VIEWPOINT

Is protein overlap in two-dimensional gels a serious practical problem?

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In a recently published article, Campostrini *et al.* [*Proteomics* 2005, *5*, 2385–2395] raised questions regarding the utility of 2-D gels in proteomics research. We believe that the authors have overlooked several key issues including the dynamic range of protein expression and the sensitivity of the analytical methods used to explore a proteome. We argue that 2-D gels have and will continue to provide meaningful quantitative data when applied to proteomic analysis and that the practical significance of spot overlap has been overstated.

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The article on spot overlap in 2-D gels by Campostrini *et al.* [1] should not go without comment because it contains oversights and some unjustified, but dogmatic claims. We believe that this paper unduly discredits the potential of 2-DE and we therefore submit this response to introduce some objective analytical science and stimulate rational discussion.

The primary concern of the authors relates to spot overlap. They maintain that in most cases where 2-DE is applied to the analysis of complex samples of biological origin, "where sample loads of *ca.* 1 mg of total protein are applied and typically at least 1000 spots are visualized", spots comprise only a single protein (or singlets as Campostrini *et al.* call them), "will be the minority, rarely exceeding 30% of all spots analyzed". Further, they suggest that their "... experimental data on the abundance of overlapping spots were in excellent agreement with theoretical data calculated on the basis of the statistical theory of spot overlapping, originally proposed by Davis and further developed by some of the authors".

We agree that both in theory and practice more than a single protein will migrate to each location on a 2-D gel and this has been discussed in several publications [1–3].

Several approaches including narrow range IPG strips, sample fractionation methods [4–6], different sample preparation conditions, and modification of conditions during electrophoresis [7] can all help to reduce the complexity of a single 2-D gel and decrease spot overlap, but meaningful data are available without the need to incorporate additional steps.

To elaborate, it has been reported that the human plasma proteome contains more than 500 000 protein forms [8] and clearly the resolution of a single gel is insufficient to allow complete separation of all of these. This would be a serious problem if each protein was equally abundant in the sample under investigation; however, the enormous dynamic range covered by proteins in biological samples works to our advantage. The abundances of human proteins reportedly vary over an enormous range of *ca.* ten orders of magnitude [8]. Consequently, it is not only the number of proteins in the sample under investigation that matters, but also their rela-

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Abbreviations: ***A***, abundance; ***I***, intensity

tive abundances and the dynamic range of the quantitative tool being employed to measure them.

For example, the measured intensity (I) at any specified location on a 2-D gel is the sum of the intensities of all the co-localized entities (*i.e.*, $I_{(total)} = I_1 + I_2 + I_3 \ldots$). Staining intensity is proportional to the total amount of protein present, but in practice most proteins at a specific location will not contribute any detectable signal because they are below the LOD. (The dynamic range of staining is limited to two to four orders of magnitude at best, depending on the approach employed.) For those proteins that are visible, in all but a few instances the measured (total) intensity will be derived from essentially one principal component (*i.e.*, $I_1 \gg I_2 \gg I_3 \ldots$) because the abundance (A) of each protein at a defined location will vary over a large range (*i.e.*, $A_1 \gg A_2 \gg A_3 \ldots$).

Campostrini *et al*. should not be surprised that sensitive analytical techniques allow us to probe a specific region of a gel and identify more than one protein, but that does not mean that quantitative determinations of that "spot" are compromised; all it means is that the LOD for the qualitative tool we have employed (the mass spectrometer) is greater than that of the detection tool employed for quantification (the stain or dye). To suggest that this is cause for "serious concern" is an overreaction. There are numerous published reports of the utility of 2-D gels for relative quantification of proteins, particularly when DIGE is employed [9–13] and these stand as testimony to the power of the 2-D gel strategy.

Improvements in detection methods will continue for the foreseeable future and will reveal that our samples are more complex than prior work indicated. Increasingly we will be able to probe deeper into the intricacies of a system and gain additional insights. For now, however, 2-DE remains our best protein separation tool, and when it is matched with appropriate detection and identification strategies, it provides reliable and powerful insights into the proteome. Most of our existing data on the human proteome has arisen from studies based on 2-DE and this will likely continue for some time.

We would be interested to know what alternative strategies the authors propose for protein separation and we would welcome the opportunity to objectively discuss the relative merits of each strategy.

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