Development of New DNA Sequencing Approaches and Investigation of Vision-related Proteins Using Synthetic Chemistry

Shenglong ZHANG

Submitted in partial fulfillment of the
requirements for the degree
of Doctor of Philosophy
in the Graduate School of Arts and Sciences

COLUMBIA UNIVERSITY

2008

ABSTRACT

Development of New DNA Sequencing Approaches and Investigation of Vision-related Proteins Using Synthetic Chemistry

Shenglong ZHANG

The fist part of this thesis presents our efforts to investigate vision-related proteins in visual cycle. The synthesis of a seven-membered ring locked analogue of 11-*cis*-retinol, tethered to a cross-linking moiety on C-15 and a lysine extended biotin on the C-3, was accomplished for its utilization as a probe to isolate retinol binding proteins that may be involved in the reisomerization of retinal from all *trans* to 11-*cis* in the visual cycle.

The second part of this thesis focuses on development of new DNA sequencing approaches using the concept of DNA sequencing by synthesis (SBS). In DNA SBS method, four nucleotide reversible terminators (A, C, G, T) are the most critical molecular tools for the efficient DNA polymerase reaction on a massive parallel DNA-sequencing chip. First, we designed and synthesized four nucleotides as reversible terminators by attaching a cleavable fluorophore to the nucleobase and capping the 3′-OH with a small reversible chemical moiety so that they are still recognized by DNA polymerase as substrates. After the cleavable

fluorescent nucleotides were incorporated into a growing DNA strand, the fluorophore and the 3′-OH capping group on a DNA extension product, were removed by photocleavage or in a chemical manner. This allows the re-initiation of DNA polymerase reaction and continuation of DNA SBS to the next cycle for incorporation of another fluorescent reversible nucleotide. The efficiency in cleaving the fluorophore and the 3′-OH capping moiety plays a crucial role in DNA SBS approach, and directly determines the read length of this methodology. To improve the efficiency of DNA SBS, we explored a variety of different chemical moieties as a 3′-OH capping group as well as the different cleavable linker bridging the nucleobase and the fluorophore.

The work led to the construction of a 4-color nucleotide library consisting of multiple functionalized nucleotides each with a suitable 3′-OH capping moiety and a cleavable fluorescent fluorophore. Also, we constructed a 3′-O-labeled nucleotide library by design and synthesis of nucleotide reversible terminators with different 3′-OH capping moieties. The above nucleotide libraries have been screened and evaluated as reversible terminators using the DNA polymerase reaction both in solution phase and on a DNA-immobilized chip. Furthermore, photocleavable nucleotide reversible terminators and fluorescent dideoxynucleotides were synthesized for the development of a chip-based Sanger-SBS hybrid DNA sequencing method. Finally, we solved the homopolymeric problems of DNA pyrosequencing by using the 3′-O-modified reversible terminators.

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.