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REVIEW

Design and application of stimulus-responsive peptide systems

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The ability of peptides and proteins to change conformations in response to external stimuli such as temperature, pH and the presence of specific small molecules is ubiquitous in nature. Exploiting this phenomenon, numerous natural and designed peptides have been used to engineer stimulus-responsive systems with potential applications in important research areas such as biomaterials, nanodevices, biosensors, bioseparations, tissue engineering and drug delivery. This review describes prominent examples of both natural and designed synthetic stimulus-responsive peptide systems. While the future looks bright for stimulus-responsive systems based on natural and rationally engineered peptides, it is expected that the range of stimulants used to manipulate such systems will be significantly broadened through the use of combinatorial protein engineering approaches such as directed evolution. These new proteins and peptides will continue to be employed in exciting and high-impact research areas including bionanotechnology and synthetic biology.
Keywords: conformational changes/stimulus-responsive peptides/bionanotechnology

Introduction

In nature, the action of proteins is frequently mediated by a significant conformational change. This conformational change is usually in response to environmental cues such as a change in pH, temperature or the binding of specific analytes. Researchers have been quick to exploit naturally available stimulus-responsive proteins or peptides to engineer stimulus-responsive systems with potential impact on various biotechnological applications, including biomaterials, nanodevices, biosensors, bioseparations, tissue engineering and drug delivery (Fig. 1). The connection between stimulus-responsive biomolecules in nature and systems engineered to respond to a stimulus is usually made by appropriately linking the natural stimulus-responsive molecule to the molecule mediating the function of interest. In addition to the use of natural stimulus-responsive peptides and proteins, novel peptide systems have also been created using rational protein design to undergo dramatic conformational changes in response to stimuli such as pH, temperature, light, redox state, physical forces or the presence of salts or metals. These rationally engineered stimulus-responsive peptide systems frequently involve stimulus-dependent self-assembly of the peptides to form larger macromolecular structures with potential utility in tissue engineering, drug delivery and biomaterials.

This review focuses on stimulus-responsive peptide systems, based on both naturally existing peptides and rationally engineered systems (Table I). The design of the stimulus-responsive systems and the value of the engineered systems in biotechnological applications are examined. Finally, the prospect of using combinatorial protein engineering approaches such as directed evolution for engineering stimulus-responsive peptide systems is discussed. The subject of engineering stimulus-responsive systems based on the insertion of entire protein domains that modulate the activity of another protein through a conformational change is not covered by this review, and interested readers are referred to a previous review published in this journal (Ostermeier, 2005). Readers interested in stimulus-responsive synthetic polymeric systems for biochemical applications are referred to elsewhere (Roy and Gupta, 2003).

Stimulus-responsive systems based on existing proteins

Through the careful study of the structures and functions of natural proteins, several peptide motifs have been identified that exhibit environmentally responsive structural behavior. Several of these peptides have been fused to other proteins, in order to make them more attractive for use in biotechnological applications (Table I). The most widely used of the stimulus-responsive natural peptides is the repeating pentameric sequence VPGVG, found in the polymeric elastin-like polypeptide (ELP) of the mammalian elastin protein. The fourth residue of this pentamer, the 'guest residue', can be varied to any amino acid except proline to alter the physicochemical properties of the ELP (Meyer and Chilkoti, 2004). When used in a highly multimeric form (20–300 pentameric repeats), the resulting ELP exhibits a sharp reversible hydrophilic–hydrophobic phase transition that is triggered by changes in temperature, pH or ionic strength. The temperature at which this transition occurs is termed the transition temperature, T_t. At temperatures below T_t, the ELP exists in an extended state and is soluble in water, while at temperatures above T_t, the ELP collapses into an ordered β-spiral that aggregates and precipitates out of solution.

Much of our knowledge about the pentameric elastin sequence and ELPs stems from studies carried out by Dan Urry. These studies include, but are not limited to, characterization of the effect of the nature and length of ELP sequences on the transition temperature, as well as analysis of the physical properties of ELPs (for examples, see Urry *et al.*, 1985, 1986, 1991, 1992). Other studies have also shed

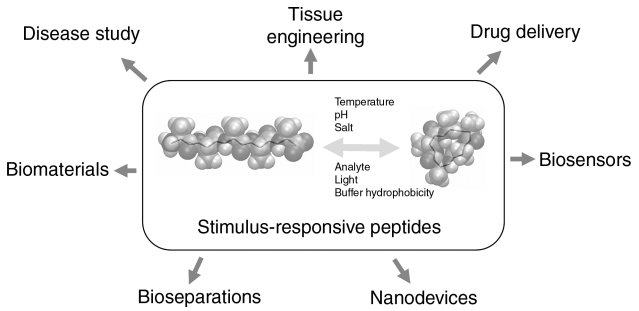

Fig. 1. Overview of applications of stimulus-responsive peptides.

light on the relationship between the sequence or length of ELPs with the transition temperature (Reiersen *et al.*, 1998; Meyer and Chilkoti, 2004).

The inverse transition temperature of ELPs has prompted the creation of synthetic ELPs that, when fused to a protein or peptide of interest, enable the reversible temperature-dependent switching of the biotechnologically useful behavior (Table I). For example, the temperature-responsive phase transition of ELPs has been exploited to develop numerous systems for purification of biomolecules. Fusion of ELPs to a protein followed by a temperature-induced precipitation process, termed as inverse transition cycling, was used to purify numerous recombinantly expressed proteins (Meyer and Chilkoti, 1999, 2004; Chilkoti *et al.*, 2002; Hyun *et al.*, 2004; McHale *et al.*, 2005; Chow *et al.*, 2006; Furgeson *et al.*, 2006). Fusion of both an ELP and a self-cleavable intein has been used to purify 10 different proteins based on temperature-induced precipitation without the introduction of extraneous tags in the final purified product (Banki *et al.*, 2005). A temperature-responsive purification of plasmid DNA has also been enabled by fusion of ELPs to a DNA-binding protein (Kostal *et al.*, 2004).

In addition to purification applications, the stimulus-responsive properties of ELPs fused to appropriate peptides or proteins have been used for remediation of toxic metals (Kostal *et al.*, 2003; Prabhukumar *et al.*, 2004) and targeted drug delivery. For the latter application, thermally responsive drug delivery was achieved either through selective aggregation of drugs (Meyer *et al.*, 2001; Raucher and Chilkoti, 2001; Furgeson *et al.*, 2006) or through thermal activation of drug-bearing cell penetrating peptides (Bidwell and Raucher, 2005; Massodi *et al.*, 2005), in hyperthermic solid tumors.

A single copy or small number (2–3) of copies of the elastin pentameric repeat sequence VPGXG has been used to modulate the functionality of proteins. Introduction of the sequence GVPGVG into the inter-helix region of an IgG-binding two-helix region of staphylococcal protein A allowed an increased α-helical structure and strengthened binding to Fc with increasing temperature (Reiersen and Rees, 1999). A similar system also permitted salt-dependent binding of an elastin peptide-containing IgG minidomain to the Fc target (Reiersen and Rees, 2000). In another study, incorporation of 1–3 copies of the VPGXG sequence into the linker region of anti-fluorescein single-chain antibodies enabled destabilization of the scFv leading to unbinding of the scFv from fluorescein at increased temperatures (Megeed *et al.*, 2006).

Another stimulus-responsive system inspired by a natural peptide is a calcium-switchable mesh with potential for application as a smart biomaterial (Ringler and Schulz, 2003). This system utilizes repeats of the characteristic calcium-binding motif GGXGXDXUX from the β-roll domain of the enzyme serralysin from *Serratia marcescens* (Baumann, 1994), where X is any amino acid and U is a large aliphatic residue. A calcium-responsive hydrogel for application in tissue engineering has also been created from the larger calcium-binding protein, calmodulin (Ehrick *et al.*, 2005).

Other natural stimulus-responsive peptides that have yet to find their way to practical applications also exist (Table I). A 36-amino acid peptide of the influenza virus protein hemagglutinin, important for membrane fusion of the virus, has been synthesized and was shown to exhibit an increased α-helical content at low pH (Carr and Kim, 1993). This peptide has potential for application in molecular motors and machines (Dubey *et al.*, 2004; Mavroidis *et al.*, 2004; Banta *et al.*, 2007). Other environmentally sensitive molecules include a 25-residue peptide from a sheep prion protein whose conformation is dependent on the hydrophobicity of the solvent (Megy *et al.*, 2004), and a 31-amino acid peptide from a marsupial prion protein, whose conformation can be modulated by divalent copper ions (Gustiananda *et al.*, 2002).

Stimulus-responsive systems based on rational design

Several peptide-based systems have rationally been engineered, both *de novo* and as modifications to existing proteins, to respond in interesting ways to various stimuli (Table I). Many of these systems are tunable via changes in temperature and/or pH. For example, an early study described the *de novo* creation of bis-amphiphilic peptides that switched states from an α-helix to a β-sheet in aqueous solution in a pH-dependent manner (Mutter *et al.*, 1991). Peptides inspired by segments of the native proteins IsK and hen egg white lysozyme that self-assemble into gels comprising β-sheet tapes were found to dissociate at pH $>$ 12 (Aggeli *et al.*, 1997). In work aimed at elucidating the formation of amyloid fibrils involved in numerous human diseases, certain 12- and 16-residue peptides with alternating hydrophobic and charged amino acids were found to exhibit a conformational change from a self-assembled β-sheet formation to an α-helical structure at temperatures $>$70°C (Fig. 2) (Zhang and Rich, 1997; Altman *et al.*, 2000). With cooling, these peptides retained the α-helix structure, and took several weeks at room temperature to partially return to the β-sheet form. These peptides were also found to reversibly undergo significant conformational changes dependent on pH. In analogous work, again aimed at amyloid study, a rationally designed 17-residue peptide with both α-helical and β-type elements was found to irreversibly change states from a coiled-coil to a β-sheet at elevated temperatures (Kammerer *et al.*, 2004), and a 29-residue peptide designed to have both α-helical- and β-sheet-like properties displayed a reversible heat-induced conversion from an α-helix to β-sheet form (Ciani *et al.*, 2002). Responsive 20-residue peptides have also been designed *de novo* with alternating hydrophobic and hydrophilic residues and a central type II′ turn structure in order to reversibly fold from a disordered state to a β-hairpin

Table I. Examples of stimulus-responsive peptides

Application	Stimulus	Motif/peptide sequence	Source	Conformational change	References
Amyloid study	Temperature, pH	ADADADADARARARAR	Designed	β-Sheet → α-helix	Zhang and Rich (1997) and Altman *et al.* (2000)
	Temperature, salt	SIRELEARIRELELRIG		α-Helix → β-sheet	Kammerer *et al.* (2004)
	Temperature	YGCVAALETKIAALE TKKAALETKIAALC			Ciani *et al.* (2002)
Alzheimer disease model	Zinc	DAEFRHDSGYEVHHQK	Amyloid β-peptide	Poorly folded helix ↔ well folded irregular 3_{10} helix	Zirah *et al.* (2006)
Biomaterials	Calcium	GGXGXDX(L/F/I)X	Serralysin	Disordered ↔ β-roll	Ringler and Schulz (2003)
	Porphyrin	IQQLKNQIKQLLKQ	Designed	Disordered ↔ α-helix	Kovaric *et al.* (2006)
Bio-mineralization	pH	CCCCGGGSRGD		ND	Hartgerink *et al.* (2001)
Bioremediation	Temperature, pH, salt	VPGXG	Elastin	Disordered ↔ β-turn	(Kostal *et al.*, 2003; Prabhukumar *et al.*, 2004)
Conformational studies	Trifluorinated alcohols, salt	GIGAVLKVLTTGL PALISWIKRKRQQ	Honey bee venom	Disordered ↔ α-helix	Raghuraman and Chattopadhyay (2006) and Schuh and Baldwin (2006)
	pH	1.EAALEAALELAAELAA 2.KAALKAALKLAAKLAA 3.KAALEAALKLAAELAA 4.EAALKAALELAAKLAA	Designed	β-Sheet ↔ α-helix	Mutter *et al.* (1991)
	Redox state	CGGEIRALKYEIARLKQAA QAKIRALEQKIAALEGGC		Dimeric coiled coil ↔ monomeric α-helix	Pandya *et al.* (2004)
	Zinc	YIHALHRKAFAKIAR LERHIRALEHAA		Trimeric coiled coil ↔ monomeric α-helix	Cerasoli *et al.* (2005)
	Redox state	YLKAMLEAMAKLMAKLMA		α-Helix ↔ β-sheet	Dado and Gellman (1993)
	Light	EACARV$_A$AACEAAARQ[a]		Disordered ↔ α-helix	Kumita *et al.* (2000)
	pH	RVIEKTNEKFHQIEKEFSE VEGRIQDLEKYVEDTKI	Hema-gglutinin		Carr and Kim (1993)
	Solvent polarity	ELALKAKAELELKAG	Designed	β-Sheet ↔ α-helix	Mutter and Hersperger (1990)
		ELLAKKALEAEALKG		Disordered ↔ α-helix	
		1.EWAVVLVAEAKHQ 2.WGKIQKLKIAKVFK 3.KVIKCKAAVLWEEKK		Disordered ↔ α-helix Disordered ↔ β-sheet	Zhong and Johnson (1992)
		1.IIPTAQETWLGVLTIMEHTV 2.LSGGIDVVAHELTHAVTDY 3.PAVHASLDKFLSSVSTVL 4.GYQCGTITAKNVTAN 5.VAEAKVAEAKVAEAK		Disordered ↔ α-helix Disordered ↔ β-sheet	Waterhous and Johnson (1994)
	pH	ETATKAELLAKYEATHK		α-Helix ↔ β-sheet	Cerpa *et al.* (1996)
	pH, salt, light	ETATKAELLAKZEATHK[b]			
	pH, temperature	IGKLKEEIDKLNR(D/N)LDDM (E/Q)DENEQLKQENKTLL KVVGKLTR	Par-4 protein	Disordered ↔ α-helix	Dutta *et al.* (2001, 2003)
	pH	1. EIAQLEYEISQLEQ 2. KIAQLKYKISQLKQ 3. EIAQLEYEISQLEQEIQALES 4. KIQALKQKISQLKWKIQSLKQ	Designed		Dong and Hartgerink (2006)
DNA purification	Temperature, pH, salt	VPGXG	Elastin	Disordered ↔ β-turn	Kostal *et al.* (2004)

Continued

Table I. *Continued*

Application	Stimulus	Motif/peptide sequence	Source	Conformational change	References
Drug delivery to solid tumors	Temperature	VPGXG			Meyer *et al.* (2001), Raucher and Chilkoti (2001), Bidwell and Raucher (2005), Massodi *et al.* (2005) and Furgeson *et al.* (2006)
Hydrogels	pH	QATNRNTDGSTDYGILQINSR	Hen egg white lysozyme	ND	Aggeli *et al.* (1997)
	Shear	KLEALYVLGFFGFFTLGIMLSYIR	IsK protein	ND	
	Hydrogen bond donor strength of solvent	KLEALYVLGFFGFFTLGIMLSYIR		β-Sheet ↔ helix/random coil	
	Salt	1. FKFEFKFEFKFE 2. FKFQFKFQFKFQ	Designed	ND	Caplan *et al.* (2000, 2002)
	Temperature	VKVKVKTKVPPTKVKTKVKV		Disordered ↔ β-hairpin	Pochan *et al.* (2003)
	Salt	FEFEFKFKFEFEFKFK		ND	Collier *et al.* (2001)
Hydrogels, tissue engineering	pH	VKVKVKVKVPPTKVKVKVKV		Disordered ↔ β-hairpin	Schneider *et al.* (2002) and Kretsinger *et al.* (2005)
Hydrogels		EIAQHEKEIQAIEKKIAQHEY KIQAIEEKIAQHKEKIQAIK		Disordered ↔ α helix	Zimenkov *et al.* (2006)
	Salt	QQKFQFQFEQQ		Disordered ↔ β-sheet	Collier and Messersmith (2003, 2004)
Modulation of protein binding	Temperature	VPGXG	Elastin	Disordered ↔ β-turn	Reiersen and Rees (1999, 2000) and Megeed *et al.* (2006)
Nanotapes	pH	QQRFEWEFEQQ	Designed	Disordered ↔ β-sheet	Aggeli *et al.* (2003) and Kayser *et al.* (2004)
Nanoropes	Salt	CKQLEDKIEELLSKA ACKQLEDKIEELLSK		Disordered ↔ α-helix	Wagner *et al.* (2005)
Nanotubes, nanowires	Solvent polarity	FF		ND	Reches and Gazit (2003)
Prion protein study		GNDYEDRYYREN MYRYPNQVYYRPVC	Sheep prion protein	β-Hairpin ↔ α helix	Megy *et al.* (2004)
Prion protein study	Copper	$(PHPGGSNWGQ)_3G$	Marsupial prion protein	ND	Gustiananda *et al.* (2002)
Protein design	Ni^{2+}, Co^{2+}, Ru(II)	bpGELAQKLEQALQKLA[c]	Designed	Poorly folded monomeric α-helix ↔ well folded trimeric α-helix	Ghadiri *et al.* (1992a)
Protein purification	Temperature, pH, salt	VPGXG	Elastin	Disordered ↔ β-turn	Meyer and Chilkoti, (1999) and Banki *et al.* (2005)
Tissue engineering	Salt	1. AEAEAKAKAEAEAKAK 2. RARADADARARADADA	Designed	β-Sheet ↔ ND	Zhang *et al.* (1995)
	Salt, pH	KLDLKLDLKLDL			Kisiday *et al.* (2002)
		1. RADARADARADARADA 2. RARADADARARADADA			Holmes *et al.* (2000), Davis *et al.* (2005), Narmoneva *et al.* (2005), Yokoi *et al.* (2005) and Ellis-Behnke *et al.* (2006)

ND not determined. [a]The two cysteines in this peptide are cross-linked by an azobenzene derivative; "A" refers to α-aminoisobutyric acid; [b]Z refers to *p*-phenylazo-L-phenylalanine; [c]"bp" refers to a 2,2′-bipyridine functionality.

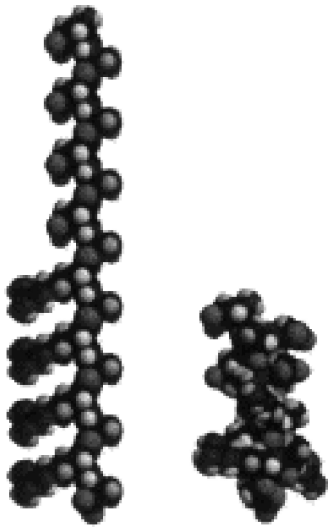

Fig. 2. Molecular models of the ionic peptide ADADADADARARARAR, which undergoes a conformational change from β-sheet (left) to α-helix (right) at high temperatures (Zhang and Rich, 1997; Altman *et al.*, 2000). Reprinted from Zhang (2002), Copyright 2002, with permission from Elsevier.

structure. This conformational shift, which leads to the formation of a hydrogel, was made responsive both to changes in pH (Schneider *et al.*, 2002) and temperature (Pochan *et al.*, 2003) by appropriately tailoring the peptide primary sequence. Variation of the solution pH has also been used to trigger the reversible assembly of a synthetic polymer-peptide amphiphile into nanotubes (Hartgerink *et al.*, 2001), and reversibly direct a 21-residue peptide to form a mechanically strong 'film state' (at neutral pH) versus a mobile 'detergent state' (at acidic pH) at a fluid–fluid interface (Dexter *et al.*, 2006).

Agents that enhance the hydrophobic effect such as salts have been used to alter the microenvironment surrounding peptides and thus trigger peptide aggregation (Table I). This has been achieved either through the design of ionic self-complementary peptides containing alternating hydrophobic and charged amino acids that assemble into macromolecular structures such as hydrogels and membranes upon salt exposure (Zhang *et al.*, 1993; Holmes *et al.*, 2000), or peptides that salt-dependently associate with 'sticky-ends', promoting self-assembly into fibers and filaments (Wagner *et al.*, 2005). Interestingly, a 'sticky-ended' peptide assembly system reliant on ionic rather than hydrophobic interactions is inhibited, not enhanced, by the presence of salt (Pandya *et al.*, 2000).

Peptides have also been designed to change their conformation in response to redox state (Dado and Gellman, 1993; Pandya *et al.*, 2004), and the α-helical content of a peptide chemically modified with an azobenzene derivative was found to be reversibly controllable by light (Kumita *et al.*, 2000). Peptides whose bulk behavior can be controlled by divalent metal ions (Cerasoli *et al.*, 2005; Dexter *et al.*, 2006) and an externally applied physical trigger, shear (Aggeli *et al.*, 1997), have also been successfully engineered. In other interesting work, 15-residue peptides N-terminally modified with pyridyl functionalities were created with the ability to self-assemble into a triple-helix metalloprotein in response to Ni^{2+}, Co^{2+} or Ru(II) metals (Ghadiri *et al.*, 1992a), or a four-helix metalloprotein in response to Ru(II) (Ghadiri *et al.*, 1992b).

An emerging area of interest in the field of stimulus-responsive peptides is the concept of enzyme-responsive peptides. For example, hydrophobic dipeptides in combination with Fmoc-modified amino acids were found to self-assemble to form hydrogels under appropriate conditions, in the presence of the protease thermolysin (Toledano *et al.*, 2006). Peptides cleavable by specific proteases offer the potential to create smart hydrogels that can be programmed to change their properties upon protease exposure (Thornton *et al.*, 2005; Zourob *et al.*, 2006).

Many designed responsive peptides that self-assemble into interesting macromolecular structures have been formulated to solve important biological problems. With an eye toward application as a scaffold for tissue engineering, ionic self-complementary peptides that salt dependently self-assemble into a macromolecular membranous matrix have been used to support the attachment of various mammalian cells (Zhang *et al.*, 1995), including the attachment of neuronal cells, enabling extensive neurite outgrowth (Holmes *et al.*, 2000). Peptide amphiphiles that self-assemble into nanotubes at low pHs were able to direct mineralization of hydroxyapatite in a process that mimics natural bone formation (Hartgerink *et al.*, 2001). Polypeptide-based diblock copolymers that self-assemble into vesicles and micelles whose stability and/or size can be controlled by pH or ionic strength have been proposed as smart, tunable alternatives to conventional lipid vesicles for drug delivery applications. Vesicles whose integrity can be manipulated by changes in pH have been constructed by linking together diblock copolypeptides comprising a hydrophobic poly-L-leucine chain and hydrophilic ethylene glycol-modified poly-L-lysine chain (Bellomo *et al.*, 2004). These vesicles were made pH-sensitive by spiking lysine residues, whose protonation state can affect chain conformation, into the hydrophobic poly-leucine block. Hybrid polypeptide-synthetic polymer diblock vesicles whose size is pH- and ionic strength-sensitive were constructed with a polybutadiene hydrophobic block and a poly-L-glutamic acid hydrophilic block (Checot *et al.*, 2005). Other self-assembling peptide systems have the potential to be used as biomaterials such as fibers, filaments, nano-ropes, nano-tapes and membranes, in addition to hydrogels for tissue engineering (Zhang *et al.*, 1993; Aggeli *et al.*, 1997; Pandya *et al.*, 2000; Wagner *et al.*, 2005). Peptides that self-assemble on surfaces can serve to reinforce surfaces or infuse the surface with new functionality (Ryadnov *et al.*, 2003; Lu *et al.*, 2004; Dexter *et al.*, 2006).

Combinatorial methods for new stimulus-responsive systems

Natural and rationally engineered peptides will continue to see use in important and diverse biotechnology applications. However, the currently available stimuli-responsive peptides may be limited by the scope and breadth of available environmental cues that can induce peptide conformational changes. Until our understanding of peptide structure–function relationships fully enables *de novo* design, engineering of novel stimulus-responsive peptide systems that populate two or more distinct equilibrium states will likely be greatly facilitated by the utilization of combinatorial methods.

Directed evolution is a combinatorial method that utilizes a selection or screening procedure to identify proteins or peptides with desired properties from randomized libraries (Kuchner and Arnold, 1997). A significant advantage of the directed evolution approach is that *a priori* knowledge of a protein's structure–function relationship is not required. The main technical challenge in directed evolution is the development of appropriate selection or screening techniques (Zhao and Arnold, 1997). This is particularly true for the directed evolution of stimulus-responsive peptides, as the determination of the onset of a conformational change in a peptide is not a readily observable event.

To attempt to overcome this barrier, we are developing protein-based conformational change sensors (CCSs). These sensors can then be used for the directed evolution of novel stimulus-responsive peptides. As a first proof of this principle, we are developing a sensor starting with an anti-fluorescein single chain antibody construct (scFv) (Jung and Pluckthun, 1997). It is well known that the properties of the artificial peptide linkers that tether the V_L and V_H domains together in the scFv format can dramatically affect the performance of the scFv (Tang *et al.*, 1996). Therefore, the binding properties of the scFv can be used to report the conformational state of the linker. This effect has recently been demonstrated when temperature-responsive fluorescein binding was observed following the insertion of a short elastin-like peptide into the scFv linker (Fig. 3) (Megeed *et al.*, 2006).

The selection of randomized peptide libraries inserted into the scFv construct for unique conformational behaviors is being performed in our laboratory using an immobilized fluorescein affinity column. This allows for the quantitative prediction of scFv binding affinities based on measured elution times using affinity chromatography models, and this prediction is being used to infer information about the conformational state of the inserted peptide linkers. Since peptides with novel stimulus responsiveness are desired, a dual positive–negative selection approach is needed. For the negative selection, peptides that have a conformation that enables fluorescein binding are recovered. For the positive selection, the desired stimulus is introduced and scFvs that exhibit weakened binding affinities in response to the stimulus of interest are eluted.

To the best of our knowledge, this will be the first demonstration of directed evolution for stimulus-responsive peptide conformational changes. Additional CCSs will be developed, which are calibrated to enable the identification of other peptides that exhibit novel triggered conformational behaviors. These methods will provide a new tool for engineering novel stimulus-responsive peptide systems for use in the various applications described in this paper.

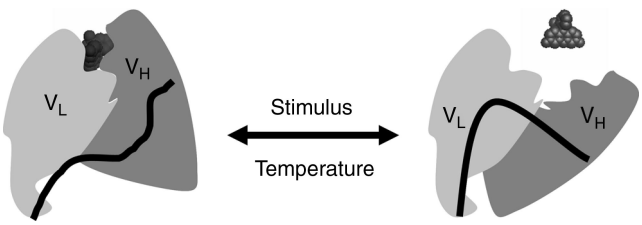

Fig. 3. The artificial peptide linker of an anti-fluorescein scFv can influence the binding properties of the antibody fragment. Megeed *et al.* (2006) have demonstrated the temperature responsive unbinding of ligand from an anti-fluorescein scFv with an elastin peptide linker. This scFv represents a convenient CCS. Immobilized fluorescein is being utilized in affinity chromatography selections for peptide linkers that exhibit novel stimulus-dependent conformational behavior.

Outlook

As our understanding of protein structures and functions continues to expand, researchers are increasingly using proteins and peptides to create new systems that have no natural counterparts. Modular assembly concepts are being used to create proteins and protein-based systems that have structural applications in addition to the chemical capabilities for which proteins are often associated. As fields such as synthetic biology and bionanotechnology continue to mature, an ever-increasing toolbox of parts will be needed for the creation of future technological advances.

Stimulus-responsive peptides will be valuable building blocks as systems are created that can respond to environmental cues. This technology can be used to make 'smart' systems, including artificial viruses, tunable tissue culture scaffolds and other bionanomachines. Rational protein design will continue to be used to create new peptides, and directed evolution will likely be used to further broaden these exciting efforts.

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