

## SUPPORTING INFORMATION

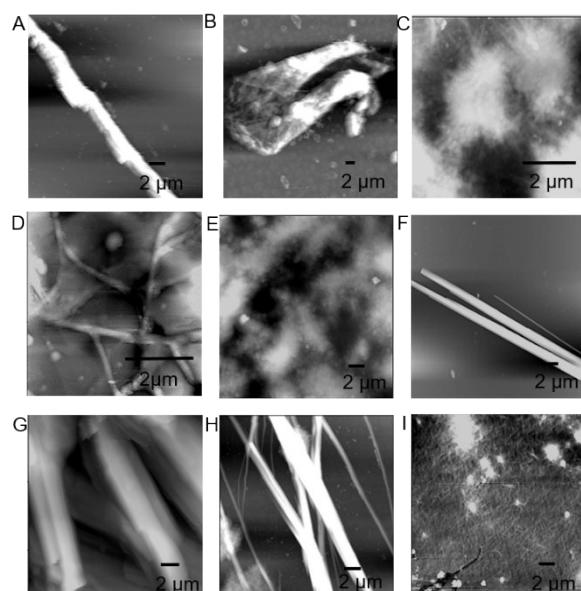
# Genetically Encoded Conductive Protein Nanofibers Secretion by Engineered Cells

Ebuzer Kalyoncu<sup>1</sup>, Recep Erdem Ahan<sup>1</sup>, Tolga Tarkan Ölmez<sup>1</sup>, Urartu Özgür Şafak Şeker<sup>1</sup>

<sup>1</sup>UNAM–National Nanotechnology Research Center and Institute of Materials Science and Nanotechnology, Bilkent University, 06800 Bilkent and Ankara, Turkey.

## Materials and Methods

### 1. Atomic Force Microscopy Images of the Peptides



**Figure S1.** Atomic force microscopy (AFM) images of the designed peptides mica respectively: Amyloid like fiber (ALF) (A), ALF3W (B), ALF3Y (C), ALF3H (D), ALF3F (E), R5T (F), R5T3W (G), R5T3Y (H), and R5T3H (I).

### 2. Cloning of the CsgA Conductive Peptides

csgAW, csgAY, csgAWY and csgAYW gene fragments listed in Supplementary Table 1 were amplified from pZa-tetO-csgA-CmR (primers were listed in Supplementary Table 2). Recombinant genes were cloned into pZa-

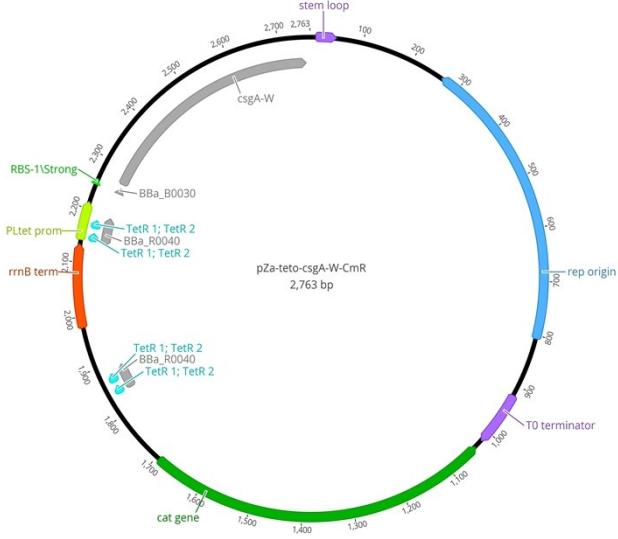
tetO-CmR through the cut ligate method using kpnI/mluI restriction sites and the plasmid constructs are represented in Figure S2, S4, S6, and S8 and sequence alignments of the constructs are shown in Figure S3, S5, S7, and S9.

**Table 1**- Gene sequences of the PCR Products

| Gene Fragment | Sequence   |
|---------------|--|
| <b>CsgAW</b>  | GGTACCATGAAACTTTAAAAGTAGCAGCAATTGCAGCAATCGTATTCTCCGGTAGCGCTCTGGCAGGTGTTCTCTCA<br>GTACGGCCGCCGGTAACCACGGTGGTGGCGGTAAATAAGCGGCCAAATTCTGAGCTGAACATTACCGATACGG<br>TGGCGGTAACTCTGCACTTGTCTGCAAACACTGATGCCGTAACTCTGACTTGAATTACCCAGCATGGCGGCCGTAATG<br>GTGCAGATGTTGGTCAGGGCTCAGATGACAGCTCATCGATGCCAACGTGGCTCGGTAAACAGCGCTACTCTTGAT<br>CAGTGGAACGGAAAAATTCTGAAATGACGGTAAACAGTCGGTGGCAACGGTGTGCTGAGTTGACCAGACTGCA<br>TCTAACTCTCCGTCAACGTGACTCAGGTTGGCTTGGTAAACAACCGGACCGCTCATCGTACTACTATCGCAAATACAAA<br>AGATGGGATGACTGGTAAACCGCGT |
| <b>CsgAY</b>  | GGTACCATGAAACTTTAAAAGTAGCAGCAATTGCAGCAATCGTATTCTCCGGTAGCGCTCTGGCAGGTGTTCTCTCA<br>GTACGGCCGCCGGTAACCACGGTGGTGGCGGTAAATAAGCGGCCAAATTCTGAGCTGAACATTACCGATACGG<br>TGGCGGTAACTCTGCACTTGTCTGCAAACACTGATGCCGTAACTCTGACTTGAATTACCCAGCATGGCGGCCGTAATG<br>GTGCAGATGTTGGTCAGGGCTCAGATGACAGCTCATCGATGCCAACGTGGCTCGGTAAACAGCGCTACTCTTGAT<br>CAGTGGAACGGAAAAATTCTGAAATGACGGTAAACAGTCGGTGGCAACGGTGTGCTGAGTTGACCAGACTGCA<br>TCTAACTCTCCGTCAACGTGACTCAGGTTGGCTTGGTAAACAACCGGACCGCTCATCGTACTACTATCGCAAATACAAA<br>GAGTATGATGACTAAACCGCGT    |
| <b>CsgAWY</b> | GGTACCATGAAACTTTAAAAGTAGCAGCAATTGCAGCAATCGTATTCTCCGGTAGCGCTCTGGCAGGTGTTCTCTCA<br>GTACGGCCGCCGGTAACCACGGTGGTGGCGGTAAATAAGCGGCCAAATTCTGAGCTGAACATTACCGATACGG<br>TGGCGGTAACTCTGCACTTGTCTGCAAACACTGATGCCGTAACTCTGACTTGAATTACCCAGCATGGCGGCCGTAATG<br>GTGCAGATGTTGGTCAGGGCTCAGATGACAGCTCATCGATGCCAACGTGGCTCGGTAAACAGCGCTACTCTTGAT<br>CAGTGGAACGGAAAAATTCTGAAATGACGGTAAACAGTCGGTGGCAACGGTGTGCTGAGTTGACCAGACTGCA<br>TCTAACTCTCCGTCAACGTGACTCAGGTTGGCTTGGTAAACAACCGGACCGCTCATCGTACTACTATCGCAAATACAAA<br>GAGTACGATGATTGGTAAACCGCGT |
| <b>CsgAYW</b> | GGTACCATGAAACTTTAAAAGTAGCAGCAATTGCAGCAATCGTATTCTCCGGTAGCGCTCTGGCAGGTGTTCTCTCA<br>GTACGGCCGCCGGTAACCACGGTGGTGGCGGTAAATAAGCGGCCAAATTCTGAGCTGAACATTACCGATACGG<br>TGGCGGTAACTCTGCACTTGTCTGCAAACACTGATGCCGTAACTCTGACTTGAATTACCCAGCATGGCGGCCGTAATG<br>GTGCAGATGTTGGTCAGGGCTCAGATGACAGCTCATCGATGCCAACGTGGCTCGGTAAACAGCGCTACTCTTGAT<br>CAGTGGAACGGAAAAATTCTGAAATGACGGTAAACAGTCGGTGGCAACGGTGTGCTGAGTTGACCAGACTGCA<br>TCTAACTCTCCGTCAACGTGACTCAGGTTGGCTTGGTAAACAACCGGACCGCTCATCGTACTACTATCGCAAATACAAA<br>GAGTGGGATGATTAAACCGCGT    |

**Table 2**- Primers that were used in this study

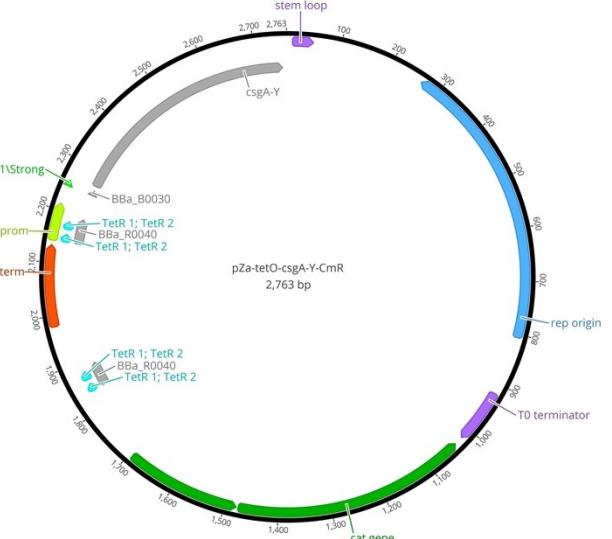
|               |  |  |
|---------------|--|--|
| EK F-W        | GGTACCATGAAACTTTAAAAGTAGCAGCAATTGC                         | Forward primer for all the PCR reactions   |
| EK R-csgA-W1  | TTCTTCCATTACGCCACAGTACTGATGAGCGGTCGC<br>GTTGTTA            | First PCR reaction to amplify csgA gene fragment from pZa-TetO-csgA-CmR and to add conductive domain sequences to 3 prime of gene fragment |
| EK R-csgA-W2  | ACCGCGTTACCACTCATCCCATTCTTCATTACGCCAC<br>CAGTACTGATG       | Second PCR reaction to elongate conductive domain sequences to 3 prime of csgA gene fragment   |
| EK R-csgA-Y1  | CTCTTGTATTGCGATAGTAGTACTGATGAGCGGTCGC<br>GTTGTTA           | First PCR reaction to amplify csgA gene fragment from pZa-TetO-csgA-CmR and to add conductive domain sequences to 3 prime of gene fragment |
| EK R-csgA-Y2  | ACCGCGTTAGTAGTCATCATCTTGTATTGCGATA<br>GTAGTACTGATGAGCGG    | Second PCR reaction to elongate conductive domain sequences to 3 prime of csgA gene fragment   |
| EK R-csgA-WY1 | CTCTTGTATTGCGATAGTAGTACTGATGAGCGGTCGC<br>GTTGTTA           | First PCR reaction to amplify csgA gene fragment from pZa-TetO-csgA-CmR and to add conductive domain sequences to 3 prime of gene fragment |
| EK R-csgA-WY2 | ACCGCGTTATAATCATCCACTCTTGTATTGCGATAG<br>TAGTACTGATGAGCGG   | Second PCR reaction to elongate conductive domain sequences to 3 prime of csgA gene fragment   |
| EK R-csgA-YW1 | CTCTTGTATTGCGATAGTAGTACTGATGAGCGGTCGC<br>GTTGTTA           | First PCR reaction to amplify csgA gene fragment from pZa-TetO-csgA-CmR and to add conductive domain sequences to 3 prime of gene fragment |
| EK R-csgA-YW2 | ACCGCGTTACCAATCATCGTACTCTTGTATTGCGATA<br>GTAGTACTGATGAGCGG | Second PCR reaction to elongate conductive domain sequences to 3 prime of csgA gene fragment   |



**Figure S2** Schematic representation of the expression vector containing the *csgAW* gene fragment

CTGGGCTTCTCGAGTCCTATCAGTGAAGAGATTGACATCCCTATCAGTGAAGAGAT  
CTGGGCTTCTCGAGTCCTATCAGTGAAGAGATTGACATCCCTATCAGTGAAGAGAT  
  
ACTGAGCACATCAGCAGGACGCCTGAGCGAAATTCTACCATTCACCTCTGGATTGGGT  
ACTGAGCACATCAGCAGGACGCCTGAGCGAAATTCTACCATTCACCTCTGGATTGGGT  
  
ATTAAGAGGGAGAAAGGTACCAGAACATTAAAAGTAGCAGCAATTGAGCAATCGTA  
ATTAAGAGGGAGAAAGGTACCAGAACATTAAAAGTAGCAGCAATTGAGCAATCGTA  
  
TTCTCCGGTAGCGCTCTGGCAGGTGTTCTCTCAGTAGCGCGCGCGCGTAACCCAGGT  
TTCTCCGGTAGCGCTCTGGCAGGTGTTCTCTCAGTAGCGCGCGCGCGTAACCCAGGT  
  
GGTGGCGGTAATAATAGCGGCCAAATTCTGAGCTGAACATTACAGTAGCGGTGGCGGT  
GGTGGCGGTAATAATAGCGGCCAAATTCTGAGCTGAACATTACAGTAGCGGTGGCGGT  
  
AACTCTGCACTTGTCTCGAAACTGATGCCGTAACCTGACTTGACTTACCCAGCAT  
AACTCTGCACTTGTCTCGAAACTGATGCCGTAACCTGACTTGACTTACCCAGCAT  
  
GGCGCGCGTAATGGTCAGATGGTGTGAGCTGAGCTGAGCTCAATCGATCTGAC  
GGCGCGCGTAATGGTCAGATGGTGTGAGCTGAGCTGAGCTCAATCGATCTGAC  
  
CAACGTTGGCTCGTAACAGCGCTACTTGTAGTGGAAACGGCAAAATTCTGAATG  
CAACGTTGGCTCGTAACAGCGCTACTTGTAGTGGAAACGGCAAAATTCTGAATG  
  
ACGGTTAACAGTTGGGGCAACGGTGTGAGCTGAGCTGACCATGCTAACCTC  
ACGGTTAACAGTTGGGGCAACGGTGTGAGCTGAGCTGACCATGCTAACCTC  
  
TCCGTCAACGTGACTCAGGTTGGCTTGGTAACAACCGCGACCGCTCATCAGTACTGGTGG  
TCCGTCAACGTGACTCAGGTTGGCTTGGTAACAACCGCGACCGCTCATCAGTACTGGTGG  
  
CGTAAATGGAAAAGAATGGGATGACTGGTAAACCGCGT 2763  
CGTAAATGGAAAAGAATGGGATGACTGGTAAACCGCGT 650

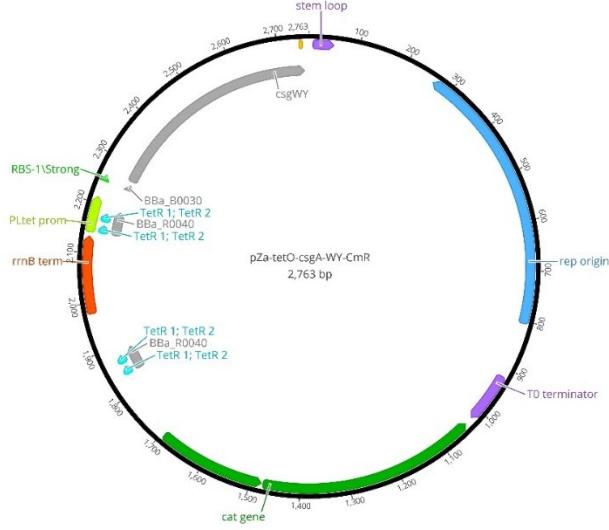
**Figure S3** Sequence analysis of *csgAW* gene fragment. Upper DNA sequence is the reference and lower one is the cloned DNA sequence.



**Figure S4** Schematic representation of the expression vector containing *csgAY* gene fragment.

CTGGGCCCTTCGAGTCCCTATCAGTGATAGAGATTGACATCCCTATCAGTGATAGAGAT  
CTGGGCCCTTCGAGTCCCTATCAGTGATAGAGATTGACATCCCTATCAGTGATAGAGAT  
ACTGAGCACATCAGCAGGACGCAGTGA  
ACTGAGCACATCAGCAGGACGCAGTGA  
ATTTAAAGAGGAGAAGGTACCATGAAACTTTAAAAGTAGCAGCAATTGCG  
ATTTAAAGAGGAGAAGGTACCATGAAACTTTAAAAGTAGCAGCAATTGCG  
TTCTCCGGTAAGCGCTTGGCAGGTGTTGTTCTCAGTACGGCGCGCGTAA  
TTCTCCGGTAAGCGCTTGGCAGGTGTTGTTCTCAGTACGGCGCGCGTAA  
GGTGGCGGTAATAATAGCGGCCAAATTCTGAGCTGA  
GGTGGCGGTAATAATAGCGGCCAAATTCTGAGCTGA  
AACTCTGCACTTGCTCTGCAA  
AACTCTGCACTTGCTCTGCAA  
GGCGGCGGTAATGGTGCA  
GGCGGCGGTAATGGTGCA  
CAACGTGGCTTGGTA  
CAACGTGGCTTGGTA  
ACGGTTAACAGTTGGTGG  
ACGGTTAACAGTTGGTGG  
TCCGTC  
TCCGTC  
CGCAAATACAAAGAGTATG  
CGCAAATACAAAGAGTATG  
2763  
651

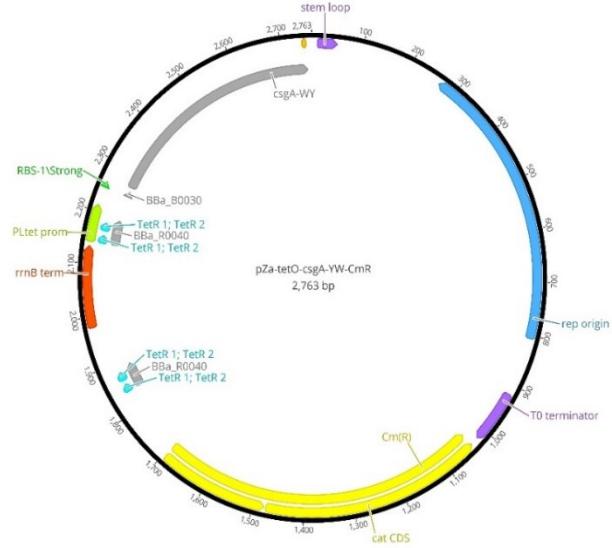
**Figure S5** Sequence analysis of *csgAY* gene fragment. Upper DNA sequence is the reference and lower one is the cloned DNA sequence.



**Figure S6** Schematic representation of the expression vector containing the *csgAWY* gene fragment.

AGGGCTTCTGAGTCCTATCAGTGTAGAGATTGACATCCCTACAGTGTAGAGATA  
TGAGCTTCTGAGTCCTATCAGTGTAGAGATTGACATCCCTACAGTGTAGAGATA  
CTGAGCACATCAGCAGGACGCACTGACCGAATTCTACCATTCACCTTGGATTTGGTA  
CTGAGCACATCAGCAGGACGCACTGACCGAATTCTACCATTCACCTTGGATTTGGTA  
TTAAAGAGGAGAAAAGGTACCATGAAACTTTAAAGTAGCAGCAATTGACGAATCGTAT  
TTAAAGAGGAGAAAAGGTACCATGAAACTTTAAAGTAGCAGCAATTGACGAATCGTAT  
TCTCGGTAGCGCTCTGGCAGGTGTTCTCAGTACGGCGGCCGGTAACCACGGT  
TCTCGGTAGCGCTCTGGCAGGTGTTCTCAGTACGGCGGCCGGTAACCACGGT  
GTGGCGTAATAATAGCGGCCAAATTCTGAGCTGAAACATTACAGTACGGTGGCGTA  
GTGGCGTAATAATAGCGGCCAAATTCTGAGCTGAAACATTACAGTACGGTGGCGTA  
ACTCTGCACTTGCTCTGCAAACGTAGCCGCTAACCTGACTTACCCAGCATG  
ACTCTGCACTTGCTCTGCAAACGTAGCCGCTAACCTGACTTACCCAGCATG  
GGCGCGTAATGGTGCAAGATGTTGGTCAGGGCTCAGATGACAGCTAACGCTGACCC  
GGCGCGTAATGGTGCAAGATGTTGGTCAGGGCTCAGATGACAGCTAACGCTGACCC  
AACGTGGCTCGGTAAACAGCGCTACTCTGATCAGTGGAACGGCAAAATTCTGAAATGA  
AACGTGGCTCGGTAAACAGCGCTACTCTGATCAGTGGAACGGCAAAATTCTGAAATGA  
CGGTTAACACGTTGGTGGCAACGGTGTGAGTTGACCAAGACTGCATCTAACCT  
CGGTTAACACGTTGGTGGCAACGGTGTGAGTTGACCAAGACTGCATCTAACCT  
CCGTCAACGTGACTCAGGTTGGCTTGGTAACACGGGACCGCTCATCAGTACTACTATC  
CCGTCAACGTGACTCAGGTTGGCTTGGTAACACGGGACCGCTCATCAGTACTACTATC  
GCAAAATACAAGAGTACGATGATTGGTAAACCGCGT 2763  
GCAAAATACAAGAGTACGATGATTGGTAAACCGCGT 653

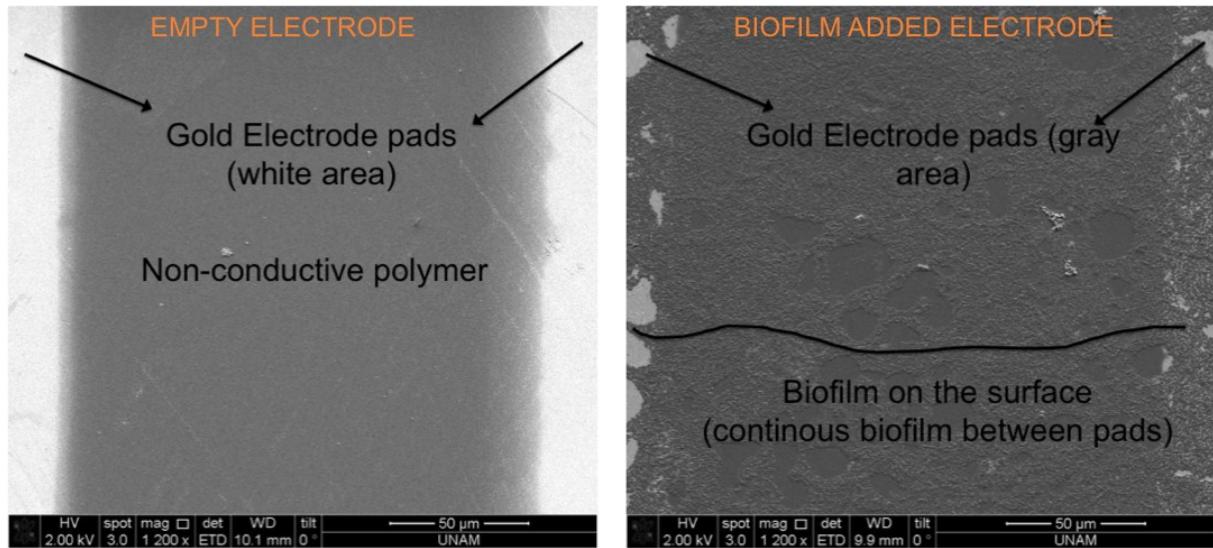
**Figure S7** Sequence analysis of *csgAWY* gene fragment. Upper DNA sequence is the reference and lower one is the cloned DNA sequence.



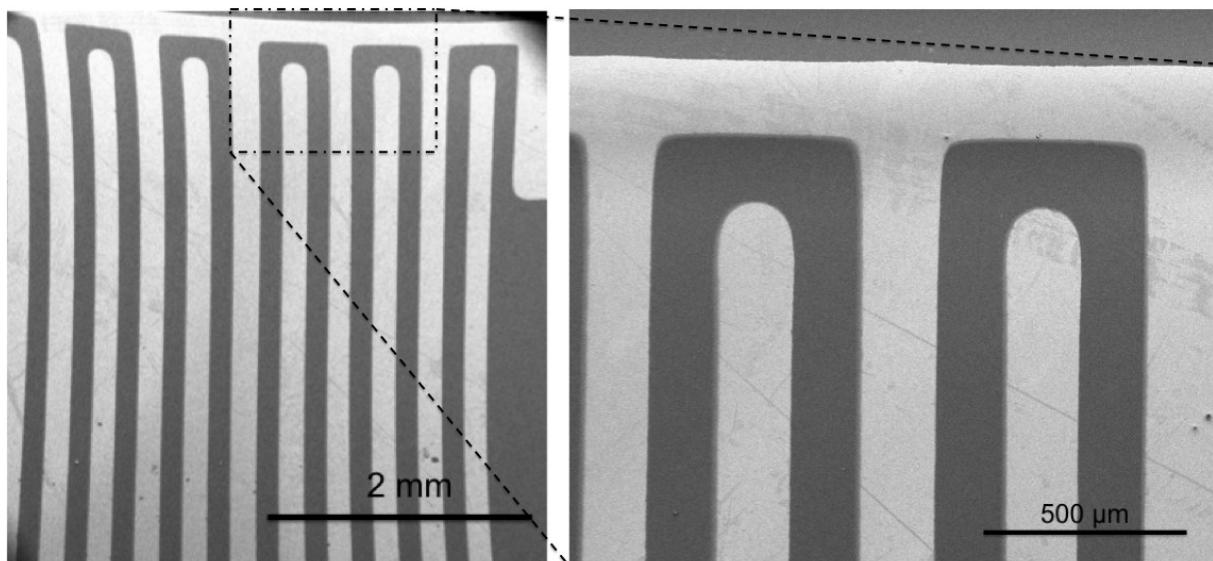
**Figure S8** Schematic representation of the expression vector containing the *csgAYW* gene fragment.

AGGGCTTCTGAGTCCTATCAGTGTAGAGATTGACATCCCTACAGTGTAGAGATA  
ACTGGGCTTCTGAGTCCTATCAGTGTAGAGATTGACATCCCTACAGTGTAGAGATA  
TACTGAGCACATCAGCAGGACGCACTGACCGAATTCTACCATTCACCTTGGATTTGG  
TACTGAGCACATCAGCAGGACGCACTGACCGAATTCTACCATTCACCTTGGATTTGG  
TATTAAGAGGAGAAAAGGTACCATGAAACTTTAAAGTAGCAGCAATTGACGAATCGT  
TATTAAGAGGAGAAAAGGTACCATGAAACTTTAAAGTAGCAGCAATTGACGAATCGT  
ATTCCTCGGTAGCGCTCTGGCAGGTGTTCTCAGTACGGCGGCCGGTAACCACGG  
ATTCCTCGGTAGCGCTCTGGCAGGTGTTCTCAGTACGGCGGCCGGTAACCACGG  
TGGTGGCGTAATAATAGCGGCCAAATTCTGAGCTGAAACATTACAGTACGGTGGCG  
TGGTGGCGTAATAATAGCGGCCAAATTCTGAGCTGAAACATTACAGTACGGTGGCG  
TAACTCTGCACTTGCTCTGCAAACGTAGCCGCTAACCTGACTTACCCAGCATG  
TAACTCTGCACTTGCTCTGCAAACGTAGCCGCTAACCTGACTTACCCAGCATG  
TGGCGCGGTAAATGGTGCAAGATGTTGGTCAGGGCTCAGATGACAGCTAACGCTGAC  
TGGCGCGGTAAATGGTGCAAGATGTTGGTCAGGGCTCAGATGACAGCTAACGCTGAC  
CCAACGTGGCTCGGTAAACAGCGCTACTCTGATCAGTGGAACGGCAAAATTCTGAAAT  
CCAACGTGGCTCGGTAAACAGCGCTACTCTGATCAGTGGAACGGCAAAATTCTGAAAT  
GACGGTTAACACGTTGGTGGCAACGGTGTGAGTTGACCAAGACTGCATCTAACTC  
GACGGTTAACACGTTGGTGGCAACGGTGTGAGTTGACCAAGACTGCATCTAACTC  
CTCGTCAACGTGACTCAGGTTGGCTTGGTAACACGGGACCGCTCATCAGTACTACTA  
CTCGTCAACGTGACTCAGGTTGGCTTGGTAACACGGGACCGCTCATCAGTACTACTA  
TCGCAAAATACAAGAGTGGGATGATTAAACCGCGT 2763  
TCGCAAAATACAAGAGTGGGATGATTAAACCGCGT 650

**Figure S9** Sequence analysis of *csgAYW* gene fragment. Upper DNA sequence is the reference and lower one is the cloned DNA sequence.



**Figure S10.** Interdigitated gold electrodes before and after the addition of the biofilms on surface for conductivity measurements.



**Figure S11.** Empty interdigitated electrode and its detail showing the successful deposition of the gold through the mask used.

### Conductivity measurement of empty interdigitated electrode

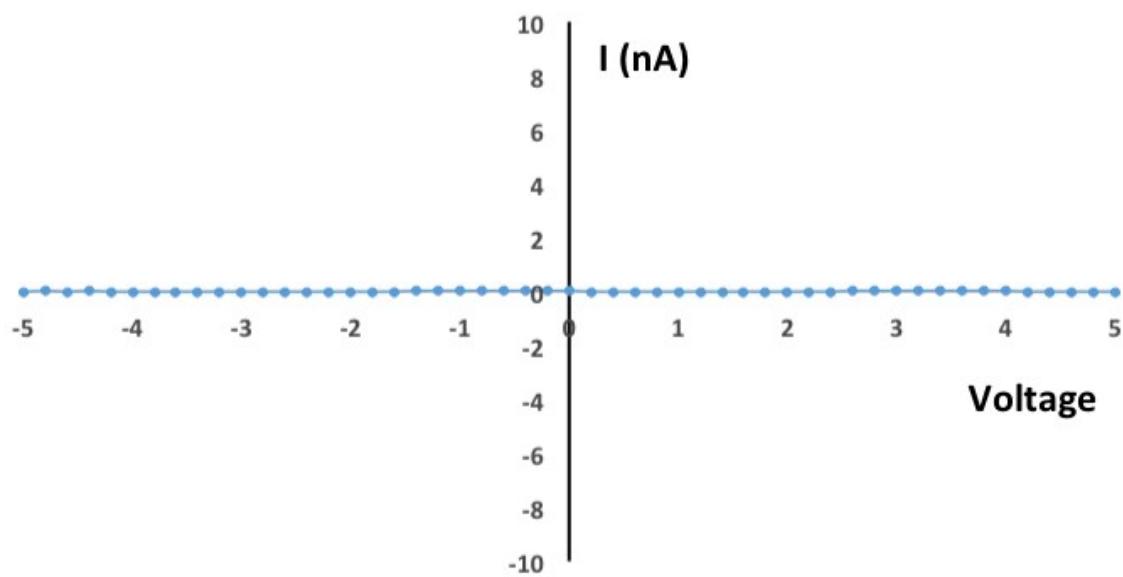


Figure S12. Conductivity measurement of the empty electrode