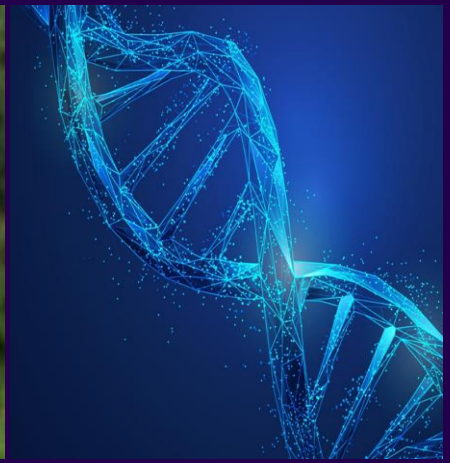


NANOBODY® und SYNTHORIN™ Moleküle –

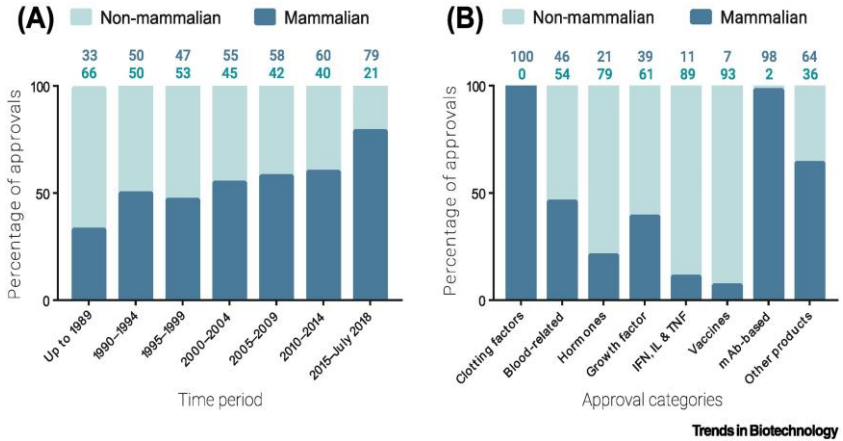
Prozesstechnische Herausforderungen neuer biopharmazeutischer Modalitäten

Dr. Thomas Sauer

Pharmaforum
23 . 03 . 2022

Microbial Expression is an Essential Technology for Biopharmaceuticals

- Microbial fermentation contributed > 50% to first wave biologics (e.g. growth factors, insulin)
- Last decade was dominated by products expressed with mammalian cell culture systems (e.g. monoclonal antibodies and other glycosylated proteins)
- Complex next generation molecules like bioconjugates and antibody fragments drive again interest in fermentation
- Market size for Microbial-produced biopharmaceuticals approx. $100 bn



Market share comparison between biotherapeutic production hosts

Trends in Biotechnology DOI: (10.1016/j.tibtech.2021.10.003)

Benefits of Microbial Expression Systems

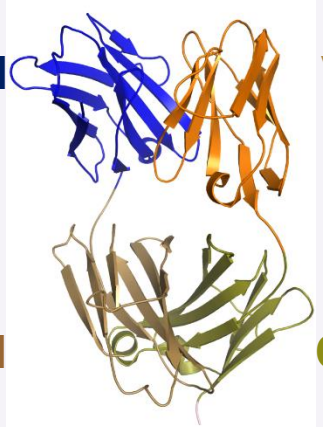
Fast development timelines

- CRISPR gen-editing technologies available for effective host design
- Fast generation of manufacturing strains
- High titer and quality
- Good scalability
- Short processing times
- Reduced manufacturing cost

NANOBODY® and SYNTHORIN™ molecules rely on new technology platforms based on microbial systems

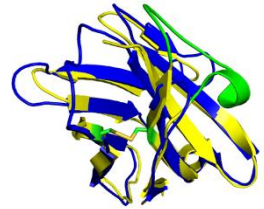
NANOBODY® Molecules are Similar to Antibody Fragements and Offer a Unique Toolbox

Fab fragment



NANOBODY® compound

Overlay



pronounced structural similarity to VH

Commonly composed of several building blocks connected by linkers of variable length:

valency: number of building blocks
specificity: number of targets addressed
paratopy: number of paratopes addressing the target

NANOBODY® Technology – A New Biopharmaceutical Perspective

Platform advantages

Mix and match

Multi-specific/multivalent molecules that adress multiple targets in a single drug molecule – flexible GS linker lenths

Multiple delivery routes

Injection | Inhalation | Ocular | Oral-to-topical

Manufacturing

High-hield, High-concentration, Low-viscosity, Microbial production

Able to bind and block challenging targets

NANOBODY® molecule against ion channels and GPCRs

Customized half-life extension

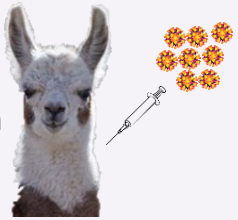
Hours/days/weeks

Albumin-binding NANOBODY® molecule

Fc

From llama to medicine

Immunization



Collect small blood sample

Selection of best NANOBODY® genes (DNA)

Clone into microorganisms

Production via fermentation

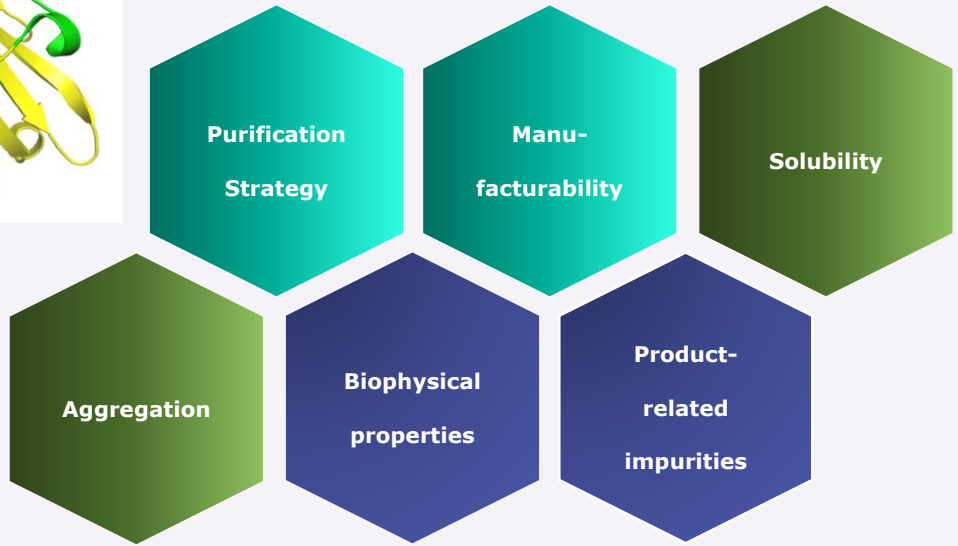
Product development

clinical studies & approved treatment

CMC Challenges

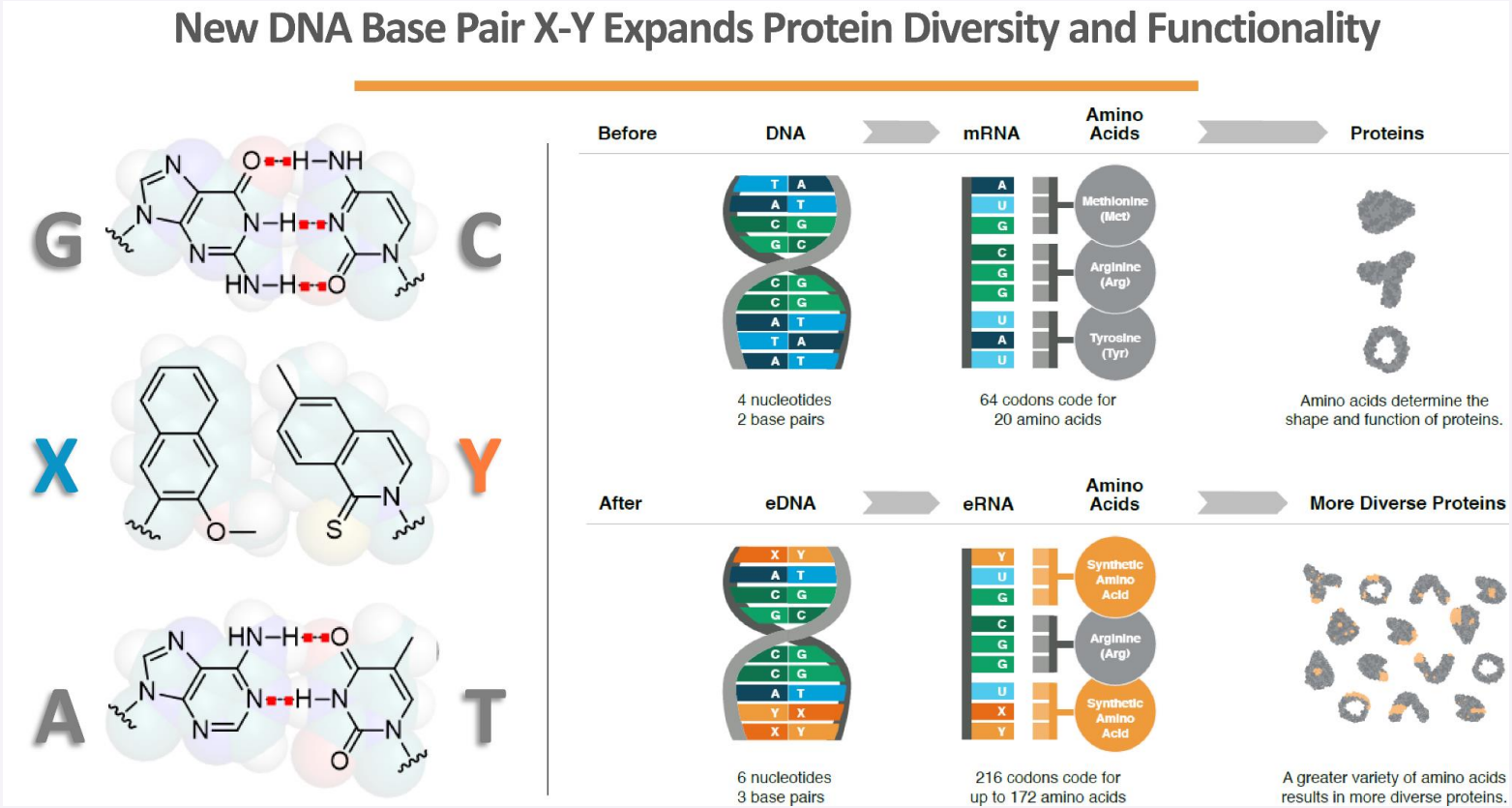
Potential byproducts require solid CMC development

- O-glycosylation
- Carbamylation
- Reduced or misassembled disulfide bridges
- Heterogeneity caused by exoproteases and imprecise cleavage
- Methoxin variants
- Pyro-glutamate variants

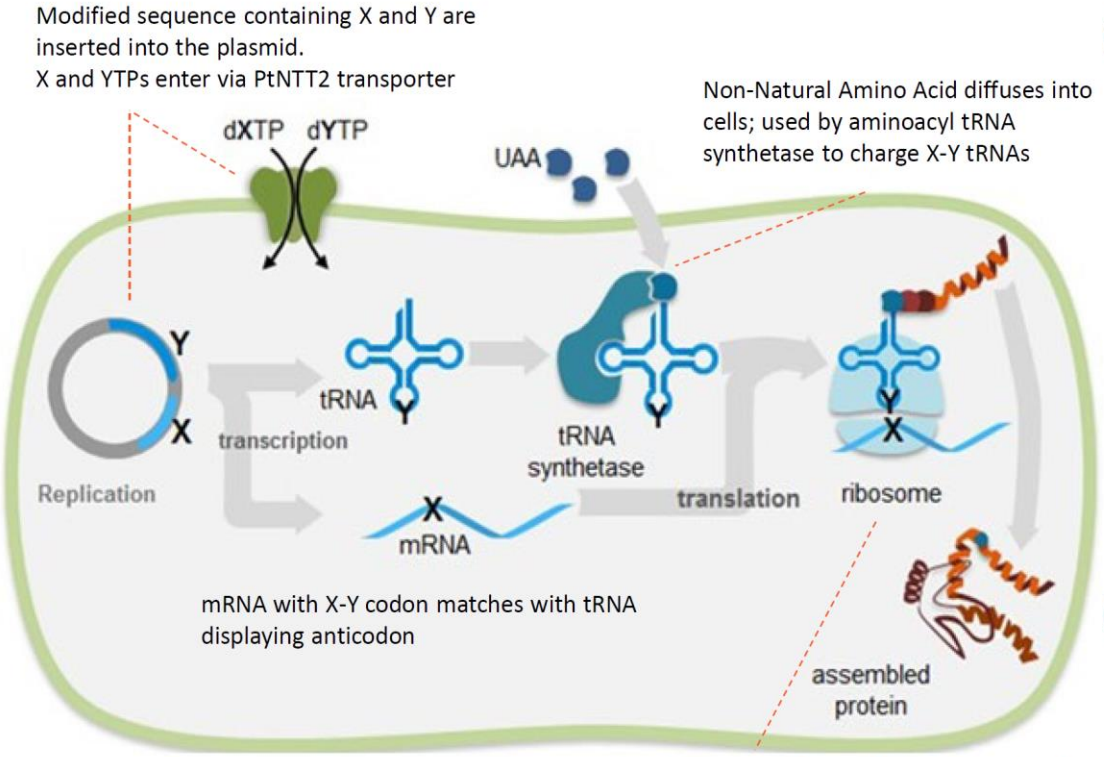


Design of a platform process

Synthorin Platform Technology – A new genetic code



A production system close to Synthetic Biology: Adapted *E. coli*



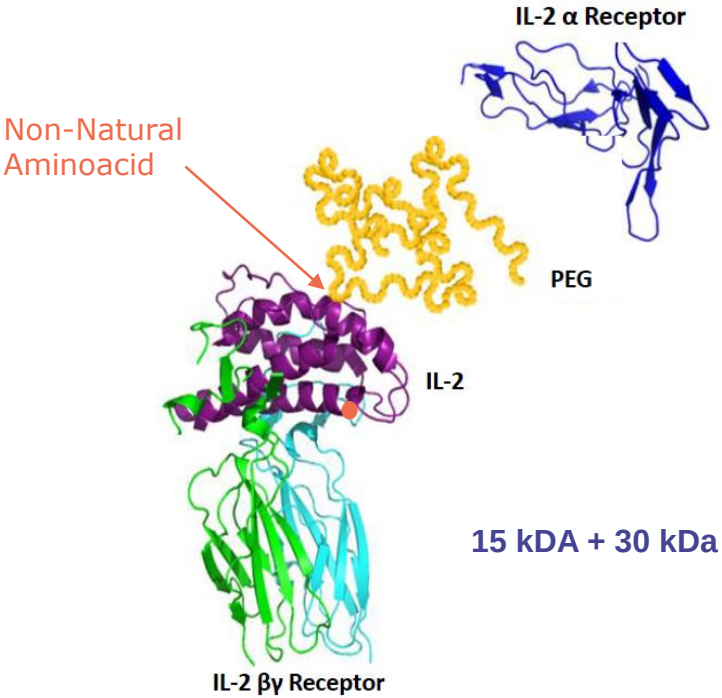
Source: *Nature* **volume 509**, pages385–388 (15 May 2014)

Strain-Encoded Modifications

- **X/Y nucleotide transporter protein PtNTT2**
  - PlacUV5- nucleotide transporter
  - Constitutive expression
  - Chlor resistance
- **tRNA synthetase**
  - pTac- Mb tRNA synthetase
  - IPTG for induction
  - Tet resistance (plasmid not shown)
- **Synthorin mRNA and tRNA**
  - pT7- Mb tRNA
  - pT7- mRNA
  - IPTG for induction
  - Zeo resistance

Supplemented Materials

- **dXTP, dYTP, XTP, YTP**
- **Non-natural amino acid**

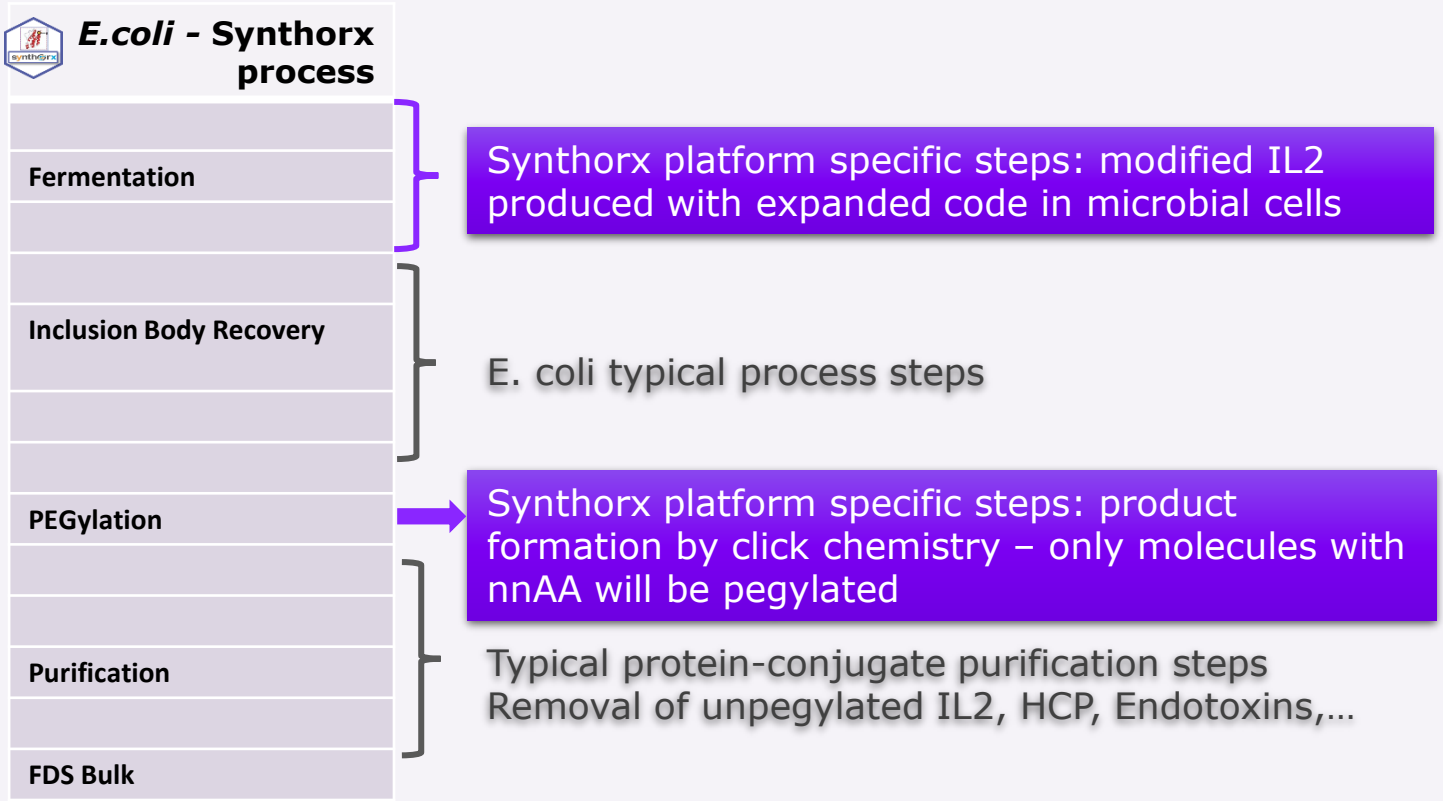


First Synthorin™ molecule:

Non-Alpha IL-2

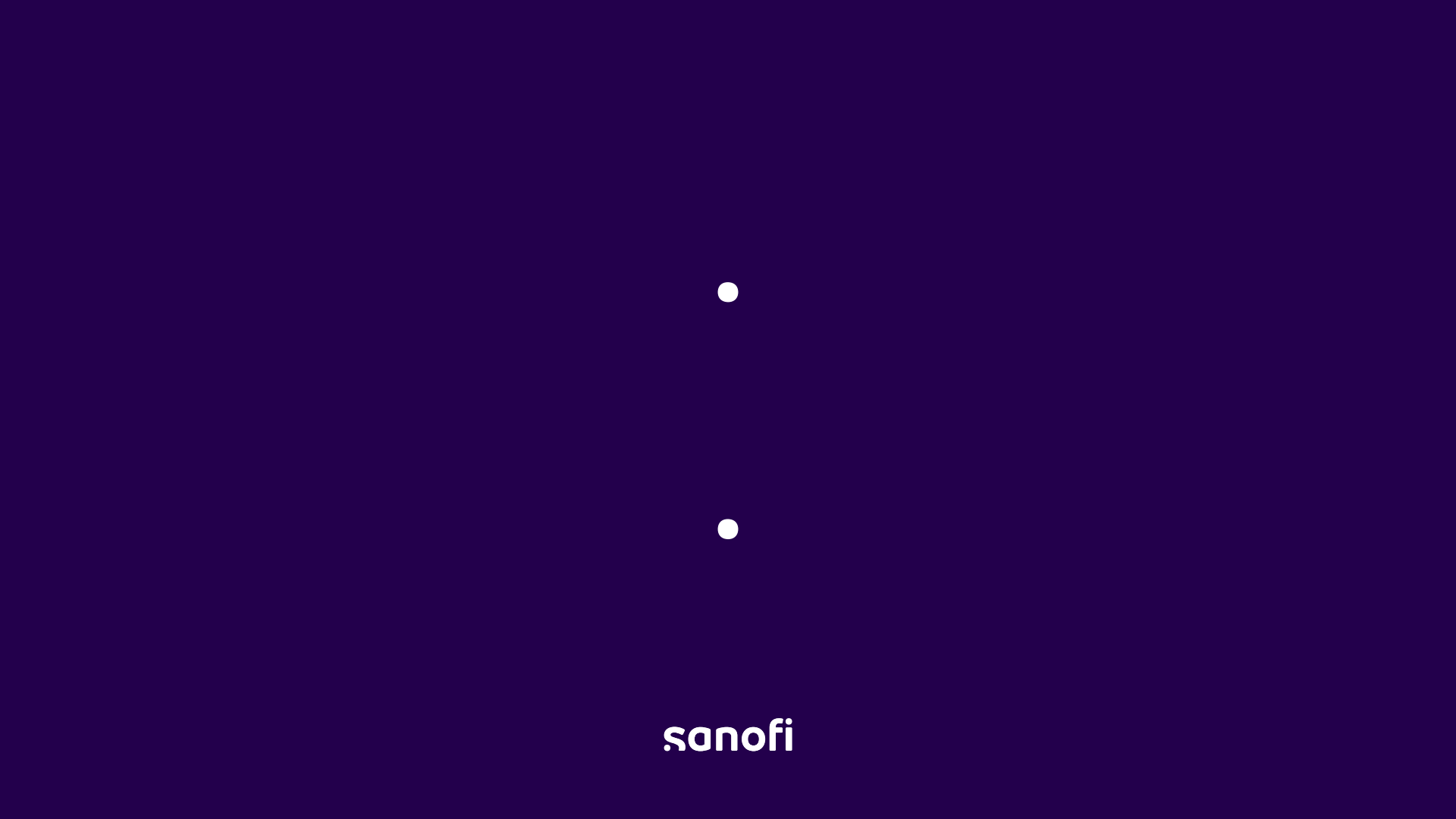
- Different types of skin, lung, head & neck, gastrointestinal cancer
- blocked binding to the IL-2 α receptor
- extends half-life of a short-acting cytokine
- Shields nAA from immune identification
- Activates NK cells

Drug Substance Process Requirements – Conceptional



Conclusion

- Next generation Biologics will significantly enlarge our toolbox to treat diseases
- NANOBODY® compounds offer a unique toolbox from nature for multispecific immune targets
- Synthorin platform enlarges the genetic code with a first application for bioconjugates
- Renaissance of Microbial Expression systems for highly engineered next generation biopharmaceuticals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