

Supplementary information for:

“Rational screening of biomineralisation peptides for colour-selected one-pot gold nanoparticle syntheses”

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Table S1. A list of 200 peptides screened as AuNP binding peptides in previous work^{*1}.

Name	Sequence	MW (kDa)	pI ^{*2}	GRAVY ^{*3}
AuP1	HFSSWETQQG	1.21	5.24	-1.45
AuP2	WTHRDASTPW	1.24	9.76	-1.22
AuP3	WYEKWQKANW	1.44	8.50	-2.05
AuP4	WMETKWQARA	1.31	8.75	-1.24
AuP5	GTWSEHQNGW	1.20	5.24	-1.78
AuP6	ETWSMQQHEW	1.36	4.51	-1.86
AuP7	WRAGQAQMOW	1.26	9.75	-1.17
AuP8	WKPWMEPQHS	1.33	6.75	-1.8
AuP9	AMQQQWEMSQ	1.27	4.00	-1.36
AuP10	RWQIEEHFAP	1.31	5.40	-1.16
AuP11	PEESQEGWMA	1.16	3.67	-1.4
AuP12	TGEWGMQGIH	1.12	5.24	-0.66
AuP13	EEPHWEEMAA	1.23	4.09	-1.42
AuP14	WWKVAIHHSK	1.27	10.00	-0.66
AuP15	RHWHSWTWEI	1.44	6.92	-1.41
AuP16	MNWKWGLESM	1.28	6.00	-0.63
AuP17	NWTAKWTQTH	1.27	8.76	-1.62
AuP18	HWIKIPPWMW	1.39	8.76	-0.21
AuP19	HWKQKVHWWG	1.39	10.00	-1.66
AuP20	WHKWWTTHGHW	1.46	8.77	-1.82
AuP21	KYWQMWMSWK	1.47	9.70	-1.23
AuP22	KWQWKQAGAQ	1.23	10.00	-1.69
AuP23	EHQQWKETWH	1.41	6.00	-2.68
AuP24	GQWQWMDAGW	1.25	5.52	-0.75
AuP25	QWTWKIQVMK	1.35	10.00	-0.67
AuP26	TGHEPMEGWG	1.10	4.51	-1.27
AuP27	HWKGEMHTDF	1.27	6.92	-1.18
AuP28	PEEGPHSLWH	1.19	5.23	-1.49
AuP29	EWVEAMGGHT	1.12	4.51	-0.47
AuP30	WPAMGWNMEQ	1.25	4.00	-0.87
AuP31	KWAIWEMKGH	1.29	8.60	-0.85
AuP32	GETWETHYSE	1.24	4.24	-1.85
AuP33	WVHKRLNWTV	1.34	11.00	-0.54
AuP34	WSWPKVKSFW	1.35	10.00	-0.67
AuP35	MLGWMHQSWQ	1.30	6.74	-0.56
AuP36	NWKWQMKWTQ	1.44	10.00	-1.98
AuP37	EWHVKWSEAI	1.28	5.40	-0.62
AuP38	LAGVPMHWYT	1.17	6.74	0.36
AuP39	QEHLSEMWGE	1.25	4.24	-1.36
AuP40	ASHQWAWKWE	1.33	6.75	-1.4
AuP41	WSEETEMWPL	1.31	3.67	-0.97
AuP42	DMVWHESWGI	1.25	5.24	0.02
AuP43	WWLQKWHGSH	1.36	8.76	-1.39
AuP44	ENHSWGGGGA	0.97	5.24	-1.17
AuP45	QRHSWGGGEA	1.08	6.75	-1.58
AuP46	WKATWAKYEK	1.31	9.53	-1.54
AuP47	TWHIMWRHAW	1.42	9.76	-0.61
AuP48	WEAKEWLHNW	1.40	5.40	-1.47
AuP49	NKGGGWQGPE	1.03	6.00	-1.85
AuP50	WGWKWEHSEA	1.32	5.40	-1.62

*1: M. Tanaka *et al.*, Acta Biomaterialia (2017). *2 and 3: The isoelectric point (pI) and the grand average of the hydropathy value (GRAVY) were analyzed using the ProtParam tool in ExPASy (<http://web.expasy.org/protparam/>).

Table S1. Continued.

Name	Sequence	MW(kDa)	pI ^{*2}	GRAVY ^{*3}
AuP51	TEKLWWHNGS	1.26	6.75	-1.40
AuP52	QTEWWTYRKG	1.35	8.59	-2.03
AuP53	AESEWSMMM	1.29	3.79	-0.29
AuP54	KLWEVSRGWG	1.22	8.75	-0.73
AuP55	WMAHAVVSPA	1.07	6.74	0.92
AuP56	SMEIQWQKPS	1.23	6.00	-1.21
AuP57	RWPWHWQEMM	1.49	6.75	-1.52
AuP58	EKSSKSMESE	1.14	4.79	-1.96
AuP59	MPWESHEISE	1.24	4.24	-1.14
AuP60	VHRGWSEMVW	1.29	6.75	-0.39
AuP61	GEHMEHMEWS	1.27	4.72	-1.52
AuP62	EWSSQRRWGN	1.31	9.60	-2.33
AuP63	SRQWQTWAAE	1.26	6.00	-1.47
AuP64	AESEHEWEVA	1.19	4.09	-1.11
AuP65	TQWHEWHWYQ	1.5	5.98	-2.16
AuP66	SQYWEHEVHQ	1.34	5.23	-1.92
AuP67	HGHMRWWWWQ	1.51	9.76	-1.65
AuP68	HIRGEHKWNW	1.36	8.76	-1.95
AuP69	EWKHPIWWER	1.47	6.76	-1.84
AuP70	PWSWHEIYLS	1.32	5.24	-0.47
AuP71	WMNWESSQKH	1.33	6.75	-1.91
AuP72	GWWARWMWMQ	1.44	9.75	-0.64
AuP73	WWERGAGRGW	1.26	9.60	-1.46
AuP74	VLWRHEWAWK	1.41	8.75	-0.80
AuP75	TKWWWMTRVQ	1.42	11.00	-0.99
AuP76	WWGMTMSKEK	1.28	8.59	-1.12
AuP77	YWASHKHWWW	1.49	8.61	-1.42
AuP78	WGEWHSEGLH	1.24	5.23	-1.30
AuP79	REWWKGFHMW	1.46	8.75	-1.35
AuP80	GGWHKLDESE	1.16	4.65	-1.63
AuP81	REWGWAKSGR	1.23	10.84	-1.80
AuP82	WQENSMEENW	1.35	3.67	-2.17
AuP83	WGMWSHAHTG	1.17	6.92	-0.68
AuP84	WHWQHAMESW	1.46	5.23	-1.85
AuP85	RRWAISLRMW	1.37	12.3	-0.41
AuP86	WEQSHVTQWE	1.33	4.51	-1.63
AuP87	GMEGHWSRWW	1.33	6.75	-1.36
AuP88	WEFRSHIHM	1.38	7.03	-1.01
AuP89	FVEGKSWGGL	1.08	6.00	0.05
AuP90	NLWMQGGIML	1.16	5.52	0.72
AuP91	WWHMQGAWAH	1.42	6.92	-1.02
AuP92	EWHHNWEEWM	1.48	4.72	-2.12
AuP93	GYHHWWGQPW	1.35	6.92	-1.63
AuP94	SMWGTQTQGH	1.11	6.74	-0.94
AuP95	AHALWIWHKT	1.26	8.76	-0.09
AuP96	WSWESPWEWH	1.43	4.51	-1.70
AuP97	HWEGHSWTIA	1.22	5.98	-0.73
AuP98	QWGRDTMWWM	1.40	5.84	-1.15
AuP99	WGVGSEMHS	1.13	5.98	-0.71
AuP100	HKWMHIQWTT	1.37	8.76	-1.06

Table S1. Continued.

Name	Sequence	MW(kDa)	pI ^{*2}	GRAVY ^{*3}
AuP101	WQKHTKWWWM	1.52	10.00	-1.69
AuP102	EEAGRWYSPK	1.22	6.14	-1.86
AuP103	KGWSMWQWGW	1.35	8.75	-1.07
AuP104	APSWVNSMEP	1.12	4.00	-0.48
AuP105	WKWWNKEVAE	1.38	6.14	-1.50
AuP106	AMQWRQWSGS	1.24	9.75	-1.16
AuP107	GHHGWVWFMM	1.29	6.92	0.18
AuP108	QSGSGRSWMM	1.13	9.75	-0.83
AuP109	NEIKWAQWQW	1.39	6.00	-1.43
AuP110	ESMHSWHWYT	1.36	5.98	-1.34
AuP111	KSWNVGGAEW	1.13	6.00	-0.83
AuP112	NWELEEHSAS	1.20	4.24	-1.41
AuP113	WHEDHAWRSL	1.34	5.99	-1.49
AuP114	GMWHIEHIWL	1.32	5.98	0.26
AuP115	GKTWDPWSGH	1.17	6.74	-1.63
AuP116	WHHWAQGWHG	1.30	7.02	-1.48
AuP117	YEAVSTTWQS	1.17	4.00	-0.62
AuP118	WWKHTATSSS	1.19	8.76	-1.09
AuP119	WKHNKTWGSS	1.23	10.00	-1.90
AuP120	YGAWKTRWWM	1.38	9.99	-0.98
AuP121	AHKWGWAAEW	1.24	6.75	-0.83
AuP122	VWAFNMMEHQ	1.29	5.24	-0.20
AuP123	WTEWTHIGAE	1.23	4.51	-0.75
AuP124	WESDTIKISH	1.22	5.32	-0.83
AuP125	TTWHGFPWAG	1.16	6.74	-0.42
AuP126	WREWSHRWPW	1.53	9.61	-2.17
AuP127	YWYWPQMGWH	1.45	6.74	-1.21
AuP128	WWKHSQVVAH	1.28	8.76	-0.62
AuP129	IHWQVMAESE	1.23	4.51	-0.30
AuP130	HWYTTSGGDS	1.11	5.08	-1.27
AuP131	GWALMHDENG	1.13	4.35	-0.79
AuP132	MVWSLHHASW	1.25	6.92	0.19
AuP133	NHKTPAWMLQ	1.23	8.76	-0.98
AuP134	RWWEMTMESL	1.37	4.53	-0.72
AuP135	SWWTLMTMGW	1.30	5.52	0.23
AuP136	WEWATKPKEG	1.23	6.14	-1.75
AuP137	VHYGSQIEWG	1.18	5.24	-0.53
AuP138	WLKWKEKRGH	1.36	10.29	-1.62
AuP139	ETEWLGHETL	1.21	4.24	-0.88
AuP140	SLMKWMALAE	1.18	6.00	0.59
AuP141	WEERWTGTYE	1.36	4.25	-1.99
AuP142	KWQEPWWWAT	1.42	6.00	-1.50
AuP143	FAQDDWLMRH	1.32	5.21	-0.88
AuP144	LWAHSEKLGG	1.10	6.75	-0.37
AuP145	SHWRKIRQMT	1.34	12.01	-1.56
AuP146	SKQMGTKWRY	1.28	10.29	-1.80
AuP147	EWEEKHHGWK	1.42	6.92	-2.43
AuP148	EWVHMWTEAL	1.30	4.51	-0.10
AuP149	LWMAGKIGPS	1.06	8.75	0.40
AuP150	WGESVKIIHT	1.17	6.75	-0.02

Table S1. Continued.

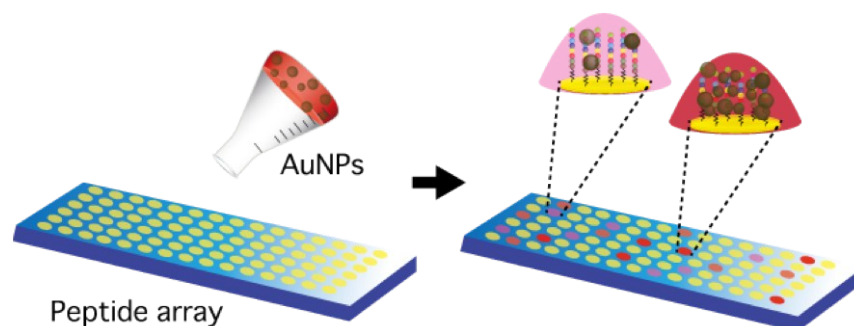
Name	Sequence	MW(kDa)	pI ^{*2}	GRAVY ^{*3}
AuP151	ETGHHIWEWM	1.33	5.23	-0.99
AuP152	VSWHLGKYKG	1.17	9.70	-0.68
AuP153	SQWPWGKETS	1.21	6.00	-1.70
AuP154	EKQWVGQGAS	1.09	6.00	-1.09
AuP155	NWTHWSTTQH	1.30	6.92	-1.81
AuP156	GHWQEIGDGV	1.10	4.35	-0.71
AuP157	HRHWEWEAWM	1.47	6.00	-1.69
AuP158	EWLHKHWWQR	1.51	8.76	-2.07
AuP159	RGEHWKAYWW	1.42	8.60	-1.77
AuP160	WIEHAWSRHL	1.33	6.92	-0.69
AuP161	GWTWQAKKMA	1.21	10.00	-0.87
AuP162	GEKSYWTGRE	1.21	6.14	-1.99
AuP163	WSQKRWEIGQ	1.32	8.75	-1.74
AuP164	WETVRSMWW	1.37	6.00	-0.69
AuP165	EGSDHPSWNQ	1.16	4.35	-2.17
AuP166	QHEVEMMWSL	1.29	4.51	-0.36
AuP167	KWWEESALHE	1.31	4.75	-1.46
AuP168	GWKWEAPWE	1.25	4.53	-1.42
AuP169	EQKAEWWTHW	1.40	5.40	-1.92
AuP170	GWSGAHWSEW	1.20	5.24	-1.00
AuP171	WEHIWHKQAW	1.42	6.92	-1.37
AuP172	SWLQWKWRGH	1.38	11.00	-1.52
AuP173	WMMWGWVHEI	1.37	5.24	0.27
AuP174	FKPAGIWWNQ	1.25	8.75	-0.56
AuP175	MTSTNEPYWA	1.20	4.00	-0.93
AuP176	RRRWEEEAHL	1.38	6.77	-2.25
AuP177	WISWEKVAHV	1.19	6.00	0.65
AuP178	LHWSSISWQH	1.28	6.92	-0.58
AuP179	ETWHSWSVKW	1.35	6.75	-1.14
AuP180	HWWEHEHEH	1.51	5.22	-2.09
AuP181	MSWHWNMTGW	1.34	6.74	-0.75
AuP182	EPNWHAWSEA	1.23	4.51	-1.43
AuP183	HQWKESHQGE	1.27	6.00	-2.64
AuP184	IWQKWSKETE	1.33	6.14	-1.71
AuP185	LWSPWAPKML	1.23	8.75	0.16
AuP186	ATADWSPMDY	1.16	3.56	-0.68
AuP187	ENWHEMAQRG	1.26	5.40	-1.93
AuP188	WMAVDRTGSG	1.08	5.84	-0.33
AuP189	WWSLEVQEPH	1.31	4.51	-0.99
AuP190	WSAVQRWHTH	1.31	9.76	-1.17
AuP191	WWHGWVQMGW	1.37	6.74	-0.50
AuP192	KGHIWKTWWH	1.38	10.00	-1.35
AuP193	PVWQGWVHEV	1.32	5.24	-0.65
AuP194	THMQAWVIWE	1.30	5.24	-0.03
AuP195	MWSHIWYRQS	1.39	8.75	-0.95
AuP196	WNEWTHNWTW	1.46	5.24	-1.87
AuP197	SEKYHRWGLG	1.23	8.60	-1.51
AuP198	WWEHGWHWGQ	1.41	5.98	-1.78
AuP199	STGHKVVWPG	1.15	8.76	-0.86
AuP200	WQERMLEQRT	1.38	6.14	-1.89

Table S2. A list of candidate peptides for color selected AuNP biomineralisation.

Red	Name(AuP)* ¹	Sequence	RGB	C_r * ²	C_g * ³	C_b * ⁴	pI* ⁵	GRAVY* ⁶
R1	116	WHHWAQGWHG	419.1	0.443	0.278	0.279	7.02	-1.48
R2	126	WREWSHRWPW	428.2	0.436	0.282	0.283	9.61	-2.17
R3	35	MLGWMHQSWQ	438.0	0.426	0.282	0.291	6.74	-0.56
R4	173	WMMWGWVHEI	440.6	0.441	0.283	0.276	5.24	0.27
R5	127	YWYWPQMGMWH	449.3	0.426	0.287	0.287	6.74	-1.21
R6	157	HRHWEWEAWM	453.2	0.415	0.302	0.283	6.00	-1.69
R7	196	WNEWTHNWTW	454.0	0.429	0.289	0.282	5.24	-1.87
R8	147	EWKWKHHGWK	454.4	0.414	0.298	0.289	6.92	-2.43
R9	106	AMQWRQWSGS	466.9	0.421	0.291	0.289	9.75	-1.16
R10	120	YGAWKTRWWM	472.1	0.411	0.295	0.293	9.99	-0.98
Average			447.6	0.426	0.289	0.285	7.33	-1.33
Green	Name(AuP)	Sequence	RGB	C_r	C_g	C_b	pI	GRAVY
G1	151	ETGHHIWEWM	524.8	0.387	0.331	0.282	5.23	-0.99
G2	65	TQWHEWHWYQ	542.5	0.388	0.331	0.280	5.98	-2.16
G3	114	GMWHIEHIWL	553.1	0.374	0.341	0.285	5.98	0.26
G4	195	MWSHIWYRQS	573.0	0.379	0.331	0.290	8.75	-0.95
G5	88	WEFRSHIHM	579.1	0.381	0.347	0.273	7.03	-1.01
G6	13	EEPHWEEMAA	583.1	0.371	0.334	0.295	4.09	-1.42
G7	84	WHWQHHEMESW	583.3	0.383	0.332	0.285	5.23	-1.85
G8	189	WWSLEVQEPH	584.0	0.364	0.335	0.301	4.51	-0.99
G9	14	WWKVANIHSK	584.9	0.371	0.336	0.293	10.00	-0.66
G10	102	EEAGRWYSPK	587.4	0.361	0.331	0.308	6.14	-1.86
Average			569.5	0.376	0.335	0.289	6.29	-1.16
Blue	Name(AuP)	Sequence	RGB	C_r	C_g	C_b	pI	GRAVY
B1	73	WWERGAGRGW	477.9	0.370	0.317	0.313	9.6	-1.46
B2	2	WTHRDASTPW	534.8	0.365	0.319	0.315	9.76	-1.22
B3	40	ASHQWAWKWE	573.3	0.359	0.328	0.313	6.75	-1.4
B4	49	NKGGGWQGPE	577.6	0.358	0.326	0.315	6.00	-1.85
B5	44	ENHSWGGA	578.6	0.373	0.314	0.313	5.24	-1.17
B6	111	KSWNVGGAEW	582.7	0.359	0.326	0.315	6.00	-0.83
B7	41	WSEETEMWPL	585.0	0.358	0.323	0.319	3.67	-0.97
B8	108	QSGSGRSWMM	588.7	0.359	0.329	0.312	9.75	-0.83
B9	9	AMQQQWEMSQ	591.6	0.362	0.327	0.311	4.00	-1.36
B10	140	SLMKWMALAE	596.4	0.355	0.331	0.315	6.00	0.59
Average			568.7	0.362	0.324	0.314	6.68	-1.01
Negative	Name(AuP)	Sequence	RGB	C_r	C_g	C_b	pI	GRAVY
N1	90	NLWMQGGIML	742.5	0.335	0.334	0.331	5.52	0.72
N2	94	SMWGTTQGHT	720.9	0.341	0.338	0.321	6.74	-0.94
N3	59	MPWESHEISE	716.2	0.338	0.337	0.326	4.24	-1.14
N4	80	GGWHKLDESE	693.0	0.342	0.338	0.321	4.65	-1.63
N5	156	GHWQEIGDGV	691.6	0.338	0.334	0.328	4.35	-0.71
N6	58	EKSSKMESE	690.3	0.335	0.334	0.332	4.79	-1.96
N7	76	WWGMTMSKEK	674.1	0.360	0.332	0.307	8.59	-1.12
N8	29	EWVEAMGGHT	667.4	0.344	0.337	0.319	4.51	-0.47
N9	131	GWALMHDENG	667.0	0.346	0.338	0.315	4.35	-0.79
N10	27	HWKGEMHTDF	665.3	0.353	0.339	0.308	6.92	-1.18
Average			692.8	0.343	0.336	0.321	5.47	-0.92

*1: The name was reported in previous paper (M. Tanaka *et al.*, Acta Biomaterialia (2017)). *2, 3 and 4: The chromaticity value for each peptide spot was calculated as $C_x = X/(R + G + B)$, where R , G and B are the averaged pixel's red, green and blue values ($x = R, G$ or B). *5 and 6: The isoelectric point (pI) and the grand average of the hydropathy value (GRAVY) were analyzed using the ProtParam tool in ExPASy (<http://web.expasy.org/protparam/>).

1. Screening of AuNP binding peptides.
(Tanaka M. et al., Acta Biomaterialia 2017)



**2. Screening of AuNP mineralisation peptides
from AuNP binding peptides with property
selection.**
(This study)

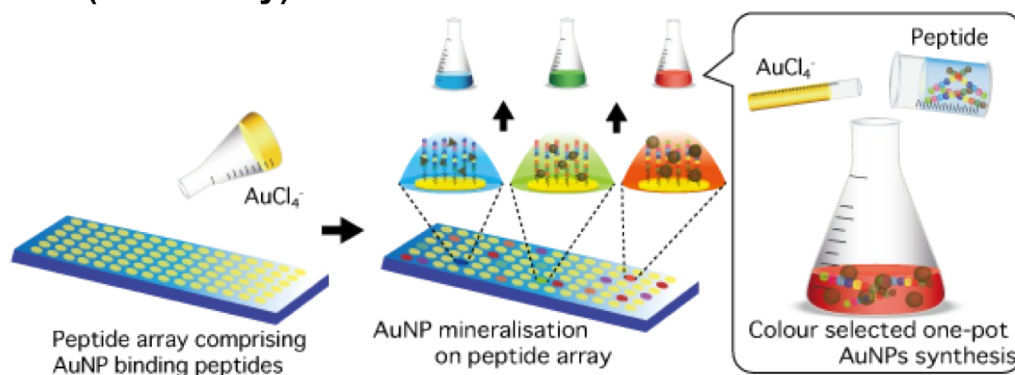


Figure S1 Schematic flow for screening of AuNP mineralisation peptides.

1) In a previous study, we screened a series of AuNP-binding peptides by designing an array based on varying the amino acid frequency informed by empirical results (Tanaka M. et al., Acta Biomaterialia 2017). 2) In this study, using the top 200 high-binding peptide sequences, biomineralisation peptides were screened with optical property selection by color imaging of peptide array.

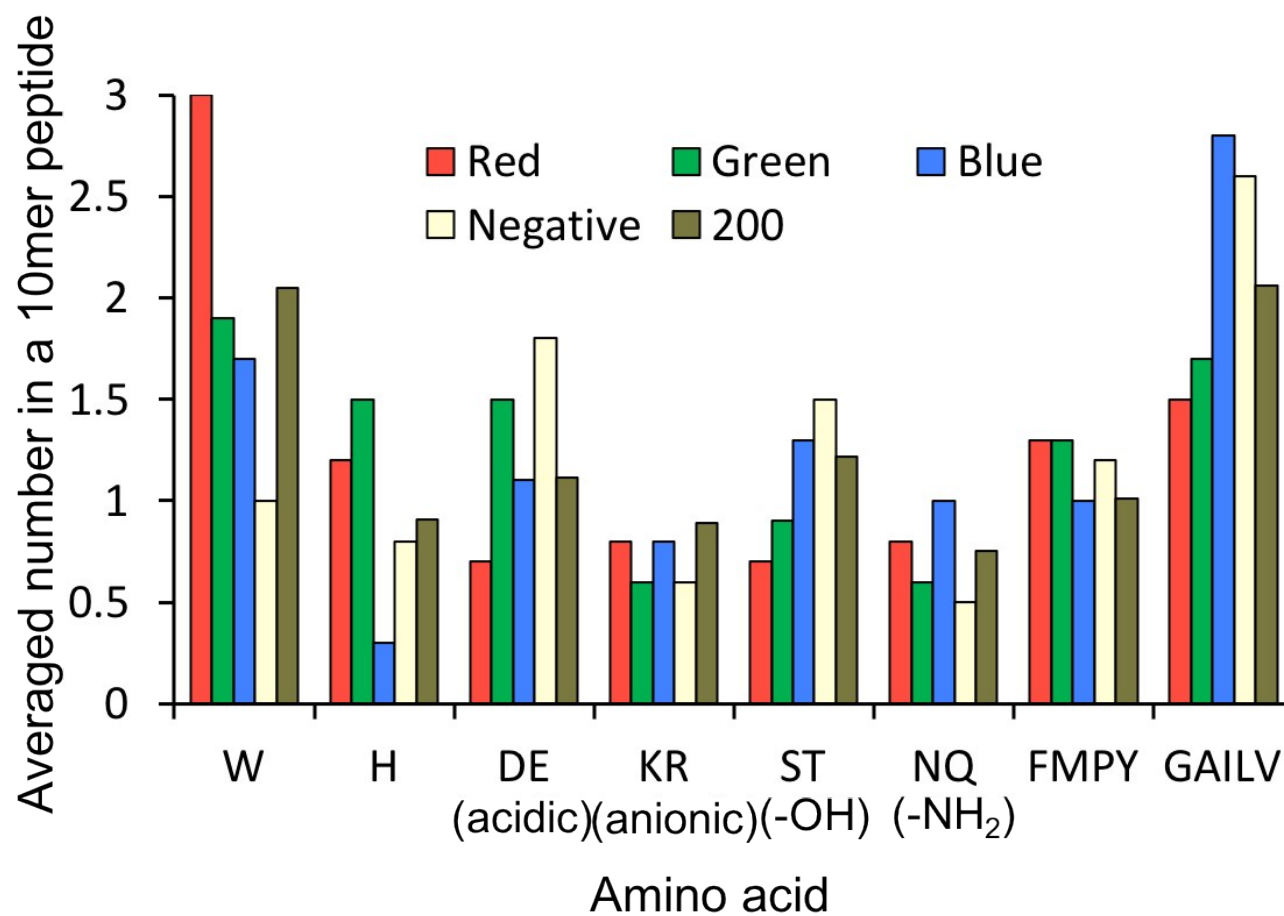


Figure S2 Amino acid frequencies of different coloured AuNP biomaterialisation peptides.

Amino acid frequencies were calculated from 10 peptide sequences for each colour and are shown as the expected number in a peptide (10 amino acids). The 19 amino acids used (cysteine was not used in this peptide library) have been categorized according to their characteristics.

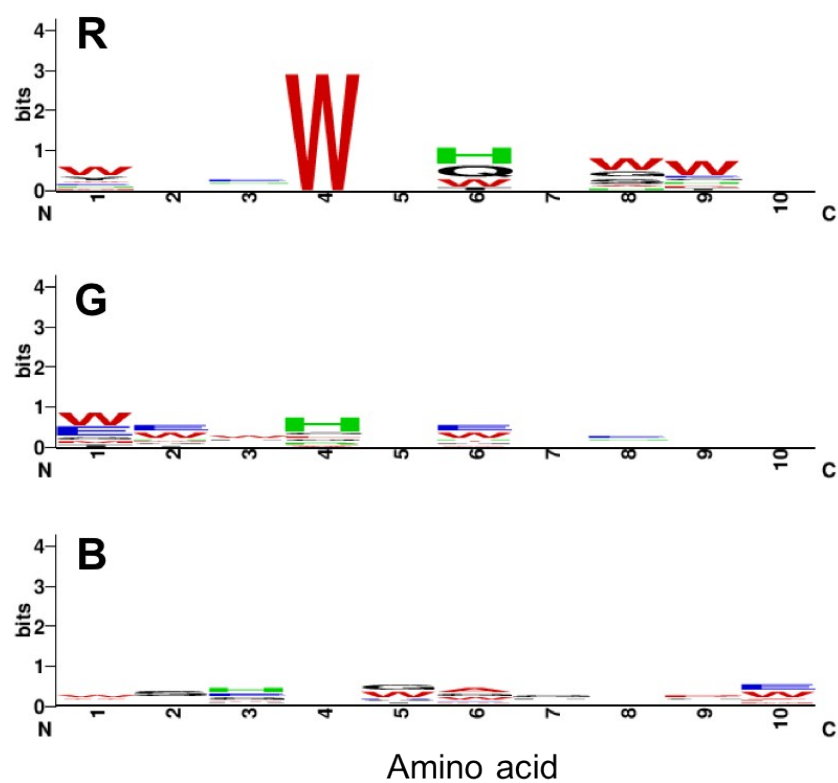


Figure S3 Amino acid sequence analysis of different coloured AuNP biomineralisation peptides. Sequence logos were obtained from 10 peptide sequences for each coloured AuNP biomineralisation (R: red, G: green and B: blue) using an online tool (<http://weblogo.berkeley.edu/logo.cgi>).

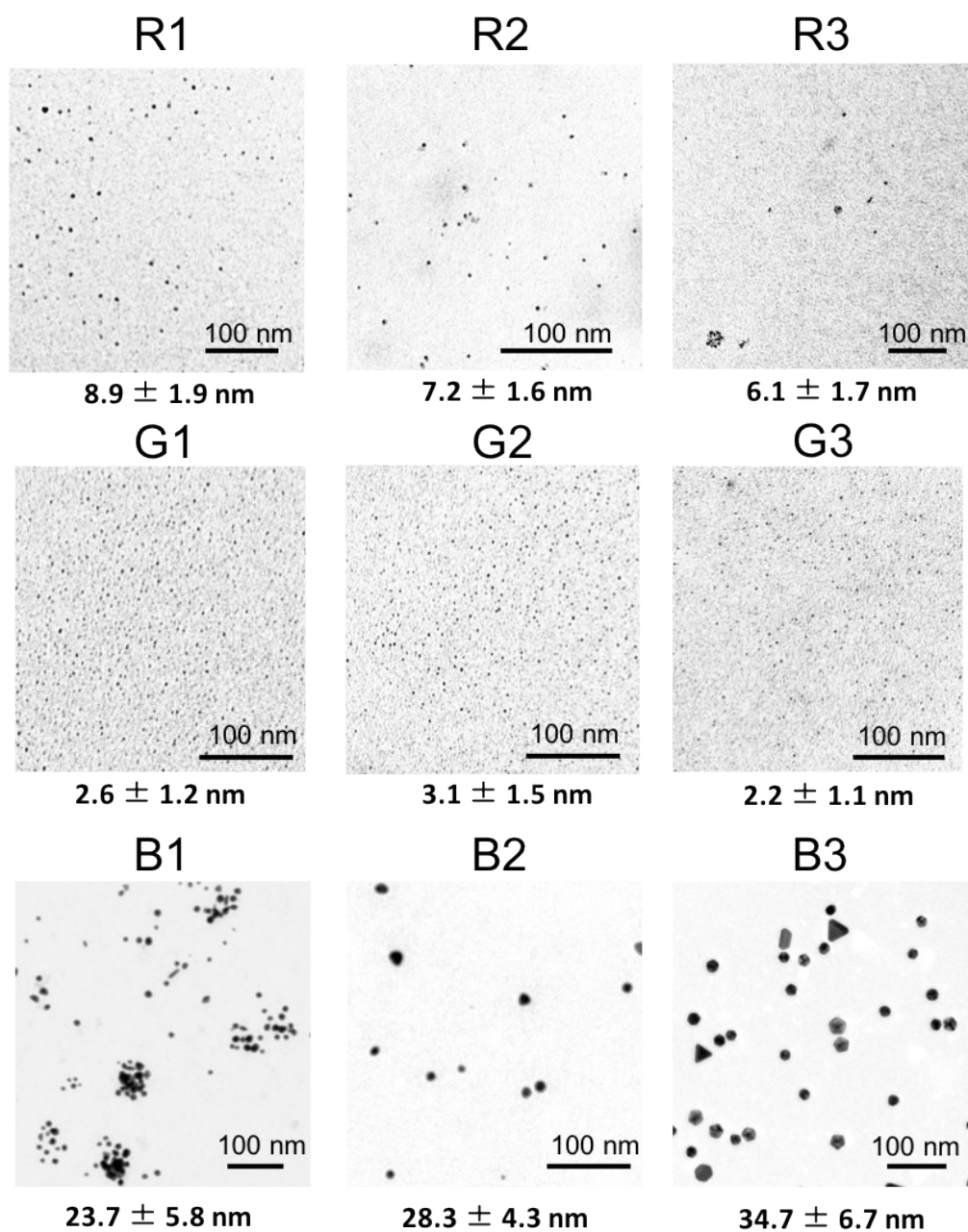


Figure S4 Representative transmission electron microscopy (TEM) images of AuNPs biomineralised by colour selected AuNP synthesis peptides. The average sizes were obtained by manual counting of more than 200 particles in TEM images. Values shown at the bottom of each image include $\pm$ SD.

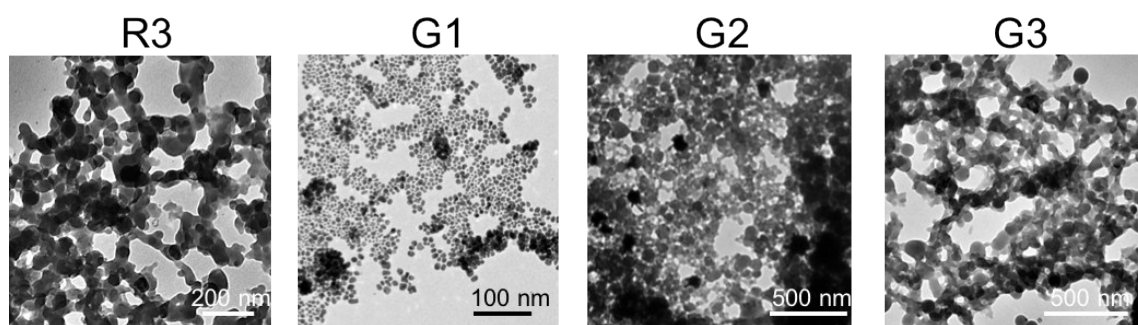


Figure S5 Representative transmission electron microscopy (TEM) images of precipitated AuNPs biomineralised by colour-selected AuNP synthesis peptides.

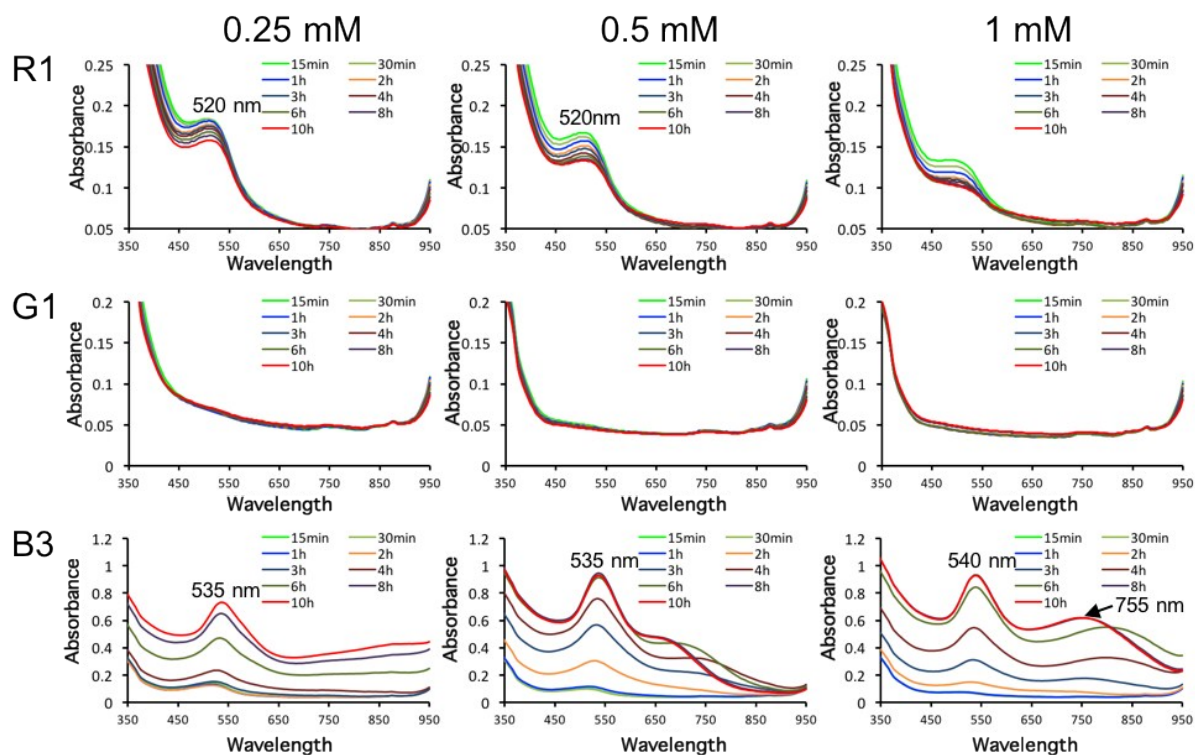


Figure S6 Time course UV-Vis absorption spectra during AuNP biomineralisation in the presence of different concentration of peptides.

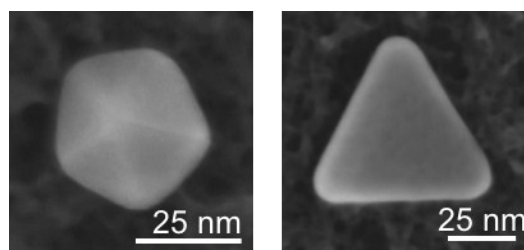


Figure S7 Representative scanning electron microscopy (SEM) images of decahedral and triangle shaped AuNPs biomineralised by B3 peptide.

Supplementary movie 1

A movie of AuNPs biomineralisation using screened peptides. The syntheses were conducted by the mixing of 0.5 mM HAuCl_4 and 0.2 mM peptide in tris-buffer saline (pH 7.4). The vials contain no, R1 (WHHWAQGWHG), G1 (ETGHHIWEWM), and B3 (ASHQWAWKWE) peptides from left to right. The movie starts by the addition of HAuCl_4 solution in each vial containing these peptides.

Materials and methods

Peptide array synthesis using spot technology

A cellulose membrane (grade 542; Whatman, Maidstone, UK) was activated using β -alanine as the N-terminal basal spacer. Activated Fmoc amino acids (0.5 M) were spotted on the membrane using a peptide auto-spotter (ResPepSL; Intavis AG, Köln, Germany), in accordance with the manufacturer's instructions. Briefly, after addition of the first residue, the remaining amino groups were blocked by 4% acetic anhydride. With each elongation step, the membrane was deprotected with 20% piperidine in N,N'-dimethylformamide (DMF) and subsequently washed thoroughly with DMF and then ethanol. After the final deprotection, side-chain protecting groups were removed with a solution of m-cresol:thioanisole:ethanedithiol:trifluoroacetic acid (1:6:3:40) for 3 h. Finally, the membrane was thoroughly washed with diethyl ether, ethanol, and Tris-buffered saline (TBS; pH 7.4), and dried without heat. The synthesized peptide list was shown in supplementary Table 1.

Evaluation of AuNP biomineralisation activity on peptide array and colour analysis

To evaluate the AuNP biomineralisation property of the peptide library, synthesized peptide array was incubated in 0.5 mM HAuCl_4 containing TBS buffer (pH 7.4) for 1 h. Then, the membrane was washed with TBS buffer and scanned (Canon, LiDE220). The R, G and B value of each peptide spot were evaluated using ImageJ software and the chromaticity value was manually calculated as $C_x = x/(R+G+B)$, where R, G and B are the averaged pixel's red, green and blue values ($x=R, G$ or B). To screen effective peptides for colour selected AuNP biomineralisation, after listing top20 peptides for red, green and blue AuNP biomineralisation according to C_x , the list was aligned by the RGB intensity for the selection of high mineralisation activity. As $(R,G,B) = (0,0,0)$ and $(R,G,B) = (255,255,255)$ means black and white colour, respectively, highly active mineralisation peptide spot reveals low summed value of RGB. Based on these two criteria, 10 candidate peptides for each colour biomineralisation were selected and named as R1-R10, G1-G10, and B1-B10. In addition, null or weak biomineralisation peptides were simply selected from the summed value of RGB and named as N1-N10.

Evaluation of AuNP biomineralization in aqueous condition using peptides

Candidate peptides were chemically synthesized (Initiator⁺ Alstra, Biotage, Japan) and purified with HPLC (purity > 90%). All purified peptides were dissolved in TBS buffer (pH 7.4), except R2 (WREWSHRWPW) and G3 (GMWHIEHIWL) peptides. As these two peptides were undissolved in TBS buffer, the peptides were dissolved in 20 μ L of 100% DMSO and added in TBS buffer. By the adding of HAuCl₄, the biomineralisation event was started.

Transmission electron microscopic (TEM) analysis was conducted using a Hitachi H7650 microscope (Hitachi, Tokyo, Japan) operating at a working voltage of 100 kV. The specimens were prepared by drop-casting 1.5 μ L of the sample dispersion onto a formvar-coated 200-mesh Cu grid. The average sizes ($\pm$ SD) were obtained by manual counting of more than 200 randomly selected particles in TEM images. UV-Vis optical absorbance of the AuNPs was measured using a microtiter plate reader (PowerScan 4, DS Pharma Biomedical Co., Ltd., Osaka, Japan).