



(86) Date de dépôt PCT/PCT Filing Date: 2001/09/06
(87) Date publication PCT/PCT Publication Date: 2002/03/14
(45) Date de délivrance/Issue Date: 2012/11/27
(85) Entrée phase nationale/National Entry: 2003/03/07
(86) N° demande PCT/PCT Application No.: DK 2001/000580
(87) N° publication PCT/PCT Publication No.: 2002/020804
(30) Priorités/Priorities: 2000/09/08 (DKPA 2000 01343);
2000/11/09 (US60/247,584)

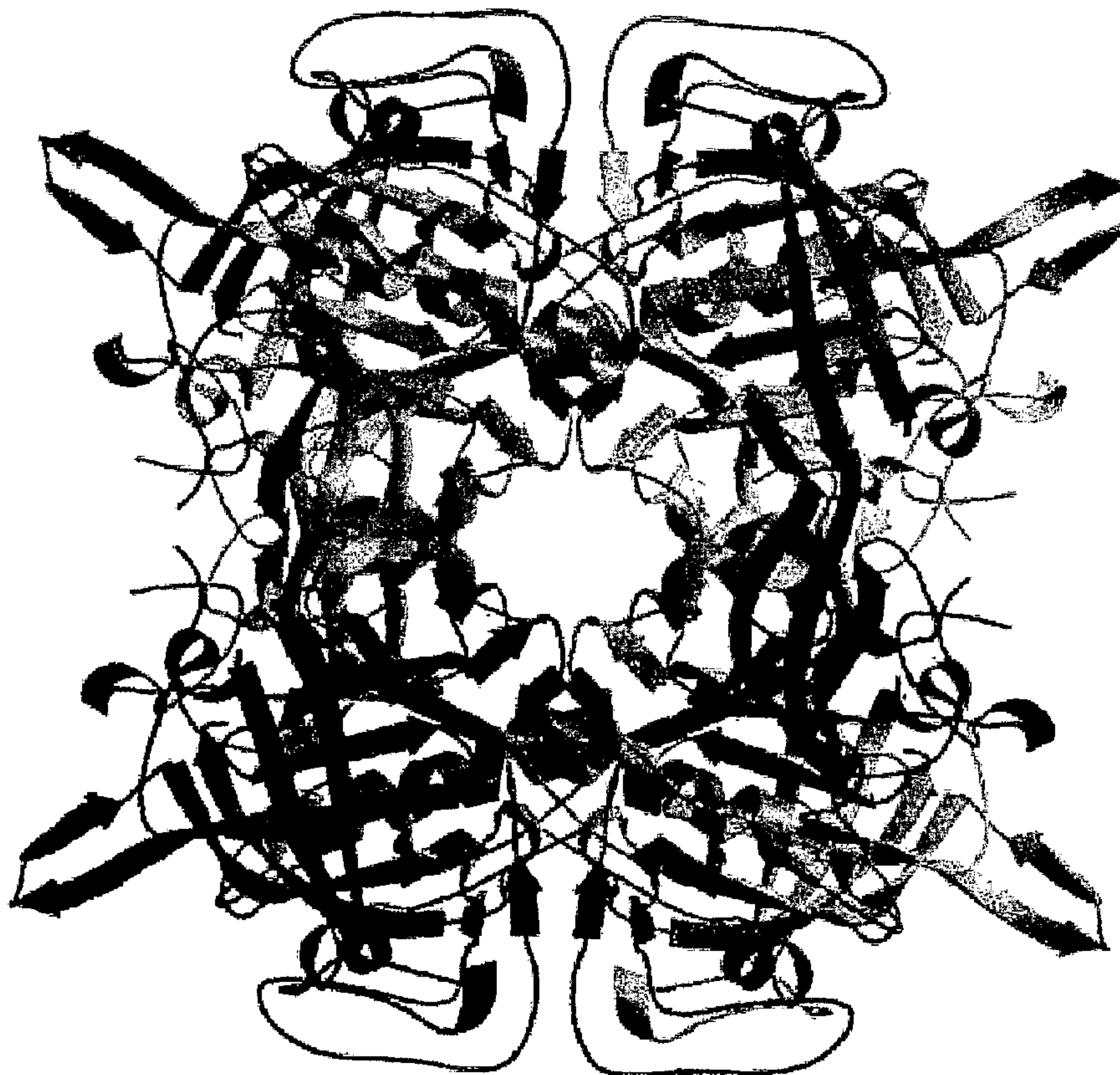
(51) Cl.Int./Int.Cl. *C12N 15/57* (2006.01),
A61P 11/00 (2006.01), *A61P 31/00* (2006.01),
C07K 1/14 (2006.01), *C07K 14/435* (2006.01),
C12N 9/48 (2006.01), *C12Q 1/68* (2006.01)

(72) Inventeurs/Inventors:
OLSEN, JOHAN GOTTHARDT, DK;
KADZIOLA, ANDERS, DK;
DAHL, SOREN WEIS, DK;
LAURITZEN, CONNIE, DK;
LARSEN, SINE, DK;
...

(73) Propriétaire/Owner:
PROZYMEX A/S, DK

(74) Agent: SIM & MCBURNEY

(54) Titre : STRUCTURE CRISTALLINE DU DIPEPTIDYL PEPTIDASE I ET L'UTILISATION DE CELUI-CI
(54) Title: DIPEPTIDYL PEPTIDASE I CRYSTAL STRUCTURE AND ITS USES



(57) Abrégé/Abstract:

The present invention relates to structural studies of dipeptidyl peptidase I (DPPI) proteins, modified dipeptidyl peptidase I (DPPI) proteins and DPPI co-complexes. Included in the present invention is a crystal of a dipeptidyl peptidase I (DPPI) and corresponding



(72) **Inventeurs(suite)/Inventors(continued):** PEDERSEN, JOHN, DK; TURK, DUSAN, SI; PODOBNIK, MARJETKA, SI; STERN, IGOR, SI

(57) **Abrégé(suite)/Abstract(continued):**

structural information obtained by X-ray crystallography from rat and human DPPI. In addition, this invention relates to methods for using structure co-ordinates of DDPI, mutants hereof and co-complexes, to design compounds that bind to the active site or accessory binding sites of DPPI and to design improved inhibitors of DPPI or homologues of the enzyme.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
14 March 2002 (14.03.2002)

PCT

(10) International Publication Number
WO 02/020804 A1(51) International Patent Classification⁷: **C12N 15/57**,
C07K 1/14, A61P 11/00, C12Q 1/68, C07K 14/435, A61P
31/00

(21) International Application Number: PCT/DK01/00580

(22) International Filing Date:
6 September 2001 (06.09.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PA 2000 01343 8 September 2000 (08.09.2000) DK
60/247,584 9 November 2000 (09.11.2000) US

Gotthardt [DK/DK]; Blågårdsgade 25, 3. tv, DK-2200 Copenhagen N (DK). **KADZIOLA, Anders** [DK/DK]; Jomsborgvej 9, 2. th, DK-2900 Hellerup (DK). **DAHL, Søren, Weis** [DK/DK]; Bolbrovej 46, DK-2960 Rungsted Kyst (DK). **LAURITZEN, Connie** [DK/DK]; Axelhøj 59, DK-2610 Rødovre (DK). **LARSEN, Sine** [DK/DK]; Stumpedyssevej 37, DK-2970 Hørsholm (DK). **PEDERSEN, John** [DK/DK]; Skovparken 8, DK-2990 Nivå (DK). **TURK, Dusan** [SI/SI]; Povsetova 33, 1000 Ljubljana (SI). **PODOBNIK, Marjetka** [SI/SI]; Polje c. XXX/19, 1260 Ljubljana-Polje (SI). **STERN, Igor** [SI/SI]; Kvedrova cesta 34, 8290 Sevnica (SI).

(74) Agent: **PLOUGMANN & VINGTOFT A/S**; Sankt Annæ Plads 11, P.O. Box 3007, DK-1021 Copenhagen K (DK).(71) Applicant (for all designated States except US):
PROZYMEX A/S [DK/DK]; Dr. Neergaards Vej 17,
DK-2970 Hørsholm (DK).

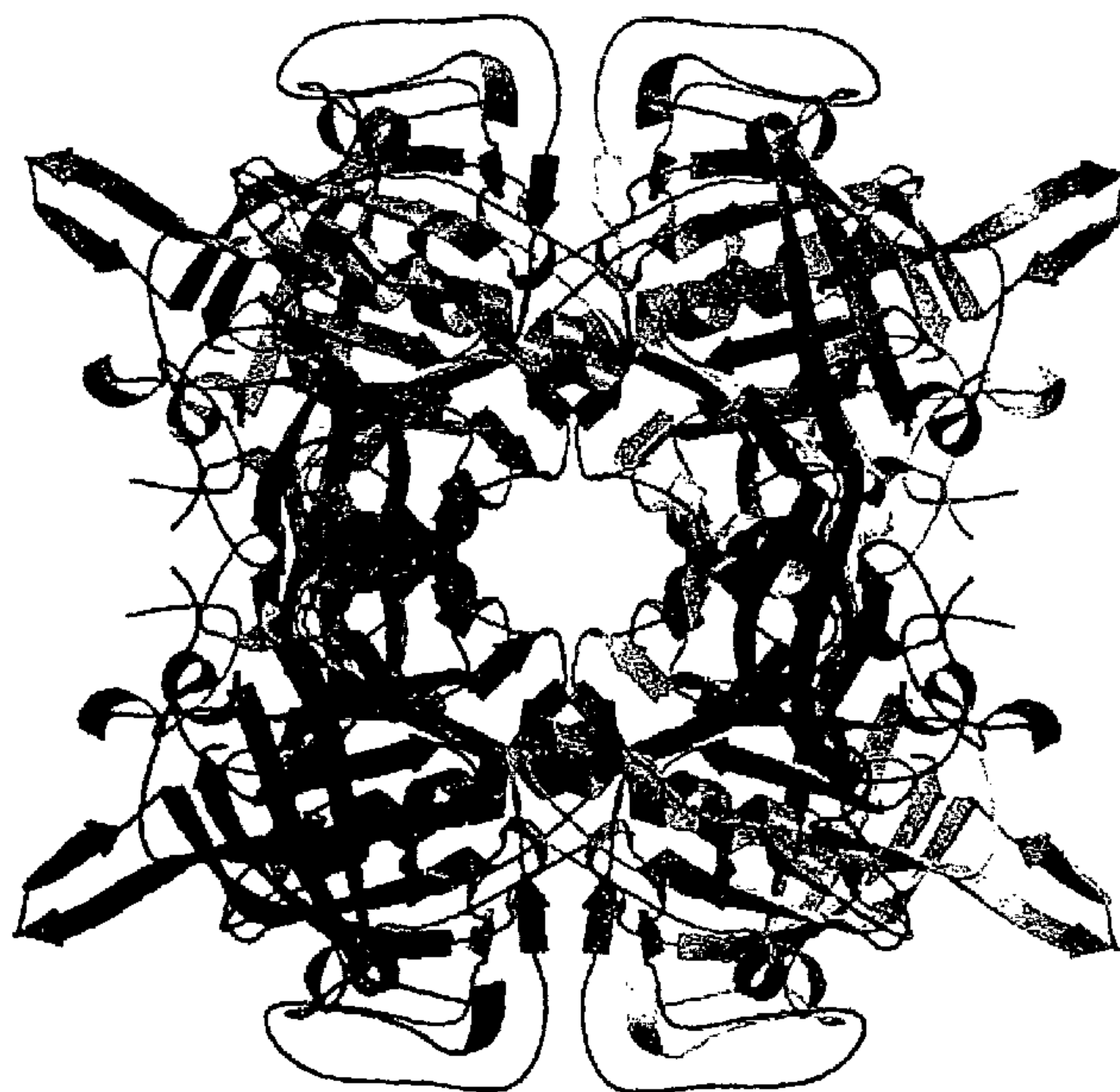
(72) Inventors; and

(75) Inventors/Applicants (for US only): **OLSEN, Johan,**

(81) Designated States (national): AE, AG, AL, AM, AT (utility model), AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ (utility model), DE (utility model), DK (utility model), DM, DZ, EC, EE (utility model), ES, FI (utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU,

[Continued on next page]

(54) Title: RAT CATHEPSIN, DIPEPTIDYL PEPTIDASE I (DPPI): CRYSTAL STRUCTURE, INHIBITORS AND ITS USES



(57) **Abstract:** The present invention relates to structural studies of dipeptidyl peptidase I (DPPI) proteins, modified dipeptidyl peptidase I (DPPI) proteins and DPPI co-complexes. Included in the present invention is a crystal of a dipeptidyl peptidase I (DPPI) and corresponding structural information obtained by X-ray crystallography from rat and human DPPI. In addition, this invention relates to methods for using structure co-ordinates of DPPI, mutants hereof and co-complexes, to design compounds that bind to the active site or accessory binding sites of DPPI and to design improved inhibitors of DPPI or homologues of the enzyme.

WO 02/020804 A1

WO 02/020804 A1

LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH,
PL, PT, RO, RU, SD, SE, SG, SI, SK (utility model), SL,
TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

— *with amended claims*

Date of publication of the amended claims: 25 July 2002

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

(48) Date of publication of this corrected version:

6 September 2002

(15) Information about Correction:

see PCT Gazette No. 36/2002 of 6 September 2002, Section II

Published:

— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A crystalline form of a dipeptidyl peptidase I-like (DPPI-like) protein, which has a crystal structure for which the structural coordinates of the back bone nitrogen, alpha-carbon and carbonyl carbon atoms of said DPPI-like protein have a root-mean-square deviation from the structural coordinates of the equivalent back bone atoms of rat DPPI of less than 2Å following structural alignment of equivalent back bone atoms, wherein
 - said DPPI-like protein, consists of a residual pro-part domain and of a catalytic domain, where said catalytic domain has structural homology to papain and consists of a light and a heavy chain,
 - said residual pro-part domain folds up as an up-and-down beta-barrel composed of eight anti-parallel beta-strands wrapped around a hydrophobic core,
 - the N-terminal residue of the residual pro-part donates a negative charge in a fixed position within the active site cleft as exemplified for rat DPPI where the free amino group of Asp1 is held in position through a hydrogen bond to the backbone oxygen atom of Asp274 and for human DPPI where the free amino group of Asp1 is held in position through a hydrogen bond to the backbone oxygen atom of Glu275, and wherein
 - said DPPI-like protein has an overall amino acid sequence that is at least 75% identical to the amino acid sequence of mature rat DPPI set forth in SEQ ID NO.1, amino acid residues 25-142, 228-389, and 395-462, and includes an amino acid sequence that is at least 30% identical to the residual pro-part domain of rat DPPI defined by amino acid residues 25-142 in SEQ ID NO: 1.
2. A crystalline form of a dipeptidyl peptidase I-like (DPPI-like) protein, which has a crystal structure for which the structural coordinates of the back bone nitrogen, alpha-carbon and carbonyl carbon atoms of said DPPI-like protein have a root-mean-square deviation from the structural coordinates of the equivalent back bone atoms of rat or human DPPI of less than 1.5Å following structural alignment of equivalent back bone atoms, wherein said DPPI-like protein, consists of a residual pro-part domain and of a

catalytic domain, where said catalytic domain has structural homology to papain and consists of a light and a heavy chain,

- said residual pro-part domain folds up as an up-and-down beta-barrel composed of eight anti-parallel beta-strands wrapped around a hydrophobic core,
- the N-terminal residue of the residual pro-part donates a negative charge in a fixed position within the active site cleft as exemplified for rat DPPI where the free amino group of Asp1 is held in position through a hydrogen bond to the backbone oxygen atom of Asp274 and for human DPPI where the free amino group of Asp1 is held in position through a hydrogen bond to the backbone oxygen atom of Glu275, and wherein
- said DPPI-like protein has an overall amino acid sequence that is at least 75% identical to the amino acid sequence of mature rat DPPI set forth in SEQ.ID.NO.1, amino acid residues 25-142, 228-389, and 395-462, and includes an amino acid sequence that is at least 30% identical to the residual pro-part domain of rat DPPI defined by amino acid residues 25-142 in SEQ ID NO: 1.

3. A crystalline form according to claim 1 or 2, wherein the DPPI-like protein has a tetrameric structure with a shape of a slightly flattened sphere with a diameter of approximately 80Å and a spherical cavity with a diameter of about 20Å in the middle.
4. A crystalline form according to any one of claims 1 to 3 comprising a protein, characterised by a space group P6₄22 and unit cell dimensions $a = 166.24\text{\AA}$ $b = 166.24\text{\AA}$ $c = 80.48\text{\AA}$.
5. A crystalline form according to any one of claims 1 to 4, wherein the free amino group of a conserved Asp1 is held in position by a hydrogen bond to the backbone carbonyl oxygen atom of Asp274.
6. A crystalline form according to claim 5, further characterised by the delocalised negative charge that said Asp1 residue carries under physiological conditions on its OD1 and OD2 oxygen atoms which are localised about 7-9Å from the sulphur atom of the catalytic Cys233 residue.
7. A crystalline form according to any one of claims 1 to 3, comprising a protein

characterised by a space group I222 and unit cell dimensions $a = 87.154\text{\AA}$ $b = 88.031\text{\AA}$ $c = 114.609\text{\AA}$.

8. A crystalline form according to any one of claims 1 to 3 and 7, wherein the free amino group of a conserved Asp1 is held in position by a hydrogen bond to the backbone carbonyl oxygen atom of Glu275.

9. A crystalline form according to any one of claims 1 to 3, 7 and 8, further characterised by the delocalised negative charge that said residue carries under physiological conditions on its ODI and OD2 oxygen atoms which are localised about 7-9Å from the sulphur atom of the catalytic Cys234 residue.

10. A crystalline form according to any one of claims 1 to 9, in which said molecule is derived from a DPPI polypeptide which is mutated prior to being crystallised.

11. A crystalline form according to any one of claims 1 to 10, in which said molecule is a chemically modified DPPI protein.

12. A crystalline form according to any one of claims 1 to 11, in which said molecule is an enzymatically modified DPPI protein.

13. A crystalline form according to any one of claims 1 to 12, which is in a covalent or non-covalent association with at least one other molecule or molecular complex.

14. A crystalline form according to any one of claims 1 to 13, which is complexed with a co-factor.

15. A crystalline form according to any one of claims 1 to 14, which is complexed with a halide.

16. A crystalline form according to claim 15, which is complexed with a chloride.

17. A heavy atom derivative of a crystalline form according to any one of claims 1 to 16.

18. A method for selecting, testing and/or rationally or semi-rationally designing a chemical compound which binds covalently or non-covalently to mature dipeptidyl peptidase I (DPPI) molecules or DPPI molecular complexes, characterised by applying in a computational analysis structure co-ordinates of a crystalline form according to any one of claims 1 to 17, and subsequently synthesizing or isolating/obtaining said chemical compound on the basis of the results of said analysis.

19. A method for identifying a potential inhibitor of mature dipeptidyl peptidase I (DPPI) molecules or DPPI molecular complexes, comprising the following steps:

- (a) using the atomic co-ordinates of a crystalline form according to any one of claims 1 to 17 to define the catalytic active sites and/or an accessory binding site of said enzyme,
- (b) identifying a compound that fits the active site and/or an accessory binding site of (a),
- (c) obtaining the compound, and
- (d) contacting the compound with a DPPI or DPPI-like protein to determine the binding properties and/or effects of said compound on and/or the inhibition of the enzymatic activity of DPPI by said compound.

20. A method for selecting, testing and/or rationally or semi-rationally designing a modified mature dipeptidyl peptidase I (DPPI) molecule or DPPI molecular complex, characterised by applying in a computational analysis structure co-ordinates of a crystalline form according to any one of claims 1 to 17, and subsequently preparing said modified DPPI molecule on the basis of the results of said analysis.

1/21

LOCUS NM_017097 1850 bp mRNA ROD 08-JUN-2000
DEFINITION Rattus norvegicus Cathepsin C (dipeptidyl peptidase I) (Ctsc), mRNA.
ACCESSION NM_017097
VERSION NM_017097.1 GI:8393217
KEYWORDS .
SOURCE Norway rat.
ORGANISM Rattus norvegicus
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Rattus.
REFERENCE 1 (bases 1 to 1850)
AUTHORS Ishidoh,K., Munõ,D., Sato,N. and Kominami,E.
TITLE Molecular cloning of cDNA for rat cathepsin C. Cathepsin C, a cysteine proteinase with an extremely long propeptide
JOURNAL J. Biol. Chem. 266 (25), 16312-16317 (1991)
MEDLINE 91358405
COMMENT REFSEQ: The reference sequence was derived from D90404.1.
PROVISIONAL RefSeq: This is a provisional reference sequence record that has not yet been subject to human review. The final curated reference sequence record may be somewhat different from this one.
FEATURES
Location/Qualifiers
source 1..1850
/organism="Rattus norvegicus"
/strain="W"
/db_xref="taxon:10116"
/clone="lambda C14"
/dev_stage="kidney"
gene 1..1850
/gene="Ctsc"
/note="CATC"
/db_xref="LocusID:25423"
/db_xref="RATMAP:41308"
sig_peptide 58..141
CDS 58..1446
/gene="Ctsc"
/EC_number="3.4.14.1"
/codon_start=1
/product="cathepsin C (dipeptidyl peptidase I)"
/protein_id="NP_058793.1"
/db_xref="GI:8393218"
/translation="MGPWTHSLRAALLLVLLGVCTVSSDTPANCTYPDLLGTWVFQVG
PRHPRSHINCSVMEPTEEKVVIHLKKLDTAYDEVGNSGYFTLIYNQGFIVLNDYKWF
AFFKYEYVKGSRASISYCHETMTGWVHDVLRNWACFVGKKMANHSEKVYVNVÁHLGGLO
EKYSERLYSHNHNFFVKAINSVQKSWTATTYEEYEKLSIRDLIRRSRSGHSGRILRPKPAP
ITDEIQQQILSLPESWDWRNVRGINFVSPVRNQESCGSCYSFASLGMLEARIRILTNN
SQTPILSPQEVVSCSPYAQGCDDGGFPYLIAGKYAQDFGVVEENCFFYTATDAPCKPKE
NCLRYYSSEYYYVGGFYGGCNEALMKLELVKHGPMVAFAFEVHDDFLHYHSGIYHHTGL
SDPFNPFELTNHAVLLVGYGKDPVTGLDYWIVKNSWGSQWGESGYFRIRRGTDCAIE
SIAMAAIPIPKL"
mat_peptide 142..1443
/product="cathepsin C propeptide"
mat_peptide 745..1443
/product="cathepsin C mature peptide"
polyA_signal 1831..1836
BASE COUNT 500 a 416 c 417 g 517 t
ORIGIN
1 gaattccggt tctagttggt gttttctctg ccatctgctc tccgggcgcc gtcaaccatg
61 ggtccgtgga cccactcctt gcgcgccgcc ctgctgctgg tgcttttggg agtctgcacc

Fig. 1

Fig. 1

2/21

```

121 gtgagctccg acactcctgc caactgcact taccctgacc tgctgggtac ctggggttttc
181 caggtgggcc ctgacatcc ccgaagtcac attaatgct cggtaatgga accaacagaa
241 gaaaaggtag tgatacacct gaagaagttg gatactgcct atgatgaagt gggcaattct
301 gggatattca ccctcattta caaccaaggc tttgagattg tggtgaatga ctacaagtgg
361 tttgcgtttt tcaagtatga agtcaaaggc agcagagcca tcagttactg ccatgagacc
421 atgacagggg ggggtccatga tgtcctgggc cggaactggg cttgctttgt tggcaagaag
481 atggcaaate actctgagaa ggtttatgtg aatgtggcac acctggagg tctccaggaa
541 aaatattctg aaaggctcta cagtcacaac cacaactttg tgaaggccat caattctgtt
601 cagaagtctt ggactgcaac cacctatgaa gaatatgaga aactgagcat acgagatttg
661 ataaggagaa gtggccacag cggaaggatc ctaaggccca aacctgccc gataactgat
721 gaaatacagc aacaaatttt aagtttgcca gaatcttggg actggagaaa cgtccgtggc
781 atcaattttg ttagccctgt tcgaaaccaa gaatcttgtg gaagctgcta ctcatctgcc
841 tctctgggta tgctagaagc aagaattcgt atattaacca acaattctca gacccaatc
901 ctgagtcctc aggaggttgt atcttgtagc ccgtatgcc aaggttgtga tgggtggattc
961 ccatacctca ttgcaggaaa gtatgcccaa gattttgggg tgggtggaaga aaactgcttt
1021 ccctacacag ccacagatgc tccatgcaaa ccaaaggaaa actgcctccg ttactattct
1081 tctgagtact actatgtggg tggtttctat ggtggctgca atgaagccct gatgaagctt
1141 gagctggtca aacacggacc catggcagtt gcctttgaag tccacgatga cttcctgcac
1201 taccacagtg ggatctacca ccacactgga ctgagcgacc ctttcaaccc ctttgagctg
1261 accaatcatg ctgttctgct tgtgggctat ggaaaagatc cagtcactgg gttagactac
1321 tggattgtca agaacagctg gggctctcaa tggggtgaga gtggctactt ccggatccgc
1381 agaggaaact atgaatgtgc aattgagagt atagccatgg cagccatacc gattcctaaa
1441 ttgtaggacc tagctcccag tgtcccatac agctttttat tattcacagg gtgatttagt
1501 cacaggctgg agacttttac aaagcaatat cagaagctta ccactaggta cccttaaaga
1561 attttgccct taagtttaaa acaatccttg atttttttct tttaatatcc tccctatcaa
1621 tcaccgaact acttttcttt ttaaagtact tggttaagta atacttttct gaggattggg
1681 tagatattgt caaatatttt tgctggtcac ctaaaatgca gccagatggt tcattgttaa
1741 aaatctatat aaaagtgcaa gctgcctttt ttaaattaca taaatcccat gaatacatgg
1801 ccaaaatagt tattttttta agacttttaa ataaatgatt aatcgatgct

```

Fig. 1 (continued)

Fig. 1 (continued)

3/21

CLUSTAL W (1.81) multiple sequence alignment of known proDPPI sequences,
Aug 2000

Scoring matrix: blosum

Opening gap penalty: 10

End gap penalty: 10

Extending gap penalty: 1.0

Gap separation penalty: 1

Rat	DTPANCTYPDLLGTWVFQVGPRHPRSHINCSVMEPTTEKVVVHLKKLDTAYDEVGNSGYF	60
Human	DTPANCTYLDLLGTWVFQVGSSGSQRDVNCSVMGPQEKVVVYLQKLDAYDDLGNNGHF	60
Dog	DTPANCTHPELLGTWVFQVGPGAS-RSVNCSVMGPPEKKVVVHLEKLDAYDNFGNTGHF	59
Bovine	DTPANCTYPDLLGTWVFQVGSSGSQRDVNCSVMGPPEKKVVVHLKKLDTAYDDFGNSGHF	60
Mouse	DTPANCTYPDLLGTWVFQVGPRSSRSINCSVMEATEEKVVVHLKKLDTAYDELGNNGHF	60
Chicken	-----AQDSLGNFGFF	11
Winter flounder	-----	
Zebrafish	-TPANCTYEDLLGTWIFSVSNVGQDKTINCSTGQTVSTVTVDLQKLSVAVDDLGHGTGF	59
S. japonicum	DTPANCSYMDAIGHWIFHVS----RYKTKCTKQLDVSQTFSMNVQYPNIVTDSYGNMGKW	56
S. mansoni	DTPANCTYEDAHGRWKFHIG----DYQSKCPEKLNSKQSVVISLLYPDIAIDFEGNRGHW	56
Rat	TLIYNQGFIEVLNDYKWFAFFKYEVKGSRAISYCHETMTGWVHDLGRNWACFVGKKMAN	120
Human	TIIYNQGFIEVLNDYKWFAFFKYKEEGSKVTTCNETMTGWVHDLGRNWACFTGKKVGT	120
Dog	TIIYNQGFIEVLNDYKWFAFFKYKEEGHKVTSYCHETMTGWVHDLGRNWACFTGKMG	119
Bovine	TIIYNQGFIEVLNDYKWFAFFKYKEEGKVTSYCHETMTGWVHDLGRNWACFTGRKTGN	120
Mouse	TLIYNQGFIEVLNDYKWFAFFKYEVGRHTAISYCHETMTGWVHDLGRNWACFVGKKVES	120
Chicken	TLIYNQGFIEVLNNYKWFAFFKYKKEGLNVTSYCHETMTGWVHDLGRNWACFTGQKISS	71
Winter flounder	-----NSARVINGYKWFAFFKYSEGGPTVTSYCDQTMPGWVHDLGNWACFVGKKVKP	54
Zebrafish	TLIYNQSFVXVINDYKWFGFFKYTHHGSQEVSYCDQTLPGXVHDLVSNXACNTGKKVQT	119
S. japonicum	TLIYNQGFIEITMNRKWLIMFAYGPNN---TYTCNKSMMPMWTHTDLCQWHCFTATKVN	113
S. mansoni	TLIYNQGFIEVTINHRKWLVI FAYKSNG---EFNCHKSMMPMWTHTDLIDSGSVCSGKIGV	113
Rat	HSEKVVNVVAHLGGLQEKYSERLYSHNHNFKAINSVQKSWTATTYEEYEKLSIRDLIRR	180
Human	ASENVYVNTAHLKNSQEKYSNRLYKYDHNFKAINAIQKSWTATTYMEYETLTLGDMIRR	180
Dog	TSEKAKVNTKHIERLQENNSNRLYKYNYEFVKAINTIQKSWTATRYIEYETLTLRDMTR	179
Bovine	TSENVNVNTARLAGLEETYSNRLYRYNHDFVKAINAIQKSWTAAPYMEYETLTLKEMIRR	180
Mouse	HIEKVNMAAHLGGLQERYSERLYTHNHNFKAINTVQKSWTATAYKEYEKMSLRDLIRR	180
Chicken	SSSDVHVRQLPLQKPRVGLSSRRFVHNDFVNAINAHQKSWRATRYEEYENFSLEELTR	131
Winter flounder	VPPRV DYKPLFSSR----LLQKPKNNMDFIDSINSVQSSWKAVAYPEHETFTLQELQR	110
Zebrafish	-----	
S. japonicum	FORMIEYKSPVLQ----LDGNQLYKVDTKFIKAINAKQNSWKATIYPEYSKYTIKEMRR	169
S. mansoni	DKFHINKLFGSKS----FG-RTLYHINPSFVGKINAHQKSWRGEIYPELSKYTIDELNR	168
Rat	SG-----HSGRIILRPKPAPITDEIQQILSLPESWDWRN--VRGINFVSPVRNQESCGSC	233
Human	SG-----GHSRKIPRPKPAPLTAEIQQILHLPTSWDWRN--VHGINFVSPVRNQASCGSC	234
Dog	VG-----GRKIPRPKPPTLTAEIHEEISRLPTSWDWRN--VRGTNFVSPVRNQASCGSC	231
Bovine	GG-----GHSRRIPRPKPAPITAEIQKILHLPTSWDWRN--VHGINFVTPVRNQSGCGSC	234
Mouse	SG-----HSQRIPRPKPAPMTDEIQQILNLPESWDWRN--VQGVNYVSPVRNQESCGSC	233
Chicken	AGG---LYSRT-SRPKPAPLTPELLKKFRLTXS-WDWRN--VNGVNYVX--RNNPVX-RY	181
Winter flounder	AGG---PASRVPMRVRPMPVRAGVAKMAAALPERFDWRN--VGGVNFLSPVRNQASCGSC	165
Zebrafish	-----	
S. japonicum	AGGSRSFAKQNVQLPKKNLTSAMMLELLALPKEFDWVNRPEGLRSPVTPVRNQKTCGSC	229
S. mansoni	AGGVKSMVTRPSVLN-RKTPSKELISLTGNLPLEFDWTSPPDGSRSPTPIRNQIGICGSC	227

Fig. 2

Fig. 2

4/21

Rat	YSFASLGMLEARIRILTNNSTPILSPQEVVSCSPYAQGCDDGGFPYLIAGKYAQDFGVVE	293
Human	YSFASMGMLEARIRILTNNSTPILSPQEVVSCSQYAQGCDDGGFPYLIAGKYAQDFGLVE	294
Dog	YAFASAMLEARIRILTNNSTPILSPQEVVSCSQYAQGCDDGGFPYLIAGKYAQDFGLVE	291
Bovine	YSFASMGMLEARIRILTNNSTPILSPQEVVSCSQYAQGCDDGGFPYLIAGKYAQDFGLVE	294
Mouse	YSFASMGMLEARIRILTNNSTPILSPQEVVSCSPYAQGCDDGGFPYLIAGKYAQDFGVVE	293
Chicken	HCSWHAEQILSKTPRAS-----	198
Winter flounder	YSFAAMGDVXGSHPKSSPNNXAPILQSR-----	194
Zebrafish	-----	
S.japonicum	YAFASAAIEARIRLASRFLQPIILSPQDIIDCSPYSEGCDGGFPYLVAGKHGEDFGFVE	289
S.mansoni	YASPSAAALEARIRLVSNFSEQPILSPQTVVDCSPYSEGCGGFPFLIAGKYGEDFGLPQ	287

Rat	ENCFPYTATDA-PCKPKENCLRYYSSEYYYVGGFYGGCNEALMKLELVKHGPMMAVAFEVH	352
Human	EACFPYTGTDS-PCKMKEDCFRYYSEYHYVGGFYGGCNEALMKLELVHHGPMMAVAFEVY	353
Dog	EACFPYAGSDS-PCKPND-CFRYYSEYHYVGGFYGGCNEALMKLELVRHGPMMAVAFEVY	349
Bovine	EDCFPYTGTDS-PCRLKEGCFRYYSEYHYVGGFYGGCNEALMKLELVHQGPMMAVAFEVY	353
Mouse	ESCFPYTAKDS-PCKPRENCLRYYSDDYYYVGGFYGGCNEALMKLELVKHGPMMAVAFEVH	352
Chicken	-----	
Winter flounder	-----	
Zebrafish	-----	
S.japonicum	EKCNPYTGKSGTCNKLLGCTRYTTDYHYIGGYGATNEDLMKLELVKNGPFPVGFVEY	349
S.mansoni	KIVIPYTGEDTGKCTVSKNCTRYTTDYSYIGGYGATNEKLMQLELISNGPFPVGFVEY	347

Rat	DDEFHYHSGIYHHTGLS---DPFNPFEELTNHAVLLVGYGKDPVTGLDYWIVKNSWGSQWG	409
Human	DDEFHYKKGIYHHTGLR---DPFNPFEELTNHAVLLVGYGTDSASGMDYWIVKNSWGTGWG	410
Dog	DDEFHYQKGIYHHTGLR---DPFNPFEELTNHAVLLVGYGTDSASGMDYWIVKNSWGSRWG	406
Bovine	DDEFHYRKGVIYHHTGLR---DPFNPFEELTNHAVLLVGYGTDAASGLDYWIVKNSWGTSWG	410
Mouse	DDEFHYHSGIYHHTGLS---DPFNPFEELTNHAVLLVGYGRDPVTGIEYWIKNWGSNNG	409
Chicken	-----	
Winter flounder	-----	
Zebrafish	-----	
S.japonicum	GDFLQYKSGVYSHTDIINNHHFNPFEELTNHAVLLVGYGIDNSSNLPYWKIKNSWGQYWG	409
S.mansoni	EDFQFYKEGIYHHTTVQTDHYNFNPFEELTNHAVLLVGYGVDKLSGEPYWKVKNWGVVEWG	407

Rat	ESGYFRIRRGTDCAIESIAMAAIPIPKL	438
Human	ENGYFRIRRGTDCAIESIAVAATPIPKL	439
Dog	EDGYFRIRRGTDCAIESIAVAATPIPKL	435
Bovine	ENGYFRIRRGTDCAIESIALAATPIPKL	439
Mouse	ESGYFRIRRGTDCAIESIAVAIPIPKL	438
Chicken	-----	
Winter flounder	-----	
Zebrafish	-----	
S.japonicum	EEGYFRILRGSDCEGVQSIAIKFDVVL--	436
S.mansoni	EQGYFRILRGTDCEGVESLGVRFPVVL--	434

Fig. 2 (continued)

Fig. 2 (continued)

5/21

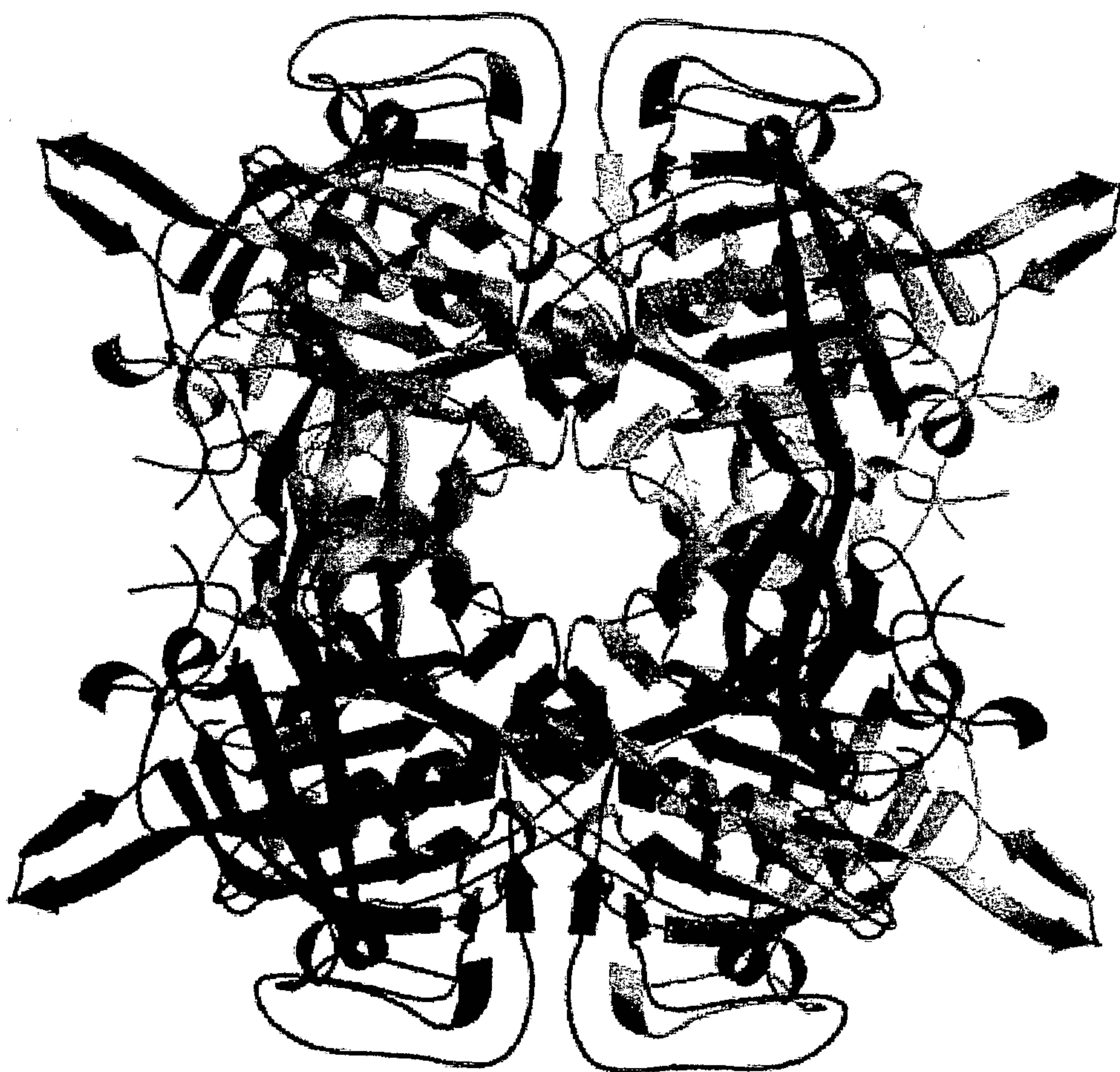


Fig. 3

Fig. 3

6/21

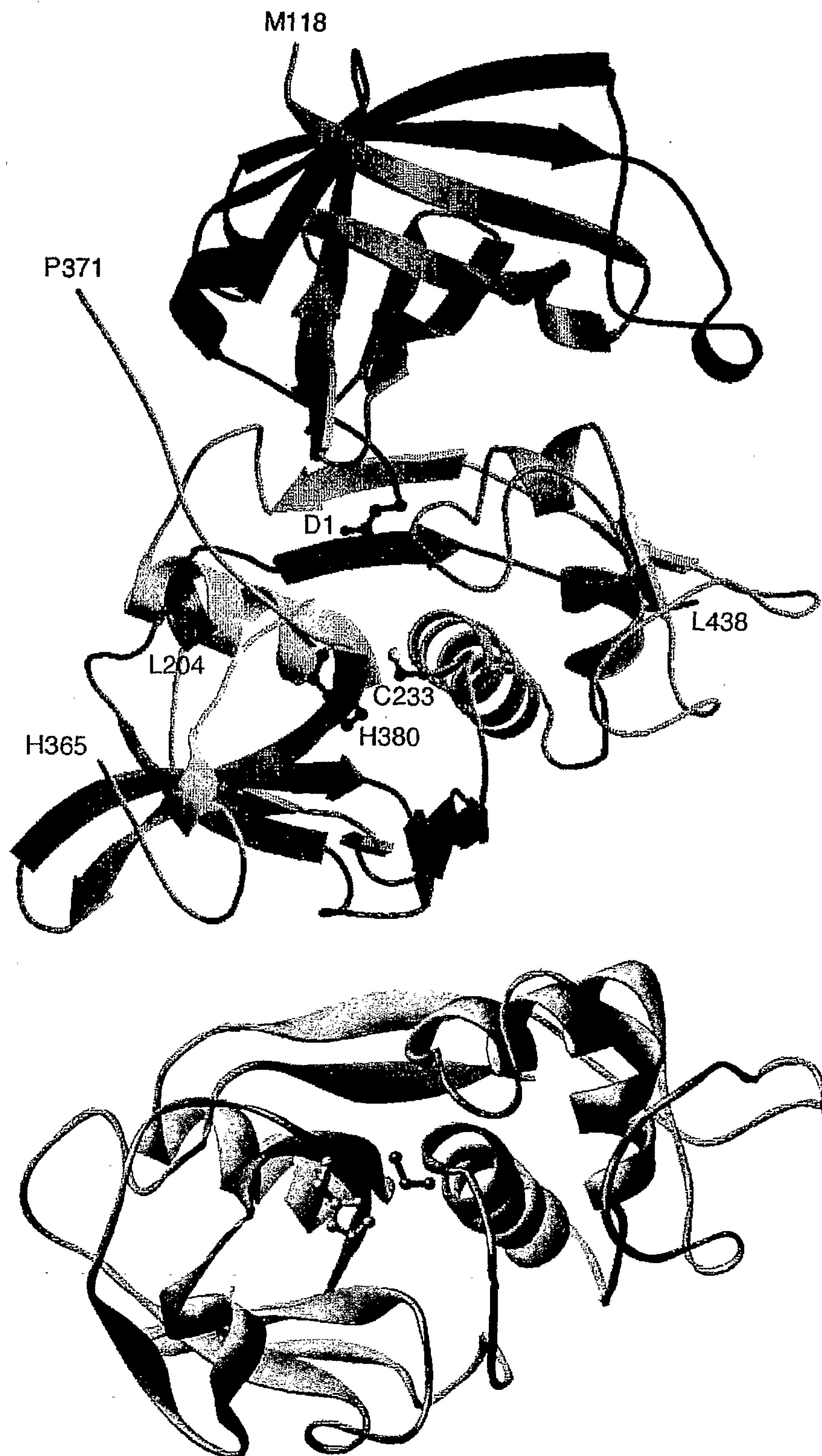


Fig. 4

Fig. 4

7/21

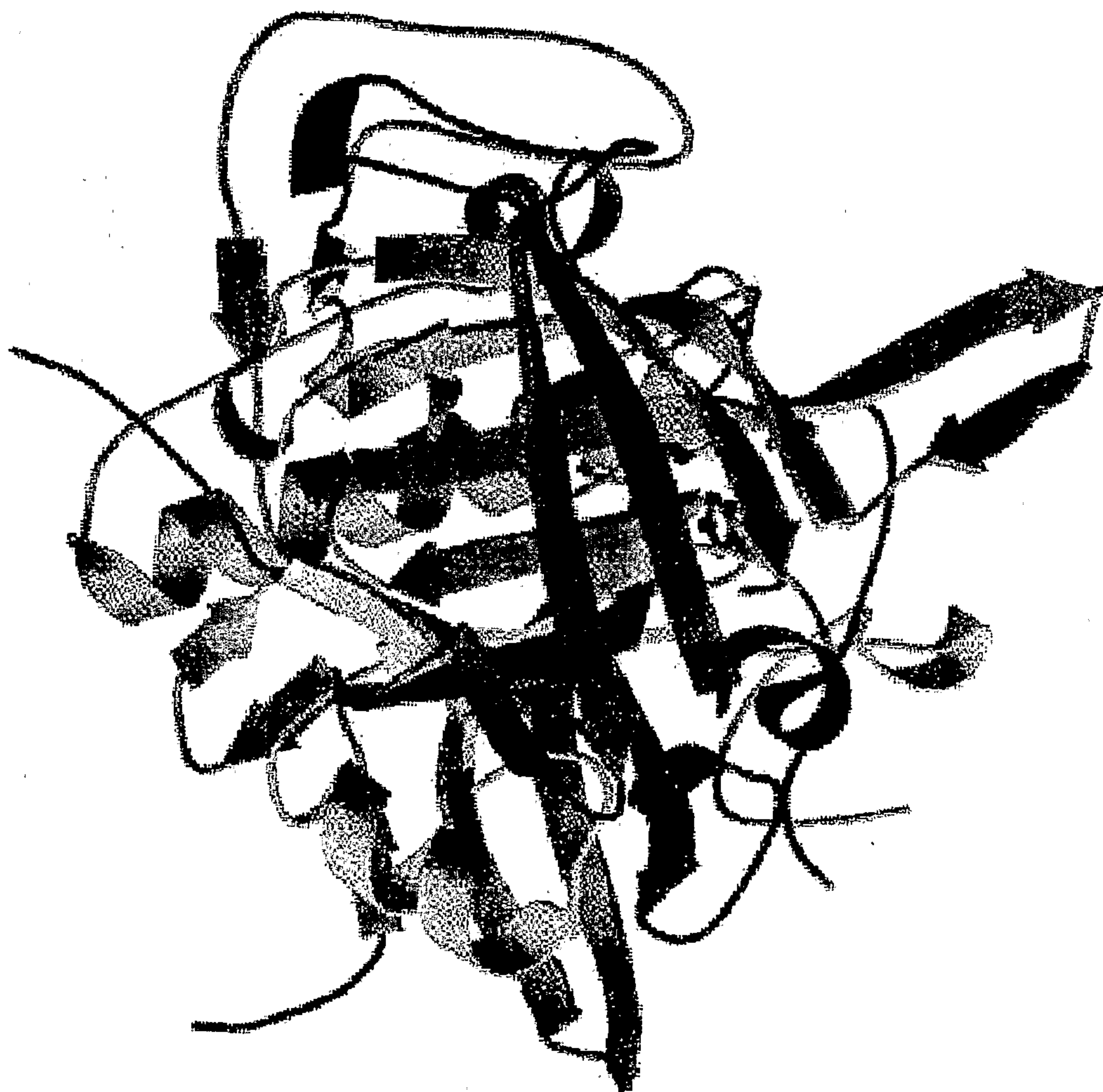


Fig. 5

Fig. 5

8/21

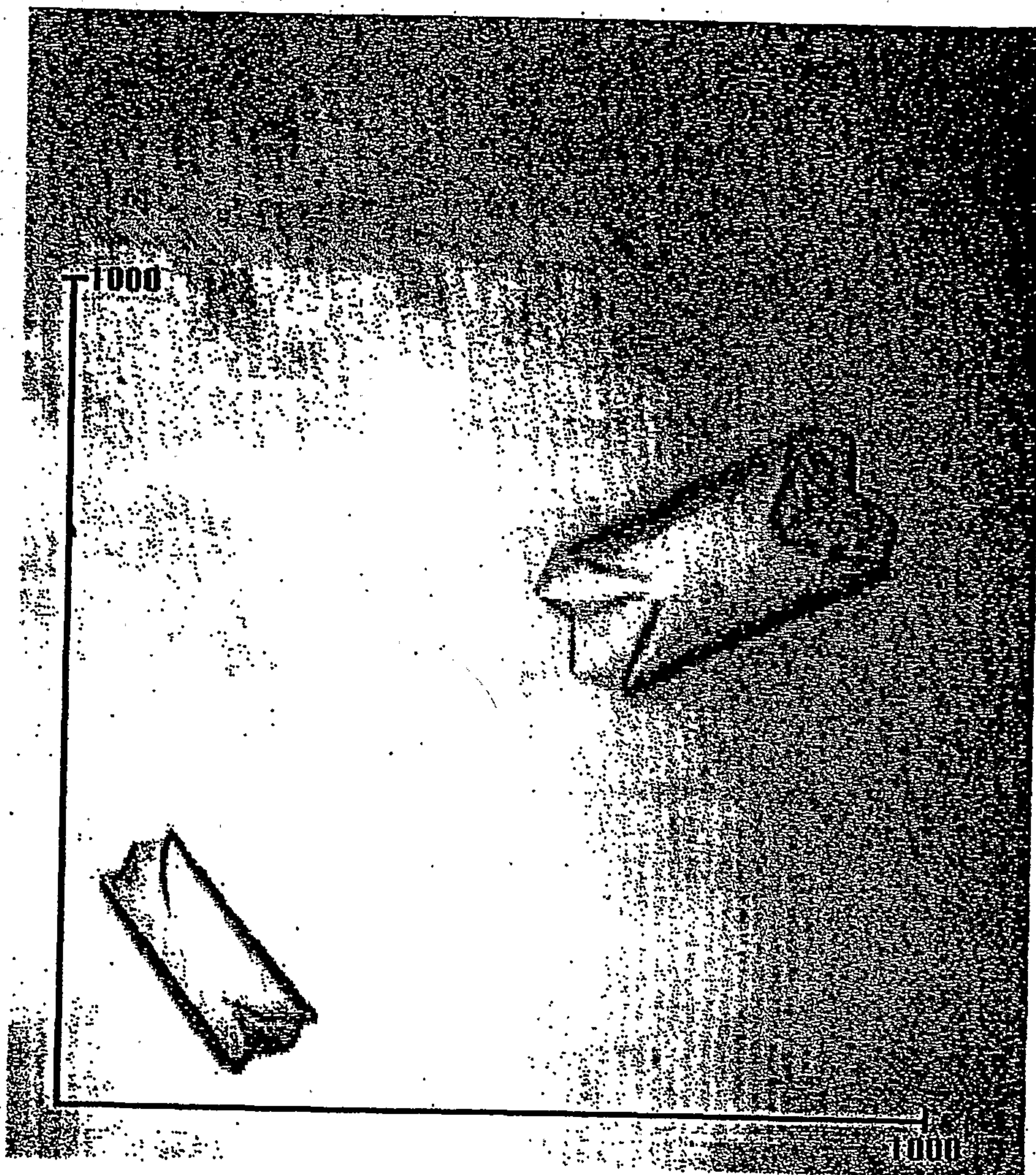


Fig. 6

Fig. 6

9/21

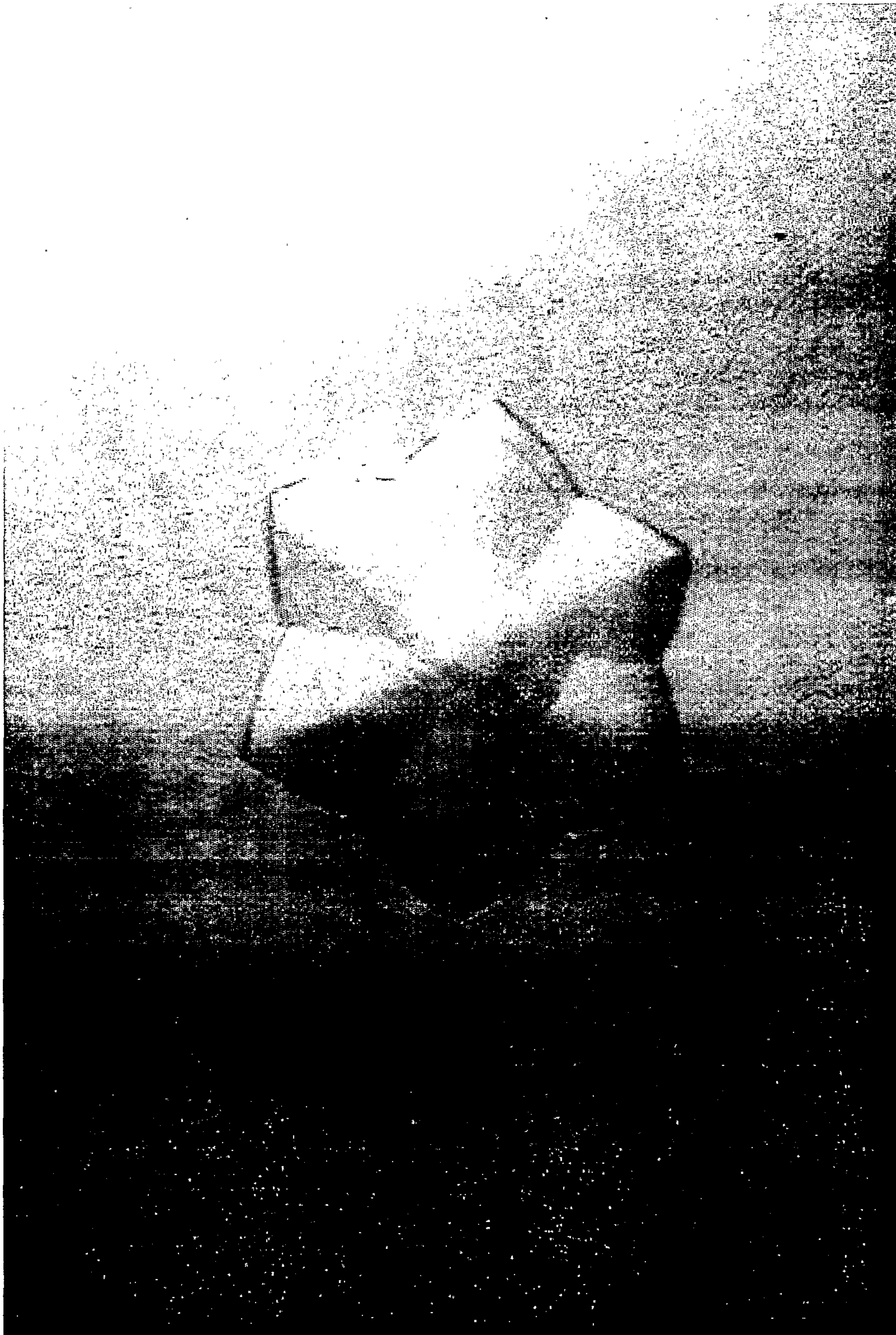


Fig. 7

Fig. 7

10/21

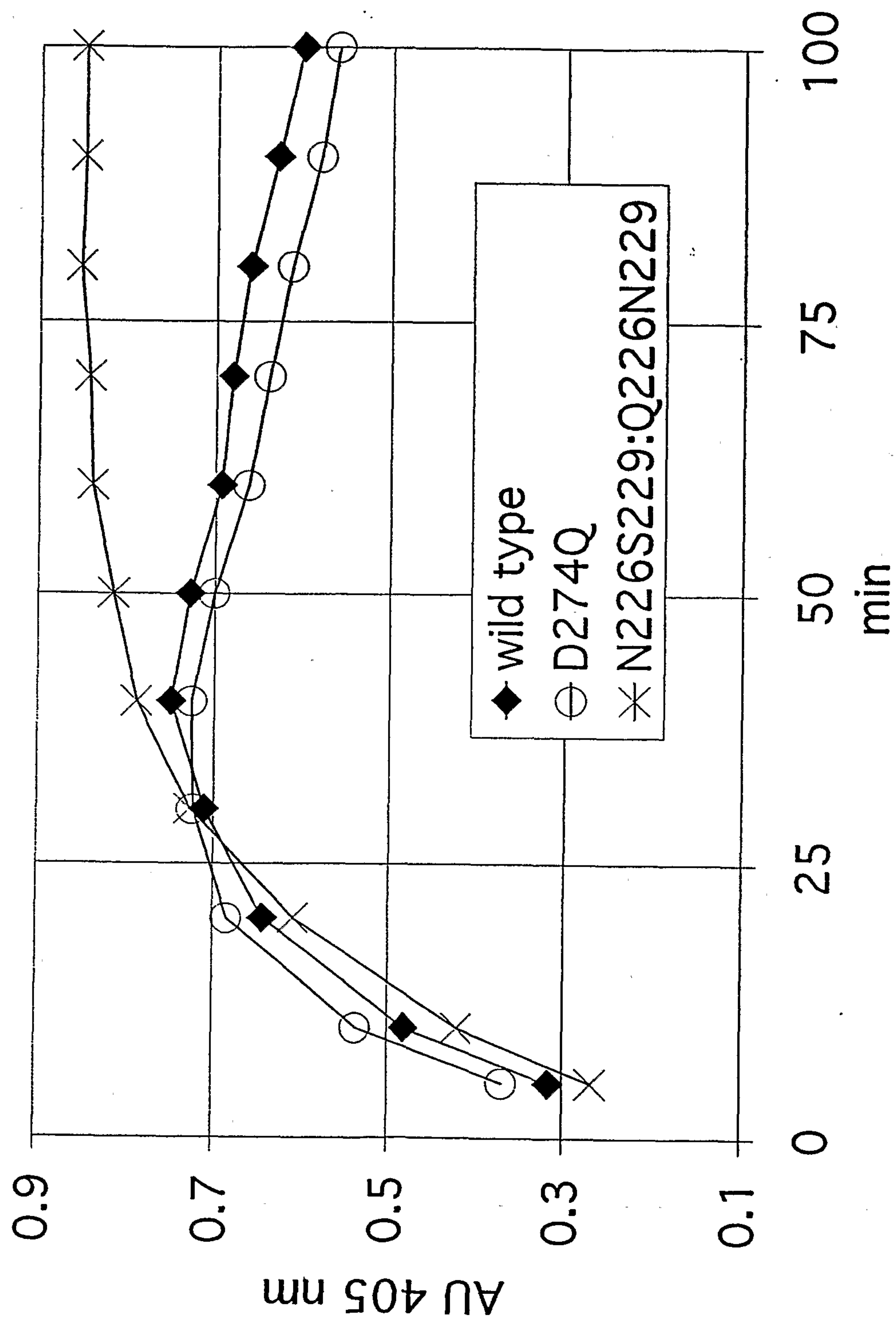


Fig. 8

Fig. 8

11/21

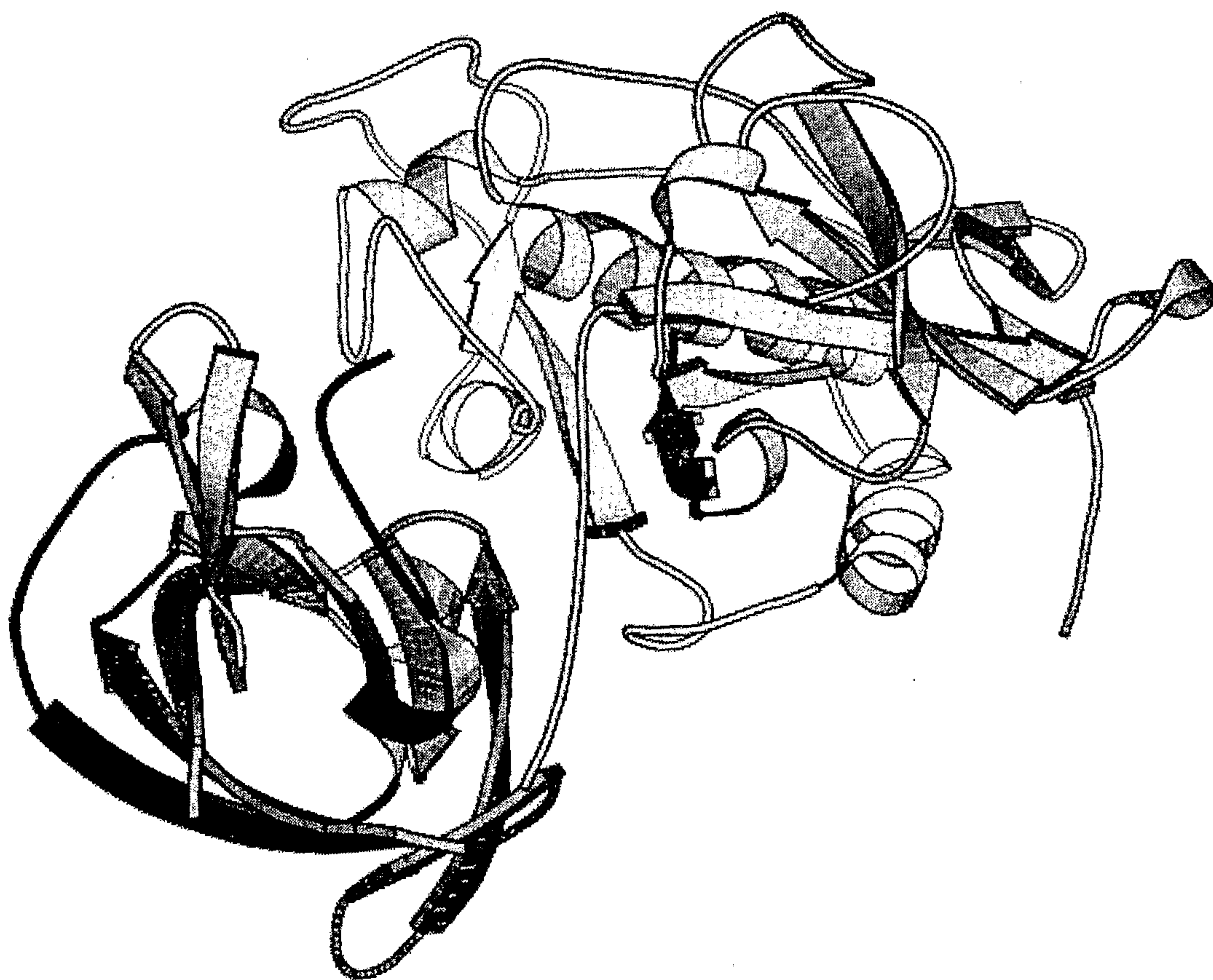


Fig. 9

Fig. 9

12/21

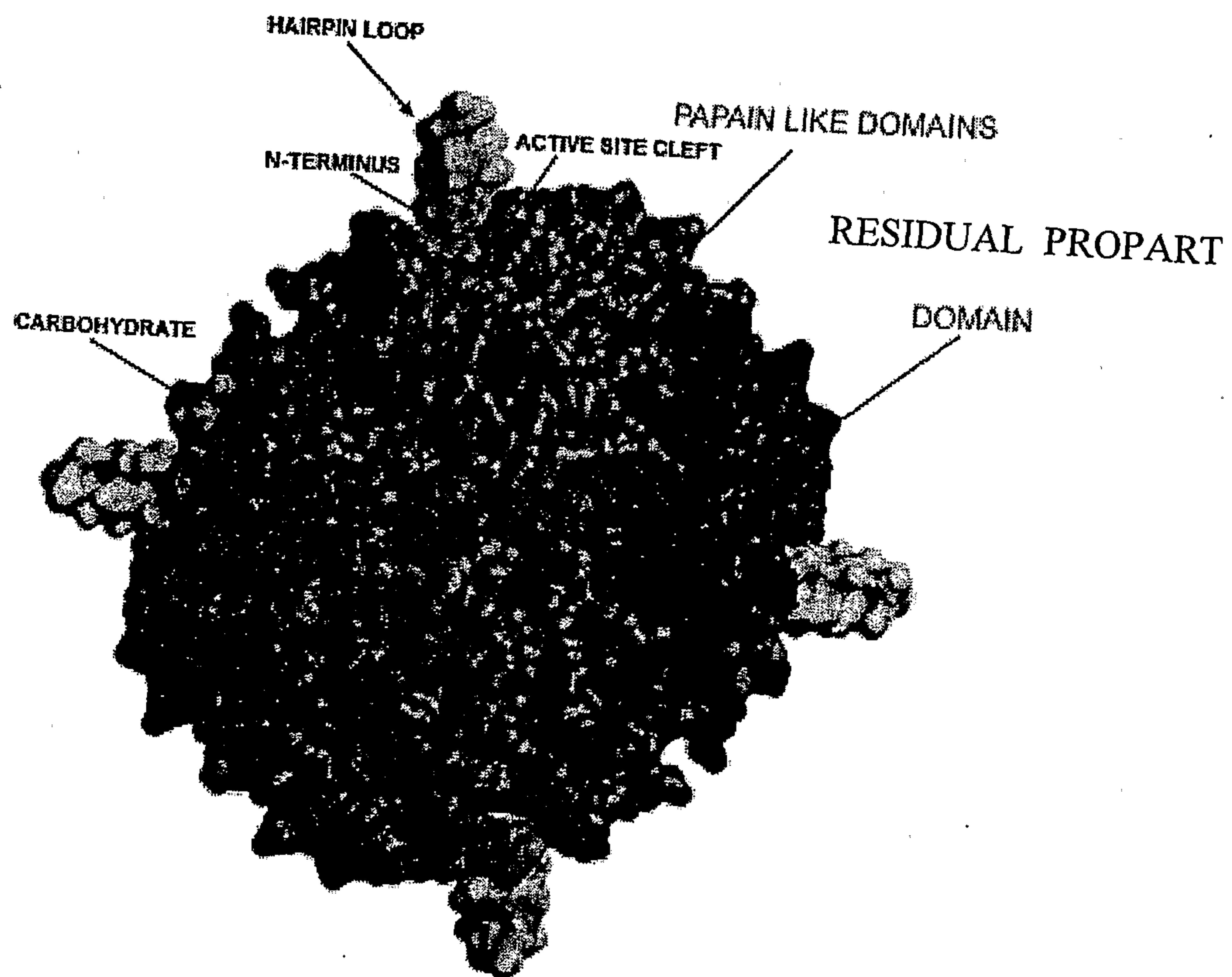


Fig. 10A

Fig. 10A

13/21

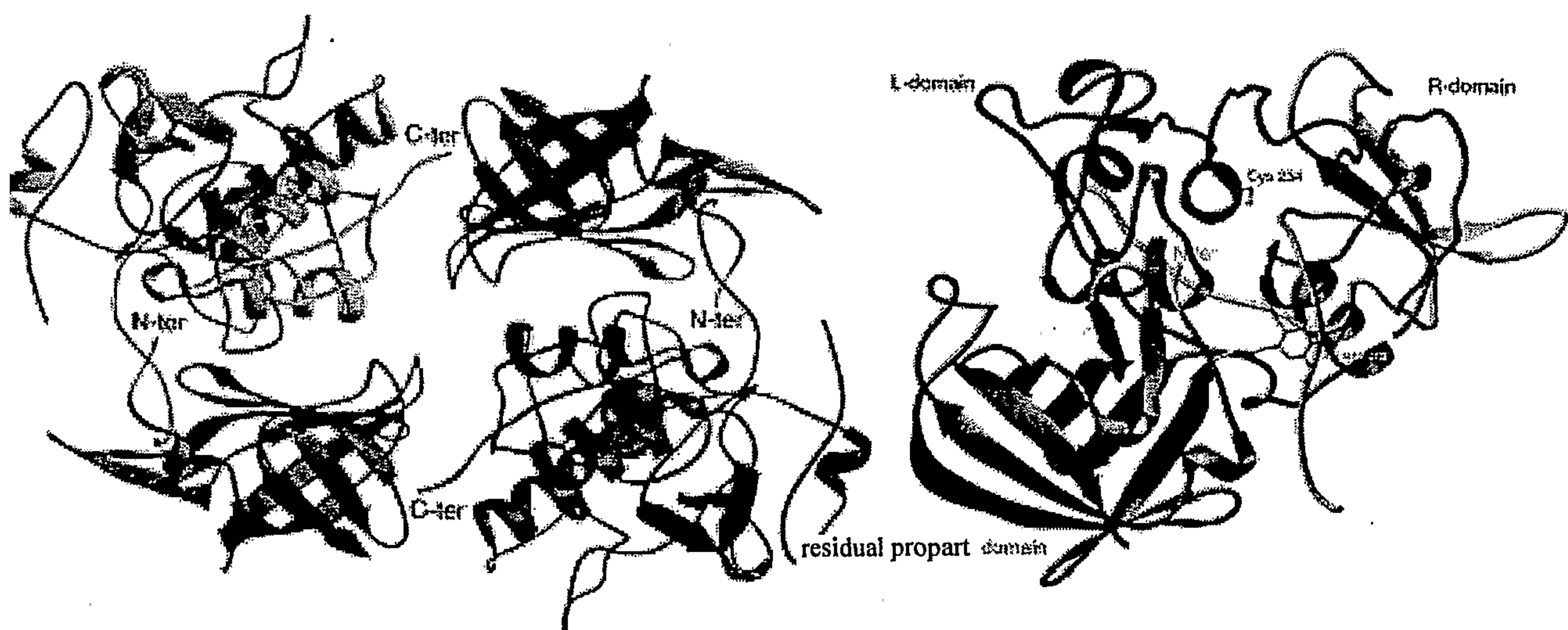


Fig. 10B

Fig. 10B

14/21

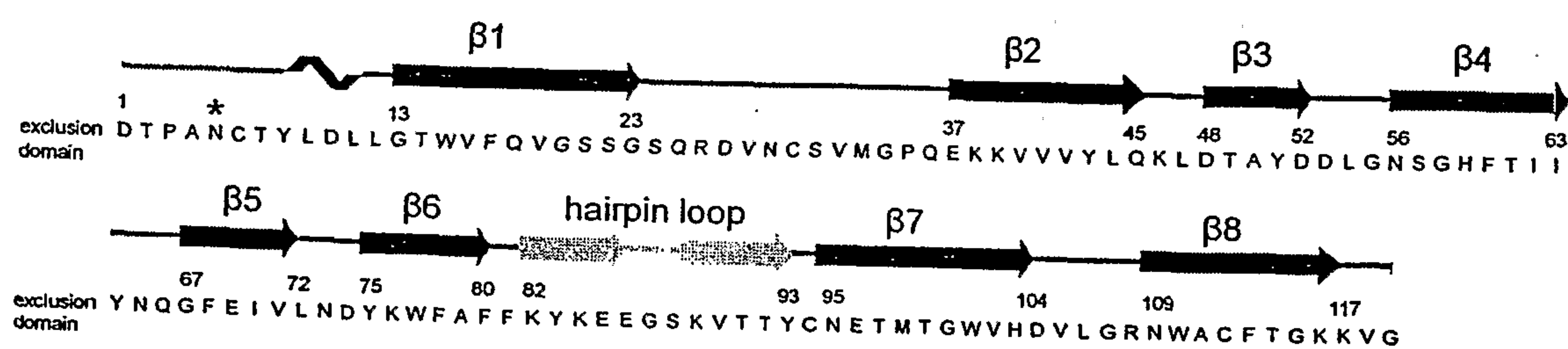


Fig. 10C

Fig. 10C

15/21

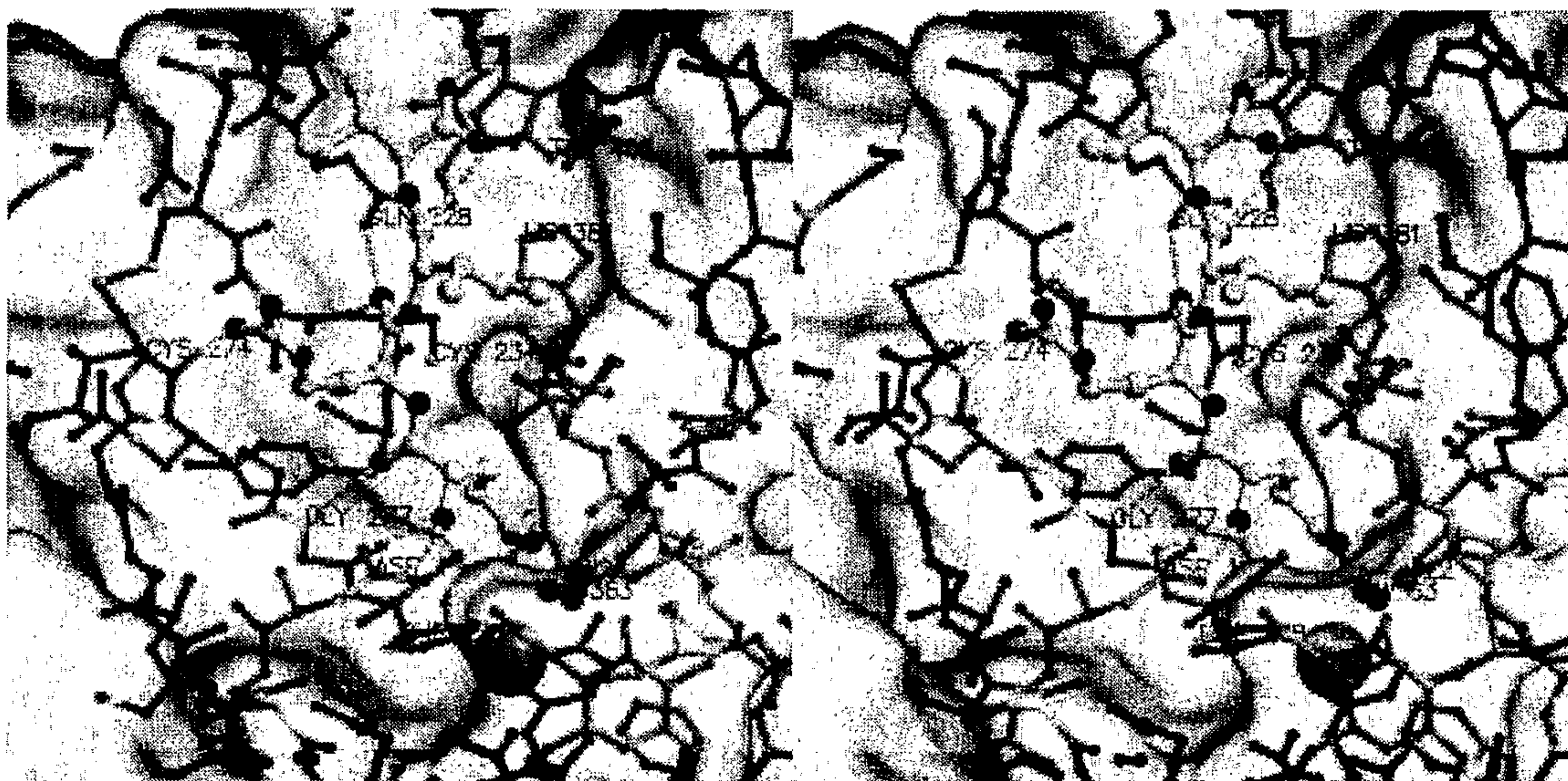


Fig. 11A

Fig. 11A

16/21

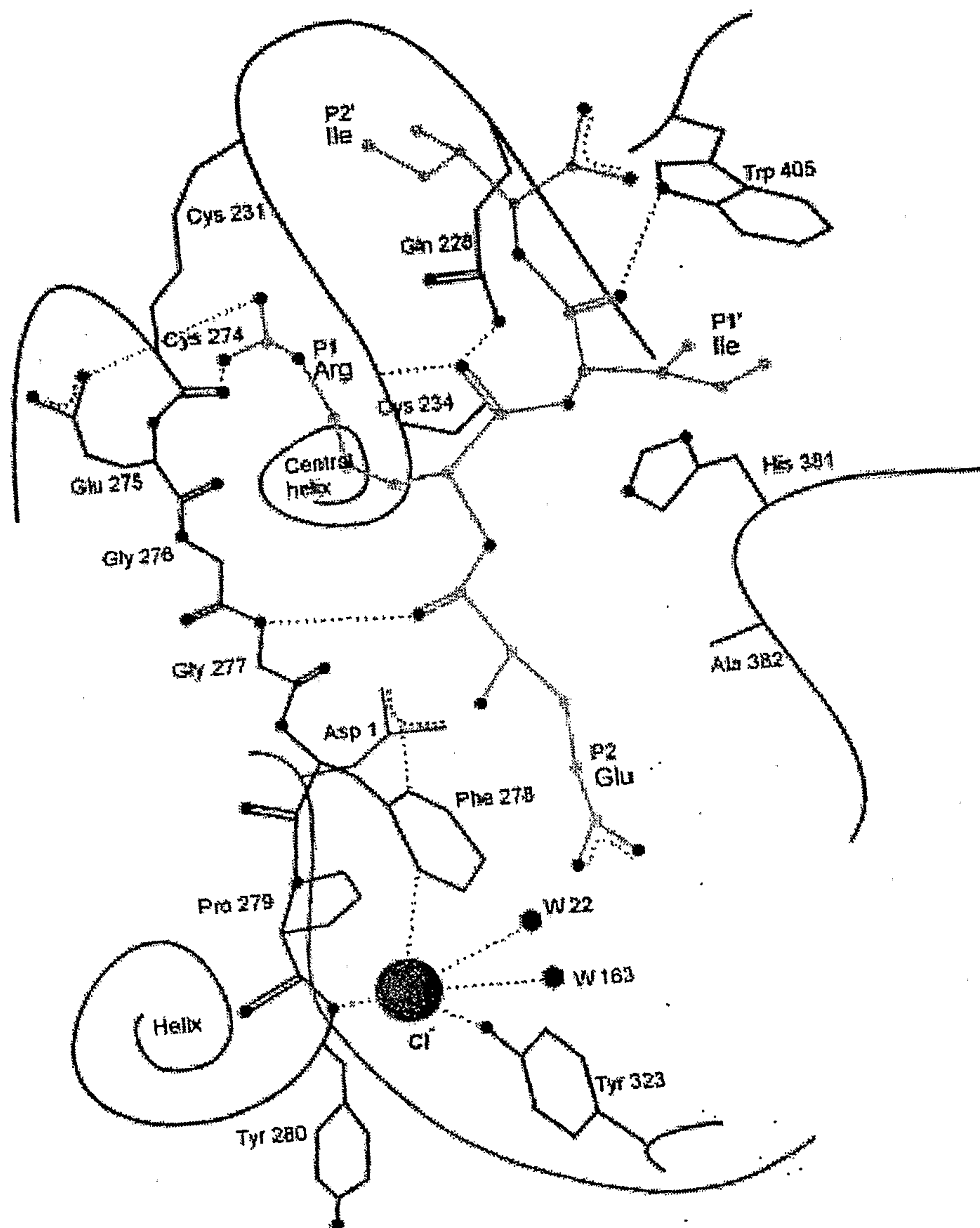


Fig. 11B

Fig. 11B

17/21

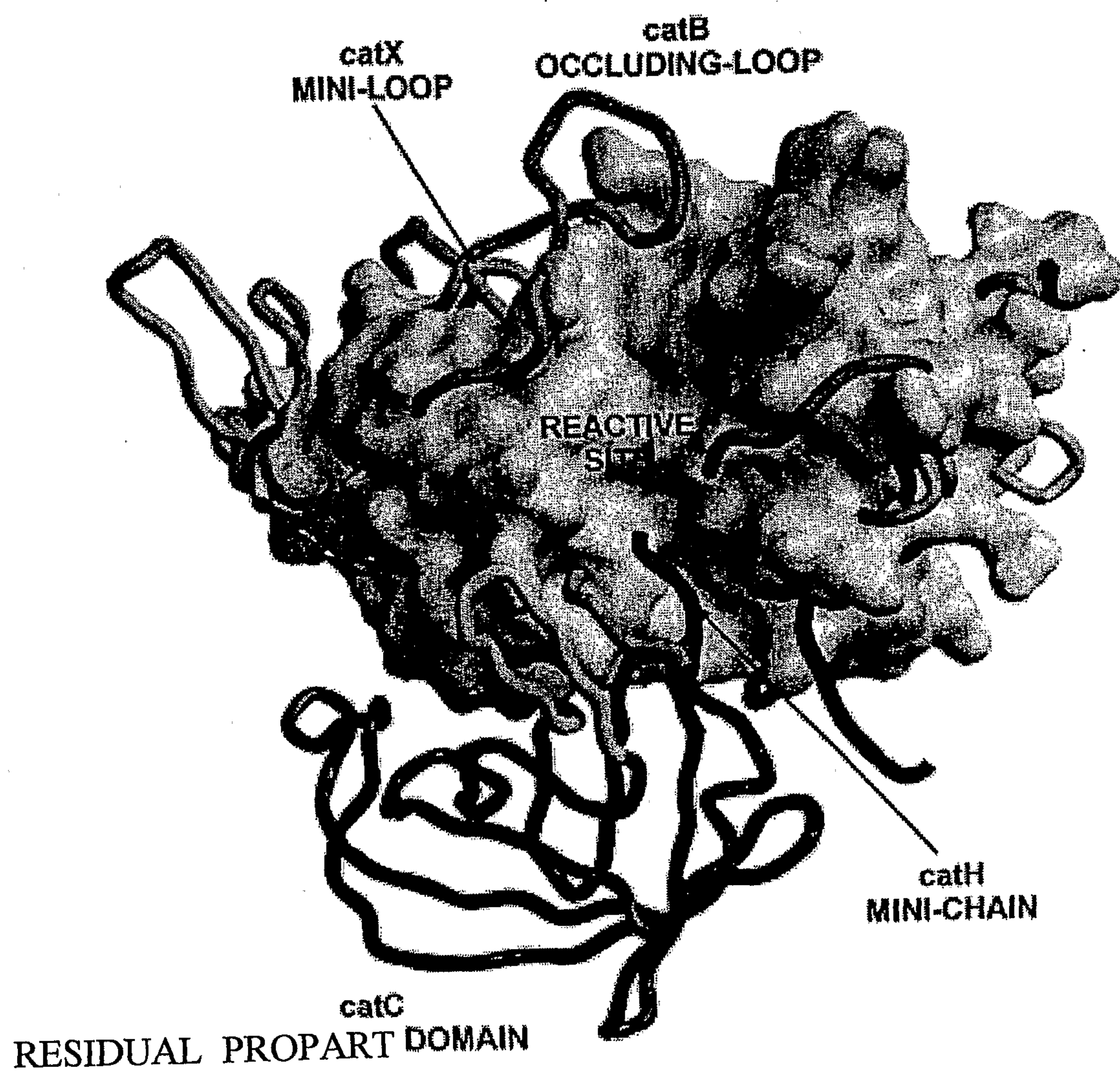


Fig. 12

Fig. 12

18/21

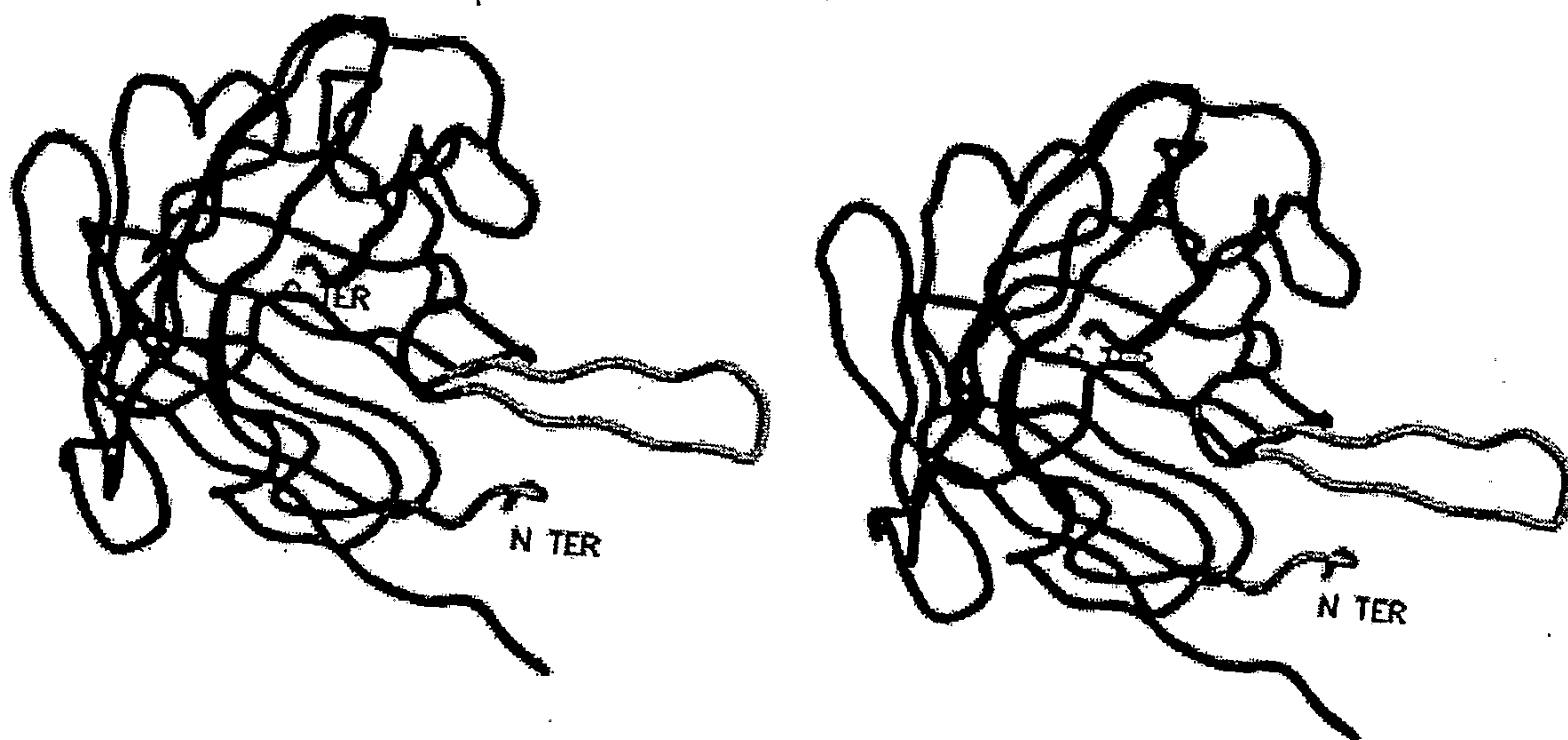


Fig. 13

Fig. 13

19/21

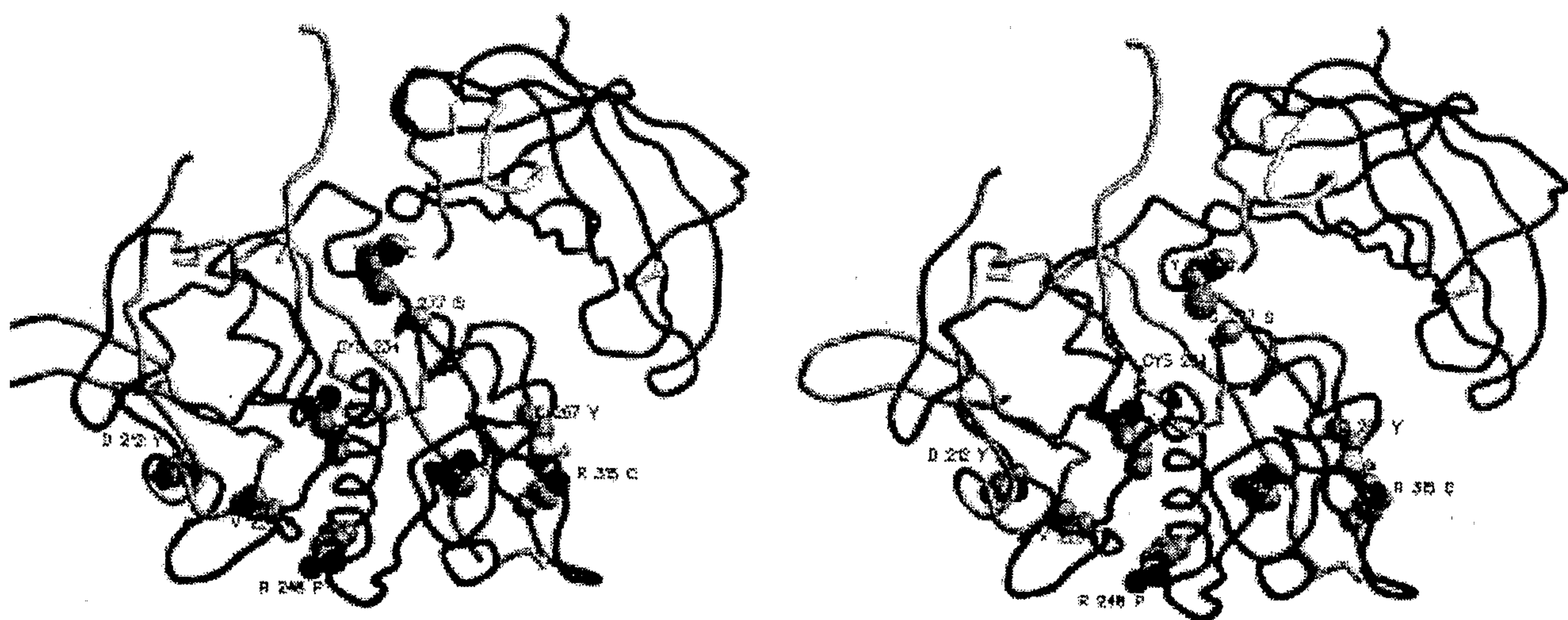


Fig. 14

Fig. 14

20/21

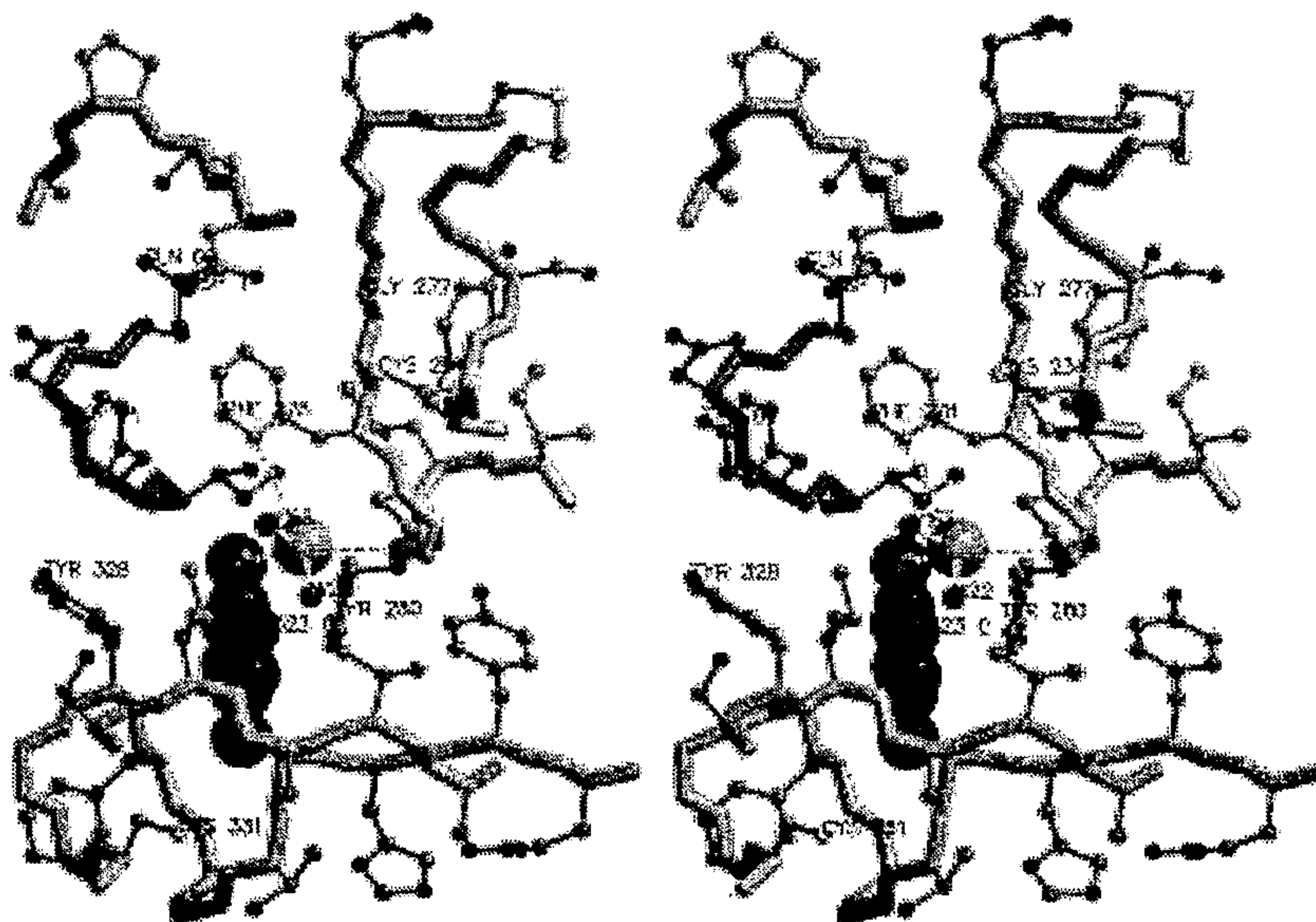


Fig. 14 (continued)

Fig. 14 (continued)

21/21

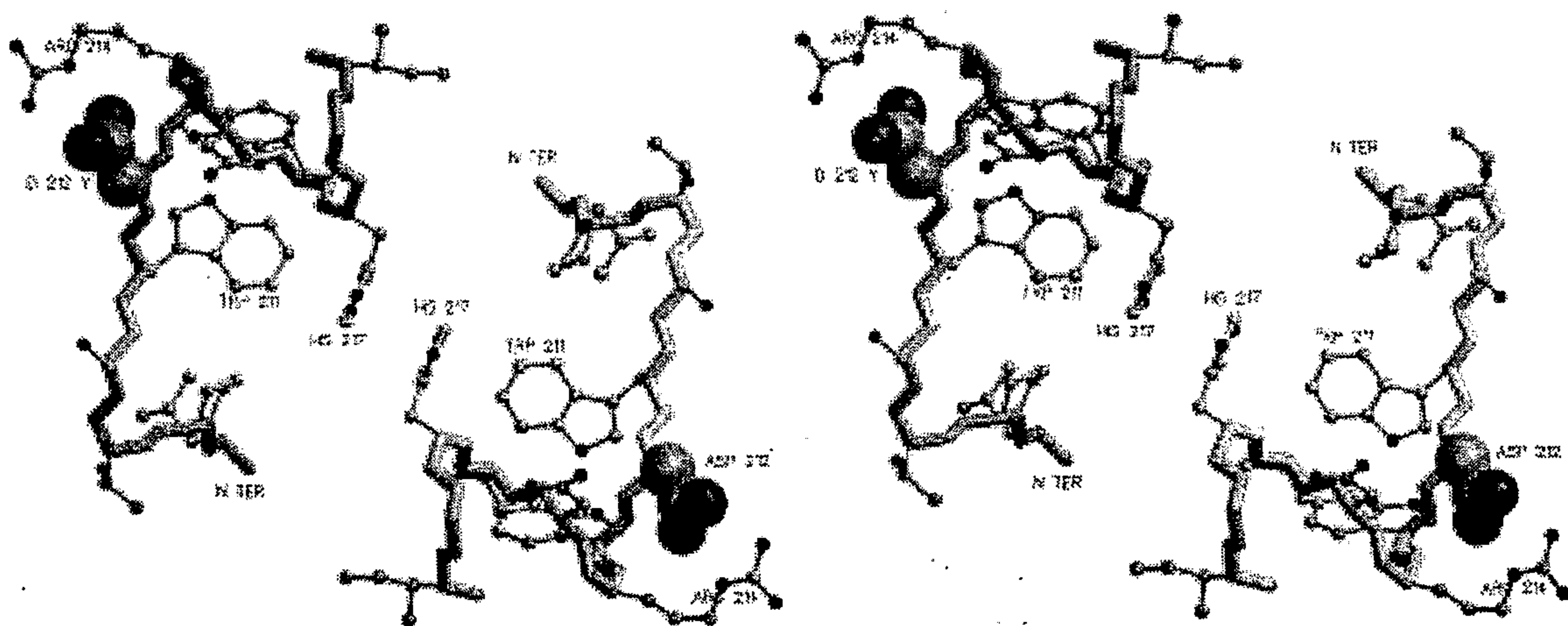


Fig. 14 (continued)

Fig. 14 (continued)

