

# Macromolecule-network electrostatics controlling delivery of the bio-therapeutic cell modulator TIMP-2

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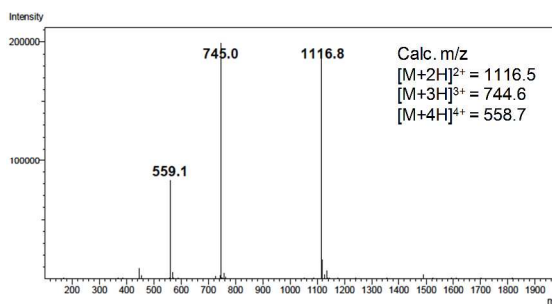
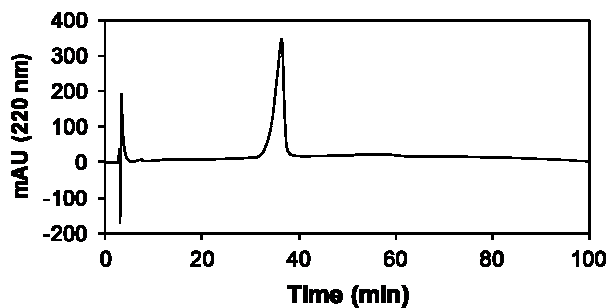
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<sup>§</sup>These authors contributed equally to this study.

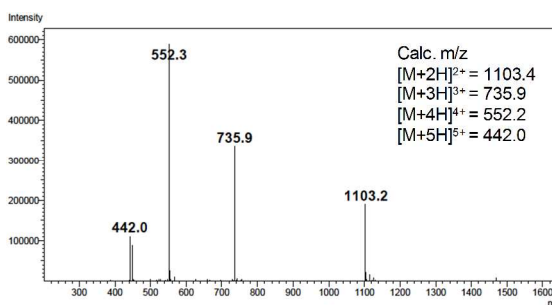
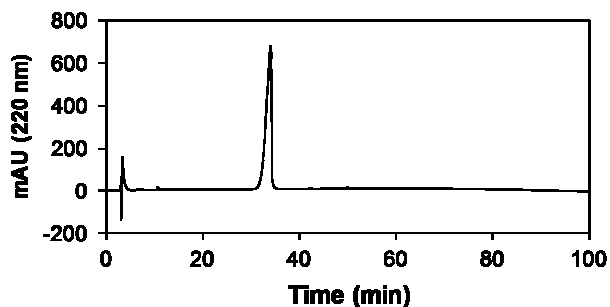
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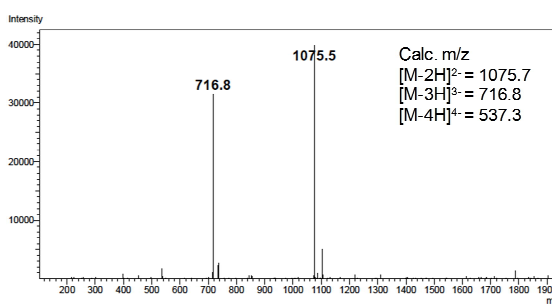
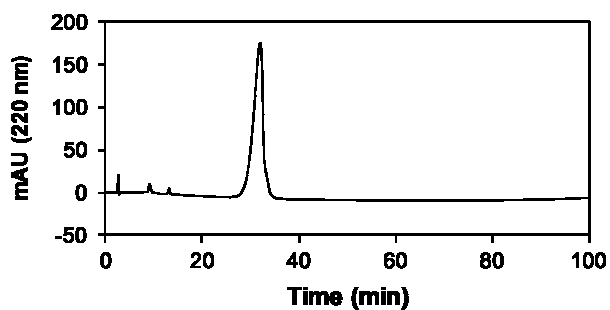
### A. MAX8



### B. HLT2



### C. AcVES3



### D. IE1

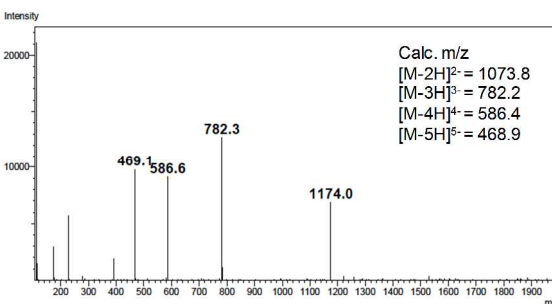
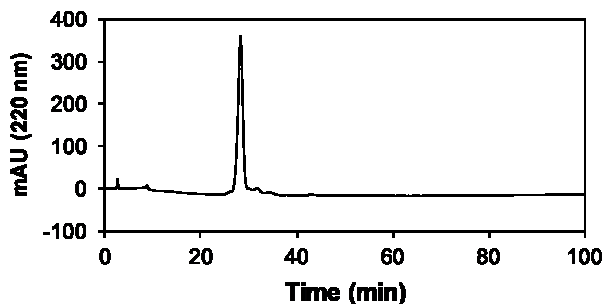
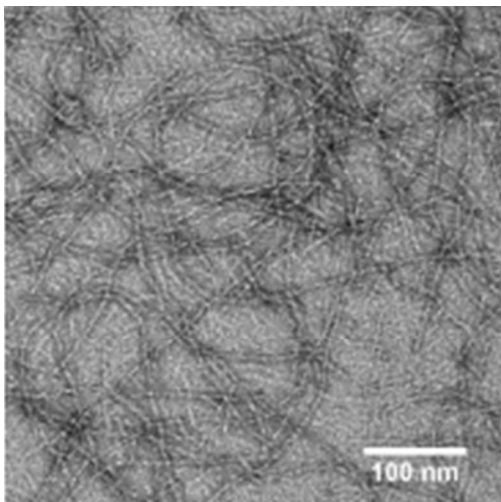
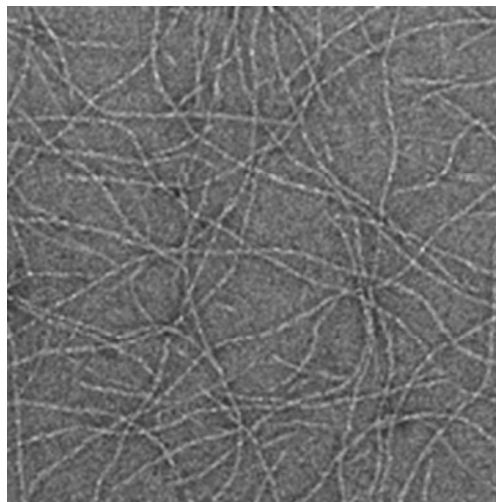


Figure S1. Analytical HPLC and ESI-mass spectra of MAX8 (A), HLT2 (B), AcVES3 (C), and IE1 (D).

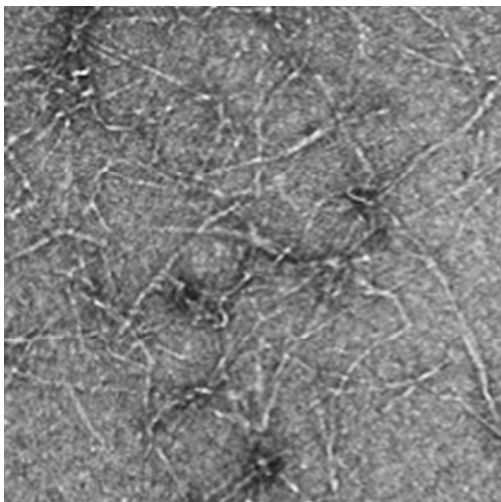
**A. MAX8**



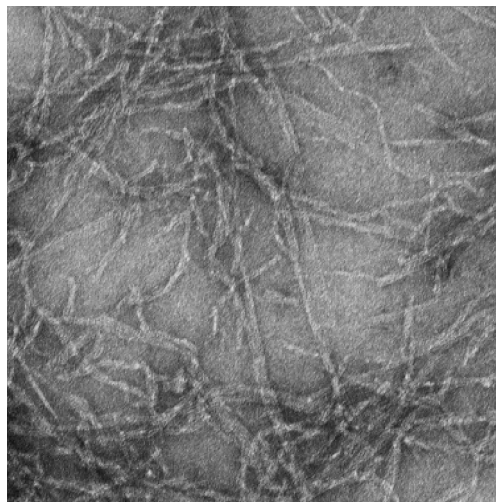
**B. HLT2**



**C. AcVES3**



**D. IE1**



**Figure S2.** TEM images of peptide fibrils, MAX8 (A), HLT2 (B), AcVES3 (C), and IE1 (D). MAX8 and HLT2 images were adapted from doi: 10.1016/j.biomaterials.2012.06.097.

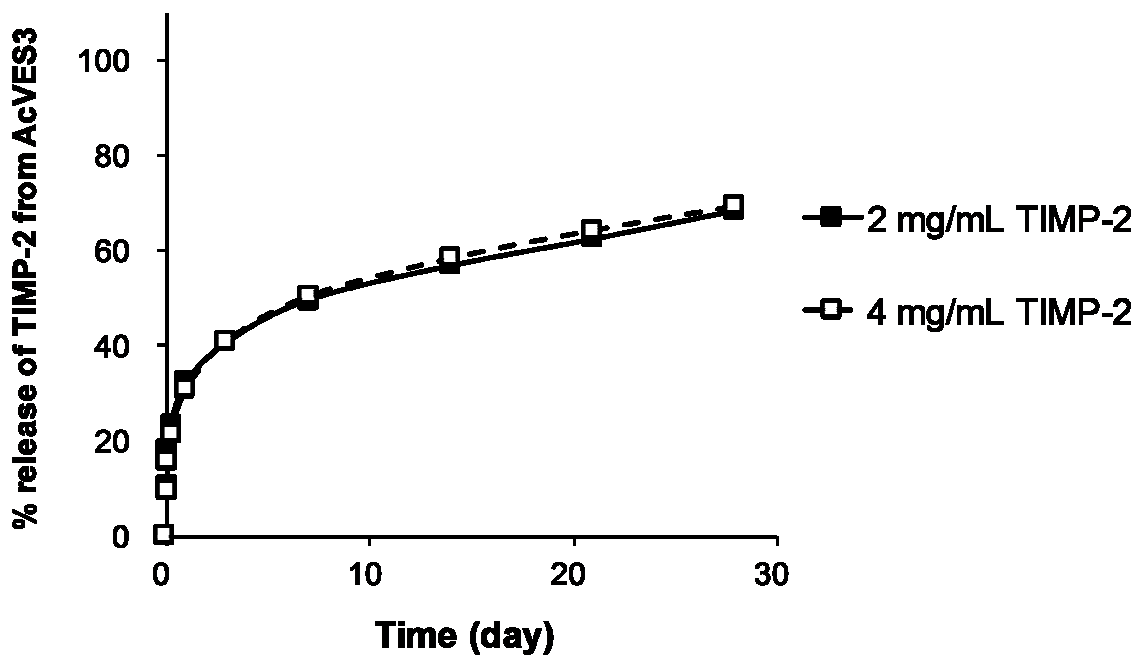
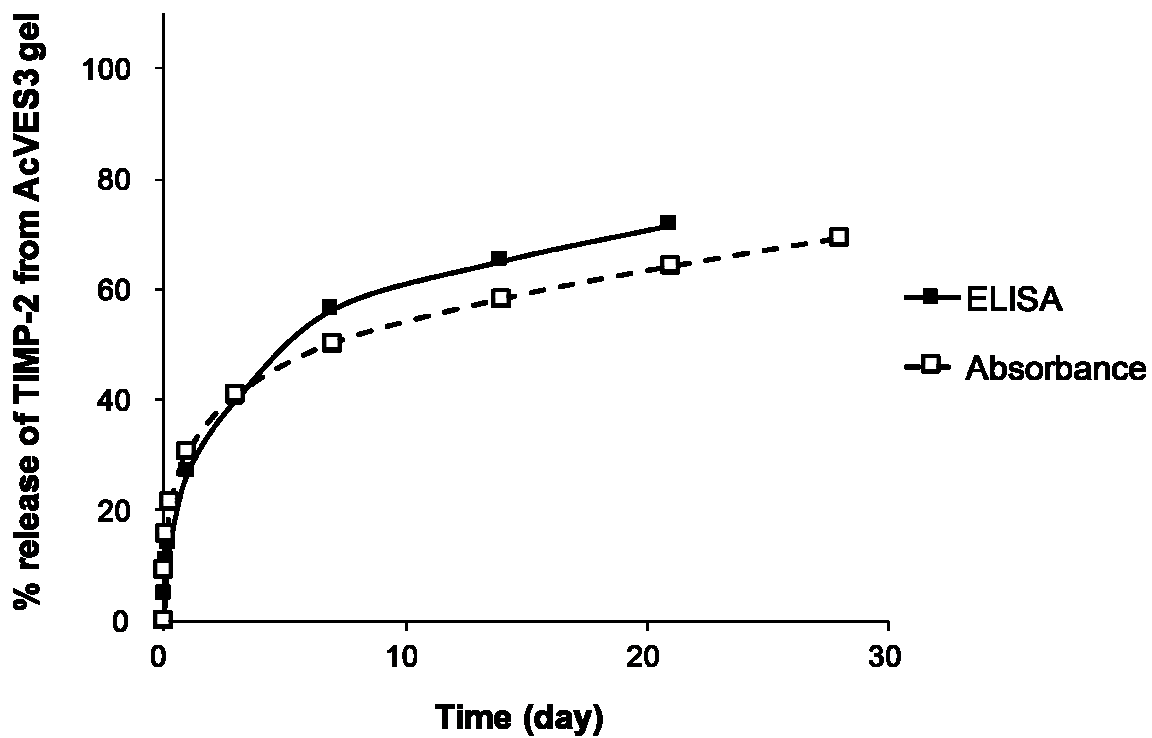
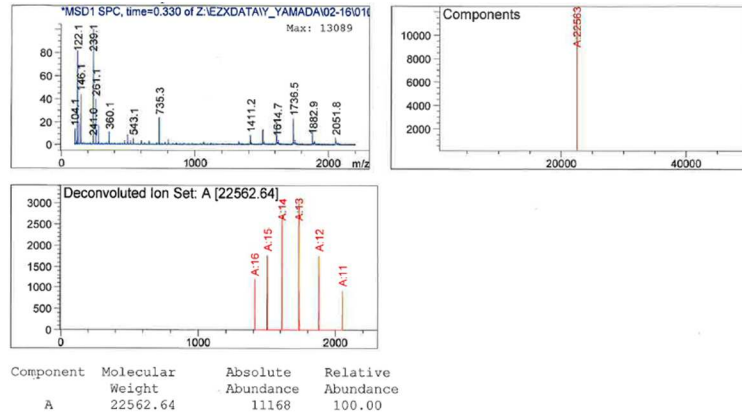


Figure S3. Cumulative release profiles of 2 mg/mL and 4 mg/mL TIMP-2 from AcVES3 gel.

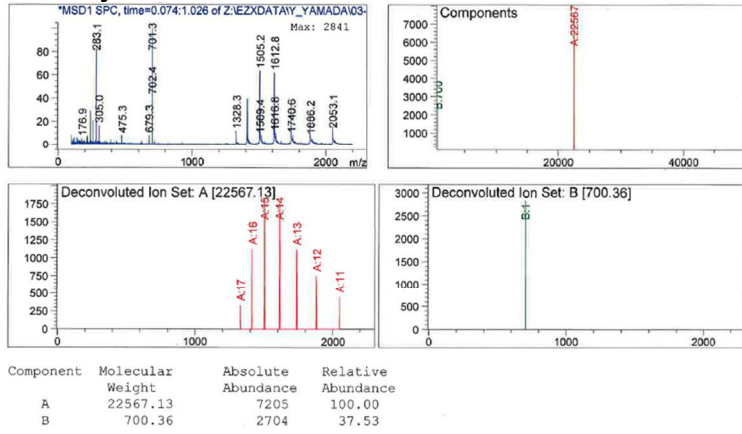


**Figure S4.** Cumulative release profiles of 4 mg/mL TIMP-2 from AcVES3 gel measured by ELISA vs. absorbance.

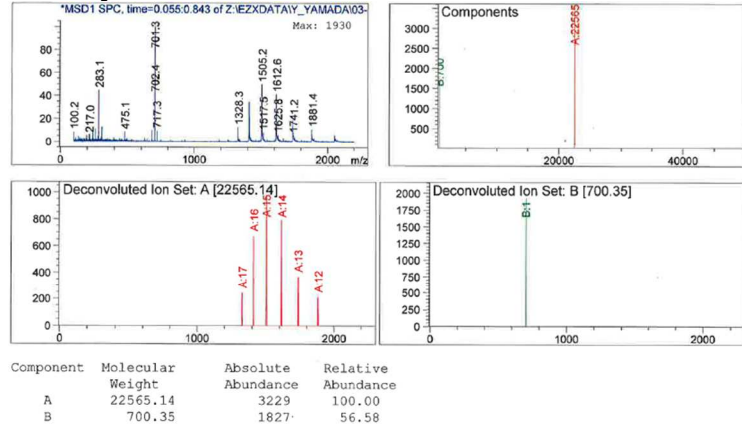
## A. Control TIMP-2



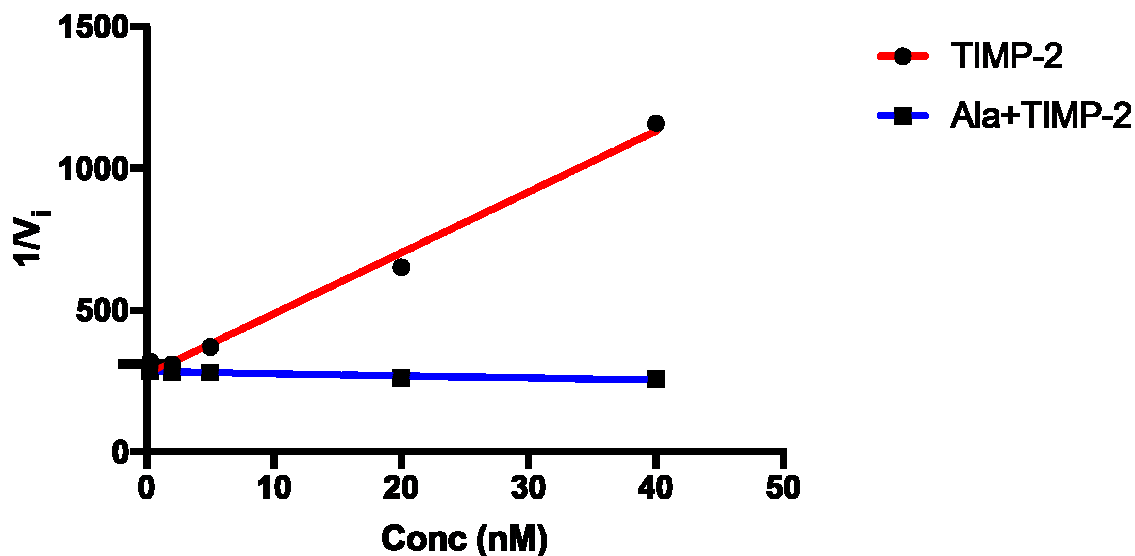
## B. Day 7



## C. Day 28



**Figure S5.** Deconvoluted mass spectra of control TIMP-2 (A) and released TIMP-2 from AcVES3 gel collected on day 7 (B) and day 28 (C). The average mass of TIMP-2 used in this study is 22565.6.



**Figure S6.** Dixon plot of MMP-2 Inhibition kinetics by TIMP-2 (red) and Ala+TIMP-2 (blue). The major tick at  $1/V_i=310$  indicates the reciprocal of the average of velocity of the control reaction (without TIMP-2). The lack of a negative slope for the Ala+TIMP-2 measurements is indicative of a complete absence of MMP-2 inhibitory activity in this assay. These findings are consistent with previous findings of a significantly decrease in  $K_{iApp}$  for this TIMP-2 mutant form, see reference <sup>62</sup>.

[1] Wingfield, P. T. P., Sax, J. K. J., Stahl, S. J. S., Kaufman, J. J., Palmer, I. I., Chung, V. V., Corcoran, M. L. M., Kleiner, D. E. D., and Stetler-Stevenson, W. G. W. (1999) Biophysical and functional characterization of full-length, recombinant human tissue inhibitor of metalloproteinases-2 (TIMP-2) produced in *Escherichia coli*. Comparison of wild type and amino-terminal alanine appended variant with implications for the mechanism of TIMP functions., *The Journal of biological chemistry* 274, 21362-21368.