Supplementary Information

Protein Corona-Induced Modification of Silver Nanoparticle Aggregation in Simulated Gastric Fluid

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Initial Particle Characterization

The initial characterization of each AgNP in MilliQ water (18 MΩ) performed using a suite techniques (Table S1). NTA and DLS were used to determine hydrodynamic diameter. Zeta potential (ZP) was determined along with DLS, showing no aggregation of particles in stock solution. TEM was used to determine particle geometric diameter and was measured from the images using Image J software. The NTA measured hydrodynamic diameter for each particle in water were used in Figures 1 and 2 for comparison to initial particle diameter. The TEM measured geometric diameter for each particle in water and subsequently dried on a TEM grid, were used in Figure 3 for comparison to initial particle diameter. The mass concentrations of each of the AgNP were measured for the consortium initially by the Nanotechnology Characterization Laboratory (NCL) in 2011.

Table S1. Properties (hydrodynamic diameter, zeta potential and projected area diameter) of initial AgNP in water measured using NTA and DLS, and zeta potential measured using DLS/ZP.

Method	C20	P20	C110	P110
NTA (nm)†	32 (± 1)	29 (± 2)	113 (± 4)	107 (± 4)
DLS (nm)†	24 (± 1)	30 (± 3)	105 (± 2)	117 (± 8)
TEM (nm)†	21 (± 4)	20 (± 3)	114 (± 9)	127 (± 15)
TEM (nm) ‡	20 (±3)	21 (± 4)	111 (± 9)	114 (± 8)
ZP (mV)†	-40 (± 3)	-25 (± 17)	-48 (± 12)	-35 (± 12)
Mass Conc. (mg/g)‡	1.1 (± 0.07)	1.09 (± 0.06)	0.98 (± 0.06)	1.1 (± 0.06)

†Also measured in Axson et al. 2015 and Bergin et al. 2016
‡Measured by the Nanotechnology Characterization Laboratory (NCL) 2011

NTA of Pepsin in SGF

SGF-P at each pH was characterized in the NTA prior to each trial (before addition of nanoparticles) and over a 2 hour time period to ensure that the pepsin was not significantly changing size within the SGF (Figure S1). Pepsin was determined not to interfere with the AgNP as it is both larger than the AgNPs and an order of magnitude lower in concentration, as shown Figure 2.

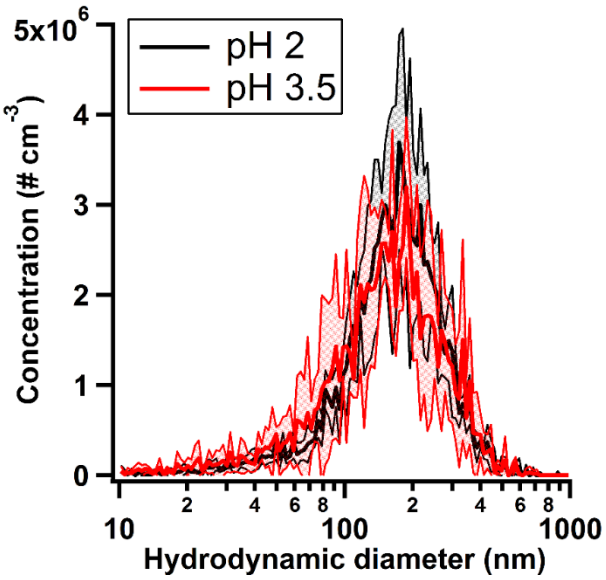

Figure S1. Pepsin size distributions measured using the NTA in SGF-P pH 2 and 3.5.

One Hour NTA Experiments

Peak hydrodynamic diameter for each AgNP determined from size distribution collected using the NTA in SGF and SGF-P for each pH. Samples were collected from the vessel at time points of 1, 10, 30, and 60 minutes during each experiment, as well as in water, to observe changes in particles during digestion in the stomach.

Table S2. The peak hydrodynamic diameter measured using NTA for all four AgNP in water and SGF/SGF-P at each pH at specified time periods during a 60 minute experiment.

Media	Time (min)	C20 (nm)		P20 (nm)		C110 (nm)		P110 (nm)	
		SGF	SGF-P	SGF	SGF-P	SGF	SGF-P	SGF	SGF-P
pH 2	1	87	254	76	167	132	155	135	145
	10	119	263	69	174	140	159	135	183
	30	150	278	58	181	153	164	138	192
	60	197	260	57	202	162	170	137	210
pH 3.5	1	74	141	61	148	129	156	120	153
	10	74	136	65	154	136	156	122	156
	30	62	145	58	175	144	160	129	158
	60	51	156	48	188	140	168	127	165
pH 5	1	72	106	46	80.7	124	144	123	142
	10	66	124	48	111	126	145	121	146
	30	58	144	43	140	130	156	121	142
	60	54	141	49	162	138	164	119	137

TEM Images of AgNP digested in SGF-P

TEM images of each of the AgNP reacted for 10 minutes in SGF-P at each pH and in water. Due to the difficulty in stopping the acidic reaction at earlier time points and damaging the TEM grid at longer time points, the 10 minute reaction time was used to examine aggregate formation.

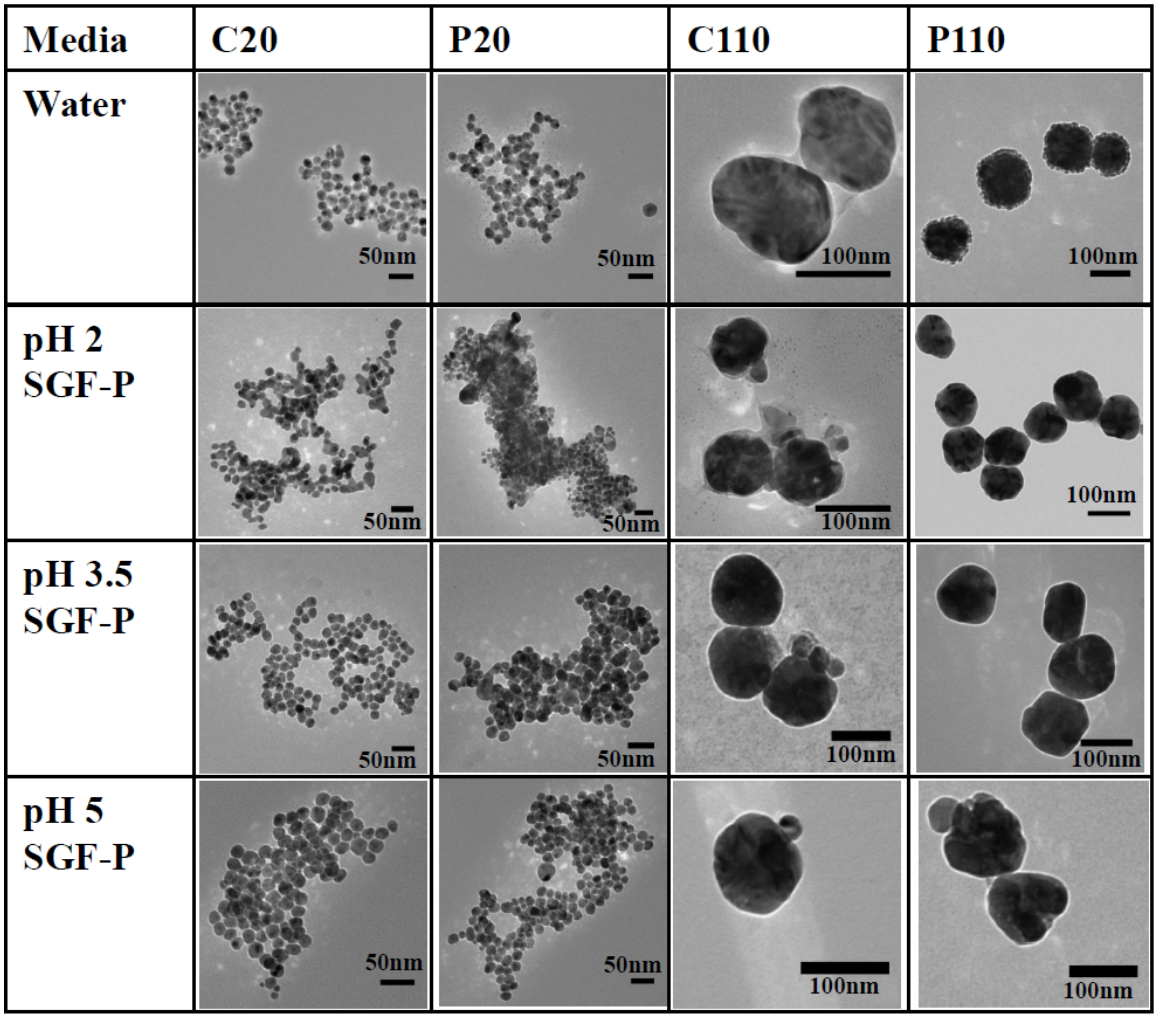

Figure S2. TEM images of all four AgNP in water for initial characterization and after 10 minutes of incubation in SGF-P at each pH.

Pepsin Tertiary Structure

Changes in pepsin tertiary structure in the presence of each AgNP in SGF at each pH was determine using UV-vis.

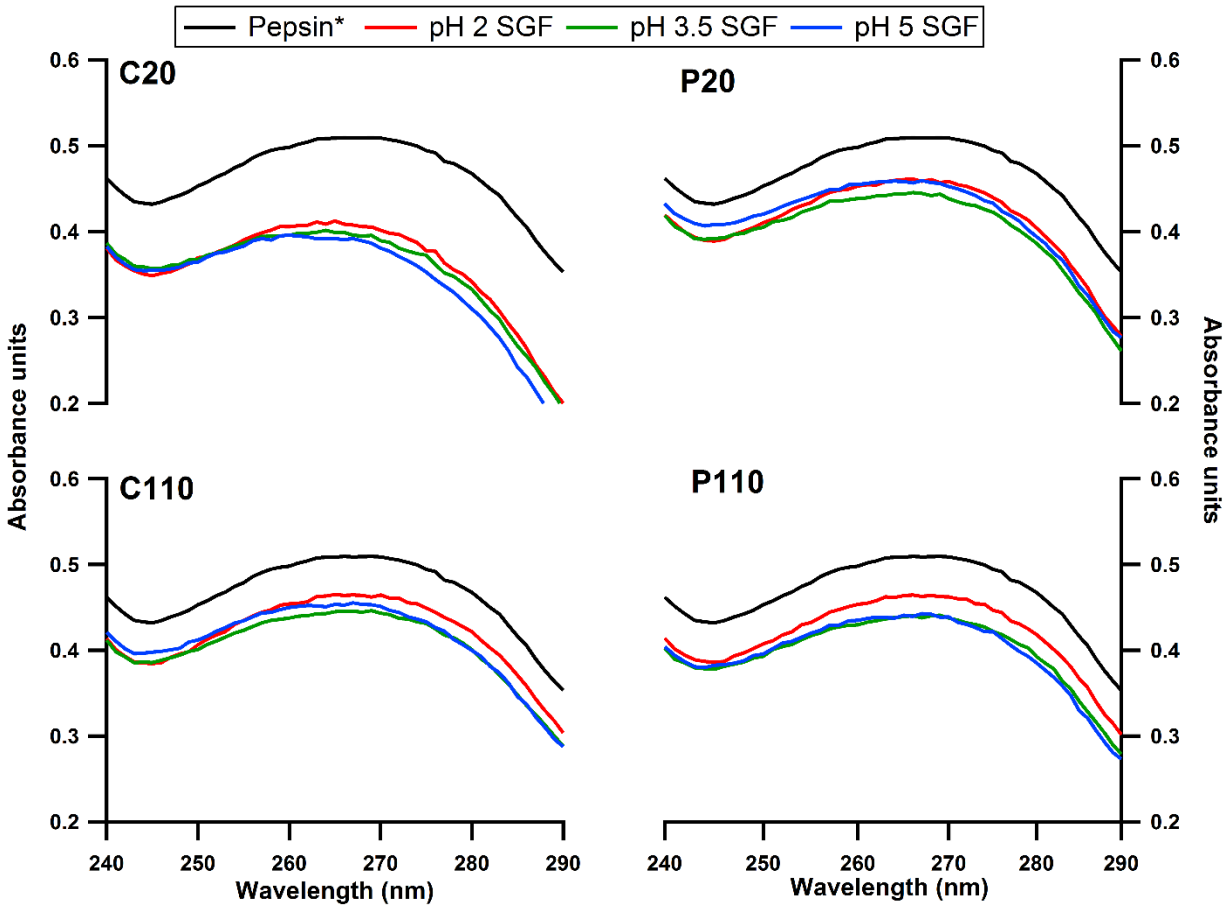

Figure S3. UV-vis spectra of pepsin after undergoing the Anson method for all four AgNP.

Influence of AgNP on Pepsin Secondary Structure

CD spectra were collected in the far-UV region from 190 - 250 nm and used to determine the changes in pepsin secondary structure upon the introduction of AgNP. Figure S4 shows the CD spectra for each particle type under the pH 2 and pH 5 conditions at 3 molar ratios. The calculated percentages of secondary structural elements are given in Table S3.

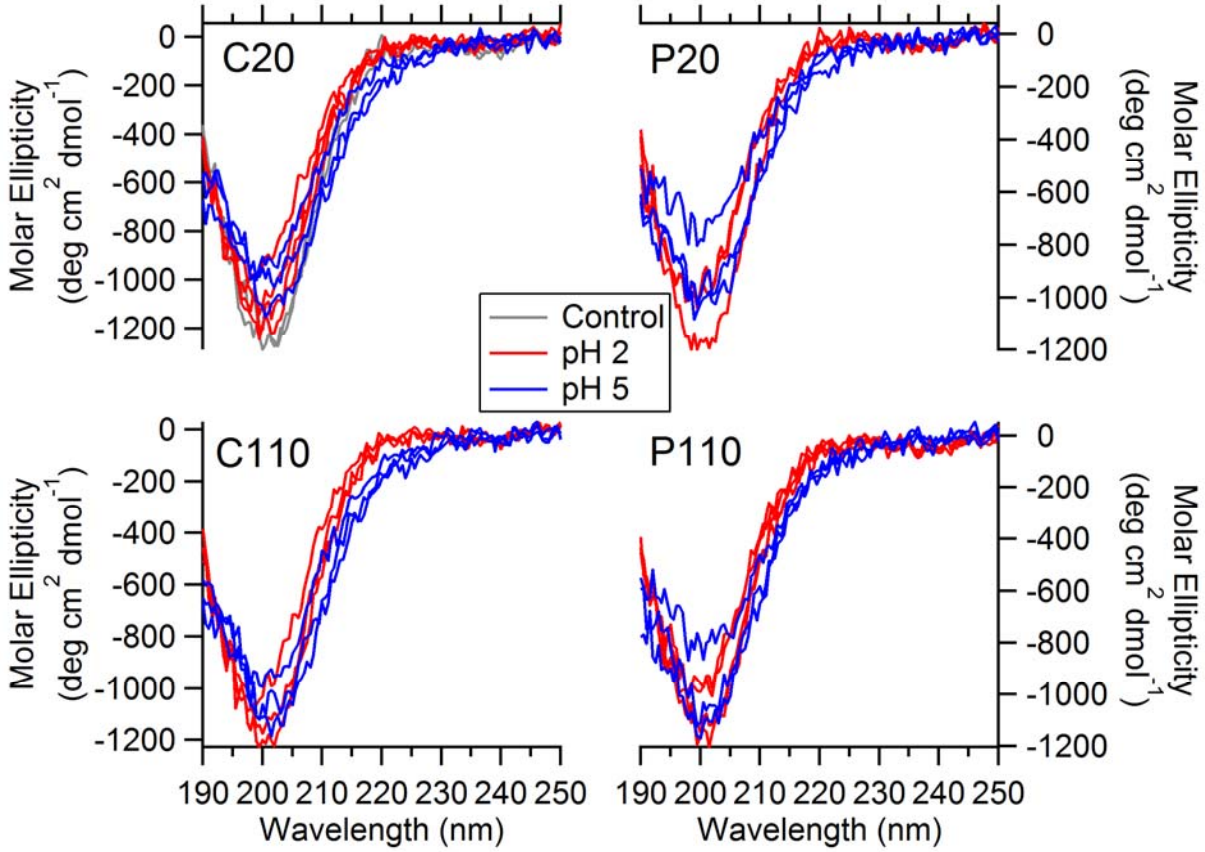

Figure S4: Molar ellipticity at 3 different AgNP:pepsin ratios and two different acidities. Control spectra are shown on the citrate 20 panel.

Table S3. The percentage of secondary structural elements in present for pepsin after incubation for 60 minutes with all four AgNP in SGF-P at pH 2 and pH 5.

Sample	Molar ratio AgNP:pepsin	pH 2				pH 5			
		α-Helix	β-Sheet	Turn	Unordered	α-Helix	β-Sheet	Turn	Unordered
Control	-	2.70	40.9	19.4	37.1	2.70	40.7	19.4	37.3
C20	158 : 1	2.70	40.9	19.4	37.0	2.60	40.9	19.4	37.1
	63.0 : 1	2.70	41.1	19.5	36.8	2.60	41.1	19.5	36.8
	31.5 : 1	2.70	40.9	19.4	37.0	2.80	41.0	19.4	36.8

P20	158 : 1	2.70	40.9	19.4	37.0	2.60	40.9	19.4	37.1
	63.0 : 1	2.70	40.9	19.4	37.0	2.60	40.7	19.4	37.3
	31.5 : 1	2.60	41.1	19.5	36.7	2.70	40.8	19.4	37.1
C110	158 : 1	2.70	41.1	19.5	36.9	2.70	40.9	19.4	37.1
	63.0 : 1	2.70	40.9	19.4	37.0	2.70	40.7	19.3	37.4
	31.5 : 1	2.70	40.9	19.4	37.1	2.60	40.6	19.2	37.6
P110	158 : 1	2.60	41.2	19.5	36.8	2.70	40.8	19.4	37.2
	63.0 : 1	2.60	41.0	19.4	37.0	2.50	40.8	19.5	37.1
	31.5 : 1	2.60	40.7	19.3	37.3	2.70	40.8	19.4	37.3

Four Hour NTA Trials

NTA trials were performed using fasted SGF and SGF-P on C20 and P20 to observe any further changes in the particles. Results indicated that C20 and P20 remained aggregated for up to 4 hours after introduction into the gastric system (Figure S4).

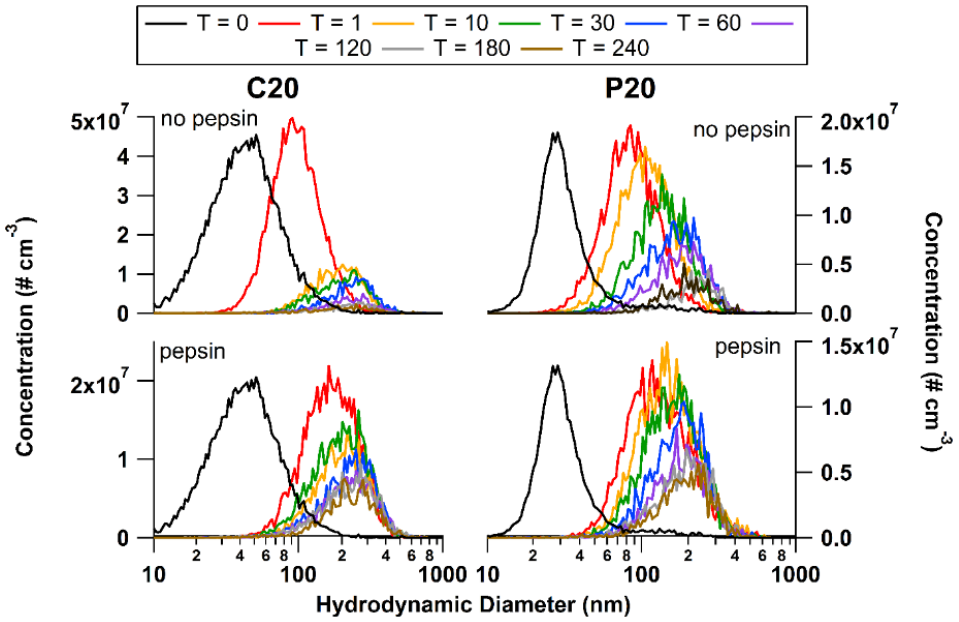

Figure S4. C20 and P20 size distributions collected using NTA for pH 2 SGF and SGF-P during a 4 hour experiment.