Supplementary Information

The Matrix Reloaded:
Specific Binding Proteins Based on Non-immunoglobulin Domains

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In the following, an extensive reference list is given for alternatives to antibodies, sorted by the different scaffolds used. Abbreviations or commercial names of the different scaffolds are indicated next to the scaffold name. In addition, we give the URLs of companies commercializing the respective scaffold.

In the last section, we also give a more comprehensive reference list of selection technologies. This, however, is still only a tiny subset, and makes no attempt to adequately reference this enormous field. We cite some original and some review articles which may guide the reader to the original publications of the numerous technologies.

Scaffolds

Fibronectin (Monobody, AdNectin, ^{10}FN3, FN3)

Koide, A., Bailey, C.W., Huang, X. & Koide, S. The fibronectin type III domain as a scaffold for novel binding proteins. *J. Mol. Biol.* **284**, 1141-1151 (1998).

Koide, A., Jordan, M.R., Horner, S.R., Batori, V. & Koide, S. Stabilization of a fibronectin type III domain by the removal of unfavorable electrostatic interactions on the protein surface. *Biochemistry* **40**, 10326-10333 (2001).

Batori, V., Koide, A. & Koide, S. Exploring the potential of the monobody scaffold: effects of loop elongation on the stability of a fibronectin type III domain. *Protein Eng.* **15**, 1015-1020 (2002).

Koide, A., Abbatiello, S., Rothgery, L. & Koide, S. Probing protein conformational changes in living cells by using designer binding proteins: application to the estrogen receptor. *Proc. Natl Acad. Sci. USA* **99**, 1253-1258 (2002).

Xu, L., Aha, P., Gu, K., Kuimelis, R.G., Kurz, M., Lam, T., Lim, A.C., Liu, H., Lohse, P.A., Sun, L., Weng, S., Wagner, R.W. & Lipovsek, D. Directed evolution of high-affinity antibody mimics using mRNA display. *Chem. Biol.* **9**, 933-942 (2002).

Richards, J., Miller, M., Abend, J., Koide, A., Koide, S. & Dewhurst, S. Engineered fibronectin type III domain with a RGDWXE sequence binds with enhanced affinity and specificity to human $\alpha v\beta 3$ integrin. *J. Mol. Biol.* **326**, 1475-1488 (2003).

Han, Z., Karatan, E., Scholle, M.D., McCafferty, J. & Kay, B.K. Accelerated screening of phage-display output with alkaline phosphatase fusions. *Comb. Chem. High Throughput Screen.* **7**, 55-62 (2004).

Karatan, E., Merguerian, M., Han, Z., Scholle, M.D., Koide, S. & Kay, B.K. Molecular recognition properties of FN3 monobodies that bind the Src SH3 domain. *Chem. Biol.* **11**, 835-844 (2004).

Lipovsek, D. & Plückthun, A. In-vitro protein evolution by ribosome display and mRNA display. *J. Immunol. Methods* **290**, 51-67 (2004).

URL (Compound Therapeutics): http://www.compoundtherapeutics.com

(formerly commercialized by Phylos)

Cytotoxic T Lymphocyte Antigen-4 (CTLA-4, Evibody)

Nuttall, S.D., Hattarki, M., Guthrie, R.E., Hudson, P.J. & Kortt, A.A. Design and expression of soluble CTLA-4 variable domain as a scaffold for the display of functional polypeptides. *Proteins* **36**, 217-227 (1999).

Hufton, S.E., van Neer, N., van den Beuken, T., Desmet, J., Sablon, E. & Hoogenboom, H.R. Development and application of cytotoxic T lymphocyte-associated antigen 4 as a protein scaffold for the generation of novel binding ligands. *FEBS Lett.* **475**, 225-231 (2000).

URL (Evogenix): http://www.evogenix.com

Tendamistat

McConnell, S.J. & Hoess, R.H. Tendamistat as a scaffold for conformationally constrained phage peptide libraries. *J. Mol. Biol.* **250**, 460-470 (1995).

Li, R., Hoess, R.H., Bennett, J.S. & DeGrado, W.F. Use of phage display to probe the evolution of binding specificity and affinity in integrins. *Protein Eng.* **16**, 65-72 (2003).

Takahashi, M., Nokihara, K. & Mihara, H. Construction of a protein-detection system using a loop peptide library with a fluorescence label. *Chem Biol* **10**, 53-60 (2003).

URL (DuPont): http://www.dupont.com

Neocarzinostatin

Heyd, B., Pecorari, F., Collinet, B., Adjadj, E., Desmadril, M. & Minard, P. *In vitro* evolution of the binding specificity of neocarzinostatin, an enediyne-binding chromoprotein. *Biochemistry* **42**, 5674-5683 (2003).

Nicaise, M., Valerio-Lepiniec, M., Minard, P. & Desmadril, M. Role of the tyrosine corner motif in the stability of neocarzinostatin. *Protein Eng.* **16**, 733-738 (2003).

Nicaise, M., Valerio-Lepiniec, M., Minard, P., Desmadril, M. Affinity transfer by CDR grafting on a nonimmunoglobulin scaffold. *Protein Sci.* **13**,1882-1891(2004).

Carbohydrate Binding Module CBM4-2

Cicortas Gunnarsson, L., Nordberg Karlsson, E., Albrekt, A.S., Andersson, M., Holst, O., Ohlin, M. A carbohydrate binding module as a diversity-carrying scaffold. *Protein. Eng. Des. Sel.* **17**, 213-221 (2004).

Lipocalins (Anticalins)

Müller, H.N. & Skerra, A. Grafting of a high-affinity Zn(II)-binding site on the β-barrel of retinol-binding protein results in enhanced folding stability and enables simplified purification. *Biochemistry* **33**, 14126-14135 (1994).

Schmidt, A.M., Müller, H.N. & Skerra, A. A Zn(II)-binding site engineered into retinol-binding protein exhibits metal-ion specificity and allows highly efficient affinity purification with a newly designed metal ligand. *Chem. Biol.* **3**, 645-653 (1996).

Beste, G., Schmidt, F.S., Stibora, T. & Skerra, A. Small antibody-like proteins with prescribed ligand specificities derived from the lipocalin fold. *Proc. Natl Acad. Sci. USA* **96**, 1898-1903 (1999).

Schlehuber, S., Beste, G. & Skerra, A. A novel type of receptor protein, based on the lipocalin scaffold, with specificity for digoxigenin. *J. Mol. Biol.* **297**, 1105-1120 (2000).

Skerra, A. Lipocalins as a scaffold. *Biochim. Biophys. Acta* **1482**, 337-350 (2000).

Schlehuber, S. & Skerra, A. Duocalins: engineered ligand-binding proteins with dual specificity derived from the lipocalin fold. *Biol. Chem.* **382**, 1335-1342 (2001).

Skerra, A. 'Anticalins': a new class of engineered ligand-binding proteins with antibody-like properties. *J. Biotechnol.* **74**, 257-275 (2001).

Götz, M., Hess, S., Beste, G., Skerra, A. & Michel-Beyerle, M.E. Ultrafast electron transfer in the complex between fluorescein and a cognate engineered lipocalin protein, a so-called anticalin. *Biochemistry* **41**, 4156-4164 (2002).

Mercader, J.V. & Skerra, A. Generation of anticalins with specificity for a nonsymmetric phthalic acid ester. *Anal. Biochem.* **308**, 269-277 (2002).

Schlehuber, S. & Skerra, A. Tuning ligand affinity, specificity, and folding stability of an engineered lipocalin variant - a so-called 'anticalin' - using a molecular random approach. *Biophys. Chem.* **96**, 213-228 (2002).

Korndörfer, I.P., Beste, G. & Skerra, A. Crystallographic analysis of an "anticalin" with tailored specificity for fluorescein reveals high structural plasticity of the lipocalin loop region. *Proteins* **53**, 121-129 (2003).

Korndörfer, I.P., Schlehuber, S. & Skerra, A. Structural mechanism of specific ligand recognition by a lipocalin tailored for the complexation of digoxigenin. *J. Mol. Biol.* **330**, 385-396 (2003).

Liu, G., Mills, J.L., Hess, T.A., Kim, S., Skalicky, J.J., Sukumaran, D.K., Kupce, E., Skerra, A. & Szyperski, T. Resonance assignments for the 21 kDa engineered fluorescein-binding lipocalin FluA. *J. Biomol. NMR* **27**, 187-188 (2003).

Skerra, A. Imitating the humoral immune response. *Curr. Opin. Chem. Biol.* **7**, 683-693 (2003).

Vogt, M. & Skerra, A. Construction of an artificial receptor protein ("anticalin") based on the human apolipoprotein D. *ChemBioChem* **5**, 191-199 (2004).

Schlehuber, S. & Skerra, A. Lipocalins in drug discovery: from natural ligand-binding proteins to "anticalins". *Drug Discov. Today* **10**, 23-33 (2005).

URL (Pieris Proteolab): http://www.pieris.biz

T-cell Receptors

Kieke, M.C., Shusta, E.V., Boder, E.T., Teyton, L., Wittrup, K.D. & Kranz, D.M. Selection of functional T cell receptor mutants from a yeast surface-display library. *Proc. Natl Acad. Sci. USA* **96**, 5651-5656 (1999).

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Holler, P.D., Holman, P.O., Shusta, E.V., O'Herrin, S., Wittrup, K.D. & Kranz, D.M. *In vitro* evolution of a T cell receptor with high affinity for peptide/MHC. *Proc. Natl Acad. Sci. USA* **97**, 5387-5392 (2000).

Kieke, M.C., Sundberg, E., Shusta, E.V., Mariuzza, R.A., Wittrup, K.D. & Kranz, D.M. High affinity T cell receptors from yeast display libraries block T cell activation by superantigens. *J. Mol. Biol.* **307**, 1305-1315 (2001).

Boulter, J.M., Glick, M., Todorov, P.T., Baston, E., Sami, M., Rizkallah, P. & Jakobsen, B.K. Stable, soluble T-cell receptor molecules for crystallization and therapeutics. *Protein Eng.* **16**, 707-711 (2003).

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Laugel, B., Boulter, J.M., Lissin, N., Vuidepot, A., Li, Y., Gostick, E., Crotty, L.E., Douek, D.C., Hemelaar, J., Price, D.A., Jakobsen, B.K. & Sewell, A.K. Design of soluble recombinant T cell receptors for antigen targeting and T cell inhibition. *J. Biol. Chem.* **280**, 1882-1892 (2005).

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Chlewicki, L.K., Holler, P.D., Monti, B.C., Clutter, M.R. & Kranz, D.M. High-affinity, peptide-specific T cell receptors can be generated by mutations in CDR1, CDR2 or CDR3. *J. Mol. Biol.* **346**, 223-239 (2005).

URL (Avidex): http://www.avidex.com/

Protein A Domain (Affibody, Protein Z)

Nilsson, B., Moks, T., Jansson, B., Abrahmsén, L., Elmblad, A., Holmgren, E., Henrichson, C., Jones, T.A. & Uhlén, M. A synthetic IgG-binding domain based on staphylococcal protein A. *Protein Eng.* **1**, 107-113 (1987).

Nord, K., Nilsson, J., Nilsson, B., Uhlén, M. & Nygren, P.-Å. A combinatorial library of an α-helical bacterial receptor domain. *Protein Eng.* **8**, 601-608 (1995).

Nord, K., Gunneriusson, E., Ringdahl, J., Ståhl, S., Uhlén, M. & Nygren, P.-Å. Binding proteins selected from combinatorial libraries of an α-helical bacterial receptor domain. *Nat. Biotechnol.* **15**, 772-777 (1997).

Gunneriusson, E., Nord, K., Uhlén, M. & Nygren, P.-Å. Affinity maturation of a *Taq* DNA polymerase specific affibody by helix shuffling. *Protein Eng.* **12**, 873-878 (1999).

Hansson, M., Ringdahl, J., Robert, A., Power, U., Goetsch, L., Nguyen, T.N., Uhlén, M., Ståhl, S. & Nygren, P.-Å. An *in vitro* selected binding protein (affibody) shows conformation- dependent recognition of the respiratory syncytial virus (RSV) G protein. *Immunotechnology* **4**, 237-252 (1999).

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Eklund, M., Axelsson, L., Uhlén, M. & Nygren, P.-Å. Anti-idiotypic protein domains selected from protein A-based affibody libraries. *Proteins* **48**, 454-462 (2002).

Henning, P., Magnusson, M.K., Gunneriusson, E., Hong, S.S., Boulanger, P., Nygren, P.-Å. & Lindholm, L. Genetic modification of adenovirus 5 tropism by a novel class of ligands based on a three-helix bundle scaffold derived from staphylococcal protein A. *Hum. Gene Ther.* **13**, 1427-1439 (2002).

Lendel, C., Wahlberg, E., Berglund, H., Eklund, M., Nygren, P.-Å. & Härd, T. ^{1}H, ^{13}C and ^{15}N resonance assignments of an affibody-target complex. *J. Biomol. NMR* **24**, 271-272 (2002).

Rönnmark, J., Hansson, M., Nguyen, T., Uhlén, M., Robert, A., Ståhl, S. & Nygren, P.-Å. Construction and characterization of affibody-Fc chimeras produced in *Escherichia coli*. *J. Immunol. Methods* **261**, 199-211 (2002).

Rönnmark, J., Grönlund, H., Uhlén, M. & Nygren, P.-Å. Human immunoglobulin A (IgA)-specific ligands from combinatorial engineering of protein A. *Eur. J. Biochem.* **269**, 2647-2655 (2002).

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Rönnmark, J., Kampf, C., Asplund, A., Höidén-Guthenberg, I., Wester, K., Pontén, F., Uhlén, M. & Nygren, P.-Å. Affibody-β-galactosidase immunoconjugates produced as soluble fusion proteins in the *Escherichia coli* cytosol. *J. Immunol. Methods* **281**, 149-160 (2003).

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Wahlberg, E., Lendel, C., Helgstrand, M., Allard, P., Dincbas-Renqvist, V., Hedqvist, A., Berglund, H., Nygren, P.-Å. & Härd, T. An affibody in complex with a target protein: structure and coupled folding. *Proc. Natl Acad. Sci. USA* **100**, 3185-3190 (2003).

Wikman, M., Steffen, A.C., Gunneriusson, E., Tolmachev, V., Adams, G.P., Carlsson, J. & Ståhl, S. Selection and characterization of HER2/neu-binding affibody ligands. *Protein Eng. Des. Sel.* **17**, 455-462 (2004).

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Engfeldt, T., Renberg, B., Brumer, H., Nygren, P.-Å. & Karlström, A.E. Chemical synthesis of triple-labelled three-helix bundle binding proteins for specific fluorescent detection of unlabelled protein. *ChemBioChem* **6**, 1043-1050 (2005).

URL (Affibody): http://www.affibody.com

Im9 (Immunity Protein 9)

Bernath, K., Magdassi, S. & Tawfik, D.S. Directed evolution of protein inhibitors of DNA-nucleases by *in vitro* compartmentalization (*IVC*) and nano-droplet delivery. *J. Mol. Biol.* **345**, 1015-1026 (2005).

Designed Ankyrin Repeat Proteins (DARPins)

Kohl, A., Binz, H.K., Forrer, P., Stumpp, M.T., Plückthun, A. & Grütter, M.G. Designed to be stable: crystal structure of a consensus ankyrin repeat protein. *Proc. Natl Acad. Sci. USA* **100**, 1700-1705 (2003).

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Lipovsek, D. & Plückthun, A. In-vitro protein evolution by ribosome display and mRNA display. *J. Immunol. Methods* **290**, 51-67 (2004).

Devi, V.S., Binz, H.K., Stumpp, M.T., Plückthun, A., Bosshard, H.R. & Jelesarov, I. Folding of a designed simple ankyrin repeat protein. *Protein Sci.* **13**, 2864-2870 (2004).

Amstutz, P., Binz, H.K., Parizek, P., Stumpp, M.T., Kohl, A., Grütter, M.G., Forrer, P. & Plückthun, A. Intracellular kinase inhibitors selected from combinatorial libraries of designed ankyrin repeat proteins. *J. Biol. Chem.* **280**, 24715-24722 (2005).

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URL (Molecular Partners): http://www.molecularpartners.com

Designed Tetratricopeptide Repeat Proteins

Main, E.R., Xiong, Y., Cocco, M.J., D'Andrea, L. & Regan, L. Design of stable α-helical arrays from an idealized TPR motif. *Structure (Camb)* **11**, 497-508 (2003).

D'Andrea, L.D. & Regan, L. TPR proteins: the versatile helix. *Trends Biochem. Sci.* **28**, 655-662 (2003).

Cortajarena, A.L., Kajander, T., Pan, W., Cocco, M.J. & Regan, L. Protein design to understand peptide ligand recognition by tetratricopeptide repeat proteins. *Protein Eng. Des. Sel.* **17**, 399-409 (2004).

Zinc Finger

Bianchi, E., Folgori, A., Wallace, A., Nicotra, M., Acali, S., Phalipon, A., Barbato, G., Bazzo, R., Cortese, R., Felici, F. & Pessi, A. A conformationally homogeneous combinatorial peptide library. *J. Mol. Biol.* **247**, 154-160 (1995).

As DNA binding molecules:

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Segal, D.J., Dreier, B., Beerli, R.R. & Barbas, C.F., 3rd. Toward controlling gene expression at will: selection and design of zinc finger domains recognizing each of the 5'-GNN-3' DNA target sequences. *Proc. Natl Acad. Sci. USA* **96**, 2758-2763 (1999).

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URL (Dyax): http://www.dyax.com

pVIII

Petrenko, V.A., Smith, G.P., Mazooji, M.M. & Quinn, T. α-helically constrained phage display library. *Protein Eng.* **15**, 943-950 (2002).

GCN4

Sia, S.K. & Kim, P.S. Protein grafting of an HIV-1-inhibiting epitope. *Proc. Natl Acad. Sci. USA* **100**, 9756-9761 (2003).

Avian Pancreatic Polypeptide (APP)

Chin, J.W., Grotzfeld, R.M., Fabian, M.A. & Schepartz, A. Methodology for optimizing functional miniature proteins based on avian pancreatic polypeptide using phage display. *Bioorg. Med. Chem. Lett.* **11**, 1501-1505 (2001).

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Volkman, H.M., Rutledge, S.E. & Schepartz, A. Binding mode and transcriptional activation potential of high affinity ligands for the CBP KIX domain. *J. Am. Chem. Soc.* **127**, 4649-4658 (2005).

Schneider, T.L., Mathew, R.S., Rice, K.P., Tamaki, K., Wood, J.L. & Schepartz, A. Increasing the kinase specificity of k252a by protein surface recognition. *Org. Lett.* **7**, 1695-1698 (2005).

WW Domain

Dalby, P.A., Hoess, R.H. & DeGrado, W.F. Evolution of binding affinity in a WW domain probed by phage display. *Protein Sci.* **9**, 2366-2376 (2000).

SH3 Domains

Hiipakka, M., Poikonen, K. & Saksela, K. SH3 domains with high affinity and engineered ligand specificities targeted to HIV-1 Nef. *J. Mol. Biol.* **293**, 1097-1106 (1999).

Hiipakka, M., Huotari, P., Manninen, A., Renkema, G.H. & Saksela, K. Inhibition of cellular functions of HIV-1 Nef by artificial SH3 domains. *Virology* **286**, 152-159 (2001).

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Panni, S., Dente, L. & Cesareni, G. *In vitro* evolution of recognition specificity mediated by SH3 domains reveals target recognition rules. *J. Biol. Chem.* **277**, 21666-21674 (2002).

SH2 Domains

Malabarba, M.G., Milia, E., Faretta, M., Zamponi, R., Pelicci, P.G. & Di Fiore, P.P. A repertoire library that allows the selection of synthetic SH2s with altered binding specificities. *Oncogene* **20**, 5186-5194 (2001).

PDZ Domains (Telobodies)

Schneider, S., Buchert, M., Georgiev, O., Catimel, B., Halford, M., Stacker, S.A., Baechi, T., Moelling, K. & Hovens, C.M. Mutagenesis and selection of PDZ domains that bind new protein targets. *Nat. Biotechnol.* **17**, 170-175 (1999).

Reina, J., Lacroix, E., Hobson, S.D., Fernandez-Ballester, G., Rybin, V., Schwab, M.S., Serrano, L. & Gonzalez, C. Computer-aided design of a PDZ domain to recognize new target sequences. *Nat. Struct. Biol.* **9**, 621-627 (2002).

Junqueira, D., Cilenti, L., Musumeci, L., Sedivy, J.M. & Zervos, A.S. Random mutagenesis of PDZ_{Omi} domain and selection of mutants that specifically bind the Myc proto-oncogene and induce apoptosis. *Oncogene* **22**, 2772-2781 (2003).

Ferrer, M., Maiolo, J., Kratz, P., Jackowski, J.L., Murphy, D.J., Delagrave, S. & Inglese, J. Directed evolution of PDZ variants to generate high-affinity detection reagents. *Protein Eng. Des. Sel.* **18**, 165-173 (2005).

URL (BioTech Studio): http://www.biotechstudio.com

TEM-1 β-lactamase

Soumillion, P., Jespers, L., Bouchet, M., Marchand-Brynaert, J., Winter, G. & Fastrez, J. Selection of β-lactamase on filamentous bacteriophage by catalytic activity. *J. Mol. Biol.* **237**, 415-422 (1994).

Legendre, D., Soumillion, P. & Fastrez, J. Engineering a regulatable enzyme for homogeneous immunoassays. *Nat. Biotechnol.* **17**, 67-72 (1999).

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Potential Selection Technologies

(Selections from complex libraries had not been reported at the time of writing)

T7 Phage Display

URL (Novagen): http://www.merckbiosciences.co.uk

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