KEY FINDINGS

- **On-chip synthesis of 864 unique PGN fragments**
- **Epitope identification of three anti-PGN antibodies**
- **Scalable tool to explore immune recognition against bacterial cell wall components across health and disease**
- **Assay transferred to industry-available customers**

Peptidoglycan microarrays for antibody biomarker discovery in immune mediated inflammatory diseases

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Background and Aims

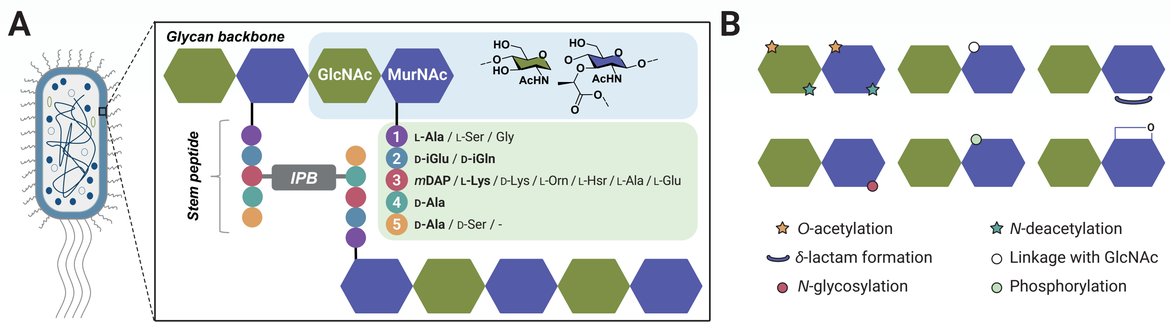

Fig. 1. Peptidoglycan (A) Schematic diagram of bacterial PGN (B) Glycan backbone variations

Peptidoglycan (PGN)

- Bacterial cell wall component
- Activates innate immunity
- Connected with brain development, autoimmunity, and inflammation[1]
- Role in adaptive immunity insufficiently characterized

Can anti-PGN antibodies serve as biomarkers?

- Development of PGN microarrays for high-throughput antibody profiling
- Parallel detection of specific anti-PGN antibodies in patient sera
- Identification of potential PGN epitopes and disease biomarkers

combinatorial Laser-Induced Forward Transfer (cLIFT) [2]

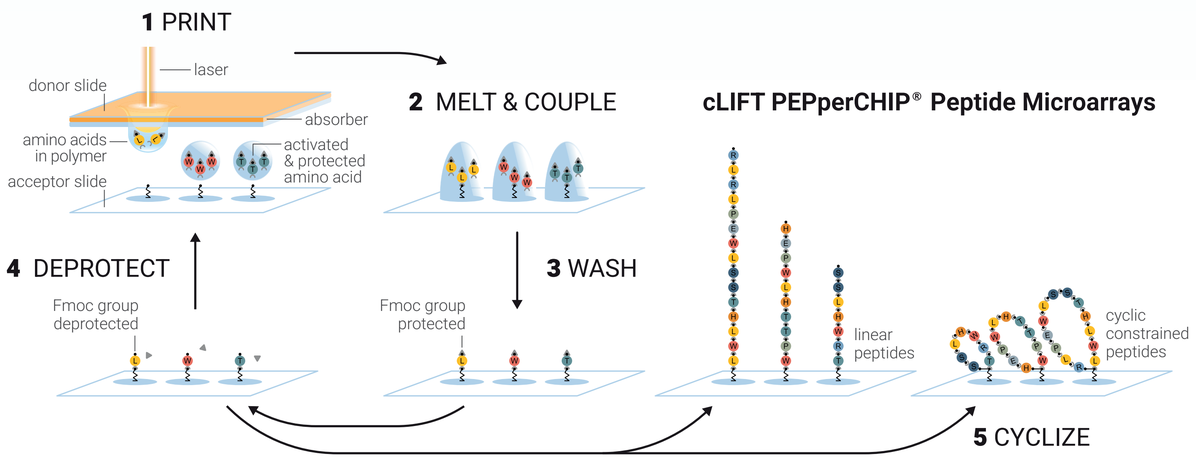

Fig. 2. Solid-phase synthesis with the cLIFT technology. Repetitive transfer of amino acids from donor glass slides onto a functionalized acceptor slide is achieved via laser transfer, reaching up to 50,000 peptide spots per microarray. Further peptide modifications such as cyclization or the attachment of glycans can be achieved to produce specialized microarrays for biological research.

Investigating Glycan Diversity [3]

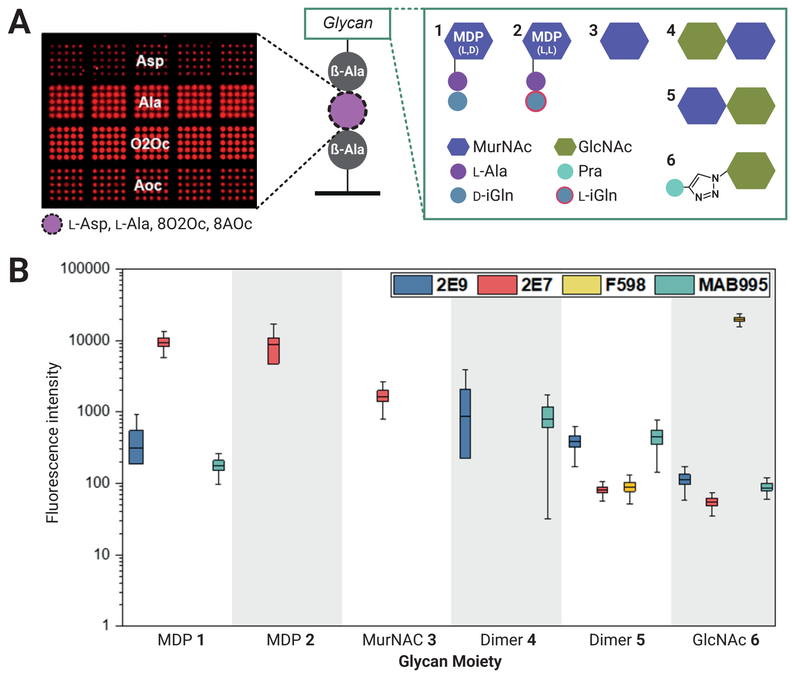

Fig. 3. Epitope identification of anti-PGN mAbs. 2E7 antibody exhibited robust and selective binding to all arrays bearing a MurNAc monomer (**1**, **2**, **3**).

Weaker but discernible interaction in the dimer **5** and GlcNAc **6**. 2E9 and MAB995 mAbs, similar binding behavior, binding selectively to MDP **1**, but not to MDP **2** or to MurNAc **3**. This highlights the importance of the amino acids in positions 1 and 2 of the PGN stem peptides. 2E9 and MAB995 bound to both dimers (**4**, **5**), indicating a second selectivity. F598 mAb high specificity for GlcNAc **6** and weak signal for dimer **5**.

Investigating Amino Acid Diversity [3]

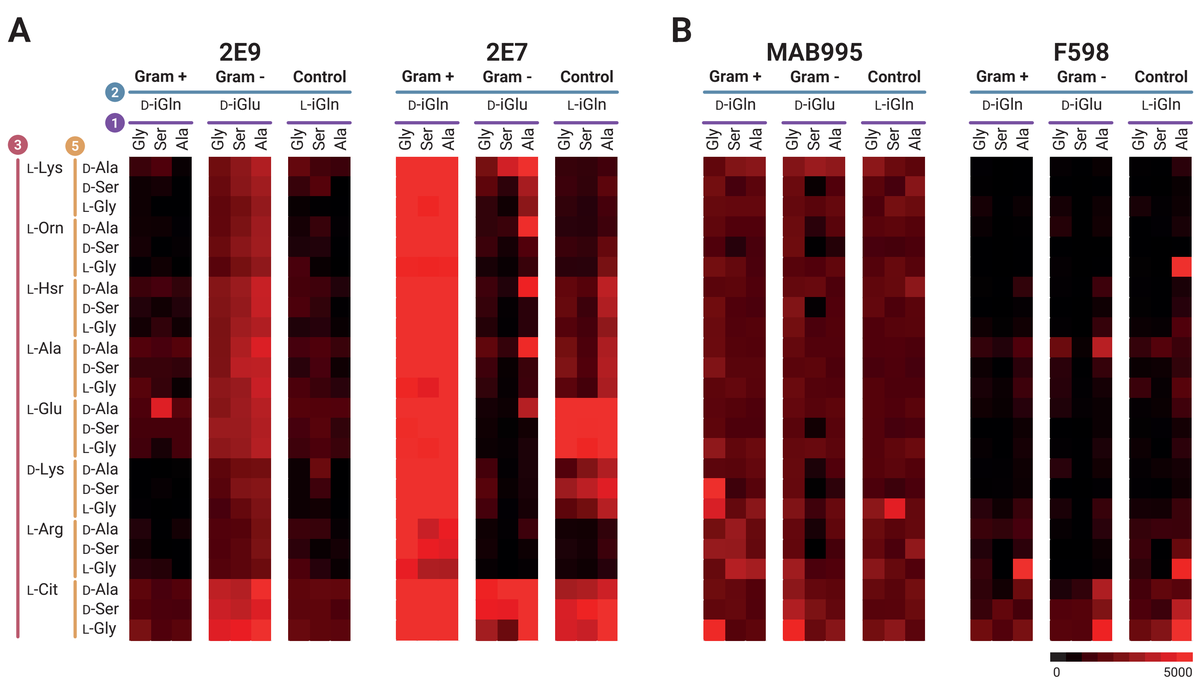

Fig. 4. Generation of combinatorial PGN microarrays. Heat maps of array screening with 216 glycosylated pentapeptides bearing (A) MurNAc **3** and (B) dimer **4**, incubated with monoclonal antibodies 2E9, 2E7, MAB995, and F598. Detection used goat anti-mouse or anti-human IgG polyclonals. MAb 2E9 preferred Gram-negative structures, 2E7 favored Gram-positive PGN. MAB995 and F598 showed minimal sensitivity to stem peptide variations, with recognition driven mainly by glycan identity and length.

Case Study with Inflammatory Disease

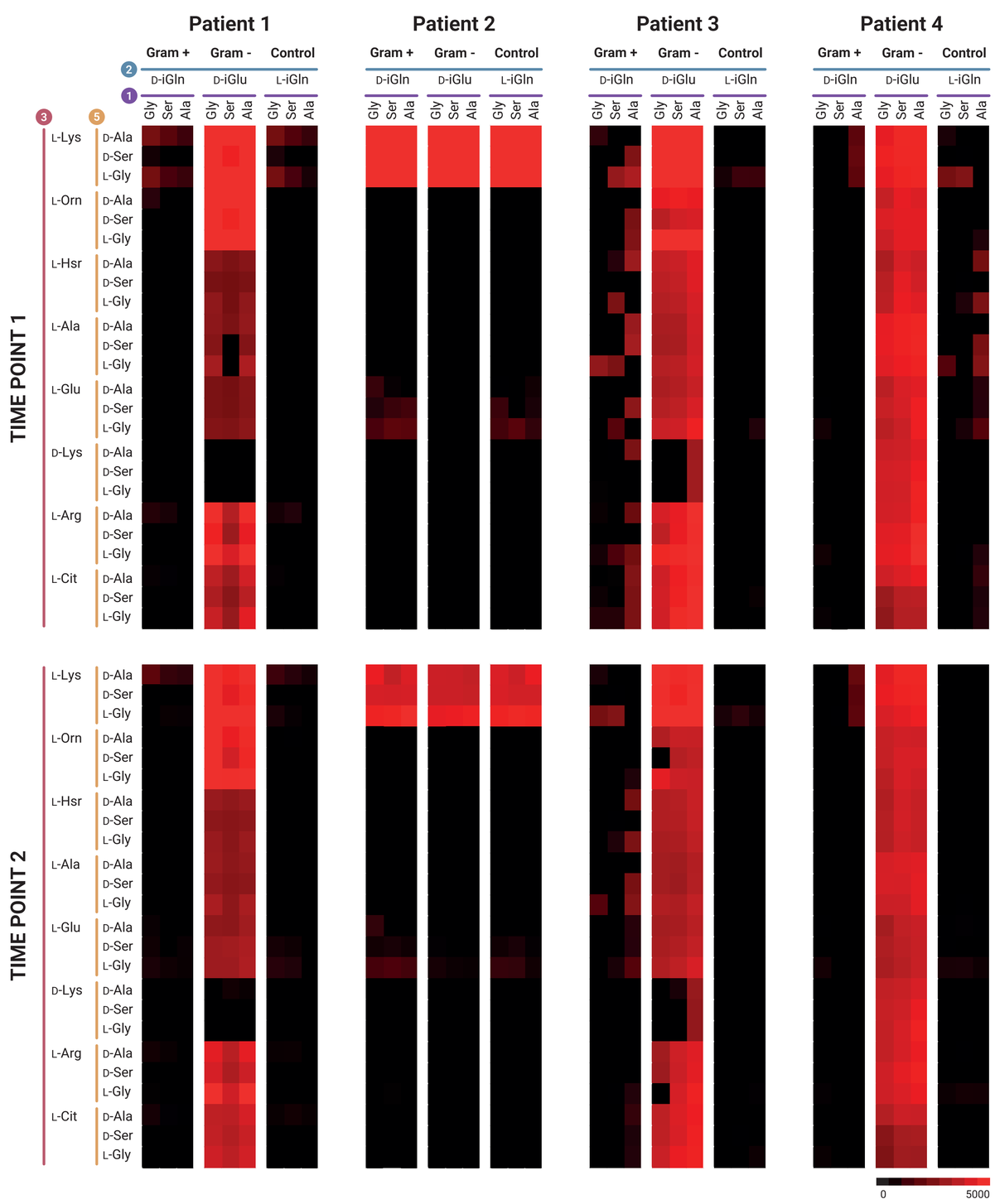

Fig. 5. Antibody profiling of patient sera. PGN microarrays for inflammatory disease screenings on two different time points (month1 vs month 3) show no drastic change between M0 and M3. Individual differences between the samples. Study is ongoing but we validate the robustness of the methodology.

Could PGN Microarrays be your next research tool?

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