Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Folate binding protein: Therapeutic natural nanotechnology for folic acid, methotrexate, and leucovorin

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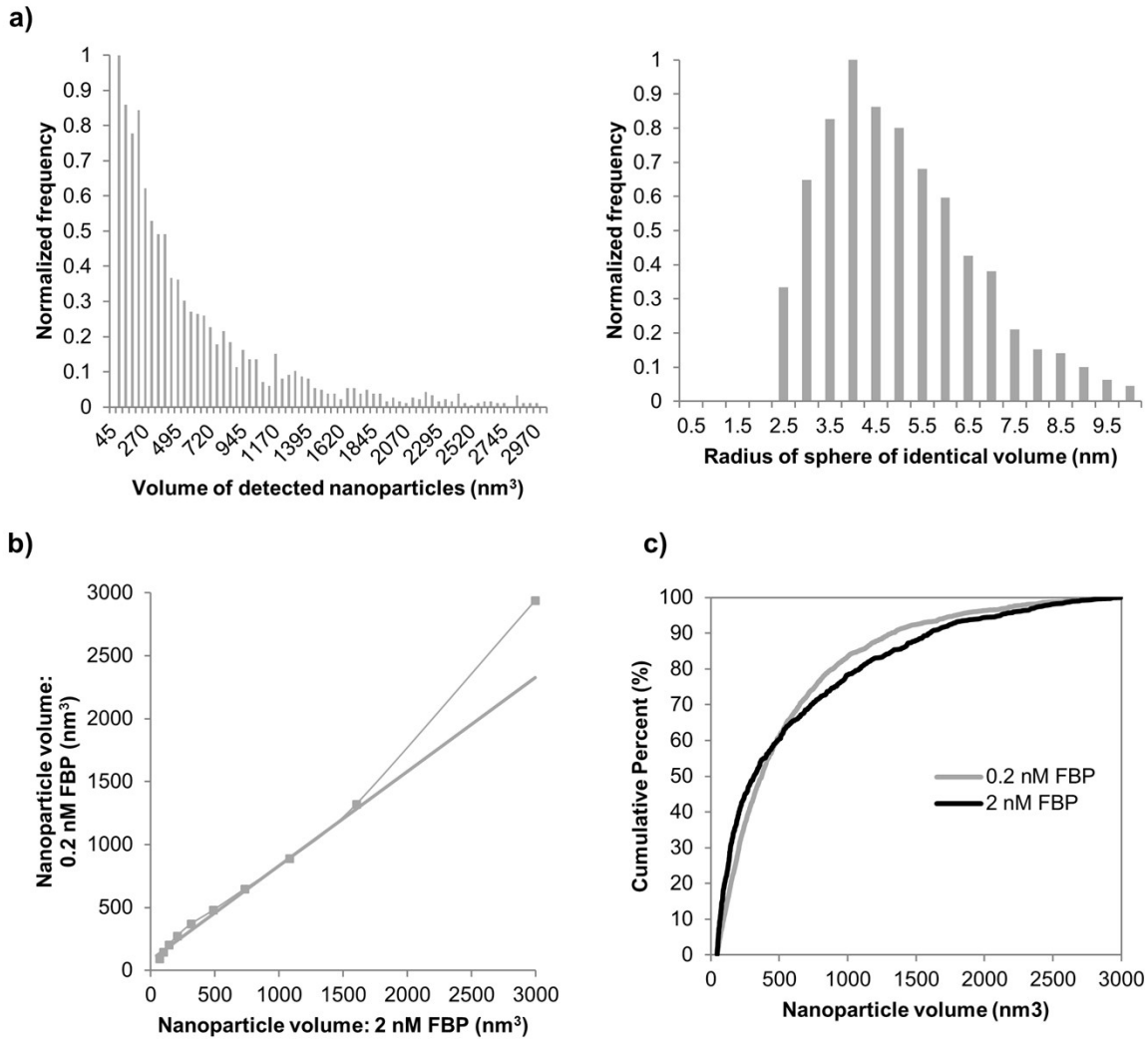
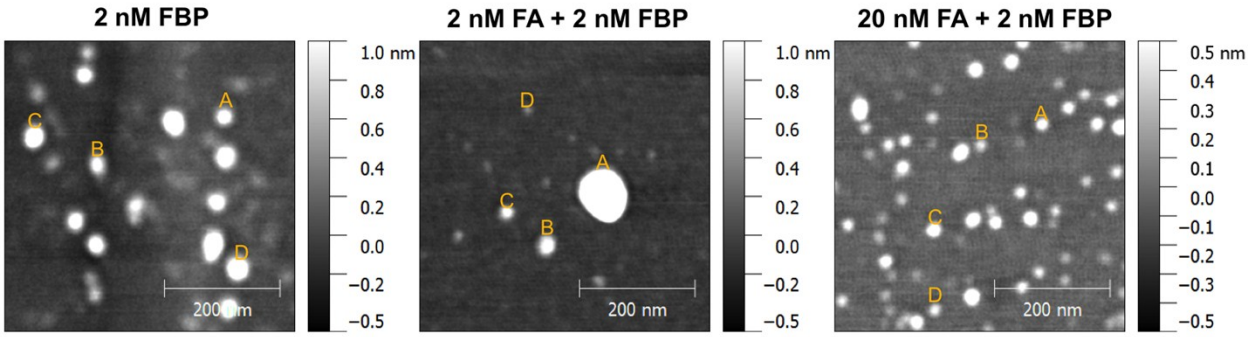


Figure S1. a) Histograms; b) QQ plots; c) and CDF plots for the FBPNP formed from 0.2 nM FBP. The K-S test comparing FBPNP at 0.2 nM and 2 nM protein rejected the null hypothesis, indicating the two nanoparticle populations are statistically different.



	Radius (nm)	#FBP
A	6.2	22
B	5.7	17
C	7.4	37
D	8.0	48

	Radius (nm)	# FBP
A	18.3	570
B	5.9	19
C	4.6	9
D	2.9	2

	Radius (nm)	# FBP
A	3.6	4
B	3.1	3
C	4.4	8
D	3.1	3

Figure S2. Representative detailed frames of AFM images showing FBP nanoparticles. Idealized spherical radii and the number of FBP comprising each selected nanoparticle are provided.

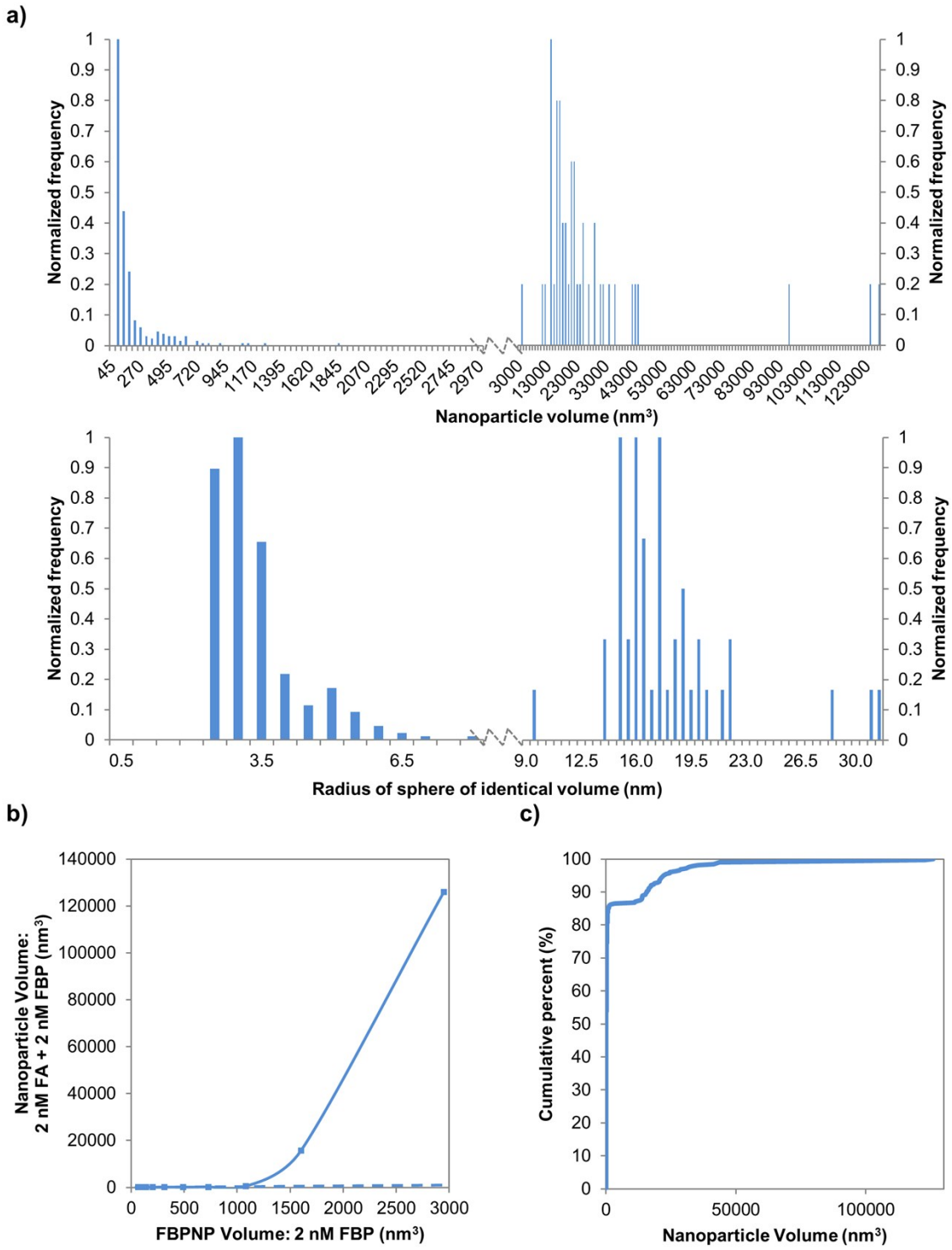


Figure S3. Histograms, QQ plots, and CDF plots of the full data set of FBPNP formed from 2 nM FA + 2 nM FBP demonstrating the biomodal distribution of FBPNP volumes.

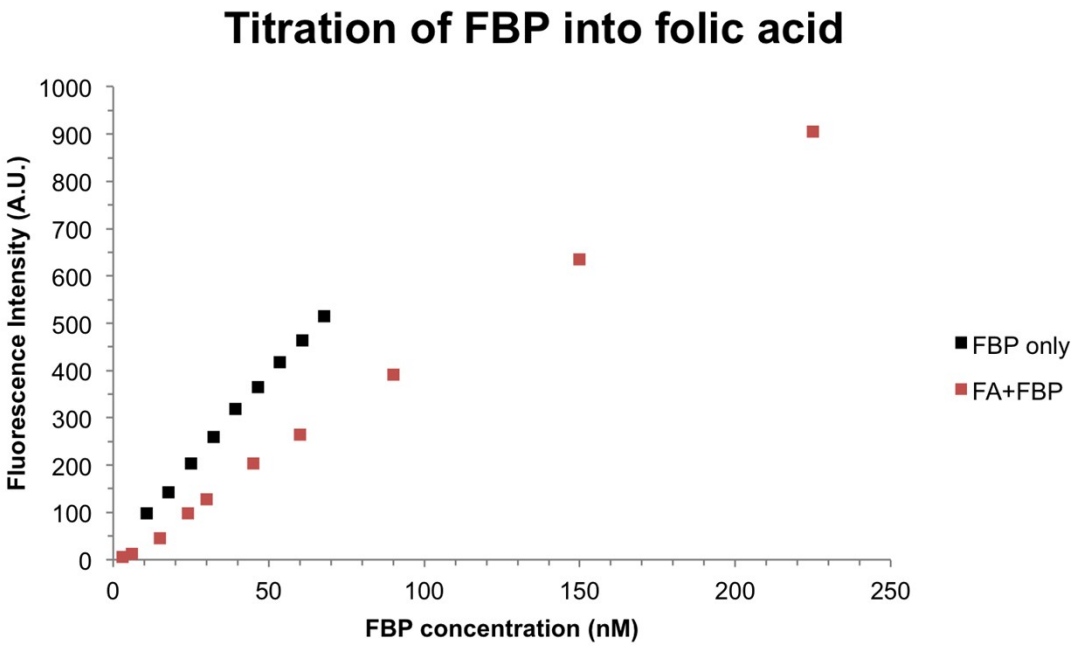


Figure S4. Titration of FBP into FA (30 nM). Trytophan fluorescence was excited at 280 nm and emission detected at 340 nm. The flatter slope of the ligated FBP fluorescence is suggests asymmetrical aggregation of apo- and holo-FBP.

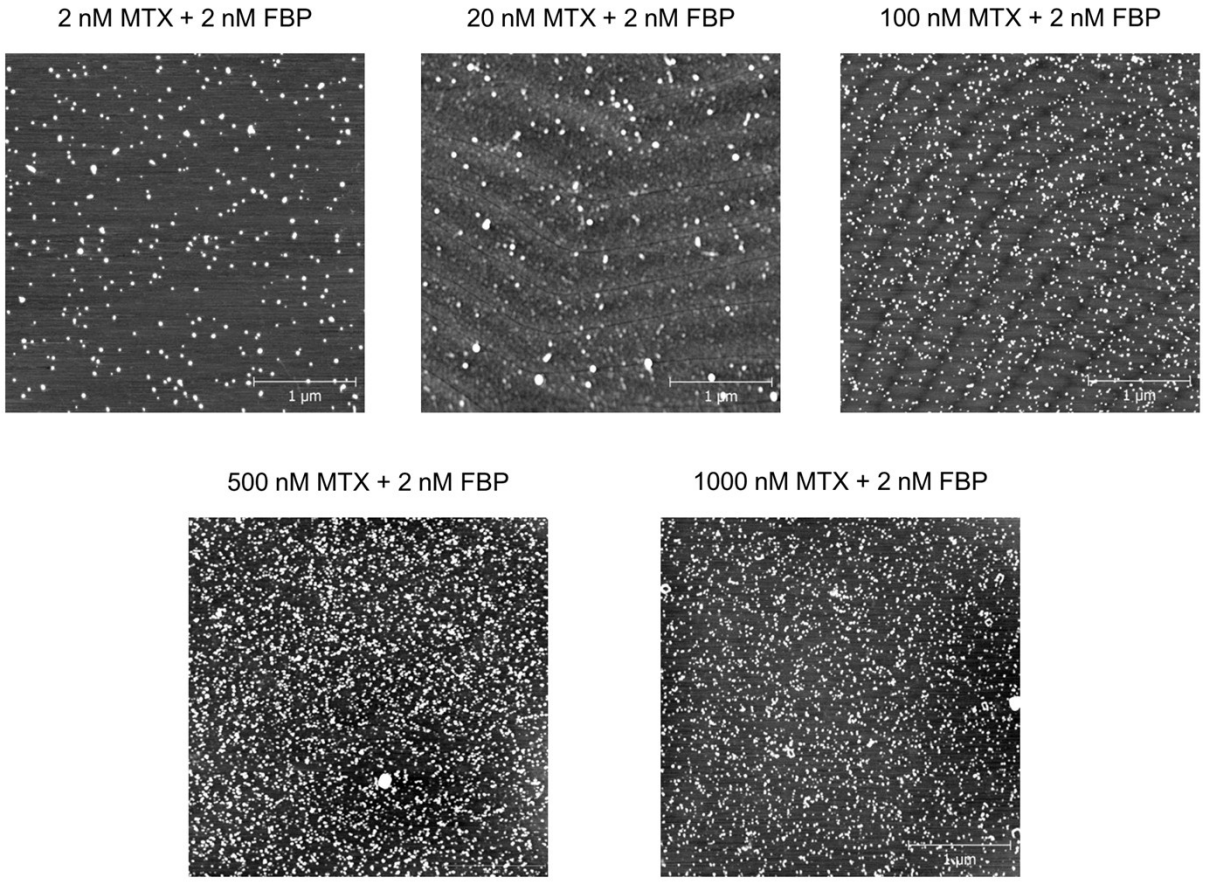


Figure S5. AFM images of FBPNP formed from a range of MTX:FBP ratios. For all samples, the FBP concentration was held constant at 2 nM in 1x PBS. AFM images were captured by spin coating the solutions onto freshly-cleaved mica.

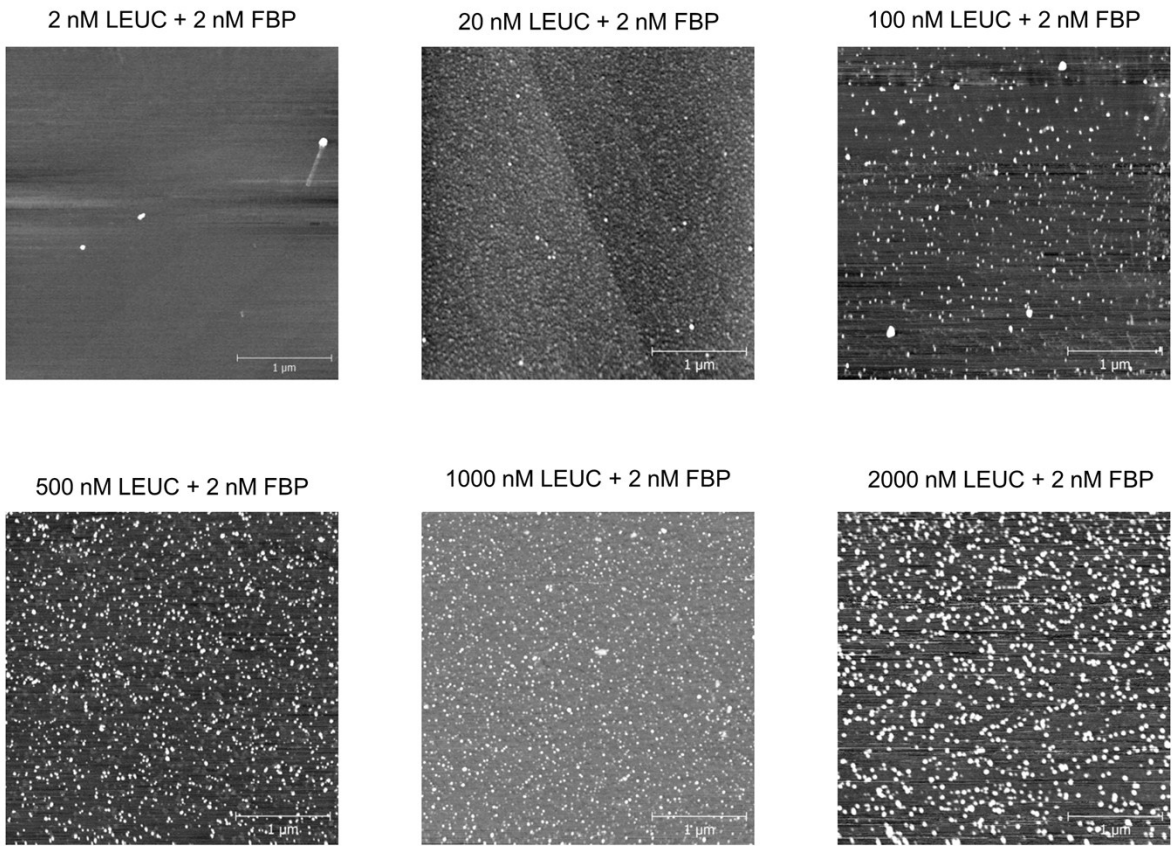


Figure S6. AFM images of FBPNP formed from a range of LEUC:FBP ratios. For all samples, the FBP concentration was held constant at 2 nM in 1x PBS. AFM images were captured by spin coating the solutions onto freshly-cleaved mica.

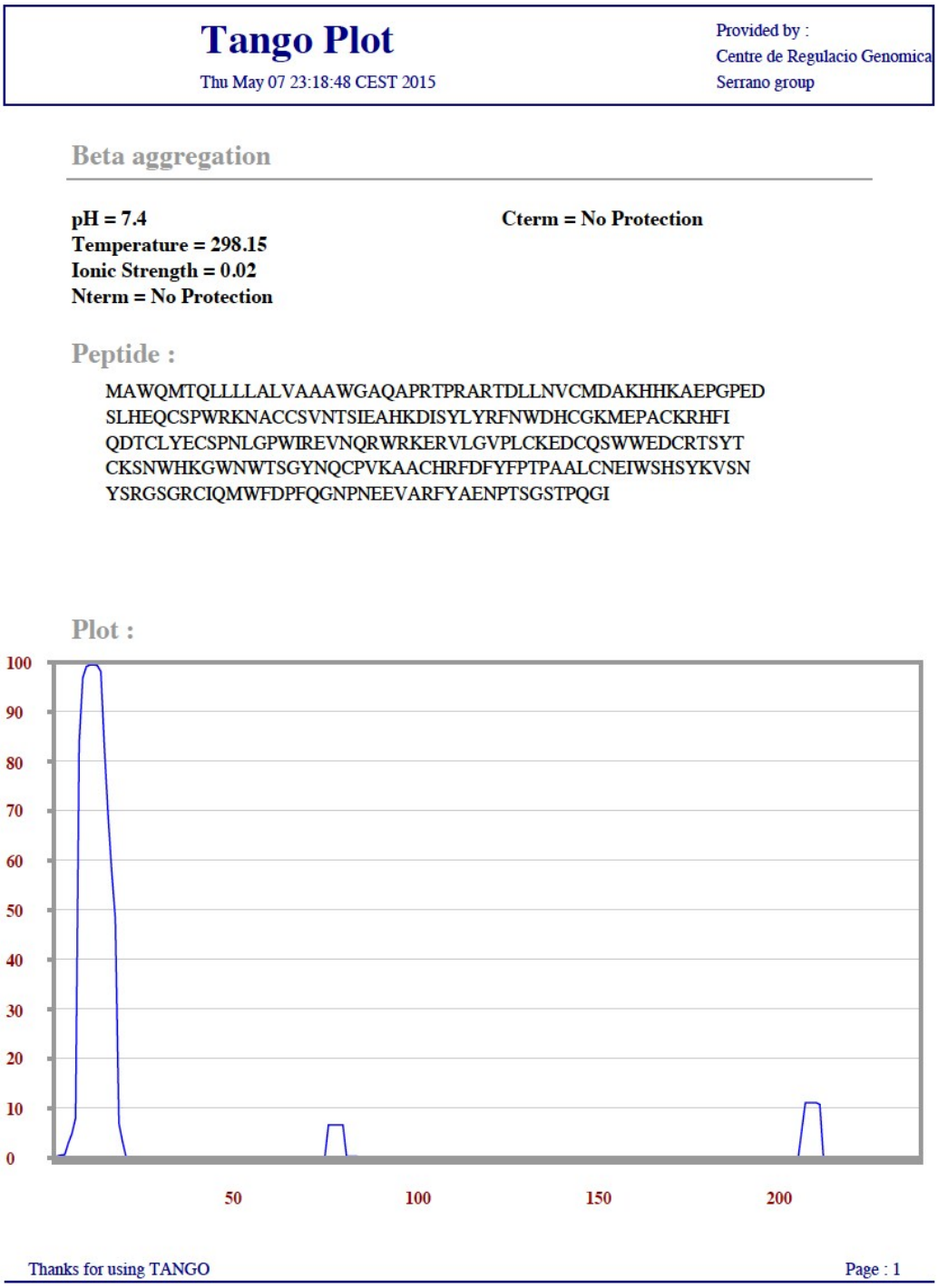


Figure S7. TANGO output for bovine apo-FBP (P02702). The N-terminus 8-18 LLLLALVAAAW sequence has ~99% aggregation tendency at 10 μM (the lowest concentration with which the code is compatible).

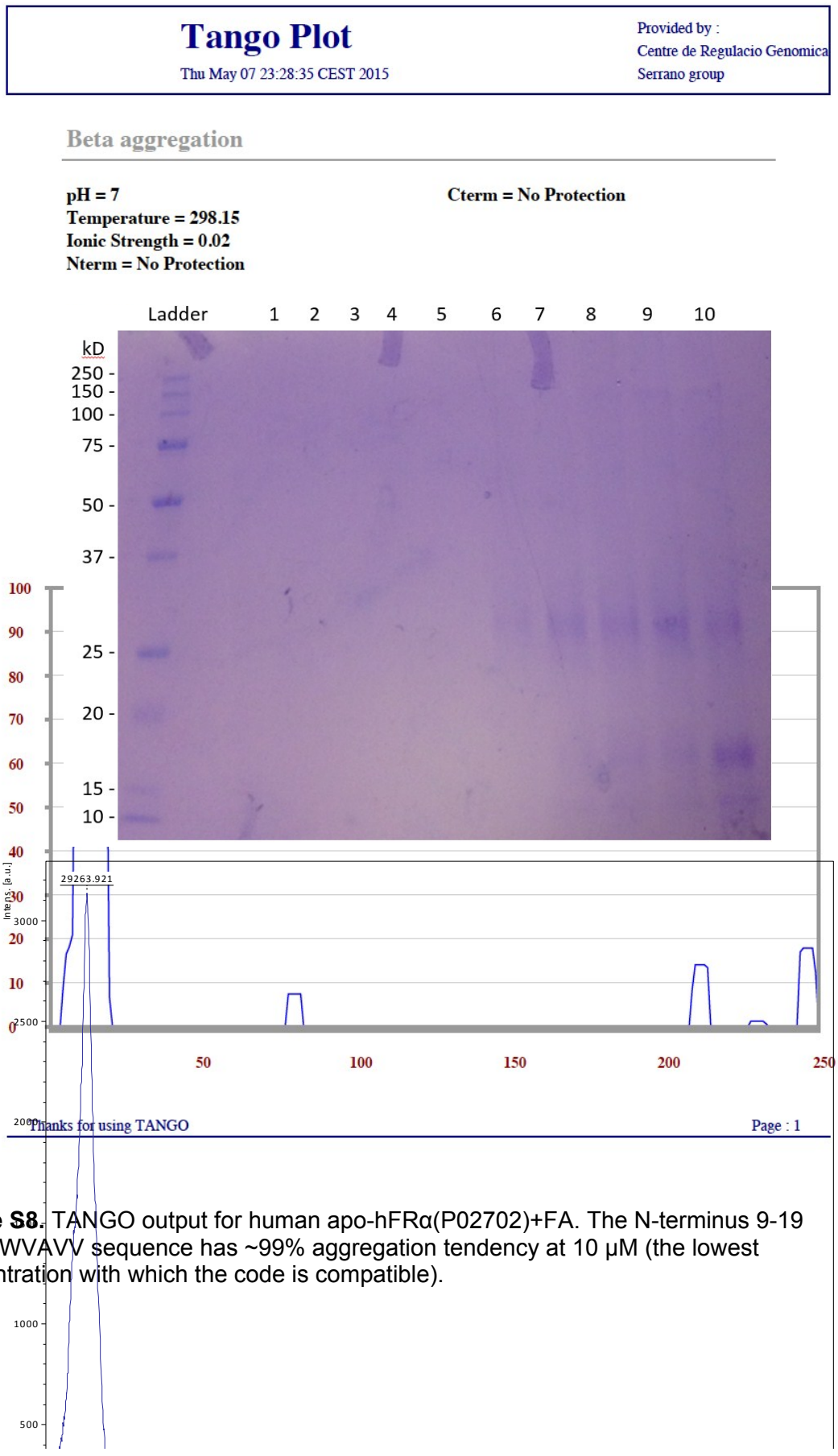


Figure S8. TANGO output for human apo-hFRα(P02702)+FA. The N-terminus 9-19 LLLLVWVAVV sequence has ~99% aggregation tendency at 10 μM (the lowest concentration with which the code is compatible).

Figure S9. SDS-PAGE and MALDI of FBP. The protein were collected by fractions and the ~29kDa fractions were pooled together. The FBP exhibited a molecular weight distribution around ~29kDa because of glycosylation at residue 68N and 160N.

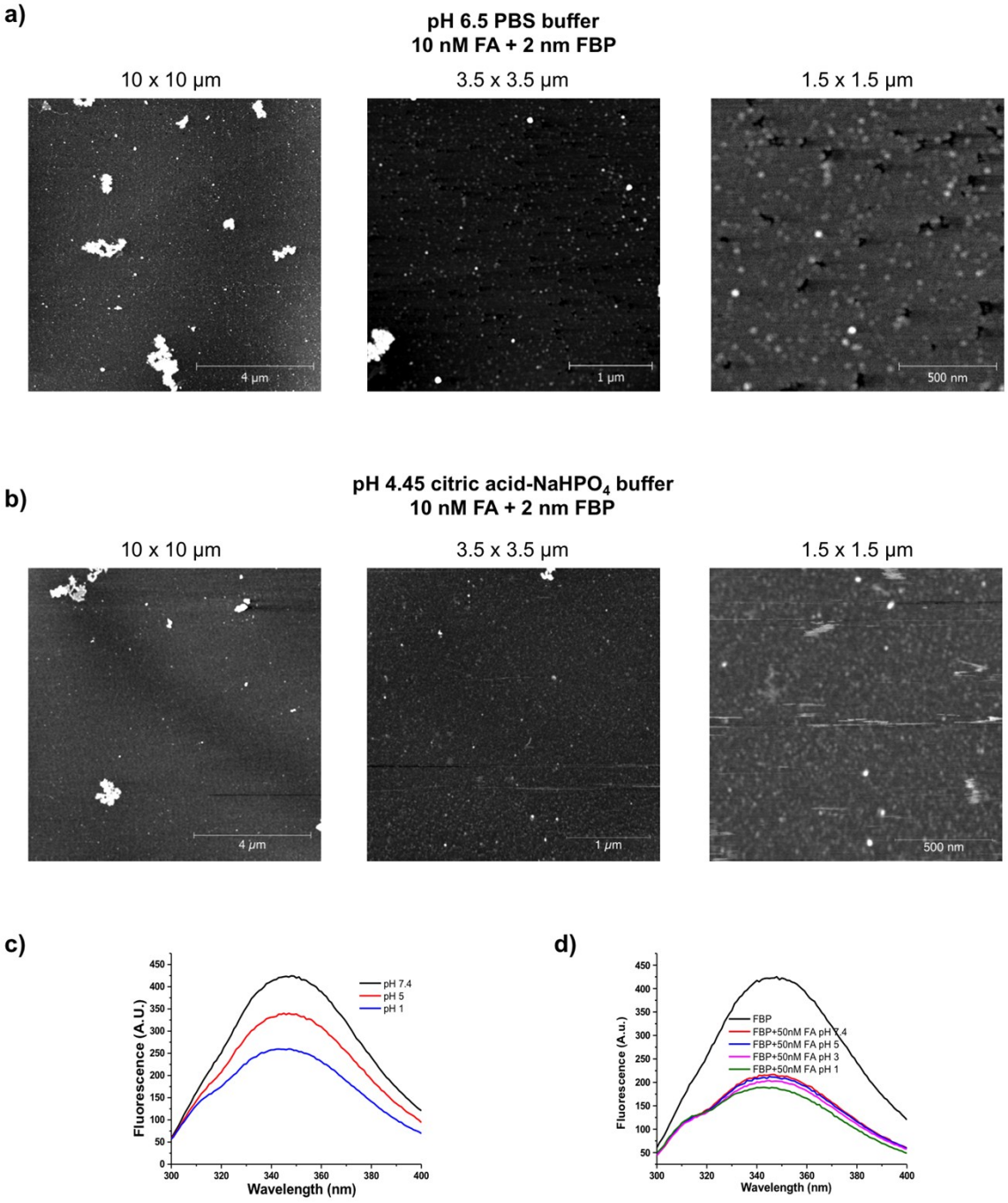


Figure S10. a) AFM images of 10 nM FA + 2 nM FBP at pH 6.5; b) AFM images of 10 nM FA + 2 nM FBP at pH 4.45. Images in both (a) and (b) show substantially less nanoparticle formation as compared to FA+FBP solution at pH 7.4; c) Tryptophan fluorescence of FBP over a range of pH values ([FBP] = 58 nM); d) Tryptophan fluorescence of FBP (58 nM) in the presence of FA (50 nM). excitation: 280 nM; emission: 340 nM