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Research article

Dynamics analysis of building block synthesis reactions for virus assembly in vitro

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Abstract: Virus assembly from structural protein monomers to virus shells is a key step of virus replication. Some drug targets were found in this process. It consists of two steps. Virus structural protein monomers firstly polymerize to building blocks, then these building blocks assemble into virus shells. So, these building block synthesis reactions in the first step are fundamental for virus assembly. Typically, virus building blocks are made up of less than six monomers. They are of five types, including dimer, trimer, tetramer, pentamer and hexamer. In this work, we develop five synthesis reaction dynamical models for these five types, respectively. Then, we prove the existence and uniqueness of the positive equilibrium solution for these dynamical models one by one. Subsequently, we also analyze the stability of the equilibrium states, respectively. We got the function of monomer and dimer concentrations for dimer building blocks in the equilibrium state. We also got the function of all intermediate polymers and monomers for trimer, tetramer, pentamer and hexamer building blocks in the equilibrium state, respectively. Based on our analysis, dimer building blocks in the equilibrium state will decrease as the ratio of the off-rate constant to the on-rate constant increases. Trimer building blocks in the equilibrium state will decrease with the increasing ratio of the off-rate constant to the on-rate constant of trimers. These results may provide further insight into the virus-building block synthesis dynamic property in vitro.

Keywords: building block; synthesis dynamics; virus structural protein

1. Introduction

A virus is a small collection of genetic materials, either DNA or RNA, which replicates only

inside living host cells of an organism. They infect all life forms, from animals and plants to microorganisms. As a host cell is infected, it is often forced to rapidly produce large batches of virus copies. Often, they kill the host cell in the replication process, and cause damage to the host organism. Some viruses can be carried by blood-sucking insects. Some viruses, including influenza viruses, chickenpox and smallpox, spread in the air by coughing and sneezing [1,2]. As is known to us all, the novel coronavirus (SARS-CoV-2) is a super virus, which has spread around the world [3]. Therefore, many researchers have tried to understand the replication mechanism of viruses to combat the spreading of viruses.

Virus assembly is a necessary step of virus replication. It usually consists of two processes. The first process is that structural proteins polymerize to building blocks. Then these building blocks assemble into virus shells. The first process is the basis and essential precondition of the second process. The aim of the first process is to form building blocks. These building blocks are often low polymers with no more than six monomers. For example, dimers are the building blocks of Moloney murine leukemia virus capsid [4]. SARS-CoV-2 spike proteins are trimers [5]. The building blocks of Mavirus are the trimeric major capsid protein [6]. The building blocks of the Hepatitis B virus capsid protein are tetramers [7]. Pentamers are the building blocks of picornavirus [7]. Hexamers are the main building blocks for HIV-1 immature capsid [8,9]. Hexamers and pentamers are the fundamental building blocks of the Rous sarcoma virus capsid [10].

Many biologists and pharmacists focus on the synthesis reactions of virus building blocks. A general approach to design 2D arrays was presented to study modular self-assembly of biomolecules by using de novo carried pseudosymmetric protein building blocks [11]. Wang and Hou [12] analyzed drug candidate building blocks to study designing drug-like compounds. Christiansen et al. [6] studied the dynamic mechanism of dissociation and re-association of the trimeric capsid building blocks. Rong and Ying [13] used building blocks to construct a functional spherical artificial virus, and mimicked the intricate morphology and the intracellular transformation of spherical viruses. Chen et al. [14] found that Zika virus induced metabolic alterations may provide building blocks for lipid droplet biogenesis and intracellular membrane rearrangements to support its replication.

However, there is not a system study on polymerization dynamic analysis of all kinds of building blocks. So, in this work, we will focus on synthesis reactions in vitro for all kinds of building blocks, including dimers, trimers, tetramer, pentamer and hexamer. Rate equations for these synthesis reactions will be developed. Then we will prove the existence and uniqueness of the positive equilibrium solution of these rate equations. Dynamic analysis of them will be carry on one by one. These results will be helpful to guide future experiments and improve the understanding of the synthesis reactions of building blocks in vitro.

2. Materials and methods

2.1. Dimer building block synthesis reaction

We consider that dimers are the building blocks of virus assembly in vitro. A dimer is polymerized by two monomers with a reaction on-rate constant. At the same time, a dimer is dissociated into two monomers with a reaction off-rate constant. Their chemical reaction is as follows.

$$M + M \underset{k_1^-}{\overset{k_1^+}{\rightleftharpoons}} M_2,$$

where M is the monomer, M_2 is the dimer, k_1^+ is the on-rate constant, $k_1^+ > 0$, k_1^- is the off-rate constant, $k_1^- > 0$.

Based on the mass conservation law and mass action law [15,16], we obtain the following rate equation system for the above chemical reaction in vitro.

$$\frac{d[M]}{dt} = -2k_1^+[M]^2 + 2k_1^-[M_2], \tag{1}$$

where $[M]$ and $[M_2]$ are the concentrations of M and M_2, respectively.

We consider that the above synthesis reaction takes place in vitro. Monomers with concentration $C_0, (C_0 > 0)$ are added to the vitro for only one time, and no dimers are added. So, the initial condition is as follows.

$$[M] = C_0, [M_2] = 0.$$

As the synthesis reaction takes place, two monomers are polymerized to a dimer. So, the concentration of dimers is equivalent to the concentration of monomers with twice their own concentration. Moreover, no monomers and no dimers will be added after the synthesis reaction takes place. So, the constrain condition is as follows.

$$2[M_2]+[M] = C_0.$$

Theorem 1. The positive equilibrium point of dimer building block synthesis reaction System (1) exists and is unique.

Proof of Theorem 1. Let $\frac{d[M]}{dt} = 0$ for Eq (1), we get

$$-2k_1^+[M]^2 + 2k_1^-[M_2]=0. \tag{2}$$

We can get the following equation from Eq (2).

$$[M_2]=\frac{k_1^+}{k_1^-}[M]^2. \tag{3}$$

Substitute the above Eq (3) to the constrain condition, we get

$$2\frac{k_1^+}{k_1^-}[M]^2+[M]-C_0=0.$$

The positive equilibrium point of monomer is as follows.

$$[M]=\frac{-1+\sqrt{1+8C_0\frac{k_1^+}{k_1^-}}}{4\frac{k_1^+}{k_1^-}}. \tag{4}$$

Add the Eq (4) to the Eq (3), we get

$$[M_2]=\frac{\left(-1+\sqrt{1+8C_0\frac{k_1^+}{k_1^-}}\right)^2}{16\frac{k_1^+}{k_1^-}}. \tag{5}$$

Therefore, based on the theorem [17], the positive equilibrium point of dimer building block synthesis reaction system (1) exists and is unique.

Remark 1. The above theorem indicates that the proposed model (1) can reflect the following biological fact: dimer building block synthesis reaction can achieve an equilibrium state and the equilibrium state is unique in vitro.

Theorem 2. The dimer building block synthesis reaction system (1) is stable.

Proof of Theorem 2. We can get the following equation by substituting the constrain condition to Eq (1).

$$\frac{d[M]}{dt}=-2k_1^+[M]^2-k_1^-[M]+k_1^-C_0. \tag{6}$$

Let $F([M])=-2k_1^+[M]^2-k_1^-[M]+k_1^-C_0$, $F'([M])=-4k_1^+[M]-k_1^-<0$.

Therefore, based on the theorem [17], the dimer building block synthesis reaction systems (1) is stable.

Remark 2. Small changes for the initial concentrations of monomers and dimers do not affect the concentrations of monomers and dimers in the equilibrium state.

Theorem 3. As $\frac{k_1^-}{k_1^+}$ increases, $[M]$ will increase in the equilibrium state, but $[M_2]$ will decrease.

Proof of Theorem 3. Let $K=\frac{k_1^-}{k_1^+}$, we can get the following equation based on Eq (4)

$$[M]=\frac{-k_1^-+\sqrt{(k_1^-)^2+8k_1^-k_1^+C_0}}{4k_1^+}=\frac{1}{4}\left(-K+\sqrt{K^2+8KC_0}\right).$$

$$\frac{d[M]}{dK}=\frac{1}{4}\left(-1+\frac{K+4C_0}{\sqrt{K^2+8KC_0}}\right)=\frac{1}{4}\left(\frac{-\sqrt{K^2+8KC_0}+K+4C_0}{\sqrt{K^2+8KC_0}}\right).$$

For

$$\frac{K+4C_0}{\sqrt{K^2+8KC_0}}=\sqrt{\frac{(K+4C_0)^2}{K^2+8KC_0}}=\sqrt{\frac{K^2+8C_0K+16C_0^2}{K^2+8C_0K}}>1.$$

$$\frac{d[M]}{dK}>0.$$

Therefore, as $\frac{k_1^-}{k_1^+}$ increases, $[M]$ will increase in the equilibrium state.

Because $[M_2]=\frac{C_0}{2}-\frac{1}{2}[M]$, $[M_2]$ will decrease.

Remark 3. This theorem reveals that dimer building blocks in the equilibrium state will decrease as the ratio of the off-rate constant to the on-rate constant increases.

2.2. Trimer building block synthesis reactions

We consider that trimers are the building blocks of virus assembly in vitro. Two monomers are polymerized to a dimer with a reaction on-rate constant. At the same time, a dimer is dissociated into two monomers with a reaction off-rate constant. A monomer and a dimer are polymerized into a trimer with a reaction on-rate constant. At the same time, a trimer is dissociated to a monomer and a dimer with a reaction off-rate constant. Their synthesis reactions are as follows.

$$M + M \underset{k_1^-}{\overset{k_1^+}{\rightleftharpoons}} M_2,$$

$$M_2 + M \underset{k_2^-}{\overset{k_2^+}{\rightleftharpoons}} M_3,$$

where M_i is the protein with *i* monomers, k_i^+ is the on-rate constant, $k_i^+ > 0$, k_i^- is the off-rate constant, $k_i^- > 0$.

The concentration of monomers will decrease for they polymerize to dimers. Moreover, they will also decrease for they polymerize to trimers with dimers. Based on the mass conservation law and mass action law [15,16], we get

$$\frac{d[M]}{dt} = -2k_1^+[M]^2 - k_2^+[M][M_2].$$

At the same time, the concentration of monomers will increase for dimers dissociate to monomers and trimers also dissociate to monomers. Based on the mass conservation law and mass action law [15,16], we get the total change rate of monomers as follows.

$$\frac{d[M]}{dt} = -2k_1^+[M]^2 - k_2^+[M][M_2] + 2k_1^-[M_2] + k_2^-[M_3].$$

With the same method, we can get the change rates of dimers and trimers. So, the rate equation system for the above chemical reactions in vitro is as follows.

$$\begin{cases} \frac{d[M]}{dt} = -2k_1^+[M]^2 - k_2^+[M][M_2] + 2k_1^-[M_2] + k_2^-[M_3] \\ \frac{d[M_2]}{dt} = k_1^+[M]^2 + k_2^-[M_3] - k_1^-[M_2] - k_2^+[M][M_2] \\ \frac{d[M_3]}{dt} = k_2^+[M][M_2] - k_2^-[M_3] \end{cases} \tag{7}$$

where $[M_i]$ are the concentration of M_i.

We consider that the above synthesis reaction takes place in vitro. Monomers with concentration $C_0, (C_0 > 0)$ are added to the vitro for only one time, and no dimers and no trimers are added. So, the initial condition is as follows.

$$[M]=C_0,[M_2]=0,[M_3]=0. \tag{8}$$

As the synthesis reaction takes place, two monomers are polymerized to a dimer. So, the concentration of dimers is equivalent to the concentration of monomers with twice their own concentration. Similarly, the concentration of trimers is equivalent to the concentration of monomers with treble their own concentration. Moreover, no monomers, no dimers and no trimers will be added after the synthesis reaction takes place. So, the constraint condition is as follows.

$$[M]+2[M_2]+3[M_3]=C_0. \tag{9}$$

Theorem 4. The positive equilibrium point of trimer building block synthesis reaction system (7) exists and is unique.

Proof of Theorem 4. Let $\frac{d[M_i]}{dt}=0,\ i=1,2,3$ for system (7), we get

$$\begin{cases} -2k_1^+[M]^2-k_2^+[M][M_2]+2k_1^-[M_2]+k_2^-[M_3]=0 \\ k_1^+[M]^2+k_2^-[M_3]-k_1^-[M_2]-k_2^+[M][M_2]=0 \\ k_2^+[M][M_2]-k_2^-[M_3]=0 \end{cases} \tag{10}$$

Add the third equation to the second equation in Eq (10), we get

$$[M_2]=\frac{k_1^+}{k_1^-}[M]^2. \tag{11}$$

Substitute the above equation to the third equation, we get

$$[M_3]=\frac{k_2^+}{k_2^-}[M][M_2]=\frac{k_2^+}{k_2^-}\frac{k_1^+}{k_1^-}[M]^3. \tag{12}$$

Substitute $[M_2]$ and $[M_3]$ to the constraint condition (9), then we get

$$[M]+2\frac{k_1^+}{k_1^-}[M]^2+3\frac{k_2^+}{k_2^-}\frac{k_1^+}{k_1^-}[M]^3=C_0.$$

$$3\frac{k_2^+}{k_2^-}\frac{k_1^+}{k_1^-}[M]^3+2\frac{k_1^+}{k_1^-}[M]^2+[M]-C_0=0.$$

Let

$$f([M])=3\frac{k_2^+}{k_2^-}\frac{k_1^+}{k_1^-}[M]^3+2\frac{k_1^+}{k_1^-}[M]^2+[M]-C_0.$$

When $[M]=0$,

$$f(0)=-C_0<0.$$

When $[M]=C_0$,

$$f(C_0)=3\frac{k_2^+}{k_2^-}\frac{k_1^+}{k_1^-}(C_0)^3+2\frac{k_1^+}{k_1^-}(C_0)^2+C_0-C_0>0.$$

Moreover,

$$f'([M])=9\frac{k_2^+}{k_2^-}\frac{k_1^+}{k_1^-}[M]^2+4\frac{k_1^+}{k_1^-}[M]+1>0,\quad \text{for } [M]\in[0,C_0].$$

Therefore, there is a unique positive point $[M^*]\in(0,C_0)$ to satisfy $f([M^*])=0$.

Substitute $[M^*]$ to Eqs (11) and (12), we can get the positive values $[M_2^*]$ and $[M_3^*]$.

Therefore, based on the theorem [17], the positive equilibrium point of trimer building block synthesis reaction System (7) exists and is unique.

Theorem 5. The trimer building block synthesis reaction system (7) is locally asymptotic stable.

Proof of Theorem 5. We can get $[M_3]=\frac{1}{3}(C_0-[M]-2[M_2])$ from the constraint condition (9). Then we substitute it to the first two equations of System (7) and get

$$\begin{cases}\dfrac{d[M]}{dt}=-2k_1^+[M]^2-k_2^+[M][M_2]+2k_1^-[M_2]+\dfrac{k_2^-}{3}(C_0-[M]-2[M_2])\\ \dfrac{d[M_2]}{dt}=k_1^+[M]^2+\dfrac{k_2^-}{3}(C_0-[M]-2[M_2])-k_1^-[M_2]-k_2^+[M][M_2]\end{cases}\tag{13}$$

The characteristic equation of the above equations is as follows

$$\begin{vmatrix}-4k_1^+[M]-k_2^+[M_2]-\dfrac{k_2^-}{3}-\lambda & -k_2^+[M]+2k_1^--\dfrac{2k_2^-}{3}\\ 2k_1^+[M]-\dfrac{k_2^-}{3}-k_2^+[M_2] & -\dfrac{2k_2^-}{3}-k_1^--k_2^+[M]-\lambda\end{vmatrix}=0.\tag{14}$$

We get the following equation by using the symbolic computational toolbox of Matlab software.

$$\lambda^2+b\lambda+c=0.\tag{15}$$

where

$$b=(4k_1^++k_2^+)[M]+k_2^+[M_2]+k_1^-+k_2^-,$$
$$c=6k_1^+k_2^+[M]^2+4k_1^+k_2^-[M]+3k_1^-k_2^+[M_2]+k_1^-k_2^-.$$

Obviously, $b>0,\ c>0$.

When $b^2-4c\geq 0$,

$$\lambda_{1,2}=\frac{-b\pm\sqrt{b^2-4c}}{2}<0.$$

When $b^2-4c<0$,

$$\lambda_{1,2}=\frac{-b\pm\sqrt{b^2-4c}}{2}.$$

The real parts of $\lambda_{1,2}$ are all negative.

Therefore, based on the theorem [17], the trimer building block synthesis reaction system (7) is locally asymptotic stable.

Theorem 6. As $\frac{k_2^-}{k_2^+}$ increases, $[M_3]$ will decrease in the equilibrium state.

Proof of Theorem 6. From Eq (12), we get

$$[M]=\left(\frac{k_1^-}{k_1^+}\frac{k_2^-}{k_2^+}\right)^{1/3}[M_3]^{1/3}.$$

Let

$$K_1=\frac{k_1^-}{k_1^+}, K_2=\frac{k_2^-}{k_2^+}.$$

From Eq (12), we get

$$[M]=\left(K_1K_2\right)^{1/3}[M_3]^{1/3}.$$

Substitute the above equation to Eq (11), we get

$$[M_2]=K_1^{-1/3}K_2^{2/3}[M_3]^{2/3}.$$

Substitute $[M]$ and $[M_2]$ to the constrain condition (9), then

$$K_1^{1/3}K_2^{1/3}[M_3]^{1/3}+2K_1^{-1/3}K_2^{2/3}[M_3]^{2/3}+3[M_3]=C_0.$$

Take the derivative of both sides for K_2, we get

$$\frac{1}{3}K_1^{1/3}K_2^{-2/3}[M_3]^{1/3}+\frac{1}{3}K_1^{1/3}K_2^{1/3}[M_3]^{-2/3}\frac{\partial[M_3]}{\partial K_2}+\frac{4}{3}K_1^{-1/3}K_2^{-1/3}[M_3]^{2/3}+$$
$$\frac{4}{3}K_1^{-1/3}K_2^{2/3}[M_3]^{-1/3}\frac{\partial[M_3]}{\partial K_2}+3\frac{\partial[M_3]}{\partial K_2}=0.$$

$$\frac{\partial[M_3]}{\partial K_2}=\frac{-\frac{1}{3}K_1^{1/3}K_2^{-2/3}[M_3]^{1/3}-\frac{4}{3}K_1^{-1/3}K_2^{-1/3}[M_3]^{2/3}}{\frac{1}{3}K_1^{1/3}K_2^{1/3}[M_3]^{-2/3}+\frac{4}{3}K_1^{-1/3}K_2^{2/3}[M_3]^{-1/3}+3}<0.$$

Therefore, as $\frac{k_2^-}{k_2^+}$ increases, $[M_3]$ will decrease in the equilibrium state.

Remark 4. Trimer building blocks in the equilibrium state will decrease with the increasing ratio of the off-rate constant to the on-rate constant of trimers.

2.3. Tetramer building block synthesis reactions

We consider that tetramers are the building blocks of virus assembly in vitro. Their synthesis reactions are as follows.

$$M+M\underset{k_1^-}{\overset{k_1^+}{\rightleftharpoons}}M_2,$$

$$M_2 + M \underset{k_2^-}{\overset{k_2^+}{\rightleftharpoons}} M_3,$$

$$M_3 + M \underset{k_3^-}{\overset{k_3^+}{\rightleftharpoons}} M_4.$$

The concentration of monomers decreases because they polymerize to dimers and they polymerize to trimers with dimers. Moreover, it also decreases because they polymerize to tetramers with trimers. Based on the mass conservation law and mass action law [15,16], we get

$$\frac{d[M]}{dt} = -2k_1^+[M]^2 - k_2^+[M][M_2] - k_3^+[M][M_3].$$

At the same time, the concentration of monomers will increase for dimers dissociate to monomers and trimers dissociate to monomers. Moreover, the concentration of monomers will also increase for tetramers dissociate to monomers. Based on the mass conservation law and mass action law [15,16], we get the total change rate of monomers as follows.

$$\frac{d[M]}{dt} = -2k_1^+[M]^2 - k_2^+[M][M_2] - k_3^+[M][M_3] + 2k_1^-[M_2] + k_2^-[M_3] + k_3^-[M_4].$$

With the same method, we can get the change rates of dimers, trimers and tetramers. So, the rate equation system for the above chemical reactions in vitro is as follows.

$$\begin{cases} \dfrac{d[M]}{dt} = -2k_1^+[M]^2 - k_2^+[M][M_2] - k_3^+[M][M_3] + 2k_1^-[M_2] + k_2^-[M_3] + k_3^-[M_4] \\ \dfrac{d[M_2]}{dt} = k_1^+[M]^2 + k_2^-[M_3] - k_1^-[M_2] - k_2^+[M][M_2] \\ \dfrac{d[M_3]}{dt} = k_2^+[M][M_2] + k_3^-[M_4] - k_2^-[M_3] - k_3^+[M][M_3] \\ \dfrac{d[M_4]}{dt} = k_3^+[M][M_3] - k_3^-[M_4] \end{cases} \tag{16}$$

We consider that the above synthesis reaction takes place in vitro. Monomers with concentration $C_0, (C_0 > 0)$ are added to the vitro for only one time, and no dimers, no trimers and no tetramers are added. So, the initial condition is as follows.

$$[M] = C_0, [M_2] = 0, [M_3] = 0, [M_4] = 0. \tag{17}$$

As the synthesis reaction takes place, two monomers are polymerized to a dimer. So, the concentration of dimers is equivalent to the concentration of monomers with twice their own concentration. Similarly, the concentration of trimers is equivalent to the concentration of monomers with treble their own concentration, and the concentration of tetramers is equivalent to the concentration of monomers with quadruple their own concentration. Moreover, no monomers, no dimers and no trimers will be added after the synthesis reaction takes place. So, the constraint condition is as follows.

$$[M] + 2[M_2] + 3[M_3] + 4[M_4] = C_0. \tag{18}$$

Theorem 7. The positive equilibrium point of tetramer building block synthesis reaction equations (16) exists and is unique.

Proof of Theorem 7. Let $\frac{d[M_i]}{dt}=0,\ i=1,2,3,4$ for system (16), we get

$$\begin{cases} -2k_1^+[M]^2-k_2^+[M][M_2]-k_3^+[M][M_3]+2k_1^-[M_2]+k_2^-[M_3]+k_3^-[M_4]=0 \\ k_1^+[M]^2+k_2^-[M_3]-k_1^-[M_2]-k_2^+[M][M_2]=0 \\ k_2^+[M][M_2]+k_3^-[M_4]-k_2^-[M_3]-k_3^+[M][M_3]=0 \\ k_3^+[M][M_3]-k_3^-[M_4]=0 \end{cases} \tag{19}$$

From the fourth equation of Eq (19) we get

$$[M_4]=\frac{k_3^+}{k_3^-}[M][M_3]. \tag{20}$$

Add the fourth equation to the third equation, we get

$$[M_3]=\frac{k_2^+}{k_2^-}[M][M_2]. \tag{21}$$

Add the fourth and the third equations to the second equation, we get

$$[M_2]=\frac{k_1^+}{k_1^-}[M]^2. \tag{22}$$

Substitute Eq (22) to Eq (21), we get

$$[M_3]=\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}[M]^3. \tag{23}$$

Substitute Eq (23) to Eq (20), we get

$$[M_4]=\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}\frac{k_3^+}{k_3^-}[M]^4. \tag{24}$$

Substitute $[M_2]$, $[M_3]$ and $[M_4]$ to the constraint condition (18), then we get

$$[M]+2\frac{k_1^+}{k_1^-}[M]^2+3\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}[M]^3+4\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}\frac{k_3^+}{k_3^-}[M]^4=C_0.$$

Let

$$f([M])=4\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}\frac{k_3^+}{k_3^-}[M]^4+3\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}[M]^3+2\frac{k_1^+}{k_1^-}[M]^2+[M]-C_0.$$

When $[M]=0$,

$$f(0)<0.$$

When $[M]=C_0$,

$$f(C_0)=4\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}\frac{k_3^+}{k_3^-}C_0^4+3\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}C_0^3+2\frac{k_1^+}{k_1^-}C_0^2+C_0-C_0>0.$$

Moreover,

$$f'([M])=16\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}\frac{k_3^+}{k_3^-}[M]^3+9\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}[M]^2+4\frac{k_1^+}{k_1^-}[M]\ +1>0.$$

Therefore, there is a unique positive point $[M^*]\in(0,C_0)$ to satisfy $f([M^*])=0$.

Substitute $[M^*]$ to Eqs (22)–(24), we can get the positive values $[M_2^*]$, $[M_3^*]$ and $[M_4^*]$.

Therefore, based on the theorem [17], the positive equilibrium point of tetramer building block synthesis reaction system (16) exists and is unique.

Theorem 8. The tetramer building block synthesis reaction system (16) is locally asymptotic stable.

Proof of Theorem 8. We can get $[M_4]=\frac{1}{4}(C_0-[M]-2[M_2]-3[M_3])$ from the constraint condition (18). Then we substitute it to the first three equations of system (16) and get

$$\begin{cases}\dfrac{d[M]}{dt}=-2k_1^+[M]^2-k_2^+[M][M_2]-k_3^+[M][M_3]+2k_1^-[M_2]+k_2^-[M_3]+\\ \qquad\dfrac{1}{4}k_3^-(C_0-[M]-2[M_2]-3[M_3])\\ \dfrac{d[M_2]}{dt}=k_1^+[M]^2+k_2^-[M_3]-k_1^-[M_2]-k_2^+[M][M_2]\\ \dfrac{d[M_3]}{dt}=k_2^+[M][M_2]+\dfrac{1}{4}k_3^-(C_0-[M]-2[M_2]-3[M_3])-k_2^-[M_3]-k_3^+[M][M_3]\end{cases}\tag{25}$$

The characteristic equation of the above equations is as follows

$$\begin{vmatrix}-4k_1^+[M]-k_2^+[M_2]-k_3^+[M_3]-\dfrac{k_3^-}{4}-\lambda & -k_2^+[M]+2k_1^--\dfrac{2k_3^-}{4} & -k_3^+[M]+k_2^--\dfrac{3k_3^-}{4}\\ 2k_1^+[M]-k_2^+[M_2] & -k_1^--k_2^+[M]-\lambda & k_2^-\\ k_2^+[M_2]+\dfrac{1}{4}k_3^-(-1)-k_3^+[M_3] & k_2^+[M]+\dfrac{1}{4}k_3^-(-2) & -\dfrac{3k_3^-}{4}-k_2^--k_3^+[M]-\lambda\end{vmatrix}=0.\tag{26}$$

We get the following equation by using the symbolic computational toolbox of MATLAB software.

$$\lambda^3+a\lambda^2+b\lambda+c=0,\tag{27}$$

where

$$\begin{aligned}a&=k_1^-+k_2^-+k_3^-+4k_1^+[M]+k_2^+[M]+k_2^+[M_2]+k_3^+[M]+k_3^+[M_3],\\ b&=k_1^-k_2^-+k_1^-k_3^-+k_2^-k_3^-+6k_1^+k_2^+[M]^2+4k_1^+k_3^+[M]^2+k_2^+k_3^+[M]^2+\\ &\quad 4k_1^+k_2^-[M]+3k_1^-k_2^+[M_2]+k_1^-k_3^+[M]+4k_1^+k_3^-[M]+k_1^-k_3^+[M_3]+\\ &\quad k_2^+k_3^-[M]+k_2^+k_3^-[M_2]+2k_2^-k_3^+[M_3]+2k_2^+k_3^+[M][M_2]+k_2^+k_3^+[M][M_3],\\ c&=k_1^-k_2^-k_3^-+6k_1^+k_2^+k_3^-[M]^2+8k_1^+k_2^+k_3^+[M]^3+4k_1^+k_2^-k_3^-[M]+\\ &\quad 3k_1^-k_2^+k_3^-[M_2]+4k_1^-k_2^-k_3^+[M_3]+4k_1^-k_2^+k_3^+[M][M_2].\end{aligned}$$

The Routh-Hurwitz matrix [18] is as follows

$$RH = \begin{pmatrix} c_{11} & c_{12} & 0 \\ c_{21} & c_{22} & 0 \\ c_{31} & 0 & \\ c_{41} & & \end{pmatrix}, \tag{28}$$

$$c_{11} = 1,$$
$$c_{12} = b,$$
$$c_{21} = a,$$
$$c_{22} = c.$$

We compute the positive or negative signs of the first column elements in the Routh-Hurwitz matrix (28) by using the symbolic computational toolbox of MATLAB software. For their expression is too complex, we only show their signs as follows.

$$\begin{aligned} &c_{11} > 0, \\ &c_{21} > 0, \\ &c_{31} = \frac{-\begin{vmatrix} c_{11} & c_{12} \\ c_{21} & c_{22} \end{vmatrix}}{c_{21}} > 0, \\ &c_{41} = \frac{-\begin{vmatrix} c_{21} & c_{22} \\ c_{31} & 0 \end{vmatrix}}{c_{31}} > 0. \end{aligned} \tag{29}$$

Based on the Routh-Hurwitz rule [18], the real parts of all eigenvalues are all negative.

Therefore, based on the theorem [17], the tetramer building block synthesis reaction system (16) is locally asymptotic stable.

2.4. Pentamer building block synthesis reactions

We consider that pentamers are the building blocks of virus assembly in vitro. Their synthesis reactions are as follows.

$$M + M \underset{k_1^-}{\overset{k_1^+}{\rightleftharpoons}} M_2,$$

$$M_2 + M \underset{k_2^-}{\overset{k_2^+}{\rightleftharpoons}} M_3,$$

$$M_3 + M \underset{k_3^-}{\overset{k_3^+}{\rightleftharpoons}} M_4,$$

$$M_4 + M \underset{k_4^-}{\overset{k_4^+}{\rightleftharpoons}} M_5.$$

The concentration of monomers will decrease for they polymerize to dimers and they polymerize to trimers with dimers. Moreover, they will also decrease for they polymerize to tetramers with trimers

and they polymerize to pentamers with tetramers. Based on the mass conservation law and mass action law [15,16], we get

$$\frac{d[M]}{dt} = -2k_1^+[M]^2 - k_2^+[M][M_2] - k_3^+[M][M_3] - k_4^+[M][M_4].$$

At the same time, the concentration of monomers will increase for dimers dissociate to monomers and trimers dissociate to monomers. Moreover, the concentration of monomers will also increase for tetramers dissociate to monomers and pentamers dissociate to monomers. Based on the mass conservation law and mass action law [15,16], we get the total change rate of monomers as follows.

$$\begin{aligned}\frac{d[M]}{dt} = &-2k_1^+[M]^2 - k_2^+[M][M_2] - k_3^+[M][M_3] - k_4^+[M][M_4] + 2k_1^-[M_2] + \\ &k_2^-[M_3] + k_3^-[M_4] + k_4^-[M_5]\end{aligned}.$$

With the same method, we can get the change rates of dimers, trimers, tetramers and pentamers. So, the rate equation system for the above chemical reactions in vitro is as follows.

$$\begin{cases}\dfrac{d[M]}{dt} = -2k_1^+[M]^2 - k_2^+[M][M_2] - k_3^+[M][M_3] - k_4^+[M][M_4] + 2k_1^-[M_2] + \\ \qquad k_2^-[M_3] + k_3^-[M_4] + k_4^-[M_5] \\ \dfrac{d[M_2]}{dt} = k_1^+[M]^2 + k_2^-[M_3] - k_1^-[M_2] - k_2^+[M][M_2] \\ \dfrac{d[M_3]}{dt} = k_2^+[M][M_2] + k_3^-[M_4] - k_2^-[M_3] - k_3^+[M][M_3] \\ \dfrac{d[M_4]}{dt} = k_3^+[M][M_3] + k_4^-[M_5] - k_3^-[M_4] - k_4^+[M][M_4] \\ \dfrac{d[M_5]}{dt} = k_4^+[M][M_4] - k_4^-[M_5]\end{cases} \tag{30}$$

The initial condition is

$$[M] = C_0, [M_2] = 0, [M_3] = 0, [M_4] = 0, [M_5] = 0. \tag{31}$$

The constraint condition is

$$[M] + 2[M_2] + 3[M_3] + 4[M_4] + 5[M_5] = C_0. \tag{32}$$

Theorem 9. The positive equilibrium point of pentamer building block synthesis reaction equations (30) exists and is unique.

Proof of Theorem 9. Let $\frac{d[M_i]}{dt}=0,\ i=1,2,3,4,5$ for System (30), we get

$$\begin{cases}-2k_1^+[M]^2 - k_2^+[M][M_2] - k_3^+[M][M_3] - k_4^+[M][M_4] + 2k_1^-[M_2] + \\ \qquad k_2^-[M_3] + k_3^-[M_4] + k_4^-[M_5] = 0 \\ k_1^+[M]^2 + k_2^-[M_3] - k_1^-[M_2] - k_2^+[M][M_2] = 0 \\ k_2^+[M][M_2] + k_3^-[M_4] - k_2^-[M_3] - k_3^+[M][M_3] = 0 \\ k_3^+[M][M_3] + k_4^-[M_5] - k_3^-[M_4] - k_4^+[M][M_4] = 0 \\ k_4^+[M][M_4] - k_4^-[M_5] = 0\end{cases} \tag{33}$$

From the fifth equation of Eq (33) we get

$$[M_5]=\frac{k_4^+}{k_4^-}[M][M_4]. \tag{34}$$

Add the fifth equation to the fourth equation of Eq (33), we get

$$[M_4]=\frac{k_3^+}{k_3^-}[M][M_3]. \tag{35}$$

Add the fifth and the fourth equations to the third equation, we get

$$[M_3]=\frac{k_2^+}{k_2^-}[M][M_2]. \tag{36}$$

Add the fifth, the fourth and the third equations to the second equation, we get

$$[M_2]=\frac{k_1^+}{k_1^-}[M]^2. \tag{37}$$

Substitute Eq (37) to Eq (36), we get

$$[M_3]=\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}[M]^3. \tag{38}$$

Substitute Eq (38) to Eq (35), we get

$$[M_4]=\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}\frac{k_3^+}{k_3^-}[M]^4. \tag{39}$$

Substitute Eq (39) to Eq (34), we get

$$[M_5]=\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}\frac{k_3^+}{k_3^-}\frac{k_4^+}{k_4^-}[M]^5. \tag{40}$$

Substitute $[M_2]$, $[M_3]$, $[M_4]$ and $[M_5]$ to the constraint condition (32), then we get

$$[M]+2\frac{k_1^+}{k_1^-}[M]^2+3\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}[M]^3+4\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}\frac{k_3^+}{k_3^-}[M]^4+5\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}\frac{k_3^+}{k_3^-}\frac{k_4^+}{k_4^-}[M]^5=C_0.$$

Let

$$f([M])=5\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}\frac{k_3^+}{k_3^-}\frac{k_4^+}{k_4^-}[M]^5+4\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}\frac{k_3^+}{k_3^-}[M]^4+3\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}[M]^3+2\frac{k_1^+}{k_1^-}[M]^2+[M]-C_0.$$

When $[M]=0$,

$$f(0)<0.$$

When $[M]=C_0$,

$$f(C_0)=5\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}\frac{k_3^+}{k_3^-}\frac{k_4^+}{k_4^-}C_0^5+4\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}\frac{k_3^+}{k_3^-}C_0^4+3\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}C_0^3+2\frac{k_1^+}{k_1^-}C_0^2+C_0-C_0>0.$$

Moreover,

$$f'([M])=20\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}\frac{k_3^+}{k_3^-}\frac{k_4^+}{k_4^-}[M]^4+16\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}\frac{k_3^+}{k_3^-}[M]^3+9\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}[M]^2+4\frac{k_1^+}{k_1^-}[M]+1>0.$$

Therefore, there is a unique positive point $[M^*]\in(0,C_0)$ to satisfy $f([M^*])=0$.

Substitute $[M^*]$ to Eqs (37)–(40) , we can get the positive values $[M_2^*]$, $[M_3^*]$, $[M_4^*]$ and $[M_5^*]$.

Therefore, based on the theorem [17], the positive equilibrium point of pentamer building block synthesis reaction system (30) exists and is unique.

Theorem 10. The pentamer building block synthesis reaction system (30) is locally asymptotic stable.

Proof of Theorem 10. We can get $[M_5]=\frac{1}{5}(C_0-[M]-2[M_2]-3[M_3]-4[M_4])$ from the constraint condition (32). Then we substitute it to the first four equations of System (30) and get

$$\begin{cases}\dfrac{d[M]}{dt}=-2k_1^+[M]^2-k_2^+[M][M_2]-k_3^+[M][M_3]-k_4^+[M][M_4]+2k_1^-[M_2]+\\ \qquad k_2^-[M_3]+k_3^-[M_4]+k_4^-\dfrac{1}{5}(C_0-[M]-2[M_2]-3[M_3]-4[M_4])\\ \dfrac{d[M_2]}{dt}=k_1^+[M]^2+k_2^-[M_3]-k_1^-[M_2]-k_2^+[M][M_2]\\ \dfrac{d[M_3]}{dt}=k_2^+[M][M_2]+k_3^-[M_4]-k_2^-[M_3]-k_3^+[M][M_3]\\ \dfrac{d[M_4]}{dt}=k_3^+[M][M_3]+k_4^-\dfrac{1}{5}(C_0-[M]-2[M_2]-3[M_3]-4[M_4])-k_3^-[M_4]-k_4^+[M][M_4]\end{cases}\tag{41}$$

The characteristic equation of the above equations is computed by using the symbolic computational toolbox of MATLAB software. Moreover, the Routh-Hurwitz matrix is also got by using the symbolic computational toolbox of MATLAB software. For characteristic equation and Routh-Hurwitz matrix are all too complex, we do not show them here. Then the first column elements of the Routh-Hurwitz matrix are all positive by using the symbolic computational toolbox of MATLAB software. Based on the Routh-Hurwitz rule [18], the real parts of all eigenvalues of the characteristic equation are all negative. Therefore, based on the theorem [17], the pentamer building block synthesis reaction system (30) is locally asymptotic stable.

2.5. Hexamer building block synthesis reactions

We consider that hexamers are the building blocks of virus assembly in vitro. Their synthesis reactions are as follows.

$$M+M\underset{k_1^-}{\overset{k_1^+}{\rightleftharpoons}}M_2,$$

$$M_2 + M \underset{k_2^-}{\overset{k_2^+}{\rightleftharpoons}} M_3,$$

$$M_3 + M \underset{k_3^-}{\overset{k_3^+}{\rightleftharpoons}} M_4,$$

$$M_4 + M \underset{k_4^-}{\overset{k_4^+}{\rightleftharpoons}} M_5,$$

$$M_5 + M \underset{k_5^-}{\overset{k_5^+}{\rightleftharpoons}} M_6.$$

The concentration of monomers will decrease for they polymerize to dimers and they polymerize to trimers with dimers. They will also decrease for they polymerize to tetramers with trimers. Moreover, they will decrease for they polymerize to pentamers with tetramers and they polymerize to hexamers with pentamers. Based on the mass conservation law and mass action law [15,16], we get

$$\frac{d[M]}{dt} = -2k_1^+[M]^2 - k_2^+[M][M_2] - k_3^+[M][M_3] - k_4^+[M][M_4] - k_5^+[M][M_5].$$

At the same time, the concentration of monomers will increase for dimers dissociate to monomers and trimers dissociate to monomers. The concentration of monomers will also increase for tetramers dissociate to monomers. Moreover, the concentration of monomers will increase for pentamers dissociate to monomers and hexamers dissociate to monomers. Based on the mass conservation law and mass action law [15,16], we get the total change rate of monomers as follows.

$$\begin{aligned}\frac{d[M]}{dt} = &-2k_1^+[M]^2 - k_2^+[M][M_2] - k_3^+[M][M_3] - k_4^+[M][M_4] - k_5^+[M][M_5] + 2k_1^-[M_2] + \\ &k_2^-[M_3] + k_3^-[M_4] + k_4^-[M_5] + k_5^-[M_6].\end{aligned}$$

With the same method, we can get the change rates of dimers, trimers, tetramers, pentamers and hexamers. So, the rate equation system for the above chemical reactions in vitro is as follows.

$$\begin{cases}\dfrac{d[M]}{dt} = -2k_1^+[M]^2 - k_2^+[M][M_2] - k_3^+[M][M_3] - k_4^+[M][M_4] - k_5^+[M][M_5] + \\ \qquad 2k_1^-[M_2] + k_2^-[M_3] + k_3^-[M_4] + k_4^-[M_5] + k_5^-[M_6] \\ \dfrac{d[M_2]}{dt} = k_1^+[M]^2 + k_2^-[M_3] - k_1^-[M_2] - k_2^+[M][M_2] \\ \dfrac{d[M_3]}{dt} = k_2^+[M][M_2] + k_3^-[M_4] - k_2^-[M_3] - k_3^+[M][M_3] \\ \dfrac{d[M_4]}{dt} = k_3^+[M][M_3] + k_4^-[M_5] - k_3^-[M_4] - k_4^+[M][M_4] \\ \dfrac{d[M_5]}{dt} = k_4^+[M][M_4] + k_5^-[M_6] - k_4^-[M_5] - k_5^+[M][M_5] \\ \dfrac{d[M_6]}{dt} = k_5^+[M][M_5] - k_5^-[M_6]\end{cases} \tag{42}$$

The initial condition is

$$[M] = C_0, [M_2] = 0, [M_3] = 0, [M_4] = 0, [M_5] = 0, [M_6] = 0.$$

The constraint condition is

$$[M]+2[M_2]+3[M_3]+4[M_4]+5[M_5]+6[M_6]=C_0. \tag{43}$$

Theorem 11. The positive equilibrium point of hexamer building block synthesis reaction equation (42) exists and is unique.

Proof of Theorem 11. Let $\frac{d[M_i]}{dt}=0,\ i=1,2,3,4,5,6$ for system (42), we get

$$\begin{cases} -2k_1^+[M]^2-k_2^+[M][M_2]-k_3^+[M][M_3]-k_4^+[M][M_4]-k_5^+[M][M_5]+2k_1^-[M_2]+ \\ \quad k_2^-[M_3]+k_3^-[M_4]+k_4^-[M_5]+k_5^-[M_6]=0 \\ k_1^+[M]^2+k_2^-[M_3]-k_1^-[M_2]-k_2^+[M][M_2]=0 \\ k_2^+[M][M_2]+k_3^-[M_4]-k_2^-[M_3]-k_3^+[M][M_3]=0 \\ k_3^+[M][M_3]+k_4^-[M_5]-k_3^-[M_4]-k_4^+[M][M_4]=0 \\ k_4^+[M][M_4]+k_5^-[M_6]-k_4^-[M_5]-k_5^+[M][M_5]=0 \\ k_5^+[M][M_5]-k_5^-[M_6]=0 \end{cases} \tag{44}$$

From the sixth equation of Eq (44) we get

$$[M_6]=\frac{k_5^+}{k_5^-}[M][M_5]. \tag{45}$$

Add the sixth equation to the fifth equation of Eq (44), we get

$$[M_5]=\frac{k_4^+}{k_4^-}[M][M_4]. \tag{46}$$

Add the sixth and the fifth equations to the fourth equation of Eq (44), we get

$$[M_4]=\frac{k_3^+}{k_3^-}[M][M_3]. \tag{47}$$

Add the sixth, the fifth and the fourth equations to the third equation, we get

$$[M_3]=\frac{k_2^+}{k_2^-}[M][M_2]. \tag{48}$$

Add the sixth, the fifth, the fourth and the third equations to the second equation, we get

$$[M_2]=\frac{k_1^+}{k_1^-}[M]^2. \tag{49}$$

Substitute Eq (49) to Eq (48), we get

$$[M_3]=\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}[M]^3. \tag{50}$$

Substitute Eq (50) to Eq (47), we get

$$[M_4]=\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}\frac{k_3^+}{k_3^-}[M]^4. \tag{51}$$

Substitute Eq (51) to Eq (46), we get

$$[M_5]=\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}\frac{k_3^+}{k_3^-}\frac{k_4^+}{k_4^-}[M]^5. \tag{52}$$

Substitute Eq (52) to Eq (45), we get

$$[M_6]=\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}\frac{k_3^+}{k_3^-}\frac{k_4^+}{k_4^-}\frac{k_5^+}{k_5^-}[M]^6. \tag{53}$$

Substitute $[M_2]$, $[M_3]$, $[M_4]$, $[M_5]$ and $[M_6]$ to the constraint condition (43), then we get

$$[M]+2\frac{k_1^+}{k_1^-}[M]^2+3\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}[M]^3+4\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}\frac{k_3^+}{k_3^-}[M]^4+5\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}\frac{k_3^+}{k_3^-}\frac{k_4^+}{k_4^-}[M]^5+6\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}\frac{k_3^+}{k_3^-}\frac{k_4^+}{k_4^-}\frac{k_5^+}{k_5^-}[M]^6=C_0.$$

Let

$$f([M])=6\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}\frac{k_3^+}{k_3^-}\frac{k_4^+}{k_4^-}\frac{k_5^+}{k_5^-}[M]^6+5\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}\frac{k_3^+}{k_3^-}\frac{k_4^+}{k_4^-}[M]^5+4\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}\frac{k_3^+}{k_3^-}[M]^4+$$
$$3\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}[M]^3+2\frac{k_1^+}{k_1^-}[M]^2+[M]-C_0.$$

When $[M]=0$,

$$f(0)<0.$$

When $[M]=C_0$,

$$f(C_0)=6\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}\frac{k_3^+}{k_3^-}\frac{k_4^+}{k_4^-}\frac{k_5^+}{k_5^-}{C_0}^6+5\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}\frac{k_3^+}{k_3^-}\frac{k_4^+}{k_4^-}{C_0}^5+4\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}\frac{k_3^+}{k_3^-}C_0^4+$$
$$3\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}C_0^3+2\frac{k_1^+}{k_1^-}C_0^2+C_0-C_0>0.$$

Moreover,

$$f'([M])=30\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}\frac{k_3^+}{k_3^-}\frac{k_4^+}{k_4^-}\frac{k_5^+}{k_5^-}[M]^5+20\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}\frac{k_3^+}{k_3^-}\frac{k_4^+}{k_4^-}[M]^4+16\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}\frac{k_3^+}{k_3^-}[M]^3+$$
$$9\frac{k_1^+}{k_1^-}\frac{k_2^+}{k_2^-}[M]^2+4\frac{k_1^+}{k_1^-}[M]+1>0.$$

Therefore, there is a unique positive point $[M^*]\in(0,C_0)$ to satisfy $f([M^*])=0$.

Substitute $[M^*]$ to Eqs (49)–(53) , we can get the positive values $[M_2^*]$, $[M_3^*]$, $[M_4^*]$, $[M_5^*]$ and $[M_6^*]$.

Therefore, based on the theorem [17], the positive equilibrium point of hexamer building block synthesis reaction system (44) exists and is unique.

Theorem 12. The hexamer building block synthesis reaction system (42) is locally asymptotic stable.

Proof of Theorem 12. We can get $[M_6]=\frac{1}{6}(C_0-[M]-2[M_2]-3[M_3]-4[M_4]-5[M_5])$ from the constraint condition (43). Then we substitute it to the first five equations of system (42) and get

$$\begin{cases} \frac{d[M]}{dt}=-2k_1^+[M]^2-k_2^+[M][M_2]-k_3^+[M][M_3]-k_4^+[M][M_4]-k_5^+[M][M_5]+2k_1^-[M_2]+ \\ \qquad k_2^-[M_3]+k_3^-[M_4]+k_4^-[M_5]+k_5^-\frac{1}{6}(C_0-[M]-2[M_2]-3[M_3]-4[M_4]-5[M_5]) \\ \frac{d[M_2]}{dt}=k_1^+[M]^2+k_2^-[M_3]-k_1^-[M_2]-k_2^+[M][M_2] \\ \frac{d[M_3]}{dt}=k_2^+[M][M_2]+k_3^-[M_4]-k_2^-[M_3]-k_3^+[M][M_3] \\ \frac{d[M_4]}{dt}=k_3^+[M][M_3]+k_4^-[M_5]-k_3^-[M_4]-k_4^+[M][M_4] \\ \frac{d[M_5]}{dt}=k_4^+[M][M_4]+k_5^-\frac{1}{6}(C_0-[M]-2[M_2]-3[M_3]-4[M_4]-5[M_5])- \\ \qquad k_4^-[M_5]-k_5^+[M][M_5] \end{cases} \tag{54}$$

The characteristic equation of the above equations is computed by using the symbolic computational toolbox of MATLAB software. Moreover, the Routh-Hurwitz matrix is also got by using the symbolic computational toolbox of MATLAB software. For characteristic equation and Routh-Hurwitz matrix are all too complex, we do not show them here. Then the first column elements of the Routh- Hurwitz matrix are all positive by using the symbolic computational toolbox of MATLAB software. Based on the Routh- Hurwitz rule [18], the real parts of all eigenvalues of the characteristic equation are all negative. Therefore, based on the theorem [17], the hexamer building block synthesis reaction system (42) is locally asymptotic stable.

3. Results and conclusions

Virus assembly is a necessary step of virus replication. It consists of two processes. The first process is that monomers polymerize to building blocks, and the second process is that building blocks polymerize into virus shells. In this work, the first process is studied. In an ordinary way, a building block consists of less than six monomers. That is, building block types include dimer, trimer, tetramer, pentamer and hexamer. We comprehensively consider all five types of building block synthesis reactions in vitro, in which one monomer is polymerized in each chemical reaction for the synthesis process of the building block. The corresponding rate equation systems are obtained. Then we prove that the positive equilibrium point of each building block synthesis reaction equation system exists and is unique. Furthermore, we prove that all building block synthesis reaction systems are locally asymptotic stable. We get the function of monomer and dimer concentrations for dimer building blocks in the equilibrium state. We also got the function of all polymers and monomers for trimer, tetramer,

pentamer and hexamer building blocks in the equilibrium state, respectively. Based on our analysis, dimer building blocks in the equilibrium state will decrease as the ratio of the off-rate constant to the on-rate constant increases. Trimer building blocks in the equilibrium state will decrease with the increasing ratio of the off-rate constant to the on-rate constant of trimers. These results can provide further insight into the virus-building block synthesis dynamic property in vitro.

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Conflict of interest

The authors declare there is no conflict of interest.

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