

**THERMAL PROTECTION AND PH-GATED RELEASE OF FOLIC ACID IN  
MICROPARTICLES AND NANOPARTICLES FOR FOOD FORTIFICATION**

**SUPPLEMENTARY INFORMATION**

Ilja Gasan OSOJNIK ČRNIVEC <sup>a</sup>, Katja ISTENIČ <sup>a</sup>, Mihalea SKRT <sup>a</sup>, Nataša POKLAR ULRIH <sup>a\*</sup>

<sup>a</sup> Chair of Biochemistry and Food Chemistry, Department of Food Science and Technology,  
Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia.

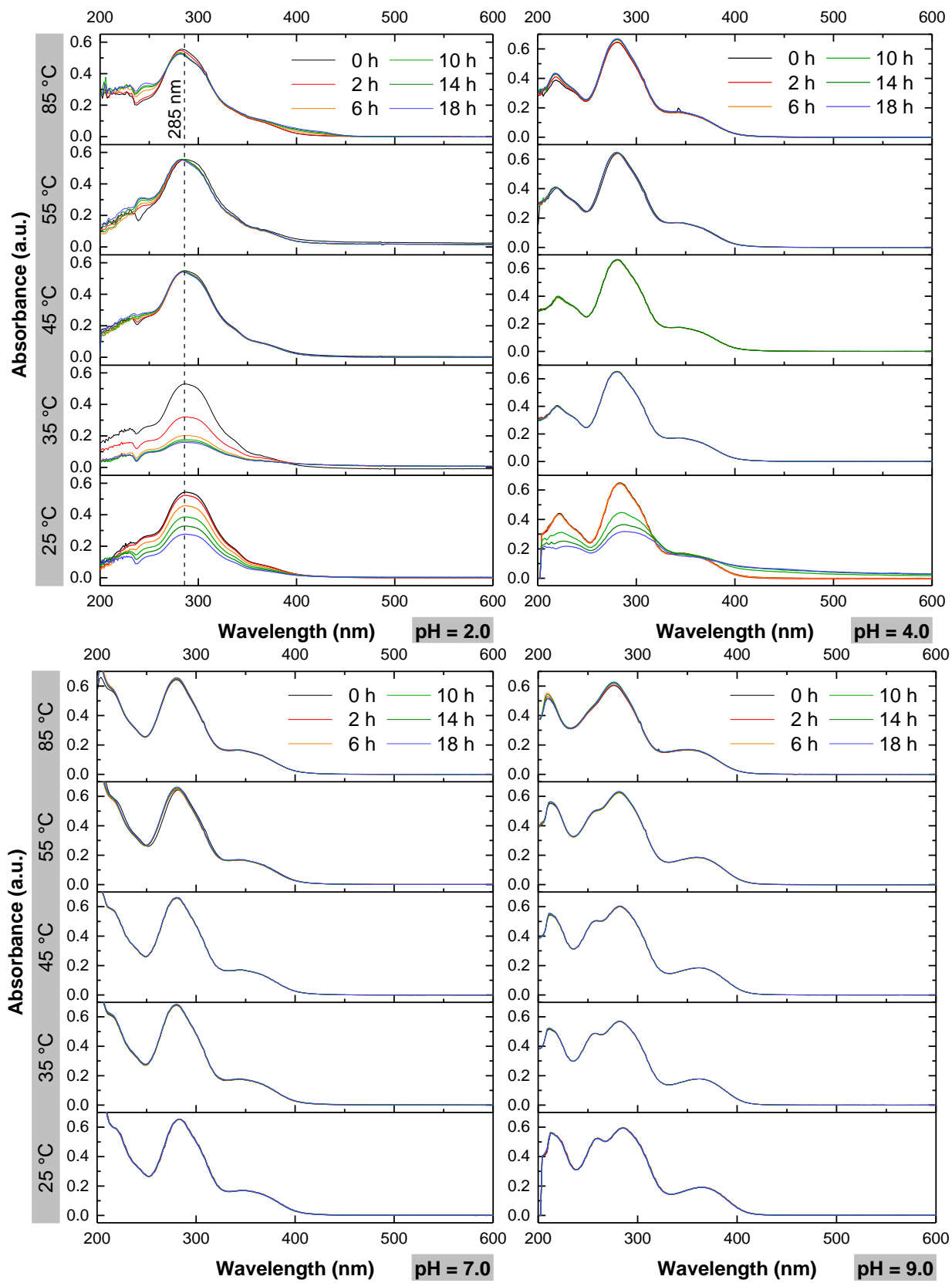
\* [natasa.poklar@bf.uni-lj.si](mailto:natasa.poklar@bf.uni-lj.si)

### Statistical analysis and release curve similarity

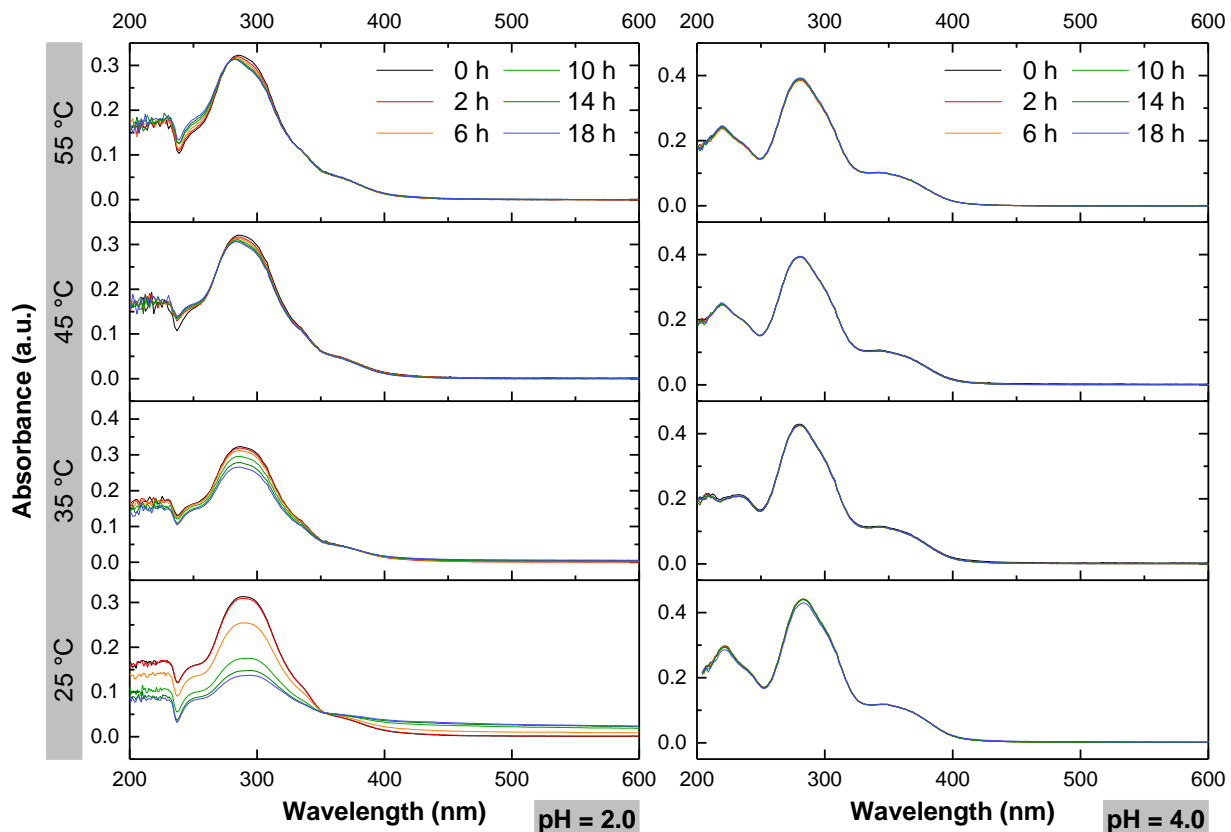
Student's t-tests were performed to differentiate between the means with a 95% confidence interval ( $P < 0.05$ ). For comparing of the release curves, the similarity factor ( $f_2$ ) was calculated according to the guidelines of the Food and Drug Administration (Evaluation 4 (1997) 15–22), according to Equation (S1), where  $n$  = number of points,  $R_t$  = release of the reference compound at the given time point (in %),  $M_t$  = release of the monitored compound at the given time point (in %). Generally,  $f_2$  values  $> 50$  are considered to ensure equivalence of the two curves.

$$f_2 = 50 \log \left\{ 100 \times \left[ 1 + \frac{1}{n} \sum_{i=1}^n (R_t - M_t)^2 \right]^{-0.5} \right\} \quad (S1)$$

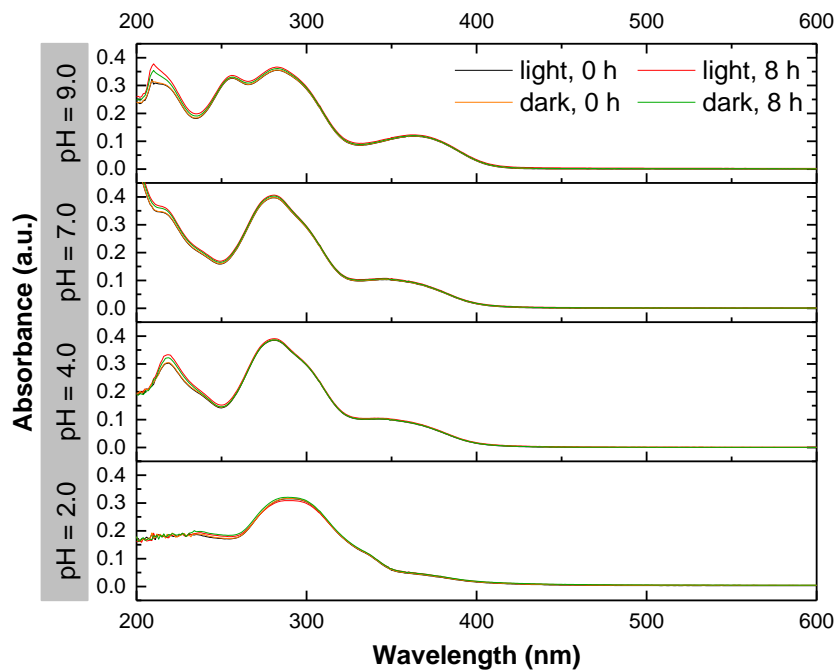
Analyses and calculations were performed with the OriginPro 2018 SR1 b9.5.1.195 (OriginLab, USA) software package.



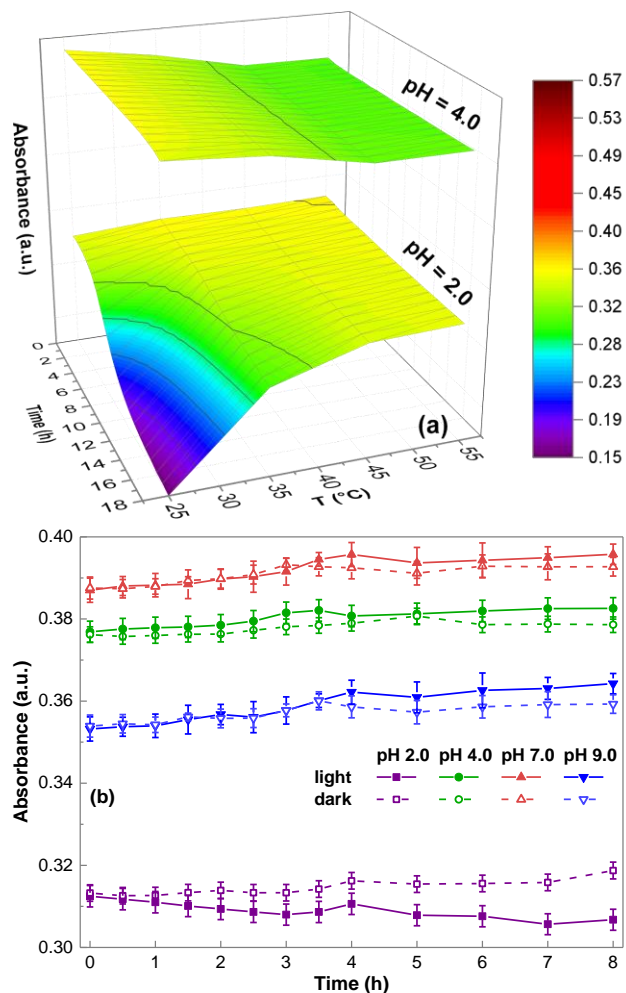
**Fig. S1** UV/Vis spectra (200-600 nm) of 25 μM folic acid over 18 h at pH 2.0 to 9.0 and at 25 °C to 85 °C, in the dark.



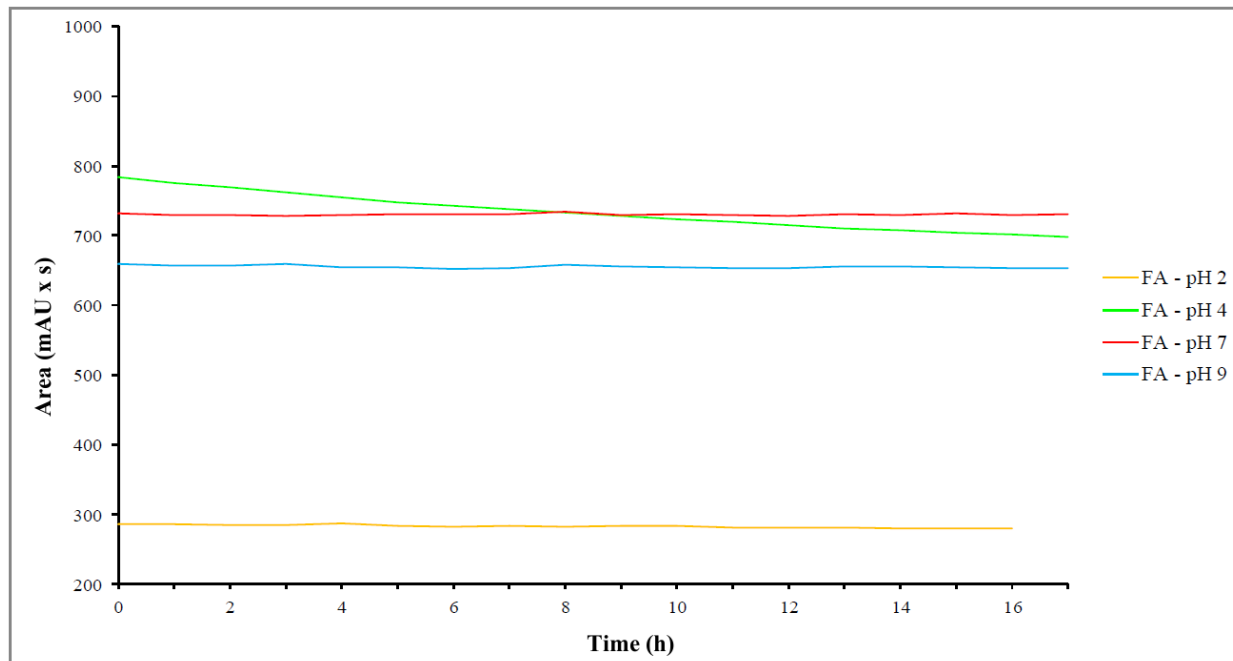
**Fig. S2** UV/Vis spectra (200-600 nm) of 15  $\mu$ M folic acid over 18 h at pH 2.0 and 4.0 and at 25  $^{\circ}$ C to 55  $^{\circ}$ C, in the dark.



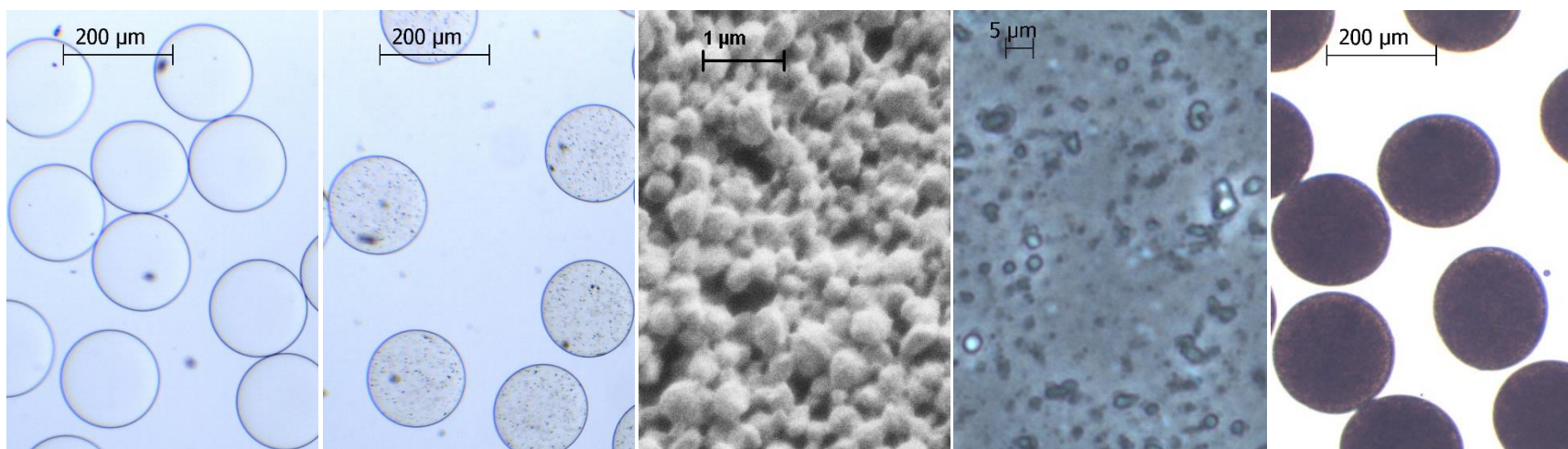
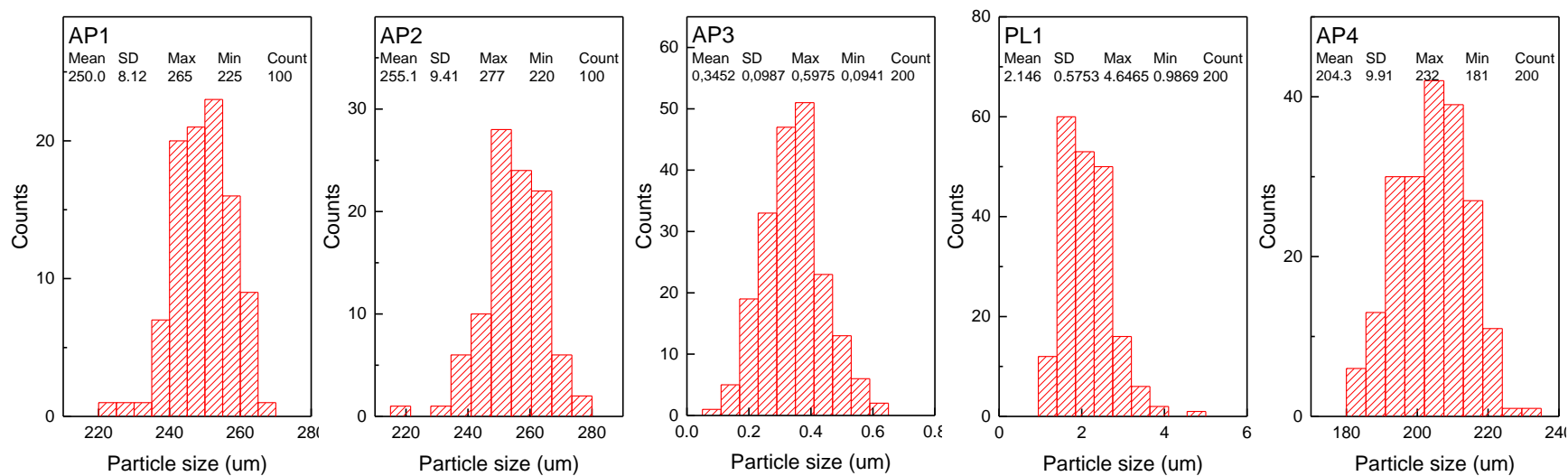
**Fig. S3** UV/Vis spectra (200-600 nm) of 15  $\mu$ M folic acid over 8 h at pH 2.0 to 9.0 and at 25  $^{\circ}$ C in the absence and presence of light.



**Fig. S4** Stability of free folic acid in aqueous solutions during exposure to environmental factors. (a) 15  $\mu\text{M}$  folic acid, pH 2-4, 25-55  $^{\circ}\text{C}$ , in the dark. (b) 15  $\mu\text{M}$  folic acid, pH 2-9, 25  $^{\circ}\text{C}$ , in the dark versus the light. Data are means (a-b)  $\pm$ SD (b) ( $n = 3$ ).



**Fig. S5** Chromatographic peak area of 25  $\mu$ M folic acid (FA) over 16 h at pH 2.0 to 9.0 at 25  $^{\circ}$ C, in the dark (diode array detector, 280 nm).



**Fig. S6** Particle size distributions (top) and corresponding morphologies (bottom) of the prepared samples. Micrographs were obtained by light microscopy (AP1, AP2, AP4, 4x magnification; PL1, 40x magnification) or electron microscopy (AP3, 1.0 kV acceleration voltage, 30  $\mu\text{m}$  aperture,  $\sim 3$  mm working distance). Scale bars, as indicated.