Supplementary Information

Engineering of Biofilms with a Glycosylation Circuit for Biomaterial Applications

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Supplementary Table 1. List of primers used in this study.

DQNAT_pet22b_F	Forward	5'AAAAAACCTAGGGGCGGTAGCGGTGATCAGAACGCGACCACTAGTTGAGATCCGGCTGCTAACAA 3'
DQNAT_pet22b_R	Reverse	5' AAAAAAGAGCTCGGCCATCGCCGGCTGG 3'
TasA-DQNAT-F	Forward	5'AAAAAAGAGCTCATGGCATTTAACGACATTAAATCAAAGGATGC 3'
TasA-DQNAT-R	Reverse	5' AAAAAACCTAGGGTGGTGGTGGTGGTGGTG 3'

Supplementary Table 2. Aminoacid sequences of proteins used in this study. Color coded aminoacids correspond to genetic parts with the same color code

TasA protein with C	MAFNDIKSKDATFASGTLDLSAKENSASVNLSNLKPGDKLTKDFQFENNGSLAIKEVLMALNYGDFKANGGSNTSPEDFLSQFEVTLLTVGKEGGNGYPKNIILDDANLKDLYLMSAKNDAAAAEKIKKQIDPKFLNASGKVNVATIDGKTAPEYDGVPKTPTDFDQVQMEIQFKDDKTKDEKGLMVQNKYQGNSIKLQFSFEATQWNGLTIKKDHTDKDGYVKENEKAHSEDKNGGSGLEHHHHHH*

terminal polyhistidine tag.	
TasA-DQNAT protein with *pelB* periplasm localization signal, DQNAT glycosylation motif at the C-ter	MKYLLPTAAAGLLLLAAQPAMAELMAFNDIKSKDATFASGTLDLSAKENSASVNLSNL KPGDKLTKDFQFENNGSLAIKEVLMALNYGDFKANGGSNTSPEDFLSQFEVTLLTVG KEGGNGYPKNIILDDANLKDLYLMSAKNDAAAAEKIKKQIDPKFLNASGKVNVATIDGK TAPEYDGVPKTPTDFDQVQMEIQFKDDKTKDEKGLMVQNKYQGNSIKLQFSFEATQ WNGLTIKKDHTDKDGYVKENEKAHSEDKNGGSGLEHHHHHHPRGGSGDQNATTS*

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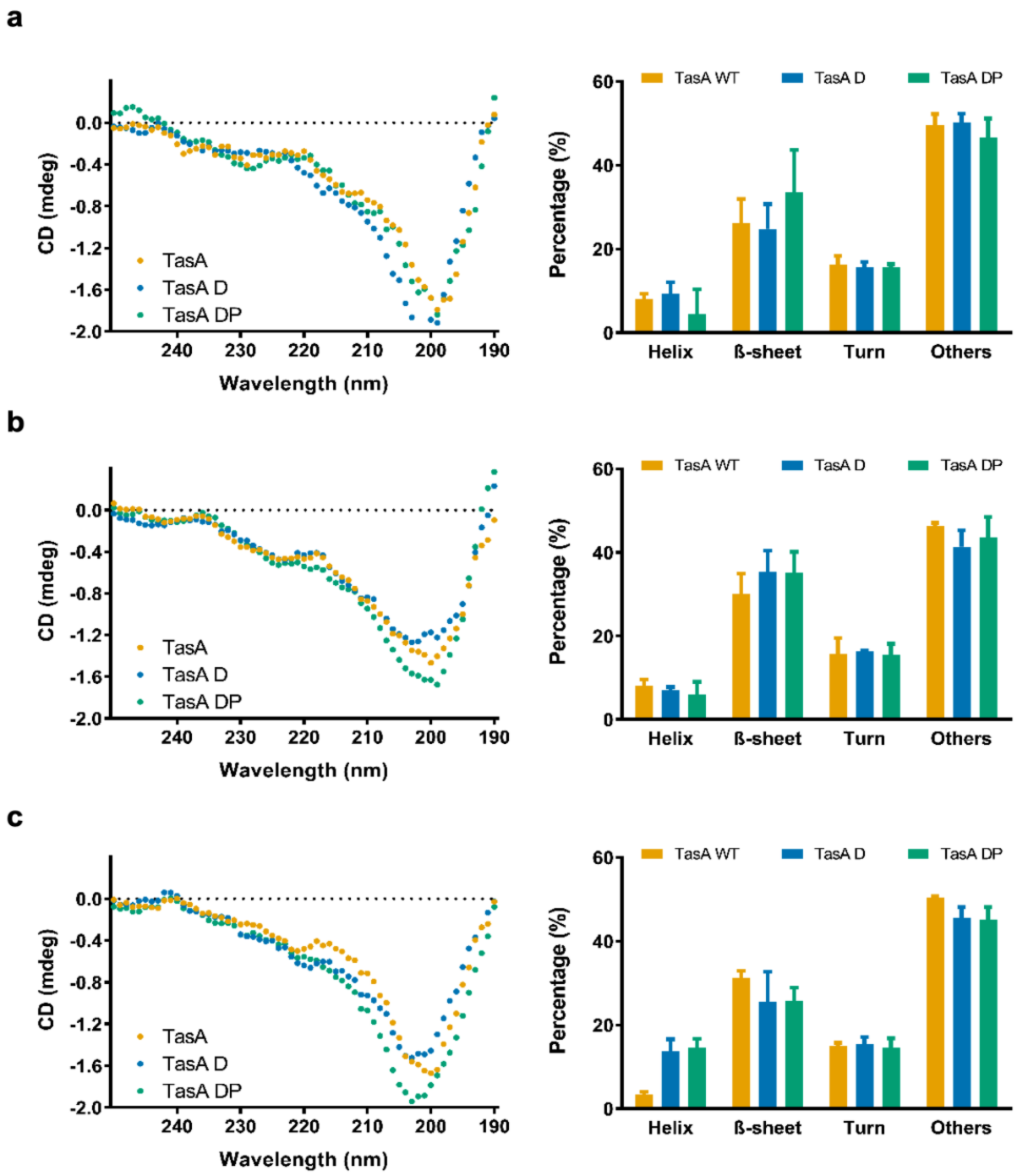
Supplementary Table 3. Desorption constant (k_d) of differently aged TasA, TasA D and TasA DP samples.

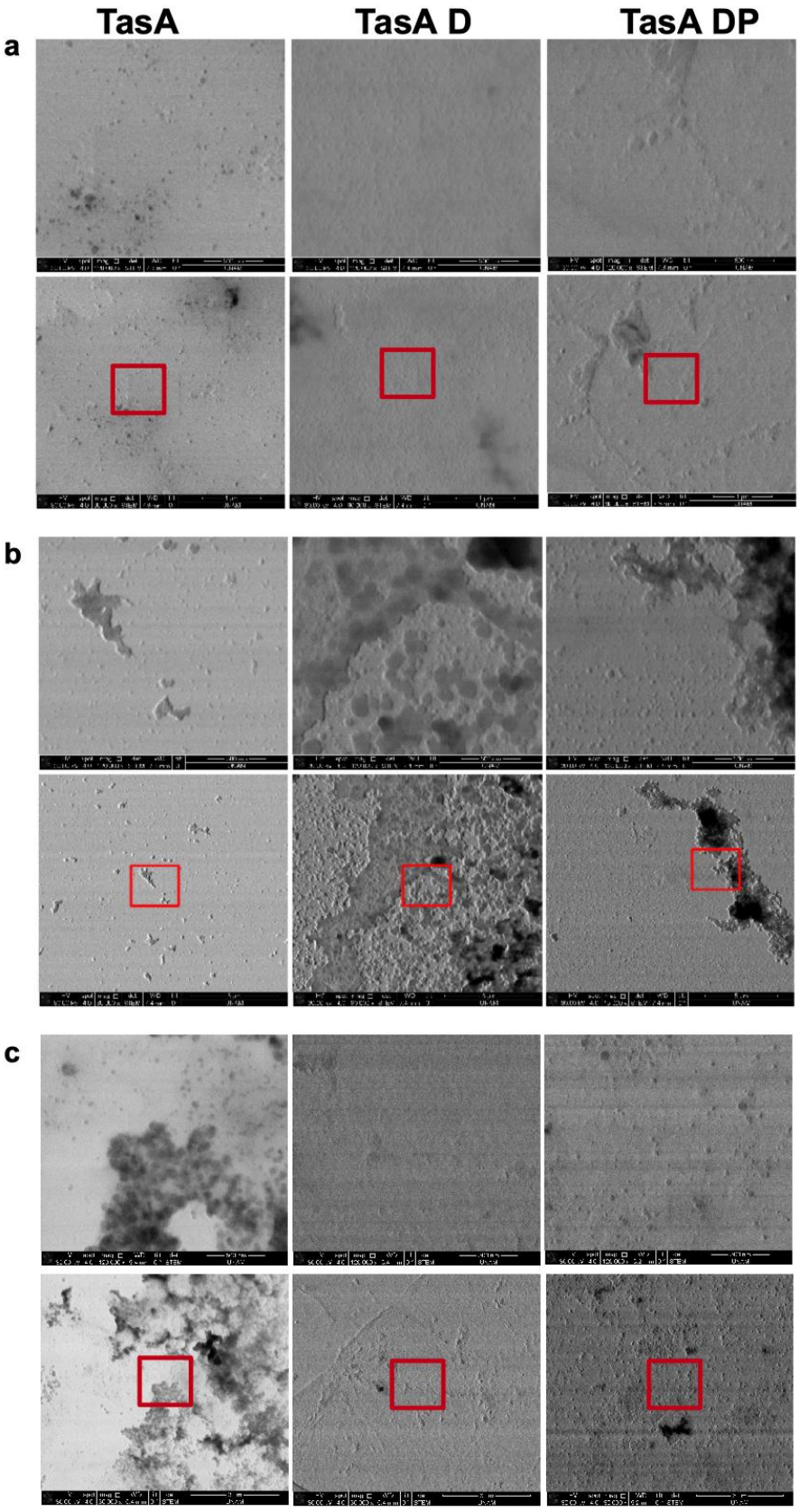
	k_d (μM), mean ± SD			
	Day 1	Day 7	Day 14	Day 28
TasA	2.94 ± 0.19	5.22 ± 0.07	5.12 ± 0.54	3.39 ± 0.09
TasA D	3.83 ± 0.06	4.24 ± 0.13	4.10 ± 0.15	3.03 ± 0.14
TasA DP	1.48 ± 0.07	1.62 ± 0.07	1.70 ± 0.18	1.32 ± 0.06

Supplementary Table 4. Gibbs free energy (ΔG^o) calculations of differently aged TasA, TasA D and TasA DP samples.

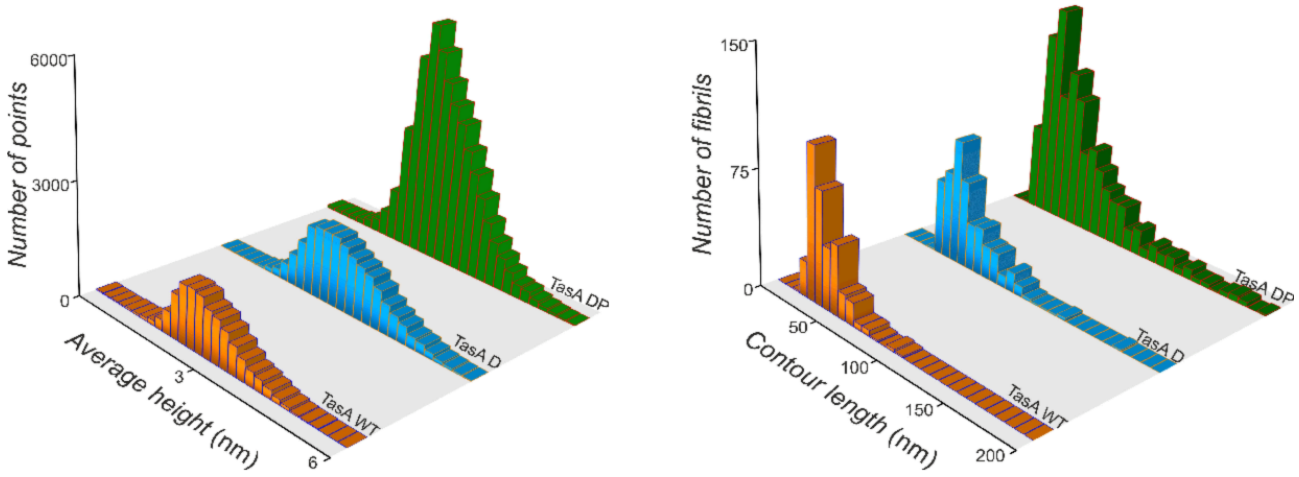
	ΔG^o (kcal/mol), mean ± SD			
	Day 1	Day 7	Day 15	Day 28
TasA	-3.28±0.02	-3.13±0.01	-3.13±0.03	-3.24±0.01
TasA D	-3.21±0.01	-3.18±0.01	-3.19±0.01	-3.27±0.01
TasA DP	-3.45±0.01	-3.43±0.01	-3.42±0.03	-3.48±0.01



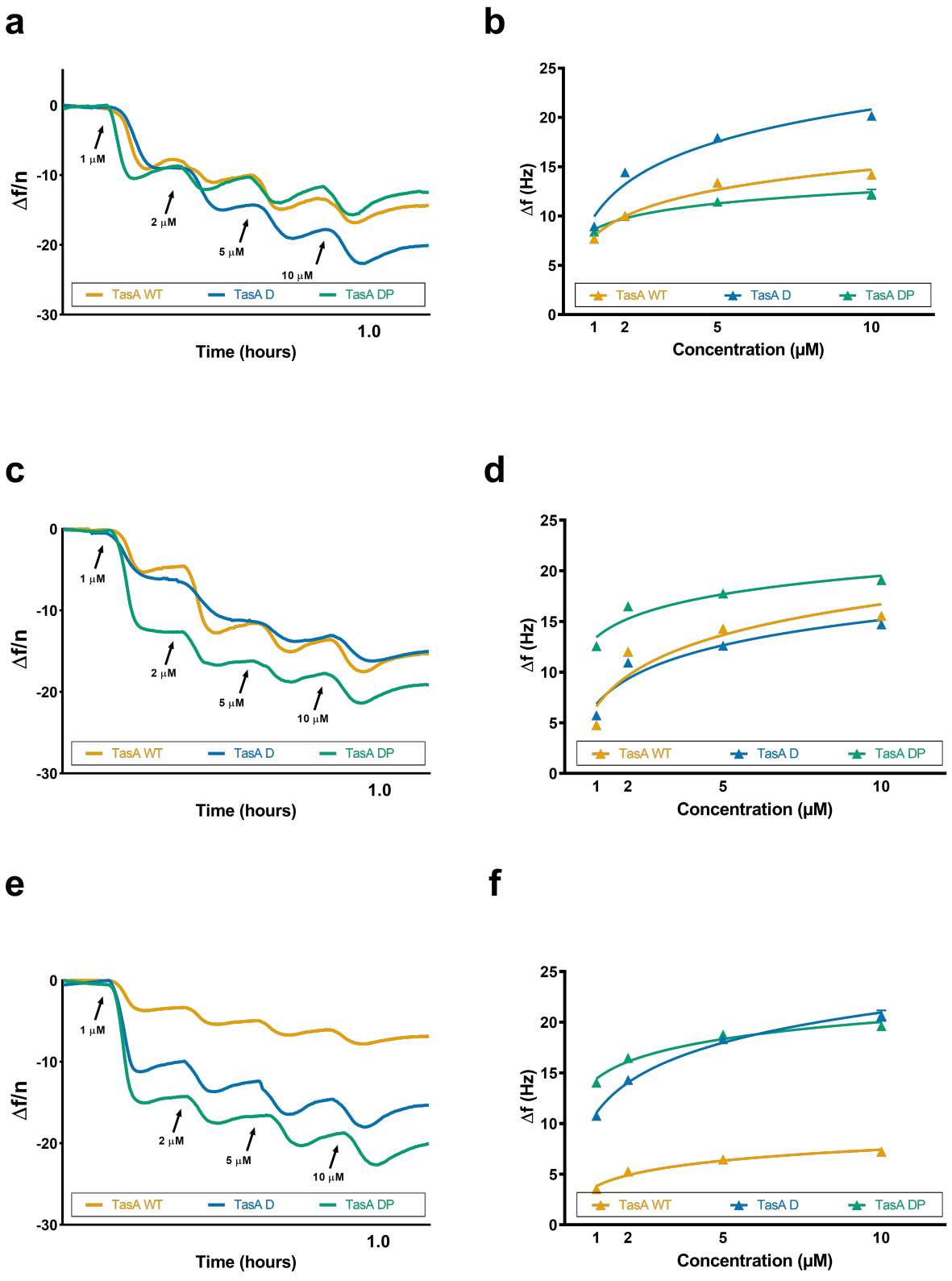
Supplementary Figure 1. Circular dichroism spectra of TasA (orange dots), TasA-D (blue dots) and TasA-DP (green dots) samples and percentages of secondary structures calculated by BestSel online tool for TasA (orange bars), TasA-D (blue bars) and TasA-DP (green bars) with aging durations of a. 1 day b. 7 days and c. 28 days. n=3.



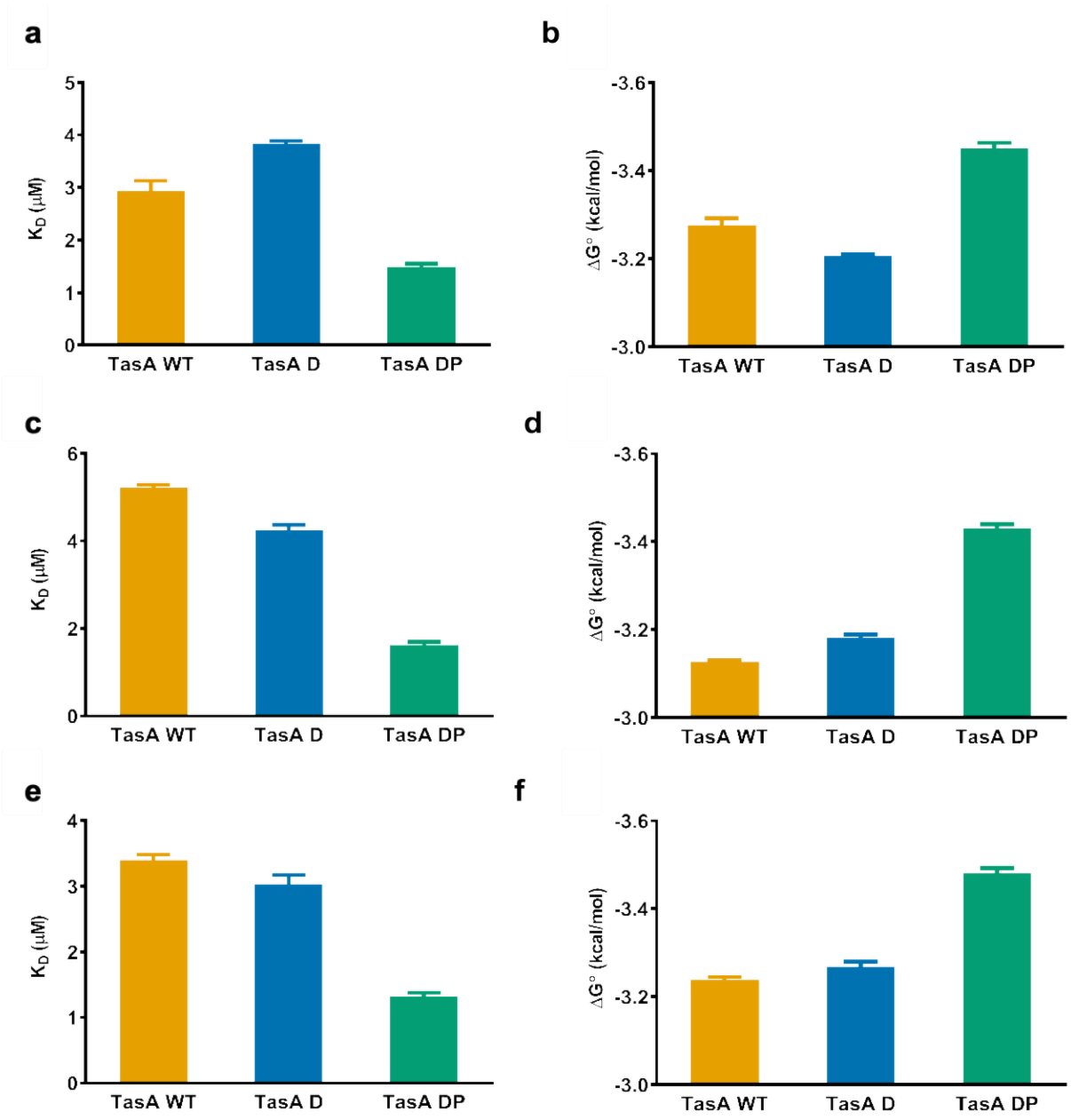
Supplementary Figure 2. STEM of images of a. 1 day b. 7 days and c. 28 days old TasA, TasA D and TasA DP samples.



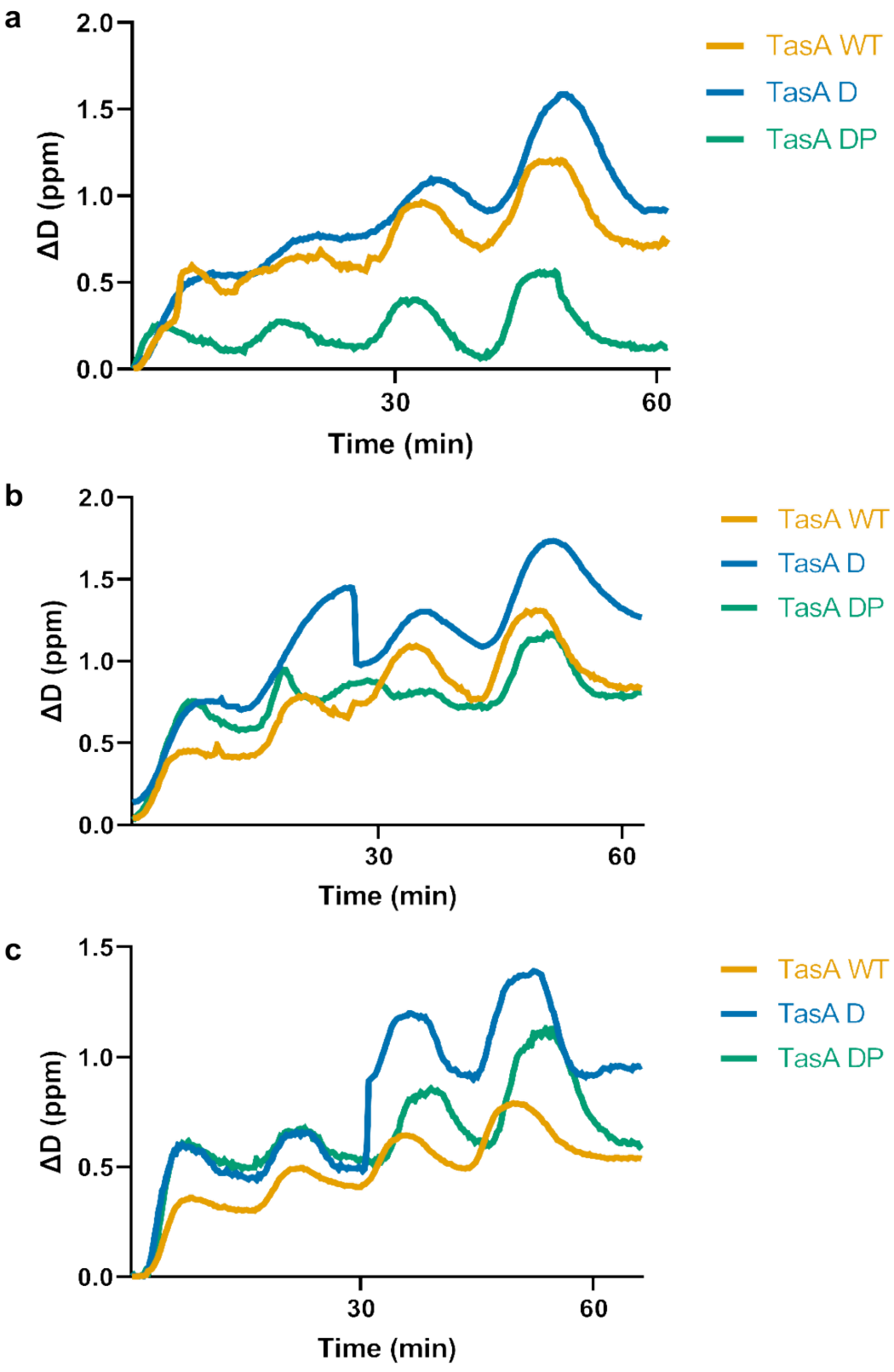
Supplementary Figure 3. Morphological characterization of 28 days old TasA, TasA D and TasA DP fibrils using Atomic force microscopy (AFM). AFM images analyzed by FiberApp software for quantification of average fibril height and contour length.



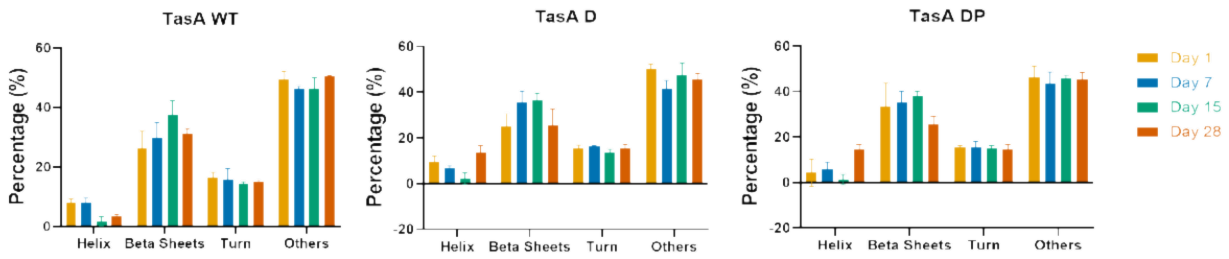
Supplementary Figure 4. QCM frequency shifts with respect to time and with respect to protein concentration for (a-b). 1 day old, (c-d). 7 days old and (e-f). 28 days old samples.



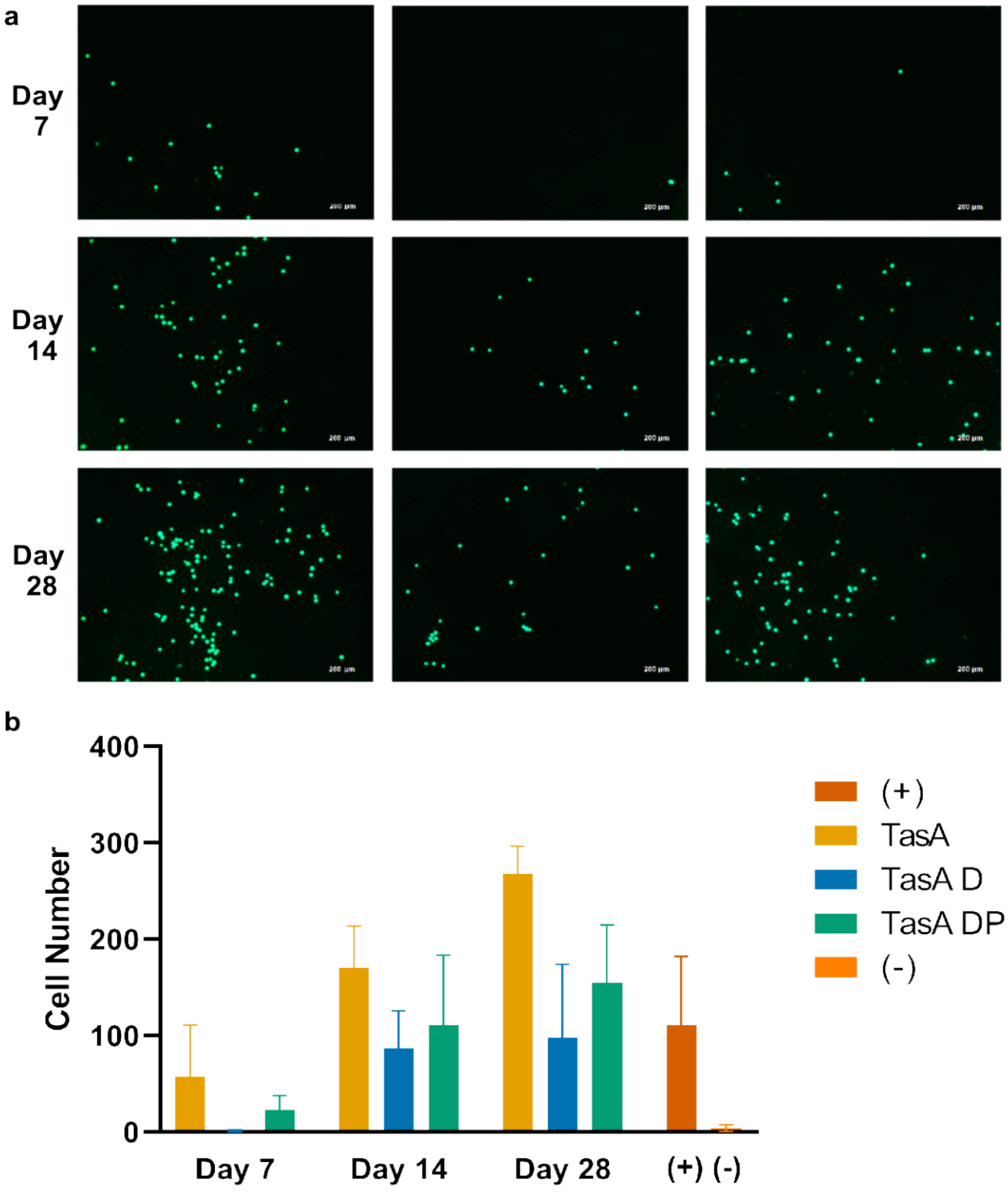
Supplementary Figure 5. k_d and ΔG^o graphs of (a-b). 1 day old, (c-d). 7 days old and (e-f). 28 days old samples. n=3.



Supplementary Figure 6. The recorded dissipation shifts in QCM-D upon adsorption with increasing concentrations of TasA, TasA-D and TasA-DP proteins with aging durations of a. 1 day b. 7 days and c. 28 days.



Supplementary Figure 7. Percentages of secondary structures calculated by BestSel online tool from CD data of TasA, TasA-D and TasA-DP with different aging durations. n=3.



Supplementary Figure 8. Adhesion of HEK293 cells on surfaces coated with differently aged TasA, TasA D and TasA DP. a. Representative images of Calcein AM labelled adhered cells and b. Quantitative analysis of adhered number of cells, n=3.