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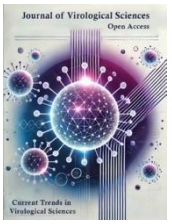

Research Article

HCV ReplicaseInteractome a Comprehensive Landscape of Host Factors Orchestrating Viral Replication

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Abstract

Hepatitis C virus (HCV), a significant global health burden, commandeers a plethora of host cellular proteins to facilitate its intricate replication cycle. The viral RNA-dependent RNA polymerase, NS5B, forms the catalytic core of the multi-protein replicase complex, a dynamic assembly anchored to modified endoplasmic reticulum membranes. Deciphering the complex web of host-virus Protein-Protein Interactions (PPIs) within the HCV replicaseinteractome is pivotal for understanding the fundamental mechanisms of viral propagation and identifying potential Achilles' heels for therapeutic intervention. This comprehensive review synthesizes the current knowledge of the HCV replicaseinteractome, encompassing the direct and indirect associations of the viral non-structural proteins (NS3, NS4A, NS4B, NS5A, and NS5B) with host cellular machinery. We meticulously examine the functional roles of these interactions across various stages of the viral lifecycle, including RNA binding, replication complex assembly, membrane remodeling, translation, and immune evasion. Furthermore, we critically evaluate the methodologies employed to map this intricate interactome and underscore the profound implications of these findings for the rational design of novel antiviral strategies. By providing a consolidated and in-depth analysis of the HCV replicaseinteractome, this review aims to foster a deeper understanding of HCV pathogenesis and pave the way for the development of innovative and targeted therapeutic interventions.

Introduction

Hepatitis C virus (HCV), a positive-sense single-stranded RNA virus belonging to the *Flaviviridae* family, remains a major global health challenge, affecting an estimated 71 million individuals worldwide and leading to chronic liver disease, cirrhosis, and hepatocellular carcinoma [1]. Upon entry into host hepatocytes, the ~9.6 kb HCV genome is translated into a single polyprotein precursor, which is subsequently cleaved by both viral and host proteases into ten structural and non-structural (NS) proteins. Among these, the non-structural proteins NS3, NS4A, NS4B, NS5A, and NS5B orchestrate the formation of a membrane-associated viral replication complex, commonly referred to as the replicase [2]. This dynamic multi-protein assembly, localized to specialized endoplasmic reticulum (ER)-derived structures known as the membranous web, is responsible for the critical process of viral RNA replication, utilizing the positive-strand genomic RNA as a template to synthesize new viral genomes.

The efficient and highly regulated replication of the HCV genome is not solely dependent on viral components but relies heavily on the hijacking and subversion of a complex network of host cellular factors. These host proteins are recruited and co-opted by the virus to facilitate a diverse array of processes essential for successful replication, including anchoring the replicase to cellular membranes, providing ranes, structural scaffolding, modulating the enzymatic activities of viral proteins, facilitating the trafficking and localization of viral RNA, and evading the host's intrinsic and adaptive immune responses [3]. Consequently, understanding the intricate web of these host-virus protein-protein interactions (PPIs), collectively termed the "HCV replicaseinteractome," is paramount for elucidating the fundamental molecular mechanisms underlying viral replication and for identifying potential host-derived vulnerabilities that can be exploited for the development of novel and targeted therapeutic interventions.

The HCV replicase is not a static entity but rather a highly dynamic and adaptable assembly whose protein composition and interactions with host factors can fluctuate throughout the viral lifecycle and in response to cellular cues [4]. Each of the five core viral NS proteins within the replicase complex plays a distinct and indispensable role in the replication process and engages with a specific and often overlapping set of host cellular factors. For instance, NS3, a bifunctional protein possessing both serine protease and RNA helicase/NTPase activities, interacts with host chaperones involved in protein folding and stability, as well as RNA-binding proteins involved in genome processing and

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immune modulation [5]. NS4A, a small hydrophobic protein, acts as a crucial cofactor for the NS3 protease and also serves as a critical anchor point for the replicase complex to the ER membrane, interacting with host proteins involved in membrane dynamics and complex stability [6] NS4B, an integral membrane protein that induces the formation of the membranous web, interacts with a diverse range of host factors involved in lipid metabolism, vesicle trafficking, and the manipulation of cellular signaling pathways [7]. NS5A, a highly phosphorylated and structurally unique protein with pleiotropic functions, engages with numerous host proteins implicated in RNA binding, replication complex assembly, interferon signaling antagonism, and virion morphogenesis [8]. Finally, NS5B, the RNA-dependent RNA polymerase (RdRp) responsible for catalyzing viral RNA synthesis, directly interacts with the viral RNA template and also engages with host factors that can modulate its enzymatic activity, processivity, and stability [9].

The systematic dissection and characterization of the HCV replicaseinteractome have been a major focus of intensive research efforts in the field of virology. A diverse arsenal of sophisticated proteomic and biochemical methodologies has been employed to identify and validate these crucial host-virus interactions, including yeast two-hybrid (Y2H) screens, co-immunoprecipitation (co-IP) assays followed by mass spectrometry (MS) analysis, affinity purification coupled to mass spectrometry (AP-MS), and proximity-based labeling techniques such as BioID and TurboID [10]. These comprehensive studies have collectively unveiled a complex and extensive network of interactions involving hundreds of distinct host cellular proteins, underscoring the profound reliance of HCV on the host cellular environment for its successful propagation.

This comprehensive review aims to provide an in-depth and up-to-date overview of the current knowledge regarding the HCV replicaseinteractome. We will meticulously discuss the key host protein interactors that have been identified for each of the core viral NS proteins within the replicase complex, critically analyze their functional roles in the intricate context of HCV replication, and thoroughly examine the profound implications of these findings for the rational design and development of novel antiviral therapies that specifically target these crucial host-virus partnerships. By providing a consolidated and critical analysis of the existing literature, this review aims to serve as a valuable resource for researchers seeking to further unravel the complexities of HCV replication and to identify innovative therapeutic strategies for combating this persistent viral infection.

Materials and Methods

The information presented in this comprehensive review was meticulously compiled through a systematic and extensive literature search utilizing relevant keywords and search terms such as "HCV replicase," "HCV interactome," "HCV protein-protein interactions," "NS3 interactome," "NS4A interactome," "NS4B interactome," "NS5A interactome," and "NS5B interactome" across prominent scientific databases including PubMed, Scopus, and Web of Science. Peer-reviewed research articles, comprehensive review articles, and relevant book chapters published up to March 2025 were carefully considered for inclusion in this review.

The inclusion criteria for the studies discussed within this review were rigorously defined to ensure the quality and relevance of the information presented: (1) studies must have unequivocally identified host cellular proteins that directly or indirectly interact with one or more of the core HCV non-structural proteins (NS3, NS4A, NS4B, NS5A, NS5B) that constitute the replicase complex; (2) the identification and validation of these interactions must have been performed using well-established and robust biochemical or proteomic techniques with appropriate controls; and (3) the studies must have provided clear experimental evidence or a strong mechanistic rationale implicating the functional relevance of these identified interactions in the context of the HCV lifecycle, with a particular emphasis on the viral RNA replication process.

Studies that primarily focused on genetic interactions without direct protein interaction data or those with limited experimental validation were generally excluded from this review.

The identified host protein interactors were systematically categorized based on their well-established and annotated cellular functions and their reported or proposed roles in the context of HCV replication. The extracted information from the selected studies was then meticulously synthesized and organized to provide a comprehensive and structured overview of the interactome for each individual viral NS protein and for the overall dynamic replicase complex.

Results

The HCV replicaseinteractome represents a highly intricate and dynamic network of host-virus protein-protein interactions that are absolutely essential for the efficient and precise replication of the viral RNA genome. The virus strategically hijacks and subverts a diverse array of host cellular proteins to create a favorable intracellular environment for its propagation. Below, we meticulously discuss the key host protein interactors that have been identified for each of the core viral NS proteins within the replicase complex and their implicated roles in the viral lifecycle.

NS3 Interactome

NS3, a multi-domain protein harboring both serine protease and RNA helicase/NTPase enzymatic activities, interacts with a diverse array of host cellular proteins that modulate its stability, enzymatic function, and involvement in viral RNA processing.

- **HSP90:** Heat shock protein 90 (HSP90), a highly conserved molecular chaperone, has been consistently shown to interact with NS3 and plays a crucial role in maintaining its proper folding, stability, and enzymatic activity [11]. Pharmacological inhibition of HSP90 leads to the proteasomal degradation of NS3 and a significant reduction in viral RNA replication.
- **FKBP8:** FK506-binding protein 8 (FKBP8), an endoplasmic reticulum (ER)-resident immunophilin, interacts with the NS3-NS4A complex and has been demonstrated to modulate the proteolytic activity of the NS3 serine protease, suggesting a role in the processing of the viral polyprotein.
- **DDX3X:** DEAD-box helicase 3, X-linked (DDX3X), a cellular RNA helicase involved in various RNA metabolic processes, has been shown to interact with NS3 and is actively recruited to the viral replication complex, where it is believed to facilitate RNA unwinding during viral RNA replication. Depletion of DDX3X has been shown to significantly inhibit HCV RNA replication.
- **G3BP1:**RasGTPase-activating protein-binding protein 1 (G3BP1), a key component of cellular stress granules, interacts with NS3 and has been implicated in the intricate regulation of viral RNA translation and replication, potentially by modulating the accessibility of viral RNA to the translational and replicational machinery.

NS4A Interactome

NS4A, a small integral membrane protein, serves as an essential cofactor for the NS3 serine protease, enhancing its proteolytic activity and substrate specificity. Furthermore, NS4A plays a critical structural role in anchoring the viral replicase complex to the ER membrane and in organizing the membranous web.

- **VAP-A/B:** Vesicle-associated membrane protein-associated protein A and B (VAP-A/B), ubiquitous ER-resident proteins involved in membrane contact site formation and lipid transport, are well-established interactors of NS4A and are absolutely essential for the formation and maintenance of the membranous web, the specialized ER-derived structures that serve as the platform for HCV RNA replication.
- **OSBP:** Oxysterol-binding protein (OSBP), a lipid-binding protein involved in sterol transport, interacts with NS4A and plays a crucial role in the recruitment of phosphatidylinositol 4-phosphate (PI4P) to the viral replication sites, a specific lipid modification of the ER membrane that is critical for efficient RNA replication.
- **PI4KIIIα:** Phosphatidylinositol 4-kinase III alpha (PI4KIIIα), a key lipid kinase responsible for the synthesis of PI4P, is recruited to the replication complex through its direct interaction with NS4A. Pharmacological inhibition of PI4KIIIα potently inhibits HCV RNA replication, highlighting the critical importance of this host factor.

NS4B Interactome

NS4B, an integral membrane protein with multiple transmembrane domains, is a key inducer of the dramatic ER membrane rearrangements that give rise to the membranous web. It interacts with a diverse array of host proteins involved in lipid metabolism, vesicle trafficking, and the modulation of cellular signaling pathways to create a replication-permissive environment.

- **ACBD3:** Acyl-CoA-binding domain-containing protein 3 (ACBD3), a peripheral membrane protein involved in lipid transport and metabolism, interacts with NS4B and plays a crucial role in the recruitment of PI4KIIIα to the viral replication complex, further emphasizing the importance of PI4P in HCV replication.
- **Rab5:** Ras-related protein Rab-5A (Rab5), a small GTPase that regulates early endosome trafficking, interacts with NS4B and has been implicated in the intricate process of membranous web formation and organization, potentially by modulating membrane curvature and fusion events.
- **FBL2:** F-box and leucine-rich repeat protein 2 (FBL2), a component of the Skp1-Cullin-F-box (SCF) E3 ubiquitin ligase complex, interacts with NS4B and has been shown to promote its ubiquitination and subsequent degradation, suggesting a potential role in regulating the steady-state levels of NS4B during the course of infection.

NS5A Interactome

NS5A, a highly phosphorylated and structurally unique protein, is a multifunctional regulator of HCV replication and virion assembly. It interacts with a remarkably diverse array of host proteins involved in various cellular processes, including RNA binding, protein trafficking, and signal transduction.

- **PI4KIIα:** Phosphatidylinositol 4-kinase II alpha (PI4KIIα), another lipid kinase responsible for PI4P synthesis, interacts with NS5A and contributes to the localized production of PI4P at the viral replication sites, highlighting the central role of this lipid modification in HCV replication.
- **hVAP-A:** Human vesicle-associated membrane protein-associated protein A (hVAP-A), a homolog of VAP-A/B, also interacts with NS5A and contributes to anchoring the replication complex to the ER membrane, providing structural support for the replication machinery.
- **Cyclophilin A:** Cyclophilin A (CypA), a peptidyl-prolyl *cis-trans* isomerase, interacts with the N-terminal domain I of NS5A and is essential for efficient HCV RNA replication in certain viral genotypes, likely by modulating the conformational dynamics of NS5A.
- **GSK-3β:** Glycogen synthase kinase 3 beta (GSK-3β), a serine/threonine kinase involved in numerous cellular signaling pathways, phosphorylates NS5A at multiple sites, and this phosphorylation has been shown to be crucial for viral RNA replication and potentially for the interaction of NS5A with other host factors.
- **AP1S3:** Adaptor-related protein complex 1 subunit sigma 3 (AP1S3), a component of the AP-1 clathrin adaptor complex involved in intracellular protein trafficking, interacts with NS5A and has been implicated in the intracellular trafficking and localization of viral proteins, potentially contributing to the assembly of infectious virions.

NS5B Interactome

NS5B, the RNA-dependent RNA polymerase (RdRp), is the central catalytic enzyme responsible for the synthesis of new viral RNA strands. While its primary function involves direct interaction with the viral RNA template, NS5B also engages with several host proteins that can modulate its enzymatic activity, processivity, and stability.

- **PCBP2:** Poly(rC)-binding protein 2 (PCBP2), a cellular RNA-binding protein involved in various RNA metabolic processes, has been reported to interact with the 3' untranslated region (UTR) of the HCV RNA and potentially with NS5B, suggesting a possible role in facilitating the initiation of RNA replication by bridging the viral RNA template to the polymerase. However, more recent studies suggest PCBP2's primary role might be in later stages of the viral lifecycle, such as genome packaging.
- **RPA:** Replication protein A (RPA), a heterotrimeric single-stranded DNA-binding protein involved in DNA replication and repair, has also been shown to interact with NS5B and may play a role in RNA unwinding or stabilization during the process of viral RNA replication.
- **PARP1:** Poly (ADP-ribose) polymerase 1 (PARP1), a nuclear enzyme involved in DNA repair and chromatin remodeling, interacts with NS5B, and its enzymatic activity has been implicated in the regulation of HCV replication, although the precise mechanism remains to be fully elucidated.
- **SETD2:** SET domain-containing protein 2 (SETD2), a histone methyltransferase involved in transcriptional regulation through histone H3 lysine 36 trimethylation (H3K36me3), interacts with NS5B and has been shown to negatively regulate HCV replication by modulating chromatin accessibility and potentially influencing the host cell's antiviral response.

Viral protein	Host interactor	Function in HCV replication
NS3	HSP90	Stabilizes NS3 and maintains its enzymatic activity
NS3	DDX3X	Facilitates RNA unwinding during viral replication
NS4A	VAP-A/B	Essential for the formation and maintenance of the membranous web
NS4A	PI4KIIIα	Recruits PI4P to replication sites, crucial for RNA replication
NS4B	ACBD3	Facilitates PI4KIIIα recruitment to the replication complex
NS4B	Rab5	Involved in the formation and organization of the membranous web
NS5A	CypA	Essential for RNA replication in certain genotypes, likely modulates NS5A conformation
NS5A	PI4KIIα	Contributes to localized PI4P production at replication sites
NS5A	GSK-3β	Phosphorylates NS5A, crucial for viral RNA replication and potentially other interactions
NS5B	RPA	Potential role in RNA unwinding or stabilization during replication
NS5B	SETD2	Negatively regulates HCV replication by modulating chromatin accessibility
NS3-NS4A	FKBP8	Modulates NS3 protease activity, influencing polyprotein processing
NS4A	OSBP	Involved in recruiting PI4P to replication sites
NS4B	FBL2	Promotes NS4B degradation, potentially regulating its levels during infection
NS5A	hVAP-A	Contributes to anchoring the replication complex to the ER membrane

Table 1: Key Host Protein Interactors of the HCV Replicase Components and Their Functions in HCV Replication.

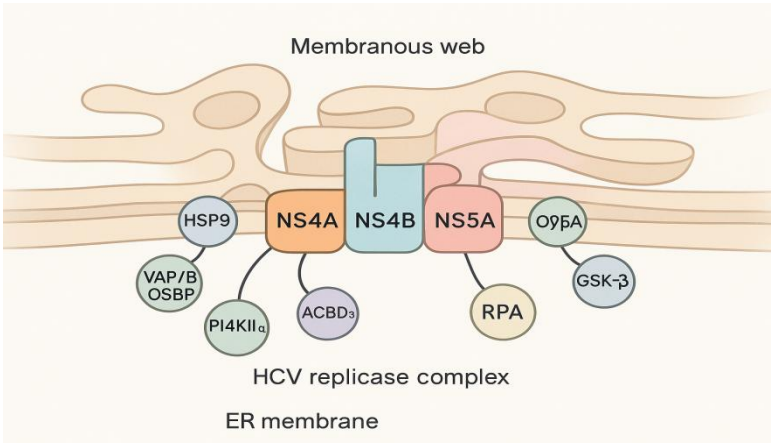

Figure 1: Schematic Representation of the HCV Replicase Complex Embedded in the Membranous Web, Highlighting Key Host Protein Interactions

Discussion

The HCV replicaseinteractome represents a highly complex and tightly regulated network of host-virus protein-protein interactions that are absolutely indispensable for the efficient and accurate replication of the viral RNA genome. The systematic identification and detailed functional characterization of these interactions have significantly advanced our fundamental understanding of the intricate molecular mechanisms underlying HCV pathogenesis. The remarkable diversity of cellular functions exhibited by the identified host factors, ranging from fundamental processes such as lipid metabolism and membrane trafficking to crucial regulatory mechanisms like RNA binding and protein folding, underscores the profound and extensive reliance of HCV on the host cellular machinery for its successful propagation within infected hepatocytes. The well-established interaction between NS4A and the ER-resident proteins VAP-A/B, coupled with the subsequent recruitment of key lipid kinases such as PI4KIIIα and PI4KIIα to the viral replication sites, unequivocally highlights the critical and central role of specific lipid modifications and dynamic membrane rearrangements in the HCV replication process.

The localized enrichment of PI4P on the ER membrane at the sites of viral replication, facilitated by these crucial host-virus interactions, creates a unique and specialized lipid microenvironment that is absolutely conducive to the proper assembly, stability, and optimal enzymatic activity of the multi-protein replicase complex. The compelling evidence implicating these host factors in HCV replication has spurred significant interest in targeting these interactions as a promising avenue for the development of novel antiviral strategies. For instance, pharmacological inhibitors of PI4KIIIα have demonstrated potent and broad-spectrum antiviral activity against various HCV genotypes in preclinical in vitro and in vivo studies.

The significant involvement of molecular chaperones, such as HSP90, in maintaining the structural integrity, proper folding, and optimal enzymatic function of essential viral proteins, particularly NS3, suggests another compelling avenue for therapeutic intervention.

HSP90 inhibitors have demonstrated antiviral efficacy against HCV by promoting the misfolding and subsequent degradation of these critical viral components. Similarly, the interaction of NS3 with cellular RNA helicases like DDX3X underscores the fundamental importance of efficient RNA unwinding for the successful progression of viral RNA replication, and specifically targeting these host-virus interactions could potentially disrupt the intricate process of viral genome amplification.

The remarkably diverse array of host protein interactors identified for NS5A, including CypA and GSK-3β, highlights the multifaceted regulatory role of this enigmatic viral protein in diverse stages of the viral lifecycle, including RNA replication, virion assembly, and modulation of host immune responses, further emphasizing its potential as a critical therapeutic target. The tremendous clinical success of direct-acting antivirals (DAAs) that specifically target NS5A has unequivocally demonstrated the therapeutic efficacy of disrupting key host-virus interactions involving this protein.

The identification of host factors that directly interact with NS5B, the viral RNA-dependent RNA polymerase, such as PCBP2 and RPA, provides valuable insights into the intricate mechanisms that govern the initiation and elongation phases of viral RNA synthesis. While highly effective direct-acting antivirals that directly target the active site of NS5B are now a cornerstone of HCV therapy, a deeper understanding of these host-polymerase interactions could potentially pave the way for the development of novel allosteric inhibitors or therapeutic strategies that indirectly interfere with polymerase function by targeting its essential host partners.

It is important to acknowledge that our current understanding of the HCV replicaseinteractome is likely to be incomplete, and the true complexity of this intricate network may be even greater than currently appreciated. The inherently dynamic nature of the replication complex, coupled with the potential for numerous interactions to be transient, context-dependent, or occurring at low stoichiometry, poses significant Furthermore, for many of the identified host-virus interactions, the precise technical challenges for comprehensive mapping efforts. molecular mechanisms and functional consequences in the context of the complete viral lifecycle remain to be fully elucidated. Future research endeavors employing cutting-edge and integrated proteomic techniques, advanced structural biology approaches, and sophisticated functional genomics studies will be absolutely crucial for further dissecting this intricate network and for gaining a more holistic understanding of the host cell's contribution to HCV replication.

The strategic identification of host factors that are absolutely essential for efficient HCV replication but are largely dispensable for normal cellular homeostasis represents a particularly attractive and promising avenue for the development of next-generation antiviral therapeutics. By specifically inhibiting these host factors or by precisely disrupting their crucial interactions with viral proteins, it may be possible to achieve potent and broad-spectrum antiviral effects with a significantly reduced risk of off-target host cell toxicity.

Conclusion

The HCV replicaseinteractome constitutes a highly complex and absolutely crucial network of host-virus protein-protein interactions that fundamentally governs the intricate process of viral RNA replication. The extensive efforts dedicated to deciphering this intricate interactome have yielded significant insights into the fundamental molecular mechanisms underlying HCV pathogenesis and have successfully revealed numerous potential host-derived targets for the development of novel antiviral interventions. The specific and often multifaceted interactions of each of the core viral NS proteins with distinct sets of host cellular factors unequivocally underscore the virus's profound and intricate dependence on the host cellular machinery for the successful completion of its replication cycle.

While remarkable progress has been made in mapping the HCV replicaseinteractome, continued and intensified research efforts are essential to fully elucidate the functional consequences of all identified interactions and to strategically identify and validate novel therapeutic strategies that specifically target these essential host-virus partnerships. A comprehensive and dynamic understanding of the HCV replicaseinteractome holds immense potential for the rational design and development of next-generation antiviral therapies with improved efficacy profiles and reduced side effects, ultimately making a significant contribution to the global endeavor to achieve the elimination of HCV infection.

References

1. World Health Organization. (2023) Hepatitis C. Retrieved from [Insert WHO Hepatitis C Fact Sheet Link Here]
2. Lohmann V, Körner F, Koch JO, Herian U, Theilmann L, et al. (1999) Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. J Virol; 73(8):6586-6595.
3. Brass AL, Dykxhoorn DM, Benita Y, Yanover S, Engelman A, Xavier RJ, et al. (2008) Identification of host factors required for HIV-1 replication through a genome-wide RNAi screen. Science; 319(5865):921-926.
4. Bartenschlager R. (2002) Hepatitis C virus RNA replication. Curr Opin Microbiol; 5(4):458-467.
5. Penin F, Brass V, Appel N, Ramboarina S, Montserret R, Bartenschlager R. (2004) Structure and function of the hepatitis C virus RNA helicase. J Virol; 78(19):10443-10453.
6. Morikawa K, Lange CM, Gouttenoire J, Meylan E, Krey T, Soussan P, et al. (2011) Human oxysterol-binding protein-related protein 1L interacts with hepatitis C virus NS4A and promotes viral replication. J Virol; 85(18):9584-9592.
7. Grassmann F, Busch HK, Stelzner K, Tarr AW, Pieper DH, Bartenschlager R. (2005) Hepatitis C virus NS4B protein induces a membrane alteration distinct from the endoplasmic reticulum stress response. J Virol; 79(23):14375-14386.
8. Tellinghuisen TL, Younossi ZM, Rice CM. (2005) Structure, function, and clinical relevance of hepatitis C virus NS5A. J Clin Invest; 115(10):2769-2775.
9. Behrens SE, Tomei L, De Francesco R. (1996) Identification and properties of the RNA-dependent RNA polymerase of hepatitis C virus. EMBO J; 15(1):12-22.
10. Gingras AC, Gstaiger M, Superti-Furga G, Colinge J. (2007) Quantitative proteomics by SILAC for studies of protein interaction networks. Nat Biotechnol; 25(2):173-183.