# AFFINITY TAGS HEAD TOWARD THE VACCINATION CLINIC

Protein purification is a tricky business. Separating a desired protein from a soup of other proteins—such as in the media used to grow cells—is particularly taxing. Generic column-based purification procedures, which exploit differences in protein size and charge, tend to give imperfect results. “You often have to use several different steps to get sufficient protein purity,” says Rebecca Ashfield, senior project manager at the Jenner Institute.

Affinity chromatography was developed as an alternative, a more targeted purification approach than these generic procedures. It has been a laboratory staple for decades. The affinity resins contain components, such as antibodies or heavy metal ions, which bind to desired proteins. When a crude mixture is passed through an affinity resin, the desired protein is trapped. Other proteins and impurities flow through the column before the target protein is released and collected.

The technique was initially limited to purifying protein types for which a suitable resin had been developed. Around 30 years ago, affinity tags were introduced to significantly expand the types of proteins compatible with this purification strategy. This approach involves attaching a short peptide sequence—known as the affinity tag—to a target protein using genetic engineering. The resin recognizes the affinity tag. It is a useful one-step technique that results in a pure protein, Ashfield says.

Typically, however, researchers avoid using affinity tags in the purification of therapeutic proteins. First, cleaving off the affinity tag during manufacture is not generally an option in line with good manufacturing practice (GMP) regulations that are in place to ensure the safety, purity, and effectiveness of pharmaceuticals. And leaving extra material on a protein destined for use as a vaccine is usually frowned upon because of the possibility of antibodies being made in the body against the affinity tag rather than the therapeutic protein, Ashfield explains.

She and her colleagues recently gained approval to leave one very short affinity tag—just four amino acids long—on a malaria vaccine candidate for clinical trials. This has opened the door to a range of novel protein-based malaria vaccine candidates that were proving impossible to purify in any other way. These agents are all either in or progressing toward clinical trials. The team is working with the assumption that the tag will be able to remain in any vaccines that are eventually licensed. The molecules could someday soon provide protection against malaria, a mosquito-borne disease that still kills about 435,000 people globally each year.

## PROGRESS ON A MALARIA VACCINE

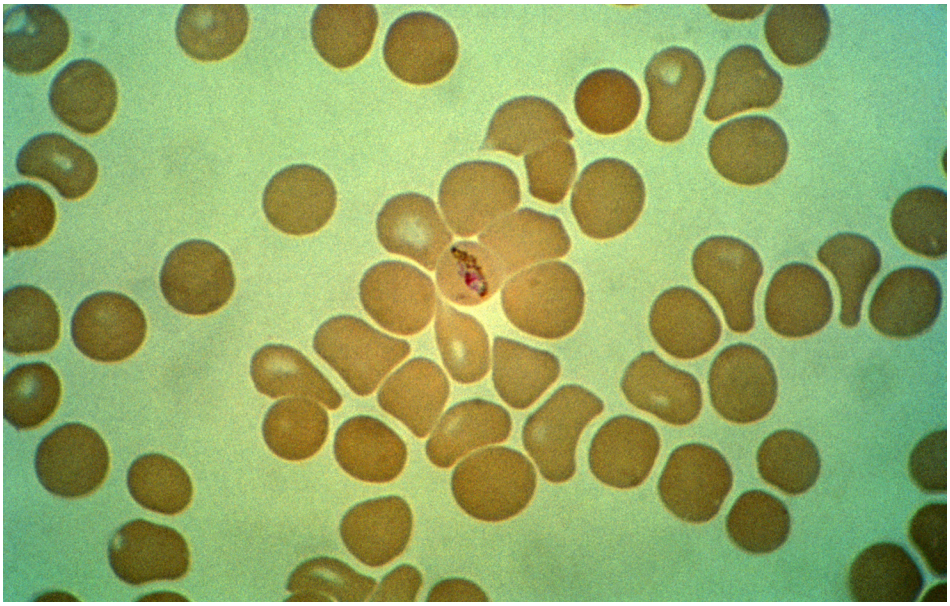
Malaria cases have dropped significantly since 2010, thanks to insecticide-treated mosquito nets, indoor residual spraying of insecticides, and antimalarial drugs. But progress has plateaued in recent years. There are still over 200 million clinical cases of malaria every year worldwide. "We desperately need a vaccine to add to the established control measures," says Simon Draper, professor of vaccinology and translational medicine and Ashfield's colleague at the Jenner Institute.

No malaria vaccines have been approved, but many are in development. The most advanced is GlaxoSmithKline's RTS,S/AS01, which targets the pre-erythrocytic stage of the malaria parasite's life cycle (when it enters and replicates in the affected person's liver). The vaccine candidate is a virus-like particle (VLP), which means it is a collection of proteins forming a particle that resembles a virus and can trigger an immune response without the risk of infection. Clinical trials showed that the particle has modest efficacy. It is being introduced in selected areas of Ghana, Kenya, and Malawi as part of a large pilot implementation program. Vaccines with greater efficacy continue to be pursued.

Another promising vaccine candidate is RH5.1.[1,2] It targets the blood-stage of the life cycle, when the parasite has left the liver and entered the blood. "It's difficult to stop the parasite invading red blood cells because it has lots of backup strategies to get into the cell," Draper explains. "So if you hit it one way, it has a backup and then another backup." In 2010, he adds, "it was discovered that there was one protein, called RH5, which the parasite absolutely needs to get into the red blood cells, and there's no backup."

RH5 is secreted by the parasite and serves as a connector, bonding with a protein on the surface of a red blood cell. RH5 thus forms a physical link between parasite and erythrocyte, giving the invader the opportunity to slip inside the red blood cell. Antibodies that block RH5 from connecting with these surface proteins disrupt the invasion process. "The idea of the vaccine is that you introduce antibodies against RH5 in the person that you vaccinated," Draper says. "Thereby if they ever get infected with malaria for real, it can't infect their red blood cells and therefore can't make them sick."

RH5 proved difficult to make in the laboratory, however. "It took us a long time to crack that problem and discover that you can make the RH5 protein in insect cells and express it into the supernatant," Draper says. "The problem with insect cells is that the supernatant that you grow the cells in is very rich. So we had difficulty purifying it using traditional technologies."



**Light micrograph showing the parasitic protozoa Plasmodium malariae (see the most central blood cell) which causes malaria in humans; Magnification 1000x at 35mm.**

***Image credit: Science Source***

At this point, the researchers approached Thermo Fisher Scientific for help. "We teamed up with them because they had this new purification system called the C-tag," Draper says. In collaboration with researchers from the VIB in Belgium, Jan Steyaert and Els Pardon, the company developed an affinity resin (CaptureSelect™ C-tag Affinity Matrix) that included an antibody that could capture any protein with a specific four-residue sequence on its C-terminus—the C-tag (See Discovering C-tag box).

The Jenner Institute scientists put a C-tag sequence on the end of the RH5 protein and tried purifying it using the CaptureSelect™ C-tag chromatography resin. "We discovered it was a fantastic means by which to pull the RH5 protein out of the rich supernatant mixture from the insect cells," Draper says. The team achieved a greater than 85% recovery and greater than 70% purity in a single step.[2]

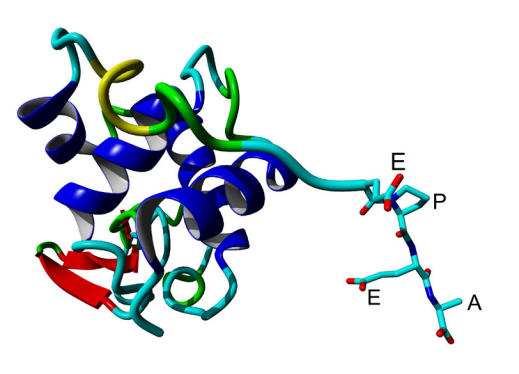
That wasn't the end of the story, however, as the CaptureSelect™ resin was approved only for research use. "To use an affinity resin for manufacturing of a therapeutic molecule—one that will be injected into patients or healthy volunteers—the quality level of the resin must be higher than for research use only," says Pim Hermans, director of ligand discovery at Thermo Fisher Scientific. His team worked to redesign its resin to significantly increase its binding capacity and meet all GMP requirements.[1]

With the regulatory hurdles cleared, RH5.1 was to be the first protein vaccine with an attached C-tag to enter clinical trials. It has completed safety and small-scale efficacy trials in the UK, and Draper hopes to start a trial in Tanzania next year. "We're confident after having a discussion with the regulatory authorities that it's not going to be an issue throughout clinical development to use this particular tag," Ashfield says.

## DISCOVERING C-TAG

Several small affinity tags are currently being used in the laboratory. Some of these, like C-tag and FLAG-tag, utilize antibody-filled chromatography resins. Others, such as polyhistidine tags, work based on the strong interaction of histidine residues with heavy metal ions, such as zinc, coupled to a resin.

The C-tag, a four-amino-acid-long sequence (glutamic acid-proline-glutamic acid-alanine), is the smallest affinity tag that can be fused at the C-terminus of any recombinant protein. A single-domain camelid antibody with high affinity and selectivity for this short peptide sequence was identified around a decade ago[4] by scientists from the VIB in a study headed by Christopher M. Dobson from the University of Cambridge.




**The C-tag, a sequence of glutamic acid (E), proline (P), glutamic acid (E), and alanine (A), is the smallest affinity tag that can be fused at the C-terminus of a recombinant protein. Here, it is fused to lysozyme.**

***Image credit: Thermo Fisher Scientific***

The team was studying how the α-synuclein protein aggregates to form the amyloid structures known to participate in neurological disorders, such as Parkinson's disease. They mixed antibody fragments with α-synuclein and looked for any resulting folding changes. "What they observed was that one of these antibodies was selectively targeting the C-terminal end of the α-synuclein protein," Hermans says. "The crystal structure showed that the four residues at the C-terminal end were largely captured by this antibody fragment, and it was really deep down in the binding pocket."

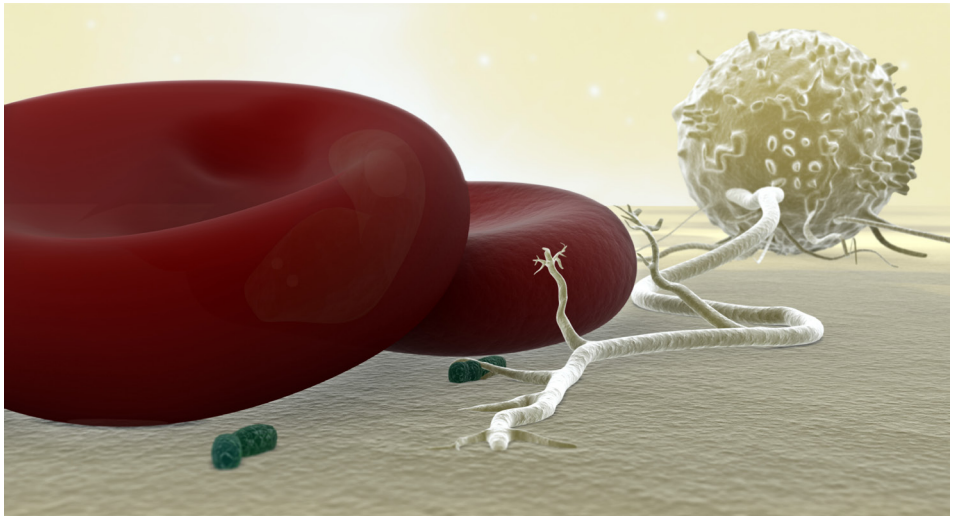
The scientists wondered if this combination of high selectivity and tight binding meant that the four amino acid sequence at the C-terminus of α-synuclein had the potential to be an antibody-based affinity tag. The VIB approached Hermans's group at BAC (now a part of Thermo Fisher Scientific). "They knew that we are making products for affinity purification," Hermans says. "And with the support of Jan Steyaert and Els Pardon this led to the development of a new type of affinity tag resin."

C-tag offers a number of advantages over other affinity tags, he adds. Its small size means that, unlike some of the larger tags, it doesn't alter protein folding and functionality. It has also been shown to punch above its weight in terms of purity achieved. For example, researchers at the Jenner Institute found that the purity of their C-tagged vaccines was far higher than observed when they used a polyhistidine-tag.[2] C-tag purification also has environmental advantages: the C-tag resin doesn't contain metal ions, so there is no heavy metal waste.

## A VACCINE TECHNOLOGY PLATFORM

At the Jenner Institute, the C-tag system is now a go-to approach for purifying protein vaccine candidates to meet GMP standards. It's much quicker and cheaper to develop the manufacturing process for a novel vaccine when researchers know generally how to purify the proteins, according to Draper. "You haven't got to work it out from scratch in a bespoke manner each time," he says.

A second malaria vaccine candidate purified with C-tag, R21c, is in clinical trials. "We were struggling to manufacture this second vaccine in a way that met GMP requirements before we tried using a C-tag," Ashfield says. Developed by Professor Adrian Hill, director of the Jenner Institute, R21c is similar in concept to GSK's RTS,S/AS01: it's a virus-like particle that targets the pre-erythrocytic stage of the malaria parasite life cycle. R21c is in early stage trials in Kenya, the UK, and Burkina Faso.[3]



**Computer artwork of the malarial parasite Plasmodium falciparum infecting blood cells.**

***Image credit: Science Source***

Draper's lab is also using the C-tag system for a second-generation RH5.1 malaria vaccine. "There are two other proteins that we now have learned form a complex with RH5, so we are also making both of those targets as well," Draper says. They will be manufactured very much like RH5.1—grown in insect cells with C-tags attached. "Our ultimate ambition is to stick those proteins onto a virus-like particle with the hope it will give us a stronger immune response," he says.

Though to this point the Jenner Institute has used the C-tag system only for malaria vaccines, Draper foresees it finding much wider clinical use. "This isn't a specific technology for malaria," he says. "If you were making a vaccine for HIV or tuberculosis or Ebola or cancer or whatever, the C-tag could potentially be useful. It could be applied to lots of different vaccines, irrespective of the disease that you're targeting."

## REFERENCES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