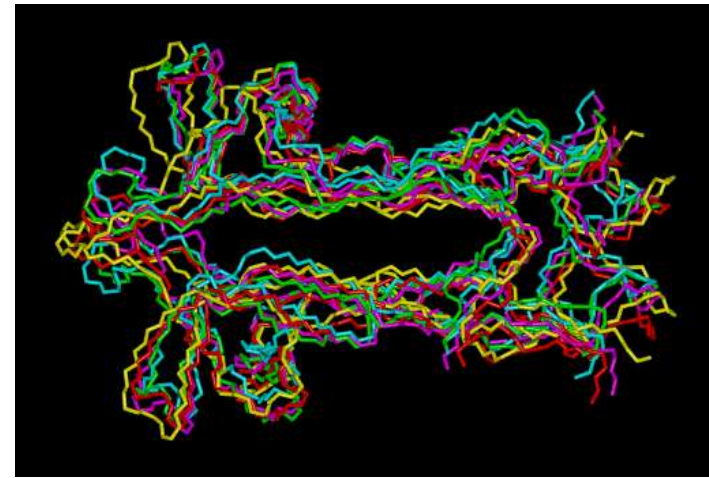
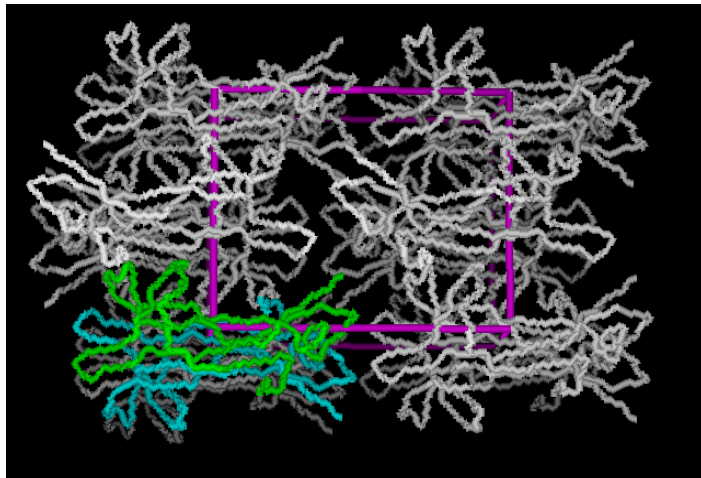


From Solid State to Solution Structure (Example: Nerve Growth Factors)



Dr. Marcus Böckmann
May 2004

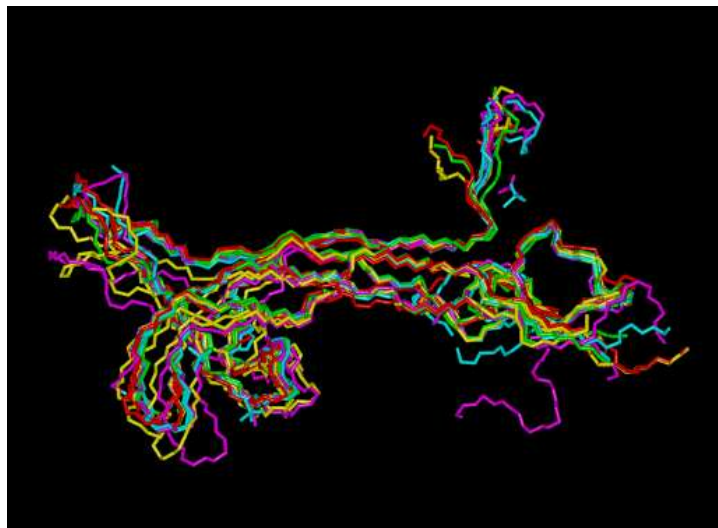
Background

- **Nerve Growth Factors**
 - **constitute neurotrophin family of growth factors**
 - **four protomers (NGF, BDNF, NT3, NT4) forming non-covalently bonded homo- and heterodimers**
 - **neurotrophins interact with tropomyosin-related kinase family of receptors (TrkA, TrkB, TrkC)**
 - **each dimer binds specifically to selected receptors**
 - **complexes located on the (aqueous) outside of membranes**
- **various crystallographic coordinate sets of dimers and complexes stored in Protein Data Bank (PDB)**
- **reduced production of BDNF is responsible for lethal disease “Veitstanz“ (Chorea Huntington)**

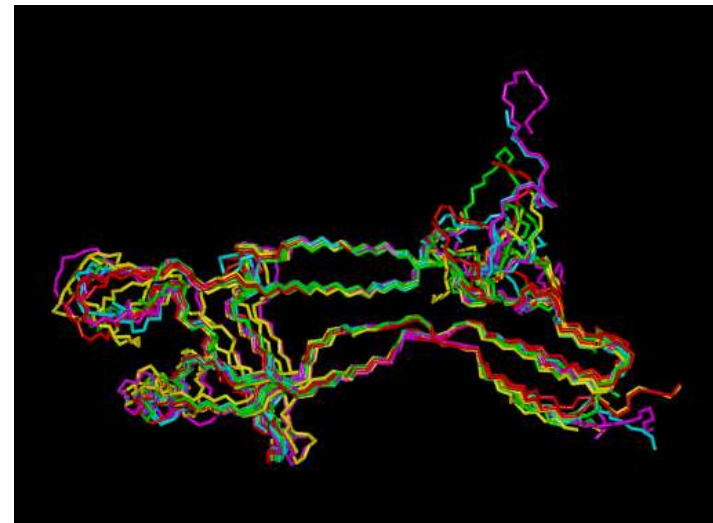
only x-ray coordinates but activity in aqueous environment

Protomer Folding

Selected backbone superposition of available protomer structures



Side View

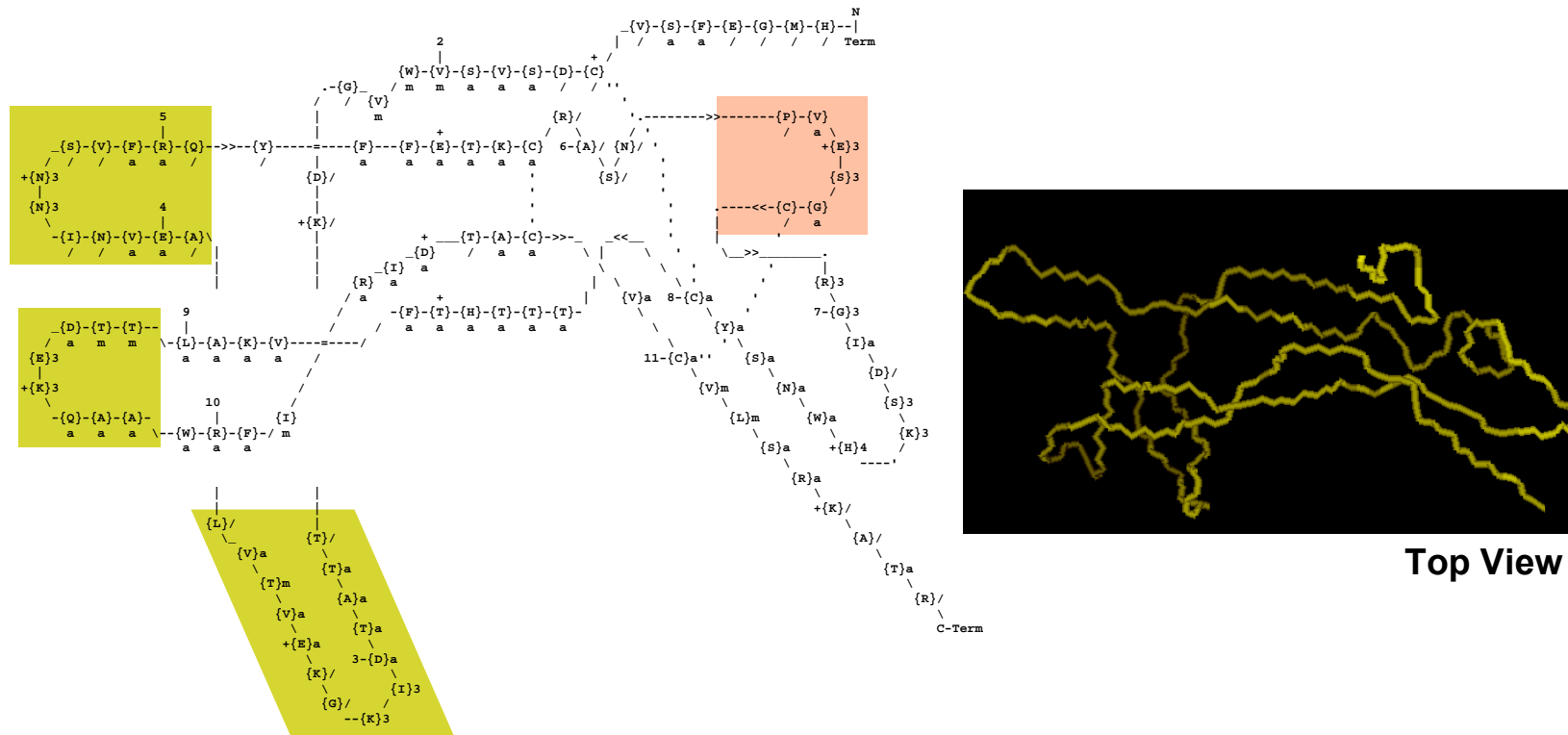


Top View

very similar folding with very diverse orientations of loops

Scheme of Protomer

Different protomers with conserved core and **varying** regions

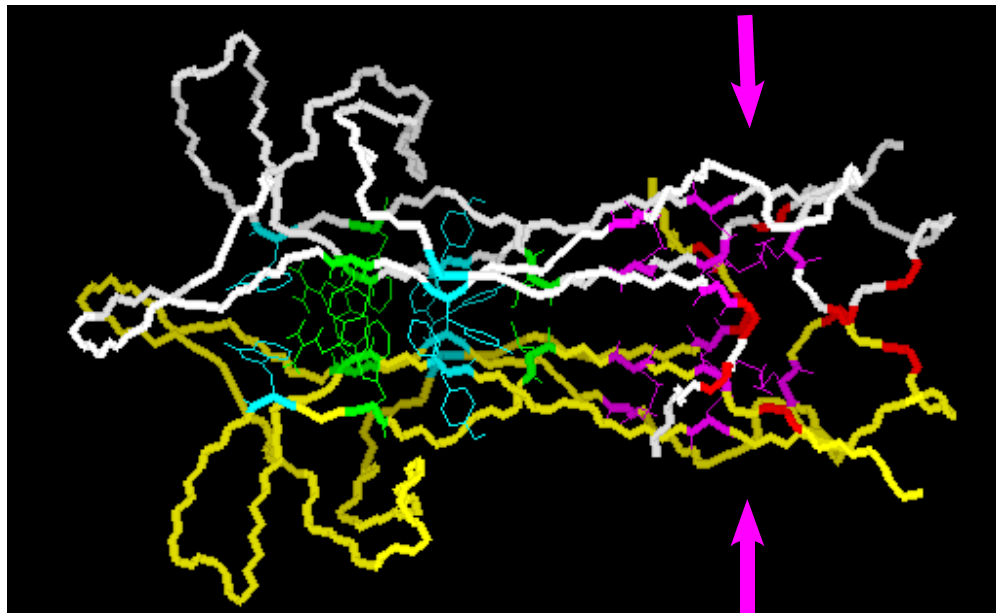


sequence variations in **finger regions** and **flexible loop** ...

Visualization of **CHEOPS** Contact Analysis

Specific dimer contacts (interactions)

Dimer of NT3 with typical, conserved **cystein-knots**



Side View

Contacts shown:

backbone to backbone
sidechain H-bond
specific van der Waals

dimer formation by non-valence (**HBO** and **VDW**) interactions only

CHEOPS Contact Analysis

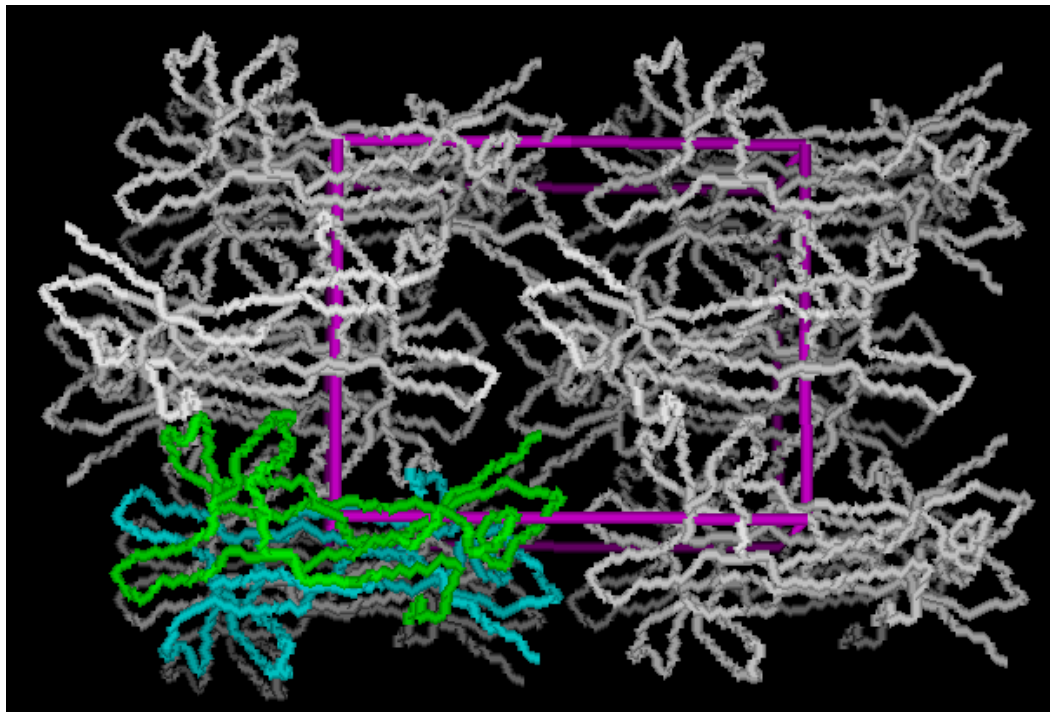
Specific dimer contacts by residue names

NUM	HINT NT3	HINT CONTACT	HINT NT3	HINT NT4	HINT BDNF	HINT 7S-NGF
	1	2	3	4	5	6
1	TYR11A	BABD	LEU112M	LEU123A	LEU113A	LEU112B
5	TRP20A	SESR	TRP101M	TRP112A	LEER	PHE101B
6	TRP20A	SESE	TRP101M	TRP112A	LEER	PHE101B
8	TYR51A	SASD	ARG87M	ARG98A	LEER	LYS88B
9	TYR51A	SRSE	TRP101M	TRP112A	LEER	PHE101B
10	TYR51A	SESE	TRP101M	TRP112A	LEER	PHE101B
12	TYR53A	SRSE	TYR85M	TYR96A	TYR86A	PHE86B
14	GLY69A	BABD	ASP71M	ASP82A	ASP72A	ASP72B
18	ASP71A	BDBA	GLY69M	GLY69A	GLY70A	GLY70B
20	TYR85A	SESR	TYR53M	PHE57A	TYR54A	PHE54B
21	ARG87A	SDSA	TYR51M	TYR55A	TYR52A	TYR52B
22	TRP99A	SESE	TRP99M	TRP110A	LEER	TRP99B
23	TRP101A	SRSE	TRP20M	TRP23A	TRP19A	TRP21B
24	TRP101A	SESE	TRP20M	TRP23A	TRP19A	TRP21B
25	TRP101A	SESR	TYR51M	TYR55A	TYR52A	TYR52B
26	TRP101A	SESE	TYR51M	TYR55A	TYR52A	TYR52B
28	THR106A	SASD	THR106M	THR117A	THR107A	THR106B
32	LEU112A	BDBA	TYR11M	LEU14A	LEU10A	PHE12B
	1	2	3	4	5	6

list of van der Waals, hydrogen bond and backbone contacts

Solid State

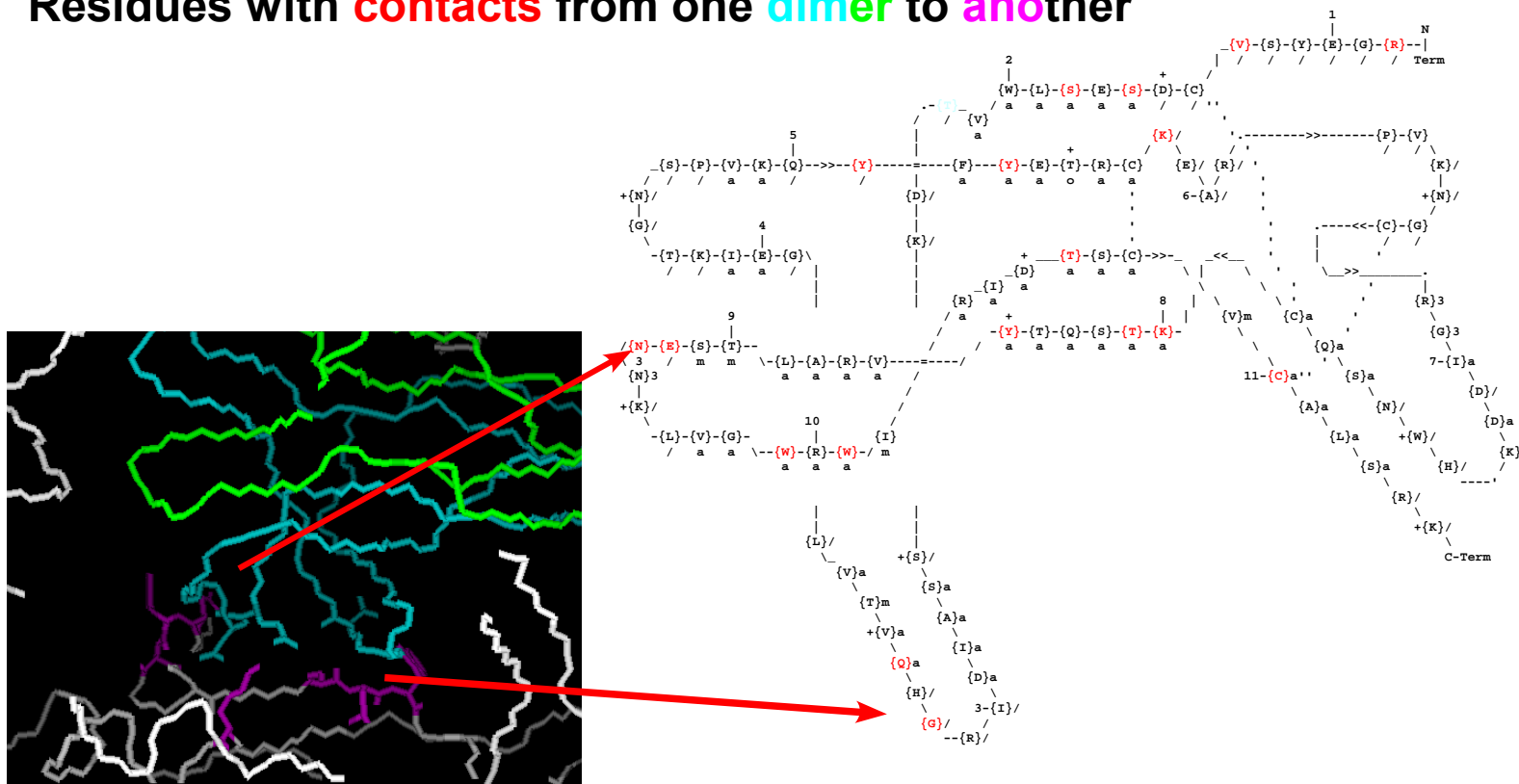
Unit Cell and crystal packing of NT3 **homodimers**
(backbones only)



crystal packing affects dimer folding

Crystal Contacts between Dimers

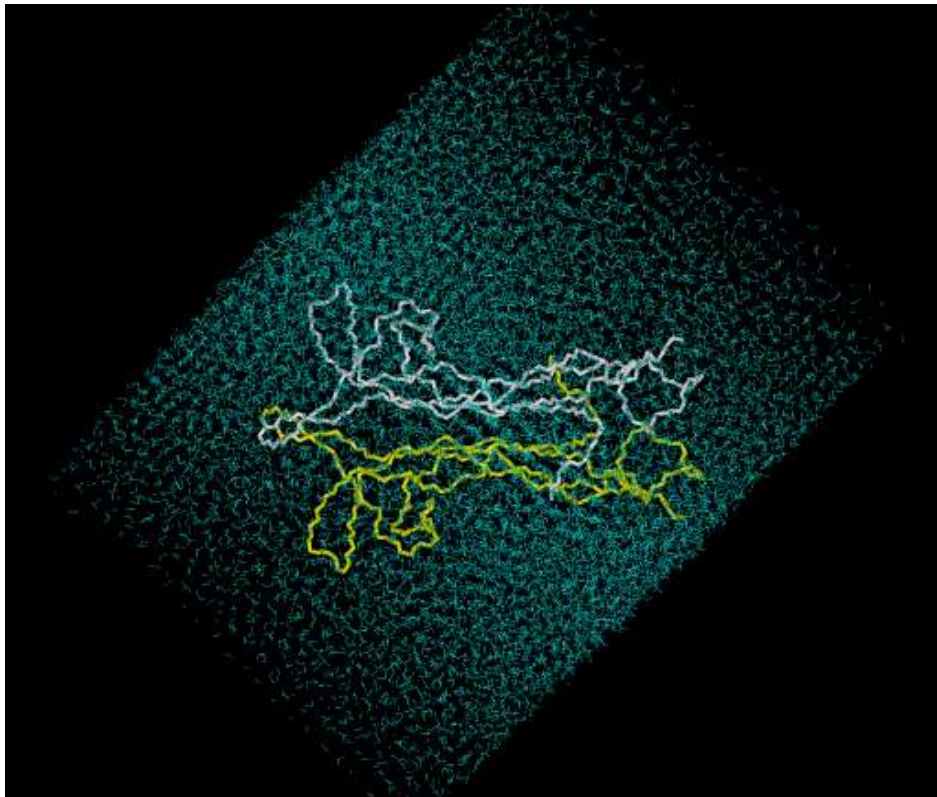
Residues with **contacts** from one **dimer** to **another**



finger regions show crystal contacts not available in solution

Molecular Dynamics Simulation

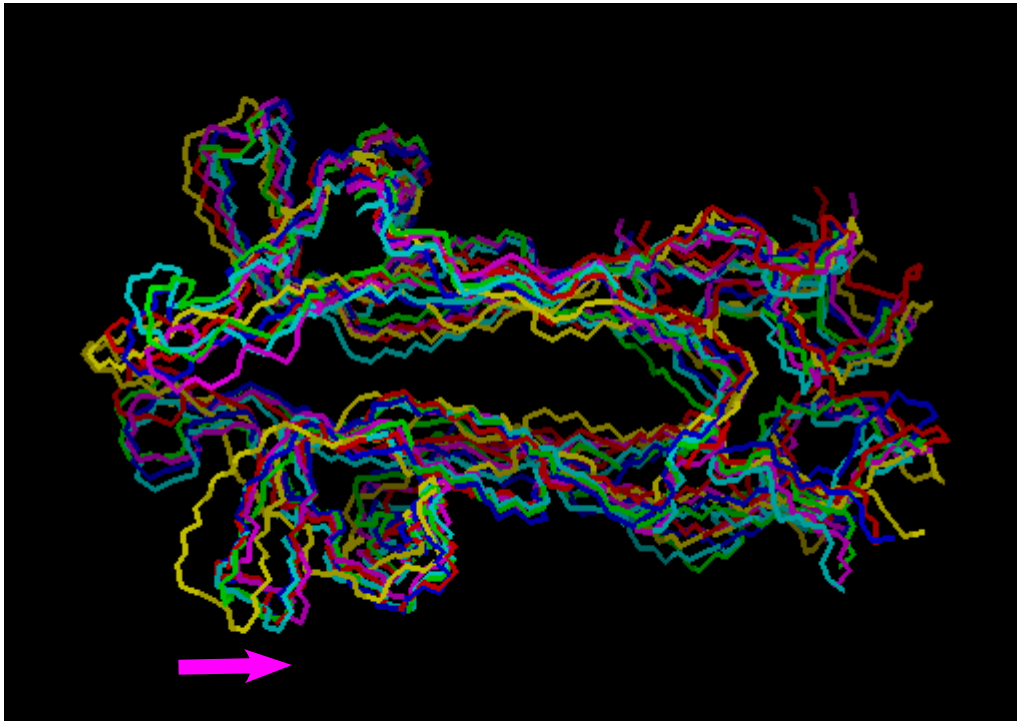
Starting structure from **CHEOPS** structure preparation



adequate solvation requires more than 10,000 water molecules

Solution Structure

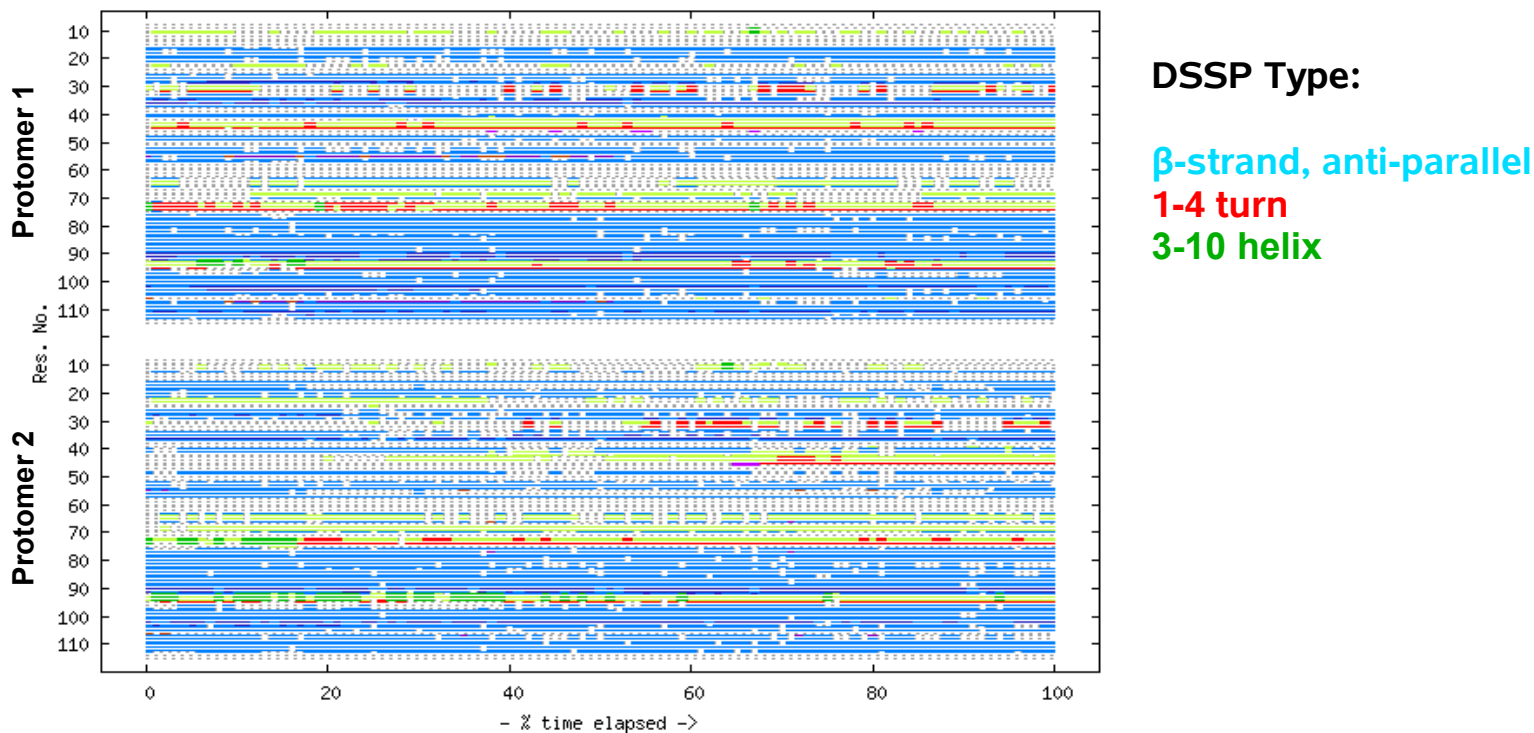
Superposition of structures at **start**, **20**, **40**, **60**, **80**, and **100 ps**



large wagging movements and **significant shift** in finger region

CHEOPS Contact Analysis

DSSP assignment for protomer in course of simulation



stable secondary structure, some regions even tightening

CHEOPS Contact Analysis

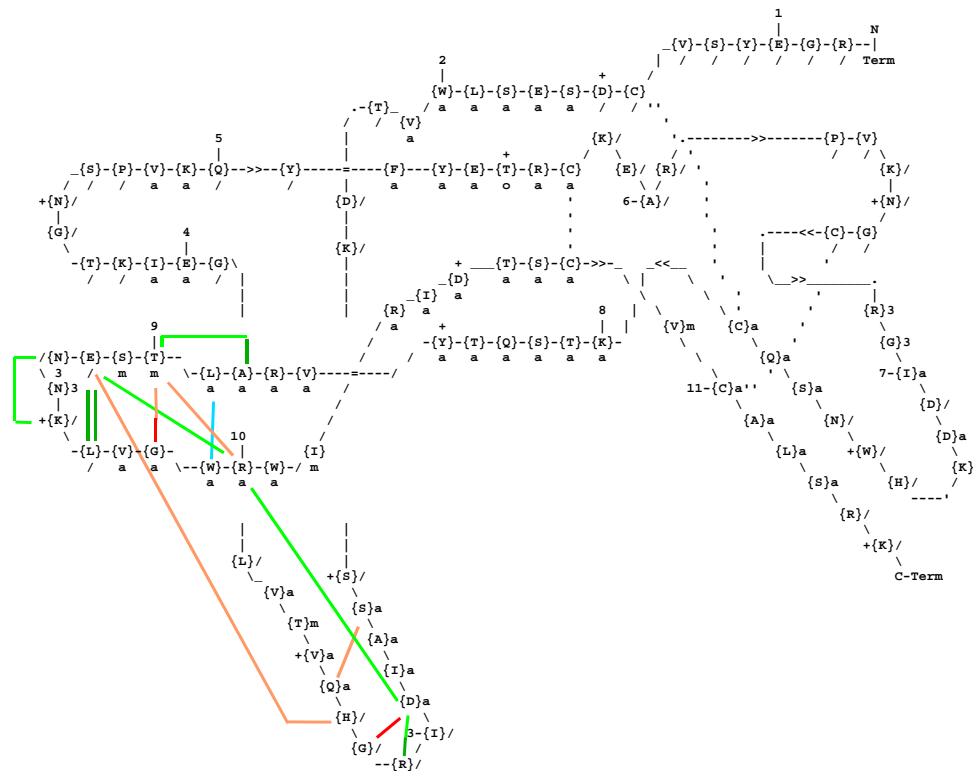
Stable, lost, and new dimer contacts during simulation

ARG8A	THR81M	SDBA	XXXXXXXX-XX-X-XXXXXXXX-----XX-----	18
ARG8A	ASP105M	SDSA	-----XX	70
GLY9A	THR81M	BDBA	-----X-XX-X-X-XX-----XXXXXXXX--XXXX--XX-XX-XXXX-XX-XXXXXXXX-XX--XXXXXXXXXXXXXX	49
GLY9A	SER82M	BDSA	-----X-X-XXXX-X--XX-X-X-X-XXXXXXXX--XXXX--XXX-XXX-XX-XX-X-XX--X-XXXX--X-X-X-	42
GLU10A	SER82M	BDSA	-----X-X-X-XXXXXXXXXX-XX-XX-----XXX-XXXXXXXX-X-XXXXXXXXXX-X-XXXXXXXX-XXXX	50
GLU10A	GLN83M	SABD	-----XX	10
SER12A	CYX110M	BDBA	-----X-X-XX-----X-XXXX-XX-XX-XXX-XXX-X-X-X-XX-XXX--X-XX--XXXX-XX--XXXX--X-	42
TRP20A	TRP85M	SESE	-XXXXXXXX-XXXXXXXXXXXXXXXX-X-XX-X--XXXX-XX-X--XX-X--XXXXXXXX-X----XXX-XX--XXX--X-X--X-XXXXXX	61
TRP20A	TRP101M	SESR	XXXXXXXXXXXXXXXXXXXX-XX-X-XXXX-X-----XXXX--XXX-XX--X-XXX-XX-----X-X-----	41
ILE41A	TRP99M	SASR	--XXXXX-XXXXXXXXXX-XXXXXXXXXXXXXXXXXXXX-X-X-X-XXXX-X-XXX-XXXXXXXXXXXX-XXX--XXX--XXXX-X-XX--XXX	75
VAL48A	ARG87M	SASR	--XXXXXXXXXX-XXXXXXXXXXXX-XXX--XXXXX-X-X-XX-X--XX-XXX-XX--XXXX--X-X-X-X--X-X-X-XXXXXXXXXXXX-	66
LYS49A	ARG87M	BASD	XX	100
TYR51A	TRP101M	SASD	XX-XX-XXX-X-X-XXXX-XXXXX--X-X-----X-----XX-X-X-X-----X-XXX-XX--X--X-X-----	35
TYR51A	TRP101M	SRSE	XXX-XX-XXXXXXXX-XXXXXXXXXXXXXXXXXXXXXXXXXXXX-X-XX-X-----X-XXXX-X-XX-X-X-----XXXXX--X--XXXXX--XX	63
TYR51A	TRP101M	SESE	XX	100
GLY69A	ASP71M	BABD	-X-----XXXXXXXXXX-XXXXXXXXXXXX-XXXX-XX--XXXXXXXXXXXXXXXX-XXXXX-X-----XXX-X-XXXXX-X	59
ASP71A	GLY69M	BDBA	-X--XXX-XXXXXXXX-XXX--XXXXXXXXXXXXXXXXXXXXXXXXXXXX-XXXX-XXXX-XXXX-X-XXX-XX-X-X-XXX-XXXXXXXXXXXX	80
TRP75A	TYR11M	SESE	-----XX-X-XXXXXXXXXXXXXXXXXXXXXXXXXXXX-XXXX-XX-X-X-X-XXXX--X-XX--XXX-----XX-XX--X-X-XXX	56
THR81A	ARG8M	BASD	XXXXXXXXXXXXXXXXXXXXXXXXXXXX-----X-----XXX--XXXX-XXXXXXXXXXXXXXXXXXXXXXXXXXXX	63
SER82A	ARG8M	SABD	-----XXX-XXX--XXXX-X-X-X-XXX--XXXXX-XXXXXX-X-X-XXXX-XXXX-XX-----XX-XX-----XXXXXXXX--	52
SER82A	GLY9M	SABD	--X-----X-XXXX--XXXXXXXXXXXXXXXX-XXXX-XX-XX--XXXXXXXX-XXXXXXXXXXXXXXXXXXXX-XXXXX-X-X-XXXX-XXX-XXX	72
TYR85A	TRP20M	SESE	-XXXXX-XXXX--XXXX--XXX-X--XXX-XXXXXXXXXX-XX-X-XXXX--XXX-X-----X-XXXX-XXXXX-X----	56
TYR85A	TYR53M	SESE	X-X-X--XXXXX-----X-X--XXXX-XXX-XXXXXX--XXX-XXX-X--XXX-XX-XX-----X-X-XX-----X----	45
ARG87A	LYS49M	SDBA	XXXXXXXXXXXXXXXXXXXX-XXXXXXXX-X-----XXX--XXXXXXXX-XXXX--X-X-----X-XXXX-XXXXXXXXXXXX	64
ARG87A	TYR51M	SDBA	-----X-XX-X-XXXXXXXX	70
TRP99A	TRP99M	SRSE	-XX-----X-X--X--XXX-X-XX-XXXXX--XXX-XXXXX-XXX-X-XXXX-XXX-XX-X-----	45
TRP99A	TRP99M	SESE	XXXXXXXX-XXXXXXXXXXXXXXXXXXXX-XXXXXXXXXXXXXXXXXXXX-XXXXXXXXXXXXXXXXXXXXXXXXXXXXX-X-XXXXXXXXXXXX	95
TRP101A	TRP20M	SRSE	XXXXXXXXXXXXXXXXXXXX-XXXXXXXXXXXXXXXXXXXX-XXXXXXXXXXXXXXXXXXXX-XXXXXXXXXXXXXXXXXXXX-XXXXXXXXXXXX-	96
TRP101A	TRP20M	SESE	XXXXXXXXXXXXXXXXXXXX-XXXXXXXXXXXXXXXXXXXX-XXXXXXXXXXXXXXXXXXXX-XXXXXXXXXXXXXXXXXXXX-XXXXXXXXXXXX	99
TRP101A	TYR51M	SDSA	XXXXX--X-X-----XX-XXXXXXXX-XX-XXXX--X-XX-XX--XXXXXXXXXX-XX-XXX-X-X-X-X-----XXXX-XXXXX	57
TRP101A	TYR51M	SESR	X-XXXXXXXX-XXXXX-----X-----X-----X-----X-----X-----X-----X-----	20
TRP101A	TYR51M	SESE	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX-XX-X-XX-X--XXXXXXXXXX-X-XXXXXXXXXXXXXXXXXX-XXXXXXXXXXXX	89
ASP105A	ARG8M	SASD	---X-XX-XXXXX-----X-----	81
CYX110A	VAL13M	BABD	X-X-----XX-X-XX-XX-XX-XXXX-XXX-X-X-----XXX-----X-----X-X-X-XXXX--XXXX-XX-XX--XXX--XXX	48
LEU112A	TYR11M	BDBA	--XXXXXXXXXXXXXXXXXXXX-XX-X-XXX--XXXXXX--XX-----X-XXXX-XXXXXXXXXXXX-X-XXXXXXXXXXXX	70

several new contacts form besides crystallographic ones

CHEOPS Contact Analysis

Selected protomer contacts **lost** and **formed**


lost

 backbone
sidechain

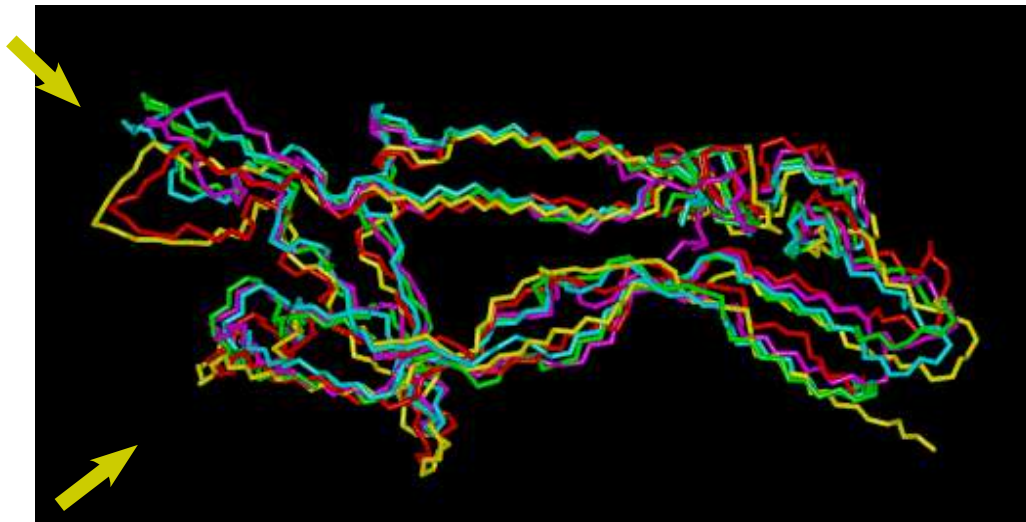
formed

 backbone
sidechain
specific v.d.W

missing 2nd dimer causes change of contacts in finger region

Solution Structure: View of Protomer

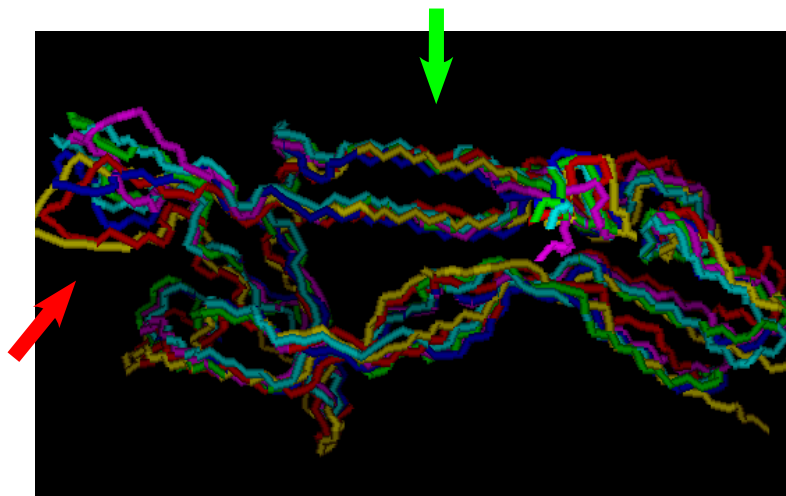
Finger regions show high flexibility



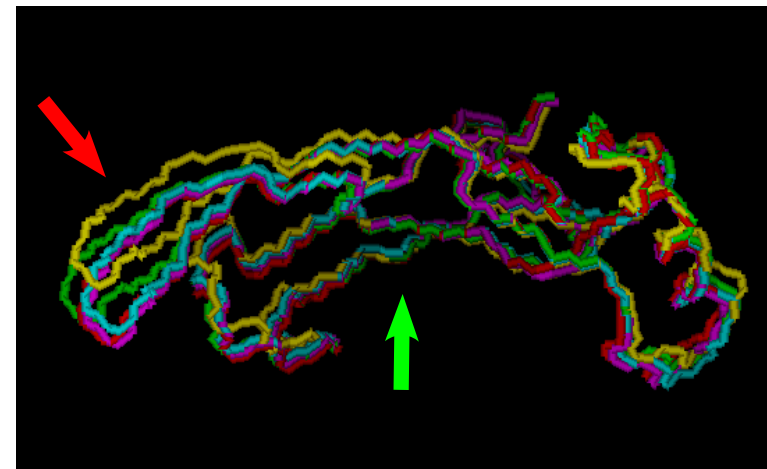
crystal structure of finger region not adequate for solution

Solution Structure: MD vs. Experiment

NMR-study supports mobility of finger region in growth factors



Protomer Superposition from MD on NT3



NMR on TGF- β 1

MD and NMR similar with **low** flexibility in core and **high** in finger regions

Conclusion

- X-ray structures for nerve growth factors:
 - very similar overall shape
 - sequence differences in finger regions contacting receptors
 - crystal packing causes inter dimer contacts for these regions
- molecular dynamics simulation for solvated dimer
 - closer to the *in vivo* environment
 - overall secondary structure conserved
 - some additional relevant contacts (interactions)
 - flexible finger regions show large movements and even shifts with respect to x-ray coordinates
 - amplitude of movements similar to NMR results on TGF
 - x-ray conformation not reliable for mechanistic analysis

CHEOPS contact analysis extracts all significant information from huge haystack of MD data

study of the specific interactions with receptors on its way