

Supporting Information

Enhanced activity of vancomycin by encapsulation in hybrid magnetic nanoparticles conjugated to a cell-penetrating peptide

Short title: Vancomycin hybrid magnetic nanoparticles

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*Cell-penetrating hexapeptide

^a Predicted by online software (Pepcalc.com)

^b Obtained from RP-HPLC

PepCalc.com - Peptide property calculator

N-terminus	Sequence	C-terminus	AA code used	Calculate!
Fmoc	arghisprophealagly	AFC	single-lette	
Disulphide connectivity				

Show abbreviations 20 standard amino acids modified amino acids unusual amino acids

Sequence submission

Single letter code: arghisprophealagly

Get a quotation

Sequence interpretation

Single letter code: Fmoc- ARGHISPROP HEALAGLY -AFC

Triple letter code: Fmoc- Ala - Arg - Gly - His - Ile - Ser - Pro - Arg - Orn - Pro - His - Glu - Ala - Leu - Ala - Gly - Leu - Tyr -AFC

Physicochemical properties

Number of residues:	18	
Molecular weight:	906.59 g/mol	<i>notes on MW</i>
Extinction coefficient:	1280 M ⁻¹ cm ¹	<i>notes on Ext. Coefficient</i>
Iso-electric point:	pH 10.93	<i>notes on pI</i>
Net charge at pH 7:	1.2	<i>notes on net charge</i>
Estimated solubility:	Poor water solubility.	<i>notes on solubility</i>

Net charge vs pH

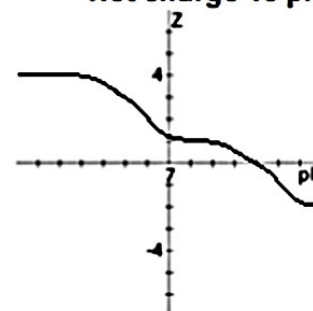


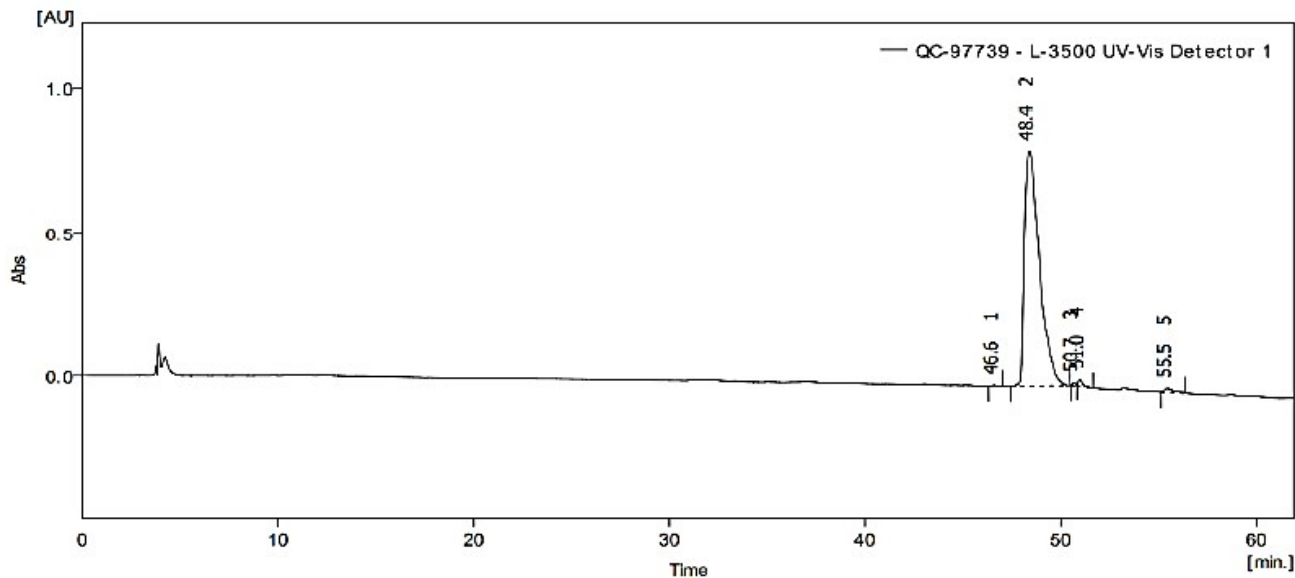
Figure S1. Physicochemical properties of synthesized CPP

Chromatogram Info:

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 Origin : Acquired, Acquisition started 12/12/2018 07:48:08 طب Acquired Date : 12/12/2018 08:50:11 طب
 Project : D:\RIGOL HPLC Data\Projects\CRGDK.PRJ By : NDN

Sample Info:

Sample ID : QC-97739 Amount : 0
 Sample : TAH pure ISTD Amount : 0
 Inj. Volume [mL] : 0 Dilution : 1



Result Table (Uncal - QC-97739 - L-3500 UV-Vis Detector 1)

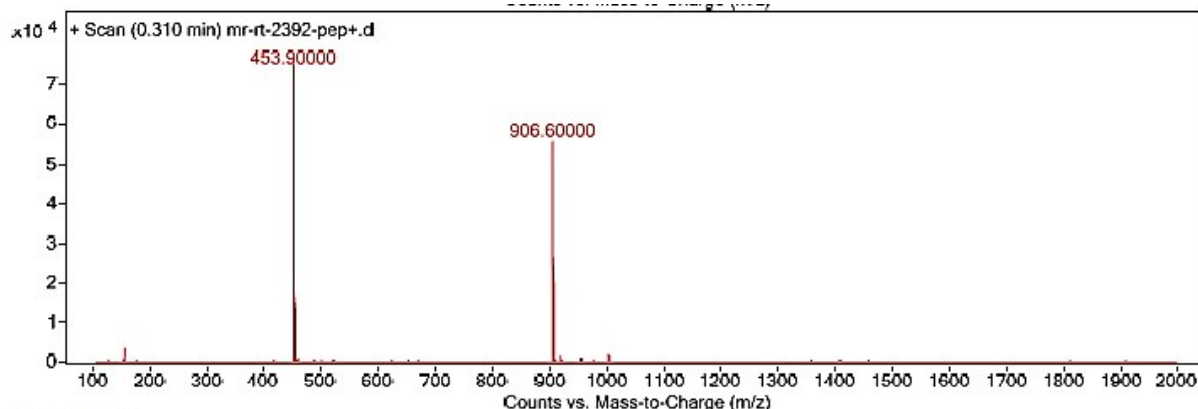
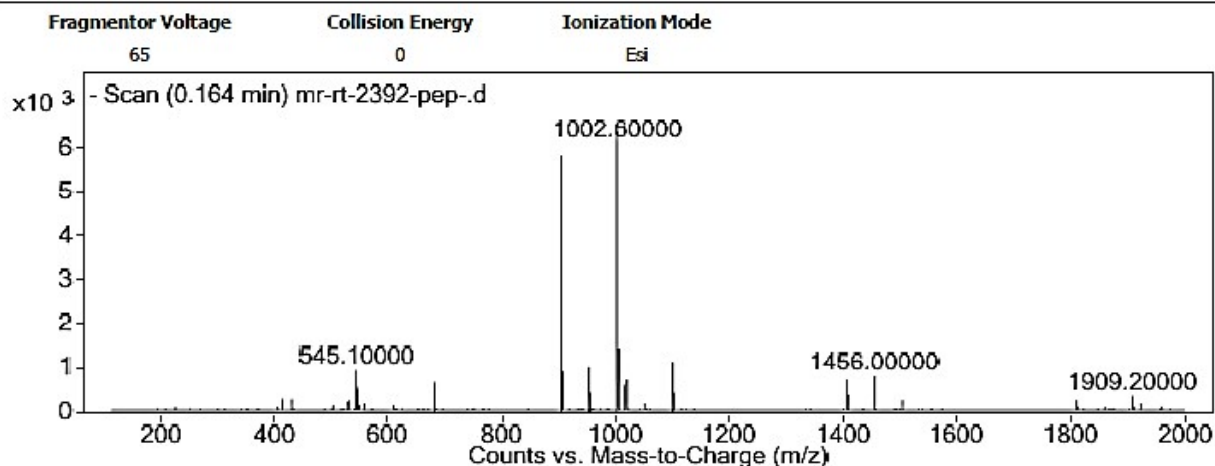
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	46.597	44.018	2.709	0.098	0.3	0.24	
2	48.410	43883.229	822.111	97.923	94.3	0.83	
3	50.683	138.138	10.878	0.308	1.2	0.26	
4	50.980	382.581	24.197	0.854	2.8	0.22	
5	55.453	366.221	12.178	0.817	1.4	0.41	
	Total	44814.186	872.072	100.000	100.0		

Table S1. Related areas and retention times of all present ingredients and impurities of CPP

Qualitative Analysis Report

Data Filename	mr-rt-2392-pep-.d	Sample Name	mr-rt-2392-pep-
Sample Type	Sample	Position	Vial 11
Instrument Name	Instrument 1	User Name	
Acq Method	General n.m	IRM Calibration Status	Success
DA Method	hashiri.m	Comment	

User Spectra



--- End of Report ---

Peak List

<i>m/z</i>	<i>z</i>	Abund.
545.1		918
904.6		5790
905.5	1	3630
906.5	1	900
953.6		989
954		934
1002.6		6359
1003.5		3477
1004.4		1396
1100.5		1109

--- End Of Report ---

Table S2. Detailed information of the synthesized CPP (LC-MS analysis).

Table S3. *In vitro* VAN release using UV-vis spectrophotometry.

Entry	Medium (pH)	Time (min)	A ^{a*} (a. u.)
1	Acetate buffer 0.1 M (5.6)	15	0.198
2	Acetate buffer 0.1 M (5.6)	30	0.322
3	Acetate buffer 0.1 M (5.6)	60	0.41
4	Acetate buffer 0.1 M (5.6)	120	0.396
5	Acetate buffer 0.1 M (5.6)	180	0.382
6	PBS 0.1 M (7.2)	15	0.09
7	PBS 0.1 M (7.2)	30	0.213
8	PBS 0.1 M (7.2)	60	0.315
9	PBS 0.1 M (7.2)	120	0.376
10	PBS 0.1 M (7.2)	180	0.418
11	PBS 0.1 M (7.2)	240	0.416
12	PBS 0.1 M (9.0)	15	0.191
13	PBS 0.1 M (9.0)	30	0.346
14	PBS 0.1 M (9.0)	60	0.414
15	PBS 0.1 M (9.0)	120	0.412
16	PBS 0.1 M (9.0)	180	0.402

^aUV-vis absorbance at 220 nm, ^{*}all samples were diluted 1/100 with medium.

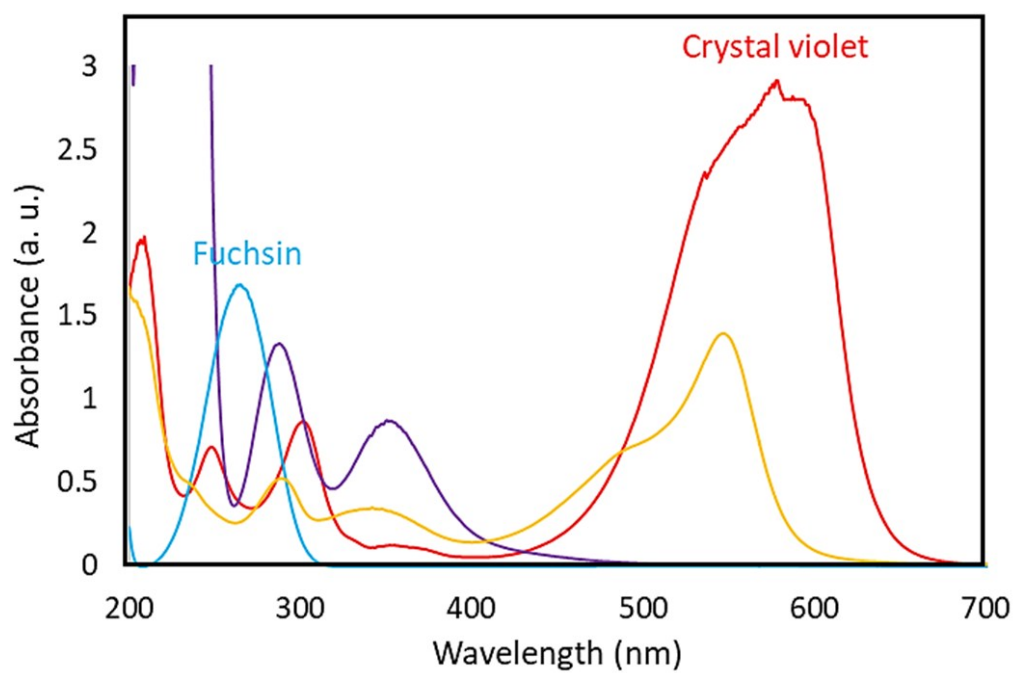
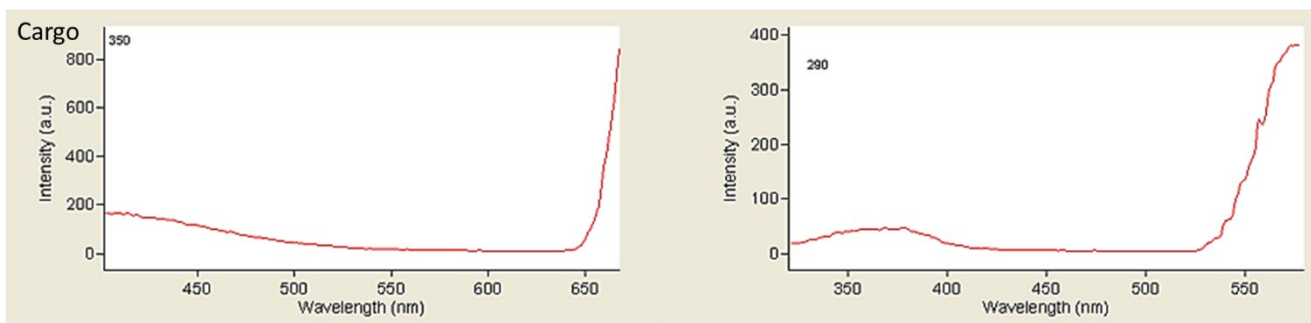


Figure S2. UV-vis spectra of fuchsin and crystal violet for cell staining.



MCCC@VAN

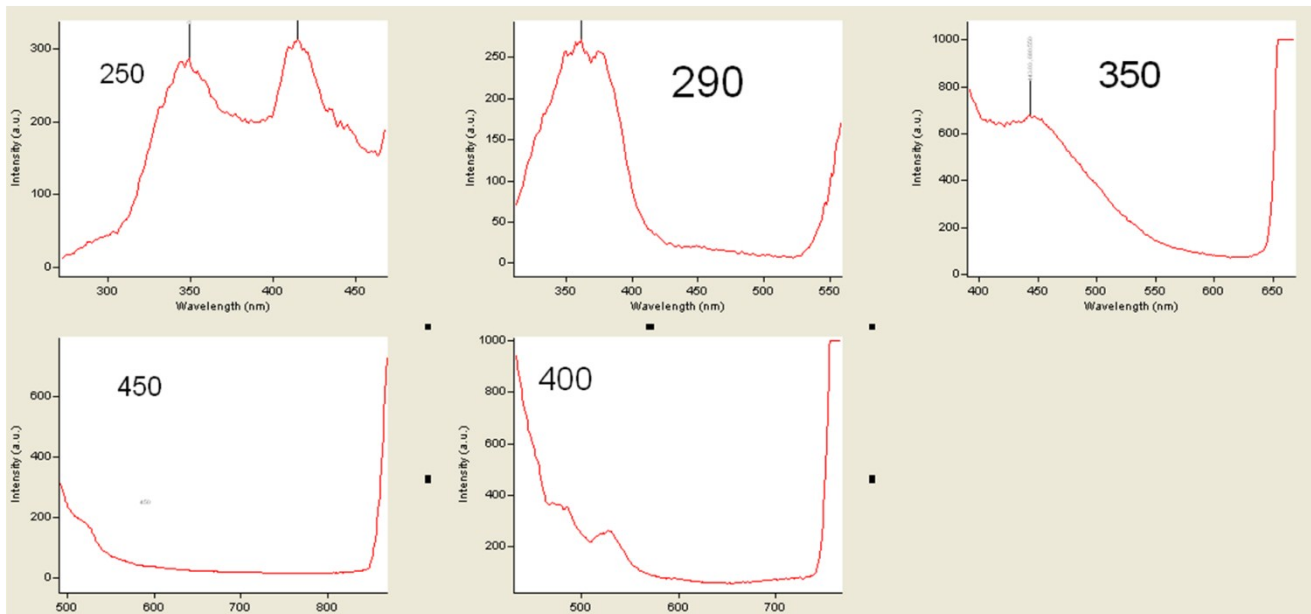


Figure S3. Photoluminescence (PL) spectra of MCCC@VAN.

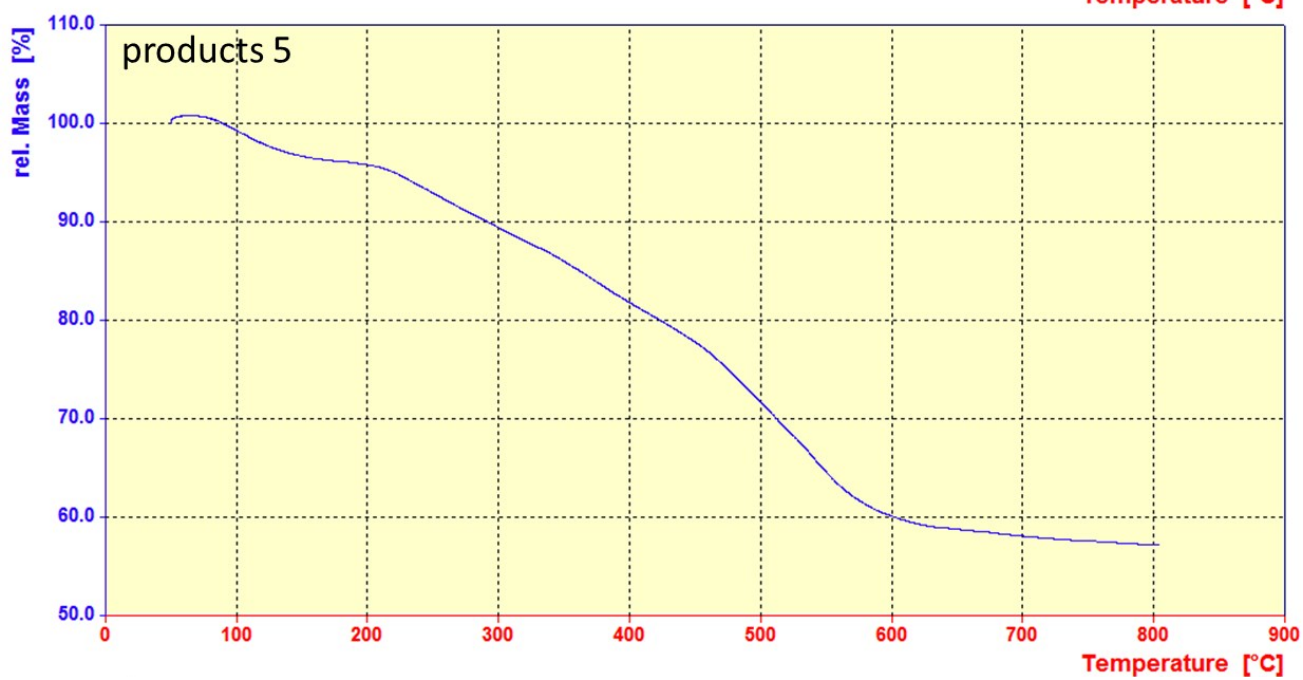
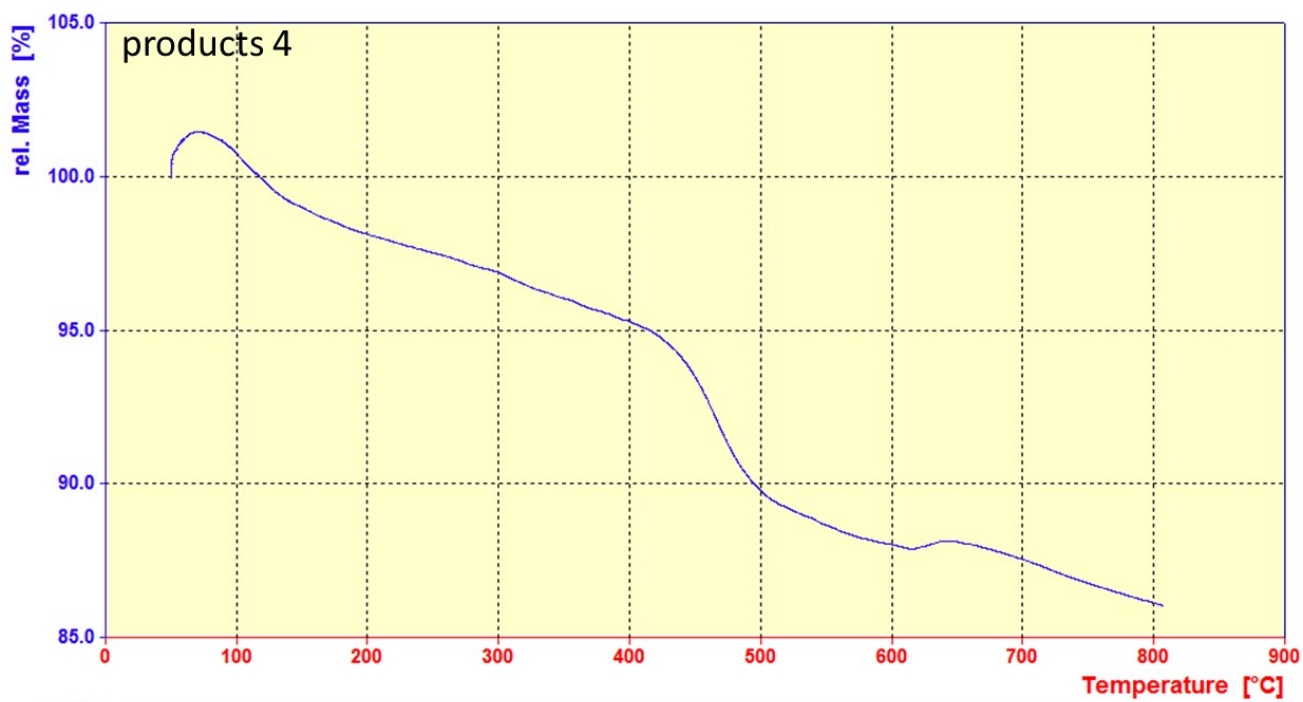


Figure S4. TGA curves of $\text{Fe}_3\text{O}_4/\text{SiO}_2/\text{PVA}/\text{SH}$ (4) and $\text{Fe}_3\text{O}_4/\text{SiO}_2/\text{PVA}/\text{SH}-12\%\text{VAN}$ (5)

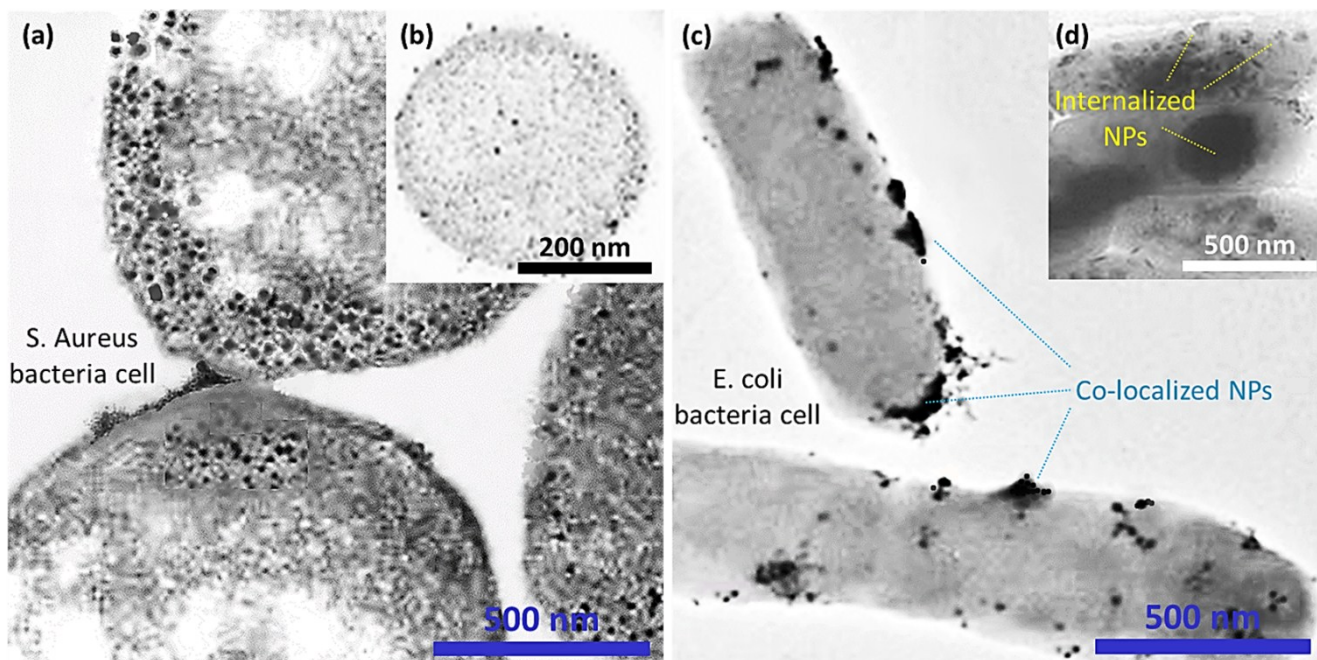
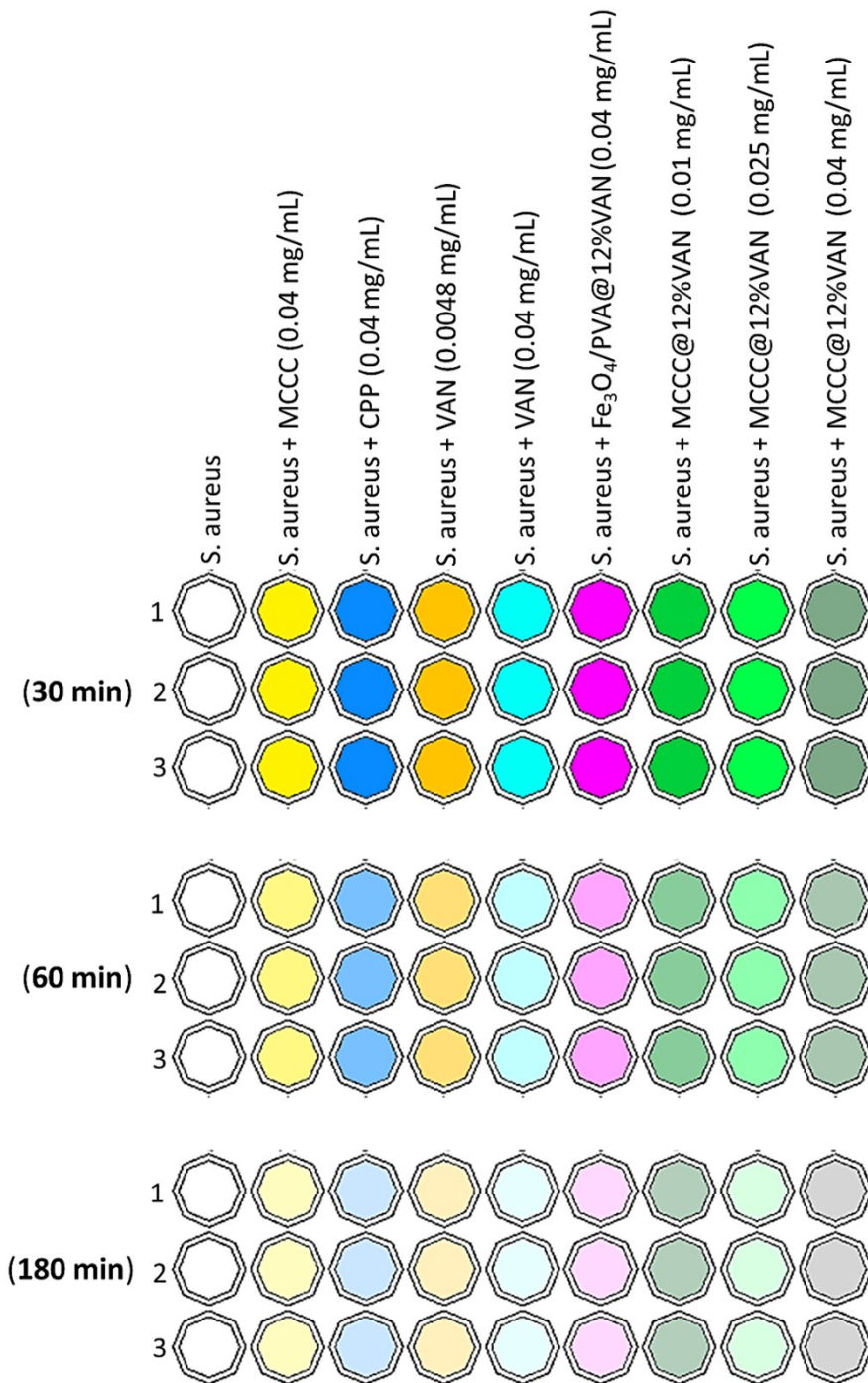


Figure S5. TEM images of the internalized MCCC@12%VAN NPs into the: *S. aureus* cells after 30 (a) and 180 min (b), and *E. coli* cells after 30 (c) and 180 min (d) [dark particles are likely co-localized and attached onto the surface of the bacteria cell, and light particles have been internalized]. In the image (d) particle accumulation has also been occurred in some areas.

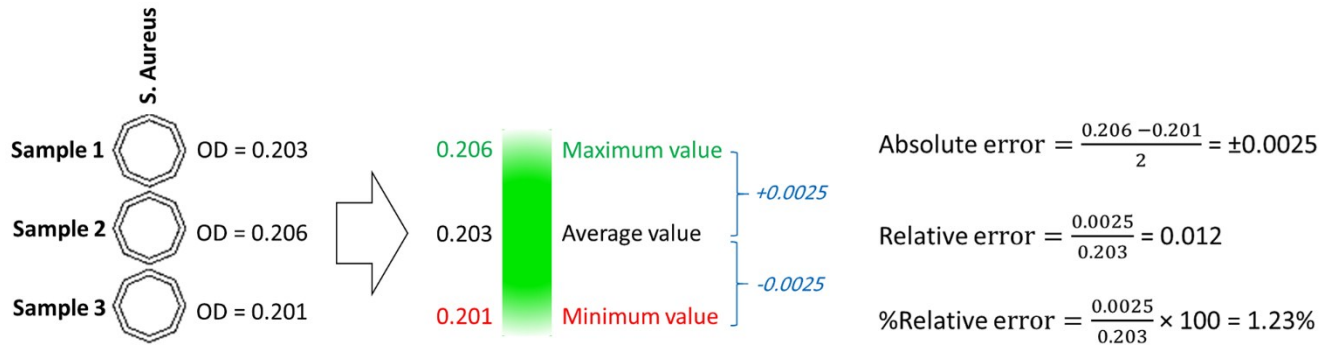
Statistical study and estimation of the errors and standard deviations for OD experiment:

To perform a statistical assessment for OD experiment, three identical samples of each condition (totally nine conditions) were tested using a 96-well plate via incubation at 37 °C, for 30, 60, and 180 min (see figure below). In this regard, standard deviation and OD error were calculated and reported in Table S4 and S5.



The calculation methods for standard deviation and the errors are explained below:

- Standard deviation for each condition has been obtained by excel software.
- To obtain error for each condition, the average OD value for three wells was calculated and the absolute error (\pm) was obtained using maximum and minimum values of OD (see following figure). For instance, for *S. aureus* (control) after 180 min incubation OD values for three identical samples are as below:



Standard deviation (calculated by excel software) = 0.0025

Absolute error = $\pm(OD_{max} - OD_{min} / 2) = \pm 0.0025$

Relative error = absolute error / real value = $0.0025 / 0.203 = 0.012$

%Relative error = (absolute error / real value) $\times 100 = 1.23\%$

Then, cell-killing potency (%CKP) of each sample is calculated by the following equation:

$$\%CKP = - (A/A_0 - 1) \times 100$$

Whereas, A is UV-vis absorbance of the desired sample, and A_0 is the UV-vis absorbance of the control (*S. aureus* and *E. coli*) at each time (30, 60, and 180 min).

Table S4. Statistical data of OD experiment on *S. aureus* cell line.

Time (min)	Conditions	OD ^a (a.u.)	STDEV ^b	%Rel. E ^c	CKP ^d (%)
30	S. aureus (control)	0.067	0.003	4.4	-
	S. aureus + MCCC (0.04 mg/mL)	0.064	0.004	6.2	4.5
	S. aureus + CPP (0.04 mg/mL)	0.062	0.005	8.0	7.5
	S. aureus + VAN (0.0048 mg/mL)	0.055	0.005	9.0	17.9
	S. aureus + VAN (0.04 mg/mL)	0.038	0.006	17.1	43.3
	S. aureus + Fe ₃ O ₄ /PVA@12%VAN (0.04 mg/mL)	0.052	0.003	5.7	22.4
	S. aureus + MCCC@12%VAN (0.01 mg/mL)	0.05	0.003	6.0	25.4
	S. aureus + MCCC@12%VAN (0.025 mg/mL)	0.017	0.003	20.5	74.6
	S. aureus + MCCC@12%VAN (0.04 mg/mL)	0.012	0.003	25	82
60	S. aureus (control)	0.167	0.003	2.0	-
	S. aureus + MCCC (0.04 mg/mL)	0.159	0.004	2.5	4.8
	S. aureus + CPP (0.04 mg/mL)	0.149	0.002	1.7	10.8
	S. aureus + VAN (0.0048 mg/mL)	0.12	0.002	2.0	28.1
	S. aureus + VAN (0.04 mg/mL)	0.036	0.004	11.1	78.5
	S. aureus + Fe ₃ O ₄ /PVA@12%VAN (0.04 mg/mL)	0.05	0.003	6.0	70
	S. aureus + MCCC@12%VAN (0.01 mg/mL)	0.041	0.002	6.2	75.5
	S. aureus + MCCC@12%VAN (0.025 mg/mL)	0.016	0.002	15.6	90.4
	S. aureus + MCCC@12%VAN (0.04 mg/mL)	0.015	0.003	23.3	91
180	S. aureus (control)	0.203	0.002	1.2	-
	S. aureus + MCCC (0.04 mg/mL)	0.19	0.015	7.8	6.4
	S. aureus + CPP (0.04 mg/mL)	0.181	0.003	1.6	10.8
	S. aureus + VAN (0.0048 mg/mL)	0.14	0.002	1.7	31.0
	S. aureus + VAN (0.04 mg/mL)	0.023	0.002	17.4	88.7
	S. aureus + Fe ₃ O ₄ /PVA@12%VAN (0.04 mg/mL)	0.043	0.002	5.8	78.8
	S. aureus + MCCC@12%VAN (0.01 mg/mL)	0.029	0.004	16.0	85.7
	S. aureus + MCCC@12%VAN (0.025 mg/mL)	0.014	0.003	21.4	93.1
	S. aureus + MCCC@12%VAN (0.04 mg/mL)	0.005	0.0002	31.0	97.5

^a OD: average optical density for three identical samples

^b STDEV: standard deviation of three identical samples

^c %Rel. E: relative error percentage for three identical samples

^d CKP: bacteria cell-killing potency during 180 min

It should be noted that in low concentrations the relation between the concentration and UV-vis absorbance may not be linear (but it was assumed linear).

Table S5. Statistical data of OD experiment on *E. coli* cell line.

Time (min)	Conditions	OD (a.u.)	STDEV	%Rel. E	CKP (%)
30	<i>E. coli</i> (control)	0.461	0.004	0.97	-
	<i>E. coli</i> + MCCC (0.04 mg/mL)	0.452	0.003	0.44	1.9
	<i>E. coli</i> + CPP (0.04 mg/mL)	0.446	0.006	1.3	3.2
	<i>E. coli</i> + VAN (0.0048 mg/mL)	0.42	0.02	4.7	8.9
	<i>E. coli</i> + VAN (0.04 mg/mL)	0.386	0.005	1.3	16.3
	<i>E. coli</i> + Fe ₃ O ₄ /PVA@12%VAN (0.04 mg/mL)	0.408	0.005	1.2	11.5
	<i>E. coli</i> + MCCC@12%VAN (0.01 mg/mL)	0.402	0.007	1.7	12.8
	<i>E. coli</i> + MCCC@12%VAN (0.025 mg/mL)	0.395	0.005	1.3	14.3
60	<i>E. coli</i> (control)	0.267	0.003	1.1	42
	<i>E. coli</i> (control)	0.484	0.005	1.1	-
	<i>E. coli</i> + MCCC (0.04 mg/mL)	0.468	0.004	0.85	3.3
	<i>E. coli</i> + CPP (0.04 mg/mL)	0.456	0.005	1.1	5.8
	<i>E. coli</i> + VAN (0.0048 mg/mL)	0.42	0.005	1.3	13.2
	<i>E. coli</i> + VAN (0.04 mg/mL)	0.285	0.005	1.9	41.1
	<i>E. coli</i> + Fe ₃ O ₄ /PVA@12%VAN (0.04 mg/mL)	0.379	0.01	2.6	21.7
	<i>E. coli</i> + MCCC@12%VAN (0.01 mg/mL)	0.34	0.005	1.4	29.7
180	<i>E. coli</i> + MCCC@12%VAN (0.025 mg/mL)	0.315	0.004	1.3	34.9
	<i>E. coli</i> + MCCC@12%VAN (0.04 mg/mL)	0.115	0.003	4.3	76.2
	<i>E. coli</i> (control)	0.558	0.007	1.3	-
	<i>E. coli</i> + MCCC (0.04 mg/mL)	0.54	0.02	3.7	3.2
	<i>E. coli</i> + CPP (0.04 mg/mL)	0.526	0.005	1.0	5.7
	<i>E. coli</i> + VAN (0.0048 mg/mL)	0.43	0.01	2.3	22.9
	<i>E. coli</i> + VAN (0.04 mg/mL)	0.2	0.02	10	64.1
	<i>E. coli</i> + Fe ₃ O ₄ /PVA@12%VAN (0.04 mg/mL)	0.31	0.03	9.9	44.4
	<i>E. coli</i> + MCCC@12%VAN (0.01 mg/mL)	0.251	0.005	2.0	55.0
	<i>E. coli</i> + MCCC@12%VAN (0.025 mg/mL)	0.204	0.004	2.2	63.4
	<i>E. coli</i> + MCCC@12%VAN (0.04 mg/mL)	0.049	0.003	7.1	91.2

Relative errors for ZOI experiment:

This test was repeated for three times for each sample. Standard deviations and relative errors were calculated via the explained method, and reported in table below:

Table S6. Statistical data of ZOI experiment.

Cell line	Sample ^a	ZOI ^b (cm)	STDEV	%Rel. E
S. aureus	CPP	Trace	-	-
	MCCC	Trace	-	-
	VAN	1.25	0.02	1.6
	Fe ₃ O ₄ /PVA@12%VAN	0.46	0.01	2.1
	MCCC@12%VAN	1.375	0.01	0.7
E. coli	CPP	Trace	-	-
	MCCC	Trace	-	-
	VAN	0.875	0.02	2.3
	Fe ₃ O ₄ /PVA@12%VAN	0.41	0.01	2.4
	MCCC@12%VAN	0.125	0.01	0.9

^a The same weight (0.01 g) was used for all samples.

^b Average value is reported for three times repeating the experiment.