of the first six N-terminal residues of the two strains. Most of these residues are deeply buried in the antibody-binding groove and establish extensive contacts.

Using the peptide as a guide, a docking complex of a whole protease monomer was generated, which suggests that mAb 1696 inhibits the HIV PR by favouring the dissociation of the active homodimer. A dissociative mechanism of protease inhibition by 1696 is consistent with the stoichiometry of the inhibition complex, as derived from the inhibition kinetic studies [3].

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CRYSTALLOGRAPHIC STUDY OF AN ANTI -CARBONIC ANHYDRASE IX MONOCLONAL ANTIBODY M75

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Carbonic anhydrase IX (CA IX) is a cell surface protein, strongly associated with certain types of human carcinomas. The predicted protein of cloned CA IX cDNA consists of the signal peptide, proteoglycan-related sequence, carbonic anhydrase domain, trasmembrane segment and a short intracellular tail (1, 2). Until now, molecular basis of involvement of CA IX in carcinogenesis has remained unclear. CA IX is a cell adhesion molecule, its carbonic anhydrase (CA) is enzymaticaly active. Structural study of a CA IX-binding monoclonal antibody (mAb) M75, complexed with its epitope peptide may contribute toward elucidation of the role of CA IX. To achieve this goal, two parallel approaches were chosen: analysis of Fab fragment, or of a smaller scFv fragment, both containing the complete antigen binding site present in mAb M75.

Monoclonal antibody M75 was obtained (3) and proved to react excellently with native and denaturated CA IX. Using synthetic oligopeptides, the epitope of mAb M75 was localized in the proteoglycan domain of CA IX, in the region of a tandem repeat and identified as amino acids PGEEDLP (4). The Fab fragment was obtained by papain cleavage. We obtained crystals of free Fab M75 and Fab M75 complexed with two different epitope peptides. The data set for Fab M75 was collected and the structure solving is underway.

Another approach is to prepare sc Fv fragment of this antibody (described in the contribution of Vlastimil Král, Milan Fábry, Magda Hořejší, Jan Zavada, Juraj Sedláček: Molecular cloning, *E. coli* expression and purification of scFv antibody fragments of diagnostic/therapeutic interest.

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A LONG WAY TO WELL DIFFRACTING PROTEIN CRYSTALS

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In experiments of protein crystallization very often spherulites, microcrystals and needles appear as a result of not having optimal crystallization conditions. Optimization of conditions is often guided by a sense of protein, however, theoretical knowledge and practical experiences are inevitable. It is well known that the best conditions for growing crystals differ from crystal nucleation conditions. Separation of these two processes in order to obtain well diffracting crystals will be presented. The importance of protein purity and homogeneity in growing crystals will be stressed and procedures improving crystal quality will be discussed. One of the topics will concern practical aspects for preparation of protein crystals for data collection at room and cryogenic temperature. All these points will be documented by our experiences with crystallization of a number of proteins, their mutants and complexes.