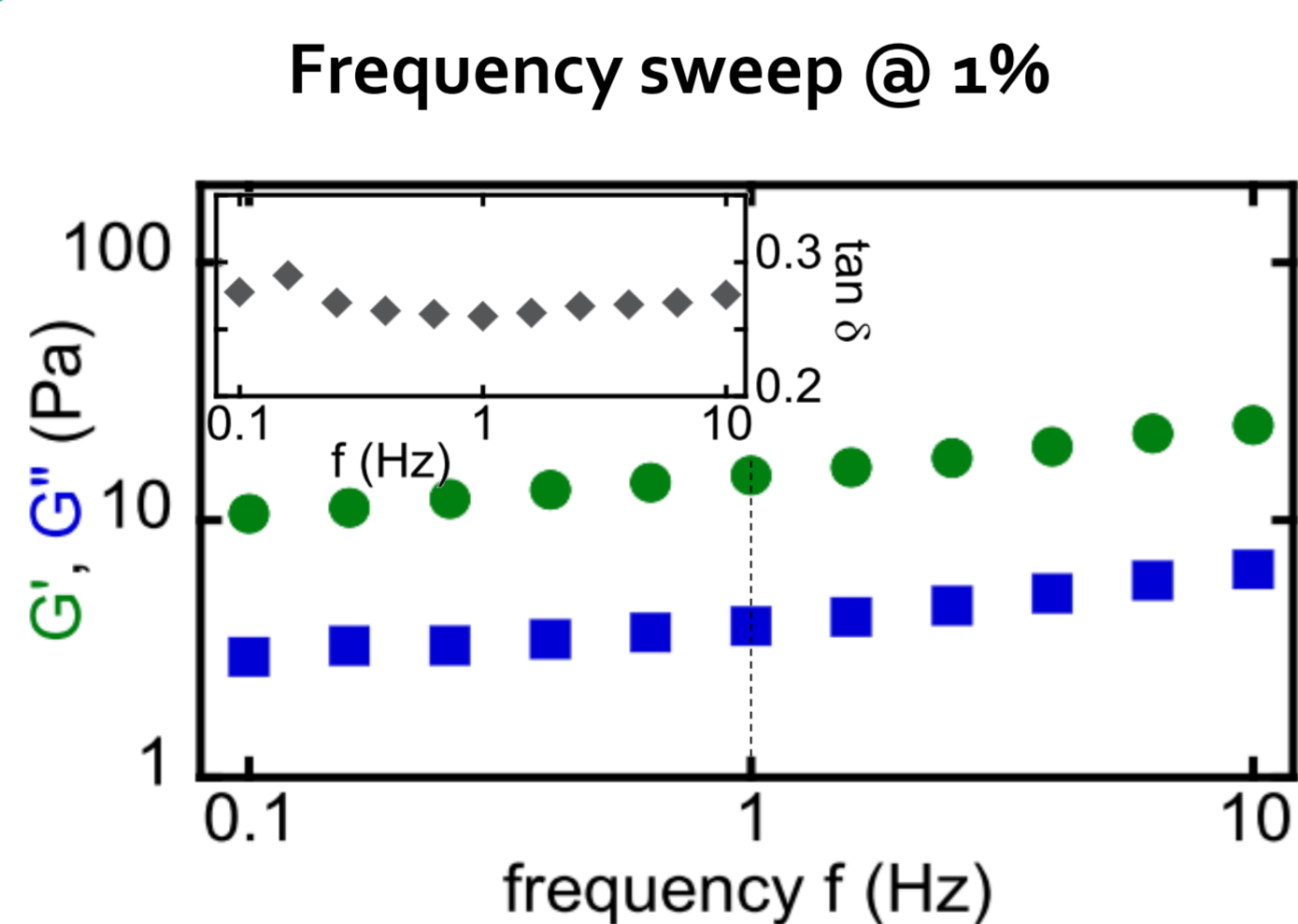
• **G' elastic modulus** $G' = \frac{\tau}{\gamma} \cos \delta$

Out of phase contribution:

• **G'' viscous modulus** $G'' = \frac{\tau}{\gamma} \sin \delta$

• **tan δ phase tangent** $\tan \delta = \frac{G''}{G'}$

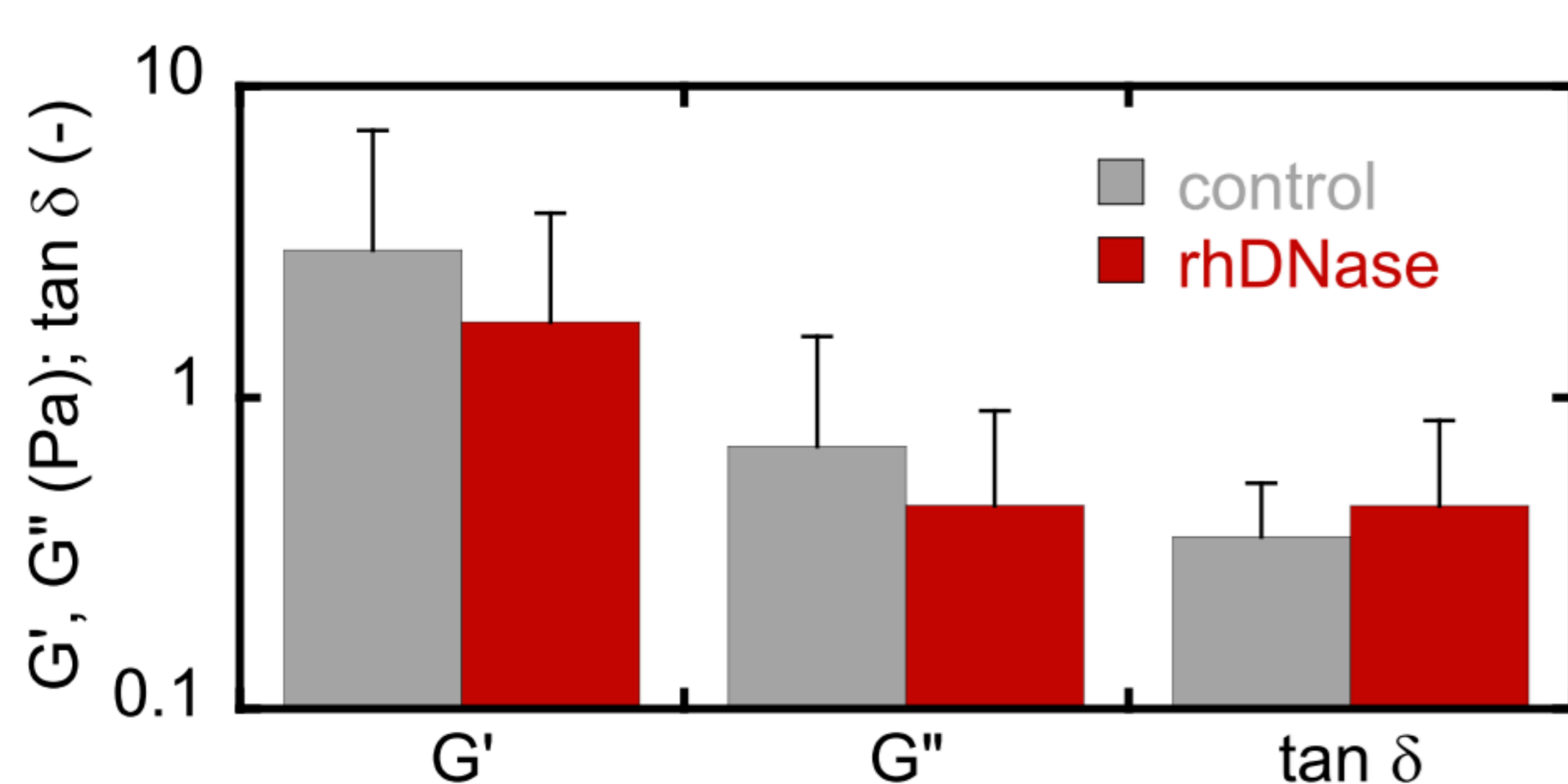
tan δ < 1 → Gel (solid-like) behaviour
tan δ > 1 → Viscous (liquid-like) behaviour



G' and G'' *a priori* depend on frequency and exerted strain.

Reference values are taken in the **linear regime** (1 Hz, 1%) where this dependency is weak.

Results



In vitro addition of rhDNase:

- Significantly **reduces the elastic modulus** G' by 41% (N = 27, p = 0.01).
- Likely **reduces the viscous modulus** G'' by 35% (N = 31, p = 0.092).
- Does not significantly affect the phase tangent (N = 23, p = 0.22).

Concluding Remarks

- 1 The **thinning effect of rhDNase** is retrieved by measuring *in vitro* the rheology of CF patients sputa. Our results are in line with those reported in highly heterogeneous sputum samples,¹⁻³ suggesting that **rhDNase likely affects both the mucus plugs and the embedding matrix** in a similar way.
- 2 Elastic and viscous moduli are both reduced in comparable proportions. **The sputum thins without globally becoming more liquid-like or gel-like.**

Perspectives

The mechanism by which rhDNase promotes clearance remains unclear with *in vitro* testing. While we evidence a global thinning effect (reduction in both G' and G''), a proper fluidisation of the mucus would imply a significant increase in tan δ, modifying the gel-like structure. Further investigations would thus be necessary to better understand the mucolytic mechanism of action.