Supplemental Table S3. Comparison of redox proteomics methodologies.

Methodology type	General comments on approach	Details	Advantages	Additional limitations (beyond general approach)
Reversible oxidation (-SOx)				
Indirect: tag switch	- lysates only - indirect method & multistep	**Classic BST:** - Block (IAM, MMTS or NEM) - reduce (DTT/TCEP) - label (biotin-HPDP/NEM) - enrich on resin	- In principle widely applicable once optimised.	- Additional steps required for quantification or use label-free. - Site ID indirect. - Many blocking reagents may crossreact with *S*-sulfenic acid.
		Variant with **CysTMT** labelling [1]	Site ID (direct) and quant. (up to 6 samples).	- No enrichment - High analytical requirements for TMT.
		Variant with **IodoTMT** labelling [2]		
		OxiTRAQ (block, reduce, label, enrich, iTRAQ labelling of eluted peptides) [3]	Site ID (indirect) and quantification.	Protocol with large number of steps.
		OxiCAT (block, reduce, label with iCAT reagents, enrich, cleave from resin with acid) [4]	- Site ID (direct) and quant. - Robust, widely used.	Only 2 samples can be compared (c.f. TMT).
		Resin-assisted enrichment variants ("label" step replaced with direct resin capture) [5]	- Site ID (indirect). - Eliminates a step.	Additional steps required for quantification or use label-free.
Indirect: detect absence of labelling	- cells possible	In cell labelling of free thiols with **iodoacetamide-alkyne** or similar e.g. caged variant. Click on biotin [6, 7]	- Site ID (direct for free thiol) and quant. possible with isotope-encoded reagent.	- indirect detection of oxidised thiols via loss of labelling
S-nitrosylation (-SNO)				
Indirect: tag switch	- lysates only - ascorbate reduction not completely selective - indirect method & multi-step	**Classic BST for NO:** [8] - Block (IAM, NEM or MMTS) - ascorbate reduction - label (biotin-HPDP/NEM)	- In principle widely applicable once optimised.	- Additional steps required for quantification or use label-free. - Site ID indirect or not always possible.
		Variant with **ICAT** at label step.	- Quantification introduced via isotopic ICAT label. - Site ID via cleavable ICAT.	
Semi-direct: block, direct label	- lysates only - requires blocking of free thiols before direct labelling - advantage: no reduction step required	**Organomercury** reagents / resin	- site ID possible	- indirect site ID (elute thiol from resin)
		Phosphine-based reagents e.g. ***SNO*TRAP** [9]	- site ID possible	- reagents may react with disulfides too - indirect site ID (elute thiol from resin)
		***S*-sulfinic acid** reagents [10]	- site ID possible	- reagent has stability issues

Methodology type	General comments on approach	Details	Advantages	Additional limitations (beyond general approach)
S-sulfenylation				
Indirect: tag-switch	- lysates only - indirect method & multistep	**Tag-switch** - block (IAM, MMTS, NEM) - reduce -SOH (arsenite) - label (biotin-HPDP/NEM etc)		Selectivity problems: -SOH is susceptible to initial blocking step
Direct labelling	- lysates - cell permeable tool has been applied for imaging	**Strained alkyne (BCN)** [11, 12]		Possible cross-reactivity of BCN reagent with thiols?
Direct labelling	Advantages: - *in vivo* possible Limitations: - most commonly used reagents (i.e. dimedone-based) react slowly. New reagents in development (see main text).	**Dimedone-based biotin probes** [13]		- only in lysates. - site ID not straightforward - additional steps required for quantification or use label-free.
		Dimedone-based click probes [14] Label, click chemistry, enrich (via biotin). Quantification can be introduced via isotope-labelled click reagents.	- Can be applied *in vivo* - Site ID (direct) and quant. possible via use of cleavable click reagents.	- additional step required (click chemistry)
		YAP1 *in vivo* genetically encoded probe [15]	- Direct labelling in cells. - Genetically-encoded protein-based probes can be localised to specific organelles.	- requires genetic manipulation – only possible for some sample types. - system could perturb local redox states - limited dose and temporal control
S-sulfinylation (-SO_2H)				
Semi-direct: block then direct labelling	- lysates only - requires blocking of free thiols before labelling - advantage: no reduction step required	**NO-Bio** [16]		- not yet applied for proteomic analysis
		Biotin-GSNO [10]		- reagent has stability issues
		NEM-based [17]	- reagent is stable	- labelled adduct only stable under acidic conditions

Methodology type	General comments on approach	Details	Advantages	Additional limitations (beyond general approach)
S-sulfhydration				
Indirect: tag-switch	- lysates only	**Tag-switch.** Block free thiols (MMTS), react -SSH sites with biotin-HPDP.		unclear if MMTS is truly selective for -SH over -SSH
Semi-direct	- lysates only (possibly parts of workflows adaptable to cells?)	**Reduction tag-switch.** Alkylation of -SH and -SSH with IAA or MSBT, followed by cleavage (via reduction) and labelling of former -SSH site with biotin. Enrichment and elution. [18]	- site ID (indirect)	Selectivity problem: other disulfides (-SSR) also released by reduction and labelled.
		Alkylation of both free thiols and persulfides with **IAM-biotin**, enrichment and selective elution of former -SSH site from resin. [19]	- more streamlined than reduction tag-switch method - site ID (indirect)	- selectivity problems if peptide also contains a disulfide site
		Labelling tag-switch. Alkylation of both free thiols and persulfides with **MSBT**, followed by selective reaction of former -SSH site with **CN-biotin** reagent. Enrichment and elution. [20]	- site ID - overcomes selectivity problem of reduction tag-switch method	
S-glutathionylation and disulfides				
Direct	- in cells	**Biotin-GSH or -GSSH** added to cells. Lysis and enrichment of sites. [21, 22]	- can be performed in cells - site identification	- not measuring endogenous level (mimics increase in oxidative stress)
		Metabolic *in situ* Azido-GSH generation. [23]	- performed in cells - enables endogenous measurement	- requires expression mutant enzyme in cells
Indirect	- in lysates - relies on complete blocking	**Enzymatic tag-switch** for -SSG. Blocking of free thiols; Grx1 enzyme system added to reduce -SSG; newly released free thiols captured on resin or biotinylated. [5]	- enzymes are highly specific and operate under physiological conditions	- only identifies Grx/Trx-susceptible modified proteins (subset) - multi-step
		Enzymatic tag-switch for -SSR. Blocking of free thiols; Trx enzyme system added to reduce -SSR; newly released free thiols captured on resin or biotinylated. [24]		
		Enzymatic capture for -SSR. Blocking of free thiols; capture of Trx-susceptible disulfides via immobilised mutant Trx; elution with DTT. [25]	- enzymes are highly specific and operate under physiological conditions - fewer steps	- only identifies Trx-susceptible modified proteins (subset)

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