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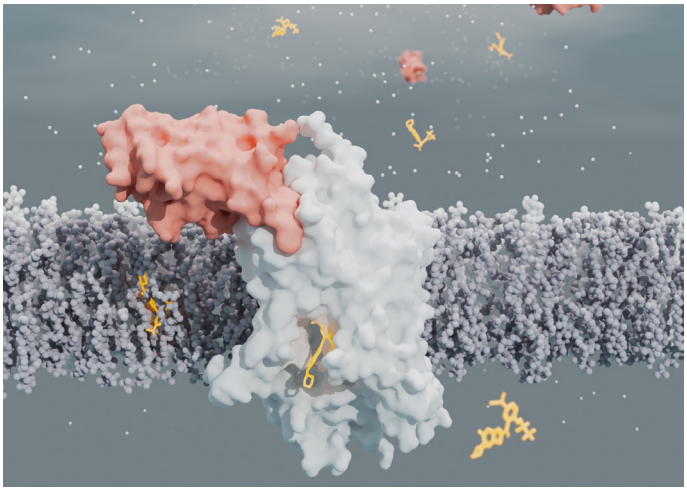
Linkster Therapeutics

SYBODY® TECHNOLOGY: A RAPID AND RELIABLE PLATFORM PRODUCING THE NEXT GENERATION OF ANTIBODY-BASED THERAPEUTICS

A recent publication in ***Nature* by Shahsavar *et al.***[1] has presented the findings of a global collaboration with Roche, EMBL Hamburg, Zurich University and Aarhus University utilising **Linkster Therapeutics' Sybody® platform**. For the first time, the structure of the glycine transporter type 1 (GlyT1) in complex with a highly selective glycine reuptake inhibitor (Cmpd1) has been determined, providing an understanding of the underlying molecular mechanism of glycine reuptake via GlyT1.

This work highlights the potential that Sybody® technology brings to the development of the next generation of antibody-based therapeutics for a wide range of difficult-to-reach targets. It adds to an increasing number of scientific publications that demonstrate the power of Sybody® technology and its potential as a platform to develop novel therapeutics.[2–5] This most recent finding will facilitate the design of new clinically efficacious GlyT1 inhibitors.



Sybody® molecule Sb7 (red) binds to GlyT1 (white) at a novel extracellular binding site, locking the transporter in an inward-open conformation that inhibits glycine uptake and enhances the binding of the GlyT1 inhibitor Cmpd1 (yellow).

> *The present study, in addition to our successful partnerships with several pharmaceutical companies, highlights the potential of Linkster's unique approach to drug discovery and development using the Sybody® as well as the Flycode® drug platforms in indications with high unmet medical need.*
>
> **Roger Dawson,** Co-Founder & Chief Executive Officer of Linkster Therapeutics

Sybody® technology

RELIABLE
100% success rate to date for the generation of target-specific antibodies

REFINED
Three libraries cover a diverse range of epitopes

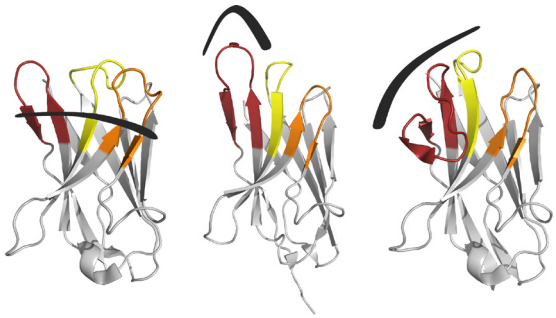
RAPID
Generation of target-specific antibodies can be achieved in 3 weeks

RELEVANT
Full control over selection conditions and generation process

The Sybody® platform: inspired by nature, improved by science

The Sybody® technology is a novel *in vitro* therapeutics discovery platform. It is based on highly stable synthetic scaffolds derived from variable heavy homodimer (VHH) camelid antibodies. Three libraries with concave, loop and convex binding site designs with complementarity-determining-region (CDR) 3 of varying lengths have been designed to facilitate exquisite epitope tractability.

By leveraging the power of ribosome and phage display technologies, the Sybody® platform delivers an experimental library diversity of 9×10^{12}. Importantly, the Sybody® library can be used to generate **disease-relevant, conformation-specific antibodies** that can target previously intractable proteins such as G protein-coupled receptors and solute carrier transporters.



The Sybody® libraries exhibit thermally stable (75–95°C) architectures and highly variable randomised surfaces (concave, loop and convex), each harbouring a diversity of 9×10^{12}. CDR1, CDR2 and CDR3 are yellow, orange and red, respectively.

Why the Sybody® approach?

Our approach builds upon advances made in the field of nanotherapy. VHH antibodies (e.g. Nanobodies®) are the antigen-binding fragment of heavy-chain only antibodies (~15 kDa) and their properties overcome many of the limitations associated with conventional antibody-based therapeutics.[6] For example, their stability and robustness make them amenable to a variety of delivery routes (e.g. inhalation, ocular and oral), and their *in vivo* half-life can be tailored to allow for the management of both acute and chronic diseases. Additionally, via direct conjugation to cytotoxic agents, they can specifically target and kill aberrant cells, which has significant advantages for the development of novel oncolytic agents.[7]

VHH antibodies have an outstanding track record in the drug discovery and therapeutics arena, and a number are under clinical evaluation for a variety of human diseases including inflammation, breast cancer, brain tumours, lung diseases and infectious diseases.[8,9] Their therapeutic potential has already translated into novel medicines such as caplacizumab, which was approved by the US Food and Drug Administration for use in patients with thrombotic thrombocytopenic purpura in 2019.[10] Importantly, the variable CDR3 of VHH antibodies facilitates access to small hydrophilic surfaces found on therapeutic targets such as membrane proteins, the binding of which by traditional antibody therapeutics is often precluded owing to their size.

Sybodies are synthetically designed VHH antibodies[8] engineered to create a new wave of biotherapeutics: multispecific, conditionally active, precision medicines that are anticipated to overcome the limitations of naturally raised antibodies and enhance tissue specificity.[11,12] Use of the *in vitro* Sybody® platform overcomes some of the known limitations of traditional antibody selection technologies and provides the following advantages.

- Full control over the Sybody® generation cascade and selection process *in vitro*, negating the need for animal immunisation and supporting the 3Rs (reduction, replacement and refinement) framework.
- Generation of conformation-specific, disease-relevant antibodies and selection of preferred epitopes on a target surface that are otherwise too unstable for use in traditional immunisation protocols.
- Compatibility with the use of conformation-inducing ligands, such as ATP, eliminating the concerns associated with ligand dissociation and host toxicity that can occur with traditional antibody generation protocols.

- Increased thermal stability compared with natural VHH precursors.
  - Concave, loop and convex Sybodies have melting temperatures of 74°C, 75°C and 95°C, respectively, representing an increase in thermal stability of 21–35°C compared with natural precursors.
- Increased epitope space and an experimental diversity of 9×10^{12} within each experimental library (concave, loop and convex).

Further, by coupling the Sybody® technology with Flycode® technology,[4] each experiment utilises **smart** *in silico* analysis and can **simultaneously** evaluate the performance of thousands of target antibodies and identify **specific** antibodies with optimal biophysical, pharmacokinetic, pharmacodynamic and biodistribution profiles. In addition, the system can identify conditional Sybodies that work in unique microenvironments, and thousands of antibodies can be screened directly in tissue or even *in vivo* in a single experiment, effectively fine-tuning the antibody for real-world development. Importantly, Flycode® technology also **reduces** *in vivo* experimentation 500-fold, further supporting the 3Rs.

Together, the Sybody® and Flycode® platforms exploit the unique architecture of highly stable synthetic scaffolds to rapidly generate high-quality, conformation-specific, disease-relevant Sybodies to previously intractable targets.

Flycode® technology

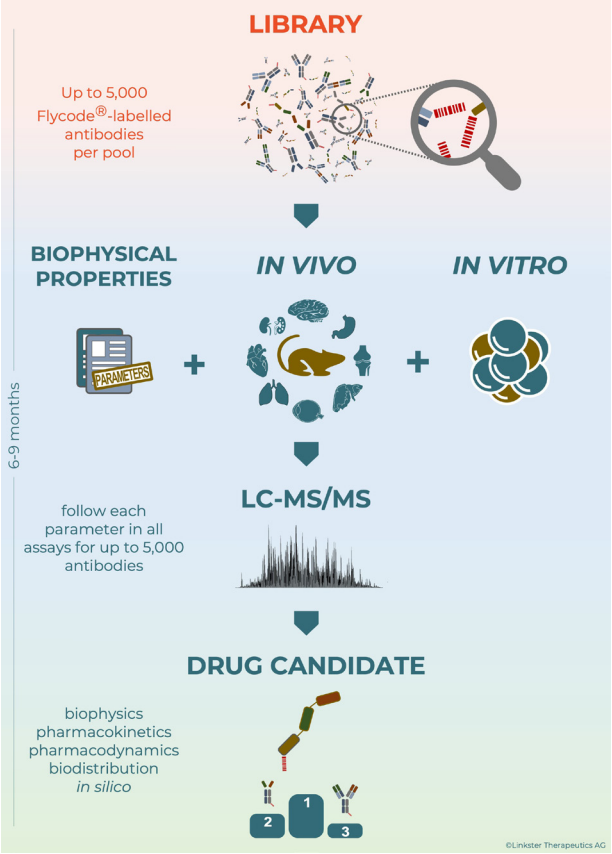
The Flycode® approach enables high-throughput *in vivo* antibody engineering by removing the need for the classical triaging cascade, a key bottleneck in the process of therapeutic antibody development.

Using this approach, thousands of antibody candidates are labelled with the revolutionary Flycode® peptide barcodes, which are designed for optimal detection by mass spectrometry. All candidates can be expressed and purified in a single batch, creating a large 'pool' of labelled antibody candidates that can be used in a single experiment against the target, directly on cells, in tissue or in living organisms. This approach enhances robustness and reliability, while reducing variability between experiments.

Multiplex screening techniques are then employed to characterise individual candidates within the 'pool' for their unique properties, enabling the identification of completely novel candidates. Importantly, pharmacokinetics and biodistribution can be screened in parallel with ligand-binding kinetics and cellular assays.

In addition, the Flycode® technology is ideally suited to *in silico* analysis and use of artificial intelligence to identify specific antibody features, enhance decision making and contextualise the data.

Consequently, the Flycode® approach greatly increases throughput and simplifies the production and property determination of thousands of candidates through expression, purification and analysis in a single experiment, saving substantial quantities of time that would typically be spent performing classical triaging. High-quality data can be obtained rapidly and, coupled with *in silico* analysis, empower novel antibody engineering and precision medicine development.



The Flycode® platform evaluates up to 5000 drug candidates per experiment from a library generated from display, immunisation, engineering or *in silico* approaches in each experiment to identify the drug candidate with the ideal biophysical *in vitro* and *in vivo* properties.

Summary

The Sybody® platform is a novel discovery engine that can rapidly generate conformation-specific antibodies to targets that are otherwise intractable using existing technology.

With a track record in the discovery arena, the *in vitro* Sybody® platform in combination with Flycode® technology is designed to deliver significant contributions to the therapeutics arena. More and completely novel types of therapeutic antibodies can be generated and screened without the need for time-consuming triage.

Flycode® technology permits the simultaneous acquisition of key therapeutic antibody parameters such as pharmacokinetics and biodistribution. Taken together, the Sybody® and Flycode® platforms are ideal differentiators to generate a new wave of multispecific, conditionally active, precision biotherapeutics.

References

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Following the successful use of VHH fragments as therapeutic compounds, Linkster Therapeutics uses its Sybody® and Flycode® technology to build a proprietary therapeutics pipeline.

At the same time, **partnering** with the pharmaceutical industry is a critical part of Linkster Therapeutic's business.

Enquiries for partnering may be addressed to business@linkstertherapeutics.com or via our LinkedIn and Twitter pages.

"The combination of Sybody® and Flycode® technology marks the beginning of an experimental journey towards the next generation of precision medicines featuring excellent biophysical, pharmacokinetic and pharmacodynamic properties."

Iwan Zimmermann, Co-Founder & Chief Scientific Officer of Linkster Therapeutics

Linkster Therapeutics

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