Ferritin-antibody fragment conjugates: protein scaffolds to modify physicochemical and pharmacokinetic properties of biotherapeutics

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INTRODUCTION

- Growing trend in the biotherapeutics field to develop molecules with a high degree of multivalency.
- Useful for receptor clustering, T-cell recruiting, agonist activation, and half-life extension.
- However, many of the currently available "molecular scaffolds" are polymer-based and raise obvious concerns with respect to biocompatibility and the accumulation of by-products.
- Protein-based scaffolds offer an attractive, "natural" alternative for modifying therapeutic agent properties and functionality
- Ferritin is a ubiquitous protein found in most human cell types as well as in invertebrates, higher plants, fungi and bacteria; its primary function is to store iron.
- In mammals, ferritins are composed of 24 subunits that form an icosahedron with an external diameter of ~12 nm.

AIMS

Here, we present preliminary results describing the development of antibody fragment (Fab)-ferritin conjugates.

Proof of concept

- Commercially available horse spleen ferritin (HSF)
- Optimization of linker chemistry using solvent exposed lysines
- Method development

Protein Engineering

- Development of recombinant ferritin
- Bioconjugation
- In depth biophysical characterization

Biological models

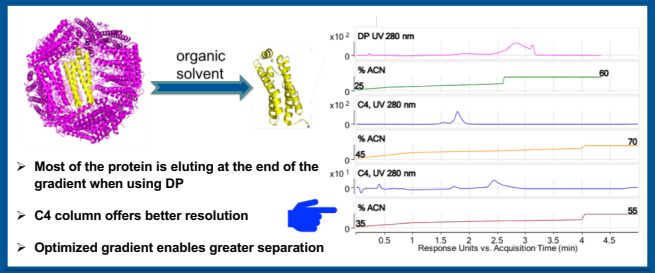
Ex vivo and in vivo analyses

METHODS/TECHNIQUES

- Maleimide-thiol chemistry
- LC/MS
- SEC-MALS
- SEC-QELS

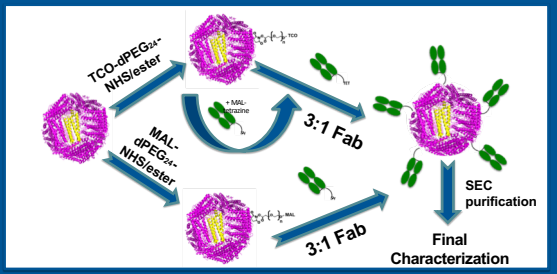
RESULTS

LC/MS method development for optimal reverse phase separation

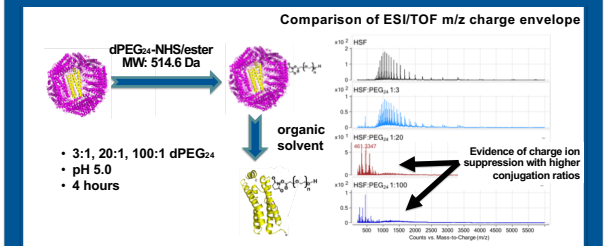


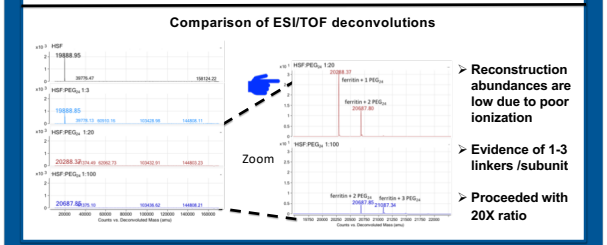
- Most of the protein is eluting at the end of the gradient when using DP
- C4 column offers better resolution
- Optimized gradient enables greater separation

Fab addition achieved using two different bifunctional chemistries



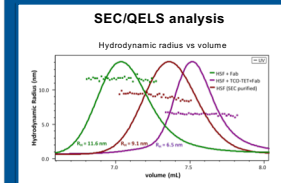
Optimization of linker chemistry using LC/MS





- Reconstruction abundances are low due to poor ionization
- Evidence of 1-3 linkers /subunit
- Proceeded with 20X ratio

Characterization of purified conjugate pools



- Both chemistries yield desired product

		TCO-TET click chemistry		Single bifunctional linker chemistry	
	HSF	HSF + PEG_{24}-TCO	HSF + PEG_{24} + TET + Fab	HSF + PEG_{37}	HSF + PEG_{24} + Fab
R_H (nm)	6.5	7.0	9.1	7.2	11.6
Number of sites*	24	26.9	6.4	17.5	16.8
Step conjugation efficiency (%)	-	112.1	23.8	80	72.9
Overall recovery (%)	-	-	26.7	-	58.3

- Neither chemistry enabled complete saturation of 24 Fabs per ferritin
- Unstable maleimide and NHS/ester reactive groups increase product heterogeneity
- Multiple surface exposed lysines led to heterogeneous conjugation mixture

CONCLUSIONS

These results confirm that Fab-ferritin conjugation can be achieved. In addition to the possible modification of Fab elimination kinetics and the potential for more prolonged therapeutic effect, the conjugates may offer other attributes well-suited for drug delivery applications that require multivalency.

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