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Chemistry & Drug Discovery

Supporting Information

Improved Bioactivity of Antimicrobial Peptides by Addition of Amino-Terminal Copper and Nickel (ATCUN) Binding Motifs

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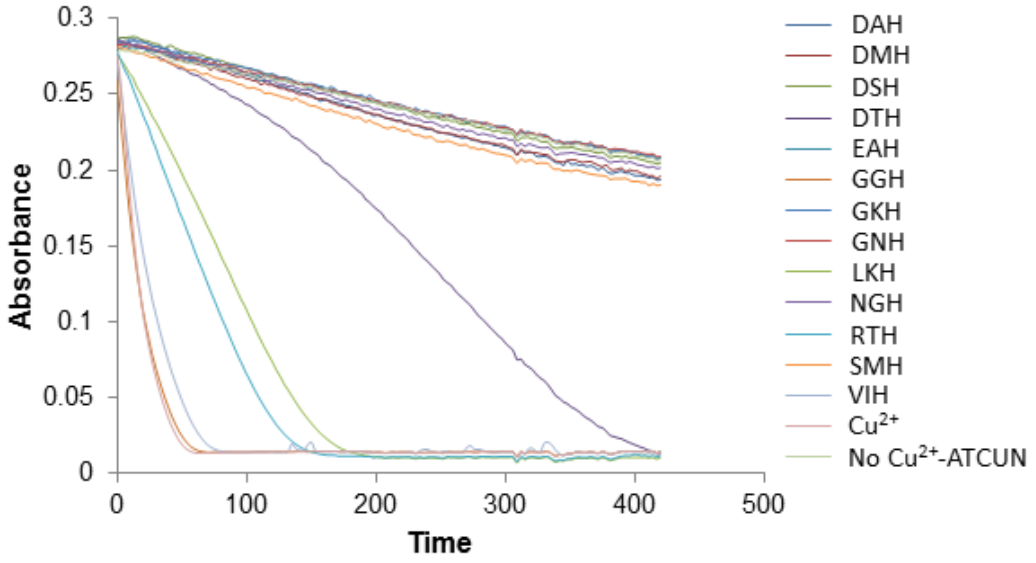
EXPERIMENTAL DETAILS

(A)



(B)

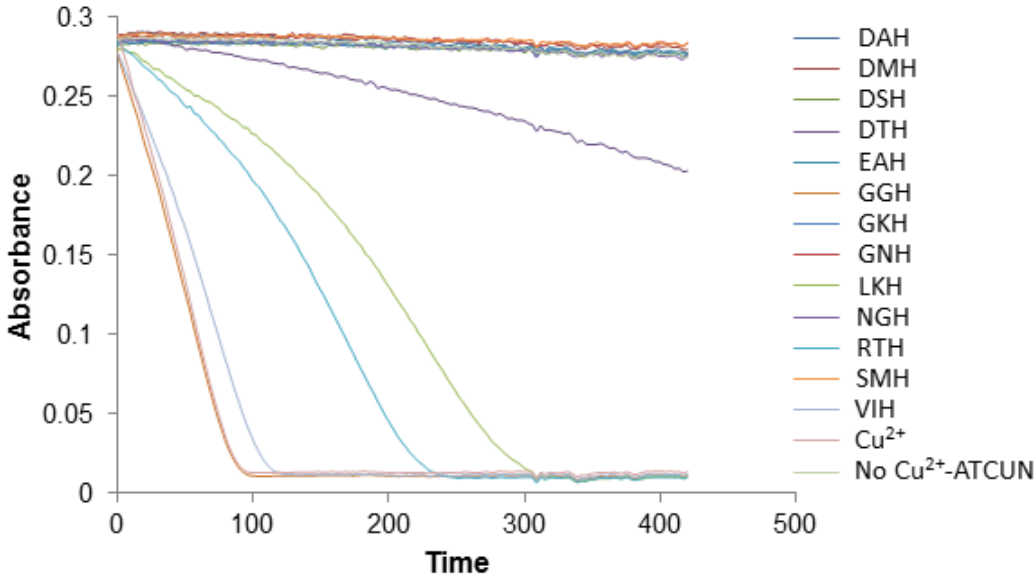


Figure S1. Rates of ascorbic acid consumption in the (A) presence and (B) absence of the H_2O_2. Reduced ascorbic acid reacts with reactive oxygen species generated by the Cu^{II}-ATCUN complexes.

Table S1. Minimum Inhibitory Concentration of ATCUN-AMPs with and without the addition of 32 μM Cu^{2+}.

Peptide	*B. subtilis*		*E. coli*	
	w/o Cu^{2+}	w/ 32 μM Cu^{2+}	w/o Cu^{2+}	w/ 32 μM Cu^{2+}
Anoplin	4	4	16	16
DAH-Anoplin	2	2	8	8
GGH-Anoplin	2	2	4	4
VIH-Anoplin	0.5	0.5	2	2
PAP	0.5	0.25	1	1
DAH-PAP	0.25	0.5	0.25	0.25
GGH-PAP	0.125	0.06	0.125	0.06
VIH-PAP	0.125	0.03	0.25	0.125
sh-Buforin	8	8	32	16
sh-DAH-Buforin	2	2	16	16
sh-GGH-Buforin	4	4	16	8
sh-VIH-Buforin	2	1	8	4

Table S2. Summary of characterization data for synthetic tripeptides. Purity was established by reinjecting purified peptides on a C_{18} analytical column using 0.1% TFA in H_2O (Buffer A) and 0.1% TFA in ACN (Buffer B) with a linear gradient of 0-95% Buffer B over 25 mins. Identities were confirmed using ESI-MS.

Tripeptide	Retention Time (mins)	% Purity	Molecular Weight	Observed $[M+H]^+$
DAH-NH_2	6.124	98.05	340.68	341.1
DMH-NH_2	6.090	96.93	400.46	401.1
DSH-NH_2	5.917	95.28	356.34	357.1
DTH-NH_2	6.123	96.94	370.37	371.1
EAH-NH_2	5.975	97.82	354.34	355.1
GGH-NH_2	6.090	98.05	268.27	269.1
GKH-NH_2	6.029	96.89	339.30	340.2
GNH-NH_2	5.935	95.29	325.32	326.1
LKH-NH_2	6.579	98.11	395.50	396.2
NGH-NH_2	5.931	97.87	325.32	326.1
RTH-NH_2	6.065	98.36	411.47	412.2
SMH-NH_2	6.091	96.29	372.45	373.1
VIH-NH_2	6.069	97.72	366.46	367.2

Table S3. Summary of characterization data of ATCUN-AMPs. Purity was established by reinjecting purified peptides on a C_{18} analytical column using 0.1% TFA in H_2O (Buffer A) and 0.1% TFA in ACN (Buffer B) with a linear gradient of 30-60% Buffer B over 30 mins. Identities were confirmed by ESI-MS. MS ion peaks in normal type are calculated whereas the ones in bold type are observed.

Peptide	Retention Time (mins)	% Purity	Molecular Weight	$[M+2H]^+$	$[M+3H]^+$	$[M+4H]^+$
Anoplin	14.38	96.34	1153.51	577.75 **577.8**	-	-
DAH-Anoplin	12.85	98.93	1476.82	739.41 **739.0**	-	-
GGH-Anoplin	12.81	97.56	1404.75	703.37 **703.1**	-	-
VIH-Anoplin	14.14	98.97	1502.94	752.47 **752.0**	-	-
Buforin II	11.25	98.34	2433.87	-	812.29 **812.2**	609.47 **609.6**
DAH-Buforin II	11.12	98.38	2757.18	-	920.06 **919.9**	690.29 **690.4**
GGH-Buforin II	11.18	98.52	2685.12	-	896.04 **896.0**	672.28 **672.3**
VIH-Buforin II	11.27	99.47	2783.3	-	928.76 **928.7**	696.82 **696.9**
sh-Buforin II	11.41	99.33	2002.43	1002.21 **1002.1**	668.47 **668.4**	-
DAH-sh-Buforin II	11.32	98.64	2325.73	1163.86 **1163.5**	776.24 **775.9**	-
GGH-sh-Buforin II	11.31	99.36	2253.67	1127.83 **1127.7**	752.22 **751.8**	-
VIH-sh-	11.36	98.39	2351.86	1176.93	784.95	-

Buforin II				**1177.0**	**784.7**	
Indolicidin	16.06	99.51	1906.29	954.14 **953.6**	636.43 **636.3**	-
DAH-Indolicidin	15.34	99.67	2229.6	1115.8 **1115.0**	744.2 **744.4**	-
GGH-Indolicidin	14.35	98.52	2157.54	1079.77 **1079.7**	720.18 **719.9**	-
VIH-Indolicidin	16.38	98.60	2255.72	1128.86 **1128.1**	752.91 **752.7**	-
PAP	11.25	97.97	1523.01	762.50 **762.2**	508.67 **508.7**	-
DAH-PAP	11.63	98.30	1846.32	924.16 **924.4**	616.44 **616.4**	-
GGH-PAP	11.48	98.05	1774.25	888.12 **887.7**	592.42 **592.4**	-
VIH-PAP	11.72	97.69	1872.44	937.22 **936.9**	625.15 **625.1**	-
Tritrpticin	14.89	99.34	1901.28	951.64 **951.1**	634.76 **634.7**	-
DAH-Tritrpticin	14.59	99.23	2224.59	1113.29**1** **113.9**	742.53 **742.3**	-
GGH-Tritrpticin	14.52	98.35	2152.52	1077.26 **1078.1**	718.51 **718.3**	-
VIH-Tritrpticin	15.09	99.26	2250.71	1126.35 **1126.6**	751.23 **750.9**	-