Supporting Information

Surface ligand density of antibiotic-nanoparticle conjugates enhances target avidity and membrane permeabilization of vancomycin-resistant bacteria

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Experimental details
All materials, unless otherwise noted, were obtained from commercial suppliers and used without further purification.

Characterization of N_3-vancomycin
^{1}H (600 MHz) and ^{13}C (125 MHz) NMR spectra were obtained using a Bruker Avance-600 spectrometer equipped with a TXI cryoprobe. Chemical shifts are reported relative to the residual solvent signals in parts per million (δ) (DMSO-d_6: ^{1}H: δ 2.50, ^{13}C: δ 39.5).
High resolution mass spectrometry (HRMS) was performed on a Bruker Micro TOF mass spectrometer using (+)-ESI calibrated to NH_4OAc.
LCMS analysis was conducted using Agilent Technologies 1200 Series instrument with a G1316A UV-Vis detector (λ= 210 nm and 254 nm), 1200 Series ELSD and 6110 quadrapole ESI-MS, using Agilent Eclipse XDB-Phenyl, 3.5 µm, 3x100mm: Flow: 1 mL/min: Column temperature RT: Scan mode, positive 100-1500: UV lamp 210 nm: Gradient timetable: 5% B for 1 min; 5% - 100 % B for 20 min, wash. The eluents are 0.05% formic acid in water and 0.05% formic acid in acetonitrile.
LCMS: R_t = 5.208 min @ 210 nm, found MS = $[M+2H]^{2+}$: 825, $[M+3H]^{3+}$: 550.
(+)-ESI-HRMS calc for $C_{74}H_{93}Cl_2N_{13}O_{26}$ $[M+2H]^{2+}$: 824.7866, found 824.7861.
See Figure S2 for NMR and S3 for LCMS.

Synthesis of N_3-NBD
NBD chloride (4-chloro-7-nitrobenzofurazan) was added to 3-azide propyl amine and caesium carbonate (Cs_2CO_3) in THF with heating to 50 ºC for 4 hours. After completion of the reaction, azide-NBD was extracted with ethyl acetate and washed with water. The water layer was again extracted with ethyl acetate, then the organic layer was dried with magnesium suphate and concentrated.

TEM imaging of bacterial cells
S. aureus strain ATCC 25923 was cultured as mentioned above. 200 µL of mid-log phase cells (10^9 cfu/mL) were incubated with 1 µL Van-NPs (final vancomycin concentration 0.05 µg/mL)

at 37 ºC under rotation for 4 hours before samples were prepared for imaging using JEOL 1011.

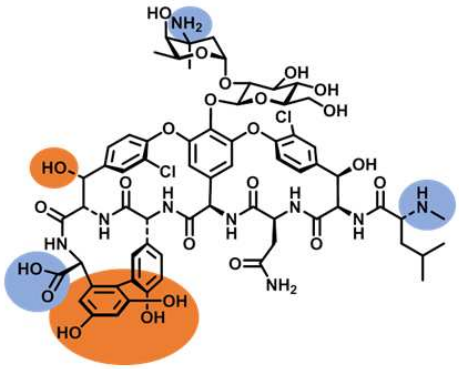


Figure S1. Potential modification sites of Vancomycin.

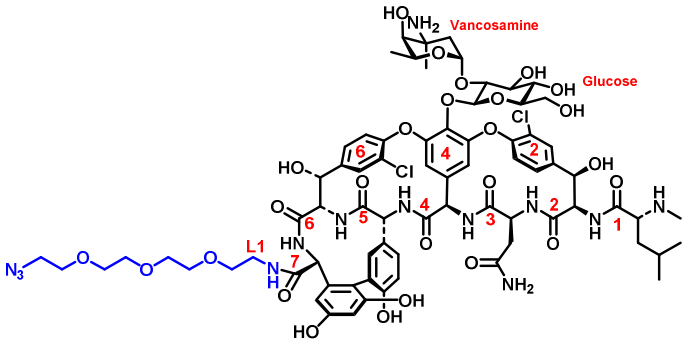


Figure S2. NMR N_3-vancomycin characterization. ^{1}H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6).[1]

Position	**Vancomycin (Williams)**		**N_3-Vancomycin (MCC_008229_01_FA salt)**	
	13C	**1H**	**13C**	**1H**
1 (D-Me-Val)				
C1	173.4		174.6	
X1	61.8	3.31 (o)	62.5	3.04 (t, 6.9)
W1		n/o		n/o
1a	40.7	1.51 (quin, 7.1) 1.47 (quin, 7.1)	41.2	1.47, m 1.39, m
1b	24.1	1.72 (non, 7.1)	24.2	1.71 (non)

1c	22.9	0.91 (d, 6.7)	23	0.88 (d, 6.5)
1d	22.5	0.86 (d, 6.7)	22.5	0.84 (d, 6.5)
1e	33.2	2.37 (s)	33.9	2.29 (s)
2				
C2	167.1		167.5	
X2	58.3	4.88 (br m)	58.1	4.87 (br s)
W2		7.93 (v br s)		7.76 (v br s)
Z2	71.1	5.16 (br s)	71.1	5.13 (br s)
Z2-OH		5.82 (br s)		n/o
2a	139.8		139.9	
2b	128.6	7.39 (br s)	128.7	7.34 (br s)
2c	127.1		126.2	
2d	149.8		149.9	
2e	124.2	7.26 (d, 8.5)	124.3	7.24 (d, 8.4)
2f	127.2	7.52 (d, 8.5)	127.2	7.49 (d, 8.2)
3 (Asp)				
C3	171.1		170.9	
X3	51.0	4.35 (br q, 5.6)	50.9	4.36 (o)
W3		6.62 (v br s)		8.20 (v br s)
3a	37.2	2.42 (o) 2.14 (dd, 15.5, 5.6)	37.6	2.36 (o) 2.13 (br d, 10.7)
C3'	170.6		168.2	
C3'-NH2		7.37 (o), 6.92 (br s)		7.32 (o) 6.86 (br s)
4				
C4	169.5		169.8	
X4	54.9	5.75 (d, 8.1)	54.9	5.73 (d, 7.8)
W4		8.25 (v br s)		8.20 (v br s)
4a	134.5		134.4	
4b	107.1	5.55 (br s)	107.1	5.52 (br s)

4c	152.1		152.1	
4d	131.9		131.9	
4e	151.3		151.4	
4f	104.6	5.21 (d, 1.4)	104.6	5.20 (s)
5				
C5	169.1		169.1	
X5	53.7	4.43 (d, 2.8)	53.8	4.42 (d, 4.5)
W5		8.64 (br d, 2.8)		8.59 (br s)
5a	126.2		126.2	
5b	135.6	7.18 (br s)	135.5	7.20 (br s)
5c	121.6		122	
5d	155.0		155.1	
5d-OH				
5e	116.2	6.72 (d, 8.5)	116.4	6.69 (d, 8.4)
5f	125.4	6.77 (dd, 8.5, 1.0)	125.3	6.74 (dd, 8.2, 1.2)
6				
C6	167.6		167.5	
X6	61.9	4.19 (d, 11.6)	62.1	4.2 (d, 11.4)
W6		6.67 (br s)		6.65 (br d, 11.7)
Z6	71.5	5.13 (br s)	71.4	5.23 (br s)
Z6-OH		5.96 (d, 6.4)		n/o
6a	142.4		142.5	
6b	127.3	7.86 (s)	127.4	7.85 (s)
6c	126.2		127.2	
6d	148.3		148.2	
6e	123.3	7.34 (d, 8.5)	123.4	7.31 (d, 8.4)
6f	127.3	7.47 (dd, 8.1, 1.0)	127.4	7.44 (d, 8.0)
7				
C7	172.5		170.3	
X7	56.7	4.42 (d, 5.6)	57.6	4.36 (d, 5.6)

W7		8.48 (br d, 5.6)		8.40 (be d, 4.3)
7a	136.2		137.6	
7b	118.0		118	
7c	156.4		156.3	
7c-OH				
7d	102.3	6.42 (d, 2.1)	102.1	6.36 (d, 1.8)
7e	157.1		157.2	
7e-OH		9.44 (s)		n/o
7f	105.8	6.26 (d, 2.1)	106.4	6.22 (d, 1.9)
G (glucose)				
G1	101.2	5.27 (d, 7.8)	101.3	5.27 (d, 7.7)
G2	78.0	3.59 (t, 8.5)	78.1	3.54 (o)
G3	77.0	3.50 (t, 8.5)	77	3.45 (o)
G3-OH		5.38 (d, 4.9)		n/o
G4	70.2	3.31 (o)	70.2	3.26 (o)
G4-OH		5.11 (br s)		5.13 (br s)
G5	76.7	3.31 (o)	76.7	3.27 (o)
G6	61.2	3.68 (dd, 10.9, 3.2) 3.57 (o)	61.2	3.66 (o) 3.51 (o)
G6-OH		4.05 (t, 5.3)		n/o
V (Vancosamine)				
V1	97.6	5.24 (d, 3.5)	96.6	5.21 (d, 3.6)
V2	33.3	1.90 (br d, 10.6) (ax) 1.75 (br d, 10.6) (eq)	33.9	1.88 (br d, 9.8, ax) 1.68 (o)
V3	53.9		53.4	
V3-NH2		n/o		n/o
V4	70.7	3.23 (br s)	70.9	3.16 (br s)
V4-OH		5.43 (br s)		n/o
V5	63.1	4.68 (q, 6.4)	63.2	4.67 (q, 6.4)

V6	15.8	1.07 (d, 6.4)	16.9	1.05 (d, 6.3)
V7	22.2	1.32 (s)	22.8	1.26 (s)
Linker				
L1 CH2-NH			38.7	3.25 (o) 3.32 (o)
L2			69	3.48 (o)
L3			69.6, 69.76, 69.79	3.55, m
L4				
L5				
L6				
L7			69.3	3.59 (t, 5.1)
L8 CH2-N3			50	3.38 (t, 5.1) 3.60 (t, 5.1)

Abbreviations br = broad; d = doublet; m = multiplet; non = nonet; o = obscured; quin = quintet; s = singlet; t = triplet; v br = very broad; q = quartet

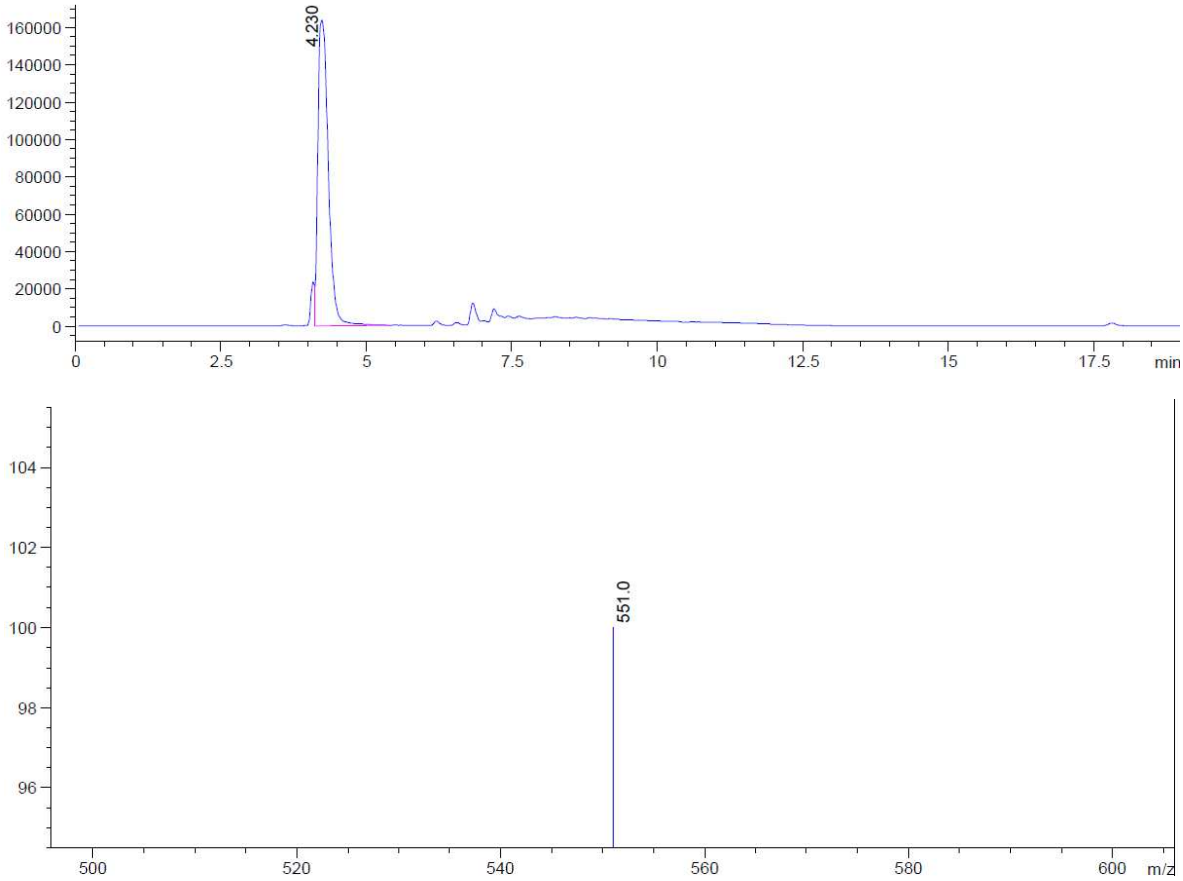


Figure S3. LC and –MS of N_3-vancomycin.

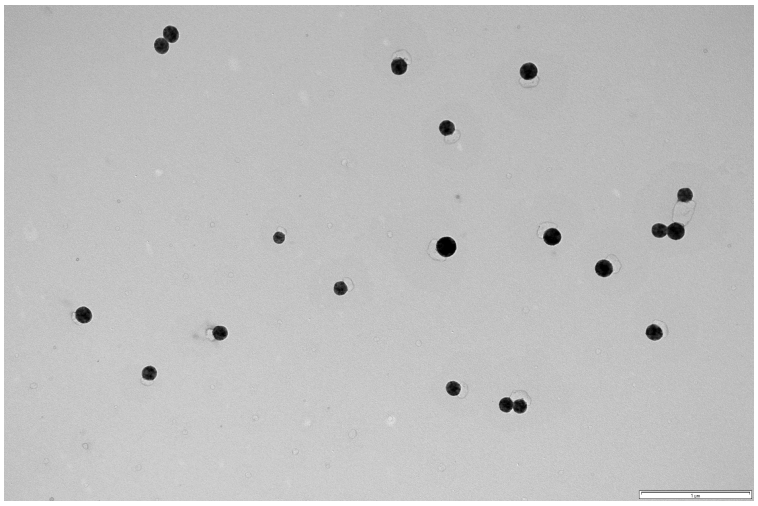


Figure S4. Transmission electron microscopy imaging (JEOL 1011) of HSA-NPs. (Scale bar is 1 µm).

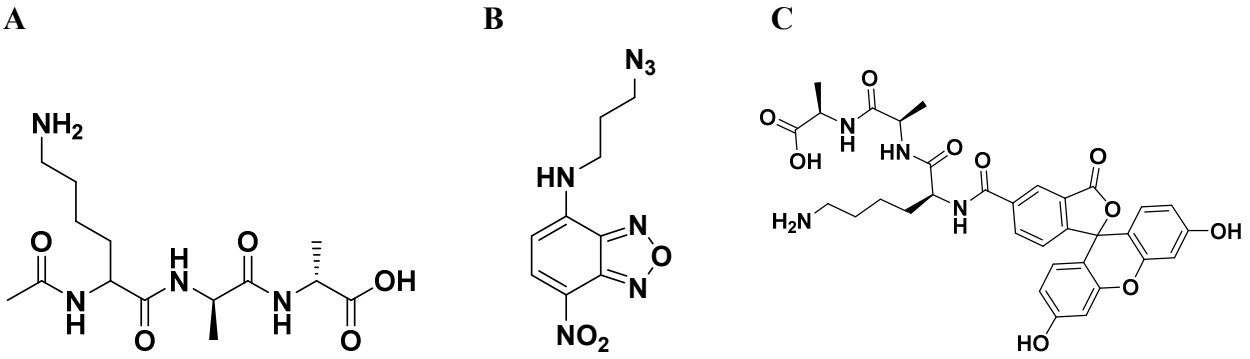
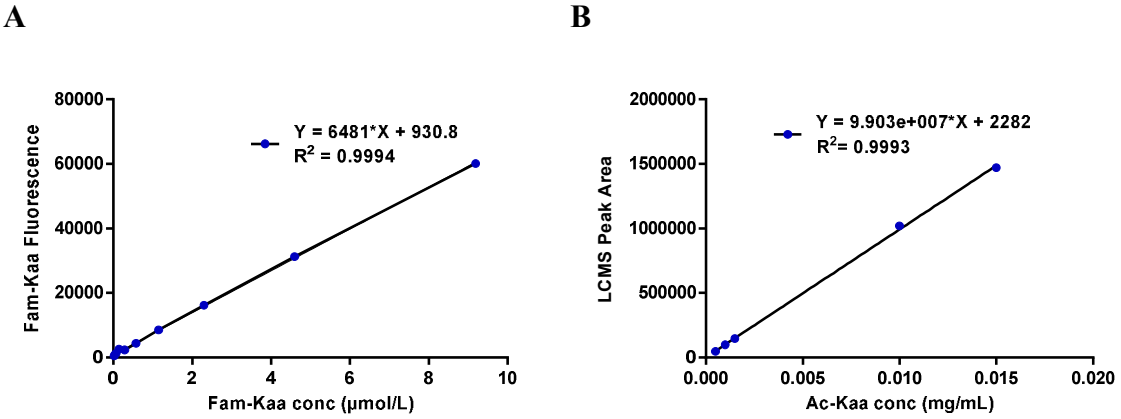
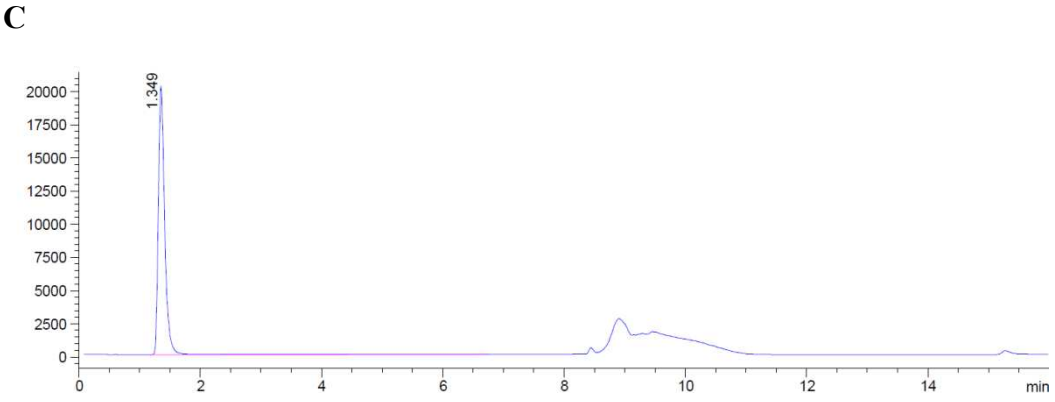


Figure S5. A) Ac-Kaa structure. **B)** N_3-NBD structure. **C)** Fam-Kaa structure.





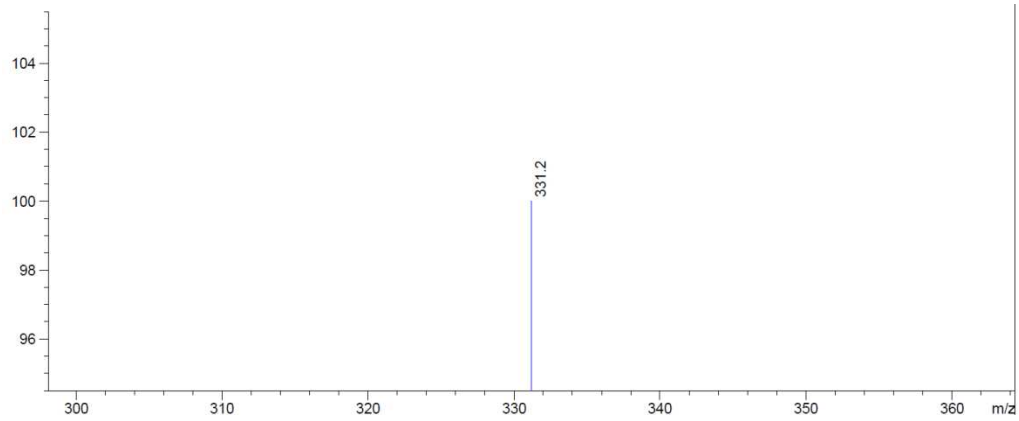


Figure S6. A) Quantification standard curve for Fam-Kaa. **B)** Quantification standard curve for LCMS of eluted Ac-Kaa. **C)** LC and –MS of eluted Ac-Kaa.

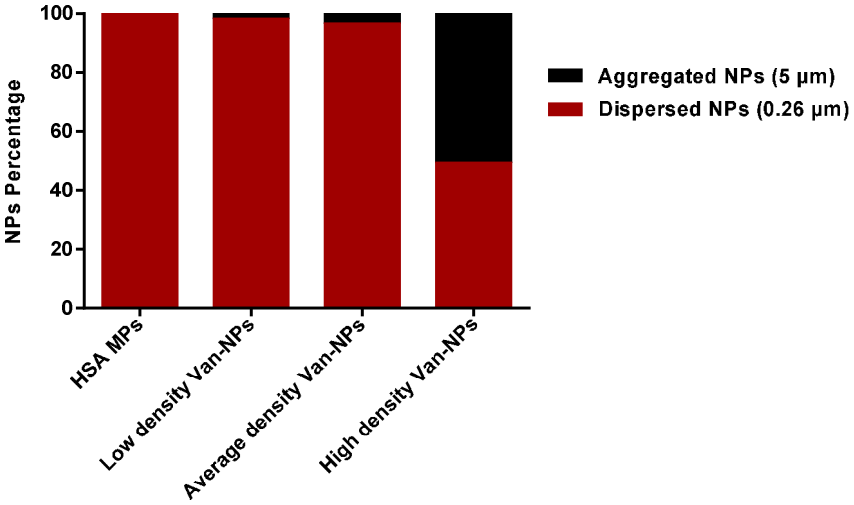
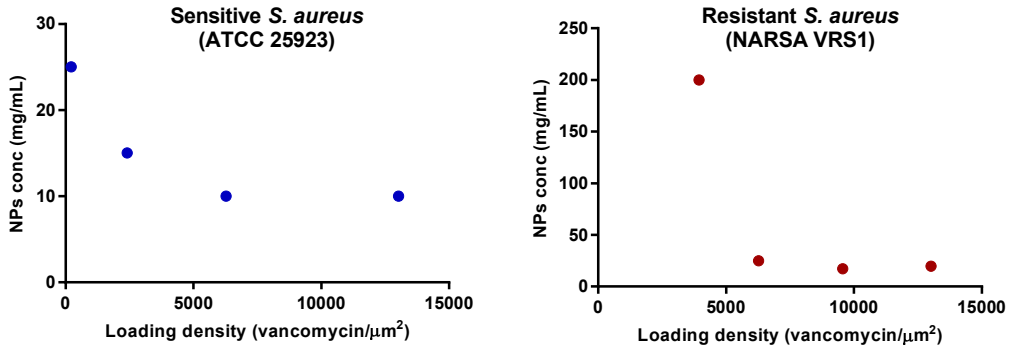


Figure S7. Fraction of aggregated and dispersed nanoparticles as determined by dynamic light scattering.

A



B

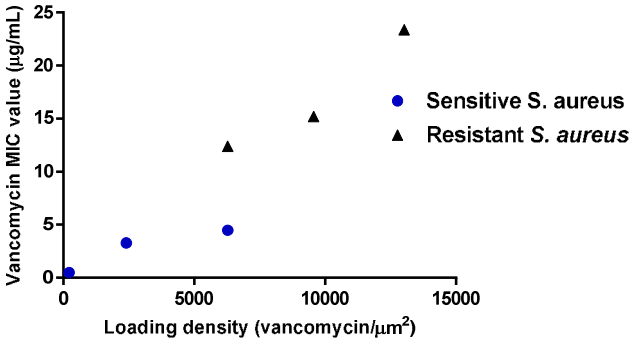


Figure S8. A) Relationship between the NP concentration required to achieve inhibition against sensitive and resistant *S. aureus* with four different Van-NPs loading densities. **B)** Relationship between MIC values against sensitive and resistant *S. aureus* with different Van-NPs loading densities.

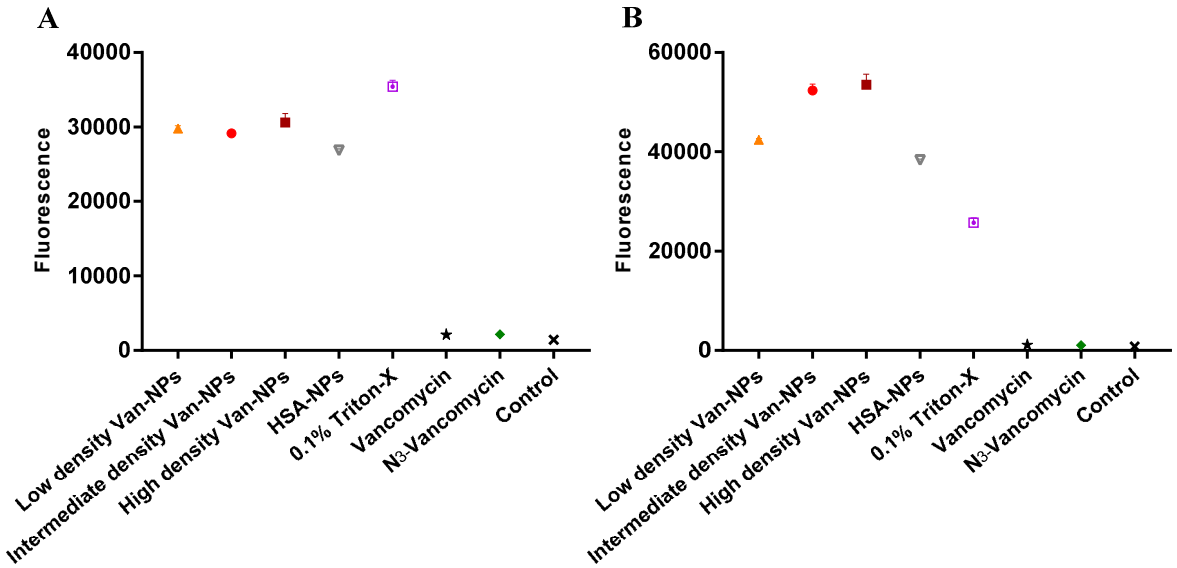


Figure S9. $DiSC_3(5)$ membrane permeabilization fluorescence values of different local densities of Van-NPs, vancomycin and N_3-vancomycin after incubation for 30 min (**A**) and 90 min (**B**). Van-NPs and vancomycin were used at MIC concentration. Controls of HSA-NPs, 0.1% Triton-X and sterile water were incubated at the same conditions. Data (n=2) are shown as means ± SD, some error bars are too small to be visible in the graph.

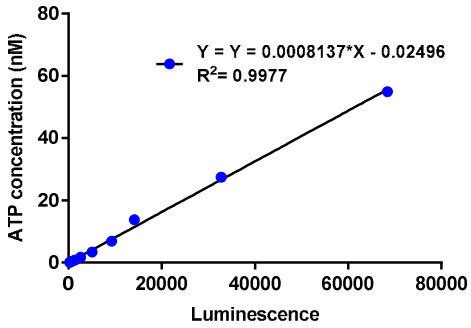


Figure S10. ATP standard curve of 1:1 serial dilution of the standard ATP.

Table S1. Estimated values of conjugated vancomycin based on fluorescence of Fam-Kaa bound to coupled N_3-vancomycin, fluorescence of N_3-NBD bound to unreacted DBCO, or concentration of Ac-Kaa bound to conjugated N_3-vancomycin then eluted and measured by LCMS.

Van-NPs input batch (mg/mL)		Vancomycin local density (Vancomycin/μm^2) estimated by		
		Fam-Kaa fluorescence of conjugated vancomycin	N_3-NBD fluorescence of reacted DBCO	LCMS analysis of eluted Ac-Kaa
Low density	**0.125**	3.05E+02	7.00E+02	2.98E+02
	0.25	2.29E+03	7.03E+02	1.21E+03
	0.5	3.10E+03	1.51E+03	2.71E+03
Intermediate density	**1**	4.92E+03	5.59E+03	5.76E+03
High density	**2**	9.56E+03	1.17E+04	1.11E+04
	3	1.29E+04	1.80E+04	1.66E+04

(Calculations were based on the estimated number of particles, 1.76E+13 particles/mL)

References

(1) Pearce, C. M., and Williams, D. H. (1995) Complete assignment of the 13C NMR spectrum of vancomycin. *J. Chem. Soc., Perkin Trans. 2*, 153-157.