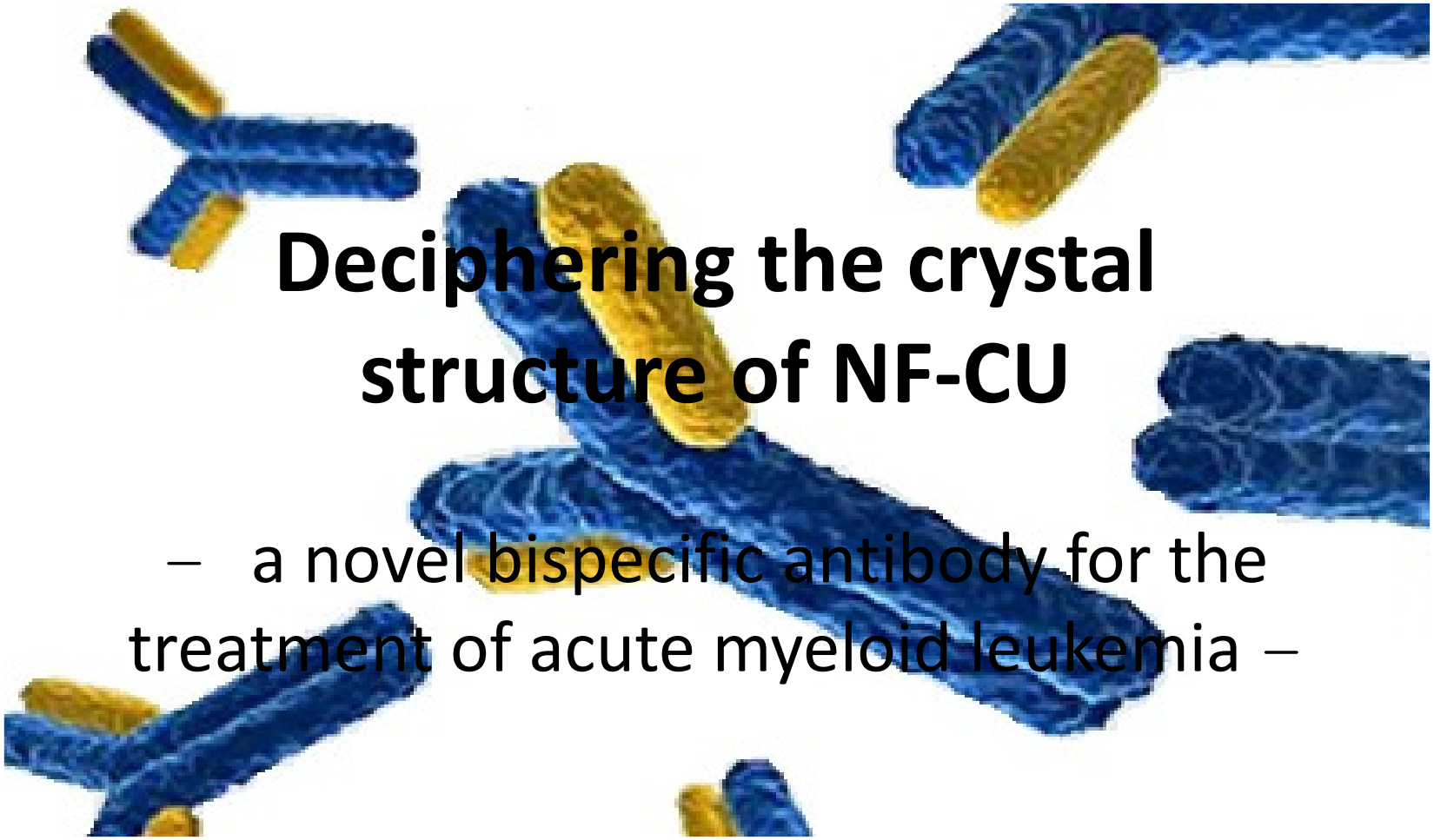


Deciphering the crystal structure of NF-CU

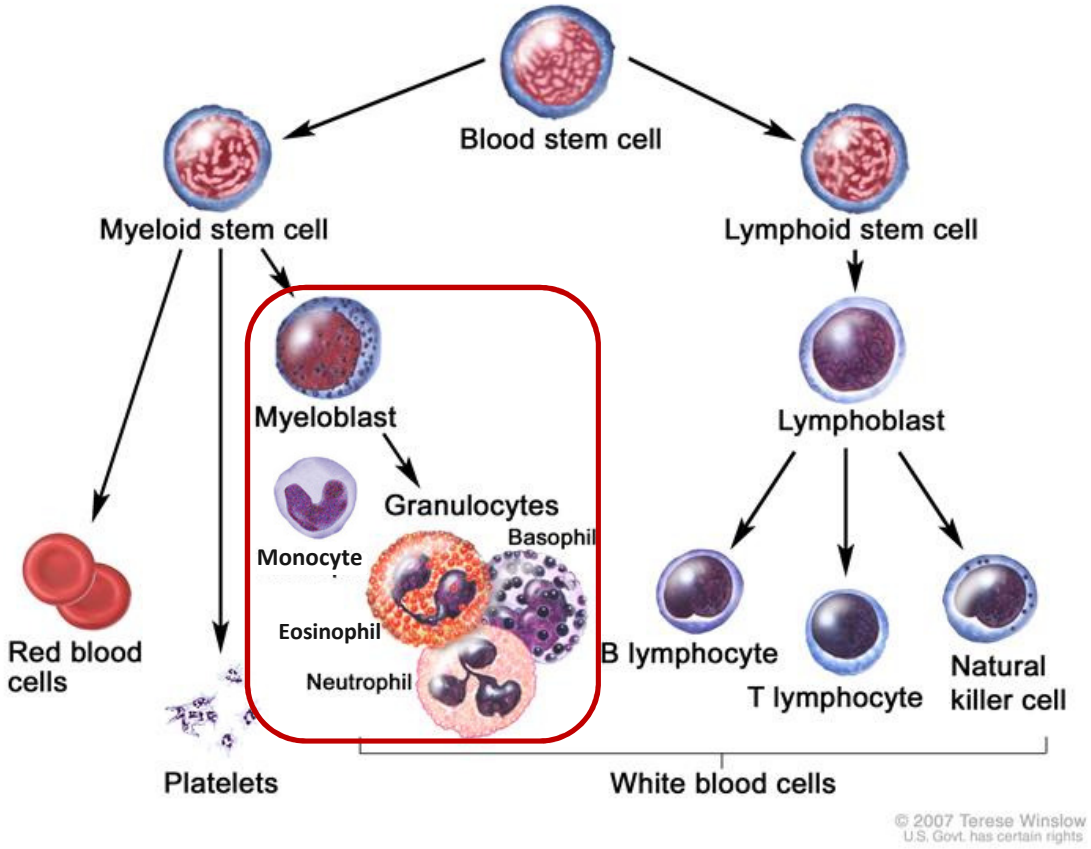
– a novel bispecific antibody for the treatment of acute myeloid leukemia –

Anne Stinn

5th European Immunology & Innate Immunity Conference
July 22, 2016

Acute myeloid leukemia (AML)

- cancer of the myeloid blood cells
  → rapid growth of abnormal white blood cells: accumulate in the bone marrow and interfere with the production of normal blood cells

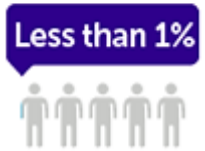


modified by: Winslow (2007)

Acute myeloid leukemia (AML)

- cancer of the myeloid blood cells
  - → rapid growth of abnormal white blood cells: accumulate in the bone marrow and interfere with the production of normal blood cells

Propotion of all cases



Percentage acute myeloid leukaemia is of total cancer cases

Age



Age that almost 6 in 10 of AML cases are diagnosed

5-year survival rate



25% of AML patients live > 5 years after diagnosis

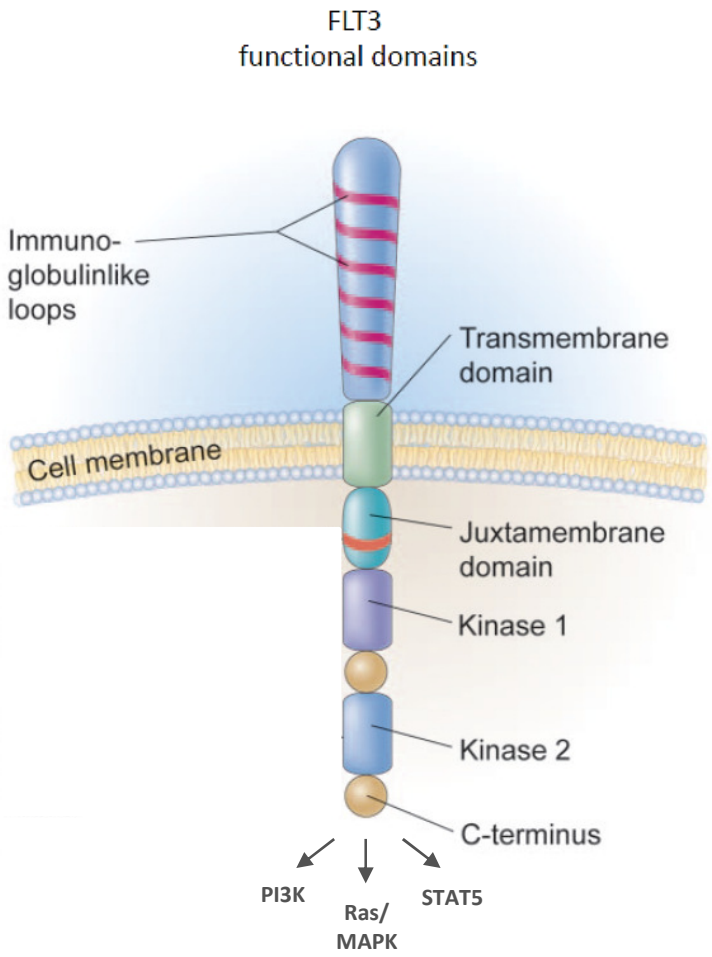
- majority of all cases mutations in the genes *NPMI1*, *CEBPA* and ***FLT3*** are reported

MAX-PLANCK-GESELLSCHAFT

FLT3 (CD135) *fms*-like tyrosine kinase 3

- primarily expressed on early hematopoietic progenitors and DCs
  → proliferation and differentiation
- type III receptor tyrosine kinase family
- five extracellular Ig-like domains **(D1-5)**

- expressed on 70 - 100% of AML and >90% ALL blasts
- Internal Tandem Duplications (ITD) or point mutations in the kinase domain are commonly occurring with AML
  → poor disease prognosis



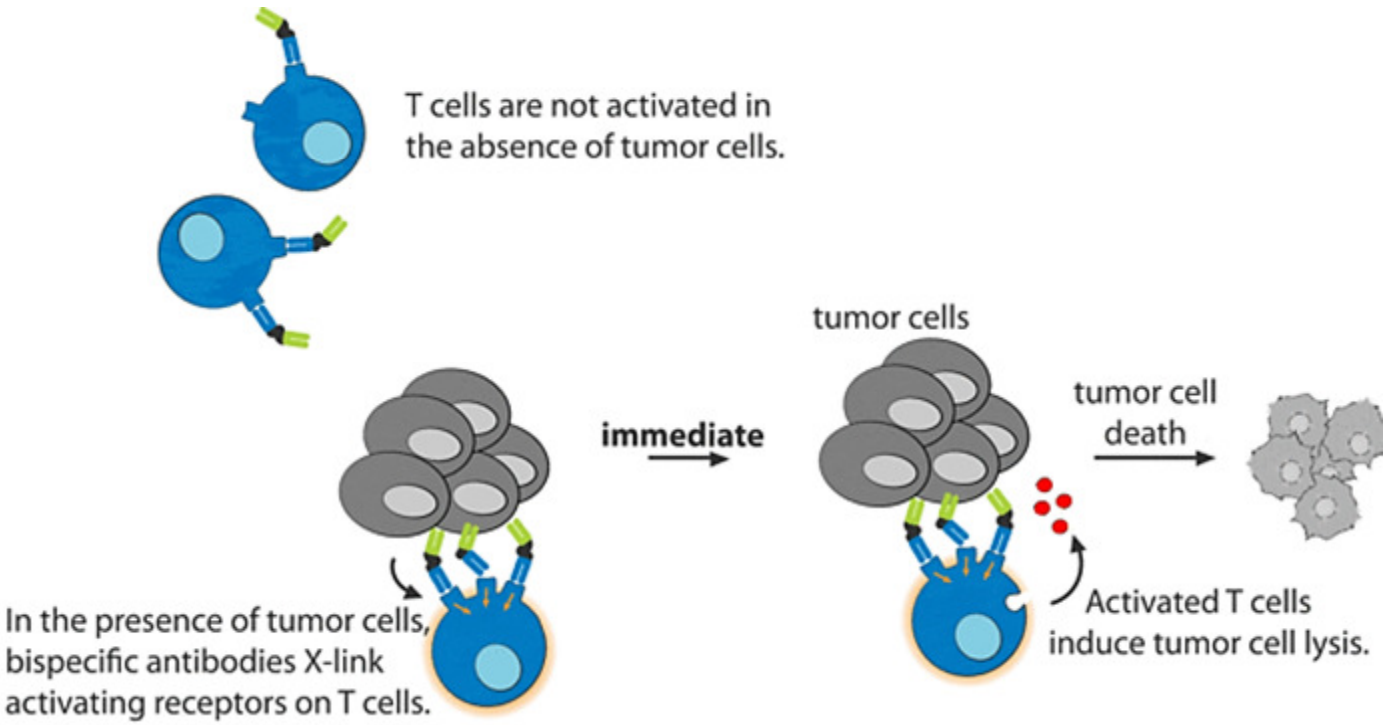
→ target molecule for treatment of AML

modified by: Litzow (2005), Blood

Immunotherapy with **bispecific** antibodies

- artificially designed antibodies that are able to bind two different antigens
  1. tumor-associated antigen (TAAs)
  2. agonistic T-cell receptor

→ **selective activation of immune cells**



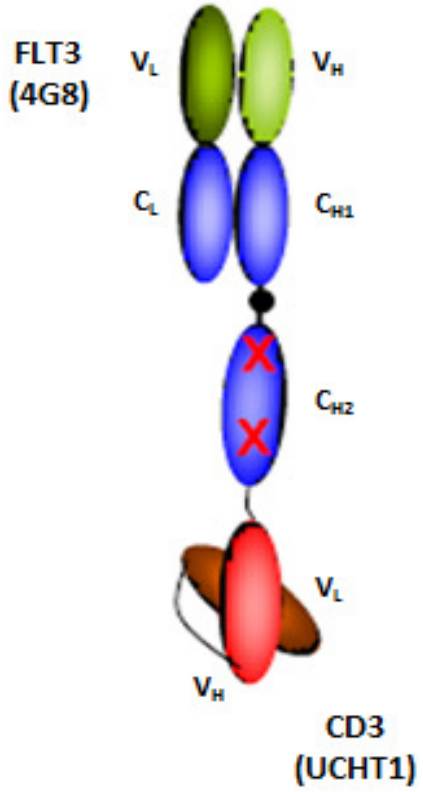
Source: SYNIMMUNE GmbH

Immunotherapy with **bispecific** antibodies

- artificially designed antibodies that are able to bind two different antigens
  1. tumor-associated antigen (TAAs)
  2. agonistic T-cell receptor

→ **selective activation of immune cells**

NF-CU
(Fabsc-format)



Source: SYNIMMUNE GmbH

MAX-PLANCK-GESELLSCHAFT

Aims

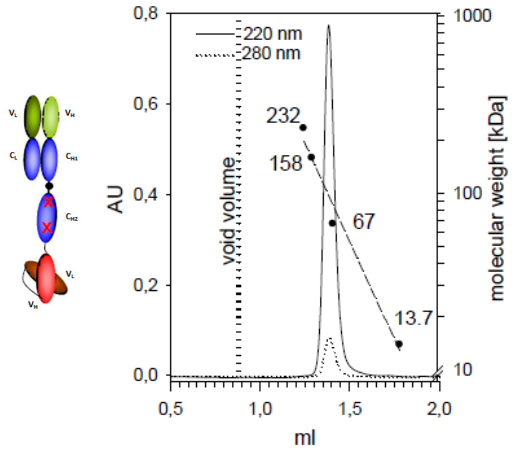
→ **insights into epitope recognition as well as molecular mechanism of T-cell activation and tumor cell death**

- solving the crystal structure of the bispecific antibody NF-CU
- co-crystallization of NF-CU with its antigens FLT3 and CD3 δ/ε

MAX-PLANCK-GESELLSCHAFT

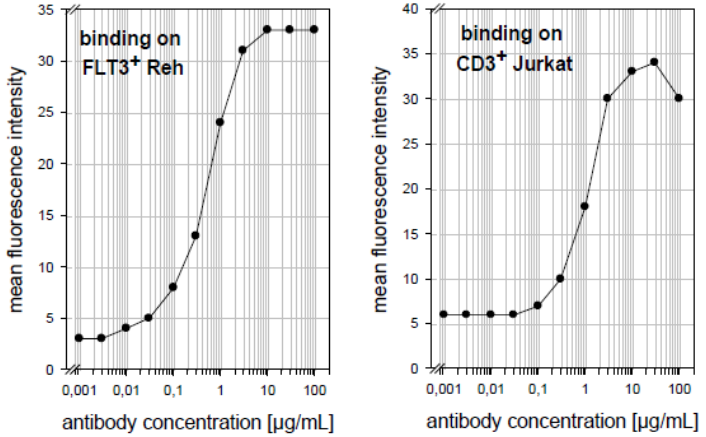
Functional characterization of the bispecific antibody **NF-CU**

Production in eukaryotic cells

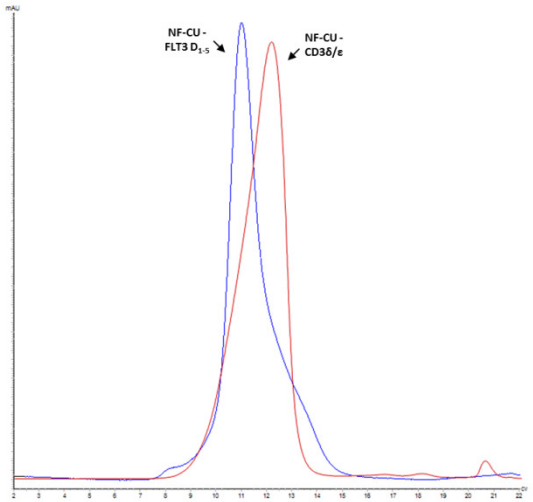


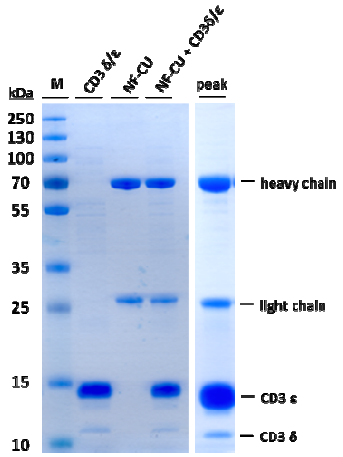
Preserved binding to both antigens, FLT3 and CD3

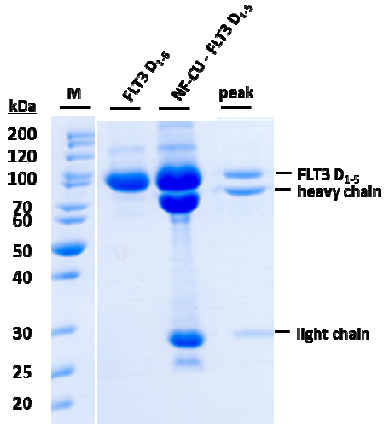
- Flow cytometry-



- Analytic gel filtration with recombinant expressed antigens-





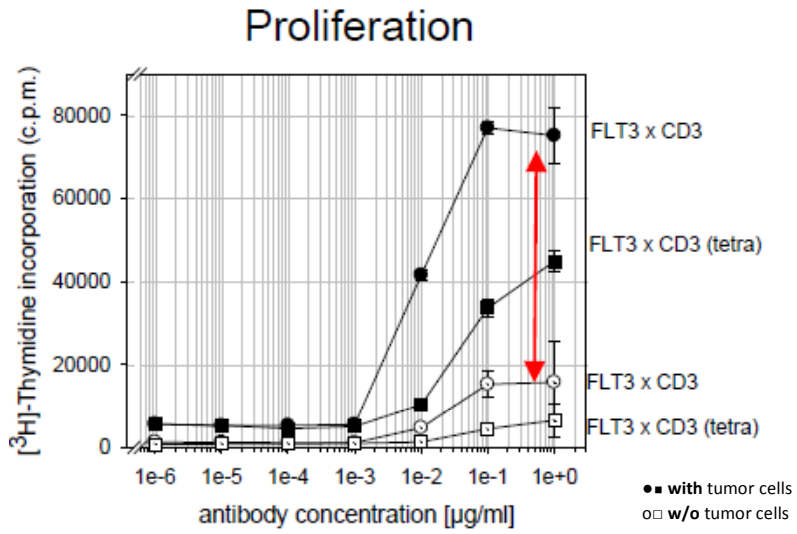


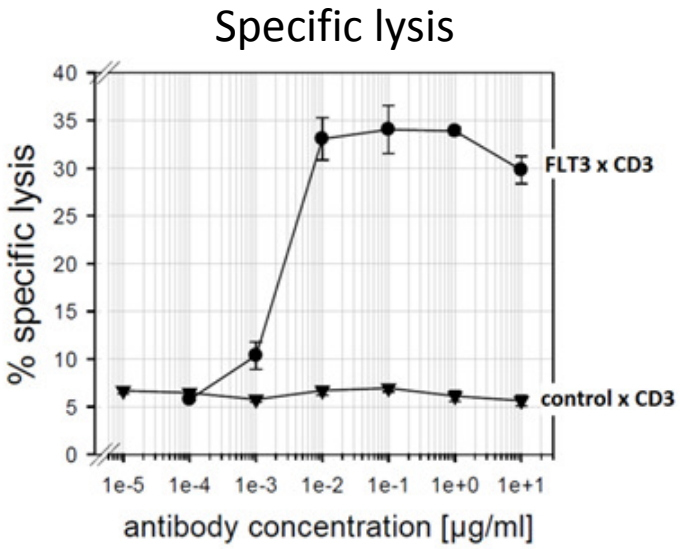
Source: SYNIMMUNE GmbH

MAX-PLANCK-GESELLSCHAFT

Target-cell restricted T-cell activation with **NF-CU**

PBMC : Reh cells





- activation of PBMCs (proliferation and cytokine release)
  → high toxicity and reduction of leukemic blasts
- no cross reactivity towards FLT3 molecules from other species

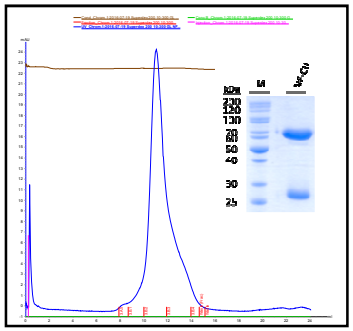
→ promising results in *in vitro* assays using laboratory cell lines as well as **material from AML patients**

Source: SYNIMMUNE GmbH

Structural investigation of **NF-CU**

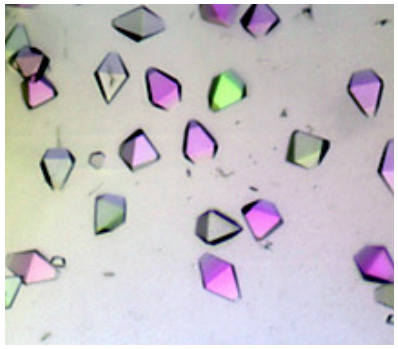
- workflow -

(recombinant) protein production and purification



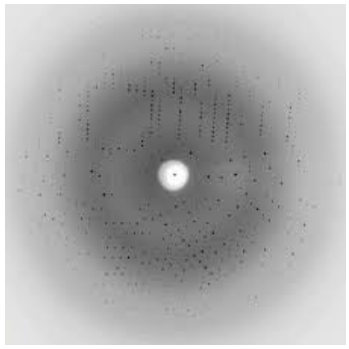
crystallization

crystals



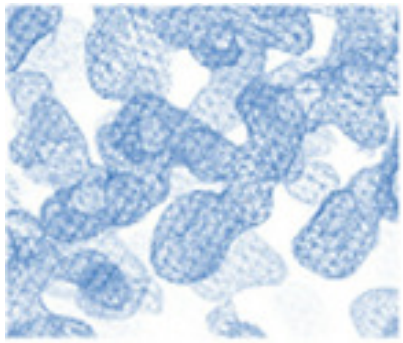
X-ray diffraction at synchrotron

diffraction pattern



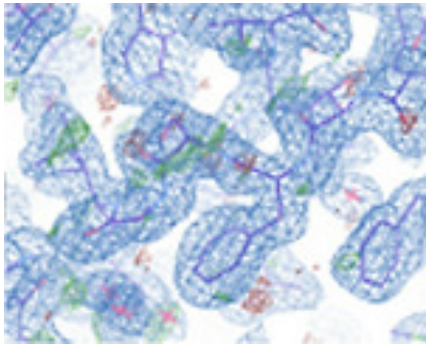
phases

refinement

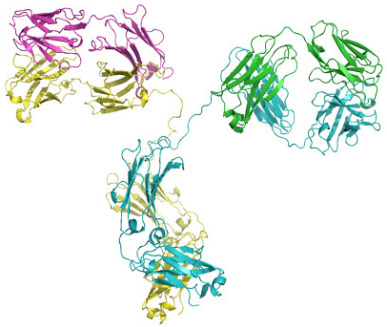


electron density map

molecular modelling/ fitting



evaluation and PDB submission



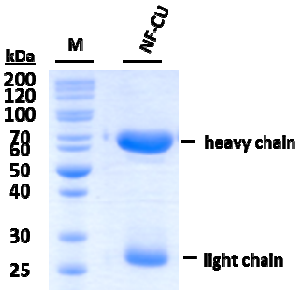
atomic model

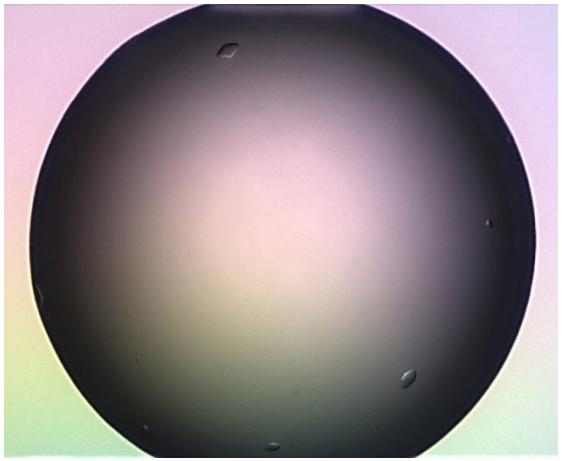
MAX-PLANCK-GESELLSCHAFT

Crystallization of the bispecific antibody **NF-CU**

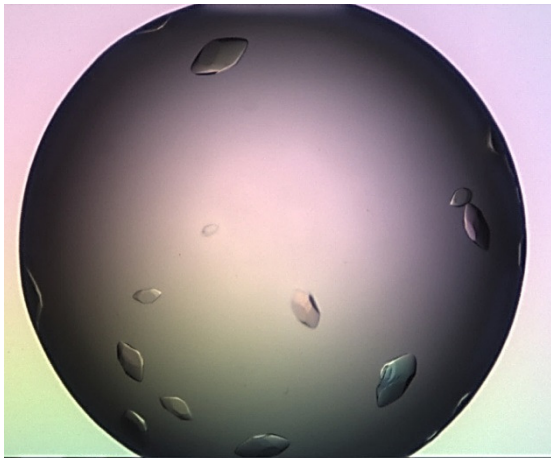
- highly pure NF-CU antibody expressed in CHO cells
- initial crystal screens (sitting drop at 20°C)
  - screened ~600 conditions

  → **1.6 M ammonium sulfate, 0.1 M MES pH 6.0** (pH Clear Screen)





1st observation (day 0)



3rd observation (day 6)

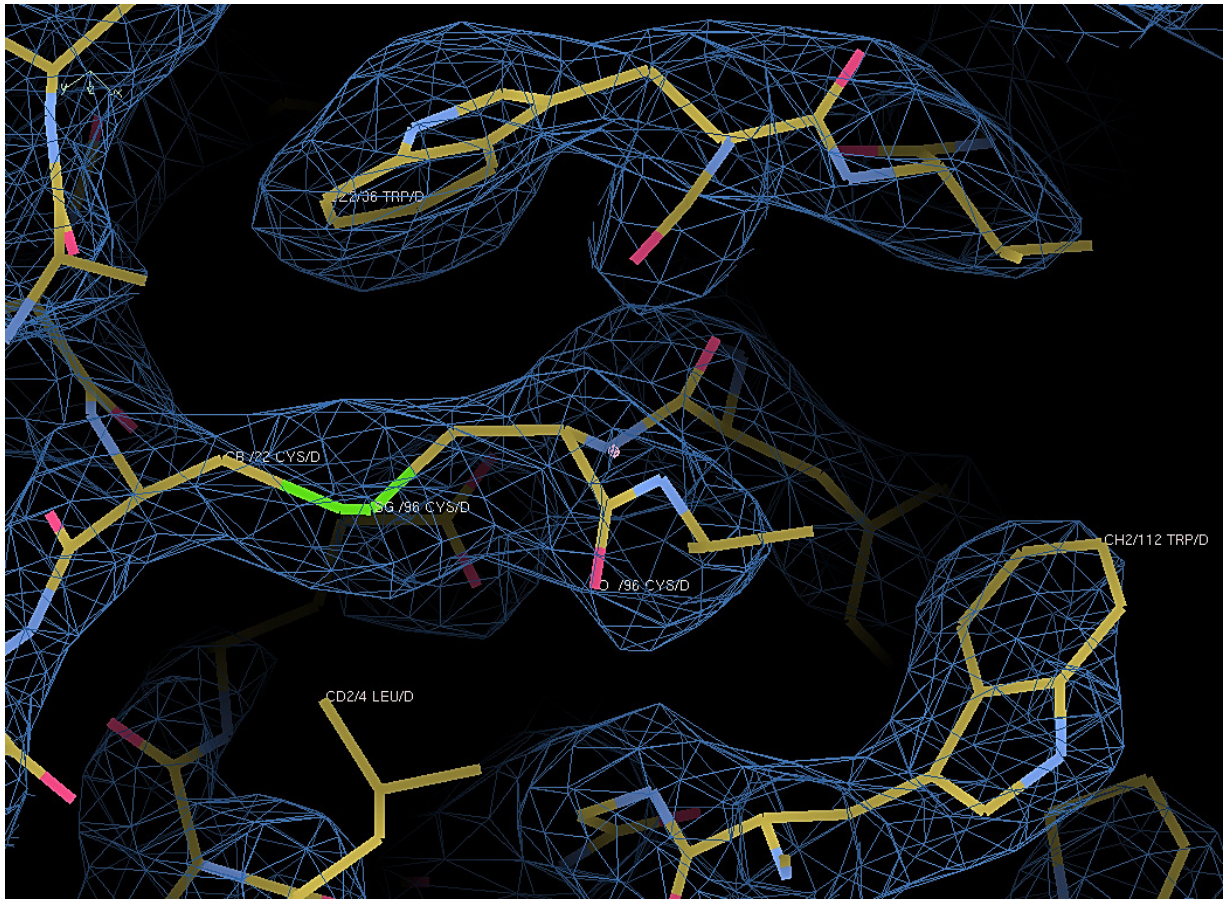
- dataset collection at the synchrotrons in Berlin (BESSY) and Hamburg (DESY)

  → **diffraction with a resolution of ~ 2.8 Å**

MAX-PLANCK-GESELLSCHAFT

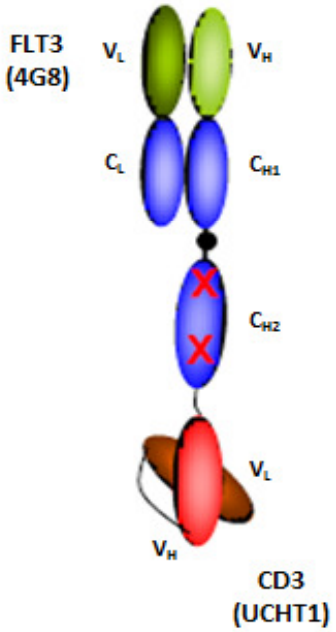
electron density map of **NF-CU**

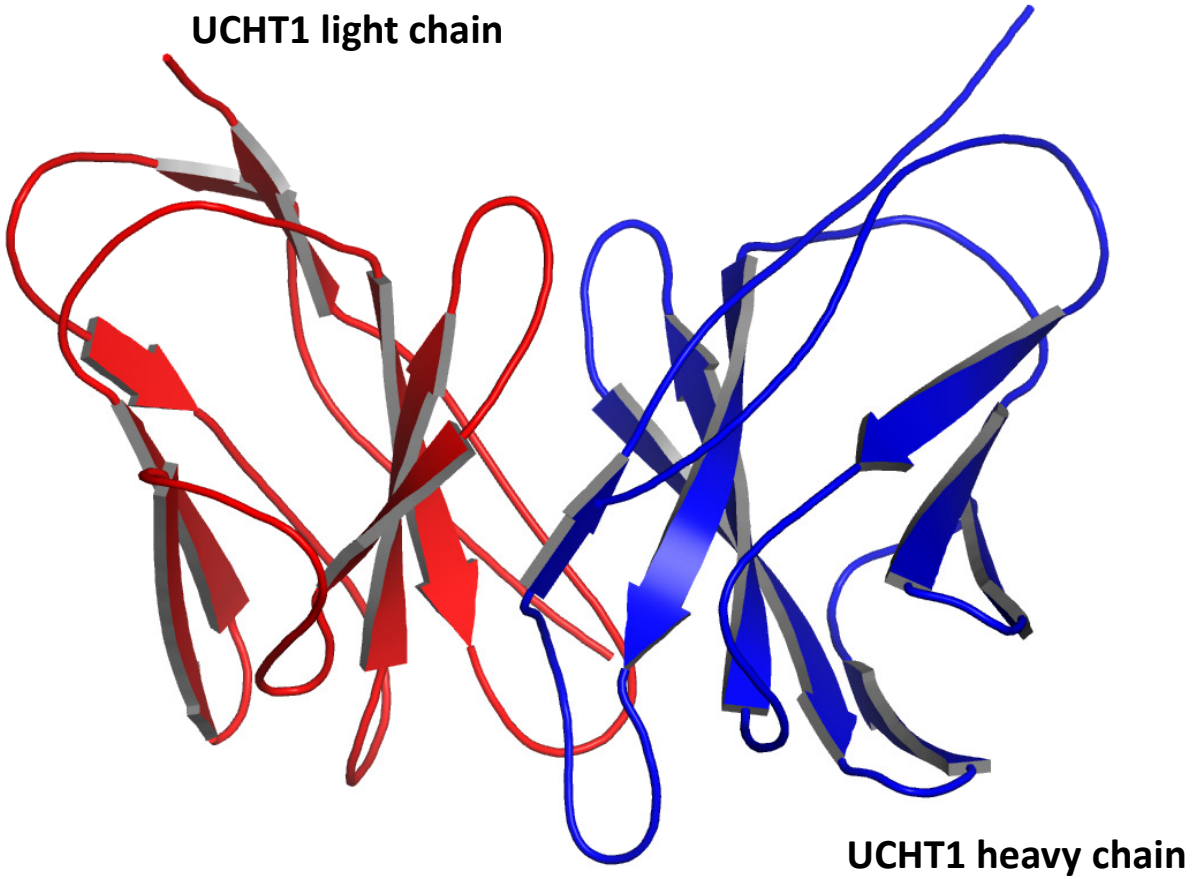
- data processing
  - space group **H32**
- molecular replacement using the crystal structure of UCHT1 (1XIW) as a template



MAX-PLANCK-GESELLSCHAFT

Preliminary model of **NF-CU** crystal structure





MAX-PLANCK-GESELLSCHAFT

Summary

NF-CU is a novel bispecific antibody for the treatment of AML.

- first structural analysis of a bispecific antibody
  - obtained nice diffracting NF-CU protein crystals (2.8 Å)
  - preliminary model of crystal structure
- co-crystallization of NF-CU with its antigens CD3 δ/ε and FLT3
  - NF-CU binds recombinantly expressed CD3 δ/ε as well as FLT3 $D_{1\text{-}5}$
    → co-crystallization plates under observation

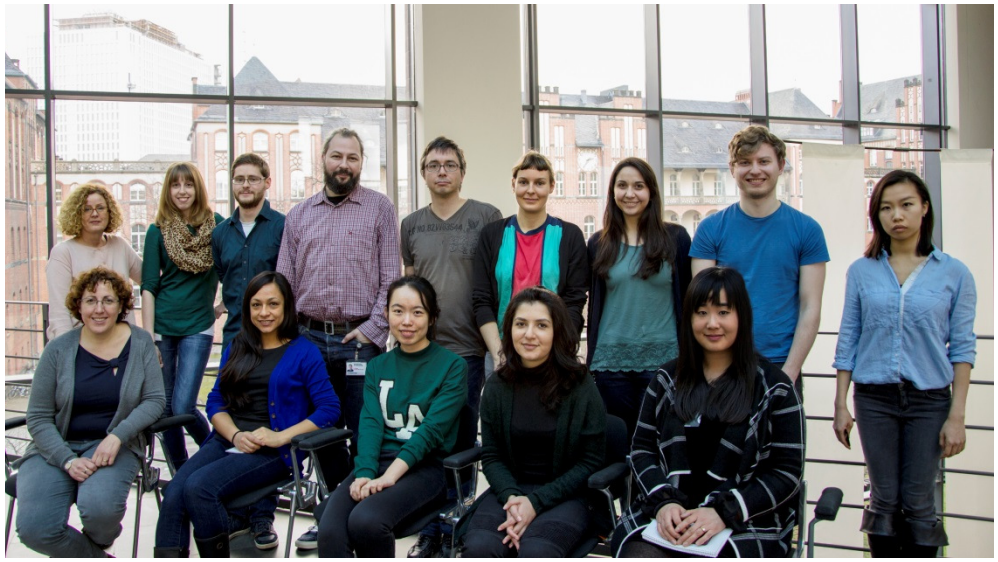
→ refinement and model evaluation of NF-CU structure model

→ determination of NF-CU binding affinities to its antigens as well as epitope mapping

MAX-PLANCK-GESELLSCHAFT

Acknowledgements

Structural Systems Biology



SYNIMMUNE Tübingen

Dr. Ludger Grosse-Hovest

Dr. Gregor Neumann

SYNIMMUNE®
ANTIBODIES FOR LIFE

MAX-PLANCK-GESELLSCHAFT

IMPRS-IDI
Infectious Diseases and Immunology

… and for your attention!