Supplementary information

Enhancing *in vitro* selection techniques to assist the discovery, understanding and use of inorganic binding peptides

S1. Polymerase Chain Reaction and agarose gel. PCR was used to investigate the presence of phage bound to the solid after each acid elution. 5 µl of non bound phage, acid elute or slurry, were added to a PCR mix containing 25 µl of JumpStart™Taq ReadyMix™ (Sigma), 0.3 µl of each primer (CMM13-01 and CMM13-02) and 0.5 µl of BSA. The composition of JumpStart™Taq ReadyMix™ was: 20 mM Tris-HCl, pH 8.3, 100 mM KCl, 3 mM $MgCl_2$, 0.002% gelatin, 0.4 mM of each dNTP (dATP, dCTP, dGTP, TTP), stabilizers, 0.1 unit/ml Taq DNA Polymerase, JumpStart Taq antibody. Water was then added to make the volume up to 50 µl. The PCR amplification was performed as follows:

Initial Denaturation	95 °C, 10 min
	35 cycles
Denaturation	95 °C, 30 secs
Annealing	57 °C, 30 secs
Elongation	72 °C, 120 secs
Final Elongation	72 °C, 5 min
Final cooling	4 °C, infinite

The PCR products were separated onto a 3% agarose gel by DNA electrophoresis. Agarose gels were prepared by suspending the agarose in TBE buffer 0.5X and melting it in a microwave oven. Ethidium bromide was added for visualization of the DNA samples. Each DNA sample (10 µl) was mixed with 2 µl of 6X loading dye (Fermentas); a 100 bp ladder (NEB) was used as marker. Gels were run (100 mA for *ca* 100 minutes), visualised and photographed using an Alpha imager equipped with a UV lamp. Quantification of the band intensities was carried out using ImageJ program (http://rsb.info.nih.gov/ij/).

S2. DNA sequencing and peptide analysis. Single-stranded DNA isolated from the phage clones was sequenced by using 96g III primer by Macrogen (Korea). The DNA sequences were translated into amino acids using the "translate" program on the proteomics server of the Swiss Institute of Bioinformatics Expert Protein Analysis System (ExPASy [http://www.expasy.ch/]). The isoelectric points of the predicted peptide sequences were calculated using "compute MW/pI," also present on the ExPASy proteomics server. Sequence alignments were performed using the CLUSTAL W sequence alignment program (available at http://www.ebi.ac.uk/clustalw/).

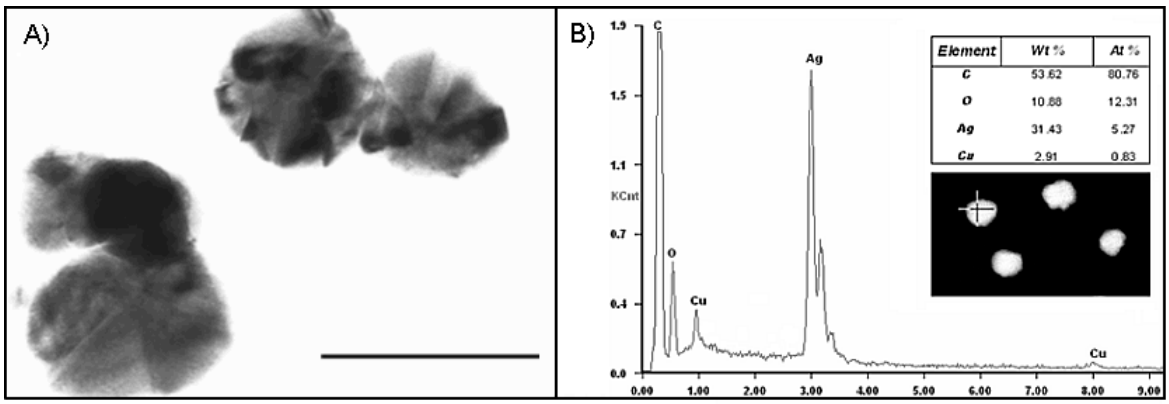

Figure 1S A) Transmission electron micrographs of silver nanoparticles synthesized in the presence of Ag- 13 clone (scale bar 100 nm). **B)** EDX analysis of the silver particles formed with the selected binding phage.

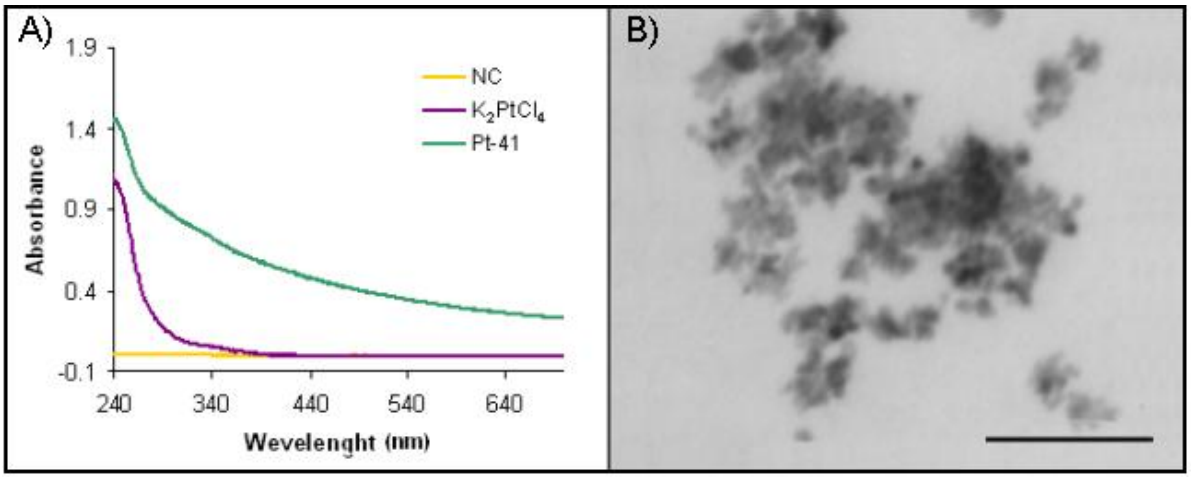

Figure 2S A) UV-vis spectrum of the colloidal dispersions. **B)** Transmission electron micrographs of platinum nanoparticles obtained in the presence of Pt-32 clone (scale bar 50 nm).

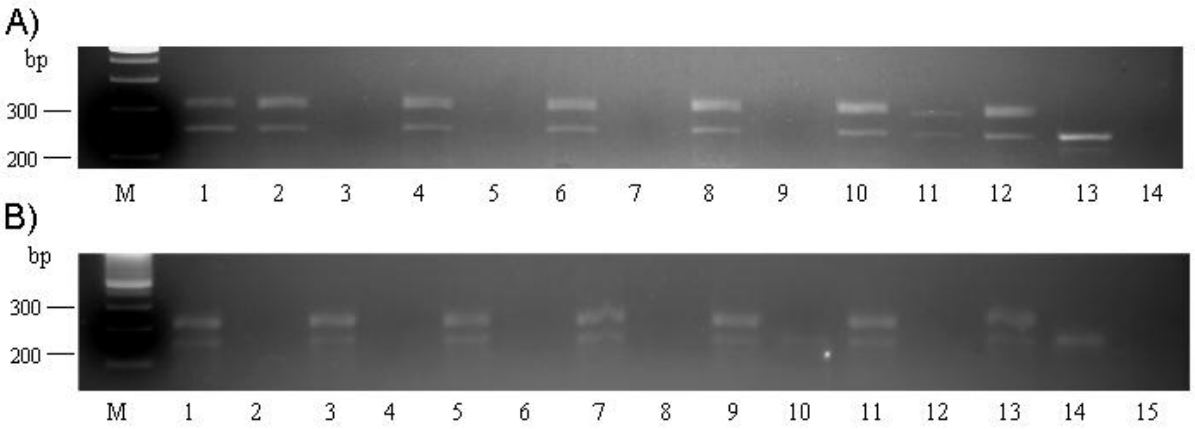

Fig 3S Agarose gel of PCR products in the A) first and B) second round of panning against celite. A) First acid eluate (lane 1); solid washed with acid buffer once (lane 2); second acid eluate (lane 3); solid washed twice with acid buffer (lane 4); third acid eluate (lane 5); solid washed three times with acid buffer (lane 6); fourth acid eluate (lane 7); solid washed four times with acid buffer (lane 8); fifth acid eluate (lane 9); solid washed five times with acid (lane 10); sixth acid eluate (lane 11); solid washed six times with acid (lane 12); wild type positive control (lane 13); negative control, PCR reaction without any phage added (lane 14); DNA marker (M). **B)** Solid washed with acid buffer once (lane 1); second acid eluate (lane 2); solid washed twice with acid buffer (lane 3); third acid eluate (lane 4); solid washed three times with acid buffer (lane 5); fourth acid eluate (lane 6); solid washed four times with acid buffer (lane 7); fifth acid eluate (lane 8); solid washed five times with acid (lane 9); sixth acid eluate (lane 10); solid washed six times with acid (lane 11); seventh acid eluate (lane 12); solid washed seven times with acid (lane 13);wild type positive control (lane 14); negative control, PCR reaction without any phage added (lane 15); DNA marker (M).

Table 1S Silver-binding peptides found using the TempliPhi method.

Sample	Sequence	Sample	Sequence
Ag-1	YFPWLPIALLK	**Ag-25**	GQMFTARPSPPG
Ag-2	LPQPAPIGGSDW	**Ag-26**	APERHIRLPPFG
Ag-3	QPLPHFILLSTS	**Ag-27**	LAVATHIRLPPL
Ag-4	TDELQPKLMLFS	**Ag-28**	LTRPNHGNTVDT
Ag-5	YHSMHPDVRKTP	**Ag-29**	MTKLGDLSLDLS
Ag-7	GSTGGQRLYMLD	**Ag-30**	SGTVESVSKIPS
Ag-9	SPSVLQPTVFAT	**Ag-31**	STIPGWPFMTAP
Ag-10	HLLASPNHALKP	**Ag-32**	APHWPLSPGNSR
Ag-11	APTHYDESPFIL	**Ag-33**	EGTVESFSKIPT
Ag-13	TSHSNWRHNKVL	**Ag-34**	MTKPETVTYKTT
Ag-15	ALANPTQSPSLI	**Ag-36**	SSSLYPKFRWGL
Ag-16	AYPRLDLRPSTV	**Ag-37**	TFKPVPSPRAWC
Ag-17	KSTVPLNFLATQ	**Ag-38**	HLQTEAPRRLTA
Ag-20	SIKFQTILPTHL	**Ag-39**	NTEGMFLKSIII
Ag-21	SHMPSASGFMRT	**Ag-40**	LHKHKCLISYTA
Ag-22	TVPPKAPRSSDL	**Ag-41**	SQSKSSYVESPL
Ag-23	NSDLSSPFLENR	**Ag-44**	AQNMNKTSVLMP

Table 2S Platinum-binding peptides found using the TempliPhi method.

Sample	Sequence	Sample	Sequence
Pt-1	FPRETNTPMTVN	**Pt-1.12**	VHPPTVSPTHYT
Pt-5	LRQSIQHFSDLT	**Pt-1.13**	ASELQYQLDPAT
Pt-7	QQQSLQHFSDLT	**Pt-1.14**	EHTNPILSHTHN
Pt-9	LPYSGELPWLRP	**Pt-1.15**	LHTNSIIQSGMR
Pt-30	HSGSYSLVPAFP	**Pt-1.16**	SAAHRWPHTNVP
Pt-32	HEKEYTNHPFVS	**Pt-1.17**	SVPPTSLAWLAH
Pt-35	LETYEMSKPLRM	**Pt-1.18**	FAHGKDSDNTPR
Pt-39	HEKEYTNHPFVS	**Pt-1.19**	TYTTPSVERGDQ
Pt-15	HPPWKHFAHYLP	**Pt-1.20**	AHRWIPDNLTPY
Pt-16	TQILPPASLSQL	**Pt-1.21**	WNGSPVMTRLLS
Pt-21	TFYDSLMGATHP	**Pt-1.22**	SILWISPSMARA
Pt-25	YPPRIQTLTGLT	**Pt-2.15**	HQVAATRDKVYS
Pt-29	QHLLGLPHPRPF	**Pt-2.4**	AQSFDQMNSAFP
Pt-41	SRLTHSNYATPT	**Pt-2.18**	NNSTMNAGSTMS
Pt-47	ATNHSLTRPVLP	**Pt-2.14**	MLTKPSVSQYKS
Pt-1.2	QSFSTNVLHTHH	**Pt-2.31**	LPAALRDSRSGS
Pt-14	EHTNPILSHTHN	**Pt-2.32**	TQGAHARPPSHV
Pt-1.1	WKSELPVQRARF	**Pt-2.51**	LSAHHTYSLPPL
Pt-2.2	NTESVKPLPPPT	**Pt-2.5**	TPPHLFATROPS
Pt-1.3	VAVLDTSLPLSA	**Pt-2.10**	KPFTAPATTPPI
Pt-1.4	FPANFLAPVTML	**Pt-2.71**	NMSAPTIWLPWA
Pt-1.5	MEKSSIMPVMRA	**Pt-2.81**	FPALKTSSSTLR
Pt-1.6	SHPWPPIFFPFP	**Pt-2.8**	MDKYPSSTLMMD
Pt-1.7	WQTHAPSSHHHL	**Pt-2.91**	VMTFPKRDQWGP
Pt-1.8	ALLIENPLSPFL	**Pt-2.41**	DVSPNETSLPSA
Pt-1.9	NYKSLATEWHQP	**Pt-2.31**	MDIKTPPSSYAPA
Pt-1.10	VGPGRPLPMWTT	**Pt-2.16**	SHQPHIRTSPPP
Pt-1.11	MDYTKGHPRQAR	**Pt-2.17**	YYGPESVARHSH

Table 3S Palladium-binding peptides found using the TempliPhi method.

Sample	Sequence
Pd-1	NQGLTHRPITLV
Pd-2	MQNPTQAPSVGL
Pd-3	DPAVYRPYYSKS
Pd-6	QDPTNSGFYGRP
Pd-8	ASTRLGVTLPSE
Pd-10	LKGANSGFYGXP
Pd-12	HKRDYGHHLFFG
Pd-18	LPLRTDPWAKAS
Pd-19	QDPTNSGFYGRR
Pd-21	HEKEYTNHPFVS

Table 4S Celite-binding peptides found using the TempliPhi method.

Sample	Sequence
Ce-5	QQQSIQHFSDLT
Ce-12	ADPAPDCFPLCT
Ce-18	FKHSVQPKNHYL
Ce-25	EAPQLYLVKSET
Ce-32	RRGLAMCLLWST
Ce-33	CVVSDEQGAMAE