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Viral amyloids: New opportunities for antiviral therapeutic strategies

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Abstract

Amyloidoses are an array of diseases associated with the aggregation of proteins into fibrils. While it was previously thought that amyloid fibril-forming proteins are exclusively host-cell encoded, recent studies have revealed that pathogenic viruses can form amyloid-like fibrils too. Intriguingly, viral amyloids are often composed of virulence factors, known for their contribution to cell death and disease progression. In this review, we survey the literature about viral proteins capable of forming amyloid-like fibrils. The molecular and cellular mechanisms underlying the formation of viral amyloid-like aggregates are explored. In addition, we discuss the functional implications for viral amplification and the complex interplay between viral amyloids, biological functions, virulence, and virus-induced pathologies.

Keywords: amyloid-like fibrils; functional amyloids; fibrillation; viruses; LLPS, phase transitions; antiviral strategies; infectious hypothesis of neurodegenerative diseases.

Introduction

Amyloidoses encompass a wide range of incurable animal diseases characterized by the deposition of amyloid fibrils either in localized organs (including the brain) or systemic tissues. Approximately 50 human proteins have been identified as contributors to amyloid fibril formation and are often linked to specific pathologies, like Alzheimer's disease (AD) and Parkinson's disease (PD). Recent successes using small molecules [1,2] or rationally designed peptides [3] hold promises for the discovery of future therapeutic approaches to prevent or even cure amyloidoses.

Most proteins, if not all, have the potential to undergo a transition to the amyloid state, the most energetically stable conformation possible [4] (**Figure 1**). The amino acid sequence contains the necessary information for structured proteins to adopt their native 3D structure, and for intrinsically disordered proteins (IDPs) to exist as disordered conformational ensembles. However, proteins also contain sequences, known as amyloid- or aggregation-prone regions (APRs), which drive the formation of an alternative structure (**Figure 1**). In this process, cellular stress typically causes partial unfolding of monomeric amyloidogenic precursors and exposure of APRs. The precursor then adopts an alternative β -rich conformation that results in aberrant assembly into straight, unbranched fibrils that are packed together to form steric zippers [5] (**Figure 1**). In the aggregates, the β -strands stack in perpendicular layers along the axis of the fibrils, creating the distinctive “amyloid fold”. The cross β -sheets, together with the connecting loops, constitute the “amyloid core”. Amyloids are typically detected using specific dyes such as thioflavin (ThT and ThS) and Congo Red.

IDPs can engage in the amyloid pathway, but unlike folded proteins, they do not require an unfolding step [6]. Approximately one-third of human proteins serving as precursors to human amyloidosis are intrinsically disordered, with A β peptides, α -synuclein, and Tau being illustrative examples.

Despite their role in protein misfolding diseases, amyloids are not systematically tied to pathologies and can have a functional role [7]. Increasing evidence suggests that viruses encode amyloidogenic proteins that form fibrillar aggregates (**Figure 2**). Several viral amyloids were shown to have functions that benefit the virus itself, supporting their classification as functional amyloids.

In this review, we focus on viral amyloids and the mechanisms driving their formation, and discuss the functional implications of amyloids for viral replication and the intricate interplay between viral amyloids, biological functions, virulence, and pathologies.

Viral amyloids

The **E7 protein of human papillomavirus (HPV)-16** was the first viral protein found to have amyloidogenic properties. HPV16 E7 is a small acidic protein, composed of a globular C-terminal domain and an intrinsically disordered N-terminal domain [8]. HPV E7 can self-assemble into defined spherical oligomers reminiscent of soluble amyloid precursors [9]. However, the functional implications of this assembly were only discussed in terms of the amyloid-cancer connection, without considering the viral disease [10].

Subsequently, **PB1-F2**, an accessory protein encoded by an alternative open reading frame in PB1 segments of **Influenza A virus (IAV)**, was shown to form amyloids [11]. PB1-F2 is an IDP involved in virulence. At early stages of infection, PB1-F2 exists as a monomer, but later it accumulates into an amyloid form. Unlike monomers, PB1-F2 amyloids disrupt cell membranes, exhibiting high cytotoxicity both when added to cells and in infected cells [11]. Although amyloid-like assemblies were linked to the membrane-lytic activity, they were not discussed in terms of the potential benefit for the virus. Another example pertains to the **nuclear export protein (NEP) from H1N1 IAV**, which forms spherical aggregates with sparse amyloid-like structures playing a role in virion budding [12].

LEF-10, a baculovirus protein, represents the first documented example of a viral protein exhibiting prion-like behavior [13]. LEF-10 or its predicted prion-forming domain can functionally replace the prion domain of the yeast Sup35 prion protein, leading to a [PSI(+)]-like phenotype. SDD-AGE analysis unveiled the presence of high-molecular-mass, SDS-resistant aggregates consistent with a fibrillar organization. In infected cells at high multiplicity of infection, LEF-10 converts into an aggregated form that inhibits viral late gene expression [13].

Members of the *Herpesviridae* family encode proteins that contain the **RIP homotypic interaction motif (RHIM)** and are capable of impairing necroptosis or apoptosis by forming hybrid amyloids with host-cell amyloids [14-16]. The RHIM is present in four human proteins (RIPK 1, RIPK3, ZBP1, and TRIF) that all play critical roles in necroptosis. When activated, RIPK1, ZBP1, and TRIF hetero-oligomerize with RIPK3 to form an amyloid-like assembly that recruits MLKL, the pore-forming effector of necroptosis. Such interactions also contribute to triggering apoptosis and pyroptosis [17]. Given the significance of these interactions in crucial cellular processes against viral infections, viruses have evolved means of disrupting RHIM-mediated signaling to ensure their survival. Herpesviruses employ RHIM mimicry to hijack these interactions.

The **M45 protein from murine cytomegalovirus (MCMV)** not only protects infected mouse cells but also safeguards human transfected cells from necroptosis induced by tumor necrosis factor receptor (TNFR) activation, with the RHIM-containing N-terminal region of M45 being sufficient to confer protection against TNFR-induced necroptosis. This region rapidly self-assembles *in vitro* into amyloid fibrils and interacts with the RHIMs of RIPK3, ZBP1, and RIPK1, forming hybrid amyloid fibrils. This property likely underlies its ability to inhibit necroptosis [14].

Varicella zoster virus (VZV, also named HHV-3) was also found to encode an amyloidogenic RHIM-containing protein, *i.e.*, the capsid triplex protein encoded by ORF 20. Similar to MCMV M45, the **RHIM of VZV ORF 20** forms hybrid amyloids with human ZBP1. Mutations in the ORF20 RHIM impair virus spread in ZBP1-expressing cells. This effect is reversed by caspase inhibition. The VZV ORF 20 RHIM was proposed to protect against ZBP1-driven apoptosis by sequestering ZBP1 in decoy amyloid assemblies during VZV infection [15].

The **ICP6 RHIM-containing protein from herpes simplex virus 1** (HSV-1, also named HHV-1) displays amyloidogenic properties *in vitro*. It interacts with host RHIM-containing proteins to form heteromeric amyloids, similar to MCMV M45 and VZV ORF 20 [16]. Like M45, the core tetrad sequence of the ICP6 RHIM is crucial for amyloid formation and interaction with host RHIM-containing proteins. The amyloid-forming ability of ICP6 RHIM is redox-dependent, suggesting a role for disulfide bonds in amyloid assembly [16]. This finding holds functional significance, given that oxidative stress is common during viral infections [18]. These findings support the idea that the formation of viral-host hybrid amyloids may represent a general viral adaptation mechanism against host immune responses, making it an attractive target for antiviral strategies.

The **NSs protein** is the major virulence factor of **Rift Valley fever virus (RVFV)**, a neuropathogenic mosquito-borne bunyavirus (*Phenuiviridae* family) [19]. NSs forms large filaments resembling amyloid deposits [20,21]. Interestingly, NMR investigations have identified one major internal disordered region within NSs [22]. The resistance of NSs to strong detergents and its interactions with the amyloid-binding dye ThS further support its ability to form amyloid-like fibrils [21]. Live-cell imaging revealed the preferential growth of fibrils through the continuous incorporation of NSs monomers rather than *via* an increase in the number of structures [21]. Using cysteine mutagenesis, the central role of redox reactions in the successive stages of NSs deposit formation was identified [21,23]. Protofibrils constitute the earliest structural intermediate required for NSs to silence interferon (IFN) responses, a prerequisite for RVFV to evade host innate immunity [21]. Importantly, only animals exposed to viruses encoding wild-type NSs or capable of forming protofibrils exhibit amyloid deposits in the brain, neuropathy, and ultimately succumb to infection [21,23]. Collectively, these observations suggest a potential connection between the formation of amyloid-like NSs fibrils, the biological functions of NSs, and the cytotoxicity and neurological disorders associated with RVFV infection [21].

The **V protein from Hendra virus** (HeV, a BSL4 pathogen within the *Henipavirus* genus, *Paramyxoviridae* family) undergoes a transition from a liquid to a gel phase. This phase transition is driven by a specific fragment, called PNT3 [24], located within the long intrinsically disordered N-terminal domain (NTD) of V [25]. *In vitro*, PNT3 forms amyloid-like fibrils, and Congo Red staining suggests their presence in transfected and infected cells [24]. The intrinsically disordered **W proteins from HeV and the related Nipah virus (NiV)**, form similar amyloid-like fibrils [24,26], consistent with the fact that V and W share the NTD and hence the PNT3 region. Both W and V play critical roles in counteracting antiviral responses against henipaviruses [27]. Therefore, their ability to form fibrils was proposed to be tied to the evasion of host innate immune responses [27,28]. Fibrillar aggregates may sequester cellular partners involved in the host innate immune responses, such as STAT and 14-3-3 proteins, thereby preventing IFN signaling and inhibiting NF- κ B-induced proinflammatory responses. Additionally, it is tempting to speculate that the ability of the NiV W protein to suppress IFN- β expression may be functionally linked to the formation of NiV W amyloids, as shown in the case of RVFV NSs amyloids [21].

Another example is the **capsid-anchor (CA) protein of Zika virus (ZIKV)**. This small transmembrane protein that plays an essential role in virus assembly and maturation, forms amyloid-like fibrils enriched in β -sheet structures *in vitro*. Moreover, these CA amyloid fibrils display cytotoxicity in mammalian cells [29].

The **nucleocapsid protein (N) of SARS-CoV-2**, responsible for packaging viral RNA and crucial for virus replication and assembly, forms amyloids in phase-separated droplets [30]. N undergoes liquid-liquid phase separation (LLPS), a process enhanced by RNA binding [31]. N possesses two low-complexity domains (LCDs) similar to those found in other proteins that undergo LLPS or form amyloid fibrils. Full-length N can both undergo phase separation and drive amyloid fibril formation in the presence of RNA. The central LCD forms amyloid fibrils with or without RNA. In the presence of RNA, the central LCD forms phase-separated droplets that bind ThS. These droplets transition to fibrous or amorphous solid-like particles, with RNA influencing their dynamics and morphology.

The X-ray structure of N fibrils unveiled a typical “amyloid fold” for all three central LCD segments [30]. Based on this structure, a peptide interfering with N self-assembly and displaying antiviral activity

in SARS-CoV-2 infected cells was designed. These findings not only provide the first atomic-resolution structures of amyloid-like fibrils of viral origin but also highlight the relevance of targeting the amyloidogenicity of SARS-CoV-2 N for drug development.

N is not the sole amyloidogenic protein within the SARS-CoV-2 proteome. *In-silico* approaches identified several APRs within ORF6 and ORF10, and two of these regions, one in ORF6 and one in ORF10, were experimentally confirmed to be amyloidogenic [32]. Considering the neuro-invasiveness of SARS-CoV-2 [33], the neurological symptoms observed in COVID-19 were hypothesized to be linked to amyloid formation [32]. The cytotoxicity and protease-resistant structure of these amyloid assemblies support the notion that they may persist in the brains of COVID-19 patients, potentially explaining the lasting neurological symptoms [32]. Whether these assemblies may also trigger aggregation of host proteins associated with amyloid-related neurodegeneration remains an open question.

Subsequent studies identified additional APRs in SARS-CoV-2 structural and accessory proteins [34]. Various peptides (see **Table 1**) and the full-length ORF10 protein were experimentally confirmed to form fibrils [34]. Furthermore, aggregates of SARS-CoV-2 NSP11 were shown to be toxic to mammalian cells. Interestingly, the corresponding fusion peptides from SARS-CoV also form amyloid-like fibrils, albeit to a lower extent than their SARS-CoV-2 counterparts [34]. In the case of SARS-CoV, the ability to form fibrils also extends to a nine-residue peptide from the extra membrane C-terminal tail of the envelope (E) protein [35]. Notably, this study also revealed that these assemblies can inhibit the fibrillation of hIAPP [35], a peptide associated with type-II diabetes, illustrating the cross-talk between viral and cellular amyloids.

The last example is provided by Dengue virus (DENV), a mosquito-borne member of the *Flaviviridae* family, for which several APRs were predicted across four serotypes. The amyloidogenicity of a peptide of the NS1 protein spanning residues 1 to 30, and of 2K and CA peptides (**Table 1**) was experimentally confirmed [36]. Notably, aggregates of NS1 peptides were also shown to cross-seed and enhance aggregation of hIAPP and *vice-versa*, further underscoring the cross-talk between virus and host amyloids and possibly explaining the increased adversity observed in diabetic dengue patients.

All these examples demonstrate that various viral families possess members capable of forming amyloid-like aggregates (**Figure 2**), which has significant implications for virus-induced pathogenesis in general and suggests that this trait likely emerged early in virus evolution. It should be noted, however, that some of the studies mentioned above only documented the ability to form amyloid-like fibrils *in vitro* or by isolated peptides. Further experimental investigations are necessary to confirm whether amyloidogenicity is retained in the cellular environment and in the context of full-length proteins. The latter point is particularly important because APRs in isolated peptides may not be functionally relevant if they are not solvent-exposed. However, their location within predicted or experimentally confirmed intrinsically disordered regions (IDRs) can provide insights: if amyloidogenic peptides occur within IDRs, which are inherently solvent-accessible, it is plausible that they may have functional relevance and could trigger the fibrillation of the entire protein. Of note, most of the described amyloidogenic viral proteins exhibit a high predicted disorder content (> 30%), and in many instances, experimental data support a disordered nature (**Table 1**).

As an additional caveat when considering data reporting the amyloidogenicity of (viral) proteins, we would like to underscore that fibrils need to meet a quite precise ensemble of biochemical and structural criteria to be considered as *bona fide* amyloids, and in several cases, the available data do not enable drawing definite conclusions in this regard. Whether some fibrillar aggregates and in particular those displaying a pronounced curly shape (see [24,26,37]) deserve to be classified in a distinct class of fibrils remains an open question and requires additional research to determine their atomic structure.

Table 1. List of viral proteins forming amyloid-like fibrils. If known, the functions of amyloid fibrils are indicated as is the experimental evidence of intrinsic disorder. Disorder and phase separation propensities were predicted with fIDPnn [38] and FuzDrop [39], respectively. Note that LLPS propensity cannot be computed for peptides of less than 45 amino acid residues. Likewise, the disorder content of peptides of less than 21 residues could not be estimated. Predicted disorder contents higher than 30% are shown in bold, as are LLPS scores higher than 50%. FP: fusion peptide. For each protein, the UniProt accession number is indicated.

Group	Virus	Protein	Fragment	UniProt Accession number	Function of amyloid fibrils	Predicted disorder (%)	Experimental disorder	LLPS propensity	Reference
I dsDNA	AcMNPV	LEF-10		Q9YKK9	Regulation of viral gene expression	30.0	-	0.24	[13]
	HHV-1 / HSV-1	ICP6		P08543	Necroptosis inhibition	13.1	-	0.82	[16]
	HHV-3 / VZV	ORF20		P09276	Apoptosis inhibition	8.3	-	0.17	[15]
	HPV-16	E7		P03129	Potential link with HPV16-induced cancer	32.7	Yes [8]	0.56	[9]
	MCMV	M45		Q06A28	Necroptosis inhibition	11.7	-	0.86	[14]
IV ssRNA(+)	DENV-1	NS1	776-806	P17763 PRO_0000264664	Unknown	69.4	Yes [36]	-	[36]
	DENV-2	NS1	776-806	P29991 PRO_0000037940	Unknown	69.0	Yes [36]	-	[36]
		2K		P29991 PRO_0000308281	Unknown	70.4	-	-	[36]
		CA		P29991	Unknown	-	-	-	[36]
	DENV-3	NS1	774-804	P27915 PRO_0000037993	Unknown	69.5	Yes [36]	-	[36]
	DENV-4	2K		Q2YHF2 PRO_0000268156	Unknown	70.4	-	-	[36]
		CA		Q2YHF2	Unknown	-	-	-	[36]
	SARS-CoV	E	55-63	P59637	Potential inhibitor of hIAPP fibrillation	17.3	-	-	[35]
		NSP11		P0C6U8-PRO_0000338265	Unknown	-	-	-	[34]
		S	798-819 (FP1)	P59594	Unknown	1.0	Yes [40]	-	[34]
		S	817-837 (FP2)	P59594	Unknown	1.1	Yes [40]	-	[34]
	SARS-CoV-2	N		P0DTC9	Unknown	39.9	Yes [41]	0.99	[30]
		NSP6		P0DTC1-PRO_0000449640	Unknown	32.6	-	0.10	[34]
		NSP11		P0DTC1-PRO_0000449645	Cytotoxicity	-	Yes [42]	-	[34]
		ORF6		P0DTC6	Cytotoxicity	32.6	-	0.36	[32]
		ORF10		A0A663DJA2	Cytotoxicity	29.8	-	-	[32,34]
		S	1-12	P0DTC2	Unknown	0.9	Yes [43]	-	[34]
	S	816-837 (FP1)	P0DTC2	Unknown	0.9	Yes [34,44]	-	[34]	
	S	835-855 (FP2)	P0DTC2	Unknown	0.9	Yes [34,44]	-	[34]	
	Zika	CA	105-123	Q32ZE1	Cytotoxicity	40.4	Yes [29]	-	[29]
V ssRNA(-)	IAV	PB1-F2		Q20MH0	Cytotoxicity	54.4	Yes [11]	0.43	[11]
		NEP		Q89733	Virion budding	22.4	No [45]	0.23	[12]
	HeV	V		O55777	Potential prevention of antiviral response	73.1	Yes [25]	0.99	[24]
	HeV	W		P0C1C6	Potential prevention of antiviral response	75.4	Yes [26]	0.99	[26]
	NiV	V		Q997F2	Potential prevention of antiviral response	70.1	Yes [25]	0.90	[24]
	NiV	W		P0C1C7	Potential prevention of antiviral response	73.3	Yes [26]	0.90	[26]
	RVFV	NSs		P21698	Antiviral response inhibition, potential destabilization of actin cytoskeleton, and inhibition of RNA synthesis	13.9	Yes [22]	0.15	[21]

IDPs and IDRs frequently act as protein-protein interaction (PPI) hubs and participate in various biological functions, and viral proteins do not constitute an exception to that rule [46]. In an attempt to shed light on possible commonalities in terms of molecular partnership, we analyzed the interactome of the viral proteins forming amyloid-like fibrils. 1053 PPIs were retrieved from IntAct [47] and UniProt databases (see **Supplementary Table S1**). Enrichment analysis was performed with FunRich [48]. Analysis of the resulting interactome reveals a limited number of shared host partners. Notably, and of particular relevance in terms of host innate immune response evasion, among these are key signal transducers of IFN signaling like STAT proteins (**Figure 3**). As a general rule, ssRNA(-) and ssRNA(+) viruses have the most intertwined network. The SARS-CoV-2 proteins exhibit the largest number of host partners, reflecting the extensive research focused on this pathogen. SARS-CoV2's partners include structural ribosome constituents (also interacting with HPV-16 E7), chaperones (also partners of RVFV NSs), and STAT proteins (also part of the interactomes of henipavirus V and W proteins). The heterogeneous nuclear ribonucleoprotein A/B (hnRNP) interacts with proteins from all three virus groups, potentially regulating viral replication and virion release [49]. Noteworthy, hnRNP A/B can undergo phase separation and possess a prion-like domain capable of forming amyloid-like assemblies [50], raising the possibility of hybrid amyloid formation.

Interplay between LLPS, phase transitions, and fibrillation

LLPS is a mechanism involved in the cell compartmentalization of biomolecules playing a critical role in various physiological and pathological processes [51]. Viruses harness LLPS to form viral factories (also known as inclusion bodies) and/or assembly compartments (see [52-55] and references therein cited). The LLPS process itself, along with the material properties of the liquid viral inclusions, have emerged as promising targets for antiviral approaches [56-60].

Liquid condensates have the potential to undergo a "maturation process", whereby they transition into more solid or jelled forms that can eventually nucleate fibrils [61]. Illustrative examples are measles virus (where maturation of viral inclusions from a liquid-like to a gel-like state was interpreted as a possible regulatory mechanism controlling organelle dynamics to optimize viral replication [62]) and the above-discussed SARS-CoV-2 N protein that *in vitro* forms amyloids in phase-separated droplets [30].

IDRs are known to drive both LLPS [61] and fibrillation *via* the formation of hydrogels [63]. Reciprocally, amyloid-like fibers can form highly stable hydrogels [64]. The ability of the HeV PNT3 region to both jellify and form fibrils [24] underscores the link between gelation and fibrillation. Although intertwined, gelation and fibrillation are not always directly interconnected. IDRs can generate amyloid-like structures not only from hydrogels but also from liquid samples. Interestingly, in the case of the RNA-processing prion-like hnRNPD protein, LLPS and the formation of amyloid-like fibrils result from two distinct and mutually exclusive pathways, with LLPS playing a protective role against fibril formation [65].

Considering the interconnections between LLPS and fibrillation, it is relevant to interrogate whether inclusion bodies are capable of nucleating fibrils and whether the few viral amyloids identified so far result from the maturation of liquid condensates or follow a distinct pathway. Both mechanisms are theoretically possible (**Figure 1**). **Table 1** shows that the predicted LLPS propensities vary widely among the viral proteins that form fibrils, ranging from 0.10 to 0.99. Only eight of these proteins display a high (> 0.5) LLPS score, making it challenging, based on the limited number of examples available, to pinpoint a prominent mechanism. Definite answers regarding the precise pathways of viral amyloid formation require further comprehensive investigations that we hope this review will foster.

The virus-amyloid connection and the infectious hypothesis of neurodegeneration

While human amyloids have traditionally been studied in the context of neurodegeneration and misfolding diseases, the discovery of functional amyloids has challenged the notion that amyloids are intrinsically toxic or pathological. Amyloids play a significant role in interacting with microbial pathogens, including viruses [66]. Human amyloids can serve both as promoters of viral infectivity and

innate restriction factors against viruses [66]. For instance, human semen amyloids enhance HIV-1 infectivity as they possess a positively charged surface that binds to the negatively charged viral envelope and cell membrane, thereby facilitating virus attachment [67].

On the other hand, human amyloids can hinder viral replication. For example, the AD-associated A β peptide plays a role in innate immunity against HSV-1 brain infections. The interaction between A β and HPV-1 glycoproteins triggers the formation of A β amyloids, which trap viral particles and exhibit an antiviral effect [68]. Reciprocally, such virus-induced A β aggregation may contribute to AD, offering a potential mechanistic explanation for the known association between herpesvirus and AD risk [66]. HSV-1-induced A β aggregation is facilitated by the presence of APRs within the viral glycoprotein, which interact specifically with homologous APRs in A β , resulting in the formation of hybrid amyloids (**Figure 1**). Exploiting the sequence specificity of amyloid interactions for therapeutic interventions to hinder viral activity is a possibility [69]. This however raises the broader question of whether viral proteins or regions with intrinsic amyloidogenic properties can trigger amyloid formation in host proteins involved in neurodegenerative diseases, such as A β and α -synuclein.

Supporting this notion, a growing body of experimental evidence suggests that one amyloidogenic protein can induce the formation of amyloids in other proteins *via* co-aggregation within amyloid fibrils [70] (**Figure 1**). The cross-talk between viral infections, amyloid formation, and neurodegeneration has been broadly discussed and has led to the “infectious hypothesis of neurodegeneration” [71]. In support of this hypothesis, HSV-1 and HIV-1 were found to induce AD-related features such as the deposition of A β peptide in amyloid plaques [72], and diverse viruses were shown to impact the aggregation of neurodegenerative disease-associated proteins [73]. An interesting example is again the SARS-CoV-2 N protein that enhances the aggregation of α -synuclein [74], potentially linking COVID-19 to PD [75]. From this perspective, the documented fibrillation capability of different SARS-CoV-2 proteins and peptides [30,32,34] may have significant implications for pathogenesis. The inherent amyloidogenicity of certain SARS-CoV-2 proteins has already been discussed in terms of its potential impact not only on AD [76,77] but also on abnormal blood clotting observed in both severe and long COVID-19, as well as in sporadic adverse vaccination outcomes, where it was connected to the amyloid formation of both the human fibrin and the viral Spike protein [78].

Conclusions

As herein discussed, there are numerous connections between viruses and amyloids, highlighting the need for collaboration between amyloid and virus research. The field of viral amyloids is still in its infancy, with a limited number of documented examples thus far. However, we anticipate that future studies will uncover more instances of viral amyloids. While the existing examples have laid the foundation for this emerging field, they have also raised several questions. Do all viral proteins form genuine amyloids? Do viral amyloids originate from the maturation of liquid droplets formed by LLPS? Can viral factories initiate fibril nucleation? Likewise, if recent studies have unveiled exciting possibilities for antiviral strategies by designing APRs that mimic viral ones, they have also set the basis for interrogating the relevance of viral amyloids for neurodegeneration and misfolding diseases.

Lastly, we want to emphasize the practical implications of research on viral amyloids. If viral amyloids act as proviral factors, this opens the doors for the design of innovative approaches exploiting fibrillation inhibitors as antiviral candidates. We hope that this review will stimulate future efforts in exploring these new captivating research avenues.

Conflict of interest statement

Nothing declared.

Credit author statement

Conceptualization: S.L.; Formal data analysis (disorder content, LLPS propensities, and interactome): F.G.; Writing - original draft: S.L.; Preparation of Figures and Tables: F.G.; Writing - Review and Editing: F.G., P.Y.L., and S.L.

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Figures

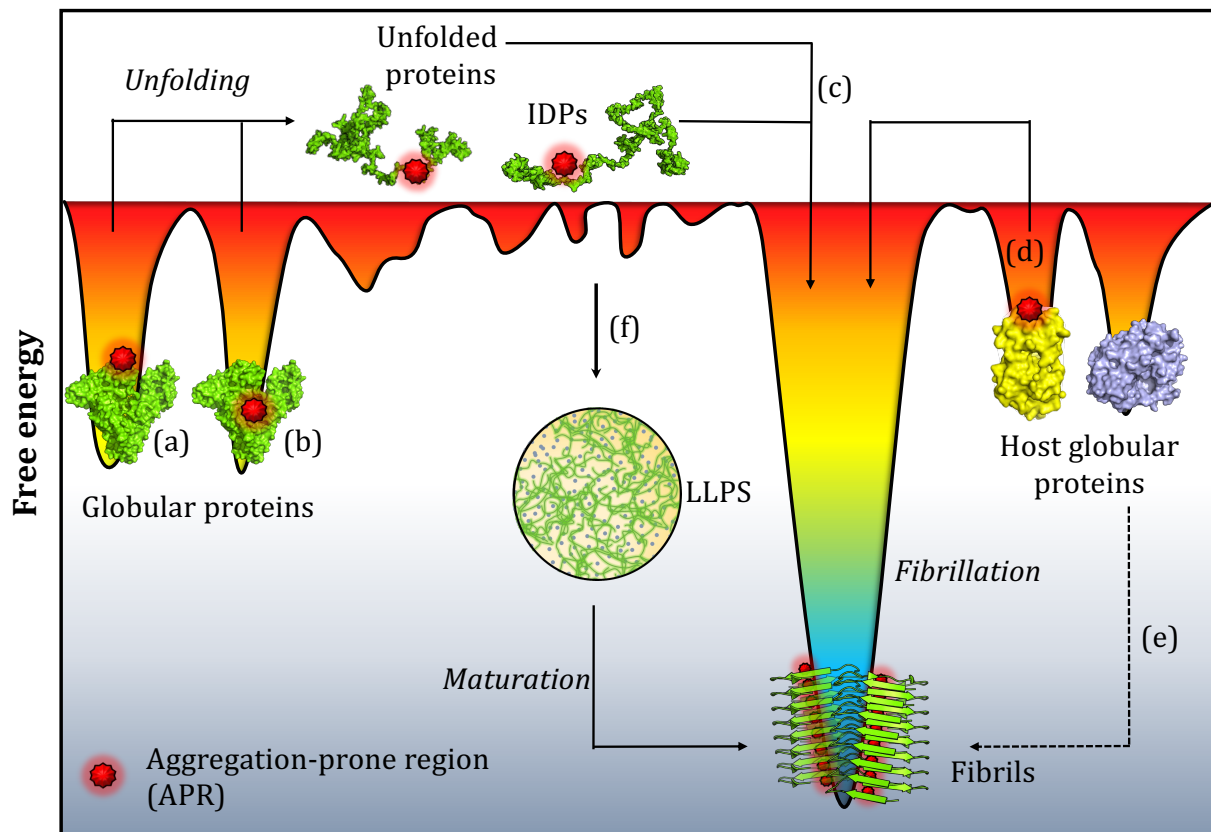


Figure 1. Amyloid formation pathways. Globular proteins containing either an exposed (a) or buried (b) aggregation-prone region (APR) need to unfold to eventually aggregate into amyloid-like fibrils. Disordered proteins (IDPs) can fibrillate either on their own (c) or in cooperation with a host cellular protein containing an APR. Host cellular proteins, devoid of APRs, can also be trapped into viral amyloids (e). Mature fibrils can also form through the maturation of liquid condensates formed by liquid-liquid phase separation (LLPS) (f).

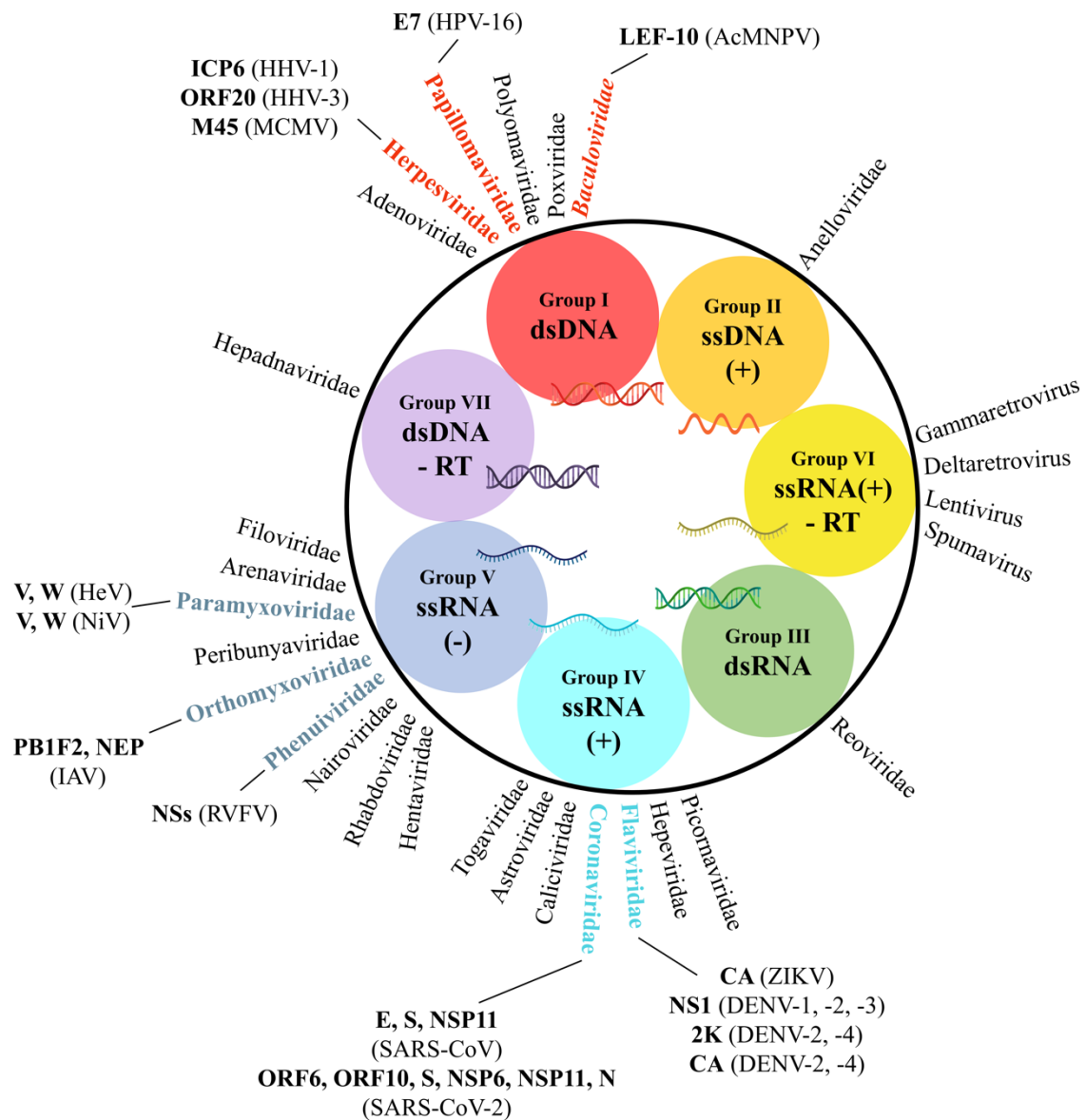


Figure 2. Distribution of viral proteins that form amyloid-like fibrils within viral families. The few examples of amyloid-forming proteins reported so far are shown within the corresponding viral family along with their virus groups (according to Baltimore classification) and the virus name indicated in brackets (for virus name abbreviations see text). Viral families for which amyloid-forming proteins have been described are shown in color, with color code according to virus group. All examples concern human viruses with the only exception of AcMNPV (an insect virus within the *Baculoviridae* family shown in italics). Abbreviations: ds, double-strand; RT, reverse transcribing; ss, single-strand.

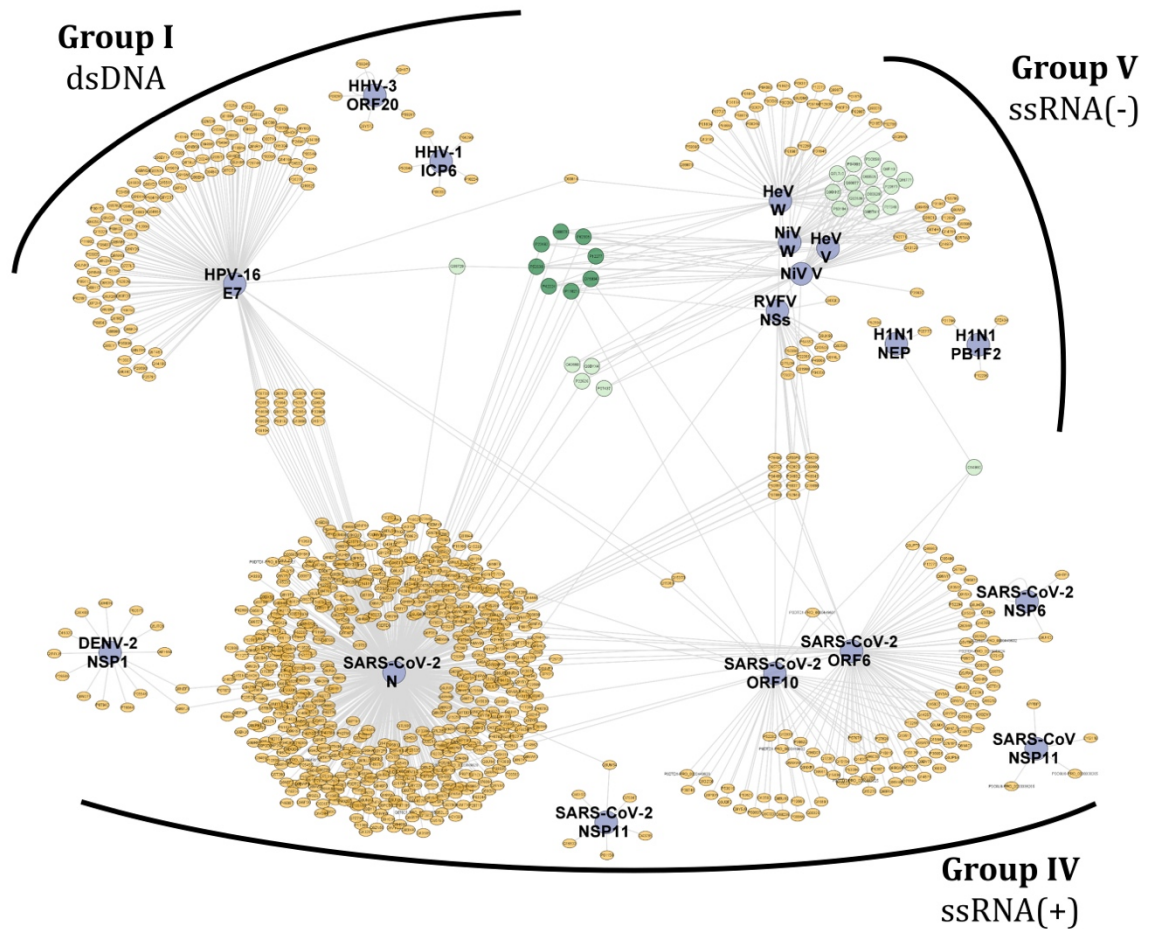


Figure 3. Protein-protein interaction (PPI) network of viral amyloid fibril-forming proteins. Viral proteins (blue nodes) are grouped by the genome type of the corresponding virus. Host partners are in yellow and named according to their UniProt accession number. Protein partners with the highest interaction degree are displayed in dark (degrees 5 and 4) and bright green (degree 3). Only viral proteins for which interaction partners are listed in IntAct are displayed. For a description of programs used to generate the interactome and corresponding references, see the text. For a detailed list of PPIs, see **Supplementary Table S1**.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

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