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Viruses as potential nanomachines

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SUMMARY

In this paper viruses are considered as very efficient nano-machines that produce numerous copies of them. Observing these nano-architectures, the question arises: which molecular forces and processes make up the set of such structures, given that they are extremely inspiring for development of new technologies at the nano level. There is a need for deep understanding of individual molecular building blocks and their structures, properties of their assemblies and dynamic behavior.

Keywords: viruses; bacteriophages; DNA nanomachines

INTRODUCTION

The high efficiency of bacteriophage-based therapy in suppressing colonies of many resistant bacteria, as in the case of cystic lung fibrosis causing their very serious infection, has made bacteriophages one of the most important new advances in molecular biology, biophysics and bio-nanotechnology. Filamentous viruses, such as bacteriophage M13, have a virion architecture that allows them to precisely build ordered two-dimensional and three-dimensional structures without damaging at the level of nanometer dimensions. This would not be possible without a detailed knowledge of the protein coat's structure and dynamics during the virus replication cycle. The results of spectroscopic studies show the critical role of protein incorporation into the membrane, both during the infectious entry of the virus into the host cell and during the assembly of a new virion in the host membrane. The protein is efficiently incorporated into the membrane using a strong C-terminal interfacial anchor. A simple tilt mechanism and subtle structural adjustment at the very end of its N-terminus provide a favorable thermodynamic association of proteins in the lipid bilayer [1, 2, 3].

Viruses, especially bacterial viruses or bacteriophages, play a key role in controlling biological systems. Advances in molecular biology over the last 50 years have been built largely due to the study of bacteriophages. Restriction endonucleases, which form the basis of molecular cloning, were developed after studies on phage infection. Many phage enzymes provide tools to the molecular biologists who study the pathways of replication, transcription, translation, and transport (Figure 1). Phage display techniques provide them with a powerful methodology for identifying and optimizing ligand antibodies and other biomolecules. Modern application of bacteriophages in engineering materials puts them in the foreground of new nano-technological devices [3, 4, 5]

BASIC CONSIDERATIONS

Filamentous bacteriophages in modern biophysics have served as a model system for the development and application of spectroscopic methods suitable for biological supramolecular assemblies. Filamentous phage envelope proteins, which can be easily synthesized in the laboratory, have two primary roles during the replication cycle as membrane proteins and as the main structural elements of phage particles, thus showing the astonishing possibility of finding two such different roles in the bacteriophage replication cycle. Learning about protein structures in different environments allows us to fully understand their latest nano-technological applications in nano-biology and bio-nanotechnology. Filamentous phages make up a family of viruses that have about ten genes. The relative simplicity of these viruses and the ease with which they can be genetically modified have made them extremely useful models for studying macromolecular structures and interactions. All filamentous strains of bacteriophages have a similar virion structure and life cycle. M13 filamentous phages are best-studied biochemically, genetically and biophysically.

M13 bacteriophage causes chronic infections, with infected cells continuing to grow and divide, albeit at a lower rate than normal. The phage is a long thread-shaped particle, 6.5 nm in diameter and 900 nm long. The flexible strand contains a circular, single-stranded viral DNA genome, protected by a long cylindrical protein coat. The major envelope proteins that make up the tube around the viral DNA, in the folding helix, are oriented so that the N end is on the outside of the envelope, and the C terminates by binding to the DNA inside the envelope. The major protein coat's hydrophobic domain is located in the central part of the protein and connects the protein coat to the adjacent protein coat to form viral particles. During reproduction, the main envelope protein is involved in

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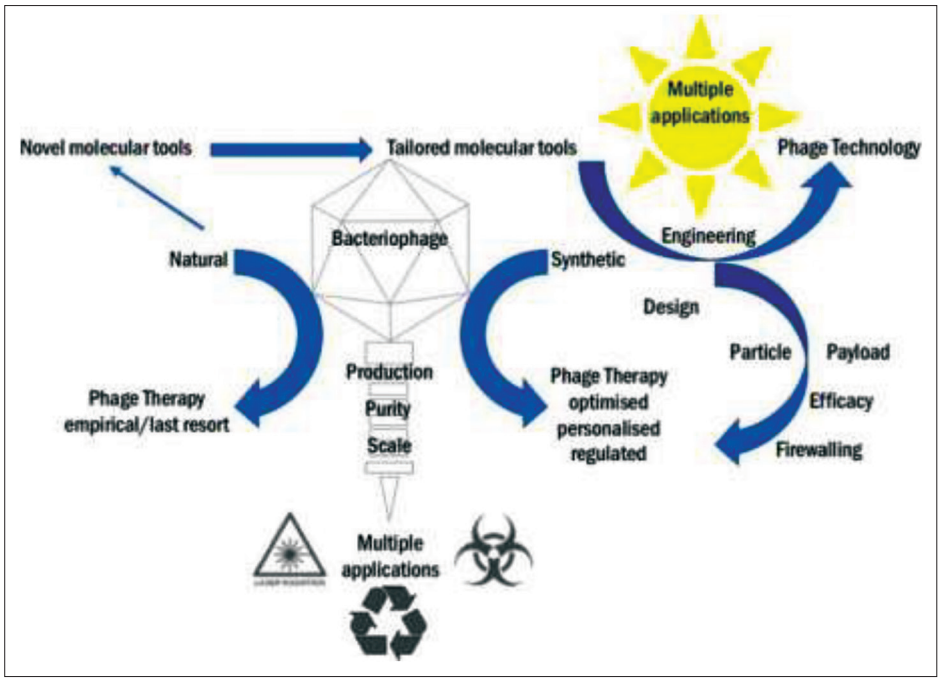

Figure 1. Bacteriophages can be envisaged as natural microbial control agents and machines for targeted synthetic genetic programming. The encoded proteins, as well as the structures of phages, offer a multitude of possibilities as outlined in this introductory chapter and detailed in the volume.
Slika 1. Bakteriofagi mogu služiti kao prirodni mikrobni kontrolni agenti, kao i nanomašine za targetirano sintetsko genetsko programiranje. Kodirani proteini, kao i struktura faga, nude mnogo mogućnosti, koje su opisane u uvodnom delu i daljem tekstu.

various molecular processes that take place in different cell environments. After entering the cell, the major envelope protein is removed from the phage particle and precipitated in the inner membrane of the host. Viral DNA enters the cell and converts to a double-stranded replicative form by the host enzyme. Progeny DNA is replicated by a cyclic mechanism, assembling with the gp5 protein to replicate and self-assemble the virus into an elongated intracellular nucleoprotein complex [5–8].

The replication of self-assembled protein (which covers and protects viral DNA inside the cell) is then replaced by envelope proteins (which cover and protect viral DNA outside the cell) in the cell membrane, where the virion is extruded through the membrane by viral and host proteins. The new envelope protein is synthesized as the envelope protein, which is the major envelope protein's precursor. It contains an additional leading amino acid sequence necessary for insertion into the cytoplasmic membrane. The envelope molecule is inserted into the membrane and then the additional leader sequence is cut off with the host peptidase. The resulting mature transmembrane protein coat is stored in the inner membrane before its use in the phage assembly process. Upon completion of the phage assembly, it is released into the medium and is ready to attack the new host cell. A key element in this whole process is the main envelope protein, which is responsible for protein-protein, protein-lipid and protein-DNA interactions during the assembly and disassembly of macromolecules [6–9].

The structure of the phage-bound coat protein has been known for many years, based on the results of fiber X-ray diffraction experiments: This structure is an almost perfect a-helix, with 4-5 flexible unstructured amino acid residues at the N-terminus exiting the phage sheath into the aqueous phase. The lysine-rich C terminus is linked to viral DNA phosphate groups, allowing phage particles to be elongated by simply inserting more DNA into the viral genome, with each protein coat subunit being approximated by a slightly curved α-helix measuring about 1 to 7 nm. Diffraction data show that the axis of the α-coil is set at a small angle to the axis of the virion, which is very precisely bounded by adjacent subunits. They form an overlapping spiral string with widely connected side chains. Envelope protein subunits possess continuous apolar domains that are held together in a virion by hydrophobic interactions between them [6–9].

The highly organized crystalline environment of the main envelope protein in the phage particle allows obtaining detailed structural and topological information about it. Based on NMR, the protein's determined structure showed that it possesses two helices in the lipid bilayer. The major coat protein is a single-coil monotopic protein that should be inserted into the membrane during the phage life cycle, have a stable thermodynamic connection to the membrane, and then leave the membrane during the assembly process. Assuming that the transmembrane coil is aligned with the normal lipid bilayer, then the L-shaped protein conformation is probably the best topology for the membrane-inserted protein due to optimal hydrophobic interactions. Because determining the topology of proteins in lipid bilayers is particularly important for virus self-assembly and for the ability of proteins to serve as a means of displaying peptides, new biophysical tools have been developed to study protein structure, topology, and lipid bilayer dynamics [5–9].

POTENTIAL PHAGES APPLICATIONS IN NANOTECHNOLOGIES

Phages could serve as probes in a new generation of real-time food safety control and environmental monitoring sensors. As detector elements, they are superior to polyclonal and monoclonal antibodies, because they are cheap, highly specific, selective and resistant to adverse environmental conditions. In addition, the unique structure of M13 bacteriophages has been used as a biological model for nanotechnology, such as the directed synthesis of semiconductor / magnetic nanowires and lithium-ion battery electrodes. Filamentous viruses enable the organization of various nanomaterials into periodically arranged hierarchical structures, such as viral rings and wires, which have an electronic, optical and biotechnological application. In materials science this approach is used to create new peptides that can bind to selected technical materials. Bacteriophages M13 with a protein coat, since they can bind to semiconductor and magnetic materials, were used as templates for the growth and organization of nanowires, serving as a template for the synthesizing of monocrystalline ZnS and CdS, and chemically arranged nanowires CoPt and FePt. The nuclear peptides incorporated into the M13 protein coat thus provide a pattern for the directed preparation of semiconductor and magnetic materials [4–7].

Some of their applications could be extremely important for tissue engineering and regenerative medicine, because such structures mimic the native extracellular matrix, which consists of a fibrous protein network and provides cells with physical support [5, 6, 7]. Recent findings indicate that the major coat proteins, which are the major building blocks of proteins, do not change their shape significantly during membrane self-organization, resulting in a very efficient mechanism for reducing energy expenditure. Any nanotechnology derived from M13 bacteriophages should take into account their basic physicochemical rules. Although many aspects of the biogeneration of nanostructures have not yet been comforted, it is expected that new advances in biophysical techniques will increase our molecular insight in the coming decades. If we understand that we are just at the beginning of a new era of bio-nanotechnology, it is clear that the future of nano-machines based on viruses is extremely bright [7, 8].

Large, multi-unit, protein complexes are initially assembled with weak interactions, which are very suitable for intracellular complexes because they are temporarily assembled and disassembled to activate mutually coordinated cellular functions. Virus particles, like other self-organizing systems, although initially formed by weak interaction, since part of their life cycle is extracellular, they must find a way of organization that guarantees them strong stability. Due to such conflicting requirements, the initial particle, usually called Procapsid or Provirion, matures over time, experiencing various conformational changes within the capsid. Some viruses, such as nodaviruses, undergo subtle autocatalytic cleavage of capsid subunits after leaving the cell, resulting in stabilizing virus particles and increasing their infectivity. In enveloped viruses, such as tetraviruses, there is a dramatic reorganization of the particle during maturation, resulting in smaller virus's autocatalytic cleavage [9, 10, 11].

DNA-BASED SYNTHETIC MOLECULAR MACHINES

DNA nano-machines are made by self-assembly, using techniques that rely on sequence-specific interactions that bind complementary oligonucleotides into a double helix. They are activated by interaction with certain signaling molecules or changes in their environment, triggering an appropriate response, to an external trigger, to serve as an intelligent molecularly sensitive drug delivery or controlled chemical synthesis. Biological molecular motors that carry cargo within cells have inspired the construction of rudimentary DNA walkers that stretch along self-installed pathways autonomously, gaining energy by catalyzing DNA or RNA fuel reactions [12, 13].

The exceptional specificity of interactions between complementary nucleotides makes DNA a useful building material, as due to the interaction between its short chains, it is possible to control the design of their basic sequences reliably. The construction of branched connections between the double helix enables the creation of complex three-dimensional objects. One way to take advantage of this extremely precise architectural control is to use self-assembled DNA patterns to position functional molecules, serving as molecular electronic circuits, optical devices, or enzyme networks. DNA is not a natural choice of material for building active structures, as it does not possess the structural and catalytic versatility of proteins and RNA, which are more suitable for that purpose [13, 14] (Figure 2).

MOLECULAR SWITCHES

The simplest active DNA nanostructures are switches or actuators, which act between two conformations, and their movement is stimulated by changes in temperature, ionic conditions or by binding of a signaling molecule, which is most often a DNA coil. Conformation changes are caused by changes in the environment, where the rotational movement is conditioned by a change in DNA twisting, in which double-stranded DNA with the sequence (CG) n is transferred from the usual right-handed helix (B-DNA) to the left-handed conformation (Z-DNA). These changes may be caused by high salt concentration and low temperature. Forster resonant energy transfer (FRET), which induced transition between the corresponding fluoro-phores and DNA helix separation on a nanometer scale with highly efficient energy transfer, mediated by dipole-dipole interaction, represents possible mechanism of the FRET based nano*machine [13–16]. Yang and co-workers converted the changes in DNA twisting into linear motion. Their device consisted of a closed loop of double-stranded DNA attached to the opposite arms of a four-legged holistic joint, whereby the holiday joint could migrate by breaking up identical base pairs in one pair of opposite arms and transforming them into another pair. The change in DNA conformation within the loop was initiated by the addition

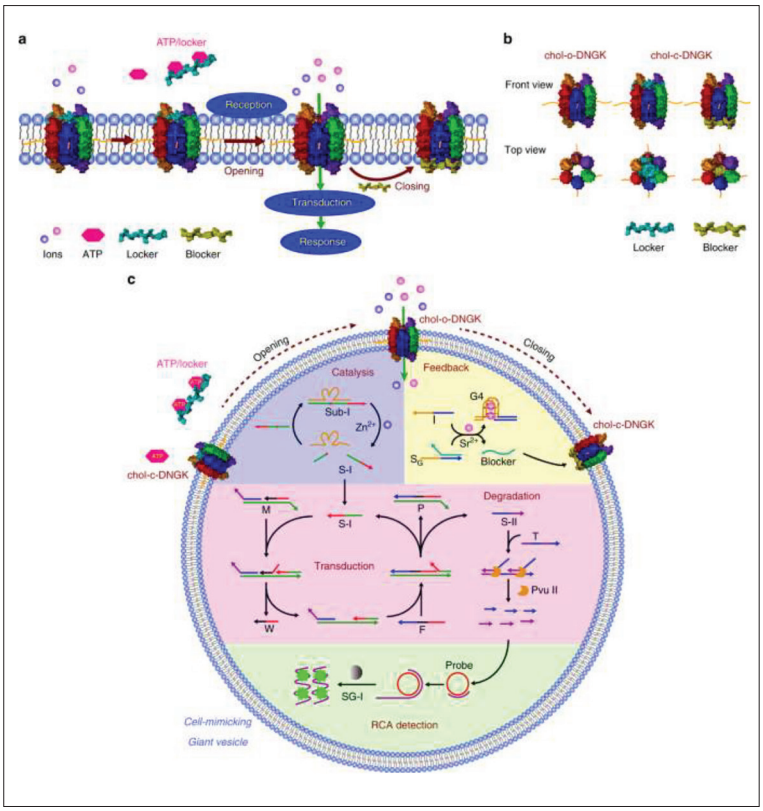

Figure 2. Biomimetic giant vesicle engineered for the construction of an artificial molecular signaling system (AMSsys)
Slika 2. Biomimetička velika vezikula stvorena za kreiranje veštačkog molekularnog signalnog sistema (AMSsys)

of ethidium bromide, which binds between adjacent base pairs, extending and partially unwinding the double helix. The resulting stress is alleviated by the migration of the node, ie the shortening of the protruding arms of the connections inside the loop, which allows its elongation without changing the total number of threads in it. A single chain of cytosine bends into an i-motif, a compact three-dimensional structure held by base pairs of protonated cytosines. In the presence of a second complementary strand of DNA, competition occurs between the i-motif and the elongated double helix formed by the hybridization of two strands [15, 16].

The transition of i-motif into a two-way transition produces mechanical work. If one surface of the silicon cantilever is coated with bonded coils containing cytosine, then a compressive surface stress bends that bends the cantilever. Such stress due to hybridization of the complementary coil with the cantilever is not expected, the effect is present at high salt concentrations, at which the interactions at a distance are comparable to the observed separation of the coils. Conformational change caused by pH-dependent binding of a single strand of DNA to a duplex to form a triple-helical structure, which is the basis of the design of a nano-mechanical actuator (Figure 2). Active DNA nanostructures are also evolving as sensors, which allow elementary logic operations to be performed on their outputs,

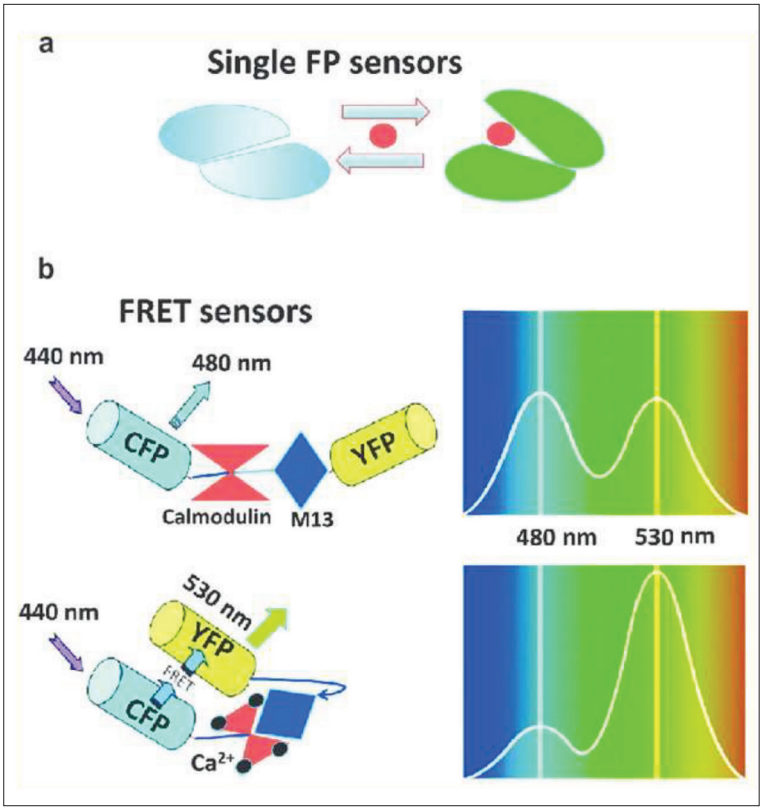

Figure 3. Basic principles of biosensors organization: (a) Single FP-based sensors consist of a protein changes its conformation and luminosity for specific binding to the ligand; (b) FRET sensors consist of two fluorescent proteins, connected by a linker containing in some cases a ligand receptor. In the absence of ligand, fluorescent proteins are far from each other and light releasing by the first protein does not excite the second one. Upon binding of the ligand linker changes its conformation and proteins come together, as a result, the radiation from the first protein excites the second one and its luminosity increases.
Slika 3. Osnovni principi organizacije biosenzora: a) Pojedinačni FP bazirani senzori sastoje se od proteina koji menja svoju konformaciju i luminoznost pri specifičnom vezivanju za ligand;
b) FRET senzori sastoje se od dva fluorescentna proteina, koja su vezana linkerom, u nekim slučajevima ligandom receptora. U odsustvu liganda, fluorescentni proteini su daleko jedan od drugog i oslobađanje svetlosti od strane jednog proteina ne rezultuje ekscitacijom drugog. Nakon vezivanja liganda, linker menja svoju konformaciju i proteini se vezuju. Kao rezultat, radijacija prvog proteina ekscitira drugi i povećava se njegova luminoznost.

which in combination with computers, can be applied to design smart drug delivery systems [15, 16, 17].

CONCLUSIONS

The bacteriophage M13 protein coat is described as the extremely inspiring in nano-machine manufacturing, because it allows the synthesis of particles of the desired functionality, assuming that no basic biophysical principle is violated, especially the ability of the protein to form a stable symmetrical coating and the ability of the protein to insert, anchor and assemble into a new particle virus. This technology is evolving on its own, so it is expected that not only phages but also others similar viruses like smart machines can produce new proteins that could improve the technology itself. This paper also described DNA-based nano-machines, as potential actuators or switches and for intelligent molecularly sensitive drug delivery systems or systems for controlled chemical synthesis.

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Virusi kao potencijalne nanomašine

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KRATAK SADRŽAJ

U ovom radu virusi su predstavljeni kao vrlo efikasne nanomašine koje proizvode brojne sopstvene kopije. Posmatrajući ove nanoarhitekture, postavlja se pitanje koje molekularne sile i procesi čine skup takvih struktura, s obzirom na to da su izuzetno inspirativne za razvoj novih tehnologija na nanonivou. Potrebno je duboko razumevanje pojedinačnih molekularnih gradivnih blokova i njihovih struktura, svojstava njihovih sklopova i dinamičkog ponašanja.

Ključne reči: virusi; bakteriofagi; DNK nanomašine

UVOD

Visoka efikasnost terapije zasnovane na bakteriofagima u suzbijanju kolonija mnogih rezistentnih bakterija, kao što je to slučaj sa cističnom fibrozom pluća koja izaziva vrlo ozbiljnu infekciju, učinila je bakteriofage jednim od najvažnijih novih dostignuća u molekularnoj biologiji, biofizici i bionanotehnologiji. Filamentozni virusi, kao što su bakteriofagi M13, imaju virionsku arhitekturu koja im omogućava preciznu izgradnju uređenih dvodimenzionalnih i trodimenzionalnih struktura bez oštećenja na nivou nanometarskih dimenzija. To ne bi bilo moguće bez detaljnog poznavanja strukture i dinamike proteinskog omotača tokom ciklusa razmnožavanja virusa. Rezultati spektroskopskih studija pokazuju kritičnu ulogu ugradnje proteina u membranu, kako tokom infektivnog ulaska virusa u ćeliju domaćina, tako i tokom sklapanja novog viriona u membrani domaćina. Protein se efikasno ugrađuje u membranu zahvaljujući snažnom C-terminalnom interfacijalnom sidru, koje jednostavnim mehanizmom nagiba i suptilnim strukturnim prilagođavanjem na samom kraju svog N-terminusa obezbeđuje povoljno termodinamičko pridruživanje proteina u lipidnom dvosloju [1, 2, 3].

Virusi, posebno bakterijski ili bakteriofagi, igraju ključnu ulogu u kontroli bioloških sistema. Napredak u molekularnoj biologiji tokom poslednjih 50 godina bazira se velikim delom na proučavanju bakteriofaga. Restrikcijske endonukleaze, koje čine osnovu molekularnog kloniranja, razvijene su nakon studija o infekciji fagom. Mnogi enzimi faga pružaju molekularnom biologu alate koji pomažu u proučavanju puteva replikacije, transkripcije, translacije i transporta. Tehnika prikazivanja faga im pruža moćnu metodologiju za identifikaciju i optimizaciju liganda antitela i drugih biomolekula. Savremena primena bakteriofaga u inženjerskim materijalima stavlja ih u prvi plan kod novih nanotehnoloških dostignuća [3, 4, 5].

OSNOVNA RAZMATRANJA

Vlaknasti bakteriofagi u savremenoj biofizici služili su kao model sistema za razvoj i primenu spektroskopskih metoda pogodnih za biološke supramolekularne sklopove. Proteini ovojnice filamentnih faga, koji se lako mogu sintetisati u laboratoriji, imaju dve primarne uloge tokom ciklusa replikacije, kao membranski proteini i kao glavni strukturni elementi čestica faga, pokazujući tako začuđujuću mogućnost da se nađu dve tako različite uloge u bakteriofagnom ciklusu replikacije. Saznanja o strukturama proteina u različitim okruženjima omogućavaju nam da u potpunosti razumemo njihove najnovije nanotehnološke primene u nanobiologiji i bionanotehnologiji.

M13 bakteriofag uzrokuje hronične infekcije, pri čemu zaražene ćelije nastavljaju da rastu i da se dele, mada sa nižom stopom od normalne. Fag je dugačka čestica u obliku niti, prečnika 6,5 nm i dužine 900 nm. Fleksibilna nit sadrži kružni, jednolančani virusni DNK genom, zaštićen dugim cilindričnim proteinskim omotačem. Glavni proteini omotača čine cev oko virusne DNK, i u preklopnom spiralnom nizu su orijentisani tako da se N kraj nalazi na spoljnoj strani omotača, a C završava vezujući se sa DNK u unutrašnjosti omotača. Hidrofobni domen glavnog proteinskog omotača se nalazi u centralnom delu proteina, i povezuje omotač proteina sa susednim proteinskim omotačem formirajući virusne čestice. Tokom reprodukcije glavni protein omotača uključen je u različite molekularske procese koji se odvijaju u različitim okruženjima ćelije. Nakon ulaska u ćeliju, glavni protein omotača se uklanja iz čestice faga i taloži u unutrašnjoj membrani domaćina. Virusna DNK ulazi u ćeliju i pretvara se u dvolančani replikativni oblik od strane enzima domaćina. DNK se replicira cikličnim mehanizmom, pri čemu se sastavlja sa proteinom gp5 za replikaciju i samoasembliranje virusa u izduženi unutarćelijski nukleoproteinski kompleks [5–8].

Replikaciono-samoasemblirani protein (koji pokriva i štiti virusnu DNK unutar ćelije) zatim se zamenjuje proteinima omotača (koji pokrivaju i štite virusnu DNK izvan ćelije) u ćelijskoj membrani, gde se virion istiskuje kroz membranu uz pomoć virusnih proteina i proteina domaćina. Novi protein se sintetiše kao protein omotača, koji je prethodnik glavnog proteina omotača. On sadrži dodatnu vodeću sekvencu aminokiselina neophodnu za umetanje u citoplazmatsku membranu. Molekul omotača se ubacuje u membranu i potom se dodatna vodeća sekvenca odseca peptidazom domaćina. Nastali zreli transmembranski proteinski omotač se skladišti u unutrašnjosti membrane pre njegove uloge u procesu faga. Nakon kompletiranja faga, on se oslobađa u medijum i spreman je za napad na novu ćeliju domaćina. Ključni element u celom ovom procesu je glavni protein omotača, koji je odgovoran za interakcije protein-protein, protein-lipid i protein-DNA tokom sastavljanja i rastavljanja makromolekula.

Struktura proteina omotača vezanog za fage poznata je već dugi niz godina, na osnovu rezultata eksperimenata rendgenske

difrakcije vlakana. Ova struktura je gotovo savršena a-spirala, na čijim se krajevima nalazi 4-5 fleksibilnih nestrukturiranih aminokiselinskih ostataka koji na N kraju izlaze iz omotača faga u vodenu fazu. C-terminus bogat lizinom povezan je sa virusnim DNK fosfatnim grupama i omogućava izduživanje čestice faga jednostavnim umetanjem više DNK u virusni genom, pri čemu se svaka podjedinica proteinskog omotača može aproksimirati pomoću jedne blago zakrivljene a-spirale dimenzija oko 1 do 7 nm. Podaci difrakcije pokazuju da je osa a-spirale postavljena pod malim uglom u odnosu na osu viriona, koji je vrlo precizno ograničen susednim podjedinicama. One čine preklapajući spiralni niz sa široko povezanim bočnim lancima. Podjedinice proteina omotača poseduju kontinuirane apolarne domene koji se drže se zajedno u virionu hidrofobnim interakcijama među njima [6–9].

Visoko organizovano kristalno okruženje glavnog proteina omotača u čestici faga omogućava dobijanje detaljnih strukturnih i topoloških informacija o njemu. Na osnovu NMR je pokazano da određena struktura proteina poseduje dve zavojnice u dvosloju lipida. Glavni protein omotača je monotopični protein koji pokriva membranu jednom zavojnicom i koji se tokom životnog ciklusa faga umeće u membranu; on ima stabilnu termodinamičku vezu sa membranom, koju napušta tokom procesa sklapanja. Ako se pretpostavi da je transmembranska zavojnica poravnata sa normalnim lipidnim dvoslojem, tada je konformacija proteina u obliku slova L verovatno najbolja topologija za membranski umetnuti protein zbog optimalnih hidrofobnih interakcija. Budući da je određivanje topologije proteina u dvoslojevima lipida posebno važno za samoasembliranje virusa i za sposobnost proteina da služi kao sredstvo za prikazivanje peptida, razvijeni su novi biofizički alati za proučavanje strukture proteina, topologije i dinamike dvosloja lipida [5–9].

POTENCIJALNA PRIMENA FAGA U NANOTEHNOLOGIJAMA

Fagi bi mogli da posluže kao sonde u novoj generaciji senzora za kontrolu bezbednosti hrane i nadzor nad životnom sredinom u realnom vremenu. Kao elementi detektora, oni su superiorniji od poliklonalnih i monoklonskih antitela, jer su jeftini, visoko specifični, selektivni i otporni na nepovoljne uslove okoline. Pored toga, jedinstvena struktura M13 bakteriofaga je iskorišćena kao biološki obrazac u nanotehnologiji, u usmerenoj sintezi poluprovodničkih / magnetnih nanožica i litijum-jonskih baterijskih elektroda. Filamentozni virusi omogućavaju organizaciju različitih nanomaterijala u periodično uređene hijerarhijske strukture, poput virusnih prstenova i žica, koje imaju elektronsku, optičku i biotehnološku primenu. U nauci o materijalima ovaj pristup se koristi za stvaranje novih peptida koji se mogu vezati za odabrane tehničke materijale. Bakteriofagi M13 sa proteinskim omotačem, budući da mogu da se vežu za poluprovodničke i magnetne materijale, korišćeni su kao predlošci za rast i organizovanje nanožica, služeći kao templejt za sintezu monokristalnih ZnS i CdS, i hemijski uređenih nanožica CoPt i FePt. Nuklearni peptidi ugrađeni u proteinski omotač M13 na taj način pružaju obrazac za usmerenu pripremu poluprovodničkih i magnetnih materijala [4–7].

Njihova primena mogla bi biti izuzetno značajna za tkivno inženjerstvo i regenerativnu medicinu, jer takve strukture imitiraju nativni ekstracelularni matriks, koji se sastoji od vlaknaste proteinske mreže i pruža ćelijama fizičku potporu [5, 6, 7].

Nedavna otkrića ukazuju da glavni proteini omotača, koji su glavni gradivni blokovi proteina, ne menjaju značajnije svoj oblik tokom membranske samoorganizacije, što ima za posledicu vrlo efikasan mehanizam za smanjenje potrošnje energije. Svaka nanotehnologija koja se bazira na bakteriofagu M13 treba da uzme u obzir njihova osnovna fizičko-hemijska pravila. I mada još uvek nisu rešeni mnogi aspekti biogeneracije nanostruktura, treba očekivati da će u narednim decenijama napredak u biofizičkim tehnikama povećati naša molekularna saznanja. Ako shvatimo da smo tek na početku nove ere bionanotehnologije, jasno je da je budućnost nanomašina na bazi virusa izuzetno svetla [7, 8].

Veliki, multijedinični, proteinski kompleksi se u početku spajaju slabim vezama, koje su vrlo pogodne za unutarćelijske komplekse, jer se privremeno sastavljaju i rastavljaju kako bi se aktivirale međusobno usklađene ćelijske funkcije. Čestice virusa, kao i drugi samoorganizujući sistemi, u početku se formiraju slabom vezom i, budući da je deo njihovog životnog ciklusa vanćelijski, oni moraju pronaći način organizacije koji im garantuje snažnu stabilnost. Zbog tako suprotstavljenih zahteva početna čestica koja se obično naziva Prokapsid ili Provirion vremenom sazreva, doživljavajući različite konformacione promene unutar kapsida. Neki virusi, kao nodavirusi, podvrgavaju se suptilnom autokatalitičkom cepanju kapsidnih podjedinica nakon izlaska iz ćelije, što dovodi do stabilizacije čestica virusa i povećanja njihove infektivnosti. Kod virusa bez ovojnice, poput tetravirusa, dramatično se reorganizuje čestica tokom sazrevanja, pri čemu dolazi do autokatalitičkog cepanja manjeg virusa [9, 10, 11].

SINTETIČKE MAŠINE ZASNOVANE NA DNK

DNK nanomašine se izrađuju samosastavljanjem, korišćenjem tehnika koje se oslanjaju na interakcije specifične za sekvence koje vezuju komplementarne oligonukleotide u dvostruku spiralu. Aktiviraju se interakcijom sa određenim signalnim molekulima ili promenama u njihovom okruženju, izazivajući odgovarajući odgovor na spoljni okidač, i služe za inteligentnu molekularno osetljivu isporuku leka ili kontrolisanu hemijsku sintezu. Biološki molekularni motori koji prenose teret unutar ćelija inspirisali su izgradnju rudimentarnih DNK šetača koji se protežu duž samoinstaliranih staza autonomno, dobijajući energiju katalizom reakcija DNK ili RNK [12, 13].

Izuzetna specifičnost interakcija između komplementarnih nukleotida čini DNK korisnim gradivnim materijalom, jer je interakcijom između njenih kratkih lanaca moguće pouzdano kontrolisati dizajniranje njihovih osnovnih sekvenci. Izgradnja razgranatih veza između dvostrukog heliksa omogućava stvaranje kompleksnih trodimenzionalnih objekata. Jedan od načina da se iskoristi ova izuzetno precizna arhitektonska kontrola je upotreba samoasembliranih DNK obrazaca za pozicioniranje funkcionalnih molekula, koji mogu da posluže kao molekularna elektronska kola, optički uređaji ili enzimske mreže. DNK nije prirodni izbor materijala za izgradnju aktivnih struktura, jer ne poseduje strukturnu i katalitičku svestranost proteina i RNK, koji su pogodniji za tu svrhu [13, 14].

MOLEKULARNI PREKIDAČI

Najjednostavnije aktivne DNK nanostrukture su prekidači ili aktuatori, koji deluju između dve konformacije, pri čemu je njihovo pomeranje podstaknuto promenama temperature, jonskih uslova ili vezivanjem signalnog molekula, koji najčešće predstavlja DNK zavojnica. Promene konformacije su izazvane promenama u okruženju, pri čemu je rotaciono kretanje uslovljeno promenom uvijanja DNK, pri kome se dvolančana DNK sa sekvencom (CG) n prebacuje iz uobičajene desnoruke zavojnice (B-DNK) u levoruku konformaciju (Z-DNK), u uslovima visoke koncentracije soli i niske temperature. Forsterov rezonantni prenos energije (FRET), koji predstavlja mogući mehanizam jedne takve biomašine, obezbeđuje prenos između odgovarajućih fluorofora i DNK zavojnica na nanometarskoj skali uz posredovanje dipol-dipol interakcije [13, 14, 15].

Jang i saradnici su pretvorili promene pri uvijanju DNK u linearno kretanje. Njihov uređaj se sastojao od zatvorene petlje dvolančane DNK pričvršćene na suprotne krakove četvorokrakog Holidejevog spoja, pri čemu Holidejev spoj može migrirati (izomerizirati) razbijanjem identičnih parova baza u jednom paru suprotnih krakova i njihovom transformacijom u drugi par. Promena konformacije DNK unutar petlje inicirana je dodavanjem etidijum-bromida, koji se veže između susednih baznih parova, produžavajući i delimično odmotavajući dvostruku spiralu. Rezultujući stres se ublažava migracijom čvora, odnosno skraćivanjem izbočenih krakova veza unutar petlje, što dopušta njeno izduženje bez promene ukupnog broja navoja u njoj. Ekološke promene u konformaciji jednolančane DNK mogu izazvati linearno kretanje, tako što se u blago kiselim uslovima jedan lanac citozina savija u i-motiv, kompaktnu trodimenzionalnu strukturu koju drže parovi baza protoniranih citozina. U prisustvu drugog komplementarnog lanca DNK javlja se konkurencija između i-motiva i produžene dvostruke zavojnice nastale hibridizacijom dve zavojnice [15, 16].

Prelazak i-motiva u dvostrani prelaz proizvodi mehanički rad. Ako se jedna površina silicijumske konzole presvuče vezanim zavojnicama koje sadrže citozin, tada dolazi do kompresivnog površinskog naprezanja koje savija konzolu. Takvo naprezanje uslovljeno hibridizacijom komplementarne zavojnice sa konzolom nije očekivano, iako je taj efekat prisutan pri visokim koncentracijama soli, gde su interakcije na daljini uporedive sa uočenim razdvajanjem zavojnica. Konformaciona promena izazvana pH zavisnim vezivanjem pojedinačnog lanca DNK u dupleks i formiranje trostruko-spiralne strukture je osnova dizajna nanomehaničkog aktuatora. Aktivne DNK nanostrukture se razvijaju kao senzori, koji omogućavaju da se elementarne logičke operacije izvode na njihovim izlazima, koji u kombinaciji sa računarima mogu biti primenjeni za dizajniranje pametnih sistema za isporuku lekova [15, 16, 17].

ZAKLJUČCI

Proteinski omotač bakteriofaga M13 opisan je kao izuzetno inspirativan u proizvodnji nanomašina, jer omogućava sintezu čestica željene funkcionalnosti, pod pretpostavkom da nije povređen nijedan osnovni biofizički princip, a posebno sposobnost proteina da formira stabilnu simetričnu oblogu i da se ubaci, usidri i sklopi u novu česticu virusa.

Ova tehnologija se razvija samostalno, pa se očekuje da ne samo fagi već i drugi slični virusi poput pametnih mašina mogu da proizvode nove proteine koji bi mogli poboljšati samu tehnologiju.

Opisane nanomašine zasnovane na DNK mogu poslužiti kao potencijalni pokretači ili prekidači za inteligentne molekularno osetljive sisteme za isporuku lekova ili sisteme za kontrolisanu hemijsku sintezu.