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Nucleic acid mutations are of tremendous importance in modern clinical work, biotechnology and in fundamental studies of nucleic acids. Therefore, rapid, cost-effective and reliable detection of mutations is an object of extensive research. Today, Forster resonance energy transfer (FRET) probes are among the most often used tools for the detection of nucleic acids and in particular, for the detection of mutations. However, multiple parameters must be taken into account in order to create efficient FRET probes that are sensitive to nucleic acid mutations. In this review, we focus on the design principles for such probes and available computational methods that allow for their rational design. Applications of advanced, rationally designed FRET probes range from new insights into cellular heterogeneity to gaining new knowledge of nucleic acid structures directly in living cells.

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