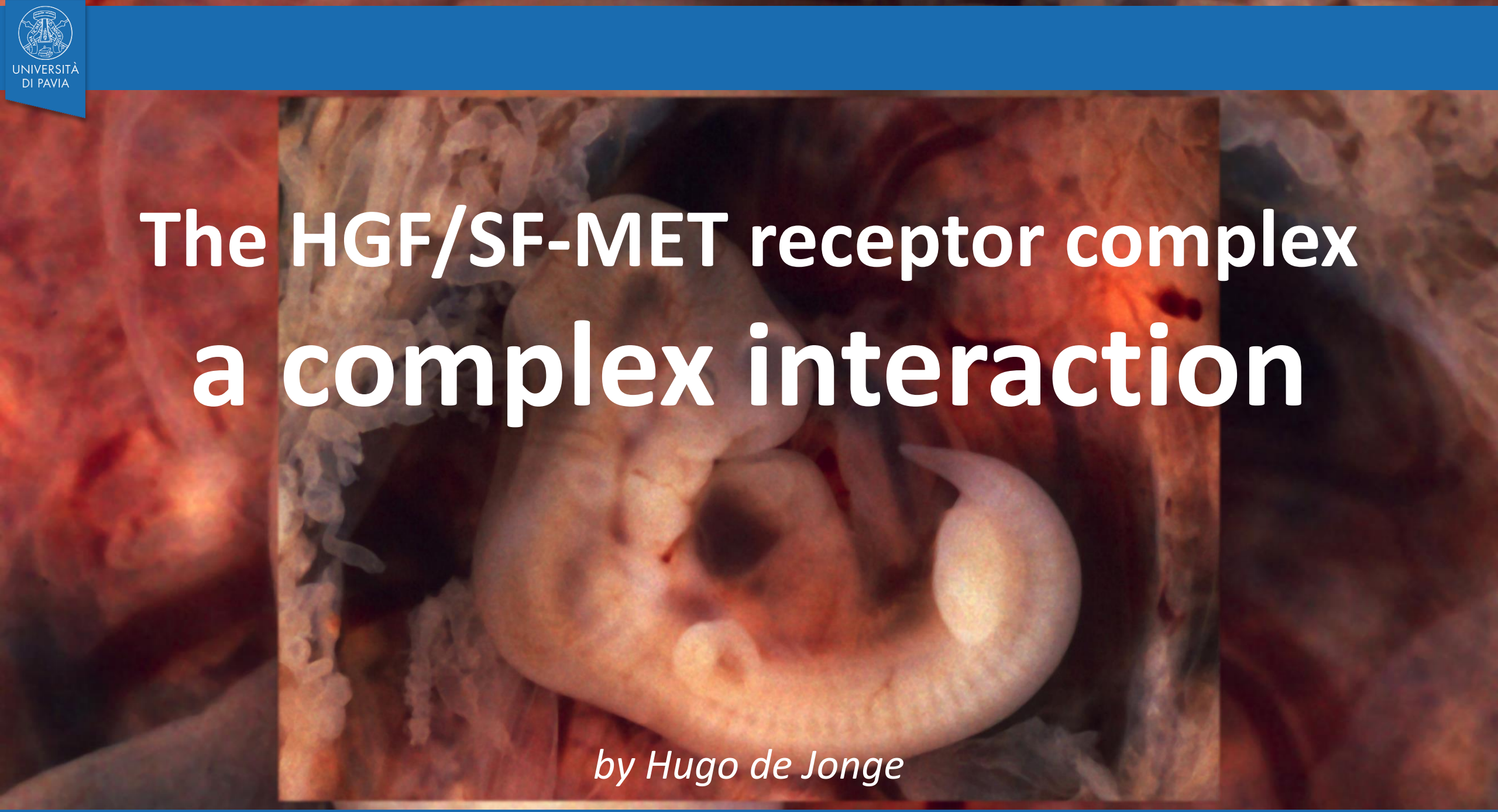
UNIVERSITÀ DI PAVIA

The HGF/SF-MET receptor complex
a complex interaction



by Hugo de Jonge

Acknowledgements

Department of Molecular Medicine

Unit of Immunology and General Pathology

- Prof Ermanno Gherardi
- Dr Luisa Iamele
- *current and past students*

Leiden Medical Centre (LUMC), The Netherlands

- Dr Cornelis Sier

Many people from former lab in Cambridge, UK

University of Pavia

The HGF/SF-MET signalling complex – a complex interaction

The importance of being able to "see" a tumour-related protein in (developing) a treatment

Let’s get interactive

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2

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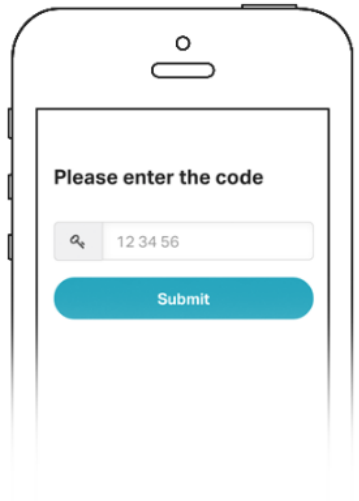


3

Enter the code 22 52 86 and vote!

The discovery of a new protein

Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 6489–6493, September 1986
Cell Biology

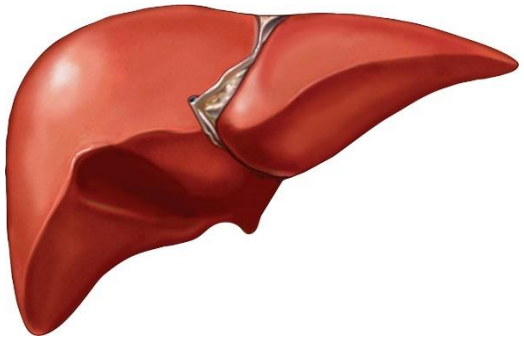
Purification and characterization of a growth factor from rat platelets for mature parenchymal hepatocytes in primary cultures

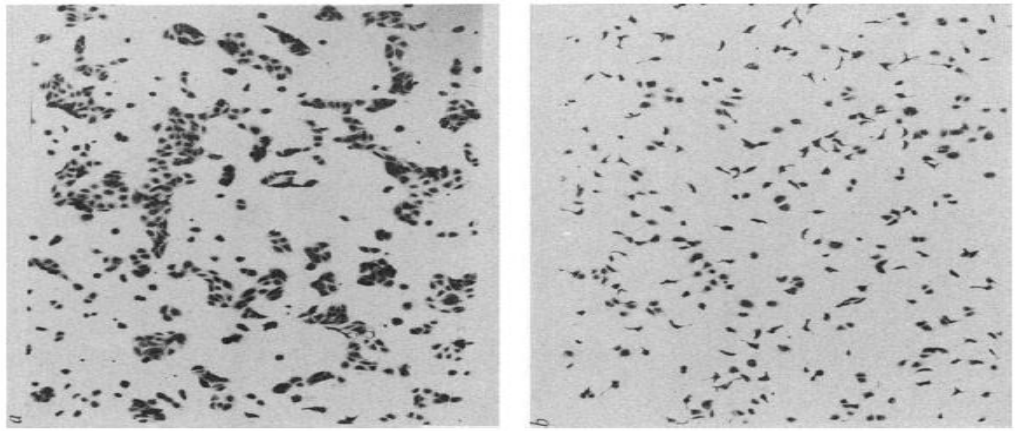
(hepatocyte growth factor/hepatotropic factor/liver regeneration)

TOSHIKAZU NAKAMURA, HIDEO TERAMOTO, AND AKIRA ICHIHARA

Institute for Enzyme Research, School of Medicine, University of Tokushima, Tokushima 770, Japan

Communicated by Van Rensselaer Potter, May 19, 1986



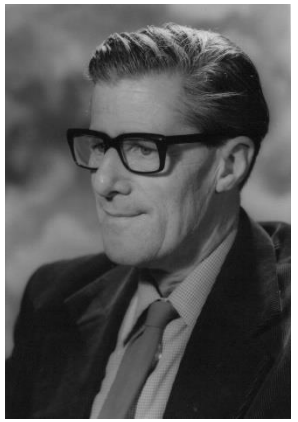


NATURE VOL. 327 21 MAY 1987

Scatter factor is a fibroblast-derived modulator of epithelial cell mobility

Michael Stoker, Ermanno Gherardi, Marion Perryman & Julia Gray

Department of Pathology, University of Cambridge, Cambridge CB2 1QP, UK and Imperial Cancer Research Fund, PO Box 123, Lincoln's Inn Fields, London WC2A 3PX, UK



Both the SAME protein! 😯

The EMBO Journal vol.10 no.10 pp.2867–2878, 1991

Scatter factor and hepatocyte growth factor are indistinguishable ligands for the *MET* receptor

Luigi Naldini, K.Michael Weidner[1], Elisa Vigna, Giovanni Gaudino, Alberto Bardelli, Carola Ponzetto, Radha P.Narsimhan, Guido Hartmann[1], Reza Zarnegar[2], George K.Michalopoulos[2], Walter Birchmeier[1] and Paolo M.Comoglio

Department of Biomedical Sciences and Oncology, University of Torino, School of Medicine, 10126 Torino, Italy, [1]Institut für Zellbiologie (Tumorforschung), Universitatsklinikum, D-4300 Essen, FRG and [2]Department of Pathology, Duke University, Durham, NC 27710, USA

Communicated by P.M.Comoglio.

Scatter Factor (SF) is a fibroblast-secreted protein which promotes motility and matrix invasion of epithelial cells. Hepatocyte Growth Factor (HGF) is a powerful mitogen for hepatocytes and other epithelial tissues. SF and HGF, purified according to their respective biological activities, were interchangeable and equally effective in assays for cell growth, motility and invasion. Both bound with identical affinities to the same sites in target cells. The receptor for SF and HGF was identified as the product of the *MET* oncogene by: (i) ligand binding and coprecipitation in immunocomplexes; (ii) chemical cross-linking to the Met β subunit; (iii) transfer of binding activity in insect cells by a baculovirus carrying the *MET* cDNA; (iv) ligand-induced tyrosine phosphorylation of the Met β subunit. SF and HGF cDNA clones from human fibroblasts, placenta and liver had virtually identical sequences. We conclude that the same molecule (SF/HGF) acts as a growth or motility factor through a single receptor in different target cells.

Key words: growth factor receptor/hepatocyte growth factor/*MET* oncogene/scatter factor/tyrosine kinase

Introduction

Scatter Factor (SF) is a secretory product of fibroblasts which dissociates epithelial cells increasing their motility and invasiveness (Stoker *et al.*,1987; Rosen *et al.*, 1990; Weidner *et al.*, 1990). It was reported to be chemotactic and not mitogenic for target cells (Gherardi *et al.*, 1989). SF might be involved in the progression of carcinoma cells to a more malignant invasive phenotype (Weidner *et al.*, 1990) and might play a role in epithelial–mesenchymal transitions during early embryonic development (Stern *et al.*, 1990).

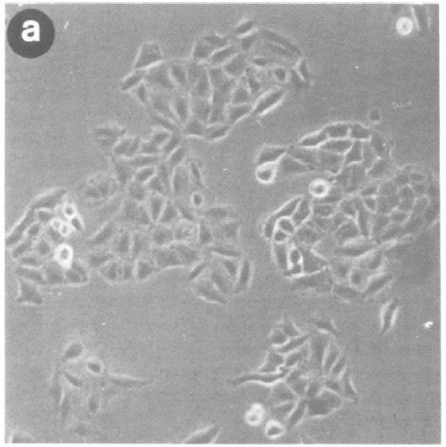
Hepatocyte Growth Factor (HGF) is a powerful mitogen for hepatocytes in primary cultures. It was isolated from several sources including rat platelets (Nakamura *et al.*, 1986), serum of human patients with hepatic failure (Gohda *et al.*, 1988), and rabbit serum (Zarnegar and Michalopoulos, 1989). HGF is considered a major mediator of liver regeneration *in vivo* (Michalopoulos, 1990). Recently, HGF was shown to stimulate the growth of other epithelial tissues, such as kidney tubular epithelium and keratinocytes (Kan *et al.*, 1991), endothelial cells and melanocytes (Rubin *et al.*, 1991).

While the biological activities of SF and HGF are apparently unrelated, purification of the molecules revealed a surprising degree of structural similarity. Both HGF and SF are disulphide-linked heterodimers of a heavy (α) subunit of 55–65 kDa and a light (β) subunit of 32 or 36 kDa. The two M_r of the β subunit of SF were ascribed to differences in glycosylation (Weidner *et al.*, 1990). The α and β subunits of HGF originate from proteolytic cleavage of a single 92 kDa precursor, as indicated by the sequence of cloned human and rat HGF cDNAs (Miyazawa *et al.*, 1989; Nakamura *et al.*, 1989; Tashiro *et al.*, 1990). When the primary sequence of several tryptic peptides derived from purified SF was compared to the deduced amino acid sequence of HGF, all the identified residues could be matched (Gherardi and Stoker, 1990; Weidner *et al.*, 1990; 1991).

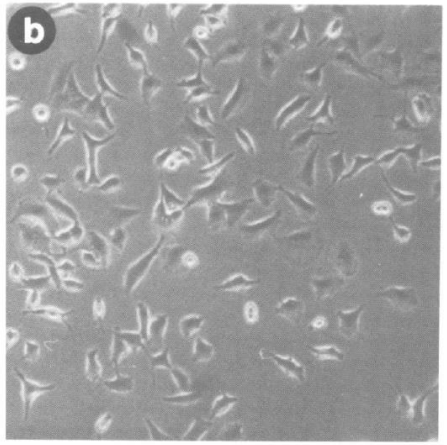
Previous work by Naldini *et al.* (1991a) and that of another laboratory (Bottaro *et al.*, 1991) has recently suggested that the HGF receptor is the product of the *MET* oncogene, a transmembrane protein endowed with tyrosine kinase activity (Cooper *et al.*, 1984; Park *et al.*, 1986, 1987). The structure of the Met protein has been investigated in a cell line (GTL16), where the gene is amplified and overexpressed (Giordano *et al.*, 1989a). The protein is a 190 kDa heterodimer ($p190^{MET}$) made of a 50 kDa subunit (α) disulphide-linked to a 145 kDa subunit (β). The molecule is synthesized as a single-chain 170 kDa precursor, which undergoes co-translational glycosylation. Disulphide rearrangements and proteolytic cleavage lead to the mature two-chain 190 kDa heterodimer (Giordano *et al.* 1989b). The α chain and the N-terminal portion of the β chain of the mature protein are exposed at the cell surface (Giordano *et al.*, 1988). The C-terminal portion of the β chain is cytoplasmic and includes a tyrosine kinase domain (Dean *et al.*, 1985; Tempest *et al.*, 1986; Gonzatti *et al.*, 1988) and phosphorylation sites involved in regulation of its activity (Ferracini *et al.*, 1991). The kinase activity is positively regulated by autophosphorylation on tyrosine (Naldini *et al.*, 1991b), and it is negatively regulated by protein kinase-C activation (Gandino *et al.*, 1990) or transient increases of intracellular Ca^{2+} concentrations (Gandino *et al.*, 1991). Stimulation of the tyrosine phosphorylation of the β subunit of the Met protein after exposure to HGF was observed both in intact cells and *in vitro* with partially purified Met protein (Bottaro et al., 1991; Naldini *et al.*, 1991a). Chemical cross-linking to the Met protein of a molecule smaller than HGF (M_r 28 kDa) but with similar binding properties was also reported (Bottaro *et al.*, 1991).

In this work we have investigated the structural and functional relationships between SF and HGF. Nucleotide sequence analysis of cDNA clones from fibroblasts, placenta

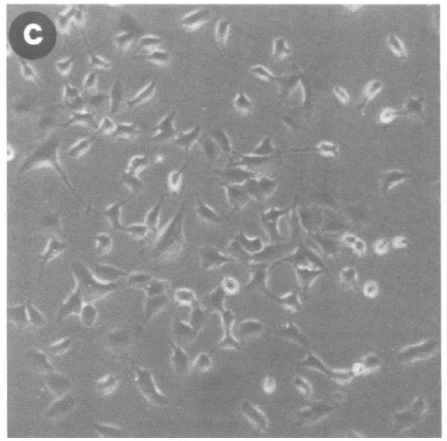
control



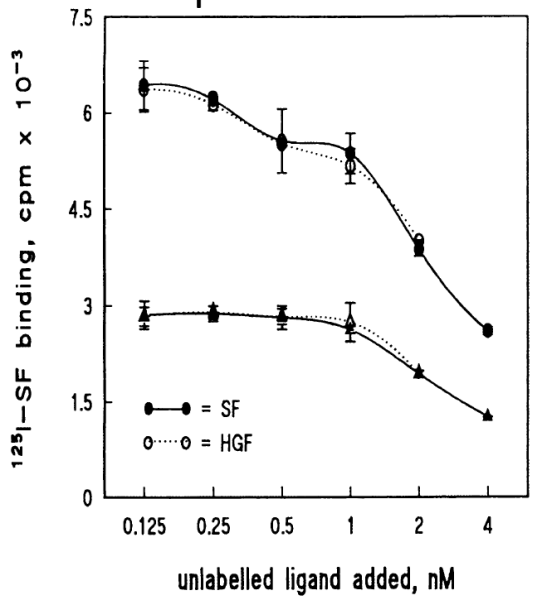
scatter factor (SF)



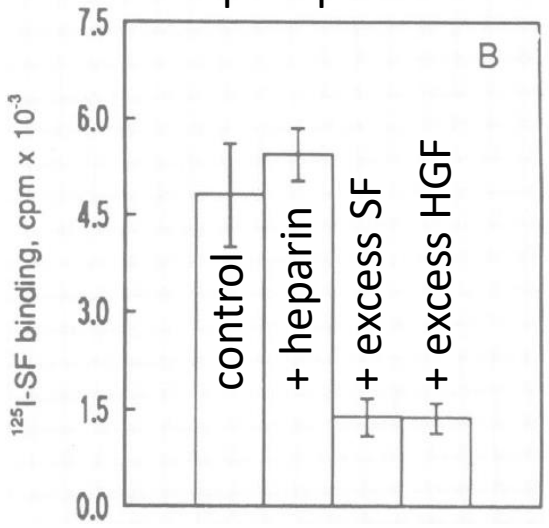
HGF



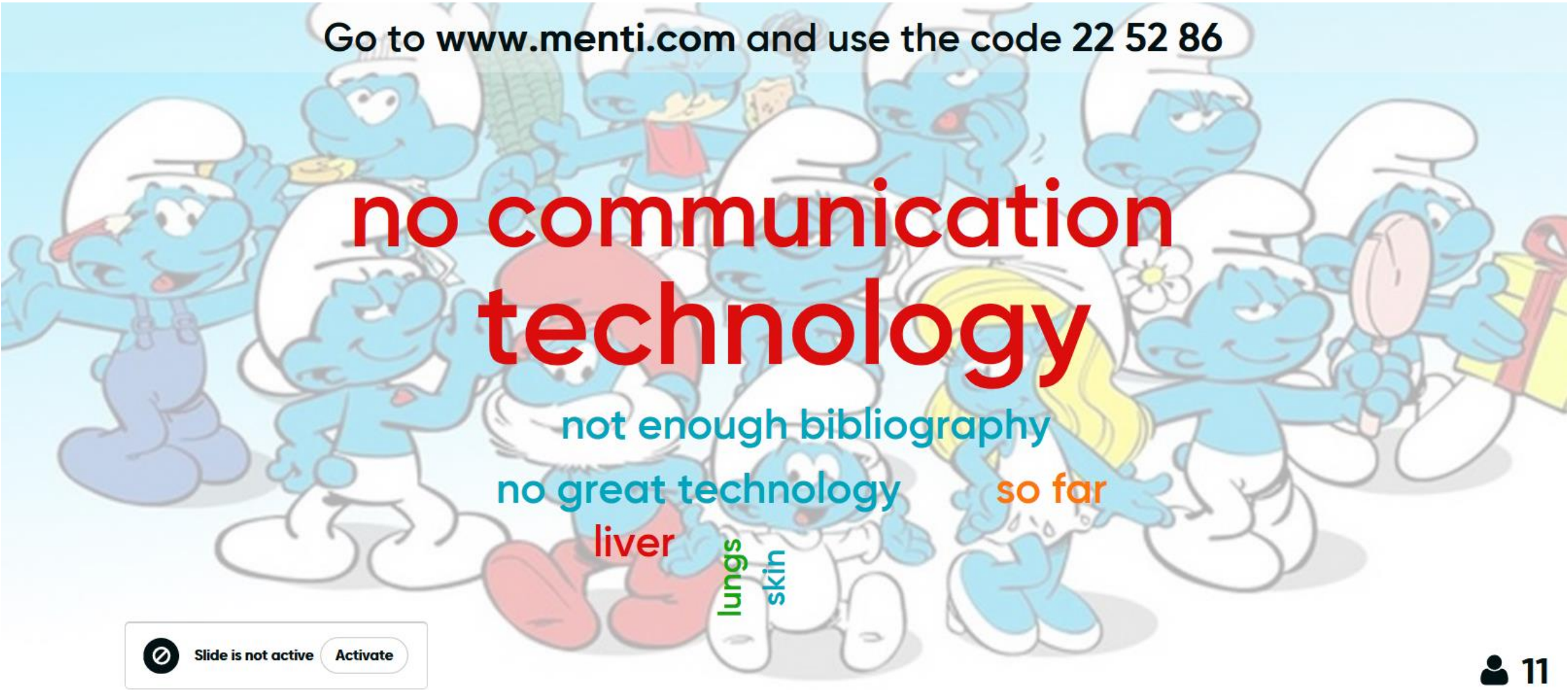
competition on cells



receptor pull down

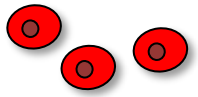


Why did it take so long?

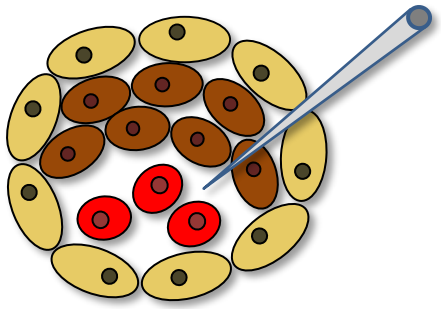


Demonstrating protein function: the “knock-out” mouse

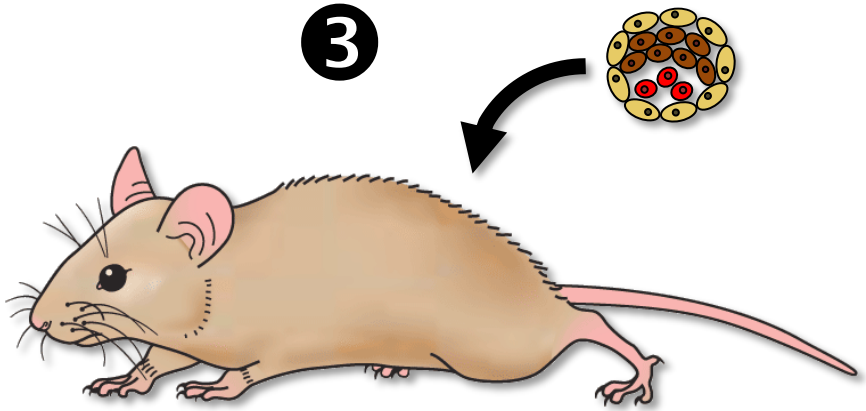
❶ **delete HGF/SF in embryonic stem cells**



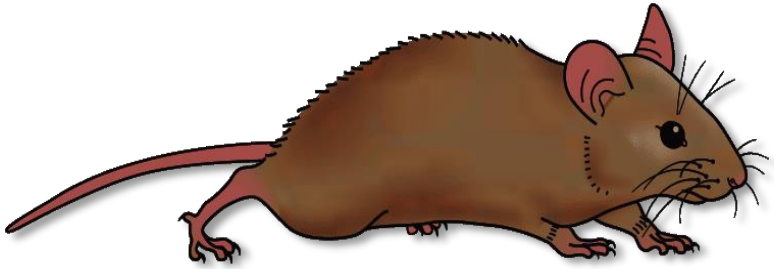
❷ **inject into blastocyst**



❸



❹

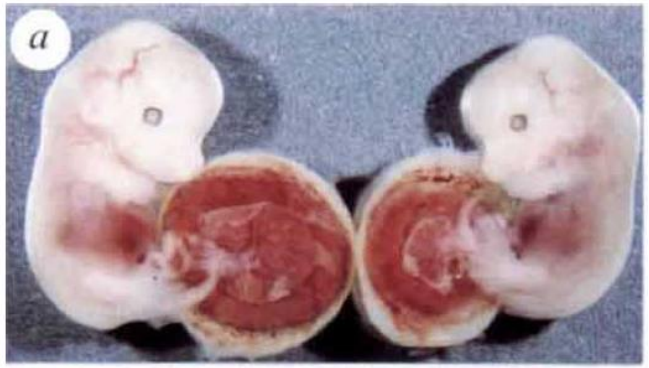


❺

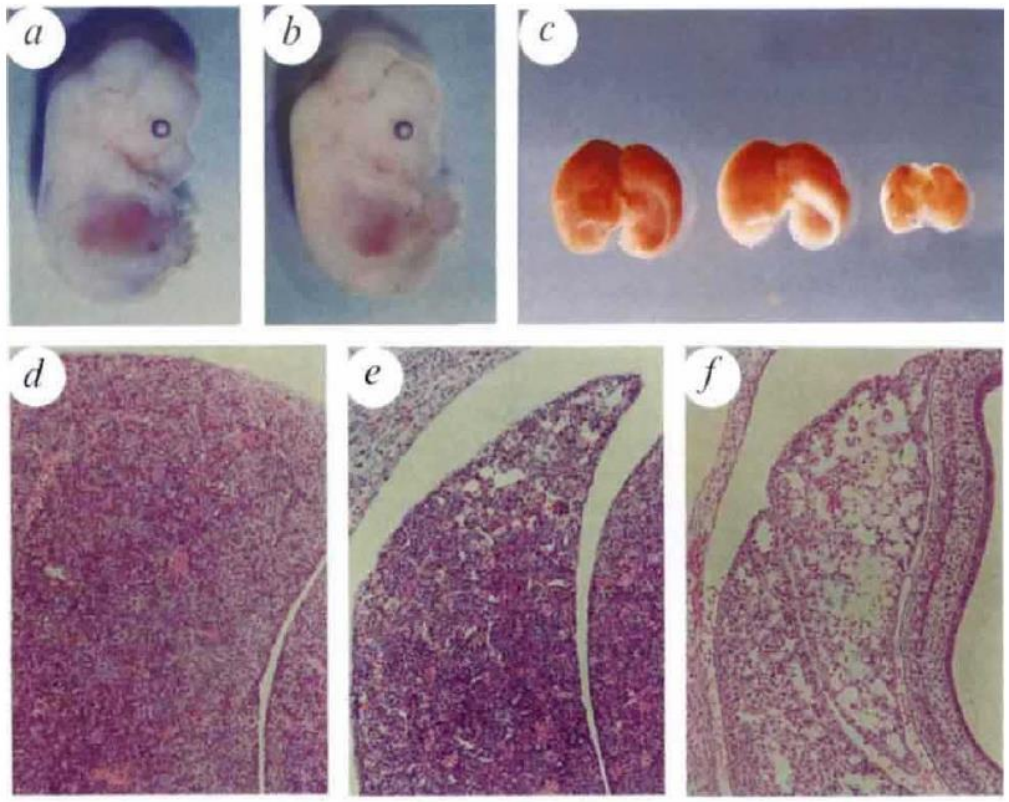
mouse with “knocked-out” HGF/SF gene

The importance of HGF/SF during embryogenesis

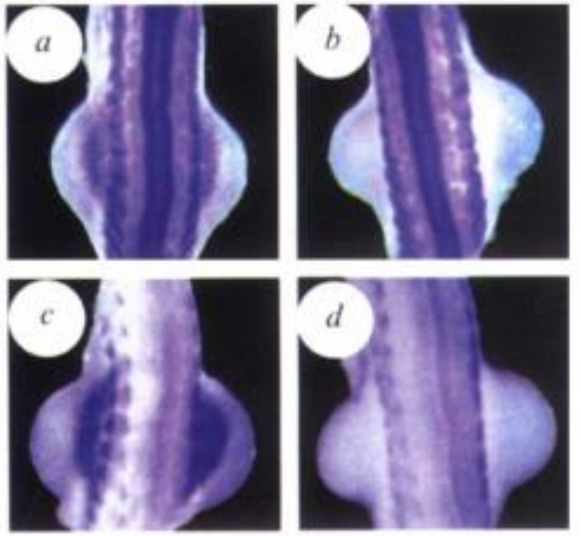
placenta



liver



myogenesis



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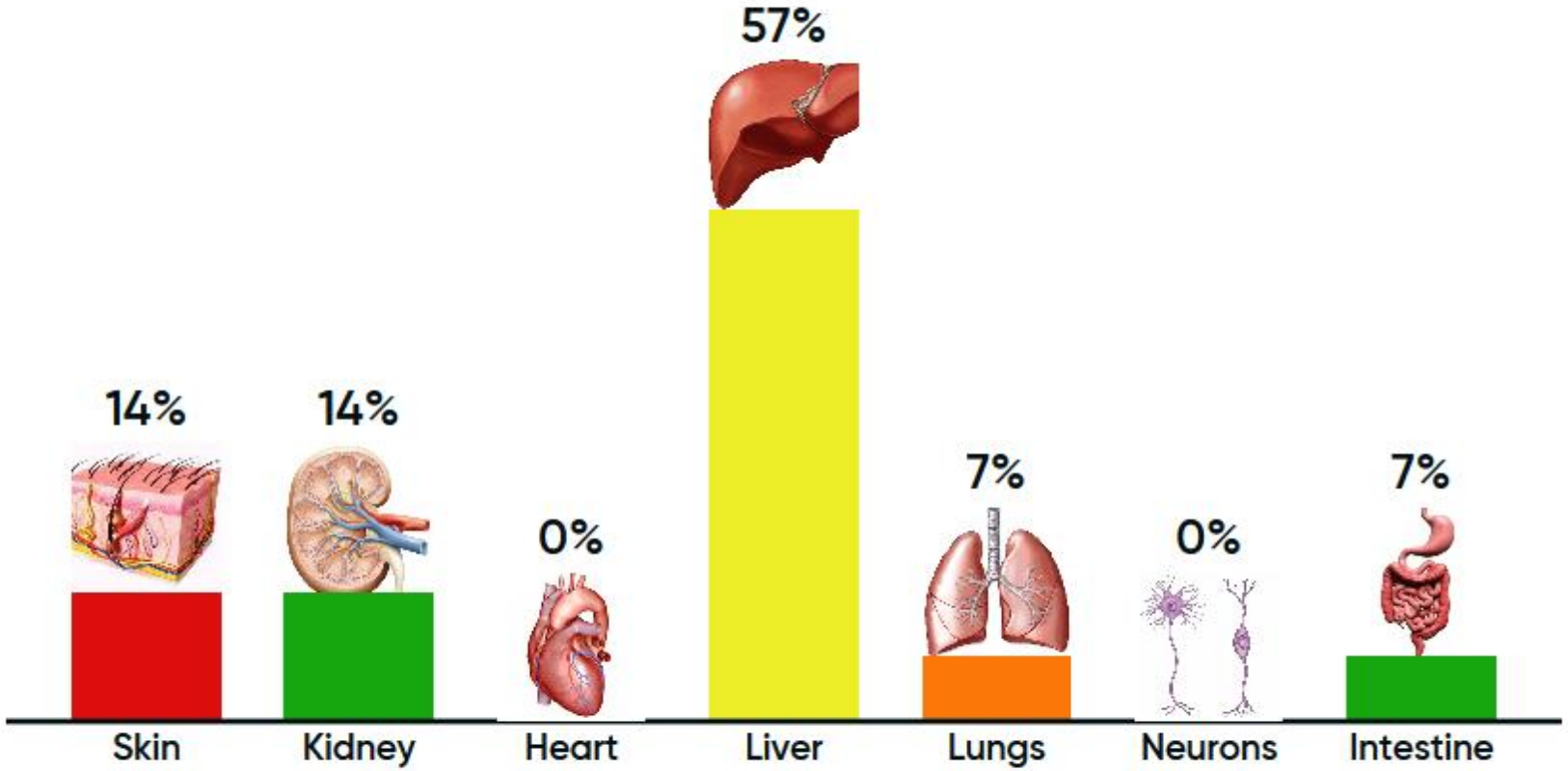
- Uehara Y, Minowa O, Mori C, Shiota K, Kuno J, Noda T, Kitamura N. Nature. 1995 373, 702-705
- Bladt F, Riethmacher D, Isenmann S, Aguzzi A, Birchmeier C. Nature. 1995 376, 768-771
- Huh CG, Factor VM, Sánchez A, Uchida K, Conner EA, Thorgeirsson SS. PNAS 2004 101 4477-4482

What about HGF/SF in adult life?

Tissue and organ regeneration after injury



Slide is not active Activate

14

How?

proliferation

differentiation

motility/migration

morphogenic

angiogenic

anti-apoptotic

Regeneration

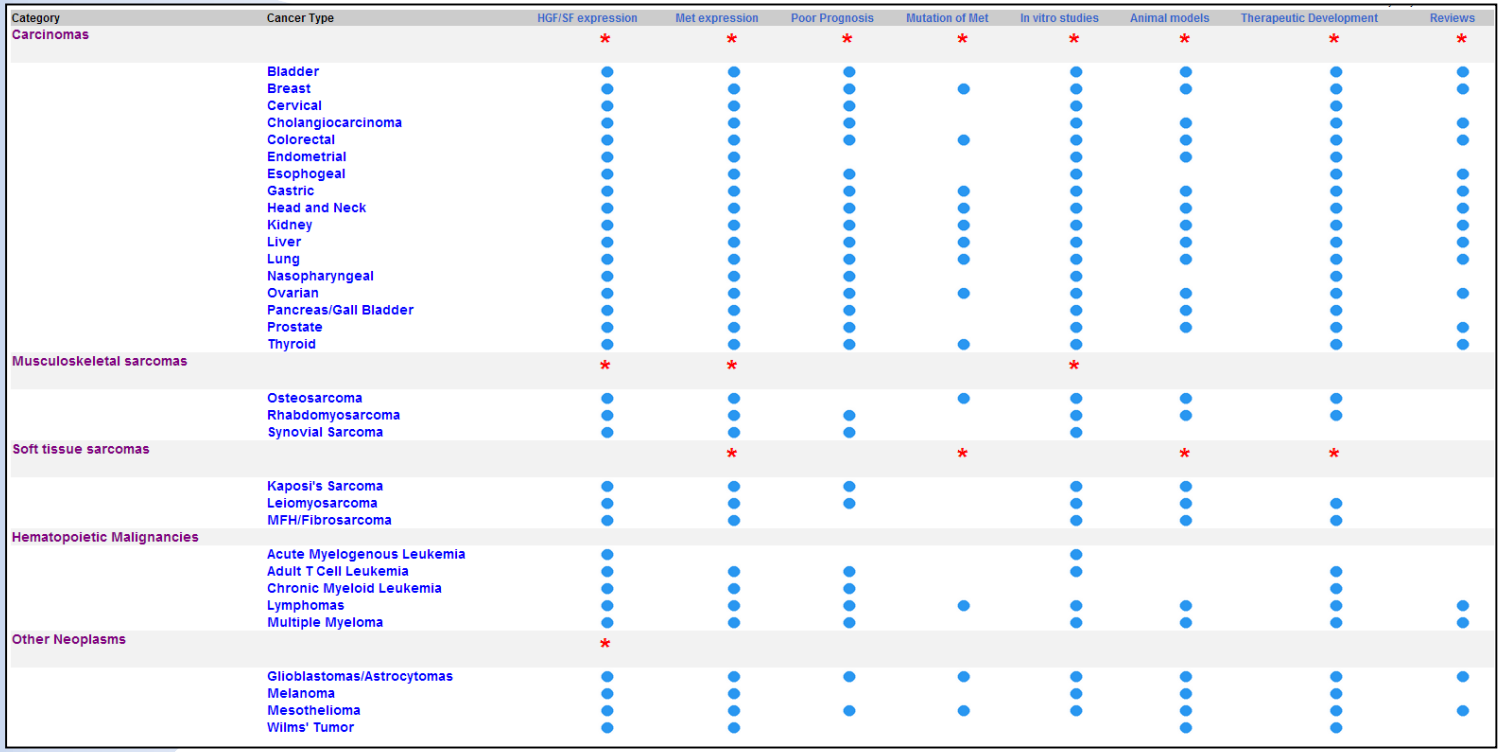
I NEED THAT !

Cancer

Thousands of papers on HGF/SF in cancer

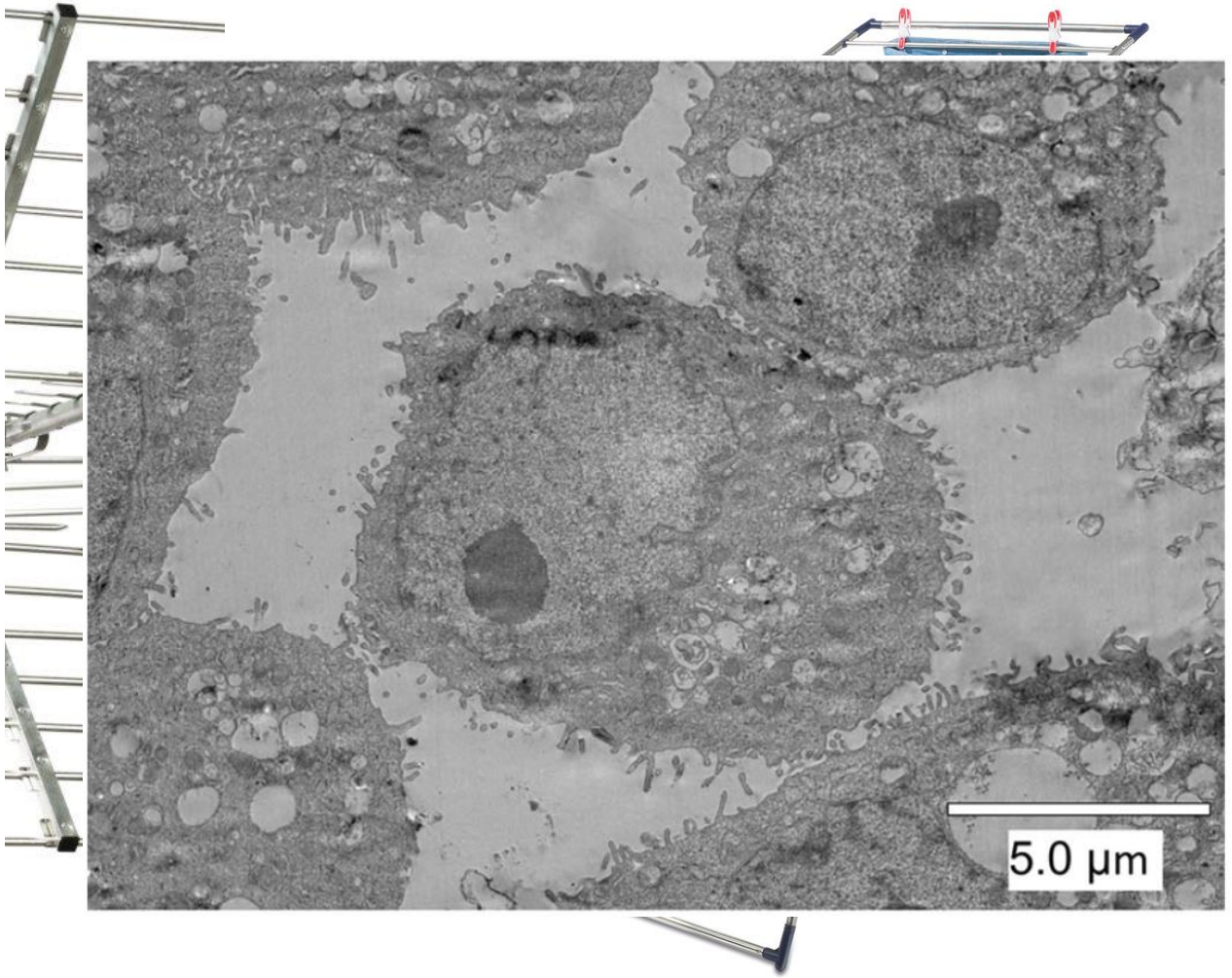
Category	Cancer Type
Carcinomas	Bladder
	Breast
	Cervical
	Cholangiocarcinoma
	Colorectal
	Endometrial
	Esophogeal
	Gastric
	Head and Neck
	Kidney
	Liver
	Lung
	Nasopharyngeal
	Ovarian
	Pancreas/Gall Bladder
	Prostate
	Thyroid
Musculoskeletal sarcomas	Osteosarcoma
	Rhabdomyosarcoma
	Synovial Sarcoma
Soft tissue sarcomas	Kaposi's Sarcoma
	Leiomyosarcoma
	MFH/Fibrosarcoma
Hematopoietic Malignancies	Acute Myelogenous Leukemia
	Adult T Cell Leukemia
	Chronic Myeloid Leukemia
	Lymphomas
	Multiple Myeloma
Other Neoplasms	Glioblastomas/Astrocytomas
	Melanoma
	Mesothelioma
	Wilms' Tumor

https://resources.vai.org/Met/Index.aspx

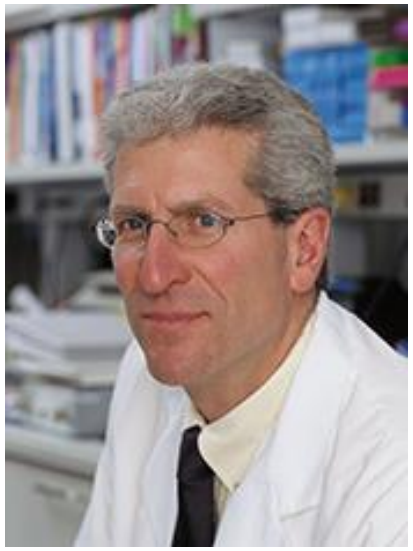


Van Andel Institute®

Every signal needs an antenna; a receptor



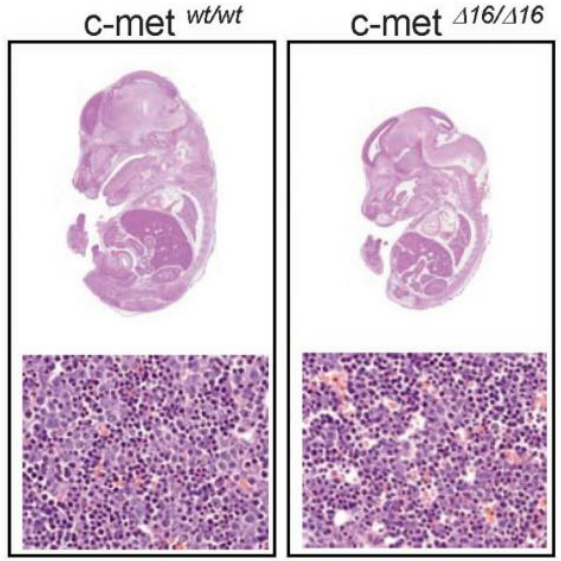
The “antenna“ for HGF/SF



Identification of the Hepatocyte Growth Factor Receptor as the c-*met* Proto-Oncogene Product

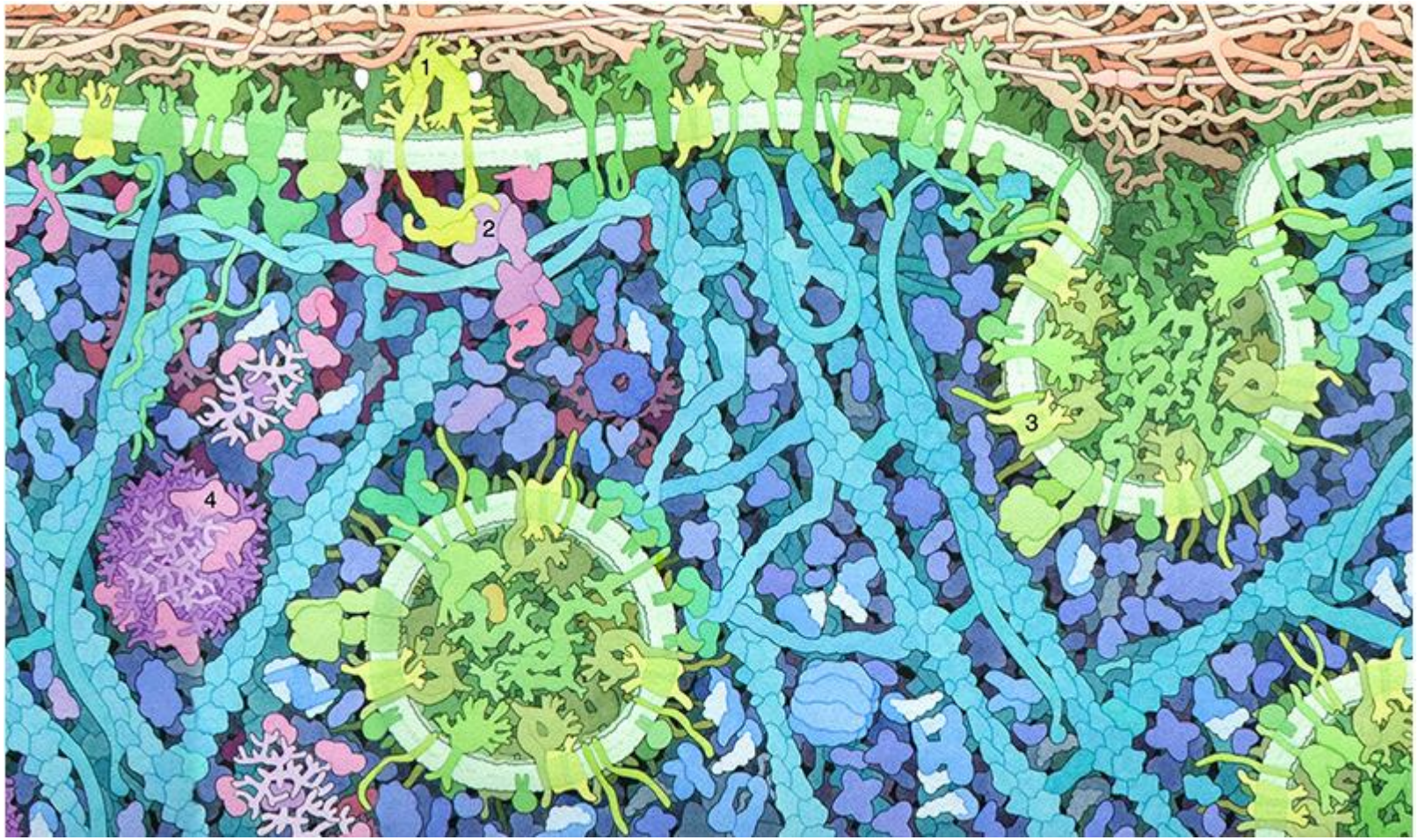
DONALD P. BOTTARO, JEFFREY S. RUBIN, DONNA L. FALETTO, ANDREW M.-L. CHAN, THOMAS E. KMIECIK, GEORGE F. VANDE WOUDE, STUART A. AARONSON

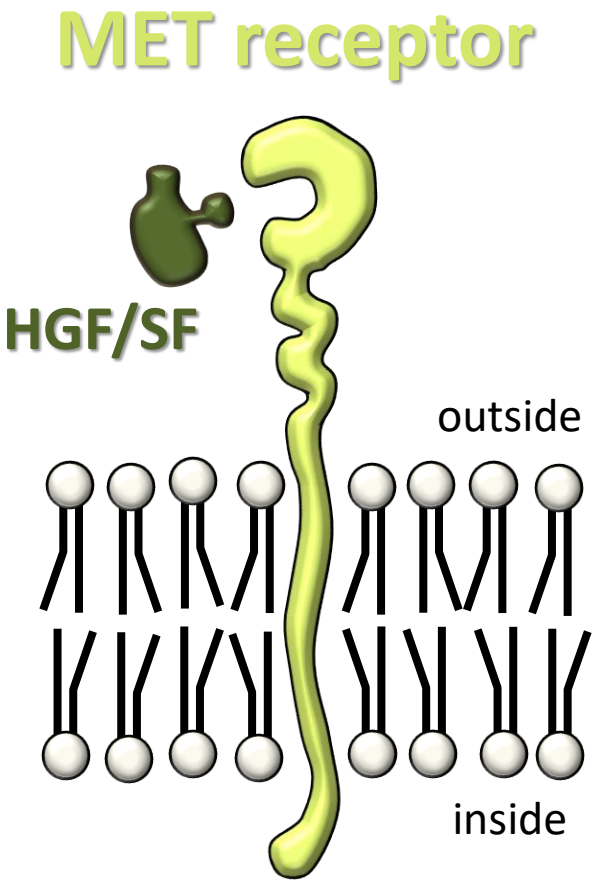
Hepatocyte growth factor (HGF) is a plasminogen-like protein thought to be a humoral mediator of liver regeneration. A 145-kilodalton tyrosyl phosphoprotein observed in rapid response to HGF treatment of intact target cells was identified by immunoblot analysis as the β subunit of the c-*met* proto-oncogene product, a membrane-spanning tyrosine kinase. Covalent cross-linking of ^{125}I-labeled ligand to cellular proteins of appropriate size that were recognized by antibodies to c-*met* directly established the c-*met* product as the cell-surface receptor for HGF.



Science 15 Feb 1991:Vol. 251, Issue 4995, pp. 802-804

The main players





Using this knowledge wisely

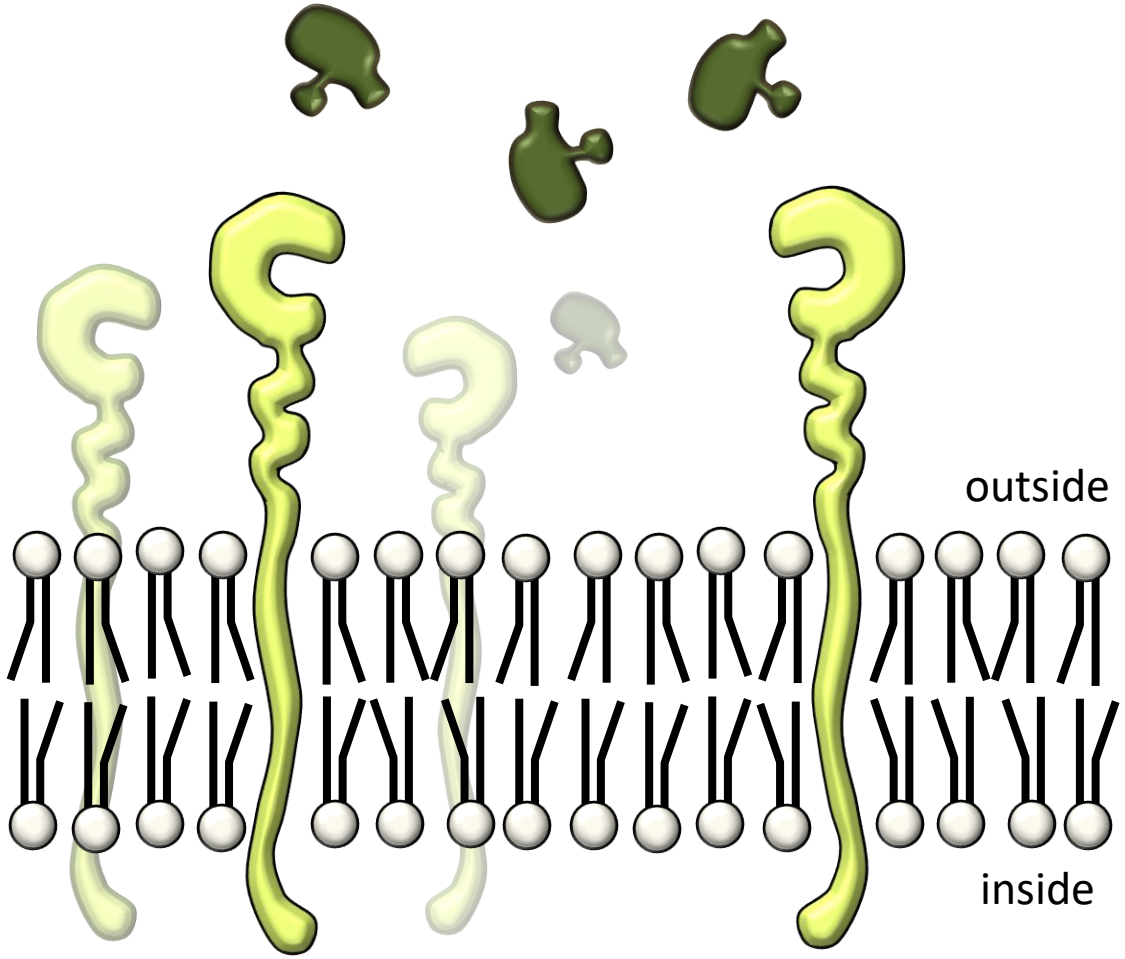
like you not I do

fight me if you dare

Regeneration

Cancer

How does it work?



biding

binding

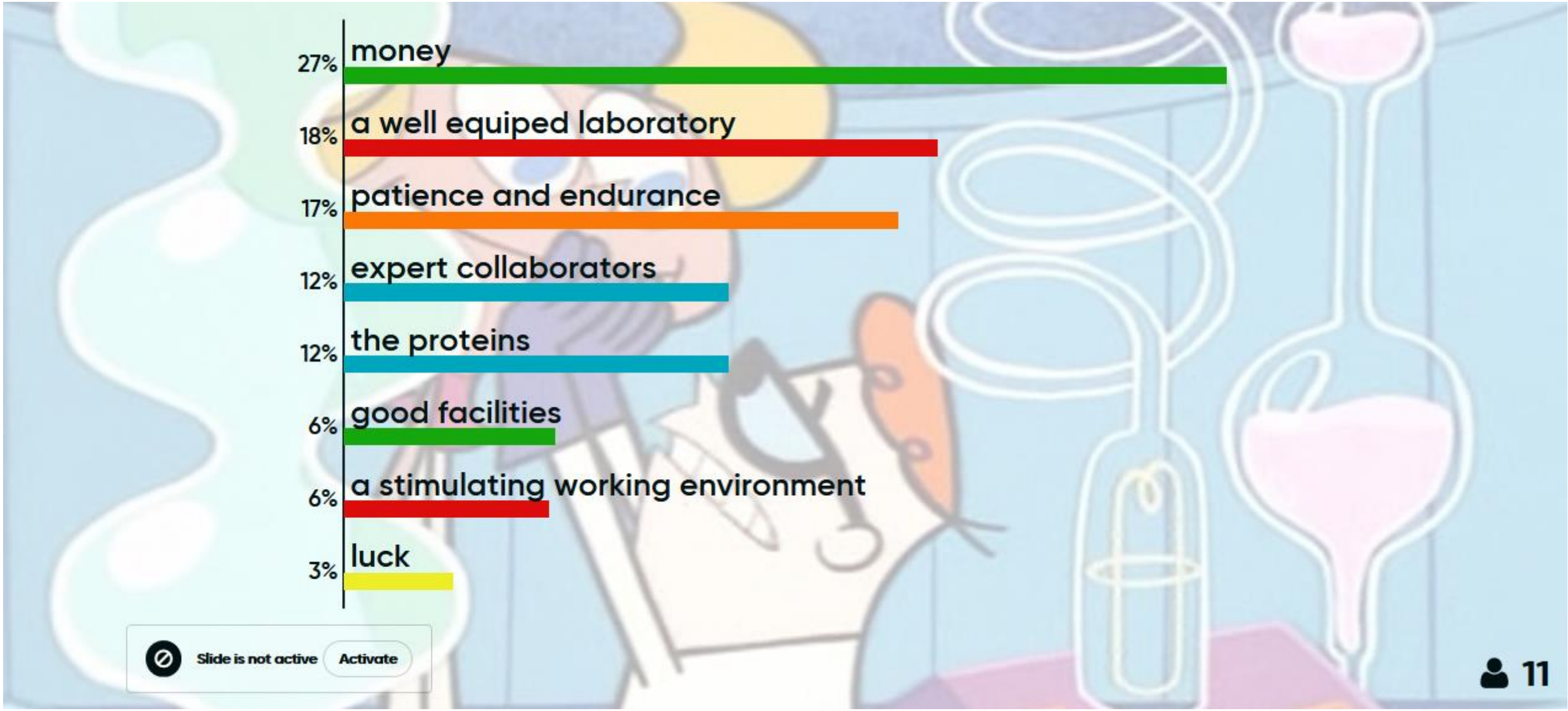
receptor ligand interacti

protein interactions

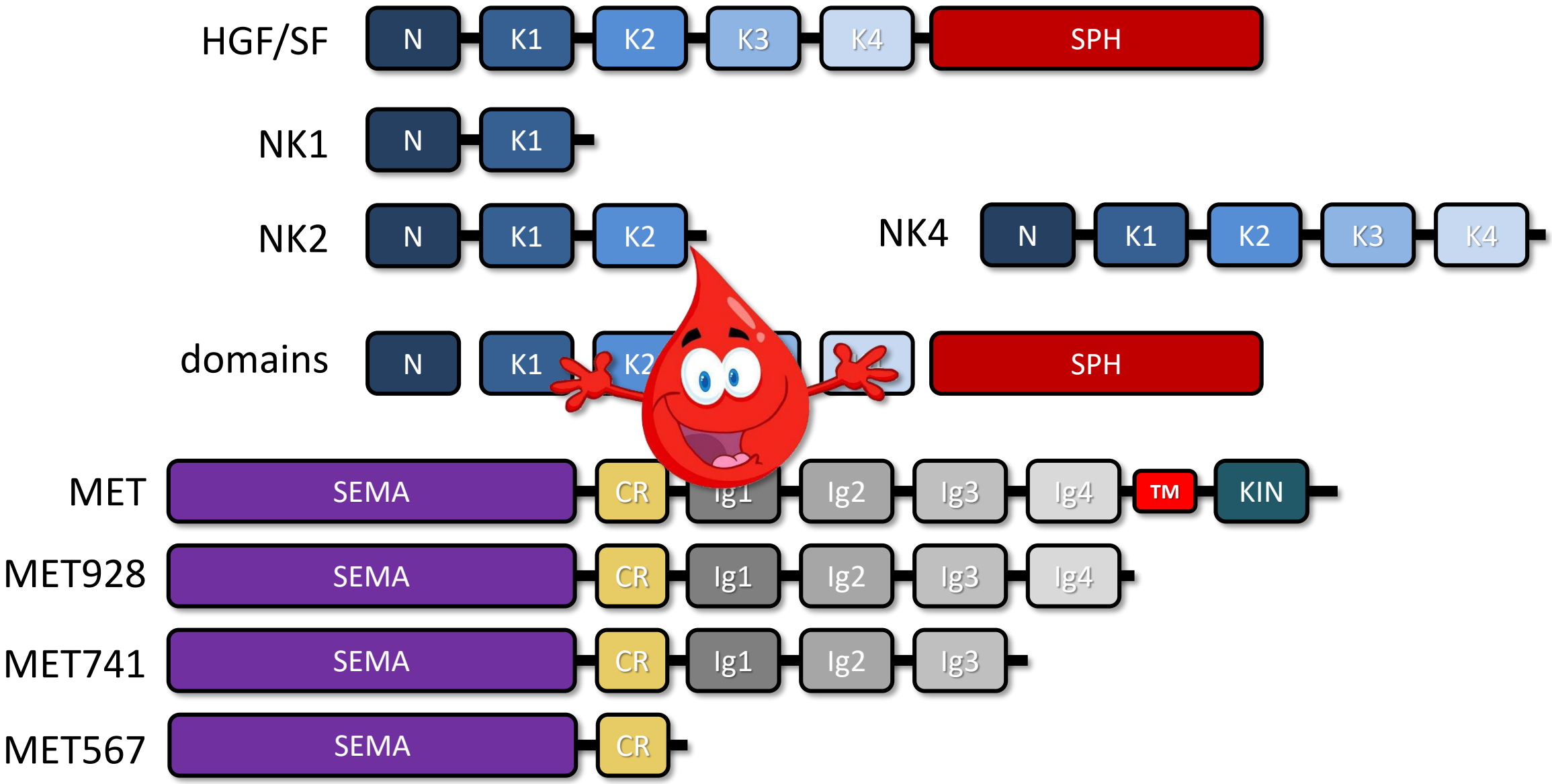
Slide is not active Activate

8

Requirements to investigate how it works

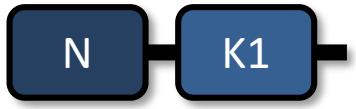


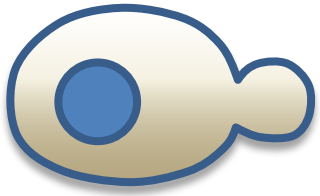
Important: soluble forms of RECEPTOR lack TRANSMEMBRANE protein



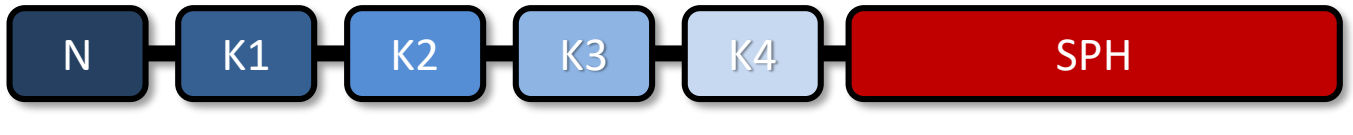
SOURCE: RECOMBINANT PROTEIN PRODUCTION

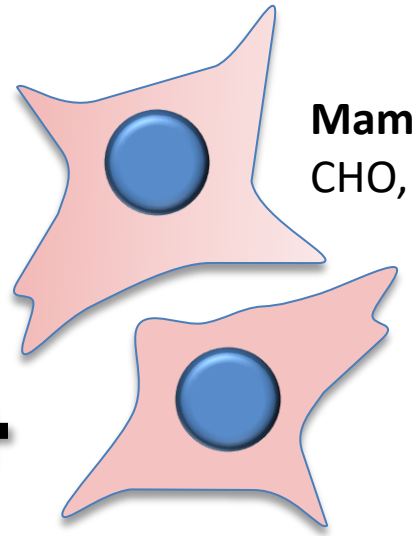
Bacteria: *E. coli* BL21, SHuffleT7





Yeast: *Pichia pastoris*

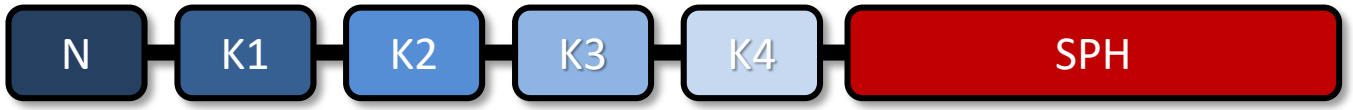




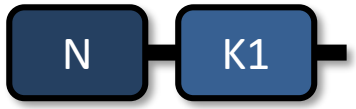
Mammalian cells:
CHO, HEK293, NS0

Example: recombinant protein production of NK1 in yeast

HGF/SF

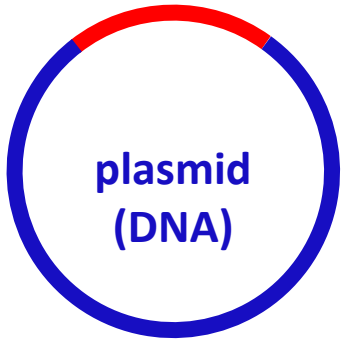


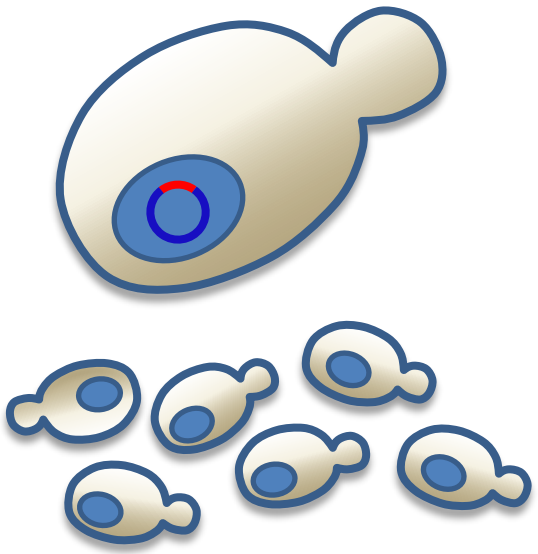
NK1:



EGQ…CSEVE (protein) = gagggacaa…cagaagttgaa (DNA)

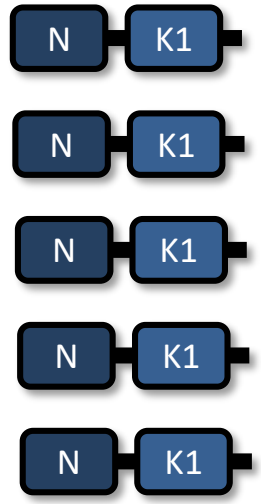
coding sequence





large scale growth

purification



Having the proteins allows you work on answers

Protein structure (alone and in complex)

- x-ray crystallography
- small-angle scattering (SAXS)
- cryo-electron microscopy

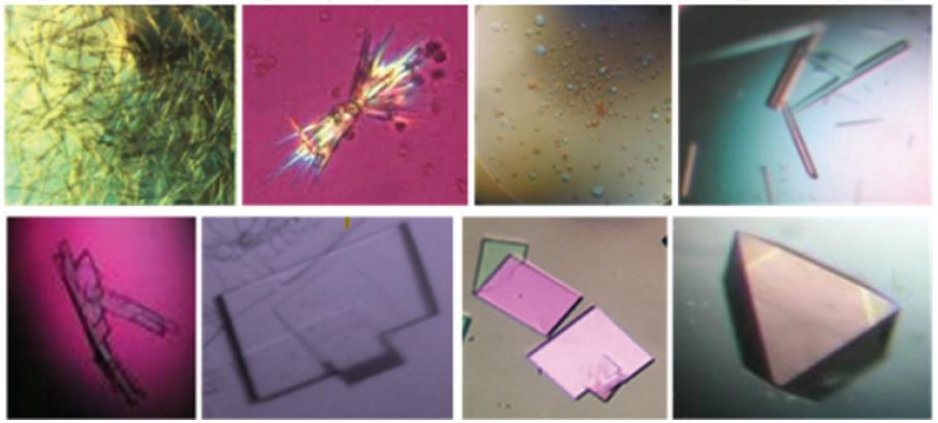
Protein function

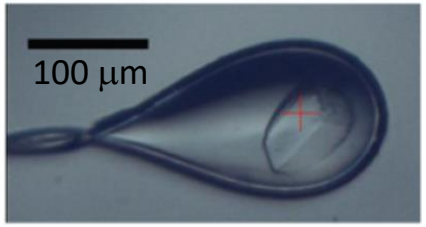
- mutational screening
- biological assays (*in vitro*)
- *in vivo* studies
- biochemical analysis (e.g. SPR)

Protein engineering

- design of ligand-based activators and inhibitors
- development of antibodies

Understanding the structure – x-ray diffraction





Requirements:

- A lot of very pure protein (many milligrams!)
- As many crystallisation conditions as possible (liquid handling robots)
- Patience…lots of patience
- Luck…lots of luck
- Some of the most complex and expensive facilities in the world

European Synchrotron Radiation Facility (ESRF)

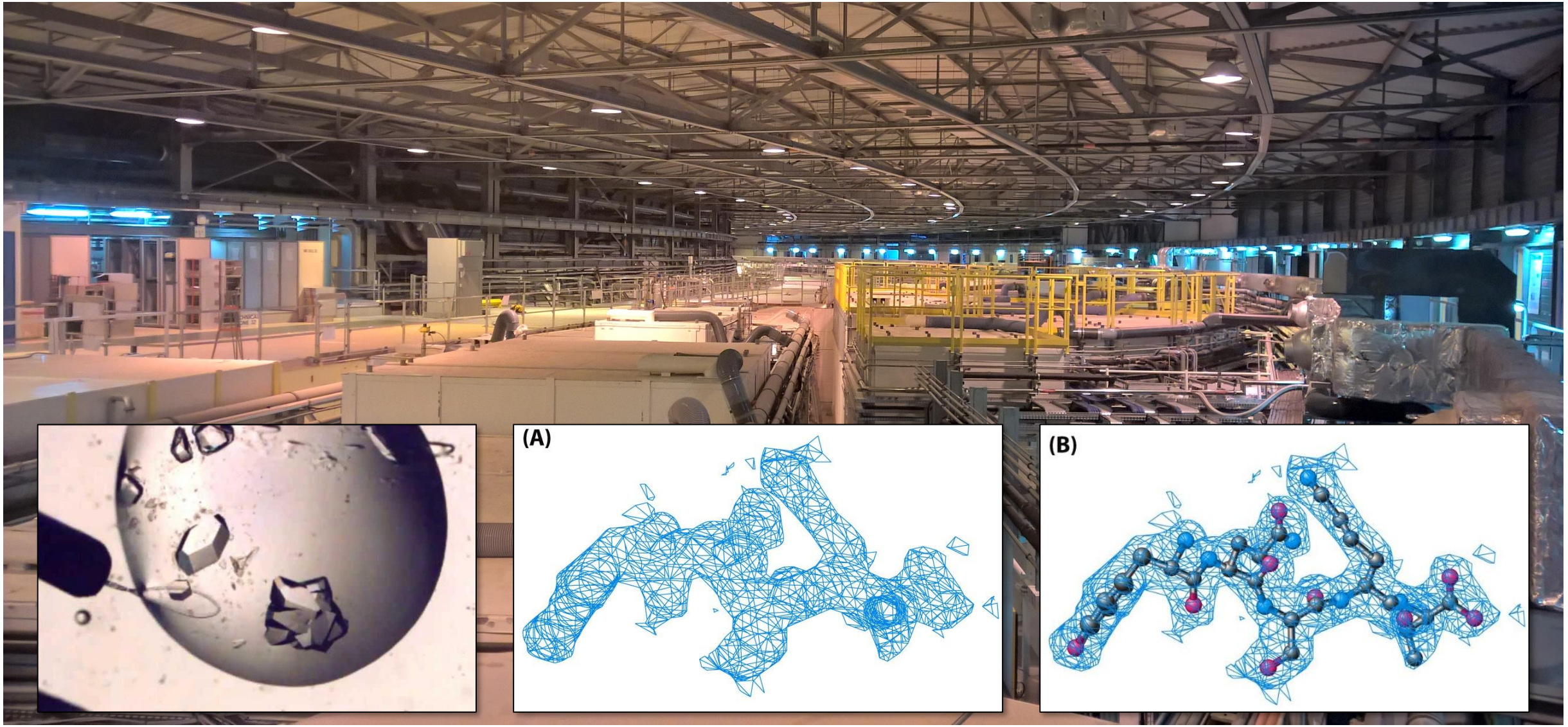
ESRF
The European Synchrotron

Supported by 22 countries:
13 member countries:
France, Germany, Italy, UK, Spain, Switzerland, Belgium, The Netherlands, Denmark, Finland, Norway, Sweden, and Russia

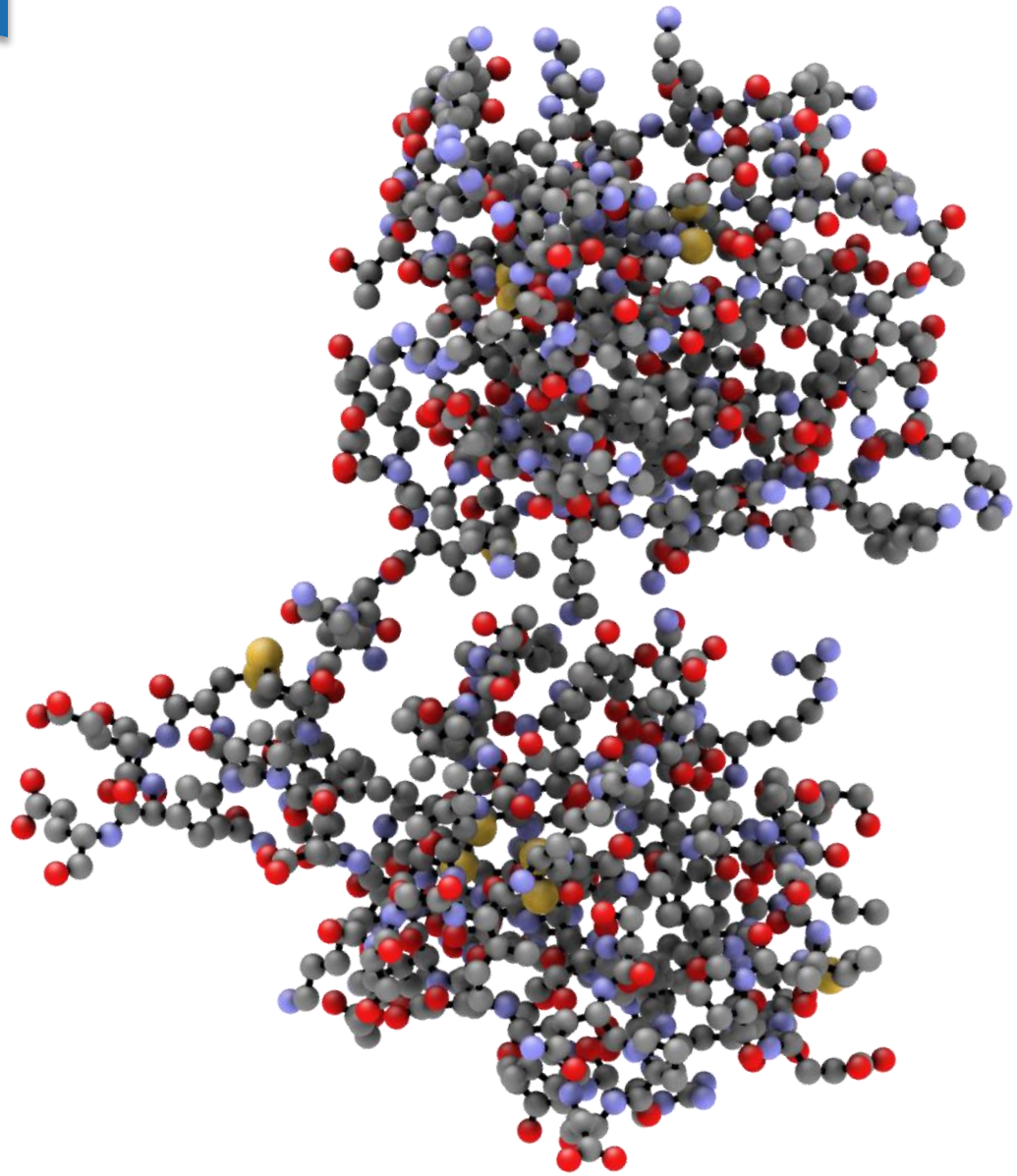
and

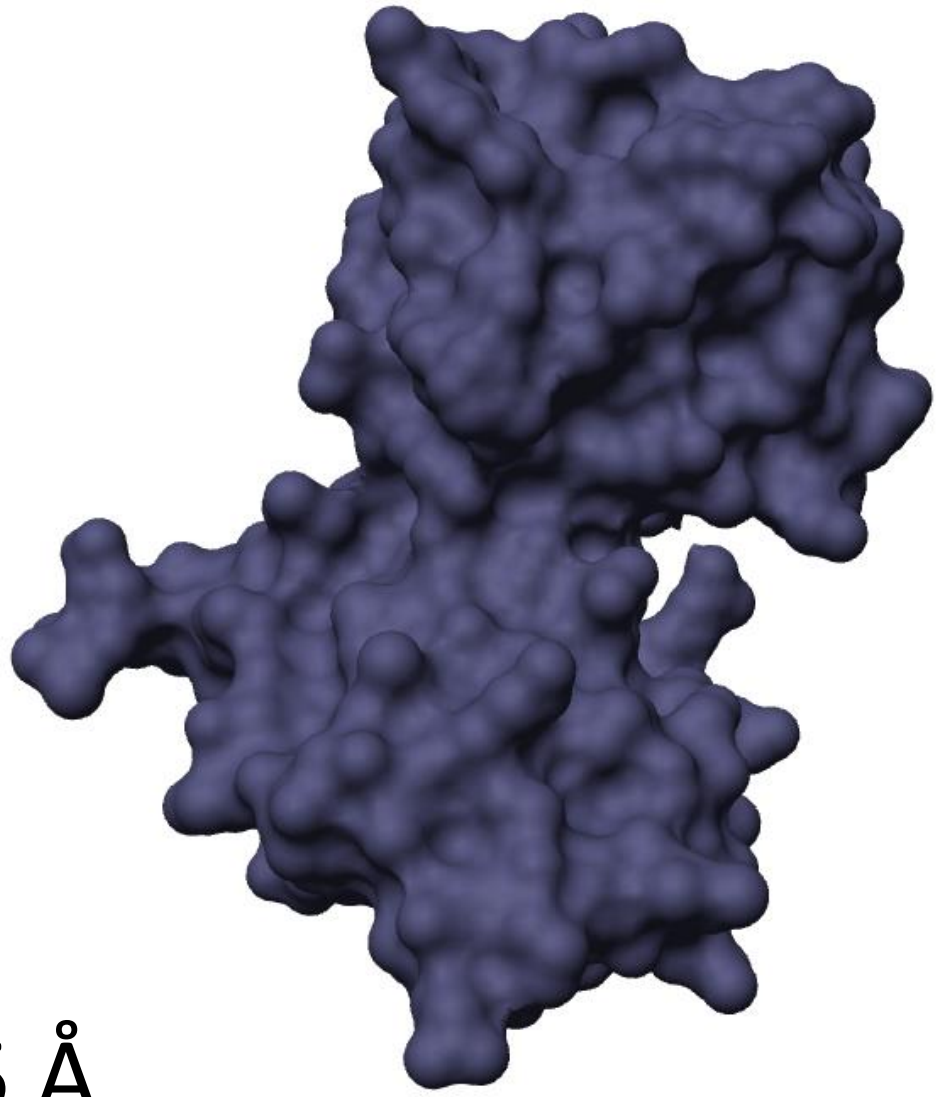
9 associate countries:
Austria, Portugal, Israel, Poland, Czech Republic, Hungary, Slovakia, India and South Africa

Using X-rays on protein crystals



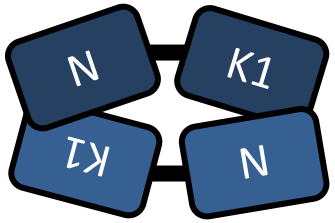
Revealing the structure of NK1



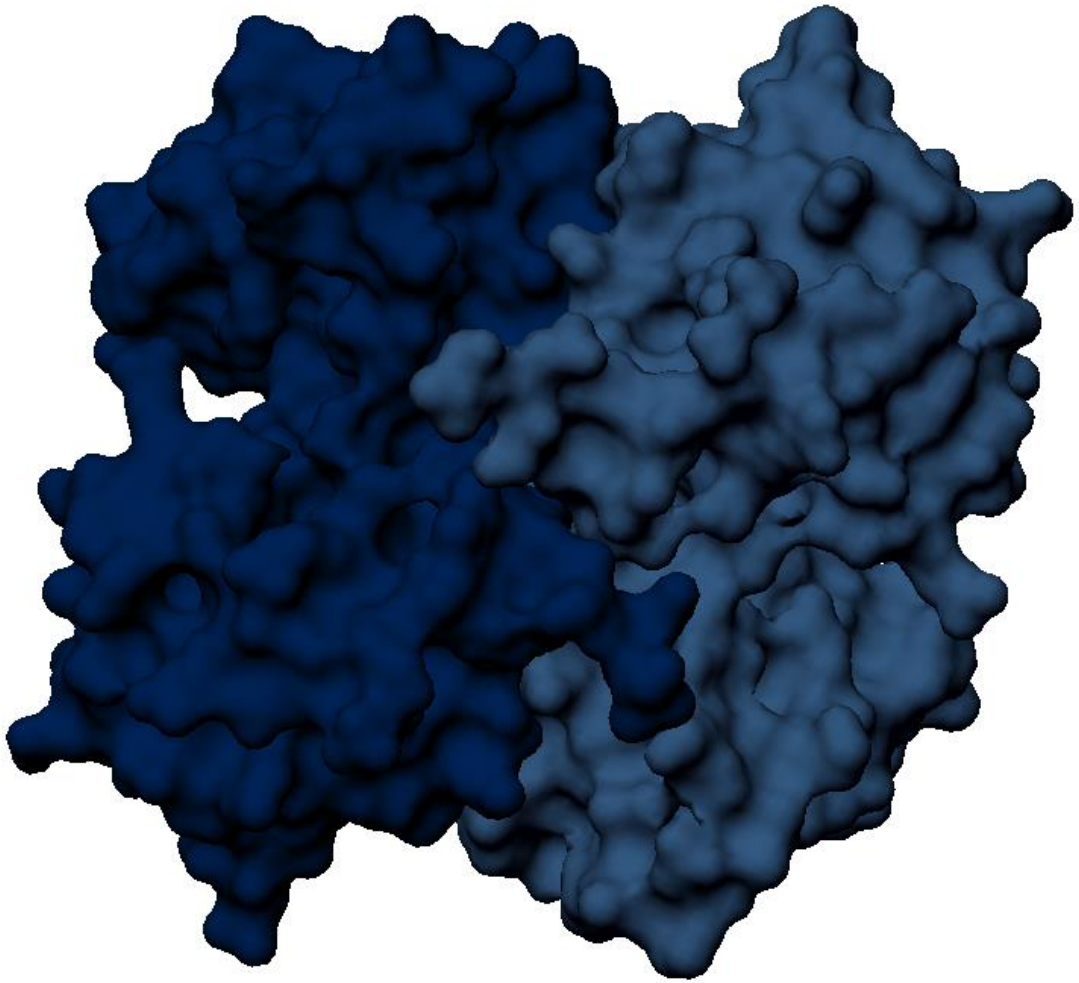


<2.5 Å

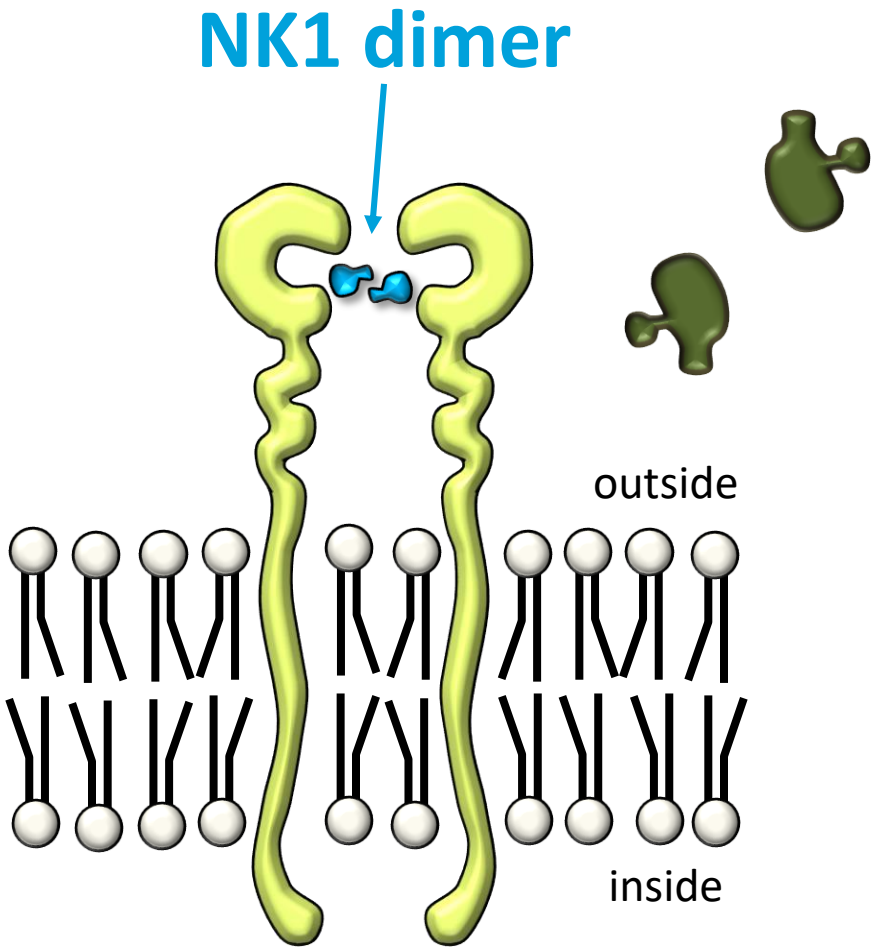
We got “more than we expected”



NK1 dimer

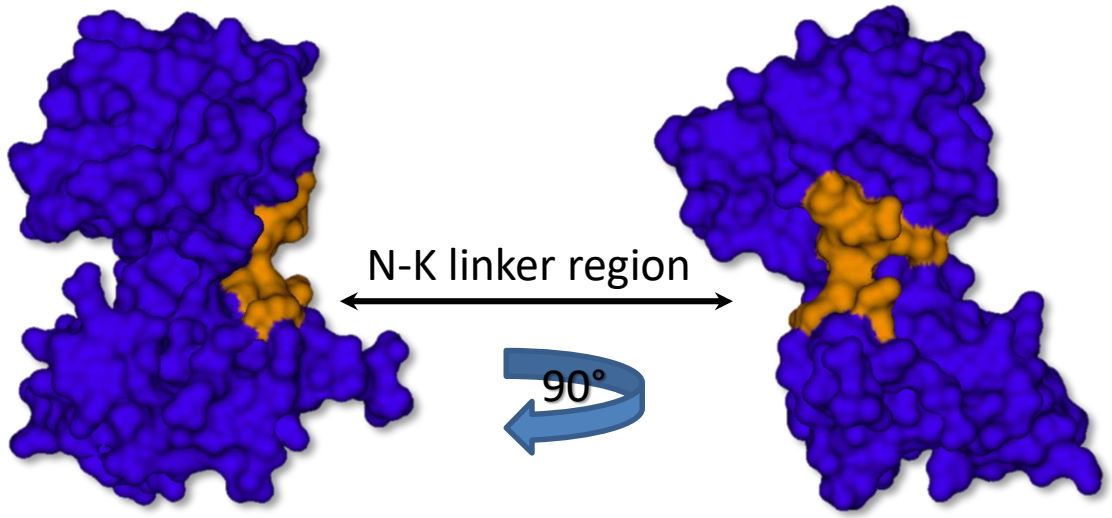


The NK1 dimer; biologically relevant?

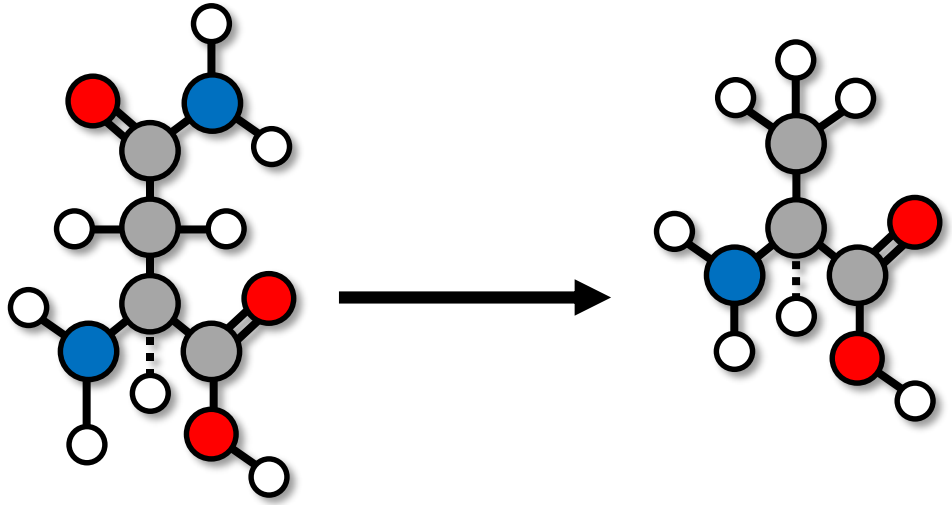


Inducing a MET receptor dimer?

Can interface mutation induce antagonism?

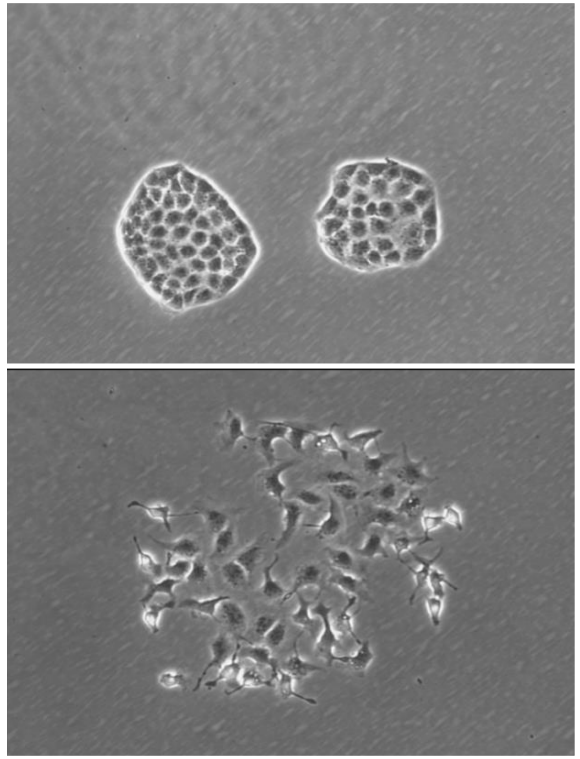


NK1 interface mutants
Y124A
N127A
Y124A, N127A
N127A, V140A
Y124A, N127A, V140A

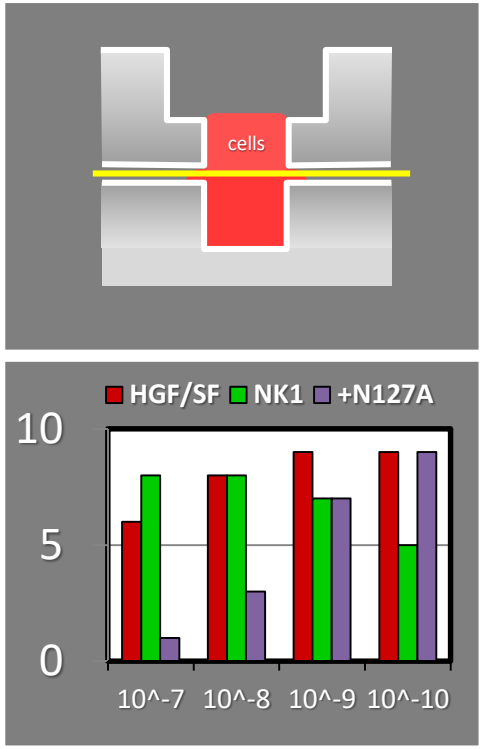


functional assays and “tools”

MDCK scatter assay

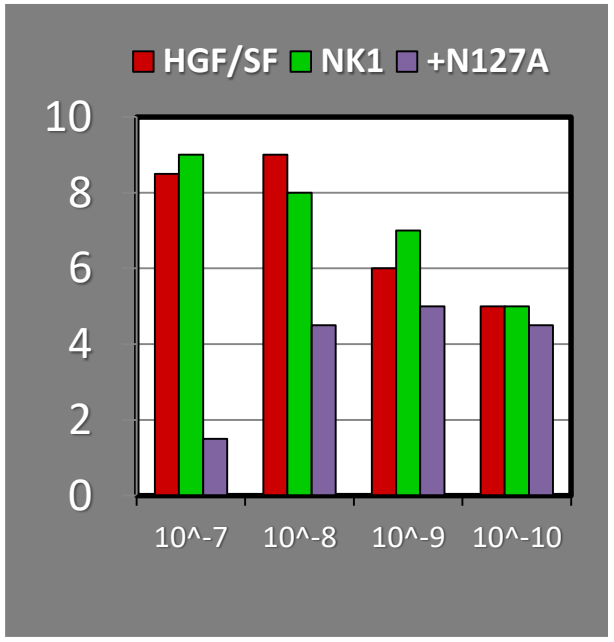


migration assay



DNA synthesis assay

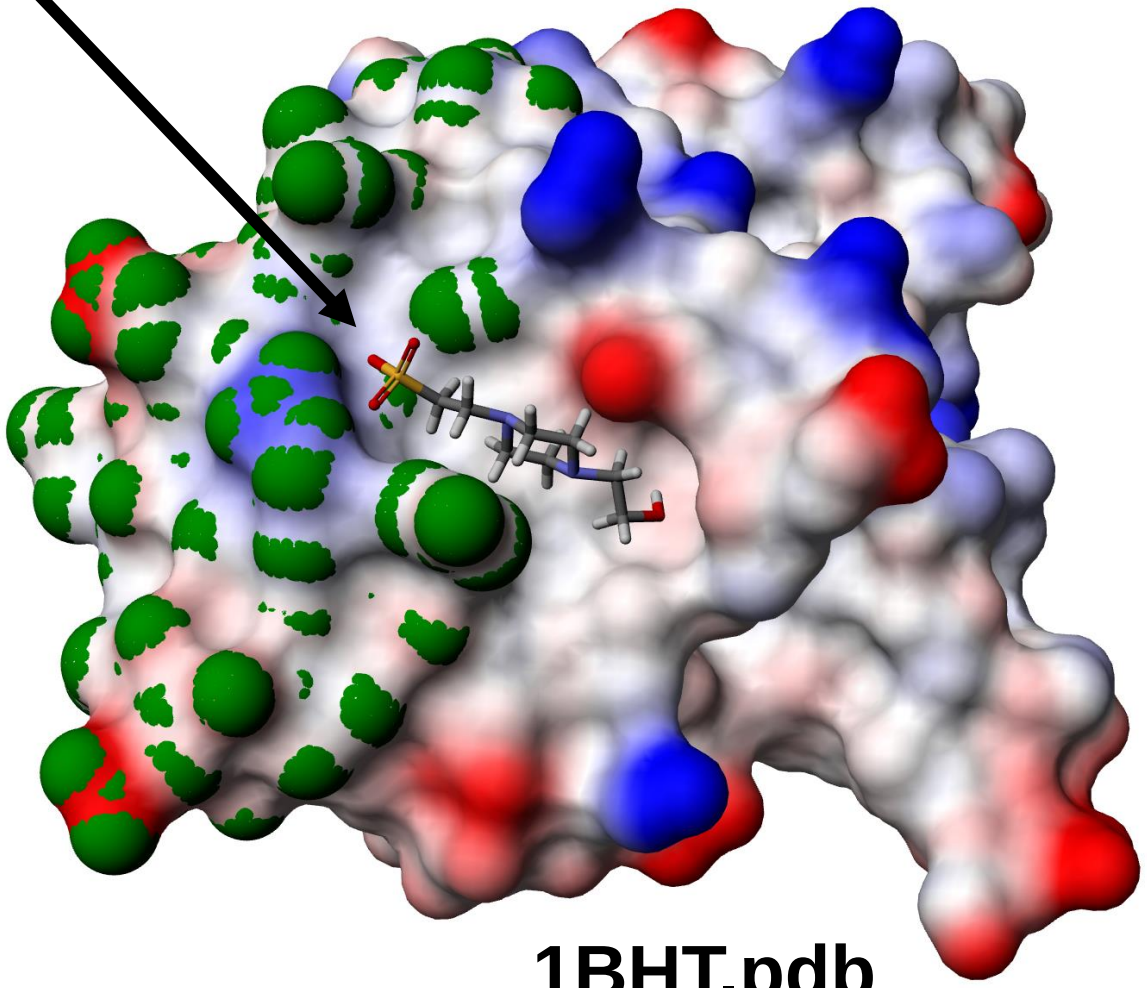
5-bromo-2’-deoxyuridine (BrdU) incorporation



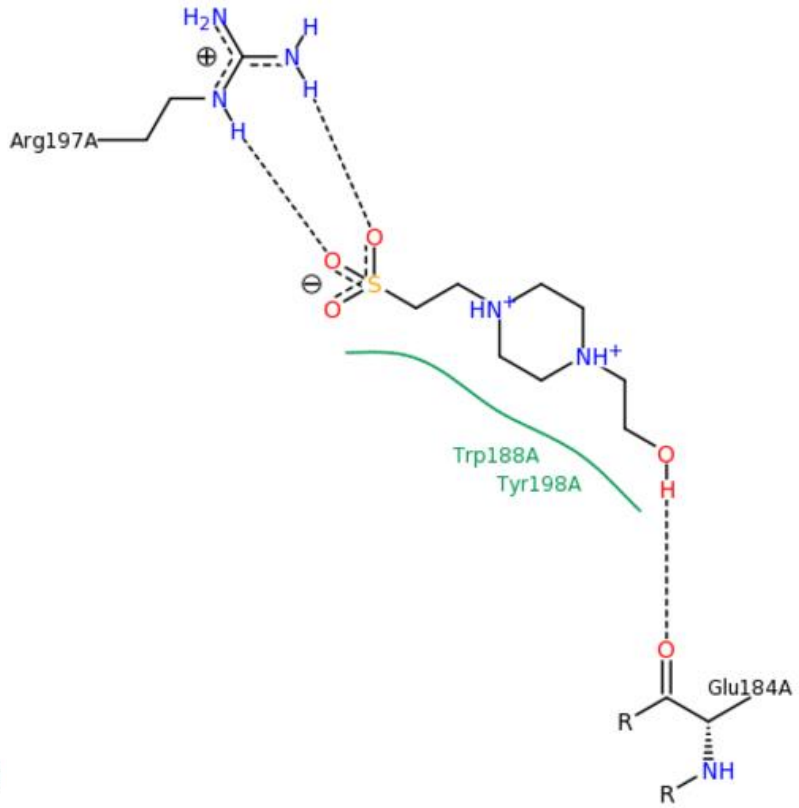
Also small buffer molecules found bound in structure

HEPES (4-(2-HYDROXYETHYL)-1-PIPERAZINE ETHANESULFONIC ACID)

receptor binding site



1BHT.pdb



Surface Plasmon Resonance selection of compounds

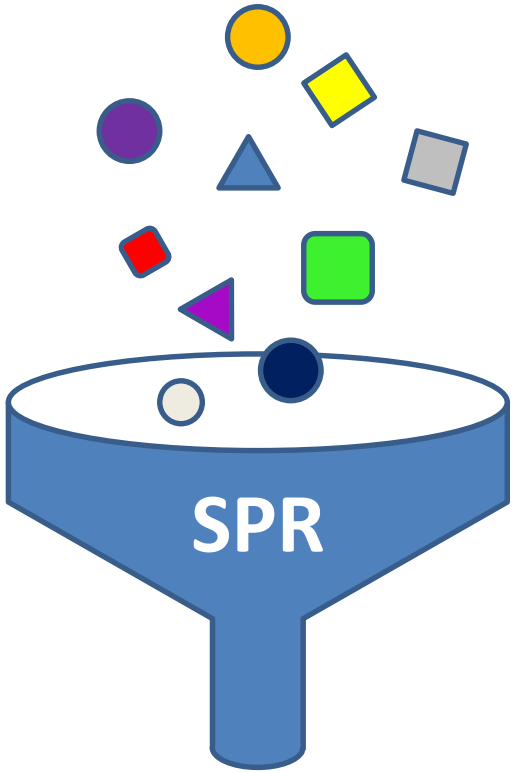
MAYBRIDGE

chemical compounds library

Biacore T200 SPR

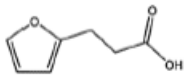
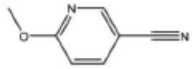
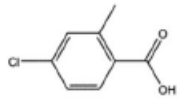
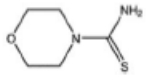
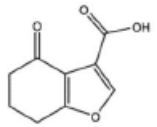
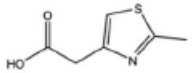
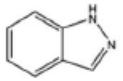
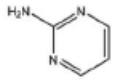
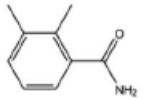
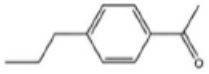
measures affinity between two molecules

1338 compounds



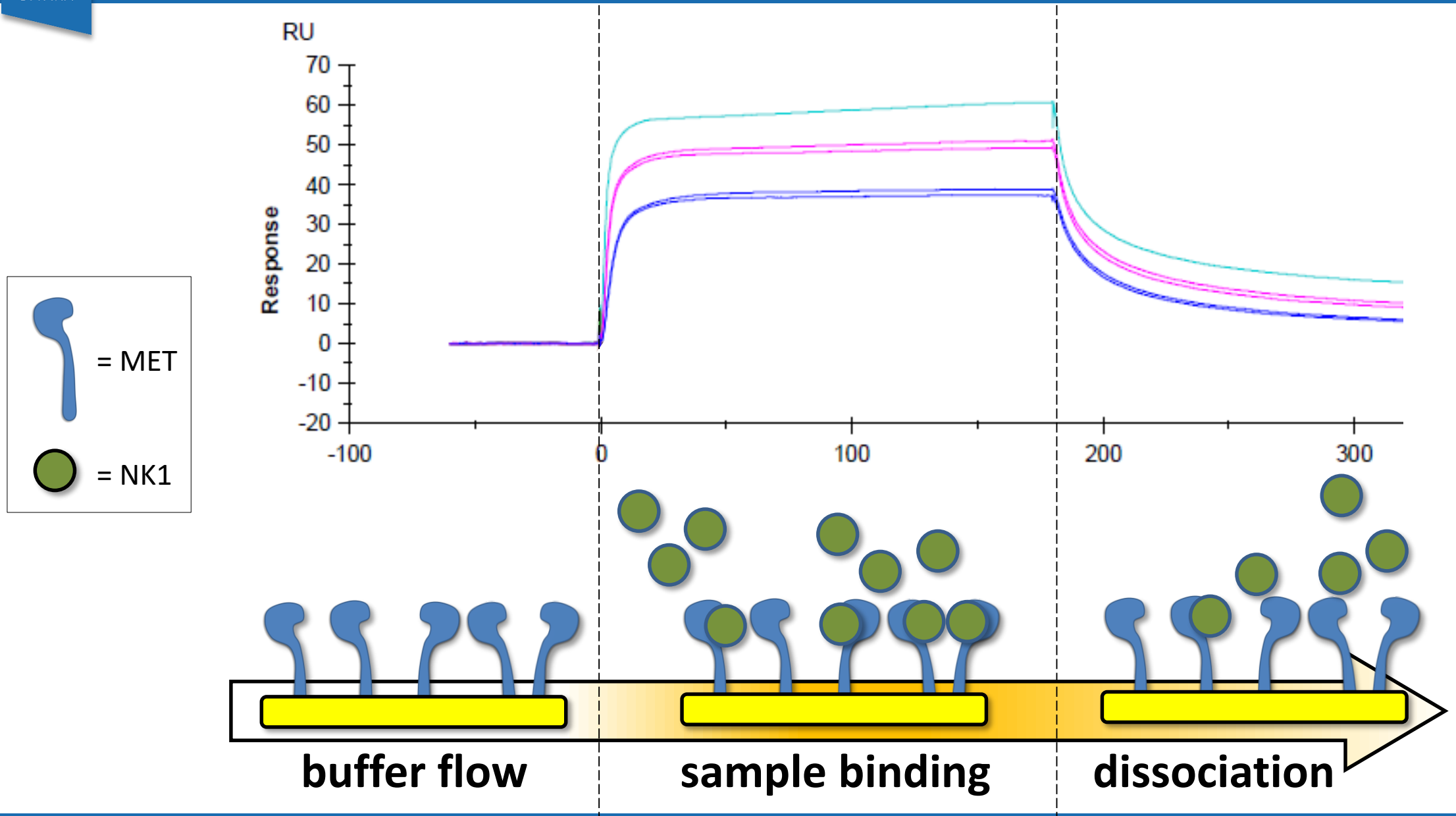
24 potential compounds

affinity of each compound for NK1

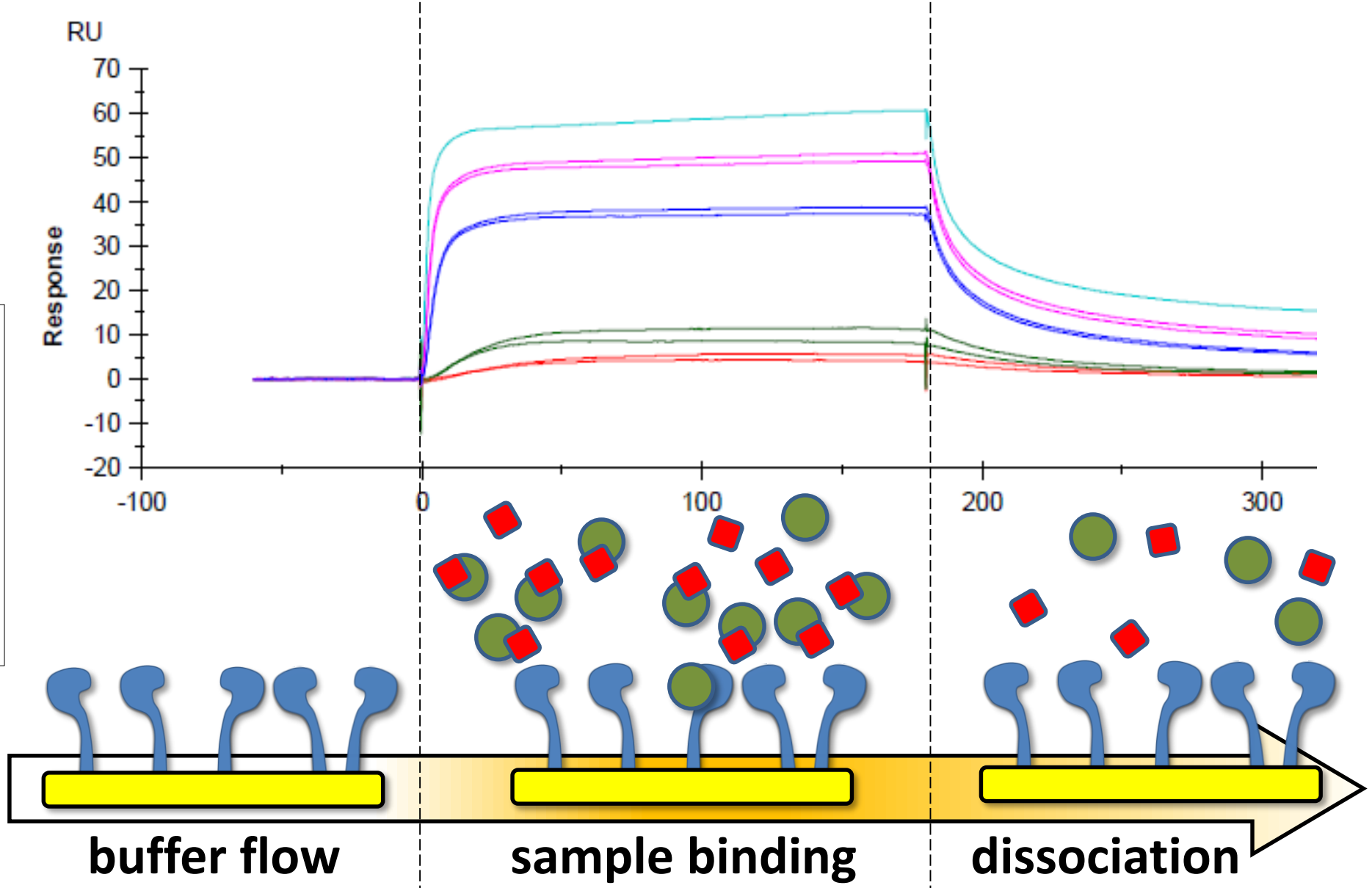
Compound name	Structure	Molecular weight [Da]	SPR response[a] [%]	K_D[b] [mM]
MB605		140.1	215	0.31 ± 0.04
MB1261		134.1	95	0.35 ± 0.04
MB1318		170.6	61	0.36 ± 0.04
MB1082		146.2	53	0.37 ± 0.04
MB417		180.2	87	0.42 ± 0.04
MB895		157.2	53	0.43 ± 0.05
AT0381		118.1	53	0.74 ± 0.06
CA023		95.1	52	0.77 ± 0.06
MB1284		149.2	90	0.86 ± 0.07
MB1315		162.2	52	0.94 ± 0.09

[a] Normalised relative value against NK1. [b] Steady-state binding constant from SPR.

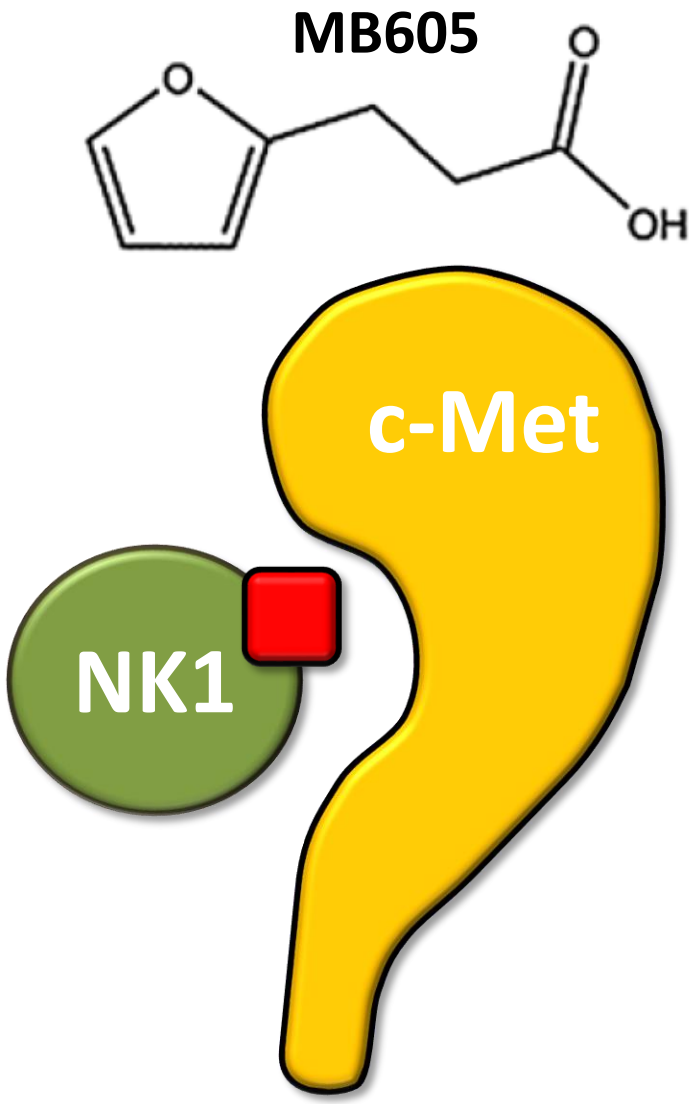
The effect of each compound on NK1-receptor binding



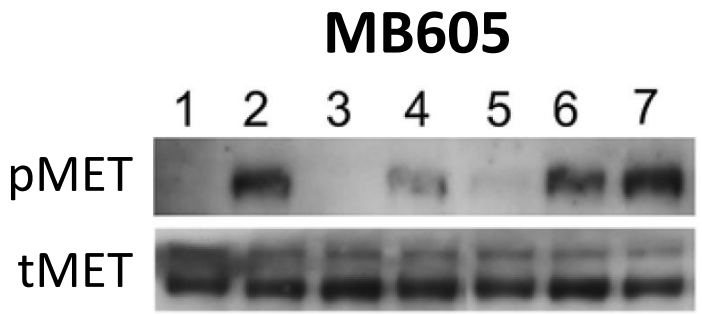
inhibition of NK1-receptor binding



Biological effects of MB605



1. no stimulus
2. 1 nM NK1
3. 0.1% DMSO
4. 100 mM HEPES + 1 nM NK1
5. 10 mM HEPES + 1 nM NK1
6. 1 mM HEPES + 1 nM NK1
7. 100 μM HEPES + 1 nM NK1



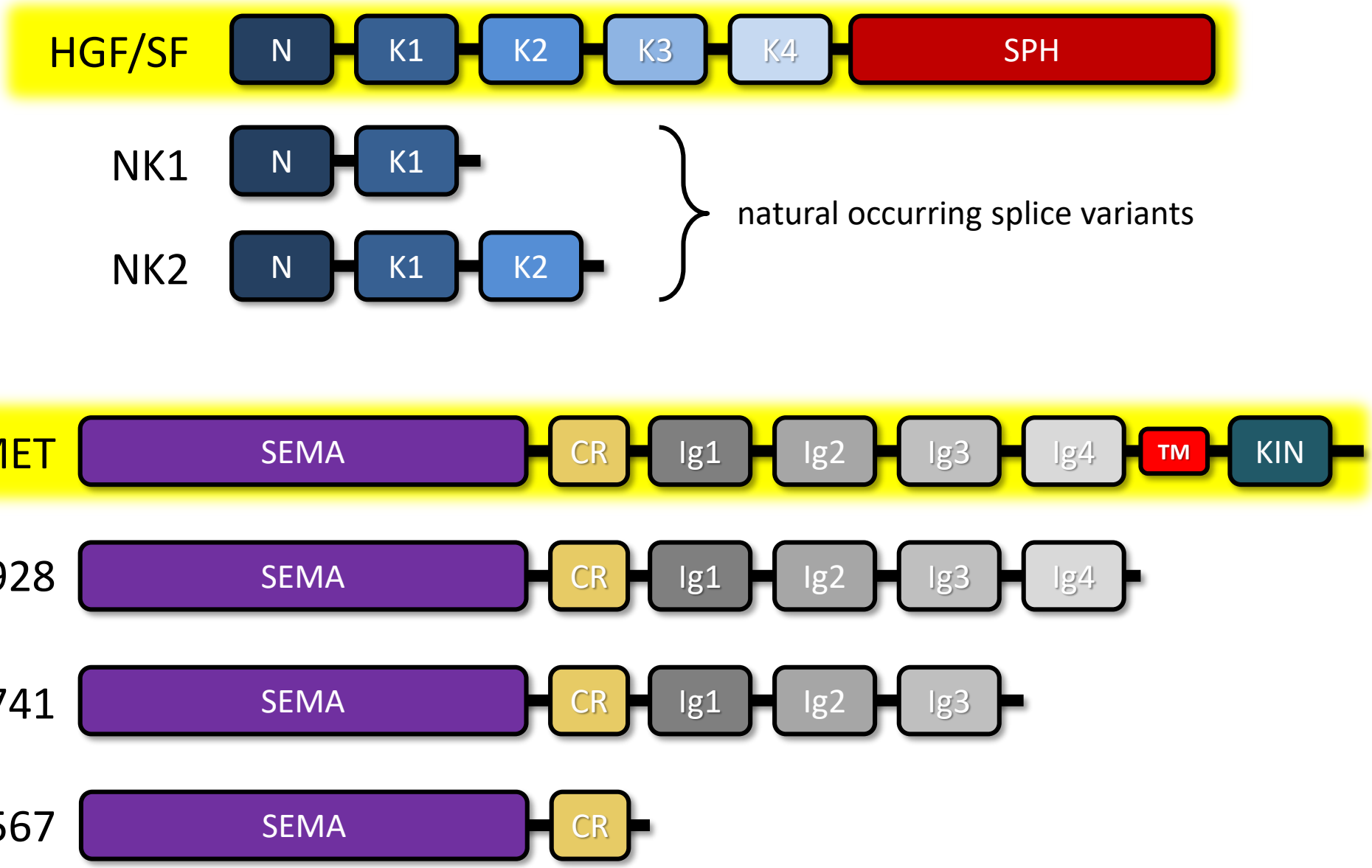
1. no stimulus
2. 1 nM NK1
3. 0.1% DMSO
4. 100 mM MB605 + 1 nM NK1
5. 10 mM MB605 + 1 nM NK1
6. 1 mM MB605 + 1 nM NK1
7. 100 μM MB605 + 1 nM NK1

Sigurdardottir *et al.*, Chem. Sci., 2015, 6, 6147

The value of being able to **“see”** a protein structure

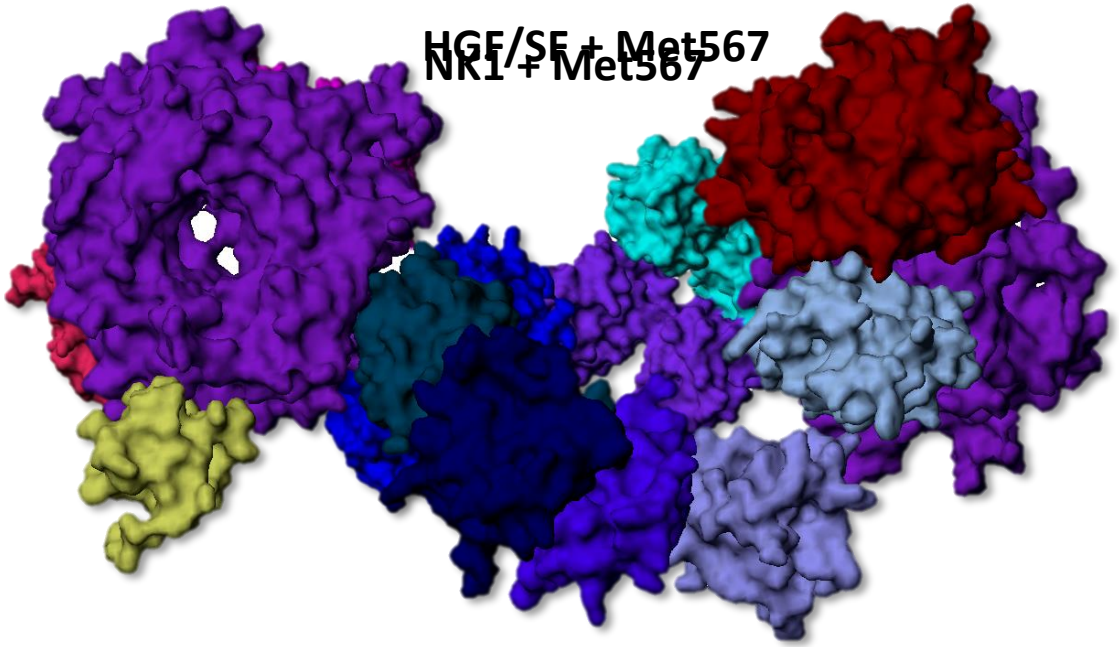
How protein structures at atomic resolution allow the development of biologically active molecules with potential clinical application.

Larger crystal structures and complexes

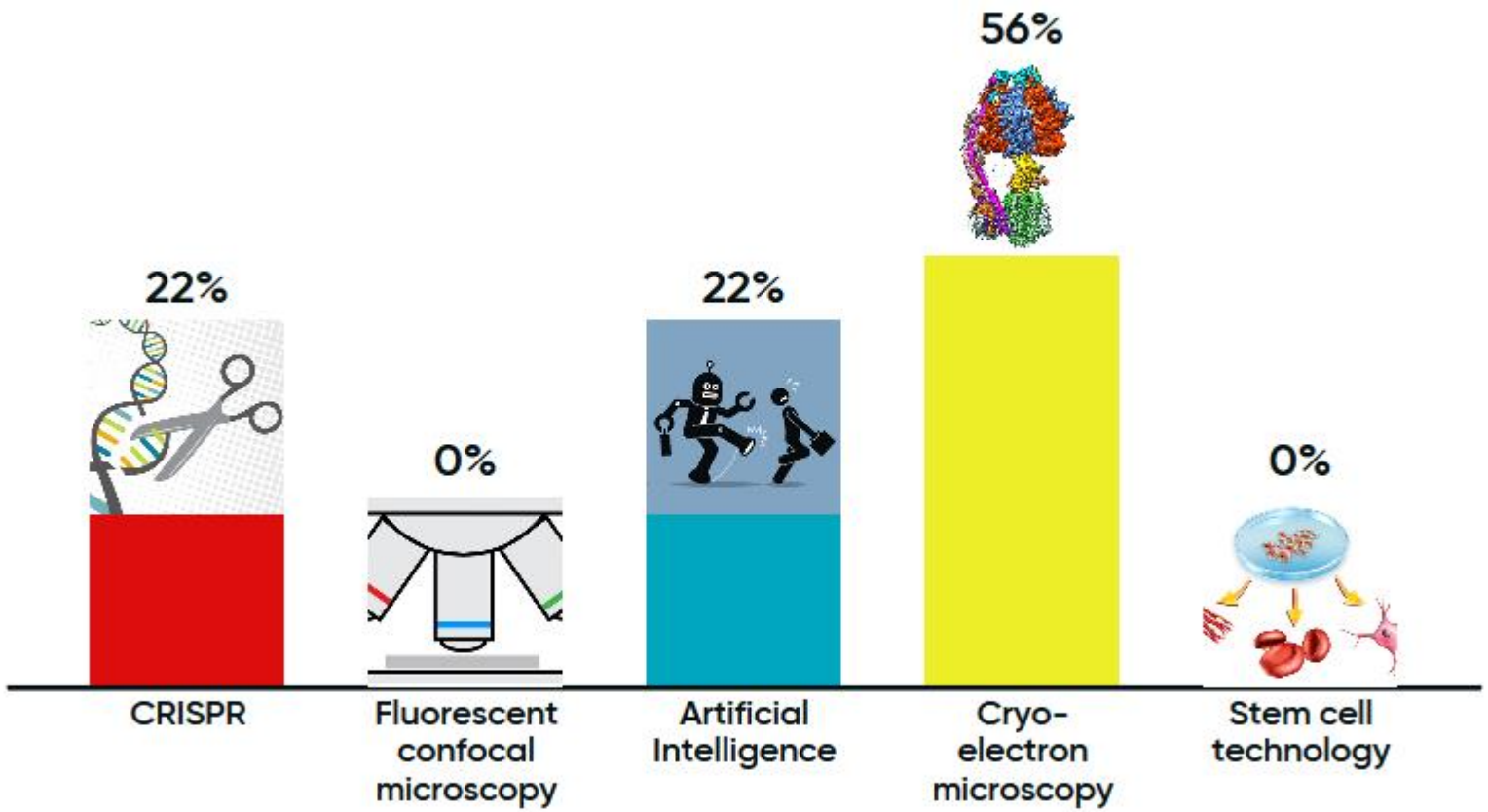


Larger crystal structures and complexes

- difficult to produce (milligrams needed!)
- difficult to keep (unstable)
- difficult to crystalise



But the future has arrived!



Slide is not active Activate

9

cryo-electron microscopy allows you to **SEE a protein**

Review

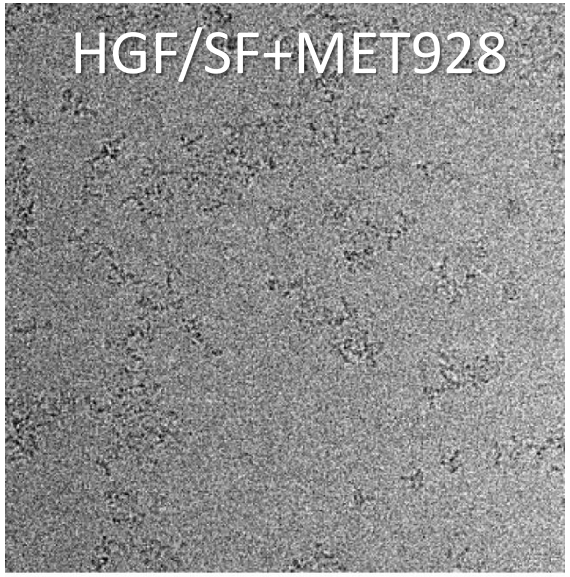
CellPress

How cryo-EM is revolutionizing structural biology

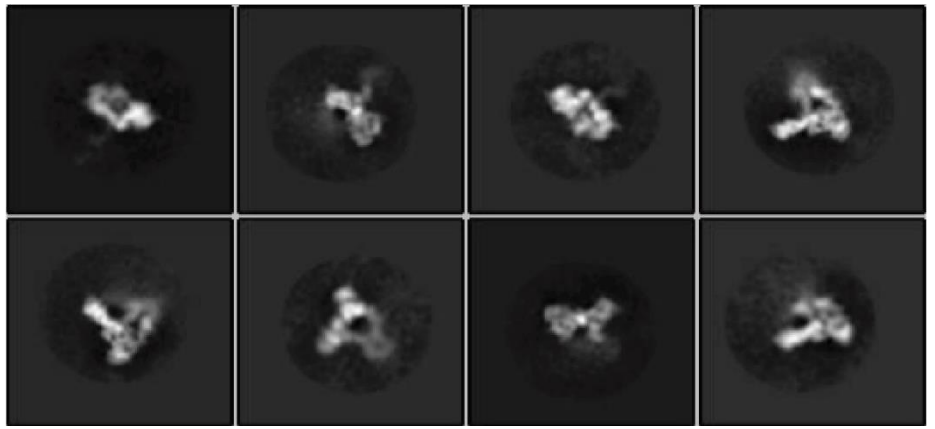
Xiao-chen Bai, Greg McMullan, and Sjors H.W Scheres

MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge Biomedical Campus, Cambridge, CB2 0QH, UK

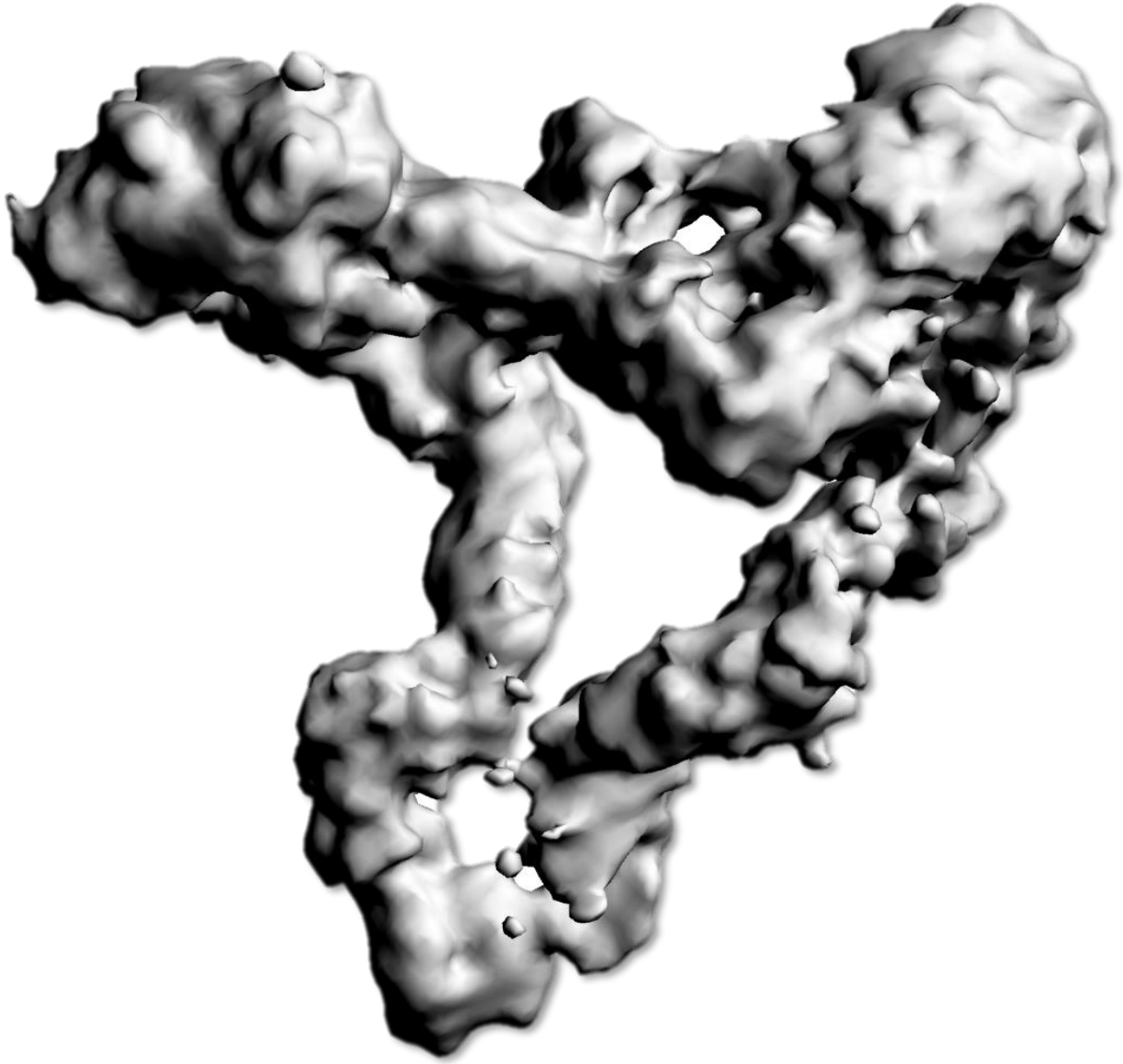
Trends Biochem Sci. 2015 Jan;40(1):49-57



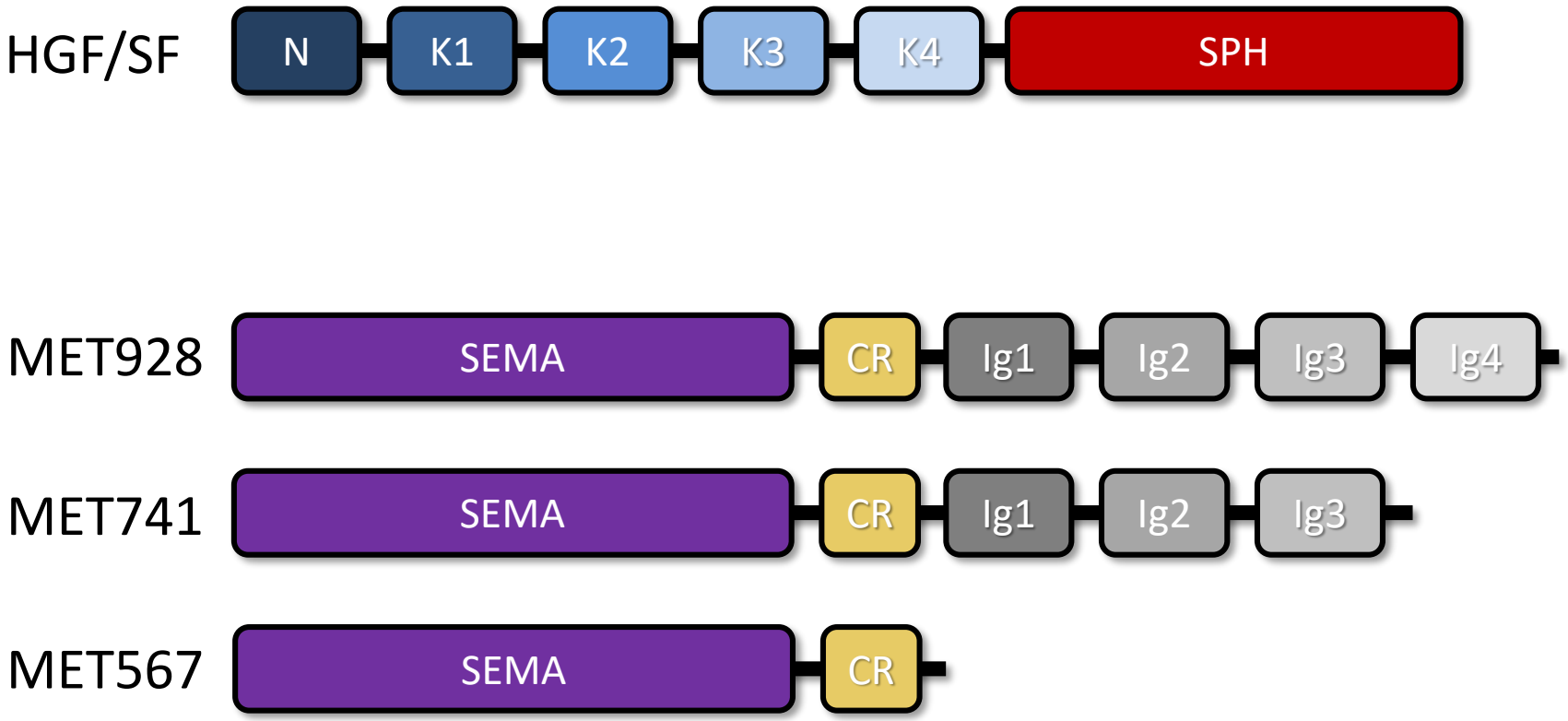
individual particles



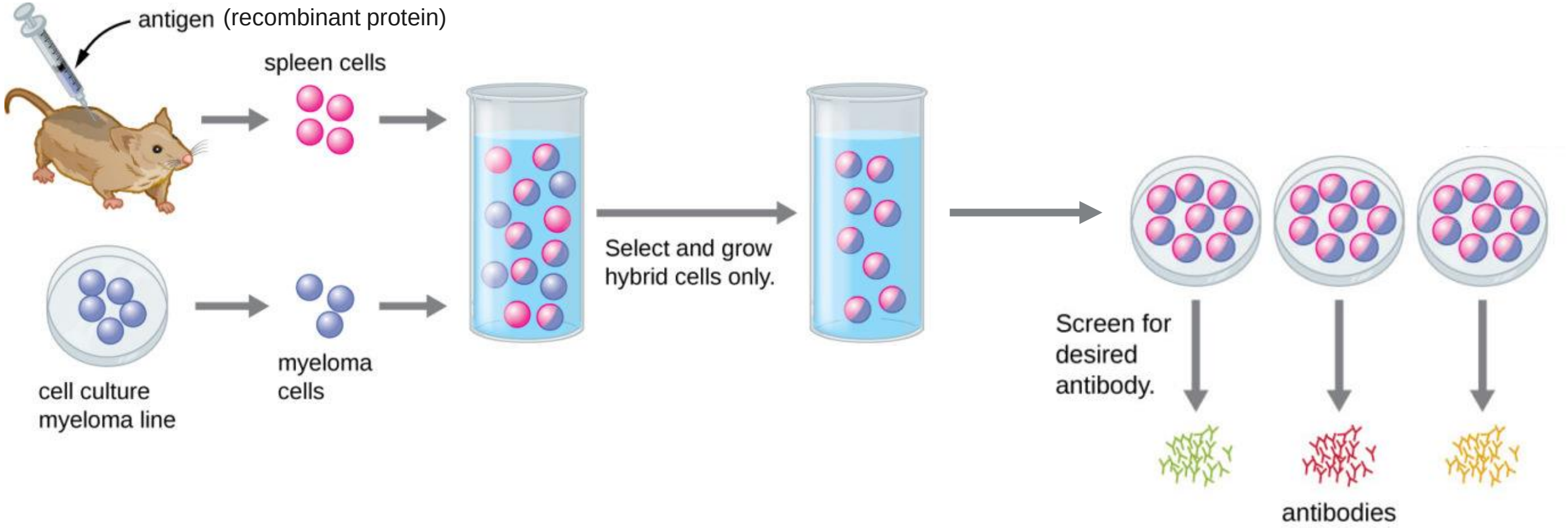
HGF/SF in complex with Met928



A second type of **SEEING a protein: in the patient**



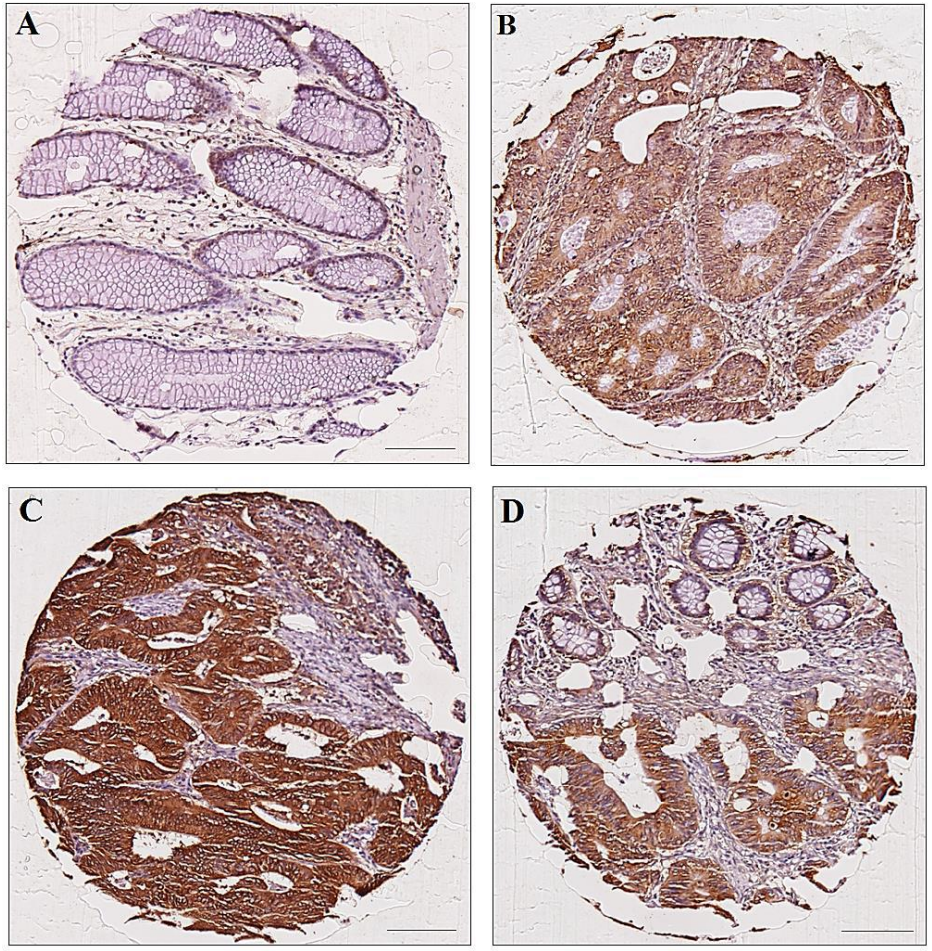
Recombinant protein as antigens for antibody production



LEIDEN UNIVERSITY MEDICAL CENTER

Cornelis Sier

Seeing MET in tumour biopsies using antibodies



A. Negative to weak staining in **normal** colorectal tissue

B. Medium positive staining in **colorectal cancer** tissue

C. Strongly positive staining in **colorectal cancer** tissue

D. TMA core showing transition of **tumour** (lower part) to **normal** (upper part) tissue.

Natasja de Vries, Bachelor Research Project, Department of Surgery, LUMC

MET as prognostic and diagnostic factor

Gastric Cancer (2016) 19:183–191
DOI 10.1007/s10120-015-0471-6

ORIGINAL ARTICLE

Prognostic impact of HER2, EGFR, and c-MET status on overall survival of advanced gastric cancer patients

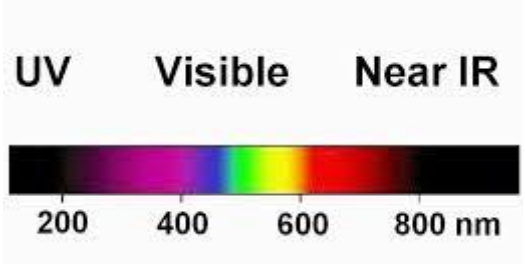
Nozomu Fuse · Yasutoshi Kuboki · Takeshi Kuwata · Tomohiro Nishina · Shigenori Kadowaki · Eiji Shinozaki · Nozomu Machida · Satoshi Yuki · Akira Ooki · Shinya Kajiura · Tetsuo Kimura · Takeharu Yamanaka · Kohei Shitara · Akiko Kawano Nagatsuma · Takayuki Yoshino · Atsushi Ochiai · Atsushi Ohtsu

Results Of the 293 patients from nine institutions, 43 (15 %) were HER2 positive, 79 (27 %) were EGFR positive, and 120 (41 %) were c-MET positive. Ten patients (3 %) showed positive co-expression of HER2, EGFR, and c-MET. After a median follow-up time of 58.4 months with 280 deaths, there was no significant difference in overall survival (OS) in terms of HER2 and EGFR status. However, there was a significant difference in OS between c-MET-positive and c-MET-negative patients [median, 11.9 months vs 14.2 months; hazard ratio, 1.31 (95 % Electronic supplementary material The online version of this confidence interval, 1.03–1.67); log-rank P = 0.024].

Seeing the tumour with fluorescently labelled antibodies **during operation**

Near-infrared fluorophores (NIRF)

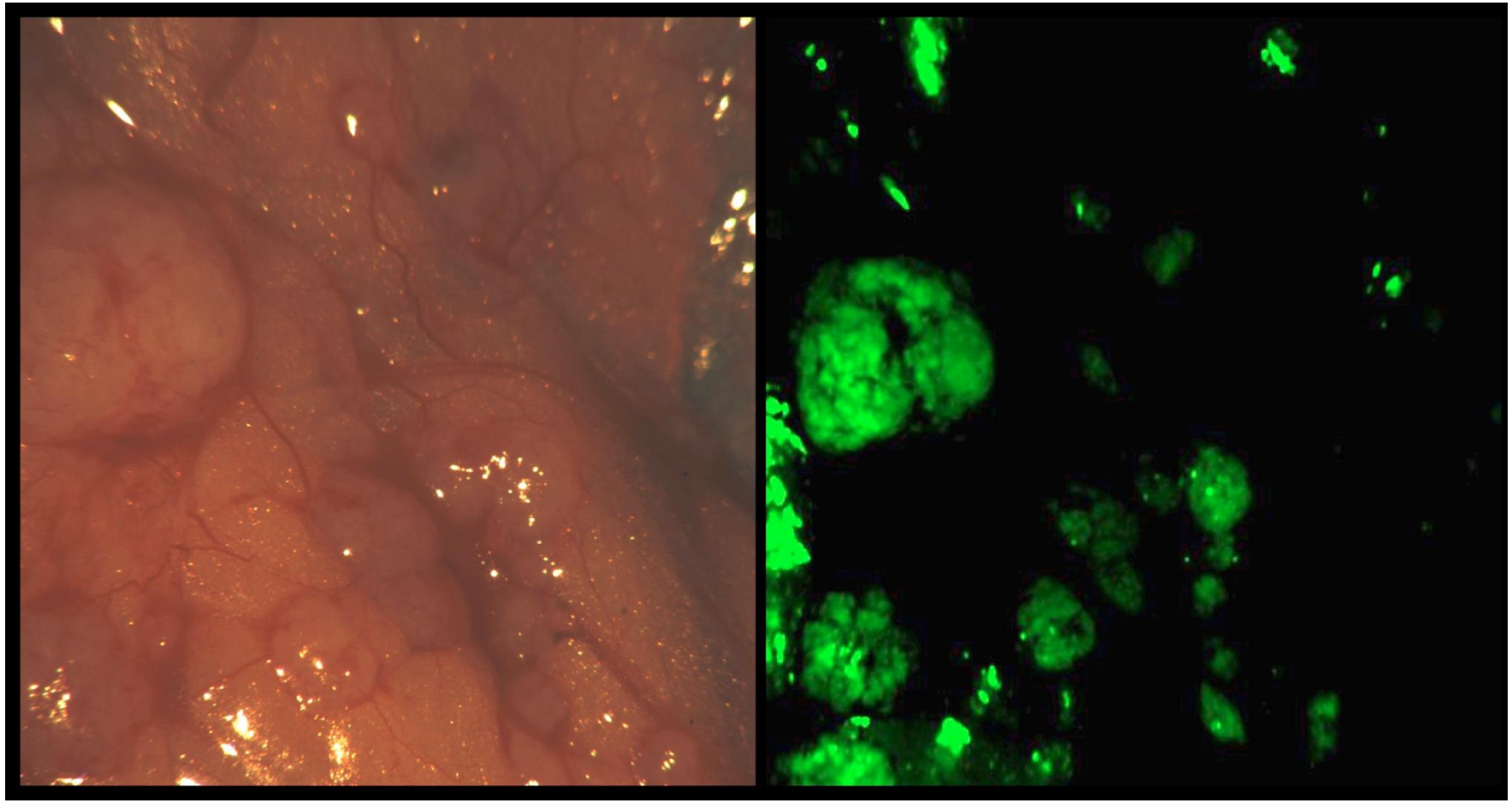
- deeper tissue penetration
- reduced auto fluorescence
- excitation wavelength does not affect surgeon's operation field



View of localised region in peritoneal cavity of an ovarian cancer patient

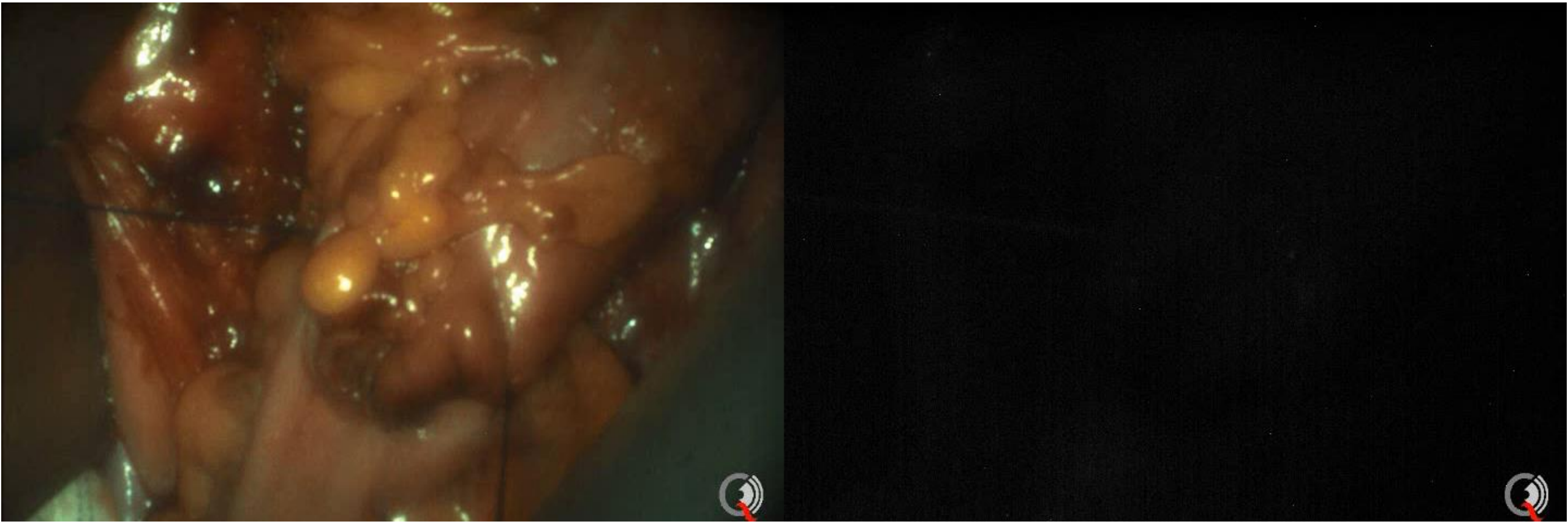
Surgeon's normal view

Fluorescence-enhanced view



Purdue University, 2011

“white stars in a black sky effect”



“Ovarian cancer is notoriously difficult to see, and this technique allowed surgeons to spot a tumour 30 times smaller than the smallest they could detect using standard techniques.“

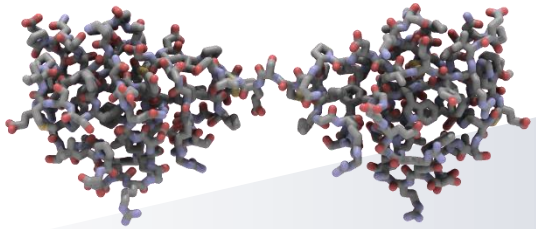
"By dramatically improving the detection of the cancer - by literally lighting it up - cancer removal is dramatically improved."

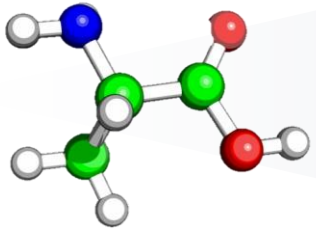
The importance of being able to “see” a protein

- Current drug design is rational and based on available high resolution structures and a molecular understanding
- New techniques now allow us to study larger protein complexes in greater detail
- Detecting the presence or absence of a specific protein in a tumour is essential for diagnosis and prognosis
- The presence or absence of a specific protein in a tumour should guide treatment (i.e. personalised medicine)
- Making specific proteins in or on the tumour visible allows more accurate surgical removal

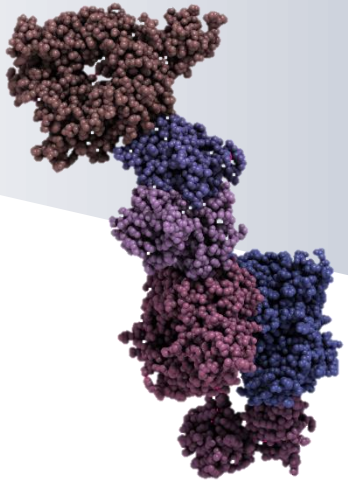
Protein structures in the fight against Cancer

Single proteins
Nanometres (nm)

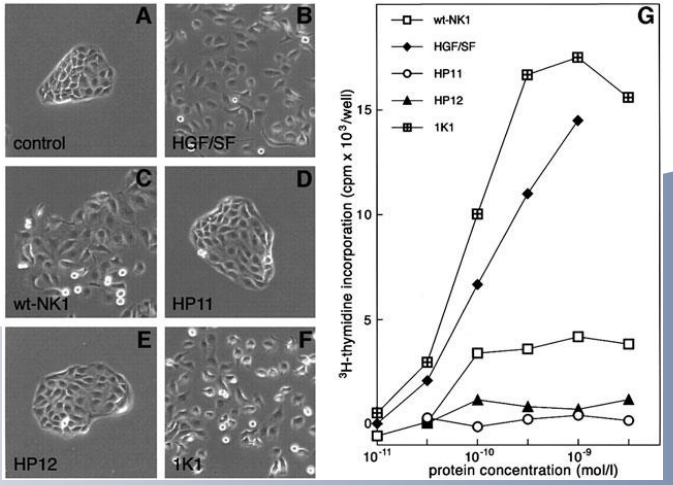




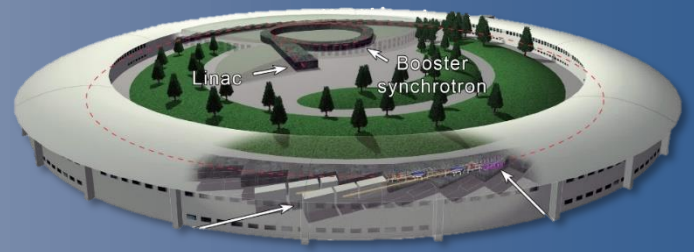
Single atom (positions)
Ångstroms (<nm)



Protein complexes
sub-micrometre (<μm)



Cells in assays
micrometres (μm)



ESRF Grenoble
(0.01 nm – 1 km)

Tumour tissue
millimetres to centimetres (mm - cm)