Medicinal chemistry

Srinivasan, Med chem (Los Angeles) 2016, 6:3
DOI: 10.4172/2161-0444.1000344

Review Article Open Access

Interface Peptide Mimetics-Rationale and Application as Therapeutic Agents

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Abstract

Biomolecular recognition via protein-protein interactions (PPI) is central to the signaling events in most physiological and pathological processes. Hence PPI are considered excellent targets for drug development. In recent years there is considerable interest in the design and development of peptide based drugs as antagonists or agonists of PPI. High potency, great selectivity and better safety profile are significant advantages of peptide therapeutics. The following is a brief review of the rationale and modifications in the design of peptide mimetics of PPI interface.

Keywords: Protein-protein interactions; Peptide drugs

Introduction

Protein-protien interaction (PPI) interface as druggable targets

PPI constitute fundamental mechanisms that support a wide variety of biological functions including cellular growth, maintenance, apoptosis, signaling pathways and metabolic activities. An associated corollary is that altered PPI implicate pathology. Hence blockade or stabilization of PPI could represent an attractive therapeutic strategy for many human diseases. The network of PPI called interactome is highly complex and expansive. PPI occurs when two or more proteins come in proximity guided by biophysical principles that govern molecular recognition. In humans, the PPI interactome is estimated to range from 14,000 binary interactions to over 650,000 multi- component interactions [1,2]. PPI binding sites called interfaces typically range in size between 1200 to 2000 A^2. They are made up of few smaller binding pockets scattered across the entire contact area [2]. Molecular recognition is commonly mediated by a subset of interface amino acids, referred to as hot spot residues located on defined secondary structures [3]. Evolutionary constraints on sequence divergence at PPI interfaces ensure that the hot spot residues are highly conserved. Since biophysical principles limit the number of ways of secondary structure packing, the types of interface structural folds are potentially limited [4,5]. Hence shared functional characteristics between structurally dissimilar proteins may be attributed to the evolutionary convergence in the interface space [2,6,7]. PPI interfaces are identified based on interatomic distance between residue pairs, the interaction between interface residues through water molecules and the buried surface area. In most PPI the hot spot residues at the interface contribute significantly to the free energy of binding, are surrounded by a ring of energetically less critical residues that occlude bulk solvent and mutation of these residues abolish PPI [6].

Collectively the convergent evolution and the minimal binding consensus suggest that the PPI interfaces are excellent targets for drug design and discovery [8-10].

Interface peptide drugs

Over the past decade considerable efforts have been directed at developing small molecule inhibitors of PPI. The efficacy of small molecule inhibitors are largely limited to a subset of PPI that present tight hydrophobic binding pockets within a relatively smaller area ranging between 300-1000 A^2 [2,9,11]. However, most PPI interface present non-contiguous distribution of hot spot residues and large surface area with little or no binding pockets. Such features not particularly favorable for development of high affinity small molecule inhibitors [7]. Although biologics with well-defined three dimensional structures such as the recombinant protein or monoclonal antibodies are capable of binding wide target space with high affinity and noteworthy selectivity, their large size often precludes efficient cellular permeability. This compromises the therapeutic efficacy of the biologics especially for intracellular targets [2,12]. Efficacious competitive PPI antagonists should be large enough to simultaneously interact with multiple hot spot patches to gain considerable part of the distributed free energy and achieve significant affinity. It is suggested that medium sized molecules with large contact surfaces could achieve nanomolar potency at PPI interfaces and function as better inhibitors [1,9].

Peptides are a hybrid class of drugs that bridge the gap between small molecules and proteins. As opposed to small molecules, peptides can bind large targets with high potency and great selectivity. Consequently peptides exhibit minimal off-target effects. Furthermore, rapid clearance of peptides precludes the accumulation of deleterious metabolites and minimizes the risk of toxicity [12,13]. In addition being considerably smaller in size than biologics, peptides are capable of better tissue penetration [2,14]. Importantly peptides are amenable to multitudes of modifications such as incorporation of non-natural building blocks and introduction of variety of functional folds which in turn exponentially increases the potential to create panels of peptide analogs with wide range of chemical diversity and functionalities [2,8,13]. Although, low proteolytic stability is a critical disadvantage, advances in peptide design and synthesis has led to the development of a rich pipeline of stable, efficacious and cost effective novel peptides with high therapeutic efficacy [10]. The potential for peptide therapeutics in the treatment of diverse medical conditions is exemplified by the rapidly growing market currently estimated at 18 billion dollars [15]. However, a commonly perceived problem associated with peptide drugs is rapid in-vivo clearance, poor bioavailability and the need for specialized delivery mechanim (10.15)

A significant percentage of PPI are mediated by protein peptide interactions [14]. Epitope mimetic design starts from mutagenesis

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Received March 11, 2016; **Accepted** March 29, 2016; **Published** March 31, 2016

Citation: Srinivasan M (2016) Interface Peptide Mimetics-Rationale and Application as Therapeutic Agents. Med chem (Los Angeles) 6: 189-194. doi:10.4172/2161-0444.1000344

and three dimensional structural data, which map the energetically important residues at the interface of interacting proteins. Advances in computational biology have propelled the development of methods to identify hot spot interface residues even if the structure of the PPI complex is not known [16]. The challenge is to recapitulate the structural and conformational properties of the target epitope, in a relatively small scaffold. The primary goal is to transform a prototype PPI peptide into a pharmacologically useful and metabolically stable drug capable of reaching the target organ [12,13]. Promising engineering strategies incorporated in peptide structure to confer selectivity and stability can be broadly classified as 1) end-group modifications, 2) peptide bond modifications and 3) peptide mimetics (Tables 1 and 2). Here we briefly discuss the modifications with examples restricted to the interface peptides of PPI other than the antigen:antibody and the peptide: major histocompatibility (human leukocyte antigen-HLA) complexes.

End group modifications

Linear peptides of residues that comprise the hot-spot patch commonly constitute the first generation inhibitors of specific PPI [10,17,18]. Since free peptides are rapidly degraded by exopeptidases, the end-protection strategy has been widely used to improve enzymatic stability. The rationale is based on the observations that the biological stability and activity of many endogenous peptides such as the neuropeptides and melanocyte-releasing hormone are attributed to

End group modifications	Acetylation, amidation, PEGylation, glycosylation.	Stabilize active conformation, secondary structure.	Minimum change to peptide structure.
Cyclization		Stabilize structure	Peptide structure modified without affecting the critical functional groups
Peptide-bond modifications	Use of D-amino acids, backbone extensions by β amino acids, methylation of amide nitrogen, hydrogen bond surrogate, stapling, hairpins, side-chain modifications with similar natural or non-natural amino acids.	Greater resistance against protease degradation, increased metabolic stability	Considerable alterations in the biochemical properties, hydrophobicity conformation, and flexibility of candidate peptide drug
Peptide mimetics	Peptide bond isosteres, organic non- peptide molecules, functional groups positioned on non-peptide scaffold	Increased stability, high affinity and selectivity	Minimal similarity with the candidate peptide drug

Table 1: Peptide modifications and effects.

Peptide modification		PPI	Indication	Status	Ref
End-group modifications	Acetylation Amidation	CD80-CD28	Autoimmune	Preclinical	[29,30]
	Acetylated peptide	GnRH:gonadotropin	Prostate cancer, endometriosis, multiple indications	Clinical Preclinical	[20]
	Acetylated	GHRH:GH	Pituitary dwarfism Retroviral lipodystrophy	Diagnostic Clinical	[23-25]
	Amidated	Amylin:receptor Amylinomimetic	Adjunct Diabetes	Clinical	[26]
	Amidated	Thymosin α1: receptor	Hepatitis B and C Cancer	Clinical	[31]
	Acetylation amidation	ATR: β-arrestin biased ligand	Heart failure	Clinical	[32-34]
	acylated	AXH:CIC	Spinocerebellar ataxis	Preclinical	[35]
	PEGylation	Exedin-4			
	Cyclization	Integrin inhibitors RGD peptides	Vitreoretinal diseases Cancer	Clinical Preclinical	[40-42]
		GLP-1: receptor	Diabetes	Clinical	[44,45]
Peptide backbone modification	Stapled peptides	ICN1:CSL:MAML1	Leukemia	Preclinical	[48]
		BID:BCL_{-XL} /BCL_{-W}	Cancer	Preclinical	[49]
		p53-MDM2	Cancer	Preclinical	[50,51]
		β-catenin-TCF	Cancer	Preclinical	[52]
		ApoE: ABCA-1	Alzheimer's disease, Coronary syndrome	Preclinical	[53]
		VDR-coactivator		Preclinical	[54,55]
	Retro-inverso	CD4, CD28	Autoimmune	Preclinical	[30]
		Icatibant	Angioedema	Clinical	[58]
		VEGF: receptor	Cancer	Preclinical	[59]
β-hairpins	β-peptides	IFN-γ:receptor	Preclinical		[63]
		Transthyretin:Aβ	Alzheimer's disease	Preclinical	[64]
		p53-Human MDM2	Cancer	Clinical	[51,65]

GnRH: gonadotropin releasing hormone; GHRH: growth hormone releasing hormone; ATR: angiotensin II receptor; AXH: AXH domain of ataxin-1; CIC: capicua transcriptional repressor; GLP: glucagon like peptide; ICN: cytoplasmic tail of NOTCH1; CSL: DNA-bound transcription factor; MNML: mastermind like; $BCL\text{-}_{XL}$: BCL-2 anti-apoptotic protein; BID: BCL-2 pro-apoptotic protein; MDM2: mouse double minute 2 protein; TCF: T-cell factor; ApoE: apolipoprotein e; ABCA-1: ATP-binding cassette transporter A1; VDR: vitamin D receptor; VEGF: vascular endothelial growth factor; Aβ: amyloid-beta.

Table 2: Interface peptide drugs. Interface peptide drugs in different stages of development: A non-exhaustive list of peptide drugs that include hot spot residues at the protein-protein interaction (PPI) interface that are being evaluated in preclinical models, clinical trials or clinical use.

amino-acetylation and carboxy amidation [8,19]. Blocking of the end-groups by acetyl and amide groups has been shown to extend the *in-vivo* half-life of synthetic peptides by several folds. Additionally, amino terminal acetylation presumably increases the peptide lipophilicity enhancing the membrane permeability and passage across the intestinal or blood brain barrier [8,16,19]. An acetate salt of a peptide analog of the gonadotropin releasing hormone (GnRH) acts as a potent inhibitor of gonadotropin secretion and has diverse clinical applications [20].

Acetate salt of a synthetic peptide amide derived from the amino-terminal sequence of the human growth hormone-releasing hormone (GHRH or GRF) (sermorelin) has been shown to block GH:GHRH interaction [21,22]. Sermorelin is used for the diagnosis of pituitary dwarfism and also has potential applications in the management of retroviral induced lipodystrophy [23-25]. Amylin, also known as islet amyloid polypeptide is a hormone co-secreted with insulin by the β- cells of the pancreas. Amylin regulates plasma glucose by slowing gastric emptying and decreasing postprandial glucagon secretion. An amidated peptide analog of human amylin, pramlintide is used as an amylin receptor agonist in the management of diabetes. Exenatide, an amidated peptide mimetic of the glucagon like peptide-1 (GLP-1), has been shown to bind GLP-1 receptors and enhance glucose dependent insulin secretion in diabetes [26,27]. Modifications such as attachment of long fatty acids to the peptides or co-synthesis with Fc fragments have been introduced to increase plasma half-life of GLP-1 analogs [28]. We have shown that end groups blocked peptides derived from the T-cell costimulatory receptor, CD28 inhibit the CD28:B7 receptor:ligand interactions and suppress disease in preclinical models of autoimmune diseases [29,30] (Figure 1A). Treatment with an amidated immunomodulatory peptide thymosin α-1 has been shown to block the endogenous peptide and exhibit antitumor activity in many human cancers [31]. Selective β-arrestin biased peptide ligand based on the sequence of angiotensin type II has been shown to block the angiotensin II receptor, reduced blood pressure and increased cardiac performance [32-34]. Autosomal dominant spinocerebellar ataxias (SCAs) are a complex group of debilitating and neurodegenerative diseases causally related to ataxin-1(ATX1) aggregation. ATX1 interacts with other transcriptional regulators such as CIC. AXH is a functional domain of ATX1 critical for dimerization. A linear derived from the amino terminus of CIC has been shown to compete dimer formation of AXH and hence prevent ATX1 aggregation [35]. Conjugation with carbohydrate moieties at the amino and carboxy termini or pegylation to block charges at the end-groups have also been shown to enhance the stability of synthetic peptides *in-vivo* [19,36].

Cyclization

Cyclization of peptides has been suggested as a strategy to overcome the metabolic instability, introduce structural constraints and reduce flexibility. Additionally the rigidity of the cyclic peptide can often increase affinity and reduce entropy loss upon interaction with its molecular target [37,38]. Linear peptides can be cyclicized head to tail, head or tail to side-chain or side-chain to side-chain. The most common approach has been to synthesize a heterodetic cyclic peptide forming a disulfide bridge between two cysteine residues [19,38,39]. Cyclic insulin peptide and integrin antagonists are some of the examples of interface peptides that exhibit high affinity and potency as PPI inhibitors [10,40,41]. Integrins are heterodimeric receptors that play

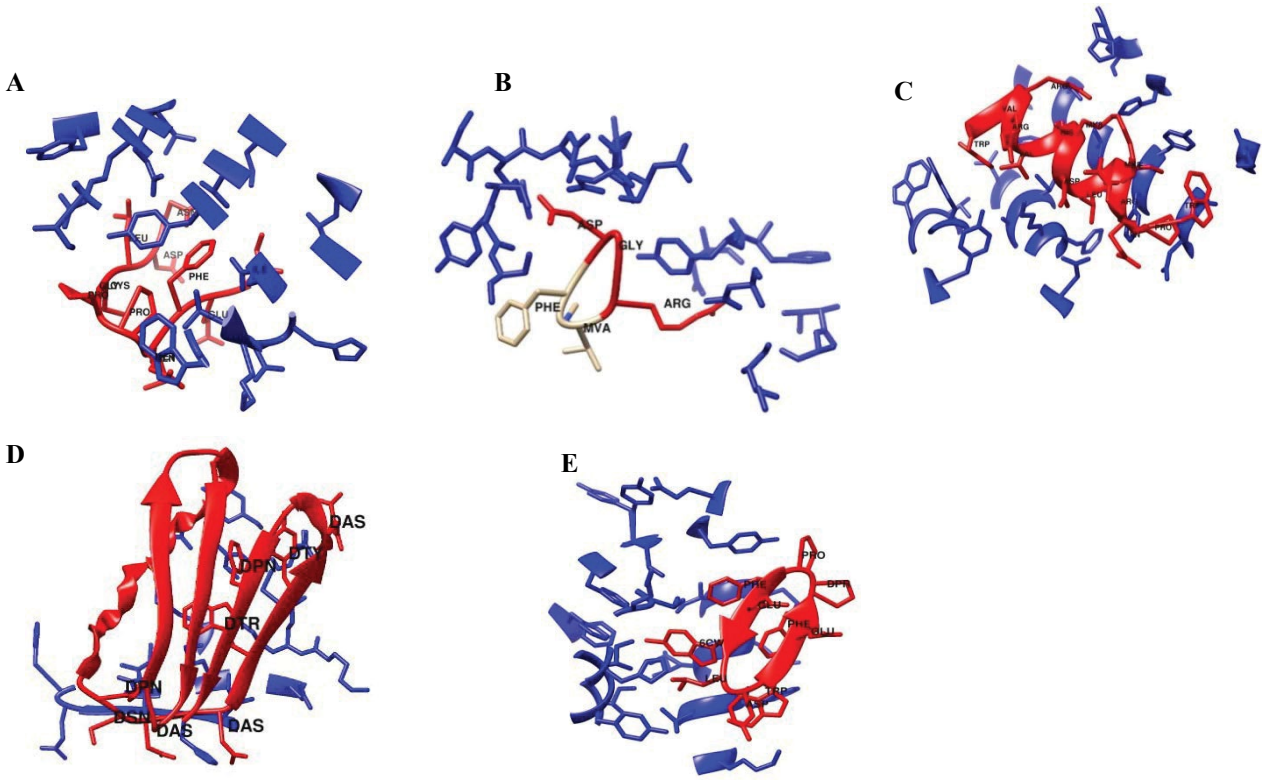

Figure 1: Molecular model or solved crystal structures of peptide drugs in the context of PPI interface. (**A**) Molecular model of end-groups blocked a CD80 competitive antagonistic peptide in complex with the solution structure of CD80 (PDB 1AH); Crystal structure of (**B**) cyclic RGD peptide in complex with the αvβ3 integrin (PDB: 1L5g); (**C**) phage display derived D- amino acid peptide in complex with vascular endothelial growth factor A (VEGF-A) (PDB:4GLS); (**D**) human MDM2 in complex with a p53 D-amino acid peptide mimetic (PDB: 3LNJ); (**E**) beta-catenin in complex with a stapled peptide inhibitor (PDB: 4 DJS). The residues of the peptide at each PPI interface are labeled.

significant roles in cell-cell or cell-extracellular matrix interactions. The integrin receptors bind the RGD tripeptide motif containing ligands and play critical roles in tumor angiogenesis and metastasis. Cylic RGD peptide c(RGDf(NMe)V) have been shown to exhibit sub-nanomolar antagonistic activity for αvβ3 receptor and nanomolar affinities for α5β1 as well as high selectivity towards the platelet receptor αIβ3 (Figure 1B). Different types of cyclized RGD peptides have been promising as inhibitors of angiogenesis in glioblastoma and head and neck cancer in clinical trials [40-42]. Covalent synthesis with the RGD peptide increased the permeability of the synthetic thymosin α-1 peptide [43]. Cyclic constraints introduced by linkage between side-chains of residues 2 and 5 in an 11-residue peptide derived from the amino terminus of the GLP-1 produced analogs that bound GLP-1 receptor at nanomolar concentrations [44,45]. Fusion of the signaling domain of IFN-γ with the cyclic peptide that recognizes the binding pocket of platelet derived growth factor β receptor (PDGFR- β) helped develop an efficacious drug for hepatic fibrosis and potentially cancer metastasis as PDGFR-β is abundantly expressed in activated fibroblasts [46].

Stapled peptides

Peptide stapling is a recently developed method to efficiently target intracellular PPI by peptide drugs. Stapling constrains the conformation of short peptides, improves stability and enhances cell permeability. It commonly involves covalently linking side chains of the amino acids on the same face of α-helix [2,8,12]. Stapled peptides are synthesized via incorporation of two α-methyl and α-alkenyl amino acids at defined positions in the peptide, followed by olefin metathesis to close the helix-spanning hydrocarbon bridge. Both the hydrocarbon bridge and the terminal methyl groups are critical for maximal effectiveness of the structurally constrained peptide agents [47]. This technology has been successfully utilized to target the multi-component NOTCH transcription factor complex. The cytoplasmic tail of NOTCH1 (ICN) engages a DNA-bound transcription factor CSL and presents a shallow groove along the interface for hosting the co-activator mastermind like 1 (MAML1) protein. The resulting ICN1:CSL:MAML1 ternary complex mediate transactivation of target genes. Stapled peptide analogs of the 16 amino-acid stretch of MAML1 acted as high affinity antagonists of NOTCH1 transcription and inhibited cancer progression in a mouse model of leukemia [48]. Similarly an all hydrocarbon stapled peptide designed to mimic the Bcl-2 homology (BH)3 interacting domain of the pro-apoptotic BID protein has been shown to adopt a stable α-helical conformation, exhibit excellent cell-permeability and inhibit tumor growth [49,50]. p53 is a transcription factor that modulates the expression of numerous target genes. Human analog of mouse double minute 2 protein (HMDM2) has been shown to act as an antagonist of p53 and also modulate the biological outcomes downstream of p53 depending on the nature of the triggering signal. Overexpression of HDM2 in tumors reduces the availability of free p53 for tumor suppression. A linear peptide derived from the transactivation domain of p53 has been shown to adopt α-helical conformation in the hydrophobic binding pocket of HMDM2. However the free peptide was unstructured and rapidly degraded. Recently phage derived stapled peptide analogs designed to stabilize the α-helical conformation of the p53 peptide in the context of the MDM2 interface has been shown to disrupt the p53:MDM2 complex and are currently being evaluated for anti-tumor effects in patients with advanced solid tumors or lymphomas expressing wild-type p53 in humans [51,52]. Wnt (Wingless and INT-1) signal transduction cascade regulates the expression of numerous genes involved in cell differentiation, proliferation, and survival. The transmission of the Wnt diffusible ligand from the plasma membrane to the nucleus is regulated by β-catenin and associated components including axin. As opposed to a linear peptide derived from the β-catenin binding domain of axin (Axin-CBD), an all hydrocarbon stapled peptide analog of the Axin-cBD has been shown to inhibit the interaction between β- catenin and the DNA-bound transcriptional regulator T-cell factor and suppress the oncogenic Wnt pathway [53] (Figure 1C). A helical peptide mimetics of the ATP-binding cassette transporter A1 (ABCA1) interacting apolipoprotein e or apolipoprotein A1 has been shown to act as the ABCA-1 agonists and prevent reverse cholesterol transfer and atherosclerosis [54]. A stapled peptide derived from the LXXXL motif of a vitamin D receptor coactivator has been shown to block the VDR:coactivator interaction and inhibit VDR mediated transcription with potential application in the treatment of Paget's disease and osteoporosis [55,56].

Retro-inverso peptides

As opposed to the rapid degradation of L-amino acid polypeptides, the proteolytic machinery is not well equipped to deal with the enantiomers or D-amino acid polypeptides [57]. Systematic inversion of the stereochemistry at the peptide backbone alpha- carbons with the use of D-amino acids when coupled with chain reversal can yield proteolytically stable retro-inverso peptide isomers. However, loss of crucial backbone hydrogen-bonding through peptide bond reversal can compromise the biological potential. Hence, partial rather than global retro-inverso isomers to stabilize hydrolysis-prone peptide bonds while maintaining the side chain topology that closely resembles the native sequence has been suggested as a better strategy for developing therapeutic peptides [58]. Icatibant is a partial retro-inverso interface peptide drug with a half-life of over 1 hr for the treatment of angioedema [59]. Developed to block the interaction between the prostaglandin and the bradykinin receptor-2 (BR-2), lcatibant is a peptide mimic of the amino terminus of BR-2 with four substituted non- natural amino acids and one D amino acid. Retro-inverso peptides derived from the ligand binding motif of the T cell co-receptor CD4 or the costimulatory molecule CD28 have been shown to prevent inflammation and ameliorate pathology in mouse models of multiple sclerosis [30]. Using mirror image phage display D-amino acid peptides have been developed as high affinity antagonists of the vascular endothelial growth factor (VEGF): receptor interactions. Select D-amino acid analog of VEGF-A has been shown to be nonimmunogenic and metabolically stable with longer half-life *in-vivo* in mice [60] (Figure 1D).

β-peptides

Amongst the secondary structures at PPI interface, β-hairpin scaffold is used by many proteins for biomolecular recognition (Figure 1D). Typically β hairpin is composed of two consecutive hydrogen-bonded antiparallel β-strands connected by a loop sequence predominantly less than five residues in length [16,61]. Specific β-hairpin epitope mimetics have been designed by transplantation of the loop sequence of a protein of known structure onto a hairpin stabilizing template [62,63]. This strategy has been used to design β hairpin peptide mimics of the protruding loop in the extracellular domain of the IFN-γ receptor to inhibit INF-γ binding [64]. Transformation of the amyloid-β (Aβ) aggregation facilitating residues from transthyretin onto a β-hairpin scaffold has been used to develop peptides that inhibit Aβ plaque and fibril formation. These Aβ peptide antagonists are evaluated as potential therapeutic agents for Alzheimer's disease [65]. Integrating structural information and data from mutagenesis β- hairpin mimic that incorporate three hot spot residues from the p53 interface on one strand of the hairpin has been shown to exhibit nanomolar affinity to HMDM2 [52,66] (Figure 1E).

Conclusions

Sequence and structural data of PPI interfaces are increasingly

adopted as valid targets for drug design and development. Considerable efforts have been directed on developing organic compounds as small molecule PPI inhibitors in the past decade, but have not been largely successful. More recently focus has turned towards developing rationally designed peptide strategies to harness PPI interface as potential therapeutic targets. Included in this review are several modifications introduced in the peptide design to develop new class of investigational agents capable of targeting and blocking several classes of proteins previously considered intractable such as multi-component complexes and PPI with extended interfaces. However, the excitement, enthusiasm and the enormous potential should be tempered with cautious expectations as many of these interface peptide mimetics are in preclinical or early phase clinical trials in humans.

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