Synchrotron Radiation and Structural Biology

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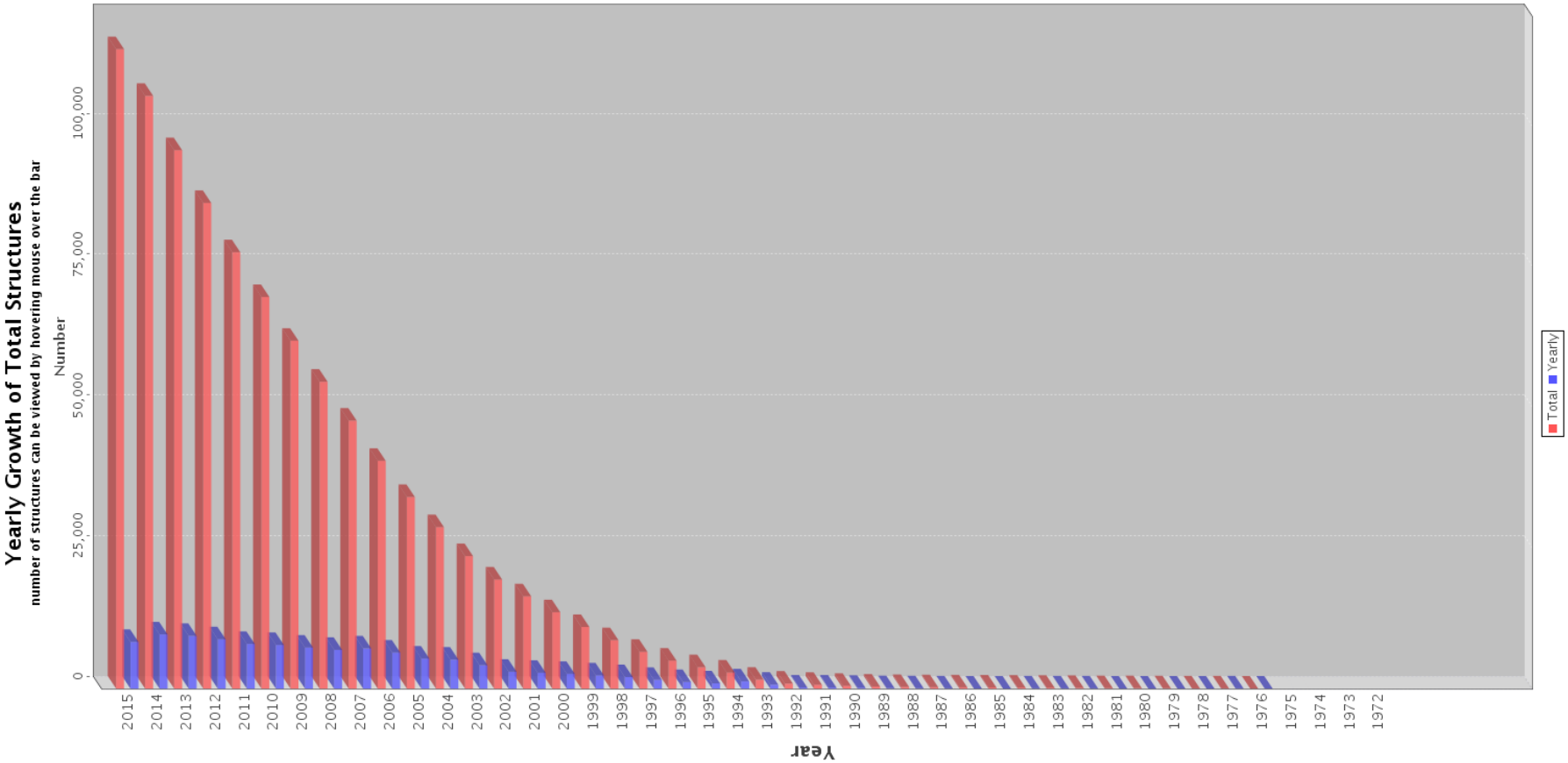
University of Copenhagen

Outline:

- Synchrotron radiation and Structural Biology from 2003 to 2013
- Malaria proteins
- Future developments
- Ways to develop a case for an ALS

Deposited structures in the Protein Data Bank

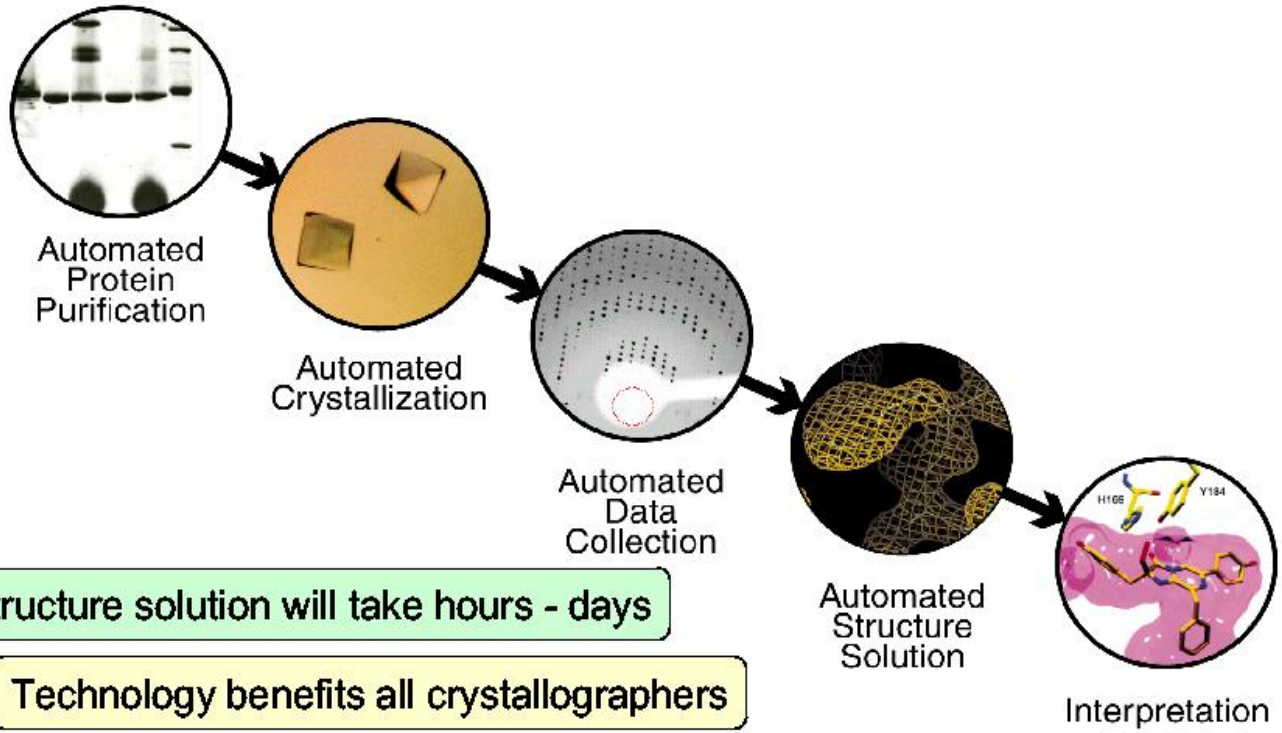


Total : 113672
X-rays: 101330

DepositionYear	Total X-Ray Structures	Total Synch Structures	Total Synch Structures(%)
1995	971	167	17.2
1996	1149	275	23.9
1997	1502	490	32.6
1998	1793	673	37.5
1999	2149	1106	51.5
2000	2457	1500	61.1
2001	2660	1722	64.7
2002	2783	1919	69.0
2003	3921	2874	73.3
2004	4459	3351	75.2
2005	5136	3851	75.0
2006	5670	4425	78.0
2007	6708	5374	80.1
2008	6120	5098	83.3
2009	7377	6147	83.3
2010	7817	6805	87.1
2011	8117	6993	86.2
2012	8775	7799	88.9
2013	9212	8451	91.7
2014	9112	8244	90.5
2015	8344	4320	51.8
Total	**106232**	**81584**	**76.8**

Nov. 3 2015

High throughput methods for X-ray crystallography

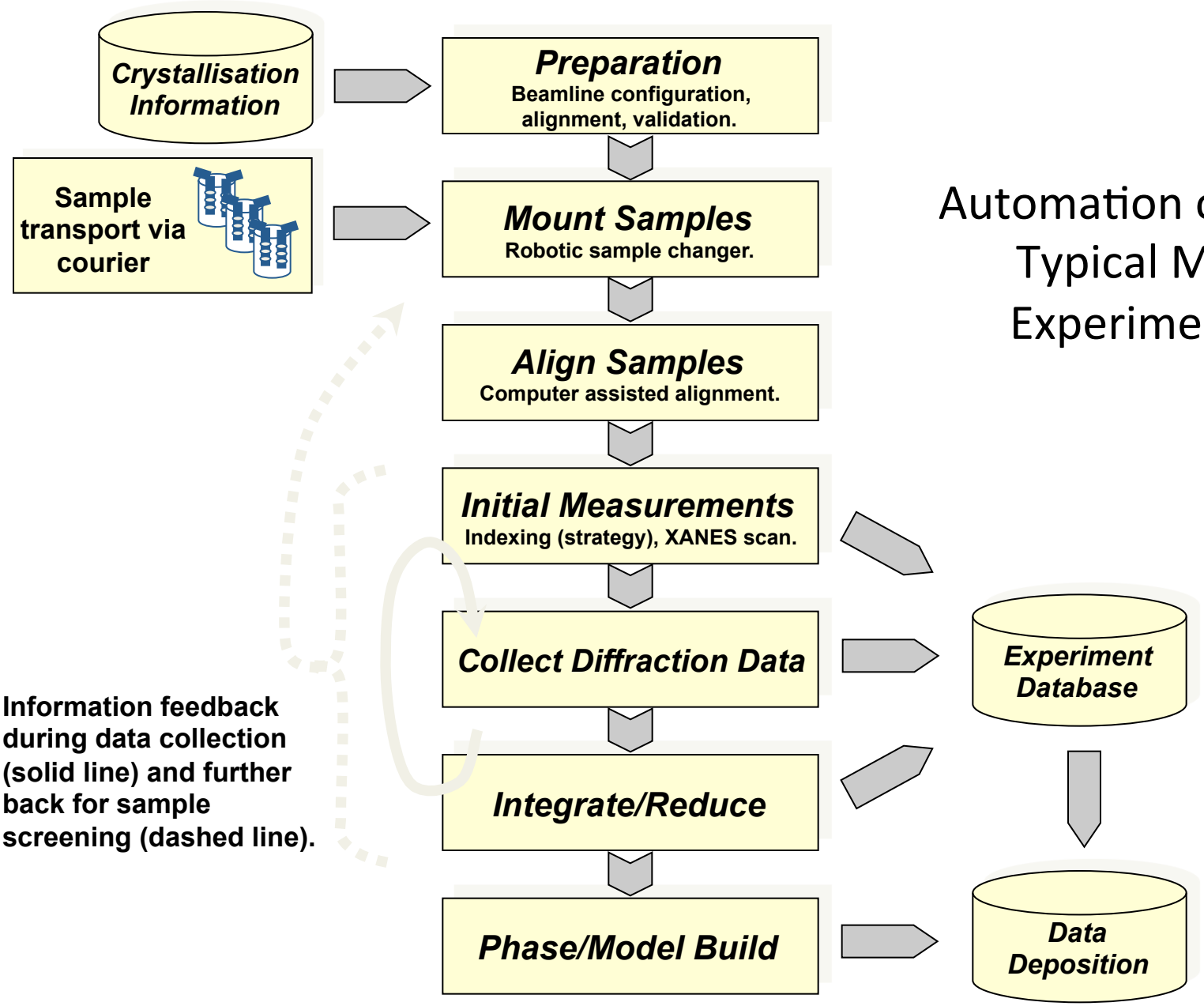


Structure solution will take hours - days

Technology benefits all crystallographers

from Paul Adams (LBL)

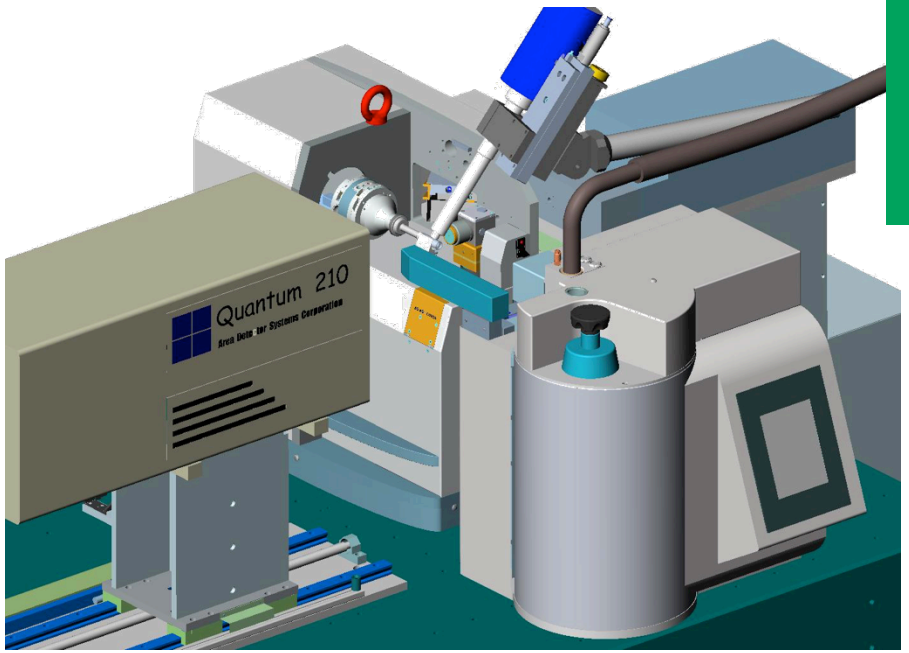
Automation of the Typical MX Experiment



Information feedback during data collection (solid line) and further back for sample screening (dashed line).

Mount and align samples

EMBL-Microdiffractometer
+
sample changer



Sample changer

Micro- or mini-diffractometer

A joint EMBL-ESRF-MRC-France project

Complementary methods

Analytical ultracentrifugation

Cryobench (spectroscopy)

Deuteration Lab

Electron Microscopy

High throughput crystallization also of membrane proteins

High Field NMR

Mass spectrometry

Size Exclusion Chromatography

Protein Sequence analysis

SAXS/SANS

Surface Plasmon Resonance

Circular Dichroism

Fluorescence spectroscopy

Light Scattering

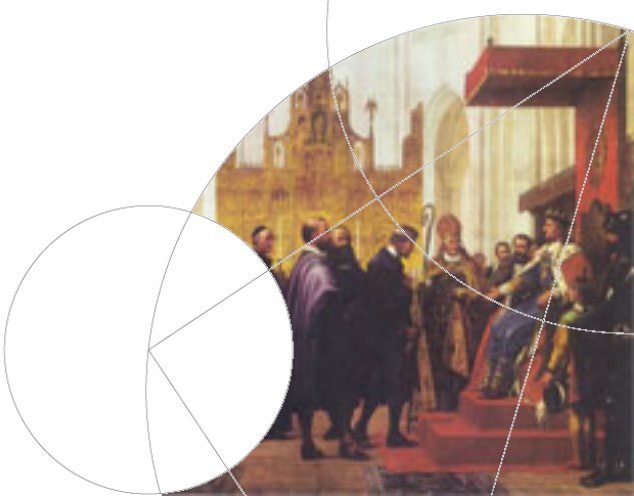
Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) Antigens

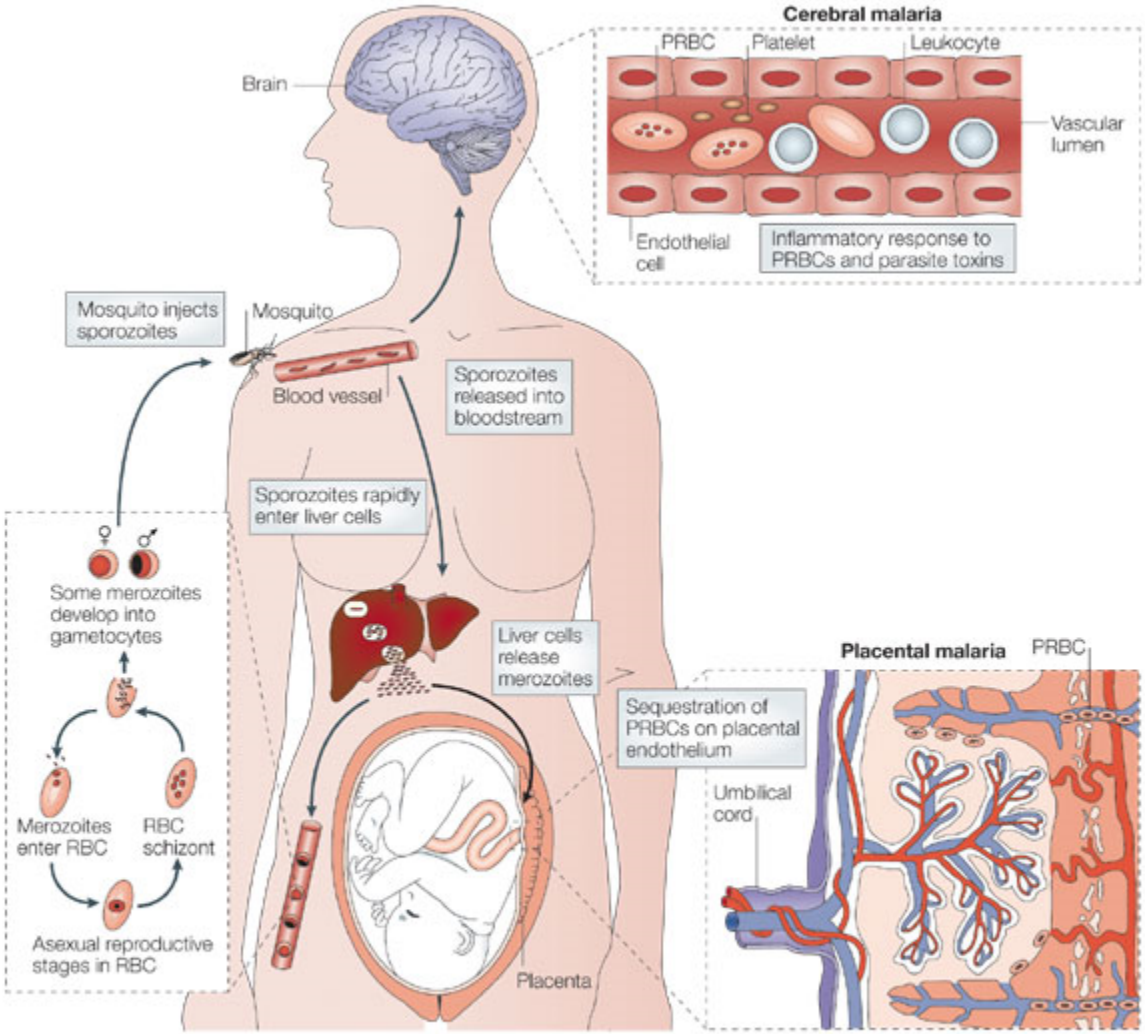
Department of Chemistry
Stig Christoffersen,
Vladyslav Soroka
Annette Langkilde

Center for Medical Parasitology (CMP)
Ali Salanti, Louise Turner, Anja T.R. Jensen
Thor G. Theander and Lars Hviid

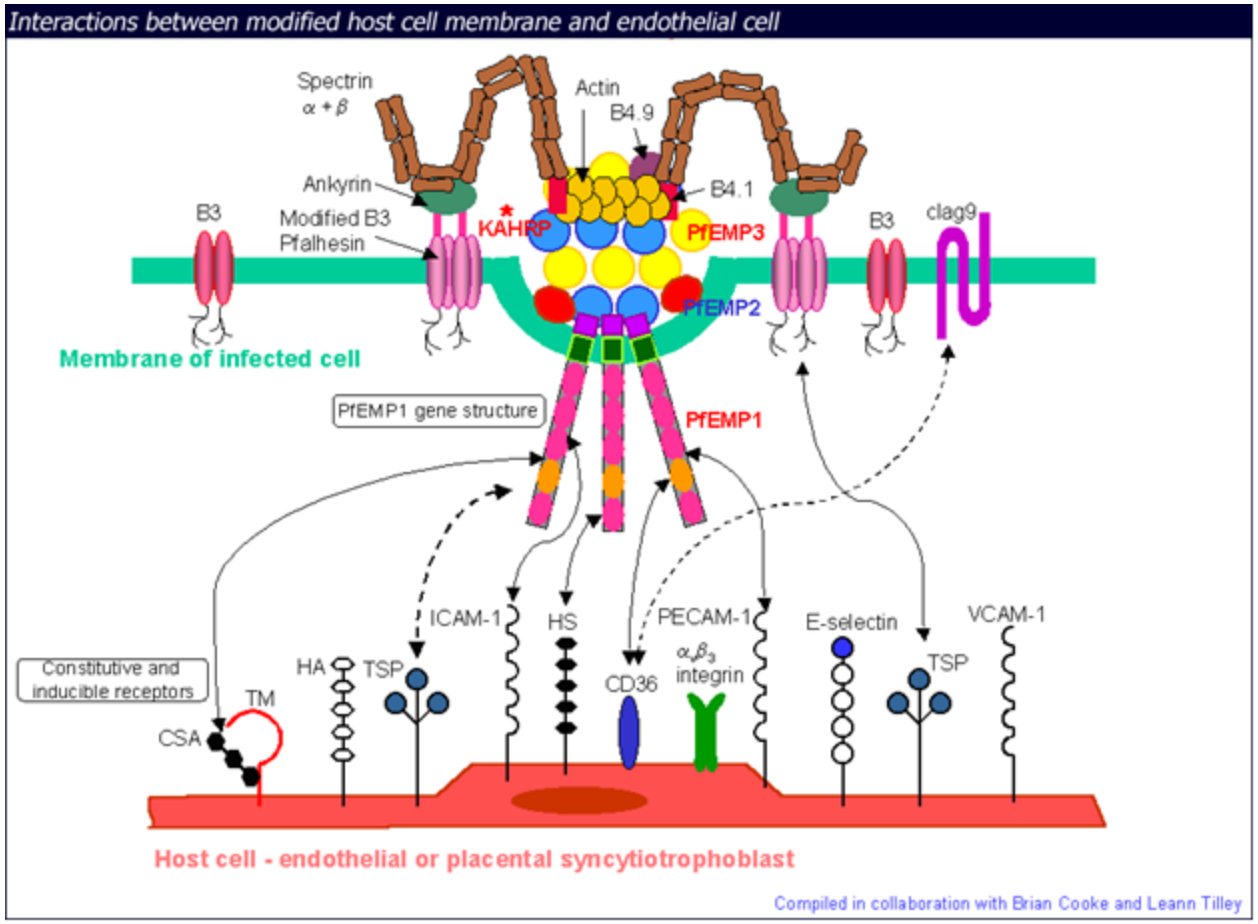
ESRF
Gordon Leonard

Funded by the “Program of Excellence” established by University of Copenhagen



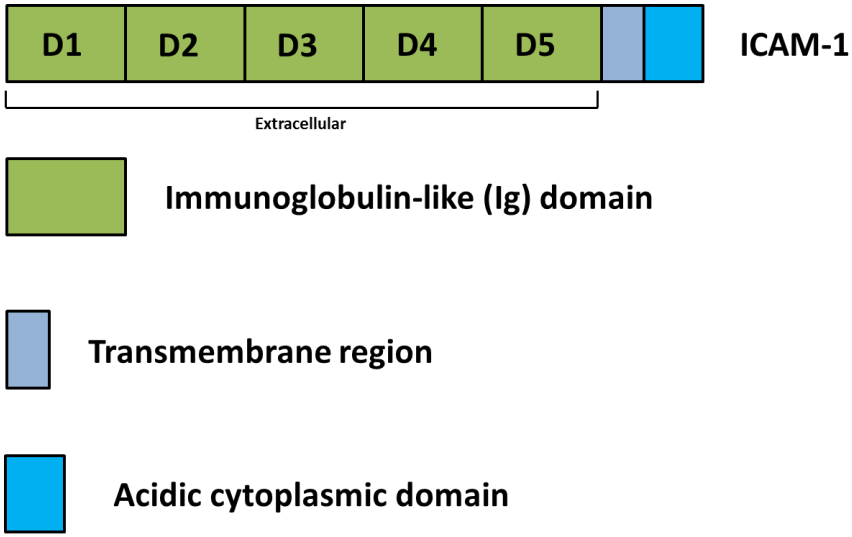


Nature Reviews | Immunology

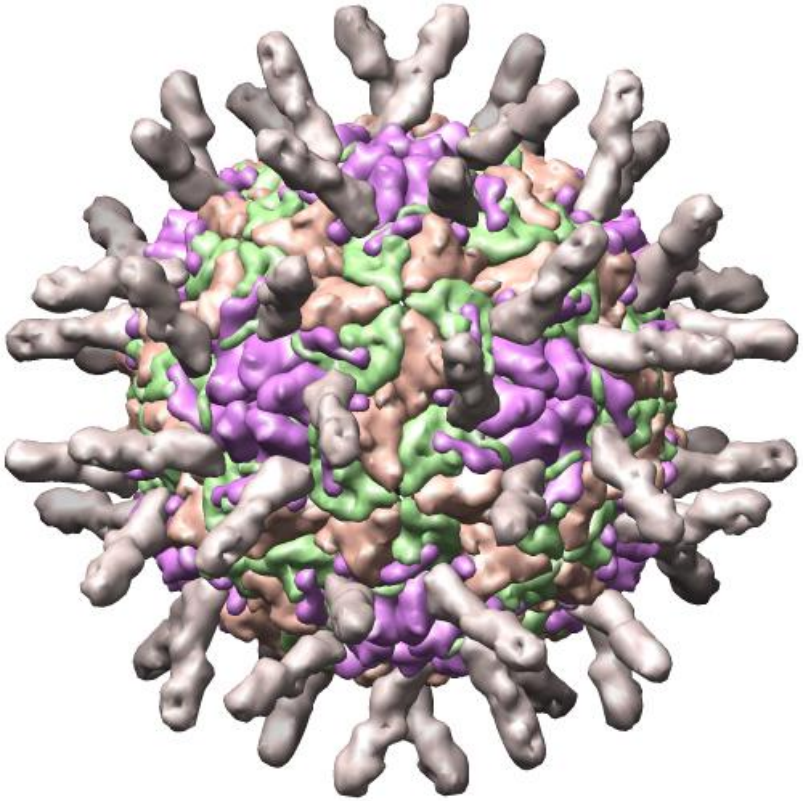


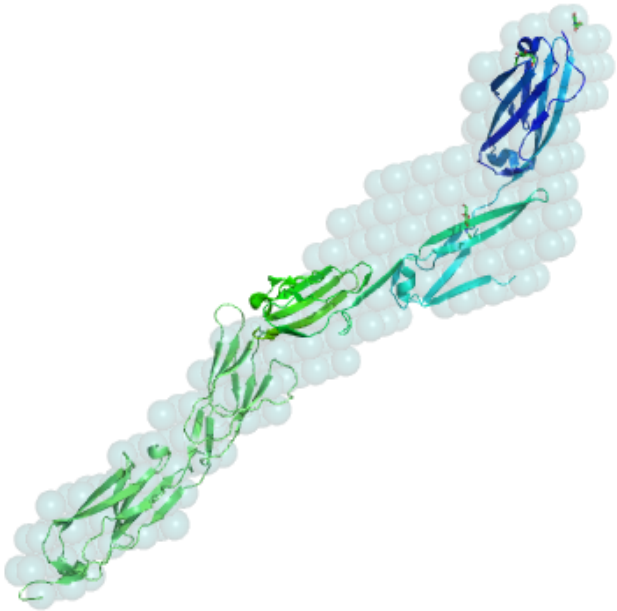
Cooke, Coppel & Wahlgren Parasitol Today 2000 **16** 416

Intercellular Adhesion molecule 1-ICAM 1



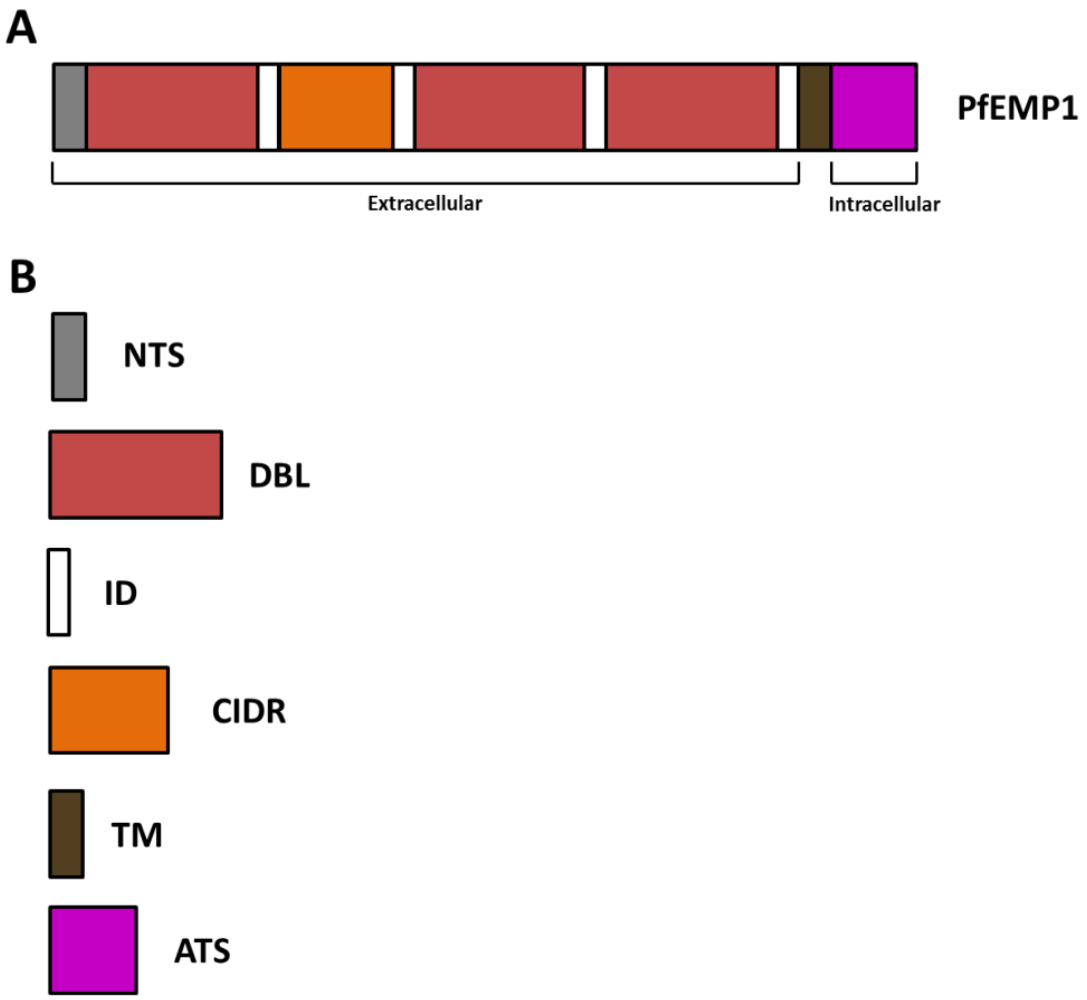
ICAM 1 glycoprotein





Involved in Cerebral Malaria

PfEMP1 proteins



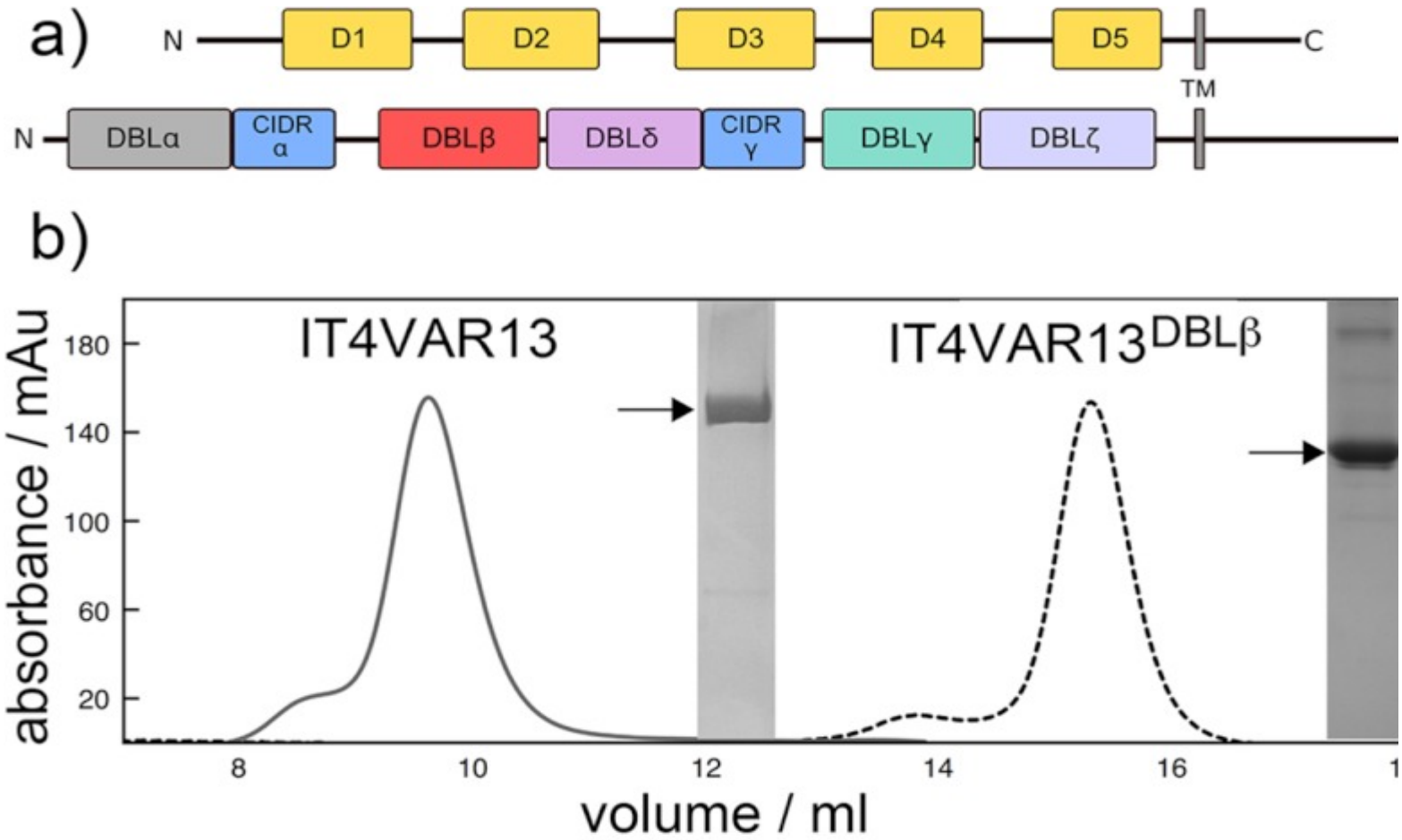
ICAM-1 receptor binding PfEMP1's involved in cerebral malaria.

VAR3 125 kDa

VAR20 280 kDa

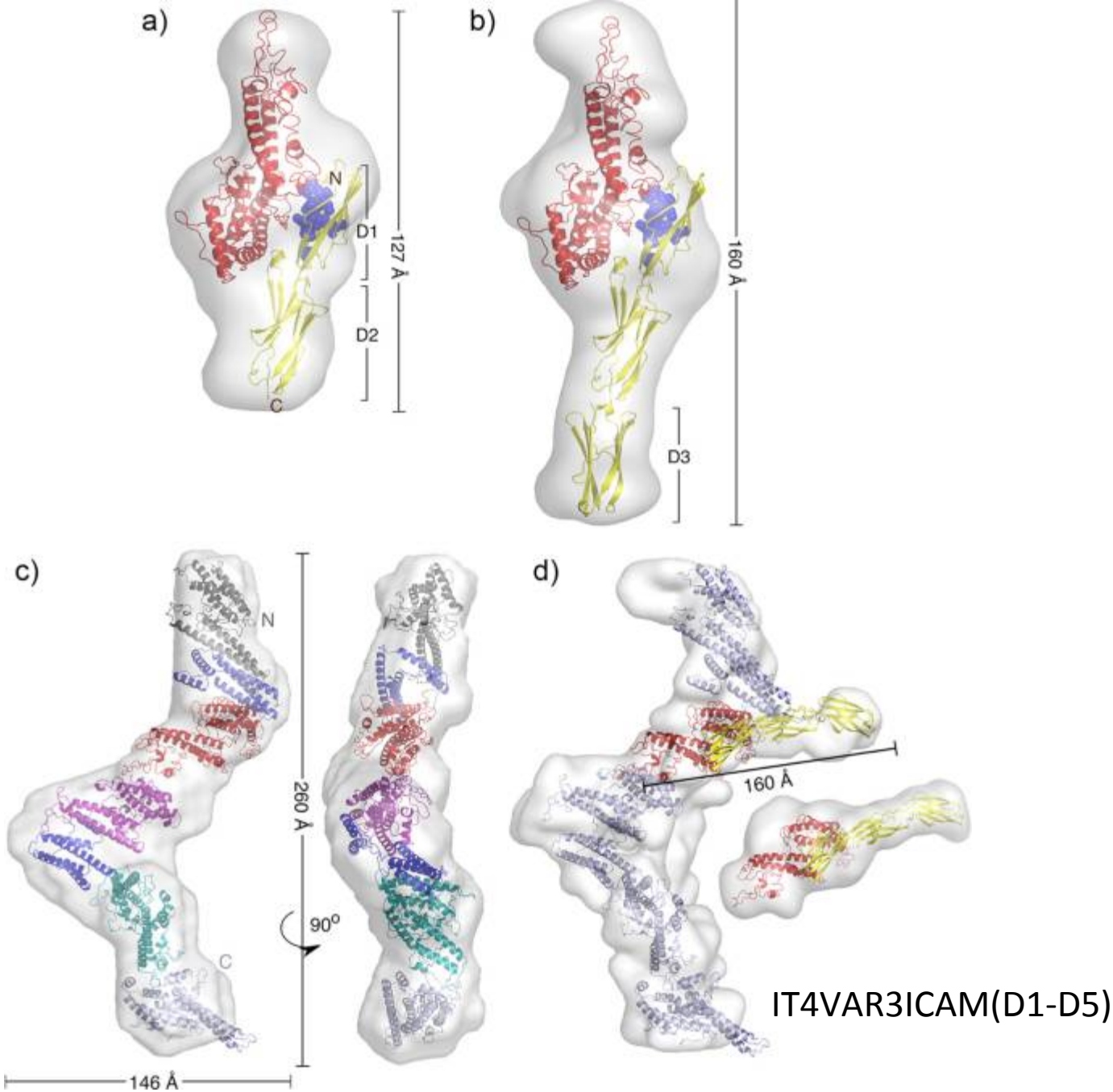
VAR13 315 kDa

Binds to the proteoglycan receptor ICAM-1



SEC and AUC shows that DBLβ interacts with ICAM-1

IT4VAR13(DBLβ)
ICAM-1 (D1-D5)



IT4VAR13 SAXS structure

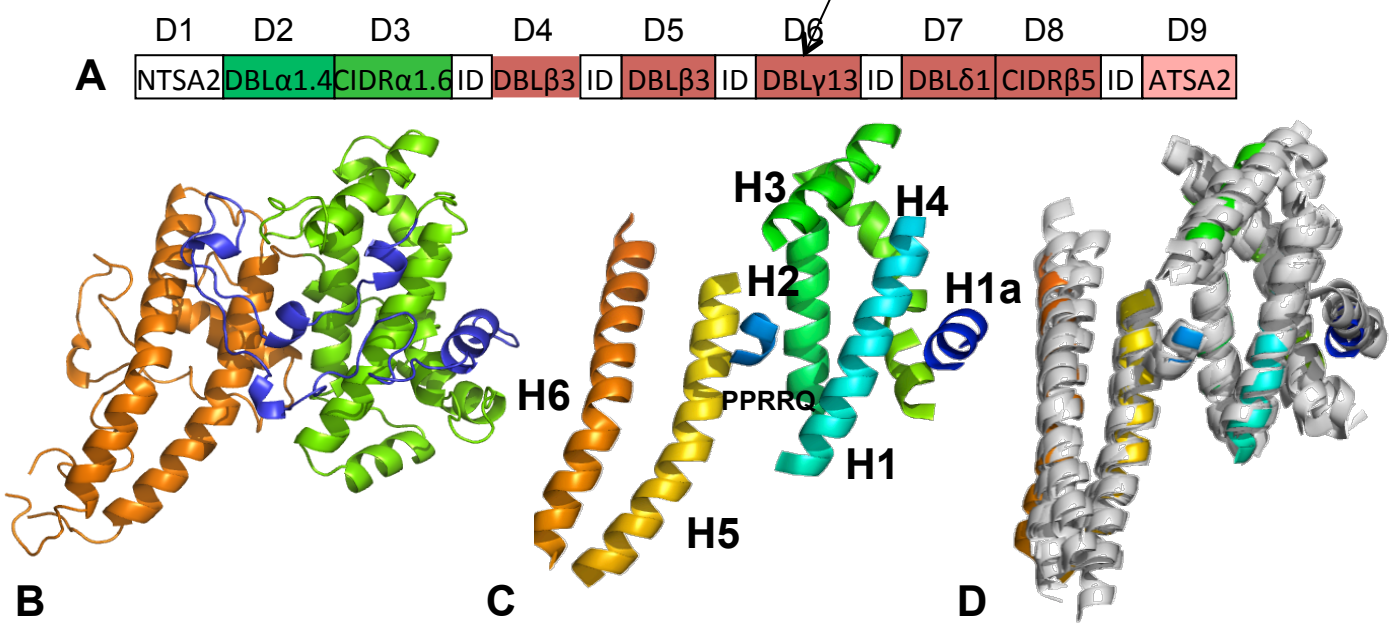
IT4VAR3ICAM(D1-D5)

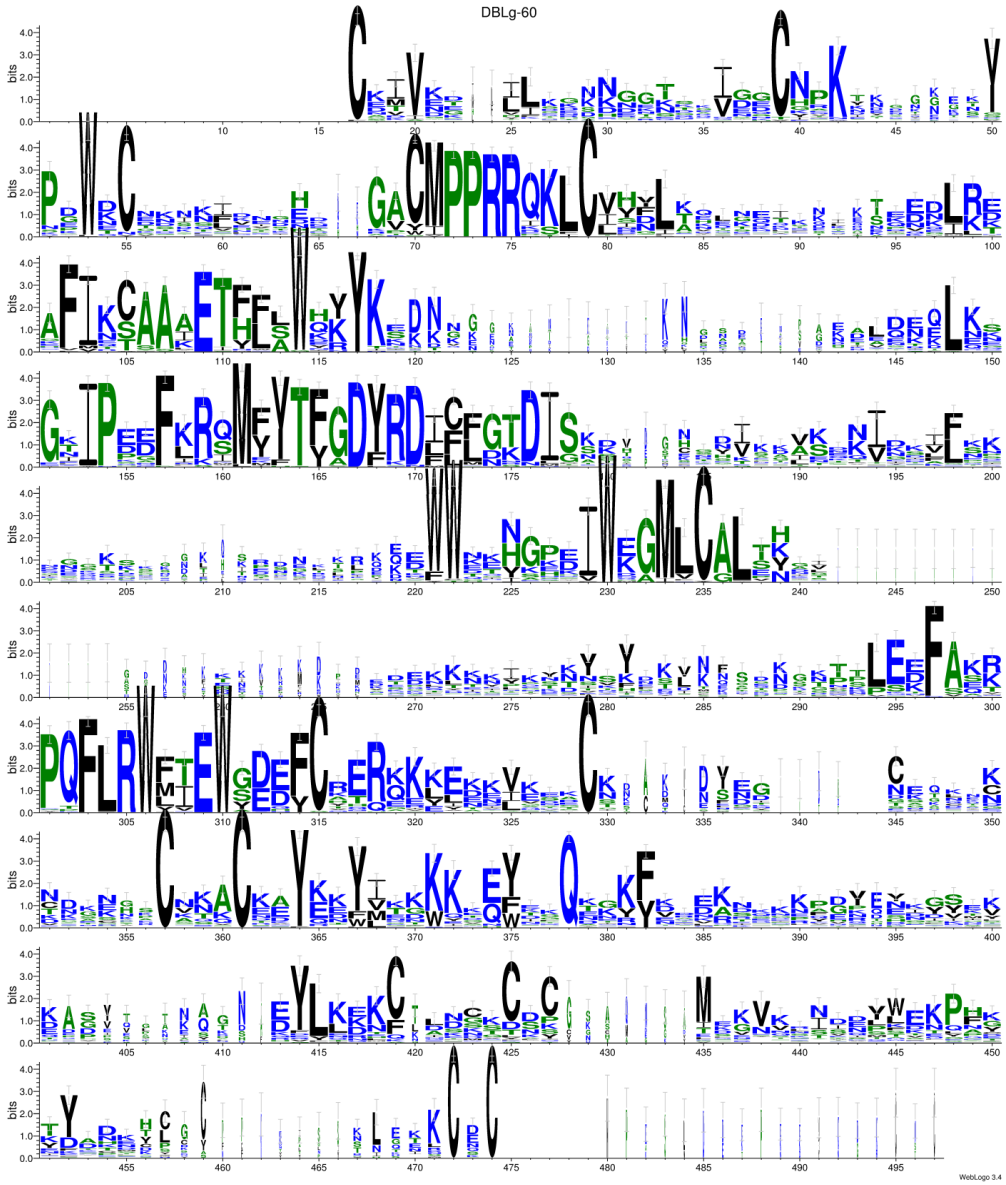
Brown, Turner, Christoffersen et al J. Biol.Chem. **288** (2013) 5992-6003

Crystallography can give a more details

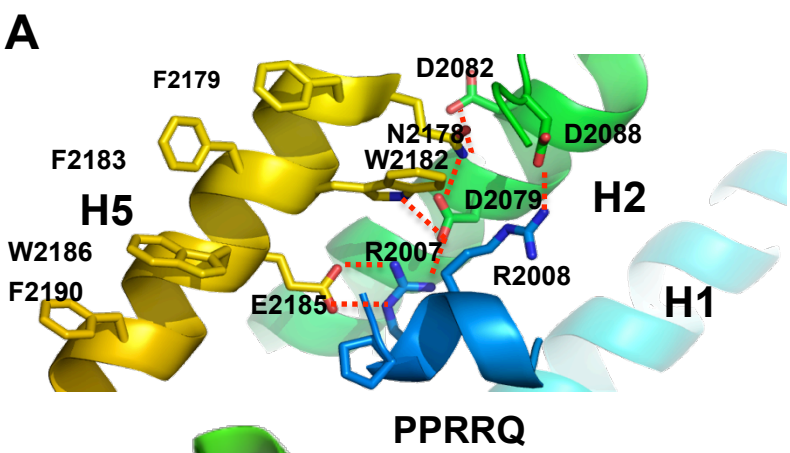
The DLBγ domain of VAR 4

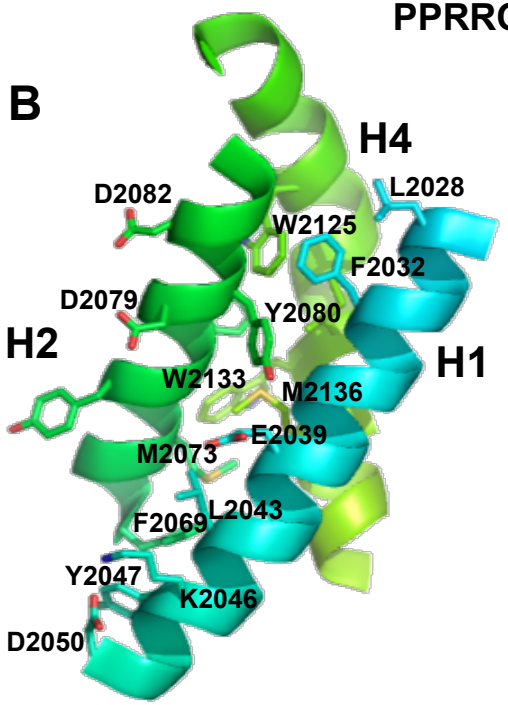
PfEMP1 protein – VAR4

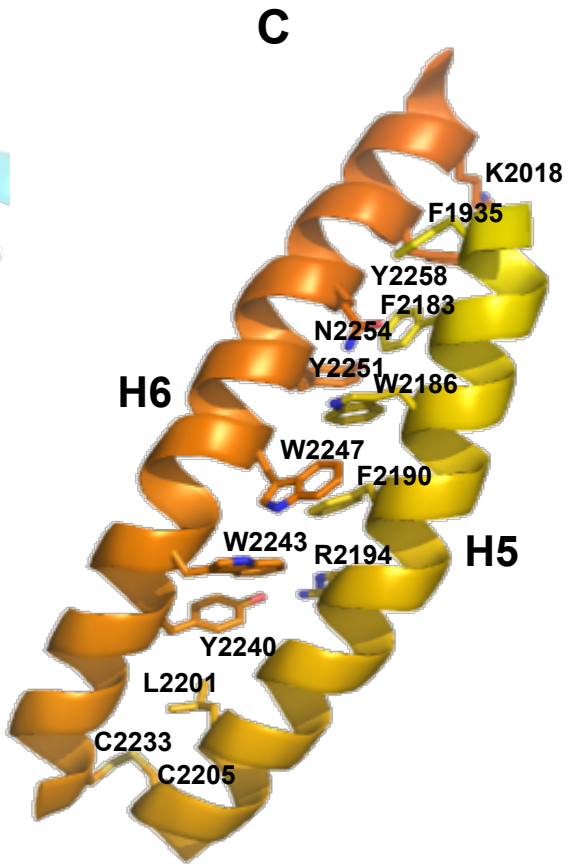




Sequence alignments of DBLγ domains



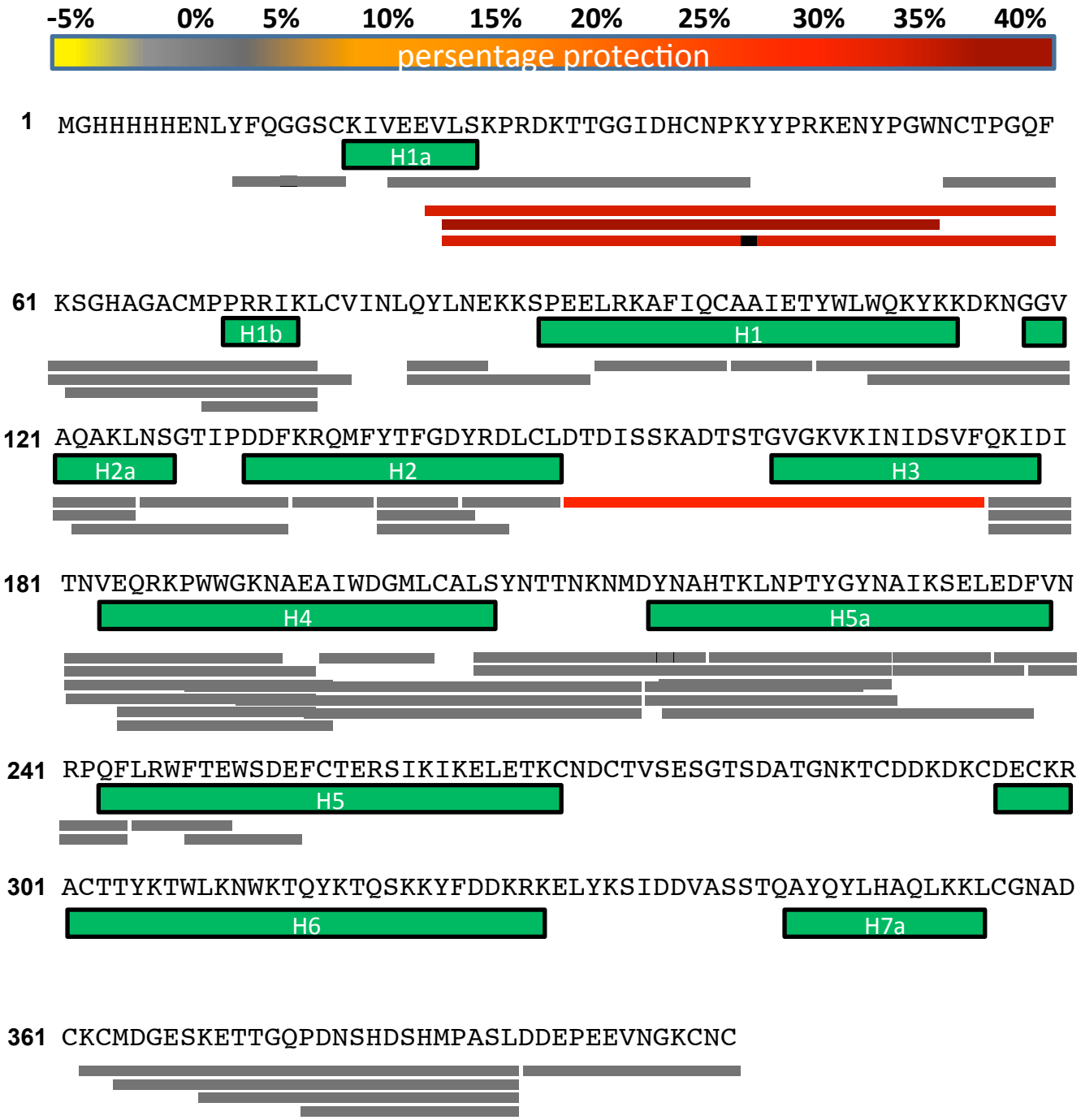


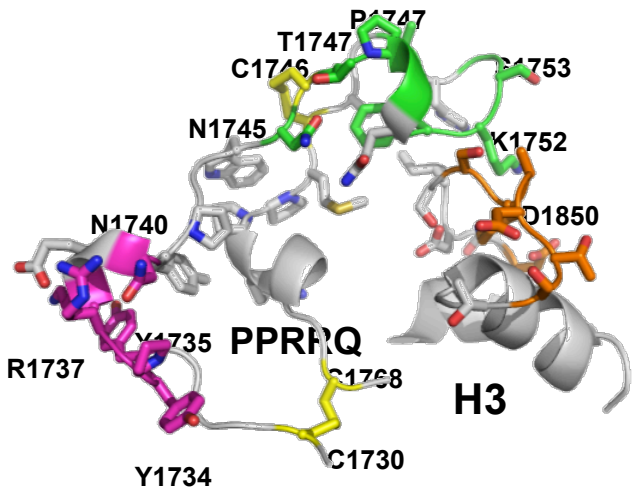


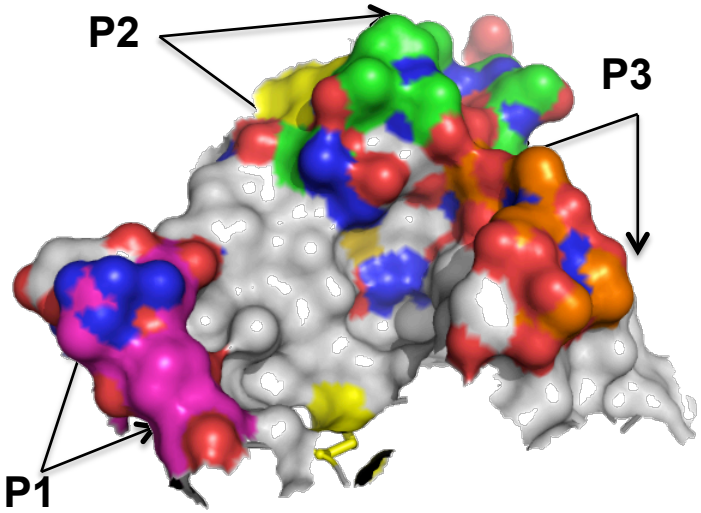
Conserved residues are found in the core domain

AB01 binds to the DBLγ domain

H/D X MS used to identify the binding sites

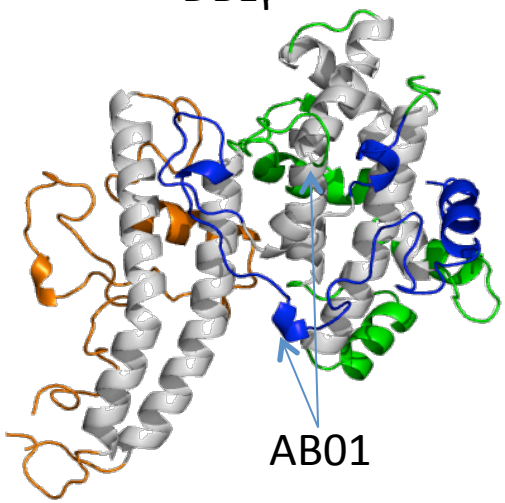

-5%
0%
5%
10%
15%
20%
25%
30%
35%
40%
persentage protection
1 MGHHHHHENLYFQGGSCKIVEEVLSKPRDKTTGGIDHCNPKYYPRKENYPGWNCTPGQF
H1a
61 KSGHAGACMPPRRIKLCVINLQYLNEKKSPEELRKAFIQCAAIETYWLWQKYKKDKNGGV
H1b
H1
121 AQAKLNSGTIPDDFKRQMFYTFGDYRDLCLDTDISSKADTSTGVGKVKINIDSVFQKIDI
H2a
H2
H3
181 TNVEQRKPWWGKNAEAIWDGMLCALSYNTTNKNMDYNAHTKLNPTYGYNAIKSELEDFVN
H4
H5a
241 RPQFLRWFTEWSDEFCTERSIKIKELETKCNDCTVSESGTSDATGNKTCDDKDKCDECKR
H5
301 ACTTYKTWLKNWKTQYKTQSKKYFDDKRKELYKSIDDVASSTQAYQYLHAQLKKLCGNAD
H6
H7a
361 CKCMDGESKETTGQPDNSHDSHMPASLDDEPEEVNGKCNC

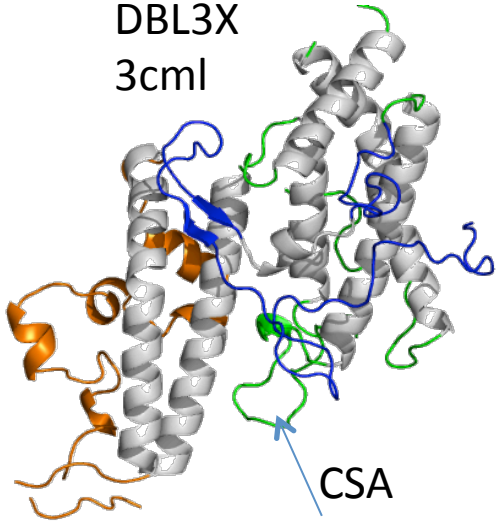


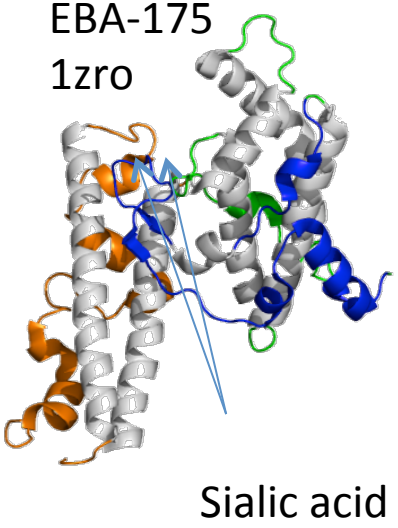


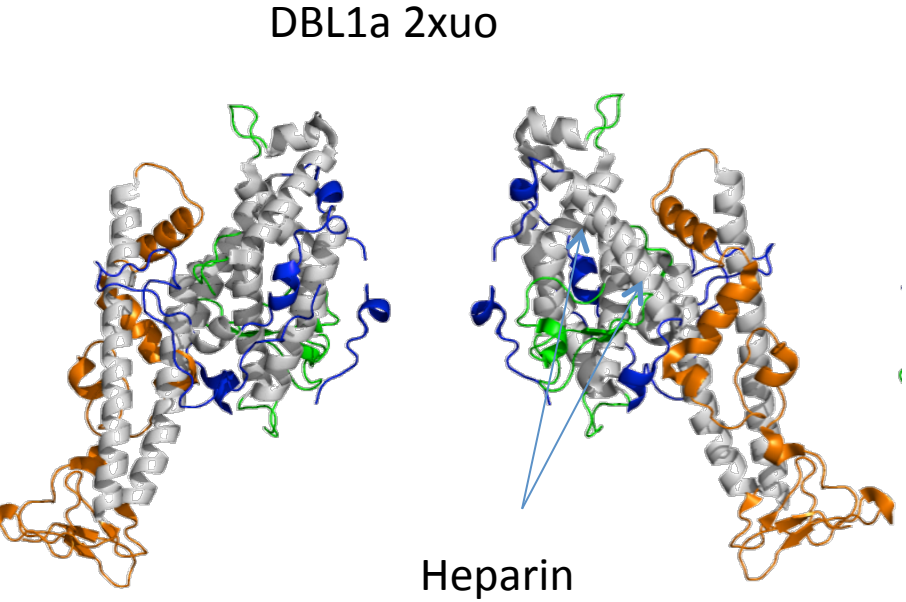
P1 and P3 from H/DX MS P2 from mimitope

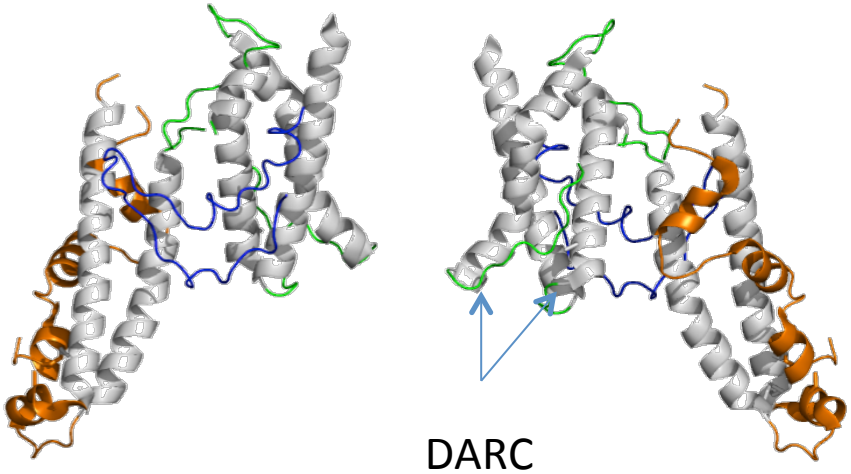
DBL domain	Ligand	Location respectively to the DBL core
DBL4g-Var4	Mab AB01	1734-YYPRKENYPGW-1745 H1a – PPRRQ loop in S1 subdomain. 1842-DTDISSKADTST-1853 H2-H3 loop in the S2.
DBL1a-Var0 (2xuo)	Heparin	K20, K32, K40 H1a helix; K226, K227, K230 H3 helix; K423, K424 H6-H7a loop; K451, K456 (H7a-H8a loop).
DBL3x-Var2-CSA (3cml)	Sulphate/ CSA	K1324, G1329, and R1467 from H1-H2 loop in S2 subdomain
EBA-175 (1zro)	Sialic acid	27-KKNDRSN-33 H1a – PPRRQ loop (S1)
PvDBP (2C6J)	DARC	91-NYQYNKEFC-98 from H1-H2 loop (S2) and 169-KEKFVWIC-176 from H4-H5 loop (S2)


DBLγ
AB01


DBL3X
3cml
CSA


EBA-175
1zro
Sialic acid

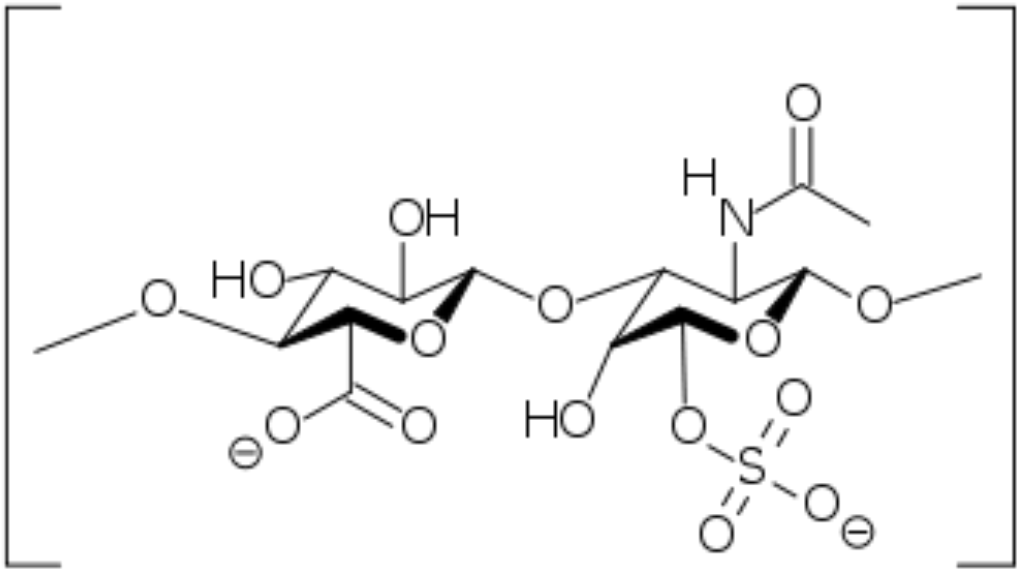

DBL1a 2xuo
Heparin


DBP P. vivax 2cbj
DARC

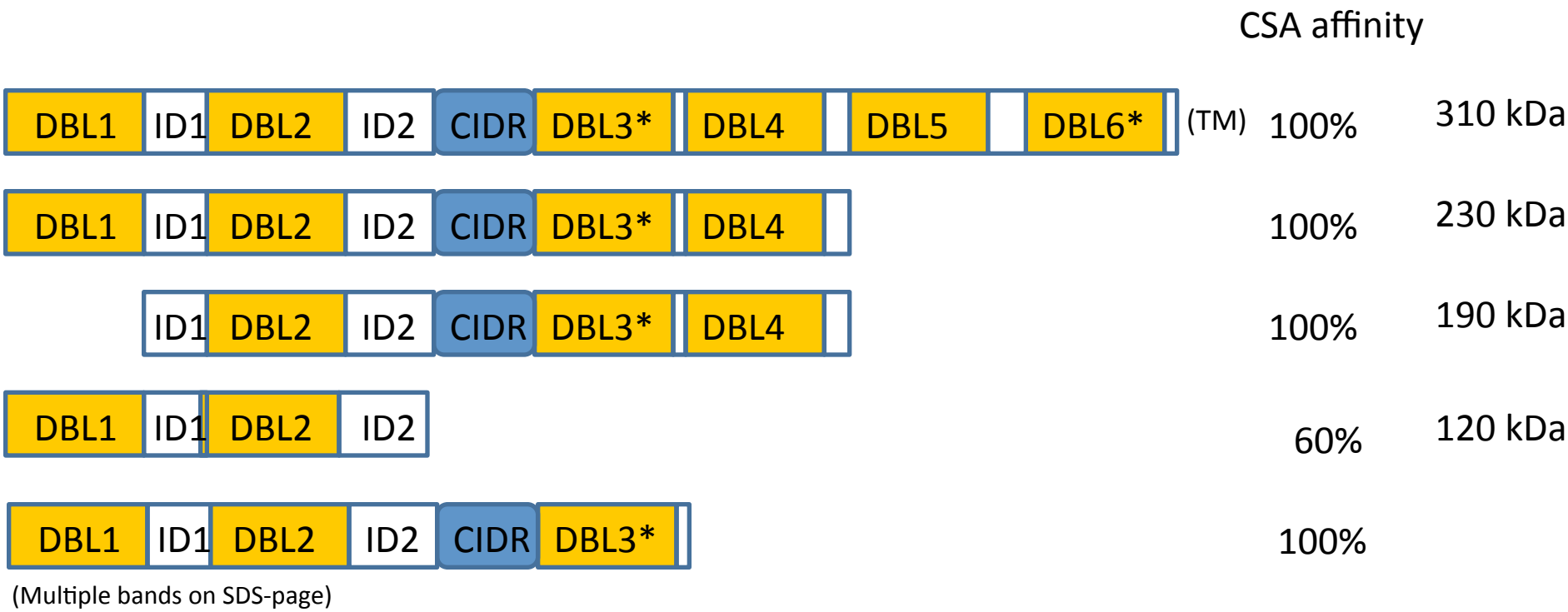
SAXS studies of VARCSA a PfEMP1 protein involved in placenta malaria

VAR2CSA- placenta malaria

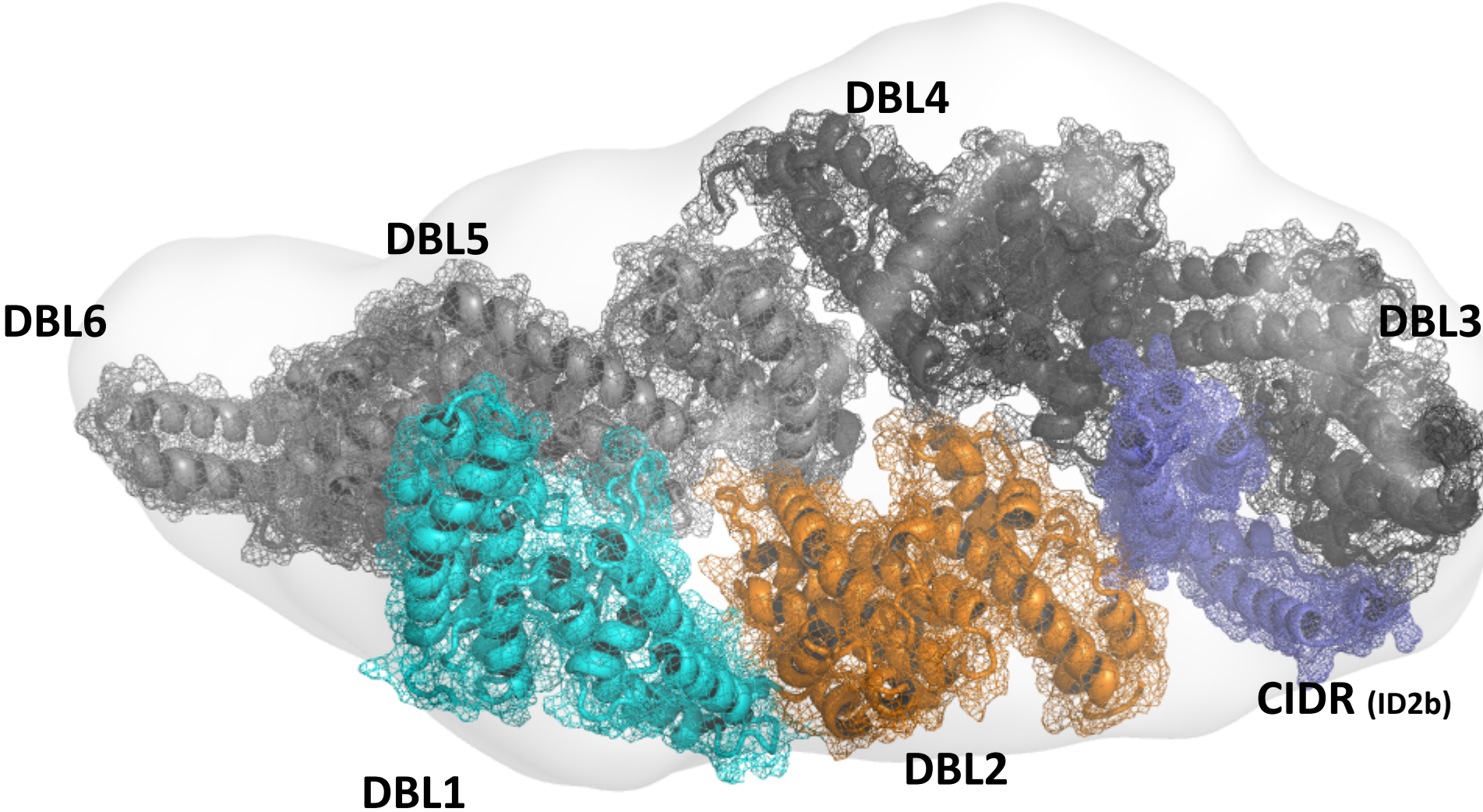
Identified as ”the most promising” vaccine candidate against **pregnancy associated malaria.** Binds specifically to Chondroitin Sulfate A (**CSA**).



Truncated Protein Constructs of VAR2CSA



- reveal CSA binding domains, and propose a model for the overall structure of VAR2CSA using SAXS

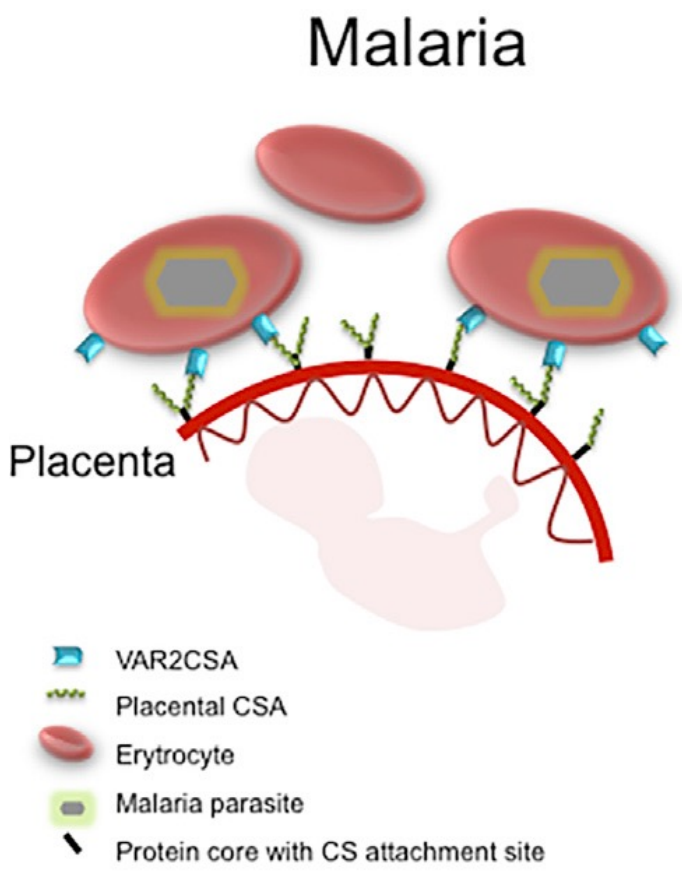


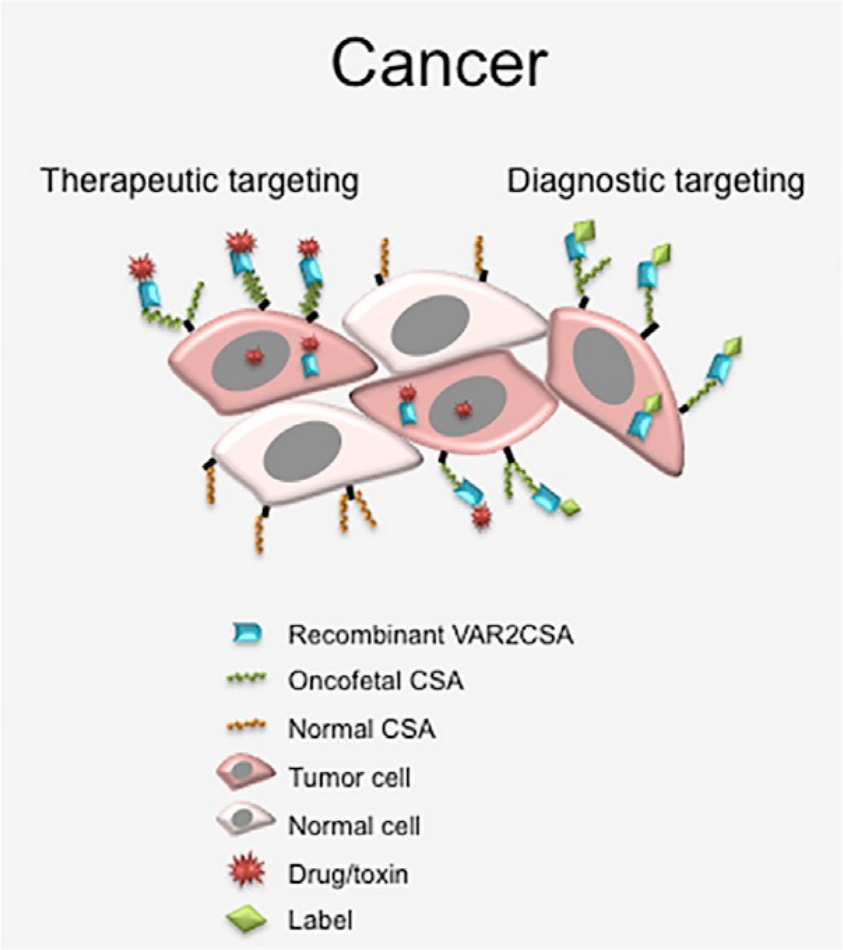
CSA-blocking antibody epitopes have been found on DBL3, DBL4 and DBL5 single do

The CSA binding was mapped to the DBL2 domain.

Clausen, Christophersen et al J.Biol. Chem. **287**, 23332 (2012)

VAR2CSA interacts with cancer cells





Salanti, Clausen et al Cancer Cell **28** (2015) 500-514

PfEMP1 proteins

- The modular PfEMP1 proteins vary in shape from compact to elongated
- The DBL domains are comprised of a helical core structure with additional loops and helices
- The interactions in the core structure involve residues that are conserved among rhe different types of DBL domains
- The interaction partners interact primarily with the additional helices and loops
- Combining results from SAXS and diffraction is important

The future for structural biology

is bright!!!

Better automation and better detectors

Impact of free electron lasers

Serial Synchrotron Crystallography

Room temperature studies

Oxidation states of metal ions in proteins

Ultimate storage rings EBS

Cryo EM

Brunnshög North of Lund, Sweden 2020

Home of the European Spallation Source (ESS) and
MAX IV the first ultimate storage ring

Interreg Öresund-Kattegat-Skagerrak

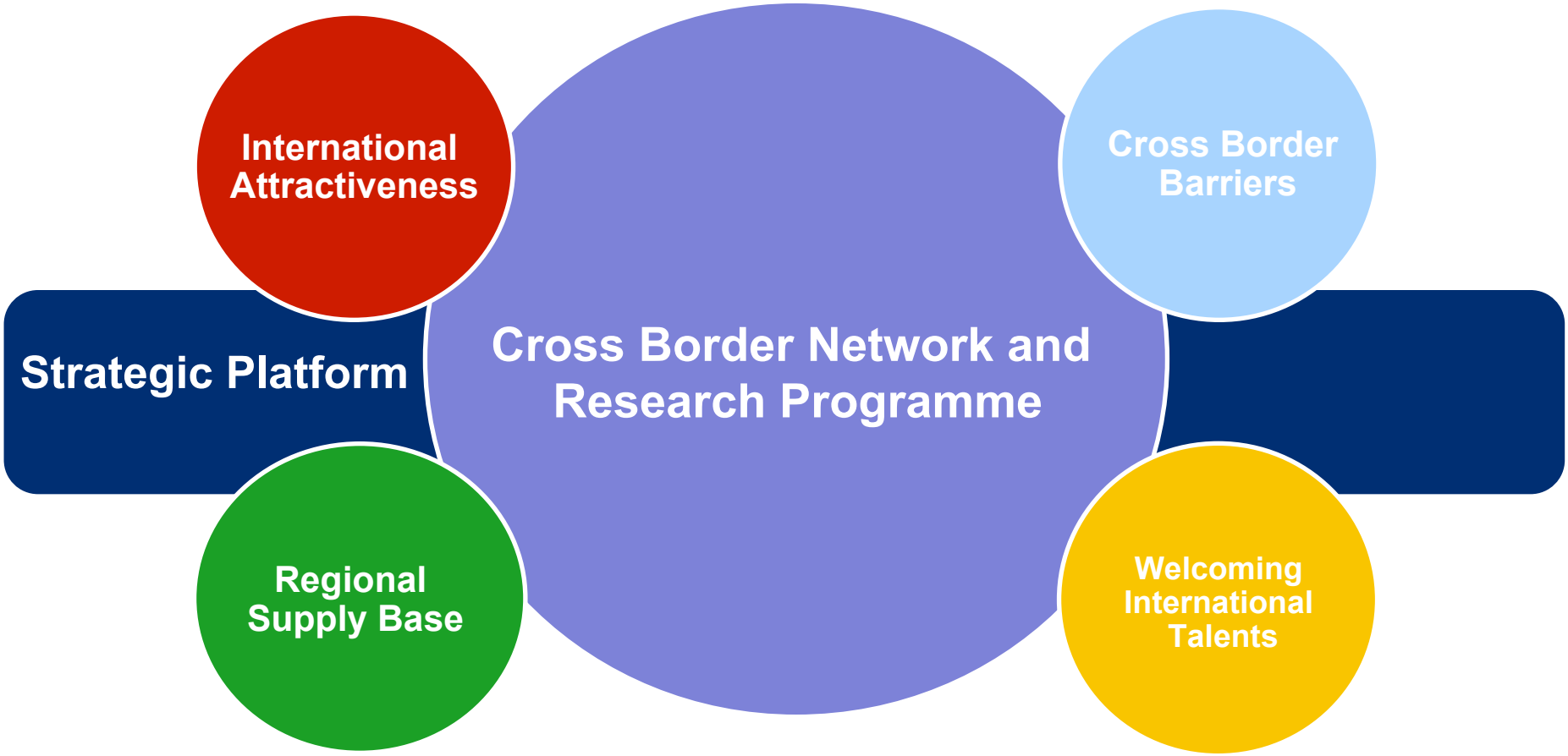
- 15 regions SE, DK, NO
- 136 MEUR for cross border cooperation
- Co-financing 50%

REGION SKANE

Interreg
EUROPEAN UNION
Öresund-Kattegat-Skagerrak
European Regional Development Fund

REGION
Region Hovedstaden

ESS & MAX IV: Cross Border Science and Society



REGION SKANE

Interreg
EUROPEAN UNION
Öresund-Kattegat-Skagerrak
European Regional Development Fund

REGION
Region Hovedstaden

LUND UNIVERSITY

CHALMERS
UNIVERSITY OF TECHNOLOGY

UNIVERSITY OF GOTHENBURG

ESS

MAX IV

DTU

Technical University of Denmark

UNIVERSITY OF OSLO

MALMÖ HÖGSKOLA

Interreg
Öresund-Kattegat-Skagerrak
European Regional Development Fund

World of synchrotrons

Thank you for your attention

There is room for one in Africa