# Biomanufacturing with the *i*CapTag™: a Novel Scalable and Tagless Protein Purification Platform


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## Background

The purification of recombinant proteins at laboratory and manufacturing scales represents a substantial difficulty in the development of new therapeutic proteins. At laboratory scales, researchers rely on affinity tags to rapidly purify diverse new targets. These approaches cannot be used at manufacturing scales however, due to the potential immunogenicity of the tags and the difficulty of removing the tags at manufacturing scales. The result is a divided world of protein purification, where tag-based methods dominate in the laboratory, but non-tag methods must later be developed for manufacturing. The disconnect between these methods can significantly delay drug development and approval, and in some cases can stop promising therapeutics from ever being commercialized. The end result is untreated diseases, high drug costs, and slow market entry for new lifesaving medicines.

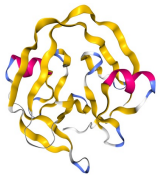


**Figure 1.** Image of Npu intein, a main component of patented *i*CapTag technology, which is able to self-assemble and under specific pH-buffer conditions also self-cleave allowing on target protein to be seamlessly purified. The two pieces of Npu are shown in **Figure 2** as a red box cartoon, that can bind together, "disassemble" during stripping step and reassemble again during target protein binding, where target protein exist as a complex and is expressed together with Npu C-terminal. On another hand cleavage of protein can be triggered by pH change.

## Definitions

**Inteins** (Intervening proteins) sequence is located between two exteins, N- and C-terminals, in nature. Interestingly, inteins naturally can splice or cleave, which led to development of intein-based purification technology here described. Moreover, when rationally engineered **inteins can self-cleave and self-assemble in predictable way. Intein-based tag technology** here presented is enabled through a C-terminal cleaving reaction induced simply by pH shift of the buffer. Note, that intein cleavage rate is pH and temperature dependent.

To address these issues, we developed a self-removing tag-based technology, known as the *i*CapTag (intein Capture Tag). It is a single platform that is designed to bridge all phases of biopharmaceuticals development from early R&D through manufacturing. It is a promising, patented and disruptive technology that is based on an engineered protein called an intein. The disruptive advantage of the intein is its ability to provide a self-cleaving tag for simple protein purification, where the tag is automatically removed during the purification process. Moreover, this product can be used with a standard laboratory equipment at laboratory and manufacturing scales.

Therefore, this technology retains all the power and convenience of conventional tag methods but has a unique ability to deliver an unmodified target protein at the end of the process. This combination of features provides strong advantages for applications in pure research and drug discovery, while also providing a platform for use in biopharmaceutical manufacturing. Thus, this single-column purification platform provides a disruptive advance in the entire drug development process.

## Method

The *i*CapTag technology is a single platform for protein purification and is designed to be used in small and large scale. Therefore, steps here described are reminiscent to those seen in ordinary R&D lab developing new biopharmaceuticals and in manufacturing (see **Figure 2**).


**Figure 2.** Steps of protein purification using *i*CapTag technology that bridge R&D and manufacturing.

### FLEXIBLILITY OF EXPRESSION SYSTEMS

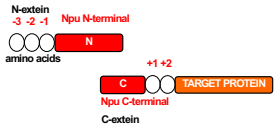
The *i*CapTag can be used with commonly used expression systems such as mammalian and bacterial host-cells to develop biopharmaceuticals in R&D labs as well as by using cell-free expression systems.
Based on internal data, the tag here described does not interfere with the secretion of expressed target protein.
At the same time, it was noted that this technology is ideal for the proteins that can be well expressed and fold.

### NEUTRALITY TO OTHER BUFFER ADDITIVES

While the pH is controlled the *i*CapTag technology was found neutral to salts, sugars and other buffer additives including protease inhibitors used for decrease of proteolytic degradation.

### CLEAVAGE RATE CONTROLLABILITY & PREDICTABILITY

The cleavage rate (k) of the target protein of interest is simply controlled by shift of pH from *e.g.*, pH 8.5 to pH 6.2.
Based on internal research, the rate of cleavage and pH sensitivity reminds mostly the same at pH 8.5 independently of first "-1" position of amino acids (a.a.) located at the beginning of Npu N-terminal. However, pH sensitivity and cleavage rate dramatically increase at pH 6.2 and depend on addition of "-1" type of a.a. At the same time, "+1" position of a.a. located at the beginning of target protein (added or naturally occurring) was found to be critical for controllability of cleavage rate (smaller & charged a.a. led to slower k, aromatic & larger a.a. led to faster k).




### ABILITY OF RESIN REGENERATION

The *i*CapTag technology can be regenerated by stripping buffer containing 150 mM phosphoric acid and 500 mM sodium chloride (pH ≤ 2.0).

### STERILIZATION BUFFERS

The *i*CapTag can be used with caustic buffers without a loss of resin activity.

### *i*CAPTAG APPLICATIONS

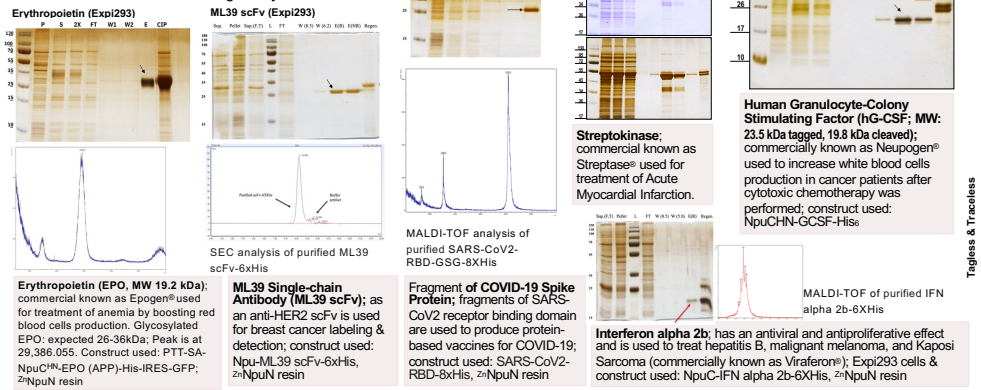
The *i*CapTag can be used for novel and well-known proteins, peptides, fragments of proteins and biosimilars. This technology is also ideal for high-throughput studies, and it can be easily implemented within existing R&D labs and CMO/CRO set-up. This technology is also scalable and can be used as a single platform from R&D to manufacturing.

### TAGLESS PROTEIN VIA ONE PLATFORM

It was shown that the beauty of the intein-based technology is that the cleavage of the target protein leads to precise and tagless release of protein. It was also shown that in some cases, the activity of the tested proteins such as GCSF (blue line) was higher than standard purchased from PeproTech (see red line shown in **Figure 3**).

## Results

Examples of protein purifications of known biopharmaceuticals using intein-based technology are shown below for R&D usage only.




**Erythropoietin (EPO, MW 19.2 kDa)**; commercial known as Epogen® used for treatment of anemia by boosting red blood cells production. Glycosylated EPO: expected 26-36kDa; Peak is at 29,386.055. Construct used: PTT-SA-NpuC$^{HN}$-EPO (APP)-His-IRES-GFP; $^{Zn}$NpuN resin

**ML39 Single-chain Antibody (ML39 scFv)**; as an anti-HER2 scFv is used for breast cancer labeling & detection; construct used: Npu-ML39 scFv-6xHis, $^{Zn}$NpuN resin

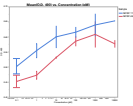
Fragment **of COVID-19 Spike Protein**; fragments of SARS-CoV2 receptor binding domain are used to produce protein-based vaccines for COVID-19; construct used: SARS-CoV2-RBD-8xHis, $^{Zn}$NpuN resin

**Streptokinase**; commercial known as Streptase® used for treatment of Acute Myocardial Infarction.

**Human Granulocyte-Colony Stimulating Factor (hG-CSF; MW: 23.5 kDa tagged, 19.8 kDa cleaved);** commercially known as Neupogen® used to increase white blood cells production in cancer patients after cytotoxic chemotherapy was performed; construct used: NpuCHN-GCSF-His$_6$

**Interferon alpha 2b**; has an antiviral and antiproliferative effect and is used to treat hepatitis B, malignant melanoma, and Kaposi Sarcoma (commercially known as Viraferon®); Expi293 cells & construct used: NpuC-IFN alpha 2b-6XHis, $^{Zn}$NpuN resin

**Figure 3.** Comparison of activity assays of GCSF purified using *i*CapTag technology vs. purchased standard (PeproTech). NFS-60 cell line was seeded at 10,000 cells/well. MTS assay was also performed for quantification of cell proliferation.



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## References


1. Belfort M, Belfort G, Derbyshire V, Wood DW, Wu W, Inventors; Rensselaer Polytechnic Institute, assignee. Genetic system and self-cleaving inteins derived therefrom, bioseparations and protein purification employing same, and methods for determining critical, generalizable amino acid residues for varying intein activity. US patent 6,933,362. August 24, 2005.
2. The Protein Data Bank H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne (2000) Nucleic Acids Research, 28: 235-242.
3. Wu WY, Miller KD, Cochrum M, Wood DW. Intein-mediated one-step purification of Escherichia coli secreted human antibody fragments. Protein expression and purification. 2011;76(2):221-228.
4. Wu C, Yu D, Cochrum M, Wood DW. Ligation-independent cloning and self-cleaving intein as a tool for high-throughput protein purification. 2015;57(2):169-174.
5. Shi C, Han TC, Wood DW. Purification of Microbially Expressed Recombinant Proteins via a Dual ELP Split Intein System. Methods Mol Biol. 2017;1495:13-25.
6. Ma B, Nalle DF, Zhu JS, Wood DW, Inventors. Compositions related to controllable intervening protein sequences (CIPS) comprising reversible zinc-binding motifs and inteins. US patent 9,796,967. October 24, 2017.
7. Lahiry A, Fan Y, Stimple SD, Raith M, Wood DW. Inteins as tools for tagless and traceless protein purification. Journal of Chemical Technology and Biotechnology. 2017;93(7):1827-1835.
8. Fan Y, Mozzi JM, Stimple SD, Han T-C, Wood DW. Column-Free Purification Methods for Recombinant Proteins Using Self-Cleaving Aggregating Tags. Polymers. 2018;10(5):468/461-468/412.
9. Wood DW, Shi C, Inventors; Ohio State Innovation Foundation, assignee. Protein Production Systems and Methods Thereof. US patent 10,066,027. September 4, 2018.
10. Izabela Gierach and David Wood, Self-Cleaving Tags Based on Split Inteins: Increased Reliability Enabling Higher Throughput Applications, American Pharmaceutical Review, 2019, 22(1), 66-69.