Supporting Information

Gemini Peptide Amphiphiles with Broad-spectrum Antimicrobial Activity and Potent Anti-biofilm Capacity

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1. Supplementary Figures.

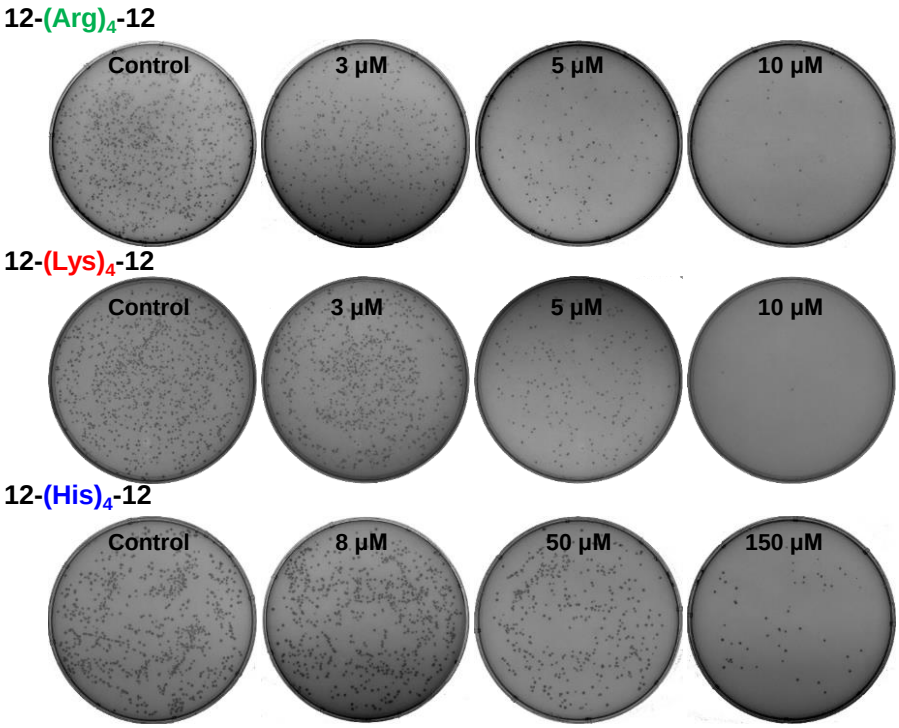


Figure S1. Number of colony forming units (CFU) of *E. coli* before (control) and after adding gemini peptide amphiphiles with different concentrations on LB agar plate

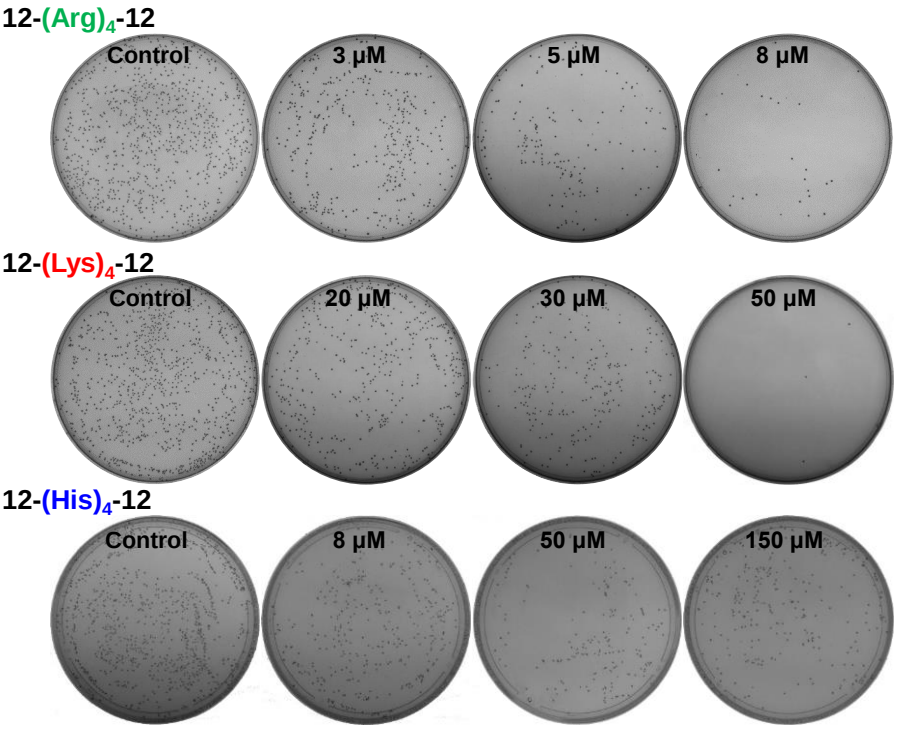


Figure S2. Number of colony forming units (CFU) of *S. aureus* before (control) and after adding gemini peptide amphiphiles with different concentrations on NB agar plate

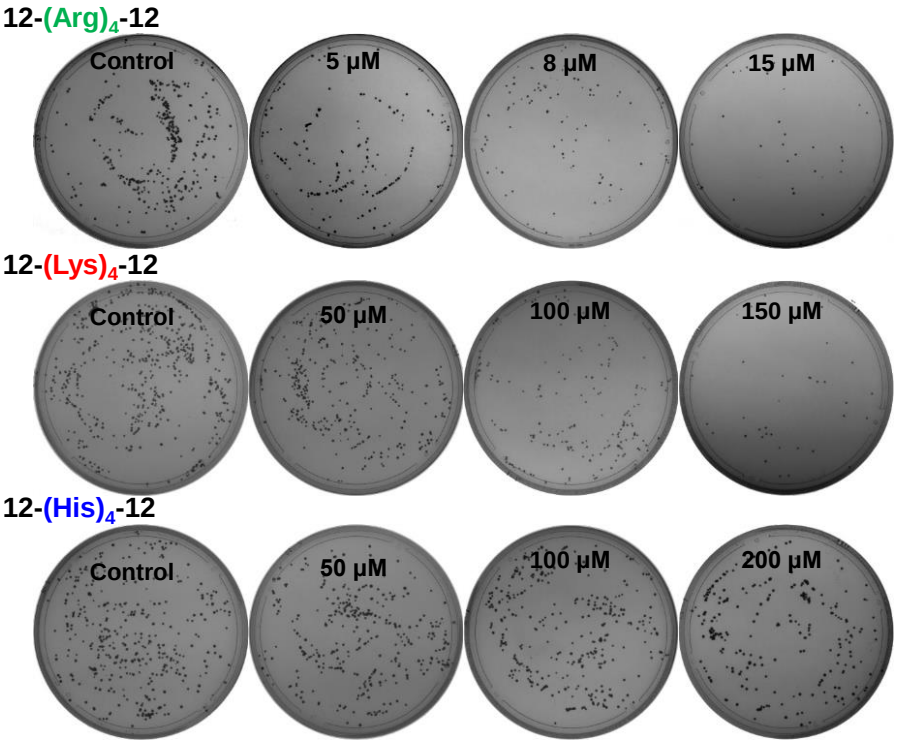


Figure S3. Number of colony forming units (CFU) of *C. albicans* before (control) and after adding gemini peptide amphiphiles with different concentrations on YPD agar plate.

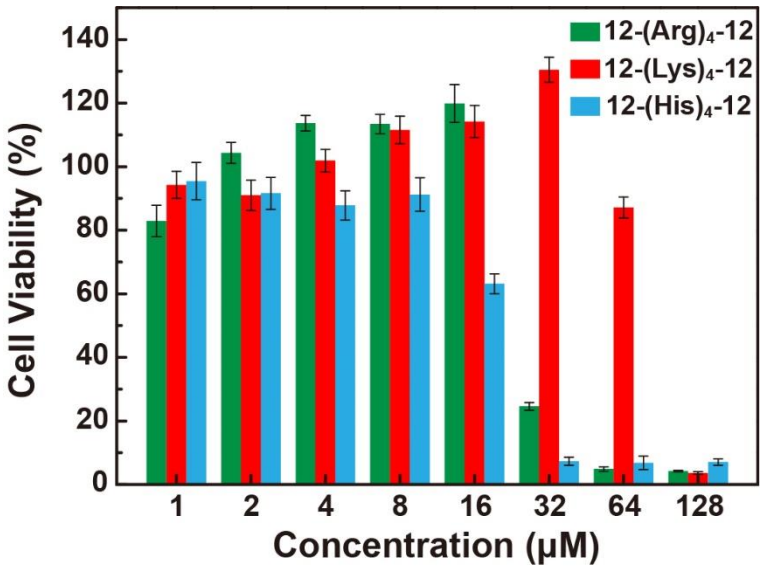


Figure S4. Cell viability of Hela cells after incubation with peptide amphiphiles at different concentrations.

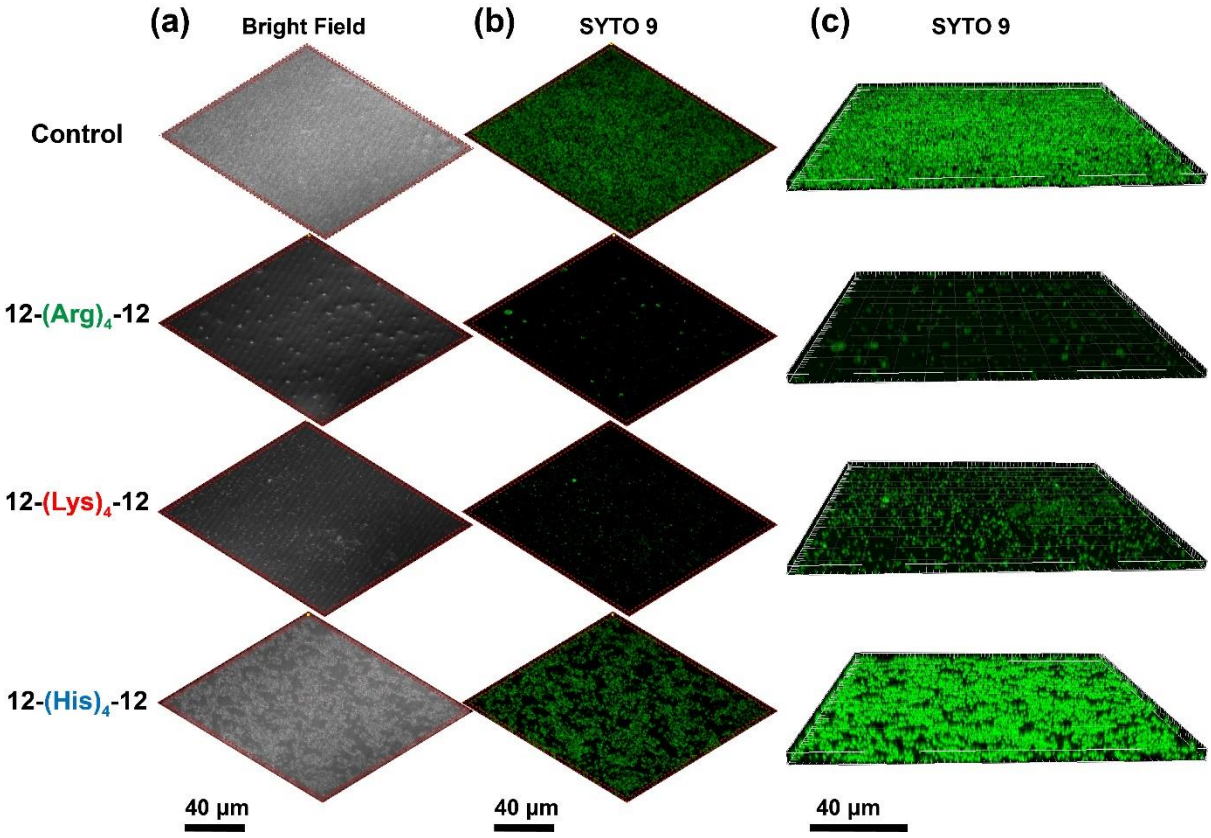
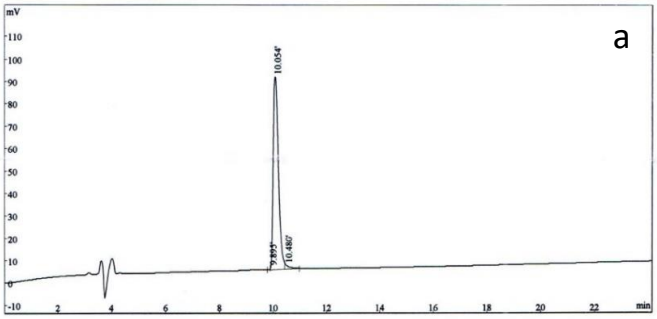
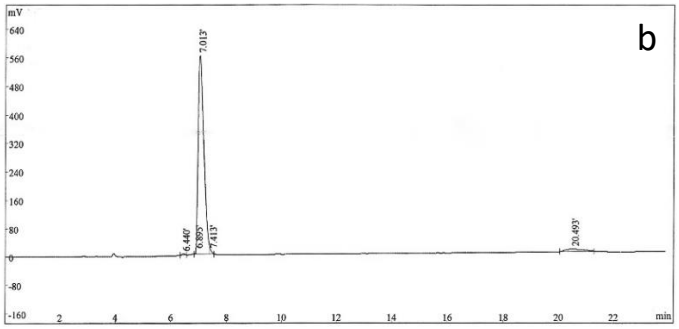


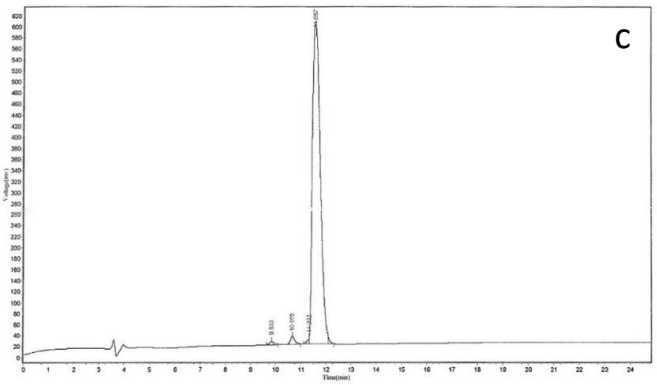
Figure S5. CLSM images of peptide amphiphiles-treated *S. aureus* biofilms. (a) Bright field, (b) SYTO 9 channel and (c) 3D images of SYTO 9 channel. Biofilms were grown for 24 h and then treated with PBS solution (control), or 50 μM 12-(Arg)$_4$-12, 100 μM 12-(Lys)$_4$-12, and 100 μM 12-(His)$_4$-12 for 2 h. Biofilms were stained with BacLight Live/Dead stain.



Rank	Time	Conc.	Area	Height
1	9.895	0.09563	1033	678
2	10.054	97.93	1057579	86199
3	10.480	1.971	21279	1459
Total		100	1079891	88336



Rank	Time	Conc.	Area
1	6.440	0.2589	19847
2	6.895	0.1097	8411
3	7.013	95.3	7303330
4	7.413	0.6202	47531
5	20.493	3.716	284790
Total		100	7663909



Peak No.	Ret Time	Height	Area	Conc.
1	9.833	5766.538	63363.188	0.4876
2	10.655	14834.970	169404.203	1.3037
3	11.332	11165.288	57105.824	0.4395
4	11.567	582093.250	12639977.000	97.2711
5	11.567	14869.438	64732.238	0.4981
Total				100.00

Figure S6. High-performance liquid chromatography of (a) 12-$(Arg)_4$-12, (b) 12-$(Lys)_4$-12 and (c) 12-$(His)_4$-12.

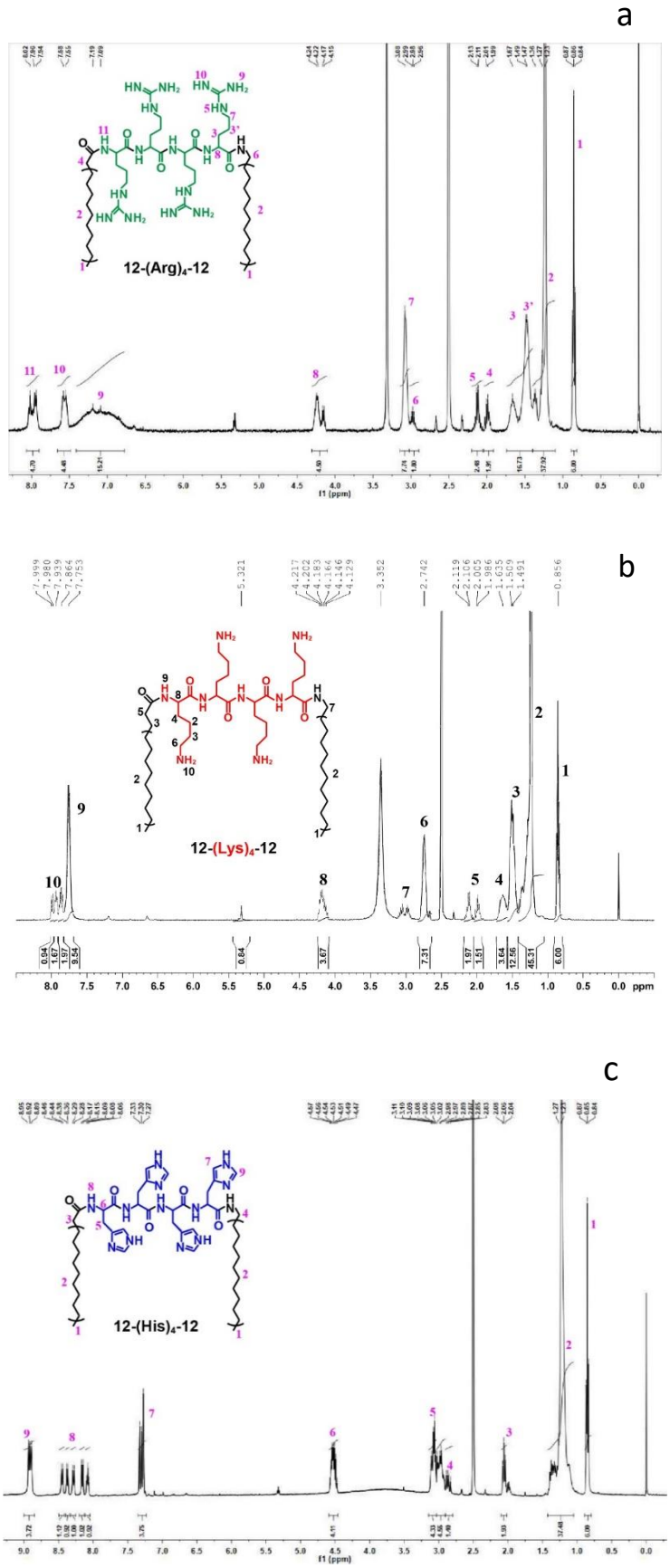


Figure S7. ^{1}H NMR spectra of (a) 12-(Arg)$_4$-12, (b) 12-(Lys)$_4$-12 and (c) 12-(His)$_4$-12.

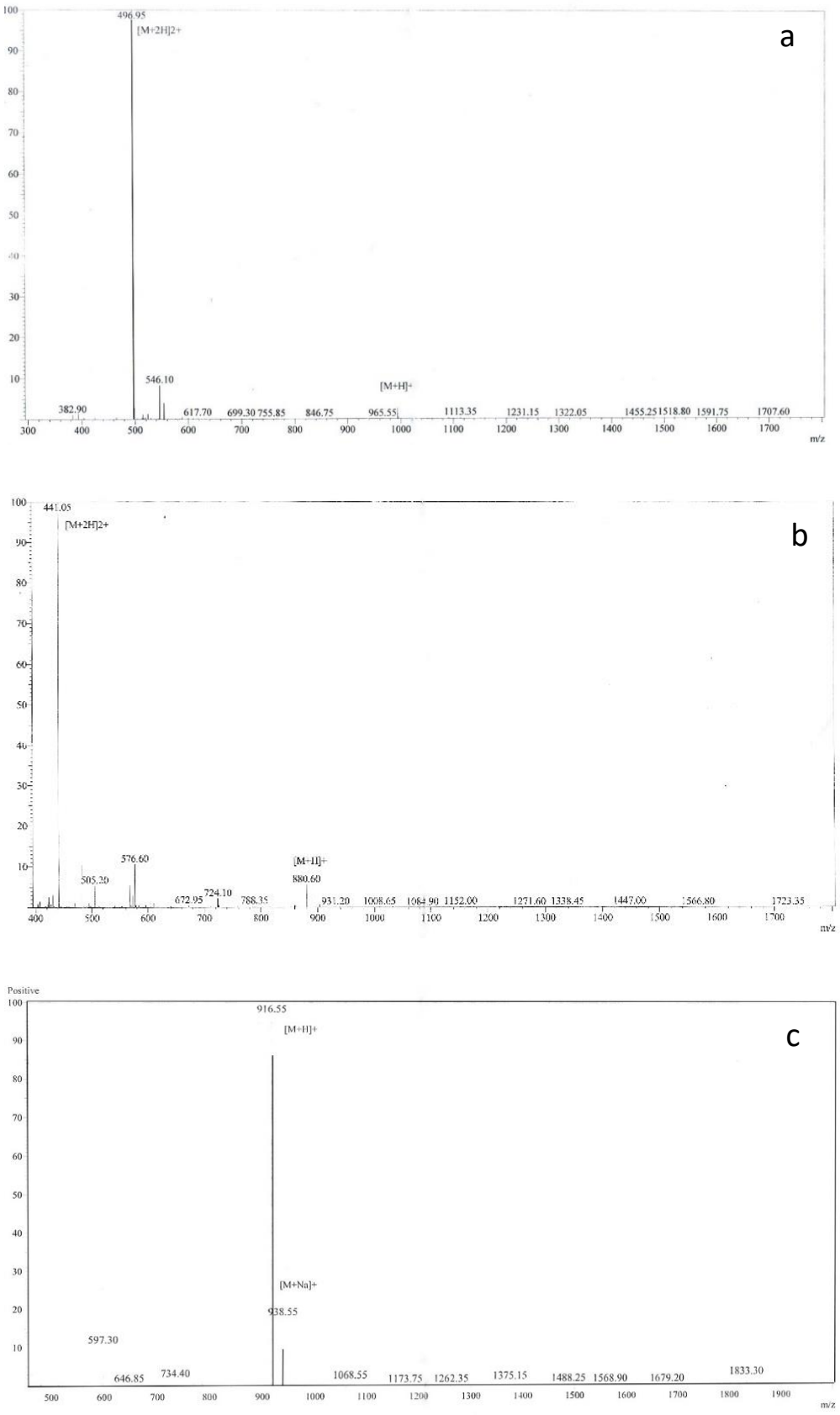


Figure S8. ESI mass spectra of (a) 12-(Arg)$_4$-12, (b) 12-(Lys)$_4$-12 and (c) 12-(His)$_4$-12.

2. ITC Analysis Process for Single Set of Identical Sites.

In the following equations,

K_b = binding constant;

N = number of binding sites;

Θ = fraction of sites occupied by ligand (the titrant);

M_t and $[M]$ are bulk and free concentration of titrand placed in the sample cells in V_0;

X_t and $[X]$ are bulk and free concentration of ligand;

V_0 = active cell volume;

ΔV_i = injection volume;

Q = the heat content;

Q_i = heat content from the completion of the i^{th} injection.

The interaction process may be described in a simplified fashion using equation

$$X+M \leftrightarrow XM \quad [1]$$

In the process, the binding constant and the concentration of ligand can be expressed as follows:

$$K_b = \frac{\Theta}{(1-\Theta[X])} \qquad [2]$$

$$X_t = [X] + N\Theta M_t \qquad [3]$$

Combining equations [2] and [3] above gives:

$$\Theta^2 - \Theta\left[1 + \frac{X_t}{NM_t} + \frac{1}{NKM_t}\right] + \frac{X_t}{NM_t} = 0 \qquad [4]$$

The total heat content Q of the solution contained in V_0 at fractional saturation Θ is:

$$Q = N\Theta M_t \Delta H V_0 \quad [5]$$

Solving the quadratic equation [4] for Θ and then substituting this into equation [5] gives:

$$Q = \frac{NM_t\Delta HV_0}{2}\left[1+\frac{X_t}{NM_t}+\frac{1}{NKM_t}-\sqrt{\left(1+\frac{X_t}{NM_t}+\frac{1}{NKM_t}\right)^2-\frac{4X_t}{NM_t}}\right] \qquad [6]$$

The heat $\Delta Q(i)$ released from the i^{th} injection can be expressed as:

$$\Delta Q(i) = Q(i) + \frac{dV_t}{V_0}\left[\frac{Q(i)+Q(i-1)}{2}\right] - Q(i-1) \qquad [7]$$

which may be used in the Marquardt algorithm to obtain best values for the fitting parameters N, K_b and ΔH. More information about the model analysis can be found in ITC Data Analysis in Origin® Tutorial Guide Version 7.0.