Bioconjugate Chemistry

Supporting information

Cross-Linked Peptide Nanoclusters for Delivery of Oncofetal Antigen as a Cancer Vaccine

Alexandra N. Tsoras and Julie A. Champion*

School of Chemical & Biomolecular Engineering and Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology. Atlanta, GA.

KEYWORDS: cancer vaccine, oncofetal antigen, peptide nanoclusters, desolvation, crosslinking

*Email : julie.champion@chbe.gatech.edu

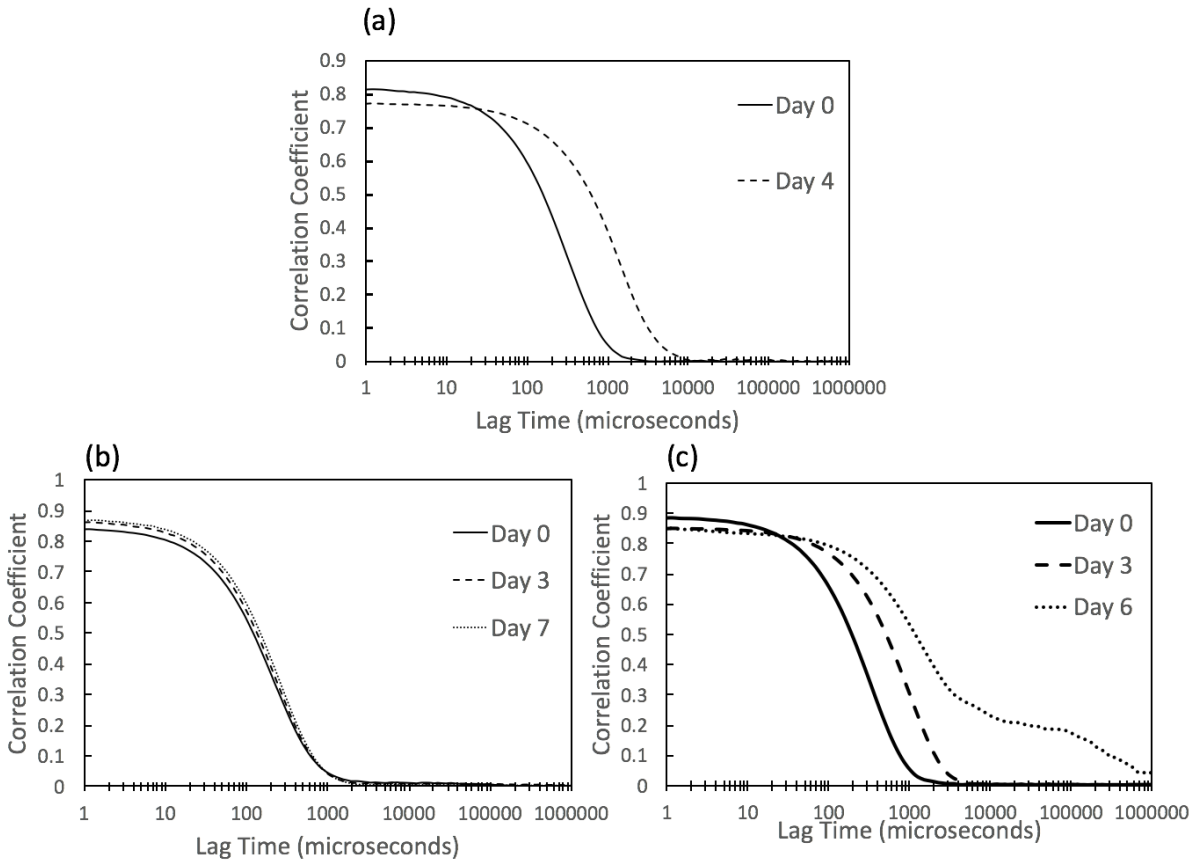

Figure S1. Correlograms reported by dynamic light scattering from (a) OFA 1C, (b) OFA 2C, and (c) OFA 3C PNC size measurements taken over several days. Consistent curves over multiple days' readings indicate stability and maintained size. Curves starting below 0.8 or above 1 are considered aggregated or too low in concentration or size to be reliable. Non-zero or spiky trends beyond the inflection point also indicate aggregation. OFA 2C demonstrates consistent, good quality correlograms, whereas 1C demonstrates elongating curves indicating particles getting larger over time, and 3C demonstrates similar trends as well as some indication of aggregation.

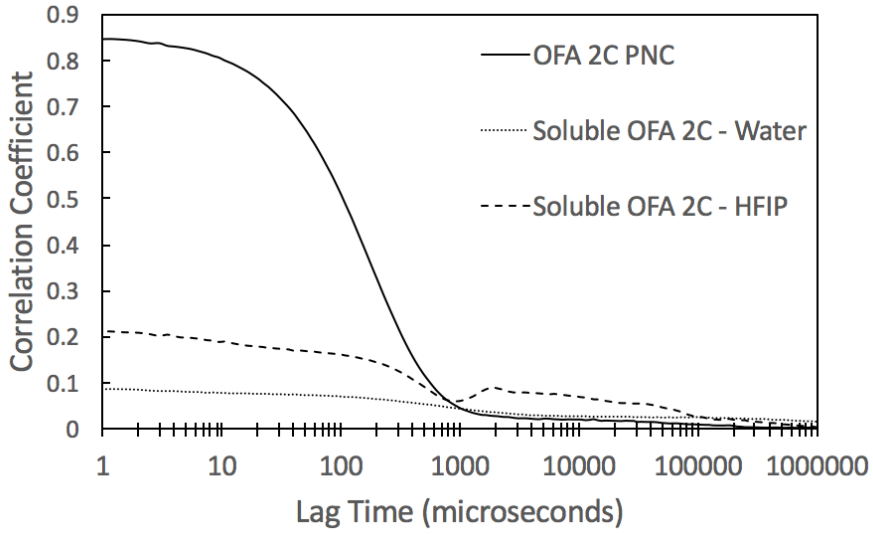

Figure S2. Dynamic light scattering (DLS) correlograms of OFA 2C in PNC form compared to OFA simply solubilized in water or HFIP. DLS reports sufficient intensity and good quality correlogram for a PNC measurement, whereas soluble OFA 2C in both water and HFIP produce insufficient scattering to report size. This indicates that the solution is contains primarily soluble OFA 2C without aggregation, the size of which is too small to be detected by DLS.

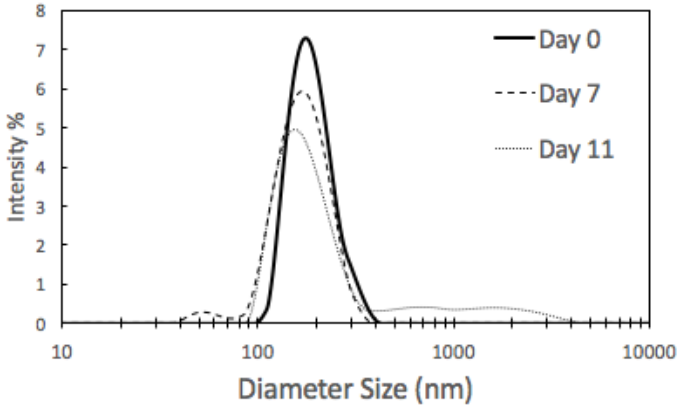

Figure S3. Dynamic light scattering size distribution peak of OFA 3C PNC that were quenched with maleimide prior to isolation. Multiple measurements were taken from the same sample over several days after synthesis. PNC were stored and measured in 1% Tween 80 solution. By quenching OFA 3C nanoparticles with approximately 1/2 the theoretical moles required to quench all thiol sites on the trithiol added during desolvation, OFA 3C PNC remained more stable in solution.

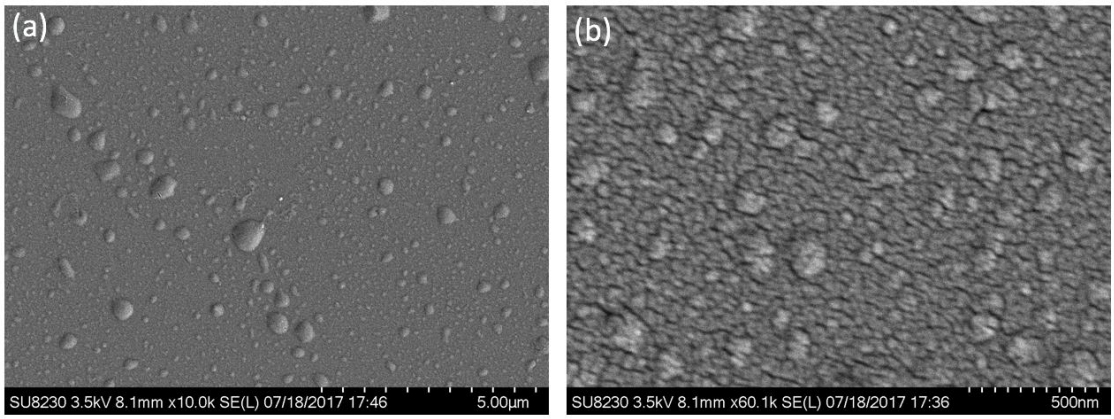

Figure S4. (a,b) Scanning electron microscopy of OFA 2C PNC drop cast in water on an aluminum stub and coated with Au/Pd. Slightly melted or cracked non-spherical images could be detected, indicating lyophilization and sputter-coating processes used to improve the imaging of the PNCs may deform or melt the PNC or leave cracked coating residue.

Table S1. Summary of characterization of PNC synthesized from OFA 2C variations.

Peptide	Size (nm)	PDI*
OFA 2C - FITC	197 ± 36	.160 ± .118
OFA 2C - biotin	222 ± 25	.145 ± .062
OFA 2C - NIR	256 ± 49	.345 ± .173

***PDI, polydispersity index**

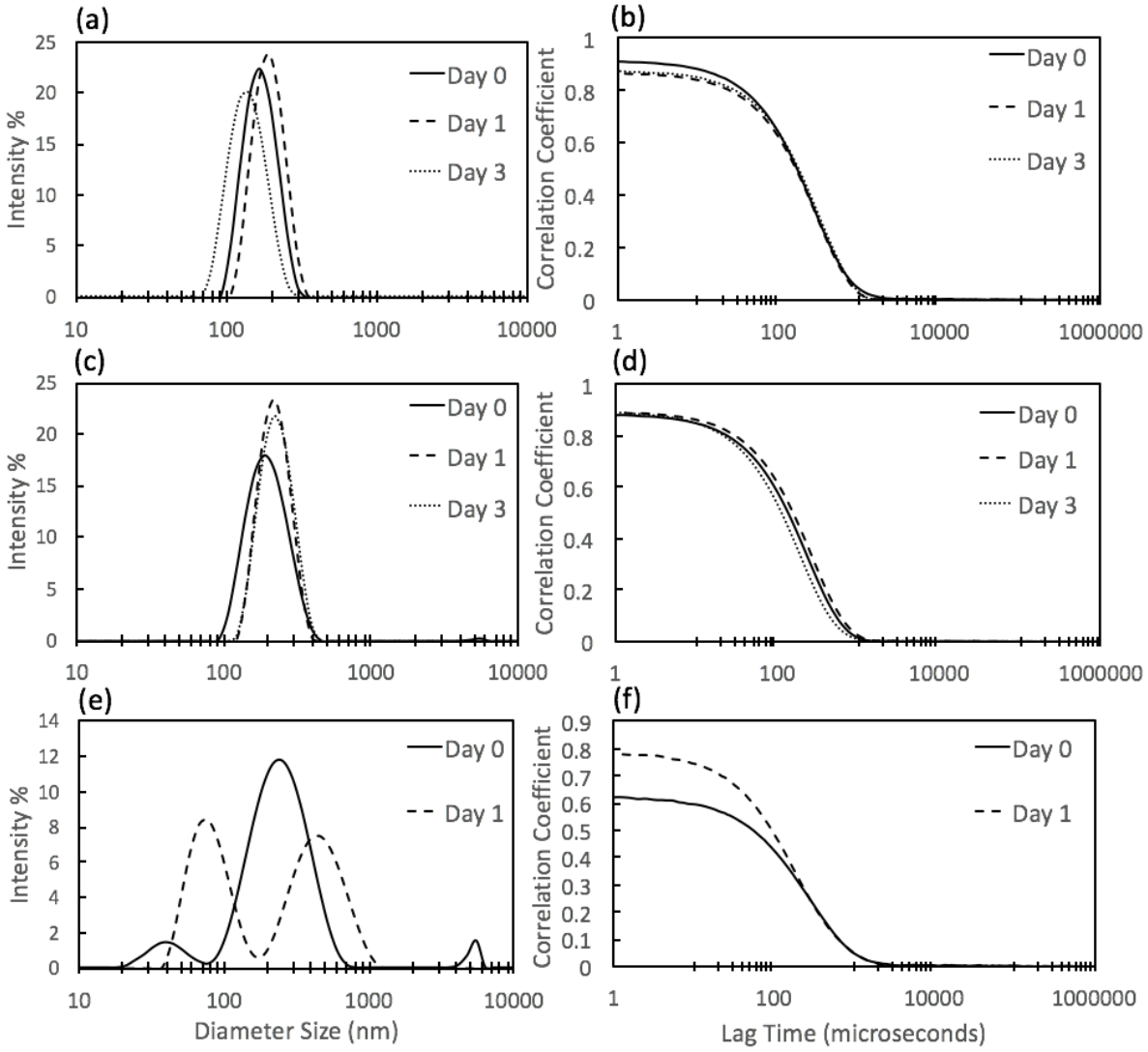

Figure S5. (a, c, e) Dynamic light scattering size distribution peaks of (a) OFA 2C + 10% OFA 2-FITC, (c) OFA 2C + 10% OFA 2-biotin, and (e) OFA 2C + 5% OFA 2-Cy5.5. Distributions and average size remain stable over at least 3 days for biotinylated and FITC PNC, and Cy5.5-tagged PNC remained within acceptable range for 1 day. (b, d, f) Dynamic light scattering correlograms of (b) OFA 2C +10% OFA 2-FITC, (d) OFA 2C + 10% OFA 2-biotin, and (f) OFA 2C + 5% OFA 2-Cy5.5. Curves starting below .8 or above 1 are considered too low in concentration/size or too aggregated, respectively, to be reliable. Non-zero or spiky trends beyond the inflection point also indicate aggregation. Correlograms demonstrate consistent, good quality measurements. OFA 2C-Cy5.5 displayed slightly low concentration at Day 0, but after 1 day improved signal in correlogram, indicating a potential equilibration in solution and acceptable quality to use *in vivo*.

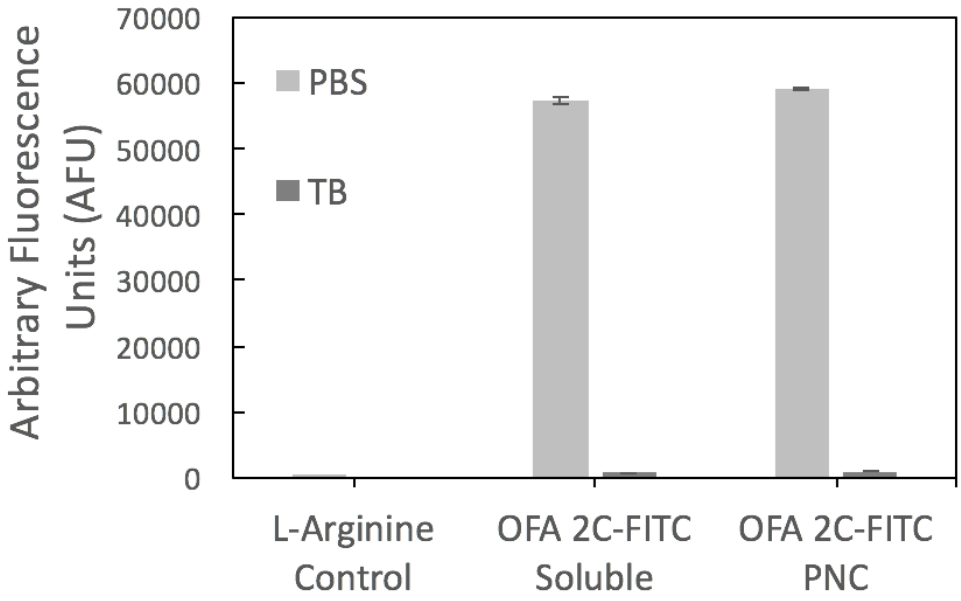

Figure S6. Fluorescence measurements of soluble and PNC OFA 2C when added to PBS or trypan blue (TB). 20 μl of 1 mg/ml 10% FITC-tagged OFA 2C PNC or soluble peptide in 150 mM L-Arginine was added to 180 μl of PBS or TB. Fluorescence measurements were immediately read from a BioTek Synergy H4 Micro Plate Reader. Quenching of TB was > 99% for peptide in both soluble and PNC form.

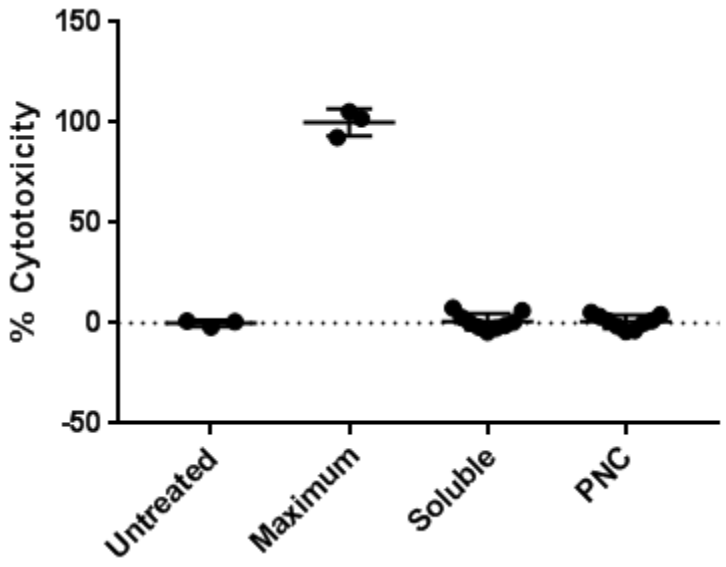

Figure S7: Percent cytotoxicity of M3 DCs incubated with soluble and PNC OFA 2C for 48 h normalized to amount of LDH spontaneously released from untreated DCs (minimum) and amount of LDH released by M3 DCs when treated with Cell Lysis Buffer for 45 minutes at room temperature (maximum).

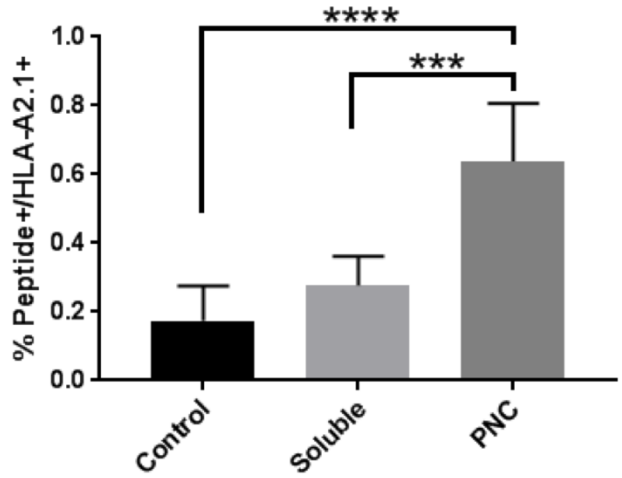

Figure S8. After 24 h of incubation with OFA 2C-biotin in PNC or soluble form and maturation cytokines, M3 DC surfaces were labeled with fluorescent streptavidin and anti-HLA-A2.1 antibody. DCs were assessed by flow cytometry to determine the percentage of cells that bound both fluorescent markers on their surfaces. ***p<.001, ****p<.0001.

Table S2. Dynamic light scattering (DLS) settings and conditions used to acquire size, polydispersity (PDI), and zeta potential (ZP) measurements for each sample reported. For all samples, averages and standard deviations were derived from average size, PDI, or ZP from three separate batches of particles that were each measured in three replicate runs in a Malvern Zetasizer Nano ZS. The average of the three replicate runs was used as the result of each batch, and the three batches were averaged to find a final average and standard deviation. General purpose (normal resolution) was used to fit the correlation function to DLS data, and the Smoluchowski equation with F(ka) = 1.5 was used for ZP calculation.

Size and PDI Measurements										
Peptide	Particle Concentration (mg/ml)*	Resuspension Medium	Refractive Index (Particle)	Refractive Index (Medium)	Viscosity (cP)	Measurement Temperature (˚C)	Cuvette Type	Laser Wavelength (nm)	Scattering Angle (˚)	Dielectric Constant
OFA 2C	1	MQ Water	1.45	1.33	0.8872	25	ZEN 0040	633	173˚	n/a
OFA 2C + 10% 2-FITC	1	150mM L-Arginine in MQ Water	1.45	1.33	0.8872	25	ZEN 0040	633	173˚	n/a
OFA 2C + 10% 2-biotin	1	150mM L-Arginine in MQ Water	1.45	1.33	0.8872	25	ZEN 0040	633	173˚	n/a
OFA 1C	1	1% Tween 80 in MQ Water	1.45	1.33	0.8872	25	ZEN 0040	633	173˚	n/a
OFA 3C	1	1% Tween 80 in MQ Water	1.45	1.33	0.8872	25	ZEN 0040	633	173˚	n/a
ZP Measurements										
OFA 2C	0.19	19% MQ water diluted with 79% 1X PBS	1.45	1.332	0.9052	25	DTS 1070	633	173˚	78.5

*Based on peptide mass.