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Peptides that bind the HIV-1 integrase and modulate its enzymatic activity – kinetic studies and mode of action

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Several peptides that specifically bind the HIV-1 integrase (IN) and either inhibit or stimulate its enzymatic activity were developed in our laboratories. Kinetic studies using 3′-end processing and strand-transfer assays were performed to study the mode of action of these peptides. The effects of the various peptides on the interaction between IN and its substrate DNA were also studied by fluorescence anisotropy. On the basis of our results, we divided these IN-interacting peptides into three groups: (a) IN-inhibitory peptides, whose binding to IN decrease its affinity for the substrate DNA – these peptides increased the K_m of the IN–DNA interaction, and were thus inhibitory; (b) peptides that slightly increased the K_m of the IN–DNA interaction, but in addition modified the V_{max} and K_{cat} values of the IN, and thus stimulated or inhibited IN activity, respectively; and (c) peptides that bound IN but had no effect on its enzymatic activity. We elucidated the approximate binding sites of the peptides in the structure of IN, providing structural insights into their mechanism of action. The IN-stimulating peptide bound IN in several specific sites that did not bind any of the inhibitory peptides. This may account for its unique activity.

Structured digital abstract

- MINT-8053571, MINT-8053597, MINT-8053615, MINT-8053633, MINT-8053651, MINT-8053669, MINT-8053687, MINT-8053705, MINT-8053723, MINT-8053741, MINT-8053759, MINT-8053777, MINT-8053795, MINT-8053814, MINT-8053836, MINT-8053854, MINT-8053872, MINT-8053890, MINT-8053908, MINT-8053926, MINT-8053944, MINT-8053962, MINT-8053980, MINT-8053998, MINT-8054037, MINT-8054145, MINT-8054163: *IN* (uniprotkb:P04585) *binds* (MI:0407) to *IN-alpha5* (uniprotkb:P04585) by *enzyme linked immunosorbent assay* (MI:0411)
- MINT-8053074, MINT-8053093, MINT-8053115, MINT-8053135, MINT-8053154, MINT-8053173, MINT-8053190, MINT-8053207, MINT-8053224, MINT-8053241, MINT-8053257, MINT-8053273, MINT-8053289, MINT-8053305, MINT-8053321, MINT-8053337, MINT-8053353, MINT-8053370, MINT-8053386, MINT-8053402, MINT-8055897: *IN* (uniprotkb:P04585) *binds* (MI:0407) to *LEDGF* (uniprotkb:O75475) by *enzyme linked immunosorbent assay* (MI:0411)
- MINT-8053418, MINT-8053442, MINT-8053458, MINT-8053474, MINT-8053506, MINT-8053523, MINT-8053539, MINT-8053555: *IN* (uniprotkb:P04585) *binds* (MI:0407) to *Rev* (uniprotkb:P69718) by *enzyme linked immunosorbent assay* (MI:0411)

Abbreviations
IN, integrase; INS, integrase-stimulating peptide; LEDGF, lens epithelium-derived growth factor; LTR, long terminal repeat; PFV, prototype foamy virus; Y2H, yeast two-hybrid.

Introduction

Much progress has been made recently in the field of anti-HIV-1 therapy, making AIDS, in many cases, a chronic disease rather than a lethal one [1–4]. Currently approved anti-HIV drugs block different stages of the HIV-1 life cycle, such as entry into cells [5,6], or inhibit viral enzymes such as the reverse transcriptase [7–12] and protease [13–18]. A major problem with the currently used anti-HIV therapy is the emergence of drug-resistant virus strains, because of the high rate of mutation [2,3,19,20]. Thus, it is important to identify new targets and, at the same time, to develop new approaches for the design of anti-HIV therapy. A promising approach is the use of peptides for inhibition or activation of certain viral or intracellular target proteins [21]. New emerging technologies have allowed the synthesis of cell-permeable peptides, as well as the synthesis of cyclic peptides, which are not readily susceptible to intracellular proteolysis and thus are metabolically stable [22–33]. For example, a peptide that bears the functional domain of the HIV envelope protein gp41, and competitively inhibits viral cell fusion and thus viral infection, is a Food and Drug Administration-approved anti-HIV drug [34].

The HIV-1 integrase (IN) mediates integration of the viral cDNA into the host chromosomal DNA, a step that is crucial for the virus life cycle [35]. This enzyme has no human homolog, so it is an ideal target for developing anti-HIV drugs [36,37]. The IN inhibitor raltegravir (MK-0518; Merck) has been approved by the Food and Drug Administration as an anti-HIV drug [36,38–40]. Another IN inhibitor, GS-9137 (Gilead), is currently in phase III of clinical trials [36,38,41–43].

The presence of the cellular protein lens epithelium-derived growth factor (LEDGF)/p75 is essential for efficient viral cDNA integration and, consequently, virus replication [44–46]. LEDGF/p75 enhances tethering of the IN–cDNA complex to the host chromatin [47]. The integration reaction proceeds via two steps: 3′-end processing, in which IN removes a GT dinucleotide from the viral DNA long terminal repeats (LTRs) [48–50], and a strand-transfer step, in which the processed viral DNA is inserted into the host chromosomal DNA [48,49,51]. Because of its central role in the replication and pathogenesis of HIV-1, inhibition of IN's enzymatic activity may be a way to block HIV-1 infection and, consequently, AIDS [36,37].

Recently, we selected, synthesized and characterized 11 peptides that interact with IN [52–58]. Two of these, LEDGF 361–370 and LEDGF 401–413, were derived from the loops binding LEDGF/p75 to IN, and were found to be inhibitory [54]. Two other IN-inhibitory peptides, Rev 13–23 and Rev 53–67, were derived from the HIV-1 Rev protein on the basis of the Rev–IN interaction [55,58–63] and following the use of a Rev-derived peptide library [58]. With the use of a yeast two-hybrid (Y2H) system and a random peptide library, five other IN-interacting peptides (IN-1 to IN-5) were selected [52]. Of these, only one peptide, IN-1, blocked IN enzymatic activity [52,57]. Screening of an IN-derived peptide library led to the discovery of an additional IN-interacting peptide, the IN-stimulating peptide (INS), which stimulated IN enzymatic activity [56,64,65]. Replacement of the C-terminal lysine of INS with glutamic acid (INS K188E) converted the stimulatory peptide into an inhibitory one [56]. Another IN-inhibitory peptide (α5) was selected previously by Zhao *et al.* [66], based on the IN dimerization domain. Its amino acid sequence highly resembles that of INS [56].

To convert some of these peptides into efficient anti-HIV drugs, it is essential to elucidate their mode of action and their effects on the kinetic parameters of the 3′-end processing and strand-transfer steps of the integration reaction. Our results obtained in the current study reveal that the IN-interacting peptides can be divided into three groups: peptides whose binding to IN decreases its affinity for DNA; peptides that slightly increase the K_m but, in addition, modify the V_{max} and K_{cat} values; and peptides that bind IN, but have no effect on its activity. Structural analysis of their interaction sites within IN provided insights into their mechanism of action.

Results and Discussion

Effect of peptides on the IN-catalyzed 3′-end processing reaction

Figure 1 summarizes the effects of the various IN-interacting peptides on the kinetic parameters of the IN-catalyzed 3′-end processing reaction. Table 1 provides a list of the 12 peptides used and their amino acid sequences. For the kinetic analysis, the enzymatic reaction was performed with different concentrations of peptides and of the DNA substrate (see Experimental procedures). The K_m and V_{max} values were calculated from the Hanes–Woolf plot (Eqn 1 [67]; see also Experimental procedures and Table S1). This equation was chosen for calculation of the kinetic parameters because it best fits the obtained results, with a minimal R^2 of 0.98.

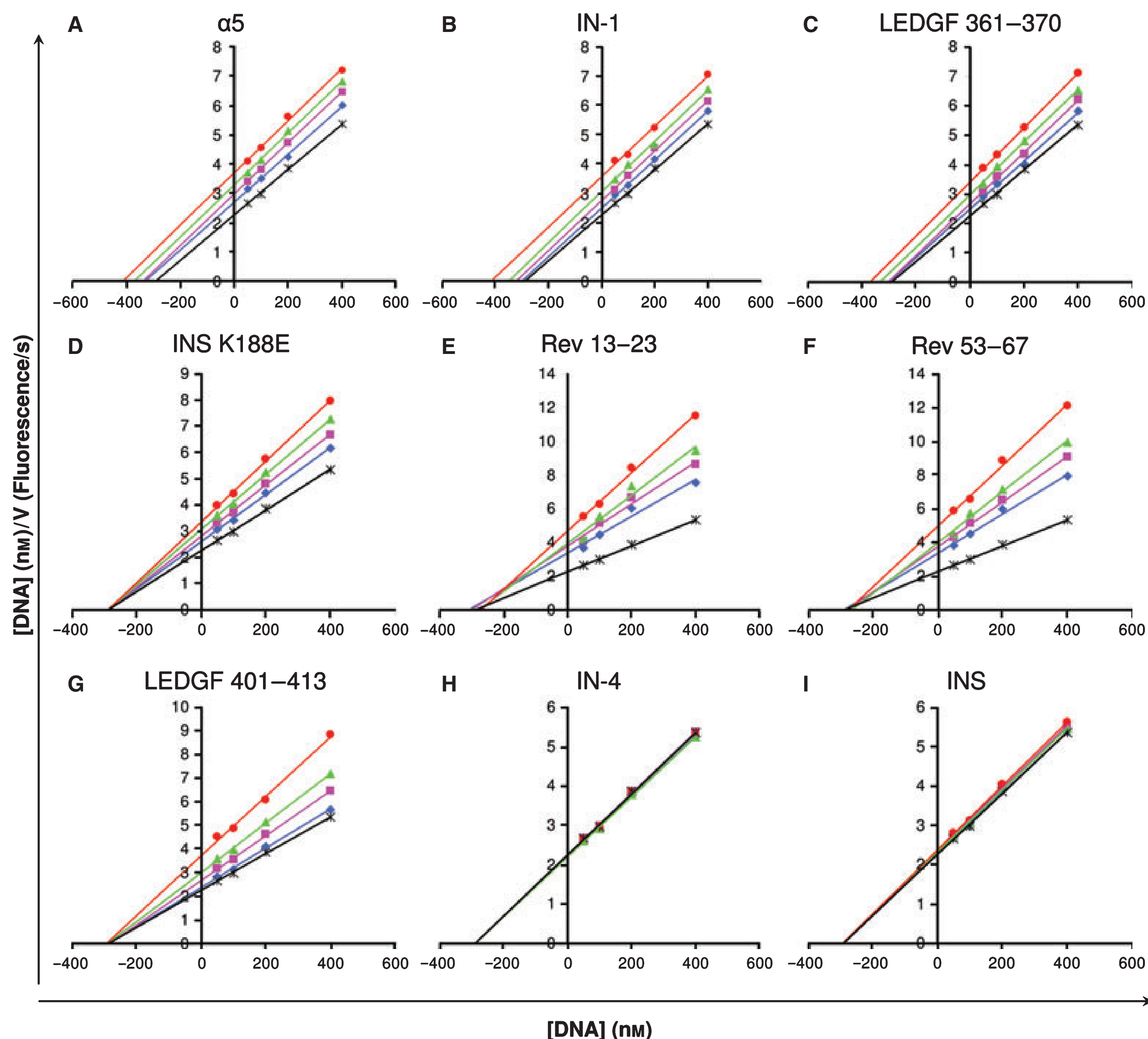

Fig. 1. Effects of the IN-interacting peptides on the 3′-end processing step: kinetic studies. IN (50 mg·L^{-1}) was incubated with the specified peptides at different IN/peptide (mol/mol) ratios [1 : 10 (◆), 1 : 100 (■), 1 : 150 (▲), 1 : 300 (●) and no peptide (*)] and with different concentrations of substrate DNA for 3′-end processing (see Experimental procedures). IN 3′-end processing activity was measured as described previously [77] and in Experimental procedures. The K_m and V_{max} values for each IN/peptide ratio were calculated and are presented in Table S1 (*t*-test, $P < 0.01$).

On the basis of the kinetic parameters, the IN-interacting peptides could be divided into three groups. The first group consisted of the IN inhibitory peptides α5, IN-1 and LEDGF 361–370, which shifted the plot while maintaining the same slope. This means that these peptides increased the K_m values of the IN–DNA interaction, decreasing the affinity between IN and its DNA substrate (Fig. 1A–C and Table S1).

The second group of inhibitory peptides includes INS K188E, Rev 13–23, Rev 53–67 and LEDGF 401–413. These peptides had a minor effect on the plot shift, meaning that they only slightly affected the K_m values of the IN–DNA interaction. However, these peptides significantly decreased the V_{max} and K_{cat} values of the 3′-end processing reaction, as inferred from the drastic change in the slope (Fig. 1D–G and Table S1). These results indicate that the inhibitory activity of this group of peptides is attributable to their effect on the turnover number of IN, and suggest a different mode of action from that of the first group.

The third group of peptides, which included IN-4 and INS, had practically no effect on the kinetic

Table 1. The selected IN-interacting peptides used in the present work.

Peptide name	Sequence	Origin	Reference
Rev 13–23	WLKTVRLIKFLY	[a]W + Rev residues 13–23	[58]
Rev 53–67	WRSISGWILSTYLGRP	[a]W + Rev residues 53–67	[58]
LEDGF 361–370	WNSLKIDNLDV	[a]W + LEDGF residues 361–370	[54]
LEDGF 401–413	WKKIRRFVSQVIM	[a]W + LEDGF residues 401–413	[54]
INS	WTAVQMAVFIHNFKRK	[a]W + IN residues 174–188	[56]
INS K188E	WTAVQMAVFIHNFKRE	[a]W + IN residues 174–187 + E	[56]
α5	HLKTAVQMAVFIHNFKR	IN residues 171–187	[66]
IN-1	WQCLTLTHRGFVLLTITVLR	Y2H peptide library	[52]
IN-2	PFSNVSSLREPNLEFELVYL	Y2H peptide library	[52]
IN-3	RCWLQMWQESFDLVAMLGDT	Y2H peptide library	[52]
IN-4	LGTGPFAHLVLWPTRALCHA	Y2H peptide library	[52]
IN-5	FVSTHFSVPASPWLLLIDIV	Y2H peptide library	[52]

[a] Labeled with tryptophan.

parameters of the 3′-end processing reaction step (Fig. 1H,I and Table S1). Similarly, the other three IN-interacting peptides (IN-2, IN-3 and IN-5) that have been selected by the Y2H system ([52] and Table 1) had no effect on the 3′-end processing step (not shown).

The effect of peptides on the strand-transfer reaction

When the effects of the various peptides on the strand-transfer step of the IN enzymatic reaction were studied, essentially the same pattern was observed (Fig. 2 and Table S2). The three peptides α5, IN-1 and LEDGF 361–370 significantly increased the K_m values for the IN–DNA interaction, with hardly any effect on the V_{max} and K_{cat} values of the enzymatic reaction, as inferred from the shift of the plots while the same slope was maintained (Fig. 2A–C and Table S2). These results further suggest that these peptides enhance the dissociation of IN and its DNA substrate or inhibit the initial IN–DNA binding, an observation that explains their inhibitory properties. A significant decrease in V_{max} and K_{cat} values and a slight increase in K_m values were inferred from the observation of the strong change in the slope and slight shift in the plot when the strand-transfer step was measured in the presence of INS K188E, Rev 13–23, Rev 53–67 and LEDGF 401–413 (Fig. 2D–G and Table S2). It is thus not surprising that this group of peptides blocked IN enzymatic activity *in vitro* and in cultured HIV-1-infected cells [52,54,58]. As expected, the nonactive IN-interacting peptide IN-4 (Fig. 2H and Table S2), as well as IN-2, IN-3 and IN-5 (not shown), had no effect on the kinetic parameters of the strand-transfer reaction step. Interestingly, despite the fact that the stimulatory INS increased the K_m value of the strand-transfer step, as was observed from the shifting of the plot, it also increased the V_{max} and K_{cat} values, as inferred from the change in the slope (Fig. 2I and Table S2). Thus, it appears that, in addition to INS stimulating the turnover number of IN, it also enhances its dissociation from its DNA substrate, allowing it to ‘hop’ between the DNA substrate molecules.

Effect of peptides on IN–DNA binding

The effects of the various IN-interacting peptides on the IN–DNA interaction were studied by fluorescence anisotropy [68,69]. Our results (Fig. 3 and Table S3) showed that the effects of the various peptides on the K_d of the IN–DNA interaction correspond to the K_m parameters obtained in the kinetics studies (Tables S1 and S2). The inhibitory peptides IN-1 [52], LEDGF 361–370, LEDGF 401–413, Rev 13–23 and Rev 53–67 induced strong dissociation of the IN–DNA complex, as reflected by the significant increase in K_d values observed in their presence (Table S3 and [52]). A much smaller effect was observed following addition of the stimulatory peptide INS and its derivative INS K188E or the inhibitory α5 peptide (Table S3). Only a minor change could be observed in the IN–DNA K_d in the presence of the nonactive peptide IN-4 (Table S3).

Dependence of the overall IN catalytic activity on its concentration – effects of IN-interacting peptides

An exponential autocatalytic effect, namely an exponential dependency of IN activity on its concentration

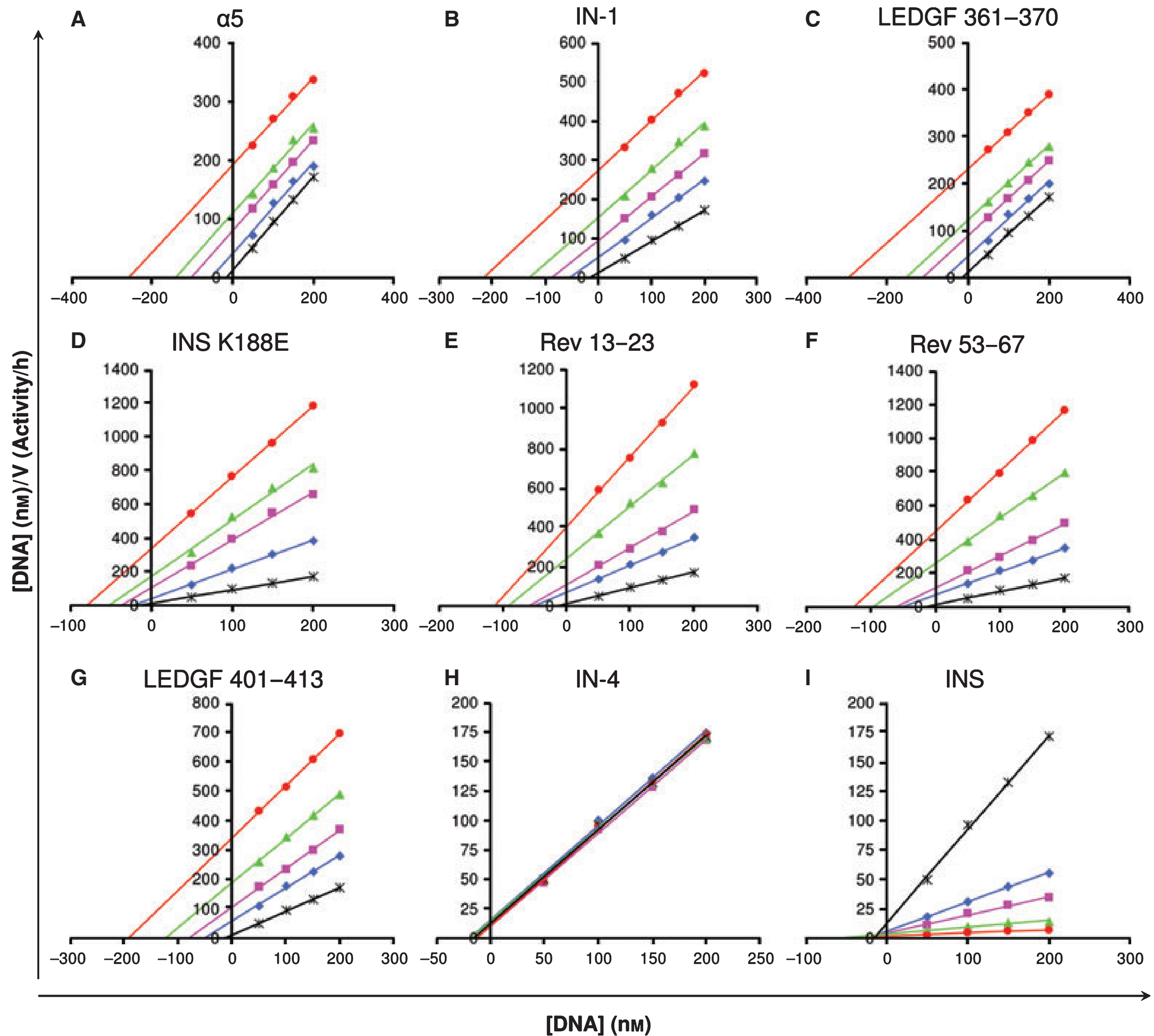

Fig. 2. Effects of the IN-interacting peptides on the strand-transfer step: kinetic studies. IN (390 nM) was incubated with different concentrations of processed LTR DNA substrate at different IN/peptide (mol/mol) ratios [1 : 10 (◆), 1 : 100 (■), 1 : 150 (▲), 1 : 300 (●) and no peptide (*)]. IN strand-transfer activity was measured as described previously [58] and in Experimental procedures. The K_m and V_{max} values for each IN/peptide ratio were calculated and are presented in Table S2 (*t*-test, $P \leq 0.01$).

(exp ^ (0.0052 × [enzyme concentration]) was observed when the dependence of IN's overall activity (3′-end processing and strand transfer) on its concentration was studied (Fig. 4). In the presence of INS, the dependence of IN's overall activity on its concentration nearly doubled (exp ^ (0.0087 × [enzyme concentration]), probably indicating enhancement of the IN–IN interaction. However, an almost linear curve was observed in the presence of all of the inhibitory peptides (Fig. 4), indicating eradication of the autocatalytic effect. This may suggest peptide-induced dissociation of the IN multimer molecule [70]. The break point observed in several of the panels of Fig. 4 is the optimum point of the reaction, namely the ideal concentrations of DNA, peptide and amount of enzyme (IN). This may slightly vary with the different peptides.

Structural analysis of the interaction of IN with the different peptides

The sites in IN that mediate its binding to the various peptides were elucidated by screening the peptides for binding a library of IN-derived peptides, using an

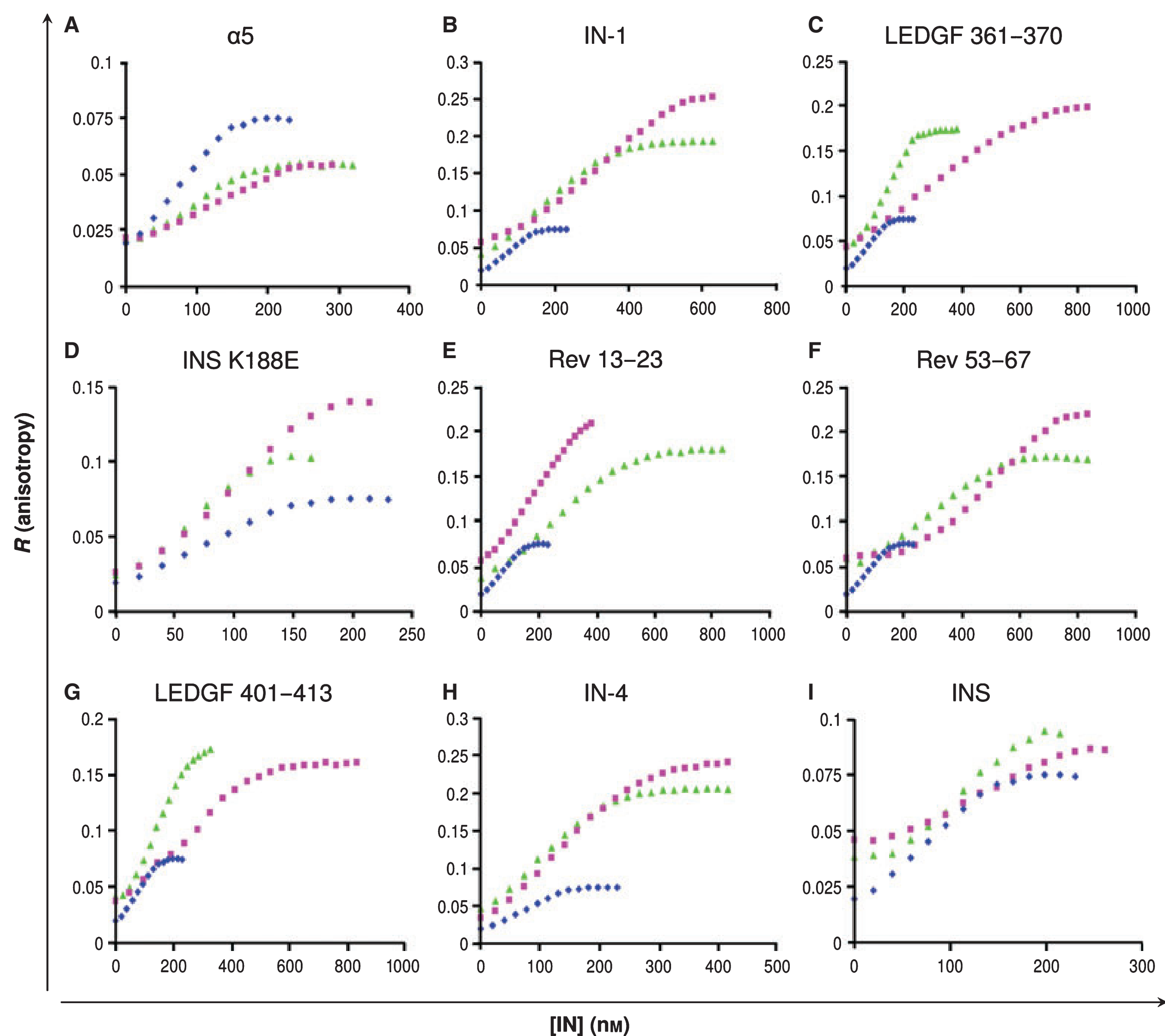

Fig. 3. IN–DNA binding affinities in the presence of the peptides. Binding of IN to DNA at different IN/peptide (mol/mol) ratios [1 : 1 (▲), 1 : 10 (■) and DNA only (◆)] was measured by fluorescence anisotropy as described in Experimental procedures. The calculated K_d values are presented in Table S3 (*t*-test, $P < 0.05$). A–I with the different peptide as indicated in the figure.

ELISA-based system (see Experimental procedures and [52,57]). The results obtained are shown in Table 2 and Fig. 5. The peptide-binding sites on IN are shown on the recently determined structure of the prototype foamy virus (PFV) intasome in its free and drug-bound forms [71]. No common sequence within IN was found to bind all of the IN-interacting peptides. There was no clear unique site in IN that bound only the inhibitory peptides or a site that bound specifically to the nonactive peptide (IN-4). This indicates that the observed peptide activity cannot be attributed solely to the masking of a specific sequence or domain within IN. In addition, the three peptide groups, with the different kinetic profiles (see above), did not have different or specific binding sites on IN.

Despite the observation that the peptide activity did not correspond with unique specific binding sites on IN, several interesting observations could be made. Several of the IN-interacting peptides uniquely bound IN at specific interfaces that participate in its dimerization, tetramerization, DNA binding or drug binding. All of the IN-interacting peptides bound IN in regions that participate in either dimerization or tetramerization. This explains why these peptides were previously shown by us to affect the IN oligomerization equilibrium and modulate its oligomeric state [52,54,55,57].

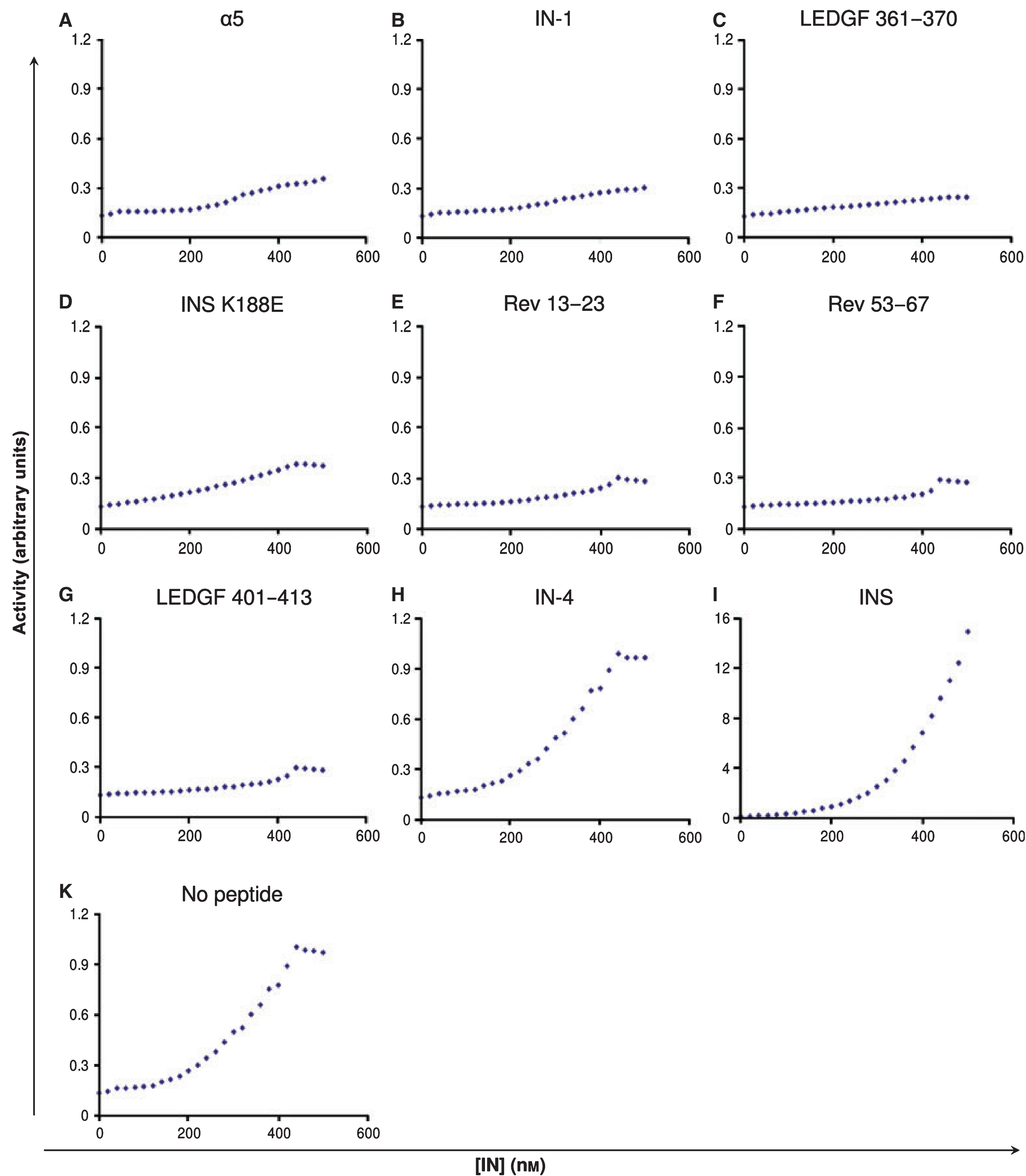

Fig. 4. Dependence of IN activity on its concentration: effects of the various peptides. Increasing concentrations of IN were incubated with unprocessed LTR DNA substrate at an IN/peptide (mol/mol) ratio of 1 : 150. IN enzymatic activity was measured as described previously [58] and in Experimental procedures (*t*-test, $P < 0.01$). A–I with the different peptide as indicated in the figure, K without peptide.

In addition, seven of the nine peptides bound IN in regions that mediate its DNA binding (Table 2). Only the two Rev-derived inhibitory peptides did not bind IN at its DNA-binding residues. However, both Rev-derived peptides bound IN in regions that are closely adjacent to its DNA-binding site (Table 2). Of the

Table 2. Summary of IN-binding domains for the various IN-interacting peptides. Binding of the various IN-interacting peptides to an IN-derived peptide library was estimated by an ELISA-based system as described in Experimental procedures. The correspondence between HIV and PFV IN is based on [80]. For localization of the binding domains on the PVF intasome (Protein Data Bank code: 3L2T [71]), see Fig. 5. DNA, number of residues participating in DNA binding [80]; Dim, number of residues participating in the dimerization interface (5-Å cut-off); Tet, number of residues participating in the tetramerization interface (5-Å cut-off).

NIH no.	Sequence	HIV IN residues	PFV IN residues	DNA	Dim	Tet	INS	INS K188E	α5	Rev 13–23	Rev 53–67	LEDGF 361–370	LEDGF 401–413	[a]IN-1	[a]IN-4
5649	ASCDKCQLKGEAMHG	38–52	94–107	4		5							+		
5650	KCQLKGEAMHGQVDC	42–56	98–116	7		7							+		
5655	QLDCTHLEGKIILVA	62–76	126–145			3			+				+		
5656	THLEGKIILVAVHVA	66–80	130–149		1	3			+		+				+
5657	GKIILVAVHVASGYI	70–84	139–153		2			+			+	+		+	+
5660	GYIEAEVIPAETGQE	82–96	151–165		2		+								
5663	GQETAYFLLKLAGRW	94–108	163–177		7		+	+	+			+	+	+	+
5664	AYFLLKLAGRWPVKT	98–112	167–181		10			+				+	+	+	+
5667	VKTIHTDNGSNFTST	110–124	179–193	1	2		+								
5669	GSNFTSTTVKAACWW	118–132	187–201				+	+	+	+		+	+	+	+
5670	TSTTVKAACWWAGIK	122–136	191–205		3		+	+	+	+					+
5671	VKAACWWAGIKQEFG	126–140	195–209	3	3				+						+
5678	MNKELKKIIGQVRDQ	154–168	223–237	3		10	+		+			+			
5679	LKKIIGQVRDQAEHL	158–172	227–242	3		15						+			
5680	IGQVRDQAEHLKTAV	162–176	231–246			12						+			
5681	RDQAEHLKTAVQMAV	166–180	235–250		2	8						+			
5682	EHLKTAVQMAVFIHN	170–184	240–254		4	5	+	+	+	+	+	+			+
5683	TAVQMAVFIHNFKRK	174–188	244–257		4	5	+	+		+	+				
5685	IHNFKRKGGIGGYSA	182–196	252–265	2	3	8	+		+			+			
5686	KRKGGIGGYSAGERI	186–200	256–269	2	6	5	+								
5692	TKELQKQITKAQNFR	210–224	304–322	2					+						+
5693	QKQITKIQNFRVYYR	214–228	308–326	2					+			+			+
5694	TKIQNFRVYYRDSRD	218–232	316–335	1		4			+			+			
5695	NFRVYYRDSRDPLWK	222–236	320–338	2		7		+				+			
5699	PAKLLWKGEGAVVIQ	238–252	340–355	4		6		+	+						+
5700	LWKGEGAVVIQDNSD	242–256	344–360	4		2						+		+	
5701	EGAVVIQDNSDIKVV	246–260	348–364	5									+		

[a] Results described in [52].

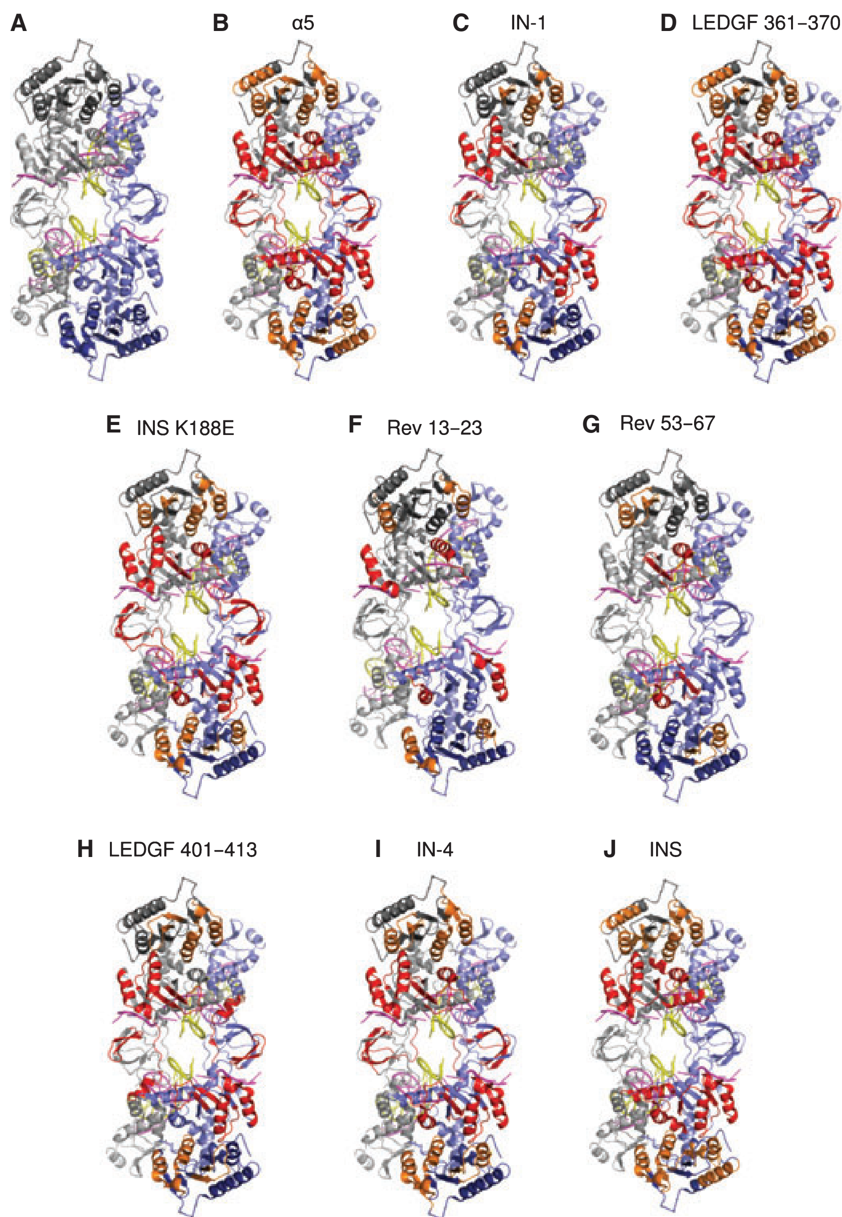

Fig. 5. Binding sites of the various peptides within the IN protein. Binding of the different peptides to an IN peptide library was determined by an ELISA-based system as described in Experimental procedures (*t*-test, $P < 0.05$). The binding sites of these peptides (summarized in Table 2) are presented as superimposed on the structure of the recently solved PFV intasome (Protein Data Bank code: 3L2T [71]). (A) The basic structure of the tetramer, which is a dimer of dimers. In the first dimer, the two monomer chains are colored light and dark respectively. In the second dimer, the two monomer chains are colored light and dark blue, respectively. DNA molecules are in yellow and magenta. (B–J) The binding sites of each peptide are colored red on chain A and in orange on chain B [70]. IN and DNA coloring is the same as in the basic tetramer (A).

above peptides, LEDGF 401–413 is the only one that bound two IN-derived peptides bearing DNA-binding residues: IN 38–56 and IN 246–260 (Table 2, Fig. 6A). LEDGF 361–370 was the only peptide that bound the IN tetramerization interface, represented by IN residues 158–180 (Fig. 6B). This explains our previous observations in which LEDGF 361–370 was shown to inhibit IN by shifting the oligomerization state towards the tetramer structure [54].

However, the most important and striking finding of the structural analysis is that the IN stimulatory peptide INS specifically bound three IN regions that did not bind any other peptide (Fig. 6C): (a) IN 82–96, bearing two dimerization interface residues; (b)

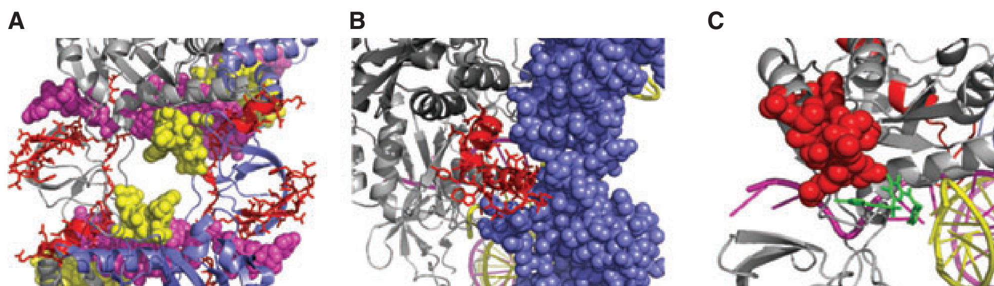

Fig. 6. IN-interacting peptides that uniquely bind IN sequences. (A) LEDGF 401–413 uniquely binds DNA-binding IN sequences. DNA chains are represented as spheres. (B) The binding site of LEDGF 361–370 is marked on one of the dimers (red). The other dimer is represented as spheres (light blue), demonstrating that the LEDGF 361–370 unique binding site on IN participates in the IN tetramerization interface. (C) INS uniquely binds the IN sequence that participates in the binding of the drug raltegravir [71]. The drug molecule is colored green and depicted as sticks. In all three cases, the unique binding site of the peptide within the IN is colored red and depicted as sticks. The IN and DNA chain coloring is the same as in Fig. 5. It should be noted that the binding sites marked are superimposed on the PFV IN structure [71].

IN 110–124, bearing one DNA-binding residue, two dimerization interface residues, one catalytic triad residue and one drug-binding residue [71]; and (c) IN 186–200, bearing two DNA-binding residues, six dimerization residues and five tetramerization residues. The unique binding sites of INS may account for its unique activity. In summary, our structural analysis showed that all of the IN-interacting peptides may potentially affect dimerization, tetramerization or DNA binding.

Summary

Our kinetic studies suggest that, of the seven inhibitory peptides studied, α5, IN-1 and LEDGF 361–370 exert their inhibitory effect by promoting dissociation between IN and its DNA substrate. Our structural studies support the view that this may result from either shifting of the oligomeric state of IN, as was suggested previously [52,54,55,57], masking of the IN–DNA interaction sites, or peptide-induced conformational changes.

On the basis of our results, some of these peptides can be developed into efficient anti-HIV drugs. Indeed, recent experiments in our laboratory demonstrated that LEDGF 361–370 was able to inhibit HIV-1 infection in a mouse model system [53]. We also recently converted this peptide to a cyclic analog [72], obtaining a more cell-permeable and metabolically stable inhibitory peptide.

Experimental procedures

Protein expression and purification

IN expression and purification were performed as described in Jenkins *et al.* [73].

Peptide synthesis, labeling and purification

Peptides were synthesized on an Applied Biosystems (ABI) 433A peptide synthesizer. Some of the peptides were also labeled with tryptophan at their N-termini for UV spectroscopy. The labeling with tryptophan did not have any effect on the activity of the peptide. However, it increased the accuracy of peptide concentration determination. Peptide purification was performed on a Gilson HPLC, with a reverse-phase C8 semipreparative column (ACE, Advanced Chromatography Technologies, London, UK) with a gradient from 5% to 60% acetonitrile in water (both containing 0.001% v/v trifluoroacetic acid). Peptide concentrations were determined with a UV spectrophotometer (Shimadzu Kyoto, Kyoto, Japan), as described previously [74]. The sequences of all the peptides are presented in Table 1.

Determination of IN activity *in vitro*

Quantitative determination of IN enzymatic activity was performed with a previously described assay system [75,76]. In this assay, the oligonucleotide substrate consists of one oligomer (5′-ACTGCTAGAGATTTTCCACACTGACTA AAAGGGTC-3′) labeled with biotin at its 3′-end, and another oligomer (5′-GACCCTTTTAGTCAGTGTGGAA AATCTCTAGCAGT-3′ for unprocessed DNA or 5′-GA CCCTTTTAGTCAGTGTGGAAAATCTCTAGCA-3′ for processed DNA) labeled with digoxigenin at its 5′-end. The final reaction mixture contained 390 nM IN, 1 μM double-stranded oligonucleotide DNA, 20 mM Hepes (pH 7.5), 10 mM $MgCl_2$, 10 mM dithiothreitol, 10% (w/v) Me_2SO, 5% (v/v) poly(ethylene glycol)-8000 and 0.1 $mg{\cdot}mL^{-1}$ BSA (Sigma, St. Louis, MO, USA) in 40 μL. When peptides were tested, IN was incubated with the peptide for 15 min prior to the addition of the DNA substrate (unless otherwise specified in the figure legends). Following a 1-h incubation at 37 °C, 60 μL of a buffer containing 20 mM Tris/HCl

(pH 8), 400 mM NaCl, 10 mM EDTA and 10 μM salmon sperm DNA was added. This overall IN reaction was followed by an immunosorbent assay on avidin-coated plates as described previously [76]. For the strand-transfer kinetics analysis, the reaction was performed with the processed DNA at different concentrations of peptide and substrate DNA. To study the dependence of IN's overall activity on its concentration, IN concentrations were varied (Fig. 4), and the unprocessed DNA was used as a substrate. K_m and V_{max} values were calculated from the Hanes–Woolf plot [67]:

$$\frac{[S]}{v} = \frac{K_m}{V_{max}} + \frac{[S]}{V_{max}} \quad (1)$$

For the autocatalytic experiment, the curve was fitted to the best R^2 value (minimum R^2 of 0.98), using SIGMAPLOT software version 11.

3′-End processing analysis

The 3′-end processing assay was performed exactly as described in He *et al.* [77]. Briefly, the reaction was performed at 37 °C in 96-well plates in a final volume of 100 μL per well. The reaction mixture contained 25 mM Pipes (pH 7.0), 10 mM β-mercaptoethanol, 5% (v/v) glycerol, 0.1 g·L^{-1} BSA, 10 mM $MnCl_2$, and 50 mg·L^{-1} purified IN. The reaction was initiated by the addition of 400 nM of the 3′-processing substrate (5′-[FAM]-ACTGCTAGAG ATTTTCCACGTGGAAAATCTCTAGCAGT-[DABCYL] -3′) or control substrate (5′-[FAM]-TGCTAGAGATTTTC CACGTGGAAAATCTCTAGCA-[DABCYL]-3′). The fluorescence signal was continuously monitored under 485-nm excitation and 535-nm emission. Enzyme-free control wells were subjected to the same reaction conditions but without IN in the reaction mixture, to monitor background signal. Substrate control wells contained all of the reagents, except for the 3′-processing substrate, which was replaced with the control substrate. These two controls were continuously monitored as the 3′-processing reaction proceeded. All of the reagents used in the assay were made fresh before each assay. K_m and V_{max} values were calculated from the Hanes–Woolf plot (Eqn 1) [67].

Fluorescence anisotropy binding studies

Measurements were performed at 10 °C in a PerkinElmer (Waltham, MA, USA) LS-55 luminescence spectrofluorometer equipped with a Hamilton microlab 500 dispenser [68,69]. The fluorescein-labeled DNA (1 mL, 0.05–0.1 μM in 20 mM Tris buffer, pH 7.4, and 185 mM NaCl) was placed in a cuvette, and the nonlabeled protein (200 μL, ~ 100 μM) was added in 20 aliquots of 10 μL each at 1-min intervals. The total fluorescence and anisotropy were measured after each addition at an excitation wavelength of 480 nm and an emission wavelength of 530 nm. Data were fitted to the Hill equation:

$$R = R_0 + \frac{\Delta R \cdot (K_a^n \cdot [\mathrm{IN}]^n)}{1 + K_a^n \cdot [\mathrm{IN}]^n} \quad (2)$$

where R is the measured anisotropy, ΔR is the amplitude of the anisotropy change from R_0 (free peptide) to peptide in complex, [IN] is the added concentration of IN, and K_a is the association constant.

In the DNA-binding experiments, a mixture of peptide (at the indicated IN/peptide ratio) and IN (4 μM) was incubated for 0.5 h and then titrated into fluorescein-labeled LTR DNA (10 nM): 5′-AGACCCTTTTAGTCAGTGTG GAAAATCTCTAGCAGT-3′.

ELISA-based binding assays

Peptide–peptide binding was estimated with an ELISA-based binding assay as described previously [78]. Briefly, Maxisorp plates (Nunc) were incubated at room temperature for 2 h with 200 μL of carbonate buffer containing 10 μg·mL^{-1} synthetic peptide from an IN peptide library spanning the full length of the HIV-1 IN subtype B consensus sequence, and containing 73 peptides, each 15 amino acids in length, with an 11 amino acid overlap between sequential peptides [AIDS Research and Reference Reagent Program of the Division of AIDS, National Institute of Allergy and Infectious Diseases, NIH: HIV-1 Consensus B Pol (15-mer) peptides (complete set)]. After incubation, the solution was removed, the plates were washed three times with NaCl/P_i, and 200 μL of 10% (w/v) BSA in NaCl/P_i was added for 2 h at room temperature. After rewashing with NaCl/P_i, biotinylated BSA–peptide conjugates dissolved in NaCl/P_i containing 10% BSA at different concentrations were added for a further 1-h incubation at room temperature. Following three washes with NaCl/P_i, the concentration of bound biotinylated molecules was estimated after the addition of streptavidin–horseradish peroxidase conjugate (Sigma), as described previously [79]. The enzymatic activity of horseradish peroxidase was estimated by monitoring the product's absorbance at 490 nm with an ELISA plate reader (Tecan Sunrise, Männedorf, Switzerland). Each measurement was performed in duplicate, and only sequences that showed a binding curve with saturation at $A > 0.1$ were selected.

Structural analysis of the peptide-binding sites on IN

The structure of the PFV intasome in the presence and absence of several drugs served as the basis for the structural analysis [71]. The sequences of PFV and HIV IN are remote, but the structural modules are similar. The PVF IN tetramer structure solved with DNA was used to pinpoint residues that contact DNA, as well as residues that

participate in dimerization and tetramerization interfaces. The correspondence between the sequences of PFV and HIV IN was based on [80].

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References

1 Fan H, Conner RF & Villarreal LP (2005) *AIDS: Science and Society*, 4th edn. Jones and Bartlett Publishers, Boston, USA.
2 Potthoff A, Brockmeyer N & Skaletz-Rorowski A (2008) Competence Network for HIV/AIDS. Further developments in therapeutic strategies for HIV-infected adults. *Eur Infect Dis* **2**, 32–34.
3 Potthoff AV & Brockmeyer NH (2009) Current therapy of HIV. *J Dtsch Dermatol Ges* **8**, 45–58.
4 Blankson JN, Persaud D & Siliciano RF (2002) The challenge of viral reservoirs in HIV-1 infection. *Annu Rev Med* **53**, 557–593.
5 Matthews T, Salgo M, Greenberg M, Chung J, DeMasi R & Bolognesi D (2004) Enfuvirtide: the first therapy to inhibit the entry of HIV-1 into host CD4 lymphocytes. *Nat Rev Drug Discov* **3**, 215–225.
6 Wild C, Greenwell T & Matthews T (1993) A synthetic peptide from HIV-1 gp41 is a potent inhibitor of virus-mediated cell–cell fusion. *AIDS Res Hum Retroviruses* **9**, 1051–1053.
7 Mitsuya H, Weinhold KJ, Furman PA, St Clair MH, Lehrman SN, Gallo RC, Bolognesi D, Barry DW & Broder S (1985) 3′-Azido-3′-deoxythymidine (BW A509U): an antiviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphadenopathy-associated virus *in vitro*. *Proc Natl Acad Sci USA* **82**, 7096–7100.
8 De Clercq E (2007) The acyclic nucleoside phosphonates from inception to clinical use: historical perspective. *Antiviral Res* **75**, 1–13.
9 De Clercq E & Holy A (2005) Acyclic nucleoside phosphonates: a key class of antiviral drugs. *Nat Rev Drug Discov* **4**, 928–940.
10 Baba M, Tanaka H, De Clercq E, Pauwels R, Balzarini J, Schols D, Nakashima H, Perno CF, Walker RT & Miyasaka T (1989) Highly specific inhibition of human immunodeficiency virus type 1 by a novel 6-substituted acyclouridine derivative. *Biochem Biophys Res Commun* **165**, 1375–1381.
11 Miyasaka T, Tanaka H, Baba M, Hayakawa H, Walker RT, Balzarini J & De Clercq E (1989) A novel lead for specific anti-HIV-1 agents: 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine. *J Med Chem* **32**, 2507–2509.
12 Pauwels R, Andries K, Desmyter J, Schols D, Kukla MJ, Breslin HJ, Raeymaeckers A, Van Gelder J, Woestenborghs R, Heykants J *et al.* (1990) Potent and selective inhibition of HIV-1 replication *in vitro* by a novel series of TIBO derivatives. *Nature* **343**, 470–474.
13 Lataillade M & Kozal MJ (2006) The hunt for HIV-1 integrase inhibitors. *AIDS Patient Care STDS* **20**, 489–501.
14 Roberts NA, Martin JA, Kinchington D, Broadhurst AV, Craig JC, Duncan IB, Galpin SA, Handa BK, Kay J, Krohn A *et al.* (1990) Rational design of peptide-based HIV proteinase inhibitors. *Science* **248**, 358–361.
15 Dorsey BD & Vacca JP (2001) Discovery and early development of indinavir. In *Protease Inhibitors in AIDS Therapy* (Ogden RC & Flexner CW eds), pp. 65–83. Marcel Dekker, New York, Basel.
16 Duncan IB & Redshaw S (2001) Discovery and early development of saquinavir. In *Protease Inhibitors in AIDS Therapy* (Ogden RC & Flexner CW eds), pp. 27–47. Marcel Dekker, New York, Basel.
17 Erickson JW (2001) HIV-1 protease as a target for AIDS therapy. In *Protease Inhibitors in AIDS Therapy* (Ogden RC & Flexner CW eds), pp. 1–25. Marcel Dekker, New York, Basel.
18 Kempf DJ (2001) Discovery and early development of ritonavir and ABT-378. In *Protease Inhibitors in AIDS Therapy* (Ogden RC & Flexner CW eds), pp. 49–64. Marcel Dekker, New York, Basel.
19 Dybul M, Fauci AS, Bartlett JG, Kaplan JE & Pau AK (2002) Guidelines for using antiretroviral agents among HIV-infected adults and adolescents. *Ann Intern Med* **137**, 381–433.
20 Martinez-Picado J, DePasquale MP, Kartsonis N, Hanna GJ, Wong J, Finzi D, Rosenberg E, Gunthard HF, Sutton L, Savara A *et al.* (2000) Antiretroviral resistance during successful therapy of HIV type 1 infection. *Proc Natl Acad Sci USA* **97**, 10948–10953.
21 Latham PW (1999) Therapeutic peptides revisited. *Nat Biotechnol* **17**, 755–757.
22 Chatterjee J, Gilon C, Hoffman A & Kessler H (2008) N-methylation of peptides: a new perspective in medicinal chemistry. *Acc Chem Res* **41**, 1331–1342.
23 Biron E, Chatterjee J, Ovadia O, Langenegger D, Brueggen J, Hoyer D, Schmid HA, Jelinek R, Gilon C, Hoffman A *et al.* (2008) Improving oral bioavailability of peptides by multiple N-methylation: somatostatin analogues. *Angew Chem Int Ed Engl* **47**, 2595–2599.
24 Chatterjee J, Ovadia O, Gilon C, Hoffman A, Mierke D & Kessler H (2009) N-methylated cyclic pentapeptides as template structures. *Adv Exp Med Biol* **611**, 109–110.

25 Baraz L, Friedler A, Blumenzweig I, Nussinuv O, Chen N, Steinitz M, Gilon C & Kotler M (1998) Human immunodeficiency virus type 1 Vif-derived peptides inhibit the viral protease and arrest virus production. *FEBS Lett* **441**, 419–426.
26 Friedler A, Friedler D, Luedtke NW, Tor Y, Loyter A & Gilon C (2000) Development of a functional backbone cyclic mimetic of the HIV-1 Tat arginine-rich motif. *J Biol Chem* **275**, 23783–23789.
27 Friedler A, Zakai N, Karni O, Broder YC, Baraz L, Kotler M, Loyter A & Gilon C (1998) Backbone cyclic peptide, which mimics the nuclear localization signal of human immunodeficiency virus type 1 matrix protein, inhibits nuclear import and virus production in nondividing cells. *Biochemistry* **37**, 5616–5622.
28 Gilon C, Halle D, Chorev M, Selinger Z & Byk G (1991) Backbone cyclization: a new method for conferring conformational constraint on peptides. *Biopolymers* **31**, 745–750.
29 Grdadolnik SG, Mierke DF, Byk G, Zeltser I, Gilon C & Kessler H (1994) Comparison of the conformation of active and nonactive backbone cyclic analogs of substance P as a tool to elucidate features of the bioactive conformation: NMR and molecular dynamics in DMSO and water. *J Med Chem* **37**, 2145–2152.
30 Hariton-Gazal E, Rosenbluh J, Zakai N, Fridkin G, Brack-Werner R, Wolff H, Devaux C, Gilon C & Loyter A (2005) Functional analysis of backbone cyclic peptides bearing the arm domain of the HIV-1 Rev protein: characterization of the karyophilic properties and inhibition of Rev-induced gene expression. *Biochemistry* **44**, 11555–11566.
31 Kasher R, Oren DA, Barda Y & Gilon C (1999) Miniaturized proteins: the backbone cyclic proteinomimetic approach. *J Mol Biol* **292**, 421–429.
32 Murray JK & Gellman SH (2007) Targeting protein–protein interactions: lessons from p53/MDM2. *Biopolymers* **88**, 657–686.
33 Qvit N, Hatzubai A, Shalev DE, Friedler A, Ben-Neriah Y & Gilon C (2009) Design and synthesis of backbone cyclic phosphorylated peptides: the IkappaB model. *Biopolymers* **91**, 157–168.
34 Kilby JM, Hopkins S, Venetta TM, DiMassimo B, Cloud GA, Lee JY, Alldredge L, Hunter E, Lambert D, Bolognesi D *et al*. (1998) Potent suppression of HIV-1 replication in humans by T-20, a peptide inhibitor of gp41-mediated virus entry. *Nat Med* **4**, 1302–1307.
35 Esposito D & Craigie R (1999) HIV integrase structure and function. *Adv Virus Res* **52**, 319–333.
36 De Clercq E (2009) The history of antiretrovirals: key discoveries over the past 25 years. *Rev Med Virol* **19**, 287–299.
37 Pommier Y, Johnson AA & Marchand C (2005) Integrase inhibitors to treat HIV/AIDS. *Nat Rev Drug Discov* **4**, 236–248.
38 Cooper DA, Steigbigel RT, Gatell JM, Rockstroh JK, Katlama C, Yeni P, Lazzarin A, Clotet B, Kumar PN, Eron JE *et al*. (2008) Subgroup and resistance analyses of raltegravir for resistant HIV-1 infection. *N Engl J Med* **359**, 355–365.
39 Grinsztejn B, Nguyen BY, Katlama C, Gatell JM, Lazzarin A, Vittecoq D, Gonzalez CJ, Chen J, Harvey CM & Isaacs RD (2007) Safety and efficacy of the HIV-1 integrase inhibitor raltegravir (MK-0518) in treatment-experienced patients with multidrug-resistant virus: a phase II randomised controlled trial. *Lancet* **369**, 1261–1269.
40 Steigbigel RT, Cooper DA, Kumar PN, Eron JE, Schechter M, Markowitz M, Loutfy MR, Lennox JL, Gatell JM, Rockstroh JK *et al*. (2008) Raltegravir with optimized background therapy for resistant HIV-1 infection. *N Engl J Med* **359**, 339–354.
41 DeJesus E, Berger D, Markowitz M, Cohen C, Hawkins T, Ruane P, Elion R, Farthing C, Zhong L, Cheng AK *et al*. (2006) Antiviral activity, pharmacokinetics, and dose response of the HIV-1 integrase inhibitor GS-9137 (JTK-303) in treatment-naive and treatment-experienced patients. *J Acquir Immune Defic Syndr* **43**, 1–5.
42 Cane PA (2009) New developments in HIV drug resistance. *J Antimicrob Chemother* **64**(Suppl 1), i37–i40.
43 Goethals O, Clayton R, Van Ginderen M, Vereycken I, Wagemans E, Geluykens P, Dockx K, Strijbos R, Smits V, Vos A *et al*. (2008) Resistance mutations in human immunodeficiency virus type 1 integrase selected with elvitegravir confer reduced susceptibility to a wide range of integrase inhibitors. *J Virol* **82**, 10366–10374.
44 Cherepanov P, Maertens G, Proost P, Devreese B, Van Beeumen J, Engelborghs Y, De Clercq E & Debyser Z (2003) HIV-1 integrase forms stable tetramers and associates with LEDGF/p75 protein in human cells. *J Biol Chem* **278**, 372–381.
45 Turlure F, Devroe E, Silver PA & Engelman A (2004) Human cell proteins and human immunodeficiency virus DNA integration. *Front Biosci* **9**, 3187–3208.
46 Llano M, Saenz DT, Meehan A, Wongthida P, Peretz M, Walker WH, Teo W & Poeschla EM (2006) An essential role for LEDGF/p75 in HIV integration. *Science* **314**, 461–464.
47 Maertens G, Cherepanov P, Pluymers W, Busschots K, De Clercq E, Debyser Z & Engelborghs Y (2003) LEDGF/p75 is essential for nuclear and chromosomal targeting of HIV-1 integrase in human cells. *J Biol Chem* **278**, 33528–33539.
48 Van Maele B, Busschots K, Vandekerckhove L, Christ F & Debyser Z (2006) Cellular co-factors of HIV-1 integration. *Trends Biochem Sci* **31**, 98–105.
49 Engelman A, Mizuuchi K & Craigie R (1991) HIV-1 DNA integration: mechanism of viral DNA cleavage and DNA strand transfer. *Cell* **67**, 1211–1221.

50 Guiot E, Carayon K, Delelis O, Simon F, Tauc P, Zubin E, Gottikh M, Mouscadet JF, Brochon JC & Deprez E (2006) Relationship between the oligomeric status of HIV-1 integrase on DNA and enzymatic activity. *J Biol Chem* **281**, 22707–22719.

51 Chen A, Weber IT, Harrison RW & Leis J (2006) Identification of amino acids in HIV-1 and avian sarcoma virus integrase subsites required for specific recognition of the long terminal repeat ends. *J Biol Chem* **281**, 4173–4182.

52 Armon-Omer A, Levin A, Hayouka Z, Butz K, Hoppe-Seyler F, Loya S, Hizi A, Friedler A & Loyter A (2008) Correlation between shiftide activity and HIV-1 integrase inhibition by a peptide selected from a combinatorial library. *J Mol Biol* **376**, 971–982.

53 Hayouka Z, Levin A, Maes M, Hadas E, Shalev DE, Volsky DJ, Loyter A & Friedler A (2010) Mechanism of action of the HIV-1 integrase inhibitory peptide LEDGF 361–370. *Biochem Biophys Res Commun* **394**, 260–265.

54 Hayouka Z, Rosenbluh J, Levin A, Loya S, Lebendiker M, Veprintsev D, Kotler M, Hizi A, Loyter A & Friedler A (2007) Inhibiting HIV-1 integrase by shifting its oligomerization equilibrium. *Proc Natl Acad Sci USA* **104**, 8316–8321.

55 Hayouka Z, Rosenbluh J, Levin A, Maes M, Loyter A & Friedler A (2008) Peptides derived from HIV-1 Rev inhibit HIV-1 integrase in a shiftide mechanism. *Biopolymers* **90**, 481–487.

56 Levin A, Hayouka Z, Helfer M, Brack-Werner R, Friedler A & Loyter A (2010) Stimulation of the HIV-1 integrase enzymatic activity and cDNA integration by a peptide derived from the integrase protein. *Biopolymers* **93**, 740–751.

57 Maes M, Levin A, Hayouka Z, Shalev DE, Loyter A & Friedler A (2009) Peptide inhibitors of HIV-1 integrase: from mechanistic studies to improved lead compounds. *Bioorg Med Chem* **17**, 7635–7642.

58 Rosenbluh J, Hayouka Z, Loya S, Levin A, Armon-Omer A, Britan E, Hizi A, Kotler M, Friedler A & Loyter A (2007) Interaction between HIV-1 Rev and integrase proteins: a basis for the development of anti-HIV peptides. *J Biol Chem* **282**, 15743–15753.

59 Levin A, Hayouka Z, Brack-Werner R, Volsky DJ, Friedler A & Loyter A (2009) Novel regulation of HIV-1 replication and pathogenicity: Rev inhibition of integration. *Protein Eng Des Sel* **22**, 753–763.

60 Levin A, Hayouka Z, Friedler A, Brack-Werner R, Volsky DJ & Loyter A (2010) A novel role for the viral Rev protein in promoting resistance to super-infection by human immunodeficiency virus type 1. *J Gen Virol* **91**, 1503–1513.

61 Levin A, Hayouka Z, Friedler A & Loyter A (2010) Nucleocytoplasmic shuttling of HIV-1 integrase is controlled by the viral Rev protein. *Nucleus* **1**, 190–201. Avilable at: http://www.landesbioscience.com/journals/nucleus/article/11300/

62 Levin A, Hayouka Z, Helfer M, Brack-Werner R, Friedler A & Loyter A (2009) Peptides derived from HIV-1 integrase that bind Rev stimulate viral genome integration. *PLoS ONE* **4**, e4155.

63 Levin A, Rosenbluh J, Hayouka Z, Friedler A & Loyter A (2010) Integration of HIV-1 DNA is regulated by interplay between viral Rev and cellular LEDGF/p75 proteins. *Mol Med* **16**, 34–44.

64 Levin A, Hayouka Z, Friedler A & Loyter A (2010) Specific eradication of HIV-1 from infected cultured cells. *AIDS Res Ther* **7**, 31.

65 Levin A, Hayouka Z, Friedler A & Loyter A (2010) Peptides derived from the HIV-1 integrase promote HIV-1 infection and multi-integration of viral cDNA in LEDGF/p75-knockdown cells. *Virol J* **7**, 177.

66 Zhao L, O'Reilly MK, Shultz MD & Chmielewski J (2003) Interfacial peptide inhibitors of HIV-1 integrase activity and dimerization. *Bioorg Med Chem Lett* **13**, 1175–1177.

67 Hanes CS (1932) Studies on plant amylases: the effect of starch concentration upon the velocity of hydrolysis by the amylase of germinated barley. *Biochem J* **26**, 1406–1421.

68 Friedler A, Hansson LO, Veprintsev DB, Freund SM, Rippin TM, Nikolova PV, Proctor MR, Rudiger S & Fersht AR (2002) A peptide that binds and stabilizes p53 core domain: chaperone strategy for rescue of oncogenic mutants. *Proc Natl Acad Sci USA* **99**, 937–942.

69 Friedler A, Veprintsev DB, Rutherford T, von Glos KI & Fersht AR (2005) Binding of Rad51 and other peptide sequences to a promiscuous, highly electrostatic binding site in p53. *J Biol Chem* **280**, 8051–8059.

70 Lam LP, Chow RY & Berger SA (1999) A transforming mutation enhances the activity of the c-Kit soluble tyrosine kinase domain. *Biochem J* **338**, 131–138.

71 Hare S, Gupta SS, Valkov E, Engelman A & Cherepanov P (2010) Retroviral intasome assembly and inhibition of DNA strand transfer. *Nature* **464**, 232–236.

72 Hayouka Z, Hurevich M, Levin A, Benyamini H, Iosub A, Maes M, Shalev DE, Loyter A, Gilon C & Friedler A (2010) Cyclic peptide inhibitors of HIV-1 integrase derived from the LEDGF/p75 protein. *Bioorg Med Chem* **18**, 8388–8395.

73 Jenkins TM, Engelman A, Ghirlando R & Craigie R (1996) A soluble active mutant of HIV-1 integrase: involvement of both the core and carboxyl-terminal domains in multimerization. *J Biol Chem* **271**, 7712–7718.

74 Kohler F, Cardon G, Pohlman M, Gill R & Schider O (1989) Enhancement of transformation rates in higher plants by low-dose irradiation: are DNA repair systems

involved in incorporation of exogenous DNA into the plant genome? *Plant Mol Biol* **12**, 189–199.
75 Craigie R, Mizuuchi K, Bushman FD & Engelman A (1991) A rapid *in vitro* assay for HIV DNA integration. *Nucleic Acids Res* **19**, 2729–2734.
76 Hwang Y, Rhodes D & Bushman F (2000) Rapid microtiter assays for poxvirus topoisomerase, mammalian type IB topoisomerase and HIV-1 integrase: application to inhibitor isolation. *Nucleic Acids Res* **28**, 4884–4892.
77 He HQ, Ma XH, Liu B, Zhang XY, Chen WZ, Wang CX & Cheng SH (2007) High-throughput real-time assay based on molecular beacons for HIV-1 integrase 3′-processing reaction. *Acta Pharmacol Sin* **28**, 811–817.
78 Rosenbluh J, Kapelnikov A, Shalev DE, Rusnati M, Bugatti A & Loyter A (2006) Positively charged peptides can interact with each other, as revealed by solid phase binding assays. *Anal Biochem* **352**, 157–168.
79 Melchior F, Paschal B, Evans J & Gerace L (1993) Inhibition of nuclear protein import by nonhydrolyzable analogues of GTP and identification of the small GTPase Ran/TC4 as an essential transport factor. *J Cell Biol* **123**, 1649–1659.
80 Krishnan L, Li X, Naraharisetty HL, Hare S, Cherepanov P & Engelman A (2010) Structure-based modeling of the functional HIV-1 intasome and its inhibition. *Proc Natl Acad Sci USA* **107**, 15910–15915.

Supporting information

The following supplementary material is available:
Table S1. Kinetic values of IN 3′-end processing activity.
Table S2. Kinetic values of IN strand-transfer activity.
Table S3. Binding of IN to DNA in the presence of the various peptides.

This supplementary material can be found in the online version of this article.

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