Technology Offer

Simple, one-step assay for reliable measurements of NAD(H) and NADP(H) in complex samples

Ref.-No.: 0105-5434-IKF

Background

NAD(H) and NADP(H) are key ubiquitous cofactors involved in a multitude of redox reactions that regulate cellular metabolism. The ratios of free NADH/NAD+ and NADPH/NADP+ have a high physiological importance as they regulate cellular redox homeostasis and could be considered as cellular metabolic readout.
Besides their role in enzymatic conversions, these factors were more recently described as transcription regulators and important signalling molecules. Their imbalance has been implicated in cancer, cardiovascular and neurodegenerative diseases.
Current methods to measure these concentrations suffer from drawbacks such as interference by light-absorbing or fluorescing molecules, or their ability to measure only total NAD or NADP.

Technology

Our scientists developed highly sensitive, highly specific, pH-independent, HTS-compatible sensors based on the enzyme sepiapterin reductase (SPR). These sensors allow simple and reliable measurement of NAD+, NADP+ and/or the ratios of NAD+/NADH and NADP+/NADPH by fluorescence or luminescence-based detection. The sensors are functional in complex samples (e.g. lysate, serum) and have various applications such as enzymatic assays and clinical tests. Given the role of NAD+ in increasing lifespan and improving health, one very exciting application for this technology is a point-of-care test in the field of ageing.
Mechanistically, the NAD sensor comprises SPR and a ligand for SPR, the ligand being capable of intramolecular binding to the active site of the enzyme. Importantly, ligand binding showed a high co-factor dependency/specificity; it binds only in the presence of the oxidized redox form of NAD. The ligand does not bind in the presence of the other redox form, or in the absence of cofactor. The binding of the ligand to SPR changes the spectroscopic properties of the sensor molecule, and this can be readily detected by fluorescence or luminescence. After rounds of optimization to the cofactor binding site, NADP(H) and NAD(H) sensors with the above-mentioned properties were obtained.

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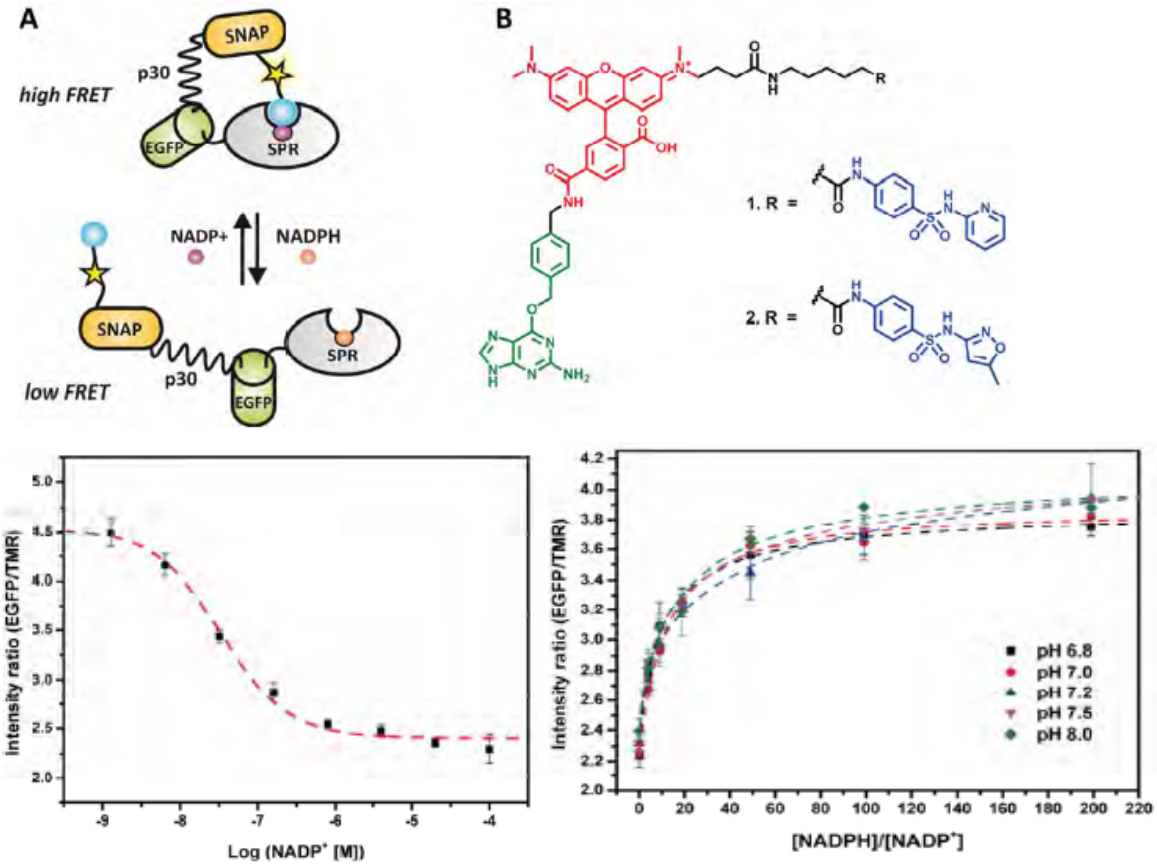

(A) Schematic description of the structure and sensing mechanism of a NADP-specific FRET sensor based on the human sepiapterin reductase as binding protein. (B) Chemical structure of the synthetic molecule BG-TMR-C6-SPY (1) and BG-TMR-C6-SMX (2) (SPY: sulfapyridine; SMX: sulfamethoxazole) used for the 20 SNAP-tag labelling. The O6-benzylguanine moiety for the SNAP-tag, the tetramethylrhodamine fluorophore and SPR ligands are depicted in green, red and blue, respectively. (C) Response curve of the sensor (SNAP-p30-EGFP-SPR labelled with BG-TMR-C6-SMX) titrated with NADP+. The FRET ratio change of this sensor is 1.8-fold between its closed and open state (improved to 8-fold in later versions). (D) Response curves of the sensor (SNAP-p30-EGFP-SPR labelled with BG-TMR-C6-SMX) titrated with ratios of NADPH/NADP+. Using sulfamethoxazole as intramolecular ligand produces a sensor insensitive to pH changes.

We are now looking for a licensing partner for this exciting technology.

Patent Information

PCT/EP2016/053280. National/regional phase entered in US/EU/JP in Aug. & Sept. 2017

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