Supplementary Methods

Phagemid cloning. The phagemid pDST22, a derivative of the vector pMorph7 (ref. 1) which is functionally equivalent to the vector pAK100 (ref. 2) was the starting point for the cloning (**Supplementary Fig. 1**). It encodes the DARPin 3a and the signal sequence of the *E. coli* PhoA (PhoAss). The *E. coli* alkaline phosphatase (Swiss-Prot database accession number P00634) has the signal sequence MKQSTIALALLPLLFTPVTKA, given in single letter amino acid code.

To allow direct experimental comparison to phagemids encoding the signal sequence of DsbA (DsbAss), a second phagemid called pDST23 was generated. The *E. coli* thiol-disulfide interchange protein DsbA (Swiss-Prot database accession number P0AEG4) has the signal sequence MKKIWLALAGLVLAFSASA, given in single letter amino acid code. To replace the PhoAss of pDST22, the oligonucleotides oDST4 (AGAGCATGCGTAGGAGAAAATAAAATGAAAAAGATTTggctggcgctggctgg), oDST5 (TCTTTGTAGTCCGCCGATGCGCTAAACGCTAAAACTAAAccagccagcgccagcc), oDST6 (GCTCTagagcatgcgtaggag (*Xba*I)), and oDST8 (GCGGATCCAtctttgtagtccgccg (*Bam*HI)) that encode the *E. coli* DsbAss were designed (Lower case letters indicate the regions designed for annealing, restriction sites are underlined and restriction enzymes given in brackets).

The oligonucleotides oDST4 and oDST5 were annealed and amplified with oligonucleotides oDST6 and oDST8 by PCR. The resulting DNA fragment encodes the DsbAss and is flanked by the restriction endonuclease sites *Xba*I and *BamH*I. This DNA fragment was digested with *Xba*I and *BamH*I and ligated into the similarly treated and dephosphorylated pDST22. The resulting phagemid pDST23 (**Supplementary Fig. 1**) was isolated and the sequence was verified by DNA sequencing.

The other phagemids used in this study are listed in **Table 1** of the publication and were obtained as follows: The coding sequences of the proteins of interest were PCR

amplified using appropriately designed PCR primers and template DNA. Thereby, either a *BamH*I or a *Bgl*II restriction site was introduced 5' to each of the coding sequences and two restriction sites (*EcoR*I and *Pst*I) were introduced 3' to each of the coding sequences. These PCR fragments were digested either with *BamH*I or *Bgl*II and either *EcoR*I or *Pst*I, and then ligated into the similarly treated and dephosphorylated phagemids pDST23 and pDST22. The open reading frame of the expression cassette for the fusion polypeptide comprising the cloned PCR product was maintained for all constructs, especially the correct reading frame for the C-terminal fusion to the C-terminal domain of phage protein 3 (CTp3) was maintained. The first and the last amino acids of the cloned proteins of interest (POI) are given in **Table 1** of the publication as well as the reference or accession number for either the GenBank or the Swiss-Prot databases. The sequence of all phagemids was verified by DNA sequencing.

For the designed ankyrin proteins (DARPins) 3a and 2_3, additional phagemids encoding the PelBss (pDST80 and pDST81, respectively), SfmCss (pDST86 and pDST87, respectively), TolBss (pDST84 and pDST85, respectively) and TorTss (pDST88 and pDST89, respectively) were generated, using the same cloning strategy with 4 oligonucleotides as described above for DsbAss, using appropriate oligonucleotides**.**

For the DARPin E3_5 and thioredoxin (TrxA), additional phagemids encoding the LamBss (pDST110 and pDST117, respectively), MalEss (pDST109 and pDST116, respectively), MglBss (pDST111 and pDST118, respectively), OmpAss (pDST103 and pDST104, respectively), PelBss (pDST105 and pDST112, respectively), SfmCss (pDST108 and pDST115, respectively), TolBss (pDST106 and pDST113, respectively) and TorTss (pDST107 and pDST114, respectively) were generated. Again, the same strategy as for DsbAss was used.

Signal sequences used are described in detail in **Supplementary Table 2** and *E. coli* strains used are described in detail in **Supplementary Table 1**.

References

1. Knappik, A. et al. Fully synthetic human combinatorial antibody libraries (HuCAL) based on modular consensus frameworks and CDRs randomized with trinucleotides. *J. Mol. Biol.* **296**, 57-86 (2000).
2. Krebber, A. et al. Reliable cloning of functional antibody variable domains from hybridomas and spleen cell repertoires employing a reengineered phage display system. *J. Immunol. Methods* **201**, 35-55 (1997).