Supporting Information

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The relationship of physicochemical properties to the antioxidative activity of free amino acids in Fenton system

Supporting Information (9 pages) include 4 tables and 3 figures.

Table S1. Hydrophobicity/hydrophilicity scales for amino acids.

Side-chain	Amino acid	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
non-polar	**Gly**	0	0	2.39	3.798	0.94	2.23	-0.16	0	-2.4	-1.131
	Ala	3.3	0.35	1.13	13.26	1.81	0.07	-0.27	0.31	-1.1	-0.605
	AABA	-	-	-0.58	-	-	-	-	0.82	-1.7	-0.247
	Pro	9.4	0.84	0.02	27.54	-	-1.22	-0.75	0.72	-0.2	-0.554
	Val	14	1.32	-2.27	44.6	4.04	-2.69	-0.4	1.22	4.1	0.050
	Nle	15.9	-	-3.71	-	-	-	-	1.7	-	0.891
	Ile	22.2	1.83	-3.48	69.53	4.92	-4.44	-0.77	1.8	8.7	0.619
	Leu	24.1	1.8	-3.66	73.84	4.92	-4.19	-1.1	1.7	9.7	0.830
	Phe	30.1	1.69	-3.62	90.17	2.98	-4.92	-1.43	1.79	10	0.644
	Trp	33.2	1.35	-4.02	100	2.33	-4.75	-1.57	2.25	9.7	0.196
polar uncharged	**Tyr**	15.2	0.39	-3.58	47.49	-0.14	-1.39	-0.56	0.96	2.5	-0.492
	DOPA	-	-	-2.57	-	-	-	-	0.56	-	-0.144
	Ser	0	-0.63	2.48	3.526	-3.4	1.96	0.42	-0.04	-4.3	-1.737
	Thr	2.8	-0.27	0.55	11.64	-2.57	0.92	0.63	0.26	-3.8	-1.277
	HYP	-	-	1.13	-	-	-	-	-	-	-1.584
	Asn	0	-0.99	2.88	1	-6.64	3.22	0.81	-0.6	-7.1	-2.216
	Gln	0.6	-0.93	1.14	5.996	-5.54	2.18	1.1	-0.22	-6	-1.858
charged	**Lys**	-3.7	-1.54	3.76	2.917	-5.55	2.84	1.7	-0.99	-9.9	-0.196
	Arg	-3.7	-1.5	3.21	10.24	-14.92	2.88	1.87	-1.01	-10	-3.412
	Asp	-1	-2.15	2.33	8.146	-8.72	3.64	0.81	-0.77	-8.3	-3.401
	Glu	-0.5	-1.95	1.07	11.12	-6.81	3.08	1.17	-0.64	-8.3	-3.689

(a) Hydrophobicity coefficients of side-chains established via RP-HPLC (pH = 7) retention times of model peptides.[21]

(b) Residue hydrophobicity calculated from retention times (reverse-phase HPLC; pH = 7.5) and normalized to Gly.[22]

(c) Hydrophilicity (z_1-scale) calculated from thin layer chromatography retention values, NMR and theoretical data.[23]

(d) Calculated coefficients for side-chain hydrophobicity in peptides using retention times (RP-HPLC) and theoretical approach.[24]

(e) Hydrophobicity calculated as free energies (kcal/mol) of transfer of amino acids side chains from dilute solution in cyclohexane to dilute aqueous solution at pH 7.[25]

(f) Calculated descriptor (principal property) of hydrophilicity (z_1).[26]

(g) Calculated descriptor (principal property) of hydrophilicity (F_4).[27]

(h) Hydrophobicity (Π) = Log P (amino acid) - Log P (Gly). Partition coefficient (P) was directly estimated in octanol/water.[28]

(i) Combined consensus scale of hydrophobicity extracted from 162 different scales (experimental and theoretical).[29]

(j) LogP (octanol/water) calculated using DruLiTo (this paper).

Table S1 (continued). Steric parameters, polarity and hydration numbers of free amino acids.

Side-chain	Amino acid	(k)	(l)	(m)	(n)	(o)
non-polar	**Gly**	2.06	0	43.2	9	5.5
	Ala	2.87	1	60.4	8.1	6.2
	AABA	4.11	2	-	-	-
	Pro	4.11	2.72	81	8	7.58
	Val	4.11	3	90.8	5.9	8.5
	Nle	6.17	4	-	-	-
	Ile	4.92	4	107.5	5.2	9.8
	Leu	4.92	4	107.5	4.9	9.7
	Phe	4.62	5.89	121.2	5.2	10.6
	Trp	7.68	8.08	143.9	5.4	-
polar uncharged	**Tyr**	4.73	6.47	123.1	6.2	-
	DOPA	4.73	7.05	-	-	-
	Ser	3.97	1.6	60.3	9.2	6.9
	Thr	4.11	2.6	76.8	8.6	-
	HYP	-	-	-	-	-
	Asn	4.58	2.95	78	11.6	-
	Gln	6.11	3.95	93.9	10.5	-
charged	**Lys**	6.89	4.77	108.5	11.3	10.5*
	Arg	7.82	6.13	127.3	10.5	-
	Asp	4.74	2.78	73.8	13	-
	Glu	5.97	3.78	85.9	12.3	7.9*

(k) STERIMOL length of the side-chain, measured in the direction in which it is attached to the glycine backbone.[28]
(l) van der Waals volume of the side chain, normalized according to equation: [V(side-chain) - V(H)] - $V(CH_2)$.[28]
(m) Molar volume (cm^3/mol) of free amino acid in the solvent at neutral pH and 25^oC.[30]
(n) Polarity.[31]
(o) Hydration number for free amino acids in water;[32] * not applied in the calculus.

Table S2. Antioxidative activities (AA) and total rate constants (k_{Fenton}) determined in this study, and previously established rate constants for reactions of FAA with $HO^{\bullet}$ produced via water radiolysis ($k_{radiolysis}$).

Side-chain	FAA	AA	k_{Fenton} ($M^{-1}s^{-1}$)*	$k_{radiolysis}$ ($M^{-1}s^{-1}$)#
non-polar	Gly	-0.095	-	1.7×10^{7}
	Ala	-0.080	-	7.7×10^{7}
	AABA	0.260	2.5×10^{9}	5.3×10^{8}
	Pro	0.349	4.0×10^{9}	6.5×10^{8}
	Val	0.542	8.9×10^{9}	8.5×10^{8}
	Nle	0.851	4.3×10^{10}	2.5×10^{9}
	Ile	0.824	3.5×10^{10}	1.8×10^{9}
	Leu	0.903	7.0×10^{10}	1.7×10^{9}
	Phe	0.840	4.0×10^{10}	6.9×10^{9}
	Trp	0.925	9.2×10^{10}	1.3×10^{10}
polar uncharged	Tyr	0.379	4.6×10^{9}	1.3×10^{10}
	DOPA	0.611	1.2×10^{10}	2.6×10^{9}
	Ser	0.059	4.7×10^{8}	3.2×10^{8}
	Thr	0.067	5.4×10^{8}	5.1×10^{8}
	HYP	0.298	3.2×10^{9}	3.2×10^{8}
	Asn	-0.104	-	4.9×10^{7}
	Gln	0.077	6.3×10^{8}	5.4×10^{8}
charged	Lys	0.396	4.9×10^{9}	3.5×10^{8}
	Arg	0.576	1.0×10^{10}	3.5×10^{9}
	Asp	-0.252	-	7.5×10^{7}
	Glu	-0.017	-	2.3×10^{8}

* k_{Fenton} (total rate constant) could not be calculated for FFA that showed AA < 0; # taken from previous reports.[9,10,19]

Table S3. Static ORP (mV) of water solutions of FAA (5 mM).

Side-chain	FAA	ORP (mV)*
non-polar	Gly	213.0
	Ala	217.8
	AABA	210.3
	Pro	208.4
	Val	221.4
	Nle	214.1
	Ile	218.2
	Leu	210.8
	Phe	244.4
	Trp	238.0
polar uncharged	Tyr	228.2
	DOPA	**78.0**
	Ser	224.2
	Thr	236.5
	HYP	216.9
	Asn	238.2
	Gln	236.0
	His	343.8
charged	Lys	238.5
	Arg	252.4
	Asp	374.1
	Glu	328.5
sulfur-containing	Cys	**170.0**
	HCY	**10.1**
	Met	239.1

* Static ORP of water was 210.9 mV.

Table S4. Contribution of functional groups to hydrophobicity/hydrophilicity of FAA (scales (a) – (j) are presented in Table S1), and coefficients of correlation (R) between contributions to hydrophobicity/hydrophilicity and contributions to AA (the calculus of contributions to AA was conducted without His; it delivered results that were almost identical compared to results presented in Figure 2c). Hydrophobicity/hydrophilicity parameters that were calculated using contributions of groups fitted well with values in the scales from the literature (mean R = 0.993).

Functional group	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
$NH_3^+CHCOO^-$	2.17	0.06	2.79	9.46	0.3	1.63	-0.17	0.08	-2.13	-1.1
CH	11.42	0.58	-1.23	29.76	0.03	-1.47	0.04	0.63	6.79	0.35
CH_2	4.55	0.39	-1.5	18.21	1.11	-1.44	-0.16	0.45	2.75	0.46
CH_3	1.47	0.38	-1.85	5.4	1.75	-1.5	-0.25	0.29	0.22	0.49
OH	-10.57	-1.2	0.83	-31.38	-4.41	2.32	0.87	-0.68	-6.95	-1.07
benzene	21.23	1.21	-5.3	56.86	2.22	-4.51	-1.12	1.2	9.1	1.26
COO^-	-9.73	-2.68	1.17	-27.13	-9.73	3.89	1.38	-1.46	-10.31	-3.13
$CONH_2$	-8.68	-1.59	1.48	-33.27	-8.05	3.23	1.35	-1.16	-8.56	-1.63
indole	26.5	0.92	-5.31	72.35	0.93	-4.94	-1.26	1.73	9.08	0.84
amino	-24.04	-3.13	6.97	-79.36	-10.28	6.96	2.48	-2.87	-18.77	-0.92
guanidino	-19.49	-2.71	4.92	-53.83	-18.54	5.57	2.49	-2.44	-16.13	-3.68
R	**0.938**	**0.920**	**-0.846**[#]	**0.947**	**0.738**	**-0.933**[#]	**-0.874**[#]	**0.918**	**0.950**	**0.746**
P[*]	**<0.0001**	**<0.0001**	**0.001**	**<0.0001**	**0.010**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.008**

[*] $P < 0.05$ – statistically significant correlation; [#] hydrophilicity.

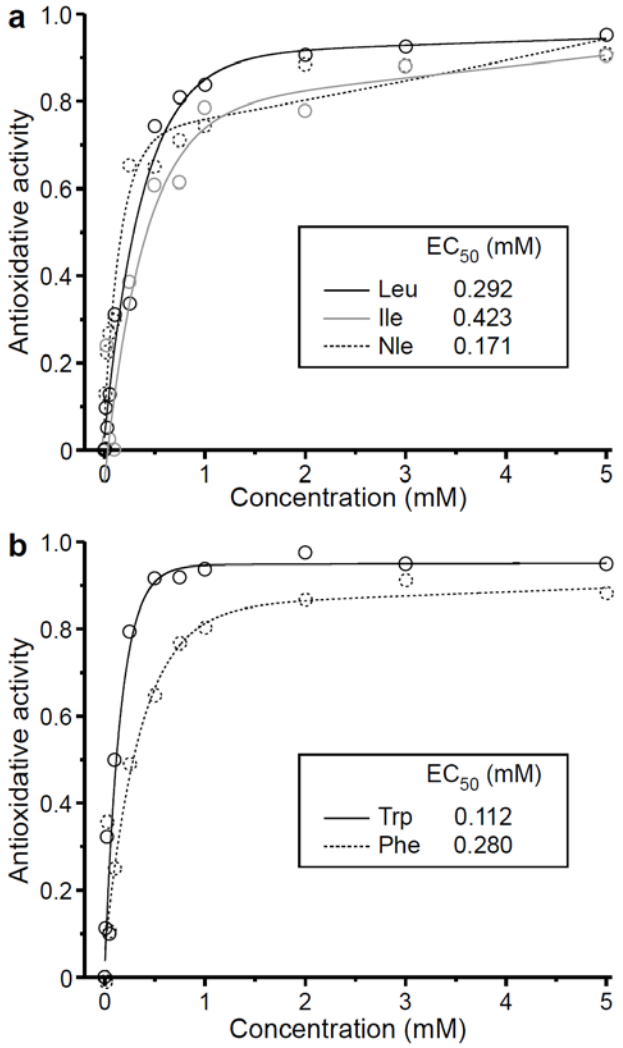

Figure S1. Concentration-dependent change of antioxidative activity. (a) Branched-chain FAA; (b) Aromatic FAA. Exponential fits with two terms (full, dashed, or gray lines) were used to extract EC_{50} (concentration of FAA at which AA = 0.5). This does not imply anything about the kinetics of reactions, but rather reflects the limitations of the method and complexity of the system, which involves radical trapping and spin-adduct decomposition. Because the calculation of EC_{50} involves interpolation, rational functions were not suitable. Smoothing spline showed a significant overshoot not reflecting the real concentration dependence.

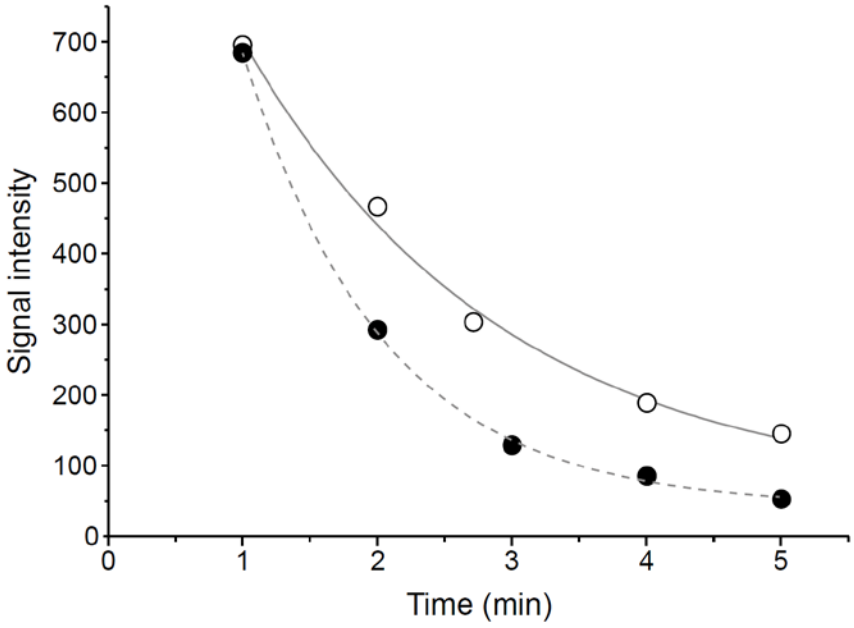

Figure S2. The decay of intensity of EPR signals of DEPMPO adducts in Cys + Fenton system (white circles; exponential fit (full line) r^2 = 0.989) and in HCY + Fenton system (black circles exponential fit (dashed line) r^2 = 0.998).

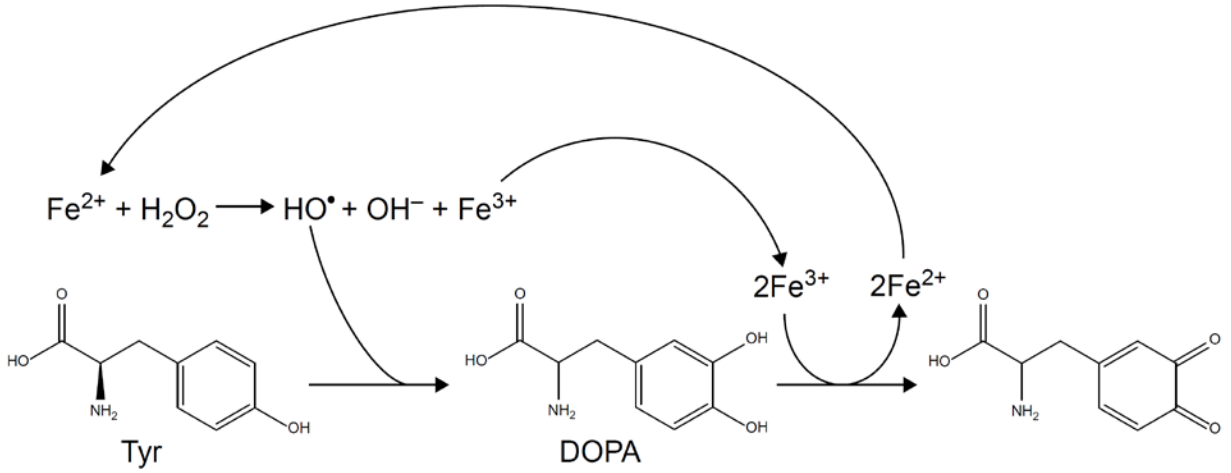

Figure S3. Scheme of pro-oxidative activity of Tyr and DOPA in the Fenton system. Tyr reacts with $^{\bullet}$OH to produce DOPA (and analogous compounds). DOPA reduces 2 Fe^{3+} to 2 Fe^{2+} via a two-step process. Firstly, DOPA forms a complex with Fe^{3+} which undergoes internal electron transfer to produce the respective semiquinone and release Fe^{2+}. Semiquinone is unstable and rapidly reduces another Fe^{3+} to Fe^{2+}. Fe^{2+} re-enters Fenton reaction thus giving rise to further production of $^{\bullet}$OH.