

DNA-Programmed Chemistry for the Detection of BCR-ABL Fusion Protein in Expressing Cells

Lawrence Haff, Yan Chang, and Yumei Huang

Ensemble Discovery Corporation, 99 Erie St., Cambridge MA 02139

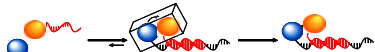
Objective: To develop simple assays employing the principle of DNA-programmed chemistry (DPC™) specific for detecting protein dimers. Specifically, employ DPC for assay of BCR-ABL fusion protein-containing cells arising in chronic myeloid leukemia.

The previously described DPC approach for detecting DNA sequences has been adapted to detecting protein homodimers, heterodimers, fusion proteins, and protein-protein interactions.

This approach offers the potential to include the measurement of proteins in their functionally-relevant state, for example BCR-ABL fusion proteins in individual cells. These cells can be detected by flow cytometry to identify subpopulations of cells responsible for minimal residual disease in CML patients.

Background and Rationale: DNA programmed chemistry (DPC) is a novel technology for synthesizing a wide array of organic compounds. The laboratory of Professor David R. Liu has demonstrated that the intrinsic binding energy from the annealing of complementary strands of DNA, each with an attached chemical moiety, creates high effective molarities that enhance, nearly 1 million-fold, both the rate and specificity of chemical reactions. DPC-facilitated reactions can be achieved with nanomolar concentrations of reactants under aqueous-neutral pH conditions at room temperature.

DNA-Programmed Chemistry (DPC)

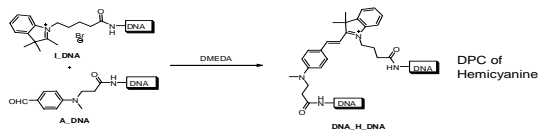
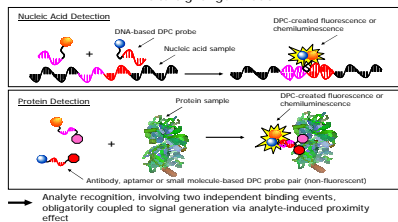


DNA hybridization increases the effective molarity and reaction specificity

- Reactants at nanomolar concentration are brought into close proximity
- reactions proceed at a localized higher concentration (millimolar to molar)
- reactions proceed in aqueous solution and physiological conditions

Based upon the fundamental principles underlying DPC and its inherent specificity, we hypothesized that this chemical approach could be used for bio-detection. We reasoned that the attachment of analyte recognition elements (e.g. antibodies, aptamers, or small molecules) to DPC-designed reactants could direct DPC to occur specifically at those adjacent sites contained within the analyte of interest. Furthermore if, for example, the reactants were non-fluorescent and the reaction product was fluorescent, then a very low non-specific background signal would be expected, facilitating the measurement of analytes in complex environments with increases in avidity, specificity and sensitivity.

A DPC-Enabled Detection System for Nucleic Acids and Proteins: *in situ* signal generation

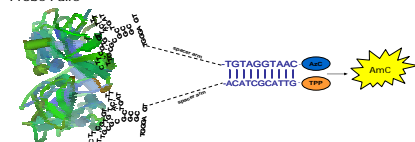


Example of Fluorescence-Producing DPC Reaction: The reaction employs a non-fluorescent indolinium precursor and an aldehyde on 3' and 5' terminal groups of complementary DNA oligonucleotides. In the presence of a catalyst, and if the oligonucleotides anneal, the fluorophore precursors react to produce an intensely fluorescent hemicyanine.

A DPC-designed probe pair is designed with each member of the pair binding independently to the protein. Each contains a recognition region and an oligonucleotide sequence which is complementary to the other. The two sequences anneal only at bulk solution concentrations much higher than those used in the assay. However, when both probes are bound to the protein simultaneously, their localized effective concentrations are increased, enabling DNA hybridization. This annealing event supports a DPC reaction generating a fluorescent product. The dual recognition serve as a point of increased avidity and specificity of the resulting ternary complex. The recognition elements can be aptamers, antibodies, or low molecular weight ligands.

Initial Proof-of-Concept Studies for DPC-Based Protein Detection: Initial studies used the homodimeric BB form of PDGF as the analyte and employed aptamers as protein recognition elements conjugated to complementary oligonucleotides in turn attached to the non-fluorescent reactants triphenylphosphine (5'-linked) and 7-azido-coumarin (3'-linked). Fluorescence generation that was strictly dependent upon the presence of PDGF was observed. The excitation and emission spectra were indicative of 7-amino-coumarin, the expected DPC product. Increasing concentrations of PDGF under conditions where the aptamer conjugates were not limiting gave proportional increases in fluorescence signal; maximal signal occurred when the ratio of complementary conjugates was 1:1.

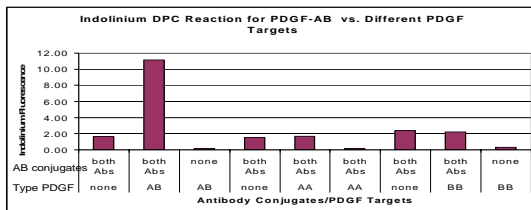
DPC-Based Detection of PDGF-BB with Aptamer-Containing Probe Pairs



- Analyte-dependent DNA hybridization within the ternary complex creates a point of avidity
- Improved specificity and affinity over singleton recognition elements
- Assay format enables detection of proteins in their functional context
 - Ideal for measuring homo or heterodimers, fusion proteins, auto-antibodies, and components of signaling pathways

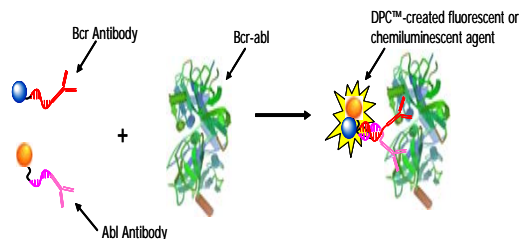


Design for antibody-based DPC Reagents: Antibody-oligonucleotide conjugates were prepared containing zip-coded oligonucleotides which anneal to complementary anti-zip-coded DPC oligonucleotides. The antibodies are not exposed to the processes necessary to synthesize the DPC chemistry on the oligonucleotides. Libraries of zip-coded antibodies can be annealed to DPC reagents simply by mixing them together.



Antibody-Based DPC Detection of Heterodimers: The same approach developed with aptamer-based DPC reagents was modified for the targeting heterodimeric proteins with pairs of anti-protein subunit antibodies.

In the above example, Indolinium DPC Reagents were prepared with anti-PDGF-A and anti-PDGF-B antibody conjugates as the affinity elements. A fluorescent response was generated in the presence of human PDGF-AB, but little response with PDGF-AA, PDGF-BB or negative controls missing antibodies.



Rationale for Developing a DPC-based Flow Cytometry Assay for Identifying BCR-ABL-Positive Cell Populations in CML Patients with Minimal Residual Disease

While methods such as PCR are sensitive indicators of the presence of minimal residual disease (MRD) in patients with CML, these methods only average the response of a minor population of cells within a large population, and provide only limited information towards understanding the molecular basis for the MRD and an individual patient's response to therapy. A DPC-based protein assay, that features dual recognition of an analyte obligatorily coupled to *de novo* signal generation, could enable the measurement of BCR-ABL in the context of a cell. In conjunction with flow cytometry, this approach could identify the population of cells responsible for the MRD and thus be invaluable for determining the best course of treatment in individual patients.

BCR-ABL Indolinium DPC Reaction and Analysis on KY01 Cells

Harvest cells and wash in PBS-BSA

Fix cells in PBS-4% formaldehyde-0.1% Saponin

Incubate cells in PBS/BSA/Saponin/IgG/50 nM blocking oligomer



Incubate cells in PBS + blockers buffer plus DMEDA catalyst 2-4 hours

Wash cells in PBS/BSA/saponin

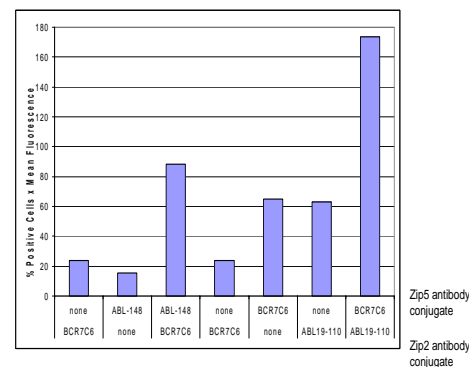
Store Cells in 0.5% Formaldehyde/PBS Buffer

Subject to FACS Analysis: excite 561 nM emit 590 nM

Process for DPC-Based Assay of BCR-ABL in KY01 Cells

- Antibodies specific for BCR and ABL selected
- Antibody conjugates containing oligonucleotide zip codes prepared
- Antizip-coded oligonucleotide reporters synthesized, each containing a nonfluorescent precursor to a fluorophore
- Antibody conjugates mixed with antizip-coded oligonucleotide reporters
- Assembly of Antibody conjugates with reporters incubated with fixed, permeabilized KY01
- Adjacent antibodies binding to BCR & ABL sites on BCR-ABL support reaction of adjacent fluorophore precursors to produce fluorophore
- Cells sorted by FACS

FACS Analysis of Indolinium DPC Reactions vs. BCR-ABL in KY01 Cells



Results from FACS Assay of KY01 Cells: The detection of BCR-ABL in KY01 cells relied upon antibody conjugates of anti-ABL-148 antibody or anti-ABL 19-110 antibody, and anti-BCR-7C6 antibody. The cells were fixed with formaldehyde and the reagents contained 0.1% Saponin to permeabilize the cells. High signal intensity required both antibody conjugates, and it did not matter which antibody carried the zip2 or zip5 sequence to anneal the DPC reporter oligonucleotides. The signal obtained with only one antibody conjugate was about equal to negative controls containing no antibody conjugates, indicating that two antibody were simultaneously required to obtain a DPC signal.

Advantages of DPC Reactions:

Specificity – two binding probes required

-increased avidity

-required localization of the two binding sites

Sensitivity: individual probes introduce no background fluorescence

Simplicity: a homogeneous format with protein targets, only simple washing steps required to assay protein dimers on/in cells

Versatility: demonstrated with soluble and immobilized proteins, cells, nucleic acids

Multiplexable: separate sets of probes may be used together generating different emission wavelengths.

Further Information

For more information call Larry Haff at Ensemble Discovery

617-492-6977 or LHaff@ensemlediscovery.com

Web site: www.ensemlediscovery.com

Or leave your business card.

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