Comprehensive Review

BAG Title: Groningen-Utrecht 1999-2000

1. List of Publications

1. Armand, S., Wagemaker, M.J.M., Sanchez-Torres, P., Kester, H.C.M., van Santen, Y., Dijkstra, B.W., Visser, J. & Benen, J.A.E. *The active site topology of Aspergillus niger endopolygalacturonase II as studied by site-directed mutagenesis.* J. Biol. Chem. **275**, 691-696 (2000).
2. Bokma, E., Barends, T., Terwisscha van Scheltinga, A.C., Dijkstra, B.W. & Beintema, J.J. *Enzyme kinetics of hevamine, a chitinase from the rubber tree Hevea brasiliensis.* FEBS Lett. **478**, 119-122 (2000).
3. Kingma, R.L., Fragiathaki, M., Snijder, H.J., Dijkstra, B.W., Verheij, H.M., Dekker, N. & Egmond, M.R. *Unusual catalytic triad of Escherichia coli outer membrane phospholipase A.* Biochemistry **39**, 10017-10022 (2000).
4. Liebeton, K., Zonta, A., Schimossek, K., Nardini, M., Lang, D., Dijkstra, B.W., Reetz, M.T. & Jaeger, K.-E. *Directed evolution of an enantioselective lipase.* Chemistry & Biology **7**, 709-718 (2000).
5. Nardini, M., Lang, D.A., Jaeger, K.-E. & Dijkstra, B.W. *Crystal structure of Pseudomonas aeruginosa lipase in the open conformation: the prototype for family I.1 of bacterial lipases.* J. Biol. Chem. **275**, 31219-31225 (2000).
6. Oubrie, A. & Dijkstra, B.W. *Structural requirements of pyrroloquinone dependent enzymatic reactions.* Protein Science **9**, 1265-1273 (2000).
7. Uitdehaag, J.C.M., van Alebeek, G.-J.W.M., van der Veen, B.A., Dijkhuizen, L. & Dijkstra, B.W. *Structures of maltohexaose and maltoheptaose bound at the donor sites of cyclodextrin glycosyltransferase give insight into the mechanisms of transglycosylation activity and cyclodextrin size specificity.* Biochemistry **39**, 7772-7780 (2000).
8. van Asselt, E.J., Kalk, K.H. & Dijkstra, B.W. *Crystallographic studies of the interactions of Escherichia coli lytic transglycosylase Slt35 with peptidoglycan.* Biochemistry **39**, 1924-1934 (2000).
9. van der Veen, B.A., Uitdehaag, J.C.M., Dijkstra, B.W. & Dijkhuizen, L. *The role of arginine 47 in the cyclization and coupling reactions of cyclodextrin glycosyltransferase from Bacillus circulans strain 251; implications for product inhibition and product specificity.* Eur. J. Biochem. **267**, 3432-3441 (2000).
10. van der Veen, B.A., Uitdehaag, J.C.M., Penninga, D., van Alebeek, G.-J.W.M., Smith, L.M., Dijkstra, B.W. & Dijkhuizen, L. *Rational design of cyclodextrin glycosyltransferase from Bacillus circulans strain 251 to Increase α-cyclodextrin production.* J. Mol. Biol. **296**, 1027-1038 (2000).
11. van der Veen, B.A., van Alebeek, G.-J.W.M., Uitdehaag, J.C.M., Dijkstra, B.W. & Dijkhuizen, L. *The three transglycosylation reactions catalyzed by cyclodextrin glycosyltransferase from Bacillus circulans (strain 251) proceed via different kinetic mechanisms.* Eur. J. Biochem. **267**, 658-665 (2000).
12. Jaeger, K.-E., Dijkstra, B.W. & Reetz, M.T. *Bacterial biocatalysts: molecular biology, three-dimensional structures, and biotechnological applications of lipases.* Annu. Rev. Microbiol. **53**, 315-351 (1999).
13. Nardini, M. & Dijkstra, B.W. α/β *Hydrolase fold enzymes: the family keeps growing.* Curr. Opin. Struct. Biol. **9**, 732-737 (1999).
14. Nardini, M., Ridder, I.S., Rozeboom, H.J., Kalk, K.H., Rink, R., Janssen, D.B. & Dijkstra, B.W. *The X-ray structure of epoxide hydrolase from Agrobacterium radiobacter AD1: an enzyme to detoxify harmful epoxides.* J. Biol. Chem. **274**, 14579-14586 (1999).

15. Oubrie, A., Rozeboom, H.J. & Dijkstra, B.W. *Active site structure of the soluble quinoprotein glucose dehydrogenase complexed with methylhydrazine: A covalent cofactor-inhibitor complex.* Proc. Natl. Acad. Sci. USA **96**, 11787-11791 (1999).
16. Oubrie, A., Rozeboom, H.J., Kalk, K.H., Duine, J.A. & Dijkstra, B.W. *The 1.7 Å crystal structure of the apo-form of the soluble quinoprotein glucose dehydrogenase from Acinetobacter calcoaceticus reveals a novel internal conserved sequence repeat.* J. Mol. Biol. **289**, 319-333 (1999).
17. Oubrie, A., Rozeboom, H.J., Kalk, K.H., Olsthoorn, A.J.J., Duine, J.A. & Dijkstra, B.W. *Structure and mechanism of soluble quinoprotein glucose dehydrogenase.* EMBO J. **18**, 5187-5194 (1999).
18. Pikkemaat, M.G., Ridder, I.S., Rozeboom, H.J., Kalk, K.H., Dijkstra, B.W. & Janssen, D.B. *Crystallographic and kinetic evidence of a collision complex formed during halide import in haloalkane dehalogenase.* Biochemistry **38**, 12052-12061 (1999).
19. Ridder, I.S. & Dijkstra, B.W. *Identification of the Mg^{2+} binding site in the P-ATPase and phosphatase members of the HAD superfamily by structural similarity to CheY.* Biochem. J. **339**, 223-226 (1999).
20. Ridder, I.S., Rozeboom, H.J. & Dijkstra, B.W. *Haloalkane dehalogenase from Xanthobacter autotrophicus GJ10 refined at 1.15 Å resolution.* Acta Crystallogr. **D55**, 1273-1290 (1999).
21. Ridder, I.S., Rozeboom, H.J., Kalk, K.H. & Dijkstra, B.W. *Crystal structures of intermediates in the dehalogenation of haloalkanoates by L-2-haloacid dehalogenase.* J. Biol. Chem. **274**, 30672-30678 (1999).
22. Rink, R., Lutje Spelberg, J.H., Pieters, R.J., Kingma, J., Nardini, M., Kellogg, R.M., Dijkstra, B.W. & Janssen, D.B. *Mutation of tyrosine residues involved in the alkylation half reaction of epoxide hydrolase from Agrobacterium radiobacter AD1 results in improved enantioselectivity.* J. Am. Chem. Soc. **121**, 7417-7418 (1999).
23. Snijder, H.J., Ubarretxena-Belandia, I., Blaauw, M., Kalk, K.H., Verheij, H.M., Egmond, M.R., Dekker, N. & Dijkstra, B.W. *Structural evidence for dimerisation regulated activation of an integral membrane phospholipase.* Nature **401**, 717-721 (1999).
24. Uitdehaag, J.C.M., Kalk, K.H., van der Veen, B.A., Dijkhuizen, L. & Dijkstra, B.W. *The cyclization mechanism of cyclodextrin glycosyltransferase as revealed by a γ-cyclodextrin-CGTase complex at 1.8 Å resolution.* J. Biol. Chem. **274**, 34868-34876 (1999).
25. Uitdehaag, J.C.M., Mosi, R., Kalk, K.H., van der Veen, B.A., Dijkhuizen, L., Withers, S.G. & Dijkstra, B.W. *X-ray structures along the reaction pathway of cyclodextrin glycosyltransferase elucidate catalysis in the α-amylase family.* Nature Struct. Biol. **6**, 432-436 (1999).
26. van Asselt, E.J., Dijkstra, A.J., Kalk, K.H., Takacs, B., Keck, W. & Dijkstra, B.W. *Crystal structure of Escherichia coli lytic transglycosylase Slt35 reveals a lysozyme-like catalytic domain with an EF hand.* Structure **7**, 1167-1180 (1999).
27. van Asselt, E.J. & Dijkstra, B.W. *Binding of calcium in the EF-hand of Escherichia coli lytic transglycosylase Slt35 is important for stability.* FEBS Lett. **458**, 429-435 (1999).
28. van Asselt, E.J., Thunnissen, A.-M.W.H. & Dijkstra, B.W. *High resolution crystal structures of the Escherichia coli lytic transglycosylase Slt70 and its complex with a peptidoglycan fragment.* J. Mol. Biol. **291**, 877-898 (1999).
29. van Santen, Y., Benen, J.A.E., Schröter, K.-H., Kalk, K.H., Armand, S., Visser, J. & Dijkstra, B.W. *1.68-Å crystal structure of endopolygalacturonase II from Aspergillus niger and identification of active site residues by site-directed mutagenesis.* J.Biol.Chem. **274**, 30474-30480 (1999).
30. van den Elsen, J.M.H., Vandeputte-Rutten, L., Kroon, J. & Gros, P., *Bactericidal antibody recognition of meningococcal PorA by induced fit: comparison of liganded and unliganded Fab structures.* J.Biol.Chem. **274**, 1495-1501 (1999).
31. Bouma, B., de Groot, P.G., van den Elsen, J.M.H., Ravelli, R.B.G., Schouten, A., Simmelink, M.J.A., Derksen, R.H.W.M., Kroon, J. & Gros, P, *Adhesion mechanism of human β2-Glycoprotein I to phospholipids based on its crystal structure.* EMBO J. **18**, 5166-5174 (1999).

32. Dekker, C., de Kruijff, B., de Korte-Kool, G., Kroon, J. & Gros, P., *Crystals of acetylated SecB diffract to 2.3 Å resolution.* J. Structural Biology **128**, 237-242 (1999).
33. Weik, M., Ravelli, R.B.G., Kryger, G., McSweeney, S., Raves, M.L., Harel, M., Gros, P., Silman, I., Kroon, J., & Sussman, J.L., *Specific and structural damage to proteins produced by synchrotron radiation.* Proc. Natl. Acad. Sci. USA **97**, 623-628 (2000).
34. P.G. de Groot, B. Bouma, B.C. Lutters, M.J. Simmelink, R.H. Derksen and P. Gros, Structure-Function Studies on 2-glycoprotein I, *J. Autoimmunity* **15**, 87-89 (2000).

Papers submitted

B. Bouma, E.G. Huizinga, M.E. Schiphorst, J.J. Sixma, J. Kroon and P. Gros, Structure of a von Willebrand Factor A3-domain - Fab Complex Points to a Location for the Collagen-Binding Site, *submitted.* (includes data from DESY-Hamburg).

Weik, M., Kryger, G., Schreurs, A.M.M., Bouma B., Silman, I., Sussman, J.L., Gros, P. & Kroon, J. Solvent behavior in flash-cooled protein crystals at cryogenic temperatures, *submitted.*

Papers in preparation

B. Bouma, Ph.G. de Groot, M.J.A. Simmelink, J. Kroon and P. Gros, Specific Binding of a Neutral Phospholipid Blocks Adhesion of $_2$-Glycoprotein I to Anionic Membranes, *in preparation.*

L. Vandeputte-Rutten, A. Kramer, N. Dekker, M. Egmond, J. Kroon and P. Gros, Structure of the Outer-membrane Proteinase OmpT from *E. coli*, *in preparation*.

B. Agianian, J.D. Clayton, K. Leonard, P.A. Tucker, B. Bullard and P. Gros, The crystal structure of the *Drosophila* sigma class GST at 1.75 Å resolution, *in preparation.*

Weik, M., Ravelli, R. B., Silman, I., Sussman, J. L. Gros, P., Kroon, J. Protein dynamics observed at the solvent glass transition temperature, *in preparation.*

F.I. Gliubich, B.W. Dijkstra and A.M.W.H. Thunnissen, The crystal structure of the endolytic membrane-bound lytic transglycosylase EmtA from Escherichia coli at 2.2 Å, *in preparation*

2. Data Collection Details

Beamline ID14-EH1

Protein name	Exp no. & type	Reso (Å)	Rmerge (%)	Compl. (%)	redun-dancy	Status
PC4	LS-1654, single	40-3.1	6.6 (26.3)	99.8 (99.5)	4.3	structure refined
OmpT-Semet	LS-1654, single	40-3.1	8.3 (40.0)	91.2 (92.6)	3.0	Manuscript in preparation
$_2$-glycoprotein I isoform	LS-1654, single	36.0–3.0	5.6 (21.4)	99.4 (99.2)	4.9	Manuscript in preparation
GST-2 Hg derivative	LS-1654, single	40.0-3.2	14.5 (41.5)	99.8 (100)	6.0	Structure under refinement
AChE	LS-1654, single , 10 d- sets @diff. Ts	40.0-3.0	5 – 6.3	97 – 99.7		Manuscript in preparation
GST-2 Sm derivative	LS-165, single	40.0-2.6	7.0 (25.4)	99.7 (99.6)	5.4	Structure under refinement

Beamline ID14-EH2

Protein name	Exp no. & type	Reso (Å)	Rmerge (%)	Compl. (%)	redun-dancy	Status
ATP-binding cassette GlcV	LS-1654, single	1.7	5.2		8	Structure being refined
Quercetinase substrate soak	LS-1654, single	2.3	6.2	92	3	Structure solved, manuscript in preparation
OmpT-Semet-ZnCl2 and high pH	LS-1654, single	40-2.95	5.3 (32.1))	98.3 (90.3)	3.4	Structure being refined
OmpT-Semet-substrate analogue	LS-1654, single	40-2.55	6.6 (31.1)	98.3 (99.0)	3.7	Structure being refined
AChE	LS-1654, single	40- 2.3 – 2.6	typically 15	typically 99		Manuscript in preparation
Halohydrin dehalo-genase (HheC)	LS-1505, single	1.8	6			Twinned
B. subtilis lipase	LS-1654, single	1.5	7.6	82.4	4	Structure solved, manuscript to be submitted

Beamline ID14-EH3

Protein name	Exp no. & type	Reso (Å)	Rmerge (%)	Compl. (%)	redun-dancy	Status
AcylCoA:isopen acyltransferase	LS-1360, native 1	1.9	4.3	97.4	3.2	Structure solved, published
botrocetin (derivative)	LS-1505, single	2.5	5.4	99.5	3.7	refinement in progress
botrocetin (derivative)	LS-1505, single	2.9	4.3	98.1	3.8	refinement in progress
botrocetin (native)	LS-1505, single	2.3	7.0	100	3.7	refinement in progress
botrocetin (derivative)	LS-1505, single	2.8	6.5	97	4.1	refinement in progress
botrocetin (derivative)	LS-1505, single	3.2	5.5	100	4.5	refinement in progress
von Willebrand factor A1	LS-1505, single	1.95	4.9	100	7.1	Publication in progress
AChE	LS-1505, single	40 – 3.0	4.1 – 5.1	99.2 – 99.4		Manuscript in preparation
$_2$-glycoprotein I isoform	LS-1505, single	38.4–3.0	6.3 (43.3)	96.3 (94.5)	3.9	Manuscript in preparation
von Willebrand Factor-A1+botrocetin	LS- 1654, single	3.2	11.2	87	4.7	refinement in progress
von Willebrand Factor-A3 S968T	LS-1654, single	1.9	8.2	98	4.0	refined
AChE	LS-1654, single	40.0-3.0	9.6 – 10.5	92.4 – 92.5		evaluation in progress
Anti-P1.4 Fab with peptide	LS-1654, single	40.0- 1.95	7.7 (38.9)	96.0 (92.1)	3.5	Structure under refinement

Beamline ID14-EH4

Protein name	Exp no. & type	Reso (Å)	Rmerge (%)	Compl. (%)	redun-dancy	Status
GlcV	LS-1654, native 1	2.0	5.7	95.6	4	Structure solved and being refined
PITP	LS-1505, native	40–2.0	6.3 (45.7)	98.3 (92.8)	3.4	phasing failed
idem	LS-1505, MAD	40–3.0	10.4 (12.4)	98.8 (98.1)	3.0	phasing failed
idem	LS-1505, MAD	40–3.0	8.1 (9.8)	98.7 (97.0)	1.8	phasing failed
idem	LS-1505, MAD	40–3.0	3.5 (5.5)	97.9 (97.6)	1.8	phasing failed
OmpT	LS-1505, single	40–2.6	7.4 (26.3)	94.3 (97.7)	1.5	Manuscript in preparation
OmpT-Semet	LS, 1654, MAD	40–2.6	5.8 (29.4)	99.4 (98.0)	3.0	Manuscript in preparation
idem	LS 1654, MAD	40–2.7	4.5 (25.5)	99.6 (99.3)	3.0	Manuscript in preparation
idem	LS 1654, MAD	40–2.7	5.9 (37.8)	99.6 (98.9)	3.0	Manuscript in preparation
OmpT-Semet	LS-1654, MAD	40–3.1	9.7 (39.5)	99.8 (100)	6.3	Manuscript in preparation
idem	LS-1654, MAD	40–3.1	7.5 (29.4)	99.8 (100)	6.3	Manuscript in preparation
idem	LS-1654, MAD	40–3.1	7.2 (18.9)	99.7 (100)	6.3	Manuscript in preparation
Anti-P1.4 Fab with peptide	LS-1654, single	40.0-2.4	7.9 (42.0)	93.9 (93.2)	2.8	Improvement of crystals

Beamline BM14

Protein name	Exp no. & type	Reso (Å)	Rmerge (%)	Compl. (%)	redun-dancy	Status
AcylCoA:isopen acyltransferase	LS-1360, MAD	2.85 (3x)	6.4	92.3	3.6 (3x)	Solved, to be published
Lytic trans-glycosylase (EmtA)	LS-1360, MAD	3.0	6.3	91.7	2.5	Failed, a new MAD was collected later on.
EmtA	LS-1505, MAD	2.75	1: 5.5 2: 6.1 3: 6.5	92.8 92.3 92.2	3.5 3.5 3.5	Solved, to be published
Halohydrin dehalo-genase (HheC)	LS-1505, MAD	2.7	5	98.2	3.4	Structure being refined
PLA2	LS-1505, single	0.97	6.4	99.8		
B. subt. Lipase, heavy atom derv.	LS-1654, two s	2.0	5.0	97.2	3.5	Structure solved, manuscript submitted
B. subt. Lipase, heavy atom derv.	LS-1654, single	2.5	12.6	98	3.3	Structure solved, manuscript submitted
SecB	LS-1505, MAD	40-3.3	7.4 (27.6)	99.7 (100.0)	2.5	phasing failed

idem	LS-1505, MAD	40-3.3	7.1 (30.2)	98.8 (99.7)	2.5	phasing failed
idem	LS-1505, MAD	40-3.3	8.9 (37.0)	98.9 (99.6)	2.5	phasing failed
OmpT-Semet	LS-1505, SAD	40–3.5	9.2	99.9	2.9	Manuscript in preparation

3. Global Summary

The Groningen protein crystallography group has a strong focus on structural research of enzyme mechanisms. Enzymes being studied comprise lipolytic enzymes (membrane phospholipase A, lipases), dehalogenases, carbohydrate converting enzymes (cyclodextrin glycosyl transferase, polygalacturonase, chitinase), bacterial enzymes involved in cell wall synthesis and degradation (penicillin binding proteins, lytic transglycosylases), and copper containing dioxygenases. In addition the group studies several other proteins involved in cellular transport, including bacterial sugar transport systems for mannitol and cellobiose and an archaeal ABC transport system for glucose.

The Utrecht protein crystallography group investigates the molecular basis of recognition and regulation with a focus on bio-medically important proteins, including human von Willebrand Factor, human platelet-surface Glycoprotein Ib, human 2-Glycoprotein I, human fucosyltransferase and protein involved in immune response to *N. meningitidis*. Furthermore, the group studies several other proteins: phosphatidylinositol transfer protein, outer-membrane proteinase OmpT from *E. coli* abd Glutathione S-transferase-2 from *Drosophila*. In collaboration with the laboratory of J. Sussman we study the mechanism of acetylcholinesterase.

4. Highlight reports

- the visualization of a covalent intermediate in the reaction pathway of cyclodextrin glycosyltransferase (Nature Struct. Biol. 6, 432-436 (1999); see also the accompanying News and Views article in the same issue of Nature Struct. Biol.). (With Dr. S. Withers, Vancouver; Prof. L. Dijkhuizen, Groningen).
- the elucidation of the crystal structure and the reaction mechanism of the biosensor enzyme glucose dehydrogenase (Proc. Natl. Acad. Sci. USA 96, 11787-11791 (1999); EMBO J. 18, 5187-5194 (1999)). (With Prof. J.A. Duine, Delft).
- the elucidation of the structure and the mechanism of activation of an integral membrane protein (Nature 401, 717-721 (1999)). (With Prof. H.M. Verheij and Dr. N. Dekker, Utrecht).
- Insight into membrane adhesion based on the structure of the heavily glycosylated 5-domain protein $_2$-glycoprotein I (EMBO J. 18, 5166-5174 (1999).
- Specific structural radiation damage observed in acetylcholinesterase (Proc. Natl Acad. Sci. 97, 623-628 (2000), with R. Ravelli, S. McSweeney, ESRF, and J. Sussman, Israel).
- Successful approach to rational vaccine design (*paper in prep.,* with Inst.for Publ Health, The Netherlands).

The X-ray structure of *E. coli* Outer Membrane Phospholipase A

Dimerization is a biological regulatory mechanism employed by both soluble and membrane proteins. Structural data on the factors that govern dimerization of membrane proteins is scarce, however. We have solved the X-ray crystal

structure of *Escherichia coli* Outer Membrane Phospholipase A (OMPLA), an integral membrane enzyme which is regulated by reversible dimerization.

The structure of monomeric OMPLA consists of a 12-stranded anti-parallel _-barrel with a convex and a flat side. The active site is located on the exterior of the _-barrel, just outside the outer leaflet ring of aromatic residues. It contains Ser144 and His142, and an asparagine (Asn156) at hydrogen bonding distance from the N_1 atom of His142.

Covalent inhibition with hexadecanesulfonylfluoride allowed the crystallization of the dimeric enzyme. In the dimer the two active sites are located at the outer leaflet side of the membrane. The dimer interactions occur mostly in the hydrophobic membrane-embedded area. The hydrophobic side chains of Leu32$^{A/B}$, Leu71$^{A/B}$, Leu73$^{A/B}$ and Leu265$^{A/B}$ exhibit a knob-and-hole interaction. In monomeric OMPLA a stabilizing hydrogen bonding network between Tyr92, Gln94 and Ser96 allows these residues to partition in the hydrophobic membrane environment. In the dimer, however, the Gln94 side-chain is additionally hydrogen bonded to Gln94 of the other monomer. Gln94 is strictly conserved among all OMPLA sequences, emphasizing the importance of this residue for functional dimerization. Thus, specific dimer interactions are obtained from a combination of surface complementarity of knob-and-hole patterns, and hydrogen bonding interactions between polar residues in the hydrophobic membrane environment.

Dimerization results in productive active sites, with two hydrophobic substrate-binding pockets created at the interface between the two monomers. Both pockets accommodate all 16 aliphatic carbon atoms of the inhibitors. Binding of the inhibitors accounts for 27% of the dimerization surface, which rationalizes the observed increase in dimer stability of the inhibited enzyme. These results present for the first time detailed structural information on activity regulation by dimerisation of a membrane protein. Despite a radically different environment, inter-molecule contacts for membrane proteins share many of the characteristics of soluble protein interactions. The results have been published by Snijder *et al.* in Nature **401**, 717-721 (1999).

Human plasma-regulator β_2-glycoprotein I: a protein and membrane adhesion molecule

$_2$-Glycoprotein I (2GPI) is present in large amount in human plasma (~200 mg/L). It is implicated in blood coagulation, clearance of apoptotic bodies and in response to oxidative stress. It is a key antigen in the autoimmune disease Anti-Phospholipid Syndrome(APS). 2GPI is heavily glycosylated (20% w/w) and consists of 5 SCR-domains, that are common to the Regulators of Complement Activation (RCA) family. The fifth domain of 2GPI has 6-residue insertion and a 9-residue extension at the C-terminus. This fifth abberrant domain has been implicated in adhesion to negatively charged membranes, which is most likely a common and essential feature of 2GPI in its various membrane ahdered functions.
We solved the structure of 2GPI to 2.7 Å resolution. The structure revealed an extended conformation of the 5 domains (with a total solvent content of the crystal of 85%). Based on the structure, we proposed a model for membrane adhesion through electrostatic interactions with a large positively charged patch on domain 5 and hydrophobic 'anchoring' by the exposed 310-318 loop that contains the critical Trp316-Lys317 for interaction with the interfacial region of the phospholipid layer. Adhesion to lipid layers likely points the N-terminal domains into the solvent, with domains 3 and 4 largely shielded by glycans and domains 1 and 2 exposed for putative protein adhesion.
Most recently, we solved the structure of 2GPI in complex with a lipid molecule; this form of the protein correlate with the abundant isoform found in patients with severe oxidative stress. Surprisingly, within the large region for 'aspecific' adhesion to negatively charged lipid layers 2GPI has a binding pocket for neutrally charged lipids. This finding sheds completely new light on the potential function(s) of 2GPI in human plasma.

The role of von Willebrand factor in platelet aggregation

Von Willebrand factor (vWF) plays a crucial role in thrombus formation after vascular damage. It forms a bridge between collagen exposed at sites of injury and glycoprotein Ib (GpIb) located on the surface of blood platelets. The A3 domain of vWF mediates binding to collagen [1,2]; the binding site for GpIb is located in the vWF-A1 domain. vWF in blood plasma does not bind spontaneously to GpIb. After vascular damage vWF becomes activated for GpIb binding, through a mechanism that is not well understood. One approach to study this activation step uses Botrocetin isolated from the venom of *Bothrops Jararaca*, that is capable of inducing the binding of vWF-A1 to GpIb.

We have determined a crystal structure of the vWF-A1 domain to 1.95 Å resolution and solved it by molecular replacement using a known structure of the domain. Next, we solved the structure of botrocetin, which is a 25 kDa protein homologous to C-type lectins. It consists of a disulfide-linked hetero-dimer composed of a 133 amino-acid _-subunit and a 125 amino-acid _-subunit. These data were used subsequently to solve the structure of the vWF-A1 – botrocetin complex by molecular replacement (to a resolution of 3.2 Å). Using a crystal of similar size a 2.3 Å data set (R-merge = 5.4%) was later collected on the micro-focus beam line ID13 using an aperture of 10 _m. Crystallographic refinement of the botrocetin-A1 complex against this 2.3 Å data set is currently in progress. Preliminary structure analysis reveals structural changes in the putative GPIb binding site of vWF-A1 induced by botrocetin binding.

[1] E.G. Huizinga, R.M. van der Plas, J. Kroon, J.J. Sixma and P. Gros, Crystal Structure of the A3 Domain of Human von Willebrand Factor: Implications for Collagen Binding, ***Structure*** **5**, 1147-1156 (1997)

[2] B. Bouma, E.G. Huizinga, M.E. Schiphorst, J.J. Sixma, J. Kroon and P. Gros, Structure of a von Willebrand Factor A3-domain - Fab Complex Points to a Location for the Collagen-Binding Site, *submitted.*

Time- and temperature resolved studies of Acetylcholinesterase

We aim at identifying enzymatic product clearance pathways in acetylcholinesterase (AChE) by trapping putative reaction intermediates using temperature controlled X-ray crystallography and chemically ‘caged’ compounds. Our experimental strategy is based on the concept of dynamical transitions in proteins, which are crucial for biological function. We hope to identify by X-ray crystallographic studies below and above a putative dynamical transition in AChE the route taken by choline, one of the reaction products, once it is cleaved from its cage by means of flash-photolysis at 100 K.

In this context, we showed that the solvent in flash-cooled AChE crystals undergoes a glass transition at 155 K – a transition from a glassy, rigid state to a fluid-like state [1]. Subsequently, we set out to identify whether this solvent glass transition triggers a dynamical transition in the protein molecules. Based on our observation that intense synchrotron radiation produces specific structural and chemical alterations to proteins [2] we designed experiments in which we use structural changes upon X-ray irradiation at different temperatures as a marker for protein dynamics. The comparison of two series of data collections at 100 and 155 K revealed conformational changes that take place at 155 but not at 100 K [3]. This is strong evidence for a correlation between conformational flexibility in the protein and the structure and dynamics of the surrounding solvent, therefore validating our experimental strategy. In the following, we went a step further in combing temperature-controlled crystallography with the use of ‘caged’ choline that has been complexed to AChE at room temperature and subsequently photocleaved at 100 K. The X-ray structure at 100 K revealed that both the cage and choline are still in place in the active site of AChE due to the ‘frozen’ environment. When the temperature was raised subsequently above 155 K, structural changes in the active site were observed that indicated disappearance of the cage and a rearrangement of the choline in the active site (manuscript in preparation). We are confident that future studies using different temperature profiles will bring us closer to the answer of how the traffic of substrates and products is organized in one of nature’s fastest enzyme.

[1] Weik, M., Kryger, G., Schreurs, A.M.M., Bouma B., Silman, I., Sussman, J.L., Gros, P. & Kroon, J. Solvent behavior in flash-cooled protein crystals at cryogenic temperatures, submitted.

[2] Weik, M., Ravelli, R. B., Kryger, G., McSweeney, S., Raves, M. L., Harel, M., Gros, P., Silman, I., Kroon, J. & Sussman, J. L. (2000). Specific chemical and structural damage to proteins produced by synchrotron radiation, *Proc. Natl. Acad. Sci. U S A* **97**, 623-8.
[3] Weik, M., Ravelli, R. B., Silman, I., Sussman, J. L. Gros, P., Kroon, J. Protein dynamics observed at the solvent glass transition temperature, manuscript in preparation

Immune response to *Neisseria meningitidis*

Neisseria meningitidis is a major cause of bacterial meningitis and sepsis in humans. Immune response depends critically on the classical pathway of complement activation.
We have initiated a rational approach to vaccine development starting from crystal structures of epitopes and their 'bactericidal' (i.e. complement activating) antibodies. For the subtype P1.16 we designed a peptide vaccine based on the structure of a linear epitope peptide bound to a bactericidal antibody against the P1.16 subtype [1]. The conformational properties of the designed peptides were analysed by molecular dynamics simulation and surface plasmon resonance binding studies. One peptide was found to have good conformational properties, and only this peptide was able to induce functional antibodies in a bactericidal assay [2]. Currently, we focus on the P1.4 subtype, which is one of the most prevalent subtypes of *N. meningitidis* in the Western countries. We have solved the structure of a Fab-fragment (of a bactericidal antibody) in complex with a linear peptide derived from the epitope sequence. We will now apply our succesful vaccine-design process to obtain an anti-P1.4 peptide-vaccine.
[1] Van den Elsen *et al*., Bactericidal antibody recognition of a PorA epitope of Neisseria meningitidis: Crystal structure of a Fab fragment in complex with a fluorescein-conjugated peptide, Protein: Structure, function and genetics **29**, 113-125, 1997
[2] Oomen *et al.*, Structure-based design of a bacterial peptide vaccine against *Neisseria meningitids,* manuscript in preparation

Structure of the membrane proteinase OmpT from *E. coli*

OmpT is a proteinase present in the outer-membrane of *Escherichia coli*. It has a unique substrate specificity, cleaving proteins preferentially between two consecutive basic amino acids. Based on its inhibitor profile, OmpT has been classified as a serine proteinase . Additionally, Ser99 and His212 have been identified to be essential active site residues.
Recently, we solved the structure of OmpT using two MAD-data sets of SeMet OmpT collected at ID14-4 at resolutions of 2.6 and 3.1 Å. Initially MAD phasing failed,most likely due to the significant amount of radiation damage built up during the experiment. Fortunately, SAD phasing using the peak data set of the second crystal (3.1 Å res.), solvent flattening and phase extension yielded a readily interpretable map at 3.1 Å resolution. Currently, the refinement to 2.6 Å resolution is nearly completed.
OmpT forms a 10-stranded -barrel similar to other bacterial outer-membrane proteins. Surprsingly, in this case the barrel extends more than 40 Å from the membrane region, yielding a vase-shaped architecture. The putative active site residues Ser99 and His212 are located on the top side of the vase, and the opening of the vase forms the substrate-binding site. However, Ser99 and His212 are 9 Å apart in the structure and therefore are unlikely to be both part of the catalytic site. The structure indicates a novel type of proteinase active site, that includes His212 (Ser99 is likely to be important for substrate binding). Crystal structures of OmpT in complex with substrate analogues and inhibitors are necessary to obtain more insight into the constellation and mechanism of the catalytic site.

5. List of BAG participants

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6. Additional comments

We refer to the list of publications for the successes obtained thanks to the BAG system and the high quality, high intensity synchrotron radiation used for our projects.