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N-terminal acetylation status of nuclear proteins upon proteotoxic stress

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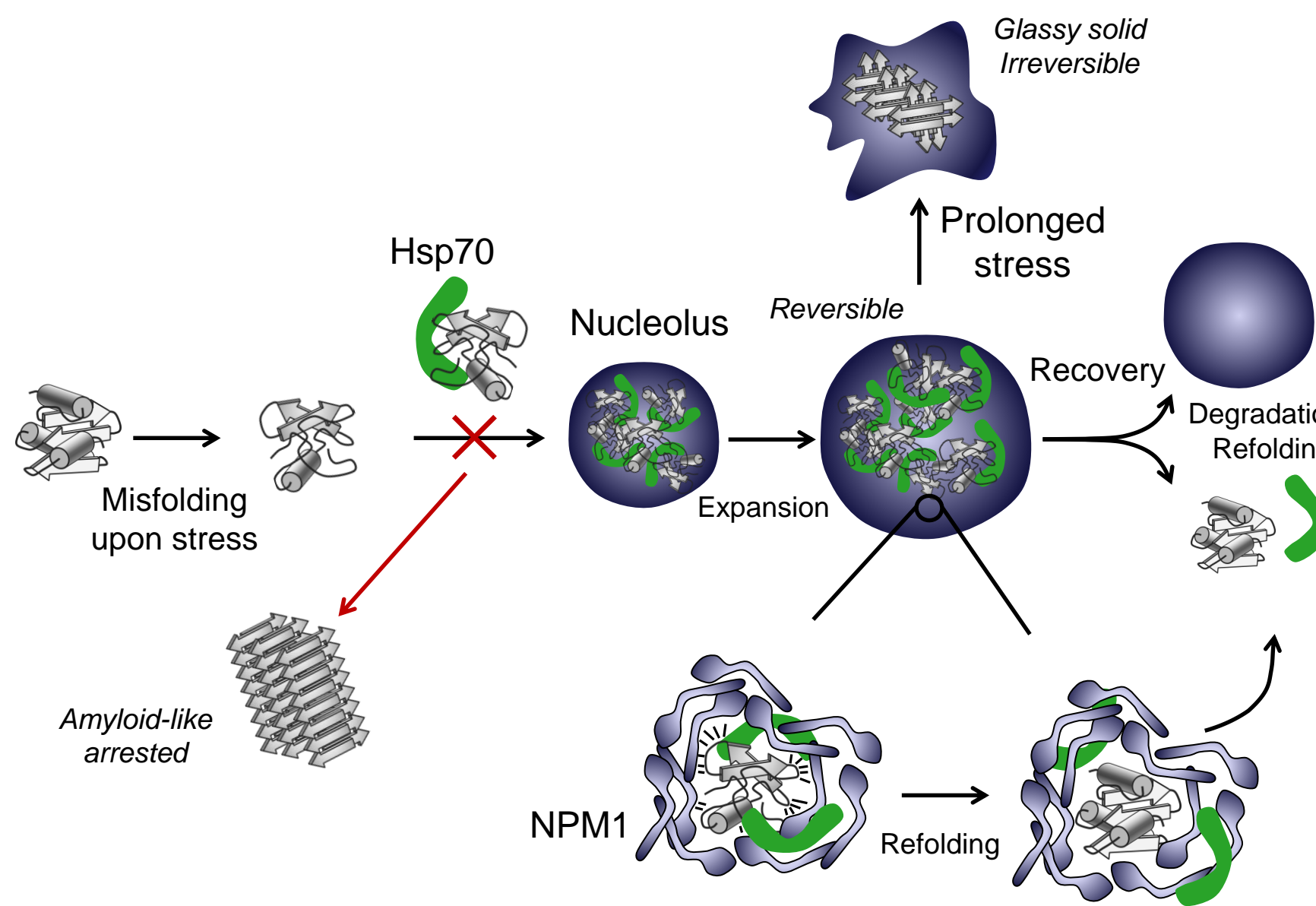
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N-(α)-terminal protein acetylation (NTA) is a widespread and essential protein modification. The reaction is catalyzed by various N-acetyltransferases (Nats) composed of a catalytic subunit alone or in complex with one or several factors. Their substrate specificities are largely determined by the first two N-terminal amino acids of the substrate protein. NTA is considered as an irreversible mark and mostly occurs co-translationally. A multiplicity of NTA roles have been uncovered such as its involvement in influencing protein's half-life. However, the majority of the proteome undergoes NTA with a high stoichiometry suggesting that NTA fulfils a broader and collective function, yet to be discovered. In this context, it has been suggested that NTA participates in maintaining protein homeostasis or proteostasis. The nucleus is rich in metastable and stress sensitive proteins suggesting that specific protein quality control strategies are in place to maintain nuclear proteostasis. Indeed

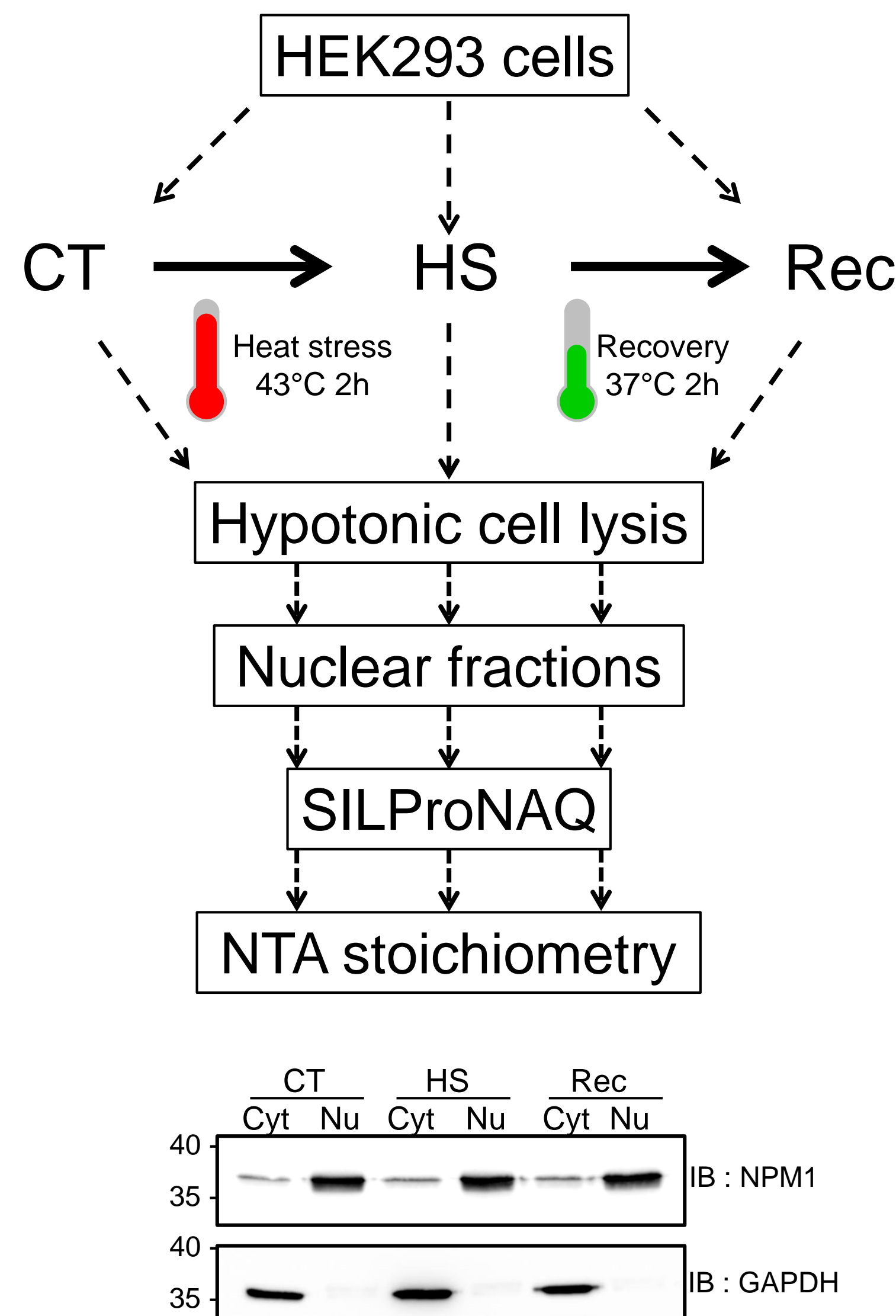
the nucleus is one of the target of pathological protein aggregation. Protein misfolding and aggregation are linked to an increasing list of diseases ranging from neurodegenerative disorders to cancer. In this context, protein N-termini may play an important role in stabilizing interactions of labile nuclear proteins and complexes in order to prevent nuclear proteostasis collapse. We thus set out to investigate NTA in HEK293 cells upon proteotoxic stress and recovery. We aimed to deliver a comprehensive nuclear N-terminal acetylome characterization and quantitate potential NTA changes upon stress, which may assist subsequent studies. To this end, we determined NTA stoichiometry of proteins from the nuclear fraction of cells subjected to stress and recovery using SILProNAQ method.

The nucleolus in nuclear protein quality control¹

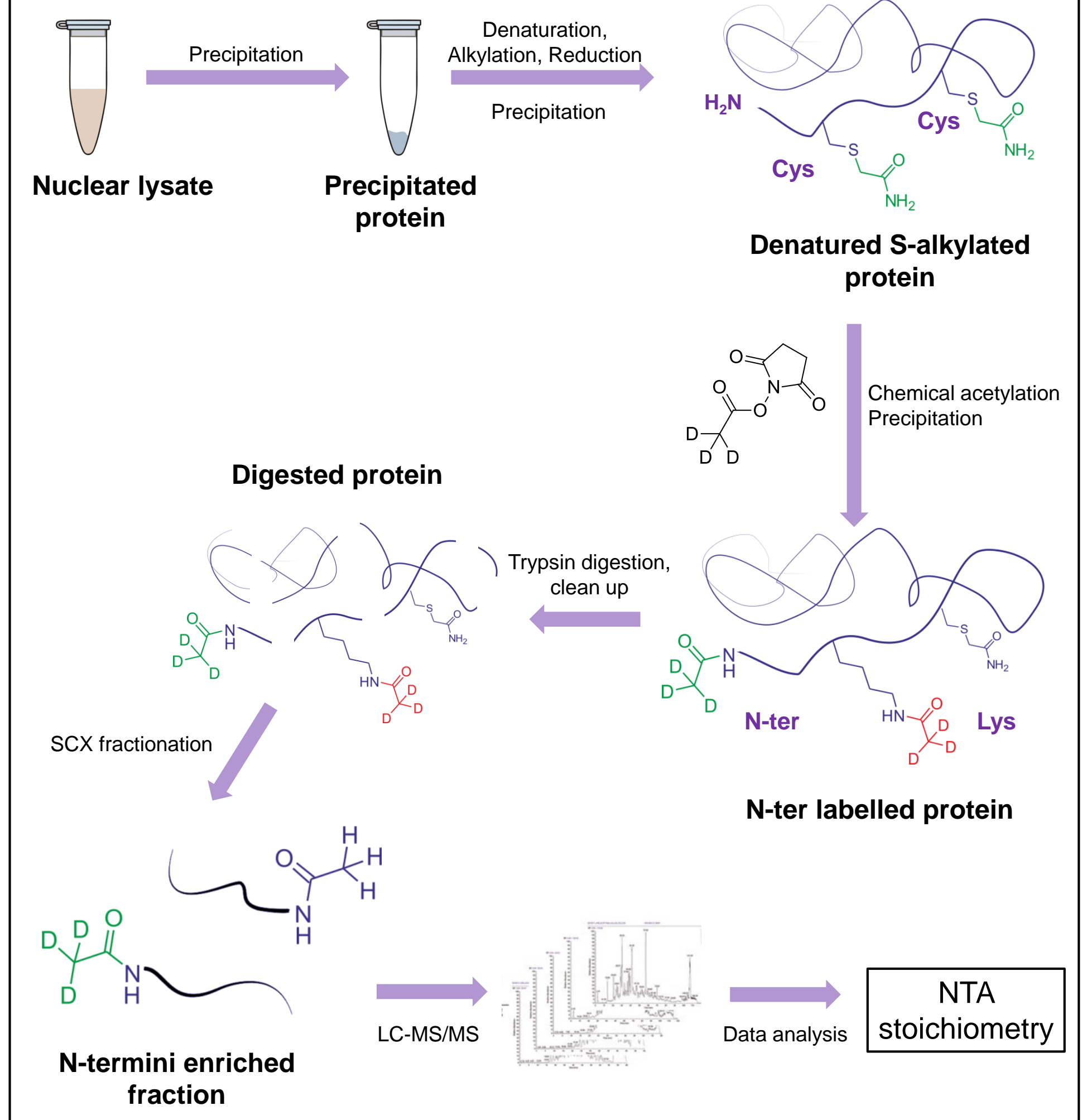


The nucleolus is a phase separated PQC compartment that prevents the irreversible aggregation of misfolded proteins. Specifically, upon heat stress, thermally unstable proteins migrate into the nucleolus, where the nucleolar granular component - one of the coexisting phases within the multiphase nucleolus - appears to exhibit non-enzymatic chaperone activity, whereby transient associations of misfolded proteins with nucleolar proteins prevent their irreversible aggregation. After stress subsides, misfolded proteins refold dependent on the activity of Hsp70 and exit the nucleolus. Dissolving the nucleolus abolished the chaperone activity of the granular component, resulting in amyloid-like aggregation of nuclear proteins. Furthermore, disease-related aggregation-prone proteins can poison the nucleolus and impair nucleolar PQC. Thus, the nucleolus harbors a novel chaperone-like capacity in a form of holdase^{1,2}.

Experimental workflow



Stable-Isotope Protein N-terminal Acetylation Quantification (SILProNAQ)³

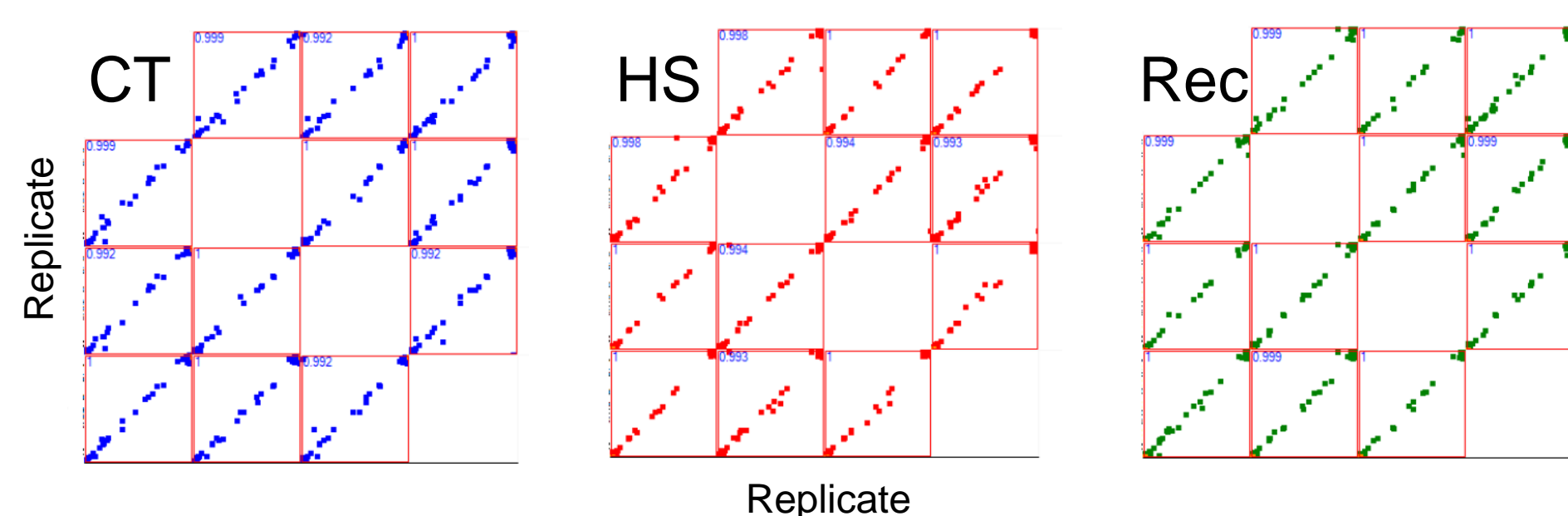


1432 N-termini identified and 770 NTA stoichiometry measured

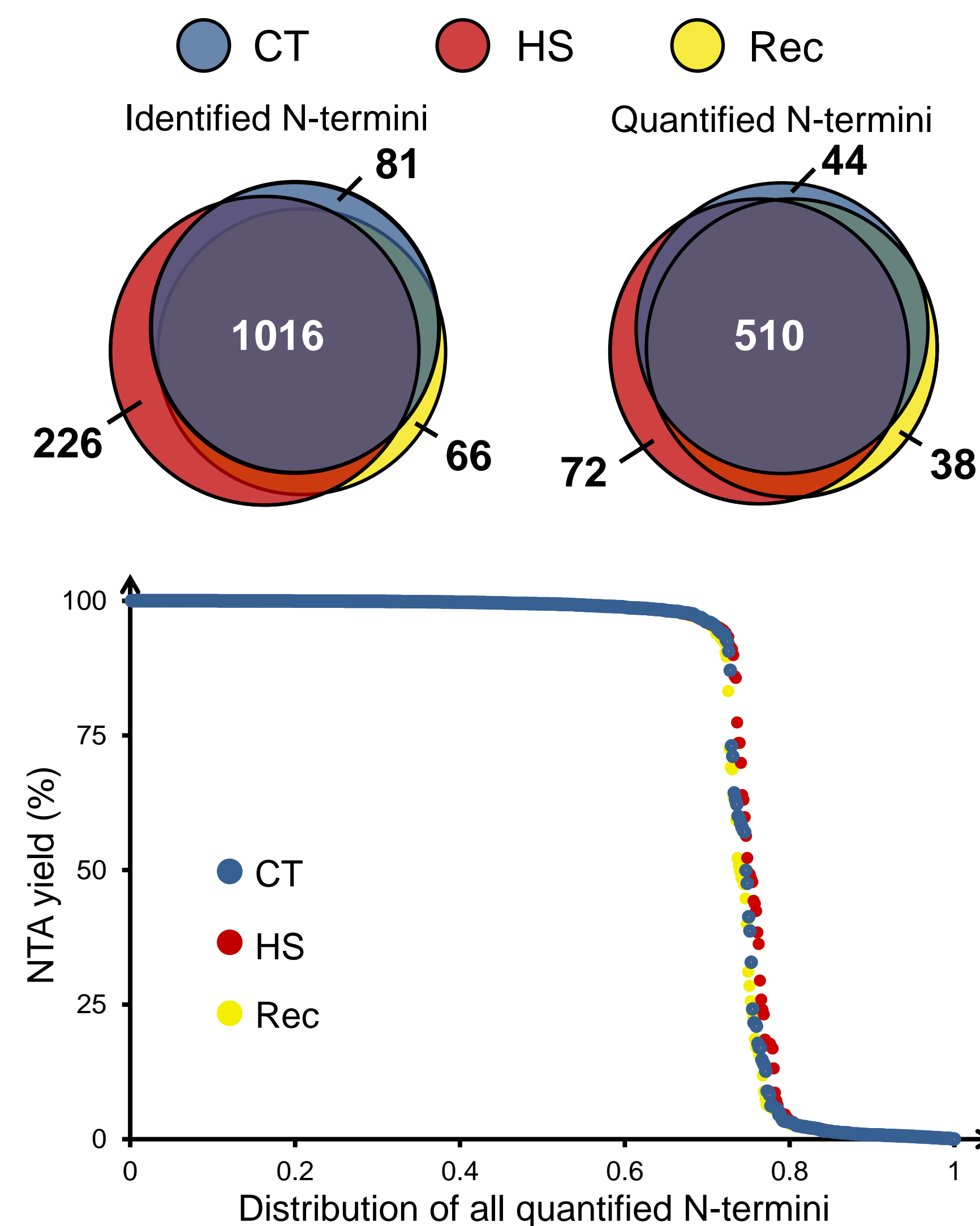
- 4 biological replicates, and 3 technical replicates

	CT (4 replicates)	HS (4 replicates)	Rec (4 replicates)	All (12 replicates)
Identified N-termini	1216	1355	1186	1557
Non-redundant proteoforms	1116	1262	1093	1432
Quantified N-termini	625	652	619	770
Full acetylation (NTA > 95%)	444	466	437	540
Partial acetylation	45	50	49	60
No acetylation (NTA < 5%)	136	136	133	170
iMet (pos. 1)	146	153	146	181
Full acetylation (NTA > 95%)	114	122	116	144
Partial acetylation	11	13	13	14
No acetylation (NTA < 5%)	21	18	17	23
+NME (pos. 2)	413	442	412	511
Full acetylation (NTA > 95%)	318	333	312	383
Partial acetylation	30	35	32	40
No acetylation (NTA < 5%)	65	74	68	88
Processed (pos. > 2)	66	57	61	78
Full acetylation (NTA > 95%)	12	11	9	13
Partial acetylation	4	2	4	6
No acetylation (NTA < 5%)	50	44	48	59

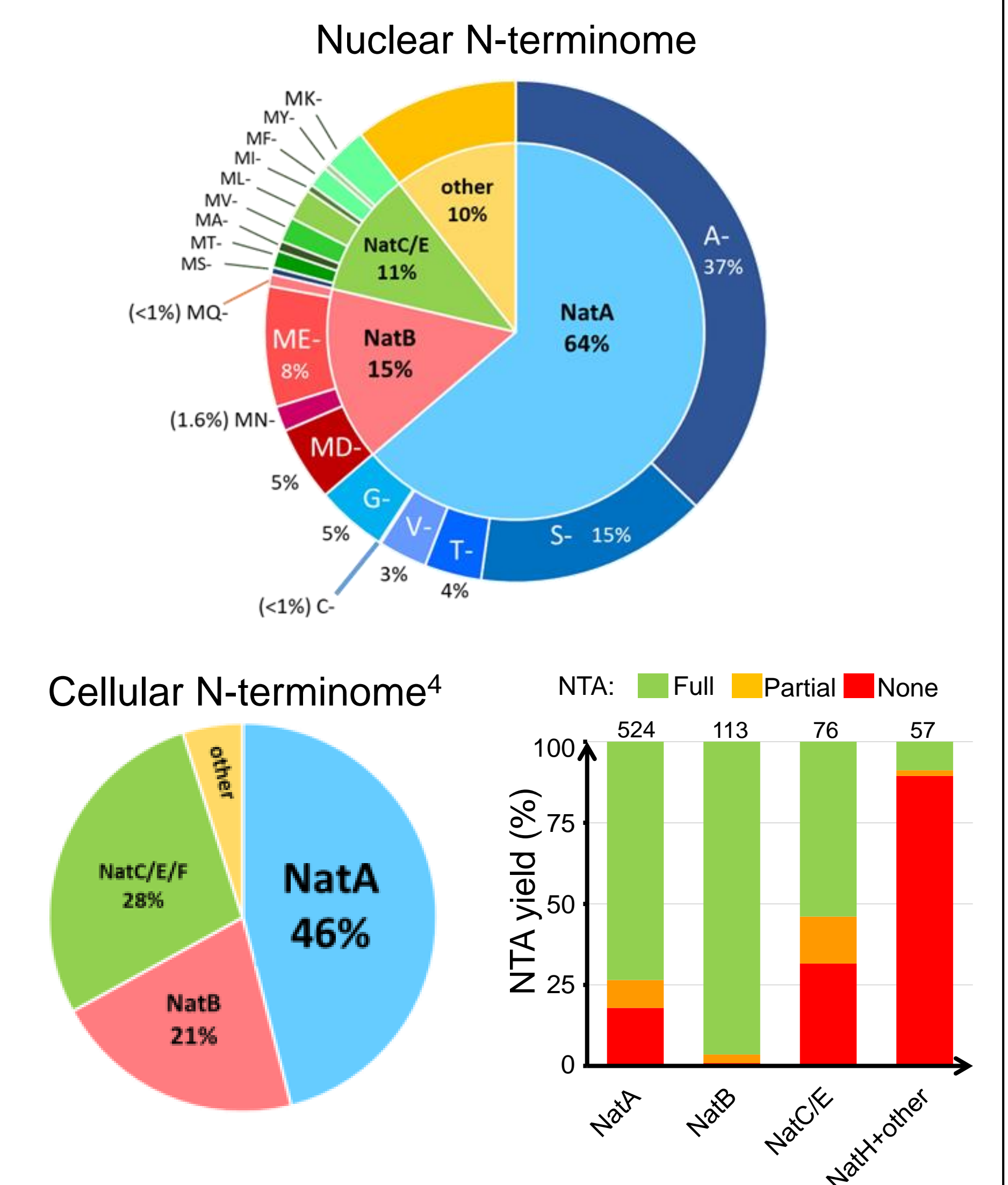
Pairwise Pearson correlation



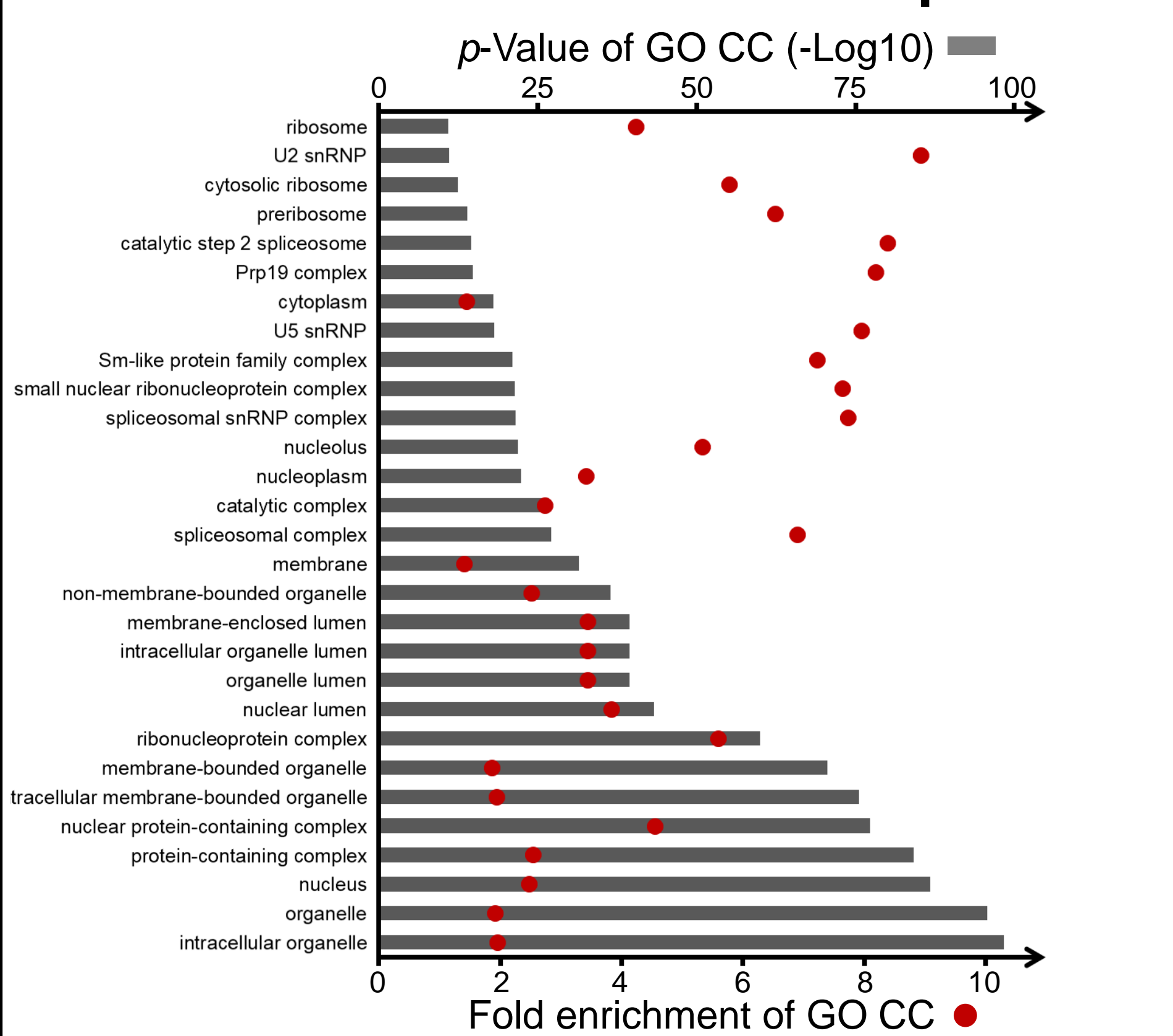
Impact of proteotoxic stress on NTA



The nuclear proteome is enriched for NatA substrates



Effective enrichment of nuclear proteins⁵



In a nutshell

- Validation of the methodology for NTA study of nuclear proteins
- Extended NTA stoichiometry for nuclear proteins
- NTA of nuclear proteins is preserved during stress and recovery
- Enrichment of NatA substrates in the nucleus as compared to whole-cell proteome.
- Set the basis for future studies

Perspectives

- Impact of downregulating NatA on nuclear protein quality control.
- Overall, a better understanding of protein modification roles in nuclear proteostasis may help in developing new strategies against proteinopathies.

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