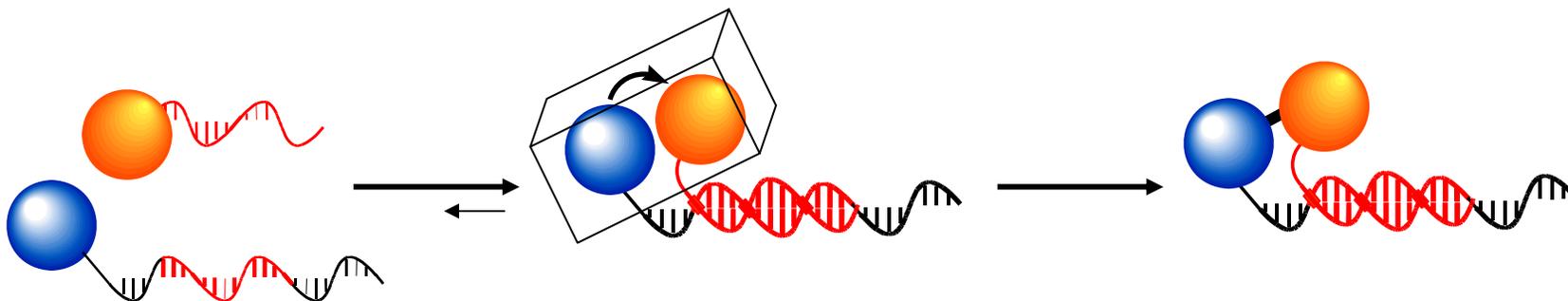


Highly Specific Detection of Proteins Using DNA Programmed Chemistry

Objectives:

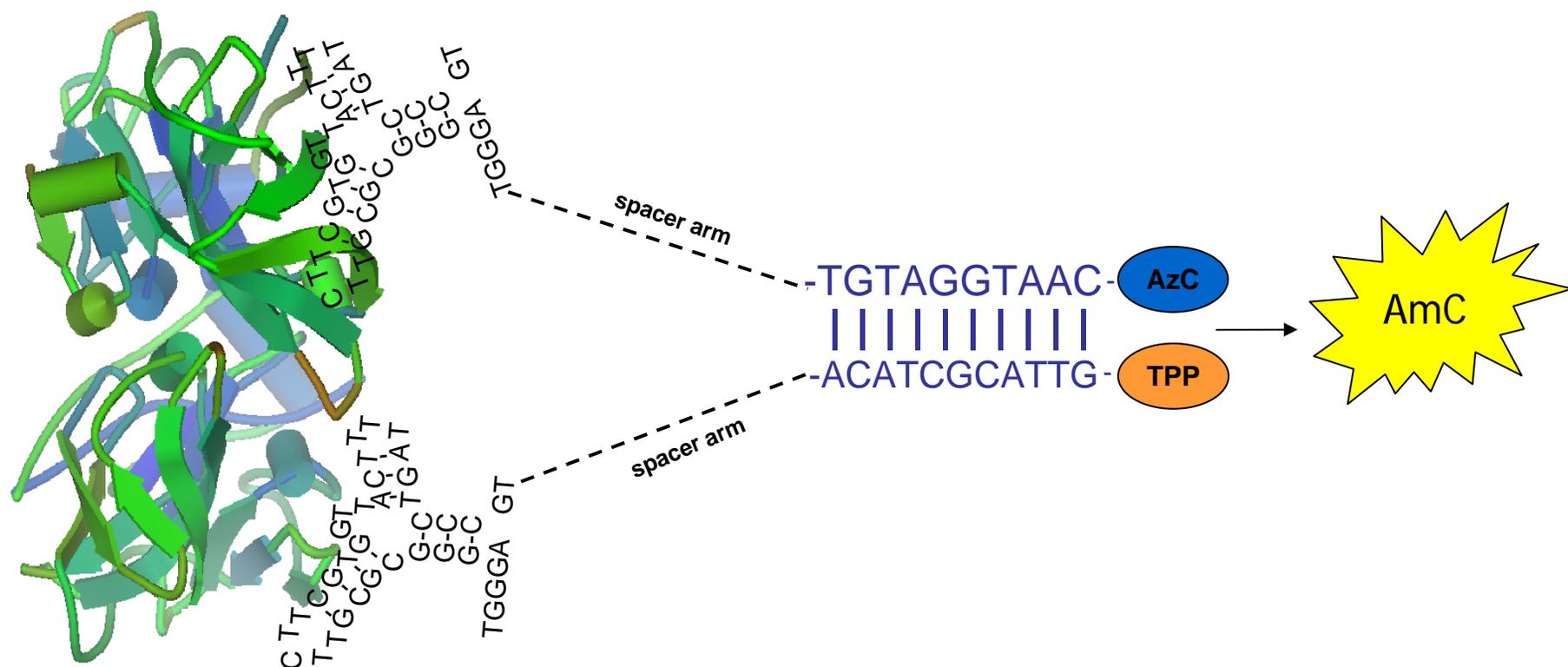
- Develop a modular assay platform broadly applicable for the specific detection of proteins in complex biological milieus
 - Simple homogeneous format for point-of-care assays
 - Flow cytometry format to identify and isolate subpopulation of cells responsible for MRD
 - Adaptable for development of *in vivo* imaging agents
- Enable the specific detection of homo- and hetero-dimers, fusion proteins, protein-protein interactions all in presence of monomeric counterparts
 - Extend scalar measurements to include the measurements of proteins specifically in their functionally-relevant or (patho)physiological context
- Complete Proof-of-concept studies using BCR-ABL fusion protein to enable highly informed mechanism-based clinical decisions

DNA Programmed Chemistry (DPC)



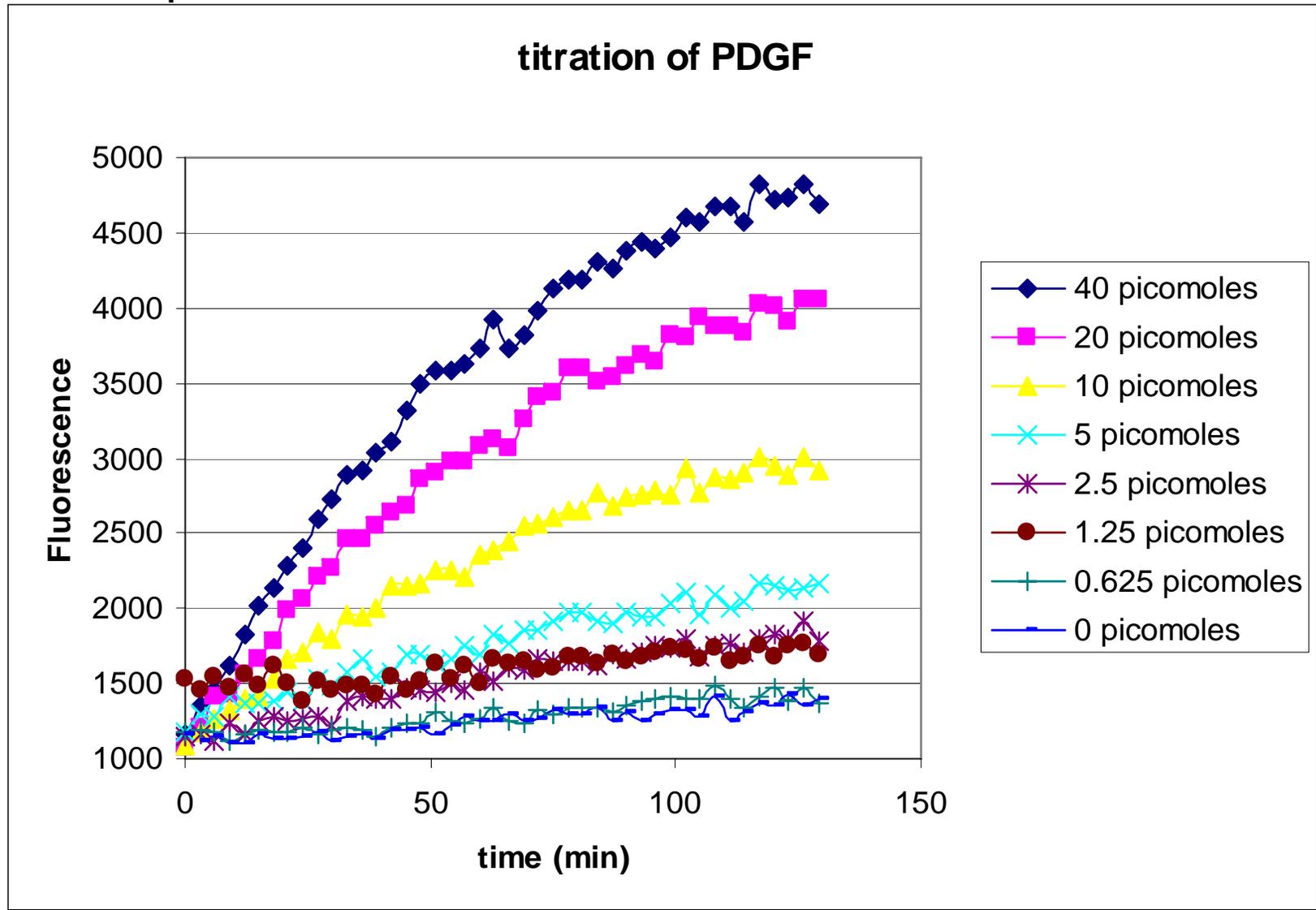
- DNA hybridization increases effective molarity and reaction specificity
 - Reactants (nanomolar) are brought into close proximity
 - Reaction proceeds at high effective concentration (millimolar-molar)
 - Analogous to single turnover enzymatic catalysis in the context of a cell
- Reactions occur in aqueous solution, often under physiologic conditions
- Potential for creating a detection signal with “zero” background
 - E.g., two non-fluorescent reactants can generate a fluorescent product
- DPC can be coupled to biological recognition elements
 - *De novo* signal generation at specific sites

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

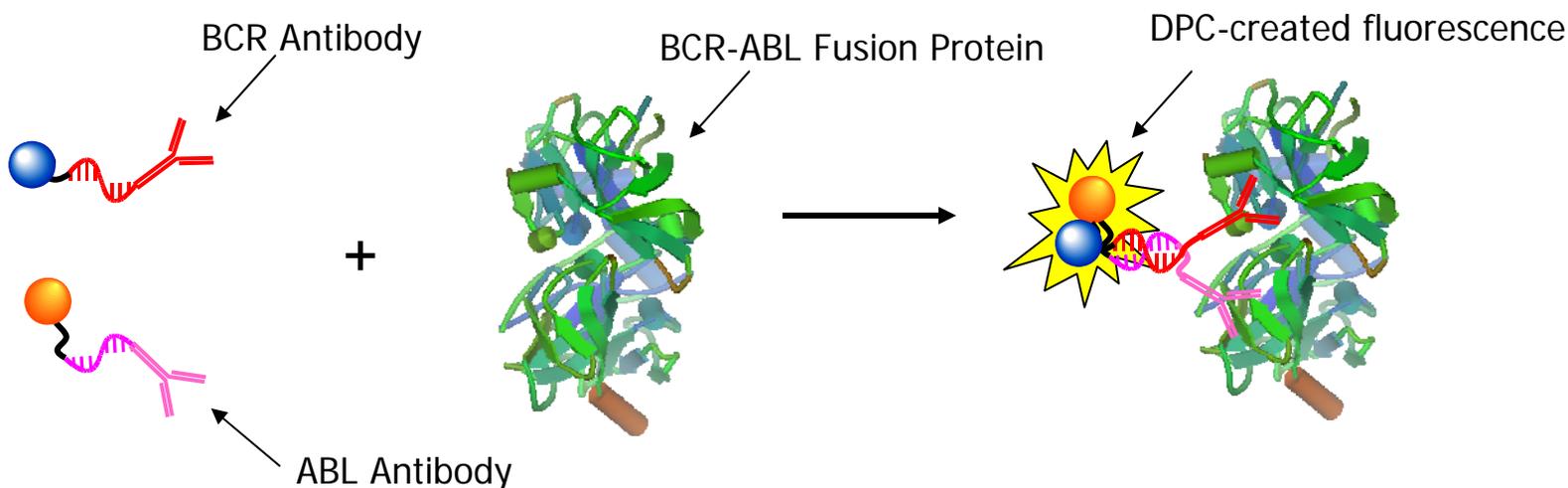


- Designed to ensure DNA hybridization occurs only in presence of analyte
 - Induced by proximity
- DNA duplex is a point of avidity within the ternary complex
 - Improved specificity and affinity over individual recognition elements
- Dual recognition is well suited for measuring homo or heterodimers, fusion proteins, auto-antibodies, and assembled components of signaling pathways in presence of monomeric counterparts

Homogeneous Assay Detection of PDGF-BB with DPC-Aptamers: PDGF- Dependent Fluorescence Generation



DPC-Enabled Chemical Synthesis at Specific Sites Should Prove To Be Useful for Both *In Vitro* and *In Vivo* Applications



Example: Clinical Application for *In vitro* Detection of BCR-ABL Fusion Protein

- When used with flow cytometry to measure minimal residual disease, should identify and isolate subpopulation of cells enabling mechanistic data-driven clinical decisions
- *In Vivo* use as a molecular imaging agent for extracellular targets should have intrinsically reduced non-specific background due to dual recognition of the target and *de novo* signal generation at the site of the target

Key Features of an Optimized DPC-Enabled Detection System

- **Specific:** Multiple factors contribute to signal generation
 - Two probes are required (dual recognition of the target)
 - Annealed probes must be localized and properly oriented
 - A chemical reaction, analogous to single turnover enzyme catalysis, occurs
- **Sensitive:** Individual probes lack ability to produce signal
 - Results in low background
 - Generates high signal/noise
- **Simple:** “Homogeneous” or “no-wash” method
 - Amenable to point-of care format
- **Adaptable:** Can be customized for multiple platforms
 - Easily incorporates broad-range of ligands and signal generation chemistries
- **Versatile:** Well-suited for both *in vivo* and *in vitro* applications
 - DPC-enabled reactions permit diverse chemistry in situ
- **Multiplexible:** Multiplexing achieved through creation of different signaling molecules
- **Cost-effective:** Economical for both nucleic acid and protein detection