Journal of
Proteomics & Bioinformatics

Ladame, J Proteomics Bioinform 2012, 5:12
DOI: 10.4172/jpb.10000e19

Editorial Open Access

Looking at the Genome and the Proteome with the Eyes of a Chemist: Engineered Molecular and Chemical Tools for Sensing and Therapeutic Applications

Sylvain Ladame*

Department of Bioengineering, Imperial College London, London, United Kingdom

It has been a major goal within biological science to achieve controlled intervention and manipulation of cellular events by strategies that target nucleic acids. This has been driven by both the considerable potential it opens up for improving the understanding of biological systems and pathways, and also the possibility of therapeutics in the longer term. One strategy is the direct alteration of genomic DNA via molecular biology (e.g. mutagenesis/gene therapy). Alternatively one may consider approaches where a secondary molecule (e.g. synthetic small molecule, natural or engineered protein) can induce temporary alterations in biology, e.g. changes in gene expression, by a molecular mechanism that involves direct interaction with DNA or RNA. Thus far, promising approaches for the development of artificial regulators of gene expression have included (i) antisense oligonucleotides or oligonucleotide mimics, and (ii) short interfering RNAs (siRNAs). Synthetic oligonucleotides were first developed to inhibit RNA translation via either formation of a steric block or via RNase H-mediated cleavage of the duplex formed. However, the efficacy of these oligonucleotides *in vivo* is limited by their rather poor cellular uptake and their metabolic instability. In order to overcome these intrinsic limitations, uncharged oligonucleotide analogues with improved pharmacological properties have been developed that include Phosphorodiamidate Morpholino Oligomers (PMOs) and Peptide Nucleic Acids (PNAs). Although these neutral analogues proved metabolically stable in cells and tissues, while retaining a very good affinity and sequence-specificity for their RNA target, they do not penetrate cells more easily than negatively charged oligonucleotides. Cell-Penetrating Peptides (CPP) was successfully used to deliver oligonucleotides but also PNAs and PMOs into cells. However, toxicity and endosomal entrapment have been identified as the two main limiting factors to the broad and systemic use of CPPs for the transfection of oligonucleotides (and analogues) into the cytosol or the nucleus of a cell.

G-rich nucleic acid sequences of the general type $G_{3+}N_{1-7}G_{3+}N_{1-7}G_{3+}N_{1-7}G_{3+}$ have the propensity to form highly stable four-stranded structures (termed G-quadruplexes or G4) *in vitro* in the presence of physiological cations, notably K^+ and Na^+. The formation of intramolecular DNA G-quadruplexes at the end of telomeres or in the promoter region of genes is now well-documented in the literature and these DNA secondary structures have been validated as very attractive therapeutic (e.g. anti-cancer) targets.

Promoter DNA quadruplexes may have evolved to play a regulatory role in nature, conversely they may not be naturally functional, but rather turn out to be elements that can be induced to play a functional role by the introduction of quadruplex-specific ligands (e.g. small molecules or proteins). There is a need to rigorously examine the existence, biophysical properties and functional role of disease-related quadruplexes, and at the same time address the possibility of a broader disease-related quadruplex paradigm that can be exploited for therapeutics. A critical and distinct feature of promoter quadruplexes that needs careful examination is their requirement to be formed within double-stranded regions of the genome. Extensive work has been carried out to specifically target such structures and few ligands are now available that strongly discriminate between quadruplex and duplex DNA and that also show biological activity *in vivo*.

Surprisingly, RNA quadruplexes have received so far much less attention than their DNA analogues although there is now growing evidences that RNA quadruplexes are involved in key biological processes. Moreover, RNA quadruplexes are also more likely to form *in vivo* than their DNA analogues given the evidence that RNA is single stranded and thus does not necessarily need to compete with complementary strands to fold into a quadruplex. While recent biological and biochemical studies unambiguously demonstrate the capacity of RNA quadruplexes to interfere with RNA translation, the possibility to regulate such processes by stabilising or inducing the formation of RNA quadruplexes *in vivo* requires the existence of ligands that can target these structures with both high affinity and specificity.

Having worked in the field of nucleic acids for over ten years, I have been particularly interested in elucidating the way they interact with either small molecules or proteins. Understanding how naturally occurring or engineered ligands bind to specific features of nucleic acids is essential in order to design probes or therapeutic drugs that target nucleic acid sequences and/or structures with both high affinity and high selectivity. My motivation is mainly driven by the fact that the chemical biology of nucleic acids has huge potential to improve human health. In the forthcoming years, I am aiming to focus on three equally promising strategies: (1) engineering fluorescent (or fluorogenic) probes that can be used to detect the presence of specific nucleic acid sequences (e.g. circulating miRNAs as biomarkers for cancer) or nucleic acid structures (e.g. DNA or RNA quadruplexes to validate these structures as suitable therapeutic targets for chemical intervention) *in vivo*; (2) engineering biologically active small molecules that target nucleic acids (or their interaction with proteins) and (3) engineering therapeutic nucleic acid structures (aptamers) that target proteins (or small biometabolites).

***Corresponding author:** Sylvain Ladame, Department of Bioengineering, Imperial College London, South Kensington Campus, London SW72AZ, United Kingdom, Tel: (+44) 02075945308; E-mail: sladame@imperial.ac.uk

Received November 26, 2012; **Accepted** November 26, 2012; **Published** November 28, 2012

Citation: Ladame S (2012) Looking at the Genome and the Proteome with the Eyes of a Chemist: Engineered Molecular and Chemical Tools for Sensing and Therapeutic Applications. J Proteomics Bioinform 5: xxii-xxiv. doi:10.4172/jpb.10000e19

In 2005, while I was working as a research associate in the group of Professor Balasubramanian (Cambridge, U.K.), we reported the first evidence of a quadruplex forming sequence occurring within the promoter region of the c-kit oncogene that proved a new target for therapeutic intervention in cancers. This first paper published in J.A.C.S. [1] was followed by a second article published in 2006 in Biochemistry [2]. Since then, increasing numbers of putative quadruplex forming sequences have been identified in oncogene promoters thus reinforcing the hypothesis that promoter quadruplexes can serve as regulatory elements of gene expression. During the same period, we demonstrated that zinc finger proteins could not only bind double-stranded DNA in a sequence-specific manner but could also be engineered to bind quadruplex DNA with high affinity (low nanomolar) and also high quadruplex versus duplex selectivity (>1000-fold). This work was published in two Biochemistry papers [3,4].

Since the start of my independent academic research career (head of the junior research group in Strasbourg, France, between 2006 and 2010 and now lecturer in the department of Bioengineering at Imperial College London, U.K., since 2010) I have been interested in various ways of demonstrating the existence of quadruplexes *in vivo* and understanding their biological function(s) in the context of a cell. This can be achieved by either developing highly specific fluorogenic probes for sensing individual quadruplexes (for recent examples see [5,6]) or linking promoter quadruplexes to naturally occurring transcription regulatory mechanisms. For instance, we have recently demonstrated that the natural protein involved in DNA damage response mechanisms and in gene regulatory mechanisms, Poly(ADP-ribose)polymerase-1 (PARP-1) could bind to the c-kit promoter quadruplex with nanomolar affinity and that this protein/DNA interaction could trigger PARP-1 catalytic activity. This first example of a functional interaction between a promoter quadruplex and a natural protein was published in 2008 in A.C.S. Chemical biology [7].

Trained as a Bioorganic Chemist but working in a Department of Bioengineering, the research pursued in my group can be qualified as "fundamentally translational and multidisciplinary". Developing chemical tools and probes to understand how nucleic acids fold in the context of a cell, to understand how they interact with small molecules and protein and how these interactions can induce a change in the level of expression of specific genes is with no doubt a question of fundamental interest. But it is also undisputable that the answers to these questions have translational consequences. Demonstrating the existence of DNA/RNA tertiary structures in cellulo, linking their formation (freely or with the assistance of one or more proteins) with a modulation of the level of expression of specific genes validates these structures (and also maybe their protein partners) as potentially valuable targets for therapeutic applications.

Building on more than 8-year experience in designing and engineering small molecules and proteins directed against nucleic acids and understanding their mode of binding *in vitro*, I am now very much interested in having a closer look at DNA and RNA folding in their "natural environment". This has obvious consequences on the nature of the probes (and/or drugs) one needs to imagine and then synthesise.

For instance, we recently reported the conception of sensitive fluorescent biosensors that are highly specific for unique DNA quadruplexes *in vitro*. The general strategy consists in targeting simultaneously the quadruplex structure itself but also its two single-stranded flanking regions in a sequence specific manner, thus allowing discrimination between one quadruplex and another. Briefly, two short Peptide Nucleic Acids (PNAs) complementary to both quadruplex flanking regions are functionalized with two non-fluorescent precursors of a fluorescent trimethine cyanine dye. Upon hybridization of the PNA probes to their single-stranded complementary DNA sequences through Watson-Crick base pairing, both moieties become in close enough proximity to react with each other when the DNA sequence between both PNAs is folded into a quadruplex structure only, while they are kept separated if the DNA remains single-stranded. Highly specific probes directed against a unique quadruplex of interest can be simply designed by adapting the PNA sequences (i.e. by making them complementary to the flaking regions of the quadruplex of interest). Whilst this rather elegant strategy has been validated *in vitro*, one step forward would be to apply this technology *in vivo*. A not so small step that involves (1) delivering the PNA probes into cells and (2) successfully detecting the characteristic fluorescent signal arising from the DNA sensing event. Few strategies are currently being investigated in our group that will be reported in due course.

Since DNA quadruplexes have been linked to ageing and cancer, a large number of small molecules have appeared in the literature that bind to and stabilise these motifs in a structure-specific manner. Selected molecules that bind promoter quadruplexes *in vitro* were also shown to inhibit the transcription of the corresponding genes *in vitro* and also *in vivo*, thus providing indirect evidences of (i) G-quadruplex formation *in vivo* and of (ii) their potential role in transcription regulatory mechanisms. Having contributed, during the past decade, to the discovery of a few original scaffolds that strongly bind and stabilise G-quadruplex DNA and are also capable of discriminating between quadruplex and duplex DNA (for a recent example from our group, [8]) we are now interested in the design of small molecules with increased specificity, i.e. molecules that can discriminate efficiently between one quadruplex and other secondary structures, including other quadruplexes. Molecules that target key interactions between quadruplexes and naturally occurring proteins rather than the quadruplex structure itself are also an attractive strategy we are currently following in our group.

Our research interests are however not restricted to quadruplexes. Fluorescent probes that target nucleic acids can have multiple purposes: (1) to identify/validate novel therapeutic targets, but also (2) to target and sense biomarkers for the diagnosis or prognosis of specific diseases. For instance, high-throughput profiling of circulating miRNAs has recently emerged as a highly promising strategy for the detection of human cancers, especially at an early stage via non-invasive or minimally invasive diagnostic tools. Validation of miRNA profiling as a reliable and cost-effective way to diagnose specific cancers requires effective tools that are sensitive, specific and quantitative. Recent efforts from our group have been focusing on the simultaneous, multi-coloured and quantitative detection of specific circulating miRNAs using original chemical probes. Developing profiling methods that are efficient and quantitative has proven to be challenging for circulating miRNA, notably because of their small size and sequence similarities. There is therefore an urgent need for reliable, highly sensitive, specific and quantitative profiling procedures that can be easily multiplexed.

In conclusion, the way biomacromolecules (DNA, RNA, proteins) fold *in vivo*, the way they interact with each other to form networks, the way these folding mechanisms and/or interaction pathways can trigger a biological response can often be explored with chemical and/or biochemical tools... at least as long as these tools are specific enough and biocompatible. The journey starts with the rational design

of the molecular probe (which structure? what properties? How can it be synthesised?) and, if successful, ends with the visualisation of the biological target or event of interest *in vivo*... the journey is often long, never follows a straight line but is almost always rewarding. Being able to carry out every step in-house, using a combination of multi-disciplinary skills acquired over the years and being surrounded by a highly stimulating environment of bioengineers, is probably what makes it even more exciting.

References

1. Rankin S, Reszka AP, Huppert J, Zloh M, Parkinson GN, et al. (2005) Putative DNA quadruplex formation within the human c-kit oncogene. J Am Chem Soc 127: 10584-10589.
2. Fernando H, Reszka AP, Huppert J, Ladame S, Rankin S, et al. (2006) A conserved quadruplex motif located in a transcription activation site of the human c-kit oncogene. Biochemistry 45: 7854-7860.
3. Patel SD, Isalan M, Gavory G, Ladame S, Choo Y, et al. (2004) Inhibition of human telomerase activity by an engineered zinc finger protein that binds G-quadruplexes. Biochemistry 43: 13452-13458.
4. Ladame S, Schouten JA, Roldan J, Redman JE, Neidle S, et al. (2006) Exploring the recognition of quadruplex DNA by an engineered Cys2-His2 zinc finger protein. Biochemistry 45: 1393-1399.
5. Meguellati K, Koripelly G, Ladame S (2010) DNA-Templated Synthesis of Trimethine Cyanine Dyes: A Versatile Fluorogenic reaction for Sensing G-Quadruplex Formation. Angew Chem Int Ed 49: 2738-2742.
6. Koripelly G, Meguellati K, Ladame S (2010) Dual sensing of hairpin and quadruplex DNA structures using multicolored peptide nucleic acid fluorescent probes. Bioconjug Chem 21: 2103-2109.
7. Soldatenkov VA, Vetcher AA, Duka T, Ladame S (2008) First evidence of a functional interaction between DNA quadruplexes and poly(ADP-ribose) polymerase-1. ACS Chem Biol 3: 214-219.
8. Percivalle C, Mahmood T, Ladame S (2012) Two-in-one: a pH-sensitive, acridine-based, fluorescent probe binds G-quadruplexes in oncogene promoters. Med Chem Commun.