

The role of human serum and solution chemistry in fibrinogen peptide-nanoparticle interactions

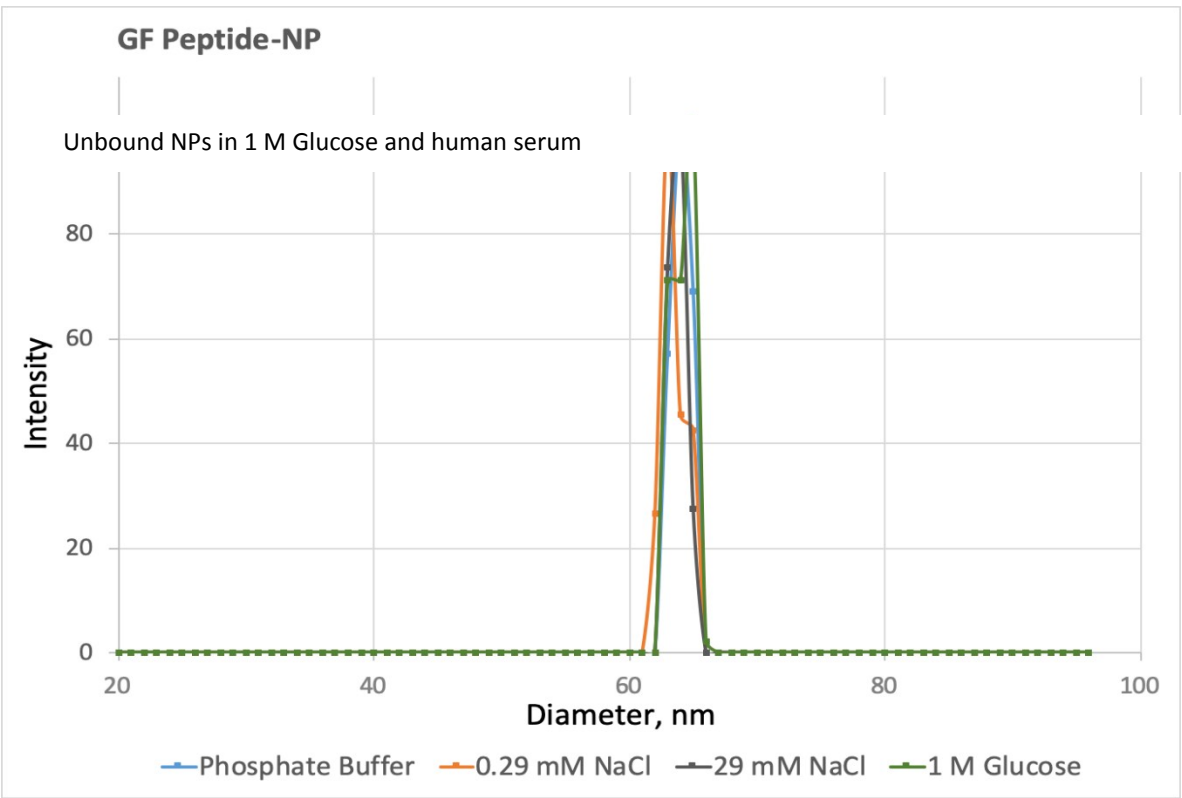
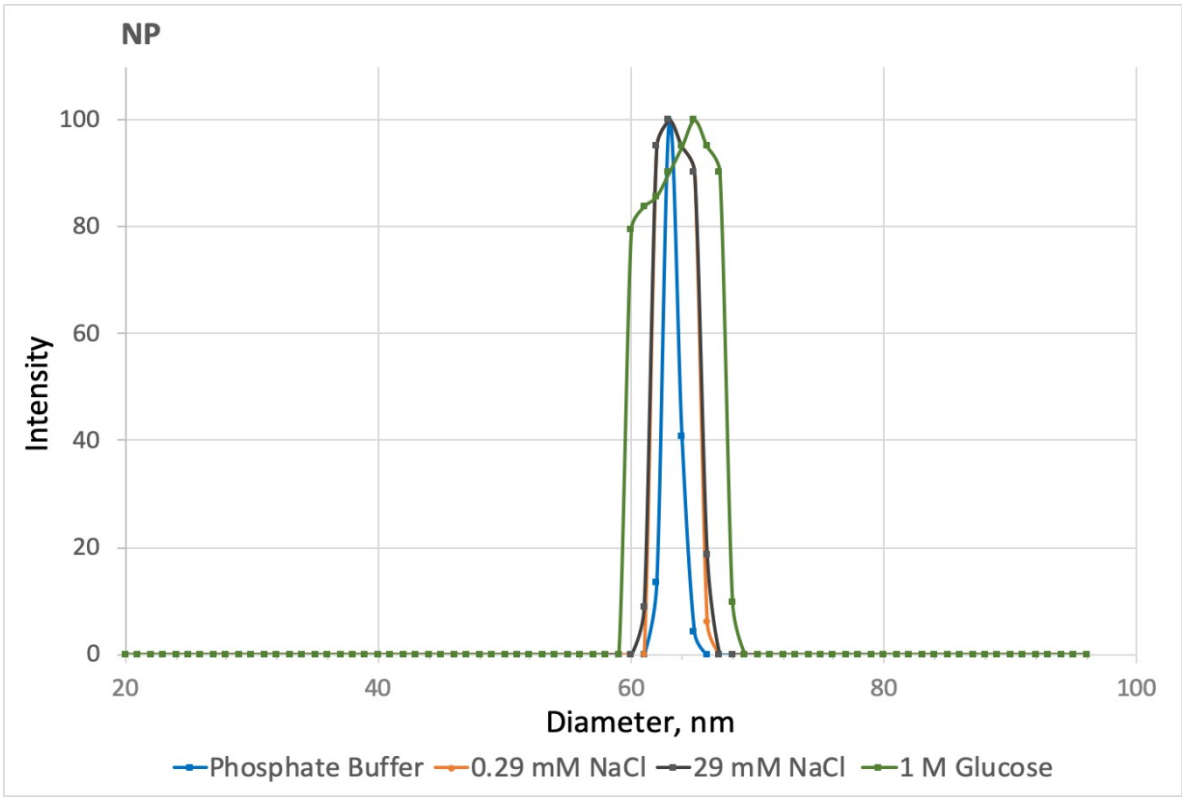
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Supplemental Information Contents	page
Figure S.1. DLS histograms of hydrodynamic diameters	2
.....	3
Figure S.2. Fluorescence anisotropy control titrations	4
Table S.1. Parameters from Langmuir adsorption isotherm fits (full titration curves in Figure 4).	5
Table S.2. ANOVA Analysis	6

Figure S.1. DLS histograms of hydrodynamic diameters



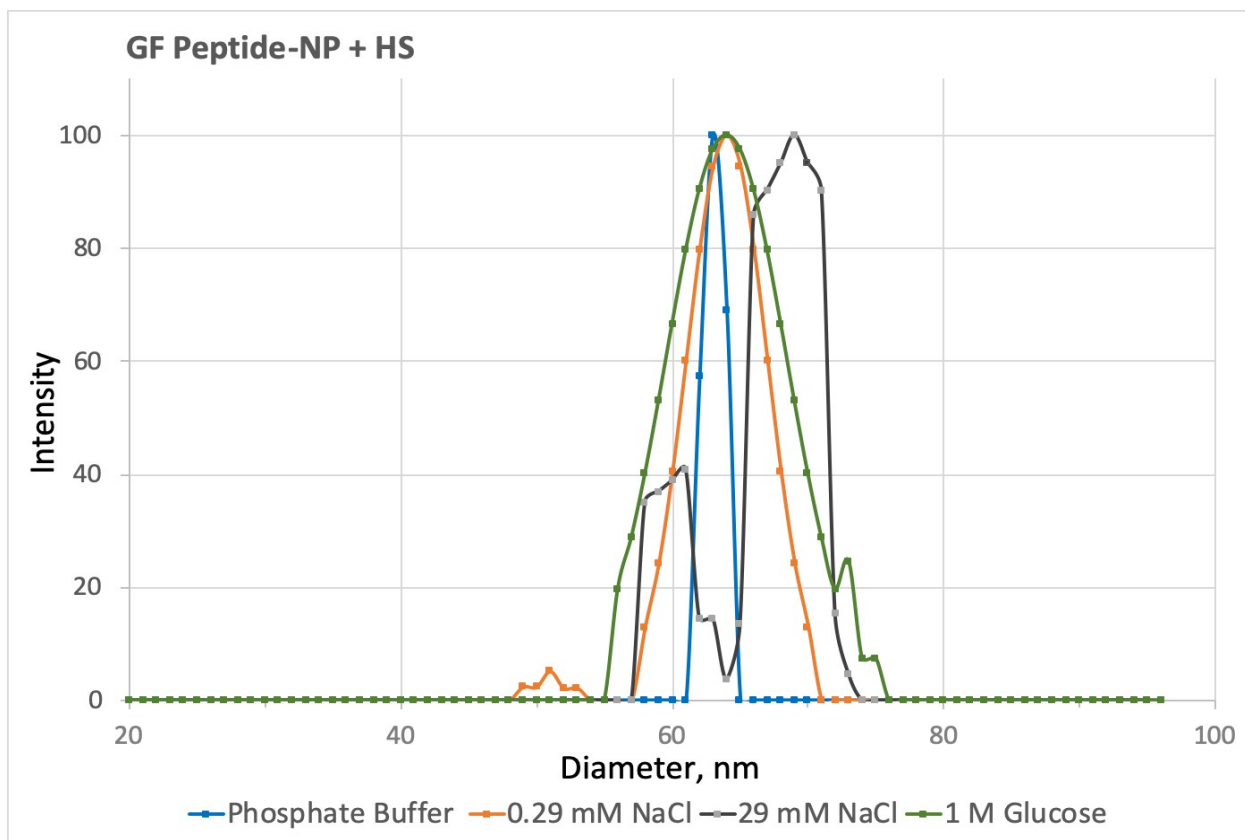
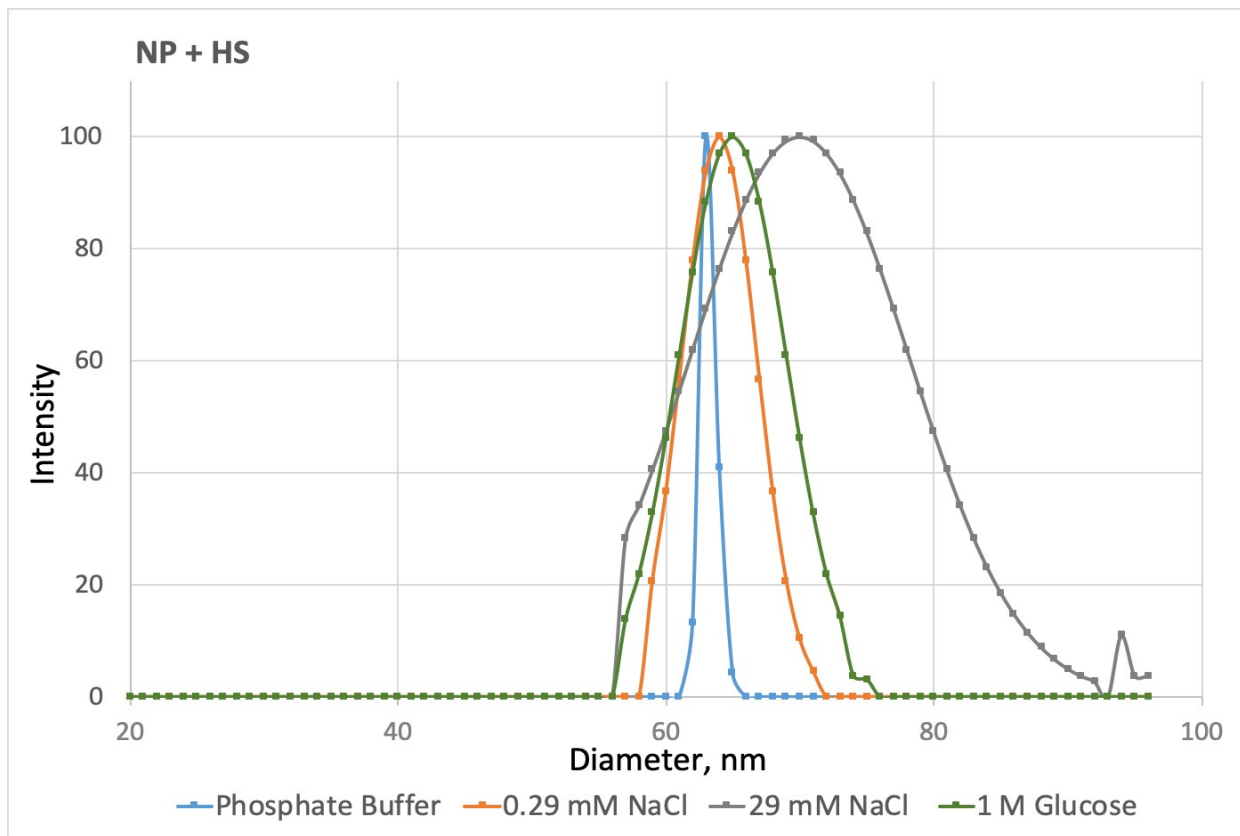


Figure S.2. Fluorescence anisotropy control titrations

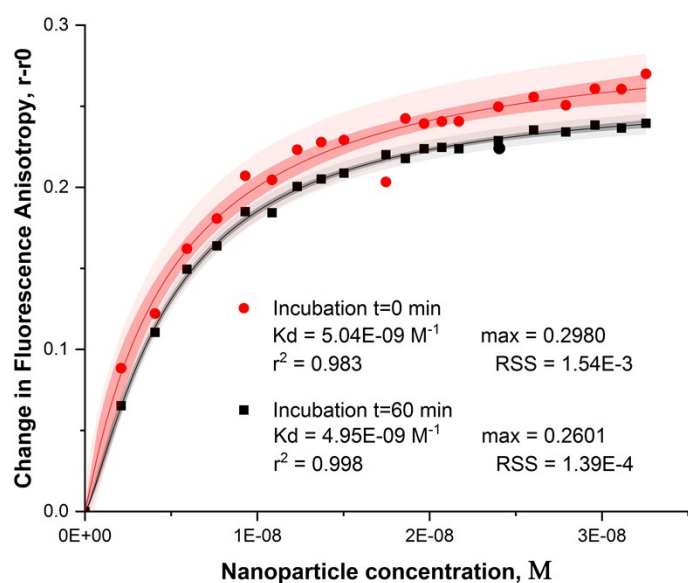


Figure S.2.a. Binding profiles for GF Peptide-NP reactions incubated for 0 min and 60 min. Longer incubation times result in smoother and more reproducible curves with improved fit parameters. RSS is the residual sum of squares for the regression analysis of the data.

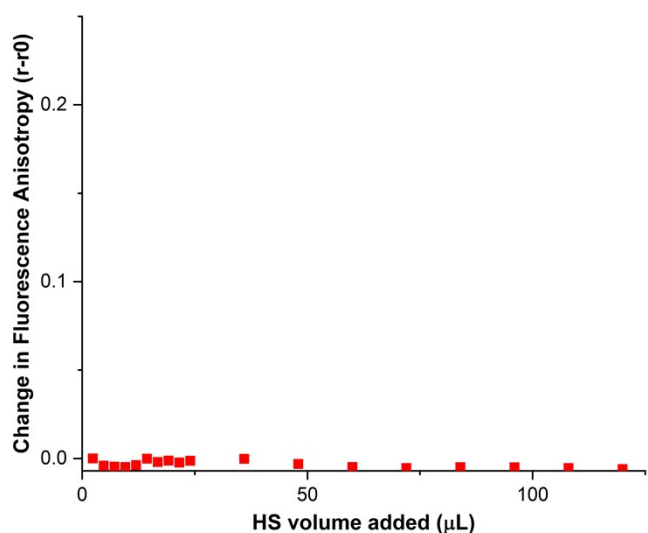


Figure S.2.b. GF Peptide titrated with increasing volumes of 1:500 human serum (HS) does not result in binding interactions characteristic of a target-ligand binding curve. These results validate that the GF Peptide-NP binding curves obtained in the presence of HS are due to Peptide-NP interactions only.

Table S.1. Parameters from Langmuir adsorption isotherm fits (full titration curves in Figure 4). Fit parameters on the top section are for assays in buffer, while the shaded, bottom section includes parameters from solutions containing 1:500 HS. Each curve was generated from n=4 measurements, RSD values are an average for all the points in each curve. The pH 7.4 and 0.29 mM NaCl solutions with HS (greyed column 3 & 6) are identical in composition (see details in experimental section), therefore averaged binding profiles and parameters reported are the same for both conditions. Thus, the parameters for the samples in 0.29 mM NaCl with HS were omitted from the table for clarity.

		pH 7.4	pH 7.2	pH 7.6	0.29 mM NaCl	29 mM NaCl	0.5 M Glucose	1.0 M Glucose
No HS	max	0.288 ± 0.003	0.294 ± 0.005	0.294 ± 0.005	0.297 ± 0.003	0.338 ± 0.006	0.253 ± 0.001	0.243 ± 0.002
	K _d , nM	5.9 ± 0.23	6.5 ± 0.35	7.6 ± 0.45	5.5 ± 0.19	18 ± 0.71	3.8 ± 0.10	4.0 ± 0.14
	r ²	0.997	0.994	0.992	0.997	0.998	0.999	0.998
	RSD	2.78	3.99	2.77	4.06	7.09	2.55	2.74
with HS	max	0.372 ± 0.017	0.372 ± 0.017	0.389 ± 0.019		0.712 ± 0.083	0.418 ± 0.026	0.340 ± 0.014
	K _d , nM	21 ± 1.8	19 ± 1.8	25 ± 2.2		80 ± 12	31 ± 3.3	20 ± 1.7
	r ²	0.991	0.989	0.991		0.994	0.991	0.991
	RSD	3.03	3.77	2.67		7.85	6.37	2.46

Table S.2. ANOVA Analysis

Homoscedasticity and Normality. Significant p-values marked with (*). Analysis conducted with samples n=3 or n=4 for each condition. Hydrodynamic diameter, Kd, and max data were log transformed for the statistical analysis.

Data	Levene's Test (Homoscedasticity)			Shapiro-Wilke's Test (Normality)	
	Df (group, residual)	F-statistic	P-value	W-statistic	P-value
K_d	5, 47	2.7095	0.03125*	0.43461	<0.001*
max	5, 47	3.5561	0.008277*	0.65237	<0.001*
hydrodynamic diameter	6, 20	0.577	0.7442	0.96058	0.3809
zeta Potential	13, 28	0.4453	0.9369	0.97258	0.4016

Dissociation constant (Kd) : 2-Way ANOVA and Tukey Test. Significant p-values marked with (*). Analysis conducted with samples n=3 or n=4 for each condition. Data was log transformed for the analysis.

ANOVA			
Parameter	Df (group, residual)	F-statistic	P-value
Conditions`	2	3.753	0.03076 *
HS	1	12.003	0.00114*
Conditions ~ HS	2	2.149	0.1279
Tukey Test (95 % Confidence Interval)			
Variance Among Conditions			
NaCl-Glu			<0.001*
pH-Glu			0.3204
pH-NaCl			0.009341*
Variance Between Human Serum ~ Non Human Serum			
HS-NonHS			<0.001*
Variance Among Conditions and HS			
Condition : HS			p-value
NaCl:No - Glu:No			0.99534
pH:No - Glu:No			0.9999
Glu:Yes - Glu:No			0.6502
NaCl:Yes - Glu:No			0.001849*
pH:Yes - Glu:No			0.7560
pH:No - NaCl:No			0.99934
Glu:Yes - NaCl:No			0.9149
NaCl:Yes - NaCl:No			0.01187*
pH:Yes - NaCl:No			0.9764
Glu:Yes - pH:No			0.69681
NaCl:Yes - pH:No			<0.001*
pH:Yes - pH:No			0.8001
NaCl:Yes - Glu:Yes			0.1856
pH:Yes - Glu:Yes			0.9983
pH:Yes - NaCl:Yes			0.02680*

Max: 2-Way ANOVA and Tukey Test. Significant p-values marked with (*). Analysis conducted with samples n=3 or n=4 for each condition. Only statistically significant Tukey test results are reported.

ANOVA

Parameter	Df (group, residual)	F-statistic	P-value
Conditions`	2	8.540	<0.001 *
HS	2	40.282	<0.001 *
Conditions ~ HS	2	2.173	0.1252
Tukey Test (95 % Confidence Interval)			
Variance Among Conditions			
Condtions		p-value	
NaCl-Glu		<0.001*	
pH-Glu		0.3203735	
pH-NaCl		0.009341*	
Variance Between Human Serum and Non Human Serum			
HS-NonHS		<0.001*	
Variance Among Conditions and HS			
Condition : HS		p-value	
NaCl:No - Glu:No		0.2211	
pH:No - Glu:No		0.4520	
Glu:Yes - Glu:No		0.004227*	
NaCl:Yes - Glu:No		<0.001*	
pH:Yes - Glu:No		<0.001*	
pH:No - NaCl:No		0.9729	
Glu:Yes - NaCl:No		0.5800	
NaCl:Yes - NaCl:No		<0.001*	
pH:Yes - NaCl:No		0.5441	
Glu:Yes - pH:No		0.1266	
NaCl:Yes - pH:No		<0.001*	
pH:Yes - pH:No		0.05948	
NaCl:Yes - Glu:Yes		0.07671	
pH:Yes - Glu:Yes		0.9999	
pH:Yes - NaCl:Yes		0.01292*	