Electronic Supplementary Material (ESI) for Soft Matter.

Supplementary information

Fmoc-phenylalanine displays antibacterial activity against Gram-positive bacteria in gel and solution phases

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Experimental Section

Bacteria used in study: *Staphylococcus aureus* (MTCC 737), *Bacillus cereus* (MTCC 430), *Enterococcus hirae* (MTCC 2728), *Bacillus subtilis* (MTCC 1305), *Pseudomonas aeruginosa* (MTCC 4673) were purchased from IMTECH, Chandigarh, India. *Mycobacterium smegmatis* (MC^2 255) was gift from Dr. Saravanan, IIT Kanpur, India. Work on pathogenic Methicillin resistant *Staphylococcus aureus* (MRSA) was done at AIIMS, New Delhi, India. Work on *Staphylococcus epidermidis* and *Corynebacterium diphtheriae* (both isolated from cow milk); *Streptococcus pneumoniae* (isolated from human throat); *Bacillus licheniformis* (isolated from cow cervical discharge); *Salmonella typhi* (isolated from chicken lungs) was done at college of veterinary and animal sciences, CSK HPKV, Palampur (HP), India.

Characterization of intracellular uptake of Fmoc-F using RP-HPLC: For intracellular localization, 50 mL of secondary culture with 1×10^8 CFU was incubated with 0.5 mM of the Fmoc-F solution. The control group was treated with phosphate buffer. After 2 h of incubation, bacterial cells were pelleted by centrifugation (2000 × g for 5 min), washed twice and re-suspended in PBS. Bacterial cell count by taking OD_{600} and dilution was done in order to get an equal number of CFU mL^{-1} in each group. For extraction of intracellular Fmoc-F, 1 mL of diluted solution was subjected to cell wall destruction using probe sonication (10 sec on: 30 sec off; 10 cycles, 12.5 mA; Ultrasonic Disintegrator, SONIPREP 150). Cellular debris was removed by centrifugation at 25000 × g for 30 min using Eppendorf centrifuge (5417R). The supernatant was collected and volume was adjusted to 1 mL with PBS. 50 μL of this supernatant was injected to HPLC column for Fmoc-F detection using RP-HPLC.

APNModel_S1:

Function name = APNModel_SurfaceTensionSzyszkowski

Brief description = APN Model: Surface Tension (Szyszkowski Equation) -USC -Al-Soufi 2011

[Independent Variables]

cSθ =

[Dependent Variables]

g =

[Fitting Parameters]

Names = cmc, r, a, K_{ad}, gθ

Meanings = cmc, relative transition width, (R T)/omega, adsorption equilibrium constant, gθ

[Formula]

cS1= nlf_APNModel_S1(cSθ,cmc,r);

g=gθ - a* ln(1+ Kad*cS1);

Table S1. Different mathematic models for Fmoc-F release from hydrogel.

	Zero order	First order	Higuchi	Korsmeyer-Pappas	Hixson-Crowell
r^2	-0.15	0.84	0.91	0.97	0.69
n	-	-	-	0.39	-

r^2 - regression coefficient; n - fickian diffusion

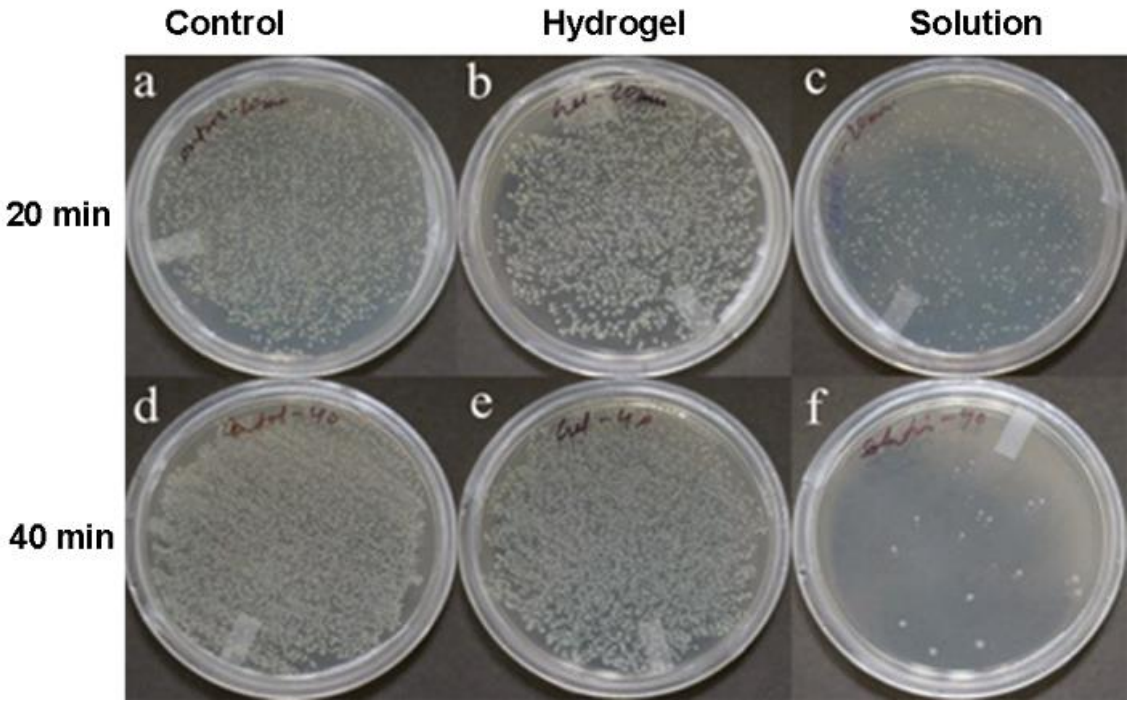

Figure S1. Antibacterial activity of Fmoc-F hydrogel and solution against *S. aureus* at 20 min and 40 min post-treatment with either Fmoc-F hydrogel or Fmoc-F solution. *S. aureus* without any treatment is considered as control.

Table S2. Percent survival of Gram-positive and Gram-negative bacteria in presence of different Fmoc-AA (1.5 mM).

Bacterial Strain [Source]	*Fmoc-* ***F*** *(%)*	*Fmoc-* ***W*** *(%)*	***Fmoc-*** ***L*** *(%)*	***Fmoc-*** ***I*** *(%)*	***Fmoc-*** ***S*** *(%)*	***Fmoc-*** ***R*** *(%)*	*Fmoc-* ***M*** *(%)*	*Fmoc-* ***Y*** *(%)*	*Fmoc-* ***P*** *(%)*	***Fmoc-*** ***N*** *(%)*
S. epidermidis[a]	17 ± 2	5 ± 2	5 ± 2	4 ± 0	10 ± 6	26 ± 2	68 ± 4	28 ± 3	82 ± 6	96 ± 0
S. pneumonia[b]	10 ± 2	27 ± 4	4 ± 2	5 ± 2	17 ± 3	93 ± 5	36 ± 5	52 ± 5	70 ± 20	106 ± 5
C. diphtheriae[a]	32 ± 7	36 ± 9	4 ± 6	12 ± 2	-	92 ± 30	76 ± 1	80 ± 21	2 ± 2	5 ± 2
B. licheniformis[c]	10 ± 0	10 ± 0	16 ± 6	29 ± 17	55 ± 11	104 ± 11	11 ± 9	13 ± 5	97 ± 12	113 ± 9
P. aerugenosa[a]	96 ± 6	86 ± 3	86 ± 4	88 ± 2	93 ± 6	96 ± 11	-	-	-	-
S. typhi[d]	93 ± 4	79 ± 6	67± 12	57 ± 3	93 ± 4	88 ± 4	-	-	-	-

Microbe source: [a]Cow milk; [b]Human throat; [c]Cow cervical discharge; [d]Chicken lungs

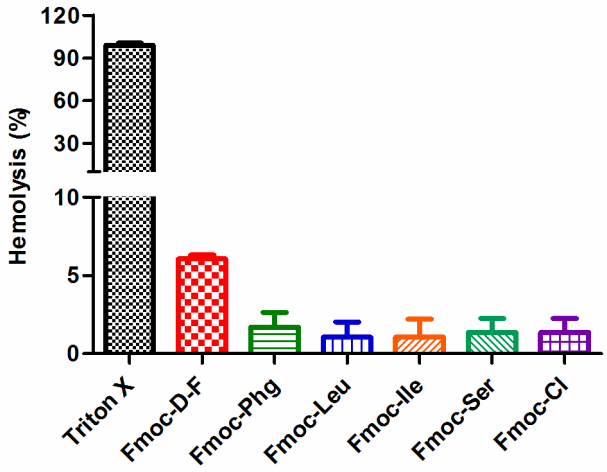

Figure S2. Hemocompatibility potential of various Fmoc-AA at 1 mM concentration. Triton-X 100 is taken as control showing complete hemolysis.

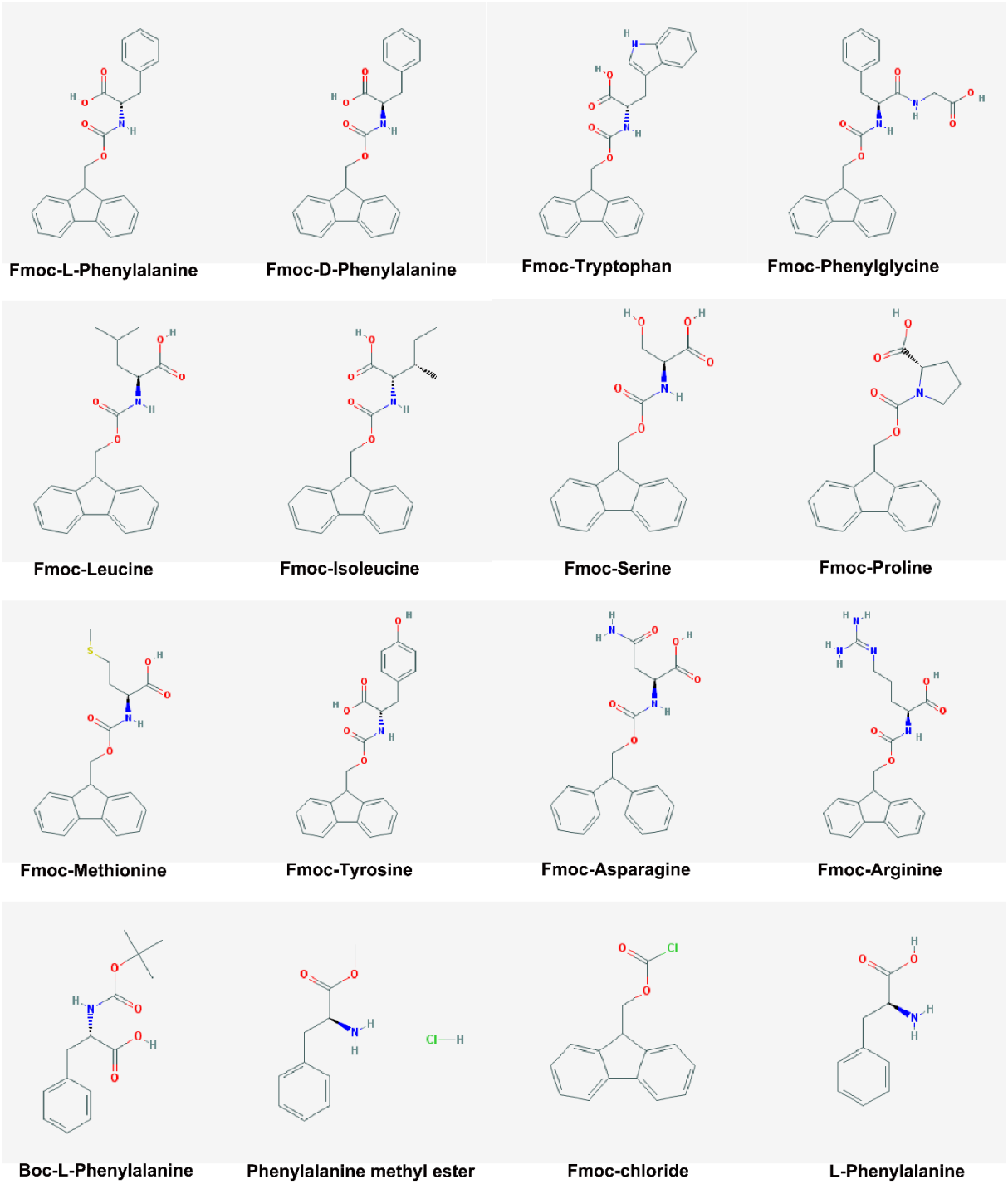

Figure S3. Chemical structures of Fmoc- AA and phenylalanine derivatives used in the study.

Table S3. APN Model fit result.

Model	APNModel_SurfaceTensionSzyszkowski (User)		
Equation	cS1= nlf_APNModel_S1(cS0,cmc,r); g=g0 - a* In(1+ Kad*cS1);		
Reduced Chi-Sqr	1.07869		
Adj. R-Square	0.98209		
		Value	Standard Error
Surface tension by stalagmometer	CMC	1.25	0.1
	r	0.1	0
	a	20	0
	Kad	1.27	0.13
	g0	71.49	0.66

CMC- critical micellar concentration; r- relative transition width; a- constant; Kad- adsorption equilibrium constant; g0- surface tension of solvent

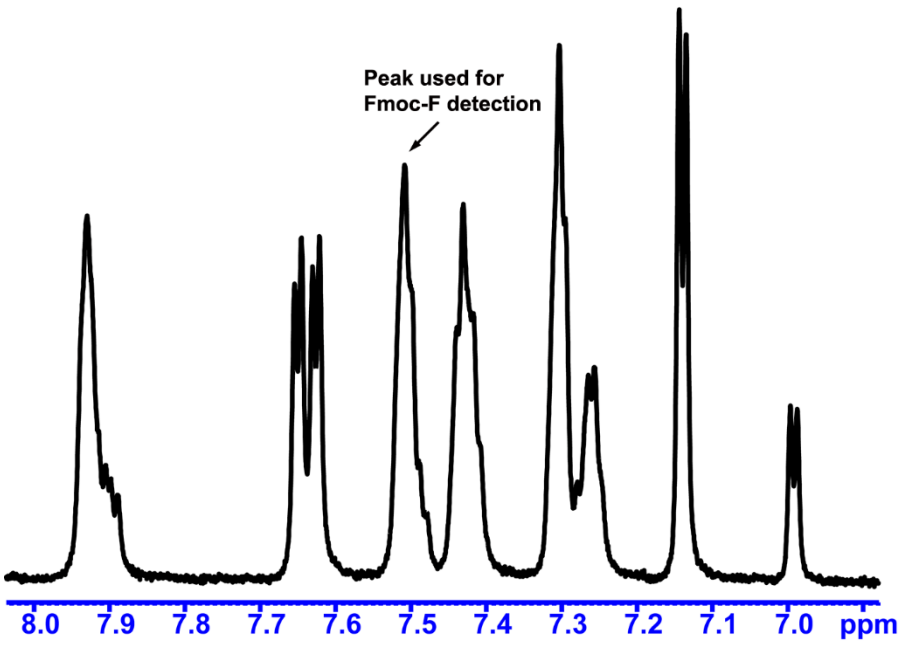

Figure S4. ^{1}H NMR spectra of Fmoc-F in phosphate buffer displaying aryl protons.

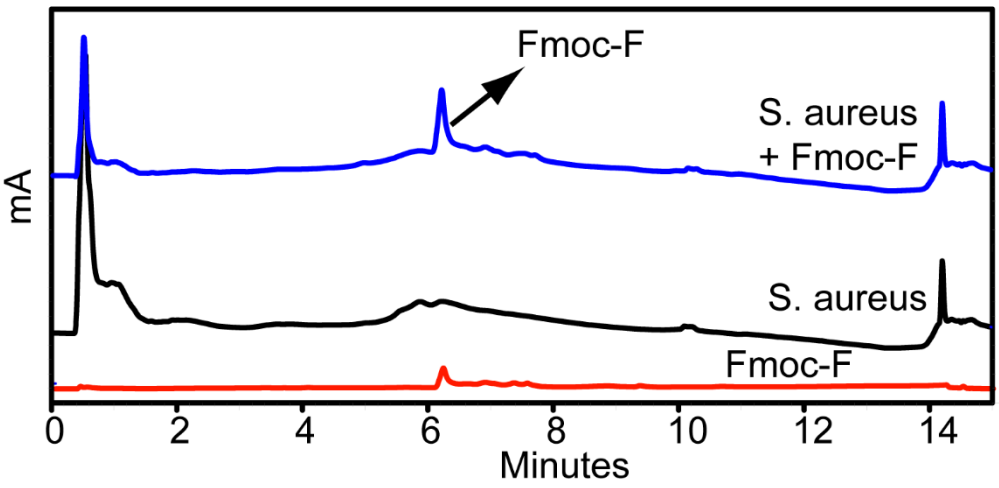

Figure S5. RP-HPLC chromatogram of Fmoc-F extracted from cytoplasm of *S. aureus* after 2 h of treatment. Cytoplasm of *S. aureus* without any treatment is considered as control.

Table S4. NMR chemical shift for betaine and glutamic acid.

S. No.	*Molecule*	*^1H ppm Chemical shift (ppm)*	*multiplicity*	*assignment*	*Peak (in ppm) used for quantification*
1	Betaine	3.26	s	CH_3	3.90
		3.90	s	CH_2	
2	Glutamic acid	2.04	m	β-CH_2	3.74
		2.11	m	β-CH_2	
		2.34	m	γ-CH_2	
		3.74	dd	α-CH	

s- singlet, m- multiplet, dd- doublet of doublet