Electronic Supplementary Material (ESI) for Soft Matter.

Quantitative analysis of biomolecule release from polystyrene-block-polyethylene oxide thin films

Matthew S. Horrocks[1,2†], Tarek Kollmetz[1,2†], Padraic O'Reilly[3], Derek Nowak[3], Jenny Malmström[1,2*]

† These authors contributed equally to the work

* Corresponding author (j.malmstrom@auckland.ac.nz)

1. Chemical and Materials Engineering, The University of Auckland, New Zealand

2. The MacDiarmid Institute for Advanced Materials and Nanotechnology, Wellington, New Zealand

3. Molecular Vista, San Jose, CA, USA

Supplementary Information

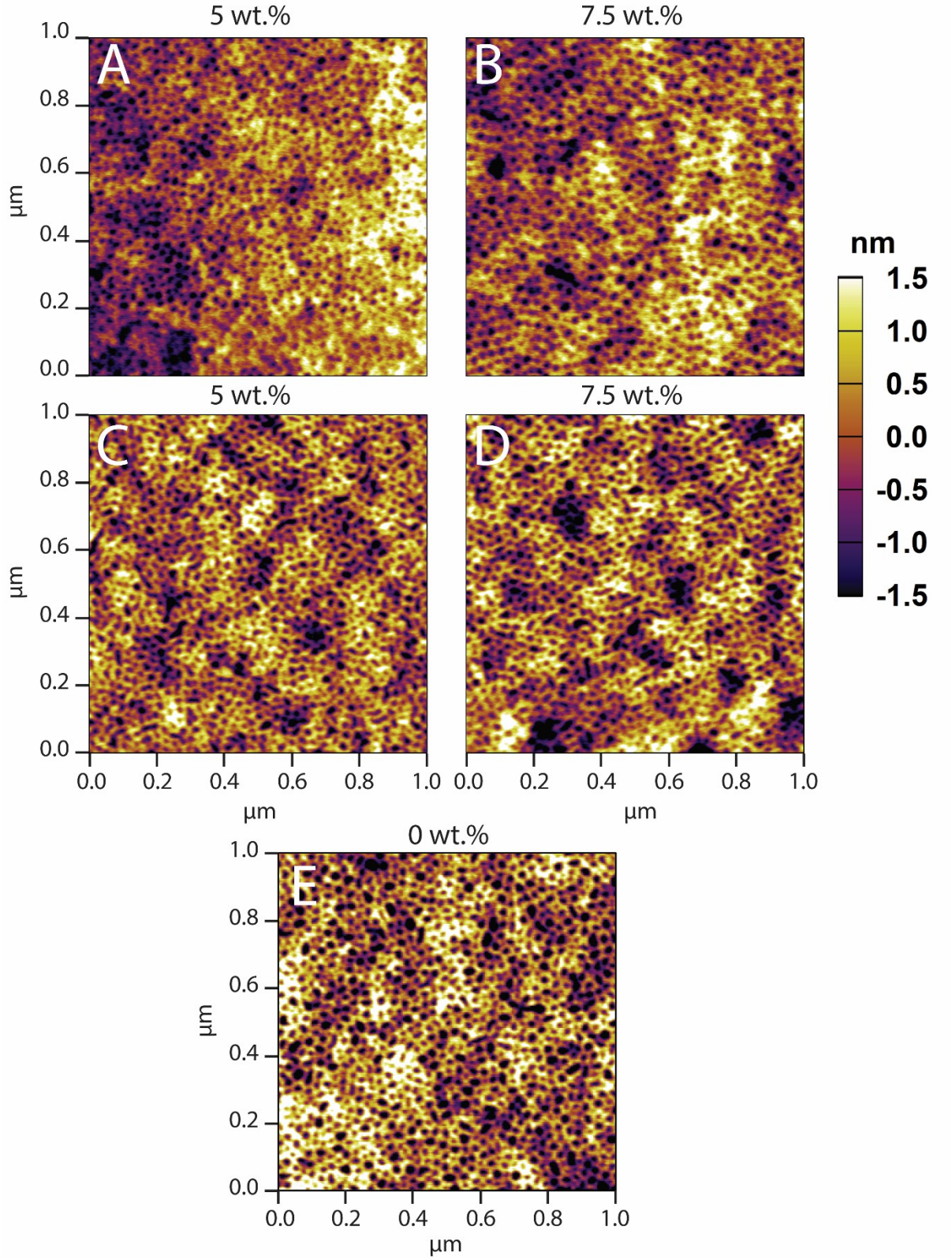

Figure S 1 AFM images for (A, B) PS-b-PEO films co-assembled with LZ, (C, D) PS-b-PEO films co-assembled with PEG-LZ, for 5 wt.% and 7.5 wt.% loading, and also films without cargo (E).

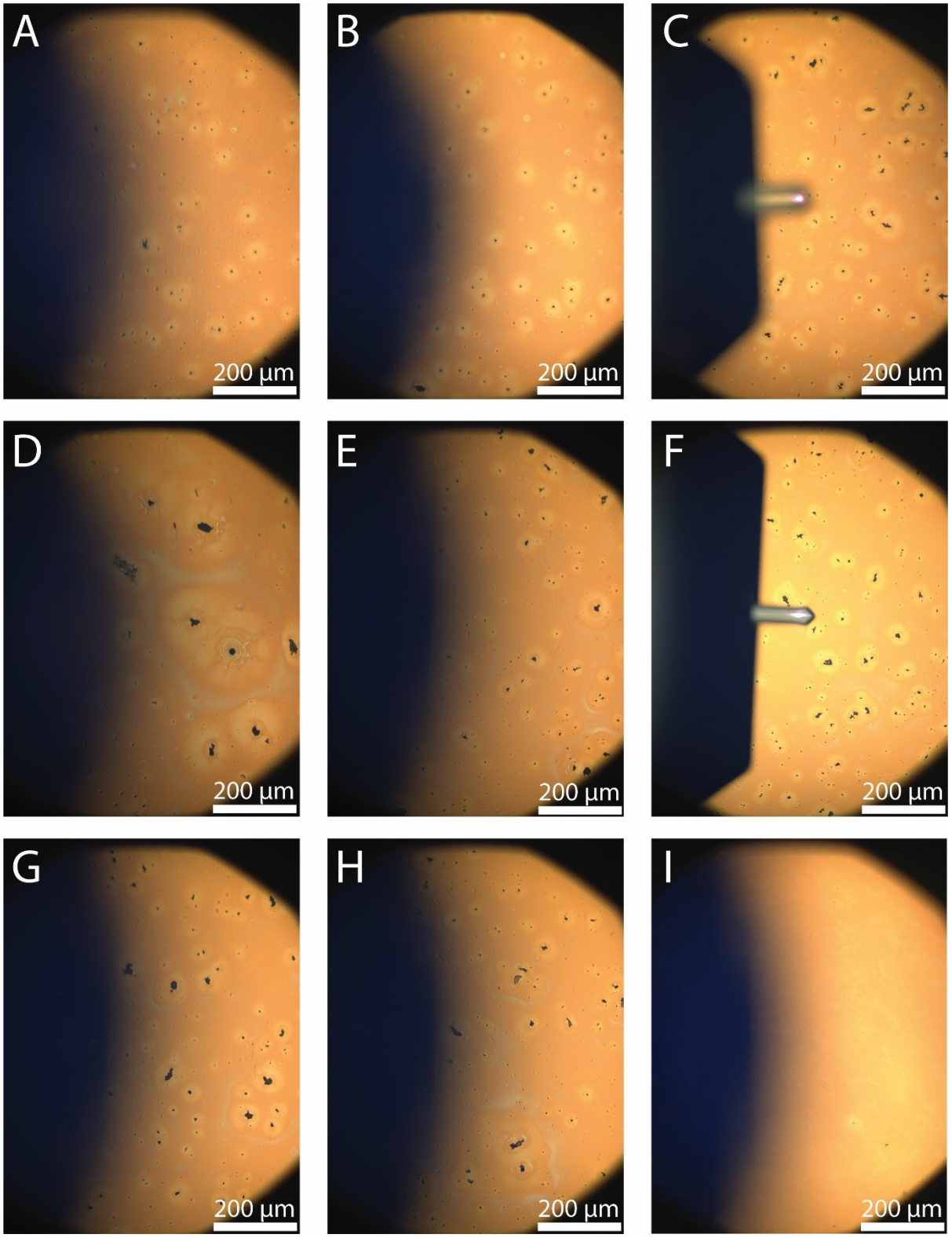

Figure S 2 Optical images taken from AFM of (A-D) LZ co-assembled PS-b-PEO films, (E-H) LZ-PEG co-assembled PS-*b*-PEO films. 4, 5, 7.5 and 10 wt.% of protein to PS-b-PEO in solution are shown in (A, B, C, D) respectively for LZ and (E, F, G, H) for LZ-PEG.

Table S 1 Characteristic beaks for polystyrene, poly(ethylene oxide), PS-b-PEO and lysozyme determined from PiFM measurements.

Material	**Characteristic Peaks**
Polystyrene	1491, 1451 cm^{-1}
Poly(ethylene oxide)	1117, 1147 cm^{-1}
PS-*b*-PEO	Combination of polystyrene and poly(ethylene oxide)
Lysozyme	1666, 1546 cm^{-1}

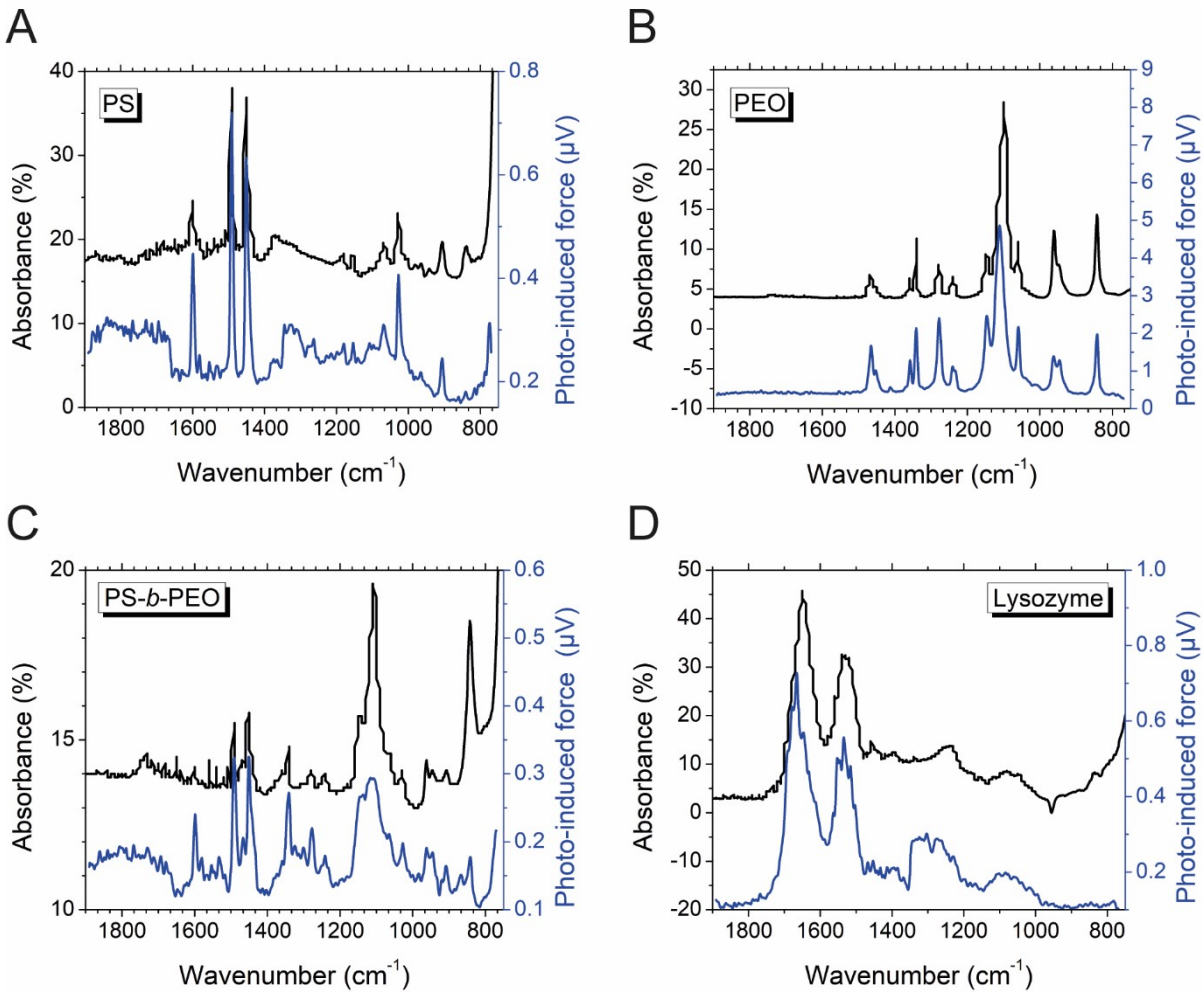

Figure S 3 Reference spectra of polystyrene (A), Poly(ethylene oxide) (B), PS-*b*-PEO (C) and lysozyme (D). The black curves represent the absorbance measured through FTIR spectroscopy, and the blue curves represent the absorbance measured through PiFM on films.

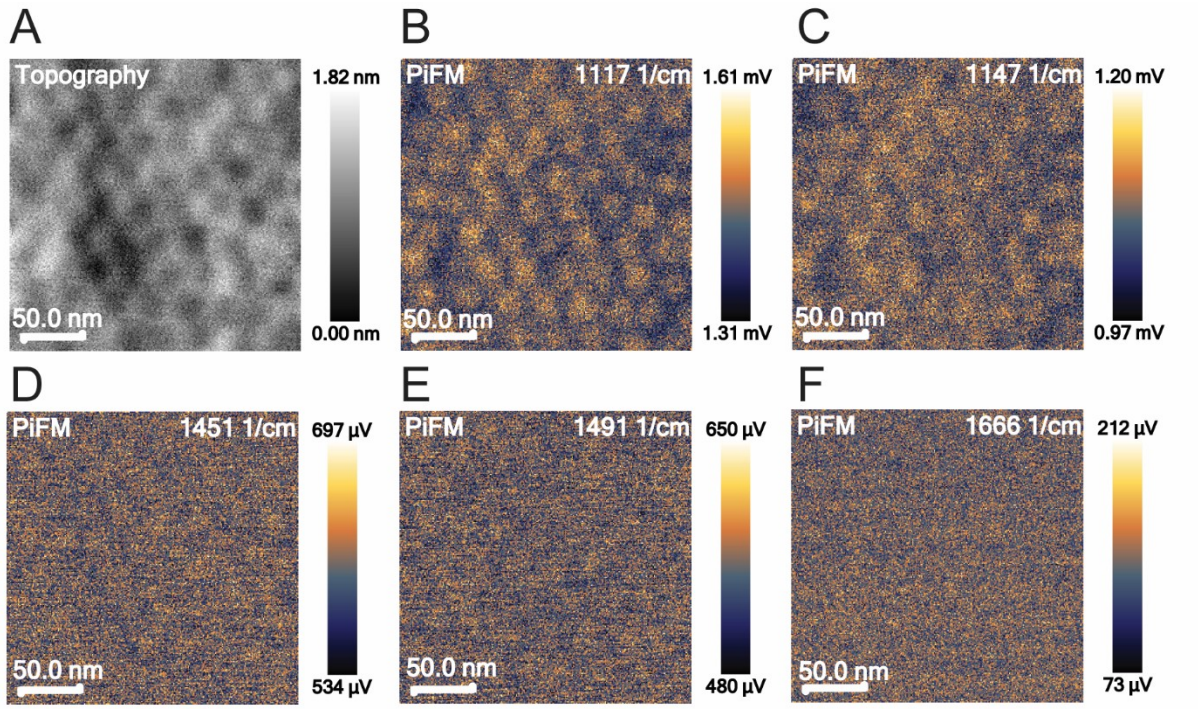

Figure S 4 Topography (A) and PiFM images of a PS-b-PEO thin-film imaged in surface-sensitive mode at 1117 (B), 1147 (C), 1451 (D), 1491 (E), and 1666 cm^{-1} (F). 1117 and 1147 cm^{-1} are attributed to PEO, 1451 and 1491 cm^{-1} are attributed to PS and 1666 cm^{-1} is attributed to lysozyme.

Estimated quantity of incorporated cargo within PS-*b*-PEO films:

The amount of co-assembled proteins was estimated based on the weight ratio of lysozyme-FITC and PS-*b*-PEO $m_{LZ/SEO}$, the density of PS-*b*-PEO ρ_{SEO} (1.06 g/cm³, product data sheet), the film thickness *h* (~60 nm), and the substrate area A of approximately 1 cm² as shown in (1) and (2):

$$m_{seo} = \rho_{seo} * V = \rho_{seo} * (A * h) \quad (1)$$

$$m_{seo} = 1.06 \frac{g}{cm^3} * 1\ cm^2 * 6 * 10^{-6}\ cm = 6360\ ng \quad (2)$$

Based on a standard protein ratio of 4 wt%, the amount of protein in films with a thickness of 60 nm was estimated to be around 254 ng/cm².

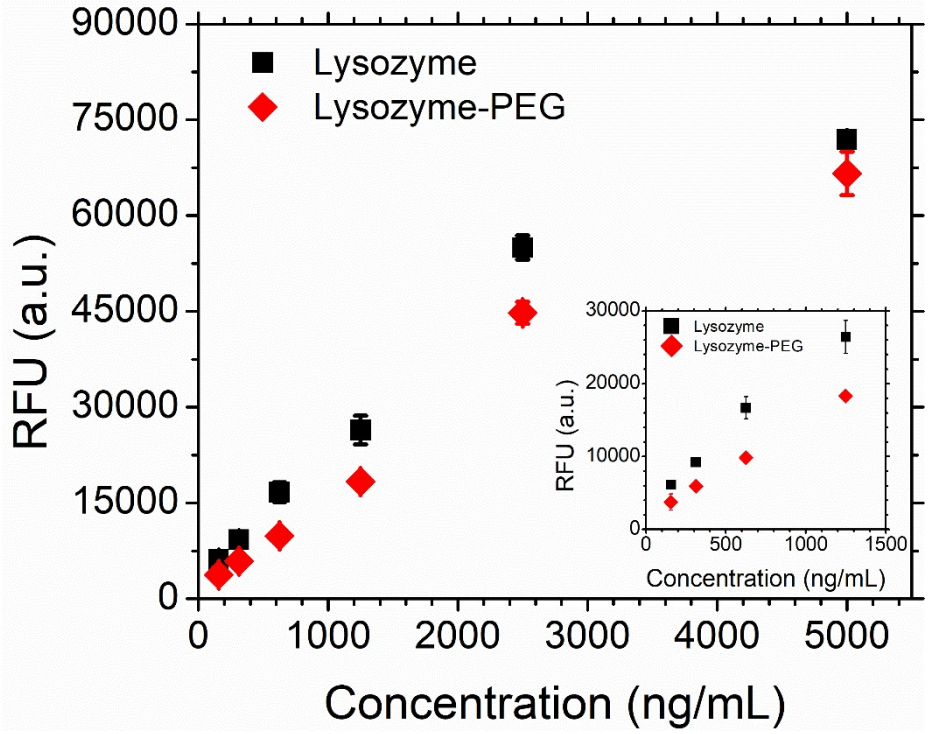

Figure S 5 RFU as a function of the lysozyme (black squares) and lysozyme-PEG (red diamonds) concentration after 60 min in a lysozyme activity assay (EnzChek). Each sample was prepared in triplicates and averaged. The error bars represent the standard deviation. The inset is a zoomed-in section of the graph in the low concentration range.

Table S 2 Individual sample and average k and n values from the fitted Korsmeyer-Peppas model for PEG-FITC-TAT, PEG-FITC-LZ, and FITC-LZ

Release condition	k	k avg	n	n avg
PEG-FITC-TAT 100min	0.671	0.681±0.075	0.096	0.095±0.024
	0.801		0.057	
	0.649		0.102	
	0.598		0.122	
FITC-LZ 100min	0.844	0.863±0.039	0.043	0.037±0.011
	0.913		0.021	
	0.892		0.031	
	0.815		0.050	
PEG-FITC-LZ 100min	0.765	0.623±0.086	0.059	0.108±0.032
	0.533		0.145	
	0.588		0.125	
	0.637		0.100	
PEG-FITC-TAT 8hr	0.822	0.820±0.008	0.038	0.040±0.002
	0.802		0.042	
	0.814		0.041	
	0.822		0.038	
PEG-FITC-LZ 8hr	0.610	0.606±0.041	0.076	0.082±0.012
	0.535		0.104	
	0.633		0.075	
	0.639		0.076	
PEG-FITC-LZ 100min from 59±7nm films	0.628	0.634±0.057	0.109	0.106±0.018
	0.714		0.082	
	0.554		0.133	
	0.610		0.114	
PEG-FITC-LZ 100min from 49±3nm films	0.677	0.681±0.029	0.095	0.094±0.01
	0.721		0.077	
	0.691		0.097	
	0.640		0.106	
PEG-FITC-LZ 100min from 45±5nm films	0.625	0.551±0.043	0.108	0.133±0.016
	0.516		0.148	
	0.531		0.143	
	0.533		0.139	