DNA base mismatch detection with bulky rhodium intercalators: synthesis and applications

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Abstract

This protocol describes the syntheses and applications of two metallointercalators, $Rh(bpy)_2(chrysi)^{3+}$ and $Rh(bpy)_2(phzi)^{3+}$, that target single base mismatches in DNA. The complexes bind mismatched DNA sites specifically and, upon photoactivation, promote strand scission neighboring the mismatch. Owing to their high specificity and sequence context independence, targeting mismatches with these complexes offers an attractive alternative to current mismatch- and SNP-detection methodologies. This protocol also describes the synthesis of these complexes and their use in marking mismatched sites. Irradiation of ^{32}P-labeled duplex DNA with either intercalator followed by denaturing PAGE allows the detection of mismatches in oligonucleotides. The protocol also outlines a method for efficient detection of single nucleotide polymorphisms (SNPs) in larger genes or plasmids. Pooled genes are denatured and re-annealed to form heteroduplexes; they are then incubated with either complex, irradiated and analyzed using capillary electrophoresis to probe for mismatches (SNP sites). The synthesis of the metallointercalators requires approximately 5–7 d. The mismatch- and SNP-detection experiments each require approximately 3 d.