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Richard M. Twyman

Principles of PROTEOMICS

SECOND EDITION

Principles of Proteomics

Second Edition

Richard M. Twyman

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Preface to the second edition

When I wrote the first edition of *Principles of Proteomics* in 2003, it was the first book that had attempted to cover the entire field of proteomics in broad strokes rather than focusing on specialized individual technologies. The first edition was published when proteomics was an emerging discipline, still unsure of its footing although confident in its abilities, with many technology platforms jostling for attention and consideration. Nearly a decade later, writing the second edition has proven a significant challenge. Although proteomics has stabilized, with certain technologies becoming unshakably established and others becoming obsolete, the cutting edge still boasts a rich and diverse source of novel technology platforms seeking to capture the proteome in ever more detail and on a scale barely conceived at the beginning of the millennium. But proteomics has also become increasingly commercialized. It is a billion-dollar industry, with many companies vying for attention, providing technologies, solutions, and contract research to other companies, who are in turn interested in using proteomics to find disease biomarkers, drug targets, vaccine candidates, novel chemical inhibitors, improved enzymes for industrial processes, and products to protect plants, the food chain, and the environment. Keeping up with the pace of change while still being aware of the fundamental aspects of proteomics, the core principles that make it possible in the first place, is a difficult task made more difficult by the dominant position of proprietary technologies, and the explosion in patents relating to proteomic technologies and strategies for processing proteomics data.

Despite the above, we must remember that proteomics is still about the global analysis of proteins. It seeks to achieve what genomics cannot—that is, a complete description of living cells in terms of all their functional components, brought about by the direct analysis of those components rather than the genes that encode them. Proteins offer a rich source of data, including sequences, structures, and biochemical and biological functions, which are influenced by modifications, subcellular localization, and, perhaps most important of all, the interactions among proteins and with other molecules. If genes are the instruction carriers, proteins are the molecules that execute those instructions. Genes are the instruments of change over evolutionary timescales, but proteins are the molecules that define which changes are accepted and which are discarded. It is from proteins that we shall learn how living cells and organisms are built and maintained and what leads to their dysfunction.

Although now firmly established, proteomics is still a difficult subject to penetrate for those not familiar with the terminology and technology, including experts in one area of proteomics venturing into another. There is still a great deal of jargon and many hyphenated acronyms that make sense once explained but otherwise remain mystifying; and there is still a high turnover of methods at the cutting edge, making it difficult to keep up. This situation is exacerbated by the increasing integration of proteomics with other areas of large-scale biology as researchers attempt to model cellular processes by looking not only at the functional components, but also at the information (genes, transcripts) and the outputs (metabolites, phenotypes) and how these are linked into networks and systems.

As I stated in the preface to the first edition, it is my hope that this book will be useful to those who need a broad overview of proteomics and what it has to offer. It is not meant to provide expertise in any particular area: there are plenty of other books that deal with specific technologies and their applications, the processing and archiving of proteomic data, and the integration of proteomics with other disciplines. The aim of this book is to pull together the different proteomics technologies and their applications, and present them in what I hope is a simple, logical, and user-friendly manner. After a brief introductory chapter providing an updated perspective on the history of proteomics since the turn of the millennium, the major proteomics technologies are discussed in more detail: two-dimensional gel electrophoresis, multidimensional liquid chromatography, mass spectrometry, sequence analysis, structural analysis, methods for studying protein interactions and modifications, and the development and applications of protein microarrays. These chapters have been broadened to account for new developments since the first edition, but I have made every effort to keep the material as concise as possible, since the brevity of the first edition was one of its strengths. I have assumed necessarily that the reader has a working knowledge of molecular biology and biochemistry. Each chapter has a short bibliography listing classic papers and useful reviews that will help the interested reader delve deeper into the literature.

The second edition would not have been possible without the help and support of the editorial team at Garland Science, so I extend special thanks to Gina Almond, David Borrowdale, and Ioana Moldovan for their dedication and assistance during the writing and revision process. I would also like to thank friends and colleagues who provided feedback on the first edition or suggestions for the second edition or who pointed out errors and omissions.

As ever, this book is dedicated with love to my parents, Peter and Irene, to my children, Emily and Lucy, and to Hannah, Joshua, and Dylan.

Richard M. Twyman

August 2013

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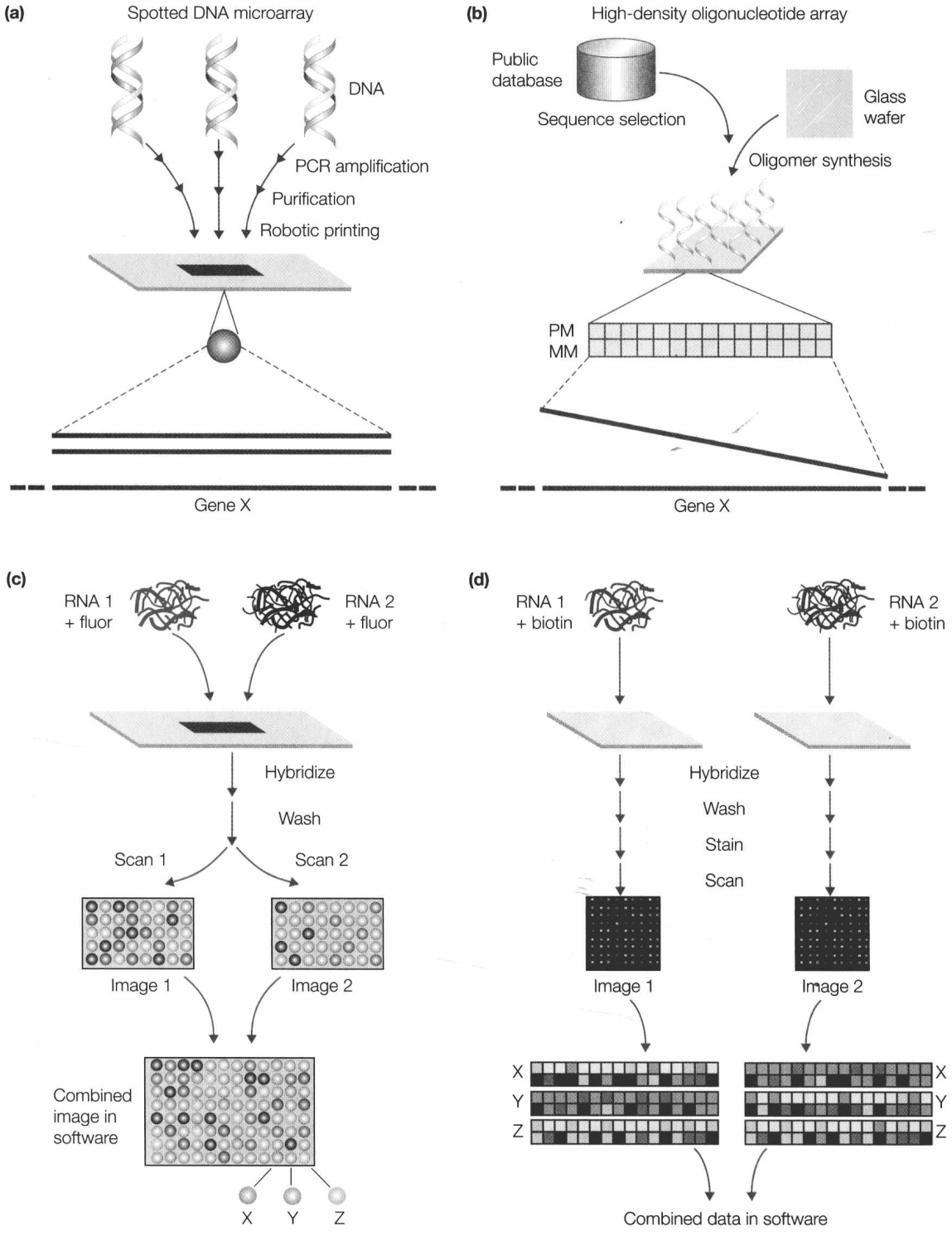


FIGURE 1.4 Expression analysis with DNA microarrays. (a) Spotted microarrays are produced by the robotic printing of amplified cDNA molecules onto glass slides. Each spot or feature corresponds to a contiguous gene fragment of several hundred base pairs or more. (b) High-density oligonucleotide chips are manufactured using a process of light-directed combinatorial chemical synthesis to produce thousands of different sequences in a highly ordered array on a small glass chip. Genes are represented by 15–20 different oligonucleotide pairs (PM, perfectly matched; MM, mismatched) on the array. (c) On spotted arrays, comparative expression assays are usually carried out by differentially labeling two mRNA or cDNA samples with different fluorophores. These are hybridized to features on the glass slide and then scanned to detect both fluorophores independently. Colored dots labeled X, Y, and Z at the bottom of the image correspond to transcripts present at increased levels in sample 1 (X), increased levels in sample 2 (Y), and similar levels in samples 1 and 2 (Z). (d) On Affymetrix GeneChips, biotinylated cRNA is hybridized to the array and stained with a fluorophore conjugated to avidin. The signal is detected by laser scanning. Sets of paired oligonucleotides for hypothetical genes present at increased levels in sample 1 (X), increased levels in sample 2 (Y), and similar levels in samples 1 and 2 (Z) are shown. (From Harrington CA, Rosenow C & Retief J (2000) *Curr. Opin. Microbiol.* 3, 285. With permission from Elsevier.)

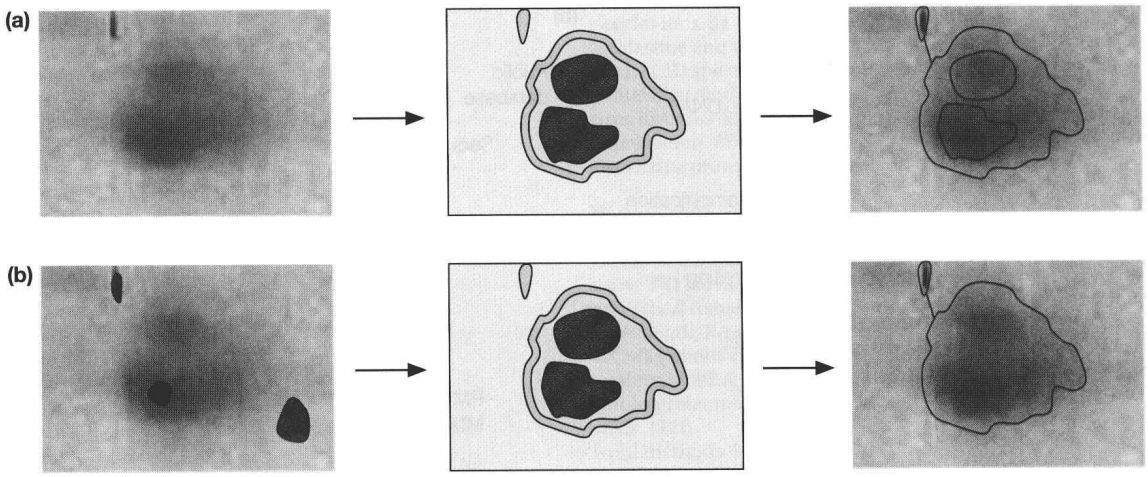


FIGURE 4.1 The watershed method for contour finding on two-dimensional gel images. (a) Any grayscale image can be considered as a topographic surface. If flooded from its minima without allowing water from different sources to merge, the image is partitioned into catchment basins and watershed lines, but in practice this leads to over-segmentation. (b) Therefore, markers (*red shapes*) are used to initiate flooding, and this reduces over-segmentation considerably. (Adapted from images by Serge Beucher, CMM/École Nationale Supérieure des Mines de Paris.)

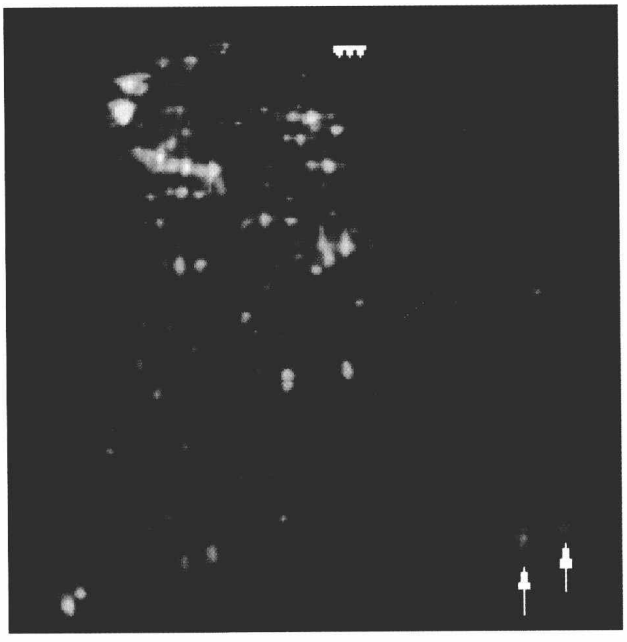
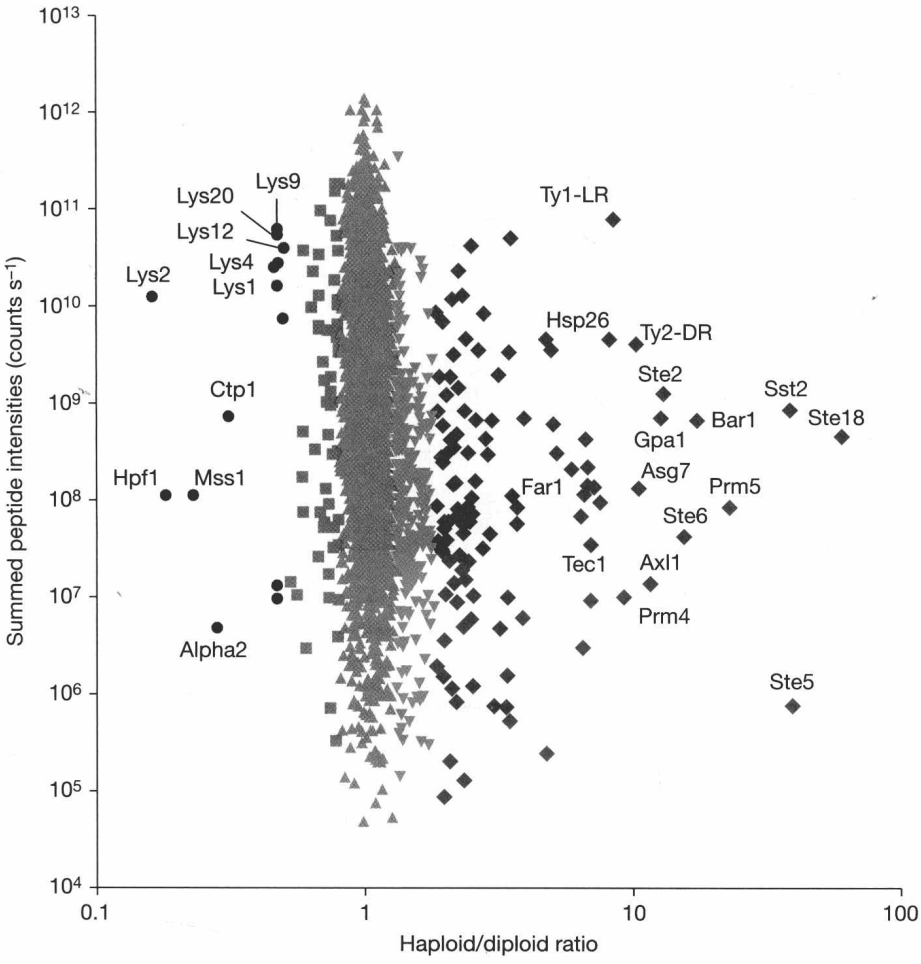


FIGURE 4.4 Two-dimensional DIGE. Overlay image of Cy3- (*green*) and Cy5- (*red*) labeled test-spiked *Erwinia carotovora* proteins. The protein test spikes were three conalbumin isoforms (*arrowheads*) and two myoglobin isoforms (*arrows*). Spots that are of equal intensity between the two channels appear *yellow* in the overlay image. As spike proteins were eight times more abundant in the Cy5 channel, they appear as *red* spots in the overlay. The gel is oriented with the acidic end to the left. (From Lilley KS, Razzaq A & Dupree P (2002) *Curr. Opin. Chem. Biol.* 6, 46. With permission from Elsevier.)



BOX 4.5 FIGURE 2 Quantitative difference between the haploid and diploid yeast proteome (overall fold change). Proteins to the left (becoming deeper *green*) are more strongly represented in haploid cells. Proteins to the right (becoming deeper *red*) are more strongly represented in diploid cells. (From de Godoy LMF, Olsen JV, Cox J et al. (2008) *Nature* 455, 1251–1254. With permission from Macmillan Publishers Ltd.)

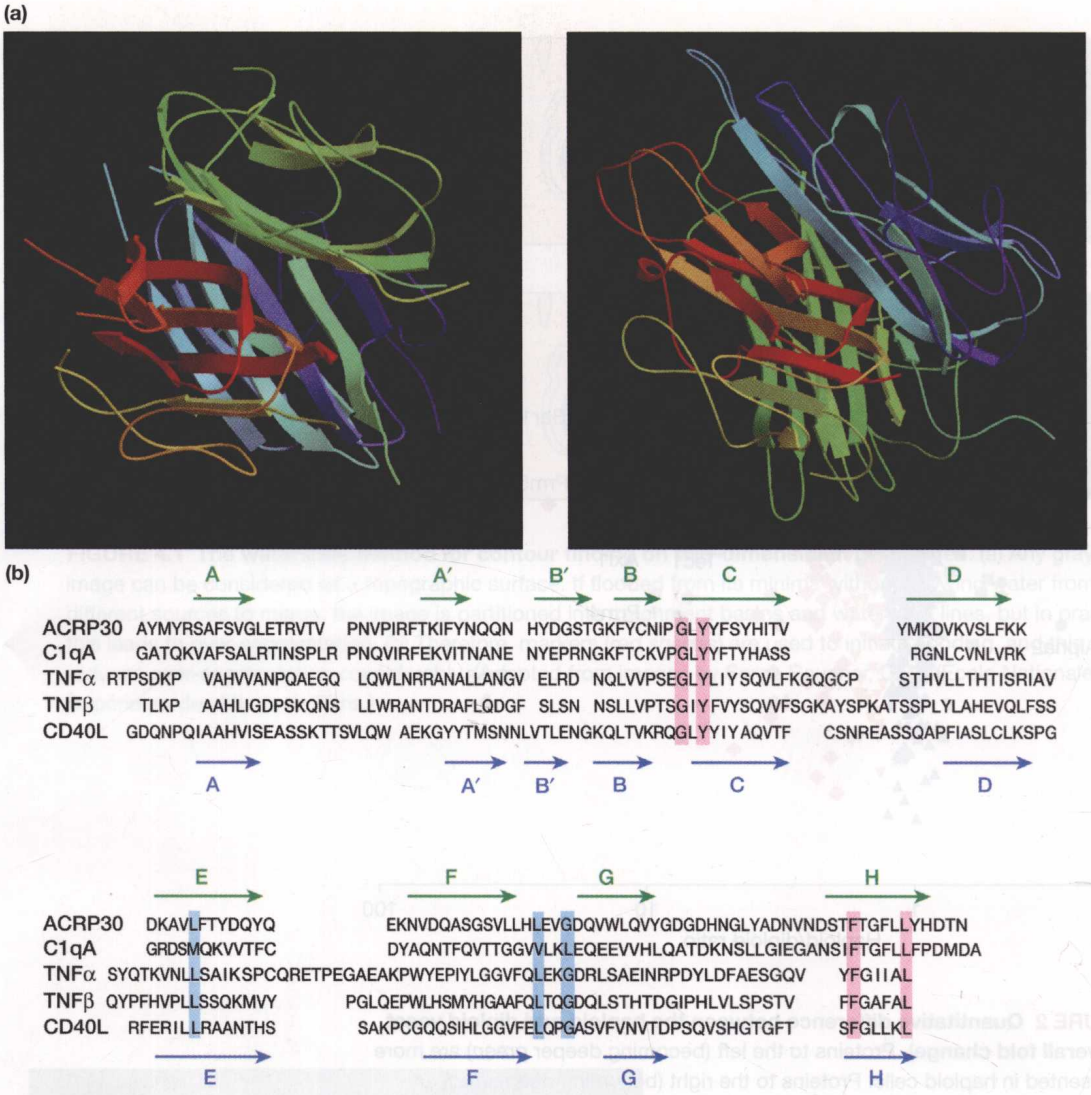


FIGURE 6.1 Identification of related proteins by structural comparison. (a) A ribbon diagram comparison of AdipoQ (left) and TNFα (right). The structural similarity is equivalent to that within the TNF family. (b) Structure-based sequence alignment between several members of the TNF family (CD40L, TNFα, and TNFβ) and two members of the C1q family (C1qA and AdipoQ, the latter labeled ACRP30). Highly conserved residues (present in at least four of the proteins) are shaded, and arrows indicate β-strand regions in the proteins. There is little sequence similarity between AdipoQ and the TNF proteins (for example, 9% identity between AdipoQ and TNFα), so BLAST searches would not identify a relationship. (Adapted from Shapiro L & Harris T (2000) *Curr. Opin. Biotechnol.* 11, 31. With permission from Elsevier. Images courtesy of Protein Data Bank.)

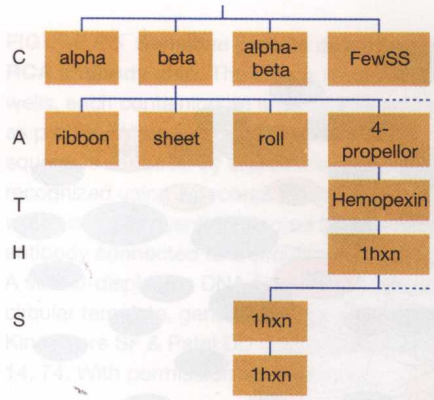
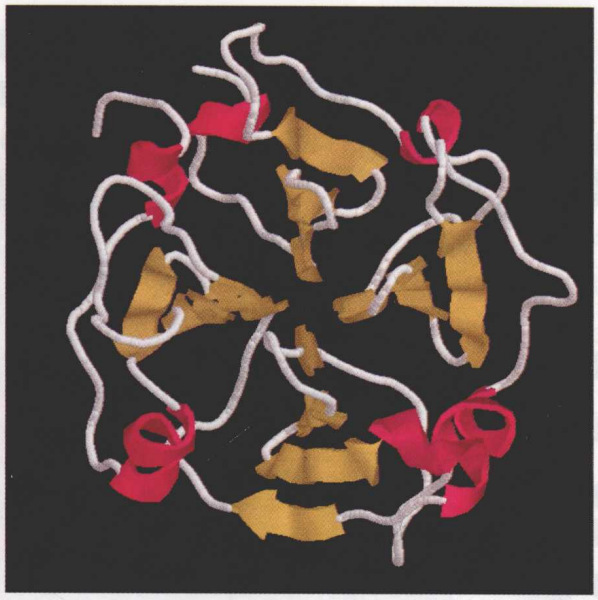


FIGURE 6.8 Structural classification of proteins using the CATH database. The protein shown is hemopexin, a protein rich in β-sheets with few α-helices. (Courtesy of Christine Orengo.)



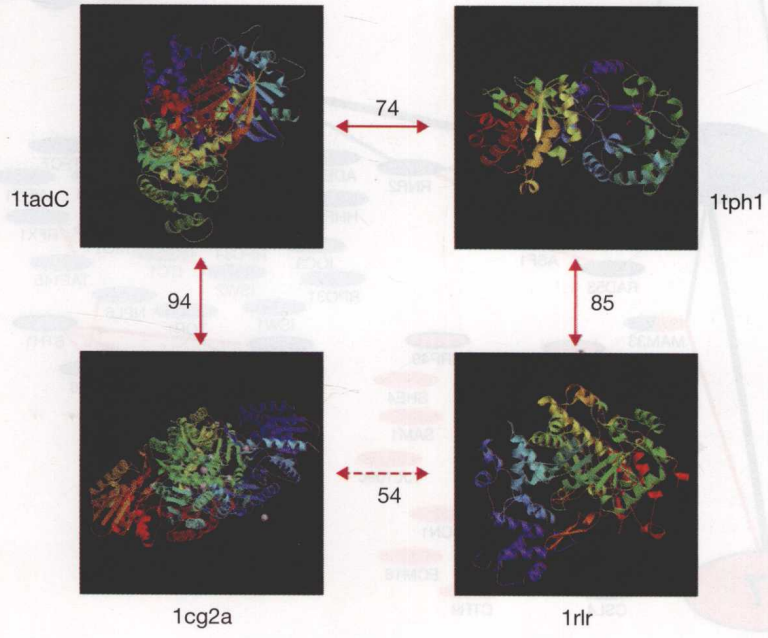


FIGURE 6.9 The Russian doll effect. Four proteins are illustrated that show continuous structural variation over fold space. Each of the proteins shares at least 74 structurally equivalent residues with its nearest neighbor, but the two extreme proteins show only 54 structurally equivalent residues when compared directly. Key: 1cg2a, carboxypeptidase G2; 1tadC, transducin-K; 1tph1, triose phosphate isomerase; 1rlr, ribonucleotide reductase protein R1. (From Domingues FS, Koppensteiner WA & Sippl MJ (2000) *FEBS Lett.* 476, 98. With permission from Elsevier. Images courtesy of Protein Data Bank.)

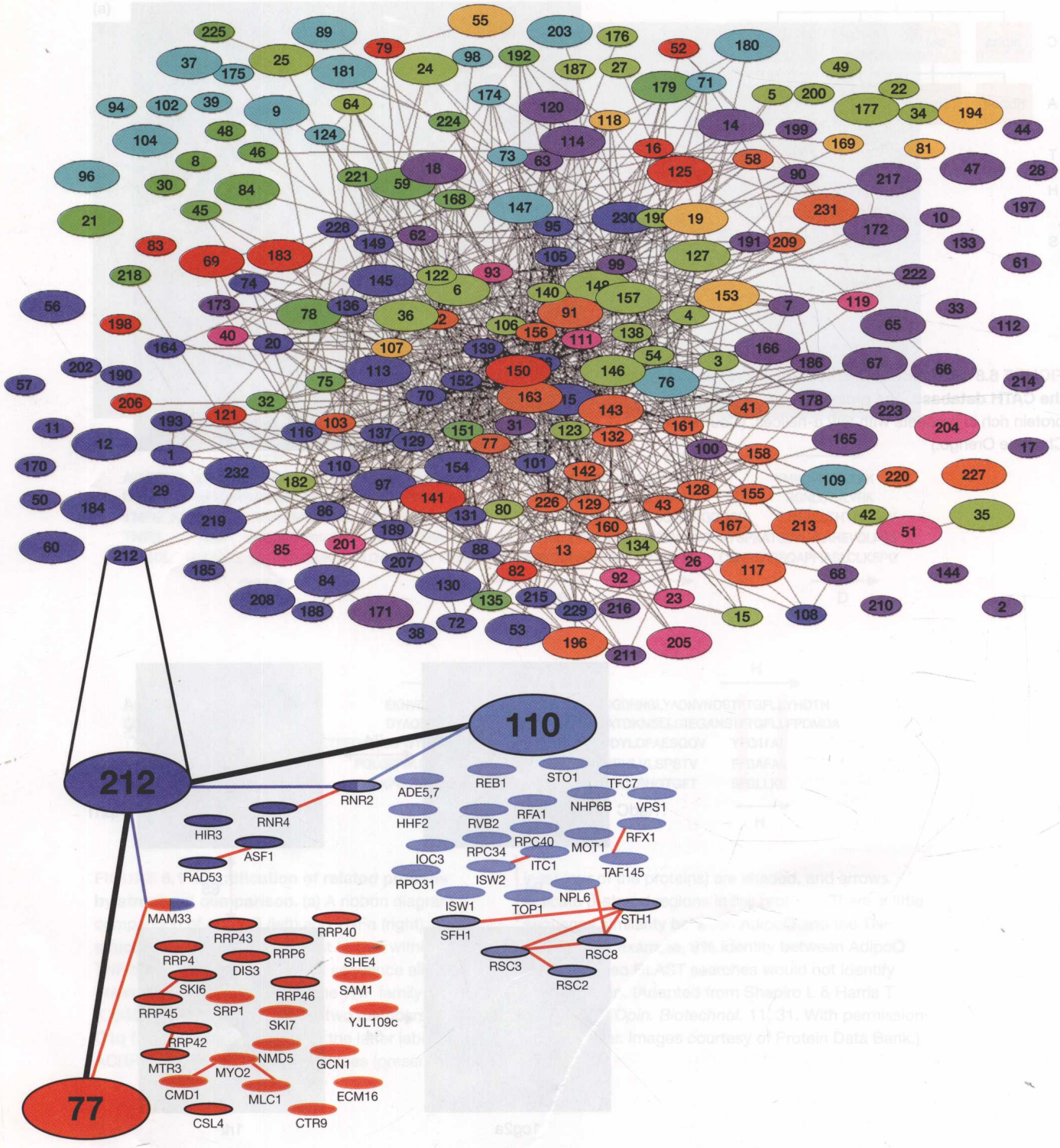


FIGURE 7.19 The protein complex network, and grouping of connected complexes. Links were established between complexes sharing at least one protein. For clarity, proteins found in more than nine complexes were omitted. The graphs were generated automatically by a relaxation algorithm that finds a local minimum in the distribution of nodes by minimizing the distance of connected nodes and maximizing the distance of unconnected nodes. In the upper panel, cellular roles of the individual complexes are color-coded: *red*, cell cycle; *dark green*, signaling; *dark blue*, transcription, DNA maintenance, chromatin structure; *pink*, protein and RNA transport; *orange*, RNA metabolism; *light green*, protein synthesis and turnover; *brown*, cell polarity and structure; *violet*, intermediate and energy metabolism; *light blue*, membrane biogenesis and traffic. The lower panel is an example of a complex (TAP-C212) linked to two other complexes (TAP-C77 and TAP-C110) by shared components. It illustrates the connection between the protein and complex levels of organization. *Red* lines indicate physical interactions as listed in the Yeast Proteome Database. (From Gavin AC, Bösche M, Krause et al. (2002) *Nature* 415, 141. With permission from Macmillan Publishers Ltd.)

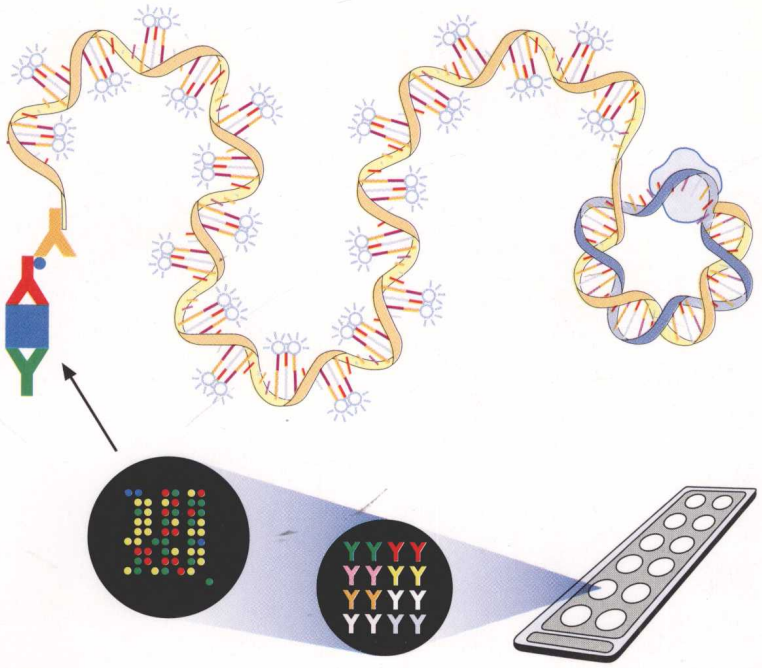


FIGURE 9.5 Sensitive protein detection using the RCA antibody chip. The chip is divided into 16 Teflon wells, each containing an array of 256 antibodies as probes. When a protein, represented by the *blue* square, is captured by one of the probes, it can be recognized using a second, biotinylated antibody (*red*), which is subsequently detected by a tertiary universal antibody connected to a circular oligonucleotide. A strand-displacing DNA polymerase can use this circular template, generating a long concatemer. (From Kingsmore SF & Patel DD (2003) *Curr. Opin. Biotechnol.* 14, 74. With permission from Elsevier.)

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