Cross-reactivity in antibody microarrays and multiplexed sandwich assays: shedding light on the dark side of multiplexing

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Immunoassays are indispensable for research and clinical analysis, and following the emergence of the omics paradigm, multiplexing of immunoassays is more needed than ever. Cross-reactivity (CR) in multiplexed immunoassays has been unexpectedly difficult to mitigate, preventing scaling up of multiplexing, limiting assay performance, and resulting in inaccurate and even false results, and wrong conclusions. Here, we review CR and its consequences in single and dual antibody single-plex and multiplex assays. We establish a distinction between sample-driven and reagent-driven CR, and describe how it affects the performance of antibody microarrays. Next, we review and evaluate various platforms aimed at mitigating CR, including SOMAmeres and protein fractionation-bead assays, as well as dual Ab methods including (i) conventional multiplex assays, (ii) proximity ligation assays, (iii) immuno-mass spectrometry, (iv) sequential multiplex analyte capture, (v) antibody colocalization microarrays and (vi) force discrimination assays.

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Introduction
Cross-reactivity (CR) to non-target proteins is ubiquitous and widespread for antibodies (Abs) \cite{1,2}, and together with a lack of Abs against many targets, arguably the biggest obstacle in establishing high-performance and large-scale multiplexed immunoassays. Unless CR is adequately addressed and suppressed, or at least mitigated, it can be devastating to the performance and reliability of immunoassays. CR is not a mainstream scientific area of research, and rather seen as an impediment, and hence only receives little attention compared to that devoted to the developments of new assay technologies and methods, such as antibody microarrays and high sensitivity assays. Ironically, progress in the development and application of novel assay technologies is often stumped by CR.

Immunoassays depend on an affinity binder — a polyclonal or a monoclonal Ab, a recombinant binder, an aptamer, or a receptor — that binds a target protein with high specificity and affinity. The binding is transduced and amplified into a detectable signal, and in the ideal scenario, the intensity of the signal is ratiometric with the concentration of target analyte. Improving immunoassays is predicated on the availability and quality of the affinity binders, and the need for more, better, and cheaper binders is widely recognized \cite{3,4}. CR of affinity binders can be tested using random peptide arrays for example, but CR to non-homologous amino-acid sequences was found to be widespread \cite{5}. The suppression of CR is further complicated by the large parameter space of possible three dimensional conformations adopted by proteins \cite{6}. The Human Protein Atlas has established a polyclonal Ab production pipeline with rigorous quality control standards, and in a Herculean effort, produced Abs against 15,000 of the ~20,000 human proteins (Human Protein Atlas; URL: http://www.proteinatlas.org). Yet, when Schwenk et al. evaluated a preselection of 11,000 affinity-purified, mono-specific Abs, only 531 Abs produced a single band on a Western blot, indicating that ~95% bound to proteins outside of the expected band \cite{7}. Whereas some of the binding might be ascribed to protein isoforms, cleaved proteins, or post-translational modifications, much is likely caused by CR. Collectively, these studies underline that affinity binders often cross-react.

The challenge of scaling up assays and enhancing their sensitivity may thus be formulated as follows: how to produce a ‘perfect assay system’ while using ‘imperfect building blocks’, that is, cross-reacting affinity binders? Or, how should a multiplexed immunoassay with ultra-high sensitivity while efficiently suppressing CR be designed? What is the trade-off, i.e. \cite{2,8}, how does multiplexing and CR affect assay performance and can it be predicted? To provide some answers to these questions, we first review and define CR in single Ab and dual Ab single-plex and multiplex assays. We review the strategies developed to mitigate CR over the last decade...
in multiplexed assays, and assess their robustness, scalability, and potential for ultrasensitive detection. Finally, we provide some suggestion for future studies and development.

Direct detection of binding can be accomplished by labeling the entire sample with biotin and incubating it with fluorescently labeled streptavidin (Figure 1a) or alternatively, by using label-free detection technologies that record a change in refractive index, mass, or conductivity at the surface [9]. However, signal arising from CR (and non-specific adsorption) is typically indistinguishable from the one arising from specific binding (Figure 1b) thus limiting the performance of this assay format. To clarify the language, we define this type of CR as sample-driven CR.

CR and non-specific binding has been studied extensively for single-plex assays, and whereas it may not be possible to eliminate it completely, it is fairly well understood and managed [10]. Dual Ab assays, also called sandwich assays, and often simply referred to as enzyme linked immunosorbent assays (ELISAs) embody an effective strategy to mitigate CR by binding two distinct epitopes on the same protein: a capture Ab (cAb) immobilizes and concentrates the analyte, while the simultaneous binding of a labeled detection Ab (dAb) transduces the binding into a detectable signal (Figure 1c). The strength of the sandwich assay stems from its tolerance to CR because a single CR (or non-specific binding) does not result in a detectable (false positive) signal (Figure 1d).

Indeed, two simultaneous spurious binding events are required to lead to detectable CR, but the odds for it to occur are very low. This point highlights the importance to distinguish between CR that leads to false positive signals and CR that does not lead to a signal, and which can be tolerated, but should not be ignored. In all cases, CR can be further minimized by seeking affinity binders with high specificity and affinity, and by developing assays protocols that minimize CR. For example, binders with low dissociation constants (and low off-binding rates) have long been used, because they can withstand harsh wash steps in ELISA and other assays, while weakly bound and cross-reacting species are washed off [4].

Recently, the limit of detection (LOD) for sandwich assays was extended to aM and even zM [11,12,13*], even outperforming nucleic acid tests with PCR amplification [14]. High performance Abs and high signal amplification, which are sometimes combined with digital assay formats that tally single binding events, are the key to these advances. Sandwich assays have also been adopted in multiplexed immunoassays — comprising both antibody microarrays on chips and dispersed bead-based assays (also called bead arrays), but as described below, new types of CR arise as a consequence of adding the dAbs as a mixture.

**Multiplexed assays and cross-reactivity: antibody microarrays and bead-based assays**

Multiplexed sandwich assays (MSAs) were proposed in 1989 by Ekins et al. a few years before the introduction of

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**Figure 1**

Cross-reactivity and its effects in single-plex immunoassays with single-AB and dual ABs (sandwich assays). (a) An ideal single-Ab assay with two specifically bound proteins that are fluorescently labeled for detection. (b) In case of non-specific adsorption (purple protein), CR (red protein), and protein-protein complexes (blue protein) additional fluorescent labels are immobilized that generate increased or even false positive signals. (c) The same assay in a sandwich format with a cAb and dAb yields the same assay result under ideal conditions and (d) following non-specific adsorption, CR, and protein-protein complex formation, highlighting its greater tolerance to CR and other spurious binding events.
DNA microarrays [15]. Since then multiplexing has progressed from 4 to ~50 targets for MSAs, while DNA microarrays were scaled from 400 to 6.4 million targets within a decade. Results obtained with MSAs are not always reproducible. In one study, a series of biomarkers for early diagnosis of Alzheimer’s disease were identified [16], but could not be validated subsequently. The failure was attributed to the variability of the antibody arrays [17]. Vulnerability to CR, which will be detailed below, can account for the difficulty of scaling up MSAs and for the lack of reproducibility.

**Reagent-driven cross-reactivity in multiplexed sandwich assays with reagent mixing**

Conventional MSAs are performed by incubating a micro-array or dispersed beads with a sample, followed by the addition of dAbs as a mixture, with the expectation that each dAb will bind to target analytes bound to the corresponding cAb. Whereas thermal agitation ensures that each reagent encounters its target, it also results in combinatorial interaction of every dAb with all other (i) analytes, (ii) cAbs and (iii) dAbs. The scenarios of possible pair-wise CR between cAb, dAb and analytes is summarized in Figure 2. The number of pair-wise interactions is defined as liability pairs, and for each scenario, it increases proportionally to the square of the number of targets N adding up to $4N(N-1)$ [18]. This sum neglects the scenario cAb-to-cAb CR which may arise in dispersed assay formats, but is less critical as the assay readout is linked to the dAb. Thus, for a 14-plex assay, 728 liability pairs exist, and for a 100-plex, 39 600; these numbers represent the vulnerability to CR. The CR for an assay with only 14 targets was determined experimentally (Figure 2c) and the results highlight its severity. In the most favorable case, CR contributes to increased background noise, compromising the LOD of the assay, but in the worst case it generates a false positive signal. The vulnerability is also expressed by the fact that a single contaminated dAb, or an additive in the mixture, can compromise all assays as it interacts with the entire array. We define CR arising because of reagent mixing as *reagent-driven* CR.

In spite of these issues, MSAs with reagent mixing have become standard both in microarray and bead-based formats. However, researchers and vendors alike devote enormous efforts in establishing and optimizing working combinations of Ab pairs, which are however limited to between one and a few tens depending on the targets and the Abs [19]. But regardless of optimization, as $N$ increases, the vulnerability increases as $\sim N^2$, and spurious CR can arise by an idiosyncratic property of the sample, or a bad reagent. The technical capability for scaling up and making larger arrays has long been available, and for example LumineX Inc. advertised 100-plex assays for over a decade, but they were not realized as MSA. A residual CR signal often remains (one vendor admitted that 10% is tolerated [20]) and is harder to minimize the larger the array is, thus imposing suboptimal assay conditions while increasing the background signal. Indeed, some Abs such as the EGF and CEA cAbs in Figure 2c broadly cross-react and cannot be used in MSAs. To minimize CR, diluting the sample 100-fold while using better signal amplification was proposed [21].

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**Figure 2**

Scenarios for reagent-driven CR arising because of reagent mixing in multiplexed sandwich assays as well as experimentally measured CR. (a) An ideal assay while (b) shows possible pair-wise cross-reactivity scenarios along with the number of combinatorial liability pairs for an assay with $N$ targets. Cross-reactive binding of (i) dAb to target protein, (ii) dAb to cAb, (iii) dAb to dAB, (iv) protein measured on the array to a cAb, and (v) protein-protein interactions of two proteins targeted on the array. These combinations apply to both microarray and bead-based assay formats and can occur simultaneously. (c) Experimental results for cross-reactivity in a 14-plex assay. 14 arrays were each incubated with one of the analytes, followed by a mixture of the 14 dAbs. The color code is shown on the right and the CR signal is shown relative to the signal of 32 ng/mL of the target protein; red indicates a CR $> 20\%$. Reproduced with permission from Ref. [18]. In an actual assay, all analytes and dAbs are mixed, and whereas the analyte concentrations may be either lower or higher, there are additional combinations that can give rise to CR.
and whereas it helps, the dilution of already low concentration analytes can make them undetectable.

End-users of commercial MSAs conducted numerous studies evaluating the performance of various kits, and initial studies with limited multiplexing found good correlations and concluded MSAs to be reproducible [22]. More recently, studies with higher number of targets and a more critical analysis found a lack of reproducibility and correlation between kits from the same [20] and from different vendors [23–26]. In addition, one study found significant differences depending on whether an assay was run in single-plex or in a multiplex format, indicating that MSAs may not be accurate [27]. Because of these findings and additional issues [28*], the use of MSAs with reagent mixing is often not recommended for quantitative analysis and clinical studies [20,23–27,28*]. The authors of these studies are seemingly unaware of the vulnerability to reagent-driven CR, which could explain the observed variability and contradictory conclusions.

**New strategies to contain cross-reactivity in multiplexed single-antibody assays**

Single-Ab arrays suffer from sample-driven CR, but unlike reagent-driven CR in MSAs, vulnerability of each spot of an array is independent of the size of the array. Simultaneously, only a single Ab is needed per target (and no matching required), and hence single-Ab arrays (and bead assays) with over 1000 Abs and sensitivities in the ng/mL have been developed. Such arrays were used for biomarker discovery studies for cancer by comparing the signal between healthy controls and patient samples [7,29,30]. But based on the results of the study by Schwenk et al. [7], CR is expected to contribute to the signal, however, an increase in CR may also be a reflection of disease progression, and an increase of signal bears a biomarker value regardless of its origin. It will be important to evaluate the robustness of these assays and whether they can be reproduced by different laboratories.

**Mitigation of cross-reactivity in single affinity-binder assays**

In an attempt to overcome the limitations of Abs, DNA-based binders called aptamers were developed. To further enhance their affinity, Gold and colleagues developed so-called SOMAmers that integrate bases with chemical side groups reminiscent of amino acids to generate a greater binding diversity [31*]. To date, over 1000 SOMAmers have been selected for both high affinity and low dissociation rate constants, and a solution-phase assay protocol compatible with plasma while minimizing CR was established (Figure 3a). The main distinction between SOMAmers and antibody arrays is the dispersed assay format with two purification steps, and the distributed binding interaction of the oligomers with the ligand [32]. The CR of SOMAmers has been studied in a limited range of conditions, and it will be interesting to compare their performance to classical Abs for a variety of applications. SOMAmers are now being routinely used to identify candidate biomarkers for various diseases [31*].

Another approach to mitigate CR is based on protein fractionation according to mass followed by large scale multiplexed assays using home-made beads (Figure 3b) [33,34*]. While target proteins are concentrated in particular fractions, cross-reacting proteins are expected to be diluted, thus increasing the signal-to-noise ratio. Furthermore, bound proteins can be cross-validated for the accurate mass. The shortcomings of this approach are the complexity of the protocol, the difficulty to quantify proteins due to the many steps, and uncertainty about contributions of residual CR to the signal. Conversely, this approach scales well and experiments with 1725 Abs were conducted making it the largest multiplexed assay reported to date [34*].

**New strategies to contain cross-reactivity in multiplexed sandwich assays**

**Proximity-induced, pair-wise recognition of specific binding only**

Proximity ligation assay (PLA) [35*], and more recently proximity elongation assay (PEA) [36] are two clever approaches that molecularly discriminate specific binding from CR. Each Ab pair of a sandwich is tagged with a DNA recognition barcode, and upon simultaneous binding to a target, the two DNAs overlap and get joined, either by DNA ligation assisted by a connector oligonucleotide [35*], or directly hybridized to each other by complementary sequences [36]. After elongation, the newly formed DNA strands are amplified by PCR and transduced into a measurable signal (Figure 3c). To prevent random linkage in the solution of complementary Abs, the sample is diluted before completing the linkage reaction. Sandwich assays of up to 24-plex were demonstrated while requiring 1 µL of sample and minute amounts of reagents. The assay protocol is however somewhat lengthy, the DNA barcodes need to be selected carefully to avoid CR, and to enable robust read-out with high multiplexing depends on a microfluidic platform that physically isolates each reaction to eliminate CR during PCR [35*]. Conversely, the low volumes allow for multiple reactions to be run in parallel, the concept of PLA and PEA is flexible and PCR amplification could be made conditional on the binding of three Abs, while new applications such as the recognition of protein complexes, or of posttranslational modifications (PTM), as well as high sensitivity assays with single molecule detection were shown [37].

**Temporal separation of reagents minimizes reagent-driven cross-reactivity**

A more radical approach to avoiding reagent driven CR is to not mix the reagents and two strategies have been proposed: temporal and spatial separation of reagents.
Six multiplexed assay formats that mitigate CR comprising two single-Ab and four sandwich assays. Purple triangles represent a CR event. (a) Cy3-labeled and biotin (B)-labeled SOMAmers (S) in a suspended assay format bind to cognate proteins (P) followed by multiple washing rounds involving streptavidin-coated beads (SB), biotinylation and photocleavage (PC) to eliminate CR (purple triangle protein). (b) Pre-fractionation concentrates and partitions proteins to enhance signal-to-noise ratio of single-Ab binding on beads. The ‘green’, ‘red’ and ‘blue’ proteins are detected in fraction II, and IV respectively, while the ‘purple (CR)’ protein is sequestered in fraction I. (c) Proximity ligation assay (PLA) uses pairs of Abs tagged with unique sequence specific reporter fragments. Signal amplification only occurs when a matched pair of Abs bind the same target analyte to create an PCR-amplifiable DNA strand, and is unaffected by cross-reacting Abs. (d) Sequential multiplex analyte capturing (SMAC) sequentially adds and retrieves sets of Ab-coated magnetic beads that are subsequently incubated with matching dAbs. (e) The antibody colocalization microarray (ACM) spatially addresses each dAb to the matched cAb spot only, thus avoiding mixing altogether, and reproducing the conditions of classical ELISA at the microscale. (f) Immuno-MS combines Ab-based purification and MS analysis. Proteins are trypsinized into peptides, isotope labeled peptides spiked in, and both captured on beads using peptide specific Abs, followed by elution and MS analysis. (g) Force-discrimination arrays use soft stamps to mechanically colocalize each dAb to the matched cAb spot, followed by dissociation. A DNA zip-probe only ruptures in the event of specific binding, but not CR, and is recorded by the transfer of the Cy3 fluorophore to the cAb spot.

In sequential multiplex analyte capturing (SMAC), batches beads each coated with different cAb are sequentially added to the sample, retrieved, and incubated in tubes with the respective dAb in solution, followed by quantification (Figure 3d) [38]. Analytes of interest are captured one-by-one; alternatively, different beads with different cAb, can be added, and multiple targets captured simultaneously. SMAC is versatile and has been used to map PTMs by incubating a mixture of beads coated with distinct Abs targeting multiple distinct PTMs, followed by incubation with a single dAb against EGFR. The complementary scenario where cAbs targeting different proteins are incubated with the sample, followed by incubation with a single dAb against a phospho-tyrosine was also shown. Whereas the number of sequential steps that can be performed with SMAC will be limited by practical considerations, the versatility and the ease of concatenating various assays in one
Spatial separation of reagents eliminates reagent-driven cross-reactivity
Reagent induced CR can be eliminated by reverting to the same conditions as in ELISAs where each dAb is only exposed to a single cAb. Haab and colleagues formed multiple replicate arrays with a variety of cAbs, and applied a single dAb or affinity binder to each of them. Whereas this strategy is not useful to multiplex protein quantification, it can be utilized to measure multiple PTMs as well as to uncover protein complexes [39,40]. This approach requires a separate array for each parameter measured, and somewhat larger sample volumes, but it is notably well suited for biomarker discovery studies in blood as large volumes can be obtained.

MSAs with spatial separation of dAb were introduced by our group and implemented by delivering each dAb to a single microarray spot with the cognate cAb. We named this technology antibody colocalization microarray (ACM) (Figure 3e) [18*]. The ACM can be seen as a microarray of single-plex microscale assays, and hence the CR is expected to be identical to the one found in a conventional sandwich ELISA. The ACM requires high precision microarray spotters and alignment to deliver both cAb and dAb solutions within <30 μm when using 100 μm-wide spots. Whereas the initial assay protocol required spotting during the experiment, we developed a technology called snap-chip for the registered transfer of reagent droplets from microarray-to-microarray [41]. cAbs and dAbs are thus pre-spotted on two distinct slides and stored. To conduct an assay, the cAb slide is incubated with the sample, then aligned and snapped with the dAb slide to transfer all dAbs simultaneously to the cAb spots. The ACM can be multiplexed and run at high-density with minute sample and reagent consumption while being scalable.

Immuno-mass spectrometry assays identify the target analyte
Mass spectrometry (MS) is biased toward high abundance molecules, but conversely has the ability to fingerprint proteins via the peptides it detects, offering a powerful tool to distinguish actual binding from CR. To detect lower abundance proteins, Ab-based enrichment was combined with MS analysis by a method called SISCAPA [42]. The sample is first digested by trypsinization, followed by spiking with synthetic, isotope-labeled proteotypic peptides that serve as reference. Next, the sample is incubated with beads functionalized with peptide-specific Abs, followed by elution and liquid chromatography–MS (Figure 3f). The sensitivity of SISCAPA can reach pg/mL, but only when using large sample volumes and the protocol is complex. A variant that uses matrix assisted laser desorption ionization is simpler and faster but at the cost of lower sensitivity [43]. The scaling up of either method is not trivial and is limited by the availability of peptide-specific Abs [42].

Whereas all methods discussed to this point aimed at reducing CR, one group leveraged CR to their advantage. Broadly cross-reacting Abs were generated using short peptide sequences (shared by many proteins, while avoiding sequences present in high abundant proteins) as the antigen. Then, using a similar protocol as SISCAPA, peptides from trypsinized samples are enriched, and the sequence of each peptide, and hence the corresponding protein, identified by MS [44*]. The accuracy of this method remains to be validated with more complex samples with large differences in protein concentration, but using this approach, large numbers of proteins can be targeted simultaneously.

Force-based discrimination immunoassays distinguish specific binding and cross-reactivity
A nanoscale force spectroscopy sensor using programmable DNA linkers has been developed to detect and quantify binding in a tug-of-war test. A dAb conjugated to a DNA-force sensor that is set to rupture only in the event of strong, specific binding of the dAb to a target analyte (but not CR) is mechanically pulled away, and the transfer of the labeled DNA probes reveals captured analytes (Figure 3g) [45,46]. This approach has already been used to probe 7 targets simultaneously. Using the snap-chip method developed for the antibody colocalization microarray, it might be possible to miniaturize and further multiplex this concept, and based on the results obtained with assays using acoustic or magnetic stringency tests [12], it might be possible to extend the LOD by several orders of magnitude.

Discussion
CR is hard to eliminate from immunoassays as Abs are imperfect and often a ‘black box’, yet assays with ever more multiplexing and higher sensitivities are sought after. Much of the discussion in this opinion is based on incidental observations of CR and reasoning. Indeed, systematic studies of CR are rare [5,18*] and the source of CR or assay interference has been difficult to identify. A synopsis of the various methods developed to mitigate CR presented in this opinion is shown in Table 1. Whereas, the discussion was largely focused on Abs, the conclusions drawn here are applicable many other types of affinity binders.

There are two fundamental routes to multiplexing, one being single Ab assays and the other dual Ab assays. Single Ab assays are easy to scale up, but are susceptible to sample-driven CR [7]. Efforts to mitigate CR include longer washing and the use of non-antibody binders [31*], as well as sample pre-fractionation [34*]. If immuno-MS methods could be adapted to work with full length...
proteins rather than peptides, it will be possible to shed light on the identity of proteins and protein complexes captured by single Ab assays and on the source of differences [7,29,30]. The tried and tested strategy to mitigate CR and improve specificity is to use two distinct parameters to capture and quantify an analyte in a sample. The two parameters are for example two distinct Abs as in the sandwich assay, or a chromatographic separation followed by affinity binding, or affinity binding followed by MS. However, one needs to consider whether these assays are scalable, and dual Ab, sandwich assays with dAbs mixtures introduce reagent-driven CR. The vulnerability to CR was shown to scale with the number of targets $N$ as $N^2$ and to be severe for a 14-plex assay. It may give us pause before deploying conventional MSAs in a clinical context with therapeutic decisions and patients at stake. A better understanding of CR and how it plays out in multiplex assays will help improve the performance of multiplexed immunoassays. Among the various methods listed in Table 1, the most promising strategies operate by differentiating specific and CR binding, as for example PLA [35], or by identifying the bound species using MS [44], or by eliminating mixing altogether as with the ACM [18]. Many of these methods might be enhanced further, for example by introducing additional stringency steps, such as force-based discrimination [46] along with greater signal amplification [11,12,13,14]. Reliable and sensitive multiplexed immunoassays will accelerate life science research, and help discover novel candidate protein biomarkers at ever lower concentrations for earlier and more accurate disease diagnosis, and also support clinical translation.

Acknowledgements
We wish to thank NSERC, CIHR and the CCS for funding, and Andy Ng for reading the manuscript. DJ acknowledges a CRC, and VL the NSERC-CREATE ISS program for support.

References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


Insightful perspective on the challenges and opportunities in establishing multiplexed assays for discovering low concentration biomarkers in complex samples such as blood.


First report on an immunoassay platform with single-molecule detection that is multiplexed. A 4-plex assay is shown, and whereas the performance is greatly improved compared to conventional assays, performance suffers from fluorescence cross-talk and reagent-driven CR.


This manuscript establishes a quantitative relation between multiplexing and cross-reactivity in MSAs with reagent mixing by means of combinatorial analysis. The results of the analysis and a series of experiments demonstrate the severity of cross-reactivity and help account for the issues encountered in such assays. The manuscript further introduces the antibody colocalization microarray that eliminates reagent-driven cross-reactivity by avoiding mixing, and hence is scalable.


Mini-review highlighting the various issues MSAs with reagent-mixing face before they may be deployed for in vitro diagnostics.


The authors introduce a chemically modified DNA as affinity binders, the production of a library with over 800 binders, and on their performance for use in biomarker studies in this study. This study highlights the potential of using chemical modifications to enhance the performance of affinity binders.


Tour de force experiments that combine protein fractionation with multiplex bead assays against 1725 proteins to overcome lack of Ab specificity, largest multiplex assay to date.


Demonstration of proximity ligation assay targeting a total of 74 proteins by using four assays that can each measure 23 and more recently up to 92 analytes simultaneoulsy. Each assay only requires 1 µl of sample, and both PEA and PLA showed high specificity while achieving a high sensitivity, exceeding the one demonstrated with other common multiplex assay platforms.


Serial multiplex analyte capture overcomes cross-reactivity by temporally separating reagents, and sequentially adding beads with cAbs and incubating them separately in tubes with the corresponding dAbs. The method was applied for simultaneous protein quantification and mapping of posttranslational modifications.


Instead of combating CR like all other methods, they exploit CR to their advantage. Abs against short peptides shared by multiple proteins are produced, and used to capture many different proteins at once from a sample. The proteins are subsequently identified using mass spectrometry.

