Supplementary Methods

Antigen cloning and production: The vector for the expression of His-tagged, non-biotinylated MBP, pQE_MBP, was constructed by inserting the PCR amplified MBP gene into pAT115, a pAT37 derivative[1], in which the *Nhe*I/*Xba*I fragment was replaced by the corresponding fragment of pQE60 (QIAgen, Germany) to remove the additional *Nco*I site. The MBP gene was amplified from pMAL-c2X (New England Biolabs) with oligonucleotides MTS23 (5'-GGGAATTCTCATGAAAACTGAAGAAGGTAAACTGG-3') and MTS24 (5'-CGGGATCCAGTCTGCGCGTCTTTCAGG-3'), and the product was cloned into pAT115 with the restriction enzymes *Bsp*HI (or *Nco*I for pAT115) and *Bam*HI.
The vectors for the expression of biotinylated, His-tagged pD (pAT222) or MBP (pAT224) were generated in several steps. The basis of the constructs is pAT194, which was itself generated in two steps. First, the *Nhe*I/*Xba*I fragment of pAT115 (contains the terminator as well as a silent chloramphenicol gene) was replaced by the sole terminator sequence of pQE30 (QIAgen), which was amplified by PCR using oligonucleotides MTS19 (5'-GGGAATTCGCTAGCAGTACTGCGATGAGTGGCAG-3') and MTS20 (5'-GCTCTAGAGCGGCGGATTTGTCC-3'), yielding pQc-pD. Second, the $lacI^q$ gene of pZS4-Int1[2] was PCR amplified using oligonucleotides MTS21 (5'-GGGAATTCGCTAGCCTAGGGAAGGCGAAGCGGCATGC-3') and MTS22 (5'-GCTCTAGAGATTTCCCTCGACAATTCGC-3'), cut with *Nhe*I/*Xba*I and inserted into *Xba*I-cut pQc-pD, yielding pAT194. This ligation allows the insertion of the $lacI^q$ fragment in both orientations. We chose the orientation opposite to the T5 expression cassette. A functional test using *E. coli* strain BL21(DE3) showed that the plasmid pAT194 provided functional lac repressor protein.

pAT194 was used to construct the biotinylation vectors. For the insertion of the N-terminal biotinylation tag[3], oligonucleotides MTS33 (5'-AATTCATTAAAGAGGAGAAATTAACTATGGCTGGTCTGAAC-3') and MTS34 (5'-TATCGTTCAGACCAGCCATAGTTAATTTCTCCTCTTTAATG-3') as well as MTS35 (5'-GATATCTTCGAAGCTCAGAAAATCGAATGGCACGAAGGTTC-3') and MTS36 (5'-CATGGAACCTTCGTGCCATTCGATTTTCTGAGCTTCGAAGA-3') were annealed separately, phosphorylated using T4 polynucleotide kinase and ligated into pAT194, previously cut with *Eco*RI/*Nco*I, yielding pAT215. A PCR was made with pAT215 as the template with oligonucleotides oli266 (5'-CGTAGCTCAGCTCATTAGTGATGGTGATGGTGATGAGAAGCTTGGGCTGCAGG TCGACCC-3') and oli267 (5'-ATCGGATCCATGGGCACCGCAACCGCGCCC-3') and the resulting PCR product was cloned into pAT215 via *Nco*I/*Bpu*1102I, yielding pAT222. pAT222 has an ORF under the control of a T5 promotor, consisting of the biotinylation tag:phage lambda protein D:multiple cloning site:His_6 tag. The phage lambda protein D in pAT222 has a truncated N-terminus. This was done to reduce the size of the flexible N-terminus in order to retain high level expression. pAT224, the vector for the production of biotinylated MBP, was generated on the basis of pAT222, replacing the protein D gene *Nco*I/*Bam*HI by a PCR amplified MBP gene of pQE-MBP (oligonucleotides MBPavitagf (5'-GAAGGTTCCATGGGGAAAACTGAAGAAGGTAAACTG-3') and MTS24).

The vectors for the expression of biotinylated, His-tagged JNK2 (pAT222_JNK2) and p38 (pAT222_p38) were generated by PCR cloning. The kinase templates[4] were amplified using oligonucleotides jnk2f (5'-TTCCGCGGATCCGGTACCTCCGACTCTAAATGTGACAGTCAG-3') and jnk2r (5'-AAACCCAAGCTTGTCGACAGCCTTCAAGG-3') or p38f (5'-

GAACAAG-3') and p38r2 (5'-AAACCCCTGCAGGGGACTCCATCTCTTCTTGGTC-3') and the PCR products were cloned into pAT222 via *Bam*HI/*Hin*dIII (JNK2) or *Bam*HI/*Pst*I (p38).

Protein production and purification: The biotinylated proteins pD, MBP, JNK2 and p38 (plasmids pAT222, pAT224, pAT222_JNK2 and pAT222_p38) were produced by *in vivo* biotinylation with plasmid pBirAcm of Avidity Inc. (Denver, Co, USA) in *E. coli* XL-1 Blue (Stratagene, USA) according to the protocols of Avidity and QIAgen. Non-biotinylated MBP for analysis and crystallization was produced in the same way as the AR proteins[5] using pQE-MBP in *E. coli* XL-1 Blue. The protein purification has been described[5]. Biotinylation was confirmed using ELISA and blotting with a streptavidin-alkaline phosphatase conjugate (Roche, Basel, Switzerland) and mass spectrometry.

Ribosome display vector (pRDV; AY327136): To create a vector suitable for ribosome display, β-lactamase (as a control insert[6]) was PCR amplified with oligonucleotides blaf (5'-TATCCATGGCGGACTACAAAGATGACGATGACAAAGGATCCCACCCAGAAACGCTG-3') and rBLA (5'-TTGCTTCTGAAGCTTTCCCCAATGCTTAATC-3'), cut with *Hin*dIII and ligated to the *Hin*dIII cut *tolA* gene[6], which was amplified with the oligonucleotides tolAfor (5'-TATACCCAAGCTTTATATGGCCTCGGGGGCCGAATTCGGATCTGGTGGCCAGAAGCAAGCTGAA-3') and tolArev (5'-GGAAGATCTCTACTACGGTTTGAAGTCCAATGG-3'). The resulting β-lactamase:tolA fusion construct was cut *Nco*I/*Bgl*II and cloned into *Nco*I/*Bam*HI cut pTFT74[7]. The sequence of the resulting plasmid pRDV was verified using standard techniques.

References:

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