



## Ontwikkelingen en toepassingen van farmaceutische producten gebaseerd op nanotechnologie

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A high-speed photograph of a water splash, showing numerous droplets in mid-air against a blue background.

**Nanobodies® -  
Inspired by nature**

# Agenda

- Y Nanowetenschap en Nanotechnologie: een kort overzicht
  
- Y Nanotechnologie in de geneeskunde
  - Nanomedicijnen
    - Celdodende kankermedicijnen ingekapseld in liposomen
    - Medicijnen met geïncorporeerde nano-antennes
    - Medicijnen met nanosensoren
    - “slimme” medicijnen: de toekomst?
    - Gecontroleerde toediening van antilichamen (focus op antilichaam therapie)
  
- Y Nanochips voor diagnostische toepassingen

# Nanotechnologie en nanowetenschap: een kort overzicht

- ✚ Samenhang van moleculen en atomen op nanometer schaal bepaalt materiaaleigenschappen waaronder
  - elektrische eigenschappen
  - optische eigenschappen
  - mechanische eigenschappen
- ✚ De natuur is “meester” in het coördineren van samenhang
  - Vervaardigt gesofisticeerde moleculaire systemen die het leven op aarde mogelijk maken.
- ✚ Nanowetenschappen en Nanotechnologie: doel
  - menselijke controle over de processen op nanoschaal
- ✚ Situering van deze wetenschappen
  - interface tussen fysica, chemie, materiaalkunde, micro-electronica, bio-chemie en biotechnologie

# Nanowetenschappen en nanotechnologie

## Y Studie, manipulatie en engineeren van materie, deeltjes of structuren op nanometerschaal

- elektrische, optische, thermische of mechanische eigenschappen zijn bepaald door de manier waarop moleculen en atomen op een nanometerschaal zich vormen tot grotere structuren.
- hebben vaak andere eigenschappen op nanometerschaal dan op macroschaal

## Y Nanotechnologie

- toepassing van nanowetenschap voor nanomaterialen of nanogroote componenten in nuttige producten.
- mogelijkheid tot generatie van custom-made materialen en producten
  - met nieuwe en verbeterde eigenschappen,
  - nieuwe nanoelectronische componenten
  - nieuwe soorten van "slimme" geneesmiddelen en sensoren
  - connecties tussen electronica en biologische systemen.

# Nanogeneeskunde

## Y Verbeterde gezondheidszorg dmv

- vroegere opsporing van ziekten
- nauwkeuriger behandeling
- nieuwe behandelmethoden
- nano biomaterialen
  - nanokorreltjes hydroxy-apatiet (is in natuurlijke vorm bestanddeel van ons bot) als coating op kunstgewricht
  - matrix van nanokorreltjes om wondes te laten genezen

## Y Miniaturisatie van medische apparatuur en en integratie met ICT

- continue “monitoring”
- diagnose en behandeling buiten het ziekenhuis

## Y Nanomedicijnen

- toegespitst op handjevol aandoeningen
  - doel: selectieve aflevering op de “zieke” plek

# Celdodende kankermedicijnen ingekapseld in liposomen/ nanopartikels

## Abbraxane: albumin-paclitaxel nanoparticles

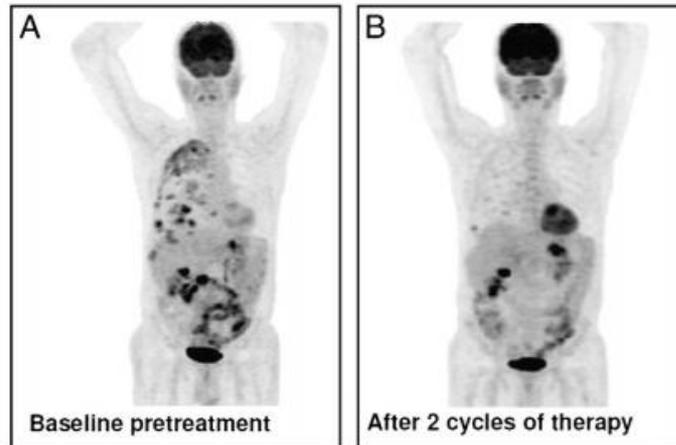
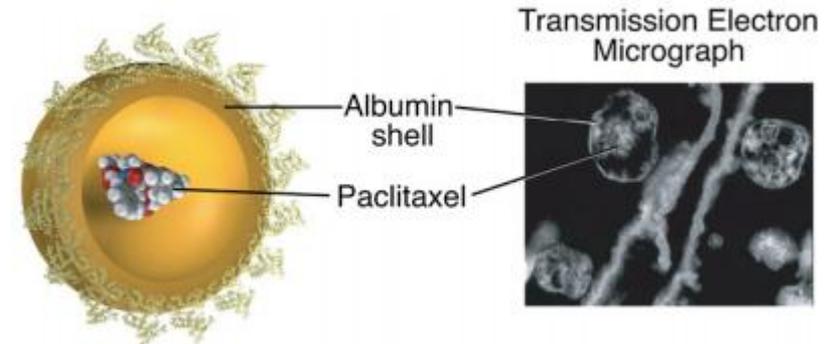
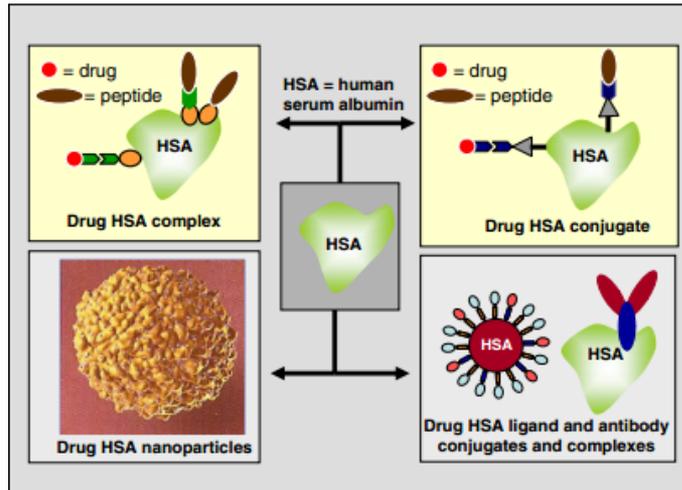
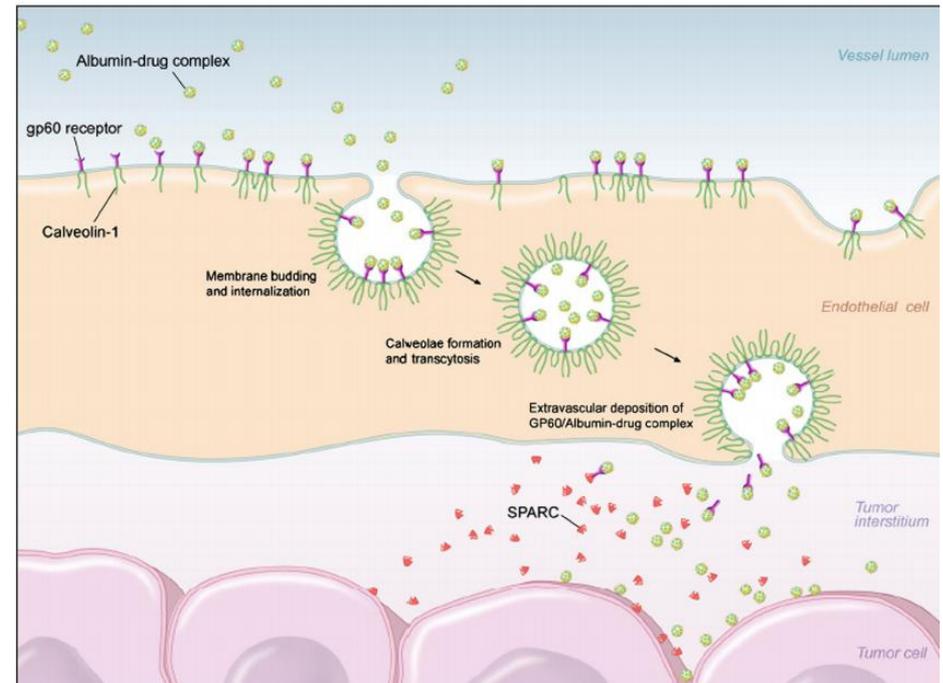


Fig. 9. After 2 cycles of the combination of Abraxane® of 125 mg/m<sup>2</sup> paclitaxel equivalents and gemcitabine at 1000 mg/m<sup>2</sup> in a patient with metastatic cancer, a significant reduction in lung and gastrointestinal metastases was observed by PET scans.

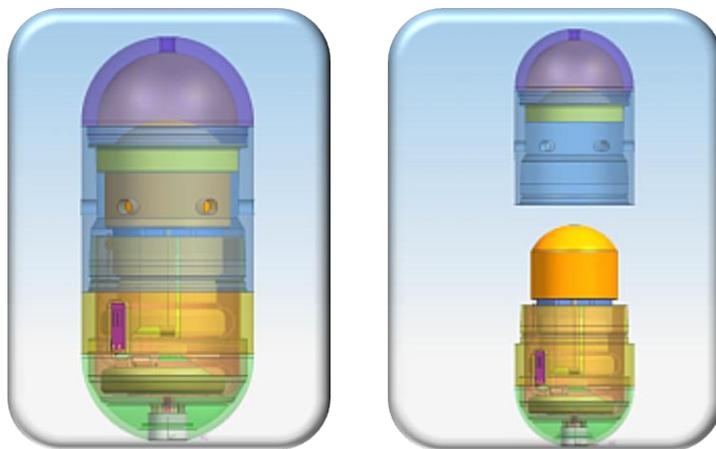


# Medicijnen met geïncorporeerde nano-antennes

- ✚ Taxol, gekoppeld aan foliumzuur en verpakt in een capsule waarin (nano-)moleculen in verwerkt zijn
  - kankercellen verbruiken foliumzuur en ze nemen dit vitamine in grote getale op
  - de ingebouwde nanomoleculen werken als antennes die de tumorcel herkennen
  
- ✚ Een variant: het paard van Troje
  - Nanopartikels met magnetische bolletjes zilveroxide en tumorspecifieke moleculen aan de buitenkant
  - Na inspuiting van het medicijn brengt men de patiënt in een magnetisch veld. Hierdoor warmen de magnetische bolletjes op en verhitten de tumorcellen zodanig dat deze afsterven

## Medicijnen met nanosensoren

- Een te slikken capsule (IntelliCap) waarmee een medicijn tegen de ziekte van Crohn gecontroleerd vrijgelaten kan worden binnen in de darm
  - in de capsule zijn “nano” detectoren ingebouwd die meerdere parameters kunnen meten, zoals temperatuur en zuurgraad
  - wanneer de capsule op de plek met de juiste parameters is aangekomen, regelt een ander nano-onderdeel dat het medicijn uit de capsule vrijkomt
  - prototype van een electronische pil



# “Slimme” medicijnen: de toekomst?

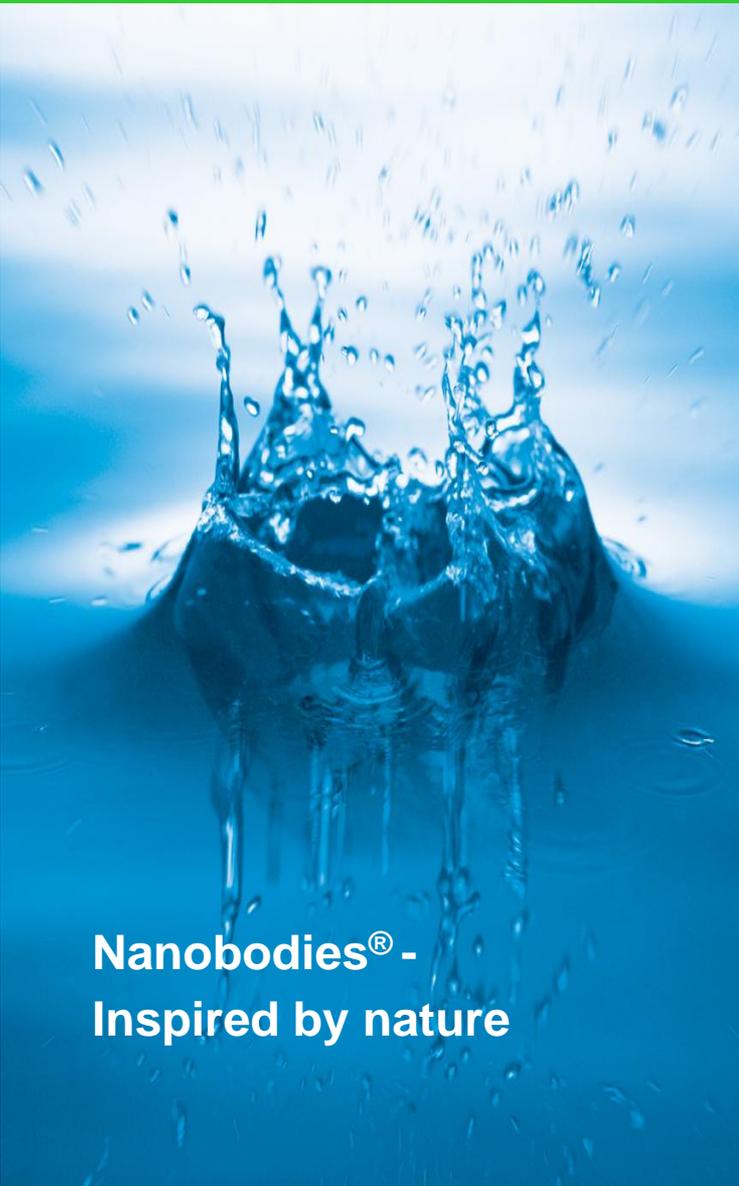
## Y Programmeerbare medicijnen

- kunnen alle functies verrichten zoals eerder vermeld
- additioneel is een computerchip ingebouwd waarmee de afgifte van het medicijn kan worden geregeld.
  - bij een bepaalde zuurgraad, temperatuur of anderszins wordt een bepaalde hoeveelheid medicijn uit de verpakking losgelaten
  - nauwkeuriger en persoonlijk afgestemde dosering van geneesmiddelen
  - medicijnen die van binnen uit computergestuurd de dosering regelen
  - met een ingebouwde computerchip is het ook technisch mogelijk om de gemeten waarden om te zetten in binaire data
  - via een draadloze verbinding kunnen gegevens naar de computer in het ziekenhuislaboratorium verstuurd worden



## Gecontroleerde toediening van medicijnen

Nanobodies<sup>®</sup> als een voorbeeld

A high-speed photograph of a water splash, showing numerous droplets and a central crown-like structure, set against a blue background.

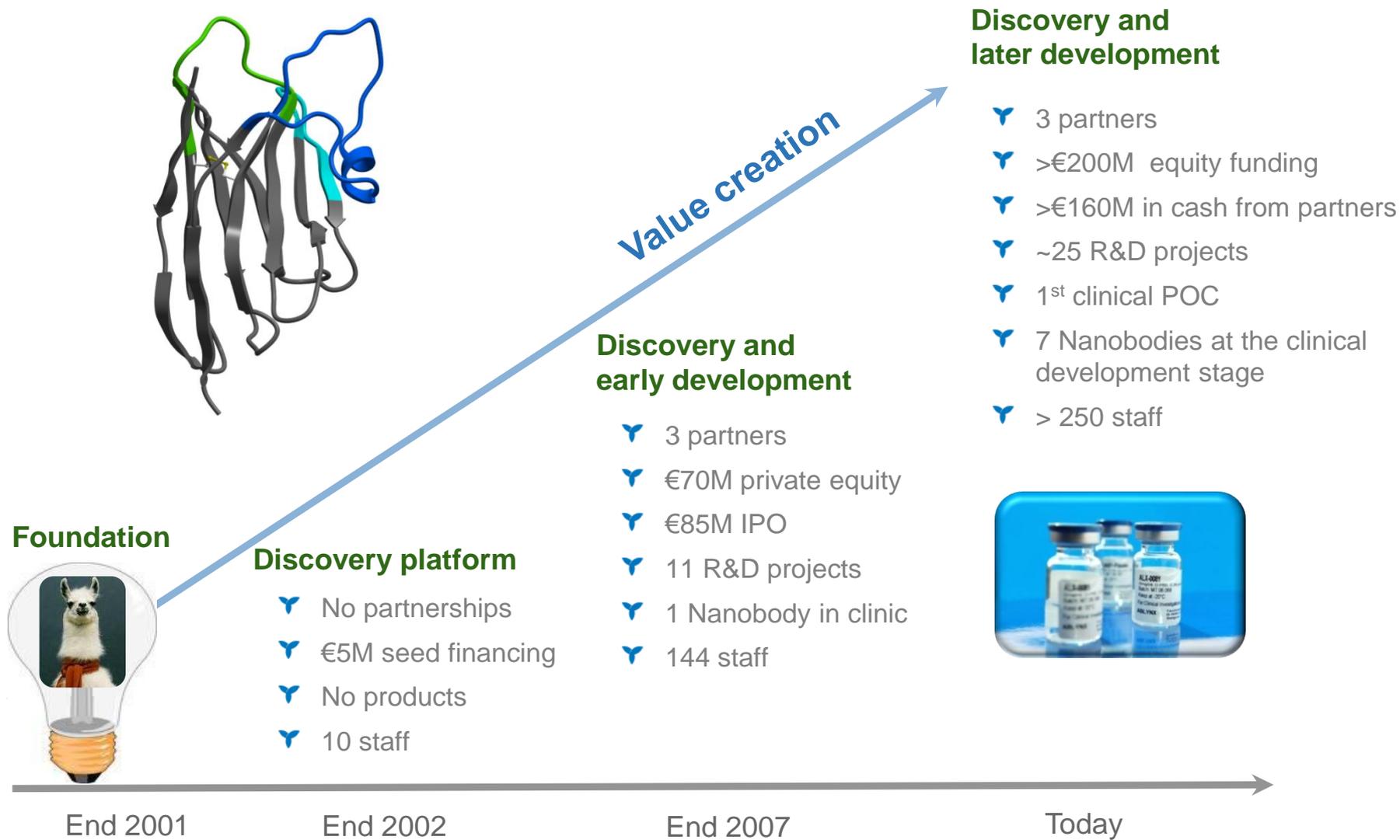
**Nanobodies<sup>®</sup> -  
Inspired by nature**

## Ablynx – een overzicht

- ✔ Drug discovery and development company based in Ghent, Belgium
- ✔ A pioneer in next generation biologics - Nanobodies®
- ✔ In total >25 Nanobody programmes – 7 in the clinical development phase
- ✔ First clinical proof-of-concept for a Nanobody achieved in May 2011
- ✔ >700 healthy volunteers and patients have now received Nanobodies
- ✔ Exclusive rights to >550 patent applications and granted patents
- ✔ 18 partnered programmes with Boehringer Ingelheim, Merck Serono and Novartis

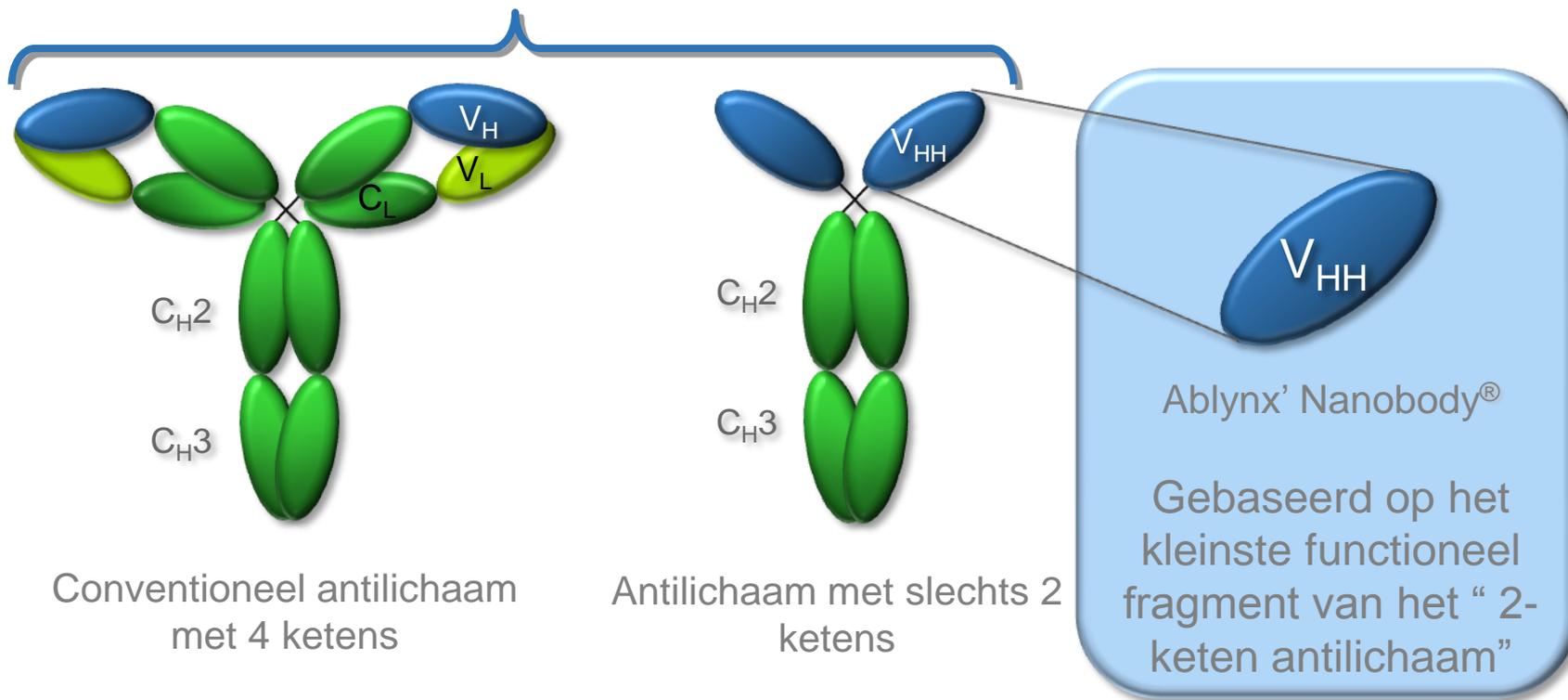


# Rapid evolution from platform to product based company



# Het Ablynx' Nanobody

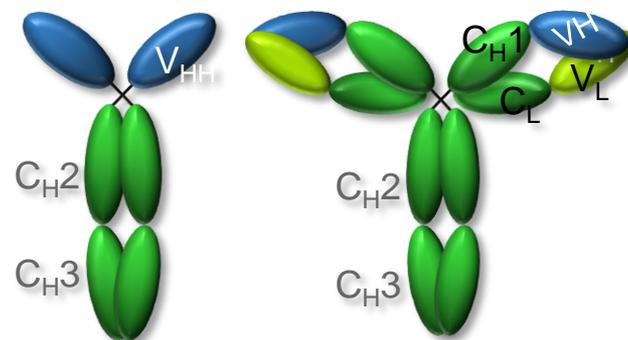
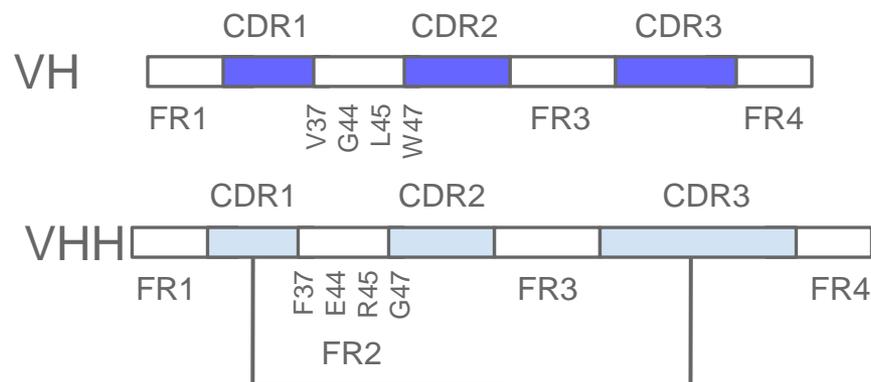
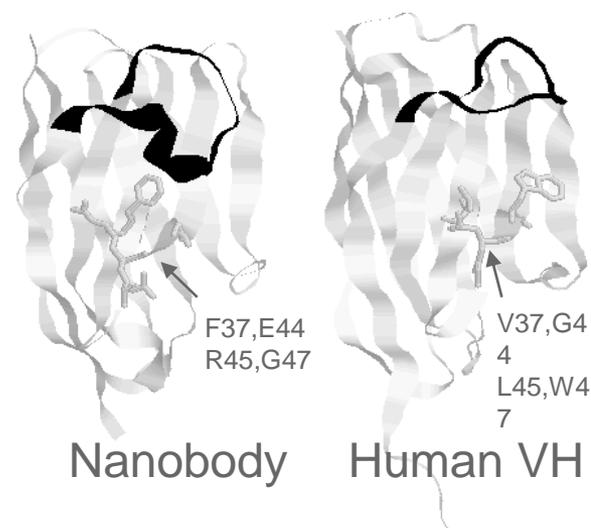
Kameel-achtigen hebben 2 vormen antilichamen



# Structural features demonstrate multiple paths to high solubility & stability

## FR2 based 'classical' hallmarks

- Phe/Tyr37, Glu/Gln44, Arg/Cys45, Gly/Ser47
- Induce local conformational changes that increases hydrophilicity of the VL interface
- Mutation to VH-type residues reduces thermodynamic stability & solubility, effects CDR-conformation, and may reinstate VL pairing
- Used for camelization of VH; only partially successful (remaining dimerization & aggregation)



# Nanobodies versus antibodies and small molecules

## Classical medicinal chemistry



- ✔ Chemical compounds
- ✔ Very small



- ✔ Complexity: low



- ✔ Stable
- ✔ Pills - tablets
- ✔ Side effects/toxicity

## Nanobodies



- ✔ Combines the best of both
  - ✔ Small

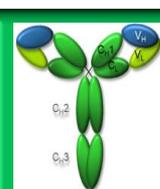


- ✔ Complexity: low



- ✔ Stable
- ✔ Beyond the needle
- ✔ Low tox, highly effective

## Monoclonal antibodies



- ✔ Biological (recombinant)
- ✔ Big



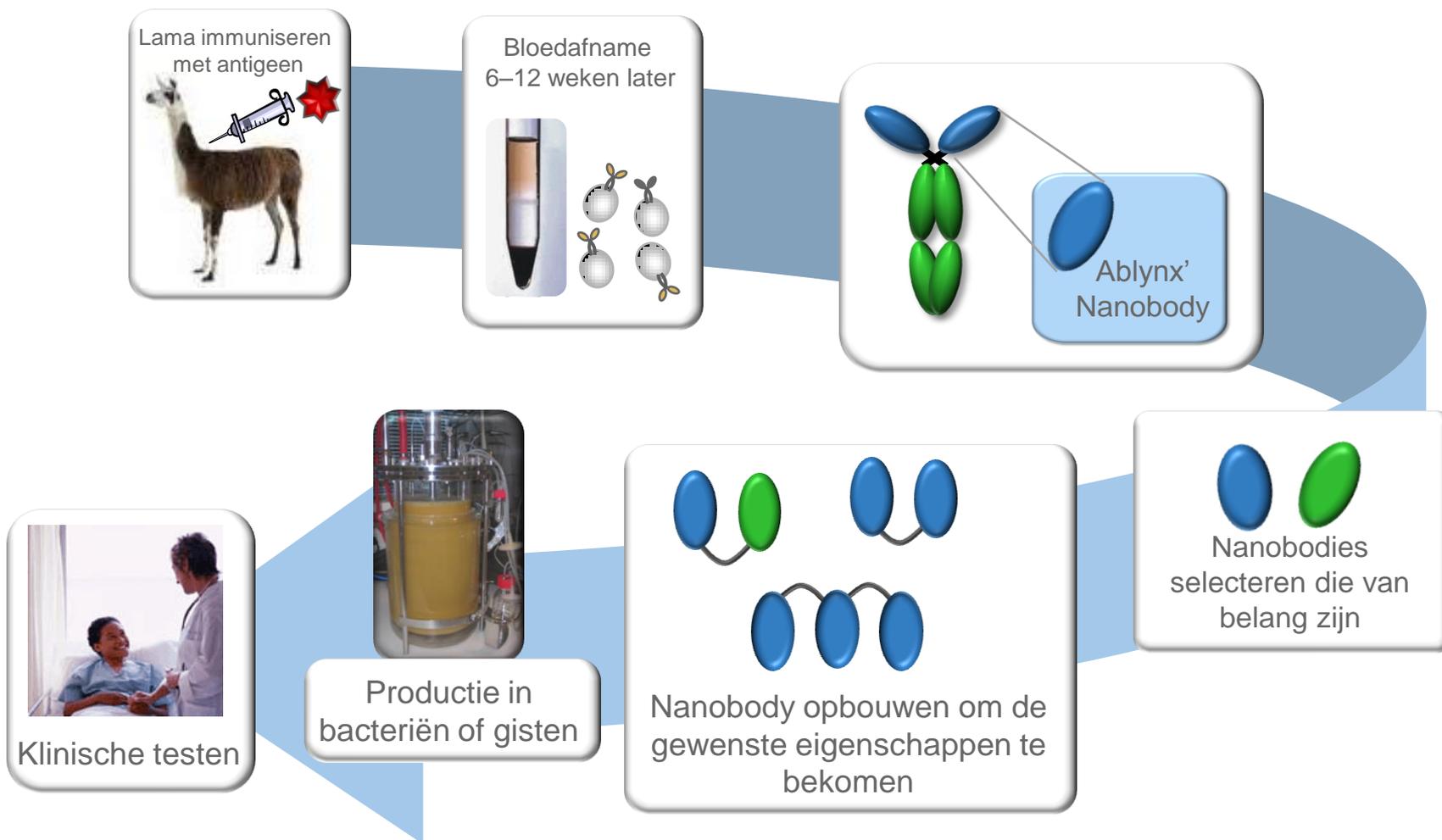
- ✔ Complexity: high



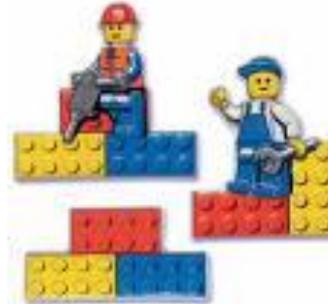
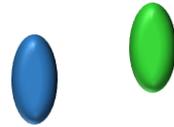
*Without fuel ☺*

- ✔ Relatively stable
- ✔ Via injection
- ✔ Low toxicity, highly effective

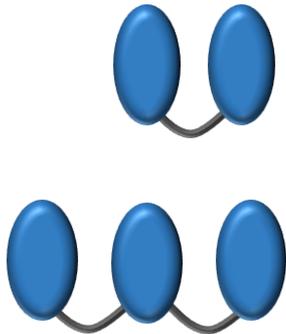
# Nanobody ontwikkelingsproces



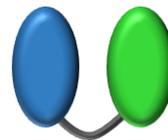
# Powerful Nanobody formatting features: multimeric, biparatopic and multispecific formats



✦ Formatting is the linking together of two or more Nanobodies which:



bind to same epitopes  
on single target

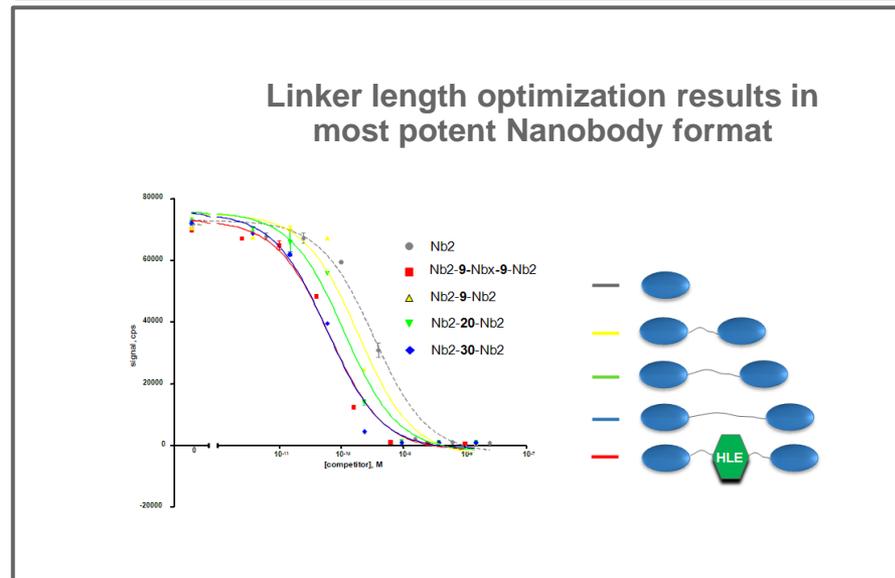
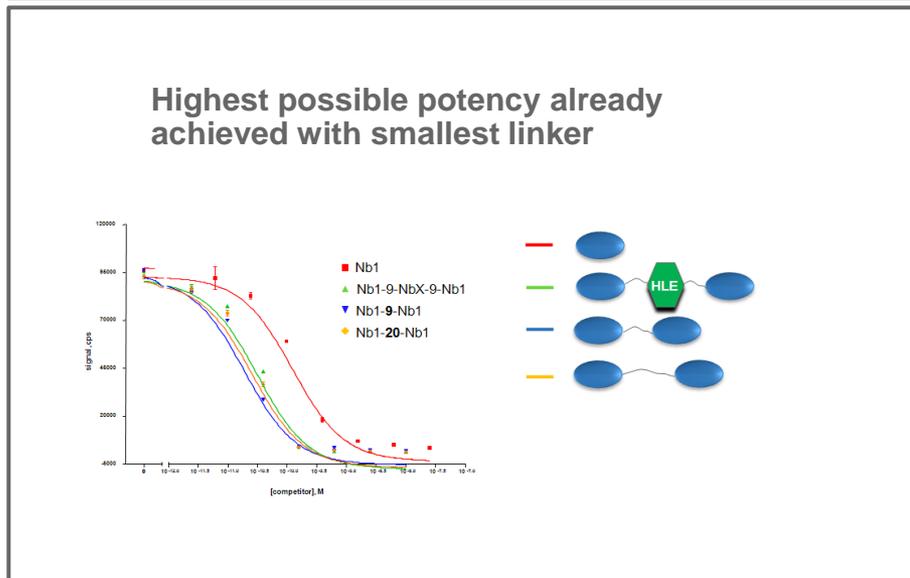
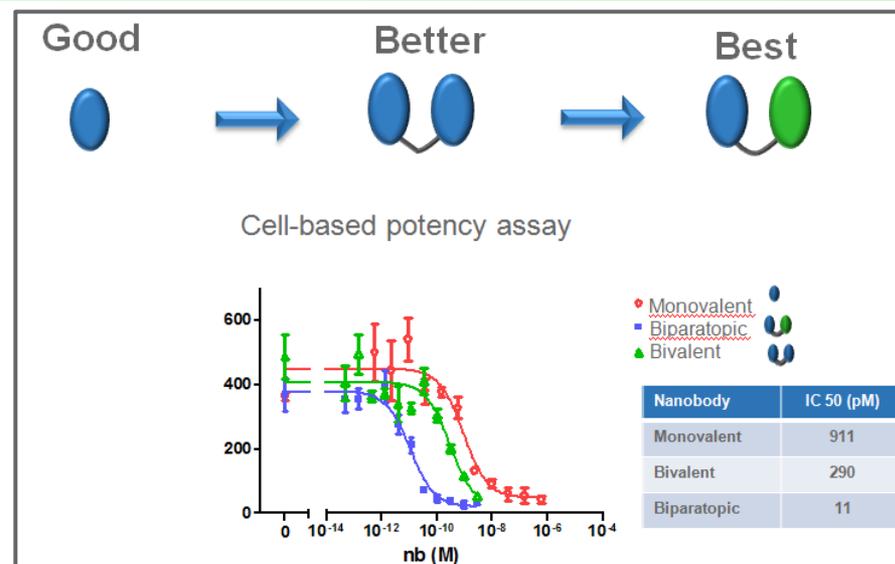
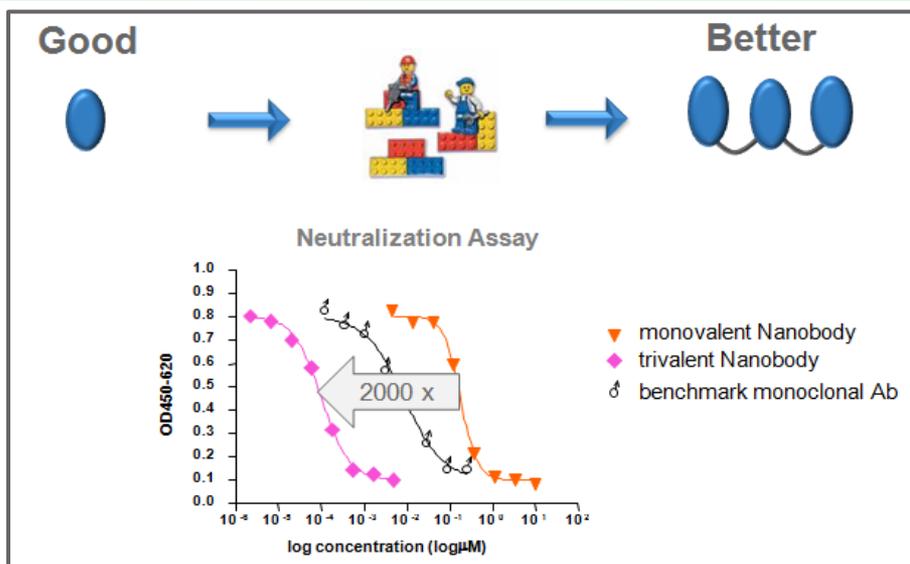


bind to different  
epitopes on target



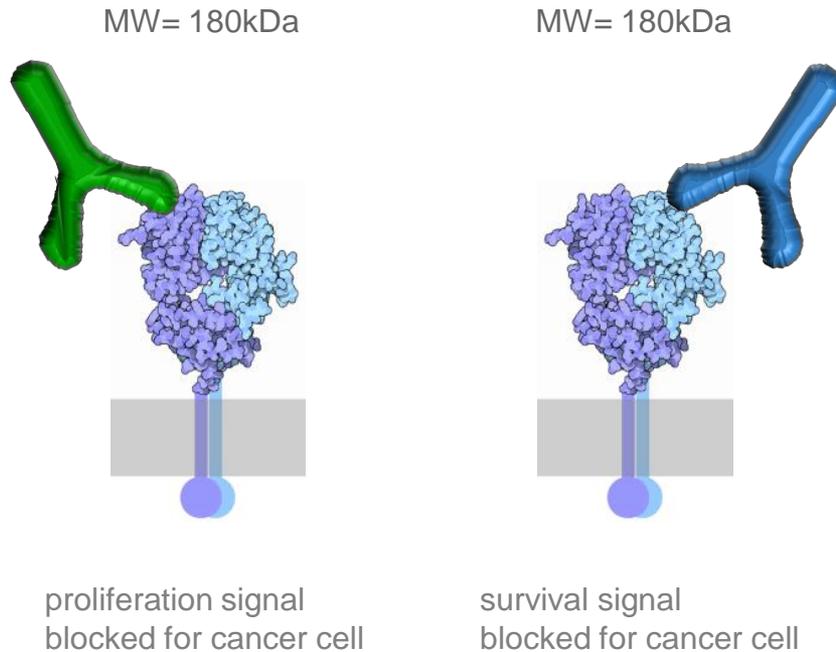
bind to different  
targets

# Using formatting to increase potency

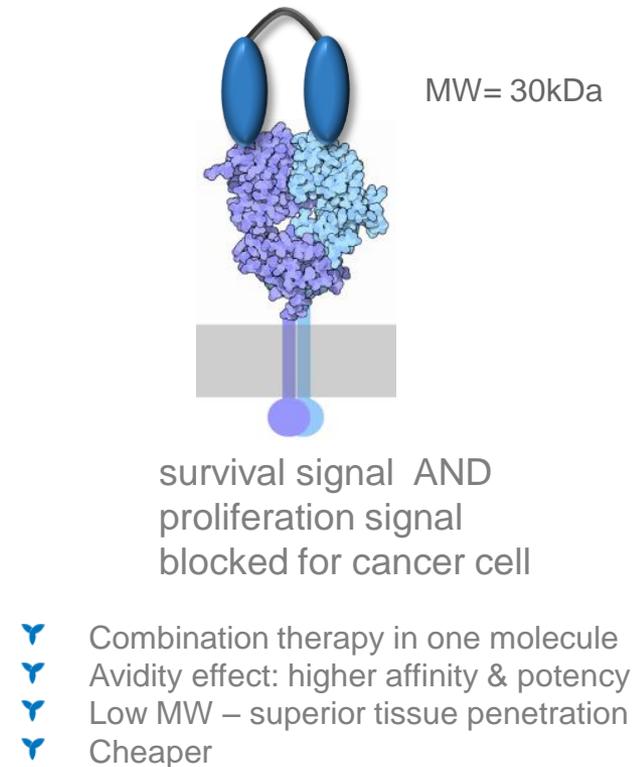


# Using biparatopic Nanobodies to obtain multi-specificity

## Herceptin and Omnitarg binding two different epitopes on receptor



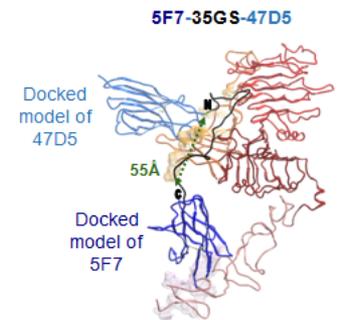
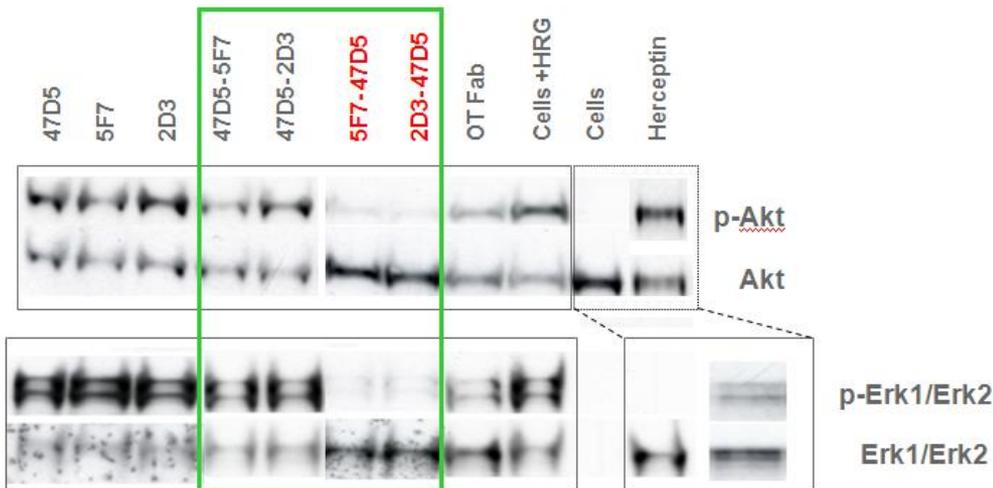
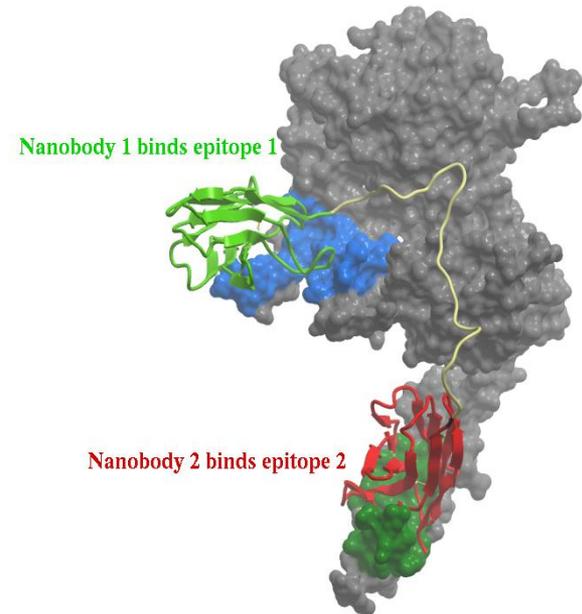
## One biparatopic Nanobody binding two different epitopes on receptor



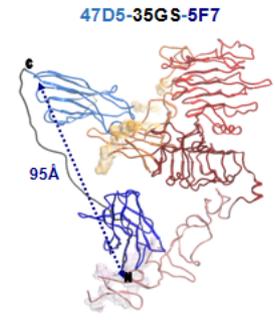
# Linker length optimization and building block orientation can result in most potent Nanobody formats

## Approach :

- combine the mechanisms of actions of Herceptin and Omnitarg, by genetically fusing Nanobodies recognizing the epitopes of the two mAbs on the extracellular domain of the HER2 receptor
- by engineering the length of the linker *intramolecular binding* can be enforced giving best-in-class potencies and avoiding receptor cross-linking



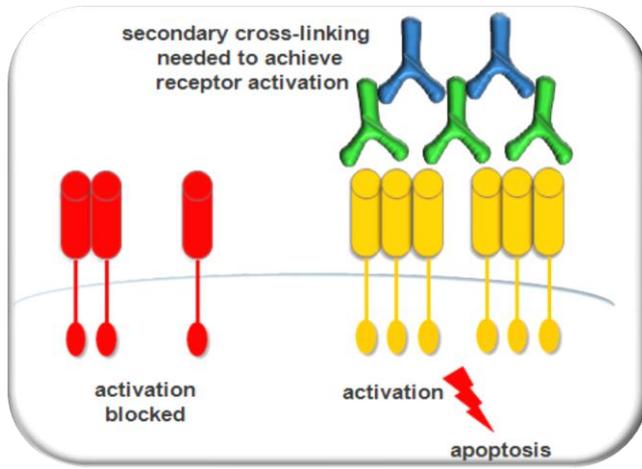
Very likely to bind intramolecularly; no energetic barrier with long linker



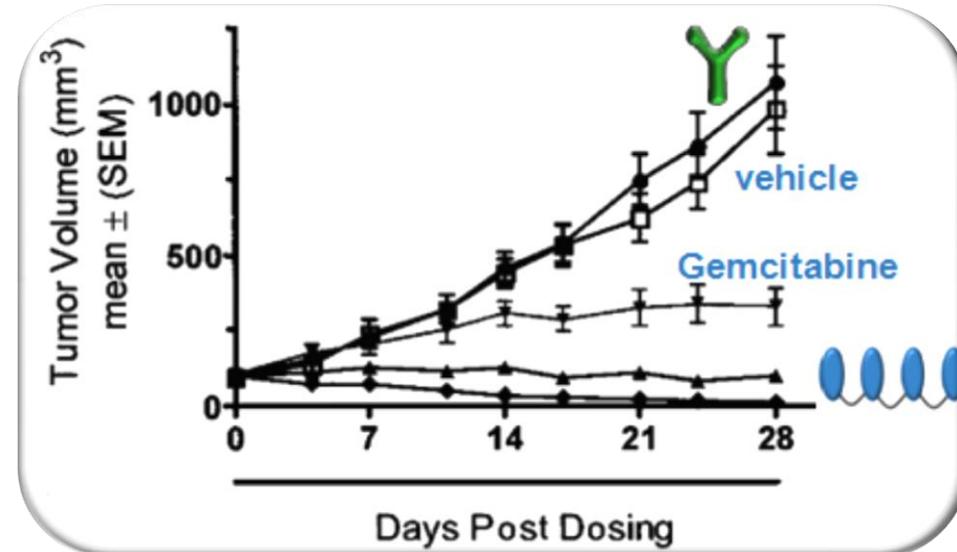
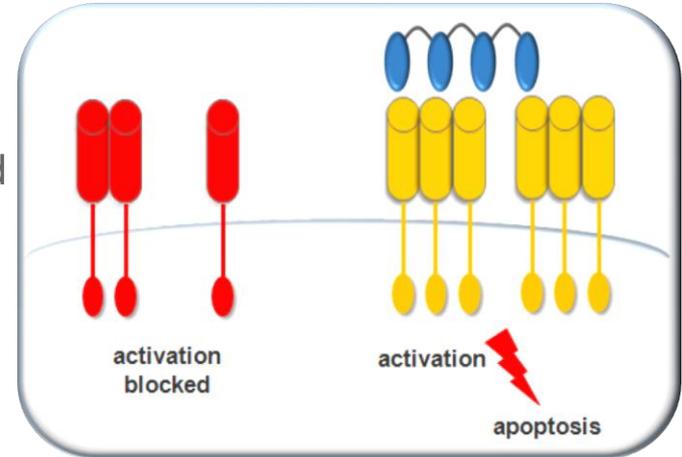
Unlikely to bind intramolecularly; high energy threshold to connect linker

**Format of building blocks impacts on potency**

# Using the formatting flexibility of Nanobodies to create agonistic formats outperforming conventional approaches



cross-linking achieved in multivalent Nanobody format



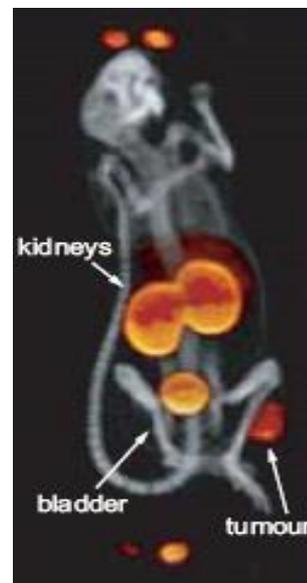
Tetrameric Nanobody regresses pancreatic TPAN-IFA tumour that is insensitive to antigen-specific mAb\*

## Nanobody formatting: Half-Life Extension (HLE)

- Nanobody is small and typically has a half-life of about 1-2 hours in the bloodstream

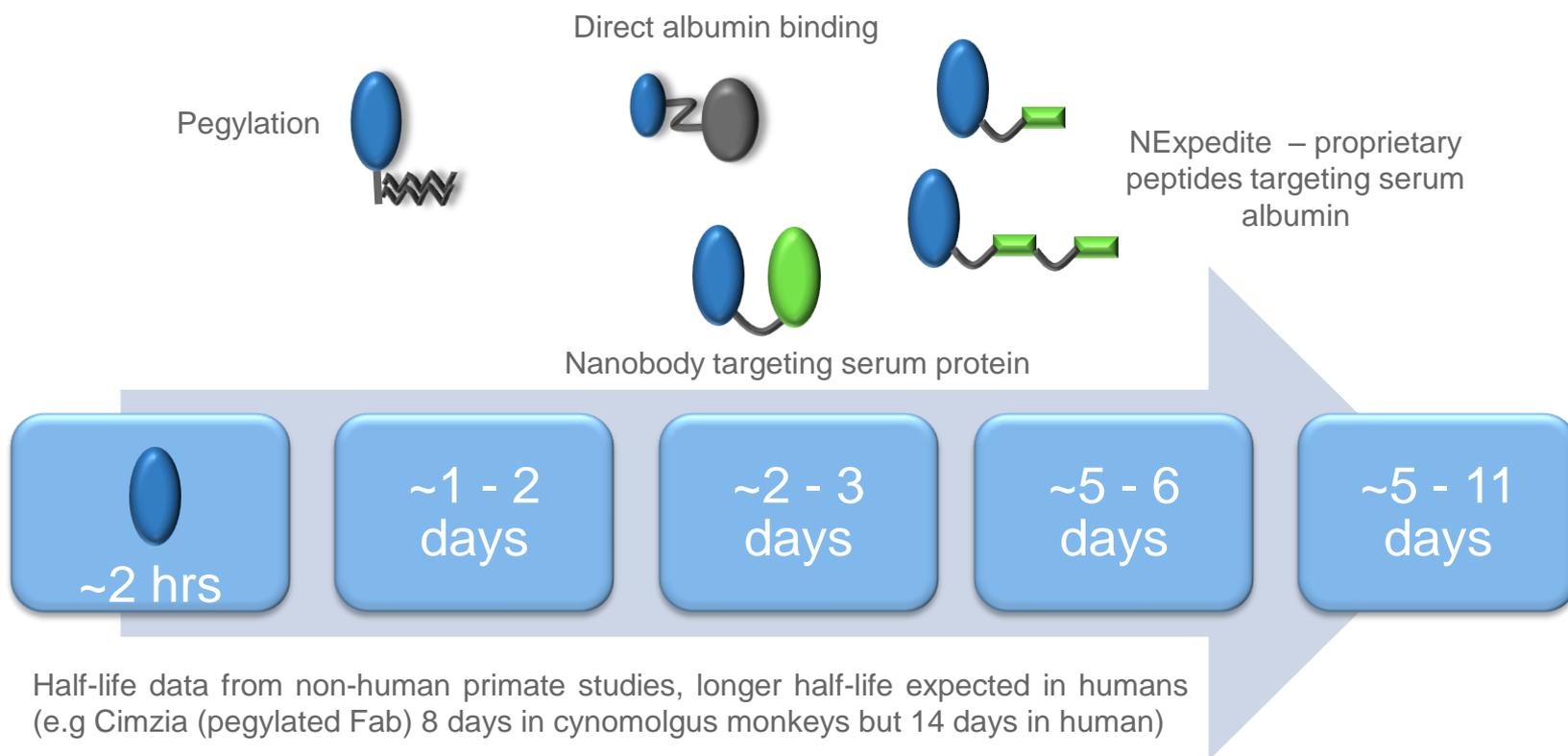


- Ideal format for
  - acute indications
  - rapid clearance wanted

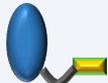
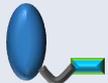
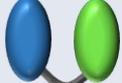


- For chronic indications, molecule should remain in circulation for days, ideally customized according to the needs

# Customized half-life - acute and chronic indications

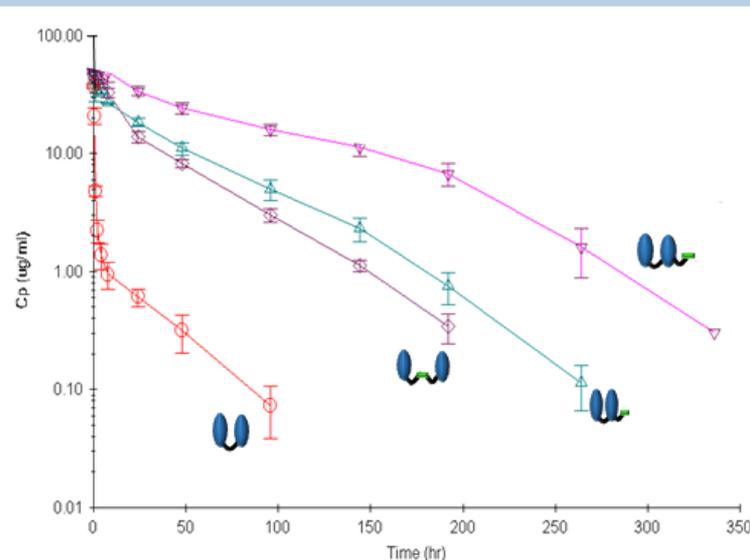


# Tailoring the half-life using different NExpedite peptides/formats – comparison with HSA binding NB

Construct	T <sub>1/2</sub> (h) in cynomolgus
	2.0 ± 0.7
	8.6 ± 0.8
	20.9 ± 4.2
	40.5 ± 7.7
	31.2 ± 3.3
	84.0 ± 14.0
	116.0 ± 18.0

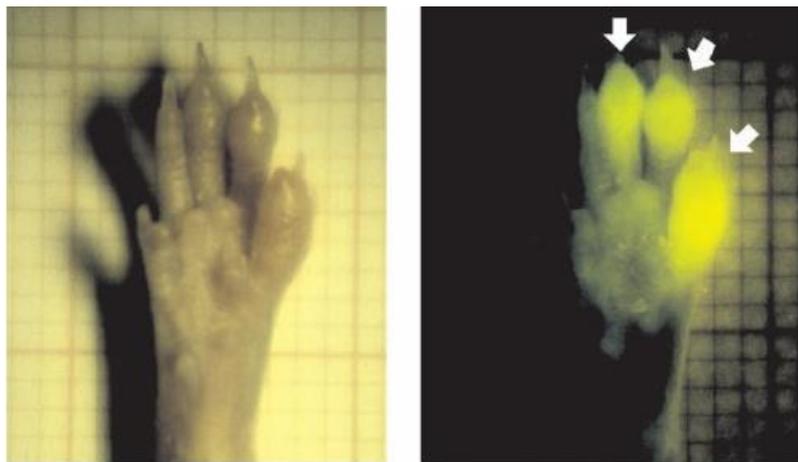
 Nanobody (~ 15 kDa)

Pharmacokinetic analysis in cynomolgus monkey: example



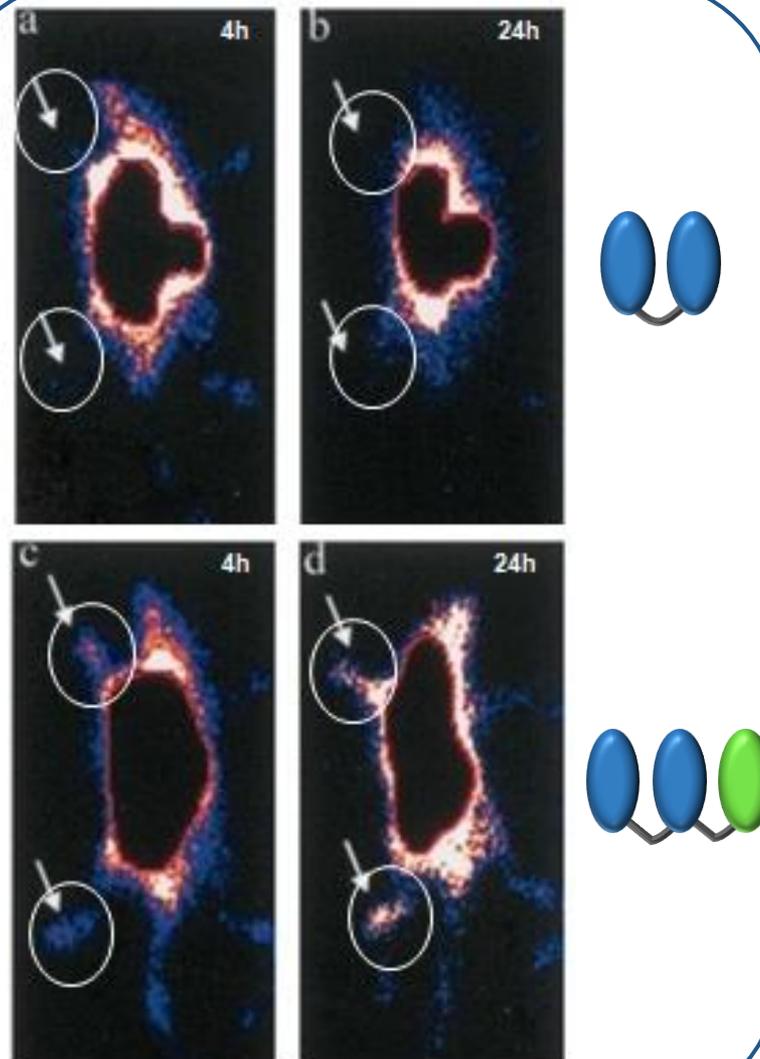
# Albumin targeting as a HLE and delivery tool (1)

- Albumin accumulates and is metabolized in inflamed joints



*Uptake of HSA labeled with aminofluorescein by inflamed toes of a mouse with CIA as determined by laseroptical imaging*

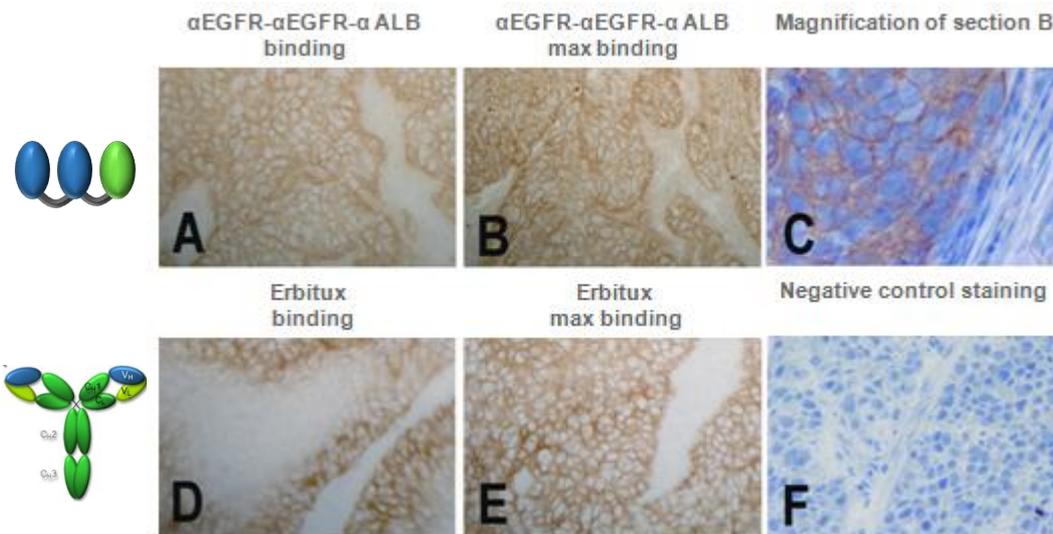
*Wunder et al., JI, 2003, 170: 4793-4801.*



*Coppieters et al. Arthr & Rheuma 2006*

# Albumin targeting as a HLE and delivery tool (2)

Albumin accumulates in and is metabolized by tumour cells



### Method:

Tumors of xenografts excised 6h after injection and cryosections incubated with Nanobody or Erbitux (Maximum uptake) or directly stained for injected antibodies (in vivo uptake)

### % Injected dose per gram (tissue) or milliliter (blood) after 24 hours

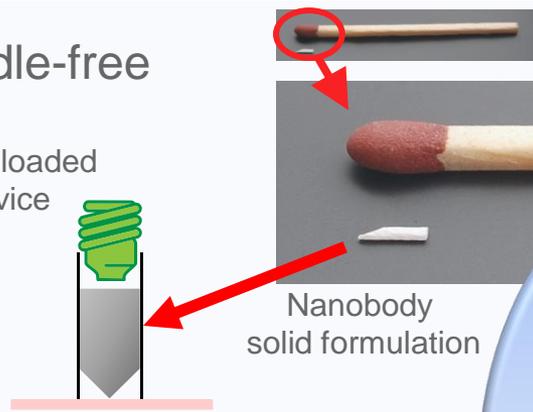
<i>Tissue</i>	<i>αEGFR-αEGFR</i>	<i>αEGFR-αEGFR-αHSA</i>	<i>Erbitux/Cetuximab</i>
Tumor	3.2 ± 0.6	35.9 ± 6.8	26.1 ± 5.3
Blood	0.0 ± 0.0	11.9 ± 0.7	13.1 ± 1.0
Liver	0.4 ± 0.1	4.6 ± 3.2	12.0 ± 1.7
Bladder	0.4 ± 0.1	4.2 ± 0.6	4.0 ± 0.5
Urine	12.2 ± 3.2	11.3 ± 9.5	10.3 ± 12.2

# Nanobodies: flexibility of administration

## Needle-free

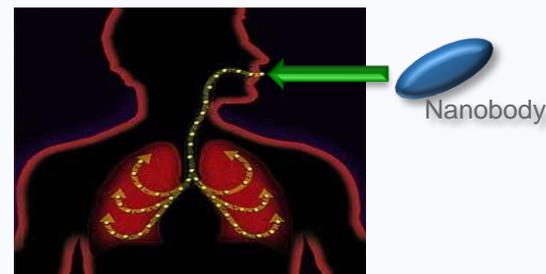
spring-loaded device

skin



Nanobody solid formulation

## Pulmonary



High stability  
High solubility  
Low propensity to aggregate

Alternative delivery options

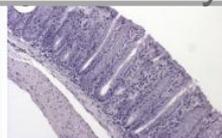
Topical

Systemic

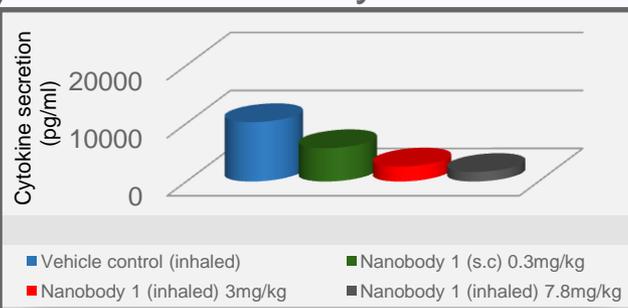
## Oral to topical

No treatment

Nanobody

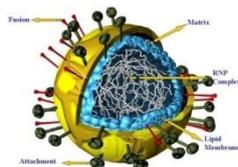


Inflammatory bowel disease

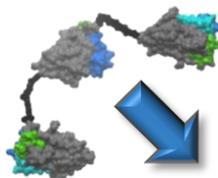


# Inhaled Nanobody for treatment of RSV infection

- RSV replicated exclusively in the lung
- Delivery direct to the lung will be a potential advantage



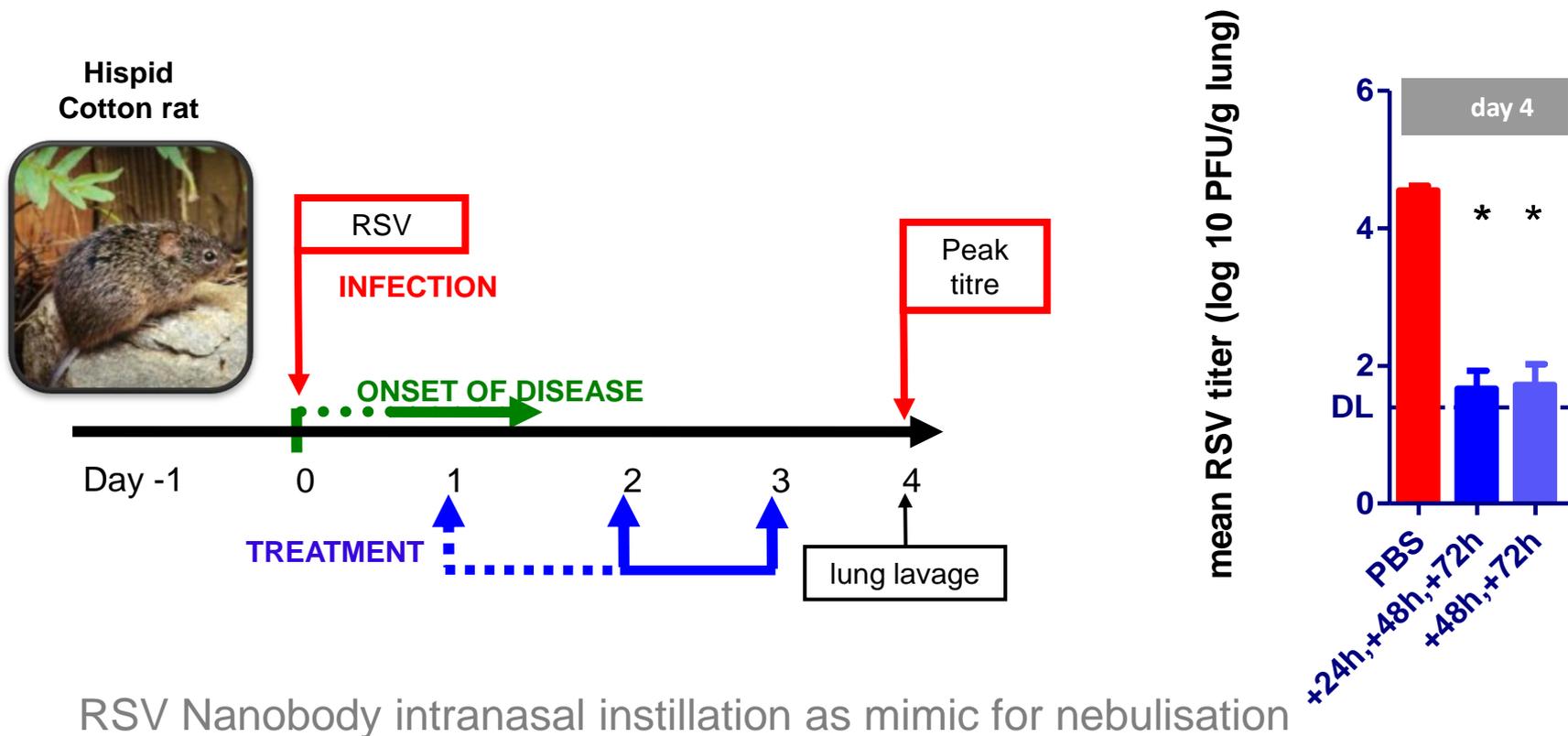
Non-treated patient:  
virus replicates in lung



Viral replication in the lung is inhibited

# ALX-0171 – proven antiviral effect in cotton rat model

- ALX-0171 significantly reduces viral replication in lung even if administered two days after infection



# RSV management in infants – Today

Duration: 1-2 weeks



Evolves to  
distressing  
symptoms

Symptomatic treatment  
including inhaled  
corticosteroid & bronchodilator

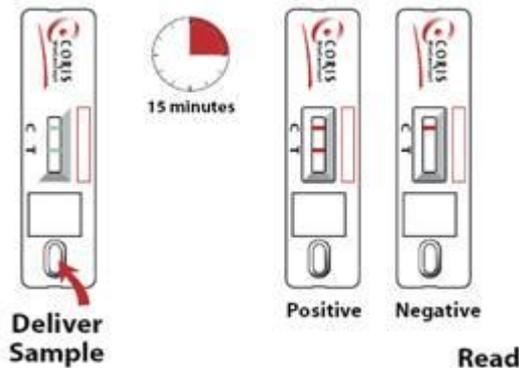
8-20%  
hospitalised

nebulisation face mask in nose breathing  
infants is an established technique

# RSV management in infants – Tomorrow Transformational ALX-0171 therapy



Shorter duration  
of symptoms

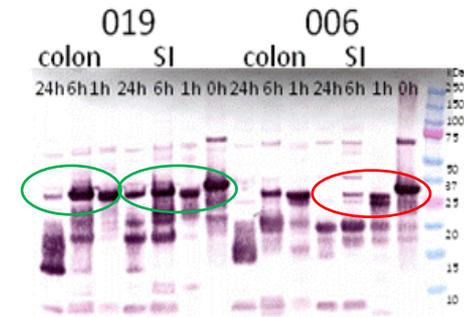


“.....In conclusion, infants hospitalised for RSV routinely have contact with health care professionals **at early time points** in their illness.....”

J.P. DeVincenzo, Antiviral Res. 2004

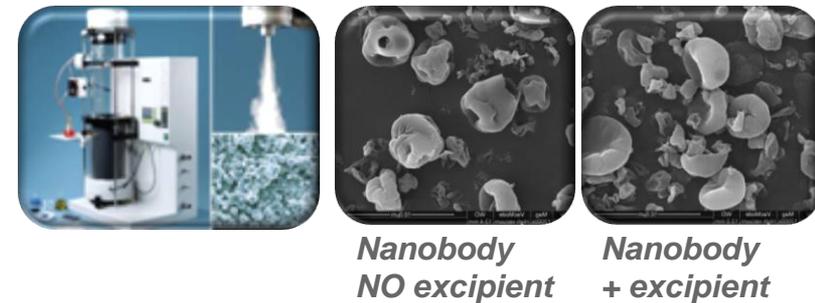
# Exploiting the robustness of Nanobodies to expand alternative delivery options

Y Nanobodies can be identified or selected for inherent resistance to pH, and proteases in the lung and intestinal tract

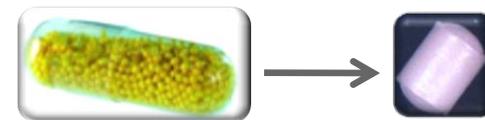


Y Successful spray-drying of Nanobodies

- Nanobody retained function
- first step in formulation for dry-powder inhalers and oral solid dosage



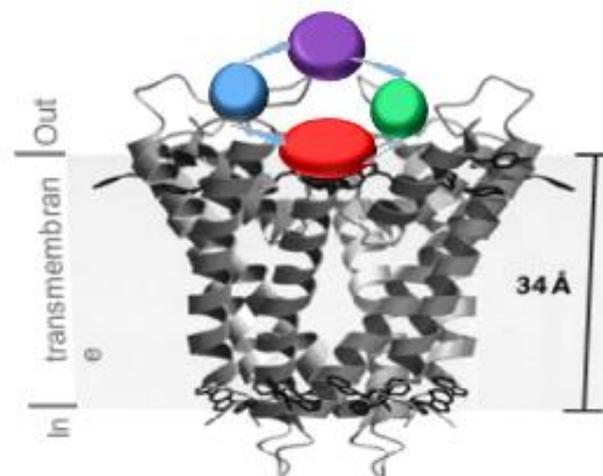
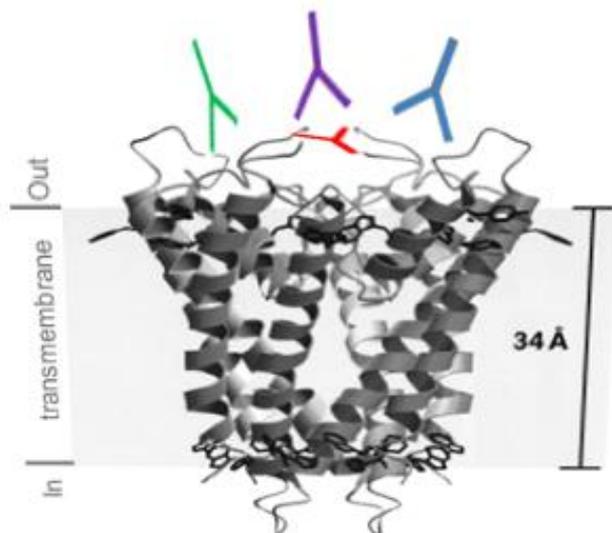
Y Spray-drying, bead coating and tableting has not significantly influenced Nanobody quality



Opportunity to develop Nanobody drugs for oral-to-topical delivery

# Extending the Nanobody platform to ion channels

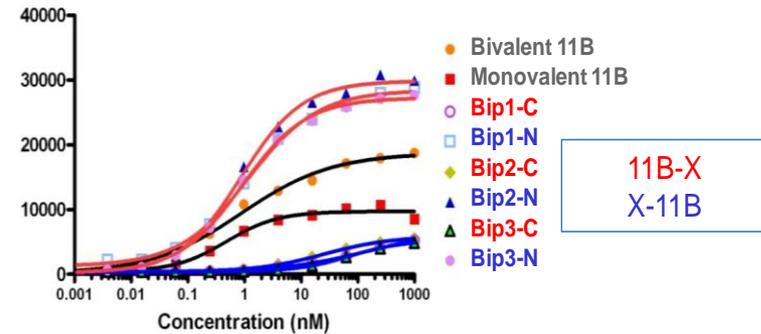
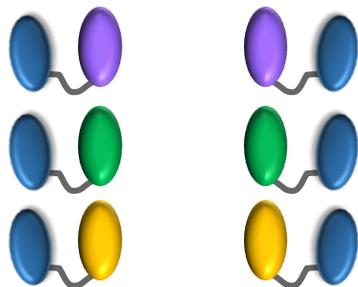
- Y The problem with small molecule drugs
  - selectivity
  - toxicity
- Y Conventional antibodies face high technical hurdles
  - blocking antibodies are polyclonal
- Y Nanobodies: a “polyclonal” in one molecule
  - formatting: combine antibody-like selectivity and polyclonal effect



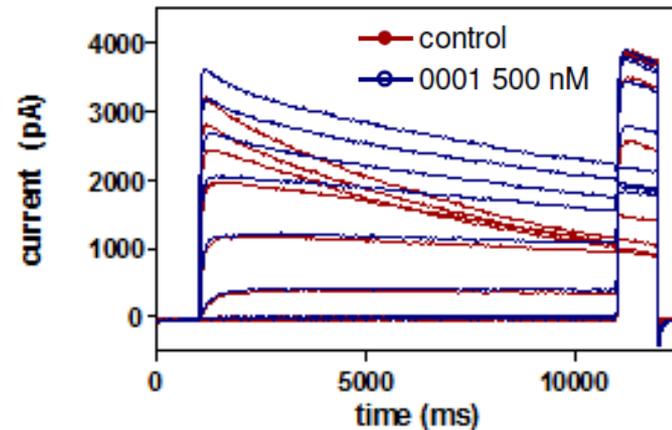
Panel of Nanobodies with on average weak binding)



Format into bivariate with strong ion channel binder (both N- and C- orientation)



**Improved binding of bivariate Nanobodies to voltage-gated IC expressing cells**

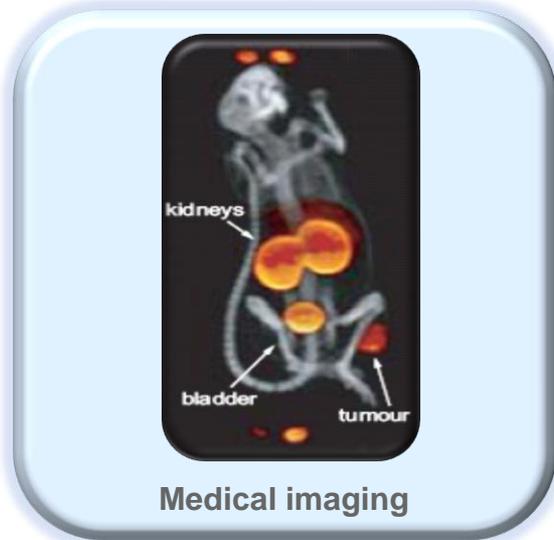


- monovalent Nanobodies have no activity
- bivariate Nanobodies have modulatory effect

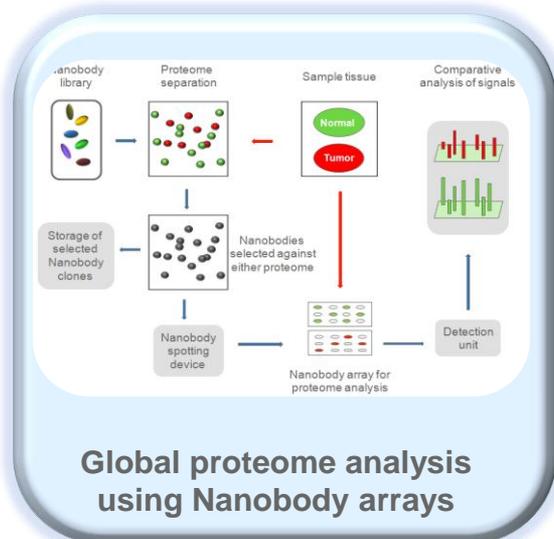
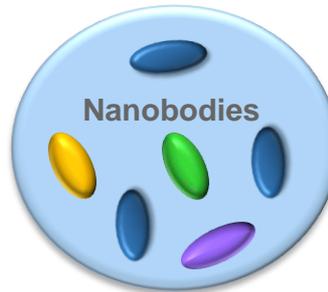


## Nanochips voor diagnostische toepassingen

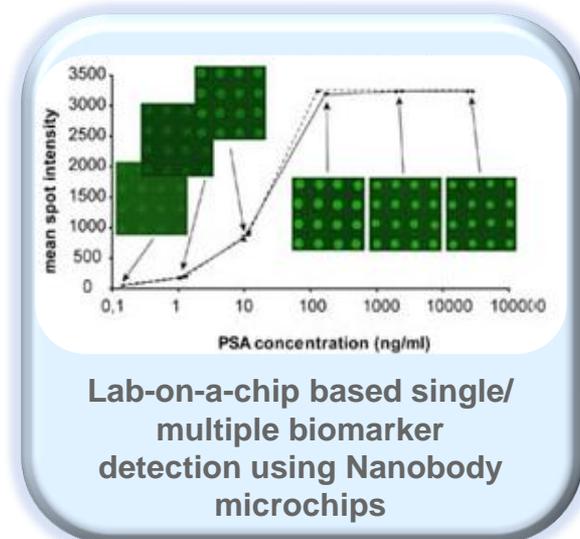
**Nanobodies® -  
Inspired by nature**



- ✔ small size
- ✔ “quick in – quick out”
- ✔ high uptake
- ✔ ease of coupling chemistry



- ✔ chemical & physical robustness
- ✔ high affinity binding
- ✔ small size
- ✔ easy microbial manufacturing





## Ontwikkelingen en toepassingen van farmaceutische producten gebaseerd op nanotechnologie

Hilde Revets, PhD, Senior Research Fellow

KVCV – KAHO Sint Lieven, Sint Niklaas

17 maart 2012

A close-up photograph of a water splash, with many small droplets in the air and a larger splash below, set against a blue background.

**Nanobodies® -  
Inspired by nature**