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Interrogating Precision Electrophile Signaling

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Graphical Abstract

Understanding the targets and signaling roles of reactive electrophilic species (RES) at a specific cellular space and time has long been hampered by the reliance of the field on the bulk administration of excess RES from outside of cells and/or animals. Uncontrolled bolus methods provide limited understanding of target engagement for these individual nonenzymatic RES-modification events. REX technologies [targetable reactive electrophiles and oxidants (T-REX) and its genome-wide variant (G-REX)] were developed as a gateway to address these limitations.

These protocols offer a new ability to both profile kinetically privileged sensors (KPSs) of RES at a systems level (G-REXTM profiling) and monitor signaling responses at the sensor

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protein-of-interest (POI)-specific level (T- REXTM delivery) with high spatiotemporal resolution. REX technologies are compatible with several model systems and are built on a HaloTag-targetable small-molecular photocaged precursor to a native RES.

ADVANTAGES:

G-REX:

Allows quantitative ranking of KPSs of the RES of interest.

Can be fully user-controlled (choice of model system, expression level, and/or locale and time of RES release).

T-REX:

Provides validation and quantitative assessment (extent of modification) of RES-sensing ability.

Can be coupled to mass spectrometry or mutagenesis of potentially sensing residues to identify the site of RES modification.

Is compatible with numerous downstream assays.

>20 000 functional human and mouse genes fused to HaloTag are commercially available, facilitating focused screens of RES sensors.

RES release is rapid ($t_{1/2} \sim 1$ min) and RES modification of KPSs is instantaneous, offering information on timescale and/or latency of downstream signal propagation from the time of POI modification.

CHALLENGES:

Overexpression of HaloTag (with or without POI fusion) is required, but expression levels can be tuned by the user.

Halo-POI fusion protein must retain native activity for T-REX.

REX probes must be custom synthesized.

Although facilitated by the commercially available Halo ORFeome library, T-REX screens are low-to-medium throughput.

UV-illumination and/or REX-probe exposure may cause artifactual responses or cellular stress, but these can be carefully controlled.

The presence of a terminal alkyne on the REX probe renders the released RES 'non-native', but the alkyne is ultimately dispensable for signaling studies.

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