Figure E1

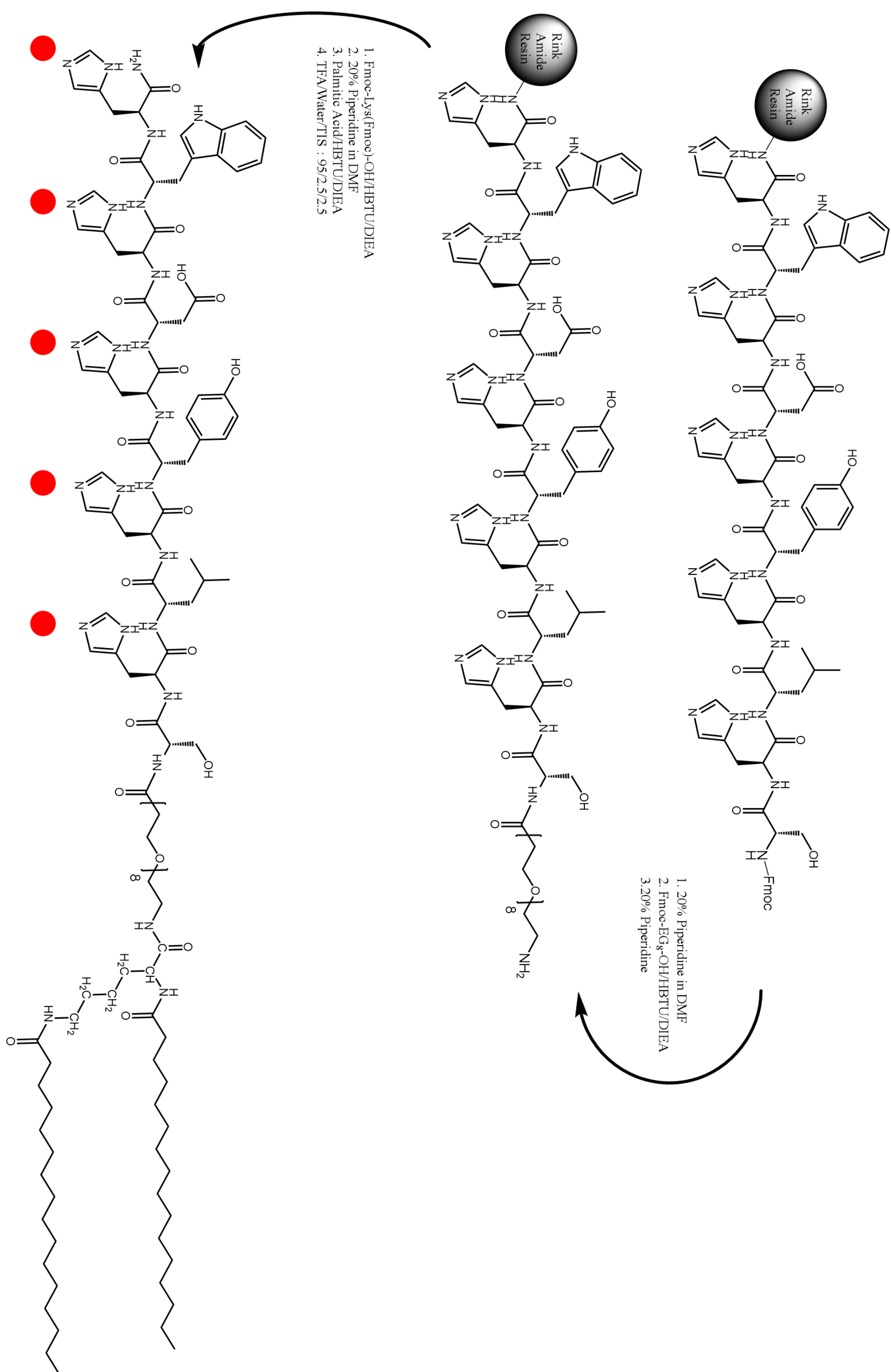
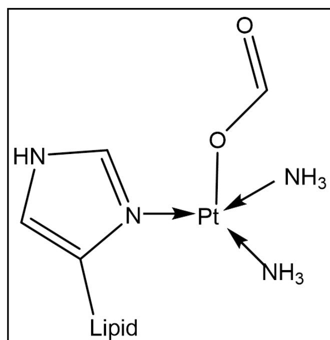


Figure E2

	Number of Platin substructures per lipid				Adducts		Exact Mass	m/z - Expected (Observed)		
Compound	A	B	C	D	H^+	Na^+	(Da)	+4	+3	+2
Cpt-Lipid	1				2		2669.43	667.85 (667.86)		
					1				890.17 (890.19)	
					0					1334.71 (1334.72)
	1	1					2896.43	724.61 (724.61)	965.81 (965.82)	1448.21 (1448.22)
	2						2943.45	735.86 (735.88)	981.15 (981.16)	
	1	2					3127.47	781.87 (781.86)		
Oxpt-Lipid			1		2		2704.49	676.62 (676.63)		
					1				901.83 (901.83)	
					0					1352.24 (1352.25)
			2				3013.57	753.39 (753.40)		
			3				3321.65	830.41 (830.42)	1106.55 (1106.54)	
			2	1		1	3375.68	849.67 (849.67)		
			4				3627.73	906.93 (906.91)	1209.24 (1209.23)	
			2	2		2	3707.73	926.93 (926.94)		
			5				3935.81	983.95 (983.95)	1311.23 (1311.23)	1966.31 (1966.31)

Note: Observed masses are listed top to bottom from most abundant

A.



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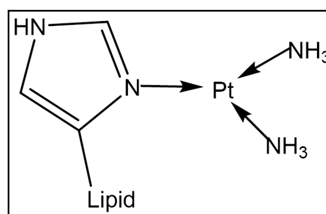

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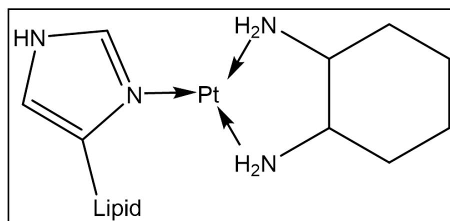

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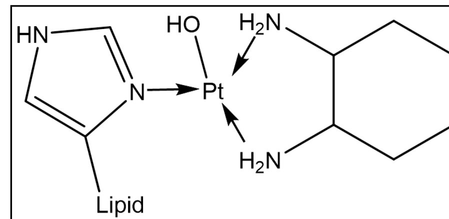

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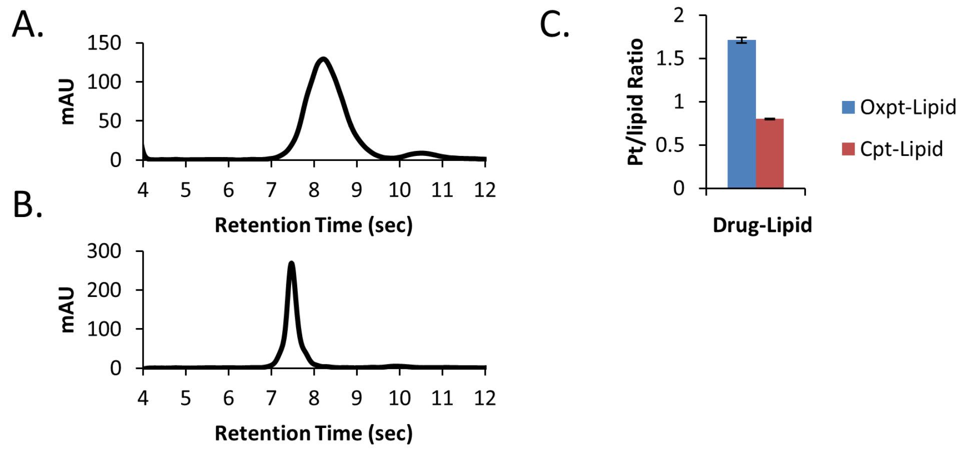

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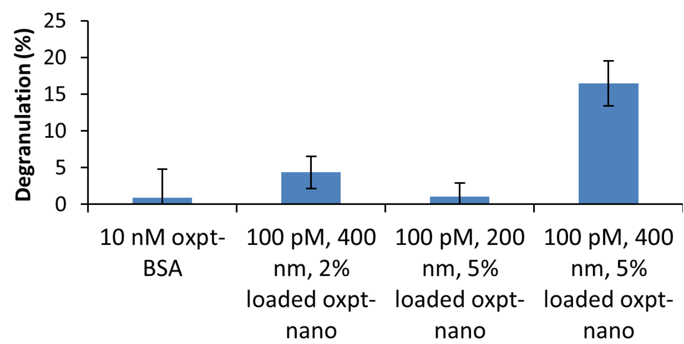

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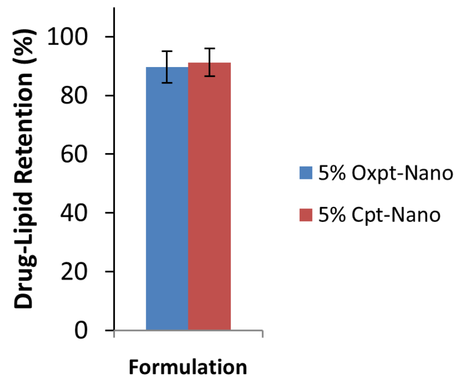


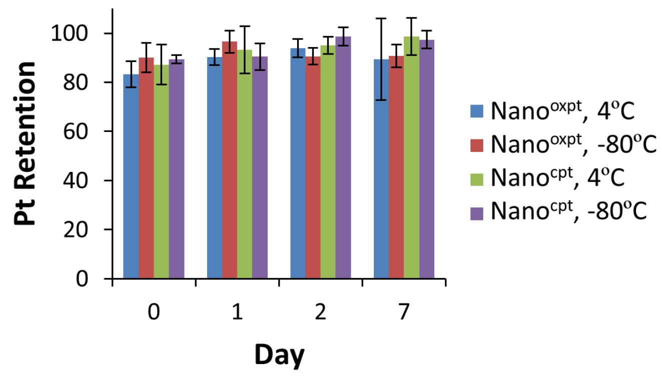
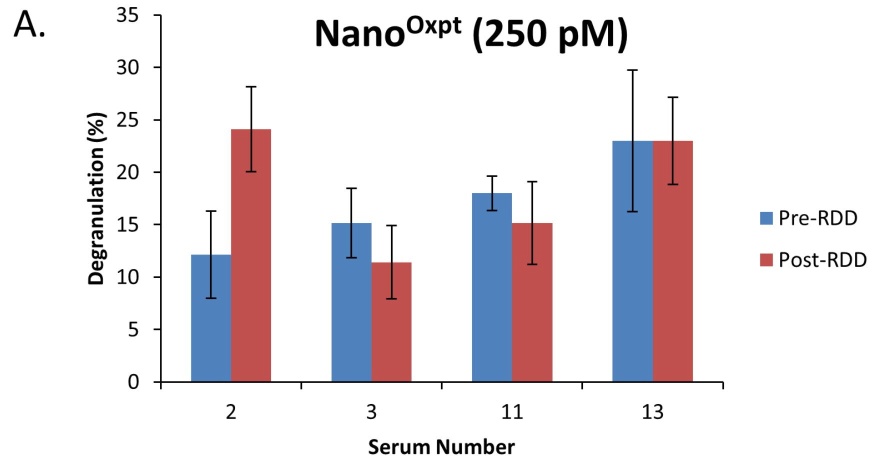


Figure E6



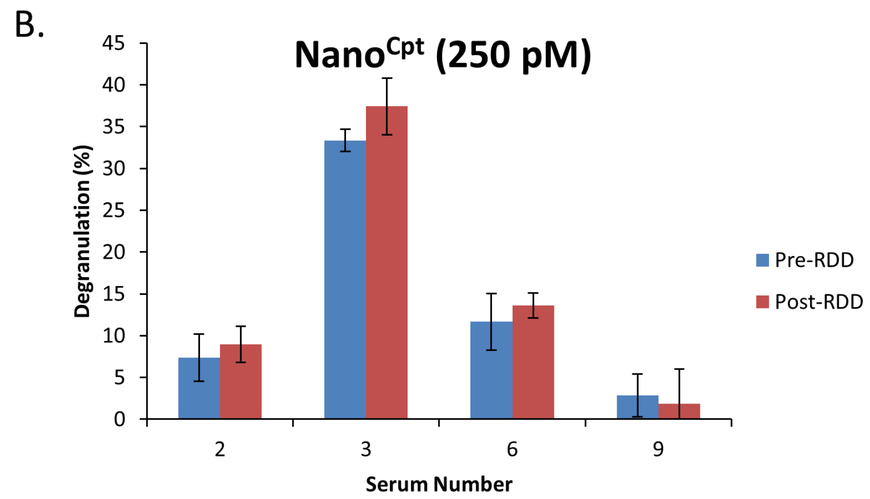
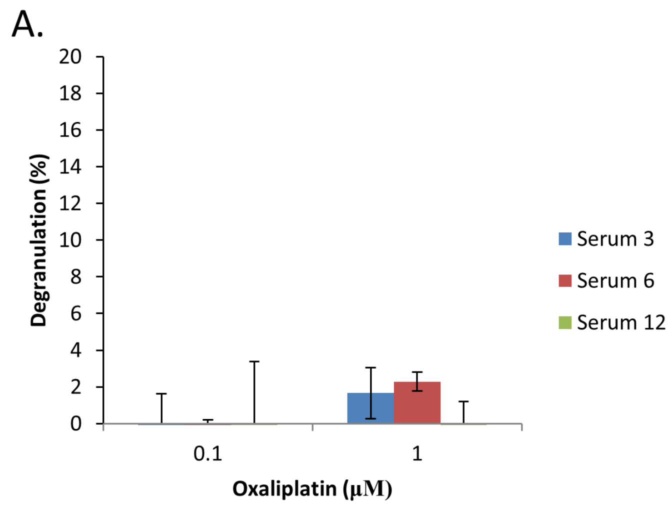


Figure E7

A.



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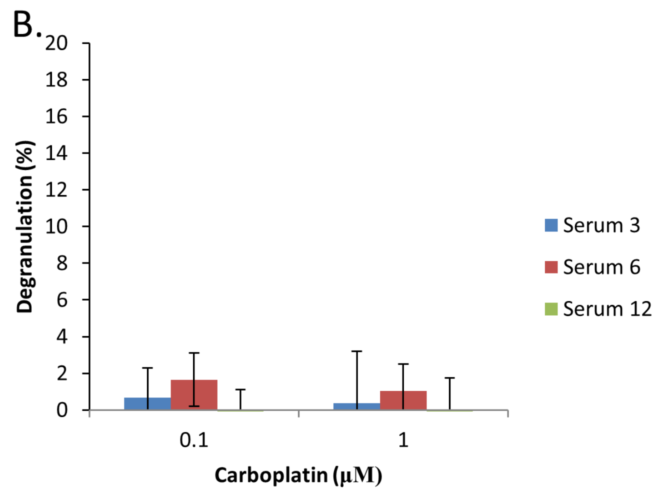
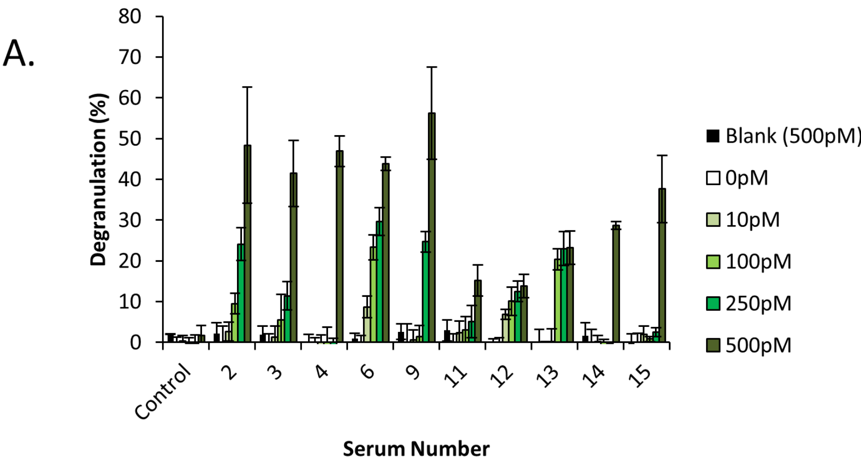
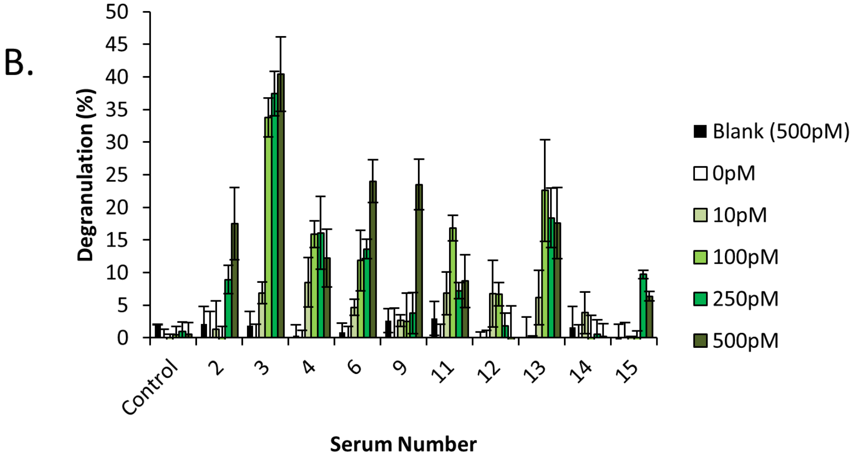


Figure E8





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13 **Supplemental Information For: Nanoallergen Platform for the Detection of Platin Drugs Allergies**
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35 ***Materials and Methods***

36 **Materials**

37 N-Fmoc-amido-dPEG$_8$-acid [Fmoc is also known as fluoren-9-ylmethoxycarbony] was purchased from
38 Quanta BioDesign. All Fmoc protected amino acids, NovaPEG Rink Amide resin, HBTU [2-(1*H*-
39 benzotriazol-1-yl)-1,1,3,3-tetramethyluroniumhexafluorophosphate], Fmoc-Arg(pfb)-OH and BSA
40 (Bovine Serum Albumin) was purchased from EMD Biosciences. DIEA (*N,N*-diisopropylethylamine), TFA
41 (trifluoroacetic acid), Triisopropylsilane (TIS), Cholesterol, Dichloromethane, 2-proponol,
42 ACN(acetonitrile), DMSO and piperidine were from Sigma and DMF (dimethylformamide) (>99.8%),
43 chloroform, Minimum Essential Media, goat anti-human IgE was purchased from Thermo Fisher. DSPC
44 (1,2-distearoyl-*sn*-glycero-3-phosphocholine), DSPE-mPEG2000 (1,2-distearoyl-*sn*-glycero-3-
45 phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (ammonium salt)), membranes and all
46 mini extruder components were purchased from Avanti Polar Lipids (Alabaster, Al, USA). Fetal Bovine
47 serum and G418 sodium salt was purchased from Gemini Bioproducts (Sacramento, CA). Cytochalasin
48 D, oxaliplatin (oxpt) and carboplatin(cpt) and 4-MUG (4-Methylumbelliferyl-β-D-glucuronide hydrate)
49 were purchased from Sigma. RBL-2H3 cells were purchased from ATCC and RBL-SX38 cells were a
50 generous gift from Dr. Jean-Pierre Kinet.

51 **Statistical Evaluation**

52 Unless otherwise stated all error bars represent the standard deviation of triplicates in a single
53 experiment. For degranulation experiments, the data is a representative experiment of several
54 experiments; all others were a single experiment. EC_{50} values and error were calculated using Origin 7
55 software. All p values were calculated using an unpaired student's t test.

56 **Synthesis and purification of Platin-Lipid (oxpt-lipid and cpt-lipid)**

57 Platin lipid conjugates were synthesized in two phases. First, peptide-lipid conjugates were synthesized
58 using Fmoc chemistry on solid support using NovaPEG Rink Amide resin as previously described.(1) The
59 synthetic scheme is described in Figure E-1. First the peptide (HWHDHYHLHS) was synthesized on the
60 solid support. Briefly, Fmoc protected amino acids with terminal acid groups were activated with HBTU
61 and a four-fold molar excess of DIEA for 5 minutes and then conjugated to the resin over 30 minutes.
62 Fmoc was deprotected with 20% piperidine in DMF. Deprotection and coupling steps were monitored
63 with Kaiser tests. An Fmoc-amido-$dPEG_8$-acid was added in a similar fashion, followed by Fmoc-Lys-
64 Fmoc-OH. Finally, a lipid tail was added by addition of a palmitic acid tail to form the peptide-lipid.
65 Peptide-lipid was then cleaved from resin using a 95/2.5/2.5 TFA/water/TIS solution for 45 minutes.
66 Peptide-lipid molecules were purified using 1200 Agilent RP-HPLC using a semi-preparative Zorbax C3
67 column. A two phase water and 70/20/10 IPA/ACN/water mix was used for purification with a gradient
68 of 60-100% IPA mix over 10 minutes at a flow rate of 3 mL min^{-1}. The product was confirmed using a
69 Bruker microTOF II mass spectrometer (m/z, expected: 2430.38, observed: 2430.38). Absorbance peaks
70 at 220nm and 280nm were collected and verified for purity with analytical injections (>95%).

71 The purified peptide lipid was then incubated with either oxpt or cpt to facilitate conjugation of the
72 reactive platinum center with nucleophilic histidine residues in the peptide lipid. Briefly, 10 mg of
73 peptide lipid was dissolved in 5 mL of DMF and 100 µL of DIEA is added. In a separate vial, oxpt or cpt is
74 dissolved: 30 mg of oxpt was dissolved in 600 µL of DMSO, or 30 mg of cpt was dissolved in 3 mL of
75 bicarbonate buffer (pH 10.0). Solutions containing peptide lipid and platin molecule (either oxpt or cpt)
76 were mixed and incubated for either 48 hours (for oxpt) or for 7 days (for cpt) at 50ºC under constant
77 stirring. After reaction, DMF was removed via rotoevaporation and oxpt-lipid or cpt-lipid purified using
78 1200 Agilent RP-HPLC using a semi-preparative Zorbax C3 column. A two phase water and 70/20/10
79 IPA/ACN/water mix was used for purification with a gradient of 60-80% IPA mix over 10 minutes at a

80 flow rate of 3 mL min^{-1}. Compounds eluted as multiple peaks, representing various combinations of

81 platin-peptide reactions (Figure E-2). These multiple peaks were mixed and collected.

82 **Nanoallergen Preparation**

83 Liposomal nanoallergens were prepared using a procedure as previously described.(2,3) Briefly, DSPC,

84 mPEG-2000-DSPC, Cholesterol, and platin-lipids (mixed at a 90:5:5:50 ratio) were dissolved in

85 chloroform, lyophilized, rehydrated in PBS at 60ºC and then extruded through a 400 nm polycarbonate

86 filter (Avanti). Nanoallergens synthesized with 5% oxpt-lipid were termed $nano^{oxpt}$ while nanoallergens

87 synthesized with 5% cpt-lipid were termed $nano^{cpt}$.

88 **Particle Characterization**

89 Liposomes were measured for size using DLS (Dynamic Light Scattering) analysis via the 90Plus

90 nanoparticle size analyzer (Brookhaven Instruments Corp.), using 658 nm light observed at a fixed angle

91 of 90º at 20ºC. Liposome samples were diluted with 0.22 μM filter sterilized PBS to a 1.25 nM liposome

92 concentration immediately after extrusion, placed in a 50 μL quartz cuvette and particle sized.

93 **Nanoallergen Stability Study**

94 In order to determine the stability of platin-presenting nanoallergens, we synthesized 10 nM solutions of

95 either $nano^{oxpt}$ or $nano^{cpt}$ and then either kept them at 4ºC or flash froze them in liquid nitrogen and

96 stored them at -80ºC for various time points. Note that flash frozen nanoallergens were extruded in a

97 90/10 v/w solution of PBS/sucrose in order to maintain particle stability. Particles were then stored for

98 0, 1, 2 or 7 days and then tested via RP-HPLC and DLS.

99 **Cell Culture**

100 RBL-SX38 (human FcεRI expressing)(4) cells were cultured in Minimum Essential Media (Gibco) with 10%

101 fetal bovine serum (Gemini BioProducts) as previously described.(5) Briefly, every 48 or 72 hours RBL

102 cells were trypsinized with 1 mL of trypsin-EDTA solution (Thermo Scientific) for 5 minutes at 37ºC after

103 achieving confluency (≈0.5 x 10^6 Cells per mL). Then cells were removed from the plate, and split 1:3

104 into fresh media.

105 **Human Sera and Preparation**

106 Human sera from oncological patients with a previous severe systemic immediate HSR to carboplatin or

107 to oxaliplatin and/or positive skin test (prick and intradermal) undergoing desensitization were obtained

108 from Brigham and Women's Hospital. (6-8)

109 All patients included in the study gave written informed consent and were recruited after approved

110 application from the Biomedical Research-Dana-Farber/Harvard Cancer Center-

111 BIDMC/BCH/BWH/DFCI/MGH/Partners Network Affiliates (DFCI Protocol Number: 13-288).

112 Blood samples were collected on the day of desensitization (DS) just before the DS and at the end of the

113 procedure. A yellow cap BD Vacutainer® SST II Advance tube was used to obtain and separate the serum

114 sample. After a clot was formed, tubes were centrifuged at 2000g for 10 minutes at 25º C. Serum was

115 collected and stored at -80º C. (7)

116 Allergic patients to oxpt or cpt were desensitized to the culprit drug based on a dose escalation (2-2.5

117 times) every 15 min. The initial concentration of the solution in a 4 bags/16 steps protocol was 1/1000,

118 and 1/100 was the first bag concentration in a 3 bags/12 steps protocol, reaching the target dose at the

119 end of the procedure .(7,9) Standard premedication used was antihistamines antiH1 and antiH2

120 (Cetirizine, Famotidine) and oncologist's premedication (steroids). Based on symptoms of the first

121 reaction: aspirin and montelukast were used to prevent flushing or bronchospasm; ativan was used for

122 anxiety

123 All patients completed the DS and no breakthrough reactions occurred.

124 In addition to these potentially allergic patient sera, control patient serum from healthy individuals were
125 purchased commercially from PlasmaLabs international. Three different control sera were used and
126 their degranulation assay results averaged.

127 Prior to degranulation assay, serum was prepared by first spinning down samples to remove precipitates
128 and diluting into RBL cell culture media (15/85: serum/media). This dilution was added to RBL-2H3 cells
129 (i.e. non-human FcεRI expressing cells) to remove anti-rat antibodies and other cytotoxic factors for 2
130 hours.(4) Serum/media mixture was then removed and spun down again to remove dead cells and other
131 precipitates.

132 **Degranulation Assays**

133 RBL-SX38 degranulation assays were performed as previously described following the standard beta
134 hexosaminidase assay, expect using nanoallergens as the allergen.(5) These assays were run in triplicate.
135 Briefly, cells at 150,000 cells per mL per added to a cell culture 96 well plate (0.1 mL per well) and
136 allowed to attach to the plate for 16 hours until plate was confluent. Cells were then incubated with
137 previously prepared serum/media mixture for 16 hours. The next morning, the plate was washed PBS
138 and incubated with cell culture media for 2-3 hours prior to assay. Then, cells were washed with
139 Tyrodes buffer (0.2 mL each well, 2 times) and 5 μL of nanoallergen solution were added into 0.095 mL
140 of Tyrodes in each well. For positive control well, a 5 μg/mL solution of anti-human IgE(Fisher) was
141 added. After incubating for one hour, 50 μL of cell supernatant solution was added to 50 μL of 1 mM 4-
142 MUG solution in citrate buffer (pH 4.5) and allowed to incubate at 37°C for 20 mins. This reaction was
143 stopped by adding a 1 mM solution of glycine (pH 10.7) and read fluorescence (ex. 365/em. 445). To
144 calculate percent degranulation, the absorbance from the triplicate was averaged and then subtracted
145 from the negative control and divided by positive control.

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177

178 Supplemental Figure Legends

179 Figure E-1. Synthetic Scheme for peptide-lipid. The peptide HWHDHYHLHS was synthesized using Fmoc
180 solid phase peptide synthesis techniques. After peptide addition, and EG_8 spacer was added followed by
181 addition of a palmitic acid tail. A red dot indicates a potential platin conjugation site.

182 Figure E-2. Predicted Platin-Lipid Structures from MS analysis. Substructures listed below table

183 Figure E-3. Purity and Platinum Ratio of oxpt-lipid and cpt-lipid. (A) RP-HPLC analysis of purified oxpt-
184 lipid. Note the broad peak indicating a mixture of several types of oxpt-lipids. (B) RP-HPLC analysis of
185 purified cpt-lipid. Note the broad peak indicating a mixture of several types of oxpt-lipids. Both
186 compounds demonstrated >95% purity. (C) Ratio of platinum atoms (as determined by ICP analysis) per
187 peptide-lipid molecules (as determined by tryptophan absorbance) for both oxpt-lipid and cpt-lipid.
188 Error bar indicate ± STD of triplicate experiments.

189 Figure E-4. Comparison of various nanoallergen formulations on degranulation sensitivities. 4
190 formulations were synthesized and then tested by RBL cell assay with patient serum 12. From left to
191 right (1) 10 nM of a BSA protein conjugated with an average of 8 oxpt per protein (oxpt-BSA), (2) 100 pM
192 of a 400 nm nanoallergen loaded with 2% oxpt-lipid, (3) 100 pM of a 200 nm nanoallergen loaded with
193 5% oxpt-lipid, (4) 100 pM of a 400 nm liposome loaded with 5% oxpt-lipid ($nano^{Oxpt}$). Error bar indicate ±
194 STD of triplicate experiments.

195 Figure E-5. Nanoallergens retain platin metabolites. (A) A solution of freshly extruded 10 nM $Nano^{oxpt}$ or
196 $Nano^{cpt}$ in PBS was tested with RP-HPLC before and after rehydration and extrusion and the peak areas
197 compared to calculate a drug-lipid retention. Both nanoallergens retain >90% of drug-lipid after
198 liposome formation. (B) A 2 nM solution of 10 nM $Nano^{oxpt}$ or $Nano^{cpt}$ was either kept at 4ºC or flash
199 frozen in a 10% sucrose solution and kept at -80ºC for 1, 2 or 7 days. Solutions were then purified with
200 LEP and pre and post-purification samples were tested with ICP. Pt retention was determined by
201 comparing pre and post purification Pt concentration. Error bar indicate ± STD of triplicate experiments.

202 Figure E-6. RDD does not alter sIgE induced degranulation to nanoallergens. Human FcεRI expressing
203 RBL-SX38 cells were primed with serum from patients before or after RDD (15% serum/85% media)
204 overnight, washed and degranulation triggered by incubating with (A)250 pM $Nano^{Oxpt}$ or (B) 250 pM
205 $Nano^{Cpt}$ and degranulation measuring using a beta-hexosaminidase assay. Error bar indicate ± STD of
206 triplicate experiments.

207 Figure E-7. Free platin drugs do not induce RBL degranulation. RBL-SX38 cells were primed with serum
208 from patients 3, 6, or 12 (15%) and then incubated with either free oxaliplatin (A) or carboplatin (B).
209 Concentrations above 1 µM demonstrated high cytotoxicity (data not shown).

210 Figure E-8. Nanoallergen Degranulation Data. RBL degranulation data for all nanoallergen-serum
211 combinations. Human FcεRI expressing RBL-SX38 cells were primed with serum from patients before or
212 after RDD (15% serum/85% media) overnight, washed and degranulation triggered with either (A)
213 $nano^{Oxpt}$ or (B) $nano^{Cpt}$. Error bar indicate ± STD of triplicate experiments.

1

Table E-1. Particle Size Stability.

	$Nano^{Oxpt}$							
	Stored at 4ºC				**Stored at -80ºC**			
	Diameter(nm)	**S.E**	**P.D**	**S.E**	**Diameter(nm)**	**S.E**	**P.D**	**S.E**
Day 0	**368.74**	**6.21**	**0.18**	**0.053**	**391.67**	**3.3**	**0.176**	**0.059**
Day 1	**367.25**	**2.91**	**0.187**	**0.012**	**387.25**	**1.72**	**0.148**	**0.022**
Day 2	**372.94**	**1.69**	**0.159**	**0.02**	**395.74**	**1.48**	**0.183**	**0.025**
Day 7	**414.17**	**1.79**	**0.23**	**0.018**	**385.92**	**1.74**	**0.166**	**0.017**

	$Nano^{Cpt}$							
	Stored at 4ºC				**Stored at -80ºC**			
	Diameter(nm)	**S.E**	**P.D**	**S.E**	**Diameter(nm)**	**S.E**	**P.D**	**S.E**
Day 0	**384.76**	**2.89**	**0.188**	**0.036**	**450.51**	**3.5**	**0.145**	**0.023**
Day 1	**398.13**	**5.99**	**0.196**	**0.015**	**443.81**	**3.54**	**0.161**	**0.008**
Day 2	**399.7**	**3.5**	**0.133**	**0.021**	**439.99**	**2.43**	**0.181**	**0.021**
Day 7	**399.37**	**3.78**	**0.135**	**0.028**	**441.07**	**2.21**	**0.188**	**0.023**

2

3 * S.E- Standard Error; P.D.- Polydispersity. Nanoallergens stored at -80℃ were extruded in a 10/90 sucrose/PBS solution (w/v) and then flash frozen in liquid nitrogen before storage.

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Table E-2. Patient Demographics

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Patient	Sex	Age	Drug	Type of cancer	Number of lifetime infusions prior to reaction	Rxn Grade	Symptoms during reaction	Skin tests	Atopy	Tryptase (ng/ml)
2	F	75	Carboplatin	Ovarian	>10	1	Flushing and itching	Positive at 1st ID (1mg/ml)	Rhinitis and asthma, drug allergy (flushing with levofloxacin)	18.2 (during 1st RDD) 5.7 (baseline)
3	F	68	Carboplatin	Uterus	16	3	Itchy palms and feet, flushing, back pain and throat itchiness	Positive at 1st ID (1mg/ml)	Seasonal rhinitis, drug allergy (spotty vision and fainting with 1st lifetime Taxol)	11.5 (during reaction) 4.4 (baseline)
4	F	64	Carboplatin	Ovarian	10	2	Pruritis, nausea, facial flushing, feeling of impending doom, hypertension, tachycardia and GI upset	Positive at 1st ID (1mg/ml)	None	5.5 (during reaction)
6	F	67	Carboplatin	Ovarian	>8	3	Throat discomfort, nausea, flushed and swollen face and hypotension	Positive at prick (10mg/ml)	None	29 (during reaction) 12.2 (baseline) 13.9 (baseline)
9	F	78	Carboplatin	Ovarian	>10	1	Nose flushing and palmar itching	Positive at 1st ID (1mg/ml)	Blisters with adhesive tape and seasonal conjunctivitis	Not done
11	M	53	Oxaliplatin	Colon	>6	1	Tingling lips and hives on wrists, elbows and neck	Positive at prick (5mg/ml)	Seasonal rhinitis	4 (baseline)
12	M	68	Oxaliplatin	Colon	>6	3	Unresponsiveness and bladder and bowel incontinence	Positive at prick (5mg/ml)	None	Not done
13	F	48	Oxaliplatin	Colon	0	3	Body flushing, hypotension and desaturation	Not done	Allergic rhinitis and conjuctivitis, eczema, chronic idiopathic urticaria, drug hypersensitivity (iron, latex, penicillin); patient under treatment with Xolair	3.8 (baseline)
14	F	60	Oxaliplatin	Pancreatic	6	2	Chest and throat tightness, shortness of breath, tongue swelling and sweating	Positive at 2nd ID (5mg/ml)	Asthma, drug allergy (irinotecan), food allergy (peach, apricot, nectarine)	Not done
15	F	54	Carboplatin	Tongue	9	2	Chest tightness, shortness of breath and whole body flushing	Positive at 1st ID (1mg/ml)	Seasonal allergies (ragweed)	5.4 (during reaction)

*Based on Brown's classification
F: female; M: male.