

# Electronic Supplementary Material

## Nanoparticles modulate Surfactant Protein A and D mediated protection against Influenza A infection *in vitro*.

DOI 10.1098/rstb.2014.0049

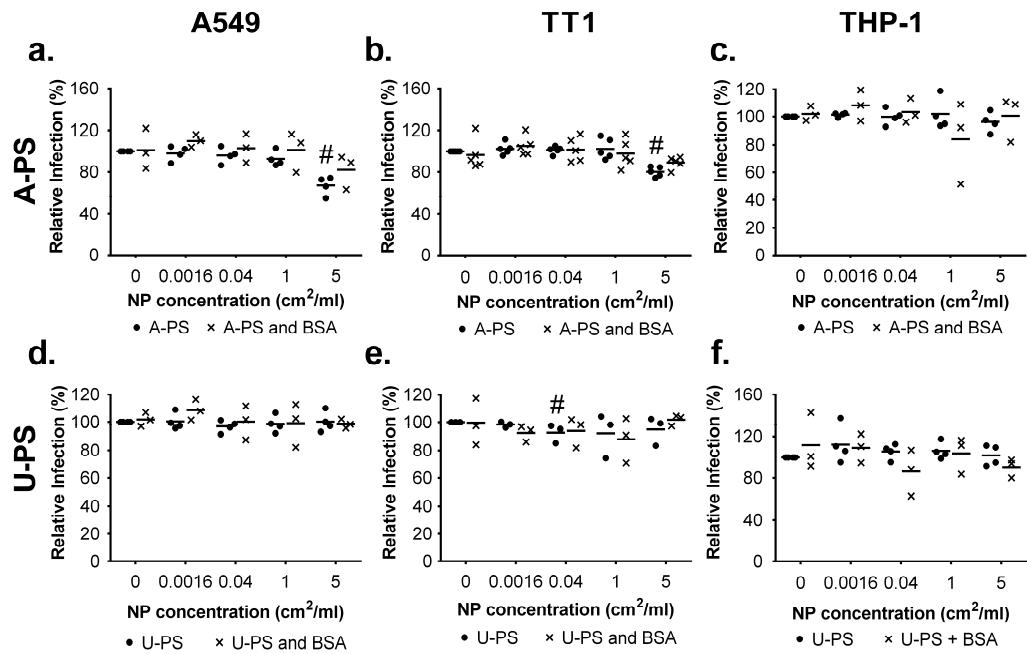
Zofi McKenzie, Michaela Kendall, Rose-Marie Mackay, Teresa D. Tetley, Cliff Morgan, Mark Griffiths, Howard W. Clark and Jens Madsen

Treatment	NP concentration (cm <sup>2</sup> /mL)				
	0	0.0016	0.04	1	5
A549 cells	Mean	100.0	100.4	97.0	98.7
	p value	-	0.219 <sup>b</sup>	0.219 <sup>b</sup>	0.219 <sup>b</sup>
U-PS + SP-A	Mean	65.5	73.5	74.1	63.4
	p value	<b>0.019<sup>a</sup></b>	<b>0.050<sup>b</sup></b>	<b>0.050<sup>b</sup></b>	<b>0.050<sup>b</sup></b>
U-PS + SP-D	Mean	44.7	48.6	44.2	45.3
	p value	<b>0.014<sup>a</sup></b>	0.386 <sup>b</sup>	0.773 <sup>b</sup>	1.000 <sup>b</sup>
U-PS + BSA	Mean	101.8	108.6	100.3	99.1
	p value	0.435 <sup>a</sup>	0.275 <sup>b</sup>	0.827 <sup>b</sup>	0.827 <sup>b</sup>
TT1 cells					
U-PS	Mean	100.0	98.4	92.7	92.2
	p value	-	0.487 <sup>b</sup>	<b>0.037<sup>b</sup></b>	0.487 <sup>b</sup>
U-PS + SP-A	Mean	73.6	67.7	63.8	74.4
	p value	<b>0.037<sup>a</sup></b>	0.513 <sup>b</sup>	0.513 <sup>b</sup>	0.127 <sup>b</sup>
U-PS + SP-D	Mean	60.1	69.1	78.8	59.7
	p value	<b>0.037<sup>a</sup></b>	0.513 <sup>b</sup>	<b>0.050<sup>b</sup></b>	<b>0.050<sup>b</sup></b>
U-PS + BSA	Mean	99.7	92.5	94.0	88.1
	p value	0.487 <sup>a</sup>	0.513 <sup>b</sup>	0.827 <sup>b</sup>	0.513 <sup>b</sup>
THP-1 cells					
U-PS	Mean	100.0	112.4	105.6	106.3
	p value	-	0.219 <sup>b</sup>	0.219 <sup>b</sup>	0.219 <sup>b</sup>
U-PS + SP-A	Mean	90.0	97.0	101.5	91.5
	p value	<b>0.014<sup>a</sup></b>	0.564 <sup>b</sup>	0.149 <sup>b</sup>	0.386 <sup>b</sup>
U-PS + SP-D	Mean	68.4	70.7	75.6	67.1
	p value	<b>0.014<sup>a</sup></b>	0.386 <sup>b</sup>	0.386 <sup>b</sup>	0.773 <sup>b</sup>
U-PS + BSA	Mean	111.7	109.2	86.2	103.9
	p value	0.435 <sup>a</sup>	0.827 <sup>b</sup>	0.275 <sup>b</sup>	0.827 <sup>b</sup>

**Table S1: Relative Infection following influenza pre-incubation with U-PS and proteins.** <sup>a</sup>p values determined using Mann-Whitney U test versus protein free control; <sup>b</sup>p values determined using Mann-Whitney U test versus nanoparticle free protein containing control.

Treatment		NP concentration (cm <sup>2</sup> /mL)				
A549 cells		0	0.0016	0.04	1	5
A-PS	Mean	100.0	98.0	96.0	92.6	66.8
	p value	-	1.000 <sup>b</sup>	0.219 <sup>b</sup>	0.219 <sup>b</sup>	<b>0.014<sup>b</sup></b>
A-PS + SP-A	Mean	53.3	52.5	53.6	60.6	46.8
	p value	<b>0.019<sup>a</sup></b>	0.513 <sup>b</sup>	0.827 <sup>b</sup>	0.513 <sup>b</sup>	0.513 <sup>b</sup>
A-PS + SP-D	Mean	37.6	45.7	42.5	42.5	33.3
	p value	<b>0.019<sup>a</sup></b>	<b>0.043<sup>b</sup></b>	0.248 <sup>b</sup>	0.248 <sup>b</sup>	0.248 <sup>b</sup>
A-PS + BSA	Mean	101.1	110.1	102.9	101.5	82.0
	p value	0.219 <sup>a</sup>	0.513 <sup>b</sup>	0.827 <sup>b</sup>	0.827 <sup>b</sup>	0.275 <sup>b</sup>
TT1 cells						
A-PS	Mean	100.0	102.5	101.2	102.5	80.2
	p value	-	0.577 <sup>b</sup>	0.095 <sup>b</sup>	0.577 <sup>b</sup>	<b>0.005<sup>b</sup></b>
A-PS + SP-A	Mean	63.0	69.0	74.0	69.7	57.3
	p value	<b>0.007<sup>a</sup></b>	0.248 <sup>b</sup>	<b>0.021<sup>b</sup></b>	0.386 <sup>b</sup>	0.386 <sup>b</sup>
A-PS + SP-D	Mean	60.9	54.0	55.2	55.5	46.6
	p value	<b>0.016<sup>a</sup></b>	0.251 <sup>b</sup>	0.602 <sup>b</sup>	0.465 <sup>b</sup>	0.076 <sup>b</sup>
A-PS + BSA	Mean	96.6	105.2	101.7	97.9	89.0
	p value	0.180 <sup>a</sup>	0.117 <sup>b</sup>	0.465 <sup>b</sup>	0.917 <sup>b</sup>	0.602 <sup>b</sup>
THP-1 cells						
A-PS	Mean	100.0	101.4	99.9	101.9	96.2
	p value	-	0.219 <sup>b</sup>	1.000 <sup>b</sup>	1.000 <sup>b</sup>	0.219 <sup>b</sup>
A-PS + SP-A	Mean	72.0	80.2	72.1	68.5	74.4
	p value	<b>0.019<sup>a</sup></b>	0.127 <sup>b</sup>	0.827 <sup>b</sup>	0.827 <sup>b</sup>	0.827 <sup>b</sup>
A-PS + SP-D	Mean	63.1	69.4	60.5	64.3	64.1
	p value	<b>0.014<sup>a</sup></b>	0.386 <sup>b</sup>	0.885 <sup>b</sup>	1.000 <sup>b</sup>	0.885 <sup>b</sup>
A-PS + BSA	Mean	102.0	108.2	103.5	84.2	100.6
	p value	0.435 <sup>a</sup>	0.513 <sup>b</sup>	0.827 <sup>b</sup>	0.513 <sup>b</sup>	0.513 <sup>b</sup>

**Table S2: Relative Infection following influenza pre-incubation with A-PS and proteins.** <sup>a</sup>p values determined using Mann-Whitney U test versus protein free control; <sup>b</sup>p values determined using Mann-Whitney U test versus nanoparticle free protein containing control. Significant values in bold.



**Figure S1: Effect of 100 nm A-PS and U-PS with BSA on influenza A infection.**  
 A-PS were pre-incubated with 5  $\mu\text{g}/\text{mL}$  BSA prior to incubation with IAV and treatment of **A.** A549, **B.** TT1 and **C.** differentiated THP-1 cells. U-PS particles were pre-incubated with 5  $\mu\text{g}/\text{mL}$  BSA prior to incubation with IAV and treatment of **D.** A549 **E.** TT1 and **F.** THP-1 cells. Horizontal line represents mean of at least three independent experiments conducted in duplicate. Statistics determined using Mann-Whitney U test where \* $p \leq 0.050$  compared to relative infection in particle free protein control; # $p \leq 0.050$  versus IAV alone.