

## Supporting Information

### Tunable layer-by-layer films containing hyaluronic acid and their interactions with CD44

Sara Amorim<sup>1,2,3,\*</sup>, Iva Paskuleva<sup>1,2</sup>, Celso A. Reis<sup>4,7</sup>, Rui L. Reis<sup>1,2,3</sup> and Ricardo A. Pires<sup>1,2,3,\*</sup>

<sup>1</sup> 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Guimarães, Portugal;

<sup>2</sup> ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal;

<sup>3</sup> The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Avepark, 4805-017 Barco, Guimarães, Portugal;

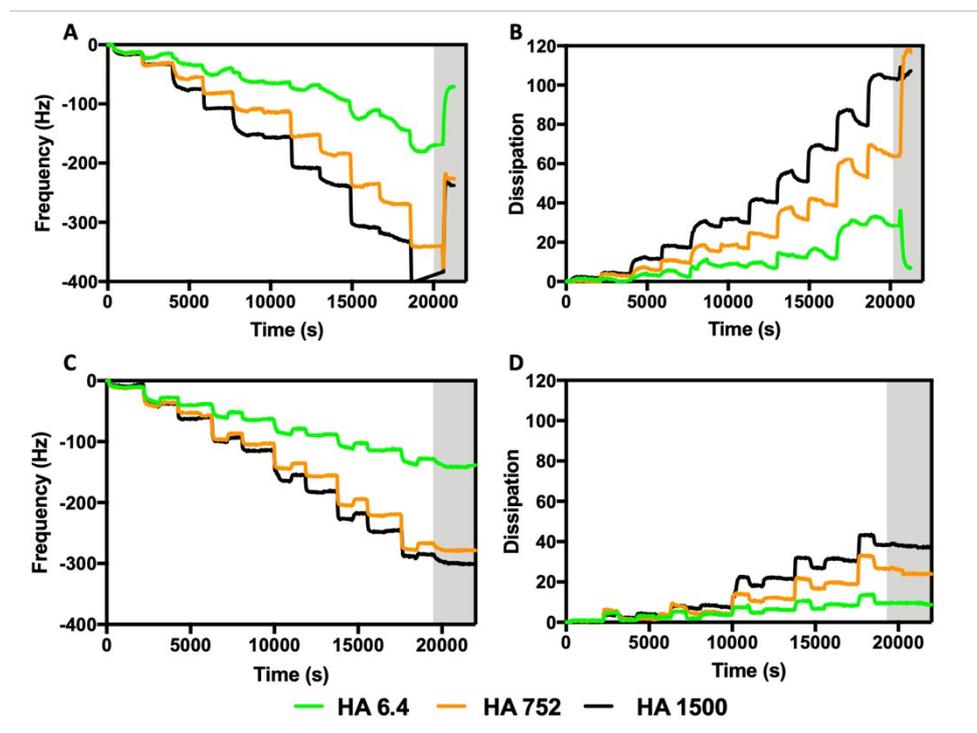
<sup>4</sup> i3S, University of Porto, Portugal;

<sup>5</sup> IPATIMUP, Porto, Portugal;

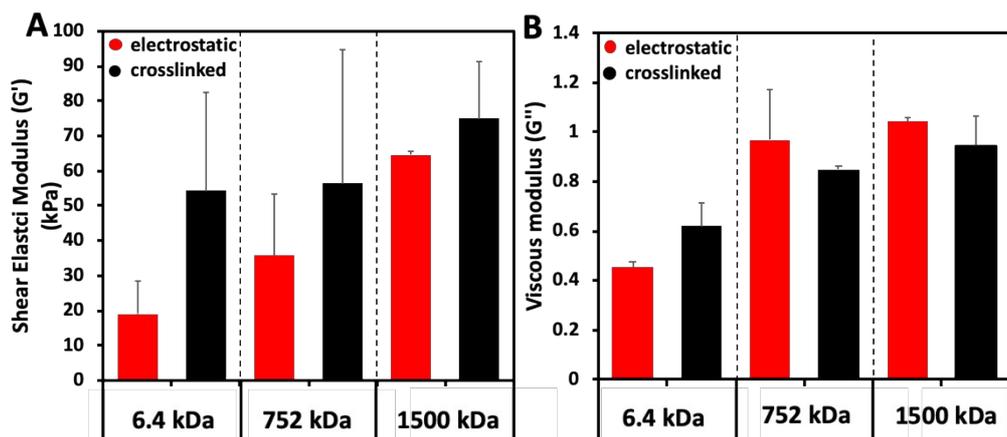
<sup>6</sup> Department of Pathology and Oncology, Faculty of Medicine, Porto University, Portugal;

<sup>7</sup> Institute of Biomedical Sciences Abel Salazar, University of Porto, Portugal.

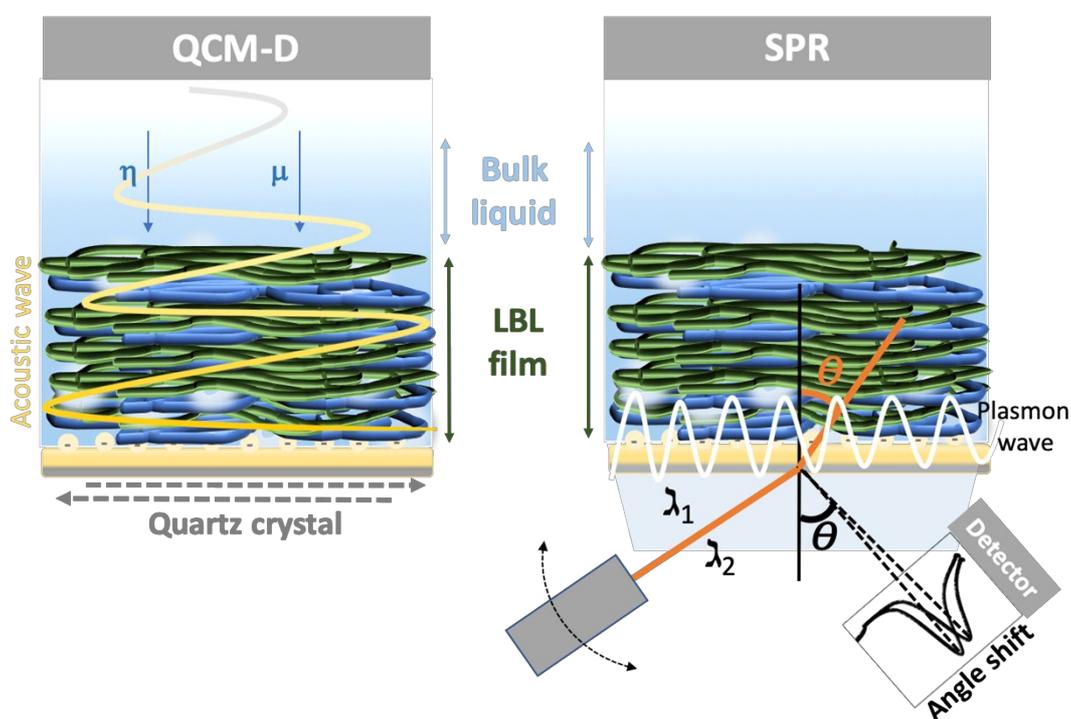
\* Corresponding authors: [rpires@i3bs.uminho.pt](mailto:rpires@i3bs.uminho.pt); [sara.amorim@i3bs.uminho.pt](mailto:sara.amorim@i3bs.uminho.pt)



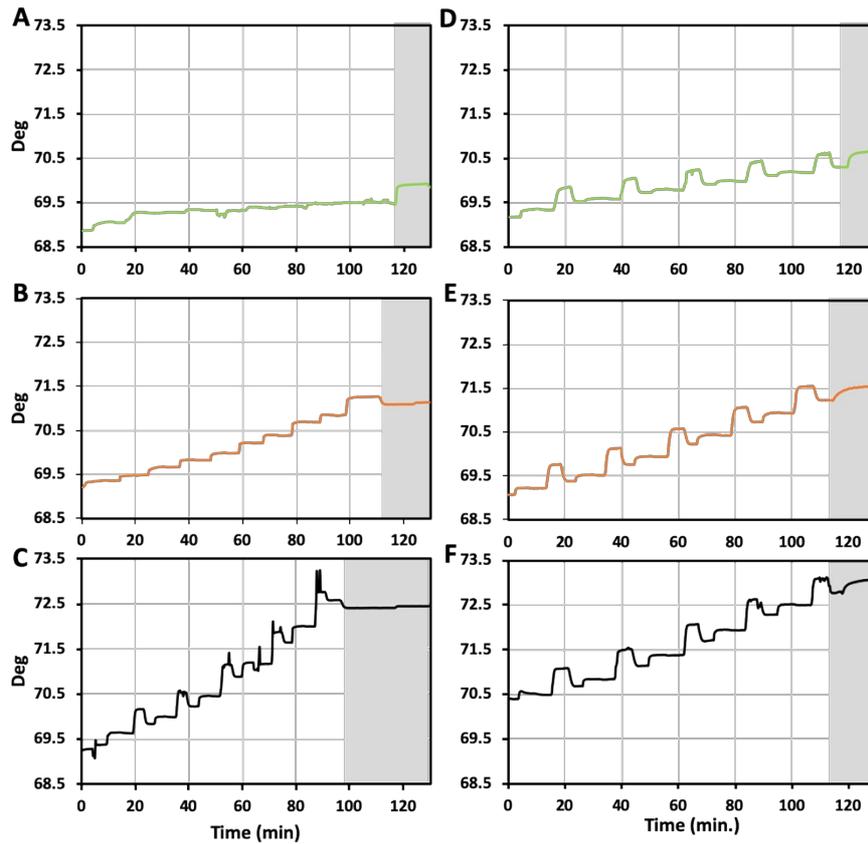
**Figure S1.** QCM-D real-time frequency and dissipation shifts for the 7<sup>th</sup> overtone, for the adsorption of PLL (30-70kDa) and HA (6.4, 752 and 1500kDa) without (A and B) and with (C and D) covalent crosslinking. Hist-tag CD44 adsorption (10 $\mu$ g/mL) after the buildup of the ten layers' film (grey zone).



**Figure S2.** Shear elastic modulus (A) and viscous modulus (B) of LbL assembly generating using HA of different  $M_w$ s, with and without covalent crosslinking. Mechanical properties obtained from Voigt modelling of the QCM-D data.



**Figure S3.** Graphical representation of the experimental design of the QCM-D and SPR techniques. In a QCM-D experiment, an acoustic sensor under a liquid environment oscillates during a specific timeframe. Shifts in the oscillation frequency are dependent on the adsorbed mass. The dissipation of the mechanical energy stored in the adsorbed layer, due to the flexibility of the molecules is related with its viscoelastic properties [1]. In the SPR measurement, the angle of incidence ( $\theta$ ) of a laser beam is altered due to changes in the refractive index ( $n$ ) near a metal surface, *i.e.* a gold layer, in response to the adsorption of a material (or the presence of a different media). Changes in  $n$  at the sensor's surface, promoted by fluctuations of the media or by the adsorption of a material, leads to a loss of the reflected light intensity originating a shift in the SPR angle[2, 3].



**Figure S4.** SPR real-time adsorption of PLL and HA (white zone), followed by CD44 protein injection (grey zone). Sensograms for PLL 30-70kDa without (**A**, **B** and **C**) and with (**D**, **E** and **F**) covalent crosslinking, using the HA of 6.4kDa (**A** and **D**), 752kDa (**B** and **E**) and 1500kDa (**C** and **F**).

**Table S1.** Theoretical radius of gyration ( $R_g$ ) and hydrodynamic radius ( $R_h$ ) of HA, as a function of its molecular weight, calculated from Eq. 3 and 4, respectively.

	HA6.4	HA752	HA1500
$R_g$ (nm)	6.8	102.4	151.9
$R_h$ (nm)	2.8	56.4	87.2

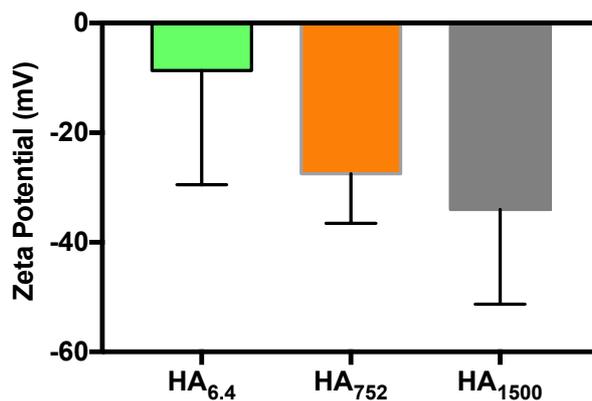


Figure S5. Zeta Potential of the LbL films using HA of  $M_w$ s.

## References

1. Sadman, K., et al., *Quantitative Rheometry of Thin Soft Materials Using the Quartz Crystal Microbalance with Dissipation*. Analytical Chemistry, 2018. **90**(6): p. 4079-4088.
2. Jung, L.S., et al., *Quantitative interpretation of the response of surface plasmon resonance sensors to adsorbed films*. Langmuir, 1998. **14**(19): p. 5636-5648.
3. Tang, Y.J., X.Q. Zeng, and J. Liang, *Surface Plasmon Resonance: An Introduction to a Surface Spectroscopy Technique*. Journal of Chemical Education, 2010. **87**(7): p. 742-746.