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TXMS: Targeted chemical cross-linking Mass Spectrometry can determine quaternary protein structures directly in complex samples

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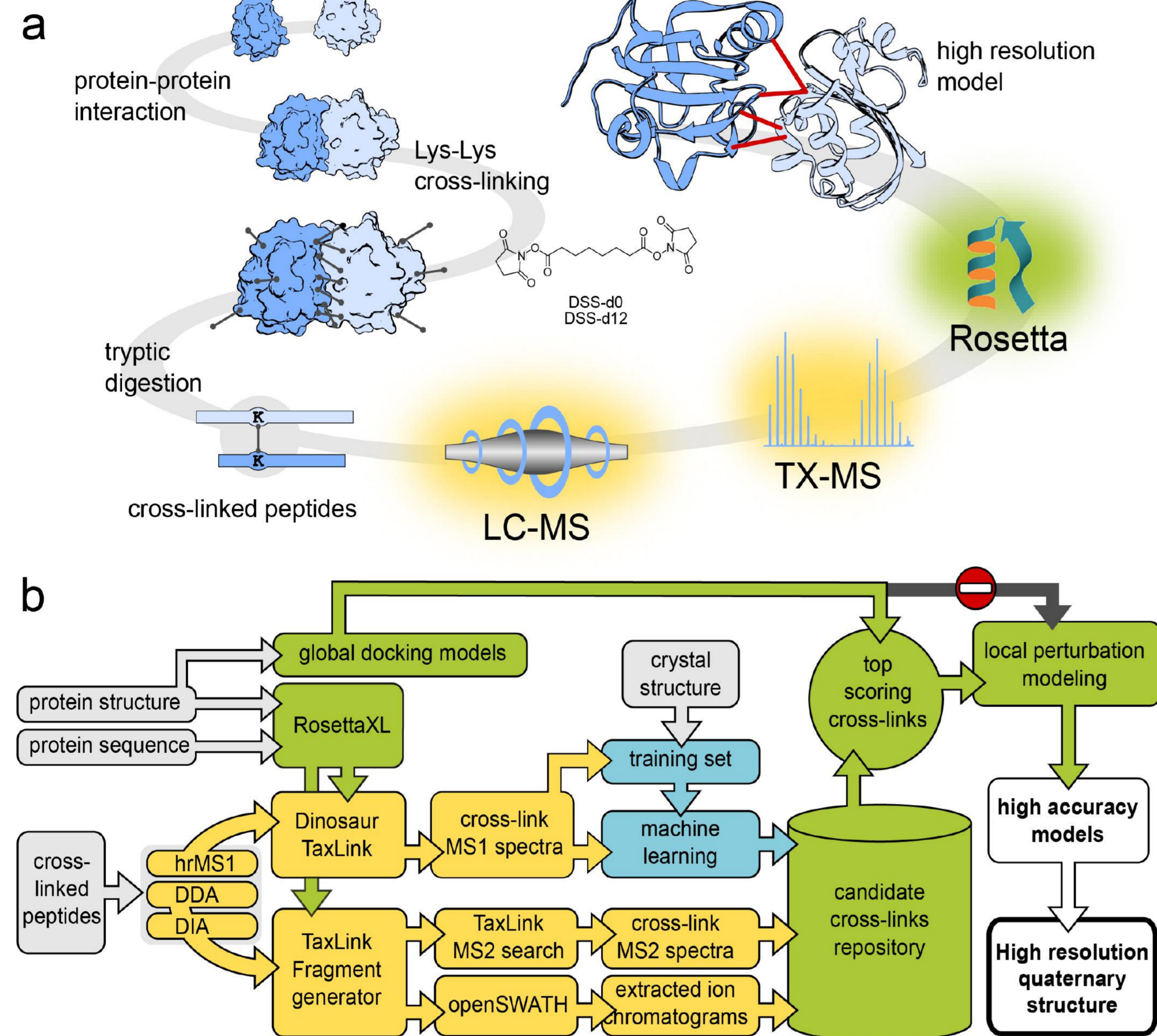
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1. Introduction

Protein-protein interactions are difficult to characterize, especially in complex samples. Protein cross-linking combined with mass spectrometry (XL-MS) and computational modeling is gaining increased recognition as a viable tool in protein interaction studies. Here, we present TX-MS, a targeted approach that combines the sensitivity of XL-MS with the power of computational modeling to rapidly determine the quaternary structures of large protein complexes using data collected on unfractionated and biologically relevant samples. By using Machine learning, TX-MS combines different MS acquisition data (high-resolution MS1 (hrMS1), data-dependent acquisition (DDA) and data-independent acquisition (DIA) and computational protein structure modeling. RosettaDock was used to produce a compendium of quaternary protein structure models which then ranked using the hrMS1 data. The best models were iteratively improved by analyzing DDA data to find fragments of XLs within cross-linkable distance. A high-resolution phase of modeling adjusted the final model which then was validated using DIA-MS data. [Hauri-Khakzad et al., Nature Communications, 2019], [Happonen et al., Nature Communications, 2019]

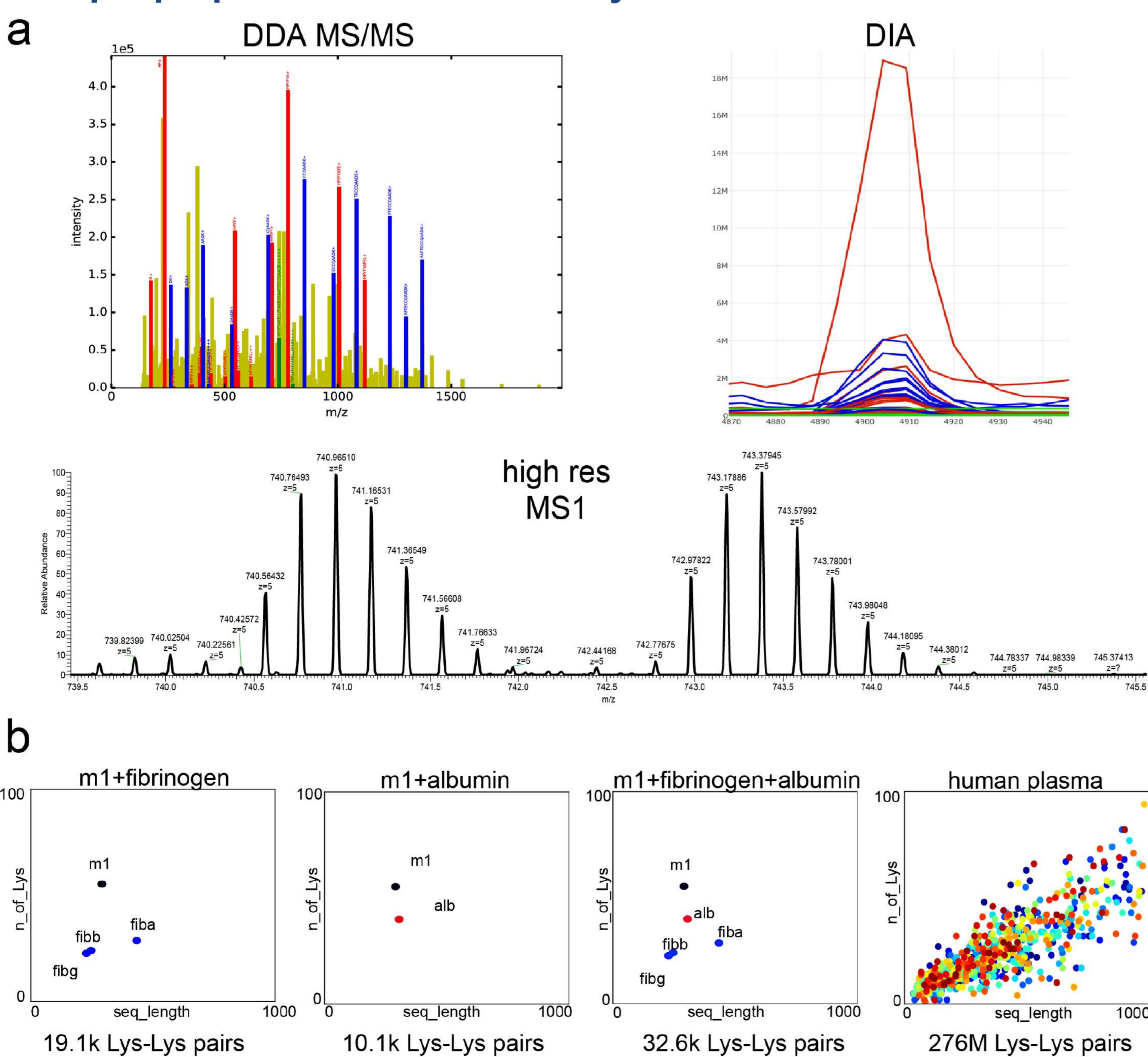
2. Methods

Flowchart



(a) The principle of targeted cross-linking mass spectrometry. (b) Schematic workflow of TX-MS. Assays for targeted XL peptides were calculated from either the protein sequence or structure. Cross-linked peptides were analyzed from LC-MS data using different acquisition modes.

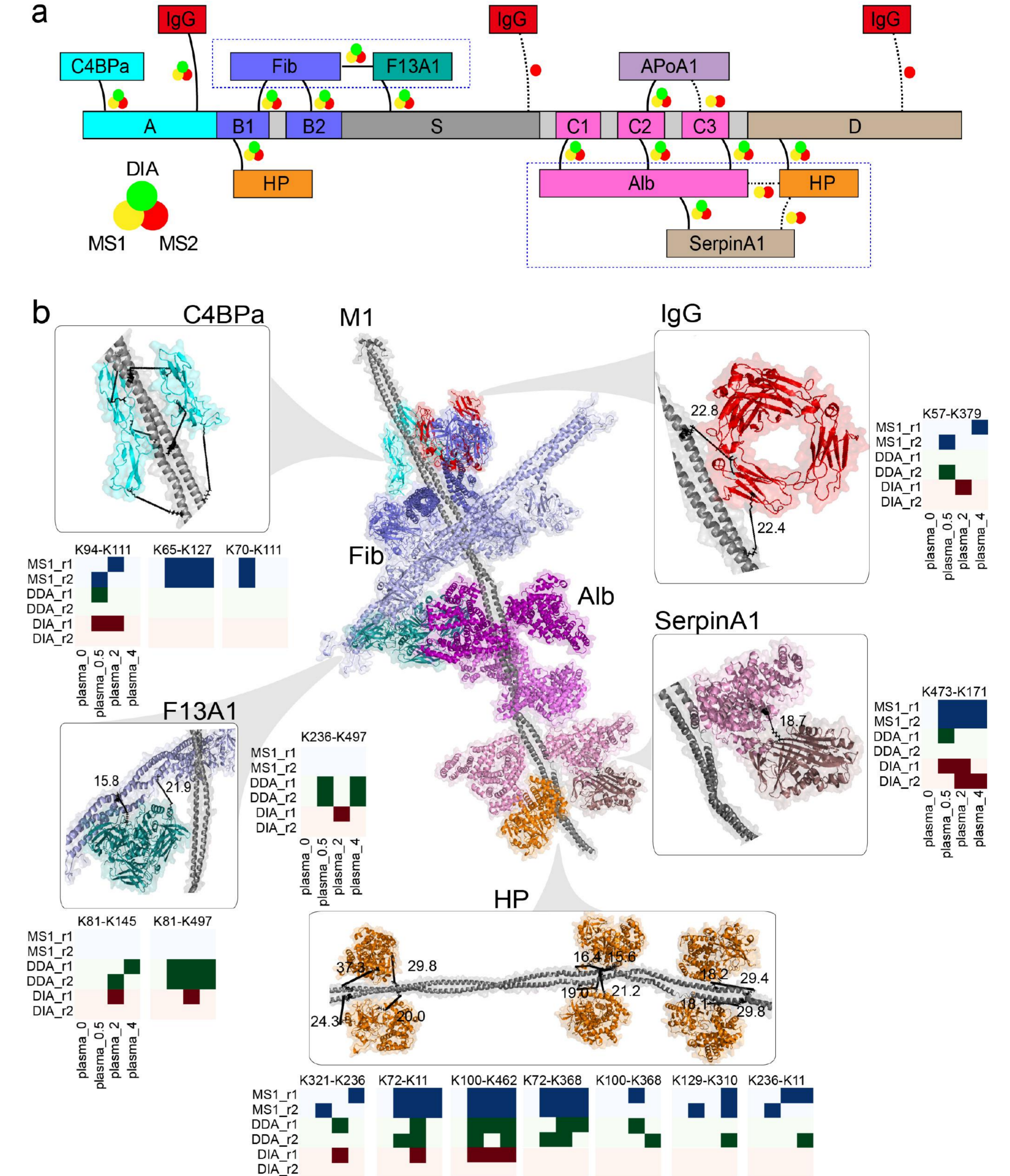
Sample preparation and data analysis



(a) Example data for the three experimental techniques. (b) The scatter plots show the number of lysines as a function of protein length of the protein identified in the sample mixture. The number of theoretical lysine-lysine pairs in each sample increases exponentially with sample complexity, from about 10,000 in the simplest mixture to over 276 million in the most complex.

3. Results

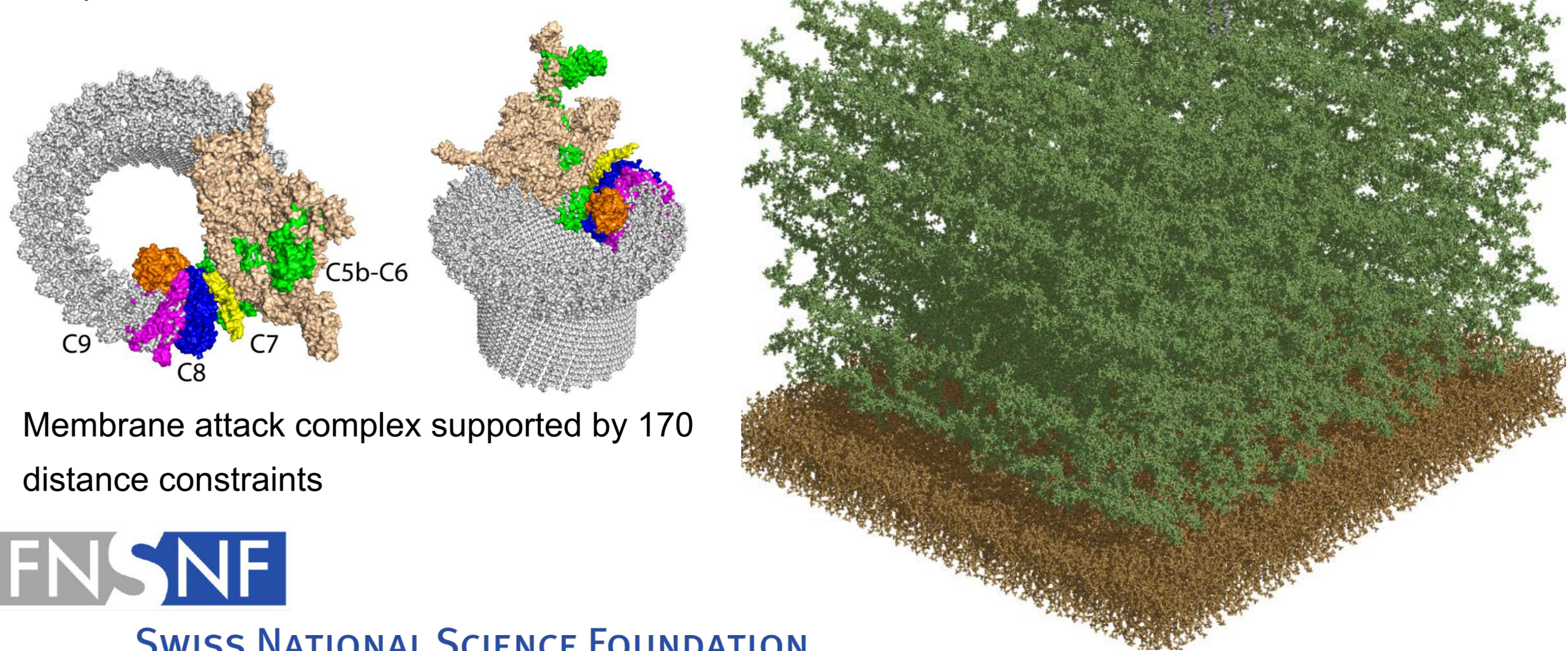
GAS M1 interactome with 15 plasma proteins



The 1.8 MDa M1 protein - human plasma protein complex model created using TX-MS with measurements directly from complex biological samples. *S. pyogenes* cells were exposed to human plasma and thoroughly washed. After application of DSS, proteins were digested with trypsin and analyzed with TX-MS. (a) Schematic binding sites for the M1 protein binders after plasma adsorption on the bacterial surface. (b) Structural model of the M1 protein after plasma adsorption on the bacterial surface.

Conclusion

We demonstrated the applicability of TX-MS by studying two large macromolecular assemblies in a single experiment by cross-linking intact bacteria in human plasma with disuccinimidyl suberate (DSS). First, we elucidated the M1 protein structural interactome on the surface of the *Streptococcus pyogenes* bacterium with 15 different plasma proteins, determining a 1.8MDa large complex supported by more than 200 XLs. Secondly, we modeled the human complement system membrane attack complex on the surface of the bacteria in the same sample, and propose here a near to atomic resolution model supported by more than 170 XLs. TX-MS is, to our knowledge, the only method capable of determining the quaternary protein structure of large protein complexes using data from unfractionated samples.



Membrane attack complex supported by 170 distance constraints

References

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