SUPPLEMENTARY MATERIAL

SUPPLEMENTARY MATERIALS AND METHODS

Evidence of the correctness of the molecular replacement solution of 3W-Actin

In Material and Methods, we briefly described the determination of the molecular replacement (MR) solution using the programs Phenix [1] and AMoRe [2]. The two programs gave the same solution. While Phenix was used in the automatic mode, because of the limited resolution we also performed a detailed analysis of the MR solution with the AMoRe package (http://mem.ibs.fr). The calculations did not require additional programming; crystal packing and rotational/translational non-crystallographic symmetry (NCS) are all part of the "hidden options" of AMoRe.

Irrespective of the resolution, the main criteria for assessing the correctness of a MR solution are a robust figure of merit, good contrast above background, and correct packing. Often, and particularly when the search model is not very accurate, a MR solution is more readily found at low resolution. A low-resolution search model can even be a ~20 Å EM map [3]. In the present case, we had a very accurate search model in the form of the high-resolution structure of crosslinked WxActin, so the confidence in the solution is even higher. Note, however, that different search models were tested, including a model containing the C-terminal helix of Tβ4 as per Irobi et al., 2004 [4], but the best-contrasted solution was obtained with the structure of crosslinked WxActin (see also Materials and Methods). In the resolution interval 15 to 8Å used in the MR search with AMoRe there are 1438 independent reflections and only 12 MR parameters to be determined.

1. Self-rotation function

Because the complex of 3W-Actin was expected to contain three actin subunits, we first performed a self-rotation search over the whole rotation space and using an integration radius of 35Å. The results are listed below. The first 12 peaks correspond to the rotational crystal symmetry of space group $P6_522$ (correlation 100%). The following 12 peaks are massive (correlation 61.7%, gray background) and link molecules related by NCS. A correct MR solution should therefore account for this strong NCS. The peaks that follow (correlation 27.6 and lower) are not contrasted above background, which already indicated the absence of a third molecule in the asymmetric unit of the crystal.

```
Self-rotation: ** EXPRESS ** Data.sca ** 15.00 8.00 ** 35 A
alpha     beta    gamma     ang      cosx      cosy      cosz   corr
180.00     0.00     0.00   180.00  0.00000  0.00000  1.00000 100.0
240.00     0.00     0.00   120.00  0.00000  0.00000 -1.00000 100.0
300.00     0.00     0.00    60.00  0.00000  0.00000 -1.00000 100.0
  0.00     0.00     0.00     0.00  0.00000  0.00000  1.00000 100.0
 60.00     0.00     0.00    60.00  0.00000  0.00000  1.00000 100.0
120.00     0.00     0.00   120.00  0.00000  0.00000  1.00000 100.0
  0.00   180.00     0.00   180.00  0.00000  1.00000  0.00000 100.0
300.00   180.00     0.00   180.00  0.50000  0.86603  0.00000 100.0
240.00   180.00     0.00   180.00  0.86603  0.50000  0.00000 100.0
180.00   180.00     0.00   180.00  1.00000  0.00000  0.00000 100.0
120.00   180.00     0.00   180.00 -0.86603  0.50000  0.00000 100.0
 60.00   180.00     0.00   180.00 -0.50000  0.86603  0.00000 100.0
```

```
210.00     0.00     0.00   150.00  0.00000  0.00000 -1.00000  61.7
270.00     0.00     0.00    90.00  0.00000  0.00000 -1.00000  61.7
330.00     0.00     0.00    30.00  0.00000  0.00000 -1.00000  61.7
 30.00     0.00     0.00    30.00  0.00000  0.00000  1.00000  61.7
 90.00     0.00     0.00    90.00  0.00000  0.00000  1.00000  61.7
150.00     0.00     0.00   150.00  0.00000  0.00000  1.00000  61.7
330.00   180.00     0.00   180.00  0.25882  0.96593  0.00000  61.7
270.00   180.00     0.00   180.00  0.70711  0.70711  0.00000  61.7
210.00   180.00     0.00   180.00  0.96593  0.25882  0.00000  61.7
150.00   180.00     0.00   180.00 -0.96593  0.25882  0.00000  61.7
 90.00   180.00     0.00   180.00 -0.70711  0.70711  0.00000  61.7
 30.00   180.00     0.00   180.00 -0.25882  0.96593  0.00000  61.7
  0.00    90.02   120.00   138.60  0.65475  0.37802  0.65453  27.6
  0.00    90.00   180.00   180.00  0.70711  0.00000  0.70711  27.6
180.00    90.02     0.00   180.00 -0.70723  0.00000  0.70698  27.6
  0.00    90.00   240.00   138.59 -0.65465  0.37796 -0.65465  27.6
.............
```

2. Multi-component molecular replacement

The standard protocol for a multi-component search using AMoRe is to search for the first molecular position, and then search for the second one while keeping the "first best" position fixed. This can be repeated by fixing the "second best" molecular position, and so on. Although computation time is no longer a serious limitation, the number of possible combinations can grow fast, and usually only a few "first best" molecular positions are explored. Alternatively, one can explore a large number of "first best" molecular positions by searching for a "second best" molecular position whose orientation is related to the fixed molecule by a self-rotation peak. In the case of the structure of 3W-Actin, only one strong self-rotation peak was observed (see above), allowing us to screen several hundred "first best" molecular positions.

As shown below, after rigid body refinement the first molecular position was already well contrasted (correlation 44.4% vs 38.8% for the second peak that was not contrasted above background).

```
   alpha   beta  gamma   tx      ty      tz      corr  rfac  corr  pack
1   13.2   36.8   77.3  0.7775  0.1488  0.2609   44.4  49.4  40.7  41.88
1   11.9   36.1   80.3  0.7922  0.1571  0.1278   38.8  51.2  41.0  42.10
1   12.0   35.0   80.2  0.7937  0.1597  0.1278   38.5  51.4  41.0  40.77
1   14.8   37.4   79.6  0.7808  0.1516  0.0424   38.2  50.6  39.8  39.43
1   14.8   37.4   79.6  0.7808  0.1515  0.0424   38.2  50.6  39.8  39.87
1   12.1   36.5   83.3  0.6091  0.3460  0.1272   38.0  52.3  41.2  40.48
1   11.2   36.1   82.8  0.7616  0.1300  0.0108   36.9  52.7  34.3  23.38
1    9.0   38.5   79.0  0.7670  0.1385  0.4802   36.8  51.2  34.8  30.15
1   15.2   33.7   77.8  0.6218  0.3571  0.1277   36.7  52.8  42.2  40.46
1   11.9   41.5   82.5  0.4652  0.5002  0.2323   36.1  52.4  40.4  46.39
1   12.3   36.2   80.6  0.6316  0.3728  0.2391   35.7  52.9  34.3  17.29
1   11.2   32.5   81.1  0.6268  0.3597  0.3645   35.6  52.5  42.0  49.00
1   12.5   39.7   81.7  0.6083  0.3490  0.2189   35.4  52.6  37.9  33.46
.............
```

AMoRe is then run by executing the command:

$AMORE/express --hkl=DATA --model=MODEL:2 --resol=15. 8. --group=P6522 ncs-self

Where; DATA is filename containing reflections, MODEL filename containing coordinates (:2 after MODEL indicates that the search is for 2 independent molecules), and --group is the space group to be tested (see below for the choice of space group).

By fixing many "first best" molecular positions and searching for a "second best" constrained by the self-rotation peak (as explained above), we obtained a very well contrasted "second best" solution (correlation 65.5% vs 50.3% for the following peak).

```
FIX 1   13.2   36.8   77.3  0.7775  0.1488  0.2609   44.4  49.4  40.7  41.88

    alpha   beta  gamma   tx      ty      tz      corr  rfac  corr  pack
2    36.2   39.4   79.1  0.2213  0.3605  0.1742   65.5  40.2  67.0  39.06
2    86.8  141.2  264.4  0.3732  0.2239  0.6228   50.3  47.6  55.1  34.70
2    84.4  142.1  260.4  0.3635  0.2236  0.7106   48.9  47.3  50.1  45.01
2    35.3   37.9   80.5  0.2238  0.3635  0.9561   48.9  47.3  50.1  45.00
2    86.4  145.5  262.0  0.3595  0.2179  0.3575   47.6  48.6  49.5  10.12
2    33.6   34.5   81.9  0.2179  0.3595  0.3091   47.6  48.6  49.6  10.13
2    36.4   38.0   80.6  0.2164  0.3583  0.3107   47.0  49.2  48.5   9.91
2    86.3  141.8  262.9  0.3613  0.2183  0.2734   46.6  48.0  47.3  48.08
2    83.9  141.8  261.3  0.3574  0.2166  0.3561   46.6  49.2  47.6   9.93
2    33.7   38.2   82.9  0.2183  0.3614  0.3933   46.6  48.0  47.3  48.07
2     9.9   36.0   79.1  0.7692  0.1423  0.2622   44.4  49.4  40.7  42.13
2    15.3   36.8   74.1  0.7764  0.1475  0.2619   44.4  49.4  40.7  42.13
.............
```

We then searched for a third molecule, by fixing the first two best molecular positions:

```
       alpha   beta  gamma   tx      ty      tz      corr  rfac  corr  pack
FIX 1   13.4   35.7   76.2  0.7735  0.1475  0.2617   44.4
FIX 2   36.2   39.4   79.1  0.2213  0.3605  0.1742   65.5  40.2  67.0  39.06
```

By testing all the orientations of the cross-rotation function, no other molecule was found. Analysis of the crystal packing for the first two solutions clearly demonstrated why a third molecule was never found (see Supporting Movies 1-4).

3. Space group selection

All possible space groups were tested (P622, $P6_122$, $P6_522$, $P6_222$, $P6_422$, $P6_322$). Below we show as an example the output for the two enantiomeric space groups $P6_122$ and $P6_522$ (notice the difference in correlation coefficients for one or two molecules in the two space groups, gray).

Space group $P6_522$:

```
Fiting:  ** EXPRESS ** Data.sca ** P6522 ** Model.pdb ** 15.00 8.00 **
1   13.4   35.7   76.2  0.7735  0.1475  0.2617   44.4  49.4  40.7  42.13
2   36.2   39.4   79.1  0.2213  0.3605  0.1742   65.5  40.1  67.0  39.03
```

Space group $P6_122$:

```
Fiting:  ** EXPRESS ** Data.sca ** P6122 ** Model.pdb ** 15.00 8.00 **
1   55.6   51.9  111.3  0.7645  0.9011  0.2317   33.2  52.5  30.6  41.21
2   90.0   46.7  110.2  0.0576  0.6368  0.6996   40.4  50.8  36.8  44.08
```

Notice that the correlation coefficient drops from 44.4% to 33.2% for the first molecule and from 65.5% to 40.4% for the dimer for the wrong space group $P6_122$.

4. Phased-translation function

Below, we also show the output of the phased-translation function when the "first best" molecular position is fixed. We see that the first peak gives a correlation of 56.7% vs 40.7% for the second one, which is not contrasted. Here again, no other solution (for a third actin subunit) was found.

```
Trying:  ** EXPRESS ** Data.sca ** P6522 ** MolB.pdb ** 15.00 8.00 **

FIX 1 13.3   36.8   77.2  0.7775  0.1488  0.2609   44.4

   alpha   beta  gamma   tx      ty      tz      corr  rfac  corr  pack
2   43.3   36.8   77.2  0.2210  0.3714  0.1732   56.7  45.0  56.9 100.00
2   43.3   36.8   77.2  0.4778  0.5295  0.9735   40.7  51.7  43.6  43.76
2   43.3   36.8   77.2  0.0103  0.2818  0.3145   39.8  51.1  41.3  35.47
2   43.3   36.8   77.2  0.3021  0.6959  0.1876   39.0  50.8  36.0  37.13
2   43.3   36.8   77.2  0.1120  0.7128  0.5086   38.8  51.6  40.6  38.82
2   43.3   36.8   77.2  0.6261  0.8309  0.8769   38.6  51.3  38.8  36.17
2   43.3   36.8   77.2  0.8703  0.4851  0.8471   38.5  51.6  39.0  36.75
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SUPPLEMENTARY MOVIE LEGENDS

Movie S1. Helix formed by the head-to-tail arrangement of actin dimers along the *c* axis of the unit cell. Crystallographically independent actin subunits are colored yellow and blue, and the W peptide is shown in red. A full turn of the helix (or repeat) comprises 12 actin subunits (i.e. 6 actin dimers).

Movie S2. In the crystal, two helices, related by the crystallographic two-fold axes, are packed tightly against one another in anti-parallel fashion. One anti-parallel pair of helices comprises 24 actin subunits, corresponding to the basic building block of the crystal lattice. The helix shown is centered at (0,0).

Movie S3. Two adjacent pairs of anti-parallel helices crossover twice in a repeat (or helical turn), corresponding to the length of the *c* axis, thus assuring the connectivity of the crystal lattice and leaving no extra-space for the missing third actin subunit. The helices shown are centered at (0,0) and (1,0).

Movie S4. Three adjacent pairs of anti-parallel helices centered at (0,0), (1,0) and (1,1).

SUPPLEMENTARY REFERENCES

1. Zwart, P. H., Afonine, P. V., Grosse-Kunstleve, R. W., Hung, L. W., Ioerger, T. R., McCoy, A. J., McKee, E., Moriarty, N. W., Read, R. J., Sacchettini, J. C., Sauter, N. K., Storoni, L. C., Terwilliger, T. C. & Adams, P. D. (2008). Automated structure solution with the PHENIX suite. *Methods Mol Biol* **426**, 419-35.
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3. Chandran, V., Fronzes, R., Duquerroy, S., Cronin, N., Navaza, J. & Waksman, G. (2009). Structure of the outer membrane complex of a type IV secretion system. *Nature* **462**, 1011-5.
4. Irobi, E., Aguda, A. H., Larsson, M., Guerin, C., Yin, H. L., Burtnick, L. D., Blanchoin, L. & Robinson, R. C. (2004). Structural basis of actin sequestration by thymosin-beta4: implications for WH2 proteins. *Embo J* **23**, 3599-608.