PROXCYS
Downstream Biosystems

Proxcys B.V.

cTRAC – Strongly increased yield in mAb production by eliminating Unit-operation

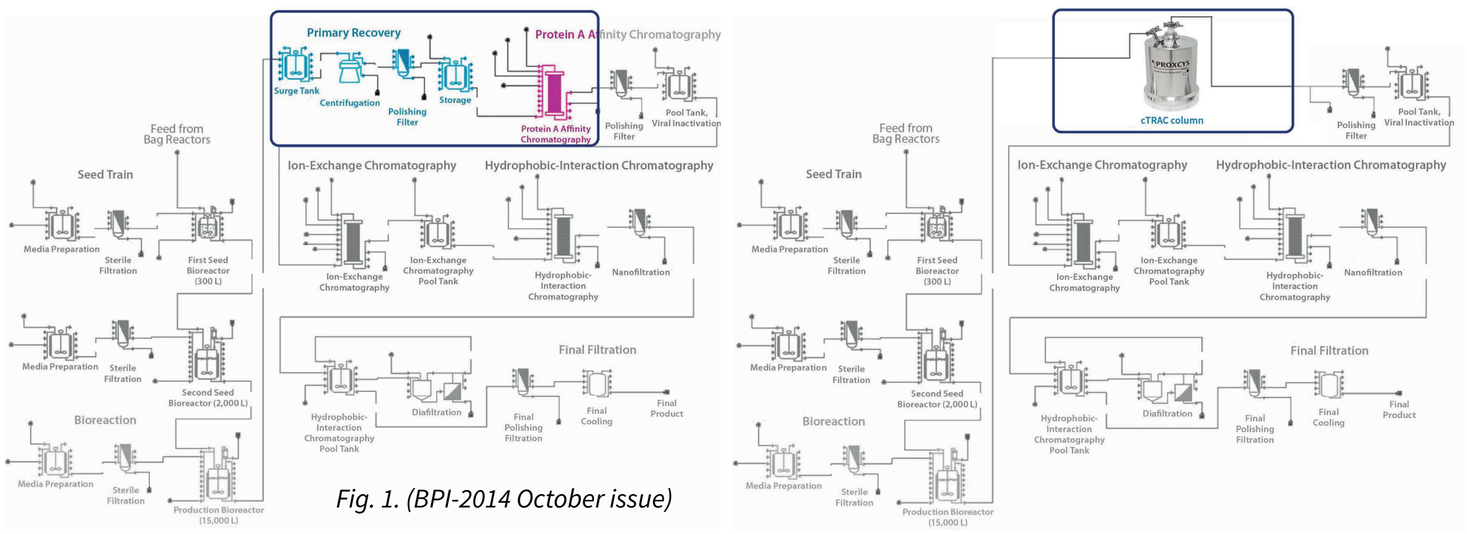

Fig. 1. (BPI-2014 October issue)

By applying cell Tolerant Radial Affinity Chromatography (cTRAC) as a link between Up-stream (USP) and Downstream (DSP), clarification losses are avoided and the net process yield of monoclonal Antibody (mAb) increases by approximately 10%. Ten percent seems small, but because of the enormously valuable mAb, the impact is significant. In Fig. 1. [1], the schematic of a generally applied 'platform production process' for the batch production of therapeutic antibodies (mAb) from Chinese Hamster Ovarian (CHO) cells is shown. A proven setup that is also used for other recombinant products from various cell lines. With the application of cTRAC-ProteinA, clarification is omitted (framed in blue in Fig.1). While maintaining the selectivity of these affinity media, the process step is assimilated into one ProteinA capture step directly from the cell culture.

The "heart" of the mAb purification process is the highly selective affinity binding of mAb to ProteinA, which purifies the mAb to >95% in one step. Follow -up steps remove host protein (Host-Cell- Protein or HCP), virus and IgG aggregates and increase the purity to >99%. During "primary recovery", the clarification step between USP and DSP, an "accepted" loss of mAb of 10 - 15% occurs [2].
Due to the costly mAb a huge loss of yield!
Omitting this clarification steps makes the process not only cheaper but also more sustainable, among others by eliminating hefty "single-use" filter installations (Fig 2.).

Fig. 2. Pall Stax

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With a very high cell density, up to $40*10^6$ CHO cells per ml, CHO cells wash through the cTRAC-ProteinA column unhindered and without damage while the mAb is bound. Hydrodynamically enabled by the funnel-shaped, radial column (Fig 3.)

Fig. 3. Radial column

Through this funnel shape, the cells are guided to the exit without opportunity for stagnation. This conduction is due to the resistance-driven focus of the flow which can be explained using the Kozeny-Carman formula (formula 1) [3].

$$\frac{150\eta}{Dp^2} * \frac{(1-\varepsilon e^2)}{\varepsilon e^3} * u * L \quad \textit{(formula 1)}$$

In capillary systems, resistance (pressure, P) increases under the influence of viscosity (η), particle diameter (Dp), superficial velocity (u), bed porosity (Ɛe), and tube length (L).

The shape of the radial column causes a gradual acceleration and resistance increase leading to flow-focusing which prevents lagging cells, and thus clogging. With the increased recovery and a reduction in costs (filters, buffers, time) there is an increase in efficiency of about 40% on the first step of mAb purification.
Thereby, the sustainability contribution of cTRAC is at least 20,000kg CO_2 per kg antibody [4].

Lab cTRAC

The mild- and low-pressure application allows for very rapid and trouble-free isolation of mAb from a small pilot cell culture even with a syringe (Fig. 4.). Obviously, this can also be done with a small peristaltic pump. For more information and the cTRAC starter kit, visit www.proxcys.com/ctrac.

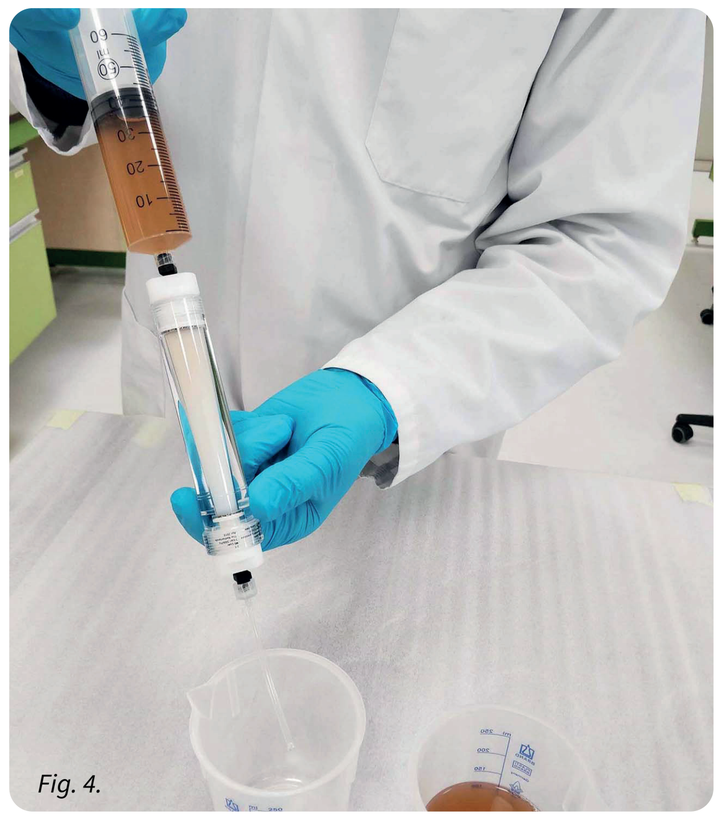
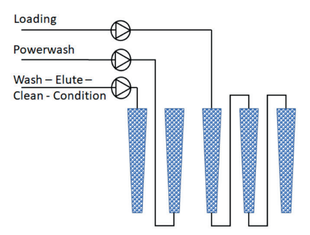
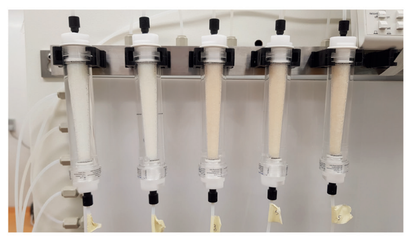


Fig. 4.

Continuous cTRAC

cTRAC columns are linear scalable to tens of liters of column volume. Because cTRAC processing is robust and gentle, it also proves to perform excellently in BioSMB mode (Fig. 5). For 3 to 5 columns connected in series, with a total bed height of 27 cm to 45 cm, flowthrough is uniform and viability above 90% (very low cell damage).





Results 8-9.08		4-14% Mini Protean TGX Precast Slab gel	
A1	Elute 2	20µl	~35ml
A2	Elute 3	20µl	~35ml
A3	Elute 4	20µl	~35ml
A4	Elute 5	20µl	~35ml
A5	Elute 6	20µl	~35ml
A6	Elute 7	20µl	~35ml
A7	Marker	7µl	
A8	FLowthru #2	20µl	1ml
A9	FLowthru #5	20µl	1ml
A10	FLowthru #11	20µl	1ml

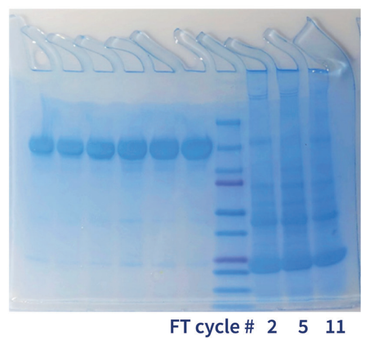


Fig. 5.

[1] Prof. M. Franzreb et.al, 2014 October-issue, BPI.
[2] Unverified generally reported data
[3] (Besselink T, et.al. 2013. Journal of Chromatography A 1271:105–114.)
[4] Proxcys Poster PPB2019 People, Planet, Profit – plasma conference.