Engineered solubility tag for solution NMR of proteins

Amy M. Ruschak, Justine D. Rose, Michael P. Coughlin, Tomasz L. Religa

Supporting Information

Molecular biology. The gene containing mutated protein A was custom synthesized by Genscript using the following E.coli codon optimized DNA sequence:

```
          |---  6 x His ---|         |-- TEV Cleavage  --||-- PA Start
       ---------|---------|---------|---------|---------|---------|---------|
     1 atgcatcaccatcaccatcacgaggcctctgagaatctttattttcaggcctctgggttt 60
     1 M  H  H  H  H  H  H  E  A  S  E  N  L  Y  F  Q  A  S  G  F   20
Restriction sites:           -StuI-                  -StuI-

       ---------|---------|---------|---------|---------|---------|---------|
    61 aacgaagaacagcaaaatgcctatgacgagatcgcccgcctgcctaatctgaatgaggag 120
    21 N  E  E  Q  Q  N  A  Y  D  E  I  A  R  L  P  N  L  N  E  E   40


       ---------|---------|---------|---------|---------|---------|---------|
   121 cagcgtaatgcctttatccaagccctgcgtgatgatccttctcaagcggctgatctgctg 180
    41 Q  R  N  A  F  I  Q  A  L  R  D  D  P  S  Q  A  A  D  L  L   60

                                     PA End  --|
       ---------|---------|---------|---------|--
   181 gcagaagcacaagccctgaacgatgcccaagcacctggttaa 222
    61 A  E  A  Q  A  L  N  D  A  Q  A  P  G  *   73
```

Subsequently, it was subcloned into pET-29a vector (Novagen) between the NdeI and BamHI restriction sites. The gene for ASC CARD was synthesized by Genscript and sub-cloned into the pETM-11 vector between the NcoI and BamHI restriction sites. The D134C C173S mutations were introduced using the QuikChange (Agilent) protocol.