



FIGURE 5. Example for a classic epitope analysis experiment with monoclonal antibody 755 raised against the human C3a receptor α -chain (Klos et al. 1988). (a) Overview of the three membranes that were analyzed for mapping (116 peptides), sizing (50 peptides), and analoging (120 peptides), summing up 286 peptides for the complete analysis. Order of peptides on the membranes as described in Fig. 3. (b) Sketch of the overlapping peptide strategy for mapping. The three active peptides are marked in bold and their common 9mer sequence is underlined. (c) Spectral diagram display of the results from the sizing membrane. Signal intensity from a peptide spot is displayed at the square corresponding to the amino-terminal residue of the respective peptide and its sequence then reads to the right: the 6mer C***RDEL**C* is the shortest peptide still strongly recognized. (d) Spectral diagram display of the results from the analog membrane. Each square represents the spot intensity obtained from the respective analog peptide. In this particular example, all residues are important for binding and are nonreplaceable except the leucine 5, which tolerates quite a number of other amino acids (but not all), a fact that would have escaped a “glycine or alanine walk” analysis. Dye units = arbitrary scale for relative intensities / C* = Cys(Acm).

Protein–Protein Interactions: A Molecular Cloning Manual, 2nd Ed., © 2005 by Cold Spring Harbor Laboratory Press, Chapter 31, Figure 5.