Supporting information

CONFORMATIONAL DYNAMICS OF TITIN PEVK EXPLORED WITH FRET SPECTROSCOPY

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The secondary structure predictions obtained on various servers are listed below.

GOR IV SECONDARY STRUCTURE PREDICTION METHOD
http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_gor4.html

```
        10
         |
WEEAYQEREVC
ccccccceeec
Sequence length :    11
GOR4 :
   Alpha helix     (Hh) :     0 is   0.00%
   3(10) helix     (Gg) :     0 is   0.00%
   Pi helix        (Ii) :     0 is   0.00%
   Beta bridge     (Bb) :     0 is   0.00%
   Extended strand (Ee) :     3 is  27.27%
   Beta turn       (Tt) :     0 is   0.00%
   Bend region     (Ss) :     0 is   0.00%
   Random coil     (Cc) :     8 is  72.73%
   Ambigous states (?)  :     0 is   0.00%
   Other states         :     0 is   0.00%


        10        20
         |         |
WEEAYQEREVIQVQKEVYEEC
ccccccchhhhhhheeeeeec
Sequence length :    21
GOR4 :
   Alpha helix     (Hh) :     7 is  33.33%
   3(10) helix     (Gg) :     0 is   0.00%
   Pi helix        (Ii) :     0 is   0.00%
   Beta bridge     (Bb) :     0 is   0.00%
   Extended strand (Ee) :     6 is  28.57%
   Beta turn       (Tt) :     0 is   0.00%
   Bend region     (Ss) :     0 is   0.00%
   Random coil     (Cc) :     8 is  38.10%
   Ambigous states (?)  :     0 is   0.00%
   Other states         :     0 is   0.00%
```

NetSurfP - Protein Surface Accessibility and Secondary Structure Predictions
http://www.cbs.dtu.dk/services/NetSurfP/

```
# Column 1: Class assignment - B for buried or E for Exposed - Threshold: 25% exposure, but
not based on RSA
# Column 2: Amino acid
# Column 3: Sequence name
# Column 4: Amino acid number
# Column 5: Relative Surface Accessibility - RSA
# Column 6: Absolute Surface Accessibility
# Column 7: Z-fit score for RSA prediction
# Column 8: Probability for Alpha-Helix
# Column 9: Probability for Beta-strand
# Column 10: Probability for Coil
E W  Sequence               1    0.711 171.044  -1.467   0.016   0.005   0.979
E E  Sequence               2    0.693 121.102   0.301   0.430   0.016   0.555
E E  Sequence               3    0.669 116.874   0.500   0.522   0.016   0.462
E A  Sequence               4    0.402  44.311  -0.160   0.622   0.015   0.363
E Y  Sequence               5    0.292  62.443   0.077   0.622   0.015   0.363
E Q  Sequence               6    0.562 100.427   1.222   0.561   0.047   0.393
E E  Sequence               7    0.664 116.018   0.837   0.605   0.105   0.290
E R  Sequence               8    0.455 104.264   0.339   0.386   0.097   0.517
E E  Sequence               9    0.673 117.486   0.036   0.268   0.043   0.689
E V  Sequence              10    0.456  70.118   0.421   0.184   0.043   0.773
E C  Sequence              11    0.750 105.356  -1.584   0.003   0.003   0.994


# Column 1: Class assignment - B for buried or E for Exposed - Threshold: 25% exposure, but
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# Column 5: Relative Surface Accessibility - RSA
# Column 6: Absolute Surface Accessibility
# Column 7: Z-fit score for RSA prediction
# Column 8: Probability for Alpha-Helix
# Column 9: Probability for Beta-strand
# Column 10: Probability for Coil
E W  Sequence               1    0.725 174.411  -1.373   0.016   0.005   0.979
E E  Sequence               2    0.701 122.412   0.322   0.339   0.016   0.645
E E  Sequence               3    0.677 118.289   0.456   0.522   0.016   0.462
E A  Sequence               4    0.433  47.661  -0.160   0.522   0.016   0.462
E Y  Sequence               5    0.314  67.016  -0.148   0.622   0.015   0.363
E Q  Sequence               6    0.562 100.445   0.833   0.660   0.049   0.291
E E  Sequence               7    0.593 103.667   0.172   0.660   0.049   0.291
E R  Sequence               8    0.397  90.913   0.590   0.751   0.050   0.199
E E  Sequence               9    0.521  91.054   0.083   0.751   0.050   0.199
B V  Sequence              10    0.241  37.119   0.450   0.779   0.100   0.120
B I  Sequence              11    0.198  36.611   0.494   0.779   0.100   0.120
E Q  Sequence              12    0.525  93.747   0.993   0.779   0.100   0.120
B V  Sequence              13    0.190  29.249   0.025   0.831   0.044   0.125
B Q  Sequence              14    0.276  49.329   0.337   0.879   0.010   0.111
E K  Sequence              15    0.508 104.434   0.620   0.938   0.007   0.055
E E  Sequence              16    0.588 102.689   1.166   0.938   0.007   0.055
B V  Sequence              17    0.169  25.975  -0.214   0.938   0.007   0.055
E Y  Sequence              18    0.296  63.255  -0.020   0.879   0.010   0.111
E E  Sequence              19    0.741 129.453   0.714   0.717   0.014   0.269
E E  Sequence              20    0.729 127.409   0.938   0.622   0.015   0.363
E C  Sequence              21    0.692  97.213  -1.939   0.016   0.005   0.979
```

Jpred3
Cole C, Barber JD & Barton GJ. Nucleic Acids Res. 2008.
http://www.compbio.dundee.ac.uk/www-jpred/

```
jp_sQjw0lW : WEEAYQEREVIQVQKEVYEEC : jp_sQjw0lW

           : 1---------11-------- :
OrigSeq    : WEEAYQEREVIQVQKEVYEEC : OrigSeq

Jnet       : ---HH---HHHHHHHHHHH-- : Jnet
jhmm       : ---HH---HHHHHHHHHHH-- : jhmm
```

```
Lupas 14   : ---------------------- : Lupas 14
Lupas 21   : ---------------------- : Lupas 21
Lupas 28   : ---------------------- : Lupas 28

Jnet_25    : ----B----BB-B---BB--- : Jnet_25
Jnet_5     : ---------------------- : Jnet_5
Jnet_0     : ---------------------- : Jnet_0
Jnet Rel   : 99711355788899986289 : Jnet Rel



Key:
Colour code for alignment:
Blue            - Complete identity at a position
Shades of red   - The more red a position is, the higher the level of
                  conservation of chemical properties of the amino acids
Jnet            - Final secondary structure prediction for query
jalign          - Jnet alignment prediction
jhmm            - Jnet hmm profile prediction
jpssm           - Jnet PSIBLAST pssm profile prediction

Lupas           - Lupas Coil prediction (window size of 14, 21 and 28)

Note on coiled coil predictions  - = less than 50% probability
                                 c = between 50% and 90% probability
                                 C = greater than 90% probability

Jnet_25         - Jnet prediction of burial, less than 25% solvent accesibility
Jnet_5          - Jnet prediction of burial, less than 5% exposure
Jnet_0          - Jnet prediction of burial, 0% exposure
Jnet Rel        - Jnet reliability of prediction accuracy, ranges from 0 to 9, bigger is
better.
```

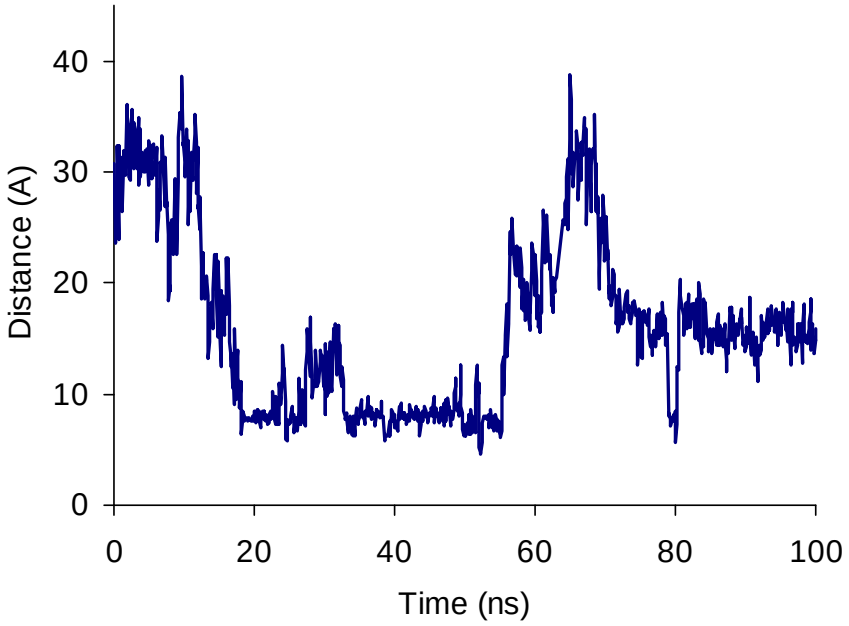
The PSIPRED Protein Structure Prediction Server
http://bioinf.cs.ucl.ac.uk/psipred/

```
Conf: 98888878639
Pred: CHHHHHHHHHC
  AA: WEEAYQEREVC

Conf: 978777788999999998759
Pred: CHHHHHHHHHHHHHHHHHHHC
  AA: WEEAYQEREVIQVQKEVYEEC

Key
Conf: Confidence (0=low, 9=high)
Pred: Predicted secondary structure (H=helix, E=strand, C=coil)
AA: Target sequence
```

Distance between the aromatic rings of the terminal residues (PEVK21**)**



Distance between the aromatic rings of the terminal residues (PEVK11**)**