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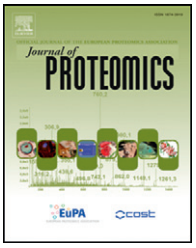

Review

Mark Twain: How to fathom the depth of your pet proteome

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ABSTRACT

The present review highlights recent progresses in the technique of combinatorial peptide ligand libraries (CPPL), a methodology that has much to offer for the detection of low- to very-low abundance proteins (nanograms/mL scale and below) in any proteome. In particular, advances in exploration of the urinary, plasma and tissue proteomes are discussed and evaluated. It is shown that when treating biological fluids, such as plasma, with CPLLs, the detection sensitivity, which in the control only reaches 10 ng/mL, can be enhanced to as high as 10 pg/mL, with an increment of sensitivity of three orders of magnitude. The possibility of using CPLLs as a two-dimensional pre-fractionation of any proteome is also evaluated: on the charge axis, CPLL capture can be implemented at no less than three different pH values (4.0, 7.2 and 9.3), thus permitting a capture of proteinaceous analytes bearing a net positive or net negative charge, respectively. When capture is performed in the absence of salts or at high levels of salts (of the Hofmeister series), one can favor the capture of hydrophilic vs. hydrophobic proteins, respectively. This would thus be a genuine 2D protocol, working on orthogonal separation principles (charge vs. hydrophobicity). As the horizon of CPLLs is expanding and its use is exponentially growing, we expect major breakthroughs in, e.g., biomarker discovery, a field that has suffered a decade of failures.

Contents

Abbreviations: CPLL, combinatorial peptide ligand library; HAP, high abundance proteins; LAP, low-abundance proteins.

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1. Introduction

We have already extensively reviewed the combinatorial peptide ligand library (CPLL) methodology in quite a large number of articles [1–11]. In one instance, detailed protocols for solid-phase peptide library users have been described [12]. Given such an extraordinary coverage, it would appear preposterous to offer yet the nth review here. Yet, we admit that, in most previous reviews, we were forced to largely cover our production, since the technique, launched in the year 2005 [13], had a rather slow growth, also due to the fact that such libraries were not yet commercially available. With the introduction of this product on the market under the trade name of ProteoMiner, the technique has experienced a steady growth and now seems to be in an exponential phase. Since very recently some articles appeared describing a quite unique in depth exploration of different proteomes, we are pleased here to cover the production of other labs and offer a glimpse at the unique enhancing power of this methodology in bringing to the limelight the low- to very-low abundance proteome, i.e. those species that, even in modern democracy, do not have a "minority speaker" to represent them.

2. Myths and legends on CPLLs

Prior to reviewing recent literature data, we have to dispel some myths that are presently plaguing the field and would confuse the user.

The first is about the capability of CPLL technology to dig deep in the proteome composition. An example of that came from Bandow in 2010 [14]. When pre-fractionating sera in search of biomarkers and comparing the performance of CPLLs vs. immuno-depletion (Seppro IgY14 System) she came to the conclusions that there was no difference between the two systems, both of them performing poorly in sera analysis. In her own words: "detectable protein spots in the different plasma fractions contained exclusively high-abundance proteins normally present in plasma at concentrations between 1 μg and 40 mg/mL". That this could be so in the case of immuno-depletion there seems to be a general consensus in current literature. For instance recently Tu et al. [15] reported a feral message for this methodology. They stated that either a top 7 or a top 14 immuno-depletion resulted in a meager 25% increase in identified proteins compared with unfractionated plasma. Although 23 low-abundance (<10 ng/mL) plasma proteins were detected, they accounted for only 5–6% of the total protein identifications in immuno-depleted plasma. In both unfractionated and immuno-depleted plasma, the 50 most abundant plasma proteins accounted for 90% of the cumulative spectral counts and precursor ion intensities, leaving little capacity to sample lower abundance proteins. Their conclusions: "untargeted proteomic analyses using current LC–MS/MS platforms – even with immuno-depletion – cannot be expected to efficiently discover low-abundance, disease specific biomarkers in plasma". Even more deadly was the message of Zhi et al. [16]: according to them immuno-depletion permits us to see 10% less proteins than in control sera! But that this could apply also to CPLLs is truly false, as it will be discussed below. So, what went wrong? An explanation is evident from Table 1, which lists most of the elution cocktails proposed in the literature. Among them, the one of Bandow [14] seems to be the poorest one, as it is based on an eluant comprising 4 M urea and 1% CHAPS. The authors here followed blindly the clearly under-optimized protocol supplied by the manufacturer. It is of common knowledge in fact that urea at 4 M levels cannot even denature proteins. Protein unfolding starts at 5 M urea and in most cases is completed in 8 to 9 M urea.

The second myth is about the strength of the interaction between peptides from CPLL and captured proteins. One should not live with the impression that the linkage between a given protein and its hexapeptide partner in the CPLL beads is a weak one, given that the peptide bait is rather short. It turns out that such binding event is quite strong and requires highly denaturing conditions to be split [1,7,10]. What has occurred has been elegantly explained by Di Girolamo et al. [17,18]. The eluant proposed in [14] will barely elute 20–25% of the captured species and certainly not those having high affinity for the hexapeptide ligands, i.e. those trace proteins that had to compete hard with the overwhelming presence of the high-abundance species (HAP) that might have had lower affinities for the same baits! As a result of the insufficient elution protocol, only the high- to medium-abundance species were desorbed,

Table 1 – Different elution systems from ProteoMiner beads.

Reference	Eluant	Results
Sihlbom et al. [34]	a) 1 M NaCl, 20 mM HEPES, pH 75. b) 200 mM glycine pH 2.4 c) 60% ethylene glycol d) Hydro-organic IPOH, ACN, TFA	Single elution (4 M urea, 1% CHAPS): 91 peaks; combined 4 elutions: 330 peaks in SELDI
Ernoult et al. [46]	a) 4 M urea, 1% CHAPS, 5% acetic acid; b) 6 M guanidine HCl, pH 6.0	320 proteins in PM; 332 immuno-depletion
Fakelman et al. [47]	4 M urea, 1% CHAPS, 5% acetic acid	Many more SELDI peaks than control
Froebel et al. [48]	a) 8 M urea, 2% CHAPS, 5% acetic acid b) 7 M urea, 2 M thiourea, 4% CHAPS, 40 mM Tris	Identical spots in 2D maps
Beseme et al. [31]	4 M urea, 1% CHAPS, 5% acetic acid	Control: 157 spots; PM treated: 557 spots, IPGs pH 4–7
De Bock et al. [49]	8 M urea, 2% CHAPS, 5% acetic acid	Control: 48 peaks; PM-treated: 136 peaks in SELDI
Bandow [14]	4 M urea, 1% CHAPS	Only high abundance proteins 1 μg to 40 mg/mL
Bandhakavi et al. [50] Candiano et al. [24]	100 mM Tris–HCl pH 6.8, 4% SDS, 10% 2-ME, boil	Untreated: 251 proteins; PM-treated: 693 proteins; saliva
Dwivedi et al. [51]	8 M urea, 2% CHAPS, 5% acetic acid	108 proteins unique to PM; 100 proteins unique to IgY; 404 total protein sera