21st Annual European Pharma Congress

May 20-22, 2019 | Zurich, Switzerland

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

Whitney Shatz[1,2], Craig Blanchette[2], Robert F. Kelley[3], Remo Perozzo[1] and **Yogeshvar N. Kalia[1]**
[1,2,3]University of Geneva and University of Lausanne, Switzerland

There is a growing trend in the biotherapeutics field to develop molecules with a high degree of multivalency. This can be 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. In contrast, protein-based scaffolds offer an attractive, "natural" alternative for modifying therapeutic agent properties and functionality. Ferritin is a ubiquitous protein found in most cell types of humans in addition to invertebrates, higher plants, fungi and bacteria; its primary function is to store iron (1). In mammals, ferritins are composed of 24 subunits that form an icosahedron with an external diameter of ~12 nm and an overall MW of ~474 kDa (2). Ferritin and its derivatives have already demonstrated their utility as "molecular cages" for applications in drug delivery (3,4). In addition, ferritin can be easily coupled covalently to biomolecules through the presence of multiple surface-exposed lysine residues (5). Site directed mutagenesis and the introduction of new amino acid residues create even more opportunities to introduce new functionality (6). Here, we present preliminary results describing the development of antibody fragment (Fab)-ferritin conjugates. In this first step, a strategy was developed for the covalent attachment of multiple Fab units to the ferritin cage, yielding a conjugate with 24 Fabs per ferritin cage (i.e. one Fab per subunit). Following optimization of the conjugation strategy, an in-depth characterization of the conjugates was performed using multiple techniques – including DLS, SEC-MALS, LC/MS, viscosity measurements, and target activity. The results confirmed that Fab-ferritin conjugation was successfully achieved. In addition to the possible modification of Fab elimination kinetics *in vivo* and the potential for more prolonged therapeutic effect, the conjugates may offer other attributes well-suited for drug delivery applications that require multivalency.

Recent Publications

1. Theil EC. Ferritin: structure, gene regulation, and cellular function in animals, plants, and microorganisms. Annu Rev Biochem. 1987;56:289–315.

2. Finazzi D, Arosio P. Biology of ferritin in mammals: an update on iron storage, oxidative damage and neurodegeneration. Arch Toxicol. 2014 Aug 15;88(10):1787–802.

Notes:

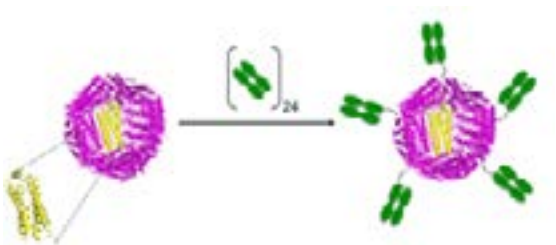

Figure 1. Schematic of ferritin conjugation with icosahedron ferritin depicted in purple, a single subunit in yellow and with addition of Fab in green.

3. He D, Marles-Wright J. Ferritin family proteins and their use in bionanotechnology. N Biotechnol. 2015 Dec 25;32(6):651–7.

4. Bhaskar S, Lim S. Engineering protein nanocages as carriers for biomedical applications. Nature Publishing Group. 2017 Mar 31;9(4):e371–18.

5. Zeng Q, Reuther R, Oxsher J, Wang Q. Characterization of horse spleen apoferritin reactive lysines by MALDI-TOF mass spectrometry combined with enzymatic digestion. Bioorg Chem. 2008 Oct;36(5):255–60.

6. Lee JM, Kim JA, Yen T-C, Lee IH, Ahn B, Lee Y, et al. A Rhizavidin Monomer with Nearly Multimeric Avidin-Like Binding Stability Against Biotin Conjugates. Angew Chem. 2016 Feb 2;128(10):3454–8.

Biography

Whitney Shatz received her M.S. in Biochemistry and Molecular Biology from the University of California in Santa Barbara, characterizing bacterial enzymes involved in the epigenetic process of DNA methylation. Since 2007, she has worked within the research organization at Genentech, supporting production and characterization of large molecule biologics. During her 11-year tenure, she has made significant contributions to the investigation of structure activity/relationship in antibody-dependent cell cytotoxicity (ADCC), as well as to the advancement of novel bispecific antibodies in a variety of disease areas. More recently, her focus has shifted to the development and characterization of protein-polymer bioconjugates for long-acting drug delivery. In addition, since 2016 she has been concurrently pursuing a doctorate in Pharmaceutical Sciences at the University of Geneva. g.

Notes: