Electronic Supplementary Material (ESI) for Analytical Methods.
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Supplementary Information

Separation and Detection of Mutans Streptococci by Magnetic Nanoparticles Stabilized with Enzyme-conjugated Polymer

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Piyaporn Na Nongkai, Suttawan Saipia,Voravee Hoven

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2 Sequence of CWBD

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6 **Fig. S2** XRD pattern of bare MNPs prepared by a solvothermal method.

7 **Fig. S3** FT-IR spectra of MNPs: (a) unmodified, (b) grafted with PAA and (c) conjugated with CWBD.

8 **Table S1** Average size and zeta potential of MNPs measured by DLS.

9 **Fig. S4** TGA (under N_2) curves of (a) unmodified MNPs, (b) PAA-grafted MNPs, and (c) CWBD-conjugated MNPs analyzed with a heating rate of 20°C /min.

10 Determination of Limit of Detection and Capture Efficiency.

11 **Fig. S5** Photographs of tested oral streptococci colonies on BHI agar plate before and after binding to CWBD-conjugated MNPs. The numbers represent the number of colony in CFU/mL.

CWBD sequence

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                                                                     80        90        100
                                                          ctc gag gat gag caa aat caa tcc tta agt gca t
                                                           XhoI  D   E   Q   N   Q   S   L   S   A   S

          110       120       130       140       150       160       170       180       190       200
ca gaa gtt att tct tct gat gcg aca tca gta tct gaa tta cca gcg aca aca gca cag ata agt cag gaa gtc aga aat aat gga caa gac agt act at
   E   V   I   S   S   D   A   T   S   V   S   E   L   P   A   T   T   A   Q   I   S   Q   E   V   R   N   N   G   Q   D   S   T   I

          210       220       230       240       250       260       270       280       290       300
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  Q   L   Q   Q   T   Q   E   Q   S   D   P   I   T   S   T   S   E   T   T   V   S   S   M   K   A   V   T   N   G   S   P   A   K

          310       320       330       340       350       360       370       380       390       400
gca aat gag act gaa aca gtt ccg tct cag gca agt act gct agt tct gtg cag act cct gat cag att tcg act gtt ccc tct gta aaa gca gaa acc a
A   N   E   T   E   T   V   P   S   Q   A   S   T   A   S   S   V   Q   T   P   D   Q   I   S   T   V   P   S   V   K   A   E   T   T

          410       420       430       440       450       460       470       480       490       500
ct tct acc gca gat caa tta caa tca aca tca tct gct cct ttg gat caa caa act gat gct aaa cgt ctt tcc aat aaa atg act cca gca agc agc gt
   S   T   A   D   Q   L   Q   S   T   S   S   A   P   L   D   Q   Q   T   D   A   K   R   L   S   N   K   M   T   P   A   S   S   V

          510       520       530       540       550       560       570       580       590       600
a caa gct cgt tct tct ctt aca caa gac aag caa gta cag gca cag gaa gtc aca agt gct gta gtg gaa gaa aaa ggg att aag cta cag tat aac ggt
  Q   A   R   S   S   L   T   Q   D   K   Q   V   Q   A   Q   E   V   T   S   A   V   V   E   E   K   G   I   K   L   Q   Y   N   G

          610       620       630       640       650       660       670       680       690       700
cag atc gct cga aat act aag att caa ttt gct gtc tgg tca gct cga aat gat caa gat gat ctt caa tgg tat acg gca aat aat atg gga gcg gcc t
Q   I   A   R   N   T   K   I   Q   F   A   V   W   S   A   R   N   D   Q   D   D   L   Q   W   Y   T   A   N   N   M   G   A   A   Y

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   A   E   F   K   N   H   R   E   Y   G   T   Y   Y   V   H   T   Y   A   N   Q   N   G   K   M   I   G   L   N   A   T   T   L   T

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  I   A   Q   P   Q   V   Q   T   N   I   Q   R   K   S   A   T   N   F   E   L   T   V   S   N   V   P   N   T   I   S   S   I   M

          910       920       930       940       950       960       970       980       990       1000
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V   P   V   W   S   D   Q   N   G   Q   D   D   I   K   W   Y   N   A   R   K   A   D   D   G   S   Y   K   A   L   I   D   T   K   N
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   H   K   N   D   L   G   H   Y   E   A   H   I   Y   G   Y   S   T   V   T   Q   S   Q   I   G   L   A   V   S   S   G   F   D   R

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  N   D   T   R   P   N   A   R   I   S   V   A   D   Y   D   Q   N   K   T   T   F   D   V   V   V   E   G   S   S   D   T   K   T

        1210        1220        1230        1240        1250        1260        1270        1280        1290        1300
gta tct gct gtt aat att gct gtt tgg tct gaa gat aaa ggt caa gat gac ctt aag tgg tat tca cca aaa att gtc aac aat aag gca act gtg acg a
V   S   A   V   N   I   A   V   W   S   E   D   K   G   Q   D   D   L   K   W   Y   S   P   K   I   V   N   N   K   A   T   V   T   I

        1310        1320        1330        1340        1350        1360        1370        1380        1390        1400
tt aat atc gct aat cat tca aat act tca gat aaa tat aat gtc cat gtt tat aca gac tac act gat ggg aca cat tct ggt act att tta ggg gct ta
   N   I   A   N   H   S   N   T   S   D   K   Y   N   V   H   V   Y   T   D   Y   T   D   G   T   H   S   G   T   I   L   G   A   Y

        1410        1420        1430        1440        1450        1460        1470        1480        1490        1500
t cag atc aat aaa ccg ctt gag aaa aat act gtt tca gct gat tta act agt gat ggc att gct ctc aaa tta gat tca aac acg gtt aca gat tat acc
  Q   I   N   K   P   L   E   K   N   T   V   S   A   D   L   T   S   D   G   I   A   L   K   L   D   S   N   T   V   T   D   Y   T

        1510        1520        1530        1540        1550        1560        1570        1580        1590        1600
aaa gta cga ttt gcc gtt tgg tcg gat caa aat ggt caa gat gat ctc aag tgg tat agt gca aat agt gat gga gcg gca act gca gct tac agt aac c
K   V   R   F   A   V   W   S   D   Q   N   G   Q   D   D   L   K   W   Y   S   A   N   S   D   G   A   A   T   A   A   Y   S   N   H

        1610        1620        1630        1640        1650        1660        1670        1680        1690        1700
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   S   G   Y   G   L   Y   H   I   H   T   Y   I   I   K   D   G   E   M   V   G   L   N   G   R   T   I   T   I   N   Q   P   S   A

        1710        1720        1730        1740        1750        1760        1770        1780        1790        1800
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  K   V   D   I   A   K   E   S   D   A   L   Y   K   V   T   V   S   N   L   P   A   Y   I   S   S   V   A   I   P   V   W   T   D

        1810        1820        1830        1840        1850        1860        1870        1880        1890        1900
aaa aac aat caa gat gat att caa tgg att ctc gcg aca aaa caa ggt gat gga acc tac gca gcg caa att cag tta gct gat cat aat ggg gaa aca g
K   N   N   Q   D   D   I   Q   W   I   L   A   T   K   Q   G   D   G   T   Y   A   A   Q   I   Q   L   A   D   H   N   G   E   T   G

        1910        1920        1930        1940        1950        1960        1970        1980        1990        2000
gc cat tat aat gtt cat gtc tat gga caa agt aaa ttt gac aat aaa acg gtt ggc tta gca gca act gat ggc ttt aat gtt gca gag aca agg aat gc
   H   Y   N   V   H   V   Y   G   Q   S   K   F   D   N   K   T   V   G   L   A   A   T   D   G   F   N   V   A   E   T   R   N   A
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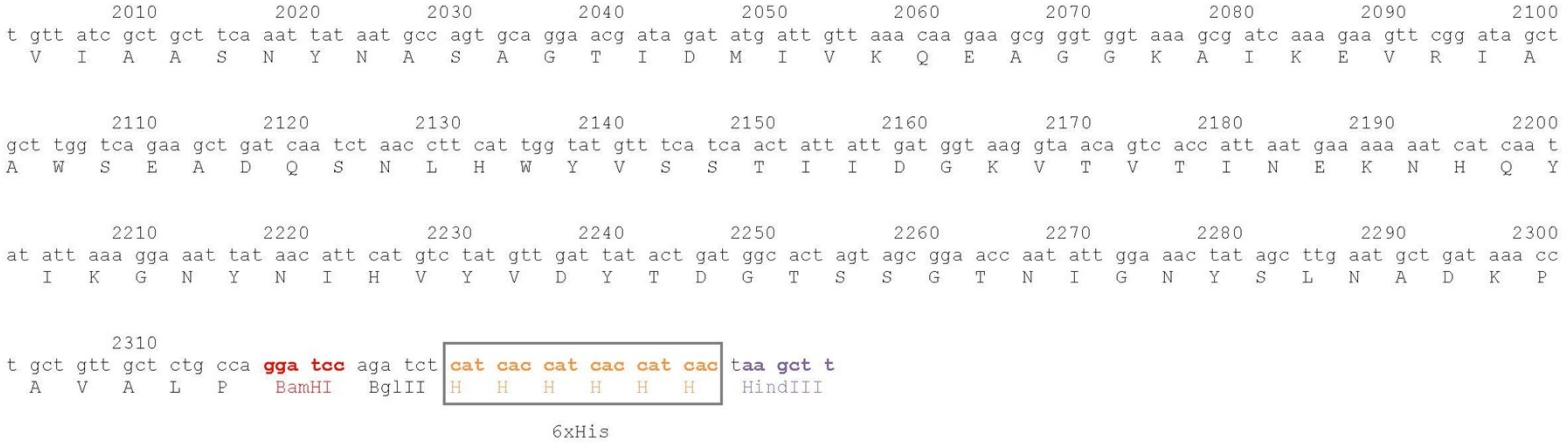
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V I A A S N Y N A S A G T I D M I V K Q E A G G K A I K E V R I A
2110 2120 2130 2140 2150 2160 2170 2180 2190 2200
gct tgg tca gaa gct gat caa tct aac ctt cat tgg tat gtt tca tca act att att gat ggt aag gta aca gtc acc att aat gaa aaa aat cat caa t
A W S E A D Q S N L H W Y V S S T I I D G K V T V T I N E K N H Q Y
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I K G N Y N I H V Y V D Y T D G T S S G T N I G N Y S L N A D K P
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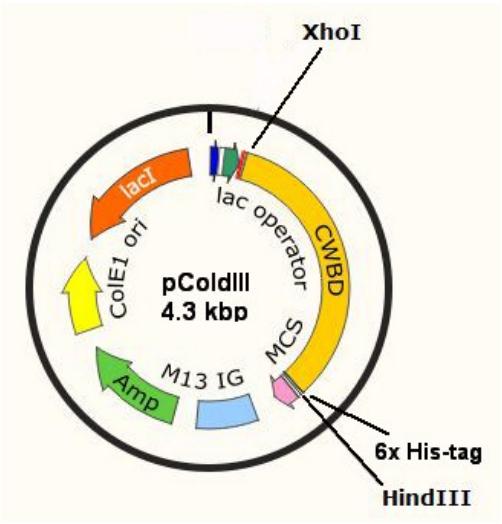
6xHis



All 4 lanes in this SDS-PAGE are CWBD

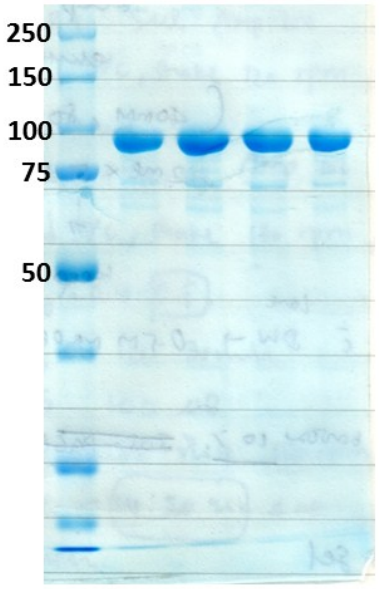


Fig. S1 Schematic plasmid map including CWBD (top) and the expressed CWBD protein on SDS-PAGE (bottom).

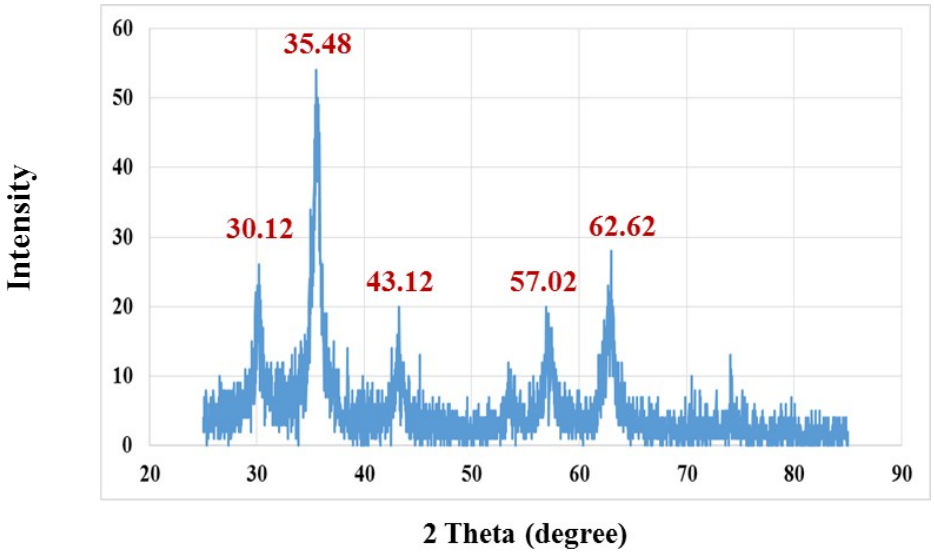


Fig. S2 XRD pattern of bare MNPs prepared by a solvothermal method.

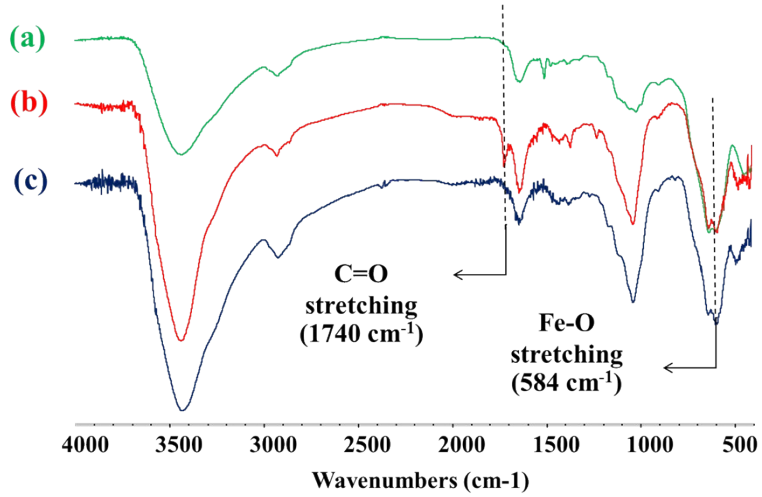


Fig. S3 FT-IR spectra of MNPs: (a) unmodified, (b) grafted with PAA and (c) conjugated with CWBD.

Table S1 Average size and zeta potential of MNPs measured by DLS.

Sample	Hydrodynamic size (nm)	PDI	Zeta potential (mV)
Bare MNPs	318 ± 18	0.30	-18.00
PAA-grafted MNPs	460 ± 21	0.37	-31.43
CWBD-conjugated MNPs	627 ± 20	0.31	-14.40

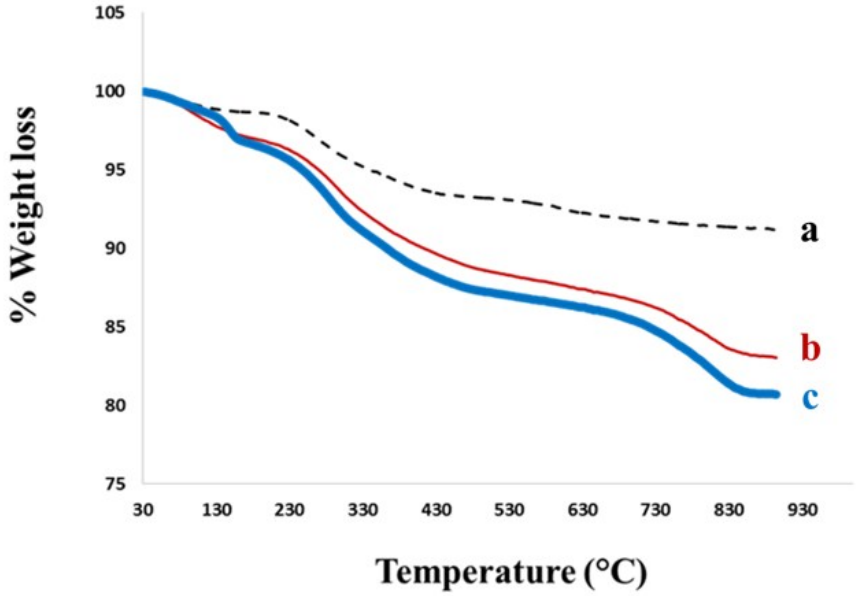


Fig. S4 TGA (under N_2) curves of (a) unmodified MNPs, (b) PAA-grafted MNPs, and (c) CWBD-conjugated MNPs analyzed with a heating rate of 20°C /min.

Determination of Limit of Detection and Capture Efficiency

I_{LOD} represents the intensity at LOD which is calculated from the equation below whereas I_0 reflects the intensity of the background and SD_0 is standard deviation of the background.

$$I_{LOD} = 3SD_0 + I_0$$

(eq.S1)

After that, LOD was calculated from I_{LOD} using linear equation of the calibration curve as shown below, where m and c are slope and intercept of the calibration curve, respectively.

$$I = mlog(CFU) + c$$

(eq.S2)

By substitute I_{LOD} and LOD into the equation, it can then be rearranged as shown below:

$$LOD = 10^{(I_{LOD} - c)/m}$$

(eq.S3)

C_{before} represents the number of colonies formed on the agar plate by diluted bacteria stock in PBS whereas C_{after} reflects the number of the unbound bacteria after contacting with the CWBD-conjugated MNPs

$$\text{Capture efficiency } (\%) = \{(C_{before} - C_{after})/ C_{before}\} \text{ x } 100$$

(eq.S4)

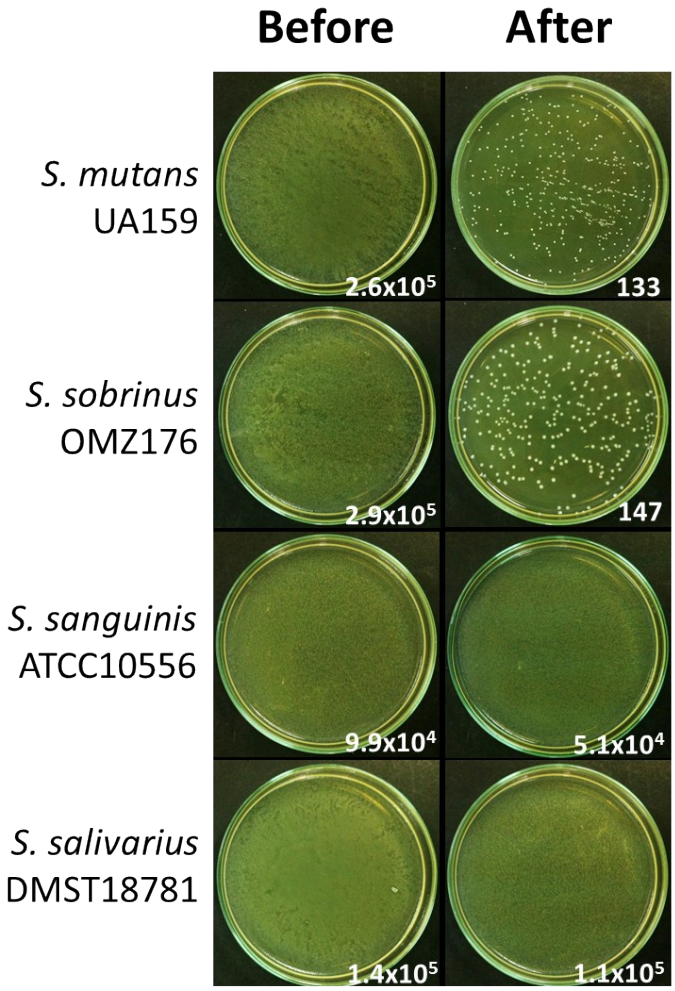


Fig. S5 Photographs of tested oral streptococci colonies on BHI agar plate before and after binding to CWBD-conjugated MNPs. The numbers represent the number of colony in CFU/mL.