

Steady State Analysis of Peptide Array-based Binding Assays

A Mathematical Model for Disease Diagnostic and Affinity Profiling of Bimolecular Interactions

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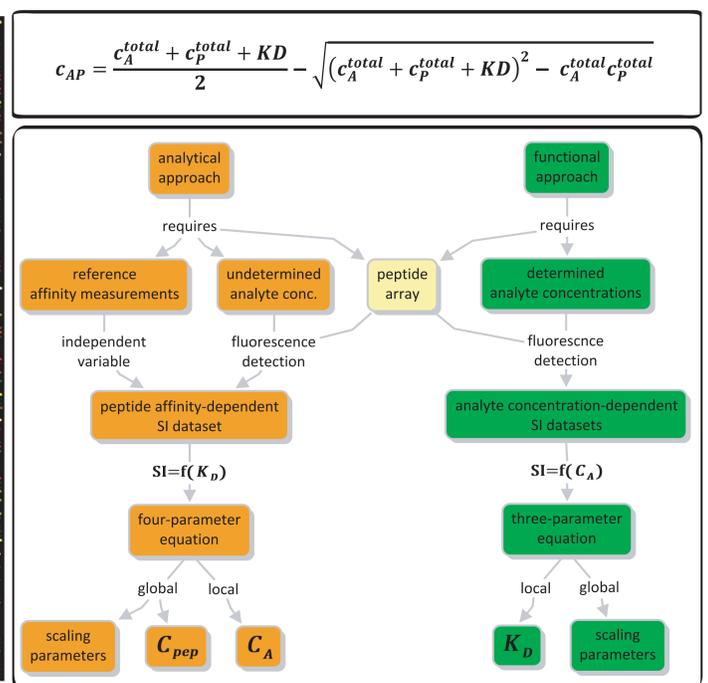
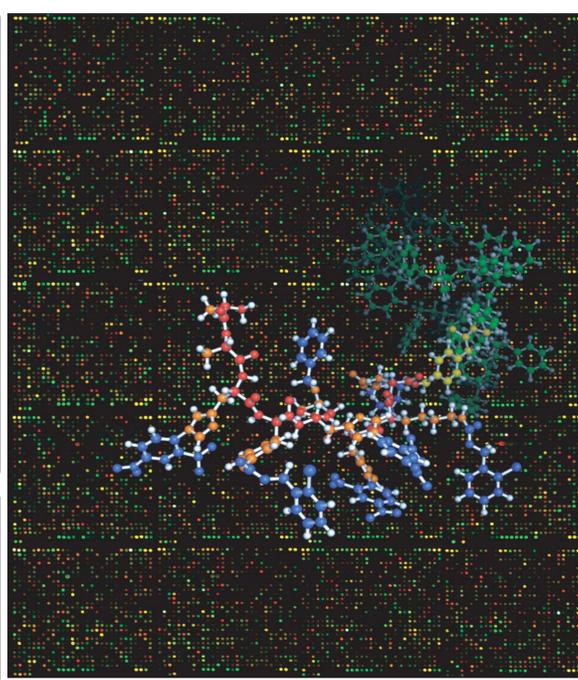
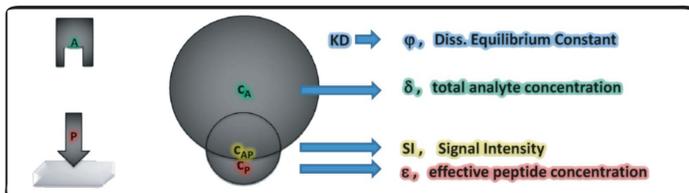
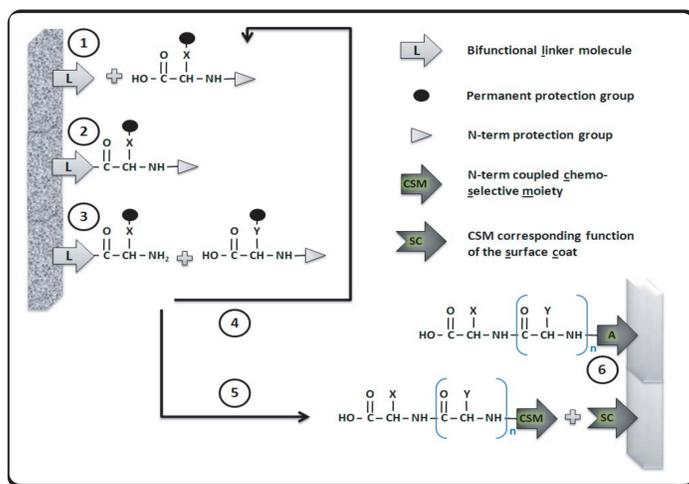
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INTRODUCTION Several techniques provide high throughput biological data. The growing pace of data gathering demands the application of mathematical tools to obtain a systems framework of biological processes. One indispensable requisite is the accuracy of measurements.

Here we describe two quantitative approaches for array-based binding assays relying in the use of analyte concentration series and miniaturization of the arrayed probes. The accuracy of measurements achieved in a case study are 90 and 75% of correlation with independent "golden standards".

PEPTIDE ARRAYS The basic point of this technology is the simultaneous display of a systematic collection of peptides on a planar support, on which nu-



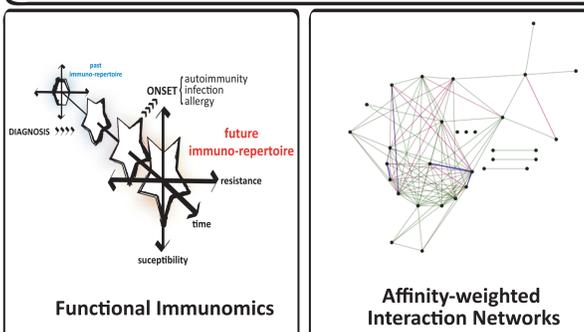
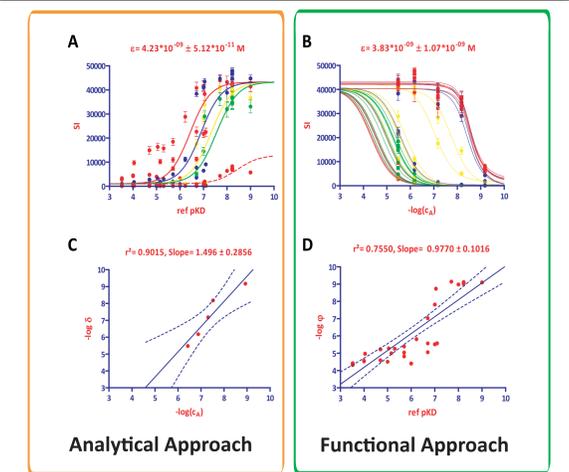
merous bimolecular interaction assays can be carried out under homogeneous conditions. The combination of SPOT peptide synthesis (left panel, 1 to 4) with appropriate [1] immobilization techniques on glass supports (left panel, 5 and 6) is wide spread. The SPOT technology provides low-scale but high-throughput synthesis, while immobilization of pre-synthesized peptides offers the benefit of a "chemical" purification step and flexible array design. Additionally, the glass support is compatible with fluorescence detection and offers the possibility to miniaturize binding assays. Beyond economy, the later point is essential for quantitative measurements at the steady-state of binding activity, as has been described by Ekins [2] and can be proven by the mass-action law.

THE MODEL The binding reaction between analyte sample (e.g. antibody in a buffered saline or serum) and arrayed probes (e.g. peptide epitopes) is analyzed by a model which simplifies the binding interaction as a three-component biochemical system: the components A, P and AP refer to a diffusible analyte in a sample solution, capture molecules, and captured analyte, respectively. Capture molecules P, in fact immobilized peptide probes, are idealized as free-diffusible components. The basic hypothesis is that all measured bimolecular interactions are carried out to chemical equilibrium.

Captured analyte AP is detected by discrete fluorescence emission from the features of the peptide array. Thus, fluorescence signal intensity SI is assumed to be directly proportional to the effective concentration of AP, c_{AP} . Clearing for c_{AP} in the resulting formalism (right panel, top) leads to the required expressions for the dependency of c_{AP} , and therefore of SI, from two experimentally adjustable variables: the affinity of interactions in terms of KD or the total analyte concentration c_A [3].

ANALYTICAL APPROACH experimental values of KD are adjusted to several independently measured peptide specific affinities to the analyte. Specially, when the effective probe concentration cannot be reliably controlled, it is important to adjust a wide range of probe-specific analyte affinities, e.g. from high pM to low mM affinities. This approach is shown here (bottom panel, A and C) to represent datasets of peptide array-derived SI generated with:

- a dilution series of analyte and



- independently measured peptide affinities for the analyte (preliminary SPR or ITC experiments).

It can be applied to analyze device production, measurement quality, and to determine the concentration of the analyte A in a sample solution for diagnosis.

FUNCTIONAL APPROACH The experimental values of c_A are adjusted by preparing a series of determined analytes concentrations. This approach is shown here (middle panel, B and D) to represent datasets of peptide array-based measurements generated by:

- a determined concentration series of total analyte
- a collection of peptide probes of undetermined affinity for the analyte.

It can be applied to statistically estimate the value of the involved affinities in terms of KD. A condition for accurate estimations is the application of analyte concentrations lower than the strongest affinity and higher than the weakest affinity (c_A and KD in M units).

MESSAGE IN A BOTTLE Such affinity profiles may provide greater reliability as reactivity profiles [4] for multivariate statistics used to predict resistance or susceptibility to pathological states [5]. Additionally, they have been used to weight SH2-domain interaction networks for relevant targets [6].

NOTES & REFERENCES

1. i.e., approaches that allow chemoselective and oriented immobilization. Several ligation and biocoupling techniques have been adapted.
2. Ekins, R. P. (1998) Ligand assays: from electrophoresis to miniaturized microarrays. Clin Chem 44, 2015-30.
3. Tapia, V., Bongartz, J., Schutkowski, M., Bruni, N., Weiser, A., Ay, B., Volkmer, R., and Or-Guil, M. (2007) Affinity profiling using the peptide microarray technology: a case study. Anal Biochem 363, 108-18.
4. reactivity profiles often refer to sophisticated transformations of raw signal intensity.
5. Quintana, F. J., Hagedorn, P. H., Elizur, G., Merbl, Y., Domany, E., and Cohen, I. R. (2004) Functional immunomics: microarray analysis of IgG autoantibody repertoires predicts the future response of mice to induced diabetes. Proc Natl Acad Sci USA 101 Suppl 2, 14615-21.
6. Jones, R. B., Gordus, A., Krall, J. A., and MacBeath, G. (2006) A quantitative protein interaction network for the ErbB receptors using protein microarrays. Nature 439, 168-74.

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